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# Ecological and metabolomic responses to environmental constraints of brown and red marine macroalgae from the intertidal zone

Fanny Lalegerie

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Fanny Lalegerie. Ecological and metabolomic responses to environmental constraints of brown and red marine macroalgae from the intertidal zone. *Vegetal Biology*. Université de Bretagne occidentale - Brest, 2020. English. NNT : 2020BRES0059 . tel-03378753

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# THESE DE DOCTORAT DE

L'UNIVERSITE  
DE BRETAGNE OCCIDENTALE

ECOLE DOCTORALE N° 598  
*Sciences de la Mer et du littoral*  
Spécialité : Biologie marine

Par

**Fanny LALEGERIE**

« **Ecological and metabolomic responses to environmental constraints of brown and red marine macroalgae from the intertidal zone** »

Réponses écologique et métabolomique aux contraintes environnementales des macroalgues marines rouges et brunes de la zone intertidale

**Thèse présentée et soutenue à Plouzané, le 10/11/2020**

**Unité de recherche : Laboratoire des Sciences de l'Environnement Marin (UMR 6539)**

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## *Remerciements*

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Je tiens en premier lieu à remercier Solène Connan et Valérie Stiger-Pouvreau, mes encadrantes de thèse avec qui j'ai passé trois années formidables. Mille mercis de m'avoir fait confiance, et d'avoir toujours été là quand j'en avais besoin. Merci pour votre écoute, pour tous vos précieux conseils, pour votre bienveillance, et pour avoir toujours su trouver les mots qu'il fallait. Nos échanges et nos petites réunions ont toujours été très constructifs et j'ai appris énormément avec vous, que ce soit sur le plan professionnel ou sur le plan personnel. Je ressors grandie de cette super aventure avec vous, et vous me manquez déjà beaucoup !

Je veux aussi remercier la Région Bretagne, l'École Doctorale des Sciences de la Mer et du Littoral (EDSML) et l'Université de Bretagne Occidentale (UBO) d'avoir financé et supporté ce projet de thèse. Je tiens également à remercier Luis Tito de Morais pour m'avoir accueilli au sein du laboratoire des sciences de l'environnement marin (LEMAR).

I would like to extend my deep gratitude to Thierry Thibaut, Dominique Davoult, Ulf Karsten, Dagmar Stengel and Nathalie Bourgougnon for agreeing to take part in my thesis jury and to evaluate my PhD thesis. I also warmly thank Dagmar Stengel, Gilles Bedoux and Sylvain Petek for having accepted to participate in my thesis committee. Thank you all for your precious advices.

I gratefully acknowledge Dagmar who welcomed me for 2 months in Ireland. It was a real pleasure to spend time in Galway in your laboratory. I very much appreciated our exchanges and I am grateful for all your help. Thank you for everything!

Je veux aussi remercier tous ceux avec qui j'ai pu échanger au cours de ces trois années, qui m'ont aidé d'une manière ou d'une autre et qui ont contribué à enrichir le contenu de cette thèse. Merci Nelly Kervarec pour ton travail sur les composés phénoliques, et pour ta bonne humeur à toute épreuve ! Merci Gérald Culioli pour tes précieux conseils sur les terpènes. Merci Fabienne Le Grand et Antoine Bideau pour votre aide sur les analyses d'acides gras. Merci Sébastien Artigaud pour les analyses MS. Merci Stéphane Cérantola et Gaëlle Simon pour m'avoir aidé sur les analyses RMN. Thank you Marianela Zanolla for your help in the field or in the laboratory in Galway. Finally, I greatly thank Ulf Karsten, Markus Ganzera and Anja Hartmann for their very helpful contribution on the quantification of MAAs.

Je tiens aussi à remercier Gauthier Schaal, pour m'avoir pris sous son aile au commencement, et sans qui je ne serais peut-être jamais arrivé jusqu'ici.

Merci aussi à l'équipe du LEBHAM, aux deux compères Erwan et Michel, à Mapou, Eric, Sirine, et aux nombreux stagiaires qui sont passés par là, avec qui on aura bien rigolé (et mangé beaucoup de gâteaux !). Merci notamment à Amandine, Mathieu et Benoit pour

m'avoir aidé sur l'analyse des terpènes ou des composés phénoliques. Merci aussi Mapou de m'avoir broyé une (très) grande partie de mes échantillons ! Et un grand merci à Sirine, pour ton aide au laboratoire, les bonnes pâtisseries tunisiennes, et pour m'avoir accompagnée au Maroc, j'en garde pleins de merveilleux souvenirs.

Ensuite je tiens à remercier mes deux acolytes algologues, la petite Leslie et Thomas (aka toto). Qu'est-ce qu'aurais été cette thèse sans vous ?! Merci de m'avoir autant fait rire et d'avoir été là du début jusqu'à la fin. J'ai adoré passer ses trois ans à vos côtés, à écouter les blagues de toto et le beau rire de Leslie ! Je garde aussi en mémoire notre super voyage en Corée. Merci pour tous ces bons moments inoubliables.

Je n'oublie pas non plus toute la troupe de doctorants/ingénieurs du LEMAR (anciens, actuels ou à venir) avec qui on aura bien rigolé. Merci au quatuor Julien M., Sarah I., Justine D., et Kévin T. pour votre soutien et tous les bons moments passés ensemble. Merci à Guillaume B., Pierre C., et Youenn J., mes super collègues de master que j'ai eu le plaisir de retrouver en thèse. Merci à tous les petits nouveaux pour les longues pauses café et les supers moments de rigolade, Amandine M., Chirine T., Luana A., Mariana V., et Valentin S.. Merci aussi à tous les anciens doctorants pour nous avoir montré le chemin à suivre, Alexandra R., Aurélien B., Fanny K., Jordan T., Marc L. (et maintenant Natalia L.M.), Nicolas D. et William H.. Merci à mon jumeau d'anniversaire Antoine B. pour nous avoir bien faire rire ! Merci aussi au douarneniste Gaëtan O.. Et bon courage à tous les futurs doctorants que je n'oublie pas, Rémi B., Nolwenn K., et Wendy D.. Merci à tous pour tous ces supers moments passés ensemble !

Enfin, bien sûr, merci à toute ma famille, mes parents, ma sœur, mon fréro, mon papy, et ma super belle-famille. Et surtout un gros merci à celui qui est à mes côtés depuis le début. Merci de m'avoir supportée, épaulée et écoutée parler des heures. Désolé pour tout ce que je t'ai fait endurer mais promis c'est (presque) fini !

## ***Publications and communications of the PhD thesis***

### ***Published articles***

**Lalegerie F.**, Lajili S., Bedoux G., Taupin L., Stiger-Pouvreau V., Connan S. (2019) Photo-protective compounds in red macroalgae from Brittany: Considerable diversity in mycosporine-like amino acids (MAAs). *Marine Environmental Research*, 147, 37-48. DOI: 10.1016/j.marenvres.2019.04.001

**Lalegerie F.**, Gager L., Stiger-Pouvreau V., and Connan, S. (2020). The stressful life of red and brown seaweeds on the temperate intertidal zone: Effect of abiotic and biotic parameters on the physiology of macroalgae and content variability of particular metabolites. In: Bourgougnon N. (Ed.), *Seaweeds around the world: state of art and perspectives. Advances in Botanical Research* (Vol. 95, pp. 247-287). Academic Press.

**Lalegerie F.**, Stiger-Pouvreau V., Connan S. (2020). Temporal variation in pigment and mycosporine-like amino acid composition of the red macroalga *Palmaria palmata* from Brittany (France): hypothesis on the MAA biosynthesis pathway under high irradiance. *Proceedings of the International Seaweeds Symposium. Journal of Applied Phycology*, 32, 2641-2656. DOI: 10.1007/s10811-020-02075-7

Gager L.\*, **Lalegerie F.\***, Connan S., Stiger-Pouvreau V (In press) Algal-derived phenolic compounds and their biological activities. In Rajauria G. and Yuan Y. (Eds.), *Recent advancements in micro and macroalgal processing: food and health perspectives*. Wiley.

### ***Articles in preparation***

**Lalegerie F.**, Stiger-Pouvreau V., Connan S. (In prep.). Research note - A cultivation experiments on the red macroalga *Palmaria palmata*: effect of UV radiation and nutrients on mycosporine-like amino acids and pigments

**Lalegerie F.**, Stiger-Pouvreau V., Zanolla M., Stengel D., Connan S. (In prep.) Phenological and metabolical study of *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) 30 years after its introduction in Brittany (France): a real threat for the native species *Palmaria palmata*?

**Lalegerie F.**, Connan S., Stiger-Pouvreau V. (In prep.). Research note - Intra-site variability in terpene composition of *Bifurcaria bifurcata* (Phaeophyceae, Fucales): evidence of microhabitat occurrence

**Lalegerie F.**, Stiger-Pouvreau V., Zanolla M., Stengel D., Connan S. (In prep.) Contrasting phenology and chemical strategies of native and invasive seaweeds: *Bifurcaria bifurcata* and *Sargassum muticum* (Sargassaceae, Fucales)

**Lalegerie F.**, Stiger-Pouvreau V., Connan S. (In prep.). Ecological and metabolomic monitoring of the brown macroalga *Halidrys siliquosa* (Sargassaceae, Fucales)

**Lalegerie F.**, Stiger-Pouvreau V., Zanolla M., Stengel D., Bideau A., Le Grand F., Connan S. (In prep.) *Halidrys siliquosa*, a species threatened by its geographical position and co-existence with the invasive *Sargassum muticum*? A comparison between France and Ireland

## ***Communications***

**6<sup>th</sup> edition of the International School of Research** (Ibn Zohr University, Agadir, Morocco, 19<sup>th</sup> – 21<sup>th</sup> April 2018) “Biodiversity, Biotechnologies, terroir product and enhancement of natural substances” - Oral presentation (« Réponses écologiques et physiologiques des macroalgues marines aux contraintes environnementales »)

**23<sup>rd</sup> International Seaweed Symposium** (International Convention Center, Jeju, Korea, April 28<sup>th</sup> – May 3<sup>rd</sup> 2019) “Seaweeds: from tradition to innovation !” - Oral presentation (Variability in the composition of mycosporine-like amino acids (MAAs) in red macroalgae from Brittany (France))

**Colloque de la Société Phycologique de France 2019** (Jussieu University, Paris, France, 14<sup>th</sup> - 15<sup>th</sup> Novembre 2019) - Oral presentation (« Mycosporine-like amino acids (MAAs): diversité chez les algues rouges intertidales de Bretagne »)

**Journées de l'EDSML** (email transmission due to Covid-19) - Poster (« Mycosporine-like amino acids (MAAs) in red macroalgae from Brittany: considerable diversity and temporal variations »).

## *List of abbreviations*

ACN: acetonitrile  
DCM: dichloromethane  
DW: dry weight  
EtOH: ethanol  
FA: fatty acids  
FAME: fatty acid methyl ester  
GC: gas chromatography  
GLOT: global radiation ( $\text{J}\cdot\text{cm}^{-2}$ )  
HPLC: high pressure liquid chromatography  
INST: insolation time (hours)  
KW: Kruskal-Wallis test  
LC: liquid chromatography  
MAAs: mycosporine-like amino acids  
MeOH: methanol  
MS: mass spectrometry  
MUFA: monounsaturated fatty acid  
PAR: photosynthetically active radiation  
PC: phenolic compounds  
PCA: principal component analysis  
PUFA: polyunsaturated fatty acid  
SFA: saturated fatty acid  
SRH: Scheirer-Ray-Hare test  
TPC: total phenolic content  
TFA: total fatty acids  
TLC: thin layer chromatography  
UVR: ultraviolet radiation



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\* The reference section available at the end of the manuscript do not include references from the publications (published or in preparation) of **Chapters 2 and 3**, and from the published review in the **part 1.2 of the General Introduction**

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# *General introduction*

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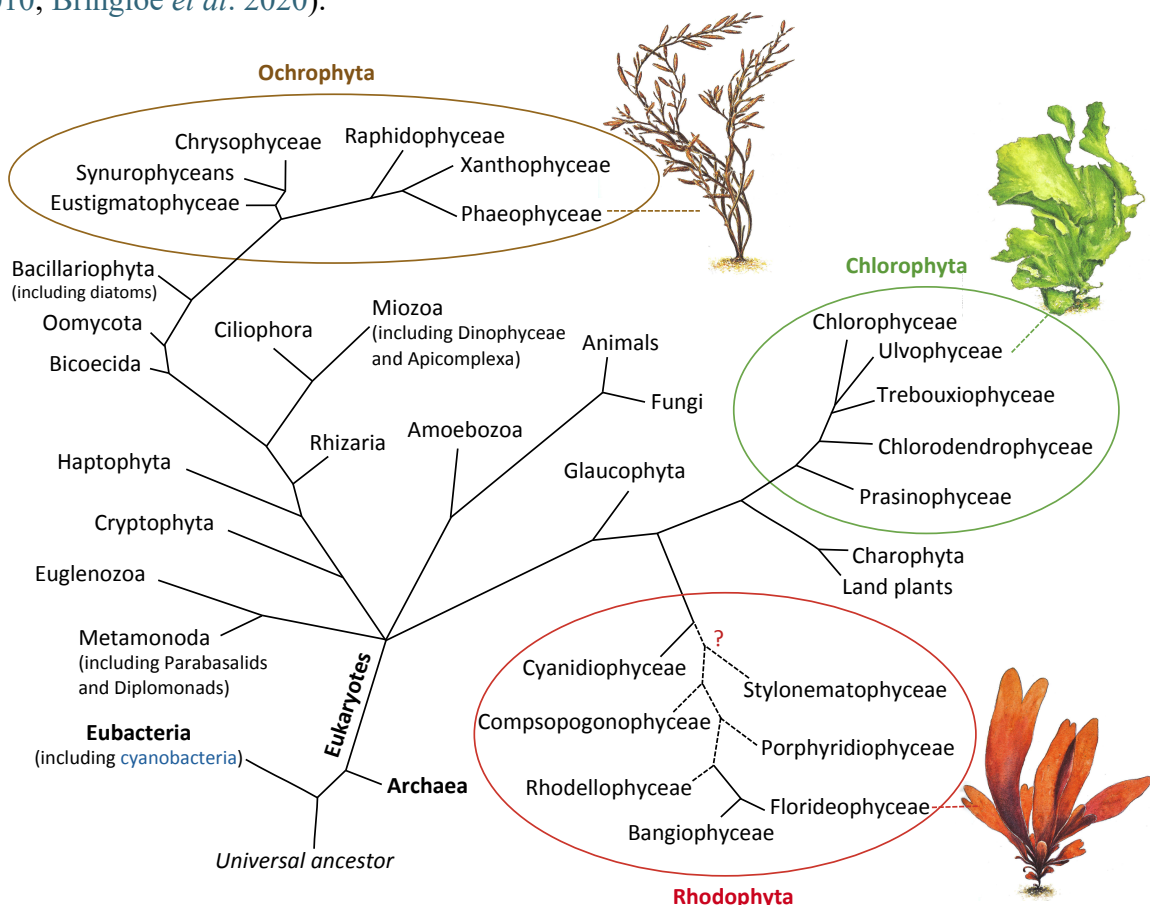
## 1. General context

### 1.1 Algae: key members of marine ecosystems

The marine environment is inhabited by a wide diversity of organisms, including algae which are at the base of the marine food chain. The term "algae" refers to a polyphyletic assemblage consisting of several evolutionary lineages that do not share a recent common evolutionary ancestor. Algae are therefore a group of organisms with a very wide diversity. Although they are encountered in a large variety of habitats exhibiting a wide range of physico-chemical conditions, algae are almost exclusively aquatic organisms (from freshwater to marine environment). In addition, they are all photosynthetic organisms and thus possess chlorophyll-*a* pigment (Lee 1999; De Reviers 2002; Stengel *et al.* 2011; Gallardo 2014). The distinction of the different algal taxonomic groups is then mainly based on their size (*i.e.* microscopic or macroscopic) and specific pigmentation (*i.e.* the green Chlorophyta, the red Rhodophyta, the brown Ochrophyta and the blue Cyanobacteria) (Fig. 1).

In this way, "microalgae" refers to unicellular and multicellular organisms, ranging from few to hundred micrometres. They constitute phytoplankton in open oceans, or microphytobenthos living on any immersed substrate. This group encompasses the prokaryote cyanobacteria (Eubacteria, known as blue algae or Cyanophyceae), and eukaryotes such as Eustigmatophyceae, Porphyridiophyceae, or Prasinophyceae, contained in Ochrophyta, Rhodophyta and Chlorophyta phyla respectively. On the contrary "macroalgae", called seaweeds if living in marine and estuarine waters, are all multicellular eukaryotes, ranging from few centimetres, such as small filamentous *Ceramium* species (Ceramiales, Florideophyceae), to size of up to 40 m as in the case of the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae). They are composed of a vegetative tissue commonly defined as a thallus that encompasses very diverse morphologies (*e.g.* foliaceous, filamentous, corticated, encrusted or calcareous). Macroalgae can be classified into three different groups according to their pigment content: brown macroalgae (all species of Phaeophyceae, belonging to Ochrophyta), red macroalgae (mainly encountered in Florideophyceae and Bangiophyceae among Rhodophyta) and green macroalgae (mostly Ulvophyceae, within Chlorophyta) (Fig. 1) (Yoon *et al.* 2010; Stengel *et al.* 2011; Gallardo 2014; Leliaert *et al.* 2012; Bringloe *et al.* 2020; Guiry and Guiry 2020). Besides the distinction of the different taxonomic groups by pigmentation, macroalgae differ considerably by their structural

organization, their biochemical composition and life cycles (Lee 1999; De Reviers 2002), some of the metabolic specifications being detailed in **section 2.3**. More particularly, the red and green algae would be derived from a primary endosymbiosis between a bacteria and a heterotrophic eukaryote, while brown algae would result from a secondary endosymbiosis with a red algae. This evolutionary difference has then resulted in specific molecular and structural differences, notably in the structure of plastids (Delwiche 1999; Larkum and Vesk 2003; Hurd *et al.* 2014). It is estimated that 2,065 different species of Phaeophyceae, 7,359 species of Rhodophyta, and 6,777 species of Chlorophyta are found in the marine environment (Guiry and Guiry 2020), including micro- and macroalgae. Due to this high diversity, the classification and relationships between the different algal lineages are complex and not yet completely understood, particularly within Rhodophyta (represented by black dotted lines in Fig. 1). Nevertheless, the development of recent molecular techniques and genome analyses are contributing to the understanding of the algal classification (Yoon *et al.* 2010; Bringloe *et al.* 2020).



**Figure 1.** Evolutionary relationships between the three major lineages of life, Archaea, Eubacteria and Eukaryotes, with the main phyla, adapted from Yoon *et al.* (2010), Leliaert *et al.* (2012), Not *et al.* (2012), Muñoz-Gómez *et al.* (2017) and Bringloe *et al.* (2020). The phyla Rhodophyta, Chlorophyta and Ochrophyta are detailed with different classes. The length of the branches is arbitrary and does not represent the evolutionary distance. The drawings of macroalgae are from Nicolas and Tréhin (2018).

On the other hand, algae are the main primary producers in marine environment, being autotrophic organisms capable of synthesizing organic matter through photosynthesis. More generally, photosynthesis by marine organisms represents ~40% of the total primary production of the Earth, with phytoplankton representing the main part of oceanic production (Duarte and Cebrián 1996; Field *et al.* 1998; Falkowski and Raven 2007; Sigman and Hain 2012). In this way, algae contribute significantly to the global carbon cycle and oceanic carbon sequestration. It has been suggested that macroalgae, generally excluded from carbon assessments, could also significantly participate to carbon sequestration, being the most obvious components of rockyshore communities (Gattuso *et al.* 1998; Krause-Jensen and Duarte 2016; Ortega *et al.* 2019). More particularly, it is estimated that macroalgae export about 43% of their production both as particulate and dissolved organic carbon (Krause-Jensen and Duarte 2016). In addition to their role in global biogeochemical cycles, some macroalgal species provide a food supply for grazers, and/or constitute a habitat/refuge for many benthic animals, enhancing the protection and substantially development of these species. In this way, some macroalgal species are sometimes described as “foundation species”. Therefore, macroalgae play an essential ecological role in the functioning of marine ecosystems, by affecting their chemical and biological environment (Lüning 1990; Little and Kitching 1996; Hurd *et al.* 2014).

In the present work, only some species of red and brown intertidal macroalgae were studied, and will be described in further detail in **Chapter 1 (Material and methods, part 1.1)**. Living in the intertidal zone, they are exposed to a highly variable environment to which they have to adapt as described in the following section.

### 1.2 The influence of environmental parameters on intertidal macroalgae

Marine macroalgae are mostly encountered on rockyshores where they contribute significantly in structuring benthic communities in intertidal or subtidal areas up to the limit of the light penetration in the seawater. The intertidal rockyshores constitute variable environments due to the alternating periods of emersion and immersion created by the tidal cycle. By consequences, macroalgae are vertically distributed on the shore according to their physiological requirements and acclimation abilities, forming horizontal « bands » or « belts ». This vertical distribution has led the biologists to define distinct vertical zones. Nevertheless it is difficult to define precise zones, which can be sometimes confused,

especially since their limits depend on hydrodynamics, or the region of the world. Consequently, there are many examples of intertidal zonation (Stephenson and Stephenson 1972; Little and Kitching 1996; Hurd *et al.* 2014), some of them being listed in Lüning (1990) or Raffaelli and Hawkins (1999). According to these last authors, we can then define:

- The high shore zone, commonly called littoral fringe (or "**supralittoral**"), is the splash zone dominated by lichens. It is extended well above the levels reached by the seawater;
- The mid-shore zone (also called "**eulittoral**") is characterized by the presence of macroalgae coexisting with suspension feeders or grazers such as limpets or mussels. It corresponds to the part of the littoral alternately emerged and immersed according to the tidal cycles. Then, Lüning (1990) and Raffaelli and Hawkins (1999) assimilated this zone to the "intertidal zone". Eulittoral is also assimilated to "mediolittoral" by some authors (see Fig. 1.4 in Lüning 1990; Cabioch *et al.* 2014). In addition, eulittoral can be divided into three more zones, *i.e.* the upper, middle and lower mid-shore, each of them experiencing different emersion duration.
- The lower shore (called "**sublittoral**" zone), which is normally submerged, and occasionally emerged at extreme low water levels. It is characterized by the development of large kelp populations (Laminariales which are brown macroalgae). The use of the term "subtidal" is then conflicting with the term "infralittoral" (see Fig. 1.4 in Lüning 1990).

In this regard, macroalgae living on the intertidal rockyshores are then subjected to a highly variable environment with in particular fluctuations in light, temperature or salinity, particularly when living in intertidal rockpools. These environmental variations then impact the physiology and ecology of the algal species. Then, owing the sessile nature of most of the intertidal species, macroalgae must adapt to these temporal variations. For this purpose, they have developed different resistance mechanisms, including the synthesis of metabolites. The impact of the different environmental parameters on the ecophysiology of red and brown macroalgae has been reviewed in a publication in the journal *Advances in Botanical Research*, available in this section.





# The stressful life of red and brown seaweeds on the temperate intertidal zone: effect of abiotic and biotic parameters on the physiology of macroalgae and content variability of particular metabolites

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## Abstract

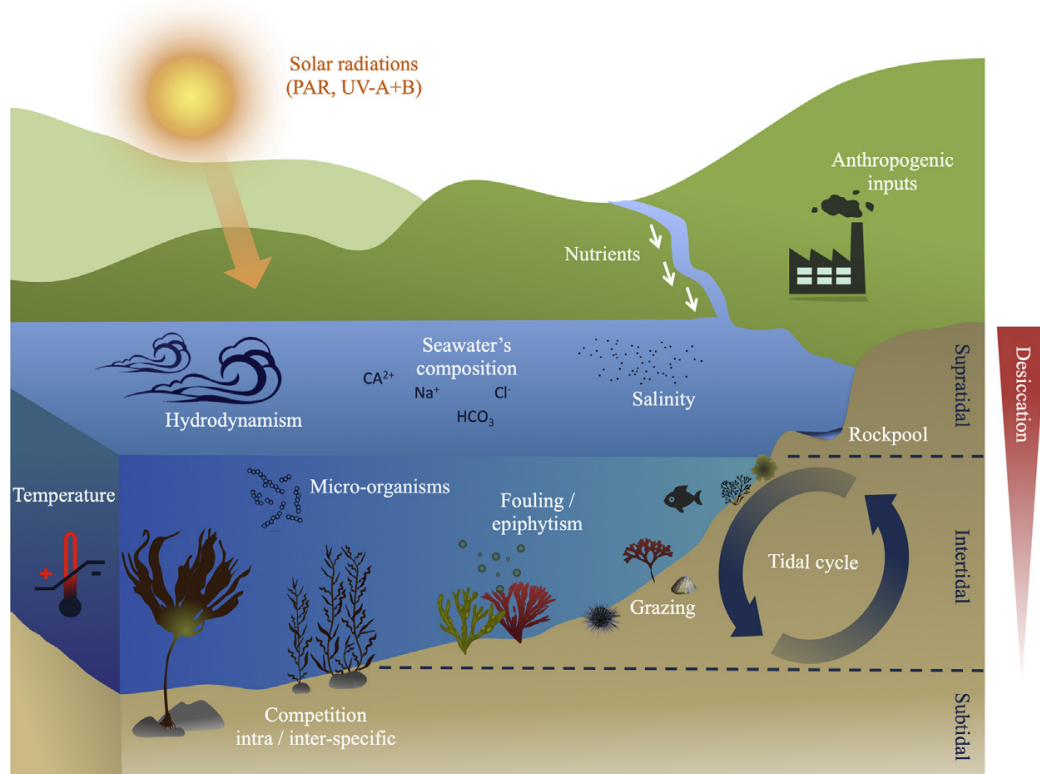
Most marine macroalgae live attached to a substrate in coastal areas characterized by a very variable environment mainly due to the tide cycle which is more or less important depending on the geographical situation. Macroalgae spread over coastal zones according to their resistance to several abiotic and biotic factors. Some species are thus present on intertidal zones and are outside the water for a more or less long time each day; on the other hand, others are always underwater if they are living in the subtidal zone or in pools in which abiotic parameters can be strongly modified during a tide cycle. During these alternating immersion/emersion phases, macroalgae deal then with important modifications of many abiotic parameters, such as light, temperature, salinity, and biotic parameters, such as grazing, fouling, pathogens. To counteract these important variations, intertidal macroalgae develop resistance mechanisms such as the synthesis of special compounds, like photoprotectors, osmolytes, antifouling molecules as examples. In this chapter, after a presentation of the main environmental parameters facing intertidal seaweeds in temperate environment, some adaptation/protection mechanisms of these algae are presented, with an emphasis on brown (structural species whose biomass is very high on intertidal zones) and red seaweeds (whose diversity is very high).



## 1. Distribution of seaweeds on the rockyshore

Algae are a highly diverse group estimated at 50,370 species including ~7,300 red (Rhodophyta) and ~2,070 brown macroalgae (Ochrophyta, Phaeophyceae), playing a key role in marine ecosystems (Guiry & Guiry, 2019). Indeed, algae are at the base of the ocean food web, and contribute to primary photosynthetic production at a level almost equivalent to that of terrestrial plants (Falkowski & Raven, 2013). Among the different groups, brown macroalgae, *i.e.* Phaeophyceae, are the most easily observed seaweeds on rocky foreshores, including Fucales and Laminariales (Lüning, 1990). Red macroalgae, *i.e.* Rhodophyta, constitute a second group with the greatest specific diversity (Krause-Jensen & Duarte, 2016; Stengel, Connan, & Popper, 2011).

Although some species such as the red macroalga *Agarophyton vermiculophyllum* (previously *Gracilaria vermiculophylla*) live on sandy or muddy substrate (Surget et al., 2017), benthic macroalgae grow mainly on the rockyshore, and are thus directly subjected to changing environmental conditions (Lüning, 1990). By consequences, algal diversity and abundance is not the same everywhere in the ocean, and more particularly in polar, temperate, and tropical regions (Bolton, 1994; Hurd, Harrison, Bischof, & Lobban, 2014). On temperate rockyshores, macroalgae are affected daily



**Fig. 1** Conceptual representation of the main abiotic and biotic factors presented in this review, influencing the ecology and physiology of seaweeds living on the rocky-shore and along the vertical zonation.

by the tidal cycle and have to deal with a whole range of stresses whose contribution varies at the different tidal levels (Fig. 1). One consequence of the alternation of emersion/immersion periods is the appearance of a vertical distribution (Lüning, 1990), which is supposed to be linked upwards to the resistance of the species to desiccation; but other various potentially stresses including high hydrodynamism, high light intensity, elevated temperature, nutrient limitation and osmotic stress resulting from emersion, also have a significant effect on intertidal macroalgae (Bird, Franklin, Smith, & Toonen, 2013; Contreras-Porcía, López-Cristoffanini, Meynard, & Kumar, 2017). Thus, rockyshores in temperate zones are divided into several levels described by Lüning (1990), in which algae will be affected by emersion for a more or less long period (Fig. 1). First of all, the supralittoral zone is the highest area of the foreshore with a dominance of lichens and halophytes but no seaweed. The mediolittoral zone is the area where the physico-chemical conditions are the most variable because it is subjected to daily emersion/immersion cycle with a tidal range up to 15 m. This level is divided into several distinct zones parallel to the shoreline at different shore heights, defined by the presence of particular species of brown seaweeds

(belts) according to their resistance to different stresses (Ar Gall & Le Duff, 2014). Thus, on the upper mediolittoral zone, most macroalgae, such as the brown seaweed *Pelvetia canaliculata*, present a lower density and size than lower foreshore species to withstand long desiccation. At the mid-mediolittoral level, there is the brown seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus* belt on sheltered sites, with a disappearance of the first species on exposed sites (Lüning, 1990). On the lower mediolittoral zone, there are the *Fucus serratus* belt and the brown seaweed *Himanthalia elongata* community, where the effects of the tide are less noticeable. Finally, at the bottom of the rocky shore, there is the subtidal level which is always submerged except in the event of very high tides. At this level, the light for photosynthesis is more limited and some species require near-permanent immersion, including *Laminaria* species.

Within this intertidal zone, seaweeds are subjected to a range of abiotic factors which can fluctuate during the day (tide/photoperiod), or with weather and seasons. Rocky foreshores are notably characterized by their wave exposure mode (*i.e.* hydrodynamism): sheltered, semi-sheltered, as opposed to exposed mode where wave intensity is much more noticeable. Communities are therefore different between shores, but there are also differences within each shore where species are positioned more or less high, depending on their resistance to different environmental parameters. As already mentioned, desiccation is the factor that influences mostly the vertical distribution of algae. Nevertheless, the impact of this factor is difficult to assess because it occurs at the same time as other stresses occurring during emersion. Desiccation is notably often associated with variation in salinity, especially in rockpools. In the Atlantic marine environment, the salinity of the water is around 35. However, this value can fluctuate, with for example a decrease in salinity in rockpools during rainy periods, or an increase during evaporation at low tide, such as in hot weather for example. As algae are photosynthetic organisms, the presence of light (quality and quantity), combined with sufficient nutrient concentration, is also an essential condition for their survival. Nevertheless, too much light and UV-radiations, combined with an increase in temperature, can be harmful. Finally, in addition to these abiotic stresses, macroalgae are subjected to biotic interactions such as competition (for light, space and available substrate), grazing and the presence of epiphytic bacteria, fungi, animals and/or algae. Among the great diversity of algae, some species are able to withstand extreme conditions, such as in rockpools on the intertidal zone, estuaries or salt marshes,

where they experience severe variable salinity, temperature and nutrient supply conditions (Lüning, 1990).

To cope with their complex and highly variable environment, algae must constantly adapt, and this adaptation is now faster as the various environmental factors tend to change more rapidly due to the global change that currently weakens coastal ecosystems. Thus, to protect and adapt themselves against these abiotic and biotic stresses, macroalgae produce a wide range of metabolites in order to ensure effective photosynthesis, respiration, growth or reproduction (Stengel et al., 2011). These include photosynthetic, photoprotective, osmoregulatory, antioxidant, antifouling or particular compounds, which can be synthesized in response to different stresses, that can act separately or in synergy. In this context, the aim of this present chapter is to review the effect of the different abiotic and biotic factors on the physiology of brown and red macroalgae. For this purpose, each parameter is detailed one by one, presenting which kind of compounds is synthesized and under which circumstances.



## 2. Effect of abiotic parameters

### 2.1 Hydrodynamism

Hydrodynamism refers to water motions, due to the tidal cycle, and to waves or ocean currents resulting from winds and differences in the densities of water masses (Hurd et al., 2014). This factor influences the distribution and the vertical zonation of algae on the shore (Fig. 1), because: (1) currents and waves affect other environmental factors as light penetration, temperature, and the availability of nutrients and all elements necessary for algal development; (2) hydrodynamism influences the distribution of fauna, indirectly affecting algae through spatial competition and potential impact of herbivores; (3) moreover, to survive on exposed sites, macroalgae must be resistant enough to remain attached to their substrate and withstand wave action, giving rise in interesting adaptations which will be developed below (Hurd, 2000; Lüning, 1990). Resulting from hydrodynamism, macroalgal species are often acclimatized to a type of wave exposure, either sheltered (low hydrodynamism), exposed (high hydrodynamism) or the intermediate, semi-exposed environment.

Hydrodynamism can affect the growth, the morphology or the phenology of seaweeds. In this sense, Kraemer and Chapman (1991) showed that juveniles of the brown alga *Egregia menziesii* grown under high

hydrodynamic conditions were about 2-times stronger and stiffer than those growing under low hydrodynamic conditions to resist hydrodynamism and not being swept away by waves and tide. In addition, seaweeds can adapt their size, shape and direction to thrive/disperse with the flow and increase their resistance to currents (Boller & Carrington, 2006). Thus, it has been shown that the red alga *Chondrus crispus* can realign its stipe and compact its thallus in response to the flow (Boller & Carrington, 2006). Moreover, nutrient uptake by macroalgae is influenced by water motion, impacting growth and photosynthesis (Gerard, 1982; Hurd, 2000). Indeed, with a high current, the nutrient absorption rate increases due to the decrease in the thickness of the diffusion boundary layer, and the acceleration of transport through it (Lüning, 1990). Thus, photosynthesis increases with hydrodynamism until enzyme saturation, as demonstrated in the brown alga *Macrocystis pyrifera* (Wheeler, 1980). In return, in order to compensate for low nutrient and gas absorption rates, algae in low hydrodynamic environments tend to have a higher surface-to-volume ratio (Stewart & Carpenter, 2003).

The reproduction can also be influenced by hydrodynamism. According to Gordon and Brawley (2004), water motion mainly affects gametes and spores release, and their dispersal distance. Thus, they have demonstrated an increase in the zoospores release of the brown alga *Alaria esculenta* under shaken conditions, and that, on the contrary, there were more gametes released by the antheridia under calm conditions.

The presence of some molecules in seaweeds could be influenced by hydrodynamism, especially polysaccharides in Phaeophyceae. In this sense, Craigie, Morris, Rees, and Thom (1984) found that the alginic acid composition in the brown alga *Saccharina longicuris* was related to the degree of wave exposure, although this hypothesis is controversial (Kraemer & Chapman, 1991). Moreover, Le Lann, Rumin, Cérantola, Culioli, and Stiger-Pouvreau (2014) showed that the brown alga *Bifurcaria bifurcata*, living in a semi-exposed/sheltered environment, synthesized more terpenes, including bifurcanone which is specific to sheltered environment. According to Rönnberg and Ruokolahti (1986), phenolic content of *F. vesiculosus* has been shown to depend on the degree of wave exposure of the habitat, with smaller fluctuations of phenolic content at exposed site compared to sheltered site, as also demonstrated in the brown alga *Sargassum muticum* by Plouguerné et al. (2006b). It is sometimes difficult to interpret the significance of the difference in composition or morphology with the exposure mode, as many other factors can interact, including desiccation and

herbivory. In this sense, [Kraemer and Chapman \(1991\)](#) did not find any variation in alginic acid content in the Laminariales *E. menziesii* exposed to different wave velocities; and [Fuller and Mathieson \(1972\)](#) showed no significant difference in carrageenan content of *C. crispus* according to the wave exposure.

## 2.2 Desiccation

In the intertidal zone, seaweeds, as being sessile organisms living attached to the substrate, are faced with desiccation for a brief or longer period depending on the position on the shore, due to the tide and the alternation of immersion and emersion. Several preliminary studies agree that the ability of algae to withstand significant water loss and mostly to rehydrate quickly defines their tolerance to drought, and not their ability to retain water ([Contreras-Porcía et al., 2017](#)). Thus, the different algal species display variable desiccation tolerance, with the most resistant species at the top of the intertidal zone ([Abe, Kurashima, Yokohama, & Tanaka, 2001](#); [Lüning, 1990](#)). Thanks to a slower water loss, a faster rehydration and a greater resistance to high water loss, macroalgae of the upper intertidal zone can lose up to 90% of their cellular water content ([Contreras-Porcía et al., 2017](#)). This rate can reach up to 96% for *Pyropia orbicularis* or *Porphyra columbina*, making these species models to study algal responses to desiccation ([Contreras-Porcía, Thomas, Flores, & Correa, 2011](#); [Fierro et al., 2017](#)). Nevertheless, a whole range of factors can influence the rate of desiccation of each species, as for example weather and seasons, as high temperatures and wind favor a high rate of water evaporation ([Lamote, Johnson, & Lemoine, 2012](#)). Desiccation also seems to be related to the exposure mode, with the most exposed species being more likely to have a lower rate of desiccation due to thicker cell walls ([Kristensen, 1968](#)).

The loss of water induced by desiccation causes morphological changes such as retraction of protoplasts and chloroplasts disorganization due to an osmotic imbalance ([Flores-Molina et al., 2014](#)). To avoid these effects, some algae regularly subjected to emersion present a small specific surface to reduce desiccation, as species with a lower surface/volume ratio lose their water content less quickly ([Lüning, 1990](#)). In this way, macroalgae in the upper intertidal zone are smaller in size to better resist to water deprivation as demonstrated for *Fucus spiralis* ([Schagerl & Möstl, 2011](#)). The formation of mucilage on the surface of some particular algae is another way that has been suggested as resistance to desiccation ([Bérard-Therriault & Cardinal, 1973](#)). Indeed, the cell wall of macroalgae is composed of some

unique polysaccharides (or phycocolloids), with a strong polyanionic character and which possess water retention properties, especially due to sulfation. The main phycocolloids are fucoidans and alginates in brown seaweeds (Bérard-Therriault & Cardinal, 1973), whereas red seaweeds contain agars, xylans and carrageenans (Kloareg & Quatrano, 1988; reviewed by; Stiger-Pouvreau, Bourgoignon, & Deslandes, 2016). Contrary to freshwater algae and terrestrial plants, polysaccharides of marine macroalgae are highly sulfated, up to 20%–38% for carrageenans, which could be a specific adaptation to marine environment and desiccation (Kloareg & Quatrano, 1988).

From a physiological point of view, it has been shown that there is a decrease in photosynthesis and chlorophyll-*a* content during emersion (Contreras-Porcía et al., 2011; Flores-Molina et al., 2014; Gao & Wang, 2012). In this sense, López-Cristoffanini et al. (2015) demonstrated in *P. orbicularis* that a drying treatment could affect the expression of proteins involved in photosynthesis. The cyclic electron flow around photosystem I has been demonstrated to play a significant role in response to drought, by protecting the photosynthetic apparatus and modulating a pH gradient for the synthesis of ATP (Gao & Wang, 2012). Photosynthesis is then completely restored after re-immersion, except if a critical water content has been reached (Dring & Brown, 1982; Lamote et al., 2012).

In general, prolonged desiccation periods can affect a whole set of physiological activities due to the fact that being out of the water starves algae for nutrients and inorganic carbon (Lamote et al., 2012). For example, desiccation causes a decrease in phosphate absorption in *F. spiralis*, *A. nodosum* and *F. serratus* (Hurd & Dring, 1991). Macroalgae experiencing periodical emersion may benefit from increasing atmospheric CO<sub>2</sub> concentrations or light (Zou & Gao, 2002), which may explain why the rate of photosynthesis during emersion can sometimes exceed the one during immersion. In addition, a long period of desiccation induces oxidative stress with an over-supply of reactive oxygen species (ROS) (Contreras-Porcía et al., 2011; Sampath-Wiley, Neefus, & Jahnke, 2008). For example, concentrations of H<sub>2</sub>O<sub>2</sub> increased up to 25-fold during desiccation in cortical cells of the brown macroalga *Lessonia spicata* (Flores-Molina et al., 2014). Molecular analysis demonstrated that some genes, such as those encoding the enzyme trehalose phosphate synthase (TPS) or catalase (CAT) which protect algae from oxidative stress, are overexpressed in *P. orbicularis* during desiccation (Fierro et al., 2017). As for higher plants, the enzyme glutathione reductase (Contreras-Porcía et al., 2017; Sampath-Wiley et al., 2008), and the



hormone abscisic acid (ABA) (Guajardo, Correa, & Contreras-Porcia, 2016) regulate oxidative stress under water deficiency in algae.

One challenge in measuring the effect of desiccation is that it occurs naturally during periods of emersion, and is thus associated with other stresses like changes in light or temperature (Matta & Chapman, 1995; Sampath-Wiley et al., 2008), which can accelerate desiccation (Binet, 1956). In particular, it has been demonstrated in the Gigartinales *Endocladia muricata* that desiccation leads to better thermotolerance, protecting the alga from high heat during low tide (Hunt & Denny, 2008). Similarly, according to Ji et al. (2015) and López-Cristoffanini et al. (2015), heat shock proteins expression is altered during desiccation, due to high temperature.

### 2.3 Light

As algae are photosynthetic organisms, light is a key factor for their growth and distribution. More particularly, algae need to absorb enough Photosynthetically Active Radiation (PAR) in their thylakoids to transform light energy into chemical energy through photosynthesis, necessary for their development. In this sense, the growth of red macroalgae such as *Gracilaria domingensis* (Ramlov et al., 2011), *C. crispus* and *Palmaria palmata* (Manríquez-Hernández, Duston, & Garbary, 2016) have been enhanced under high photon flux intensity, indicating that light can be a limiting factor. There is a photosynthetic light compensation point, which corresponds to the photon fluence rate, for which the amount of oxygen produced by photosynthesis accurately compensates for the oxygen absorption related to respiration (Hurd et al., 2014; Lüning, 1990). On the other hand, if the light increases well beyond the compensation point, the light saturation can be reached and photosynthesis is thus maximum ( $P_{max}$ ). At this point, the enzymatic reactions limit the photosynthesis, and if the light increased again, photosynthesis is then reduced (photoinhibition).

To achieve photosynthesis, all algal species have chlorophyll-*a* in their chloroplasts to capture light photon energy. Depending on the taxonomic group of algae, there are also other chlorophylls that absorb light at other wavelengths (Table 1). In addition, the different classes of seaweeds are characterized by their carotenoid composition including fucoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin,  $\alpha$ -carotene, and  $\beta$ -carotene (Lüning, 1990) (Table 1). The composition and quantities of carotenoids also varied widely between algal species, although no link has ever been demonstrated with phylogeny (Schubert, Garcia-Mendoza, & Pacheco-Ruiz, 2006). In red seaweeds, lutein is the most common

**Table 1** Pigment composition and presence of phenolic compounds or mycosporine-like amino acids (MAAs) within the red and brown macroalgae according to [Stengel, Connan, & Popper \(2011\)](#).

Group	Chlorophylls	Other pigments	MAAs	Phenolic compounds
Rhodophyta	Chlorophyll- <i>a</i>	phycocyanin, phycoerythrin, allophycocyanin, $\alpha$ -carotene, $\beta$ -carotene, violaxanthin, antheraxanthin, zeaxanthin, lutein	Yes	Few
Phaeophyceae	Chlorophyll- <i>a</i> Chlorophyll- <i>c</i> <sub>1</sub> , <i>c</i> <sub>2</sub>	$\beta$ -carotene, fucoxanthin, violaxanthin, antheraxanthin, zeaxanthin, neoxanthin	No	Many

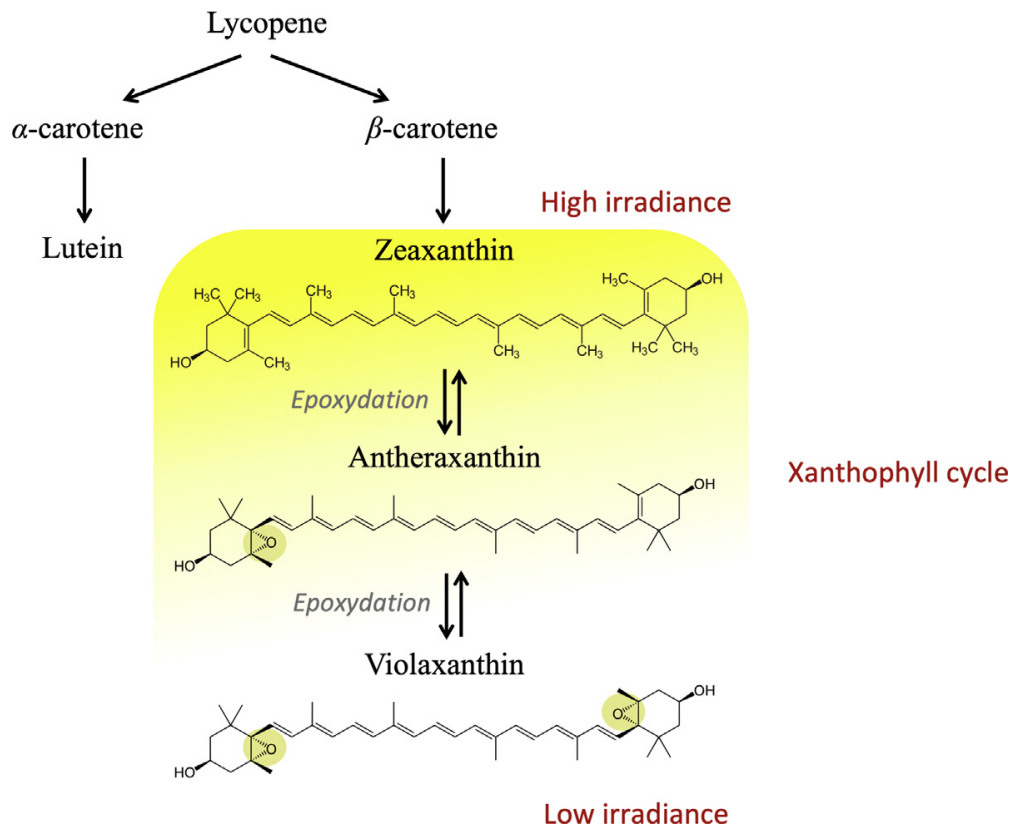
carotenoid and can represent up to 99% of the total carotenoid content ([Lalegerie et al., 2019](#); [Schubert, García-Mendoza, & Pacheco-Ruiz, 2006](#)). In order to optimize photosynthesis in the presence of low light intensity, red algae also possess extra pigments called phycobiliproteins (*i.e.* phycocyanins, phycoerythrins and allophycocyanins in the phycobilisome) ([Dumay, Morancais, Munier, Le Guillard, & Fleurence, 2014](#)).

Irradiance in the ocean is highly variable, since it depends on weather, algal position on the shore, latitude, water turbidity, seasons and time of the day (day/night and tidal cycles) ([Hanelt & Figueroa, 2012](#); [Hurd et al., 2014](#)). However, any modification of the light, whether by the duration of irradiance, the intensity or the nature of the radiations, will have an effect on the physiology and distribution of seaweeds ([Hurd et al., 2014](#)). In particular, light is an environmental signal for reproduction and growth, since it has been demonstrated for example that the release and accumulation of gametes are light-induced (especially with the blue radiations) in several species like the brown macroalga *Dictyota dichotoma* ([Hanelt & Figueroa, 2012](#)). However, the amount of light first affects photosynthesis and the amount of chlorophyll-*a*. Thus, [Torres, Chow, and Santos \(2015\)](#) observed a 55% increase in chlorophyll-*a* content from 1000 to 60  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  in *Gracilaria tenuifrons*, to enhance light energy capture at low irradiance. On the contrary,

many studies have shown that chlorophyll-*a* level is lower under high light, particularly in summer due to the increasing irradiance. It has been observed, for example, a 50% decrease in chlorophyll-*a* in *A. nodosum* during summer (Stengel & Dring, 1997), and a threefold decrease from February to July in *P. palmata* (Guihéneuf, Gietl, & Stengel, 2018). Similarly, chlorophyll-*a* content increases with water depths with light attenuation and fluctuation in the composition of the light spectrum, as shown in *Porphyra umbilicalis* and *C. crispus* (Ramus, Beale, Mauzerale, & Howard, 1976).

Although light is vital for algae, too much radiation, especially in the UV-A and UV-B range, can be harmful, mainly for the early development stages (Karentz, 2001; Wiencke et al., 2000). UV-radiation can cause a decrease in growth, as observed in the red seaweeds *Crassiphycus birdiae* (Ayres-Ostrock & Plastino, 2014) and *Kappaphycus alvarezii* (Schmidt, Maraschin, & Bouzon, 2010). Above a certain threshold, extreme irradiances can cause photodamages such as DNA alterations, synthesis of reactive oxygen species (ROS), photo-oxidation, or degradation of photosynthetic pigments (Karentz, 2001; Karsten, 2008; van de Poll, Eggert, Buma, & Breeman, 2001). UV radiation is notably reported to be responsible for damage to chloroplasts, thylakoids and mitochondria in *K. alvarezii* (Schmidt et al., 2010) and *P. palmata* (Holzinger, Lütz, Karsten, & Wiencke, 2004). A very strong visible light can also lead to photoinhibition consisting in the reduction of the photosynthesis, which could be temporary or deleterious if very strong or long lasting (Franklin, Osmond, & Larkum, 2003; Hanelt & Nultsch, 2003). For example, photoinhibition can reach 95% in *C. crispus* (Yakovleva & Titlyanov, 2001). Under UV radiation, this phenomenon of inhibition of photosynthesis may be related to the activity of Rubisco and D<sub>1</sub> protein of photosystem II (Bischoff, Hanelt, & Wiencke, 2000; Schmidt et al., 2010).

For their own protection, algae are able to produce photoprotective compounds that will dissipate excessive energy (Bhatia, Sharma, Sharma, & Purohit, 2011; Rastogi, Sinha, Singh, & Häder, 2010). In this sense, carotenoids can play both photosynthetic and photoprotective roles (Hashimoto, Uragami, & Cogdell, 2016; Young & Frank, 1996). In particular, in some seaweeds, they protect the cells against excessive light energy in photosystem II and oxidative stress via the xanthophyll cycle (Fig. 2): when the amount of light is too high, violaxanthin is converted by de-epoxidation to antheraxanthin and then zeaxanthin, which reduces excess energy (Karentz, 2001; Ursi, Pedersen, Platino, & Snoeijs, 2003).



**Fig. 2** The xanthophyll cycle in macroalgae according to [Latowski, Kuczyńska, and Strzałka \(2011\)](#) and [Takaichi \(2011\)](#).

Mycosporine-like amino acids (MAAs) are a second group of photoprotective compounds found in red macroalgae ([Sinha, Singh, & Häder, 2007](#)), which are reported to be effective against UV radiation (see Chapter 8). [Yakovleva and Titlyanov \(2001\)](#) for example, showed that repeated exposure to UV radiation resulted in the accumulation of MAAs, coupled with less photoinhibition in *C. crispus*. Some species such as *Furcellaria lumbricalis* or *Heterosiphonia plumosa* are not able to produce MAAs, while others like *P. palmata*, or *C. crispus* have been reported to synthesize up to 7 or 8 MAAs ([Lalegerie et al., 2019](#)). Shinorine, palythine and porphyra-334 are the MAAs most widely distributed among red algae. Several studies have suggested that MAAs could also be synthesized in response to other environmental stresses and more particularly with respect to nutrient limitation ([Oren & Gunde-Cimerman, 2007](#)).

Beyond the well-known photoprotective compounds mentioned above, it has also been demonstrated that phenolic compounds of brown seaweeds (phlorotannins) could play a role in the UV-protection ([Schoenwaelder, 2002](#)). For example, [Pavia, Cervin, Lindgren, and Åberg \(1997\)](#) have found

that a 50% increase in UV radiation during 2 weeks resulted to a 30% increase in phlorotannin concentration of *A. nodosum*.

## 2.4 Temperature

Temperature is the major factor controlling survival, growth, reproduction and thus geographical distribution of seaweeds (Yarish, Breeman, & van den Hoeck, 1986). However, in temperate zones, temperature can change according to the latitude, depth, season, but also according to the tide, with emersion and immersion phases. Thus, according to Eggert (2012), seaweeds respond to the temperature in three different ways: genetic adaptation to the constant temperature of the living environment; phenotypic acclimatization; or short-term physiological regulation in response to the temporal temperature shocks. In summer and winter at low tide, the seawater temperature highly varies, forcing intertidal organisms to be euryceous and highly tolerant to any variation in temperature. Intertidal seaweeds living on the upper shore have thus been found to have temperature tolerance ranges broader than subtidal species, and can tolerate exposure to high temperatures for prolonged time periods (Einav, Breckle, & Beer, 1995). For example, the sublittoral brown alga *Laminaria* spp. can tolerate a temperature up to 20 °C, whereas *F. spiralis* from upper littoral is resistant up to 28 °C (Lüning, 1990). Moreover, the temperature tolerance of seaweed depends on latitude, with southern perennial kelp more resistant to short heat shock than the northern one (Pereira, Engelen, Pearson, Valero, & Serrão, 2015).

The temperature can affect the growth and reproduction, for which optimal temperature and timing must be particularly important (Molenaar & Breeman, 1997). Indeed, growth and reproduction can be under direct photoperiodic control or endogenous circannual rhythm (Lüning & Tom Dieck, 1989). For example, Molenaar and Breeman (1994) showed that the life history of the red alga *Phyllophora pseudoceranooides* is regulated by temperature and daylength, with the production of spores in winter. In addition, Yarish, Breeman and van den Hoeck (1986) showed that gametophytes of *Polyneura bonnemaisonii* (previously *P. hilliae*) have a smaller survival rate at low temperatures than tetrasporophytes, which limits its distribution.

Moreover, the temperature can impact the photosynthesis and the other metabolic pathways of seaweeds, as enzymatic activities are temperature-dependent. In this sense, the response of thermal acclimation of photosynthesis appears to be highly comparable to that described for photoacclimation (Huner, Öquist, & Sarhan, 1998). However, temperature and light have been shown to have interactive effects on photosynthesis and its regulation

(Savitch, Maxwell, & Huner, 1996). Moreover, temperature sensitivity affects proteins and membranes: quantitatively (concentration changing), qualitatively or through modulation (modify the protein environment) (Eggert, 2012). More particularly, it has been demonstrated that membrane fluidity decreases with a decrease in temperature (Eggert, 2012; Inaba et al., 2003; Szalontai, Nishiyama, Gombos & Murata, 2000). Heat stress affects membrane-associated processes because high temperatures cause fluidization of membranes and finally disintegration of the lipid bilayer (Los & Murata, 2004). In addition, Pakker et al. (2000) demonstrated that the reparation of DNA damages induced by UV-B radiation is dependent on the temperature in *P. palmata*.

The production of metabolites in macroalgae is also temperature dependant. Notably, high temperature induces the production of heat shock proteins. For example, Ireland et al. (2004) showed an induction of these molecules in *F. serratus* exposed to 42 °C, maximum after 2 h. Tanniou (2014) studied the impact of water temperature on total phenolic content during 30 days at 11° and 22 °C on *S. muticum*: a decrease in phenolic level was observed at 22 °C compared to those grown at 11 °C. According to Connan, Deslandes, and Ar Gall (2007), air-temperature correlated significantly with the phenolic content in uppershore *P. canaliculata* and midshore *A. nodosum*, but not for the lowshore *B. bifurcata*. Temperature can also influence pigment content of the algae. Indeed, Rothäusler, Gómez, Karsten, Tala, and Thiel (2011) showed the highest carotenoid level at the coolest temperature (12 °C) compared to ambient (17 °C) and warm (20 °C) temperatures and independently of irradiance, in *M. pyrifera* being afloat for 15 days. Vanitha and Chandra (2012) found no correlation between pigments (R-phycoerythrin, R-phyococyanin, allophyococyanin and chlorophyll-*a*) and temperature but some variations appeared maybe due to other factors (light or humidity) in red algae (*Gracilaria corticata*, *Grateloupia lithophila*, *Gelidium* sp. and *Bryocladia thwaistesii*). On the contrary, Kim, Kraemer, Neefus, Chung, and Yarish (2007) showed an influence of temperature on the phycoerythrin content in several *Porphyra* species (*P. leucosticta*, *P. linearis*, *P. umbilicalis*); with higher contents at low temperature (10 °C compared to 15 °C and 20 °C), but not for *P. amplissima*. In a similar manner, Guihéneuf, Gietl, and Stengel (2018) found an increase in chlorophyll-*a* and phycobiliprotein concentrations in *C. crispus* and *P. palmata* when temperature and irradiance decreased in autumn. Conversely, Ding, Ma, Huang, and Chen (2013) showed an increase in chlorophyll *a* content when water temperature increases from 15 until 25 °C in *Hypnea cervicornis*.

## 2.5 Salinity

The ranges of salinity encountered in marine habitats differ greatly with respect to space and time. Salinity is different according to the oceans and seas. More particularly, saltmarshes, estuaries and near-shore waters are environments with extreme salinity variations. In marine waters, intertidal algae are also subjected to variations in salinity according to the season, day, or even hours, as in the rockpools of the intertidal zone. Notably, at low tide seaweeds experienced extreme dilutions by rainwater or, on the contrary, extreme salinities by evaporation. In the intertidal zone, Fucales can withstand variations in salinity ranging from 0.1 to 3.5 times that of seawater, while subtidal algae, like the red macroalgal genera *Antithamnion* or *Plocamium*, are less tolerant, between 0.5 and 1.5 times that of seawater (Zaneveld, 1969). Macroalgae living in environments with variable salinity must maintain a higher osmotic pressure in their cell medium than in their environment; an osmotic pressure of 2.6–3 MPa is typical of seaweeds (Karsten, 2007).

Some algal species are euryhaline as they are able to tolerate a large salinity range, while stenohaline species cannot survive at a salinity too far from their salinity optimum (Bird & McLachlan, 1986). Karsten (2007) demonstrated that the euryhaline *Fucus distichus* was not affected by hypo- or hypersaline stresses (salinities ranging from 5 to 60) while *A. esculenta*, *Saccharina latissima* and *Laminaria solidungula* demonstrated a strong loss of pigments (bleaching) or even high mortality under hyposaline stress; intertidal species are thus mostly euryhaline while subtidal are stenohaline. Schubert, Feuerpfeil, Marquardt, Telesh, and Skarlato (2011) showed a decline in abundance of macroalgae with salinity until 7.2. Moreover, Stratil, Neulinger, Knecht, Friedrichs, and Wahl (2014) demonstrated that salinity was important in structuring algal-associated epibacterial communities: they showed a disappearance of epiphytic and epilithic betaproteobacteria with an increase in salinity, from 5 to 25.

Faced to variations in salinity, algae respond to osmotic shock by regulating either their concentrations of ions, such as  $K^+$ ,  $Na^+$ ,  $Cl^-$  or by synthesizing organic osmolytes, *i.e.* molecules with low molecular weight in order to be easily and rapidly exchange with the surrounding water to equilibrate osmotic pressure with their own tissues (Karsten, 2007). In general, osmolytes are concentrated in algal vacuoles and/or are located in the cytoplasm (Karsten, 2007). The last author drew a schematic functioning of the osmotic adjustment in macroalgae, listing two phases in this

phenomenon: a phase I which includes rapid water flows through the cell-wall, entering (hypoosmotic shock) or leaving (hyperosmotic shock) depending on the osmotic shock, and a phase II which represents metabolic control, including at the beginning an ionic regulation which is very rapid, followed by a slow ionic regulation involving osmolytes.

Variations in salinity affect different aspects of macroalgal metabolism, in particular photosynthesis, respiration, ionic fluxes and algal growth, as demonstrated in the brown macroalga *Dictyopteria membranacea* by Gessner (1969), with a photosynthetic optimum at a salinity between 28 and 55. During readjustment to hyperosmotic shock, rapid changes in mineral concentration often precede a slower synthesis of organic osmolytes, such as mannitol in brown algal species (Munda, 1978). Mannitol is the main product resulting from photosynthesis, which accumulates in Phaeophyceae where it represents a significant part of the dry matter of the algae: 23% and 19.5% in the Fucales *P. canaliculata* and *Halidrys siliquosa*, respectively (Reed, Davison, Chudek, & Foster, 1985). The accumulation of mannitol and volemitol has been shown in the uppershore *P. canaliculata* (Reed et al., 1985) while at the bottom of the mediolittoral zone, *H. elongata* synthesizes mannitol and altritol (Wright & Reed, 1985). Red algae produce mainly floridoside for their osmoregulation (Reed, 1990). In *Porphyra/Pyropia* spp., different forms of isofloridoside are present simultaneously with floridoside for the osmoregulation of each species (Reed, Collins, & Russell, 1980). The average floridoside content varies between 1.5 and 8% of the dry matter of the algae, but can reach 22%–30% in some species (Kerjean et al., 2007; Kirst, 1990). Floridoside is a small carbohydrate synthesized very early in photosynthesis, as demonstrated in the red alga *Solieria chordalis* (Goulard, Diouris, Quéré, Deslandes, & Floc'h, 2001). Simon-Colin, Bessieres, and Deslandes (2002) highlighted the major role of floridoside in the osmoregulation of the red macroalga *Grateloupia turuturu* (formerly *Grateloupia doryphora*), demonstrating an increase at high salinity and a decrease under hypohaline stress, and returning at its initial state after 3 days of cultivation of the macroalga. Under controlled conditions, early after the hypo- or hyper-haline stress, Kerjean et al. (2007) demonstrated that the floridoside content can reach high values around 39 mg g<sup>-1</sup> DW in *Mastocarpus stellatus*.

As a general rule, red algae do not synthesize alditol, *i.e.* non-cyclic polyol, except few Ceramiales, which accumulate mannitol, as *Caloglossa leprieuri*, whose mannitol contributes 23% of the internal osmotic pressure (Karsten et al., 1992a). *Bostrychia* (previously *Stictosiphonia*) *arbuscula* use a



combination of D-dulcitol and D-sorbitol as organic osmolytes (Karsten, West, & Zuccarello, 1992b). Some amino acids are also involved in the osmoregulation of some species, generally characterized by low concentrations of basic amino acids and a dominance of alanine, aspartic and glutamic acids that can represent more than 50% of free amino acids (FAA; Fattorusso & Piattelli, 1980). Other amino acids are involved in the osmoregulation of red algae: proline (54.8% FAA in *Polysiphonia nigrescens* and 65.3% FAA in *Halopitys incurvus*; Fattorusso & Piattelli, 1980); taurine, a sulfur amino acid derivative, such as *A. vermiculophyllum* (Surget et al., 2017), *Gracilariopsis lemaneiformis* (Broberg, Kenne, & Pedersen, 1998), *Grateloupia* sp. (Impellizzeri et al., 1975) and *C. crispus* (Harnedy & Fitzgerald, 2011). In marine algae, there are also other compatible molecules that are accumulated in response to osmotic stress, such as quaternary ammoniums (betaines) found in some red algae, such as *M. stellatus*, *Calliblepharis jubata* and the brown alga *Dilophus fasciola* (Blunden et al., 1992), and dimethylsulfoniopropionate (DMSP), a tertiary sulfonium found in some Rhodophyta, such as *Porphyra*, *Polysiphonia* species and in minority in brown algae (Reed, 1983).

## 2.6 Seawater chemical composition

To achieve their photosynthesis and growth, algae need nutrients that are essential to their development. As a result, several experiments have shown that nutrient enrichment leads to higher growth and nutrient content in tissues, such as in *A. vermiculophyllum* (Abreu, Pereira, Buschmann, Sousa-Pinto, & Yarish, 2011). More particularly, many culture experiments have demonstrated that carbon (C), hydrogen (H), oxygen (O), nitrogen (N), magnesium (Mg), copper (Cu), manganese (Mn), and zinc (Zn) are essential for algae (Hurd et al., 2014). Nitrogen as nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), ammonium ( $\text{NH}_4^+$ ), or urea ( $\text{CO}(\text{NH}_2)_2$ ), and phosphorus as phosphate ( $\text{H}_3\text{PO}_4^{3-}$ ) are often limiting factors for primary production and algal growth in natural environment since they occur at low concentrations in seawater (Lüning, 1990). Some seaweeds could thus produce hyaline hairs under low nutrient conditions to increase nutrient uptake rate (Harrison & Hurd, 2001; Wallentinus, 1984). Different algal species do not have the same affinity for each form of nutrients available in the marine environment:  $\text{NH}_4^+$  is taken up more preferentially and quickly by most seaweeds, such as *F. serratus* (Brenchley, Raven, & Johnston, 1997) and *Pelvetiopsis limitata* (Fujita, Wheeler, & Edwards, 1989) or *C. crispus* (Corey, Kim, Duston, Garbary, & Prithiviraj, 2013). On the other hand, *P. palmata* showed similar affinity for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Martínez & Rico, 2004).

Seaweed physiology depends on nutrients, whose concentrations are very variable in the marine environment: concentration of dissolved nutrients in surface waters are generally important in high latitudes, in coastal upwelling zones (Levitus, Conkright, Reid, Najjar, & Mantyla, 1993), and generally close to the coastlines and in estuaries or eutrophic sites. In addition, the nutrient availability can vary greatly depending on the time of the day or year. Indeed nutrient inputs are dependent on tidal cycle, since algae have no longer nitrogen source during emersion, affecting thus the nutrient seaweed content. For example, in *Porphyra umbilicalis*, the N content in tissues decreased during emersion (Kim, Kraemer, & Yarish, 2013). More specifically, species living at the upper intertidal zone need to be adapted to a longer nutrient deficiency, by modifying their uptake rate, uptake efficiency or enzymatic activities (Phillips & Hurd, 2003). In this sense, Benes and Bracken (2016) have demonstrated that the absorption rate of *F. vesiculosus* is 18% higher when living in the upper intertidal zone. In addition, algae are subjected to seasonal fluctuations in the nutrient content in the seawater. Macroalgae are particularly affected by very low ambient N and P concentrations during summer (Fujita et al., 1989). To cope with these variations, some species adapt their nutrient uptake, such as *Fucus gardneri* whose nitrate reductase activity increased sevenfold between February and March (Hurd, Berges, Osborne, & Harrison, 1995). Similarly, relative preferences for each N source vary according to the time of year in *Bostrychia arbuscula* and the two brown algae *Scytothamnus australis* and *Xiphophora gladiata*: in winter  $\text{NH}_4^+$  is preferred over  $\text{NO}_3^-$  and urea, while in summer  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are equally absorbed (Phillips & Hurd, 2003). In addition, nutrient concentrations depend on several abiotic factors: it has been shown that nutrient uptake depends on water motion (*i.e.* hydrodynamism), since it decreases the size of the water boundary layer of the seaweed (Hurd, 2000).

To cope with the temporal variation of nutrients, some species have the ability to incorporate and store more or less important quantities of nutrients in their cells, for a longer or shorter time in N-rich molecules such as proteins, amino acids and pigments (Gagné, Mann, & Chapman, 1982). According to Naldi and Wheeler (1999), proteins can contribute up to 90% to total N storage in algae. In *P. palmata*, N from phycoerythrin represents up to 8.3% of the thallus N content (Martínez & Rico, 2002). Levels of these compounds increase with N concentration and are quickly degraded under N limitation, as demonstrated in *Porphyra/Pyropia* spp. (Korbee, Huovinen, Figueroa, Aguilera, & Karsten, 2005; Pereira, Kraemer, Yarish, & Sousa-Pinto, 2008). Recently, mycosporine-like amino acids have been

also suggested as a N source for *P. palmata* in summer (Guihéneuf, Gietl, & Stengel, 2018). As a result, algal growth is not directly related to nutrient concentrations in the surrounding environment. Thus, to ensure their reproduction during summer, some species store nutrients in winter, when nutrients are high but not light and temperature. Thanks to these internal reserves, *Laminaria longicuris* can extend its growth several months after the decrease in N in the environment (Gagné et al., 1982).

The fast absorption or resistance to low nutrient contents can lead to competition between species: some species can take advantage during nutrient peaks, modifying the composition of algal communities since slow-growing benthic macrophytes are replaced by opportunistic ephemeral macroalgae with fast growth and high nutrient demands (Pedersen & Borum, 1996). If concentrations are too high, nutrients can be responsible for seasonal algal blooms (Heisler et al., 2008). This phenomenon of excessive algal biomass production is increasing from year to year due to eutrophication of the marine environment and nutrient inputs near agricultural lands or factories. Many algal blooms have been reported worldwide, mainly due to cyanobacteria, diatoms or some green algae, but there are also massive seasonal strandings of red and brown seaweeds (Teichberg, Martinetto, & Fox, 2012). Recently, nitrogen sources from the Amazon and Congo rivers are suspected to stimulate the growth and proliferation of pelagic *Sargassum* spp. in the North Atlantic Ocean (Ody et al., 2019; Oviatt, Huizenga, Rogers, & Miller, 2019). These developments modify community structures, impacting biodiversity and local economy (Teichberg et al., 2012).

Similar to nutrients, metals occur naturally in seawater at low concentrations; but elevated levels may occur in coastal waters due to contamination near mines, harbors, and industrial activity (e.g. Nriagu, 1990) which then can pose a threat to marine life if they are bioaccumulated. However, the metal bioavailability depends on many factors such as the salinity or the acidity of seawater. Among trace metals, copper is one of the most toxic: for example, for brown macroalgae, copper in excess inhibits photosynthesis (Connan & Stengel, 2011a), restricts growth (Nielsen, Brownlee, Coelho, & Brown, 2003), and disrupts gametophyte development (Garman, Pillai, & Cherr, 1994). Copper also affects the distribution of other algal compounds such as proteins (Contreras, Moenne, Gaillard, Potin, & Correa, 2010), free fatty acids (Ritter et al., 2008), phenolic compounds (Connan & Stengel, 2011b), and induces oxidative stress (Contreras, Mella, Moenne, & Correa, 2009).



### 3. Effect of biotic parameters

#### 3.1 Microfouling

Any substrate immersed in aqueous solution is immediately colonized by organic molecules (forming the primary film) that are consumed by bacteria followed by microalgae and fungi (Lewis, 1998). These microorganisms constitute the microfouling that will be used by other benthic macroorganisms such as invertebrates or macroalgae for their settlement. In the marine environment, over 4000 organisms, including algae, are able to form fouling (Chambers, Stokes, Wahsh, & Wood, 2006; Yebra, Kiil, & Dam-Johansen, 2004). Macroalgae represent a living substrate on which other organisms, plant or animal, can attach themselves. The community of the epiphytic bacteria is mainly host specific (according to the algal species) and is changing according to the part of the thallus, site and/or season (for example: Bengtsson, Sjøtun, and Øvreås (2010) and Mancuso, D'Hondt, Willems, Airoidi, and De Clerck (2016) on brown seaweed; and de Oliveira et al. (2012) for red macroalgae). The physiological state of the basiphyte will also influence the settlement of epiphytic bacteria: Marzinelli et al. (2015) observed that microbial communities were strongly associated with the physiological condition of *Ecklonia radiata* (healthy vs. stressed). The epiphytic bacteria interact with seaweeds, thereby modulating the health of their host. They may have positive effect on the basiphyte as being involved in their morphology and physiology. For example, when cultured in an axenic environment, some seaweed species will develop abnormal morphologies which become normal again when bacteria are added, suggesting the synthesis by the bacteria of compounds necessary for the macroalgal morphology (Egan et al., 2013). Weinberger et al. (2007) have also observed that secondary compounds produce by the bacteria are important in the algal spore release and settlement. Moreover, cyanobacteria are often abundant on benthic macroalgae probably due to their nitrogen fixing function that will help the macroalga in relatively poor nitrogen environment (de Oliveira et al., 2012). This strong relationship was emphasized by Egan et al. (2013) who suggested the use of the term holobiont for the association seaweed-bacteria, similarly to the coral.

On the other hand, biofilms can reduce the access of their host to light, gases and nutrients and alter the interaction with other fouling epibionts, consumers and pathogens (Goecke, Labes, Wiese, & Imhoff, 2010; Wahl, Goecke, Labes, Dobretsov, & Weinberger, 2012). To limit or reduce the microfouling, macroalgae will produce metabolites. For example, some

red and brown macroalgae are able to produce oxidative burst against epiphytic bacteria. Küpper, Kloareg, Guern, and Potin (2001), and Küpper, Muller, Peters, Kloareg, and Potin (2002) reported that brown macroalgae from the orders Laminariales and Desmarestiales and the species *Pylaiella littoralis* produce rapidly H<sub>2</sub>O<sub>2</sub> in response to the degradation of cell wall alginates and thus the liberation of alginate oligomers by bacteria. Similarly, red macroalgae and especially *Gracilaria conferta*, produce a similar oxidative burst in response to agar oligomers, which resulted in a 60% reduction of agarolytic bacterial pathogens (Weinberger & Friedlander, 2000). Plouguerné et al. (2008) worked on two invasive species, *G. turuturu* and *S. muticum*, which presented clean surface, without epiphytes. They demonstrated the importance of lipidic molecules as potential defense against microfouling: cholesteryl formate and glycolipids in respectively *G. turuturu* (Plouguerné, Kikuchi, Oshima, Deslandes, & Stiger-Pouvreau, 2006a) and *S. muticum* (Plouguerné et al. 2010). Hellio, Bergé, Beaupoil, Le Gal, and Bourgougnon (2002) carried out screenings on French intertidal seaweeds and demonstrated that extracts of red and brown species, especially *Vertebrata* (formerly *Polysiphonia*) *lanosa*, *C. crispus*, *Undaria pinnatifida* and *S. muticum*, are a source of antifouling molecules.

### 3.2 Epiphytes and endophytes

Following the development of microorganisms at the surface of the macroalgae (first step of the biofouling), a secondary colonization occurs with macroorganisms such as other algae or animals, that settle on the algal surface. Other organisms will colonize the subsurface and enter into the seaweed thallus. The first group of species is called epiphytes as being epibiont of an alga (called basiphyte), and the other endophytes. The carrying of epiphytes is common on marine algae, ranging from obligate relationships (such as *Vertebrata lanosa* on *A. nodosum*; Garbary, Miller, & Scrosati, 2014), to species that colonize any available space. Five anatomical relationships between epiphyte and their host have been detected (reviewed in Potin, 2012) from the weakly attachment to the host surface with no tissue damage (Type I), up to the deep penetration of the epiphyte into the host cortex, reaching the medullary tissue, and causing the destruction of the host's cells in the area around the infection (Type V).

As for microfouling, the abundance and diversity of epiphytes follow a seasonal pattern (e.g. higher diversity and biomass of epiphytes on *F. vesiculosus* in spring-summer; Rindi & Guiry, 2004). Spatial variation in the epiphyte community on the stipes of *Laminaria hyperborea* was recorded in

Scotland, with differences with the depth along a stipe and according to the host position on the shore (Whittick, 1983). The age of the algal part also affected the epiphyte abundance, with epiphyte densities greatest on the oldest tissue; surface rugosity also affected fouling (Jennings & Steinberg, 1997). The epiphytes, as they live at the surface of the seaweeds, will reduce the amount of light reaching the basiphyte thus impacting the algal photosynthesis (Sand-Jensen, 1977), compete for nutrients and gases, and reduce water motion surrounding the host, thus impacting the host growth (Buschmann & Gómez, 1993). Epiphytes will add weight to the host alga promoting the detachment of the entire, or part of it (e.g. Buschmann & Gómez, 1993; Kraberg & Norton, 2007). Epiphytes also decrease the reproductive effort of algae (e.g. *V. lanosa* on *A. nodosum*; Kraberg & Norton, 2007). Finally, epiphytes will release metabolites in the surrounding water that can promote or be detrimental to the host algae (see Harlin, 1987 for a review).

On the other hand, epiphytes may also have a positive effect on the basiphyte by, for example, reducing the desiccation experienced at low tide by the host or protecting the basiphyte from grazers (Karez, Engelbrecht, & Sommer, 2000; see following section). On the other side, as for the primary foulers, the seaweed host can exhibit a variety of defense against the epiphyte attachment (Ducker & Knox, 1984), including the production of a mucilaginous covering, a rapid growth, a sloughing of outer cell walls (Filion-Myklebust & Norton, 1981; Moss, 1982), allelopathy, or the release of toxic chemicals such as phenolic compounds (Rönnerberg & Ruokolaihti, 1986). However, this activity of phenolic compounds is sometimes controversial (Jennings & Steinberg, 1997). The algal host will thus modify its metabolism and produce compounds to eliminate or control the epi- and endophytes growing on or in its thallus. For example, red and brown macroalgae are known to produce terpenes with antifouling activity against microorganisms (see previous section) but also against animals. Schmitt, Hay, and Lindquist (1995) identified the diterpenes pachydictyol A and dictyol E in *Dictyota menstrualis* which were active against the settlement of algal epiphyte and bryozoan larvae (*Bugula neritina*). The red macroalga *Delisea pulchra* produces halogenated furanones which were the most effective natural product against the settlement of barnacles (de Nys et al., 1995).

Many filamentous epiphytes can also develop into endophytes through notably the expansion of rhizoids or the filaments embedded into the host thallus (Chirapart, Praiboon, Boonprab, & Puangsombat, 2018; Leonardi et al., 2006). A benthic diatom, *Pseudogomphonema* sp. has also been found

living and reproducing into the red macroalga *Neoabbottiella* sp. (Klochkova et al., 2014). Generally, endophytes are bacteria, fungi or other algae that live into their host, but these relationships are species-specific from being innocuous up to pathogenic (Schoenrock, Amsler, McClintock, & Baker, 2013). There is also many metabolite exchanges between the endophyte and the seaweed host. Endophytes induce morphological modifications (such as kelp twisted stipes and blades; Lein, Sjøtun, & Wakili, 1991) of the host up to the development of galls (Apt, 1988). The brown algal endophyte *Laminariocolax aecidioides* (previously *Streblonema aecidioides*) causes tissue thickening in *S. latissima* (Peters & Schaffelke, 1996), but has no impact on the sorus maturation of *U. pinnatifida* (Gauna, Parodi, & Cáceres, 2009). The red macroalga *C. crispus* and *Mazzaella laminarioides* both developed severe lesions and cellular damages when infected by the green alga *Ulvella* (previously *Acrochaete*) *operculata* (Correa & McLachlan, 1992) and *Undaria ramosa* (previously *Endophyton ramosum*) (Correa, Flores & Garrido, 1994), respectively. As against bacterial fouling (see previous section), *C. crispus* resisted to the infection by *Undaria operculata* through the setting up of an oxidative burst, resulting from the contact between carrageenan and the green algal filaments (Weinberger et al., 2005); burst which is stronger in the gametophyte than the sporophyte of *C. crispus* (Bouarab, Potin, Correa, & Kloareg, 1999). One positive reported effect of the presence of epiphyte or endophyte for their host is the decrease in algal palatability for grazers (Amsler, Amsler, McClintock, & Baker, 2009; Karez et al., 2000), although both can sometimes attract grazers to the basiphyte (Campbell, Vergés, & Steinberg, 2014; Wahl, Hay, & Enderlein, 1997).

### 3.3 Grazing

In the coastal communities, grazing is a structuring element because herbivores are able of significantly affecting the amount of seaweed biomass available (Lubchenco & Gaines, 1981). For example, uncontrolled urchin populations may impact a seaweed community up to completely remove the macroalgae and leave “urchin barrens” where only encrusting algae may survive such as in Norway or Alaska (Steneck et al., 2002). Grazers reduced the growth and reproduction of seaweeds (e.g. *F. gardneri*; Dethier, Williams, & Freeman, 2005) and induced the breaking down of seaweeds by removing parts that weakened their thalli and making them more vulnerable to hydrodynamism (reviewed in Iken, 2012).

Some macroalgae modify their morphology in response to a herbivore presence: for example, transplantation of the brown macroalga *Padina*

*sanctae-crucis* (previously *P. jamaicensis*) into a grazing-protected area resulted in a rapid morphological shift from prostrate, highly branched turf to an erect foliose morphology (Lewis, Norris, & Searles, 1987). Another algal morphological change to decrease grazing is the inclusion of calcium carbonate into the thallus often concomitant with the synthesis of chemical defense (Paul & Hay, 1986). Brown macroalgae produce phenolic compounds whose role as anti-grazing molecule has been either observed (e.g. Geiselman & McConnell, 1981) or not (e.g. Kubanek, Lester, Fenical, & Hay, 2004) depending on the algal or herbivore species. Brown seaweeds belonging to Dictyotales are known to produce many defensive secondary metabolites with many having anti-herbivorous activity (reviewed in Amsler & Fairhead, 2005): for example, *Dictyota* spp. produce terpenes and notably pachydictyol-A (diterpene alcohol) which showed anti-grazing activity against many fishes, sea urchins and amphipods (Hay & Fenical, 1992). Flöthe, Molis, Kruse, Weinberger, and John (2014) studied the molecular response of *F. vesiculosus* to grazing by the isopod *Idotea baltica*: algal defense was induced by the grazing and they found up to ~500 genes that were up (e.g. lipid, carbohydrate metabolism, defense and stress response) or down-regulated (such as photosynthesis) and indicative thus of algal metabolic changes. These changes were also herbivore specific as the grazing by a periwinkle (*Littorea obtusata*) showed different transcriptome changes (Flöthe, Molis, & John, 2014). The same isopod produced also inducible defense in three red macroalgae, *F. lumbricalis*, *Delesseria sanguinea* and *P. pseudoceranoides*, in feeding experiments (Rohde & Wahl, 2008). *D. pulchra* produced halogenated polyketide metabolites or furanones (de Nys, Wright, Konig, & Sticher, 1993), which protect the alga not only against fouling (see previous section) but also grazers (Wright, de Nys, Poore, & Steinberg, 2004). A rapid response of two kelp species (*L. spicata* and *Laminaria digitata*) to grazing by their main herbivores was observed by Ritter et al. (2017): seaweeds produced first sulfur containing amino acids followed by free fatty acids liberation and eicosanoid oxylipins synthesis likely representing metabolites related to stress. Sometimes the production of defense metabolites by the seaweed does not need a physical contact between the seaweed and the grazer: for example, *A. nodosum* when grazed will produce and release compounds in the surrounding seawater that will enable neighboring *A. nodosum* thalli to produce defense compounds even if they were not grazed (Toth & Pavia, 2000).



## 4. Conclusion

Macroalgae in rocky shores are subjected to various environmental factors, such as hydrodynamism, desiccation, light, salinity and temperature (Fig. 1). However, these different disturbances vary during the day (tidal cycle or nycthemeral cycle), the season, or the geographical area, impacting the distribution of algae, whether between shores, or within the same shore (vertical zonation). To cope with this highly variable environment, seaweeds must therefore have a high adaptative ability to respond to significant changes that can occur in just a few hours. They are thus able to adapt their photosynthesis, respiration or reproduction; and to synthesize a wide range of compounds, including pigments (photosynthetic or photoprotective), mycosporine-like amino acids, lipids, proteins, polysaccharides, or phenolic compounds, specific to each species (Table 2). Nevertheless, different stresses

**Table 2** Examples of molecules synthesized by marine macroalgae, with their chemical structures.

Compounds	Chemical structure
Pigments	<p>Chlorophyll-<i>a</i>, Lutein, Fucoxanthin</p>
Carbohydrates	<p>Floridoside, Mannitol, K-carrageenan</p>
Lipids and fatty acids	<p>Palmitic acid, Eicosapentaenoic acid</p>
Terpenes	<p>Bifurcane, Eleganolone, Eleganediol</p>
Phenolic compounds	<p>Phloroglucinol, Fuhalol, Gallic acid</p>
MAAs	<p>Porphyra-334, Shinorine, Asterina-330</p>

can act at the same time on the shore, and have a synergistic effect, making it complex to attribute the synthesis of a compound to a particular factor. In order to better understand the ecological and physiological responses of algae, it is therefore important to carry out field monitoring to study algae in their own environment with all factors; and to carry out culture experiments in the laboratory to test the impact of different stresses one by one or in combination.

In addition, by biometism, the study and understanding of the synthesis of these protective compounds can provide inspiration for valorization in various fields such as medicine, cosmetics (reviewed by [Stiger-Pouvreau & Guérard, 2018](#)), fertilizer, antifouling paints ([Hellio, De La Broise, Dufosse, Le Gal, & Bourgougnon, 2001](#)) or biofuel. Thus, algae have a high potential since they are a highly diversified resource that is much less studied than terrestrial plants. In this sense, there are, for example, cosmetic products with algal photoprotective compounds to reduce skin aging and the development of skin cancer: for example, the MAAs shinorine and porphyra-334 extracted from *Porphyra umbilicalis* in Helioguard 365 (Mibelle group, Switzerland), a commercial formulation used to protect the skin from UV radiation ([La Barre, Roullier, & Boustie, 2014](#)).

Finally, human activities are increasing, resulting in a stronger anthropogenic effect on the environment: they generate significant greenhouse gas emissions, which are mainly responsible of global warming and ocean acidification. These changes disrupt the physiology and ecology of many algal species ([Walther, 2010](#)). In particular, the geographical distribution of algae is guided by climatic limits; climate change induces recurrent exposition of species to extreme temperatures, variable in time and leading to not only a switch of species to northern latitudes, but also a succession of scattered local extinctions (*e.g.* in Portugal; [Lima, Ribeiro, Queiroz, Hwakins, & Santos, 2007](#)). Local communities are restructuring as species are added or deleted and some species may develop in areas where they could not previously survive or reproduce. In this sense, review of the different seaweed assemblages of each region have already been made ([Garbary, 2001](#); [Lüning, 1990](#); [van den Hoeck, 1984](#)), but it is important to carry out periodically local checklists as some species are expected to migrate or disappear with human activity and climate change. In particular, in recent years, the massive strandings of algae and the proliferation of invasive algae have increased, especially in Brittany ([Stiger-Pouvreau & Thouzeau, 2015](#)), weakening native algal fields. Climate change also affects interactions among marine organisms: for example, elevated temperature reduced herbivore defenses in *F. vesiculosus*

(Weinberger et al., 2011) and facilitate bacterial infections (Harley et al., 2012). Seaweed thus have to cope with these rapid changes in their environment.

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Macroalgae living in the intertidal zone are exposed to many environmental variations impacting their physiology, ecology and subsequently their distribution as described in the previous review (Lalegerie *et al.* 2020). However, these environmental conditions tend to evolve as a result of global change, which algal populations will have to cope with to avoid disappearance as described in the next section. In this respect, it is important to differentiate between acclimation and adaptation (see **Box 1**). Only the acclimation abilities are studied in this work, contributing to a better understanding of the potential adaptation of algal populations in response to global change.

### Box 1 – Acclimation versus adaptation

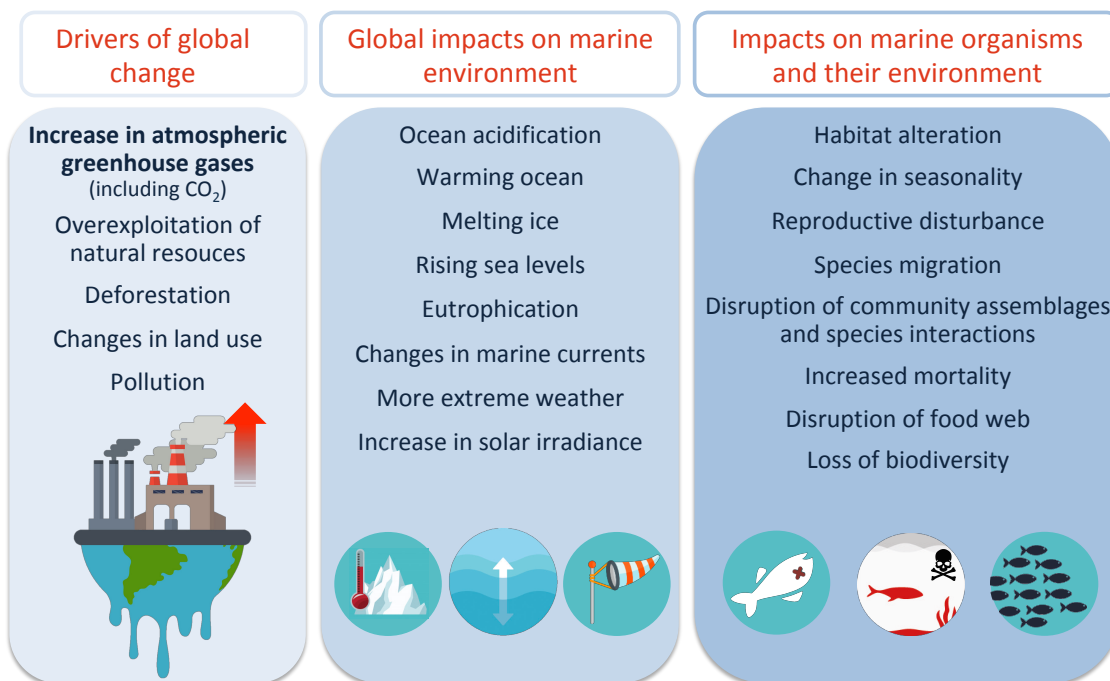
All organisms, including macroalgae, deal with environmental variations in their natural habitat, to which they respond at different scales (*i.e.* acclimation or adaptation) depending on the duration and degree of amplitude of these variations (Raven and Geider 2003). In this regard, acclimation can be defined as a physiological, biochemical, morphological change in an individual due to its exposure to a new environment. It involves, for example, the synthesis of secondary metabolites such as photoprotective pigments or osmolytes. It is a temporary (*i.e.* hours to days) and reversible phenomenon. Then, the degree of acclimation depends on the genetic constraints of the species considered. In other words, each species has its own level of phenotypic plasticity, that can be defined as the capacity of a genotype (*i.e.* genetic heritage) to produce different phenotypes (*i.e.* the observable characteristics or traits of an organism) through the expression of one or more genes in response to environmental change.

On the other hand, adaptation is a process of evolution by natural selection, which takes place within a species after several generations. This implies a modification of the genotype that takes place over a longer time scale (*i.e.* millions of years) compared to acclimation (Pigliucci 2005). Adaptation is thus the response of species to sustained changes in environmental conditions, such as those caused by global change. This phenomenon of adaptation leads then to a high genetic, specific, or chemical diversity.

## 1.3 The global change and its impact on macroalgae

### 1.3.1 What is the global change?

Marine organisms are currently threatened and weakened by a combination of environmental changes observable on a global scale, resulting mostly from anthropogenic factors (Harley *et al.* 2006; Duarte 2014) (Fig 2). Indeed, human activities lead to air and water pollution, waste production, eutrophication, overexploitation resulting in a scarcity of resources (*e.g.* water, energy, animals and plants), habitat modification/degradation (*e.g.* deforestation, establishment of new structures), etc. More particularly, fossil fuel combustion (for industrial factories or transport) is a major source of air pollution, representing around 40 billions tonnes of carbon dioxide (CO<sub>2</sub>) generated per year (IPCC 2018).



**Figure 2.** Conceptual model of drivers of global change (caused mainly by human activity), with consequences on marine environment and marine organisms and ecosystems (non-exhaustive list), adapted from Duarte (2014).

All these disturbances associated with human activities then intensify and evolve with demographic pressure, the globalisation of exchanges, technological evolution or changes in social behaviour (*e.g.* desertification of rural areas coupled to an urbanisation). Then, all these modifications of natural environment at the scale of Earth lead to ongoing changes and a deep disruption of ecosystems worldwide (including marine ecosystems), defined as global change. In this way, in marine environment, there is already an ocean acidification, sea level rise,

change in marine currents or increase in UV radiation for example, subsequently impacting marine organisms (Fig. 2) (Duarte 2014).

More particularly, there is currently an ongoing rise in the average temperature of Earth's climate system due to an increase in greenhouse gases caused by human activities (Cicerone and Nurse 2014). In fact, there is a natural phenomenon called "greenhouse effect" whereby part of the solar energy arriving on Earth is absorbed by the Earth's surface (~50%), resulting in an increase in the temperature of the Earth's surface (Mitchell 1989) (Fig. 3). The Earth's surface then emits in return infrared radiation (IR), of which a part is trapped by greenhouse gases (or « heat-trapping gases ») presents in Earth's atmosphere, such as carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) or ozone (O<sub>3</sub>). Then, human activities, through the use of fossil fuels or deforestation, increase the emission of greenhouse gas, adding to those naturally present in the atmosphere. This enhances the greenhouse effect, unbalancing climate system and causing consequently global warming (Mitchell 1989). It is coupled to uncertain effects on ozone layer, modifying the attenuation of UV radiation (Bais *et al.* 2018).

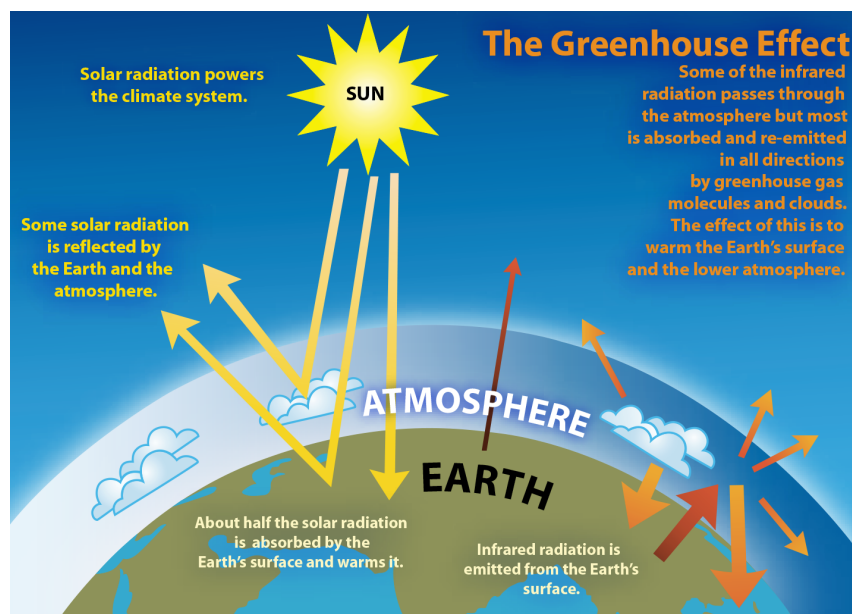


Figure 3. Schematic representation of greenhouse effect, from Solomon *et al.* (2007).

By absorbing more than 90% of the excess heat, the ocean is then particularly affected by carbon emissions and global warming (Jewett and Romanou 2017), causing also indirect effects such as melting ice, rising sea levels, ocean acidification, changes in marine currents, or more extreme weather (*i.e.* more intense precipitations, wind, storms). In this way, it has already been measured a rise in the mean sea level, sea-surface temperature or mean ocean-

surface pH over the last century (Fig. 4). For example, the average sea-surface temperature is 0.80°C higher than it was at the pre-industrial time period (*i.e.* 1800) (IPCC 2019). This global warming is coupled to an increase in atmospheric CO<sub>2</sub> which is about 20 ppm per decade since 2000, *i.e.* 10 times faster than any sustained rise in CO<sub>2</sub> during the past 800,000 years (IPCC 2018) (Fig. 4).

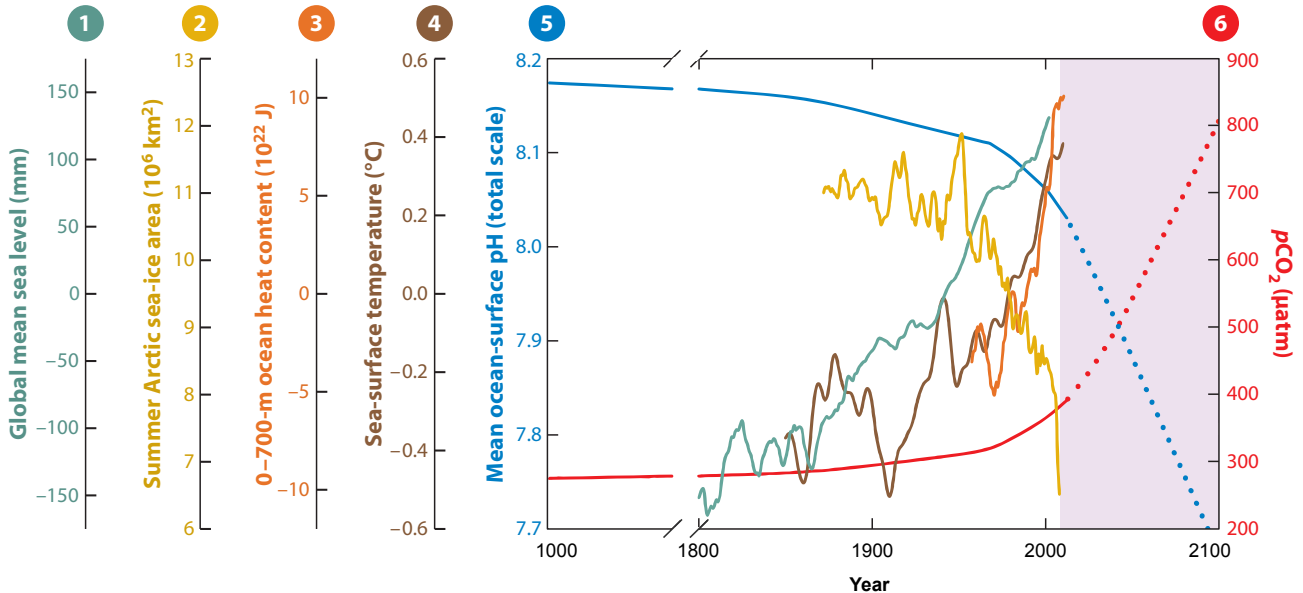


Figure 4. Temporal evolution in ① global mean sea level, ② summer Arctic sea-ice area, ③ 0–700-m ocean heat content, ④ sea-surface temperature, ⑤ mean ocean-surface pH, and ⑥ atmosphere  $p_{\text{CO}_2}$ . Light purple shaded region denotes projected changes in pH and  $p_{\text{CO}_2}$  consistent with the Intergovernmental Panel on Climate Change’s twenty-first-century A2 emissions scenario with rapid population growth. From Doney *et al.* (2012).

The phenomenon of climate change and more largely global change is then likely to get worse in the coming years as the World's population is steadily increasing. Indeed, global human population is growing at 1.1% per year, and it is estimated that it will pass the threshold of nine billion people by 2050 (Asadian *et al.* 2018). In this regard, one objective is to reduce our CO<sub>2</sub> emissions in an attempt to limit / slow down climate change, which involved the implementation of climate policies. Then, Intergovernmental Panel on Climate Change (IPCC) defined different emission scenarios (classified in 4 groups), according to demography, economy, and societal and environmental proprieties (IPCC 2000). The last projections of the IPCC estimate that the concentration of CO<sub>2</sub> equivalents in 2100 should be between 430 and > 720 ppm depending on the scenarios and mitigation policy (IPCC 2014). In parallel, ocean pH will decline, between 0.04 and 0.29 pH units by 2081–2100, relative to 2006–2015, depending on emission scenarios (IPCC 2019). Similarly, the global surface

temperature change for the end of the 21<sup>st</sup> century (2081–2100) is projected to be between 1.5°C and 2°C above pre-industrial levels (IPCC 2019). This is expected to be coupled with marine heatwaves which are set to become even more frequent, intense and longer-lasting under future climate change scenarios (IPCC 2019). The Paris Agreement within the United Nations Framework Convention on Climate Change (UNFCCC) signed in 2016 aims then to limit the rise in average global temperatures below 2°C, and as close as possible to 1.5°C, to reduce risks and impacts of climate change (IPCC 2018).

### 1.3.2 The consequences of global change on macroalgae

All various environmental disturbances attributable to human activities affect marine organisms in terms of their photosynthesis, ecology, phenology, and physiology, sometimes even at the molecular level (Doney *et al.* 2012; Harley *et al.* 2012; Ji *et al.* 2016). In this regard, these significant environmental variations related to global change have repercussions on the composition, abundance and distribution of different marine organisms worldwide, including algal populations (Dring *et al.* 1996). This leads then to changes in species interactions and to the trophic chain, influencing the coastal ecosystem as a whole (Lotze and Worm 2002; Häder *et al.* 2007) (Fig. 2). More particularly, a temperature increase of the seawater has a strong impact on macroalgae as it is the main factor controlling their growth, reproduction, or recruitment, and that their survival is directly dependent on the physiological tolerance of their life cycle stages to this environmental parameter. For example, there is already a weakening of the reproductive capacity of some kelp populations worldwide, *i.e.* in Brittany (Oppliger *et al.* 2014), as well as in other regions of the world, such as along the Iberian Peninsula (Voerman *et al.* 2013), or in Australia (Wernberg *et al.* 2011). In this way, thermal changes lead to modifications in the biogeographical distribution patterns of species as described by Bartsch *et al.* (2012). More specifically, the distribution of some large brown macroalgae has already changed, leading to a significant modification of the ecosystem with potential impacts on commercial species (Smale *et al.* 2020). Recently, it has been reported for example the local extinction of *Durvillaea antarctica* in New-Zealand (Thomsen *et al.* 2019), the first observation of *Laminaria ochroleuca* in Ireland (Schoenrock *et al.* 2019), or the migration of *Carpodesmia tamariscifolia* from Southern to Northern Ireland (Hiscock *et al.* 2004). At the same time, red macroalgae are also impacted, as shown by the recent observation in Northern Europe of the red *Centroceras clavatum* with warm-water affinity (Le Duff and Ar Gall 2015). Similarly, Gallon *et al.* (2014) showed in Brittany that the

diversity of red macroalgal species associated with kelp was significantly modified from 1990 to 2010, probably due to an average increase of 0.7°C in coastal waters. More particularly, species with a cold-water affinity and therefore at the Southern limit of their range are perhaps the species most threatened by the predicted increase in temperature. Thus, there are three possibilities: (1) they could **adapt** to new conditions at a genetic and molecular level; (2) they could **migrate** through pelagic part of their life cycle and see their range shifting northwards due to the increase in seawater temperature; and (3) they could **disappear** from macroalgal communities in more southern regions or disappear completely (*i.e.* species extinction) (Bartsch *et al.* 2012). This would leave a vacant ecological niche, which could then be colonised by other species, either native or introduced.

In this regard, the number of introduction (see **Box 2** for more informations on introduced species) is likely to increase with global change, more particularly in marine environment due to the overexploitation of the resources, pollutions and the intensification of maritime transport (Occhipinti-Ambrogi and Galil 2010; Early *et al.* 2016). In this way, although there is currently insufficient data on the impact of global change on the expansion of introduced / invasive marine macroalgal species, some studies have suggested a potential relationship between global change and increasing number of exotic species. More particularly, changes in the distribution of some marine species linked to ocean acidification or global warming have already been observed, leading to substantial changes in ecological niches (Hughes 2000; Hiscock *et al.* 2004; Occhipinti-Ambrogi and Galil 2010; Ji *et al.* 2016; Schoenrock *et al.* 2019; Thomsen *et al.* 2019; Smale 2020). Furthermore, it is possible that global change enhances the number of invasive species by modifying the propagation speed, growth, or reproductive success of introduced species.

### Box 2 - Case study of introduced species face to global change

The introduction of species and the spread of invasive species constitute an important ecological and economic threat, particularly in marine environments since oceans / seas are highly connected (Ruiz *et al.* 1997; Mooney and Cleland 2001). Then, Boudouresque and Verlaque (2002) defined:

- **Introduced species** (also called alien, or non-indigenous species): species arriving in an area where it was previously absent (through human activity, whether accidental or not), with a geographical discontinuity with the area of origin.
- **Naturalized species**: non-native species settled / permanently established in their new environment (*i.e.* able to reproduce without human intervention).
- **Invasive species**: an introduced species that creates negative ecological impacts on local ecosystems and species, and / or economic problems in the new colonized geographical area.

To date, 1,223 non-indigenous species have been reported in the Europe's seas (EEA 2019), introduced by different vectors (*i.e.* transfer mechanisms), such as shipping, aquaculture activity, constructions of marine structures (*e.g.* Suez Canal), aquarium trade, biological experiments, or tourism (Molnar *et al.* 2008; Minchin *et al.* 2009; Katsanevakis *et al.* 2013). Macroalgae represent then a significant part of these marine species (Schaffelke *et al.* 2006; Tsiamis *et al.* 2019), including invasive species such as *Undaria pinnatifida*, *Sargassum muticum*, *Caulerpa taxifolia*, *Agarophyton vermiculophyllum* or *Grateloupia turuturu* (Streftaris and Zenetos 2006; Stiger-Pouvreau and Thouzeau 2015). A particular attention is paid to invasive species since their spread induces alterations to the receiving ecosystems, by modifying habitats, species communities, and a wide range of ecosystem functions (Wallentinus and Nyberg 2007; Molnar *et al.* 2008; Katsanevakis *et al.* 2014). More particularly, invasive species threaten the distribution and survival of native species through competition for space, light and nutrient availability. In most cases, invasive species lead subsequently to a loss of biodiversity (Schaffelke and Hewitt 2007; Katsanevakis *et al.* 2014; Davidson *et al.* 2015). Conversely, few studies reported positive impact following the introduction of species. For example, it has been demonstrated that the introduction of the red *A. vermiculophyllum* has led to the increase in macrofauna and meiofauna diversity in Brittany mudflats (Davoult *et al.* 2017). Ecological impacts are then usually accompanied by a significant economic loss, due to the negative impacts for aquaculture, fisheries, or tourism, coupled with the costs of eradication/limitation efforts (Boudouresque and Verlaque 2002; Pimentel *et al.* 2005; Kettunen *et al.* 2009). In this way, the global cost resulting from invasive species was estimated at €12.5 billion per year in Europe (Kettunen *et al.* 2009). To limit the loss caused by invasive species, three strategies are then possible: (1) eradicate, (2) limit the proliferation of the species, or (3) prevent possible future introductions by species monitoring / inventories and the implementation of global legislation (Minchin *et al.* 2009; Stiger-Pouvreau and Thouzeau 2015).



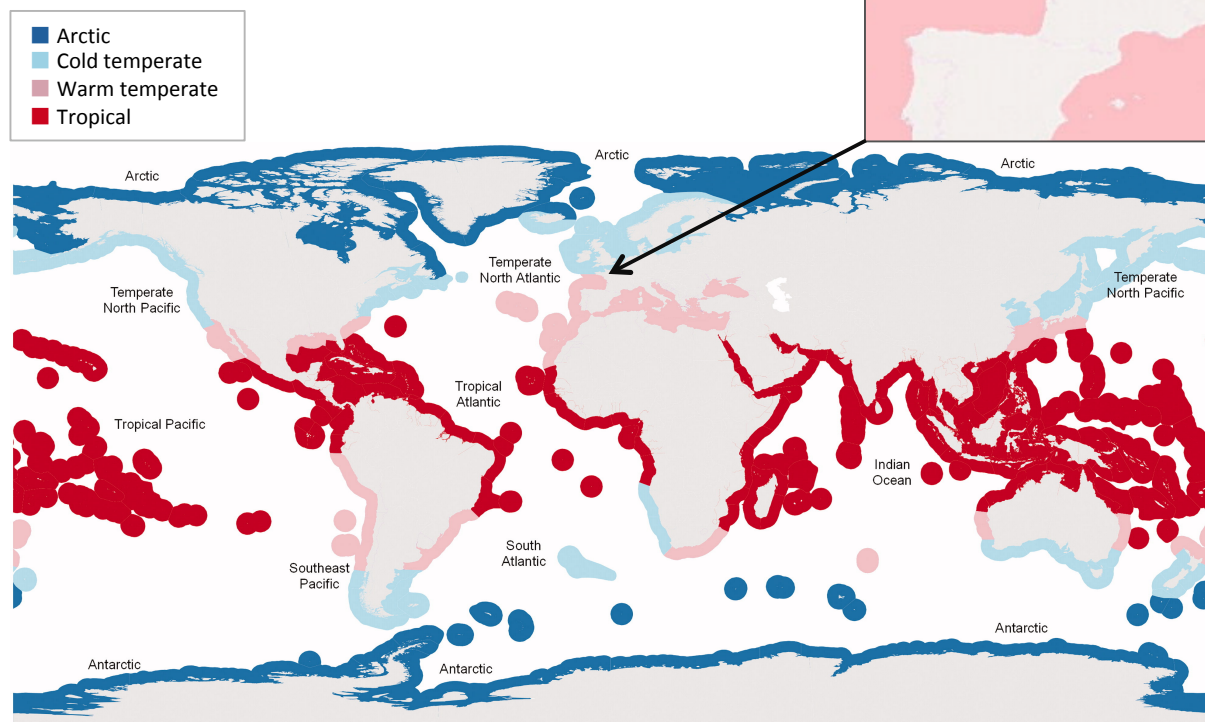
The increase in the number of species introductions could further disrupt existing ecosystems and impact native species already threatened by climate change (Occhipinti-Ambrogi and Savini 2003). Indeed, by modifying environmental conditions, the global change will lead both to the weakening / disappearance of native species, and to the introduction of new species that will refill the niches left vacant. In other words, global change will lead to changes in the current interactions / co-habitation between introduced and native species, and create competitive disadvantages for some species. Thus, unfavourable conditions could decrease the tolerance of some native species which would then encounter difficulties in adapting both to environmental changes (*e.g.* increasing temperature) and to competition with new introduced species. In this extend, species at the limit of their distribution may be the most threatened. Nevertheless, it is difficult to differentiate the impact of both biological invasions and climate change on native species, as these two anthropogenic forces are key elements impacting ecosystems.

Moreover, the phenotypic plasticity of introduced/invasive species could also be modified by climate change. More particularly, global change could impact the production of particular metabolites by native and invasive macroalgal species, key components of their acclimation, although little is actually known. The invasive potential of some species could be enhanced, and actual non-harmful species could become threatened under favourable conditions, up to supplant native species in the worst case. Conversely, species that are currently invasive could see their fitness level decreasing, becoming disadvantaged compared to native species under stressful conditions (Davidson *et al.* 2011). These uncertain conditions promote the monitoring of introduced / invasive species with comparisons with native species.

## 2. Context of the thesis

### 2.1 Brittany (France): a biogeographic transition zone

Marine organisms are distributed worldwide according to their tolerance limit to different physico-chemical or biological factors, and to their migration events from one region to another as described by Lüning (1990). Thus, on a global scale, the current marine species distribution is usually divided into different biogeographical regions. In this regard, by reviewing over 230 works on the basis of taxonomic configurations, Spalding *et al.* (2007) defined 12 realms, 62 provinces and 232 ecoregions which cover all coastal and shelf waters. More generally, temperature is usually one of the main factors driving geographical subdivisions since it affects the physiology and phenology, and notably the reproduction, of marine organisms. In this way, in an attempt to simplify, 4 subdivisions are generally defined: Arctic, cold temperate, warm temperate and tropical waters (Fig. 5) (Bringloe *et al.* 2020).



**Figure 5.** Marine biogeographic subdivisions, modified from Bringloe *et al.* (2020), initially adapted from Spalding *et al.* (2007). Brittany (France) is located at the limit between cold and warm temperate regions.

Brittany, located at the North-West of France, constitutes a transition zone between 2 ecoregions, *i.e.* cold-temperate and warm-temperate, from North Atlantic (Spalding *et al.* 2007) (Fig. 5). As a result, the Breton flora and fauna are characteristic of these two subdivisions,

resulting in a high specific diversity in Brittany. This diversity is also favoured by the length of Brittany's coastline (which extends between 2,500 and 3,000 km) including a high proportion of rocky shores, and a large tidal amplitude, allowing an important development of very diverse macroalgal populations. In this way, it has been recorded more than 650 species of macroalgae on Brittany's coastline (Cabioc'h *et al.* 2014; Dizerbo and Herpe 2007; Burel *et al.* 2019), constituting one of the greatest specific diversity in the world (Keith *et al.* 2014).

In the context of climate change with the temperature rise predicted by IPCC (see **section 1.3.1**), Brittany represents an interesting transition zone, *i.e.* a privileged study site: due to its biogeographical feature and high specific diversity, Brittany could be the centre of important changes in intertidal macroalgal distribution caused by global change. For example, in Brittany, the diversity of red macroalgal species associated with kelp was strongly modified from 1990 to 2010 (Gallon *et al.* 2014), probably due to an average increase of 0.7°C in coastal water temperature. In that respect, a significant number of migrations could be observed in this region in the coming years, with species from the south moving northwards. More particularly on a local scale, some macroalgal species have their northern or southern limit of distribution in Brittany waters. In this way, some Southern species with warm-water affinity not yet recorded in Brittany could extend their distribution up to this region. On the contrary, some cold-water affinity species that have their Southern limit of distribution in Brittany, such as the brown macroalgae *Alaria esculenta* (Castric-Fey *et al.* 2001) or *Laminaria digitata* (Lüning 1990; Dizerbo and Herpe 2007) could migrate beyond the Northern limit of Brittany, and/or see their populations disappear in this area (Oppliger *et al.* 2014).

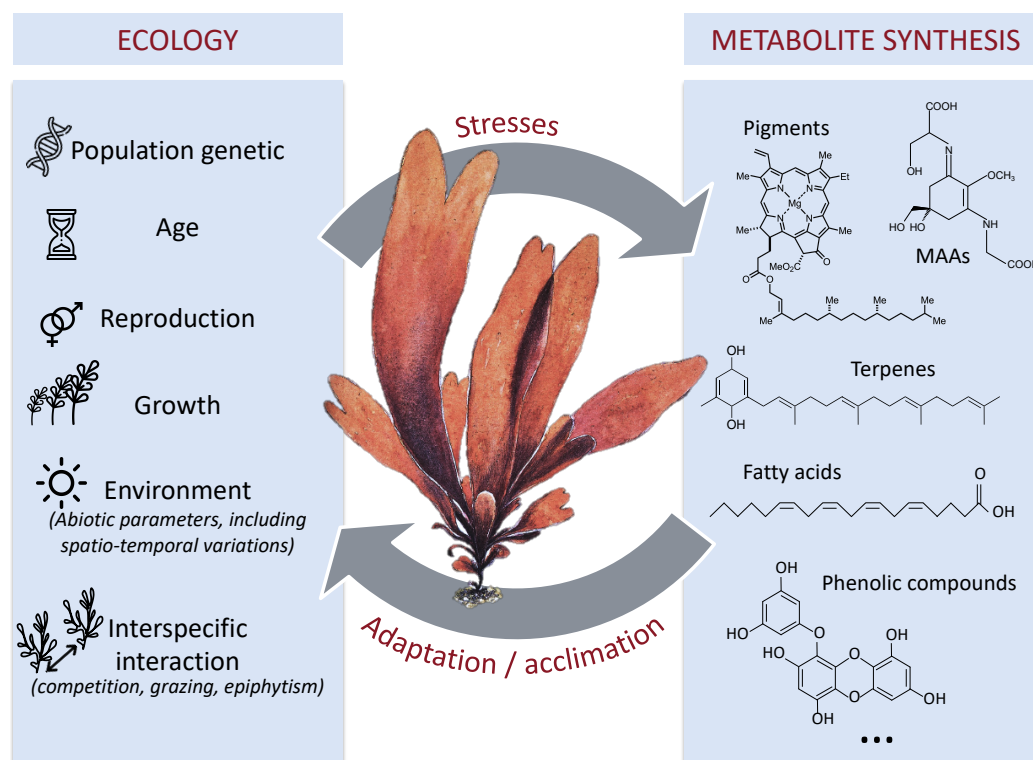
Furthermore, Brittany is one of the major points for the marine species introduction in Europe as evidenced by the number of alien species in this region compared to the rest of the world (Molnar *et al.* 2008). These introductions, involuntary or not, were induced mainly by aquaculture (*i.e.* seashell / oyster imports), and shipping activities (the main introduction event being in 1944 during the Second World War) (Stiger-Pouvreau and Thouzeau 2015). Among the introduced species, some have successfully established, constituting perennial populations; and some of them have become invasive, such as the brown macroalgae *Sargassum muticum* or *Undaria pinnatifida*, and the red algae *Agarophyton vermiculophyllum* or *Grateloupia turuturu* (Strefataris and Zenetos 2006; Stiger-Pouvreau and Thouzeau 2015).

Then, in the context of climate change which will lead to the migration of species from South to North, there is an additional risk of perturbations of native species in Brittany, due to its position as a transition zone (Fig. 5). Such a situation could disrupt the existing diversity, and threaten the native algal species, more particularly those at their limit of distribution range.

## 2.2 An "Eco-metabolomic" approach to study the adaptive responses of macroalgal populations

Recent works emphasize the importance of coupling ecological and metabolomic data, which is defined as "eco-metabolomics" (Jones *et al.* 2013; Peters *et al.* 2018). In this way, a such integrated approach could provide a better understanding of adaptive abilities, and interactions within and between algal species. Indeed, natural macroalgal populations are exposed to various biotic or abiotic stresses, *e.g.* inter-species competition, or fluctuations in environmental parameters such as light, temperature or salinity that will in turn affect their growth or reproduction (reviewed in Lalegerie *et al.* 2020). When studying algal populations in their natural environment, all these factors interact simultaneously, and it is then difficult to determine which environmental factor impacts the survival and development of the alga. Thus, and in the context of global change, it is difficult to study the acclimation / adaptation of a species based only on ecological data, since ecological variations can be the result of variations of a multitude of biotic or abiotic factors. Another way to study the acclimation of species to different environmental stresses is to study their metabolome. Indeed, macroalgae have the ability to accumulate a wide variety of metabolites, such as examples, pigments, phenolic compounds, or fatty acids, which act as key components of their ecological adaptation / acclimation to different biotic or abiotic stresses (see review **section 1.2**). Nevertheless, each compound can also be synthesized in response to a multitude of ecological variables. For example, the composition of phenolic compounds depends on the stage of development, grazing, salinity or nutrient content (see **section 2.3.4**). Thus, to understand how macroalgae adapt to / interact with the environment, it is useful to take all variables into consideration by combining the ecology of populations with the metabolomic composition of individuals, in order to have a global overview of their adaptability (Fig. 6). The objective is then to study as many metabolites as possible in order to obtain a global metabolic profile to link to ecology, especially since the role of some metabolites is not always completely understood. Such an approach can help to determine which factor impacts the ecology of macroalgal species, and to have a global understanding of its future evolution in the context of

global change. More particularly, it contributes to determine whether one species could be less adaptable than another one and then disappear.



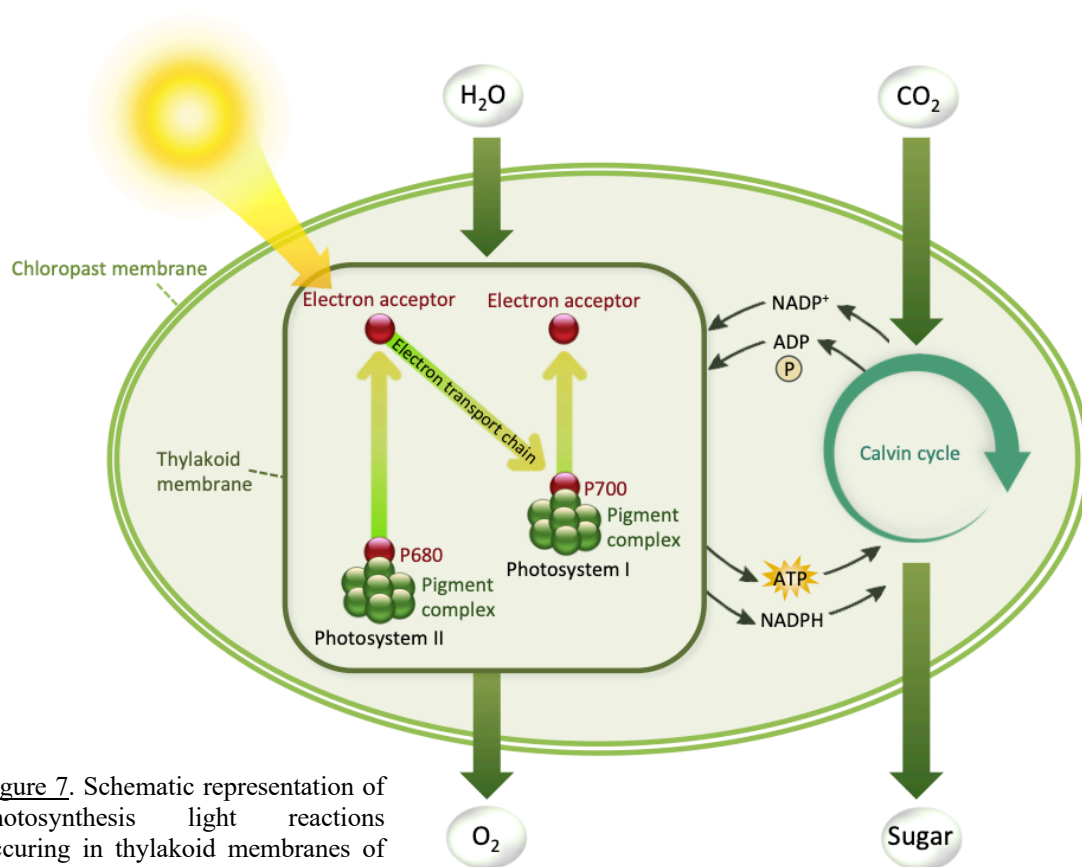
**Figure 6.** Schematic representation of eco-metabolomic, *i.e.* integrated approach coupling the study of ecology with the analysis of various metabolites to get a global overview of adaptive/acclimation abilities of macroalgal species. The drawing of macroalga is from [Nicolas and Tréhin \(2018\)](#).

### 2.3 Overview of the metabolites studied

In the context of global change, and among the wide range of primary and secondary metabolites synthesized by red and brown macroalgae ([Stengel \*et al.\* 2011](#)), it was chosen to study preferentially the compounds involved in photoprotection and acclimation to temperature fluctuations. In this regard, the different algal metabolites studied were: pigments, mycosporine-like amino acids (MAAs), fatty acids, and phenolic compounds. In addition, a first investigation on terpenes has been carried out, as these compounds represent major secondary metabolites of many brown macroalgae. An overall description of each of these compounds is provided in this section. The impact of different biotic or abiotic factors on the synthesis of these compounds is described in the **section 1.2** (Review published in ABR).

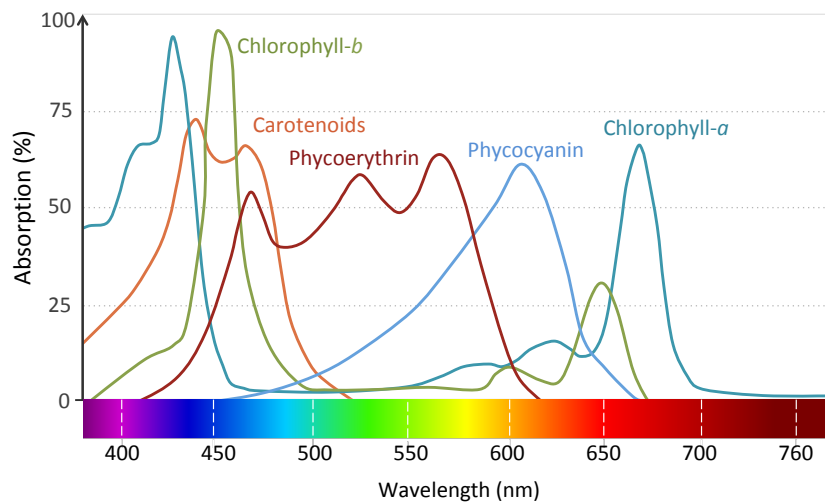
### 2.3.1 Pigments

As macroalgae are photosynthetic organisms, they require light for their development and reproduction. They thus possess pigments, whose physiological role is to capture light energy. This light absorption takes place at the surface of thylakoid membranes, within structures called photosystems (PS) (Fig. 7). There are two photosystems (*i.e.* PSI and PSII), and both of them are involved in the photochemistry of photosynthesis, *i.e.* light reactions. Photosystems are then both composed of two parts: a light-harvesting complex (composed of pigments) that enhance the photon absorption, and a reaction centre where the light energy is then transmitted as excitation energy to an electron acceptor molecule and transformed into chemical energy (*i.e.* ATP and NADPH). The PSI reaction centre is called P700, while the PSII reaction centre is called P680 (Lee 1999; De Reviere 2002; Hurd *et al.* 2014). At the end of the photosynthetic reaction, glucose and oxygen are produced, according to the following global equation:



**Figure 7.** Schematic representation of photosynthesis light reactions occurring in thylakoid membranes of chloroplasts. © F. Lalegerie

The three main groups of pigments involved in the photosynthesis of macroalgae are: chlorophylls, carotenoids and phycobiliproteins (De Reviers 2002; Hurd *et al.* 2014), absorbing light at different wavelengths to enhance the capture of light energy (Fig. 8). However, not all pigments are involved in photosynthesis to the same extent, and some of them are rather involved in photoprotection, *i.e.* attenuating the radiation reaching the photosynthetic tissues and/or chloroplasts (Mimuro and Akimoto 2003).



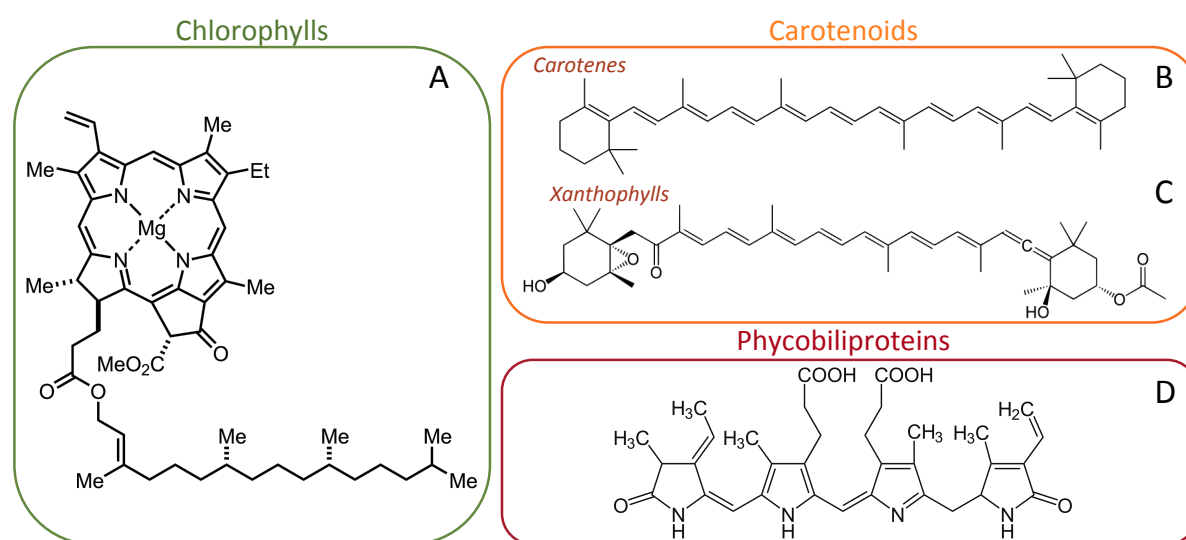
**Figure 8.** Absorption spectra of main algal photosynthetic pigments, and visible light spectrum, adapted from Gantt (1975).

### 2.3.1.1. Chlorophylls

Chlorophylls, which are responsible for the green colour, are pigments soluble in organic / less polar solvents (*e.g.* alcohol, methanol, acetone), and composed of a tetrapyrrole ring with magnesium, coupled to a long apolar phytol chain (Fig. 9a). Then, all algae possess chlorophyll-*a*, which is essential for photosynthesis, being present in light-harvesting system and reaction centre of photosystems I and II. Chlorophyll-*a* has then different functions: (1) light absorption within light-harvesting system, (2) transfer of light energy to the adjacent molecules by resonance transfer, up to a specific chlorophyll-*a* pair in the reaction centre, and (3) charge separation by this pair of chlorophyll-*a* (*i.e.* conversion of light energy into chemical energy, by electron transfer) (De Reviers 2002; Hurd *et al.* 2014).

Depending on their phylum, algae possess other types of chlorophylls (*i.e.* *b*, *c*, or *d*) distinguished by their molecular structure (Lee 1999; De Reviers 2002). Their role is also to capture light, but as accessory photosynthetic pigments. In this way, chlorophyll-*b*, found in Chlorophyta, Euglenophyceae and Charophyta, differs from chlorophyll-*a* by the presence of

an aldehyde (-CHO) instead of a methyl (-CH<sub>3</sub>) group. On the other hand, chlorophyll-*c* (divided into subtypes *c*<sub>1</sub>, *c*<sub>2</sub> or *c*<sub>3</sub>), is distinguished by the absence of phytol chain, and occurs in Phaeophyceae, diatoms or dinoflagellates, but not in red and green algae (Scheer 1991; De Reviere 2002; Zapata *et al.* 2006). In addition, it has been reported that some species of red algae may also contain chlorophyll-*d*, although some authors associated this presence to contamination by cyanobacteria (Holt and Morley 1959; Landrau and Welschmeyer 2000; Murakami 2004). All chlorophylls exhibit then absorptions peaks at 400 - 450 nm in the blue, and then at 650 – 700 nm in the red (Hurd *et al.* 2014) (Fig. 8). In addition, chlorophylls are generally accompanied by the presence of derivatives, such as for example chlorophyllid and pheophytin in red and brown macroalgae. It has been demonstrated then the involvement of pheophytin in the photosynthesis as primary electron acceptor of photosystem II (Klimov 2003), while chlorophyllid correspond to a degradation of chlorophyll-*a* in damaged tissues, or improperly stored samples (Steinman *et al.* 2017).



**Figure 9.** Examples of pigment structures, among the 3 classes found in macroalgae, *i.e.* chlorophylls, carotenoids (subdivided in carotenes and xanthophyll), and phycobiliproteins. (A) Chlorophyll-*a*, (B)  $\beta$ -carotene, (C) fucoxanthin and (D) phycoerythrobilin, one of the chromophores forming phycobiliproteins (*e.g.* phycoerythrin), adapted from Lüning (1990).

