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Kévin Drouet

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Ecole Doctorale des Sciences de l'Environnement d'Ile de France (ED 129)

Impact de la température sur la biogéographie et la phénologie des dinoflagellés benthiques toxiques du genre *Ostreopsis* en Méditerranée et en Atlantique

Thèse présentée par

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En vue de l'obtention du grade de
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Cette thèse est dédiée à mes parents

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A bercé mon cœur, pour la vie »
Charles Trenet, *La Mer*, 1946

Sommaire

Préambule

Introduction..... 20

1. Les microalgues : rôle et diversité.....	21
1.1. Phytoplancton et production primaire.....	21
1.2. Diversité des microalgues.....	22
1.3. Espèces toxiques et Harmful Algal Blooms.....	23
2. Les microalgues : Ecologie.....	25
2.1. Facteurs influençant la composition des communautés microalgales.....	25
2.2. Variabilité spatio-temporelle des microalgues.....	26
2.3. La température : Un facteur de contrôle clé des dynamiques d'efflorescences.....	29
2.3.1. Relation entre température et physiologie des microalgues.....	29
2.3.2. Température et distribution des microalgues.....	32
2.3.3. Température et phénologie des microalgues.....	33
3. Impacts des changements environnementaux d'origine anthropique sur les microalgues.....	35
3.1. Réchauffement global.....	35
3.2. Acidification des océans.....	37
4. Recensement, expansion et surveillance des HABs.....	40
5. Le modèle <i>Ostreopsis</i> spp.....	45
5.1. Taxonomie.....	45
5.1. Ecologie d' <i>Ostreopsis</i> spp., dinoflagellé benthique toxique.....	46
5.3. Distribution.....	50
5.4. Toxicité.....	52
5.5. Méditerranée et Atlantique : cas d'études de la thèse.....	54

Objectifs de la thèse..... 57

Chapitre 1 : Le dinoflagellé benthique toxique *Ostreopsis* cf. *ovata* en Méditerranée nord-occidentale : Relation entre températures printanières et phénologie des efflorescences..... 61

Contexte de l'étude..... 62

Contribution des auteurs..... 62

1. Introduction.....	65
2. Material and Methods.....	68
2.1. Sampling sites.....	68
2.2. Sampling process and cell quantification.....	68
2.3. Hydroclimatic parameters.....	70
2.4. Calculation and Statistics.....	70
3. Results.....	71
3.1. Spatial variability.....	71
3.2. Interannual variability.....	73

3.3.	Relations between environmental factors and <i>Ostreopsis cf. ovata</i> blooms.....	75
4.	Discussion.....	79
4.1.	Net growth rate.....	80
4.2.	Variations in bloom phenology.....	81
4.3.	Spring SST anomalies: a tool for managers and modelers.....	83
5.	Conclusion.....	84

Chapitre 2 : Suivi de l'expansion du dinoflagellé benthique nuisible *Ostreopsis cf. siamensis* dans le Golfe de Gascogne en Atlantique Nord-Est..... 87

Contexte de l'étude.....	88	
Contribution des auteurs.....	89	
1.	Introduction.....	91
2.	Material and methods.....	95
2.1.	Study area.....	95
2.2.	Field sampling of epiphytic microalgal cells and eDNA.....	98
2.3.	<i>Ostreopsis</i> spp. cell quantification.....	99
2.4.	DNA extraction and amplification from cultures and environmental samples.....	99
2.5.	Real time PCR of eDNA from environmental samples and plastic polymer substrates.....	101
2.6.	Advection and connectivity among sampled areas.....	102
3.	Results.....	104
3.1.	Light microscopy analysis of fixed samples.....	104
3.2.	Isolation of strains and species identification.....	105
3.3.	Environmental DNA (eDNA) analysis.....	108
3.4.	Connectivity analysis.....	110
4.	Discussion.....	113
4.1.	<i>Ostreopsis cf. siamensis</i> eDNA analysis.....	113
4.2.	Geographical and niche ranges.....	115
4.3.	Potential effect of global changes on <i>O. cf. siamensis</i> expansion.....	118
5.	Conclusions.....	120
6.	Appendix.....	122

Chapitre 3 : Acclimatation thermique et réponses physiologiques en fonction de la température chez *Ostreopsis cf. siamensis* et *Ostreopsis cf. ovata* du nord-est de l'Atlantique et du nord-ouest de la Méditerranée..... 131

Contexte de l'étude.....	132	
Contribution des auteurs.....	132	
1.	Introduction.....	135
2.	Material and methods.....	138
2.1.	Culture conditions.....	138
2.1.1.	<i>Ostreopsis</i> strains and maintenance of stock cultures.....	138
2.1.2.	Thermal acclimation experiment.....	140
2.2.	Cell abundance quantification and growth rate calculation.....	141

2.3.	Estimations of microalgal toxicity.....	141
2.3.1.	<i>Ostreopsis cf. siamensis</i> toxin extraction and identification by LC-MS/MS.....	141
2.3.2.	<i>Artemia franciscana</i> toxicity bio-assay.....	142
2.4.	Maximum quantum yield of PSII.....	143
2.5.	Pigment analyses through High-Performance-Liquid-Chromatography (HPLC).....	143
2.6.	Reactive Species of Oxygen (ROS) production.....	144
2.7.	Nitrites (NO ₂ -) excretion.....	145
2.8.	Statistical analysis and modeling.....	145
3.	Results.....	146
3.1.	Growth rates.....	146
3.2.	<i>Ostreopsis cf. siamensis</i> toxicity.....	149
3.3.	Physiological stress indicators.....	152
3.3.1.	Fv/Fm.....	152
3.3.2.	ROS production.....	153
3.3.3.	Nitrites excretion.....	153
3.3.4.	Pigment composition.....	154
4.	Discussion.....	155
4.1.	Optimal growth temperature for <i>O. cf. siamensis</i>	155
4.2.	Acclimation strategy towards temperatures.....	157
4.3.	Thermal niche and potential distribution limits.....	160
4.4.	Toxicity and temperature.....	163
4.5.	Risk repartition.....	166
5.	Conclusion.....	167
	Discussion générale et perspectives.....	171
	Références bibliographiques.....	191
	Annexes.....	213

Listes des articles publiés, soumis ou en préparation

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Drouet K., Lemée R., Guilloud, E., Malestroit P., Schmitt S., Laza-Martinez A., Seoane S., Boutoute M., Réveillon D., Hervé F., Siano R. and Jauzein C.. Thermal acclimation and eco-physiological responses towards temperature in *Ostreopsis* cf. *siamensis* and *Ostreopsis* cf. *ovata* from NE Atlantic and NW Mediterranean Sea. In preparation.

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Siano R., Jauzein C., Seoane S., Laza-Martinez A. Lemée R. and **Drouet K.**. *Ostreopsis* cf. *siamensis* : un nouveau risque d'HAB dans le Golfe de Gascogne ? Journées REPHY, February 2019, Nantes.

Liste des figures

Figure 1 : Schéma de la pompe biologique du carbone dans les océans.....	22
Figure 2 : Comparaison de la gamme de taille du phytoplancton relatif à des objets macroscopiques.....	23
Figure 3 : Colorations d'eaux côtières suite à des efflorescences des dinoflagellés <i>Karenia brevis</i> (gauche) et du genre <i>Noctiluca</i> (droite).....	24
Figure 4 : Moyennes annuelles de la concentration globale de la chlorophylle-a à la surface des océans en 1998 et 2007.....	27
Figure 5 : Distribution de la richesse spécifique du phytoplancton.....	28
Figure 6 : Schéma des processus physiques et biologiques influençant le cycle annuel du phytoplancton.....	29
Figure 7 : Modèle de température cardinale avec inflexion (« Cardinal Temperature Model with Inflexion », CTMI) représentant le taux de croissance de la diatomée <i>Asterionella formosa</i> en fonction de la température.....	30
Figure 8 : Gradient latitudinal des températures optimales pour la croissance des espèces phytoplanctoniques marines et estuariennes.....	33
Figure 9 : Changements temporels dans le timing des pics de blooms saisonniers du dinoflagellé <i>Ceratium fusus</i> et de la diatomée <i>Cylindrotheca closterium</i> entre 1958 et 2002.....	34
Figure 10 : Paramètres physiques influençant la biomasse globale du phytoplancton.....	36
Figure 11 : Prévisions de l'évolution du climat à travers le projet d'intercomparaison des modèles couplés (CMIP5) et selon différents scénarios.....	38
Figure 12 : Représentation de l'évolution de la température des eaux de surfaces à travers le projet d'intercomparaison des modèles couplés (CMIP5) et l'utilisation du scénario RCP8.5 en moyennant 25 modèles climatiques intégrés dans le portail de la NOAA Climate Change (https://www.esrl.noaa.gov/psd/ipcc/ocn/).....	39
Figure 13 : Evolution du nombre d'évènements recensés liés aux toxines de type PSP entre 1970 et 2005.....	42
Figure 14 : Potentiels scénarios d'élargissement de la fenêtre temporelle de développement d' <i>Alexandrium catenella</i> à Sequim Bay (Puget Sound, Etats-Unis) suite à une augmentation de +2, +4 et +6°C de la température de surface la température de surface.....	43
Figure 15 : Effets des différents facteurs environnementaux (température, stratification, acidification, eutrophisation, prédation) impactant différents types d'HABs.....	44
Figure 16 : Dessins originaux de la première description morphologique d' <i>Ostreopsis siamensis</i> dans le Golfe de Siam.....	46
Figure 17 : Arbre phylogénétique (Maximum Likelihood) des espèces d' <i>Ostreopsis</i> à partir des domaines D1 – D3 de la grande sous-unité de l'ADNr.....	47
Figure 18 : Observations de cellules d' <i>Ostreopsis</i> spp.....	48
Figure 19 : Schéma du cycle de vie des dinoflagellés.....	49
Figure 20 : Répartition mondiale des différentes espèces d' <i>Ostreopsis</i> selon des données d'entre 1995 et 2019.....	51
Figure 21 : Structure chimique de la palytoxine et de ses analogues retrouvés dans différentes espèces d' <i>Ostreopsis</i>	53
Figure 22: Map of the sampling sites (from 1 to 5) in Larvotto, Monaco. In the top-left corner is the map of the NW Mediterranean Sea, with the location of Monaco, between France and Italy.....	69

Figure 23: Inter-site variability of benthic cell abundance during the <i>Ostreopsis cf. ovata</i> bloom monitoring of summer 2017 in Larvotto Beach.....	72
Figure 24: Correlation between benthic and planktonic cell abundances of <i>Ostreopsis cf. ovata</i> in Larvotto beach.....	73
Figure 25: Evolution of benthic abundances of <i>Ostreopsis cf. ovata</i> and SST during summer in Larvotto (Monaco) from 2007 to 2019.....	75
Figure 26: Representation of environmental parameters on the first factorial space of the Principal Component Analysis (PCA).....	76
Figure 27: Variation of positive growth rates with sea surface temperatures.....	77
Figure 28: Weekly cumulative sum of SST anomalies in Point B (Villefranche-sur-Mer) corresponding to each year of survey.....	78
Figure 29: Correlation between bloom starting day and cumulative sum of SST anomalies.....	79
Figure 30: Map of the study area and sampling sites along the Bay of Biscay during the 2018 sampling campaign.....	97
Figure 31: Abundances of epiphytic <i>Ostreopsis</i> spp. cells per gram of macroalgae fresh weight (cell/gFW) in each sampling site in relation to water temperature and salinity across the Bay of Biscay.....	104
Figure 32: Spatial distribution of <i>Ostreopsis cf. siamensis</i> in the Bay of Biscay.....	106
Figure 33: Temporal distribution of <i>Ostreopsis cf. siamensis</i> inferred from environmental DNA analyses carried out on plastic polymer substrates collected in 2017 and 2018 in Brest and La Tremblade.....	110
Figure 34: Summer connectivity from areas D (green), H (light blue) and K (dark blue) in 2011 and 2013.....	112
Figure 35: Map of origin locations of strains of <i>Ostreopsis cf. siamensis</i> (yellow) and <i>Ostreopsis cf. ovata</i> (orange) used in this study.....	139
Figure 36: Mean growth rates of <i>O. cf. siamensis</i> and <i>O. cf. ovata</i> at each site at 22°C.....	147
Figure 37: Growth rates of <i>O. cf. siamensis</i> as a function of temperature.....	148
Figure 38: Percentage of mortality of <i>Artemia franciscana</i> nauplii after (A) 24 h and (B) 48h of exposure to 0, 4, 40 and 400 cells/mL of six different strains of <i>Ostreopsis cf. siamensis</i> (H1, H8, H10, Z1, Z7 and Z8) grown at 22°C.....	151
Figure 39: LC30 after 48 h of exposure of <i>A. franciscana</i> nauplii to <i>Ostreopsis cf. siamensis</i> (strains H and Z) under various temperature conditions.....	151
Figure 40: Fm (black dots) and Fv/Fm (white squares) values relatively to the cell concentration of <i>O. cf. siamensis</i> for the pre-experiment in exponential phase of growth, under graded temperature levels.....	152
Figure 41: Mean ratios of percentage of excreted NO ₂ ⁻ (in µmolN) relatively to consumed NO ₃ ⁻ (in µmolN) for acclimated cultures of <i>O. cf. siamensis</i> in exponential phase of growth, under graded temperature levels.....	154
Figure 42: Weekly average of coastal SST in Brest and San Sebastian over the 2000 – 2019 period according to data provided by the SOMLIT and the Aquarium of San Sebastian, respectively.....	162
Supplementary figure 1: <i>Ostreopsis cf. siamensis</i> connectivity matrix showing the “mean transit time” (days) between the releasing (particle deployment) areas (x-axis) and the receiving areas (y-axis) in spring-summer 2011.....	123
Supplementary figure 2: <i>Ostreopsis cf. siamensis</i> connectivity matrix showing the “mean transit time” (days) between the releasing (particle deployment) areas (x-axis) and the receiving areas (y-axis) in spring-summer 2011.....	123

Supplementary figure 3: <i>Ostreopsis cf. siamensis</i> connectivity matrix showing the “mean transit time” (days) between the releasing (particle deployment) areas (x-axis) and the receiving areas (y-axis) in spring-summer 2013.....	124
Supplementary figure 4: <i>Ostreopsis cf. siamensis</i> connectivity matrix showing the “mean transit time” (days) between the releasing (particle deployment) areas (x-axis) and the receiving areas (y-axis) in spring-summer 2013.	124
Supplementary figure 5: <i>Ostreopsis cf. siamensis</i> connectivity matrix showing the “mean transit time” (days) between the releasing areas, in spring-summer from 2010 to 2014.....	125
Supplementary figure 6: <i>Ostreopsis cf. siamensis</i> connectivity matrix showing the “mean transit time” (days) between the releasing (particle deployment) areas in spring-summer from 2010 to 2014. The data showed are based on simulations that used the temperature limitation of 16°C.....	125
Supplementary figure 7: rt-PCR cell-based standard curves.....	128
Supplementary figure 8: Dissociation curves (A) and amplification plots (B) of rt-PCR performed on environmental samples.....	128

Liste des tableaux

Table 1: Sampling site information and environmental data collected during the sampling campaign.....	96
Table 2: Information of the 64 new isolated, sequenced and genetically identified <i>Ostreopsis cf. siamensis</i> strains.....	107
Table 3: Information of strains of <i>Ostreopsis cf. siamensis</i> and <i>Ostreopsis cf. ovata</i> used in this study.....	139
Table 4: LC50 at 24 h and 48 h of exposure and LT50 at 400 cells/mL for each strain at various temperatures on <i>Artemia franciscana</i> nauplii.....	150
Supplementary table 1: Correlation values between Lagrangian indices.....	122
Supplementary table 2: Correlation values of mean transit time between different years.....	122
Supplementary table 3: qPCR analysis and sequences identification data of collected samples from artificial substrates.....	126 – 127

Préambule



Le projet Européen CoCliME (“Co-development of Climate Services for adaptation to changing Marine Ecosystems”) fait partie du consortium ERA-NET « ERA4CS » (“European Research Area for Climate Services”) et a été financé par un ensemble de partenaires gouvernementaux européens d’Irlande (EPA), d’Allemagne (BMBF), de Roumanie (UEFISCDI), de Norvège (RCN), de Suède (FORMAS) et de France (ANR), ainsi que par une bourse de l’Union Européenne (690462).

Ce projet, initié en Septembre 2017 et se terminant en fin d’année 2020, a pour but de produire des services climatiques afin de soutenir une prise de décision éclairée et pertinente face aux impacts écologiques et socio-économiques liés aux changements climatiques dans les écosystèmes marins côtiers. Pour atteindre ces objectifs, le consortium CoCliME a rassemblé une équipe transdisciplinaire de scientifiques naturels et sociaux ainsi que des décideurs et des utilisateurs de services climatologiques. Leur interaction dynamique a visé à identifier les vulnérabilités et solutions communes et prioritaires liées au changement climatique dans six zones côtières européennes à travers des études de cas dans les eaux Atlantique, de la Mer Baltique, Noire, Méditerranée, du Nord et de Norvège.

Dans ces zones, le consortium CoCliME s’est concentré sur les indicateurs de l’état des écosystèmes côtiers (prolifération d’algues nuisibles, biotoxines et agents pathogènes marins, biodiversité microbienne marine) pouvant être fortement influencés par le changement climatique et avoir des impacts directs sur la santé humaine (intoxication d’origine alimentaire et problèmes de santé liés à la qualité de l’eau), la prospérité économique (pêche, aquaculture, tourisme) et le bien-être social (loisirs).

Les services et informations produits par cette structure pourront également servir aux différents acteurs impliqués dans les objectifs de développement durable des Nations Unies, la Directive-Cadre Stratégie pour le Milieu Marin, la Planification de l’Espace Maritime, les exigences nationales en matière de surveillance et de rapport ainsi que dans la planification de l’adaptation au climat pour assurer la protection et l’utilisation durable du milieu marin et des écosystèmes côtiers pour les générations futures.

Dans le cadre du projet CoCliME, ma thèse s’est donc focalisée sur l’étude des microalgues benthiques nuisibles du genre *Ostreopsis* pouvant impacter la santé publique, l’économie et les écosystèmes marins, et cela principalement dans les eaux européennes d’Atlantique et de Méditerranée. J’ai ainsi pu participer aux échanges entre les différents acteurs scientifiques de ce projet lors d’assemblées générales, ainsi qu’à un atelier de formation scientifique développé et animé par des membres du consortium CoCliME (« qPCR Workshop », à l’AWI, Bremerhaven, Allemagne).

Introduction

1. Les microalgues : rôle et diversité

Les microalgues sont des organismes unicellulaires pouvant être trouvées dans différents compartiments océaniques et considérées comme pélagiques lorsqu'elles se trouvent flottant dans la colonne d'eau, ou bien benthiques lorsqu'elles se développent sur le *benthos*, le fond des océans. Les microalgues benthiques peuvent vivre à la surface de substrats abiotiques tels que des roches ou d'autres substrats artificiels, mais également coloniser des surfaces biotiques telles que des macroalgues, et sont alors considérées comme épiphytes.

1.1. Phytoplancton et production primaire

Le terme « plancton » trouve son origine dans *L'Odyssée* d'Homère où il désignait les animaux « errants » (du grec ancien *planktós*) à la surface des flots. Ainsi, le phytoplancton désigne l'ensemble des micro-organismes végétaux (du grec ancien *phyton*) vivant en suspension dans la zone euphotique des océans et milieux aquatiques et dérivant au grès des courants, qu'ils soient motiles ou non. Le phytoplancton est composé de micro-organismes eucaryotes et procaryotes photoautotrophes, c'est-à-dire capables de réaliser la photosynthèse et produisant de la matière organique et du dioxygène à partir d'eau, de dioxyde de carbone et d'énergie lumineuse. Le processus de photosynthèse chez le phytoplancton est responsable d'environ 45% de la production totale de dioxygène atmosphérique (Field, 1998; Geider *et al.*, 2001) bien que le phytoplancton ne représente pas plus de 1% de la biomasse photosynthétique totale, le reste correspondant aux végétaux terrestres. En plus de produire une quantité considérable de dioxygène nécessaire au processus de respiration des autres organismes, le phytoplancton est à la base du cycle du carbone dans les océans à travers la production de matière organique résultant de la photosynthèse, appelée la production primaire (Falkowski, 1994). Le CO₂ atmosphérique fixé par le phytoplancton est ainsi converti en matière organique carbonée afin d'accroître sa propre biomasse. Au sein de la chaîne trophique des océans, cette biomasse est transférée aux échelons supérieurs notamment à travers le broutage du phytoplancton par le zooplancton (Figure 1), puis par la prédation du zooplancton par les consommateurs supérieurs successifs (Legendre and Le Fèvre, 1991). Le phytoplancton produit également du carbone organique sous forme particulaire ou dissoute (Figure 1), généralement issu de la mort de ce dernier, qui, en sédimentant vers le fond des océans, peut être reminéralisé par les communautés bactériennes ou accumulé dans les

sédiments tapissant les fonds des océans. Ce long et important processus est décrit comme la pompe biologique qui participe à la régulation de la quantité de CO₂ atmosphérique et océanique.

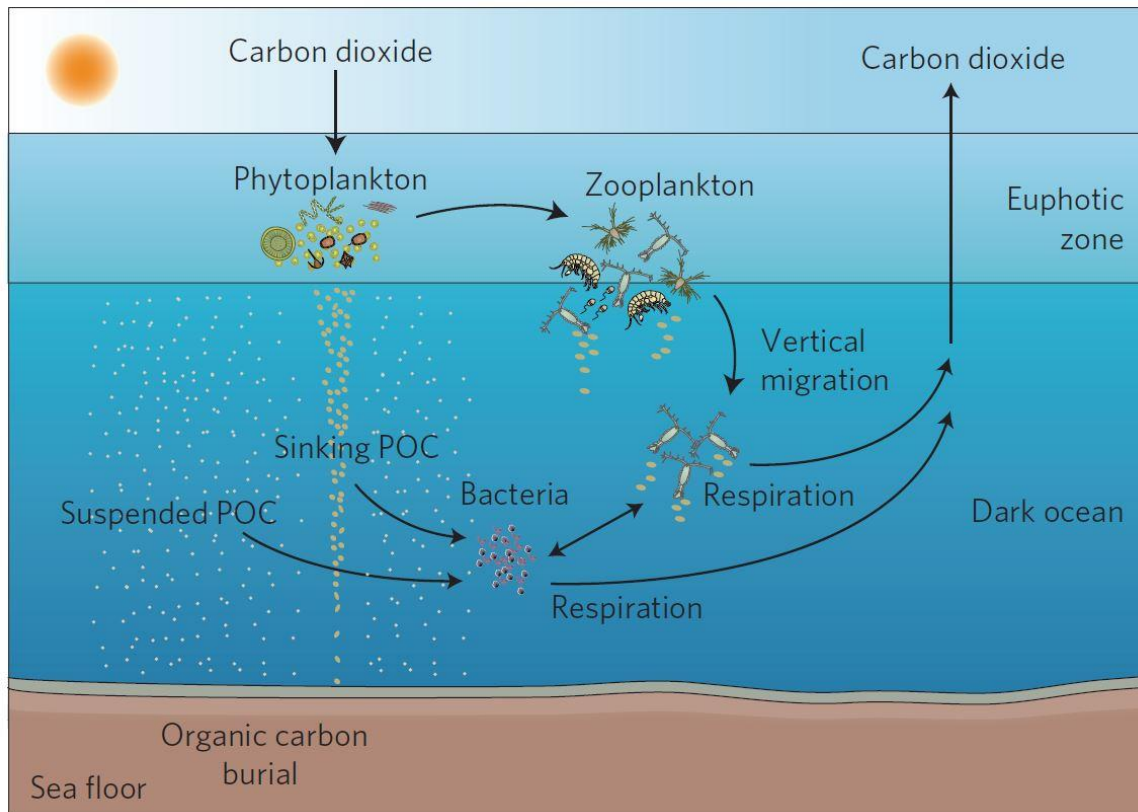


Figure 1 : Schéma de la pompe biologique du carbone dans les océans (d'après Herndl and Reinthaler, 2013).

1.2. Diversité des microalgues

Il existe une très grande variété d'organismes phytoplanctoniques qui se répartissent entre les deux principaux domaines des procaryotes et eucaryotes. Parmi les taxons de phytoplancton les plus représentés chez les protistes marins, les diatomées, les dinoflagellés, les haptophytes et les cyanobactéries dominent les communautés des écosystèmes côtiers et du plateau continental (Simon *et al.*, 2009). En plus d'être classés selon différents taxa, les organismes phytoplanctoniques sont également divisés par classes de taille : Le picophytoplancton (0.2 – 2 µm), le nanophytoplancton (2 – 20 µm), le microphytoplancton (20 – 200 µm) et le mésophytoplancton (2 – 20 mm) (Figure 2). C'est au sein du microphytoplancton que sont notamment répertoriés la plupart des diatomées et des dinoflagellés.

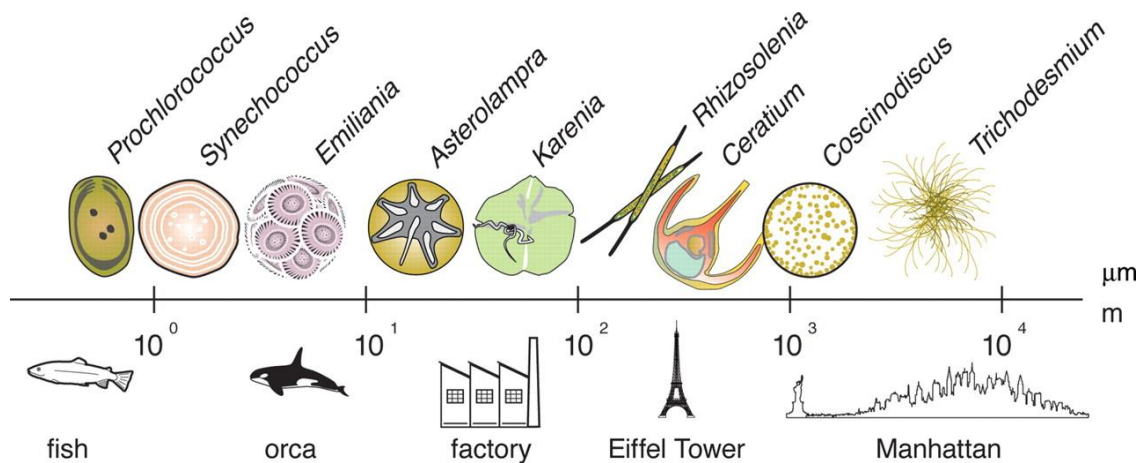


Figure 2 : Comparaison de la gamme de taille du phytoplancton relatif à des objets macroscopiques (d'après Finkel *et al.*, 2010).

Grâce à l'avènement des techniques de biologie moléculaire et suite aux avancées dans les techniques d'observation microscopique, la phylogénie moléculaire a permis de mettre en évidence des relations phylogénétiques proches entre organismes autrefois considérés comme phylogénétiquement éloignés selon des critères morphologiques et trophiques (Caron *et al.*, 2012). Récemment, le développement de l'écologie fonctionnelle en tant que discipline à part entière dans l'écologie générale des organismes tente d'apporter de nouvelles réponses pour expliquer et décrire la diversité observée au sein des communautés phytoplanctoniques. Basée sur une approche systémique et intégrative, elle associe les traits physiologiques des organismes à leur fonction au sein de leur écosystème pour étudier les dynamiques communautaires et le rôle de chaque organisme (Litchman and Klausmeier, 2008).

1.3. Espèces toxiques et Harmful Algal Blooms

Certaines espèces de microalgues sont capables de produire des composés toxiques et d'ainsi affecter de manière négative l'ensemble de la faune et de la flore marine évoluant dans le même écosystème. Tous les phénomènes négatifs associables aux proliférations d'espèces de microalgues nuisibles rentrent dans la catégorie de « Harmful Algal Blooms » (HABs), ou efflorescences algales nuisibles, qui résultent d'un développement épisodique massif entraînant la prolifération en très grande quantité de cellules microalgales, toxiques ou non. Ces épisodes sont le plus souvent associés à des espèces appartenant aux groupes des diatomées, des dinoflagellés et des cyanobactéries et peuvent être observés aussi bien en

milieu marin qu'en milieu lacustre. Dans certains cas, des efflorescences associées à certaines espèces peuvent se traduire par une coloration de l'eau pouvant s'étendre sur une zone relativement étendue en raison de la forte concentration cellulaire ainsi que de la forte teneur en pigments (Figure 3).



Figure 3 : Colorations d'eaux côtières suite à des efflorescences des dinoflagellés *Karenia brevis* (gauche) et du genre *Noctiluca* (droite) (Crédits : Schmidt P., Sun C. in Kudela *et al.*, 2017).

Les mortalités de la faune et de la flore associées à ces épisodes d'efflorescences sont souvent causées par la production de phycotoxines par les microalgues responsable des blooms (Smayda, 1997; Granéli and Turner, 2006). Elles peuvent aussi provenir de phénomènes d'anoxie survenant après le bloom dus à la consommation quasi-totale de l'oxygène par les bactéries dégradant la matière organique (Pitcher and Probyn, 2011; Lim *et al.*, 2014).

En plus de représenter une menace pour les écosystèmes, certaines phycotoxines produites lors d'évènements HABs représentent également un risque majeur pour l'économie et la santé humaine. De manière générale, l'exposition humaine aux phycotoxines se fait par ingestion d'organismes ayant été exposé et ayant accumulé de tels composés, comme c'est le cas chez de nombreux mollusques (Picot *et al.*, 2011). Les phycotoxines sont alors classées selon les symptômes provoqués et selon la nature chimique des composés toxiques (James *et al.*, 2010). Les principales classes de phycotoxines sont : (i) les toxines amnésiantes (« Amnesic Shellfish Poisoning », ASP) comme l'acide domoïque produit notamment par certaines espèces de diatomées du genre *Pseudo-nitzschia*, (ii) les toxines paralysantes (« Paralytic Shellfish Poisoning », PSP) comme les saxitoxines produites par les dinoflagellés du genre *Alexandrium* et par *Gymnodinium catenatum*, (iii) les toxines diarrhéiques (« Diarrhetic

Shellfish Poisoning », DSP) comme l'acide okadaïque produit par des espèces du genre *Dinophysis* et *Prorocentrum*, (iv) les neurotoxines (« Neurotoxique Shellfish Poisoning », NSP) comme les brevétoxines produites par le dinoflagellé *Karenia brevis* et les toxines associées à la ciguatera (« Ciguatera Fish Poisoning », CFP) comme les ciguatoxines produites par les dinoflagellés du genre *Gambierdiscus*. D'autres phycotoxines comme celles produites par les dinoflagellés du genre *Ostreopsis*, affectant la santé humaine par contact, respiration d'aérosols ou ingestion des toxines en créant des irritations de la peau ou des voies respiratoires, n'ont pas encore été associées à une de ces catégories.

Les coûts économiques associés aux efflorescences toxiques touchent de nombreux secteurs. Parmi eux, l'industrie de la pêche est directement impactée par la contamination de produits destinés à la consommation et les interdictions de vente associées (Jin *et al.*, 2008). L'industrie du tourisme peut subir des pertes suite à la fermeture de zones récréatives exposées à la présence de toxines (Dyson and Huppert, 2010; Bechard, 2020). Le domaine de la santé publique est impacté par la prise en charge et le traitement des patients intoxiqués (Hoagland *et al.*, 2009). Enfin, la prévention, le monitoring et le management de tels évènements représente un coût économique parfois très important (Hoagland *et al.*, 2002). A titre d'exemple, aux Etats-Unis, le coût moyen annuel associé aux épisodes de HABs est estimé à pas moins de 83 millions de dollars (Ritzman *et al.*, 2018). L'impact des phycotoxines sur la santé humaine est également au cœur du problème associé aux HABs (Backer and McGillicuddy, 2006).

2. Les microalgues : Ecologie

2.1. Facteurs influençant la composition des communautés microalgales

La composition et la diversité des communautés microalgales est susceptible de varier dans l'espace et dans le temps selon un ensemble de facteurs biotiques et abiotiques. La limitation des ressources nécessaires au développement des espèces est un des principaux facteurs contrôlant la composition des communautés. Ce contrôle par les ressources est dit « bottom-up » (par le bas). Il concerne principalement la disponibilité en nutriments et la lumière (Tilman *et al.*, 1982; Burson *et al.*, 2018). D'autres facteurs physico-chimiques, tels que la température, la turbulence ou la circulation des masses d'eaux, peuvent influencer la

disponibilité de ces ressources (Finkel *et al.*, 2010), mais aussi directement jouer sur la croissance algale. Un autre type de contrôle majeur de la composition des communautés microalgales est le contrôle dit « top-down » (par le haut) qui est lié à la prédation des espèces par d'autres organismes, notamment le zooplancton, responsable du broutage des microalgues (Armstrong, 1994). D'autres interactions biotiques que le broutage peuvent également jouer sur les pertes de biomasse algales et le déclin des efflorescences, dont, par exemple, les infections parasitaires et virales (Chambouvet *et al.*, 2008; Vardi *et al.*, 2012). La combinaison des deux types majeurs de contrôle, bottom-up et top-down, ainsi que l'ensemble des facteurs environnementaux influençant la physiologie ou la lyse des microalgues, détermine ainsi la composition et la diversité des communautés (Granéli *et al.*, 1999).

2.2. Variabilité spatio-temporelle des microalgues

Dans les océans, les processus associés à la distribution et à la saisonnalité des microalgues sont complexes et différents selon les zones géographiques. La répartition des microalgues varie à la fois dans le temps et dans l'espace sous l'influence de nombreux paramètres physico-chimiques et processus biologiques. De manière générale, la distribution globale de la biomasse microalgale est estimée à travers l'observation de la chlorophylle-*a*, pigment présent chez les organismes photosynthétiques. Grâce aux progrès des techniques d'observation et au couplage des données collectées *in situ* avec les images satellites, la concentration en chlorophylle-*a* dans les couches de surface peut ainsi être estimée (Figure 4) et rapportée en biomasse phytoplanctonique. Ces observations montrent qu'en moyenne les plus fortes concentrations de phytoplancton se trouvent aux niveaux des zones tempérées et subpolaires, dans les hautes latitudes, tandis que les régions tropicales et subtropicales présentent généralement des concentrations en biomasse phytoplanctonique moins importante (McClain, 2009). De fortes concentrations en phytoplancton sont également observées dans les zones d'« upwelling », où les remontées d'eaux profondes chargées en nutriments sont favorables au développement des espèces phytoplanctoniques (Abbott and Zion, 1985).

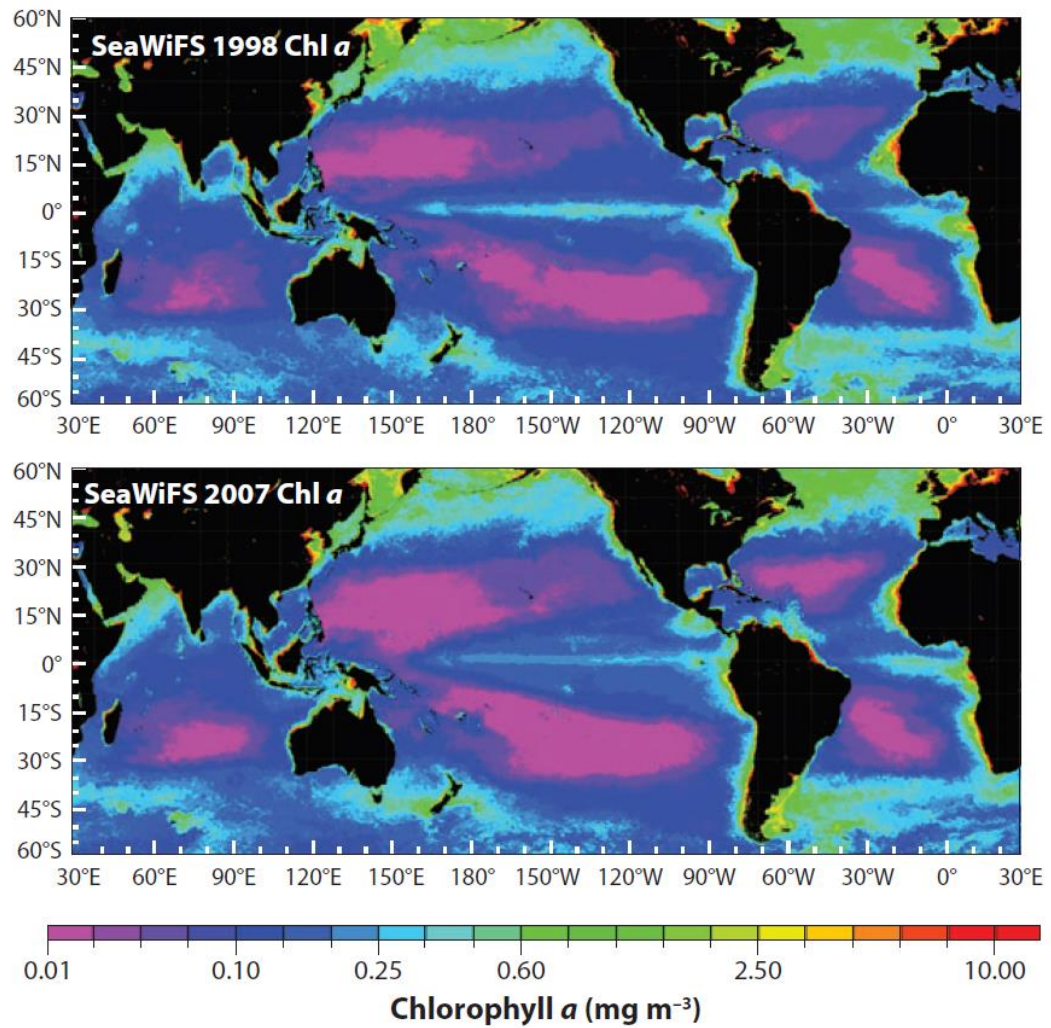


Figure 4 : Moyennes annuelles de la concentration globale de la chlorophylle- a à la surface des océans en 1998 et 2007 (d'après les données satellitaires SeaWiFS, extrait de McClain, 2009).

Cependant, la distribution géographique de la diversité du phytoplancton semble suivre un gradient latitudinal avec un plus grand nombre d'espèces et donc une plus grande richesse spécifique dans les eaux tropicales situées aux faibles latitudes (Figure 5). Cette répartition de la diversité est également en lien étroit avec de nombreux facteurs environnementaux, dont la température des océans (Righetti *et al.*, 2019).

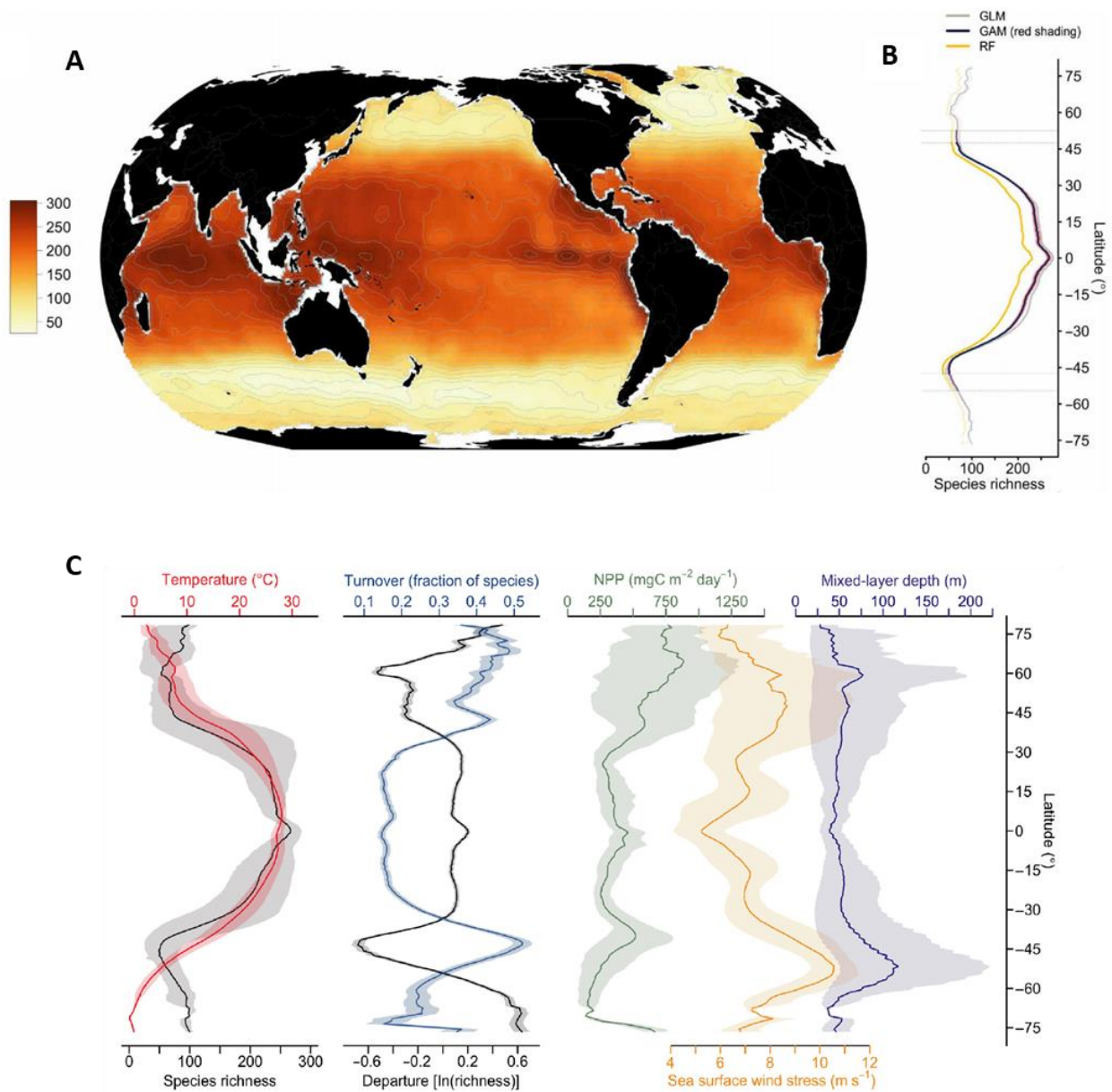


Figure 5 : Distribution de la richesse spécifique du phytoplancton. (A) Moyenne annuelle de la richesse spécifique du phytoplancton par mois et (B) selon un gradient latitudinal. (C) Tendances latitudinales de la richesse spécifique du phytoplancton en fonction de variables environnementales (Température, turnover des espèces, production primaire nette, force des vents de surface et profondeur de la couche de mélange). (d'après Righetti *et al.*, 2019).

La variabilité saisonnière du phytoplancton s'explique par la variation des processus affectant la croissance du phytoplancton au cours des saisons et qui peuvent également varier selon les régions. La succession des saisons entraîne des variations cycliques de réchauffement et de refroidissement, de variation de la photopériode ainsi que de quantité de nutriments. Les propriétés physiques et biogéochimiques des couches d'eaux évoluent également avec

l'alternance des saisons et impactent la disponibilité des ressources nécessaires au développement du phytoplancton (Figure 6).

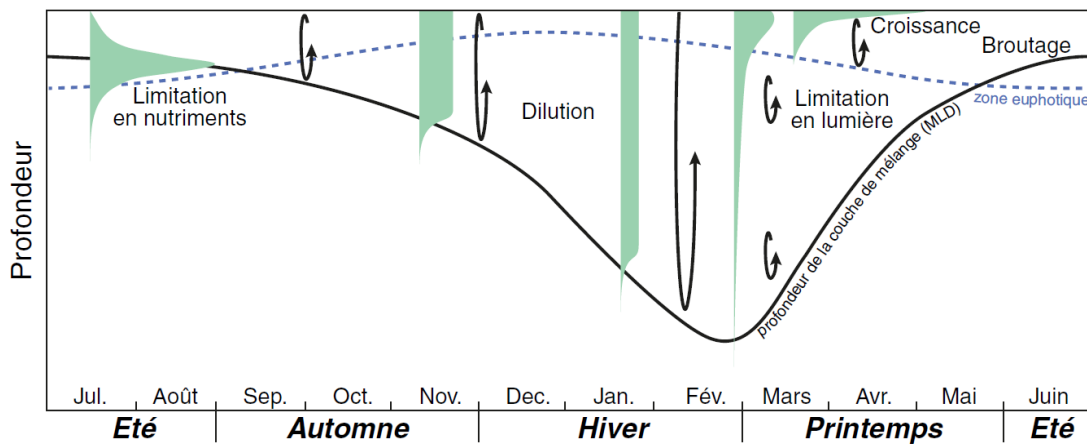


Figure 6 : Schéma des processus physiques et biologiques influençant le cycle annuel du phytoplancton. La concentration en phytoplancton est représentée par les profils verts (adapté par N. Mayot, 2016 d'après Chiswell *et al.*, 2015).

De manière générale, dans les zones tempérées et subpolaires, la croissance du phytoplancton suit une variation saisonnière, avec des pics de biomasse observés au printemps. A cette saison, les températures et la durée d'exposition à la lumière augmentent, accompagnées par une remontée de la profondeur de la couche de mélange, une diminution de la turbulence et la mise en place de la stratification des eaux de surface (Chiswell *et al.*, 2015).

2.3. La température : Un facteur de contrôle clé des dynamiques d'efflorescences

2.3.1. Relation entre température et physiologie des microalgues

Chez les microorganismes photosynthétiques, la température est un des facteurs primordiaux régulant de nombreux mécanismes physiologiques. De manière générale, la température de l'eau est considérée comme l'un des paramètres environnementaux les plus importants régissant la croissance du phytoplancton (Eppley, 1972), mais est également impliquée dans d'autres processus biologiques tels que la germination ou la production de toxines chez certaines espèces nuisibles.

De manière générale, l'évolution du taux de croissance net des microalgues en fonction de la température suit une relation exponentielle (Bissinger *et al.*, 2008) jusqu'à un seuil au-delà duquel le taux de croissance chute jusqu'à être nul (Rosso *et al.*, 1993). La relation exponentielle entre croissance cellulaire et température résulte de la somme de différents processus biologiques n'observant pas forcément une telle relation eux-mêmes avec ce paramètre (Montagnes *et al.*, 2003). L'allure de la courbe obtenue en fonction d'un gradient thermique est spécifique à chaque microorganisme phytoplanctonique et définit une gamme de températures, appelée « niche thermique », dans laquelle l'espèce est capable de se développer.

