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Aurélien Baud

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Aurélien Baud. Base biologique et génétique de la domestication de l'algue rouge *Palmaria palmata*. Biodiversité. Sorbonne Université, 2023. Français. NNT : 2023SORUS666 . tel-04514998

HAL Id: tel-04514998

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Thèse de Doctorat de Sorbonne Université

Ecole Doctorale 227 « Science de la Nature et de l'Homme : Evolution et Ecologie »

MNHN-Sorbonne université

En vue de l'obtention du grade de Docteur de Sorbonne Université

Bases biologiques et génétiques de la domestication de l'algue rouge *Palmaria palmata*



Présenté et soutenu publiquement par Aurélien Baud

Le 15 Décembre 2023

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Remerciements

Tout d'abord, je tiens à remercier mon jury de thèse, Stacy Krueger-Hadfield, Ronan Sulpice, Line Le Gall et Sylvain Huchette pour avoir accepté d'évaluer ce travail ainsi que les membres de mon comité de thèse, Line Le Gall, Didier Aurelle, Catherine Le Blanc, Bertrand Jacquemin et Myriam Valero pour la qualité des échanges que nous avons eus au cours de ces dernières années.

Un grand merci à Philippe, Christophe et Myriam pour leur encadrement, leur disponibilité et pour m'avoir accompagné dans cette grande aventure qui m'a permis de concrétiser un rêve d'enfance : devenir biologiste marin. Je vous remercie également pour votre soutien tout au long de cette thèse, ainsi que pour la préparation intensive du concours de l'Ecole Doctorale. Bien que le début de ma thèse ait connu de nombreux rebondissement digne d'une série Netflix, ces mésaventures m'ont permis de rencontrer des personnes formidables, passionnés par leur métier. A ce propos, je remercie Loan et Tristan pour leur soutien et leur aide sur le terrain et au labo. Un grand merci à Bertrand qui a joué un rôle essentiel dans la conception de ce projet de thèse.

Merci aux membres de l'équipe ABIE et de L'IRL pour toutes nos discussions et nos échanges scientifiques ainsi que pour vos interventions sur le terrain.

Merci à Stéphane Mauger pour m'avoir formé au développement et à l'analyse des marqueurs microsatellites et pour son aide aussi bien sur le terrain qu'au labo.

Merci à Emeline, Elodie, Laurence et Sylvie de m'avoir formé aux techniques et pour l'entretien des cultures de macroalgues.

Merci aux membres du RAS pour la mise en place et l'entretien des système de cultures. Je souhaiterais remercier plus particulièrement Dan, pour son aide inestimable sur le terrain et au laboratoire, sans son ingéniosité, il aurait été impossible de développer tous les systèmes de culture essentiels à la réalisation de cette thèse.

Merci à Laura pour son excellent travail de laboratoire durant son stage.

Merci à Sylvain Huchette et Alexandre Berthelot pour m'avoir permis de réaliser un changement d'échelle des cultures de palmaria et m'a permis d'avancer dans mes réflexions.

Je souhaiterais remercier mes compagnons de bureau, Samuel, Lisa, Yoanis et Guilhem, pour leur soutien tout au long de la thèse ainsi que dans la création de « meme ». Merci également à mes collègues et amis de la Station sans qui cette aventure n'aurait pas été aussi épique : Jean, Jeremy, Erwan, Martin, Louison, Emma, Pierre, Lucie, Roman, Louise, Zofia, Aga, Kevin, Sonia, Yasmine, Pauline, Elisa, Julie, Clarisse, Elias, Pélagie, Aline, PG, Bertille, Camille P.

Merci aux « Océanauts » pour leurs soutiens, pour tous ces bons moments passés ensemble mais aussi pour leurs conseils.

Merci à SR, dont les souvenirs de nos nombreuses aventures m'ont accompagné et réconforté dans les moments difficiles.

Un immense merci à mon épouse, ma fille, mes parents, mes beau-parents, mes frères et toute ma famille, sans qui je n'aurais pas trouvé la force et la volonté d'arriver au bout de cette aventure. Camille, du sommet du Buet au fond des Calanques tu m'as suivi jusqu'au bout de la terre, sans ton soutien cette thèse n'aurait jamais abouti. Talia, malgré les innombrables heures passées au laboratoire, sur le terrain ou derrière un écran à m'arracher mes derniers cheveux, les systèmes de cultures conçus, les hypothèses formulées, les expérimentations créées, vous resterez, toi et ton petit frère, mes plus belles créations.

Le travail présenté ici résulte des nombreuses interactions que j'ai eues avec vous durant ces cinq dernières années, un grand Merci à toutes et à tous.

« Ces algues sont véritablement un prodige de la création, une des merveilles de la flore universelle » - “Pr. Pierre Aronnax”, Vingt Mille Lieues sous les mers de Jule Vernes.

« Nothing makes sense in biology except under the light of Evolution” - Theodosius Dobzhansky

Introduction générale

1. Le processus de domestication

Si la notion de domestication est déjà très complexe en soi, la distinction entre domestication et sélection l'est plus encore car il est possible de domestiquer sans sélectionner, alors que l'inverse ne se conçoit pas (Zeder, 2015). Donc, dans un premier temps, il est important de définir ces deux concepts et les replacer dans l'historique de l'agriculture, de l'élevage d'animaux et de la culture de végétaux terrestres et aquatiques.

1.1 Définition

Initié il y a plus de 12 000 ans chez les plantes et les animaux, le processus de domestication a marqué un changement de paradigme pour l'espèce humaine (Diamond, 2002). D'un point de vue biologique, la domestication peut être définie comme un processus coévolutif issu d'un mutualisme (Bates et al., 2021; Zeder, 2015, 2020), dans lequel une espèce (le domesticateur) crée un environnement dans lequel elle gère l'espèce domestiquée. Cette relation, qui permet d'améliorer la survie et la reproduction des espèces partenaires, se stabilise au cours du temps et peut être considéré comme un processus coévolutif qui découle d'une interaction écologique (mutualisme) (Rindos, 2013; Zeder, 2020). Depuis l'apparition de l'agriculture, les espèces animales et végétales domestiquées ont progressivement accru leur adaptation et leur dépendance à l'environnement artificiel de l'agriculture. Dans ce contexte, il est important de savoir ce qu'est une espèce domestiquée. En d'autres termes, la question est de savoir à partir de quel degré d'évolution et de dépendance les espèces sont reconnues comme des espèces domestiquées (Purugganan, 2022).

L'étape de pré-domestication, qui est plus ou moins longue, consiste à modifier l'environnement de l'espèce à domestiquer pour améliorer sa production (Larson et al., 2014). Durant cette première étape, le domesticateur modifie, de façon volontaire ou involontaire, les pressions de sélection, favorisant les individus capables de prospérer dans ce nouvel environnement (Darwin, 1868 ; Milla et al., 2014). Il est reconnu depuis longtemps qu'il existe d'autres espèces que l'espèce humaine qui font de la domestication, comme par exemple les fourmis qui pratiquent l'élevage ce qui a conduit à la domestication de certaines espèces. Le faible nombre de plantes et d'animaux terrestres aujourd'hui domestiqués par l'espèce humaine, par rapport à l'énorme réservoir d'espèces sauvages, reflète la complexité de ce processus, dont la réussite dépend essentiellement des caractéristiques intrinsèques de l'espèce à domestiquer (Diamond, 2002). Il y a donc des espèces plus faciles à domestiquer que d'autres que l'on appellera ici, les espèces domesticables. Par exemple, on peut penser que les chevaux

et les ânes étaient des espèces “domesticables” alors que les zèbres ne le sont clairement pas. L’un des points clé pour initier la domestication est la transmission des caractères héréditaires impliqués dans ce processus d’une génération à l’autre. Dans ce sens, la maîtrise de la reproduction permet de sélectionner des individus présentant des traits d’intérêt permettant de survivre dans ce nouvel environnement et donc de s’affranchir des populations sauvages (Larson 2014). En retour, cette étape du processus de domestication s’accompagne souvent par une modification profonde de l’environnement de l’espèce à domestiquer ainsi qu’une modification du comportement de l’espèce domesticatrice. Chez l’homme par exemple, ce changement a eu comme conséquence de bouleverser complètement le mode de vie et les sociétés humaines au Néolithique en passant du stade chasseur/cueilleur des populations nomades au stade cultivateur sédentaire (Zeder, 2015).

1.2 Domestication des espèces aquatiques.

D’un point de vue historique, on estime que l’homme a commencé à cultiver des végétaux et à élever des animaux il y a un peu plus de 10 000 ans avec le développement des sociétés grégaires (Troy et al., 2001). En parallèle avec la culture d’annuelles à graines ou à fruits comme le haricot ou le blé, les premières espèces animales domestiquées furent la chèvre, le mouton et le bœuf (Troy *et al*, 2001) ; la domestication du porc, de la poule et du cheval n’apparaissant que 1000 à 4000 ans plus tard (Anthony et al., 1991; Giuffra et al., 2000; West & Zhou, 1988). L’élevage et la domestication des espèces aquatiques sont beaucoup plus récents : la domestication apparaît avec la carpe (*Cyprinus carpio*) et le poisson rouge (*Carassius auratus*) il y a environ 3 500 ans (Balon, 1995; Liao & Huang, 2000). D’une manière générale, la domestication des espèces terrestres s’est développée très rapidement au contraire de celle des espèces aquatiques. On estime que 2 000 ans ont suffi à domestiquer les espèces terrestres actuellement cultivées et élevées. Néanmoins, depuis la révolution industrielle, la domestication de nouvelles espèces terrestres a fortement ralenti alors, qu’au contraire, elle connaît une progression fulgurante chez les espèces aquatiques (Fig. 1, d’après (Duarte et al., 2007) ; 97 % des espèces aquatiques (soit 430 espèces) actuellement élevées, ont été domestiquées au cours du dernier siècle, dont 25% ces 20 dernières années. Ce rythme de domestication est environ 100 fois plus élevé que celui qui a été observé chez les plantes ou les animaux terrestres aux périodes les plus fastes. Au total, et malgré une relation plus ancienne avec l’homme, on estime qu’à l’heure actuelle, 0,08% des plantes et 0,0002% des animaux terrestres connus ont été domestiqués contre 0,17% et 0,13% des plantes et des animaux marins (Groombridge & Jenkins, 2000).

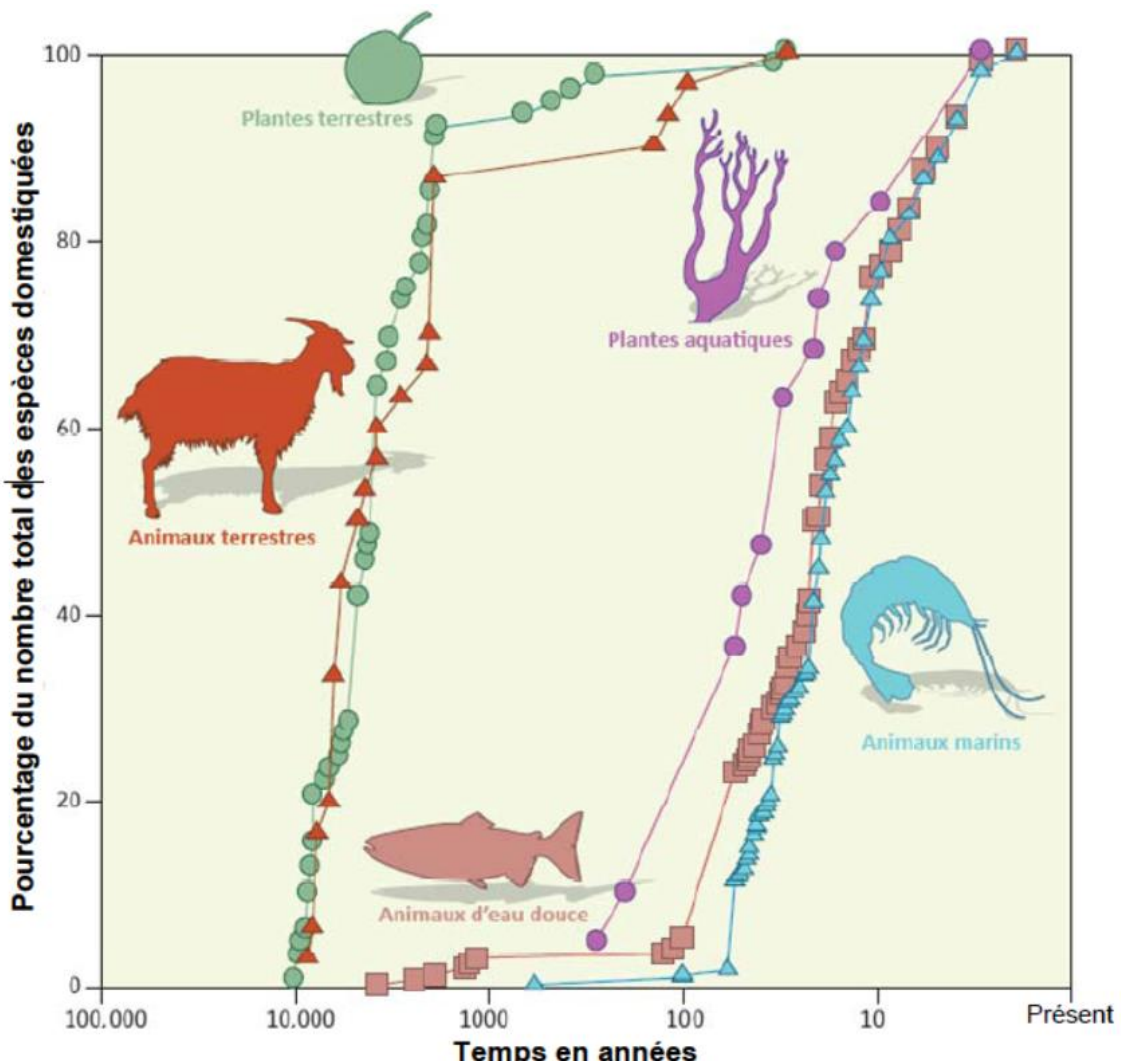


Figure 1 : Historique de l'évolution du pourcentage du nombre total des espèces domestiquées par catégorie (d'après Duarte et al., 2007).

Cette disparité s'explique, en premier lieu, parce que la domestication des espèces terrestres s'est principalement concentrée sur les mammifères et les oiseaux et très peu sur les invertébrés (i.e. les abeilles ou les escargots) alors qu'un large éventail de taxons aquatiques ont pu être domestiqués (i.e. mollusques, crustacés, vertébrés, échinodermes, méduses et vers (Olive, 1999) FAO, 2018). On considère que plus de 3 000 espèces aquatiques sont potentiellement comestibles contre seulement 200 espèces terrestres (Pullin, 1996 ; FAO, 2018), ouvrant ainsi de vastes perspectives de domestication. En second lieu, les embranchements du vivant sont bien plus diversifiés dans l'océan que sur terre, ce qui augmente d'autant les chances d'y trouver des espèces offrant des critères favorables à leur exploitation, e.g. absence de toxicité, croissance rapide, cycle de vie court, tolérance à l'aliment et aux conditions de captivité (Diamond, 2002). Cependant, le nombre d'espèces de

végétaux aquatiques domestiquées est resté relativement faible par rapport aux nombres d'espèces de végétaux terrestres (Duarte et al., 2007)

Enfin, la domestication des espèces aquatiques a pu bénéficier de la rapide avancée des connaissances techniques et scientifiques associée à une demande sociétale croissante en produits aquacoles et à la stagnation des pêches mondiales due à la surexploitation des ressources marines (Pauly et al., 2002). Cette conjoncture a permis un essor mondial de l'aquaculture, e.g. : la biomasse sauvage capturée de poissons, crustacés, mollusques, algues stagne depuis plus de 30 ans au-dessous de 90 millions de tonnes pour les pêcheries, mais ce tonnage a été progressivement dépassé par celui de l'aquaculture, soit 120 millions de tonnes, dont cependant 40 % est d'origine continentale (FAO, 2018). Sur ce volume, les algues marines contribuent à près de la moitié des productions aquacoles marines (FAO, 2018).

1.3 Domestication des macroalgues marines

Bien que les premières traces d'utilisation des macroalgues par l'homme remontent à plus de 14 000 a.v JC (Dillehay et al., 2008), la domestication des macroalgues est un processus plus récent que celle des plantes et animaux terrestres (Duarte et al., 2007). Les premières étapes consistaient à exploiter les populations sauvages de macroalgues présentes sur l'estran (Valero et al., 2017). Il faut attendre le 17^e siècle avant de voir apparaître les premières cultures de nori (*Pyropia*) par captage de spores sur des bambous au Japon et en Corée (Wheeler et al. 1979, Hwang & Park, 2020) ou également par décapage de surface de roches en préalable de l'apparition des frondes de nori en Chine. Cependant, le véritable essor de l'algoculture s'est produit à partir de la deuxième moitié du 20^e siècle avec les premiers programmes de sélection génétique sur *Saccharina japonica* (Loureiro et al., 2015; Zhang et al., 2007). Un autre aspect qui explique l'initiation tardive du processus de domestication des macroalgues est la diversité et la complexité de leur cycle de reproduction (Fig. 2). En effet, sous le terme de macroalgues sont regroupées trois grandes lignées évolutives distinctes qui ont divergées il y a plus de un milliard d'années (Fig. 9): le phylum des Chlorophyta (algues vertes), le phylum des Rhodophyta (algues rouges) et celui des Phaeophyceae (algues brunes) (Chapman et al. , 1998). Une grande diversité de cycles diphasiques montrant l'alternance de générations gamétophyte et de sporophyte a émergé au cours de l'évolution de ces différentes lignées (Liu et al., 2017). L'existence de certaines phases microscopiques dans leur cycle de reproduction a limité fortement la maîtrise de leur reproduction et donc l'amélioration des méthodes de culture. Par exemple, l'identification de la phase diploïde microscopique filamenteuse du cycle de reproduction de *Porphyra umbilicalis* en 1949 par Drew a permis l'industrialisation de la culture de cette espèce (Drew, 1949). Ses expériences sont

rapidement répétées par le phycologue japonais Sokichi Segawa sur les espèces asiatiques de *Pyropia* spp. (identifiées comme *Porphyra*), qui à son tour révolutionne la culture de la nori qui auparavant souffrait de récoltes incertaines. Déjà en 1953, Fusao Ota et d'autres biologistes marins japonais avaient développé des techniques de semis artificiels en se basant sur les recherches de Kathleen Mary Drew-Baker (Mouritsen, 2013). Pour les algues brunes, les travaux de Sauvageau sur les Laminaires et de la Tilopteridales, *Saccorhiza polyschides* ont amené à la première découverte d'un cycle de vie hétéromorphe (1915). Cette avancée scientifique significative a suscité beaucoup d'intérêt pour la culture d'algues brunes et ces travaux ont été repris comme bases de la domestication de *Saccharina japonica* (anciennement *Laminaria japonica*) en République Populaire de Chine au début des années 1950 par le Professeur Tseng et ses collaborateurs (Cheng, 1969). L'acquisition de connaissances fondamentales sur le cycle de reproduction des macroalgues est donc essentielle pour mener à bien le processus de domestication. Plus récemment, le transfert des méthodes calquées sur l'agriculture vers l'algoculture (culture intensive, sélection de cultivars, transfert d'espèces) pose aujourd'hui certains problèmes liés à la durabilité de ces pratiques (dépression de consanguinité, maladie, introduction d'espèces à caractère invasive) (Loureiro et al. 2015, Valero et al 2017).

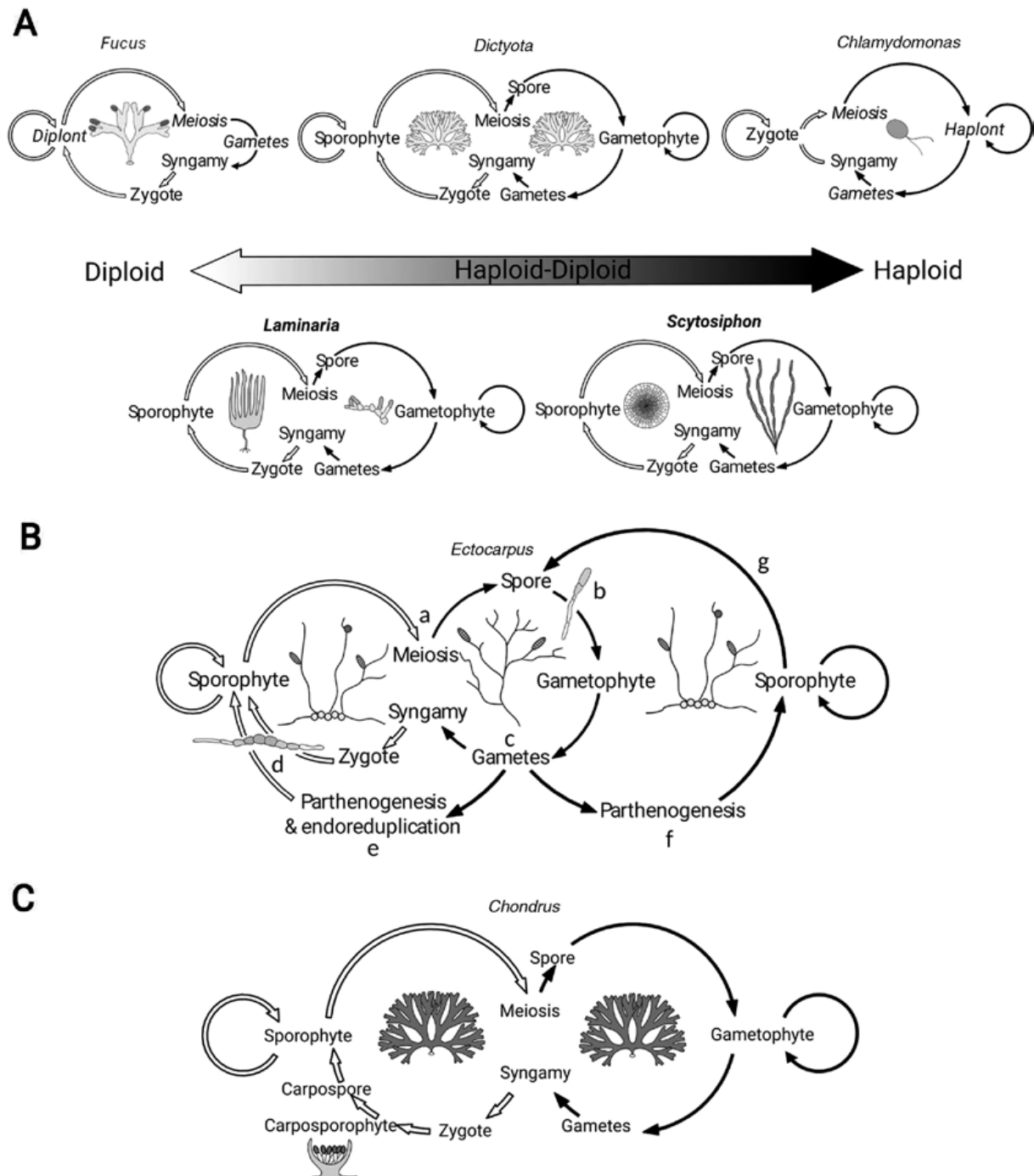


Figure 2 : Diversité des cycles de reproduction chez les macroalgues (Liu et al. 2017). Les stades diploïdes et haploïdes sont marqués respectivement par des flèches blanches et noires. (A) Cycle de vie des algues brunes. Les fucales sont caractérisées par un cycle de vie diplontique. La méiose dans le tissu reproducteur est immédiatement suivie d'une gamétogénèse et d'une syngamie produisant un zygote diploïde. La plupart des algues telles que *Dictyota*, *Scytosiphon* et *Laminaria* présentent un cycle de vie diplohaplontique où un gamétophyte haploïde alterne avec un sporophyte diploïde. Ici, après la méiose, la spore résultante se développe en un organisme multicellulaire. Les phases haploïde et diploïde peuvent être de morphologie identique (isomorphe) (*Dictyota*), ou les deux phases peuvent se développer différemment (hétéromorphe), le gamétophyte (*Laminaria*) ou le sporophyte (*Scytosiphon*) étant microscopique. De nombreuses algues unicellulaires (*Chlamydomonas*) sont caractérisées par un cycle de vie haplontique où la formation d'un zygote est immédiatement suivie d'une division méiotique. (B) Cycle de vie diplohaplontique simplifié

d'*Ectocarpus siliculosus*. La méiose (a) a lieu dans le sporophyte (diploïde) pour produire des spores haploïdes. La première division cellulaire des spores en germination est asymétrique (b) et elles se transforment en gamétophytes multicellulaires. Les gamétophytes produisent des gamètes mâles et femelles (c) morphologiquement identiques mais physiologiquement différenciés, qui fusionnent pour former un zygote. Après une première division cellulaire symétrique (d), le zygote se transforme en sporophyte diploïde. Alternativement, les gamètes qui ne rencontrent pas de partenaire du sexe opposé se transforment en parthénosporophytes diploïdes au moyen d'une parthénogenèse combinée à une endoreduplication (e) ou en parthénosporophytes haploïdes (f). Cette dernière produit des méiospores via un événement apoméiotique non réducteur (g). (C) Cycle de vie de l'algue rouge *Chondrus* montrant trois stades distincts : le gamétophyte, le sporophyte et le carposporophyte, qui se développe de manière parasitaire sur le gamétophyte après fécondation. Les boucles reliant une génération à elle-même désignent une reproduction asexuée médiée par la reproduction végétative (par exemple fragmentation, formation de propagules) (*Fucus*, *Dictyota*), la formation de spores asexuées (mitospores) (*Dictyota*, *Ectocarpus*, *Laminaria*), la mitose (*Chlamydomonas*) ou le développement parthénogénétique de gamètes (femelles) non fécondés (*Scytosiphon*, *Laminaria*, *Ectocarpus*).

1.3.1. Les cultures d'algues dans le monde

Selon les statistiques de la FAO (FAO, 2018), parmi les sept taxons d'algues les plus cultivés, trois sont utilisés principalement pour l'extraction d'hydrocolloïdes : *Euचेuma spp.* et *Kappaphycus alvarezii* pour les carraghénanes, et *Gracilaria/Gracilariopsis spp.* pour l'agar-agar; *Saccharina japonica*, *Undaria pinnatifida*, *Pyropia spp.* et *Sargassum fusiforme* représentent les plus importantes productions essentiellement pour l'alimentation humaine et quelques autres applications comme aliments fonctionnels (Fig. 3). Les principaux pays producteurs sont la Chine, l'Indonésie et les Philippines, qui cultivent aussi une plus grande diversité d'espèces d'algues (7, 6 et 4 respectivement, FAO, 2014, 2016). Alors que la consommation d'algues en Asie du Sud-Est reste commune et traditionnelle, dépendante surtout du goût et du prix, l'utilisation des algues comme aliment dans les pays non asiatiques, comme les marchés européens et américains ont introduit des paramètres tels que la valeur nutritionnelle et la « valeur santé », avec une forte préférence des consommateurs pour produits biologiques, durables et équitables (à faibles impacts tant sur l'environnement que sur la biodiversité) (Chapman et al., 2015; Gomez Pinchetti, 2016). Cependant, sur le marché mondial, la part de la production d'algues marines utilisée à des fins alimentaires et « autres utilisations », c'est-à-dire autres que pour les hydrocolloïdes, est toujours inférieure à 1% de la production totale de biomasse (Fig. 3).

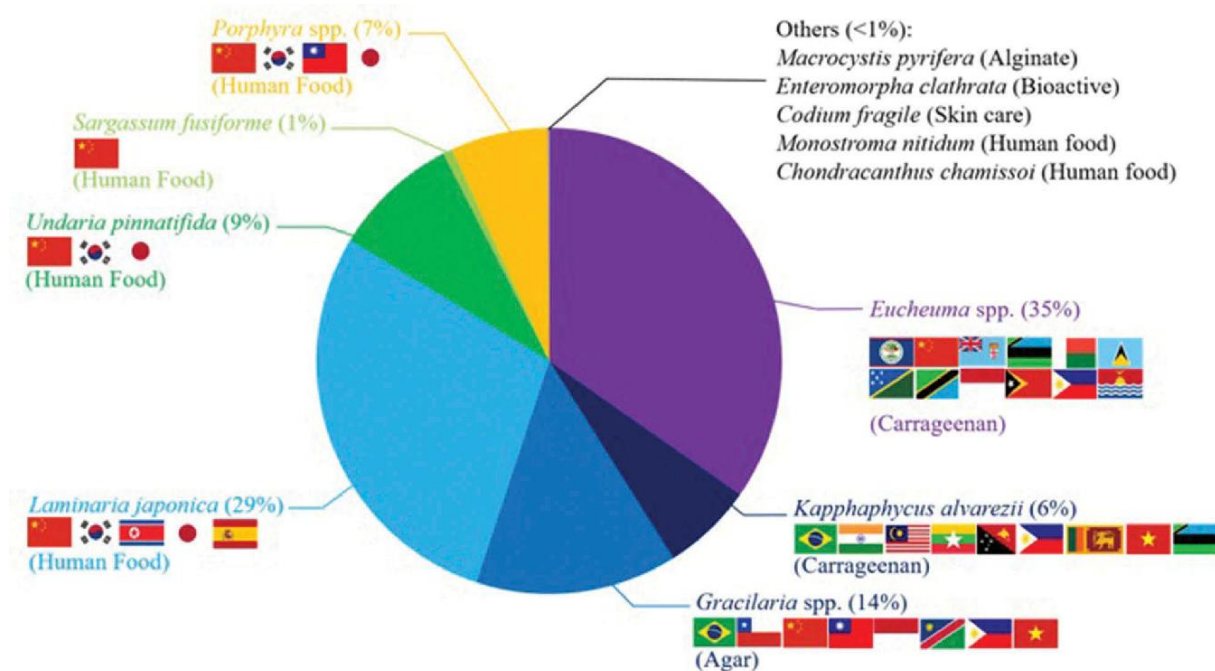


Figure 3: Pourcentage de production aquacole d’algues par espèce et par pays pour l’année 2014. Les principales utilisations réelles de chaque espèce ont été incluses. Source : FAO (2016). Extrait de (Buschmann et al., 2017)

La culture des algues peut être pratiquée sur terre ou en mer. Même si les technologies de culture des algues dans ces deux systèmes ne sont pas fondamentalement différentes, il existe des différences évidentes dans les méthodes de culture en raison de ces différents environnements.

1.3.1.1 Cultures à terre

La culture d'algues à terre se fait principalement dans des systèmes fermés tels que des réservoirs d'eau, des étangs, des lagunes et des pipelines (Sara et al. 2020, Hafting et al., 2012). La méthode de culture convient à un large éventail de genres d’algues qui se reproduisent surtout par bouturage, mais elles peuvent aussi propager des plantules issues d’écloseries par reproduction sexuée ou développement de spores. Ces systèmes peuvent disposer d’équipements simples et facilement accessibles qui permettent un suivi en temps réel et une régulation efficace des conditions de culture des algues (nutriments, lumière, pH, CO₂ et salinité) pour produire davantage de produits ciblés.

Actuellement, les exploitations terrestres de cultures d’algues ne produisent pas en masse en raison du prix élevé de l’espace. Ils peuvent cependant produire toute l’année.

Les fermes terrestres peuvent également cultiver des espèces plus difficiles, voire impossibles à cultiver en mer. Tout comme dans une serre de cultures végétales terrestres, tout peut être contrôlé.

Si la prise d'eau de mer commence à aspirer de l'eau pauvre en nutriments ou polluée, l'ensemble du système peut passer à l'eau de mer fertilisée ou artificielle. C'est ce contrôle qui sera crucial si le changement climatique se poursuit au rythme actuel. Si les océans deviennent plus acides ou trop chauds, l'aquaculture terrestre pourrait être la seule option à la condition de trouver des systèmes de thermorégulation soutenables basées sur des énergies renouvelables. L'avantage supplémentaire de produire toute l'année est que le produit peut être récolté à tout moment.

Les principaux tonnages actuellement produits dans des fermes à terre sont les algues vertes du genre *Ulva* sur tous les continents et des algues rouges comme *Chondrus crispus* au Canada et en France, *Gracilaria spp.* en Californie et *Palmaria mollis* dans l'Oregon (Pereira et al., 2024) (Fig. 4).



Figure 4 Deux installations de cultures de macroalgues à terre en France. Gauche Raceways de cultures d'Ulves de l'entreprise France Haliotis à Plouguerneau (opérationnelle depuis Juillet 2021). Droite : Ferme historique de la société Innovalg en Vendée cultivant du *Chondrus crispus* et des diatomées (en opération depuis 1989), source www.idealg.org.

1.3.1.2 Les cultures en pleine mer

L'aquaculture côtière en eaux peu profondes est pratiquée depuis au moins quatre siècles en Asie dans la zone maritime proche de la terre et constitue la principale méthode de culture d'algues, des estrans jusqu'à une profondeur de mer de 5 à 50 m, à l'aide de pieux fixes posés sur le fond ou d'autres systèmes d'ancrage. Ces baies sont suffisamment riches en nutriments d'origine terrestres et bénéficient d'un hydrodynamisme et d'un régime de vent modérés. Pour répondre à la demande croissante de produits à base d'algues, le domaine de la culture des algues s'est étendu vers l'off-shore et il existe une méthode de culture en eau profonde en mer qui repose sur des radeaux flottants ou de longues cordes pour fabriquer des radeaux de culture (Fig.5). La mariculture n'occupe pas ainsi de ressources foncières rares.

La culture en mer à une certaine distance du rivage est une alternative moins coûteuse à la culture à terre dans des réservoirs. Les diverses méthodes de culture et récolte des algues varient selon l'espèce cultivée. Elles peuvent être soit naturellement fixées au fond de l'océan ou suspendues à des réseaux flottants de cordes ou de filets, technique populaire à cause de son coût modéré. Les semences peuvent être attachées directement aux cordes ou transplantées après une période de croissance initiale en éclosiers. C'est une technique qui a fait ses preuves en Asie et reste à un coût abordable si la productivité est bonne, mais elle présente aussi des défis importants. En effet, ce type de ferme marine est à la merci des conditions climatiques que l'on peut retrouver en mer et qui peuvent emporter les infrastructures et les algues elles-mêmes. Pour réduire les risques et minimiser les pertes, ce type d'installations doit être à la fois intensif et réparti en différents lieux. On recherche habituellement des lieux où les courants sont suffisamment forts pour transporter des quantités importantes de nutriments. Un autre problème courant de cette technique est la contamination par des organismes, qui viennent se réfugier dans ces grandes laminaires, causant ainsi des pertes de biomasse et obligeant les producteurs dans certains cas à récolter prématurément les algues, avant qu'elles aient atteint leur teneur optimale en polysaccharides, par exemple. Les changements climatiques et les phénomènes comme El Niño, qui peuvent affecter la température et la qualité de l'eau, sont aussi des menaces pour la culture en mer. Il s'agit globalement d'une technique relativement peu coûteuse, mais qui comporte son lot de menaces et de défis.

Les cultures d'algues rouges tropicales (*Euclima/Kappaphycus* et *Gracilaria*) et des Gracilaires au Chili s'opèrent avec des techniques très différentes de celles des algues multipliées par reproduction sexuée ou par collecte de spores.

Parmi les nombreux systèmes développés à partir d'expériences sur le terrain et en laboratoire, un seul type, le système tie-tie, qui se manifeste sous trois formes de base : lignes tendues fixées sur des pieux, flottantes et en radeaux, s'est propagée dans toutes les régions de l'Asie du Sud-Est, de l'Afrique de l'Est où elles ont été transférées (Ask & Azanza, 2002). Le système s'est répliqué principalement parce que (1) il était simple, (2) les matériaux nécessaires étaient facilement disponibles et peu coûteux, et (3) les algues se développaient très bien sur des cycles de 30 à 45 jours (Fig.6). Les sacs en filets et les filets tubulaires les filets sont aussi utilisés aujourd'hui par les entreprises comme Sea6 Energy qui vise à développer de nouveaux types de fermes d' Euclimoides.

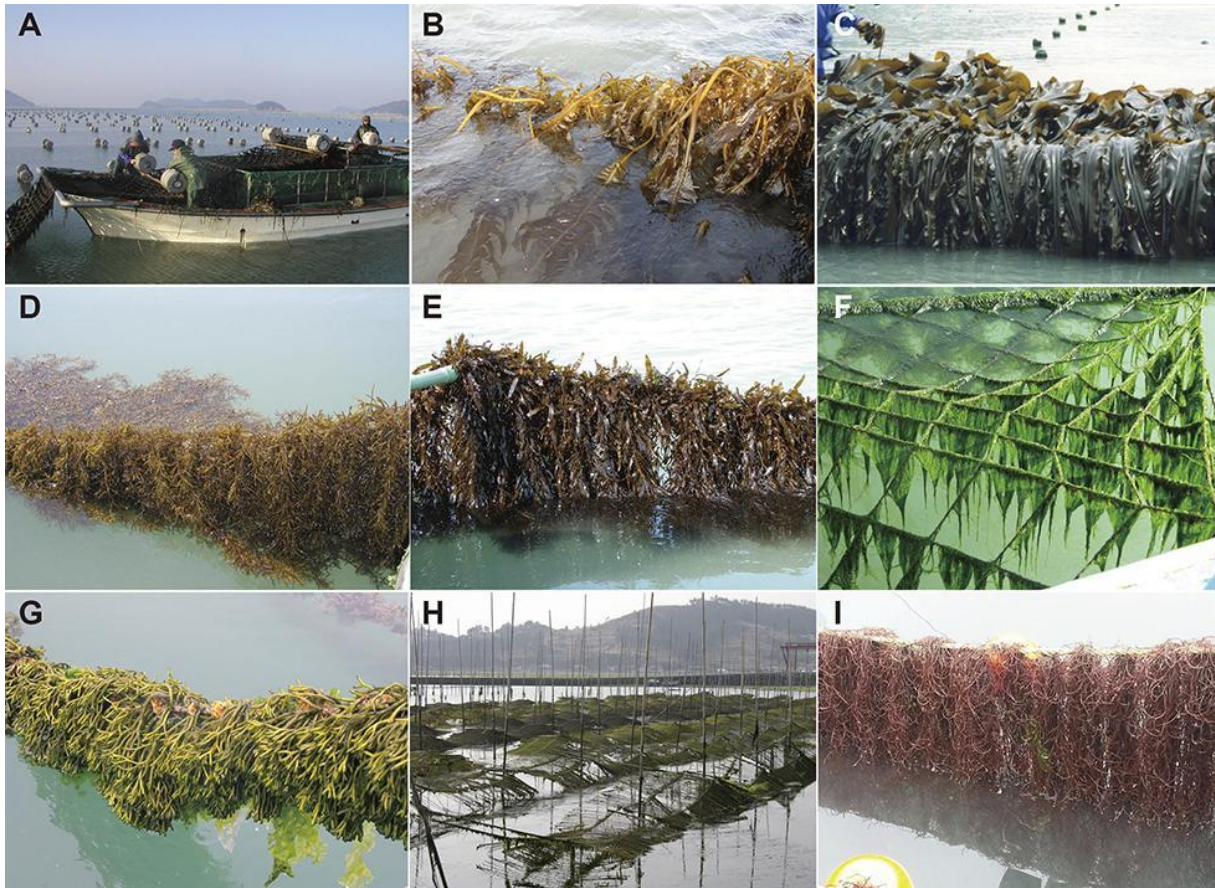


Figure 5: Exemples d'espèces d'algues marines cultivées en pleine mer en Corée du Sud. (A) *Pyropia* sp., (B) *Undaria pinnatifida*, (C) *Saccharina japonica*, (D) *Sargassum fusiforme*, (E) *Sargassum fulvellum*, (F) *Ulva* sp., (G) *Codium fragile*, (H) *Capsosiphon fulvescens* and (I) *Gracilariopsis chorda*. (Hwang et al., 2020).



Figure 6: Pratiques de la culture d'espèces d'algues rouges tropicales cultivées en Asie du Sud-Est et en Afrique de l'Est : *Kappaphycus alvarezii* et *Eucheuma spinosum*. (Crédit photo : Leila Hayashi)

1.3.2. Les cultures d'algues en Europe

En Europe, aujourd'hui, la majorité de la production de macroalgues provient de l'exploitation des populations sauvages (FAO, 2018). Une première espèce d'algues brunes domestiquée en Asie a été introduite en France et en Espagne dans les années 1980 pour démarrer sa culture en mer. En 1983, elle a été introduite de manière volontaire pour des tests de culture en Bretagne : baie de Lampaul (Ouessant), île de Groix, île de Sein et dans l'estuaire de la Rance (St Malo). Des populations spontanées ont ensuite été signalées en Manche, de Ouessant jusqu'aux Pays-Bas, et sur les côtes anglaises. Des populations se sont également installées sur la façade Atlantique, en France, du Guilvinec à la Rochelle et en Espagne en Asturies et en Galice (Voisin, 2007)). En 2012, ces connaissances ainsi qu'une réglementation européenne donnant un cadre législatif à l'aquaculture d'espèces exotiques ou localement absentes conduisent l'Ifremer à formuler un avis réservé quant à la mise en place de nouvelles cultures de l'espèce (Lemoine et al., 2012). Sinon seules deux espèces de laminaires, *Saccharina latissima* et *Alaria esculenta* sont actuellement cultivées en mer et font l'objet de programme d'amélioration variétale (Kraan & Guiry, 2006). Cependant pour le moment, leurs cultures pour la production de biomasse reposent encore sur l'utilisation de géniteurs sauvages (Barbier et al.,

2020). L'augmentation de la demande en biomasse ainsi que l'impact du changement climatique qui menace l'exploitation des populations naturelles nous incitent aujourd'hui à passer du stade récoltant au stade algoculteur pour préserver cette ressource (Buschmann et al., 2014).

Le développement de la production aquacole de macroalgues est actuellement en cours dans 13 pays européens, mais il reste à un stade précoce de développement en Europe en termes de volumes de production et de nombre d'unités de production (Araújo et al., 2021). Selon les statistiques officielles, la production aquacole d'algues contribue à moins de 1 % de la production européenne totale de biomasse d'algues (Araújo et al., 2019), représentant dans cette étude 32 % des unités de production de macroalgues cartographiées. La plupart des unités de production sont situées en mer (au large ou dans les eaux côtières) avec seulement 24 % des entreprises exerçant des activités terrestres (Araújo et al. 2021) (Figure 7).

L'aquaculture d'algues est considérée comme un moyen possible de répondre à la demande croissante de l'industrie de transformation pour des rendements de biomasse traçables, de haute qualité et prévisibles. En outre, il est largement reconnu qu'une transition de la récolte d'algues sauvages vers l'aquaculture est nécessaire pour répondre à la demande croissante tout en évitant la surexploitation des ressources en algues sauvages. De plus, on constate un intérêt croissant pour les pratiques agricoles respectueuses de l'environnement dans lesquelles les macroalgues joueront un rôle clé dans le développement émergent de l'aquaculture multitrophique intégrée (IMTA), actuellement promue aux niveaux européen et national (Alexander et al., 2016; Buck et al., 2018; Kleitou et al., 2018). En Europe, la Norvège est le pays qui compte le plus grand nombre d'entreprises d'aquaculture d'algues. Ceci est le résultat de la récente stratégie norvégienne encourageant les instituts de recherche, les industries et les pouvoirs publics à développer une bioéconomie basée sur la production et la transformation d'algues cultivées (Stévant et al., 2017). Les premières licences pour la culture d'algues en mer en Norvège ont été accordées en 2014. En 2019, la surface allouée à la culture d'algues était de 834 ha, ce qui correspond à un potentiel de production d'environ 48 000 tonnes (FW), avec une production enregistrée de 111 tonnes pour une valeur d'environ 0,43 million d'euros (Gjertsen et al., 2020). Le potentiel estimé n'a pas encore été atteint puisque seule une partie des entreprises titulaires d'un permis sont actuellement en activité et la plupart ont encore réduit leurs capacités de production (Broch et al., 2019). Bien que la culture à grande échelle ne soit pas encore une réalité, la côte norvégienne dispose des conditions optimales pour étendre davantage la superficie et l'emplacement des sites de culture (Stévant et al., 2017).

D'autres pays, comme le Danemark, comptent actuellement une part égale d'entreprises d'aquaculture et de récolte, avec des entreprises commerciales se destinant à la production de

Laminaires à grande échelle dans le cadre de l'IMTA depuis 2012 (Marinho et al., 2015) ou dans les îles Faroe (Bak et al., 2018)

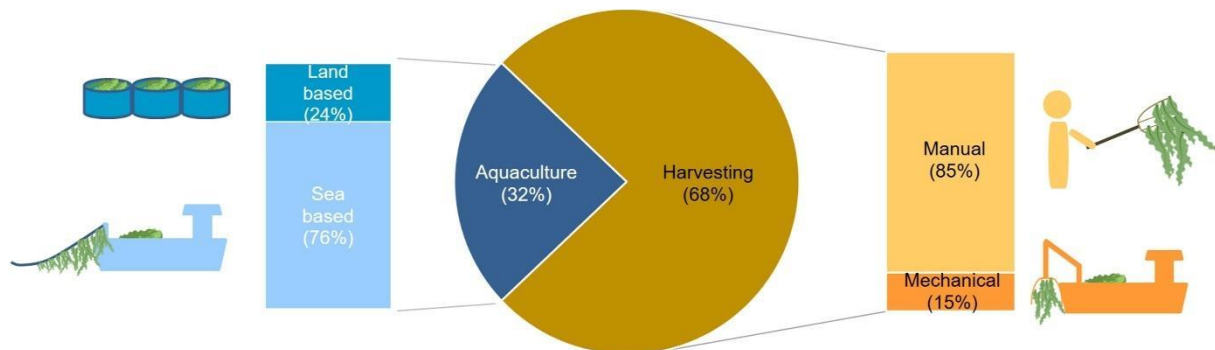


Figure 7 : Production de macroalgues en Europe (part selon le nombre d'entreprises utilisant ces méthodes) extrait d'Araújo et al. (2021).

Dans des pays comme la France, l'Espagne et le Portugal, certaines entreprises aquacoles sont déjà établies à une échelle entièrement commerciale avec des installations de production basées en mer (côtières) ou à terre. Au Portugal, l'entreprise AlgaPlus a réussi à développer un modèle original de productions en bassins à terre dans le système lagunaire d'Aveiro (<https://genialproject.eu/2020/08/28/a-future-of-seaweed-farming-in-europe/>).

En Espagne, le développement de l'aquaculture à l'échelle commerciale d'espèces d'algues alimentaires suscite un intérêt croissant depuis le début des années 2000 (Peteiro et al., 2016). Cette attention a stimulé le développement d'initiatives de pisciculture commerciale pour deux espèces d'algues : *S. latissima* et *U. pinnatifida*, cette dernière étant interdite par la réglementation environnementale nationale sur les espèces exotiques envahissantes (Lemoine et al., 2012). Actuellement, certaines licences sont accordées (au moins quatre autorisations) pour la culture commerciale de *S. latissima* dans des systèmes marins et terrestres. Bien que le nombre de licences soit encore faible, on s'attend à ce que l'aquaculture d'algues se développe de manière significative au cours des années à venir, en particulier la culture d'espèces très demandées et à forte valeur commerciale et pour lesquelles l'extraction des stocks sauvages n'est actuellement pas autorisée en raison de contraintes environnementales et de mesures de protection (par exemple pour *S. latissima* et *P. palmata*) ou la faible disponibilité de biomasse sauvage (par exemple *Porphyra* sensu lato). D'autres espèces d'algues telles que *Ulva* spp. et *Gracilaria/Gracilariopsis* spp. Sont également actuellement cultivés dans des systèmes à terre. Les autres pays disposant d'installations de culture sont l'Estonie, les Îles Féroé, l'Islande, l'Irlande, la Suède, les Pays-Bas et le Royaume-Uni (Araujo et al., 2021).

Différentes techniques de culture sont utilisées en fonction de l'espèce cible et des conditions disponibles. Chaque technologie de culture présente ses propres avantages et défis car les systèmes de culture européens sont loin de rivaliser avec les productions algales asiatiques (Barbier et al., 2019).

1.3.3. Les défis de la domestication des macroalgues marines

La domestication de nouvelles espèces est donc nécessaire pour effectuer cette transition. Elle représente aujourd'hui un défi majeur qui va bien au-delà de la compréhension et de la maîtrise de la reproduction de l'espèce cible. En effet, les connaissances accumulées sur plusieurs millénaires de domestication des espèces terrestres, le développement des outils de biologie moléculaire, le recul critique sur les pratiques de l'algoculture intensive et le changement climatique nous incitent à intégrer l'ensemble de ces paramètres dans la domestication d'une espèce dans le but de pratiquer une algoculture durable (Valero et al. 2017). Cela nous oblige à intégrer dans le processus de domestication des connaissances fondamentales sur la génétique des populations et l'écologie de l'espèce cible. En plus d'acquérir des connaissances fondamentales sur le cycle de reproduction, il est important d'évaluer l'état de sa ressource génétique actuelle afin de prévenir les risques de pollution génétique ou pour prendre en compte l'effet de l'adaptation locale dans la sélection de souches. Les méthodes de conservation, adaptée à la particularité des espèces devraient permettre d'une part de s'affranchir de l'utilisation de géniteurs sauvages pour ensemercer les cultures et d'autre part de pouvoir constituer une collection de génotypes d'intérêt. Finalement, il est important de prendre en considération le contexte socio-économique dans le développement des cultures afin de limiter les conflits d'usages. Ces aspects représentent les bases biologiques et génétiques de la domestication d'une nouvelle espèce. Ces bases se fondent sur l'acquisition de connaissances fondamentales et l'application de ces dernières dans le cadre d'un développement durable de la culture de macroalgues (Fig.8)

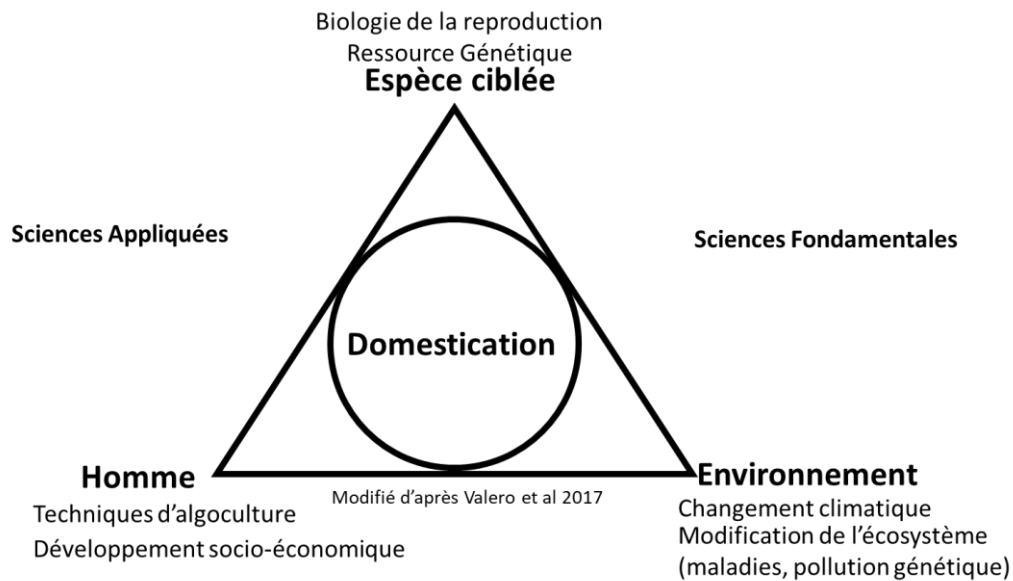


Figure 8 : Processus de domestication d'une nouvelle espèce (modifié d'après Valero et al. 2017).

2. La ressource génétique et la sélection génétique

La sélection artificielle est l'aspect le mieux connu du processus de domestication. Elle est appliquée intentionnellement sur des caractères choisis par le sélectionneur, mais peut entraîner, de façon fortuite, des caractères non désirés génétiquement corrélés au caractère choisi (Arnoux, 2019). C'est pourquoi, les possibilités de connaître les ressources génétiques d'une espèce dans la région où va se développer une culture d'algues représente un atout très important pour accélérer la domestication d'une nouvelle espèce.

2.1 Un atout pour le processus de domestication

La ressource génétique en agriculture désigne la part de la biodiversité présentant des traits d'intérêt pour l'activité humaine (Deplazes-Zemp, 2018). Plus précisément, il s'agit de la diversité intraspécifique (phénotypique ou génétique observée entre populations ou entre individus) impliquée dans la survie dans un environnement modifié tel que les systèmes de cultures. La caractérisation de cette ressource génétique est essentielle à la fois pour mener à bien le processus de domestication et de sélection, mais aussi pour mieux comprendre les impacts écologiques des activités humaines sur la diversité génétique des populations sauvages. Dans ce contexte, une des premières étapes de la domestication est de caractériser et d'inventorier la diversité et la structure génétique des populations sauvages sur une partie de l'aire de distribution de l'espèce afin d'assurer d'une part la durabilité des exploitations naturelles et d'autre part de sélectionner des populations d'algues génétiquement hétérogènes pour l'algoculture ce qui permettrait la mise en place de stratégie de conservation des ressources génétiques (Thomas et al., 2015).

2.2 Développement d'outils de marqueurs génétiques

L'étude et l'utilisation de la ressource génétique nécessite le développement de marqueurs moléculaires appropriés (Bonhomme & Gerard, 1998; Ollivier et al., 2000). Malgré les récents progrès effectués dans le séquençage haut débit, le coût et les moyens informatiques restreignent l'accès aux données (Halewood, et al., 2018a). Aujourd'hui le développement de cultures de nouvelles espèces d'algues est souvent freiné par le manque de connaissance sur leur biologie et sur leur diversité génétique (Halewood et al., 2018b). C'est pour cela qu'il est nécessaire de développer des nouvelles méthodes permettant d'acquérir rapidement et à moindre coût des marqueurs génétiques adaptés à l'étude de la structure génétique des populations naturelles (Mauger et al., 2023), Article 1). Ces marqueurs permettent également d'étudier le mode de reproduction de ces espèces qui peut-être soit sexuée soit asexuée ou les deux.

3. La reproduction des algues

Dans tous les processus de domestication, la compréhension des systèmes de reproduction de l'espèce domestiquée est capitale pour maîtriser la propagation de l'espèce en vue d'améliorer sa production.

3.1 La reproduction sexuée

La reproduction sexuée implique l'alternance d'une phase haploïde et d'une phase diploïde. La méiose permet de passer de l'état diploïde ($2n$) à l'état haploïde ($1n$) et la fécondation qui consiste à la fusion de deux cellules haploïdes (ou gamètes) permet de restaurer la diploïdie en formant un zygote. Les cycles de vie sexuée peuvent être regroupés en trois grands types, en fonction du nombre de divisions mitotiques dans chaque phase (Valero et al., 1992). Les animaux ont un cycle de vie où le stade multicellulaire est diploïde et la phase haploïde est limitée aux gamètes (c'est ce qu'on appelle le cycle diploïde ou diplontique). En revanche, dans le cycle haploïde (haplontique) seul le zygote est diploïde et les mitoses se font sous la forme haploïde pour donner des individus haploïdes multicellulaires. Ce cycle de vie se retrouve dans la plupart des champignons et chez certaines algues vertes (Figure 2). Le cycle de vie de la plupart des macroalgues est haploïde-diploïde (haplodiplontique), car le développement multicellulaire se produit à la fois dans les phases haploïde et diploïde (cycle diphasique), ce qui donne des organismes qui peuvent vivre librement et être écologiquement distinctes (Bell, 1997; Coelho et al., 2007; Hughes & Otto, 1999; Valero et al., 1992). Cette stratégie, appelée alternance des générations (Drew, 1949), est apparue indépendamment dans au moins deux grandes lignées d'eucaryotes - les Heterokontophyta et la lignée verte (Rhodophyta, Chlorophyta) (Bourdareau et al., 2021). La phase haploïde est appelée gamétophyte (génération gamétophytique),

car elle produit des gamètes. La phase diploïde est appelée tétrasporophyte (génération sporophytique ou tétrasporophytique) car elle génère des spores haploïdes par méiose et qui se développent en gamétophyte.

3.2 La reproduction asexuée

La clonalité est un phénomène très répandu dans le règne végétal mais également chez les lichens, les champignons, les algues et certains groupes d'animaux. La croissance clonale des plantes peut se manifester de nombreuses manières, notamment par la formation de ramets sur des tiges rampantes aériennes et souterraines (comme chez le fraisier ou la fougère), par le drageonnage des racines (comme chez le Robinier) ou par la production végétative de plantules et de bulbilles sur les parties aériennes de la plante. La caractéristique commune de tous les types de clonalité est la production de descendants génétiquement identiques à la plante mère. En d'autres termes, la clonalité se caractérise par le fait que les individus sont généralement produits sans passer par le processus méiotique.

Chez les algues, la reproduction asexuée peut être considérée comme un phénomène qui s'ajoute ou se substitue, au moins partiellement, à la reproduction sexuée normale (Feldmann & L'Hardy-Halos, 1977). Deux mécanismes principaux peuvent être distingués : 1) la reproduction par spores spécialisées (spores directes), par apogamies avec des gamètes se comportant comme des spores haploïdes (parthénogenèse ou androgenèse) ou par apoméioses (spores diploïdes ne faisant pas de méiose) et 2) la reproduction par fragments de thalle.

Chez les algues rouges, on note une grande diversité de spores directes comme par exemple chez diverses Némalionales et Cryptonémiales (Van Den Hoek et al., 1972). L'apomixie, c'est-à-dire la reproduction sans fécondation ni méiose (apogamie et apoméiose) est relativement courante chez les algues rouges, et a été observée dans près de 40 genres (Hawkes 1990, West et al. 2001). Certaines espèces apomictiques peuvent être obligatoires, c'est-à-dire totalement dépourvues de reproduction sexuelle, mais l'apomixie facultative est un phénomène plus courant chez les algues rouges (Hawkes dans (Cole & Sheath, 1990). Dans le cas de l'apomixie facultative, les tétraspores se développent surtout en tétrasporophytes, mais aussi parfois en gamétophytes (Maggs 1988). Bien que l'apomixie des algues rouges soit reconnue depuis longtemps et soit, chez certaines espèces, plus fréquente que la reproduction sexuée (Maggs & Stegenga, 1998), les causes de l'apomixie sont encore inconnues, même si de plus en plus d'arguments suggèrent que les espèces apomictiques strictes seraient des hybrides (Dudgeon et al., 2017 ; Kamiya, 2004; Kamiya et al., 2011; Kamiya & West, 2008).

La fragmentation végétative, c'est-à-dire la capacité des fragments d'algues séparés du thalle adulte à se fixer, à s'attacher secondairement et à croître comme un clone, est une stratégie de reproduction importante couramment utilisée par les macroalgues marines (Cecere et al., 2011; Kilar & Mclachlan,

1986; Smith & Walters, 1999; Walters et al., 2002). De nombreuses espèces capables de se fragmenter, ou de se cloner, sont particulièrement efficaces pour s'établir dans des nouveaux milieux (De Gruyter et al., 2001 ; Kilar & Mclachlan, 1986). Il s'agit notamment des exemples bien connus d'invasion d'algues, comme l'algue verte *Caulerpa taxifolia* en Méditerranée (Meusnier et al., 2001), *Gracilaria vermiculophylla* (Krueger-Hadfield et al., 2016) ou comme l'algue rouge *Polysiphonia morrowi* (Geoffroy et al., 2012) . Au fil du temps, le processus de fragmentation peut influencer la structure génétique et la dynamique de la population (Cecere et al., 2011; Coffroth & Lasker, 1998, De Meeûs et al. 2007). Bien que la fragmentation ait été documentée comme un trait important du cycle de vie de certaines algues (Cecere et al., 2011; Guillemain et al., 2008; Krueger-Hadfield et al., 2023), peu de recherches ont été menées pour comprendre le processus de recrutement de ces fragments sur le terrain.

3.3 Domestication et reproduction des macroalgues

La compréhension des cycles de reproduction complexe des macroalgues est un point clé dans le développement de méthodes de conservation, de gestion et de domestication adaptées à ces organismes d'importance culturelle, écologique et économique. Identifier les modes de reproduction de l'espèce à domestiquer, qu'il soit sexué ou asexué, est nécessaire pour passer de l'étape de "cueilleur" à celui d'agriculteur, ou dans notre cas d'algoculteur (Valero et al., 2017). L'identification de moyens de propagation végétative (reproduction asexuée) permet d'isoler rapidement les génotypes d'intérêt, adaptés aux conditions environnementales particulières imposées par les nouveaux systèmes de cultures. La multiplication clonale devrait théoriquement permettre de maintenir indéfiniment les clones en cultures (J. P. Van Der Meer, 1983). Cependant, ce mode de reproduction a ses limites et ne permet pas l'amélioration variétale par croisement, contrairement à la reproduction sexuée qui, par le biais de la recombinaison lors de la méiose et de la fusion des gamètes, permet de maintenir la diversité génétique dans une population cultivée et donc de maintenir le potentiel adaptatif et évolutif de cette dernière, face aux aléas de l'environnement marin (Valero et al., 2017). Par la diversité de leur cycle de reproduction diphasique, de nombreuses espèces de macroalgues sont capables aussi bien de se reproduire de façon sexuée ou asexuée, par propagation végétative, cependant, l'utilisation de la reproduction sexuée (production de spores et de gamètes) est souvent limitée par la méconnaissance des facteurs biotiques et abiotiques impliqués dans l'initiation des phases de reproduction des gamétophytes et des sporophytes (Liu et al., 2017). Au cours des processus de domestication des macroalgues, plusieurs méthodes de culture ont été développées en adéquation avec les traits biologiques (cycle de vie et mode de reproduction) des espèces domestiquées. Les premières étapes de la domestication ainsi que le changement drastique

d'environnement lié à la culture peuvent conduire à des modifications profondes du mode de reproduction. C'est ce qui a été observé par exemple chez *Kappaphycus sp.* et *Gracilaria chilensis* qui ont perdu la possibilité de se reproduire de façon sexuée après quelques générations seulement de culture (Guillemin et al. 2008, Valero et al. 2017). Chez ces espèces, les cultures sont composées d'un nombre restreint de clones sélectionnés (intentionnellement ou pas) pour la production de biomasse. La diversité génétique s'en trouve considérablement réduite contrairement aux populations sauvages qui présentent une diversité génétique importante et qui se reproduisent essentiellement de manière sexuée (Guillemin et al. 2008).