### 2.3.1.2 Carotenoids

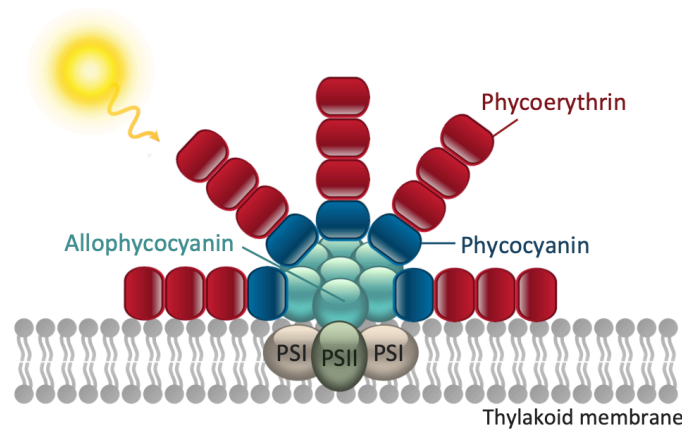
Carotenoids are a large group of apolar pigments, representing approximately 750 natural compounds to date (Takaichi 2011; Whang *et al.* 2018). These pigments characterized by their yellow-orange colour have a maximum absorption between 440 and 460 nm (Lee 1999; De Reviere 2002). Algal carotenoids are then divided into two groups: (1) carotenes, forming



long hydrocarbon chains, and (2) xanthophylls, structurally similar but with additional oxygen atoms (Fig. 9,c). The composition of carotenoids varies depending on the algal groups due to specific enzymes mediating each step of their biosynthesis, explaining why carotenoid composition is sometimes referred to phylogeny (Schubert *et al.* 2006; Takaichi 2011). In this way, red macroalgae have been reported to contain  $\alpha$ -carotene and  $\beta$ -carotene, in addition to the xanthophylls zeaxanthin, antheraxanthin, violaxanthin, lutein (Schubert *et al.* 2006; Esteban *et al.* 2009). Conversely, brown macroalgae do not possess  $\alpha$ -carotene or lutein, and are known for their high fucoxanthin content, in addition to zeaxanthin, antheraxanthin, and violaxanthin (Haugan and Liaaen-Jensen 1994; De Reviers 2002). Carotenoids have then several physiological roles: they participate in the photosynthesis by capturing light within light harvesting system, and may also act as important photoprotective agents (De Reviers 2002; Mimuro and Akimoto 2003). Indeed, some carotenoids participate in the dissipation of solar energy and the deactivation of reactive oxygen species, avoiding photo-oxidation (Rastogi *et al.* 2010). More particularly, there is a reversible oxygen uptake phenomenon in carotenoids, known as the xanthophyll cycle: when the amount of light is too high, violaxanthin is converted into antheraxanthin and then zeaxanthin, which reduces excess energy around photosystem II (Siefermann-Harms 1987; Goss and Jakob 2010; Janhs and Holzwarth 2012) (see figure 2 of the review available in **section 1.2**).

### 2.3.1.3 Phycobiliproteins

One of the characteristics of red algae is the presence of phycobilisomes at the surface of their thylakoids, forming spherical structures, not found in brown or green algae (Fig. 10). They are particular supramolecular complexes containing phycobiliproteins, supernumerary pigments of red algae, also found in Cyanobacteria and Cryptophytes (De Reviers 2002; Dumay *et al.* 2014). Unlike chlorophylls or carotenoids, these pigments are polar and water-soluble, due to their chemical structure composed of proteins and covalently bound chromophores, *i.e.* phycobilins belonging to tetrapyrroles not cyclized, such as phycoerythrobilin or phycourobilin for example (Fig. 9d). Phycobiliproteins optimize photosynthesis of red algae by absorbing light energy at additional wavelengths than chlorophylls or carotenoids (Glazer *et al.* 1982; Gantt 1990; Lobban and Harrison 1994) (Fig. 8).



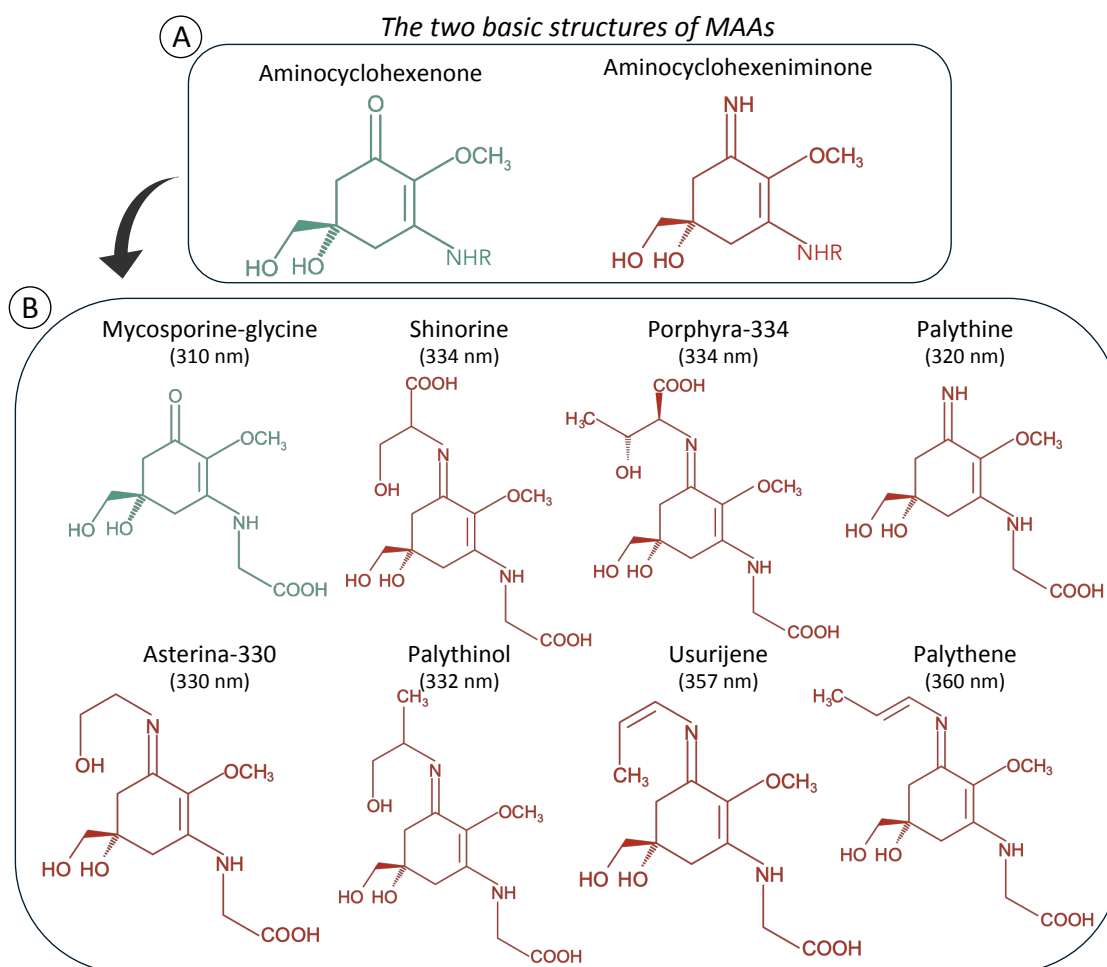
**Figure 10.** Schematic representation of phycobilisome in red macroalgae, adapted from Gantt (1990).

In red macroalgae, there are three main types of phycobiliproteins organized in a specific order within phycobilisomes: in the centre, allophycocyanins (turquoise blue,  $\lambda_{\max} = 650 - 655$  nm), and then phycocyanin (blue,  $\lambda_{\max} = 610 - 620$  nm) and phycoerythrin (red,  $\lambda_{\max} = 490 - 570$  nm), all stacked, forming cylindrical discs, as illustrated in the figure 10 (Dumay *et al.* 2014). The phycoerythrin gives the red colour to the alga as it can constitute a major proportion of the red algal proteins, representing up to 12% of total protein content in *Palmaria palmata* for example (Galland-Irmouli *et al.* 1999).

### 2.3.2 Mycosporine-like amino acids (MAAs)

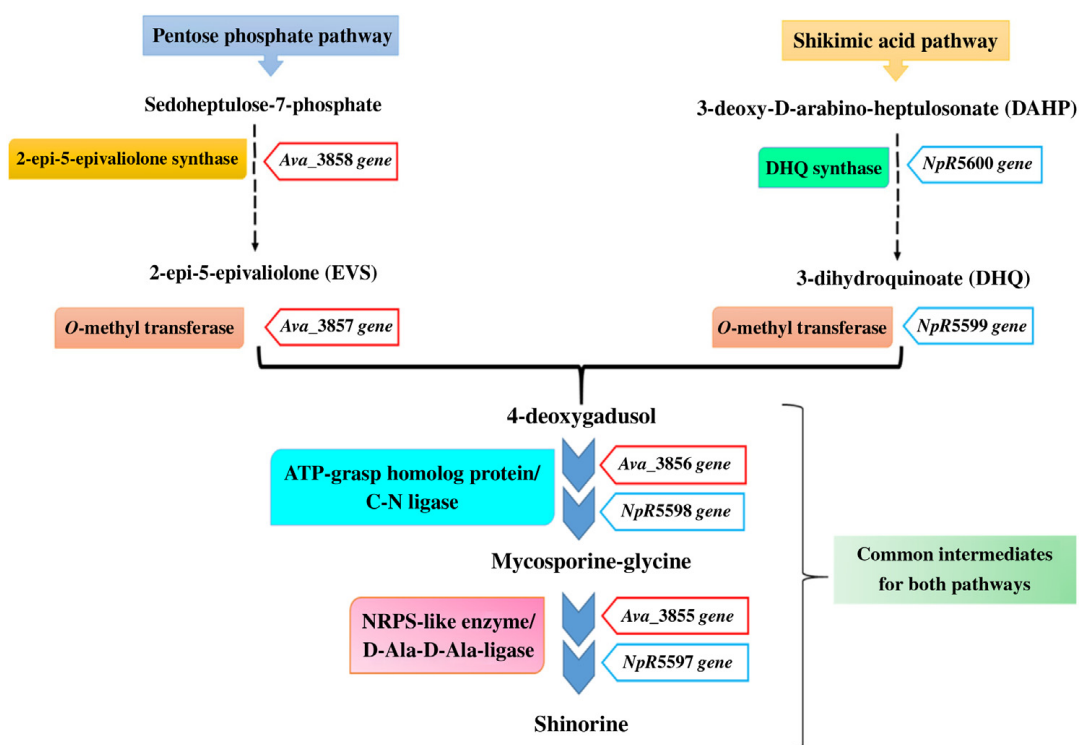
Mycosporines were first discovered in 1965 in the fungus *Ascochyta pisi* (Leach 1965). Since then, the presence of mycosporine-like amino acids (MAAs) has been detected in a wide range of marine and some terrestrial organisms (Bandaranayake 1998; Shick and Dunlap 2002). There are notably widely distributed in macroalgae, phytoplankton, Cyanobacteria, corals and lichens (Gröniger *et al.* 2000; Sinha *et al.* 2007; Bedoux *et al.* 2020; Sun *et al.* 2020). In some organisms, the presence of MAAs has been suggested to be the result of food consumption or bacterial association (Dunlap and Shick 1998; Shick and Dunlap 2002; Sinha *et al.* 2007). Among macroalgae, it has been shown that red macroalgae have the greatest diversity and proportion of MAAs (Karentz 2001; Sinha *et al.* 2007; Carreto and Carignan 2011). In this way, Sun *et al.* (2020) reported recently that 486 species of Rhodophyta contained MAAs, while conversely very few brown algae have been reported to contain MAAs, and their presence in this Class being generally associated with the presence of red or green epiphytes.

MAAs are low molecular weight compounds (< 400 Da), soluble in water, with a maximum absorption ( $\lambda_{\max}$ ) between 310 and 360 nm (Rastogi *et al.* 2010). These natural compounds are then generally described as UV-absorbing compounds, as their involvement in photoprotection has already been largely demonstrated (Bandaranayake 1998; Huovinen *et al.* 2004; Korbee *et al.* 2006; Singh *et al.* 2008). Nevertheless, it seems that MAAs could be multi-functional compounds, as some studies have also demonstrated or suggested other physiological functions, such as antioxidant, osmoregulatory or thermoregulatory (Dunlap and Yamamoto 1995; Oren and Gunde-Cimerman 2007; De la Coba *et al.* 2009; Bhatia *et al.* 2011; Wada *et al.* 2015). Through their molecular structures, two groups of MAAs can be distinguished: those consisting of an aminocyclohexenone ring, and those with an aminocyclohexenimine ring. To these two rings are bond different additional functional groups, resulting in about 30 MAAs detected in red macroalgae to date (Sinha *et al.* 2007; Sun *et al.* 2020) (Fig. 11).



**Figure 11.** (A) Aminocyclohexenone and aminocyclohexenimine rings, forming the basic structure of MAAs. (B) Some examples of MAAs found in red macroalgae with their absorption maxima, adapted from Sinha *et al.* (2007), Karentz (2001) and Chrapusta *et al.* (2017).

However, these compounds are relatively understudied and some recent papers suggest that there are still many MAAs to be discovered (Hartmann *et al.* 2015; Orfanoudaki *et al.* 2019). Moreover, their synthesis pathway in macroalgae is not entirely elucidated (Bedoux *et al.* 2020; Belcour *et al.* 2020) although current studies suggest two possible pathways (Fig. 12). The first one, which is the most reported, is the shikimate pathway usually used for the synthesis of aromatic amino acids and which involves the 3-deoxy-D-arabino-heptulosonate (DAHP). The other potential synthesis pathway is then derived from the metabolic pathway of pentose phosphate, and involves the sedoheptulose-7-phosphate. Both of the two synthesis pathways lead to the formation of 4-deoxygadusol, the initial precursor of MAAs. Then, mycosporine-glycine would correspond to the first synthesised MAAs, and would then be transformed into secondary MAAs such as shinorine or porphyra-334 (Rastogi *et al.* 2010; Bhatia *et al.* 2011). Furthermore, different hypotheses have been made on the relationships between the different MAAs, and their inter-conversion in red macroalgae (Carreto *et al.* 2005; Sinha *et al.* 2007; Bhatia *et al.* 2011).



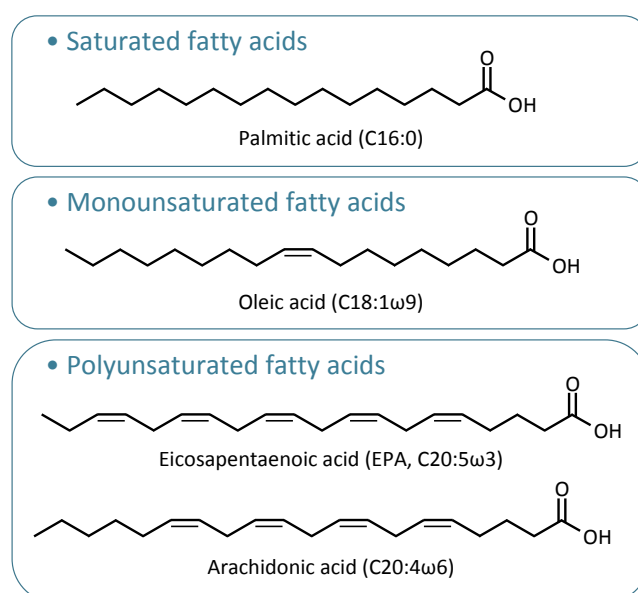
**Figure 12.** Representation of the two hypothetical MAAs biosynthesis pathways, according to Singh *et al.* (2020), modified from Jain *et al.* (2017).

### 2.3.3 Fatty acids

Fatty acids are a subgroup of lipids with long hydrocarbon chain (14 or more carbon atoms), with a terminal carboxyl group. They are divided in three groups according to the number of double bonds within hydrocarbon chain, *i.e.* saturated (SFA, no double bond), monounsaturated (MUFA, one) or polyunsaturated (PUFA, two or more) fatty acids (Fig. 13). Each fatty acid is named in accordance with the number of carbon atoms, the number of double bonds, and the position of the first double bond, counting from the methyl end. MUFA include n-9 ( $\omega$ 9) fatty acids, while n-6 ( $\omega$ 6) and n-3 ( $\omega$ 3) are PUFA (Holdt and Kraan 2011; Stengel *et al.* 2011).

In general, red and brown seaweeds are rich in PUFA of 20 carbons, and contain mainly eicosapentaenoic acid (20:5 $\omega$ 3, known as EPA) and arachidonic acid (20:4 $\omega$ 6) (Mišurcová *et al.* 2011; Kornprobst 2014), which can represent up to 50% of the total fatty acid content (Kumari *et al.* 2013). In this way, macroalgae have a higher PUFA level than terrestrial plants, despite a low lipid content (Fleurence *et al.* 1994; Bedoux *et*

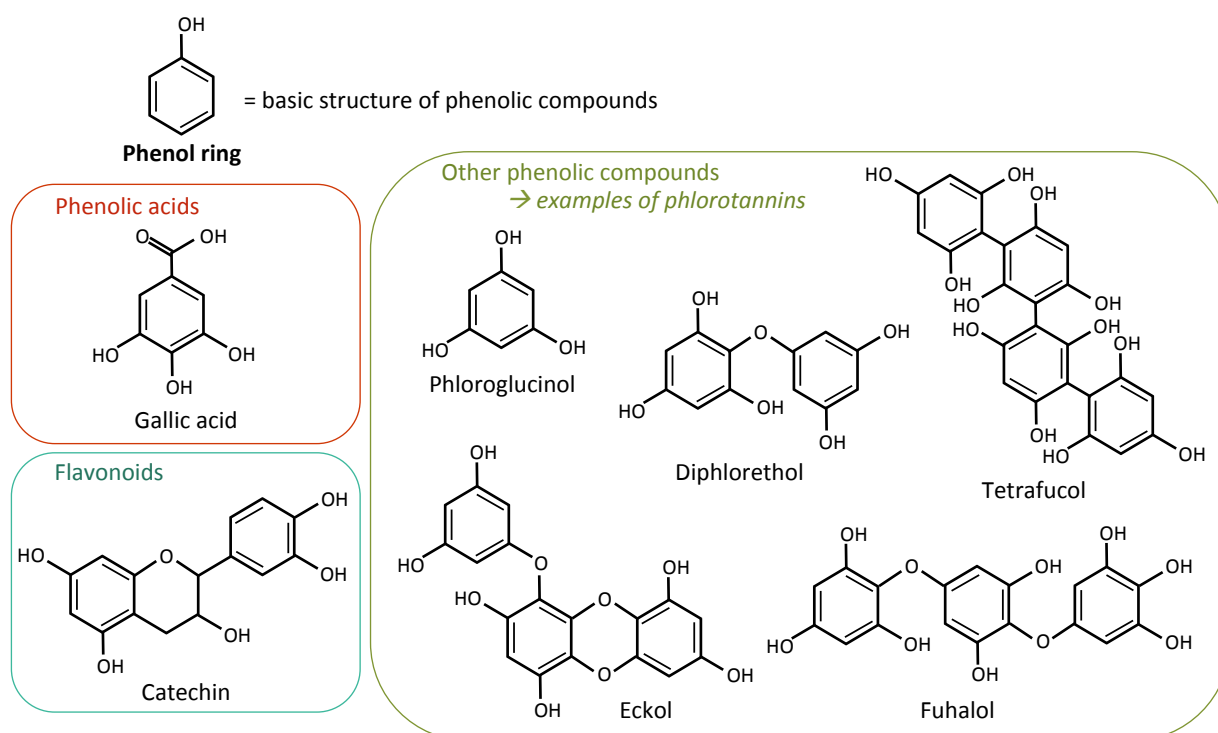
*al.* 2014). In addition, macroalgae possess palmitic (C16:0) and oleic (18:1 $\omega$ 9) acids that are the main SFA and MUFA, respectively (see structures in Fig. 13). Then, the fatty acid level and composition are influenced by different environmental parameters such as temperature (Al-Hasan *et al.* 1991; Floreto *et al.* 1993), light level (Hotimchenko 2002), or salinity (Kumar *et al.* 2010), and thus exhibit seasonal variations (Kendel *et al.* 2013; Schmid *et al.* 2014, 2017). More particularly, PUFA can represent a high proportion of glycolipids and phospholipids, the major constituents of cellular membrane of algae (Fleurence *et al.* 1994; Hold and Kraan 2011). In this way, unsaturation of fatty acids is involved in membrane fluidity and acclimation to temperature change (Los *et al.* 2013).



**Figure 13.** Examples of saturated, monounsaturated and polyunsaturated fatty acids encountered in marine macroalgae.

### 2.3.4 Phenolic compounds

Phenolic compounds are a heterogeneous group, comprising all compounds constituted of one or more hydroxyl groups (–OH) bond to an aromatic hydrocarbon group. This includes a wide range of metabolites, from the simplest molecule, to the most complex / polymerized polyphenol (Fig. 14). Polyphenols can be then divided in three main groups: phenolic acids (*i.e.* phenolic ring coupled to a carboxylic acid), flavonoids (*i.e.* C6-C3-C6 compounds), and others phenolic compounds (*i.e.* lignin, stilbenes and tannins) (Cikoš *et al.* 2019; Gómez and Huovinen 2020).



**Figure 14.** Structure of phenol ring, constituting the basic structure of phenolic compounds. In addition, some examples of molecules representatives of the three main groups of phenolic compounds (*i.e.* phenolic acids, flavonoids and others) are illustrated, adapted from Stiger-Pouvreau *et al.* (2014) and Gómez-Guzmán *et al.* (2018). Examples from the last class of compounds are exclusively phlorotannins.

Among macroalgae, brown seaweed are the most prolific in the synthesis of phenolic compounds, which can constitute up to 25% of their dry weight (Ragan and Glombitza 1986; Murray *et al.* 2018). Brown macroalgae have specific phenolic compounds, *i.e.* phlorotannins, produced by the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) units, and located in specific vesicles within cells called physodes for soluble phlorotannins (Ragan and Glombitza 1986; Stiger-Pouvreau *et al.* 2014; Gómez and Huovinen 2020). Synthesized via the acetate-malonate pathway, phlorotannins are classified into different groups according to

their chemical structures (*i.e.* type of links between phloroglucinol units): fucols, fuhals, phlorethols, fucophlorethols, isofuhals and eckols (Fig. 14). In this way, there are for example ether (Aryl-O-Aryl) links in phlorethols, or dibenzodioxin links in eckols (Amsler and Fairhead 2005; Kornprobst 2014; Stiger-Pouvreau *et al.* 2014). Moreover, there are derivative phlorotannins, *e.g.* halogenated or sulphated compounds. This leads to a very wide range of compounds, with a molecular weight ranging from 126 Da (*i.e.* phloroglucinol monomer alone), to over 1,000 kDa (Ragan and Glombitza, 1986). In addition, brown macroalgae contain large variety of meroditerpenes, which can be included within phenolic compounds as their molecular structure comprises a phenolic ring (see next **section 2.3.5**). Conversely, red macroalgae contain low level of phenolic compounds. They contain mainly bromophenols, and small phenolic compounds such as catechin, epicatechin or catechol (Kornprobst 2014; Cikoš *et al.* 2019). These compounds are structurally close to gallic acid, commonly used in quantification of phenolic content in red algae (see structures in Fig. 14). In addition, red algae contain mycosporine-like amino acids which are sometimes considered as phenolic compounds (Stengel *et al.* 2011) (see previous **section 2.3.2**).

Finally, phenolic compounds are secondary metabolites as they are synthesized in response to a combination of biotic or abiotic factors, such as herbivory, pathogens, or fluctuating light, salinity and temperature. It was also demonstrated that the content of phenolic compounds could vary with the age and stage of development (Pavia *et al.* 2003; Stiger *et al.* 2004). These compounds also have a primary role within algal cells (notably for brown macroalgae) as they contribute to the structure and strength of the cell-wall (Schoenwaelder 2000). Then, phenolic compounds have a high antioxidant potential due to the large number of hydroxyl groups in their structure. The different types of phenolic compounds found in marine algae as well as many biological properties and their potential for use in the medical and cosmetic industries have been the subject of a book chapter whose abstract is available in **APPENDIX 1** (Gager L.\*, Lalegerie F.\*, Connan S., Stiger-Pouvreau V. (In press) Algal-derived phenolic compounds and their biological activities. In: Rajauria G. and Yuan Y. (Eds.), *Recent advancements in micro and macroalgal processing: food and health perspectives*. Wiley).

### 2.3.5 Terpenes

Terpenes are apolar compounds constituted by isoprene units (2-methylbuta-1,3-diene, general formula  $C_5H_8$ ), forming hydrocarbonated chain (Fig. 15). They can have diverse structures and are thus linear, cyclic or more complex (*i.e.* rearranged), resulting in many metabolites (Bourgougnon and Stiger-Pouvreau 2011; Gaysinski *et al.* 2015). Terpenes are divided in several groups according to the number of isoprene units and carbon atoms (Amico 1995; Valls and Piovetti 1995), as described in Table 1. In addition, the basic structure of the terpenes can be modified / rearranged: such as the removal of the methyl group of isoprene unit, or the addition of functional groups/atoms, notably oxygen. In this case, terpenes are defined as terpenoids. More particularly, the linear terpenic chain can be coupled to a quinone (or a derivative), forming a meroditerpene (Fig. 15); or to a halogenated element, such as bromine, forming a bromoterpene.

Table 1. Classification of terpenes according to the number of isoprene units and carbon atoms

	Isoprene units	Carbon atoms
Monoterpenes	2	10
Sesquiterpenes	3	15
Diterpenes	4	20
Sesterterpenes	5	25
Triterpenes	6	30
Tetraterpenes	8	40
Polyterpenes	> 8	> 40

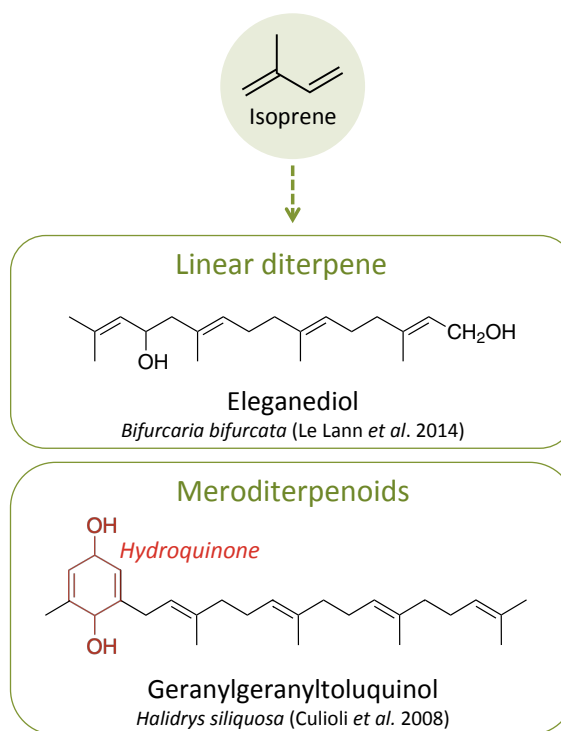


Figure 15. Chemical structure of isoprene unit, with two examples of terpenes found in brown macroalgae.

Among macroalgae, brown species represent the richest source of terpenes, notably species from the two orders Dictyotales and Fucales. More particularly, among Fucales, many species from Sargassaceae have been reported to contain numerous linear diterpenes and cyclic sesquiterpenes, with meroterpenes (Bourgougnon and Stiger-Pouvreau 2011; Kornprobst 2014). On the contrary, red macroalgae exhibit generally a terpenic composition not as rich as brown macroalgae. Indeed, only species belonging to the Orders Ceramiales and Gigartinales



contained halogenated terpenes, including monoterpenes, bromoditerpenes, or sesquiterpenes (Kornprobst 2014). Terpenes are defensive compounds, mainly synthesized in response to grazers, epiphytes or pathogens (Bourgougnon and Stiger-Pouvreau 2011). In this way, they are generally studied for their antifouling activities, inhibiting the settlement or development of microalgae, bacteria, or fungi (Hellio *et al.* 2001; Maréchal *et al.* 2004; Mokrini *et al.* 2008; Gouveia *et al.* 2013).

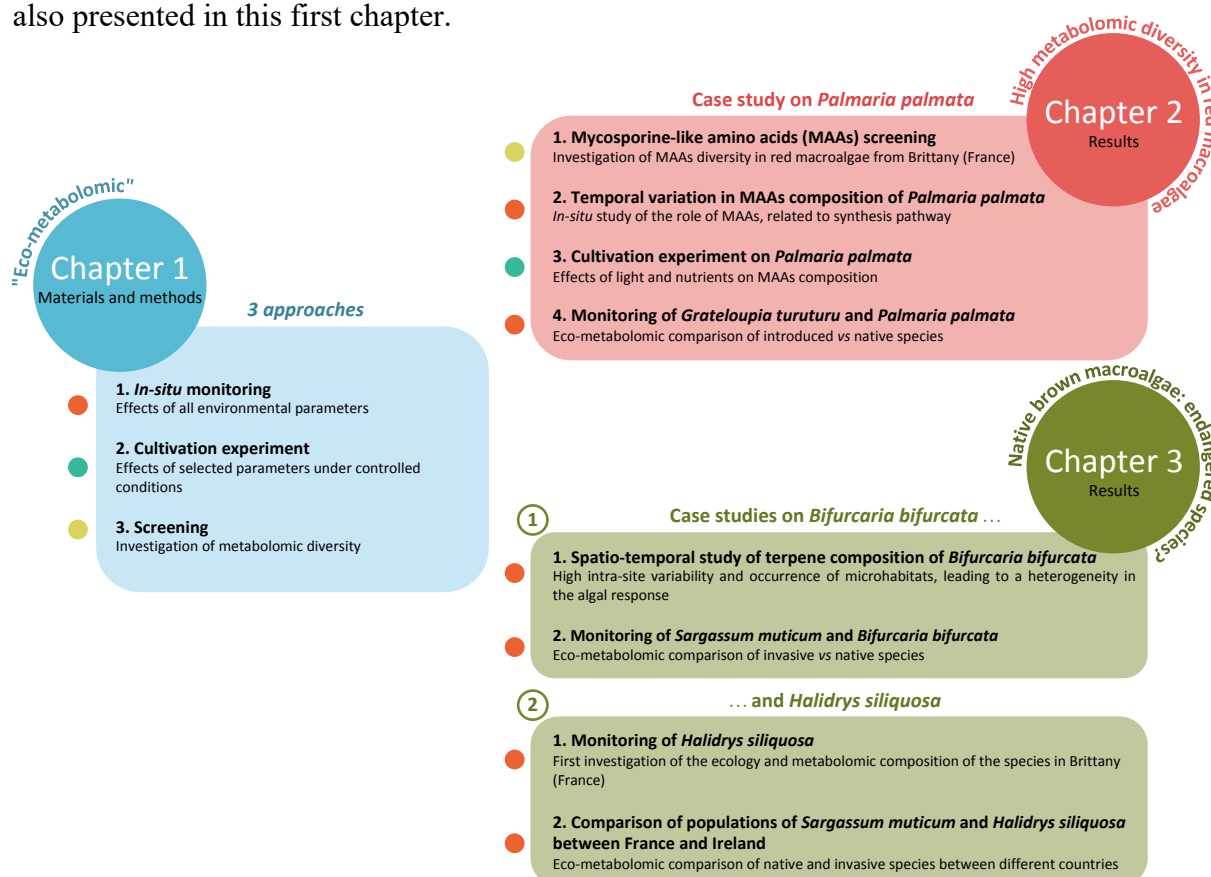
## 2.4 Objectives and organisation of the thesis manuscript

As presented in the first part of the introduction, marine macroalgae living attached to the rocky littoral zone experienced a range of biotic or abiotic factors. These various environmental parameters affect then marine algae in terms of their ecology and physiology, *e.g.* photosynthesis, growth, reproduction, or stress responses, altering the development and the composition / distribution of marine macroalgal communities. Furthermore, macroalgae will have to cope with global warming and global changes, resulting in environmental disturbances, which are becoming more and more intense. They will thus have to adapt to these new environmental conditions or they risk to disappear or see their area of distribution regressed. In this regard, one of the objectives of this thesis is to **study acclimation abilities of some intertidal algal populations monitored in Brittany**, to provide a better understanding of their becoming in relation with the global changes which will occur on this flora.

Therefore, as described in the second part of this introduction, one feature of this thesis was to **combine the ecology of populations with the metabolomic composition of individuals**. Such integrated approach can be considered as "eco-metabolomic", providing a global overview of adaptive abilities of marine macroalgae. In this way, as many metabolites as possible have been studied during this PhD work, to obtain "metabolomic profiles" (not global/complete profile but including major primary (pigments, fatty acids) and secondary (phenolics, MAAs, terpenes) metabolites of brown and red macroalgae), applied to ecology and phenology of seaweeds. Indeed, macroalgae adapt (or not) to fluctuating environment by producing a wide range of primary and/or secondary metabolites, playing an important role in their acclimation, adaptation and consequently in the structure of coastal communities. Furthermore, these compounds, of different natures depending on the algae, are synthesized in response to different stresses such as high light radiation, temperature increase, or salinity and

desiccation, and could therefore be key compounds in the adaptation of intertidal macroalgae to global change. On the other hand, another objective of this thesis was to **investigate a comparison in adaptive responses to environmental constraints between native and introduced / invasive species**. Indeed, due notably to the increase in global marine commercial exchanges, it is likely that the number of introductions will also increase; and due to global change, the phenotypic plasticity of native / invasive species will evolve as described previously. In this way, the invasive potential of some species could be enhanced, while native species could be more vulnerable and disadvantaged compared to introduced species.

In this context, the thesis manuscript is divided in three chapters as schematically shown in figure 16. In a **first chapter**, a description of the three approaches using in the "eco-metabolomic" initiative is provided, *i.e.* (1) *in-situ* monitoring, (2) cultivation experiment under controlled conditions, and (3) screening to explore metabolomic diversity. Then, the three approaches require the extraction and analysis of different algal metabolites, which are also presented in this first chapter.



**Figure 16.** Schematic presentation of the outlines of the thesis manuscript. The coloured dots (orange, blue, yellow) represent the three approaches described in chapter 1 (materials and methods), and that are used in chapters 2 and 3.

Then the results are presented under the format of scientific publications (published or not yet submitted), distributed in two different chapters. In this way, **Chapter 2** focuses on red macroalgae (and more particularly *Palmaria palmata*), which are known for their high specific and metabolic diversities (Stengel *et al.* 2011). This chapter consists of 4 publications with the following content:

- (1) Screening of mycosporine-like amino acids (MAAs) in red macroalgal species collected on Brittany shores, in order to investigate the diversity of these compounds
- (2) Temporal variation in MAAs composition of the red alga *Palmaria palmata*, a native species which exhibits a high diversity in these compounds
- (3) Cultivation experiment carried out on *Palmaria palmata*, to study *ex-situ* the impact of light and nutrients on the synthesis of MAAs
- (4) *In-situ* monitoring of the population biology and metabolomic composition of the native *Palmaria palmata*, in comparison with the introduced *Grateloupia turuturu*

On the other hand, the **Chapter 3** focuses on brown macroalgae which may represent an important biomass on temperate rockyshores, some species forming large canopies (Lüning 1990; Little and Kitching 1996). More specifically, this chapter focuses on three species of Sargassaceae, *i.e.* two native species (*Bifurcaria bifurcata* and *Halidrys siliquosa*) that lives in the same habitat as the invasive *Sargassum muticum*. In this way, this chapter is divided in two parts: the **part 1** focuses on *Bifurcaria bifurcata*, afterwards the **part 2** concerns *Halidrys siliquosa*. This latter is a cold-water affinity species which could be particularly threatened in its population sustainability face to both global change and cohabitation with introduced species. More particularly, although some individuals of *H. siliquosa* are found on the intertidal zone (and especially in rockpools), this species lives preferentially in the subtidal zone, and then could see its intertidal populations disappear. Then, the parts 1 and 2 of Chapter 3 consist of two publications each: (i) a preliminary assessment of the ecology and/or metabolomic composition, and therefore the acclimation capacities of the native species monitored in Brittany, and (ii) a comparison with the invasive species *Sargassum muticum*. This chapter includes a comparison of the French and Irish populations of *H. siliquosa* in relation to its limit of distribution.

Finally, a general conclusion on results obtained in both chapters 2 and 3 is provided at the end of the PhD manuscript.

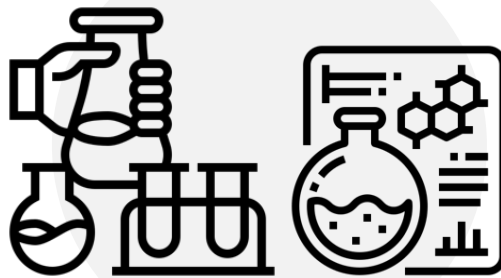
# *Chapter 1*

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## Material and methods

*An eco-metabolomic study integrating  
different approaches*

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**This PhD work is based on three different approaches described in this chapter 2:**

**1. *In-situ* monitoring** of native and invasive species, to study spatio-temporal variations in the ecology and metabolomic composition of the natural populations, with all environmental parameters fluctuating together. This part includes the description of the studied species, *i.e.* intertidal marine macroalgae, whether native or introduced in Brittany (France).

**2. Cultivation experiment**, to study the impact of specific environmental factors on selected species, under controlled conditions in the laboratory.

**3. Screening**, to explore the metabolomic diversity between species, such as mycosporine-like amino acids diversity in red macroalgae in the present study. This term can also be assimilated to spatio-temporal monitoring. In this regard, within the manuscript, this part refers only to comparison between a wide range of species.

Then, the three approaches require the extraction and analysis of different algal metabolites, which will be presented in part **4.** of this chapter.

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### ***1. In-situ monitoring of native and introduced species***

For the first approach of this work, five species of macroalgae (including native and introduced species) were monitored *in situ*, in order to acquire knowledge about the ecophysiology and acclimation abilities of their populations:

- a couple of red seaweeds: the native *Palmaria palmata* and the introduced *Grateloupia turuturu*, both with a similar foliose morphology.
- a trio of brown macroalgae: the native *Bifurcaria bifurcata* and *Halidrys siliquosa*, and the invasive *Sargassum muticum*, all belonging to Sargassaceae.

All species were monitored for one year on three sites in Brittany. Each month, different ecological variables were measured, and samples were collected for metabolomic analysis in the laboratory. In addition, a second short monitoring at two contrasted seasons were carried out in Ireland, to compare French and Irish populations of *Halidrys siliquosa* (which has its Southern limit of distribution in France), and *Sargassum muticum* (which has different introduction time between the two countries).

## 1.1. Species monitored

### 1.1.1. *Palmaria palmata*

#### 1.1.1.1. Description

*Palmaria palmata* (Linnaeus) F. Weber & D. Mohr 1805 is a red macroalga, more commonly known as “Dulse”, or “Dillisk” in Gaelic. It was first named *Rhodymenia palmata* (Linnaeus) Greville before being assigned in the Palmariaceae family (Guiry 1974) (Fig. 17). This species is a perennial seaweed, which can live several years, reaching 40-50 cm length and 2-3 cm width. *Palmaria palmata* is a foliose seaweed, typically divided into lobes from the centre of the frond, sometimes forming a large clump. It is attached to the substrate by a discoid holdfast, connected to the thallus by a small stipe. Young thalli are often thinner than older ones, which carry often more epiphytes (Werner and Dring 2011).

Phylum: Rhodophyta  
 Subphylum: Eurhodophytina  
 Class: Florideophyceae  
 Subclass: Nemaliophycidae  
 Order: Palmariales  
 Family: Palmariaceae  
 Genus: *Palmaria*  
 Species: *Palmaria palmata*



Figure 17. Classification and illustration of a thallus of *Palmaria palmata* in intertidal rockpools (Portsall, Brittany, France; October 2017). © F. Lalegerie

#### 1.1.1.2 Distribution

This seaweed is mainly distributed in Arctic Ocean, North Atlantic, North Sea and English Channel. However it has also been reported in Canada and Japan. It is one of the commonest red macroalgae from the Northern Hemisphere, found in the intertidal zone and shallow subtidal up to 20-m deep (Grote 2017). In the intertidal zone, living in sheltered to exposed environments, it is sometimes found in rockpools, or as an epiphyte on brown seaweeds such as *Himanthalia elongata*, *Fucus* spp. or *Laminaria* spp. (Werner and Dring 2011).

### 1.1.1.3 Life cycle

*Palmaria palmata* is a perennial dioecious species with the male and female reproductive parts being separated on two distinct thalli (Fig. 18). Moreover *P. palmata* presents a sexual dimorphism, the male gametophyte consisting of macroscopic blades with pinkish reproductive areas, whereas the female gametophyte is characterized by a microscopic crustose thallus (Le Gall *et al.* 2004a). Furthermore, and contrary to many red macroalgal species, its life cycle is digenetic, with the succession of a haploid gametophytic and a diploid sporophytic generation, with no carposporophyte observed in natural populations. In addition its life cycle is often described as isomorphic because the male gametophyte and the tetrasporophyte are morphologically similar (Le Gall *et al.* 2004a), although it is heteromorphic by the female gametophyte.

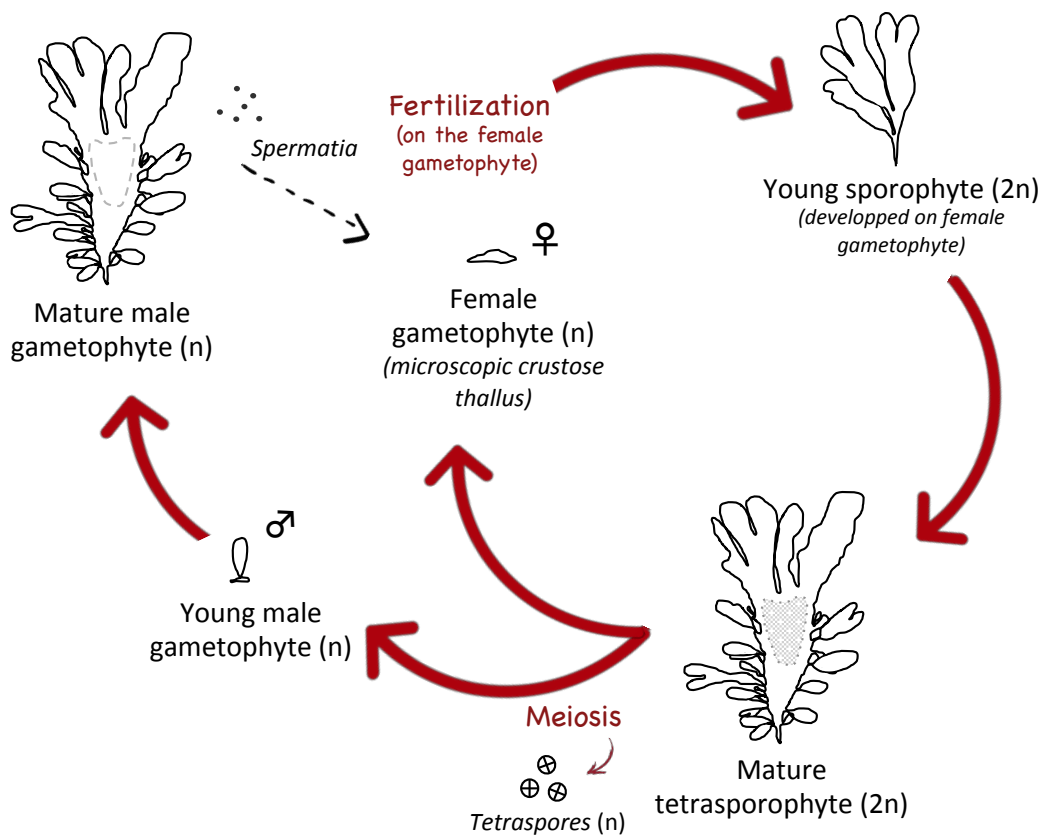


Figure 18. Life cycle of *Palmaria palmata*, adapted from van der Meer and Todd (1980).

### 1.1.1.4 Biochemical composition and valorisation

*Palmaria palmata* could form large stands which are exploited by professional harvesters in Brittany (Philippe 2013). It is one of the limited number of seaweeds authorized for human

consumption, and one of the most popular, known as dulse in numerous countries. It is notably consumed in France, Canada, Ireland, or Iceland where it is used directly fresh as sea vegetable, or under dried flakes (Mouritsen *et al.* 2013). This consumption is associated to its high protein composition, which can represent 10 – 30 % DW (Morgan *et al.* 1980; Galland-Irmouli *et al.* 1999; Bjarnadóttir *et al.* 2018). *Palmaria palmata* is also rich in dietary fibres representing 33 % DW (Lahaye *et al.* 1993), and in vitamins and minerals (Morgan *et al.* 1980). It is also an important source of eicosapentaenoic acid (EPA), which would have multiple healthy effects on human health (Mishra *et al.* 1993). Many activities of *P. palmata* extract have been reported such as antioxidant (Yuan *et al.* 2005; Beaulieu *et al.* 2016; Harnedy *et al.* 2017), antibacterial (García-Bueno *et al.* 2014), antihypertensive (Fitzgerald *et al.* 2014), or antiinflammatory (Banskota *et al.* 2014; Lee *et al.* 2017). In addition to its use in human food, *P. palmata* has also a high potential for valorisation in the cosmetic field, in particular for skin protection against UV radiation and dehydration. In this way, *P. palmata* has been reported to be rich in antioxidants, mycosporine-like amino acids which have UV-protection properties (Kartsen and Wiencke 1999; Yuan *et al.* 2009), and in glycine and arginine (Morgan *et al.* 1980), two natural moisturizing factors. There are already some trade extract or cosmetic products containing *P. palmata*, such as the INCI 223751-74-4 (Bedoux *et al.* 2014). Additionally, *P. palmata* is used in aquaculture, specifically for its bioremediation potential (Corey *et al.* 2013; Grote 2016), or as food supplement for the commercial exploitation of abalones (Grote 2017; Roussel *et al.* 2019, 2020) or urchins (Cook and Kelly 2007). Because of its commercial importance, the industry of *P. palmata* is growing, representing a requirement estimated between 750 and 1,200 tonnes per year in Ireland (Walsh and Watson 2011). In Brittany, *Palmaria palmata* can be harvested all year with approximately 180 to 310 tonnes per year, without restriction and depending of the industrial demand (Philippe 2013). Nevertheless, depending on sites, natural stocks are sometimes limited and influenced by seasons. As a result, this increasing demand is leading to many studies for several years to develop culture techniques of *P. palmata* (*e.g.* in tanks or open sea culture) in order to obtain larger biomass (Grote 2017).

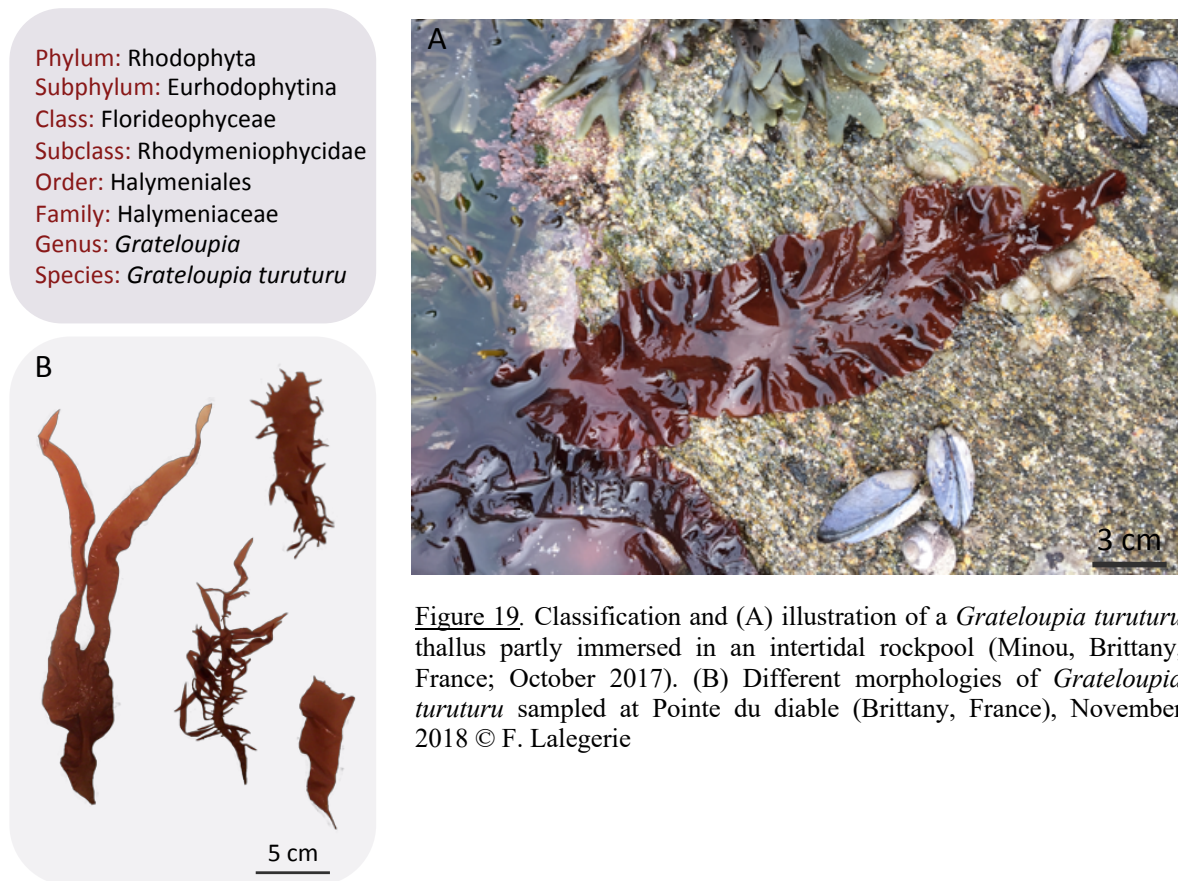
### 1.1.2. *Grateloupia turuturu*

#### 1.1.2.1 Description

*Grateloupia turuturu* Yamada 1941 is a red macroalga belonging to the Halymeniaceae



(Fig. 19). This species has been first misidentified in France, England and Rhode Island and formerly assimilated to *G. doryphora* (Montagne) M. Howe 1914 on morphological criteria, before being revised by Gavio and Fredericq (2002) by comparative molecular and morphological analysis. Its thallus is foliaceous, consisting of one or several blades distinguishable by a soft and slippery feel to the touch, growing from a single holdfast (Simon *et al.* 2001). Usually measuring less than 50 cm (Denis *et al.* 2010), *G. turuturu* can nevertheless reach considerable size, up to 3 m recorded in Callot Island (Brittany, France) in 1999 (Simon *et al.* 2001). It is therefore considered to be one of the largest red macroalgae in the world (Simon *et al.* 2001). Moreover, this species shows a high degree of polymorphism, with one or more large blades, whole or divided, and with more or less marginal proliferations (Simon *et al.* 2001; Araújo *et al.* 2011) (Fig. 19).

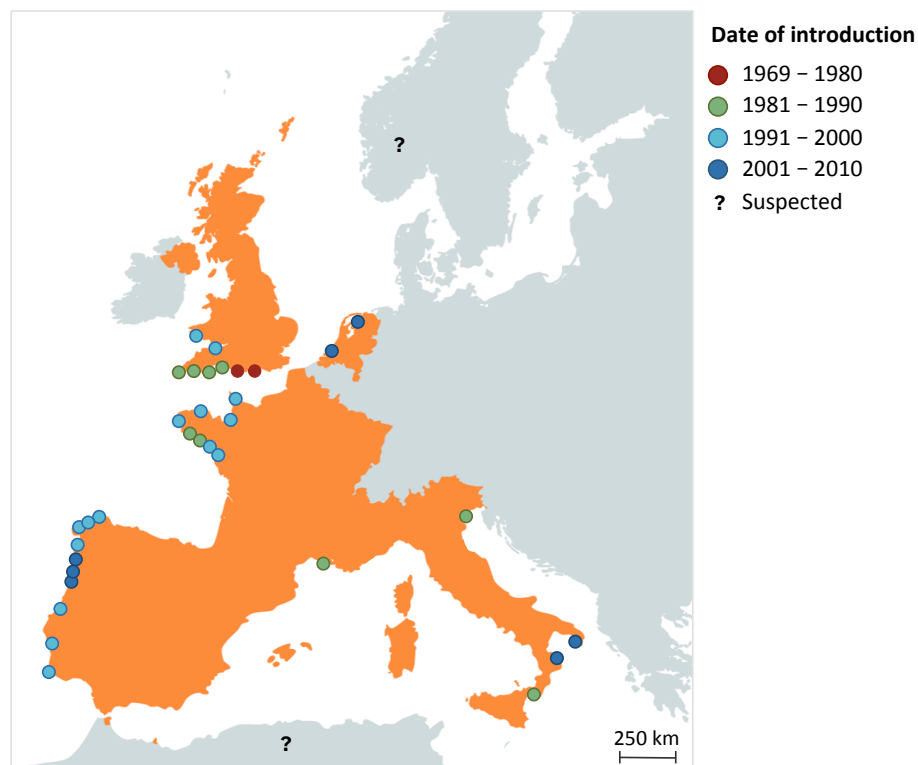


**Figure 19.** Classification and (A) illustration of a *Grateloupia turuturu* thallus partly immersed in an intertidal rockpool (Minou, Brittany, France; October 2017). (B) Different morphologies of *Grateloupia turuturu* sampled at Pointe du diable (Brittany, France), November 2018 © F. Lalegerie

### 1.1.2.2 Distribution

Originated from the North Pacific (*i.e.* native from Japan), *G. turuturu* has a worldwide distribution, including in Europe (Fig. 20). It would have been first introduced in the 1970s in Portsmouth (United Kingdom), through the import of oyster spat for oyster farming (Farnham

and Irvine 1973). Since then, it has spread to other areas along Atlantic, North Sea, Mediterranean coasts and also along Pacific coast, and has been reported for example in North America (Villalard-Bohnsack and Harlin 1997), Spain and Portugal (Bárbara and Cremades 2004; Araújo *et al.* 2011), Venice Lagoon and Mar Piccolo basin of Toronto (Cecere *et al.* 2011), Australia (Saunders and Withall 2006), New-Zealand (D'Archino *et al.* 2007), or Mexico (Aguilar-Rosas *et al.* 2012). More recently, it was recorded in Israel (Katsanevakis *et al.* 2014), and in southern Brazil (de Azevedo *et al.* 2015), constituting its first records in Eastern Mediterranean and South-Western Atlantic, respectively. Moreover, it should be noted that as this species is relatively understudied, there are probably other introduction points not yet reported in France or in Europe.



**Figure 20.** Distribution map of the red macroalga *Grateloupia turuturu* in Europe, with main sites of introduction, adapted from Stiger-Pouvreau and Thouzeau (2015); Bárbara and Cremades (2004); Araújo *et al.* (2011); Bunker *et al.* (2017), and updated with data from European Alien Species Information Network (EASIN, available <https://easin.jrc.ec.europa.eu/easin>). The reported dates of introduction are displayed in different colours, although the species may have been present in previous years.

In France, *G. turuturu* has been reported for the first time in 1982 in the Etang de Thau (France) (Riouall *et al.* 1985), and then in Brittany at Fort Bloqué in 1989 (Cabioch *et al.* 1997). Its distribution area then expanded at Callot Island (Carantec) in 1992, before spreading to the south of Brittany and Normandy (Simon *et al.* 2001). *Grateloupia turuturu*

lives from shallow subtidal to low intertidal areas, generally in sheltered locations or areas exposed to rippling. It is commonly found in rockpools where water persists at low tide and where conditions are generally unfavourable to the majority of macroalgal species due to extreme conditions (Simon *et al.* 2001).

### 1.1.2.3 Life cycle

*Grateloupia turuturu* is a monoecious species with a trigenetic reproductive cycle, with the succession of gametophyte, carposporophyte (*i.e.* microscopic form attached to the gametophyte, which as a granulose aspect), and tetrasporophyte (*i.e.* with a rougher surface, described as "orange skin" (Fig. 21).

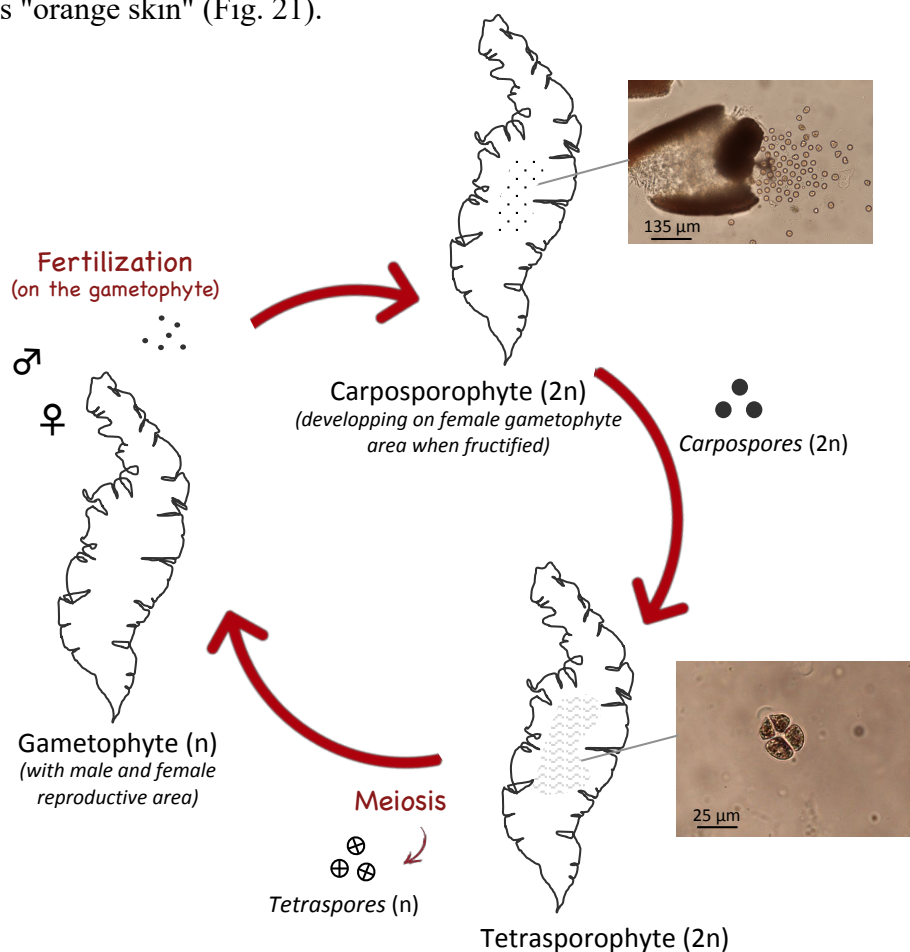


Figure 21. Life cycle of *Grateloupia turuturu*, adapted from Simon *et al.* (2001) and Cardoso *et al.* (2019).

At maturity, the gametophyte liberates male and female gametes from different parts on the same thallus. The gametophyte is haploid whereas carposporophytes and tetrasporophytes are diploid. Then, each stage is visible throughout the year, in varying proportions (Simon *et al.* 2001; Araújo *et al.* 2011).

#### 1.1.2.4 Invasive potential

Known as the "Devil's Tongue", it has been suggested that the introduced *G. turuturu* could be invasive, including in Brittany (Simon *et al.* 2001). It is therefore described as one of the five most-threatening introduced species in the world (Nyberg and Wallentinus 2005; Koerich *et al.* 2020). Indeed, several studies have demonstrated that *G. turuturu* shows multiple recruitment strategies (Harlin and Villalard-Bohnsack 2001) and that it can withstand wide variation in environmental parameters, supporting its invasiveness. More particularly, this species is described as euryhaline and eurythermal (Simon *et al.* 1999). Then, some studies showed that *G. turuturu* could compete with native macroalgae such as *Chondrus crispus* and could be responsible for the disruption of native assemblages (Mathieson *et al.* 2007; Janiak and Whitlach 2012). However, more recent studies suggest that *G. turuturu* may not be invasive in Brittany, but rather that it could become invasive only on the condition of future changes of one or several environmental parameters (Stiger-Pouvreau and Thouzeau 2015).

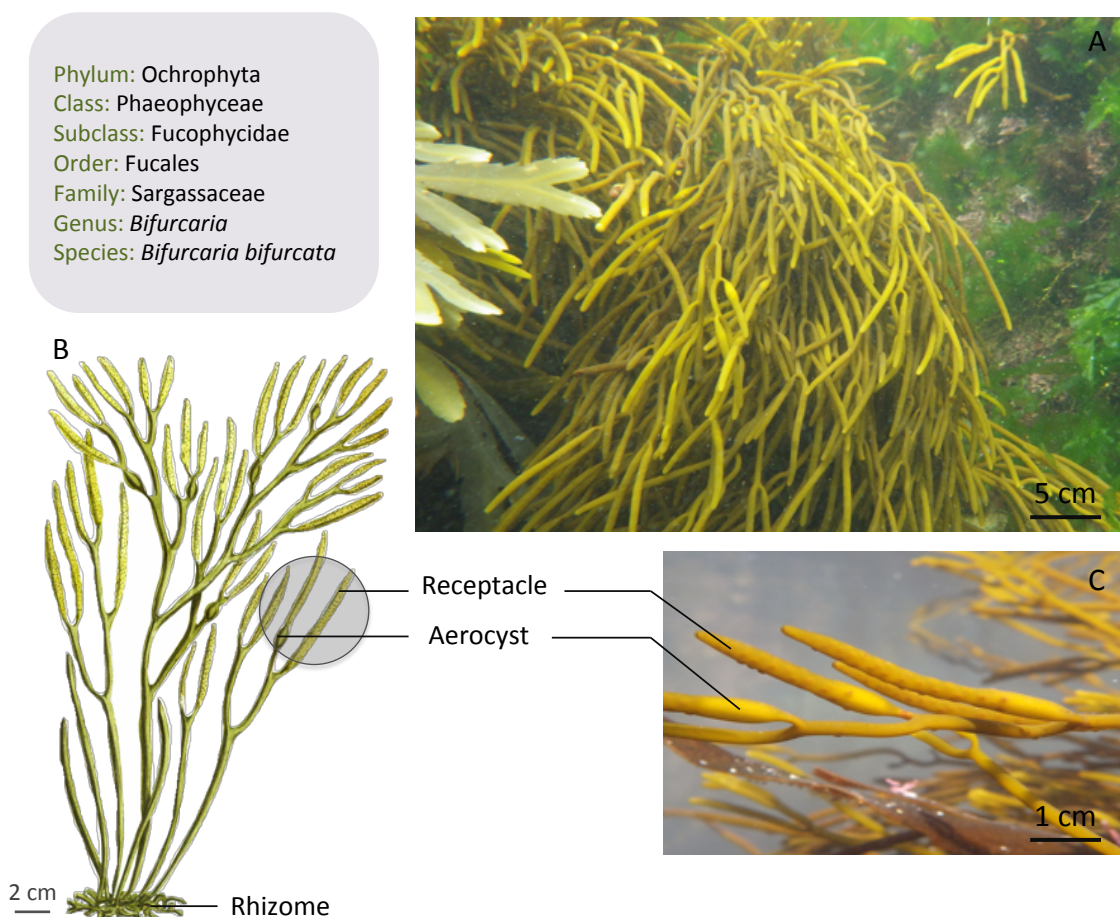
#### 1.1.2.5 Biochemical composition and valorisation

*Grateloupia turuturu* contains significant levels of floridoside (Simon-Colin *et al.* 2004), mycosporine-like amino acids (Figuerola *et al.* 2007; De Ramos *et al.* 2019) or more particularly high value R-phycoerythrin (Denis *et al.* 2010). Moreover it has been demonstrated that extracts from *G. turuturu* presents antibacterial (Pang *et al.* 2006; García-Bueno *et al.* 2014), or antifouling activities (Plouguerné *et al.* 2008). Cholesteryl formate was isolated from French populations of *G. turuturu* and demonstrated interesting antimicrofouling activities (Plouguerné *et al.* 2006). In this way, *G. turuturu* could have a commercial interest although it is currently poorly valorised (Denis *et al.* 2009b). However, to facilitate the access to the metabolites of interest, it may require an enzymatic digestion of its thallus as this access is limited by the presence of anionic polysaccharides in the cell wall (Denis *et al.* 2009a). In addition, *G. turuturu* is used as a source of hybrid carrageenan polysaccharides (food additive E407) (Cardoso *et al.* 2019). More particularly, it is use in human nutrition, mostly consumed in Asian countries, due to its composition rich in proteins and dietary fibres, which can represent 20 and 60 % of its dry weight, respectively (Denis *et al.* 2010; García-Bueno *et al.* 2014). In addition, it has a potential nutritive interest due to its high level in eicosapentaenoic acids (EPA), representing 12 % of its fatty acids content, similarly to other edible red seaweeds (Denis *et al.* 2010; Kendel *et al.* 2013).

### 1.1.3. *Bifurcaria bifurcata*

#### 1.1.3.1 Description

*Bifurcaria bifurcata* R. Ross 1958 is a temperate brown macroalga belonging to the Fucales order and the Sargassaceae family (Fig. 22). It is a perennial seaweed with a cylindrical shape of 3-4 mm in diameter, *i.e.* dichotomous branches which can reach 50 cm in length. Unlike many species of macroalgae, it is not attached to the substrate by a disc or holdfast but by a pseudo-rhizome (Le Lann *et al.* 2008). In addition, sometimes the thallus may present some aerocysts, *i.e.* oblong air bladders (Fig. 22). Due to the presence of the pseudo-rhizome, it is difficult to determine the number of individuals accurately. In *B. bifurcata*, the notion of individual does not exist; we then considered almost each patch of fronds as an individual (Le Lann *et al.* 2012).



**Figure 22.** Classification and (A) illustration of thalli of *Bifurcaria bifurcata* in an intertidal rockpool (Minou, Brittany, France; September 2018). (B) Schematic illustration of the morphology of a thallus of *Bifurcaria bifurcata*, identifying the different parts (drawn adapted from William Henry Harvey (Phycologia Britannica, plate LXXXIX, 1846-1851)), with (C) an illustration of fertile receptacles and some aerocysts (Minou, Brittany, France; February 2019) © F. Lalegerie

### 1.1.3.2 Distribution

This species is widespread on the rocky foreshores of the English Channel and the North-East Atlantic Ocean (*i.e.* from north-western Ireland to south Morocco), particularly common on the British Isles, French and Spanish coasts (de Valera 1961; Valls *et al.* 1995). *Bifurcaria bifurcata* lives on the middle and lower tidal zones where it coexists with the brown macroalga *Himanthalia elongata*, down to the upper limit of the subtidal zone. It can be found on exposed or sheltered shores, sometimes emerged at low tide, and commonly found in intertidal rockpools (Le Lann *et al.* 2012).

### 1.1.3.3 Life cycle

*Bifurcaria bifurcata* has monoecious thalli, *i.e.* a single type of individual that produces both male and female gametes. Its life-cycle is similar to other Fucales species, with receptacles located at the apex of the thallus (Fig. 23). *Bifurcaria bifurcata* possess hermaphrodite conceptacles, *i.e.* producing both male and female gametes (Jensen 1974). It reproduces mainly in winter, although receptacles can be visible most of the year (Le Lann *et al.* 2012). To date, there is very limited information on the biology and phenology of *B. bifurcata*, due to the limited number of ecological studies on its populations.

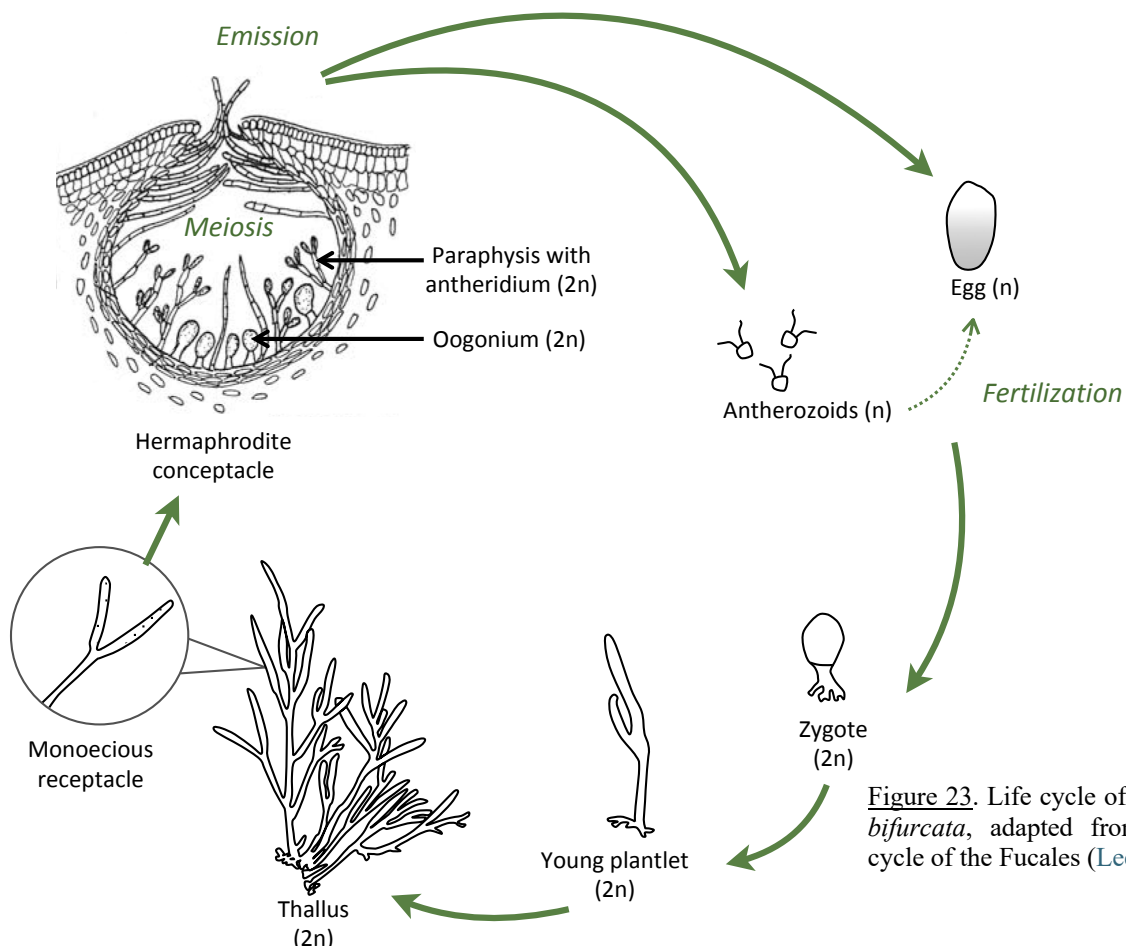


Figure 23. Life cycle of *Bifurcaria bifurcata*, adapted from the life cycle of the Fucales (Lee 1999).

### 1.1.3.4 Biochemical composition and valorisation

Although *B. bifurcata* remains largely unexploited and that its commercial use is very limited compared to other macroalgal species, it appears to have an industrial potential in different sectors, including food and cosmetics (Pais *et al.* 2019). To my knowledge, only an aqueous extract has been marketed by GELYMA (France) for its anti-ageing and photoprotective properties (Stiger-Pouvreau and Guérard 2018). Nevertheless, this species has revealed a rich variety of acyclic terpenoids in its lipid extracts (Valls and Piovetti 1995; Valls *et al.* 1995; Culioli *et al.* 1999, 2000, 2001, 2004; Göthel *et al.* 2012), recently reviewed by Pais *et al.* (2019). Some of them are studied for their antifouling and antimicrobial properties (Hellio *et al.* 2001; Muñoz *et al.* 2013; Pais *et al.* 2019). Moreover, some crude aqueous, ethanolic or methanolic extracts have demonstrated high antioxidant (Agregán *et al.* 2017; Le Lann *et al.* 2008), antimicrobial (Maréchal *et al.* 2004; Horta *et al.* 2014) or antitumoral activities (Di Guardia *et al.* 1999; Moreau *et al.* 2006; Zubia *et al.* 2009). In particular, it has a phenolic content that can represent 4-5 % DW (Connan *et al.* 2007; Le Lann *et al.* 2008). More generally, *B. bifurcata* contains more than 70 % of moisture, a high fat content representing 6 % DW, and a protein content of 8 % DW (Alves *et al.* 2016; Lorenzo *et al.* 2017). It was furthermore shown that its chemical composition could vary between seasons (Culioli *et al.* 2002; Le Lann *et al.* 2012), or the location, depending on abiotic factors such as hydrodynamism or substrate (Valls *et al.* 1995; Le Lann *et al.* 2014).

### 1.1.4. *Halidrys siliquosa*

#### 1.1.4.1 Description

*Halidrys siliquosa* (Linnaeus) Lyngbye 1819 is a brown macroalga, belonging to the Fucales order and the Sargassaceae family (Fig. 24). It forms large fronds up to 2 - 3 m long and 5 m width, with laterals from 0.5 to 1 cm wide. These fronds are flattened and coriaceous. *H. siliquosa* has alternate branching in one plane (*i.e.* distichous), the lower laterals being the longest. It is attached to the substrate by a discoid holdfast. Some branches on the thallus have permanent air-bladders having a silicic shape with transverse divisions, whereas others branches bear receptacles, very similar to air-bladders but ephemeral and with a granular surface (Moss and Lacey 1963; Stiger-Pouvreau *et al.* 2014).

Phylum: Ochrophyta  
 Class: Phaeophyceae  
 Subclass: Fucophycidae  
 Order: Fucales  
 Family: Sargassaceae  
 Genus: *Halidrys*  
 Species: *Halidrys siliquosa*

**Figure 24.** Classification and illustration of a thallus of *Halidrys siliquosa* in an intertidal rockpool (Minou, Brittany, France; October 2018). © F. Lalegerie



#### 1.1.4.2 Distribution

The distribution area of *H. siliquosa* is restricted to the North-East Atlantic, in addition to the North Sea and the English Channel. It has a Nordic/boreal affinity and thus is generally found in Ireland, Great Britain and Norway. Its southern limit of distribution is Spain and Portugal being absent in the Mediterranean sea (Moss and Lacey 1963; Margalet and Navarro 1990; Lima *et al.* 2009). In this way, seawater temperature and light could be an important factor conditioning its latitudinal distribution (Moss and Sheader 1973; Larsson *et al.* 1986). It is preferentially found in sheltered or semi-sheltered environments in rockpools of the lower intertidal zone, at the same level as *Himanthalia elongata*. In addition, it can also be found in the subtidal zone up to 15 m deep, at the same level as the *Laminaria* spp. never emerged (Moss and Lacey 1963). In France, it often coexists with *Sargassum muticum* or *Cystoseira/Carpodesmia* spp. (pers. obs.) in intertidal rockpools and the subtidal area.

#### 1.1.4.3 Life cycle

There are limited studies on the life cycle of the perennial *Halidrys siliquosa* although close to the classical cycle of species from Fucales (see the cycle of *B. bifurcata* Fig. 23). Indeed, the cycle of *H. siliquosa* could present some specificities. Notably, according to Moss and Elliot (1957), it appears that *H. siliquosa* could possess a haploid thallus, with meiosis during the germination of the zygote; or that oogonium develop parthenogenetically, as no reduction division was observed in the oogonium and antheridium. As a result, it could be distinct from the majority of the Fucales which have diploid thallus (Heesch *et al.* 2019). In this way, Bell



(1997) suggested that not all Fucales were diploid and that there may be exceptions. By contrast to Moss and Elliot (1957), the study of Naylor (1958) suggests that *H. siliquosa* could possess a diploid thallus, comparable to the classical cycle of the Fucales (Heesch *et al.* 2019). Then, further investigations are needed to clarify the life cycle of *H. siliquosa*. In any case, *H. siliquosa* is a monoecious species, *i.e.* only one individual producing both male and female gametes (Stiger-Pouvreau *et al.* 2014), and from the same conceptacles (Hardy and Moss 1978). According to Moss and Lacey (1963), *H. siliquosa* has a reproductive phenology as follows: the development of the reproductive tissues (*i.e.* receptacles) between approximately August and December, the gamete release between December and March, and germling development and vegetative growth of young thalli between March and July. Then, new sporophytes reach maturity and develop reproductive tissues in 1-2 years (Moss and Lacey 1963).

#### 1.1.4.4 Biochemical composition and valorisation

Its biomass being much reduced compared to other species of Fucales, *Halidrys siliquosa* is still poorly valorised nowadays. Nevertheless, it appears that it may have an economic interest because of its potential antioxidant (Zubia *et al.* 2009; Le Lann *et al.* 2016; Gager *et al.* 2020), antibacterial and antimicrobial (Hornsey and Hide 1974; Spavieri *et al.* 2010; Buseti *et al.* 2015), antiparasitic (Spavieri *et al.* 2010; Vonthron-Senecheau *et al.* 2011), photoprotective (Le Lann *et al.* 2016), or antitumoural activities (Zubia *et al.* 2009). To my knowledge, there is only one patent registered by the company Gelyma (France) (Pellegrini and Pellegrini 2009) on the use of an aqueous extract of *H. siliquosa* for a cosmetic and/or dermatological application, due to its antibacterial properties. Furthermore, *H. siliquosa* is a species rich in terpenes such as monomethyl hydroquinols (Amico 1995), tetraprenyltoluquinol-related metabolites (Culioli *et al.* 2008), or polyprenyl hydroquinol mono-methyl ethers (Higgs and Mulheim 1981), some of them with potential antifouling activities (Hellio *et al.* 2001; Culioli *et al.* 2008; Buseti *et al.* 2015). In addition, *H. siliquosa* has a high content of phenolic compounds, which can represent almost 20 % DW (Stiger-Pouvreau *et al.* 2014). More specifically, Stiger-Pouvreau *et al.* (2014) and Le Lann *et al.* (2016) reported the occurrence of trifuhalol, tetrafuhalol, diphlorethol and triphlorethol in *H. siliquosa*.

### 1.1.5. *Sargassum muticum*

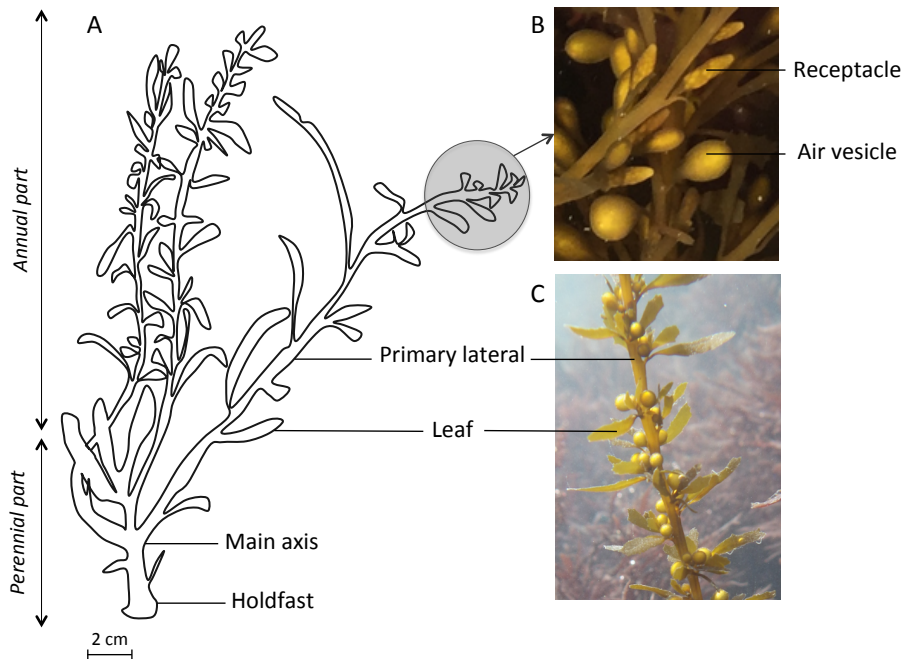
#### 1.1.5.1 Description

*Sargassum muticum* (Yendo) Fensholt 1955 is a brown macroalga belonging to the Fucales order and the Sargassaceae family (Fig. 25). The thallus of this species can be divided into two different parts: a perennial part (*i.e.* holdfast and main axis) and an annual part (*i.e.* primary and secondary laterals, bearing leaves, air-bladders, and receptacles at maturity) (Critchley 1983) (Fig 26.). The laterals are reduced in winter, but can reach several meters in length in summer; thalli up to 5 m have thus been observed (Sabour *et al.* 2013).

Phylum: Ochrophyta  
 Class: Phaeophyceae  
 Subclass: Fucophycidae  
 Order: Fucales  
 Family: Sargassaceae  
 Genus: *Sargassum*  
 Species: *Sargassum muticum*



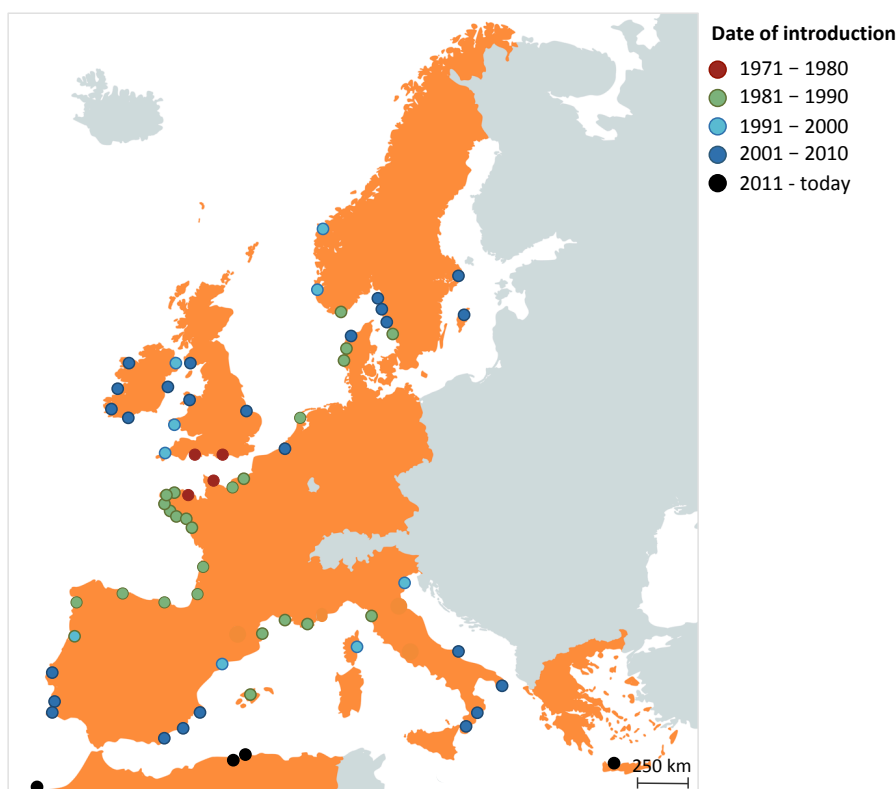
**Figure 25.** Classification and illustration of thalli of *Sargassum muticum* in an intertidal rockpool (Minou, Brittany, France; March 2018). © F. Lalegerie



**Figure 26.** (A) Schematic illustration of the morphology of *Sargassum muticum*, identifying the different parts of the thallus, according to Critchley (1983). Illustrations of (B) the receptacles, and (C) primary lateral (Pointe du diable, Brittany, France; May and February 2018 respectively). © F. Lalegerie.

### 1.1.5.2 Distribution

Originally from Japan and Pacific Ocean, *S. muticum* was accidentally introduced into Europe in 1970s, probably following the transfer of the oyster *Crassostrea gigas* (Critchley 1983; Stiger-Pouvreau and Thouzeau 2015). It was first reported at Bembridge, Isle of Wight, in 1971 (Farnham 1980). Since then, it has spread along Atlantic coasts, Mediterranean, and North Sea, from Norway to Morocco (Fig. 27) (Engelen *et al.* 2015; Stiger-Pouvreau and Thouzeau 2015; Thibaut *et al.* 2016). Recently, it was reported for the first time on the Algerian coast in Cherchell and Sidi Fredj (Benali *et al.* 2019), suggesting that it could expand further in the Mediterranean Sea.



**Figure 27.** Distribution map of *Sargassum muticum* in Europe, with main sites of introduction, adapted from Stiger and Thouzeau (2015), Engelen *et al.* (2015) and Thibaut *et al.* (2016), and updated with data from European Alien Species Information Network (EASIN, available <https://easin.jrc.ec.europa.eu/easin>). The reported dates of introduction are displayed in different colours, although the species may have been present in previous years.

*Sargassum muticum* is particularly well-established in France since the country constitutes one of the first introduction area in Europe. It was reported first in 1977 in Pointe du Hoc (Normandy), and in Brittany and the Thau Lagoon in 1981 (Critchle 1983). Generally, *S. muticum* lives in a wide range of habitats in the intertidal zone, sometimes in rockpools, on sheltered or exposed sites (Plouguerné *et al.* 2006). Nevertheless, it seems to settle

preferentially in areas characterized by low hydrodynamic conditions (Andrew and Viejo 1998). *Sargassum muticum* is also found in subtidal zone where it forms small forests never too large, probably due to insufficient light availability (Belattmania *et al.* 2020).

### 1.1.5.3 Life cycle

*Sargassum muticum* has a monogenetic and monoecious life cycle (Fig. 28): only one generation is present and there is only one type of individual (Engelen *et al.* 2015). Diploid individuals bear receptacles (*i.e.* reproductive structures regrouping conceptacles) containing both male and female gametes produced within different conceptacle. These receptacles are visible from spring to early autumn (Fig. 26b).

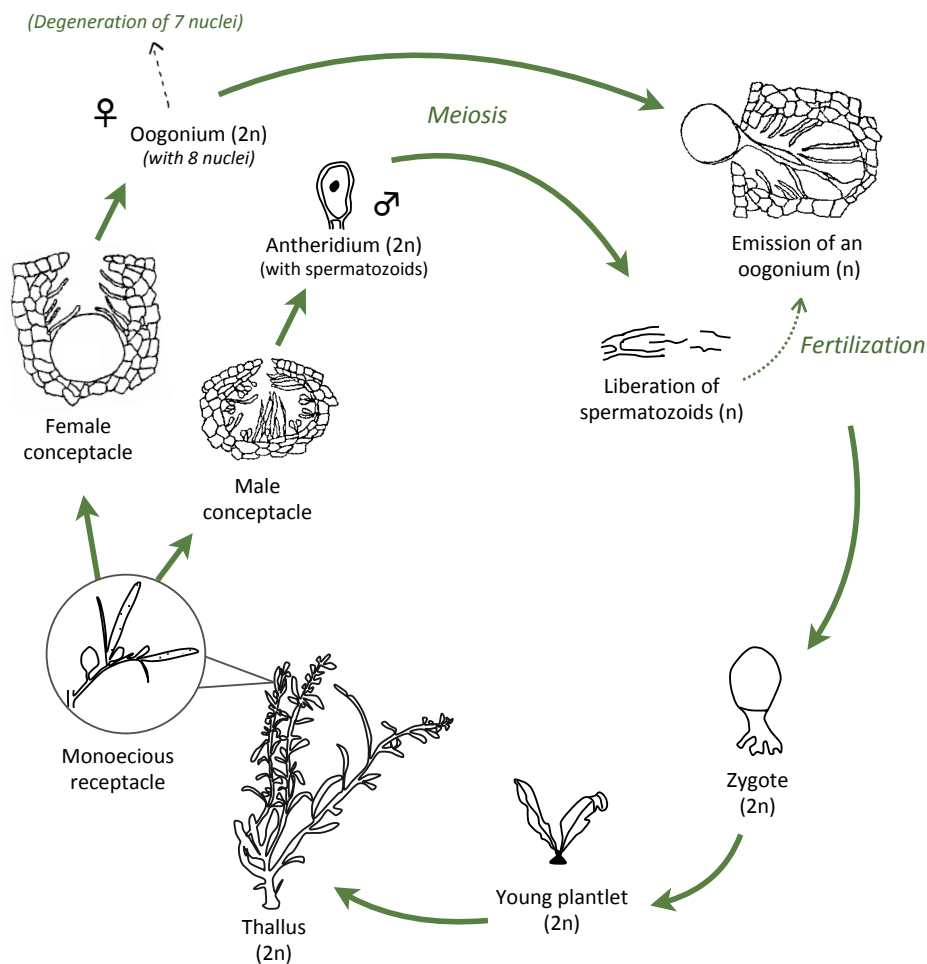


Figure 28. Life cycle of *Sargassum muticum*, adapted from Stiger (1997).

Each conceptacle then produces haploid gametes, male (*i.e.* spermatozooids within antheridia) or female (*i.e.* oosphere/egg within oogonia). In addition, *S. muticum* can reproduce asexually, by fragmentation of its thallus. This species is thus known to have a high dispersal capacity

due to the detachment of its laterals which can float over long distances before releasing its gametes far from their origin (Engelen *et al.* 2015). In addition, *S. muticum* exhibits high seasonality, with individuals reduced in size in winter and then a fast growth in spring reaching several meters (Le Lann *et al.* 2012).

#### 1.1.5.4 Invasive potential

*Sargassum muticum* is an alien species considered nowadays to be one of the top 10 species being the most widespread and disturbing marine invaders in Europe (Nyberg and Wallentinus 2005; Engelen *et al.* 2015; Stiger-Pouvreau and Thouzeau 2015). Its high dispersion is due in part to its high dissemination capacity, and its high plasticity allowing it to adapt to a wide range of environments (Engelen *et al.* 2015; Benali *et al.* 2019). In addition, it has a fast-growth rate, leading it to rapidly reach a high biomass. Then, *S. muticum* competes with native species for space and available resources (Stæhr *et al.* 2000; Engelen *et al.* 2015), including several other members of Sargassaceae, such as *Carpodesmia* (previously *Cystoseira*) *tamariscifolia*, *Bifurcaria bifurcata* and *Treptacantha* (*Cystoseira*) *baccata* (Le Lann *et al.* 2012) or *Halidrys siliquosa* (Stæhr *et al.* 2000; Britton-Simmons 2004; Sánchez *et al.* 2005). This competition therefore leads to the decrease in diversity and abundance of some native species. However, few studies highlighted rather no or limited effect of *S. muticum* on some native species suggesting more complex interactions, as with *Bifurcaria bifurcata* (Sánchez and Fernández 2006; Olabarria *et al.* 2009). Moreover, *S. muticum* huge development can cause a hindrance to certain activities such as navigation (*e.g.* by the obstruction of navigation channels, or the accumulation of thalli in ships' propellers), fishing (*e.g.* by fouling fishing gear / nets) or aquaculture (*e.g.* making maintenance and access to the oyster farms more difficult) (Engelen *et al.* 2015). In response to its ecological and economical negative impacts, various measures (*e.g.* hand removal, trawling, cutting or chemical eradication) have been intended to limit or eradicate *S. muticum*, without success to this day (Kraan 2007).

#### 1.1.5.5 Biochemical composition and valorisation

Because of its invasive nature and unsuccessful eradication, some studies have attempted to find a way to valorise *S. muticum*, largely underexploited until now. Only few studies report on its chemical composition (Milledge *et al.* 2016). Nevertheless, the potential extraction of valuable components such as fucoxanthin and fatty acids were explored (Conde *et al.* 2015).

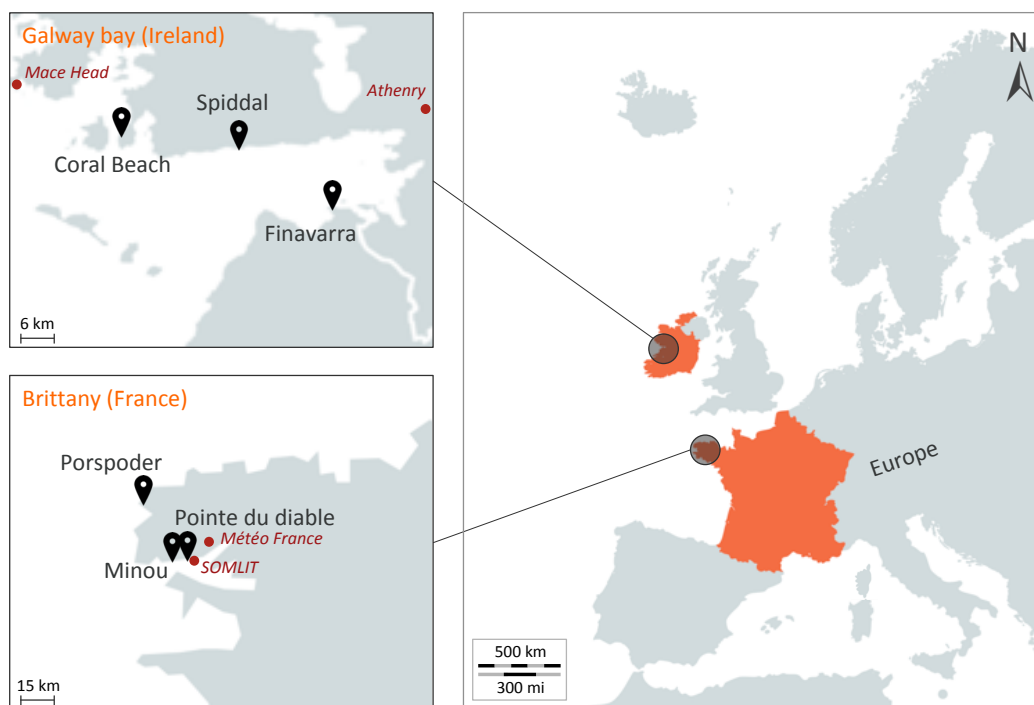
According to Balboa *et al.* (2013), *S. muticum* could be used more particularly for its production of alginate or sulphated polysaccharides (*i.e.* fucoidan), with an appropriate extraction protocol to increase the yield. In this way Flórez-Fernández *et al.* (2019) obtained alginate gels from *S. muticum* comparable to those prepared from several *Laminaria* species. In addition, it would appear that *S. muticum* could be used as a fertilizer or adsorbent as heavy metals removal (Milledge *et al.* 2016). Additionally, Song *et al.* (2016) also showed that ethyl acetate fraction of *S. muticum* prevents UVB-induced wrinkle formation, suggesting a potential interest for cosmeceutical and pharmaceutical markets. *Sargassum muticum* demonstrated also a high accumulation of secondary metabolites with anti-inflammatory (Jeon *et al.* 2019), antitumoural (Liu *et al.* 2016; Pérez-Larrán *et al.* 2020) or antibacterial (Hellio *et al.* 2001; Plouguerné *et al.* 2008) activities. Plouguerné *et al.* (2010) highlighted in *S. muticum* the production of glycolipids with antifouling activities. Among fatty acids, palmitic acid constitutes 21.5 % of the total fatty acids of *S. muticum*, and demonstrated to have antifouling activity (Bazes *et al.* 2009; Milledge *et al.* 2016). More particularly, French populations of *S. muticum* synthesizes a high amount of phenolic compounds, representing 6 % DW with antioxidant activities (Connan *et al.* 2006). These phenolic compounds were later identified as phloretols (Stiger-Pouvreau *et al.* 2014; Tanniou *et al.* 2013). Moreover, *S. muticum* was found notably to have the highest levels of both  $\alpha$  and  $\gamma$ -tocopherol among 16 algal species (Farvin and Jacobsen 2013). In addition, the chemical composition of *S. muticum* appeared to be highly correlated with environmental variations and different seasons (Balboa *et al.* 2016). Connan *et al.* (2006) highlighted a high intra-thallus variation in the phenolic content of the invasive species, with the basal part producing higher content compared to the apical part. All these elements will have to be taken into account for a future valorisation of the biomass of *S. muticum* and requires further research on the ecophysiology and the seasonal biochemical composition of this species.

## 1.2. Site description and environmental data

The French populations of red (*i.e.* *Palmaria palmata* and *Grateloupia turuturu*) and brown (*i.e.* *Bifurcaria bifurcata*, *Halidrys siliquosa* and *Sargassum muticum*) macroalgae were monitored during one year, between February 2018 and February 2019. Ecological measurements were performed each month on three sites, and was coupled to collection of samples for metabolomic analysis. A second short monitoring was carried out in February and

June 2019 to compare French and Irish populations of *S. muticum* and *H. siliquosa*. This second monitoring was carried out on the same sites than the first monitoring in France, and then on three sites in Galway Bay (Ireland) (Fig. 29).

Concerning France, environmental data, *i.e.* seawater temperature ( $^{\circ}\text{C}$ ), pH, salinity, nutrient concentrations ( $\mu\text{M}$ ), oxygen level ( $\text{mL}\cdot\text{L}^{-1}$ ), measured at sea surface in open sea, were provided by "Service d'Observation en Milieu Littoral, INSU-CNRS, Saint-Anne du Portzic" ( $48^{\circ}21'32.13''\text{ N} - 4^{\circ}33'07.19''\text{ W}$ ). In addition, sunshine duration (hours) and irradiance ( $\text{J}\cdot\text{cm}^{-2}$ ) were provided by "Météo France" (station Brest-Guipavas,  $48^{\circ}27'00''\text{ N} - 4^{\circ}22'59''\text{ W}$ ). For Ireland, global irradiance was provide by the meteorological service Met éireann (Athenry station), while other data (sea temperature, pH, salinity, nitrate content) were provide by the compass Mace Head observation buoy of Marine Institute. These environmental data will be detailed in the results of Chapters 2 and 3.



**Figure 29.** Location of the different sites (black), and stations for environmental measurements (red), in France and in Ireland

### 1.2.1. Sites in Brittany (France)

Three sites were chosen for the monitoring of macroalgal species on the Brittany coast (France) were: Minou ( $48^{\circ}20'20.314''\text{ N} - 4^{\circ}37'3.779''\text{ W}$ ), Pointe du diable ( $48^{\circ}21'15.606''\text{ N} - 4^{\circ}33'30.067''\text{ W}$ ) and Porspoder ( $48^{\circ}28'56.644''\text{ N} - 4^{\circ}46'6.812''\text{ W}$ ) (Fig. 29).

### 1.2.1.1 Pointe du diable

Located in the Bay of Brest in Plouzané (Finistère, Brittany), the Pointe du diable is a rocky site with two large rockpools that are emerged at low tide. One rockpool was used for the red macroalgal monitoring, and another one for the brown seaweed monitoring (sampled between 10 and 30-cm deep). The two rockpools are located next to each other, at the bottom of a cliff, at the *Fucus serratus* belt level. In addition, the site is exposed to strong hydrodynamic conditions. A high diversity of macroalgae can be observed at this site, with notably a covering of red macroalgae such as *Chondrus crispus*, or *Calliblepharis jubata* (pers. obs.; Fig. 30). Regarding the brown seaweeds, individuals of *B. bifurcata* are restricted to the edges of the rockpool (Le Lann *et al.* 2012), whereas *S. muticum* covers much of the pool in the spring, before the large development of *Ulva* spp. in late spring - summer.

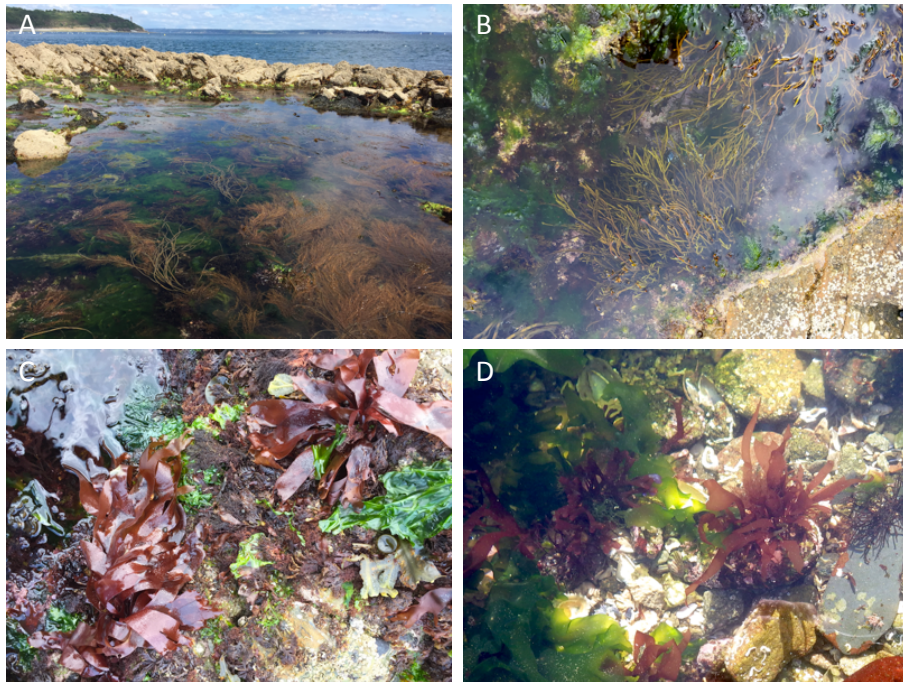


Figure 30. Illustration of Pointe du diable site (Brittany, France): (A) overview of one of the two large rockpools where the species were monitored, showing *Sargassum muticum* in the foreground; (B) *Bifurcaria bifurcata*, on the edges of the pool; (C) *Palmaria palmata*; (D) *Grateloupia turuturu*. © F. Lalegerie

### 1.2.1.2 Minou

Minou is located at the entrance of the Bay of Brest in Plouzané (Finistère, Brittany), nearby Pointe du diable site (Fig. 29). It is a sandy cove, below the Petit Minou lighthouse. This beach, exposed to the waves is only emerged at low tide. Its sand has been used for a long time to amend the soil of the municipality. The rocky part where the seaweeds grow is located



at the intertidal level, at the edge of the beach, below a short cliff. Macroalgae are located in small rockpools, from 20-cm to 1-m width, and from 5 to 50-cm deep (Fig. 31). The site is characterized by the presence of numerous mussels and grazers (e.g. *Aplysia* sp.). In addition, there is a relatively large quantity of calcareous macroalgae (i.e. *Corallina* sp.), and in summer green macroalgae due to a freshwater inflow. *Grateloupia turuturu* is well present, distributed in a scattered way, mainly in water flows, whereas *Palmaria palmata* is limited to a few rare individuals. *Sargassum muticum* and *Bifurcaria bifurcata* are the two predominant brown macroalgae at this site, living together in rockpools or in separated ones.



**Figure 31.** Illustration of Minou site (Brittany, France): (A) overview of rockpools where the species were monitored; (B) *Grateloupia turuturu*, in the centre of a small pool; (C) *Sargassum muticum*; (D) *Bifurcaria bifurcata*, on the edge of the pool. © F. Lalegerie

### 1.2.1.3 Porspoder

This site is located on the western extremity of Brittany (France), between Lanildut and Porspoder, and more precisely at Porsmeur Cove (Fig. 29). The sampling area is located in the intertidal zone in cohabitation with *Fucus serratus*, along a rocky tide channel, largely covered by coarse sand. The site is sheltered, as it is protected from the waves by a rocky barrier. The channel is only emerged at spring tides (coefficient > 85, i.e. under a water height of 1.5 m), and never completely emerged at low tide. The site is characterized by the presence of large amount of *Fucus serratus*, and *Cystoseira/Carpodesmia* spp., in addition to *H. siliquosa*, *B. bifurcata* and large individuals of *S. muticum* in summer (Fig. 32). Moreover,

there is a freshwater input, although the presence of large quantities of green macroalgae has not been observed. The important algal harvesting by seaweed harvesters (known as "goémoniers") based in Lanildut, the first harbour for seaweed harvest in Europe, attests to the richness and large quantities of macroalgae on this site (Philippe 2013).



**Figure 32.** Illustration of Porspoder site (Brittany, France): (A) overview of the channel where the species were monitored; (B) *Palmaria palmata* epiphyte on *Fucus serratus*; (C) *Bifurcaria bifurcata* attached on a rock at the edge of the channel; (D) *Halidrys siliquosa*. © F. Lalegerie

### 1.2.2. Sites in Galway Bay (Ireland)

The French population of *Sargassum muticum* and *Halidrys siliquosa* were compared with Irish populations, monitored on three sites in the Galway Bay (Ireland): Finavarra (53°09'15.9'' N – 9°07'02.9'' W), Coral beach (53°14'55.5'' N – 9°37'48.7'' W) and Spiddal (53°14'25.1'' N – 9°18'35.5'' W) (Fig. 29).

#### 1.2.2.1 Finavarra

Finavarra, Co. Clare, is located approximately 45 km of Galway city, on the south side of the Galway Bay (Fig. 29). It is a sheltered / semi-exposed site characterized by a sandy substrate, mixed with coarse rocks, partially or completely covered by sand. Finavarra is a site with diversified habitats, with more large rocky portions, especially at the upper shore. The site is never completely uncovered at low tide, forming large shallow rockpools. Moreover, this site is known to have a rich macroalgal diversity, representing a biodiversity hotspot for

seaweeds (Rae *et al.* 2013). In summer, *S. muticum* can form a large canopy, covering a large part of the intertidal rockpools (Fig. 33). In parallel, *H. siliquosa* is located in the upper sublittoral zone, where few randomly located individuals are growing.

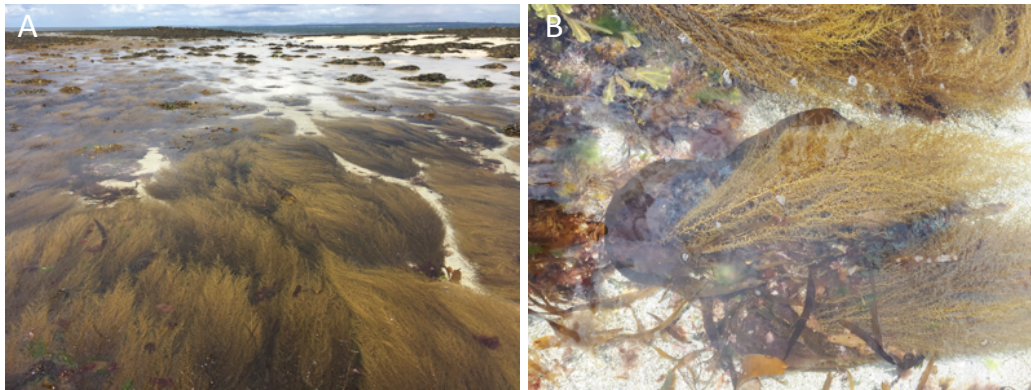


Figure 33. Illustration of Finavarra site (Galway, Ireland): (A) overview of the channel where the species were monitored; (B) *Sargassum muticum*. © F. Lalegerie

### 1.2.2.2 Coral Beach

Carraroe Coral Beach, Co. Galway, is located at the southern edge of Connemara, along the west coast of the Cheathrú Rua peninsula, approximately 45 km west of Galway city (Fig. 29). It is characterized by a substrate composed by coralline algae known as maerl, almost entirely composed of *Phymatolithon calcareum*. It is a moderately exposed site, with many rockpools and emergent rock on the intertidal zone where coexist different brown macroalgal species such as *S. muticum*, *Fucus* spp., or lower *H. siliquosa* and *Laminaria* sp. (Fig. 34). It is a popular diving and snorkelling location, in part due to its clear water and usually good underwater visibility.

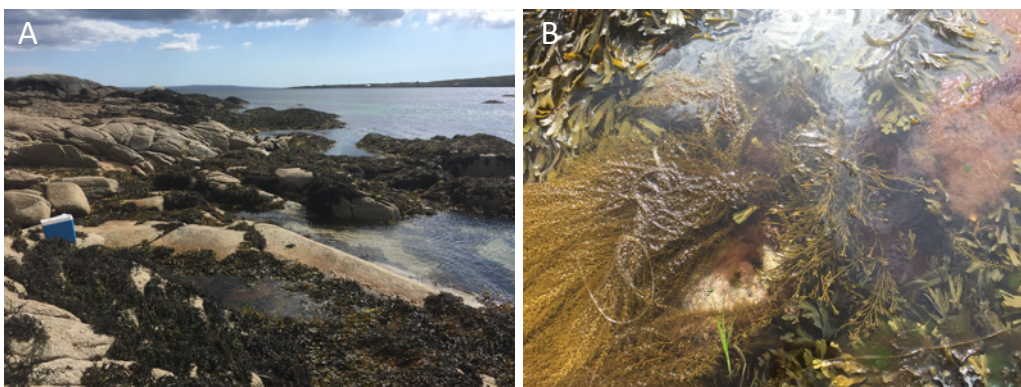
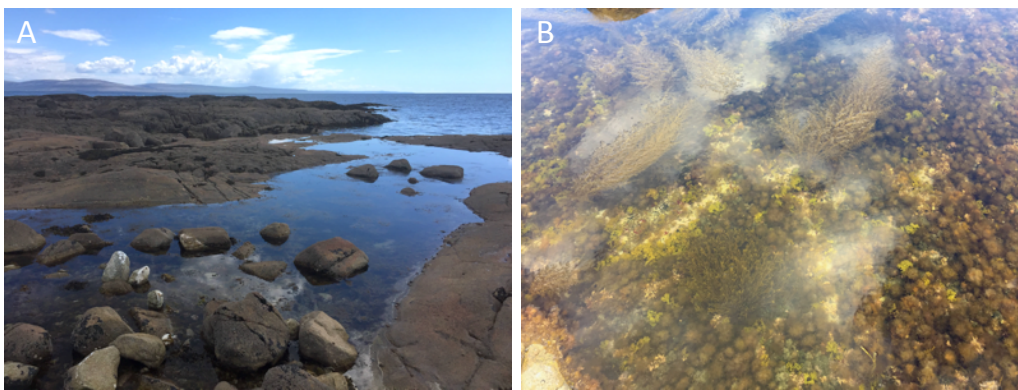


Figure 34. Illustration of Coral Beach site (Galway, Ireland): (A) overview of the rockpools where the species were monitored; (B) *Sargassum muticum* coexisting with *Halidrys siliquosa*. © F. Lalegerie

### 1.2.2.3 Spiddal

Spiddal (Irish: An Spidéal), Co. Galway, is located 15 km west of Galway city, along North coast road of Galway Bay. The studied area is next to Bay fishing, approximately 1 km from Spiddal beach (Fig. 29). The tidal zone consists of flat rocky area with some large shallow rockpools mainly emerged at low tide (Fig. 35). The site, exposed to waves, is characterized by the presence of many red macroalgal species, such as *Chondrus crispus*, *Polysiphonia* spp. or *Corallina* spp. forming a wide algal cover. At this site, seaweeds exhibited yellowish, lighter colour. In addition, there were many individuals of *S. muticum* and *H. siliquosa*, reduced in size due to shallow water depth in rockpools.



**Figure 35.** Illustration of Spiddal site (Galway, Ireland): (A) overview of the large rockpools where the species were monitored; (B) *Sargassum muticum* coexisting with *Halidrys siliquosa*. © F. Lalegerie

## 1.3. Ecological measurements

For both monitoring, *i.e.* in France and Ireland, a pre-sampling was conducted in order to determine the minimum sampling area for each species, using 50 x 50-cm quadrats in France, and 100 x 100-cm quadrats in Ireland, subdivided into 10 x 10-cm areas ( $n = 6$ ). The minimum areas for each species were then obtained when there was no statistical difference in the number of individuals when the sampling area increased (Table 2). The number of replicates (*i.e.* quadrats) was fixed to four since it was not possible to analyse more quadrats, to allow the monitoring of the five species over the duration of a tide (with one site monitored per day, *i.e.* three field days per month). The quadrats were then randomly positioned in each population during the monitoring.

**Table 2.** Size of the quadrats used for each species monitored, with a number of replicates by species fixed at 4.

<b>Species</b>	<b>Size of quadrats</b>
<i>Palmaria palmata</i>	0.09 m <sup>2</sup>
<i>Grateloupia turuturu</i>	0.09 m <sup>2</sup>
<i>Bifurcaria bifurcata</i>	0.16 m <sup>2</sup>
<i>Halidrys siliquosa</i>	0.16 m <sup>2</sup> France / 1.00 m <sup>2</sup> Ireland
<i>Sargassum muticum</i>	0.09 m <sup>2</sup> France / 0.25 m <sup>2</sup> Ireland

**Ecological variables measured were:**

- density (*i.e.* number of individuals per quadrat)
- maximum length (*i.e.* from the base to the apical part)
- maximum circumference for brown macroalgae or width for the foliaceous thallus of red macroalgae

In addition, the reproductive stage was recorded, corresponding to gametophyte, carposporophyte, or tetrasporophyte for red macroalgae; or to the relative abundance of receptacles by individuals (*i.e.* no, few or many) for brown macroalgae to estimate the maturity rate as the percentage of individuals bearing both few and many receptacles. For all species, the undifferentiated stage corresponded to thalli without reproductive structures, which can be either not distinguishable gametophytes or sporophytes for red macroalgae and immature individuals for brown macroalgae. For each individual, some additional indications were recorded, *i.e.* the colour of the thallus, the presence of epiphytes (in term of species and abundance), traces of grazing, and the possible degradation of the thallus.

In addition, at each sampling, algal material was collected for metabolomic analyses, in areas adjacent to the quadrats. After collection, all algal samples were cleaned to remove epiphytes, and frozen immediately before being freeze-dried and ground into a fine powder; samples for metabolomic analyses were processed dried and within two months after collection.

## 2. Cultivation experiment under controlled conditions of the red alga *Palmaria palmata*

In order to study the effect of specific environmental factors without all other surrounding factors interfering, a cultivation experiment under controlled conditions was carried out on the red macroalga *Palmaria palmata*. More particularly, this culture experiment was carried out in order to study the impact of two environmental parameters, light (quality only) and nutrients, on the production of mycosporine-like amino acids (MAAs), whose synthesis and potential diverse roles are still unclear. Then, the species *P. palmata* was chosen for its high diversity in MAAs, compared to *Grateloupia turuturu* with only few MAAs, or brown algae that do not contain MAAs. Samples were collected on March 2017 in Portsall, and cultivated over a period of 21 days. Two light conditions (photosynthetically active radiation (PAR) or PAR + UV-radiations) and three nitrate concentrations ( $\text{NaNO}_3$  at 0, 100 and 300  $\mu\text{M}$ ) were tested, in triplicate for each condition (each replicate corresponding to an individual small tank containing one thallus) (Fig. 36). The nutrient levels tested were determined according to previous published cultivation experiments carried out on MAAs content of other red algae (Figueroa *et al.* 2003; Peinado *et al.* 2004; Huovinen *et al.* 2006; Korbee *et al.* 2005). Light intensity was either 42.6 or 63.4  $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$  under PAR and PAR+UV respectively, which was lower than the levels tested in previous studies (usually between 5 and 300  $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ , see the references cited in the previous sentence), but corresponded to the maximum limit of our equipment. Each tank was equipped with a bubbling system and water and nutrients were renewed every 3 days.

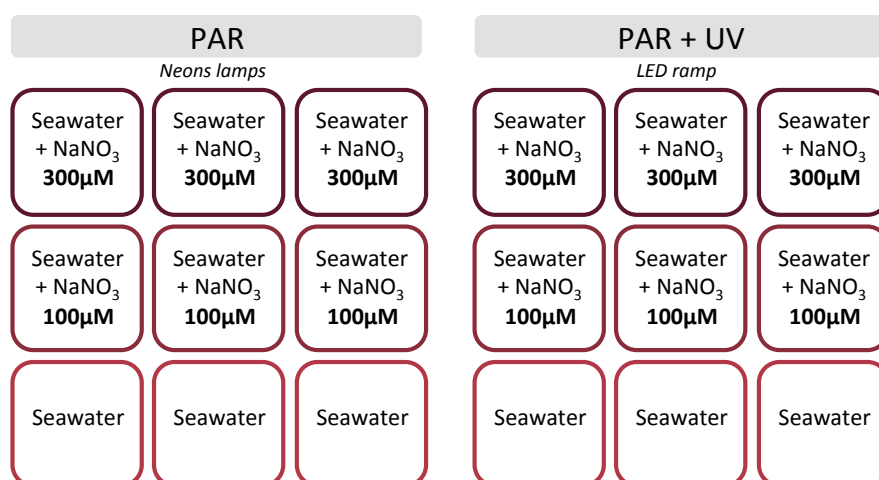
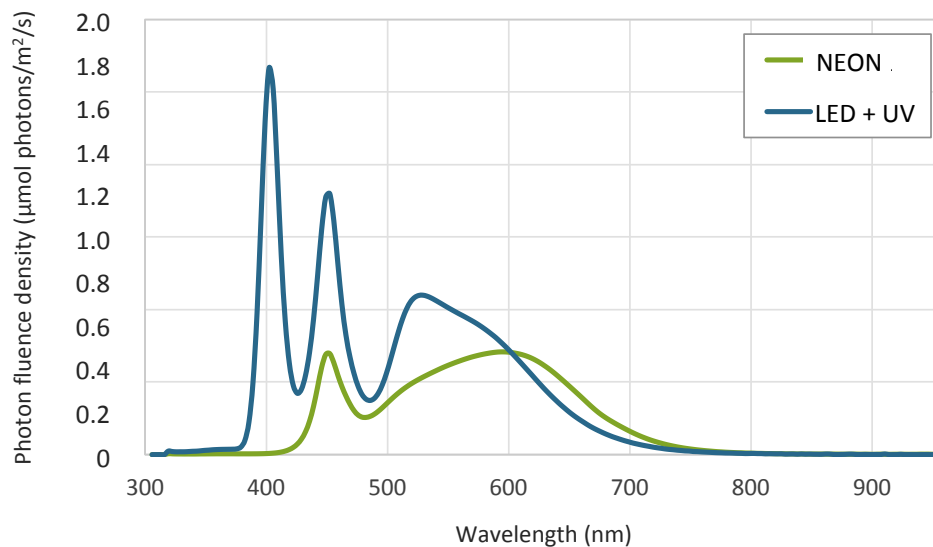


Figure 36. Schematic representation of the culture experiment carried out on the red macroalga *Palmaria palmata* in March 2017.

Algae were artificially illuminated by PAR (400 - 700nm), or by PAR+UV radiation (addition of UVA 315 - 400 nm), with a photoperiod of 16 h light : 8 h dark. The PAR radiation was provided by neon bulbs, while the PAR+UV radiation was provided by a DMX LED lighting ramp (Nicolaudie) associated with ESAPro software. In order to see only the effect of the UV radiation, the PAR provided by the neon bulbs has been reproduced as best as possible on the LED ramp (Fig. 37). The MAAs and pigments were extracted on samples at day 0 (D0: start of culture experiment) and then for each condition after 7, 15 and 21 days of culture. Before the beginning of the experiment, the seaweeds have been acclimatised for 3 days.

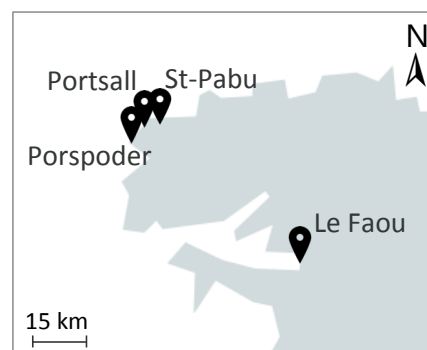


**Figure 37.** Spectra of light sources used in the culture experiment using *Palmaria palmata*. The spectra were obtained using a TriOS RAMSES-ACC spectroradiometer.

### 3. Screening of mycosporine-like amino acids in red macroalgae from Brittany (France)

In order to investigate the diversity in mycosporine-like amino acids (MAAs), a screening was carried out in a large number of red macroalgal species collected on Brittany shores (France). As some macroalgae are present for only a part of the year, two samplings were carried out at two different periods, *i.e.* in February and June 2017. Macroalgae were collected at low tides on four different locations in Brittany (Fig. 38): Porspoder ( $48^{\circ}28'58''\text{N} - 4^{\circ}46'5''\text{W}$ ), Portsall ( $48^{\circ}33'53''\text{N} - 4^{\circ}42'5''\text{W}$ ), Saint-Pabu ( $48^{\circ}34'34''\text{N} - 4^{\circ}38'45''\text{W}$ ) and Le Faou ( $48^{\circ}17'44''\text{N} - 4^{\circ}10'56''\text{W}$ ).

These sites were chosen for their different geographical location and topography in order to increase the number of species collected. Thus, Portsall, with a north-western exposure, is a sheltered, mostly rocky site, surrounded by sand and intertidal pools. Saint-Pabu is a North exposed site characterized by a sandy substrate. Porspoder is a site, which despite its western exposure, remains sheltered due to the presence of two large rocky advances. Finally, the Faou stands out from the three previous sites since a river flows into the bay of Brest,



**Figure 38.** Location of the different sites (Brittany, France) where red algae were collected for the screening of mycosporine-like amino acids.

forming a sheltered but muddy estuary at low tide. Thus, 40 species of red macroalgae were collected across the four different sites: 21 species were found both in winter and summer and 19 species were found only at one season. All species belong to Rhodophyta and Florideophyceae class, except *Porphyra dioica* (Bangiophyceae) (Table 3). These included species from upper and lower intertidal, with different morpho-anatomical groups (Fig. 39). In parallel to the MAAs screening, the pigment composition (*i.e.* chlorophylls, carotenoids and phycobiliproteins) was also analysed (see **Part 4** for the extraction and analysis methods of algal metabolites).



**Table 3.** List and taxonomic classification of the 39 red macroalgal species belonging to Florideophyceae sampled for the screening of MAAs, in addition to the Bangiophyceae *Porphyra dioica* J.Brodie & L.M. Irvine 1997 (Order: Bangiales; Family: Bangiaceae).

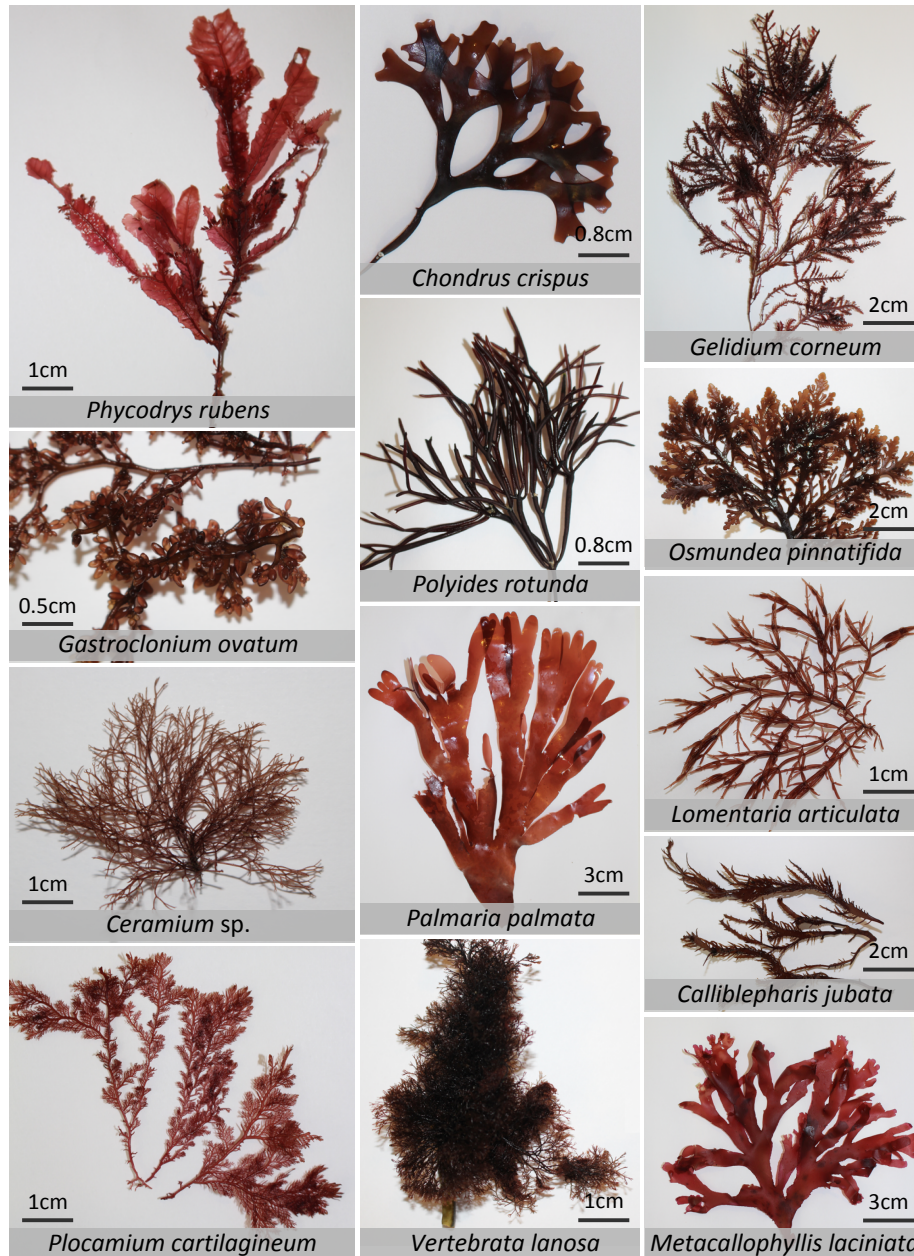
ORDER	FAMILY	SPECIES	
Bonnemaisoniales	Bonnemaisoniaceae	<i>Asparagopsis armata</i> Harvey 1855	
		<i>Bonnemaisonia hamifera</i> Hariot 1891	
Cerariales	Ceramiaceae	<i>Callithamnion tetricum</i> (Dillwyn) S.F. Gray 1821	
		<i>Callithamnion tetragonum</i> (Withering) S.F. Gray 1821	
		<i>Ceramium echionotum</i> J. Agardh 1844	
		<i>Ceramium virgatum</i> Roth 1797 (previously <i>C. nodulosum</i> (Lightfoot) Ducluzeau 1806)	
		<i>Ceramium secundatum</i> Lyngbye 1819	
	Dasyaceae	<i>Plumaria plumosa</i> (Hudson) Kuntze 1891	
	Delesseriaceae	<i>Heterosiphonia plumosa</i> (J. Ellis) Batters 1902	
		<i>Hypoglossum hypoglossoides</i> (Stackhouse) Collins & Hervey 1917	
		<i>Membranoptera alata</i> (Hudson) Stackhouse 1809	
	Rhodomelaceae	<i>Phycodrys rubens</i> (Linnaeus) Batters 1902	
		<i>Bostrychia scorpioides</i> (Hudson) Montagne 1842	
		<i>Osmundea hybrida</i> (A.P. de Candolle) K.W. Nam 1994	
<i>Osmundea pinnatifida</i> (Hudson) Stackhouse 1809			
Wrangeliaceae	<i>Vertebrata lanosa</i> (Linnaeus) T.A. Christensen 1967		
	<i>Bornetia secundiflora</i> (J. Agardh) Thuret 1855		
Corallinales	Corallinaceae	<i>Griffithsia corallinoides</i> (Linnaeus) Trevisan 1845	
Gelidiales	Gelidiaceae	<i>Corallina</i> sp. Linnaeus 1758	
	Cystocloniaceae	<i>Gelidium corneum</i> (Hudson) J.V. Lamouroux 1813	
	Dumontiaceae	<i>Calliblepharis jubata</i> (Goodenough & Woodward) Kützing 1843	
	Furcellariaceae	<i>Dilsea carnosa</i> (Schmidel) Kuntze 1898	
	Gigartinales	Furcellariaceae	<i>Furcellaria lumbricalis</i> (Hudson) J.V. Lamouroux 1813
		Gigartinaceae	<i>Chondracanthus acicularis</i> (Roth) Fredericq 1993
		Gigartinaceae	<i>Chondrus crispus</i> Stackhouse 1797
		Gigartinaceae	<i>Gigartina pistillata</i> (S.G. Gmelin) Stackhouse 1809
	Kallymeniaceae	<i>Metacallophyllis laciniata</i> (Hudson) A. Vergés & L. Le Gall 2017	
	Phylloporaceae	<i>Mastocarpus stellatus</i> (Stackhouse) Guiry 1984	
<i>Ahnfeltiopsis devoniensis</i> (Greville) P.C. Silva & De Cew 1992			
Sphaerococcaceae	<i>Ahnfeltiopsis devoniensis</i> (Greville) P.C. Silva & De Cew 1992		
Polyidaceae	<i>Sphaerococcus coronopifolius</i> Stackhouse 1797		
Gracilariales	Gracilariaceae	<i>Polyides rotunda</i> (Hudson) Gaillon 1828	
		<i>Gracilaria gracilis</i> (Stackhouse) M.Steentoft, L.M. Irvine & W.F. Farnham 1995	
Palmariales	Palmariaceae	<i>Agarophyton vermiculophyllum</i> (Ohmi) Gurgel, J.N. Norris & Fredericq 2018	
Plocamiales	Plocamiaceae	<i>Palmaria palmata</i> (Linnaeus) F. Weber & D. Mohr 1805	
		<i>Plocamium cartilagineum</i> (Linnaeus) P.S. Dixon 1967	
Rhodymeniales	Champiaceae	<i>Champia parvula</i> (C. Agardh) Harvey 1853	
		<i>Chylocladia verticillata</i> (Lightfoot) Bliding 1928	
	Lomentariaceae	<i>Gastroclonium ovatum</i> (Hudson) Papenfuss 1944	
		<i>Lomentaria articulata</i> (Hudson) Lyngbye 1819	

The diversity of MAAs was estimated by the development of a new index. To this end, the presence and absence of each MAA for each species was coded in a table, as 0 or 1 respectively. A distance matrix was then calculated by comparing species two by two using the following index of MAA similarity:

$$\text{Index of MAA similarity} = \frac{M}{M+N}$$

with M the number of matches between the two species (presence and absence) and N the total number of MAAs which were present in either species. The closer the index was to 1,

the more similar the two species were in terms of their MAAs composition. A dendrogram was then made from this matrix with the R program (R Development Core team, 2008) to represent species that had similar MAA compositions (hclust with single method, hclust package). In order to highlight any links between the MAAs composition of the different algal species with their phylogeny, a phylogenetic analysis was also performed using *rbcL* and *cox1* genes sequences obtained from the Genbank (available on <https://www.ncbi.nlm.nih.gov/genbank>) (see **Chapter 2, section 1** for more details).



**Figure 39.** Illustration of the different morphologies of some red macroalgae screened for their MAAs composition (Portsall, Brittany, France; March 2017). © F. Lalegerie

## 4. Extraction, analysis and protocol development of the different algal metabolites

The different approaches of the manuscript described above include the analysis of different algal metabolites, meaning:

- For **Part 1** (“Eco-metabolomic” monitoring of native and invasive species) on brown and red macroalgae: **apolar pigments, phenolic compounds, fatty acids**, with in addition **phycobiliproteins** and **mycosporine-like amino acids** for red algae, or **terpenes** for brown algae.
- **Part 2** (Cultivation experiment) and **part 3** (screening) on red algae: **mycosporine-like amino acids, apolar pigments** and **phycobiliproteins**.

For each metabolites, the different protocols of extraction and analysis are described, as well as protocol optimisation. The general description of each compound is available in **section 2.3 of Chapter 1**.

### 4.1. Apolar pigments from red and brown macroalgae

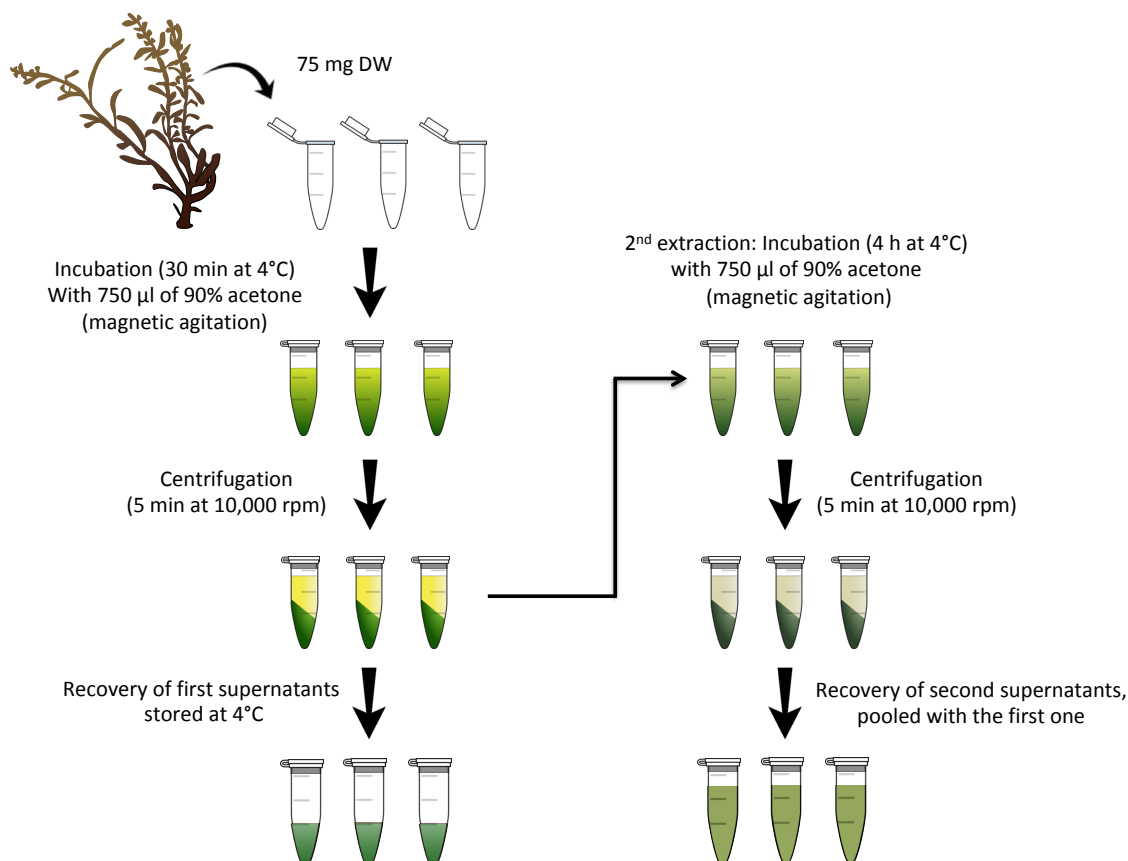
#### 4.1.1 Extraction

Apolar pigments (*i.e.* chlorophylls and carotenoids) were extracted using 75 mg of dry seaweed powder with 750  $\mu$ L 90 % aqueous acetone according to the protocol described in [Schmid and Stengel \(2015\)](#). Two successive extractions were carried out in the dark (Fig. 40): after a first extraction of 30 min at 4 °C under magnetic agitation, samples were centrifuged at 10,000 rpm for 5 min (Eppendorf Centrifuge 5810, Germany) and the supernatant was collected. The remaining pellets were then mixed with 90 % aqueous acetone for 4 h at 4 °C. After this second extraction, samples were centrifuged and both supernatants were pooled and filtered before High Pressure Liquid Chromatography (HPLC) analysis performed directly after extraction.