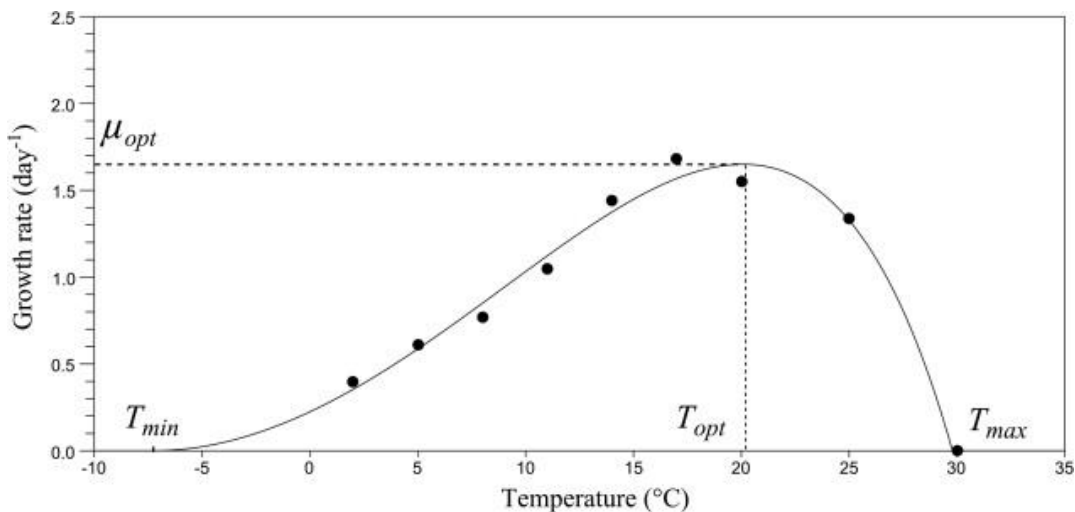


Figure 7 : Modèle de température cardinale avec inflexion (« Cardinal Temperature Model with Inflexion », CTMI) représentant le taux de croissance de la diatomée *Asterionella formosa* en fonction de la température (d'après Bernard and Rémond, 2012).

La régulation de la croissance algale par la température est en partie le fait d'un contrôle direct de la vitesse des réactions enzymatiques cellulaires, mais d'autres processus interviennent également. En ce qui concerne l'activité photosynthétique, l'influence de la température joue à plusieurs niveaux, à la fois morphologiques (composition en pigments ou lipides) et réactionnels. Chez le phytoplancton, les très faibles températures réduisent la fluidité membranaire, les transferts d'électrons ainsi que les activités catalytiques des enzymes (Raven and Geider, 1988; Falkowski and Raven, 2007), tandis que les températures optimales spécifiques favorisent la fixation du carbone et la biosynthèse (Li, 1980). Au-dessus de ces températures optimales, la demande en composés organiques pour le maintien de la croissance de la cellule devient d'abord plus forte que la production, ce qui induit une baisse

du taux de croissance ; sous des températures encore plus élevées, le stress cellulaire provoque une augmentation de la fluidité membranaire et du taux de dégradation des enzymes (Raven and Geider, 1988). L'enzyme ribulose-1,5-biphosphate carboxylase/oxygénase, plus connue sous le nom de « RuBisCO » est une enzyme catalysant la carboxylation et l'oxydation du ribulose-1,5-biphosphate afin de produire de la matière organique à partir de carbone inorganique en fixant le CO₂ à travers le processus de photosynthèse. L'activité de la RuBisCO est modulée par l'irradiance (MacIntyre *et al.*, 1996), mais également par la température (Li *et al.*, 1984; Sage and Kubien, 2007). Bien que l'activité de la RuBisCO augmente avec la température, des températures trop élevées peuvent inhiber son activité, réduisant par conséquent l'activité photosynthétique des cellules (Lesser, 1996; Crafts-Brandner and Law, 2000; Salvucci and Crafts-Brandner, 2004). Cette inhibition de l'activité photosynthétique est aussi attribuée aux effets des températures élevées sur les composants impliqués dans le transport d'électrons au sein du photosystème II (PSII) situé dans la membrane des thylakoïdes, allant jusqu'à inhiber l'activité du PSII (Havaux, 1993; Rokka *et al.*, 2000). En utilisant le ratio F_v/F_m comme indicateur de l'activité du PSII, de nombreuses études ont montré un effet de la température sur l'efficacité photosynthétique des microalgues, en observant des limitations de cette efficacité sous des températures basses ou hautes (Jauzein and Erdner, 2013; López-Rosales *et al.*, 2014; Aquino-Cruz *et al.*, 2018). Enfin, les profils pigmentaires des microalgues sont connus pour varier en fonction de la température, mettant en exergue des bascules possibles entre chlorophylles (pigments capteurs de lumière) et caroténoïdes (pigments photo-protecteurs) (Zapata *et al.*, 2012; López-Rosales *et al.*, 2014). De manière générale, l'acclimatation des microalgues aux fortes températures induit des modulations de l'activité et la quantité d'enzymes des thylakoïdes ainsi que de la quantité et du type de pigments photosynthétiques, tandis que l'acclimatation aux faibles températures implique des modifications majeures des lipides membranaires (Raven and Geider, 1988).

Comme la croissance cellulaire, la germination des kystes de microalgues, et notamment de dinoflagellés, est un processus très influencé par la température de l'eau (Kremp and Anderson, 2000). De nombreuses études ont montré la nécessité d'atteindre une certaine température pour induire le processus de germination (Bravo and Anderson, 1994; Shikata *et al.*, 2007). Pour les dinoflagellés toxiques en particulier, la température régulerait à

la fois l'état de dormance et le déclenchement de la germination des kystes quiescents, jouant ainsi un rôle clé dans la saisonnalité des efflorescences (Fischer *et al.*, 2018; Lopez *et al.*, 2019).

La production de toxines chez certaines espèces de phytoplancton peut aussi être influencée par la température, augmentant ou diminuant la toxicité selon les espèces. A titre d'exemple, (Navarro *et al.*, 2006) ont montré en laboratoire que la toxicité du dinoflagellé *Alexandrium catenella* était plus élevée à 10°C comparé à 16°C, tandis que (Lim *et al.*, 2006) ont observé une augmentation du taux de production de toxines chez *Alexandrium minutum* et *Alexandrium tamiyavanichii* entre 15°C et 25°C. Globalement, la toxicité chez les espèces responsables de HABs liés à la production de toxines tend à être plus élevée lorsque les cellules sont exposées à des températures de croissance optimales et donc relativement plus faible lorsqu'elles sont exposées à un stress thermique (Pezzolesi *et al.*, 2012; Vidyaratna and Granéli, 2012; Aquino-Cruz *et al.*, 2018).

2.3.2. Température et distribution des microalgues

La théorie attribuée à (Baas Becking, 1934) stipulant que « tout est partout, mais l'environnement sélectionne » peut s'appliquer à la distribution des espèces phytoplanctoniques dans les océans. En effet, l'absence de barrières physiques empêchant la dispersion des cellules par les courants et les masses d'eau conférerait une distribution ubiquitaire aux microalgues. Les capacités d'adaptations des espèces face aux paramètres environnementaux locaux ainsi que les processus de sélections sont alors responsables de la structuration des communautés (Fenchel and Finlay, 2004; de Wit and Bouvier, 2006). Ainsi, la distribution géographique d'une espèce s'articule autour de sa niche écologique, selon le concept défini par (Hutchinson, 1957), représentant l'ensemble des facteurs biotiques et abiotiques favorables à son développement (Holt, 2003; Sexton *et al.*, 2009). La gamme de température favorisant la croissance des microalgues, définissant leur niche thermique et incluant la température optimale de croissance spécifique à chaque espèce, est donc déterminante dans la distribution des espèces à l'échelle du globe (Figure 8). L'augmentation de la température des océans pourrait influencer la croissance et la distribution actuelle du phytoplancton, provoquant un déplacement des espèces vers les pôles malgré le potentiel d'adaptation thermique observé chez de nombreuses espèces phytoplanctoniques (Thomas *et al.*, 2012; Boyd *et al.*, 2013).

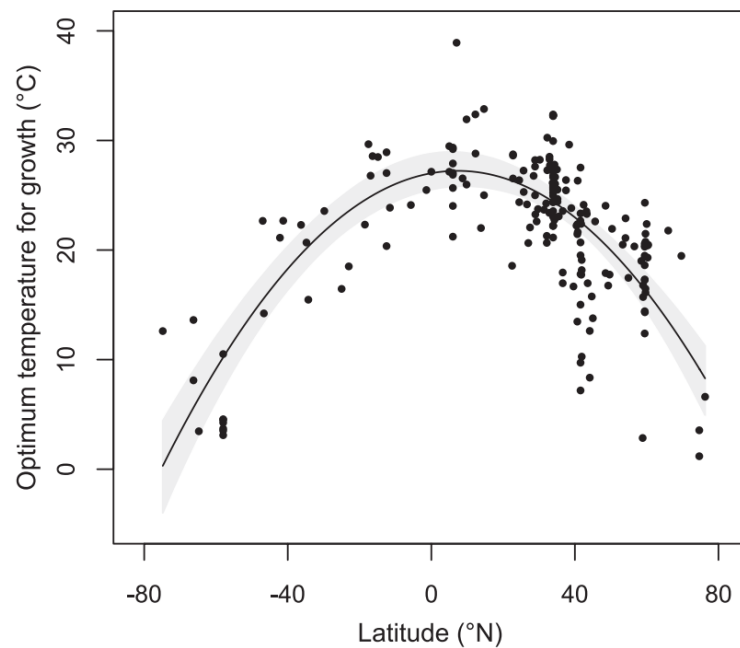


Figure 8 : Gradient latitudinal des températures optimales pour la croissance des espèces phytoplanctoniques marines et estuariennes (d'après Thomas *et al.*, 2012).

2.3.3. Température et phénologie des microalgues

La phénologie correspond à l'étude des phénomènes périodiques, notamment dans le monde du vivant, se produisant de manière cyclique et en relation avec des facteurs environnementaux. Le développement des efflorescences de microalgues est régi par un ensemble de paramètres environnementaux saisonniers aussi bien biotiques qu'abiotiques, et correspond donc par définition un phénomène cyclique, le plus souvent annuel. La température est un facteur prépondérant dans le développement des efflorescences de microalgues. De manière générale, au printemps, couplée à l'allongement de la durée de la photopériode, à la stratification progressive des eaux de surface et à une disponibilité suffisante en éléments nutritifs, l'augmentation de la température agit sur le processus de germination des cellules phytoplanctoniques en état de dormance ainsi que sur leur taux de croissance, favorisant l'apparition d'une efflorescence.

Au niveau des hautes latitudes, des eaux plus chaudes observées pendant les périodes de blooms estivaux sont associées à une extension de la période de croissance et donc à un allongement des épisodes d'efflorescences (Racault *et al.*, 2012). Le phénomène inverse est observé dans les régions tropicales et subtropicales où la durée moyenne des efflorescences diminue en présence de fortes températures et du renforcement de la stratification.

Toutefois, l'évolution des températures de surface en tant que conséquence du réchauffement climatique peut entraîner des variations dans la phénologie des blooms de microalgues. Par exemple, Edwards *et al.* (2006) ont observé des variations phénologiques, notamment en termes d'intensité, dans les efflorescences de microalgues se produisant au niveau de régions exposées au réchauffement des températures de surfaces en Mer du Nord. Similairement, (Edwards and Richardson, 2004) ont montré l'évolution de la temporalité des blooms saisonniers de phytoplancton entre 1958 et 2002, en relation avec l'évolution des températures sur cette période (Figure 9), révélant notamment que les dinoflagellés observaient des pics d'efflorescences de plus en plus précoces à travers le globe.

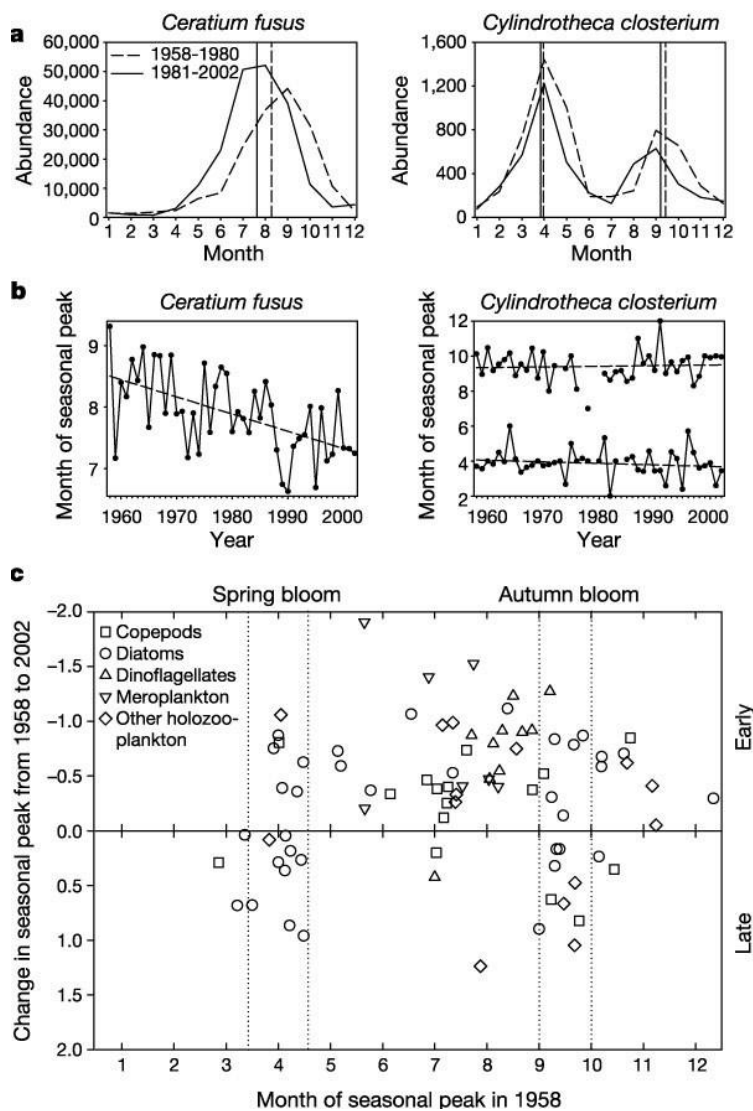


Figure 9 : Changements temporels dans le timing des pics de blooms saisonniers du dinoflagellé *Ceratium fusus* et de la diatomée *Cyndrotheca closterium* entre 1958 et 2002. (a) Dynamique des blooms au cours des périodes 1958 – 1980 et 1981 – 2002. (b) Variabilité interannuelle du timing d'apparition des pics entre 1958 et 2002. (c) Variations de la temporalité des pics de bloom saisonniers de 66 taxa d'organismes planctoniques entre 1958 et 2002 (d'après Edwards and Richardson, 2004).

3. Impacts des changements environnementaux d'origine anthropique sur les microalgues

L'équilibre des microalgues dans les océans est fragile et leur distribution, composition et développement, en plus d'être affectés par les variations naturelles des conditions environnementales, sont exposés à des changements induits par les activités humaines. Parmi les influences anthropiques affectant les communautés microalgales, les phénomènes d'eutrophisation et de changements climatiques sont les plus importants (Heisler *et al.*, 2008; Hallegraeff, 2010). Le contexte actuel de changements climatiques implique, en particulier des modifications directes de la température de l'eau, associées à une acidification des océans.

3.1. Réchauffement global

L'augmentation de la température des océans associée au réchauffement climatique est un des facteurs importants impactant l'écologie des espèces phytoplanctoniques. Selon (Boyce *et al.*, 2010), la biomasse totale du phytoplancton serait en constant déclin depuis le début du siècle dernier, allant jusqu'à diminuer de 1% chaque année, et ce en lien avec le réchauffement des eaux de surface et l'augmentation de la stratification limitant la disponibilité des nutriments notamment dans les zones tropicales et subtropicales (Figure 10). Le réchauffement pourrait également être responsable d'une diminution de la diversité du phytoplancton dans les zones tropicales où les espèces seraient plus sensibles au réchauffement des océans (Thomas *et al.*, 2012). Cependant, le déplacement des espèces vers des plus hautes latitudes pourrait entraîner une hausse de la diversité dans les zones subtropicales et tempérées.

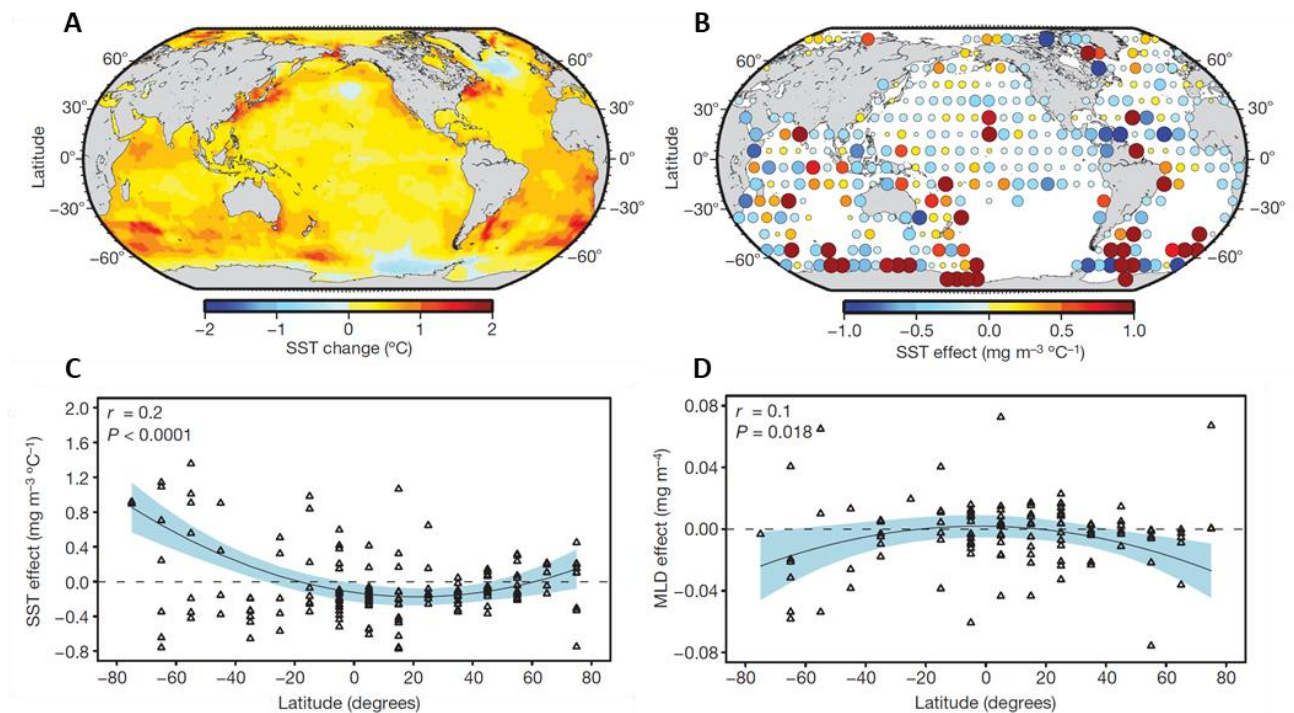


Figure 10 : Paramètres physiques influençant la biomasse globale du phytoplancton. (A) Evolution de la température de surface entre 1899 et 1999. (B) Estimation de l'effet des changements de températures sur la concentration en Chlorophylle. Effets de (C) la température des eaux de surfaces et (D) de la profondeur de la couche de mélange sur la concentration en Chlorophylle en fonction de la latitude (d'après Boyce *et al.*, 2010).

Depuis la fin de l'ère préindustrielle, l'augmentation de la température mondiale atmosphérique et océanique combinées a été estimée à près de +0.85°C (Pachauri *et al.*, 2014), et cette augmentation s'accélère. Depuis 1880 où les données de température globales ont commencé à être mesurées et compilées, le taux moyen d'augmentation de la température globale a augmenté d'environ +0.07°C par décennie jusqu'en 1980 où ce taux a plus que doublé pour atteindre +0.18°C (NOAA, 2019). Les cinq dernières années ont été recensées comme les plus chaudes jamais enregistrées depuis 1880. Les émissions de gaz à effet de serre d'origine anthropique, dont l'accumulation dans l'atmosphère piège l'excédent de chaleur absorbé par la terre et les océans, en sont la principale cause et sont en constante augmentation (Figure 11A). L'océan absorbant plus de 90% de l'excès de chaleur présent sur Terre, la quantité de chaleur dans les océans (« Ocean Heat Content », OHC) est également en constante augmentation et s'est notamment accélérée depuis les années 1980. En effet, les 75 premiers mètres des océans se sont en moyenne réchauffés d'environ 0.11°C par décennie entre 1971 à 2010. Le Groupe d'experts Intergouvernemental sur l'Evolution du Climat (GIEC/IPCC), notamment chargé d'étudier et de prédire l'évolution du réchauffement

climatique, a modélisé les prévisions de température atmosphérique et océaniques au cours du XXI^{ème} siècle en prenant en compte plusieurs scénarios dépendant de la quantité de gaz à effet de serre émis (IPCC 2014 ; Figure 11B). Selon la projection « RCP8.5 » (« Representative Concentration Pathway ») représentant le scénario tenant compte du taux d'émission de CO₂ actuel, la température des 100 premiers mètres de la surface des océans pourrait potentiellement augmenter de +2°C d'ici à 2100. Cependant, selon une récente étude de (Cheng *et al.*, 2019), ces prévisions seraient susceptibles de sous-estimer la vitesse du réchauffement des océans et donc pourrait sous-estimer l'augmentation de ces températures à moyen et long terme. D'un point de vue spatial, les différentes régions du globe ne se réchaufferaient pas avec la même intensité (Figure 12), ce qui pourrait impacter la répartition des espèces.

3.1. Acidification des océans

L'augmentation de CO₂ atmosphérique, en plus d'engendrer un effet de serre et donc une élévation de la température globale, est également responsable du processus d'acidification des océans (Doney *et al.*, 2009). En effet, les océans absorbent près de 30% des émissions de CO₂ atmosphérique d'origine anthropogénique et, bien qu'ayant considérablement réduit la concentration en gaz à effet de serre dans l'atmosphère et donc atténué l'effet de réchauffement climatique, l'absorption du CO₂ a un impact sur la chimie de l'eau. Depuis le début de la révolution industrielle, le pH moyen des océans est passé de 8.2 à 8.1, ce qui pourrait paraître relativement faible. Or, l'échelle de mesure du pH est logarithmique, ce qui signifie qu'une différence d'une unité de pH correspond à une différence d'un facteur 10 en concentration en ions hydrogène, responsables de l'acidité de l'eau. D'ici la fin du siècle, les projections actuelles estiment que le pH pourrait avoir diminué de 0.2 à 0.4 par rapport à aujourd'hui, et donc atteindre des valeurs entre 7.9 et 7.7 (IPCC 2014, Figure 11C). L'augmentation de la concentration en ions hydrogène dans les océans réduit la disponibilité des ions carbonates indispensables à de nombreux organismes marins dont certaines espèces de phytoplancton. De plus, réchauffement et acidification des océans sont liés, car la solubilité du CO₂ atmosphérique dans l'eau dépend de sa température. Ainsi, plus les océans se réchauffent, moins ils seront capables d'absorber et de réguler la quantité de CO₂ atmosphérique, entraînant un renforcement de l'effet de serre déjà présent.

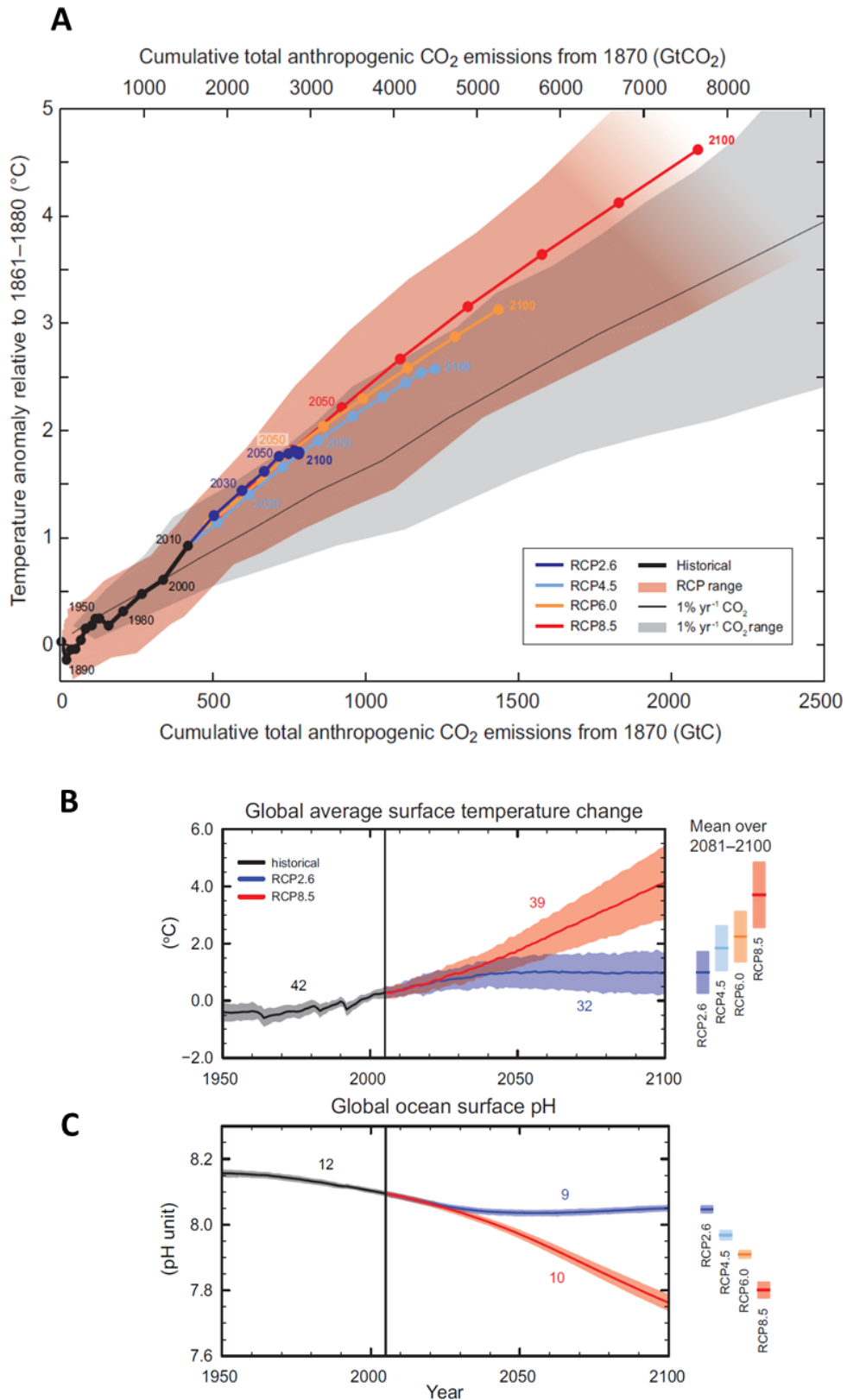


Figure 11 : Prévisions de l'évolution du climat à travers le projet d'intercomparaison des modèles couplés (CMIP5) et selon différents scénarios. (A) Prévisions de la relation entre anomalies de températures en émissions de CO₂ anthropogénique relatives à la période 1861 – 1880 selon différents scénarios d'émission. Prévisions de l'augmentation (A) des températures de surface et (B) du pH à la surface des océans d'ici à 2100 relatif à la période 1986 – 2005 selon les scénarios RCP2.6 et RCP8.5 (adapté d'après le rapport IPCC 2014, Pachauri *et al.*, 2014).

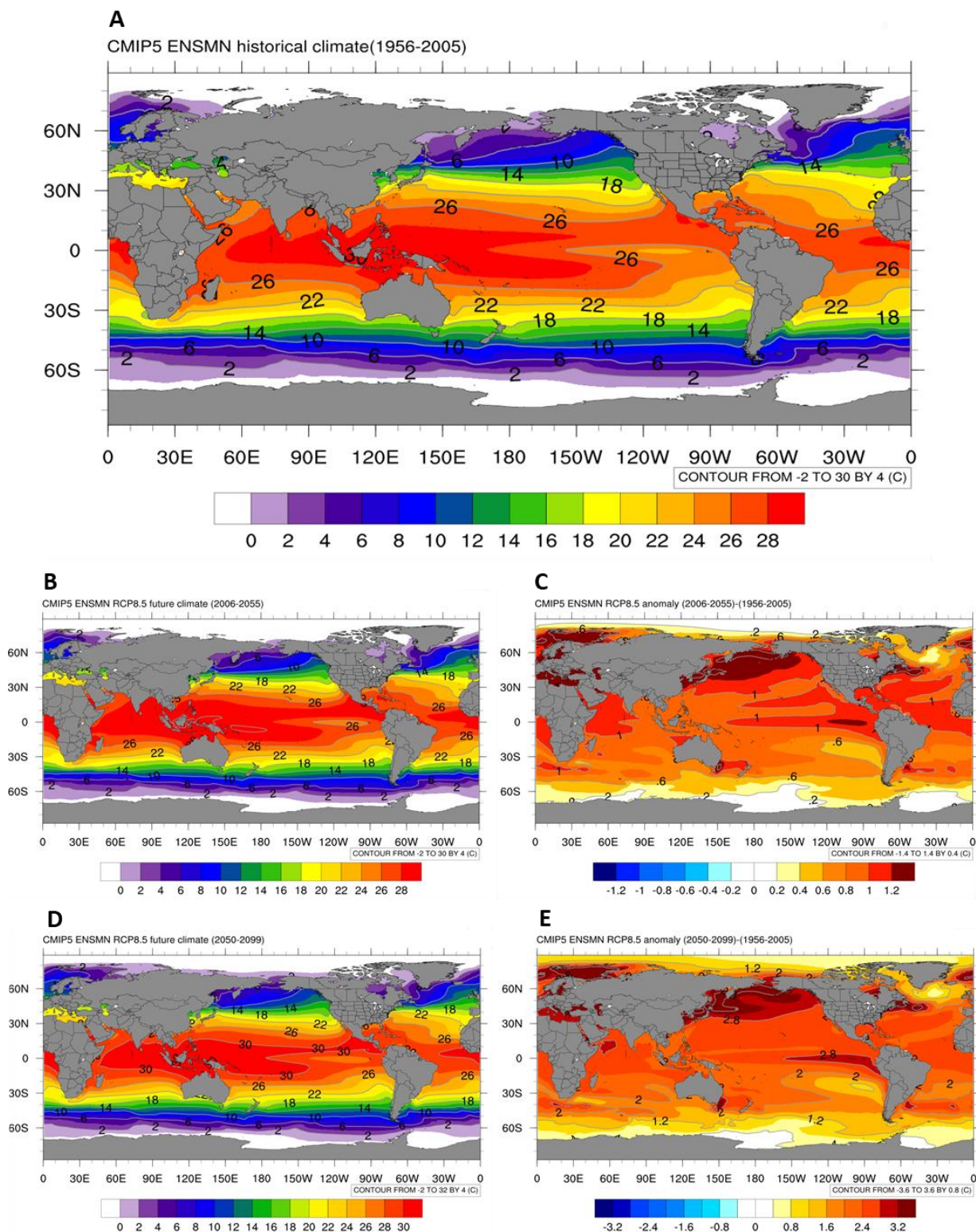


Figure 12 : Représentation de l'évolution de la température des eaux de surfaces à travers le projet d'intercomparaison des modèles couplés (CMIP5) et l'utilisation du scénario RCP8.5 en moyennant 25 modèles climatiques intégrés dans le portail de la NOAA Climate Change (<https://www.esrl.noaa.gov/psd/ipcc/ocn/>). (A) Moyenne historique annuelle des températures des eaux de surface entre 1956 et 2005. Projection des futures moyennes annuelles (B) et des anomalies (C) de températures des eaux de surface entre 2006 et 2055 par rapport à la période 1956 – 2005. Projection des futures moyennes annuelles (D) et des anomalies (E) de température des eaux de surface entre 2050 et 2099 par rapport à la période 1956 – 2005.

Ce processus d'acidification est connu pour affecter un grand nombre d'organismes marins tels que les coraux, les échinodermes et les mollusques (Wittmann and Pörtner, 2013), mais également la structure des communautés phytoplanctoniques (Dutkiewicz *et al.*, 2015). L'effet de l'acidification a notamment été observé chez certains groupes d'organismes phytoplanctoniques comme les coccolithophoridés. Ces eucaryotes unicellulaires capables de former des structures calcaires et jouant un rôle prépondérant dans le cycle du carbone dans les océans sont grandement impactés par la baisse du pH dans les océans, induisant un manque de disponibilité des ions carbonates nécessaires à leur développement (Meyer and Riebesell, 2015). De manière générale, les microalgues sont relativement adaptées pour se développer dans des milieux à pH faible (Berge *et al.*, 2010), mais l'effet de l'acidification sur la physiologie des microalgues semble varier d'une espèce à l'autre (Kremp *et al.*, 2012).

4. Recensement, expansion et surveillance des HABs

Depuis plusieurs décennies, une augmentation de la fréquence d'observation des efflorescences algales toxiques a été observée dans de nombreuses régions du globe (Figure 13). Si des modifications des pratiques d'observation du phytoplancton (intensités des suivis et optimisation des techniques) peuvent jouer dans cette tendance (Van Dolah, 2000; Anderson *et al.*, 2012), une partie de l'expansion géographique des HABs à travers le globe serait liée à la stratégie adaptative de ces espèces vis-à-vis des changements environnementaux (Smayda, 2002; Kudela and Gobler, 2012). Or, si les pressions environnementales liées aux changements climatiques représentent un danger pour les écosystèmes marins, elles pourraient influencer le développement des HABs en particulier. Différents groupes d'experts se sont récemment réunis afin d'envisager si les changements globaux pouvaient, entre autres, être responsables de l'intensification des HABs et de leur expansion présente et future (Wells *et al.*, 2015; Berdalet *et al.*, 2017a; Kudela *et al.*, 2017). Le rapport spécial IPCC de 2019 sur les océans et la cryosphère a pour la première fois établi un lien direct entre changements climatiques et HABs.

Plusieurs études ont mis en relation l'intensification des phénomènes de HABs avec l'atteinte de températures se rapprochant des températures optimales de croissances des espèces (Paerl and Huisman, 2008; Moore *et al.*, 2009). Les températures optimales

favoriseraient ainsi un développement plus rapide et plus fréquent des espèces de microalgues toxiques (Vandersea *et al.*, 2018; Gobler, 2020; Tester *et al.*, 2020). Des études modélisant le développement d'*Alexandrium catenella* (ex *A. fundyense*), de *Dinophysis acuminata* et de *Cochlodinium polykrikoides* en relation avec l'augmentation des températures suggèrent également une intensification de leurs efflorescences ainsi qu'une potentielle expansion de leurs limites de distribution (Gobler *et al.*, 2017; Griffith *et al.*, 2019). Ces tendances concerneraient à la fois les espèces toxiques planctoniques et benthiques. En effet, (Tester *et al.*, 2020) suggèrent que certains genres de dinoflagellés benthiques comme *Gambierdiscus*, *Fukuyoa* et *Ostreopsis* vont voir leur aire de répartition s'étendre de plus en plus vers les hautes latitudes en réponse à l'élévation des températures. Pour les espèces se trouvant en zone tropicale où les températures correspondent déjà aux températures optimales de croissance, la migration vers des habitats plus froids ou plus profonds pourrait servir de protection face à de trop fortes températures. Une forte baisse de la croissance de ces espèces pourrait en effet être observée dans les zones où la température dépasserait les 30 – 31°C correspondant aux températures limites de croissance de ces espèces. Pour les espèces se trouvant dans des régions subtropicales ou tempérées, les habitats observant un réchauffement et dont la température serait propice à la croissance pourrait conduire à la colonisation de ces nouveaux habitats favorables.

En plus d'impacter la distribution spatiale des espèces responsables d'HABs, l'augmentation des températures pourrait influencer la temporalité de ces événements. En effet, l'élargissement de la fenêtre temporelle de températures optimales pourrait favoriser le développement d'épisodes toxiques sur une plus longue plage temporelle comme suggéré par (Moore *et al.*, 2008) (Figure 14). L'arrivée de températures favorables plus tôt au cours de l'année pourrait être également un facteur favorisant le développement précoce d'efflorescences (Moore *et al.*, 2009).

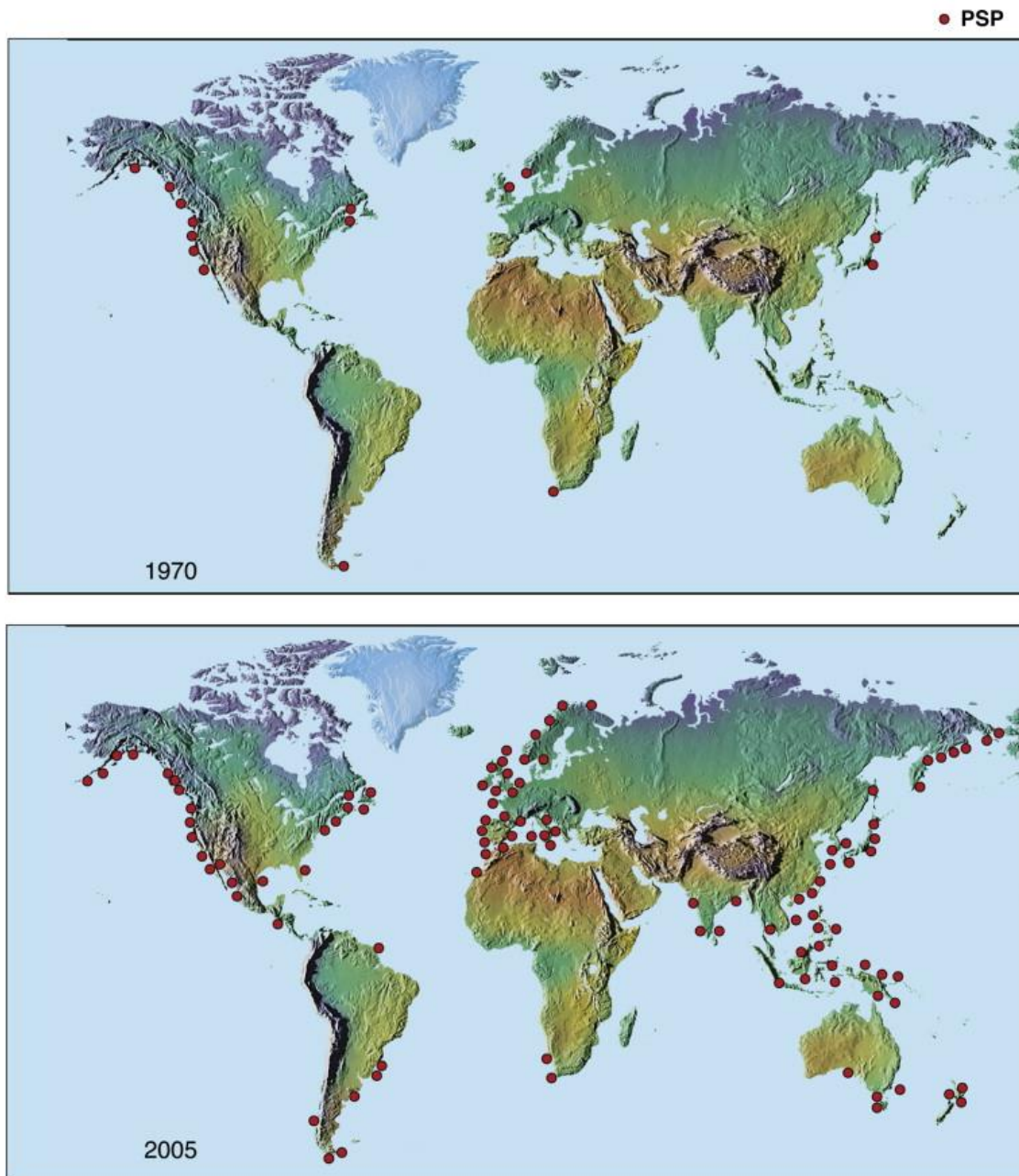


Figure 13 : Evolution du nombre d'évènements recensés liés aux toxines de type PSP entre 1970 et 2005 (d'après Anderson, 2009).

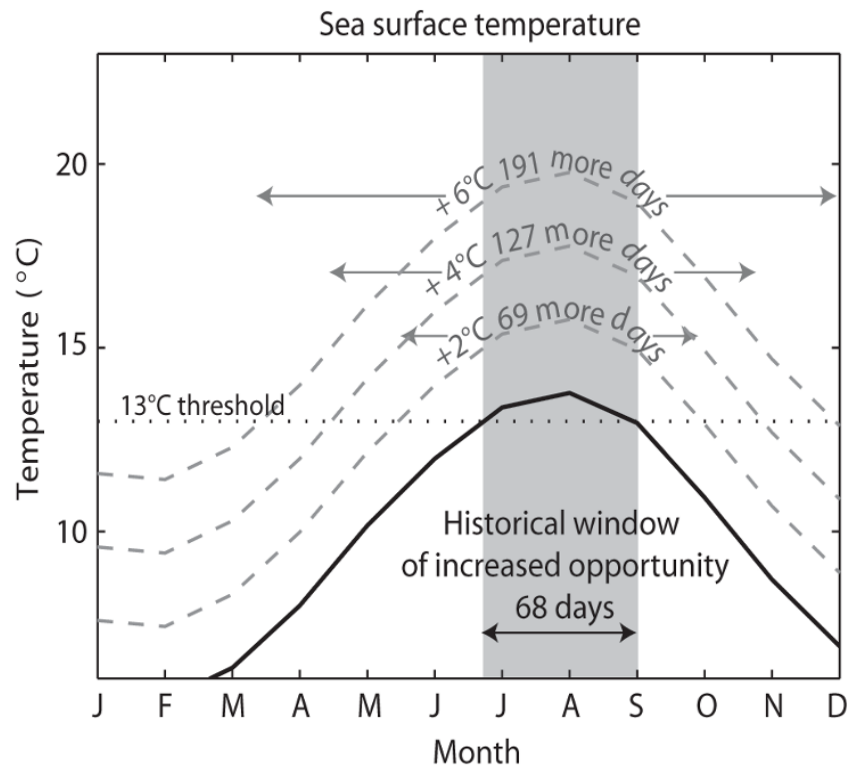


Figure 14 : Potentiels scénarios d'élargissement de la fenêtre temporelle de développement d'*Alexandrium catenella* à Sequim Bay (Puget Sound, Etats-Unis) suite à une augmentation de +2, +4 et +6°C de la température de surface la température de surface (d'après Moore *et al.*, 2008).

L'effet direct de l'acidification des océans sur la formation des HABs est relativement méconnu (Wells *et al.*, 2015). A titre d'exemple, certaines espèces nuisibles des diatomées du genre *Pseudo-nitzschia* pourraient être favorisées par la diminution du pH, comme *P. multiseriata* (Sun *et al.*, 2011) et *P. fraudulenta* (Tatters *et al.*, 2012), tandis que d'autres pourraient être défavorisées (Lundholm *et al.*, 2004). L'effet de l'augmentation de la concentration en CO₂ sur la toxicité des microalgues semble également varier au sein des espèces voire même des souches. (Kremp *et al.*, 2012) ont observé une augmentation de la toxicité de certaines souches d'*Alexandrium ostenfeldii* face à une augmentation en pression partielle de CO₂. (Hattenrath-Lehmann *et al.*, 2015) ont également observé une augmentation de la toxicité chez certaines souches d'*Alexandrium fundyense* provenant de Northport Bay (New-York) mais pas chez celles provenant de la baie de Fundy (Canada). Cependant, les effets notables de l'acidification des océans sur les espèces toxiques de phytoplancton pourraient ne pas être irréversibles étant donné leur potentiel d'adaptation et de dévolution relativement élevé face aux variations de pH en milieu marin (Collins *et al.*, 2014).

De par la variabilité de réponses physiologiques et phénologiques des différentes espèces de microalgues nuisibles face aux changements climatiques, il reste tout de même

difficile d'établir et déterminer les liens directs existants entre changements climatiques et développement des HABs et de prédire leur évolution future. Cependant, il semblerait tout de même que l'effet combiné des pressions d'origine climatiques ainsi que d'origine anthropique agissant à la fois sur la température, le pH, la concentration en nutriments, la couche de mélange ainsi que sur les organismes prédateurs du phytoplancton, favoriserait le développement d'espèces toxiques et pourrait donc promouvoir l'induction de phénomènes d'HABs (Wells *et al.*, 2015).

		Environmental Factor				
		↑ T°C	↑ Stratification	↑ OA	↑ Cultural Eutroph.	Grazing
HAB Type	Diatoms (e.g., <i>Pseudo-nitzschia</i> spp.)	↕ +	↓ ++	↕	↓	↕
	Toxic Flagellates (e.g., <i>Alexandrium</i> , <i>Pyrodinium</i> , <i>Gymnodinium</i>)	↑	↑ ++	↕	↑	↕
	Benthic (e.g., <i>Gambierdiscus</i> spp.)	↕ ++	↑ ++	?	↑	↕
	Fish Killing (e.g., <i>Heterosigma</i> spp.)	↑	↑ ++	?	↑ +	↑ +
	High Biomass (e.g., mixed spp.)	↕	↕	↕	↑ ++	↕
	Cyanobacteria (e.g., <i>Nodularia</i> spp.)	↑ +	↑ ++	↕	↑ ++	?
	Cell Toxicity	?	?	↕	↕	↕

Figure 15 : Effets des différents facteurs environnementaux (température, stratification, acidification, eutrophisation, prédation) impactant différents types d'HABs. Les flèches vers le haut et vers le bas représentent respectivement un effet d'augmentation ou de diminution. Les « + » et « ++ » représentent respectivement une forte et très forte probabilité d'observer l'effet décrit (d'après Wells *et al.*, 2015).

Les écosystèmes déjà exposés aux pressions liées aux changements climatiques et à celles liées aux pollutions anthropogéniques vont devoir faire face à l'augmentation des risques liés aux HABs (Griffith and Gobler, 2020). La combinaison de ces pressions biotiques et abiotiques représente une menace globale pour l'équilibre de la faune et la flore marine et nécessite d'être suivi et étudié attentivement. Des programmes de surveillance ainsi que des programmes de recherche internationaux tels que GEOHAB, GlobalHAB et S-3 EUROHAB ont

été développés en ce sens, afin d'améliorer les connaissances relatives aux microalgues toxiques, à leur développement et à leur devenir face aux changements environnementaux d'origine anthropique (Berdalet *et al.*, 2017a; Kudela *et al.*, 2017).

En France, des réseaux de surveillance comme le REPHY (http://envlit.ifremer.fr/surveillance/phytoplankton_phycotoxines) ou PHENOMER (www.phenomer.org) ont été développés afin de surveiller la dynamique du phytoplancton et des efflorescences nocives en milieu côtier. Les réseaux et programmes de surveillance permettent non seulement d'observer et de surveiller le développement d'épisodes toxiques, mais également d'accumuler des données d'observations environnementales précieuses à la compréhension de ces phénomènes.