Les méthodes d'algoculture, détaillées dans la section 1.3.1 ci-dessus, peuvent être classées en deux catégories : la culture en mer et la culture à terre (en bassins). Les méthodes de culture en mer sur support utilisent à la fois la reproduction sexuée (Ex : culture de laminaire sur filière) ou la propagation végétative (Ex : culture de *Kappaphycus sp.* en lagon sur filière ou de *Gracilaria chilensis* à même l'estran par bouturage dans la vase) (Andersen, 2005) Les méthodes de culture à terre reposent principalement sur la capacité des espèces cultivées à se propager de façon végétative par bouturage. Elle se déroule en général dans des bassins dans lesquels les thalles clonaux sont maintenus en suspension par bullage ou autre moyen mécanique. Ces deux méthodes ont leur avantage et inconvénient, l'utilisation de surface terrestres, de système de pompage et filtration, de nutriment, voire d'éclairage rend la production en bassin à terre plus énergivore et généralement plus onéreuse. La culture en mer qui permet de produire une grande quantité de biomasse est plus difficile à contrôler, et reste dépendante des aléas climatiques. Les méthodes de culture de macro algues sont donc diverses et souvent spécifiques au trait d'histoire de vie de l'espèce cultivée. Dans le cadre de la domestication d'une nouvelle espèce, il est donc nécessaire d'identifier ou de développer des méthodes de culture adaptées au trait biologique de l'espèce cible voir même des populations locales. Cela nécessite d'acquérir des connaissances fondamentales sur le cycle de vie, le mode et le système de reproduction de l'espèce.

4. Collection de souches pour la culture et la conservation

4.1 Intérêt des collections

Les collections *ex situ* de plantes sont des outils importants pour prévenir l'extinction de certaines espèces (Mounce et al., 2017), ainsi que pour fournir du matériel pour la restauration des populations (Nevill et al., 2016). Elles permettent de fournir du matériel génétique en grandes quantités, sans épuiser le matériel local et sans avoir d'impact sur les populations naturelles (Broadhurst et al., 2015). Il est donc primordial de constituer des collections génétiquement diversifiées et représentatives de

la variation génétique des espèces cibles (Bragg et al., 2021). L'objectif est donc généralement de constituer des collections d'individus diversifiés (adaptables) et présentant des traits particuliers (adaptés). Ce qui devrait permettre d'avoir du matériel capable de s'adapter aux conditions changeantes (Bürger & Lynch, 1995 ; Stange et al., 2021) tout en ayant assez de diversité génétique pour éviter les problèmes de dépression de consanguinité, dus à l'utilisation d'un nombre trop faible de géniteurs apparentés issus des collections pour la restauration (Charlesworth & Willis, 2009).

La possibilité de conserver des souches permet donc de s'affranchir de la ressource naturelle et d'initier la sélection variétale de l'espèce cible. Chez les plantes, les collections sont généralement constituées de graines en dormance ou d'individus plantés (jardins botaniques).

4.2 Collection de macroalgues

Chez les algues dépourvues d'organe de résistance, la collection est constituée d'individus gamétophytiques ou tetrasporophytiques. La phase du cycle de reproduction utilisée pour la conservation dépend des traits biologiques et varie fortement d'une espèce à une autre (Wade et al., 2020). Chez certaines espèces, l'hétéromorphisme impliquant la présence d'une phase microscopique permet d'utiliser cette dernière pour la conservation à longs termes, comme c'est le cas chez les laminaires avec la phase gamétophytique ou chez *Pyropia* avec la phase diploïde filamenteuse. Chez d'autres espèces, capables de propagation végétative comme *Gracilaria gracilis* ou *Kappaphycus*, les individus entiers de n'importe quelle phase du cycle de reproduction peuvent être utilisés pour la conservation de l'espèce en collections. La prise en considération des interactions biotiques (parasitisme, épiphytisme, prédation) est nécessaire pour développer des méthodes d'isolement des souches unialgales (Kawai et al. 2005 dans Andersen, 2005) et d'assurer la survie de l'espèce cible dans les écosystèmes modifiés que sont les systèmes de culture. Dans ce cadre, les connaissances des caractéristiques liées à la croissance et la reproduction sont nécessaires pour développer les méthodes de conservation et de culture adaptées pour chaque espèce. La maîtrise de la conservation en culture de ces individus sous forme de collections ouvre donc des perspectives intéressantes pour la restauration, l'amélioration et la conservation de l'espèce en question. Il s'agit d'une étape essentielle dans le processus de domestication. Cette étape nécessite une parfaite connaissance de la biologie et de l'écologie de l'espèce cible (Valero et al. 2017). Cependant, la prise en compte de la notion d'holobionte dans la biologie de l'espèce amène encore d'autres questions qui ne sont pas prises en compte dans les collections actuelles de ressources génétiques (Hoffmann et al. 2024).

5. *Palmaria palmata*, une nouvelle espèce d'algue rouge à domestiquer

Palmaria palmata (L.) Weber & Mohr, 1805 (Rhodophyta, Eurhodophytina, Florideophyceae, Nemaliophycidae, Palmariales, Palmariaceae) est une algue rouge qui se trouve à la base des Florideophyceae (Figure 9).

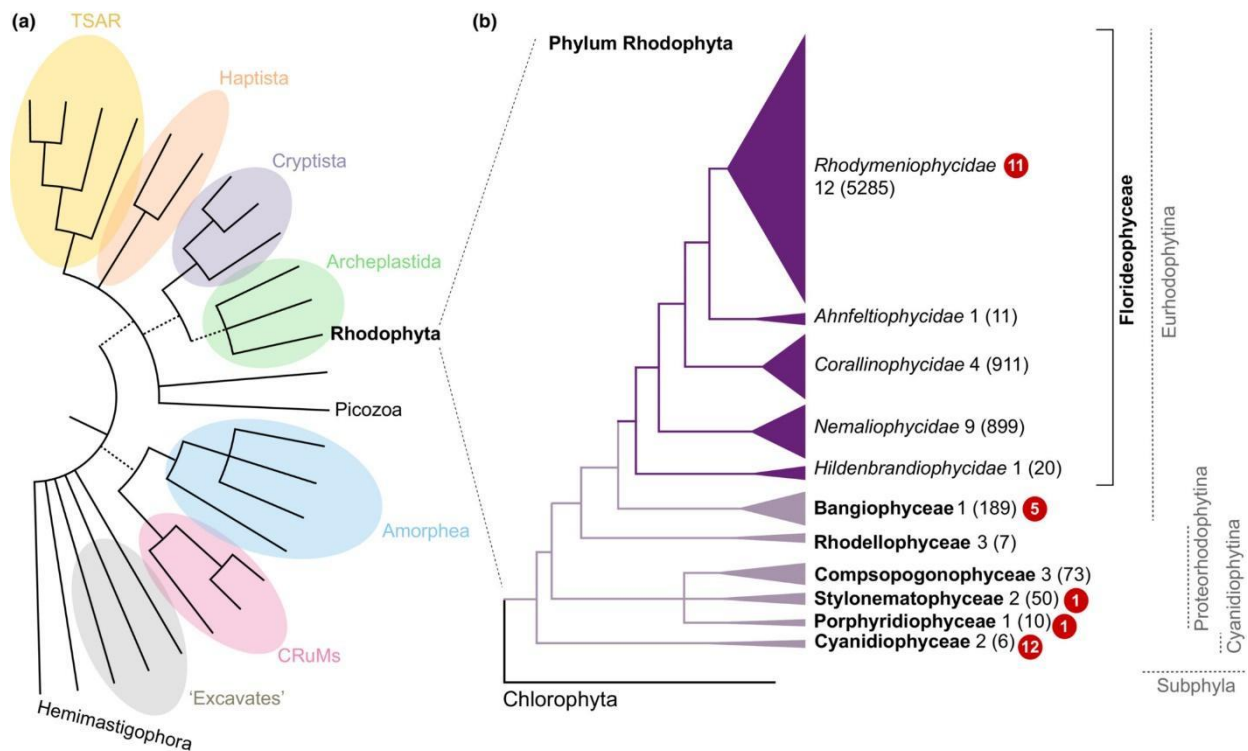


Figure 9 (a) L'arbre évolutif des eucaryotes (basé sur Burki et al., 2020) avec la position des Rhodophyta marquée en gras. (b) Phylogénie des Rhodophyta (d'après Yang et al., 2016). Les classes sont indiquées en gras, les sous-classes en italique, les nombres correspondent au nombre d'ordres et le nombre d'espèces est donné entre parenthèses (d'après Guiry & Guiry, 2023). Le nombre de séquences génomiques disponibles par classe est indiqué dans les cercles rouges. *P. palmata* appartient à la sous-classe des Nemaliophycidae (Borg et al., 2023).

Elle vit fixée sur les substrats rocheux du littoral de l'Atlantique nord ou en épiphyte sur *Fucus serratus* et *Laminaria hyperborea* (Grote, 2019 ; Stévant et al., 2023). Sa distribution s'étend, dans l'Atlantique Est, du nord du Portugal au Svalbard et en Atlantique Ouest, de l'Etat du Maine (USA) jusqu'au Groenland (figure 10) (Lüning, 1990, Stévant 2023).

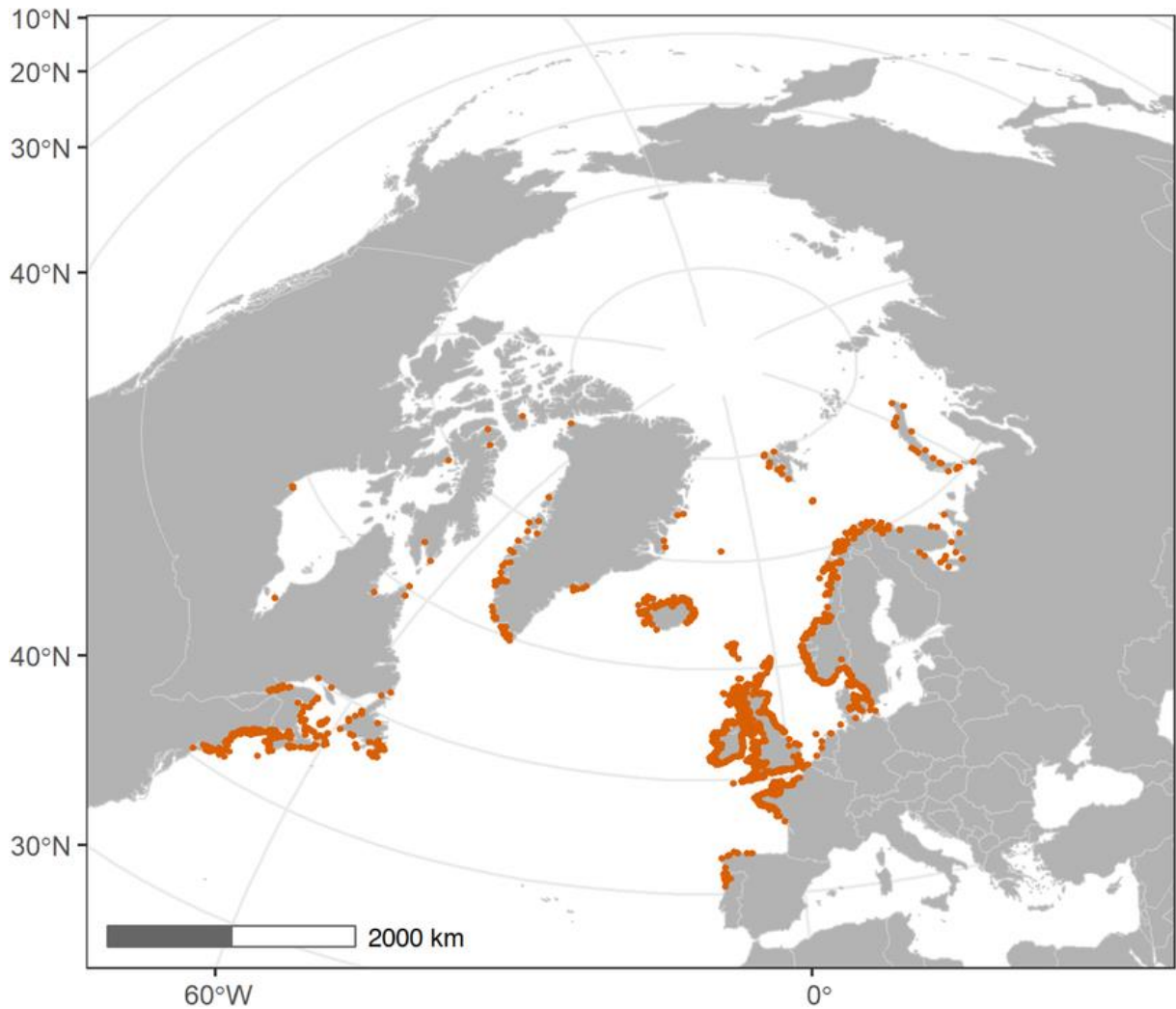


Figure 10: Distribution géographique de *Palmaria palmata* basée sur les rapports d'observation et de collecte de l'espèce provenant des bases de données du Système d'information sur la biodiversité océanique (OBIS 2022) et du Centre mondial d'information sur la biodiversité (GBIF 2022), des sources de répartition d'AlgaeBase (Guiry et Guiry 2022) ainsi que des enregistrements d'observations à partir de la littérature citée et des observations personnelles des auteurs de Stévant et al., 2023). Selon des études taxonomiques récentes, *P. palmata* est principalement confinée à l'Atlantique Nord.

Plus connue sous le nom de dulse (*dillisk* en gaélique), elle est consommée crue ou séchée depuis plusieurs millénaires en Europe. Elle est actuellement utilisée dans l'alimentation humaine et animale (Mouritsen et al., 2013). Historiquement, cette algue était principalement consommée pendant les périodes de famine (Garineaud, 2017). Depuis une vingtaine d'années, la demande en dulse pour l'alimentation humaine n'a cessé d'augmenter, car elle fait aujourd'hui partie des « new health food ». Elle est notamment très prisée dans la nouvelle cuisine nordique comme un aliment riche en protéines

(jusqu'à 30% de son poids sec) et en fibres (xylanes et mannanes sulfatés) qui la différencie de toutes les autres algues rouges (agarophytes et carraghénophytes) (Mouritsen et al., 2013). De nouveaux secteurs comme la médecine ou la cosmétique commencent également à s'y intéresser pour ses qualités anti-diabétiques, cardio-protectrices et anti-inflammatoires (Harnedy & FitzGerald, 2013). Elle constitue par ailleurs un fourrage de prédilection pour les élevages d'ormeaux et d'oursins (Cook & Kelly, 2007 ; Le Gall et al., 2004).

Aujourd'hui, la majorité de sa production est assurée par une récolte manuelle dans les populations sauvages (Hafting et al., 2012; Stévant 2023). Cette production est soumise à des variations inter-annuelles qui sont liées aux aléas climatiques (voir Figure 11) et des variations saisonnières liées à sa dynamique de croissance. Après la période de reproduction qui démarre à la fin de l'automne et plusieurs épisodes d'arrachage partiel des frondes dus aux tempêtes hivernales, *P. palmata* rentre en phase de croissance du printemps jusqu'à l'automne. Durant cette phase, *P. palmata* génère de nouvelles repousses à partir des thalles qui ont résisté aux assauts de l'hiver (Faes & Viejo, 2003).

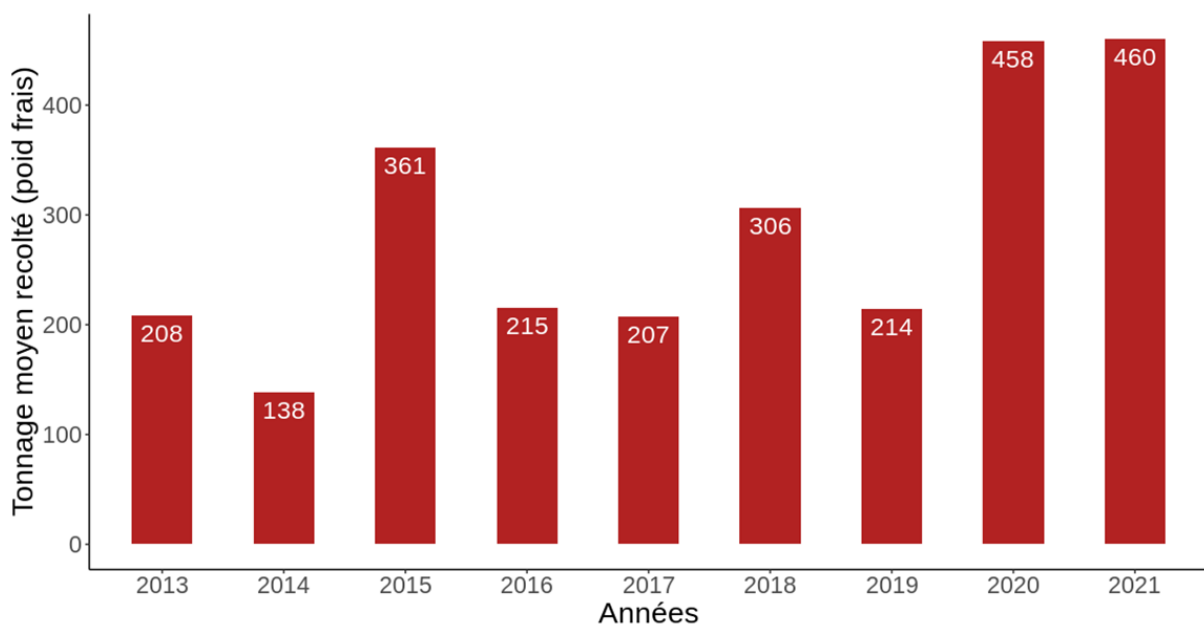


Figure 11: Tonnage récolté de *P. palmata* en tonnes fraîches en Bretagne entre 2013 et 2021 (CRPMEM de Bretagne, 2023)

En Bretagne, où elle est très abondante, l'augmentation de la demande et les restrictions de ramassage dans certaines zones conduisent à une forte pression de récolte sur certaines populations (Garineaud, 2017). En effet, même si on la retrouve sur l'ensemble du littoral Normano-Breton (de Piriac au Cap Lévi) elle est très peu abondante dans le sud de la Bretagne. Elle est également moins abondante dans le golfe de Saint-Malo où l'on retrouve un morphotype de plus petite taille. Les populations les plus abondantes se situent dans la partie nord-ouest de la péninsule bretonne où se concentre l'ensemble

de la pression de récolte (Figure. 12). Finalement, 90% de la biomasse de *P. palmata* "produite" en Europe est récoltée dans cette région.

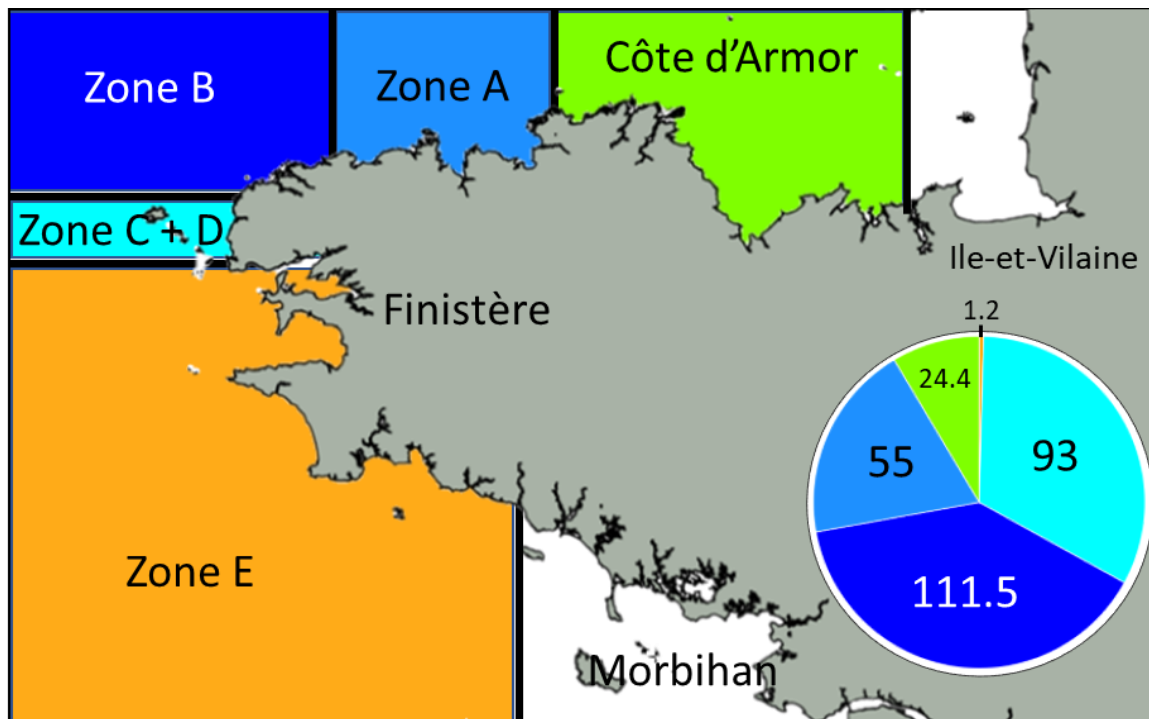


Figure 12: Répartition de l'effort de récolte de *P. palmata* dans la région Bretagne. Valeur moyenne en tonnes d'algues fraîches récoltées sur une année entre 2013 et 2021 (CRPMEB de Bretagne, 2023)

Pour répondre à cette demande croissante, plusieurs tentatives de développement de la culture de *P. palmata* ont été menées ces 20 dernières années, avec plus ou moins de succès (Edwards & Dring, 2011 ; Le Gall et al., 2004; Sanderson, 2015; Schmedes, 2020; Werner & Dring, 2011) . Ces échecs peuvent être attribués au manque de connaissances sur la ressource génétique de cette espèce et à son cycle de reproduction atypique. Sans description de la ressource génétique, il n'est pas possible d'identifier des génotypes d'intérêt et sans maîtrise du cycle de reproduction, il n'est pas possible de s'affranchir des géniteurs sauvages, qui sont souvent peu performants dans l'environnement modifié par l'homme pour la culture (Larson et al., 2014). Et finalement, le développement de méthodes de conservation des génotypes d'intérêt est nécessaire pour initier la sélection variétale de ces espèces d'intérêt économique.

6. Ressource génétique chez *P. palmata*

Seulement deux études sur la phylogéographie de *P. palmata* ont été effectuées en utilisant des marqueurs mitochondriaux (Li et al., 2015; Provan et al., 2005). Ces études suggèrent un début de

spéciation entre les populations Est et Ouest de l'Atlantique Nord et la présence de zones refuges des deux côtés de l'Atlantique lors de la dernière glaciation. Cependant, les deux études citées précédemment utilisent des marqueurs génétiques peu adaptés à l'étude de la diversité intraspécifique. Pour caractériser la ressource génétique de *P. palmata*, il est donc nécessaire de développer des marqueurs adaptés à l'étude de la diversité et de la structure génétique des populations naturelles. Les marqueurs de type microsatellites sont utilisés en génétique des populations depuis près de 30 ans (Mauger, Baud et al., 2023, article 1). Même à l'ère du séquençage haut débit, l'emploi de ce type de marqueurs reste d'actualité pour leur facilité d'utilisation et leur coût réduit. De plus, des études comparatives ont montré que ces marqueurs permettent d'obtenir des résultats similaires à ceux obtenus avec des SNPs (Single Nucleotides Polymorphisms) produit par séquençage nouvelle génération (Guzinski et al., 2018).

Chez les macroalgues, la dispersion des propagules, impliquées dans la reproduction sexuée ou asexuée, dépend principalement de l'action des courants marins. Ces derniers dispersent de façon passive les spores ou thalles fertiles sur des distances variables en fonction des traits d'histoire de vie des espèces (van den Hoek, 1987). Une étude récente, basée sur des données empiriques croisée avec des modèles océanographiques, a démontré l'importance de la connectivité océanographique dans la répartition de la diversité intraspécifique chez plusieurs espèces de macroalgues (Legrand et al., 2023). Il est donc nécessaire de prendre en considération cette composante importante du milieu marin lors de l'étude de structure et de la diversité génétique d'une espèce. Dans le cas de *P. palmata*, les résultats obtenus par Provan et al, 2005 et Liu et al. 2015 suggère que sa capacité de dispersion est limitée, un trait commun aux algues rouges. En effet, chez les algues rouges, les spores et les spermaties sont dépourvue de flagelle, les rendant complètement tributaires de l'environnement pour se disperser (Morrow, 2004) . De plus, des études sur les spores d'algues rouges ont montré que ces dernières sont plus denses que l'eau de mer et se fixe rapidement (Destombe et al., 1992 ; Okuda & Neushul, 1981). Cependant, la dérive de thalles fertiles décrochés pendant les tempêtes pourrait permettre une dispersion sur des distances plus grande (van den Hoek, 1987).

L'impact de l'environnement (niveau de marée, courant océanique) et des traits d'histoire de vie (capacité de dispersion) sur la structure et la diversité génétique des populations bretonnes n'ont jamais été étudiés avec des marqueurs adaptés. Les populations naturelles de *P. palmata* sur le littoral breton ont la caractéristique d'être présente à deux niveaux de marée distincts. Les populations de l'étage médiolittoral, présentent sur la roche et en épiphyte sur *Fucus serratus* sont régulièrement exondées et sont les plus exposées à la récolte. Les populations infralittorales supérieures, sont seulement exondées aux grandes marées de vive eau et sont principalement présentes uniquement en épiphyte sur *Laminaria hyperborea*. (Fig. 13)

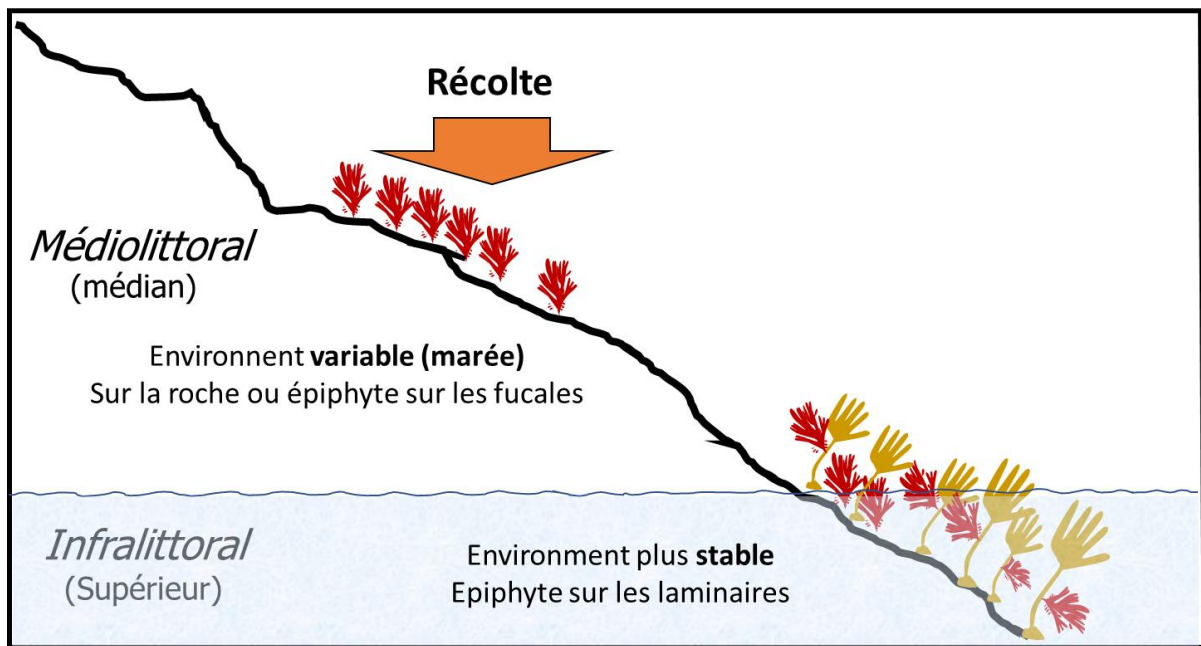


Figure 13 : Contexte écologique de *P. palmata* le long du gradient de marée

Le littoral breton est également sous l'influence de nombreux courants marins qui ont probablement un effet sur la capacité de dispersion de *P. palmata* (Garreau, 1993, Salomon & Breton, 1993). Ces paramètres environnementaux devront être pris en compte, avec les traits d'histoire de vie, dans l'étude de la structure et de la diversité génétique de *P. palmata*.

La température est l'un des facteurs environnementaux qui joue un rôle majeur dans la distribution des macroalgues (Bartsch et al., 2012 ; Breeman, 1988). Comprendre les phénomènes d'adaptation locale liés à ce paramètre abiotique permettrait d'anticiper la résilience des populations sauvages au changement climatique, mais aussi de sélectionner des génotypes d'intérêt pour la culture ou la restauration des populations menacées (Valero et al., 2017).

Les eaux de surface des côtes bretonnes sont caractérisées par une mosaïque de températures différentes comme l'illustre la figure 14 qui représente les températures moyennes mesurées par imagerie satellite sur 20 ans dans cette région. Le réchauffement enregistré sur la période étudiée a montré qu'il était plus intense dans le nord-est (Gallon et al., 2014).

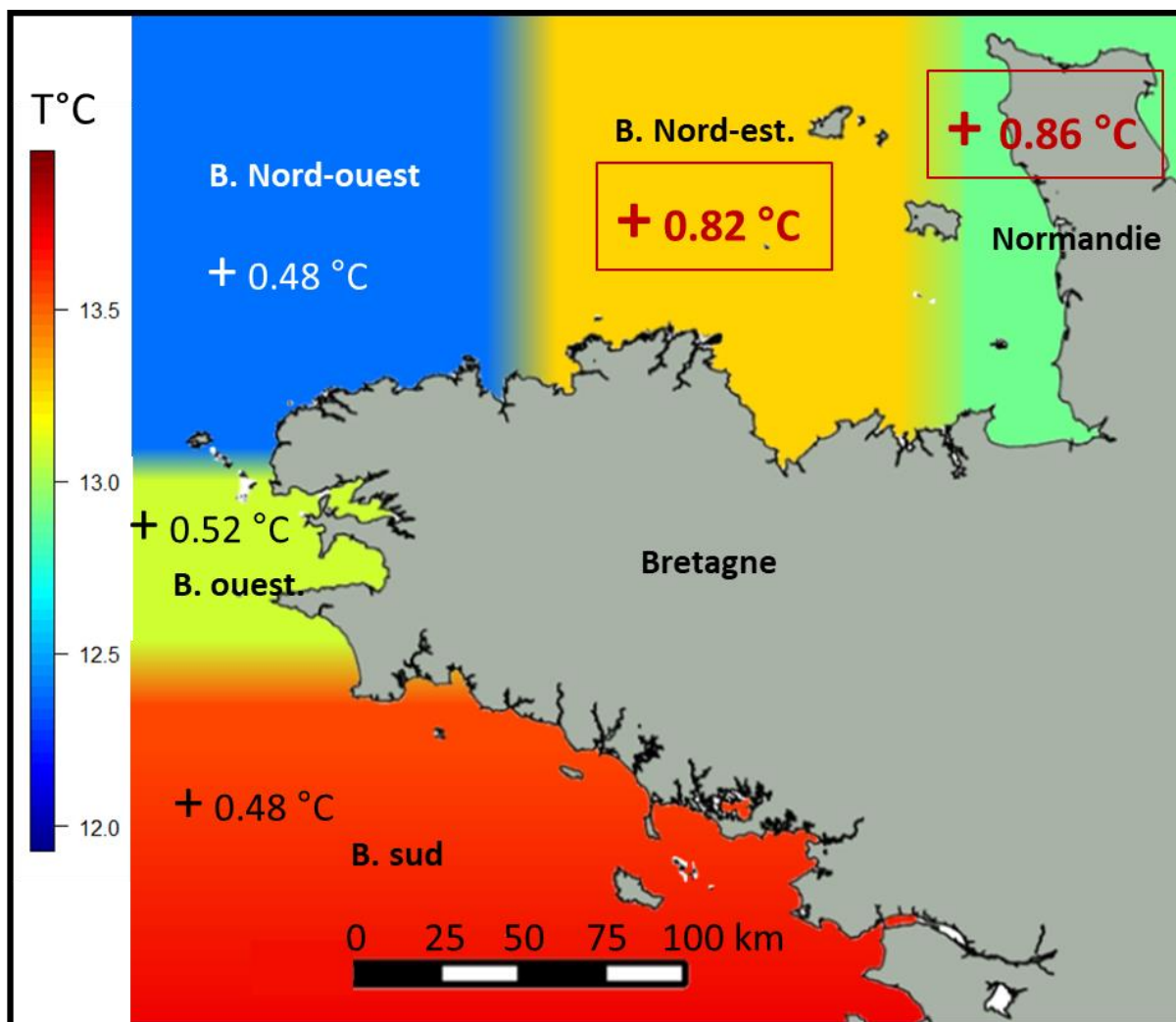


Figure 14: Température de surface moyenne des côte Normando-Bretonne mesurée par satellites. Les valeurs indiquées pour chaque zone correspondent au réchauffement enregistré entre 1992 et 2012. D'après les données de Gallon et al. 2014.

Cette forte hétérogénéité environnementale (température, réchauffement, courant et marée) pourrait favoriser un processus d'adaptation locale à l'échelle régionale ou le long du gradient de marée. L'utilisation d'expérimentations en jardin en commun permettra d'étudier ce processus (Blanquart et al., 2013). Cependant, il est d'abord nécessaire de compléter les données obtenues par imagerie satellite, en caractériser la température in situ à l'échelle locale et régionale à l'aide d'un suivi sur plusieurs années.

7. Biologie de la reproduction de *P. palmata*

7.1 Cycle des Florideophyceae

Les cycles de vie haploïde-diploïde des algues rouges sont parmi les plus complexes et les plus diversifiés (Borg et al., 2023). En effet, la plupart des Florideophyceae ont un cycle de vie sexué où alternent deux types d'individus : les tétrasporophytes diploïdes et des gamétophytes dioïques (mâle et femelle séparés) haploïdes. La troisième phase (multicellulaire) qui se développe en épi/endophyte sur la femelle après la fécondation et qui produit des spores est appelée le carposporophyte. Les gamétophytes mâles libèrent des spermatis (gamètes mâles non mobiles) tandis que les gamétophytes femelles produisent un carpogone (l'ovule) qui est retenu sur le thalle femelle. Après la fécondation, le carposporophyte se développe et reste attaché au thalle gamétophytique femelle dans une structure appelée cystocarpe, qui consiste en un tissu carposporophytique diploïde entouré d'un tissu maternel haploïde (Fredericq & Hommersand, 1989). Les carpospores sont finalement libérées et germent en un tétrasporophyte diploïde qui produit des spores haploïdes par méiose. Ces tétraspores haploïdes germent ensuite pour donner un gamétophyte mâle ou femelle, achevant ainsi le cycle de vie (Fig. 15).

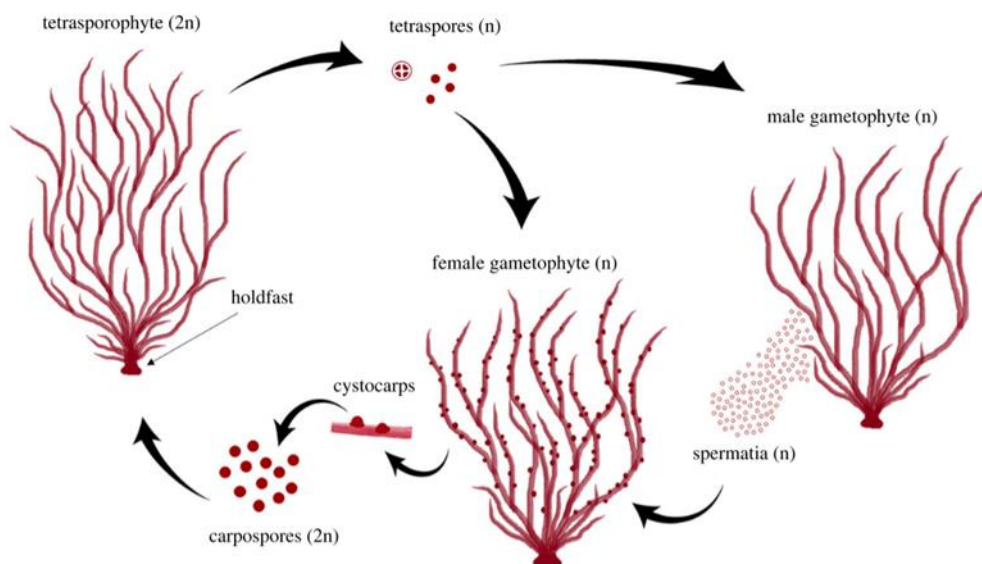


Figure 15 : Cycle de reproduction isomorphe de *Gracilaria gracilis* modifié d'après (Lavaut et al., 2023).

Étant donné que trois étapes distinctes caractérisent cette stratégie de cycle de vie, elle a été classiquement appelée cycle de vie "triphase" dans la littérature (Drew, 1955, Fredericq & Hommersand, 1989), chacune des trois "phases" représentant des étapes distinctes du développement plutôt qu'un changement de ploïdie.

Le carposporophyte qui produit des spores diploïdes génétiquement identiques a été considéré comme une phase de multiplication du zygote qui aurait évolué pour compenser l'inefficacité relative des fécondations due à l'absence de flagelle chez les spermatis (Searles, 1980). Il a été suggéré que l'évolution de la phase carposporophyte était une innovation majeure, jouant un rôle central dans le succès et la diversification explosive des Florideophyceae (Lee et al., 2018). Bien que la signification évolutive de cette troisième phase du cycle de vie ne soit pas encore claire, il est probable qu'elle améliore le succès reproductif. Diverses études suggèrent que le carposporophyte, dont le développement dépend du gamétophyte femelle, pourrait jouer un rôle de médiateur dans la compétition entre les spermatozoïdes par le biais du choix de la femelle (Engel et al., 1999; Richerd et al., 1993). En effet, Fredericq & Hommersand (1989) suggèrent que le carposporophyte pourrait jouer un rôle dans le rejet des fécondations incompatibles ou disharmonieuses, cependant, on peut s'interroger sur l'absence de cette phase carposporophytique chez les Nemaliophycidae, dont fait partie notre espèce modèle *Palmaria palmata*.

7.2 Cycle de *P. palmata*

Le cycle sexué de *P. palmata*, qui a représenté longtemps une énigme pour les phycologues, se caractérise premièrement par une absence de cystocarpe et par un dimorphisme sexuel prononcé entre individus mâles et femelles. Après fécondation, le zygote n'est pas libéré et germe sur la femelle qui est microscopique pour donner un tetrasporophyte (figure 16).

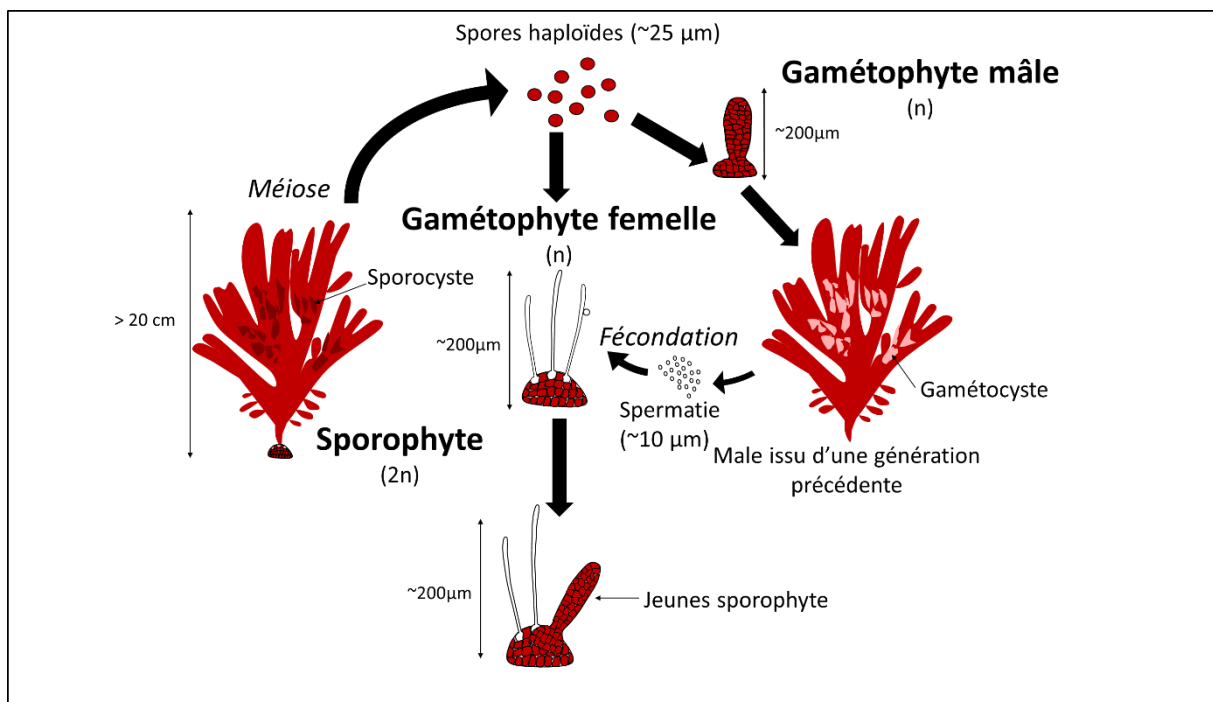


Figure 16: Cycle de vie de *Palmaria palmata*. Modifié depuis van der Meer & Todd 1984

À maturité, les sporophytes diploïdes génèrent des spores par méiose. Après fixation, les spores se développent en gamétophytes mâle ou femelle (1:1 ratio). Les gamétophytes mâles deviennent fertiles un an après la germination de la spore. En revanche, les femelles sont fertiles deux à trois semaines, les cellules reproductives des gamétophytes femelles (carpogones) développent une extension cellulaire appelée trichogyne sur laquelle se fixent les spermatis (fig. 17). Le nouveau sporophyte se développe sur la femelle et finit par la recouvrir entièrement. En effet, dans ce cycle les gamétophytes mâles et les tetrasporophytes ont la même morphologie (isomorphe) tandis que le gamétophyte femelle est microscopique (dimorphisme sexuel).

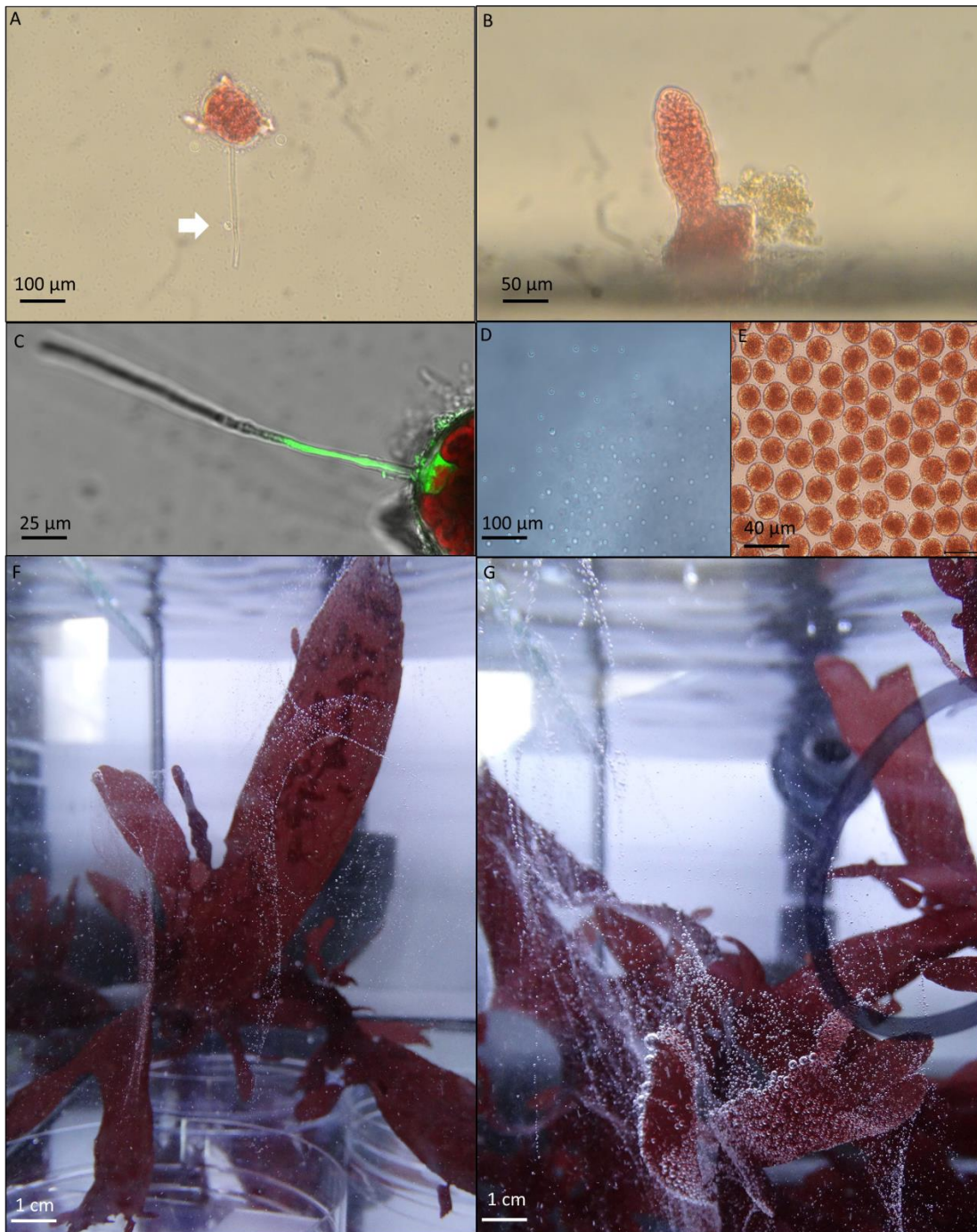


Figure 17 : Photographies des différentes étapes du cycle de reproduction A : Photo d'un gamétophyte femelle en cours de fécondation, la flèche montre une spermatie collée au trichogyne. B : Photo d'un jeune gamétophyte mâle. C : Prise de vue au microscope confocal d'un gamétophyte femelle. Les cellules corticales du thalle sont mises en évidence par la fluorescence naturelle de la chlorophylle-a (en rouge). Le trichogyne montre une fluorescence verte correspondant à la carbocyanine lipophile DiOC18 (DiO) qui se fixe sur les lipides membranaires des cellules (crédit Sébastien Collin). D: spermatis. E : tetraspores. F: Tetrasporophyte en train de libérer ses spores. G: Gamétophyte mâle en train de libérer ses spermatis.

Certaines études basées sur la sporulation et la survie des spores ont été réalisées chez cette espèce afin d'améliorer la densité des ensemencements et par conséquent les rendements des cultures en mer (Schmedes, 2020). Cependant, ces méthodes restent encore à optimiser dans le but d'assurer la rentabilité économique des cultures. La fécondation et les mécanismes biologiques et physiques impliqués dans la gamétogenèse et la sporogénèse restent relativement peu étudiés. À ce jour, seuls les travaux de (Pang & Lüning, 2006) et les travaux de Schmedes et al. en 2020 se sont intéressés aux facteurs physiques et aux mécanismes biologiques impliqués dans la maturation des gamétophytes mâles et des tétrasporophytes de *P. palmata*. Ils ont notamment mis en évidence expérimentalement au laboratoire l'effet de la photopériode et de la température sur la gamétogenèse et la sporogénèse. Ces résultats concordent avec les observations de terrain effectuées par Le Gall en 2002 qui montrent que la saison de reproduction de *P. palmata* s'étend d'octobre à mai en fonction des régions d'études entre la Normandie et la Bretagne. Le déclenchement de la saison de reproduction en automne correspond à la diminution de photopériode et de température propre aux régions tempérées et froides de l'hémisphère nord. D'autres mécanismes abiotiques ou biotiques pourraient également influencer le déclenchement de la reproduction de cette espèce, comme par exemple, la taille des individus ou le cycle des marées.

Pour ce qui est de la fécondation, cette dernière a été tout particulièrement étudiée par van der Meer & Todd (1980) durant les travaux qui ont mené à l'élucidation du cycle de reproduction de *P. palmata*. Cependant, les applications de ces connaissances pour la culture restent encore limitées. Seuls les travaux de Schmedes (2020) ont implémenté une étape de fécondation des gamétophytes femelles sur des supports de culture lors de la phase d'éclosion. Étant donné que le déterminisme du sexe chez *P. palmata* est génétique, le nombre de spores portant le chromosome mâle est équivalent au nombre de spores portant le chromosome femelle, cela se traduit par un sex-ratio 1 :1 après la germination (van der Meer 1990). Toutes choses étant égales, si onensemence des cordes avec des spores et si toutes les femelles sont fécondées, le nombre de tétrasporophytes devrait être égal au nombre de mâles. La fécondation des gamétophytes femelles devrait donc doubler le nombre de plantules observées sur les supports de cultures. C'est ce qui a été observé par Schmedes et collaborateurs sur des plantules âgées de 19 jours, sans information sur le succès de la fécondation des femelles. En revanche, ce n'est pas ce qui est observé dans les populations naturelles de Bretagne nord et de Normandie dans lesquelles on observe trois fois plus de sporophytes que de gamétophytes mâles (Le Gall, 2002). Différentes hypothèses non exclusives peuvent être proposées pour expliquer ces génération-ratios : 1) soit les femelles ont une meilleure survie que les mâles et 2) soit les sporophytes ont une meilleure survie que les gamétophytes mâles. Chez différentes algues rouges

(Florideophyceae), il a été montré que les variations de la fréquence des individus haploïdes et diploïdes dans les populations sont dues à des différences de fertilité et de survie (De Wreede & Klinger, 1988 ; Santelices, 1990 ; Richerd et al, 1993). La prédominance des tétrasporophytes chez *Palmaria palmata* semble indiquer qu'il y a quelque chose dans cette espèce qui rend la phase diploïde plus adaptée. Les individus échantillonnés dans les populations de *P. palmata* pour estimer les ratios de ploïdies proviennent de plusieurs évènements de sporulations (plusieurs saisons de reproduction). Ces résultats pourraient correspondre à des différences de valeurs sélectives des phases haploïdes et diploïdes concernant donc des stades de développement plus avancés que ceux étudiés expérimentalement. Le suivi, sur plusieurs semaines, de la croissance de sporophytes (issus de gamétophytes femelles fécondés) et de gamétophytes mâles issus de la même génération en laboratoire et en mer devrait permettre de mieux comprendre le ratio de ploïdie observée dans les populations naturelles. Il est également nécessaire d'approfondir les connaissances sur le ratio de ploïdie dans les populations naturelles de *P. palmata*.

Jusqu'à l'élucidation de son cycle de vie en 1980 par Van der Meer et Todd, *P. palmata* était considéré comme une espèce qui pratiquait uniquement la reproduction asexuée. En effet, la reproduction sexuée était considérée comme impossible car on ne trouvait pas de femelles macroscopiques dans les populations. La culture des tétraspores au laboratoire a permis de mettre en évidence que les femelles étaient microscopiques et les fécondations pouvaient se faire en boîte de Petri, ce qui a complètement changé la vision du cycle de *P. palmata*. Bien que la fragmentation de méristèmes apicaux donne lieu à la formation de nouveaux thalles, on peut s'interroger sur leur capacité à générer un nouveau disque de fixation dans la nature (voir chapitre III,2.2). En l'absence d'un organe spécialisé (stolon) ou de spores asexuées(monospores) on ne connaît pas l'importance de la propagation végétative par rapport à la reproduction sexuée dans les populations naturelles. Par ailleurs, l'utilisation de la propagation végétative par fragmentation pour la production de biomasse pose certains problèmes. En effet, dans le cadre d'une étude pilote sur la culture de *P. palmata*, Werner et Dring ont conclu en 2011 que la production par croissance végétative n'était pas viable économiquement. De plus, dans le cas de *P. palmata*, la propagation végétative est peu adaptée à la culture en mer. La faible capacité de rattachement des thalles limite les possibilités de cultiver *P. palmata* par propagation végétative en mer, de la même façon que les exploitations de *Kappaphycus sp.* Des essais de culture en mer réalisés par Martinez et collaborateurs en 2006 ont montré que la culture par propagation végétative sur corde n'était pas faisable, car les thalles placés sur les cordes finissent par se dégrader. Bien que la sélection de génotypes pourrait permettre d'améliorer grandement les rendements de la culture de *P. palmata* (voir chapitre III. 3), la propagation végétative offre peu de perspectives d'amélioration variétale et de capacité d'adaptation à la variabilité de

l'environnement marin. La maîtrise de la propagation végétative de la souche sélectionnée est cependant nécessaire pour générer une biomasse de géniteurs sélectionnés dans le but de réaliser des ensemencements et des croisements, ce sujet est traité dans le chapitre III.

Le dimorphisme sexuel qui caractérise le cycle de reproduction de *P. palmata* suggère que les femelles d'une saison de reproduction donnée sont fécondées par les mâles de la saison précédente. Cela a potentiellement pour effet de limiter la probabilité d'autofécondation (croisement frères/sœurs). Cependant, la faible capacité de dispersion propre aux algues rouges (Morrow, 2004 ; Searles, 1980), principalement dû à l'absence de flagelle chez les spores et les gamètes, pourrait contrebalancer l'effet du décalage générationnel sur le système de reproduction en facilitant la reproduction entre individus apparentés (consanguinité). La particularité du cycle de reproduction de *P. palmata* ainsi que le peu d'information disponible sur son système de reproduction souligne la nécessité d'étudier ces aspects à l'aide d'outils adaptés. Les marqueurs génétiques de type microsatellites sont des outils de choix pour étudier le système de reproduction d'une espèce. L'étude du mode et du système de reproduction ainsi que la maîtrise de la reproduction sexuée de cette espèce est donc nécessaire pour assurer la rentabilité des cultures en mer et mener à bien sa domestication.

8. Méthodes de collection adaptées à la biologie de *P. palmata*

8.1 Propagation végétative de *P. palmata*

Même si les apex permettent de générer rapidement de nouvelles frondes (Morgan et al., 1980 ; Titlyanov & Titlyanova, 2006a ; b), la capacité de *P. palmata* à générer des nouvelles pousses sur l'ensemble de son thalle est un atout majeur pour son maintien en collection. En effet, il est possible de produire de la biomasse à partir de n'importe quelle partie du thalle.

L'utilisation de ce mode de reproduction par propagation végétative permet de conserver des individus de façon clonale. Aucune donnée n'est disponible sur la durée de vie d'un individu dans le milieu naturel et encore moins en laboratoire. Cependant, des observations effectuées sur des souches clonales d'algues rouges préservées pendant plus de 40 ans par propagation végétative ont montré la perte de capacité à produire des propagules au cours du temps (West, 2005 dans Anderson 2005). De façon similaire, la sélection massale "inconsciente" réalisée par les aquaculteurs-fermiers en bouturant les thalles de *Gracilaria chilensis* a entraîné en une dizaine d'années une perte de la capacité à se reproduire de façon sexuée (Guillemin et al. 2008). L'impact à long terme de la conservation en collection se doit donc d'être étudié.

L'utilisation de ce mode de production végétatif permet de tester les premières étapes de la "domesticabilité" de cette espèce, c'est-à-dire sa capacité à croître dans des conditions contrôlées de laboratoire. Cette première étape de sélection est essentielle pour assurer l'utilisation des souches en culture, en mer et à terre. En effet, la croissance végétative des souches sélectionnées doit être suffisamment importante pour permettre la production de géniteurs pour ensemercer les supports ou assurer la production de biomasse en bassins.

8.2 Méthodes de conservation en culture adaptées à *P. palmata*

Quelques études se sont intéressées à la physiologie de *P. palmata* et ont permis de définir les optimums de température, intensité lumineuse, photopériode et nutriments pour la culture par propagation végétative. *P. palmata* est connue pour se développer entre 6 et 18°C avec des variations en fonction de la localité (Matos et al., 2006 ; Morgan & Simpson, 1981). Une tolérance au changement de salinité a été observée par Schmedes en 2020 et semble également varier localement. Concernant la lumière, *P. palmata* est capable de s'acclimater à des intensités lumineuses variables comme la montre des études en laboratoire et sur le terrain. En effet, *P. palmata* est présente à la fois dans l'espace médiolittoral, où elle est surexposée au rayonnement lumineux (2000 $\mu\text{mol photons}/\text{sec}/\text{m}^2$), et dans l'espace infralittoral où elle se développe jusqu'à 20m en fonction de la turbidité (Sagert & Shubert, 2000 ; Stévant et al. 2023). Elle est décrite comme une espèce plutôt photophile, son seuil de saturation a été estimé à 113 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ (Sagert & Schubert, 2000).

Dans notre étude, nous avons défini des conditions standard de culture en nous basant sur les données moyennes de terrain. La température est fixée à 13°C, l'intensité lumineuse est de 100 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ et la photopériode longue de 16 : 8 d'alternance lumière/obscurité. Pour la conservation des souches, la température a été fixée à 10°C et l'intensité lumineuse à 25 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ en conservant une photopériode longue pour éviter le déclenchement de la reproduction.

Peu de données existent sur les interactions biotiques chez *P. palmata*. Les expérimentations de culture effectuées ces 20 dernières années sur *P. palmata* ont mis en évidence l'existence de différents parasites ou épiphytes. En éclosion, l'oomycète *Olpidiopsis sp.* occasionne d'importants dégâts sur le développement des jeunes plantules (Badis et al., 2019). Par ailleurs, il a été montré que la présence de l'algue rouge filamenteuse *Acrochaetium sp.* et d'algues brunes filamenteuses de l'ordre des *Ectocarpales* impactent fortement la croissance des cultures en bassins (Werner and Dring, 2011). La

gestion de ces contaminations est un point clé dans l'obtention de souches unialgales pour la conservation à long terme.

8.3 Sélectionner des souches d'intérêt pour la domestication de *P. palmata*

La définition des critères de sélection d'une espèce dépend des méthodes de culture employée pour la production. Par la complexité du cycle de reproduction de *P. palmata* plusieurs traits phénotypiques devront être sélectionnées chez les tétrasporophytes et les gamétophytes mâles afin d'assurer sa propagation dans les différents systèmes de culture. Le critère de sélection principal est la capacité de croissance végétative des souches dans un environnement modifié. En effet, la culture de dulse en mer ou à terre nécessite une phase de croissance végétative. Pour la culture en bassin, il s'agit du moyen principal utilisé pour produire de la biomasse. Dans le cadre de la culture en mer, une phase de propagation végétative des géniteurs utilisés pour l'ensemencement est nécessaire afin d'assurer la qualité de l'ensemencement des supports de culture. De plus, les plantules se développant sur les supports de culture devront être en capacité de croître rapidement par croissance végétative pour lutter contre les espèces indésirables et ainsi assurer la production de biomasse commercialisable. L'acquisition d'une première collection de souches sauvages sélectionnée sur ce critère servira de base pour initier la sélection variétale par croisement et ainsi améliorer la production de *P. palmata* en mer et à terre.

Objectifs de la thèse

Cette thèse se situe à l'interface entre recherche fondamentale et appliquée. Les objectifs sont de poser les bases biologiques et génétiques de la domestication chez l'algue rouge *Palmaria palmata* en vue d'améliorer les connaissances sur sa reproduction et sur la diversité génétique des populations afin de mieux appréhender les méthodes de propagation et de conservation de cette espèce. L'étude s'est focalisée sur les populations des côtes bretonnes qui sont soumises à une pression de récolte croissante et elle a été réalisée en concertation avec les récoltants et les aquaculteurs. Pour parvenir à ces grands objectifs, trois grands axes d'étude ont été développés dans cette thèse et leurs résultats sont synthétisés sous la forme de 6 articles ou projets d'articles répartis dans 3 chapitres :

Chapitre I : Estimation de la ressource génétique dans les populations naturelles.

La première étape nécessaire pour étudier la ressource génétique a été de développer des marqueurs microsatellites spécifiques à *P. palmata* en utilisant une méthode basée sur l'utilisation du séquençage

haut débit. Il a ainsi été possible d'identifier rapidement les motifs microsatellites, et d'obtenir des marqueurs pour *Palmaria palmata* ainsi que pour 5 autres espèces d'algues non modèles. (Article n°1).

Ces marqueurs ont été utilisés pour caractériser la diversité et la structure génétique des populations médiolittorales et infralittorales le long des côtes Normano-bretonnes, (Article n°2)

Pour étudier le processus d'adaptation locale à la température le long des côtes bretonnes, un suivi *in situ* de la température a été réalisé sur deux années. L'adaptation locale à la température a été étudiée expérimentalement au laboratoire en "jardins communs" en utilisant des individus provenant de populations du nord-est et du nord-ouest de la Bretagne soumises à des températures contrastées en fonction des saisons. (Article n°3)

Chapitre II : Système et mode de reproduction chez *P. palmata*.

L'étude de la reproduction de *P. palmata* a été réalisée dans 17 localités réparties le long de son aire de distribution. Les marqueurs microsatellites ont été utilisés pour analyser le mode et le système de reproduction à différents niveaux bathymétriques sur l'estran. (Article n°4)

Des expérimentations en conditions contrôlées ont été réalisées dans le but de lever les derniers verrous sur la maîtrise du cycle de reproduction. Les facteurs biotiques et abiotiques, impliquées dans le déclenchement de la reproduction, ont été étudiés sur des souches isolées en laboratoire. Des essais de fécondation contrôlée sur des supports de culture ont également été réalisés. (Article n°5)

Chapitre III : Constitution d'une collection de souches et sélection de phénotypes d'intérêt.