In addition, in order to increase the number of sample extractions in the same day and optimize the analysis by HPLC, a duration of 15 h for the second extraction (instead of 4 h) was tested in order to keep it overnight. Extractions of 4 h or 15 h did not show any significant difference (Student test,  $p$ -value = 0.869), and both extraction times were therefore used. For this test, the extraction was made on the red macroalga *Chondrus crispus*, and the

chlorophyll-*a* content was approximately determined by spectrophotometry using the trichroic equation of Ritchie (2008) (expressed in  $\mu\text{g}\cdot\text{mL}^{-1}$ ):

$$\text{Chlorophyll-}a = -0.3002 (A_{630} - A_{750}) - 1.7538 (A_{647} - A_{750}) + 11.9092 (A_{664} - A_{750})$$

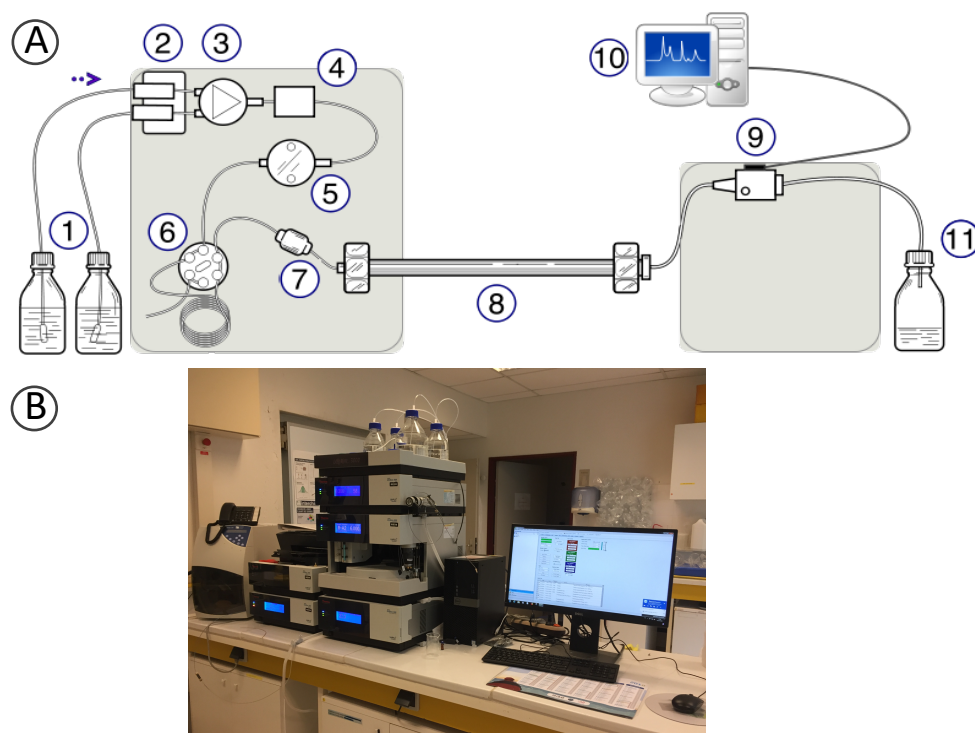


**Figure 40.** Schematic representation of the extraction procedure of the apolar pigments. © F. Lalegerie

#### 4.1.2 Analysis by high pressure liquid chromatography (HPLC)

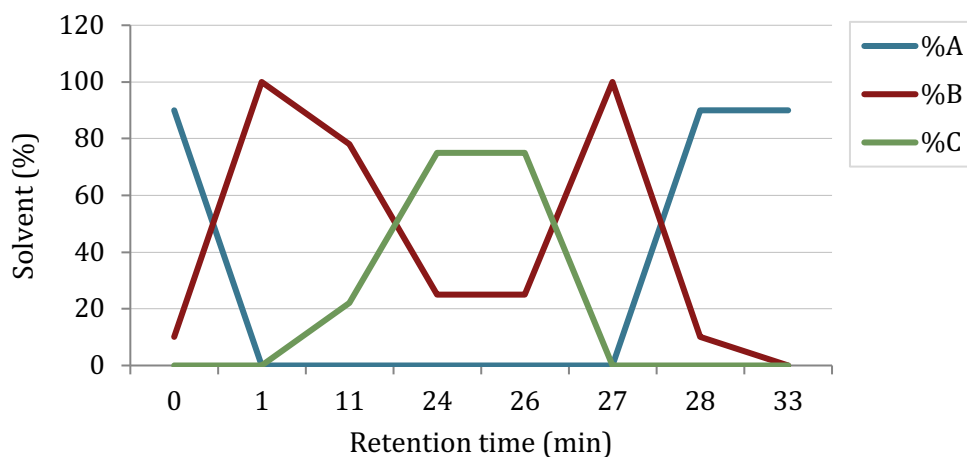
Pigments were analysed by high pressure liquid chromatography (HPLC), a separation technique based on the polarity and size of the molecules. It has the advantage of being fast, automatic, and highly accurate (Dong 2006). The molecules to be separated are dissolved and then injected into a mobile phase (solvent/s or eluent/s). Due to high pressure, the mobile phase is pushed through the chromatographic chain to a stationary phase (column, solid support) that retains the molecules more or less according to their affinity with this phase. Once separated, the molecules are detected at the end of the chain by one or more detector(s), the separated molecules having different retention times (Fig. 41a). Among the different detectors are for example the Diode Array Detector (DAD, for absorbance measurement), the Fluorescence Detector (FLD), the Evaporative Light Scattering Detector (ELSD) or a Mass

Spectrometer (MS). A chromatogram is then obtained on which each peak represents a molecule which can then be quantified using standards according to its area.



**Figure 41.** (A) Schematic representation of a High Pressure Liquid Chromatography system (HPLC). (1) Solvents, (2) solvent degasser, (3) gradient valve, (4) mixing valve for delivery of the mobile phase, (5) high-pressure pump, (6) injection system, (7) guard-column, (8) analytical column, (9) detector, (10) data acquisition, (11) waste (Picture by Yassine Mrabet). (B) Illustration of the Dionex Ultimate 3000 system used at the laboratory. © F. Lalegerie

In this work, a Dionex Ultimate 3000 HPLC system (ThermoScientific) was used (Fig. 41b) equipped with a pump (LPG-3400SD), an autosampler (WPS-3000TSL Analytical), a column compartment (TCC-3000SD), and a diode array detector (DAD-3000). For pigment analysis, an ACE C<sub>18</sub> column was used (dimension 150 × 4.6 mm, granulometry 3 μm, pore size 100 Å, carbon load 15.5 %, maximal temperature 60 °C, pH range 1.5 to 10), protected with a guard-column. The column and injector temperatures were set at 30 ± 1 °C and 5 ± 1 °C, respectively. HPLC was programmed to dilute ¾ the samples with ammonium acetate buffer (0.5 M, pH 7.2) just before the injection of 6 μL, to avoid pigment degradation. The mobile phase consisted of three solvents through a gradient applied with a flow rate of 1.0 mL.min<sup>-1</sup> (Fig. 42). Data acquisition was performed at 435, 470 and 665 nm, according to the wavelength of maximum absorbance ( $\lambda_{\max}$ ) of pigments (chlorophylls, carotenoids).



**Figure 42.** Solvent gradient used for HPLC analysis of apolar pigments. The solvents are: (A) methanol : ammonium acetate buffer 0.5 M (80 : 20), (B) acetonitrile : water MilliQ water (87.5 : 12.5), (C) 100% ethyl acetate.

For pigment analysis, the identification was obtained by comparison of retention times and absorption spectra with commercial standards of chlorophyll-*a* (Sigma, USA), and chlorophyll-*b*, chlorophyll-*c*<sub>2</sub>, chlorophyll-*c*<sub>3</sub>, fucoxanthin, pheophytin-*a*,  $\alpha$ -carotene,  $\beta$ -carotene, lutein, zeaxanthin, antheraxanthin, and violaxanthin (DHI, Denmark) (Fig. 43). Pigment concentrations were then quantified using standard curves, obtained through the injection of different concentrations of the standards. Only peaks with an area larger than 0.4 mAU.min for  $\alpha$ - and  $\beta$ -carotene and 1 mAU.min for the other pigments were quantified. Chromeleon 7 software (Thermo Scientific Dionex, France) was used for HPLC control and data acquisition.

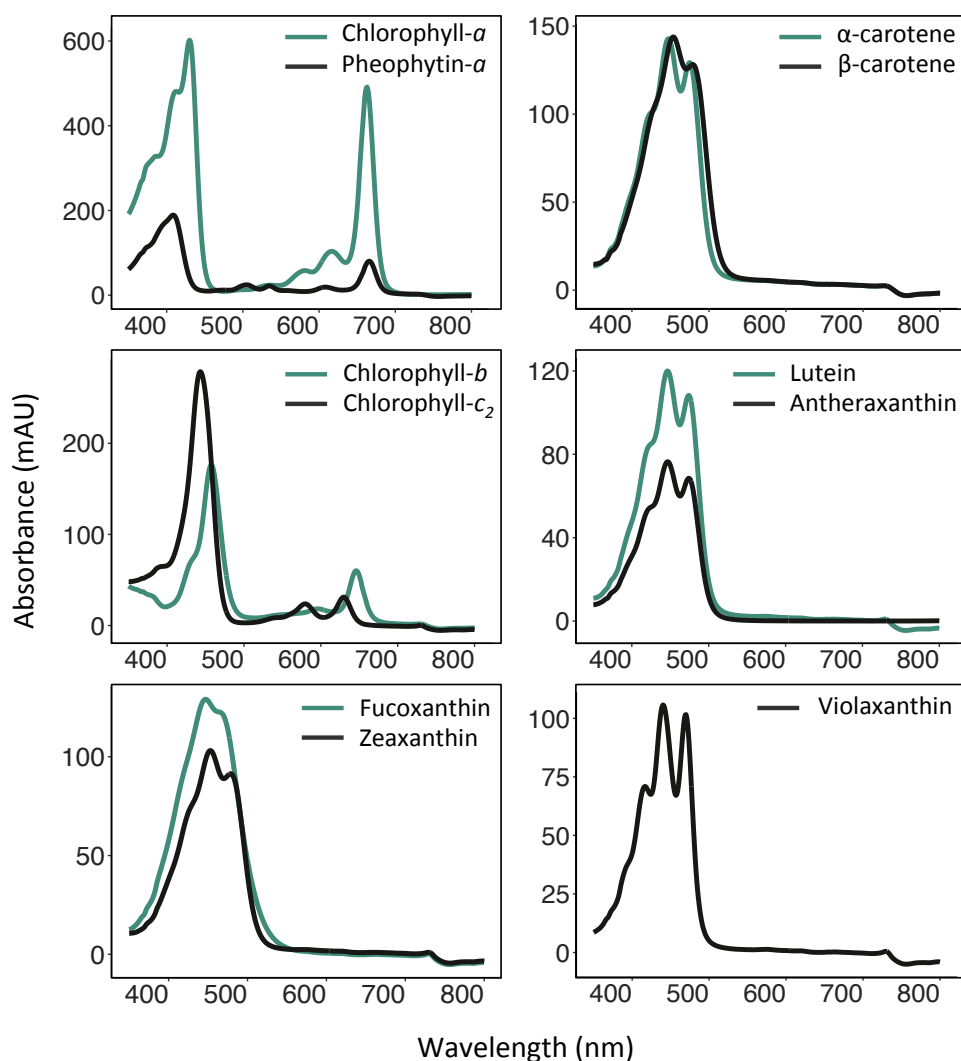


Figure 43. Absorption spectra of pigment standards analysed by HPLC. The retention times of each pigment for identification are as follows: chlorophyll- $c_2$  4.5 min, fucoxanthin 6.6 min, violaxanthin 8.2 min, antheraxanthin 10.0 min, lutein 12.5 min, zeaxanthin 12.8 min, chlorophyll- $b$  18.5 min, chlorophyll- $a$  20.0 min, pheophytin- $a$  22.6 min,  $\alpha$ -carotene 23.8 min and  $\beta$ -carotene 23.9 min.

## 4.2. Phycobiliproteins from red macroalgae

### 4.2.1 Extraction

The extraction of phycobiliproteins (*i.e.* phycoerythrin or PE; phycocyanin or PC) was performed on 75 mg DW of algal powder added to 1.5 mL of phosphate buffer (0.1 M, pH 6.8), using a protocol adapted from Sun *et al.* (2009) (Fig. 44). Two successive extractions of 15 min at 4 °C were performed in the dark, and samples were then centrifuged for 20 min at 10,000 rpm (miniSpin plus, Eppendorf). Supernatants were recovered to measure absorbance.

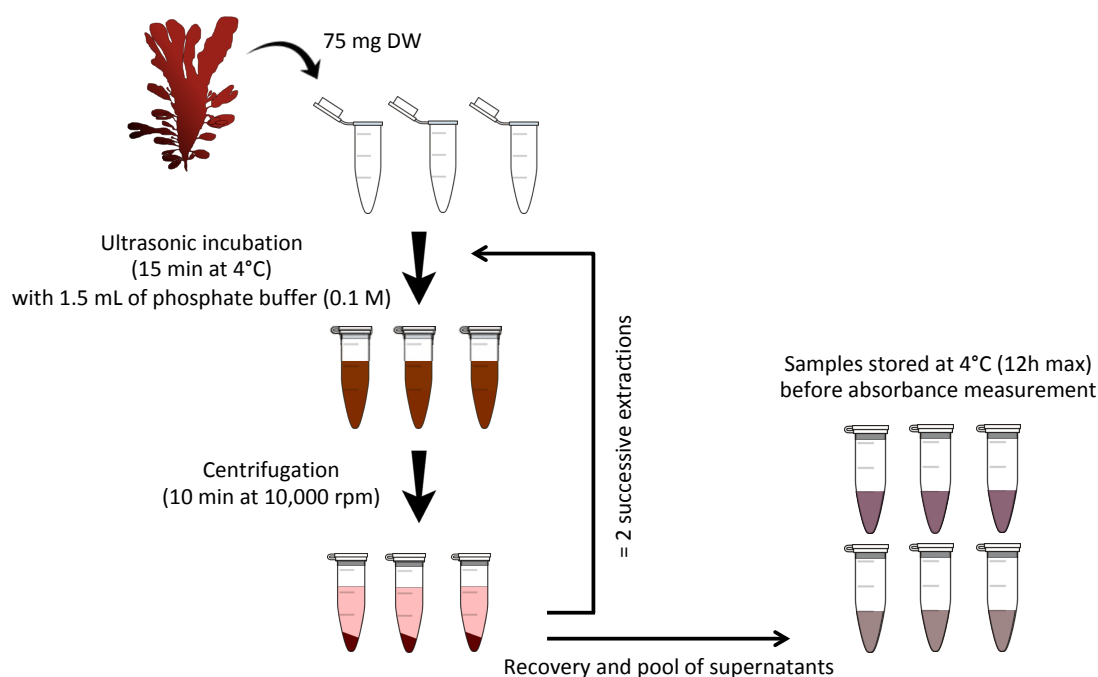


Figure 44. Schematic representation of the extraction procedure of the phycobiliproteins. © F. Lalegerie

Beforehand, three concentrations of phosphate buffer were tested: 6, 50 and 100 mM, as well as the number of successive extraction required (Fig. 45). Two extractions appeared to be sufficient, and no significant difference was found between the different phosphate buffers (ANOVA,  $p$ -value = 0.140 and 0.921 for PE and PC, respectively). The 100 mM phosphate buffer was chosen for further analysis due to lower variability among replicates. In addition, attempts have been made to extract both apolar pigments and phycobiliproteins from the same algal powder of *Chondrus crispus*. However, following the extraction of chlorophyll-*a*, the results showed no phycobiliproteins, even after the addition of a 90% acetone evaporation step between the extraction of the two groups of pigments. Conversely, it was possible to extract chlorophyll-*a* after the extraction of phycobiliproteins, but to a lesser level (Fig. 46), and thus despite the

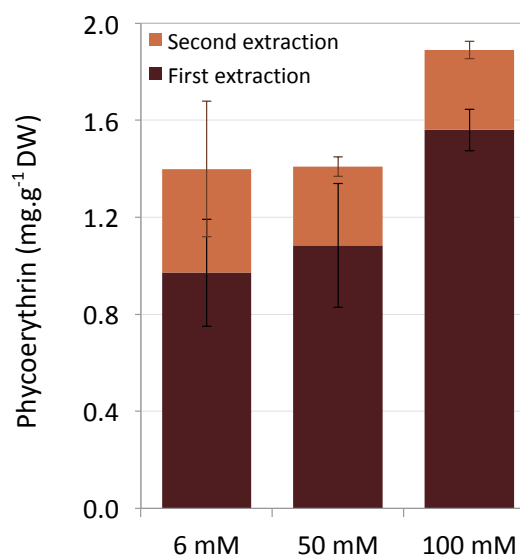
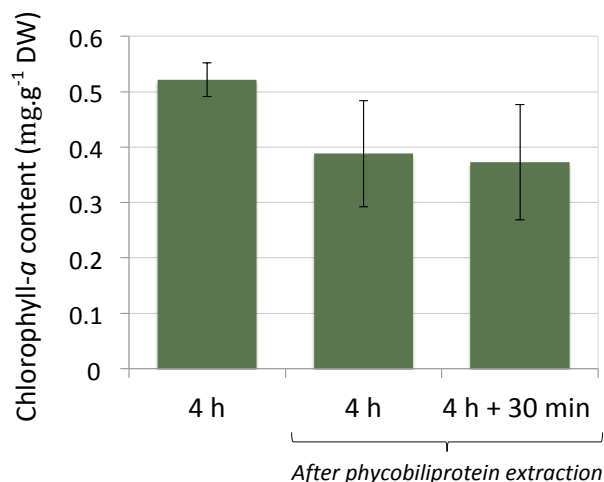


Figure 45. Phycoerythrin content (mg.g<sup>-1</sup> algal DW) of the red macroalga *Chondrus crispus* (sampled on January 2017, Portsall, Brittany, France) according to the concentration of the phosphate buffer (6, 50 or 100 mM). Results are displayed as the stacked cumulative contents of two successive extractions.



addition of a 30 min extraction step for chlorophyll-*a*. Thus the two types of pigments were extracted on separated algal powders.



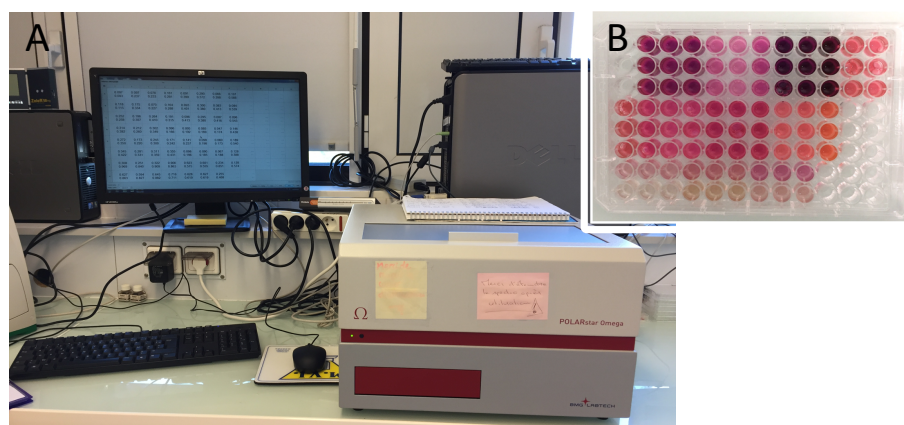
**Figure 46.** Comparison of chlorophyll-*a* content (mg.g<sup>-1</sup> algal DW) of the red macroalga *Chondrus crispus* (sampled on January 2017, Portsall, Brittany, France) obtained after classical extraction (see **section 4.1.1**), or following the extraction of phycobiliproteins carried out on the same powder, with or without an additional 30 min extraction time.

#### 4.2.2 Analysis by spectrophotometry

The samples were placed in a 96-well plate and absorbance was measured using a microplate reader (POLARStar Omega, BMG Labtech) (Fig. 47). The amounts of PE and PC were then estimated using the following equations from **Beer and Eshel (1985)**:

$$[\text{PE}] \text{ (mg.mL}^{-1}\text{)} = [(A_{565} - A_{592}) - (A_{455} - A_{592}) \times 0.20] \times 0.12$$

$$[\text{PC}] \text{ (mg.mL}^{-1}\text{)} = [(A_{618} - A_{645}) - (A_{592} - A_{645}) \times 0.51] \times 0.15$$

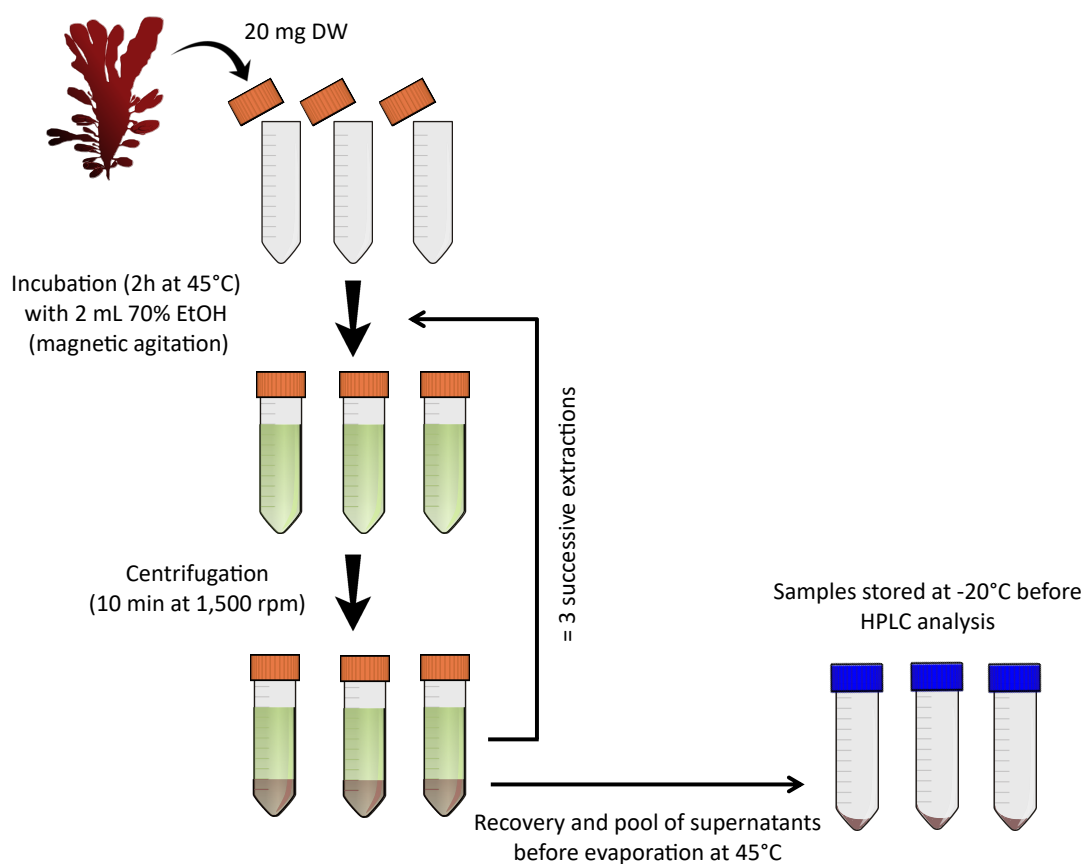


**Figure 47.** Illustrations of (A) the spectrophotometer used (POLARStar Omega, BMG Labtech), with (B) an example of 96-well plate filled with phycobiliprotein extracts. © F. Lalegerie

### 4.3. Mycosporine-like amino acids from red macroalgae

#### 4.3.1 Extraction

Twenty mg DW of finely ground algae were extracted for 2 h with 2 mL of 70 % aqueous ethanol (v/v) at 45 °C with magnetic stirring in the dark. After centrifugation (centrifuge 5810, Eppendorf) at 1,500 rpm for 10 min, the supernatant was recovered. The pellet was re-extracted twice following the same procedure and the combined supernatants were evaporated to dryness under vacuum (miVac, Genevac, France) at 45 °C. The evaporated extracts were then stored at –20 °C before analysis by HPLC (Fig. 48).



**Figure 48.** Schematic representation of the extraction procedure of the mycosporine-like amino acids. © F. Lalegerie

In order to find the best solvent to use for the extraction of MAAs, a pre-test was carried out using *Chondrus crispus* collected in January 2017 in Portsall (Brittany, France). Different extraction solvents were tested according to their use in the bibliography: MeOH 25 %, MeOH 5 %, EtOH 15 %, and EtOH 70 %. All solvents were tested with three successive extractions during 2 h at 45 °C (Fig. 48). In addition, another extraction condition was tested with a single extraction step: 12 h at 4 °C with MeOH 5 % to see the potential effect of

temperature. The different solvents exhibited similar results (Fig. 49), with the exception of MeOH 5 % at 4 °C for 12 h, with which the total MAAs was significantly lower (ANOVA,  $p$ -value = 0.0144). Ethanol was chosen as the extraction solvent because it provided reliable data and this solvent had the advantage to be less toxic than methanol which is commonly used in the literature (e.g. Briani *et al.* 2018; Gröniger *et al.* 2000; Guihéneuf *et al.* 2018; Pandey *et al.* 2017; Yuan *et al.* 2009). Then, EtOH 70 % was chosen over EtOH 15 % because the re-solubilisation and filtration of extracts for HPLC analysis was facilitated.

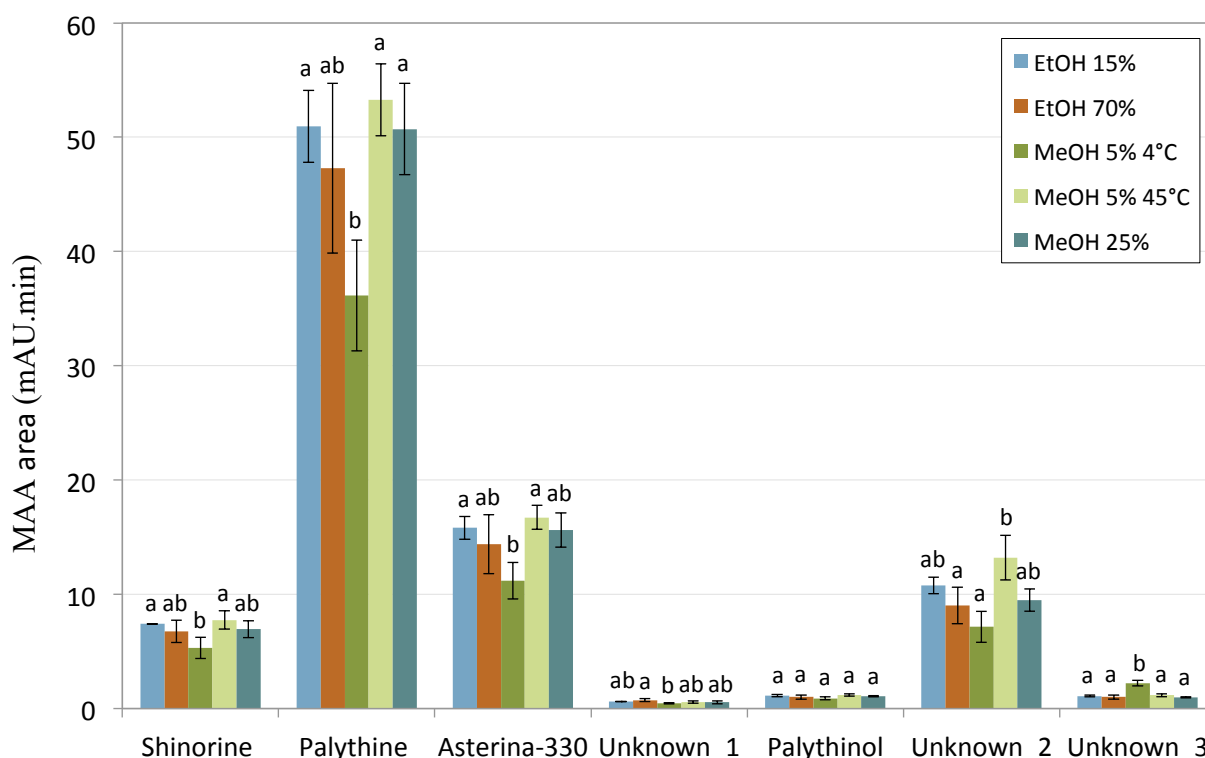
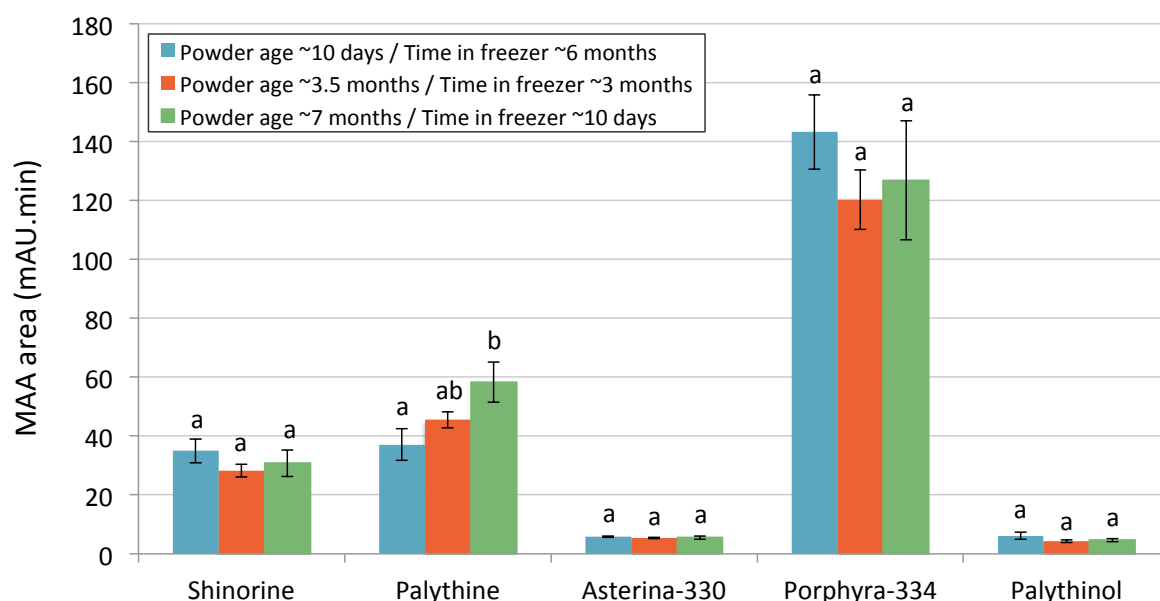


Figure 49. Effect of the extraction solvent and temperature on the different MAAs extracted from *Chondrus crispus* (sampled in January 2017, Portsall, Brittany, France), and analysed by HPLC. Letters indicate the significant differences between the different conditions for each MAA (ANOVA,  $p$ -value > 0.05). Unknown MAAs had a maximum absorption at 320, 356 and 334 nm respectively.

The conservation of MAAs was tested using *Palmaria palmata* harvested in March 2018 at Porspoder and Pointe du diable. Thus, different HPLC analyses were carried out at different times after sampling (*i.e.* extraction 10 days, 3.5 or 7 months after harvesting), and after extraction (*i.e.* storage of extracts for 10 days, 3 or 6 months in the freezer at -25 °C). Results showed no significant difference, except a slight increase in palythine after seven months of conservation of the extracts in the freezer (ANOVA,  $p$ -value = 0.008) (Fig. 50). Thus, the algal powder seems to keep well over time, but the HPLC analyses must be performed within three months after extraction. This result is in accordance with Gröniger and Häder (2000)

who demonstrated that these compounds are highly stable, particularly with respect to extreme pH, elevated temperature, and high irradiances.



**Figure 50.** MAAs composition of *Palmaria palmata* (harvested in March 2018, Porspoder, Brittany, France) according to the time between sampling (*i.e.* powder age) or extraction (*i.e.* time in a freezer), and HPLC analysis. Letters indicate the significant differences between the different times, for each MAA (ANOVA,  $p$ -value > 0.05).

### 4.3.2 Analysis by HPLC

For HPLC analysis, extracts were dissolved in 500  $\mu$ L of 2.5 % aqueous methanol with 0.1 % acetic acid (diluted in MilliQ water), centrifuged for 5 min at 10,000 rpm (miniSpin plus, Eppendorf), and filtered through 0.2  $\mu$ m-pore syringe filters. MAAs analyses were performed using a Dionex Ultimate 3000 HPLC (Thermo Scientific, Germany) equipped with a Diode-Array Detector (DAD) (see **section 4.1.2** for more details). MAA separation was performed using an Agilent (USA)  $C_{18}$  column (Zorbax Eclipse XDB, dimension 4.6  $\times$  250 mm, granulometry 5  $\mu$ m, pore size 80  $\text{\AA}$ , carbon load 10 %, maximal temperature 60  $^{\circ}$ C, pH range 2.0 to 9.0), equipped with a guard-column. For one analysis, 20  $\mu$ L of extract were injected and the operating parameters were as follow: isocratic elution of 0.1 % acetic acid in Milli-Q water as the mobile phase; a flow rate of 1  $\text{mL}\cdot\text{min}^{-1}$ ; a run time of 20 min; a column temperature of  $25 \pm 1$   $^{\circ}$ C; an injector temperature of  $5 \pm 1$   $^{\circ}$ C.

Beforehand, several HPLC columns were tested as well as several solvents in order to optimize the separation of MAAs. Four different solvent conditions were tested (diluted in

MilliQ water): isocratic elution of acetic acid 0.1 % in MeOH 5 %; isocratic elution of acetic acid 0.1 % in MeOH 2.5 %; acetic acid 0.1 % + gradient of MeOH from 0 to 5 %; isocratic elution of acetic acid 0.1 %. Three HPLC columns were tested: Agilent Zorbax Eclipse XDB C18, Agilent Zorbax Eclipse plus Phenyl-Hexyl, and ACE® C18. The analytical method with Zorbax C18 column and isocratic elution of 0.1 % acetic acid was chosen as it gave the best separation of the MAAs (Fig. 51).

HPLC data were collected using Chromeleon 0.7 software (Thermo Scientific Dionex, France). MAAs were detected / analysed at 320, 330, 332, 334 and 360 nm according to the maximum absorption of each MAA. In addition, data analysis was performed with 3D field to allow absorption spectra acquisition between 190 at 400 nm in the event that there may be unknown MAAs at other wavelengths. As there are no MAAs commercial standard available, it was not possible to use a calibration curve to determine the MAA concentrations. In this way, MAAs can be quantified on the basis of their molar extinction coefficients (available in Karsten *et al.* 1998) and their wavelengths of maximum absorption, using the formulas of Pellilo *et al.* (2004). However, these formulas were obtained under different analytical conditions than those used in the present work. Thus, in order to avoid an approximate quantification that could be incorrect, I have chosen to express the results mainly as peak area (mAU.min) of each MAA assigned on the chromatogram. Then, a collaboration with Pr. Ulf Karsten (University of Rostock, Germany), Dr. Ganzera and Dr. Hartmann (University of Innsbruck, Austria) allowed to obtain preliminary quantification results on the basis of standards that they obtained by purification of algal extracts. These preliminary results are not presented in this manuscript since they require more identification and quantification tests using LC-MS.

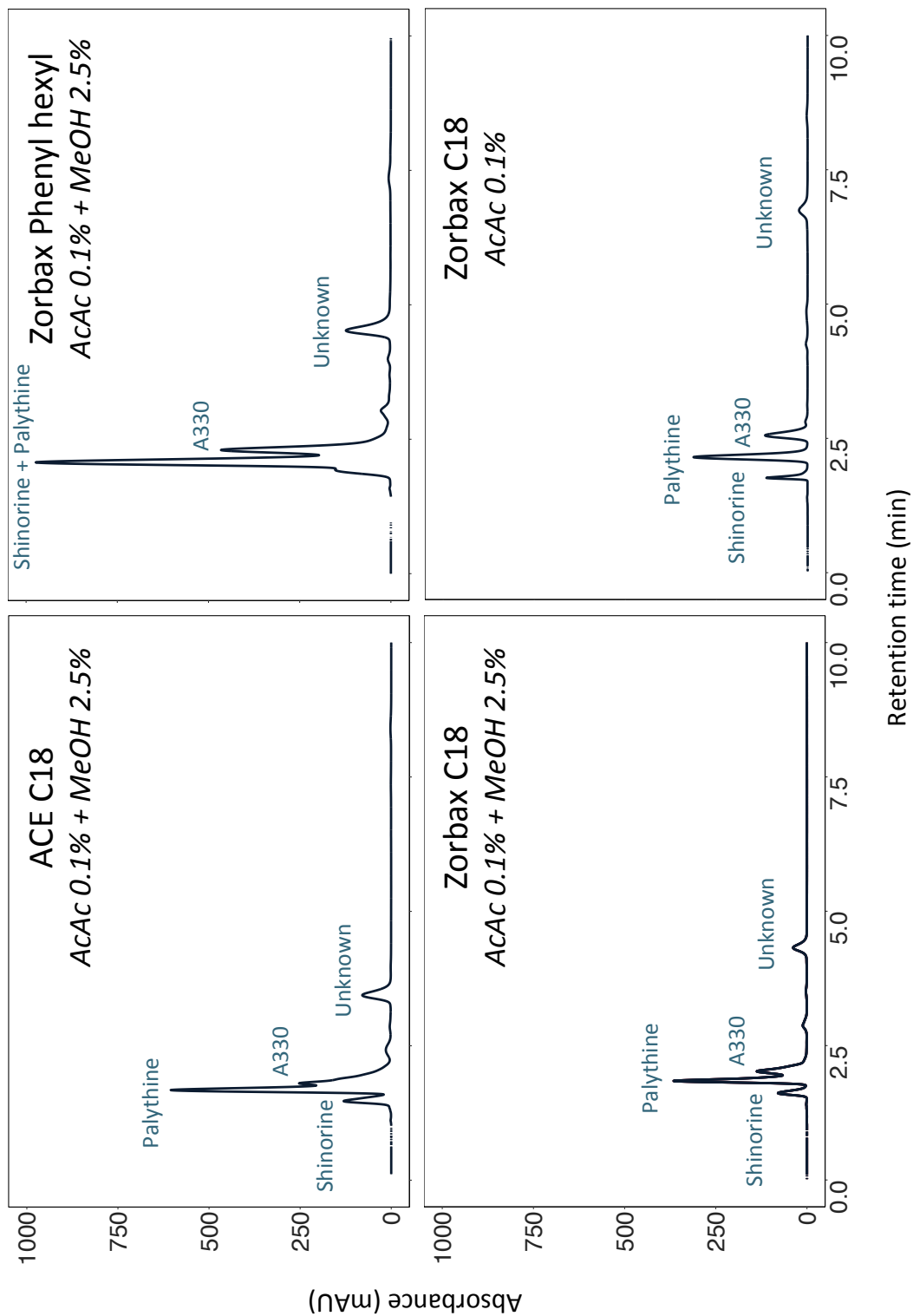
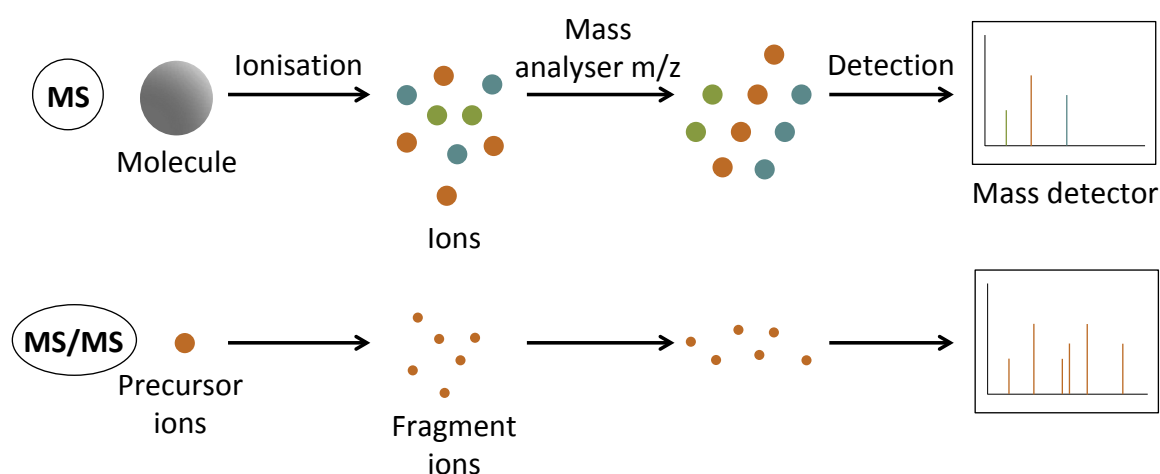


Figure 51. HPLC chromatograms (at 330 nm) of MAAs extracted using MeOH 25 % from *Chondrus crispus* (harvested in January 2017, Portsall, Brittany, France), according to the different columns and two solvent conditions tested, *i.e.* isocratic elution of acetic acid 0.1% in methanol 2.5 % , or isocratic elution of acetic acid 0.1 % .

### 4.3.3 Identification by mass spectrometry

For identification, some MAAs extracts were analysed by LC-MS. Indeed, HPLC analysis can be coupled to a Mass Spectrometer (MS) detector to identify separated molecules by mass measurement. The analysis by spectrometer can be divided into 4 steps: (1) the molecules, previously separated by HPLC are injected into the spectrometer, and (2) fragmented by an ionisation process. Then, (3) the ions (or fragments) are separated in the gas phase according to their mass-to-charge ratio ( $m/z$ ) with a time-of-flight analyser; and (4) a detector measures the abundance of each ion, transforms it into an electric signal, allowing to obtain a mass spectrum, *i.e.* the abundance of each ion as a function of its  $m/z$  ratio at a time  $t$  (Fig. 52). This process can be in positive or negative mode depending on the charge of the ions studied. In positive mode, only positive ions are collected, from which the mass of an  $H^+$  ion is subtracted. Then, if the mass of an ion does not allow for its identification, it can be re-fragmented to obtain an MS/MS (or  $MS^2$ ) spectrum, *i.e.* a fragmentation profile. From the fragmentation profiles, it is possible to obtain additional information of the structure of the molecules. Then, the masses and fragmentation profiles are compared with those available in the bibliography. If the data do not allow identification, a fragment can be fragmented again  $n$  times, to obtain an  $MS^n$  spectrum. There are also different types of mass spectrometers, depending on the type of ionization (*e.g.* electrospray (ESI), or Matrix Assisted Laser Desorption Ionisation (MALDI)), or analysers (*e.g.* Time-of-Flight (TOF) or Orbitrap).



**Figure 52.** Schematic representation of operating principles of a mass spectrometer, functioning in MS or MS/MS mode.

In this way, the same LC method with Dionex Ultimate 3000 and Zorbax C<sub>18</sub> column was used and coupled to an ESI-Q-TOF-MS (Dionex, Ultimate 3000, Bruker, micrOTOF- QII) system (Bruker Daltonik GmbH, Bremen, Germany) at the University of South Brittany (UBS, France), in collaboration with Gilles Bedoux and Laure Taupin (Marine Biotechnology and Chemistry Laboratory LBCM, UBS, France). The LC method was adapted with a flow reduced to 0.5 mL.min<sup>-1</sup>. Then, source parameters were: positive mode; source temperature, 200 °C; capillary voltage, 4.5 kV; nebulizer gas (N<sub>2</sub>) at 2.8 bars and dry gas (N<sub>2</sub>) at 12 L.min<sup>-1</sup>. Mass spectra acquisition was set at 0.5 Hz from m/z 50 to 1000. MS/MS analyses were performed with a collision energy of 30 eV and an isolation width of 2 m/z. All raw data were collected with Compass data Analysis Version 4.1

From 70 % EtOH extracts, six MAAs have been identified by fragmentation: shinorine, palythine, asterina-330 from *Chondrus crispus*, and porphyra-334, usurijene, and palythene from *Palmaria palmata* (Fig. 53). To this end, fragmentation profiles have been compared with those available in bibliography, e.g. in Volkman and Gorbushina (2006), Cardozo *et al.* (2008), Roullier *et al.* (2011), and Shukla *et al.* (2016). In addition, palythanol was identified later (molecular mass = 302 g.mol<sup>-1</sup>) with a LTQ Orbitrap XL (Thermo Fischer Scientific) mass spectrometer at the LEMAR (in collaboration with Sebastien Artigaud), from 70 % EtOH extract of *Palmaria palmata*. Similarly, LC method with Dionex Ultimate 3000 and Zorbax C<sub>18</sub> column was adapted with a flow reduced to 0.4 mL.min<sup>-1</sup>. Then source parameters for MS analysis were: positive mode; source temperature, 300 °C; ESI voltage, 3.9 kV; capillary voltage, 35 kV; sheath gas flow at 6 L.min<sup>-1</sup>. Full scan MS spectra (m/z 100–2000) were acquired in the OrbiTrap with a resolution of 60,000. Normalized collision energy was set to 10 eV with an activation time of 30 ms and isolation width of 2 m/z. From this identification, the individuals peaks of all chromatograms of this PhD work were identified by comparison with the retention times and the absorbance spectra corresponding to the seven identified MAAs (see **Appendix 2 in section 1 of Chapter 2**). For peaks that have not yet been elucidated, their identification was not established and described as unknown MAAs.



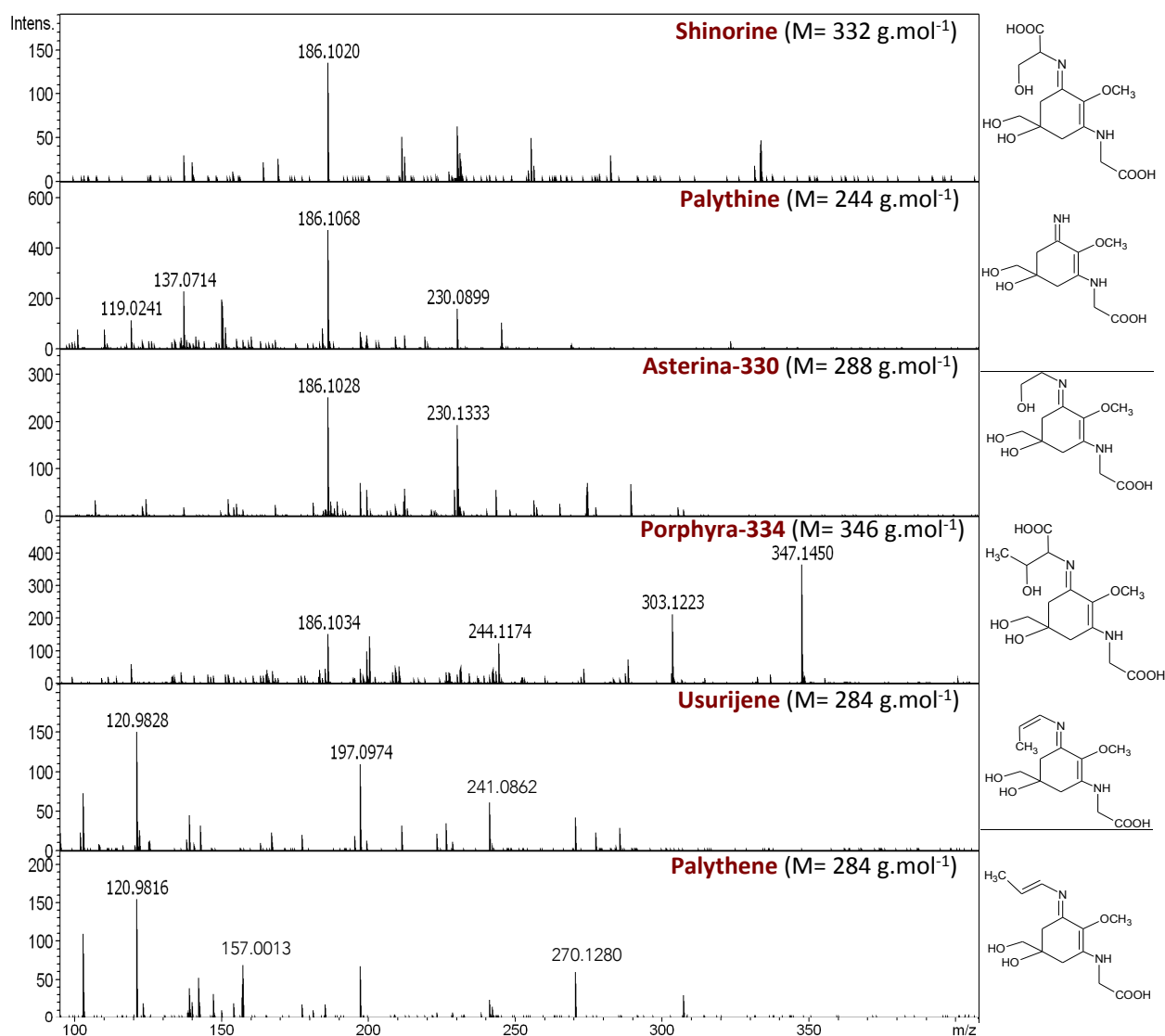


Figure 53. Fragmentation profiles of the six MAAs identified by LC-MS/MS with ESI-Q-TOF detector, with comparison of MS<sup>n</sup> main fragments available in bibliography (Volkman and Gorbushina 2006; Cardozo *et al.* 2008; Roullier *et al.* 2011; Shukla *et al.* 2016).

## 4.4. Fatty acids in red and brown macroalgae

### 4.4.1 Extraction

Fatty acids were extracted by direct transmethylation according to the protocol of Schmid *et al.* (2014), before analysis by Gas-Chromatography (GC). For each sample, 40 mg DW of algal powder underwent extraction with 2 mL of 2 % H<sub>2</sub>SO<sub>4</sub> diluted in MeOH, during 2 h at 80 °C under magnetic stirring and nitrogen atmosphere. Obtained extracts were cooled to

room temperature, and 1 mL of distilled water and 2 mL of 100 % hexane were added. Samples were then vortexed and centrifuged 2 min at 3,000 g (Rotina 38R, Hettich, Germany). The upper layer containing fatty acids was recovered for gas chromatography analysis (Fig. 54a).

The protocol was then adapted as all samples were not analysed on the same analytical platform, and equipment for extraction and requirements for the GC equipment were not the same, *i.e.* initial protocol carried out at the Martin Ryan Institute (National University of Galway, Ireland), and adapted protocol at LIPIDOCEAN platform from the LEMAR (IUEM-UBO, Plouzané, France). The method was modified as follows: samples were incubated during 90 min. After cooling, 2 mL of 100 % hexane and 1.5 mL of distilled water saturated with hexane were added. Samples were centrifuged and the lower aqueous phase has been discarded. This step of rinsing with water was repeated 3 times, before recovering the hexane upper layer for gas chromatography analysis (Fig. 54b). The adapted protocol was used for samples of *Halidrys siliquosa* monitored during one year in France, and for samples from the comparison of *S. muticum* and *H. siliquosa* between France and Ireland populations.

#### 4.2 Analysis by Gas chromatography (GC)

Gas chromatography (GC) is an analytical technique allowing separation, detection and quantification of each compound present in a liquid sample. In this way, the principle of GC is similar to liquid chromatography (described in **sections 4.1.2**), with a gaseous mobile phase. The GC system is then composed of three main components: the injector which vaporizes the sample by heat (*i.e.* conversion of the volatile extraction solvent to vapour); the column which separates the compounds in the mobile phase (*i.e.* gas maintained at an appropriate flow rate), and the detector which detects the different compounds and converts their concentrations in electric signal. Then, the separation of each compound in the mobile phase and the column depends of the affinity of each compound with the internal structure of the column (*i.e.* stationary phase). It results in a chromatogram on which the signal intensity of the different compounds is displayed on the horizontal axis according to their retention time, similarly to an HPLC chromatogram (McNair *et al.* 2019).

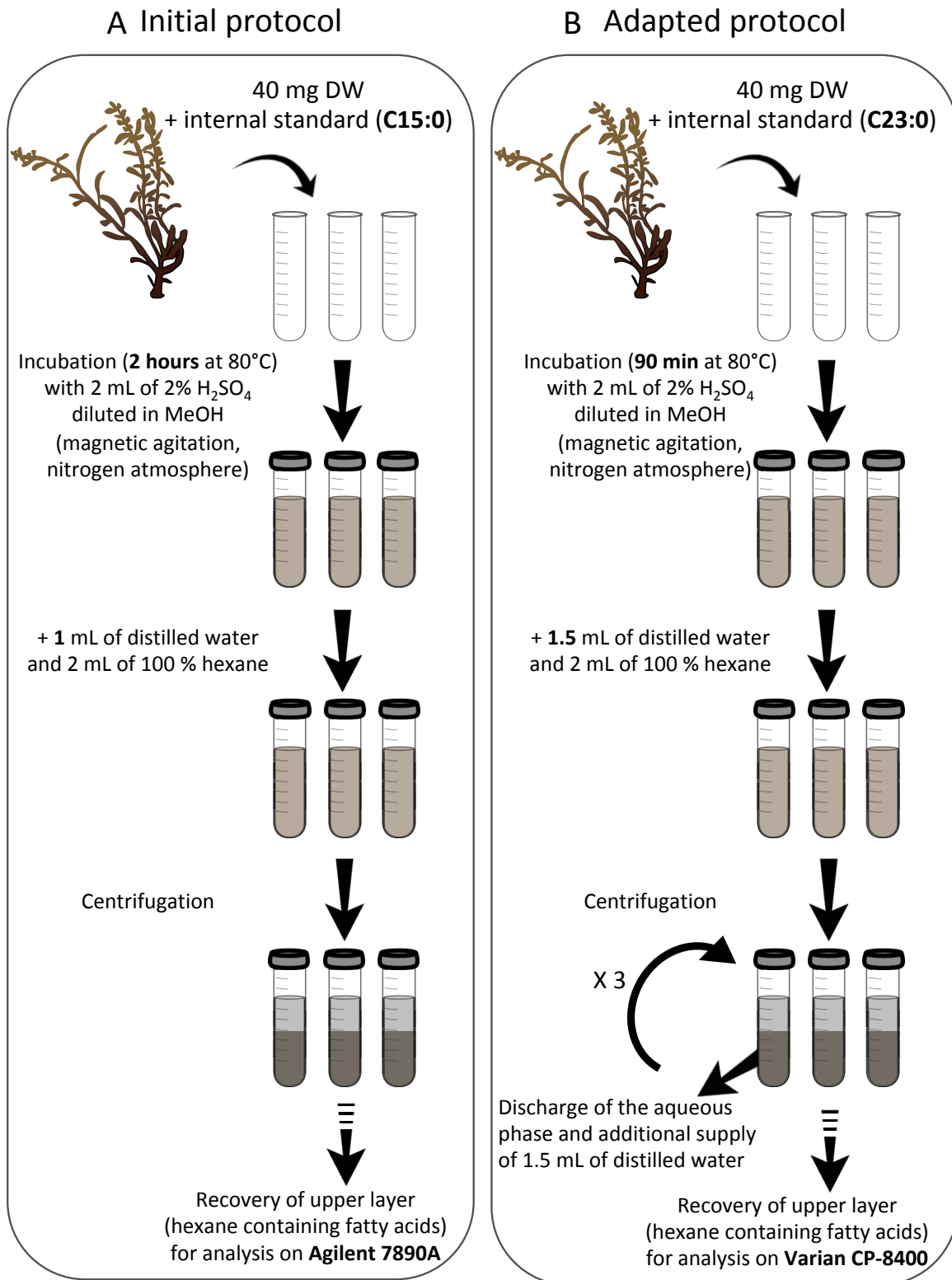


Figure 54. Schematic representation of the extraction procedure of the fatty acids, (A) for the samples of the French monitoring of *Sargassum muticum*, *Bifurcaria bifurcata*, *Palmaria palmata*, and *Grateloupia turuturu*, and (B) for the French monitoring of *Halidrys siliquosa*, and the comparison of *S. muticum* and *H. siliquosa* between France and Ireland. © F. Lalegerie

The fatty acid samples from the initial protocol (Martin Ryan Institute, Galway, Ireland) were analysed by an Agilent 7890A GC / 5975C MSD series equipped with 7583B injector, a flame-ionization detector (FID) and a silica capillary column (DB-Wax, Agilent, 0.25 mm x 30 m x 0.25  $\mu$ m). Hydrogen was used as vector gas at 1.2 mL.min<sup>-1</sup> constant flow, the detector temperature was set at 300 °C, and samples were injected at 250 °C. The temperature profile was then: 140 °C (1 min), then increased by 15 °C.min<sup>-1</sup> to 200 °C, and 2 °C.min<sup>-1</sup> to 250 °C. The fatty acids were then identified using FAME (fatty acid methyl esters) of Fish Oil (Menhaden Oil, Supelco, Bellefonte, PA, USA) and FAME mix (Supelco™ 37 Component FAME Mix, Supelco, Bellefonte, PA, USA); and quantification was made using a known amount of internal standard, *i.e.* pentadecanoic acid (C15:0) added initially to each sample.

In a second part, the fatty acid analysis from the adapted protocol (LIPIDOCEAN platform of the LEMAR) were analysed on a Varian CP-8400 system equipped with a flame-ionization detector. Samples (2  $\mu$ L) were injected on two different columns, one polar (Zebron ZB-Wax, Agilent, 0.25 mm x 30 m x 0.2  $\mu$ m) and one apolar (ZB-5HT, Agilent, 0.25 mm x 30 m x 0.2  $\mu$ m), with hydrogen as vector gas at 2 mL.min<sup>-1</sup> constant flow. The injector and detector temperatures were set at 220 °C and 280 °C, respectively and the oven temperature was programmed according to the following gradient: 0 °C to 150 °C at 50 °C min<sup>-1</sup>, then increased to 170 °C by a rate of 3.5 °C.min<sup>-1</sup>, then to 185 °C by 1.5 °C.min<sup>-1</sup>, to 225 °C by 2.4 °C.min<sup>-1</sup>, and finally increased to 250 °C by 5.5 °C.min<sup>-1</sup> and kept at this final temperature for 15 min. Fatty acids were also identified with a commercial standard mixture (Supelco 37-components FAME Mix, Sigma-Aldrich) and each fatty acid was quantified using tricosanoic acid (C23:0) as internal standard.

## 4.5. Phenolic compounds from red and brown macroalgae

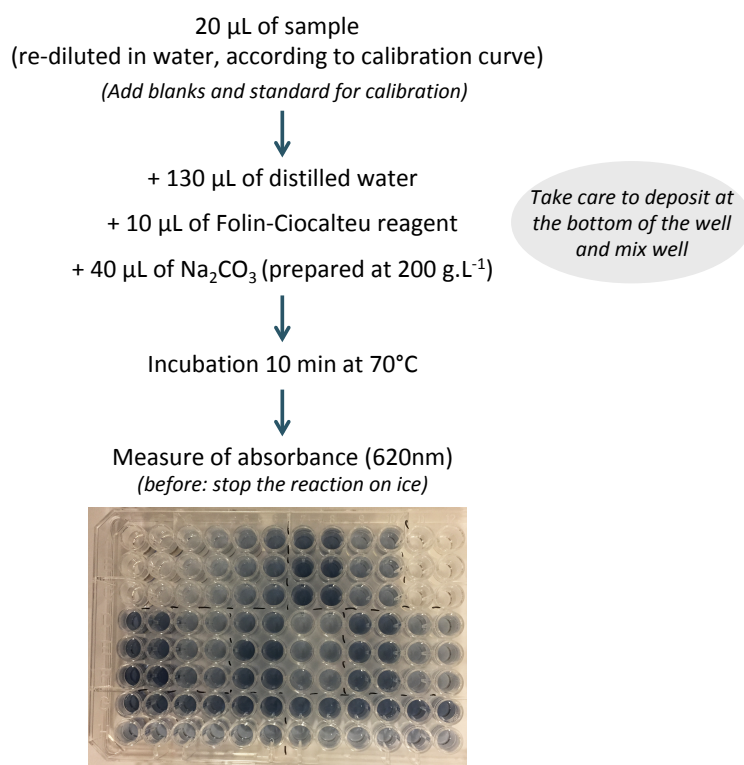
### 4.5.1 Extraction

Phenolic compound were extracted using 15 mg DW of algal powder mixed with 1 mL of 70 % EtOH. The samples were kept in an ultrasonic bath (Sonicator 88155, Fisher Bioblock Scientific, France) during 15 min at 4 °C, and then under magnetic stirring during 2 h at 40 °C in the dark. Samples were then centrifuged for 10 min at 8,000 rpm (Eppendorf Centrifuge 5810, Germany), the supernatant was recovered, and samples were subjected to a second extraction. At the end, supernatants were pooled and evaporated at 40 °C using a centrifugal

concentrator (miVac, Genevac, France). Beforehand the number of successive extractions required was tested: two extractions appeared to be sufficient and was subsequently applied to all analyses (data not shown).

#### 4.5.2 Analysis by spectrophotometry

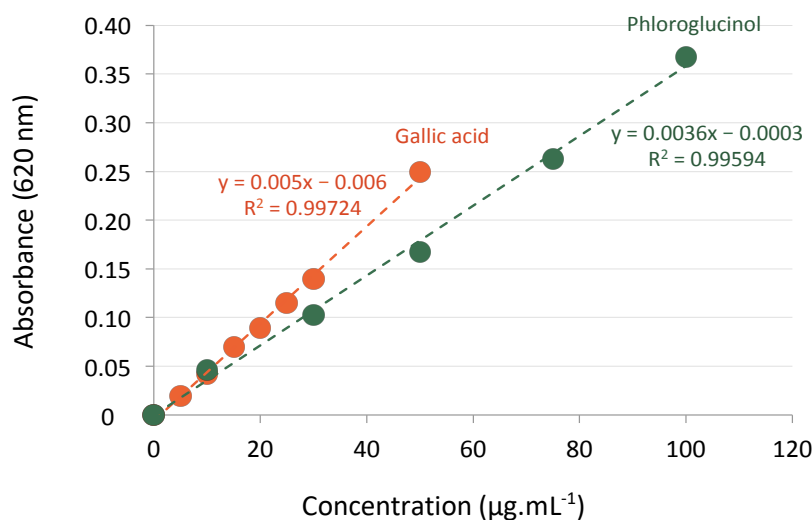
The total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric assay adapted from *Zubia et al. (2009)*. In this way, the wells of the microplates were filled with 20  $\mu\text{L}$  of sample (diluted in distilled water), adding to 130  $\mu\text{L}$  of distilled water, 10  $\mu\text{L}$  of Folin-Ciocalteu reagent and 40  $\mu\text{L}$  of sodium carbonate ( $\text{Na}_2\text{CO}_3$ , 200  $\text{g}\cdot\text{L}^{-1}$ ). Microplates were then incubated for 10 min at 70  $^\circ\text{C}$ , and placed on ice to stop the chemical reaction (Fig. 55).



**Figure 55.** Schematic representation of the quantification procedure of phenolic compounds using the Folin-Ciocalteu assay, according to the protocol of *Zubia et al. (2009)*.

The total phenolic content (TPC) was then determined by measuring the absorbance of each sample (in triplicate) at 620 nm (Multiskan FC, Thermo Scientific, USA) and comparing to a standard curve of phloroglucinol (1,3,5-trihydroxybenzene) for brown macroalgae, or gallic acid for red macroalgae (prepared at 100  $\mu\text{g}\cdot\text{mL}^{-1}$ ) (Fig. 56). In this way, each sample was diluted with distilled water in order to obtain a concentration corresponding to either calibration curves: several dilutions were tested between 0.01 and 2  $\text{mg}$  of  $\text{extract}\cdot\text{mL}^{-1}$ , and

then fixed to 2 mg of extract.mL<sup>-1</sup> for red and 0.25 mg of extract.mL<sup>-1</sup> for brown macroalgae.

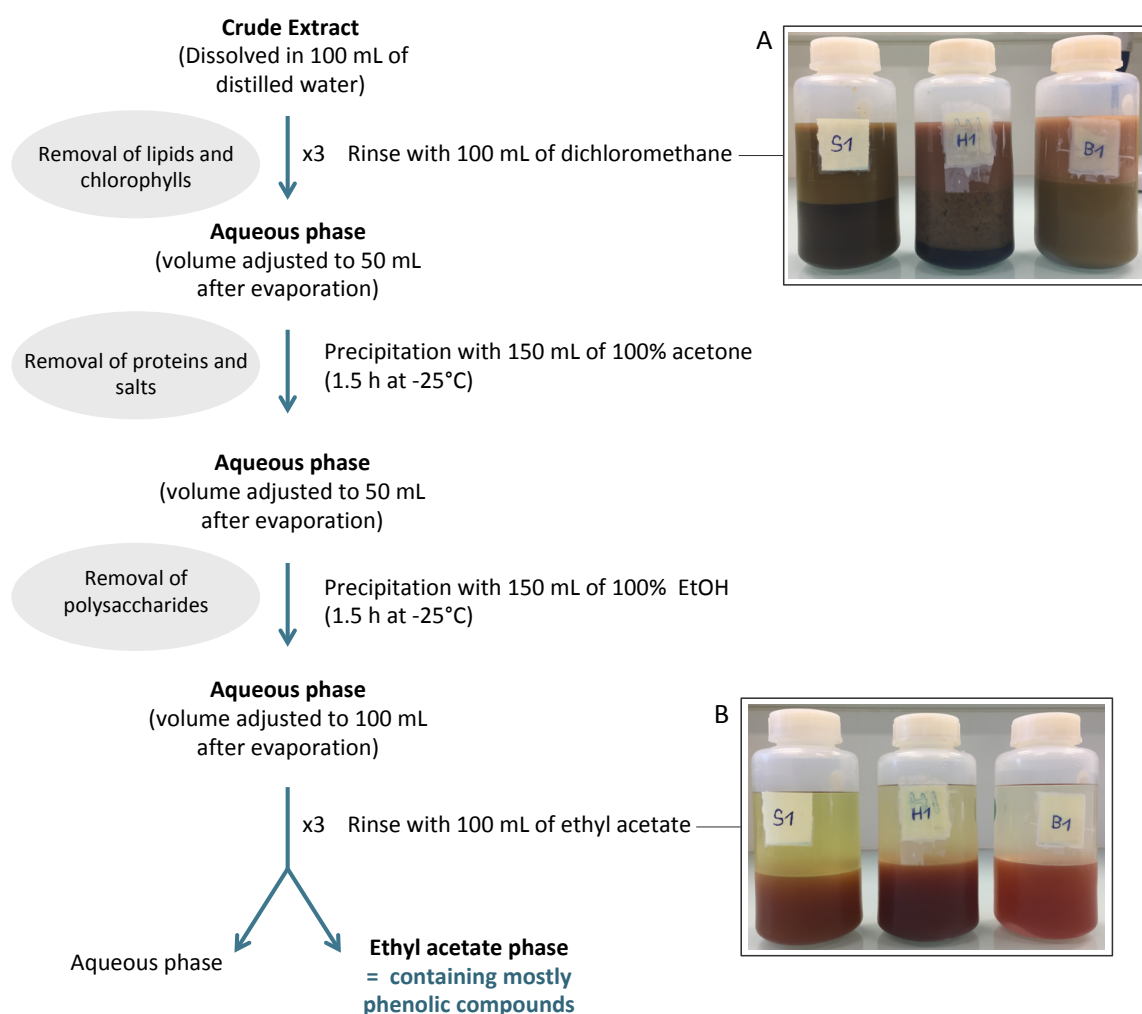


**Figure 56.** Calibration curves of the standards phloroglucinol and gallic acid, used for the quantification using the Folin-Ciocalteu assay of phenolic compounds produced by brown and red macroalgae, respectively.

#### 4.5.3 Purification and analysis of phlorotannins by mass spectrometry and HPLC

The Folin-Ciocalteu assay provided only an approximate quantification of phenolic compounds, and does not provide any information on the nature of these compounds. Then, in order to identify and characterize the structure of the phenolic compounds of the different species studied, a liquid/liquid purification was conducted on some samples and some initial tests were conducted by mass spectrometry (MALDI-TOF) or by HPLC, as exploratory investigations for future identification / characterisation. The results are still not satisfactory as semi-purified fractions still have compounds, other than phenolic compounds (such as fatty acids, amino acids, polysaccharides, etc.), and analytical methods need then to be further developed. Nevertheless, some initial data are presented here but analysis of phenolic compounds by HPLC or mass spectrometer was not used in the rest of the manuscript. These tests were performed using the brown seaweeds monitored, *i.e.* *Bifurcaria bifurcata*, *Sargassum muticum*, *Halidrys siliquosa*, with in addition the Fucaceae *Fucus serratus* Linnaeus 1753, a brown macroalga not studied during my PhD thesis but commonly used in the laboratory and containing bigger phlorotannins compared to Sargassaceae species (Ragan and Glombitza 1986).

Liquid-liquid extraction is a method used to separate compounds, based on their relative solubility in two different non-miscible solvents. Phlorotannins are usually extracted using water (polar) or aqueous ethanol, and then concentrated in an ethyl acetate/water mixture (less polar) (Ragan and Glombitza 1986; Stiger-Pouvreau *et al.* 2014; Creis *et al.* 2018). In this way, 15 g DW of algal powder were extracted with 150 mL of 50 % ethanol, under agitation (Julabo GmbH SW22, Germany) at 40 °C during 2 h. Samples were then centrifuged to recover the supernatant. The pellet was subjected to two additional similar extractions with 150 mL of 50 % ethanol during 1 h. Then, the supernatant was evaporated at 40 °C (Heidolph Rotary Evaporator Laborator 4000 efficient, Germany). The crude extract was then subjected to purification with dichloromethane and ethyl acetate according to Stiger-Pouvreau *et al.* (2014) (Fig. 57).

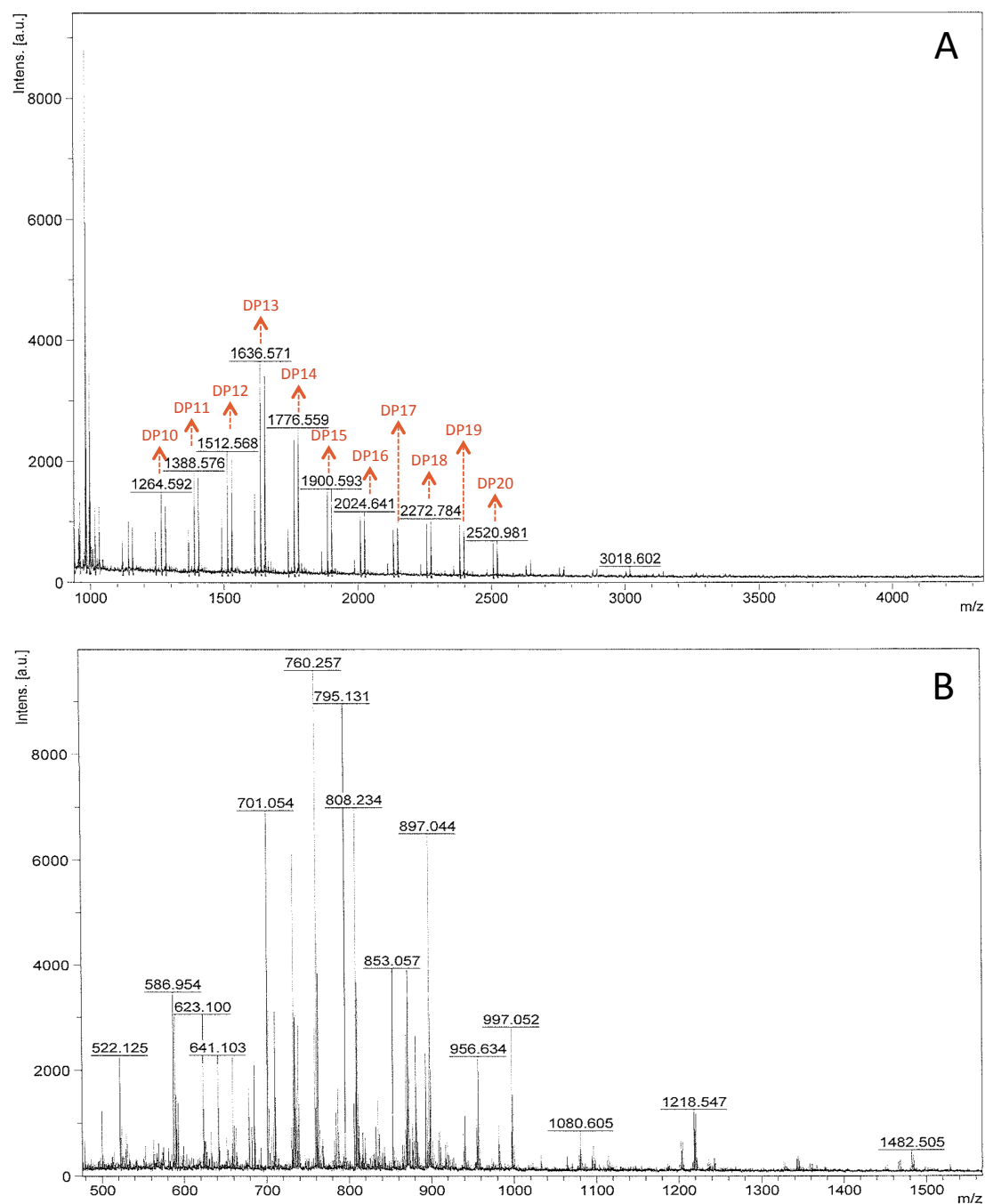


**Figure 57.** Schematic representation of the liquid/liquid purification procedure of phenolic compounds, according to the protocol of Stiger-Pouvreau *et al.* (2014). Illustrations correspond to (A) the first rinse with dichloromethane, and (B) third rinse with ethyl acetate of extracts of *Sargassum muticum* (S1), *Halidrysa siliquosa* (H1) and *Bifurcaria bifurcata* (B1).

Ethyl acetate fractions were then analysed by mass spectrometer (see **section 4.3.3** for description), without preliminary separation by HPLC. Studies were performed on a spectrometer MALDI-TOF (Matrix Assisted Laser Desorption Ionisation-Time Of Flight) BRUKER (Daltonics, Bruker Autoflex III SmartBeam) at the Mass Spectrometry platform of the University (UBO) in collaboration with Nelly Kervarec. It was equipped with a laser under nitrogen, at 337 nm, at about 20 kV, in positive mode by reflectron. The signals were recorded between 400 and 4,420 Da, with about 4,000 shots. The system was calibrated by Bruker tryptic-digested BSA (Bovine Serum Albumin) MS standard. After several tests to develop the method according to preliminary experiments and bibliography (Vissers *et al.* 2017; Le Lann *et al.* 2016), the best analyses conditions so far have been obtained with a DHB matrix (2,5-dihydroxybenzoic acid, signal at 273 Da), prepared on the day of experiment to avoid any degradation. It was prepared at 10 mg.mL<sup>-1</sup> in TA30 (ACN : H<sub>2</sub>O 30 : 70, v : v). Then samples were prepared from 2 mg of purified dry extract solubilized in 1 mL of MeOH. Each sample (30 µL) were then mix with 30 µL of matrix (ratio 1 : 1). The mix was homogenized and deposited (2 x 1 µL) on MTP384 target (Bruker Daltonics).

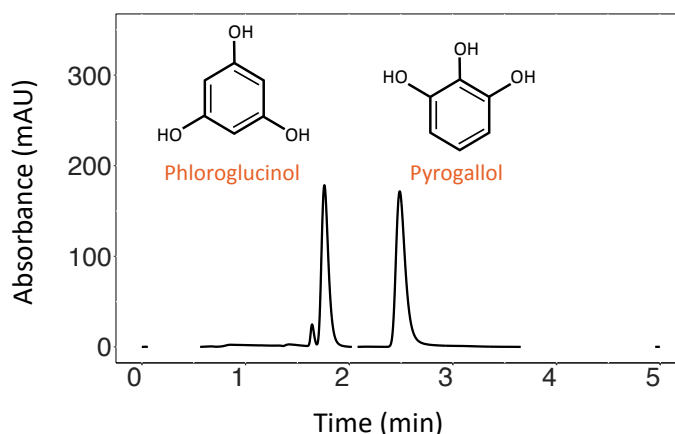
One mass spectrum from a purified extract from *Fucus serratus* presents interesting preliminary results and is shown in Figure 58a. Indeed, the mass of phloroglucinol, *i.e.* the monomer of phenolic compounds (see section 2.3.4 of the general introduction), is 126 Da, and corresponds to one degree of polymerization (DP1). Then, in *Fucus serratus*, oligomers with a degree of polymerization between DP10 (1,264 Da) and DP20 (2,520 Da) were observed. Then, the mass difference between the peaks or different DP was 124 Da, which corresponds to the mass of phloroglucinol less two hydrogen (H) atoms, characteristic of a covalent bond. Only the mass difference between DP13 and DP14 was of 140 Da, which could correspond to phloroglucinol with H<sub>2</sub>O. Similarly, ethyl acetate semi-purified extract obtained from *Bifurcaria bifurcata* was also analysed by mass spectrometer. However, despite peaks between 522 and 997 Da, results did not allow to observe oligomers which requires to adapt the method for smaller phenolic compounds (Fig. 58b).





**Figure 58.** Mass spectra of ethyl-acetate semi-purified fractions/extracts from two brown macroalgae, (A) *Fucus serratus* (Fucaceae, Fucales) and (B) *Bifurcaria bifurcata* (Sargassaceae, Fucales) (harvested in January 2020 at Corn ar Gazel, Saint Pabu, Brittany, France; purified in February 2020), analysed by MALDI-TOF in June 2020.

In parallel, HPLC analyses were conducted in order to separate phenolic compounds, and then identify PC by comparison with standards such as phloroglucinol or pyrogallol (Fig. 59), or by coupling this separation with a mass spectrometer detector.



**Figure 59.** HPLC chromatograms (with a detection at 270 nm) of two standards of phenolic compounds found in brown macroalgae, *i.e.* phloroglucinol and pyrogallol (prepared at 1 mg.mL<sup>-1</sup>, 6 μL injected), with their chemical structures.

HPLC analyses were performed using a Dionex Ultimate 3000 HPLC (Thermo Scientific, Germany) equipped with a diode-array detector (DAD) (see **section 4.1.2** for more details). Separation was done using a Waters XSelect HSS PFP XP column (dimension 3 × 150 mm, granulometry 2.5 μm, pore size 100 Å, carbon load 7 %, maximal temperature 60 °C, pH range 2.0 to 8.0), equipped with a guard-column. After method development according to [Kirke \*et al.\* \(2017\)](#), the optimal parameters so far were as follow: gradient elution of (A) H<sub>2</sub>O + formic acid 0.1% and (B) acetonitrile + formic acid 0.1%; a flow rate of 0.7 mL.min<sup>-1</sup>; a run time of 15 min; a column temperature of 32 ± 1 °C; an injector temperature of 5 ± 1 °C; detection between 200 and 300 nm. The solvent gradient was first tested at 66, 40 and 28 min, before being reduced at 16 min: 0 min 99.5 : 0.5 (A : B, v : v), 1 min 99.5 : 0.5 (A : B, v : v), 10 min 70 : 30 (A : B, v : v), 11.5 min 10 : 90 (A : B, v : v), 13 min 99.5 : 0.5 (A : B, v : v), 15 min 99.5 : 0.5 (A : B, v : v). Crude extracts, semi-purified ethyl acetate fractions and standards were diluted and filtered through 0.2 μm-pore syringe filters before injection.

This method allowed a good detection and separation of phloroglucinol and pyrogallol (Fig. 59). Then, calibration curves for phloroglucinol and pyrogallol were obtained through the injection of different amounts of both standards (data not shown). The analysis of semi-purified ethyl acetate fractions resulted in chromatograms with many peaks in contrast to the analysis of crude extracts (Fig. 60). These preliminary tests are promising but require further development and verification of peak composition (*i.e.* presence of phenolic compounds) by coupling the HPLC system with a mass spectrometer detector.

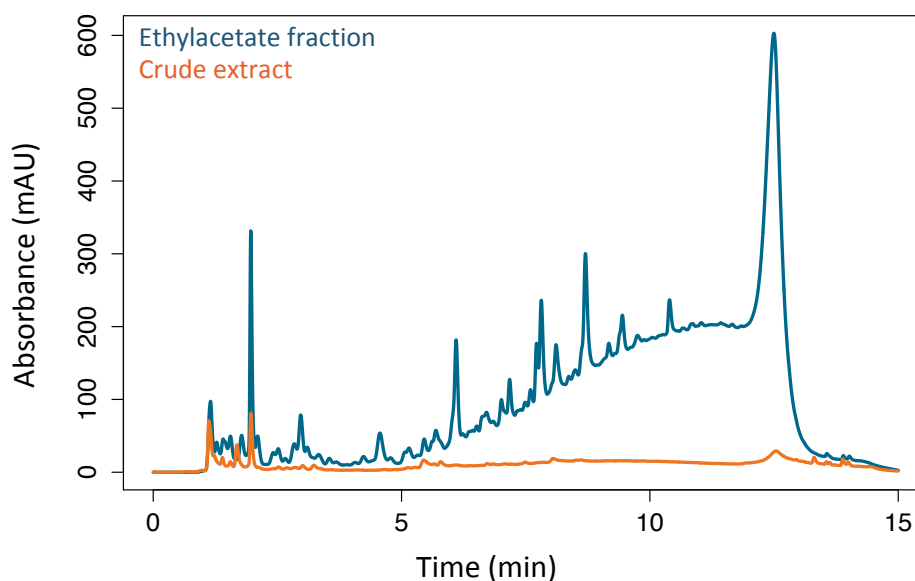


Figure 60. HPLC chromatogram (at 270 nm) of a semi-purified ethyl acetate fraction and the corresponding crude extract (prepared at 5 mg.mL<sup>-1</sup>, 20 µL injected) of *Sargassum muticum*.

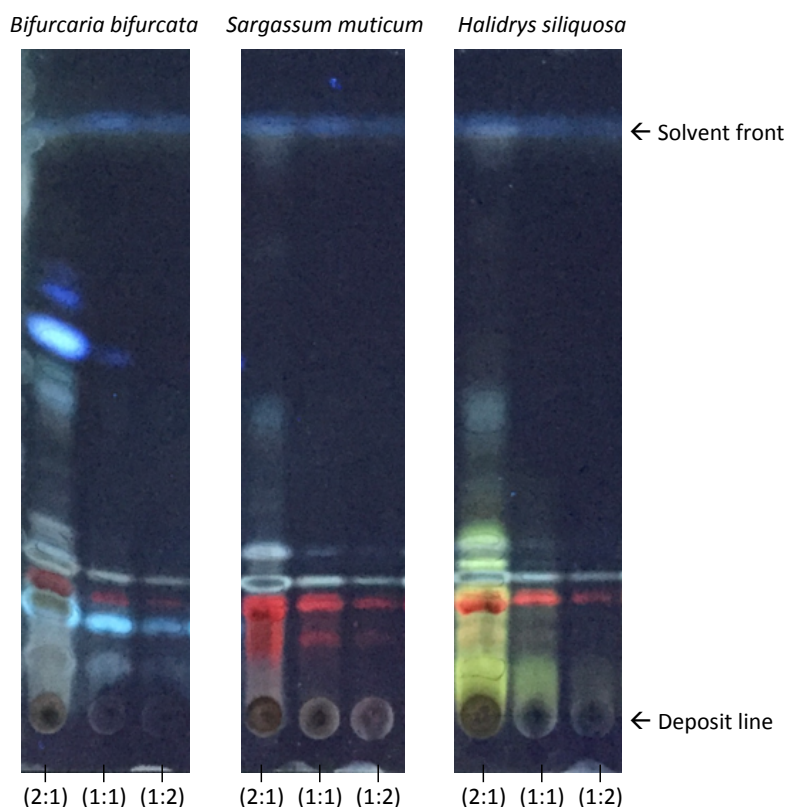
## 4.6. Terpenes from brown macroalgae

### 4.6.1 Extraction

Terpenes were extracted by maceration and analysed by thin layer chromatography (TLC). Thus, 4 mL of a mixture of dichloromethane : methanol (DCM : MeOH, 2 : 1, v : v) were added to 200 mg of seaweed powder for 15 min in an ultrasound bath in the dark, according to a protocol adapted from *Le Lann et al. (2014)*. Then extracts were centrifuged 10 min at 2,500 rpm. The supernatants were recovered, and a second extraction was carried out in the same way. Finally, both supernatants were combined, evaporated (miVac, Genevac), and stored at -80 °C before analysis.

In order to optimize the extraction method, three ratios of solvents DCM : MeOH have been tested beforehand: 2 : 1, 1 : 1 and 1 : 2 (v : v). In addition, four different extraction times were tested: 15 min ultrasound; 15 min ultrasound + 1 h maceration; 30 min ultrasound; 30 min ultrasound + 1 h maceration. Extraction with 15 min of ultrasound was chosen for the analyses since no difference was observed between the different extraction times. Then, the ratio DCM : MeOH (2 : 1) allowed to visualize more spots on the TLC, and was therefore chosen for analyses (Fig. 61).

Figure 61. Thin layer chromatography (exposed to UV light at 365 nm, after revelation using an agent specific to lipid compounds) of terpenes of the three species of brown macroalgae (harvested in May 2018 in Porspoder, Brittany, France), according to different ratios of DCM : MeOH (v : v): 2 : 1, 1 : 1, 1 : 2.



#### 4.6.2 Analysis by thin-layer chromatography (TLC)

Terpene samples were analysed by thin-layer chromatography (TLC). TLC is a technique used to separate different compounds in a mixture: samples are deposited on a TLC plate composed of silica (TLC Silica gel 60 with fluorescence indicator F<sub>254</sub>, Merck), corresponding to the stationary phase. Then, the plate is brought into contact with a solvent mixture (*i.e.* eluent, corresponding to the mobile phase), which moves up the plate by capillary action, carrying the sample mixture. The different compounds of the sample are then eluted at different levels on the plate and separated depending on their affinity with the stationary phase.

In this way, the dried extracts were dissolved in 1 mL of DCM : MeOH 2 : 1 (v : v). Then, 30 µL of each sample were deposited on the plate, according to a protocol adapted from [Le Lann \*et al.\* \(2014\)](#). Elution was performed using a mixture of hexane : diethyl ether : glacial acetic acid 80 : 20 : 2 (v : v : v). To get a better separation, 500 µL of 100 % MeOH was added in the mobile phase just before migration. After migration, the plates were observed with UV light at 365 and 254 nm. In a second step, each plate has been stained with a mixture

of copper sulphate (3 g), orthophosphoric acid (8 mL) and distilled water (92 mL) (revealing agent, which is specific to lipid compounds) to allow the visualization of additional apolar compounds, according to *Le Lann et al. (2014)*. The plates were then dried for 15 min in an oven at 100 °C before a second reading under UV light. Then, for each spot, the retardation factor ( $R_f$ , *i.e.* the ratio of the distance travelled by the centre of the spot to the distance of the solvent front) was measured, in order to compare the profiles between the different TLC plates.

A test was performed with or without the addition of a filtration step of the crude extract prior to TLC analysis, which may influence the elution of compounds. Nevertheless, no difference was visualised with and without filtration, so this additional step has not been maintained (data not shown). Also, purification tests (by solid phase extraction, SPE) and analyses of terpenes by nuclear magnetic resonance (NMR) and HPLC have been carried out in order to characterize and/or identify the different terpenes, but these last identification tests are not presented in the thesis manuscript as they have not been successful so far.

## *Chapter 2*

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High metabolomic diversity in red macroalgae: key components in the future adaptation of native species to global change?

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Case study on *Palmaria palmata*



## *Foreword to chapter 2*

Red macroalgae represent a very diverse group of species known for their richness in secondary metabolites. In this regard, some of these metabolites could have an active role in the future adaptation of red algae to global change. More particularly, mycosporine-like amino acids are secondary metabolites widely encountered in red macroalgae. It has then already been shown that these compounds are involved in photoprotection, notably against UV radiation. However the other roles and synthesis pathway of these understudied compounds have not yet been fully elucidated. In this regard, this chapter consists of four publications on red macroalgae, with a particular interest on *Palmaria palmata*. The last part consists of a comparison of the acclimation abilities of the native *P. palmata*, with the introduced *Grateloupia turuturu*, usually described as invasive in Brittany and which possesses potentially a different eco-metabolomic strategy. An abstract summarising the main results is available at the beginning of each publication, and a conclusion of Chapter 2 is available on page 230.

### **(1) Is there a large metabolic variation between species that would indicate differences in their adaptation?**

→ **PUBLICATION** [Lalegerie F., Lajili S., Bedoux G., Taupin L., Stiger-Pouvreau V., Connan S. (2019) Photo-protective compounds in red macroalgae from Brittany: Considerable diversity in mycosporine-like amino acids (MAAs). *Marine Environmental Research*, 147, 37-48]

### **(2) Could MAAs have an important role in the future adaptation of red macroalgae to climate change?**

→ **PUBLICATION** [Lalegerie F., Stiger-Pouvreau V., Connan S. (2020). Temporal variation in pigment and mycosporine-like amino acid composition of the red macroalga *Palmaria palmata* from Brittany (France): hypothesis on the MAA biosynthesis pathway under high irradiance. *Journal of Applied Phycology*, 32, 2641-2656]

### **(3) MAAs: key compounds in the adaption to changes in light and nutrients?**

→ **PUBLICATION** [Lalegerie F., Stiger-Pouvreau V., Connan S. (In prep.). Research note - A cultivation experiments on the red macroalga *Palmaria palmata*: effect of UV radiation and nutrients on mycosporine-like amino acids and pigments]

### **(4) Do introduced species have a better adaptability and represent a threat to native species?**

→ **PUBLICATION** [Lalegerie F., Stiger-Pouvreau V., Zanolla M., Stengel D., Connan S. (In prep.) Phenological and metabolomic study of *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) 30 years after its introduction in Brittany (France): a real threat for the native species *Palmaria palmata*?]

## Photoprotective compounds in red macroalgae from Brittany: Considerable diversity in mycosporine-like amino acids (MAAs)

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Publié dans *Marine Environmental Research* 147, 37-48

### RESUME

Pour faire face aux stress biotiques et abiotiques subis dans leur environnement, les macroalgues marines ont développé certains mécanismes de défense, notamment la synthèse de molécules photoprotectrices contre la lumière et les rayons UV particulièrement nocifs. L'objectif de cette étude était de cribler certaines algues rouges, un groupe phylogénétique très diversifié, pour la production de molécules photoprotectrices. La teneur et la composition en pigments (*i.e.* chlorophylle-*a*, phycobiliprotéines et caroténoïdes) et la composition des acides aminés de type mycosporine (MAAs) ont été étudiées chez 40 espèces de macroalgues rouges collectées en Bretagne (France), à deux périodes distinctes (*i.e.* février et juillet 2017). Une forte variabilité interspécifique a été démontrée en termes de contenu pigmentaire et de composition en MAAs. Vingt-trois MAAs potentiels ont été détectés par HPLC, et six ont été identifiés par LC-MS (*i.e.* shinorine, palythine, asterina-330, porphyra-334, usuriène et palythène). Il s'agit de la première étude à faire état de la composition en pigments et en MAAs dans un groupe diversifié d'algues rouges de Bretagne, incluant des espèces pour lesquelles la composition en MAAs n'avait jamais été étudiée auparavant. Néanmoins, les résultats suggèrent que certaines espèces d'algues rouges sont plus susceptibles de faire face à des niveaux élevés de rayonnement lumineux puisque des espèces telles que *Bostrychia scorpioides*, *Porphyra dioica*, *Agarophyton vermiculophyllum* (previously *Gracilaria vermiculophylla*) et *Vertebrata lanosa* vivent dans des environnements exposés à des niveaux plus élevés de rayonnement, et possèdent divers MAAs en plus de pigments photoprotecteurs.





## Photo-protective compounds in red macroalgae from Brittany: Considerable diversity in mycosporine-like amino acids (MAAs)



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### ARTICLE INFO

#### Keywords:

Algae  
Diversity  
HPLC  
MAAs  
Metabolites  
Photo-protection  
Pigments  
Rhodophyta  
Screening

### ABSTRACT

To cope with the biotic and abiotic stresses experienced within their environment, marine macroalgae have developed certain defence mechanisms including the synthesis of photo-protective molecules against light and particularly harmful UV radiation. The aim of this study was to screen selected red algae, a highly diverse phylogenetic group, for the production of photo-protective molecules. The pigment content and composition (i.e. chlorophyll-*a*, phycobiliproteins and carotenoids) and the composition of mycosporine-like amino acids (MAAs) were studied in 40 species of red macroalgae collected in Brittany (France), at two distinct periods (i.e. February and July 2017). A high inter-specific variability was demonstrated in terms of pigment content and MAA composition. Twenty-three potential MAAs were detected by HPLC, and six were identified by LC-MS (i.e. shinorine, palythine, asterina-330, porphyra-334, usurijene and palythene). This is the first study to report on the composition of pigments and MAAs in a diverse group of red seaweeds from Brittany, including some species for which the MAA composition has never been studied before. Nevertheless, the results suggested that some species of red algae are more likely to cope with high levels of light radiation since those species such as *Bostrychia scorpioides*, *Porphyra dioica*, *Gracilaria vermiculophylla* and *Vertebrata lanosa* are living in environments exposed to higher levels of irradiation, and had various MAAs in addition to their photo-protective pigments.

### 1. Introduction

Macroalgae are known to play a key role in coastal ecosystems as being at the basis of the food web and providing refugium for many animal species (Lüning, 1990). In particular, seaweeds produce diverse compounds under natural conditions, which could potentially be used in health, cosmetic, or food sectors (Holdt and Kraan, 2011; Stengel et al., 2011; Stiger-Pouvreau and Guérard, 2018; Surget et al., 2017). However, both the concentration and composition of these bioactive compounds can fluctuate within individuals, with their habitat and with season. In the context of climate change, the environmental parameters impacting particularly intertidal seaweeds are in a state of flux. To cope with changing environmental conditions, seaweeds have developed particular adaptive, metabolic responses and are thus able to resist and adapt to different abiotic stresses (Davison and Pearson, 1996; Gantt, 1990).

Irradiance is one of the factors which can change over the intertidal range in coastal areas, in term of quantity and quality because it varies during the day and with the tide, the turbidity of the water, the climate,

the depth or seasons (Sagert et al., 1997). Light is also very important for seaweeds as algae are photosynthetic organisms (Gantt, 1990; Lobban and Harrison, 1994) with some living in the intertidal zone and thus exposed directly to the sunlight during emersion phases. One of the consequences of climate change is the amount of ultraviolet (UV) radiation reaching the Earth's surface (Bischof et al., 2006; Thomas et al., 2012). UV radiations are notably at the origin of DNA alterations, the synthesis of reactive oxygen species (ROS) and, in seaweeds, the photo-inhibition mechanism or the degradation of photosynthetic pigments (Karentz, 2001; Karsten, 2008; Van de Poll et al., 2001). However, due to human activities and ozone depletion, the protective filter that constitutes the atmosphere gradually loses its effectiveness, which means that the amount of UV to which the organisms are exposed tends to increase (Carreto et al., 2018; McKenzie et al., 2007). Although some authors agree that the ozone layer will probably not fully recover before several decades, the hole in this layer would be resorbing at mid-latitudes due to the implementation of some policies tending to reduce greenhouse gas emissions, but this would not be the case at high latitudes and the evolution of the UV level remains complex (McKenzie

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<https://doi.org/10.1016/j.marenvres.2019.04.001>

Received 14 December 2018; Received in revised form 29 March 2019; Accepted 1 April 2019

Available online 06 April 2019

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et al., 2011; Wilmouth et al., 2018).

Different types of macroalgae have developed various strategies to protect themselves from harmful UV radiation. These include the synthesis of photo-protective molecules (Bhatia et al., 2011; Le Lann et al., 2016; Rastogi et al., 2010; Sinha et al., 2007). Among the algal photo-protective compounds, this study focuses on pigments and mycosporine-like amino acids (MAAs), synthesized by red algae (Rhodophyta). MAAs were discovered in 1965 in the terrestrial fungus *Ascochyta pisi* (Leach, 1965). Since then, the presence of MAAs has been demonstrated in a wide range of marine organisms from bacteria to fish (Bandaranayake, 1998; La Barre et al., 2014; Rosic et al., 2015), including different species of algae (Bedoux et al., 2014; Sinha et al., 2007). MAAs are low molecular weight molecules (< 400 Da), soluble in water, with a high molar extinction coefficient (between 28,000–50,000 M<sup>-1</sup> cm<sup>-1</sup>) and a maximum of absorption ( $\lambda_{\text{max}}$ ) between 310 and 362 nm (Rastogi et al., 2010). In addition, it has been demonstrated that red algae, which constitute a well-marked phylogenetic group, have the greatest diversity and the highest proportion of MAAs (Carreto and Carignan, 2011; Karentz, 2001; Sinha et al., 2007). Until now, about 20 MAAs have been identified in various red macroalgae (Sinha et al., 2007). However, as some recent studies have shown (Briani et al., 2018; Hartmann et al., 2016), many MAAs have yet to be identified. The major role of MAAs in photo-protection against UV radiation has already been widely demonstrated (Bedoux et al., 2014; Huovinen et al., 2004; Singh et al., 2008), and functionality could be linked to their individual structures (Woolley et al., 2018). It appears that some MAAs may also have various functions such as anti-oxidants (De la Coba et al., 2009; Torres et al., 2018; Wada et al., 2015), osmolytes, nitrogen storage, or protective agents against desiccation or temperature variations (Bhatia et al., 2011; Oren and Gunde-Cimerman, 2007).

Pigments are part of a second significant group of photo-protective compounds in macroalgae. There are three main families of pigments: chlorophylls, carotenoids and phycobiliproteins (reviewed in Stengel et al., 2011). They do not all have a role in photo-protection, such as chlorophylls and phycobiliproteins which harvest light for photosynthesis. Among the chlorophylls, there is only chlorophyll-*a* in red algae (Stengel et al., 2011). One characteristic of red algae is the presence of phycobiliproteins (reviewed by Dumay et al., 2014). These are “secondary pigments” which also capture solar energy and transfer it to chlorophyll *a* for the photosynthesis; they are particularly effective under low light conditions (Gantt, 1990; Lobban and Harrison, 1994). There are 3 main groups of phycobiliproteins in the Rhodophyta: phycoerythrin (PE,  $\lambda_{\text{max}}$  = 540–570 nm) which provides their red colour, phycocyanin (PC,  $\lambda_{\text{max}}$  = 610–620 nm) and allophycocyanin (APC,  $\lambda_{\text{max}}$  = 650–655 nm) which both have a blueish hue (Dumay et al., 2014). Finally, there are two groups of carotenoids: carotenes ( $\alpha$ -carotene,  $\beta$ -carotene in red algae) and xanthophylls (i.e. zeaxanthin, antheraxanthin, violaxanthin and lutein in red algae) which convey a yellowish colouration. These last pigments have several physiological roles (Karsten, 2008; Young and Frank, 1996): they take part in photosynthesis through light harvesting in thylakoids (Hashimoto et al., 2016) but they also have an important role in photo-protection (Mimuro and Akimoto, 2003). Indeed, they also may participate in the dissipation of solar energy (Young et al., 1997) and to the de-activation of reactive oxygen species (ROS) (Rastogi et al., 2010).

According to the nature, quality and quantity of photo-protective compounds, and also their morphology (blade, filamentous, crustose) and life cycle, different species of macroalgae are then more or less sensitive to UV radiation (Davison and Pearson, 1996; Roleda et al., 2004). In this context, the aim of the present study was to investigate the inter-specific variations in red algal photo-protective compounds within a diverse, representative group of temperate and local red seaweeds. Thus, a screening was carried out on a large number of red seaweeds sampled from the coast of Brittany (France). In order to study the maximum number of species, seaweeds were sampled during two

distinctly different seasons, i.e. winter and summer, as some species occur only during a part of the year. Candidate species for analysis were also collected from the upper subtidal zone (i.e. emerged only at spring tides for maximum 1 h; Ar Gall and Le Duff, 2014) and along the intertidal zone (i.e. with different maximum emersion times from 1 h in the lower intertidal zone to 12 h in the upper intertidal zone during spring tides; Ar Gall and Le Duff, 2014). A MAA diversity index was determined for each species, in order to compare and provide hierarchical comparisons within the red macroalgae collected in this study. Moreover, in order to test the hypotheses of a relationship between the diversity of MAAs and individual red algal taxonomy, various orders/families of red macroalgae were specifically targeted for collection.

## 2. Materials and methods

### 2.1. Sampling sites

Macroalgae were collected at low tides on four different sites in Brittany (France): Porspoder (48°28'58"N – 4°46'5"W), Portsall (48°33'53"N – 4°42'5"W), Saint-Pabu (48°34'34"N – 4°38'45"W) and Le Faou (48°17'44"N – 4°10'56"W). Portsall, exposed North-West, is a sheltered and mainly rocky site, surrounded by sand and intertidal pools. Despite its western exposure, Porspoder remains sheltered due to the presence of two large rocky overhangs. The site chosen at Saint-Pabu, was exposed to the North; it is characterized by sandy dunes. Finally, Le Faou differs from the three previous sites because a river flows there into the Bay of Brest, forming a sheltered but muddy estuary at low tide. Two samplings periods were carried out: in February (winter) and July (summer) 2017.

### 2.2. Macroalgal samples

Forty species of red macroalgae were collected across the four different sites: 21 species were found both in winter and summer and 19 species were found on the shore only for one season. All species belong to the class of Florideophyceae, except *Porphyra dioica* (Bangiophyceae). These included species from different morpho-anatomical groups (i.e. MAG, according to Steneck and Dethier, 1994): filamentous algae (MAG 2); corticated or polysiphonous filaments algae (MAG 2.5); foliose algae (MAG 3); corticated foliose algae (MAG 3.5); corticated algae (MAG 4); and articulated calcareous algae (MAG 6) (Appendix 1).

After collection, algal samples were brought back to the laboratory into sampling bags. Samples were stored in a fridge and washed within 4 h of sampling with filtered seawater to remove residual sediments and salt; visible epiphytes were removed by hand. Seaweeds were then stored in the freezer before being freeze-dried ( $\beta$ -1-8 LD plus Christ) and the entire thallus was milled into a fine powder (MM400 Retsch). The dried powder was kept in darkness at room temperature until analyses.

### 2.3. Extraction and assay of chlorophyll-*a* and carotenoids

Pigments were extracted from 75 mg dry weight (DW) of algal powder in 750  $\mu$ L of 90% acetone, according to Schmid and Stengel (2015). Two successive extractions of 30 min and 4 h were carried out at 4 °C, under magnetic agitation. Samples were then centrifuged at 10,000 rpm for 5 min (miniSpin plus, Eppendorf), and the supernatants combined and filtered for HPLC (High Pressure Liquid Chromatography) analysis (Dionex Ultimate 3000, ThermoScientific). Pigments were separated using an ACE C<sub>18</sub> column (150 × 4.6 mm, 3  $\mu$ m) with a guard-column, an injection volume of 6  $\mu$ L and a run-time of 33 min per sample. Before injection, each sample was automatically diluted 3/4 with ammonium acetate buffer (0.5 M, pH 7.2). Separation was achieved using a solvent gradient described in Table 1, delivered at a flow rate of 1.0 mL.min<sup>-1</sup>. A photo-diode array detector (DAD3000, ThermoScientific) was used for the detection of pigments at 435, 470 and

**Table 1**

HPLC solvent gradient. (A) methanol:ammonium acetate buffer 0.5 M (80:20), (B) acetonitrile:milliQ water (87.5:13.5), (C) ethylacetate 100%.

Time (min)	% A	% B	% C
0	90	10	0
1	0	100	0
11	0	78	22
24	0	25	75
26	0	25	75
27	0	100	0
28	90	10	0
33	90	10	0

650 nm. The identification and quantification of each pigment was based on spectral comparisons and calibration using commercial standards: chlorophyll *a* (Sigma, USA), and  $\alpha$ -carotene,  $\beta$ -carotene, lutein, zeaxanthin, antheraxanthin, violaxanthin (DHI, Denmark). Only peaks with an area larger than 0.4 mAU.min for  $\alpha$ - and  $\beta$ -carotenes and 1 mAU.min for the other pigments were considered in the analysis. HPLC data were collected using Chromeleon 0.7 software (Thermo Scientific Dionex, France).

#### 2.4. Extraction and assay of phycobiliproteins

Tissue concentrations of phycoerythrin (PE) and phycocyanin (PC) were determined using a method adapted from Sun et al. (2009). Two successive extractions of 15 min at 4 °C were performed from 75 mg DW of algal powder, with 1.5 mL of phosphate buffer (0.1 M, pH 6.8). Subsequently, samples were centrifuged for 20 min at 10,000 rpm (miniSpin plus, Eppendorf) and supernatants were used to measure absorbances at 455, 565, 592, 618 and 645 nm using a spectrophotometer (POLARStar Omega, BMG LABTECH). The concentrations of PE and PC (mg.g<sup>-1</sup> DW) were estimated using the following equations from Beer and Eshel (1985):

$$[PE] = [(A_{565} - A_{592}) - (A_{455} - A_{592}) \times 0.20] \times 0.12$$

$$[PC] = [(A_{618} - A_{645}) - (A_{592} - A_{645}) \times 0.51] \times 0.15$$

#### 2.5. Extraction and assay of mycosporine-like amino acids (MAAs)

20 mg DW of finely ground algae were extracted for 2 h with 2 mL of 70% aqueous ethanol (v/v) at 45 °C with magnetic stirring. After centrifugation (centrifuge 5810, Eppendorf) at 1,500 rpm for 10 min, the supernatant was recovered. The pellet was re-extracted twice following the same procedure and the combined supernatants were evaporated to dryness under vacuum (miVac, Genevac, France) at 45 °C. The evaporated extracts were then stored at -20 °C before analysis.

Prior to HPLC analysis, extracts were dissolved in 500  $\mu$ L of 2.5% aqueous methanol with 0.1% acetic acid, centrifuged for 5 min at 10,000 rpm (miniSpin plus, Eppendorf), and filtered through 0.2  $\mu$ m-pore syringe filters. MAAs analyses were performed using a Dionex Ultimate 3000 HPLC (Thermo Scientific, Germany) equipped with a diode-array detector (DAD). MAA separation was performed using a Zorbax Eclipse XDB C<sub>18</sub> column, 5  $\mu$ m, 4.6  $\times$  250 mm (Agilent, USA) equipped with a guard-column. For one analysis, 20  $\mu$ L of extract were injected and the operating parameters were as follow: 0.1% acetic acid in Milli-Q water as the mobile phase; a flow rate of 1 mL.min<sup>-1</sup>; a column temperature of 25  $\pm$  1 °C; an injector temperature of 5  $\pm$  1 °C; a run time of 20 min. MAAs were detected at 320, 330, 334 and 360 nm. HPLC data were collected using Chromeleon 0.7 software (Thermo Scientific Dionex, France). Individual peaks were identified by online absorption spectra and retention time (Rt).

For identification, MAAs extracts were also analyzed by LC-MS using a LC-ESI-Q-TOF-MS (Dionex, Ultimate 3000, Bruker, micrOTOF-QII) system (Bruker Daltonik GmbH, Bremen, Germany). The same LC

method was used, only the flow was reduced to 0.5 mL.min<sup>-1</sup>. Source parameters were: positive mode; source temperature, 200 °C; capillary voltage, 4.5 kV; nebulizer gas (N<sub>2</sub>) at 2.8 bars and dry gas (N<sub>2</sub>) at 12 L.min<sup>-1</sup>. Mass spectra acquisition was set at 0.5 Hz from m/Z 50 to 1000. MS/MS analyses were performed with a collision energy of 30 eV and an isolation width of 2 m/Z. All raw data were collected with Compass data Analysis Version 4.1. The quantification of the identified MAAs was accomplished using their molar extinction coefficients  $\epsilon$  at the wavelengths of maximum absorption, according to Pelillo et al. (2004), and the molar extinction coefficient of Karsten et al. (1998b). For MAAs whose molecular structure has not yet been elucidated, their quantification was not established. Each MAA was then numbered in the order in which it appeared during the run.

Finally, the diversity of MAAs within each seaweed species tested was estimated by the development of a new index. The presence and absence of each MAA for each species was coded in a table, as 0 or 1 respectively. A distance matrix was then calculated by comparing species two by two using the following index:

$$Index = \frac{M}{M + N}$$

where 'M' is the number of matches between the two species (presence and absence) and 'N' the total number of MAAs which were present in only one of the two species. The closer the index was to 1, the more similar the two species were in terms of the composition of their MAAs. A species with many weak indices thus represented a specific composition. A dendrogram was made from this matrix with the R program (R Development Core team, 2008) to represent species that had similar MAA compositions (hclust with single method, hclust package). In order to highlight any links between the composition of MAAs by algal species with their phylogeny, a phylogenetic analysis was performed using *rbcL* and *cox1* genes sequences, as retrieved from GenBank (Accession numbers for GenBank sequences are listed in Appendix 1 and are available on <https://www.ncbi.nlm.nih.gov>). Sequences were aligned using the ClustalW programme in the BioEdit alignment editor (Hall, 1999). A phylogenetic tree was built using MEGA version 7 (Kumar et al., 2016), based on the Neighbor-Joining clustering method. Evolutionary distances were computed using the Maximum Composite Likelihood. The tree was subjected to a bootstrap test (5000 replicates) to estimate robustness at each branch (pvclust, pvclust package).

#### 2.6. Statistical analyses

All data were statistically analyzed with the R program (R Development Core team, 2008). All extractions were performed in triplicate, and results expressed as average  $\pm$  standard deviation (SD). Comparisons were carried out using a Student's t-test (2 samples) or an ANOVA (more than 2 samples). Beforehand, the conditions of application were controlled using the Shapiro test for compliance with a normal distribution, and the F-test or Bartlett's test for the homogeneity of variance (Dytham, 2011). The non-parametric Wilcoxon test (2 samples) or Scheirer-Ray-Hare test (SHR, more than 2 samples) were used when at least one of the conditions of application was not met (to replace the t-test or the ANOVA, respectively). If possible, when significant results were highlighted, *post-hoc* tests (i.e. multiple comparisons) were performed: a Tukey *post-hoc* test after an ANOVA (TukeyHSD), or a non-parametric *post-hoc* test after a Scheirer-Ray test (kruskalmc, pirms package) (Dytham, 2011). Principal Component Analysis (PCA) was carried out (package FactoMineR) to highlight species which stood out.

### 3. Results

#### 3.1. Pigments: chlorophyll *a*, carotenoids and phycobiliproteins

The chlorophyll *a* content (Fig. 1) varied significantly among the

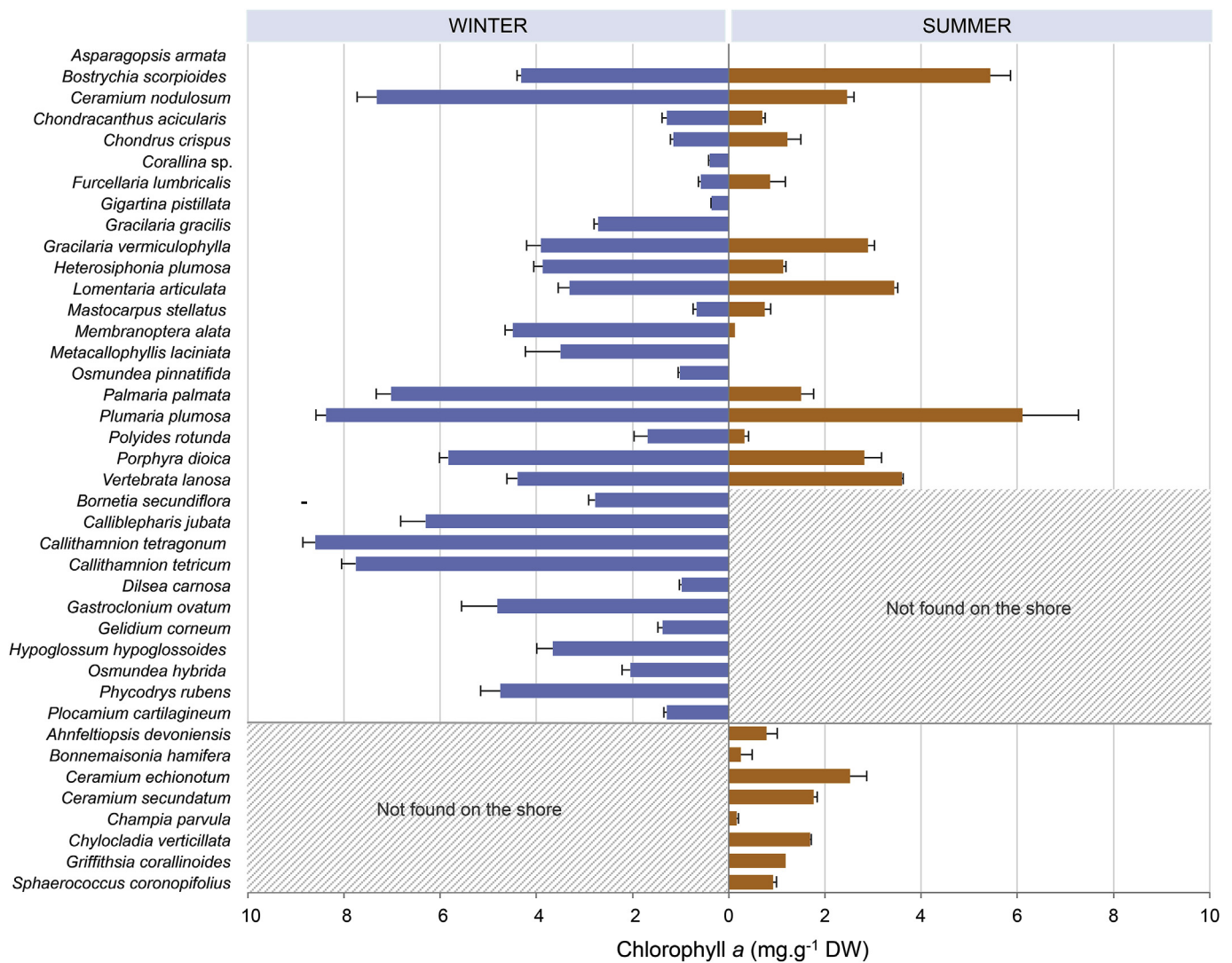


Fig. 1. Chlorophyll a concentrations (mg.g<sup>-1</sup> DW) (mean ± standard deviation, n = 3) of 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that chlorophyll-a level was below the limit of detection or quantification.

analyzed red algal species (SHR test, p-value < 0.0001). In some species, such as *Mastocarpus stellatus* or *Furcellaria lumbricalis*, chlorophyll a concentrations did not exceed 1 mg.g<sup>-1</sup> DW -whatever the season. However, *Callithamnion tetragonum* and *Plumaria plumosa* for example contained in winter 8.60 ± 0.26 and 8.37 ± 0.21 mg.g<sup>-1</sup> DW of chlorophyll a, respectively. Moreover, a seasonal variability was demonstrated with chlorophyll a concentrations decreasing in summer (SHR test, p-value = 0.0040). For example, *Palmaria palmata* contained 7.02 ± 0.31 mg.g<sup>-1</sup> DW of chlorophyll a in February, and 1.50 ± 0.27 mg.g<sup>-1</sup> DW in July. Conversely, *Bostrychia scorpioides* was the only species with a significantly increased concentration in summer (i.e. 5.44 ± 0.43, as compared to 4.32 ± 0.09 mg.g<sup>-1</sup> DW in winter).

Similarly, total concentration of carotenoids changed with species (SHR test, p-value < 0.0001) (Fig. 2). For example, *Chondrus crispus* and *F. lumbricalis* contained less than 0.2 mg.g<sup>-1</sup> DW of carotenoids, whereas *Vertebrata lanosa*, *Porphyra dioica* or *B. scorpioides* contained up to 0.78, 0.84 and 0.65 mg.g<sup>-1</sup> DW of total carotenoids, respectively. A difference in term of composition was also observed (Fig. 2): overall, lutein was the most common carotenoid in 31 species out of 40. However, the amount of lutein remained low as compared to chlorophyll a, which constituted 90.1% on average of the total liposoluble pigment content. Moreover, there was no seasonal variation in the carotenoid composition as a whole, but the concentration was seen to

decrease in summer (SHR test, p-value = 0.0012) (Fig. 2), except for *V. lanosa* in which the concentration increased (e.g. 0.78 ± 0.01 and 0.66 ± 0.04 mg.g<sup>-1</sup> DW in summer and winter, respectively). *Gigartina pistillata*, *Metacallophyllis laciniata* and *Membranoptera alata* did not contain any detectable levels of carotenoids in summer. However, although there were seasonal variations in chlorophyll a and overall carotenoid amounts, the ratio of carotenoids/chlorophyll a appeared to be constant between both seasons (SHR test, p-value = 0.4919) (data not shown).

The different studied red algae contained variable levels of phyco-biliproteins similarly to their chlorophyll a or carotenoid amounts (SHR test, p-value < 0.0001) (Fig. 3). For example, *Bornetia secundiflora* and *Membranoptera alata* contained 27.39 ± 3.13 and 19.67 ± 0.15 mg.g<sup>-1</sup> DW of PE in winter, respectively, whereas *C. crispus*, *Mastocarpus stellatus* or *V. lanosa* contained less than 5.0 mg.g<sup>-1</sup> DW (Fig. 3). *P. plumosa* was more differentiated from the other species with high levels of PE in both seasons (23.18 ± 0.49 mg.g<sup>-1</sup> DW in winter and 13.58 ± 1.36 mg.g<sup>-1</sup> DW in summer). Moreover, it would appear that, on the whole, the PE content decreased in summer (SHR test, p-value < 0.0001). *Bostrychia scorpioides* was the only species for which the PE concentration really increased in summer (6.62 ± 0.22 mg.g<sup>-1</sup> DW in summer, compared to 5.32 ± 0.50 mg.g<sup>-1</sup> DW in winter). The results were similar for

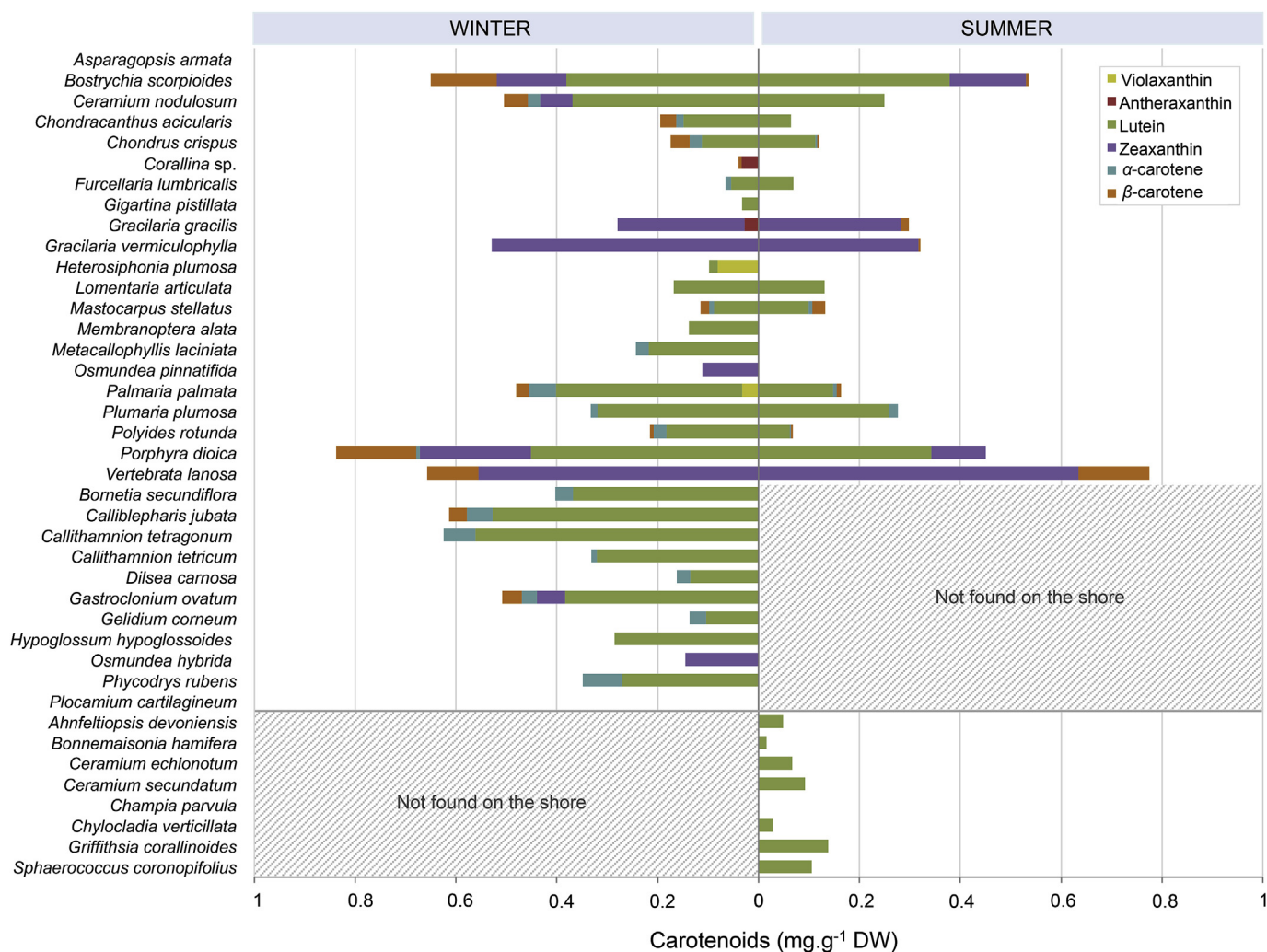


Fig. 2. Carotenoid concentrations ( $\text{mg.g}^{-1}$  DW) (mean,  $n = 3$ ) in 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that carotenoids level was lower than the limit of detection or quantification.

phycocyanin (data not shown).

A Principal Component Analysis (PCA) of all pigments (Fig. 4a and b) pointed out six species: *B. scorpioides*, *Porphyra dioica*, *V. lanosa*, *Gracilaria vermiculophylla*, *Gracilaria gracilis* and *Plumaria plumosa*. This last species contained the highest concentration of chlorophyll *a* and phycoerythrin. The five other species were the only ones containing zeaxanthin with a concentration of  $0.5 \mu\text{g.g}^{-1}$  DW or greater. *G. gracilis* also contained antheraxanthin. Finally, the species belonging to similar MAG showed different pigment content (chlorophyll *a*, carotenoids and phycoerythrin) and composition. Moreover, no link between pigment composition of the red seaweeds studied here and their phylogeny, morphology, position on the shore or sampling site could be demonstrated.

### 3.2. Mycosporine-like amino acids

HPLC analyses showed 23 different peaks that may correspond to MAAs (Fig. 5 and Appendix 2), and six (i.e. shinorine, palythine, asterina-330, porphyra-334, usujirene and palythene) were elucidated by LC-MS. The composition and concentration of various MAAs showed a difference between the various species of red macroalgae sampled in this study. These observations were independent of the sampling season. Amongst the 21 species of algae commonly occurring in both seasons considered, six did not contain any MAA (these included: *F. lumbricalis*, *Metacallophyllis laciniata*, *Polydides rotunda*, *Osmundea*

*pinnatifida*, *Membranoptera alata* and *Plumaria plumosa*). For the eight species which could be collected only in summer, no MAAs were detected in four and for those harvested only in winter, five did not contain any detectable MAAs. At the same time, *Mastocarpus stellatus* contained a high concentration of only shinorine (i.e.  $3.12 \pm 0.26$  and  $1.62 \pm 0.07 \text{ mg.g}^{-1}$  DW, respectively in winter and in summer). One should note that three species, i.e. *Palmaria palmata*, *B. scorpioides* and *G. vermiculophylla*, contained eight different MAAs, visible as separate peaks on the chromatograms (data not shown). A chromatogram of the MAAs analyzed from *P. palmata* (Fig. 6) showed the presence of six identified MAAs (i.e. shinorine, palythine, asterina-330, porphyra-334, usujirene and palythene) and two other molecular structures that may correspond to MAAs. Moreover, some unidentified MAAs were present in only one algal species, such as *B. scorpioides*, which contained four unidentified (new) MAAs (i.e. MAA\_4, MAA\_13, MAA\_18 and MAA\_23), *G. pistillata* which had MAA\_14 and MAA\_22, and *V. lanosa* MAA\_1 (Fig. 5).

Based on HPLC chromatograms, shinorine was the predominant MAA identified among all sampled red macroalgae in this study. Indeed, with the exception of *V. lanosa*, shinorine was the only MAA common to all of those species which appeared able to produce MAAs. Shinorine was found to be in sizeable quantities in *M. stellatus*, *G. gracilis*, and *Ceramium nodulosum* with  $3.12 \pm 0.26$ ,  $4.00 \pm 0.06$  and  $1.92 \pm 0.11 \text{ mg.g}^{-1}$  DW, respectively for species harvested in winter (February). These amounts were approximately halved in July with

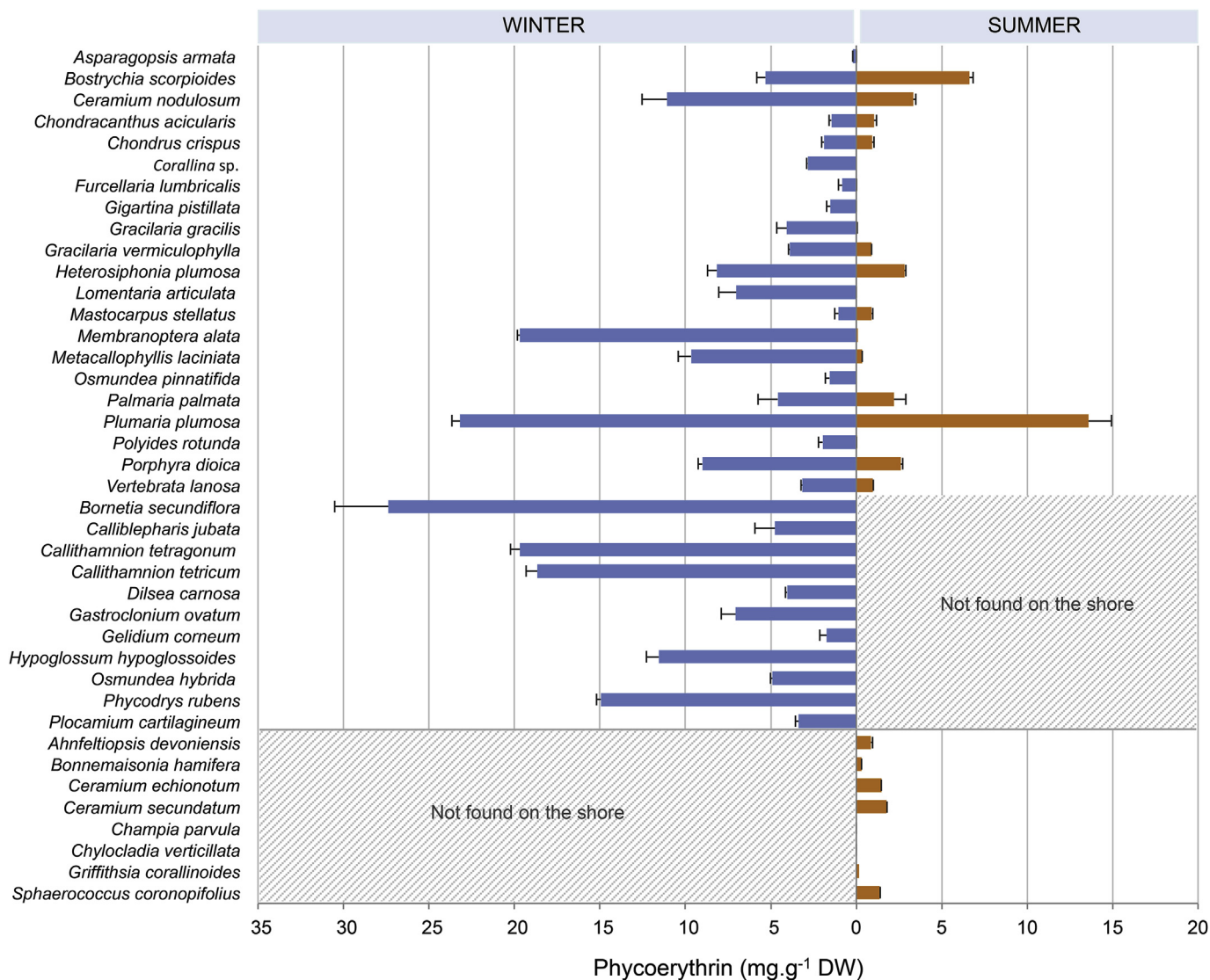


Fig. 3. Phycoerythrin concentrations (mg.g<sup>-1</sup> DW) (mean ± standard deviation, n = 3) in 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that phycoerythrin level was lower than the limit of quantification.

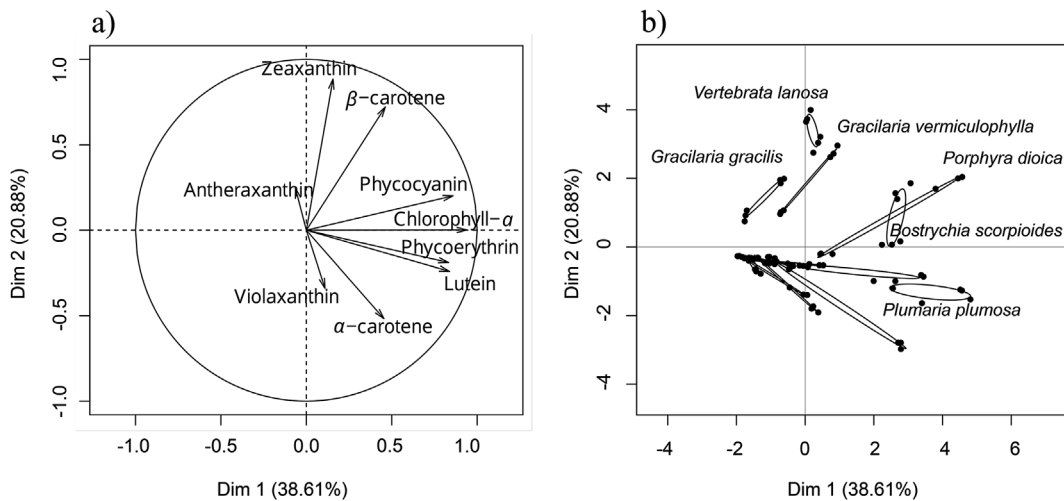


Fig. 4. Principal Component Analysis (PCA) of pigment contents, for 21 species of red macroalgae collected in winter and summer (A: variables; B: samples). Ellipses were drawn around species that had a confidence level of 95% or more. Dimension 1 was principally characterized by chlorophyll a level and dimension 2 by zeaxanthin presence and level (correlation coefficient = 0.93 and 0.88, respectively).