5. Le modèle *Ostreopsis* spp.

5.1. Taxonomie

Le dinoflagellé toxique du genre *Ostreopsis* Schmidt a été pour la première fois décrit dans le Golfe de Siam (Thaïlande) par Schmidt en 1901 (Figure 16). Depuis, près de 11 espèces différentes ont été décrites incluant *O. siamensis*, *O. ovata*, *O. lenticularis*, *O. mascarenensis*, *O. heptagona*, *O. fattorussoi*, *O. labens*, *O. marina*, *O. belizeana*, *O. caribbeana*, et *O. rhodesae* (Parsons *et al.*, 2012; Chomérat *et al.*, 2019). La forte variabilité morphologique au sein d'une même espèce complique les études phylogéographiques et rend l'identification génétique des espèces indispensable (Chomérat *et al.*, 2020a) (Figure 17). Les différents clades moléculaires ont été associés à des distributions géographiques spécifiques, ce qui renforce l'idée d'une distribution cryptique du genre *Ostreopsis* à travers le globe. Par exemple, l'holotype *Ostreopsis siamensis* Schmidt (Schmidt, 1901) observé dans le Golfe de Siam a seulement été décrit morphologiquement, et jamais génétiquement caractérisé. Depuis, des souches correspondant morphologiquement à *O. siamensis* ont été isolées dans différentes régions du globe (Faust *et al.*, 1996; Rhodes, 2011) et ont d'abord été associées à *O. siamensis*. Elles ont depuis été renommées « *O. cf. siamensis* » en raison de leurs divergences génétiques avec des souches isolées récemment dans des régions proches du Golfe de Siam, correspondant morphologiquement à *O. siamensis* et génétiquement caractérisées cette fois-ci (Tawong *et al.*, 2014; Chomérat *et al.*, 2020b).

Ostreopsis Schm. nov. gen.

Body flattened, oyster-shaped. Apex excentric, marked by a narrow, slit-shaped area. Longitudinal girdle small, not proceeding to apex, only to be seen on the inferior valve. 3 apical plates (one of which being reduced) and 1 antapical plate are present. Structure of plates porous.



Fig. 7. *Ostreopsis siamensis* Schmidt. Sketch of a specimen in lateral view. Arrangement of plates not indicated.

In its shape this peculiar genus is nearest to *Pyrophacus* Stein of the genera hitherto known; the number of apical- and antapical plates reminds of *Gonyaulax* Diesing, but from both those genera it is easily distinguished by the above features.

23. *O. siamensis* Schm. n. sp., figs. 5, 6, 7.

Body flat, oyster-shaped, somewhat convexo-concave, in a transversal section view triangular or roundish. Superior valve convexe, with 3 apical plates (one of which being very small) and 7 præmedian plates; inferior valve a little concave, with 1 antapical and 4 larger postmedian plates (and sometimes with small accessory plates). Longitudinal girdle short, only on the inferior valve. Structure coarsely porous, like that of *Ceratium tripos*. Length of sagittal axis about 90 μ .

2 (r) — 3 (rr) — 6 (rr).

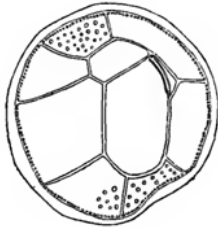


Fig. 5. *Ostreopsis siamensis* Schmidt. Arrangement of plates in the superior valve.

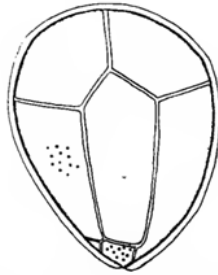


Fig. 6. *Ostreopsis siamensis* Schmidt. Another specimen. Arrangement of plates in the inferior valve.

Figure 16 : Dessins originaux de la première description morphologique d'*Ostreopsis siamensis* dans le Golfe de Siam (d'après Schmidt, 1901).

3.1. Ecologie d'*Ostreopsis* spp., dinoflagellé benthique toxique

Ostreopsis spp. fait partie du groupe des dinoflagellés. Ce groupe de microorganismes unicellulaires regroupe des organismes photosynthétiques, hétérotrophes ou bien mixotrophes, vivant majoritairement dans les océans mais aussi dans les estuaires ou eaux douces. Les dinoflagellés possèdent généralement deux flagelles, localisés au niveau du sulcus et du cingulum, qui leur confèrent une certaine mobilité. La plupart sont équipés d'une thèque formée d'un assemblage spécifique de plaques cellulodiques, souvent caractéristiques de l'espèce (Figure 18). La reproduction des dinoflagellés se fait généralement de manière asexuée par fission binaire de cellules haploïdes (Figure 19), mais certaines espèces peuvent également se reproduire de manière sexuée en produisant des gamètes fusionnants entre elles et formant une cellule diploïde appelée « planozygote » (Pfiester and Anderson, 1987). Cette dernière peut entrer en phase de kyste dormant benthique appelé « hypnozygote » pouvant résister à des conditions environnementales défavorables pendant plusieurs mois voire plusieurs années (Rengefors and Anderson, 1998; Kim *et al.*, 2002). La germination de ces kystes de résistance, une fois les conditions favorables réunies, peut alors être à l'origine de la formation d'efflorescences (Rengefors and Anderson, 1998; Kim *et al.*, 2002). Certains stress environnementaux comme des carences en éléments nutritifs, des changements de températures ainsi que des interactions allélopathiques peuvent également induire la

formation de kystes temporaires chez certains dinoflagellés (Fistarol *et al.*, 2004) ayant une période d'enkystement plus courte que les kystes de résistance, de quelques heures à quelques jours (Wang *et al.*, 2007).

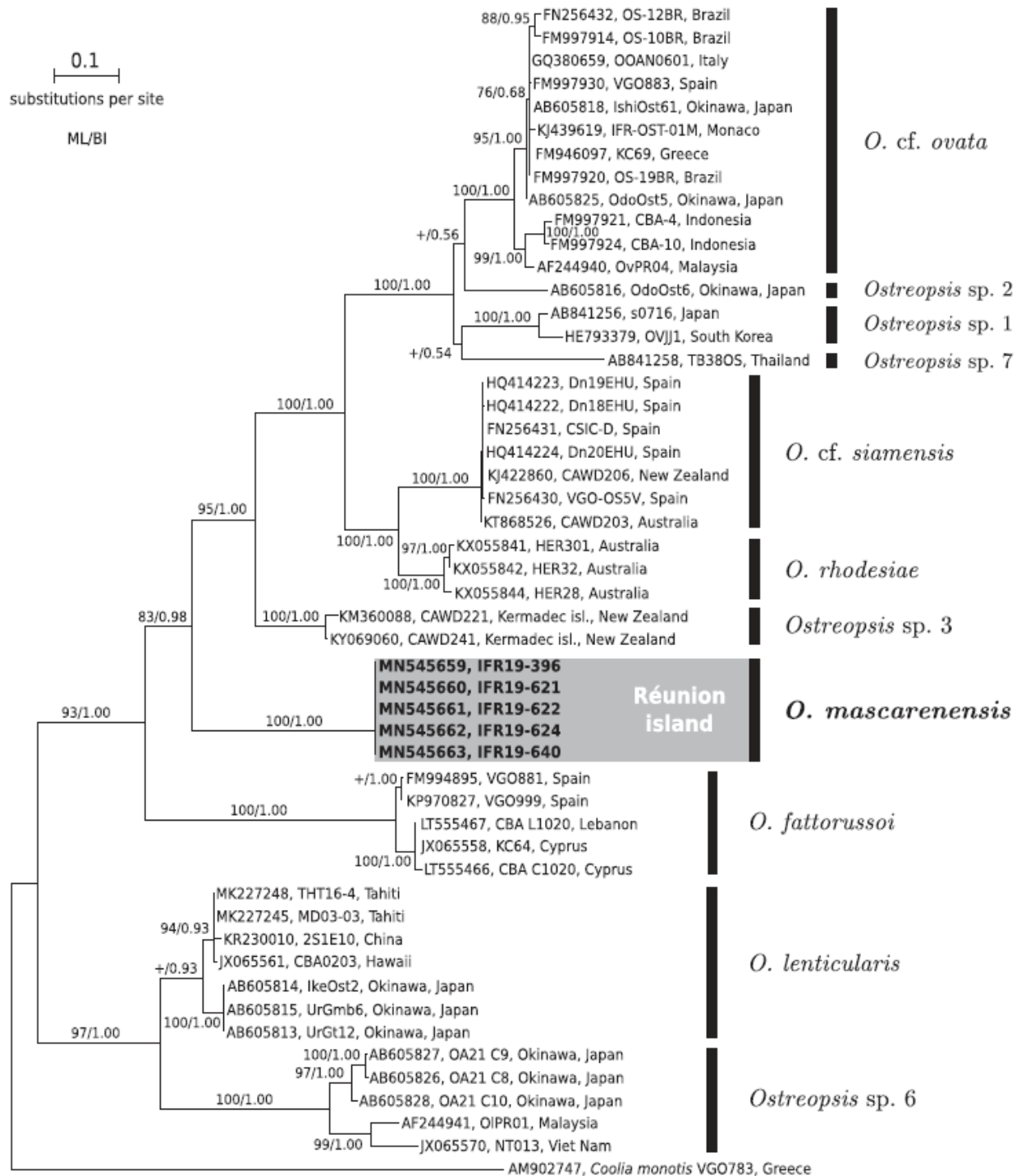


Figure 17 : Arbre phylogénétique (Maximum Likelihood) des espèces d'*Ostreopsis* à partir des domaines D1 – D3 de la grande sous-unité de l'ADNr (d'après Chomérat *et al.*, 2020a).

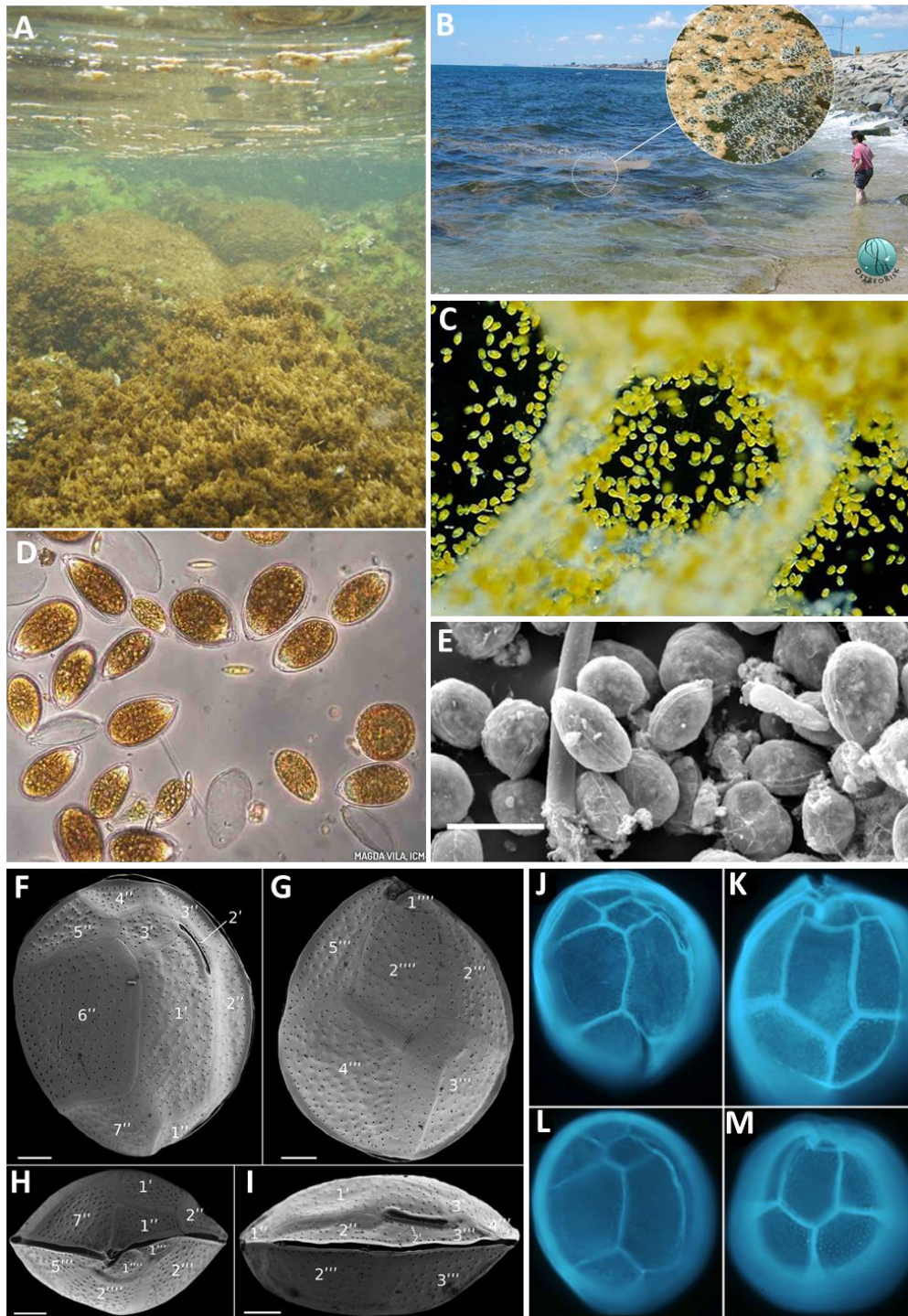


Figure 18 : Observations de cellules d'*Ostreopsis* spp.. (A) Bloom d'*Ostreopsis* cf. *ovata* sur les côtes N-O Méditerranéennes colonisant des macroalgues (crédits : Mangialajo L.). (B) Formation de fleurs d'eau en surface suite à un bloom d'*Ostreopsis* cf. *ovata* à Sant Andreu de Llavaneres (crédits : ostreorisk.icm.csic.es). (C) Observations en microscopie optique de cellules d'*Ostreopsis* cf. *ovata* dans leur matrice mucilagineuse (crédits : Duro R.). (D) Observations en microscopie optique (x100) de cellules d'*Ostreopsis* cf. *ovata* (crédits : Vila M.). (E) Image de microscopie électronique à balayage d'une culture d'*O.* cf. *ovata* (Crédits : Perini F.). Images de microscopie électronique à balayage d'une souche d'*O. lenticularis* de Tahiti en vue (F) apicale, (G) antapicale, (H) ventrale et (I) latérale, la barre blanche équivalent à 10 μ m (adapté d'après Chomérat *et al.*, 2019). Observations en microscopie à épifluorescence de cellules d'*O. lenticularis* de la souche THT16-4 de Tahiti en vue (J) apicale et (K) antapicale et de la souche MGR17-1 de Mangareva en vue (L) apicale et (M) antapicale après marquage au Solophényl-Flavine 7GFE500 (crédits : Chomérat N., IFREMER/projet TATOO).

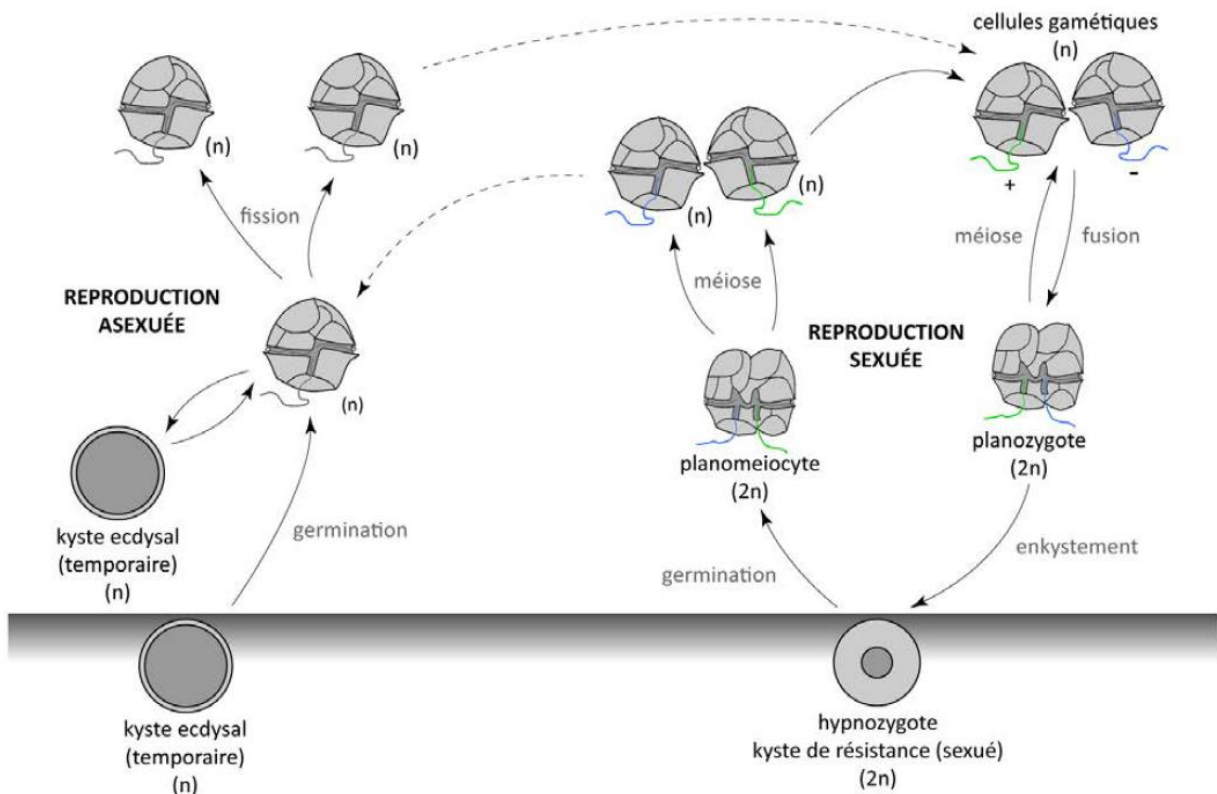


Figure 19 : Schéma du cycle de vie des dinoflagellés (d'après Le Bescot, 2014).

Ostreopsis spp., à l'instar d'autres dinoflagellés comme *Gambierdiscus* spp., *Coolia* spp. et *Prorocentrum* spp., est un dinoflagellé marin benthique, c'est-à-dire se développant à la surface de substrats biotiques ou abiotiques. Plus exactement, les cellules d'*Ostreopsis* spp. sont dites « tychoplanctoniques », ce qui signifie que leur remise en suspension dans la partie pélagique des océans est possible notamment dû à une faible adhérence au substrat et un intense hydrodynamisme local (Aligizaki and Nikolaidis, 2006).

Ostreopsis spp. est également capable de former des efflorescences, souvent toxiques, de manière saisonnière lorsque les conditions environnementales sont favorables (Mangialajo et al., 2008). De nombreux paramètres environnementaux sont connus pour influencer le développement d'*Ostreopsis* spp. comme la température, la lumière, l'hydrodynamisme et le substrat (Accoroni and Totti, 2016). En effet, de manière générale, les blooms formés par les différents espèces d'*Ostreopsis* sont principalement observés en milieu côtier dans des zones et à des périodes où les températures de surfaces sont relativement élevées (Mangialajo et al., 2011), dans des habitats faiblement ou modérément exposés à l'hydrodynamisme et peu profonds (Vila et al., 2001; Shears and Ross, 2009; Accoroni et al., 2012). Ces efflorescences montrent une répartition en patchs sur le fond. Les cellules y sont le plus souvent épiphytes à

la surface de macroalgues (Cohu *et al.*, 2013) bien qu'elles aient également été observées sur une large diversité de substrats abiotiques, naturels ou artificiels. L'influence d'autres paramètres physico-chimiques tels que la salinité (Parsons and Preskitt, 2007; Accoroni *et al.*, 2015a; Blanfuné *et al.*, 2015) et les nutriments (Shears and Ross, 2009; Cohu *et al.*, 2011) sur le développement des efflorescences d'*Ostreopsis* est relativement contrastée et semble également varier selon les espèces et zones géographiques.

Au cours d'intenses efflorescences d'*Ostreopsis* spp., la forte concentration des cellules benthiques et planctoniques peut entraîner l'apparition d'agrégats denses à la surface de l'eau, appelés également « fleurs d'eau ». Ces agrégats sont composés d'un mélange de cellules en très forte concentration, agglutinées dans une gangue muqueuse. Si des filaments muqueux sont produits par les cellules d'*Ostreopsis* spp. afin de permettre leur adhésion aux substrats (Honsell *et al.*, 2013), le mucilage produit pourrait aussi avoir d'autres rôles liés à sa viscosité. Il pourrait, en particulier, avoir un intérêt allélopathique en créant une zone extracellulaire capable de concentrer les molécules bioactives (Barone, 2007; Giussani *et al.*, 2015). Lorsque le mucus est produit en grande quantité, il piège également l'oxygène produit par les cellules lors de la photosynthèse, permettant l'accumulation de microbulles de gaz et ainsi la migration en surface d'agrégats sous forme de fleurs d'eau.

3.2. Distribution

Les espèces d'*Ostreopsis* ont longtemps été décrites comme étant pantropicales, c'est-à-dire distribuées dans les zones tropicales et subtropicales de chaque hémisphère. Cependant, depuis plusieurs décennies, la présence ainsi que des efflorescences d'*Ostreopsis* spp. ont été régulièrement observées au-delà de cette zone, dans les régions tempérées (Rhodes, 2011). La répartition actuelle connue du genre *Ostreopsis* est large à l'échelle du globe. Concernant les zones tropicales, *Ostreopsis* spp. est présent aussi bien dans les zones tropicales d'Asie du sud-est (Pin *et al.*, 2001; Tawong *et al.*, 2014) que dans l'océan Indien (Chomérat *et al.*, 2020b), dans la mer des Caraïbes (Boisnoir *et al.*, 2018) et sur les côtes Atlantiques d'Amérique du sud (Nascimento *et al.*, 2012). Au niveau des régions tempérées, des efflorescences d'*Ostreopsis* sont observées en mer du Japon (Sato *et al.*, 2011; Selina *et al.*, 2014), en Océanie (Shears and Ross, 2009; Chomérat *et al.*, 2019) ou en mer Méditerranée (Totti *et al.*, 2010; Mangialajo *et al.*, 2011; Illoul *et al.*, 2012) (Figure 20). Comme pour les autres organismes responsables

d'HABs, l'augmentation apparente de l'aire de répartition du genre *Ostreopsis* et du nombre d'évènements toxiques recensés à l'échelle du globe pourrait être en partie dû à l'intérêt croissant porté à ce groupe depuis une dizaine d'années (Van Dolah, 2000). De plus, l'évolution récente et constante des nouvelles techniques de biologie moléculaire et des techniques d'échantillonnage rendent l'observation et l'étude d'*Ostreopsis* spp. sensiblement plus accessible. En effet, la standardisation des méthodes d'échantillonnage (Jauzein *et al.*, 2018), l'utilisation de plus en plus fréquente de substrats de type artificiels (Tester *et al.*, 2014; Jauzein *et al.*, 2016) ainsi que le développement de techniques moléculaires basées sur l'ADN environnemental (Penna *et al.*, 2007; Battocchi *et al.*, 2010; Casabianca *et al.*, 2013; Vassalli *et al.*, 2018) ont permis l'amélioration de l'effort de monitoring des microalgues benthiques nuisibles.

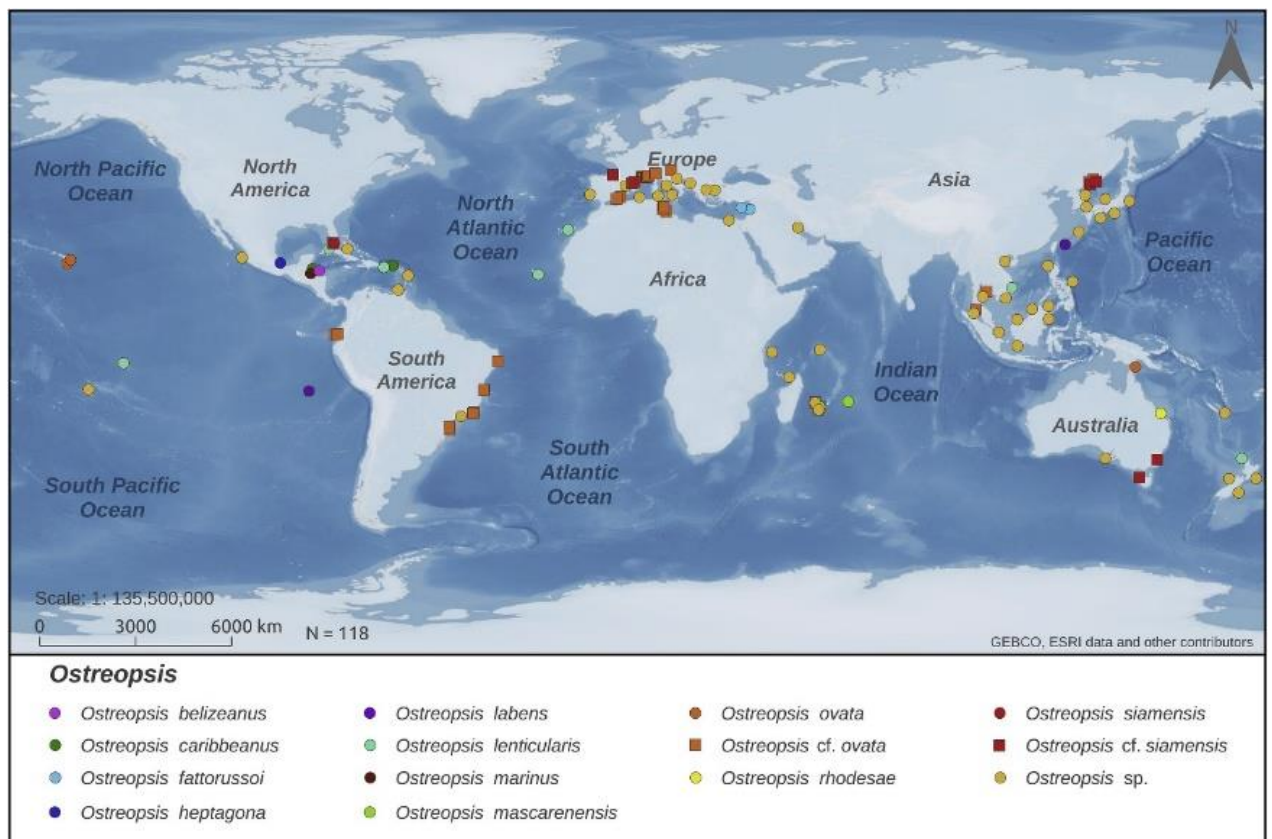


Figure 20 : Répartition mondiale des différentes espèces d'*Ostreopsis* selon des données d'entre 1995 et 2019 (d'après Tester *et al.*, 2020).

Les changements climatiques, et notamment le réchauffement des océans, constituent l'autre hypothèse majeure expliquant cet accroissement du développement des espèces d'*Ostreopsis* dans le monde (Granéli *et al.*, 2011; Wells and Karlson, 2018). Tester *et al.* (2020)

suggèrent que les phénomènes d'HABs liés à des microalgues benthiques, dont *Ostreopsis* spp., risqueraient d'être favorisés par l'augmentation des températures de surface, et qu'une expansion de leur limite de répartition vers de plus hautes latitudes serait attendue. Cependant, les liens directs existant entre efflorescences d'*Ostreopsis* spp. et changements climatiques restent encore relativement méconnus. Des connaissances supplémentaires doivent être accumulées, à la fois à partir de suivis d'efflorescences *in situ* sur le long terme et lors d'expérimentations en milieu contrôlé en laboratoire. Les expérimentations menées sur des souches monoclonales en laboratoire peuvent en particulier permettre d'envisager les différences de comportement entre espèces et entre souches.

3.3. Toxicité

Certaines espèces d'*Ostreopsis* sont capables de produire des métabolites secondaires toxiques, essentiellement dérivés de la palytoxine comme les ovatoxines, ostreocines, mascarenotoxines et autres composés analogues (Pavaux *et al.*, 2020a) (Figure 21). La palytoxine, neurotoxine isolée pour la première fois à partir du genre de corail mou tropical *Palythoa*, est la toxine non-bactérienne d'origine biologique la plus toxique jamais recensée. Une production de palytoxine n'a été pour l'instant identifiée que pour 7 espèces d'*Ostreopsis* sur les 11 décrites, dont *O. fattorussoi* (Accoroni *et al.*, 2016a), *O. lenticularis* (Chomérat *et al.*, 2019), *O. mascarenensis* (Lenoir *et al.*, 2004), *O. ovata* (Ciminiello *et al.*, 2010), *O. cf. ovata* (Sechet *et al.*, 2012), *O. siamensis* (Terajima *et al.*, 2019) et *O. cf. siamensis* (Verma *et al.*, 2016). Des effets allélopathiques ont cependant été observés chez *O. labens* et *O. rhodesae* malgré l'absence de détection de toxines connues (Pavaux *et al.*, 2020a).

Lors d'efflorescences, la production de toxines chez certaines espèces d'*Ostreopsis* peut avoir des effets sur la santé humaine et impacter négativement les activités économiques de la zone côtière ainsi que les écosystèmes marins concernés. Plusieurs épisodes d'intoxication associés à des blooms toxiques d'*O. cf. ovata* ce sont notamment produits en Méditerranée Nord-Occidentale comme par exemple le long des côtes italiennes (Gênes, été 2005 et 2006, Brescianini *et al.*, 2006), espagnoles (Almeria à l'été 2006 et Sant Andreu de Lllaneres en 2004 ; Barroso García *et al.*, 2008; Vila *et al.*, 2008) et algériennes (en 2009, Illoul *et al.*, 2012). Un des épisodes les plus marquants est sans doute celui s'étant produit à Gênes en 2005, où plus de 200 personnes se trouvant à proximité des plages ont ressentis des symptômes liés à la contamination de toxines produites par *O. cf. ovata*, dont 20 ont dû être

admises à l'hôpital. Une étude menée le long des côtes françaises par (Tichadou *et al.*, 2010) a également permis de recenser près de 47 cas d'infections correspondant à 9 épisodes de bloom provoqués par *Ostreopsis* spp. entre 2006 et 2009. Les symptômes décrits dans l'ensemble de ces études correspondent à des irritations cutanées induites par contact direct avec les cellules ou le mucus, mais également des irritations des muqueuses et voies aériennes ainsi que des nausées, fièvres, étourdissements, toux et maux de têtes induits par les toxines transportées sous forme d'aérosols. A ce jour, malgré le fait que des traces de palytoxine aient été retrouvées dans des macroinvertébrés marins (Biré *et al.*, 2013, 2015; Brissard *et al.*, 2014), il reste difficile d'associer les épisodes d'intoxication par ingestion de fruits de mer avec les efflorescences d'*Ostreopsis* spp. et les toxines produites.

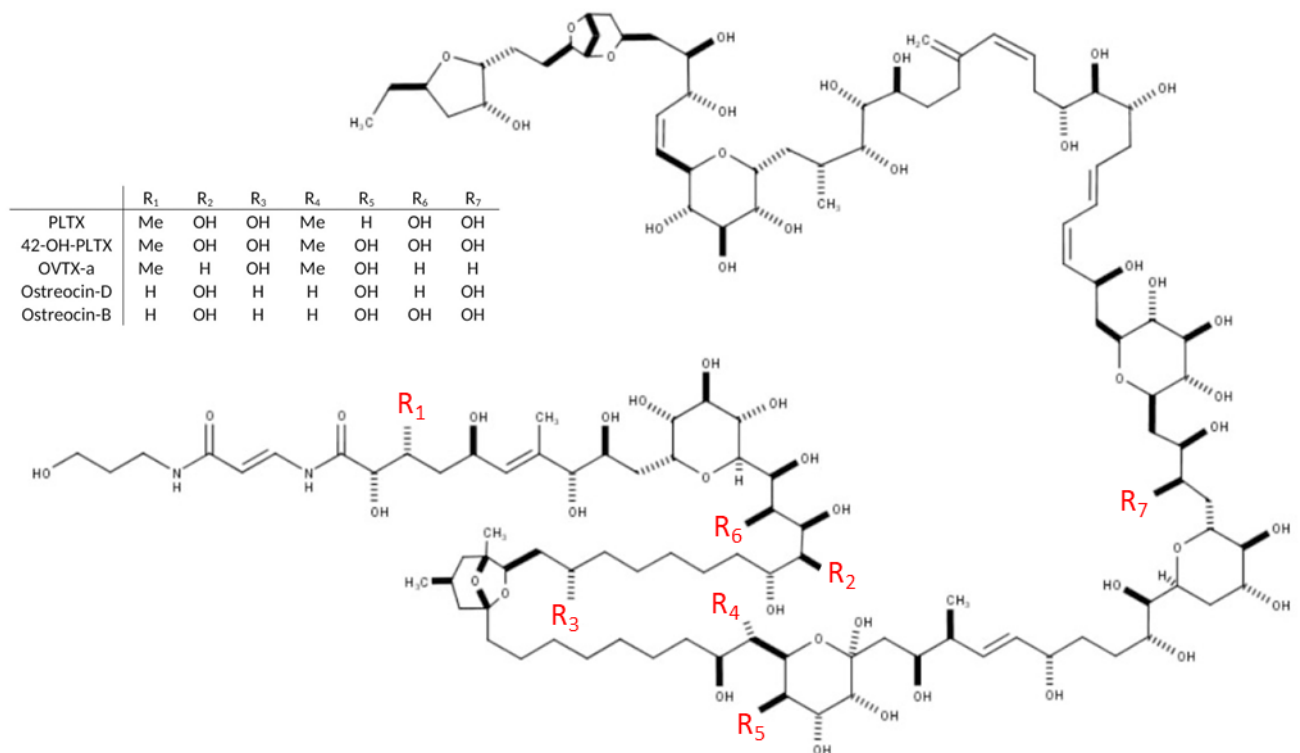


Figure 21 : Structure chimique de la palytoxine et de ses analogues retrouvés dans différentes espèces d'*Ostreopsis* (adapté d'après Pavaux *et al.*, 2020a).

En plus d'affecter la santé humaine, les cellules d'*Ostreopsis* spp. peuvent, via la production de molécules allélopathiques, impacter la survie d'autres organismes marins vivant au sein du même écosystème (Pavaux *et al.*, 2020a). En effet, de fortes mortalités de macroorganismes invertébrés ont été observées durant des épisodes d'efflorescence d'*Ostreopsis* (Granéli *et al.*, 2002; Shears and Ross, 2009). Les microalgues compétitrices

peuvent également être impactées. Accoroni *et al.* (2016b) ont ainsi observé l'effet d'un bloom d'*O. cf. ovata* sur la composition des communautés microphytobenthiques en mer Adriatique, diminuant fortement la diversité mais favorisant la présence d'espèces de diatomées motiles. Ternon *et al.* (2018) ont également observé en laboratoire qu'il existait des interactions allélopathiques entre *O. cf. ovata* et *Licmophora paradoxa*, une diatomée fréquemment associée avec *Ostreopsis* spp. dans le milieu. Concernant l'effet sur de potentiels prédateurs, une étude menée par Pavaux *et al.* (2019) a montré qu'*O. cf. ovata* était capable d'affecter la fertilité et la fécondité du copépode meiobenthique *Sarsamphiascus cf. propinquus*, malgré le fait que ce copépode soit relativement résistant aux toxines produites par *O. cf. ovata*, tandis que Guidi-Guilvard *et al.* (2012) ont observé l'effet négatif *in situ* d'*O. cf. ovata* sur la meiofaune associée. Enfin, des études en laboratoire ont également observé des effets d'*Ostreopsis* spp. sur des macroorganismes comme l'oursin *Lytechinus variegatus* (Neves *et al.*, 2018) ou la méduse *Aurelia* sp. (Giussani *et al.*, 2016).

3.4. Méditerranée et Atlantique : cas d'études de la thèse

Depuis deux décennies, l'augmentation des observations d'efflorescences associées à *Ostreopsis* spp. a été observée dans les zones tempérées, et plus particulièrement en Méditerranée. En effet, depuis sa première identification dans la rade de Villefranche-sur-Mer en 1972 (Taylor, 1979), *Ostreopsis* spp. a été observé dans plusieurs zones du pourtour Méditerranéen, notamment dans la partie Nord-Occidentale le long des côtes Catalanes (Vila *et al.*, 2001), des côtes françaises (Mangialajo *et al.*, 2008), en Mer Adriatique (Accoroni *et al.*, 2011) et en Mer Tyrrhénienne (Tognetto *et al.*, 1995), mais également sur les côtes Algériennes et Tunisiennes (Turki *et al.*, 2006; Illoul *et al.*, 2012), en Grèce (Aligizaki and Nikolaidis, 2006) et au Liban (Accoroni *et al.*, 2016a). Parmi les espèces d'*Ostreopsis* observées en Méditerranée, *O. cf. ovata* semble être la plus répandue, notamment dans la partie Nord-Occidentale, tandis que *O. cf. siamensis* et *O. fattorussoi* ont surtout été observé respectivement en Méditerranée Occidentale (Espagne, Tunisie, Italie) et Orientale (Chypre, Liban) (Battocchi *et al.*, 2010; Accoroni and Totti, 2016; Accoroni *et al.*, 2016a). Compte tenu du nombre élevé d'intoxication associés à des épisodes d'efflorescences toxiques causés par *O. cf. ovata* recensés par le passé, de nombreuses zones à risque en Méditerranée sont très étudiées et surveillées afin de prévenir les risques sanitaires associés à *Ostreopsis* spp.

En dehors de la Méditerranée, les côtes Atlantique de la péninsule Ibérique ainsi que le Golfe de Gascogne constituent également une zone observant l'émergence du risque lié à *Ostreopsis* spp.. En effet, ce n'est que relativement récemment que *Ostreopsis* spp. a été détecté sur la façade Atlantique de la péninsule Ibérique (Amorim *et al.*, 2010; Laza-Martinez *et al.*, 2011) et, contrairement à la Méditerranée, le morphotype d'*Ostreopsis* spp. le plus répandu dans cette zone semble être *O. cf. siamensis*. Les deux morphotypes *O. cf. siamensis* et *O. cf. ovata* ont été observé au sud du Portugal (David *et al.*, 2013; Santos *et al.*, 2019) tandis que seul *O. cf. siamensis* a été observé au nord de la péninsule formant des efflorescences dans le sud-est du Golfe de Gascogne (Laza-Martinez *et al.*, 2011). Cependant, le risque toxique représenté par *O. cf. siamensis* dans ces régions reste encore à déterminer, notamment dû au fait que, malgré l'observation en laboratoire d'effets allélopathiques sur des nauplii d'*Artemias franciscana* (Laza-Martinez *et al.*, 2011), les études menées sur des souches d'*O. cf. siamensis* appartenant au clade Atlantique/Méditerranée n'ont pas confirmé la présence de toxines connues habituellement retrouvées chez les autres espèces d'*Ostreopsis* (Ciminiello *et al.*, 2013).

Méditerranée et Golfe de Gascogne représentent deux régions tempérées où *Ostreopsis* spp. se développe. Pourtant, ces deux régions ont des caractéristiques environnementales bien différentes. Elles représentent donc des zones clés pour étudier les préférences environnementales des deux espèces *O. cf. siamensis* et *O. cf. ovata*. En effet, les eaux du Golfe de Gascogne relativement plus froides, moins salées et observant un fort hydrodynamisme causé par les marées pourraient être moins favorables au développement d'*O. cf. ovata* que les eaux chaudes et stratifiées du nord-ouest de la Méditerranée, et inversement pour *O. cf. siamensis*. Malgré leurs différences, ces deux régions sont exposées aux changements climatiques, et particulièrement à l'augmentation de la température de l'eau (deCastro *et al.*, 2009; Costoya *et al.*, 2015; Pastor *et al.*, 2020). Compte tenu du potentiel effet du réchauffement des eaux de surface sur le développement des HABs et notamment des microalgues benthiques comme *Ostreopsis* spp., la zone du Golfe de Gascogne, où *O. cf. siamensis* est présent dans la partie sud, pourrait servir de zone témoin de la potentielle expansion vers de plus hautes latitudes de cette espèce. En parallèle, le nord-ouest de la Méditerranée pourrait servir de zone témoin de l'impact du réchauffement d'une zone observant déjà des températures élevées.

Objectifs de la thèse

Mon projet de thèse s'est inscrit dans le cadre du projet Européen « CoCliME » (Co-development of Climate Services for adaptation to changing Marine Ecosystems) qui a pour but premier d'étudier les réponses d'indicateurs biologiques, comme les HABs, pouvant impacter la santé humaine ainsi que les activités socio-économiques, face aux changements climatiques. Dans ce contexte, l'objectif principal de mes travaux de thèse a été d'étudier les effets potentiels de la température, en lien donc avec le réchauffement climatique, sur l'écologie et la physiologie des espèces nuisibles *Ostreopsis cf. siamensis* et *Ostreopsis cf. ovata*. Pour répondre à cet objectif, je me suis intéressé à trois aspects distincts de l'influence de la température sur *Ostreopsis* spp. : 1) la phénologie des efflorescences (*O. cf. ovata*), 2) la distribution d'espèce le long d'un gradient latitudinal (*O. cf. siamensis*) et 3) les adaptations physiologiques à un gradient de température (*O. cf. ovata* et *O. cf. siamensis*). Deux sites d'étude ont permis d'aborder ces approches complémentaires : (i) la Mer Méditerranée où des efflorescences massives d'*O. cf. ovata* ont été étudiées à partir de suivis pluri-annuels et (ii) le Golfe de Gascogne présentant un fort gradient latitudinal et comportant la dernière limite Nord d'aire de répartition d'*Ostreopsis* rapportée en Atlantique Nord-Est.

Au cours du premier chapitre de cette thèse, j'ai cherché à **comprendre si l'augmentation de la température des eaux de surfaces pouvait avoir un effet sur la phénologie des blooms d'*Ostreopsis cf. ovata* en Méditerranée nord-occidentale**. Pour cela, j'ai utilisé les données biologiques et environnementales relatives aux blooms saisonniers d'*O. cf. ovata* acquises au cours du suiviestival de la plage du Larvotto, à Monaco. Ce monitoring, ayant débuté durant l'été 2007 et toujours en cours, constitue aujourd'hui l'une des plus longues séries temporelles jamais acquises sur les efflorescences d'*O. cf. ovata* au monde. Pour étudier les données des années 2007-2019 et comprendre l'effet des paramètres environnementaux sur les différents traits phénologiques des blooms observés, j'ai utilisé une approche d'analyse multivariée et focalisé mon intérêt sur le rôle des températures printanières sur le timing des efflorescences.

Au cours du second chapitre de cette thèse, j'ai étudié le **lien entre température et distribution d'*O. cf. siamensis* en analysant s'il pouvait justifier la répartition des blooms dans le Golfe de Gascogne**. Nos connaissances concernant la présence d'*Ostreopsis* spp. dans cette zone indiquaient que seule l'espèce *O. cf. siamensis* était présente dans la partie sud-est du Golfe. Nous avons alors planifié et conduit une campagne d'échantillonnage à travers toute l'étendue du Golfe et même au-delà, entre Comillas (Espagne) et Roscoff (Bretagne), entre

août et septembre 2018 afin de déterminer, pour la première fois dans cette zone, la distribution d'*Ostreopsis* spp. L'originalité de cette campagne résidait dans l'utilisation d'une approche de biologie moléculaire basée sur la détection d'ADN environnemental, en plus de l'approche classique basée sur l'observation microscopique, pour déterminer la présence d'*Ostreopsis* spp. Afin de valider la distribution observée et de s'affranchir des limites liées notamment à l'utilisation de l'ADN environnemental, nous avons couplé à nos analyses moléculaires une analyse de modélisation de transport lagrangien pour envisager la connectivité physique entre différentes zones de prélèvement. Ainsi, le couplage entre approche moléculaire et approche de modélisation a permis la détermination de zones géographiques clés qui pourraient permettre de suivre une potentielle future expansion des efflorescences d'*O. cf. siamensis* dans le Golfe de Gascogne.

Enfin, au cours du troisième chapitre de cette thèse, j'ai **étudié les réponses physiologiques adaptatives d'*O. cf. siamensis* et d'*O. cf. ovata* face à des changements de température**. La détermination des températures optimales et favorables à la croissance algale sont primordiales pour la compréhension de l'effet de la température sur la phénologie des efflorescences, l'aire de répartition des espèces et leurs réponses à des températures changeantes. J'ai donc cherché à définir en laboratoire la niche thermique de nombreuses souches d'*O. cf. siamensis* et d'*O. cf. ovata* afin d'étudier la variabilité entre espèces et entre souches (variabilité intra-spécifique) de ce paramètre. J'ai également étudié des aspects particuliers de la morphologie et du métabolisme cellulaire afin d'envisager la plasticité physiologique des cellules. Ces approches écophysologiques ont permis de définir des stratégies et moyens que la cellule d'*Ostreopsis* peut utiliser pour optimiser sa croissance sous des températures non-optimales.

Le dernier chapitre de cette thèse consiste en une discussion générale discutant de l'influence générale de la température sur l'écologie du dinoflagellé benthique toxique *Ostreopsis* spp. ainsi que des perspectives envisagées pour améliorer et compléter ces travaux de thèse.

Chapitre 1

Le dinoflagellé benthique toxique *Ostreopsis* cf. *ovata* en Méditerranée nord-occidentale :
Relation entre températures printanières et
phénologie des efflorescences

Contexte de l'étude

La fréquence d'observation d'efflorescences algales nuisibles ainsi que leur distribution semblent être en constante augmentation depuis plusieurs décennies, et parmi les facteurs environnementaux susceptibles d'en être à l'origine, les changements climatiques sont avancés comme en étant la principale cause (Hallegraeff, 2010; Wells and Karlson, 2018; Tester *et al.*, 2020). Les dinoflagellés benthiques toxiques du genre *Ostreopsis*, présents dans les régions tropicales, subtropicales et tempérées du globe (Rhodes, 2011), sont capables de former de telles efflorescences dont les conséquences sur la santé humaine peuvent être importantes. En effet, les efflorescences de *Ostreopsis cf. ovata*, espèce très présente en Mer Méditerranée, ont été impliquées dans plusieurs épisodes d'intoxication massives sur l'homme via l'exposition direct à des composés toxiques dérivés de la palytoxine et produits par les cellules (Brescianini *et al.*, 2006; Vila *et al.*, 2008; Illoul *et al.*, 2012). Ces efflorescences saisonnières sont régulièrement observées sur les côtes Méditerranéennes nord-occidentales (Mangialajo *et al.*, 2011), et notamment à Monaco, où un suivi temporel multiparamétrique des blooms estivaux d'*O. cf. ovata* est conduit depuis 2007. (Cohu *et al.*, 2011), en s'appuyant sur les deux premières années de monitoring à Monaco, ont observé une forte variabilité phénologique entre ces deux efflorescences et ont suggéré que les températures printanières pourraient influencer la phénologie des efflorescences.

Ce premier chapitre de thèse est centré sur l'analyse de cette série temporelle, aujourd'hui constituée de treize années de suivi, afin de mettre en évidence les relations entre les différents paramètres environnementaux mesurés et les traits phénologiques des efflorescences d'*O. cf. ovata*.

Contribution des auteurs

Dans cette étude basée sur le suivi des efflorescences d'*O. cf. ovata* mis en place à la plage du Larvotto à travers la collaboration entre R. Lemée et V. Davenet-Sbirrazuoli, j'ai compilé les données d'abondance d'*O. cf. ovata* issues des échantillons collectés et analysés par R. Lemée, C. Jauzein, A-S. Pavoux, S. Marro ainsi que les stagiaires volontaires impliqués lors de chacune des treize saisons du suivi. J'ai également rassemblé et compilé les données environnementales fournies par la Direction de l'Environnement de Monaco et du SOMLIT. Avec l'aide de S. Gasparini, j'ai ainsi pu analyser ces données en procédant à des analyses de

données uni/multivariées (ACP, somme cumulée des anomalies, corrélations) et statistiques afin de mettre en relation phénologie des efflorescences et paramètres environnementaux. J'ai écrit le chapitre de thèse principalement sous la supervision de R. Lemée et en collaboration avec mes deux autres co-encadrants C. Jauzein et R. Siano.