Un échantillonnage le long des côtes bretonnes a été réalisé afin de constituer une collection de souches de gamétophytes mâles et de sporophytes. Cette collection a servi de base pour développer un système de culture versatile, adapté à la biologie particulière de *P. palmata*. Ce système permet de maintenir en culture et de caractériser le phénotype des souches. Une première étape de sélection a été effectuée sur la croissance végétative des individus collectés dans les populations sauvages. (Article n°6)

Chapitre I : Etudes de la ressource génétique des populations Bretonnes de *P.palmata*

Dans ce chapitre, j'ai étudié la ressource génétique de *P. palmata* dans les populations du littoral breton à différents niveaux de marée, le médiolittoral et l'infralittoral. Ces connaissances sont aujourd'hui nécessaires pour franchir une étape supplémentaire dans le processus de domestication. La mise en évidence de processus d'adaptation locale avec la caractérisation de la structure génétique permettra d'apporter des connaissances essentielles pour commencer la sélection variétale de l'espèce. Les résultats ainsi obtenus seront également utiles à la gestion des populations exploitées et à l'estimation de leur capacité de résilience au réchauffement des eaux.

La première partie de ce chapitre décrit le développement de marqueurs génétiques de type microsatellites qui ont ensuite été utilisés pour caractériser le système de reproduction et la structure génétique de *P. palmata*. (Article n°1)

Les côtes du littoral breton ont la particularité de présenter une forte hétérogénéité environnementale (température et courantologie), de plus les populations de *P. palmata* sont présentes à deux niveaux de marée bien distincts dans cette région. Dans la seconde partie de ce chapitre, je me suis donc intéressé à l'étude de la structure génétique des populations médiolittorales et infralittorales de *P. palmata* le long des côtes bretonnes en relation avec le contexte environnemental et les traits d'histoire de vie particuliers de cette espèce. (Article n°2)

Finalement, dans la troisième partie de ce chapitre, j'ai caractérisé la température *in situ* le long des côtes bretonnes à deux niveaux de marée et j'ai étudié expérimentalement le processus d'adaptation locale à la température dans les populations du nord de la Bretagne (Article n°3)

1. Développement de marqueurs génétiques (Article n°1)

L'article n°1 ci-dessous détaille la méthode employée pour développer des marqueurs génétiques de type microsatellites en utilisant une méthode innovante chez six espèces de macroalgues, dont *Palmaria palmata*. Cet article a été publié dans *Algal Research* le 9 septembre 2023.



Contents lists available at ScienceDirect

Algal Research

journal www.elsevier.com/locate/algal

Genetic resources of macroalgae: Development of an efficient method using microsatellite markers in non-model organisms

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ARTICLE INFO

Keywords:

Genetic diversity
Microsatellite markers
Seaweed
Rhodophyta
Ochrophyta ddRAD

ABSTRACT

Red and brown seaweeds are species with high ecological and economic importance. Here we report the feasibility of cost-effective molecular marker development in 6 species from different phyla. Microsatellite markers of two brown seaweed species *Alaria esculenta* and *Pylaiella littoralis*, and of four red seaweed species *Calliblepharis jubata*, *Gracilaria gracilis*, *Gracilaria dura* and *Palmaria palmata* were identified and characterized in genomic sequences obtained using Double-Digest Restriction site Associated DNA (ddRAD). A total of 64,623,186 reads were generated from two runs of multiplexed Illumina Miseq sequencing, from which 30,636 reads containing microsatellite motifs including 15,443 where primer pairs could be designed. Five hundred seventy-six primers pairs were selected for amplification trials to determine levels of polymorphism. Of these 576 loci, 338 could be amplified and 142 of the 338 loci were polymorphic. A total of 28 usable polymorphic markers were developed in *A. esculenta*, 18 in *P. littoralis*, 11 in *C. jubata*, 14 in *G. gracilis*, 21 in *G. dura* and 13 in *P. palmata*. The overall number of alleles per locus ranged from 2 to 22. These 105 new microsatellite markers will be useful for further studies of population genetics, breeding programs and conservation genetics of these species. Compared with traditional approaches, our study yielded thousands of microsatellite loci for six different species in a short time and with affordable costs. This study, based on the use of ddRADseq, provided preliminary data about the genetic structure and reproduction mode of these six non-model species based on a small number of individuals from two geographically-distant populations and on the genetic structure and reproduction mode of two non-model species, i.e. detection of clonality for two red algae, *C. jubata* and *G. dura* and detection of highly genetically divergent populations corresponding probably to different cryptic species under the name *P. littoralis*.

Introduction

Evaluation of genetic resources is crucial for the sustainable conservation of economically or ecologically important species. It is also a key step in the domestication process of new species [1,2]. In the context of global change, most of the seaweed biomass in Europe comes from harvesting wild populations (FAO, 2018), questioning the sustainability of the wild genetic resources [3]. Moreover, the development of seaweed aquaculture since the 1950s in China for the Japanese kelp, or more recently in the 1980s in South East Asia and East Africa for tropical red seaweeds, has led to long-distance translocations of a few genotypes. These aquaculture practices are raising concern about their possible detrimental effects on local adaptation of wild populations that may be impacted by human-mediated gene-flow when

located in the vicinity of farms [4]. There is therefore, an urgent need for genetic markers to conduct genetic studies on an increasing number of seaweed species that may be negatively impacted by intensive exploitation of their natural populations, the future development of aquaculture or the impact of global change. For example, recent studies reveal that marine heat waves in recent years have played a role in the decline of several macroalgal species on the coast of northern Spain [5,6]; a possible influence of latitudinal patterns of genetic diversity on the vulnerability of the kelp *Ecklonia radiata* to a marine heatwave on the South-Eastern Australian coast [7]; multi-decadal decline in the cover of the giant kelp *Macrocystis pyrifera* at the southern limit of its Australian range [8]. These studies highlight the need to step up coastal monitoring efforts in order to analyse the impact of extreme temperature events on algal genetic biodiversity.

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<https://doi.org/10.1016/j.algal.2023.103251>

Even if genome-wide studies are more accessible as a result to next generation sequencing (NGS) methods, there are still numerous non-model, but nonetheless valuable, algae species for which genomic data are not available. This restricts the possibility to search for molecular markers *in silico*. Double Digested Restriction site Associated DNA sequencing (ddRADseq) has already been used to efficiently develop molecular markers such as Single Nucleotide Polymorphism (SNPs) [9] and, more recently, Short Sequence Repeats (SSRs) or microsatellite markers for non-model species [10,11]. For over twenty years, microsatellite markers have set the standard in the field of population genetic markers, offering the possibility of population analysis over time and providing useful insights based on the results of the many studies carried out with this type of molecular marker. These markers have the characteristic of being transferable between related species of the same genus, opening the possibility of studying the same locus in different species [12]. They also provide excellent results in paternity analysis, where SNPs have not yielded accurate information [13]. Although SNPs have better genome coverage than microsatellites, the bioinformatic skills required, the computational power and the cost of reagents limit their application to large numbers of individuals. Moreover, development of SNPs, also requires a greater number of sample replications because of the high error rate compared to microsatellite markers. Recent studies have indicated that, when addressing genetic structure or patterns of genetic diversity, a study with a dozen of microsatellite markers gave similar results to those obtained with thousands of SNP markers [13,14]. Microsatellite markers are therefore better suited (cheaper) for large sample studies and require less bioinformatic skills and computer power than SNPs [12,15], making them more accessible. The ability of microsatellite markers to uncover genetic variations at the same locus beyond time and species, along with their accessibility, makes them a gold standard in population genetic studies.

Since 2010, data on whole genome sequencing have been accumulating for seaweeds facilitating the search for microsatellites (i.e., for *Ectocarpus* species 7, referred to as *Ectocarpus siliculosus* [16]; for *Saccharina japonica* [17]; for *Ecklonia radicata* [18]). However, developing new microsatellite markers for a non-model species can be challenging when there is a lack of genomic data for *in silico* research (see for review: [19]) because the traditional microsatellite marker isolation approach, such as generation of an enriched library followed by cloning and Sanger sequencing or NGS pyrosequencing, is a costly, labour-intensive and often inefficient process. Although the use of expressed sequence tag (EST) databases has proved to be a valuable source for the development of SSR markers that are potentially transferable between taxa [20], there are still some taxonomic groups where this type of sequence remains rare. Other Methods to develop microsatellite markers rapidly and cost-effectively are needed for population genetic studies of non-model species. An efficient method was developed during the last ten years for non-model animals and plants [10,11], based on the generation of double-digest restriction site associated DNA (ddRADseq; [9]) from genomic DNA of a few individuals of the species of interest using Illumina sequencing technology. Microsatellite markers are mined *in silico* using tools that require limited bioinformatic skills and computer power compared to SNP development.

Marine macroalgae are a valuable resource with a wide range of applications, from ecosystem services, for example by acting as carbon sinks and fish nurseries, to food and feed production [21,22]. As the demand for seaweed biomass increases, new tools such as species-specific genetic markers are needed for the sustainable exploitation of wild resources as well as for the implementation of cultivation of those valuable species [4]. Since ddRADseq allows DNA tagging for sample-pooled sequencing, the aim of this study was to extend the method of Mori et al. [11] to multiple species using a maximum of two sequencing runs. In this way, it was possible to increase yields and reduce the cost of microsatellite marker development for economically important non-model species of seaweeds. We focused our study on 6 seaweed species, commonly found along the European coast, which are known for their socio-economic, ecological and scientific values. *Palmaria palmata* and *Alaria esculenta* are used for human consumption, cosmetics and animal feed [23,24]. *Calliblepharis jubata* and *Pylaiella littoralis* are used

for cosmetics [25]. *Gracilaria gracilis* and *Gracilaria dura* are known for their high agar content and serves as model species to test hypotheses related to the evolution of sex [26–29]. Except for *A. esculenta* none of those six North Atlantic species are cultivated at large scales [30] but several cultivation trials have been conducted in the past twenty years for *P. palmata* [31], and more recently for *P. littoralis* and *C. jubata* [32]. The development of microsatellite markers, which was performed for individuals from two geographically-distinct populations, provided a set of new preliminary data on the genetic structure and mode of reproduction of the six non-model species: the two brown seaweeds (Phaeophyceae): *A. esculenta* and *P. littoralis* and the four red algae (Rhodophyta): *P. palmata*, *C. jubata*, *G. gracilis* and *G. dura*.

Materials and methods

Sample collection and DNA extraction

A total of 275 individuals from six species of seaweed were collected from thirteen sampling locations (Table 1). The ploidy levels were known (either using phenological observations or flow-cytometry analyses) prior to genotyping for all study species except *G. dura* and *C. jubata*. Only later after genotyping, could ploidy levels could be assessed for all individuals. Upon collection, a piece of tissue was cut out from a spot that was free of algal and animal epiphytes and stored in silica gel. No specific treatments were carried out to remove bacteria from the tissue but we estimate the percentage of bacterial DNA after sequencing using the KAIJU software version 1.9.2 developed by Menzel et al., 2016 [33]. Total genomic DNA was extracted from 15 to 20 mg of dry tissue using the Nucleospin 96 plant kit (Macherey-Nagel, Germany). The extraction was performed according to the manufacturer's instructions except that samples were left in the PL1 lysis buffer at 65 °C for 15 min rather than 30 min. The extracted DNA was eluted into 120 µL of the supplied elution buffer.

DNA library preparation, sequencing and assembly

Two individuals of each species of seaweed (one per locality, except Quitao for *G. dura*) were used to construct the genomic libraries according to the methods described by Peterson et al. [9] with the following modifications (Table 1). Double-digest restriction site associated DNA was generated with *Pst*I and *Hha*I (New England Biolabs, USA) restriction enzymes in a total reaction volume of 50 µL using 300 ng of high-quality genomic DNA by incubating for 3 h at 37 °C (Fig. S1). Adapter ligation was performed following the instructions of Peterson et al. [9] with a unique P1 adaptor for each sample (Table 1) and ligation reactions were inactivated using NucleoMag NGS clean-up beads (Macherey-Nagel, Germany) with a final elution into 40 µL of water. Individual PCR amplifications were carried out in 40 µL reaction volumes containing 10 µL of Adapter-ligated DNA, 170 nM of indexed primer mix at 10 µM, 200 µM of dNTPs (Thermo Fisher Scientific Inc., USA), 1× of Q5® Reaction Buffer, 1× of Q5® High GC Enhancer, 0.8 Unit of Q5® High-fidelity DNA Polymerase (New England Biolabs, USA). DNA amplifications were carried out in a Bio-Rad DNA Engine Peltier Thermal Cycler using the following conditions: initial denaturation phase at 98 °C for 30 s, followed by 15 cycles of denaturation at 98 °C for 20 s, annealing at 60 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 10 min. PCR products were purified with NucleoMag NGS clean-up beads (Macherey-Nagel, Germany) with a final elution in 40 µL of water. The pooling was done before the size selection to generate two libraries with 6 samples each to obtain about 8 million reads per sample (Illumina performance specifications for Miseq v3 kits is between 22 and 25 million reads per run), Lib1 (*A. esculenta*, *P. littoralis* and *C. jubata*) and Lib2 (*G. dura*, *P. palmata* and *G. gracilis*). The Sage Science Pippin-Prep (sagescience.com) method was used to carry out the size selection to obtain the fragments of interest of between 350 and 550 bp (between 480 and 680 bp with the full library construction fragments).

Table 1
Geographical location of the populations used in the study, sample size (n), sexual phenotypes and barcodes used for ddRADseq.

Species	Populations	n	Phase (ploidy level)	Latitude (N)	Longitude (W)	P1 adaptor Sequence 3'-5'
<i>Alaria esculenta</i>	Roscoff, France	24	Sporophyte (2n)	48.7316	-3.9907	CTGGTT
	Plouguerneau, France	24	Sporophyte (2n)	48.6365	-4.5674	AAGATA
<i>Pylaiella littoralis</i>	Wissant, France	24	Sporophyte (2n)	50.9045	1.6326	ACTTCC
	Ile de Aransa, Portugal	24	Sporophyte (2n)	41.1571	-8.6892	TTACGG
<i>Calliblepharis jubata</i>	Roscoff, France	24	Tetrasporophyte (2n)	48.4344	-3.5920	AAGGAA
	Le Havre, France	24	Tetrasporophyte (2n)	49.7105	-1.8719	ATTCAT
<i>Gracilaria gracilis</i>	Gris-Nez, France	24	Tetrasporophyte (2n)	50.8727	1.5924	CTGGTT
	Porto, Portugal	24	Tetrasporophyte (2n)	41.1532	-8.6804	AAGATA
<i>Gracilaria dura</i>	Locquirec, France	12	Mixed phase (3n)	48.6954	-3.6528	AACGAA
	Coroso, Spain	15	Mixed phase (3n)	42.5691	-8.9778	ATTCAT
	Quitao, Portugal	8	Mixed phase (3n)	41.1945	-8.7090	Not used for sequencing
<i>Palmaria palmata</i>	Roscoff, France	24	Tetrasporophyte (2n)	48.7261	-3.9685	ACTTCC
	Belle-Ile, France	24	Tetrasporophyte (2n)	47.3781	-3.2252	TTACGG

Size selection and quality of both libraries were checked using a Bioanalyzer and High Sensitivity DNA Chips (Agilent Technologies), and the final concentrations were estimated through quantitative PCR (qPCR) using a KAPA Library Quantification Kit (Roche Molecular Systems, Inc) and a LightCycler® 480 System (Roche Molecular Systems, Inc). Sequencing was performed in two runs on a MiSeq sequencer (Illumina, San Diego, CA, USA) using a Miseq Reagent Kit v3 600 cycles (2 × 300 paired-end reads) and libraries at 15pM mixed with 5 % of Phix control v3 (Illumina, San Diego, CA, USA).

To obtain clean, high-quality reads, we discarded low quality raw sequences with adapter contamination or $N > 10$ %. We used STACKS software version 2.52 [34] to demultiplex the pooled samples based on a unique P1 adaptor (Table 1). Overlapping paired-end sequencing reads were assembled using the FLASH v1.2.11 software [35]; the minimum required overlap length between two reads to provide a confident overlap (m) was fifteen and the maximum overlap length expected in approximately 90 % of read pairs (M) was three hundred. To construct the reference sequences, the dDocent pipeline version 2.8.7 [36] was run using default parameters and procedures as described in the user manual, except that percent clustering similarity was set at 95 % for the reference assembly.

Microsatellite loci screening and primer design

Microsatellite motifs were identified using the QDD pipeline program version 3.1.2 [37] and all reference sequences with sizes >80 bp (PIPE1 in QDD) were used for detection. Both perfect and imperfect di-, tri-, tetra-, penta- and hexanucleotide motifs were targeted. Dinucleotide motifs with at least 4 repeats and longer motifs with at least 3 repeats were selected (PIPE1 in QDD). Reads with at least 95 % identity were clustered as consensus sequences, while those that could not be grouped were classified as singletons using Blast+ [38] (PIPE2 in QDD). Primers were designed using the PRIMER v3.0 software [39]. Duplicate primers were removed and unique primers of the putative loci were retained. Primers were mainly selected according to the type and size of the microsatellite motif, including also the following criteria: primer length between 18 bp and 27 bp; 50 to 75 % GC content; a distance >20 bp between the primer motif and the repeated motif and amplicon sizes between 100 bp and 450 bp (PIPE3 in QDD). In order to test these primers cost-effectively for amplification, we reduced the number of selected microsatellite regions to 96 (the capacity of a microtiter plate) for each species by selecting the ones with the highest number of repeats for each motif category.

Amplification trials and levels of polymorphism of microsatellite loci

For each species, positive amplification of 96 primer pairs was tested using DNA from 8 random individuals. Polymorphic level and genetic diversity characterization of the primer pairs that amplified positively were tested using between 35 and 48 individuals per species (Table 1–Fig. 1).

Amplifications were carried out in 10 µL reaction volume with 2 µL of DNA template diluted 1:100 and following the method of Guzinski et al. [40]. The PCR products labeled, with the four different colors, were pooled (24 pools in total) and diluted 1:10 with water. Next, 2 µL of the diluted PCR product pool was added to 10 µL of loading buffer made up of 0.5 µL of the SM594 size standard [41] and 9.5 µL of Hi-Di formamide, denatured at 95 °C for 3 min, and run in an ABI 3130 XL capillary sequencer (Applied Biosystems, USA). Genotypes were scored manually in Genemapper version 4.0 (Applied Biosystems).

Genetic analyses

The results obtained for the populations of diploid individuals were analyzed with the following software. Prior to the genetic analysis, we tested for null alleles using MICRO-CHECKER v2.2.3 [42]. GENEPOP v4.7.5 [43] was used to test for linkage disequilibrium (global test employing Fisher's method, the following Markov chain parameters were used: 100,000 for dememorization, 1000 batches and 50,000 iterations per batch). HIERFSTAT v0.5–10 R package [44] was used to obtain the number of observed alleles per locus (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e), the within-population deviation from Hardy-Weinberg equilibrium (F_{is}) and its significance per locus and for all loci (tested with a permutation test; based on 10,000 permutations). The Polymorphic information content (PIC) was calculated with CERVUS v3.0.7 [45].

A modified analysis was used for the triploid individuals (*G. dura*): AUTOTET v1 software [46], a program for the population analysis of allotetraploid microsatellite data, was used for the genetic analysis and F_{is} index; its significance per locus and all loci (tested with a permutation test; based on 10,000 permutations) was determined using SPAGeDi v1.5d software [47]. The Polymorphic information content (PIC) was calculated using "polysat" v.1.7 R package according to Clark and Jasieniuk [48].

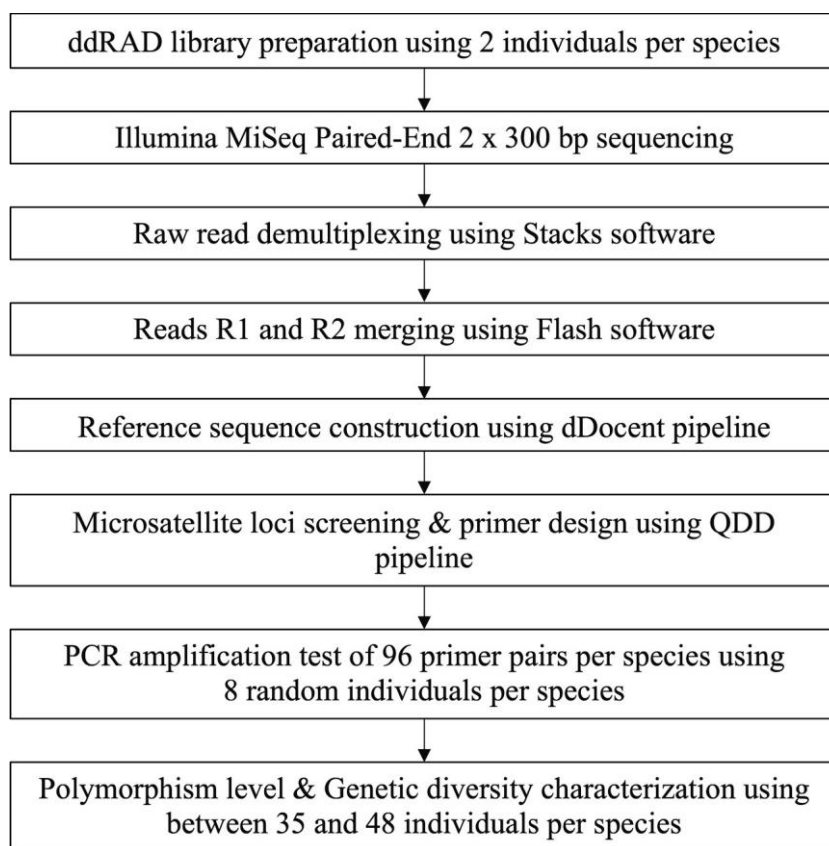


Fig. 1. Workflow and experimental set-up used for microsatellite markers development using ddRADseq.

The number of distinct Multilocus Genotypes (*MLG*), the genotypic richness (*R*) and the Genotype accumulation curves (based on 10,000 permutations) were obtained with the “poppr” v2.9.3 R package [49].

Finally, to estimate the level of genetic differentiation between localities, pairwise F_{ST} [50] values were computed in HIERFSTAT v0.5–10 R package [44]. The significance of the comparisons was estimated by performing 10,000 bootstraps over loci, with the comparisons judged significant if the bootstrap-generated confidence intervals did not overlap zero.

Results

ddRAD sequencing and assembly

The sequencing procedure generated a total of 64.6 million reads (6.3 to 14.4 million per species) for the six studied species [51] (Table S1). The percentage of bacterial DNA in the reads was <5 % with an average of 2.94 % (Table S2). After editing/trimming, 377.7 Mb of high-quality sequences (41.9 to 111.4 Mb per species) were available, which were assembled into 1,351,981 contigs (134,653 to 400,288 contigs per species). We obtained an average contig length of 294.0 bp (217.2 to 406.4 bp per species), an average contig N50 size of 279.3 bp (173 to 407 bp per species) and an average GC content of 51.2 % (49.9 % to 53.8 % per species) (Table S1).

Microsatellite loci development and characterization

Among the 1,351,981 assembled contigs, a total of 30,636 microsatellite motifs were found in the six species (1755 to 11,487 per species) using the QDD pipeline (Table S1). The microsatellite sequences contained 14,288 (46.6 %) di-, 14,529 (47.4 %) tri-, 861 (2.8 %) tetra-, 481 (1.6 %) penta- and 477 (1.6 %) hexanucleotide motifs (Table S3). After choosing regions with flanking sequences of sufficient length and requiring a product size of between 100

and 450 bp, we obtained 15,443 primer pairs (752 to 6592 per species – Table S1) corresponding to 7327 (47.4 %) di-, 6997 (45.3 %) tri-, 525 (3.4 %) tetra-, 316 (2.0 %) penta- and 278 (1.8 %) hexanucleotide motifs (Table S3). A total of 576 primer pairs were tested for all species (96 primer pairs each, focusing on the ones with the highest number of repeats), 338 were amplified including 104 (30.7 %) di-, 77 (22.7 %) tri-, 63 (18.6 %) tetra-, 67 (19.8 %) penta- and 27 (7.9 %) hexanucleotide motifs (Table S3). The 338 loci retained after the amplification tests (see Table S1 for details per species) were subsequently checked for level of polymorphism within the sample of 8 individuals per species. A total of 142 out of these 338 putative loci including 31 for *A. esculenta*, 28 for *P. littoralis*, 15 for *C. jubata*, 20 for *G. gracilis*, 28 for *G. dura* and 20 for *P. palmata* produced PCR products of the expected size, were polymorphic (Table S1) and corresponding to 54 (38.0 %) di-, 28 (19.7 %) tri-, 27 (19.0 %) tetra-, 27 (19.0 %) penta- and 6 (4.2 %) hexanucleotide motifs (Table S2). Therefore, the rate of success for each of the five motif categories in terms of acquisition of polymorphic microsatellite was 33.5 % for di-, 19.1 % for tri-, 23.5 % for tetra-, 26.0 % for penta- and 12.2 % for hexanucleotide repeats (Table S3).

Genetic diversity

All the loci analyzed in this study (142 microsatellite markers for 6 species) were selected for their amplification success and their polymorphism in each species. This allowed us to confirm the ploidy levels for all individuals in the 4 species for which ploidy levels were known prior to genotyping (i.e., *A. esculenta*, *P. littoralis*, *P. palmata* and *G. gracilis*). For *G. dura* and *C. jubata*, the ploidy level was determined after genotyping and triploid individuals were detected in *G. dura*. Data for the number of alleles, null allele frequency, genetic diversity indices and departure from random mating (F_{IS}) are given in Table S4 for each species, locus and population. However, among the loci that correctly amplified, several markers (3 in *A. esculenta*, 10 in *P. littoralis*, 4 in

C. jubata, 6 in *G. gracilis*, 7 in *G. dura* and 7 in *P. palmata*) were discarded because they appeared to be either monomorphic within populations, or because they showed a significant frequency of null alleles, or an extreme departure from random mating suggesting non-Mendelian inheritance (F_{IS} values >0.6 or F_{IS} values < -0.6). Overall, from our study, 28 markers appeared to be useful for *A. esculenta*, 18 for *P. littoralis*, 11 for *C. jubata*, 14 for *G. gracilis*, 21 for *G. dura* and 13 for *P. palmata* (corresponds to the markers in bold in the Table S4).

The level of diversity was highly variable across species (Table S4). The mean number of alleles (N_a) computed over all loci and populations (i.e. global, Table S4) was higher in the two brown seaweeds *A. esculenta* (6.7) and *P. littoralis* (8.0) compared to *C. jubata* (3.8), *G. gracilis* (3.4), *G. dura* (6.2) and *P. palmata* (5.7). However, this trend was not retrieved for the other estimate of gene diversity (H_e). The highest H_e value was observed in the red alga *G. dura* (0.758) and explained by fixed heterozygosity compared to all other species: the two brown seaweeds *A. esculenta* (0.535) and *P. littoralis* (0.496) and the three remaining red algae *C. jubata* (0.344), *G. gracilis* (0.412) and *P. palmata* (0.489).

Finally, polymorphic information content (PIC) was higher for *P. littoralis* (0.678) and *G. dura* (0.725) compared to *A. esculenta* (0.532), *C. jubata* (0.335), *G. gracilis* (0.421), and *P. palmata* (0.478).

Differences in the level of clonality were observed among species. No signal of clonality could be detected in *A. esculenta*, *P. littoralis*, *G. gracilis* and *P. palmata* as all multilocus genotypes (MLG) were distinct ($n = MLG$) and genotype richness (R) was maximum and equal to 1 (Table 2). In contrast, the number of multilocus genotypes (MLG) were not distinct in *G. dura* and *C. jubata* ($n > MLG$) and the mean values of genotypic richness (R) over populations were low: 0.378 and 0.848 respectively (Table 2). For these two partially clonal species, F_{IS} values were significantly negative, revealing heterozygous excess, while for the four others sexually reproducing species, F_{IS} values were not significantly different from 0, revealing random mating (Table 2). The genotype accumulation curves showed that between five and twelve loci were sufficient to differentiate all non-clonal individuals for each of the studied populations (Fig. 2).

Finally, the pattern of genetic differentiation among populations was highly significant ($P < 0.001$) for all studied species (Table 2). However, the magnitude of F_{ST} values were very different among species ranging from 0.118 in *P. palmata* to 0.482 in *P. littoralis* (Table 2).

Table 2

Mean genetic diversity among loci for the populations sampled for each of the 6 species.

Species	Populations	<i>n</i>	<i>nLoc</i>	<i>N_a</i>	<i>A</i>	<i>MLG</i>	<i>R</i>	<i>H_e</i>	<i>F_{IS}</i>	<i>Loc dist</i>	<i>F_{ST}</i>
<i>Alaria esculenta</i>	Roscoff, France	24	28	4.1	4.24 ± 0.15	24	1	0.505	-0.031	50	0.136*
	Plouguerneau, France	24	28	5.8	3.32 ± 0.10	24	1	0.566	-0.006		
<i>Pyliella littoralis</i>	Wissant, France	24	18	5.3	4.09 ± 0.14	24	1	0.556	-0.083	2200	0.482*
	Ile de Aransa, Portugal	24	18	3.7	2.97 ± 0.20	24	1	0.436	-0.008		
<i>Calliblepharis jubata</i>	Roscoff, France	24	13	2.9	2.42 ± 0.17	20	0.826	0.346	-0.131\$	510	0.132*
	Le Havre, France	24	13	2.5	2.29 ± 0.12	21	0.870	0.394	-0.285\$		
<i>Gracilaria gracilis</i>	Gris-Nez, France	24	14	2.9	2.65 ± 0.10	24	1	0.464	-0.024	2150	0.262*
	Porto, Portugal	24	14	2.8	2.28 ± 0.17	24	1	0.362	-0.075		
<i>Gracilaria dura</i>	Locquirec, France (a)	12	28	3.8	3.43 ± 0.36	7	0.636	0.655	-0.186\$	1470 (a-b)	0.263* (a-b)
	Coroso, Spain (b)	15	28	3.2	2.86 ± 0.31	4	0.214	0.674	-0.390\$	1600 (a-c)	0.299* (a-c)
	Quitao, Portugal (c)	8	28	2.5	2.54	3	0.286	0.617	-0.350\$	130 (b-c)	0.279* (b-c)
<i>Palmaria palmata</i>	Roscoff, France	24	13	5.1	3.51 ± 0.22	24	1	0.514	0.023	300	0.118*
	Belle-Ile, France	24	13	3.9	3.12 ± 0.19	24	1	0.465	-0.014		

n Number of genotyped individuals. *nLOC* Number of loci retained as suitable polymorphic markers (i.e., polymorphic, no-significant *Nf*, no-significant *F_{IS}* for no clonal species).

N_a Means of number of alleles per locus.

A Standardized allelic richness for a common sample size of 8 individuals.

MLG Number of distinct Multilocus Genotypes.

R Genotypic richness ($(G-1)/(N-1)$) (Dorken and Eckert, 2001). *H_e*

Expected heterozygosity.

F_{IS} Inbreeding coefficient as defined by Weir and Cockerham (1984) calculated overall polymorphic loci, *F_{IS}* values significantly different from zero are shown with \$. *Loc Dist* distance between two localities in km.

* *F_{ST}* Pairwise estimates of genetic differentiation obtained with HIERFSTAT (Goudet 2014), $P < 0.001$.

Discussion

The development of NGS has improved our capacity to identify and develop microsatellite markers (for review see [52]). In particular, polymorphic loci in model and non-model, plant or animal, species can now be identified rapidly using ddRAD sequence data [10,11,53,54]. The ddRADseq method [9] was largely developed during the last 10 years for population genetics studies in many organisms to search for SNPs and to decrease the cost of whole genome sequencing for genotyping. It is still the object of continuous innovations [55] like those we have developed for multiple seaweed species. However, even if several studies have developed ddRADseq methods for SNP discovery in seaweeds in recent years (firstly in the brown algal model *Ectocarpus* [56] and mostly in the kelp or large brown algal species *Undaria pinnatifida* [14]; *Saccharina latissima* [40]; *Ecklonia radiata* [57]; *Sargassum thunbergii* [58] and *Durvillaea antarctica* [59]), none of them was exploited to discover microsatellites because markers were already available.

In this study, implementing the protocol developed by Mori et al. [11] with only two multiplexed libraries for the 6 species, we successfully developed around 20 microsatellite markers per species in different brown and red seaweeds of economic and ecological interest. This highly efficient method allows rapid identification of microsatellite motifs distributed throughout the genome, ultimately revealing polymorphic markers in the target species with an efficiency of about 25 %. Interestingly, in *G. gracilis*, >50 % of the microsatellite loci already identified using classical library cloning methods [60,61] were found in this study confirming good coverage of the genome.

Characteristics of microsatellites between species

Our data yielded ten times more microsatellite motifs in brown than in red seaweeds but this difference could not be clearly linked to their genome size (Table S1). However, for *Gracilaria* and *Calliblepharis*, it may be important to consider their small genome size for future development of microsatellite markers by, for example, adapting the coverage (e.g., sequencing 2 species per run instead of 3 or more).

The occurrence and the frequency of different types of microsatellite motifs seem to be characteristic of a species, which can be distant or closely related [12,62,63]. In our study, the distribution of microsatellite marker repeats nucleotide types (di, tri, tetra, penta and hexanucleotide) was similar to that obtained for the red alga *G. lichenoides*

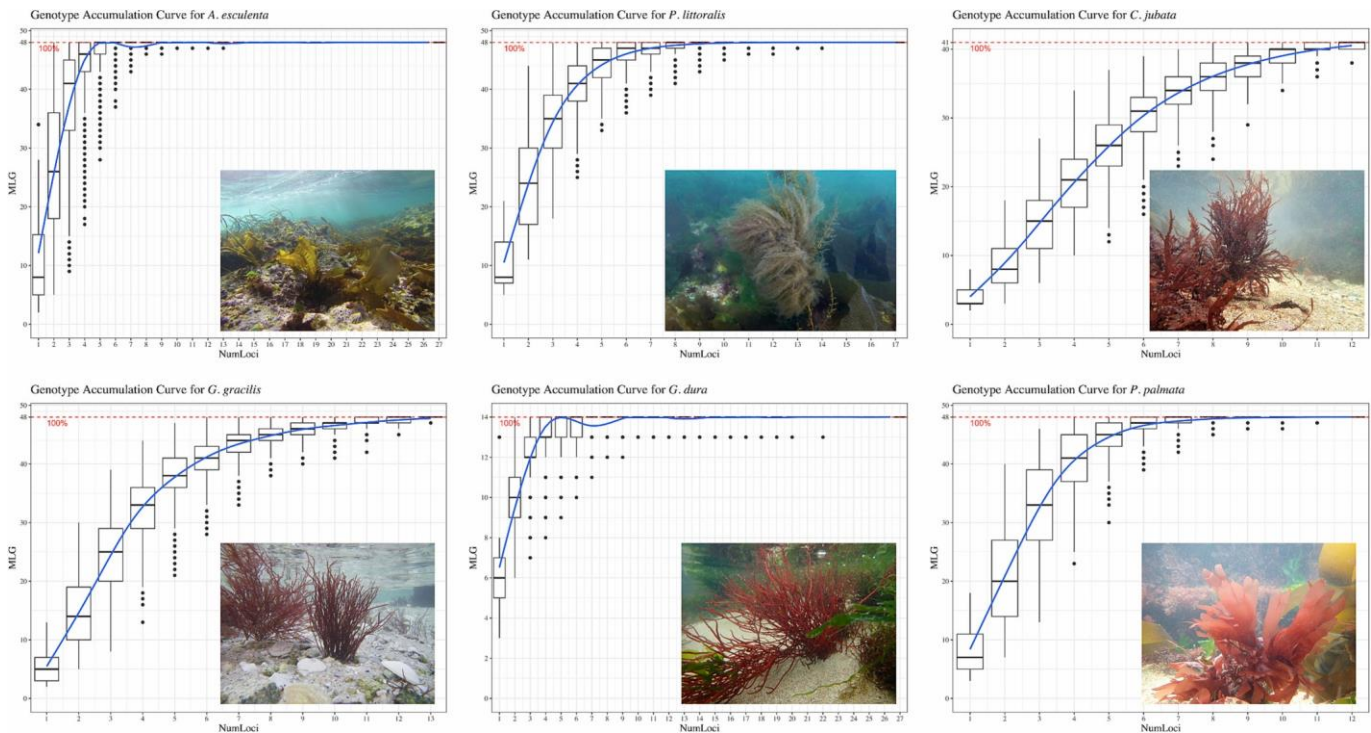


Fig. 2. Accumulation curves of the genotypes of the 6 species based on 10,000 permutations with “poppr” v2.9.3 R package. Photo credits: © Destombe C. for *G. gracilis* and *G. dura*; © Baud A. for *A. esculenta* and *P. palmata*; © Thomas W. for *P. littoralis*.

[64]. Dinucleotide and trinucleotide repeats were found to be the most abundant motif, on average 46.6 % and 47.4 % respectively (Table S3), probably because short repeats are the starting point for subsequent extensions of Short Tandem Repeats (STRs) [65] which are the most variable type of DNA sequence in the genome [66]. The most common class of polymorphic marker contained dinucleotide motifs (35 %–40 % of the polymorphic markers) for each species except for *A. esculenta* and *P. littoralis* for which polymorphic markers with tetranucleotide and pentanucleotide motifs were the most abundant, respectively (32.3 % and 35.7 % of the polymorphic markers respectively). In contrast to what was found for plants by Zhu et al. [63], hexanucleotide repeats in our study were the least abundant type of polymorphic markers. This could be due either to the phylogenetic distance between algae and land plants [63] or to an artefact related to the size of the fragments present in the ddRADseq libraries (partial genome vs complete genome) or to the types of restriction enzymes used for library construction.

The consequent yields and largely sufficient genome coverage imply that the method used in this study could be extended to >6 species. Future studies should be conducted to find the adequate balance between numbers of species and genome coverage, accounting for genome structure and length.

New perspectives of using next-generation sequencing for population-level studies

Several methods have been developed in many organisms to lower the cost and/or streamline next-generation sequencing for population-level studies [55]. Future developments for seaweeds will depend of many contingencies. With the need to develop knowledge on new species of seaweed to domesticate or to conserve in natural populations, only a few laboratories are developing approaches, depending on the means available for these studies. Large whole-genome sequencing efforts such as the Darwin tree of life in the UK, Atlasea in France and the ERGA (European Reference Genome Atlas) project at the EU level will offer new opportunities to enlarge the search for genetic markers for novel species. For the development of multiple PCR, synthesizing unique barcodes for each sample or even a subgroup of samples is expensive. Moreover, multi-, or single-level multiplexing and demultiplexing of samples can be cumbersome, time consuming, and costly. Therefore, approaches for targeted sequencing-based genetic screening such as the use of high multiplex PCR coupled to NGS (Hi-Plex, [67]) can be developed for only a few species of seaweeds and mainly models of economic importance [68].

Limited population genetics data could provide useful information on genetic diversity and reproductive systems

The preliminary results of the population genetic analysis, even though they were carried out on a few populations, provided interesting information about the reproductive systems and diversity of the study species. Indeed, two of the four red algal species, *C. jubata* and *G. dura*, showed strong signatures of asexual reproduction (heterozygous excess, low genotypic richness, repeated multilocus genotypes). It is known that most Rhodophyta species are able to reproduce vegetatively either by thallus fragmentation or propagule dispersal [69–71], however, the significance of asexual reproduction in the field remains poorly known except for *Phymatolithon calcareum* (maerl beds) [72], cultivated *G. chilensis* in farms [73] and the introduced species *G. vermiculophylla* [74]. Our results for *C. jubata* suggest that this species partially undergoes natural fragmentation. Indeed, the rhizoids could play a central role in vegetative propagation [71], by detaching from the parent thallus to give a new individual. However, no field observations had been made until now. To our knowledge, our results confirm this hypothesis for the first time. In contrast, in the genus *Gracilaria*, such specialized organs have never been observed and vegetative multiplication occurs by spontaneous fragmentation of the thallus. Cuttings are routinely used to reproduce macroalgae in aquaculture [75]. However, cuttings are not generally capable of giving rise to a new perennial individual [27]. In both *G. chilensis* and *G. vermiculophylla* species for which there is strong evidence for asexual reproduction [73,74], it was shown that vegetative fragmentation could be facilitated by farming practices (in *G. chilensis*) and explained rapid colonization during biological invasion (in *G. vermiculophylla*). In the case of *G. dura*, as the individuals are attached to rocks, the fragmentation hypothesis can be rejected. Instead, sexual reproduction could occur either by apomixis or apogamy, as observed in some triploid individuals of the red alga *Caloglossa leprieurii* [76]. Further investigation is therefore needed to better understand how and by what means this species reproduces asexually. The results obtained for *G. gracilis* and *P. littoralis*, confirmed that individuals reproduced sexually by outcrossing [77,78]. Finally, our results suggested the occurrence of random mating systems in natural populations of *A. esculenta* and *P. palmata*.

The genetic diversity observed in this study was of the same order of magnitude as that observed previously for the same or closely related species. Overall, genetic diversity tended to be higher in brown than in red algae, probably due to differences in their effective population sizes. For example, the genetic diversity indices found in *A. esculenta* ($H_e = 0.505$ and $H_e = 0.566$) was close to that recorded for other kelp beds from the same region *Saccharina latissima* ($H_e = 0.549$), *Laminaria digitata* ($H_e = 0.534$) and *Laminaria hyperborea* ($H_e = 0.591$) [79,80]. In the filamentous alga *P. littoralis*, genetic diversity ($H_e = 0.556$) was slightly reduced compared with that found in a previous study ($H_e = 0.608$) maybe because we used cross-amplification of microsatellite markers from *Ectocarpus siliculosus* [78]. In *G. gracilis*, we studied the same population using 14 newly developed markers (GN2, located at Cape Gris Nez) that was genotyped 20 years ago using seven microsatellite markers. Lower genetic diversity values were observed in our study ($H_e = 0.514$ in 2001, [77] vs $H_e = 0.464$ in 2021, this study) suggesting that temporal changes could have happened in this population. Finally, these markers were used to estimate differentiation between populations (F_{ST}). Even though the scale of distance between populations varied greatly between species (from 50 to 2200 km), it was possible to detect significant genetic differentiation even between populations separated by distances of 50 km. Finally, in *Pylaiella* where the occurrence of two cryptic

species along the French coast has been reported by Geoffroy et al. [78], the high values of genetic differentiation (F_{ST} close to 0.5) observed between the two studied populations suggest that they belong to different cryptic species.

Conclusion

We were able to rapidly develop species-specific molecular markers for six phylogenetically distantly-related species of red and brown seaweeds with relatively low cost (in time and money). These markers are suitable for studying the diversity and genetic structure, and can also be used to detect and decipher clonality processes involved in the reproduction of these species. This work, which uses this rapid and inexpensive method, could facilitate evolutionary analyses in a wide variety of non-model taxa. The applications of these new species-specific genetic markers are numerous, such as breeding, selection and conservation programs, genetic resources studies, management of harvested stocks and monitoring of the origin of processed products. In addition, the use of ddRAD sequencing in the microsatellite development process still allows for the detection of SNPs with the generated data.

Supplementary data to this article can be found online at

<https://doi.org/10.1016/j.algal.2023.103251>. **CRedit** authorship

contribution statement

S. Mauger: original concept, laboratory work, bioinformatics analysis, analysis of molecular data, led manuscript drafting and editing; A. Baud: sampling, led manuscript drafting and editing; G. Le Corguill'e: bioinformatics analysis, editing manuscript; G. Tanguy: laboratory work and editing manuscript; E. Legeay: laboratory work and editing manuscript; E. Creis: sampling and editing manuscript; M. Valero: drafting and editing manuscript; P. Potin: funding and supervision, drafting and editing manuscript; C. Destombe: funding and supervision, sampling and editing manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated during this study are openly available in a Dryad Digital Repository <https://doi.org/10.5061/dryad.63xsj3v6w>.

Acknowledgments

We would like to thank J. Mark Cock and the three anonymous reviewers for their comments on the manuscript. This work benefited from the support of the French Government through the National Research Agency with regards to an investment expenditure program IDEALG (ANR-10-BTBR-04), the EU Horizon 2020 project GENIALG (Grant Agreement No 727892), the European Maritime and Fisheries Fund (EMFF) through the POLISTR project and R'egion Bretagne (PhD grant ARED Domdulse from ED227 Sorbonne-Universit'e/MNHN to AB) and FUI 23 project OCEACTIF from the French Ministry of Research to CD and PP. We are grateful to the Roscoff Bioinformatic platform (ABIIMS) for bioinformatics support, to the Genomer platform, Biogenouest genomics and EMBRC France partner core facility for its technical support. The authors are deeply indebted to the Service Mer & Observation (SMO) of Roscoff, Alexandre Geoffroy and Dan Potin for sampling.

Ethical consent

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

All authors read and approved the manuscript for the publication in *Algal Research*.

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2. Etude de la structure génétique des populations bretonnes de *P. palmata* (Article n°2)

L'article 2 ci-dessous regroupe les travaux réalisés au cours de ma thèse sur la structure et la diversité génétique au sein des populations armoricaines de *P. palmata*. Il est prévu pour une soumission pour publication début 2024 dans la revue *Evolutionary applications*.

Article n°2: Seascape and shore level habitat shape the fine-scale genetic structure of natural populations of the red alga *Palmaria palmata* along the Brittany coast.

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Abstract

In marine organisms, genetic differentiation among populations is influenced by life history traits and by a range of spatial, oceanographic, and environmental factors that are attributed to the seascape. The relative influences of these factors may vary in different locations and can be measured using seascape genetic approaches. Dispersal of most marine organisms can be either facilitated or limited by oceanographic features regardless of the geographic distances. Here, we combined a biological and a seascape genetic approach to populations of the red alga, *Palmaria palmata*, at a fine spatial scale along the Brittany coast, western France, a complex seascape of rocky shores with strong, multidirectional currents greatly influenced by extreme tidal ranges (up to 11 m). This study uses 11 highly polymorphic microsatellite loci to explore the reproductive system and the fine genetic structure of 11 sites with natural populations of *P. palmata*. As both the intertidal and subtidal subpopulations were compared, the genetics of the 11 markers were investigated in the diploid tétrasporophytes, which dominate *P. palmata* wild populations, and exhibited high levels of polymorphism. Thus, all frond genotypes were distinguished and we established that dispersal was limited in this species, leading to substructuring populations over a few meters and a notable genetic differentiation over a few tens of meters between the intertidal and subtidal levels. These results

support the relatively poor dispersion ability of the species. Our findings add to the growing evidence for the key role of seascape attributes in driving spatial patterns of gene flow. There was significant genetic structuring over small spatial scales implicating dispersal and recruitment bottlenecks and highlighting the importance of implementing local-scale conservation and management measures.

Introduction

Estimating gene flow between populations is a major challenge for understanding past and ongoing evolutionary processes, as well as adapting population management of terrestrial and marine organisms. The extent of gene flow among populations influences the genetic structure which refers to the distribution in space of the genetic diversity. Understanding the processes that generate genetic structure is highly informative for a broad range of conservation practices (Beger et al., 2014; Frankham et al., 2010; Magris et al., 2014; Palumbi, 2004) such as identifying conservation units (Jahnke et al., 2020; Palumbi, 2003), their spatial boundaries (e.g., Nielsen et al., 2017), and areas that are important stepping stones or the source of recruits (e.g., Coolen et al., 2020; Jahnke et al., 2019). The geographic distance between populations, currents and ocean barriers, species dispersal capacity, and the availability of environments to colonize can all influence genetic structure. Historically, isolation by distance (IBD) has been the simplest model used to explain genetic differentiation, whereby gene flow decreases with distance from the original population (Wright, 1943). However, in the marine environment, genetic differentiation often cannot be explained simply as a function of geographic distance (Riginos & Liggins, 2013). Dispersal of most marine organisms can be either facilitated or limited by oceanographic features (such as water currents) regardless of the geographic distances. In general, species with low mobility or poor propagules dispersal ability tend to have stronger genetic structure due to lower gene flow between geographically close populations compared to species with high dispersal potential. (e.g., Dawson et al. 2014, Haye et al. 2014). Dispersal of marine species depends primarily on physical factors (such as ocean currents and seascape configuration) and biological factors (such as season, reproductive cycle, and propagule types) (Hohenlohe 2004, Ruggeri et al. 2016). Because of the importance of currents, this environment has long been considered highly dispersive thanks to occurrences of long pelagic larvae phases of most marine species life cycle (Palumbi 2004). However, studies have demonstrated the existence of smaller-scale genetic structure in intertidal organisms suggesting limited dispersal potential of these organisms, often related to the tidal gradient (Billot et al., 2003; Krueger-Hadfield et al. 2013;; Johannesson et al., 2010; Engel et al., 2004; Zardi et al., 2011).

In seaweed, the dispersal distances of spores and gametes are generally considered to be very limited (Santelices, 1990). In kelps (*Lessonia spicata*, *Alaria esculenta*, *Macrocystis pyrifera*, and *Postelsia palmaeformis*) new sporophytes have been shown to be recruited within a few meters of the parental sporophyte (Parada et al. 2016, Anderson & North, 1966; Dayton, 1973). However, different rare long-distance dispersal events have also been demonstrated, which could maintain genetic homogeneity within species (Kinlan et al., 2005; Waples et al., 2008). The few studies that focused on red seaweed dispersal abilities show limited to strongly limited dispersion range (Krueger-hadielf et al., 2013; Shum and Palumbi, 2020)

In this study, given the distribution of *P. palmata* along the tidal gradient, its peculiar life cycle, the complexity of Brittany seascape, we propose to address the relationship between landscape dynamics (shore levels and regional context) and population genetic structure for this ecologically and economically important species of red algae along Brittany Coast.

Brittany is an ideal region to explore the potential drivers of population genetic structure, owing to its particularity to hold a complex seascape that may shape genetic structure on a fine and on a larger scale. This seascape can be divided in three biogeographic sub-regions as South, North-West and North-East Brittany on the basis of biogeographic studies, annual ocean circulations, sea surface temperatures and population density. Indeed, Brittany corresponds to the limit between the northern European Sea and the Lusitanian biogeographical provinces according to Spalding et al. (2007). Brittany coast surface circulation is dominated by tidal currents and residual circulations coming from the North Atlantic Current, which subdivide in two streams off Brittany western coast. The first stream flows southward into the Bay of Biscay, delimiting North and South Brittany. While the second one flow northward into the English Channel and form gyres around the English Channel Islands, isolating the gulf of Saint Malo from the main flow (Salomon & Breton 1993, Jegou and Salomon 1991) therefore delimiting North West from North East Brittany. According to Gallon et al 2014, which studied Brittany sea surface temperatures over a 20 years survey, this region consists of a mosaic of contrasting conditions. North-West Brittany is colder and presents less annual thermic amplitude than North-East and South Brittany.

P. palmata is commonly found along the rocky shores of the North Atlantic Ocean from Maine to Northern Portugal (Lüning 1990). Light being a major factor limiting its vertical distribution, the species is most abundant in the first 10 meters below the surface, it can be observed to a maximum depth of around 20 m when the water is exceptionally clear (Stevant et al. 2023). The upper limit of distribution in the intertidal zone is probably due to the physical effects of exondation (desiccation and rise in temperature) as well as grazing by molluscs (Cabioc'h et al. 2014). Although *P. palmata* spans most of

the lower intertidal zone in Brittany, its density is typically disjointed with peaks in the *Fucus serratus* belt and in the *Laminaria hyperborea* belt with a disruption of his presence in the *L. digitata* belt (Cabioc'h et al. 2014). This discontinuous pattern in density with intertidal zone is relatively stable throughout the year, and is likely related to desiccation stress during periods of emersion and the biotic interactions and light irradiance when *P. palmata* is epiphytic on brown algae.

Palmaria palmata has a distinct life cycle compared to the majority of Florideophyceae with a strong sexual dimorphism and a lack of dispersion phases after fertilization because the lack of a carposporophytic phase in the Palmariales (van der Meer & Todd 1980). Moreover, *P. palmata* is known to be harvested by hand-picking on shores for millennia in Europe and North-Eastern American coasts, where it is mostly used for human and animal consumption (Garineaud, 2017; Mouritsen et al., 2013, Garbary et al. 2012). Today, in addition to its interest in human food, this alga is also used in cosmetics and medicines (Harnedy & FitzGerald, 2013; Yuan et al., 2005). Wild populations remain the main source of biomass (Grote, 2019) and today up to 361 tonnes of fresh biomass are harvested yearly from Brittany intertidal populations (CRPMEM, 2020). However, several programs are underway in Europe and North-East America to develop its commercial aquaculture (Stevant et al., 2023). Aside from the biological and physical differences between tidal levels that could favor microgeographic genetic patterns and restrain gene flow between the three sub-regions delimited by temperature patterns in Brittany by reproductive isolation. Moreover, a report from Regional Committee for Maritime Fisheries and Marine Farming of Brittany (CRPMEMB) showed that 95% of the harvested *P. palmata* biomass between 2013 and 2018 come from North-West Brittany intertidal populations, suggesting the presence of larger and denser populations in this region. In regard to the *P. palmata* life history trait, we expect to find significant genetic differentiation along tidal gradients and between the predefined region. We also expect to observe differences in dispersion across the seascape between intertidal and subtidal levels of *P. palmata* populations. Moreover, the increase in harvesting pressure over years and the impact of heatwaves in coastal North East Atlantic highlight the need to understand the genetic diversity and structure of this exploited species in order to adapt the management of the populations at Brittany scale and to possibly select some genotypes within the different populations for a selective breeding program. Recently, we developed microsatellite loci for *P. palmata* and five other species of red and brown algae (Mauger et al., 2023), and here we use eleven polymorphic markers for population genetic measures, to investigate genetic structure and diversity of *P. palmata* populations across shore levels and along the Brittany coast.

Material and methods

Sampling, phenotyping and genotyping

Sampling was carried out October 2019 to February 2022 during spring tides. Thallus of *Palmaria palmata* were collected randomly at two different tide levels, using the brown algal belts as height references. Thirty thallus were collected at the *Fucus serratus* level (middle intertidal level, hereafter referred as intertidal level, between 1.62 and 3.13 meters), and 30 thallus at the *Laminaria hyperborea* level (upper subtidal level, hereafter referred as subtidal level, between 0.75 and 1.32) (Table 1). This sampling method was applied to 12 sites along the coast of Brittany (France) in the three predefined regions mentioned in the introduction: North-East, North-West and South (Fig.1).

The difference in altitude between the levels varies between 0.7 and 1.81 m above theoretical sea level (Table 1) . To avoid sampling the same thallus twice, fronds separated by at least one meter, or found as epiphytes on different *L. hyperborea* stipes, were collected. The ploidy of each thallus was determined using flow cytometry (Guillemin et al. 2008). Two apical fragments (2-3 cm long) of each thallus were preserved in silica gel for DNA extraction.

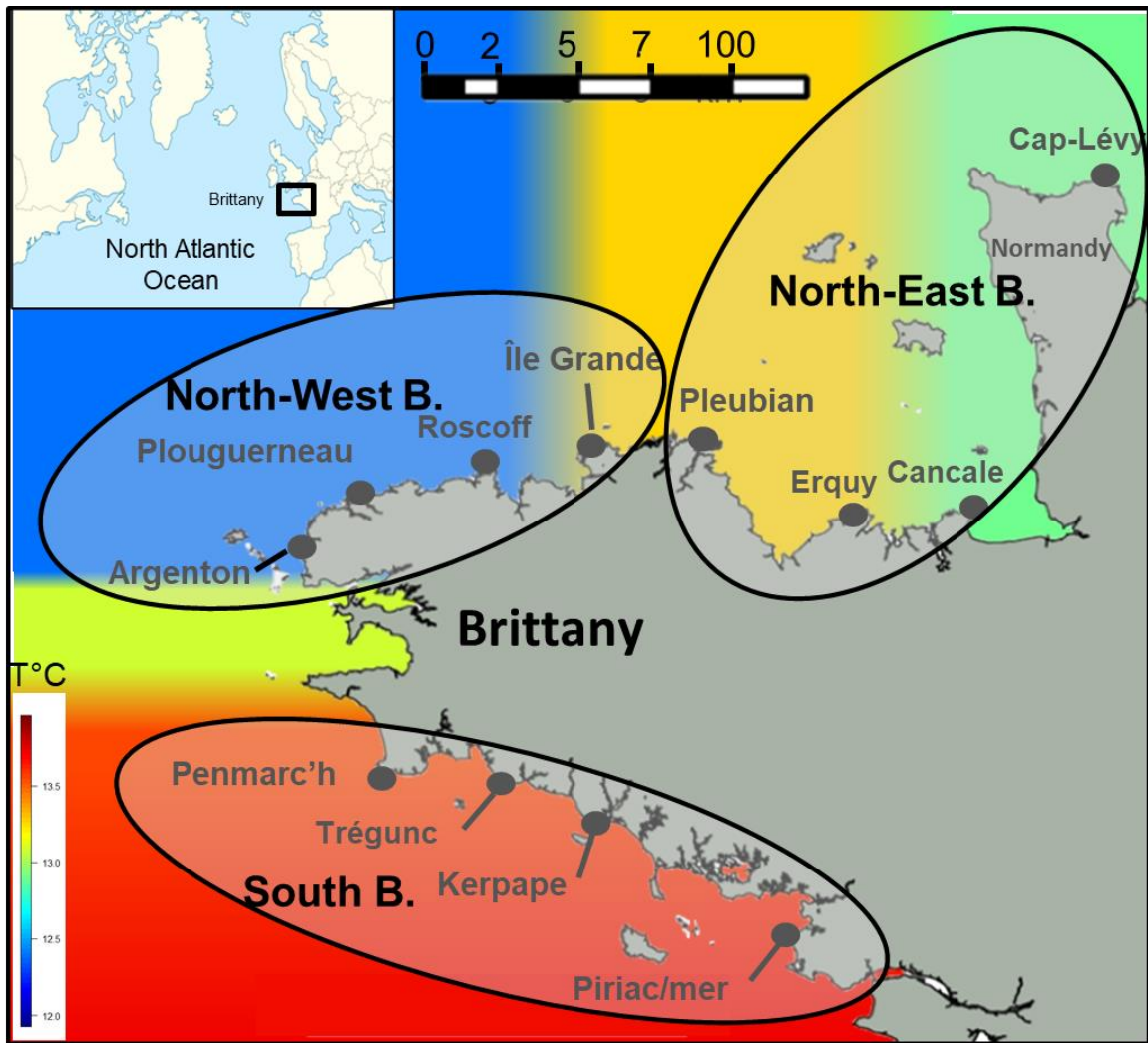


Fig. 1 : Sampling regions and sites of *P. palmata* in Brittany and thermal context. Sample sites are indicated by black dots. Predefined regions are indicated by grey ellipses. Thermal regions are indicated by colors which represent average sea surface temperature measured by satellite (Gallon et al., 2014)

DNA extraction was performed using Nucleospin® 96 plant kit (Macherey-Nagel, Duren, Germany) with a slight modification of the digestion step, reduced to 15 min to avoid extraction of PCR inhibitors. Genotyping of 507 diploid thallus using 11 microsatellite markers (Ppalm_02, Ppalm_05, Ppalm_07, Ppalm_08, Ppalm_09, Ppalm_13, Ppalm_14, Ppalm_16, Ppalm_17, Ppalm_19 and Ppalm_20, Mauger et al., 2023).

Table 1: Sampling site characteristics and genetic diversity

Site/region	Code	Lat.	Long.	Intertidal					Subtidal						
				h	Month/year	Nd	Ar (sd)	He (sd)	Pa	Δh	h	Month/year	Nd	Ar(sd)	He(sd)
Cap Lévy	CAL	49,69814444	-1,468066667	2.89	Nov.2019	25	2.85 (1.2)	0.535 (0.213)	1	--	--	19	3.04 (1.22)	0.529 (0.186)	2
Cancale	CAN	48,71291667	-1,843211111	3.13	Nov.2019	22	2.8 (1.06)	0.502 (0.174)	0	1.81	1.32	25	2.96 (1.22)	0.521 (0.174)	2
Erquy (§)	ERQ	48,65633611	-2,428652778	2.85	Mar.2020	28	3.05 (1.52)	0.51 (0.239)	4	1.58	1.27	19	3.33 (1.37)	0.538 (0.204)	10
Pleubian (§)	PLE	48,87329444	-3,121002778	1.7	Nov.2019	28	2.96 (1.19)	0.48 (0.216)	3	0.76	0.94	15	3.15 (1.38)	0.482 (0.267)	3
North -East	NE	--	--	--	--	103	4.73 (2.67)	0.554 (0.156)	11	--	--	78	5.5 (3.2)	0.569 (0.17)	18
île Grande	GRA	48,8108	-3,575680556	2.36	Mar.2020	26	3.17 (1.13)	0.488 (0.231)	7	1.31	1.05	20	2.75 (1.07)	0.489 (0.195)	3
Roscoff (h)	ROS	48,72609722	-3,968505556	2.42	Oct.2019	27	3.12 (1.37)	0.434 (0.235)	7	1.37	1.05	20	3.2 (1.44)	0.447 (0.236)	4
Plouguerneau (h)	PLO	48,63490833	-4,561961111	2.08	Oct.2019	24	2.7 (1.25)	0.413 (0.236)	2	0.98	1.1	14	3.19 (1.79)	0.483 (0.278)	1
Argenton (h)	ARG	48,52988056	-4,766941667	1.95	Sept.2019	18	2.91 (1.32)	0.427 (0.235)	1	0.94	1.01	19	3.29 (1.6)	0.461 (0.267)	1
North-West	NW	--	--	--	--	95	5.5 (2.87)	0.463 (0.224)	21	--	--	73	5.31 (3.22)	0.498 (0.205)	10
Penmarc'h	PEN	47,99851389	-4,381813889	1.62	Nov.2019	24	3.08 (1.7)	0.433 (0.253)	4	0.63	0.99	28	3.08 (1.48)	0.447 (0.21)	3
Trégunc	TRE	47,832725	-3,895422222	1.60	Oct.2020	12	3.14 (1.67)	0.528 (0.239)	1	0.70	0.9	19	3.43 (1.83)	0.501 (0.25)	5
Kerpape	KER	47,69955556	-3,415944444	--	Fev.2022	15	2.99 (0.98)	0.452 (0.203)	2	--	--	19	3.14 (1.4)	0.46 (0.253)	7
South	S	--	--	--	--	51	5.75 (3.46)	0.536 (0.21)	10	--	--	66	6.31 (3.87)	0.531 (0.222)	16
Total		--	--	--	--	249	8.72 (5.38)	0.544 (0.171)	11	--	--	217	9.35 (6.75)	0.569(0.177)	17

§: Sites where individual presented a specific morphology close to *P. palmata var sarniensis* only at intertidal level (ref) ; α Harvested Sites , only at intertidal level; h: Altitude of the site in meters; Δh delta of altitudes between the two tidal levels in meters ; Δh :altitude differences between intertidal and subtidal level within site Nd: number on genotyped Sporophytes Ar: allelic richness estimated over the lowest effectif numbers (n=12 for sites and n=51 for region); He: expected heterozygosity, sd: standard deviation; Pa number of Private Allele.