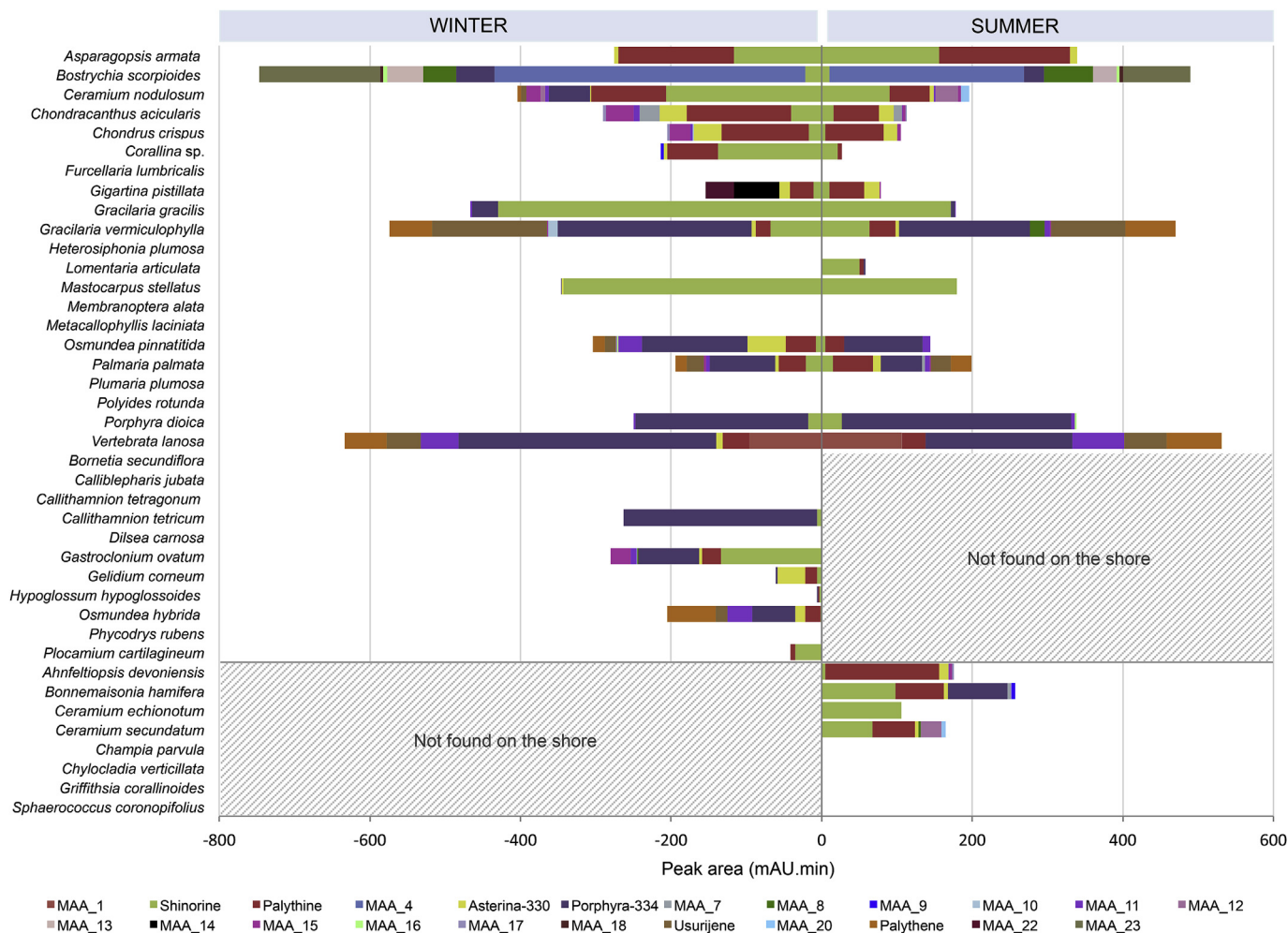


Fig. 5. Mycosporine-like amino acids composition (mAU.min) (mean, n = 3) in 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that MAAs level was lower than the limit of detection or quantification.

1.62 ± 0.07, 1.47 ± 0.10 and 0.86 ± 0.06 mg.g<sup>-1</sup> DW, respectively for the same algae (t-test, p-value = 0.0004). The next most common MAA was palythine which was found in 19 different species.

Moreover, no large modifications in the level of MAAs (Wilcoxon test, p-value = 0.7804) and their composition was observed between both sampling periods in the different species (Fig. 5). However, some

changes were noticed such as in *O. pinnatifida* with the presence of asterina-330, usujirene and palythene in those thalli collected in winter (February), but not in those collected in summer (July). The same was observed for *G. pistillata* with the presence of two new MAAs (i.e. MAA\_14 and MAA\_22) in the winter samples.

In winter and summer, the analysis of the diversity indices for the

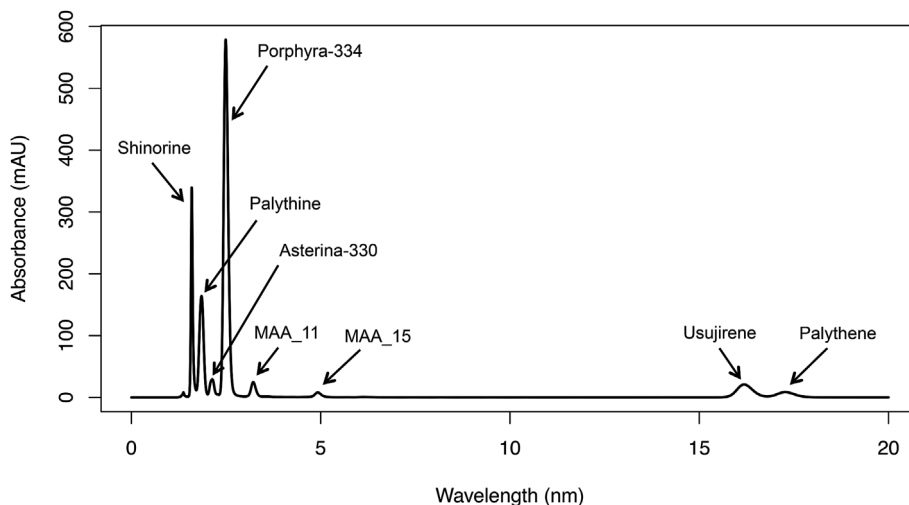
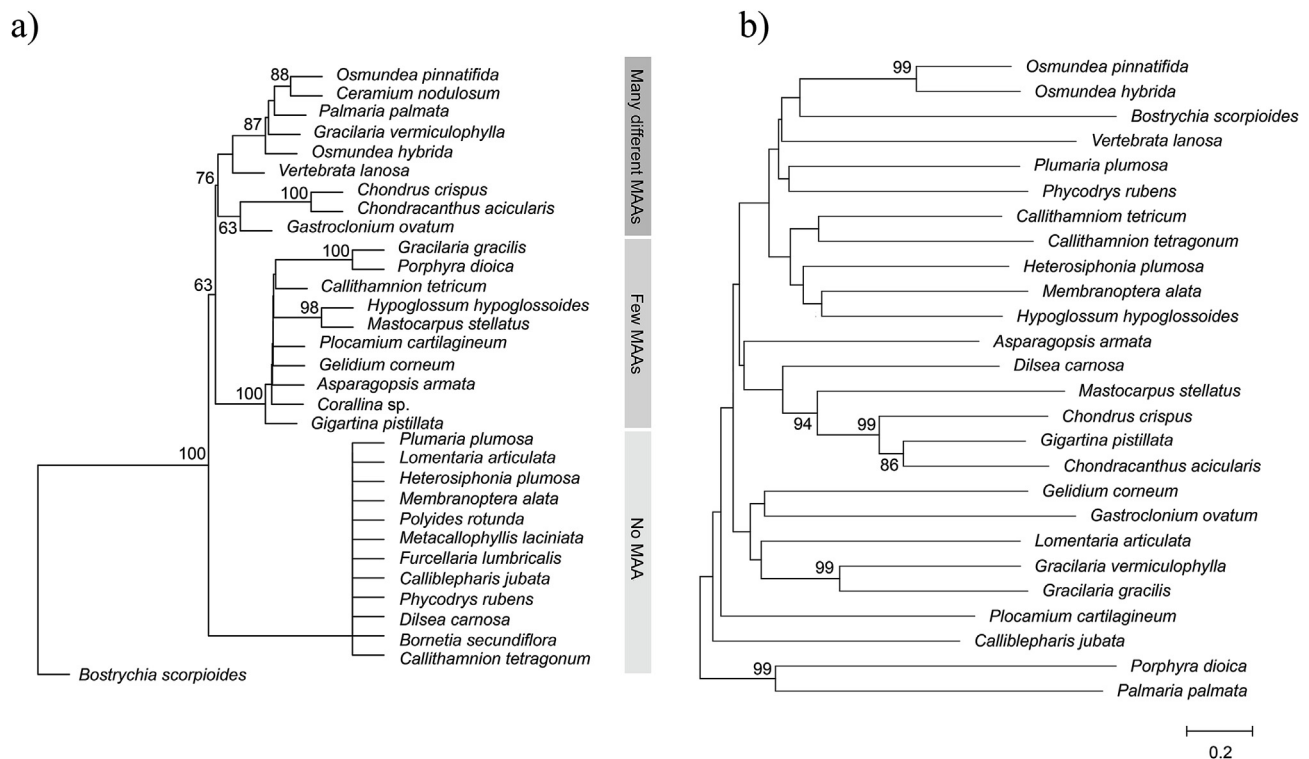


Fig. 6. Chromatogram at 330 nm of MAAs extracted from the red macroalga *Palmaria palmata* collected in February 2017 (winter) from Porspoder (Brittany, France).



**Fig. 7.** (A) Dendrogram based on the diversity indices of MAAs, obtained from hierarchical cluster analysis (single linkage method); (B) Neighbor-joining phylogenetic tree based on *rbcL* and *cox1* genes sequences, for the 32 macroalgal species collected from Brittany during winter. The most relevant boot-strap values are shown next to the branches. Some species are not represented on the phylogenetic tree due to missing sequences on GenBank (see Appendix 1).

MAAs highlighted three groups of species (Fig. 7A and Appendix 3A): those with no MAA (Group 1), those with few MAAs (between 1 and 5; Group 2) and those with many MAAs (6 or more; Group 3). Only *B. scorpioides* seemed to stand out and did not correspond to any group, even though it contained eight different MAAs. Shinorine was present in all species of Groups 2 and 3, except in *V. lanosa* which was also the only species having MAA\_1. Moreover, asterina-330 and palythine were also two MAAs found in some species from Group 2, and in all species from Group 3 (with the exception of *V. lanosa* and *B. scorpioides*). Finally, there were some MAAs which were only found in Group 3, notably usujirene and palythene which were present in six of the nine species of the Group 3 in winter. The high diversity in MAAs did not seem to be related to phylogeny (Fig. 7B and Appendix 3B), anatomy and morphology, the height on the shore nor the sampling site (Appendix 1). Hence, species which are close phylogenetically (i.e. *G. vermiculophylla* and *G. gracilis*, or *Callithamnion tetricum* and *C. tetragonum* in our study), morphologically (*M. stellatus* and *C. crispus*), or the only two species harvested from a muddy habitat (i.e. *B. scorpioides* and *G. vermiculophylla*) did not have the same MAAs profile (see Appendix 1 and Fig. 5). No link was also found between the MAAs and the fact that a species was introduced or indigenous to Brittany (data not shown).

#### 4. Discussion

As intertidal macroalgae are organisms directly exposed to solar radiation, they have developed some protective mechanisms in order to survive, such as the synthesis of photo-protective compounds. In this sense, the objective of this study was to collect many different red algal species from a temperate shore in order to have a first overview of the specific distribution of these compounds, and more particularly of mycosporine-like amino acids for which data are not actually available for some species.

Firstly, a high inter-specific variability has been demonstrated in terms of pigment composition and concentration (Figs. 1–3). Some

species had a high content of chlorophyll-*a* (*P. plumosa* or *Callithamnion* sp.) while others had more carotenoids (*P. dioica*, *B. scorpioides*, *V. lanosa*), or others had low concentrations of both pigments (*C. crispus* or *G. pistillata*). Three groups were observed from the carotenoids composition, as suggested by Schubert et al. (2006): species rich in lutein, species rich in zeaxanthin, and those with violaxanthin or antheraxanthin (Fig. 2). Lutein was the major carotenoid, as already illustrated (Esteban et al., 2009; Marquardt and Hanelt, 2004).

Furthermore, the quantity of photosynthetic pigment (i.e. chlorophyll-*a* and phycobiliproteins) decreased in the summer samples (Figs. 1 and 3) due to an increase in irradiance. Indeed, the increase in light energy availability leads species to reduce their pigment complement to harvest the same quantity of light (Ak and Yücesan, 2012; Ramus et al., 1976). Another explanation would be linked to the nitrogenous nature of chlorophyll-*a* and phycobiliproteins (Huovinen et al., 2006; Lüning, 1990; Parjikolaei et al., 2013). They are used for nitrogen storage in winter and as a nitrogen source when limitations occur in summer (Barufi et al., 2011; Lapointe and Duke, 1984; Surget et al., 2017). Stack et al. (2017) demonstrated that the protein content of *Porphyra dioica* doubled in winter months, perhaps because of phycobiliproteins acting as a nitrogen storage. In general, the impact of N concentration on pigments has already been widely demonstrated in many species such as in *Palmaria palmata* (Corey et al., 2013; Parjikolaei et al., 2013).

The aim of the second part of this study was to elucidate the MAAs composition of 40 red macroalgae. The extraction method in 70% ethanol, coupled with the extract analysis by HPLC, provided reliable data, and had the advantage of being non-toxic, unlike using methanol which is commonly used in the literature (e.g. Briani et al., 2018; Gröniger et al., 2000; Guihéneuf et al., 2018; Pandey et al., 2017; Yuan et al., 2009). The use of a less-toxic solvent such as ethanol allowed for the valorization of extracted MAAs as they have a significant potential for biotechnology applications in for example human health and cosmetics (Chrapusta et al., 2017; Lawrence et al., 2018; Pangestuti et al.,



2018). For example, Helioguard 365 (Mibelle group, Switzerland) is a commercial formulation used to protect the skin or hair from UV radiation and based on the MAAs shinorine and porphyra-334 extracted from the red macroalga *Porphyra umbilicalis* (La Barre et al., 2014; Schmid et al., 2006).

The results of the screening revealed a high diversity in MAA composition in the 40 studied species, including some species for which the MAA composition had not been reported before, e.g. *Plumaria plumosa*, *Bornetia secundiflora*, *Dilsea carnosa*, *Gastroclonium ovatum*, *Hypoglossum hypoglossoides*, *Champia parvula* and *Chylocladia verticillata*. It was already demonstrated that some species of the Rhodophyta have the capacity to accumulate a high degree of diversity and concentration of MAAs (e.g. Rastogi et al., 2010; Sinha et al., 2007), but this present study is the first to report on MAA profiles from as many species of red alga from a temperate region, here from Brittany. Based on their absorption maxima and retention time, 23 potential MAAs were identified by HPLC analyses in the 40 red seaweeds harvested along the Brittany coasts. Shinorine and palythine were found to be the most common MAAs in this study (Fig. 5), which is in agreement with Karentz (2001). The composition in MAAs of each species coincided with the literature, even for poorly studied species such as *Asparagopsis armata* (Figueroa et al., 2008). For example, in accordance with the bibliography, shinorine and porphyra-334 are found in *P. dioica* (Guihéneuf et al., 2018); shinorine, palythine and porphyra-334 in *C. nodulosum*; or species contained no MAA such as *Phycodryx rubens* and *F. lumbricalis* (Karsten et al., 1998b).

The development of a diversity index for MAAs allowed for the identification of three groups of species (Fig. 7A): i.e. species without MAA (e.g. *Plumaria plumosa*, *H. plumosa*, *M. alata*, *Polyides rotunda* or *F. lumbricalis*), species with a few MAAs (e.g. *Porphyra dioica*, *Gracilaria gracilis*, *C. tetricum*), and some with more than six MAAs (e.g. *Palmaria palmata*, *B. scorpioides*, *Gracilaria vermiculophylla*, *V. lanosa*). These observations suggested a huge diversity of mycosporine-like amino acids among different members of Rhodophyta, with no apparent link with phylogeny, or morphology, position on the shore or sampling site. Similarly to our study, the distribution of various MAAs in other publications is not related to a phylogenetic pattern (Karentz et al., 1991; McClintock and Karentz, 1997), but more to an acclimatization to environmental variations (Briani et al., 2018).

Furthermore, 23 MAAs of which some potentially new and as yet unidentified were observed in the 40 species knowing that approximately 20 MAAs have been reported so far in red algae, suggesting that there may be in this study some MAAs that have never been yet identified. *B. scorpioides* was the species that stood out the most because it had four, unique, as yet un-identified MAAs (i.e. MAA\_4, MAA\_13, MAA\_18 and MAA\_23). This species also did not fit into any of the three groups based on the diversity index (Fig. 7A). Only six MAAs were identified by LC-MS in the studied species (i.e. shinorine, palythine, porphyra-334, asterina-330, usurijene, palythene). In addition, we propose that MAA\_11 corresponded to palythanol by comparing the results with the literature: indeed, palythanol is a MAA often observed in many red macroalgae in the literature (Sinha et al., 2007) and MAAs\_11 is the fifth most common MAA in the red algae studied here. MAA\_11 is present for example in *P. palmata*, which should contain palythanol according to the literature (Gröniger et al., 2000; Karsten and Wiencke, 1999; Yuan et al., 2009). It is difficult to identify MAAs based only on the absorbance spectra and retention times of known MAAs in the bibliography, especially since some MAAs are present in very small quantities which makes it impossible to detect them in LC-MS. The lack of commercial standards makes this study particularly complex, in addition to making impossible the quantification of the different MAAs. In addition, some peaks have similar absorption maxima and retention times. For example, usurijene and palythene only differ by their cis- or trans-conformation and less than one minute separated them (Carreto and Carignan, 2011). In order to identify other MAAs, more biomass for the extractions (if the available biomass

allowed it), or the development of a purification protocol to concentrate each MAA would therefore be necessary.

Moreover, there was no large seasonal difference in terms of the composition and concentration of MAAs (Fig. 5). This is an unexpected result as the role of photo-protection by MAAs has been widely demonstrated (e.g. Conde et al., 2000; Singh et al., 2008). We expected that the concentration of total MAAs would increase in relation to the intensity of radiation in the marine ecosystem (Karsten et al., 1998a; Torres et al., 2016). However, it is possible that a possible increase in MAAs content occurred between the two sampling periods, February and July, indicating that two sampling periods are not enough to study seasonal variation in MAAs content. This is suggested by Guihéneuf et al. (2018), who found that the concentration of MAAs increased between February and May in *P. palmata* and *C. crispus* collected on Irish shores and not during summer as expected. Another explanation is that, as MAAs are nitrogenous compounds, they are used in summer as a source of nitrogen (Karentz, 2001). In this sense, a number of studies have already demonstrated the positive effect of nitrogen on concentrations of MAAs (Figueroa et al., 2014; Korbee et al., 2005; Peinado et al., 2004).

Thus, the variability observed in pigments and MAAs composition suggests that all species do not seem to have the same protective capabilities against solar radiation. In an attempt to obtain a first explanation of this diversity, we tried to relate this composition to phylogeny, or morphology, position on the shore or sampling site, which was not successful. To date, few studies have examined the link between pigment composition and phylogeny, and no clear relationship has been found (Vandervelde, 1973; Wang et al., 2018). The MAAs composition would also not be related to a phylogenetic pattern (Karentz et al., 1991; McClintock and Karentz, 1997). Thus there is currently no explanation for the composition (presence/absence) in MAAs between the different species that seems to be random. However, it has been experimentally demonstrated that an increase in UV radiation could have an impact on the levels of MAAs, for example in *Palmaria palmata* or *Chondrus crispus* (Hoyer et al., 2002; Kräbs et al., 2004); or that the MAAs concentration depended on the season (Guihéneuf et al., 2018), nutrient concentrations and pH (Briani et al., 2018), latitude (Karsten et al., 1998b) or depth (Franklin et al., 1999; Karsten and Wiencke, 1999). For example, the total MAA concentration in *P. palmata* and *Devalerea ramentaceae* collected at different depths decrease with sampling depth, although the composition remains the same (Hoyer et al., 2001; Karsten et al., 1999).

In our study, four species stand out due to their diversified compositions of MAAs: *B. scorpioides*, *G. vermiculophylla*, *V. lanosa* and *P. dioica*, which are the sampled species the most exposed to solar radiation due to their relatively high position on the shore. Indeed, *B. scorpioides* lives at the top of muddy estuaries (Sánchez de Pedro et al., 2014); *G. vermiculophylla* lives at the surface of the intertidal muddy estuaries (Roleda et al., 2012; Surget et al., 2017); *P. dioica* is located on the upper intertidal, and *V. lanosa* is an obligate epiphyte on the brown macroalga *Ascophyllum nodosum* (Garbary et al., 2014) which has a wide range of coverage on intertidal rockyshores at mid-tide level. These four red algal species were characterized by the presence of several MAAs and a high concentration of carotenoids, in particular zeaxanthin, a pigment synthesized under higher light conditions (Rmiki et al., 1996; Schubert and Garcia-Mendoza, 2008). Thus, they stood out from the others on the Principal Component Analysis of pigments (Fig. 4). Another species, *G. gracilis*, also stood out the PCA since it contained antheraxanthin, in accordance with Rmiki et al. (1996) and Schubert et al. (2006). Conversely, *P. plumosa*, with no observed MAA, was the single species with the highest concentrations of chlorophyll *a* and phycoerythrin. This alga lives in shaded areas (Yakovleva et al., 1998), which may explain its necessity to have high concentrations of pigments which absorb light energy, rather than act as photo-protectants.

## 5. Conclusions

This study highlights the occurrence of numerous MAAs in red macroalgae from Brittany, with variations in the number and level of MAAs per species. Thus, this research contributes to reinforce the few existing databases on the MAAs composition of red algae as described by Gröniger et al. (2000) and Sinha et al. (2007), knowing that the identification of these molecules is difficult due to the lack of commercial standards. Here we attempted to identify the factors controlling this MAAs diversity: MAAs composition was no related to a phylogenetic pattern; however, the algal species most exposed to light radiation were those with a high level and diversity of photoprotective compounds which could protect their photosynthetic mechanisms against UV-radiation. Nevertheless, it remains complex to provide conclusions about the actual impact of the different biotic and abiotic factors on the synthesis of each MAA. Although MAAs seem to be photo-protective, they may play other and as yet unknown roles in macroalgal metabolism. Subsequently, it is necessary to select few species and carry out macroalgal cultures under controlled conditions in order to understand factors responsible for the production of each MAAs. Thus, the impact of temperature, UV radiation, nutrients or pH could be tested one by one or in combination, allowing to highlight a change in the ratio of MAAs for example. This would result in a better understanding of the synthesis and function of these compounds.

## Declaration of interest

None.

## Authors' contributions

F.L. performed the analysis of the samples collected in winter, drafted the manuscript and conducted the statistical analyses. S.L. performed the analysis of the samples collected in summer and contributed to the writing of the manuscript. G.B. and L.T. performed the LC-MS analyses. V.S-P designed the diversity index, helped in carrying out the phylogenetic analyses and contributed to the interpretation of the results. S.C. designed and supervised the project. All authors reviewed and approved the final manuscript.

## Acknowledgements

We thank the European Institute for Marine Studies (IUEM), the University of Western Brittany (UBO, Brest) and the region Bretagne for funding Fanny Lalegerie's PhD, and the region Bretagne for the post-doctoral fellowship of Sirine Lajili through the SAD 2016 - MABIOPA (9648) project. Authors thanks T. Burel for the identification of some studied species.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2019.04.001>.

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## Supplementary data

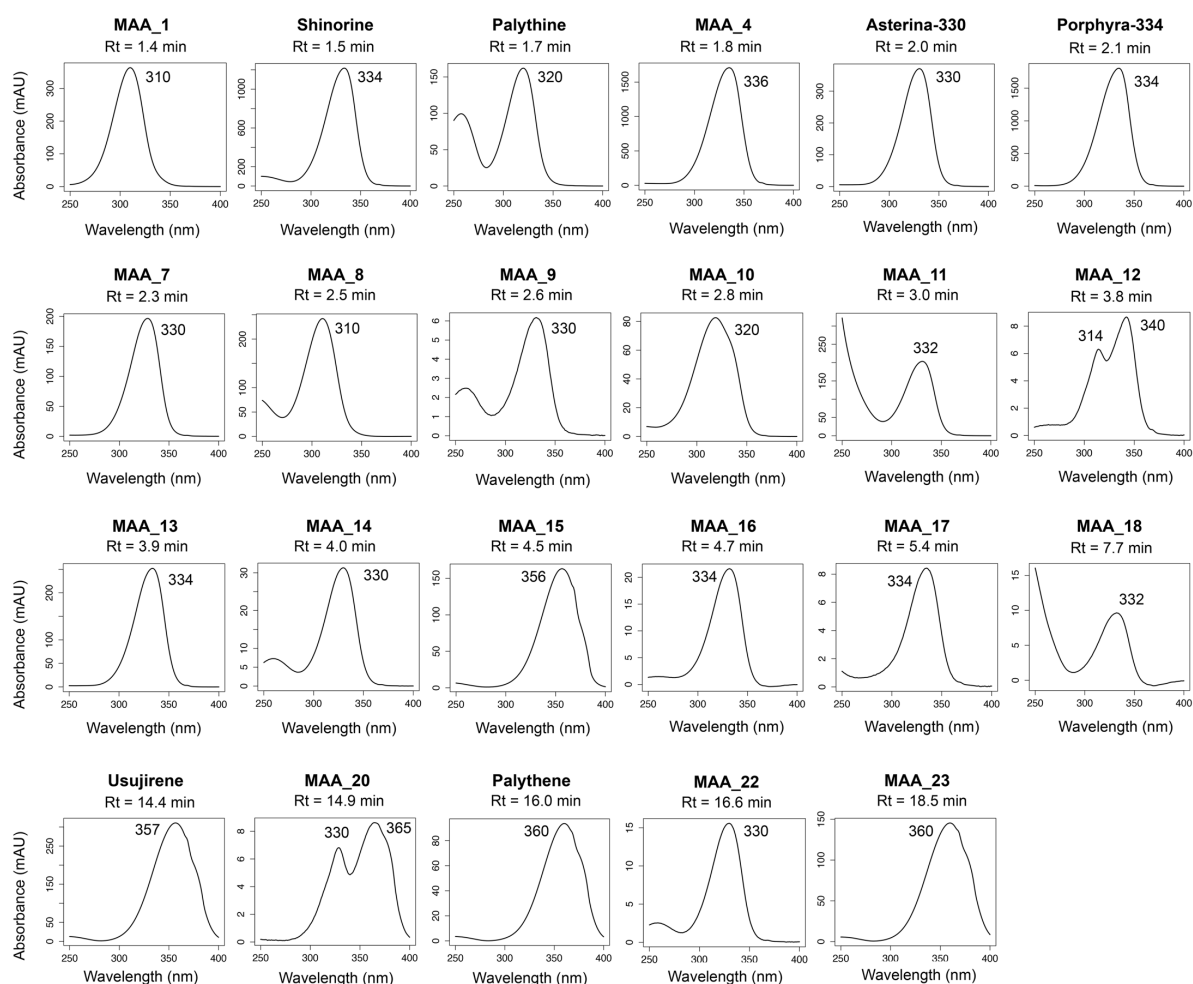
**Appendix 1.** List of the 40 red macroalgal species collected in February and/or July 2017, with their associated GenBank accession numbers (for the genes *rbcL* and *cox1*), morpho-anatomical group (*i.e.* MAG; according to Steneck and Dethier, 1994), localisation on the shore, substrate and sampling site. The different MAGs are: (2) filamentous algae; (2.5) corticated or polysiphonous filaments algae; (3) foliose algae; (3.5) corticated foliose algae; (4) corticated algae and (6) articulated calcareous algae.

Species	Accession numbers		MAG	Localisation	Substrate	Sampling sites
	<i>rbcL</i>	<i>cox1</i>				
<i>Ahnfeltiopsis devoniensis</i>	KU640212.1	KF641876.1	4	Lower intertidal	Rocky	Portsall
<i>Asparagopsis armata</i>	GQ337068.1	KJ960343.1	2.5	Lower intertidal	Rocky	Portsall
<i>Bonnemaisonia hamifera</i>	FJ195604.1	KJ960353.1	2.5	Lower intertidal	Rocky	Portsall
<i>Bornetia secundiflora</i>	No data		2.5	Upper subtidal	Rocky	Portsall
<i>Bostrychia scorpioides</i>	AY920825.1	MF094019.1	2.5	Upper intertidal	Muddy	Le Faou
<i>Calliblepharis jubata</i>	KC121138.1	KJ960410.1	4	Lower intertidal	Rocky	Portsall
<i>Callithamnion tetragonum</i>	AF439301.1	MF447481.1	2	Lower intertidal	Rocky	Portsall
<i>Callithamnion tetricum</i>	AF439300.1	KJ960434.1	2	Lower intertidal	Rocky	Portsall
<i>Ceramium echionotum</i>	AF439313.1	KJ960509.1	2.5	Lower intertidal	Rocky	Portsall
<i>Ceramium nodulosum</i>	No data		2.5	Lower intertidal	Rocky	Portsall
<i>Ceramium secundatum</i>	KT250273.1	KT250269.1	2.5	Lower intertidal	Rocky	Portsall
<i>Champia parvula</i>	EF613312.1	HQ422864.1	2.5	Lower intertidal	Rocky	Portsall
<i>Chondracanthus acicularis</i>	KJ202090.1	KJ202081.1	4	Lower intertidal	Rocky	Portsall
<i>Chondrus crispus</i>	KF026483.1	GU645233.1	4	Lower intertidal	Rocky	Portsall
<i>Chylocladia verticillata</i>	No data		2.5	Intertidal	Rocky	Portsall
<i>Corallina</i> sp.	No data		6	Lower intertidal	Rocky	Porspoder <sup>a</sup> ; St-Pabu <sup>b</sup>
<i>Dilsea carnosa</i>	KT310705.1	KY572820.1	3.5	Upper subtidal	Rocky	Portsall
<i>Furcellaria lumbricalis</i>	No data		4	Intertidal	Rocky	Portsall
<i>Gastroclonium ovatum</i>	KU726714.1	KJ960700.1	2.5	Lower intertidal	Rocky	Portsall
<i>Gelidium corneum</i>	HM629821.1	KJ960706.1	4	Lower intertidal	Rocky	Porspoder
<i>Gigartina pistillata</i>	AY294375.1	KJ960717.1	4	Lower intertidal	Rocky	Porspoder <sup>a</sup> ; Portsall <sup>b</sup>
<i>Gracilaria gracilis</i>	AY049400.1	KF714853.1	4	Lower intertidal	Rocky	Portsall
<i>Gracilaria vermiculophylla</i>	JQ768774.1	JQ794759.1	4	Upper intertidal	Muddy	Le Faou
<i>Griffithsia corallinoides</i>	No data		2.5	Lower intertidal	Rocky	Portsall
<i>Heterosiphonia plumosa</i>	AF083379.1	KJ960780.1	2.5	Upper subtidal	Rocky	Portsall <sup>a</sup> ; St-Pabu <sup>b</sup>
<i>Hypoglossum hypoglossoides</i>	AF257368.1	KJ179930.1	3.5	Lower intertidal	Rocky	Portsall
<i>Lomentaria articulata</i>	KU726701.1	KU707860.1	2.5	Lower intertidal	Rocky	Portsall
<i>Mastocarpus stellatus</i>	U02992.1	KY572683.1	4	Lower intertidal	Rocky	Portsall
<i>Membranoptera alata</i>	JQ864359.1	KJ960846.1	3.5	Lower intertidal	Rocky	Portsall
<i>Metacallophyllis laciniata</i>	No data		3.5	Upper subtidal	Rocky	Portsall

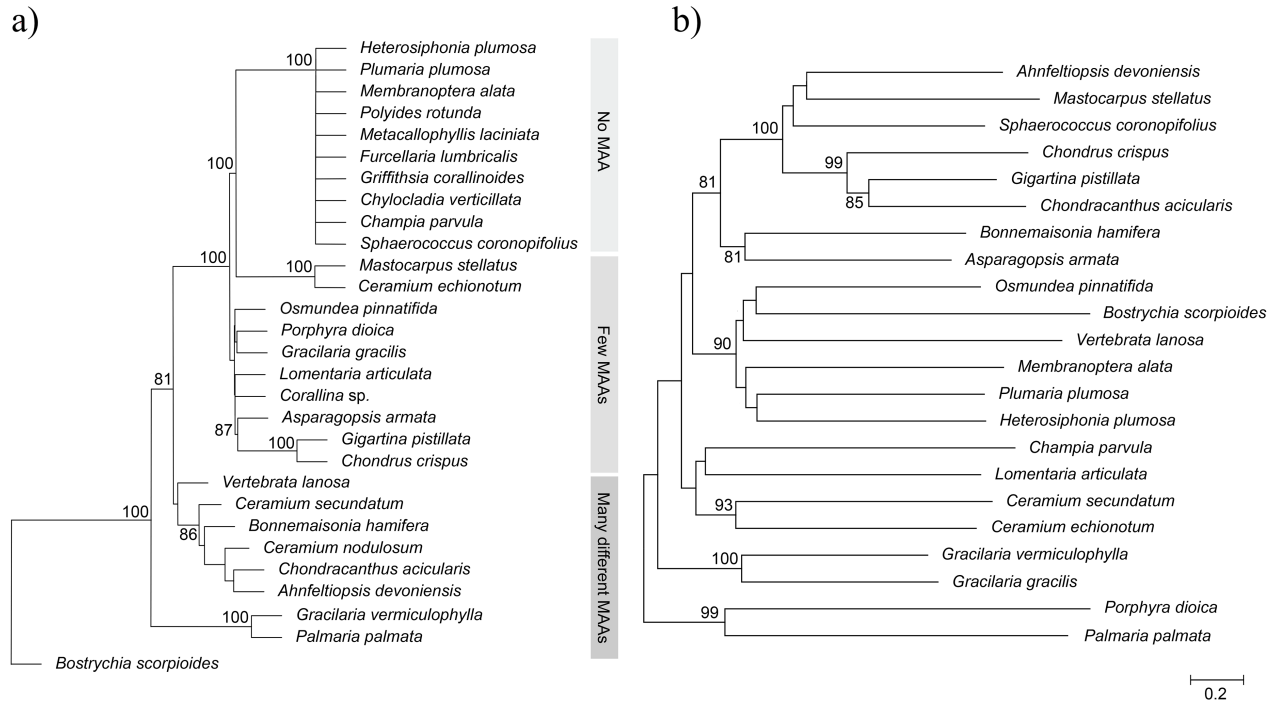
<i>Osmundea hybrida</i>	AF281878.1	KX258831.1	4	Lower intertidal	Rocky	Portsall
<i>Osmundea pinnatifida</i>	JX828140.1	KU566536.1	4	Lower intertidal	Rocky	Porspoder <sup>a</sup> ; Portsall <sup>b</sup>
<i>Palmaria palmata</i>	LN999410.1	KY572816.1	3.5	Lower intertidal	Rocky	Porspoder <sup>a</sup> ; Portsall <sup>b</sup>
<i>Phycodryis rubens</i>	JX110932.1	KY572841.1	3.5	Upper subtidal	Rocky	Porspoder
<i>Plocamium cartilagineum</i>	HQ224543.1	JF271583.1	4	Lower intertidal	Rocky	Portsall
<i>Plumaria plumosa</i>	KU381993.1	HQ412551.1	2.5	Lower intertidal	Rocky	Portsall
<i>Polyides rotunda</i>	No data		4	Lower intertidal	Rocky	Portsall
<i>Porphyra dioica</i>	JN703282.1	JN847313.1	3	Upper intertidal	Rocky	Portsall <sup>a</sup> ; St-Pabu <sup>b</sup>
<i>Sphaerococcus coronopifolius</i>	AY294376.1	KJ961109.1	4	Upper subtidal	Rocky	St-Pabu
<i>Vertebrata lanosa</i>	MF120849.1	KX344122.1	2.5	Intertidal	Rocky	Portsall

<sup>a</sup>Samples collected in winter; <sup>b</sup>Samples collected in summer

**Appendix 2.** List of the 23 different peaks analyzed in 40 red macroalgae species, that may correspond to MAAs, with their absorption spectra,  $\lambda_{max}$  and their retention time (Rt).



**Appendix 3.** (A) Dendrogram based on the diversity indices of MAAs, obtained from hierarchical cluster analysis (single linkage method); (B) Neighbor-joining phylogenetic tree based on *rbcL* and *cox1* genes sequences, for the 29 macroalgal species collected in summer. The most relevant boot-strap values are shown next to the branches. Some species are not represented on the phylogenetic tree due to missing sequences on GenBank (see Appendix 1).



## ENGLISH

The previous screening highlighted a high diversity in MAAs composition within red macroalgal species collected in Brittany. In order to understand why such a high diversity, one species was selected to investigate the roles of the different MAAs. In this regard, the red alga *Palmaria palmata* was chosen since it showed a composition rich in MAAs in the previous screening. The temporal variability in its MAA composition was thus monitored *in-situ* during one year on different sites in Brittany. In this regard, what are the environmental factors that influence the synthesis of MAAs? Do all MAAs show the same seasonal pattern? Is it possible to link the temporal variations to the MAA synthesis pathway? The results have led to a publication in the *Journal of Applied Phycology* and are presented in this second part.

## FRANÇAIS

Le précédent screening a mis en évidence une grande diversité dans la composition en MAAs chez les espèces de macroalgues rouges collectées en Bretagne. Afin de comprendre pourquoi une telle diversité, une espèce a été sélectionnée pour étudier les rôles des différents MAAs. À cet égard, l'algue rouge *Palmaria palmata* a été choisie car elle présentait une composition riche en MAAs lors du précédent screening. La variabilité temporelle de sa composition en MAAs a donc été suivie *in-situ* pendant un an sur différents sites en Bretagne. Ainsi, quels sont les facteurs environnementaux qui influencent la synthèse en MAAs ? Tous les MAAs présentent-ils le même schéma saisonnier ? Est-il possible de relier les variations temporelles à la voie de synthèse des MAAs ? Les résultats ont donné lieu à une publication dans *Journal of Applied Phycology* et sont présentés dans cette deuxième partie.





# Temporal variation in pigment and mycosporine-like amino acid composition of the red macroalga *Palmaria palmata* from Brittany (France): hypothesis on the MAA biosynthesis pathway under high irradiance

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Received: 26 July 2019 / Revised and accepted: 18 February 2020  
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## Abstract

Changes in pigment (chlorophyll-*a*, carotenoids and phycobiliproteins) and mycosporine-like amino acid (MAA) composition were studied in the red edible macroalga *Palmaria palmata*, harvested between February 2018 and February 2019 at 2 sites from Brittany (France). The results occurring in natural conditions revealed a high seasonal variability that can be related to a combination of environmental parameters, including light, nutrients, temperature, salinity and pH, which may have synergistic or antagonistic effects. A decrease in pigments was observed in summer, matching with the increase in irradiance at the same period. On the contrary, the increase in light from February to May has caused an increase in the total MAA content, confirming the UV-photoprotective role attributed to these compounds. Analysis of the results coupled with the biosynthesis pathway suggests that some MAAs are favoured in the occurrence of high irradiance. In this sense, asterina-330 was synthesized only between March and July and not the rest of the year, supporting its importance in photoprotection. In addition, analysis revealed that nutrient availability could have a significant influence on MAAs since their synthesis seemed to be limited in summer, despite significant exposure to light. However, this decrease could be due to another potential role of MAAs as a nitrogen source for macroalgae. Thus, the diversity of these compounds and the different synthesis pathways could be explained by different roles including photo-protection and nitrogen source, making MAA multi-purpose protective agents which could play a key role in the adaptation of this species to its changing environment.

**Keywords** Rhodophyta · HPLC · MAAs · Nutrient storage · Photo-protection · Pigments · Synthesis pathway

## Introduction

*Palmaria palmata* (Linnaeus) F. Weber & D. Mohr, commonly known as ‘dulse’, is an edible red macroalga (Rhodophyta, Palmariaceae) distributed along the rocky shores of North Atlantic Ocean (Li et al. 2015). It has been traditionally consumed as a source of proteins or vitamins and for its potential

positive effects on human health (Galland-Irmouli et al. 1999; MacArtain et al. 2007; Bjarnadóttir et al. 2018). Notably, seaweeds, including dulse, are considered as a source of antioxidant, anti-microbial, anti-inflammatory or anti-cancer compounds (Yuan et al. 2005; Cornish and Garbary 2010; Wang et al. 2010; Lee et al. 2017). In addition, *P. palmata* contains a supernumerary pigment specific to red algae, phycoerythrin (PE), which has a high commercial value due to its valorization in various sectors such as the medical and food industries (Sekar and Chandramohan 2008; Dumay et al. 2013). Thus, *P. palmata* is one of the most known and commercialized edible seaweeds, with *Porphyra/Pyropia* spp., *Himantalia elongata*, *Undaria pinnatifida* or *Ulva* spp. (McHugh 2003).

However, although there is a growing interest in edible algae (Mendis and Kim 2011), species living in the intertidal zone, such as *P. palmata*, are vulnerable to a variety of abiotic and biotic parameters such as fluctuations in light,

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**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s10811-020-02075-7>) contains supplementary material, which is available to authorized users.

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temperature, desiccation or nutrient inputs (Fogg 2001; Hanelt et al. 2003; Hurd et al. 2014; Ji et al. 2016) or grazing and competition for light (Edwards and Connell 2012; Iken 2012). In this sense, it is essential to investigate ecophysiological responses of seaweeds to the different environmental parameters at the level of molecules, in order to understand the vulnerability and the potential capacity of the seaweeds to adapt face to a changing environment (Figueroa and Korbee 2010). More specifically, algae from intertidal rocky shores are particularly exposed to solar and UV radiations during the emergence phases, and it is partly their resistance to desiccation and their sensitivity to light that define their specific vertical zonation (Johansson and Snoeijs 2002; Häder et al. 2007). UV radiation is particularly harmful in the early stages of algal development (Huovinen et al. 2000; Wiencke et al. 2006; Roleda et al. 2007) and can cause DNA damages or synthesis of reactive oxygen species (ROS) (Van de Poll et al. 2001). It can also affect growth, photosynthesis rate and nutrients uptake of algae (Van de Poll et al. 2001; Zheng and Gao 2009; Xu and Gao 2012).

However, high solar irradiance neither is the unique environmental factor which occurs in the intertidal zone nor it is the only factor that can have an effect on the physiological and ecological responses of algae. Several factors are involved at the same time in the natural environment and light can have antagonistically or synergistically effects with temperature, salinity or nutrient level (Figueroa and Korbee 2010). For example, an increase in the seawater temperature has an effect on intertidal rocky shore populations by modifying their photosynthesis or their growth. Notably, seawater temperature has fundamental effects on enzymes, modifying the rate of metabolic reactions (Fogg 2001; Eggert 2012). In this sense, *P. palmata* grew well around 10 °C, and an increase of a few degrees in the seawater temperature could have consequences on its distribution (Morgan and Simpson 1981). Similarly, nutrients can limit the growth of seaweeds (Godillo 2012), which explains why some species are able to store N and P, sometimes for several months such as *Macrocystis* (Brown et al. 1997) or *Laminaria* (Gagne et al. 1982). Thus, addition of N commonly results in an increase in N-containing compounds such as chlorophyll and phycobiliproteins (Lapointe and Duke 1984). More broadly, seaweeds must adapt their energy source for photosynthesis according to the daily and seasonal fluctuations in nutrients (Godillo 2012). Thus, N and P uptake kinetics and content in *P. palmata* tissues have been reported to be dependent on nutrient exposure levels (Martínez and Rico 2004; Corey et al. 2013).

In response to its various environmental stresses, algae have a strong potential of acclimatization, including among others photoinhibition, photoprotection, DNA repair or synthesis of protective agents (Van de Poll et al. 2001; Bischof et al. 2007). For that, seaweeds have a high chemical diversity and are able to synthesize different compounds, whose

composition and concentration change and acclimate in response to environmental variations (Stengel et al. 2011). Among molecules produced by algae, we were interested in the pigments and mycosporine-like amino acids (MAAs). There are different types of algal pigments, which can be either photosynthetic by capturing sufficient light energy for photosynthesis or photoprotective by dissipating solar energy and deactivating ROS (Rastogi et al. 2010). Thus, it is possible to distinguish chlorophyll-*a*, carotenoids (i.e. lutein, zeaxanthin, violaxanthin, antheraxanthin,  $\alpha$ - and  $\beta$ -carotene in red algae) and phycobiliproteins (i.e. phycoerythrin and phycocyanin), whose composition and levels vary according to space and/or time (Stengel et al. 2011). In addition, some red macroalgae are known to synthesize mycosporine-like amino acids, a class of at least 20 UV-screening compounds with a low molecular weight, soluble in water and with a maximum absorption between 310 and 360 nm (Rastogi et al. 2010; Lalegerie et al. 2019). To our knowledge, the first report of MAAs in *P. palmata* was by Karsten et al. (1998a). The photoprotective role of MAAs is widely recognised since it has been shown that strong solar radiation or different radiation treatments can cause an accumulation of MAAs (Figueroa et al. 2003; Barufi et al. 2012). However, MAAs could be multifunctional secondary metabolites as some studies have reported other potential roles such as anti-oxidants (Wada et al. 2015; Torres et al. 2018), osmolytes (Oren 1997), nitrogen storage (Korbee et al. 2005a; Zheng and Gao 2009) or protective molecules against desiccation or temperature variations (Wright et al. 2005; Oren and Gunde-Cimerman 2007). Thus, in the event of a possible increase in UV radiation, combined with an increase in anthropisation and eutrophication, an accumulation of MAAs can be expected.

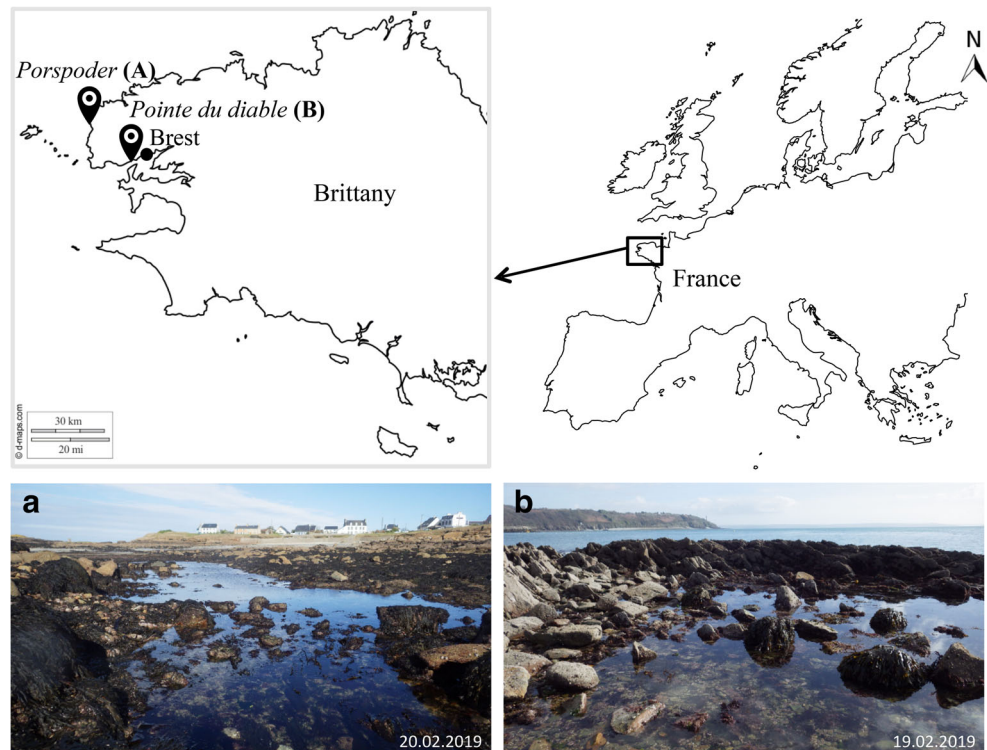
In this context, the objectives of the present study are to follow the composition of pigments and MAAs of *P. palmata*, in relation with multiple environmental parameters. Thus, the results will give new insights into the ecophysiological responses of *P. palmata*, especially in relation to light and nutrients. In particular, one of the challenges is to determine whether it is possible for some MAAs to have different or additional roles within the alga, which could explain such diversity of these compounds in red macroalgae.

## Materials and methods

### Seaweed collection

Samples of the red macroalga *Palmaria palmata* were collected in two different sites from Brittany (France): Porspoder (48° 28' 56.644" N–4° 46' 6.812" W) and Pointe du Diable (48° 21' 15.606" N–4° 33' 30.067" W) (Fig. 1). Sampling was carried out in triplicate every month during low tide at each site, from February 2018 to February 2019. Each replicate

**Fig. 1** Locations of the two sampling sites, Porspoder (a) and Pointe du Diable (b), in Brittany (France). The photographs were taken in February 2019



sample consisted of several thalli randomly collected a few metres apart. After collection, all samples were brought back to the laboratory and hand cleaned to remove epiphytes. Samples were then directly stored in a freezer ( $-20\text{ }^{\circ}\text{C}$ ) before freeze-drying ( $\beta 1-8$  LD plus, Christ) and grinding into a fine powder (MM400, Retsch). The dried powder was kept in darkness at room temperature until analysis, performed in the 2 months following the harvest.

Both sites differ in their hydrodynamism and substrate conditions: Porspoder is a sheltered site, with a sandy area protected by a rocky over-hang, with individuals of *P. palmata* living in a 20-cm-deep rocky tide channel largely covered by coarse sand (Fig. 1a); Pointe du Diable is a more exposed rocky site, characterized by the presence of tide pools, with individuals sampled in 30-cm deep steep-sided rocky tide pools (Fig. 1b). In addition, water temperature ( $^{\circ}\text{C}$ ), pH, salinity and nutrients (nitrates  $\text{NO}_3^-$  and phosphate  $\text{PO}_4^{3-}$  expressed in  $\mu\text{M}$ , and mentioned as N and P later in the manuscript) data were provided by ‘Service d’Observation en Milieu Littoral, INSU-CNRS, Saint-Anne du Portzic’, near Pointe du Diable site ( $48^{\circ} 21' 32.13'' \text{N}-4^{\circ} 33' 07.19'' \text{W}$ ). Daily sunshine duration (hours) and irradiance ( $\text{J. cm}^{-2}$ ) data from Brest-Guipavas were provided by Météo France ( $48^{\circ} 27' 00'' \text{N}-4^{\circ} 22' 59'' \text{W}$ ).

### Chlorophyll and carotenoids extraction and analysis

Chlorophyll-*a* and carotenoids were extracted according to Schmid and Stengel (2015). Extractions were carried out

using 75 mg dry weight (DW) of algal powder, in the presence of 750  $\mu\text{L}$  of a mixture of acetone:water (90:10), for 30 min at  $4\text{ }^{\circ}\text{C}$  under magnetic agitation. After this first extraction, samples were centrifuged at 10,000 rpm for 5 min (miniSpin plus, Eppendorf) and the supernatant was collected. The remaining pellets were again mixed with 90% acetone for 4 h at  $4\text{ }^{\circ}\text{C}$ . After the second extraction, samples were centrifuged and both supernatants were combined and filtered through 0.2  $\mu\text{m}$ -pore syringe filters.

HPLC analysis was performed directly after extraction on a Dionex Ultimate 3000 system equipped with a diode array detector (Thermo Scientific). Pigments were separated using an ACE  $\text{C}_{18}$  column ( $150 \times 4.6$  mm, 3  $\mu\text{m}$ ) protected with a guard column; the mobile phase consisted of (A) methanol:ammonium acetate buffer 0.5 M (80:20), (B) acetonitrile:Milli-Q water (87.5:12.5) and (C) ethylacetate 100%. A flow rate of  $1.0\text{ mL. min}^{-1}$  was used with the following gradient: 0 min 90:10 (A:B, v/v), 1 min 0:100 (A:B, v/v), 11 min 78:22 (B:C, v/v), 24 min 25:75 (B:C, v/v), 26 min 25:75 (B:C v/v), 27 min 100:0 (B:C, v/v), 28 min 90:10 (A:B, v/v) and 33 min 90:10 (A:B, v/v). The column temperature was set at  $30 \pm 1\text{ }^{\circ}\text{C}$ , and the injector temperature was  $5 \pm 1\text{ }^{\circ}\text{C}$ . Each sample was automatically diluted 3/4 with ammonium acetate buffer (0.5 M, pH 7.2), just before injection (volume of injection = 6  $\mu\text{L}$ ) to avoid degradation of pigments during the analysis and to achieve better separation. Pigments were identified by comparison of retention times and absorption spectra with commercial standards. They were quantified using standard curves, obtained by injections of different

concentrations of the commercial standards. Peaks below 1 mAU. min (or 0.4 mAU. min for  $\alpha$ - and  $\beta$ -carotene) were not taken into account for the quantification. HPLC data were collected using Chromeleon 0.7 software (Thermo Scientific Dionex, France).

### Phycobiliproteins extraction and analysis

The extraction of phycobiliproteins was made on 75 mg DW of algal powder with 1.5 mL of phosphate buffer (0.1 M, pH 6.8) added to extract phycoerythrin (PE) and phycocyanin (PC), using a protocol adapted from Sun et al. (2009). Two successive extractions of 15 min at 4 °C were performed, and samples were then centrifuged for 20 min at 10,000 rpm (miniSpin plus, Eppendorf). Supernatants were recovered to measure absorbances using a spectrophotometer (POLARStar Omega, BMG Labtech), and to estimate the amounts of PE and PC (mg. g<sup>-1</sup> DW) using the following equations from Beer and Eshel (1985):

$$[\text{PE}] = [(A_{565} - A_{592}) - (A_{455} - A_{592}) \times 0.20] \times 0.12$$

$$[\text{PC}] = [(A_{618} - A_{645}) - (A_{592} - A_{645}) \times 0.51] \times 0.15$$

### Mycosporine-like amino acid extraction and analysis

The mycosporine-like amino acids extraction and analyses were performed according to Lalegerie et al. (2019). Each sample consisted to 20 mg of dried seaweed powder added to 2 mL of 70% aqueous ethanol (v/v), macerated for 2 h at 45 °C with magnetic stirring. Then, the extract was centrifuged (centrifuge 5810, Eppendorf) at 1500 rpm for 10 min and the supernatant was transferred in Eppendorf tube. The pellet was re-extracted twice following the same procedure and the combined supernatants were evaporated to dryness using a miVac Duo Centrifugal Concentrator (Genevac, France) at 45 °C. The evaporated extracts were then stored at -20 °C before HPLC analysis. The extracts were shown to be stable and can be preserved in freezer for 4 to 5 months (data not shown).

Dried extracts were re-dissolved in 500  $\mu$ L of 2.5% aqueous methanol in 0.1% acetic acid in Milli-Q water, vortexed and filtered through 0.2  $\mu$ m-pore syringe filters. Filtrates were then analysed using a Dionex Ultimate 3000 HPLC system (Thermo Scientific, Germany). MAAs separation was achieved using a Zorbax Eclipse XDB C<sub>18</sub> column, 5  $\mu$ m, 4.6  $\times$  250 mm (Agilent, USA) protected with a guard-column. The HPLC eluent was 0.1% acetic acid in Milli-Q water, pumped isocratically at a flow of 1 mL. min<sup>-1</sup>, with an injection volume of 20  $\mu$ L. The column temperature was set at 25  $\pm$  1 °C and the injector temperature at 5  $\pm$  1 °C. MAAs were detected with a diode array detector at 320, 330, 334 and 360 nm, and data were collected using Chromeleon 0.7

software (Thermo Scientific Dionex, France). Identification was done by absorption spectra, retention time and previous LC-MS analyses (Lalegerie et al. 2019), and quantification was made using the published molar extinction coefficients  $\epsilon$  of Karsten et al. (1998a), according to Pelillo et al. (2004).

### Statistical analyses

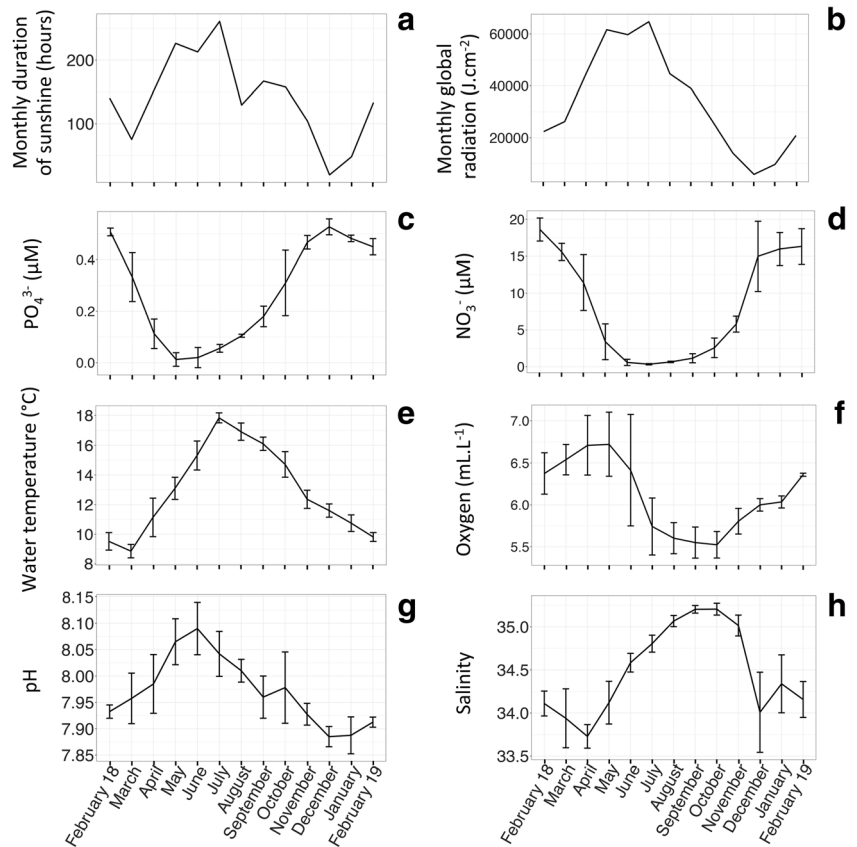
All data are expressed as mean  $\pm$  standard deviation (SD) of triplicate extractions. Comparisons were carried out using a Student's *t* test (2 samples) or an ANOVA (more than 2 samples), with a confidence level of 95%. The conditions of application were checked using the Shapiro test (normal distribution) and the *F* test or Bartlett's test (homogeneity of variance). The non-parametric Wilcoxon test (2 samples) or Scheirer-Ray-Hare (SHR, more than 2 samples) test was used when at least one of the conditions of application was not met. Post-hoc tests, i.e. multiple comparisons, were performed using a Tukey post-hoc test after an ANOVA (TukeyHSD) or a non-parametric post-hoc test after a Scheirer-Ray test (kruskalmc, pirms package). Principal component analyses (PCA) were carried out (package FactoMineR) to highlight which environmental factor influences MAA and pigment compositions. Daily light data (sunshine duration as the total number of hours; and global radiation corresponding to light intensity receive each day in J. cm<sup>-2</sup>) were accumulated over the 30 days prior to harvest, in the aim to establish a link with the composition of MAAs and pigments. In addition, to study the relationship between the different variables, Pearson's correlation coefficients were calculated (or Kendall's correlation coefficient if the normality was not respected). Finally, to establish potential links between MAAs and the synthesis pathway, a correlation matrix was generated using the 'corrplot' package. All the statistical analyses were carried out using the R program (R Development Core team 2008).

## Results

### Seasonal variability in environmental parameters

There was a significant seasonal variation for all environmental parameters, with light having the widest range of variation all along the monitoring period (Fig. 2a, b). Indeed, there was an increase in the monthly duration of sunshine and monthly global radiation from March to July, with a maximum reached in July (260.9 h and 64,732 J. cm<sup>-2</sup>, respectively). The light then gradually decreased from August until it reached a minimum value in December (19.5 h and 5874 J. cm<sup>-2</sup>, respectively). In addition, the seawater temperature increased in summer with a maximum of 17.83  $\pm$  0.34 °C in July, and the minimum was in March with 8.86  $\pm$  0.45 °C (Fig. 2e). Similarly, the pH increased in summer with a maximum of

**Fig. 2** Seasonal variations of **a** monthly duration of sunshine (hours), **b** monthly global radiation ( $J \cdot cm^{-2}$ ) **c** phosphates level ( $\mu M$ ), **d** nitrate level ( $\mu M$ ), **e** seawater temperature ( $^{\circ}C$ ), **f** oxygen level ( $mL \cdot L^{-1}$ ), **g** pH and **h** salinity, measured between February 2018 and February 2019. The light data were provided by ‘Météo France’, and other data were provided by ‘Service d’Observation en Milieu Littoral, INSU-CNRS, Saint-Anne du Portzic’



$8.09 \pm 0.05$  in June before decreasing (Fig. 2g). Salinity also increased in summer, slightly delayed compared to pH or temperature, i.e. continuously from April reaching a maximum in October ( $35.20 \pm 0.04$  (Fig. 2h). In contrast, the nutrients followed a reverse pattern as they decreased in summer (Fig. 2c, d). Indeed,  $NO_3^-$  and  $PO_4^{3-}$  were low between April and November, with a minimum in May–June for  $PO_4^{3-}$  ( $< 0.02 \mu M$ ), and between June and September for  $NO_3^-$  ( $< 2.00 \mu M$ ). Similarly, the oxygen level decreased after May, down to a minimum in October ( $5.52 \pm 0.16 mL \cdot L^{-1}$ ) (Fig. 2f).

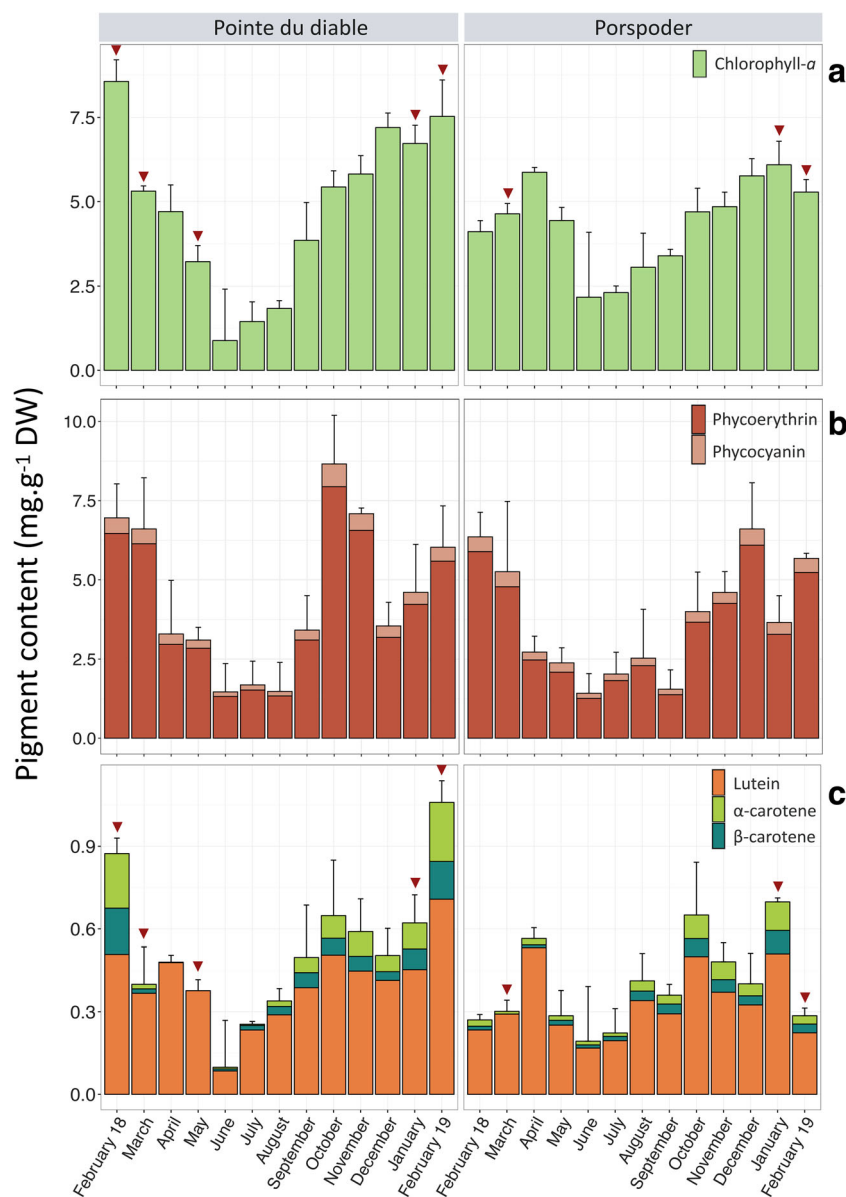
### Seasonal variability in pigments (chlorophyll-*a*, phycobiliproteins and carotenoids)

Seasonal variations in the pigment composition of *P. palmata* were observed for samples collected at both sites, Porspoder and Pointe du Diable (Fig. 3). Thus, in the samples collected from Pointe du Diable, the content of chlorophyll-*a* ranges from  $0.882 \pm 1.528 mg \cdot g^{-1} DW$  in June to  $8.556 \pm 0.643 mg \cdot g^{-1} DW$  in February 2018. Similarly, for the samples collected at Porspoder, chlorophyll-*a* content ranged from  $2.170 \pm 1.918$  to  $6.092 \pm 0.699 mg \cdot g^{-1} DW$ , in June 2018 and January 2019, respectively (Fig. 3a). Thus, although no significant difference between sites was detected (*t* test, *p*-value = 0.314), the chlorophyll-*a* content was significantly different between months (one-way ANOVA, *p*-value  $< 0.0001$ ), due to

the decrease in chlorophyll-*a* during the summer (June, July and August). The analysis of phycoerythrin and phycocyanin also revealed a temporal variation with a decrease in contents in summer (Fig. 3b), with a minimum in June ( $1.288 \pm 0.629$  and  $0.153 \pm 0.073 mg \cdot g^{-1} DW$  respectively). As for chlorophyll-*a*, there was no difference between sites in the phycobiliproteins contents (*t* test, *p*-value = 0.318 for PC and *p*-value = 0.166 for PE).

Similarly, lutein and  $\alpha$ - and  $\beta$ -carotene concentrations varied between months (Kruskal-Wallis test, *p*-value  $< 0.0001$ ) (Fig. 3c). Indeed, total carotenoid content was reduced in June 2018, to  $0.098 \pm 0.170 mg \cdot g^{-1}$  and  $0.193 \pm 0.199 mg \cdot g^{-1} DW$ , for the samples from Pointe du Diable and Porspoder respectively. This seasonal variability was higher for the lutein content with the lowest concentration in June ( $0.085 \pm 0.147$  and  $0.168 \pm 0.170 mg \cdot g^{-1} DW$  for the samples collected from Pointe du Diable and Porspoder respectively). In parallel, the proportion of  $\alpha$ - and  $\beta$ -carotene decreased, reaching less than 12% between March and May. Unlike other pigments, lutein showed a difference between sites (*t* test, *p*-value = 0.023). This difference was also observed for chlorophyll-*b* (expressed in area), a pigment specific to green algae not quantified due to the absence of a standard (Wilcoxon test, *p*-value = 0.028). In this sense, it should also be noted that both fucoxanthin and chlorophyll-*c*<sub>2</sub>, pigments found in brown algae, were also detected in some

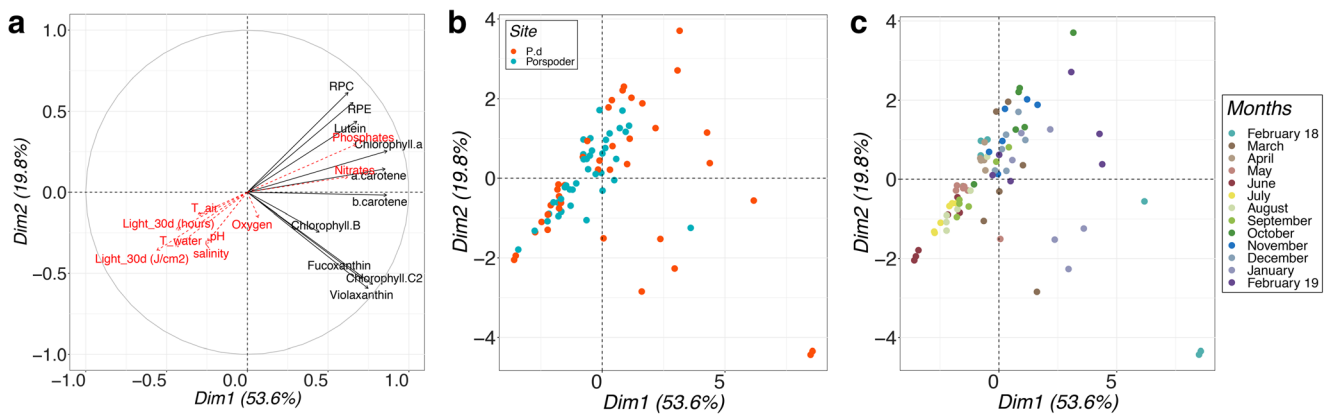
**Fig. 3** **a** Chlorophyll-*a*, **b** phycobiliproteins (phycoerythrin and phycocyanin), and **c** carotenoids (lutein,  $\alpha$ - and  $\beta$ -carotene) contents ( $\text{mg} \cdot \text{g}^{-1}$  DW) (mean  $\pm$  standard deviation (SD),  $n = 3$ ) of the red macroalga *Palmaria palmata* collected at 2 sites (Pointe du Diable and Porspoder) between February 2018 and February 2019. For carotenoids and phycobiliproteins, results are displayed as the stacked cumulative contents (SD of the cumulative concentrations). The red inverted triangle symbol indicates the presence of microscopic epi- or endophytes on *P. palmata* (presence of fucoxanthin, chlorophyll-*c* and chlorophyll-*b*)



samples collected in winter as indicated on Fig. 3, which may result in an overestimation of some pigments.

In addition, correlation analyses on all samples collected at both sites revealed a significant positive correlation between chlorophyll-*a* and lutein contents (Pearson's correlation,  $r = 0.744$ ,  $p$ -value  $< 0.0001$ ) and between chlorophyll-*a* and phycoerythrin or phycocyanin (Pearson's correlation,  $r = 0.665$  and  $r = 0.636$  respectively,  $p$ -value  $< 0.0001$ ). A PCA of all pigments showed that light and nutrients are the main factors contributing in the pigment composition, with the first two principal axes explaining 73.4% of the total variance in the pigments profile (Fig. 4). In this sense, light, both duration sunshine and global radiation, had negative effect on pigments whereas nutrients had a positive one. Thus, the results confirm that the overall pigment composition changed during the year (Fig. 4c), due to the

fluctuations in light and nutrients. More particularly, the decrease in chlorophyll-*a* (Fig. 3a) occurred at the same time as the increase in monthly irradiance and the decrease in nutrients (Fig. 2a-d). In consequence, chlorophyll-*a* was negatively correlated to global irradiance accumulated over 30 days before harvest (Kendall's correlation,  $\tau = -0.666$ ,  $p$ -value  $< 0.0001$ ) and to nutrients (Kendall's correlation,  $\tau = 0.600$  for nitrates and  $\tau = 0.556$  for phosphates,  $p$ -value  $< 0.0001$ ). However, a combination of multiple environmental parameters could affect this composition, including seawater temperature, pH and salinity (Fig. 4a). In addition, the individuals harvested in February 2018 and in January/February 2019 at Pointe du Diable stand out (Fig. 4b, c), which can be explained by the presence of epiphytes observed on thalli at this period (data not shown). In this sense, the axes of chlorophyll-*b*, fucoxanthin, chlorophyll-*c*<sub>2</sub> and



**Fig. 4** Principal component analysis (PCA) of pigment contents of the red macroalga *Palmaria palmata*, for samples collected between February 2018 and February 2019, at Pointe du Diable (P.d) and Porspoder (Brittany, France). **a** Variable factor map with pigments represented by the black lines and the environmental parameters by the red lines in [Supplementary variables](#). **b** Plot of individuals depending of the sampling site. **c** Plot of individuals depending of the sampling month. The

violaxanthin in Fig. 4a are in the same direction than the samples harvested in February 2018. Thus, pigment composition in regard to the two sites did not differ on the PCA analyses (Fig. 4b), except when the samples showed epiphytes, which were more numerous at Pointe du Diable.

### Seasonal variability in mycosporine-like amino acids

Analysis of mycosporine-like amino acids (MAAs) revealed the presence of 8 MAAs in *P. palmata*, including 7 identified: shinorine, palythine, asterina-330, porphyra-334, palythanol, usuriene and palythene. In addition to these 7 MAAs, there was an unknown MAA with a maximum absorption at 355 nm (Supplementary Fig. 1).

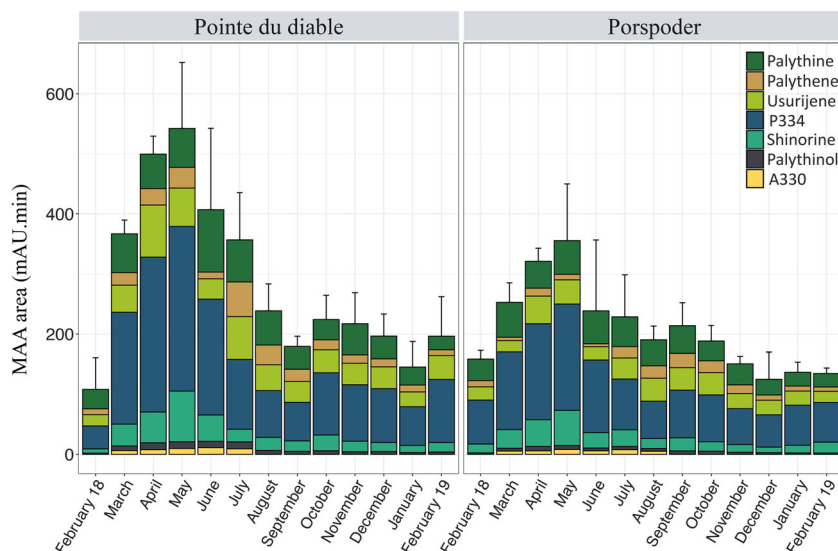
Knowing that for each MAA, the area of the peak varies according to the quantity of the molecule, the total MAA area/content exhibited significant variations between months (Kruskal-Wallis,  $p$ -value < 0.0001). Indeed, the total MAA area/content increased between February and May, by 5 for the samples from Pointe du Diable and by 2 for the samples from Porspoder (Fig. 5). This increase in total MAA content was higher for the samples collected from Pointe du Diable, resulting to a maximum of  $542.222 \pm 112.399$  mAU. min in May, while the maximum total MAA content was  $355.817 \pm 59.974$  mAU min in May for the samples from Porspoder. After May, the total MAA content decreased despite a maximum content of palythine in June ( $103.456 \pm 42.862$  mAU. min) and palythene in July ( $57.630 \pm 20.333$  mAU. min). This decrease in MAA content in summertime (June and July) occurred under high irradiance but at the same time as the decline of nutrient content and oxygen level (Fig. 2c–f). A principal component analysis showed that light and nutrients are the main factors contributing to the MAA composition, with the first two principal axes explaining

environmental parameters are seawater temperature in degree Celsius ( $T_{\text{water}}$ ), air temperature in degree Celsius ( $T_{\text{air}}$ ), oxygen concentration in milligrams per litre, nitrates and phosphates levels ( $\mu\text{M}$ ), salinity, pH, duration of sunshine as the total number of hours accumulated over the 30 days prior to harvest (Ligh\_30d (hours)) and global radiation corresponding to light intensity accumulated over the 30 days prior to harvest (Ligh\_30d ( $\text{J. cm}^{-2}$ ))

72.3% of the total variance (Fig. 6a). Contrary to pigments, light had positive influence on MAAs, whereas nutrients had a negative effect. Thus, 3 groups differed according to the time of year (Fig. 6c, d): (1) March to June with high light radiation and high oxygen water concentration, (2) July to October with high light radiation but low oxygen and nutrients and (3) the rest of the year with low light. No correlation was revealed between the total MAA area and global radiation accumulated over 30 days before harvest (Kendall's correlation,  $\tau = 0.360$ ,  $p$ -value < 0.0001) or nutrients (Kendall's correlation,  $\tau = -0.292$  for nitrates and  $\tau = -0.496$  for phosphates,  $p$ -value < 0.001). The total MAA content was not related to the chlorophyll-*a* content too (Kendall's correlation,  $\tau = -0.285$ ,  $p$ -value < 0.0001), suggesting that a combination of environmental parameters would affect the composition of MAAs, which can act at different intervals. In addition, although there was a significant difference in the total MAA area between the samples of Pointe du Diable and Porspoder (Wilcoxon test,  $p$ -value = 0.012), the PCA did not show any difference in the composition in MAAs between the 2 sites (Fig. 6b) since the composition and the ratio between the MAAs were similar in samples from both sites (Fig. 5).

Moreover, the MAA composition and the proportion of each MAA area changed between months (Fig. 5). Thus, the proportion of porphyra-334 and shinorine (as a percentage of the total MAA area) increased between February 2018 and May, from 35 to 51% and from 7 to 16% respectively. In addition, analyses showed the appearance of asterina-330 between March and August, whereas this MAA was not present (at least not detected) the rest of the year. In order to highlight potential relationships between MAAs, a correlation matrix was carried out and revealed only positive correlations between the different MAAs (Fig. 7a). More particularly, there were significant positive correlations between palythanol and

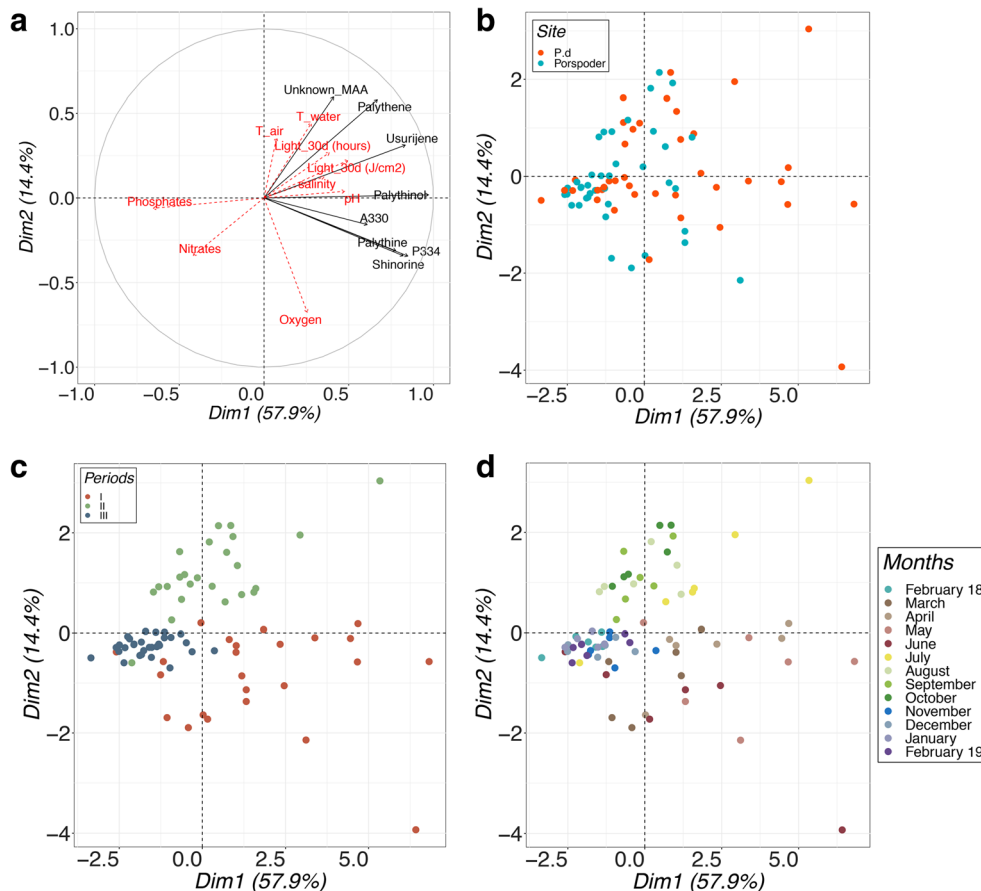
**Fig. 5** Mycosporine-like amino acid (MAA) composition of the red macroalga *Palmaria palmata* (mAU. min) (mean,  $n = 3$ ), collected between February 2018 and February 2019, from Pointe du Diable and Porspoder (Brittany, France). Results are displayed as the stacked cumulative content of each MAA



asterina-330 (Fig. 7c) (Kendall correlation,  $\tau = 0.590$ ,  $p$ -value  $< 0.0001$ ), between shinorine and porphyra-334 (Fig. 7b) (Kendall correlation,  $\tau = 0.714$ ,  $p$ -value  $< 0.0001$ ) and between palythinol and usurijene (Fig. 7d) (Kendall correlation,  $\tau = 0.704$ ,  $p$ -value  $< 0.0001$ ).

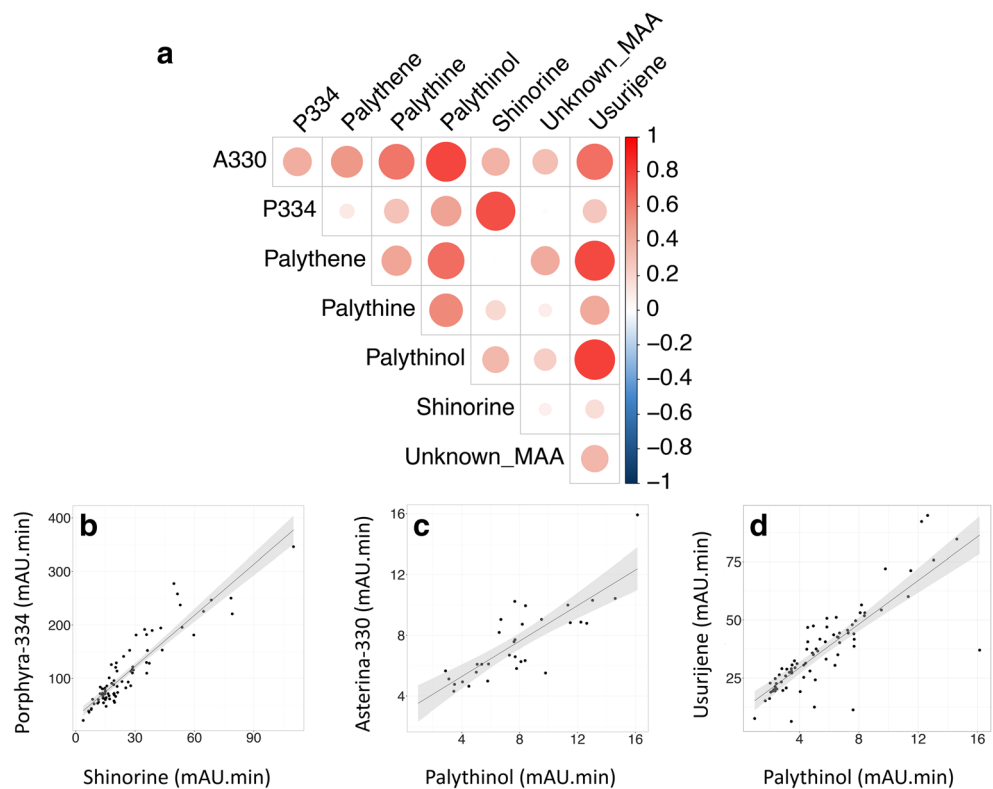
As no commercial standard is available, MAAs were quantified using the published molar extinction coefficients, except for the usurijene for which there is no reference (Supplementary Table 1). Thus, porphyra-334 was the MAA with the highest concentration, from  $0.391 \pm 0.164 \text{ mg. g}^{-1}$  DW in February 2018 to  $2.638 \pm 0.652 \text{ mg. g}^{-1}$  in May for

**Fig. 6** Principal component analysis (PCA) of MAA composition of the red macroalga *Palmaria palmata*, for samples collected between February 2018 and February 2019, from Pointe du Diable (P.d) and Porspoder (Brittany, France). **a** Variable factor map with MAAs represented by the black lines, and the environmental parameters by the red lines in supplementary variables. **b** Plot of individuals depending of the sampling site. **c** Plot of individuals depending to the time of the year (period I: March to June/period II: July to October/period III: November to February). **d** Plot of individuals depending of the sampling month. Refer to Fig. 4 for the description of environmental parameters





**Fig. 7** **a** Matrix correlation between the different MAAs (expressed in areas) of the red macroalga *Palmaria palmata* collected from Pointe du Diable and Porspoder between February 2018 and February 2019. The positive correlations are represented in red and the negative correlations in blue, with the size of the circle proportional to the Kendall's correlation coefficients. To examine correlations, linear regressions were performed between **b** shinorine and porphyra-334, **c** palythinol and asterina-330 and between **d** palythinol and usurijene areas (mAU. min) of *Palmaria palmata*



the samples collected at Pointe du Diable, representing between 33% of the total MAA area in July /August and up to 50% between March and May (Fig. 5).

## Discussion

The results of this study clearly demonstrate a high seasonal variability in the pigment and MAA content and composition in *P. palmata* collected on Brittany shores. These seasonal changes occurring in natural conditions can be explained by a combination of several environmental parameters, which may have synergic effects. Indeed, macroalgae, and more especially species living in the intertidal zone, are subjected to considerable environmental stresses, including notably wide variations in light, temperature, nutrients or salinity (Fogg 2001; Hurd et al. 2014).

In this sense, the seasonality in the pigment composition of marine macroalgae is well known (Sampath-Wiley et al. 2008; Esteban et al. 2015; Ismail and Osman 2016) and matches with the increase in light intensity ( $J. cm^{-2}$ ) and sunshine duration (hours) occurring in summer in the studied region (Brittany, France) (Fig. 2a, b). Indeed, during winter, the low sun radiations result in the fact that macroalgae require more chlorophyll-*a* to absorb enough light to maintain sufficient electron transport for photosynthesis. On the contrary, the chlorophyll-*a* content decreases under high irradiance when a maximum of photosynthetic activity has been reached to

avoid photodamage and photoinhibition (Raven and Hurd 2012). Thus, an important decrease in chlorophyll-*a* content in *P. palmata* was observed in June for both studied sites, whereas higher concentrations were observed in autumn and winter (Fig. 3a). Such pattern has been already demonstrated in many red macroalgae, including *P. palmata* (Sampath-Wiley et al. 2008; Schmid et al. 2017; Surget et al. 2017; Guihéneuf et al. 2018). Similarly, phycoerythrin and phycocyanin, photosynthetic accessory pigments whose contents were correlated with chlorophyll-*a*, had a lower concentration under high irradiance and a maximum content under low irradiance (Fig. 3b), as already demonstrated in *P. palmata* (Sagert and Schubert 2000; Martínez and Rico 2002; Guihéneuf et al. 2018). The fact that this species bleached in summer seems to support these results (Lalegerie, pers. observ.). In addition, analysis of the carotenoid composition revealed a decrease in lutein and  $\alpha$ -/ $\beta$ -carotene contents under high irradiance and the absence of zeaxanthin in *P. palmata* (Fig. 3c). Although the absence of zeaxanthin has already been demonstrated (Morgan et al. 1980; Esteban et al. 2009), there are few studies on the effect of light and seasonal variation on the carotenoid composition of *P. palmata*. Thus, the decrease in carotenoid contents in summer supports the fact that lutein and carotenes act as accessory light-harvesting pigments in this species (Hashimoto et al. 2016) and not as a photoprotective pigment, as it is sometimes described for higher plants (Niyogi et al. 1997; Telfer 2005; Jahns and Holzwarth 2012). This is confirmed by the correlation between lutein and chlorophyll-*a*

levels. In this sense, the photoprotective role of carotenoids in red algae still needs to be elucidated (Schubert and García-Mendoza 2008; Esteban et al. 2009).

In addition of light effect, a principal component analysis revealed an influence of nutrients on pigment concentrations (Fig. 4a). PCA results were confirmed by correlations between chlorophyll-*a* and nitrate or phosphate levels. Such results have already been demonstrated in many macroalgal species, including *P. palmata* (Martínez and Rico 2002; Corey et al. 2013; Parjikolaei et al. 2013). According to Martínez and Rico (2002), *P. palmata* would take advantage of high nitrogen and phosphorus concentrations in winter to create reserves to allow its growth in early summer, which is in agreement with our results, with low phosphate and nitrate values recorded in summer (Fig. 2c, d). Thus, due to their nitrogen nature/chemical structure, chlorophyll-*a* and phycobiliproteins would be a nitrogen storage for macroalgae, especially in winter when nutrient supply is high and a nitrogen source in summer when nutrient levels in the water are low (Barufi et al. 2011; Lapointe and Duke 1984; Stack et al. 2017), which would further reduce their concentration in addition to strong light radiations. Moreover, P and N were oriented in the same direction on the PCA (Fig. 4a) since both nutrient levels evolve in the same way in seawater (Fig. 2). However, it is possible that phosphates do not affect pigment level or have an indirect effect on pigments by decreasing concentrations of other phosphate-containing compounds involved in the photosynthesis, such as Rubisco (García-Sánchez et al. 1996). Similarly, as nutrient decrease and light increase occurred simultaneously, the decrease in carotenoids could be affected by both factors. Thus, Parjikolaei et al. (2013) have already demonstrated by culture experiments the effect of nutrients on carotenoid level of *P. palmata*, despite the fact that carotenoids do not contain nitrogen or phosphate in their structure.

Moreover, according to the PCA analysis, other environmental factors could affect pigment content by synergic effect with light or nutrients, more particularly seawater temperature and pH, whose axes are oriented in the same direction on the variable factor map (Fig. 4a). In this sense, it is sometimes difficult to distinguish the effect of light from the effect of temperature since the increase in these two factors occurs at the same time in natural environment (Duarte and Ferreira 1995; Liu and Dong 2001; Marinho-Soriano 2012; Ismail and Osman 2016). Nevertheless, some laboratory experiments have already demonstrated the important role of temperature on photosynthesis (Davison 1991; Kübler and Davison 1995; Zucchi and Necchi Jr 2001; Necchi 2004; Ding et al. 2013). Indeed, a change in temperature leads to a modification of the kinetics and quantity of enzymes involved in photosynthesis (Fogg 2001; Eggert 2012), affecting the electron transport rate and causing a modification in photosynthetic activity (Davison 1991; Hanelt et al. 2003; Eggert 2012). For red seaweeds, Kübler et al. (1991) have demonstrated in two

*Lomentaria* species (*L. baileyana* and *L. orcadensis*) that high temperature leads to an inhibition of photosynthesis due to the disruption of energy transfer between phycobilisomes and the photosystem II (PSII). Thus, each species has its own optimal photosynthesis temperature range which reflects seawater temperature in their natural environment (Dawes 1989; Necchi 2004). However, although many studies focus on the relationship between temperature and photosynthesis, the link between temperature and chlorophyll content is rarely discussed despite the fact that some studies have shown variations in chlorophyll content with temperature (Torres et al. 1991; Nejrup et al. 2013). As with photosynthesis, it can be assumed that there could be a temperature range for which pigment synthesis is optimal. Pakker et al. (2000) previously demonstrated that *P. palmata* could tolerate a temperature of 25 °C for few hours at low tide, like it occurs some days in summertime in temperate countries, but it could not support this temperature permanently. According to Morgan and Simpson (1981), the optimal growth seawater temperature for *P. palmata*, and therefore its optimal photosynthesis temperature, appears to be 10 °C.

Moreover, a variety of environmental parameters act simultaneously on rocky shores and sometimes their effect on the composition of macroalgae is amplified by their association. For example, light and temperature have an indirect effect on pigment content through the enhancement of salinity and desiccation stress (Beauchamp 2012). In addition, it has already been established that photosynthesis and chlorophyll-*a* content could be influenced by pH associated with the carbon dioxide (CO<sub>2</sub>) level in seawater, since CO<sub>2</sub> is the substrate for photosynthesis (Middelboe and Hansen 2007; Zou and Gao 2010; Gauna et al. 2013). According to Sebök et al. (2017), high CO<sub>2</sub> concentrations result in a significant increase in light-harvesting efficiency of *P. palmata*. Similarly, according to Wu et al. (2008), faced with an increase in inorganic carbon, algae have a similar response to an increase in light, resulting in a decrease in pigment content and bleaching. Nevertheless, it is difficult to make a link between pH and pigment content without culture experiments because pH is relatively constant through one year in environmental habitat contrary to others parameters. It is likely that pH does not directly influence pigment concentration but acts indirectly by influencing other cellular compounds or mechanisms involved in photosynthesis.

Finally, no difference in pigment composition was detected between both sampling sites, with the exception of lutein and chlorophyll-*b*. The presence of these two pigments were linked to the abundance in green algal epiphytes or endophytes (for which lutein is a major pigment according to Takaichi 2011) that still remained at the surface of the thalli after cleaning, influencing the positioning of contaminated individuals on the PCA in the direction of epiphytic pigments (Fig. 4a, c). It should be noted that both fucoxanthin and

chlorophyll- $c_2$  were also detected in some samples collected in winter as indicated on Fig. 3; both pigments are found in brown algae and thus epiphytes or endophytes of *P. palmata* (Stengel et al. 2011). Since the content of chlorophyll- $a$ , phycobiliproteins and  $\alpha$ -/ $\beta$ -carotene did not vary, this suggests that light is not a divergent factor between the two sites.

In a second part, the analysis of the mycosporine-like amino acids revealed the presence of 8 MAAs in *P. palmata* including shinorine, palythine, porphyra-334, asterina-330, palythanol, usuriene and palythene. This composition for *P. palmata* was in accordance with the literature, with porphyra-334 being the most important MAA (Karsten et al. 1998a; Yuan et al. 2009; Guihéneuf et al. 2018; Lalegerie et al. 2019). In addition to these 7 MAAs, mycosporine-glycine has also already been detected in *P. palmata*, but the unknown MAA in this study does not have the same absorption maximum (Karsten and Wiencke 1999) (Supplementary Fig. 1b). Thus, although the presence of MAAs in red algae is already widely demonstrated, *P. palmata* appears as one of the species with the greatest diversity in MAAs, such as *Agarophyton vermiculophyllum* (previously *Gracilaria vermiculophylla*), *Vertebrata lanosa* or *Bostrychia scorpioides* (Sinha et al. 2007; Lalegerie et al. 2019).

Moreover, as for pigments, a seasonal variability has been observed in the MAA content, with a maximum content in May. This increase is related to the increase in monthly irradiance occurring at the same time. Then, shinorine and porphyra-334 would be most responsible for this variability in MAA content in this species. These results confirm the photoprotective role attributed to MAAs (Franklin et al. 2001; Karsten et al. 1999; Conde et al. 2000) and their potential antioxidant activity by reducing excess light/quenching the ROS produced under high light (De la Coba et al. 2009; Wada et al. 2015). Indeed, several studies have already shown that MAA content would be affected by PAR and UV radiations or negatively correlated with water depth (Karsten et al. 1998b; Karsten and Wiencke 1999; Hoyer et al. 2001). In addition, some studies had already shown a variability in the MAA content of *P. palmata*. For example, Aguilera et al. (2002) had demonstrated an increase in MAA content in *P. palmata* samples collected between 3 and 4 m depth between June and August in an Arctic fjord in Norway, due to the increase in sun radiation caused by sea-ice break up. Similarly, Guihéneuf et al. (2018) studied seasonal changes in MAA composition of three red macroalgae, *P. palmata*, *Chondrus crispus* and *Porphyra dioica* collected in Galway Bay (Ireland) between July 2014 and July 2015, with respect to several environmental parameters. Thus, in accordance with the results of the present study, they observed an increase in the MAA content of *P. palmata* in relation with the increasing irradiance, with a maximum in April 2015. However, as in Guihéneuf et al. (2018), our results suggest that the MAA content decreased in summer (from May in this study) and

was low in June and July, and this, despite a maximum in the asterina-330 and palythene contents in June and July, respectively. This result is unexpected for photoprotective molecules, especially since according to environmental data, the maximum monthly irradiance and sunshine duration were in July (Fig. 2a, b). This decrease suggests the influence of other environmental factors, in opposition to the light. One possible explanation for the decrease in MAA content from May could be that the total MAA content, and more especially shinorine and porphyra-334, was limited by the low seawater nutrient concentrations as already discussed in Guihéneuf et al. (2018). A decrease in MAA content relative to phosphate concentration has already been suggested by Briani et al. (2018) and could be explained by the fact that the synthesis of MAAs via the shikimate pathway requires some intermediates containing phosphate, as 3-deoxy-D-arabinoheptulosinate-7-phosphate (DAHP) (Shick and Dunlap 2002). Some authors suggested also that the synthesis of MAAs could occur via the pentose phosphate pathway, which is phosphate dependant (Rosic 2012; Pope et al. 2015). More particularly, as nitrogen compounds, it can be supposed that, despite a high light stress, seaweeds are unable to synthesize MAAs in summer because of the low nitrogen availability in seawater. Some studies previously highlighted evidences about the regulation of MAAs by synergistic effect between light and nutrients. Notably, nitrogen and ammonium availabilities have already been shown to be limiting factors in the accumulation of MAAs in different species, such as *P. palmata* (Morgan and Simpson 1981), *Porphyra* sp. (Korbee et al. 2005a) and *Grateloupia lanceola* (Huovinen et al. 2006). However, a second hypothesis is that MAAs could be used as a nitrogen source in summer when nutrients are limited in seawater. This could highlight a second role of these molecules, in addition to be photo-protective. This idea of a second role is supported by the fact that the total MAA content was different between the 2 sites. Indeed, there was a two-fold increase in the total MAA content between February 2018 and May at Porspoder, while it was five-fold at Pointe du Diable. This difference can be explained by the fact that Porspoder is characterized by a lower hydrodynamism than Pointe du Diable. Thus, the nutrient level at Porspoder is lower maybe due to a lower water mixing and MAAs could then be used as nitrogen source for the individuals from this site. Thus, as already suggested, MAAs can be considered as multipurpose compounds with various roles for seaweeds: photo-protective agent, antioxidant compounds and nitrogen source.

Because of light and nutrient effects, 3 groups stand out according to the time of year according to PCA analysis (Fig. 6c, d): first, (1) from March to June, there is an increasing light radiation/sunshine duration and still a relatively high level of nutrients and  $O_2$ . Thus, there is a significant photosynthesis with a relatively high level of pigments, and an increase in the synthesis of MAAs for their photoprotective role/antioxidant

activity. Then, (2) from July to October, there is still high light intensities but the levels of nutrients and oxygen are decreasing. Thus, the pigment level decreases and photosynthesis is reduced to avoid photo-damages. In parallel, the algal metabolism is slowed down, and the amounts of nutrients in the water are no longer sufficient to synthesize MAAs, whose pool formed during spring is slowly reduced. Then, (3) in winter, the light decreases which results in an increase in the level of pigments and the stabilisation of MAA concentrations. In addition, MAAs could also be affected by multiple other environmental stresses that could have a synergic effect with light, including seawater temperature and pH (Fig. 4a).

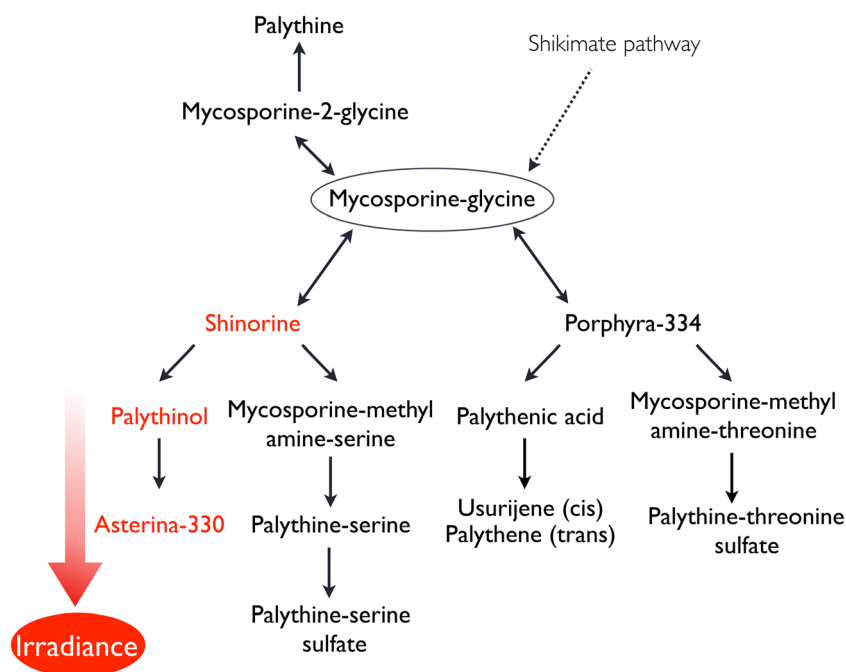
There are few studies investigating the factors controlling the synthesis and accumulation of each individual MAA in macroalgae. In this sense, the analysis of correlations between MAAs revealed relationships (Fig. 7) in agreement with the hypothetical biosynthesis pathway of the MAAs (Fig. 8). Notably, shinorine and porphyra-334, which were both correlated together (Fig. 7b), would be equally synthesized from mycosporine-glycine in response to an increased irradiance. We should have also detected mycosporine-2-glycine in an equivalent way. This last MAA was not detected in the samples, which can be explained by the fact that it was directly transformed into palythine (Fig. 8), which had a maximum content in June. In comparison, Franklin et al. (2001) demonstrated that the growth of *Chondrus crispus* under UV radiations lead to the synthesis of shinorine rather than palythine. In addition, Karsten and Wiencke (1999) showed that *P. palmata* synthesized shinorine, porphyra-334 and palythine in equivalent amounts for samples collected at a depth of 4 or 10 m,

while palythine was more synthesized for samples collected at a depth of 2 m. Similarly, according to the same authors, UV-A seemed to enhance shinorine synthesis while porphyra-334 seemed to be independent of the amount of UV-A or UV-B. The use of white, blue, red or green lights would also change the proportion of some MAAs (Korbee et al. 2005b).

Moreover, the results of this present study showed that asterina-330 was significantly synthesized between March and August but not the rest of the year and that its content was correlated with the one of palythanol. Thus, according to the synthesis pathway of MAAs, it seems necessary to have enough light to synthesize enough shinorine, then palythanol and finally asterina-330 (Fig. 8). Thus, this synthesis pathway would be favoured under high irradiance. Torres et al. (2015) had already demonstrated the accumulation of palythanol under high PAR in *Gracilariopsis tenuifrons*.

All these results confirm that there are different synthesis pathways for the different MAAs as the concentration of the individual MAA can change independently, depending on several factors, such as the quantity and the quality of light. However, all the correlations were positive which suggests that in response to an environmental change, the synthesis of all MAAs increases and decreases simultaneously. Thus, MAAs such as palythanol and usurijene may appear correlated while there is no apparent link in their synthesis pathways. In addition, with high light intensity, the overall increase in the synthesis of some MAAs precursors at the base of the synthesis pathway may result in the appearance or increase in the synthesis of other MAAs by interconversion, which can for example explain the appearance of asterina-330.

**Fig. 8** Hypothetic pathways for the biosynthesis of some MAAs in red algae (adapted from Carreto et al. 2005; Sinha et al. 2007; Bhatia et al. 2011)



Nevertheless, each MAA could have different role, which would explain such high diversity and the different synthesis pathways. In this sense, it has already been shown that MAAs can also act as protective agents against desiccation and osmotic stresses in cyanobacteria (Oren 1997; Oren and Gunde-Cimerman 2007). According to Wada et al. (2015), the different structures of MAAs, substituted, derivated or hybrid MAAs could have been diversified during the environmental adaptation of organisms and be linked to their antioxidant activity. Thus, MAAs could be key substances in the acclimatation of *P. palmata* to different stress conditions including extreme UV radiation and other potential factors such as temperature, CO<sub>2</sub> or nutrients. This study is the first to compare the seasonal composition and biosynthesis pathway of MAAs in a red macroalga. To date, the MAA synthesis pathway has been studied mainly in cyanobacteria or phytoplankton, but rarely in macroalgae (Portwich and Garcia-Pichel 2003; Callone et al. 2006; Rosic 2012; Pathak et al. 2019). Further research including culture experiments is needed to understand the factors responsible for the biotransformations of the different MAAs and to determine the genes and enzymes involved in their synthesis. The latter are still unknown, particularly in red macroalgae which are known for their high diversity in MAAs (Rastogi et al. 2017; Lalegerie et al. 2019), with new MAAs regularly discovered (Hartmann et al. 2015; Orfanoudaki et al. 2019).

**Acknowledgements** This study is part of the Ph.D. thesis work carried out by the first author within the Laboratoire des Sciences de l'Environnement Marin (LEMAR UMR 6539) set at the European Institute for Marine Studies (IUEM), marine Institute from the University of Western Brittany (UBO, Brest). We thank UBO and the Région Bretagne, through the Doctoral School of Marine and Coastal Sciences, for funding Fanny Lalegerie's PhD. We thank Marie-Aude Poullaouec for the sample grinding.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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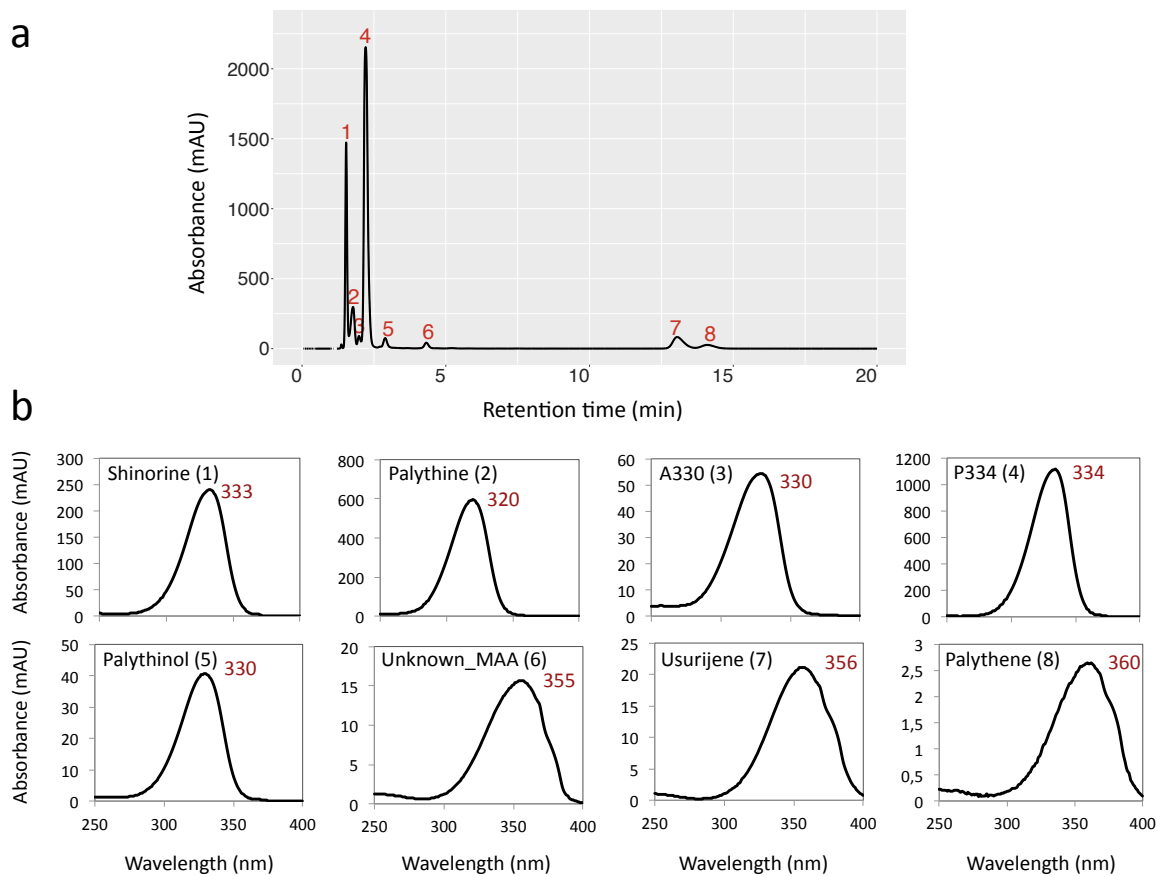
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## Supplementary data

**Supplementary Figure 1.** (a) HPLC chromatogram (at 330 nm) of MAAs extracted from the red macroalga *Palmaria palmata* collected in May 2018 from Pointe du Diable (Brittany, France). (b) The absorption spectra of the 8 MAAs detected are represented with their maximum absorption ( $\lambda_{\text{max}}$ ) between 320 and 360 nm.



**Supplementary Table 1.** Quantification of MAAs from the red macroalga *Palmaria palmata* collected between February 2018 and February 2019 from Pointe du Diable and Porspoder (Brittany, France). Results are expressed in mg. g<sup>-1</sup> DW (mean ± standard deviation, n=3) and were made according to Pelillo *et al.* (2004), using the published molar extinction coefficients  $\epsilon$  of Karsten *et al.* (1998a) for MAAs for which data were available.

	Shinorine	P334	A330	Palythine	Palythene	Palythinol	
Pointe du diable	February 18	0.066 ± 0.034	0.391 ± 0.164	-	0.273 ± 0.145	0.069 ± 0.044	0.018 ± 0.009
	March	0.311 ± 0.040	1.756 ± 0.099	0.049 ± 0.001	0.503 ± 0.031	0.136 ± 0.078	0.061 ± 0.006
	April	0.474 ± 0.036	2.614 ± 0.167	0.064 ± 0.018	0.486 ± 0.178	0.196 ± 0.074	0.100 ± 0.018
	May	0.738 ± 0.214	2.638 ± 0.652	0.076 ± 0.005	0.513 ± 0.052	0.231 ± 0.033	0.092 ± 0.015
	June	0.382 ± 0.175	1.869 ± 0.428	0.087 ± 0.028	0.820 ± 0.282	0.078 ± 0.079	0.088 ± 0.032
	July	0.190 ± 0.053	1.146 ± 0.328	0.074 ± 0.009	0.569 ± 0.050	0.392 ± 0.139	0.095 ± 0.027
	August	0.203 ± 0.042	0.802 ± 0.150	-	0.484 ± 0.125	0.232 ± 0.091	0.057 ± 0.014
	September	0.162 ± 0.020	0.652 ± 0.143	-	0.313 ± 0.072	0.144 ± 0.027	0.041 ± 0.002
	October	0.189 ± 0.147	0.814 ± 0.632	-	0.218 ± 0.171	0.097 ± 0.078	0.040 ± 0.031
	november	0.156 ± 0.20	0.912 ± 0.210	-	0.413 ± 0.037	0.093 ± 0.024	0.035 ± 0.010
	December	0.135 ± 0.034	0.880 ± 0.145	-	0.306 ± 0.021	0.091 ± 0.011	0.038 ± 0.007
	January	0.113 ± 0.035	0.667 ± 0.218	-	0.254 ± 0.074	0.081 ± 0.026	0.025 ± 0.007
	February 19	0.146 ± 0.062	1.077 ± 0.408	-	0.192 ± 0.064	0.064 ± 0.016	0.034 ± 0.012
Porspoder	February 18	0.134 ± 0.008	0.731 ± 0.114	-	0.295 ± 0.018	0.072 ± 0.05	0.021 ± 0.002
	March	0.285 ± 0.016	1.306 ± 0.093	0.045 ± 0.002	0.488 ± 0.052	0.040 ± 0.019	0.041 ± 0.004
	April	0.407 ± 0.075	1.607 ± 0.303	0.048 ± 0.007	0.373 ± 0.059	0.094 ± 0.039	0.064 ± 0.013
	May	0.521 ± 0.217	1.721 ± 0.557	0.064 ± 0.020	0.452 ± 0.178	0.063 ± 0.030	0.054 ± 0.013
	June	0.216 ± 0.084	1.113 ± 0.610	0.044 ± 0.012	0.419 ± 0.099	0.032 ± 0.033	0.037 ± 0.020
	July	0.244 ± 0.062	0.821 ± 0.218	0.061 ± 0.016	0.399 ± 0.059	0.122 ± 0.065	0.045 ± 0.016
	August	0.152 ± 0.026	0.618 ± 0.054	0.042 ± 0.007	0.354 ± 0.075	0.143 ± 0.060	0.039 ± 0.011
	September	0.203 ± 0.039	0.807 ± 0.157	-	0.384 ± 0.049	0.168 ± 0.027	0.049 ± 0.010
	October	0.148 ± 0.036	0.796 ± 0.152	-	0.275 ± 0.077	0.139 ± 0.046	0.045 ± 0.013
	november	0.119 ± 0.023	0.615 ± 0.092	-	0.291 ± 0.041	0.100 ± 0.047	0.030 ± 0.006
	December	0.084 ± 0.029	0.532 ± 0.215	-	0.214 ± 0.055	0.059 ± 0.018	0.023 ± 0.006
	January	0.112 ± 0.024	0.652 ± 0.107	-	0.185 ± 0.015	0.057 ± 0.005	0.020 ± 0.003
	February 19	0.164 ± 0.024	0.661 ± 0.079	-	0.183 ± 0.035	0.049 ± 0.018	0.018 ± 0.003



## ENGLISH

The *in-situ* monitoring of *Palmaria palmata* (previous section) highlighted a temporal variation in its MAA composition. Indeed, results showed that the total MAA content increased simultaneously with the increase in light from February to May, confirming the photoprotective role attributed to these compounds. More particularly, results revealed that the MAA asterina-330 is synthesised only during a part of the year (*i.e.* between March and July), supporting its importance in the photoprotection of *P. palmata*. Then, analysis showed a decrease in MAA content after May, despite high irradiance occurring in summer. This result suggested that the synthesis of MAAs could be influenced by other environmental parameters, and more particularly nutrient level which was limited in summer. In this regard, the next part consists of a cultivation experiment, to test the impact of particular factors on the MAA composition of the red alga *Palmaria palmata*, under controlled conditions. Then, can the synthesis of a particular MAA be induced? More particularly, do some MAAs have a specific role in the acclimation of *P. palmata* to UV radiation and/or nutrient level?

## FRANÇAIS

Le suivi *in-situ* de *Palmaria palmata* (section précédente) a mis en évidence une variation temporelle de sa composition en MAAs. En effet, les résultats ont montré une augmentation de la teneur totale en MAAs simultanément à l'augmentation de la lumière de février à mai, confirmant le rôle photoprotecteur attribué à ces composés. Plus particulièrement, les résultats ont révélé qu'un MAA, l'astérina-330, n'est synthétisé que pendant une partie de l'année (*i.e.* entre mars et juillet), ce qui confirme son importance dans la photoprotection de *P. palmata*. Ensuite, l'analyse a montré une diminution de la teneur en MAAs après mai, malgré une intensité lumineuse élevée en été. Ce résultat suggère que la synthèse des MAAs pourrait être influencée par d'autres paramètres environnementaux, et plus particulièrement par les concentrations en nutriments qui sont limitées à cette période de l'année. À cet égard, la partie suivante consiste en une expérience de culture en conditions contrôlées, pour tester l'impact de facteurs particuliers sur la composition en MAAs de l'algue rouge *Palmaria palmata*. Ainsi, la synthèse d'un MAA en particulier peut-elle être induite ? Plus particulièrement, certains MAAs ont-ils un rôle spécifique dans l'acclimatation de *P. palmata* face aux rayonnements UV et/ou à la teneur en nutriments ?

## **Research note - A cultivation experiment on the red macroalga *Palmaria palmata*: effect of UV radiation and nutrients on mycosporine-like amino acids and pigments**

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In preparation, to be submitted to: *Journal of experimental marine biology and ecology*

### **RESUME**

L'effet combiné de la lumière (*i.e.* avec ou sans radiations UV) et des nutriments (*i.e.* eau de mer enrichie avec 0, 100 ou 300  $\mu\text{M}$  de  $\text{NaNO}_3$ ) sur la synthèse d'acides aminés de type mycosporine (MAA) et de pigments a été étudié sur la macroalgue rouge *Palmaria palmata* cultivée pendant 21 jours dans des bacs de 1 L. Les données ont montré une diminution de la teneur totale en MAAs et en pigments, probablement due aux conditions expérimentales, *i.e.* à une quantité insuffisante de l'intensité lumineuse appliquée pendant l'expérience par rapport à l'environnement naturel. Ensuite, les résultats ont suggéré un effet positif de l'azote sur les teneurs en MAAs et en pigments, car l'apport en nitrate a limité la diminution de leurs concentrations. Néanmoins, il est possible que l'apport en nutriments ait également été insuffisant. À cet égard, deux hypothèses se présentent : (i) le manque d'azote a conduit à une synthèse limitée des MAAs, et/ou (ii) les MAAs sont potentiellement utilisés comme source d'azote par les macroalgues rouges. Enfin, aucun effet des UV n'a été détecté sur la synthèse des MAAs connus pour leur rôle photoprotecteur, ce qui pourrait être dû à la limitation de leur synthèse dans les conditions de culture, et/ou à une quantité insuffisante de rayonnement UV. L'usurijène est le seul MAA qui n'a montré aucune variation entre les différents apports en nitrates, et qui présentait même une teneur légèrement plus élevée au 21<sup>e</sup> jour par rapport au début de l'expérience, sous exposition aux rayons UV. Ceci suggère une voie d'induction ou de synthèse spécifique de cet MAA. Les données ont donc confirmé l'hypothèse précédente sur l'effet des nutriments et de la lumière sur la synthèse des MAAs, tout en apportant de nouvelles informations sur la synthèse spécifique de l'usurijène.

**Mots-clés:** Expérience de culture, MAAs, Pigment, radiations UV, Ajout de nitrates, Macroalgues rouges, Usurijène

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## ABSTRACT

The combined effect of light (i.e. with or without UV radiation) and nutrients (i.e. enriched seawater with 0, 100 or 300  $\mu\text{M}$  of  $\text{NaNO}_3$ ) on the accumulation of mycosporine-like amino acids (MAAs) and pigments was investigated on the red macroalga *Palmaria palmata* cultivated for 21 days in 1-L tanks. Data showed a decrease in both total MAA and pigment contents probably due to experimental conditions, i.e. insufficient amount of light radiation applied during the experiment compared to the natural environment. Then, results suggested a positive effect of nitrogen on MAA and pigment contents, as nitrate supply has limited the decrease in their concentrations. Nevertheless, it is possible that the nutrient supply may have been insufficient too. In this regard, there are two hypotheses: (i) nitrogen starvation led to a limited synthesis of MAAs, and / or (ii) MAAs are potentially used as a nitrogen source for red macroalgae. Lastly, no effect of UV has been detected on the synthesis of MAAs known as photoprotective compounds, which could be due to the limitation of their synthesis under cultivation conditions, and/or by an insufficient amount of UV radiation. Usurijene was the only MAA showing no variation between different nitrate supplies, and that exhibited a slightly higher content at day 21 than at the beginning of the experiment when exposed to UV radiation, suggesting a specific induction or synthesis pathway of this MAA. The data therefore confirmed the previous hypothesis on the effect of nutrients and light on the synthesis of MAAs, while providing new insights on the specific synthesis of usurijene.

**Keywords:** Cultivation experiment, MAAs, Pigment, UV radiation, Nitrate supply, Red macroalgae, Usurijene

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## INTRODUCTION

Mycosporine-like amino acids (MAAs) constitute a group of secondary metabolites with a maximal absorption in the UV region (310 – 360 nm) (Sinha *et al.* 2007; Bedoux *et al.* 2020). In this way, they are generally described as UV-screening substances. Many studies have then demonstrated a synthesis and accumulation of MAAs under UV exposition and/or high irradiance, coupled to a decrease in photosynthetic pigments. For example, Peinado *et al.* (2004) demonstrated a 29 % and 5 % increase of MAAs in *Pyropia columbina* exposed to UV-A and UV-B, respectively. Similarly, Torres *et al.* (2015) demonstrated a correlation

between palythanol amount and irradiance in *Gracilariopsis tenuifrons*. More recently, Álvarez-Gómez *et al.* (2019) showed a 30 %, 40 % and 50 % increase in total MAA content in *Halopithys incurva*, *Gracilariopsis longissima*, and *Crassiphycus corneus* respectively, when exposed to photosynthetically active radiation (PAR) + UV-A + UV-B treatment. Moreover, Karsten *et al.* (1998) showed the induction of the synthesis of shinorine, palythine and palythene in *Chondrus crispus* when exposed for 24 h to PAR. In the same way, it has been demonstrated that the synthesis of asterina-330 in wild/*in situ* populations of *Palmaria palmata*, is induced under high irradiance (Lalegerie *et al.* 2020).

In this context, although their photoprotective role is widely accepted, MAAs could also have secondary bioactivities such as being osmoregulatory, thermoregulatory or antioxidant compounds (Bedoux *et al.* 2020). Indeed, the role of each individual MAA is not completely understood, as well as their synthesis pathway not completely elucidated (Bedoux *et al.* 2020; Belcour *et al.* 2020). In this way, the synthesis and accumulation of some MAAs could be induced under specific environmental conditions, several studies suggesting that MAAs are not only dependent on light, but also of other environmental parameters such as salinity or temperature (Oren and Gunde-Cimerman 2007). Then, MAAs exhibit seasonal variations depending on each algal species (Jofre *et al.* 2020; Guihéneuf *et al.* 2018; Lalegerie *et al.* 2020). More particularly, MAAs are nitrogenous compounds, and their synthesis could be affected by nutrient availability, as for example in *Porphyra umbilicalis* and *Neopyropia leucosticta* (Korbee *et al.* 2005), or *Grateloupia lanceola* (Huovinen *et al.* 2006), whose MAAs content appeared to depend on the ammonium level in the seawater. Similarly, Figueroa *et al.* (2008) demonstrated in *Asparagopsis armata* that the content of individual MAA was dependant of nitrogen (N) level, and that shinorine percentage decreased with N fluxes, compared to palythine which increased. More recently, in a previous field study we demonstrated that the accumulation of MAAs under high irradiance in summer could be limited by low nutrient content in seawater occurring at the same period (Lalegerie *et al.* 2020). In this context, the MAA and pigment accumulation under combined exposition to UV radiation and nutrient supply was investigated in the red alga *Palmaria palmata*, known for its high diversity in MAAs (Lalegerie *et al.* 2019), in order to contribute to the general understanding of the synthesis of these compounds.

## MATERIAL AND METHODS

*Palmaria palmata* (Linnaeus) F. Weber & D. Mohr was collected at Portsall (Brittany, France, 48°33'53''N – 4°42'5''W) in March 2017. Thalli were PAR-acclimated within three days and maintained during 21 days in 1-L tanks filled with seawater, and supplied with 0, 100 or 300  $\mu\text{M}$  of  $\text{NaNO}_3$ . Several thalli ( $n=3$ ) were cultivated for each treatment and used as replicates. Tanks were artificially illuminated with photosynthetically active radiation (PAR, 400-700nm), or with PAR+UV radiation (addition of UVA 315-400 nm), with a 16 h light : 8 h dark cycle. Light intensity (PAR and PAR+UV) was less than 65  $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ , which was lower than in-situ conditions and light conditions commonly used in laboratory experiments (Peinado *et al.* 2004; Bonomi-Barufi *et al.* 2012; 2020; Parjikolaei *et al.* 2013;), but corresponded to the maximum limit of our equipment. The seawater was changed every three days, and samples were collected at day 0 (start of experiment), 7, 15 and 21, and freeze-dried before metabolomic analyses. Water temperature in tanks varied between 15 ° and 18 °C during the experiment. MAAs and apolar pigments were extracted with 70 % ethanol and 90 % acetone respectively, and analysed by HPLC according to the protocols described in Lalegerie *et al.* (2019). In addition phycobiliproteins (*i.e.* phycoerythrin and phycocyanin) were extracted with a phosphate buffer (0.1 M), and quantified by spectrophotometry, according to Lalegerie *et al.* (2019).

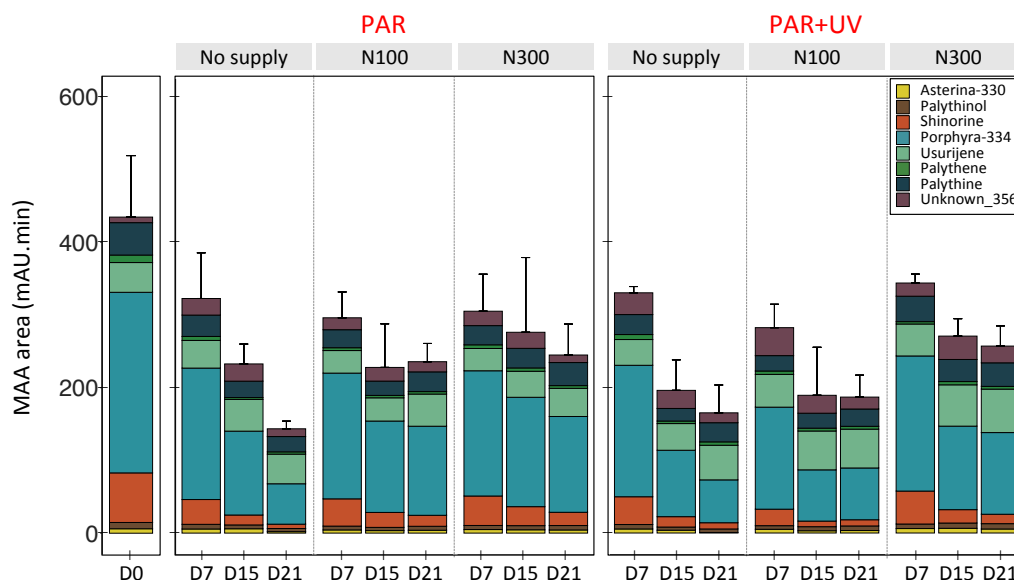
The effect of light and/or nutrient were analysed by Student's test or multi-way ANOVA, according to the "day", "light", and/or "nutrient" factors. The Tukey post-hoc test was performed following ANOVA in order to compare the different groups. Statistical analyses were performed using R software (version 3.6.1) (R Core Team 2019) through the integrated development environment Rstudio (version 1.2.5001) (Rstudio Team 2019). The normality and homogeneity of variances were checked with Shapiro test and F-test respectively. Results are expressed as mean  $\pm$  standard deviation ( $n=3$ ).

## RESULTS AND DISCUSSION

In all samples, whatever the duration or cultivation conditions, eight MAAs were identified in *Palmaria palmata*: shinorine, palythine, asterina-330, porphyra-334, palythanol, usurijene, palythene, and an unknown MAA with a  $\lambda_{\text{max}}$  of 356 nm (described as Unknown\_356) (Fig. 1). Porphyra-334 was the main MAA representing in average  $49.51 \pm 9.19$  % of the total MAA area, as already described in Lalegerie *et al.* (2020). Then, the total MAA area varied



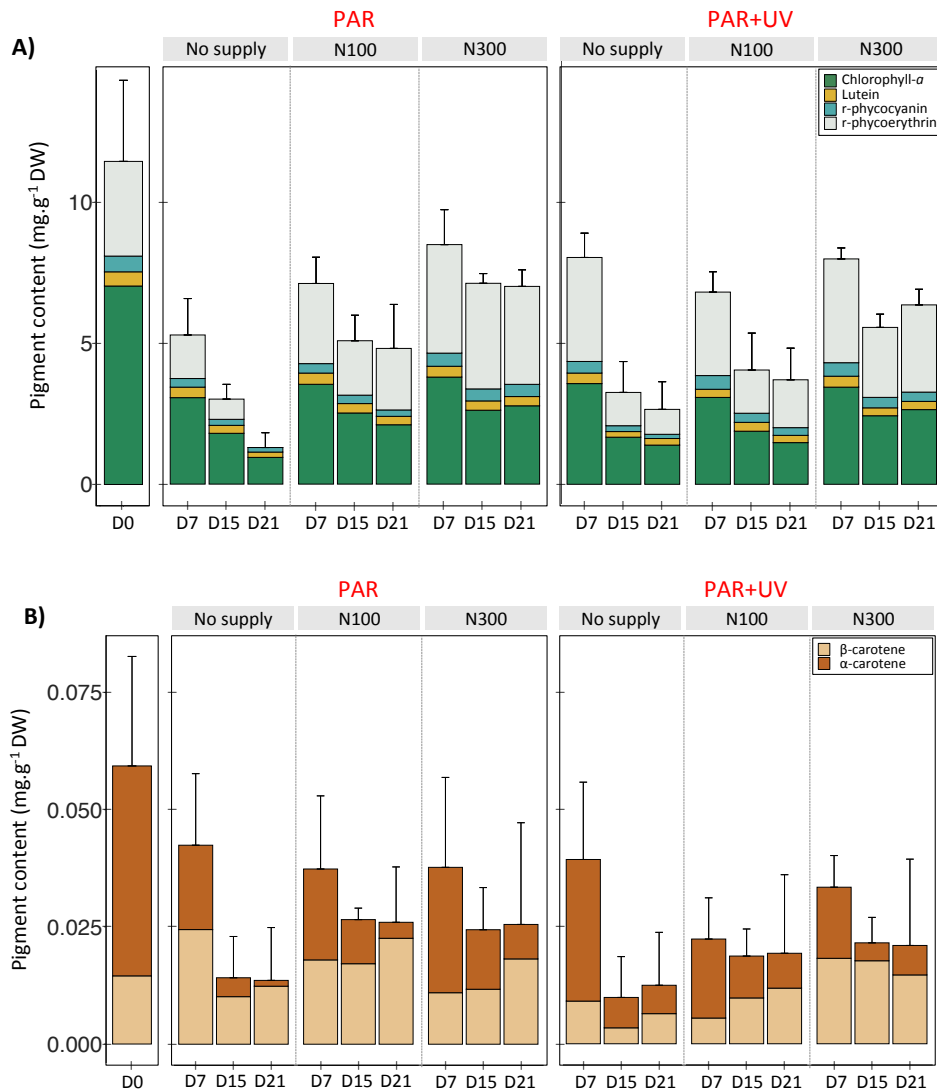
significantly with cultivation time, whatever the culture condition (Multi-Way ANOVA,  $p$ -value  $< 0.001$  for the factor ‘day’,  $p$ -value = 0.646 for the factor ‘light’,  $p$ -value = 0.008 for the factor ‘nutrient’ and  $p$ -value  $> 0.1$  for all interactions) (Fig. 1).