Le travail accompli dans cette partie de la thèse est ici présenté sous forme de chapitre de thèse rédigé en anglais. La rédaction d'un article scientifique basé sur ce chapitre est prévue à court terme. Le manuscrit sera soumis à la review *Harmful Algae* dans le cadre d'un numéro spéciale consacré au projet CoCliME.

The benthic toxic dinoflagellate *Ostreopsis cf. ovata* in the NW Mediterranean Sea: Relationship between spring temperature and phenology

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Abstract:

Harmful Algal Blooms associated with the benthic toxic dinoflagellate *Ostreopsis cf. ovata* are responsible for ecological, sanitary and economic issues in different temperate areas and need better understanding. Every summer, from 2007 to 2019, *Ostreopsis cf. ovata* blooms have been monitored in Larvotto beach (Monaco, NW Mediterranean Sea), allowing for the achievement of one of the longest time series of *Ostreopsis cf. ovata* blooms worldwide. Five sites were sampled each year from mid-June to end August in order to characterize bloom dynamics.

Blooms phenology (timing, length and maximum cell abundance) was highly variable across the study period. Variations of net growth rate estimated during the phases of bloom development were analyzed as a function of Sea Surface Temperature (SST). The highest growth rates were not associated with the maximal temperature records (27.5°C) but were associated to temperature ranging between 21°C and 25°C. Many authors suggested that global warming might have influenced *Ostreopsis cf. ovata* expansion from tropical areas to temperate waters, such as the Mediterranean Sea. Current results do not directly support this hypothesis, but suggest a more complex role of temperature in bloom dynamics than a simple facilitation factor for algal growth, at least in temperate areas.

In order to characterize in more details the role of SST on *O. cf. ovata* bloom phenology, SST anomalies were calculated as differences between temperature values and the mean SST value over the 13 years of survey. A strong positive correlation was observed between positive anomalies of SST during the spring and the bloom precocity. This result indicates that blooms occurred earlier in the season when spring was warmer. The relation between SST and net growth rate as well as between SST anomalies and precocity of the blooms should be of great interest for managers and modelers. Predicting the distribution and the phenology of *Ostreopsis cf. ovata* in European coastal waters is a crucial challenge, especially in a context of global warming.

Keywords: *Ostreopsis cf. ovata*; Benthic HABs; Phenology; Sea Surface Temperature; NW Mediterranean Sea

1. Introduction

Over the past decades, reports of Harmful Algal Blooms (HABs) have increased in intensity, distribution and frequency (Shears and Ross, 2009; Hallegraeff, 2010; Berdalet *et al.*, 2017b), asking ecological, sanitary and economic questions. Causes of these increases are still a matter of debate, involving climate changes (Glibert *et al.*, 2014; Kibler *et al.*, 2015; Moore *et al.*, 2008; Tester *et al.*, 2020; Wells and Karlson, 2018), anthropogenic effects (Anderson *et al.*, 2002; Davidson *et al.*, 2014; Fu *et al.*, 2012; Glibert *et al.*, 2005; Heisler *et al.*, 2008) and increase in worldwide monitoring programs (Anderson *et al.*, 2012; Van Dolah, 2000). A mix of those hypothesis is probably at the origin of the phenomena.

Amongst the numerous organisms responsible of harmful events, toxic species belonging to the marine dinoflagellate genus *Ostreopsis* Schmidt are found in tropical, sub-tropical and temperate areas (Faust *et al.*, 1996; Rhodes, 2011). Since its first report in the Mediterranean Sea in 1972 in Villefranche-sur-Mer, France (Taylor, 1979), *Ostreopsis* spp. have been observed all across Northern Mediterranean coasts (Mangialajo *et al.*, 2011). Among *Ostreopsis* species, *Ostreopsis cf. ovata* (Fukuyo) and *Ostreopsis cf. siamensis* (Schmidt) are the most widespread in NW Mediterranean Sea. The first bloom of *Ostreopsis cf. ovata* in this area was observed along the Tuscany coasts in 1998 (Sansoni *et al.*, 2003). Along French Mediterranean coasts, recurrent blooms have only been associated with the species *Ostreopsis cf. ovata* (Blanfuné *et al.*, 2015; Cohu *et al.*, 2011; Fricke *et al.*, 2018; Mangialajo *et al.*, 2011; Tichadou *et al.*, 2010). In Western Mediterranean Sea, toxic outbreaks resulting from *Ostreopsis* spp. bloom episodes mostly occurred along the Northern coasts (Barroso García *et al.*, 2008; Brescianini *et al.*, 2006; Pfannkuchen *et al.*, 2012; Vila *et al.*, 2008), although toxic events have been observed in Southern coast (Illoul *et al.*, 2012). Unlike other HABs, such as some species of the genus *Prorocentrum*, *Gambierdiscus* or *Alexandrium*, causing human health issues through phycotoxin contamination of the food chain, *Ostreopsis cf. ovata* affects human health through direct contact inducing skin irritations (Tichadou *et al.*, 2010; Tubaro *et al.*, 2011) or by inhaling aerosols responsible for respiratory symptoms (Durando *et al.*, 2007; Vila *et al.*, 2016). Ecological issues such as mass mortality of invertebrates (Sansoni *et al.*, 2003; Totti *et al.*, 2010) and invertebrates and fish contamination (Aligizaki *et al.*, 2008; Biré *et al.*, 2013; Biré *et al.*, 2015; Brissard *et al.*, 2014) are also associated with *Ostreopsis* blooms. Production of toxins belonging to the palytoxin group

(Ciminiello *et al.*, 2006, 2012, 2014) seems to be the major cause for *Ostreopsis* toxicity, although toxicity level may vary according to local environmental conditions (Pistocchi *et al.*, 2011).

Mediterranean blooms of *Ostreopsis cf. ovata* are a recurrent phenomenon observed during summer-autumn period (Mangialajo *et al.*, 2008, 2011; Totti *et al.*, 2010; Vila *et al.*, 2001). The fact that in certain areas those episodes are observed in particular time periods of the year only suggests that specific environmental conditions are required for such blooms to occur. Studies showed the importance of hydrodynamics on *Ostreopsis* development (Barone, 2007; Shears and Ross, 2009; Totti *et al.*, 2010). Low wave actions to moderately shaken waters seem to favor cells settlement (Vila *et al.*, 2001), as strong winds and currents would favor cells dispersion and impact cell growth. The influence of nutrients availability on *Ostreopsis* blooms is still discussed. No clear relationship was found between nutrients concentrations and cell abundances during blooms periods (Accoroni *et al.*, 2012; Cohu *et al.*, 2011; Pistocchi *et al.*, 2011; Vila *et al.*, 2001), although nitrate/phosphate ratios may be involved in the bloom onset (Accoroni *et al.*, 2015a). Blooms of *Ostreopsis cf. ovata* have been observed in salinity near 37-38 in NW Mediterranean Sea (Mangialajo *et al.*, 2008; Vila *et al.*, 2001) but also in salinity ranging from 31 to 39 in Northern Adriatic Sea (Accoroni *et al.*, 2015a), although some authors suggested a negative impact of low salinities associated to freshwater inflows (Blanfuné *et al.*, 2015; Carnicer *et al.*, 2015). Laboratory studies suggested a strain-specific response towards salinity levels (Tawong *et al.*, 2015) although Mediterranean strains of *O.cf. ovata* seem to prefer high level of salinity (Pezzolesi *et al.*, 2012). Depth and substratum also appear to be a specific factor as *Ostreopsis* spp. cells are mainly found in shallow waters (Cohu and Lemée, 2012; Totti *et al.*, 2010) and higher abundances were found on ramified host macroalgae (Accoroni *et al.*, 2015b; Cohu *et al.*, 2013). The presence of ramified host macroalgae combined to a sheltered shallow habitat contributes for the settlement and the growth of *O. cf. ovata*.

Amongst environmental parameters prone to affect *Ostreopsis* spp. development, seawater temperature (closely associated to period of the year) is considered as one of the most important (Granéli *et al.*, 2011; Mangialajo *et al.*, 2008; Sansoni *et al.*, 2003). Blooms of *Ostreopsis cf. ovata* in NW Mediterranean Sea are not observed at the same time in each area. Peaks of bloom are usually recorded in mid-summer (July) along the Ligurian Sea coasts (Cohu *et al.*, 2011; Mangialajo *et al.*, 2008), while they appear later in autumn (September-October)

in the Northern Adriatic Sea (Monti *et al.*, 2007; Totti *et al.*, 2010) or can last from August to November in Catalan coasts (Vila *et al.*, 2016). Authors suggested that relatively high temperatures were needed for *Ostreopsis* spp. to grow (Hallegraeff, 2010; Granéli *et al.*, 2011) while others proposed the exceeding of a certain temperature threshold during long enough period of time (David *et al.*, 2012) as the main factor triggering the bloom. According to previous observations in NW Mediterranean Sea, blooms of *Ostreopsis cf. ovata* have been occurring in a wide range of temperatures, from 16.8°C to 30°C (Accoroni and Totti, 2016; Mangialajo *et al.*, 2011). Contradictory observations between studies on the effect of sea temperature on *O. cf. ovata* in Mediterranean Sea suggest a more underlying and complex role of temperature. Laboratory studies on different strains of *Ostreopsis cf. ovata* from Mediterranean Sea showed that optimal temperatures for growth were between 20°C and 26°C (Pezzolesi *et al.*, 2012; Scalco *et al.*, 2012; Carnicer *et al.*, 2016).

In a context of global warming, the study of optimum temperatures and *in situ* niche of *Ostreopsis cf. ovata* could be used as a tool to plan monitoring programs and to help prediction of blooms. The use of a long-term monitoring dataset is one attempt to study the relation between *O. cf. ovata* and temperature at a local scale (Ligurian Sea). Since summer 2007, a monitoring program was implemented in Monaco in order to study the evolution of seasonal blooms of *O. cf. ovata* and the assessment of its sanitary risks in recreational waters. Since then, this monitoring enabled to build the world's longest long-term dataset ever acquired on *O. cf. ovata*. In addition to the cell abundance monitoring in Monaco, the proximity of this study area to the Bay of Villefranche-sur-Mer, where hydroclimatic parameters have been weekly collected for many decades is an opportunity to combine biological and ecological data in order to study the relationships between environmental factors and *O. cf. ovata* bloom phenology. Based on data collected during the first two years of this monitoring program, Cohu *et al.* (2011) reported a distinct pattern in bloom timing between 2007 and 2008, corresponding to different hydroclimatic conditions: a warmer spring and a colder summer were observed in 2007 compared to 2008. With thirteen years of collected data on *O. cf. ovata* in Monaco, the present study gives expectations to determine relationships between hydroclimatic factors and bloom phenology with a high robustness and an opportunity to confirm such relationship between temperature and timing.

The aim of this study was then to have a better understanding of the ecology of *Ostreopsis cf. ovata* in NW Mediterranean Sea by seeking for links between environmental

factors and the bloom phenology of the species, based on the combination of our ongoing monitoring program in Monaco and environmental long-term dataset associated to the area. Following the previous observations of Cohu *et al.* (2011), we will focus more on the role of water temperature on *O. cf. ovata* bloom phenology.

2. Material and Methods

2.1. Sampling sites

Located between the French and the Italian borders on the Mediterranean coast, Monaco is a highly touristic area whose population increases drastically during the summer season. The Larvotto (43°44.71' N – 7°26.04' E) is a small sheltered beach surrounded by residential buildings, shops and restaurants potentially exposed to *Ostreopsis cf. ovata* toxins during bloom events (Figure 22). However, no highly toxic outbreak has ever been reported at this location, even if sporadic skin and mucosal irritations have been observed (Tichadou *et al.*, 2010). The beach is South-Est orientated and artificial rocky dykes were built as a barrier from waves and swell. Such structures constitute ideal substrates for the development of ramified macroalgae, especially Phaeophyceae and Florideophyceae, such as *Halopteris sp.*, *Dictyota sp.* and *Ellisolandia sp.*.

2.2. Sampling process and cell quantification

In NW Mediterranean Sea, blooms of *Ostreopsis cf. ovata* are always observed in early summer, when the sea temperature increases and wind conditions are calm. In order to quantify the abundance of epiphytic *Ostreopsis cf. ovata* cells, a monitoring program started in 2007 and was then conducted every year, generally from mid-June until the end of August. Sampling was carried out once a week during this period, between 9 am and 11 am, in five sites located on rocky substrate areas at the Larvotto Beach (see Figure 22). The evolution of the bloom was monitored both through the benthic cells stock and planktonic cells quantification. Samples of the most representative macroalgae (usually *Dictyota sp.* Lamouroux or *Ellisolandia elongata* Hind and Saunders or *Halopteris scoparia* Sauvageau) of each sampling sites were collected at 0.5-meter depth using 250 ml plastic flask, as well as water above each macroalgae at 0.3-meter depth for planktonic cells quantification such as

described by Cohu *et al.* (2011). In order to avoid any disturbance of benthic stock cells due to resuspension, planktonic sampling was always performed before benthic sampling. Samples were then fixed with acidic Lugol (1% vol./vol.) and kept in dark at 4°C. Macroalgae samples were vigorously shaken and filtered through a 500 µm meshed filter in order to separate epiphytic cells from the macroalgae. Macroalgae were rinsed twice with 100 ml of 0.2 µm filtered seawater to detach as much microalgae cells as possible then drained and placed onto a tin foil for fresh weight measurement (Jauzein *et al.*, 2018). Epiphytic *Ostreopsis cf. ovata* cell abundances were then estimated in 1 ml Sedgwick Rafter counting cells using a light microscope (Axioscope 5, Zeiss, Oberkochen, Germany) and allow to calculate the number of cells per gram of fresh weight of macroalgae (cell g⁻¹ FW). Planktonic cell abundances were quantified using an inverted light microscope after sedimentation in 50 ml column following the Utermöhl method (Utermöhl, 1958).

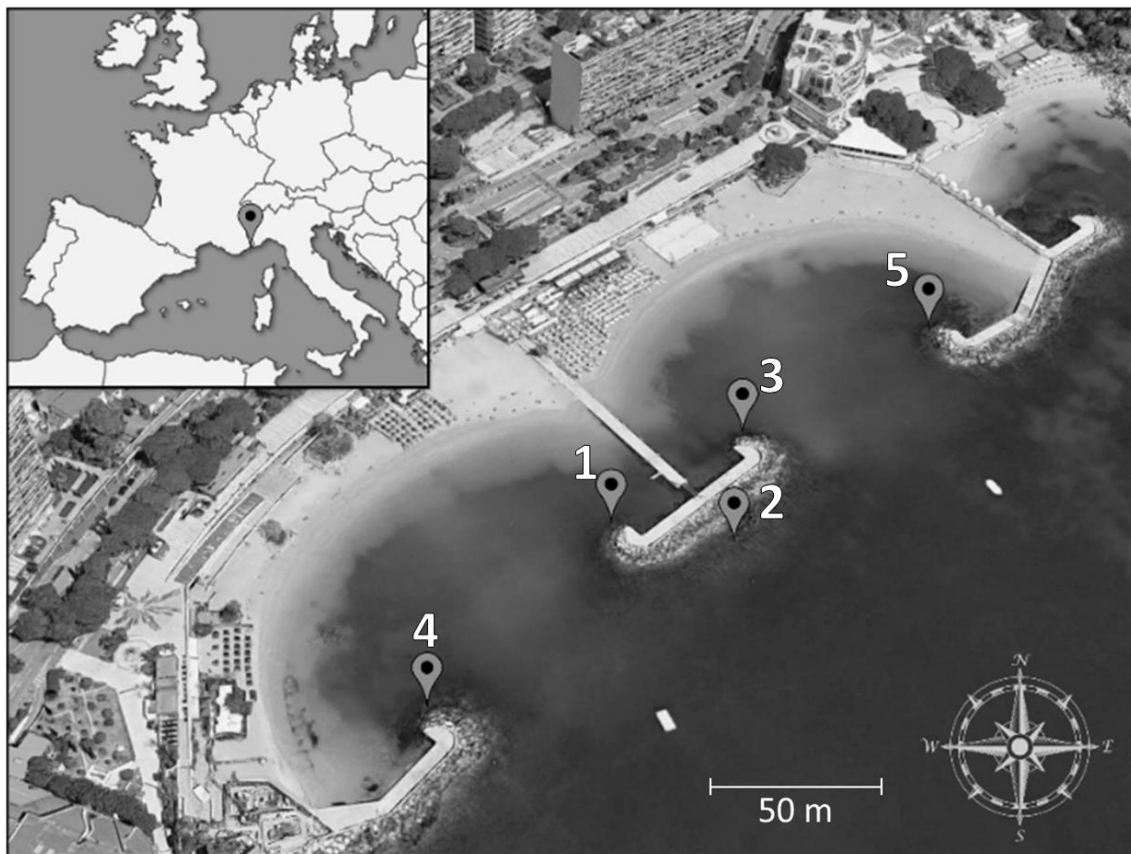


Figure 22: Map of the sampling sites (from 1 to 5) in Larvotto, Monaco. In the top-left corner is the map of the NW Mediterranean Sea, with the location of Monaco, between France and Italy.

2.3. Hydroclimatic parameters

During the sampling period, temperature of seawater was measured *in situ* at each sampling sites ($\pm 0.1^\circ\text{C}$). The “Direction de l’Environnement” of Monaco provided hydroclimatic data from their meteorological program. On the other side, long-term hydrological data came from Point B in Villefranche-sur-Mer ($43^\circ41.10'$ N - $7^\circ18.94'$ E) collected by the SOMLIT, a French national coastline monitoring network (<http://somlit.epoc.u-bordeaux1.fr>). Located 12 km away from Monaco, Villefranche Bay can be considered as a reference area for the western part of the Ligurian Sea, including Monaco. These data were collected once a week in the bay, all year long, during the whole study period.

2.4. Calculation and Statistics

Cell abundance for each sampling dates was calculated as the mean cell abundance between the five sampling sites. The difference in cell abundance between two sampling dates (i.e. net growth rate = μ) is the result of the combination of many parameters such as cell growth rate, cell dispersion, cell accumulation, predation and cell mortality rate. Net growth rates were calculated between each week of sampling using the following equation:

$$\mu = \frac{\text{Ln}N2 - \text{Ln}N1}{t2 - t1}$$

Where μ is the net growth rate (d^{-1}) between sampling day 1 and sampling day 2, N1 the mean Cell abundance at sampling day 1 (cell g^{-1} FW), N2 the mean Cell abundance at sampling day 2 (cell g^{-1} FW), T1 the sampling day 1 and T2 the sampling day 2.

Data from the monitoring of year 2007 were not included in all our following analyses as the sampling monitoring started too late in early July when the bloom was already declining. However, cell abundances recorded at the first day of sampling were the highest ever recorded in Monaco, suggesting that the peak of the bloom would have occurred at least during June. Starting date, maximum abundances and maximum growth rates could not be estimated for this year.

In order to categorize the different years of monitoring according to their sea temperature variations, a mean annual temperature profile was calculated over the thirteen

years monitoring period. For each year, weekly SST anomalies were calculated as differences between temperature values and the mean SST value previously calculated. Cumulative sums of SST anomalies were performed starting from the first week of each year. Data were from Villefranche Bay.

Variations of net growth rate estimated during the phases of bloom development were analyzed as a function of Sea Surface Temperature (SST) using a non-parametric “Loess” local polynomial regression model (Cleveland, 1979). The non-parametric Spearman correlation test was used to examine links between the abundance of benthic and planktonic cells, as well as between sea surface temperature and *Ostreopsis cf. ovata* abundances. A Wilcoxon signed-rank (non-parametric) test was applied on temperature data collected both in Monaco and Villefranche Bay to verify that the temperatures at both locations were matching. A non-parametric Friedman ANOVA was performed to assess the benthic abundance spatial variability between sampling sites for both each year and for the whole monitoring period as well as a *post-hoc* pairwise Wilcoxon signed-rank test in order to look at pairwise comparisons between sampling sites. Environmental data such as water temperature, salinity, pH, wind speed and rainfall (provided by the Direction de l’Environnement de Monaco) were used to perform a Principal Component Analysis (PCA) to describe relationships between both cell abundance and net growth rates with these environmental factors. The multivariate analysis was performed using 81 samples points where environmental data were available. Weekly average values of environmental factors were calculated and associated with both corresponding benthic abundance and net growth rates values issued from monitoring samples. Factorial axis of the PCA were built based on linear combination of environmental variables in order to represent the ordination between environmental variables on an Euclidean space. All statistical analyses were performed using R studio (R Core Team, 2019).

3. Results

3.1. Spatial variability:

Monitoring of bloom events are conducted through periodic and steady counting of *Ostreopsis cf. ovata* cells. The global estimation of the benthic cells stock in a given area is complex as the cell concentration may depend on the presence of certain types of macroalgae and may

also vary according to small scale environmental variations. In order to give a reliable estimation of the cell abundance of the area, five different sites were sampled at each sampling date (Figure 23). Along our thirteen years of monitoring program, we observed clear spatial variations in cell abundance between the five different sites at the same sampling date (Figure 23). However, the Friedman ANOVA showed no significant differences between the five sampling sites over the thirteen years of monitoring (N = 141, Fc = 6.355, df = 4, p-value = 0.174). A Friedman ANOVA was also performed for each year of monitoring and overall significant differences between sites were only found during summer 2008 (N = 11, Fc = 14.0, df = 4, p-value = 0.0075) and 2017 (N = 11, Fc = 17.82, df = 4, p-value = 0.0013). Pairwise Wilcoxon signed-rank tests revealed no significant pairwise differences between sites during summer 2008 while in 2017 site 4 significantly differed from both sites 2, 3 and 5 and site 5 differed from sites 1, 3 and 4. In 2017, the site 4 exhibited significantly lower cell abundances than the other sites while the site 5 exhibited significantly higher cell abundances. Even if statistical differences were observed between sites for 2 out of 13 years, the mean (for benthic and planktonic abundances) of the 5 sites was used in order to study the phenology of *O. cf. ovata*.

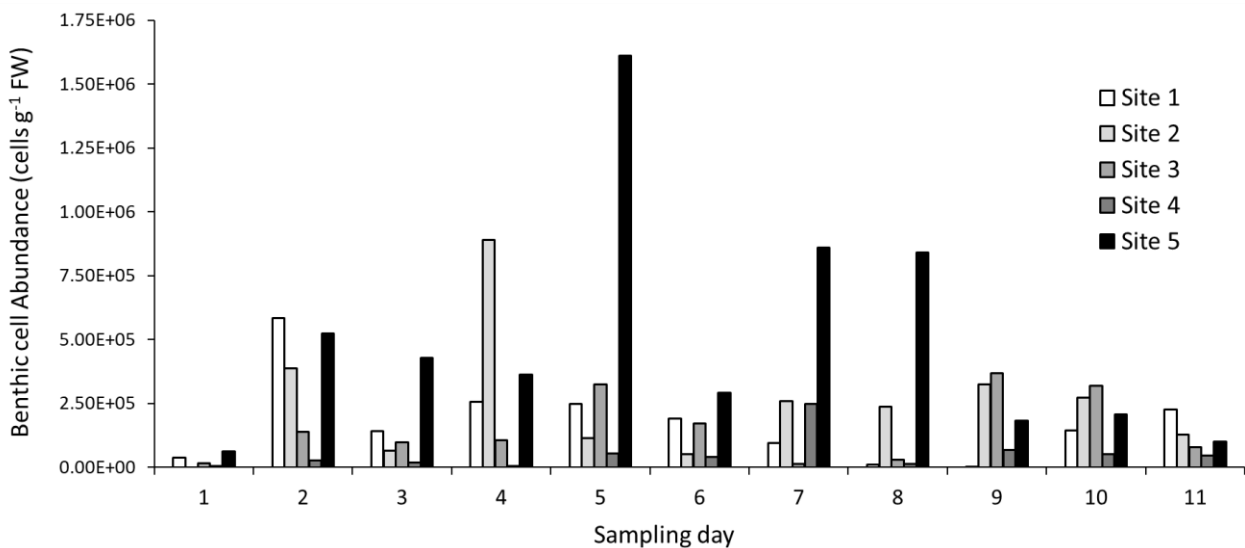


Figure 23: Inter-site variability of benthic cell abundance during the *Ostreopsis cf. ovata* bloom monitoring of summer 2017 in Larvotto Beach.

3.2. Interannual variability:

Since 2007, blooms of *O. cf. ovata* at the Larvotto occurred every year. Blooms were constituted by an increase in both benthic and planktonic cell abundances until the maximum peak was reached during the summer (between June and August), followed by a decrease to a very low level of cell concentration, marking the end of the bloom. For two out of thirteen years of survey, the maximum peak of benthic cells was followed by a second and smaller peak in late summer.

Log transformed data of the mean abundances over the five sampling sites (Figure 24) showed a positive correlation between benthic and planktonic abundances of *Ostreopsis cf. ovata* (Spearman test, $N = 147$, $r_s = 0.805$, p -value < 0.01). This species is considered as epibenthic and thus the stock of cells is on the bottom. Moreover, planktonic cells could be easily concentrated or dispersed by hydrodynamics. For those reasons, all the following analysis were focused exclusively on benthic abundances. All along the study period, highly variable phenological features in *Ostreopsis cf. ovata* blooms were observed in Monaco (Figure 25). Each summer bloom was differing from one another in at least one of the three following criteria: timing, duration and maximum abundance.

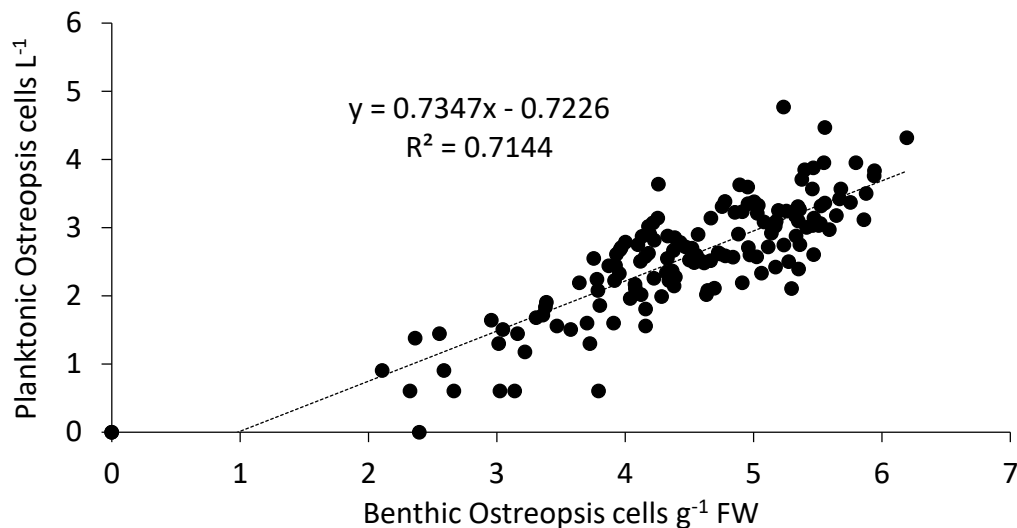


Figure 24: Correlation between benthic and planktonic cell abundances of *Ostreopsis cf. ovata* in Larvotto beach.

A threshold of 200 000 cells g⁻¹ FW was chosen to define the start of a bloom. This value was close to the lowest maximum abundance found during the thirteen blooms recorded (212 300 cells g⁻¹ FW, in 2015). For the whole study period, the average bloom starting day was 191 (Julian day ± 10.6). Delays in the beginning of the bloom timing appeared as early as 15 days prior to the average starting day (Julian day 177, in 2015), while the latest bloom occurred 25 days later (Julian day 214, 2016).

The duration of the event was also variable over the years. As samples were collected once a week, the bloom was considered lasting one week when more than the 200 000 cells g⁻¹ FW was quantified for two sampling days in a row. When abundance higher than the threshold was reported only once, the duration of the bloom was defined as one day. The average length of the bloom was 8.2 days (± 7.5), with years 2011 and 2016 having the longest blooming period (28 and 16 days), while years 2007, 2009 and 2015 were having the shortest (1 day).

The average value of the highest peak of each year was 570 878 (± 391 871) cells g⁻¹ FW. The highest value ever recorder was 1 565 514 cells g⁻¹ FW (174 % more than the average) in 2007 and the minimum was 212 300 cells g⁻¹ FW (63 % less than the average) in 2013.

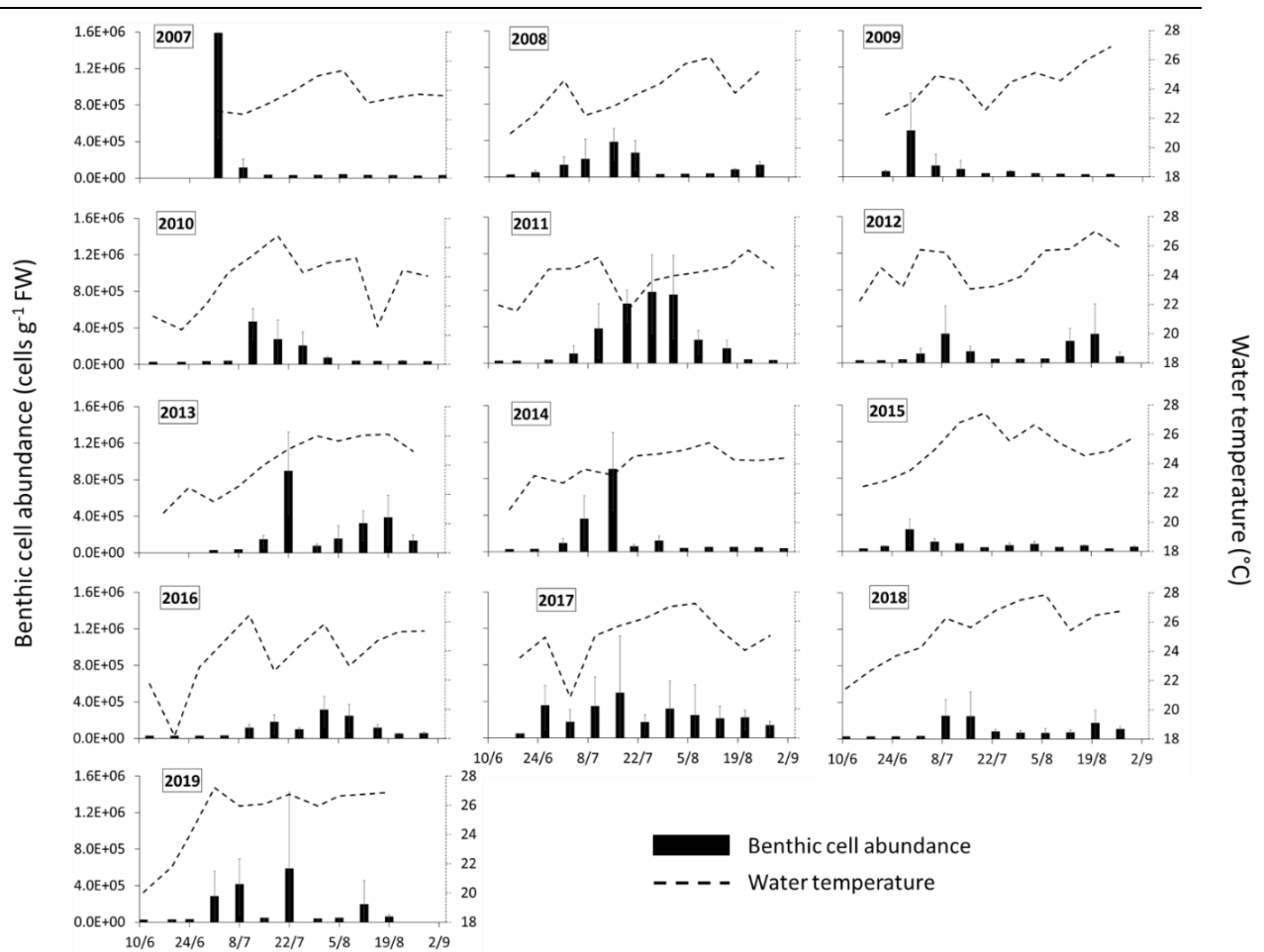


Figure 25: Evolution of benthic abundances of *Ostreopsis cf. ovata* and SST during summer in Larvotto (Monaco) from 2007 to 2019.

3.3. Relations between environmental factors and *Ostreopsis cf. ovata* blooms

The two first axis forming the first factorial space of the PCA performed on environmental factors explain more than 55% of the total inertia of projected variables (Figure 26). This indicates a relatively good representation of the relationship between the different environmental parameters (SST, water salinity, wind speed, pH and rain). The first factorial axis of the PCA (29.34 % of the total inertia) is mainly explained by rainfall (34.60 %) and SST (30.66 %) whereas the second factorial axis (26.12 % of the total inertia) is constructed by salinity (45.84 %) and wind speed (41.47 %). Unfortunately, the relations of cell abundance and net growth rate (here represented as supplementary variables in the first factorial space) with environmental parameters were not clear and no direct correlations were found. Indeed,

benthic cell abundance and growth rates were not well represented in the factorial space described by the combination of environmental factors which could not explain the variations among these phenological features observed during monitoring.

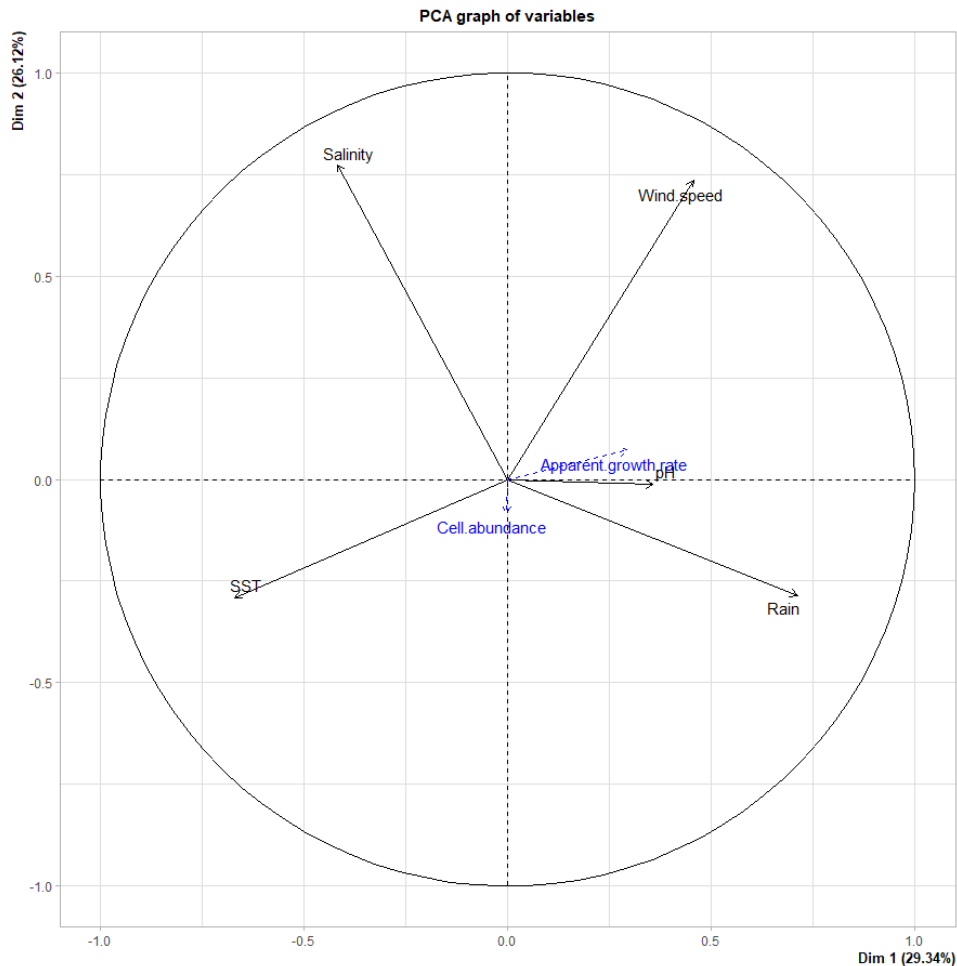


Figure 26: Representation of environmental parameters on the first factorial space of the Principal Component Analysis (PCA). Variables used to create the factorial space were SST, Salinity, Rain, Wind speed and pH. Cell abundance and apparent (=net) growth rate (blue arrows) were added as supplementary variables to the analysis.

Throughout the entire time series, the presence of *Ostreopsis cf. ovata* benthic cells was observed when sea surface temperatures (SST) ranged from 18.2°C to 27.9°C, with an average of $24.3 \pm 1.8^\circ\text{C}$. The mean SST during blooming periods (i.e. when benthic cell abundance was superior to 200 000 cells g^{-1} FW) was $25 \pm 1.5^\circ\text{C}$, while minimum and maximum for each year were in average respectively $24 \pm 1.5^\circ\text{C}$ and $25.2 \pm 1.7^\circ\text{C}$. Average SST when cell densities first exceeded the threshold of 200 000 cells g^{-1} FW (i.e. the beginning of a bloom) was $24.9 \pm 1.4^\circ\text{C}$.

Positive net growth rates (NGR) were recorded between 18.2°C and 27.9°C. A negative correlation was found between temperature and NGR (Spearman, $N = 133$, $r_s = -0.27$, p -value < 0.01), but the relation was not linear and highest positive NGR were not associated with maximal temperature records (Figure 27). The maximum NGR (0.53) occurred at a temperature of 23.9°C. These variations are confirmed by the local regression “loess” model, defining the optimal growth temperature as 23.5°C. When considering each maximum NGR per year, the window of optimum temperature could be estimated between 21.0°C and 24.7°C (Figure 27).

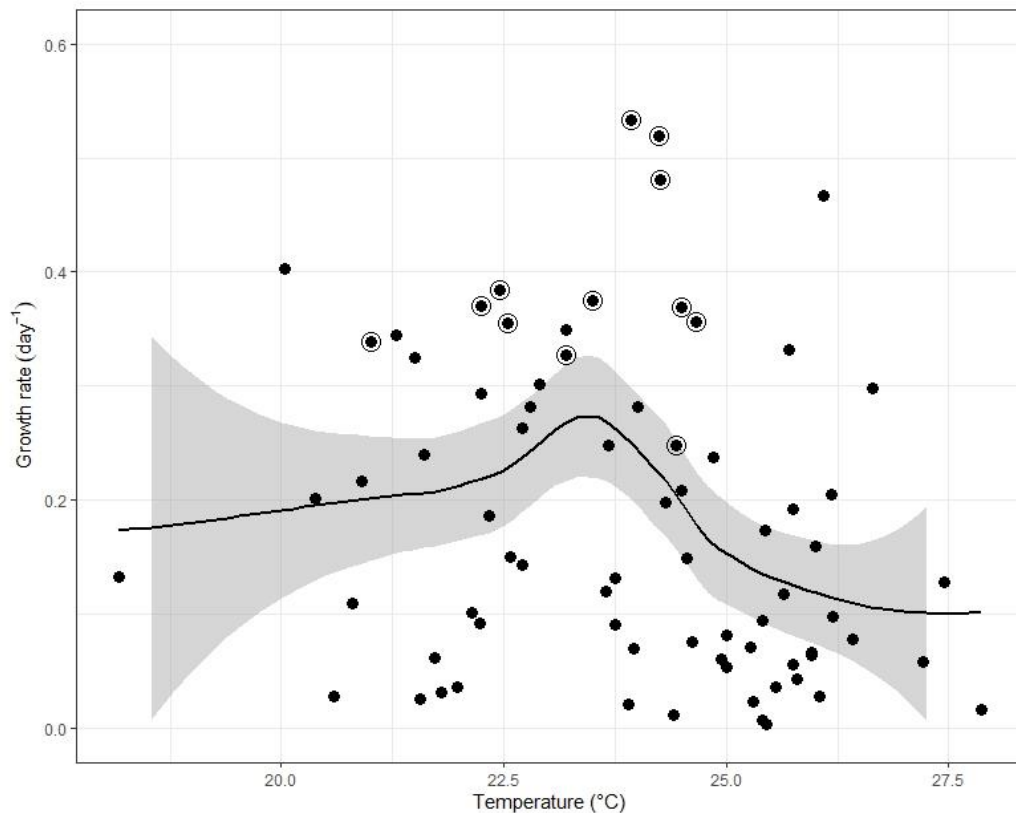


Figure 27: Variation of positive growth rates with sea surface temperatures. Circled dots represent the highest positive growth rate value for each year of survey. Black line is the loess regression model with 95% confident interval in grey.

Comparison of summer SST values from Larvotto with values from Villefranche Bay showed no significant differences (Wilcoxon signed-rank test: $N = 125$, $W = 3869$, p -value = 0.645). This allows the use of SST values recorded in Villefranche Bay as a proxy for SST values for Monaco area. Using data collected weekly in this Bay, it was also possible to describe the annual general hydroclimate of the area. From 2007 to 2019, the mean annual SST was 18.8°C (± 4.3), while the mean of lowest annual values was 13.4°C (± 0.4) and 26.3°C (± 0.9) for the

mean of highest annual temperatures. Cumulative sums of SST anomalies represented the comparison of heat accumulation in surface waters between years of survey (Figure 28). Years with higher values of SST anomalies were considered warmer, while years with lowest values of SST anomalies were considered colder. Interannual differences of the sum of SST anomalies were also found at seasonal and monthly scales, separating years with a warmer spring season from years with a colder one. Mean cumulative sum of SST anomalies by month prior to the bloom period showed strong positive correlations with bloom timing in April, May, June and spring season (mean of April-May-June). Amongst the different phenological features of interest previously detailed, strong positive correlations were found only between bloom timing and positive anomalies of SST (Figure 29A). Blooms of *Ostreopsis cf. ovata* tend to start earlier when SST anomalies are higher. These were observed in monthly averaged SST anomalies for April, May, June and the whole spring season (April-May-June averaged), but not for earlier months (Figure 29B). No other significant correlations were observed between SST anomalies and both duration and maximum abundance of the bloom.

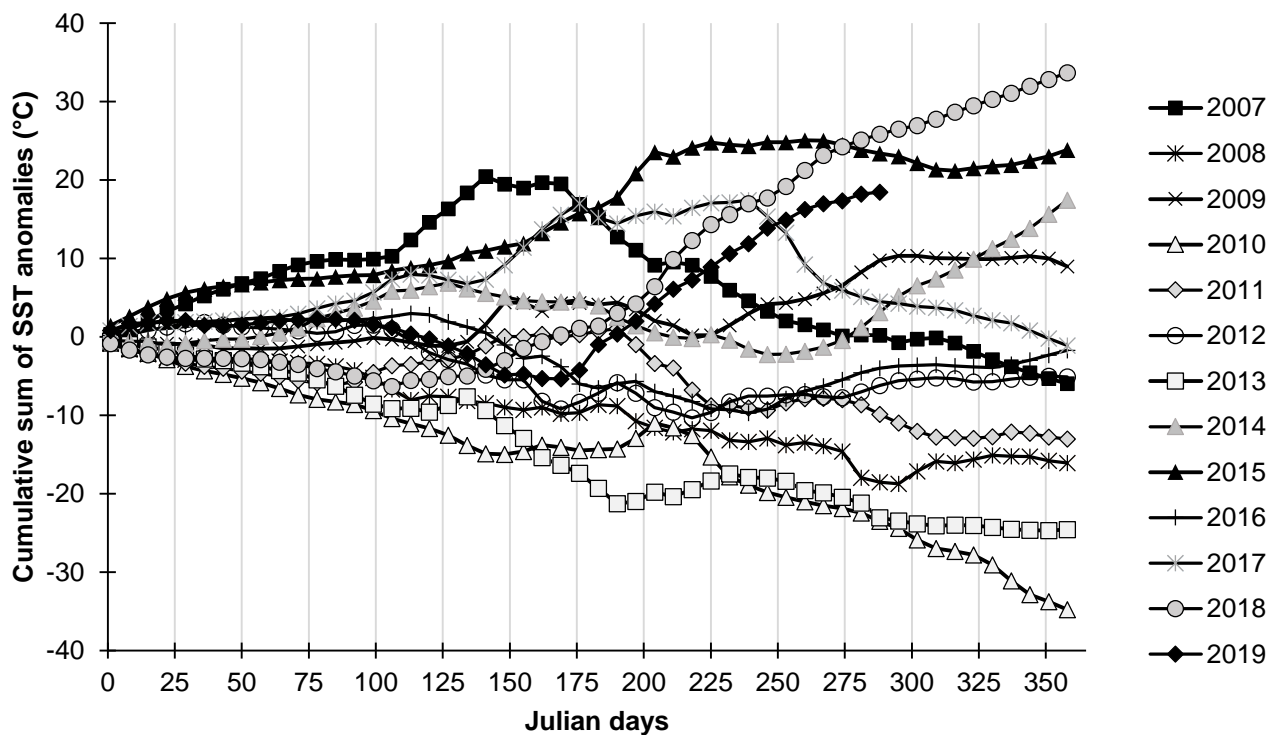


Figure 28: Weekly cumulative sum of SST anomalies in Point B (Villefranche-sur-Mer) corresponding to each year of survey.

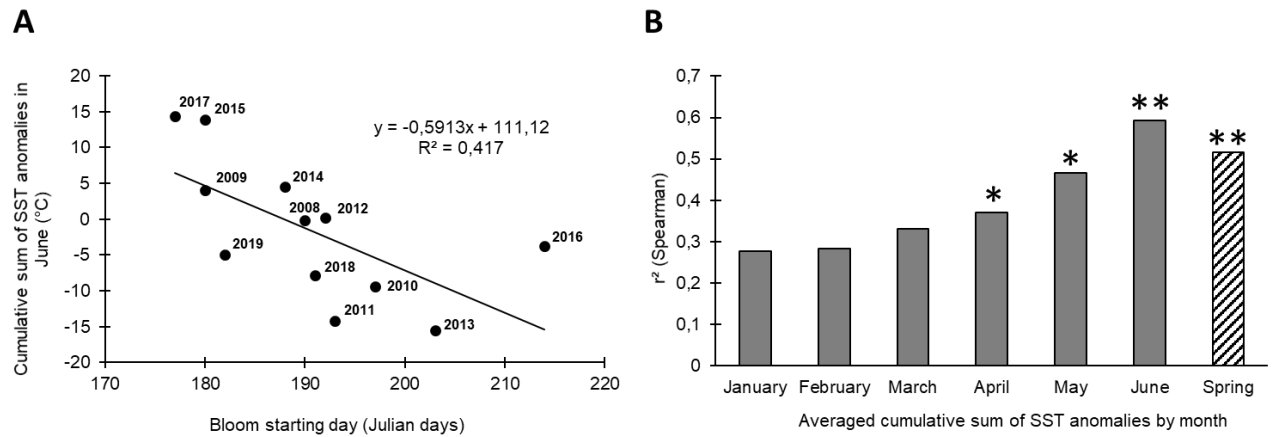


Figure 29: Correlation between bloom starting day and cumulative sum of SST anomalies: (A) Example of cumulative sum of SST anomalies averaged for June compared to bloom starting days for each year of the survey (2007 excluded). (B) Strength of correlations represented by the Spearman r_s^2 for each averaged month and spring season (*: $p < 0.05$; **: $p < 0.01$).