Labeled PCR products were diluted 1:10 with water. Next, 2 μL of the diluted PCR product pool was added to 10 μL of loading buffer made up of 0.5 μL of the SM594 size standard (Mauger et al., 2012) and 9.5 μL of Hi-Di formamide, denatured at 95°C for 3 min, and run in an ABI 3130 XL capillary sequencer (Applied Biosystems, USA). Genotypes were scored manually in Genemapper version 4.0 (Applied Biosystems).

Genetic analysis

Due to the low number of gametophytes detected in some populations, genetic analyses were carried out only on diploid thallus, i.e. a total of 466 thallus. The site of Piriac was not included in genetic analyses due to the high number of missing data. We first tested for genetic differentiation between tidal levels using analysis of molecular variance (AMOVA) with 10000 permutations (Arlequin v3.5.2.2). As significant genetic differentiations between tidal levels were observed (Table 3.a), analyses were performed separately for each level (249 thallus for the intertidal and 217 for the subtidal).

Genetic diversity index such as mean unbiased heterozygosity (H_e) and mean allelic richness (A_r) over the 11 locus was calculated using Hierfstats R package (v 0.5-11, Goudet et al., 2022) for tidal level and within site and region at both tidal levels. In order to estimate allelic richness, we used the rarefaction methods from El Mousadik et al. 1996 with $n=12$ individuals for sites, $n= 51$ for regions and $n=217$ for tidal levels. As genetic diversity index did not follow normal distribution, the effect of region and site was assessed with a non-parametric test PERMANOVA using adonis function of Vegan R package (2.6-4, Oksanen et al., 2022).

To complete the first AMOVA analysis done between tide level, the genetic differentiation between pair of tidal levels in each sites (F_{st}). In order to assess if this genetic differentiation was significant we estimated the mean F_{st} over all pair of tidal level within sites according to Krueger Hadfield et al. 2011. Significance of overall mean F_{st} between tidal level within site was obtained by combining probability of pairwise F_{st} using Fisher formula $-2\sum \ln(p)$ (Fisher, 1925). We then estimated F_{st} between a pair of sites respectively between intertidal sites and between subtidal sites. AMOVA on 10 000 permutations was done in order to assess genetic variation between region ($n=3$) and between sites ($n=11$) within both studied tidal levels using Arlequin v3.5.2.2 (Excoffier & Lischer, 2010) .

Pattern of genetic structure was investigated using discriminant analysis of principal component (Dapc), optimal numbers k of genetic cluster and probability of assignment to a priori defined site was tested using Akaike information criterion (AIC, Akaike, 1973)) and snapclust function of adegenet R package (v2.1.10; Jombart,2008). We also used STRUCTURE software v2.3.4 (Hubisz et al., 2009) with 50 000 burning period and 10x6 MCMC on 10 runs for each K tested. Best numbers of k clusters for STRUCTURE runs were found using the Evanno methods with the STRUCTURE HARVESTER online (Earl

& von Holdt 2011). For each K chosen, we used CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007) to aggregate the 10 runs. Pophelper v2.3.1 (Francis, 2017) R package was used for rendering the graph.

To study the relation between geographic distance and genetic distance and how it was affected by tide level, isolation by distance (IBD) was tested between sites separately for each tide level using a Mantel test between pairwise genetic distance matrix and pairwise geographic distance matrix. The Pearson correlation value (z) and the probabilities of this correlation to be significant was estimated over 10000 permutations. Geographic distances were estimated using Google Earth for measuring the

Results

Genetic diversity

Permanova analysis did not reveal any significant pattern of genetic diversity. Overall loci, no significant differences in expected heterozygosity and Allelic richness were found between tidal levels, sites or regions. However, it is interesting to note that intertidal populations hold lowest genetic diversity ($A_r = 8.72$; $H_e = 0.544$) than subtidal ones ($A_r = 9.35$; $H_e = 0.569$). Populations from the North West Brittany region were less genetically diversified than the populations from the other two regions. In intertidal populations as a whole, genetic diversity was highest in Trégunc ($A_r = 3.14$; $H_e = 0.528$) and lowest in Plouguerneau ($A_r = 2.7$; $H_e = 0.413$), while at subtidal level it was highest in Erquy ($A_r = 3.33$; $H_e = 0.538$) and lowest in Roscoff ($H_e = 0.447$).

However, differences in the number of private alleles have been observed between tidal levels and between regions. Overall, the number of private alleles were higher in the subtidal ($PA = 17$) than in the intertidal ($PA = 11$), but these results differed between regions. For the intertidal, the number of private alleles was higher in north-west Brittany ($PA = 21$) than in the north-east ($PA = 11$) and the south ($PA = 10$). On the other hand, for the subtidal, the number of private alleles was lower in north-west Brittany ($PA = 10$) than in the north-east ($PA = 18$) and the south ($PA = 16$). (Table 1)

Genetic differentiation and structure along the tidal gradient and across Brittany seascape.

Mean genetic differentiation between pairs of populations was significant (mean pairwise $F_{st} = 0.045$ Fisher test $p < 0.01$). Genetic differentiation between intertidal and subtidal populations was significant (AMOVA, mean $F_{st} = 0.005$ (table 3.a)), with values of F_{st} ranging from 0.022 (Plouguerneau) to 0.109 (Île Grande). Only 3 sites out of 11 showed no difference according to depth (Cap Lévy, Cancale and Penmarc'h (Table 2.a).

Table 2: Genetic differentiation between pair of sites (pairwise Fst). (a) Between Tidal level within sites (b) Between intertidal sites and (c) between subtidal sites.

(a)	North East B.				North West B.				South B.		
	Cap Lévy	Cancale	Erquy	Pleubian	Île Grande	Roscoff	Plouguerneau	Argenton	Penmarc'h	Trégunc	Kerpape
Fst int/sub	<i>0.004</i>	<i>0.003</i>	0.075	0.056	0.135	0.046	0.022	0.036	-0.004	0.070	0.055
P-values	0.281	0.197	<0.001	<0.001	<0.001	<0.001	0.041	0.005	0.846	0.001	0.004
(b)	North East B.				North West B.				South B.		
	Cap Lévy	Cancale	Erquy	Pleubian	Île Grande	Roscoff	Plouguerneau	Argenton	Penmarc'h	Trégunc	Kerpape
Cap Lévy		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cancale	0.149		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Erquy	0.151	0.075		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pleubian	0.147	0.098	0.043		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Île Grande	0.138	0.163	0.104	0.073		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Roscoff	0.140	0.121	0.048	0.039	0.054		<0.001	<0.001	<0.001	<0.001	<0.001
Plouguerneau	0.138	0.152	0.073	0.065	0.104	0.017		<0.001	<0.001	<0.001	<0.001
Argenton	0.091	0.147	0.116	0.116	0.095	0.058	0.083		<0.001	<0.001	<0.001
Penmarc'h	0.261	0.091	0.092	0.133	0.172	0.134	0.172	0.191		<0.001	<0.001
Trégunc	0.275	0.204	0.158	0.230	0.227	0.213	0.267	0.228	0.184		<0.001
Kerpape	0.268	0.235	0.140	0.144	0.189	0.187	0.227	0.209	0.181	0.230	
(c)	North East B.				North West B.				South B.		
	Cap Lévy	Cancale	Erquy	Pleubian	Île Grande	Roscoff	Plouguerneau	Argenton	Penmarc'h	Trégunc	Kerpape
Cap Lévy		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cancale	0.101		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Erquy	0.088	0.108		0.169	<0.001	0.012	0.206	0.008	<0.001	<0.001	<0.001
Pleubian	0.072	0.129	0.022		<0.001	0.387	0.025	0.223	<0.001	<0.001	<0.001
Île Grande	0.124	0.105	0.077	0.115		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Roscoff	0.108	0.155	0.037	0.005	0.152		0.070	0.290	<0.001	<0.001	<0.001
Plouguerneau	0.089	0.122	0.020	0.032	0.129	0.019		0.817	<0.001	<0.001	<0.001
Argenton	0.091	0.126	0.038	0.010	0.150	0.006	-0.008		<0.001	<0.001	<0.001
Penmarc'h	0.218	0.077	0.169	0.176	0.192	0.199	0.178	0.175		<0.001	<0.001
Trégunc	0.238	0.172	0.202	0.190	0.288	0.216	0.205	0.188	0.120		<0.001
Kerpape	0.213	0.161	0.162	0.158	0.188	0.206	0.211	0.207	0.126	0.198	

Non significant values ($p > 0.05$) are indicated in italics. Fst values are scaled by color from low (blue) to high (orange). Framed Fst values correspond to predefined regions. Upper part of the matrix shows p-values of the pairwise Fst.

Comparison between sites of populations located at the same intertidal level showed significant mean pairwise Fst values ($F_{st} = 0.146$, $p < 0.001$) (Table 2.b) ranging from 0.017 (Roscoff/Plouguerneau) to 0.275 (Trégunc/Cap Lévy). For the subtidal level, the mean pairwise Fst between sites was 0.132 and ranged from 0.032 (Pleubian/Plouguerneau) to 0.288 (Trégunc/Île Grande) (Table 3.c). Seven values of

Fst per pair were not significant ($p>0.05$), indicating that there is no genetic differentiation between these pairs of sites. Whatever the bathymetric level studied, the highest Fst values were observed between sites geographically distant between the north and south of Brittany.

AMOVA analyses revealed significant genetic differentiation between individuals within sites, between sites within regions and between regions at both intertidal and subtidal levels (Table 3.b and 3.c.).

Table 3: Results of the AMOVA (Analysis of MOlecular Variances) testing the effect of the factor (a) tidal level (b) Region and sites at the intertidal level and (c) at the subtidal level. df, degree of freedom.

Source of variation	df	Sum of squares	Variance components	Percentage of variation (%)	F-statistic	P-value
(a) Between tidal level						
Among tidal level	1	9.946	0.01441	0.53	FST : 0.005	<0.001
Among individuals within tidal level	464	1512.753	0.55072	20.22	FIS : 0.203	<0.001
Within individuals	466	1006	2.1588	79.25	FIT : 0.207	<0.001
Total	931	2528.698	2.72393			
(b) Regions and sites at the intertidal level						
Among regions	2	65.795	0.1083	3.91	FCT : 0.039	0.001
Among sites within region	8	124.985	0.29015	10.49	FSC : 0.109	<0.001
Among individuals within site	238	607.051	0.18194	6.58	FIS : 0.077	<0.001
Within individuals	249	544.5	2.18675	79.03	FIT : 0.210	<0.001
Total	497	1342.331	2.76714			
(c) Regions and sites at the subtidal level						
Among regions	2	61.353	0.13051	5.4	FCT : 0.054	0.009
Among sites within region	8	90.518	0.23169	9.59	FSC : 0.101	<0.001
Among individuals within site	206	476.088	0.25694	10.63	FIS : 0.125	<0.001
Within individuals	217	390	1.79724	74.38	FIT : 0.256	<0.001
Total	433	1017.959	2.41638			

The region factor explained 3.91% of the genetic variation in the intertidal area and 5.4% in the subtidal area, while the sites explained 10.49% of the genetic variation in the intertidal area and 9.59% in the subtidal zone.

Clustering by DAPC was highly congruent with the patterns of pairwise genetic differentiation amongst the sampling sites.

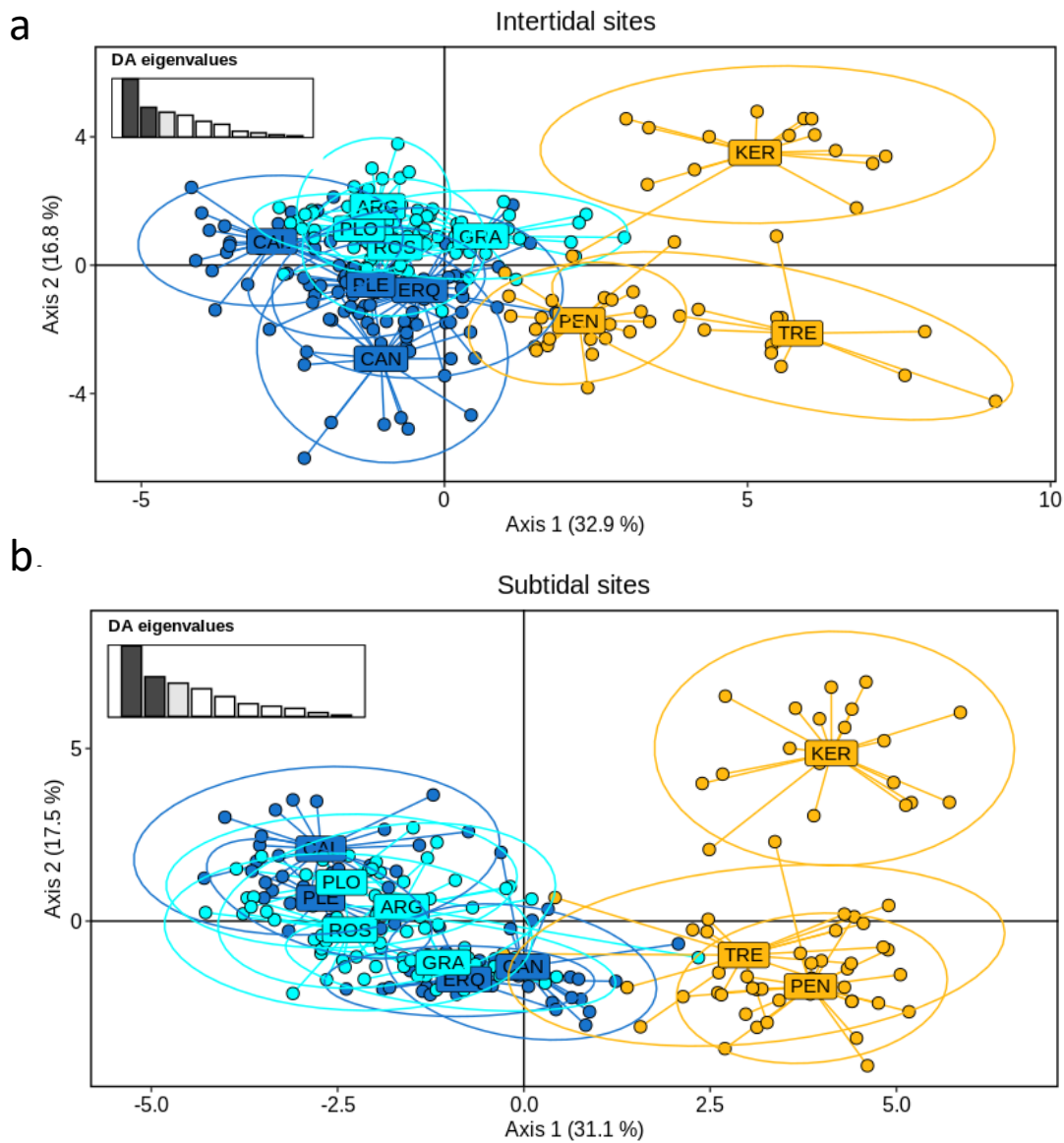


Figure 2 : Scatterplot of the first two components (Axes 1 and 2) of the discriminant analysis of principal components (DAPC) performed on (a) 249 thallus among intertidal sites and on (b) 217 thallus among subtidal levels with 11 microsatellite loci using sampling location as prior clusters. Sampling regions are shown by different colours (Blue for North east, Cyan for North west and yellow for South). Sites are indicated by the first three letters of the site names in the centroid of the inertia ellipse. Each point corresponds to a single individual.

The two types of populations, intertidal and subtidal, showed very similar DAPC grouping patterns, although with a clearer subdivision for the intertidal level. In accordance with the first axis, the populations followed a more or less marked latitudinal gradient: the most southerly and most northerly populations were grouped together. For the intertidal populations, the two first axes of the DAPC explained more than 49% of the variability. The first axis separated populations from the north and the south. The southern sites of Kerpape and Trégunc stand out from the Penmarc'h site which was closer to the northern sites (Fig. 2.a). The second axis separated the populations from the north-western to the north-eastern.

For the subtidal populations, the two first axes of the DAPC explained up to 48% of the genetic variation, and separated the two distinct northern and southern clusters. However, differentiation between northern sites and between southern sites was less marked than in intertidal (Fig. 2.b).

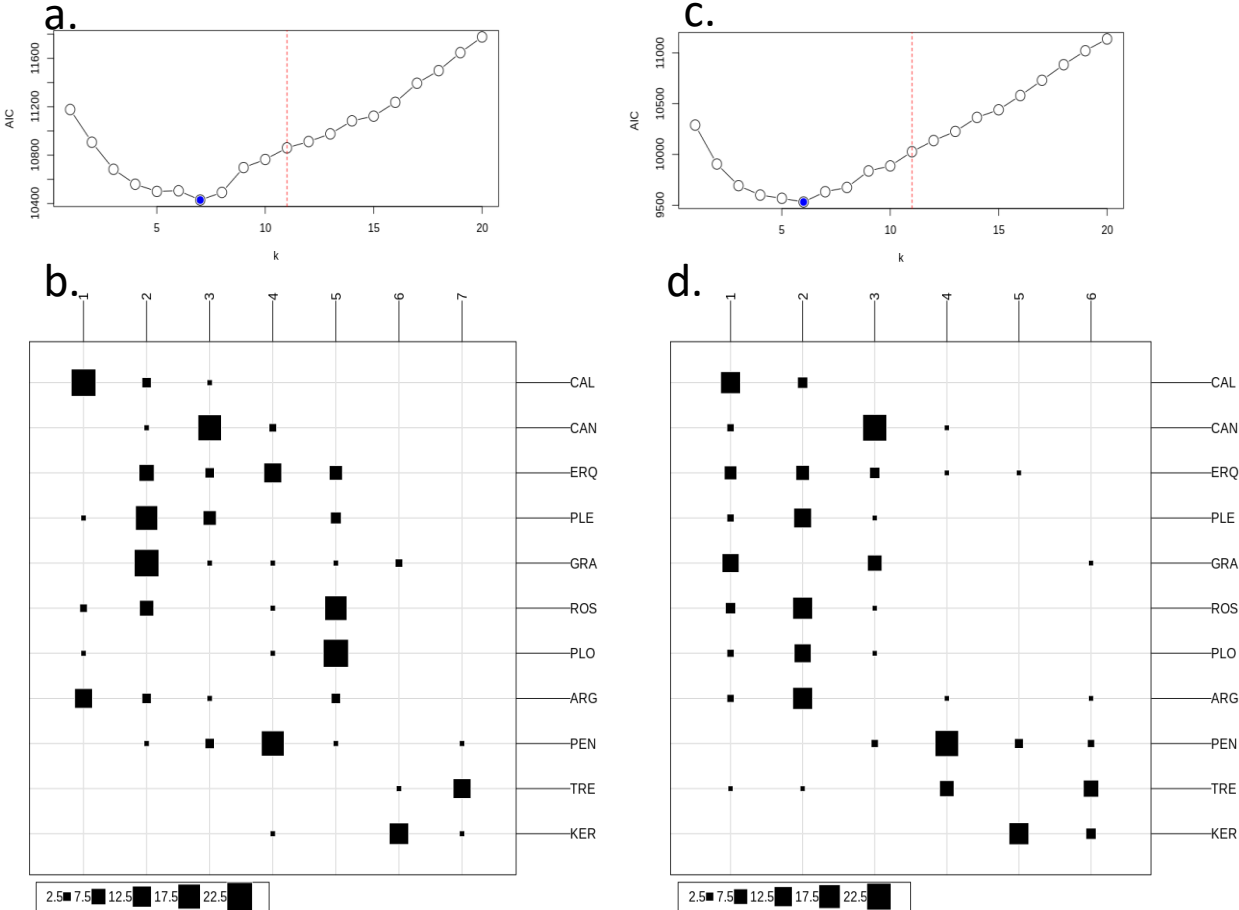


Figure 3: Estimation of Optimal k clusters in (a) intertidal sites and (b) subtidal sites using Akaike information criterion. Group membership probabilities to cluster are inferred depending on sampling sites.

The most parsimonious number of snapclust clusters according to the AIC criterion was K=7 for the intertidal level and K=6 for the subtidal level. (Fig. 3 a and c). The seven genetic clusters defined for the intertidal level grouped the sites according to their geography: a southern Brittany cluster composed of only 3 populations clearly separated (KER, TRE, PEN); a north-western Brittany cluster (GRA, ROS, PLO, ARG); and a cluster grouping localities from the eastern section (PLE to CAL)

The probability of assignment to estimated cluster and a priori defined population showed that the northern intertidal site was spread among 5 genetic clusters, while northern subtidal sites were spread among only 3 genetic clusters (Fig. 3 c and d). Similar results were obtained with STRUCTURE, estimation of optimal k numbers of genetic clusters with Evanno et al methods gave K=2, 4 and 6 for intertidal sites and K=2 and 3 for subtidal sites (see Supplementary Information Fig. S1).

Isolation by distance

Mantel test for IBD indicated a significant correlation between genetic distance and geographic distance at both intertidal and subtidal level. However, correlation value was higher in intertidal ($z = 0.475$) (Fig 4.a) than in subtidal ($z=0.385$) (Fig 4.b).

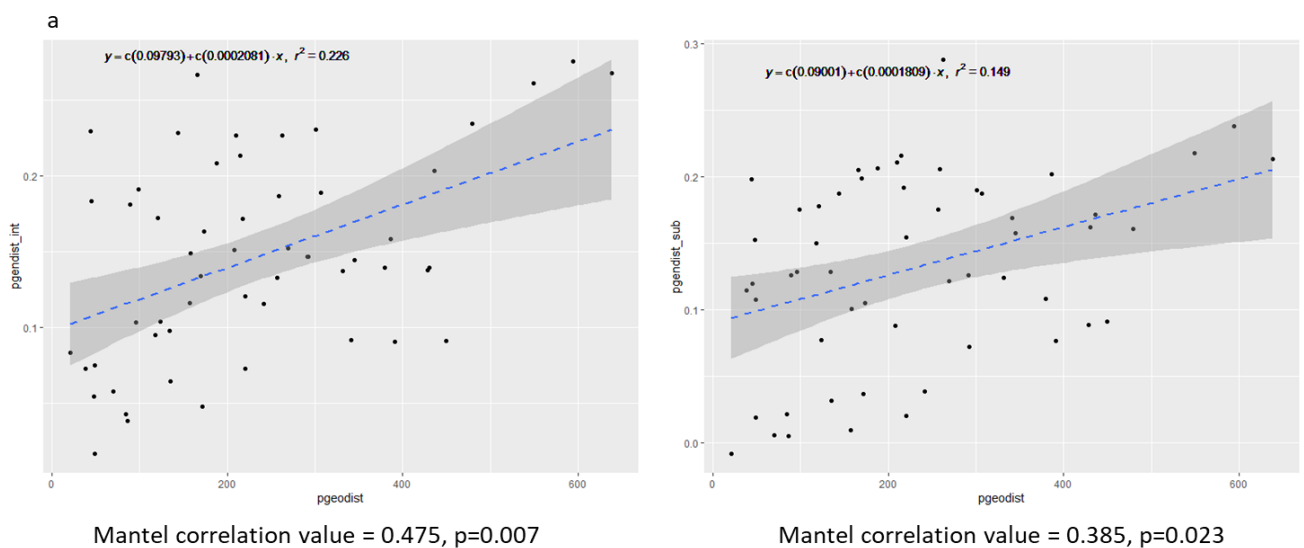


Figure 4 : Correlation between genetic and geographic distance at studied tide levels and results of the Mantel test for (a) intertidal sites and (b) subtidal sites.

Discussion

As the environment rapidly changes under anthropogenic-driven climate change, understanding population connectivity is a priority for marine conservation and resource management. Therefore, studying genetic structure of *Palmaria palmata* at different scales of the intertidal seascape provides a better understanding of the complexity of connectivity patterns for this species also submitted to high commercial harvesting pressure along the coasts of Brittany (France). Regardless of the region studied, our results show genetic differentiation within sites between intertidal and subtidal populations separated by a few tens of meters. In addition, large-scale comparisons between populations along the coast of Brittany show greater genetic divergence with increasing geographical distance. A clear discontinuity was found between southern and northern Brittany, but also, to a lesser extent, between northwestern and northeastern Brittany. Our study confirms the importance of the dominant ocean currents to explain the pattern of gene flow for this species and shows that intertidal populations found themselves more genetically isolated from each other than subtidal ones in this complex seascape.

Genetic connectivity of *P. palmata* populations along the Brittany coast

In the marine environment, most genetic studies have focused on the influence of physical factors such as habitat discontinuities, currents and geographical barriers on the population structure of intertidal invertebrates. It was demonstrated that species with no pelagic larval dispersal tend to be highly genetically structured suggesting very low gene flow between populations (Palumbi et al. 1994) while longer pelagic development should result in greater dispersal and better-connected populations (Bohonak 1999, Burton 1983). Moreover, several studies of marine invertebrate species have reported a decrease in genetic structure with increasing tidal height (Kelly and Palumbi 2010). In this study, we demonstrated that the populations along the Brittany coasts belong to 3 major genetic groups: the North-East, North-West and South regions. Our results demonstrated a clear separation between North-West and South regions resulting from the low level of contemporary gene flow. The North-West group was composed of the 4 populations (Ile Grande, Roscoff, Plouguerneau, Argenton) and the Southern group was composed of the 3 populations (Penmarc'h, Tregunc and Kerpape) located at the southern edge of our sampling range. Interestingly, this separation between the North-West and the Southern groups has been also found in two kelp species, *Laminaria digitata* and *L. hyperborea* (Couceiro et al. 2013, Robuchon et al. 2014, Neiva et al. 2020) and in *Fucus serratus*, *Fucus ceranoides* and *Ascophyllum nodosum* (Hoarau et al., 2007; Olsen et al., 2010, Neiva et al., 2012). In particular, it is interesting to note that since *P. palmata* is epiphytic on the stipes of *L. hyperborea* in the subtidal level and in *F. serratus* in the intertidal environment, we can assume that these two couple of species

share a long common history. These previous findings, like the results of the present study, are consistent with the existence of a refugium in the English Channel (Provan et al. 2005), however, the possibility of a second refuge in the Bay of Biscay cannot be excluded. More recent phylogeography studies on Atlantic populations of *P. palmata* using a few mitochondrial and nuclear gene markers by Li et al. (2015) also suggested significant genetic differentiation in *P. palmata* both at a trans-Atlantic continental scale and at a European basin scale, and that multiple glacial refugia may have existed for this species. This research revealed very limited genetic exchange between populations after their separation. Apart from a few migrants (0.394) from Bergen (Norway) to the English Channel, no gene flow was detected among pairwise comparisons that could be related to the weak dispersal ability of *P. palmata*. Ecological studies have shown that members of the genus *Palmaria* have a single spore-releasing phase (the diploid sporophyte), and the haploid spores settle quickly (< 12 h). The female crusts are overgrown by the diploid sporophyte after fertilization, with no carpospores formed for release, which would minimize the chance of successful recruitment and persistence (van der Meer & Bird, 1985). In addition, these authors suggested that wave action, competition and grazing may contribute to the low rates of survival of new migrants.

The fact that this discontinuity is observed in different species of algae with different life histories suggest that connectivity between populations from the south and north of Brittany is mainly determined by the hydrodynamic characteristics of the region, constituting a marine barrier to dispersal (Ayata et al. 2010). This has also been reported for other invertebrate species, the worm *Pectinaria koreni* (Jolly et al. 2005) and the great scallop *Pecten maximus* (Morvezen et al., 2016) and the fish *Pollachius pollachius* (Charrier et al. 2006). In our study, a slight variation in the delimitation between these two main genetic groups is observed for the Penmarc'h location, which is identified here as a transition zone. This is confirmed by the DAPC and STRUCTURE analysis, where Penmarc'h is in either the North-West or South group depending on the depth of the population. This result suggests that this oceanographic barrier is permeable, depending on tidal currents and seasonal variations (Ayata et al. 2010). Southern populations of Tregunc and Kerpape remain strongly isolated from each other probably because of the discontinuity of the rocky substratum (Billot et al. 2003; Valero et al. 2011; Couceiro et al. 2013). Such effect of habitat discontinuity has been demonstrated in kelp (Alberto et al. 2010; Fraser et al. 2010) and in some red macroalgae (*Mazzaella laminarioides* and *Gigartina skottsbergii*) (Faugeron et al. 2001, 2004). To a lesser extent, the north-west and north-east of Brittany form two groups. In fact, the sites in the north-east, Cap Lévy, Cancale and Ile Grande form a genetic group. The geographical distance and the configuration of the residual currents of the gyres described in the Bay of Saint Malo (Garreau, 1993) seem to explain the isolation of Cap Lévy and

Cancale. On the other hand, it is difficult to understand why Ile Grande stands out as a genetically isolated site.

Impact of tide level and seascape on genetic structure

For the intertidal and subtidal levels, the region factor explains 3.91% and 5.4% of the genetic variation respectively (AMOVA). Analysis of F_{st} values between populations confirms that genetic structuring is slightly higher in the intertidal than in the subtidal (cf. DAPC and STRUCTURE analysis), suggesting that gene flow is greater at the subtidal level than at the intertidal level. Considering the overall F_{st} values between sites in the three regions studied, as well as the assignment probabilities (Figure 4), the north-western sites of Roscoff, Plouguerneau and Argenton appear to stand out as a homogeneous genetic region. The lower F_{st} (<0.09) at intertidal level and the non-significant F_{st} at subtidal level observed between these three sites support the hypothesis of denser populations exposed to stronger hydrodynamics, favoring dispersion between sites in this region and resulting in regional genetic homogeneity. It can be seen that the F_{st} values between the intertidal sites are similar to the F_{st} values between the tidal levels within these sites. Stronger pattern of isolation by distance between intertidal sites was detected (mantel test). Cap Lévy, Cancale and Ile Grande stand out as genetically isolated sites. The geographical distance along with residual currents pattern of gyres described in Saint Malo Bay explain why Cap Lévy and Cancale are isolated. However, it is difficult to understand why Île Grande, in addition to presenting a significant inbreeding signal, stands out as a genetically isolated site. Previous studies performed on more than 15 species of kelp (Valero et al. 2011) demonstrated that the intertidal species were generally more structured than subtidal ones. This pattern can be explained by the fact that intertidal species, which are only submerged for part of the day, may have fewer opportunities to spread their propagules in the water column (Kelly and Palumbi 2010). Rhodophyta species are generally considered as poor dispersers, since spores and male gametes (female gametes are not dispersed and remain on the female) are short-lived (Destombe et al. 1992; Engel & Destombe 2002). Recent DNA metabarcoding studies carried out on more than 500 marine species from kelp forests have revealed that red algae is one of the groups with the finest scale genetic structure (1-3 km) (Shum and Palumbi, 2020). In the intertidal zone, it has been documented that two species of red algae typically found in this zone (*Gracilaria gracilis* and *Chondrus crispus*) display reduced gene flow between high shore and low shore populations (Engel et al. 2004 and Krueger-Hadfield et al. 2013). These authors suggest that tidal emersion and immersion cycle, probably drives gene flow between populations since low-shore pools are more often submerged than emerged. In our study, the genetic differentiation observed between intertidal and subtidal populations of *P. palmata* at most sites suggests that intertidal populations are more isolated than subtidal populations.

Pairwise F_{st} values between populations located at the same tidal level are slightly lower in the subtidal than in the intertidal (with the exception of Ile Grande). These results suggest that submerged populations receive more spores and gametes from other sites than populations subject to exondation. This result highlights the importance of immersion time on dispersal reinforcing the results obtained by Engel et al. 2004 in *G. gracilis*. The fact that the values of genetic differentiation between populations from the same site located at different heights and separated by a few tens of meters are higher in *P. palmata* (F_{st} from 0.024 to 0.109) than in *C. crispus* (from 0.025 to 0.030) (Krueger-Hadfield et al. 2013) and *G. gracilis* (from 0.002 to 0.012) (Engel et al. 2004) suggests that *P. palmata* has the lowest dispersal capacity, probably linked to its unique life cycle showing only one type of spore (Van der Meer & Todd 1980).

Furthermore, the contrasting environmental conditions driven by differences in emersion time could lead to local adaptation of the populations. Indeed, in *P. palmata*, which is distributed over strong environmental gradients at relatively small spatial scales, the increase in tidal height leads to an increase in stress factors such as desiccation, wave energy and temperature (Menge and Branch, 2001). As a result of this adaptation, gene flow between these ecologically divergent habitats could be reduced, leading to less successful establishment of immigrants from different environments (Nosil et al 2005). Further investigations is needed, to uncover how selective pressure along the tidal gradient impacts gene flow.

Applications in harvest management and domestication:

The study of genetic structure and diversity on a small or regional scale is necessary to refine management methods adapted to the exploitation of natural populations of economically important algae. In Brittany, *Palmaria palmata* is harvested for human consumption and most of the harvests come from the intertidal populations of western Brittany. The populations of *P. palmata* that we studied along the Brittany coast show, on the whole, great genetic diversity (He and allelic richness), which could guarantee a high evolutionary potential for recovery despite exploitation. However, in the most heavily harvested intertidal populations (Argenton, Plouguerneau and Penmarc'h) we detected, even if the diversity values remain relatively high, one of the lowest genetic diversities (Ar and He) compared with the subtidal populations and other sites. A certain harvesting effect cannot be overlooked, but it should be stressed that actual harvest management policy, such as closing harvest during reproduction seasons and avoiding “uprooting” whole thallus, are for now efficient. However, a recent report shows important inter-annual variations of the collected biomass had been observed despite the increase in harvest effort over last years (CRPMEM, 2020). Thus, with the increasing frequency of extreme weather events, could threaten the wild intertidal populations in the near future

and even triggering local seasonal extinction events as it was observed in the north of Spain (Diaz-Caneja, pers. Com.) and in the south of Brittany (on field personal observations). On top of that, the genetic isolation of intertidal populations observed in North-West, should raise awareness over careful monitoring of those economically essential populations. The genetic markers developed herein will help to monitor wild populations for a durable exploitation by allowing to assess the impact of climate change and harvest on genetic diversity and structure over years. Further investigation with adapted sampling methods should be conducted along with structure in charge of *P. palmata* stock management for the better good of the people living on these natural resources.

This work is also a step forward in the domestication process of this valuable seaweed. The results presented here underline the need to select local strains. Indeed, considering the high level of genetic structure and the environmental heterogeneity, processes of local adaptation could occur within genetic clusters.

Conclusion

Our results demonstrated that dispersal was limited in *P. palmata*, leading to sub-structuring populations with a notable genetic differentiation over a few tens of meters. The within-sites genetic differentiation observed along tidal gradients in most of the sites raise interesting hypotheses on how reproductive isolation and differences in selective pressure may impact gene flow between tidal levels. Overall, intertidal populations found themselves more genetically isolated from each other than subtidal ones. This is a consequence of low dispersal abilities and daily isolation from currents due to tide cycle. Strong pattern of genetic structure occurs at both tidal levels between predefined regions, indicating that hydrodynamics strongly influences the genetic structure.

Acknowledgments

This work benefited from the support of the European Maritime and Fisheries Fund (EMFF) through the POLISTR project and Région Bretagne (PhD grant ARED Domdulse from ED227 Sorbonne-Université/MNHN to AB). We are grateful to the Roscoff Genomer platform for its technical support and to the EMBRC France partner core facility for its technical support, especially Gaetan Schires and Sébastien Henry. The authors are also deeply indebted to the Service Mer & Observation (SMO) of Roscoff.

AUTHOR CONTRIBUTIONS

Conceptualization, M.V., C.D and P.P.; methodology, A.B. and S.M.; data analysis A.B, S.M., C.D. and M.V.; resources, A.B, D.P., C.D. and M.V.; data curation, A.B., S.M., C.D. and M.V.; writing—original draft preparation, A.B., P.P., C.D. and M.V.; writing—review and editing, A.B., P.P., C.D. and M.V.; supervision, P.P., C.D. and M.V.; funding acquisition, M.V. and P.P. All authors have read and agreed to the published version of the manuscript.

Supplementary information

The following supplementary material is accessible below

Supplementary fig. S1. **Barplot of assignation probabilities estimated by DPCA with sampling sites as prior.**

Availability of data and materials

Genotypic data will be deposited and made available in a Dryad Digital Repository

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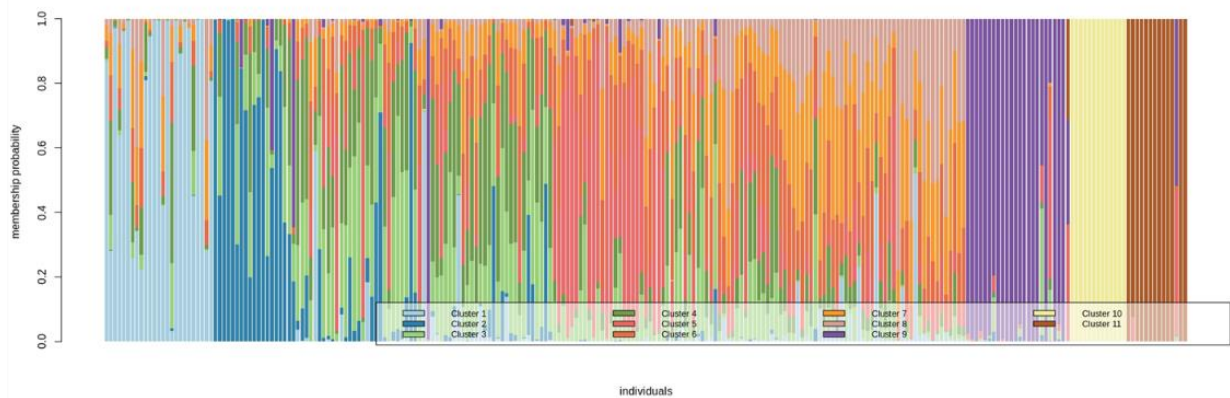
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Supplementary information:

Intertidal



Subtidal

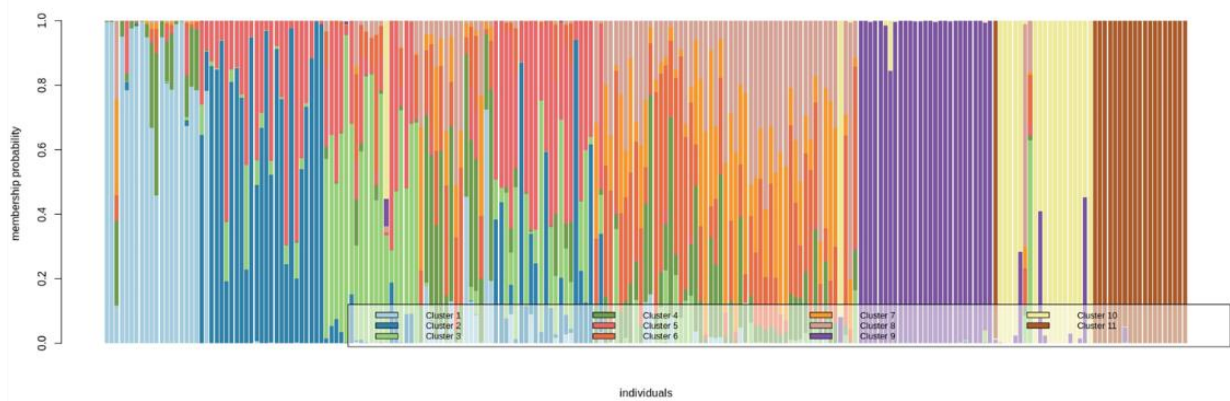


Fig. S1: Barplot of assignment probabilities estimated by DPCA with intertidal (up) and subtidal (down) sampling sites. Each individual is represented by a vertical line divided into K coloured segments, the length of which indicates the individual's membership fraction to each of K clusters. Individuals are grouped according to their sampling locality (ordered along a north to south gradient) for the regional-scale analysis.

3. Etude du processus d'adaptation locale dans les populations du nord de la Bretagne (Article n°3)

L'article 3 ci-dessous présente les travaux réalisés au cours de ma thèse pour tester une hypothèse sur un processus d'adaptation locale à la température au sein des populations de Bretagne-Nord-Est et Nord-Ouest de *P. palmata*. Ce manuscrit ne sera pas soumis en l'état pour publication, mais après des corrections de mes co-auteurs début 2024.

Article 3 : Uncovering process of local adaptation in the intertidal and subtidal populations of the red seaweed *Palmaria palmata*

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Abstract

Understanding the importance of population structuring in the landscape is necessary to predict the response of species to environmental changes. *Palmaria palmata* is a stenothermic red alga that thrives in the cold temperate to arctic waters of the North Atlantic Ocean and whose meridional limit of distribution appears to be linked to temperature. Using a common garden experiment, we tested the response of haploid and diploid thallus from different intertidal and subtidal populations of the species to temperature. The response of the thallus was estimated using the measure of photosynthetic parameters F_v/F_m . On average, no difference was detected between haploid and diploid thallus whereas the response to temperature varied between populations separated by about 100 kilometers along the latitudinal gradient. The performance of the populations was then influenced by the geographic origin of the population. The results suggest a pattern of local adaptation corresponding to a temperature-dependent adaptation of the populations. Our study confirms the

importance of taking local population structure into account when predicting responses to global environmental change. Moreover, these results raise interesting hypotheses concerning the resilience of this species to climate change and its use in aquaculture.

Introduction

Local adaptation in seaweed

Local adaptation, which occurs when environmental heterogeneity is present in a species' range, results from the complex interaction of evolutionary forces such as genetic drift, migration and selection (Blanquart et al., 2013; Kawecki & Ebert, 2004). This key process generates intraspecific biodiversity potentially at the origin of speciation phenomena (Kirkpatrick & Barton, 1997; Levene, 1953). Seaweeds make an essential contribution to coastal ecosystems as primary producers and habitat-forming species (Pfister et al., 2019). They also support local economic activities either directly (harvesting) or indirectly (nurseries) (Lefcheck et al., 2019). Their ability to adapt rapidly to environmental change and extreme events such as heat waves will have a major impact on ecosystems and the economic activities that depend on them (Coleman et al., 2020).

Understanding local adaptation is essential for predicting the resilience of populations to climate change or for selecting strains that are better adapted to aquaculture conditions. Moreover, information on local adaptation and genetic resource management is important for defining source populations in restoration projects to ensure the long-term success of restocking actions (McKay et al., 2005). Seaweed distribution patterns are shaped by vicariant events, dispersal and climatic limits (Breeman & Pakker, 1994). Temperature is the main factor limiting the range of distribution of seaweed (Assis et al., 2018; Jueterbock et al., 2018; Martínez et al., 2018). Reproduction and growth are critically dependent on temperature (Breeman, 1990), which will have an impact on genetic diversity and population adaptability (Coleman et al., 2020; Gurgel et al., 2020). A recent study carried out on the kelp *Macrocystis pyrifera*, which lives in the subtidal area, revealed evidence of local adaptation to temperature in the early stages of sporophyte development (Solas et al., 2023). These results are consistent with the spatial genetic structure of the range of this giant seaweed in South America (Camus et al., 2018; MacAya & Zuccarello, 2010). Among seaweed-dominated habitats, the intertidal rocky shores are subject to regular exposure to atmospheric conditions and are therefore exposed to numerous extreme climatic events such as heatwave (Helmuth et al., 2011). This habitat is characterized by contrasting short- and large-scale environmental heterogeneity, making it more likely to host processes of local adaptation. Its strong gradients of abiotic (complex hydrodynamics, temperature, desiccation) and biotic (grazing, epiphytism) selective pressures have provided good examples of habitat-driven divergence along tidal gradients, or even along the coast. Classic examples

of such a process have been described in the gastropod *Littorina saxatilis* with the high/low shore ecotypes (Johannesson et al., 2010) and in the *Fucus* brown seaweed species complex (*F. spiralis*, *F. guiryi*, *F. vesiculosus*), where temperature, desiccation and light have been shown to be the main factors driving habitat divergence (Zardi et al., 2011). Due to its important role in growth and reproduction, temperature has been identified as one of the main drivers of seaweed species distribution (Bartsch et al., 2012; Breeman, 1988), making it an excellent factor to study local adaptation processes. With the exception of the *Fucus* genera, the process of local adaptation to temperature in intertidal seaweed remains poorly understood.

Why *Palmaria palmata* in Brittany?

Palmaria palmata is a stenothermic red alga that thrives in the cold temperate to arctic waters of the North Atlantic Ocean and whose meridional limit of distribution appears to be linked to temperature. Various experimental studies have confirmed its sensitivity to temperature. For example, *P. palmata* from New Brunswick (Canada) stopped growing above 18°C (Morgan & Simpson, 1981) and thallus from northern Portugal did not survive above 21°C when grown in outdoor tanks (Matos et al., 2006). Its sexual life cycle is haploid-diploid, with diploid tetrasporophytes and haploid gametophytes, only the male of which is macroscopic and of the same size as the tetrasporophyte. The female gametophyte is microscopic and, unlike most Florideophyceae, do not develop a cystocarp, a reproductive structure sheltering carpospore produced by clonal reproduction of the zygote after fertilization. As the male gametophyte and the tetrasporophyte have a similar morphology and live together in the same environment, we could suppose that selection acts in the same way on the haploid and diploid stages, however different studies reported different responses between ploidy levels in isomorphic red seaweeds (Destombe et al., 1993). Theoretical studies suggest that haploids may be more sensitive than diploids to certain environmental conditions (Otto & Goldstein, 1992; Valero et al., 1992). In Brittany, the ecological distribution of this species on rocky coasts extends from the middle intertidal level, where it is epilithic or epiphyte on *Fucus serratus*, to the upper subtidal level, where it is found as an epiphyte on the stipes of the kelp *Laminaria hyperborean* (Stévant et al., 2023). These two distinct habitats exhibit specific patterns of temperature variations in relation to the tidal cycle. Thallus in the intertidal zone are more frequently exposed to intense thermal stress than thallus in the upper subtidal zone, which grow in a more buffered environment.

The Brittany coast has a heterogenous mosaic of sea surface temperature (SST). A study of the SST over 20 years (1992-2012) using satellite monitoring showed significant differences in the variation and the mean annual temperature between North-West Brittany, North-East Brittany and South Brittany. North-West Brittany is characterized by less variation in temperature over the seasons than

the other two regions studied (Gallon et al., 2014). This heterogeneity in temperature along the northern coast of Brittany and along the tidal gradient could be a favorable ground for the emergence of local adaptation processes. However, sea surface temperature is mainly assessed by satellite and does not provide accurate data on intertidal temperature patterns, such as temperature variation along the tidal gradient, highlighting the need for in situ monitoring.

Palmaria palmata is an appropriate model for studying local adaptation at different geographical scales. In this study, after monitoring in situ temperature variations at intertidal and subtidal levels, we aimed to estimate the maximum thermal tolerances of the populations of Brittany for haploids and diploids. Finally, the heat stress tolerance of *P. palmata* was studied along regional gradients using common garden experiments.

Material and methods

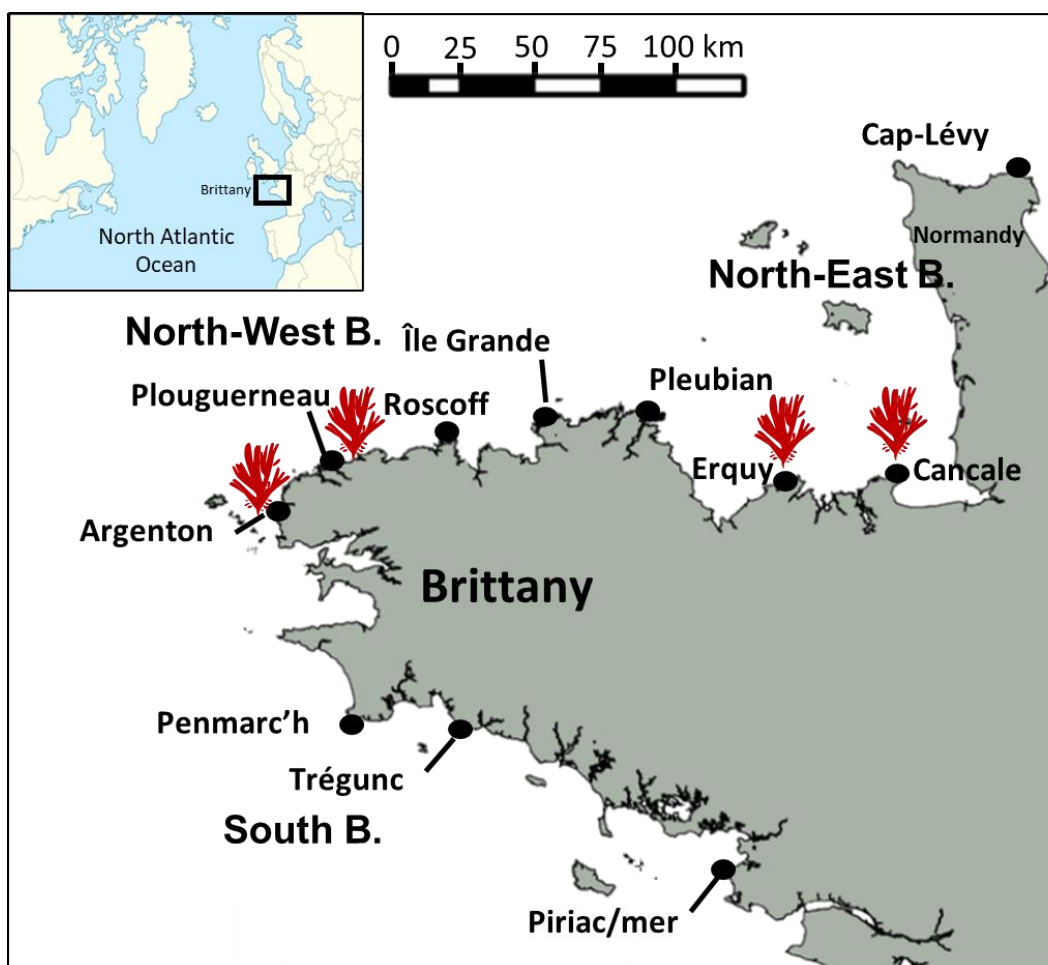


Figure 1 : In situ monitoring and sampling sites in Brittany. Monitored localities are indicated by a black dot. Sampling sites for biological material are indicated by a *Palmaria* emoji.

Table 1 : Temperature monitoring site and supplementary variable

Site	lat.	Long.	lvl	h	Code	Mean	Max	Min	Range	DTR
Cap Lévy*	49.698207	-1.468163	Intertidal	2.89	CAL_int	12.65	23.99	0.72	23.26	12.31
Cancale	48.71291667	-1.843211111	Intertidal	3.13	CAN_int	13.91	25.87	-1.02	26.88	16.23
			Subtidal	1.32	CAN_sub	13.93	23.33	06.03	17.30	15.30
Erquy	48.65633611	-2.428652778	Intertidal	2.85	ERQ_int	13.80	30.77	0.08	30.69	14.37
			Subtidal	1.27	ERQ_sub	13.81	32.12	6.66	25.46	13.33
Pleubian	48.87329444	-3.121002778	Intertidal	1.7	PLE_int	13.76	29.14	4.40	24.74	14.44
			Subtidal	0.94	PLE_sub	13.78	28.69	4.38	24.32	14.42
Île Grande	48.8108	-3.575680556	Intertidal	2.36	GRA_int	13.59	37.21	1.86	35.35	11.41
			Subtidal	01.05	GRA_sub	13.48	25.36	7.95	17.41	9.34
Roscoff	48.72609722	-3.968505556	Intertidal	2.42	SB_int	13.13	27.92	1.34	26.58	9.51
			Subtidal	01.05	SB_sub	13.17	29.09	7.75	21.35	8.73
Plouguerneau	48.63490833	-4.561961111	Intertidal	02.08	PLO_int	12.98	26.72	1.43	25.30	7.69
			Subtidal	1.1	PLO_sub	12.98	29.82	6.56	23.26	6.34
Argenton	48.52988056	-4.766941667	Intertidal	1.95	ARG_int	13.10	28.99	2.21	26.78	7.37
			Subtidal	01.01	ARG_sub	13.04	28.00	6.81	21.19	6.84
Rade de Brest	48.329429	-4.452378	Intertidal	02.07	RAD_int	13.86	33.65	01.04	32.61	10.74
Penmarc'h	47.79851389	-4.381813889	Intertidal	1.62	PEN_int	13.88	26.97	0.72	26.25	15.08
			Subtidal	0.99	PEN_sub	13.84	27.73	4.14	23.59	14.13
Trégunc	47.832725	-3.895422222	Intertidal	1.6	TRE_int	13.86	24.65	3.35	21.30	13.66
			Subtidal	0.9	TRE_sub	13.91	25.16	6.91	18.25	13.29
Piriac*	47.375959	-2.562633	Intertidal	1.97	PIR_int	13.55	33.7	0.19	33.51	15.87
			Subtidal	0.75	PIR_sub	13.59	26.28	6.306	19.97	15.22

Lat. : Latitude; Long.: Longitude; lvl: tidal level ; h: logger elevation from theoretical 0; Other column of the table describe respectively mean, maximum, minimum and range of temperature in degree Celsius over the studied period; DTR: Daily temperature range over the studied period in degree Celsius.

In situ temperature monitoring

To monitor in situ temperature at intertidal and subtidal levels, we deployed 21 Onset Hobo UTBI-001 TidbiTv52 data loggers along the coast of Brittany (Figure 1). For each site we used the brown seaweed belts as a height indicator to place the logger on the bedrock, *Fucus serratus* for intertidal level and *Laminaria hyperborea* for the subtidal level. The loggers were set to measure temperature in situ every 10 min. Temperature monitoring was carried out for each site from April 2020 to October 2022, with the exception of Cap Lévy and Piriac where the loggers were placed in March 2021. The altitude of each logger was determined retrospectively on the basis of the observed time of major temperature variations and sea level forecast calculations by the French Navy Hydrographic and Oceanographic Service (Service hydrographique et océanographique de la Marine – SHOM). Intertidal loggers were

located between 1.6 m and 3.13 m above the zero while subtidal loggers were installed between 0.75 and 1.32 m.

Data analysis

Data were extracted from loggers with Onset HOBOWare software (v3.7.23) and processed with R software (R Core Team, 2022) . Box plots were plotted using Plotly software while statistical analyses such as Principal Component Analysis (PCA) were performed using FactorMineR package. Each temperature record between 18/10/2020 and 18/10/2022 was used as a variable for each site, with a total of 105119 variables for all sites except Cap Lévy and Piriac which were discarded due to missing data. We used latitude, longitude, temperature range, mean daily temperature range and overall mean temperature as additional variables and tested their correlation with selected Axis (see Table 1). As data did not follow normal distribution, Kruskal Wallis tests and Friedman tests were used to study the effect of sites and ploidy on the response of heat stress using R basic stats (R Core Team, 2022) .

Common garden experimental (CGE) design.

Sampling site and cultivation

Four sampling sites were selected on the basis of the results of the in-situ temperature monitoring. According to these data we assigned the Argenton and Plouguerneau sites to the “cold” group, and Cancale and Erquy sites to the “warm” group. At each site, a dozen of tetrasporophytes and male gametophytes were collected at spring 2022 for intertidal sites and at spring 2023 for subtidal sites. The ploidy level was assessed by binocular observation of reproductive structure and by flow cytometry when the thalli were vegetative (Guillemin et al., 2008). The sampled thallus were then acclimated to the control temperature of 13°C in 300L tank in an open system (filtered seawater to 5µm) for at least a week. Later, the apices of thallus were cut in order to create pools of genetically different thallus and cultivated at 13°C during five days for acclimatization before starting the heat stress experiment. For a given site and ploidy, we used pools composed of the same thallus to test differences in response to heat stress.

Monitoring of physiological status

To monitor seaweed physiological status, we measured the maximum quantum yield of Photosystem II (dark adapted) as F_v/F_m , using a Walz Junior-PAM (Waltz, Germany). After 5 min of dark adaptation, samples were illuminated with modulated excitation light, provided by a combination of photodiodes. Since photosynthesis is stress sensitive, the quantum yield decreased under sub-optimal (stressful) conditions. We also assigned a bleaching score to each apex, on a scale from 0 (healthy) to 4 (dead seaweed jelly) see figure 2a.

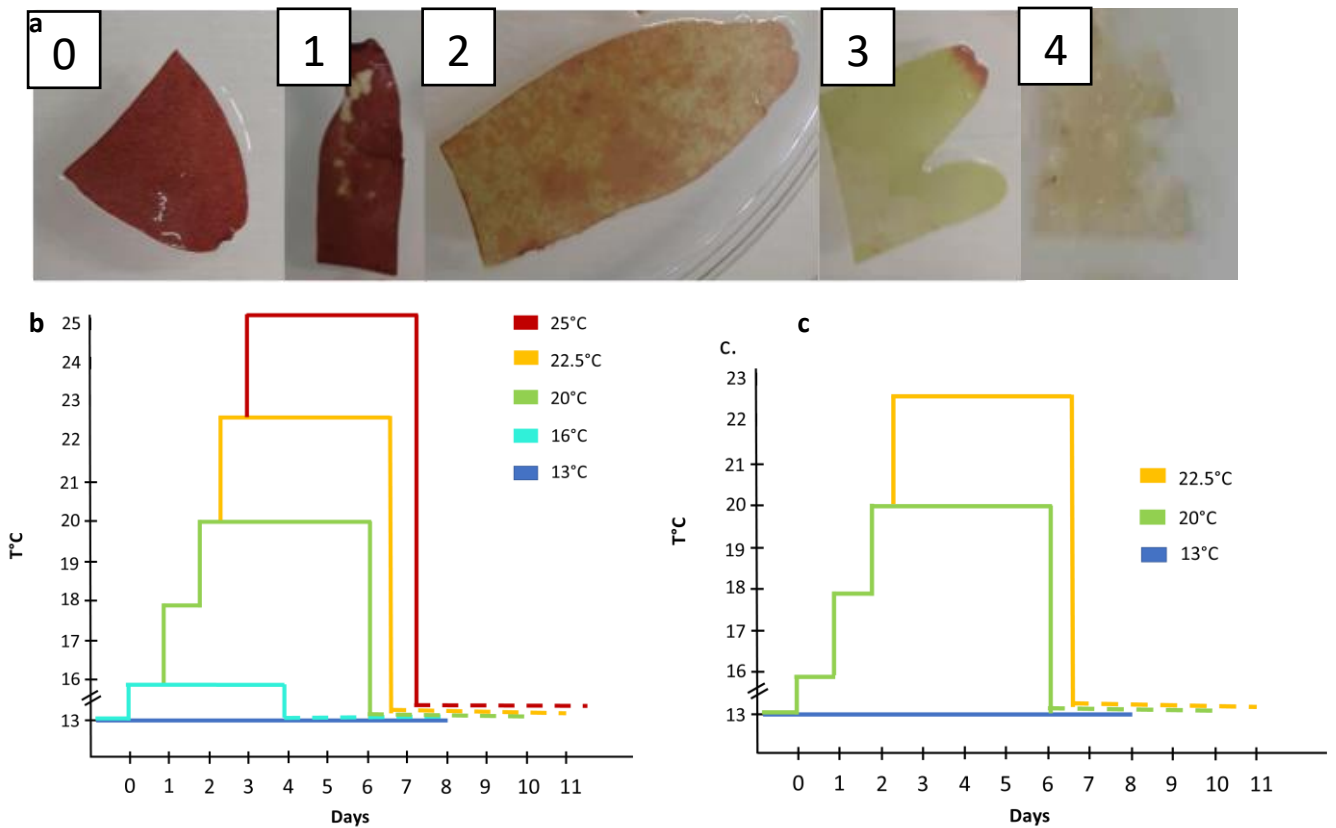


Figure 2: (a) Bleaching score scale and Common garden experimental design to test lethal temperature (b) and heat stress resilience (c)

CGE n°1: Assessing lethal temperature

To study the thermal limits of gametophytic and tetrasporophytic thallus of *P. palmata* from Northern Brittany, whole thalli were sampled in the two sites of Cancale and Argenton. Then, pools of apices from male gametophytes and of tetrasporophytes were cultivated in 1L balloon filled with Provasoli(Provasoli et al., 1956; Starr & Zeikus, 1993) enriched seawater (filtered at 1 μ m). These cultures were exposed for 4 days to 13 °C (control), 16 °C (mean summer temperature for the cold group), 20°C (mean summer temperature for the warm group), 22.5°C and 25°C (negative control). The temperature gradually increased over 3 days (figure 2). Then apices were set to recover for 4 days at 13°C. For each combination of temperature*sites*ploidy tested, 4 replicates consisting of 5 apical meristems were used. We measured photosynthetic activity and bleaching score for each meristem before stress (t0), after stress (t1) and after recovery (t2) (see figure 2b). The culture enriched seawater was changed at each measurement.

CGE n°2 : Local adaptation to heat stress at the intertidal level

In order to assess local adaptation to heat stress, we studied the response to thermal stress of pools of tetrasporophytes and male gametophytes from intertidal populations of Argenton, Plouguerneau and Erquy/Cancale). The pools were exposed for 4 days at 20°C and 22.5°C, with another pool kept at 13 °C for control purposes. The temperature gradually increased over a period of two to three days. For each combination of temperature*sites*ploidy , 4 replicates consisting of 5 meristems were grown in 1 L flasks filled with Provasoli-enriched seawater (filtered at 1µm). We measured photosynthetic activity and bleaching score at t0 (before stress), t1 (after stress) and t2 (after recovery). The enriched seawater medium was renewed at each measurement (see figure 2c).

CGE n°3 :Local adaptation to heat stress at the subtidal level

In order to study the response to heat stress of tetrasporophytic thallus from the subtidal populations, samplings were performed in Argenton and Cancale populations. Thallus were exposed to the same heat stress. We choose those sites because they present the greatest differences in average temperature and heat stress response at intertidal level. We also focused on tetrasporophytes because they were much easier to sample as this phase of the life cycle account for 70% of thallus in wild populations ((Le Gall, 2002); Baud et al, article 4). For each combination of site *temperature we used pools of 40 apical meristems collected from 20 thallus that were cultivated in 10L tanks (Provasoli enriched seawater, filtered at 1µm). After one week at 13°C, pools of tetrasporophytes were exposed to 20°C and 22.5°C for 4 days (plus 2 days of temperature increase) and then returned at 13°C for recovery. Photosynthetic activity and bleaching score were recorded before stress (t0), after stress (t1) and after recovery (t2) (figure 2c).