**Figure 1.** MAAs areas (mAU.min, injection volume = 20  $\mu$ L) measured in *Palmaria palmata* during cultivation experiment, on 1<sup>st</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 21<sup>st</sup> days (*i.e.* D0, D7, D15 and D21), under PAR or PAR+UV light, according to three nitrate levels (*i.e.* NaNO<sub>3</sub> at 0 (no supply), 100 (N100) or 300  $\mu$ M (N300)).

This temporal variation was mainly due to the difference between the initial date (D0) and the 7<sup>th</sup> day (D7) of cultivation (test post-hoc Tukey HSD,  $p$ -value  $< 0.001$ ). Indeed, the total MAA area decreased from  $434.55 \pm 84.61$  mAU.min to in average  $313.96 \pm 39.20$  mAU.min within the first week. This decrease was caused by a reduction of all MAAs, except usurijene which did not exhibit significant temporal variations (Multi-Way ANOVA,  $p$ -value = 0.240 for the factor ‘day’,  $p$ -value = 0.009 for the factor ‘light’,  $p$ -value = 0.709 for the factor ‘nutrient’ and  $p$ -value  $> 0.7$  for all interactions). Then, the total MAA content decreased more slowly, with ultimately no significant variation between the last two sampling points (15<sup>th</sup> and 21<sup>st</sup> day), except in seawater with no nitrate supply under PAR conditions (Student’s test,  $p$ -value = 0.019).

Moreover, four apolar pigments have been identified in *P. palmata* by HPLC analysis, *i.e.* chlorophyll-*a*, lutein,  $\alpha$ -carotene and  $\beta$ -carotene, in addition to the water-soluble pigments analysed by spectrophotometry, *i.e.* phycocyanin and phycoerythrin (Fig. 2).

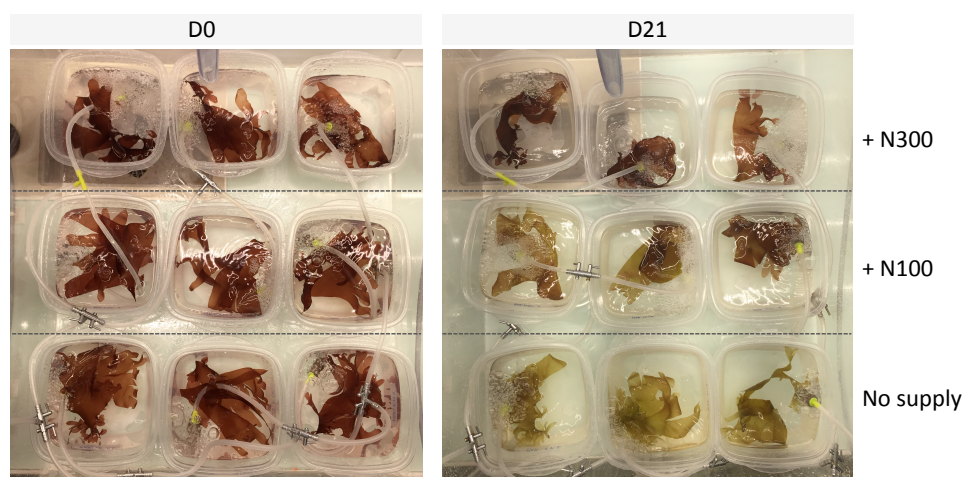


**Figure 2.** Pigment content (mg.g<sup>-1</sup> DW), i.e. (A) chlorophyll-*a*, lutein, r-phycoerythrin, r-phycocyanin, and (B) α-carotene, β-carotene measured in *Palmaria palmata* during cultivation experiment, on 1<sup>st</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 21<sup>st</sup> days (i.e. D0, D7, D15 and D21), under PAR or PAR+UV light, according to three nitrate levels (i.e. NaNO<sub>3</sub> at 0 (no supply), 100 (N100) or 300 μM (N300)).

Similarly to MAAs, the total pigment content declined between the initial date and the 7<sup>th</sup> day of cultivation, under all conditions (Multi-Way ANOVA,  $p$ -value < 0.001 for the factor ‘day’,  $p$ -value = 0.785 for the factor ‘light’,  $p$ -value < 0.001 for the factor ‘nutrient’,  $p$ -value > 0.09 for all interactions). This decrease was almost due exclusively to chlorophyll-*a*, which represented in average  $87.27 \pm 7.24$  % of the total liposoluble pigment content, varying from  $7.03 \pm 3.32$  to  $3.42 \pm 0.45$  mg.g<sup>-1</sup> DW during the first week (Fig. 2). Then, from the 7<sup>th</sup> day and until the end of cultivation, a very clear difference in the colour of *P. palmata* thalli was visible (Fig. 3). Indeed, *P. palmata* became increasingly less red during the cultivation experiment, due to a decrease in phycoerythrin (Fig. 2A), which is the pigment characteristic

of the colour of red macroalgae (Dumay *et al.* 2014). This difference was statistically confirmed with a lower phycoerythrin content over time (Two-way ANOVA,  $p$ -value  $< 0.001$  for the factors ‘day’ and ‘nutrient’,  $p$ -value = 0.14 for the interaction) (Fig. 2A). The same differences were found for phycocyanin, whose content was correlated with phycoerythrin (Pearson’s correlation,  $r = 0.80$ ,  $p$ -value  $< 0.001$ ).

Considering these decreases, occurring mainly during the first week of cultivation, it seems that the synthesis of both MAAs and pigments in *P. palmata* have been limited by the cultivation conditions. Indeed, the irradiance in the cultivation room was lower than *in situ* conditions (data not shown) and has probably led to a decrease in photosynthetic activity and subsequently in photosynthetic and photoprotective compounds. In parallel, it is possible that this decrease was also caused by the difference in temperature between the cultivation room and *in situ* conditions at the collection time, *i.e.* 15-18°C measured in the tanks, compared to not more than an *in situ* 11°C in March 2017 (data not shown). Some studies have already shown a negative effect of temperature on chlorophyll-*a* and MAAs content (Guihéneuf *et al.* 2018), which could be related to a modification of the chemical kinetics and a slowing down of the photosynthetic metabolism (Eggert, 2012). Beyond the light and temperature conditions, the most likely hypothesis is that the algae suffered from a nitrogen deficiency under cultivation conditions that impacted the entire algal metabolism, including photosynthesis. In this regard, a significant effect of the nutrient concentrations in the culture medium was observed on the total pigment content (Two-way ANOVA,  $p$ -value  $< 0.001$  for the factors ‘nutrient’ and ‘day’,  $p$ -value = 0.109 for the interaction) or total MAA area (Two-way ANOVA,  $p$ -value  $< 0.005$  for the factors ‘nutrient’ and ‘day’,  $p$ -value = 0.094 for the interaction). Thus, chlorophyll-*a* decreased from  $7.03 \pm 3.32$  mg.g<sup>-1</sup> DW at the first day, to  $1.17 \pm 0.55$  mg.g<sup>-1</sup> DW at day 21 in the seawater with no supply, compared to  $2.71 \pm 0.35$  mg.g<sup>-1</sup> DW at day 21 with a supply of 300 µM of NaNO<sub>3</sub>. Similarly, the addition of nitrate maintained a phycoerythrin content equivalent to the initial level, *i.e.*  $3.35 \pm 1.25$  mg.g<sup>-1</sup> DW at D1 and then  $3.28 \pm 0.35$  mg.g<sup>-1</sup> DW at the 21<sup>st</sup> day with a supply of 300 µM of NaNO<sub>3</sub> (Fig. 2). Conversely, the phycoerythrin content declined in thalli cultivated in non-enriched seawater with in average  $0.44 \pm 0.55$  mg.g<sup>-1</sup> DW on day 21, until becoming undetectable in some samples. By consequence, *P. palmata* thalli became green after 21 days in the tanks without the addition of NaNO<sub>3</sub> (Fig. 3).



**Figure 3.** Cultivation tanks of *Palmaria palmata*, at initial date (D0) and after 21 days (D21) of cultivation under PAR light conditions, exposed under three nitrate levels, *i.e.*  $\text{NaNO}_3$  at 0 (no supply), 100 (N100) or 300  $\mu\text{M}$  (N300).

In the same way, the levels of MAAs decreased during cultivation, but the addition of nitrate resulted in a less significant decrease. Total MAA area reached a decline of 42 % of the initial value in the 300- $\mu\text{M}$  nitrate supply condition, compared to 64 % in seawater with no supply. Thus, the experiment highlighted a very clear positive effect of nitrate content on the MAAs and pigment contents, as suggested by previous studies on macroalgae (Peinado *et al.* 2004; Korbee *et al.* 2005; Bonomi-Barufi *et al.* 2011; Figueroa *et al.* 2014), including *P. palmata* (Parjikolaei *et al.* 2013). Indeed, the content of photosynthetic pigments decreases with low nutrient levels, since photosynthetic activity is limited by Calvin's cycle (Bonomi-Barufi *et al.* 2011). Then, the effect of nitrate can be explained by the fact that MAAs and most pigments contain nitrogen in their chemical structures, a starvation leading to a limited synthesis of these compounds. Such limitation could then explain the high decline observed within the first week of cultivation, which may be due to a limited nutrient content in the tank compared to *in situ* conditions. In this way, results from the present cultivation experiment are in accordance with our previous *in situ* study (Lalegerie *et al.* 2020), highlighting the relationship between MAAs content and nutrient variations measured on wild/*in situ* populations of *P. palmata*. Then, these photoprotective compounds could be reduced in summer despite high light exposure, due to a synthesis limited by the nutrient content in seawater with no supply. However, in order to determine if MAAs could act as nitrogen source and storage and not only as photoprotectors as suggested in Bonomi-Barufi *et al.* (2012) or Lalegerie *et al.* (2020), it would be necessary to repeat the experiment with much higher nitrate contents, in order to look at a possible induction of the synthesis of these

compounds (*i.e.* an increase compared to day 0). In this way, although all MAAs have been positively affected by nitrate supply, usurijene was the only one that showed no temporal variation, with no difference between nitrate supply (Two-way ANOVA,  $p$ -value = 0.273 for the factor ‘day’,  $p$ -value = 0.729 for the factor ‘nutrient’ and  $p$ -value = 0.990 for the interaction). Likewise, it exhibited a slightly higher content at day 21 with in average  $47.49 \pm 15.26$  mAU.min compared to  $40.90 \pm 12.83$  mAU.min at day 0, suggesting a different reactivity of this MAA, which may not serve as a source of nitrogen unlike other MAAs.

On the other hand, no significant difference was observed between PAR and PAR+UV, on the total MAAs (Two-way ANOVA on samples from day 7 to day 21,  $p$ -value = 0.723 for the factor ‘light’,  $p$ -value = 0.046 for the factor ‘nutrient’ and  $p$ -value = 0.5394 for the interaction), or total pigment content (Two-way ANOVA on samples from day 7 to day 21,  $p$ -value = 0.836 for the factor ‘light’,  $p$ -value < 0.001 for the factor ‘nutrient’ and  $p$ -value = 0.085 for the interaction). This is contrary to our expectations, as many studies show an impact of UV on the content of MAAs or photoprotective pigments of red macroalgae (Kräbs *et al.* 2002; Peinado *et al.* 2004; Bonomi-Barufi *et al.* 2012), including *P. palmata* (Karsten and Wiencke 1999). Karsten *et al.* (1998) showed for example that shinorine accumulation in *C. crispus* is stimulated by UVR. In this regard, one hypothesis is that MAA content may have declined due to the lack of UV-B, as Bonomi-Barufi *et al.* (2020) demonstrated the importance of UV-A and UV-B together in MAAs regulation. Then, the decrease in MAAs could be explained by an insufficient amount of UV radiation compared to the natural environment, and/or by the limited synthesis of these compounds due to the restricted nutrient contents as described above. However, among all MAAs, usurijene was the only one that exhibited a higher content under PAR+UV than PAR (Two-way ANOVA on samples from day 7 to day 21,  $p$ -value = 0.006 for the factor ‘light’,  $p$ -value = 0.683 for the factor ‘nutrient’ and  $p$ -value = 0.094 for the interaction), suggesting once again a different behaviour of this MAA, which could have a different synthesis pathway less limited by nutrient content, or a specific role in photoprotection. Notably, Conde *et al.* (2003) indicated a different photoreactivity of cis-usurijene than its isomer trans-palythene. In conclusion, the data presented indicate an effect of both UV radiation and nitrate content on the MAAs content, with a particular interest in usurijene which could have a distinctive role in photoprotection.

## Acknowledgements

Fanny Lalegerie's PhD fellowship was provided from Region Bretagne and the Doctoral School of Marine and Littoral Sciences of the Université Bretagne Loire (UBO, Brest, France) and supervised by both S.C. and V.S.-P.

## Authors' contributions

F.L. performed the analysis of the samples, drafted the manuscript and conducted the statistical analyses. V.S-P and S.C designed and supervised the project. All authors reviewed and approved the final manuscript.

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**ENGLISH**

The previous parts highlighted the effect of different environmental parameters (*i.e.* light and nutrients) on the synthesis of MAAs by the native red alga *Palmaria palmata*. More particularly, results suggested that the species is able to induce the synthesis of specific MAAs (*i.e.* asterina-330 and urusijene) under certain conditions (*i.e.* high PAR and UV radiation respectively). In this sense, the high diversity in MAAs of *P. palmata* could be the result of a relatively high acclimation potential and appears to be an ecological advantage. Nevertheless, the role of MAAs and the diversity of these compounds is not fully elucidated and does not allow us to confirm the latter hypothesis. In this regard, the next section consists of a comparison of the acclimation capacities of the native species with the introduced species *Grateloupia turuturu*, supposed to possess a higher plasticity and adaptability. In this way, do the two species have the same metabolomic composition and the same ecological strategy? Is the introduced species able to better acclimate / adapt to strong environmental variations, especially in the context of global change? And finally, does the introduced species represent a threat to the native species?

**FRANÇAIS**

Les parties précédentes ont mis en évidence l'effet de différents paramètres environnementaux (*i.e.* la lumière et les nutriments) sur la synthèse des MAAs par l'algue rouge indigène *Palmaria palmata*. Plus particulièrement, les résultats suggèrent que l'espèce est capable d'induire spécifiquement la synthèse de certains MAAs (*i.e.* l'astérina-330 et l'urusijène) dans certaines conditions (*i.e.* sous forte exposition aux rayonnements PAR et UV respectivement). En ce sens, la grande diversité en MAAs de *P. palmata* pourrait être le résultat d'un potentiel d'acclimatation relativement élevé et semble être un avantage écologique. Néanmoins, le rôle des MAAs et la diversité de ces composés ne sont pas entièrement élucidés et ne permettent pas de confirmer cette dernière hypothèse. A cet égard, la section suivante consiste en une comparaison des capacités d'acclimatation de l'espèce native avec l'espèce introduite *Grateloupia turuturu*, supposée posséder une plasticité et une adaptabilité plus élevées. Ainsi, les deux espèces ont-elles la même composition métabolomique et la même stratégie écologique ? L'espèce introduite est-elle capable de mieux s'acclimater / adapter à de fortes variations environnementales, notamment dans le contexte du changement global ? Enfin, l'espèce introduite représente-elle une menace pour l'espèce native ?

## **Phenological and metabolomic study of *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) 30 years after its introduction in Brittany (France): a real threat for the native species *Palmaria palmata*?**

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In preparation, to be submitted to: **Biological invasion**

### **RESUME**

La macroalgue rouge *Grateloupia turuturu* est généralement décrite comme invasive en Europe, bien qu'elle reste peu étudiée et que les données sur son comportement invasif soient limitées. Ainsi, près de 30 ans après son introduction en Bretagne (France), cette étude apporte de nouveaux éléments sur la phénologie de cette espèce, suivie dans la zone intertidale en Bretagne. Plus intéressant encore, les résultats fournissent une comparaison écologique et métabolomique avec l'algue native *Palmaria palmata*, en comparant différents sites où les deux espèces co-existent ou non. Cette approche permet d'étudier les différentes capacités d'acclimatation des deux espèces, et d'évaluer le potentiel invasif de *G. turuturu*. Les résultats ont révélé une grande plasticité de *G. turuturu*, avec la présence d'individus fertiles et de juvéniles tout au long de l'année, contrairement à l'espèce native *P. palmata* qui semble être très dépendante de la température. Par ailleurs, la stratégie écologique de *G. turuturu* est couplée à sa capacité à synthétiser et à accumuler rapidement de grandes quantités de métabolites secondaires, augmentant sa tolérance aux changements saisonniers par rapport à *P. palmata*. D'autre part, *P. palmata* présentait une taille et une densité réduites lorsqu'elle coexistait avec *G. turuturu* (*i.e.* pas plus de 35 cm et 15 ind. / quadrat, par rapport à un maximum de 64 cm et 25 ind. / quadrat sans *G. turuturu*), ce qui suggère potentiellement un impact négatif de l'espèce introduite sur l'espèce native. Néanmoins, les populations intertidales de *G. turuturu* restent relativement réduites, et l'espèce introduite semble plutôt profiter des perturbations ou des conditions défavorables pour se développer, contrairement à des espèces natives telles que *P. palmata* qui en sont incapables. *Grateloupia turuturu* ne

semble donc pas avoir un comportement invasif en Bretagne, bien que des études à long terme soient nécessaires pour confirmer cet état, car ses impacts futurs restent inexplorés dans le contexte du changement global.

**Mots-clés:** Suivi écologique, Espèce invasive, Acide aminés de type mycosporine, Pigments, Plasticité, Macroalgues rouges

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## ABSTRACT

The red macroalga *Grateloupia turuturu* is generally described as invasive in Europe, although it remains largely understudied and there is limited information on its invasive behaviour. In this way, nearly 30 years after its introduction in Brittany (France), this study provides new insights on the phenology of this species, monitored in the intertidal zone. More interestingly, results provide an ecological and metabolomic comparison with the native *Palmaria palmata*, by comparing different sites where both species co-exist or not, to investigate the different acclimation abilities of both species, and evaluate the potential invasiveness of *G. turuturu*. Results revealed a high plasticity of *G. turuturu*, with the presence of fertile individuals or juveniles throughout the year, contrary to the native *P. palmata* which appeared to be highly dependent of temperature. Then, the ecological strategy of *G. turuturu* was coupled with its ability to rapidly synthesize and accumulate high amounts of secondary metabolites, increasing its tolerance to seasonal changes compared to *P. palmata*. On the other hand, *P. palmata* exhibited reduced size and density when coexisting with *G. turuturu* (*i.e.* no more than 35 cm and 15 ind. / quadrat, compared to up to 64 cm and 25 ind. / quadrat without *G. turuturu*), suggesting a potential negative effect of the introduced species. Nevertheless, the intertidal populations of *G. turuturu* were relatively reduced, and the introduced species rather appears to take advantage of disturbances or unfavourable conditions to develop, when native species such as *P. palmata* cannot. *Grateloupia turuturu* therefore did not appear to have invasive behaviour in Brittany, although long-term studies are required as its future impacts remain unexplored in the context of global change.

**Keywords:** Ecological monitoring, Invasive species, Mycosporine-like amino acids, Pigments, Plasticity, Red macroalgae

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## 1. Introduction

For many years, the number of introduced species in marine ecosystems has been growing steadily, emphasising the potential risk of the spread of invasive species (Bax *et al.* 2003; Seebens *et al.* 2016). More particularly in Europe and Brittany (France), many species have been accidentally introduced from Asian countries causing important ecological and economic impacts (Stiger-Pouvreau and Thouzeau 2015). These negative effects explain the numerous studies carried out on the evolution of the number of introductions, the global impact and the management possibilities of invasive species (*e.g.* Schaffelke and Hewitt 2007; Williams and Smith 2007; Molnar *et al.* 2008; Stiger-Pouvreau and Thouzeau 2015; Tsiamis *et al.* 2019). Nevertheless, it is sometimes difficult to determine the long-term invasive potential of an introduced species (Pyšek and Richardson 2007), and studies generally focus on the ecological effects of invasive species rather than on the biology, acclimation abilities and actual evolution of the introduced species.

In this context, *Grateloupia turuturu* (Yamada) 1941 is a red macroalga originated from Japan, reported to be introduced in Atlantic Ocean (along European seas and North America; Villalard-Bohnsack and Harlin 1997; Simon *et al.* 2001; Araújo *et al.* 2011), Mediterranean Sea (Cecere *et al.* 2011), and other areas of Pacific Ocean (*e.g.* Mexico, New-Zealand and Tasmania; Saunders and Withall 2006; D'Archino *et al.* 2007; Aguilar-Rosas *et al.* 2012). More recently, it was recorded in southern Brazil (de Azevedo *et al.* 2015), constituting its first record in south-western Atlantic Ocean. Many studies have then suggested that *G. turuturu* could be invasive (Simon *et al.* 2001; Villalard-Bohnsack and Harlin 1997; Araújo *et al.* 2011), being responsible of disrupting native assemblage, by reducing the abundance and richness of native species (Mathieson *et al.* 2007; Janiak and Whitlatch 2012). In this sense, *G. turuturu* possesses ecological traits promoting its invasiveness, *i.e.* (1) multiple reproductive modes coupled to a rapid growth (Harlin and Villalard-Bohnsack 2001), (2) high dispersal rate (Simon *et al.* 2001), and (3) large tolerance to environmental variations (Simon *et al.* 1999). More particularly, *G. turuturu* is known to be resistant to wide variations in temperature and salinity. Indeed, Simon *et al.* (1999) demonstrated that *G. turuturu* tolerated temperature between 5 and 25 °C, similarly to Villalard-Bohnsack and Harlin (1997) who reported the presence of the introduced species in seawater at 4 °C in Rhode Island. In addition, *G. turuturu* can survive to a salinity ranging from 12 to 52 (Simon *et al.* 1999), in particular through the synthesis of osmoregulatory compounds such as

floridoside or N-methyl-L-methionine sulfoxide (Simon-Colin *et al.* 2002). This species presents also a defense against fouling of its surface in producing defense metabolites like cholesteryl formate (Plouguerné *et al.* 2006b, 2008) and furanone-like compounds in gland cells-like structures (Plouguerné *et al.* 2007). Within this context, *G. turuturu* presents a large distribution in Brittany, living from subtidal to intertidal areas, as well as tidepools where conditions are generally unfavourable to the majority of red foliaceous macroalgal species due to extreme and very variable physico-chemical conditions (Simon *et al.* 2001).

However, except these few biological data, *G. turuturu* (known before as *G. doryphora*, Gavio and Fredericq 2002) is a species relatively understudied, with most research dating back 20 years or so. In Brittany, *G. turuturu* was first reported in South Brittany at Fort Bloqué in 1989 and then on the north coast at Callot Island (Carantec) in 1992, before spreading to the south of Brittany and Normandy (Simon *et al.* 2001). Early studies suggested that *G. turuturu* would spread further and that it could potentially become invasive in Brittany (Simon *et al.* 2001). Nevertheless, to our knowledge, limited study has reported so far ecological impact of *G. turuturu*, and it would seem that the introduced species has not caused the disappearance of any native species in Brittany, contrary to Long Island Sound (USA) (Janiak and Whitlatch 2012) or Portugal (Freitas *et al.* 2016) where habitats dominated by *G. turuturu* demonstrated a reduced species richness and abundance. In this sense, Stiger-Pouvreau and Thouzeau (2015) described *G. turuturu* as being very discrete in the colonized area, and suggested that it could become invasive only if one or several environmental parameters evolve. Thus, about 30 years after its introduction, the aim of the present study was to provide new information on the ecology and biology of *G. turuturu*, in order to re-evaluate its invasive potential. More interestingly, this investigation will bring new elements on its phenology and on its tolerance to different environmental stresses through a one-year study of its ecology and biochemical composition. Indeed, recent works emphasize the importance of coupling ecological and metabolomic data (defined as “eco-metabolomics”) (Jones *et al.* 2013; Peters *et al.* 2018), in the aim to better characterise adaptive abilities, and interactions within and between species. In addition, the results will be compared with a native species, in order to assess the potential negative effect of *G. turuturu* on native populations from Brittany.

In this way, *G. turuturu* will be compared with *Palmaria palmata* (Linnaeus) F. Weber & D. Mohr 1805, a red macroalgal species morphologically similar to the invasive one. Both

species are commonly encountered in the same habitat, *i.e.* from mid-intertidal to shallow subtidal, where they compete for resources and space. Therefore, one objective of the present study was to determine whether *G. turuturu* could be a threat to *P. palmata*, by comparing the native species in habitat associated or not with the introduced species. In this sense, according to Liu and Pang (2010), *G. turuturu* could more likely tolerate greater oxidative stress, caused by high salinity and heat stress, than *P. palmata*. Thus, *G. turuturu* is generally described as a euryhaline and eurythermal species, contrary to *P. palmata*. More particularly, *P. palmata* is a cold water species widely localised in North Atlantic Ocean (Karsten and Wiencke 1999; Grote 2017), and has in particular been the subject of numerous studies in Arctic Ocean (Karsten and Wiencke 1999; Aguilera *et al.* 2002; Van de Poll *et al.* 2002). On the contrary, *G. turuturu* is distributed from warm temperate to tropical waters worldwide (Villalard-Bohnsack and Harlin 1997). In addition, the tolerance of *G. turuturu* to variations in light could then exceed those of *P. palmata*. Indeed, previous studies showed that *P. palmata* is highly sensitive to high light, and more particularly to UV radiations that induce DNA damages (Cordi *et al.* 1997; Bischof *et al.* 2000; Holzinger *et al.* 2004). In the current context of global change, *P. palmata* could therefore be more likely impacted than the introduced species, and its distribution area could then be reduced. Its regression could have an important economic impact since *P. palmata*, commonly known as "Dulse", is widely cultivated, natural populations are exploited, and used as human food (Galland-Irmouli *et al.* 1999; Mouritsen *et al.* 2013; Schiener *et al.* 2017) and animal feed (Roussel *et al.* 2019).

In this context, both species were monitored during one year (from February 2018 to February 2019), on 3 sites in Brittany (France): one site where both species co-exist, and two sites with either *G. turuturu* or *P. palmata*. Monthly, ecological data (*i.e.* density, length and width of individuals, presence or absence of reproductive parts on individuals) were measured for both species, and samples were brought back to the laboratory for metabolomic analysis (*i.e.* pigments, mycosporine-like amino acids, phenolic compounds, fatty acids). We hypothesized that:

- (1) The invasive *G. turuturu* and the native *P. palmata* have different ecological strategies
- (2) Both species exhibit seasonal variations in their phenology, with a metabolomic composition in accordance to their specific/own ecological strategy
- (3) When exposed to the same *in situ* conditions, *G. turuturu* has a greater tolerance to environmental stressor compared to *P. palmata*

- (4) Despite a high plasticity, *G. turuturu* should not be considered an invasive species that could affect *P. palmata*

## 2. Materials and methods

### 2.1. Sampling and environmental conditions

Field work was undertaken monthly at low tides between February 2018 and February 2019 on the Brittany coasts (France). Thalli of *Grateloupia turuturu* and *Palmaria palmata* were collected at Pointe du diable (48°21'15.606'' N – 4°33'30.067'' W), an exposed site with many rockpools where both species coexist. The two macroalgal species were also monitored on another site where the second species was missing: *G. turuturu* at Minou (48°20'20.314'' N - 4°37'3.779'' W), an exposed site; and *P. palmata* at the sheltered site Porspoder (48°28'56.644'' N – 4°46'6.812'' W) where individuals live in a 20-cm-deep rocky channel. Environmental data were provided by « Météo France » from Brest-Guipavas (48°27'00'' N – 4°22'59'' W), and « Service d'Observation en Milieu Littoral, INSU-CNRS, Saint-Anne du Portzic », recorded near Pointe du diable (48°21'32.13'' N – 4°33'07.19'' W) (Supplementary Table 1). After collection, all algal samples were brought to the laboratory, cleaned and preserved at - 20 °C. Metabolomic analyses were performed on fine powder obtained by freeze-drying ( $\beta$ 1-8 LD plus Christ) and grinding (MM400 Retsch).

### 2.2. Ecological measurements

Ecological data were recorded monthly on each site, in close but distinct areas from sampling zone for metabolomic analysis. For both species, ecological measurements were carried out using four replicate quadrats of 0.09 m<sup>2</sup> randomly positioned, and by monitoring all individuals within each of them. Minimal area for both species was determined using 50 x 50-cm quadrats subdivided into 10 x 10-cm areas (n = 6): the quadrat sizes for both species were obtained when there was no statistical difference in the number of individuals by increasing the sampling area. As the minimal area was the same for both species, it will not be mentioned again in the rest of the study and will be described as “quadrat”. All the data collected were then pooled, constituting a database containing 939 individuals for *P. palmata* and 968 for *G. turuturu*, over the whole studied period. The different ecological variables measured for both species were: density (*i.e.* number of individuals per quadrat), maximum



thallus length (*i.e.* from the base to the apical part), maximum blade width (excluding the proliferations), reproductive status, and the potential presence of epiphytes. *Grateloupia turuturu* has a trigenetic life cycle that is isomorphic, *i.e.* with two generations (tetrasporophytes, and gametophytes) having similar morphologies. On the contrary *P. palmata* has a digenetic life cycle, with the tetrasporophyte and male gametophyte having similar morphologies (erect frond), in addition to the female gametophyte being a microscopic crust. Then, different phenological states have been recorded according to morphological features visually apparent. Thus, tetrasporophytes of *P. palmata* are characterized by the presence of fertile dark areas containing haploid tetraspores, and the male gametophyte by pinkish reproductive areas not easily observable on the fronds of young thallus (Le Gall *et al.* 2004). Concerning *G. turuturu*, tetrasporophytes present rough surface similar to “orange skin”, and fructified gametophytes bear carposporophytes then having a granular surface (Simon *et al.* 2001). For both species, the undifferentiated stage corresponds then to thallus without reproductive structures, which can correspond to not distinguishable gametophytes or immature tetrasporophytes.

### 2.3. Chlorophyll and carotenoids extraction and analysis

Chlorophyll-*a* and carotenoids were extracted using 75 mg dry weight (DW) of algal powder mixed with 750  $\mu$ L 90 % aqueous acetone according to the protocol of Schmid and Stengel (2015). Pigments were separated according to the protocol already described in Lalegerie *et al.* (2019) using an HPLC Dionex Ultimate 3000 system equipped with a diode array detector (ThermoScientific) and an ACE C<sub>18</sub> column (150  $\times$  4.6 mm, 3  $\mu$ m). Identification and quantification of pigments were then determined using standard curves obtained from commercial standards (chlorophyll-*a* from Sigma (USA), and pheophytin-*a*,  $\alpha$ -carotene,  $\beta$ -carotene, lutein, zeaxanthin, antheraxanthin, violaxanthin from DHI (Denmark)). HPLC data acquisition was done by Chromeleon 7 software (Thermo Scientific Dionex, France).

### 2.4. Phycobiliproteins extraction and analysis

Phycobiliproteins were extracted from 75 mg DW of algal powder mixed with 1.5 mL of phosphate buffer (0.1 M, pH 6.8) according to the protocol of Sun *et al.* (2009), already adapted in Lalegerie *et al.* (2019). Phycobiliproteins were quantified using the following equations from Beer and Eshel (1985):

$$[\text{Phycoerythrin}] = [(A_{565} - A_{592}) - (A_{455} - A_{592}) \times 0.20] \times 0.12$$

$$[\text{Phycocyanin}] = [(A_{618} - A_{645}) - (A_{592} - A_{645}) \times 0.51] \times 0.15$$

### 2.5. Mycosporine-like amino acids extraction and analysis

The mycosporine-like amino acids (MAAs) were extracted and analysed according to Lalegerie *et al.* (2019). Briefly, 20 mg DW of algal powder was added to 2 mL of 70 % aqueous ethanol (v/v). MAAs were analysed by HPLC performed on a Dionex Ultimate 3000 HPLC system (Thermo Scientific, Germany) equipped with Zorbax Eclipse XDB C<sub>18</sub> column (5 µm, 4.6 × 250 mm; Agilent, USA). Identification was done by comparison of absorption spectra and retention time of MAAs identified by previous LC-MS analysis (Lalegerie *et al.* 2019). Without commercial standards available, no pseudo-quantification has been carried out. In order to be able to compare species, the peak area of each MAA was divided by the injection volume, and results are thus expressed in mAU.min.µL<sup>-1</sup>.

### 2.6. Phenolic compounds extraction and quantification

Phenolics were extracted from 15 mg DW of algal powder mixed with 1 mL of 70 % ethanol. The samples were kept in an ultrasonic bath (Sonicator 88155, Fisher Bioblock Scientific, France) during 15 min at 4 °C, and then under magnetic stirring during 2 h at 40 °C. Samples were then centrifuged for 10 min at 8,000 rpm (Eppendorf Centrifuge 5810, Germany); the supernatant was recovered, and samples were subjected to a second extraction. At the end, supernatants were pooled and evaporated at 40 °C using a centrifugal concentrator (miVac, Genevac, France). The total phenolic content (TPC) was then quantified using the Folin-Ciocalteu colorimetric assay adapted from Zubia *et al.* (2009): 20 µL of sample, adding to 130 µL of distilled water, 10 µL of Folin-Ciocalteu reagent and 40 µL of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>, 200 g.L<sup>-1</sup>). TPC (expressed in gallic acid equivalent (GAE)) were determined by measuring the absorbance of each sample (in triplicate) at 620 nm (Multiskan FC, Thermo Scientific, USA), and compared to a standard curve of gallic acid (Sigma, USA).

## 2.7. Fatty acids extraction and analysis

Fatty acids extraction and analysis were performed according to the protocols of Schmid *et al.* (2014). For each sample, 40 mg DW of algal powder were extracted with 2 mL of methanol containing 2 % H<sub>2</sub>SO<sub>4</sub>, during 2 h at 80 °C under magnetic stirring. Then, 1 mL of distilled water and 2 mL of 100 % hexane was added to the extracts. After vortexing and centrifugation, the hexane upper layer were analysed using an Agilent 7890A GC / 5975C MSD series equipped with a 7583B injector, a flame-ionization detector and a silica capillary column (DB-Wax, Agilent, 0.25 mm x 30 m x 0.25 µm) with hydrogen as vector gas at 1.2 mL.min<sup>-1</sup> constant flow. The temperature was programmed at 140 °C for 1 min, then increased to 200 °C by a rate of 15 °C.min<sup>-1</sup>, and then to 250 °C at a rate of 2 °C.min<sup>-1</sup>. The injector and detector temperatures were set at 250 °C and 300 °C, respectively. Fatty acids were identified using FAME of Fish Oil (Menhaden Oil, Supelco, Bellefonte, PA, USA) and FAME mix (Supelco™ 37 Component FAME Mix, Supelco, Bellefonte, PA, USA); and quantification was made using a known amount of internal standard, *i.e.* pentadecanoic acid C15:0 (5 mg.mL<sup>-1</sup>) added initially to each sample.

## 2.8. Statistical analyses

Statistical analyses were carried out using R software (version 3.6.1) (R Core Team 2019) and Rstudio (version 1.2.5001) (Rstudio Team 2019). Data were collected in triplicate monthly over the studied period (one replicate corresponding to several thalli). The size class distributions adapted to each population at each site were obtained using the Sturges rule. For metabolomic data, the monthly values were averaged per season as follows: February 2018 to March (Winter I), April to June (Spring), July to September (Summer), October to December (Autumn) and January to February 2019 (Winter II); this pooling corresponded to environmental data in Supplementary Table 1. For fatty acid analysis, seasonal averages were calculated only from 7 months analysed by GC-MS (*i.e.* February 18, May, June, July, August, November and February 19). Student's t-test and ANOVA (1 or more factors) (or the non-parametric Wilcoxon, Kruskal-Wallis (KW) or Scheirer-Ray-Hare (SRH) tests respectively) were applied to test the effect of 'species', 'sampling periods' or 'sites' on ecological and metabolomic data. After ANOVA, post-hoc test were performed (Tukey's HSD, or the non-parametric KW (kruskalmc, pircmess)). In addition, Principal Component Analyses were performed with FactoMineR, in order to highlight differences between sites,

seasons or species on different metabolite compositions. Finally, Pearson's correlation (or non-parametric Kendall rank correlation) were carried out to investigate potential relationships among data.

### 3. Results

#### 3.1. *Grateloupia turuturu* ecology

For both sites, most thalli of *G. turuturu* were fertile over the year, with no significant seasonal variations in the proportions of the different reproductive state (Table 1). Indeed, there was a year-round presence of tetrasporophytes (SRH test,  $p$ -value = 0.068 for the factor months,  $p$ -value = 0.501 for sites, and  $p$ -value = 0.213 for the interaction months x sites), or carposporophytes (SRH test,  $p$ -value = 0.129 for the factor months,  $p$ -value = 0.823 for sites, and  $p$ -value = 0.187 for the interaction months x sites). More particularly, high proportions of tetrasporophytes were recorded in May (in average  $65.5 \pm 29.2$  % per quadrat) and July ( $51.7 \pm 42.3$  %), at Pointe du diable and Minou respectively. This increase occurred at the same time as the increase in light and temperature and the decrease in nutrient levels (Supplementary Table 1). There was a second increase in the proportions of fertile individuals in winter, representing  $50.0 \pm 57.7$  % of total individuals in February 2019 at Pointe du diable, and  $62.5 \pm 47.9$  % in January at Minou. Then, a peak in the proportion of carposporophytes was observed in July at Pointe du diable (in average  $39.8 \pm 13.5$  % per quadrat), and time-delayed in February 2019 at Minou ( $43.8 \pm 51.5$  %). In this way, the proportion of sporophytes was greater and more homogeneous throughout the year at this last site, with in average  $36.12 \pm 18.81$  % of fertile individuals over the year, compared to  $19.92 \pm 15.95$  % at Pointe du diable (Table 1). Furthermore, a significant proportion of small individuals (< 5.3 cm) were observed almost continuously throughout the year at both sites (Table 1). More particularly, an important recruitment was observed over a long period at Pointe du diable, recruits representing  $69.0 \pm 22.6$  % of the total number of individuals between August and November. Then, a significant proportion of juveniles was observed over a shorter period at Minou, *i.e.* between June and August with  $40.6 \pm 2.0$  % of recruits among total individuals. An increase in density occurred simultaneously with recruitment for both sites (SRH test,  $p$ -value < 0.001 for the factors sampling months and sites,  $p$ -value = 0.341 for the interaction sites x months), *i.e.* a maximum in October ( $27.8 \pm 18.0$  individuals / quadrat)

and August ( $19.3 \pm 5.3$  individuals / quadrat) at Pointe du diable and Minou respectively (Table 1).

**Table 1.** Temporal size (cm) class distribution (number of individuals) of *Grateloupia turuturu* and *Palmaria palmata* collected monthly between February 2018 and February 2019, at Pointe du diable, and then at Minou or Porspoder respectively (Brittany, France). Monthly average density (number of individuals per quadrat) and length (cm) are displayed in bold. Monthly pie charts display the proportion of each reproductive stage recorded. The maximum density of each site appears in red, and the individuals of the 3 upper size classes (*i.e.* representative of maximal length) in blue.

		February 18	March	April	May	June	July	August	September	October	November	December	January	February 19			
<i>Grateloupia turuturu</i>	Minou	(0-5.3]	2	1	6	2	3	10	30	4	17	0	5	2	2		
		(5.3-10.6]	4	3	3	3	3	0	32	26	15	4	8	1	3		
		(10.6-15.9]	3	0	0	1	1	4	5	11	23	4	1	2	1		
		(15.9-21.2]	0	0	1	1	0	5	7	8	13	5	2	2	1		
		(21.2-26.5]	0	0	0	0	0	1	1	4	0	2	3	0	0		
		(26.5-31.8]	0	0	0	0	0	1	1	1	2	0	0	0	0		
		(31.8-37.1]	0	0	0	0	0	3	0	2	0	0	0	0	0		
		(37.1-42.4]	0	0	0	0	0	0	1	2	0	0	0	0	0		
		(42.4-47.7]	0	0	0	0	0	1	0	0	1	0	0	0	0		
		(47.7-53.0]	0	0	0	0	0	0	0	3	0	0	0	0	0		
		<b>Mean density</b>	<b>2.3 ± 1.5</b>	<b>1.0 ± 0.0</b>	<b>2.5 ± 1.3</b>	<b>1.8 ± 1.0</b>	<b>1.8 ± 1.0</b>	<b>6.0 ± 5.8</b>	<b>19.3 ± 5.3</b>	<b>15.3 ± 6.3</b>	<b>17.8 ± 8.4</b>	<b>3.8 ± 1.0</b>	<b>4.8 ± 3.6</b>	<b>1.8 ± 1.0</b>	<b>1.8 ± 1.0</b>		
		<b>Mean length</b>	<b>8.6 ± 3.4</b>	<b>8.1 ± 2.9</b>	<b>6.1 ± 4.2</b>	<b>9.3 ± 4.7</b>	<b>6.1 ± 3.7</b>	<b>14.1 ± 13.9</b>	<b>8.0 ± 6.5</b>	<b>15.6 ± 11.7</b>	<b>11.5 ± 7.0</b>	<b>14.9 ± 5.9</b>	<b>10.8 ± 7.8</b>	<b>11.1 ± 5.6</b>	<b>8.2 ± 5.5</b>		
		<i>Grateloupia turuturu</i>	Pointe du diable	(0-5.3]	13	20	8	0	4	9	28	53	94	65	24	30	0
(5.3-10.6]	15			16	3	0	5	12	36	30	16	6	14	19	1		
(10.6-15.9]	3			6	5	3	5	9	5	2	1	2	7	9	3		
(15.9-21.2]	0			4	0	2	5	5	1	0	0	0	0	6	0		
(21.2-26.5]	0			2	2	4	4	4	0	0	0	0	0	1	3		
(26.5-31.8]	0			0	2	2	6	0	0	0	0	0	0	0	0		
(31.8-37.1]	0			0	1	2	2	0	0	0	0	0	0	0	0		
(37.1-42.4]	0			0	1	2	2	0	0	0	0	0	0	0	0		
(42.4-47.7]	0			0	1	3	2	1	0	0	0	0	0	0	0		
(47.7-53.0]	0			0	0	0	0	1	0	0	0	0	0	0	0		
<b>Mean density</b>	<b>7.8 ± 3.9</b>			<b>12.0 ± 6.8</b>	<b>5.5 ± 3.0</b>	<b>4.3 ± 2.8</b>	<b>8.5 ± 8.4</b>	<b>10.3 ± 4.4</b>	<b>17.5 ± 16.3</b>	<b>19.0 ± 5.4</b>	<b>27.8 ± 18.0</b>	<b>18.3 ± 13.4</b>	<b>11.3 ± 3.3</b>	<b>16.3 ± 5.2</b>	<b>1.8 ± 1.5</b>		
<b>Mean length</b>	<b>6.3 ± 3.2</b>			<b>7.6 ± 6.6</b>	<b>12.8 ± 11.2</b>	<b>27.7 ± 11.0</b>	<b>19.9 ± 12.0</b>	<b>12.9 ± 10.6</b>	<b>6.6 ± 3.1</b>	<b>4.7 ± 2.6</b>	<b>3.6 ± 2.0</b>	<b>3.6 ± 2.3</b>	<b>6.4 ± 3.8</b>	<b>7.0 ± 5.9</b>	<b>18.0 ± 6.9</b>		
<i>Palmaria palmata</i>	Pointe du diable			[0-4.0]	0	4	5	0	1	1	11	7	6	0	0	16	NA
		(4.0-8.0]	1	0	3	2	0	5	6	20	22	2	8	15	NA		
		(8.0-12.0]	1	0	4	6	3	9	10	12	19	5	10	5	NA		
		(12.0-16.0]	3	0	1	4	2	6	9	5	10	5	1	3	NA		
		(16.0-20.0]	1	3	2	1	3	3	2	1	3	0	1	0	NA		
		(20.0-24.0]	0	1	0	1	2	3	0	1	0	0	2	0	NA		
		(24.0-28.0]	0	0	0	0	1	3	1	0	0	0	0	0	NA		
		(28.0-32.0]	0	0	0	0	4	1	0	0	0	0	0	0	NA		
		(32.0-35.5]	0	0	0	1	0	1	0	0	0	2	0	0	NA		
		<b>Mean density</b>	<b>1.5 ± 3.0</b>	<b>2.0 ± 2.0</b>	<b>3.8 ± 2.1</b>	<b>3.8 ± 2.2</b>	<b>4.0 ± 2.7</b>	<b>8.0 ± 2.0</b>	<b>9.8 ± 4.5</b>	<b>11.5 ± 4.2</b>	<b>15.3 ± 15.6</b>	<b>3.5 ± 3.3</b>	<b>5.5 ± 7.7</b>	<b>9.8 ± 8.2</b>	NA		
		<b>Mean length</b>	<b>13.0 ± 4.0</b>	<b>10.0 ± 10.2</b>	<b>8.3 ± 5.2</b>	<b>13.6 ± 7.4</b>	<b>19.5 ± 8.3</b>	<b>14.9 ± 7.5</b>	<b>9.8 ± 5.5</b>	<b>8.0 ± 4.3</b>	<b>9.1 ± 4.0</b>	<b>15.4 ± 8.8</b>	<b>10.8 ± 4.4</b>	<b>5.9 ± 3.9</b>	NA		
		<i>Palmaria palmata</i>	Porspoder	(0-6.4]	5	0	32	27	11	32	37	28	14	2	3	1	1
				(6.4-12.8]	4	1	6	14	20	19	30	15	13	5	5	12	8
(12.8-19.2]	5			1	0	10	4	6	7	13	7	5	6	10	13		
(19.2-25.6]	1			2	0	7	7	4	11	3	8	2	3	10	10		
(25.6-32.0]	1			1	1	3	6	3	10	3	6	8	3	9	7		
(32.0-38.4]	0			0	0	1	2	1	3	1	3	0	5	3	2		
(38.4-44.8]	0			1	0	1	0	2	0	1	5	3	1	6	0		
(44.8-51.2]	0			0	0	0	0	0	0	0	2	1	2	2	2		
(51.2-57.6]	0			0	0	0	0	1	0	0	0	0	0	1	0		
(57.6-64.0]	0			0	0	0	0	2	0	1	0	0	0	0	0		
<b>Mean density</b>	<b>4.0 ± 1.8</b>			<b>6.0 ± 3.2</b>	<b>19.5 ± 7.8</b>	<b>15.8 ± 7.8</b>	<b>12.5 ± 7.2</b>	<b>17.5 ± 7.0</b>	<b>24.5 ± 18.1</b>	<b>16.3 ± 11.0</b>	<b>14.5 ± 7.0</b>	<b>6.5 ± 4.0</b>	<b>7.0 ± 5.5</b>	<b>13.5 ± 12.1</b>	<b>10.8 ± 9.6</b>		
<b>Mean length</b>	<b>11.8 ± 7.1</b>			<b>24.3 ± 10.0</b>	<b>3.7 ± 5.1</b>	<b>11.3 ± 8.8</b>	<b>13.8 ± 9.1</b>	<b>11.8 ± 13.8</b>	<b>11.7 ± 9.6</b>	<b>10.8 ± 10.9</b>	<b>18.7 ± 13.4</b>	<b>22.6 ± 12.1</b>	<b>22.3 ± 13.1</b>	<b>23.6 ± 12.6</b>	<b>20.4 ± 9.7</b>		

Reproductive stage: Tetrasporophyte (green), Undifferentiated (red), Carposporophyte (blue)

Moreover, there was also a significant temporal variation in the length of *G. turuturu*, which was different between sites (SRH test,  $p$ -value  $< 0.001$  for sampling sites, months, and the interaction sites x months), as for the width of *G. turuturu* which was positively correlated to the length (Kendall's correlation,  $\tau = 0.62$  for Pointe du diable and  $\tau = 0.59$  for Minou,  $p$ -value  $< 0.001$ ). Indeed, the length of *G. turuturu* was higher from April to July at Pointe du diable, reaching a maximum of  $27.6 \pm 10.9$  cm in May. On the opposite, individuals at Minou had greater length from July to October, simultaneously with recruitment, reaching a maximum of  $15.6 \pm 11.7$  cm in September (Table 1). Then, maximum length was almost twice higher at Pointe du diable. Nevertheless, over the year, the average thallus length of *G. turuturu* was smaller at Pointe du diable than Minou (Wilcoxon test,  $p$ -value  $< 0.001$ ) with in average  $7.6 \pm 7.8$  cm and  $11.2 \pm 8.8$  cm respectively, contrary to density (Wilcoxon test,  $p$ -value  $< 0.001$ ), with in average  $12.3 \pm 10.4$  individuals / quadrat at Pointe du diable, compared to  $6.1 \pm 7.3$  individuals / quadrat at Minou.

### 3.2. *Palmaria palmata* ecology

*Palmaria palmata* showed seasonal variations in its reproduction, with a similar pattern at both sites (SRH test,  $p$ -value  $< 0.001$  for the factor months,  $p$ -value = 0.961 for sites, and  $p$ -value = 0.084 for the interaction months x sites). Indeed, a high proportion of tetrasporophytes was recorded in June and then between November and March (Table 1). Tetrasporophyte proportion reached thus a maximum in February 18 and June at Pointe du diable (33.3 and 36.5 % respectively), and March and June at Porspoder (66.7 and 71.9 % respectively). No male or female gametophytes were recorded. On the other hand, a slightly different temporal pattern in recruitment periods were observed at both sites. Indeed, at Pointe du diable, 3 short recruitment periods were observed, *i.e.* between March and April, from August to October and then in January. On the contrary, individuals from Porspoder exhibited a larger recruitment period, with many new juveniles observed between April and October (Table 1). The maximum recruitment was recorded in January for Pointe du diable with 16 individuals shorter than 4.0 cm; and in August for Porspoder with 37 individuals shorter than 6.4 cm. There was a temporal variation in the density of *P. palmata*, slightly different between sites (SRH test,  $p$ -value  $< 0.001$  for sampling sites,  $p$ -value = 0.016 for months and  $p$ -value = 0.562 for the interaction sites x months). Indeed, due to lower recruitment at Pointe du diable, the density gradually increased from February 2018, until reaching a maximum of

15.3 ± 15.6 individuals per quadrat in October. This temporal pattern was slightly different for Porspoder, whose density increased in April and August, with 19.5 ± 7.8 and 24.5 ± 18.1 individuals per quadrat, respectively.

Moreover, thallus length of *P. palmata* exhibited seasonal variations, different between sites (SRH test,  $p$ -value = 0.006 for sampling sites, and  $p$ -value < 0.001 for months and the interaction sites x months). The same pattern was observed for the width which was positively correlated to the length (Kendall's correlation,  $\tau$  = 0.55 for Pointe du diable and  $\tau$  = 0.51 for Porspoder,  $p$ -value < 0.001). Thus, the length of *P. palmata* increased in summer at Pointe du diable, and in winter for Porspoder, reaching a maximum of 19.5 ± 8.3 cm in June and 23.6 ± 12.6 cm in January, respectively (Table 1). Then, the arrival of new recruits in (March) / April caused a decrease in averaged thallus length, which reached a minimum of 8.3 ± 5.2 cm and 3.7 ± 5.1 cm at Pointe du diable and Porspoder, respectively (Table 1). A slight second decrease in thallus length was observed in September coinciding both with the second arrival of recruits, and the dislocation of the old thallus (*pers. obs.*).

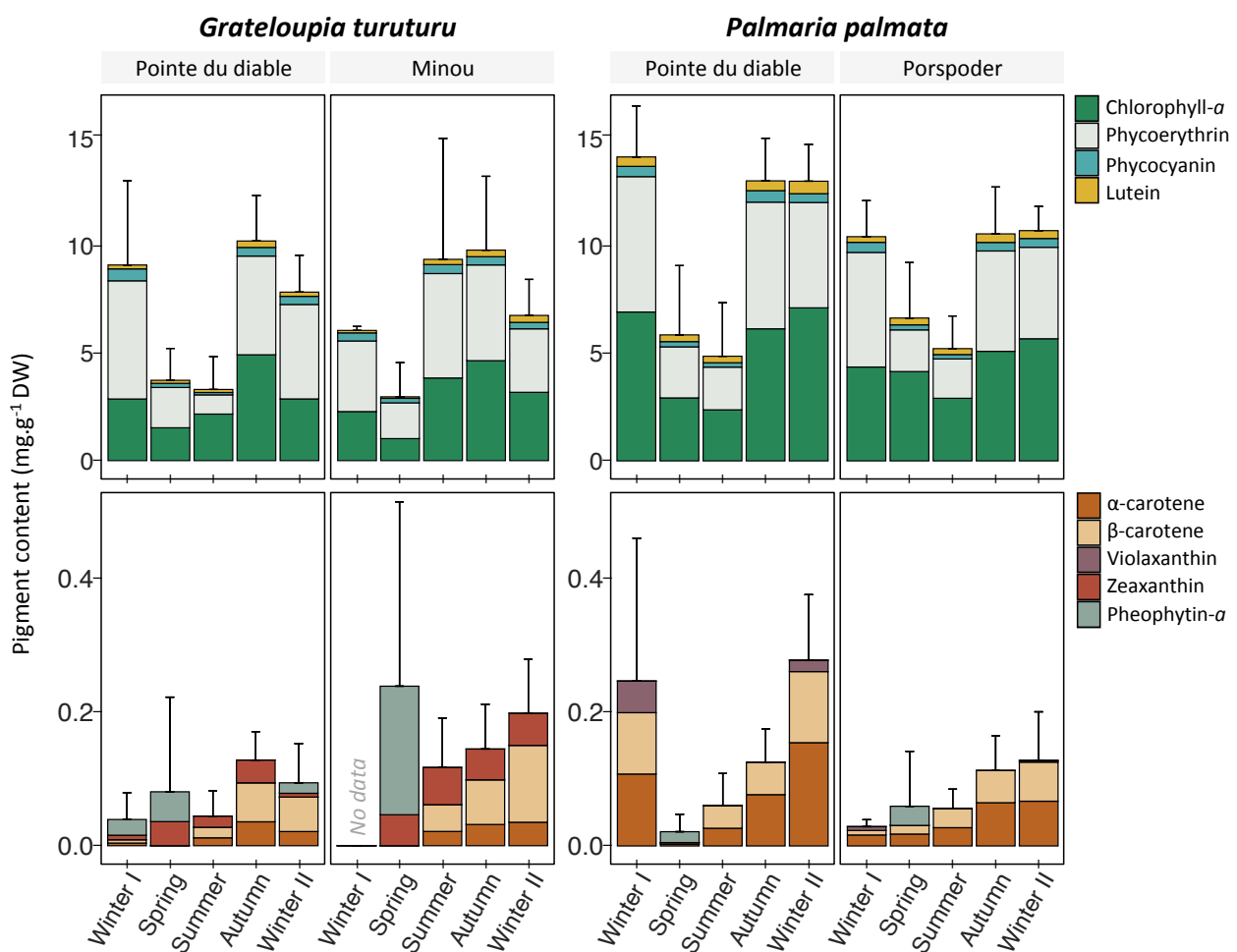
Finally, the thallus length of *P. palmata* was significantly smaller at Pointe du diable than Porspoder (Wilcoxon test,  $p$ -value < 0.001), with 10.4 ± 6.5 cm and 14.6 ± 12.1 cm, respectively. The same result was observed for the density (Wilcoxon test,  $p$ -value < 0.001) with in average 6.02 ± 6.74 individuals / quadrat at Pointe du diable, compared to 13.11 ± 9.77 individuals / quadrat at Porspoder. More particularly, *P. palmata* did not exceed 35 cm at Pointe du diable, compared to 65 cm at Porspoder.

### 3.3. Temporal variability in pigment content (chlorophyll-*a*, phycobiliproteins and carotenoids)

HPLC analysis revealed the presence of 5 common pigments in *G. turuturu* and *P. palmata*, in addition to phycoerythrin and phycocyanin analysed by spectrophotometry (Fig. 1). *G. turuturu* differed significantly from *P. palmata* by the presence of zeaxanthin and the absence of violaxanthin. Among shared pigments, the content of chlorophyll-*a* was significantly lower in *G. turuturu* with in average 3.2 ± 1.6 mg.g<sup>-1</sup> DW compared to 4.6 ± 2.0 mg.g<sup>-1</sup> DW in *P. palmata* (Student's test,  $p$ -value < 0.001), as well as  $\alpha$ -carotene and lutein in a lesser extent (Wilcoxon test,  $p$ -value < 0.001 for both pigments).

Furthermore, although principal component analysis (PCA) revealed no difference in the pigment composition between either species or the different sampling sites (data not shown),

results showed seasonal variations in the pigment composition for both species: there was a significant decrease in chlorophyll-*a* in spring and summer for both species (SRH test, *p*-value < 0.005 for seasons, species, and the interaction species x seasons), coinciding with the global increase in light radiation and temperature, and decrease in nutrient contents between February and July (Fig. 1 and Supplementary Table 1). Only individuals of *G. turuturu* collected at Minou exhibited a limited decrease in chlorophyll-*a* in summer due to an earlier re-increase in September compared to October at Pointe du diable. In addition there was a second slight decrease in winter 2019 for *G. turuturu* at both sites that coincided with a slight increase in global irradiation intensity from December to February (Supplementary Table 1).



**Figure 1.** Temporal variations in pigment composition ( $\text{mg.g}^{-1}$  DW) of *Grateloupia turuturu* and *Palmaria palmata* collected monthly from February 2018 to February 2019 (Brittany, France). Results are displayed as the stacked cumulative contents (with standard deviation of the cumulative contents).

The maximum content of chlorophyll-*a* for *G. turuturu* was in Autumn, *i.e.* November for both sites with in average  $5.2 \pm 0.2 \text{ mg.g}^{-1}$  DW, whereas it was in winter for *P. palmata*, *i.e.*



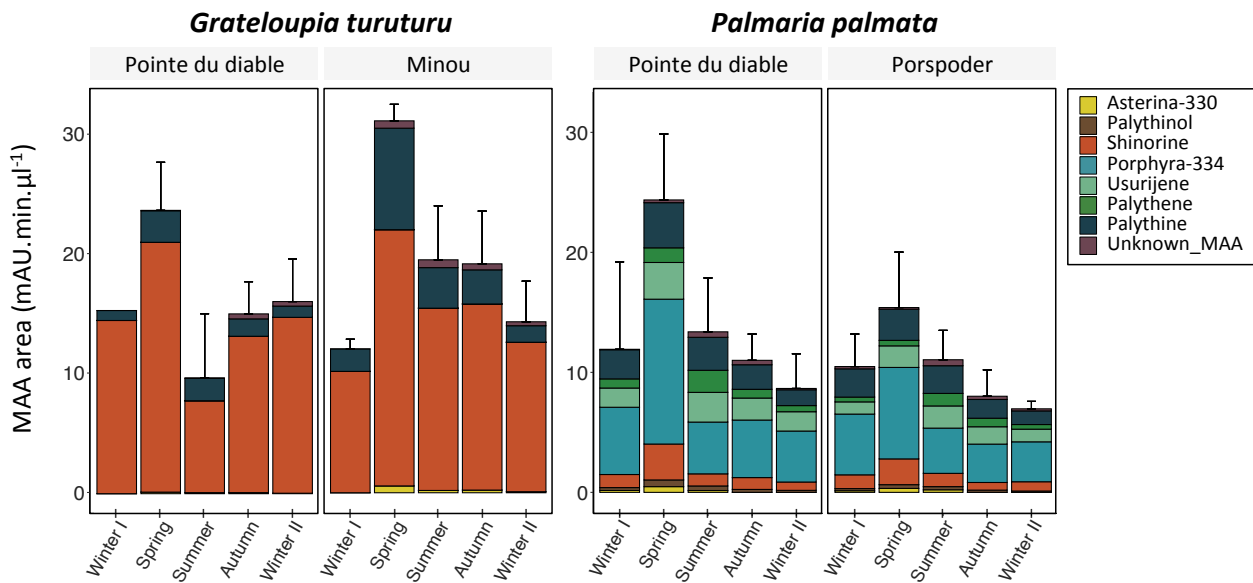
between December and February for both sites, with in average  $6.4 \pm 1.0 \text{ mg.g}^{-1} \text{ DW}$ . Decreases in chlorophyll-*a* coincided with the presence of pheophytin-*a* for both species, whose content was higher in *G. turuturu*, reaching  $0.13 \pm 0.23 \text{ mg.g}^{-1} \text{ DW}$  in June at Pointe du diable and  $0.19 \pm 0.52 \text{ mg.g}^{-1} \text{ DW}$  in May at Minou, compared to only  $0.03 \pm 0.08 \text{ mg.g}^{-1} \text{ DW}$  in *P. palmata* (Fig. 1).

Phycobiliproteins, lutein and  $\alpha$ -carotene followed a similar pattern as chlorophyll-*a*, with which they were correlated for both species (Kendall's correlation,  $\tau = 0.48, 0.49, 0.61$  and  $0.55$  for phycoerythrin, phycocyanin, lutein and  $\alpha$ -carotene respectively,  $p$ -value  $< 0.001$  for all pigments). The same seasonal variation was also observed for  $\beta$ -carotene, except that this pigment did not follow the second decline in winter 2019 for *G. turuturu* collected at Minou (SRH test,  $p$ -value  $< 0.0001$  for the factors seasons and sites,  $p$ -value =  $0.026$  for the interaction seasons x sites) (Fig. 1). In addition, zeaxanthin in *G. turuturu* also slightly varied with months (KW test,  $p$ -value =  $0.003$ ), reaching a maximum content in May and then in October, with  $0.59 \pm 0.02$  and  $0.55 \pm 0.02 \text{ mg.g}^{-1} \text{ DW}$  respectively. On the contrary violaxanthin in *P. palmata* was observed only from January to March at both sites, reaching a maximum content in February 2018 at Pointe du diable with  $0.07 \pm 0.03 \text{ mg.g}^{-1} \text{ DW}$  (Fig. 1).

### 3.4. Seasonal variability in mycosporine-like amino acids

HPLC analysis of mycosporine-like amino acids (MAAs) revealed a different composition between both species (Fig. 2). Indeed, *G. turuturu* had shinorine, asterina-330, palythine, and an unknown MAA (with a maximum absorption at 355 nm), whereas *P. palmata* exhibited the same MAAs, with in addition porphyra-334, usurijene, palythene, and palythanol. Then, shinorine was the main MAA of *G. turuturu* representing in average  $83.62 \pm 8.26 \%$  of its total MAA area, compared to porphyra-334 in *P. palmata*, not exceeding more than  $43.07 \pm 7.50 \%$ . For each species, the same MAAs were observed on the different sampling sites. However, the area of palythine and in a lesser extent asterina-330 were significantly higher at Minou for *G. turuturu* (Wilcoxon test,  $p$ -value  $< 0.001$ ; and Student's test,  $p$ -value =  $0.026$  respectively). Regarding *P. palmata*, the total MAA area was slightly greater at Pointe du diable due to higher level of all MAAs except shinorine (Wilcoxon test,  $p$ -value =  $0.415$ ), palythine (Wilcoxon test,  $p$ -value =  $0.057$ ) and the unknown MAA (Wilcoxon test,  $p$ -value =  $0.754$ ) (Fig. 2). Nevertheless, for both species, the proportions of each MAA were

significantly the same between the different sites (Wilcoxon test,  $p$ -value > 0.05 for all MAAs).



**Figure 2.** Temporal variations in mycosporine-like amino acid composition (mAU.min.µl<sup>-1</sup>) of *Grateloupia turuturu* and *Palmaria palmata* collected monthly from February 2018 to February 2019 (Brittany, France). Results are displayed as the stacked cumulative contents (with standard deviation of the cumulative contents).

Furthermore, results showed seasonal variations in MAAs composition for both species (Fig. 2), which could be due to a combination of environmental parameters including light and nutrients (PCA analysis, Supplementary Fig. 1). Indeed, the total MAA area of *G. turuturu* increased in spring (ANOVA,  $p$ -value < 0.005 for seasons, sites and the interaction months x seasons), reaching  $31.14 \pm 1.38$  mAU.min.µL<sup>-1</sup> for Minou, and  $23.73 \pm 4.02$  mAU.min.µL<sup>-1</sup> for Pointe du diable at this season. This seasonal increase was due to both shinorine and palythine (ANOVA,  $p$ -value < 0.03 for seasons, sites and the interaction months x seasons, for both MAAs), whose areas increased in average from 11.23 to 21.12 mAU.min.µL<sup>-1</sup>, and from 1.62 to 4.84 mAU.min.µL<sup>-1</sup> respectively. Total MAAs area then highly decreased in summer, down to  $19.51 \pm 4.52$  for Minou, and  $9.70 \pm 5.37$  mAU.min.µL<sup>-1</sup> for Pointe du diable. Then, asterina-330 was recorded only some months, *i.e.* those for which the total MAA area increased, with a maximum in May.

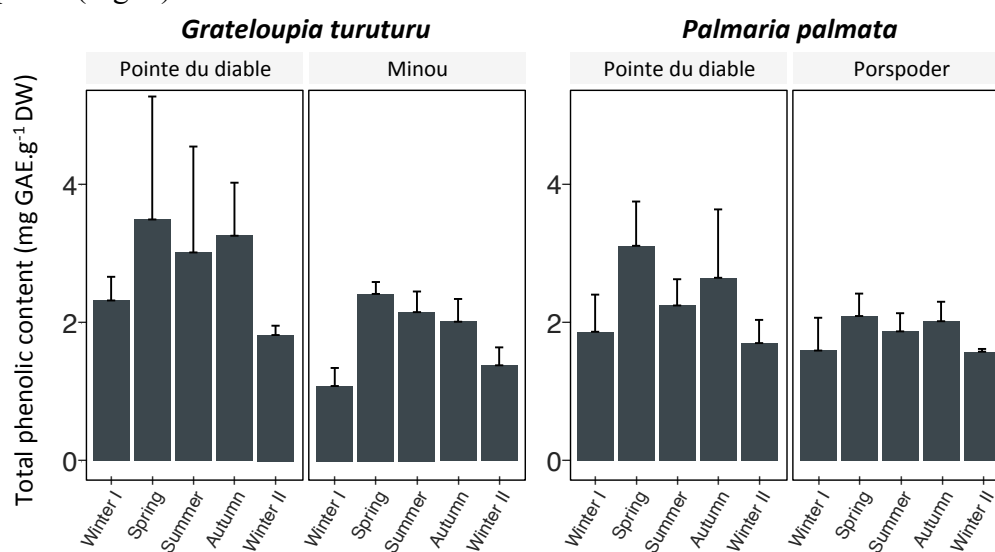
As for *G. turuturu*, the total MAA area of *P. palmata* increased in spring, before decreasing in summer (SRH test,  $p$ -value < 0.001 for seasons,  $p$ -value = 0.013 for sites and  $p$ -value = 0.863 for the interaction months x seasons). Thus, it reached a maximum in May with  $27.74 \pm 5.59$  mAU.min.µL<sup>-1</sup> in Pointe du diable and  $18.13 \pm 4.39$  mAU.min.µL<sup>-1</sup> in Porspoder. This

increase was mainly due to shinorine (SRH test,  $p$ -value < 0.001 for seasons,  $p$ -value = 0.410 for sites and  $p$ -value = 0.391 for the interaction months x seasons) and porphyra-334 (SRH test,  $p$ -value < 0.001 for seasons,  $p$ -value = 0.035 for sites and  $p$ -value = 0.546 for the interaction months x seasons) whose areas increased from  $1.12 \pm 0.65$  to  $2.57 \pm 1.16$  mAU.min. $\mu\text{L}^{-1}$ , and from  $5.33 \pm 2.30$  to  $9.85 \pm 3.47$  mAU.min. $\mu\text{L}^{-1}$ , respectively between winter and spring. Others MAAs followed a similar pattern, and more particularly asterina-330 which has been detected only from March to July-August, with a maximum of  $0.45 \pm 0.08$  mAU.min. $\mu\text{L}^{-1}$  in May.

For both species, only the unknown MAA did not follow the same seasonal pattern than other MAAs, reaching a maximum of  $0.75 \pm 0.30$  mAU.min. $\mu\text{L}^{-1}$  in October at Pointe du diable and  $1.24 \pm 0.55$  mAU.min. $\mu\text{L}^{-1}$  in July at Minou for *G. turuturu*. For *P. palmata*, its content progressively increased from June for both sites, to a maximum of  $0.85 \pm 0.03$  mAU.min. $\mu\text{L}^{-1}$  in October, before significantly decreasing again to  $0.05 \pm 0.04$  mAU.min. $\mu\text{L}^{-1}$  in November.

### 3.5. Seasonal variability in total phenolic content

For both species, total phenolic content (TPC) was significantly different between sampling sites (SRH test,  $p$ -value < 0.002 for sites and species,  $p$ -value = 0.976 for the interaction species x sites). Indeed, TPC was greater at Pointe du diable than Minou for *G. turuturu* with  $2.89 \pm 1.29$  and  $1.88 \pm 0.50$  mg.g<sup>-1</sup> DW respectively. In the same way, TPC of *P. palmata* was higher at Pointe du diable with  $2.40 \pm 0.80$  mg.g<sup>-1</sup> DW, compared to  $1.87 \pm 0.36$  mg.g<sup>-1</sup> DW at Porspoder (Fig. 3).

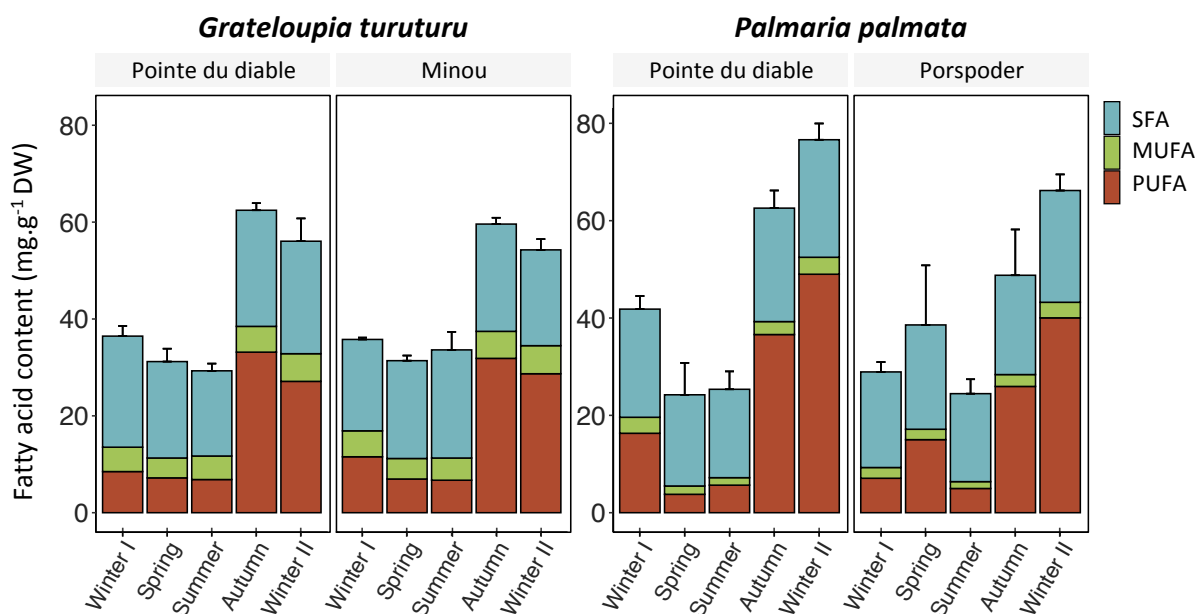


**Figure 3.** Temporal variations in total phenolic content (TPC) (mg Gallic acid equivalent.g<sup>-1</sup> DW) of *Grateloupia turuturu* and *Palmaria palmata* collected monthly from February 2018 to February 2019 (Brittany, France).

However, for both species and all sites, TPC exhibited a quite similar seasonal pattern (SRH test,  $p$ -value < 0.001 for sites and seasons,  $p$ -value = 0.262 for the interaction sites x seasons), increasing in spring and autumn (Fig. 3). TPC reached thus a maximum content in April at Pointe du diable for both species, representing  $5.57 \pm 1.64$  mg.g<sup>-1</sup> DW for *G. turuturu*, and  $3.66 \pm 0.43$  for *P. palmata*. In this way, *G. turuturu* showed higher seasonal increase than *P. palmata*. For both species, TPC was not correlated to seawater temperature (Kendall's correlation,  $\tau = 0.11$  and  $p$ -value = 0.16 for *P. palmata*,  $\tau = 0.13$  and  $p$ -value = 0.12 for *G. turuturu*), and not meaningfully to monthly global radiation (Kendall's correlation,  $\tau = 0.21$  and  $p$ -value = 0.009 for *P. palmata*,  $\tau = 0.15$  and  $p$ -value = 0.07 for *G. turuturu*).

### 3.6. Seasonal variability in fatty acid content

The total fatty acid (TFA) content was significantly the same between both species and the different sampling sites (SRH test,  $p$ -value = 0.061 for species,  $p$ -value = 0.005 for sites and  $p$ -value = 0.596 for the interaction species x sites). TFA was on average  $43.63 \pm 32.40$  mg.g<sup>-1</sup> DW for Pointe du diable and  $45.99 \pm 12.71$  mg.g<sup>-1</sup> DW for Minou in *G. turuturu*; and  $44.32 \pm 23.40$  mg.g<sup>-1</sup> DW for Pointe du diable and  $43.09 \pm 17.32$  mg.g<sup>-1</sup> DW for Porspoder in *P. palmata* (Fig. 4).



**Figure 4.** Temporal variation in fatty acid composition (mg.g<sup>-1</sup> DW) of *Grateloupia turuturu* and *Palmaria palmata* collected from February 2018 to February 2019 (Brittany, France), according to the different types: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), and PUFA (polyunsaturated fatty acids). Results are displayed as the stacked cumulative contents (with standard deviation of the cumulative contents).

Both species had the same main fatty acids, *i.e.* myristic acid (C14:0), palmitic acid (C16:0) and eicosapentaenoic acid (20:5n-3) (see supplementary Table 2 for detailed composition). However, species differed by the presence of palmitoleic acid (C16:1n-7) and unknowns FA\_11 and FA\_13 in *G. turuturu*, compared to the presence of docosahexaenoic acid (C22:6n-3), and unknowns FA\_8 and FA\_14 in *P. palmata*. In addition, among shared fatty acids, PCA analysis revealed that *G. turuturu* had more C16:0, linoleic acid (C18:2n-6), and arachidonic acid (C20:4n-6), whereas *P. palmata* contained slightly more C14:0, and stearic acid (C18:0) (Fig. 5 and supplementary Table 2).

More generally, monounsaturated fatty acids (MUFA) was significantly twice higher in *G. turuturu* (Wilcoxon test,  $p$ -value < 0.001) with in average  $4.93 \pm 0.66 \text{ mg.g}^{-1} \text{ DW}$  compared to  $2.20 \pm 0.77 \text{ mg.g}^{-1} \text{ DW}$  in *P. palmata*. This difference was due to the specific presence of palmitoleic acid (C16:1n-7) in *G. turuturu* and higher level of oleic acid (C18:1n-9) and vaccenic acid (C18:1n-7) (Wilcoxon test,  $p$ -value < 0.001 for both fatty acids). On the contrary there was no significant difference in polyunsaturated fatty acid (PUFA) (Wilcoxon test,  $p$ -value = 0.847) or saturated fatty acids (SFA) content (Wilcoxon test,  $p$ -value = 0.501) between both species.

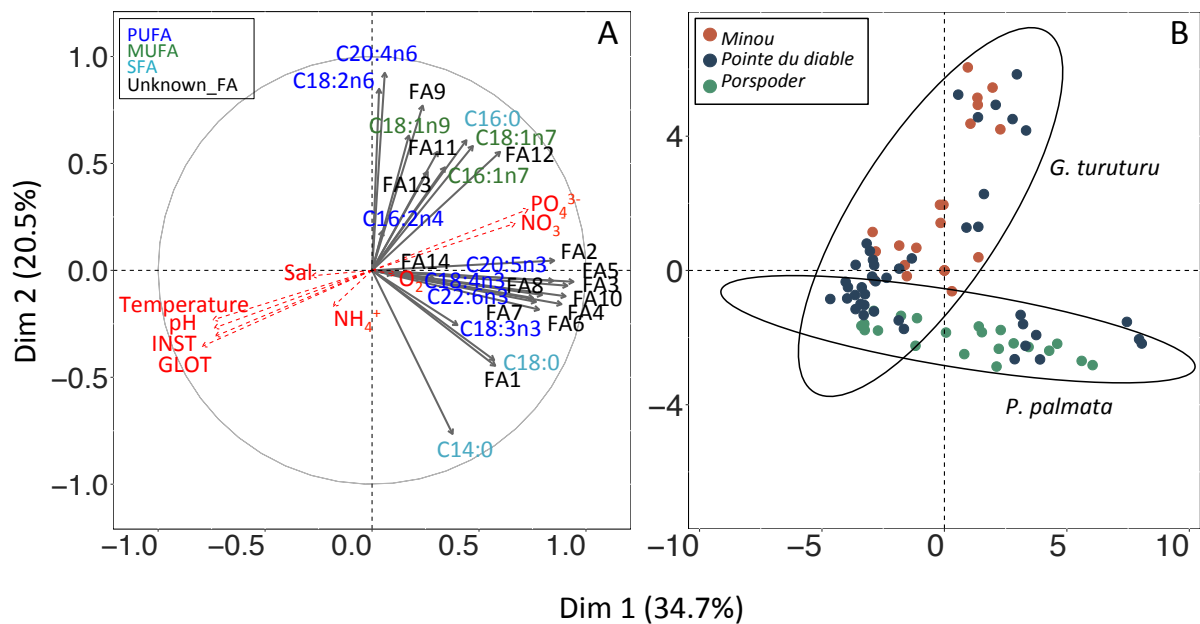


Figure 5. Principal component analysis on fatty acid composition ( $\text{mg.g}^{-1} \text{ DW}$ ) of *Palmaria palmata* and *Grateloupia turuturu* collected monthly from February 2018 to February 2019 (Brittany, France). A: Variable; B: individuals according to sampling site, with ellipses drawn around both species (confidence level of 95%).

Furthermore, results showed temporal variation in fatty acid composition for both species (Figs. 4 and 5). Indeed, *G. turuturu* exhibited a significant increase in TFA content in autumn

and winter 2019 for both sites, whereas it was almost stable the rest of the year (SRH,  $p$ -value  $< 0.001$  for months,  $p$ -value = 0.635 for sites, and  $p$ -value = 0.761 for the interaction months x sites). Thus, among months analysed, TFA content reached a maximum value in November with  $68.82 \pm 1.30 \text{ mg.g}^{-1} \text{ DW}$  for Pointe du diable, and  $65.34 \pm 1.64 \text{ mg.g}^{-1} \text{ DW}$  for Minou. This increase was due to a higher PUFA content (ANOVA,  $p$ -value  $< 0.001$  for months,  $p$ -value 0.307 for sites, and  $p$ -value = 0.541 for the interaction months x sites) whose proportion increased from 18.81 to 53.27 % of TFA between August and November, mainly due to C20:5n-3 and C20:4n-6.

Concerning *P. palmata*, TFA content showed a decrease in spring and summer, and conversely an increase in autumn and winter (ANOVA,  $p$ -value = 0.579 for sites, and  $p$ -value  $< 0.001$  for seasons and the interaction sites x seasons). In addition, there was a slight additional increase in TFA in May for Porspoder (increasing the average TFA content in spring), but not for Pointe du diable (Fig. 4). TFA thus reached a maximum in February 2019 for Pointe du diable with  $85.52 \pm 3.36 \text{ mg.g}^{-1} \text{ DW}$ , while TFA exhibited two peaks for Porspoder, *i.e.* in February 2019 and May with  $72.80 \pm 4.08$  and  $52.84 \pm 6.01 \text{ mg.g}^{-1} \text{ DW}$  respectively. As for *G. turuturu*, these seasonal variations were due to an increase in PUFA content (ANOVA,  $p$ -value = 0.439 for sites, and  $p$ -value  $< 0.001$  for months and the interaction months x sites) whose proportion increased from 13.54 to 62.16 % of TFA between June and February 2019.

#### 4. Discussion

Through a one-year *in-situ* monitoring, the present study adds new information on the understudied phenology of the introduced macroalga *Grateloupia turuturu*, around 30 years after its arrival in Brittany (Simon *et al.* 2001). In addition, results provide an ecological comparison with the native *Palmaria palmata*, a morphologically similar species which lives in the same habitat as *G. turuturu*. More particularly, the present study compared both species in an intertidal habitat where they co-exist, with 2 other locations with either *G. turuturu* or *P. palmata*, to evaluate the potential effects and invasive behaviour of the introduced species. Ecological data were combined with metabolic composition, showing temporal and spatial variations, with intra- and inter-specific differences.

#### **4.1. *G. turuturu*: a species with multiple reproductive strategies reflecting a potential invasive behaviour?**

*Grateloupia turuturu* exhibited fertile individuals all year round, with two major reproductive peaks, one in summer (between May and August), and another one in winter (in January - February) as already described by Simon *et al.* (2001) along the Brittany coasts, and Araújo *et al.* (2011) in the northern Portuguese coast. The reproductive abilities of *G. turuturu* at two contrasting seasons suggested that this species is highly tolerant to environmental variations, and more particularly to increasing light and temperature. This characteristic could be related to its presence in both subtidal and upper intertidal zones, and its distribution from temperate to tropical regions (Hoffmann 1987; Villalard-Bohnsack and Harlin 1997; Liu *et al.* 2017). On the other hand, the presence of fertile fronds throughout the year is consistent with the multiple reproductive strategies of an introduced species (Harlin and Villalard-Bohnsack 2001) and the fact that there is a continuous renewal of individuals with several populations occurring at the same time (Simon *et al.* 2001). Indeed, *G. turuturu* can reproduce both by spores and vegetative propagation, and thus demonstrated four different recruitment strategies described by Harlin and Villalard-Bohnsack (2001): (1) spores develop into small crusts and then into new individuals, (2) production of new crusts by old crusts or filaments, (3) new plantlets from old crusts, and (4) individuals regenerate from old damaged blades. According to Wang *et al.* (2012), the development of juvenile seedlings from original carpospores requires then approximately 60 days. As a result of high fertility year-round, small individuals were recorded throughout the year, with in particular a massive recruitment from August to November at Pointe du diable, and from June to August at Minou. The reproductive potential of *G. turuturu* with different recruitment periods and the presence of fertile individuals or juveniles throughout the year enhance its survival and spread, which is consistent with the potential invasive nature of this species. This invasiveness has not yet been expressed for the French populations but could do so depending on the evolution of certain environmental parameters in Brittany due to global change.

#### **4.2. *G. turuturu*: a high adaptive ability allowing this species to survive in unfavourable habitats?**

Ecological data of *G. turuturu* revealed differences between locations, suggesting the influence of one or several abiotic factors, or competition with native species. Indeed, the

introduced species notably exhibited differences in its reproductive rate, with more fertile individuals at Minou than Pointe du diable (36.12 and 19.92 %, respectively). In addition there was an imbalance in the carposporophytes to tetrasporophytes ratio, individuals from Minou exhibiting a 1:1 ratio, whereas the proportion of carposporophytes was slightly higher at Pointe du diable. This spatial variability in reproduction and ratio could be the result of the different requirements and physiological tolerance of the different life phase to environmental conditions, and more particularly to their differences in mortality and reproductive output leading to the dominance of one phase (Fierst *et al.* 2005; Araújo *et al.* 2011). In this respect, it has been shown for example in *Chondrus ocellatus* (Li *et al.* 2010) that carposporophytes are more likely to withstand extreme thermal conditions. However it is difficult to really conclude on the tolerance of each phase since the reproduction of macroalgae is controlled by the combination of several abiotic and biotic factors (Hoffmann 1987; Liu *et al.* 2017; Lalegerie *et al.* 2020a). Then, spatial difference in reproduction could be due to higher recruitment observed at Pointe du diable, reducing the proportion of fertile individuals and modifying ratios, a larger number of recruits not requiring the same reproductive efficiency.

Moreover, results revealed spatial differences in the recruitment and growth of *G. turuturu*, similarly to Simon *et al.* (2001). Indeed, the introduced species exhibited different phenology between both sites, *i.e.* discrepancy between the periods of recruitment and maximal length at Pointe du diable but not at Minou. In this way, failure in recruitment or a limited growth can cause a discrepancy between these two periods of development. In this regard, individuals from Minou exhibited a lower density and maximal length compared to Pointe du diable, suggesting a limited development of *G. turuturu* at this site that may have caused a delay in its phenology. This limited development could be due to the reduced depth of the rockpools at this site. In addition, Harlin and Villalard-Bohnsack (2001) suggested that the extreme temperatures and not the average temperature condition the survival of *G. turuturu*, as extremes conditions can surpass limits of biological tolerance. Consequently, it is possible that the seawater of the rockpools from Minou may have reached a higher temperature in summer, causing a limited recruitment. On the contrary, the phenological delay between both sites could be due to higher macroalgal diversity and biomass at Pointe du diable (*pers. obs.*). Indeed, *G. turuturu* may have delayed its recruitment or growth phase in order to not increase its biomass and thus limit clutter. Subsequently, spatial variations could therefore be the result of intra- and inter-specific competition for space or resources. In this way, previous works have already suggested that some species can regulate their length or density to limit



intraspecific competition, such as *Sargassum muticum* (Arenas *et al.* 2002), *Turbinaria ornata* (Stiger and Payri 2005) or *Fucus* spp. (Steen and Scrosati 2004).

Finally, the high spatial variability in growth and reproduction might also be related to multiple reproductive strategy of *G. turuturu*. More particularly, the constant renewal of individuals showed that *G. turuturu* has a strong capacity to adapt and modulate its phenology over the year, which might enhance phenological differences between sites. It is possible to consider that all individuals of *G. turuturu* do not follow a specific seasonal pattern, explaining phenological differences with and within the different studies previously reported on the ecology of *G. turuturu* (Harlin and Villalard-Bohnsack 2001; Simon *et al.* 2001; Araújo *et al.* 2011). This supports the idea that *G. turuturu* possesses a large plasticity, and thus the ability to survive under unfavourable conditions contrary to many algal species, demonstrating once again its invasive potential. More specifically, *G. turuturu* is living in rockpools, where conditions are particular and highly variable, and creating potential microhabitats different with and within sites.

#### **4.3. *Palmaria palmata*: a native species with lower plasticity, threatened when associated with the introduced *G. turuturu*?**

Results revealed that *Palmaria palmata* was mainly fertile in winter (*i.e.* between November and March), coinciding with previous studies within various regions (Faes and Viejo 2003; Le Gall *et al.* 2004; Pang and Lüning 2006). By consequence, the native *P. palmata* appeared to have a lower reproductive plasticity compared to the introduced *G. turuturu* which exhibited fertile individuals all year-round. More particularly, reproduction of *P. palmata* appeared to be favoured by low light and temperature conditions, as confirmed by environmental parameters recorded in the present study (Supplementary Table 1), and the almost total absence of fertile individuals from July to October. In addition, a significant depigmentation and degradation of the thallus has been observed at the end of summer at both sites (*pers. obs.*), suggesting again low tolerance of *P. palmata* to prolonged exposure to high light and temperature. The geographic distribution of *P. palmata* from cold temperate to Polar Regions (Karsten and Wiencke 1999; Grote 2017) could therefore be in part related to its tolerance to these both environmental parameters. However, a second one-off reproductive period was observed in summer at both sampling sites, with a high proportion of tetrasporophytes recorded in June at both sites. To our knowledge, few studies reported the presence of fertile

individuals in summer *in situ*. However, this result is in accordance with Pang and Lüning (2006) who reported the possibility to induce two phases of spore release in tank cultivation, *i.e.* in January and June, by changing light/dark regime and temperature. The presence of tetrasporophyte in June could be then a possible disruption related to a change in environmental conditions occurred at both sites, modifying the development of tetrasporophytes or causing an imbalance in the ratio of male and female gametophytes which are usually fertile simultaneously (Le Gall *et al.* 2004).

Results also revealed a spatial difference in growth and recruitment of *P. palmata*. First, at Pointe du diable, three short recruitment periods were observed, while individuals from Porspoder exhibited a larger recruitment period, between April and October. Secondly, individuals from Porspoder exhibited higher density and length. Notably, *P. palmata* did not exceed 35 cm at Pointe du diable, compared to 65 cm at Porspoder. In this respect, the development of *P. palmata* could be limited at Pointe du diable due to the great algal diversity and abundance at this site. More particularly, the high recruitment of *G. turuturu* between August and January at Pointe du diable may have limited the development of *P. palmata* which exhibited then a maximum length in summer, compared to winter at Porspoder. On the other hand, the development of *P. palmata* may have been limited by the hydrodynamic conditions which were more important at Pointe du Diable than Porspoder. In this respect, a sudden decrease in density was observed in November at both locations, which may be related to hydrodynamism with the frequent winter storms (*pers. obs.*), suggesting the importance of this factor in the distribution of the native species. In conclusion, it cannot be excluded that the native species is affected by the presence of the introduced species at Pointe du diable, although spatial difference and reduced density at this site could be due to a combination of factors leading to unfavourable conditions for the native species. The ecological data suggest then that *P. palmata* may be challenged to manage both its acclimation to environmental variations and its cohabitation with other species. More particularly, it could be greatly impacted in the context of global warming as the native species appeared to be highly dependent to light and temperature.

#### 4.4. Pigment composition: the evidence of different light sensitivity between *G. turuturu* and *P. palmata*?

Beyond ecological data, this present study revealed differences in the metabolic profiles of *G. turuturu* and *P. palmata*, including spatial differences, highlighting then different adaptive abilities which might be related to their specific plasticity and development strategies. First, *G. turuturu* exhibited higher zeaxanthin and pheophytin-*a* at Minou than Pointe du diable. Zeaxanthin contributed to the xanthophyll cycle, *i.e.* a photoprotective mechanism commonly found in red and brown macroalgae, in which violaxanthin is indirectly converted into zeaxanthin (Karentz 2001; Ursi *et al.* 2003; Goss and Jakob 2010). Pheophytin-*a* derived from the degradation of chlorophyll-*a* (Steinman *et al.* 2017), and could have a photoprotective role (Hou 2014). In addition, this pigment composition at Minou was coupled to a greater MAA content of *G. turuturu* (*i.e.* palythine and asterina-330), known for their photoprotective role (Singh *et al.* 2008). In this regard, individuals from Minou may have experienced higher light radiation due to shallow rockpools at this site, demonstrating then the ability of *G. turuturu* to adapt to high light levels. On the contrary, *P. palmata* showed higher violaxanthin and MAA contents at Pointe du diable than Porspoder. Similarly to *G. turuturu*, individuals from Porspoder may have been less exposed to light radiation, since they live in a channel with a relatively deeper water depth. Then, the reduced development and recruitment of *P. palmata* at Pointe du diable could be related to its low tolerance to high light, despite the synthesis of photoprotective compounds.

On the other hand, results suggested that *G. turuturu* is more sensitive to light than *P. palmata*. Indeed, pigment analyses revealed a decrease in chlorophyll-*a* and other photosynthetic pigment in spring and summer for both species, due to high irradiance prevailing at this period, to reduce photosynthesis and avoid photodamages (Raven and Hurd 2012; de Ramos *et al.* 2019). However, a second slight decrease was observed in winter 2019 for *G. turuturu*, but not for *P. palmata*, caused by a slight second gradual increase in global radiation and insolation between December 2018 and February 2019. More globally, *G. turuturu* displayed a lower level of photosynthetic pigments over the year compared to *P. palmata* and appeared thus to be more sensitive. On the contrary, *G. turuturu* appeared to synthesize more photoprotective pigments, including pheophytin-*a*, and zeaxanthin. It can therefore be inferred that *G. turuturu* is more sensitive to high light, but that in parallel it is conversely able to synthesize a greater amount of sunscreen metabolites to cope with

increasing light stress and improve its tolerance. This result is in accordance with its multiple reproductive strategies and fertile individuals and juveniles throughout the year, requiring/inducing enhanced photoprotection. In this sense, it has been demonstrated that the growth rate of young sporophytes of the red algae *Mastocarpus stellatus* or *Chondrus crispus* were more sensitive to UV radiations than gametophytes (Roleda *et al.* 2007), which could be similar in *G. turuturu*. In conclusion, despite higher sensitivity to high light, the capacity of *G. turuturu* to increase its contents in photoprotective pigments shows that this species could be physiologically better equipped to cope with global change than *P. palmata*.

#### 4.5. Mycosporine-like amino acids: two different synthesis strategies?

As pigment data showed more photoprotective pigments and a greater sensitivity to increasing light in *G. turuturu*, it was expected also different MAAs profiles between both species considering their photoprotective role (Singh *et al.* 2008; Bhatia *et al.* 2011). In this way, the MAAs analysis revealed that *G. turuturu* contained four MAAs (shinorine, palythine, asterina-330 and an unknown MAA), compared to eight in *P. palmata* (*i.e.* the same as *G. turuturu* with in addition porphyra-334, palythinol, usurijene, and palythene). These profiles were in accordance with composition previously reported (Karsten *et al.* 1998; Yuan *et al.* 2009; Guihéneuf *et al.* 2018 for *P. palmata*; and Figueroa *et al.* 2007 for *G. turuturu*). However, despite this difference in composition, both species exhibited the same seasonal variations, with an increase in the total MAA area in spring (including the appearance of asterina-330) and then a decrease in summer, related to changes in both light and nutrient conditions (see more details in Lalegerie *et al.* (2020b)). By consequence, seasonal variations in MAAs does not appear to be impacted by the ecology and reproductive strategies of both species. It has already been shown for example that sporophytes and gametophytes do not differ in composition, as in *Chondracanthus chamissoi* (Véliz *et al.* 2019) or *Bangia* spp. (Boedeker and Karsten 2005), although both studies showed a slightly higher levels of MAAs in gametophytes. Only the unknown MAA (assumed to be a MAA by its maximum absorption at 355 nm) exhibited a different seasonal pattern between both species, following approximately the density of each species. It could therefore be indirectly related to intra-competition or to the age of the individuals. However, further investigations are needed since, to our knowledge, no study has so far shown a similar result.

Moreover, despite similar seasonal pattern, *G. turuturu* appeared to preferentially accumulate a great amount of one MAA (*i.e.* shinorine representing in average 83.62 % of its total MAA area), compared to *P. palmata* which exhibited a greater diversity (with porphyra-334 as the main MAA, not exceeding more than 43.07 %). Thus, although it is not possible to properly compare the content in MAAs between both species due to the lack of commercially available standards, this difference in the number of MAAs suggests 2 different strategies of synthesis pathways between the native and introduced species: we may assume that *P. palmata* has potentially additional synthesis pathways and that it synthesizes several MAAs due to their different potential roles (see Bhatia *et al.* 2011 for a review). Conversely, only one main MAA has been observed in *G. turuturu*, either (i) because the transformation between the different MAAs is too rapid to be observed compared to the native species, (ii) or because *G. turututu* possess different synthesis pathways. In both cases, this could be a strategy for *G. turuturu* to be able to accumulate huge amount of shinorine and respond to increasing light more quickly. Then, as MAAs are nitrogenous compounds, it is possible that the native species has not the capacity to accumulate huge amount of one single MAA, due to the reduced nutrient availability in seawater during spring / summer (Lalegerie *et al.* 2020b). In this way, *P. palmata* might have lower nutrient storage than *G. turuturu* because of its distinct reproductive strategies described above. In summary, the fact that both species exhibited different MAAs profiles, one favouring the rapid accumulation of one or few compounds, and the other one favouring the synthesis of multiple compounds with potential synergic effects, suggests that both species may not have the same abilities to cope with future environmental changes.

#### 4.6. Phenolic compounds: biological multi-purpose agents?

Phenolic compounds are known to be involved in many protective mechanisms (*e.g.* antioxidant, photoprotective, or antimicrobial) and to fluctuate with many parameters, both biotic and abiotic (Freile-Pelegrín and Robledo 2013; Gager *et al.* in press). In this way, total phenolic content (TPC) exhibited monthly variations, with a similar pattern for both species and all sampling sites. Amplitude differences in the seasonal pattern between sampling sites could be due to the characteristics of each location whatever the season. More particularly, the greater hydrodynamic conditions at Pointe du diable for both species could be responsible of the higher TPC at this location as already demonstrated in brown macroalgae (Rönnerberg and

Ruokolahti 1986; Plouguerné *et al.* 2006a) but never studied in red seaweeds. More particularly, *P. palmata* showed at Pointe du diable a reduced length and density, and could thus synthesize more TPC in response to potential unfavourable conditions caused by a greater hydrodynamism.

Then, two months presented slightly higher phenolic concentrations: one in April, and the second one around October. The first seasonal peak in TPC could be due to the increasing light and temperature in spring, supporting the idea of photoprotective and antioxidant roles of phenolic compounds. However, the peak in TPC did not coincide with the seasonal maxima of both parameters (supplementary Table 1). Similarly, no environmental parameter (among those recorded) appeared to be related to the second increase in TPC. Thus, it is possible that the changes in TPC were also related to the ecological phenologies of both species. In this sense, *G. turuturu* exhibited higher recruitment at Pointe du diable, with a maximum density in October. Concerning *P. palmata*, both increases in TPC occurred when almost no fertile individuals were observed; and simultaneously with recruitment or increasing growth at Pointe du diable, which were indirectly dependent on light, temperature and nutrient levels. Then, TPC could vary with the stage of development or the part of the thallus in red macroalgae, as already demonstrated in brown species (Pavia *et al.* 2003; Stiger *et al.* 2004; Connan *et al.* 2006). Another hypothesis is that seasonal increases in TPC could be related to the growth and development of other native or invasive species at the same locations. Indeed, some species were common to the three sampling sites, including *Sargassum muticum* and *Bifurcaria bifurcata*. Interestingly, these brown macroalgae were monitored over the same period as the present study (Lalegerie *et al.* in prep) and exhibited an opposite seasonal pattern in TPC with however, different types of phenolic compounds with molecular structure based on phloroglucinol in brown seaweeds and phenolic acids as caffeic acid or halogenated phenolic compounds in red seaweeds (Gager *et al.* in press). This opposite pattern was then supposed to be related to maximum light in summer and the reproductive period of *S. muticum*. As a result, *P. palmata* and *G. turuturu* could be dependent from other species, including *S. muticum* which is invasive in Brittany. Finally, whatever the biotic or abiotic factor(s) that caused the seasonal peaks in TPC, results showed a greater seasonal increases in *G. turuturu* than *P. palmata* at Pointe du diable, suggesting once again a higher accumulation and thus a better tolerance of the introduced species to environmental changes.

#### 4.7. Fatty acids: a composition consistent with the ecology and geographical distribution of both species?

As PUFA are an important part of photosynthetic apparatus, it was assumed that their concentrations increase under low light, with photosynthetic rate (Wacker *et al.* 2015; Schmid *et al.* 2017). It was also reported that PUFA level increase at low temperatures, since they are responsible for the membrane fluidity, and thus maintain the function of photosystems (Hotimchenko 2002; Wacker *et al.* 2015; Schmid *et al.* 2017; Harwood 2019). In this way, both species showed a similar significant increase in TFA in winter due to polyunsaturated fatty acids (PUFA), and more particularly to arachidonic acid (C20:4n-6) and eicosapentaenoic acid (C20:5n-3). Their specific composition were then in accordance with composition previously reported (see Denis *et al.* 2010; Hotimchenko 2002; Kendel *et al.* 2013 for *G. turuturu*; and Mishra *et al.* 1993; Fleurence *et al.* 1994; Schmid *et al.* 2017 for *P. palmata*). Moreover, both species showed a high content of PUFA in February 2018 compared to February 2019, which could be related to a difference in light and temperature between both winters. Then, unlike *G. turuturu*, who showed no spatial variation, *P. palmata* exhibited an additional increase in PUFA content in May, at Pointe du diable but not at Porspoder. This increase could be due to a temporary nutrient starvation at Pointe du diable, which could be another (direct or indirect) factor responsible of its limited development at this site (see section 4.4). It has been shown for example that a phosphate limitation can lead to an imbalance in membrane lipid, and more particularly phospholipids and glycolipids (Goss and Wilhelm 2009; Harwood 2019), leading to a change in the unsaturation level.

On the other hand, despite similar TFA and PUFA content between both species, *P. palmata* exhibited a greater seasonal decrease in summer compared to *G. turuturu*, which could be related to their specific acclimation to high temperature. Indeed, *P. palmata* could be more sensitive to high temperature occurring in summer, related to its geographical distribution from cold temperate to Polar Regions (Karsten and Wiencke 1999; Grote 2017), compared to the tropical distribution of *G. turuturu* (Villalard-Bohnsack and Harlin 1997). In this sense, Liu and Pang (2010) showed that *G. turuturu* could be better acclimatized to high temperature due to higher activities of superoxide dismutase (SOD) and peroxidase (POD), giving it more resistance to oxidative stress. More generally, *G. turuturu* is known to be tolerant to a wide range of temperature (from 5 to 25 °C) (Simon *et al.* 1999, 2001), compared to other species, and notably *P. palmata* whose optimal growth is between 6 and 10 °C (Corey *et al.* 2012).

Their different thermal acclimation could be then related to their specific polyunsaturated fatty acid composition, *G. turuturu* synthesizing more arachidonic acid (C20:4n-6). On the other hand, it is possible that PUFA of *G. turuturu* has been maintained at a relatively higher content to limit salt stress caused by high temperature and evaporation in rockpools during summer. Indeed, it has been shown in the cyanobacterium *Synechococcus* sp. that an increased unsaturation of fatty acids could enhance tolerance to salt stress by protecting photosystems against NaCl-induced inactivation (Allakhverdiev *et al.* 2009), which could be similar in the introduced species. Finally, results highlighted a 2-fold higher monounsaturated fatty acids (MUFA) content in *G. turuturu*, compared to *P. palmata*, related in part to the specific presence of palmitoleic acid. This specificity could be the result of the reproductive strategy of *G. turuturu*, with many fertile individuals all year-round. Indeed, MUFA could represent a large part of storage lipids, used for growth, or reproduction (Guschina and Harwood 2013). In this way, it is possible that the gametophytes of *G. turuturu* had a higher MUFA content than sporophytes, as demonstrated in *Chondrus crispus* (Tasende 2000).

#### 4.8. Conclusion and perspectives: *G. turuturu*, a real threat to native species?

Data obtained in this study supported the hypothesis that the introduced species *G. turuturu* exhibited a different ecological phenology than *P. palmata*, in accordance with their specific metabolomic composition. Indeed, *G. turuturu* was fertile throughout the year, enhancing its survival and spread. Then, it appeared to have the ability to accumulate huge amount of secondary metabolites (*i.e.* pigments, MAAs, or phenolic compounds), increasing its tolerance to seasonal changes, and enhancing the survival of its juveniles and fertile individuals over the year. In addition, results revealed a high plasticity of *G. turuturu* between sampling sites, enhancing its ability to survive to different / extreme conditions, which is consistent with its potential invasive nature. On the contrary, *P. palmata* is a perennial species, showing a high reproduction in winter, due to a high sensitivity to high temperature. Then, the native species appeared to have a limited growth and recruitment at Pointe du diable, where it co-exist with the introduced *G. turuturu*. However, although it cannot be excluded that *P. palmata* is affected by the presence of the introduced species at Pointe du diable, spatial difference could be due to a combination of factors leading to unfavourable conditions for the native species, including higher hydrodynamism, coupled to a greater algal diversity and abundance at this site. In this way, despite a high physiological tolerance, the



intertidal populations of *G. turuturu* in Brittany were relatively reduced, and appeared far from its common description, as one of the five most-threatening introduced species in the world (Nyberg and Wallentinus 2005; Koerich *et al.* 2020). Indeed, *G. turuturu* was often limited to a few individuals and rarely exceeded 30 cm in length (Table 1). Then, it would appear then unlikely that this introduced species would have competitive or harmful impact on *P. palmata* or other native species at these locations in Brittany. In this sense, previous studies have already suggested that *G. turuturu* may have no invasive behaviour, and that it would rather take advantage of disturbances and unfavourable environmental conditions to establish in the niche left vacant by other species (Harlin and Villalard-Bohnsack 2001; Cecere *et al.* 2011; Mulas and Bertocci 2016). Similarly to our results, Petrocelli *et al.* (2020) concluded recently that *G. turuturu* is not invasive in the Mar Piccolo of Taranto (Mediterranean Sea), based on observations carried out from 2008 to 2018. Nevertheless, the great physiological tolerance of the introduced species could be an ecological advantage compared to native species, to cope with future environmental changes. For example, Bertocci *et al.* (2015) demonstrated that the spores of *G. turuturu* could survive at 30 °C, unlike those of *Chondrus crispus*, another red algal species commonly cohabiting with *G. turuturu*. Then, although *G. turuturu* does not appear to be invasive for the moment in Brittany, a future change in one or more environmental parameters could increase its invasiveness, hence the interest in monitoring introduced species over the long term, which is still a requirement today, especially for this type of discrete species. In this regard, *G. turuturu* could replace some native species such as *P. palmata* which may be challenged to manage both their acclimation to environmental variations (*e.g.* increasing temperature) and their cohabitation with other opportunistic species.

## 5. Acknowledgements

Fanny Lalegerie's PhD fellowship was provided from Region Bretagne and the Doctoral School of Marine and Littoral Sciences of the Université Bretagne Loire (UBO, Brest, France) and supervised by both S.C. and V.S.-P. We thank Marie-Aude Poullaouec for her help in sample grinding and the phenolic compounds extractions. Finally we thank Leslie Gager, Mathieu Helias and all the others colleagues of the Institute for helping at field sampling.

## 6. Authors' contributions

F.L. performed the analysis of the samples, drafted the manuscript and conducted the statistical analyses. V.S-P and S.C designed and supervised the project. M.Z. and D.B.S. supervised the extraction and analysis of fatty acids.

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## Supplementary data

**Supplementary Table 1.** Seasonal variations of environmental data measured between February 2018 and February 2019 (Brittany, France). The light data were provided by « Météo France », and other data were provided by « Service d'Observation en Milieu Littoral, INSU-CNRS, Saint-Anne du Portzic ». Data are expressed in °C for temperature, mL.L<sup>-1</sup> for oxygen level, µM for nutrient levels, J.cm<sup>-2</sup> for light radiation and hours for insolation. The maximum value of each parameter is displayed in red.

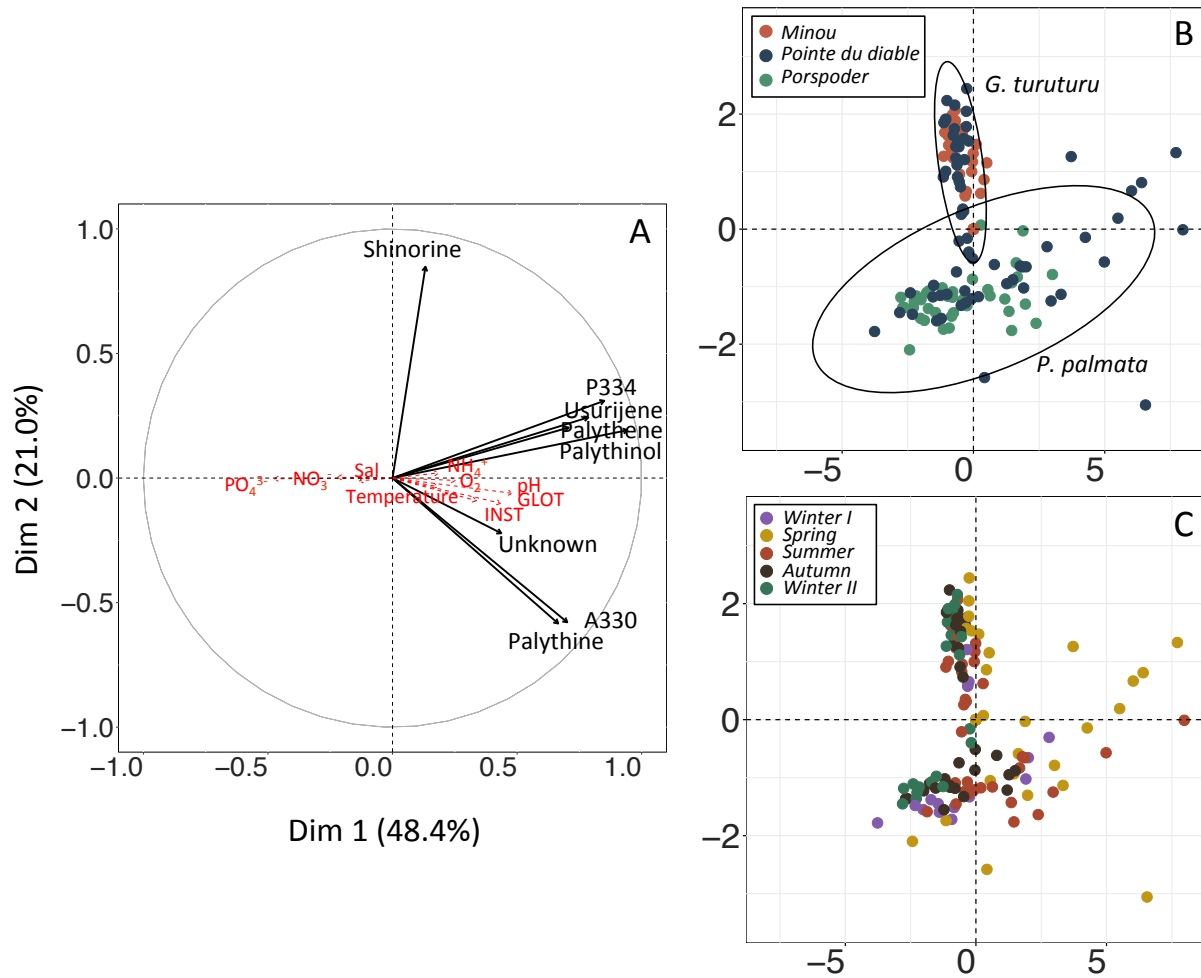
	Seawater temperature	Salinity	Oxygen	pH	Ammonium	Nitrates	Phosphates	Global irradiation	Daily insolation
<b>February 2018</b>	9.52 ± 0.59	34.11 ± 0.14	6.38 ± 0.25	7.93 ± 0.01	0.53 ± 0.28	<b>18.62 ± 1.56</b>	0.51 ± 0.02	800.18 ± 367.18	5.01 ± 3.30
<b>March</b>	8.86 ± 0.45	33.94 ± 0.34	6.54 ± 0.18	7.96 ± 0.05	0.46 ± 0.08	15.57 ± 1.17	0.33 ± 0.09	847.23 ± 377.69	2.43 ± 2.47
<b>April</b>	11.14 ± 1.30	33.73 ± 0.14	6.71 ± 0.35	7.99 ± 0.06	0.52 ± 0.18	11.43 ± 3.78	0.11 ± 0.06	1479.93 ± 592.42	5.04 ± 4.29
<b>May</b>	13.10 ± 0.75	34.12 ± 0.25	<b>6.72 ± 0.38</b>	8.07 ± 0.04	1.07 ± 0.71	3.40 ± 2.43	0.01 ± 0.03	1988.55 ± 672.75	7.30 ± 4.99
<b>June</b>	15.31 ± 0.98	34.58 ± 0.11	6.41 ± 0.66	<b>8.09 ± 0.05</b>	0.70 ± 0.62	0.60 ± 0.43	0.02 ± 0.04	1990.97 ± 849.18	7.09 ± 6.32
<b>July</b>	<b>17.83 ± 0.34</b>	34.80 ± 0.10	5.74 ± 0.34	8.04 ± 0.04	0.42 ± 0.15	0.34 ± 0.09	0.06 ± 0.02	<b>2088.13 ± 694.69</b>	<b>8.42 ± 5.25</b>
<b>August</b>	16.91 ± 0.57	35.07 ± 0.07	5.60 ± 0.19	8.01 ± 0.02	0.57 ± 0.09	0.66 ± 0.09	0.11 ± 0.01	1440.29 ± 642.42	4.17 ± 4.50
<b>September</b>	16.10 ± 0.45	<b>35.20 ± 0.04</b>	5.55 ± 0.19	7.96 ± 0.04	0.93 ± 0.49	1.16 ± 0.62	0.18 ± 0.04	1301.10 ± 489.21	5.56 ± 4.27
<b>October</b>	14.71 ± 0.86	35.20 ± 0.07	5.52 ± 0.16	7.98 ± 0.07	1.03 ± 0.37	2.58 ± 1.33	0.31 ± 0.13	861.55 ± 414.77	5.08 ± 3.73
<b>November</b>	12.36 ± 0.61	35.02 ± 0.12	5.81 ± 0.15	7.93 ± 0.02	1.01 ± 0.14	5.80 ± 1.08	0.47 ± 0.03	468.80 ± 241.71	3.47 ± 2.68
<b>December</b>	11.60 ± 0.44	34.01 ± 0.46	6.00 ± 0.07	7.89 ± 0.02	<b>1.08 ± 0.29</b>	14.98 ± 4.75	<b>0.53 ± 0.03</b>	189.48 ± 123.76	0.63 ± 0.95
<b>January</b>	10.75 ± 0.56	34.34 ± 0.34	6.04 ± 0.07	7.89 ± 0.04	0.45 ± 0.08	15.98 ± 2.23	0.48 ± 0.01	313.71 ± 161.69	1.55 ± 1.96
<b>February 2019</b>	9.82 ± 0.30	34.16 ± 0.21	6.36 ± 0.02	7.91 ± 0.01	0.45 ± 0.11	16.33 ± 2.41	0.45 ± 0.03	746.25 ± 411.01	4.76 ± 4.16

**Supplementary Table 2.** Monthly fatty acid composition (mg.g<sup>-1</sup> DW) and total fatty acids (TFA) content in *Palmaria palmata* and *Grateloupia turururu* collected monthly between February 2018 and February 2019 (Brittany, France). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids. n.d. = not detected (under the detection level).

		<i>Grateloupia turururu</i>						
		February 18	May	June	July	August	November	February 19
<b>SFA</b>	C14:0	1.77 ± 0.17	2.00 ± 0.46	1.84 ± 0.23	1.95 ± 0.19	2.04 ± 0.18	1.83 ± 0.03	1.78 ± 0.10
	C16:0	18.5 ± 1.94	18.48 ± 0.79	15.71 ± 0.12	16.59 ± 2.2	18.07 ± 3.33	20.69 ± 1.07	19.15 ± 1.82
	C18:0	0.63 ± 0.19	0.52 ± 0.02	0.46 ± 0.10	0.50 ± 0.12	0.76 ± 0.33	0.53 ± 0.04	0.57 ± 0.10
	C16:1n-7	1.09 ± 0.23	0.42 ± 0.10	0.52 ± 0.05	0.46 ± 0.03	0.52 ± 0.06	1.01 ± 0.16	1.09 ± 0.22
	C18:1n-9	2.83 ± 0.42	2.70 ± 0.16	3.16 ± 0.16	3.41 ± 0.38	3.31 ± 0.5	3.19 ± 0.18	3.26 ± 0.58
	C18:1n-7	1.27 ± 0.38	0.85 ± 0.21	0.83 ± 0.13	0.82 ± 0.12	0.92 ± 0.03	1.25 ± 0.20	1.39 ± 0.37
	C16:2n-4	0.14 ± 0.03	n.d.	n.d.	n.d.	n.d.	n.d.	0.19 ± 0.02
	C18:2n-6	0.92 ± 0.09	0.60 ± 0.12	0.82 ± 0.16	0.75 ± 0.16	0.73 ± 0.20	1.32 ± 0.08	1.43 ± 0.20
	C18:3n-3	n.d.	n.d.	n.d.	n.d.	0.31 ± 0.08	n.d.	0.48 ± 0.31
	C18:4n-3	n.d.	n.d.	n.d.	n.d.	n.d.	0.35 ± 0.17	0.84 ± 0.50
	C20:4n-6	4.15 ± 1.23	2.01 ± 0.47	3.26 ± 0.81	3.26 ± 1.09	2.43 ± 0.64	10.36 ± 0.99	8.80 ± 1.72
	<b>C20:5n-3</b>	<b>4.79 ± 1.02</b>	<b>3.71 ± 1.03</b>	<b>4.56 ± 2.29</b>	<b>3.62 ± 1.58</b>	<b>2.57 ± 0.70</b>	<b>20.66 ± 2.00</b>	<b>16.48 ± 2.23</b>
C22:6n-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
<b>MUFA</b>	FA_1	0.12 ± 0.02	0.19 ± 0.05	0.10 ± 0.01	0.20 ± 0.03	0.21 ± 0.09	0.33 ± 0.10	0.18 ± 0.08
	FA_2	0.17 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.16 ± 0.02	0.16 ± 0.02	0.16 ± 0.03
	FA_3	0.32 ± 0.05	0.22 ± 0.03	0.21 ± 0.02	0.19 ± 0.04	0.25 ± 0.09	0.37 ± 0.05	0.32 ± 0.04
	FA_4	0.25 ± 0.03	0.17 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.18 ± 0.06	0.28 ± 0.04	0.23 ± 0.03
	FA_5	0.27 ± 0.03	0.19 ± 0.03	0.17 ± 0.02	0.15 ± 0.02	0.20 ± 0.08	0.32 ± 0.05	0.26 ± 0.02
	FA_6	0.44 ± 0.09	0.33 ± 0.03	0.18 ± 0.05	0.17 ± 0.04	0.28 ± 0.14	0.37 ± 0.05	0.36 ± 0.14
	FA_7	0.72 ± 0.16	0.51 ± 0.07	0.28 ± 0.06	0.28 ± 0.07	0.45 ± 0.25	0.60 ± 0.09	0.58 ± 0.21
	FA_8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	FA_9	0.55 ± 0.17	0.48 ± 0.04	0.20 ± 0.07	0.26 ± 0.07	0.35 ± 0.11	0.72 ± 0.06	0.86 ± 0.07
	FA_10	0.38 ± 0.05	0.28 ± 0.04	0.22 ± 0.04	0.21 ± 0.03	0.30 ± 0.09	0.44 ± 0.05	0.35 ± 0.04
	FA_11	0.30 ± 0.06	0.26 ± 0.03	0.30 ± 0.12	0.27 ± 0.06	0.21 ± 0.01	0.53 ± 0.04	0.65 ± 0.07
	FA_12	1.34 ± 0.40	1.05 ± 0.09	0.29 ± 0.09	0.42 ± 0.12	0.62 ± 0.22	1.36 ± 0.12	1.81 ± 0.18
FA_13	0.31 ± 0.07	0.37 ± 0.06	0.36 ± 0.06	0.36 ± 0.13	0.31 ± 0.07	0.59 ± 0.02	0.49 ± 0.06	
FA_14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
% SFA	57.92 ± 6.45	67.25 ± 4.57	58.07 ± 5.74	60.88 ± 6.23	65.95 ± 4.41	37.78 ± 1.02	39.04 ± 3.43	
% MUFA	14.38 ± 0.68	12.68 ± 0.92	14.56 ± 1.76	15.08 ± 1.56	15.24 ± 2.29	8.94 ± 0.57	10.44 ± 0.70	
% PUFA	27.70 ± 5.93	20.07 ± 3.95	27.37 ± 7.41	24.04 ± 6.34	18.81 ± 3.52	53.27 ± 0.83	50.52 ± 3.40	
<b>TFA</b>	<b>41.3 ± 1.90</b>	<b>35.25 ± 2.26</b>	<b>33.68 ± 3.44</b>	<b>33.99 ± 4.28</b>	<b>34.79 ± 4.63</b>	<b>67.08 ± 2.32</b>	<b>61.41 ± 3.65</b>	

		<i>Palmaria palmata</i>						
		February 18	May	June	July	August	November	February 19
<b>SFA</b>	C14:0	5.06 ± 0.49	4.72 ± 0.48	4.18 ± 0.65	4.11 ± 0.15	4.35 ± 0.34	4.90 ± 0.49	5.57 ± 0.17
	C16:0	14.96 ± 1.16	15.94 ± 1.36	13.96 ± 1.73	12.98 ± 0.92	13.66 ± 1.13	16.12 ± 1.46	16.97 ± 0.79
	C18:0	0.91 ± 0.32	0.77 ± 0.19	0.60 ± 0.19	0.59 ± 0.08	0.53 ± 0.08	0.83 ± 0.11	1.02 ± 0.27
	C16:1n-7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	C18:1n-9	1.83 ± 0.47	1.63 ± 0.23	1.00 ± 0.19	0.9 ± 0.10	0.94 ± 0.12	1.68 ± 0.24	2.26 ± 0.35
	C18:1n-7	0.92 ± 0.16	0.68 ± 0.15	0.53 ± 0.28	0.43 ± 0.04	0.68 ± 0.14	0.87 ± 0.21	1.08 ± 0.20
	C16:2n-4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	C18:2n-6	0.66 ± 0.40	0.23 ± 0.06	0.26 ± 0.07	n.d.	0.34 ± 0.18	0.31 ± 0.14	0.57 ± 0.14
	C18:3n-3	1.47 ± 0.20	n.d.	n.d.	n.d.	0.38 ± 0.17	0.52 ± 0.03	0.84 ± 0.32
	C18:4n-3	1.00 ± 0.50	0.27 ± 0.08	n.d.	n.d.	n.d.	0.40 ± 0.27	1.21 ± 0.49
	C20:4n-6	0.55 ± 0.24	0.54 ± 0.17	0.46 ± 0.36	0.31 ± 0.05	0.38 ± 0.11	0.91 ± 0.27	0.92 ± 0.11
	<b>C20:5n-3</b>	<b>8.34 ± 3.76</b>	<b>12.52 ± 9.48</b>	<b>3.85 ± 6.65</b>	<b>3.26 ± 2.16</b>	<b>5.48 ± 2.05</b>	<b>28.72 ± 7.66</b>	<b>39.75 ± 6.23</b>
C22:6n-3	0.94 ± 0.20	0.83 ± 0.14	0.49 ± 0.14	0.43 ± 0.07	0.61 ± 0.09	0.83 ± 0.10	1.22 ± 0.12	
<b>MUFA</b>	FA_1	0.30 ± 0.14	0.34 ± 0.14	0.30 ± 0.10	0.28 ± 0.05	0.34 ± 0.03	0.45 ± 0.07	0.70 ± 0.11
	FA_2	0.16 ± 0.03	0.13 ± 0.05	0.1 ± 0.03	0.09 ± 0.02	0.11 ± 0.03	0.19 ± 0.02	0.22 ± 0.03
	FA_3	0.44 ± 0.08	0.34 ± 0.14	0.26 ± 0.10	0.17 ± 0.05	0.20 ± 0.05	0.36 ± 0.05	0.48 ± 0.03
	FA_4	0.35 ± 0.06	0.27 ± 0.10	0.21 ± 0.07	0.16 ± 0.04	0.16 ± 0.06	0.28 ± 0.03	0.38 ± 0.04
	FA_5	0.32 ± 0.05	0.27 ± 0.12	0.18 ± 0.07	0.14 ± 0.05	0.15 ± 0.05	0.34 ± 0.06	0.44 ± 0.06
	FA_6	0.39 ± 0.07	0.34 ± 0.15	0.23 ± 0.06	0.25 ± 0.12	0.28 ± 0.06	0.68 ± 0.18	0.63 ± 0.21
	FA_7	0.60 ± 0.13	0.51 ± 0.26	0.33 ± 0.10	0.36 ± 0.22	0.42 ± 0.17	1.20 ± 0.34	0.98 ± 0.32
	FA_8	0.74 ± 0.17	0.76 ± 0.20	0.25 ± 0.08	0.20 ± 0.03	0.22 ± 0.04	0.70 ± 0.19	1.08 ± 0.25
	FA_9	0.30 ± 0.08	0.24 ± 0.03	0.17 ± 0.02	n.d.	n.d.	0.16 ± 0.04	0.41 ± 0.19
	FA_10	0.54 ± 0.13	0.40 ± 0.12	0.29 ± 0.11	0.23 ± 0.06	0.26 ± 0.09	0.44 ± 0.05	0.57 ± 0.05
	FA_11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	FA_12	0.78 ± 0.12	0.98 ± 0.30	0.36 ± 0.07	0.27 ± 0.03	0.31 ± 0.01	0.86 ± 0.30	1.30 ± 0.44
FA_13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
FA_14	0.44 ± 0.15	0.46 ± 0.24	0.34 ± 0.17	n.d.	n.d.	0.36 ± 0.07	0.56 ± 0.11	
% SFA	60.66 ± 8.60	60.15 ± 15.19	80.18 ± 15.27	77.76 ± 7.84	69.35 ± 6.72	40.08 ± 6.15	33.14 ± 2.23	
% MUFA	7.77 ± 0.61	6.37 ± 1.20	6.28 ± 0.66	5.83 ± 0.65	5.99 ± 0.52	4.64 ± 0.50	4.70 ± 0.39	
% PUFA	31.58 ± 8.65	33.48 ± 16.32	13.54 ± 15.73	16.40 ± 8.37	24.66 ± 6.97	55.28 ± 6.55	62.16 ± 2.57	
<b>TFA</b>	<b>40.74 ± 7.56</b>	<b>42.88 ± 12.06</b>	<b>27.53 ± 10.45</b>	<b>24.81 ± 2.41</b>	<b>29.17 ± 3.02</b>	<b>61.65 ± 10.19</b>	<b>79.16 ± 7.73</b>	



**Supplementary Figure 1.** Principal component analysis on mycosporine-like amino acid composition (mAU.min) of *Palmaria palmata* and *Grateloupia turuturu* collected monthly from February 2018 to February 2019 (Brittany, France). (A: Variable; B: individuals according to sampling site, with ellipses drawn around both species (confidence level of 95%); C: individuals according to sampling seasons).



## Conclusion of chapter 2

The screening of mycosporine-like amino acids carried out on 40 red macroalgal species (**part 1**) highlighted a high diversity, with species producing between 0 (e.g. *Polyides rotunda*, *Osmundea pinnatifida*, or *Membranoptera alata*) and 8 MAAs (i.e. *Palmaria palmata*, *Bostrychia scorpioides* and *Agarophyton vermiculophyllum*), including many unknowns among the 23 compounds detected. The results include profiles of some species for which MAA composition has never been studied before, suggested that there are still many MAAs to be discovered, as also suggested by recent studies (Hartmann *et al.* 2015; Orfanoudaki *et al.* 2019). On the other hand, the MAAs composition did not appear to be related to phylogeny, but rather to an acclimation to environmental conditions.

In a second part, in order to investigate the roles of the different MAAs that could explain such a high diversity, we selected one species, i.e. *Palmaria palmata*, whose temporal MAA composition was monitored *in-situ* during one year (**part 2**). Results then seemed to confirm the photoprotective role of these compounds as an increase of total MAA content was observed in May, coupled to increasing irradiance at this period. Although this increase was apparently due to an overall increase in the content of all MAAs, results suggested that the synthesis of some MAAs could be induced under certain conditions. Notably the presence of asterina-330 was observed only between March and August, highlighting its important role in the photoprotection of the species. In addition results showed that nutrient availability could have a significant influence on MAAs since their synthesis appeared to be limited in summer, despite significant exposure to light.

In this regard, a cultivation experiment conducted on *P. palmata* (**part 3**) confirmed the positive effect of nitrate content on MAAs. Two hypotheses could then be made: (i) the synthesis of MAAs is limited under nitrogen starvation as they are nitrogenous compounds; (ii) MAAs could be used as nitrogen source by macroalgae, to enhance their metabolism when nutrients are insufficient in seawater. In this way, MAAs could be multifunctional secondary metabolites, acting both as photoprotectors and nitrogen source. Nevertheless further investigations are needed to confirm this last hypothesis. On the other hand, the cultivation experiment did not show a global effect of UV radiation. Conversely, contrary to what was expected, the content of all MAAs (including asterina-330) decreased throughout the cultivation period, which could be related to the insufficient nutrient and/or light

conditions in the laboratory. Only usurijene exhibited a slightly higher content at the end of the experiment than at the beginning when exposed to UV radiation, suggesting a specific induction or synthesis pathway of this MAA and its possible involvement in UV photoprotection in *P. palmata*. Once again, there are different hypotheses that could explain this result:

- (i) Usurijene could be synthesized under a different pathway than other MAAs, involving different precursors or intermediates, which would not have been limited under laboratory conditions.
- (ii) Contrary to other MAAs, the usurijene would not serve as a source of nitrogen for macroalgae (if MAAs have actually this role), although the chemical structure of this MAA also contains nitrogen.
- (iii) Usurijene would be highly dependent on the high UV-A exposure (315-400nm), whereas the other MAAs would be favoured under high photosynthetically active radiation (400-700nm). The synthesis of different MAAs would thus vary depending on both the quantity and quality of the light. Previously, Kräbs *et al.* (2002) had already suggested that the different synthesis pathways of MAAs in *Chondrus crispus* might be induced by different wavelengths bands. In this sense, the amount of UV may have been sufficient to induce the synthesis of usurijene, while the limited amount of PAR may have been insufficient to induce to the synthesis of the other MAAs. Notably, the cultivation experiment showed a decrease in asterina-330 level, which on the contrary exhibited an increase in concentration under strong light radiation under *in-situ* conditions (part 2).

In any cases, it appears that there are different synthesis pathways for each MAA beyond shikimate and pentose phosphate pathways. In particular, it is possible that some MAAs may be the result of chemical modifications of other MAAs by substitution, condensation dehydration, decarboxylation, oxidation or reduction reactions (Bedoux *et al.* 2020). For example, the presence of sulphated MAAs or MAAs glycosides has been reported in corals and cyanobacteria, respectively (Wada *et al.* 2015; Bedoux *et al.* 2020). Indeed, new MAA structures could be induced under certain triggering factors and/or in certain species, leading to a whole metabolomic network as outlined by Belcour *et al.* (2020). In this regard, further cultivation experiments are required to clarify the different synthesis pathways / possible MAA inductions in red macroalgae. In the context of global change, the synthesis of some

MAAs (e.g. asterina-330 or usurijene) could be preferentially induced. More interestingly, the modification of environmental conditions could lead to an adaptation, *i.e.* the multiplication of modifications in the MAAs structure enhancing the diversity of these compounds. In this way, the high diversity of MAAs in *P. palmata* or between species (observed in **part 1**) could already be the result of previous specific adaptations.

Finally, in the last **part 4**, the ecological and metabolomic composition of *P. palmata* was compared with the introduced red alga *Grateloupia turuturu*. Indeed, introduced species are known for their high phenotypic plasticity, and it is unclear how their invasive potential will evolve in the context of global change, and whether they constitute a threat to native species. In this sense, the native and the introduced species exhibited different ecological and metabolomic strategies. *Grateloupia turuturu* showed fertile individuals throughout the year with spatial difference in recruitment, reproduction or growth, suggesting a strong capacity to adapt and modulate its phenology. This was coupled to the ability to rapidly synthesize and accumulate high amounts of secondary metabolites (including MAAs, photoprotective pigments or phenolic compounds), increasing its tolerance to environmental changes. On the contrary, the native *P. palmata* showed lower plasticity and appeared to be highly dependent on temperature as it was mainly fertile in winter. In addition, MAAs data suggested that *P. palmata* favours the synthesis of multiple compounds with potential synergic effects, rather than the rapid accumulation of few compounds observed in *G. turuturu*. It is possible that the two species do not possess the same precursors / intermediates / enzymes and resulting synthesis pathways, leading in a different MAAs diversity and thus metabolic strategies.

Then, *P. palmata* appeared to have a limited development, when co-existing with the introduced *G. turuturu*. However this could be the result of a combination of factors leading to unfavourable conditions for the native species. Indeed, despite high plasticity, *G. turuturu* did not appear to be a threat for *P. palmata* or other native species in Brittany since its abundance remains quite limited in the intertidal zone. The introduced species rather appears to take advantage of disturbances or unfavourable conditions that affect the development of the native species. Thus in the context of global change, *G. turuturu* is likely to adapt easily, while *P. palmata* may be challenged to manage both its acclimation to environmental variations and its cohabitation with other species. However, the question of the outcome of both species cannot be completely solved given the multiplicity of interactions between macroalgal populations and their environment.