4. Discussion

Understanding the relation between environmental parameters and phenology of *Ostreopsis cf. ovata* in NW Mediterranean Sea could be of great help for manager if it can predict timing, and/or duration or intensity of blooms. The majority of studies focusing on *Ostreopsis cf. ovata*'s phenology in the Mediterranean Sea are generally based on the observation of a single bloom event (Accoroni *et al.*, 2011; Battocchi *et al.*, 2010; Illoul *et al.*, 2012; Totti *et al.*, 2010; Vila *et al.*, 2001) and does not allow the study of interannual variability. Another force of the present study is the measure of the *in situ* thermal niche, which is of great interest not only for managers of HABs, but also for modeler in the context of global change and especially the warming of seawater.

Focused was made on benthic cells. In our study, a strong correlation between epiphytic and planktonic cells was found. This correlation is similar to those already found by Mangialajo *et al.* (2008) in Genoa (Italy), Cochu *et al.* (2011) in Monaco and Jauzein *et al.* (2018) in Villefranche-sur-Mer (France), all in NW Mediterranean regions. Epiphytic cells of *Ostreopsis cf. ovata* constitute the main stock of the population, potentially involved in skin irritations by contact. Planktonic cells are mainly issued from the resuspension of benthic cells (Totti *et al.*, 2010) and could be linked to intoxications by inhalation, which would be

promoted by the transport of toxic compounds in aerosols caused by winds (Ciminiello *et al.*, 2014).

As many other studies (Carnicer *et al.*, 2015; Accoroni and Totti, 2016), relation between benthic *Ostreopsis cf. ovata* development and *in situ* ecological parameters are difficult to highlight, even from multi-parametric approaches. Two main results emerge from the analysis of the long-term dataset: highest temperatures were not associated with highest growth rates of *O. cf. ovata* and spring SST are driving the timing of the bloom.

4.1. Net growth rate

Highest positive net growth rates of benthic *Ostreopsis cf. ovata* during bloom episodes were not observed when highest temperatures were recorded. This indicates that relatively high temperatures are not optimal for *Ostreopsis* growth, at least in Monaco. Instead, the Loess regression model indicates a moderate temperature (23.5°C) to be the most suitable for *Ostreopsis cf. ovata* growth. Numerous *in situ* studies have tried to understand the link between sea surface temperature and the bloom development, often resulting in different observations depending on the studied areas (Accoroni and Totti, 2016). For instance, Mangialajo *et al.* (2008) and Aligizaki and Nikolaidis (2006) observed highest abundances at temperatures exceeding 26°C respectively in Ligurian Sea and in Greece, whereas (Totti *et al.*, 2010) and (Accoroni *et al.*, 2011) recorded highest abundances when temperatures were respectively below 22°C and 23°C in Northern Adriatic Sea. In most cases, these studies were focused on the cell abundances and did not find any direct correlation between sea surface temperature and cell abundances (Cohu *et al.*, 2011; Totti *et al.*, 2010; Vila *et al.*, 2001). Based on growth rate study, our observations defined a narrower range of optimum temperature for *Ostreopsis cf. ovata* growth in NW Mediterranean Sea, estimated between 21.0°C and 24.7°C, using each maximum net growth rate for each bloom event. Laboratory studies observed different temperature niches based on growth rate depending on the strain and the area studied. Pezzolesi *et al.* (2012) showed that strains from Tyrrhenian Sea exhibited maximum growth rate at 20°C, whereas Granéli *et al.* (2011) showed an optimal temperature of 30°C for strains isolated from Adriatic Sea. Given the differences found in *Ostreopsis cf. ovata* temperature niche between locations and strains across Mediterranean Sea, effects of

temperature on bloom development are likely to vary depending on local environmental parameters and strains.

Although it is known that sea temperature is a determinant factor for cells development of benthic dinoflagellates (Pistocchi *et al.*, 2011; Parsons *et al.*, 2012), observations of *Ostreopsis cf. ovata* abundances and growth rates during blooms are sometimes also related to other environmental factors (Carnicer *et al.*, 2015; Accoroni and Totti, 2016). It is admitted that blooms of *Ostreopsis cf. ovata* are generally favored by low levels of turbulence as highest abundances are recorded in sheltered sites, even though blooms also occur in exposed sites (Barone, 2007; Totti *et al.*, 2010; Accoroni and Totti, 2016). However, ACP analysis in the present study was not able to demonstrate any relationship between *Ostreopsis cf. ovata* abundances or net growth rate and available parameters, such as wind speed, rain, pH and salinity.

4.2. Variations in bloom phenology

Monitoring of *O. cf. ovata* blooms in Monaco during 13 consecutive summer seasons revealed strong variations in phenological features across the different years. Those variations in bloom timing, bloom length and bloom intensity reflect the complexity behind *Ostreopsis cf. ovata* bloom formation and how interactions between environmental factors would shape bloom events. Despite all of the analyses done in the present study, no relation was found between ecological parameters and bloom length or intensity. For the bloom initiation, a strong positive correlation was found between SST anomalies accumulation and the bloom starting day. This relation indicates that *O. cf. ovata* blooms are likely to occur earlier when spring temperatures are warmer. This correlation is even stronger and more significant when approaching summer season: Starting from April, temperatures anomalies can be summed to predict the most likely period for epiphytic abundances of *Ostreopsis cf. ovata* to reach the 200 000 cells g⁻¹ FW threshold. However, variations in SST in May and June have a stronger impact on the bloom timing and may reject the prediction from April. The accuracy of the prediction gets stronger when approaching the bloom event.

The reasons behind the occurrence of an early bloom could be linked either to an early germination of resting cysts or to higher growth rates favored by optimal environmental conditions, as well as both conditions occurring simultaneously. For instance, Lau *et al.* (2017)

showed that the initiation of an intense bloom of *Alexandrium minutum* in northeast of Peninsular Malaysian waters was linked to an active excystment process. Studies on the effects of environmental factors on cysts germination in dinoflagellates revealed the importance of several parameters such as light exposure (Anderson *et al.*, 1987; Moore *et al.*, 2015), nutrient availability (Bravo and Anderson, 1994; Binder and Anderson, 2004) and a mandatory period of dark-cold exposure (Montresor and Lewis, 2006). Temperature also appears to be one of the main factors favoring excystment of resting dinoflagellate cells (Anderson, 1980; Bravo and Anderson, 1994; Moore *et al.*, 2015; Ní Rathaille and Raine, 2011) as dinoflagellate cysts need sea temperature to reach a threshold in order to induce excystment. For *O. cf. ovata* blooms in the Mediterranean Sea, the excystment does not seem to be triggered only by the reach of such specific temperature threshold, as no relationship between the sea temperature and the bloom starting date was found. Instead, using the sum of temperature anomalies, effects of heat accumulation in surface waters and time of exposure to an optimal temperature threshold were revealed. Accoroni *et al.* (2015a) suggest both temperature and Nitrate/Phosphate ratio could be responsible for initiating cell excystment. Another study by Accoroni *et al.* (2014) indicates that cysts of *Ostreopsis cf. ovata* from Tyrrhenian Sea needed a temperature of 25°C to germinate. However, blooms occurring in Tyrrhenian Sea are observed from late summer to beginning of fall, after highest temperatures are recorded. In Monaco, blooms of *O. cf. ovata* start before maximum temperatures are reached.

Length and intensity of *O. cf. ovata* bloom events also varied across years. The highest epiphytic cell abundance observed reached more than 1 500 000 cells g⁻¹ FW (in 2007) while the lowest maximum abundance barely exceeded 200 000 cells g⁻¹ FW (in 2013), lasting from less than a week (only one sampling day, in 2009 and 2015) up to 4 weeks (5 consecutive sampling days, in 2011). Both length and intensity can be limited by factors such as strong hydrodynamics, nutrient depletion and temperature decrease. Other parameters could favor long lasting and intense dinoflagellate's bloom. Garces (2002) suggested that temporary cyst would act as population stock in blooms of *Alexandrium taylori*. Following this, a slow and wide excystment process in the temporary cyst stock would continuously provide new vegetative cells to the blooming population, thus allowing for a longer lasting bloom episode. Intense blooms could be the result of the combination between a high production of biomass

due to favoring growth conditions and a low rate of cell loss controlled by biotic (grazers, parasites) and abiotic (hydrodynamics) factors.

Finally, variations in *Ostreopsis cf. ovata* bloom phenology could also be linked to the high variability of abundance within a low spatial scale. This was already observed by Cohu *et al.* (2011), who conducted the first two years of our monitoring program in this area. Those low spatial scale variations amongst our 5 sites in Monaco may be explained by the spatial and temporal evolution of the diversity of biotic substrates (Totti *et al.*, 2010), as well as local hydrodynamic conditions.

4.3. Spring SST anomalies: a tool for managers and modelers

The correlation between spring temperature anomalies and bloom timing represents a useful tool for the prediction of future HABs of *Ostreopsis cf. ovata* in Monaco area. Using SST data acquired during the beginning of the year allow to predict a temporal window for the start of a bloom event and thus can guide the monitoring effort (see mathematical relation in Figure 8A). This prediction would noticeably help sanitary authorities in charge of toxic outbreaks in their management strategies facing this seasonal HAB.

This relation may also be important for modelers. Modeling the dynamics of coastal HABs is crucial, especially in a context of climate change where HABs occurrences seem to be increasing and expanding. Many authors are trying to understand and to predict blooms of toxic dinoflagellates (Maguire *et al.*, 2016; Moita *et al.*, 2016; Ruiz-Villarreal *et al.*, 2016) and toxic cyanobacteria (Hamilton *et al.*, 2009; Rigosi *et al.*, 2015) using models based on ecological observations. For now, the accuracy in modelling and predicting such complex phenological events seems to be limited by the lack of data collected both on the species itself and on the environmental factors, and also by the numerous unanswered questions about *Ostreopsis cf. ovata* ecology (Asnaghi *et al.*, 2012). While our study provides data on the realized temperature niche of *Ostreopsis cf. ovata* in NW Mediterranean Sea and the links between its bloom phenology and spring SST temperatures, more models of *Ostreopsis cf. ovata* blooms need to be designed, improved and adapted to local environmental variables in order to minimize risks of exposition.

Concerning the impact of climate change, many studies consider global warming as potential factors for benthic HABs species to expand their geographic ranges, seasonal occurrences and bloom windows (Wells *et al.*, 2015; Gobler *et al.*, 2017; Nakada *et al.*, 2018;

Gobler, 2020; Tester *et al.*, 2020). In Mediterranean Sea, the projection foresees SST to increase, on average, by 0.45°C per decade by 2050 (Alexander *et al.*, 2018). As *Ostreopsis cf. ovata* already develop in the north parts of the NW Mediterranean Sea, an extension toward the north is not possible. Moreover, an increase in SST temperature may be noxious to *O. cf. ovata* development, since higher temperature are not associated to the highest growth rate in the present study. On the other hand, global warming would favor an earlier bloom, as optimal temperature conditions would be reached earlier. Global warming is not the only consequence of global change and a more specific study between *Ostreopsis cf. ovata* and future ecological conditions in the Mediterranean Sea is needed.

5. Conclusion

The phenology of *O. cf. ovata* blooms seems to be governed by a complex assemblage of environmental factors and is still lacking of knowledge on how these interact and affect the progress of the bloom. Although no clear pattern was identified concerning the bloom pattern in relation with a combination of environmental parameters, sea temperature was identified as a crucial hydroclimatic factor. The optimal temperature growth favoring blooms of *O. cf. ovata* in Monaco has been observed to be ranging from 21°C to 25°C. In addition, this study revealed a clear relation between spring temperatures and the timing of the bloom of *O. cf. ovata* in NW Mediterranean. Using one of the longest time series of *O. cf. ovata* blooms, it is suggested that the accumulation of positive sea surface temperature anomalies induces an early start of the bloom onset. This confirms the previous observations made by Cochu *et al.* (2011) who suggested an important impact of spring sea surface temperature anomalies on the period of bloom appearance. Considering the increasing sea temperature trend observed worldwide due to global changes, these results could be of a great interest for the prediction and management of *O. cf. ovata* blooms in recreational waters. The pursuit of data collection of this time series is needed to improve the present knowledge and to further investigate the effect of combined environmental parameters on *O. cf. ovata* bloom phenology.

Acknowledgement

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Authors' contribution

R.L. and V. D-S designed and established the monitoring program at the Larvotto Beach (Monaco). R.L., C.J., A-S.P., S.M. and other volunteers were involved in the sampling and counting analyses of collected samples. K.D. and S.G. processed to the data analysis. K.D. analyzed the results and prepared the figures. K.D. and R.L. wrote the manuscript.

Chapitre 2

Suivi de l'expansion du dinoflagellé benthique nuisible *Ostreopsis* cf. *siamensis* dans le Golfe de Gascogne en Atlantique Nord-Est

Contexte de l'étude

Le long des côtes Atlantiques de la Péninsule Ibérique, la présence des deux espèces *O. cf. ovata* et *O. cf. siamensis* a été observée le long des côtes portugaises, mais seule *O. cf. siamensis* a été observée formant des efflorescences, et ce uniquement dans la zone sud-est du Golfe de Gascogne, en Mer Cantabrique (Laza-Martinez *et al.*, 2011; David *et al.*, 2013; Santos *et al.*, 2019). Cette zone, à défaut d'étude ayant été conduite dans la partie centrale et nord du Golfe, est pour le moment considérée comme la limite Nord de répartition d'*O. cf. siamensis* sur les côtes Atlantiques Européennes. Une relation entre températures de surface et présence d'*O. cf. siamensis* a été suggérée par (David *et al.*, 2012), laissant penser que le développement de cette espèce ne pourrait que se produire dans des eaux suffisamment chaudes. Un tel lien entre températures et développement d'*O. cf. siamensis* pourrait faire écho au risque anticipé d'une augmentation des HABs benthiques à de plus hautes latitudes, en réponse au réchauffement global des océans (Tester *et al.*, 2020). Dans un tel schéma, le risque sanitaire lié à la présence d'espèces d'*Ostreopsis* pourrait potentiellement évoluer dans le futur, et ce en particulier dans le Golfe de Gascogne où un réchauffement des eaux associé aux changements climatiques a pu être mis en évidence (deCastro *et al.*, 2009; Costoya *et al.*, 2015). Le Golfe de Gascogne possède un gradient de température à la fois latitudinal et longitudinal. Il pourrait donc constituer une région témoin permettant d'observer l'expansion d'une espèce de microalgue toxique vers des eaux en cours de réchauffement.

A partir de cet état de l'art, notre étude a visé à établir la distribution d'*O. cf. siamensis* dans la totalité du Golfe de Gascogne. Pour cela, nous avons mené une campagne d'échantillonnage en août et septembre 2018 en sondant 40 sites distribués le long des côtes espagnoles et françaises du Golfe jusqu'à l'entrée de La Manche. A chaque site, des prélèvements benthiques classiques pour l'observation microscopique ont été couplés à l'analyse d'échantillons d'ADN environnementaux collectés grâce à l'utilisation, pour la première fois, de substrats artificiels. L'utilisation d'outils moléculaires a permis d'établir la présence d'*Ostreopsis* spp. même à l'état de trace dans les communautés. Cette campagne fut aussi l'occasion d'isoler des souches d'*O. cf. siamensis* à partir de plusieurs sites localisés le long des côtes espagnols. De plus, des échantillons de microplastiques provenant de suivis temporels situés en rade de Brest et à La Tremblade ont pu être utilisés. Ils ont permis d'évaluer la fenêtre temporelle de développement d'*Ostreopsis* spp. à ces sites, en apportant une approche temporelle à notre étude spatiale. Couplée à ces approches de terrain et de

génétique, une analyse de simulation de suivi de particules basée sur une approche de modélisation Lagrangienne a été conduite afin d'évaluer la connectivité physique entre différentes zones d'échantillonnage et de tenter d'expliquer les résultats de distribution observés.

Contribution des auteurs

Lors de cette étude, j'ai planifié la campagne d'échantillonnage et parcouru les côtes françaises du Golfe de Gascogne aux côtés de R. Siano et C. Jauzein en utilisant un camping-car comme laboratoire mobile. A. Laza-Martinez et S. Seoane (University of the Basque Country, Espagne) ont mené l'échantillonnage sur les côtes espagnoles et isolé les souches d'*O. cf. siamensis*. J'ai été en charge de l'observation microscopique et des analyses moléculaires (extractions de l'ADN environnemental, analyses PCR et rt-PCR) des échantillons en laboratoire. S. Hariri, M. Sourisseau et M. Plus (Ifremer Brest) ont été en charge de la partie modélisation tandis que D. Herviot-Heath (Ifremer Brest) et C. Lecadet (Ifremer La Tremblade) nous ont prodigué les échantillons associés aux microplastiques et ont supervisé leurs analyses. J'ai ensuite compilé et analysé les résultats ainsi que rédigé ce chapitre principalement sous la supervision de R. Siano et en collaboration avec mes deux autres co-cadrants C. Jauzein et R. Lemée. Après avoir pris en compte les avis et corrections de tous les co-auteurs, le manuscrit associé à ce travail a été soumis à la revue *Environmental Microbiology*. Il est actuellement en train d'être révisé par les rapporteurs sélectionnés par le journal. Dans la thèse, ce travail est présenté dans le même format que le manuscrit soumis à la revue.

Monitoring the expansion of the harmful benthic dinoflagellate *Ostreopsis cf. siamensis* towards the warming waters of the Bay of Biscay, North-East Atlantic

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Running title: *Ostreopsis* expansion monitoring

Abstract:

In a future scenario of increasing temperatures in North-Atlantic waters, the risk associated with the expansion of the harmful, benthic dinoflagellate *Ostreopsis cf. siamensis* has to be evaluated and monitored. Microscopy observations and spatio-temporal surveys of environmental DNA (eDNA) were associated with Lagrangian particle dispersal simulations to: i) establish the current colonization of the species in the Bay of Biscay, ii) assess the spatial connectivity among sampling zones that explain this distribution, and iii) identify sentinel zones to monitor future expansion. Throughout a sampling campaign carried out in August-September 2018, microscope analysis showed that the species develops in the south-east of the bay where optimal temperatures foster blooms. Real-time-PCR analyses revealed its presence across almost the whole bay to the western English Channel. An eDNA time-series collected on plastic samplers showed that the species occurs in the bay from April to September. Due to the water circulation, colonization of the whole bay from the southern blooming zones is explained by continuous inter-site connectivity. Key areas in the middle of the bay permit continuous dispersal connectivity towards the north. These key areas are proposed as sentinel zones to monitor *O. cf. siamensis* invasions towards the warming water of the North-East Atlantic.

Key-words: Climate change; Harmful Algal Blooms; Dinoflagellate; Invasive species; Environmental DNA; Real-time PCR; Lagrangian modeling; Risk management

1. Introduction

Climate change is considered to be a driver of marine species distribution (Perry, 2005; Thomas *et al.*, 2012). In a future scenario of seawater temperature variation, the expansion of harmful algae into new areas (Hallegraeff, 2010; Kudela and Gobler, 2012; McLeod *et al.*, 2012; Gobler, 2020; Tester *et al.*, 2020) and changes in their seasonal occurrences are expected (Moore *et al.*, 2008; Ralston *et al.*, 2014; Nakada *et al.*, 2018). Harmful algal blooms (HABs) refer to the development of noxious phytoplankton species at levels causing deleterious impacts on humans or marine biota. Risks to human health are generally associated with the consumption of contaminated seafood (Jeffery *et al.*, 2004; Etheridge, 2010) and direct toxin exposure to cells or aerosols (Cheng *et al.*, 2005). Fauna and flora mortality could be linked to toxin exposure, but also to hypoxia or anoxia due to bacterial degradation after the bloom (Pitcher and Probyn, 2011; Lim *et al.*, 2014). In recent decades, reports of HABs events have been increasing (Shears and Ross, 2009; Hallegraeff, 2010; Berdalet *et al.*, 2017b), raising awareness of sanitary, economic and ecological impacts. Discussion of the causes of these increases are ongoing; they could be linked to a combination of factors such as climate change (Moore *et al.*, 2008; Glibert *et al.*, 2014; Kibler *et al.*, 2015; Wells and Karlson, 2018; Tester *et al.*, 2020), anthropogenic eutrophication (Glibert *et al.*, 2005; Heisler *et al.*, 2008; Davidson *et al.*, 2014), ballast water dispersion (Hallegraeff and Bolch, 1991; Smayda, 2007) and the worldwide improvement in knowledge and monitoring of HABs (Van Dolah, 2000; Anderson, 2009; Anderson *et al.*, 2012). Given the key role played by temperature in the growth and distribution of marine phytoplankton (Eppley, 1972; Wells *et al.*, 2015), HAB geographic ranges are expected to expand in temperate zones (Paerl and Paul, 2012; Carnicer *et al.*, 2016; Gobler *et al.*, 2017). For instance, Kudela and Gobler (2012) witnessed the recent global expansion of *Cochlodinium* sp. while Vandersea *et al.* (2018) observed an increase in *Alexandrium catenella* blooms associated with positive sea surface temperature anomalies (>1-2 °C) in the Gulf of Alaska. The potential global expansion of HAB species due to climate change represents a threat to human health and marine ecosystems and risk assessment is essential for effective management (Berdalet *et al.*, 2017a; Brown *et al.*, 2019).

The benthic dinoflagellate genus *Ostreopsis* Schmidt (1901) is considered to be a case study regarding the impact of climate change on harmful algal species (Tester *et al.*, 2020). Its

expansion beyond the genus's traditional geographical range due to ocean warming is expected in temperate regions where specific habitat requirements are met (Tester *et al.*, 2020). Although *Ostreopsis* species have been detected in relatively cold waters, blooms of this genus have mostly been observed across the world in warm waters of tropical and temperate regions (Shears and Ross, 2009; Rhodes, 2011; Selina *et al.*, 2014), notably in the Mediterranean Sea (Mangialajo *et al.*, 2011; Blanfuné *et al.*, 2015; Accoroni and Totti, 2016). Toxic outbreaks resulting from some *Ostreopsis* species blooms affect human health through direct contact, inducing skin irritation (Tichadou *et al.*, 2010; Tubaro *et al.*, 2011), or through inhalation of aerosols, responsible for serious respiratory symptoms (Durando *et al.*, 2007; Vila *et al.*, 2016), presumably linked to the production of toxins belonging to the palytoxin group (Ciminiello *et al.*, 2006, 2012, 2014; Sechet *et al.*, 2012). While the genus is relatively easy to detect morphologically, with observation using light microscopy, identification at species level can be difficult because unambiguous morphological synapomorphies seem elusive. Eleven named *Ostreopsis* species have been described so far but the high degree of morphological variability within the same species (Parsons *et al.*, 2012; Chomérat *et al.*, 2019) makes genetic identification essential and complicates phylogeographical and species-specific studies (Penna *et al.*, 2010; Verma *et al.*, 2016; Zhang *et al.*, 2018; Chomérat *et al.*, 2020b). Analysis of the global distribution of *Ostreopsis* species is even more problematic considering that molecular clades have been associated with geographical distribution (Penna *et al.*, 2010, 2014; Sato *et al.*, 2011; Tawong *et al.*, 2014), highlighting a cryptic distributional pattern of *Ostreopsis* species around the world.

This genetic clade-specific distribution has been reported for the type species *Ostreopsis siamensis* Schmidt, which was first described in the tropical waters of the Gulf of Siam (Thailand) (Schmidt 1901), but never genetically characterized. Since then, isolates morphologically attributable to *O. siamensis* have been detected in tropical, sub-tropical and temperate areas in the Atlantic, Pacific and Indian Oceans (Faust *et al.*, 1996; Rhodes, 2011). The taxonomic classification "*O. cf. siamensis*" is conventionally used in the literature, although no sequences from this clade have been clustered with *O. siamensis* strains recently isolated in areas close to the Gulf of Siam (Tawong *et al.*, 2014; Chomérat *et al.*, 2019). The latitudinal distribution of the clade *O. cf. siamensis* across the world seems to be limited to between the 43rd and 44th parallel in the northern hemisphere, where it has been detected in the North-West Mediterranean Sea (Mangialajo *et al.*, 2008) and in North-East Atlantic (Laza-

Martinez *et al.*, 2011), and to the 41st parallel in the southern hemisphere in New-Zealand and Tasmania (Pearce *et al.*, 2001; Shears and Ross, 2009). In the North-East Atlantic, both *O. cf. ovata* and *O. cf. siamensis* have been observed along the south-western coasts of the Atlantic Iberian Peninsula (Amorim *et al.*, 2010; Santos *et al.*, 2019), but only *O. cf. siamensis* has been observed in the northern part of the coast line of the Cantabrian Sea, located in the south of the Bay of Biscay (Laza-Martinez *et al.*, 2011; David *et al.*, 2013; Seoane and Siano, 2018). To date, no survey of *Ostreopsis* species across the whole of the Bay of Biscay has been performed. This tide-affected bay, exposed to ocean warming (deCastro *et al.*, 2009; Costoya *et al.*, 2016), represents a key zone in monitoring potential *Ostreopsis* spp. expansion into warming waters in the future that could offer new ecological niches for *Ostreopsis* species. Moreover, this area offers favorable habitats for *Ostreopsis* spp. settlement and development, such as shallow and sheltered habitats where wide ramified macroalgae coverage serves as biotic substrate (Vila *et al.*, 2001; Totti *et al.*, 2010; Cohu *et al.*, 2013). The toxicity of *O. cf. siamensis* has been poorly investigated so far. Although toxicity bioassays have revealed a toxic effect in Mediterranean and Atlantic strains (Cagide *et al.*, 2009; Laza-Martinez *et al.*, 2011) potentially imputed to the production of palytoxin-like compounds, traces of these analogs have been exclusively found in South-East Australian strains (Ciminiello *et al.*, 2013; Verma *et al.*, 2016). Other non-identified allelopathic compounds could thus be responsible for *O. cf. siamensis* toxicity. Although *O. cf. siamensis* represents a lower threat to human health than *O. cf. ovata* (Pavaux *et al.*, 2020a), this species is still considered a potentially HAB risk species worldwide.

Monitoring of *Ostreopsis* spp. has generally been conducted through benthic sampling of macroalgae followed by light microscopy observation of epiphytic cells (Jauzein *et al.*, 2018). However, this method seems inadequate in areas where cells are not abundant enough to be easily detected. Molecular methods based on the analysis of environmental DNA (eDNA) are now used to detect the presence of rare species of microorganisms, especially for the conservation and monitoring of endangered species (Jerde *et al.*, 2011; Taberlet *et al.*, 2012; Thomsen and Willerslev, 2015; Barnes and Turner, 2016), but also for monitoring harmful algal species (Galluzzi *et al.*, 2004; Fitzpatrick *et al.*, 2010; Garvetto *et al.*, 2018; Huang *et al.*, 2019). Standard and quantitative real time PCR (rt-PCR) methods, based on the analysis of species-specific Internal Transcribed Spacer (ITS)-5.8S and Large Sub Unit (LSU) ribosomal DNA, have been developed and applied to the monitoring of *Ostreopsis* species (Penna *et al.*, 2007;

Battocchi *et al.*, 2010; Casabianca *et al.*, 2013, 2014). However, when dealing with eDNA-based monitoring approaches for species repartition assessment, the origins of intracellular DNA (DNA extracted from cells) or extracellular DNA (free DNA molecules derived from cell lysis) and its degree of persistence in marine environments are key issues that could complicate the interpretation of genetic surveys.

Ostreopsis species are tychoplanktonic microorganisms, which means that cells can be resuspended in the water column from their epiphyte substrates and hydrodynamically dispersed. The dispersal and introduction of planktonic species into new environments can then be achieved by water mass dynamics and currents (Coleman *et al.*, 2013). The advection of water parcels is responsible for the connectivity of different physical environments (Jönsson and Watson, 2016) and constrains the biogeographic distribution of planktonic species (Tremblay *et al.*, 2008; McManus and Woodson, 2012; Ginders *et al.*, 2016). Species dispersal areas can be investigated through particle transport modeling. In particular, the Lagrangian approach can be used to identify the temporal and spatial trajectories of planktonic organisms (van Sebille *et al.*, 2018). Therefore, this approach seems appropriate for the study of *Ostreopsis* spp. dispersal in its planktonic stage form.

In order to monitor the expansion of *Ostreopsis* spp. towards the potentially warming waters of the NE Atlantic, the first baseline distribution survey of *Ostreopsis* spp. in the Bay of Biscay was carried out to detect its presence, even under very low levels of abundance. Areas favorable to *O. cf. siamensis* development and areas likely to be colonized due to water mass advection were identified using a Lagrangian approach. The innovative association between eDNA monitoring and hydrological connectivity has enabled the identification of sentinel zones to monitor potential future expansion and development of the species in relation to climate changes.

2. Material and methods

2.1. Study area

The Bay of Biscay (North-East Atlantic Ocean) extends from the northwestern territories of the Iberian Peninsula (Galicia, Spain), covering the whole Cantabrian Sea, to the northwestern territories of Brittany (Bay of Brest, France). A total of 40 sites, along both the French and Spanish coasts of the Bay, were sampled from August to September 2018 (Table 1). The sampling temporal window and track direction (northward in France and eastward in Spain) were chosen in order to sample waters during the warmest period of the year. French sites and Spanish sites were sampled in parallel. Spanish sites were sampled in one week starting from Hondarribia to Comillas (Figure 30); whereas French sites were sampled over four weeks from Hendaye to Roscoff (Figure 30), using a camping van as a mobile laboratory for rapid sample processing.

Prior to sampling, the benthic facies and habitats of the studied area were carefully examined using satellite images to select sites maximizing the chances of observation and collection of *Ostreopsis* spp. cells. The environmental features identified as potential sites for the development of *Ostreopsis* spp. were sheltered habitats providing low exposure to wave action and the presence of rocky substrates that support macroalgal settlement. Several sites that did not strictly meet these criteria (beach sites located in French Aquitaine) were also sampled in order to maintain distances of less than 100 km between the sampling sites. To ensure the consistency of inter-site sample comparisons, and because little is known about *Ostreopsis* spp. resistance to air exposure at low tide, samples were collected in the lower intertidal zone (sub-mesolittoral zone) at low tide. The sub-mesolittoral zone is generally (80% of the time) immersed, even during low tides. In North Atlantic waters, this zone is characterized by the presence of distinctive species of macroalgae, such as *Fucus serratus* Linnaeus or *Ascophyllum nodosum* Le Jolis in colder waters and *Gelidium corneum* Lamouroux or *Halopteris scoparia* Sauvageau in warmer waters. These seaweeds were used as landmarks of the lower intertidal zone at low tides during sampling.

Site ID	Country	Site locality	Coordinates	Sampling date	Habitat type	Wave exposure	Temperature (°C)	Salinity (PSU)	Duration of artificial substrate exposure (h)
1	Spain	Comillas	43.391687°N -4.288888°E	28/08/2018	Rocky substrate/sandy bottom	Low	22.5	35.0	24.3
2	Spain	Santander	43.469985°N -3.727045°E	28/08/2018	Rocky substrate	Medium	21.1	34.8	24.5
3	Spain	Castro	43.375686°N -3.212714°E	30/08/2018	Rocky substrate / sandy bottom	Low	22.3	34.8	24.6
4	Spain	Zierbena	43.353911°N -3.078913°E	30/08/2018	Rocky substrate / sandy bottom	Sheltered	22.6	34.8	24.7
5	Spain	Lekeitio	43.363350°N -2.498764°E	30/08/2018	Rocky substrate/sandy bottom	Medium	23.5	32.0	24.6
6	Spain	Mutriku	43.309862°N -2.381035°E	30/08/2018	Rocky substrate	Sheltered	25.9	34.6	24.5
7	Spain	San Sebastian	43.319686°N -2.004416°E	28/08/2018	Rocky substrate / sandy bottom	Sheltered	24.9	34.8	24.0
8	Spain	Hondarribia	43.391588°N -1.790892°E	28/08/2018	Rocky substrate	Medium	23.7	34.2	24.3
9	France	Hendaye	43.380289°N -1.756551°E	28/08/2018	Rocky substrate	Medium	23.5	33.7	NA
10	France	Plage Abbadia	43.381051°N -1.733057°E	28/08/2018	Rocky substrate	Low	24.7	28.4	NA
11	France	Saint-Jean-de-Luz Ouest	43.396656°N -1.664600°E	27/08/2018	Rocky substrate / sandy bottom	Low	23.3	32.5	26.8
12	France	Saint-Jean-de-Luz Est	43.398342°N -1.664600°E	27/08/2018	Rocky substrate	Medium	24.8	33.3	26.2
13	France	Biarritz	43.493184°N -1.549430°E	30/08/2018	Rocky substrate / sandy bottom	High	23.0	34.0	24.8
14	France	Bayonne	43.527494°N -1.524644°E	30/08/2018	Rocky substrate / sandy bottom	High	23.3	32.8	24.9
15	France	Capbreton	43.648780°N -1.446800°E	30/08/2018	Rocky substrate / sandy bottom	High	23.7	34.3	24.9
16	France	Vieux-Boucau	43.785063°N -1.413639°E	01/09/2018	Estuary	Low	23.8	6.8	24.7
17	France	Contis-les-bains	44.089298°N -1.325365°E	01/09/2018	Estuary	High	18.8	3.0	23.5
18	France	Cap Ferret	44.630290°N -1.243460°E	03/09/2018	Rocky substrate	High	21.0	34.0	24.5
19	France	Le Tottoral	45.482604°N -1.152316°E	03/09/2018	Sandy	Sheltered	20.0	36.0	24.0
20	France	Fouras	46.003350°N -1.121211°E	05/09/2018	Sandy	Medium	25.7	37.0	23.8
21	France	Ile de Ré (Fort du Groin)	46.231126°N -1.411441°E	05/09/2018	Rocky substrate / sandy bottom	Medium	21.1	35.8	24.3
22	France	Jard-sur-Mer	46.408523°N -1.577400°E	08/09/2018	Rocky substrate / muddy bottom	Medium	19.3	34.6	25.5
23	France	Les Sables d'Olonne	46.488590°N -1.799913°E	08/09/2018	Rocky substrate / muddy bottom	Medium	16.1	35.2	25.5
24	France	Le Prégneau	46.628567°N -1.878315°E	08/09/2018	Rocky substrate / muddy bottom	Medium	15.5	35.0	25.6
25	France	La Caillaudière	46.692708°N -1.968054°E	08/09/2018	Rocky substrate / muddy bottom	Medium	14.0	35.1	25.5
26	France	Noirmoutier	47.026724°N -2.248401°E	10/09/2018	Rocky substrate / sandy bottom	Low	18.4	34.5	24.8
27	France	Pornic	47.101201°N -2.078190°E	10/09/2018	Sandy	Medium	20.6	34.9	24.8
28	France	Pointe St Gildas	47.131156°N -2.232927°E	10/09/2018	Rocky substrate	Low	20.5	34.5	24.9
29	France	Lérat	47.364712°N -2.531667°E	12/09/2018	Rocky substrate / sandy bottom	Low	17.5	35.0	24.0
30	France	Ile du Béchét	47.451917°N -2.495008°E	12/09/2018	Rocky substrate	Low	20.7	35.2	23.3
31	France	Damgan	47.515778°N -2.577584°E	12/09/2018	Rocky substrate / muddy bottom	Low	20.0	24.8	23.4
32	France	Lorient	47.698625°N -3.441273°E	14/09/2018	Rocky substrate	Sheltered	18.5	35.2	23.7
33	France	Concarneau	47.860375°N -3.914161°E	14/09/2018	Rocky substrate / sandy bottom	Medium	18.1	34.9	24.0
34	France	Douarnenez-Tréboul	48.102643°N -4.348145°E	16/09/2018	Rocky substrate / sandy bottom	Low	16.5	35.4	24.2
35	France	Crozon-Raguenez	48.228184°N -4.433185°E	16/09/2018	Rocky substrate / sandy bottom	Low	17.6	35.3	24.2
36	France	Roscanvel	48.312875°N -4.546529°E	16/09/2018	Rocky substrate	Low	17.3	35.3	24.2
37	France	Plougonvelin	48.348803°N -4.699510°E	18/09/2018	Rocky substrate	Medium	16.3	35.2	24.9
38	France	St Anne	48.361506°N -4.551664°E	18/09/2018	Rocky substrate	Sheltered	17.5	35.8	24.7
39	France	Plougarnec	48.626055°N -4.511130°E	20/09/2018	Rocky substrate	Medium	15.0	31.5	24.0
40	France	Roscoff	48.728051°N -3.986287°E	20/09/2018	Rocky substrate	Low	16.8	35.4	24.1

Table 1: Sampling site information and environmental data collected during the sampling campaign.



Figure 30: Map of the study area and sampling sites along the Bay of Biscay during the 2018 sampling campaign. Black points represent the 40 sites sampled during the campaign. Red frames represent the areas used for connectivity analyses. Orange squares represent the two sites used for plastic polymer temporal sampling.

2.2. Field sampling of epiphytic microalgal cells and eDNA

Three macroalgal samples with surrounding water were collected per site at a depth of about 0.1-0.5 m, using 250 ml plastic flasks. After shaking the bottle, 15 ml of each sample was subsampled and used for observation of living cells, within a few hours of sampling, with a portable light microscope (Bresser® MicroSet, 10x40). Some *Ostreopsis* cells were isolated from these subsamples to start the establishment of monoclonal cultures. Macroalgae were drained, placed onto a piece of tin foil to measure the fresh weight, and photographed for taxonomic identification. The remaining water was fixed with acid Lugol's solution (2% vol/vol) and stored in dark glass bottles until the end of the sampling period.

To complete the conventional *Ostreopsis* spp. sampling of macroalgae, an eDNA approach was adopted, modifying the artificial substrate method developed by Jauzein *et al.* (2016) to our study and sampling strategy. eDNA was collected using 2 mm mesh nylon screens, an innovation applied in this study to enhance the possibility of collecting rare DNA traces of *Ostreopsis* spp. in the environment during substrate immersion. Artificial substrates were placed in the sub-mesolittoral zone of the selected sites at low tide and recovered after two tidal cycles (approximately 24 h) (Table 1). At each station three frames, corresponding to three replicates, were anchored to a breeze block. During recovery the frames were carefully removed from the water and the screens were detached and immediately placed into 250 ml plastic flasks filled with seawater that had been collected locally and previously filtered through 0.22 µm nitrocellulose filters (Merck Millipore diameter 47 mm, Merck Group, Darmstadt, Germany). The plastic flasks were vigorously shaken in order to detach cells and particles from the screen and the water was then filtered through 3 µm polycarbonate filters. Filters were stored in 5 ml cryotubes and flash frozen in liquid nitrogen in a dry shipper container where they were kept until the end of the sampling campaign. At the completion of the campaign, samples were stored at -80 °C until molecular biology analysis. In order to avoid cross-station contamination, new screens were deployed at each station and all plastic frames and breeze blocks were decontaminated using bleach then rinsed thoroughly with sea water before being re-used. Due to adverse weather conditions during the first sampling day, screens could not be recovered at sites 9 and 10 (Hendaye and Plage Abbadia) and only one sample was recovered at site 8 (Hondarribia).

The eDNA spatial survey was supplemented with a temporal sampling strategy conducted at two reference stations to assess the temporal distribution of *Ostreopsis* spp. at these sites. A total of 118 eDNA samples collected during a monitoring program aimed at studying the structure and dynamics of microbial communities associated with plastic substrates were analyzed. Four different types of plastic polymers (Nylon [NY], Low Density Polyethylene [LDPE], Polypropylene [PP] and Polyethylene Terephthalate [PET]) were fixed into cages placed in the tidal zone of two sampling sites located in the Bay of Biscay: La Tremblade (La Floride: 45.80388° N; -1.16193° E; near site 20) and Brest (Pointe-du-Château: 48.33294° N; -4.32296° E; near sites 36 and 38). The plastic samples were collected after 15 days of deployment, from December 2017 to December 2018. Plastic substrates with attached biofilms were collected in 5 ml cryotubes, flash frozen in liquid nitrogen and stored at -80 °C until molecular biology analysis.

2.3. *Ostreopsis* spp. cell quantification

The Lugol-fixed samples of epiphytic cells collected from macroalgae were used to estimate benthic cell abundances of *Ostreopsis* spp.. One ml of seawater was analyzed in a Sedgewick Rafter Chamber using an inverted microscope (Axiovert 40 CFL, 10x40, Zeiss, Oberkochen, Germany). Cell abundances were estimated as the number of cells per gram of fresh weight of macroalgae (cells g/FW) in order to relate the abundance of epiphytic cells to macroalgal biomass.

2.4. DNA extraction and amplification from cultures and environmental samples

A total of 67 *Ostreopsis* spp. strains isolated from seven different sites during the sampling campaign were maintained in culture (K medium, 22 °C, 100 $\mu\text{E. m}^{-2} \text{ s}^{-1}$). Cultures were developed to produce biomass for accurate genetic species identification and for the calibration of genetic analysis. For DNA extraction, 10 mL of each culture was centrifuged (3000 g/10 min) and DNA was extracted from the cell pellet using the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Extracted genomic DNA

was eluted in a final volume of 100 µl of DNase-free water (Millipore, Merck Group, Darmstadt, Germany) and stored at -20 °C until molecular analysis.

Total DNA from the environmental sample filters was extracted using the lysis buffer-based NucleoSpin® Plant II DNA extraction kit (Macherey Nagel, Hoerd, France), following the manufacturer's instructions. Total genomic DNA was eluted in a final volume of 100 µl of DNase-free water, quantified using the Quant-iT™ PicoGreen® dsDNA assay kit (Invitrogen™, Waltham, MA, USA) through a spectrophotometer (BioTek® FLx800, Winooski, VT, USA) and stored at -20 °C until molecular analysis.

DNA from the biofilms that had developed on plastic polymer substrate samples collected during the temporal surveys was extracted with a chemical extraction method using Cetrimonium Bromide (CTAB) / Phenol-Chloroform-Isoamyl Alcohol (PCI). Plastic polymers were cut into small pieces and then incubated and agitated (300 rpm) at 60 °C for 210 min in 1200 µl of filtered CTAB extraction buffer solution (0.1 M Tris pH=8.0; 1.4 M NaCl; 20 mM EDTA pH=8.0; 2g CTAB; 20% SDS; 20 mg/ml proteinase K; β mercapto-ethanol). After incubation, the samples were centrifuged (10 min, 12000 g) and Phenol:Chloroform:Isoamyl Alcohol (25:24:1) was added (vol/vol) to the supernatant. Samples were then centrifuged (10 min, 12000 g) to recover the aqueous phase. DNA precipitation was obtained by adding 0.1 volume of sodium acetate (3 M pH=5.2) and 0.6 volume of isopropanol and storing samples overnight at -20 °C. DNA was recovered by centrifugation (30 min, 14000 g, 4 °C) and washed with 0.5 mL of 70% ethanol. After centrifugation (20 min, 14000 g, 4 °C), the DNA was dried and resuspended in 50 µl of DNase-free water and stored at -20 °C until molecular analysis.

The amplification of genomic DNA extracted from each of the 67 cultures was performed following the protocol described in Penna *et al.* (2007). Standard PCR using eukaryotic primers targeting the ITS1-5.8S-ITS2 region of the ribosomal DNA (rDNA) was performed using a peqSTAR® thermocycler (VWR™ Peqlab, Radnor, PA, USA) as follows: 50 µl of mixture containing 200 µM of each dNTP (Promega, Madison, WI, USA), 2 mM of MgCl₂, 0.5 µM of primer ITS1F and ITS4R, 1x GoTaq® Flexi Buffer reaction (Promega), 1.25 U of GoTaq® Polymerase (Promega) and 5 µl of template DNA. Thermal cycling conditions comprised 4 min at 94 °C for denaturation, followed by 30 cycles of hybridization (94 °C for 1 min, 50 °C for 2 min, 72 °C for 3 min), ending with 72 °C for 7 min for final elongation. Amplification products were purified using NucleoSpin® Gel and PCR Clean-up kit (Macherey Nagel, Hoerd, France) following the manufacturer's instruction. Amplified products were purified (QIAquick PCR

Purification Kit, Qiagen) and sent to GATC Biotech (www.eurofinsgenomics.eu/en/custom-dna-sequencing/gatc-services) for Sanger sequencing. After quality control, all sequences were analyzed on the National Center for Biotechnology Information website (<https://www.ncbi.nlm.nih.gov/>) using the tool Nucleotide Blast. For species identification sequences were edited using BioEdit v7.2.5 software (Hall, 1999) and multiple sequence alignment was achieved using the ClustalW program (Thompson *et al.*, 1994) with reference sequences obtained from the BLAST database (Altschul *et al.*, 1990) in GenBank and following recent phylogenetic studies of the genus *Ostreopsis* (Santos *et al.*, 2019).

2.5. Real time PCR of eDNA from environmental samples and plastic polymer substrates

The real-time PCR (rt-PCR) protocol described in Penna *et al.* (2007) was used to determine the presence of *Ostreopsis* spp. DNA in eDNA samples collected in the sampling campaign and the temporal surveys. Three sets of species-specific ITS-5.8 rDNA region primers were used to target *Ostreopsis* spp., *Ostreopsis cf. siamensis* and *Ostreopsis cf. ovata*. Rt-PCR was conducted using the Platinum[®] SYBR[®] Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, California, USA) and performed on a Stratagene Mx3000P (Agilent Technologies, Santa-Clara, California, USA) thermocycler in a final volume of 25 µl. The reaction mixture was composed of 20 µM of each primer, 25 µM of MgCl₂, 12.5 µl of SybrGreen Mastermix solution and 2 µl of DNA template. The rt-PCR assays were performed using 96-well plates containing genomic *Ostreopsis cf. siamensis* DNA dilutions (standard curve), eDNA extracts and both negative (DNase-free water) and positive (*O. cf. siamensis* and *O. cf. ovata* cultures) controls. All extracts were run in triplicate. Thermal cycling conditions consisted of 10 min at 95 °C for the initial denaturation step, followed by 40 cycles of hybridization (95 °C for 15 s, 60 °C for 15 s, 72 °C for 15 s) and ended with a final cycle of 95 °C for 1 min, 60 °C for 30 s and 95 °C for 30 s. Acquisition of the rt-PCR data and subsequent analysis were carried out using StepOne Software ver. 2.3 (Applied Biosystem, Carlsbad, California, USA). Standard curves were constructed for eDNA relative quantification using a 5-point tenfold dilution series (5.0 ng/µl to 5.0 x 10⁻⁴ ng/µl) using DNA extracted from a culture of *Ostreopsis cf. siamensis* Os-3E (GenBank accession number: MT952070) established from cells isolated in the Cantabrian Sea during a previous field study. The threshold cycle (Ct) for each sample reaction was

determined. Initial concentrations of *Ostreopsis* spp. DNA in environmental samples were estimated by interpolating each Ct value from samples using the slope of the standard curve and relative eDNA concentrations of *O. cf. siamensis* were calculated from standard curve analysis. Averaged values from the three replicates were used for ecological analysis. Relations between the mean eDNA concentration estimation and both benthic cell abundance and number of positive replicates per site were assessed using the non-parametric Spearman correlation test. Analyses were conducted in RStudio (R Core Team, 2019).