Data analysis

Statistical analyses were carried out using R basic stats software(R Core Team, 2022) and Plotly R package for graph design(Plotly Technologies Inc., 2015). The Shapiro test was first performed to verify the normal distribution of our data. As the data did not follow normal distribution, we used Kruskal-Wallis analysis to test the effect of site and ploidy on measured Fv/Fm and bleaching scores at each temperature tested.

Results

In situ Temperature

The PCA analysis presented in figure 3a revealed major differences between sites and minor differences between tidal levels. The first axis, which explained 57.3 % of the variation in the data, was correlated with the mean daily temperature ranges (DTR). The second axis, which explained 21.3 % of the dataset variation, was correlated with latitudes (figure 3b). From axis 1, three main groups can be

distinguished, the low temperature amplitude or “cold” group (Argenton, Plouguerneau and Roscoff) ($9.51 > DTR > 6.34$), the moderate temperature amplitude or “temperate” group (Trégunc, Penmarc’h, Île Grande and Cap Lévy) ($16.33 > DTR > 13.33$) and the high temperature amplitude group or “warm” group (Erquy, Cancale and Piriac). Locally, significant differences in daily mean temperature between tide levels was observed in Île Grande, Piriac and Penmarc’h. Looking at additional variables, mean temperature was similar at all sites with slight variations (mean range). However, as expected, temperature ranges between tidal levels were extremely different. In intertidal sites, temperatures of up to 35°C with a maximum at 37.21°C and a minimum at -1.02 °C were measured, while at subtidal sites, temperatures of up to 25.46°C with a maximum at 32.12°C and a minimum at 4.14 °C were measured. These extreme values were measured during winter and summer spring tides when loggers were exposed to the atmospheric air temperature. Most of these extreme values were recorded at sites in the north east, south and in the site of Île Grande in the north west.

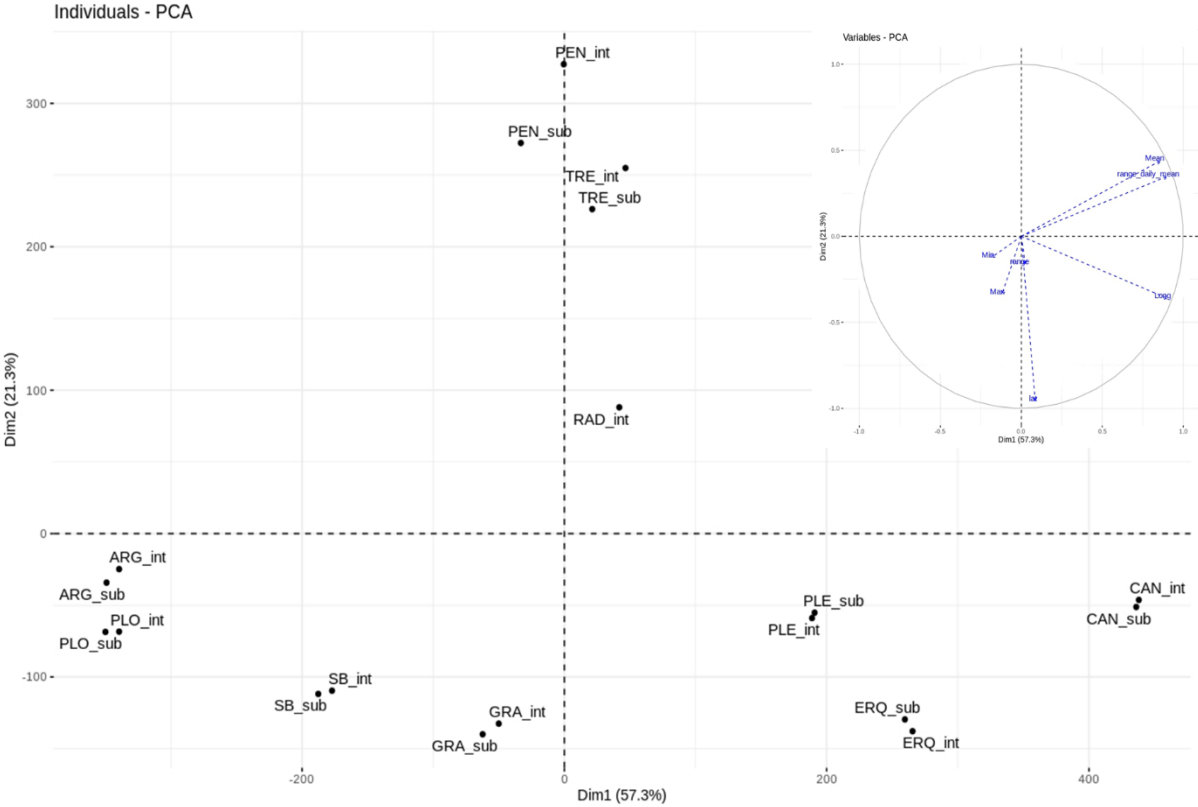


Figure 3: PCA on temperature over a 2 years period (October 2020 to October 2022) within each site at intertidal and subtidal level and inserted diagram of correlation to retained axis of additional variable (see Table 1). Cap lévy and Piriac site had not been used for this analysis because of incomplete datasets.

To perform the common garden experiments, we choose thallus from the populations of Cancale and Erquy (“Warm” group) that show wide variations of temperature over the season, with a mean daily temperature of 5.6°C in winter and 21.8°C in summer. In contrast, Argenton and Plouguerneau

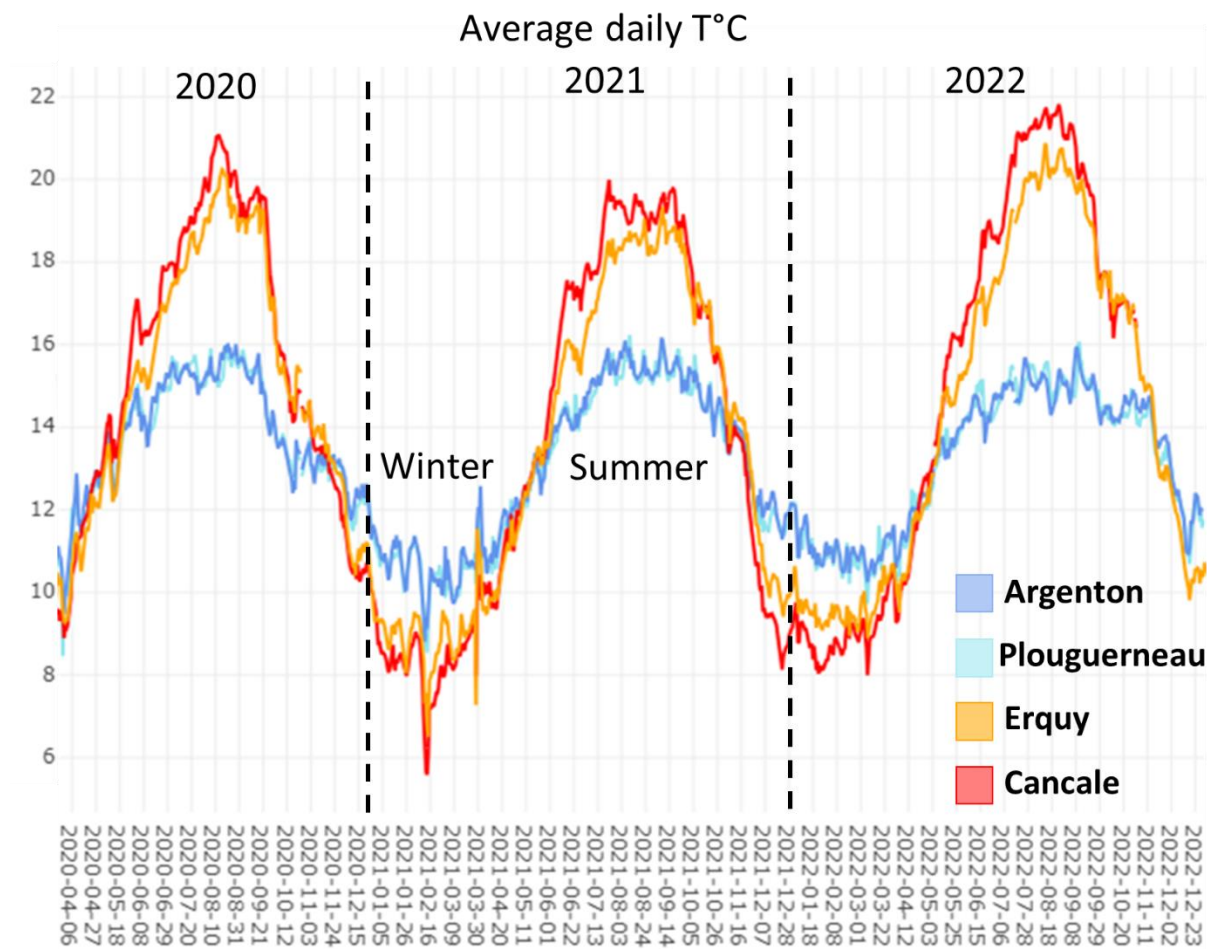


Figure 4: Seasonal variation of mean daily temperatures at studied sites over a three year periods. In situ monitoring started earlier (April 2020) in those sites.

have more stable conditions throughout the year, with an average daily minimum temperature of 8.4°C in winter and maximum 16.2°C in summer (Figure 4).

Ploidy effect

Experiments in the common garden showed that, overall, the level of ploidy had no effect on the response to temperature stress in CGE n°1 (Kruskal-Wallis, $p=0.83$) and CGE n°2 (Kruskal-Wallis, $p=0.09$) (Figure 5). As overall ploidy did not have any effect on heat stress response it was then decided to pool tetrasporophytes and male gametophytes together in statistical analysis in order to increase statistical power.

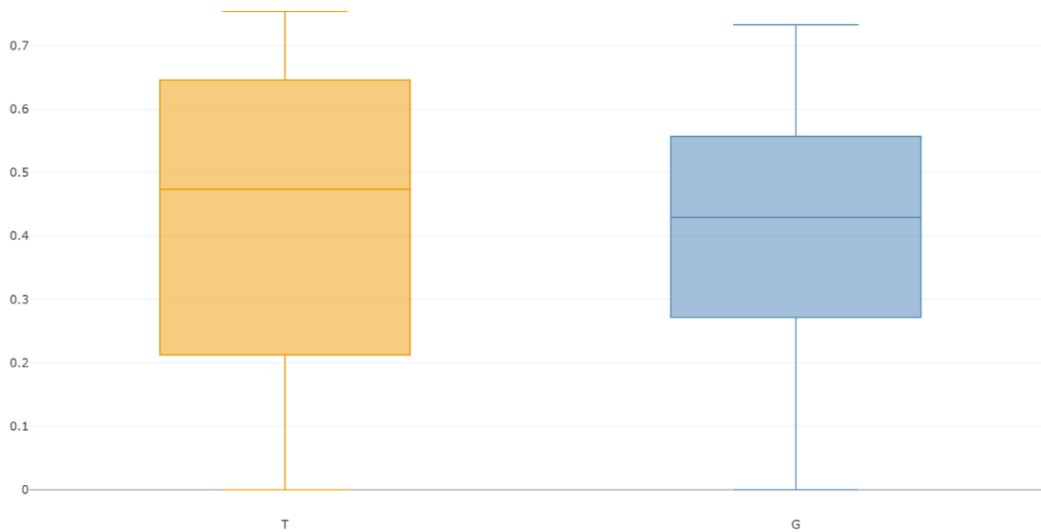


Figure 5: Fv/Fm value overall for tetrasporophytes (T, in orange) and male gametophytes (G; in Blue), after 4 days at 22.5°C and 4 days of recovery. Result from CGE n°2.

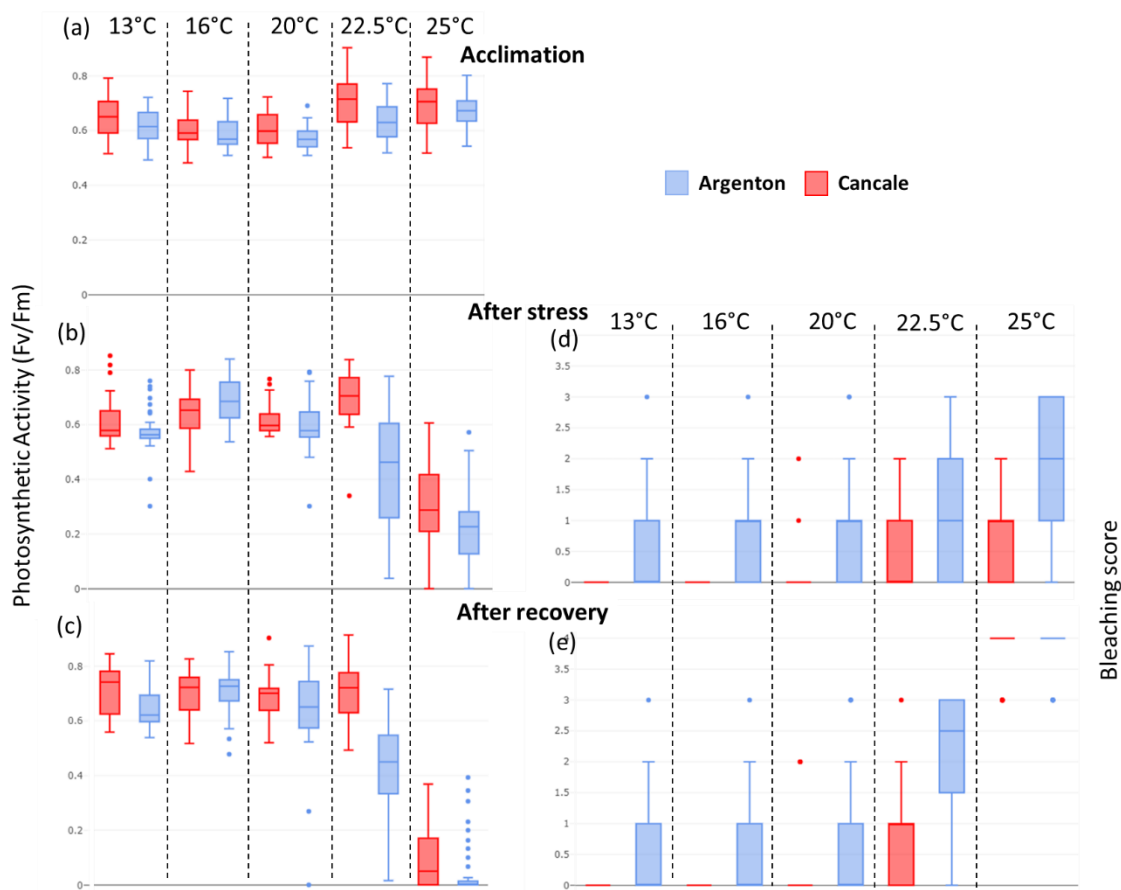


Figure 6: CGE n°1 : Effect of temperature on measured physiological parameters (Photosynthetic activity and bleaching score): after acclimation at 13°C (a), after stress (b,d) and after recovery(c,e). Site origin of the tested thallus are indicated in colours (Red for Cancale, Blue for Argenton)

Lethal temperature

No significant effect of temperature on photosynthetic parameters was observed for thallus exposed to 13°C, 16°C and 20°C. However, at 25°C and after recovery, thallus from both locations appeared to have been stressed beyond recovery, with mean photosynthetic activities close to 0 and mean bleaching scores close to 4 were monitored for thallus of Argenton (mean Fv/Fm =0.049, mean BS =3.83) and Cancale (mean Fv/Fm =0.091, mean BS =3.8).

However, at 22.5 °C and after recovery significant lower mean photosynthetic activities (Fv/Fm = 0.440) and higher Bleaching Score (BS = 2.15) were observed for thallus from Argenton (Kruskal-Wallis, $p < 0.001$). At this temperature and after recovery, thallus from Cancale did not appear to have been affected by heat stress (mean Fv/Fm = 0.709, mean BS = 0.8). Furthermore, the variations between thallus from the same site after stress and after recovery were higher at Argenton than at Cancale for bleaching scores and Fv/Fm (Figure 6).

Local adaptation to temperature

Intertidal sites

After treatment at 22.5°C, the algae showed differences in the recovery of their photosynthetic activity depending on their geographical origin (“cold” group vs “warm” group). Thallus from Argenton (mean Fv/Fm =0.315, mean BS =2.14) and Plouguerneau (mean Fv/Fm = 0.235) have significantly lower mean photosynthetic activities than thallus from Cancale (mean Fv/Fm = 0.599) and Erquy (mean Fv/Fm =0.462) (Kruskal-Wallis, $p < 0.001$). However, this pattern of differences between “cold” and “warm” groups was not observed with the bleaching score. Only Cancale showed lower mean bleaching score (BS = 1.5) at 22.5°C. In contrast, Argenton, Plouguerneau and Erquy showed a similar high mean bleaching score value (2.14, 3.4 and 2.35 respectively). The level of variation in photosynthetic activity between thallus at the same site was higher in Argenton, Plouguerneau and Erquy than in Cancale. (Figure 7).

Subtidal Sites

At the end of the acclimation period, thallus from Cancale showed lower Fv/Fm values than those from Argenton. However, over time, thallus from Cancale showed signs of recovery to stress caused by the acclimation phase at 13°C, even when exposed to high temperatures. At 22.5°C and after recovery, significant differences in physiological responses were observed between thallus from Argenton and Cancale (Kruskal-Wallis, $p < 0.001$). A significant effect of sites was also observed for thallus at 13°C and 20°C. Higher mean photosynthetic activities and lower mean bleaching score were measured in Cancale thallus (mean Fv/Fm = 0.571, mean BS=0.12) than in Argenton ones

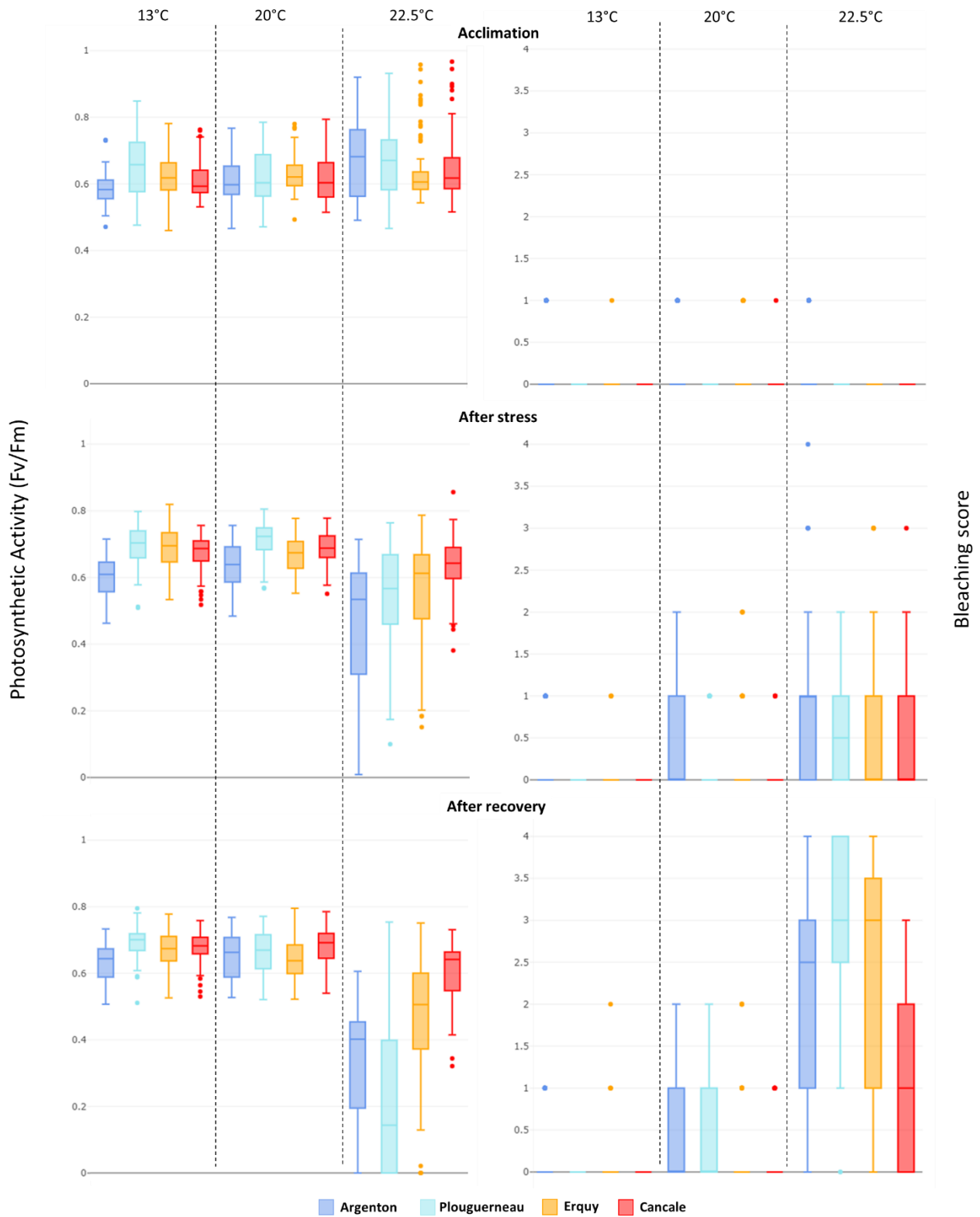


Figure 7: : CGE n°2: Effect of temperature and site origin on measured physiological parameters at intertidal level(Photosynthetic activity and bleaching score).

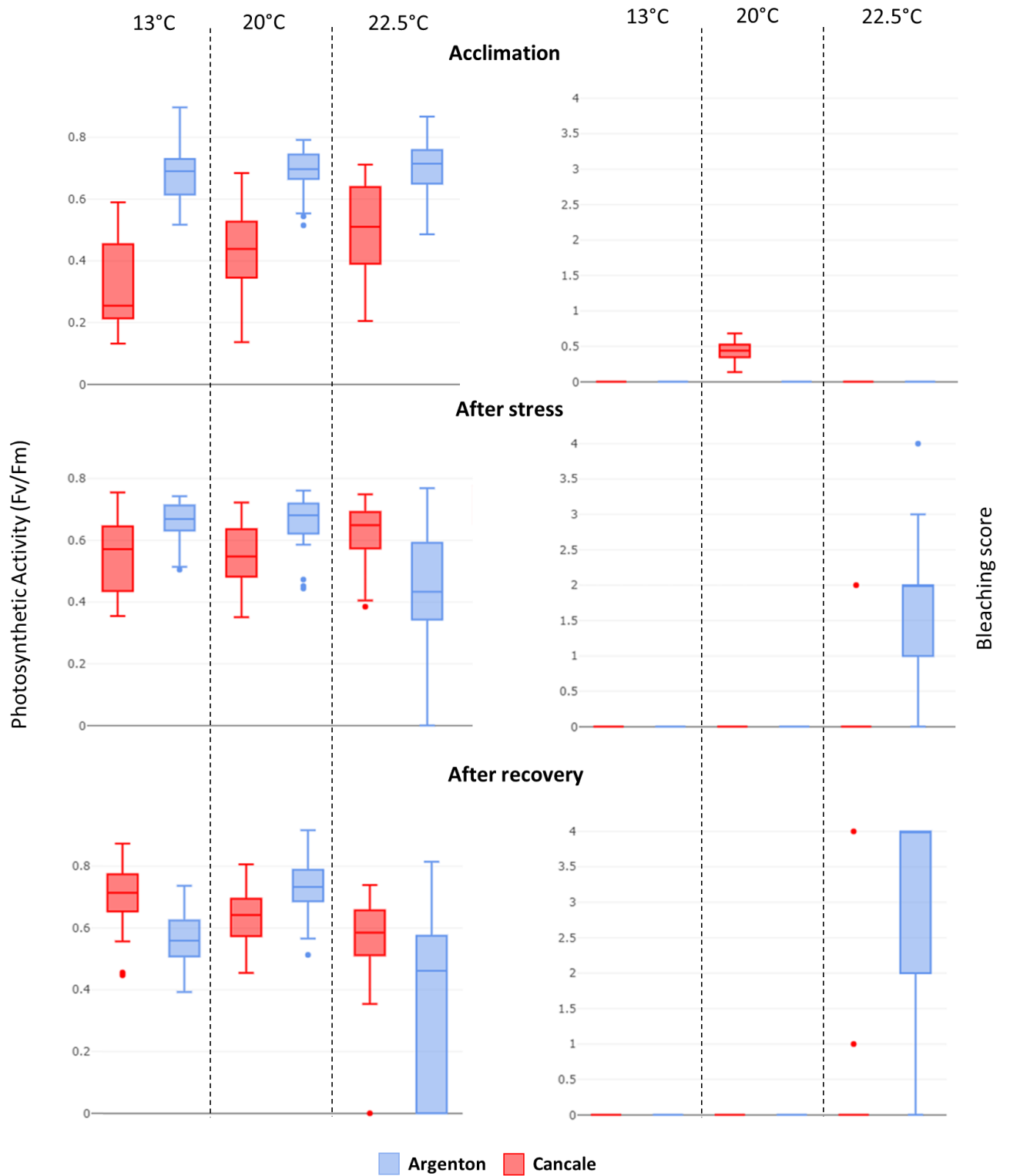


Figure 8: : CGE n°3: Effect of temperature and site origin on measured physiological parameters (Photosynthetic activity and bleaching score)

(mean Fv/Fm = 0.339, mean BS = 3.02). The variations in the measurements of physiological parameters among thallus was higher in Argenton than in Cancale (Figure 8).

Discussion:

Our study on the red alga *P. palmata* provides evidence of local adaptation to temperature along the North Brittany coast. The lethal temperature, which is close to 22.5°C, varies according to the population of origin and does not depend on the ploidy level of the thallus.

Temperature across Brittany coast seascape

The field temperature records performed between 2021 and 2022 using in situ temperature probes confirmed the existence of three main regions along the Brittany coast (south, north-west and north-east) in accordance with the previous SST data (Gallon et al. 2014). The mean daily temperatures observed were however higher than those reported by Gallon et al. In 2014 (Mean T° ~12.75 in North-West B.; Mean T°C ~13.25 in North east B.; Mean DTR ~7.5°C in North-West B.; North east B. Mean DTR ~13°C) with in situ values reaching up to 21.8°C degrees during one week in the summer of 2022. These results confirm the existence of heat waves in this region. The striking differences in seasonal temperature patterns between north-west and north-east Brittany could be explained by the different topographies and oceanographic conditions of these two regions.

The north-west of Brittany rocky shores corresponds to the Iroise Sea, which is characterized by the Ushant tidal front which is the dominant feature of the summer season hydrological structure in this area. It separates tidally mixed coastal waters from thermally stratified open Celtic Sea waters preventing too much Summer heating (Leboyer et al., 2007). In addition, this region is characterized by mixing effects that bear a very strong influence on the thermal structure of the warm-water intrusions (Le Boyer et al., 2009). By contrast north-east Brittany is more exposed to continental climatic conditions (characterized by higher temperatures throughout the year) and is much shallower, this region is also known as a large bay featuring some of the greatest tidal amplitudes and strongest currents in the world (Garreau, 1993; Salomon & Breton, 1993). Furthermore, the Erquy and Cancale study sites are located close to large bays, the bay of St Brieuc and the bay of Mont Saint Michel respectively. These particular areas, where the open strands reach 250 km², act as a huge heat exchanger at rising tide, accentuating temperature variations in winter and summer. Despite these heat waves, *P. palmata* is still present along the coast of north-east Brittany. However, the distribution of *P. palmata* could in the near future be restricted to the north-west, where summer temperature amplitudes are lower. Indeed, if summer temperatures exceed 22.5 degrees, *P. palmata* will no longer

be able to survive and reproduce and, given climate change forecasts, this could happen in the near future, particularly if a hot summer like that those of 2022 (1 month above 21°C) becomes the norm.

Ploidy

The absence of significant differences in the response to heat stress between male gametophytes and tetrasporophytes suggests that the same genes involved in thermal tolerance could be expressed in both haploid and diploid phases. On average, diploids have no advantage in survival compared to haploids. This result comforts some other experimental work on isomorphic red algae, which failed to reveal any significant differences between (Hannach & Santelices, 1985; Littler et al., 1987; Zhang & van der Meer, 1987; Huges & Otto, 1999).

However, different hypotheses have been proposed to explain what are the consequences of having one or two copies of a homologous genome within a cell (for review see Valero et al., 1992). One of these hypotheses suggest that possessing one more nuclear DNA copy per cell confers to diploid thallus an advantage in terms of cellular metabolism and DNA complementation. According to this hypothesis, diploids could benefit from a better cellular regulation and metabolism (Raper & Flexer, 1970; Maynard-Smith, 1978) and should then be more vigorous and tolerant to stress. For example, it has been shown that under toxic and mutagen conditions, diploid juveniles of *G. gracilis* have a higher growth and sometimes a greater viability than haploid ones (Destombe et al., 1993). Overall, the experimental conditions we used here did not allow us to point out any differences in responses between male fronds and tetrasporophytic fronds, except in the Plouguerneau population where diploids showed better survival to stress. As this population is located in a site with the lowest DTR (daily temperature range) value, we can hypothesize that thallus from this site are particularly sensitive to temperature stress, which would result in a strongest response from haploids compared to diploids.

Lethal temperature

Our results show that the lethal temperature for *P. palmata* in Brittany is between 22.5°C and 25°C and that its thermal tolerance, estimated over a short period (4 days) is around 22.5°C. In the longer term in the field, the Cancale population, which was exposed to temperatures above 21°C for more than a month in 2022, was still present at spring 2023. This last summer thermal event did not therefore result in the extinction of the population. Furthermore, in our study, we only tested temperature stress experimentally, without considering other factors such as light intensity or desiccation. For example, dehydration has been shown to increase high-temperature tolerance in some intertidal seaweeds (e.g. (Davison & Pearson, 1996; Hunt & Denny, 2008). Further investigation

of the long-term temperature tolerance combined with light intensity should be tested to assess more sharply *P. palmata* lethal temperature.

In addition, our study showed differences in response between thallus from different populations. Thallus from populations in north-west Brittany are less tolerant to heat than those from the north-east populations. These results are consistent with the observations in populations in New Brunswick (Canada), in which thallus stop growing at 18°C (Morgan & Simpson, 1981). On the other hand, thallus from the populations in north-east Brittany show a resistance to thermal stress closer to that of populations in Portugal, which can continue to grow at 17°C and survive temperatures of up to 21°C (Matos et al., 2006).

Ongoing Local adaptation process across seascape

Local adaptation is the result of selection by the local environment, favouring phenotypes that improve the selective values of individuals, i.e. their survival and reproduction. More precisely, local adaptation can result from genetic trade-offs, in which a local allele has an advantage in terms of fitness in its site of origin and a disadvantage elsewhere (Hall et al., 2010). The local environment and genetic structure play a major role in the formation of phenotypic differences within a species, either through phenotypic plasticity or through the evolution of local adaptation. Using common garden experiments, we demonstrated the differences in response to thermal stress between the different populations of *P. palmata* between north-west and north-east Brittany, whatever their bathymetric level. This result constitutes a strong evidence for local adaptation. Such a hypothesis was proposed by Matos et al in 2006, whose study described the tolerance of Portuguese populations to thermal stress. These authors hypothesized that Portuguese populations, regularly exposed to hot weather events, could be better adapted to these events than, for example, populations in Canada. This hypothesis was based on a comparison of two regions that are very far apart geographically (around 4,500 km) and genetically. Indeed, it is estimated that amphi Atlantic gene flow in *P. palmata* stopped between 320 000 to 720 000 years ago (Provan et al., 2005; Li et al, 2015).

In our study, the differences of responses between populations separated by only a hundred kilometers or so are genetically differentiated (Baud et al. article 2). This recent study of the genetic structure of *P. palmata* populations along the Brittany coast revealed that gene flow between populations is restricted. The fact that *P. palmata* populations are generally of small size and located on a narrow altitudinal fringe of the foreshore, and that gene flow is limited, could be parameters that promote local adaptation (Blanquart et al., 2013). This suggests that local adaptation processes could occur between genetically isolated populations subject to contrasting environmental conditions.

Why not acclimation?

A recent study has shown that the brown alga *Fucus guiryi* displays differences in thermal tolerance depending on the season, leading to changes in vulnerability to warming from one season to the next (Sánchez de Pedro et al., 2023). These authors suggest that transgenerational plasticity can occur in nature on a seasonal scale, as a natural 'priming' mechanism. Unlike adaptation to particular environmental factors, which occurs over a long-time scale and is a speciation mechanism, acclimation is a phenomenon that occurs in a few hours or days and involves changes in gene expression and the synthesis of new proteins that also lead to some epigenetic modifications on histone proteins and/or DNA methylation (Jueterbock et al., 2021). We cannot reject the hypothesis that the results we obtained in our experiments are solely the result of adaptation, as they could correspond in part to the acquisition of a memory of thermal stress. This process has already been described in plants to confer tolerance for months or years, however, transcriptional networks and epigenetic regulation during high-temperature stress is not yet fully understood during plant heat stress (Ohama et al., 2017).

Recent studies have shown that such a process can occur in the red seaweed *Bangia fuscopurpurea*, where thallus that had already been exposed to sublethal temperature showed greater resilience when re-exposed a week later and showed improved temperature resilience (Kishimoto et al., 2019). In our study, thallus were collected in spring, which is the time of year when temperature is lower and homogeneous along the north Brittany coast (see figure). Sampling in spring therefore ensures that thallus have not been exposed to heat stress for at least 6 months. The stress memory of *Bangia fuscopurpurea* seems to fade when thallus are exposed to heat stress more than 2 weeks after the first episode of thermal stress. Moreover, no study has confirmed that such phenomena last more than a few weeks. It is also interesting to note that *P. palmata* produce new blades from old thallus every spring, which means that the apical tissues used in our experiment were probably not exposed to heat stress last summer in the field. However, to ensure that acclimation (or priming) is not responsible for the heat stress response observed here, we suggest repeating this experiment with thallus grown in the lab from spores to try to avoid a potential maternal effect.

Application for aquaculture & management

These results are of great interest for the domestication and restoration of wild populations of this species. Indeed, the fact that some thallus from north-western group survived heat stress, resulting in the high variability observed in these populations, raises hopes that these economically important populations will be resilient to future marine heat waves. In addition, the identification of strains capable of withstanding temperatures of up to 22.5 °C make it possible to extend the production of vegetative crops of this seaweed in tanks during summer.

Conclusion

In conclusion, we have detected geographical variations in the thermal sensitivity of fronds of *P. palmata*, with relevant implications for conservation, restoration and aquaculture development. We have demonstrated that this species cannot tolerate a temperature above 22.5 °C for a maximum of 4 days and that this tolerance depends on its population of origin. This highlights the sensitivity of this species to warming and should raise awareness of the future management of wild stocks of *P. palmata*. In addition, our results provide an initial insight into a probable ongoing process of local adaptation on a short scale (around 100 km) across the distributions of *P. palmata* along the Brittany coast, with slight differences along the tidal gradient. These results raise interesting hypotheses concerning the resilience of this species to climate change and its use in aquaculture. Further studies based on long-term experiments will make it possible to separate the phenotypic and genotypic components of these responses and improve our understanding of the physiology and ecology of this species.

Acknowledgments

This work benefited from the support of the European Maritime and Fisheries Fund (EMFF) through the POLISTR project to PP and Région Bretagne (PhD grant ARED Domdulse from ED227 Sorbonne-Université/MNHN to AB). We are grateful to the EMBRC France partner core facility for its technical support, especially Gaetan Schires and Sébastien Henry. The authors are also deeply indebted to the Service Mer & Observation (SMO) of Roscoff.

AUTHOR CONTRIBUTIONS

Conceptualization, A.B., M.V., C.D and PP.; methodology, A.B., D.P. and L.F.; data analysis A.B, D.P., L.F., resources, A.B, D.P., C.D. and M.V.; data curation, A.B., D.P., C.D. and M.V.; writing—original draft preparation, A.B., P.P., C.D.; writing—review and editing, A.B., P.P., C.D. and M.V; supervision, P.P., C.D. and M.V.; funding acquisition, M.V. and P.P. All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

Temperature data will be deposited and will be available in a repository at <http://>

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Chapitre II : Etude du système de reproduction et contrôle du cycle de reproduction

Ce chapitre est dédié à l'étude de la reproduction des populations naturelles et de la maîtrise des différentes étapes du cycle de vie particulier de *P. palmata* en laboratoire. Ces connaissances sont indispensables pour mener à bien la domestication en permettant de maîtriser la reproduction des souches sélectionnées dans le chapitre 3 afin d'inciter la sélection variétale par croisement.

Dans ce chapitre, j'ai donc en premier lieu étudié le système de reproduction à l'aide des marqueurs microsatellites ainsi que les ratios de ploïdie des populations sauvages de *P. palmata* à l'étage médiolittoral et infralittoral dans plusieurs sites en Bretagne et deux sites éloignés (Irlande & Nord et détroit du Pas de Calais). (Article n°4)

Dans un second temps, je me suis intéressé aux facteurs environnementaux impliqués dans le contrôle du déclenchement de la reproduction dans les populations naturelles et en laboratoire. J'ai également étudié la fécondation en milieu contrôlé afin d'améliorer les connaissances sur cette étape cruciale du cycle de reproduction. (Article n°5)

1. Etudes du système de reproduction dans les populations naturelle (Article n°4)

L'article n°4 ci-dessous regroupe mes travaux de thèse sur l'étude de la reproduction au sein des populations de *P. palmata*. Il est prévu pour une soumission pour publication dans les prochains mois dans la revue *Journal of Phycology*.

Article n° 4: Stage structure and mating system in the haploid-diploid red alga *Palmaria palmata*: effect of sexual dimorphism and absence of cystocarp.

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Abstract:

Genetic diversity and its distribution are essential to ensure the adaptive potential of a species. The mode of reproduction (sexual or asexual reproduction) and the mating system (inbreeding or outcrossing) have a considerable impact on the distribution of this genetic diversity within and between populations. Red algae reproduce with a haploid diploid sexual cycle in which gametophyte individuals (which produce gametes) and tetrasporophyte individuals (which produce meiotic spores) alternate, and also vegetatively by cuttings thanks to their unique ability to draw their resources directly from the entire surface of the thallus (no roots compared with angiosperms). Here we have studied the balance between sexual and asexual reproduction in the red alga *P. palmata*, which has a marked sexual dimorphism in its sexual cycle. The relative frequency of diploid vs haploid thallus was studied and the pattern of genetic structure was compared in haploid and diploid stages at different geographic scale at the intertidal and subtidal levels: a total of 269 haploid thallus and 628 diploid thallus located in seventeen geographic locations at intertidal and subtidal levels were genotyped using eleven microsatellite loci. The genetic data show that reproduction in *P. palmata* is sexual. The sexual dimorphism and the absence of a cystocarp could explain the dominance of diploids in the populations. The differences in population composition between intertidal and subtidal levels suggest that diploids are more resistant to environmental conditions than haploids.

Introduction

The mode of reproduction (sexual vs. asexual reproduction) and the breeding system (inbreeding vs. outcrossing) of a species have a considerable impact on the partitioning of genetic diversity within and among populations (Hamrick & Godt, 1996, Krueger Hadfield et al, 2021; Stoeckel et al. 2021). In plants, Hamrick and Godt (1996) demonstrated that significant differences in genetic diversity existed between species with different life history traits. These specific features, which affect genetic diversity and its distribution, are therefore essential for ensuring the adaptive potential of a species and, in particular, its resilience to climate change. For example, clonal populations, in which recombination is absent, are less genetically diverse than sexual ones. Similarly, inbreeding populations show lower diversity than populations of species with outcrossing systems. The mode of reproduction and the distribution of diversity will therefore have an impact on the evolution of the population by modulating the relative influences of drift, migration, selection and mutation (Orive & Krueger-Hadfield, 2021). For species that exhibit both types of reproduction, the balance between sexual reproduction and clonal reproduction will strongly influence their ecological success (Halkett et al., 2005; Silvertown, 2008). The importance of sexual versus asexual reproduction within populations has been studied in different algal groups: Chlorophyta (e.g. (Van Der Strate et al., 2002), Phaeophyceae (Couceiro et al., 2015; Steen et al., 2019) and Rhodophyta (Engel et al., 2004; Heiser, 2022; Krueger-Hadfield et al., 2013; Krueger-Hadfield et al., 2016; Pardo et al., 2019; Sosa et al., 1998). In this latter phylum, it has been shown that some species reproduce mainly sexually, such as *Gracilaria gracilis*, in which outcrossing prevails (Engel et al., 2004), or *Chondrus crispus*, which shows high levels of self-fertilization (Krueger-Hadfield et al., 2013; Krueger-Hadfield et al., 2015) while other species such as *Phymatolithon calcareum* or *Chondria tumulosa* mainly reproduce by asexual means (Pardo et al., 2019).

Clonality is a widespread phenomenon in the plant kingdom, but also in lichens, fungi, algae and certain groups of animals (Whitehead et al., 2018). In algae, asexual reproduction can be considered as a phenomenon that adds to, or replaces, at least partially, sexual reproduction (Feldmann & L'Hardy-Halos, 1977; Krueger-Hadfield et al., 2021). Two main mechanisms can be distinguished: 1) reproduction by specialized spores (direct spores) or by apogamy with gametes behaving like haploid spores (parthenogenesis or androgenesis) or by apomeiosis (diploid spores not undergoing meiosis) and 2) reproduction by thallus fragmentation.

Apomixis, i.e., reproduction without fertilization or meiosis (apogamy and apomeiosis) is relatively common in Rhodophyta, and has been observed in nearly 40 genera (Hawkes 1990, West et al. 2001). Some apomictic species may be obligate, i.e. totally lacking in sexual reproduction, but facultative

apomixis is a more common phenomenon in red algae (Hawkes 1990) characterized by the relative rates of asexual versus sexual reproduction. Vegetative fragmentation, i.e. the ability of algal fragments separated from the adult thallus to give a new individual is an important reproductive strategy commonly used by marine macroalgae (Cecere et al., 2011; Kilar & McLachlan, 1986; Smith & Walters, 1999; Walters et al., 2002). Many species that are able to fragment are particularly effective at establishing themselves in new environments. This is particularly true of certain invasive species (Ceccarelli et al., 1996; Kilar & McLachlan 1986; Krueger-Hadfield et al., 2016). In haplodiplontic sexual life cycles, characterized by the alternation of isomorphic haploid gametophyte and diploid tetrasporophyte thallus, clonal processes reproduce one or other of the phases. For example, the dominance of clonal multiplication of the diploid stage (tetrasporophyte) has been observed in *G. chilensis* and *G. vermiculophylla* following vegetative fragmentation facilitated by agricultural practices and biological invasion, respectively (Guillemin et al., 2013; Krueger-Hadfield & Hoban, 2016). This dominance in natural estuarine populations of free-floating or mud-buried plants could be the result of a better fitness (or growth rate) of diploid thallus compared with haploid ones (Guillemin et al., 2013). Ecological differences between isomorphic haploid and diploid thallus have been demonstrated experimentally in various species of red algae in the orders Gigartinales and Gracilariales (Destombe et al., 1993; Hannach & Santelices, 1985; Thornber, 2006). This could partly explain (along with the respective fertility of thallus) the relative frequency of diploids compared with haploids in natural populations (Destombe et al., 1989; Kain (Jones) & Destombe, 1995).

In sexual species of Rhodophyta, meiosis and syngamy generate an alternation of haploid and diploid thallus. This life cycle indicates that the recruitment of one stage depends on the survival, fecundity and migration, as well as the effectiveness of selection, of the other stage. A demographic model of the population dynamics and structure of the haploid-diploid red alga *G. gracilis* shows that the dynamics of sex ratios and ploidy depend essentially on the survival of adults, but also to a lesser extent on their fertility (Engel et al. 2004). One of the special features of the red algal Florideophyceae cycle is the formation of the cystocarp. This macroscopic hemispherical fruiting body is developed after fertilization of the female gamete which is retained on the female gametophyte. In *G. gracilis*, the zygote multiplies by mitosis, producing thousands of genetically identical diploid spores (carpospores). The carpospores are eventually released from the cystocarp and germinate into a free-living diploid tetrasporophyte that produces haploid spores through meiosis. These haploid tetraspores then germinate into either a male or female gametophyte, completing the life cycle. Zygotic amplification in the cystocarp has been proposed to compensate for the inefficiency of fertilization by non-motile spermatia and the lack of dispersal of the female gamete (Searles, 1980). In fact, this phase of zygote

multiplication in the cystocarp can be considered an effective dispersal strategy which could also compensate for the poor germination success of carpospores observed in the field (Destombe et al. 1989).

The relative frequency of haploid and diploid thallus within populations varies between species. In *Chondrus crispus* (Collén et al., 2014) many intertidal populations are dominated by gametophytes. While, in Gracilariales, Ceramiales and Plocamiales, tetrasporophytes are dominant (Cecere et al., 2000; Heiser et al., 2023; Kain (Jones) & Destombe, 1995). Numerous hypotheses linked to the fitness of haploid and diploid thallus have been proposed to explain these variations (for a review see Thornber 2006 and Krueger-Hadfield 2020).

The life cycle of the red alga *Palmaria palmata* has long been regarded as an enigma, because in natural populations only male and tetrasporophyte blades were observed. The Palmariales were formerly regarded as being strictly asexual with tetrasporophytes reproducing by apomeiotic tetrasporangia spores germinating to yield only male gametophytes and tetrasporophytes (West and Hommersand 1981). Female gametophytes were unknown until produced in culture by van de Meer & Todd (1980). From tetrads isolated from field-collected tetrasporophytes these authors showed a 2:2 segregation of large haploid male plants with flat fronds producing spermatia and small haploid female plants as simple discs (0.1mm diam) producing numerous carpogonia. After fertilization, the diploid tetrasporophyte blades developed directly from the female gametes. As a result, the sexual cycles of Palmariales are very different from that of other Florideophyceae, as they are characterized by strong sexual dimorphism and the absence of a cystocarp (Figure 1). Sexual dimorphism is generally explained by the different reproductive requirements of females and males, which results in sex-specific selection pressures on traits that influence viability and fertility. Theory suggests that trait divergence in females or males is facilitated by the presence of sex chromosomes (Mank, 2009; Rice, 1984). In *P. palmata* the sex segregation observed in culture strongly suggested the occurrence of the UV sexual chromosome system (Borg et al., 2023), male gametophyte has V chromosome, female gametophyte U chromosome and tetrasporophyte is heterozygote UV. Given that genes located on sex chromosomes spend a different amount of time evolving in females and males, they are expected to gain fitness advantages disproportionately in either sex. We can thus hypothesize that the large male gametophyte would rather have a dispersal role (Keddy, 1981) while the microscopic female would ensure the survival of the young tetrasporophyte, drawing resources for the first development stages from the female disc, thus shunting the cystocarp. In addition, one may wonder about the importance of vegetative reproduction in nature in this species, which can be cut very easily in culture and in which

a few cases of fixation disc formation have been observed under in vitro culture conditions (personal observations).

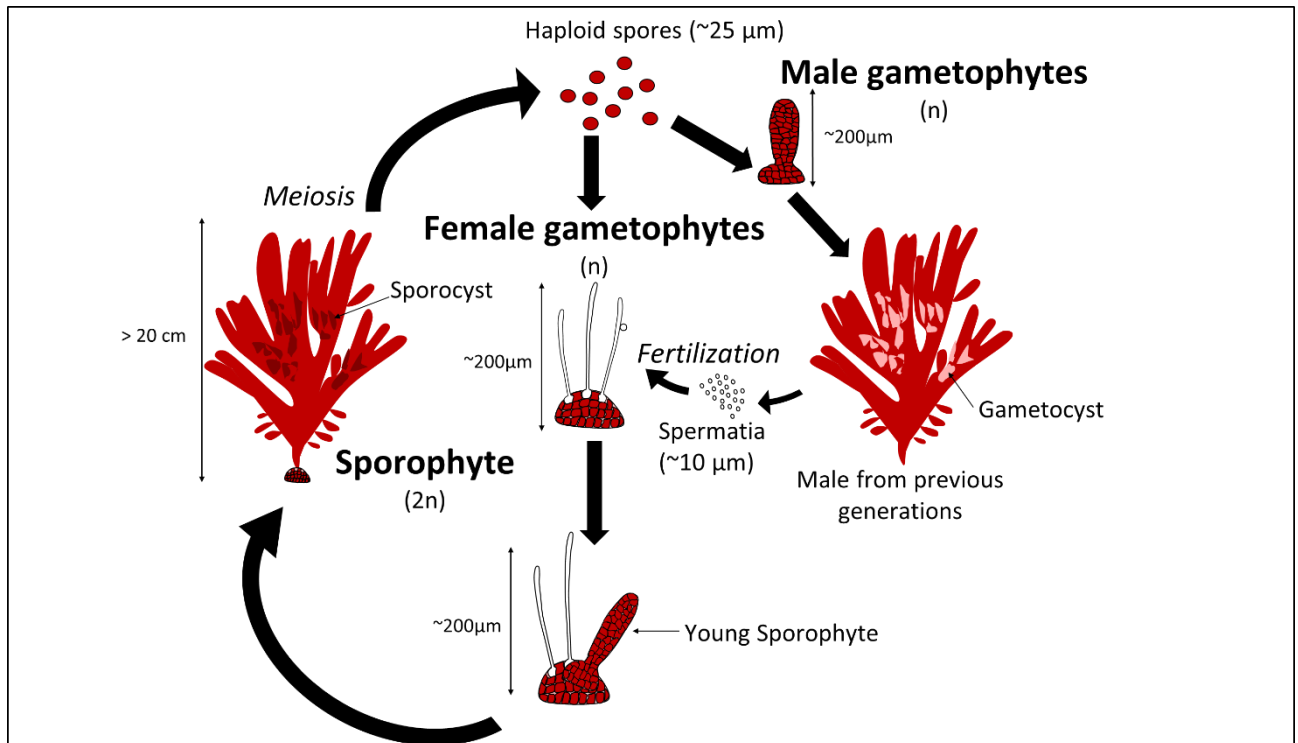


Figure 1: Life cycle of *Palmaria palmata*. (Modified from Van Der Meer & Todd 1980)

The mature diploid tetrasporophyte generates haploid tetraspores by meiosis. After release and attachment, these spores develop into male and female gametophytes (ratio 1:1). Two to three weeks after germination, the female gametophytes, which remain microscopic, give rise to reproductive cells (carpogonium) surmounted by a cellular extension called a trichogyne, on which spermata stick. After fertilization, a new sporophyte develops on the female gametophyte. The male gametophyte and the tetrasporophyte are isomorphic, while there are marked sexual dimorphism between the male and female.

Studies of red algal reproductive system variation using molecular genetic tools have predominantly focused on taxa showing isomorphic life cycle with cystocarps either living either in intertidal (*Gracilaria gracilis* Engel et al. 2004, *G. chilensis*, Guillemain et al. 2008, *Chondrus crispus* Krueger-Hadfield et al. 2013) or in subtidal species (*Phymatolithon calcareum* Pardo et al. 2019, *Plocamium sp* Heiser et al. 2023). More on, a recent papper compared reproductive system and population genetics of several red algae species along with other haplodiplontic taxa, emphasising the need to account for

life history trait when studying such species (Krueger-Hadfield et al 2021). However, there is very little information on a species such as *P. palmata*, showing atypical life history and located at the boundary between the subtidal and the intertidal.

Material and methods

Sampling, assessing ploidy and genotyping

Sampling was carried out in 17 locations, mainly along the coast of Brittany, between October 2019 and February 2022 (Figure 2). When possible, localities were sampled at two different bathymetric levels (intertidal and subtidal, herein referred as sites), taking an average of 30 thallus per site, in order to study the differentiation between populations along the tidal gradient. At the Cap Gris Nez, Noirmoutier and Rade de Brest location, *P. palmata* was not found at the subtidal level, whereas at Belle-île, *P. palmata* was absent at the intertidal level. At the intertidal level, fronds were sampled at random, maintaining a distance of about 1 m between samples. In the subtidal zone, a single frond was sampled per kelp stipe. The difference in altitude for the intertidal levels varies between 1.60 m and 3.13 m above theoretical sea level and for the subtidal levels between 0.75 m to 1.32m (Table 1). Depending on the sampling season, the type of individual (male gametophyte or tetrasporophyte) was determined in the laboratory by observation under a binocular microscope if they were fertile. For non-fertile thallus, we used flow cytometry to determine the ploidy of the thallus (according to Guillemain et al. 2008). This enabled us to approximate the ploidy ratio (ratio of male gametophyte to tetrasporophyte) in the different locations sampled and at different bathymetric levels.

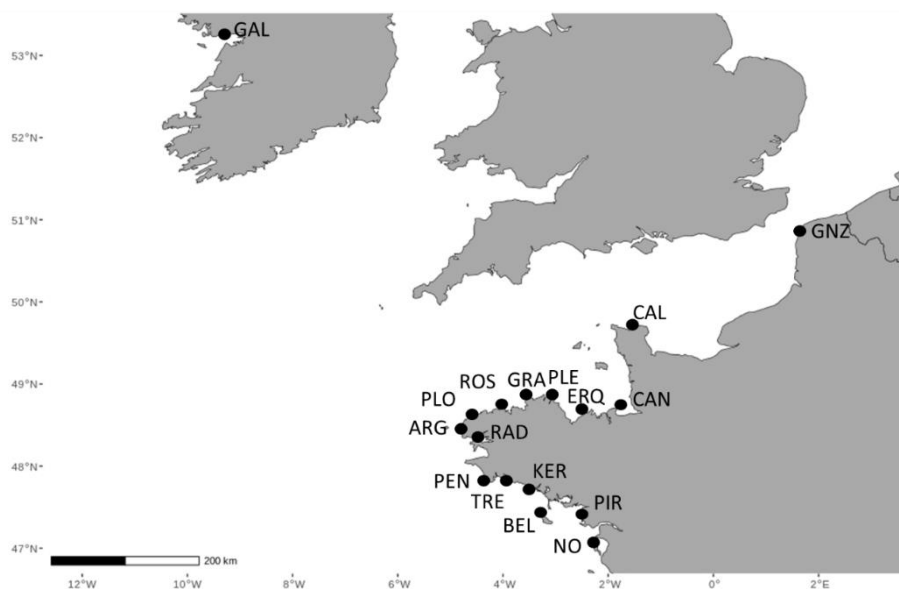


Figure 2: Study locations, see Table 1 for locations code names.

DNA extraction was performed using Nucleospin® 96 plant kit (Macherey-Nagel, Duren, Germany) with a slight modification of the digestion step, reduced to 15 min to avoid extraction of PCR inhibitors. We genotyped 628 diploids and 260 haploids using 11 microsatellite markers (Ppalm_02, Ppalm_05, Ppalm_07, Ppalm_08, Ppalm_09, Ppalm_13, Ppalm_14, Ppalm_16, Ppalm_17, Ppalm_19 and Ppalm_20, Mauger et al., 2023). Labeled PCR products were diluted 1:10 with water. Next, 2 µL of the diluted PCR product pool was added to 10 µL of loading buffer made up of 0.5 µL of the SM594 size standard (Mauger et al., 2012) and 9.5 µL of Hi-Di formamide, denatured at 95°C for 3 min, and run in an ABI 3130 XL capillary sequencer (Applied Biosystems, USA). Genotypes were scored manually in Genemapper version 4.0 (Applied Biosystems).

Testing Ploidy level

Considering that sex is genetically determined (expected sex ratio of 1:1) and that females are microscopic (van der Meer & Todd 1980), it is possible to correct the expected haploid frequency in the population by considering that we had as many males as females. This allowed us to test, using the binomial distribution, whether the estimated gametophyte/tetrasporophyte ratio differed from the predicted ratio of $\sqrt{2}:1$, which was hypothesized if there were no differences in fitness between ploidy levels (Destombe et al., 1989; Thornber & Gaines, 2004). The significance of the difference in mean diploid frequencies between tidal levels was investigated using Student's t test.

Genetic analysis

Null allele is the result of mutation in primers sequences leading to the non-amplification of an alleles at a given loci. We used the frequencies of non-amplification in haploid genotype to assess for null allele at a given loci (Krueger-Hadfield et al., 2013)

We used summary statistic in order to study the reproduction mode and system of *P. palmata* in Brittany and additional location. We estimated genotypic richness (R) and the distribution of clonal membership (Pareto- β), which test evenness of clone repartition among the studied thallus at a given site and allow to estimate reproduction mode of a species. Comparaison of Pareto-B values between several haplodiplontic species in a recent study showed that low Pareto- β value (<0.7) are associated with clonal reproduction, while higher Pareto- β (>2) value are associated with high level of sexual reproduction and intermediate value are relevant of partial clonal reproduction (Krueger-Hadfield et al., 2021; Stoeckel et al., 2021). Then Linkage disequilibrium overall loci was estimated (\bar{r}_D) as observation positive and negative linkage disequilibrium can be link to partial clonality of selfing. Inbreeding coefficient (Fis), expected heterozygosity (He) and equivalent for haploid (H) were also estimated by locus and overall al locus for each study site. Genetic differentiation between Haploids

and diploids populations for a given sites (F_{st}) and the index cited above were calculated with HapDip GenPop software (Krueger-Hadfield & Stoeckel, unpublished)

For genetic differentiation between diploid thallus with less than 3 missing loci were used ($n=601$) to increase statistical power. We estimated pairwise F_{st} with Arlequin software using Reynolds method over 10 000 permutations. Discriminant analyses on principal component (Dapc) were conducted on the same dataset with Adegenet Rpackage using location as prior to asses genetic structure of the studied populations. Those data are presented as supplementary material because genetic structure was not the focus of the studies but can help us interpret potential geographical pattern of reproductive system variation.

Statistic test on genetic diversity were conduct using R basic stat package (R core team, 2023) and Ggplot2 R package was used for graphic rendering.

Results

Sporophyte /male gametophyte ratio

During the breeding season between October 2019 and February 2022, out of the 897 thallus sampled in 17 different localities, observation with a binocular magnifying glass showed that only 37% (239) of thallus were reproductive (i.e., 59% and 68% respectively in the intertidal and subtidal sites), 10% (90) of fronds bore male organs (gametangia) recognizable by the white part of the thallus and 27% (239) were tetrasporophytes with tetraspore sori (Table 1). On average, tetrasporophytes represented more than 72.6% of reproductive thallus in the populations (i.e. 72% and 73.5% respectively in the intertidal and subtidal sites).

Table 1: Phenology of sampled individuals and diploid frequencies

Region	Site	Code	h	Month/Year	Intertidal				Subtidal				P-value		
					N	Nt	Ng	Df	cDf	P-value	Δh	h		Month/year	Ns
Ireland	Galway	GAL	--	Apr.2021	30	16	5	0.7	0.54	NS	--	--	0.6	0.46	NS
North English Channel	Gris_Nez	GNZ	--	Apr.2021	38	8	5	0.6	0.49	NS	--	--	--	--	--
North -East B.	Cap Lévy	CAL	2.89	Nov.2019	30	0	0	0.9	0.82	***	--	--	0	0.46	NS
	Cancale	CAN	3.13	Nov.2019	30	0	0	0.7	0.58	**	1.81	1.32	30	0.71	***
	Erquy (\$)	ERQ	2.85	Mar.2020	30	18	2	0.9	0.88	***	1.58	1.27	30	0.50	NS
	Pleubian (\$)	PLE	1.7	Nov.2019	30	0	0	0.9	0.88	***	0.76	0.94	30	0.33	NS
North-West B.	Île Grande	GRA	2.36	Mar.2020	30	26	4	0.9	0.76	***	1.31	1.05	30	0.50	NS
	Roscoff (€)	ROS	2.42	Oct.2019	30	15	0	0.9	0.82	***	1.37	1.05	30	0.7	0.58
	Plouguerneau (€)	PLO	2.08	Oct.2019	30	9	0	0.8	0.67	**	0.98	1.1	30	0.35	NS
	Argenton (€)	ARG	1.95	Sept.2019	30	0	0	0.6	0.40	NS	0.94	1.01	30	0.6	0.46
	Rade de Brest (\$)	RAD			30	19	8	0.7	0.54	NS	--	--	--	--	--
South B.	Penmarc'h	PEN	1.62	Nov.2019	30	5	0	0.8	0.71	***	0.63	0.99	30	0.88	***
	Trégunc	TRE	1.6	Oct.2020	30	12	18	0.4	0.25	**	0.70	0.9	30	0.54	NS
	Kerpage	KER	--	Fev.2022	29	13	13	0.5	0.35	NS	--	--	30	0.58	**
	Belle-Île	BEL	--	--	--	--	--	--	--	--	--	--	26	0.37	NS
	Piriac	PIR	1.97	Mar.2020	24	0	0	0.7	0.50	NS	1.2200	0.75	30	0.46	NS
	Noirmoutier	NO		Aug. 2021	30	0	0	0.8	0.62	**	--	--	--	--	--

\$. Sites where individual presented a specific morphology close to *P. parvata var. sarriensis* only at intertidal level (ref); €. Harvested Sites (only at intertidal level); €. Altitude of the site in meters; Δh: delta of altitudes between the two tidal levels in meters; N: number of sampled individuals on the fields; NT: numbers of reproductive tetrasporophyte; Ng: numbers of reproductive gametophyte; P: probability to observe a deviation of corrected diploid frequency from expected diploid frequencies; cDf: corrected diploid frequency by doubling of male effective to account for sexual dimorphisms; p: probability to observe a deviation of corrected diploid frequencies from expected diploid frequencies of 0.41, ***p<0.001, **p<0.05, N.S.p<0.05

Flow cytometry analysis showed that each site had male gametophyte and tetrasporophyte thallus. The frequency of tetrasporophytes in the population averaged 70%. These values fluctuate between populations and range from 40% in Trégunc to 90% in five populations in North Brittany. The frequency of tetrasporophyte was higher in intertidal than in subtidal populations (i.e., 74% vs 66% respectively). For the intertidal populations, tetrasporophytes were more frequent in the populations of North Brittany than in those of South Brittany (0.825 +/- 0.116 vs 0.637 +/- 0.106 respectively) (Kruskal-Wallis test $p=0.012$) while no clear difference was observed for the subtidal populations (Fig. 3).