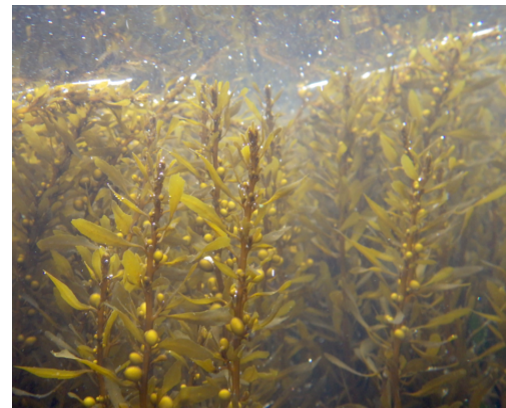
## *Chapter 3*

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Native brown macroalgae: endangered species in the context of global change and cohabitation with introduced species?

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Case studies on *Bifurcaria bifurcata* and *Halidrys siliquosa*





## *Foreword to chapter 3*

The evolution of environmental conditions through human activities and climate change induces ecophysiology adaptation of macroalgae to promote their survival. In particular, changes in interspecific interactions and biogeographical distribution of species are reported, with an increasing number of migration of species affecting marine ecosystems as a whole. In this context, the aim of this chapter is to apprehend the adaptive processes of three species of Sargassaceae, *i.e.* two species native to Brittany (*Bifurcaria bifurcata* and *Halidrys siliquosa*), and one introduced (*Sargassum muticum*). In this regard, this chapter consists of two parts. The first one **(1)** focuses on *B. bifurcata*, a species very abundant on Brittany shores, and forming a characteristic algal belt, and colonising also rockpools. As it participates in the structuring of algal communities, its disappearance in the context of global change could thus have a significant ecological impact. On the contrary, the second part **(2)** is a case study on *H. siliquosa*, a cold-water affinity species poorly present on intertidal shores of Brittany. It could be particularly threatened in its adaptability both to global change and to cohabitation with introduced species. Each part then consists of two publications, *i.e.* a first article on a one-year *in-situ* monitoring of the native species, and then a comparison of their acclimation abilities with the invasive *S. muticum*. In this way, part **(2)** includes a comparison of populations between France (where *H. siliquosa* is weakened by its “Southern” position and coexists with the well-established introduced *S. muticum*), and Ireland (where *S. muticum* is at an earlier stage of invasion). An abstract summarising the main results is available at the beginning of each publication, and a conclusion of Chapter 3 is available on page 348.

### 1. Case study on *Bifurcaria bifurcata*

#### **(1.1) *Bifurcaria bifurcata*: a native species highly sensitive to environmental changes, threatened despite high abundance?**

→ **PUBLICATION** [Lalegerie F., Connan S., Stiger-Pouvreau V. (In prep.). Research note - Intra-site variability in terpene composition of *Bifurcaria bifurcata* (Phaeophyceae, Fucales): evidence of microhabitat occurrence]

#### **(1.2) Can global change have an effect on the invasive potential of certain species and their threat to native species?**

→ **PUBLICATION** [Lalegerie F., Stiger-Pouvreau V., Zanolla M., Stengel D., Connan S. (In prep.) Contrasting phenology and chemical strategies of native and invasive seaweeds: *Bifurcaria bifurcata* and *Sargassum muticum* (Sargassaceae, Fucales)]

## 2. Case study on *Halidrys siliquosa*

### **(2.1) *Halidrys siliquosa*: a weakened species in the intertidal zone?**

→ **PUBLICATION** [Lalegerie F., Stiger-Pouvreau V., Connan S. (In prep.). Ecological and metabolomic monitoring of the brown macroalga *Halidrys siliquosa* (Sargassaceae, Fucales)]

### **(2.2) *Halidrys siliquosa*: a native species threatened in Brittany by its geographical position and co-existence with the introduced species *Sargassum muticum*?**

→ **PUBLICATION** [Lalegerie F., Stiger-Pouvreau V., Zanolla M., Stengel D., Bideau A., Le Grand F., Connan S. (In prep.) *Halidrys siliquosa*, a species threatened by its southern limit and co-existence with the invasive *Sargassum muticum*? A comparison between France and Ireland]

## **Research note – Intra-site variability in the terpene composition of *Bifurcaria bifurcata* (Phaeophyceae, Fucales): evidence of microhabitat occurrence**

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Almost ready for submission to: [Journal of experimental marine biology and ecology](#)

### **RESUME**

*Bifurcaria bifurcata* est une macroalgue brune abondante en milieu tempéré et vivant le long de la côte atlantique, connue pour produire une large gamme de diterpènes acycliques. Des études antérieures ont fait état d'une grande variation géographique dans sa composition terpénique, avec différents chémotypes observés à grande échelle, *i.e.* entre la France et le Maroc, puis à moyenne échelle, *i.e.* entre différentes localisations au sein d'un même pays. De plus, la composition en terpènes pourrait faire l'objet de variations temporelles. Cependant, il est difficile de déterminer quels facteurs sont responsables de ces variations spatio-temporelles, qui pourraient expliquer la grande diversité en terpènes de *B. bifurcata*. Dans ce contexte, dans le but d'étudier plus en détail la composition terpénique de cette espèce, des échantillons ont été collectés chaque mois entre février et décembre 2018, sur trois sites en Bretagne (France), et analysés par chromatographie sur couche mince (CCM). Les résultats ont été comparés à ceux de deux autres espèces de Sargassaceae (*Sargassum muticum* et *Halidrys siliquosa*), afin de déterminer si les variations sont spécifiques à *B. bifurcata*. Les résultats ont mis en évidence des profils différents entre les trois espèces, avec une variation spatio-temporelle observée uniquement chez *B. bifurcata*. De ce fait, *B. bifurcata* présentait deux profils CCM différents, en fonction de l'exposition aux vagues, *i.e.* préférentiellement le profil 1 pour les sites exposés, et seulement le profil 2 pour les sites abrités. Plus intéressant encore, les sites exposés ont montré à la fois la présence du profil 1 et du profil 2, démontrant la présence de microhabitats au sein des sites exposés, entraînant la présence des deux profils parmi les réplicats d'un même site. Ainsi, cette étude rapporte pour la première fois une variabilité géographique à petite échelle dans la composition terpénique de *B. bifurcata*, qui semble particulièrement sensible à l'hydrodynamisme, contrairement aux deux autres espèces de Sargassaceae.

**Mots-clés:** *Bifurcaria bifurcata*, Macroalgues brunes, *Halidrys siliquosa*, Hydrodynamisme, Microhabitat, *Sargassum muticum*, Terpènes

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## ABSTRACT

*Bifurcaria bifurcata* is a temperate brown macroalga living along Atlantic coast, known to produce a wide range of acyclic diterpenes. Previous studies have reported a high geographical variation in its terpene composition, with different chemotypes observed at large scale, *i.e.* between France and Morocco, and then at medium scale, *i.e.* between different locations countrywide. In addition, temporal fluctuations could occur in terpene composition. However, little is known regarding the factors responsible for these spatio-temporal variations, and the resulting high diversity in terpenes of *B. bifurcata*. In this context, in the aim to further investigate terpene composition of *B. bifurcata*, samples were collected monthly between February and December 2018, at three sites in Brittany (France), and analysed using thin layer chromatography (TLC). Results were compared with two other Sargassaceae species (*Sargassum muticum* and *Halidrys siliquosa*), to consider whether variations are specific to *B. bifurcata*. Results highlighted different profiles between the three species, with spatio-temporal variations only for *B. bifurcata*. Then, *B. bifurcata* exhibited two different TLC profiles, depending on wave exposure, *i.e.* preferentially the profile 1 in exposed sites, and only profile 2 in sheltered site. More interestingly, profile 2 was also observed in some samples from the exposed sites showing the effect of microhabitats within exposed sites resulting in the presence of both profiles among replicates. In this way, the present study reports for the first time small-scale geographical variability in terpene composition of *B. bifurcata*, that seems to be particularly dependent on hydrodynamism unlike other closely Sargassaceae species.

**Keywords:** *Bifurcaria bifurcata*, Brown macroalgae, *Halidrys siliquosa*, Hydrodynamism, Microhabitat, *Sargassum muticum*, Terpenes

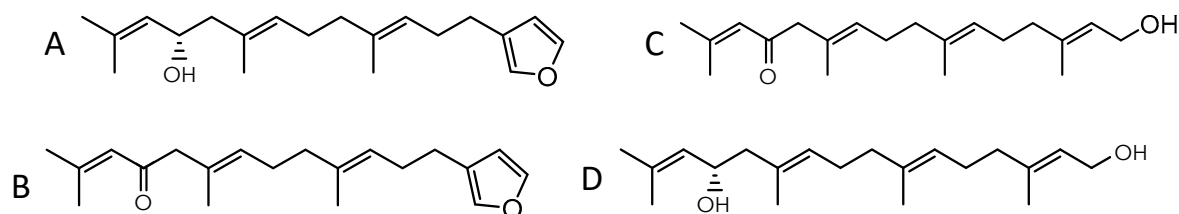
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## INTRODUCTION

In marine environment, brown seaweeds (Phaeophyceae), such as *Bifurcaria bifurcata* R. Ross, constitute a wide and diverse source of secondary metabolites, including terpenes (Gaysinski *et al.* 2015). These latter are defensive compounds, mainly synthesized in response

to grazers, epiphytes or pathogens, constituting allelopathic agents (Bourgougnon and Stiger 2011). Many studies have thus reported an antifouling, antimicrobial or antibacterial activity of terpenes, for example in *B. bifurcata*, *Halidrys siliquosa* or *Cystoseira/Treptacantha* spp. (Hellio *et al.* 2001; Maréchal *et al.* 2004; Mokrini *et al.* 2008; Gouveia *et al.* 2013).

Among brown algae, Sargassaceae (Fucales, Phaeophyceae) and Dictyotaceae regroup the most prolific species concerning the synthesis of terpenes (Kornprobst 2014; Bourgougnon and Stiger-Pouvreau 2011; Gaysinski *et al.* 2015). More particularly, *Bifurcaria bifurcata* is known for its richness in linear diterpenes contained in lipophilic extracts (see recent reviews of Muñoz *et al.* (2013) and Pais *et al.* (2019) for complete description). Indeed, this species living in intertidal rockpools of North-East Atlantic coasts (*i.e.* from Ireland to Morocco) has been reported to contain for example eleganediol (Biard *et al.* 1980a), eleganolone (Hougaard *et al.* 1991; Göthel *et al.* 2012a,b), bifurcane and bifurcanone (Le Lann *et al.* 2014), or geranylgeraniol and their derivatives (Valls *et al.* 1986; Culioli *et al.* 1999, 2001, 2004; Ortalo-Magné *et al.* 2005) (Fig. 1). Some of these terpenes have shown interesting activities, such as antimicrobial (Biard *et al.* 1980b), antitumoral (Di Guardia *et al.* 1999), or antiprotozoal (Gallé *et al.* 2013; Smyrniotopoulos *et al.* 2017).



**Figure 1.** Four examples of diterpenes found in *Bifurcaria bifurcata*: bifurcane (A), bifurcanone (B), eleganolone (C), and eleganediol (D) according to Le Lann *et al.* (2014).

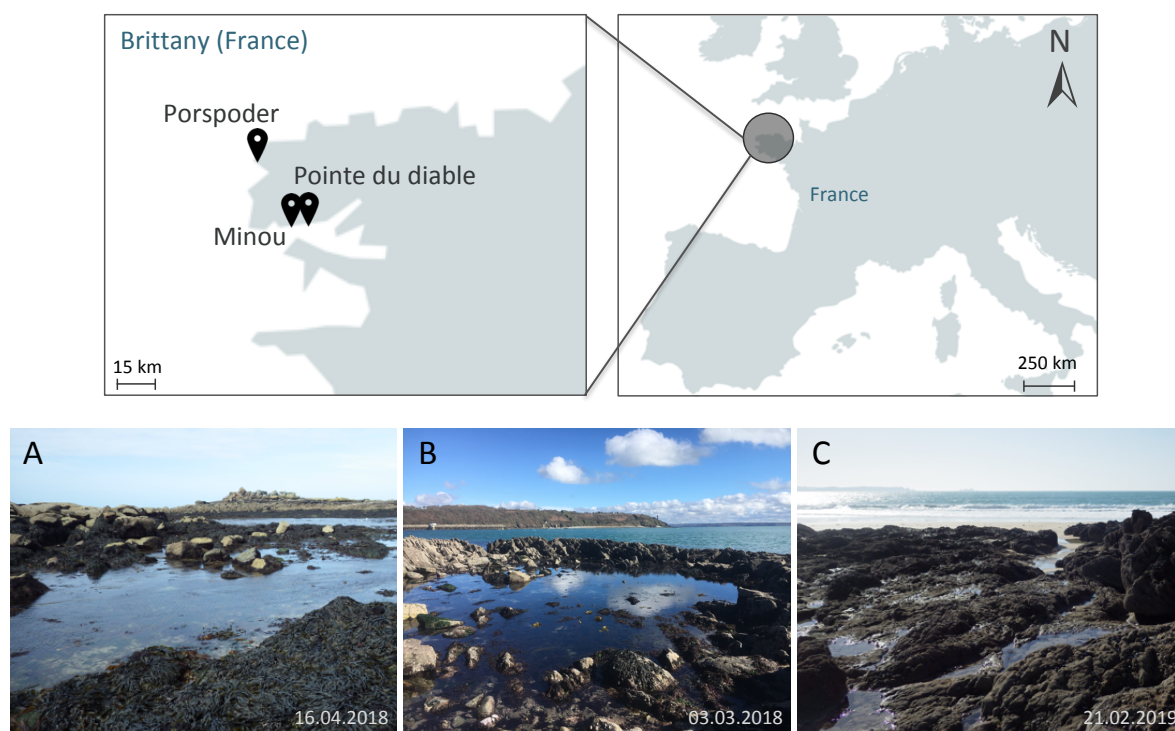
Furthermore, some studies have reported temporal and spatial variations in the terpene composition of *B. bifurcata*. Notably, Valls *et al.* (1995) demonstrated four geographical chemotypes along French Atlantic coasts. Then, Culioli *et al.* (2002) demonstrated a quantitative variation in the concentration of terpenes of *B. bifurcata* collected at Roscoff (France), while Valls *et al.* (1993) showed a relatively constant diterpene concentration of *B. bifurcata* collected in Morocco, suggesting that temporal variation could also depend on geographical location. Le Lann *et al.* (2014) suggested furthermore that the terpene composition of *B. bifurcata* could change with seasons and sampling sites, according to hydrodynamic conditions. It would thus appear that there is a wide diversity in the terpene

composition of *B. bifurcata*, coupled with a great spatial and temporal variability. Nevertheless, none of these studies investigated fine-scale / intra-site variability, resulting from the occurrence of microhabitats (*i.e.* small-sized habitat with physical and/or chemical characteristics different from those of the larger habitat in which the microhabitat is included). Indeed, previous studies mainly focused on chemical characterization, which requires to harvest a large biomass at each site without replicates, which can hide intra-population variability. However, such variability could help to understand the diversity and variability in the terpene composition of this species.

In this context, the terpene composition of *Bifurcaria bifurcata* was investigated by collecting samples monthly at 3 sites in Brittany (France), between February and December 2018. Then, terpene composition was studied by thin layer chromatography (TLC). Such approach allowed to obtain spatio-temporal chemical profiles, including replicates, in order to highlight potential inter- or intra-sites variations. In addition, results were compared with *Sargassum muticum* (Yendo) Fensholt 1955 and *Halidrys siliquosa* (Linnaeus) Lyngbye 1819, two Sargassaceae species synthesizing mainly meroditerpenes (Culioli *et al.* 2008; Gaysinski *et al.* 2015). In this way, results will allow to consider whether variability similar to *B. bifurcata* also occurred in these two taxonomically related species.

## MATERIAL AND METHODS

A monthly sampling from February to December 2018 was conducted at three sites in western Brittany (France), *i.e.* Minou (48°20'20.314'' N - 4°37'3.779'' W), Pointe du Diable (48°21'15.606'' N - 4°33'30.067'' W) and Porspoder (48°28'56.644'' N - 4°46'6.812'' W) (Fig. 2). Minou and Pointe du Diable are two exposed sites located at the entrance of the Bay of Brest, about 6 km apart. Minou is mostly a sandy site with small rockpools, whereas Pointe du Diable is a rocky site with large rockpools. Additionally, Porspoder, located further northwest, is exposed to the waters of the English Channel. This site is sheltered, with a wide channel similar to a large pool never completely emerged and protected from wave exposure by the presence of a large rocky barrier (Fig. 2). For all sites, samples were collected in triplicate in rockpools at low tide. Each replicate corresponded to about ten thalli, collected within 1-2 m of each other. Then, the different replicates were harvested in distant areas within each site (5-8 m).



**Figure 2.** Location of the sampling sites in Brittany (France), with illustrations of Porspoder (A), Pointe du Diable (B), and Minou (C). © F. Lalegerie

Terpenes were extracted by maceration, with 4 mL of a mixture of dichloromethane : methanol (DCM : MeOH, 2 : 1, v : v) added to 200 mg DW of algal powder. Samples were kept for 15 min in an ultrasound bath in the dark, according to a protocol adapted from Le Lann *et al.* (2014). Then extracts were centrifuged 10 min at 2,500 rpm (centrifuge 5810, Eppendorf); the supernatant was recovered, and a second extraction was carried out in the same way with again 4 mL DCM : MeOH. Finally, both supernatants were combined, evaporated (miVac, Genevac), and stored at -80 °C before analysis by Thin Layer Chromatography (TLC).

Dried extracts were dissolved in 1 mL of DCM : MeOH 2 : 1 (v : v). Then, 30 µL of each sample were deposited on a TLC plate (TLC Silica gel 60 with fluorescence indicator F<sub>254</sub>, Merck), according to a protocol adapted from Le Lann *et al.* (2014). Elution was performed using an apolar mixture of hexane : diethyl ether : glacial acetic acid 80 : 20 : 2 (v : v : v). To get a better separation, 500 µL of 100 % MeOH was added in the mobile phase just before migration. After migration, different components were revealed by observation with UV light at 365 nm and 254 nm. In a second step, each plate has been vaporized with a specific reagent, *i.e.* a mixture of copper sulphate (3 g), orthophosphoric acid (8 mL) and distilled water (92 mL) to allow the visualization of additional apolar compounds, according to Le

Lann *et al.* (2014). The plates were then dried for 15 min in an oven at 100 °C before a second reading under UV light. Then, for each spot, the retardation factor ( $R_f$ , *i.e.* the ratio of the distance travelled by the centre of the spot to the distance of the solvent front) was measured, in order to compare the profiles between the different TLC plates.

## RESULTS AND DISCUSSION

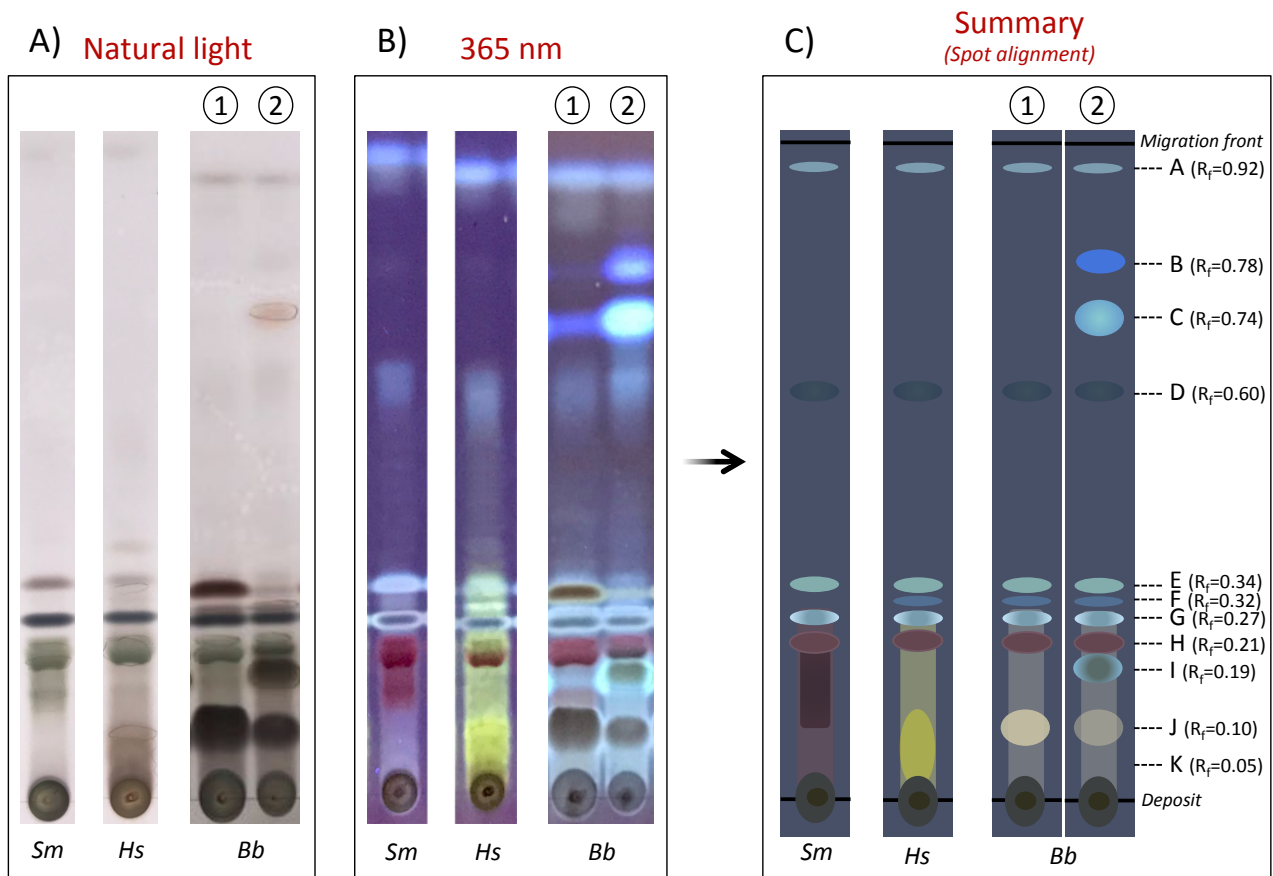
### 1. Interspecific variability

The TLC profiles revealed that the brown macroalgae *Sargassum muticum*, *Halidrys siliquosa* and *Bifurcaria bifurcata* had a different terpene composition, with two different profiles observed for *B. bifurcata* (Fig. 3, profiles 1 and 2). Despite specific differences, five spots were common to the three species, *i.e.* spots A, D, E, G, and H. Among these similarities, spots A, E, G and H were visible before reagent application, and could be pigments or other fluorescent compounds such as sterols or fatty acids. More particularly, some of these common spots could be  $\beta$ -carotene, pheophytin-*a*, chlorophyll-*a* or fucoxanthin which are all synthesized by brown algae (Stengel *et al.* 2011).

Then, *B. bifurcata* was the only species to possess the spots B, C, I and J in the profile 2, and appeared thus to contain more spots, *i.e.* the presence of ten spots, compared to five and seven spots in *S. muticum* and *H. siliquosa*, respectively. This result is in accordance with the high terpene diversity reported in *B. bifurcata* (Muñoz *et al.* 2013; Pais *et al.* 2019). Among the two different profiles, samples from *B. bifurcata* could possess eleganediol, eleganolone, bifurcane, bifurcanone or bifurcanol, commonly reported in French populations (Valls *et al.* 1995; Le Lann *et al.* 2014). On the other hand, *H. siliquosa* was the only one to possess spot K, which could correspond to a toluquinol derivative according to Culioli *et al.* (2008). In this regard, this spot could be specific to *H. siliquosa* and be used as a chemotaxonomic marker similarly to previous studies (Culioli *et al.* 2000; Teixeira *et al.* 2001; Jégou *et al.* 2012). Finally, *S. muticum* appeared to be less rich in lipid derivatives that could be related to its chemistry but also to its ecology. Indeed, *S. muticum* is an invasive species with pseudo-perennial life history, *i.e.* annual loss of its laterals that does not require protection of long-lived structure compared to the two native species, *B. bifurcata* and *H. siliquosa* (Critchley 1983; Baer and Stengel 2010; Le Lann *et al.* 2012). In this way, our results highlighted differences between species, which could be related to a combination of factors, including



taxonomic classification, or ecology, coupled to specific acclimation to environmental parameters.



**Figure 3.** TLC profiles of crude extracts of three brown macroalgae *Sargassum muticum* (Sm), *Halidrys siliquosa* (Hs) and *Bifurcaria bifurcata* (Bb), after revelation. (A) and (B) are photographs of TLC plates visualized under natural light and UV radiation (365 nm) respectively, and (C) is a schematic summary of specific composition after comparison of all TLC plates and spot alignment and following the calculation of the retardation factors ( $R_f$ ). The highlighted spots have been numbered from A to K. Sm and Hs samples were collected at Porspoder on 18/05/2018 and Bb samples were collected at Minou on 14/08/2018 in Brittany (France).

## 2. Spatio-temporal variability

Results showed no significant spatio-temporal changes for *S. muticum* and *H. siliquosa*, as profiles were similar for all months at all sites, with no additional or missing spots over time. Then, based on the observation of TLC plates, several spots seemed to vary in intensity over time. Notably, the intensity of spot G seemed to increase from February to May when observed under 265 nm. Nevertheless, TLC is not a quantitative method and further investigations are thus needed to quantify the concentrations corresponding to each specific spot. Interestingly, two different profiles were obtained all along the study period in *B. bifurcata* (Figure 3), which showed temporal and spatial variations contrary to *S. muticum*

and *H. siliquosa*. Indeed, all samples from Porspoder exhibited the profile 2 (*i.e.* with the additional spots B, C and I, and with the spot J having a lower intensity compared to the profile 1; Table 1). On the contrary, samples from Minou and Pointe du diable exhibited both profiles 1 and 2 (Table 1).

**Table 1.** Spatio-temporal variation in the occurrence of different TLC profiles (1 and 2) in the brown macroalga *Bifurcaria bifurcata* collected monthly at three sites in Brittany (France) between February and December 2018. The presence is represented by (+) and the absence by (-).

	Pointe du diable		Minou		Porspoder	
	Profil 1	Profil 2	Profil 1	Profil 2	Profil 1	Profil 2
February	+	+	+	+	-	+
March	-	+	-	+	-	+
April	+	-	-	+	-	+
May	+	+	+	+	-	+
June	+	+	-	+	-	+
July	+	+	-	+	-	+
August	+	-	+	+	-	+
September	+	-	-	+	-	+
October	+	+	-	+	-	+
November	+	+	+	+	-	+
December	+	+	+	+	-	+

In this sense, previous studies already highlighted geographical variations in the terpene composition of *B. bifurcata*. First, Valls *et al.* (1995) revealed a large-scale spatial variability, between samples collected in Morocco and France. The authors defined four geographical chemotypes according to the main constituents, *i.e.* (a) eleganediol and in a lesser extent bifurcanol and bifurcarenonol, (b) eleganediol, (c) bifurcadiol, or (d) bifurcane. Then, chemotypes a and c were observed only in samples from Morocco, while chemotype d was reported only in France. In the same way, medium-scale variations have also been demonstrated. Indeed, Valls *et al.* (1993) showed three different profiles between different Moroccan locations, *i.e.* from north to south (1) eleganediol in high proportion at Rabat, (2) mainly eleganolone with in addition eleganediol at El Jadida, and (3) mainly 12(S)-hydroxygeranylgeraniol in samples from Oualidia. Afterwards, the same authors (Valls *et al.* 1995) demonstrated the existence of two chemotypes in *B. bifurcata* collected along Brittany coasts, *i.e.* type I and type II with eleganolone or bifurcane as main diterpenes, respectively. Then, type I was observed at St Briec and Lorient, and were thus distinguishable from Roscoff where type II was observed. Among brown macroalgae, it has also been shown that

*Styopodium zonale* (Dictyotales) produces meroditerpene that differs depending on different locations along Brazilian coast, highlighting 3 chemotypes (Soares *et al.* 2015). However, the same authors demonstrated that this spatial variation could depend on the different stages in the life cycle of this alga (Soares *et al.* 2003), with immature individuals producing atomaric acid, compared to stypoldione in fertile individuals. Then, Le Lann *et al.* (2014) suggested that medium-scale spatial variability could also be related to hydrodynamic conditions. Indeed, by pooling replicates within a site, they showed that *B. bifurcata* synthesized bifurcanone and eleganolone when living in a sandy and sheltered site, compared to bifurcane and eleganediol in rocky and exposed sites. As this last study was conducted on sites common to the current study (*i.e.* the sheltered site Porspoder and the exposed site Pointe du Diable), we can then supposed that spots B and C from the present study could correspond to bifurcanone and eleganolone.

Furthermore, our results revealed a high variability between replicates collected within the same site, highlighting for the first time small-scale variability in the terpene composition of *B. bifurcata*. More precisely, Minou showed 82 % of replicates with profile 2, compared to 48 % at the most exposed site Pointe du Diable. In this way, profile 2 appeared to be favoured by sheltered conditions, whereas profile 1 is more frequent under increasingly wave exposure (Fig. 3). Then, the presence of two profiles within the same site suggests that the metabolic composition of some species may vary due to the existence of microhabitats within a site. Wave exposure appears to be responsible to intra-site variability. Indeed, within an exposed site such as Pointe du Diable or Minou, there can be more sheltered areas, especially since sampling have been carried out in intertidal rockpools, where the topography is more complex and where some algal replicates may be protected by rocks.

A temporal variation has also been observed in the proportion of the two profiles, with a decrease in occurrence of profile 2 between April and September at Pointe du Diable, compared to the increasing occurrence of profile 1 in winter at Minou (Table 1). Similarly, Le Lann *et al.* (2014) showed the occurrence of bifurcanone (suspected of belonging to profile 2 of the present study) only in winter, in samples of *B. bifurcata* collected on sheltered site. This temporal variation could be then the result of a less wave exposure during the summer, favouring profile 1. In conclusion, the chemical composition of algal species can be influenced by the existence of microhabitats within a site, caused by the small-scale variation of some particular environmental factors. In this case, hydrodynamism appeared to have a

significant impact, influencing the terpene composition of *B. bifurcata*, contrary to *S. muticum* or *H. siliquosa*, suggesting then a species-specific response to these environmental variations. Besides, the terpene composition of *B. bifurcata* could have been influenced by many other biotic and abiotic factors, such as the presence of grazers or epiphytes, which may require further investigations.

## Acknowledgements

We thank the European Institute for Marine Studies (IUEM), the University of Western Brittany (UBO, Brest) and the region Bretagne for funding Fanny Lalegerie's PhD. Authors thanks Gerald Culioli for his precious advices. We thank Marie-Aude Poullaouec for the sample grinding.

## Authors' contributions

F.L. drafted the manuscript and supervised the extraction / analysis of terpenes. A.G. performed the extractions of terpenes and analysis by TLC. V.S-P. and S.C. designed and supervised the project. All authors participated in the collection of samples.

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## ENGLISH

The previous study revealed a high degree of heterogeneity in the terpene composition of the brown alga *Bifurcaria bifurcata*, not observed in *Halidrys siliquosa* or *Sargassum muticum*. Indeed, while studies had already shown variability in terpene profiles of *B. bifurcata* on a large scale (*i.e.* France / Morocco) or on a medium scale (*i.e.* between different sites in Brittany, France), our study showed a variability on a small scale, with differences in profiles between replicates collected on the same site. This variability could be related to hydrodynamic conditions varying from one habitat to another, with the presence of microhabitats within the same site. As a result, this species, very abundant on the Breton rockyshores, appears to be particularly sensitive to environmental conditions compared to other Sargassaceae. Then, it is unclear whether this specific response is limited to hydrodynamic conditions or whether other environmental factors may have a significant effect on *B. bifurcata*. In this respect, the following part consists of a one-year monitoring of the ecology and metabolomic composition of *B. bifurcata*. More interestingly, the results are compared with those of the invasive species *S. muticum* (known for its high plasticity), in order to study the differences in acclimation between the two species. Moreover, *S. muticum* is a well-established species in France, which could have a significant effect on the distribution of *B. bifurcata*, particularly in the context of global change.

## FRANÇAIS

L'étude précédente a révélé un degré élevé d'hétérogénéité dans la composition terpénique de l'algue brune *Bifurcaria bifurcata*, non observé chez *Halidrys siliquosa* ou *Sargassum muticum*. En effet, alors que des études avaient déjà montré une variabilité des profils terpéniques de *B. bifurcata* à grande échelle (c'est-à-dire entre la France et la Maroc) ou à moyenne échelle (c'est-à-dire entre différents sites de Bretagne, France), notre étude a montré une variabilité à petite échelle, avec des différences de profils entre les répliquats collectés sur un même site. Cette variabilité pourrait être liée à des conditions hydrodynamiques variant d'un habitat à l'autre, avec la présence de microhabitats au sein d'un même site. En conséquence, cette espèce, très abondante sur les rivages bretons, semble être particulièrement sensible aux conditions environnementales par rapport aux autres Sargassaceae. Par ailleurs, il est difficile de déterminer si cette réponse spécifique se limite aux conditions hydrodynamiques ou si d'autres facteurs environnementaux peuvent avoir un effet significatif

sur *B. bifurcata*. À cet égard, la partie suivante consiste en un suivi *in-situ* d'un an de l'écologie et de la composition métabolomique de *B. bifurcata*. Plus intéressant encore, les résultats sont comparés à ceux de l'espèce introduite *S. muticum* (connue pour sa grande plasticité phénotypique), afin d'étudier les différences d'acclimatation entre les deux espèces. De plus, *S. muticum* est une espèce bien établie en France, et pourrait ainsi avoir un impact significatif sur la distribution de *B. bifurcata*, notamment dans le contexte du changement global.



## **Contrasting phenology and chemical strategies of native and invasive seaweeds: *Bifurcaria bifurcata* and *Sargassum muticum* (Sargassaceae, Fucales)**

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### **RESUME**

Introduite en Europe dans les années 70s, *Sargassum muticum* est une macroalgue brune invasive qui est maintenant largement répandue sur les côtes Nord-Est de l'océan Atlantique, démontrant sa grande capacité d'adaptation à différents environnements. Près de 50 ans après sa première introduction, cette étude a pour objectif de comparer sa phénologie et ses capacités d'adaptation à celles des populations natives de l'algue brune *Bifurcaria bifurcata* en Bretagne, en utilisant une approche couplant à la fois l'écologie et la métabolomique. Les données écologiques et la composition en métabolites primaires et secondaires (pigments, composés phénoliques et acides gras) des deux espèces ont été suivies et comparées sur trois sites pendant un an. Les résultats ont montré que *B. bifurcata* est une espèce à croissance lente avec une reproduction hivernale tandis que *S. muticum* a démontré un taux de reproduction élevé et une croissance rapide au printemps-été. En outre, contrairement à *B. bifurcata*, *S. muticum* a présenté une seule cohorte chaque année, en raison de la rupture et de la perte ultérieure de ses latérales à la fin de sa période de reproduction, permettant sa forte dissémination. La meilleure plasticité chimique de *S. muticum* en réponse aux variations de lumière et de température est probablement liée à sa stratégie écologique. Plus particulièrement, les analyses métabolomiques suggèrent une plus grande capacité de stockage et de protection de cette espèce invasive pendant sa période de reproduction, favorisant sa survie en réduisant le risque de mortalité dans les premiers stades de son développement. Les résultats indiquent par ailleurs que l'hydrodynamisme est un facteur important dans la distribution de cette espèce puisque ce facteur influence la période de détachement des latérales. Cette étude écologique, combinée à une importante analyse

métabolomique, contribue à la compréhension à long terme des stratégies d'adaptation de l'espèce invasive *S. muticum* et de l'espèce native *B. bifurcata* qui coexistent en Bretagne.

**Keywords:** Acides gras, Espèce native versus invasive, Communautés macroalgales, Pigments, Composés phénoliques, Phénologie

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## ABSTRACT

Introduced to Europe in the 1970s, *Sargassum muticum* is an invasive brown macroalga which is now widespread on north-east Atlantic coastlines, demonstrating its high adaptability to different environments. Nearly 50 years after its first introduction, this study aimed to compare its phenology and adaptive abilities to those of native populations of *Bifurcaria bifurcata* in Brittany using both ecological and metabolomic approaches. Ecological data and composition of primary and secondary metabolites (pigments, phenolic compounds and fatty acids) of the two species were monitored and compared at three sites for one year. Results showed that *B. bifurcata* is a slow-growing species with winter reproduction while *S. muticum* demonstrated a high reproductive rate and rapid growth in spring-summer. In addition, unlike *B. bifurcata*, *S. muticum* presented a single cohort each year, due to the rupture and subsequent loss of its laterals at the end of its reproductive period to allow dissemination of germlings. The higher chemical plasticity of *S. muticum* in response to variations in light and temperature is likely to be related to its ecological strategy. More particularly, metabolomic analyses suggest a greater storage capacity and protection of this invasive species during its reproductive period that may aid its survival by reducing the risk of mortality in the early stages of development. Results further indicate that hydrodynamics is an important factor regulating its distribution by delaying the detachment of the laterals. This present metabolomic analysis, combined with an in-depth ecological assessment contributes to the long-term understanding of the adaptive strategies of the invasive *Sargassum muticum* and the native *Bifurcaria bifurcata* that coexist in Brittany.

**Keywords:** Fatty acids, Native versus invasive species, Macroalgal communities, Pigments, phenolic compounds, Phenology

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## 1. Introduction

Through anthropogenic or natural vectors, the introduction of species and the spread of invasive species constitute a growing ecological and economic threat, particularly in marine environments since oceans and seas are highly connected (Ruiz *et al.* 1997; Mooney and Cleland 2001). Among marine organisms, seaweeds (marine macroalgae) represent a significant part of non-indigenous species in EU coastal waters (Schaffelke *et al.* 2006; Tsiamis *et al.* 2019). About twenty invasive macroalgal species have been observed on the French Atlantic and Mediterranean coasts, including examples of brown (*Undaria pinnatifida*, *Colpomenia peregrina*, *Sargassum muticum*), green (*Caulerpa taxifolia*, *Codium fragile*) and red (*Agarophyton vermiculophyllum* or *Grateloupia turuturu*) species (Streftaris and Zenetos 2006; Stiger-Pouvreau and Thouzeau 2015). The spread of non-native macroalgae has led to competition with native communities and species; and this is likely to increase as a result of climate change and human activities, more particularly in marine environment due to the overexploitation of the resources and the intensification of maritime transport (Occhipinti-Ambrogi and Galil 2010; Early *et al.* 2016). Indeed, changes in the distribution of some marine species linked to ocean acidification or global warming have been already observed, leading to substantial changes in ecological niches (Hughes 2000; Hiscock *et al.* 2004; Occhipinti-Ambrogi and Galil 2010; Ji *et al.* 2016; Schoenrock *et al.* 2019; Thomsen *et al.* 2019; Smale 2020).

Exotic species may have a significant ecological impact as they threaten the distribution and survival of native species through competition for space, light and nutrients. The survival of each species thus depends on their ecological strategy and their ability to adapt rapidly to a changing environment based on their phenotypic plasticity. A higher phenotypic plasticity an ability to reproduce sexually and asexually, and a rapid growth rate leading to their expansion have already been demonstrated for some invasive species (Sakai *et al.* 2001; Davidson *et al.* 2011). As a result of biological introduction and inter-specific competition, invasive species lead to a loss of biodiversity, changes in the species composition and habitat alteration (Schaffelke and Hewitt 2007), as well as economic impacts (Kettunen *et al.* 2009, Pimentel *et al.* 2005).

Among the invasive species in Brittany, the brown seaweed *Sargassum muticum* (Yendo) Fensholt (1955), established in subtidal habitats and intertidal rockpools, is considered to be one of the most widespread and disturbing marine invaders in Europe (Engelen *et al.* 2015). It

was accidentally introduced from Japan into Europe in the 1970s at the same time as the importation of the Pacific oyster *Crassostrea gigas* for aquaculture (Stiger-Pouvreau and Thouzeau 2015). Since then it has spread from Norway to Morocco (Engelen *et al.* 2015; Stiger-Pouvreau and Thouzeau 2015), with drifting specimens recently been reported from the Algerian coast (Benali *et al.* 2019). This species is characterized by a perennial part (*i.e.* holdfast and main axis) and an annual part (*i.e.* primary and secondary laterals, bearing leaves, air vesicles, and receptacles at maturity) (Critchley 1983). Due to this morphological characteristic, *S. muticum* is reduced in size in winter to its small perennial part, whereas in summer, laterals can reach several meters (Le Lann *et al.* 2012). Its proliferation is due in part to its rapid reproductive/growth rate, to both sexual and asexual reproduction, its high dispersal capacity, and its high plasticity (Engelen *et al.* 2015; Benali *et al.* 2019). Thus, *S. muticum* can rapidly reach a high biomass causing competition with native species (Stæhr *et al.* 2000; Engelen *et al.* 2015), and a hindrance to certain activities such as navigation, fishing or aquaculture (Engelen *et al.* 2015).

As a result of its dominance in some intertidal areas and its ecological and economic impacts, *Sargassum muticum* is a relatively well-studied species, with some comparative ecological studies between the invasive and others native species (Arenas *et al.* 1995; Le Lann *et al.* 2012). Indeed, many studies have already demonstrated that the proliferation of *S. muticum* could have negative effect on distribution/abundance of some native species, such as *Saccharina latissima* (Stæhr *et al.* 2000; Pedersen *et al.* 2005). On the contrary, some studies showed no or only limited effect of *S. muticum* on native species, suggesting more complex interactions, for example with *Bifurcaria bifurcata* (R. Ross) (1958) (Sánchez and Fernández 2005, 2006; Sánchez *et al.* 2005; Olabarria *et al.* 2009). Indeed, the presence of a *B. bifurcata* canopy inhibited *S. muticum* early stages of recruitment and development and, by contrast, the native species could benefit *S. muticum* once successfully recruited (Sánchez and Fernández 2006). More recently, abundance of *B. bifurcata* was reported to increase slightly following removal of *S. muticum* in rockpools (Sánchez and Fernández 2018) suggesting a dynamic interaction.

Whilst most previous studies have focused only on ecological observations, a combined approach, incorporating a metabolomic comparison, may provide an integrative overview of the specific acclimation abilities of the species since key molecules can accumulate in response to biotic and abiotic stressors (Lalegerie *et al.* 2020a). For example, seaweeds can

produce photoprotective pigments in response to high light, modify their fatty acid composition regarding to a change in temperature, or increase their phenolic content when subjected to oxidative stress induced by an increase in light or temperature (Lalegerie *et al.* 2020a). The aim of this study was thus to compare populations of invasive *S. muticum* with those of a native species in Brittany, *B. bifurcata*, both belonging to the Sargassaceae. To this end, an ecological monitoring (*i.e.* size distribution, circumference, reproduction, and epiphytes) was carried out for one year at three sites in Brittany where samples were collected monthly for metabolomic analysis (*i.e.* pigments, fatty acids, phenolic compounds). By coupling spatio-temporal ecological and metabolomic data, this study enabled the comparative assessment of the phenology and ecological strategy of the two, native vs. invasive, species. We hypothesized that the fast-growth strategy of the invasive *S. muticum* might involve better protection to limit the negative impacts of failed reproduction on its survival. Specifically, do the two species exhibit differential acclimation to environmental variations? Is one either species more affected by a particular biotic or abiotic factor? And, finally, is the metabolomic composition consistent with the ecology of each species?

## 2. Materials and methods

### 2.1 Sampling sites, environmental and ecological data collection, and algal sampling

Populations of *Sargassum muticum* and *Bifurcaria bifurcata* were monitored monthly at spring low tides between February 2018 and February 2019, at three sites in Brittany (France): Minou (48°20'20.314'' N - 4°37'3.779'' W), Pointe du diable (48°21'15.606'' N – 4°33'30.067'' W) and Porspoder (48°28'56.644'' N – 4°46'6.812'' W) where both species co-occurred. Minou is an exposed and sandy site at the entrance to the Bay of Brest, fringed by rocky coastline and characterized by the presence of many rockpools 10 to 30 cm deep. Pointe du diable was also exposed, with dominant waves, entirely rocky and characterized by the presence of tide pools larger than those at Minou. Finally, Porspoder is sheltered, with macroalgal communities living in a sheltered rocky channel largely covered with sand, and protected from high hydrodynamic conditions by a rocky overhang. Environmental data (temperature, pH, salinity and nutrient contents) recorded near Pointe du diable (48°21'32.13'' N – 4°33'07.19'' W) were provided by « Service d'Observation en Milieu Littoral, INSU-CNRS, Saint-Anne du Portzic ». In addition, daily sunshine duration (hours) (insolation time - INST) and irradiance (global radiation- GLOT) ( $J.cm^{-2}$ ) data were provided by Météo France

from Brest-Guipavas (48°27'00" N – 4°22'59" W) (Supplementary Fig.1).

For both species, on each sampling occasion, ecological measurements were carried out on all individuals within four permanent replicate quadrats (0.09 m<sup>2</sup> and 0.16 m<sup>2</sup> for *S. muticum* and *B. bifurcata*, respectively), arbitrarily positioned. Minimal area for both species was determined using 50 x 50-cm quadrats subdivided into 10 x 10-cm areas (n = 6): the quadrat sizes were obtained when there was no statistical difference in the number of individuals by increasing the sampling area. As *B. bifurcata* has a clonal growth, each clump / patch of fronds was considered as an individual as described in Le Lann *et al.* (2012). Variables measured were: density (*i.e.* number of individuals (or clumps for *B. bifurcata*) per quadrat), maximum length (*i.e.* from the base to the apical part of the longest lateral branch), maximum circumference, the relative abundance of receptacles per individual (*i.e.* 'none', 'few' (1-99) or 'many' (≥100)), presence of epiphytes (in term of species and relative abundance, *i.e.* 'none', 'few' (only a part of the thallus covered), or 'many' (thallus fully covered)). Reproductive index was then estimated as the percentage of individuals bearing few and many receptacles. On each sampling occasion, algal materials were also collected for metabolomic analyses, in areas adjacent to the quadrats. After collection, all algal samples were cleaned to remove epiphytes, and frozen immediately before freeze-drying (β1-8 LD plus Christ) and grinding into a fine powder (MM400 Retsch); samples for metabolomic analyses were processed dried and within two months of collection.

## 2.2 Chlorophyll and carotenoids extraction and analysis

Pigments were extracted using 75 mg of dry seaweed powder with 750 µL 90 % aqueous acetone according to the protocol described in Schmid and Stengel (2015) and Lalegerie *et al.* (2019). Two successive extractions were carried out and High Pressure Liquid Chromatography (HPLC) analysis was performed directly after extraction. Pigments were separated according to Lalegerie *et al.* (2019) on an HPLC Dionex Ultimate 3000 system equipped with a diode array detector (ThermoScientific) and an ACE C<sub>18</sub> column (150 × 4.6 mm, 3 µm) protected with a guard-column. Identification of pigments was obtained by comparison of retention times and absorption spectra with commercial standards of chlorophyll-*a* (Sigma, USA), and chlorophyll-*c*<sub>2</sub>, chlorophyll-*c*<sub>3</sub>, pheophytin-*a*, α-carotene, β-carotene, zeaxanthin, antheraxanthin, violaxanthin (DHI, Denmark). Pigment concentrations were then quantified using standard curves, obtained by injecting different concentrations of

the standards. Chromeleon 7 software (Thermo Scientific Dionex, France) was used for HPLC control and data acquisition.

### 2.3 Fatty acids extraction and analysis

Fatty acids methyl esters (FAMES) were extracted and analysed according to Schmid *et al.* (2014). Thus 40 mg dry weight (DW) of algal powder was added to 2 mL of methanol containing 2 % H<sub>2</sub>SO<sub>4</sub> and samples were incubated under magnetic stirring during 2 h at 80 °C in a dry bath heater (Sigma Aldrich Corning LSE, UK). In order to avoid oxidation, samples were closed under nitrogen flow. After this reaction called transmethylation, samples were cooled to room temperature, and FAMES were extracted by the addition of 1 mL of distilled water and 2 mL of 100 % hexane. After vortex mixing, samples were centrifuged 2 min at 3,000 g (Rotina 38R, Hettich, Germany). The hexane upper layer containing fatty acids was then analysed by Gas Chromatography. Analyses were performed on an Agilent 7890A GC / 5975C MSD series equipped with 7583B injector, a flame-ionization detector and a silica capillary column (DB-Wax, Agilent, 0.25 mm x 30 m x 0.25 µm), with hydrogen as vector gas at 1.2 mL.min<sup>-1</sup> constant flow. The temperature was programmed at 140 °C for 1 min, then increased to 200 °C by a rate of 15 °C.min<sup>-1</sup>, and then to 250 °C at a rate of 2 °C.min<sup>-1</sup>. The injector and detector temperatures were set at 250 °C and 300 °C, respectively. FAMES were identified by comparisons of the retention times with FAME of Fish Oil (Menhaden Oil, Supelco, Bellefonte, PA, USA) and FAME mix (Supelco<sup>TM</sup> 37 Component FAME Mix, Supelco, Bellefonte, PA, USA). Fatty acids were then quantified by comparing peak area with the peak corresponding to a known amount of internal standard, *i.e.* pentadecanoic acid C15:0 (5 mg.mL<sup>-1</sup>) added prior to transmethylation. FA contents were expressed in milligrams per gram DW of seaweed, and total fatty acids (TFA) refers to the sum of each individual fatty acid concentration.

### 2.4 Phenolic compounds extraction and quantification

Phenolic compounds were extracted from 15 mg DW of algal powder in 1 mL of 70 % ethanol. The extraction was carried out with an ultrasonic bath (Sonicator 88155, Fisher Bioblock Scientific, France) during 15 min at 4 °C followed by 2 h at 40 °C under magnetic stirring. Then, samples were centrifuged for 10 min at 8,000 rpm (Eppendorf Centrifuge 5810, Germany) and supernatants were recovered. Samples underwent a second successive extraction, after which the supernatants were pooled and evaporated at 40 °C using a

centrifugal concentrator (miVac, Genevac, France). Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric assay modified from Zubia *et al.* (2009). Thus, 20  $\mu\text{L}$  of sample was added to 130  $\mu\text{L}$  of distilled water, 10  $\mu\text{L}$  of Folin-Ciocalteu reagent and 40  $\mu\text{L}$  of sodium carbonate ( $\text{Na}_2\text{CO}_3$ , 200  $\text{g}\cdot\text{L}^{-1}$ ). Then microplates were incubated for 10 min at 70 °C, after which the chemical reaction was stopped by placing the microplate on ice. Then, the absorbance of each sample was measured in triplicate at 620 nm (Multiskan FC, Thermo Scientific, USA). TPC was determined using a standard curve of phloroglucinol (1,3,5-trihydroxybenzene) and was expressed in milligrams per gram of the dried seaweed powder ( $\text{mg}\cdot\text{g}^{-1}$  DW).

## 2.5 Statistical analyses

Each species at each site was harvested in independent triplicates each month, with one replicate corresponding to a dozen individual thalli. For some data, the monthly values were averaged per season as follows: February 2018 to March (Winter 2018), April to June (Spring), July to September (Summer), October to December (Autumn) and January to February 2019 (Winter 2019); this pooling corresponded to meteorological data in supplementary Fig. 1 (see Lalegerie *et al.* 2020b for more details). The number of size classes was obtained according to Sturges rule with the number of classes =  $1 + 3.3 \log_{10}(n)$ , where  $n$  is the number of individuals. Statistical analyses were performed using R software (version 3.6.1) (R Core Team 2019) through the integrated development environment Rstudio (version 1.2.5001) (Rstudio Team 2019), and results are expressed as mean  $\pm$  standard deviation. Student's t-tests or ANOVAs were carried out in order to compare two groups or more respectively within a single variable, with a confidence level of 95 %. Non-parametric Wilcoxon test (two groups), Kruskal-Wallis (KW; more than two groups, one factor) or Scheirer-Ray-Hare tests (SRH; more than two groups, multifactorial) were used when at least one of the conditions of application was not respected, *i.e.* when the hypothesis of normal distribution (Shapiro test) and/or the homogeneity of variance (F-test) were not respected. In the case of multiple comparison (more than 2 groups), Tukey post-hoc tests after an ANOVA (TukeyHSD), or a non-parametric post-hoc test after a SRH test (kruskalmc, pircmess package) were carried out in order to figure out which group differs in the sample. Principal Component Analyses (PCA) were carried out with the packages FactoMineR and explor, in order to highlight differences between sites, seasons or species in pigment and fatty acid compositions. Finally, Pearson's correlations between ecological, metabolomic and



environmental variables were carried out (or Kendall's correlation if the normality was not respected) to highlight potential relationships between the different compounds or ecology variables, in link to a specific acclimatization response.

### 3. Results

#### 3.1 Environmental data

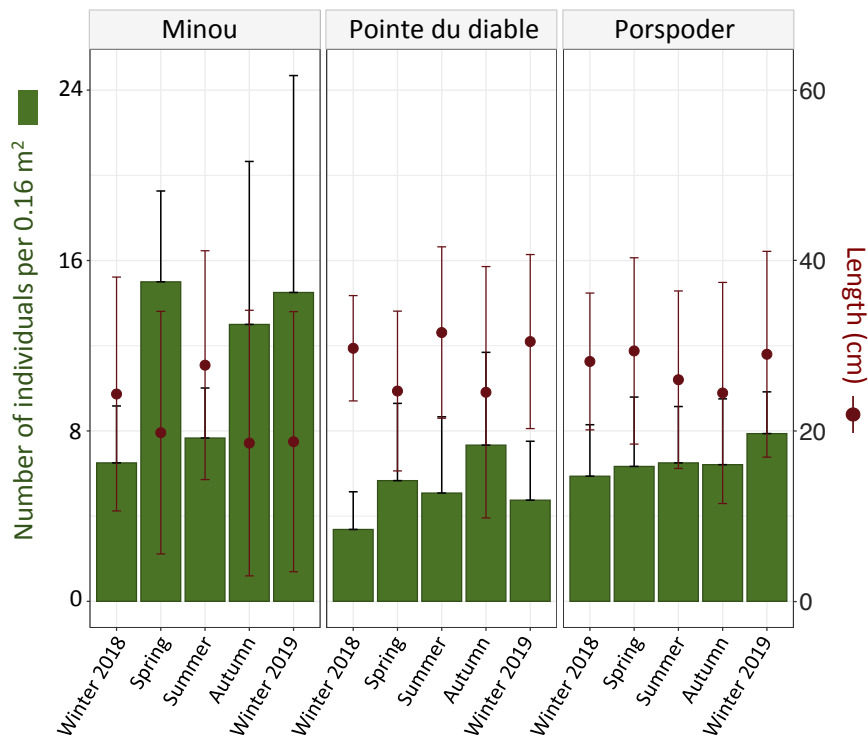
All environmental parameters exhibited strong seasonal variation across the monitoring period (supplementary Fig. 1). Notably, there was a significant increase in daily global radiation and duration of sunshine from spring to summer, reaching a maximum in July ( $2088.13 \pm 694.69 \text{ J.cm}^{-2}$  and  $8.42 \pm 5.25 \text{ h}$  respectively). In parallel, water temperature and pH also increased from spring to summer, with a maximum of  $17.83 \pm 0.34 \text{ }^\circ\text{C}$  in July and  $8.09 \pm 0.05$  in June respectively. Salinity also increased from spring, up to a maximum in autumn, *i.e.*  $35.20 \pm 0.04$  in October. On the contrary, there was a decrease in nutrient levels from spring to summer, with a minimum in July for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  ( $0.34 \pm 0.09 \text{ } \mu\text{M}$  and  $0.42 \pm 0.15 \text{ } \mu\text{M}$  respectively), and May for  $\text{PO}_4^{3-}$  ( $0.01 \pm 0.03 \text{ } \mu\text{M}$ ). Similarly, water oxygen levels were low in summer, with in average  $5.65 \pm 0.26 \text{ mL.L}^{-1}$ .

#### 3.2 *Bifurcaria bifurcata* ecology

Maximal length of *Bifurcaria bifurcata* exhibited temporal variation (Fig. 1) and differed among sites (SRH test,  $p$ -value  $< 0.001$  for sampling sites, seasons and the interaction sites x seasons), with a slight increase in summer at Minou and Pointe du diable (KW,  $p$ -value  $< 0.0001$  for both sites), but not at Porspoder (KW,  $p$ -value = 0.050). Thallus circumference followed a similar pattern (SRH test,  $p$ -value  $< 0.001$  for sampling sites, seasons and the interaction site x season), with an increase in summer only for Minou and Pointe du diable (KW,  $p$ -value  $< 0.0001$  for both sites).

Moreover, despite the high standard deviations observed resulting from the important heterogeneity in size class at all sites, the averaged maximum thallus length of *B. bifurcata* was significantly greater at Pointe du diable and Porspoder sites than at Minou (KW,  $p$ -value  $< 0.001$ ), with a mean maximal length of  $27.39 \pm 11.71$ ,  $27.26 \pm 11.30$  and  $20.88 \pm 14.96 \text{ cm}$  respectively (Fig. 1). By contrast, the number of individuals was significantly higher at Minou (KW,  $p$ -value  $< 0.001$ ), with in average  $11.46 \pm 6.72$  individuals /  $0.16 \text{ m}^2$ , compared to  $5.42 \pm 3.58$  and  $6.56 \pm 2.75$  individuals /  $0.16 \text{ m}^2$  at Pointe du diable and Porspoder, respectively

(Fig. 1). The maximum length was also positively correlated to the circumference for all sites (Kendall's correlation,  $\tau = 0.512$ ,  $p$ -value  $< 0.0001$ ).



**Figure. 1** Temporal number of individuals per 0.16 m<sup>2</sup> (bars) and average thallus length in cm (points) of *Bifurcaria bifurcata* recorded monthly between February 2018 and February 2019 at Minou, Pointe du diable and Porspoder (Brittany, France). Due to the clonal growth, each individual correspond to a patch of fronds.

In parallel, two major recruitment events were observed at both Minou and Pointe du diable, *i.e.* between April and June, and between October and December (Fig. 2 and Supplementary Fig. 2), causing an increase in the density, *i.e.* number of individuals / 0.16 m<sup>2</sup>, and a decrease in the mean thallus length at the same time (Fig. 1). At Porspoder, recruitment peaks were less pronounced (Supplementary Fig. 3), inducing a number of individuals and an averaged length more homogeneous throughout the year (Fig. 1).

*Bifurcaria bifurcata* presented a high fertility index throughout the year at all sites, with an average of  $68.64 \pm 23.89$ ,  $60.75 \pm 39.91$  and  $65.89 \pm 22.19$  % of fertile individuals at Minou, Pointe du diable and Porspoder, respectively. There was a slight decrease in the proportion of fertile individuals between late spring and summer, with no fertile individuals present in July and August at Pointe du diable and Porspoder (SRH test,  $p$ -value  $< 0.001$  for the factor months,  $p$ -value = 0.855 for sites, and  $p$ -value = 0.155 for the interaction months x sites) (see Fig. 2 for Minou, and Supplementary Figs. 2 and 3 for Pointe du diable and Porspoder).

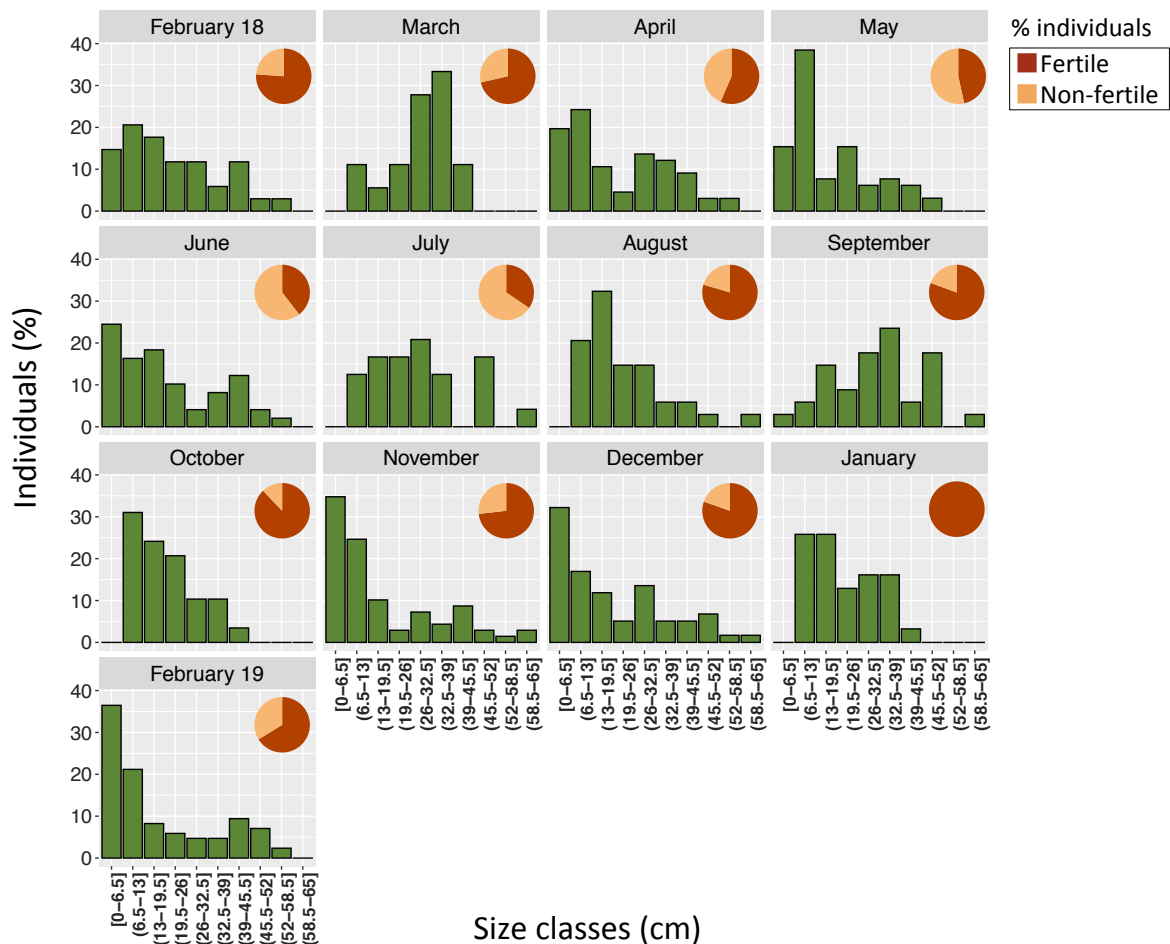
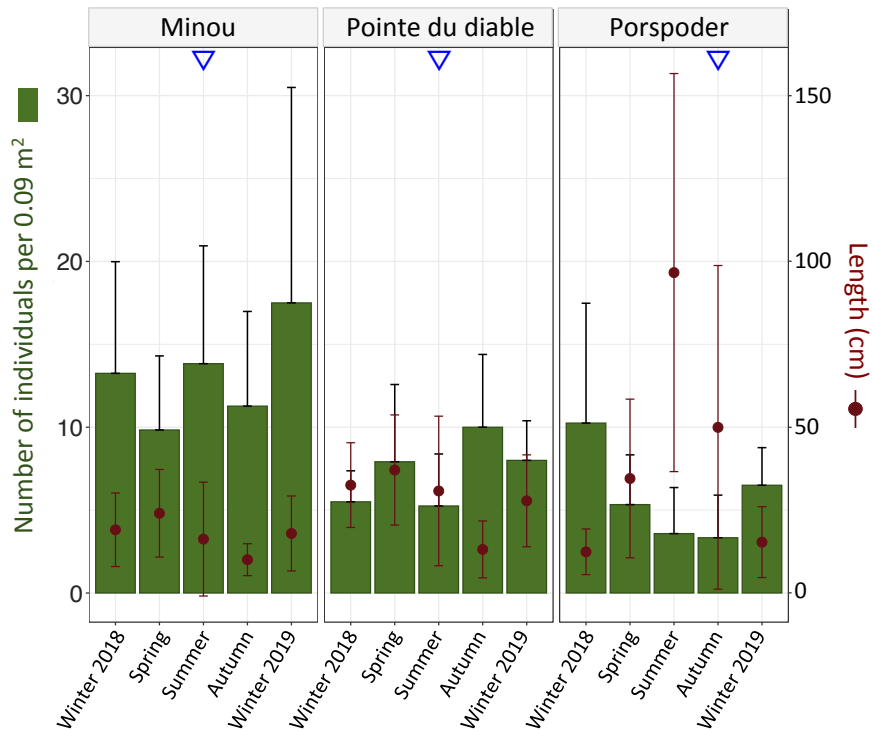


Figure 2. Temporal size (cm) class distribution (%) of the native *Bifurcaria bifurcata* collected monthly between February 2018 and February 2019 at Minou. Monthly pie charts display the fertility index, *i.e.* the proportion of mature individuals, according to the presence of receptacles on thalli.

### 3.3 *Sargassum muticum* ecology

*Sargassum muticum* exhibited a strong temporal variation in the length of its laterals, increasing from spring to summer until reaching a maximum mean length of  $43.70 \pm 21.60$  cm in August at Pointe du diable,  $39.80 \pm 12.70$  cm in July at Minou and  $120.03 \pm 67.80$  cm in August at Porspoder (Fig. 4). Laterals were increasingly colonized by algal epiphytes over time (data not shown). After reaching a maximum size up to  $96.64 \pm 60.03$  cm in summer at Porspoder, which was 2.6 and 4-fold longer than the maximum length in spring at Pointe du diable and Minou, respectively, laterals were mostly broken (*i.e.* missing) by September at Minou and Pointe du diable, and later in December at Porspoder (Fig. 3 and Supplementary Figs. 4 and 5). The mean circumference exhibited a similar temporal pattern to thallus length and was larger at Porspoder (KW,  $p$ -value = 0.001), reaching  $6.43 \pm 4.56$  cm in summer at Porspoder, and only  $3.08 \pm 2.46$  cm in spring and  $3.93 \pm 3.50$  cm in summer at Minou and Pointe du diable, respectively (data not shown). However, conversely to length and

circumference, the number of individuals at Porspoder was smaller (KW,  $p$ -value < 0.001), with an average of  $12.82 \pm 7.65$ ,  $7.50 \pm 3.99$  and  $4.98 \pm 3.72$  individuals per  $0.09 \text{ m}^2$  at Minou, Pointe du diable and Porspoder, respectively.



**Figure 3.** Temporal number of individuals per  $0.09 \text{ m}^2$  (bars) and averaged thallus length in cm (points) of *Sargassum muticum* recorded monthly between February 2018 and February 2019 at Minou, Pointe du diable and Porspoder (Brittany, France). The massive presence of recruits and the annual breakage of long laterals caused a decrease in the mean size, which corresponds to the seasons marked by the symbol  $\nabla$  at the top of the graph.

Almost simultaneously to the detachment of laterals, the number of successful recruits increased substantially (from August to November at Minou, and September to November at Pointe du diable and later from December to February at Porspoder) which led to a fast increase in the number of individuals per quadrat at the same period (Fig. 3 and supplementary Figs. 4, 5). The detachment of the laterals and the presence of new individuals caused a decrease in the average thallus length, in summer for Minou and Pointe du diable and in autumn for Porspoder (KW,  $p$ -value < 0.001 for all sites) (Fig. 4).

Contrary to *B. bifurcata*, the invasive *S. muticum* reproduced for a shorter period (Fig. 4), between May and August at Minou and Pointe du diable, and between April and October at Porspoder (SRH test,  $p$ -value < 0.001 for the factor months,  $p$ -value = 0.011 for sites and  $p$ -value = 0.273 for the interaction months x sites). Fertility index reached its maximum in July for Minou and Pointe du diable with  $87.86 \pm 14.45 \%$  and  $93.75 \pm 12.0 \%$  respectively, and in

August for Porspoder with 100 % of mature individuals. No receptacles were detected during the rest of the monitoring period (See Fig. 4 for Minou, and Supplementary Figs. 4 and 5 for Pointe du diable and Porspoder, respectively).

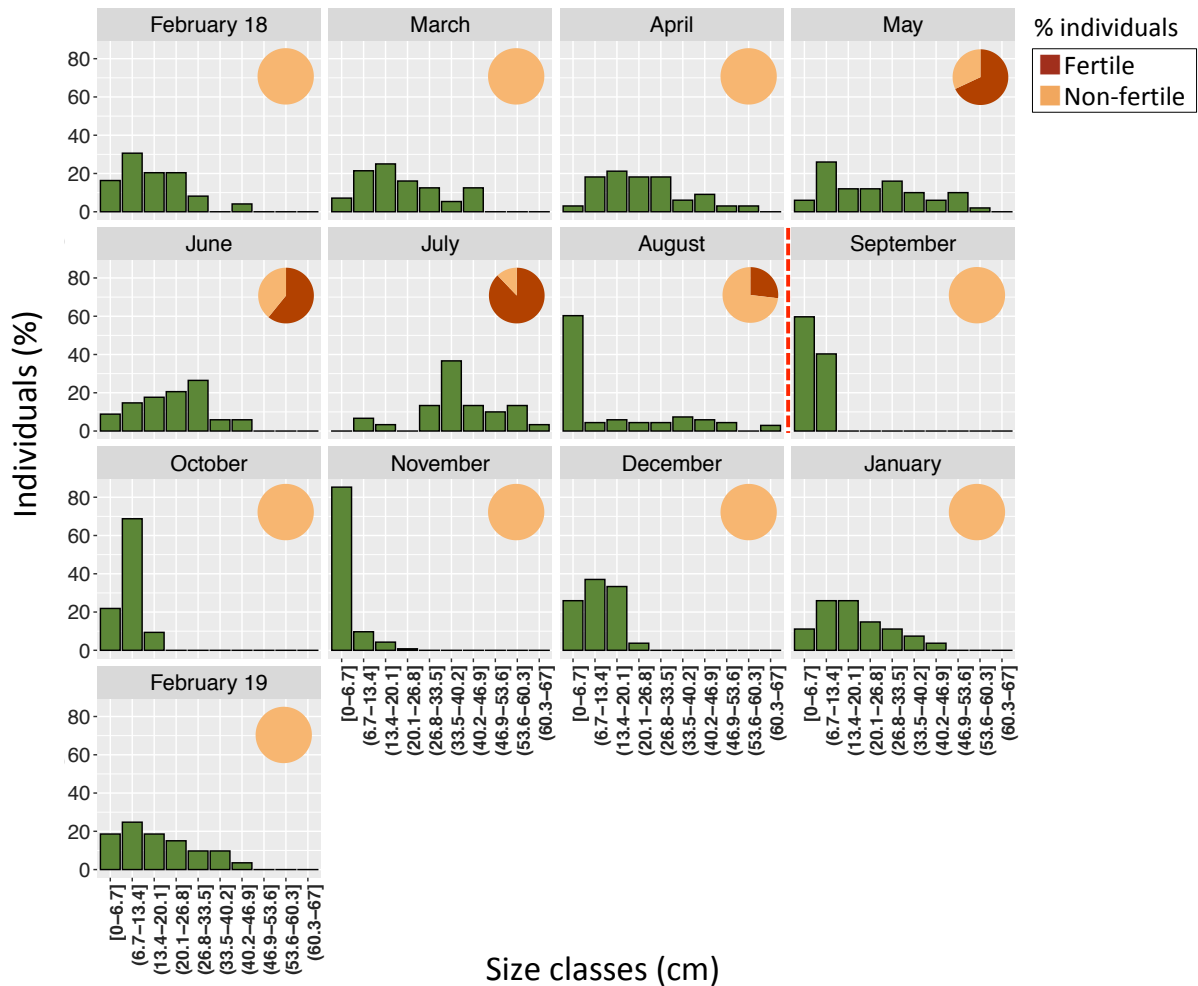
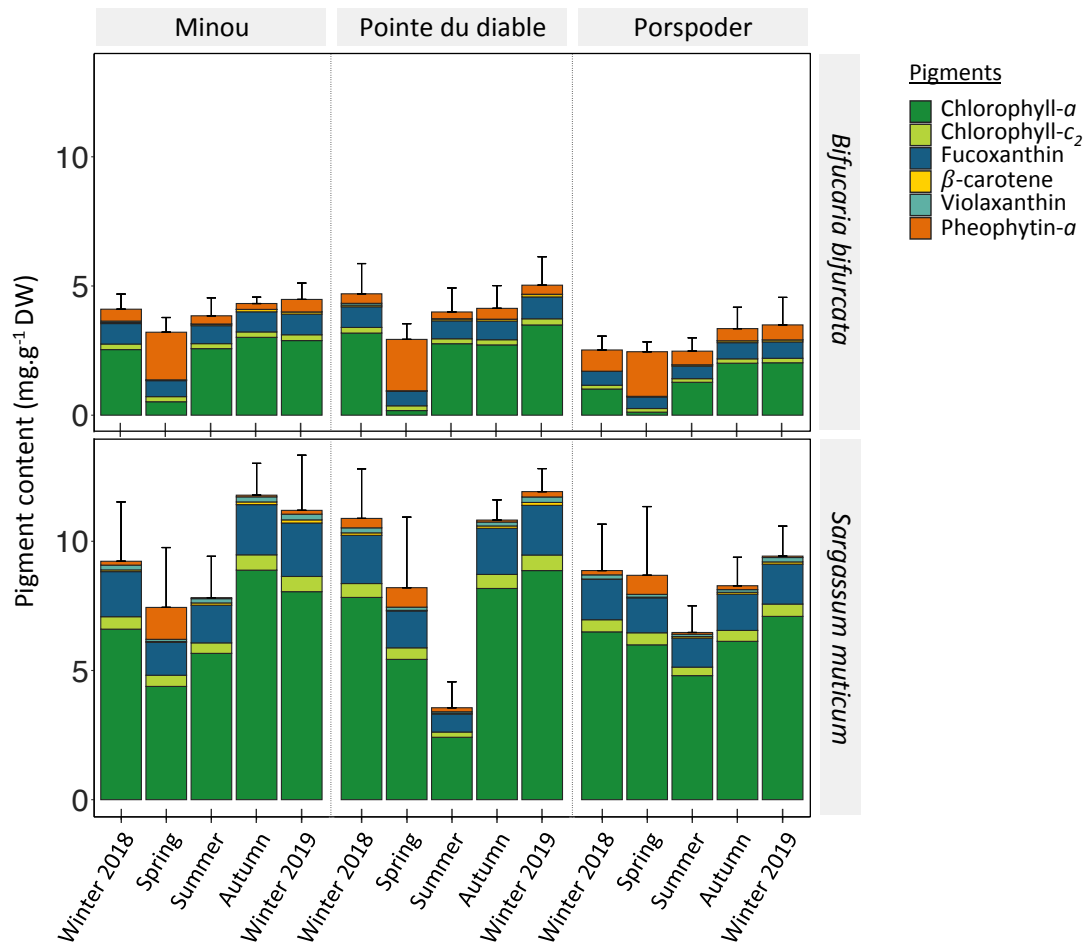


Figure 4. Temporal size (cm) class distribution (%) of the invasive *Sargassum muticum* collected monthly between February 2018 and February 2019 at Minou. Monthly pie charts display the fertility index, *i.e.* the proportion of mature individuals, according to the presence of receptacles on thalli. The red dashed line represents a break in the thallus length of *S. muticum*, due to a detachment of the laterals from the old, mature main axes and the emergence of a new generation.

### 3.4 Pigment composition

Pigment analysis revealed the presence of 6 pigments in both species, *Bifurcaria bifurcata* and *Sargassum muticum*: chlorophyll-*a* was the main pigment, with fucoxanthin and pheophytin-*a* representing, on average  $59.99 \pm 22.63$  %,  $18.05 \pm 2.88$  % and  $14.61 \pm 22.00$  % of the total pigment content, respectively (Fig. 5). Chlorophyll-*c*<sub>2</sub>,  $\beta$ -carotene and violaxanthin were also detected in both species but to a lesser extent (on average  $5.33 \pm 0.74$  %,  $1.14 \pm$

0.76 % and  $0.86 \pm 0.89$  % of the total pigment content, respectively) (Fig. 5). Minor traces of  $\alpha$ -carotene, zeaxanthin, antheraxanthin or lutein were detected in *S. muticum* samples in late summer (data not shown).



**Figure 5.** Temporal pigment content (chlorophyll-*a*, chlorophyll-*c*<sub>2</sub>, fucoxanthin,  $\beta$ -carotene, violaxanthin and pheophytin-*a*) of *Bifurcaria bifurcata* and *Sargassum muticum* collected monthly from February 2018 to February 2019 at Minou, Pointe du diable and Porspoder (Brittany, France). Results are displayed as the stacked cumulative contents (with standard deviation of the cumulative contents).

Pigment composition differed slightly between the invasive *S. muticum* and the native *B. bifurcata*, but there was no difference between sampling sites (Fig. 5 and Supplementary Fig. 6). Chlorophyll-*a* content was significantly higher in *S. muticum* (Wilcoxon test,  $p$ -value  $< 0.001$ ), with a mean content of  $6.29 \pm 2.23$  mg.g<sup>-1</sup> DW compared to  $1.95 \pm 1.32$  mg.g<sup>-1</sup> DW for *B. bifurcata*. Similarly, the contents of violaxanthin, fucoxanthin and chlorophyll-*c*<sub>2</sub> were higher in *S. muticum*, following the same temporal pattern (Wilcoxon test,  $p$ -value  $< 0.001$  for all variables) (Fig. 5). On the contrary, the pheophytin-*a* content was significantly higher in *B. bifurcata* (Wilcoxon test,  $p$ -value  $< 0.001$ ) with a mean content of  $0.75 \pm 0.76$  mg.g<sup>-1</sup> DW

compared to  $0.31 \pm 0.51 \text{ mg.g}^{-1} \text{ DW}$  for *S. muticum*, representing an average of  $24.69 \pm 26.15 \%$  and  $4.54 \pm 9.12 \%$  of the total pigment content respectively (Fig. 5). The contents of pheophytin-*a* and chlorophyll-*a* were negatively correlated for *B. bifurcata* (Kendall's correlation,  $\tau = -0.65$ ,  $p$ -value  $< 0.001$ ), but not for *S. muticum* (Kendall's correlation,  $\tau = -0.07$ ,  $p$ -value = 0.27). In *B. bifurcata*, pheophytin-*a* was the main pigment quantified in spring for the three sites (Fig. 5). Moreover, although the content of  $\beta$ -carotene was not significantly different between both species (Wilcoxon test,  $p$ -value = 0.337), the proportion of this pigment was significantly twice as high in *B. bifurcata* (Wilcoxon test,  $p$ -value  $< 0.001$ ), with  $1.53 \pm 0.82 \%$  compared to  $0.75 \pm 0.44 \%$  for *S. muticum*.

For both species, pigment contents and proportions (Fig. 5) varied seasonally, coinciding with the global increase in light radiation, temperature, ammonium, and the decrease in phosphate and nitrate contents (Supplementary Fig. 1). Chlorophyll-*a* content decreased significantly in spring for *B. bifurcata*, and in summer for *S. muticum* (SRH test,  $p$ -value  $< 0.001$  for species and season,  $p$ -value = 0.058 for the interaction) (Fig. 5). This decrease was substantially greater for *B. bifurcata* (45 % decrease in content between winter 2018 and spring), than *S. muticum* (10 %). As a result, chlorophyll-*a* content was even below the limit of quantification for the majority of *B. bifurcata* samples collected in May and June. On the contrary, an increase in pheophytin-*a* content was detected in spring for both species (SRH test,  $p$ -value  $< 0.001$  for species and season,  $p$ -value = 0.744 for the interaction), which was greater for *B. bifurcata*. Thus, the proportion of pheophytin-*a* represented  $63.46 \pm 19.90 \%$  of the total pigment content in spring for *B. bifurcata*, and only  $13.44 \pm 12.46 \%$  for *S. muticum*. According to the PCA analysis (Supplementary Fig. 6a,b), pheophytin-*a* appeared then to be, mainly positively, affected by light intensity and ammonium level.

### 3.5 Fatty acids

In *S. muticum*, there was a significant increase in TFA content in May and February 2019 (One-way ANOVA,  $p$ -value  $< 0.0001$ ), reaching  $87.34 \pm 6.06 \text{ mg.g}^{-1} \text{ DW}$  in May before decreasing to  $63.64 \pm 5.97 \text{ mg.g}^{-1} \text{ DW}$  in August and increasing again up to  $86.68 \pm 7.37 \text{ mg.g}^{-1} \text{ DW}$  in February 2019. The proportion of PUFA also varied over time (KW,  $p$ -value  $< 0.0001$ ), with a maximum in May and February 2019, mainly due to linolenic acid (C18:3n-3), C20:4n-3, C20:4n-6 and eicosapentaenoic acid (C20:5n-3) (Table 1 and Supplementary Fig. 7a,b). By contrast, the proportion of SFA and MUFA decreased in May and February 2019 (KW and One-Way ANOVA respectively,  $p$ -value  $< 0.0001$ ).

**Table 1.** Temporal total fatty acids (TFA) content and fatty acid composition (% of the total fatty acids content) in *Bifurcaria bifurcata* and *Sargassum muticum*, collected monthly between February 2018 and February 2019 at Minou, Pointe du diable and Porspoder (Brittany, France). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids. n.d. = not detected (under the detection level).

% Fatty acids	<i>Bifurcaria bifurcata</i>						<i>Sargassum muticum</i>					
	February 18	May	August	November	February 19		February 18	May	August	November	February 19	
SFA												
C14:0	4.35 ± 0.28	4.25 ± 0.69	4.71 ± 0.43	5.02 ± 0.73	4.64 ± 0.48		3.28 ± 0.24	3.53 ± 0.32	3.62 ± 0.49	3.09 ± 0.17	2.50 ± 0.17	
C16:0	24.25 ± 1.25	25.07 ± 0.92	25.53 ± 1.11	23.57 ± 0.70	23.13 ± 0.71		33.38 ± 2.31	27.19 ± 1.63	30.86 ± 1.45	27.38 ± 1.36	25.66 ± 2.12	
C18:0	n.d.	n.d.	n.d.	n.d.	n.d.		0.81 ± 0.14	0.62 ± 0.08	0.95 ± 0.37	0.64 ± 0.14	0.60 ± 0.11	
MUFA												
C16:1 n-7	3.92 ± 0.79	2.94 ± 0.59	3.53 ± 0.81	3.65 ± 0.64	3.45 ± 0.49		6.33 ± 0.89	4.29 ± 0.42	6.74 ± 0.87	5.93 ± 1.78	4.36 ± 0.89	
C18:1 n-7	n.d.	n.d.	n.d.	n.d.	n.d.		0.50 ± 0.38	0.48 ± 0.24	0.90 ± 0.17	0.91 ± 0.97	0.31 ± 0.21	
C18:1 n-9	15.74 ± 1.87	14.41 ± 0.71	15.06 ± 0.75	14.71 ± 0.94	14.67 ± 0.62		11.18 ± 0.52	8.66 ± 0.87	9.79 ± 0.33	8.70 ± 0.87	8.72 ± 0.99	
C20:1 n-9	0.56 ± 0.68	0.30 ± 0.60	1.05 ± 0.81	0.67 ± 0.81	1.14 ± 0.17		n.d.	n.d.	n.d.	n.d.	n.d.	
C22:1 n-9	n.d.	n.d.	n.d.	n.d.	n.d.		2.58 ± 0.20	1.52 ± 0.19	1.71 ± 0.24	1.93 ± 0.18	1.82 ± 0.08	
PUFA												
C16:2 n-4	n.d.	n.d.	n.d.	n.d.	n.d.		0.55 ± 0.06	0.51 ± 0.04	0.58 ± 0.10	0.70 ± 0.33	0.54 ± 0.04	
C18:2 n-6	3.92 ± 0.12	4.04 ± 0.28	4.26 ± 0.27	3.99 ± 0.36	3.93 ± 0.32		6.80 ± 0.93	5.99 ± 0.64	5.63 ± 0.57	5.57 ± 0.63	6.12 ± 0.86	
C18:3 n-3	7.21 ± 0.75	7.61 ± 0.48	7.37 ± 0.45	7.02 ± 0.59	7.39 ± 0.84		6.34 ± 0.56	9.41 ± 0.58	8.95 ± 0.8	9.08 ± 0.88	9.03 ± 1.19	
C18:4 n-3	6.87 ± 1.33	7.93 ± 0.78	6.01 ± 0.57	7.03 ± 0.81	7.66 ± 1.02		4.17 ± 0.47	7.34 ± 0.70	4.33 ± 0.47	7.18 ± 1.36	8.39 ± 1.26	
C20:4 n-3	2.21 ± 0.33	2.12 ± 0.21	1.89 ± 0.23	2.13 ± 0.29	2.11 ± 0.17		0.31 ± 0.18	0.72 ± 0.16	0.44 ± 0.06	0.46 ± 0.21	0.61 ± 0.07	
C20:4 n-6	17.87 ± 0.83	17.81 ± 0.46	17.45 ± 0.63	18.73 ± 0.63	17.8 ± 0.58		9.47 ± 1.04	12.53 ± 1.11	11.71 ± 1.03	12.01 ± 0.92	13.56 ± 0.29	
C20:5 n-3	7.19 ± 0.79	8.17 ± 0.70	6.94 ± 0.49	7.35 ± 1.11	7.53 ± 0.57		4.16 ± 0.55	9.45 ± 0.75	7.12 ± 0.57	8.04 ± 0.99	9.57 ± 1.15	
Unknown_FA1	n.d.	n.d.	n.d.	n.d.	n.d.		2.12 ± 0.43	0.96 ± 0.16	1.54 ± 0.15	1.39 ± 0.36	1.35 ± 0.51	
Unknown_FA2	n.d.	n.d.	n.d.	n.d.	n.d.		1.28 ± 0.41	1.36 ± 0.53	0.86 ± 0.35	1.40 ± 0.35	1.25 ± 0.43	
Unknown_FA3	n.d.	n.d.	n.d.	n.d.	n.d.		1.33 ± 0.23	1.28 ± 0.28	0.80 ± 0.08	1.33 ± 0.16	1.29 ± 0.16	
Unknown_FA4	n.d.	n.d.	n.d.	n.d.	n.d.		1.09 ± 0.18	0.80 ± 0.07	0.65 ± 0.07	1.00 ± 0.08	0.90 ± 0.09	
Unknown_FA5	1.18 ± 0.6	0.88 ± 0.54	0.91 ± 0.81	1.10 ± 0.89	1.20 ± 0.48		n.d.	n.d.	n.d.	n.d.	n.d.	
Unknown_FA6	1.02 ± 0.96	0.96 ± 0.43	1.02 ± 0.99	1.10 ± 1.07	1.17 ± 0.58		n.d.	n.d.	n.d.	n.d.	n.d.	
Unknown_FA7	n.d.	n.d.	n.d.	n.d.	n.d.		2.55 ± 0.14	1.91 ± 0.15	1.98 ± 0.24	1.95 ± 0.12	1.83 ± 0.18	
Unknown_FA8	n.d.	n.d.	n.d.	n.d.	n.d.		1.78 ± 0.30	1.45 ± 0.09	0.82 ± 0.19	1.31 ± 0.46	1.59 ± 0.29	
Unknown_FA9	2.40 ± 1.14	2.13 ± 0.92	3.10 ± 1.17	2.35 ± 1.20	2.46 ± 0.55		n.d.	n.d.	n.d.	n.d.	n.d.	
Unknown_FA10	1.32 ± 1.14	1.37 ± 1.19	1.18 ± 1.49	1.58 ± 1.39	1.74 ± 1.53		n.d.	n.d.	n.d.	n.d.	n.d.	
% SFA	28.59 ± 1.18	29.33 ± 0.98	30.24 ± 1.27	28.59 ± 0.83	27.77 ± 0.80		37.47 ± 2.53	31.34 ± 1.92	35.43 ± 1.85	31.11 ± 1.44	28.75 ± 2.35	
% MUFA	20.22 ± 2.72	17.65 ± 1.11	19.64 ± 1.19	19.03 ± 0.98	19.25 ± 0.82		20.59 ± 1.37	14.96 ± 1.41	19.15 ± 1.10	17.47 ± 2.14	15.21 ± 1.93	
% PUFA	45.27 ± 3.08	47.68 ± 2.07	43.92 ± 0.92	46.25 ± 1.49	46.41 ± 1.86		31.8 ± 2.85	45.95 ± 2.58	38.77 ± 2.75	43.04 ± 2.34	47.82 ± 3.30	
<b>TFA (mg·g<sup>-1</sup> DW)</b>	<b>57.05 ± 3.75</b>	<b>60.75 ± 3.17</b>	<b>65.22 ± 4.16</b>	<b>66.33 ± 3.40</b>	<b>62.97 ± 2.72</b>		<b>59.53 ± 2.85</b>	<b>87.34 ± 6.06</b>	<b>63.64 ± 5.87</b>	<b>70.33 ± 9.69</b>	<b>86.68 ± 7.36</b>	

C14:0 = myristic acid, C16:0 = palmitic acid, C18:0 = stearic acid, C16:1n-7 = palmitoleic acid, C18:1n-7 = vaccenic acid, C18:1n-9 = oleic acid,

C20:1n-9 = eicosenoic acid, C22:1n-9 = erucic acid, C16:2n-4 = hexadecadienoic acid, C18:2n-6 = linoleic acid, C18:3n-3 = linolenic acid,

C18:4n-3 = octadecatetraenoic acid, C20:4n-3 = eicosatetraenoic acid, C20:5n-3 = eicosapentaenoic acid



In *B. bifurcata*, TFA content varied over time (One-way ANOVA,  $p$ -value  $< 0.0001$ ), increasing slightly from  $57.05 \pm 3.75 \text{ mg.g}^{-1} \text{ DW}$  in February 2018 to a maximum in November with  $66.33 \pm 3.40 \text{ mg.g}^{-1} \text{ DW}$ . Moreover, a slight decrease in the proportion of PUFA was observed in August (KW,  $p$ -value = 0.006), whereas the proportion of SFA (One-way ANOVA,  $p$ -value  $< 0.0001$ ) and MUFA (KW,  $p$ -value = 0.022) increased slightly during the same period (Table 1). Despite this, PCA analysis (Supplementary Fig. 7c,d) did not reveal any difference in the fatty acid composition of *B. bifurcata* over time contrary to *S. muticum*, due to a more moderate variation. Then, PUFA content of *B. bifurcata* appeared to be linked to temperature and possibly nutrients or salinity (Supplementary Fig. 7c,d); this was in contrast to *S. muticum* where measured environmental factors did not appear to influence FA composition, nor PUFA levels (Supplementary Fig. 7a,b).

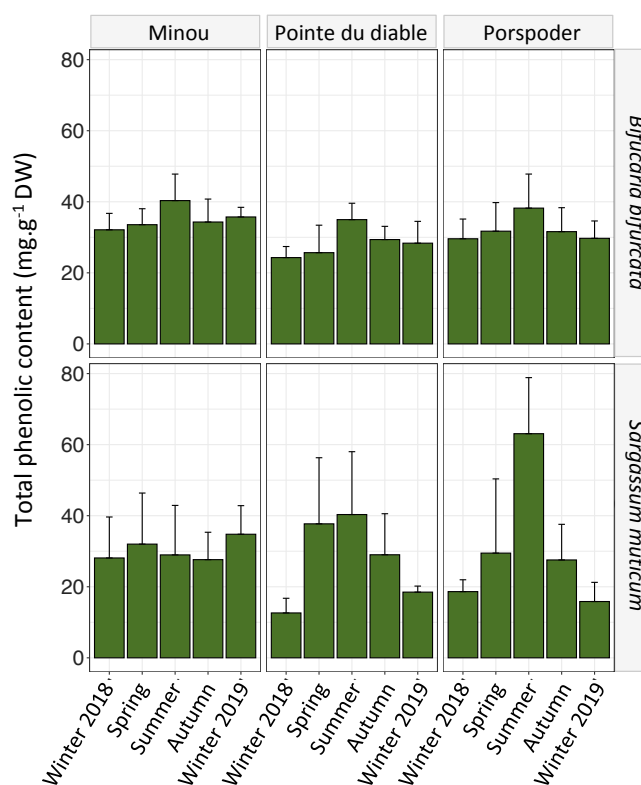
In both species, three main fatty acids were palmitic acid (C16:0), arachidonic acid (C20:4n-6) and oleic acid (C18:1n-9), representing in average  $24.31 \pm 1.29 \%$ ,  $17.93 \pm 0.74 \%$  and  $14.92 \pm 1.13 \%$  of the the total fatty acid (TFA) content of *B. bifurcata* and  $28.89 \pm 3.33 \%$ ,  $11.85 \pm 1.63 \%$  and  $9.41 \pm 1.23 \%$  of TFA content of *S. muticum* (Table 1).

Then, although there were no spatial differences in TFA content (One-way ANOVA,  $p$ -value = 0.106 for *B. bifurcata* and  $p$ -value = 0.550 for *S. muticum*) or in fatty acid composition (Supplementary Fig. 8) in either species, composition differed between the two species. Only *B. bifurcata* contained eicosenoic acid (C20:1n-9) and 4 other unknown FAs while only *S. muticum* contained hexadecadienoic acid (C16:2n-4), stearic acid (C18:0), vaccenic acid (C18:1n-7), erucic acid (C22:1n-9) and 6 unknown FAs (Table 1). In addition, *B. bifurcata* contained more myristic acid (C14:0), eicosatetraenoic acid (C20:4n-3), C20:4n-6 and C18:1n-9 (Wilcoxon and Student's test respectively,  $p$ -value  $< 0.001$  for both variables), while *S. muticum* had higher levels of C16:0, palmitoleic acid (C16:1n-7), and linoleic acid (C18:2n-6) (Wilcoxon test,  $p$ -value  $< 0.001$  for all variables) (see detailed composition in Table 1, and PCA analysis in Supplementary Fig. 8).

### 3.6 Phenolic compounds

Total phenolic content (TPC) differed significantly across the two species and seasons (SRH test,  $p$ -value = 0.0179 for species and  $p$ -value  $< 0.0001$  for seasons) (Fig. 6). For both species, TPC increased in summer at all sites, however this increase was greater for *S. muticum*, coinciding with its reproductive period between May and August (Figs. 4 and 6). For *S.*

*muticum*, TPC ranged from  $19.79 \pm 9.51$  mg.g<sup>-1</sup> DW in winter 2018 to  $44.13 \pm 21.91$  mg.g<sup>-1</sup> DW in summer. In *B. bifurcata*, TPC was less variable and only ranged from  $28.67 \pm 5.43$  mg.g<sup>-1</sup> DW in winter 2018, to  $37.86 \pm 7.64$  mg.g<sup>-1</sup> DW in summer.



**Figure 6.** Temporal total phenolic content (TPC) (mg.g<sup>-1</sup> DW) in *Bifurcaria bifurcata* and *Sargassum muticum* collected monthly between February 2018 and February 2019 at Minou, Pointe du diable and Porspoder (Brittany, France).

In addition, there were differences between sampling site which depended on season (SRH test,  $p$ -value = 0.0278 for sampling sites,  $p$ -value < 0.0001 for seasons and  $p$ -value = 0.0203 for the interaction site x season). Indeed, although the TPC of *S. muticum* increased in summer at all sites, this seasonal variation was smaller at Minou (Fig. 6). TPC in *S. muticum* declined then in August at Minou and Pointe du diable, and in December at Porspoder. For both species, average TPC was also slightly lower at Pointe du diable, although this spatial difference depended on seasons and was mainly due to lower winter values. Regardless of season, TPC of *S. muticum* did not differ between sites with, on average,  $29.49 \pm 16.65$  mg.g<sup>-1</sup>,  $30.12 \pm 11.38$  mg.g<sup>-1</sup> and  $33.02 \pm 21.77$  mg.g<sup>-1</sup> for Pointe du diable, Minou and Porspoder respectively (KW,  $p$ -value = 0.749). Finally, TPC was positively correlated to temperature for both species (Kendall's correlation,  $\tau = 0.382$  for *B. bifurcata* and  $\tau = 0.432$  for *S. muticum*,  $p$ -

value  $< 0.0001$  for both species), and to the global radiation accumulated over 30 days before sampling only for *S. muticum* (Kendall's correlation,  $\tau = 0.454$  and  $p$ -value  $< 0.0001$ ).

## 4. Discussion

The objective of this present study was to evaluate differences in phenology and acclimation abilities of two co-existing macroalgal species, the native *Bifurcaria bifurcata* and the invasive *Sargassum muticum*. By coupling ecological and metabolomic data, the results provide an integrative comparison of their ecological strategies where they co-exist within the same habitat, *i.e.* rockpools in intertidal areas of Brittany (France), almost 50 years after the introduction of *S. muticum* (Critchley *et al.* 1983).

### 4.1 Ecological segregation in the reproductive period: adaptation to environmental variations and competition for resource availability?

Because of the invasive nature of *S. muticum*, it was expected that this species would have an ecological strategy and then phenology different from the native species. In this study, *S. muticum* and *B. bifurcata* demonstrated strongly seasonal, but different, reproductive strategies. Indeed, *B. bifurcata* was fertile over a long period from autumn to spring, whereas *S. muticum* had a high reproductive rate in spring / summer over a shorter period. This difference in seasonality has already been described in previous studies, whether or not the two species live together (De Valera 1961; Arenas *et al.* 1995; Baer and Stengel 2010; Plouguerné *et al.* 2006; Martínez *et al.* 2012; Le Lann *et al.* 2012). *B. bifurcata* is described as a slow-growing species that takes advantage of high nutrient levels in winter to reproduce (Martínez *et al.* 2012). *B. bifurcata* also stores N reserves in order to be more tolerant to nutrient limitation during summer that allows this perennial species to spread its reproductive period until spring (Martínez *et al.* 2012). On the contrary, *S. muticum* is a fast-growing species, which grows very quickly as soon as the environment presents all conditions needed for its reproduction and growth (Olabarria *et al.* 2018). Thus, as observed in the present study, *S. muticum* showed a rapid reproduction and growth period in spring / summer, following the seasonal increase in light and temperature, and when nutrient levels were still sufficiently high in the water. Some studies have already shown that the reproduction and growth of *S. muticum* was favoured by warm temperatures (Norton 1977; Cacabelos *et al.* 2013), and that N availability impacts its growth and biomass (Steen and Rueness 2004; Sánchez and Fernández 2006; Incera *et al.* 2009; Vieira *et al.* 2017). Therefore, the temporal segregation in

the phenologies of *S. muticum* and *B. bifurcata* could be a coincidental and not linked to invasive or native characteristics; however, it appears to prevent competition for resource availability, and then allow them to co-exist in the same habitat. Similar time segregation strategies between native and invasive species have already been shown between *Cystoseira nodicaulis* and *S. muticum* (Arenas *et al.* 1995), and between *B. bifurcata* and *Ulva intestinalis* (Martínez *et al.* 2012).

#### **4.2 *S. muticum*: a fast-growing species dependant on hydrodynamic conditions?**

Compared to *B. bifurcata*, the ecology of *S. muticum*, which differed between exposed and sheltered sites, suggests a potential effect of hydrodynamic conditions. Indeed, *S. muticum* reached larger sizes at the sheltered site Porspoder, up to 120 cm on average, in August. By contrast, the length of its thalli did not exceed 39 cm and 43 cm at exposed sites, respectively in July at Minou and in August at Pointe du diable. In addition, although the laterals were longer at the sheltered site Porspoder, there were fewer individuals at this site. There could be a compensation between the high length of *S. muticum* and its density at Porspoder, to regulate its biomass and intra-species competition as suggested by Arenas *et al.* (2002) and Baer and Stengel (2010), to limit its mortality under crowded conditions. Such results on the influence of hydrodynamic conditions on length and density have also been observed by Andrew and Viejo (1998), Plouguerné *et al.* (2006), and Le Lann *et al.* (2012), and therefore constitutes an important factor in the survival of the species.

Then, the mean length of *S. muticum* decreased significantly in autumn when the fertile laterals became detached from the thallus at the end of the reproductive period which allows its germlings to be dispatched over several meters to kilometers (Engelen *et al.* 2015). However, this detachment of the laterals of *S. muticum* occurred later at the sheltered site Porspoder, contrary to the two exposed sites Pointe du diable and Minou. Thus, greater wave exposure could weaken the laterals, causing an early detachment. Then, due to the later break of the laterals at Porspoder, reproduction was more extended in time and the observation of recruits was delayed. Contrary to the perennial *B. bifurcata* which exhibited several cohorts at the same time, only one cohort was visible for *S. muticum* due to its ecological strategy of lateral detachment, with a mass arrival of recruits at the end of the summer, replacing the previous cohort, and an increase in laterals between each recruitment (Baer and Stengel 2010). Thus, a delay detachment of the laterals due to hydrodynamic conditions could impact the reproduction and ultimately the survival of *S. muticum*, explaining why the invasive

species benefits from growth on sheltered coasts or protection within rockpools at exposed sites (Andrew and Viejo 1998; Engelen *et al.* 2015). Furthermore, early detachment was likely enhanced by a massive epiphyte occurrence at exposed sites, leading to an excessive weight of laterals in accordance with Baer and Stengel (2014). In this way, Serebryakova *et al.* (2018) demonstrated a seasonal shift in bacterial composition associated to *S. muticum*, supporting the idea of a behavioural change in the host during summer. This is in accordance with the detection of lutein and  $\alpha$ -carotene in late summer, two pigments specific to red macroalgae, or cyanobacteria. Finally, in addition to hydrodynamics, the abundance of *S. muticum* could also depend on depth and substratum, as suggested by Thomsen *et al.* (2006). In this sense, it is possible that the length of *S. muticum* thalli was limited by the shallow depth of rockpools at Minou and Pointe du diable, or that the settlement of *S. muticum* recruits may have been limited at the sandy site Porspoder.

#### 4.3 Light acclimation potential: improved tolerance for *S. muticum*?

Results revealed differences in pigment composition between both species, indicating a differential strategy in light acclimation for the species. Chlorophyll-*a* decreased in spring-summer in both species, but significantly more in *B. bifurcata*. This decrease in chlorophyll-*a* content, the main pigment responsible of light absorption with fucoxanthin in brown macroalgae (Mimuro and Akimoto 2003), suggests a greater reduction in photosynthetic activity and thus photo-acclimation to avoid photodamages on photosystem II (PSII) (Franklin *et al.* 2003) in *B. bifurcata* where chlorophyll-*a* was not even detectable in the majority of samples collected in May and June. In addition, *B. bifurcata* showed a very high level of pheophytin-*a* in spring in contrast to *S. muticum* (Fig. 5). Pheophytin-*a* is a degradation product of chlorophyll-*a* due to the elimination of central  $Mg^{2+}$  (Steinman *et al.* 2017), with a potential protective role of P680 (*i.e.* primary electron donor of PSII) against photo-induced damage (Hou *et al.* 2014). In this way, its presence revealed the vulnerability and thus acclimation of *B. bifurcata* to increased light irradiance. Alternatively, the difference in pigment composition between both species could be related to their differential reproductive strategies. Since *S. muticum* reproduced in spring-summer, a greater decrease in pigments due to a change in biomass composition may have been predicted (*i.e.* increase of reproductive materials compared to photosynthetic tissues). However, this was not observed since the decline in photosynthetic pigments in *S. muticum* was less pronounced than in the native species. It can therefore be suggested that its receptacles were less light-sensitive

requiring less acclimation. Additionally, the increasingly density of epiphytes on *S. muticum* from spring to summer at all sites could have caused a light reduction, and protected receptacles against harmful radiation, as already suggested for seagrass by Sand-Jensen (1977). Although violaxanthin was detected in both species, there was no increase in the content of photoprotective pigments, such as antheraxanthin or zeaxanthin component of xanthophyll cycle in Phaeophyceae (Goss and Jakob 2010). Therefore, overall, pigment data suggest that *S. muticum* appears to be more tolerant to light than *B. bifurcata*, and/or potentially has other photoprotective metabolites or mechanisms, such as phenolic compounds (Franklin *et al.* 2003; Bischof *et al.* 2006).

#### **4.4 Phenolic compounds: is *S. muticum* protected against strong environmental variation?**

Results highlighted an increase in phenolic contents in summer for both species, reaching an average of 38 and 44 mg.g<sup>-1</sup> DW for *B. bifurcata* and *S. muticum*, respectively. This can in part be explained by the protective role of these particular aromatic compounds against high light (Schoenwaelder 2002; Swanson and Druehl 2002; Le Lann *et al.* 2008) and temperature (Tanniou *et al.* 2014). However, the seasonal increase was greater for *S. muticum* and coincided with its reproductive period; by producing more phenolic compounds than *B. bifurcata*, the invasive *S. muticum* may thus have additionally increased its tolerance to high light and temperature, as previously suggested by Plouguerné *et al.* (2006). However, as this increase was site-specific (less distinct at Minou), other factors may have triggered and controlled phenol production. Previous studies have already shown seasonal variations in TPC (Connan *et al.* 2004; Abdala-Díaz *et al.* 2006; Plouguerné *et al.* 2006; Gager *et al.* 2020) in response to abiotic factors such as salinity (Polo *et al.* 2014 ; Connan and Stengel 2011), nutrient content (Yates and Peckol 1993), tidal cycle (Connan *et al.* 2007); or biotic factors such as grazing (Svensson *et al.* 2007; Olabarria *et al.* 2018), or the stage of development (Pavia *et al.* 2003; Stiger *et al.* 2004). Connan *et al.* (2006) also showed that phenolic content could vary with the part of the thallus in temperate brown macroalgae. A differential chemical defence strategy of both species could be hypothesized, with *Bifurcaria bifurcata* favouring a constitutive defence (*i.e.* by producing phenolic compounds that vary little during the year), and *Sargassum muticum* presenting an inducible defence, with a production of phenolic compounds in response to the increase in light and temperature, and reproductive status.

#### 4.5 Fatty acids: a reproductive adaptation of *S. muticum*?

*B. bifurcata* and *S. muticum* exhibited a seasonal variation in their fatty acid composition, with a shift between PUFA and SFA/MUFA contents, not occurring at the same time for both species. Thus, PUFA content increased and SFA/MUFA contents decreased in winter at low water temperatures for *B. bifurcata* (Table 1), to allow an increase in membrane fluidity (Los *et al.* 2013). More specifically, an increase in PUFA could facilitate a higher fluidity of thylakoids membranes to enhance photosynthesis in winter (Gombos *et al.* 1994), as they can represent a large part of phospholipids and glycolipids, the main constituents of membrane (Khotimchenko 2003; Sanina *et al.* 2004; Kendel *et al.* 2013; Santos *et al.* 2020). However, the opposite temporal pattern was observed for *S. muticum*, with a reduction in SFA/MUFA in spring, which was associated with PUFA increase mainly due to linolenic acid (C18:3n-3), eicosatetraenoic acid (C20:4n-3), arachidonic acid (C20:4n-6) and eicosapentaenoic acid (C20:5n-3), similarly to the results of Balboa *et al.* (2016). The same trend has already been shown in other native species such as the Sargassaceae *Cystoseira indica* (Fariman *et al.* 2016) or the Fucaceae *Fucus serratus* and *Ascophyllum nodosum* (Schmid *et al.* 2017). One possible explanation for the seasonal differentiation between *S. muticum* and *B. bifurcata* is the potential use of SFA and MUFA for the reproduction in *S. muticum*, since its maximal fertility rate was also observed in spring. In this sense, SFA and MUFA could represent a large part of neutral lipids, *i.e.* carbon storage lipids which can be used for growth, or reproduction (Guschina and Harwood 2013), also suggested as a fuel for algal spores swimming (Reed *et al.* 1999). The observed increase in PUFA in response to high light could also photoprotective mechanism of *S. muticum* receptacles in summer. Some studies previously demonstrated a dependency of fatty acid composition on light (Floreto *et al.* 1993; Khotimchenko 2002), where unsaturation could be involved in repair of damages to photosystem II (Los *et al.*, 2013). This seasonal shift between the two species could be thus an adaptive response of *S. muticum* contributing to its storage capacity and protection against high light during its reproductive period in summer; this is in contrast to *B. bifurcata* where no link could be established between TFA content and summer environmental conditions.

#### 4.6 *S. muticum* and *B. bifurcata*: towards a long-term successful coexistence?

Because of its rapid growth, *S. muticum* thalli become very large in summer (as observed at Porspoder; *pers. obs.*), and can rapidly overgrow other macroalgae, modifying primary production and associated communities (Stæhr *et al.* 2000; Salvaterra *et al.* 2013; Vaz-Pinto

*et al.* 2014). Whilst Sánchez and Fernández (2018) showed that the abundance of *B. bifurcata* increased without *S. muticum* 30 years after its invasion of Spain, in the present study *S. muticum* did not seem to limit the development of the native species or conversely (*pers. obs.*). This suggests that after almost 50 years of *S. muticum* introduction in Brittany (Critchley *et al.* 1983), a balanced interaction between the invasive and native species has been reached in these sampling areas. In this way, Fernández (2020) described the invasion of *S. muticum* in Spain as a ‘boom-bust’ process, with a phase of rapid expansion (‘boom’), followed by a decline period (‘bust’). However, the interaction between the two species could be more complex, depending on environmental variations and adaptive abilities of both species, with interactions depending on location (Sánchez and Fernández 2006). It is therefore important to monitor the evolution of invasive species over a long period, especially since marked seasonal variations of *S. muticum* have already been observed around the world and especially in Europe (see Arenas *et al.* 1995 in Spain; Wernberg *et al.* 2001 and Pedersen *et al.* 2005 in Denmark; Baer and Stengel 2010 in Ireland; and Tanniou *et al.* 2014 along a latitudinal gradient from Portugal to Norway), attesting a high adaptability of this species which could expand to the detriment of native species, particularly in the context of global change. In this sense, Atkinson *et al.* (2020) suggested that marine heatwaves could enhance the performance of *S. muticum*, whereas it have a negative impacts on native seaweeds such as *Fucus serratus*. Then, Méndez-Sandín and Fernández (2016) demonstrated a decrease in the biomass of *B. bifurcata* monitored on the North coast of Spain between 1977 and 2007 which could be due to an increase in temperature over the last years, suggesting that this species could be substituted in the long-term.

#### **4.7 Long-term co-existence of the invasive *Sargassum muticum* with native species in Brittany: temporal trends and evolution of phenology?**

The invasive *Sargassum muticum* has been present on Brittany coastlines for almost 50 years (Critchley *et al.* 1983) and has previously shown fluctuations in population dynamics. The comparison with previous monitoring data at Pointe du diable indicates that its density has increased since 2003 a, and that, on the contrary, its average length decreased from  $47.6 \pm 1.5$  to  $26.3 \pm 17.7$  cm (Table 2). The ecological data seem thus suggest that it has stabilized its density and consolidated its presence in 2006-2008 and 2017-2018, compared to 2003-2004 (Table 2); the number of individuals stabilized at around 84 – 92 individuals / m<sup>2</sup>, while the length has decreased to around 22-26 cm. In addition, and similarly to our results, Le Lann *et*



*al.* (2012) also revealed a greater length of *S. muticum* at Porspoder, with an average of  $58.99 \pm 2.49$  cm between December 2006 and May 2008, compared to  $40.71 \pm 46.63$  cm between February 2017 and February 2018 in this study.

**Table 2.** Comparative ecological data obtained from different monitoring of *Sargassum muticum* collected at Pointe du diable (France) between 2003 and 2019.

Publications	Plouguerné <i>et al.</i> (2006)	Le Lann <i>et al.</i> (2012)	Present study
Sampling period	Feb 2003 – June 2004	Dec 2006 – May 2008	Feb 2018 – Feb 2019
<b>Mean density over the sampling period (ind / m<sup>2</sup>)</b>	25.6 ± 2.0	93.9 ± 1.3	83.3 ± 44.3
<b>Mean length over the sampling period (cm)</b>	47.6 ± 1.5	22.4 ± 0.9	26.3 ± 17.7
Reproductive period	May to September	June to August	May to August
<b>Mean TPC over the sampling period (% DW)</b>	1.39 ± 0.01	0.21 ± 0.01	2.95 ± 1.67
Maximum seawater temperature (°C)	23.5 (June 2003)	16.3 (Summer 2007)	18.2 (July 2017)
Minimum seawater temperature (°C)	8.3 (February 2003)	10.0 (Winter 2008)	8.7 (February 2018)

Concerning *B. bifurcata*, Le Lann *et al.* (2012) showed similar results to our study, with a homogeneous thallus length over the year. The native species exhibited thus an average length of  $25.46 \pm 3.02$  cm at Pointe du diable and  $30.19 \pm 3.25$  cm at Porspoder between December 2006 and May 2008. According to our results, average thallus length was similar 10 years later, with  $27.39 \pm 11.71$  cm and  $27.26 \pm 11.30$  cm at Pointe du diable and Porspoder, respectively. By contrast, density had increased over time, with on average  $3.49 \pm 0.75$  ind/0.25 m<sup>2</sup> in 2007 (Le Lann *et al.* 2012), compared to  $5.42 \pm 3.58$  ind/0.16 m<sup>2</sup> in the present study. Consequently, there was no evidence that the native species had regressed since 2012, and cohabitation with *S. muticum* appears to have stabilized although there are no data available for *B. bifurcata* before 2012, which does not exclude the possibility that the native species may have regressed between 2003 and 2012.

Potential variations in environmental parameters throughout the years also need to be considered as variability in terms of density, length and maturity of individuals of *Sargassum muticum* depend on climatic parameters (Plouguerné *et al.*, 2006; Le Lann *et al.* 2012). More particularly, the heatwave during Spring-Summer 2003 may have favoured the development and thus thallus lengths of *S. muticum* as suggested by Atkinson *et al.* (2020). In addition, TPC was higher in 2018 compared to previous results from Plouguerné *et al.* (2006) and Le

Lann *et al.* (2012). This difference could be due to elevated light levels and temperatures throughout 2018; an increase in seawater temperature has been observed over previous decades in the English Channel (L'Hévéder *et al.* 2016) and Bay of Biscay (Costoya *et al.* 2015). These results thus highlight the importance of integrating a metabolomic approach in our understanding of the ecology and evolution of algal species. In conclusion, this study has emphasizes the importance of long-term ecological monitoring, and more particularly in the context of global warming which could impact the phenology and biomass of *S. muticum*, to the detriment of *B. bifurcata* which seems to be less resistant to an increase in seawater temperature.

## 5. Acknowledgements

Fanny Lalegerie's PhD fellowship was provided by Region Bretagne and the Doctoral School of Marine and Littoral Sciences of the Université Bretagne Loire (UBO, Brest, France) and supervised by both S.C. and V.S.-P. We thank Marie-Aude Poullaouec and Benoit Le Fourn for their help in the phenolic compounds extractions. We thank Leslie Gager, Celine Hubert, Mathieu Helias and all the others colleagues of the Institute for helping at field sampling.

## 6. Author's contributions

F.L. performed the analysis of the samples, drafted the manuscript and conducted the statistical analyses. V.S.-P. and S.C. designed and supervised the project. M.Z. and D.B.S. supervised the extraction and analysis of fatty acids. All authors reviewed and approved the manuscript.

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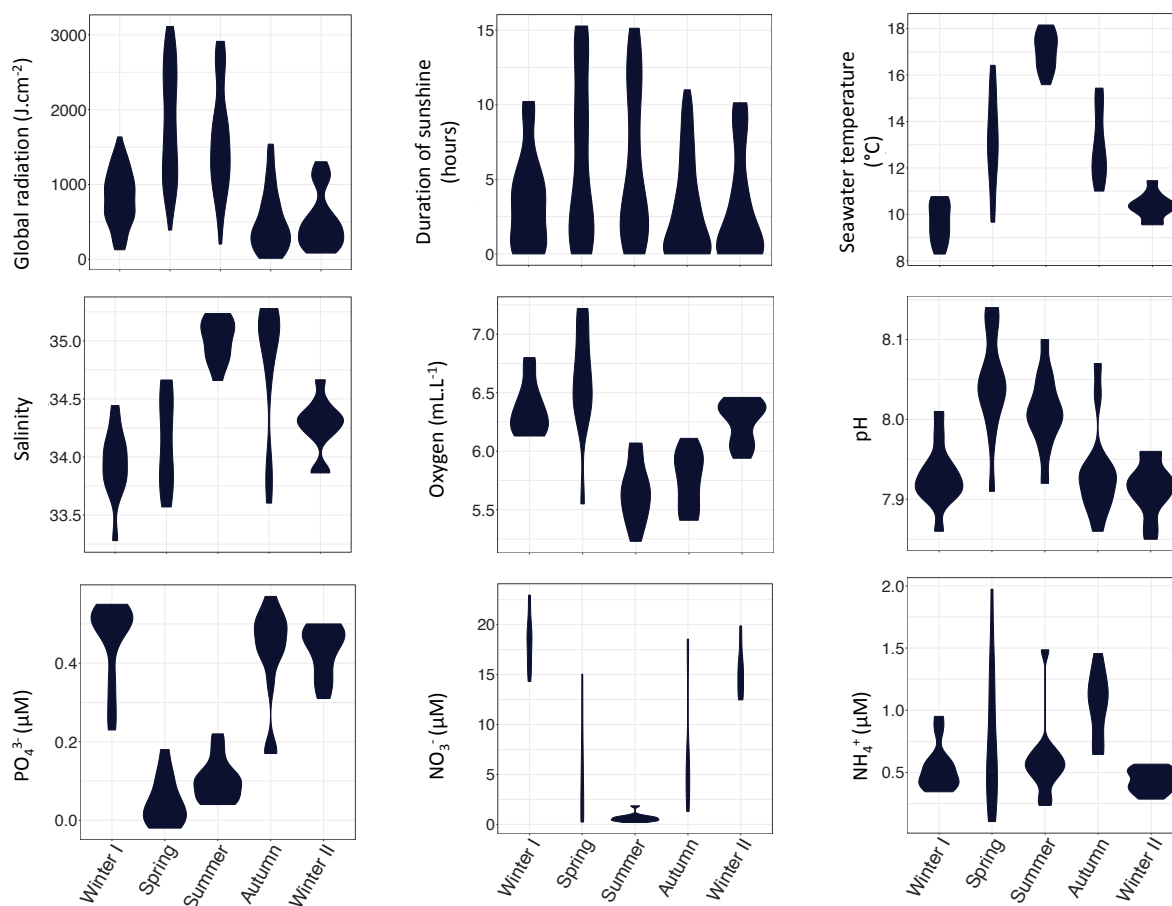
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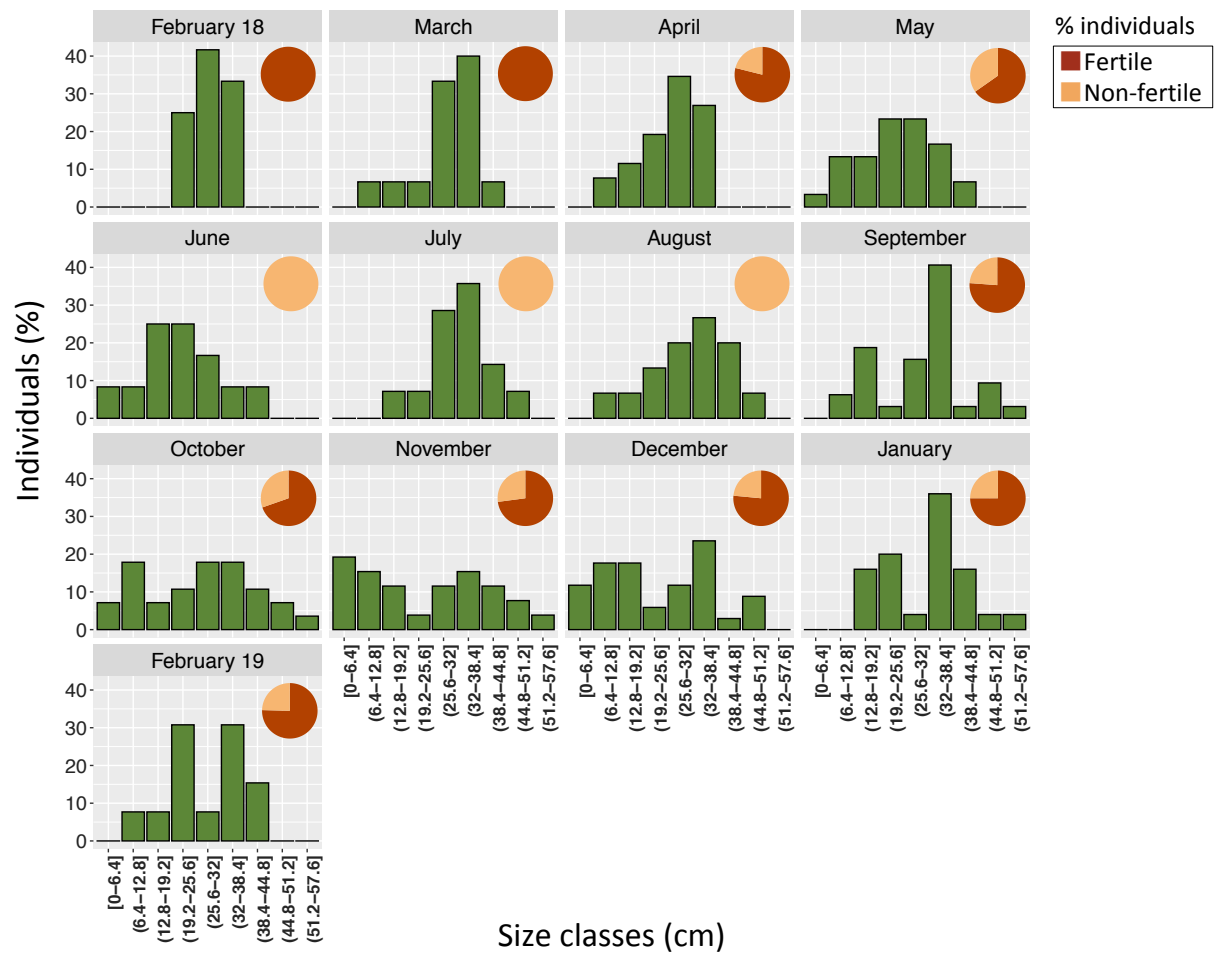
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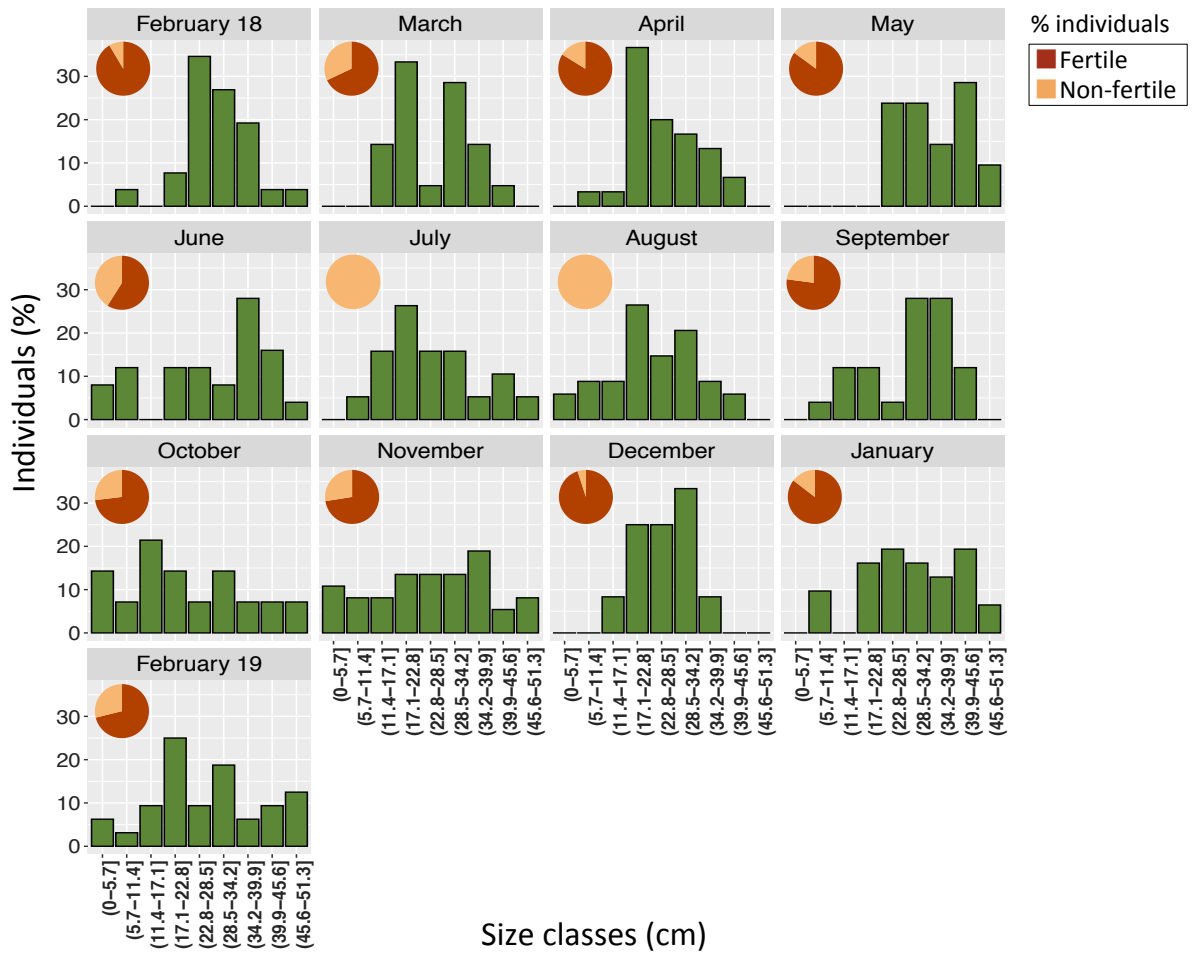
## Supplementary data



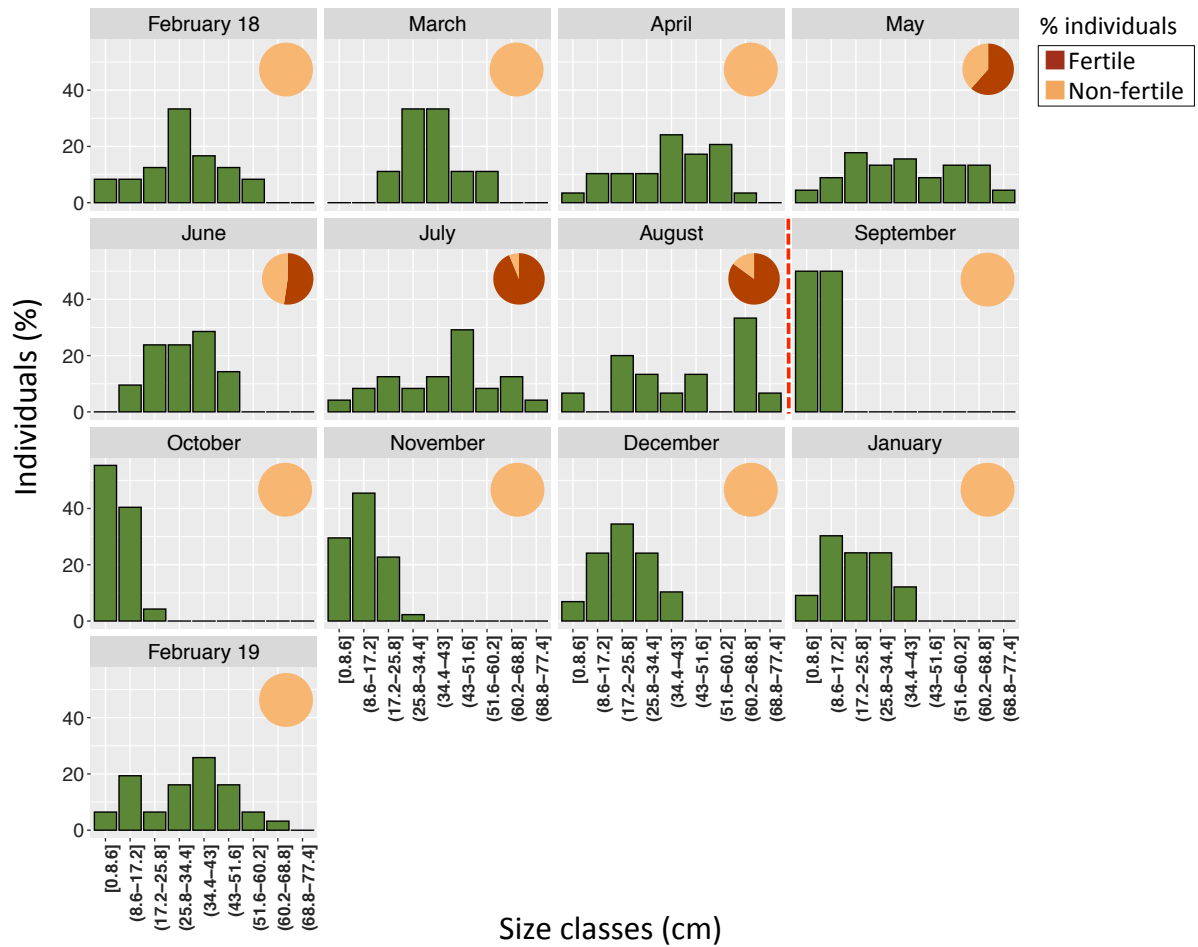
**Supplementary Figure 1.** Seasonal variations of (a) mean daily global radiation ( $\text{J}\cdot\text{cm}^{-2}$ ), (b) mean daily duration of sunshine (hours), (c) seawater temperature ( $^{\circ}\text{C}$ ), (d) salinity, (e) oxygen ( $\text{mL}\cdot\text{L}^{-1}$ ), (f) pH, (g) phosphates ( $\mu\text{M}$ ), (h) nitrates ( $\mu\text{M}$ ), and (i) ammonium ( $\mu\text{M}$ ) levels. Data were provided by «Service d’Observation en Milieu Littoral, INSU-CNRS, Saint-Anne du Portzic», except light data which were provided by «Météo France» (Brest-Guipavas station).