In order to verify the taxonomy of the amplification products, rt-PCR samples exhibiting a positive signal observed at the corresponding primer melting temperature of *O. cf. siamensis* control samples (79.8 °C) were sent for Sanger sequencing (Eurofins Genomics©). The eDNA amplification products from artificial substrates samples were identified to species level using reverse primers only (*Ostreopsis* R in Penna *et al.* (2007)), which amplifies the most discriminating part of the ITS1-5.8S- rDNA at the species level. Samples of the eDNA amplification products from the plastic polymer substrates were identified to species level using both forward and reverse primers (*Siamensis* F and *Ostreopsis* R in Penna *et al.* (2007)) in order to maximize the chances of obtaining the longest sequence possible. After quality control, these sequences were analyzed using the tool Nucleotide Blast (<http://www.ncbi.nlm.nih.gov>) (Supplementary Material 1).

2.6. Advection and connectivity among sampled areas

In our Lagrangian framework, the ocean circulation was generated with the state-of-the-art ocean general circulation model MARS3D (Lazure and Dumas, 2008) and two configurations (C4 and C1) with different resolutions and external forcing were considered, both covering the entire Bay of Biscay. Detailed descriptions of C4 and C1, including validation processes, can be found in Lazure *et al.* (2009) and in Charria *et al.* (2017), respectively. Briefly, C4 and C1 have, respectively, 30 and 100 vertical levels in generalized sigma coordinates and their horizontal resolutions are 4 and 1 km. The C4 simulation covers the period 1958-2014 with ERA40 and ERA40-Interim ECMFW reanalysis (Uppala *et al.*, 2005; Dee *et al.*, 2011). Boundary conditions were provided by ORCA25 (NEMO configuration) and tides by FES2004 (Lyard *et al.*, 2006) with a larger barotropic model. C1 obviously covers a smaller period (2003-2012), all boundary conditions were derived from the DRAKKAR global configuration ORCA12_L46_MJM88

(Molines *et al.*, 2014) and some sponge layers were used. Tidal data was provided in the same way as those for C4. No validated configuration was available for the sampling period (2018) since configuration set-up and *in situ* data processing have not yet been performed for that year. Existing validated simulations from 2010 to 2014 were thus chosen to catch a part of the inter-annual variability and to describe the average patterns of advection along the Bay of Biscay.

Connectivity between the 14 coastal areas (from A to N), including almost all the sites sampled during the campaign (Figure 30), was assessed using different Lagrangian indices. Particle trajectories were computed with ocean circulations and an offline Lagrangian mass-preserving scheme from Ichthyop software (V3.3, www.ichthyop.org, Lett *et al.*, 2008). Four Lagrangian indices based on the transit time (TR) between two areas were estimated: "mean", "minimum", "median" and "most frequent value" (the value that appeared most often). Correlations were calculated between the different Lagrangian indices and the mean TR was retained and used for subsequent analysis because this index exhibited the highest correlation with the other indices (>0.91, see Supplementary Material 2). In addition, mean TR correlations were also performed between the simulated years (2010 to 2014) to analyze the inter-annual variability over this five-year period. 2011 and 2013 appeared as the two years with the most different connectivity patterns and have been retained for the following analyses (Supplementary Material 3).

In order to cover the entire potential reproductive period (summer), particles were released daily (≈ 11000 particles per day at a random initial time of the day, for a total of 1.10^6 particles per area) during a restricted period of ninety days (June-August). Although positive relative cell buoyancy has been observed in nature (pelagic cells of *Ostreopsis* are mainly observed in surface layers in floating mucilaginous aggregates), it was considered as a first approximation that particles exhibited neutral buoyancy and were transported with water masses after their release in the surface layer from benthic substrates. Assuming that cell survival during water transport might be influenced by seawater temperature, two different particles datasets were used. The first scenario included all numerical trajectories without any biological filter and the second filtered trajectories which had not encountered a cold temperature threshold (fixed at 16 °C). Given the dearth of data regarding *O. cf. siamensis* thermal preferendum in temperate areas, this filter of 16 °C was based on data available for the well-studied *O. cf. ovata* as this species has been reported to form blooms in water

temperatures ranging from 17° C to 29° C (Accoroni and Totti, 2016; Tester *et al.*, 2020). Therefore, 16 °C was considered to be a reasonable non-optimal temperature for *O. cf. siamensis*.

3. Results

3.1. Light microscopy analysis of fixed samples

The presence of epiphytic *Ostreopsis* spp. cells was analyzed from macroalgae samples collected in the 40 sampling sites (Figure 30, Table 1) of the spatial survey carried on in the Bay of Biscay during August-September 2018. The species was recorded at 13 consecutive sampling sites from Comillas (Spain, Site 1) to Biarritz (France, Site 13) (Fig. 31, Fig. 32A). *Ostreopsis* spp. cells were found in sites characterized by rocky substrates and the presence of macroalgae, even in wave-exposed conditions (Biarritz). No *Ostreopsis* spp. cells were found north of Biarritz (from Site 14 to Site 20) in coastal zones corresponding to sandy ecosystems. In the middle (from Site 21 to Site 26) and in the north (From Site 28 to Site 40) of the Bay of Biscay, despite the presence of rocky substrates and extensive macroalgae coverage, no *Ostreopsis* cells were observed under light microscopy. In stations where *Ostreopsis* cells were observed, the maximum mean cell abundance was found in Hondarribia (Spain, Site 8) with $291\,073 \pm 37\,712$ cells/gFW while the lowest abundance was found in Biarritz with 9 ± 16 cells/gFW.

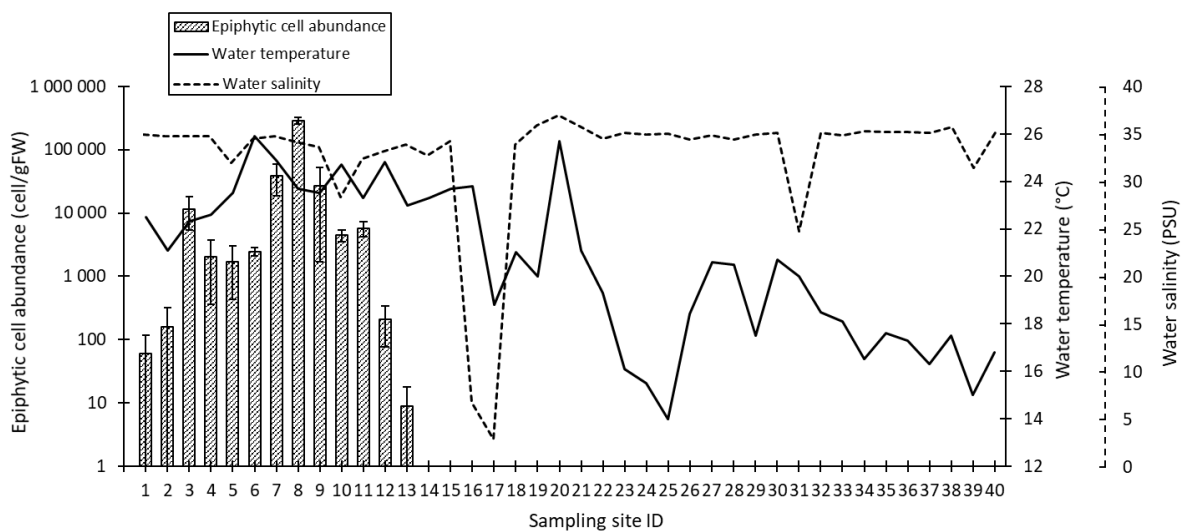


Figure 31: Abundances of epiphytic *Ostreopsis* spp. cells per gram of macroalgae fresh weight (cell/gFW) in each sampling site in relation to water temperature and salinity across the Bay of Biscay.

Ostreopsis spp. cell observations were coincident with sea temperatures ranging from 21.1 °C to 25.9 °C and salinities ranging from 28.4 to 34.8. Water salinities recorded in Sites 16 and 17 were extremely low (respectively 6.8 and 3.0) as these samples were collected in estuary habitats in order to verify the absence of cells in low salinity conditions.

In terms of biological substrates, taxonomic identification of macroalgae was successful for 81 of the 111 samples collected. The presence of epiphytic *Ostreopsis* spp. cells was confirmed on nine different genera of macroalgae. High levels of benthic abundances were recorded on *Gelidium* spp., *Centroceras* spp., *Halopteris* spp., *Cystoseira tamariscifolia* and *Corallina* spp. (> 15 000 cell/gFW). However, the relationship between cell abundances and type of macroalga could not be assessed since the macroalgae collected differed among sites.

3.2. Isolation of strains and species identification

Monoclonal cultures were obtained from sites where living *Ostreopsis* spp. cells could be observed in freshly collected benthic samples. In total, 67 monoclonal *Ostreopsis* spp. strains were established from seven Spanish sites (Table 2). Of these, 64 sequences from the amplified products were unambiguously assigned to *Ostreopsis cf. siamensis* (Table 2), exhibiting a percent identity greater than 97% with the highest similar sequence following Nucleotide Blast. The three remaining sequences were either not long enough or DNA sequencing failed to provide usable data for identification. Sequence lengths varied from 245 bp to 435 bp. Fifty-one new strain sequences were most similar to the sequence KT868527.1 (Australian strain CAWD203), four to the sequence MH478526.1 (Portuguese strain IPMA01) and eight to the sequence MH790464.1 (Spanish strain IRTA-SMM-16-84) (Table 2).

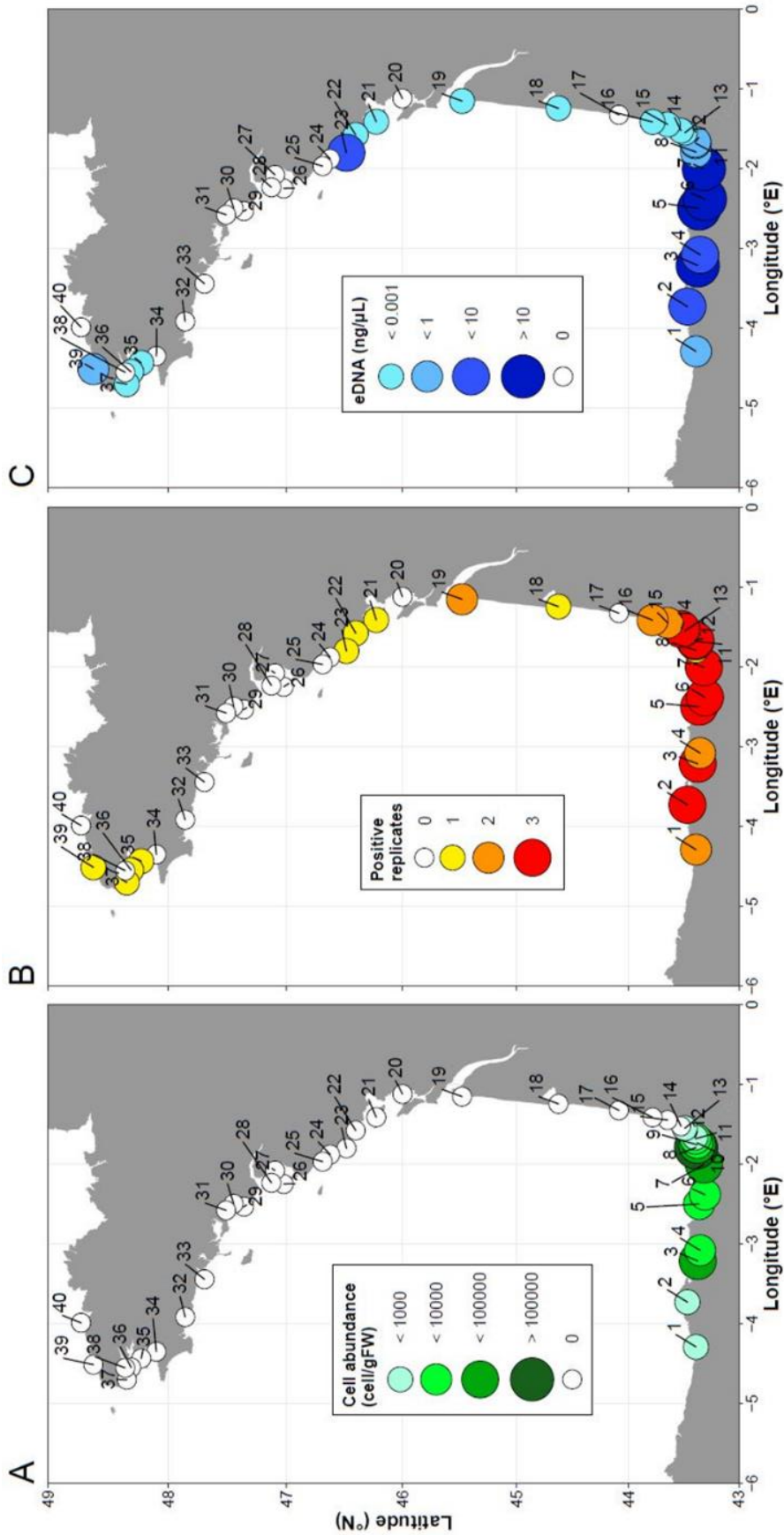


Figure 32: Spatial distribution of *Ostreopsis cf. siamensis* in the Bay of Biscay. Abundance of epiphytic cells assessed by light microscopy counting (A), eDNA detection (B) and estimation (C) of *O. cf. siamensis* assessed by rt-PCR analyses.

Isolated strain ID	Number of isolated strains	Site ID	Isolation locality	Accession number	Highest similar sequence after nucleotide blast	Identified species
SB1, SB3, SB4, SB5, SC1, SC2, SC5, SD3, SD4, SD5, SD6	11	2	Santander	MT952006; MT952007; MT952008; MT952009; MT952010; MT952011; MT952012; MT952015; MT952016; MT952017; MT952018	KT868527.1	<i>Ostreopsis cf. siamensis</i>
SC6, SD2	2	2	Santander	MT952013; MT952014	MH790464.1	<i>Ostreopsis cf. siamensis</i>
C2, C3, C4, C6, C8, C9, C10, C11	8	3	Castro	MT952059; MT952060; MT952061; MT952063; MT952065; MT952066; MT952067; MT952068	KT868527.1	<i>Ostreopsis cf. siamensis</i>
C1, C5	2	3	Castro	MT952058; MT952062	MH790464.1	<i>Ostreopsis cf. siamensis</i>
C7	1	3	Castro	MT952064	MH478526.1	<i>Ostreopsis cf. siamensis</i>
Z1, Z2, Z3, Z4, Z5, Z7, Z8	7	4	Zierbena	MT952050; MT952051; MT952052; MT952053; MT952054; MT952056; MT952057	KT868527.1	<i>Ostreopsis cf. siamensis</i>
Z6	1	4	Zierbena	MT952055	MH790464.1	<i>Ostreopsis cf. siamensis</i>
LA4	1	5	Lekeitio	MT952035	MH478526.1	<i>Ostreopsis cf. siamensis</i>
LD3	1	5	Lekeitio	MT952036	KT868527.1	<i>Ostreopsis cf. siamensis</i>
MC3	1	6	Mutriku	MT952069	KT868527.1	<i>Ostreopsis cf. siamensis</i>
D2, D3, D4, D5, D7, D8, D9, D10, D11, D12, D13, D14, D16, D17	14	7	San Sebastian	MT952019; MT952020; MT952021; MT952022; MT952024; MT952025; MT952026; MT952027; MT952028; MT952029; MT952030; MT952031; MT952033; MT952034	KT868527.1	<i>Ostreopsis cf. siamensis</i>
D6, D15	2	7	San Sebastian	MT952023; MT952032	MH478526.1	<i>Ostreopsis cf. siamensis</i>
H2, H3, H4, H6, H7, H8, H9, H12, H13	9	8	Hondarribia	MT952038; MT952039; MT952040; MT952042; MT952043; MT952044; MT952045; MT952048; MT952049	KT868527.1	<i>Ostreopsis cf. siamensis</i>
H1, H5, H10	3	8	Hondarribia	MT952037; MT952041; MT952046	MH790464.1	<i>Ostreopsis cf. siamensis</i>
H11	1	8	Hondarribia	MT952047	MH478526.1	<i>Ostreopsis cf. siamensis</i>

 Table 2: Information of the 64 new isolated, sequenced and genetically identified *Ostreopsis cf. siamensis* strains.

3.3. Environmental DNA (eDNA) analysis

eDNA samples were collected from both nylon screens (spatial survey in 40 sites) and plastic polymer substrates (temporal survey in 2 sites during 13 months) of the Bay of Biscay (Figure 30). Of the 112 and 118 eDNA samples collected, respectively, 45 and 5 were positive for *O. cf. siamensis*. All samples were negative for *O. cf. ovata*. All positive rt-PCR products sequenced were associated with the sequence MH478557.1 (Portuguese strain IO96-08), with a percent identity that was always higher than 96% for nylon screen samples and higher than 92% for plastic polymer substrate samples and with an e-value always lower than 1×10^{-10} (Supplementary Material 4). Data from rt-PCR products positive for *O. cf. siamensis* and sequencing were used for ecological analyses.

The cell-based standard curves covered linear detection over five orders of magnitude, with a mean rt-PCR efficiency of 82.5% (slope = -3.828) for *O. cf. siamensis* (Supplementary Material 5). The slope (-3.828) and intercept (+20.40) of the mean standard curve were used to estimate *O. cf. siamensis* eDNA concentrations in field samples from cycle threshold (Ct) values derived from the fluorescence intensity of the rt-PCR assays. No amplification was observed in the negative control templates (DNase-free water) whereas all the positive control templates were successfully amplified and were used to define the primer melting temperature associated with *O. cf. siamensis* (Supplementary Material 6). The Ct values obtained ranged from 16.8 to 39.8, corresponding, respectively, to 306 and 2×10^{-5} ng/ μ l.

Given the possible sources of bias in *Ostreopsis* spp. eDNA quantification by rt-PCR that have not been completely addressed in this study (sampling bias, DNA extraction yield, PCR inhibition and rt-PCR efficiency), rt-PCR data were interpreted as a tool to verify the presence of *O. cf. siamensis* in our samples and, in a semi-quantitative way, to suggest a potential distribution pattern of the species in the study area. *Ostreopsis cf. siamensis* eDNA was found, and semi-quantified, in artificial substrate samples collected in 23 of the 40 sampling sites. Comillas (Spain, Site 1) and Plouguerneau (France, Site 39) represented, respectively, the westernmost and northernmost sites where *O. cf. siamensis* was detected across the study area (Figure 32B). *Ostreopsis cf. siamensis* eDNA was detected at all sites (1-13) where cells had been observed under light microscopy (Figure 32A, 32B); the presence of the species was confirmed in the three replicates collected for genetic analysis at almost all of

these sites (Figure 32B). The highest *O. cf. siamensis* eDNA concentrations were estimated to occur at Sites 3, 5, 6 and 7 (Figure 32C).

Whereas no *O. cf. siamensis* cells were observed by light microscopy in any of the sites located at a higher latitude than Site 13 (Figure 32A), its presence was confirmed by rt-PCR analyses in 1-2 replicates of the samples collected in the middle of the bay (Sites 14-23, with the exception of Sites 17 and 20). In these samples, low eDNA concentrations were estimated, with the exception of Site 23 (Les Sables d'Olonne) where *O. cf. siamensis* eDNA concentrations were close to those measured in the south of the bay (Figure 32C). Some positive findings were observed up to the northernmost stations of the study area (Sites 35-39, with the exception of Sites 38 and 40) but only in one sample replicate and at very low concentrations ($<10^{-2}$ ng/ μ l). No correlation was observed between *O. cf. siamensis* cell abundance and mean eDNA estimation (Spearman test, $r_s = 0.518$, p-value > 0.05), probably due to methodological bias. However, when *O. cf. siamensis* eDNA was detected, a positive correlation was found between the number of positive replicates per site and mean eDNA estimation (Spearman, $r_s = 0.653$, p-value $\ll 0.01$).

Ostreopsis cf. siamensis eDNA was detected on different plastic substrates collected at the temporal monitoring sites of La Tremblade and Brest, confirming the presence of the species in these areas. The presence of *O. cf. siamensis* was detected in two samples collected in late spring (April-June) 2018 at both sites, and in one sample collected at the end of September – beginning of October 2018 in La Tremblade (Figure 33). No relationship between the type of polymer and *O. cf. siamensis* eDNA was found as the species was detected at least once on every polymer used during the monitoring period. At the time of the spatial survey (August-beginning of September 2018), *O. cf. siamensis* was not detected in La Tremblade or Brest.

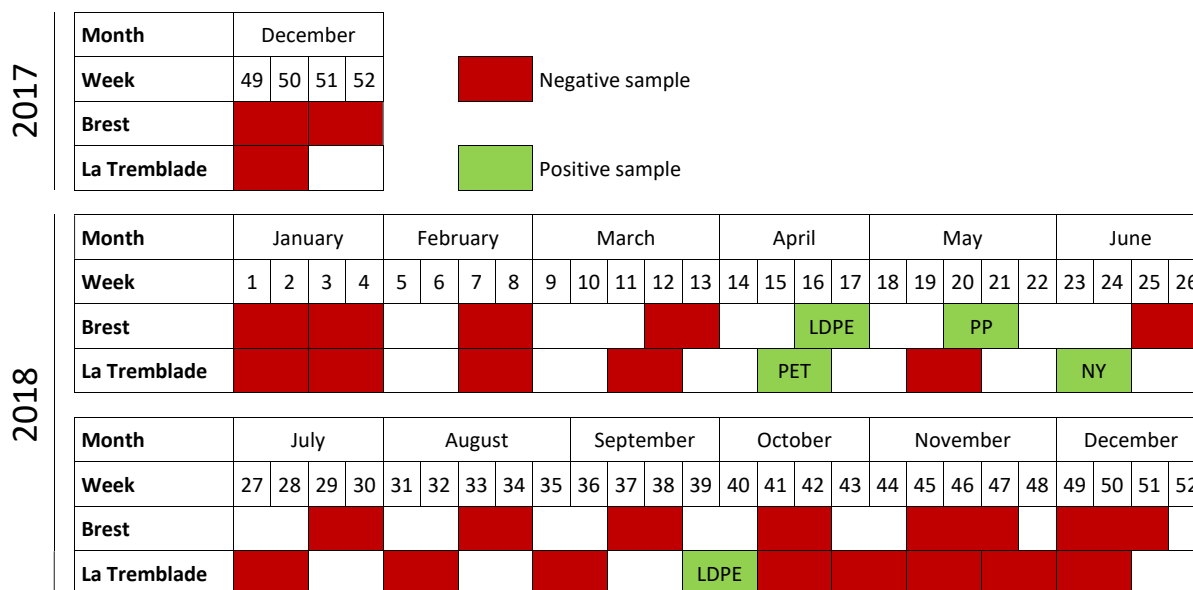


Figure 33: Temporal distribution of *Ostreopsis cf. siamensis* inferred from environmental DNA analyses carried out on plastic polymer substrates collected in 2017 and 2018 in Brest and La Tremblade. Plastic polymer substrates were immersed for two weeks, indicated with number for each year. Positive detection corresponds to the validation of *O. cf. siamensis* sequence from rt-PCR products. The type of polymer substrate of positive samples is indicated (LDPE = Low Density Polyethylene; PP = Polypropylene; PET = Polyethylene Terephthalate; NY = Nylon).

3.4. Connectivity analysis

The connectivity among sampled areas (Figure 30) was analyzed using a Lagrangian framework (i.e. particle tracking), which is classically used to study subpopulations and/or marine protected area connectivity (Mitarai *et al.*, 2009; Pujolar *et al.*, 2013). Within the transport time considered (90 days), none of the areas appeared to be connected to all the others (Figure 34, Supplementary Material 3). Particles released from Area A could only reach Areas B, C, D and E, Area E being the farthest away. Equally, particles that could reach Area N must have been released from Area K or north of this area (Figure 34). Nonetheless, particle movement patterns were generally fast enough to permit at least five successive areas in both directions to be connected at a seasonal scale. The large numbers of connections between areas from B to H along the south and west of the region are due to the presence of regular cyclonic and anticyclonic eddies. Because of this circulation, particles released in these areas

remain in the surface mixed layer where the effects of mesoscale and sub-mesoscale processes on the distribution patterns of numerical particles are considerable. Connection times between Area E to Areas D and F in the southeast Bay of Biscay are faster (between 3 and 5 days) than those recorded at other stations due to the continental slope current. This current increases the mixing activity of the particles by generating high turbulent flows. From Areas F to H, even if a northward connection was observed to the closest station, the main direction of connectivity is southward, and particles can reach Area B. A break in the connectivity time scale is observed around the stations I, J or K for particles with such behavior. The mean TR showed that particles needed fewer than 13 days in 2011 and 8 days in 2013 to move from Station H to Station I but needed more than 85 days in both years to travel from H to J. The least well-connected cluster was observed from Areas L to N. Finally, estimated connectivity from Areas I, J and K was also very variable between the two simulated years. For example, Area I was connected mainly with southward areas in 2011 whereas in 2013, some particles reached Areas K and L. Overall advection also appeared to be stronger in 2013 than in 2011.

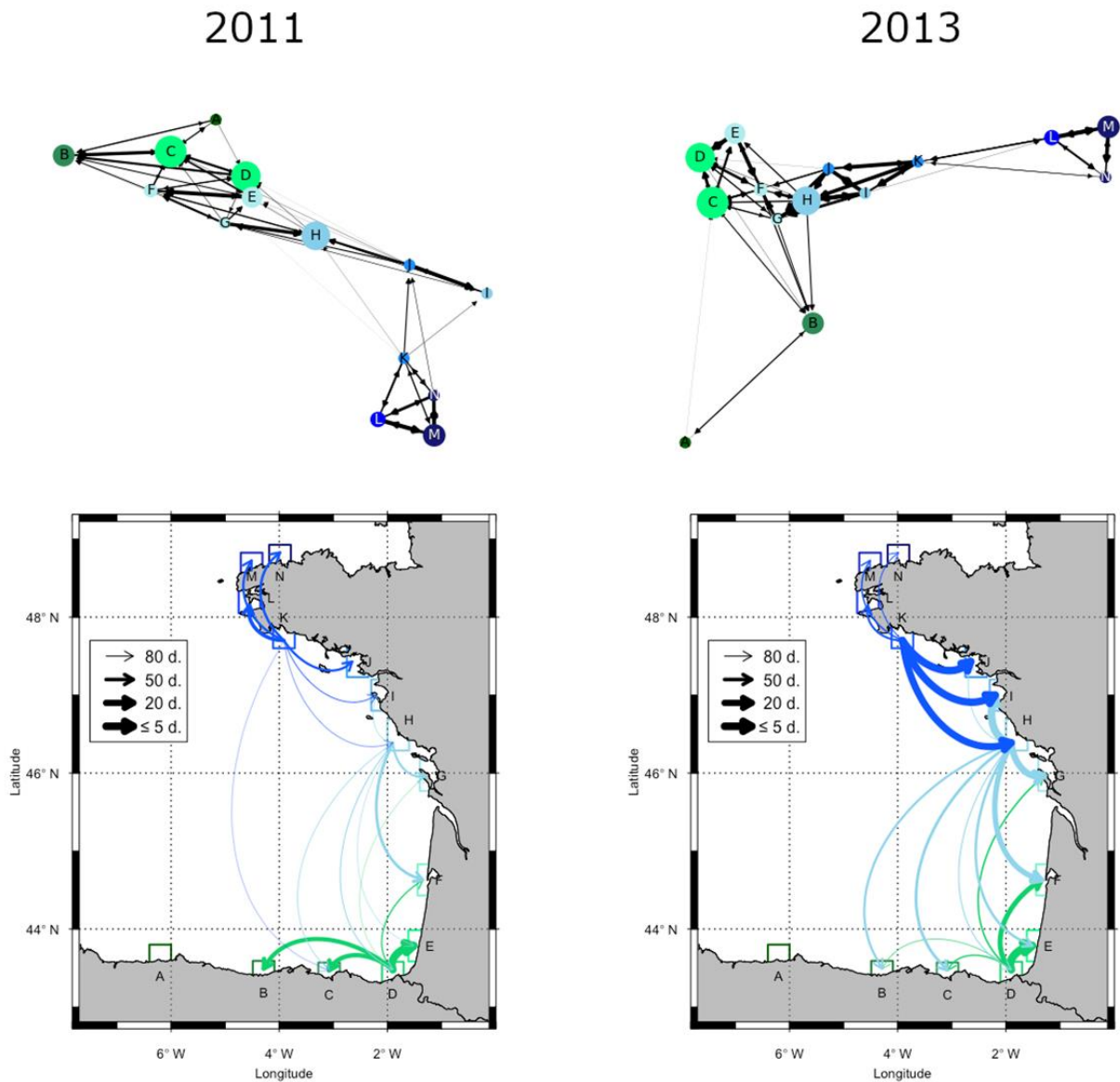


Figure 34: Summer connectivity from areas D (green), H (light blue) and K (dark blue) in 2011 and 2013. The networks (directed graph) illustrate relationships between stations for each year. In the maps the nodes are *Ostreopsis cf. siamensis* areas (from A to N) considered in the analysis and the edges width are proportional to the mean transit time of particles between pairs of stations.

4. Discussion

4.1. *Ostreopsis cf. siamensis* eDNA analysis

Environmental DNA refers to the intracellular (originating from living organisms) and extracellular (resulting from the organism's death) DNA that can be extracted from environmental samples without the need for prior isolation of the targeted organism (Taberlet *et al.*, 2012). Over the last decade eDNA analyses have proved their value to ecological and biodiversity studies (Taberlet *et al.*, 2012; Thomsen and Willerslev, 2015; Lacoursière-Roussel and Deiner, 2019). For instance, the use of eDNA to demonstrate the presence of a target species in an ecosystem is now used more and more, especially for monitoring species and detecting rare species (Jerde *et al.*, 2011; Bohmann *et al.*, 2014; Mächler *et al.*, 2014). The greater accuracy of eDNA detection compared to classical identification surveys saves time, in addition to collecting relevant new information concerning ecological communities and species distribution. In this study, standard benthic macroalgae sampling allowed the identification of sites where *O. cf. siamensis* is developing in large enough concentrations permitting classical observations by light microscopy. Dinoflagellate cells were observed in the south-eastern part of the bay, between Comillas (Spain) and Biarritz (France). These observations corroborate the previous reports of *O. cf. siamensis* in the Bay of Biscay (Laza-Martinez *et al.*, 2011; David *et al.*, 2013; Seoane and Siano, 2018). In the latest studies, the western and northern limits of *Ostreopsis* cell development have been established as, respectively, Santander (Cantabria) and Saint-Jean-de-Luz (French Basque Country), while in our study some cells were observed over a slightly larger spatial range (from Comillas (Site 1) to Biarritz (Site 13)). Nevertheless, the detection of *O. cf. siamensis* eDNA by rt-PCR revealed a wider distribution area of this species in the Bay of Biscay, demonstrating the complementarity of eDNA analyses with classical monitoring surveys. rt-PCR analysis followed by species identification through PCR product sequencing allowed the unambiguous attribution of eDNA rt-PCR products to *O. cf. siamensis*. Although no cells were observed further north than Biarritz (Site 13), rt-PCR analyses confirmed the presence of *O. cf. siamensis* eDNA across the whole of the Bay of Biscay and even further to the north, in Plouguerneau (Site 39), in the English Channel.

In this first attempt to describe *O. cf. siamensis* distribution across the Bay of Biscay using eDNA, it was important to evaluate issues associated with the use of this molecular tool. The transport and persistence of extracellular eDNA in marine waters and aquatic environments have been identified as potential sources of false negative and/or positive detection of target organisms (Goldberg *et al.*, 2018), i.e. eDNA could be transported and detected in the sampled area but not the original organism to which the DNA belongs. The majority of studies related to eDNA transport in aquatic environments have been carried out in rivers, where stream flow is mostly unidirectional. These studies have demonstrated that the eDNA of organisms might be retrieved relatively far from the original organism that was its source (Deiner and Altermatt, 2014; Shogren *et al.*, 2017). In the marine environment, where currents are multidirectional and water transport is variable across seasons and years, the study of eDNA transport is much more complicated and has been barely addressed so far. Using Lagrangian particle tracking, Andruszkiewicz *et al.* (2019) estimated that eDNA could be detected relatively far away from the original source (within 40 km northward approximately 4 days after shedding). Conversely, Jeunen *et al.* (2019) showed that eDNA dispersal can be limited within strongly connected marine coastal habitats, demonstrating that the study of eDNA transport is dependent on the specific water circulation of the studied area. The issue of eDNA persistence in marine waters is also still debated. The decay rate of eDNA seems to be influenced by both biotic and abiotic factors, including temperature, pH, UV and the microbial community in lotic (Barnes *et al.*, 2014; Strickler *et al.*, 2015; Lance *et al.*, 2017; Seymour *et al.*, 2018) and marine ecosystems (Sassoubre *et al.*, 2016; Andruszkiewicz *et al.*, 2017). In marine systems, Collins *et al.* (2018) estimated macroorganism (fish and crab) eDNA to be detected for up to 48 h. As for microorganisms and planktonic species, studies conducted on bacterial eDNA (*Salmonella* spp. and *Legionella* spp.) also observed a faster rate of degradation associated with warmer temperatures, resulting in the complete degradation of eDNA within a few days (Palmer *et al.*, 1993; Dupray *et al.*, 1997).

Lagrangian-based particle transport simulations in the Bay of Biscay showed that the minimum estimated particle-transport time between two relatively close areas in the southern part of the bay (< 60 km) was 2-5 days (i.e. between Areas D and E in 2011 and 2013). This indicates that for relatively close areas in the south of the bay, estimated particle-transport time is expected to be very similar to the estimated eDNA persistence time, suggesting that positive detection of *O. cf. siamensis* eDNA in this area could correspond to

free cells or to advected extracellular DNA produced from the large population of fixed cells. Conversely, estimated particle-transport time across areas located in the north of the Bay and in the English Channel was always greater than five days (between 20 and 80 days). This time, being longer than eDNA persistence estimates in the marine environment, suggests that even in the northern part of the bay, *O. cf. siamensis* eDNA detections could not be related to advected extracellular DNA but only to the presence of living cells, definitely indicating that this species is part of the microflora of the Bay of Biscay.

4.2. Geographical and niche ranges

The study of *Ostreopsis* spp. distribution at a regional level in the NE Atlantic contributed to the analysis of species distribution partitioning within the genus. Among all the eDNA samples collected and analyzed, *O. cf. ovata* was never detected, confirming the hypothesis of David *et al.* (2013) stating that the distribution of *O. cf. ovata* in the NE Atlantic is limited to south of the Iberian Peninsula (Portugal). In contrast, *O. cf. siamensis* seems to occur infrequently in the Mediterranean Sea, where *O. cf. ovata* is known to be very abundant (Penna *et al.*, 2010, 2012; Mangialajo *et al.*, 2011; Accoroni and Totti, 2016). This niche partitioning could be explained by the ability of *O. cf. siamensis* to be more resistant to cooler temperatures, lower salinities and/or tidal cycles than *O. cf. ovata*, as David *et al.* (2013) and Santos *et al.* (2019) observed *O. cf. siamensis* at higher latitudes than *O. cf. ovata* in the West of the Iberian Peninsula. In temperate waters both sea temperature and, to a lesser extent, salinity are known to be factors affecting the growth of *O. cf. ovata* (Pistocchi *et al.*, 2011; Pezolesi *et al.*, 2012; Scalco *et al.*, 2012; Accoroni and Totti, 2016; Carnicer *et al.*, 2016). Thus, being less well-adapted to environmental conditions of the Bay of Biscay than *O. cf. siamensis*, *O. cf. ovata* would not be able to develop at higher western latitudes despite its potential northward advection.

The environmental survey performed in this study made it possible to identify the geographical range of *O. cf. siamensis* in the Bay of Biscay, a concept related to the ecological niche theory (Peterson, 2011). Species' geographic range is considered as the basic unit of biogeography and represents the area where a species is found (Brown *et al.*, 1996). This range corresponds to the realized niche in the niche concept developed by Hutchinson (1957), where species are found within a space where environmental conditions are favoring their

development (Holt, 2003; Sexton *et al.*, 2009). However, dispersal by water circulation can promote the colonization of new ecosystems by microorganisms. Indeed, it is believed the apparent lack of dispersal barriers would confer marine planktonic microorganisms a ubiquitous distribution, especially in species forming resting stages with high dispersal potential. Local environmental constraints and random selection processes would then shape the community structure (Baas Becking, 1934; Fenchel and Finlay, 2004; de Wit and Bouvier, 2006). The distribution of high potential dispersal species can then be explained by the “source-sink” dynamics theory (Pulliam, 1988, 2000), deriving from Hutchinson’s niche theory, which suggests that populations issued from a suitable habitat (source) can feed environmentally non-suitable habitats (sinks). Hence, in a scenario where dispersal is high enough, the geographic range of a species can extend beyond its niche range (Hargreaves *et al.*, 2014), which is considered as the zone where environmental factors favor species development. A well-established population of *O. cf. siamensis* has already been documented along the Cantabrian Sea coasts (Laza-Martinez *et al.*, 2011). Along the Atlantic coasts of the Iberian Peninsula David *et al.* (2012) linked the presence of *O. cf. siamensis* with sea surface temperature. This was confirmed by Santos *et al.* (2019), who observed higher *O. cf. siamensis* cell abundances following periods of positive sea surface temperature anomalies and low sea state in Lagos Bay (Portugal). In this study, optimal temperature conditions for the occurrence of local *O. cf. siamensis* blooms were found between Santander (Site 2) and Saint-Jean-de-Luz (Site 12). We consider that, so far, this area represents the niche range of the species in the Bay of Biscay, i.e. the optimal zone for its development where the species can bloom. Comillas (Site 1) and Biarritz (Site 13), where cells were observed albeit in very low abundances, represent the edges of this niche range. This limit could potentially extend westward as no site was sampled after Comillas. Following water circulation, the northward dispersion of southern-based populations enables the colonization of the northern areas of the bay, extending the geographical range of *O. cf. siamensis* at least to the north of the Bay of Biscay (Brittany) and the English Channel.

Based on Lagrangian integration, different approaches have been used in the literature to quantify the connectivity of different marine areas (Mitarai *et al.*, 2009; Rossi *et al.*, 2014; Jönsson and Watson, 2016; Costa *et al.*, 2017). The mean and median values of transit time for all particles traveling from one station to another has been applied in this study following the proposal by Costa *et al.* (2017) of calculating “betweenness”, which is the shortest paths

between different sites within variable ocean dynamics. Due to its oceanographic characteristics, connectivity between sites located along the coasts of the Bay of Biscay are mainly driven by tidal forcing, wind regimes and oceanic topography, such as continental slopes (Charria *et al.*, 2013). In spring and summer, the period set as the time of particle release in our simulations, the prevailing wind regime generates currents that flow south and west-southwest, increasing particle travel time towards northern areas. The presence of weak residual currents and vortices with low dispersion rates ensures connectivity between the areas in this part of the Bay. Conversely, in the winter winds are stronger and southwesterly, higher rivers flow increases the relevance of densities gradient circulation and the Iberian Poleward Current runs close to the coast from the Atlantic coasts of the Iberian Peninsula towards the north (Frouin *et al.*, 1990; Peliz *et al.*, 2005). These observed seasonal patterns are also strongly dependent on weather variability (Charria *et al.*, 2017). Lagrangian particle-tracking simulations demonstrated that, due to its specific ocean dynamics, some key areas were responsible for maintaining continuous dispersal connectivity along the coasts of the Bay of Biscay. Area D, located in the south of the Bay, represents the area where the main *O. cf. siamensis* source population is established and from where cells are dispersed towards northern sites. Area H, located in the middle of the bay, links the southern and northern areas while Area K, located in the north of the bay, is the only area connecting both the English Channel and the Bay of Biscay. Hence, dispersal of *O. cf. siamensis* towards the northernmost areas can only be assured if *O. cf. siamensis* cells rest in intermediate “shelter” areas of the bay (Areas H and K) before eventually being dispersed northward. Northern coasts of Brittany along the English Channel (Site 39) and the 48th parallel were identified as the northernmost area where *O. cf. siamensis* could be detected. However, the application of water circulation models connecting the Bay of Biscay, the English Channel and the Celtic Sea could reveal even higher dispersion in the NE Atlantic.

eDNA analysis of the temporal survey data also confirmed the presence of *O. cf. siamensis* in La Tremblade (near Site 20) and in Brest (near Sites 36 and 38) and helped define the time window for the presence of this species in these areas. The ability of marine microorganisms to adhere to, and develop on plastic and synthetic surfaces has been extensively described and represents a potential dispersion vector for microbial communities colonizing drifting plastic substrates (Zettler *et al.*, 2013; Reisser *et al.*, 2014; Oberbeckmann *et al.*, 2015). Plastic colonization has also been observed for dinoflagellates (Masó *et al.*, 2003,

2016; Casabianca *et al.*, 2019, 2020) and has been used for monitoring through artificial substrate sampling methods (Tester *et al.*, 2014; Jauzein *et al.*, 2016; Parsons *et al.*, 2017). The temporal survey carried out on plastic substrates was used in this study to describe the temporal dynamic of *O. cf. siamensis*. Although *O. cf. siamensis* eDNA was found between April and June both in Brest and La Tremblade, and also at the end of September in La Tremblade, the species was not found at these sites during the period of the spatial sampling survey (August-September). This could indicate that the screens used in the artificial substrate method might be more accurate for the collection of rare eDNA than plastic polymer substrates despite no relationship was observed between the type of plastic polymer used and the presence of the species. However, positive samples found during the temporal surveys suggest a favorable window of development larger than expected prior to the sampling campaign. Coupling temporal and spatial analyses, we can therefore infer that *O. cf. siamensis* can be present, in high enough abundance to be detected using eDNA analyses, in the Bay of Biscay from spring (April-June) to the end of summer (August-September). However, more specific monitoring is needed to assess more precisely the phenology of the species and, for this purpose, artificial substrates seem to be an adequate tool.

4.3. Potential effect of global changes on *O. cf. siamensis* expansion

Environmental conditions, and especially the water temperature, in the north of the Bay of Biscay are not optimal for *O. cf. siamensis* development. However, global ocean warming induced by climate change may foster *O. cf. siamensis* expansion in new areas whose habitat characteristics are suitable for the development of the species (Tester *et al.*, 2020). Yet, if both earth and ocean climates are warming at a global scale since the last century and are related to the large increase in anthropogenic gas emissions (Levitus, 2001; Pierce *et al.*, 2006; Alexander *et al.*, 2018), not all oceans are warming at the same rate. Since 1950 a general increase in North Atlantic Ocean sea surface temperatures (SST) have been observed (Levitus, 2000; Saba *et al.*, 2016; Cheng *et al.*, 2017) simultaneously with multidecadal oscillations corresponding to successive cycles of warming and cooling periods (Polyakov *et al.*, 2005). As part of the North Atlantic, the Bay of Biscay has been affected by both global warming and multidecadal oscillations (deCastro *et al.*, 2009; Costoya *et al.*, 2016). A non-homogenous long-term increasing trend of both surface and intermediate water temperatures of the bay

was demonstrated, with the south-eastern area exhibiting both stronger increase in SST and seasonality than the northern area (Koutsikopoulos *et al.*, 1998; Planque *et al.*, 2003; González-Pola, 2005).

Due to the major role played by sea temperature in phytoplankton species ecology (Eppley, 1972; Wells *et al.*, 2015), ocean warming is expected to affect microalgae distribution and phenology (Fu *et al.*, 2012; McLeod *et al.*, 2012; Paerl and Paul, 2012), including benthic species (Tester *et al.*, 2020). Eco-evolutionary models of thermal adaptation mechanisms in phytoplankton species suggest a poleward shift in their thermal niche (Thomas *et al.*, 2012) and simulation models forecast the expansion of potentially toxic species towards higher latitudes (Gobler *et al.*, 2017). Selected species of the genus *Ostreopsis* spp. are likely to expand beyond their traditional pantropical geographical range, colonizing temperate areas at higher latitudes (Tester *et al.*, 2020). Many laboratory studies have suggested that relatively high temperatures were more suitable for optimal *Ostreopsis* growth, although the thermal niche seems to be species and strain dependent (Granéli *et al.*, 2011; Scalco *et al.*, 2012; Tanimoto *et al.*, 2013; Tawong *et al.*, 2015; Carnicer *et al.*, 2016). In the Mediterranean Sea, *in situ* observations by Carnicer *et al.* (2015) suggested that water temperature was the main ecological driver among all the environmental variables influencing the growth of *O. cf. ovata*. However, very little is known about the thermal niche of *O. cf. siamensis* populations in the Bay of Biscay, except the aforementioned observations by David *et al.* (2012) who hypothesized that the presence of the species was linked with the presence of relative warm waters (19.5 °C) for at least three consecutive months. In our study, *O. cf. siamensis* cells were observed in the southern part of the Bay at temperatures that were always above 21 °C. However, the occurrence of the species has been reported in this study in the cooler northern part of the Bay. Following the global long-term trend of warming waters worldwide, and specifically in the North-East Atlantic, future expansion of *O. cf. siamensis* distribution from the south-eastern area to the warming northern parts of the Bay of Biscay could be expected. However, the species' physiological adaptations towards evolving sea temperatures will certainly play a role in this expansion, as shown for other HABs species (Flores-Moya *et al.*, 2012). This adaptive process has been poorly studied in *O. cf. siamensis* and predictive models applying such data to achieve a conclusive forecast of the potential expansion of this species are needed.

In order to monitor *O. cf. siamensis* expansion towards higher latitudes, an environmental managing strategy should be undertaken. The inter-site connectivity revealed by our Lagrangian particle-tracking simulations in the Bay of Biscay identified the geographic range limit (Area D) of *O. cf. siamensis* as well as two areas (H and K) that are crucial for the maintenance of a continuous connection between the southern and northern parts of the Bay. We propose that these three areas, corresponding to the zones of San Sebastian/Hondarribia/Hendaye (Sites 7, 8, 9; Area D), Les Sables d'Olonne (Site 23; Area H), and Concarneau (Site 33; Area K), should be identified as sentinel areas for future studies conducted on the potential expansion of *O. cf. siamensis*. These areas will be used to establish the recrudescence of bloom phenomena (Area D) as well as the occurrence of potential new blooming areas (H and K). The use of a specific seasonal monitoring program should be developed with tools adapted to detection of the species. Both eDNA samplers (artificial substrate/plastic sampler substrates) and microscopic analysis could be employed, and the identification of high abundances of living cells in microscopical samples instead of eDNA could be used as a proxy of potential species development.

5. Conclusions

The effect of sea temperature warming on the expansion of HAB phenomena is a global threat. This study contributes to the assessment of this cause-effect link, using a study area of the North-East Atlantic, the Bay of Biscay, sensitive to global warming, and a harmful benthic dinoflagellate, *O. cf. siamensis*, whose distribution is expected to be affected by global water warming. Our innovative eDNA sampling strategy (artificial substrates) allowed us to determine that the species is present in the whole bay and even in the English Channel (48th parallel). Within this extended geographical range, the niche range, i.e. the zone where environmental conditions are suitable and where the species can bloom, is limited to the south of the Bay. In addition, the temporal eDNA survey suggested the presence of the species to extend from April to September. Our Lagrangian model simulations of particle dispersal did not only help to demonstrate that cells can be transported from the south to the north of the Bay of Biscay, but also revealed that key zones in the middle of the bay formed a staging post to allow this distribution across the bay. We believe that expansion toward the north of the Bay is currently limited by temperature, but the thermal tolerance of *O. cf. siamensis* to lower

and higher temperatures is yet to be demonstrated. In order to monitor such an expansion, we propose to establish specific monitoring programs at three different latitudes, corresponding to the three most closely connected areas of the bay (San Sebastian/Hondarribia/Hendaye, Les Sables d'Olonne and Concarneau). This monitoring could be enlarged to northern areas of the English Channel (England, Wales, Ireland), where water circulation could promote species dispersal and colonization.