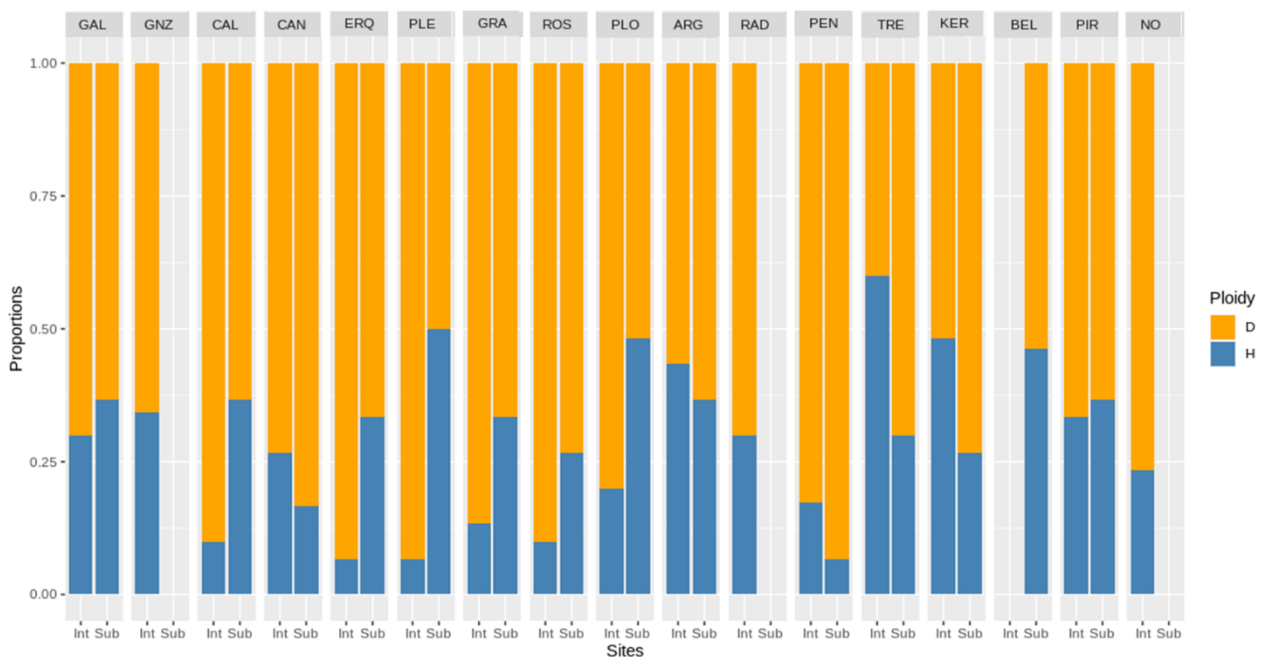


Figure 3: Relative frequencies of diploid tetrasporophytes (in yellow) and male haploid gametophytes (in blue) estimated by flow cytometry in different intertidal and subtidal locations within geographical site.

When correcting for sexual dimorphism by doubling male gametophyte proportions, significant deviation from the hypothesized 2:1 ratio was observed in almost all intertidal sites except for Galway, Cap gris Nez, Argenton, Rade de Brest, Kerpape and Piriac. The significant deviations measured in other populations showed tetrasporophyte excess except in Trégunc where male gametophyte excess was estimated. At subtidal level, significant deviation showing tetrasporophyte excess were estimated at only four locations, Cancale, Roscoff, Penmar'ch and Kerpape (Table1).

Null allele

We detected non-amplification in haploid genotype at every locus, with frequencies between 0.02 and 0.09. To avoid overestimations of heterozygosity deficiencies or impact of null allele on genotypic richness and Pareto- β estimation the thallus with incomplete genotypes were removed from the

dataset. The results presented below were obtained from the analyses of the complete genotypes of 465 diploids (29 sites) and 146 haploids (15 sites) and sites with less than 5 thallus were then not used. We also observed three thallus assigned as tétrasporophytes by sores observation that were homozygote at all 11 locus (Table 2).

Reproductive system

Results of the genetic analyses are presented in Table 2. Genotypic richness in diploid populations was equal to 1 (no repeated multilocus genotype) in all the locations except for Penmarc'h subtidal sites where one repeated multilocus genotype was observed. Among the fifteen studied haploid populations, genotypic richness was inferior to 1 in four intertidal sites (Cap gris Nez, Argenton, Rade de Brest and Trégunc) and in two subtidal sites (Argenton and Belle-Île). Almost all diploid populations showed Pareto- β values superior to 2 except for Piriac intertidal sites (Pareto- β = 1.58). However, in haploid populations four sites showed Pareto- β value inferior to 2, intertidal site of Cancale and subtidal sites of Roscoff, Argenton and Trégunc. Intertidal site of cap-Gris-nez showed Pareto- β value close to 0.7. No significant linkage disequilibrium ($\bar{r}D > 0.05$ or < -0.05) was observed in most of the studied diploid and haploid populations. Except for the intertidal haploid populations of Cancale ($\bar{r}D = -0.06$) and the subtidal haploid populations of Argenton ($\bar{r}D = 0.154$). Finally, No F_{st} value between haploid and diploid populations over 0.05 were observed.

Mean Expected heterozygosity in diploid population varied between 0.320 (intertidal site of Rade de Brest) and 0.516 (intertidal site of Cap Lévy). Mean H in haploid populations varied between 0.207 (intertidal sites of cap Gris-Nez) and 0.403 (subtidal site of Pleubian)

Multilocus F_{is} (inbreeding coefficient) varied greatly between sites, 13 out of the 29 of the studied sites presented positive value of F_{is} (> 0.1), two presented negative value of F_{is} (< -0.1) and the remaining 14 sites showed F_{is} value close to 0. As represented on Figure 4, F_{is} value are highly variable between locus at a given sites and between site, fixed locus was observed in some site.

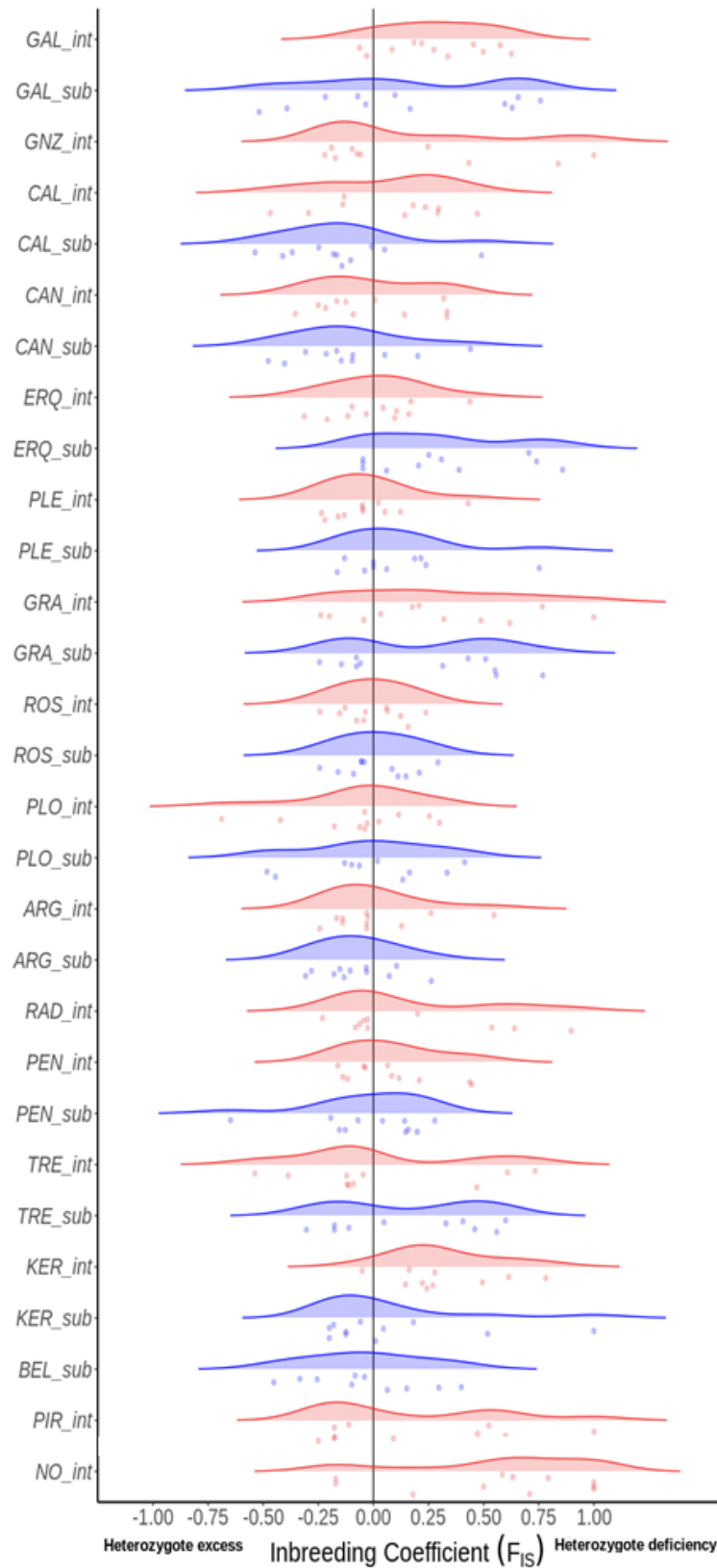


Figure 4: Inbreeding coefficient (F_{IS}) of *P. palmata* in studied sites and locations. Point below density plot represent single locus values. Red indicate intertidal site, blue indicate subtidal site.

Discussion

Our results showed that *P. palmata* propagates mainly by sexual reproduction, as suggested by ploidy ratio (presence of both phases) and population genetic structures. The predominance of sporophytes in the populations, suggest that diploid phase is fitter, at least in certain environmental conditions such as the intertidal environment.

Ploidy bias

In our study, sporophytes are dominant and represent about 70% of sampled blades and confirm previous observations performed in Roscoff and Cap Levy (Le Gall, 2002). These results are partly due to the fact that this species has a unique haploid-diploid life cycle and that only tetrasporophytes and male gametophytes were considered in this study, female gametophytes being microscopic (van der Meer & Todd, 1980). If we assume a balanced sex ratio (van der Meer & Chen, 1979) as well as similar survival rates between young males and females, the number of gametophytes should be doubled, so the diploid frequency would be close to 0.54. In Rhodophyta, the relative frequency of diploid versus haploid thallus in field populations has received considerable attention (see review by Thornber 2006 or Krueger-Hadfield et al. 2020 and in a recent report on *Plocamium sp.* by Heiser et al., 2023). Although these frequencies vary between species and between populations within species, there are some major trends, for example, in the Gracilariales and Ceramiales, diploid thallus are generally dominant (Fierst et al., 2005; Kain & Destombe, 1995) while in some other taxa like *Chondrus sp.*, *Mazzaella sp.* and *Plocamium sp.* haploids are generally predominant (Collén et al., 2014, Bellgrove & Aoki, 2008, Thornnber and Gaines 2004).

The variability of diploid frequency in a population depends on the fertility and the survival of each type of thallus. For species with an isomorphic life cycle, Destombe and collaborators (1989) developed a simple theoretical model. They assumed that if survivorship rates of haploid and diploid adult thallus are equal, the frequency of diploids depends only on the relative fertility of female and diploid plants, and on the survivorship rate of juveniles. Because the aim of the model was to look at the relative proportion of haploid and diploid thallus, the female fertility was then considered as the number of diploid spores produced by the female via the cystocarp. Since multiple carpospores are produced asexually from a single fertilized gamete, haploid fecundity is effectively increased. If the two phases have equal demographic rates, the model predicts a stable distribution with a ratio of $\sqrt{2}:1$ (60% haploids and 40% diploids) (Destombe et al., 1989, Thornnber and Gaines, 2004). Deviations from this distribution are then expected when the demographic rates (mortality, fecundity) between phases vary widely. In our study, the frequencies observed in *P. palmata*, assuming a sex ratio of 1:1, would

therefore be on average 46% haploids and 54% diploids. The mean values of diploids remain much higher than the theoretical one of 0.41 (ratio of $\sqrt{2}$ gametophyte: 1 sporophyte) expected at equilibrium for bi-phasic species with 2 ecologically equivalent phases (Destombe et al. 1998, Thornber 2006). In *P. palmata*, due to the absence of a cystocarp and to the tiny size of the female gametophyte, we expect an extremely low female fertility compared with that of tetrasporophytes. This would give haploids an advantage. However, it can be assumed that the viability of young diploids growing directly on females is much higher than that of haploid spores, which are dispersed and can germinate in unsuitable environments. This could compensate for the imbalance in fertility between the gametophyte and the tetrasporophyte.

This diploid dominance among algal populations has often been attributed to the better fitness of the diploid phase, probably due to heterosis and the masking of deleterious recessive alleles (de Wreede and Klinger in (Lovett Doust & Lovett Doust, 1988), Valero et al., 1992). We can therefore assume that the two phases do not have the same characteristics and that in this species, which has been described as pseudo-perennial due to its holdfast surviving several years (Stévant et al., 2023), sporophytes could survive better than gametophytes. In fact, as the zygote develops directly on the female gametophyte to give a new sporophyte, we should expect the same number of male gametophytes and sporophytes, but this is not the case. The survival of sporophytes would therefore be on average three times higher than that of male gametophytes. These differences between gametophytes and sporophytes can also result from a wide range of factors such as differences in growth rate, light and temperature optima, stress resistance and/or herbivore selectivity or host interactions. These hypotheses are supported by the fact that in our study, in North Brittany, the proportions of diploids were higher in intertidal than in subtidal areas, which could correspond to a better stress tolerance of diploids to desiccation. For example, demographic studies carried out on *G. gracilis* showed that the survival of adult thalli was the most important factor in determining ploidy ratios (Engel et al., 2001). Better survival of tetrasporophytes has been observed in *G. gracilis* (Destombe et al., 1989) and *G. vermiculophylla* (Krueger-Hadfield & Ryan, 2020). This must be investigated further in laboratory experiment by studying sex and ploidy ratio on a single generation, by estimating early stage survival rates of gametophytes or by modeling as suggested by Faes and Viejo in 2003 after a population dynamic and structure survey of a northern Spain *P. palmata* population. Studies combining field surveys with demographic studies should be performed to determine the relative effects of mortality on population structure.

Life history traits of *P. palmata* and mating system

The presence of both phases of the life cycle (gametophyte and sporophyte) in all populations studied, the quasi absence of clones (out of more than 465 thallus analyzed, only one repeated multilocus genotype were observed i.e in Penmarc'h), the Pareto- β superior to 2 in most of the sites, the absence of linkage disequilibrium and the absence of genetic differentiation between phase suggest that *P. palmata* reproduces essentially by sexual reproduction.

The thallus in the *P. palmata* populations studied were probably the result of sexual reproduction (i.e. germination of meiotic spores and zygotes). In fact, no genetic signatures of asexual reproduction were observed, such as linkage disequilibrium, differences in allele frequencies between life history stages, an excess of heterozygotes and repeated MLGs (Guillemin et al. 2008). The MLG values were similar to those found in *G. gracilis* (mean $D = 0.94$, Engel et al. 2004), which reproduces sexually. For half of *P. palmata* populations, the F_{is} values were not significantly different from zero, this result confirms, like the other dioicous Rhodophyta species, that *P. palmata* is an obligate outcrosser (Engel et al. 2004, Krueger-Hadfield 2013) and deviations from panmixia might be link to environmental factor.

Geographical pattern of F_{is} is observed in this species, North-West sites present non-significant F_{is} value indicating that random mating (panmixia) occurs in those sites while North-East and South sites present heterozygous deficiencies. This could be due to the effect of population density or environmental factors increasing dispersion, which will increase probabilities of random mating between Individuals. In the Chilean intertidal populations of the red alga *Mazzaella laminarioides*, geographical patterns of F_{is} have been described (Guillemin et al., 2016). These authors observed significant positive F_{is} in peripheral populations of *M. laminarioides* (ranging from 0.120 to 0.450) and panmixia in central populations. They attributed this pattern to differences in population density. The absence of differences in genetic diversity index observed between sites, or tidal gradient, does not indicate that significant differences in effective population size occurs in the studied *P. palmata* populations. However, as 95% of the *P. palmata* harvested in Brittany occur within the North-West coast, population density is probably higher than in other studied regions. In addition, environmental factors favoring dispersion such as higher water motions, specifics of the North West coast of Brittany (Guillou et al., 2023), could favor random mating by increasing small scale dispersion of spores and spermatia.

In haploid-diploid red algae species, mating between male and female gametophytes from the same tetrasporophyte parental individual is entirely analogous to self-fertilization (Krueger-Hadfield et al. 2016)). However, in our study, the time lag between the maturity of the females, which produce carpogonium a few weeks after the haploid spores germinate, and the maturity of the males, whose

fronds are reproductive after a year, makes self-fertilization unlikely but not impossible. In our study, we observed limited evidence of null alleles. While null alleles may artificially increase F_{is} values at some loci, they do not explain the values obtained in some populations of *P. palmata*. The results obtained show that in most cases, the deficit in heterozygotes is not due to selfing but rather to the genetic sub-structure. Such a tendency is probably the consequence of the low dispersal capacity of this species. Red algae are generally considered to be poor dispersers as the spores and gametes are short-lived, red algal spores lack flagella and spore viability is usually less than 48 h with dispersal distances from 2–10 m (Santelices 1990) and spermatia in at least one species, being viable for less than 6 h after release (Destombe et al.1990). In *P. palmata*, it is interesting to note that in addition to sperm dispersal, unlike other species of Florideophyceae, dispersal is ensured by a single type of spore, tetraspores. These characteristics may favor a fine-scale genetic structure through limited dispersal (due to short-lived tetraspores and non-flagellated male gametes) as suggested by the high F_{st} value observed between diploid populations (See table S1 and Figure S1; but also, Baud et al., article n°2, Chap 2). More on clumped dispersion of aggregate spore and spermatia, linked by a dense mucilage have been observed in laboratory conditions (low water motions). The mucilage was so dense that the spore aggregate was visible to the naked eye (Figure 4).

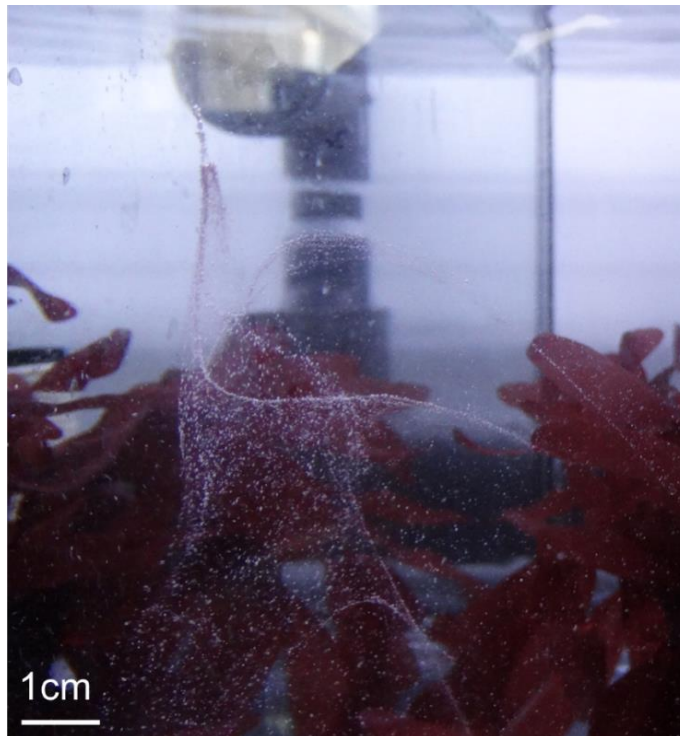


Figure 4: Massive spore release triggered by desiccation stress due to low tide conditions. Sporophytes were collected in March 2019 at Roscoff during a low spring tide and rehydrated in the laboratory. The presence of air microbubbles in the water enabled the mucilage to be visualized. The red coloration of the mucilage is due to the high density of spores.

The signal of selfing observed in Île Grande suggests that dispersal of haploid spores is strongly limited leading to inbreeding effect. As the sporophyte is perennial (Faes & Viejo, 2003), it is possible that microscopic short-lived female gametophytes (van der Meer & Todd 1980) sired by one sporophyte are fertilized by perennial male gametophytes sired by the same sporophyte one year before. Lifespan of sporophyte and male or female gametophytes over years could increase probability of inbreeding, but there is currently not enough data available on population dynamics for this species to conclude further.

How can the evolution of the *P. palmata* life cycle be explained? The interpretation of the life cycle in terms of natural and sexual selection is based on the different properties of spores and gametes (Bell, 1997). These two classes of propagules have not only different, but opposite functions. Spores, which can germinate directly and develop into new thallus, are adapted to dispersal. Gametes, on the other hand, are adapted to fusion. The different types of gametes produced correspond to the two sexes: the female sex produces relatively few gametes, which are large and generally non-motile, while the male sex produces many gametes, which are smaller and often mobile. In the red algae, female gametes are retained on the female and non-flagellated male gametes are dispersed. Recent phylogenetic studies on red algae pointed out that sexual reproduction co-occurs with a zygote

amplification step (the cystocarp). Phylogenetic results suggest that this life cycle modification arose around 700 Ma (Yang et al. 2016). Searles (1980) hypothesized that this evolution of this typical stage of the life history in which the female nurtures and protects the zygote is probably a response to low rates of fertilization due to the absence of flagella. In the Florideophyceae, there is a great diversity of types of cystocarps including various complex cell fusion phenomena that characterize each of these subclasses (Yang et al. 2016). Lee et al. (2016) suggest that the cystocarp innovation could have played a central role in the success and radiation of the Florideophyceae. In the Palmariales, the cystocarp is absent, the female is microscopic and the male is large. These different stages suggest that another reproductive strategy has been selected. In the majority of Florideophyceae, the females are the same size as the males, which makes it easier to capture sperm which are either dispersed passively by water movement (Brawley & Johnson, 1992) or transported by animals, as in *G. gracilis* (Lavaut et al., 2022), and also to participate in the efficient dispersal of carpospores.

In the Palmariales, the reproductive strategy would consist of ensuring the production of a tetrasporophyte, by favoring fertilization on the microscopic female, as the carpogonia in these species have long trichogynes (van der Meer & Todd, 1980) and by ensuring germination of the zygote on the female to the detriment of dispersal. The large blade of male assures the high production of sperm. This could explain the strong genetic structuring observed in the populations. However, given the small number of species observed in this group, we can assume that this strategy could be considered as an evolutionary dead end compared to the other taxa of Florideophyceae.

Acknowledgements

We are grateful to Stacy Krueger-Hadfield and Solenn Stoeckel to providing use access to HapDip GenPop software. This work benefited from the support of the French Government through the National Research Agency with regards to an investment expenditure program IDEALG (ANR-10-BTBR-04), the EU Horizon 2020 project GENIALG (Grant Agreement No 727892), the European Maritime and Fisheries Fund (EMFF) through the POLISTR project to MV and PP and Région Bretagne (PhD grant ARED Domdulse from ED227 Sorbonne-Université/MNHN to AB). We are grateful to the EMBC France partner core facility for its technical support, especially Gaetan Schires and Sébastien Henry. The authors are deeply indebted to the Service Mer & Observation (SMO) of Roscoff for sampling.

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Supplementary Materials:

Table S1: Genetic differentiations between diploid populations of the studied locations and levels.

CAL_int	GAL_int	ERQ_int	PEL_int	GRA_int	ROS_int	PIO_int	ARG_int	PTN_int	TRE_int	KER_int	CAL_sub	GAM_sub	ERQ_sub	PEL_sub	GRA_sub	ROS_sub	PIO_sub	ARG_sub	FEN_sub	TRE_sub	KER_sub	GAZ_sub	RAD_sub	BEL_sub	PRJ_sub	NO_sub	GAL_sub	
0.152	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.725	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.154	0.072	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.146	0.106	0.036	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.136	0.166	0.133	0.088	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.135	0.135	0.095	0.087	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.139	0.139	0.095	0.097	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.134	0.134	0.095	0.097	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.083	0.134	0.119	0.112	0.092	0.087	0.087	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.270	0.083	0.129	0.122	0.118	0.119	0.146	0.191	0.207	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.299	0.221	0.179	0.253	0.254	0.230	0.282	0.252	0.242	0.218	0.218	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.254	0.205	0.127	0.094	0.155	0.140	0.179	0.177	0.177	0.218	0.218	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.004	0.118	0.130	0.133	0.110	0.121	0.124	0.088	0.213	0.213	0.213	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.056	0.124	0.135	0.135	0.104	0.104	0.104	0.104	0.104	0.104	0.104	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.126	0.126	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.114	0.142	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
0.126	0.136	0.096	0.074	0.135	0.109	0.095	0.180	0.184	0.263	0.263	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	0.152	
0.149	0.165	0.120	0.070	0.105	0.046	0.078	0.084	0.184	0.264	0.264	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	
0.102	0.134	0.088	0.060	0.022	0.021	0.022	0.062	0.167	0.247	0.247	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	
0.141	0.099	0.070	0.020	0.027	0.049	0.036	0.162	0.251	0.339	0.339	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	
0.185	0.129	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119
0.255	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175
0.260	0.197	0.150	0.140	0.174	0.185	0.238	0.248	0.321	0.357	0.357	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141	
0.183	0.183	0.134	0.130	0.134	0.138	0.155	0.248	0.321	0.357	0.357	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248
0.227	0.172	0.120	0.080	0.121	0.154	0.222	0.205	0.220	0.294	0.294	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191
0.264	0.169	0.090	0.088	0.121	0.154	0.222	0.205	0.220	0.294	0.294	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191
0.267	0.206	0.165	0.193	0.276	0.256	0.281	0.291	0.225	0.275	0.275	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238
0.241	0.198	0.168	0.124	0.281	0.269	0.277	0.333	0.358	0.377	0.377	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258	0.258
0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275
0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327	0.327
0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343	0.343

Fst values are scaled by color from low (blue) to high (orange).
Upper part of the matrix show p-values of the pairwise Fst

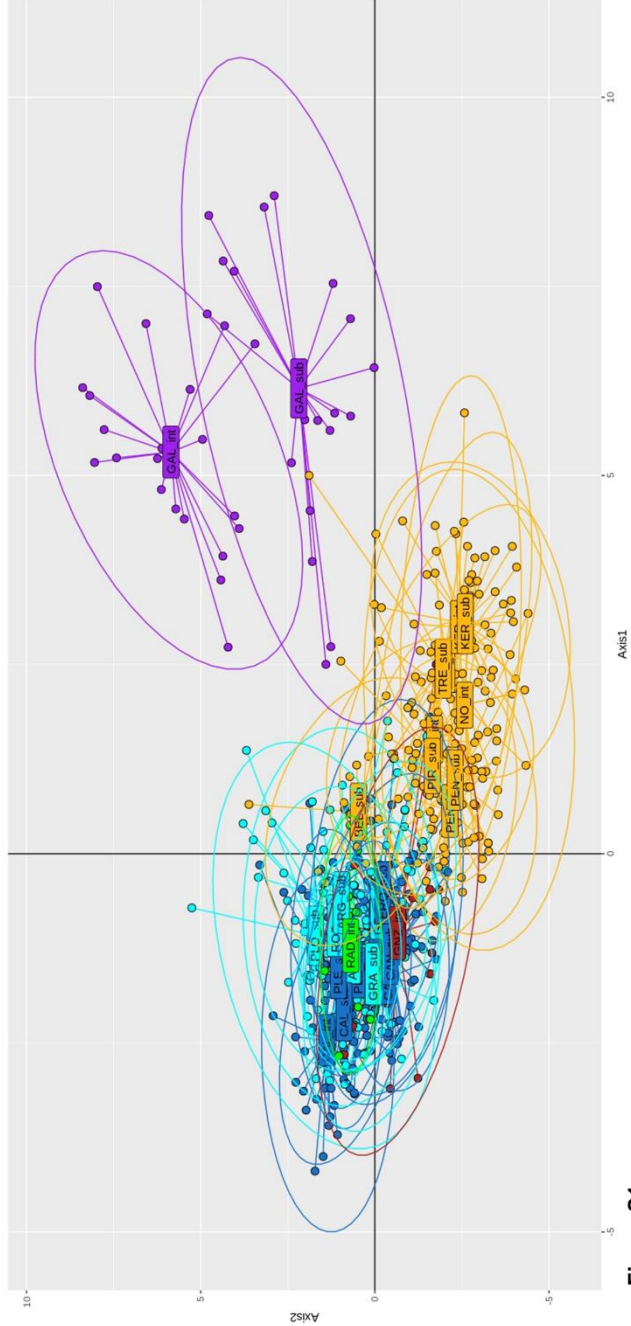


Figure S1 : Scatterplot of the first two components (Axes 1 -22.1% and 2 -14.8%) of the discriminant analysis (DAPC) performed on 601 individuals with 11 microsatellites loci using sampling location as prior clusters. Sampling regions are shown by different colours (Blue for North east Brittany, Cyan for North west Brittany, yellow for South Brittany, green for Rade de Brest, red for Northern English Channel and purple for Iriande). Sites by the first three letters, tidal level after the underscore of the site names in the centroid of the inertia ellipse. Each point corresponds to a single individual.

2.Maitrise du cycle de reproduction en laboratoire (Article n°5)

L'article 5 ci-dessous regroupe les travaux réalisés au cours de ma thèse pour mettre au point les techniques de contrôle des phases du cycle de vie de *Palmaria palmata* ainsi que pour comprendre les bases du succès reproducteur. Il n'a pas vocation à être publié en l'état mais il sera soumis pour publication dans un journal d'aquaculture avec des expériences complémentaires sur la fécondation contrôlée et des tests en conditions de culture en écloséries à plus grande échelle.

Article n°5 : Improved control of the life cycle of selected strains of the red alga *Palmaria palmata* and fertilization success in hatchery conditions.

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Abstract

Increasing interest in marine resources and climate changes threatening exploitation of seaweed wild populations forces us to domesticate new species of seaweed. Control of sexual reproduction is a milestone in the domestication process of a new species such as the red alga *Palmaria palmata*. This economically important species is harvested on foreshore for food, feed and cosmetic markets. Given the unique and complex life cycle of the species, further knowledge on its sexual reproduction is needed in order to unlock the development of its large scale at-sea cultivation and initiate selective breeding by crosses. Herein, we investigate the effect of temperature variations and thalli size on controlled induction of sporogenesis and gametogenesis of selected strains over several laboratory experiments. Thallus size was shown to be the principal factor controlling sporocyst and gametocyst production in the selected strains. We then studied fertilization success in hatchery conditions and showed that further knowledge on reproduction timing is required to fully control this step of the life cycle. Moreover, no differences between sporophyte and gametophyte growth were observed in the studied periods (three first months of the life stage life). Reproduction control (sporogenesis and

gametogenesis) of a selected strain opens the door to selective breeding of this economically important seaweed, which will be a step further toward its domestication.

1. Introduction

Control of sexual reproduction, a milestone in the domestication process

Through the study of land plant and animals domestication, that started over ten thousand years ago and more recently for seaweed which domestication started about four centuries ago, consensus have been made on the requirement of reproduction control to initiate the domestication process of a new species (Larson et al., 2014; Valero et al., 2017; Zeder, 2015). Identifying sexual or asexual reproduction mode of a species allowed humans to select and maintain a phenotype of interest in the targeted species by isolating the cultivated populations from the wild populations (Diamond, 2002). Identification of asexual reproduction in the targeted species allowed farmers to quickly select superior genotypes that will be fixed and maintained in the new environment created by humans for the cultivation of the targeted species (J. P. Van Der Meer, 1983). The use of asexual reproduction has its limits, the adaptability of the selected strain is restrained to their plasticity and lack adaptability in a changing environment. However, sexual reproduction allows improvement of the cultivated populations by crosses between different selected strains and to maintain diversity, and then adaptability to environmental changes in cultivated populations (Valero et al., 2017). Therefore, controlling sexual reproduction of selected strains is a necessary step toward achieving the domestication process of a new species.

A new species to domesticate

Recent interest in marine resources have led to increasing exploitation of wild populations, in order to ensure supply for this expanding market and protect wild populations of exploited seaweed domestication of new species is needed (ref). *Palmaria palmata* (L.) Weber & Mohr, commonly referred to as dulse, is a well-known and highly valued red macroalga distributed along both sides of the North Atlantic Ocean, from the Arctic to cold-temperate regions (Lüning, 1990). The natural populations of *P. palmata* on the Breton coasts, its major region of commercial harvesting, have the particularity of being present at two distinct levels of the coastline (Baud et al., article 2). They are present in the mid-littoral area (tidal zone), where they develop attached to the rock or as epiphytes on Fucales. They are also present in the sublittoral (below the tidal zone) where they are found exclusively as epiphytes on the stipes of *Laminaria hyperborea* (Stévant et al., 2023). *P. palmata* is a species of commercial importance with historical records of use as food dating back several centuries

in the Viking sagas and in Ireland (Mouritsen et al., 2013) to the current harvesting of dulse by hand-picking on the foreshore in Western Europe as well as Canada (New Brunswick and Nova Scotia) and USA (Maine). Because the demand for *P. palmata* increases and future sustainable commercial developments cannot rely solely on wild-harvested biomass, significant efforts have been made by research and industrial actors to cultivate the species (Grote, 2019; Stévant et al., 2023). However, this species has a unique life cycle which have limited the development of efficient at-sea cultivation methods, today its production is mainly based on harvesting of wild populations or on small-scale cultivation by vegetative propagation in a few land-based farms (Stévant et al., 2023).

Life cycle of P. palmata

Palmaria palmata is considered pseudo-perennial since the fronds grow, reproduce and senesce from a holdfast that survives multiple years. It has a complex heteromorphic diplohaplontic life cycle (Fig. 1) which is unique among the Rhodophyta and had remained a mystery for phycologists for a long time. Foliose male gametophytes and tetrasporophytic thalli were described by van der Meer & Chen in 1979, but the life history of *P. palmata* was first revealed by van der Meer & Todd, 1980, by crossing a red-pigmented female gametophyte with a mutant green male. After fertilization and development, a green diploid tetrasporophyte could be observed attached to the red-pigmented female haploid thallus. The life history of *P. palmata* has been further described in the scientific literature, focusing on improving the cultivation of the species (Grote, 2019; Werner & Dring, 2011). According to Pueschel, 1979 and van der Meer and Todd (1980), haploid tetraspores develop within the sori of the frond of diploid tetrasporophytes through meiosis. The haploid tetraspores germinate into either microscopic female or blade-like male gametophytes of a similar morphology to diploid tetrasporophytes. The female gametophyte develops oogonia with trichogynes (i.e. hair-like structures) to catch spermatia (i.e. non-motile male gametes) released from a mature male gametophyte (at least one year old). The zygote resulting from the fertilization of a female gamete by a spermatium grows into a diploid tetrasporophyte from the encrusting female gametophyte, which is overgrown at an early stage as the tetrasporophyte develops its own discoid holdfast. As opposed to the commonly observed pattern within the Rhodophyta, the carpogonium (female sexual organ) does not develop into a carposporophyte following fertilization, i.e. a diploid microscopic structure releasing diploid carpospores into the environment from which like the majority of macroalgal species, *P. palmata* relies on the production and release of spores for the reproduction, dissemination and colonization of substrata.

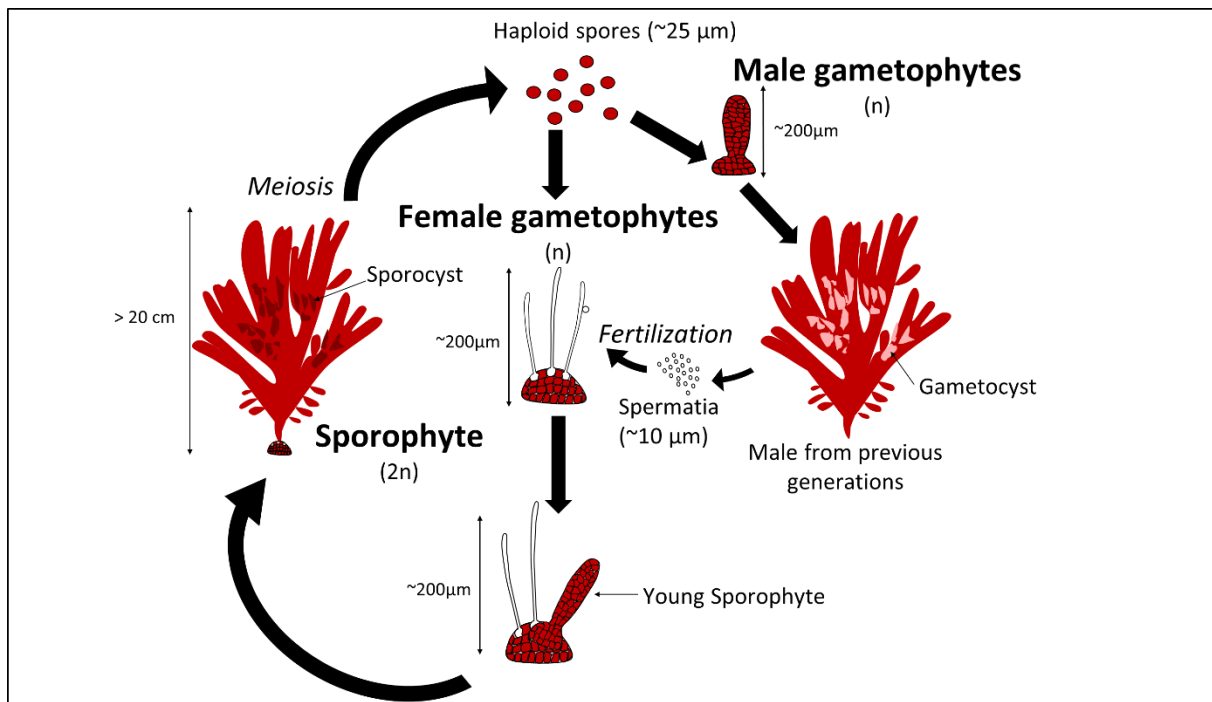


Figure 1: Life cycle of *Palmaria palmata*. Modified from van der Meer & Todd (1980). At maturity diploid sporophyte will generate spores by meiosis. After fixation, spores develop either in haploid male or female gametophytes (1:1 ratio). Male gametophytes will have to grow during at least one year to produce spermatia. However, between two and three weeks after germination, reproductive cells of the female gametophytes develop a cellular extension known as a trichogyne which will allow spermatia from older male gametes to bind on it for fertilization. Then a new sporophyte will develop over the female gametophyte, eventually overgrowing it.

At sea cultivation of P. palmata

At sea cultivation trials attempted during the past two decades were based on seeding cultivation substrates by sporulation of fertile wild sporophytes collected during the reproductive season. This practice led to the development of only male gametophytes on the seeded support in the absence of female fertilization in hatchery conditions. One of the major problems of these first trials was low spore survival and heterogeneity of substrate seeding density. Those issues were partially resolved by the work of Schmedes et al. (2019) who significantly improved spore settlement homogeneity and survival. Another issue of those first trials was the dependence on the supply of fertile wild sporophytic thallus at the beginning of Fall to ensure Winter and Spring at sea cultivation after a few months of hatchery. This issue could be resolved by controlling sporogenesis induction to produce seeded support early in Fall and ensure longer at-sea growth phase (Werner and Dring 2011; Schmedes 2020). Moreover, control of gametogenesis would ensure the supply of fertile male sporophytes and then female fertilization to increase at sea cultivation yields (Schmedes 2020).

Control of sporogenesis and gametogenesis for at-sea cultivation and domestication

In wild populations, to become fertile and induce the maturation of spores, *P. palmata* has specific environmental requirements regarding temperature and light conditions, which influence the timing of reproduction (phenology). The development and maturation of tetrasporangial tissue (Fig. 5) and gametangial was triggered by short day conditions, i.e. 8 h of light per day and a temperature of 10 °C or below (Pang & Lüning, 2006; Schmedes, 2020). However, development of tetrasporangia was also reported under a photoperiod of 12:12 h (light:dark) but at a lower irradiance, i.e. 5 to 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, than those used in the above-mentioned studies (Werner and Dring 2011a). This suggests that a prolonged low exposure to PAR could be the actual trigger of fertility induction in *P. palmata*. This photoperiodic control of the reproductive development combined with a rather specific temperature requirement narrows the reproductive window, as well as the distribution of the species to temperate and arctic regions which are characterized by a strong seasonality. As argued by Pang and Lüning (2006), this timing may be a strategy to ensure vigorous vegetative growth in Spring under optimal irradiance and daylength conditions. The seasonality of tetrasporophyte and male gametophyte maturation has been investigated in several studies. In mid-Norway (Trondheimsfjord), mature tetrasporophytes were found from October to May, with the occurrence of mature specimens peaking from December to February (up to 50% of all thallus). Mature male gametophytes were observed during a slightly shorter time frame, i.e. from November to April, with a peak in January ((Bøe, 2019; Schmedes, 2020). Monthly samples of *P. palmata* in the Isle of Man revealed a similar pattern of mature tetrasporophytes and male gametophytes being observed from November to July (Kain 1982). This is also supported by observations of mature specimens (tetrasporophytes and male gametophytes) from late autumn to late spring along the Eastern coast of the North Atlantic (Edwards & Dring, 2011; Faes & Viejo, 2003; Le Gall, 2002; Pang & Lüning, 2006; Schmedes, 2020; Werner & Dring, 2011) and Greenland ((Rosenvinge, 1898; Jónsson 1904). However, Bird and McLachlan (1992) describes *P. palmata* as fertile in summer to late autumn for the Maritime Provinces of Canada, although “well-developed and fertile fronds are found throughout the year”.

Considering major differences of environmental conditions between tide levels, such as temperature and light intensity, differences in reproduction periods could be observed in the wild populations of *P. palmata* along the tidal gradient. This must be investigated by in situ monitoring of temperature to characterize difference in variations between tidal level and phenology studies of intertidal and subtidal populations of *P. palmata* in Brittany at the beginning of the reproduction season. Moreover, previous experiments were made only on wild thallus, however in the context of the domestication process the control of reproduction allows us to free ourselves from wild populations for seed

production (Valero et al., 2017). This is achieved by controlling reproduction of selected strains and ensuring their viability over multiple seasons. However, no data on *P. palmata* life span, minimum size required for maturation or ability to reproduce over several seasons is available for this species. Conservation of red algae strain by vegetative propagation also seems to inhibit reproduction capacity after several years of storage (West, in Andersen, 2005). This must be tested by inducing maturity on clonal strains over multiple years to ensure viability for spore production over seasons. Identifying abiotic (temperature variation) and biotic factor (size) involved in early induction of the reproduction could help improve time of maturity induction in selected sporophytes and male gametophytes strains to improve hatchery of *P. palmata* and initiate varietal improvement by crosses.

Controlled fertilization of female gametophytes and life cycle phase fitness

The work carried out by van der Meer and Todd in 1980 highlighted that the reproductive cycle of *P. palmata* is characterized by marked sexual dimorphism and the absence of a zygote multiplication phase. The spores germinate into male and female gametophytes (50% male and 50% female). Male gametophytes quickly develop a thallus while female gametophytes remain microscopic. After fertilization, the tetrasporophyte develops directly on the female gametophyte (Van der Meer & Todd, 1980). The characteristics of the reproduction cycle of *P. palmata* imply that in the context of seeding of culture substrate by sporulation, only the male gametophytes (50% of the spores) reach a sufficient size for harvesting. A study by Schmedes carried out in 2020 showed that the addition of a solution of spermatia to a culture of male and female gametophytes of *P. palmata* significantly increased the number of seedlings present on their culture medium. In order to validate this technique for improving sea cultivation, it is necessary to study the ploidy ratio of thalli developing on substrates exposed to a spermatia solution. Considering the delays in tetrasporophyte growth in those hatchery conditions, we might expect differences in size during the first month of seedling growth. However, tetrasporophytes being diploid are supposed to hold a better fitness than haploid phases (Destombe et al., 1989, 1993; Guillemin et al., 2008; Otto & Goldstein, 1992; Valero et al., 1992). Then difference in size of thalli from the same sporulation and fertilization success should be investigated to test differences in fitness/vigor between phases.

In this study, we aimed to improve control of the unique life cycle of *P. palmata* in hatcheries. We first studied field temperature variation and phenology of *P. palmata* wild populations along the tidal level. Based on those observations, we experimentally tested the effect of temperature variations and size on sporogenesis of a selected strain. Then we investigated fertilization success and the growth of the different life cycle phases in hatchery and farm conditions.

2. Material and Methods

2.1 Temperature and phenology monitoring along the tide gradient

Sampling for determining the phenology variation along the tide gradient was carried out at the beginning of October 2019 in Roscoff and Plouguerneau during spring tides. Thirty thallus were randomly collected in intertidal populations and as well in subtidal populations. We used brown seaweed belts as height indicators. Intertidal populations of *P. palmata* develop on *Fucus serratus* and are separated to subtidal populations, which develop on *Laminaria hyperborea*, by the belt of *Laminaria digitata*. Back from the field, reproductive status evaluation and ploidy assessment of all thallus was done using binocular lens checking the presence of sporocysts or gametocysts. Determination of vegetative individual ploidy was done using flow cytometry following (Guillemin et al., 2008). On field qualitative observations of reproductive structure were then made on the same sites at both tide level in October 2020, 2021 and 2022.

Temperature monitoring was conducted in Roscoff subtidal population (h= 1.05 m) and intertidal population (h= 2.42 m) as well as in Plouguerneau subtidal population (h =1.1m) and intertidal populations (h =2.08 m) from February 2020 to October 2022. Temperature was measured every ten minutes over the monitoring periods using Onset HOBO probes. Daily temperature mean, variations, maximum and minimum were estimated using Rstudio basic stats (ref package), graphic was generated using Plotly package (ref).

2.2 Seaweed material for reproduction control

Isolates of reference strains, collected in Roscoff in 2017 and in Plouguerneau in 2019 for collection purposes, were used to study the reproductive cycle and vegetative growth of *P. palmata* are the following :

SB7-AUG is a tetrasporophyte collected in November 2017 from the Sainte Barbe site in Roscoff and placed in the collection by Drion in 2017 This strain, stored for 1 ½ years in a 40 ml flask at 13°C under a light intensity of less than 10 $\mu\text{m}^2/\text{sec}^{-1}$ without renewing the medium and under a long day photoperiod (16 hours day/8 hours night), was successfully returned to culture. This isolated strain was selected for its rapid vegetative growth in lab conditions. During the vegetative growth phase preceding our experiments, it shows daily growth rates of up to 14%.

PLO-26-G is a male gametophyte isolated in the mediolittoral population of Plouguerneau in October 2019. This strain was notably selected on its ability to produce marginal regrowth during the conservation phase in a 12-well plate and its rapid vegetative growth (Daily growth rate up to 15.5 %). After more than 2 years of conservation in 12-well plates, the strains were successfully returned to culture.

These strains are preserved in the collection of *P. palmata* culture isolates described in Article 6-Chapter 3

To obtain enough material of unialgal clonal cultures, thallus apices from the collection were grown at 13°C in different volumes. Right after isolation the apex were cultivated in 1L flasks of enriched seawater (PES 1mL/L; (Provasoli et al., 1956)), under a photoperiod of 12:12, and a light intensity of 45 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. When total weight of clonal thalli reached 10 g they were transfer in 10L tanks with the same culture conditions as 1L flask. When culture biomass reached 40 g, they were transferred to 40 L cylinder with continuous 5 μm filtered seawater income and then to 300L cylinder with the same conditions. In 40 L and 300 L cylinders, photoperiods were set to long days (16:8) and 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to ensure vegetative growth of thalli.

2.3 Determination of reproductive developmental stages of selected strains

Sporogenesis

In order to assess the maturity stage of sporocyst development, we based our scales on the description of *P. palmata* tetrasporangia ultrastructure by Pueschel in 1979 (Cole & Sheath, 1990) , which was also used by Schmedes in 2020 for the same purpose. This description recognized three majors phases, (i) formation of the tetrasporocyst by mitosis of an apical cell of the cortex, (ii) tetrasporocysts undergoing meiosis and (iii) empty tetrasporocysts after spore release.

Our scale relies on surface tissue observation by using binocular lenses. Thalli without any reproduction structure were assigned as Non-fertile (nf), thalli with a majority of forming tetrasporocyst which are recognizable by large empty cells were assigned as Stage 0, thalli with a majority of tetrasporocysts undergoing meiosis which were characterized by deep red coloration were assigned as Stage 1+ (Fig.2).

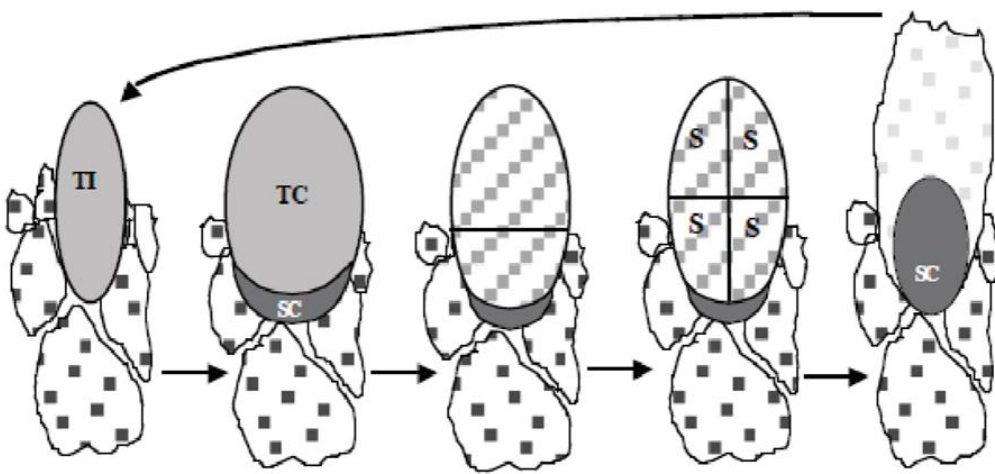
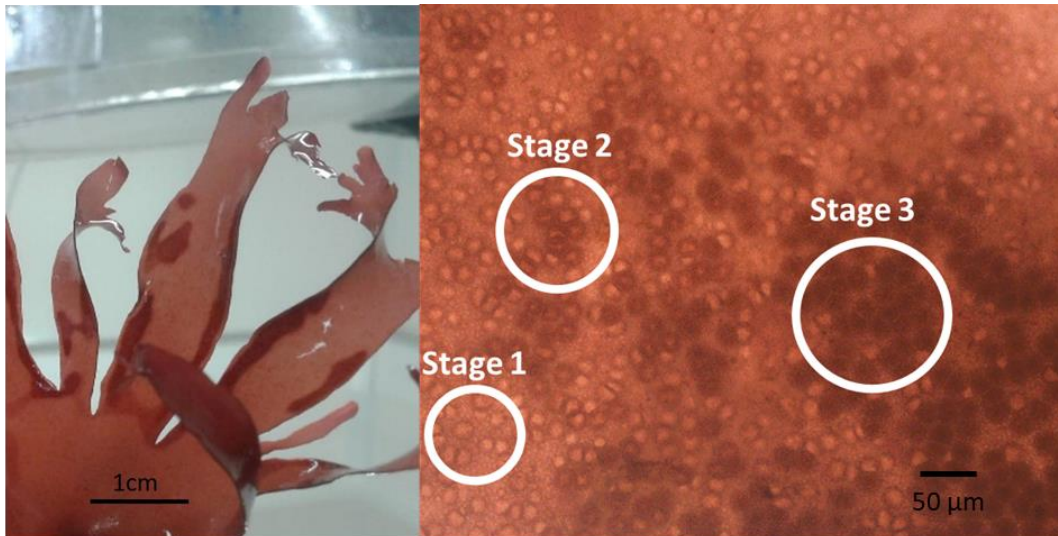


Figure 2 : SB7-AUG thalli after 56 days of induction see sporogenesis experiment 1 (Left) and Microscopic view of the 3 tetraspores development stages (100x) of a maturing *Palmaria palmata* sorus bearing fertile tetrasporangia. (Below) Diagrammatic illustration of the tetrasporangial development in Palmariaceae. TI: terrasporiangular initial develops by cell enlargement of apical cell in cortex filament and develop into tetrastorocyte (TC) by mitose. Stalk cell (SC). Meiosis results in four tetraspores (S) within the tetrasporangia embedded in the cortex. After sporangial rupture, a second sporangium forms, indicated by the arrow (Cole and Sheath,1990) Diagram from Schmedes, 2020.

Viability of spore released from induced sporophytic strain was evaluated by observation of gametophytes development from spore solution obtained by incubating fertile fragment in 140ø petri dishes for 5 days with autoclaved seawater enriched with Provasoli medium's.

Gametogenesis

Except the work of Shmedes in 2020 few data are available on how spermatia form from cortical cells during gametogenesis, in an attempt to further describe this process we did observation of fertile male gametophytes using Confocal laser scanning microscope (CLSM). We used Lipophilic carbocyanine DiOC18 (DiO), which binds to the membrane lipids of the cells of spermatia and cells undergoing differentiation and show green fluorescence (ref). Observation of live spermatia release from fertile gametophytes using this probe, 0.1 mg/mL final (10 min), was done using a CLSM and chloroplasts were localized from the autofluorescence of chlorophyll (red). CLSM acquisitions were conducted on a Leica inverted TCS SP8 equipped with a compact light supply unit (solid-state lasers 405/488/552/638 nm), LIAchroics beam splitter, regular PMTs detector, and objectives HC PL APO 40x/1.10 W motCORR CS2, HC PL APO 20x/0.75 IMM CORR CS2) on the MerImage platform at Roscoff Biological Station.

2.4 Improved control of sporogenesis and gametogenesis.

In order to estimate the impact of temperature variations and size but also viability of strain over years, laboratory experiments were carried in 2020, 2022 and 2023.

A first experiment was carried out in 2020 on SB7-AUG to ensure viability of the strain to reproduce, with a stable temperature (8°C), low intensity artificial light (25 $\mu\text{mol}/\text{m}^2/\text{sec}$), a short photoperiod (8 hours day, 16 hours night) and regular renewal of the sea water (2 volumes per day, filtered at 1 μm). Presence of sorus and maturation stage of thalli were observed at the end of the experiment, thalli were grouped by reproduction stage and fresh weight of each group were measured. Fragment of thalli bearing sorus were used to produce spore solution to observe spore settling and gametophyte development in Petri dishes.

During winter 2022 a second experiment was carried out on male gametophytes and sporophytes to test the effect of temperature variations conditions. First PLO 26 G Thalli of approximately 30 cm were incubated in a 300 L cylinder supplied with seawater filtered at 5 μm (volume renewed 4 times per day) In January 2022. The culture cylinder, placed outdoors, was exposed to natural light with a short photoperiod (8h days:16h nights) and an average temperature of 8°C with daily variations of 2°C. Then sporophyte strain SB7-AUG, with 30 cm thalli and a slightly different photoperiod, was carried out in a 300L cylinder also exposed to natural light in February 2022. Presence of sorus and gametocyte on thalli were checker regularly after day 20 of cultivation.

Finally, a third experiment was conducted in full controlled conditions in 2023 in order to test if either size thalli or temperature variation have significant effect on sporogenesis and gametogenesis of

selected strains. Fragments of thalli from the sporophyte (strain SB7-AUG) and the male gametophyte (strain PLO-26-G) were incubated in 200 L cylinders continuously supplied with seawater filtered at 5 μm (2 changes per day) between February 28, 2023 and April 17, 2023. Two different temperature conditions, a “stable” condition (13°C) and a variable condition (13°C/9°C, daily variations) were tested with a short photoperiod and low light (approx. 25 $\mu\text{mol}/\text{m}^2/\text{dry}$) provided by LED lighting. Temperature variations were ensured by the addition, twice a day, of cold sea water from a tank thermostated at 8°C. The temperature profile of the two conditions was monitored using a HOBO probe. For each condition and ploidy, around sixty thalli were incubated. The maximum size, fresh weight and tissue maturity of the sporophytes were measured. The effect of maximum fragment size and fresh weight on the degree of maturity of thalli was tested respectively with a non-parametric Kruskal-Wallis test and an ANOVA.

Production of thalli of PLO26G strains by vegetative propagations resulted in almost identical thalli length, the effect of size could not be assessed for male gametophytes, we only noted apparition of gametocyst by visual control in the two tested temperature conditions.

2.5 Fertilization trials on seeded support

Around twenty tetrasporophytes collected at the end of October 2022 from the population of Sainte Barbe in Roscoff were used for this experiment. The spores were seeded on ropes (\varnothing 5 mm in Kuralon) placed on PVC frames at the bottom of the culture tank. The fertile fragments of tetrasporophyte thallus with sori were cut and cleaned with absorbent paper in order to limit the intake of contamination into the cultures. The seeding tank with a capacity of 50L was filled with FSW at 5 μm enriched with Provasoli ES and illuminated by cold white LEDs with a low light intensity (10-15 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$). The FSW medium of the hatchery tanks was changed weekly. The fragments of fertile thalli were incubated for five days, to ensure the homogeneous distribution of the spores and bubbling aeration was added to the culture system. Four weeks after seeding, fragments of fertile male gametophytes carrying gametocysts were added to the cultures. The presence of spermata in the culture tanks was observed the day after the addition of male gametophytes, and the fragments of fertile thalli were removed. After 45 days in the hatchery, the seeded ropes were transferred to a conical culture cylinder of 200L capacity supplied with 5 μm seawater in order to monitor well development and growth of male gametophytes and sporophytes. Another part of these supports was deployed on the seaweed farm within Morlaix bay in December 2022 in coculture with seedlings of *Saccharina latissima* and *Laminaria hyperborea*. In April 2023, random tissues sampled were collected on thalli along the cultivation line in the seaweed farm for ploidy determination by flow cytometry, using the same protocol as Guillemin et al., 2008. In June 2023, all thallus present on a 30-centimeter

ropes segment, cultivated in the 200L conical cylinder, were detached and placed on a backlight translucent plate in order to be imaged to estimate their surface area using the ImageJ software (Collins, 2007). Tissue samples of all thallus were used to determine ploidy of thalli using flow cytometry using protocol developed by Guillemin et al, 2008.

3. Results and Discussion

3.1 Temperature variation and phenology of *P. palmata* along the tide gradient.

Mean and variation temperature profile in the studied sites and tide level from September to November in 2020 and 2021 are presented in Figure 3 and 4. This period corresponds to the maturation season triggered by lower temperature and shortening photoperiod.

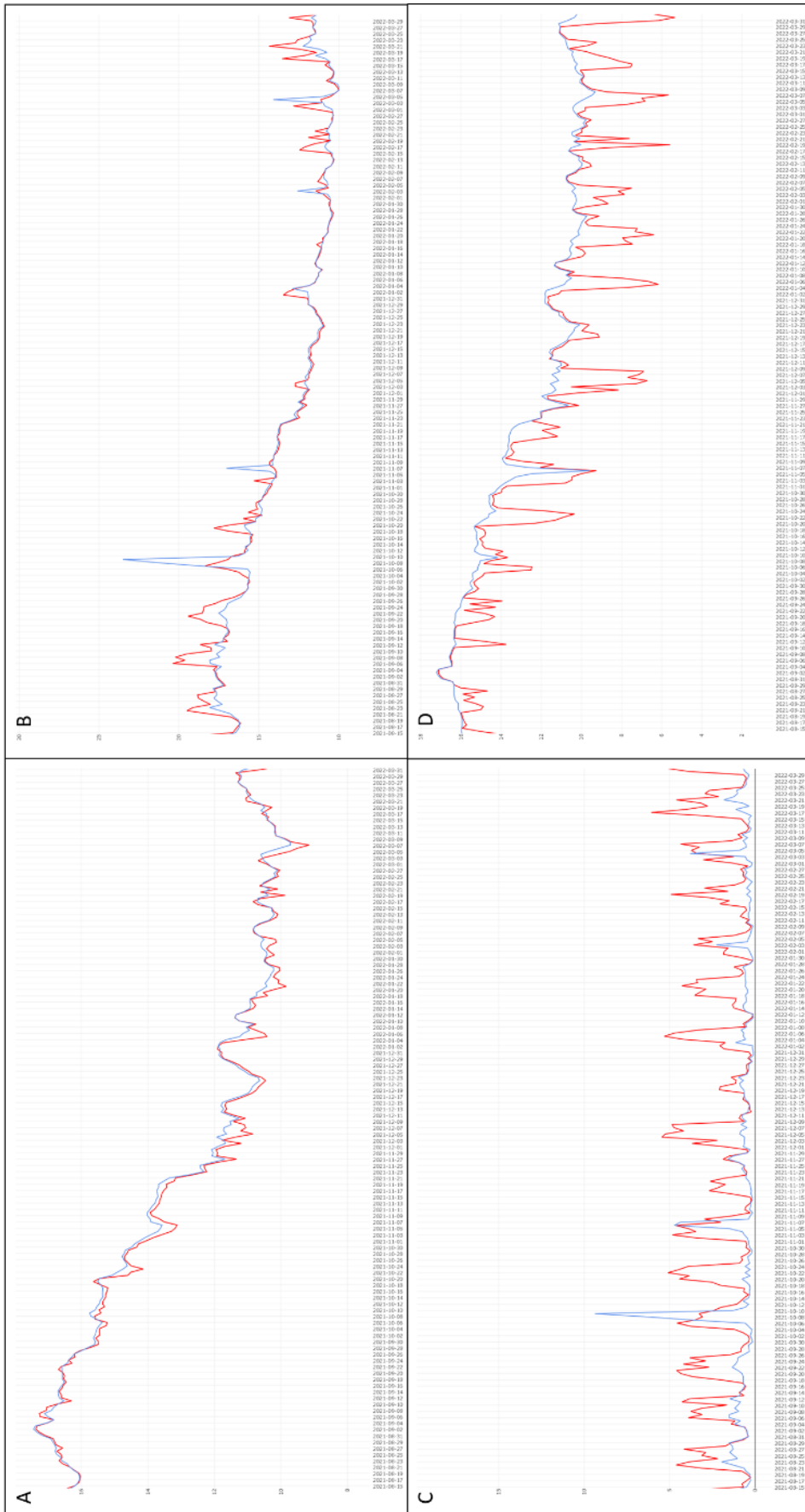


Figure 3 Temperature monitoring using HOBO probes in the intertidal (Red) and the upper sublittoral (Blue) between August 2021 and March 2022 at Roscoff site. A: daily maximum T°C; B: daily mean T°C; C: daily minimum T°C; D: daily T°C range.