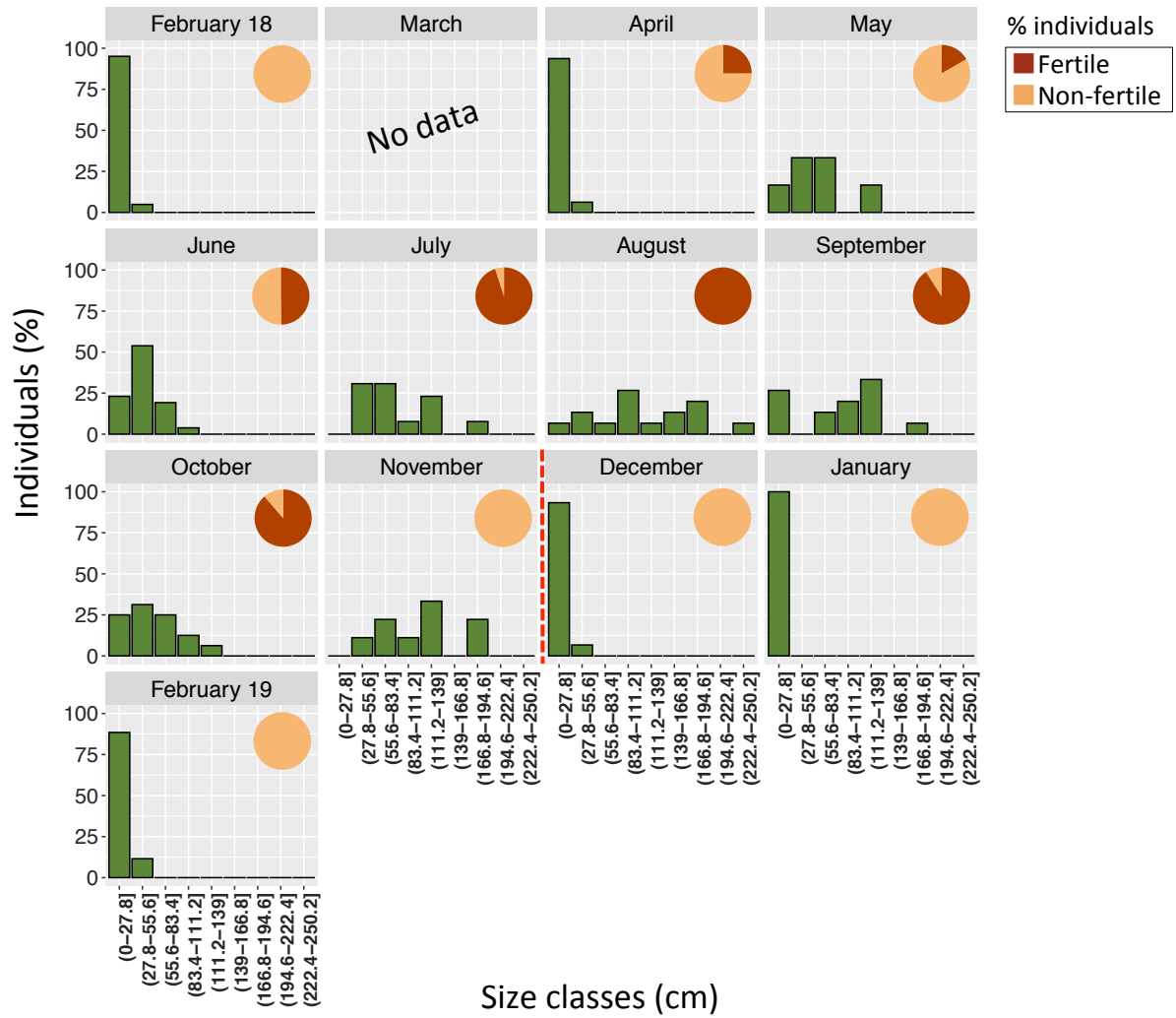
Supplementary Figure 2. Temporal size (cm) classes distribution (%) of the native *Bifurcaria bifurcata* collected monthly between February 2018 and February 2019 at Pointe du diable. Monthly pie charts display the fertility index, *i.e.* the proportion of mature individuals, according to the presence of receptacles on thalli.



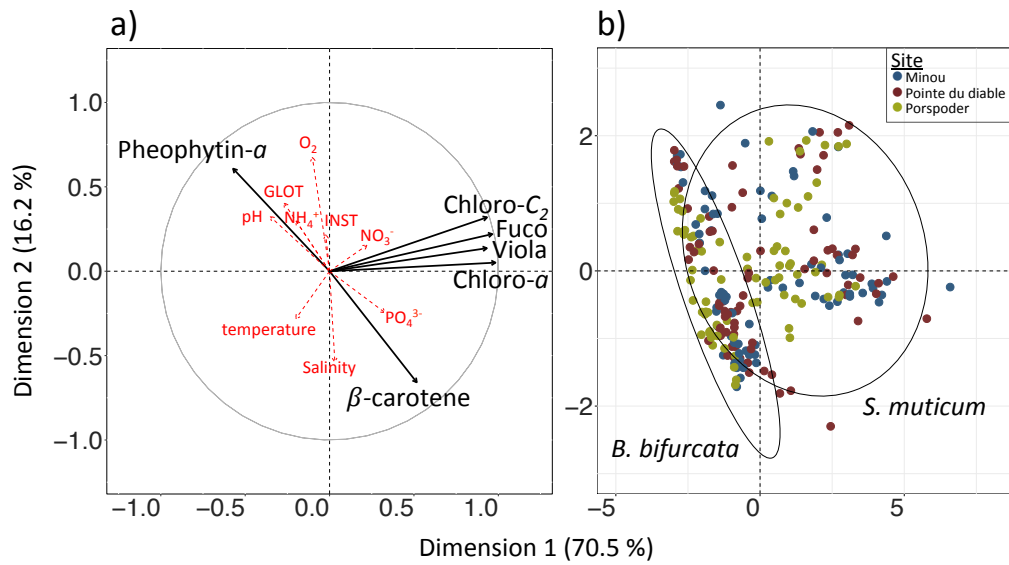
Supplementary Figure 3. Temporal size (cm) class distribution (%) of the native *Bifurcaria bifurcata* collected monthly between February 2018 and February 2019 at Porspoder. Monthly pie charts display the fertility index, *i.e.* the proportion of mature individuals, according to the presence of receptacles on thalli.



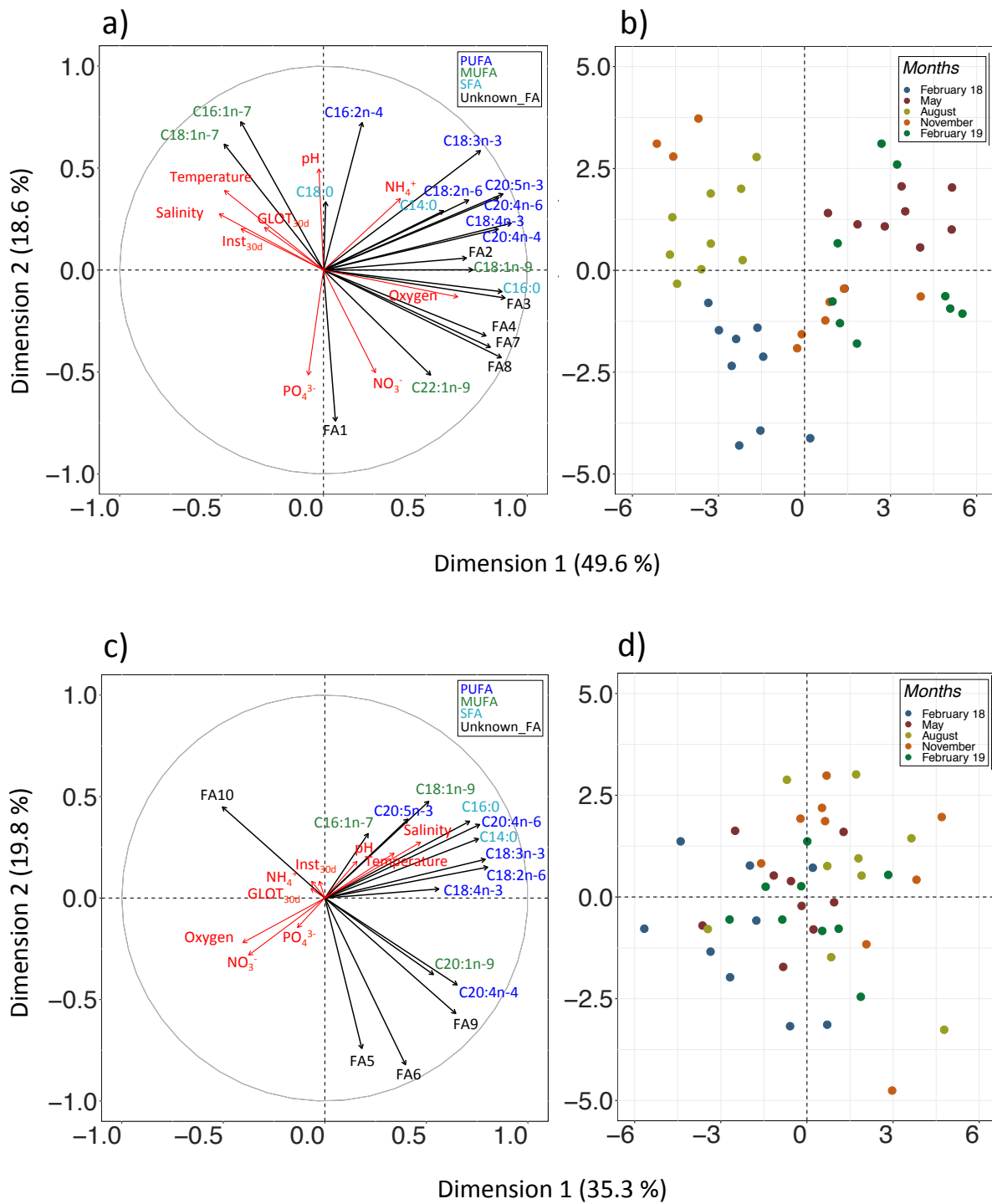
**Supplementary Figure 4.** Temporal size (cm) class distribution (%) of the invasive *Sargassum muticum* collected monthly between February 2018 and February 2019 at Pointe du diable. Monthly pie charts display the fertility index, *i.e.* the proportion of mature individuals, according to the presence of receptacles on thalli. The dashed red line represents a break in the thallus length of *S. muticum*, due to a detachment of the laterals from the main axes, and consecutively to the liberation of zygotes in the environment in previous months, the emergence of a new generation.



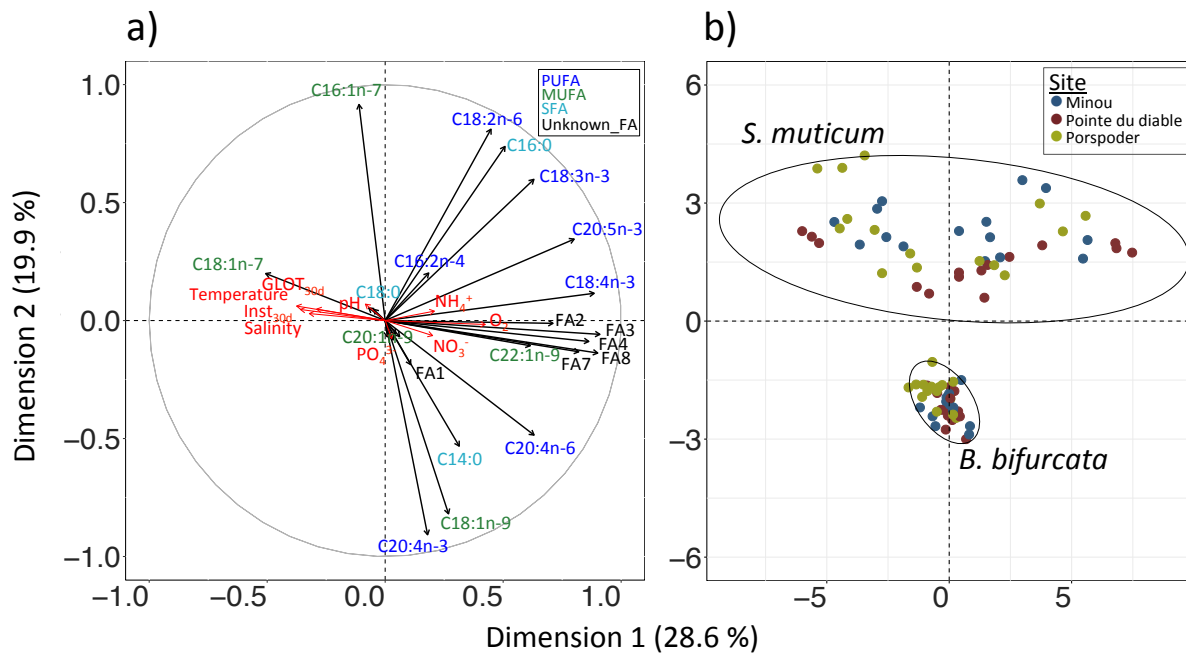
**Supplementary Figure 5.** Temporal size (cm) class distribution (%) of *Sargassum muticum* collected monthly between February 2018 and February 2019 at Porspoder. Monthly pie charts display the fertility index, *i.e.* the proportion of mature individuals, according to the presence of receptacles on thalli. The dashed red line represents a break in the thallus length of *S. muticum*, due to a detachment of the laterals from the main axes, and consecutively to the liberation of zygotes in the environment in previous months, the emergence of a new generation.



Supplementary Figure 6. Principal Component Analyses (PCA) of pigment contents of *Sargassum muticum* and *Bifurcaria bifurcata* samples collected between February 2018 and February 2019 at Porspoder, Pointe du diable and Minou (Brittany, France). (a) Variable factor map and (b) Individual factor depending on the sampling sites. Ellipses were drawn around each species (confidence level of 95 %). Supplementary environmental variables are indicated by red arrows.



**Supplementary Figure 7.** Principal Component Analyses (PCA) of fatty acids contents for samples collected between February 2018 and February 2019 at Porspoder, Pointe du diable and Minou (Brittany, France). (a) Variable factor and (b) individuals' factor map for *Sargassum muticum* depending on the sampling month. (c) Variable factor and (d) individuals' factor map for *Bifurcaria bifurcata* depending on the sampling month. The fatty acids variables are indicated by black arrows (specify by the type PUFA (blue), MUFA (green), SFA (light blue) or unknown\_fatty acid (black)), and supplementary environmental variables by the red arrows.



**Supplementary Figure 8.** Principal Component Analyses (PCA) of fatty acid content of *Sargassum muticum* and *Bifurcaria bifurcata* samples collected between February 2018 and February 2019 at Porspoder, Pointe du diable and Minou (Brittany, France). (a) Variable factor map and (b) Individual factor depending on the sampling sites. Ellipses were drawn around each species (confidence level of 95 %). The fatty acids variables are indicated by black arrows (specify by the type PUFA (blue), MUFA (green), SFA (light blue) or unknown\_fatty acid (black)), and supplementary environmental variables by the red arrows.



## ENGLISH

The first part of **Chapter 3 (subsections 1.1 and 1.2)** provided new insights into the ecology and acclimation capacities of *B. bifurcata*, a brown algal species very abundant in Brittany, and involved in the structuring of communities. In this way, the results suggest that the native populations of *B. bifurcata* are in good ecological status, despite a reduced plasticity compared to the invasive species *S. muticum*, which appeared to be more likely to cope with increasing light or temperature. In this regard, it is not clear how native and invasive algal populations and their interaction will evolve in the context of global change. More particularly, native species more weakened than *B. bifurcata* could see their populations disappear to the detriment of species possessing a great adaptability such as *S. muticum*. In this regard, the **part 2 of Chapter 3** consist of a study of another brown algal species native to Brittany, *Halidrys siliquosa*. Indeed, the latter is a cold-water affinity species with its French southern limit of distribution near Brittany, and that could be particularly threatened in its population sustainability face to both global change and cohabitation with introduced species. More particularly, its intertidal populations appeared to be particularly threatened as *H. siliquosa* lives preferentially in the subtidal zone. In this regard, the next section (**subsection 2.1**) corresponds to a one-year monitoring of intertidal populations of *H. siliquosa* near their southern limit in Brittany. This will provide a first insight of the ecology and metabolomic composition of this species largely understudied, before a geographical comparison between France and Ireland in a second part (**subsection 2.2**).

## FRANÇAIS

La première partie du **Chapitre 3 (sections 1.1 and 1.2)** a permis d'apporter de nouveaux éléments sur l'écologie et les capacités d'acclimatation de *B. bifurcata*, une espèce d'algue brune très abondante en Bretagne, et impliquée dans la structuration des communautés. Ainsi, les résultats suggèrent que les populations natives de *B. bifurcata* sont en bon état écologique, malgré une plasticité réduite par rapport à l'espèce introduite *S. muticum*, qui semble plus à même de faire face à une augmentation de la lumière ou de la température. À cet égard, il est difficile de déterminer comment les populations d'algues natives et introduites et leur interaction vont évoluer dans le contexte du changement global. Plus particulièrement, des espèces natives plus affaiblies que *B. bifurcata* pourraient voir leurs populations disparaître au détriment d'espèces possédant une plus grande adaptabilité comme *S. muticum*. A cet égard, la **partie 2 du Chapitre 3** portait sur l'étude d'une autre espèce d'algue brune originaire de

Bretagne, *Halidrys siliquosa*. En effet, cette dernière est une espèce ayant une affinité d'eau froide qui, en France, a sa limite sud de distribution près de la Bretagne, et qui pourrait être particulièrement menacée face au changement global et à la cohabitation avec des espèces introduites. Plus particulièrement, ses populations intertidales semblent être particulièrement menacées car *H. siliquosa* vit préférentiellement dans la zone subtidale. Dans ce contexte, la section suivante (**section 2.1**) correspond à un suivi *in situ* d'un an des populations intertidales de *H. siliquosa* près de leur limite sud en Bretagne. Cela permettra d'obtenir un premier aperçu de l'écologie et de la composition métabolomique de cette espèce largement sous-étudiée, avant une comparaison géographique entre la France et l'Irlande dans une deuxième partie (**section 2.2**).

## **Ecological and metabolomic monitoring of the brown macroalga *Halidrys siliquosa* (Sargassaceae, Fucales)**

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In preparation, to be submitted to: *Journal of phycology*

### **RESUME**

*Halidrys siliquosa* est une espèce que l'on trouve couramment sur les estrans rocheux le long des côtes Nord-Ouest de l'Europe. Cependant, cette espèce est largement sous-étudiée et son écologie peu documentée. Ainsi, *H. siliquosa* a été suivie sur la zone intertidale pendant un an en Bretagne (France), afin de fournir de nouvelles informations sur sa phénologie et sa reproduction. De plus, une analyse métabolomique a été menée afin d'obtenir un profil temporel intégrant à la fois l'écologie et la composition métabolomique de l'espèce, afin de mieux évaluer ses capacités d'adaptation face aux variations environnementales. Ainsi, les résultats ont mis en évidence une saisonnalité modérée sans variations significatives de longueur, de circonférence ou de densité entre les mois. De plus, plusieurs cohortes d'individus matures ont été enregistrées tout au long de l'année, ce qui suggère une reproduction potentiellement permanente. L'analyse métabolomique a révélé une acclimatation à la lumière et aux températures élevées, avec un niveau important de phéophytine-*a* en mai couplé à une diminution des acides gras polyinsaturés (AGPI), mais aucune variation significative de la teneur en composés phénoliques. Les résultats ont été discutés en comparaison avec des études précédentes réalisées sur des populations nordiques, liées à une régression potentielle des populations intertidales françaises. En effet, *H. siliquosa* est une espèce ayant une affinité pour les eaux froides, et qui se développe préférentiellement en zone subtidale. De ce fait, les populations de la zone intertidale pourraient régresser avec l'augmentation de la température de l'eau de mer, en particulier près de sa limite sud. Dans ce sens, les populations intertidales françaises ont montré une densité limitée couplée à une plus grande teneur en AGPI que les populations du nord, suggérant un stress et une fragilité plus importante. Ainsi le changement global pourrait entraîner une régression future de *H. siliquosa* dans la zone intertidale, proche de sa limite sud de répartition en France.

**Mots-clés:** *Halidrys siliquosa*, Acides gras, Changement global, Suivi, Pigments, Aire de distribution

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## ABSTRACT

*Halidrys siliquosa* is a species commonly found on rocky shores along the north-western coast of Europe. However, the species is largely understudied, and its ecology poorly documented. In this way, *H. siliquosa* has been monitored in the intertidal zone during one year in Brittany (France), in order to provide new insights on its phenology and reproductive behaviour. In addition, a metabolomic approach has been used to obtain an integrative ecological and metabolomic temporal profile and assess the adaptive abilities of the species to environmental variations. In this way, results highlighted a moderate seasonality with no significant variations in length, circumference or density between months. Moreover, several cohorts with mature individuals were recorded throughout the year, suggesting potentially a permanent reproduction. Metabolomic analysis revealed an acclimation to high light and temperature, with an important level of pheophytin-*a* in May coupled to a decrease in polyunsaturated fatty acids (PUFA), but no significant variation in phenolic content. Results were discussed in comparison with previous studies carried out on Northern populations linked with a potential regression of French intertidal populations. Indeed, *H. siliquosa* is a cold-water species preferentially found in subtidal areas; with increasing seawater temperature, considering the intertidal zone, its distribution range could regress, particularly near its southern limit. In this way, French intertidal populations exhibited a limited density coupled to a greater PUFA content than Northern populations, suggesting an important stress and weakness that could lead to a future regression of *H. siliquosa* in the intertidal zone, near to its French southern limit.

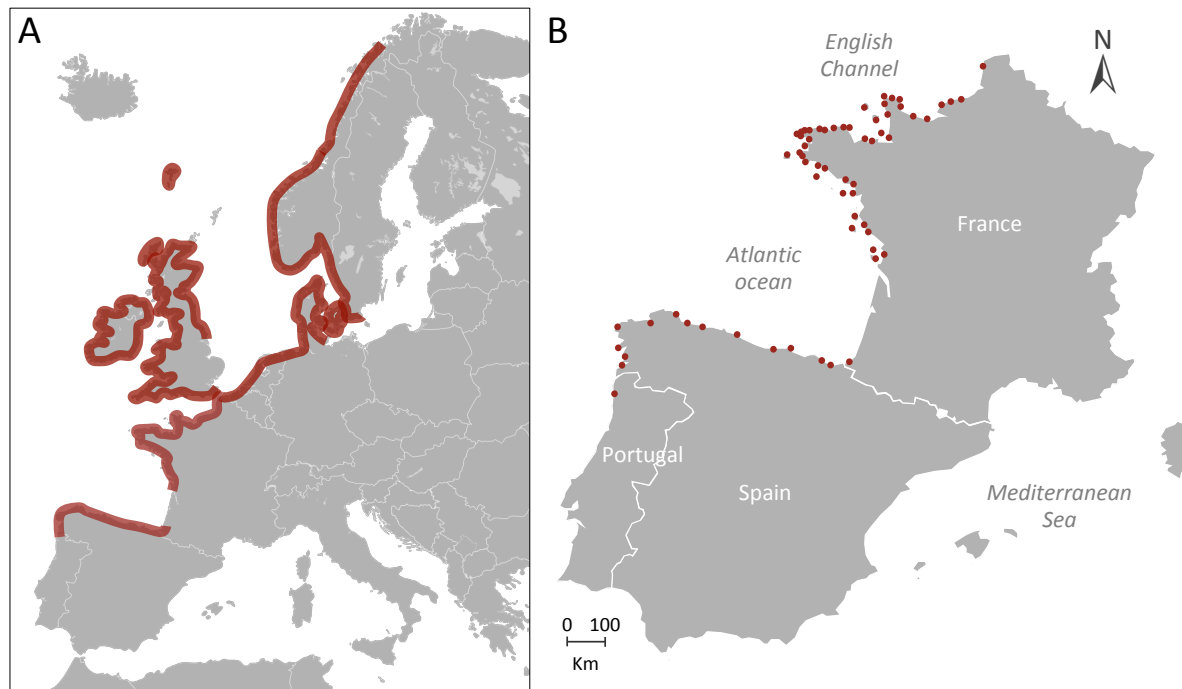
**Keywords:** *Halidrys siliquosa*, Fatty acids, Global warming, Monitoring, Pigments, Distribution areas

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## 1. Introduction

*Halidrys siliquosa* (Linnaeus) Lyngbye 1819 is a perennial brown macroalga belonging to Fucales order and Sargassaceae family. This species is living on rocky shores from Northern Arctic to northern Spain, and commonly in France, Britain, Ireland, Denmark, Netherlands,

Norway, and Sweden (Margalet and Navarro 1990; Lüning 1990; Guiry and Guiry 2020) (Fig. 1). In Brittany, this large macroalga with alternately branched fronds lives in subtidal zone, in sheltered to moderately exposed areas, coexisting with other macroalgal species such as *Laminaria* spp., and Sargassaceae like *Cystoseira* spp. or *Sargassum muticum*. In addition, it is also commonly found in large intertidal rockpools, where it can form large forest with *Himanthalia elongata* or occasional stands, never emerged (Moss and Lacey 1963).



**Figure 1.** (A) Distribution map showing the southern limit of *Halidrys siliquosa* in south-western Europe, with (B) a zoom on France and Spain, with dots corresponding to the main sites where the species was reported, based on data from Guiry and Guiry (2020), Dizerbo and Herpe (2007) and Margalet and Navarro (1990).

Little information has been reported so far on the biology and ecology of *H. siliquosa*, thus largely understudied. Generally, this species is further investigated for its richness in meroditerpenoids and their antifouling activity against several marine bacteria (Higgs and Mulheirn 1981; Hellio *et al.* 2001; Culioli *et al.* 2008; Buseti *et al.* 2015). Moss and Lacey (1963) were the first to study the development of *H. siliquosa* in Great Britain and reported information on its growth and reproductive cycle. In this way, thalli of *H. siliquosa* reach maturity in 1-2 years; and reproductive tissues development and gamete discharge occur preferentially in autumn and winter, respectively. Wernberg *et al.* (2001) together with Pedersen *et al.* (2005) were among the few authors to document the ecological phenology of Danish populations of *H. siliquosa*. They described a moderate seasonality in the populations

from Denmark, with a slight increase in biomass occurring in summer despite a thallus length around 30 cm throughout the year.

In France, the intertidal populations of *H. siliquosa* are restricted to the Bay of Biscay and the English Channel, while they are completely absent on the Southern French coasts along Mediterranean Sea (Fig. 1). In this way, *H. siliquosa* is a northern/boreal species with cold water affinity which has its historical southern limit in northern Portugal, with fragile populations occurring in France. More particularly, most distribution sites in south-western Europe (Fig. 1) are located in southern Brittany (France) but populations are also observed further south towards the Gironde, with some individuals reported in Spain in the Galician province (Lima *et al.* 2009). More recently, *H. siliquosa* was reported attached in tide pools at Praia do Carreço on the Northern Portuguese coast (Lima *et al.* 2009), although this was restricted to few individuals, in contrast to the large forests observed in Ireland or Norway.

In the context of global change, many environmental factors are expected to evolve. More particularly, an increase of few degrees in the seawater temperature as expected by IPCC (2014) could have a major adverse impact on Nordic affinity/boreal species. In this way, *H. siliquosa* is therefore one of the species likely to see its geographical distribution modified / regressed, or to disappear in France. More specifically, *H. siliquosa* is a species living preferentially in the subtidal zone, and populations in the intertidal zone could thus even more likely disappear as temperature and light conditions are less favourable at this shore level. An increase in temperature may in particular have adverse effects on winter gamete release of *H. siliquosa*. According to Lüning (1990), *H. siliquosa* has a temperature maximum for survival not exceeding 25 °C. Moss and Shearer (1973) showed that *H. siliquosa* reproduces preferentially between 3 and 10 °C and that its germination success is considerably reduced above 20 °C. In addition, co-habitation with the increasing number of non-native species with global change could further weaken *H. siliquosa*. In this way, Stæhr *et al.* (2000) already reported that the expansion of *Sargassum muticum* in Limfjorden (Denmark) between 1984 and 1997 resulted in more than a 50 % decrease in *H. siliquosa* cover.

This context emphasizes the importance to study the ecology and monitor this species, especially in France where no data are available and where *H. siliquosa* populations are mostly restricted to few individuals. This could provide new information on its phenology at this Southern location, and see if populations have different patterns than in Northern locations. Beyond the ecology aspect, there are very few studies on the metabolomic

composition of *H. siliquosa*, except some reports on its composition in terpenes (Culioli *et al.* 2008), fatty acids (Fleurence *et al.* 1994; Biancarosa *et al.* 2018), or phlorotannins (Zubia *et al.* 2009; Stiger-Pouvreau *et al.* 2014; Le Lann *et al.* 2016; Gager *et al.* 2020). Moreover, almost no study reported on the temporal variations of its metabolome. Coupling the spatio-temporal study of both ecological and metabolomic data will provide an overview of its adaptive abilities, essential to the understanding of its ecology.

In this way, an ecological monitoring was carried out on *H. siliquosa* for almost one year on one site in Brittany, providing data on its length, size class distribution, circumference, and maturity status over the sampling period. In addition, samples were collected monthly to study its metabolomic composition in pigments, fatty acids, and phenolic compounds. We then had few hypothesis: do intertidal populations of *H. siliquosa* exhibit a seasonality in their ecology? Do they have an ecological pattern similar to northern populations? And, is it possible to relate its phenology to metabolite composition which could demonstrate an ecological adaptation of *H. siliquosa* to environmental variations? More particularly, we hypothesized that this species exhibits high variation in fatty acid and pigment contents to support high variation in seawater temperature and light occurring in intertidal rockpools.

## 2. Material and methods

### 2.1 Sampling site, environmental and ecological data collection

*Halidrys siliquosa* was monitored at Porspoder (48°28'56.644" N – 4°46'6.812" W) where the species lives in a large rockpool. Environmental data (temperature, pH, salinity and nutrient contents) were recorded at Saint-Anne du Portzic (Brittany, France) (48°21'32.13" N – 4°33'07.19" W) and were provided by « Service d'Observation en Milieu Littoral, INSU-CNRS ». In addition, daily sunshine duration (hours) (INST) and irradiance (GLOT) ( $\text{J}\cdot\text{cm}^{-2}$ ) data were provided by Météo France from Brest-Guipavas (48°27'00" N – 4°22'59" W) (see Fig. 2 in Lalegerie *et al.* (2020) for details on environmental variations).

### 2.2 Ecological monitoring

Ecological measurements were carried out monthly between April 2018 and February 2019, on all individuals within four replicate quadrats of  $0.16 \text{ m}^2$ , randomly positioned. Ecological variables measured were: density, maximum length (*i.e.* from the base to the apical part of the longest branch), maximum circumference, maturity (*i.e.* presence or absence of receptacles),

and presence of epiphytes. Maturity rate was estimated as the proportion of individuals bearing receptacles.

### 2.3 Metabolomic monitoring

The metabolomic monitoring of *H. siliquosa* started earlier (in February 2018) than the ecological one and ended in February 2019. On each sampling occasion, algal samples were collected for metabolomic analyses, in areas adjacent to the quadrats. After collection, all algal samples were cleaned to remove epiphytes, and frozen immediately before being freeze-dried ( $\beta$ 1-8 LD plus Christ) and ground into a fine powder (MM400 Retsch); samples for metabolomic analyses were processed dried and within two months of collection.

### 2.4 Chlorophyll and carotenoids extraction and analysis

Pigments were extracted using 75 mg of dry seaweed powder with 750  $\mu$ L 90 % aqueous acetone according to the protocol described in Schmid and Stengel (2015) and Lalegerie *et al.* (2019). Two successive extractions were carried out and High Pressure Liquid Chromatography (HPLC) analysis was performed directly after extraction. Pigments were separated according to Lalegerie *et al.* (2019) on an HPLC Dionex Ultimate 3000 system equipped with an ACE C<sub>18</sub> column (150  $\times$  4.6 mm, 3  $\mu$ m) and a diode array detector (ThermoScientific). Identification and quantification of pigments were then obtained by comparison with commercial standards of chlorophyll-*a* (Sigma, USA), and chlorophyll-*c*<sub>2</sub>, pheophytin-*a*,  $\beta$ -carotene, fucoxanthin, and violaxanthin (DHI, Denmark).

### 2.5 Fatty acids extraction and analysis

For fatty acids analysis, only the samples collected in February 2018, May, August, November and February 2019 were processed. Fatty acids were extracted by direct transmethylation according to a protocol adapted from Schmid *et al.* (2014), and described in Lalegerie *et al.* (in prep). Fatty acid analysis was performed on a Varian CP-8400 system equipped with a flame-ionization detector and two different columns, one polar (Zebron ZB-Wax, Agilent, 0.25 mm x 30 m x 0.2  $\mu$ m) and one apolar (ZB-5HT, Agilent, 0.25 mm x 30 m x 0.2  $\mu$ m). Fatty acids were then identified by comparison with a commercial standard mixture (Supelco 37-components FAME Mix, Sigma-Aldrich) and each fatty acid was quantified by comparing peak areas with tricosanoic acid (C23:0) preliminarily added as



internal standard. Fatty acids results are expressed in percentage of the total fatty acids (TFA) content. Data were collected using Galaxie 1.9.3.2 software (Agilent).

## 2.6 Phenolic compounds extraction and quantification

Phenolic compounds were extracted from 15 mg DW of algal powder in 1 mL of 70 % ethanol, according to the protocol already described in Lalegerie *et al.* (in prep). Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric assay modified from Zubia *et al.* (2009) and described in Lalegerie *et al.* (in prep). TPC was determined using a standard curve of phloroglucinol (1,3,5-trihydroxybenzene) (Sigma, USA).

## 2.7 Statistical analyses

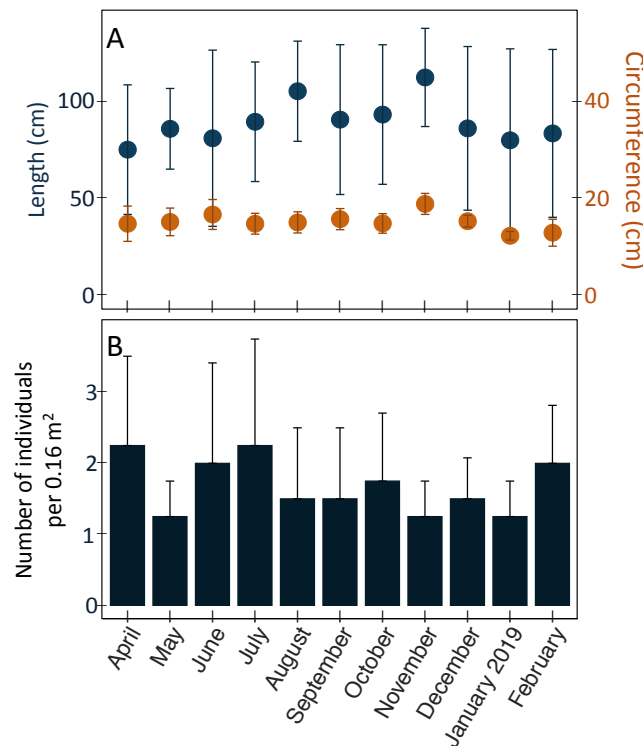
*Halidrys siliquosa* was harvested in independent triplicates each month, with one replicate corresponding to a dozen fragments of 1-4 thalli. The number of size classes was obtained according to Sturges rule with the number of classes =  $1 + 3.3 \log_{10}(n)$ , where  $n$  is the number of individuals. Monthly metabolic data were averaged per season as follows: February 2018 to March (Winter 2018), April to June (Spring), July to September (Summer), October to December (Autumn) and January to February 2019 (Winter 2019); this pooling corresponded to meteorological data (see Lalegerie *et al.* (2020) for more details). Only the fatty acids data were not averaged by season since only 5 months were analysed. Statistical analyses were performed using R software (version 3.6.1) (R Core Team 2019) through the integrated development environment Rstudio (version 1.2.5001) (Rstudio Team 2019), and results are expressed as mean  $\pm$  standard deviation. One-way ANOVAs were carried out in order to compare samples of the different months within a single variable, with a confidence level of 95 %. Non-parametric Kruskal-Wallis test (KW; more than two groups, one factor) was used when at least one of the conditions of application was not respected, *i.e.* when the hypothesis of normal distribution (Shapiro test) and/or the homogeneity of variance (F-test) were not met. Then, Tukey post-hoc tests after an ANOVA (TukeyHSD), or a non-parametric post-hoc test after a Kruskal-Wallis test (kruskalmc, pigrmess package) were carried out in order to figure out which group differ in the sample. Principal Component Analyses (PCA) were carried out with the packages FactoMineR and explor, in order to highlight temporal variations in pigment and fatty acid compositions. Finally, Pearson's correlations between ecological, metabolomic and environmental variables were carried out (or Kendall's correlation if the normality was not met) to highlight potential relationships between the

different data, linked to a specific acclimation response.

### 3. Results

#### 3.1 *Halidrys siliquosa* ecology

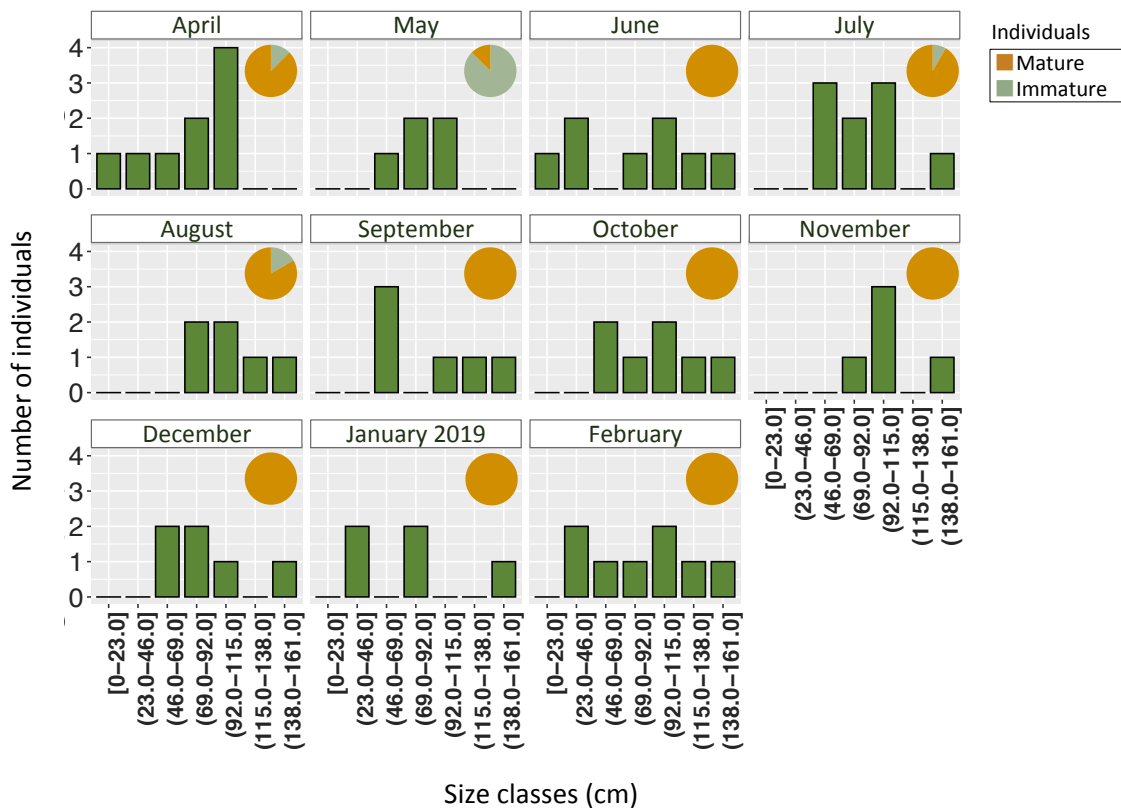
*Halidrys siliquosa* exhibited no significant temporal variation in its thallus length (One-Way ANOVA,  $p$ -value = 0.847), ranging from  $74.89 \pm 33.31$  cm in April to  $112.00 \pm 25.23$  cm in November. Similarly, the circumference did not change along the sampling period (One-Way ANOVA,  $p$ -value = 0.935), ranging from  $14.72 \pm 9.07$  cm in April to  $18.80 \pm 5.40$  cm in November (Fig. 2A). Both measurements were positively correlated (Pearson's correlation,  $r = 0.519$ ,  $p$ -value < 0.0001), with in average  $88.01 \pm 35.42$  cm in length and  $15.05 \pm 6.04$  cm in circumference over the year.



**Figure 2.** Temporal averaged thallus length and circumference in cm (A) and number of individuals per 0.16 m<sup>2</sup> (B) of *Halidrys siliquosa* recorded monthly between April 2018 and February 2019 at Porspoder (Brittany, France).

The density also did not vary (KW test,  $p$ -value = 0.792), never exceeding more than four individuals per quadrat. Nevertheless, a slight increase in density was observed in April, June and February 2019 (Fig. 2B). Size class analysis showed that these months corresponded to periods of recruitment with individuals smaller than 27 cm (Fig. 3). *Halidrys siliquosa* exhibited also a high maturity rate throughout the year, with in average  $79.55 \pm 38.02$  % of

mature individuals. Only May showed a low rate of maturity, with  $12.50 \pm 25.00$  % of mature individuals (KW test,  $p$ -value = 0.0001).



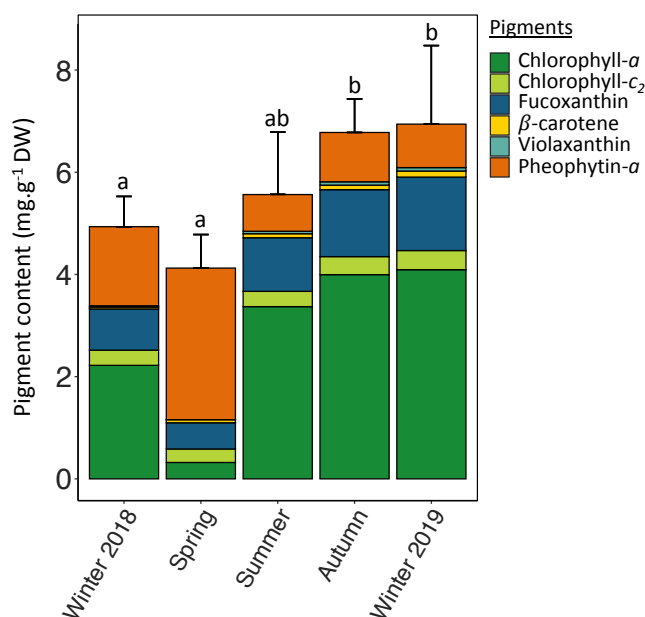
**Figure 3.** Temporal size (cm) class distribution of *Halidrys siliquosa* collected monthly between April 2018 and February 2019 at Porspoder (Brittany, France). Monthly pie charts display the maturity rate, *i.e.* the proportion of mature individuals, according to the presence of receptacles on thalli.

## 3.2 *Halidrys siliquosa* composition

### 3.2.1 Pigment composition

*Halidrys siliquosa* contained 6 pigments, *i.e.* chlorophyll-*a*, chlorophyll-*c*<sub>2</sub>, fucoxanthin,  $\beta$ -carotene, violaxanthin and pheophytin-*a*, representing in average  $3.19 \pm 1.62$ ,  $0.32 \pm 0.06$ ,  $1.09 \pm 0.37$ ,  $0.08 \pm 0.04$ ,  $0.05 \pm 0.04$ , and  $1.16 \pm 0.95$  mg.g<sup>-1</sup> DW respectively (Fig. 4). The data revealed a temporal variation in pigment composition (Fig. 4 and Supplementary Fig. 1): chlorophyll-*a* exhibited a minimum content in Spring, *i.e.* in May with  $0.32 \pm 0.53$  mg.g<sup>-1</sup> DW, compared to a maximum content in Winter 2018, *i.e.* in January with  $5.36 \pm 1.14$  mg.g<sup>-1</sup> DW (One-way ANOVA,  $p$ -value = < 0.001). Violaxanthin and fucoxanthin followed a similar temporal pattern to chlorophyll-*a* (KW test,  $p$ -value = 0.001 for violaxanthin; One-way ANOVA,  $p$ -value < 0.001 for fucoxanthin), with no violaxanthin detected in May, and a

minimum content of fucoxanthin in February 2018 and May, both exhibiting in average 0.51 mg.g<sup>-1</sup> DW.



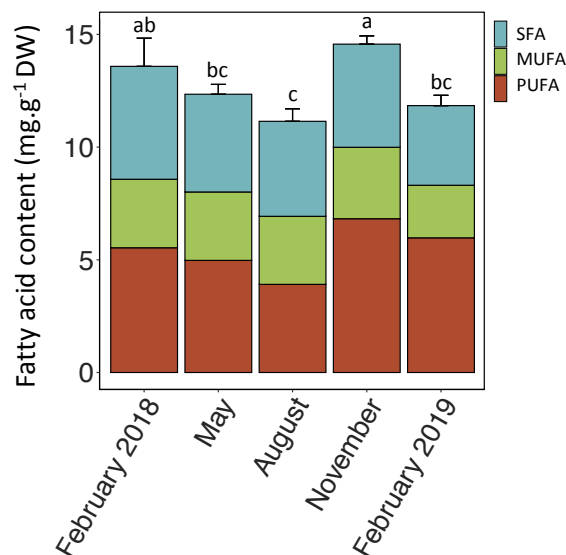
**Figure 4.** Seasonal pigment content (chlorophyll-*a*, chlorophyll-*c*<sub>2</sub>, fucoxanthin, β-carotene, violaxanthin and pheophytin-*a*) of *Halidrys siliquosa* collected monthly from February 2018 to February 2019 at Porspoder (Brittany, France). Results are displayed as the stacked cumulative contents (with standard deviation of the cumulative contents). Letters indicate the significant differences in total pigment content between sampling seasons (ANOVA, *p*-value < 0.05).

This temporal pattern was quite similar for β-carotene and chlorophyll-*c*<sub>2</sub>, although less pronounced (KW test, *p*-value = 0.002 for β-carotene; One-way ANOVA, *p*-value = 0.007 for chlorophyll-*c*<sub>2</sub>). On the contrary, pheophytin-*a* exhibited a maximum content in May with  $2.97 \pm 0.10$  mg.g<sup>-1</sup> DW (One-way ANOVA, *p*-value = < 0.001), representing  $73.28 \pm 12.95$  % of the total pigment content at this period. Moreover, the contents of pheophytin-*a* and chlorophyll-*a* were negatively correlated (Kendall's correlation,  $\tau = -0.67$ , *p*-value < 0.001). Finally, according to the PCA analysis (Supplementary Fig. 1), the temporal variation of chlorophyll-*a* appeared to be negatively affected by light intensity and ammonium level, while the opposite can be observed for pheophytin-*a*.

### 3.2.2 Fatty acid composition

The main fatty acids in *Halidrys siliquosa* were palmitic acid (C16:0), oleic acid (C18:1n-9), arachidonic acid (C20:4n-6), and myristic acid (C14:0), representing in average  $23.10 \pm 2.87$ ,  $18.98 \pm 2.49$ ,  $13.94 \pm 2.41$  and  $7.21 \pm 0.83$  % of the total fatty acid (TFA) content (see detailed composition in Supplementary Table 1). TFA content varied over time (One-way

ANOVA,  $p$ -value < 0.001), decreasing slightly from February to August, reaching then a minimum content of  $11.31 \pm 0.54$  mg.g<sup>-1</sup> DW. Then, TFA increased in November, reaching then its maximum content of  $14.74 \pm 0.37$  mg.g<sup>-1</sup> DW (Fig. 5).

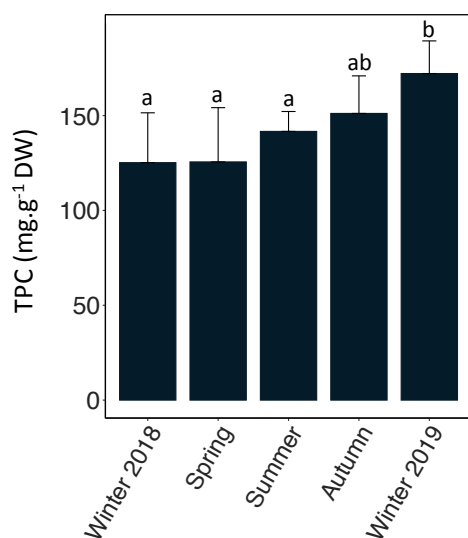


**Figure 5.** Temporal fatty acid content (mg.g<sup>-1</sup> DW) of *Halidrys siliquosa* collected from February 2018 to February 2019 at Porspoder (Brittany, France). Results are displayed as the stacked cumulative contents of the different type of fatty acids (with standard deviation of the cumulative contents), *i.e.* SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), and PUFA (polyunsaturated fatty acids). Letters indicate the significant differences in total fatty acid (TFA) between sampling months (ANOVA,  $p$ -value < 0.05).

Polyunsaturated fatty acids (PUFA) followed a similar pattern (One-way ANOVA,  $p$ -value = 0.004), representing in average  $41.98 \pm 6.18$  % of the TFA content. Thus, it reached a minimal content in August with  $3.91 \pm 0.61$  mg.g<sup>-1</sup> DW, compared to a maximum in November with  $6.82 \pm 0.36$  mg.g<sup>-1</sup> DW (Fig. 5). To a lesser extent, saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) also exhibited a monthly variation (One-way ANOVA,  $p$ -value < 0.001 for both variables), different from PUFA: they reached a minimum content in February 2019, representing  $3.53 \pm 0.15$  and  $2.33 \pm 0.07$  mg.g<sup>-1</sup> DW for SFA and MUFA, respectively. Then, SFA content was maximum in February 2018 with  $5.01 \pm 0.17$  mg.g<sup>-1</sup> DW, while MUFA content was maximum in November with  $3.17 \pm 0.09$  mg.g<sup>-1</sup> DW. According to PCA analysis (Supplementary Fig. 2), these temporal variations are related to overall fatty acids, and not to some particular one. PUFA appeared to be negatively related to temperature and global radiation, whereas SFA and MUFA seemed to be related to a combination of factors, including nutrients or salinity.

### 3.2.3 Phenolic content

*Halidrys siliquosa* exhibited a slight temporal variation of total phenolic content (TPC) (One-way ANOVA,  $p$ -value = 0.005), with a maximum content recorded in Winter 2019 with in average  $172.17 \pm 17.21$  mg.g<sup>-1</sup> DW (Fig. 6). Then, TPC was minimum in Winter 2018, reaching  $125.19 \pm 16.28$  mg.g<sup>-1</sup> DW. These temporal variation appeared to be not related to any particular environmental factors among those recorded. More particularly, TPC was not clearly correlated to global irradiance (GLOT) (Kendall's correlation,  $\tau = -0.28$ ,  $p$ -value = 0.03) or daily light duration (INST) (Kendall's correlation,  $\tau = -0.25$ ,  $p$ -value = 0.04).



**Figure 6.** Seasonal total phenolic content (TPC; mg.g<sup>-1</sup> DW) in *Halidrys siliquosa* collected monthly between February 2018 and February 2019 at Porspoder (Brittany, France). Letters indicate the significant differences in TPC between sampling seasons (ANOVA,  $p$ -value < 0.05).

## 4. Discussion

Commonly found on rocky shores of north-west Europe, *Halidrys siliquosa* is a brown macroalga largely under-investigated. In this way, the present study represents an important contribution to the understanding of its ecological phenology by a one-year *in situ* monitoring. *Halidrys siliquosa* is a northern/boreal species with cold water affinity whose distribution area could regress with increasing temperature, particularly in Brittany (France), close to its southern limit. Results are thus discussed in comparison with previous studies carried out on Northern populations in association with a potential regression of French intertidal populations. More interestingly, ecological data are compared for the first time with the temporal metabolomic composition of *H. siliquosa* in the aim to add information on its adaptive abilities to predicted environmental changes.

#### 4.1 Ecological description of French intertidal populations of *H. siliquosa*

*Halidrys siliquosa* showed no significant temporal variations in length and circumference, exhibiting in average 88 and 15 cm, respectively. Similarly, its density did not show significant variations over the studied period. In this way, *H. siliquosa* seems comparable to other perennial Sargassaceae native from Brittany such as *Bifurcaria bifurcata* or *Treptacantha* (previously *Cystoseira*) *baccata* which also demonstrated a moderate seasonality (Le Lann *et al.* 2012). This low seasonality indicates a slow-growth strategy of this species: *H. siliquosa* has been reported to have a maximal growth rate of 2 cm per month between July and August in populations from Northumberland coast (United Kingdom) (Moss and Lacey 1963), and to have a maximum daily productivity of 0.2 g DW.m<sup>-2</sup> in June in populations from Denmark (Pedersen *et al.* 2005). By comparison, fast-growing species can reach significantly higher rates, as for example the invasive *Sargassum muticum* which showed an elongation of 2 cm per day in North Spain (Arenas *et al.* 1995) and up to 8 cm per day in the Lagoon of Venice (Sfriso and Facca 2013).

Moss and Lacey (1963) and Pedersen *et al.* (2005) demonstrated a higher growth rate between late spring and summer on thalli monitored *in-situ* attached to a boulder, or in translucent PVC cages, respectively. More particularly, in the initial description of the development of *H. siliquosa*, Moss and Lacey (1963) assimilated this rapid growth to the summer development of young meiosporophytes, before their winter reproduction at the end of their first year of development. Then, the results from the present study showed a tendency to increasing length from June to August, similarly to Wernberg *et al.* (2001) who showed a tendency to increasing biomass in summer. However, this temporal variation remained limited and not significant, and therefore did not appear to be a phase of rapid growth similar to that described by Moss and Lacey (1963) on a one-individual study. If such a fast-growth phase exists in the intertidal populations of *H. siliquosa*, it would indicate that the size of large individuals is compensated by the presence of small individuals, resulting in no variation in average length over the year. In this sense, results demonstrated several recruitment periods with individuals less than 27 cm, *i.e.* in April, June and February 2019. Nevertheless, these recruitment periods were relatively limited with no variation in density and never more than 4 individuals per quadrat, suggesting rather a slow growth of perennial individuals of *H. siliquosa*. The slight temporal variation in size could thus correspond rather to a decrease in size in winter, related to winter storms and increasing hydrodynamics which would weaken thalli.

In addition, *H. siliquosa* appeared to be mature throughout the year as receptacles were observed every month, with in average 80 % of mature individuals. Moss and Lacey (1963) showed an increasing number of receptacles from August to January, suggesting a fertility and consequently reproduction preferentially in winter. This result is in accordance with the reduced maturity rate observed in May in the present study. However, our observations suggest that *H. siliquosa* can reproduce at any time of the year, as several cohorts were observed simultaneously, requiring further investigations. The slow-growth strategy of *H. siliquosa* is then probably coupled to a low metabolic demand as described in *B. bifurcata* (Martínez *et al.* 2012), as it needs to allocate energy towards growth and reproduction throughout the year.

#### **4.2 Temporal metabolomic composition of *H. siliquosa*, in relation to its ecology and sensitivity to increasing temperature and light**

The present study constitutes the first investigation of the metabolomic variations in phenolic content, pigments and fatty acids compositions studied in *H. siliquosa* over a one-year monitoring. In this way, similarly to ecological data, results revealed moderate temporal variations in total phenolic content. These secondary metabolites are synthesized by macroalgae in response to a broad variety of biotic or abiotic factors, such as life stage, salinity, nutrient enrichment, light exposure, or herbivorous grazing (Stiger-Pouvreau *et al.* 2014; Lalegerie *et al.* 2019). Thus, the fact that slight seasonal variation was observed may be related to the ecology of *H. siliquosa*. Indeed, as the perennial species exhibited no temporal variations in its average length and density, TPC followed this phenology, in accordance with its slow-growth strategy. In addition, phenolic compounds could be / provide a permanent protection of year-round mature individuals of *H. siliquosa*, against several environmental stresses including high light radiation (Le Lann *et al.* 2016; Gager *et al.* 2020) or oxidative stresses (Jiménez-Escrig *et al.* 2001; Le Lann *et al.* 2016; Gager *et al.* 2020).

Conversely, pigment analyses demonstrated a high seasonality. More particularly, *H. siliquosa* showed a high content of pheophytin-*a*, negatively correlated with chlorophyll-*a*. Pheophytin-*a* is a degradation product of chlorophyll-*a* (Steinman *et al.* 2017), and although the degradation pathway is still poorly understood, this could represent a potential photoprotective and antioxidant defence system in macroalgae. In a lesser extent, it was demonstrated that pheophytin-*a* can be obtained by thermal degradation of chlorophyll-*a* (Samide and Tutunaru 2017), suggesting a potential impact of high temperature on the



photosynthetic apparatus. Pheophytin-*a* increased in May with increasing irradiance and temperature, representing 73 % of the total pigment content. In addition, the high decrease in chlorophyll-*a* in May and then in February 2019 corresponded to the two months with the highest cumulative monthly duration of sunshine (see Fig. 2 in Lalegerie *et al.* 2020 for environmental data), and coincided with the low maturity rate observed in May. This result indicates the high sensitivity of *H. siliquosa* to high light explaining in part why this species is preferentially found in subtidal areas. More particularly, Moss and Sheader (1973) demonstrated that high light intensities inhibited growth of *H. siliquosa* and that the germination of its zygote was inhibited at 22 °C.

Finally, the analysis of fatty acids demonstrated also a temporal variation, different between polyunsaturated (PUFA), and saturated (SFA) / monounsaturated (MUFA) fatty acids. Notably, a decrease in the total fatty acids (TFA) content occurred in May - August, compared to a maximum content in November. This temporal variation was due to the PUFA, representing 42 % of the TFA content. Indeed, PUFA are essential structural components of the cell membrane and are involved in the membrane fluidity which is indirectly dependant to *in situ* temperature fluctuations (Los *et al.* 2013). In this way, high temperatures occurring in summer cause an increase in the membrane fluidity, which ultimately leads to a disintegration of the lipid bilayer (Eggert 2012). More particularly in May, given the intertidal habitat where *H. siliquosa* lives, the seawater temperature exceeded 12 °C (see Fig. 2 in Lalegerie *et al.* (2020) for environmental data), while considering a temperature more extreme in intertidal rockpools.

On the contrary, PUFA content was greater in November due to low temperature, enhancing photosynthesis by modifying the structure of thylakoid membrane (Los *et al.* 2013; Hotimchenko 2002), and by accelerating the recovery of photosynthesis (Gombos *et al.* 1994). More particularly, Fleurence *et al.* (1994) showed that *H. siliquosa* contained 55 % of glycolipids (known to be rich in PUFA), whose role is to maintain the stability of cell membrane. Then, Biancarosa *et al.* (2018) showed a PUFA content of 3 mg.g<sup>-1</sup> in *H. siliquosa* samples harvested in October 2014 along Norwegian coasts, compared to a level twice higher in our samples collected in November. This result is not in accordance with the role of PUFA in membrane fluidity, and thus does not support the finding of Colombo *et al.* (2006) who reported that cold-water species have significantly higher PUFA contents than warm-water species. This difference in latitude could then be explained by the fact that unsaturated fatty

acid chains are mostly susceptible to oxidative damage associated for example with salinity fluctuations or desiccation stress (Miyashita *et al.* 2013; Kumar *et al.* 2014). Fatty acids data suggests thus a high sensitivity of *H. siliquosa* to high temperature and indicates a higher stress in French intertidal populations than in Northern populations.

### 4.3 Comparison with Northern populations: towards a regression of the distribution area of *H. siliquosa*?

*Halidrys siliquosa* exhibited a moderate seasonality with a length, circumference and density not significantly different between months. In this way, French intertidal populations appeared to have a phenology similar to subtidal (*i.e.* 3-m depth) populations from Limfjorden (Denmark) described in Wernberg *et al.* (2001) and Pedersen *et al.* (2005), notwithstanding the difference in latitude and depth. However, despite no apparent difference in phenology, *H. siliquosa* has a very limited density on intertidal areas in France, restricted to few individuals in rockpools (*i.e.* no more than 4 individuals par quadrat). This low density explains why *H. siliquosa* is rarely found on the southern intertidal coasts of Finistère (Brittany, France), limiting this study to a single site. In this way, the populations studied are likely to suffer more from high temperatures than populations from subtidal areas, or from higher latitudes. Then, this hypothesis is in accordance with the metabolomic analysis that revealed a high sensitivity of the perennial species to high light and temperature with an important decrease in fatty acids in May coupled to a high level of pheophytin-*a*. More particularly, data revealed a higher PUFA content than populations from Norway (Biancarosa *et al.* 2018), suggesting a potential greater oxidative stress in the populations studied.

Afterwards, an increase in temperature of a few degrees or in UV radiation as expected by IPCC (2014) could have an irreversible effect on French intertidal populations. More particularly, as meiosporophytes of *H. siliquosa* have a one-year vegetative growth before reaching their sexual maturity at the end of the second year (Moss and Lacey 1963), populations would therefore take at least two years to begin recovery if removed. Then, the population could be greatly weakened, or even be led to their disappearance in the eventuality of seasonal increase of the seawater temperature, due to recurring heat waves. Obviously, intertidal populations of *H. siliquosa* are in any event threatened with disappear, especially as the species has a low potential for dispersal success (Wernberg *et al.* 2001), which limits its distribution range. Then, one hypothesis is that southern intertidal populations have adapted / could adapt through permanent reproduction (and not only in winter as described by Moss and

Lacey 1963), since several cohorts have been observed simultaneously, coupled with mature individuals all year round. Such a strategy could prevent the risk of disappearance due to high temperatures and thus ensure its long-term survival. However, such a hypothesis remains very hypothetical and requires further study of the reproduction of *H. siliquosa*. In addition, cultivation experiments could be carried out to test the tolerance of French populations to temperature variations, and assess its adaptive capacity in the context of climate change.

## 5. Acknowledgements

Fanny Lalegerie's PhD fellowship was provided by Region Bretagne and the Doctoral School of Marine and Littoral Sciences of the Université Bretagne Loire (UBO, Brest, France) and supervised by both S.C. and V.S.-P.

## 6. Author's contributions

F.L. performed the analysis of the samples, drafted the manuscript and conducted the statistical analyses. V.S.-P. and S.C. designed and supervised the project. A.B. and F.L.G. carried out the analysis of fatty acids by GC-MS.

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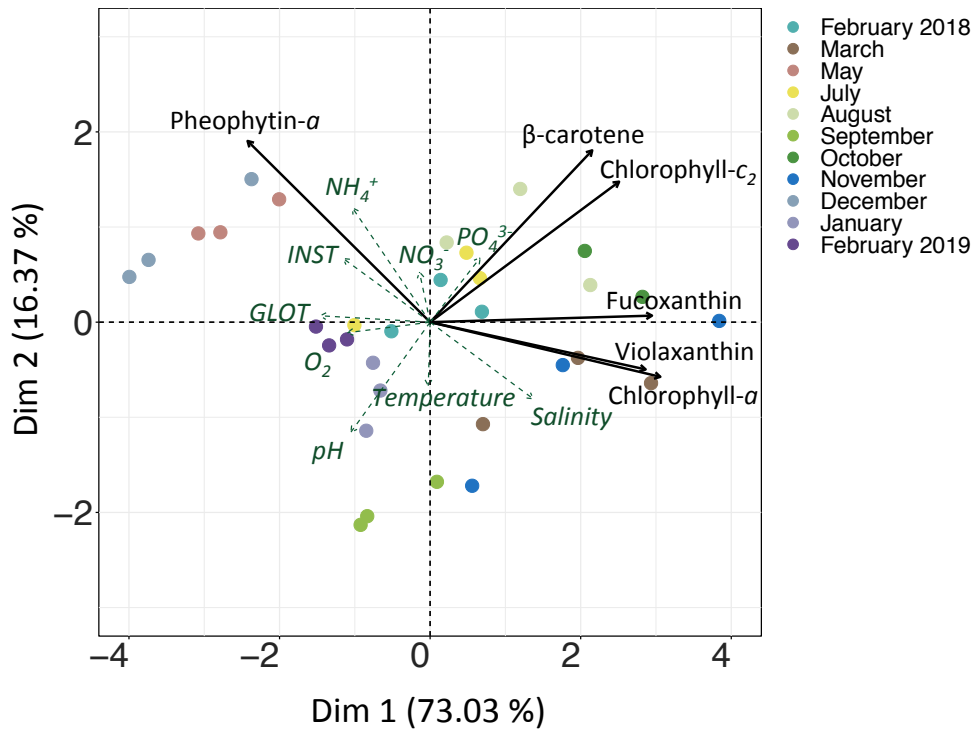
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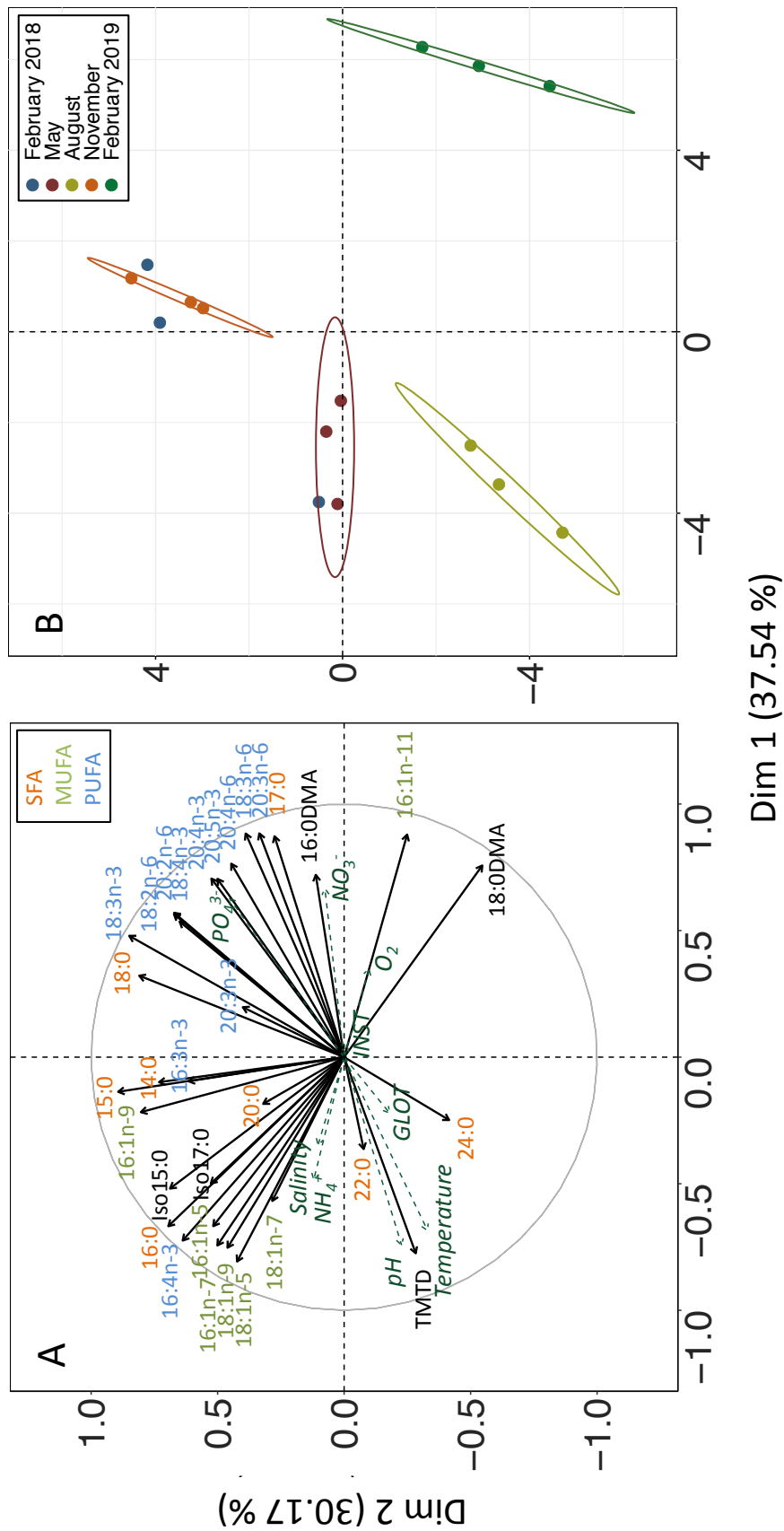
## Supplementary data



Supplementary Figure 1. Principal Component Analysis (PCA) of pigment contents of *Halidrys siliquosa* collected monthly between February 2018 and February 2019 at Porspoder (Brittany, France). Individuals are represented by dots, coloured according to the sampling months. The pigment variables are indicated by black arrows and supplementary environmental variables by the dotted arrows.

**Table 1.** Temporal fatty acid composition (mg.g<sup>-1</sup> DW) in *Halidrys siliquosa* collected between February 2018 and February 2019 at Porspoder (Brittany, France). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, TFA = total fatty acids. The main fatty acids are written in bold. The first fatty acids in black correspond to branched fatty acids and dimethyl acetal.

Fatty acids	February 2018	May	August	November	February 2019
iso15:0	0.05 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
iso17:0	0.05 ± 0.01	0.07 ± 0.01	0.04 ± 0.00	0.06 ± 0.01	0.03 ± 0.00
TMTD	0.04 ± 0.01	0.03 ± 0.00	0.05 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
C16:0DMA	0.04 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
C18:0DMA	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.05 ± 0.00
<b>C14:00</b>	<b>1.18 ± 0.07</b>	<b>0.84 ± 0.01</b>	<b>0.85 ± 0.02</b>	<b>0.96 ± 0.02</b>	<b>0.81 ± 0.06</b>
<b>C15:00</b>	0.06 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.04 ± 0.01
<b>C16:00</b>	<b>3.35 ± 0.08</b>	<b>3.08 ± 0.07</b>	<b>2.97 ± 0.08</b>	<b>3.13 ± 0.10</b>	<b>2.29 ± 0.08</b>
<b>C17:00</b>	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
<b>C18:00</b>	0.19 ± 0.04	0.14 ± 0.01	0.14 ± 0.01	0.20 ± 0.01	0.16 ± 0.00
<b>C20:00</b>	0.09 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
<b>C22:00</b>	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
<b>C24:00</b>	0.05 ± 0.01	0.07 ± 0.00	0.07 ± 0.01	0.06 ± 0.02	0.06 ± 0.01
<b>C16:1n-5</b>	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
<b>C16:1n-7</b>	0.33 ± 0.01	0.43 ± 0.02	0.30 ± 0.02	0.35 ± 0.01	0.17 ± 0.01
<b>C16:1n-9</b>	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
<b>C16:1n-11</b>	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.12 ± 0.00
<b>C18:1n-5</b>	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
<b>C18:1n-7</b>	0.04 ± 0.01	0.07 ± 0.02	0.05 ± 0.00	0.06 ± 0.00	0.04 ± 0.00
<b>C18:1n-9</b>	<b>2.54 ± 0.13</b>	<b>2.42 ± 0.03</b>	<b>2.57 ± 0.05</b>	<b>2.64 ± 0.07</b>	<b>1.97 ± 0.08</b>
<b>C16:3n-3</b>	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
<b>C16:4n-3</b>	0.08 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.01 ± 0.00
<b>C18:2n-6</b>	<b>1.10 ± 0.23</b>	<b>0.59 ± 0.03</b>	<b>0.52 ± 0.05</b>	<b>0.87 ± 0.04</b>	<b>0.85 ± 0.10</b>
<b>C18:3n-3</b>	<b>1.05 ± 0.16</b>	<b>0.97 ± 0.07</b>	<b>0.76 ± 0.07</b>	<b>1.16 ± 0.05</b>	<b>0.98 ± 0.08</b>
<b>C18:3n-6</b>	0.08 ± 0.03	0.04 ± 0.00	0.03 ± 0.01	0.09 ± 0.00	0.10 ± 0.01
<b>C18:4n-3</b>	0.80 ± 0.20	0.83 ± 0.12	0.57 ± 0.13	1.19 ± 0.10	0.91 ± 0.07
<b>C20:2n-6</b>	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
<b>C20:3n-3</b>	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
<b>C20:3n-6</b>	0.08 ± 0.02	0.06 ± 0.00	0.04 ± 0.01	0.07 ± 0.01	0.09 ± 0.00
<b>C20:4n-3</b>	0.10 ± 0.02	0.09 ± 0.01	0.08 ± 0.02	0.15 ± 0.01	0.12 ± 0.01
<b>C20:4n-6</b>	<b>1.64 ± 0.33</b>	<b>1.65 ± 0.20</b>	<b>1.37 ± 0.26</b>	<b>2.27 ± 0.14</b>	<b>2.08 ± 0.02</b>
<b>C20:5n-3</b>	0.54 ± 0.18	0.59 ± 0.10	0.41 ± 0.12	0.87 ± 0.05	0.74 ± 0.09
<b>SFA</b>	5.01 ± 0.17	4.34 ± 0.08	4.22 ± 0.08	4.58 ± 0.09	3.53 ± 0.15
<b>MUFA</b>	3.04 ± 0.12	3.03 ± 0.06	3.02 ± 0.03	3.17 ± 0.09	2.33 ± 0.07
<b>PUFA</b>	5.54 ± 1.16	4.97 ± 0.52	3.91 ± 0.61	6.82 ± 0.36	5.98 ± 0.34
<b>TFA</b>	<b>13.77 ± 1.24</b>	<b>12.54 ± 0.43</b>	<b>11.31 ± 0.54</b>	<b>14.74 ± 0.37</b>	<b>12.02 ± 0.47</b>



Supplementary Figure 2. Principal Component Analysis (PCA) of fatty acids contents of *Halidrys siliquosa* collected between February 2018 and February 2019 at Porspoder (Brittany, France). (a) Variable factor and (b) individuals factor map depending on the sampling month. The fatty acids variables are indicated by black arrows (specified by the FA type: SFA (light green), MUFA (purple), PUFA (blue), and supplementary environmental variables by the dotted arrows. Ellipses were drawn around sampling months (confidence level of 95 %).



**ENGLISH**

The one-year monitoring of *Halidrys siliquosa* revealed a high sensitivity of the native species to high light and temperature, among intertidal populations of Brittany. In addition, *H. siliquosa* exhibited a very limited density in France, never exceeding more than four individuals per 0.16 m<sup>2</sup>. In this way, the French population of *H. siliquosa* appeared to be weakened in the intertidal zone, suggesting an important stress which could be due to a too high temperature in this habitat, perhaps close to the tolerance limit of this Nordic species. In this regard, the next section of the **Chapter 3** is a geographical comparison of the acclimation abilities of *H. siliquosa* between France and Ireland, where the native species may be less weakened and present different acclimation abilities. In addition, results are compared with the invasive *Sargassum muticum*, which is well-established in France, and at an earlier stage of invasion in Ireland. Indeed, the native species could be all the more threatened as it has to cope both with global change and its co-habitation with introduced species. Thus, is *Halidrys siliquosa* likely to disappear near its southern limit due to increased temperature? And are introduced species likely to impact further on its survival, *i.e.* particularly in France where the invasive *S. muticum* is established for a longer period of time?

**FRANÇAIS**

Le suivi d'un an d'*Halidrys siliquosa* a révélé une grande sensibilité des populations intertidales de l'espèce native de Bretagne face aux variations de lumière et de température. De plus, l'espèce a montré une densité très limitée en France, ne dépassant jamais plus de quatre individus par 0,16 m<sup>2</sup>. Ainsi, les populations françaises de *H. siliquosa* semblent être fragilisée dans la zone intertidale, ce qui suggère un stress important qui pourrait être dû à une température trop élevée dans cet habitat, peut-être proche de la limite de tolérance de cette espèce nordique. À cet égard, la section suivante du **Chapitre 3** est une comparaison géographique des capacités d'acclimatation de *H. siliquosa* entre la France et l'Irlande, où l'espèce native pourrait être moins affaiblie et présenter des capacités d'acclimatation différentes. En outre, les résultats sont comparés avec l'espèce invasive *Sargassum muticum*, qui est bien établie en France et à un stade plus précoce d'introduction en Irlande. En effet, l'espèce native pourrait être d'autant plus menacée qu'elle doit faire face à la fois au changement global et à sa cohabitation avec des espèces introduites. Ainsi, *Halidrys siliquosa* est-elle susceptible de disparaître près de sa limite sud en raison de l'augmentation de la

température ? Risque-t-elle d'être d'autant plus fragilisée par sa co-habitation avec des espèces introduites qui pourraient avoir un impact important sur sa survie, notamment en France où l'espèce invasive *S. muticum* est établie depuis plus longtemps ?

## ***Halidrys siliquosa*, a species threatened by its geographical position and co-existence with the invasive *Sargassum muticum*? A Comparison between France and Ireland**

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In preparation, to be submitted to: *Estuarine, coastal and shelf science*

### **RESUME**

En raison du changement global, on observe une augmentation constante du nombre de migrations, d'introductions ou d'extinctions d'espèces, ce qui entraîne des changements dans les communautés macroalgales. Les espèces natives doivent ainsi faire face à la fois au changement climatique et à la concurrence avec les espèces introduites. Dans ce contexte, l'objectif de cette étude était d'étudier l'écologie et les capacités d'adaptation de l'espèce native *Halidrys siliquosa* et de l'espèce invasive *Sargassum muticum*, en France où l'espèce nordique *H. siliquosa* est potentiellement affaiblie par sa position géographique, et sa cohabitation avec *S. muticum*, et en Irlande où *S. muticum* est à un stade d'introduction plus précoce. Les deux espèces ont été suivies sur trois sites dans chaque pays, en février et juin 2019. Les données écologiques ont révélé des différences phénologiques, *H. siliquosa* présentant une croissance lente, par rapport à une croissance rapide de *S. muticum*. Ensuite, les résultats ont montré une faible densité d'*H. siliquosa* en France, qui semblait affaiblie au niveau de ses populations. De même, *S. muticum* présentait une densité plus élevée en Irlande, avec une population qui n'est peut-être pas encore complètement stabilisée. Par ailleurs, *H. siliquosa* a montré une teneur plus importante en pigments photoprotecteurs que l'espèce invasive, notamment en France, ce qui suggère une forte sensibilité à l'augmentation de la lumière. De plus, *S. muticum* a montré une saisonnalité élevée dans sa composition en acides gras et composés phénoliques contrairement à *H. siliquosa*, notamment en France. Cette augmentation coïncidait avec sa période de reproduction et suggérait une plus grande acclimatation à l'augmentation de la lumière et de la température de l'espèce introduite par rapport à l'espèce native. Ainsi, *S. muticum* semblait plus susceptible de s'adapter à de fortes

variations environnementales que *H. siliquosa*. Plus spécifiquement, l'aire de répartition d'*H. siliquosa* pourrait se réduire puisque ses populations intertidales françaises sont déjà affaiblies, et pourraient disparaître complètement si elles ne peuvent pas s'adapter à la fois au changement climatique et à la coexistence avec des espèces invasives.

**Mots-clés:** Co-habitation, Ecophysiologie, Acides gras, Comparaison géographique, Espèce invasive, Analyses métabolomiques

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## ABSTRACT

Due to global change, there is a constant increase in the number of species migrations, introductions, or extinctions, causing changes in algal communities, resulting for native species to cope with both climate change and competition with non-indigenous species. In this context, the aim of this study was to investigate the ecology and adaptive abilities of the native *Halidrys siliquosa* and the invasive *Sargassum muticum*, in France where the Nordic *H. siliquosa* is weakened by this geographical location, and co-exists with the well-established introduced *S. muticum*, and Ireland where *S. muticum* is at an earlier stage of invasion. Both species were monitored at three sites in each country, in February and June 2019. Ecological data revealed phenological differences, with a slow-growth *H. siliquosa*, compared to a fast growth *S. muticum*. Then, results showed a low density of *H. siliquosa* in France, which appeared weakened at its southern populations. Similarly, *S. muticum* exhibited higher density in Ireland, with a population that may have not yet stabilised. In addition, *H. siliquosa* synthesized more photoprotective pigments than the invasive species, particularly in France, suggesting a high sensitivity to increasing light. By contrast, *S. muticum* exhibited a high seasonality in fatty acid and phenolic contents, particularly in France. This increase coincided with its reproductive period and suggested a greater acclimation to increasing light and temperature than the native species. In this way, *S. muticum* appeared more likely to adapt to high environmental changes than *H. siliquosa*. More particularly, the distribution range of *H. siliquosa* could be reduced since its French intertidal populations are already weakened, and could completely disappear if they cannot adapt to both climate change and coexistence with invasive species.

**Keywords:** Co-existence, Ecophysiologie, Fatty acids, Geographical comparison, Invasive species, Metabolomic analyses

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## 1. Introduction

One of multiple consequences of climate change and anthropogenic inputs is the migrations, regressions or local extinctions of native species, which lead to a switch in the structure of communities and are thus considered to be one of the greatest threat to marine biodiversity (Harley *et al.* 2012; Mineur *et al.* 2015). More particularly, species are affected by recurrent exposition to extreme temperatures, to which many organisms have to adapt their physiology and phenology, or to migrate to northern latitudes according to their specific resilience and plasticity (Gallon *et al.* 2014; Lima *et al.* 2007). In this sense, migrations or changes in the distribution area of many species have already been reported, such as in Ireland with the progressive disappearance of cold-water species *Alaria esculenta* and *Laminaria hyperborea* (Simkanin *et al.* 2005), the northwards extension of the distribution of *Carpodesmia* (previously *Cystoseira*) *tamariscifolia* (Hiscock *et al.* 2004), or the recent report of *Laminaria ochroleuca* whose northern limit was so far in Brittany, France (Schoenrock *et al.* 2019). In addition to these natural migrations, there are also intentional or accidental introductions of species through human activities that facilitate the global movement of non-native species, for example through maritime transport, fishing or aquaculture (Mineur *et al.* 2015). As a result, thousands of non-indigenous macroalgal species have been identified in marine ecosystems worldwide, such as *Sargassum muticum*, *Saccharina japonica*, or *Undaria pinnatifida* (Schaffelke *et al.* 2006; Molnar *et al.* 2008; Tsiamis *et al.* 2019).

Due to these migrations, native species have to cohabit with non-indigenous ones and compete for space, light, or nutrient availability. Thus both native and introduced species must adapt locally and find strategies to successfully coexist and survive (Schaffelke and Hewitt 2007; Vaz-Pinto *et al.* 2014). In this way, different macroalgal species have variable stress tolerance and plasticity under severe environmental conditions, which can result in physiological or morphological acclimation, up to genetic adaptation (Harley *et al.* 2012; Asadian *et al.* 2018). Invasive species usually have a better physiological and phenotypical plasticity compared to native species, conferring them an ecological advantage in their interaction (Zanolla *et al.* 2015; Pyšek and Richardson 2008; Davidson *et al.* 2011). Notably, seaweeds can accumulate large amount of metabolites in response to biotic and abiotic factors and seasonal fluctuations (Lalegerie *et al.* 2020), including phenolic compounds, fatty acids or pigments. Some of these biomolecules (level or composition) could be an advantage for non-indigenous species in their adaptation to their new environment. For example, it has been

demonstrated that the invasive success of *Caulerpa racemosa* was correlated to its capacity to reorganise its photosynthetic apparatus and adjust its light harvesting pigment content in relation to available light (Raniello *et al.* 2004). Similarly, non-native populations of *Agarophyton vermiculophyllum* were more resistant to elevated temperature due to increasing level of heat shock proteins compare to native seaweed populations (Hammann *et al.* 2016), or to withstand higher salinity variations through the synthesis of taurine (Surget *et al.* 2017).

Among non-indigenous species, *Sargassum muticum* (Yendo) Fensholt (1955) is a brown macroalga native from Japan and accidentally introduced into Europe in 1970s (Farnham 1980; Critchley *et al.* 1983; Stiger-Pouvreau and Thouzeau 2015). It is now a dominant species widespread in temperate macroalgal communities, becoming one of the most disturbing invasive species in many countries from Norway to Morocco and Mediterranean Sea (Sabour *et al.* 2013; Engelen *et al.* 2015; Stiger-Pouvreau and Thouzeau 2015). Established from subtidal to intertidal areas, *S. muticum* is a pseudo-perennial species (Critchley 1983), owing its large expansion to its rapid reproduction and growth, a large distance of dispersal and a high plasticity to environmental changes (Engelen *et al.* 2015; Benali *et al.* 2019). Therefore, *S. muticum* can rapidly reach large size and biomass, competing with many native species, including several other members of Sargassaceae, such as *Carpodesmia tamariscifolia*, *Treptacantha* (previously *Cystoseira*) *baccata*, *Bifurcaria bifurcata* or *Halidrys siliquosa* (Stæhr *et al.* 2000; Britton-Simmons 2004; Sánchez *et al.* 2005; Olabarria *et al.* 2009; Le Lann *et al.* 2012), impacted thus intertidal and upper subtidal algal assemblages. In France, *S. muticum* is a well-established species since the country constitutes its first introduction area in Europe, with the first record in 1977 in Pointe du Hoc (Normandy, France) and then in Brittany in 1981 (Critchley *et al.* 1983). In contrast, *S. muticum* is only at the beginning of its installation in Ireland and its expansion is less advanced due to the insular position of this country (Kraan 2007; Monagail and Morrison 2020). First individuals were thus reported almost 30 years later, *i.e.* in 1995 for Northern coast (Strangford Lough, Co. Down), and in 2001 for Western coast (Cashel Bay, Co. Galway) (Kraan 2007; Baer and Stengel 2010). Irish populations of *S. muticum* are thus less established compared to France, and are likely to evolve. This could lead this brown macroalga to outcompete and replace native species, especially in the current context of climate change which impacts the growth / survival of all algal species (Asadian *et al.* 2018). Among native species living commonly in habitats invaded by *S. muticum*, *Halidrys siliquosa* (Linnaeus) Lyngbye 1819 is distributed from North Sea along Scandinavian Peninsula, to

Northeast Atlantic ocean along French and Portuguese coasts (Lima *et al.* 2009). *H. siliquosa* is taxonomically and morphologically close to *S. muticum* since they both belong to Sargassaceae, and that they have both coarsely branched thallus (Wernberg *et al.* 2001; Pedersen *et al.* 2005). It is a perennial macroalga, found commonly in large rockpools of intertidal to upper subtidal area where it can be dominant, without forming a large canopy similar to those of *S. muticum* (Tyler-Walters and Pizzolla 2008). In addition, *H. siliquosa* is a species with a high potential for valorisation since it is rich in bioactive compounds (Kornprobst 2014; Stiger-Pouvreau *et al.* 2014; Le Lann *et al.* 2016), such as phenolic compounds and linear diterpenoids, exhibiting high anti-fouling and antibacterial activities (Higgs and Mulheim 1981; Hellio *et al.* 2001; Culioli *et al.* 2008). However, to date there is to our knowledge very limited information available on its ecology, phenology or adaptive abilities, except few studies from Denmark (Wernberg *et al.* 2001; Pedersen *et al.* 2005), hence the need to acquire more data. Moreover, contrary to *S. muticum* which has a large geographical distribution, *H. siliquosa* is a boreal species with a Nordic affinity, and progressively declining in density at its southern limit, and therefore completely absent on the southern French coast (Lima *et al.* 2009). In France, its current southern limit thus corresponds to Brittany, which constitutes a biogeographical transition zone between cold-temperate and warm-temperate regions for many species (Gallon *et al.* 2014). However, this southern limit is likely to evolve and shift to higher latitudes with increasing temperature. Thus, French populations of *H. siliquosa* will probably have to acclimate to warmer temperatures, otherwise this species will disappear. It will lead to changes in its co-habitation with other species, including the introduced *S. muticum*, which could, on the contrary, develop further. In addition, the relatively recent introduction of *S. muticum* in Ireland is likely to result in a decline of northern populations of *H. siliquosa* which could thus disappear in both countries. In this sense, Stæhr *et al.* (2000) already reported that the expansion of *S. muticum* in Limfjorden (Denmark) between 1984 and 1997 resulted in a more than 50% decrease in *H. siliquosa* cover.

In this context, the aim of the present study was to compare and contrast the adaptive abilities of the introduced *S. muticum* and the native *H. siliquosa* between France (Brittany) and Ireland (Bay of Galway), where populations of both species are likely to evolve. To this end, ecology (*i.e.* reproduction, size distribution, et circumference) of both species were monitored over two sampling months (February and June 2019), at three sites in France and three sites in Ireland, and samples were brought back to laboratory for metabolomic analyses (pigments,

phenolic compounds, fatty acids). Although reports on the phenology of *S. muticum* and its ecological impacts following its expansion are numerous, to date no study compares the competitive ability of the algal invader and co-occurring native species between two countries, across a latitudinal range. By coupling both ecological and metabolomic data, results will provide a further vision of the ecophysiology and acclimation process of both species, and thus their potential vulnerability to environmental changes, in order to better predict the evolution of their distribution range. In this way, are there ecological or metabolomic differences between French and Irish populations? More particularly, does the time of invasion of *S. muticum* have any effect on these acclimation abilities? And is *Halidrys siliquosa* likely to disappear due to increased temperature and its co-habitation with *S. muticum*, whether in France or in Ireland?

## 2. Materials and methods

### 2.1 Seaweed sampling and environmental conditions

Both *Sargassum muticum* and *Halidrys siliquosa* (Phaeophyceae, Sargassaceae) were collected at two different seasons (*i.e.* winter as February 2019 and spring as June 2019), simultaneously in two countries, France and Ireland. In order to compare the acclimation performance of *H. siliquosa* and *S. muticum* under the same conditions, sites were chosen where the two species co-exist. Samples were thus harvested at low spring tides, at three sites on the Brittany coasts (France): the exposed sites Minou (48°20'20.314'' N - 4°37'3.779'' W) and Pointe du diable (48°21'15.606'' N - 4°33'30.067'' W), and the sheltered site Porspoder (48°28'56.644'' N - 4°46'6.812'' W). Similarly, samples were collected at three sites within the Galway Bay (Ireland): the exposed sites Coral beach (53°14'55.5'' N - 9°37'48.7'' W) and Spiddal (53°14'25.1'' N - 9°18'35.5'' W), and the sheltered site Finavarra (53°09'15.9'' N - 9°07'02.9'' W). Each sample constituted of a dozen thalli collected in areas adjacent to the ecological study areas, with three independent replicates per species and per site. All samples were cleaned of epiphytes and frozen on the day of collection, before being freeze-dried and ground into a fine powder. Samples were further sent in France where all metabolomic analyses were carried out. In addition, environmental data were provided by « Service d'Observation en Milieu Littoral, INSU-CNRS, Saint-Anne du Portzic » (sea temperature, pH, salinity and nutrient contents), or « Météo France » (sunshine duration and irradiance from station Brest-Guipavas) for France; and from the Irish meteorological service Met éireann (irradiance), or compass Mace Head observation buoy of Marine Institute (sea



temperature, pH, salinity, nitrate content) for Ireland.

## 2.2 Ecological measurements

Ecological measurements have been carried out for both species in quadrats of 0.09 m<sup>2</sup> and 0.16 m<sup>2</sup> in France, and 0.25 m<sup>2</sup> and 1.00 m<sup>2</sup> in Ireland, for *S. muticum* and *Halidrys siliquosa* respectively (n = 4). The size of the quadrats was selected following preliminary sampling studies, and were adapted to each population's size. Thus, each minimum sampling areas was determined by using quadrats subdivided into 10 x 10-cm areas (n = 6), and counting the number of individuals until there was no significant difference by increasing the area. For both species, different ecological variables were measured: density (*i.e.* number of individuals per quadrat), length of individuals (*i.e.* size from the holdfast until the top of the longest lateral), maximum circumference, reproductive stage (*i.e.* presence or absence of receptacles), and general aspect of individuals (*i.e.* colour and the absence or presence of epiphytes). In addition, the number of primary laterals was counted for *S. muticum* (for morphological descriptions, see Critchley, 1983, for *S. muticum*, and Moss and Lacey, 1963, for *H. siliquosa*).

## 2.3 Pigment extraction and analysis

Pigment extraction and analysis were carried out according to Schmid and Stengel (2015), adapted and already described in Lalegerie *et al.* (2019). Thus, pigments were extracted from dried powder (75 mg) in a 90 % aqueous acetone solution (750 µL), with two successive extractions of 30 min and 4 h at 4 °C under magnetic agitation. Pigments were then directly analysed by High Pressure Liquid Chromatography (HPLC) using an ACE C<sub>18</sub> column (150 × 4.6 mm, 3 µm) on a HPLC Dionex Ultimate 3000 system. Separation was performed using a mobile phase of (A) methanol:ammonium acetate buffer 0.5 M (80:20), (B) acetonitrile:MilliQ water (87.5:12.5), (C) ethylacetate 100 %, according to the following gradient described in Lalegerie *et al.* (2019). Before injection, pigments have been diluted ¾ with ammonium acetate buffer (0.5 M, pH 7.2). Quantification was obtained using standard curves of chlorophyll-*a* (Sigma, USA), chlorophyll-*c*<sub>2</sub>, β-carotene, fucoxanthin, zeaxanthin, violaxanthin and pheophytin-*a* (DHI, Denmark).

## 2.4 Fatty acids extraction and analysis

Fatty acids were extracted by direct transmethylation according to a protocol adapted from

Schmid *et al.* (2014), by adding 2 mL of a mixture of H<sub>2</sub>SO<sub>4</sub>:methanol (2:98) to 40 mg of dry seaweed powder. Samples were incubated during 90 min at 80 °C after addition of an internal standard (2.3 µg of tricosanoic acid C23:0). After cooling, 2 mL of hexane 100 % and 1.5 mL of distilled water saturated with hexane were added. Samples were centrifuged and the lower aqueous phase has been discarded. This step of rinsing with water was repeated 3 times, before recovering the hexane upper layer containing fatty acids for analyses using Gas Chromatography. Fatty acid analysis was performed on a Varian CP-8400 system equipped with a flame-ionization detector. Samples (2 µL) were injected on two different columns, one polar (Zebron ZB-Wax, Agilent, 0.25 mm x 30 m x 0.2 µm) and one apolar (ZB-5HT, Agilent, 0.25 mm x 30 m x 0.2 µm), with hydrogen as vector gas at 2 mL.min<sup>-1</sup> constant flow. The injector and detector temperatures were set at 220 °C and 280 °C, respectively and the oven temperature was programmed according to the following gradient: 0 °C to 150 °C at 50 °C min<sup>-1</sup>, then increased to 170 °C by a rate of 3.5 °C.min<sup>-1</sup>, then to 185 °C by 1.5 °C.min<sup>-1</sup>, to 225 °C by 2.4 °C.min<sup>-1</sup>, and finally increased to 250 °C by 5.5 °C.min<sup>-1</sup> and kept at this final temperature for 15 min. Fatty acids were identified by comparison with a commercial standard mixture (Supelco 37-components FAME Mix, Sigma-Aldrich) and each fatty acid was quantified by comparing peak areas with tricosanoic acid (C23:0) as internal standard. Fatty acids results are expressed in percentage of the total fatty acids (TFA) content. Data were collected using Galaxie 1.9.3.2 software (Agilent).

### 2.5 Phenolic compounds extraction and analysis

Phenolic compound were extracted from dried powder (15 mg) in ethanol 70 % (1 mL), with two successive extractions, one of 15 min at 4 °C in an ultrasonic bath (Sonicator 88155, Fisher Bioblock Scientific, France), and a second of 2 h at 40 °C under magnetic stirring following Lalegerie *et al.* (in prep.). Then, samples were centrifuged for 10 min at 8,000 rpm (Eppendorf Centrifuge 5810, Germany), the supernatants were recovered and the extraction was repeated a second time. Finally, the supernatants were pooled and evaporated at 40 °C using a centrifugal concentrator (miVac, Genevac, France). The Folin-Ciocalteu colorimetric assay was performed to quantify the total phenolic content (TPC), according to Zubia *et al.* (2009). Thus, a microplate well contained 20 µL of sample, 130 µL of distilled water, 10 µL of Folin-Ciocalteu reagent and 40 µL of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>, 200 g.L<sup>-1</sup>). Microplates were incubated for 10 min at 70 °C, and then put on ice to stop the chemical reaction. Finally, the absorbance of each sample was measured at 620 nm with a Multiskan FC (Thermo

Scientific, USA). A standard curve of phloroglucinol (1,3,5-trihydroxybenzene) was achieved to determine the TPC of each sample in mg PGE.g<sup>-1</sup> DW (milligrams of phloroglucinol equivalent per gram of dried seaweed powder).

## 2.6 Statistical analyses

Statistical analyses were performed using R software (version 3.6.1) (R Core Team, 2019) through the integrated development environment Rstudio (version 1.2.5001) (Rstudio Team, 2019). All analyses were carried out in triplicate and results are expressed as means ± standard deviation. As the aim of this publication was to make a global comparison of the ecological and metabolomic differences of *H. siliquosa* and *S. muticum* between France and Ireland, the data for the different sites in each country have been pooled and averaged. Only data on the size class distribution are shown per site, due to the specific structuring of each population. Data comparisons were then performed using a Student's t-test or ANOVA with a confidence level of 95 %. However, if the data did not meet the requirements for parametric tests (*i.e.* normal distribution and homoscedasticity), the non-parametric Wilcoxon (two groups), Kruskal-Wallis (> two groups, one factor) or Scheirer-Ray-Hare (> two groups, several factors) tests were used. The ANOVA-multiple comparison analyses were followed by a Tukey post-hoc test (TukeyHSD), or a non-parametric post-hoc Scheirer-Ray-Hare test (kruskalmc, pirms package). In addition, Principal Component Analyses (PCA) were carried out with the packages FactoMineR and explor, to compare pigment and fatty acid composition in relation to species, country or season. Finally for ecological data, the number of size classes was obtained according to Sturges rule using the following equation: number of classes =  $(1 + 3.3 \log_{10}(n))$ , where  $n$  is the number of individuals. In addition, the measured densities (*i.e.* individuals / quadrat) was normalized into estimated densities (*i.e.* individuals / m<sup>2</sup>) in order to compare data between species / countries. However, as this method does not consider the population structure, both data are presented.

## 3. Results

### 3.1 Environmental data

The environmental parameters (Table 1) recorded in February and June 2019 highlighted differences between France and Ireland. Notably, France exhibited a higher global irradiation in May, reaching 60,696 J.cm<sup>-2</sup>, compared to 57,194 J.cm<sup>-2</sup> in June in Ireland. In this regard, a decrease in global irradiation was observed between May and June for France, compared to

an increase in Ireland. Moreover, the temperature was greater in summer for France, with 15.14 °C in June, compared to 13.55 °C in Ireland at the same period. Then, both countries showed a decrease in nutrient content in the summer. However, the nitrate content showed a greater variation in France, ranging from 16.33 to 0.25 µM between February and May respectively, compared to 13.02 to 4.59 µM in Ireland.

**Table 1.** Environmental conditions in France and Ireland for both sampling months, *i.e.* February and June 2019. Data from the months prior to the seaweed harvest were included to provide an overview of the environmental variations in both countries at both sampling dates.

<b>France</b>				
	January	February	May	June
Monthly insolation (hours)	48.02	133.36	203.40	164.88
Global irradiation (J.cm <sup>-2</sup> )	9725	20895	60696	53994
Seawater temperature (°C)	10.75 ± 0.56	9.82 ± 0.30	13.71 ± 0.91	15.14 ± 0.07
Salinity	34.44 ± 0.29	34.16 ± 0.21	34.68 ± 0.13	34.88 ± 0.06
Oxygen (mL.L <sup>-1</sup> )	6.04 ± 0.07	6.36 ± 0.02	6.53 ± 0.31	5.83 ± 0.16
pH	7.89 ± 0.04	7.91 ± 0.01	8.09 ± 0.04	8.06 ± 0.05
NO <sub>3</sub> <sup>-</sup> (µM)	15.98 ± 2.23	16.33 ± 2.41	0.25 ± 0.06	0.29 ± 0.11
NH <sub>4</sub> <sup>+</sup> (µM)	0.45 ± 0.08	0.45 ± 0.11	0.25 ± 0.08	0.29 ± 0.08
PO <sub>4</sub> <sup>3-</sup> (µM)	0.48 ± 0.01	0.45 ± 0.03	0.05 ± 0.01	0.10 ± 0.02

<b>Ireland</b>				
	January	February	May	June
Global irradiation (J.cm <sup>-2</sup> )	6339	12890	53337	57194
Seawater temperature (°C)	9.66 ± 0.37	8.78 ± 0.44	11.92 ± 0.62	13.55 ± 0.89
Salinity	33.35 ± 0.70	33.36 ± 0.52	34.29 ± 0.28	34.38 ± 0.22
pH	7.96 ± 0.07	8.03 ± 0.01	8.12 ± 0.05	8.06 ± 0.06
NO <sub>3</sub> <sup>-</sup> (µM)	10.83 ± 0.22	13.02 ± 1.23	4.59 ± 0.73	4.64 ± 0.56

### 3.2 Ecological study

The results highlighted large differences in densities (*i.e.* number of individuals per quadrat) between the native *Halidrys siliquosa* and the invasive *Sargassum muticum*, with specific differences between countries and seasons (Table 2). In this way, the density of *H. siliquosa* was similar between the two seasons (Scheirer-Ray-Hare test,  $p$ -value = 0.373 for the factor ‘month’,  $p$ -value < 0.001 for ‘country’, and  $p$ -value = 0.625 for the interaction), with an increasing trend in the summer. Then, according to the measured density, the native species had a higher density in Ireland with an average of  $5.84 \pm 4.04$  individuals per quadrat compared to only  $0.92 \pm 1.35$  individuals per quadrat in France (Wilcoxon test,  $p$ -value < 0.0001) (Table 2). However, when adjusted to 1 m<sup>2</sup> for both countries, the estimated density did not reveal any spatial difference (Wilcoxon test,  $p$ -value = 0.094).

**Table 2.** Seasonal measured density (*i.e.* individuals per quadrat), estimated density (*i.e.* estimated number of individuals adjusted to 1 m<sup>2</sup>), averaged thallus length (cm) and circumference (cm) of *Halidrys siliquosa* and *Sargassum muticum* recorded in France and Ireland, in February and June 2019. The data are expressed in respect to the size of the quadrats (m<sup>2</sup>) used, *i.e.* adapted to each population.

		Quadrat (m <sup>2</sup> )	Measured density (ind / quadrat)	Estimated density (ind / m <sup>2</sup> )	Length (cm)	Circumference (cm)	
<b><i>H. siliquosa</i></b>	February	0.16	0.83 ± 1.03	5.25 ± 6.58	70.10 ± 47.64	12.40 ± 7.64	<b>FRANCE</b>
	June		1.00 ± 1.65	6.33 ± 10.35	56.13 ± 18.19	8.71 ± 4.76	
<b><i>S. muticum</i></b>	February	0.09	14.17 ± 11.42	157.33 ± 126.73	21.01 ± 13.60	1.89 ± 1.57	
	June		5.17 ± 2.08	57.42 ± 23.33	54.56 ± 38.93	5.03 ± 2.73	
<b><i>H. siliquosa</i></b>	February	1.00	4.18 ± 1.60	4.18 ± 1.60	65.35 ± 34.59	17.35 ± 7.88	<b>IRELAND</b>
	June		7.14 ± 4.90	7.14 ± 4.90	37.33 ± 25.38	10.64 ± 6.15	
<b><i>S. muticum</i></b>	February	0.25	10.75 ± 5.67	43.00 ± 22.70	29.83 ± 12.12	2.08 ± 0.87	
	June		6.58 ± 3.23	26.33 ± 12.93	72.66 ± 32.12	6.87 ± 3.89	

On the other hand, *S. muticum* exhibited a lower number of individuals in June, whatever the country, either with the measured (Scheirer-Ray-Hare test,  $p$ -value = 0.001 for the factor ‘month’,  $p$ -value = 0.606 for ‘country’, and  $p$ -value = 0.427 for the interaction) or the estimated density (Scheirer-Ray-Hare test,  $p$ -value = 0.012 for the factor ‘month’,  $p$ -value < 0.0001 for ‘country’, and  $p$ -value = 0.781 for the interaction). In this way, there was for example an average of  $14.17 \pm 11.42$  individuals per quadrat in February compared to  $5.17 \pm 2.08$  individuals per quadrat in June in France (Table 2). Then, according to the measured density, *S. muticum* had a similar density between France and Ireland (Wilcoxon test,  $p$ -value = 0.611) with in average  $9.67 \pm 9.25$  and  $8.67 \pm 4.99$  individuals per quadrat respectively. Nevertheless, when adjusted to 1 m<sup>2</sup> for both countries, the estimated density revealed a higher density in France, with in average  $107.38 \pm 102.69$  ind / m<sup>2</sup>, compared to  $34.67 \pm 19.97$  ind / m<sup>2</sup> in Ireland. Besides, *S. muticum* had a higher density than *H. siliquosa*, particularly in France where the populations of the native species remained limited (Kruskal-Wallis on measured density,  $p$ -value < 0.0001 for France and  $p$ -value = 0.0137 for Ireland).

Then, the size class distribution was investigated, highlighting differences between both species, with seasonal and spatial disparities. In this way, the Irish populations of *H. siliquosa* was quite homogeneous between the two seasons with individuals between [0 – 17.4 cm] and [104.4 – 121.8 cm], corresponding to several cohorts. Only the Irish site Finavarra appeared to stand out with large individuals above 64.0 cm in February, and a majority of smaller individuals in June between 0 and 34.8 cm (Fig. 1). As a result, the mean length of *H. siliquosa* tended to decrease in summer in Ireland, ranging from  $66.35 \pm 34.59$  cm in February to  $37.33 \pm 25.38$  cm in June (Table 2). In parallel, the limited density of *H. siliquosa*

in France not allowed to obtain meaningful distributions, and data are thus presented only for one site (*i.e.* Porspoder). The data showed no significant seasonal variation in length for the latter site (Student's test,  $p$ -value = 0.128), although larger individuals were observed in February, similarly to Finavarra. More generally, results suggested that the native species exhibited a similar length between France and Ireland (Scheirer-Ray-Hare test,  $p$ -value < 0.001 for the factor 'month' and 'country',  $p$ -value = 0.019 for the interaction) (Table 2 and Fig. 1).

Contrary to *H. siliquosa*, a single cohort was visible for *S. muticum*, with a high proportion of juveniles in February (*i.e.* ranging from [0 - 20.4 cm]), compared to larger individuals in summer for both countries (Fig. 1). This increase in length was greater at Coral beach and Finavarra in Ireland, or Porspoder in France, with individuals exceeding 122 cm at these sites. In this way, *S. muticum* showed a seasonal pattern opposite to *H. siliquosa*, increasing significantly in summer whatever the country. It varied for example from  $29.83 \pm 12.12$  cm in February to  $72.66 \pm 32.12$  cm in June in Ireland.

Then, average data suggested that the invasive species had a higher length in Ireland than France, whatever the season (Scheirer-Ray-Hare test,  $p$ -value < 0.001 for the factor 'country and 'month,  $p$ -value = 0.826 for the interaction) (Table 2). This was coupled to a slightly higher number of laterals in Ireland, similar between both seasons (Scheirer-Ray-Hare test,  $p$ -value = 0.002 for the factor 'country,  $p$ -value = 0.096 for 'month, and  $p$ -value = 0.727 for the interaction), *i.e.* in average  $4.55 \pm 3.58$  laterals per individual in Ireland, compared to  $3.45 \pm 2.67$  laterals per individual in France (data not shown). Nevertheless, size class distribution showed that *S. muticum* reached a maximum size in France, with individuals reaching up to 200 cm in June at Porspoder. Besides, mean circumference exhibited the same trends than mean length (Table 2), as both variables were correlated (Kendall's correlation,  $\tau = 0.43$  for *H. siliquosa*,  $\tau = 0.56$  for *S. muticum* and  $p$ -value < 0.0001 for both species). Finally, while *H. siliquosa* had a high maturity rate in both sampling seasons with almost 100 % individuals with receptacles for both countries, *S. muticum* on the contrary was mature only in June for both countries, with in average  $72.22 \pm 11.75$  % of mature individuals in France compared to  $60.79 \pm 0.11$  % in Ireland (data not shown).

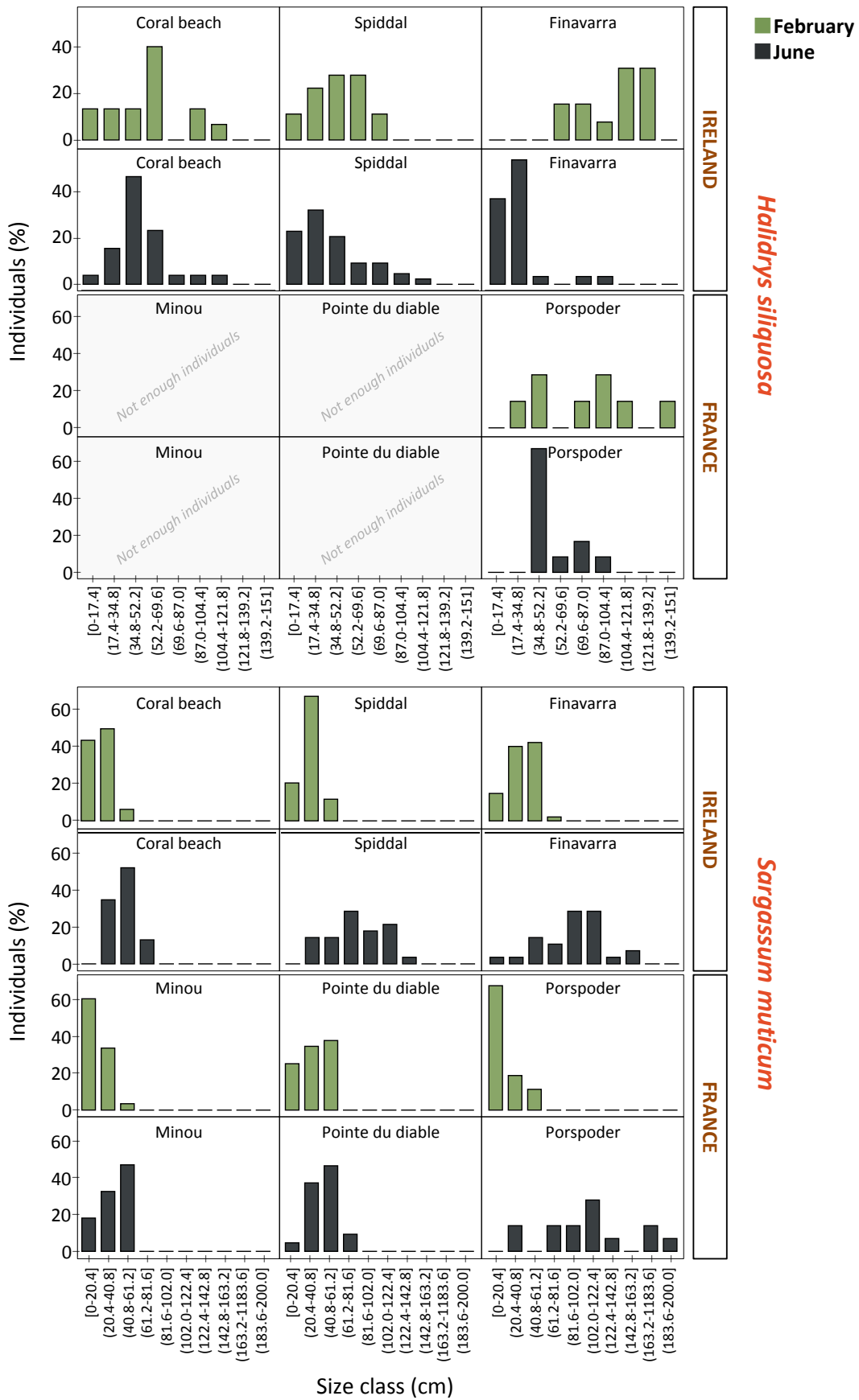


Figure 1. Seasonal size class distribution (%) of *Halidrys siliquosa* and *Sargassum muticum* recorded in France and Ireland, in February and June 2019.

### 3.3 Metabolomic analyses

#### 3.3.1 Pigment content and composition

Then, results highlighted a clear seasonal distinction between France and Ireland, whatever the species. Then chlorophyll-*a* and violaxanthin decreased in June in Ireland for both species, whereas it increased in France (Student's test,  $p$ -value < 0.05 for both variables whatever the country or species). The same pattern was observed for zeaxanthin, although it was only significant for *S. muticum* (Student's test,  $p$ -value = 0.01418 for France and  $p$ -value = 0.002 for Ireland). On the contrary, pheophytin-*a* content tended to increase in June whatever the country, although this was significant only for *H. siliquosa* (Student's test,  $p$ -value < 0.05 for France and Ireland).

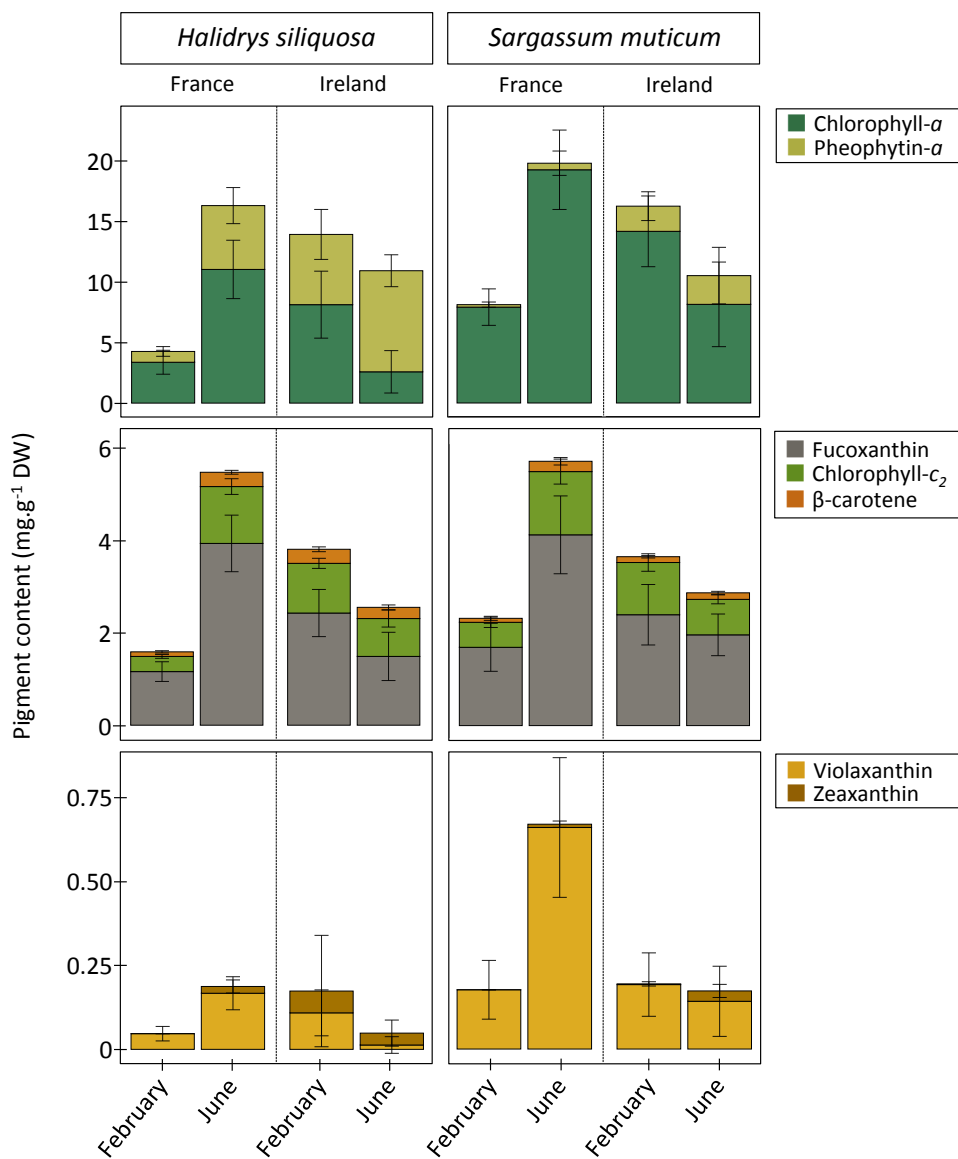


Figure 2. Pigment composition (mg.g<sup>-1</sup> DW) of *Halidrys siliquosa* and *Sargassum muticum* collected in France and Ireland, in February and June 2019. Results are displayed as the stacked cumulative contents (with SD for each pigment).



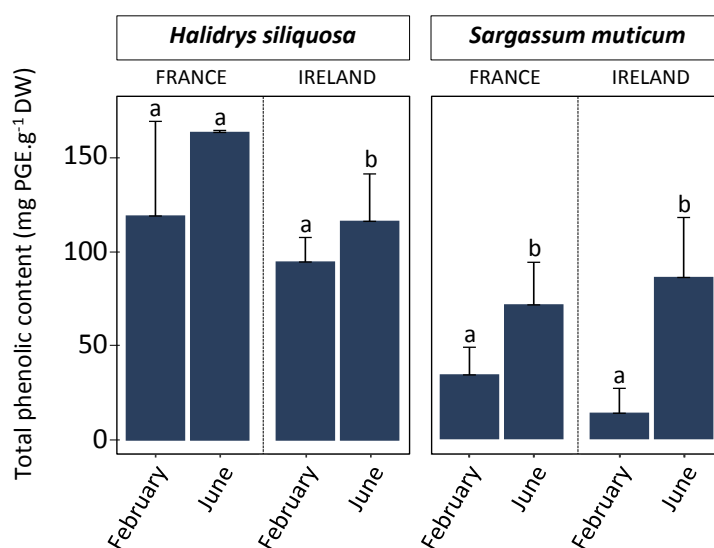
In terms of proportions (Supplementary Table 1), chlorophyll-*a* and pheophytin-*a* were however perfectly correlated for both species (Kendall's correlation,  $\tau = -0.85$ ,  $p$ -value  $< 0.001$ ). More generally, pheophytin-*a* content was greater in Ireland for both species, whatever the season (Scheirer-Ray-Hare test,  $p$ -value  $< 0.0001$  for the factors 'species' and 'country',  $p$ -value = 0.790 for the interaction), in relation to the different environmental conditions (Table 1).

On the other hand, results showed differences in the pigment composition between both species together with spatial variations. Notably, *H. siliquosa* tended to exhibit a high level of zeaxanthin in Ireland (Kruskal-Wallis,  $p$ -value = 0.050), contrary to the French populations which showed zeaxanthin only in June, in a lower level. On the contrary, the native species showed a relatively high level of violaxanthin in June in France, while the Irish population showed almost no violaxanthin in June (Wilcoxon test,  $p$ -value = 0.036). Concerning *S. muticum*, statistical results suggested that French populations contained more violaxanthin, due to an important increase in June (Wilcoxon test,  $p$ -value = 0.005). On the contrary, Irish populations did not exhibit significant seasonal variations in violaxanthin level, but contained slightly more zeaxanthin (Wilcoxon test,  $p$ -value = 0.048). More generally, *H. siliquosa* tended to have more zeaxanthin than *S. muticum*, although the difference was not significant considering its low content (Scheirer-Ray-Hare test,  $p$ -value = 0.699 for the factor 'species',  $p$ -value = 0.017 for 'country',  $p$ -value = 0.750 for the interaction). In addition, the native species contained also a higher level of pheophytin-*a* than *S. muticum* (Scheirer-Ray-Hare test,  $p$ -value  $< 0.0001$  for the factors 'species' and 'country',  $p$ -value = 0.790 for the interaction) with  $5.04 \pm 3.28$  mg.g<sup>-1</sup> DW and to  $1.29 \pm 1.63$  mg.g<sup>-1</sup> DW respectively. On the contrary, *S. muticum* had a higher chlorophyll-*a* content whatever the country, with a mean of  $12.27 \pm 5.47$  mg.g<sup>-1</sup> DW compared to  $5.33 \pm 3.60$  mg.g<sup>-1</sup> DW for *H. siliquosa* (ANOVA,  $p$ -value  $< 0.001$  for the factor 'species',  $p$ -value = 0.267 for 'country' and  $p$ -value = 0.297 for the interaction). In the same way, *S. muticum* tended also to have more violaxanthin since it followed the same pattern and was well positively correlated to chlorophyll-*a* for both species (Kendall's correlation,  $\tau = 0.77$ ,  $p$ -value  $< 0.001$ ).

### 3.3.2 Phenolic compounds

The results highlighted a significant difference in the total phenolic content (TPC) between species (Fig. 3). In this way, *H. siliquosa* exhibited no significant variations in TPC between France and Ireland (Wilcoxon test,  $p$ -value = 0.146), or between sampling months (Wilcoxon

test,  $p$ -value = 0.122). Despite this statistical result, the native species tended to produce more TPC in June, particularly in France where TPC ranged from  $118.92 \pm 50.24$  mg PGE  $\cdot g^{-1}$  DW in February to  $163.30 \pm 25.18$  mg PGE  $\cdot g^{-1}$  DW. Similarly, TPC increased highly in June for *S. muticum* (Student's test,  $p$ -value < 0.0001), with no significant difference between France and Ireland (Student's test,  $p$ -value = 0.835). Thus, TPC of the invasive species varied in average from  $24.93 \pm 17.14$  mg PGE  $\cdot g^{-1}$  DW in February to  $78.70 \pm 28.06$  mg PGE  $\cdot g^{-1}$  DW in June. In this sense, the seasonal increase in TPC during summer was twice higher for *S. muticum*, matching with its reproductive period. By contrast, whatever the country, the average TPC was higher in *H. siliquosa* with  $113.33 \pm 34.75$  mg PGE  $\cdot g^{-1}$  DW, compared to  $52.63 \pm 35.72$  mg PGE  $\cdot g^{-1}$  DW for *S. muticum* (ANOVA,  $p$ -value < 0.001 for the factor 'species',  $p$ -value = 0.366 for 'country' and  $p$ -value = 0.178 for the interaction).

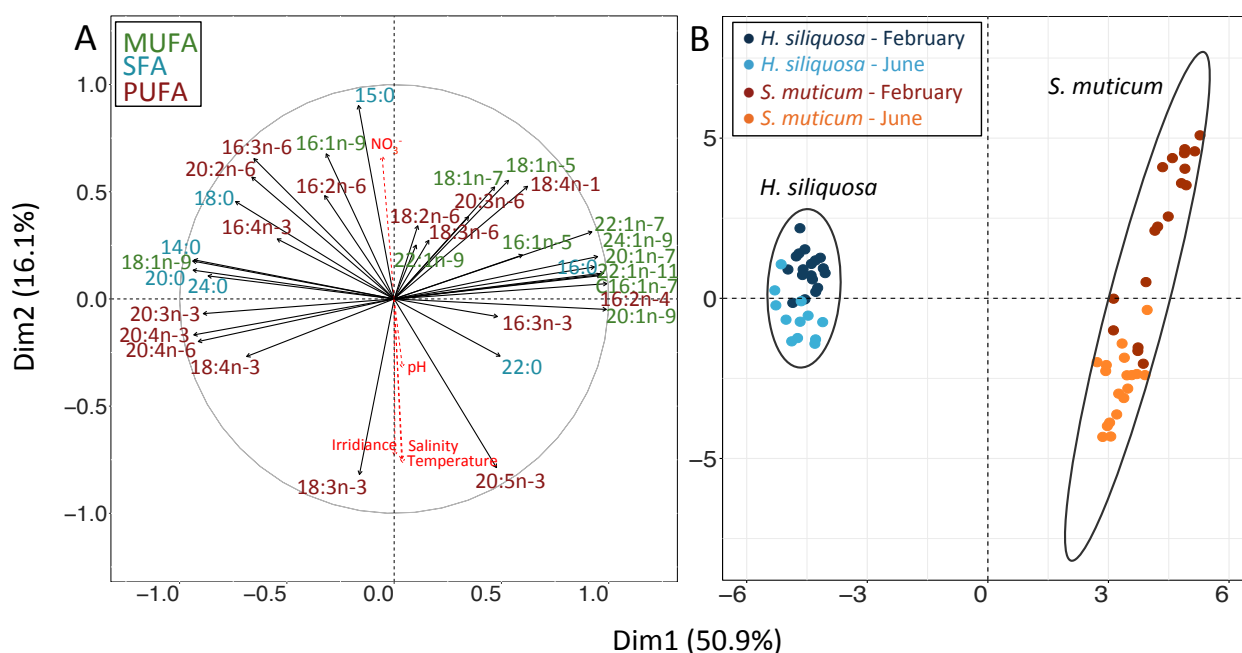


**Figure 3.** Total phenolic content (mg PGE.  $g^{-1}$  DW) in *Halidrys siliquosa* and *Sargassum muticum* collected in France and Ireland, in February and June 2019. Letters indicate the significant difference in TPC between sampling month, for each groups 'species x sites' (Wilcoxon test,  $p$ -value < 0.05).

### 3.3.3 Fatty acid content and composition

A principal component analysis (PCA) revealed differences in fatty acid composition between the native and the invasive species (Fig. 4a,b), but not between countries (data not shown). Indeed, despite their limited proportions, five fatty acids were present only in *S. muticum*, *i.e.* cetoleic acid (C22:1n-11), 13-eicosenoic acid (C20:1n-7), nervonic acid (C24:1n-9), 15-docosenoic acid (C22:1n-7) and 17-octadecetetraenoic acid (C18:4n-1) (see supplementary Table 2 for detailed fatty acid composition). In addition, among shared fatty acids, PCA highlighted that *S. muticum* had higher levels of palmitic acid (C16:0), palmitoleic acid

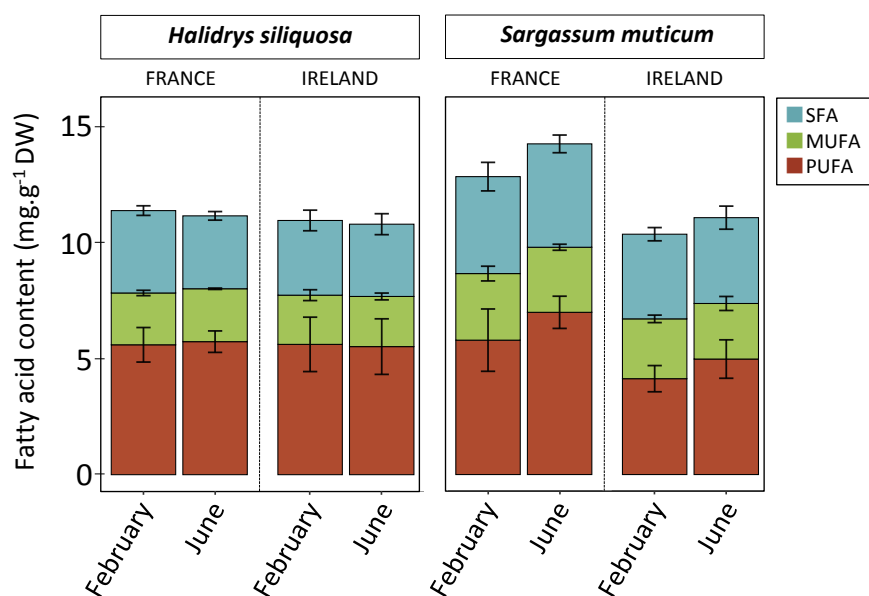
(C16:1n-7), hexadecadienoic acid (C16:2n-4) and eicosenoic acid (C20:1n-9) (Kruskal-Wallis,  $p$ -value < 0.001 for all variables), while *H. siliquosa* contained more myristic acid (C14:0), eicosatetraenoic acid (C20:4n-3), arachidonic acid (C20:4n-6) and oleic acid (C18:1n-9) (Kruskal-Wallis,  $p$ -value < 0.001 for all variables). Despite these differences, the main fatty acids were similar for both species, including (C16:0), (C20:4n-6) and (C18:1n-9), representing in average  $27.10 \pm 2.05$  %,  $12.88 \pm 1.47$  % and  $9.55 \pm 0.88$  % of the total fatty acid (TFA) content of *S. muticum*, and  $19.99 \pm 1.04$  %,  $18.73 \pm 1.62$  % and  $17.43 \pm 1.79$  % of the TFA content of *H. siliquosa*, respectively.



**Figure 4.** Principal component analysis on fatty acid (%) (A variable, B individuals) compositions of *Halidrys siliquosa* and *Sargassum muticum* collected in February and June 2019, on different sites in France and Ireland. SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), and PUFA (polyunsaturated fatty acids). Ellipses were drawn around both species (confidence level of 95%).

Furthermore, results revealed significant differences in total fatty acid (TFA) content depending on species and/or country (Fig. 5 and supplementary Table 2). Indeed, results showed that TFA content of *H. siliquosa* was similar between France and Ireland (Student's test,  $p$ -value = 0.337), whereas TFA content was significantly higher in France for *S. muticum*, compared to Ireland (Student's test,  $p$ -value < 0.001). In this sense, *S. muticum* had higher content of PUFA in France than in Ireland (ANOVA,  $p$ -value < 0.0001 for the factor 'country',  $p$ -value = 0.0134 for 'month' and  $p$ -value = 0.469 for the interaction), with in average  $6.40 \pm 1.21$  and  $4.56 \pm 0.82$  mg.g<sup>-1</sup> DW, respectively. Then, in France, *S. muticum* had a higher TFA content than *H. siliquosa* at the two seasons, with in average  $13.99 \pm 1.78$

and  $11.53 \pm 0.89$  mg.g<sup>-1</sup> DW in June and February, respectively (ANOVA,  $p$ -value < 0.001 for the factor ‘species’,  $p$ -value = 0.165 for ‘month’ and  $p$ -value = 0.219 for the interaction). This difference was due to the higher content of both saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in *S. muticum* (Student’s test,  $p$ -value < 0.001 for SFA and MUFA,  $p$ -value = 0.0347 for PUFA). On the contrary, the TFA content was statistically the same between both species in Ireland, with in average  $11.04 \pm 1.32$  and  $11.05 \pm 1.74$  mg.g<sup>-1</sup> DW for *S. muticum* and *H. siliquosa*, respectively (ANOVA,  $p$ -value = 0.987 for the factor ‘species’,  $p$ -value = 0.709 for ‘month’ and  $p$ -value = 0.482 for the interaction).



**Figure 5.** Fatty acid composition (mg.g<sup>-1</sup> DW) of *Halidrys siliquosa* and *Sargassum muticum* collected in France and Ireland, in February and June 2019, according to the different types: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), and PUFA (polyunsaturated fatty acids).

Finally, PCA analysis revealed a seasonal distinction in the fatty acid composition for both species (Fig. 4a,b). Indeed, *H. siliquosa* exhibited an increase in (C20:5n-3) and a decrease in (C18:3n-3) in June (Fig. 4a,b). However, the TFA content was not significantly different between the sampling months, whatever the country (ANOVA,  $p$ -value = 0.411 for the factor ‘country’,  $p$ -value = 0.756 for ‘season’, and  $p$ -value = 0.985 for the interaction), such as the proportion of PUFA (ANOVA,  $p$ -value = 0.222 for the factor ‘country’,  $p$ -value = 0.567 for ‘season’, and  $p$ -value = 0.314 for the interaction). Concerning *S. muticum*, despite an equivalent TFA content at both sampling months (ANOVA,  $p$ -value < 0.001 for the factor ‘country’,  $p$ -value = 0.075 for ‘season’, and  $p$ -value = 0.480 for the interaction), the proportion of PUFA (in particular (C20:4n-6), (C20:5n-3), (C18:3n-3)) increased in June (ANOVA,  $p$ -value < 0.001 for the factors ‘country’ and ‘month’,  $p$ -value = 0.639 for the interaction),

reducing the proportion of MUFA (ANOVA,  $p$ -value  $< 0.001$  for the factors ‘country’ and ‘month’,  $p$ -value = 0.687 for the interaction) (Fig. 5).

#### 4. Discussion

Global change and human activities have significant impacts on marine ecosystems, including a predicted northward shift in the distribution of many species that are threatened by rising temperatures. More particularly, species which are at the limits of their distribution are the most threatened, and are likely to migrate, adapt or disappear. In this respect, the present study provides a geographical comparison of ecology and adaptive abilities of the Northern affinity species *Halidrys siliquosa* between France and Ireland. More interestingly, results are compared with the invasive *Sargassum muticum*, which is well-established in France, and at an earlier stage of invasion in Ireland. By coupling ecological and metabolomic data measured at two different seasons (February and June 2019), this study contributes to understand whether the Nordic species *H. siliquosa* is weakened by its southern position in France, and especially when co-existing with the invasive species *S. muticum* in intertidal rockpools.

##### 4.1 Phenological patterns: the same reproductive strategy for the native and invasive species?

The results confirmed differences between the phenology of both species, with a seasonal distinction in their ecology due to their specific reproductive period, whatever the country. Thus, *H. siliquosa* showed receptacles in February and June in both countries, coinciding with the previous studies demonstrating a large reproductive period. Indeed, *H. siliquosa* is described as a perennial macroalga (Wernberg *et al.* 2001; Pedersen *et al.* 2005), with a reproduction cycle as follows, according to Moss and Lacey (1963) carried out on English population: the development / maturation of the reproductive tissues (*i.e.* receptacles) between approximatively August and December, the gamete release between December and March, and germling development and vegetative growth of young thallus between March and July. Then, as the density and length of *H. siliquosa* did not show seasonal variation in the present study, this species exhibited a slow growth. Only the sheltered sites Finavarra (Ireland) and Porspoder (France) showed a seasonal difference, with small individuals in June compared to February. In this respect, *H. siliquosa* could grow more rapidly at these two sheltered sites; or else display a more limited recruitment.