Acknowledgement

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Authors' contribution

K.D., R.S., C.J. and R.L. designed and planned the sampling campaign. K.D., R.S., C.J., A.L-Z. and S.S. conducted the spatial sampling campaign. C.L. organized the plastic polymer temporal survey and collected the samples. K.D. analyzed all samples. S.H., M.S. and M.P. designed and performed the modeling analyses. K.D., R.S., C.J., D.H-H., S.H., M.S. and M.P. analyzed the results and prepared the figures. K.D. and R.S. wrote the manuscript. All authors reviewed and accepted the final version of the manuscript.

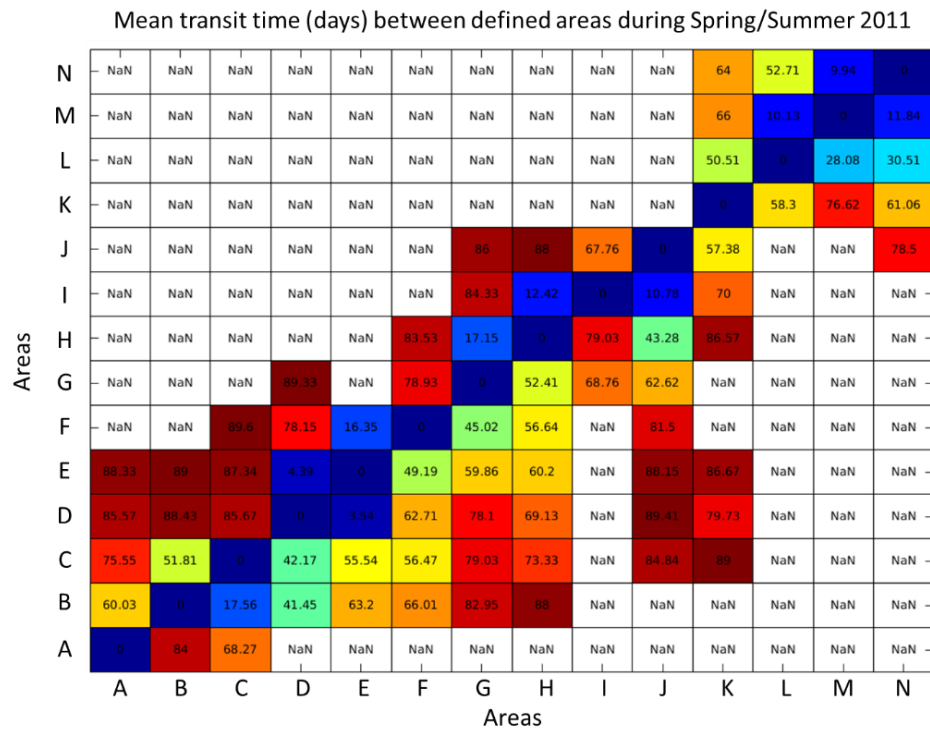
6. Appendix

Correlation	“mean transit time”	“median transit time”	“most frequent transit time”	“minimum time”
“mean transit time”	-	0.954260	0.915440	0.961930
“median transit time”	0.954260	-	0.887575	0.924950
“most frequent transit time”	0.915440	0.887575	-	0.883345
“minimum transit time”	0.961930	0.924820	0.883345	-

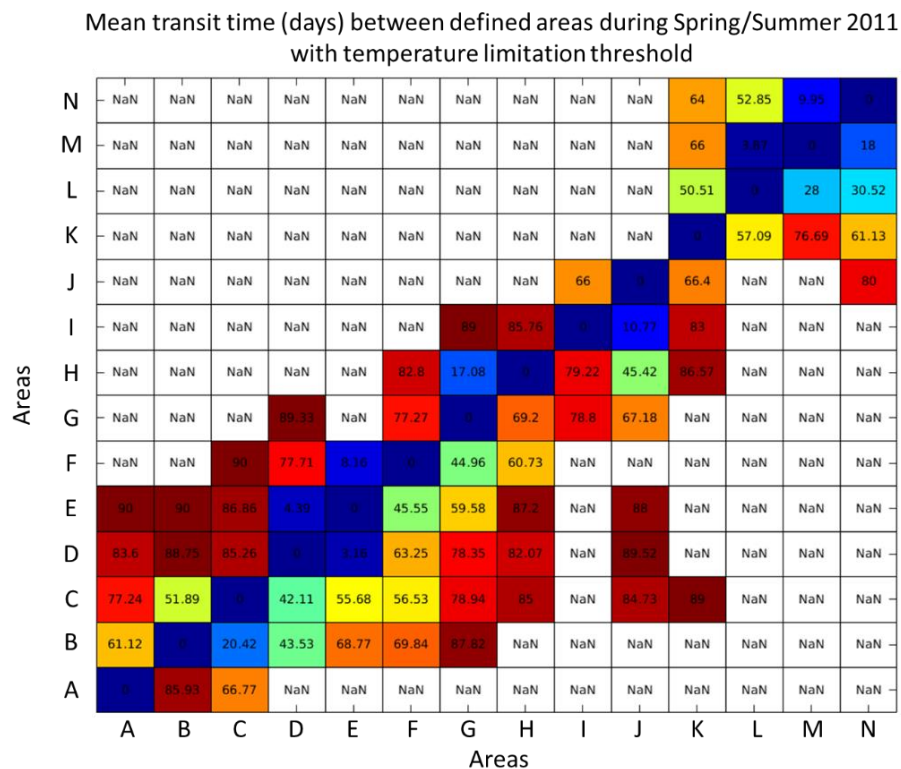
Supplementary table 1: Correlation values between Lagrangian indices

Correlation	Mean 2010	Mean 2011	Mean2012	Mean2013	Mean2014
Mean2010	-	0.9493150	0.9337400	0.9107550	0.9285750
Mean2011	0.9493150	-	0.9645000	0.9249550	0.9676500
Mean2012	0.9337400	0.9645000	-	0.9146700	0.9285750
Mean2013	0.9107550	0.9249550	0.9146700	-	0.9169500
Mean2014	0.9285750	0.9676500	0.9285750	0.9169500	-

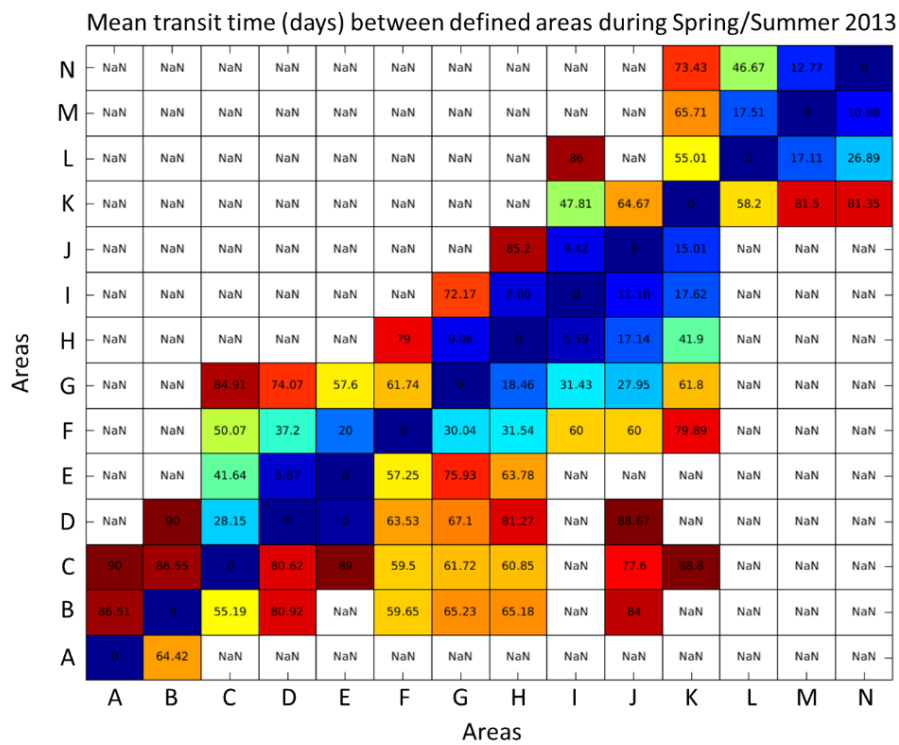
Supplementary table 2: Correlation values of mean transit time between different years



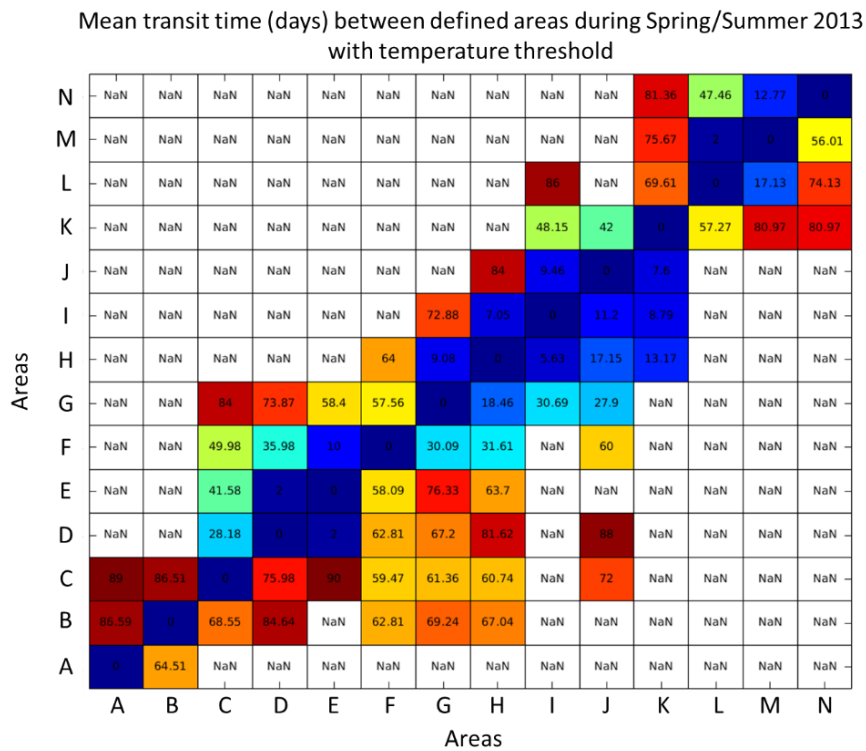
Supplementary figure 1: *Ostreopsis cf. siamensis* connectivity matrix showing the “mean transit time” (days) between the releasing (particle deployment) areas (x-axis) and the receiving areas (y-axis) in spring-summer 2011. Model horizontal resolution = 4 km.



Supplementary figure 2: *Ostreopsis cf. siamensis* connectivity matrix showing the “mean transit time” (days) between the releasing (particle deployment) areas (x-axis) and the receiving areas (y-axis) in spring-summer 2011. The data showed are based on simulations that used the temperature limitation of 16°C. Model horizontal resolution = 4 km.

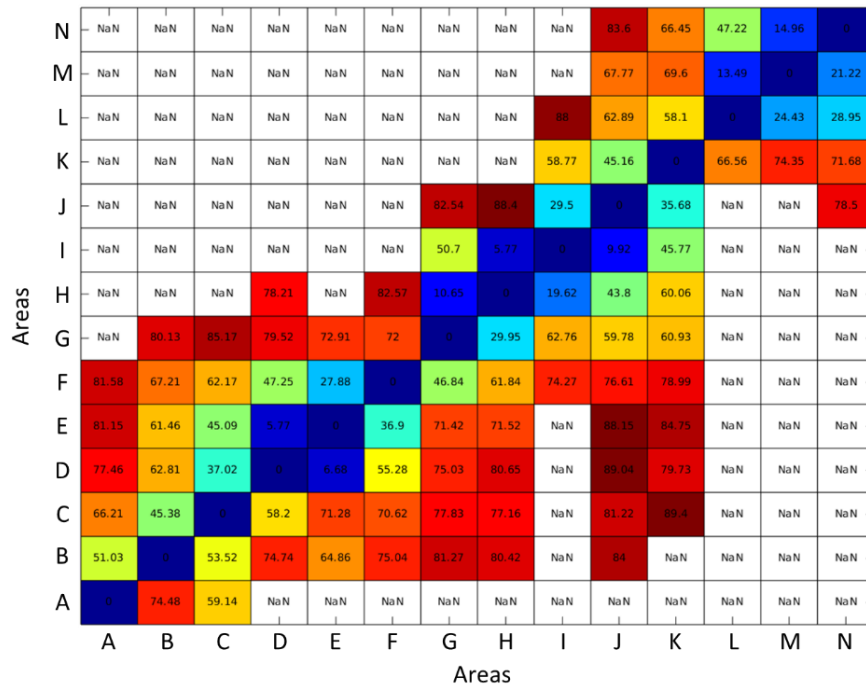


Supplementary figure 3: *Ostreopsis cf. siamensis* connectivity matrix showing the “mean transit time” (days) between the releasing (particle deployment) areas (x-axis) and the receiving areas (y-axis) in spring-summer 2013. Model horizontal resolution = 4 km.



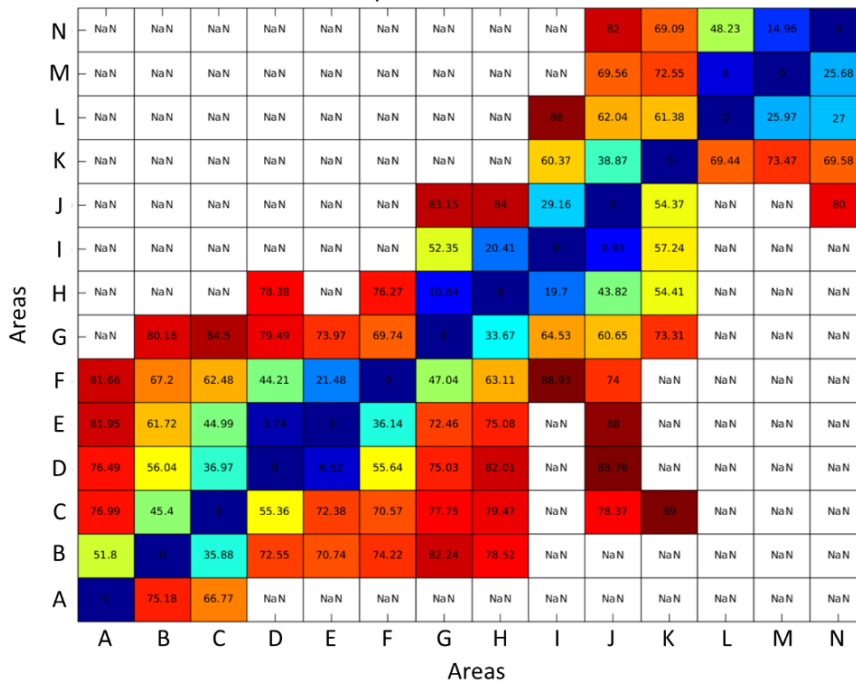
Supplementary figure 4: *Ostreopsis cf. siamensis* connectivity matrix showing the “mean transit time” (days) between the releasing (particle deployment) areas (x-axis) and the receiving areas (y-axis) in spring-summer 2013. The data showed are based on simulations that used the temperature limitation of 16°C. Model horizontal resolution = 4 km.

Mean transit time (days) between defined areas during Spring/Summer during 2010-2014

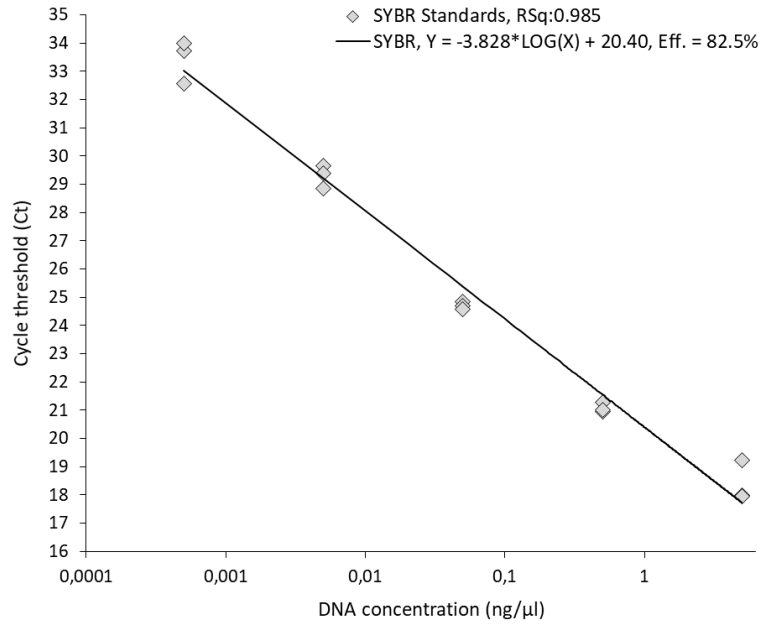


Supplementary figure 5: *Ostreopsis cf. siamensis* connectivity matrix showing the "mean transit time" (days) between the releasing areas, in spring-summer from 2010 to 2014. Model horizontal resolution = 4 km.

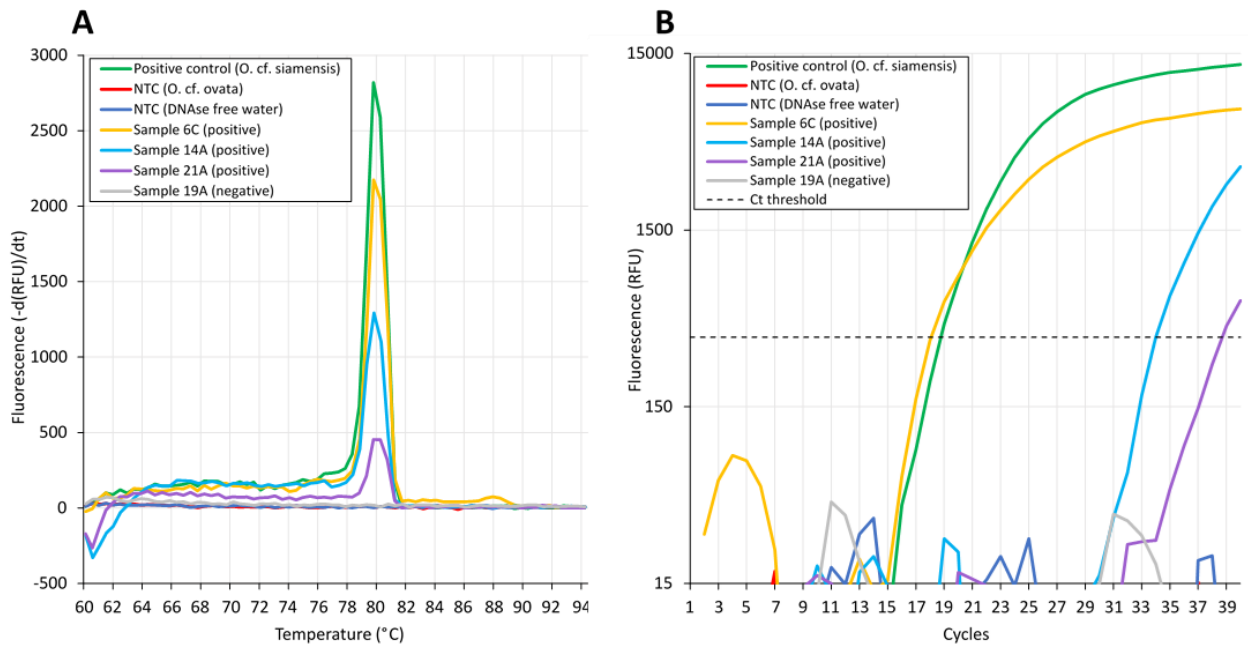
Mean transit time (days) between defined areas during Spring/Summer during 2010-2014 with temperature limitation threshold



Supplementary figure 6: *Ostreopsis cf. siamensis* connectivity matrix showing the "mean transit time" (days) between the releasing (particle deployment) areas in spring-summer from 2010 to 2014. The data showed are based on simulations that used the temperature limitation of 16°C. Model horizontal resolution = 4 km.



Supplementary figure 7: rt-PCR cell-based standard curves



Supplementary figure 8: Dissociation curves (A) and amplification plots (B) of rt-PCR performed on environmental samples.

Chapitre 3

Acclimatation thermique et réponses physiologiques en fonction de la température chez *Ostreopsis* cf. *siamensis* et *Ostreopsis* cf. *ovata* du nord-est de l'Atlantique et du nord-ouest de la Méditerranée

Contexte de l'étude

La température est un facteur déterminant dans le développement des microalgues (Eppley, 1972), et notamment dans la formation d'efflorescences associées à des espèces nuisibles (Wells *et al.*, 2015). Des études de laboratoires ont montré que la croissance et la toxicité de souches d'*O. cf. ovata* de Méditerranée étaient influencées par la température (Pistocchi *et al.*, 2011; Pezolesi *et al.*, 2012), mais aucune étude d'écophysiologie et écotoxicologique n'a été réalisée sur des souches d'*O. cf. siamensis* provenant des côtes Atlantiques européennes. Or, une caractérisation fine de la réponse physiologique d'*Ostreopsis* spp, et d'*O. cf. siamensis* en particulier, à la température pourrait permettre (i) d'envisager la tolérance thermique de l'espèce (pouvant être interprétée en termes de niche écologique ou de contexte de réchauffement global), (ii) de comprendre les mécanismes physiologiques impliqués dans la réponse au stress thermique et (iii) d'étudier les liens directs ou indirects entre température et toxicité.

Dans ce cadre, notre étude vise à caractériser la réponse physiologique des cellules d'*Ostreopsis* face à une acclimatation douce à différents paliers de température, variant de 14°C à 28°C. Un effort particulier a été déployé pour analyser de nombreuses souches d'*Ostreopsis*, afin de pouvoir envisager une différence entre souches d'une même espèce, voir la formation d'écotypes associés à des localités précises. Au final, 9 souches d'*O. cf. siamensis* isolées dans le sud-est du Golfe de Gascogne et 3 souches d'*O. cf. ovata* provenant de Méditerranée nord-occidentale ont été testées. Les paramètres ciblés visaient à caractériser les capacités de croissance des cellules (taux de croissance, paramètres photosynthétiques), la réponse au stress et à l'acclimatation thermique (excrétion de nitrites, profils pigmentaire et lipidique, accumulation d'espèces réactives de l'oxygène) ainsi que la toxicité.

Contribution des auteurs

Au cours de cette étude basée sur les souches d'*O. cf. siamensis* isolées par A. Laza-Martinez et S. Seoane ainsi que sur les souches d'*O. cf. ovata* fournies par la collection MCCV (EMBRC), j'ai planifié cette expérience d'acclimatation thermique en laboratoire sous la supervision de C. Jauzein. Les expériences de culture ont été réalisées avec l'aide de C. Jauzein, d'E. Guilloud (stagiaire en alternance à l'Ifremer de Brest pendant une année), de G. Heude (stagiaire M1) et de P. Malestroit (technicienne Ifremer Brest). Après avoir recueilli la biomasse nécessaire

aux analyses de profils lipidiques, pigmentaires et toxiniques, ces mesures ont été respectivement confiées à M. Boutoute, S. Schmitt et D. Réveillon et F. Hervé. Certaines analyses sont encore à ce jour en attente. Le présent chapitre de thèse a donc été rédigé à partir des données acquises jusqu'à maintenant, mais ce set de données sera prochainement complété et finalisé, en particulier par les approches d'analyses pigmentaire et de lipidomique. J'ai écrit le chapitre de thèse sous la supervision de C. Jauzein et en collaboration avec mes deux autres co-encadrants R. Siano et R. Lemée. Une fois l'ensemble des données acquises, ce travail est destiné à être valorisé en tant qu'article dans un journal scientifique de rang A.

Thermal acclimation and eco-physiological responses towards temperature in *Ostreopsis* cf. *siamensis* and *Ostreopsis* cf. *ovata* from NE Atlantic and NW Mediterranean Sea

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Running title: *Ostreopsis* eco-physiological responses towards temperature

Abstract:

Reports of the benthic dinoflagellate *Ostreopsis* spp. have been increasing in the last decades, especially in temperate areas. Evidences of the effects of the increasing sea temperature trend due to global warming on its physiology and its distribution are still lacking and need to be investigated. In this study, acclimated eco-physiological responses of strains of *O. cf. siamensis* from the Bay of Biscay (NE Atlantic) and Mediterranean *O. cf. ovata* strains towards changing temperatures were assessed. *O. cf. siamensis* cultures were exposed to temperatures ranging from 14°C to 28°C and acclimation was successful for temperatures ranging from 16°C to 25°C with the highest growth rates were reported at 22°C (0.67 – 1.06 d⁻¹). Variations in toxicity assessed through the *Artemia franciscana* bioassay revealed a decrease in toxicity when exposed to low temperatures (16°C and 19°C), whereas highest toxicity levels were recorded at temperatures corresponding to the highest growth rates (22°C). Among potential physiological adaptation strategies, the role of non-assimilatory nitrogen-uptake was shown for *O. cf. siamensis* exposed to cold temperatures and other strategies (modifications of pigments or lipids contents) are under investigation. This work provides evidences that moderate to relatively high temperatures are necessary to allow for bloom development of *O. cf. siamensis* strains in the Bay of Biscay. Experiments also showed that the species has the capacity to cope with thermal stress conditions all over the Bay of Biscay, with a thermal niche allowing for growth even at latitudes as high as the Bay of Brest.

Key-words: Temperature; Acclimation; Harmful Algal Blooms; Benthic dinoflagellate; Growth; Toxicity

1. Introduction

Among microalgae species responsible for harmful algal bloom development, the toxic benthic dinoflagellate *Ostreopsis* spp. represents an emerging threat for human health. Blooms of this genus have mainly been observed in tropical and temperate areas (Shears and Ross, 2009; Rhodes, 2011; Selina *et al.*, 2014), and especially in the Mediterranean Sea (Mangialajo *et al.*, 2011; Blanfuné *et al.*, 2015; Accoroni and Totti, 2016). To date, a total of eleven *Ostreopsis* species have been identified and characterized. The description of *Ostreopsis* species was traditionally based upon morphological features but the high degree of morphological plasticity within and among species made the genetic identification mandatory for the proper identification and characterization of species, as well as for inter-comparison of species-specific studies (Parsons *et al.*, 2012; Chomérat *et al.*, 2019; Chomérat *et al.*, 2020b). For instance, *O. siamensis* and *O. ovata* were described for the first time only based on morphological features and not genetically characterized. Thus, species morphologically associated with these two taxa are now referred as *O. cf. siamensis* and *O. cf. ovata*, respectively, contributing to the confusion between different clades. Blooming episodes formed by massive development of certain species of *Ostreopsis* have been resulting in toxic outbreaks affecting human health through skin irritations induced by direct contact (Tichadou *et al.*, 2010; Tubaro *et al.*, 2011) or through serious respiratory symptoms caused by aerosol inhalation (Durando *et al.*, 2007; Illoul *et al.*, 2012; Vila *et al.*, 2016). Apart from human health, *Ostreopsis* sp. also exhibit allelopathic effects on marine fauna (Pavaux *et al.*, 2019, 2020a, 2020b). Several *Ostreopsis* species are known to produce a high diversity of palytoxin analogs such as ovatoxins, ostreocins and mascarenotoxins (Pavaux *et al.*, 2020a). Concerning *O. cf. siamensis*, traces of palytoxin-like compounds have only been found in strains from South-Est Australia (Verma *et al.*, 2016) but not in strains from Atlantic or Mediterranean Sea (Ciminiello *et al.*, 2013). However, toxicity bioassays revealed active toxicity in these strains (Cagide *et al.*, 2009; Laza-Martinez *et al.*, 2011), suggesting that other non-identified allelopathic compounds could be responsible for *O. cf. siamensis* toxicity and may represent a threat for human health.

The development of *Ostreopsis* species and their blooms phenology is known to be driven by an assembly of biotic and abiotic factors influencing physiological mechanisms and growth ability. Environmental parameters such as hydrodynamics, biotic substratum,

irradiance, salinity, nutrients availability and sea temperature are suggested to be the most influencing factors (Parsons *et al.*, 2012; Accoroni and Totti, 2016). Among these, the temperature can play a major role in both the definition of the ecological niche (David *et al.*, 2012; Drouet, *et al.*, Submitted; Scalco *et al.*, 2012) and the phenology of blooms (Carnicer *et al.*, 2015; Drouet *et al.*, in prep.). In both cases, temperature can regulate the presence of the species in the water column by controlling the germination of resting stages (if they are part of the life cycle of the species) (Bravo and Anderson, 1994; Bravo *et al.*, 2012; Fischer *et al.*, 2018) and/or directly influencing microalgal growth (Tian, 2006; Pezolesi *et al.*, 2012). In temperate areas in particular, *Ostreopsis* spp. blooms are recurrent phenomena that seem to be favored by high temperatures: they mainly occur during the summer period, with maximal abundances often corresponding to relatively high temperatures, but not necessarily the highest (Cohu *et al.*, 2011; Laza-Martinez *et al.*, 2011b; Selina *et al.*, 2014; Totti *et al.*, 2010). Extensive laboratory studies conducted on *O. cf. ovata* observed the highest growth rates of Mediterranean strains at temperatures ranging between 20°C and 26°C (Pistocchi *et al.*, 2011; Pezolesi *et al.*, 2012; Scalco *et al.*, 2012), whereas Asian or Brazilian strains exhibited highest growth rates at temperatures ranging from 25°C to 30°C (Nascimento *et al.*, 2012; Yamaguchi *et al.*, 2012; Tawong *et al.*, 2015). Such differences in thermal ranges show strain-specific variations in the relationship between growth abilities and temperature. They may represent physiological adaptations of cells to the specific environmental conditions of isolation sites, and thus the existence of ecotypes. Several studies reported another interesting relationship between temperature and *Ostreopsis* cell metabolism: it seems to influence the toxin production with higher cellular concentrations of toxins recorded at temperatures more favorable to algal growth, at least for *O. cf. ovata* (Pistocchi *et al.*, 2011; Pezolesi *et al.*, 2012; Scalco *et al.*, 2012). However, it is still unclear if such temperature effect is either direct on toxin synthesis pathways or indirect through modifications of growth rate.

Anthropic activities might be responsible for some regulations of HABs development through climate change, and the worldwide increase of sea temperature in particular. For benthic species (including *Ostreopsis* spp.), Tester *et al.* (2020) recently described a potential migration of their ecological niche from tropical areas towards higher latitudes and an extension of their habitat in temperate areas in response to global warming. In the face of this expected change and maybe increase in occurrence of harmful events, elaborating risk assessment and management for the mitigation of HABs in recreational waters are crucial

(Anderson, 2009; Berdalet *et al.*, 2016; Berdalet *et al.*, 2017b). Areas where *Ostreopsis* species are known to be highly present and to form blooms are exposed to sea temperature increase and the current distribution of the species in these areas could thus evolve in the future. While *O. cf. ovata* has been extensively observed and studied in the Mediterranean Sea (Mangialajo *et al.*, 2011; Accoroni and Totti, 2016), only *O. cf. siamensis* has been described forming blooms in the Bay of Biscay, NE Atlantic (Laza-Martinez *et al.*, 2011; David *et al.*, 2013; Santos *et al.*, 2019). As both of these areas are exposed to SST warming (deCastro *et al.*, 2009; Costoya *et al.*, 2015; Pastor *et al.*, 2020), investigations regarding the physiological responses of local HABs species towards increasing temperatures are needed.

Phytoplankton species have several ways to respond to changes in environmental conditions. At the population level, species can take advantage of the high genetic diversity observed in the field, and over the development of HABs in particular (Dia *et al.*, 2014): in response to environmental stress, a selection of advantageous genotypes can occur, favoring clones with high fitness. At the intra-population level, each clone is characterized by specific characteristics associated with growth capacities (e.g. efficiency to take up nutrients, Jauzein *et al.*, 2008) or response abilities to stress. Indeed, at the cellular scale, cells facing changing or unfavorable growth conditions can modify some aspects of their morphology or metabolism in a strain-specific range of physiological plasticity, in order to optimize their growth over an acclimation process. Variations of temperature, in particular, induce a stress that can generate a disruption in microalgal cell homeostasis, which is the equilibrium of physiological process and composition of an organism in balance with its environment (Borowitzka, 2018). This disruption leads to a regulation of cell physiological functions in order to restore homeostasis. Temperature is known to affect the functionalities of enzymes in charge of photosynthesis (Salvucci and Crafts-Brandner, 2004) and can thus directly influence the photosynthesis activity and growth of microalgal cells (Ras *et al.*, 2013). Cells can also use mechanisms, such as changes in pigment composition (Anning *et al.*, 2001; López-Rosales *et al.*, 2014), lipid content (Ding *et al.*, 2019; Thompson *et al.*, 1992b) and nutrient pathways (Glibert *et al.*, 2016), to generate an acclimation response to stress and optimize growth.

The aim of this study was to investigate how different clones of *O. cf. siamensis* and *O. cf. ovata* can acclimate to various temperature levels and to define the influence of temperature on their toxicity. From a long laboratory experiment, growth abilities and physiological adaptations of cells were characterized over a large temperature gradient, using

strains isolated from the Bay of Biscay (NE Atlantic) and the Bay of Villefranche-sur-Mer (NW Mediterranean Sea). They allowed for the definition of strain-specific favorable and optimal temperatures that give crucial information about the thermal niche at the clone, population and species level. They also revealed strategies of physiological changes that both *O. cf. siamensis* and *O. cf. ovata* can use for their response to thermal stress, including the release of toxin compounds.

2. Material and methods

2.1. Culture conditions

2.1.1. *Ostreopsis* strains and maintenance of stock cultures

Strains of *O. cf. siamensis* were isolated from five different sites located along the southeastern coast of the Bay of Biscay (North-East Atlantic). The geographical repartition of these isolation sites straddled the frontier between Spain and France (Figure 35). Three or four strains of *O. cf. siamensis* were used per site, reaching 17 strains in total (Table 1). For *O. cf. ovata*, three strains were used, all originated from the same site in the bay of Villefranche-sur-Mer (France, North-Western Mediterranean; Figure 1) and obtained from the Mediterranean Culture Collection of Villefranche (MCCV, <http://www.embrc-france.fr>) (Table 3). Strains of *O. cf. siamensis* and *O. cf. ovata* were grown in K media (Keller *et al.*, 1987) without silicate using autoclaved and filtered seawater at salinities of 35 and 38, respectively. Stock cultures were maintained at 22°C, under a 12:12 light/dark cycle with an irradiance intensity of 150-200 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$. The temperature of 22°C, known to be favorable for the growth of both *O. cf. siamensis* and *O. cf. ovata* in their respective isolation sites, was considered as a control condition for the rest of the experiment.



Figure 35: Map of origin locations of strains of *Ostreopsis cf. siamensis* (yellow) and *Ostreopsis cf. ovata* (orange) used in this study

Strain ID	Location	Location ID	Latitude (°N)	Longitude (°E)	Country	Isolation date	Species
C3	Castro	C	43.375686	-3.212714	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
C5	Castro	C	43.375686	-3.212714	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
C11	Castro	C	43.375686	-3.212714	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
Z1	Zierbena	Z	43.353911	-3.078913	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
Z6	Zierbena	Z	43.353911	-3.078913	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
Z7	Zierbena	Z	43.353911	-3.078913	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
Z8	Zierbena	Z	43.353911	-3.078913	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
D8	Donostia	D	43.319686	-2.004416	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
D9	Donostia	D	43.319686	-2.004416	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
D13	Donostia	D	43.319686	-2.004416	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
H1	Hondarribia	H	43.391588	-1.790892	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
H7	Hondarribia	H	43.391588	-1.790892	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
H8	Hondarribia	H	43.391588	-1.790892	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
H10	Hondarribia	H	43.391588	-1.790892	Spain	August 2018	<i>Ostreopsis cf. siamensis</i>
SJL1	Saint-Jean-de-Luz	SJL	43.398342	-1.664600	France	August 2019	<i>Ostreopsis cf. siamensis</i>
SJL2	Saint-Jean-de-Luz	SJL	43.398342	-1.664600	France	August 2019	<i>Ostreopsis cf. siamensis</i>
SJL3	Saint-Jean-de-Luz	SJL	43.398342	-1.664600	France	August 2019	<i>Ostreopsis cf. siamensis</i>
MCCV54	Villefranche-sur-Mer (Rochambeau)	R	43.693011	7.308466	France	August 2014	<i>Ostreopsis cf. ovata</i>
MCCV98	Villefranche-sur-Mer (Rochambeau)	R	43.692956	7.308602	France	June 2017	<i>Ostreopsis cf. ovata</i>
MCCV99	Villefranche-sur-Mer (Rochambeau)	R	43.692956	7.308602	France	June 2017	<i>Ostreopsis cf. ovata</i>

Table 3: Information of strains of *Ostreopsis cf. siamensis* and *Ostreopsis cf. ovata* used in this study

2.1.2. Thermal acclimation experiment

The growth and biomass production of *Ostreopsis* strains were carried out in 25 cm², 75 cm² and 150 cm² polystyrene culture flasks (TPP® Cell culture flask, Sigma-Aldrich), testing gradual temperatures that ranged from 14°C to 28°C. Under the control temperature of 22°C, all the 20 strains were used and characterized by estimations of growth rate and toxicity, using both chemical analyses in a search for palytoxin-like compounds and biotests on *Artemia franciscana*. Among these strains, nine *O. cf. siamensis* strains (Z1, Z7 and Z8 from Zierbena; H1, H8 and H10 from Hondarribia; SJL1, SJL2 and SJL3 from Saint-Jean-de-Luz) and three *O. cf. ovata* strains (MCCV 54, MCCV 98 and MCCV 99 from Rochambeau) were selected. They were characterized in more details in terms of growth abilities and physiology and gradually acclimated to increasing (25°C, 28°C) and decreasing (19°C, 16°C, 14°C) levels of temperature until cultures were no longer capable of surviving.

Estimations of growth rates were used to validate acclimation at each step of the thermal gradient. They were conducted for successive cultures, but never for the first cell transfer after a change in temperature. Additional parameters, based on direct measurements or production and collection of biomasses, were only estimated on cultures that were considered acclimated, once a high and stable growth rate was measured over three successive cell transfers. In the end, these additional estimations were performed 25 days or later after any change of temperature. They focused on the characterization of growth abilities (photosynthetic parameters), on potential signs of cellular stress and acclimation to non-optimal growth conditions (profiles of pigments and lipids, potential accumulation of reactive oxygen species, nitrite excretion) and on toxicity using biotests on *A. franciscana*. All these additional parameters were estimated during the exponential phase of growth of the cultures.

The acclimation experiment was conducted in two successive sets, characterizing three strains from two sites each. A full set of measurements was completed for the six *O. cf. siamensis* strains H1, H8, H10, Z1, Z7 and Z10, originating from either Hondarribia (H) or Zierbena (Z). The second set of the experiment is still ongoing. It will allow for the characterization of three *O. cf. siamensis* strains from Saint-Jean-de-Luz (SJL1, SJL2, SJL3) and the three *O. cf. ovata* strains from Rochambeau (M54, M98, M99). Only the results of the first set of acclimations are shown and discussed in the present manuscript.

2.2. Cell abundance quantification and growth rate calculation

Every two or three days, cultures were homogenized after two gentle washes of the bottom of the flask (using a pipette and the culture itself) and a subsequent gentle agitation of the flask. Sub-samples (4 mL) were then collected and immediately fixed with acidic Lugol (2% vol./vol.). They were kept less than three months at room temperature before analysis. Cell quantification was performed in triplicates using either a Utermöhl chamber (Utermöhl, 1958) under an inverted light microscope (AxioVert 135, x100, Zeiss, Oberkochen, Germany) or a Nageotte hemocytometer (Hecht Assistent, Sondheim vor der Rhön, Germany) and a light microscope (Olympus® BX51, x100, Tokyo, Japan), depending on the volume used for observation. Successive phases of microalgal growth were identified from the temporal variations of cell counts. The maximal growth rate (μ_{\max}) was specifically calculated over the exponential growth phase, from a linear regression of the logarithm of cell concentrations expressed as a function of time, following the equation:

$$\mu = \frac{\ln N_1 - \ln N_0}{t_1 - t_0}$$

Where μ is the growth rate (d^{-1}) and N_0 and N_1 are the cell abundances ($cell.mL^{-1}$) at time t_0 and t_1 (d). Replicates of growth rate estimations were obtained successively one after another, over successive cell transfers and growth curves.

2.3. Estimations of microalgal toxicity

2.3.1. *Ostreopsis* cf. *siamensis* toxin extraction and identification by LC-MS/MS

Cultures of *O. cf. siamensis* were grown at 22°C in 150 mL for biomass production. Microalgal cells were collected during the exponential phase. Sub-samples of the cultures were taken for cell concentration estimations, in order to calculate the toxin content per cell. Cells were harvested by centrifugation (3000 g for 10 min) and, in order to get sufficient biomass for toxin analyses, the cell pellets of between 5 and 10 batches of culture were pooled to obtain between 1.3 et 11 million cells depending on the strain. They were stored at -80°C until extraction. Toxin extraction was performed according to Chomérat *et al.* (2019). Pellets were

recovered in 1.5 mL MeOH 50%, homogenized, sonicated in an ice bath for 10 min, centrifuged and ultrafiltered (0.2 μm , Nanosep MF, Pall, at 10 000 g). Toxin analyses were conducted using three LC-MS/MS methods that allowed for the detection of 2 palytoxins (palytoxin and 42-OH palytoxin), 12 ovatoxins (-a to -k) and other palytoxin-like compounds including 4 ostreocins (-a, -b, -d and -e1) and 2 mascarenotoxins (-a and -c) (Chomérat *et al.*, 2019). The separation of toxins was performed following García-Altare *et al.* (2015) a liquid chromatography system (UFLC XR, Shimadzu) using a Poroshell 120EC-C18 column (100 x 2.1 mm x 2.7 μm , Agilent) at 25°C and an injection volume of 5 μL of sample extract. Mobile phases composing the elution gradient were composed of water (eluent A) and acetonitrile 95% (eluent B), both containing 0.2% acetic acid. The elution gradient consisted in 28-29% B over 10 min, 29-30% B over 5 min, 30-100% B over 1 min and maintained 5 min, then 100-28% B over 1 min and finally maintained 4 min for equilibration. Detections of toxins were then performed following Chomérat *et al.* (2019) on a triple quadrupole/ion trap QTRAP mass spectrometer (QTRAP 4000, ABSciex) using Multiple Reaction Monitoring (positive mode of acquisition). Quantification was performed relative to the only available standard Palytoxin (Wako Chemicals GmbH, Germany) with a 6-point calibration curve and assuming similar molar responses between the standard and the PLTX-like molecules. Limit of detection and quantification were 20 and 30 ng mL⁻¹ for PLTX standard.

2.3.2. *Artemia franciscana* toxicity bio-assay

The toxicity of *Ostreopsis* strains was assayed using the *Artemia franciscana* bio-assay. Approximately 100 mg of *A. franciscana* cysts (Sep-Art Artemia cysts, Ocean Nutrition®, Newark, CA, USA) was hatched in 1 L of filtered seawater at salinity of 35 and a temperature of $22 \pm 1^\circ\text{C}$, under continuous light exposition ($100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and slight moderate aeration. After 40 hours of incubation, *Artemia franciscana* larvae had moulted into metanauplii instars (stages 2-3), considered to be the most sensitive stage regarding ecotoxicological bio-assays (Nunes *et al.*, 2006). Hatched nauplii were separated from remaining cysts by attracting them with light. They were collected close to the surface using a Pasteur Pipette. Incubations of the bio-assay were conducted in 12-wells plates. Five nauplii were transferred per well, each well being previously filled with 3 mL of filtered seawater and a defined concentration of *Ostreopsis* cells. Four different concentrations of *O. cf. siamensis* and *O. cf. ovata* were tested: 0 (for

blank values), 4, 40 and 400 cells.mL⁻¹. Three replicated incubations were performed for each *Ostreopsis* concentration, on the same plate. Plates were placed in the dark to reduce nauplii mortality due to their strong positive phototactism and to limit *Ostreopsis* cell division in order to control levels of microalgal cell concentrations. Nauplii mortality was measured at 2, 4, 6, 8, 24 and 48 hours of exposure to *Ostreopsis* cells using a binocular magnifier (Olympus® SZX10, x10, Tokyo, Japan). The death of nauplii was validated when no movement or reaction was observed after 10 seconds. Some bio-assays were performed in replicates in order to verify the stability of estimations of mortality rate using the defined protocol; this was assessed for six strains à 22°C, three à 25°C and three at 19°C.

2.4. Maximum quantum yield of PSII

The fluorescence-based maximum quantum yield of photosystem II (F_v/F_m) of *Ostreopsis* cells was determined using a handled Pulse Amplitude Modulation (PAM) fluorometer (Aquapen-C AP-C 100, Photon Systems Instruments, Drassov, Czech Republic). Measurements of *in vivo* fluorescence were performed with saturation pulses of blue light at 440 nm on dark-adapted (15 minutes) cells. Values of F_v/F_m were acquired from the manufacturer's software using the following equation:

$$\frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m}$$

Where F_v is the variable fluorescence; F_0 and F_m are the minimum and maximum, respectively, *in vivo* fluorescence in a dark-adapted state (Genty *et al.*, 1989).

A short experiment was conducted in order to assess the variability of the fluorescence signal and the F_v/F_m measurement depending on the concentration of *Ostreopsis* cells. For this purpose, a dilution cascade was performed from a dense culture of *O. cf. siamensis*, maintained at 22°C, and measurements were done on graded concentrations ranging from 22000 to 2750 cell.mL⁻¹.

2.5. Pigment analyses through High-Performance-Liquid-Chromatography (HPLC)

One culture of 150 mL corresponding to approximately 1x10⁵ to 6x10⁵ cells in exponential phase was filtered on 25 mm filters (0.22 µm pore size) to collect biomass for pigment

analyses. Filters were immediately stored at -20°C and kept at this temperature until analysis. Extractions of pigments from filters were performed in the dark. Filters were placed into glass test tubes and 3 mL of a mix solution (internal standard, composed of methanol and tocopherol) was added. Tubes were vortexed and placed at -20°C during 30 minutes, sonicated for 10 seconds then placed again at -20°C for 30 minutes. Extracts were vortexed and filtered through 25 mm diameter Polyvinylidene fluoride (PVDF) syringe filters for cellular debris removal. Then, 500 µL of filtrate were added to 500 µL of tetrabutylammonium acetate (TBAA) buffer in 2 mL amber tubes and vortexed (Mantoura and Llewellyn, 1983). Final extracts were analyzed through HPLC using an Agilent HPLC-1100 (Agilent Technologies, Santa-Clara, CA, USA) coupled to the automatic sample changer Agilent 1260 infinity, according to (Van Heukelem and Thomas, 2001). Optical densities for solvent (blank, 70% methanol – 30% TBAA) and extracts were measured for large diversity of pigments: 19'-but-fucoxanthin; 19'-hex fucoxanthin, Alloxanthin, Antheraxanthin, Astaxanthin, beta-carotene, Canthaxanthin, Chlorophyll-a, Chlorophyll-b, Chlorophyll-c2, Chlorophyll-c2-MGDG, Chlorophyll c3, Chlorophyllide-a, Chlorophyllide-b, Diadinoxanthin, Diatoxanthin, Divinyl Chlorophyll-a, Fucoxanthin, Lutein, Neoxanthin, Peridinin, Pheophytin-a, Pheophorbide-a, Prasincoxanthin, Violaxanthin, Zeaxanthin, Mixed pigments MIX-1 and Dincoxanthin. Pigment quantifications were estimated using standard curve determined for each pigment.