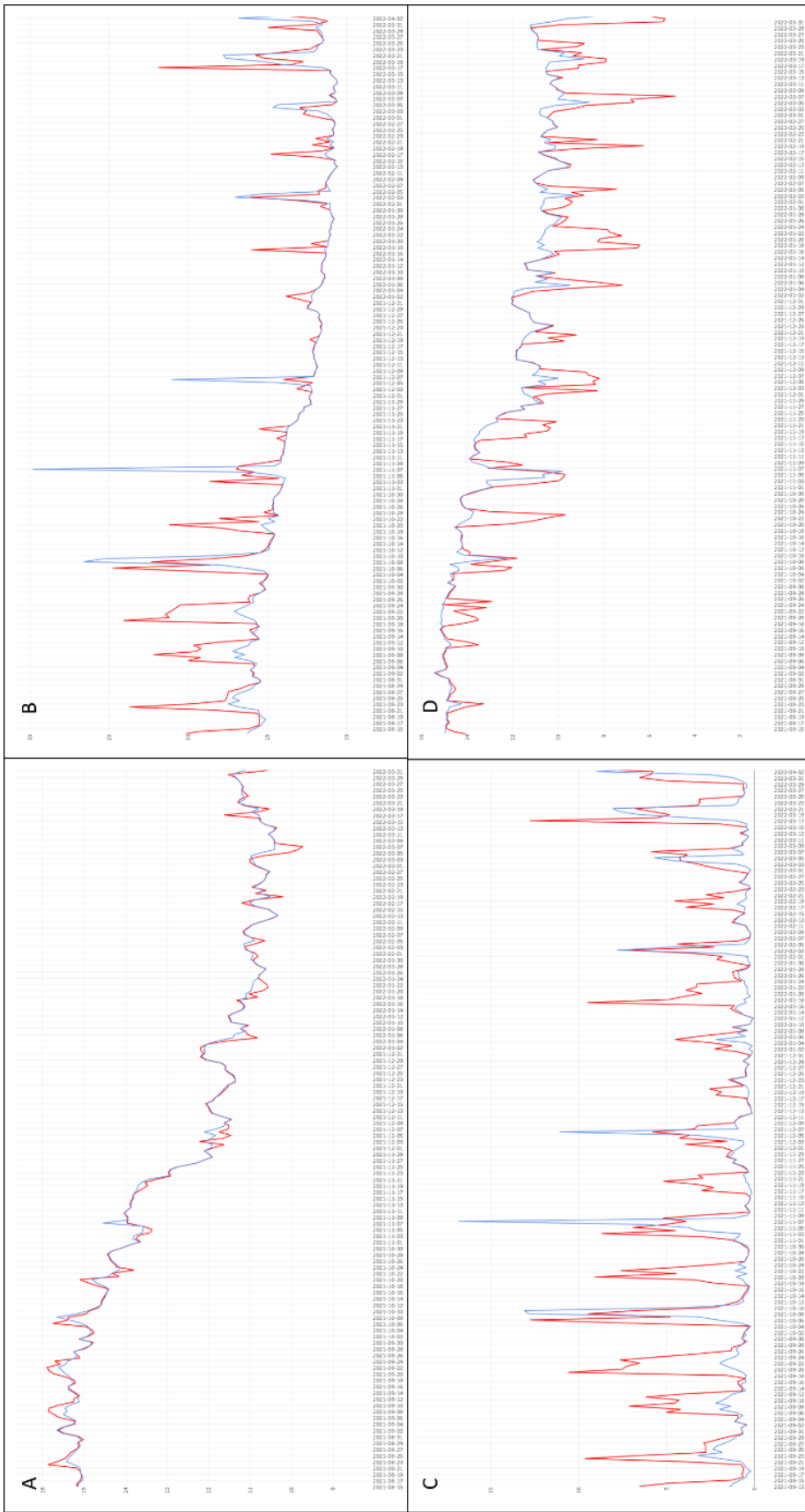


Figure 4 Temperature monitoring using HOBO probes in the intertidal (red) and the upper sublittoral (blue) between August 2021 and March 2022 at Plouguerneau site. A: daily mean T°C; B: daily maximum T°C, C: daily T°C range, D: daily minimum T°C.

As shown on Fig. 3 and Fig.4, temperature mean, min, max and range greatly differed between intertidal and subtidal level during the studied period which correspond to the progressive drop in T° and photoperiods leading to fertility of Brittany wild populations. These different patterns observed create contrasting temperature conditions for the induction of maturity depending of the tidal level.

Over the thirty thallus randomly collected at the intertidal level, 15 were identified as fertile sporophytes in Roscoff, and 9 were identified as fertile sporophytes in Plouguerneau. At the subtidal level, all thallus were vegetative in both sites.

After flow cytometry analysis of vegetative thallus, diploid frequency in Roscoff and Plouguerneau were respectively 0.9 and 0.8 at intertidal level. However, at subtidal level, diploid frequency was 0.7 in Roscoff and 0.5 in Plouguerneau.

Qualitative field observations in the same sites carried out in October of 2020, 2021 and 2022 indicated that mid-littoral populations of *P. palmata* have an earlier reproduction period than sub-littoral populations. In Ushant, fertile sporophytes were observed at infralittoral (~6m) in July (personal observation).

3.2 Gametogenesis

As shown in Figure 5, the sperm production zone (shown by DiO green fluorescence) does not display any particular structure. Cortical cells (characterized by the natural fluorescence of Chlorophyll-a in red, marked as A 1) are progressively transitioning to reproductive spermatia by losing chloroplast (A 2) and are then released.

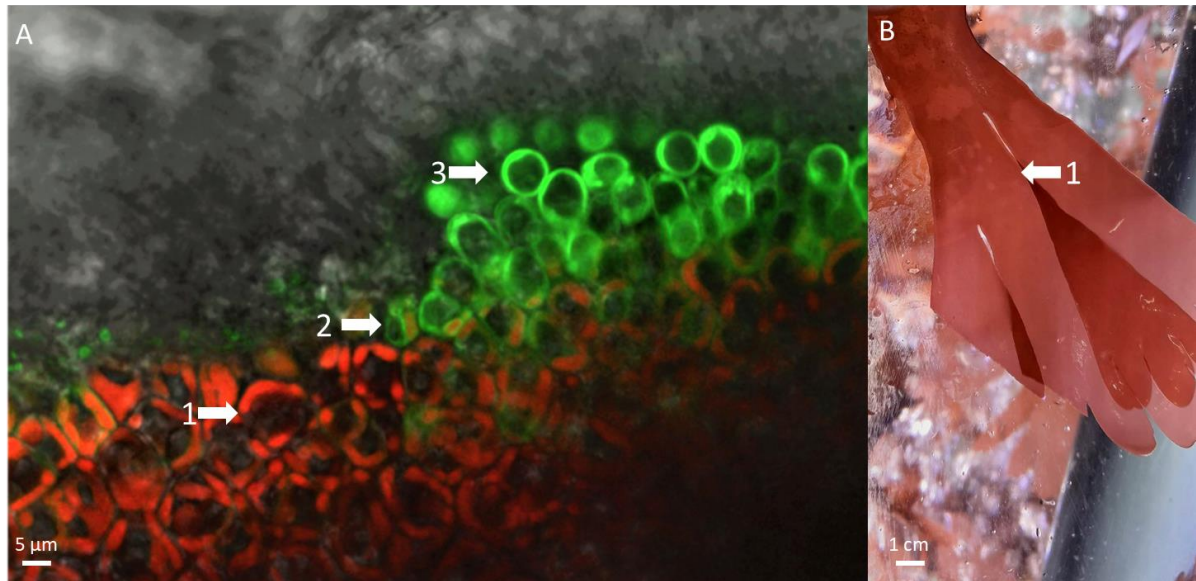


Figure 5: A: Confocal microscope image of the sperm production zone in a male gametophyte of *P. palmata*. 1 : The cortical cells of the thallus are highlighted by the natural fluorescence of Chlorophyll-a (in red). 2 : cells undergoing differentiation show green fluorescence corresponding to the lipophilic carbocyanine DiOC18 (DiO) which binds to the membrane lipids of the cells, fading chloroplast are seen on the right part of the cells. 3: Fully formed spermatia ready to disperse. Credit: Sébastien Collin. B: Photo of mature male gametophyte thalli of PLO-26-G strain in winter 2022, after 28 days of exposure to short day conditions. 1: White areas correspond to the presence of spermatocysts on the thallus surface.

Observations of mature male gametophytes in the field seem to indicate that there is a difference in visual appearance between the areas of sperm production depending on their maturity (before and after emission of sperm). In addition, mass sperm release events (clumps of sperm visible to the naked eye) have been observed in the laboratory on wild thallus. It is therefore possible that spermatia are emitted continuously into the environment, in small quantities and in very large quantities following stress (emersion during the tide). However, monitoring the development of these sperm production zones during gametogenesis experiments seems difficult to implement. Indeed, the difficulty in identifying areas which have already emitted spermatia, areas which produce spermatia complicates the acquisition of the state of maturity of male gametophytes.

3.3 Controlling sporogenesis and gametogenesis of selected strains

3.3.1 First experiment

During sporogenesis, the development of the sori is not homogeneous over the entire thallus, it occurs progressively from the inside to the outside of the mature zone, the most mature cells being found in the center of the sori (Figure 2). This experiment made it possible to obtain sori in 50 days on a selected strain, produced by vegetative propagation in lab after 2 years of conservation in collection. The thalli used for this experiment measured between 2 and 12 cm. A release of spores made it possible to obtain viable male and female gametophytes (development of fronds in males and trichogynes in females). The smallest thalli (between 5 and 2 cm) did not have reproductive tissues.

3.3.2 Second experiment : “Natural” conditions

After only 28 days of induction in those “natural like” conditions, gametocysts were visible on all male gametophyte’s thalli of PLO-26-G (Figure 5 B) and developing sori (mainly at Stage 0) were observable on every sporophyte thalli of SB7-AUG. Production of spermatia was observed in after observation of a sample of water from the 300L cultivation tank, density was estimated at $0.7 \cdot 10^9$ cells/ml using malassé cell for counting. Massif spore release was observed on every thalli of SB7-AUG, which add similar size of 28 +/- 2cm after 39 days of expositions to “natural like” conditions. Spores settlement and development of viable gametophytes from this sporulation event was observed.

The results of these experiments suggest that temperature variations could induce an early onset of sporogenesis and gametogenesis in *P. palmata*. Daily temperature variations of 2°C seem to be sufficient to halve the incubation time of sporophytes and gametophytes. However, the size of the thalli used as well as the quality of the light differs greatly between this experiment and the first experiment. This conclusion led us to design the third experiment in order to test the effect of size and temperature variations on the previously tested strains.

3.3.3. Third experiments: Impact of temperature variations caused by tides and thallus size on the initiation of reproduction in the red alga *Palmaria palmata*

Gametogenesis

Gametocyte development were observed on every sixty thalli, which had similar size, in both variables and stable conditions. Visual mark of stress, ie depigmentation of the thalli, was observable for the

one cultivated in variable conditions. Liberation of spermata was observed in water sample of the cultivation tank from both treatments.

Sporogenesis

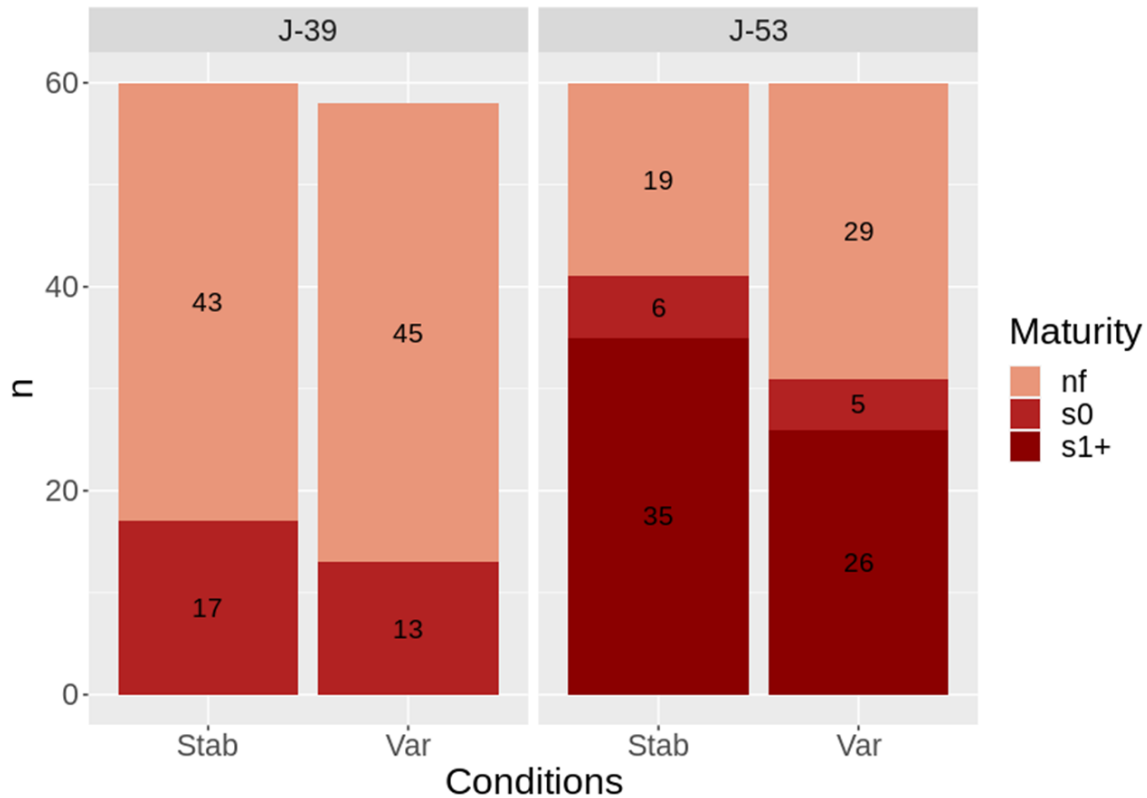


Figure 6: Stacked plots of the proportions of vegetative thalli and thalli carrying reproductive structures depending on the conditions tested: no temperature variation (Stab); temperature variations of 2°C twice a day (Var). The degree of maturity of the thalli is indicated by a color code. Nf: non-fertile thalli; s0: thalli mainly carrying sporocysts at the early stage of development (see chapter II -2.1.2)

After 39 days of culture, reproductive development is visible on the thalli of sporophytes and male gametophytes in both conditions. As shown in Figure 6, the proportion of sporophyte thalli bearing reproductive tissues at stage 1 did not vary between the two conditions tested. We therefore decided to group the two conditions together to increase the power of the statistical tests on the effect of the maximum size and weight of the fragments on the degree of maturity of the thalli. All male gametophyte thalli present fertile tissues, whereas in the sporophyte the presence of fertile tissue depends on the size of the thalli. Indeed, as shown in Figure 7 and 8, the thalli carrying stage 1 sporocysts on D 39 have a maximum size (Kruskal-wallis test, $p < 0.001$) and a fresh weight (ANOVA, $p < 0.001$) significantly superior to thalli in the vegetative state.

At day 53, the proportions of thalli carrying reproductive structures are greater for the stable temperature condition. Discoloration of thalli exposed to daily temperature variations is visible, indicating that thallus grown in this condition are experiencing stress. As for the previous measurements, the thalli carrying sporocysts at stage 0, 1, 2 and 3 (denoted s1+) have a maximum size (Kruskal-Wallis test, $p < 0.001$) and a fresh weight (ANOVA, $p < 0.001$) significantly higher than vegetative thalli in both conditions tested.

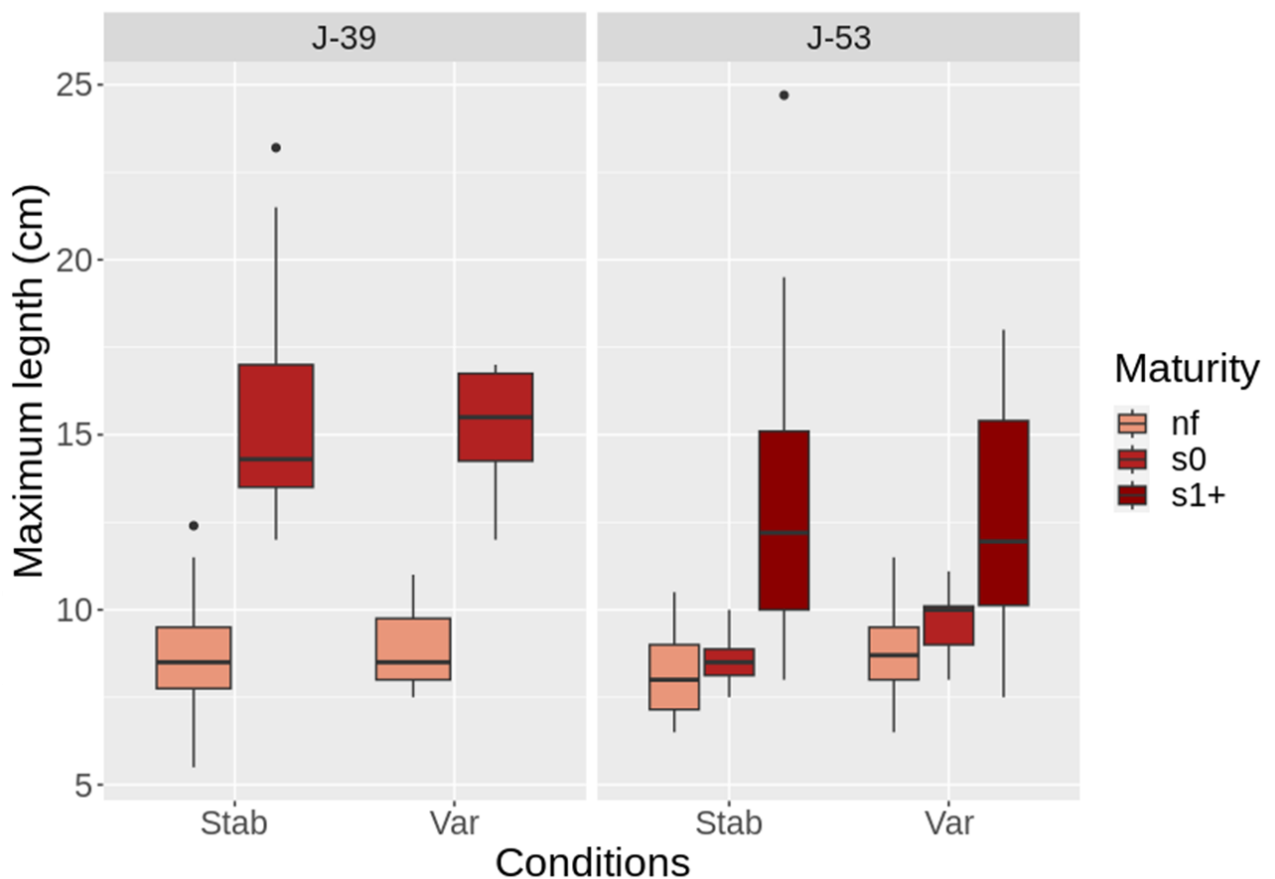


Figure 7: Box plots of the relation between maximum length and state of maturity of the clonal thalli of the SB7-AUG strain after 39 and 53 days of exposure to a temperature of 13°C and a photoperiod 8D/16N.

The appearance of reproductive structures in sporophytes and male gametophytes, compared to experimentation in natural conditions, is relatively late. This is likely due to a difference in water quality. Indeed, the tests which led to the appearance of reproductive structure in 28 days were carried out during a period when the coastal waters which supply the Station network are rich in nutrients and the average size of thalli used was 30 cm. Monitoring of the nitrate concentration of water from the SBR seawater reserve revealed significant seasonal variations. These observations agree with the work of Schmedes et al in 2020 which showed importance of nutrient supply in sore formations.

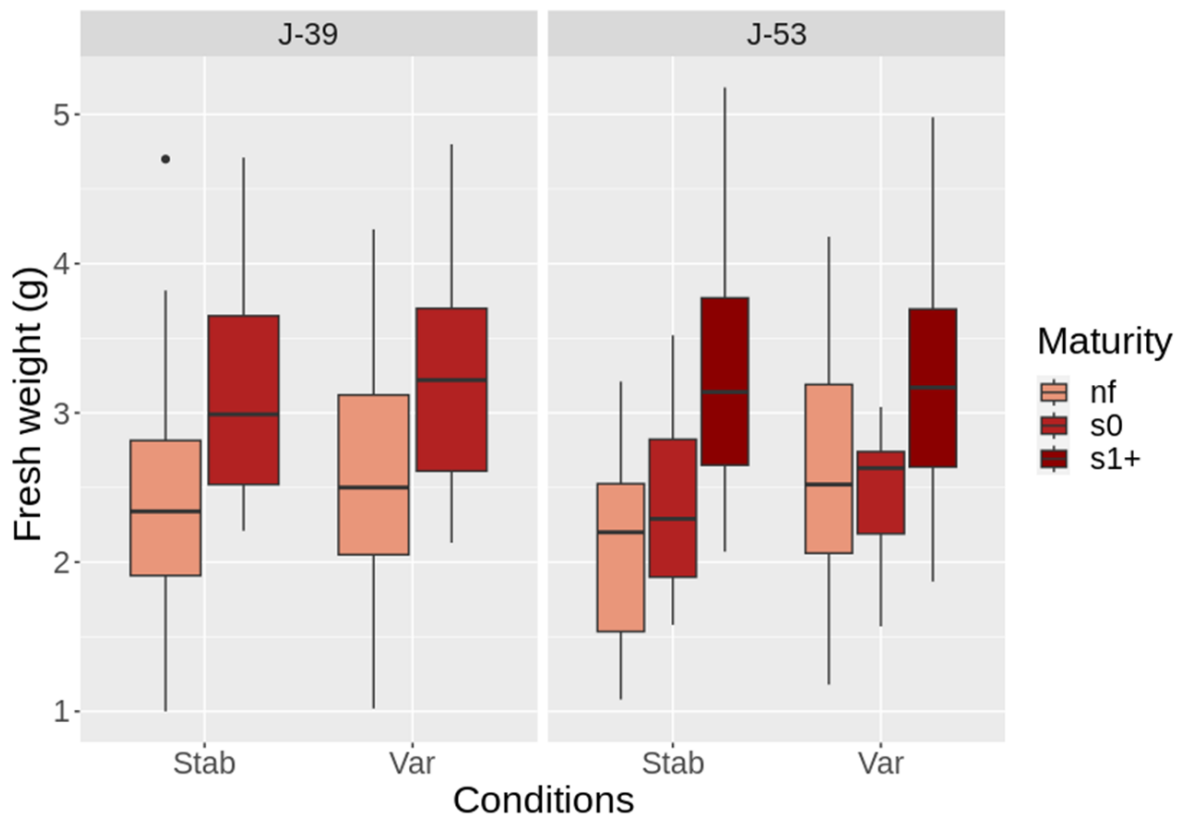


Figure 8: Maximum fresh weight and state of maturity of the clonal thalli of the SB7-AUG strain after 39 and 53 days of exposure to a temperature of 13°C and a photoperiod P:8/16

which shows the importance of nutrients in the development of reproductive structures. This difference cannot be attributed to inter-individual variability given that we used the same sporophyte strain as in the 2022 experiments. Furthermore, in each experiment, the clonal strains were produced by vegetative propagation in a long photoperiod at 13°C in order to standardize the physiological conditions of the thalli before the induction of maturity.

Despite this, the daily variation in temperature did not favor the development of reproductive structures, our results indicate that the most important factor in the development of sporocysts and gametocysts, in the tested conditions, is the size of the thallus. The results show a positive correlation between size and speed of production of reproductive structures. It is likely that other stress factors, link to intertidal environment, are involved in the early onset of reproduction in mid-coastal populations. The greater light intensity and desiccation stress experienced by thallus at this stage could be the key factors favoring the early development of reproductive structures. To confirm this, it is necessary to integrate all these parameters into future experiments, which should use tidal simulators. However, the application of this type of module on an industrial scale is difficult to implement and will

certainly pose profitability problems. It therefore seems preferable to increase the size of the cuttings in order to limit the maturation time of the thalli. We have shown here that it is possible to induce sporogenesis and gametogenesis in selected clonal strains. The induction time would strongly depend on the size of the thallus.

The formation of tetrasporangial sori in *Palmaria palmata* appears to be governed by meristematic tissues as observed in kelp species (e.g. *L. digitata*, *Saccharina latissima*) in which the meristem produces inhibitors of sporangium development during the season of rapid growth (Buchholz & Lüning, 1999; Pang & Lüning, 2004)). In these species, suboptimal growth conditions under low irradiance and temperature result in the decrease in the production of these inhibitors leading to sori formation. Likewise, isolating the meristem from the rest of the blade induces the development of sporangia (Pang & Lüning, 2004). Titlyanov & Titlyanova, (2006) observed a similar pattern in *P. palmata* where the formation of tetraspores in submeristematic fragments was stimulated by isolation from the meristem. However, cutting the marginal edges (meristem) of *P. palmata* fronds does not systematically result in sorus formation (Schmedes, 2020). Micrographs of fertile tissue of *P. palmata* are shown in Fig. 4. Sporangium formation is initiated by the differentiation of outer cortex cells into sporangial initials i.e. tetrasporocytes, and stalk cells (Pueschel 1979). Tetrasporocysts undergo meiosis in a cruciate cleavage to form four tetraspores. Although the knowledge related to tetrasporangial formation is limited, available information revealed that cleavages in Rhodophyta generally occur either successively or simultaneously i.e. the first cleavage is completed before the next or all cleavages are completed at the same time (Pueschel & Cole, 1982). *Palmaria palmata*, on the other hand, is characterized by an intermediate process in which the second cleavage starts just before the completion of the first division (Pueschel 1979). Following the release of tetraspores, the primary stalk cells form new stalk cells and tetrasporocysts, and the entire process of tetrasporogenesis is then repeated. High levels of tyramine have been measured in fertile sporophytic tissue compared to the concentrations measured in non-fertile fronds suggesting that this biogenic polyamine is involved in tetrasporogenesis in *P. palmata* (Schmedes 2020). Low levels of putrescine were measured and both spermine and spermidine were not detected, in contrast with studies reporting the influence of these polyamines in the maturation of reproductive structures in other red algae species (García-Jiménez & Robaina, 2012; Guzmán-Uriöstegui et al., 2002). More research is needed to properly understand the physiological mechanisms of sorus formation and maturation in *P. palmata*.

Controlling the reproduction of selected clonal strains opens the way to varietal improvement by crossing.

3.4. Fertilization trials

Around fifty thallus were collected from the Roscoff-Ar Pourven seaweed farm in April 2023. Tissue samples were taken for culture and determination of ploidy by flow cytometry as detailed in the Material and methods. Coculture of *P. palmata* with *L. hyperborea* and *S. latissima* resulted in competition for space between these species. *P. palmata* which is not forming a holdfast was completely invaded by kelps, which explains the low densities of *P. palmata* in the co-cultures. Of the 56 thallus sampled, 41 were identified as haploid males and 15 as diploid tetrasporophytes, a ratio of 2.73/1. Only thalli larger than 2 cm were collected. Since females were fertilized one month after sporulation, it is likely that the sporophytes were smaller than the gametophytes. In June 2023, a segment of 30 cm rope on which male gametophytes and sporophytes from the same sporulation/fertilization event were attached was cultivated in the laboratory in a 200L cylinder. On a set of 77 thallus, flow cytometry analysis reveals 49 male gametophytes and 24 tetrasporophytes, a ratio of 2.02/1. Analysis of the surface area of thallus shows that there is no relationship between ploidy and size of thallus (figure 9). The largest thallus were kept in culture in a 40L cylinder supplied with running 5µm FSW.

Our results show that there are twice more males than tetrasporophytes on the ropes. Given the hypothesis which estimates the ploidy ratio after fertilization of the female gametophytes at 1:1, the ploidy ratio observed here deviates significantly (binomial law, $p < 0.001$) of the expected ratio. This discrepancy is not due to a difference in growth given that tetrasporophytes are not smaller than male gametophytes. This discrepancy could be due to a deficit in fertilization; it is likely that the incubation time of the females in the spermatia solution was not sufficient despite the relatively high spermatia concentrations. However, experiments carried out on the fertilization of *G. gracilis* show that an incubation of one to two hours is enough to fertilize females (Lavaut et al., 2022). It is also likely that one month after sporulation, some of the females are no longer fertile as suggested by Van der Meer & Todd in 1980 which estimated that female gametophytes lost reproduction cells after 6 to 8 months. Finally, it is likely that other abiotic or biotic factors must be considered in the fertilization process. A recent study showed the direct involvement of the isopod *Idotea baltica* in the fertilization success of female gametophytes of the red alga *Gracilaria gracilis* (Lavaut et al., 2022). Such a co-evolutive process with animals could also exist in other red algae such as *P. palmata*

As the ploidy ratios observed in these experiments are different from those observed in the natural environment where sporophytes dominate male gametophytes. It will be interesting in future experiments to test the optimal timing and incubation time of female gametophytes in order to optimize the fertilization success and obtain the expected ratio.

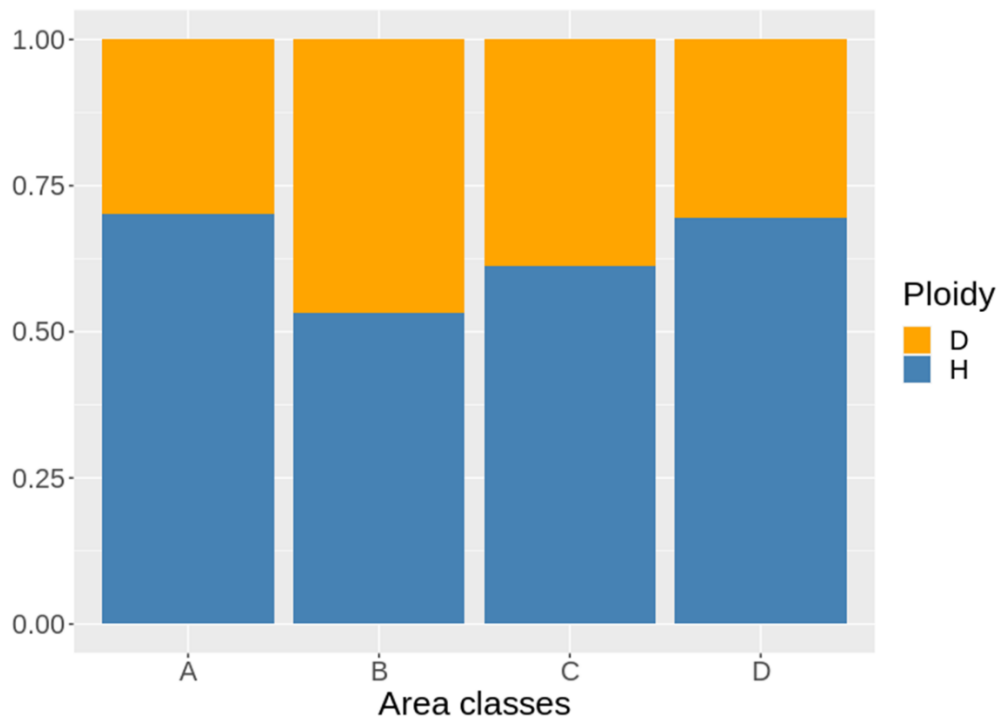


Figure 9: Stacked bar plot of diploid sporophytes to haploid male gametophytes ratio resulting from crossing, and cultivated in the laboratory, depending of surface area of the thalli classes size (cm²). A : 0 to 1 cm² ; B : 1 to 5 cm² ; C : 5 to 10 cm² , D : more than 10 cm²

4. Conclusion and prospects

During these experiments aimed at controlling the reproductive cycle of *P. palmata* both in the laboratory and in hatchery conditions, we have demonstrated that the triggering of sporogenesis and gametogenesis is controlled by the size of the thallus. Furthermore, we showed that the induction of fertility in a sporophytic or gametophytic clonal strain could be induced artificially several years in a row. We also showed that the fertilization of female gametophytes under laboratory conditions requires particular attention to the incubation

time and the age of the females, and require further investigations before being included in hatchery methods.

Acknowledgements

This work benefited from the support of the French Government through the National Research Agency with regards to an investment expenditure program IDEALG (ANR-10-BTBR-04), the EU Horizon 2020 project GENIALG (Grant Agreement No 727892), the European Maritime and Fisheries Fund (EMFF) through the POLISTR project to MV and PP and Région Bretagne (PhD grant ARED Domdulse from ED227 Sorbonne-Université/MNHN to AB). We are grateful to the EMBRC France partner core facility for its technical support, especially Gaetan Schires and Sébastien Henry. The authors are deeply indebted to the Service Mer & Observation (SMO) of Roscoff for sampling. We also thanks J-C Leclerc for given us the opportunity experiment on Sébastien Jonas seaweed farm in Morlaix Bay through the Kelp Me! Project.

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Chapitre III : Culture à long terme pour établir une collection de matériel génétique et sélectionner des souches d'intérêt de l'algue rouge *Palmaria palmata* (Article n°6).

Ce chapitre s'est penché dans une première partie sur la mise en place d'une collection par l'isolement de souches sauvages issues de plusieurs populations Normano-bretonnes en testant les méthodes d'isolement déjà connues pour cette espèce afin de retenir la plus efficace. Des gamétophytes mâles et des tétrasporophytes ont été utilisés pour la collection provenant de toutes les populations bretonnes échantillonnées dans le Chapitre I. De plus, l'échantillonnage a intégré le contexte écologique particulier des populations de *P. palmata* le long de l'estran en prélevant des individus à deux niveaux bathymétriques différents comme défini dans le Chapitre I. Une telle collection a permis d'étudier l'importance de la ploïdie, de l'origine géographique et du niveau bathymétrique dans la capacité d'acclimatation des individus sauvages aux conditions de laboratoire et d'écloseries. Elle a également permis d'identifier et de développer des systèmes de conservation ou de culture adaptée adaptés au mode de reproduction par propagation végétative chez cette espèce. Notamment, dans le but de minimiser les changements fréquents de milieu de culture dans un grand nombre d'échantillons, un système de culture à faible intervention (SyCFI), basé sur les travaux initiés par Augustin Drion dans le cadre d'un stage de master en 2017, a été développé.

La seconde partie du chapitre avait pour objectif dans un premier temps d'utiliser le système de culture, développé pour phénotyper les souches de la collection sur les critères détaillés en introduction, principalement la croissance végétative. Cela a permis d'étudier également la répartition de la ressource génétique entre les différentes populations et niveaux bathymétriques étudiés.

L'article 6 ci-dessous regroupe ces deux parties et n'a pas vocation à être publié en l'état mais il sera soumis pour publication après des expériences complémentaires pour valider le SyCFI et conforter des tests en conditions de culture en bassins à plus grande échelle.

Article n°6: Long-term culture maintenance to establish a germplasm collection and select interesting strains of the red alga *Palmaria palmata*.

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1. Introduction

Ex situ plant collections are important tools for preventing the extinction of certain species (Mounce et al., 2017), as well as providing material for population restoration (Nevill et al., 2016) and developing improved varieties from a large pool of genetic resources (Odong et al., 2013). They make it possible to provide genetic material in large quantities, without depleting local material and without having an impact on natural populations (Broadhurst et al., 2015). It is therefore essential to constitute collections that are genetically diverse and representative of the genetic variation of the target species (Bragg et al., 2021). The objective is therefore generally to constitute collections of diversified thallus (adaptable) and presenting particular traits (adapted). This should make it possible to obtain material capable of adapting to changing conditions ((Bürger & Lynch, 1995; Stange et al., 2020), while at the same time having sufficient genetic diversity to avoid the problems of inbreeding depression caused by using too few related genitors from restoration collections (Charlesworth & Willis, 2009).

The possibility of preserving strains therefore makes it possible to free ourselves from the natural resource and to initiate the varietal selection of the target species. In plants, collections generally consist of dormant seeds or planted thallus (botanical gardens). In algae lacking a resistance organ, the collection is made up of gametophytic or tetrasporophytic thallus. The conservation in culture of these

thallus in the form of a collection therefore opens interesting perspectives for the restoration, improvement and conservation of the species in question. This is an essential step in the domestication process. This step requires perfect knowledge of the biology and ecology of the target species (Valero et al., 2017).

Culture studies of seaweeds are necessary to observe their development from unicellular reproductive cells to multicellular thallus, or to elucidate the whole life cycle that often cannot be observed from field-collected specimens (Kawai et al, 2005 in Robert A. Andersen, 2005). Such studies began in the nineteenth century; however, it was often difficult to complete life histories using natural seawater and culturing at ambient temperatures (Kawai et al, 2005 in Robert A. Andersen, 2005). The first discovery of a heteromorphic life history in kelps was made by Sauvageau (1915) in France, who cultured *Saccorhiza bulbosa* J. Agardh, a member of the order Tilopteridales with a life cycle similar to those of large kelps of the order Laminariales. This very significant discovery by Sauvageau aroused a great deal of interest in culturing brown algae. It was evident that the cycle of development of many of brown, but also red and green algae could not be ascertained unless they were grown in culture. This was illustrated later one with the elucidation of the life cycle sporophytic phase of nori crops (the red algae *Porphyra* and *Pyropia spp.*) by Kathleen Drew, 1949) and more recently by the discovery of the life cycle of the red alga *Palmaria palmata* by van der Meer & Todd, 1980).

Gradually, because of the improvement of culture media and the use of controlled-temperature incubation chambers, unialgal culture techniques became well established, and by the 1960s culture studies of macroalgae became popular (Bold 1942, Tatewaki 1966). In addition to their use in studies of early development and life histories, unialgal, clonal, and axenic cultures of macroalgae have become essential for many studies of morphogenesis, morphological development, nutritional physiology, responses to various chemicals, crossing experiments, extracting various compounds without contamination (including for molecular biology such as genomic DNA, cDNA libraries, Northern blotting, etc.), long-term strain preservation, exchange of research materials, mass culture and preparation of mariculture seed-stock, and so on. It is especially important to maintain stock cultures (backup cultures) of all cultivars in the lab. Stock cultures should be kept at lowered temperatures and light levels to minimize growth and need for culture changes (West, 2005 in Andersen, 2005). For long-term maintenance, cold temperate plants should be kept at 5-10°C, warm temperate plants at 15-20°C. Illumination is best kept at 1-10% of normal culture conditions, which is at or near compensation level (generally 27 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$).

Taking into consideration biotic interactions (parasitism, epiphytism, predation) is necessary to develop methods for isolating unialgal strains (Kawai et al., 2005) and ensuring the survival of the

target species in modified ecosystems such as cropping systems. In this context, knowledge of the characteristics linked to growth and reproduction is necessary to develop conservation and cultivation methods adapted for each species (Kawaiï et al., 2005, West, 2005). Few data exist on biotic interactions in *P. palmata* (Stévant et al., 2023). Cultivation experiments carried out over the last 20 years on *P. palmata* have highlighted the existence of different parasites or epiphytes. In hatchery conditions, the oomycete *Olpidiopsis* sp. caused significant damage to the development of young seedlings (Badis et al., 2019). Furthermore, it has been shown that the presence of the filamentous red alga *Acrochaetium* sp. and filamentous brown algae of the order Ectocarpales have a strong impact on the growth in inland tank cultures (Werner & Dring, 2011).

Even if the apices allow new fronds to be quickly generated (Morgan et al., 1980; Titlyanov et al., 2006; Titlyanov & Titlyanova, 2006), the ability of *P. palmata* to generate new shoots throughout its thallus is a major asset for its maintenance in the collection. Indeed, it is possible to produce biomass from any part of the thallus. The use of this method of reproduction by vegetative propagation makes it possible to conserve thallus clonally. No data is available on the lifespan of an individual in the natural environment and even less in the laboratory. However, observations made on clonal strains of red algae preserved for more than 40 years by vegetative propagation have shown the loss of capacity to produce propagules over time (West, in Anderson 2005). Similarly, the “unconscious” mass selection carried out by farmers by cutting the thalli of *Gracilaria chilensis* resulted in a loss of the capacity to reproduce sexually in around ten years (Guillemin et al., 2008). The long-term impact of conservation in collections should be studied.

The use of this vegetative production method could be considered as a first step of the domestication of this species, that is its ability to grow under controlled laboratory conditions. This first selection step is essential to ensure the use of the strains in culture, at sea and on land-based culture facilities. Indeed, the vegetative growth of the selected strains must be sufficiently significant to allow the production of genitors to seed the culture substrates or ensure the production of biomass in ponds.

Some studies have focused on the physiology of *P. palmata* and it has been possible to define the optimum temperature, light intensity, photoperiod and nutrient for cultivation by vegetative propagation (Grote, 2019). *P. palmata* is known to grow between 6 and 18°C with variations depending on the locality (Matos et al., 2006; Morgan & Simpson, 1981 Chapter I article 3). Tolerance to salinity changes was observed by (Schmedes, 2020) and also appears to vary locally. Concerning light, *P. palmata* is able to acclimatize to varying light intensities as shown by laboratory and field studies. Indeed, *P. palmata* is present both in the mid-littoral region, where it is overexposed to light radiation (2000 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$), and in the upper sub-littoral zone where it grows up to 20m depending

on turbidity (Sagert & Shubert, 2000; Stévant et al. 2023). It is described as a species photophilic, its saturation threshold has been estimated at $113 \mu\text{mol photons photons.m}^{-2}.\text{sec}^{-1}$ (Sagert & Schubert, 2000).

Due to the complexity of the reproduction cycle of *P. palmata*, several phenotypic traits will have to be selected in tetrasporophytes and male gametophytes in order to ensure its propagation in different cultivation systems. The main selection criterion is the vegetative growth capacity of the strains in a modified environment. Indeed, growing dulse at sea or on land requires a vegetative growth phase. For pond culture, this is the main means used to produce biomass. In the context of cultivation at sea, a vegetative propagation phase of the broodstock used for seeding is necessary in order to ensure the quality of seeding on ropes or support nets. In addition, the seedlings developing on the culture substrate must be able to grow rapidly through vegetative growth to compete with undesirable species and thus ensure the production of marketable biomass. The acquisition of a first collection of wild strains selected on this criterion will serve as a basis for initiating selective breeding by crossing and thus improving the production of *P. palmata* at sea and on land.

In this paper as defined in Kawai et al. (2005), a unialgal culture is a culture that includes only one species of alga (bacteria may be present). An axenic culture is unialgal and free of bacteria. A clonal culture is a culture of a single genome set (e.g., cultures derived from a single vegetative cell or tissue or from a reproductive cell) and propagated vegetatively. Here, we describe the techniques for establishing and maintaining unialgal cultures of *Palmaria palmata* and to develop selection methods for the best clones. We also discuss the problem of epiphytes that we have encountered. Finally, we describe the development of a Low Intervention Culture System (LICSy). This LICSy was initially designed to provide a system with an easy and fast renewal of culture medium in a large number of flasks that could accommodate the conservation of at least one hundred of strains of *P. palmata*. It appeared that this system could also provide a powerful phenotyping tools to select fast-growing strains of *P. palmata*.

2. Material and Methods

2.1. Algal material sampling

Sampling was carried out from October 2019 to October 2020 (except Noirmoutier population sampled during Summer 2021) during spring tides. Thallus of *Palmaria palmata* were collected randomly at two different tide levels, using the brown algal belts as height references. Thirty thallus

were collected at the *Fucus serratus* level (middle intertidal level, hereafter referred as intertidal level, between 1.62 and 3.13 meters), and 30 thallus at the *Laminaria hyperborea* level (upper subtidal level, hereafter referred as subtidal level, between 0.75 and 1.32) (Table 1). This sampling method was applied to 14 sites along the coast of Brittany (France) in the three predefined regions mentioned in Article 2 from Chapter I: North-East, North-West and South Brittany.

The difference in altitude between the levels varies between 0.7 and 1.81 m above theoretical sea level (Table 1). To avoid sampling the same individual twice, fronds separated by at least one meter, or found as epiphytes on different *L. hyperborea* stipes, were collected. The ploidy of each individual was determined using flow cytometry (Guillemin et al. 2008). Two apical fragments (2-3 cm long) of each individual were preserved in silica gel for genetic studies.

In some sites, the ecological conditions did not allow the growth of *P. palmata* at the two levels studied and only 6 thallus were taken, this is the case for the sites of Belle Île (infralittoral), Rade de Brest (medio-littoral) and Noirmoutier (medio-littoral). The identification of the ploidy of thallus was carried out by observation of reproductive structures (tetrasporocysts or male gametocysts) or by flow cytometry for vegetative thallus. From February 2021 and in certain sites, additional sampling campaigns, following the same protocol, were carried out to supplement the loss of thallus preserved in the laboratory in 12-well plates. These thallus were both isolated in 12-well dishes and/or placed in the culture system developed in part 2.3.

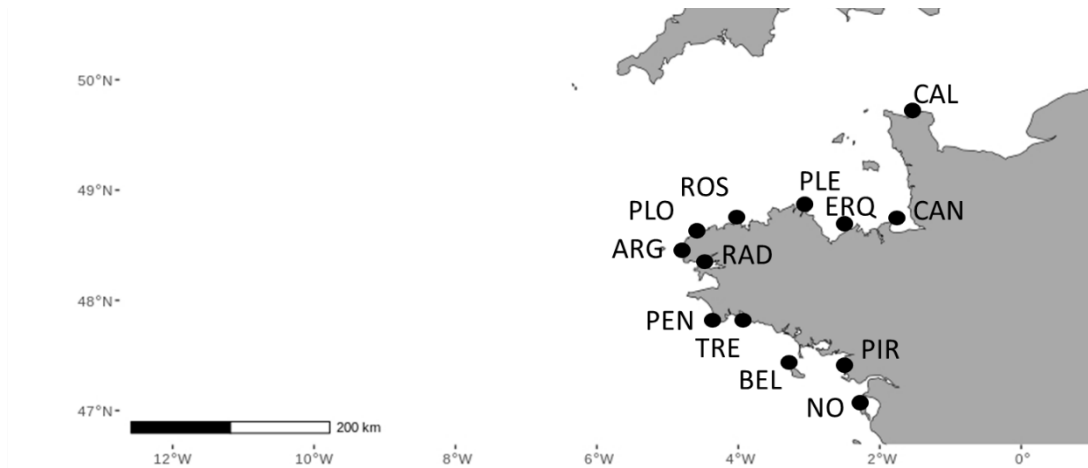


Figure 1 : Sampling sites along the Brittany coasts and margin regions. CAL= Cap Lévi; CAN= Cancale ; ERQ=Erquy ;PLE=Pleubian; ROS= Roscoff; PLO=Plouguerneau ; ARG= Argenton; RAD = Rade de Brest ; PEN = Penmarc'h ; TRE= Trégunc; BEL = Belle île; PIR = Piriatic ; NO = Noirmoutier

2.2 Culture conditions

In our study, we defined standard cultivation conditions based on average field data. The temperature was set at 13°C, the light intensity was 100 $\mu\text{mol photons.m}^2.\text{sec}^{-1}$ and the long days photoperiod was 16: 8. For the conservation of the strains, the temperature was set at 10°C and the light intensity at 25 $\mu\text{mol photons.m}^2.\text{sec}^{-1}$ while maintaining a long photoperiod to avoid triggering reproduction.

2.3. Collections of static culture in controlled cabinets.

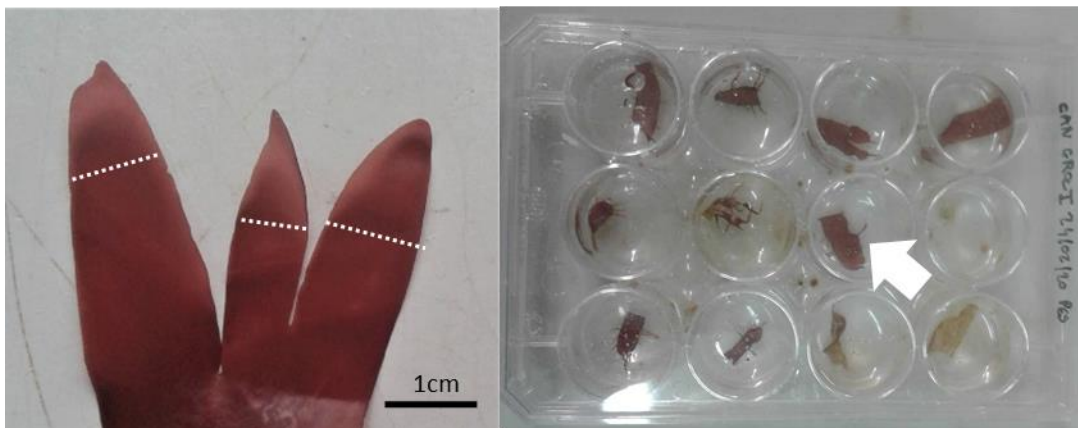


Figure 2: Left: apical meristems of *P. palmata*, the dotted lines mark the areas where the meristems are cut. Right: the collection of strains in a 12-well plates, with arrow indicate a thalli fragment initiating marginal growth.

For each individual collected from the field, between 24 and 12 healthy apical meristems (Figure 2) were cut out with a scalpel, rubbed with absorbent paper and rinsed in two baths of sterile sea water. For the Piriatic site, the physiological state of the thallus sampled in the field did not allow healthy

meristems to be taken. The thallus were therefore cultured in a 10 L container with seawater filtered at 1 μm enriched with Provasoli Enrichment Solution (PES) until healthy meristems were obtained. Once cleaned, the meristem apices were placed in 12-well cell culture boxes containing enriched sea water and nutrient with the addition of PES (Provasoli et al., 1956) then and stored in a thermostatically controlled cabinet at 10°C, under a with a long photoperiod in long days (P: 16/8) and a low light intensity (25 $\mu\text{mol photons.m}^2.\text{sec}^{-1}$). The culture medium was changed every month between October 2019 and May 2021, then once every 4 to 6 months between May 2021 and December 2023. For this last period, the quantity of medium added to the sterile seawater was doubled in order to space out environmental changes over time. As changes occurred, the most contaminated strains or meristems were eliminated from the collection.

The number of apical meristems having survived at the time of the assessment was reported as the number of meristems initially placed in the collection for a given individual. This allows us to estimate the fitness of thallus kept in the collection through a meristem survival rate comprised of 1 (survival of all meristems) and 0 (loss of all meristems). The level of contamination was estimated visually by strain using the following scale: 0 = no contamination; 1= a species of epiphytes observed; 2 = several species of epiphytes observed and 3 = epiphytes covering the majority of the apex meristems. Statistical tests were carried out in R, with the Rstats package (R core team, 2023). The data does not follow a normal distribution (Shapiro test, $p < 0.001$), the effect of geographical origin, bathymetric level as well as ploidy on the survival rate of thallus was estimated with a Kruskal-Wallis test.

2.4. Development of Low Intervention Culture System (LICSy)

The LICSy was initially designed to provide a system for easy and rapid renewal of culture medium in a large number of flasks capable of containing at least a hundred strains of *P. palmata*. Handling hundreds of flasks for the change of media is time consuming and the automation of this medium renewal either with a recirculation system or an open flow system could improve the conditions for long-term maintenance of a large collection of *P. palmata* strains. After several trials, the following system was designed.

It consists of 250 mL flasks which were custom made by a glass craftsman (Lyon, France) to fit a cap that contain a glass tube reaching the bottom of the flask connected to the medium flow. On the lateral side, an outflow directs the running medium to the retention tank.

The system was automated with a seawater tank connected to a 1 μm filtration cartridge and a UV-C generator before entering the TECO TANK-500 chiller set-up at 13°C.

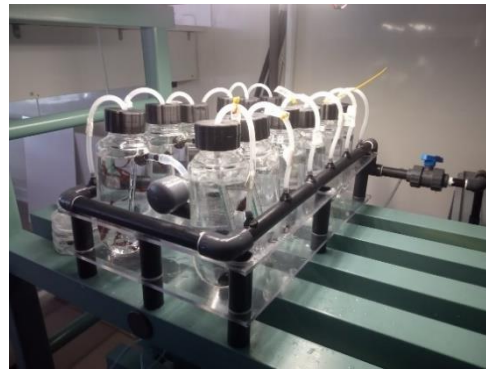
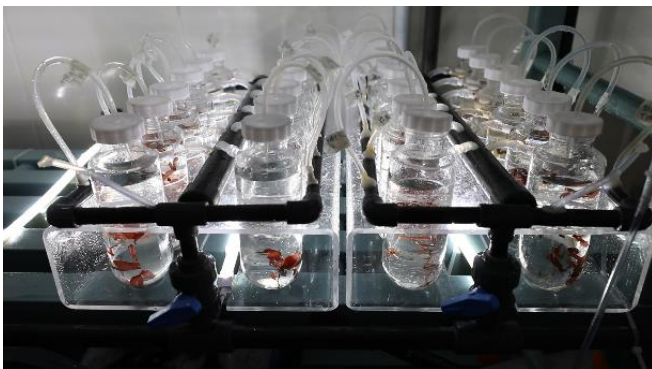
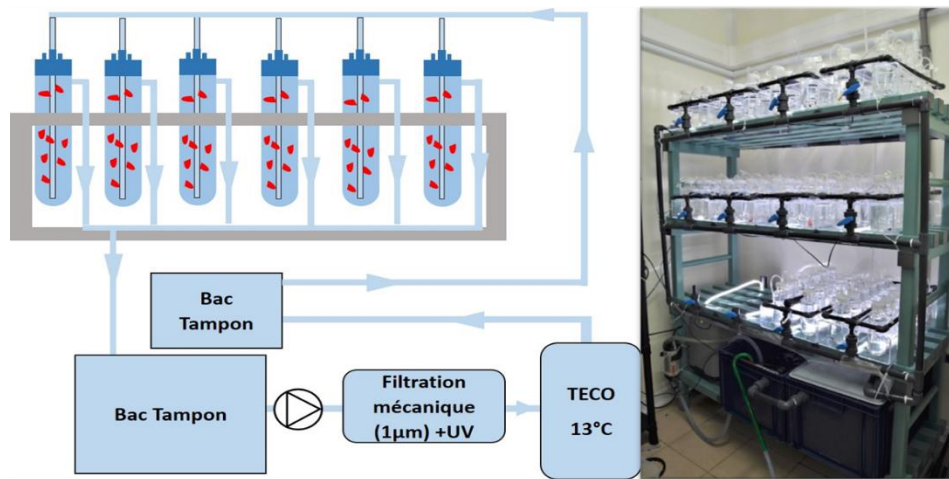


Figure 3: Low Intervention Culture System (LICSy) developed for the conservation of *P. palmata* strains (left). A) Schematic drawing of the LICSy. B) Picture of the collection of 120 strains of *P. palmata* in culture in the LICSy (right). C) Detailed picture on two rack systems fitting 12 bottles containing strains from a given population.

In February 2021, the 11 populations initially kept in thermostatically controlled cabinets were duplicated and transferred to the LICSy (Figure 3). For each strain, 3 healthy meristems were selected, the rest were kept in a thermostatically controlled cabinet as back-up in new 12-well dishes. Until December 2021, the LICSy operated in a closed circuit for technical reasons. In January 2022, the LICSy was switched to open circulation.

The percentage of daily growth rate (DGR) of the meristems was calculated according to the following formula: $r = [\ln(W_t/W_0)/t] * 100$, with W_t the fresh weight at time t , W_0 the weight initial and t the number of days between two weighings (Yong et al., 2013). The results were analyzed using the R software (R Core Team, 2022), the growth rate data not following a normal law (Shapiro test, $p < 0.001$)

we tested the effect of the origin of the populations, the bathymetric level and ploidy with a Kruskal-Wallis test. Graphics were rendered using Ggplot2 R package(Wickham, 2014).

3.Results and Discussion

3.1. Assessment of the collection in 12-well plates

In February 2020, a first assessment of the state of conservation of the strains was carried out on the thallus kept in the collection since the end of 2019, only the number of living meristem apices was noted. In March 2021, a second assessment of the state of conservation was carried out for all the strains preserved for at least one year. The number of surviving meristems, the presence of contamination, as well as the presence of marginal regrowth were reported for thallus collected in the sites of Cap Lévy, Cancale, Erquy (mediolittoral only), Pleubian, Roscoff, Plouguerneau, Argenton, Rade from Brest, Penmarc'h, Trégunc and Belle île.

The results of measured survival rates measured in February 2020 are shown in Figure 4 (a), (b), and (c). No significant effect of ploidy (Kruskal-Wallis, $p=0.216$) or bathymetric level on the survival rate was measured (Kruskal-Wallis, $p=0.305$). On the other hand, a significant effect of geographical origin was measured (Kruskal-Wallis, $p<0.001$)

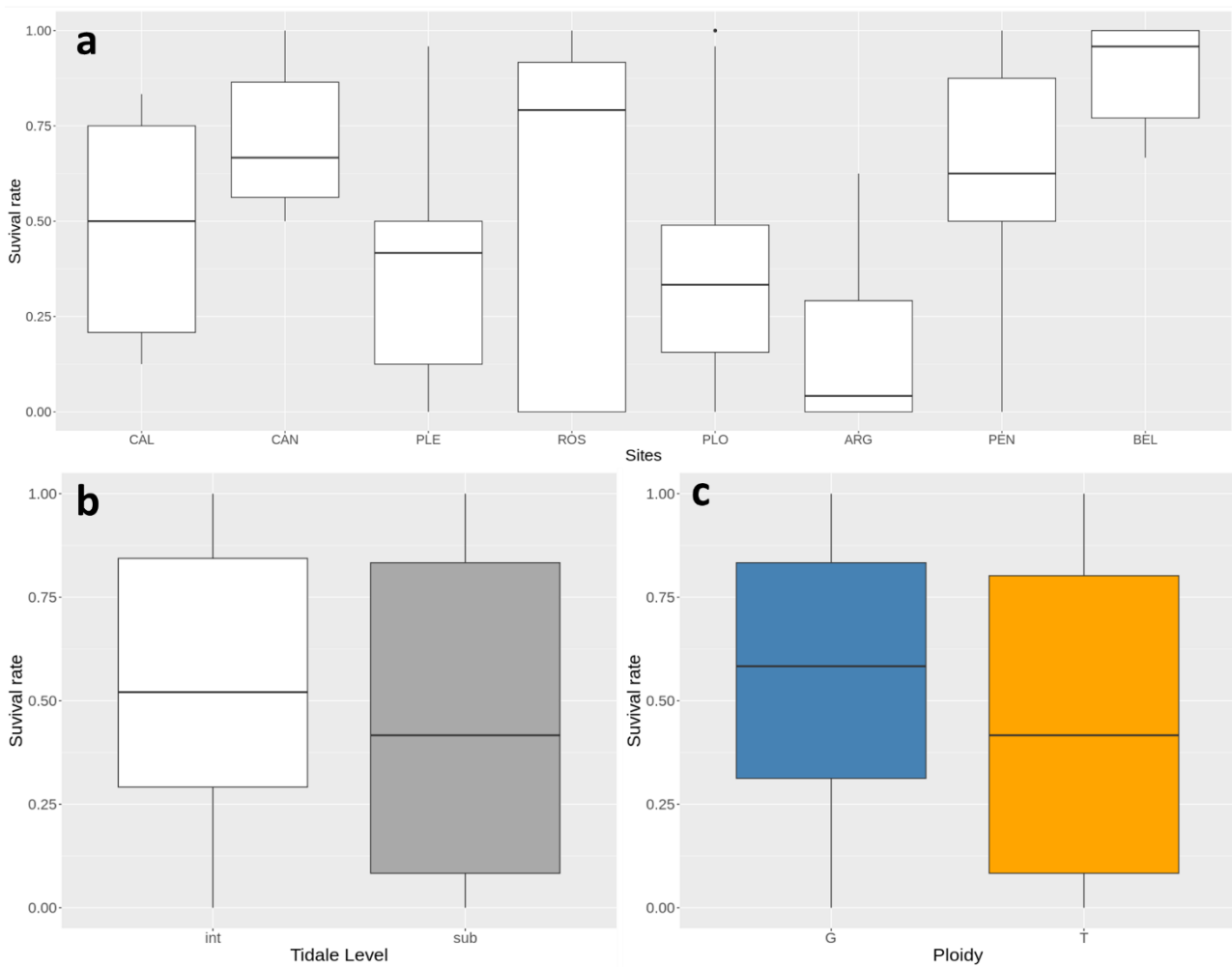


Figure 4 Box plots of the survival rates of preserved strains kept in collections in 12-well plate cultures between October 2019 and February 2020 depending on (a) sampling site, (b) bathymetric level and (c) ploidy. Int= mid-littoral level; Sub = sublittoral level; G= male gametophyte (haploid); T=tetrasporophyte (diploid)

In February 2020, a first assessment of the state of conservation of the strains was carried out on the thallus kept in the collection since the end of 2019, only the number of living meristem apices was noted. In March 2021, a second assessment of the state of conservation was carried out for all the strains preserved for at least a year. The number of surviving meristems, the presence of contamination, as well as the presence of marginal regrowth were reported for thallus collected in the sites of Cap Lévy, Cancale, Erquy (mediolittoral only), Pleubian, Roscoff, Plouguerneau, Argenton, Rade from Brest, Penmarc'h, Trégunc and Belle île. Survival rates for each site, bathymetric level and ploidy measured in March 2021 are shown in Figure 5 a, b c. No significant effect of ploidy (Kruskal-Wallis, $p=0.212$) or bathymetric level on survival rate was measured (Kruskal-Wallis, $p=0.688$). On the other

hand, a significant effect of geographical origin was measured (Kruskal-Wallis, $p < 0.001$). The average contamination score for the entire collection was 1.6, indicating that the majority of strains were contaminated with one or even two species of epiphytes. 77% of thallus kept in collections over this period produced marginal regrowth.