In contrast to *H. siliquosa*, *S. muticum* was mature only during the summer and showed a considerable seasonal variation in length and density. Indeed, *S. muticum* is described as a pseudo-perennial species, with annual laterals growing from a perennial holdfast, with only one cohort occurring at the same time (Critchley 1983; Baer and Stengel 2010). Thus, *S. muticum* commonly reproduces in the spring-summer over a short reproductive period, approximatively between May and August (Baer and Stengel 2010; Le Lann *et al.* 2012, Lalegerie *et al.* in prep). Then, the detachment of annual laterals and the arrival of new recruits occur almost simultaneously in late summer / early fall, reducing the average length of *S. muticum*. The same pattern was observed in the present study, with large size classes in June before the detachment of laterals, and small size classes in February with the growth of new recruits produced the previous summer (Fig. 2). Similar ecological results were already described across different countries in Europe, including Atlantic populations in Spain (Arenas *et al.* 1995), France (Le Lann *et al.* 2012; Lalegerie *et al.* in prep.), or Ireland (Baer and Stengel 2010); populations of North sea in Denmark (Wernberg *et al.* 2001; Pedersen *et al.* 2005); or Mediterranean populations in Italy (Sfriso and Facca 2013). Then, *S. muticum* showed a greater length in summer on sheltered sites (*i.e.* Finavarra and Porspoder), which may have affected the density of *H. siliquosa*. Moreover, as a result of its shorter reproductive period, *S. muticum* is known to be a fast-growing species allowing recruits of the new generation to develop rapidly and reach sexual maturity in only few months, unlike *H. siliquosa* which is not fertile until the end of its second years old (Moss and Lacey 1963). It was shown that *S. muticum* has an average growth rate of 1 - 4 cm per day (Wernberg *et al.* 2001; Steen and Rueness 2004; Vaz-Pinto *et al.* 2014), with a maximum average length in mid-summer between 120 - 170 cm (Wernberg *et al.* 2001; Baer and Stengel 2010; Lalegerie *et al.* in prep). Recently Sfriso and Facca (2013) even reported a record in Venice Lagoon, with an individual's growth rate of 8.33 cm per day, reaching up to 485 cm in length. Conversely, *H. siliquosa* has a lower growth rate, resulting in a more constant size throughout the year (*pers. obs.*; Wernberg *et al.* 2001).

The different ecological phenology between both species implies then different nutrient requirements. In this way, some studies demonstrated that high nutrient availability increase growth and biomass of algal species, including *H. siliquosa* (Wernberg *et al.* 2001) and *S. muticum* (Steen and Rueness 2004; Sánchez and Fernández 2006; Incera *et al.* 2009; Vieira *et al.* 2017). Thus, *H. siliquosa* could take advantage of high nutrient levels in the winter to reproduce and store N reserves, contrary to the invasive species which reproduces in spring -

summer. In this sense, Wernberg *et al.* (2001) reported that the N:P (nitrogen to phosphorus) ratio was higher in January for *S. muticum* and in May for *H. siliquosa*. Thus, these authors suggested that P could be limiting in spring and N in summer, particularly for *Sargassum muticum*. However, Sfriso and Facca (2013) demonstrated with one year study that N:P ratio of *S. muticum* was close from those collected in water column (ratio of around 30), suggesting no limitation of growth. Then, considering only germlings under controlled conditions, Steen (2003) demonstrated that their growth was stimulated by a nutrient enrichment. In this sense, further culture experiments are also required to test nutrient limitation on both species, especially for the adult thalli.

#### **4.2 *Halidrys siliquosa*: a native species more sensitive than *S. muticum* to environmental fluctuations?**

Results revealed differences in metabolomic composition between both species, which could be specific adaptive responses to seasonal constraints / variations in biotic and abiotic parameters. In this way, pigment analyses showed a higher content in zeaxanthin and pheophytin-*a* in *H. siliquosa*, compared to *S. muticum* which contained more chlorophyll-*a* and violaxanthin among both countries, suggesting then a higher light sensitivity and photo-protection in the native species. Indeed, violaxanthin and zeaxanthin are both pigments involved in the xanthophyll cycle in Phaeophyceae (Goss and Jakob 2010): under low irradiance, brown seaweeds produce preferentially violaxanthin, whereas it is converted into antheraxanthin and then zeaxanthin under extreme irradiance, protecting thylakoid membrane lipids against photooxidation. In addition, some studies suggest that pheophytin-*a*, which is a degradation product of chlorophyll-*a* (Steinman *et al.* 2017), could have a photo-protective role on P680 (photosystem II primary donor) by unidirectional photo-damages (Hou 2014). Pigment data thus suggest a higher sensitivity of *H. siliquosa* to high light in June, contrary to *S. muticum*. In this way, Moss and Lacey (1963) showed that high light can inhibited the growth of *H. siliquosa*, whereas it reached a maximal length at 10 °C in total darkness. Conversely, *S. muticum* seems to be more resistant to light, since it reproduces with increasing light occurring in spring (Baer and Stengel 2010; Lalegerie *et al.* in prep), potentially involving other photoprotection mechanisms. However, to our knowledge, there are actually no data on the impact of light on the reproduction of *S. muticum*, which would require further investigation by culture experiments.

On the other hand, *S. muticum* showed a clear increase in the proportion of polyunsaturated

fatty acids (PUFA) in June, mainly due to linolenic acid (C18:3n-3), arachidonic acid (C20:4n-6) and eicosapentaenoic acid (C20:5n-3), contrary to *H. siliquosa* whose TFA and PUFA content did not change between both contrasted seasons (*i.e.* sampling months), maybe due to its less pronounced phenological changes between seasons. To our knowledge there are no study on the seasonal variation in the fatty acid composition of *H. siliquosa*. On the contrary, a such seasonal pattern in fatty acid composition of *S. muticum* (with a PUFA increase coupled to saturated (SFA) and monounsaturated (MUFA) fatty acids decrease in spring – summer) has already been demonstrated by Balboa *et al.* (2016) and Lalegerie *et al.* (in prep). Indeed, SFA and MUFA could be used as carbon storage for growth and reproduction (Guschina and Harwood 2013), whereas PUFA could be used as a multi-protective agent for the reproductive parts, *i.e.* receptacles, of the alga. In this sense, Los *et al.* (2013) suggested that unsaturation along aliphatic chains of fatty acids could be a chemical photo-protective mechanism by repairing damages of the photosystem II. In addition, PUFA which increased during summertime in *S. muticum*, could represent an antifouling strategy in this species. Indeed, an antibacterial activity of linoleic acid has been demonstrated, possibly by inhibiting bacterial fatty acid synthesis (Nieman 1954; Zheng *et al.* 2005).

Similarly, phenolic compounds showed a seasonal increase in June for *S. muticum*, whereas the difference was not significant for *H. siliquosa*, as already suggested by Gager *et al.* (2020). Phenolic compounds could thus represent multi-protective agents for the receptacles of *S. muticum*, against seasonal variations in light, temperature, salinity, nutrients or grazing as demonstrated in other brown macroalgae (Pavia *et al.* 1997; Connan *et al.* 2004; Plouguerné *et al.* 2006; Le Lann *et al.* 2012; Stiger-Pouvreau *et al.* 2014; Tanniou *et al.* 2013; Le Lann *et al.* 2016; Gager *et al.* 2020). Then, despite no seasonal variation in our study for *H. siliquosa*, this last species produced significantly higher total phenolic content (TPC) than *S. muticum*. In this sense, many studies have already demonstrated high level of phlorotannins in this native species (Zubia *et al.* 2009; Gager *et al.* 2020), with mainly diphlorethol, triphlorethol, trifuhalol and tetrafuhalol as phlorotannins, contrary to *S. muticum* which possesses only phlorethols (Tanniou *et al.* 2013; Stiger-Pouvreau *et al.* 2014). It has been shown that the high phenolic content of *H. siliquosa* could be correlated to antioxidant (Jiménez-Escrig *et al.* 2001; Le Lann *et al.* 2016; Gager *et al.* 2020) and photo-protective (Le Lann *et al.* 2016; Gager *et al.* 2020) activities, suggesting a global protection of the alga over the year contrary to *S. muticum*. Due to its pseudo-perennial ecological strategy with the detachment of its laterals, *S. muticum* avoids using energy in the protection and maintenance



of long-lived structures contrary to *H. siliquosa*, which leads to a seasonal protection of its receptacle with an increase in PUFA and TPC. Then, the invasive species can allocated more energy to reproduction and subsequent elongation along the year, responsible in part of its rapid expansion.

#### **4.3 Metabolomic composition: Do the adaptive abilities of both species differ between France and Ireland?**

Metabolomic data exhibited only few differences between France and Ireland, in response to the different environmental conditions between both countries (Table 1). In particular pigment composition revealed a clear geographical difference by exhibiting a higher seasonal photoinhibition in Ireland for both species, with higher pheophytin-*a* and zeaxanthin contents in June, compared to French populations which contained more violaxanthin and chlorophyll-*a*. However this difference could be due to the different nutrient and light conditions between both countries (Table 1). More particularly, there was a divergence in irradiance occurring in June, *i.e.* a significant increase in the duration of sunshine between May and June in Ireland, while the opposite was recorded in France. Pigment data thus revealed a monthly rapid light acclimation for both species.

Furthermore, results revealed geographical difference in fatty acids composition of *S. muticum*, which had higher polyunsaturated fatty acids content in France, especially in June. The same pattern was not observed for *H. siliquosa*, suggesting that this geographical difference was not correlated to abiotic environmental parameters. Then, depending on the potential roles given to PUFAs described in section 4.2, the higher rate of unsaturation found for populations in France could be related to the slightly higher rate of maturity of *S. muticum* ( $72.22 \pm 11.75\%$  in France compared to  $60.79 \pm 0.11\%$  in Ireland), or to a higher level of epiphytes. In this sense, Pedersen *et al.* (2016) hypothesized that invasive species could be less epiphytic grazed in the new area that they colonize (as native species fail in recognize the newly introduced species), which could explain the lower PUFA level in Ireland where *S. muticum* was less affected by epiphytes (*pers. obs.*). However, the results only included two sampling months, therefore it would be interesting to follow both species throughout a full year, which could highlight more differences in the specific metabolic acclimations between the two countries for a broader view.

Finally, contrary to *S. muticum*, *H. siliquosa* tended to exhibit a slightly higher TPC content in

France, particularly in June, although the difference was not significant. In this way, the results suggest that *H. siliquosa* populations experience greater stress in France. In this sense, it is possible that the native species, which has a northern affinity, may be weakened at its southern limit by higher temperatures, especially in summer. In this regard, it has been shown that *H. siliquosa* reproduces preferentially between 3 and 10 °C and that its germination success is considerably reduced over 20 °C (Moss and Sheader 1973), explaining its gamete release in winter (Moss and Lacey 1963). On the contrary, Steen and Rueness (2004) reported that *S. muticum* germlings had a considerably higher growth rate at 17 °C than at 7 °C (*i.e.*  $\sim 0.21 \text{ day}^{-1}$  and  $\sim 0.05 \text{ day}^{-1}$  respectively), supporting the fact that its reproduction occurs / is favoured by warm temperature occurring in spring-summer. Phenolic compounds could be thus a key component in the acclimation of *S. muticum* to increasing temperature, related to its ecological phenology.

#### 4.4 Geographical variation in population ecology: Towards an evolution of the French and Irish populations?

Despite similar reproductive phenology and acclimation abilities, ecological data revealed spatial differences in the density of the native *H. siliquosa*. Indeed, it exhibited a higher density in Ireland whatever the seasons, with an average of  $5.84 \pm 4.04$  individuals per quadrat compared to only  $0.92 \pm 1.35$  individuals per quadrat in France (Table 2). Noted that, as *H. siliquosa* was distributed in patches with a limited number of individuals in France, the estimated density (*i.e.* adjusted to  $1\text{m}^2$ ) is not representative of the observed density, and it is more appropriate to compare densities by keeping the size of the quadrats adapted to each population (*i.e.* measured density). Besides, *S. muticum* exhibited a similar density between both countries when comparing the measured densities (*i.e.* per specific size quadrat), although the adjusted density (*i.e.* per  $\text{m}^2$ ) tended to be higher in Ireland. This difference in the density of *H. siliquosa* could then be explained by increasing temperature with climate change, or by the co-habitation between the native and invasive species, and the fact that *S. muticum* is still in its earlier invasion stage in Ireland compared to France (Kraan 2007; Baer and Stengel 2010; Monagail and Morrison 2020). By contrast, *S. muticum* may not yet have stabilised its population in Ireland, which can still increase or decrease in future years. Indeed, in Ireland, attached individuals of *S. muticum* were first reported in 1995 (Kraan 2007; Baer and Stengel 2010), while the invasive species is more established in France as it was reported almost 30 years earlier, *i.e.* in the 1970s (Engelen *et al.* 2015; Stiger-Pouvreau

and Thouzeau 2015). In this sense, Steen (2003) concluded from experimental cultures that *S. muticum* germlings presented a low growth and low tolerance to competition at low temperature, which could weaken the species to its expansion in the North of Europe. Then, the lower number of individuals of *H. siliquosa* in France could be due to a competition for space, and the pressure exerted by the well-established invasive species. However, there are no previous data on the ecology and density of *H. siliquosa* in France to support these hypotheses, therefore emphasising the importance of monitoring species over a long term. Nevertheless, a competition for space between the two species has already been suggested by Bartsch and Tittley (2004) in Helgoland. In addition, despite a higher density of *H. siliquosa* in Ireland, *S. muticum* reached a larger size in summer than the native species, contrary to the French populations, which could then lead to a competition for light or nutrient availability. Thus, the data suggest that the Irish population could tend to evolve, with the decline of either species, despite different phenologies and reproductive strategies. In this sense, many studies have already reported that *S. muticum* could lead to the regression of native species, such as *Laminaria* spp., or *Fucus* spp. (Stæhr *et al.* 2000; Brittons-Simmons 2004; Sánchez *et al.* 2005). More specifically, Stæhr *et al.* (2000) have reported that the expansion of *S. muticum* in Limfjorden (Denmark) between 1984 and 1997 resulted in a decrease of *H. siliquosa* cover over 50 %. According to Wernberg *et al.* (2001) and Pedersen *et al.* (2005) which previously compared the phenology and biomass dynamic of *H. siliquosa* and *S. muticum* in Limfjorden (Denmark), the competitive success of *S. muticum* could be caused by its pseudo-perennial life history, *i.e.* similarly to our results, a high seasonality for *S. muticum* with a fast-growing compared to the native species. In addition, Pedersen *et al.* (2005) highlighted a fast decomposition of *S. muticum*, increasing turnover of organic matter and associated nutrients. These authors suggested then that the effect of the invasion of *S. muticum* were similar to those from ephemeral algae following eutrophication, impacting native species. Similarly, the ecology of both species could evolve in France and Ireland, and even faster with global change, since their phenologies could be affected by environmental shifts. In this way, our results and those from previous studies suggested that increasing temperature, irradiance or nutrients availability could favour the reproduction of *S. muticum*. More particularly, seawater temperature from Brittany (France) has already increased by 0.7 °C on average over the past two decades (Gallon *et al.* 2014), and seawater temperature on Ireland coast is predicted to increase by the 2050s, with more than 2 °C higher than the actual temperature, causing changes in the distribution and abundance of species (Hiscock *et al.* 2004). On the contrary,

metabolomic results suggested a higher sensitivity of *H. siliquosa* to an increase in light and temperature, the main factor controlling algal growth and reproduction (Agrawal 2012; Lalegerie *et al.* 2020). Climate change could thus lead to the regression of the population of *H. siliquosa* in the long term. More specifically, the southern populations of *H. siliquosa* in France, which is a species with Nordic/boreal affinity (Dizerbo and Herpe 2004), could then completely disappear from the intertidal zone (rockpools) and be definitively substituted by *S. muticum* in this upper area. However, Lima *et al.* (2009) published the first record of *H. siliquosa* in tide pools along the Portuguese coast, going against all climatological predictions.

In conclusion, this study provided for the first time the evidence that native and invasive populations of brown Sargassaceae exhibit geographical differentiation, suggesting a potential disappearance of the Nordic *H. siliquosa* at its southern limit in France, and a potential regression in Ireland in the long term, if the seawater temperature pursues its increase in the Northwest part of Europe. In addition, our results supported the hypothesis that the competitive success of *S. muticum* could be caused by its pseudo-perennial life history and that invasive species could further weaken native species at the edge of their southern distribution range in the context of global change.

## 5. Acknowledgements

Fanny Lalegerie's PhD fellowship was provided from Region Bretagne and the Doctoral School of Marine and Littoral Sciences of the Université Bretagne Loire (UBO, Brest, France). F. Lalegerie and S. Connan's mobility grants to Ireland were provided by « Direction Europe et Internationale » from UBO. We thanks Thomas Burel and Leslie Gager for helping on field monitoring.

## 6. Author's contribution

F.L. performed the analysis of the samples, drafted the manuscript and conducted the statistical analyses. V.S.-P. and S.C. designed and supervised the project. M.Z. and D.B.S. contributed to the field sampling in Ireland. A.B and F.L.G supervised the extraction and analysis of fatty acids.

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## Supplementary data

**Table 1.** Ratio of the different pigments (%) with respect to the total pigment concentrations in *Halidrys siliquosa* and *Sargassum muticum* collected in France and Ireland, in February and June 2019. The results correspond to the average of the 3 sites.

Pigment	<i>Halidrys siliquosa</i>				<i>Sargassum muticum</i>			
	France		Ireland		France		Ireland	
	February	June	February	June	February	June	February	June
Chlorophyll- <i>a</i>	56.42 ± 8.11	50.08 ± 7.53	44.79 ± 12.07	17.44 ± 9.7	74.69 ± 2.61	73.44 ± 1.15	70.42 ± 4.43	58.5 ± 19.13
Pheophytin- <i>a</i>	15.83 ± 7.38	24.12 ± 7.26	33 ± 12.98	63.68 ± 11.65	1.9 ± 2.03	1.96 ± 3.46	10.43 ± 5.54	18.99 ± 19.74
Violaxanthin	0.77 ± 0.23	0.76 ± 0.24	0.59 ± 0.35	0.08 ± 0.14	1.61 ± 0.43	2.53 ± 0.65	0.94 ± 0.39	0.99 ± 0.62
Zeaxanthin	<i>n.d.</i>	0.09 ± 0.08	0.34 ± 0.85	0.25 ± 0.28	<i>n.d.</i>	0.04 ± 0.04	0.01 ± 0.03	0.23 ± 0.16
β-carotene	1.68 ± 0.25	1.41 ± 0.02	1.73 ± 0.39	1.8 ± 0.08	0.78 ± 0.2	0.86 ± 0.25	0.64 ± 0.11	1.06 ± 0.23
Chlorophyll- <i>c</i> <sub>2</sub>	5.62 ± 0.37	5.62 ± 0.28	6.04 ± 0.39	6.06 ± 0.18	5.13 ± 0.19	5.25 ± 0.34	5.69 ± 0.29	5.76 ± 0.5
Fucoxanthin	19.69 ± 1.27	17.92 ± 0.38	13.5 ± 1.65	10.68 ± 2.04	15.88 ± 1.64	15.91 ± 2	11.88 ± 1.25	14.48 ± 0.76

**Table 2.** Seasonal fatty acid composition (% of the total fatty acids content) and total fatty acids (TFA) content (mg.g<sup>-1</sup> DW) in *Halidrys siliquosa* and *Sargassum muticum* collected in France and Ireland, in February and June 2019. The results correspond to the average of the 3 sites. The fatty acids with the highest proportions are in bold. SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids. nd = not detected

% Fatty acids	<i>Halidrys siliquosa</i>				<i>Sargassum muticum</i>			
	France		Ireland		France		Ireland	
	February	June	February	June	February	June	February	June
iso15:0	0.22 ± 0.06	0.48 ± 0.01	0.39 ± 0.22	0.33 ± 0.08	0.51 ± 0.12	0.41 ± 0.03	0.5 ± 0.06	0.43 ± 0.06
iso17:0	0.30 ± 0.03	0.38 ± 0.04	0.25 ± 0.04	0.32 ± 0.03	1.30 ± 0.17	0.93 ± 0.27	1.15 ± 0.11	0.92 ± 0.09
TMTD	0.29 ± 0.06	0.21 ± 0.01	0.20 ± 0.05	0.18 ± 0.05	0.50 ± 0.07	0.28 ± 0.09	0.58 ± 0.10	0.19 ± 0.06
16:0DMA	0.35 ± 0.03	0.36 ± 0.02	0.30 ± 0.03	0.27 ± 0.03	0.46 ± 0.06	0.33 ± 0.06	0.46 ± 0.06	0.24 ± 0.02
18:0DMA	0.44 ± 0.02	0.42 ± 0.01	0.41 ± 0.03	0.34 ± 0.05	0.79 ± 0.09	0.49 ± 0.06	0.86 ± 0.16	0.36 ± 0.05
<b>14:0</b>	<b>6.99 ± 0.47</b>	<b>5.17 ± 0.15</b>	<b>6.32 ± 0.59</b>	<b>5.71 ± 0.41</b>	<b>2.56 ± 0.29</b>	<b>3.26 ± 0.17</b>	<b>3.08 ± 0.29</b>	<b>2.69 ± 0.15</b>
15:0	0.35 ± 0.03	0.30 ± 0.00	0.33 ± 0.03	0.29 ± 0.01	0.31 ± 0.03	0.23 ± 0.02	0.39 ± 0.03	0.21 ± 0.01
<b>16:0</b>	<b>20.63 ± 1.29</b>	<b>19.77 ± 0.09</b>	<b>19.56 ± 1.12</b>	<b>19.86 ± 0.54</b>	<b>26.88 ± 2.89</b>	<b>25.29 ± 0.73</b>	<b>28.65 ± 1.59</b>	<b>27.55 ± 0.59</b>
18:0	1.13 ± 0.16	0.82 ± 0.06	1.01 ± 0.14	0.94 ± 0.14	0.64 ± 0.15	0.51 ± 0.04	0.76 ± 0.05	0.67 ± 0.07
20:0	0.69 ± 0.08	0.64 ± 0.02	0.66 ± 0.06	0.68 ± 0.07	0.33 ± 0.03	0.31 ± 0.01	0.34 ± 0.02	0.44 ± 0.02
22:0	0.49 ± 0.05	0.45 ± 0.01	0.55 ± 0.07	0.49 ± 0.07	0.53 ± 0.08	0.63 ± 0.04	0.57 ± 0.04	0.87 ± 0.09
24:0	0.51 ± 0.05	0.49 ± 0.04	0.61 ± 0.12	0.55 ± 0.07	0.27 ± 0.08	0.27 ± 0.05	0.23 ± 0.11	0.32 ± 0.04
16:1n-5	0.05 ± 0.02	0.06 ± 0.01	0.06 ± 0.02	0.08 ± 0.03	0.10 ± 0.03	0.11 ± 0.01	0.15 ± 0.09	0.11 ± 0.01
<b>16:1n-7</b>	<b>1.39 ± 0.07</b>	<b>1.33 ± 0.06</b>	<b>1.47 ± 0.19</b>	<b>1.21 ± 0.10</b>	<b>5.01 ± 0.85</b>	<b>4.39 ± 0.48</b>	<b>5.59 ± 0.48</b>	<b>4.26 ± 0.44</b>
16:1n-9	0.21 ± 0.02	0.27 ± 0.02	0.26 ± 0.05	0.24 ± 0.05	0.22 ± 0.02	0.16 ± 0.02	0.27 ± 0.02	0.15 ± 0.01
18:1n-5	0.07 ± 0.03	0.09 ± 0.01	0.10 ± 0.02	0.11 ± 0.02	0.17 ± 0.05	0.12 ± 0.02	0.26 ± 0.08	0.10 ± 0.01
18:1n-7	0.21 ± 0.07	0.30 ± 0.01	0.26 ± 0.06	0.25 ± 0.10	0.36 ± 0.16	0.25 ± 0.11	0.45 ± 0.09	0.33 ± 0.09
<b>18:1n-9</b>	<b>17.26 ± 1.14</b>	<b>17.81 ± 1.28</b>	<b>16.92 ± 1.84</b>	<b>17.99 ± 2.40</b>	<b>9.34 ± 1.02</b>	<b>8.86 ± 0.32</b>	<b>9.93 ± 0.99</b>	<b>10.06 ± 0.41</b>
20:1n-7	n.d	n.d	n.d	n.d	1.75 ± 0.31	1.34 ± 0.11	2.14 ± 0.16	1.50 ± 0.19
20:1n-9	0.08 ± 0.02	0.15 ± 0.01	0.10 ± 0.04	0.09 ± 0.04	2.13 ± 0.23	1.97 ± 0.08	2.30 ± 0.11	2.40 ± 0.12
22:1n-11	n.d	n.d	0.02 ± 0.02	n.d	2.20 ± 0.12	1.73 ± 0.16	2.48 ± 0.14	1.99 ± 0.15
22:1n-7	n.d	n.d	n.d	n.d	0.10 ± 0.01	0.06 ± 0.00	0.14 ± 0.02	0.07 ± 0.02
22:1n-9	0.10 ± 0.02	0.11 ± 0.02	0.07 ± 0.03	0.08 ± 0.02	0.11 ± 0.04	0.11 ± 0.02	0.09 ± 0.02	0.05 ± 0.02
24:1n-9	n.d	n.d	n.d	n.d	0.26 ± 0.06	0.16 ± 0.01	0.28 ± 0.04	0.19 ± 0.05
16:2n-4	0.10 ± 0.02	0.08 ± 0.02	0.12 ± 0.03	0.06 ± 0.04	0.47 ± 0.03	0.39 ± 0.04	0.52 ± 0.07	0.37 ± 0.03
16:2n-6	0.08 ± 0.02	0.09 ± 0.01	0.09 ± 0.02	0.10 ± 0.02	0.05 ± 0.04	0.05 ± 0.01	0.09 ± 0.01	0.09 ± 0.02
16:3n-3	0.21 ± 0.01	0.23 ± 0.03	0.18 ± 0.01	0.20 ± 0.03	0.25 ± 0.06	0.24 ± 0.03	0.24 ± 0.03	0.23 ± 0.04
16:3n-6	0.18 ± 0.02	0.13 ± 0.00	0.16 ± 0.02	0.14 ± 0.01	0.11 ± 0.02	0.07 ± 0.01	0.13 ± 0.01	0.08 ± 0.01
16:4n-3	0.09 ± 0.01	0.14 ± 0.02	0.05 ± 0.01	0.10 ± 0.07	0.04 ± 0.05	0.01 ± 0.02	0.06 ± 0.02	0.01 ± 0.02
<b>18:2n-6</b>	<b>7.50 ± 0.75</b>	<b>3.76 ± 0.17</b>	<b>6.36 ± 1.16</b>	<b>4.46 ± 0.36</b>	<b>6.08 ± 0.82</b>	<b>5.58 ± 0.23</b>	<b>6.46 ± 0.82</b>	<b>6.06 ± 0.38</b>
<b>18:3n-3</b>	<b>8.05 ± 0.28</b>	<b>7.78 ± 0.44</b>	<b>7.73 ± 0.44</b>	<b>7.89 ± 0.32</b>	<b>8.00 ± 1.26</b>	<b>9.26 ± 0.30</b>	<b>6.08 ± 0.53</b>	<b>7.78 ± 0.21</b>
18:3n-6	0.71 ± 0.14	0.46 ± 0.01	0.58 ± 0.13	0.37 ± 0.05	0.71 ± 0.06	0.52 ± 0.12	0.61 ± 0.07	0.47 ± 0.08
18:4n-1	n.d	n.d	n.d	n.d	0.05 ± 0.09	0.04 ± 0.05	0.23 ± 0.10	0.08 ± 0.05
<b>18:4n-3</b>	<b>6.87 ± 1.04</b>	<b>8.89 ± 0.55</b>	<b>7.07 ± 0.71</b>	<b>7.84 ± 1.07</b>	<b>6.58 ± 1.38</b>	<b>6.00 ± 0.55</b>	<b>4.38 ± 0.71</b>	<b>4.29 ± 0.25</b>
20:2n-6	0.37 ± 0.05	0.31 ± 0.02	0.38 ± 0.04	0.38 ± 0.05	0.26 ± 0.04	0.23 ± 0.04	0.33 ± 0.04	0.20 ± 0.05
20:3n-3	0.17 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.11 ± 0.01	0.14 ± 0.02	0.11 ± 0.01	0.11 ± 0.02
20:3n-6	0.75 ± 0.09	0.49 ± 0.02	0.71 ± 0.14	0.59 ± 0.06	0.79 ± 0.12	0.66 ± 0.04	0.83 ± 0.13	0.67 ± 0.09
20:4n-3	0.93 ± 0.09	1.09 ± 0.03	1.09 ± 0.05	1.12 ± 0.14	0.54 ± 0.08	0.64 ± 0.06	0.45 ± 0.09	0.61 ± 0.06
<b>20:4n-6</b>	<b>16.66 ± 1.00</b>	<b>19.18 ± 0.41</b>	<b>19.48 ± 0.85</b>	<b>19.89 ± 0.87</b>	<b>11.72 ± 0.65</b>	<b>14.22 ± 0.95</b>	<b>11.56 ± 0.80</b>	<b>14.03 ± 0.67</b>
<b>20:5n-3</b>	<b>5.55 ± 0.85</b>	<b>7.57 ± 0.28</b>	<b>5.98 ± 0.43</b>	<b>6.67 ± 0.97</b>	<b>7.41 ± 1.27</b>	<b>9.75 ± 1.29</b>	<b>6.27 ± 1.00</b>	<b>8.82 ± 0.54</b>
% SFA	30.79 ± 1.71	27.64 ± 0.15	29.03 ± 0.87	28.52 ± 0.93	31.53 ± 3.43	30.5 ± 0.93	34.02 ± 1.77	32.75 ± 0.85
% MUFA	19.38 ± 1.1	20.11 ± 1.33	19.27 ± 2.13	20.05 ± 2.43	21.74 ± 1.85	19.25 ± 0.90	24.07 ± 1.45	21.23 ± 0.69
% PUFA	48.24 ± 2.64	50.40 ± 1.18	50.15 ± 2.86	49.99 ± 3.04	43.16 ± 4.38	47.82 ± 1.64	38.36 ± 2.62	43.88 ± 1.14
TFA (mg.g <sup>-1</sup> )	11.58 ± 0.99	11.38 ± 0.64	11.14 ± 1.82	10.96 ± 1.77	13.34 ± 2.09	14.63 ± 1.20	10.76 ± 0.90	11.33 ± 1.64



## Conclusion of chapter 3

The aim of this chapter was to establish spatio-temporal eco-metabolomic profiling and assess the acclimation abilities and potential changes in distribution of native / invasive species face to future environmental changes. In this way, two native species from Brittany were studied, *i.e.* ***Bifurcaria bifurcata* (part 1)**, and ***Halidrys siliquosa* (part 2)**. These two native species exhibited similarities in their phenology and composition:

- a moderate seasonality in ecological data (*i.e.* no significant variation in length, circumference or density over the year for *H. siliquosa*, or very slight decrease in length in spring and autumn for *B. bifurcata* due to recruitment), due to their perennial life cycle;
- the observation of mature individuals almost all year-round (although reproduction seemed to occur preferentially in winter), coupled to the simultaneous occurrence of several cohorts;
- a high sensitivity to increasing light and temperature, resulting in high level of pheophytin-*a* in spring (and zeaxanthin for *H. siliquosa*), coupled to a significant decrease in chlorophyll-*a* and polyunsaturated fatty acids (PUFA);
- a moderate seasonality in phenolic content (*i.e.* very slight increase in winter for *H. siliquosa* and in summer for *B. bifurcata*).

These results were then compared with ecological and metabolomic phenology of the invasive ***Sargassum muticum***. This species exhibited:

- a high seasonality in ecology due to its pseudo-perennial life cycle, *i.e.* an increase in length from spring to summer, and then the detachment of annual laterals from perennial holdfasts at the end of the summer, allowing the dissemination of germlings;
- only one cohort occurred at the same time due to its pseudo-perennial strategy;
- a spatial difference in the length / detachment of the laterals, which have been shown to be dependent on hydrodynamic conditions, causing a delay in recruitment;
- a high reproduction over a short period in spring / summer that seems to be favoured under warm temperature, leading to the arrival of juveniles in late summer / early fall;
- high metabolomic variations, *i.e.* a significant increase in PUFA and phenolic content in summer, coinciding with the reproductive period.

In this way, the results demonstrated ecological variations between the native species and the invasive *S. muticum*, with differential phenology. Indeed, native species have perennial life cycle, involving a slow-growth strategy, resulting subsequently in low seasonal changes in populations. On the contrary, invasive species generally present a high-growth strategy, taking advantage of favourable conditions to develop, reaching high size / sexual maturity in only few months as observed with *S. muticum*. This illustrates the variability of ecological strategies/responses within algal communities, as species do not all have the same acclimation abilities. In this regard, the reproduction and growth of *S. muticum* seem to be favoured by warm temperature as already suggested (Norton 1977; Cacabelos *et al.* 2013).

These different strategies result in differences in metabolomic composition. In this way, native species have to maintain / provide energy towards growth and reproduction throughout the year, involving storage, low metabolomic demand and subsequently limited biomass renewal. Likewise, the presence of several cohorts during the year and the maintenance of a constant population require permanent protection. In this regard, native species reproduced preferentially in winter, suggesting a greater sensitivity of fertile individuals to high light and/or temperature. Then, phenolic compounds exhibited reduce seasonality and could be the result of a constant protection of individuals over the year. However, native species showed also an increase in photoprotective compounds in spring and summer, indicating the ability to induce punctually a greater stress tolerant strategy, *i.e.* against high light. Then, it is unclear how native algal populations would cope with prolonged stress, particularly in the context of climate change.

On the other hand, *S. muticum* is a pseudo-perennial species that avoid using energy in the protection and maintenance of long-lived laterals, allowing to allocate more energy for reproduction or growth / development of young life stages in spring-summer. More particularly, this strategy allows *S. muticum* to seasonally accumulate rapidly high amount of metabolites. In addition, this implies probably a greater metabolomic variability between and within individuals / thallus, with a protection localised / concentrated only on the reproductive parts / young recruits. Indeed, since *S. muticum* presents only one cohort at the same time, such a metabolomic strategy enhanced its protection and aids its survival by reducing the risk of mortality in the early stages of development, and therefore avoiding its populations disappearance if recruitment fails. In this regard, *S. muticum* showed a lower decrease in

chlorophyll-*a* and a limited synthesis of photoprotective compounds in spring / summer compared to native species, suggesting a less light-sensitivity. On the contrary, *S. muticum* exhibited a high seasonal increase in phenolic content in response to increasing light and temperature, compared to both native algae. More interestingly, *S. muticum* showed an increase in PUFA in summer, at the opposite to the seasonal pattern of native species. Thus these compounds could have an important role in the reproduction, growth or protection of the invasive species. Consequently, *S. muticum* appeared to have a greater plasticity than native species.

In this context, the invasive species appear to be more resistant to cope with important environmental changes, contrary to native species which exhibited a limited plasticity. It has been suggested for example that the heatwave during Spring-Summer 2003 may have favoured the development and thus thallus length of *S. muticum* (Atkinson *et al.* 2020). Thus, increasing temperature could favour the reproduction of *S. muticum* over a longer period, and subsequently enhance its invasive potential. On the contrary, the present results suggested that native species could be greatly weakened with the rise in temperature and the increasingly frequent occurrence of long and strong heatwaves. In addition, the analysis of the terpene composition of *B. bifurcata* in **part 1.1** has shown that this species is particularly sensitive to hydrodynamic conditions, and could be threatened by changes in climatic conditions. Nevertheless, it is difficult to assess the acclimation / adaptive potential of each species given the complexity of the marine environment, including multiple stressor effects, various interactions with and within communities, and the different levels of response (*i.e.* individuals, populations or ecosystems). Thus, although *S. muticum* appears to be more resilient to high environmental changes, some evidence suggests that it may also be weakened. Notably, an increase in the occurrence / intensity of storms and hydrodynamic conditions could weaken the invasive species, by causing early lateral detachment and disrupting its recruitment.

More generally, the phenology and interactions between species could be altered by environmental shifts. As a result, algal populations will have to adapt to new environmental conditions, otherwise they will disappear. In this way, the synthesis of metabolites could be a key factor in the adaptation of macroalgae as already described in **Chapter 2** with red macroalgae. Furthermore, the data provided in this chapter are not enough to determine how

the algae will adapt given the richness of metabolites and the wide range of biological functions undertaken by each of these metabolites (including pigments, phenolic compounds and fatty acids), which can vary according to many biotic or abiotic factors. This enhance the necessity to perform cultivation experiments, or to study more metabolites based on the "eco-metabolomic" approach. In this respect, a cultivation experiment on brown seaweeds has been initiated, but cancelled because of the COVID-19 (Fig. 62).

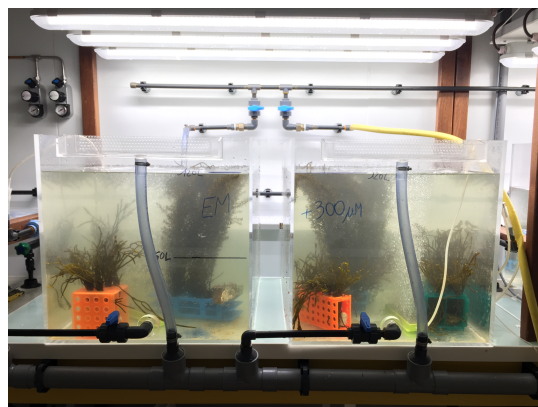


Figure 62. Illustration of the cultivation experiment initiated on brown macroalgae in March 2020, but cancelled due to COVID-19.

Another way of adapting is to migrate to other geographical areas with more favourable conditions. In this respect, **part 2.2** of this chapter included a geographical comparison of the population of *H. siliquosa* and *S. muticum* between France and Ireland, monitored in January and June 2019. Indeed, *H. siliquosa* is a cold-water affinity species with its French southern limit of distribution near Brittany, and that could be weakened by climate change. On the other hand, *S. muticum* presents a different level of invasion between the two countries (*i.e.* well-established in France compared to an earlier stage of invasion in Ireland). Then, results showed notably:

- a limited density of French populations of *H. siliquosa*, compared to a similar density between the French and Irish populations of *S. muticum*;
- a 10 % higher reproductive rate in France for *S. muticum*, coupled with a higher summer increase in PUFA content;
- no seasonal or spatial differences in maturity rate or fatty acid content for *H. siliquosa*;
- a limited growth of *S. muticum* on exposed sites whatever the country, contrary to *H. siliquosa*;
- a spatial difference in seasonal pattern of pigment composition whatever the species, due to a greater light irradiance in June in Ireland, resulting in the synthesis of zeaxanthin in June by Irish population of *H. siliquosa*, contrary to violaxanthin by French populations. Similarly, there was a high increase in violaxanthin and chlorophyll-*a* content in June for French population of *S. muticum*, while Irish populations exhibited no significant seasonal change.



According to these results, *H. siliquosa* seems to be weakened in France as suggested by limited density. This could be the result of a combination of factors, including higher temperatures / light radiation throughout the year, or more challenging competition with the invasive species *S. muticum*. Indeed, *S. muticum* was introduced in France 30 years earlier than in Ireland (Critchley 1983; Kraan 2007). Then results from **part 1.2** showed ecological and metabolic data for French *S. muticum* similar to 2006 (Le Lann *et al.* 2012) suggesting that this species has stabilised its population in Brittany, unlike Irish populations. In this way, although *S. muticum* exhibited similar length and density between France and Ireland, its reproductive rate was 10 % higher in France than Ireland, coupled with a higher PUFA content. This suggests a reduced plasticity of invasive species in Ireland. Likewise, the Irish populations of *S. muticum* could be threatened by an intensification of hydrodynamic conditions as suggested by intra-sites variability. Thus, it is possible that both *H. siliquosa* and *S. muticum* will be led to evolve to adapt their populations to changing environment, modifying the interaction between the two species in both countries. In this sense, the French populations of *H. siliquosa* may be challenged to manage both climate change and co-habitation. Thus, three possibilities can be considered: (i) *H. siliquosa* will adapt / increase the synthesis of protective compounds, to the detriment of its reproduction or growth. Nevertheless, the data did not show any metabolic difference in favour of the French populations; (ii) The French intertidal populations will disappear, restricting *H. siliquosa* to subtidal populations, or reducing its distribution range; (iii) *H. siliquosa* will migrate further northwards where it could however be confronted with more competition from *S. muticum*. This remind the importance of monitoring species over a long term as it is not possible to predict with certainty how the populations will evolve. In this sense Stæhr *et al.* (2000) have reported that the expansion of *S. muticum* in Limfjorden (Denmark) between 1984 and 1997 resulted in a decrease of *H. siliquosa* cover over 50 %. The monitoring of populations is all the more important as there may be microhabitats, *i.e.* a small habitat constituting a subset of a larger habitat, which has different physical or chemical characteristics. Indeed, the spatio-temporal variations in terpenes composition of *B. bifurcata* (**part 1.1**) highlighted difference between and within sites depending on hydrodynamic conditions. In this way, these microhabitats can contribute to the local adaptation of species, or create refuge areas for species threatened by climate change.

In conclusion, although it is impossible to predict future adaptation of algal populations to

future environmental changes with certainty, all publications in this chapter have provided a comprehensive overview of the phenotypic and metabolic variations of some native or invasive brown algal species in Brittany. Then, results obtained in this chapter participate to a better understanding of the acclimation abilities of these Breton algal populations.



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***General conclusion and  
perspectives***

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This project, based on the study of five macroalgal species, three native and two introduced ones species living in Brittany (France), has considered the following problematics:

- What is the current status of the algal populations of studied species in Brittany? *i.e.* using an ecological approach and an "eco-metabolomic" approach, is there any spatio-temporal variability in the ecology and metabolomic composition of these populations?
- Are there differences in acclimation between native and introduced species, related to different environmental factors? More particularly, will introduced species experience an increase in their invasive potential to the detriment of native species?
- Could global change have an effect on the synthesis and metabolomic composition of species? In other words, could certain metabolites have a key-role in the acclimation of macroalgal populations and thus participate in their future adaptation?
- Finally, are some species more likely than others to disappear in a context of global change? In particular, how will evolve native species near their limit of distribution range, knowing that these species will have to cope with multiple changes?

In this way, I decided to study both red (**Chapter 2**) and brown (**Chapter 3**) macroalgae, which constitute two distinct taxonomic groups and have thus a potentially different metabolic composition and adaptive capacities (Stengel *et al.* 2011). More particularly, the **Chapter 2** focused on red macroalgae and their production of mycosporine-like amino acids, compounds whose different roles and synthesis pathways have not yet been fully elucidated (Bedoux *et al.* 2020; Belcour *et al.* 2020). These secondary metabolites, otherwise very diversified, could actively participate in the future adaptation of red algae. The second part of this work (**Chapter 3**) focused on Sargassaceae species, a diversified family of brown macroalgae, including two common species along the rockyshores of Brittany and the widespread invasive *Sargassum muticum*.

### ***Effects of environmental fluctuations on secondary metabolites and the need to combine the ecology with metabolomics***

The acclimation potential of the different macroalgal species depends in part on their metabolome, which constitutes a key component in their subsequently adaptation. In this regard, a screening of mycosporine-like amino acids in red algae (**Chapter 2**) revealed a high

heterogeneity in the metabolic composition between species, with some species showing a greater diversity than others. The species with the highest metabolic diversity seem to be more likely to acclimate and adapt to environmental changes, although some changes could favour the rapid accumulation of specific compounds. On the other hand, there is currently limited data on the effect of global change on the production of metabolites, and whether species will develop new metabolic abilities/functions or induce the synthesis of particular compounds to enhance their survival. In addition, all species do not possess the same precursors / intermediates / enzymes and resulting synthesis pathways, leading in a different metabolic diversity and thus metabolic strategy. More particularly, the global change could lead to new metabolic pathways or "pathway drift" through divergent gene expression. In this way, new metabolites structures could be induced in some species under certain triggering factors. As suggested by [Belcour \*et al.\* \(2020\)](#) which combined genomic and metabolomic data from the red alga *Chondrus crispus*, this could lead to various complex metabolic network, as discussed in **Chapter 2** regarding MAAs. Consequently, it is difficult to predict the future adaptation of algal populations, which will be different for each species. This adaptation could even be different within species (*i.e.* inter-population heterogeneity) as secondary metabolites act as a chemical interface between organisms and their specific surrounding environment. This feature supports the interest of studying complementary approaches such as eco-metabolomic, and then to study the becoming of marine macroalgae by coupling ecology and metabolomic.

Indeed, macroalgae inhabit a complex environment, and experience a diverse range of abiotic and biotic environmental fluctuations, anthropogenic stresses and threats from invasive species. Given the multiplicity of interactions that can occur under these conditions, it is therefore difficult to interpret the ecological responses and determine which factors impact the different algal populations and how they will adapt locally. In this regard, metabolites are involved in many biological processes. The study of global metabolic profiles, coupled to ecological data can then provide a better understanding of adaptive abilities of macroalgae. In this perspective, and in order to respond to the different problematics, I choose to carry out ecological and metabolomic monitoring on different natural algal populations of Brittany (France) and Galway bay (Ireland), based on the emergent "eco-metabolomic" approach ([Jones \*et al.\* 2013](#); [Kumar \*et al.\* 2016](#); [Belghit \*et al.\* 2017](#); [Peters \*et al.\* 2018](#)). In this regard, I have tried to study as many algal metabolites as possible (*i.e.* chlorophylls, carotenoids,

phycobiliproteins, MAAs, fatty acids, phenolic compounds and terpenes), although I was aware that this represents a limited subset of the overall metabolomic composition of macroalgae (*i.e.* more than 10,000 metabolites), and that this does not constitute a global profile in any way. So far, there are few *in-situ* studies that link the ecology of algae to so many compounds. Then, to link ecology to metabolomics, cultivation experiments are required to (i) determine *ex-situ* which particular environmental factors impact the ecology of species, and could be responsible of the synthesis of specific metabolites, and (ii) clarify the different metabolite synthesis pathways. In this sense, a cultivation experiment was carried out on the red alga *Palmaria palmata*, which showed the effect of light and nutrients on the production of pigments and MAAs. More particularly, this experiment demonstrated that shinorine and usurijene could have a key role in the adaptation of this species to increasing light radiation (**Chapter 2**). In this way, the "eco-metabolomic" approach allowed to bring new elements regarding to the acclimation processes of the different studied species.

This "eco-metabolomic" approach has required the development of extraction and analysis techniques for the different studied metabolites (**Chapter 1**). In this sense, all protocols developed have achieved satisfactory results, although there are still improvements to achieve. In particular, the extraction and analysis of MAAs using 70 % ethanol and milliQ water + 0.1 % acetic acid, respectively, provided reliable results, constituting an eco-responsible / green method, without methanol as commonly used in the literature. However, it was not possible to purify and identify / quantify all MAAs, so further assays are needed to obtain standards for these compounds (not commercially available). In this respect, a collaboration with Pr. Ulf Karsten (University of Rostock, Germany), Dr. Markus Ganzera and Dr. Anja Hartmann (University of Innsbruck, Austria) has provided encouraging initial results (not presented in this manuscript). In the same way, the TLC analysis of terpenes did not allow to identify / quantify these compounds, which requires the development of purification methods by solid phase extraction (SPE), followed by an analysis by HPLC for example. Regarding the phenolic compounds, their separation and identification using LC-MS have started during this PhD (see **section 4.5 of Chapter 1**) and are to be continued.

### *Current status of native algal populations in Brittany (France)*

In this project, three macroalgal species native to Brittany were particularly studied: the red *Palmaria palmata*, and two brown macroalgae, *Bifurcaria bifurcata* and *Halidrys siliquosa*. So far, previous studies on *P. palmata* have mostly focused on its tank / semi-controlled cultivation and its nutritional value due to its economic importance for human consumption or aquaculture (Galland-Irmouli *et al.* 1999; Le Gall *et al.* 2004b; Beaulieu *et al.* 2016). Similarly, the ecology of *B. bifurcata* populations is little reported (Connan *et al.* 2007; Le Lann *et al.* 2012; Méndez-Sandín and Fernández 2016), as previous studies focused generally only on its terpene composition in terms of biological activities and potential valorisation (Hellio *et al.* 2001; Alves *et al.* 2016; Pais *et al.* 2019), and this despite its ecological importance, through its participation in the structuring of intertidal communities in Brittany. Likewise, to my knowledge, few studies have reported on the ecology of *H. siliquosa*, except some studies carried out in Denmark (Wernberg *et al.* 2001, 2004; Pedersen *et al.* 2005), and none in Brittany. In this way, the results obtained from this work, by the study of the eco-metabolomic variations occurring in the natural populations of these three species, provide new knowledge on their acclimation abilities.

The three studied native macroalgae have in common to be perennial species, reproducing preferentially in winter. They also displayed spatial and temporal variations in their metabolic profiles and ecological pattern, suggesting different environmental acclimation abilities. Based on these data, natural populations of *P. palmata* and *B. bifurcata* appeared in good ecological status, *i.e.* exhibiting regular growth with a seasonal renewal of populations, relatively high density, thalli without physical damage or permanent depigmentation, and low epiphytism. In this way, the two species did not appear currently threatened by environmental changes or any anthropogenic pressures. Nevertheless, the results suggested that unfavourable conditions could impact the populations of *P. palmata* or *B. bifurcata* that exhibited low plasticity and appeared to be dependent of light, temperature, nutrient or hydrodynamic conditions. More particularly, *B. bifurcata* seemed to be highly affected by hydrodynamic conditions, resulting in a heterogeneity of terpene profiles within and between sampling sites. Despite that, Martínez *et al.* (2012) predicted for example an increase in the occurrence of *B. bifurcata* along North-Western Iberian Peninsula, which could be favoured by warm temperature. More generally, this species has shown an expansion of its range both towards the Mouth of Portugal and the English Channel (Martínez *et al.* 2012). In this way, the impact



of future environmental changes on native populations of macroalgae remain unclear, some of them being susceptible to not be able to withstand increasingly intense and prolonged stresses. Furthermore, native species that seem to have low plasticity could nevertheless succeed in adapting to future environmental conditions through their metabolome. In this regard, results revealed that *P. palmata* possesses a high metabolic diversity and subsequently adaptation potential. Indeed, it had a high diversity in MAAs, some of them having potentially different/complementary roles as demonstrated *in-situ* and *ex-situ*.

In contrast to *P. palmata* and *B. bifurcata*, *H. siliquosa* exhibited a very limited density on the intertidal studied sites in Brittany. More generally, this species is rarely observed in the bay of Brest, and intertidal populations are often restricted to few individuals (*i.e.* 1 or 2 individuals per 0.25 m<sup>2</sup> for studied sites). A comparison carried out between France and Ireland revealed a greater abundance / biomass in Irish populations. This ecological difference was coupled to a variation in *H. siliquosa* metabolome, *i.e.* a slightly higher phenolic content in French populations, compared to higher amount of zeaxanthin and pheophytin-*a* in Irish populations. These metabolomic differences reflect physiological responses of *H. siliquosa* to differential environmental conditions between France and Ireland. Considering this, acclimation in France did not seem to compensate for the stressful environmental conditions as the density of the native species remained limited in the intertidal zone. It indicates then less adaptability and / or resilience among Southern populations than in Northern populations. In this way, *H. siliquosa* is a cold-water affinity species which appeared to be more weakened in Brittany due to its geographical position. Indeed, it is likely that *H. siliquosa* is weakened by warm temperatures that can have a significant effect on its reproduction (Moss and Sheader 1973), and subsequently its recruitment and density. In this way, despite spatial acclimation, this species is challenged in maintaining good ecological conditions near its southern limit in France considering multiple stresses caused by global change. The results suggest that *H. siliquosa* is progressively declining in density in Brittany and that its intertidal populations are threatened with extinction. For future studies, it would be interesting to study the subtidal populations of *H. siliquosa* which may have different acclimation capacities, and which are potentially less threatened.

### *Invasive potential of introduced species*

Another objective of this thesis work was to compare the acclimation abilities of native species to those of introduced ones in the context of global change, as introduced species may constitute a threat to native ones. In this way, two introduced macroalgal species were studied: the red *Grateloupia turuturu* and the brown *Sargassum muticum*, both originating from Japan.

Concerning red macroalgae, in agreement with previous studies (Simon *et al.* 1999, 2001), the results revealed that *G. turuturu* has a high plasticity, exhibiting spatial and temporal variations in its ecology and metabolome. This high plasticity reflects its ability to adapt to very diverse / changing environments. Therefore, *G. turuturu* possesses the ability to rapidly synthesize and accumulate high amounts of secondary metabolites (*e.g.* zeaxanthin, shinorine or phenolic compounds), increasing its tolerance to environmental changes. As a result, fertile individuals and recruits were observed throughout the year, despite spatial difference in growth, recruitment and the ratio between tetrasporophytes and carposporophytes. Moreover, *G. turuturu* exhibited high level of monounsaturated fatty acids over the year at all sites compared to *P. palmata*, what may contribute to its permanent reproduction / adaptability. Furthermore, the introduced species appeared to be more sensitive to high light than to high temperature, as fatty acids showed no spatial differences, contrary to pigment composition. Indeed, *G. turuturu* exhibited high levels of MAAs, pheophytin-*a* and zeaxanthin in spring, particularly at Minou where it may have been exposed to a greater level of light radiation due to shallow depth of the rockpools. In this sense, de Ramos *et al.* (2019) demonstrated a low resistance of *G. turuturu* to UV radiation in Brazil, which could be a factor controlling its expansion. On the other hand, although *G. turuturu* has shown a strong plasticity and capacity to adapt to different environmental constraints, it presently exhibited in the studied intertidal sites a relatively low density population, generally limited to a few individuals. In this sense, *G. turuturu* did not seem as invasive and threatening in Europe as it is described in the literature (Simon *et al.* 2001; Nyberg and Wallentinus 2005; Araújo *et al.* 2011; Koerich *et al.* 2020). Our results are in accordance with the recent study by Petrocelli *et al.* (2020) which concluded that *G. turuturu* is not invasive in Mediterranean Sea, based on observations carried out from 2008 to 2018. Thus, it would seem that this species experienced a period of rapid expansion following its introduction, before decreasing and stabilising to a low but stable level 30 years after its arrival in Brittany. Thus, the kinetics of introduction of

*G. turuturu* seem to have followed the "boom and bust model" described by Boudouresque and Verlaque (2012). In this sense the introduced species appeared rather to take advantage of disturbances or unfavourable conditions to develop, while other native species such as *P. palmata* have more difficulty in maintaining when exposed to the same conditions. This hypothesis is to be confirmed by other *in-situ* studies of *G. turuturu* populations elsewhere in Europe. Moreover, *G. turuturu* being a species very tolerant to wide temperature variation (Simon *et al.* 1999, 2001; Bertocci *et al.* 2015), it can be assumed that it will be less weakened than some native species by climate change, and that its invasive potential may evolve.

Similarly to *G. turuturu*, concerning brown macroalgae, results showed also a high plasticity of *S. muticum*, with temporal and spatial variations in ecology and metabolome. Indeed, it has a pseudo-perennial life cycle, *i.e.* increasing in length from spring to summer, and then exhibiting the detachment of annual laterals from perennial holdfasts at the end of summer, allowing a broader dissemination of germlings. As a result, only one cohort occurred among the populations of *S. muticum*. This strategy allows to allocate more energy to growth and reproduction, rather than maintaining long-lived structures. Thus it can rapidly reach large size, enhancing its invasive potential. This strategy then requires enhanced protection to limit the negative impacts of failed reproduction or recruitment on its survival. In this sense, pigment data suggested that *S. muticum* is less light-sensitive. In addition, it exhibited a significant increase in phenolic content simultaneously with its reproductive period, then in summertime, and a seasonal pattern in fatty acid composition opposite to that of the native species (*i.e.* an increase in PUFA content in May with increasing light and temperature), participating in reducing the risk of failed reproduction / mortality in the early stages of development. On the other hand, ecological and metabolomic data in this project are comparable to those obtained in a previous study in 2006 (Le Lann *et al.* 2012). In this way, *S. muticum* seems to have stabilised its populations in Brittany, nearly 50 years after its first introduction, and did not seem to have a direct negative effect on the other native species studied. This common and abundant species could then be considered as a naturalized species rather than an invasive species. Recently, Fernández (2020) also concluded that *S. muticum* can be considered as an addition to the algal flora, after reporting 30 years of observations in northern Spain. Nevertheless, the results suggested that the growth and / or reproduction could be favoured by high light and temperature. Thus, although *S. muticum* appears to be

stable, its invasive potential could evolve in the context of climate change. On the contrary, results about spatial differences indicate that hydrodynamic is an important factor regulating its distribution by delaying the detachment of the laterals. In this regard, an increase in hydrodynamic conditions could create a failure in recruitment and threaten the survival of populations. In this way, long-term studies are required as its future impacts remain unexplored in the context of global change.

### *Conclusion and perspectives*

Global change and human activities lead to ongoing changes on climate system and profound disturbance on ecosystems. As a result, species are expected to evolve and adapt to new living conditions through their ecology, phenology, or physiology, sometimes even at the molecular or genetic level. Each species will then adapt differently, some species presenting a high acclimation ability / plasticity being more likely to adapt to future environmental conditions than others. In this sense, I demonstrated a high spatial and temporal variability in the ecology and metabolomic composition of brown and red macroalgae in the rocky foreshores of Brittany, demonstrating the adaptive capacity of these organisms, especially when introduced. Hence, this work enhances our understanding of the future adaptation of algal populations to global changes, confirming that some species are more likely to be threatened by global change and anthropogenic inputs. Nevertheless, the evolution of native / invasive species remains unclear. For example, *H. siliquosa* has recently been reported in eulittoral rockpools on the Portuguese coast, extending southward its previously known geographical distribution, contrary to any prediction (Lima *et al.* 2009). The evolution of algal populations is more than uncertain as the human population continues to grow and as the resulting human pressure will make macroalgae more vulnerable to environmental changes, leading in modifications in their resilience / adaptive abilities. Besides, macroalgae will adapt to different space and time scales, *i.e.* from local to global scale, with species that will adapt faster than others. For example, Liesner *et al.* (2020) showed variations in physiological responses and genetic diversity of *Laminaria digitata* across its latitudinal distribution (*i.e.* from France to Norway). Similarly, Martínez *et al.* (2012) suggested that *B. bifurcata* would display delay responses and adapt later than *Himanthalia elongata* in northern Spain. This delay in the response of organisms limits the possibility of automatically transferring knowledge from one organism to another, or from one population to another. In this regard, the results from this PhD work were obtained for the populations of Brittany, which is a particular geographical area,

constituting actually a transition zone between cold-temperate and warm-temperate regions (Spalding *et al.* 2007; Figure 5 in the **section 2.1 of the Introduction**). In addition, the results showed inter-site variations within this geographical area, and highlighted the presence of microhabitats within sites, *i.e.* localized / small-scale habitat, with particular environmental conditions differing from those in the surrounding area.

Furthermore, predicting the adaptation and evolution of species is one of the most complex exercise, since global change is progressive / gradual. Ecosystems are constantly evolving, and species are constantly adapting, resulting in a continuous turnover of species. More particularly, the concept of global warming emerged in public debates in the 1950s (Fleming 2005), and since then it has already led to many adaptations / migrations / introductions of species that no one had predicted before. Nevertheless, although ecosystems are undergoing a progressive evolution of environmental conditions, the impacts of climate change on ecosystems and biodiversity are intensifying worldwide over the last 20 years. It can thus be assumed that these changes may become too rapid for species to track and adapt. Notably, it is expected that heatwaves will become more frequent, intense and longer (IPCC 2019) that only some species will be able to cope with. As a result, the number of species introductions and extinctions is likely to increase, becoming a prominent threat for the marine flora worldwide. In this sense, I have shown that species at the limit of their distribution areas are the first to be threatened. The latter are the most threatened as they are / will be weakened by both climate change, and competition with many introduced species. In particular, it is not known how the invasive potential of introduced species will evolve as discussed above. In particular, some harmless species could become more threatening for native species with the modification of certain environmental parameters that would favour their development. This study therefore supports the importance of monitoring the species at different scales of time and space, while reinforcing the eco-metabolomic approach by analysing more compounds, or obtaining complete metabolomic fingerprints/profiles in order to better apprehend the adaptation capacities of different species.

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# *Appendix*

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## Appendix 1.

### **Algal-derived phenolic compounds and their biological activities**

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Gager L.\*, Lalegerie F.\*, Connan S., Stiger-Pouvreau V (In press) Algal-derived phenolic compounds and their biological activities. In Rajauria G. and Yuan Y. (Eds.), *Recent advancements in micro and macroalgal processing: food and health perspectives*. Wiley.

#### **RESUME**

Les algues marines, qui représentent un groupe polyphylétique, possèdent une forte richesse nutritionnelle puisqu'elles produisent une grande variété de molécules, notamment des protéines, des polysaccharides, des fibres alimentaires, des acides aminés, des minéraux, des vitamines et des composés aromatiques. Parmi eux, les composés phénoliques sont des métabolites présents dans divers organismes, y compris les algues marines, et sont connus pour être synthétisés en réponse à différentes conditions environnementales. Ils protègent notamment ces organismes contre les herbivores ou les épiphytes, les infections bactériennes ou les rayons UV. Une fois consommés, les composés phénoliques peuvent être bénéfiques pour la santé humaine et la prévention des risques de maladies chroniques car ils possèdent une large gamme de bioactivités. Ils peuvent avoir des activités antioxydantes, antimicrobiennes, anti-inflammatoires, antitumorales, anti-vieillessement ou pro-minéralogiques, ou agir comme modulateurs du risque de maladies cardiovasculaires. Cette revue présente les composés phénoliques rencontrés dans les algues marines, c'est-à-dire les micro- et les macroalgues, leurs structures chimiques, ainsi que leurs activités biologiques et leur valorisation potentielle pour les industries médicales et cosmétiques.

**Mots-clés:** Micro et macro-algues marines, Polyphénols, Bioactivités, Antioxydant, Anti-inflammatoire, Anti-microbien, Lutte contre le cancer, Maladies cardiovasculaires, Cosmétique

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## ABSTRACT

Marine algae, representing a polyphyletic group, are considered as a rich food source as these organisms produce a great variety of molecules including proteins, polysaccharides, dietary fiber, amino acids, minerals, vitamins, and aromatic compounds. Among them, phenolic compounds are metabolites present in various organisms, including marine algae, and are known to be synthesized in response to different environmental conditions. In particular, they protect these organisms against herbivores or epiphytes, bacterial infection, or UV radiation. Once consumed, they may be beneficial to human health and chronic disease risk prevention since they have a wide range of bioactivities. They can exhibit antioxidant, antimicrobial, anti-inflammatory, antitumor, anti-aging, or pro-mineralogenic activities, or act as modulators of cardiovascular disease risk. This review presents phenolic compounds encountered in marine algae, i.e. micro- and macroalgae, their chemical structures, together with their biological activities and their potential valorization for medical and cosmetic industries.

**Keywords:** Marine macro- and microalgae, Polyphenols, Bioactivities, Antioxidant, Anti-inflammatory, Anti-microbial, Cancer prevention, Cardiovascular diseases, Cosmetic

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## Appendix 2.

### Objectifs et organisation du manuscrit de thèse

Comme présenté dans la première partie de l'introduction, les macroalgues marines benthiques vivant sur les estrans rocheux sont exposées à une large gamme de facteurs biotiques ou abiotiques. Ces divers paramètres environnementaux affectent les algues marines au niveau de leur écologie et de leur physiologie, comme par exemple leur photosynthèse, leur croissance, leur reproduction ou leur réponse aux stress, modifiant le développement et la composition / distribution des communautés de macroalgues marines. En outre, dans un futur proche, les macroalgues vont devoir faire face au changement global et notamment le réchauffement climatique, qui entraîne des perturbations environnementales de plus en plus intenses. Elles vont devoir s'adapter à ces nouvelles conditions environnementales, sous peine de disparaître ou de voir leur aire de répartition régresser. A cet égard, l'un des objectifs de cette thèse était d'**étudier les capacités d'acclimatation de certaines populations de macroalgues intertidales de Bretagne**, afin de mieux comprendre leur devenir en relation avec les changements globaux qui impactent cette flore.

Par ailleurs, comme décrit dans la deuxième partie de l'introduction, l'une des caractéristiques de cette thèse était de **combinaison l'écologie des populations avec la composition métabolomique des individus**. Cette approche intégrée peut être considérée comme une démarche "éco-métabolomique", fournissant une vue d'ensemble globale des capacités d'adaptation des macroalgues marines. Ainsi, le plus grand nombre possible de métabolites a été étudié au cours de ce travail de thèse, afin d'obtenir des "profils métabolomiques", non complets, mais incluant les principaux métabolites primaires (*i.e.* pigments, acides gras) et secondaires (*i.e.* phénoliques, MAA, terpènes) des macroalgues brunes et rouges, appliqués à l'écologie et à la phénologie des algues marines. En effet, les macroalgues s'adaptent (ou non) aux fluctuations de l'environnement en produisant une large gamme de métabolites primaires et/ou secondaires, jouant un rôle important dans leur adaptation et par conséquent dans la structuration des communautés côtières. En outre, ces composés, de nature différente selon les algues, sont synthétisés en réponse à différents stress environnementaux tels qu'un rayonnement lumineux élevé, une augmentation de la température, ou la salinité et la dessiccation, et pourraient donc être des composés clés dans l'adaptation des macroalgues

intertidales au changement global. D'autre part, un autre objectif de cette thèse était de **comparer les réponses adaptatives aux conditions environnementales entre les espèces natives et les espèces introduites / invasives**. En effet, il est probable que le nombre d'introductions d'espèces augmente dans les prochaines années, notamment du fait de l'accroissement des échanges commerciaux marins mondiaux. De plus, en raison du changement global, la plasticité phénotypique des espèces natives / introduites est appelée à évoluer comme décrit dans l'introduction générale. Ainsi, le potentiel invasif de certaines espèces introduites pourrait être renforcé, tandis que les espèces natives pourraient être plus vulnérables et désavantagées par rapport aux espèces introduites.

Dans ce contexte, le manuscrit de thèse est divisé en trois chapitres comme le montre la figure 63. Dans un **premier chapitre**, une description des trois approches utilisées en lien avec le principe d'"éco-métabolomique" est fournie, *i.e.* (1) suivi *in-situ*, (2) expérience de culture en conditions contrôlées, et (3) screening pour explorer la diversité métabolomique. Les trois approches nécessitent l'extraction et l'analyse de différents métabolites algaux, qui sont également présentés dans ce premier chapitre.

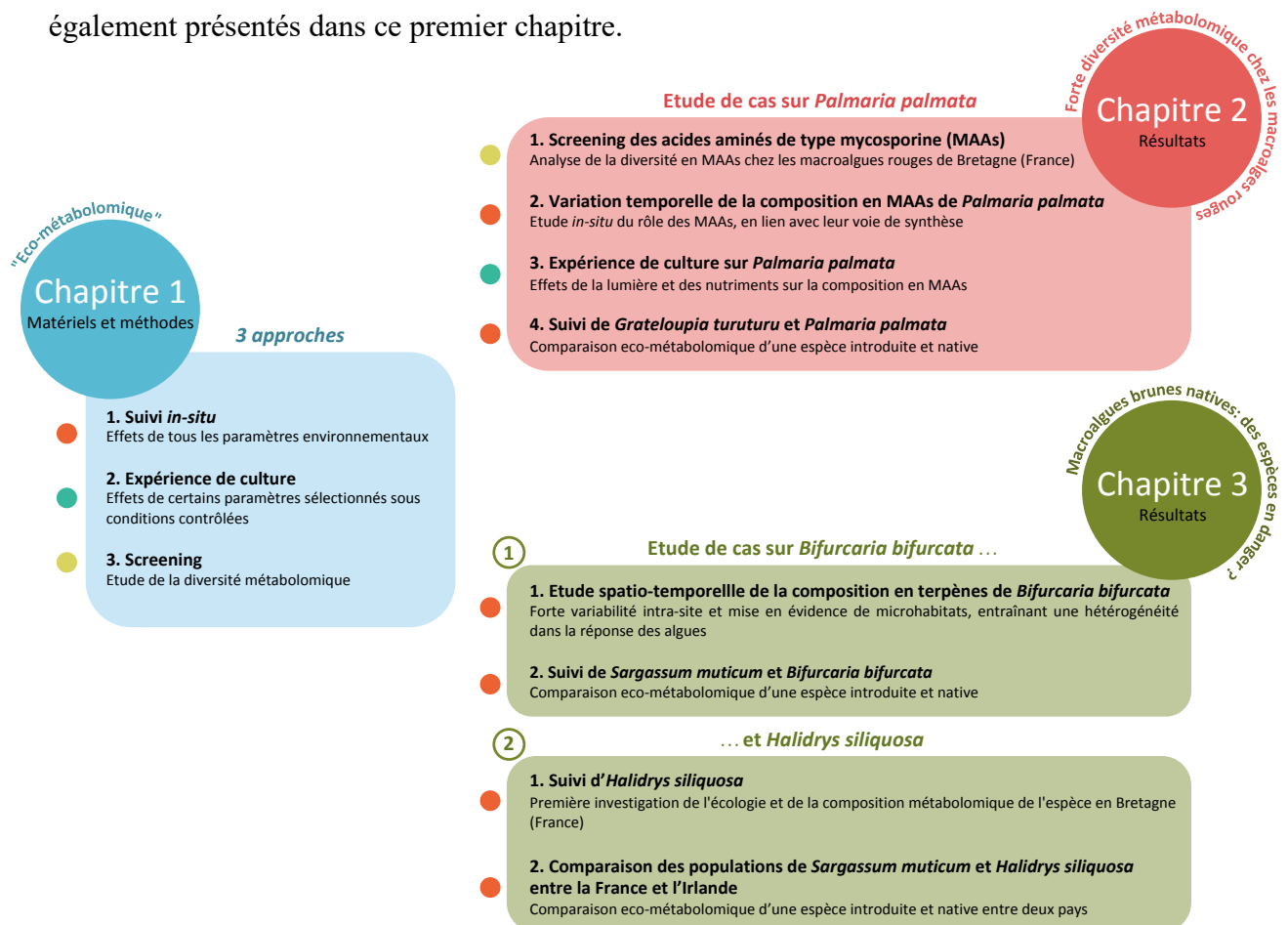


Figure 63. Représentation schématique du plan du manuscrit de thèse. Les points de couleur (orange, turquoise, jaune) représentent les trois approches décrites dans le chapitre 1 (matériels et méthodes), et qui sont utilisées dans les chapitres 2 et 3 (résultats).

Les résultats sont présentés sous la forme de publications scientifiques (publiées ou non), réparties en deux chapitres distincts. Ainsi, le **Chapitre 2** est consacré à l'étude de macroalgues rouges, qui sont connues pour leur grande diversité spécifique et métabolique (Stengel *et al.* 2011). Certains de ces métabolites pourraient jouer un rôle actif dans la future adaptation des algues rouges au changement global. Plus particulièrement, les acides aminés de type mycosporine sont des métabolites secondaires largement rencontrés dans les macroalgues rouges. Il a ainsi déjà été démontré que ces composés sont impliqués dans la photoprotection, notamment contre les rayonnements UV. Cependant, les autres rôles et voies de synthèse de ces composés peu étudiés n'ont pas encore été entièrement élucidés. À cet égard, le présent chapitre se divise en quatre publications sur les macroalgues rouges, avec un intérêt particulier pour *Palmaria palmata* :

**(1) Inventaire des acides aminés de type mycosporine (MAAs) chez des espèces de macroalgues rouges collectées sur les côtes bretonnes - Y a-t-il une forte variation métabolique entre les espèces d'algues rouges qui indiquerait des différences dans leur adaptation ?**

→ PUBLICATION [Lalegerie F., Lajili S., Bedoux G., Taupin L., Stiger-Pouvreau V., Connan S. (2019) Photo-protective compounds in red macroalgae from Brittany: Considerable diversity in mycosporine-like amino acids (MAAs). *Marine Environmental Research*, 147, 37-48]

**(2) Variation temporelle de la composition en MAAs de l'algue rouge *Palmaria palmata*, une espèce native qui présente une grande diversité de ces composés - Les MAAs pourraient-ils jouer un rôle important dans la future adaptation des macroalgues rouges face au changement climatique ?**

→ PUBLICATION [Lalegerie F., Stiger-Pouvreau V., Connan S. (2020). Temporal variation in pigment and mycosporine-like amino acid composition of the red macroalga *Palmaria palmata* from Brittany (France): hypothesis on the MAA biosynthesis pathway under high irradiance. *Journal of Applied Phycology*, 32, 2641-2656]

**(3) Expérience de culture réalisée sur *Palmaria palmata*, pour étudier *ex-situ* l'impact de la lumière et des nutriments sur la synthèse des MAAs - Les MAAs, des composés clés dans l'adaptation des algues rouges aux changements de lumière et de nutriments ?**

→ PUBLICATION [Lalegerie F., Stiger-Pouvreau V., Connan S. (In prep.). Research note - A cultivation experiments on the red macroalga *Palmaria palmata*: effect of UV radiation and nutrients on mycosporine-like amino acids and pigments]

**(4) Suivi *in-situ* de la biologie des populations et de la composition métabolomique de l'espèce native *Palmaria palmata*, en comparaison avec l'espèce introduite *Grateloupia turuturu* - Est-ce que les espèces introduites ont une meilleure adaptabilité et représentent une menace pour les espèces indigènes ?**

→ PUBLICATION [Lalegerie F., Stiger-Pouvreau V., Zanolla M., Stengel D., Connan S. (In prep.) Phenological and metabolomic study of *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) 30 years after its introduction in Brittany (France): a real threat for the native species *Palmaria palmata*?]

Dans un second temps, le **Chapitre 3** est axé sur les macroalgues brunes qui peuvent représenter une biomasse importante sur les estrans rocheux tempérés, certaines espèces pouvant former de grandes canopées (Lüning 1990; Little & Kitching 1996). Plus précisément, l'objectif de ce chapitre est d'appréhender les processus d'adaptation de trois espèces de Sargassaceae, *i.e.* deux espèces natives (*Bifurcaria bifurcata* et *Halidrys siliquosa*) qui vivent dans le même habitat que l'espèce introduite *Sargassum muticum*. En effet, l'évolution des conditions environnementales en réponse aux activités humaines et au changement climatique induit une acclimatation / adaptation écophysiological des macroalgues pour favoriser leur survie. En particulier, des changements dans les interactions interspécifiques et la distribution biogéographique des espèces ont déjà été rapportés, avec un nombre croissant de migrations d'espèces, affectant les écosystèmes marins dans leur ensemble. Ainsi, ce chapitre est ensuite divisé en deux parties. La **première partie (1)** focalise sur *B. bifurcata*, une espèce très abondante sur les estrans bretons, constituant une ceinture algale caractéristique en bas de l'étage médio-littoral, tout en colonisant les mares intertidales. Sa disparition dans le contexte du changement global pourrait ainsi avoir un impact écologique important étant donné qu'elle participe à la structuration des communautés algales. La **deuxième partie (2)** de ce chapitre est une étude de cas sur *Halidrys siliquosa*, une espèce ayant une affinité pour les eaux froides. Plus particulièrement, *H. siliquosa* est une espèce qui vit préférentiellement dans la zone subtidale, et qui pourrait voir ses populations intertidales disparaître avec le réchauffement climatique. A cet égard, les populations intertidales de *H. siliquosa* sont fragilisées en Bretagne, puisqu'elles sont proches de sa limite sud d'aire de répartition en France. Cette espèce pourrait être d'autant plus menacée du fait qu'elle pourrait avoir des difficultés à s'adapter à la fois au changement global et à sa co-habitation avec des espèces introduites. Les **parties 1 et 2 du chapitre 3** sont ensuite constituées de deux publications chacune, *i.e.* un premier article constituant une première étude *in-situ* d'un an de l'écologie et/ou de la composition métabolomique, et donc des capacités d'acclimatation de chaque espèce native suivie en Bretagne (**1.1** et **2.1**); puis une comparaison de leurs capacités d'acclimatation avec l'espèce introduite *S. muticum* (**1.2** et **2.2**). La partie (**2.2**) comprend une comparaison des populations entre la France (où *H. siliquosa* est affaiblie par cette position méridionale et sa co-habitation avec l'espèce introduite *S. muticum* qui est établie depuis longtemps dans ce pays), et l'Irlande (où *S. muticum* est à un stade d'invasion plus précoce).



## 1. Etude de cas sur *Bifurcaria bifurcata*

**(1.1) Etude de la composition terpénique de *Bifurcaria bifurcata* - une espèce native très sensible aux changements environnementaux, qui pourrait être menacée malgré sa forte abondance ?**

→ PUBLICATION [Lalegerie F., Connan S., Stiger-Pouvreau V. (In prep.). Research note - Intra-site variability in terpene composition of *Bifurcaria bifurcata* (Phaeophyceae, Fucales): evidence of microhabitat occurrence]

**(1.2) Suivi *in-situ* de l'écologie et de la composition métabolomique de l'espèce native *Bifurcaria bifurcata*, en comparaison avec l'espèce introduite *Sargassum muticum* - Le changement global peut-il avoir un effet sur le potentiel invasif de certaines espèces et augmenter la menace qu'elles représentent pour les espèces indigènes ?**

→ PUBLICATION [Lalegerie F., Stiger-Pouvreau V., Zanolla M., Stengel D., Connan S. (In prep.) Contrasting phenology and chemical strategies of native and invasive seaweeds: *Bifurcaria bifurcata* and *Sargassum muticum* (Sargassaceae, Fucales)]

## 2. Etude de cas sur *Halidrys siliquosa*

**(2.1) Première investigation de l'écologie et de la composition métabolomique des populations bretonnes d'*Halidrys siliquosa* - une espèce fragilisée dans la zone intertidale ?**

→ PUBLICATION [Lalegerie F., Stiger-Pouvreau V., Connan S. (In prep.). Ecological and metabolomic monitoring of the brown macroalga *Halidrys siliquosa* (Sargassaceae, Fucales)]

**(2.2) Comparaison des populations françaises et irlandaise de *Halidrys siliquosa* et *Sargassum muticum* - *Halidrys siliquosa*, une espèce native menacée en Bretagne par sa localisation géographique et sa coexistence avec l'espèce introduite *Sargassum muticum* ?**

→ PUBLICATION [Lalegerie F., Stiger-Pouvreau V., Zanolla M., Stengel D., Bideau A., Le Grand F., Connan S. (In prep.) *Halidrys siliquosa*, a species threatened by its geographical position and coexistence with the invasive *Sargassum muticum*? A comparison between France and Ireland]

## Résumé du chapitre 2

Le screening des acides aminés de type mycosporine réalisée sur 40 espèces de macroalgues rouges (**partie 1**) a mis en évidence une grande diversité, avec des espèces produisant entre 0 (e.g. *Polyides rotunda*, *Osmundea pinnatifida*, ou *Membranoptera alata*) et 8 MAAs (i.e. *Palmaria palmata*, *Bostrychia scorpioides* et *Agarophyton vermiculophyllum*), incluant de nombreux composés inconnus parmi les 23 détectés. Les résultats obtenus ont permis d'obtenir des profils en MAAs de certaines espèces pour lesquelles la composition en MAAs n'avait jamais été étudiée auparavant, ce qui laisse supposer qu'il reste encore de nombreux MAAs à découvrir, comme le suggèrent également des études récentes (Hartmann *et al.* 2015; Orfanoudaki *et al.* 2019). D'autre part, la composition en MAAs ne semble pas être liée à la

phylogénie, mais plutôt à une acclimatation aux conditions environnementales.

Dans une deuxième partie, afin d'étudier les rôles des différents MAAs qui pourraient expliquer une si grande diversité, nous avons sélectionné une espèce riche en MAAs, *i.e. Palmaria palmata*, dont la composition temporelle en MAAs a été suivie *in-situ* pendant un an (**partie 2**). Les résultats semblent confirmer le rôle photoprotecteur de ces composés, puisqu'une augmentation de la teneur totale en MAAs a été observée en mai, couplée à une augmentation de l'intensité lumineuse à cette période. Bien que cette augmentation soit apparemment due à une augmentation globale de la teneur de tous les MAAs, les résultats ont suggéré que la synthèse de certains MAAs pouvait être induite sous certaines conditions. En particulier, la présence d'astérina-330 n'a été observée qu'entre mars et août, ce qui souligne son rôle important dans la photoprotection de l'espèce. En outre, les résultats ont montré que la disponibilité en nutriments pourrait avoir une influence significative sur les MAAs, puisque leur synthèse semble être limitée l'été, malgré une exposition importante à la lumière.

À cet égard, une expérience de culture menée sur *P. palmata* (**partie 3**) a confirmé l'effet positif de la teneur en nitrate sur les MAAs. Deux hypothèses ont alors pu être formulées : (i) la synthèse des MAAs est limitée en cas de carence en azote car il s'agit de composés azotés ; (ii) les MAAs pourraient être utilisés comme source d'azote par les macroalgues, pour améliorer leur métabolisme lorsque les nutriments sont insuffisants dans l'eau de mer. De cette façon, les MAAs pourraient être des métabolites secondaires multifonctionnels, agissant à la fois comme photoprotecteurs et comme source d'azote. Néanmoins, des recherches supplémentaires sont nécessaires pour confirmer cette dernière hypothèse. D'autre part, l'expérience de culture n'a pas montré d'effet global du rayonnement UV. À l'inverse, contrairement à ce qui était attendu, la teneur de tous les MAAs (y compris l'astérina-330) a diminué tout au long de la période de culture, ce qui pourrait être lié à une quantité insuffisante des nutriments et/ou aux conditions de lumière en laboratoire. Seul l'usurijène présentait une teneur légèrement plus élevée à la fin de l'expérience sous exposition aux rayonnements UV, ce qui suggère une voie d'induction ou de synthèse spécifique de cet MAA et son implication possible dans la photoprotection UV chez *P. palmata*. Une fois de plus, différentes hypothèses pourraient expliquer ce résultat :

- (i) l'usurijène pourrait être synthétisé par une voie différente de celle des autres MAAs, impliquant différents précurseurs ou intermédiaires, qui n'auraient pas été

limités par les conditions en laboratoire.

- (ii) Contrairement aux autres MAAs, l'usurijène ne servirait pas de source d'azote pour les macroalgues (si les MAAs présentent effectivement ce rôle), bien que la structure chimique de cet MAA contienne aussi de l'azote.
- (iii) L'Usurijène serait très dépendant de l'exposition aux UV-A (315-400 nm), alors que la synthèse des autres MAAs serait favorisée par un rayonnement photosynthétique actif (PAR) élevé (400-700nm). La synthèse des différents MAAs varierait donc à la fois par la quantité et la qualité de la lumière. Précédemment, *Kräbs et al. (2002)* avaient déjà suggéré que les différentes voies de synthèse des MAAs chez *Chondrus crispus* pourraient être induites par différentes longueurs d'onde. En ce sens, la quantité d'UV pourrait avoir été suffisante pour induire la synthèse de l'usurijène, tandis que la quantité limitée de PAR pourrait avoir été insuffisante pour induire la synthèse des autres MAAs. En particulier, l'expérience de culture a montré une diminution de la teneur en astérina-330, qui au contraire avait montré une augmentation sous un fort rayonnement lumineux dans des conditions *in-situ* (**partie 2**).

Dans tous les cas, il semble qu'il existe différentes voies de synthèse selon le MAA au-delà des voies du shikimate et du pentose phosphate. En particulier, il est possible que certains MAAs soient des dérivés d'autres MAAs, obtenus par modifications chimiques *via* des réactions de substitution, de déshydratation par condensation, de décarboxylation, d'oxydation ou de réduction (*Bedoux et al. 2020*). Par exemple, la présence de MAAs sulfatés ou de MAAs glycosides a été rapportée dans des coraux et des cyanobactéries, respectivement (*Wada et al. 2015; Bedoux et al. 2020*). En effet, de nouvelles structures de MAAs pourraient être induites sous certains facteurs déclenchants et/ou chez certaines espèces, conduisant à tout un réseau métabolomique tel que décrit par *Belcour et al. (2020)*. À cet égard, des expériences de culture supplémentaires sont nécessaires pour clarifier les différentes voies de synthèse / les éventuelles inductions de MAAs possibles chez les macroalgues rouges. Dans le contexte du changement global, la synthèse de certains MAAs (par exemple l'astérina-330 ou l'usurijène) pourrait être préférentiellement induite. Plus intéressant encore, la modification des conditions environnementales pourrait conduire à une adaptation, c'est-à-dire à la multiplication des modifications de structure des MAAs, ce qui augmenterait la diversité de ces composés. Ainsi, la grande diversité des MAAs chez *P. palmata* ou entre espèces

(observée dans la **partie 1**) pourrait déjà être le résultat d'adaptations spécifiques antérieures.

Enfin, dans la dernière **partie 4**, la composition écologique et métabolomique de *P. palmata* a été comparée à celle de l'algue rouge introduite *Grateloupia turuturu*. En effet, les espèces introduites sont connues pour leur grande plasticité phénotypique. Or on ne sait pas comment leur potentiel invasif va évoluer dans le contexte du changement global, et si elles constituent une menace pour les espèces natives. En ce sens, l'espèce native et l'espèce introduite ont montré des stratégies écologiques et métabolomiques différentes. *Grateloupia turuturu* a montré des individus fertiles tout au long de l'année avec des différences spatiales dans le recrutement, la reproduction ou la croissance, ce qui suggère une forte capacité à s'adapter et à moduler sa phénologie. Cette capacité est associée à sa faculté à synthétiser et à accumuler rapidement de grandes quantités de métabolites secondaires (incluant les MAAs, pigments photoprotecteurs ou composés phénoliques), augmentant sa tolérance aux changements environnementaux. Au contraire, l'algue native *P. palmata* présentait une plasticité plus faible et semblait fortement dépendante de la température, étant principalement fertile en hiver. En outre, les données sur les MAAs suggèrent que *P. palmata* favorise la synthèse d'une multitude de composés ayant potentiellement des effets synergiques, plutôt que l'accumulation rapide de quelques composés comme observé chez *G. turuturu*. Il est possible que les deux espèces ne possèdent pas les mêmes précurseurs / intermédiaires / enzymes ni les mêmes voies de synthèse qui en résultent, ce qui entraîne une diversité différente en MAAs et donc des stratégies métaboliques différentes.

Enfin, *P. palmata* a montré un développement limité en présence de *G. turuturu*. Toutefois, cela pourrait être le résultat d'une combinaison de facteurs conduisant à des conditions défavorables pour l'espèce native. En effet, malgré une grande plasticité, *G. turuturu* ne semble pas être une menace acuellement pour *P. palmata* ou d'autres espèces natives en Bretagne puisque son abondance est restée limitée dans la zone intertidale. L'espèce introduite semble plutôt profiter des perturbations ou des conditions défavorables qui affectent le développement de l'espèce indigène. Ainsi, dans le contexte du changement global, *G. turuturu* est susceptible de s'adapter facilement, tandis que *P. palmata* pourrait être mis au défi de gérer à la fois son acclimatation aux variations environnementales et sa co-habitation avec d'autres espèces. Cependant, la question du devenir de ces deux espèces ne peut être complètement résolue étant donné la multiplicité des interactions entre les populations de macroalgues et leur environnement.

## Résumé du chapitre 3

L'objectif de ce chapitre était d'établir un profil éco-métabolomique spatio-temporel et d'évaluer les capacités d'acclimatation et les changements potentiels dans la distribution des espèces natives / introduites face aux futurs changements environnementaux. Ainsi, deux espèces indigènes de Bretagne ont été étudiées, *i.e.* ***Bifurcaria bifurcata*** (partie 1), et ***Halidrys siliquosa*** (partie 2). Ces deux espèces natives ont démontré des similitudes dans leur phénologie et leur composition :

- une saisonnalité modérée des données écologiques (*i.e.* pas de variation significative de la taille des individus, de la circonférence ou de la densité pour *H. siliquosa*, ou légère diminution de la taille des individus au printemps et en automne pour *B. bifurcata* liée au recrutement), en raison de leur cycle de vie pérenne ;
- l'observation d'individus matures presque toute l'année (bien que la reproduction semble se produire préférentiellement en hiver), couplée à la présence simultanée de plusieurs cohortes ;
- une grande sensibilité à l'augmentation de la lumière et de la température, se traduisant par un niveau élevé de phéophytine-*a* au printemps (et de zéaxanthine pour *H. siliquosa*), couplé à une diminution significative de la chlorophylle-*a* et des acides gras polyinsaturés (AGPI) ;
- une saisonnalité modérée de la teneur en composés phénoliques (*i.e.* une très légère augmentation en hiver pour *H. siliquosa* et en été pour *B. bifurcata*).

Ces résultats ont ensuite été comparés à la phénologie écologique et métabolomique de l'espèce invasive ***Sargassum muticum***. L'espèce a montré :

- une forte saisonnalité au niveau de son écologie en raison de son cycle de vie pseudo-pérenne, *i.e.* une augmentation de la taille des individus du printemps à l'été, puis le détachement annuel des latérales à la fin de l'été, permettant une plus grande dissémination
- la présence d'une seule cohorte à la fois en raison de sa stratégie pseudo-pérenne ;
- une différence spatiale dans la taille des individus / le détachement des latérales, liée aux conditions hydrodynamiques, pouvant entraîner un retard dans le recrutement ;
- une reproduction élevée sur une courte période au printemps / été, qui semble être favorisée par des températures croissantes, suivie de l'arrivée des juvéniles à la fin de

l'été / au début de l'automne ;

- de fortes variations métabolomiques, *i.e.* une hausse significative de la teneur en AGPI et en composés phénoliques en été, coïncidant avec sa période de reproduction.

Ainsi, les résultats ont démontré des variations écologiques entre les espèces natives et l'espèce invasive *S. muticum*, avec une phénologie différentielle. En effet, les espèces natives ont un cycle de vie pérenne, impliquant une stratégie de croissance lente, ce qui entraîne par la suite de faibles variations saisonnières des populations. Au contraire, les espèces invasives présentent généralement une stratégie de croissance élevée, profitant de conditions favorables pour se développer, atteignant une taille élevée et une maturité sexuelle en quelques mois seulement comme observé avec *S. muticum*. Cela illustre la variabilité des stratégies / réponses écologiques au sein des communautés d'algues, les espèces ne présentant pas toutes les mêmes capacités d'acclimatation. À cet égard, la reproduction et la croissance de *S. muticum* semblent être favorisées par des températures élevées, comme cela a déjà été suggéré (Norton 1977; Cacabelos *et al.* 2013).

Ces différentes stratégies entraînent des différences de composition métabolomique. Ainsi, les espèces natives doivent maintenir / fournir de l'énergie pour la croissance et la reproduction tout au long de l'année, ce qui implique un stockage, une faible demande métabolomique et, par conséquent, un renouvellement limité de la biomasse. De même, la présence de plusieurs cohortes pendant l'année et le maintien d'une population constante nécessitent une protection permanente. À cet égard, les espèces natives ont montré une reproduction se produisant préférentiellement en hiver, suggérant une plus grande sensibilité des individus fertiles à une lumière et/ou une température élevée. Ensuite, la synthèse des composés phénoliques a montré une saisonnalité réduite ce qui pourrait être le résultat d'une protection constante des individus tout au long de l'année. Cependant, les espèces natives ont également montré une hausse de la teneur en composés photoprotecteurs au printemps et en été, ce qui indique la capacité à induire ponctuellement une stratégie de tolérance au stress plus importante. Par ailleurs, il est difficile de prédire exactement comment les populations d'algues natives vont s'adapter face à des stress persistants / de plus en plus long, en particulier dans le contexte du changement climatique.

A l'inverse, *S. muticum* est une espèce pseudo-pérenne qui évite d'utiliser de l'énergie pour la

protection et la maintenance de longues latérales tout au long de l'année. Ceci lui permet d'allouer plus d'énergie à la reproduction ou à la croissance / au développement de ses jeunes stades de vie au printemps-été. Plus particulièrement, cette stratégie permet à *S. muticum* d'accumuler rapidement, de façon saisonnière, une quantité élevée de métabolites. Cela implique probablement une plus grande variabilité métabolomique entre et au sein des individus / thalles, avec une protection localisée / concentrée uniquement sur les parties reproductrices / jeunes recrues. En effet, puisque *S. muticum* ne présente qu'une seule cohorte à la fois, une telle stratégie métabolomique renforce sa protection et favorise sa survie en réduisant le risque de mortalité dans les premiers stades de développement, et évite ainsi la disparition de ses populations en cas d'échec du recrutement. À cet égard, *S. muticum* a montré une diminution plus faible de la chlorophylle-*a* et une synthèse limitée de composés photoprotecteurs au printemps / été par rapport aux espèces natives, ce qui suggère une sensibilité différente à la lumière. Au contraire, *S. muticum* a présenté une forte augmentation saisonnière de la teneur en composés phénoliques en réponse à l'augmentation de la lumière et de la température, par rapport aux deux algues natives. Plus intéressant encore, *S. muticum* a montré une augmentation en AGPI en été, à l'opposé du schéma saisonnier des espèces natives. Ces composés pourraient donc jouer un rôle important dans la reproduction, la croissance ou la protection de l'espèce invasive. De façon générale, *S. muticum* semblait avoir une plus grande plasticité que les espèces natives.

Dans ce contexte, l'espèce invasive semble être plus résistante pour faire face à d'importantes modifications de l'environnement, contrairement aux espèces natives qui présentaient une plasticité plus limitée. Il a été suggéré par exemple que la vague de chaleur du printemps-été 2003 pourrait avoir favorisé le développement et donc la longueur du thalle de *S. muticum* (Atkinson *et al.* 2020). Ainsi, l'augmentation de la température pourrait favoriser la reproduction de *S. muticum* sur une plus longue période, et par conséquent accroître son potentiel invasif. À l'inverse, nos résultats suggèrent que les espèces natives pourraient être fortement affaiblies par l'augmentation de la température et l'apparition de plus en plus fréquente de longues et fortes vagues de chaleur. Par ailleurs, l'analyse de la composition terpénique de *B. bifurcata* dans la **partie 1.1** a montré que cette espèce est particulièrement sensible aux conditions hydrodynamiques, et pourrait être menacée par des changements de conditions climatiques. Néanmoins, il est difficile d'évaluer le potentiel d'acclimatation / adaptation de chaque espèce étant donné la complexité du milieu marin, notamment les effets

de multiples facteurs de stress, les diverses interactions avec et au sein des communautés, et les différents niveaux de réponse (*i.e.* au niveau des individus, des populations ou des écosystèmes). Ainsi, bien que *S. muticum* semble être plus résistante, certains éléments suggèrent qu'elle pourrait également être affaiblie face à de forts changements environnementaux. Notamment, une augmentation de la fréquence / intensité des tempêtes et des conditions hydrodynamiques pourrait affaiblir l'espèce invasive, en provoquant un détachement précoce de ses latérales, perturbant son recrutement.

Plus généralement, la phénologie et les interactions entre les espèces pourraient évoluer / être modifiées par les changements environnementaux. En conséquence, les populations d'algues vont devoir s'adapter à de nouvelles conditions environnementales, au risque de disparaître. Plus particulièrement, la synthèse de métabolites pourrait être un facteur clé dans l'adaptation des macroalgues, comme cela a déjà été décrit au **Chapitre 2** avec les macroalgues rouges. En outre, les données fournies dans ce chapitre ne sont pas suffisantes pour déterminer comment les algues vont s'adapter étant donné la richesse des métabolites et le large éventail de fonctions biologiques assurées par chacun de ces composés (incluant les pigments, les composés phénoliques et les acides gras), variant en fonction de nombreux facteurs biotiques ou abiotiques. Cela renforce la nécessité de réaliser des expériences de culture ou d'étudier davantage de métabolites selon l'approche "écométabolomique". À cet égard, une expérience de culture sur les algues brunes a été lancée, mais a été annulée en raison de la COVID-19 (Fig. 64).

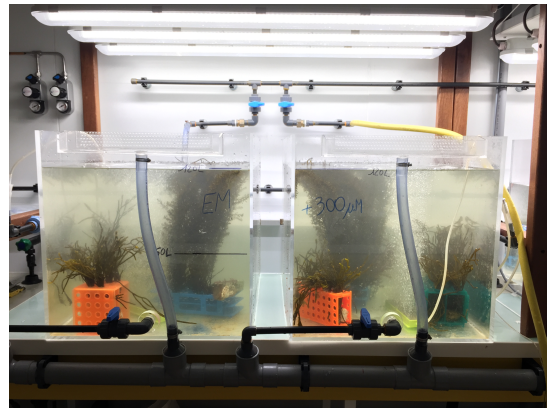


Figure 64. Illustration de l'expérience de culture sur les macroalgues brunes initiée en mars 2020, mais annulée en raison de la COVID-19.

Une autre façon de s'adapter pour les macroalgues est de migrer vers d'autres zones géographiques offrant des conditions plus favorables. À cet égard, la **partie 2.2** de ce chapitre comprenait une comparaison géographique des populations de *H. siliquosa* et *S. muticum* entre la France et l'Irlande, suivies en janvier et juin 2019. En effet, *H. siliquosa* est une espèce ayant une affinité pour les eaux froides, dont la limite sud de répartition se situe près de la Bretagne, et qui pourrait être affaiblie par le changement climatique. D'autre part,



*S. muticum* présente un niveau « d'invasion » différent entre les deux pays (*i.e.* bien établi en France par rapport à une introduction plus tardive en Irlande). Les résultats ont ainsi montré :

- une densité limitée des populations françaises de *H. siliquosa*, par rapport à une densité similaire entre les populations françaises et irlandaises pour *S. muticum* ;
- un taux de reproduction plus élevé de 10 % en France pour *S. muticum*, associé à une augmentation estivale plus importante de la teneur en AGPI ;
- aucune différence saisonnière ou spatiale dans le taux de maturité ou la teneur en acides gras pour *H. siliquosa* ;
- une croissance limitée de *S. muticum* sur les sites exposés quel que soit le pays, contrairement à *H. siliquosa* ;
- une différence spatiale dans le schéma saisonnier de la composition pigmentaire quelle que soit l'espèce, due à une intensité lumineuse plus importante en juin en Irlande, résultant à la synthèse de zéaxanthine par les populations irlandaises de *H. siliquosa*, contrairement à de la violaxanthine par les populations françaises. De même, il a été constaté une forte augmentation de la teneur en violaxanthine et en chlorophylle-*a* en juin pour la population française de *S. muticum*, alors que les populations irlandaises n'ont pas présenté de changement saisonnier significatif.

Selon ces résultats, *H. siliquosa* semble être plus fragilisée en France, comme suggéré par sa faible densité. Cela pourrait être l'effet d'une combinaison de facteurs, notamment des températures ou un rayonnement lumineux plus élevés tout au long de l'année, ou une concurrence plus difficile avec l'espèce invasive *S. muticum*. En effet, *S. muticum* a été introduite en France 30 ans plus tôt qu'en Irlande (Critchley 1983; Kraan 2007). Par ailleurs, les résultats de la **partie 1.2** ont montré des données écologiques et métabolomiques pour les populations françaises de *S. muticum* similaires à celles obtenues en 2006 (Le Lann *et al.* 2012), suggérant que cette espèce a stabilisé sa population en Bretagne, contrairement aux populations irlandaises. Par ailleurs, bien que *S. muticum* ait présenté une taille des individus et une densité similaires entre la France et l'Irlande, son taux de reproduction était 10 % plus élevé en France qu'en Irlande, associé à une teneur en AGPI plus élevée. Cela suggère une plasticité réduite de l'espèce invasive en Irlande. De même, les populations irlandaises de *S. muticum* pourraient être menacées par une intensification des conditions hydrodynamiques comme le suggère la variabilité intra-site observée. Ainsi, il est possible que *H. siliquosa* et *S. muticum* soient toutes deux amenées à évoluer du fait d'une adaptation de leurs populations

à un environnement changeant, modifiant l'interaction entre les deux espèces dans les deux pays. En ce sens, les populations françaises de *H. siliquosa* pourraient être amenées à faire face à la fois au changement climatique et la co-habitation avec d'autres espèces invasives. Ainsi, trois possibilités peuvent être envisagées : (i) *H. siliquosa* va s'adapter / augmenter la synthèse de composés protecteurs, au détriment de sa reproduction ou de sa croissance ; néanmoins, les données n'ont pas montré de différence métabolique en faveur des populations françaises ; (ii) les populations françaises intertidales disparaîtront, limitant *H. siliquosa* aux populations subtidales, ou réduisant son aire de distribution ; (iii) *H. siliquosa* va migrer plus au nord où elle pourrait cependant être confrontée à une plus grande concurrence avec *S. muticum*. Cela rappelle l'importance de faire des suivis de populations sur le long terme car il n'est pas possible de prévoir avec certitude leur évolution. Dans ce sens, [Stæhr et al. \(2000\)](#) ont rapporté que l'expansion de *S. muticum* dans le Limfjorden (Danemark) entre 1984 et 1997 a entraîné une diminution de la couverture de *H. siliquosa* de plus de 50 %. Le suivi des populations est d'autant plus important qu'il peut y avoir sur un même site des microhabitats, c'est-à-dire un habitat de petite taille constituant un sous-ensemble d'un habitat plus grand, qui présentent des caractéristiques physiques ou chimiques différentes. En effet, les variations spatio-temporelles de la composition en terpènes de *B. bifurcata* (**partie 1.1**) ont mis en évidence des différences entre et au sein des sites en fonction des conditions hydrodynamiques. Ainsi, ces microhabitats peuvent contribuer à l'adaptation locale des espèces, ou créer des zones de refuge pour les espèces menacées par le changement climatique.

En conclusion, bien qu'il soit impossible de prévoir avec certitude l'adaptation des populations d'algues face aux futurs changements environnementaux, toutes les publications de ce chapitre ont fourni un aperçu des variations phénotypiques et métaboliques de certaines espèces d'algues brunes natives ou invasive en Bretagne. De ce fait, les résultats obtenus dans ce chapitre participent à une meilleure compréhension des capacités d'acclimatation de ces populations d'algues bretonnes.

## Conclusion générale et perspectives

Ce travail de thèse, basé sur l'étude de cinq espèces de macroalgues, *i.e.* trois espèces natives et deux introduites en Bretagne (France), a pris en compte les problématiques suivantes :

- Quel est l'état actuel des populations d'algues des espèces étudiées en Bretagne ? Plus particulièrement, en utilisant une approche écologique et "éco-métabolomique", existe-t-il une variabilité spatio-temporelle dans l'écologie et la composition métabolomique de ces populations ?
- Y a-t-il des différences d'acclimatation entre les espèces natives et les espèces introduites, liées aux différents facteurs environnementaux ? Plus particulièrement, y a-t-il un risque que le potentiel invasif des espèces introduites augmente, au détriment des espèces natives ?
- Le changement global pourrait-il avoir un effet sur la synthèse et la composition métabolomique des espèces ? En d'autres termes, certains métabolites pourraient-ils avoir un rôle clé dans l'acclimatation des populations de macroalgues et ainsi participer à leur adaptation future ?
- Enfin, certaines espèces sont-elles plus susceptibles que d'autres de disparaître dans un contexte de changement global ? En particulier, comment vont évoluer les espèces natives proches de leur limite d'aire de répartition, sachant que ces espèces devront faire face à de multiples changements ?

De cette façon, j'ai étudié à la fois des macroalgues rouges (**Chapitre 2**) et brunes (**Chapitre 3**), qui constituent deux groupes taxonomiques distincts et qui présentent donc une composition métabolique et des capacités d'adaptation potentiellement différentes (Stengel *et al.* 2011). Plus particulièrement, le **Chapitre 2** était axé sur les macroalgues rouges et la production d'acides aminés de type mycosporine (MAAs), composés dont les différents rôles et voies de synthèse n'ont pas encore été entièrement élucidés (Bedoux *et al.* 2020; Belcour *et al.* 2020). Ces métabolites secondaires, par ailleurs très diversifiés, pourraient participer activement à la future adaptation des algues rouges au changement global. La seconde partie de ce travail (**Chapitre 3**) a porté sur des espèces de Sargassaceae, une famille diversifiée de macroalgues brunes. Deux espèces natives communes le long des côtes rocheuses de Bretagne et l'espèce invasive très répandue *Sargassum muticum* ont été étudiées.

## *Les effets des variations environnementales sur les métabolites secondaires et la nécessité de combiner l'écologie avec la métabolomique*

Le potentiel d'acclimatation des différentes espèces de macroalgues dépend en partie de leur métabolome, qui constitue un élément clé dans leur adaptation ultérieure. À cet égard, un screening des acides aminés de type mycosporine (MAAs) chez de nombreuses algues rouges (**Chapitre 2**) a révélé une grande hétérogénéité dans la composition métabolique entre les espèces, certaines espèces présentant une plus grande diversité que d'autres. Les espèces présentant la plus grande diversité en MAAs semblent être plus susceptibles de s'acclimater et s'adapter aux changements environnementaux, bien que certains changements puissent favoriser l'accumulation rapide de composés spécifiques. D'autre part, il existe actuellement peu de données sur l'effet du changement global sur la production de métabolites, et sur la question de savoir si les espèces développeront de nouvelles capacités / fonctions métaboliques ou induiront la synthèse de composés particuliers pour améliorer leur survie. En outre, toutes les espèces ne possèdent pas les mêmes précurseurs / intermédiaires / enzymes et les mêmes voies de synthèse qui en résultent, ce qui entraîne une diversité métabolique différente et donc des stratégies métaboliques différentes. Plus particulièrement, le changement global pourrait conduire à de nouvelles voies métaboliques par des divergences dans l'expression génétique. De cette manière, de nouvelles structures métabolomiques pourraient être induites chez certaines espèces par l'intermédiaire de certains facteurs déclenchants. Comme suggéré par [Belcour et al. \(2020\)](#) qui ont combiné des données génomiques et métabolomiques obtenues à partir de l'algue rouge *Chondrus crispus*, cela pourrait conduire à des réseaux métaboliques complexes, comme indiqué dans le **Chapitre 2** à l'égard des MAAs. Par conséquent, il est difficile de prévoir comment vont s'adapter les populations d'algues, d'autant plus que cette adaptation sera différente pour chaque espèce. Cette adaptation pourrait même être différente au sein des espèces (*i.e.* hétérogénéité inter-populationnelle) puisque les métabolites secondaires agissent comme une interface chimique entre les organismes et leur environnement spécifique. Cette caractéristique soutient l'intérêt d'utiliser des approches complémentaires telles que l'éco-métabolomique, et d'étudier le devenir des macroalgues marines en couplant à la fois l'écologie et la métabolomique.

En effet, les macroalgues vivent dans un environnement complexe et subissent une large gamme de variations environnementales (abiotiques et biotiques) et de stress anthropiques,

tout en étant menacées par les espèces introduites. Étant donné la multiplicité des interactions qui peuvent se produire dans ces conditions, il est donc difficile d'interpréter les réponses écologiques et de déterminer quels facteurs ont un impact sur les différentes populations d'algues et comment elles s'adapteront localement. À cet égard, les métabolites sont impliqués dans de nombreux processus biologiques. L'étude de profils métaboliques globaux, couplée à des données écologiques, peuvent alors permettre de mieux comprendre les capacités d'adaptation des macroalgues. Dans cette perspective, et afin de répondre aux différentes problématiques, j'ai choisi de réaliser un suivi écologique et métabolomique sur différentes populations naturelles d'algues de Bretagne (France) et de la baie de Galway (Irlande), en m'appuyant sur l'approche émergente d'"éco-métabolomique" (Jones *et al.* 2013; Kumar *et al.* 2016; Belghit *et al.* 2017; Peters *et al.* 2018). Dans cette optique, j'ai essayé d'étudier le plus grand nombre possible de métabolites d'algues (*i.e.* chlorophylles, caroténoïdes, phycobiliprotéines, MAAs, acides gras, composés phénoliques et terpènes), bien que je sois consciente que cela représente un sous-ensemble limité de la composition métabolomique globale des macroalgues (*i.e.* plus de 10 000 métabolites), et que cela ne constitue en aucun cas un profil global. Jusqu'à présent, il existe peu d'études *in-situ* qui lient l'écologie des algues à autant de composés. Ensuite, pour relier l'écologie à la métabolomique, des expériences de culture en conditions contrôlées au laboratoire sont nécessaires pour (i) déterminer *ex-situ* quels facteurs environnementaux particuliers ont un impact sur la physiologie des espèces, et pourraient être responsables de la synthèse de métabolites spécifiques, et (ii) clarifier les différentes voies de synthèse des métabolites. Dans ce sens, une expérience de culture a été réalisée sur l'algue rouge *Palmaria palmata*, ce qui a permis de montrer l'effet de la lumière et des nutriments sur la production de MAAs et de pigments. Plus particulièrement, cette expérience a démontré que la shinorine et l'usurijène pourraient jouer un rôle clé dans l'adaptation de cette espèce à l'augmentation du rayonnement lumineux (**Chapitre 2**). Ainsi, l'approche "éco-métabolomique" a permis d'apporter de nouveaux éléments concernant les processus d'acclimatation des différentes espèces étudiées.

Cette approche "éco-métabolomique" a nécessité le développement de techniques d'extraction et d'analyse des différents métabolites étudiés (**Chapitre 1**). En ce sens, tous les protocoles développés ont permis d'obtenir des résultats satisfaisants, bien qu'il y ait encore des améliorations à apporter. En particulier, l'extraction et l'analyse des MAAs utilisant respectivement de l'éthanol à 70% et de l'acide acétique à 0,1% ont donné des résultats

fiables, constituant une méthode éco-responsable, sans méthanol contrairement à ce qui est couramment utilisé dans la littérature. Cependant, il n'a pas été possible de purifier et d'identifier / quantifier tous les MAAs, de sorte que des analyses supplémentaires sont nécessaires pour obtenir des standards pour ces composés (non disponibles dans le commerce). À cet égard, une collaboration avec le Pr. Ulf Karsten (Université de Rostock, Allemagne), le Dr. Markus Ganzera et le Dr. Anja Hartmann (Université d'Innsbruck, Autriche) a fourni des premiers résultats encourageants (non présentés dans ce manuscrit). De même, l'analyse CCM des terpènes n'a pas permis d'identifier / quantifier ces composés, ce qui nécessite la mise au point de méthodes de purification par extraction en phase solide (SPE), suivie d'une analyse par LC-MS par exemple. Concernant les composés phénoliques, leur séparation et leur identification par LC-MS a été initiée au cours de ce travail de thèse (voir **section 4.5 du Chapitre 1**) et doivent être poursuivies.

### **Situation actuelle des populations d'algues natives de Bretagne (France)**

Dans ce projet, trois espèces de macroalgues natives de Bretagne ont été particulièrement étudiées : la macroalgue rouge *Palmaria palmata*, et deux macroalgues brunes, *Bifurcaria bifurcata* et *Halidrys siliquosa*. Jusqu'à présent, les études déjà réalisées sur *P. palmata* étaient des expériences de cultures en bacs / conditions semi-contrôlées, et se sont surtout concentrées sur sa valeur nutritionnelle en raison de son importance économique pour la consommation humaine ou l'aquaculture (Galland-Irmouli *et al.* 1999; Le Gall *et al.* 2004b; Beaulieu *et al.* 2016). De la même façon, l'écologie des populations de *B. bifurcata* est peu documentée (Connan *et al.* 2007; Le Lann *et al.* 2012; Méndez-Sandín & Fernández 2016), les études précédentes portant généralement uniquement sur sa composition en terpènes en termes d'activités biologiques et de valorisation potentielle (Hellio *et al.* 2001; Alves *et al.* 2016; Pais *et al.* 2019), et ce malgré son importance écologique, par sa participation à la structuration des communautés intertidales en Bretagne. De même, à ma connaissance, peu d'études ont fait état de l'écologie de *H. siliquosa*, à l'exception de quelques études réalisées au Danemark (Wernberg *et al.* 2001, 2004; Pedersen *et al.* 2005), et aucune en Bretagne. Ainsi, les résultats obtenus par l'étude des variations éco-métabolomiques des populations naturelles de ces trois espèces apportent de nouvelles connaissances sur leurs capacités d'acclimatation.

Les trois macroalgues natives étudiées ont en commun d'être des espèces pérennes, se reproduisant préférentiellement en hiver. Elles présentent également des variations spatiales et temporelles de leur profil métabolique et écologique, ce qui suggère différentes capacités d'acclimatation aux conditions environnementales. Sur la base de ces données, les populations naturelles de *P. palmata* et *B. bifurcata* sont apparues en bon état écologique, c'est-à-dire présentant une croissance régulière avec un renouvellement saisonnier des populations, une densité relativement élevée, des thalles sans dommage physique ni dépigmentation permanente, et un faible épiphytisme. Ainsi, les deux espèces ne semblaient pas actuellement menacées par les changements globaux ou les pressions anthropiques. Néanmoins, les résultats suggèrent que des conditions défavorables pourraient avoir un impact sur les populations de *P. palmata* ou *B. bifurcata* qui présentent une faible plasticité et semblent dépendre de la lumière, de la température, des conditions nutritives ou de l'hydrodynamisme. Plus particulièrement, *B. bifurcata* semblait être fortement affectée par l'exposition à des conditions hydrodynamiques plus importantes, résultant à une hétérogénéité des profils terpéniques au sein et entre les sites d'échantillonnage. Au contraire, [Martínez et al. \(2012\)](#) ont prédit par exemple une augmentation de la présence de *B. bifurcata* le long des côtes Nord-Ouest de la péninsule ibérique, qui pourrait être favorisée par une température élevée. Plus généralement, cette espèce a montré une expansion de son aire de répartition à la fois vers le sud du Portugal et en Manche ([Martínez et al. 2012](#)). Ainsi, l'impact des futurs changements environnementaux sur les populations natives de macroalgues reste incertain, certaines d'entre elles étant susceptibles de ne pas pouvoir résister à des stress de plus en plus intenses et prolongés. En outre, les espèces natives qui semblent avoir une faible plasticité pourraient malgré tout réussir à s'adapter aux conditions environnementales futures grâce à leur métabolome. À cet égard, les résultats ont révélé que *P. palmata* possède une grande diversité métabolique et donc un potentiel d'adaptation élevé. En effet, cette espèce a montré une grande diversité en MAAs, certains d'entre eux ayant des rôles potentiellement différents/complémentaires, comme cela a été démontré *in-situ* et *ex-situ*.

Contrairement à *P. palmata* et *B. bifurcata*, *H. siliquosa* a montré une densité très limitée en zone intertidale des sites étudiés en Bretagne. Plus généralement, cette espèce est rarement observée en baie de Brest, et les populations intertidales sont souvent limitées à quelques individus (soit 1 ou 2 individus par 0,25 m<sup>2</sup> pour les sites étudiés). Une comparaison effectuée entre la France et l'Irlande a révélé une plus grande abondance / biomasse dans les

populations irlandaises. Cette différence écologique a été couplée à une variation du métabolome de *H. siliquosa*, c'est-à-dire un contenu phénolique légèrement plus élevé dans les populations françaises, par rapport à une quantité plus importante de zéaxanthine et de phéophytine-*a* dans les populations irlandaises. Ces différences métabolomiques reflètent les réponses physiologiques de *H. siliquosa* aux conditions environnementales différentes entre la France et l'Irlande. Considérant ceci, l'acclimatation en France ne semble pas compenser les conditions environnementales stressantes car la densité des espèces natives reste limitée dans la zone intertidale. Elle indique donc une adaptabilité et/ou une résilience moindre dans les populations du Sud que celles du Nord. Ainsi, *H. siliquosa* est une espèce ayant une affinité pour les eaux froides qui semble plus affaiblie en Bretagne en raison de sa localisation géographique. En effet, il est probable que *H. siliquosa* soit affaiblie par des températures chaudes qui peuvent avoir un effet significatif sur sa reproduction (Moss & Sheader 1973), et, par la suite, son recrutement et sa densité. Ainsi, malgré l'acclimatation spatiale, cette espèce est confrontée à la difficulté de maintenir de bonnes conditions écologiques à sa limite sud en France compte tenu des multiples stress causés par le changement climatique. Les résultats suggèrent que la densité de *H. siliquosa* diminue progressivement en Bretagne et que ses populations intertidales sont menacées d'extinction. Pour de futures études, il serait intéressant d'étudier les populations subtidales de *H. siliquosa* qui peuvent avoir des capacités d'acclimatation différentes, et qui sont potentiellement moins menacées.

### **Espèces introduites et potentiel invasive**

Un autre objectif de ce travail de thèse était de comparer les capacités d'acclimatation des espèces natives à celles des espèces introduites dans le contexte du changement global, les espèces introduites pouvant constituer une menace pour les espèces natives. Ainsi, deux espèces de macroalgues introduites ont été étudiées : l'algue rouge *Grateloupia turuturu* et l'algue brune *Sargassum muticum*, toutes deux originaires du Japon.

En ce qui concerne les macroalgues rouges, en accord avec les études précédentes (Simon *et al.* 1999, 2001), les résultats ont révélé une plasticité élevée de *G. turuturu*, qui a montré des variations spatiales et temporelles de son écologie et de son métabolome. Cette plasticité élevée reflète sa capacité à s'adapter à des environnements très divers / changeants. Par conséquent, *G. turuturu* possède la capacité de synthétiser et d'accumuler rapidement de grandes quantités de métabolites secondaires (par exemple, zéaxanthine, shinorine ou



composés phénoliques), ce qui augmente sa tolérance aux changements environnementaux. De ce fait, des individus et des recrues fertiles ont été observés tout au long de l'année, avec une différence spatiale dans la croissance, le recrutement et le ratio tétrasporophytes / carposporophytes. De plus, *G. turuturu* a présenté un niveau élevé d'acides gras monoinsaturés tout au long de l'année sur tous les sites par rapport à *P. palmata*, ce qui pourrait contribuer à sa reproduction permanente. En outre, l'espèce introduite semble être plus sensible à la lumière qu'à la température, car les acides gras ne présentent pas de différences spatiales, contrairement à la composition pigmentaire. En effet, *G. turuturu* présentait des niveaux élevés en MAAs, de phéophytine-*a* et de zéaxanthine au printemps, en particulier au Minou où cette espèce a pu être exposée à un niveau plus élevé de rayonnement lumineux en raison de la faible profondeur des mares. Dans ce sens, de Ramos *et al.* (2019) ont démontré une faible tolérance de *G. turuturu* face aux rayonnements UV au Brésil, ce qui pourrait être un facteur de contrôle de son expansion. D'autre part, bien que *G. turuturu* ait montré une forte plasticité et une capacité d'adaptation aux différentes contraintes environnementales, cette espèce a montré une densité de population relativement faible dans les zones intertidales étudiées, généralement limitée à quelques individus. En ce sens, *G. turuturu* ne semble pas aussi invasive et menaçante en Europe telle qu'elle est décrite dans la littérature (Simon *et al.* 2001; Nyberg & Wallentinus 2005; Araújo *et al.* 2011; Koerich *et al.* 2020). Nos résultats sont en accord avec l'étude récente de Petrocelli *et al.* (2020) qui a conclu que *G. turuturu* n'est pas invasive en mer Méditerranée, sur la base d'observations de 2008 à 2018. Ainsi, il semblerait que cette espèce ait connu une période d'expansion rapide après son introduction, avant de décroître et de stabiliser sa densité à un niveau plus bas mais stable 30 ans après son arrivée en Bretagne. Ainsi, la cinétique de l'introduction de *G. turuturu* semble avoir suivi le "modèle de l'expansion et de l'effondrement" décrit par Boudouresque & Verlaque (2012). En ce sens, l'espèce introduite semble plutôt profiter des perturbations ou des conditions défavorables pour se développer, alors que d'autres espèces natives comme *P. palmata* pourraient avoir plus de mal à se maintenir lorsqu'elles sont exposées aux mêmes conditions. Cette hypothèse doit être confirmée par d'autres études *in-situ* des populations de *G. turuturu* ailleurs en Europe. De plus, *G. turuturu* étant une espèce très tolérante aux grandes variations de température (Simon *et al.* 1999, 2001; Bertocci *et al.* 2015), on peut supposer qu'elle sera moins affaiblie que certaines espèces natives par le changement climatique, et que son potentiel d'invasion pourrait évoluer.

Comme pour *G. turuturu*, les résultats ont également montré une plasticité élevée chez l'algue brune introduite *S. muticum*, avec des variations temporelles et spatiales de son écologie et de son métabolome. En effet, cette espèce a un cycle de vie pseudo-pérenne, *i.e.* elle grandit du printemps à l'été, avant le détachement annuel de ses latérales à la fin de l'été, permettant une plus large dissémination des jeunes recrues. En conséquence, une seule cohorte a été observée parmi les populations de *S. muticum*. Cette stratégie permet d'allouer plus d'énergie à la croissance et à la reproduction, plutôt qu'au maintien de structures pérennes tout au long de l'année. Ainsi, cette espèce peut rapidement atteindre une grande taille, augmentant son potentiel invasif. Cette stratégie nécessite ensuite une protection renforcée pour limiter les conséquences négatives d'un échec de recrutement sur sa survie. En ce sens, les données obtenues sur les pigments suggèrent que *S. muticum* est moins sensible à la lumière. De plus, elle a présenté une augmentation significative de sa teneur en composés phénoliques simultanément à sa période de reproduction en été, et une variation saisonnière de la composition en acides gras opposé à celle de l'espèce native (*i.e.* une augmentation du contenu en AGPI en mai avec l'augmentation de la lumière et de la température), participant à réduire le risque de mortalité des premiers stades de développement. D'autre part, les données écologiques et métabolomiques de cette étude sont comparables à celles obtenues lors d'une étude précédente en 2006 (Le Lann *et al.* 2012). Ainsi, *S. muticum* semble avoir stabilisé ses populations en Bretagne, près de 50 ans après sa première introduction, et ne semble pas avoir eu d'effet négatif direct sur les autres espèces natives étudiées. Cette espèce commune et abondante pourrait alors être considérée comme une espèce naturalisée plutôt que comme une espèce invasive. Récemment, Fernández (2020) a également conclu que *S. muticum* pourrait être considérée comme un nouvel élément de la flore algale locale, après avoir rapporté 30 ans d'observations dans le nord de l'Espagne. Néanmoins, les résultats suggèrent que la croissance et/ou la reproduction de cette espèce pourraient être favorisées par une lumière et une température élevées. Ainsi, bien que *S. muticum* semble être stable aujourd'hui, son potentiel invasif pourrait évoluer d'avantage dans le contexte du changement climatique. Au contraire, les résultats indiquant des différences spatiales suggèrent que l'hydrodynamisme est un facteur important, régulant la distribution de cette espèce en provoquant un détachement différé de ses latérales. A cet égard, une future augmentation des conditions hydrodynamiques pourrait créer un échec du recrutement et menacer la survie de ses populations. De ce fait, des études à long terme sont nécessaires car ses impacts futurs restent inexplorés dans le contexte du changement global.

## Conclusion et perspectives

Le changement global et les activités humaines entraînent des modifications permanentes du système climatique et des perturbations profondes des écosystèmes. En conséquence, les espèces sont amenées à migrer, ou à évoluer et à s'adapter à de nouvelles conditions de vie par leur écologie, leur phénologie ou leur physiologie, parfois même au niveau moléculaire ou génétique. Chaque espèce va alors s'adapter différemment, les espèces présentant une capacité d'acclimatation / plasticité élevée étant plus susceptibles de s'adapter aux conditions environnementales futures que d'autres. De ce contexte, le nombre d'introductions et d'extinctions d'espèces va probablement augmenter, devenant une menace importante pour la flore marine dans le monde entier. En ce sens, ce travail de thèse a démontré une grande variabilité spatiale et temporelle dans l'écologie et la composition métabolomique des macroalgues brunes et rouges des estrans rocheux de Bretagne, démontrant la capacité d'adaptation de ces organismes, surtout les espèces introduites. Ainsi, ces travaux améliorent notre compréhension de l'adaptation future des populations d'algues aux changements mondiaux, confirmant que certaines espèces sont plus susceptibles d'être menacées par le changement global et les apports anthropiques. Néanmoins, l'évolution des espèces natives / introduites reste incertaine. Par exemple, l'espèce nordique *H. siliquosa* a récemment été signalée dans des mares rocheuses eulittorales des côtes portugaises, étendant son aire de répartition géographique vers le sud, allant à l'inverse de toutes les prédictions (Lima *et al.* 2009). L'évolution des populations d'algues est d'autant plus incertaine que la population humaine continue de croître et que la pression anthropique grandissante qui en résulte va rendre les macroalgues plus vulnérables aux changements environnementaux, entraînant des modifications de leur résilience / capacités d'adaptation. Par ailleurs, les macroalgues vont s'adapter à différentes échelles spatiales et temporelles, c'est-à-dire de l'échelle locale à l'échelle mondiale, avec des espèces qui s'adapteront plus rapidement que d'autres. Par exemple, Liesner *et al.* (2020) ont montré des variations dans les réponses physiologiques et la diversité génétique de *Laminaria digitata* à travers sa distribution latitudinale (*i.e.* de la France à la Norvège). De même, Martínez *et al.* (2012) ont suggéré à travers une étude menée au nord de l'Espagne que *B. bifurcata* pourrait s'adapter plus tardivement que *Himanthalia elongata* du fait d'une réponse plus tardive. Ce retard dans la réponse des organismes limite la possibilité de transférer automatiquement les connaissances d'un organisme à un autre, ou d'une population à une autre. A cet égard, les résultats de ce travail de thèse ont été obtenus pour les populations intertidales de Bretagne, qui est une zone géographique particulière,

constituant une zone de transition entre les régions tempérées froides et tempérées chaudes (Spalding *et al.* 2007; Figure 5 dans la **section 2.1 de l'introduction**). De plus, les résultats ont montré des variations inter-sites dans cette zone géographique, et ont mis en évidence la présence de micro-habitats au sein d'un même site, *i.e.* des habitats localisés / à petite échelle, dont les conditions environnementales particulières diffèrent de celles de la zone environnante.

Par ailleurs, prévoir l'adaptation et l'évolution des espèces est un exercice complexe car le changement global est progressif / graduel. En effet, les écosystèmes sont en constante évolution et les espèces s'adaptent constamment, ce qui entraîne un renouvellement continu des espèces. Dans ce sens, le concept de réchauffement climatique est apparu dans les débats publics dès les années 50 (Fleming 2005), et depuis, ce phénomène a déjà conduit à de nombreuses adaptations / migrations / introductions d'espèces que personne n'avait prévues auparavant. Néanmoins, bien que les écosystèmes subissent une évolution progressive des conditions environnementales, les impacts du changement climatique sur ces écosystèmes et la biodiversité s'intensifient dans le monde entier depuis 20 ans. On peut donc supposer que ces changements pourraient devenir trop rapides pour que les espèces puissent s'y adapter. Il est prédit notamment des vagues de chaleur de plus en plus fréquentes, plus intenses et plus longues (IPCC 2019), auxquelles seules certaines espèces pourront faire face. En ce sens, cette étude a montré que les espèces situées à la limite de leur aire de répartition sont les premières à être menacées. Celles-ci sont d'autant plus menacées qu'elles sont / seront affaiblies à la fois par le changement climatique et par la concurrence avec de nombreuses espèces introduites. De plus, on ne sait pas comment le potentiel invasif des espèces introduites va évoluer comme expliqué précédemment. Dans ce sens, certaines espèces discrètes jusqu'à présent pourraient devenir plus menaçantes pour les espèces natives du fait de la modification de certains paramètres environnementaux qui pourrait favoriser leur développement. Cette étude soutient donc l'importance de surveiller les espèces à différentes échelles de temps et d'espace, tout en renforçant l'approche éco-métabolomique en analysant davantage de composés, ou en obtenant des empreintes/profils métabolomiques complets afin de mieux appréhender les capacités d'adaptation des différentes espèces.

**Titre :** Réponses écologique et métabolomique aux contraintes environnementales des macroalgues marines rouges et brunes de la zone intertidale

**Mots clés :** Acclimatation, Acides aminés de type mycosporine, Changement global, Culture, Espèces invasives, Florideophyceae, Sargassaceae, Suivi écologique

**Résumé:** Les macroalgues marines de la zone intertidale vivent dans un environnement très variable, actuellement menacé par les activités humaines qui entraînent des changements profonds des écosystèmes marins dans le monde entier. À cet égard, les populations d'algues doivent s'adapter à un environnement en constante évolution, soit en migrant, soit en produisant des métabolites particuliers par exemple, au risque de disparaître. En outre, chaque espèce présente une capacité d'adaptation différente, les espèces ayant une plasticité phénotypique élevée étant plus susceptibles de s'adapter aux futures conditions environnementales que d'autres. Dans ce contexte, l'objectif de ce travail était d'étudier les capacités d'acclimatation de cinq espèces de macroalgues (native ou introduite) en Bretagne (France), à travers un suivi d'un an combinant à la fois des données écologiques et métabolomiques. La première partie focalisait sur les macroalgues

rouges et les acides aminés de type mycosporine. Les résultats suggèrent que ces composés très diversifiés, dont la voie de synthèse n'est pas complètement élucidée, pourraient être des métabolites secondaires multifonctionnels. Ainsi, ils pourraient jouer un rôle clé dans l'adaptation future de certaines espèces d'algues rouges telles que l'espèce native *Palmaria palmata*, par rapport à l'espèce introduite *Grateloupia turuturu*. La deuxième partie s'est ensuite concentrée sur trois espèces de macroalgues brunes (Sargassaceae), et a montré que l'espèce indigène *Halidrys siliquosa* est plus menacée que l'autre espèce indigène (*i.e.* *Bifurcaria bifurcata*) dans le contexte du changement global. En effet, c'est une espèce d'affinité d'eau froide qui est d'autant plus menacée qu'elle doit faire face à la fois au changement global et à la cohabitation avec des espèces introduites comme *Sargassum muticum*.

**Title:** Ecological and metabolomic responses to environmental constraints of brown and red marine macroalgae from the intertidal zone

**Keywords:** Acclimation, Mycosporine-like amino acids, Global change, Cultivation, Invasive species, Florideophyceae, Sargassaceae, Ecological monitoring

**Abstract:** Marine intertidal macroalgae live in a highly variable environment, currently threatened by human activities which lead to ongoing changes on marine ecosystems worldwide. In this regard, algal populations have to adapt to an evolving environment to avoid disappearance, by migrating or producing particular metabolites for example. Besides, each species has a different adaptive capacity, the species presenting a high phenotypic plasticity being more likely to adapt to future environmental conditions than others. In this context, the aim of this work was to study the acclimation abilities of five macroalgal species (either native or introduced) from Brittany (France), through a one-year monitoring combining both ecological and metabolomic data. The first part focused on red macroalgae and mycosporine-like

amino acids. Results suggested that these highly diverse compounds, whose synthesis pathway is not completely elucidated, could be multifunctional secondary metabolites. Thus, they could play a key role in the future adaptation of some red algal species such as the native *Palmaria palmata*, compared to the introduced *Grateloupia turuturu*. The second part then focused on three species of brown macroalgae (Sargassaceae) and showed that the native *Halidrys siliquosa* is more threatened than the other native species (*i.e.* *Bifurcaria bifurcata*) in the context of global change. Indeed, it is a cold-water affinity species that is all the more threatened as it has to cope both with global change and co-habitation with introduced species such as *Sargassum muticum*.