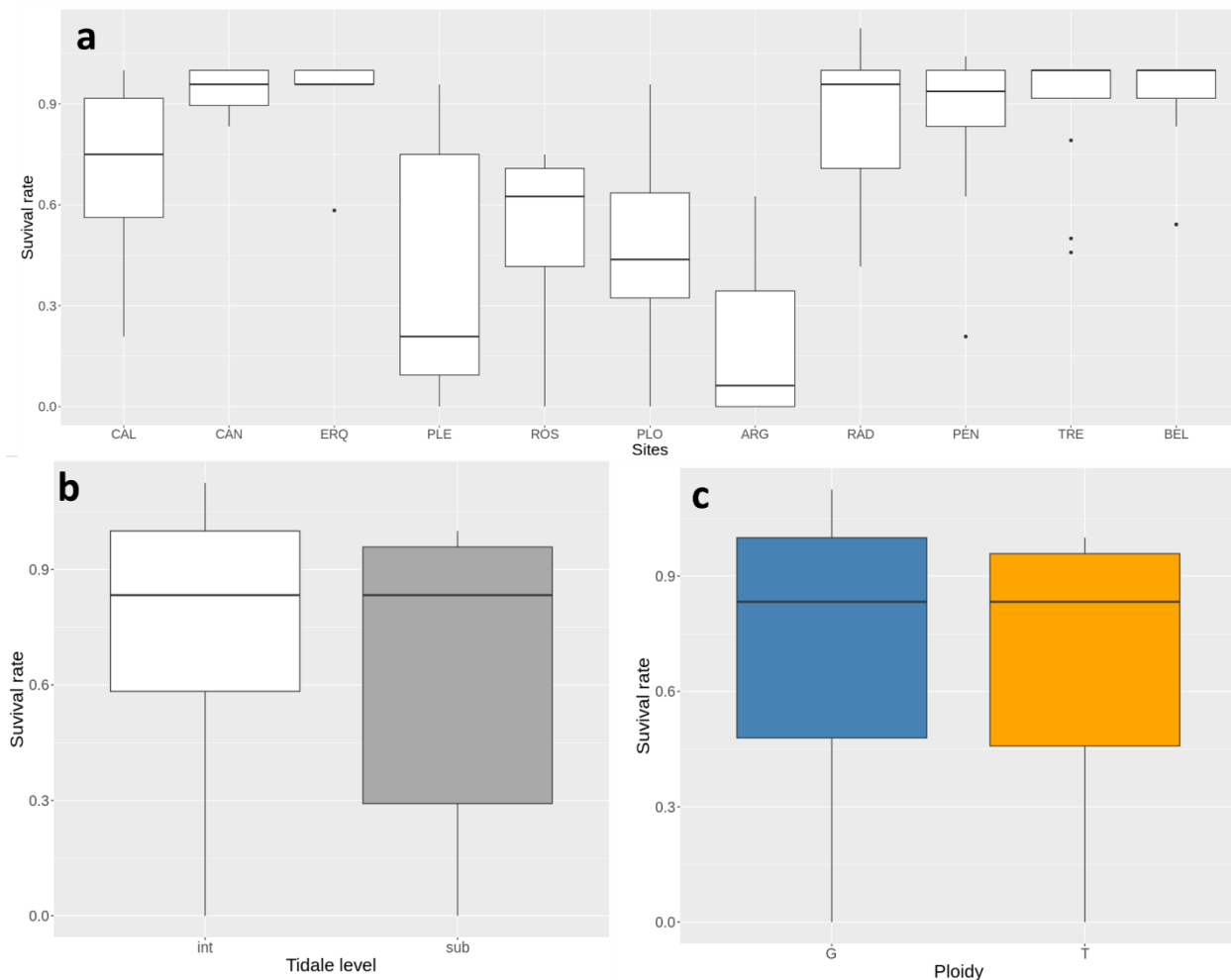


Figure 5: Box plots of the survival rates of preserved strains kept in collections in 12-well plate cultures between March 2020 and March 2021 depending on (a) sampling site, (b) bathymetric level and (c) ploidy. Int= mid-littoral level; Sub = sub-littoral level; G= male gametophyte (haploid); T=tetrasporophyte (diploid)

The significant difference in survival rates observed between sites highlights the importance of the geographic origin of the strains selected for domestication. However, in most sites there is very high variability in response with at least one fragment of an individual showing good survival rates. It is therefore possible to select suitable strains in all sites, the sampling effort must be adapted according to the functions of the sites. In addition, the majority of strains are capable of producing marginal

regrowth from the apical meristem. This supports the hypothesis that a fraction of any population is capable of adapting to growing conditions. The absence of difference in survival rates between phases of the reproductive cycle indicates that under the conditions tested, haploids and diploids have the same fitness. The bathymetric level does not seem to influence the acclimation capacity of strains to culture conditions despite the fact that intertidal populations are more often exposed to environmental variations.

The main problem with this method of conservation is the appearance and rapid development of contaminations if the basic biological material is still bearing some dormant spores of epiphytic macroalgae and diatoms. Although many new shoots have been observed, their growth remains limited by the low volume of water (2 ml for 12-well plates) in which they are stored. Some strains quickly find themselves covered by epiphytes of the genus *Ectocarpus* sp (Stramenopiles) or *Acrochaetium* sp (Rhodophytes), which makes them unusable. The presence of microalgae (diatoms) was also observed in the collection. The strain isolation protocol therefore remains to be perfected to limit the presence of epiphytes. However, the little space used by this type of collection and the possibility of preserving strains without intervention for several months makes it a good means of long-term conservation of strains previously cleared of all epiphytes. In order to obtain uni-algal strains from wild thallus carrying epiphytes, it is preferable to develop a culture system capable of accommodating a large number of strains in an individualized manner, but which does not limit the growth of marginal shoots. This system will allow the production of healthy apices and meristems which can thus be isolated. The uni-algal strains thus produced can be transferred to 12-well or 6-well plates or sterile plastic culture flasks for long-term storage. Such a system will also make it possible to effectively select strains that exhibit increased vegetative growth.

3.2 Isolation and conservation of uni-algal strains.

Following the isolation problems encountered with the 12-well plate collection, we have tested different means of limiting the propagation of epiphytes and thus obtaining uni-algal strains. The identification and cutting of healthy meristems under a binocular microscope made it possible to isolate a uni-algal strain from a culture contaminated by a filamentous red alga (*Acrochaetium* sp.). These tests were carried out on the SB7-AUG strain. Cutting meristems under a binocular microscope is therefore an effective technique, but very time-consuming, because the cultures must be restarted with very little biomass. Other tests to control contamination were carried out (hyper- and hypo-saline stress, nutrient depletion, exposure to monochrome light, use = use of ethanol or chlorine at 1%) but all without success. Bacterial contamination can also hinder the growth of uni-algal strains. This was the case for SB7-AUG, the problem was resolved with treatment by soaking the meristems in an iodine

solution (1%) for 3 min. However, the recovery of growth of SB7-AUG was very slow. New tests were carried out with wild thallus.

Following this, we carried out conservation tests with SB7-AUG in 6-well plates with 10ml of sterile filtered seawater enriched with Provasoli ES (double dose). This method appears suitable for the long-term conservation of *P. palmata*. A change of medium every 4 to 6 months is sufficient to maintain the strains. However, it is essential to obtain a unialgal strain before transfer to a 6-well plate in order to ensure the sustainability of the culture.

This test also revealed the incredible morphological plasticity of *P. palmata* in culture. As shown in Figure 6, a change in morphology was observed in the SB7 strains stored in 6-well plates. Some strains from the collection in 12-well plates reacted in the same way. When kept in small volumes with a limited nutrient source, the morphology of *P. palmata* strains appears to converge toward a terete form contrasting with cross-sections that are flattened in the same strain kept in larger volumes. However, when they are placed in more favorable growing conditions (T=13°C, open circuit, high light intensity) they return to their initial morphologies.



Figure 6 : Morphological variations related to culture conditions in the tetrasporophyte strain SB7-AUG.

These marginal proliferations observed during the conservation phase in a 6-well box improve the efficiency of vegetative propagation. This process makes it possible to maintain SB7-AUG in the collection and to regularly produce biomass by vegetative propagation.

3.3. Development of a low intervention cultivation system (LICSy).

In order to characterize inter-individual and inter-population variability, we undertook to phenotype all the strains in the collection using the LICSy. This experiment was designed to identify the most efficient strains for vegetative propagation. To do this, we followed, from May 2021 to January 2022,

the vegetative growth of the strains stored in the LICSy by measuring the fresh weight of each individual once a month. The acquisition of several growth rate values per strain over time allowed us to better understand the variability in acclimatization to culture conditions between each individual in the collection.

As shown on Figure 7, the Daily Growth Rates of all the strains cultivated in the LICSy during the period of the experiment decreased. The Daily Growth Rate of all the strains decreased sharply in November 2021.

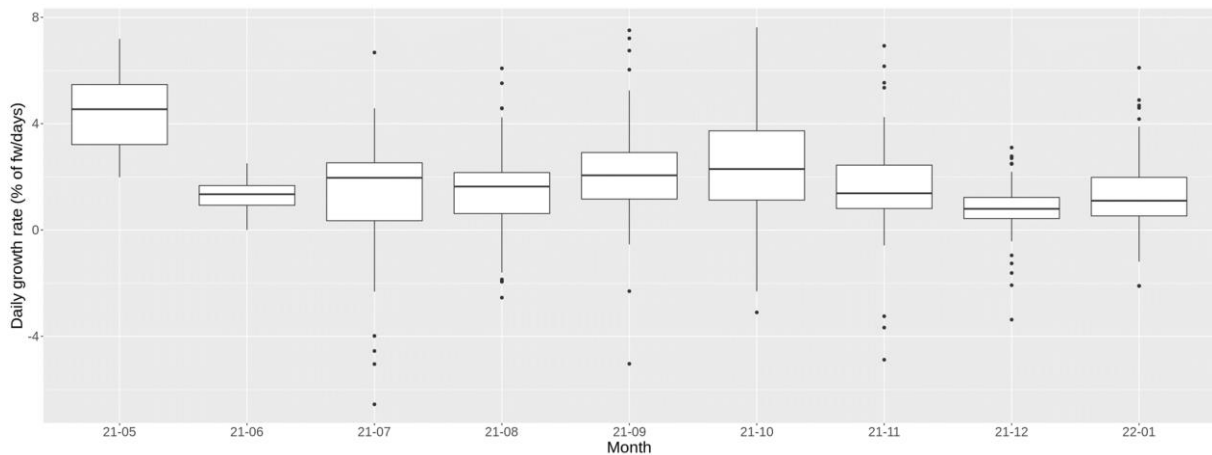


Figure 7: Daily growth rate (in % biomass gain per day) of all the strains kept in the LICSy from May 2021 to January 2022.

This reduction in the growth rate corresponds to contamination of all the strains by the filamentous red alga *Acrochaetium sp.* Indeed, during this period, the LICSy was operating in a close recirculation flow, and following an error in handling the filter cartridge 1 μ m mechanical cartridge has been replaced by a 5 μ m cartridge. This likely allowed fragments or spores of *Acrochaetium sp.*, present at the surface of some strains, to spread throughout the system. This incident highlights the need to isolate unialgal strains and to use the open flow culture system in order to limit the spread of contamination.

Figure 8 shows the growth rates according to geographic origin, bathymetric level and ploidy of strains conserved between February 2021 and January 2022.

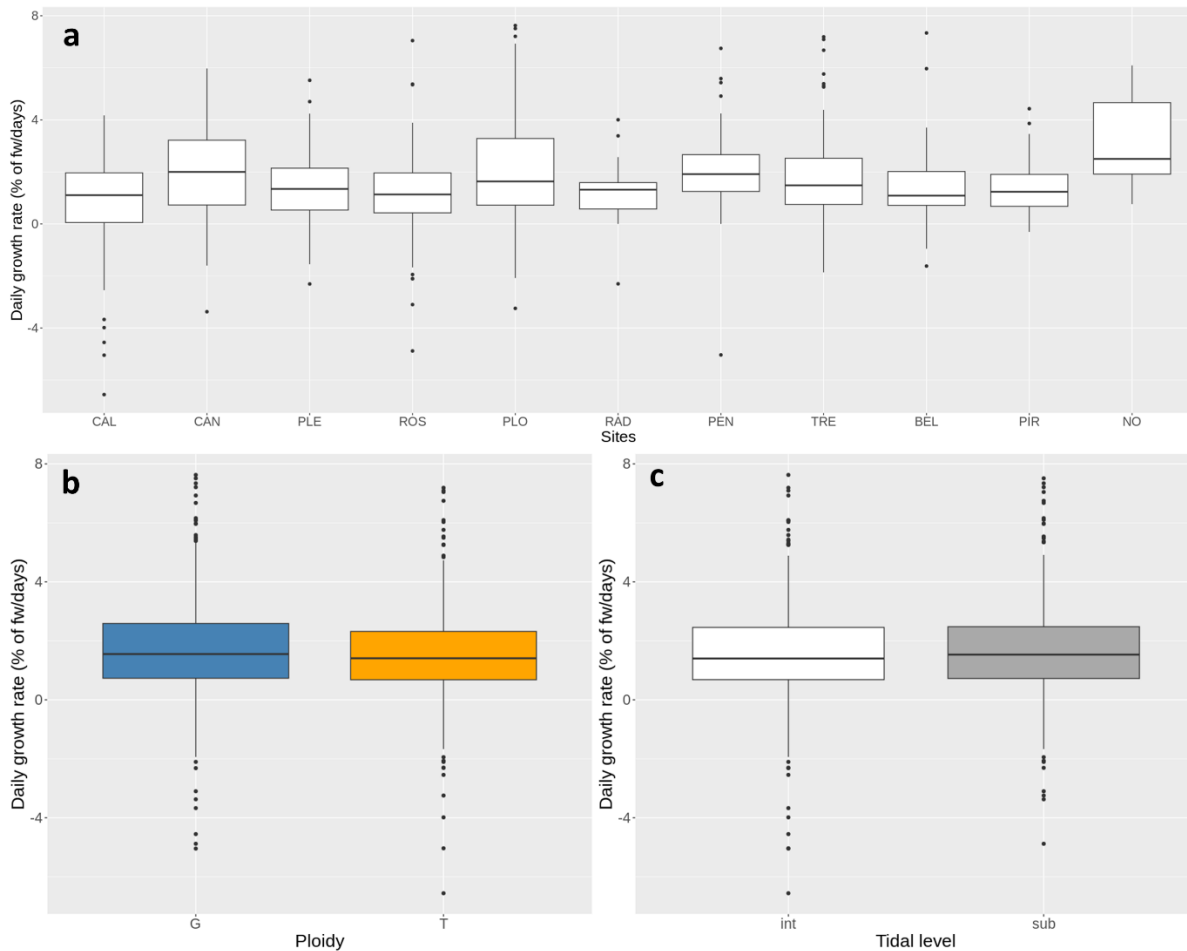


Figure 8: Box plots of the daily growth rate of strains stored in the LICSy between February 2021 and January 2022 as a function of (a) sampling site, (b) bathymetric level and (c) ploidy. Int= mid-littoral level; Sub = sublittoral level; G= male gametophyte (haploid); T=tetrasporophyte (diploid).

As for the experiment carried out in a 12-well plate, the ploidy (Kruskal-Wallis, $p = 0.108$) and the bathymetric level (Kruskal Wallis, $p = 0.536$) have no effect on the growth rate of the strains. The average growth rate of the strains depends mainly on the geographical origin (Kruskal Wallis, $p < 0.001$). It is also interesting to note the strong inter-individual variability was observed during this experiment, illustrated in Figure 9.

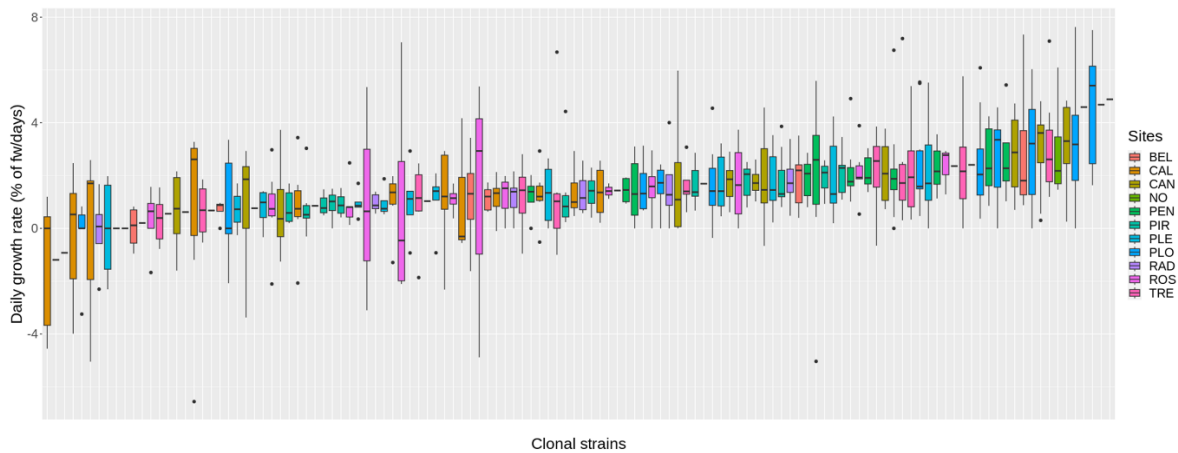


Figure 9: Box plots of the daily growth rate of all clonal strains stored in the LICSy from May 2021 to January 2022. The box plots are classified in ascending order based on the average daily growth rate measured during the experiment.

All of the strains which present daily growth rates greater than 0 (right part of the graph) correspond to strains of interest which have been selected for cultivation by vegetative propagation at 13°C. This result allows us to visualize the share of genetic resources available in Breton populations. It is interesting to note that even if certain populations show low DG rates, we still observe at least one individual who presents a phenotype of interest for cultivation. It should be taken into consideration that only 12 thallus were phenotyped per site. With a targeted sampling effort from a given population, for example a population close to a seaweed farm, it seems possible to isolate several strains of interest with this culture system.

When the LICSy was switched to an open flow circulation, we noted the appearance of benthic diatoms, despite filtration at 1µm. Treatment with germanium oxide (GeO₂) made it possible to control their propagation for a certain time, but can also impact the growth of the strains (Tatewaki and Mizuno, 1979) and it is costly to apply on the volume of the LICSy (+/- 100L). The proliferation of these benthic diatoms can cause significant nutrient starvation stress in the most contaminated strains. In certain cases, it triggers necrosis of the thallus. However, regular treatment with freshwater helped to limit the proliferation of diatoms.

The LICSy appears to be more suitable for vegetative growth of strains rather than for long-term conservation. As shown by the results on the vegetative growth of strains kept in collections, it is a great phenotyping tool. It is also very practical for generating healthy meristems in wild thallus in order to select and transfer new strains into 6-well dishes or glass flasks for long-term conservation. With the aim of optimizing and expanding the collection of strains, it therefore seems preferable to use the LICSy in an open flow system to cultivate healthy meristems from wild thallus collected from natural

populations. Monitoring the daily growth of thallus over one or two months will make it possible to select the strains of interest for vegetative propagation. Once the strains have been selected, they will be isolated under a binocular microscope to obtain unialgal strains and transferred to a 6-well dish or in flasks for long-term storage. For conservation in larger volumes, Erlen-Meyer flasks or sterile plastic flasks used more conventionally in culture collections will of course be usable for static conservation but this type of bottle requires regular interventions and significant maintenance which limits the number of strains conserved depending on available human resources (West,2005). The LICSy can also be used to reinitiate the growth of these strains preserved in 6-well plates.

Obtaining unialgal strains is essential to ensure the conservation of the strains in a thermostatically controlled cabinet, however the control of epiphytes in the culture environment in tanks and at sea is difficult. Therefore, it is necessary to put in place methods that allow contamination to be managed. In land-based cultivation systems, the mechanical filtration of seawater is limited to around a hundred microns in order to guarantee sufficient flow to feed the crops. It is therefore impossible to prevent the appearance of epiphytes in crops. However, it is possible to control the proliferation of these epiphytes by adjusting the density of the crops. Maintaining vegetative cultures at a high density makes it possible to limit the growth of epiphytes on the thalli and the walls of the culture system (Morgan et al., 1980; Morgan & Simpson, 1981; Pang & Lüning, 2004). In addition, observations made on our unialgal strains tested in a culture tank indicated that a clean strain tends to better resist to epiphytism than wild thallus which are hosting microscopic propagules of numerous organisms. Treating the biomass with fresh water at the time of inoculation also limits the proliferation of contamination during the first week of cultivation, particularly diatoms.

4.Conclusion

The various experiments presented in this chapter made it possible to set up a culture system and a methodology adapted to the conservation and selection of strains of interest for the cultivation of *P. palmata*. Managing contamination and obtaining uni-algal strains remains one of the key points in maintaining a collection adapted to the development of the culture. The results obtained underline the importance of the geographical origin of populations in the selection process. The selected strains could serve as a basis for initiating varietal selection by crossing. The low-intervention Cultivation System developed could also be used to select strains based on other criteria such as temperature resistance for future restoration programs of natural populations. This cultivation system can also be used for any species capable of reproducing by vegetative propagation of meristems.

Acknowledgements

This work benefited from the support of the French Government through the National Research Agency with regards to an investment expenditure program IDEALG (ANR-10-BTBR-04), the EU Horizon 2020 project GENIALG (Grant Agreement No 727892), the European Maritime and Fisheries Fund (EMFF) through the POLISTR project to MV and PP and Région Bretagne (PhD grant ARED Domdulse from ED227 Sorbonne-Université/MNHN to AB). We are grateful to the EMBRC France partner core facility for its technical support. The authors are also deeply indebted to the Service Mer & Observation (SMO) of Roscoff for sampling.

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Discussion Générale

Cette thèse a permis d'approfondir les connaissances fondamentales sur la ressource génétique, la biologie de la reproduction et les méthodes de maintien en culture de l'algue rouge *P. palmata* qui pourront être appliquées pour mener à bien sa domestication. L'utilisation d'une méthode innovante a permis le développement de marqueurs microsatellites chez 6 espèces de macroalgues dont *P. palmata* (Article n°1) pour laquelle nous disposons à présent de 11 marqueurs génétiques polymorphes adaptés à l'étude de la diversité intra-spécifique. Ces marqueurs ont permis d'étudier la génétique des populations sauvages de *P. palmata* en Bretagne, mettant en évidence une forte structuration génétique à l'échelle régionale (notamment entre le nord et le sud) et locale (différentiation entre niveaux de marée) dans un contexte océanographique complexe et des populations plus fortement impactées par les activités de récolte et/ou les impacts du changement climatique (Article n°2). Le suivi in situ de la température et les expérimentations en jardins communs ont permis de mettre en évidence la présence d'un processus d'adaptation locale à la température dans les populations du nord de la Bretagne (Article n°3). L'étude de la phénologie des populations m'a permis de mettre en évidence que la phase diploïde (tetrasporophyte) est plus abondante que la phase haploïde (gamétophytes) dans les populations naturelles, ce qui est probablement lié à une différence de fitness entre haploïdes et diploïdes (survie, durée de vie). L'utilisation des marqueurs microsatellites montre que *P. palmata* se reproduit exclusivement de façon sexuée. Elle s'effectue entre individus apparentés dans la majorité des populations étudiées, à l'exception du nord-ouest de la Bretagne (Article n°4). Des expérimentations en conditions contrôlées mettent en évidence que la taille des individus est un facteur important dans le déclenchement de la reproduction chez cette espèce et qu'il était possible d'induire la reproduction de souches isolées sur plusieurs années. Des essais de fécondation en condition d'écloserie montrent que davantage d'informations sur la fertilité des femelles et sur la durée de vie des spermatis sont nécessaires afin de maîtriser parfaitement cette étape du cycle de reproduction (Article n° 5). Finalement, j'ai mis en place des méthodes d'isolement et de conservation de souches adaptées au cycle très particulier de cette espèce par rapport aux autres Florideophyceae en développant un système de culture versatile qui permet la conservation et la sélection de souche d'intérêt pour l'aquaculture. En me basant sur la capacité de propagation végétative, j'ai sélectionné plusieurs souches d'intérêt pour l'aquaculture (Article n°6).

1. Bases biologiques

Les travaux effectués durant cette thèse ont permis d’approfondir les connaissances fondamentales sur la reproduction de *P. palmata* dans les populations naturelles et en laboratoire.

1.1 La reproduction sexuée

Les travaux effectués dans cette thèse permettent de mieux comprendre le mode et le système de reproduction au sein des populations naturelles et d’approfondir les connaissances sur la reproduction sexuée en laboratoire. Cependant, certains aspects fondamentaux de la reproduction nécessitent encore d’être étudiés, comme la différence de fitness entre haploïde et diploïde, la biologie particulière du gamétophyte femelle ou encore les conséquences du système de reproduction sur l’adaptation locale dans les populations étudiées.

1.1.1 Différence de fitness entre les phases du cycle de reproduction

La fitness ou valeur sélective individuelle est définie comme le nombre de descendants (et de gènes) légués à la génération suivante. Elle dépend donc du succès des individus c’est-à-dire de la fertilité et de la survie (au stade juvénile ou adulte) (Lenormand et al., 2016). Chez les macroalgues, la phase diploïde du cycle de reproduction est souvent considérée comme ayant un meilleur fitness que la phase haploïde (pour revue voir Valero et al. 1992). La dominance de la phase diploïde observée dans les populations naturelles de *P. palmata* est en accord avec ces observations. De même la différence de fréquences relatives des diploïdes entre les populations médiolittorales et infralittorales renforce cette hypothèse. En effet, on observe moins d’haploïdes dans la zone médiolittorale, qui est soumise aux stress environnementaux que dans la zone infralittorale (Article n°4). Les diploïdes auraient une meilleure survie que les haploïdes sur le terrain en revanche, les résultats des expérimentations au laboratoire ne montrent pas de différences significatives ni dans la croissance végétative (Article 6 et 5), ni dans la résilience au stress thermique (Article n°3) ou le temps d’induction de la maturité entre les différentes phases du cycle de reproduction (Article n°5). Il est donc probable que le ratio de ploïdie observé dans les populations naturelles résulte d’une différence de durée de vie entre les deux phases. Afin de tester cette hypothèse, il serait nécessaire de réaliser un suivi individu-centré de la dynamique des populations de *P. palmata* sur plusieurs années pour estimer la survie des différentes phases du cycle de reproduction dans le milieu naturel ainsi que la durée de vie moyenne des individus.

1.1.2 Biologie des femelles

Le faible taux de fécondation des femelles observé en éclosion et l’étude du système de reproduction dans les populations naturelles soulèvent plusieurs questions concernant la biologie des gamétophytes femelles chez *P. palmata*. La durée de vie des femelles a été estimée en culture à environ 6 à 8 mois (van der Meer & Chen, 1979)). Ces auteurs observent que les gamétophytes non fécondés peuvent

produire des thalles parthénogénétiques capables de produire des spores (van der Meer & Chen 1979). Cependant, la fréquence de ce phénomène dans les populations sauvages n'est pas connue. Un suivi de la survie de gamétophytes femelles que nous avons isolés en 2021 en plaques 48 puits et conservées à 10°C, avec une intensité faible durant plus de 2 ans, montre qu'environ 1,5% des gamétophytes femelles non fécondées produisent des thalles dressés de quelques millimètres

Le processus de parthénogenèse a été étudié chez l'algue brune *Ectocarpus siliculosus* chez qui la production de parthénosporophyte est accompagnée d'une diploïdisation du thalle (Bothwell et al., 2010). Les thalles femelles fertiles de *P. palmata* observés par van der Meer & Chen en 1979 portait des sores, semblable aux sporophytes, capable de produire des spores non viables. Durant l'étude du système de reproduction de *P. palmata*, nous avons détecté 3 tétrasporophytes, homozygotes au 11 locus, identifiés par cytométrie en flux ou par la présence de sores de sporocystes (Article n°4). Ces individus pourraient être issus de parthénogenèse. Quoiqu'il en soit, nous disposons de trop peu d'information sur le développement des thalles femelles pour en tirer des conclusions. Il serait donc intéressant d'étudier le devenir des femelles non fécondées dans le milieu naturel. Les gamétophytes femelles de *P. palmata* sont également capables de produire plusieurs trichogynes et donc être fécondées par plusieurs mâles ce qui donnerait des tétrasporophytes apparentés (ayant la même mère) partant du même pied. Cela pourrait contribuer à expliquer les taux élevés de consanguinité observés dans les populations naturelles (Article 3). La croissance des thalles femelles conservés en collection et l'estimation de leur ploïdie en cytométrie en flux pourrait nous permettre de mieux comprendre le phénomène de parthénogenèse. Ce type d'individus stériles pourrait avoir un intérêt pour la culture par propagation végétative en limitant les risques d'introduction de génotypes étrangers dans les populations naturelles.

1.1.3 Environnement et système de reproduction

L'étude du système de reproduction des populations bretonnes de *P. palmata* a révélé que la reproduction sexuée a lieu entre individus apparentés dans un peu moins de la moitié des populations étudiées (Article n° 4). Cette variation régionale pourrait être liée à des facteurs environnementaux ou biologiques comme la faible capacité de dispersion de *P. palmata*, responsables également de la forte structuration génétique des populations étudiées (Article n°2). En effet, les populations du nord-ouest de la Bretagne, qui présentent une reproduction panmictique, sont exposées à un plus fort hydrodynamisme (Garreau, 1993 ; Guillou et al., 2023). La dispersion limiterait la différenciation génétique entre les sites. Néanmoins, l'impact de l'autofécondation et de l'allofécondation chez cette espèce devrait être étudiés au laboratoire dans des conditions contrôlées. L'observation de fort taux de consanguinité dans les sites distants de Galway et du Cap Griz-Nez suggère que ces populations font

de l'autofécondation. Il serait intéressant d'étendre l'étude du système de reproduction à l'aide de marqueurs microsatellites, à l'ensemble de l'aire de répartition de *P. palmata*.

1.2 La reproduction asexuée

L'étude du mode de reproduction suggèrent que la reproduction asexuée dans les populations naturelles de *P. palmata* est négligeable. En revanche, en culture cette algue se reproduit très bien par bouturage, la reproduction végétative étant donc purement induite par l'homme. Cette modification du mode de reproduction a déjà été observée durant la domestication d'autres algues rouges comme *Kappaphycus sp.* et *Gracilaria chilensis* chez lesquelles la production de biomasse est assurée par la propagation végétative des thalles (Valero et al. 2017, Guillemin et al., 2008). Cependant, nous avons observé qu'au laboratoire les fragments de thalle étaient capables de générer des nouveaux disques de fixation à une très faible fréquence. La maîtrise de la reproduction asexuée chez cette espèce reste essentielle au développement de sa culture, notamment pour la conservation et la production de biomasse de géniteurs sélectionnés pour la culture en mer. Il est donc nécessaire d'approfondir les connaissances sur les facteurs impliqués dans la génération de repousses marginales.

2. Bases génétiques

2.1 Génétique des populations à l'échelle de l'aire de distribution de *P. palmata*

L'étude de la génétique des populations et de l'adaptation locale effectuée dans le cadre de cette thèse s'est focalisée sur une partie relativement restreinte de l'aire de répartition de *P. palmata* (Figure 17). Malgré cela, une forte différenciation génétique a été observée entre les populations le long des côtes de Bretagne et peut être attribuée à la faible capacité de dispersion résultant des traits d'histoire de vie particulier de cette espèce (Article n°2).

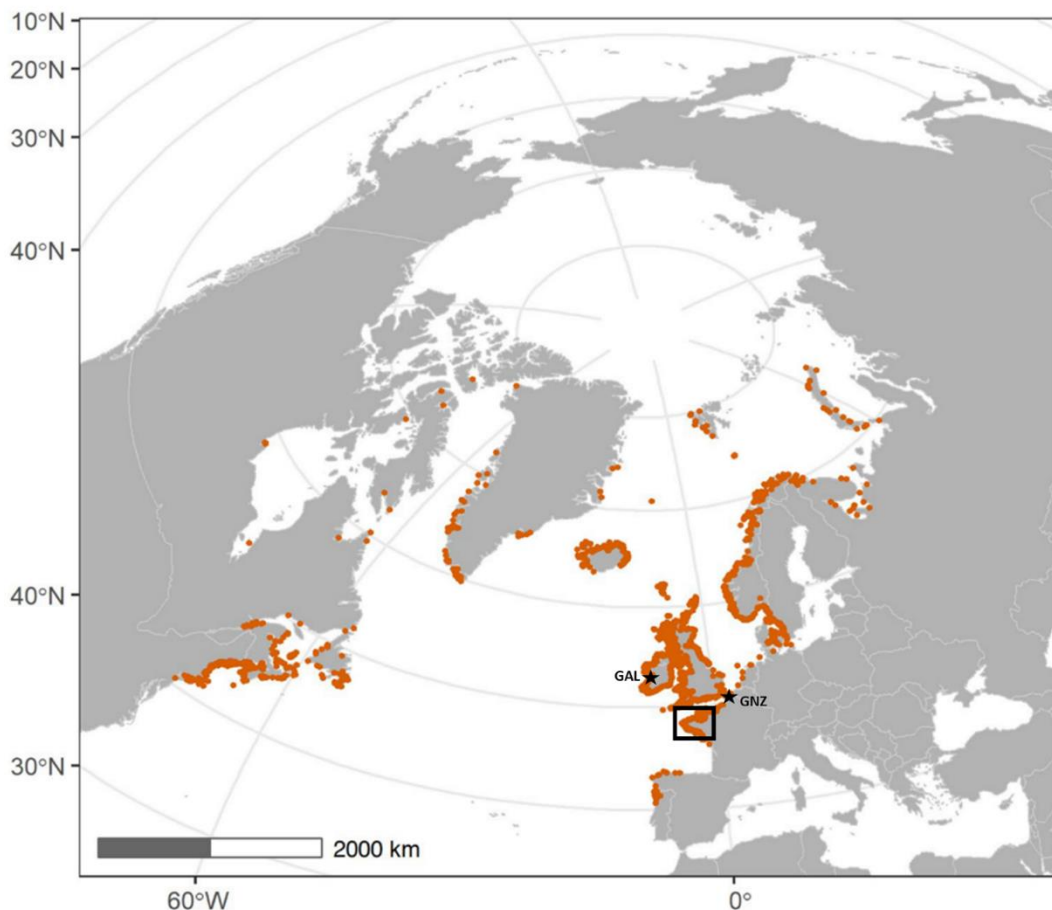


Figure 17 : Région d'échantillonnage et aire de répartition de *P. palmata*. GAL= Galway ; GNZ= Cap Gris-Nez. Adapté de Stevant et al., 2023.

L'étude de la différenciation génétique entre sites de Bretagne et le site échantillonné en Irlande (Galway) (Article n°4, matériel Supp.,) révèle une forte différenciation génétique à l'échelle de l'Europe et probablement de l'aire de répartition de *P. palmata* (figure X). Il serait intéressant d'élargir cette étude à l'ensemble de l'aire de répartition, en utilisant les marqueurs développés dans cette thèse (Article n°1). Les populations de *P. palmata* de part et d'autre de l'Atlantique sont caractérisées par des lignées mitochondriales distinctes dont l'isolement remonterait de 348 000 à 716 000 ans (Li et al. 2015). Cependant, le fort polymorphisme intraspécifique propre aux motifs microsatellites pourrait limiter l'utilisation de ce type de marqueurs génétiques, développés à partir d'individus d'Atlantique Nord-Est, pour génotyper les populations nord-américaines. L'article n°2 a également révélé l'existence d'une différenciation génétique significative à plus petite échelle (quelques dizaines de mètres) entre niveaux de marée dans les populations bretonnes mais également à Galway (Figure 18). Il sera nécessaire d'implémenter cet aspect dans les futures études de génétique des populations de *P. palmata* à l'échelle de son aire de distribution.

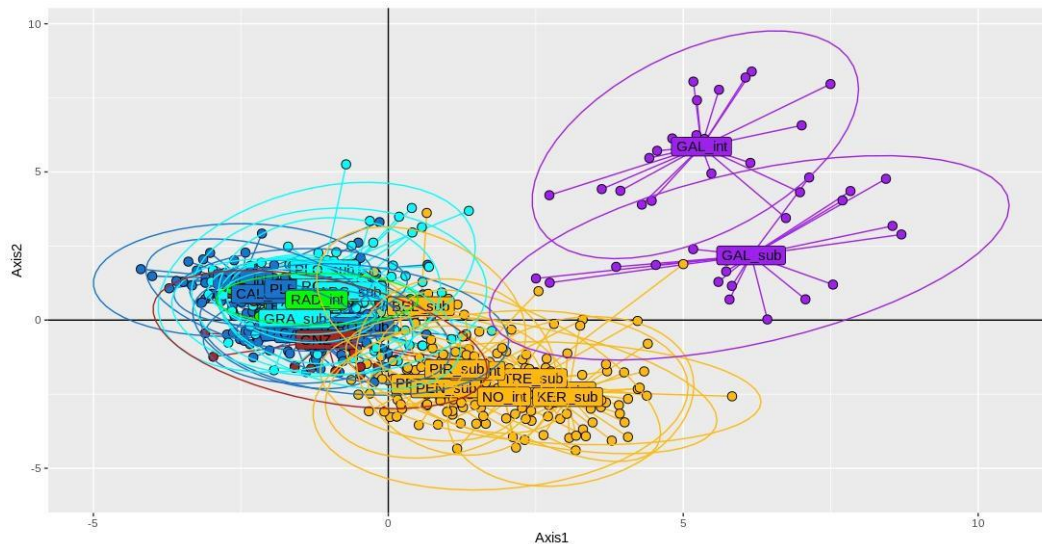


Figure 18 : Analyse discriminante par composante principale (DAPC) sur l'ensemble des populations géotypées durant la thèse (Article n°4, matérielles supplémentaires, voir Table 1 pour la correspondance des codes). Les couleurs indiquent les régions dans lesquelles ont été échantillonnés les sites. En bleu : Nord-Est Bretagne, en Cyan : Nord-Ouest Bretagne, En Orange : sud Bretagne, en Vert : Rade de Brest, en violet : Irlande (Galways) et en rouge : nord de la Manche (Cap Griz Nez).

La différenciation génétique observée entre les populations bretonnes suggère qu'il devrait y avoir une forte différenciation à l'échelle de l'aire de répartition (Articles n° 2 & 3). En effet, les populations étudiées dans l'article 3, nord-est et nord-ouest de la Bretagne, sont séparées seulement d'environ deux cents kilomètres, ce qui est peu comparé à la distribution latitudinale de *P. palmata* le long des côtes de l'atlantique Nord-Est (4000 km entre le nord de la Norvège et le nord du Portugal). De nouvelles expérimentations en jardin commun seront nécessaires pour mettre en évidence ce processus d'adaptation locale à l'échelle de l'aire de distribution de *P. palmata*. Il sera nécessaire de tester une gamme de températures plus large, notamment des températures plus froides auxquelles sont exposées les populations en limite nord de l'aire de répartition.

2.2 Résilience au changement climatique, gestion et restauration des populations sauvages

L'estimation de la température létale de *P. palmata* au seuil de 22,5°C (sur 4 jours d'exposition), la mise en évidence d'une adaptation locale à la température, la forte différenciation génétique observée entre les populations étudiées nous permet de mieux anticiper la réponse des populations du littoral face au changement climatique ainsi que de proposer des recommandations pour la gestion et la restauration des populations exploitées.

Les différents seuils de létalité d'exposition aux vagues de chaleurs observées entre les populations du Nord-ouest (20°C > température létale > 22.5°C) et du Nord-est (22.5°C > température létale > 25°C)

de la Bretagne ainsi que la forte différenciation génétique observée entre ces mêmes populations suggèrent que la réponse au changement climatique, notamment le réchauffement des eaux, ne sera probablement pas homogène sur l'ensemble de la région étudiée. En effet, les conditions particulières (stabilité annuelle et pluriannuelle de la température et fort hydrodynamisme), qui permettent aux populations du nord-ouest de la Bretagne de prospérer, font de cette partie du littoral une potentielle zone refuge pour de nombreuses espèces (Gallon et al. 2014). Cependant, le processus d'adaptation locale limite la capacité de ces populations à survivre aux vagues de chaleur, si la température dans cette région dépasse trop régulièrement le seuil de 20°C, les dommages sur les populations sauvages pourraient être irréversibles et profondément impacter la récolte de cette espèce à l'échelle européenne. La densité des populations qui s'y développe actuellement font la zone principale de récolte de *P. palmata* en Europe (Stévant et al 2023 ; CRPEM, 2020). La forte différenciation génétique observée le long du littoral breton et le long du gradient de marée limite les possibilités de recolonisation en cas d'extinctions locales au niveau médiolittoral, phénomènes déjà observés de façon saisonnière dans le sud de la Bretagne. Cependant, l'étude de la diversité génétique et du système de reproduction des populations sauvages n'indique pas d'effet notable de la récolte. Une étude spécifique permettrait d'évaluer l'effet de la récolte sur les populations sauvages, notamment le suivi de la génétique des populations exploitées sur plusieurs années.

Finalement, l'estimation de la valeur sélective des individus issus de croisement, entre apparentés ou non, permettra d'étudier l'impact du système de reproduction sur le maintien de l'adaptation locale dans le contexte de programmes de restauration ou de la domestication. De tels mécanismes ont récemment été étudiés chez *Macrocystis pyrifera* le long des côtes du Chili. Les résultats de cette étude montrent l'importance de considérer les effets de la vigueur hybride et de la dépression de consanguinité dans le maintien de l'adaptation locale, particulièrement dans le cadre de programmes de restauration (Solas et al., 2023).

3. Perspective pour la domestication de *Palmaria palmata*

Ces travaux de thèse ont permis d'apporter des connaissances fondamentales sur la structure et la diversité génétique des populations, le système de reproduction, la biologie de la reproduction et l'écologie de cette espèce, qui sont essentielles dans l'aboutissement du processus de domestication. L'application des connaissances acquises durant cette thèse et durant les 20 dernières années d'essais de culture devrait participer au développement de la culture économiquement viable de cette espèce, en initiant la sélection variétale et en améliorant les méthodes de culture en mer pour maximiser la production.

3.1 Programme de sélection et amélioration variétale par croisements

L'ensemble des essais de culture de *P. palmata* effectués ces 20 dernières années reposait sur l'utilisation de géniteurs sauvages. Cependant, comme l'a montré l'étude de l'acclimatation des souches aux conditions de culture présentées dans l'article 6, seulement une fraction des génotypes sauvages sont capables de survivre dans un environnement modifié. Les connaissances accumulées sur la maîtrise de la reproduction sexuée (induction de la maturité, fécondation, amélioration de la densité des supports de culture) et la propagation végétative, complété par les travaux de cette thèse (maîtrise de la reproduction de souches sélectionnées, conservation et caractérisation de la ressource génétique) permettent d'initier la sélection de génotypes à partir des populations sauvages et l'amélioration variétale par croisements afin d'améliorer la production de biomasse en culture.

3.1.1 Sélection

La mise en place d'un programme de sélection de souches devra prendre en compte les contraintes écologiques propres aux écosystèmes modifiés que sont les systèmes de culture à terre et en mer. La première étape de sélection devra se faire sur des souches sauvages et sur leur vitesse de croissance végétative, que ce soit pour la culture en bassin ou en mer. En effet, cette étape est primordiale pour assurer la rentabilité de la culture en bassin et la capacité à produire une biomasse de géniteurs suffisante pour l'ensemencement de support pour la culture en mer.

La sélection des premiers géniteurs sauvages s'effectuera en prenant en compte nos résultats sur la génétique des populations, qui suggèrent de focaliser les premières étapes de sélection en prenant des génotypes (mutants) présents dans les populations locales. *P. palmata* possède une capacité de dispersion limitée qui crée des différences génétiques significatives en fonction de la hauteur des populations sur l'estran. De plus, l'adaptation locale à l'échelle régionale suggère que la sélection devrait se restreindre aux populations locales afin d'éviter les risques de mal adaptation. À cela s'ajoute des différences écologiques majeures entre niveaux de marée qui pourraient impacter la capacité d'adaptation des individus sauvages aux systèmes de culture en mer ou à terre. Les individus présents dans la zone médiolittorale, régulièrement exondés et exposés à de forts stress abiotiques, pourraient se montrer plus résistants aux variations de température propres à la culture en bassin. Quant aux populations de l'infralittoral supérieur, rarement exondées et fixées sur les stipes de laminaires, elles se développent dans un environnement plus proche des cultures en mer sur filière. Il serait donc préférable de commencer par la sélection des souches locales dans les populations médiolittorales et infralittorales sur la base de leur croissance végétative avant de les tester dans différents systèmes de culture. En effet, la croissance végétative est le premier trait à sélectionner pour assurer la production de géniteurs par propagation végétative en bassins. D'autres traits comme

la longueur des thalles ou la reproduction précoce pourront ensuite être sélectionnées (Schmedes, 2020). Des connaissances supplémentaires sur les processus physiologiques impliqués dans la production de repousses marginales sont également nécessaires afin d'améliorer la densité des cultures par propagation végétative et sécuriser la production des géniteurs sélectionnés. Le pool de souches sauvages sélectionnées servira de bases pour initier un programme d'amélioration variétale par croisement. Ce pool de souches pourra par ailleurs être renforcé par l'ajout des souches déjà sélectionnées pour la production végétative et actuellement conservées en collection à la Station Biologique de Roscoff.

3.1.2 Croisement

Les croisements, prenant en compte le décalage générationnel dû au dimorphisme sexuel seront effectués. Comme l'illustre la figure 19, un ou plusieurs tétrasporophytes sauvages, sélectionnés sur la croissance végétative et amenés à maturité par l'induction artificielle de la sporogenèse, devront être utilisés pour produire les gamétophytes mâles et femelles. Ces dernières seront fécondées par un ou plusieurs gamétophytes mâles sauvages, sélectionnés sur la croissance végétative et amenés à maturité par l'induction artificielle de la gamétogenèse afin de féconder les femelles et produire de nouveaux sporophytes.

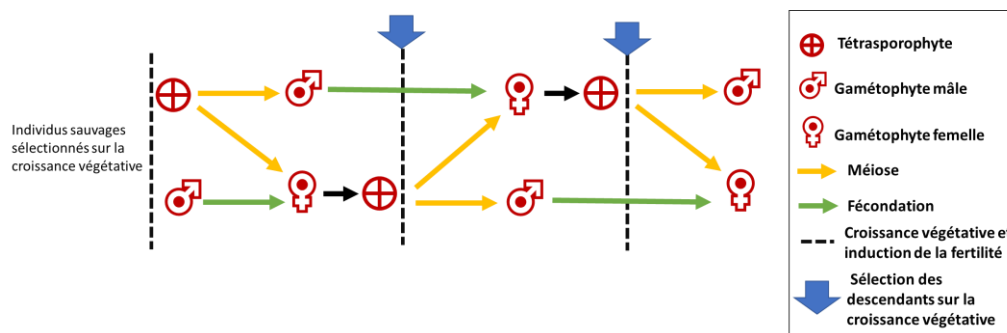


Figure 19 : Amélioration variétale par croisements de l'algue rouge *P. palmata*

Les descendants (tétrasporophytes issus de croisements et gamétophytes mâles issus de méioses) devront alors être sélectionnés sur leur capacité à survivre et à se développer sur les supports de culture en mer ou en bassin selon les besoins. Les thalles de cette pseudo-génération F1 qui aura été sélectionnée, seront utilisés pour la deuxième phase de croisement après une étape de croissance végétative et d'induction de la maturité.

L'origine des individus utilisés pour les croisements nécessite plus de connaissances sur les effets de l'allo fécondation ou de la consanguinité sur la fitness (survie et reproduction) des individus. Le futur programme d'amélioration variétale devra implémenter les problématiques liées à la différenciation entre populations et entre niveaux de marée en testant les rendements en bassin et sur filières des

descendants issus de croisements entre géniteurs apparentés ou non. Les programmes de sélection variétale sur les laminaires ont montré qu'il était possible d'obtenir des cultivars en 6 à 7 générations sur la base de croisements successifs entre gamétophytes apparentés (Zhang et al. 2007). Cependant, des études récentes montrent la fragilité de tels cultivars face aux changements environnementaux ou aux maladies ce qui suggèrent la nécessité de conserver le potentiel évolutif des populations cultivées en conservant une certaine diversité lors de la sélection des souches (Valero et al. 2017).

3.2 Production de supports ensemencés avec des souches sélectionnées.

Le développement d'un système d'écloserie et de cultures en mer et en bassins, adaptés à cette espèce, sera basé sur l'utilisation de souches sélectionnées qui permettront d'améliorer la production en mer.

3.2.1 Support de culture

Traditionnellement, la culture en mer s'effectue par ensemencement de spores sur des cordelettes en écloserie qui sont déployées en mer sur des cordes porteuses. Ces méthodes sont fortement inspirées de la culture des laminaires. Cependant, ces méthodes pourraient être améliorés car contrairement aux laminaires, les spores de *P. palmata* ne sont pas moiles, ce qui engendre une hétérogénéité des ensemencements.

Le problème de densité et d'homogénéité des supports de culture en mer a été résolu par le développement d'une méthode innovante d'ensemencement par Schmedes et al. (2019). Basée sur la capacité de fixation des spores et de gamétophytes, cette étude a montré que l'ajout de bullage durant la phase de sporulation permet d'assurer l'homogénéité de la fixation des spores sur le support de culture. Ensuite les gamétophytes qui se développent sur les fonds du bassin d'ensemencement peuvent être remis en suspension pour se fixer sur le support de culture, augmentant ainsi la densité d'ensemencement. De plus, la fécondation des gamétophytes femelles améliore également la densité des plantules sur les supports de culture (Schmedes, 2020).

Cependant, les observations de terrain effectuées durant cette thèse et la littérature suggère que *P. palmata* est un épiphyte opportuniste (Stévant et al., 2023), qui occupe l'espace disponible lors du recrutement de spores non motiles seulement si le substrat est disponible. Contrairement à la laminaire, cette espèce ne développe ni crampon, ni stipe, limitant sa capacité à occuper l'espace. L'utilisation de cordelettes ensemencées pour la culture de *P. palmata*, même avec des densités correctes, expose les cultures en mer aux espèces opportunistes capables d'occuper rapidement l'espace comme *Saccorhiza polyschides*. Les stipes de *L. hyperborea* paraissent être un bon exemple de support de culture. Leur rugosité assure la fixation optimale des spores et leur exposition au courant assure à la dulce un apport en nutriments régulier. Bien que cette relation semble simplement résulter

de phénomènes physiques, il serait intéressant d'étudier le rôle des communautés bactériennes associées aux algues brunes dans la fixation et la survie des spores de *P. palmata* dans le milieu naturel. L'environnement dans lequel se développe *P. palmata* dans cet étage du littoral sont plus proches des conditions de cultures sur filière. Le meilleur moyen de limiter la compétition pour l'espace sur les filières semble être donc d'effectuer l'ensemencement directement sur les cordes porteuses.

Des supports de culture alternatifs pourraient également être testés dans le but de tirer profit de la possibilité de régénération de cette espèce au fil des saisons. Des filets pourraient être ensemencés et déposés à même le fond, à une profondeur compatible avec la croissance de *P. palmata*, pour créer des champs artificiels exploitables sur plusieurs années.

3.2.2 Écloserie adaptée à l'utilisation de souches sélectionnées.

Il est nécessaire de repenser les systèmes d'écloserie pour la production de supports ensemencés avec des souches de *P. palmata* sélectionnées, dont la maturité aura été induite artificiellement. Ce système d'écloserie devra intégrer les résultats des travaux de Schmedes (2020). Un système composé de deux bassins avec une circulation en série, alimenté en eau de mer filtrée peut être envisagé. Le premier bassin, adapté à la culture de boutures, contiendra les géniteurs (sporophytes et gamétophytes mâles sauvages ou issus de croisements sélectionnés) dont la maturité aura été préalablement induite dans le même bassin en simulant des conditions hivernales. Les observations faites lors de la maturation de cultures clonales suggèrent que la libération de spores ou de spermaties est synchrone à l'échelle de la culture clonale si cette dernière présente des thalles de tailles homogènes. Le suivi de la maturité des tetrasporophytes et des gamétophytes permettra d'anticiper la période la plus propice à l'ensemencement des supports. Un second bassin, adapté à l'accueil des supports d'ensemencement, recueillera les spores et de spermaties. Dans un premier temps, le bassin n°1 sera chargé de tetrasporophytes matures pour ensemençer les supports, après une semaine d'ensemencement les sporophytes seront remplacés par des gamétophytes mâles matures afin de féconder les gamétophytes femelles issus de la sporulation. Le bassin n°2 sera muni d'un système d'agitation par bullage dans le but d'améliorer la croissance et l'homogénéité des plantules sur les supports de culture. Finalement et après quelques semaines d'écloserie, les gamétophytes et sporophytes fixés sur les parois et le fond du bac d'écloserie seront remis en suspension pour accroître la densité d'ensemencement selon la méthode GMA (Germination Maceration Agitation) de Schmedes et al. (2019). Ce système permettra de réaliser la production végétative de thalles de géniteurs et l'induction de leur maturité dans le bassin n°1 et l'ensemencement de support dans le bassin n°2

Conclusion générale

Les connaissances fondamentales obtenues durant cette thèse contribuent à développer les bases biologiques et génétiques de la domestication de l'algue rouge *Palmaria palmata*. L'étude du mode et du système de reproduction montre que dans la nature, cette espèce se reproduit essentiellement de façon sexuée et que les populations sont composées en majorité d'individus diploïdes. L'utilisation des marqueurs génétiques et des expérimentations, ont permis d'étudier la ressource génétique des populations naturelles et de mettre en évidence une forte structuration génétique des populations ainsi qu'un processus d'adaptation locale à la température. Ces résultats ouvrent des perspectives sur l'étude de ce processus à l'échelle de l'aire de répartition de *P. palmata* ainsi que pour la gestion et la restauration des populations sauvages exploitées. La caractérisation de la ressource génétique, la maîtrise du cycle de reproduction en laboratoire de souches sélectionnées et le développement de méthodes de conservation de ces souches en collection ouvrent de nouvelles perspectives pour la domestication de cette espèce. L'utilisation de la ressource génétique permettra d'orienter la sélection et les croisements de souches issues des populations sauvages. Les méthodes de conservation en collection et la maîtrise de l'induction de la reproduction permettront d'utiliser ces génotypes sélectionnés pour la production de plantules à la demande et d'initier l'amélioration variétale par croisements (Figure 19).

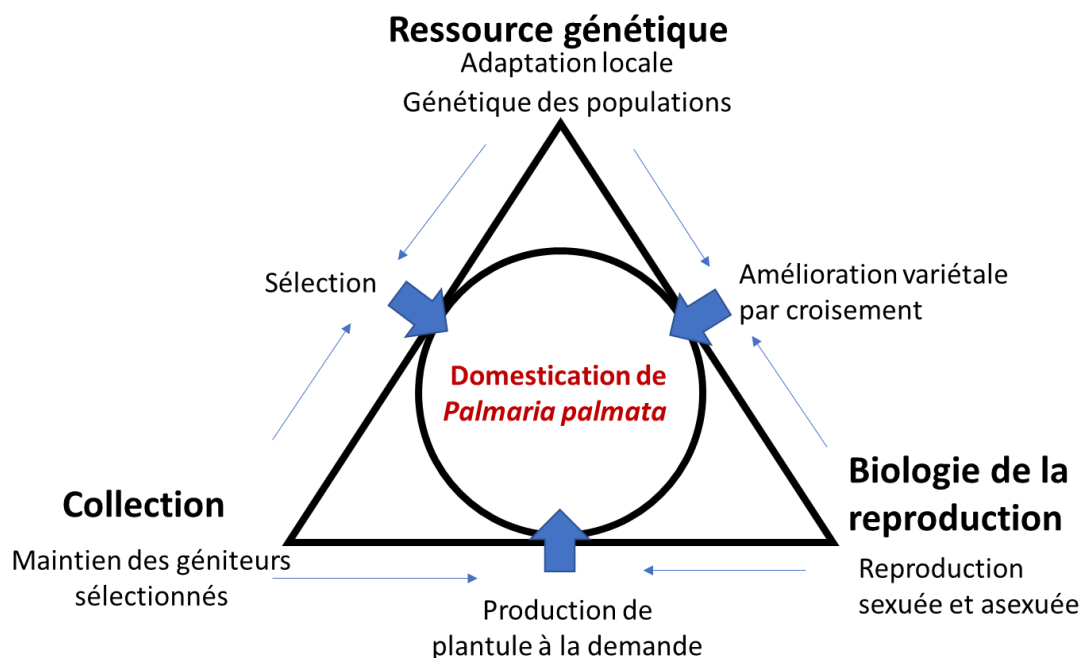


Figure 19 : Prochaines étapes de la domestication de l'algue rouge *Palmaria Palmata*

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Résumé :

L'algue rouge *Palmaria palmata* utilisée dans l'industrie alimentaire, est récoltée à marée basse dans la zone de balancement des marées. La demande croissante pour cette algue nécessite de franchir un pas pour trouver une alternative économique efficace à la récolte. Plus de 20 années d'essais de culture ont permis d'identifier des méthodes d'ensemencement et de propagation végétative adaptées à la biologie particulière de cette algue rouge, mais sans développement commercial. L'objectif central de mes travaux de thèse était donc de lever les verrous de la domestication de cette espèce.

J'ai abordé les premières étapes du processus de domestication chez *P. palmata*. Ce processus long et complexe nécessitait des connaissances approfondies de sa ressource génétique, de sa reproduction ainsi que des méthodes de conservation de souches en collection. La caractérisation de la ressource est une étape indispensable pour développer une aquaculture durable. Elle nécessite le développement d'outils adaptés afin d'étudier la diversité et la structure génétique des populations sauvages. La compréhension du mode et du système de reproduction de l'espèce est primordiale afin de maîtriser sa propagation en culture, de développer des collections de géniteurs pour initier des programmes de sélection variétale.

La structure génétique des populations de *P. palmata* a été étudiée en utilisant des marqueurs génétiques de type microsatellites que nous avons développé au sein du laboratoire. L'étude des populations le long des côtes bretonnes et à différents niveaux bathymétriques m'a permis de mettre en évidence des différenciations génétiques significatives entre populations séparées par quelques dizaines de mètres. Ces résultats suggèrent d'une part que la dispersion est limitée chez cette espèce et d'autre part que sa structure génétique est fortement influencée par l'isolement spatio-temporel des populations dû au balancement des marées. A plus grande échelle, les résultats confirment l'importance des courants océaniques dominants dans la dispersion de cette espèce. Des expérimentations en jardin commun que j'ai réalisées au laboratoire révèlent des différences de réponses à la température entre populations en fonction de leur origine géographique. Ces résultats ainsi que la différenciation génétique entre populations suggèrent l'existence de phénomènes d'adaptation locale au sein de cette espèce.

Contrairement à la majorité des Florideophyceae, le cycle des *Palmariales* est diphasique. Il se caractérise par l'absence de cystocarpe (phase de multiplication du zygote) et par un dimorphisme important entre mâle et femelle. Les femelles sont microscopiques et les mâles et les tétrasporophytes ont la même forme et peuvent atteindre jusqu'à un mètre de long. L'étude de la biologie des populations en utilisant des outils de génétique des populations m'a permis de mieux comprendre la reproduction chez cette espèce. Les résultats de la phénologie, des génération-ratios et des indices génétiques (F_{is} , Beta Pareto) montrent que cette espèce se reproduit par voie sexuée. Les croisements expérimentaux réalisés au laboratoire et les observations de terrain suggèrent que les tétrasporophytes présentent une meilleure valeur sélective que les haploïdes. Par ailleurs, j'ai pu montrer expérimentalement que l'initiation de structures reproductrices chez cette espèce dépendait de la taille du thalle.

Enfin, dans le but de conserver des souches d'intérêt pour l'algoculture et l'expérimentation, un système de culture versatile capable d'accueillir jusqu'à 120 souches a été développé. Ce système de culture révèle une forte différence d'acclimatation des souches sauvages aux conditions de laboratoire et une grande plasticité morphologique en fonction des conditions de culture.

Ces travaux ont donc permis d'établir les bases biologiques et génétiques nécessaires à l'avancement du processus de domestication de cette espèce d'intérêt commercial.

Abstract :

The red seaweed *Palmaria palmata* used mainly in food industry, is harvested in wild populations thriving on rocky foreshores. The increasing market size for this species underline the need to transition from harvester to cultivation. More than 20 years of applied research resulted in seeding and vegetative propagation methods adapted to the particular biology of this species without commercial upscaling. The main aim of this thesis work was to unlock the domestication process of this species.

I studied the first step of the domestication process of *P. palmata*. This long and complex process required better knowledge of its genetic resource, its reproduction and strain collection conservation methods. Investigating natural resource is necessary to develop durable cultivations. Thus, development of adapted tools to study genetic diversity and structure of wild populations is needed. Understanding reproduction mode and system is also primordial to master its propagation within culture or develop breeding program base on selected strain.

We developed microsatellite genetic markers to study genetic structure of *P. palmata* natural populations. Studies of those populations along Brittany coast, at two tidal levels, allowed to highlight significative genetic differentiation between population distant of few dozen meters. Those result first suggest that dispersion is limited for the species and secondly, its genetic structure is highly impacted by spatial and temporal isolation resulting from tide cycles. At a larger scale, the results corroborate the importance of dominant oceanic currents in this species dispersion. The common garden experiment I conducted in the laboratory revealed differences in response to temperature between populations depending on their geographical origins. Those results, along with the genetic differentiation occurring between populations, suggest the occurrence of local adaptation process ongoing within this species regional distribution range.

Contrary to most of the Florideophyceae, *Palmariales* life cycle is biphasic. It is characterized by the absence of cystocarp (zygotic multiplication phase) and by a marked dimorphism between male and female. The females are microscopic while males and tetrasporophytes are isomorphic et can reach up to one meter in length. Study of population biology using population genetic tools allowed me to better understand this species reproduction. The results of phenology, generation ratio and genetic index (Fis, Beta Pareto) show that this species reproduce sexually. The experimental crosses conducted in the laboratory along with the field observations suggest that tetrasporophytes present a better fitness than haploids. I have also experimentally demonstrated that the triggering of reproductive structures depend on thallus length.

Finally, in order to ensure conservation of strain of interest for cultivation and experimentation, we developed a versatile cultivation system able to keep up to 120 strains with reduced intervention. This system was also use to phenotype strain and revealing high differences of acclimation abilities of wild strains to lab conditions as well as great morphological plasticity depending on lab cultivation conditions.

This work permitted to establish the biologic and genetic bases necessary to advance the domestication process of this species of commercial interest.