Filters were collected for every experimental condition. Some analysis have not been finalized in time, however. Only the pigment profile of control samples (at 22°C) are described in the present manuscript.

2.6. Reactive Species of Oxygen (ROS) production

Accumulation of ROS in *Ostreopsis* cells was assayed using the membrane permeable non-fluorescent probe 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (carboxy-H₂DCFDA, Invitrogen™, Carlsbad, CA, USA). Sub-samples of 0.5 mL of cultures in exponential phase were used for running incubations with carboxy-H₂DCFDA at a final concentration of 5 µM, for 30 min at room temperature. After incubation, the green fluorescence produced by the oxidation of carboxy-H₂DCFDA with ROS was immediately observed under a VivaTome fluorescence microscope (x100, Zeiss, Oberkochen, Germany). More than 100 cells were carefully observed

per sample and the percentage of positively stained cells, showing a high accumulation of intracellular ROS, was calculated.

2.7. Nitrites (NO₂⁻) excretion

Sub-samples of approximately 20 mL of culture in exponential phase were taken and filtered on 0.22 µm. Each filtrate was collected and stored at -20°C until analysis. Concentrations of nitrate and nitrite were estimated following the method defined in Aminot and K erouel (2007) and using a SEAL AutoAnalyzer AA3 HR (SEAL Analytical, Mequon, WI, USA).

2.8. Statistical analysis and modeling

For toxicity analysis, mortality rates of *A. franciscana* were used to calculate LC₅₀ (and LC₃₀) after 24 and 48 h of exposure, which is the cell concentration of *Ostreopsis* cells causing 50% (and 30%) of nauplii mortality. These values were estimated following probit regression models using a sigmoidal log-response curve from the R package "ecotox".

For the analysis of variations of growth rate as a function of temperature, the data set was fitted to several growth models: four of them are described by (Tian, 2006) and so called the "Thebault", "Beta", "exponential product" and the "modified exponential" non-linear functions, the last one was defined by Bernard and R mond (2012). Among those models, the non-linear "exponential product" function (Kamykowski and McCollum, 1986) generated the best fit ($r^2 = 0.76$) compared to others ($r^2 \leq 0.29$). This function is described by the following equation:

$$\mu(T) = (1 - e^{-a(T-T_{min})})(1 - e^{-b(T_{max}-T)})$$

Where μ is the growth rate at a T given temperature (°C), T_{min} and T_{max} are the lowest and highest temperatures under which the corresponding biological rate is zero, and a and b are constants.

In this study, differences in growth rates, toxicity, pigment amounts and F_v/F_m among *Ostreopsis* strains under the different temperatures tested were assessed using non-parametric (Kruskal-Wallis and Friedman) ANOVAs. *Post-hoc* multiple pairwise comparison tests (Dunn's and Wilcoxon's signed-rank tests using Benjamini-Hochberg correction) were also performed when significant differences were observed ($p < 0.05$). Relationships between

variables were tested using the non-parametric Spearman correlation. All statistical analyses were conducted in R Studio (R Core Team, 2019).

3. Results

3.1. Growth rates

Under the temperature of 22°C, the 17 strains of *O. cf. siamensis* and the 3 strains of *O. cf. ovata* were characterized by mean growth rate in exponential phase that ranged from 0.5 d⁻¹ (*O. cf. ovata* strain M99) to 1.26 d⁻¹ (*O. cf. siamensis* strain Z6) (Figure 36). Out of these 20 strains, 5 showed μ_{\max} higher than 0.9 d⁻¹ and were all of the species *O. cf. siamensis*. These strains did not originate from the same site but were isolated from either Zierbena, Donostia or Hondarribia, three Spanish sites. For these sites in particular, a strong variability between strains was noted in terms of growth abilities, with, for example, values of strain specific μ_{\max} ranging from 0.65 to 1.26 d⁻¹ for the site of Zierbena or from 0.75 to 1.19 d⁻¹ for Donostia. In the end, when pooling μ_{\max} values of the three to four strains per site, the non-parametric Kruskal-Wallis ANOVA revealed an overall significant difference between the sites in terms of growth abilities (Chi² = 13.1, df = 5, p < 0.05). The post-hoc Dunn's multiple comparison test failed to identify significant difference between groups of strains, however, even though some comparisons between strains of *O. cf. ovata* (site of Rochambeau) and *O. cf. siamensis* (sites of Zierbena or Donostia) were approaching significance (p = 0.0543).

The six strains used for the first set of acclimations were able to growth and be maintained at temperatures ranging from 16°C to 25°C, with a maximal growth rate obtained at 22°C (Figure 37). Out of this range, some strains showed a drastic response to the last change in temperature (+3°C or -2°C), characterized by a sudden stop of swimming activity and cell division that ended in cell death after few weeks. Few strains showed some growth abilities under 14°C and 28°C, with an activation of cell division observed over two or three successive culture transfers. Under 28°C, no measurement of growth rate was done on the two strains (Z7 and Z8) that were able to growth over few weeks before dying. Two successive estimations of growth rate were measured at 14°C for five strains (H1, H8, H10, Z1, Z7, Z8) and averaged at 0.09 d⁻¹ ± 0.03 d⁻¹ (Figure 37), but these strains did not reach acclimation either and the cultures finally stopped their growth and died.

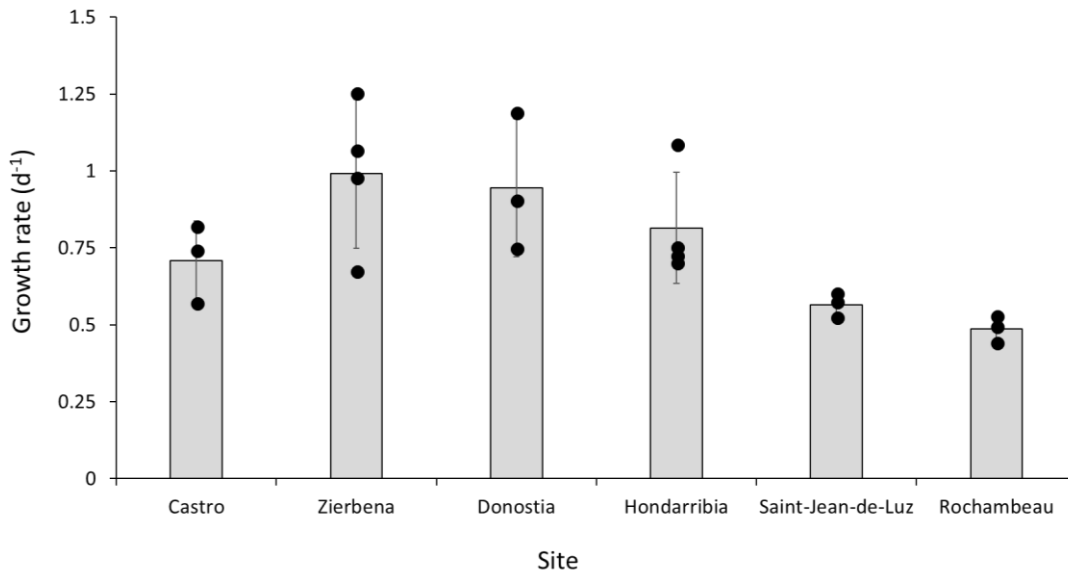


Figure 36: Mean growth rates of *O. cf. siamensis* and *O. cf. ovata* at each site at 22°C. Black dots represent the average growth rate of each strain obtained from three to four replicated estimations.

Maximal abundances reached in cultures followed the same pattern than growth rate as a function of temperature. Highest cell concentrations were obtained at 22°C (always exceeding 10,000 cells/mL), high levels of maximal abundance were observed at 19 and 25°C (always exceeding 5,000 cells/mL), intermediate values were recorded at 16°C (comprised between 1,600 and 7,500 cells/mL) and cell concentrations never exceeded 500 cells/mL at 14°C.

A first set of analysis of variations of growth rate as a function of temperature was conducted for each site, H or Z, using strain specific μ_{\max} data of H1, H8 and H10 for H and Z1, Z7 and Z8 for Z. For both sites H and Z, the non-parametric Friedman's ANOVAs revealed significant overall differences in growth rate depending on the temperature (H: $\text{Chi}^2 = 12$, $\text{df} = 4$, $p < 0.05$; Z: $\text{Chi}^2 = 10.2$, $\text{df} = 4$, $p < 0.05$). However, post-hoc multiple comparison test failed to identify which temperatures were leading a significant difference (Wilcoxon signed-rank, $p > 0.05$). Similar patterns of variations in mean growth rate was observed as a function of temperature for the two sites and no significant difference between the mean growth rates of sites H and Z was noted for each temperature (Wilcoxon signed-rank test, $W = 9$, $p > 0.05$). Thus, growth rates of the six strains were then pulled together in order to give a mean representation of growth capacities of *O. cf. siamensis* in the Bay of Biscay in relation to temperature (Figure 37). With this larger set of data ($n = 6$), an overall significant difference was found between *O. cf. siamensis* growth rates depending on temperature using the non-

parametric Friedman's ANOVA ($\text{Chi}^2 = 22$, $\text{df} = 4$, $p < 0.001$). Post-hoc multiple comparisons Wilcoxon signed-rank tests showed a significant decrease ($p < 0.05$) in growth rates at each temperature step, from 22°C to 14°C, and between 22°C and 25°C. Growth rates at 25°C showed no significant difference with the ones measured at 16°C and 19°C.

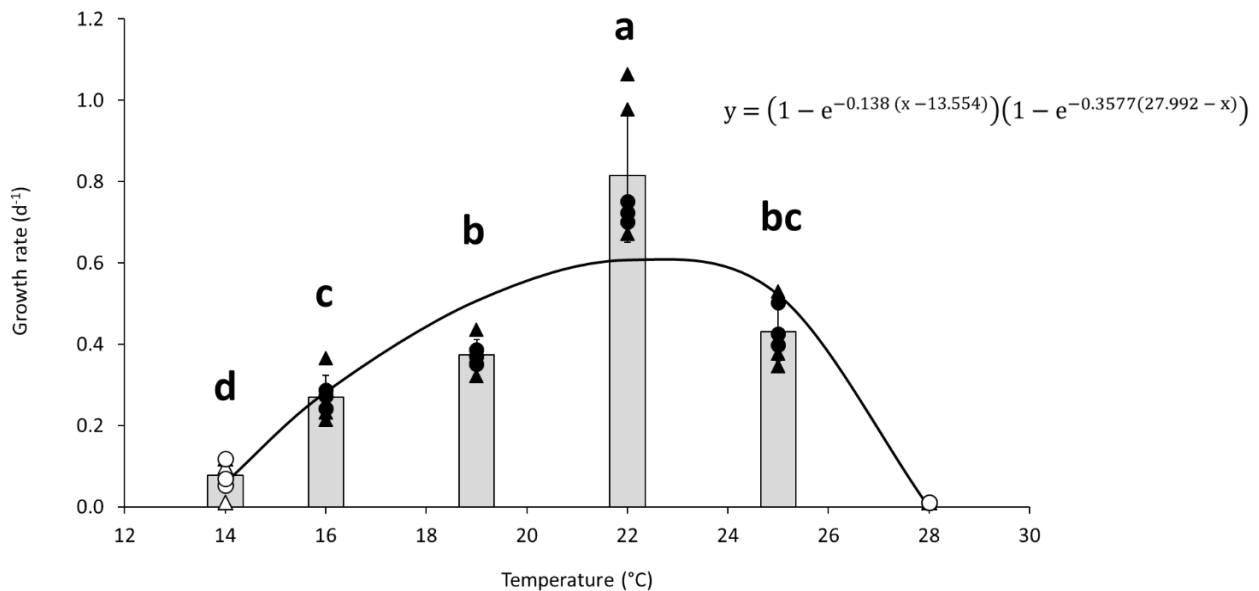


Figure 37: Growth rates of *O. cf. siamensis* as a function of temperature. Dots and triangles represent the mean growth rate of each of the three strains from sites H and Z, respectively. Black symbols are associated with values of acclimated cultures whereas white symbols are for non-acclimated ones. Values at 14°C correspond to growth rate measurements and values at 28°C were arbitrarily fixed at 0.01d⁻¹. Bars and standard deviations represent the mean response of the six strains, H1, H8, H10, Z1, Z7 and Z8. Different letters are indicating a significant difference (Wilcoxon signed-rank, $p < 0.05$) between mean growth rates of the pooled data set ($n=6$). The black line represents the non-linear exponential product function describing the growth response.

In order to go further on the analysis of variations of growth rate of *O. cf. siamensis* as a function of temperature, a growth model was run on the data set of μ_{max} that pooled results from the six strains. This set was complemented with the non-acclimated values of μ_{max} at 14°C and a theoretical growth rate of 0.01 d⁻¹ at 28°C in way to simulate the poor growth abilities of strains under these extreme temperature levels. The best fit was obtained with a model using an exponential product function. The convergence of the model was optimal for the following values of parameters: 13.55°C and 27.99°C for T_{min} and T_{max} (the low and high temperatures under which $\mu = 0$), respectively, and the two constants $a = 0.138$ and $b = 0.357$ (Figure 37). The optimal temperature associated with the highest growth rate was estimated at 22.45°C by the model.

3.2. *Ostreopsis* cf. *siamensis* toxicity

The analyses conducted through LC-MS/MS on the 17 strains of *Ostreopsis* cf. *siamensis* grown at 22°C did not reveal any presence of classical toxins associated with the *Ostreopsis* genus. The bioassay on *A. franciscana* nauplii did reveal a toxicity in every strains of *O. cf. siamensis*, however, with estimations of LC₅₀ lower than 25 cell/mL over 48h of exposure for all strains except one (Table 4). For the strain SJL1, a correct estimation of LC₅₀ could not be obtained due a mortality of *Artemias* strongly higher at 40 cell/mL than 400 cell/mL of *Ostreopsis*. For the *O. cf. ovata* strains tested with the bioassay at 22°C, two strains were characterized with a LC₅₀ lower than 4 cell/mL over 48h of exposure and one with a LC₅₀ lower than 50 cell/mL (Table 4). No death of *A. franciscana* nauplii was observed in any control conditions (filtered sea water and K-Si medium) over the incubation duration.

During the acclimation experiment, *O. cf. siamensis* toxicity on *A. franciscana* nauplii was assessed for 16°C, 19°C, 22°C and 25°C, the four levels of temperature for which cultures were successfully acclimatized. Mortality of *A. franciscana* was observed for every strain, at least after 48 h of exposure, at each of the three *O. cf. siamensis* cell concentrations and for every temperature tested. No death of *A. franciscana* nauplii was observed in any control conditions. A positive significant correlation was found between mortality and both cell concentration (Spearman, $r_s = 0.322$, $p < 0.001$) and time of exposure (Spearman, $r_s = 0.637$, $p < 0.001$) (Figure 38).

Temperature	Strain	LC ₅₀ (cell/mL)	
		24 h	48 h
16°C	H1	(NaN)	162.92
	H8	(2894)	111.46
	H10	(13652)	302.33
	Z1	(762)	(3541)
	Z7	(787622)	(1074)
	Z8	(NaN)	(2603)
19°C	H1	(3496697)	(238)
	H8	(5134)	(1121)
	H10	(NaN)	34.08
	Z1	(NaN)	28.52
	Z7	(580102)	6.54
	Z8	(16052)	(492)
22°C	H1	117.37	0.59
	H8	195.17	4.53
	H10	396.35	3.55
	Z1	79.00	0.83
	Z7	(3258)	0.37
	Z8	50.52	1.69
	H7	17.42	2.03
	Z6	35.53	4.27
	C3	55.29	6.98
	C5	(312)	2.44
	C11	55.10	0.05
	D8	32.67	4.04
	D9	(8223)	7.74
	D13	236.15	1.03
	SJL1	(NaN)	(19819)
	SJL2	295.46	22.89
SJL3	(17813)	24.40	
MCCV54	(1576)	46.00	
MCCV98	(510)	1.07	
MCCV99	(2977)	0.04	
25°C	H1	(1359)	0.94
	H8	(1435)	18.20
	H10	53.22	3.41
	Z1	(1081)	2.72
	Z7	325.28	3.49
	Z8	80.65	0.34

Table 4: LC₅₀ at 24 h and 48 h of exposure and LT₅₀ at 400 cells/mL for each strain at various temperatures on *Artemia franciscana* nauplii. LC₅₀ values in brackets were calculated from the probit regression-model with mortality values not reaching at least 50% after 48 h and exhibiting nauplii death only after 48 h.

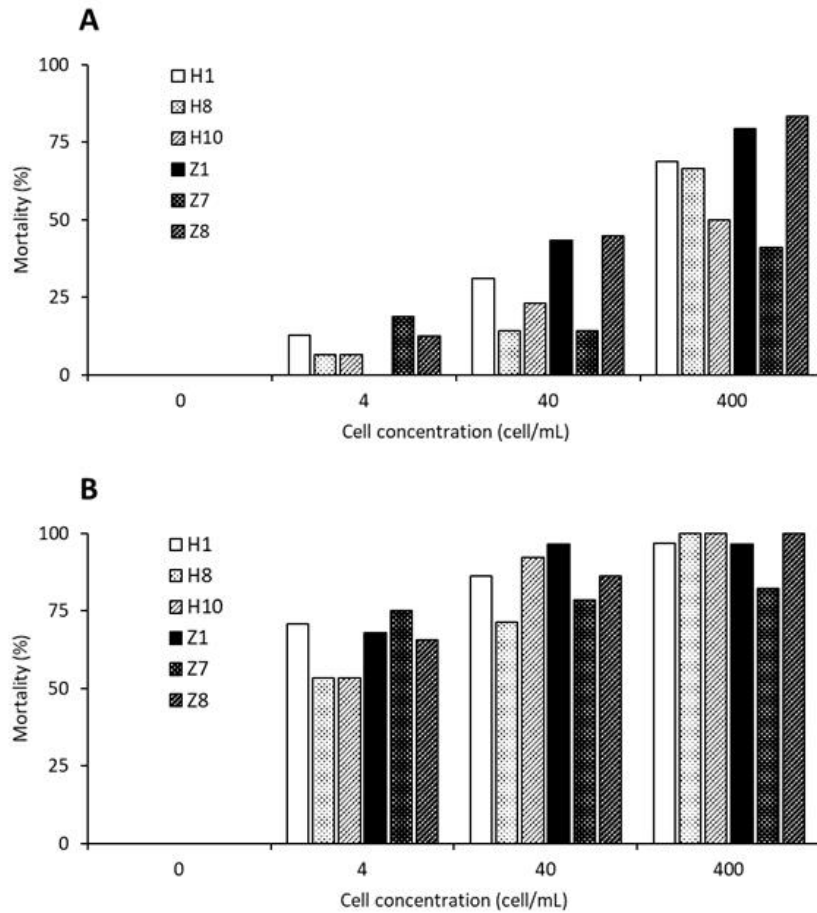


Figure 38: Percentage of mortality of *Artemia franciscana* nauplii after (A) 24 h and (B) 48h of exposure to 0, 4, 40 and 400 cells/mL of six different strains of *Ostreopsis cf. siamensis* (H1, H8, H10, Z1, Z7 and Z8) grown at 22°C. Data correspond to averages of measurements that were done over three replicated incubations.

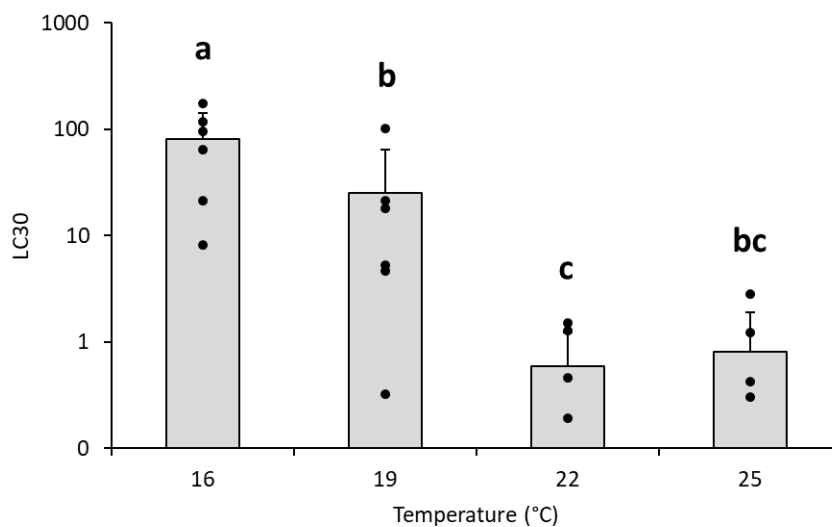


Figure 39: LC₃₀ after 48 h of exposure of *A. franciscana* nauplii to *Ostreopsis cf. siamensis* (strains H and Z) under various temperature conditions. Dots represent strain specific values and bars the average response of the six strains, with associated standard variations. Letters indicate a significant difference (Wilcoxon signed-rank, $p < 0.05$) between means of groups.

3.3. Physiological stress indicators

3.3.1. F_v/F_m

A pre-experiment based on a dilution cascade of a dense *O. cf. siamensis* culture allowed for the estimation of the stability and precision of F_v/F_m measurements as a function of *Ostreopsis* cell density using the Aquapen-C AP-C 100. Between 2,250 cell/mL and 22,000 cell/mL, variations of F_m as a function of cell concentration showed a strong positive linear correlation (Pearson, $r^2 = 0.9985$, $p < 0.001$) and F_v/F_m values remained stable only above a F_m threshold of 1000 (Figure 40). Under this threshold, F_v/F_m ratio was underestimated of more than 5%.

Measurements of F_v/F_m were performed in exponential phase for the strains used during the acclimation experiment and for the temperatures 16°C, 19°C, 22°C and 25°C. Even if dense subsamples were used for all estimations, F_m values obtained at 16°C never exceed 400 and was lower than 1000 for some cases at 19°C and 25°C. In order to avoid using data that were significantly underestimated, values of F_v/F_m were only compared between 19°C, 22°C and 25°C. Among those three temperatures and after pooling together the F_v/F_m values of the six strains, the non-parametric Friedman's ANOVA revealed no significant difference ($\text{Chi}^2 = 1.3$, $\text{df} = 2$, $p > 0.05$) in F_v/F_m values measured.

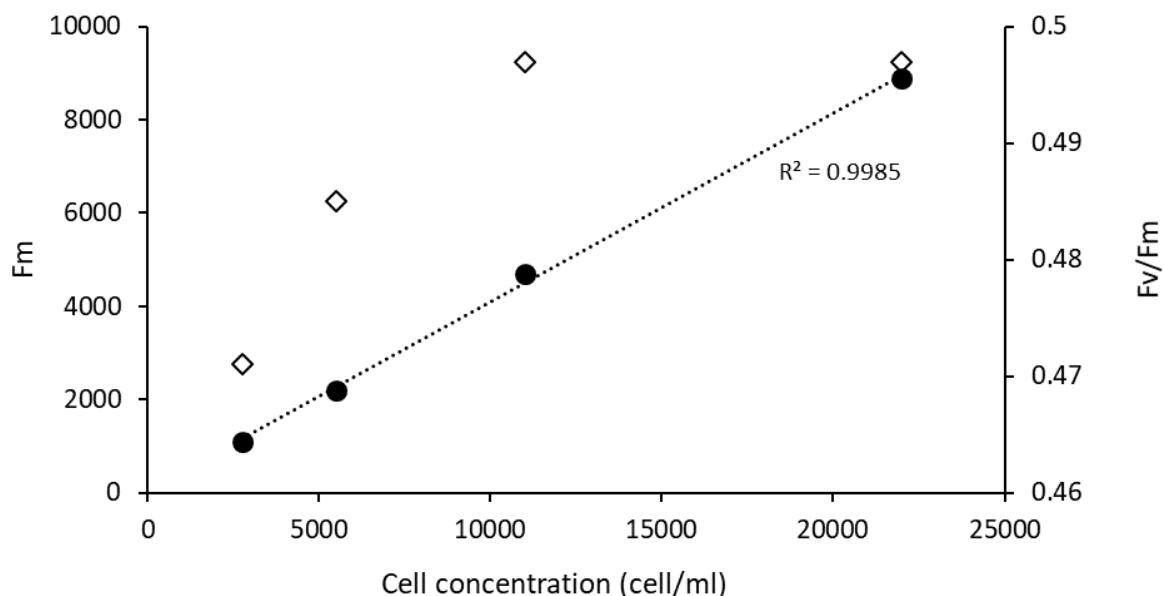


Figure 40: F_m (black dots) and F_v/F_m (white squares) values relatively to the cell concentration of *O. cf. siamensis* for the pre-experiment in exponential phase of growth, under graded temperature levels. The dotted line represents the linear correlation (Pearson, $r^2 = 0.9985$, $p < 0.001$) between the F_m value and the cell concentration.

3.3.2. ROS production

Microscopic observations of intracellular ROS accumulation were performed for the six strains used during the first set of acclimations (H1, H8, H10, Z1, Z7 and Z8) at 16°C, 19°C, 22°C and 25°C. For every samples, the percentage of positively stained cells, that showed a high accumulation of intracellular ROS, never exceed 5%. Thus, *O. cf. siamensis* cells acclimated to temperatures ranging from 16°C to 25°C had a metabolism that avoided strong oxidative stress.

3.3.3. Nitrites excretion

Concentrations of nitrites (NO_2^-) and nitrates (NO_3^-) measured during the exponential phase of growth of acclimated cultures of the six strains (H1, H8, H10, Z1, Z7 and Z8) were compared to values obtained for the initial enriched medium (K-medium). Differences allowed for estimation of the quantity of nitrogen taken up by the cells as NO_3^- since the cell transfer and the quantity of NO_2^- excreted over the same duration. Estimations of nitrite excretion was expressed as the ratio between the quantities (in μmolN) of excreted NO_2^- relatively to consumed NO_3^- (Figure 41).

The non-parametric Friedman's ANOVA revealed an overall significant difference in NO_2^- excretion / NO_3^- consumption ($\text{Chi}^2 = 8.4$, $\text{df} = 3$, $p < 0.05$) among temperatures. The post-hoc multiple comparison Wilcoxon signed-rank test showed no significant difference ($p > 0.05$), however, failing to identify which temperatures affected this ratio more than one another (Figure 40). This mainly came from a strong variability of response between strains. When considering strain specific values of ratio, it could be noted that four cultures showed values out of the range of the control conditions at 22°C: they corresponded to cultures maintained at 19°C (from sites Z (Z1, Z7) and H (H1)) and at 16°C (H1).

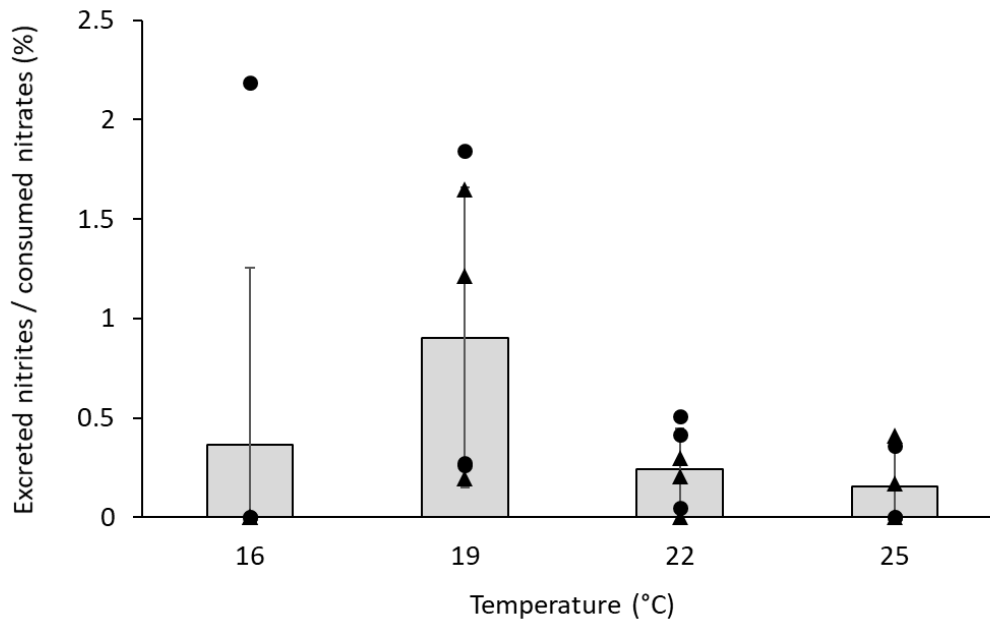


Figure 41: Mean ratios of percentage of excreted NO_2^- (in μmolN) relatively to consumed NO_3^- (in μmolN) for acclimated cultures of *O. cf. siamensis* in exponential phase of growth, under graded temperature levels. Dots and triangles represent the mean ratios of strains from sites H and Z, respectively. Bars and standard deviations represent the average response of the six strains.

3.3.4. Pigment composition

Different chlorophylls and carotenoids were identified in the pigment profile of *O. cf. siamensis*, with an overall profile corresponding to chloroplast Type 1 (Zapata *et al.* 2012): chlorophyll c_2 (chl c_2) was present in addition to chlorophyll a (chl a) and carotenoids included peridinin (peri), diadinoxanthin (Dd), dinoxanthin (Dn) and β -carotene. Diatoxanthine (Dt) was also measured, showing cellular concentrations seven times lower than Dd. Other specific ratios between chlorophylls and carotenoids were as followed: chl c_2 /chl a = 0.53, peri/chl a = 1.74, Dd/chl a = 0.53 and Dt/(Dd+Dt) = 0.13.

4. Discussion

4.1. Optimal growth temperature for *O. cf. siamensis*

The characterization of optimal growth temperature is crucial for the investigation of species ecology, especially in a context of global warming where marine phytoplankton communities and productivity will be affected by rising temperatures (Behrenfeld *et al.*, 2006; Morán *et al.*, 2010). For the first time, a thermal acclimation experiment was conducted on *O. cf. siamensis* and identified the temperature of 22°C as the most optimal for the growth of the six strains tested (all isolated from the Bay of Biscay), both in terms of growth rate and maximum yield. In balanced growth conditions, a modeling approach can be used to describe microalgal growth as a function of temperature (Tian, 2006; Bernard and Rémond, 2012; Grimaud *et al.*, 2017) and can allow for the estimation of crucial parameters, such as the minimal, maximal and optimal temperatures when using asymmetric thermal growth curve (Kingsolver, 2009). Non-monotonous functions describing asymmetric curves are particularly adapted to the description of biological rates observing an optimal value above which rates are decreasing (Tian, 2006) and should thus be preferred over monotonous functions such as the Eppley curve (Eppley, 1972; Bissinger *et al.*, 2008). The “exponential product” (Kamykowski and McCollum, 1986) model used in this study estimated a theoretical temperature for optimal growth of 22.45°C for *O. cf. siamensis*, which is very close to 22°C where maximum growth rates were recorded. This optimal temperature around 22°C is in the low range of values used for characterizing other *Ostreopsis* species from laboratory studies. Previous works reported maximum growth rates of *Ostreopsis* cells at temperatures ranging from 22°C to 30°C for *Ostreopsis cf. ovata* and *Ostreopsis* sp.1, sp.6 and sp.7 (Yamaguchi *et al.*, 2012; Tanimoto *et al.*, 2013; Tawong *et al.*, 2015; Carnicer *et al.*, 2016). In accordance with these reports, Drouet *et al.* (in prep) reported a range from 21°C to 25°C for estimations of maximum net growth rates over a long-term monitoring dataset on *O. cf. ovata* blooms in the Mediterranean Sea (Monaco).

Growth abilities of 17 strains of *O. cf. siamensis* were characterized at 22°C and showed a strong variability between strains, with μ_{\max} varying between 0.57 and 1.25 d⁻¹. For the Spanish sites of Zierbena, Hondarribia and Donostia in particular, five strains showed $\mu_{\max} > 0.9$ d⁻¹, corresponding to high values compared to rates usually reported for dinoflagellates.

Dinoflagellate growth rates are mostly lower than 1.0 d^{-1} , when diatoms and various flagellate taxa are known to often exceed this threshold value (Smayda, 1997; Sarthou *et al.*, 2005; Litchman *et al.*, 2007). On a global point of view, low growth ability is reported as one of the main functional traits of dinoflagellates. It may be partly explained by poor abilities of dinoflagellate cells to take up inorganic nitrogen sources, even if this competitive disadvantage can be partly compensated by other functional traits of this phytoplankton group such as mixotrophy or active migration ability (Litchman *et al.*, 2007). Out of these general assumptions, extremely high growth rates ($> 1 \text{ d}^{-1}$) were reported for some dinoflagellate species (Smayda, 1997), and more precisely for some clones of dinoflagellates. For instance, optimal growth rates of several clones of *Alexandrium catenella* and *Alexandrium tamarense* ranged from 0.28 to 1.0 d^{-1} and from 0.23 to 1.03 d^{-1} , respectively (Smayda, 1997; Laabir *et al.*, 2011). Thus, for a same species, many clones can fit to the general assumptions defined by the trait-based approach, but some can show specific competitive abilities. This can be the case for strains of dinoflagellates able to growth at μ_{max} close to or higher than 1.0 d^{-1} , such as the ones highlighted in the present study for *O. cf. siamensis*: these strains show growth abilities as high as the ones characterizing other phytoplankton groups, such as diatoms, coccolithophorids or green algae (Litchman *et al.*, 2007). Maximal growth ability is crucial for the species competitiveness in the field. It drives the overall dominance of diatoms over dinoflagellates in early spring, with diatoms showing significantly higher growth rate and allowing them to develop faster with a velocity strategy (Litchman *et al.*, 2007). With some clones able to compete with such strategy, *O. cf. siamensis* in the Bay of Biscay can use strain diversity and high growth abilities of some clones as significant competitive advantages, promoting a successful colonization and extension of its habitat.

When considering data reported in previous laboratory studies on *Ostreopsis* cells, such a competitive advantage in terms of maximal growth ability cannot be clearly defined for *O. cf. ovata* yet, and probably not in coastal waters of the Mediterranean Sea. Indeed, for strains of *O. cf. ovata* isolated from the Mediterranean Sea, maximum growth rates values ranged from 0.32 d^{-1} to 0.83 d^{-1} between 20°C and 26°C (Guerrini *et al.*, 2010; Pezolesi *et al.*, 2012; Scalco *et al.*, 2012; Jauzein *et al.*, 2017). Only one strain of *O. cf. ovata* from Japan was characterized by a μ_{max} higher than 1 d^{-1} , reaching 1.03 d^{-1} between 25°C and 30°C (Yamaguchi *et al.*, 2012). Growth abilities of strains of *O. cf. ovata* used in the present study will be characterized over a large gradient of temperature, and at the optimal temperature for

growth in particular. When grown at 22°C, growth rates were estimated between 0.31 and 0.85 d⁻¹, a range fully in accordance with previous reports done for Mediterranean strains of *O. cf. ovata*.

4.2. Acclimation strategy towards temperatures

Any change of temperature induces a cellular response in microalgal cells, associated with a disruption of homeostasis and activation of stress pathways (Borowitzka, 2018). In response, cells can modify their metabolism and regulate physiological functioning during an acclimation process in order to restore homeostasis. If a new state of equilibrium can be reached, cells are able to grow under the new environmental conditions; if not, cells die, potentially after the formation of quiescent stages, such as temporary cysts in *Ostreopsis* cells (Bravo *et al.*, 2012). A well-known indicator of transient stress response in phytoplankton cells is the intracellular accumulation of ROS (Lesser, 2006; Cirulis *et al.*, 2013). The over-production of ROS is commonly detected in marine organisms in response to environmental stressors such as thermal shock (e.g. for *Alexandrium tamarense* in Jauzein and Erdner, 2013). It is linked to both the role of ROS as signaling molecules in stress pathways and/or to the disruption of production sites of ROS such as the photosynthesis machinery (Jauzein and Erdner, 2013; Lesser, 2006). In the present study, six strains of *O. cf. siamensis* were slowly acclimated to various temperature levels and were able to grow and be maintained between 16°C and 25°C, without showing any signs of intracellular accumulation of ROS. These stable growth abilities coupled with a lack of over-production of ROS allowed for a validation of acclimation over the range 16°C – 25°C.

For the six strains of *O. cf. siamensis* tested, moving away from the optimal temperature of 22°C induced a net decrease in growth rate associated with acclimation to non-optimal growth conditions. Different pathways were studied in order to define physiological modifications allowing for such acclimation to temperature. They mainly dealt with photosynthesis and its disruption, through measurements of photosystem II efficiency and activation of specific nitrogen fluxes helping for the regulation of reducing power (such as NAD(P)H). Other morphologic criteria will bring complementary data on these processes. They correspond to analysis of pigment profile and lipid composition. If the associated samples

were collected all over the temperature gradient, they have unfortunately not all been analyzed yet.

Limitation of photosynthetic efficiency, estimated from F_v/F_m measurements, can be observed in phytoplankton cells in response to various stressors, including thermal stress (Aquino-Cruz *et al.*, 2018; Jauzein and Erdner, 2013; López-Rosales *et al.*, 2014). In the present study, difficulties to obtain appropriate estimations using the PAM-technology were noted for *Ostreopsis* cells and limited the survey to the first set of non-optimal temperatures, 19°C and 25°C. No significant differences in F_v/F_m values were observed under these temperatures, compared to the optimal temperature of 22°C. This shows that the efficiency of photosystem II was not significantly reduced at least under these temperatures. Thus, other steps in the photosynthesis or other nutritive pathways have to be considered for explaining the significant decrease in growth rate noted under any non-optimal thermal conditions.

Apart from modifications of the direct components of the photosynthesis machinery, acclimation of cells to environmental changes can rely on other pathways, including nitrogen fluxes. An excretion of nitrite was observed for some strains of *O. cf. siamensis* maintained at temperature lower than the optimal temperature of 22°C. Such release of nitrite for microalgal cultures growing on nitrate is known as a non-assimilatory mode of nitrogen (N) uptake, linked with the cellular response to stressful conditions. Indeed, when growing under non-optimal growth conditions, and in particular high light and cool temperatures, the photosynthesis rate of microalgal cells can exceed assimilatory capacity and induce an accumulation of reducing power. When grown on nitrate, microalgal cells can use this N-source for regulating its energy balance, using the reduction of nitrate into nitrite, and even the subsequent reduction of nitrite into ammonium or dissolved organic nitrogen, as a sink of excess reductant (Glibert *et al.*, 2016). The subsequent release of N in a more reduced state allows the cells to restore or maintain its redox homeostasis. Significant excretion of nitrite was observed for *O. cf. ovata* cells growing on nitrate in a previous study (E. Ternon, com pers.), suggesting that this species was able to use such non-assimilatory pathway for regulating cell homeostasis. Results of the present study confirm that *O. cf. siamensis* is another species able to do it. Additional experiments will have to be done, however, in order to fully define the range of this strategy in growth success of *Ostreopsis* cells, between species, strains and over various environmental conditions.

Results dealing with pigment and lipid contents in *O. cf. siamensis* cells over the acclimation experiment are not available yet but are highly promising. Indeed, these both types of cellular components may play a significant role in the thermal stress response of dinoflagellates. Concerning pigment content and composition, they are known to be affected by irradiance (Dubinsky and Stambler, 2009) but also by other environmental factors such as temperature (Kana *et al.*, 1997; Thompson *et al.*, 1992a, 1992b). As an example, López-Rosales *et al.*, (2014) characterized effects of temperature on pigments composition of the dinoflagellate *Prorocentrum belizeanum*. Under thermal stress (above 25°C for this species), they observed a decrease in the cellular content of most of the photosynthetic (chlorophylls) and photoprotective (carotenoids) pigments (including diadinoxanthin (Dd)) with increasing temperature, but also an increase in dinoxanthin (Dn) concentration. This particular process is probably related to an interconversion between Dd and Dn, similar to the one often observed between Dd and diatoxanthin (Dt) and involved in the photoprotection process of phytoplankton cells (Tchernov *et al.*, 2004). In other taxa, Anning *et al.* (2001) reported variations in the carotenoid:chlorophyll-a ratio of the diatom *Chaetoceros calcitrans* in response to temperature shifts. Such variations in pigment composition and concentrations allow to balance the energy flow between carbon fixation and light absorption after exposure to sub-optimal temperatures (Anning *et al.*, 2001). Pre-results from the present study revealed the presence of carotenoid pigments (Peridinin (Pd), Dn, Dd, Dt) in the pigment profile of *O. cf. siamensis* strains grown under 22°C, as well as photosynthetic pigments (Chl-a, Chl-C2). This combination of pigments is characteristic of type I chloroplasts observed in dinoflagellate species (Zapata *et al.*, 2012) and allows for modifications of the carotenoid:chlorophyll ratio in response to changes in temperature. Interestingly, it also includes specific carotenoid pigments such as Dd, Dt and Dn. In some phytoplankton groups such as dinoflagellates, diatoms or haptophytes, Dd can be converted into Dt in order to increase the photoprotective process (Kuczynska *et al.*, 2015) and a similar process may occur between Dd and Dn (López-Rosales *et al.*, 2014). Thus, in the present study, higher values of the ratios carotenoids/chlorophylls, Dt/(Dd+Dt) and/or Dn (Dd+Dn) are expected at 25°C compared to 22°C and would define physiological adaptations to high temperatures, including the diadinoxanthin cycle.

A characterization of lipids will also be performed in the frame of this study and represents another promising approach in studying physiological adaptations to temperature.

Indeed, for instance, galactolipids, lipids composing the majority of dinoflagellates photosynthetic membranes, are known to be implied in thermotolerance and stabilization of photosynthesis (Sakurai *et al.*, 2007; Mizusawa *et al.*, 2009) and their composition seems to be affected by temperature (Leblond *et al.*, 2015). The saturation of lipids in thylakoid membranes is also used as an indicator of thermal tolerance in marine phytoplankton species: an increase in the production of saturated fatty acids can be observed with increasing temperature (Thompson *et al.*, 1992b; Renaud *et al.*, 2002; Ding *et al.*, 2019), indicating interruptions in desaturation processes.

4.3. Thermal niche and potential distribution limits

Even if the optimal growth temperature of *O. cf. siamensis* strains from the Bay of Biscay was described in this study around 22°C, cells were able to grow from 16°C to 25°C exhibiting relatively high growth rates for non-optimal temperatures (0.23 – 0.37 d⁻¹ at 16°C; 0.35 – 0.53 d⁻¹ at 25°C). At the two extreme temperatures of the gradient tested (14°C – 28°C), none of the strain used was able to acclimate even if some showed transient activation of cell division. Thus, those two temperatures can be considered as not suitable for the growth of *O. cf. siamensis* in the Bay of Biscay. The use of a growth model for simulating variations of growth rate as a function of temperature did not allowed for a more precise definition of the thermal niche of *O. cf. siamensis*. Indeed, the exponential product function used to fit the data set defined theoretical values of minimum and maximum growth temperatures (under which growth is no longer possible) as 13.5°C and 29°C, values under which cultures could not be maintained under acclimation. In the end, the acclimation experiment carried out in the present study allows to define the temperature range 16°C – 25°C as suitable for the growth of *O. cf. siamensis* in the Bay of Biscay, when 14°C and 28°C represent highly stressful conditions under which cells cannot grow on a long term.

Sea temperature plays a major role in phytoplankton species growth (Eppley, 1972; Bissinger *et al.*, 2008) and thus on its repartition and evolution (Thomas *et al.*, 2012; Tester *et al.*, 2020). The geographic range limits of a species are generally associated with its niche limits, which means species stop occurring in habitats where abiotic and biotic factors are not suitable (Brown *et al.*, 1996; Holt, 2003). Following this idea, species temperature niche would shape geographic range. In the Bay of Biscay, *O. cf. siamensis* populations are mostly

implemented in the southeastern part where warm sea surface temperatures (SST) are found and allow those populations to bloom when suitable environmental conditions occur (Laza-Martinez *et al.*, 2011; David *et al.*, 2012). In a recent study by Drouet *et al.* (submitted), *O. cf. siamensis* cells were observed from Comillas (Spain) to Biarritz (France). In this area, annual surveys of SST allowed for the definition of a temporal window during which temperature is favorable for the growth of *O. cf. siamensis*. A dataset issued from the Aquarium of San Sebastian (Spain) revealed that temperatures suitable for *O. cf. siamensis* were found over 25 consecutive weeks during the "summer-autumn" period. This represents a large window, creating enough time for high developments of *O. cf. siamensis* as long as other environmental factors are not inducing strong growth limitations. On the other hand, this SST monitoring also revealed that the winter period is characterized by a long period (19 consecutive weeks on average) during which sea temperature drops under the 14°C threshold defined as highly stressful for *O. cf. siamensis*. Cells of *O. cf. siamensis* are regularly observed in this area (Santos *et al.*, 2019), despite the fact that vegetative cells would not be able to survive under the cold temperatures of winter. Thus, the species has to rely on another form than growing vegetative cells to cope with winter conditions, probably such as resting cysts that are common quiescent stages of dinoflagellates (Pfiester, 1989). The formation of resting cysts was identified for *O. cf. ovata* (Bravo *et al.*, 2012; Accoroni *et al.*, 2014). This type of cyst is issued from sexual reproduction and can remain in a dormant stage over several months or even years (Delebecq *et al.*, 2020). Field surveys on the benthic compartment or crossing experiments between strains in the laboratory will have to be conducted in order to validate the presence of resting cysts in the cell cycle of *O. cf. siamensis*.

When considering the North part of the Bay of Biscay, the compilation of average weekly SST over 2000 – 2018 in the Bay of Brest (dataset issued from SOMLIT, French national coastal monitoring program) shows that SST higher than 16°C are observed over 14 consecutive weeks in average, from July to October (Figure 42). Such a time window is sufficient for allowing bloom development of *O. cf. siamensis*, at least when considering potential limitation by temperature only and through a low growth rate. Although they did not observe cells further North from Biarritz, Drouet *et al.* (submitted) found eDNA traces of *O. cf. siamensis* in several sites along the coasts of the Bay of Biscay and even in the western English Channel, including Brest, at this period of the year (> 16°C). In this study, authors also used a characterization of particle transport in the Bay of Biscay to suggest that *O. cf. siamensis*

eDNA detections observed probably corresponded to viable cells and not to extracellular eDNA (Drouet *et al.*, 2020b). The range of the thermal niche of *O. cf. siamensis* estimated in the present study support this hypothesis. It shows that even the North part of the Bay of Biscay (including the Bay of Brest) offers conditions that coincide with the thermal niche of the species. Thus, when considering only temperature as a controlling factor, growth and maintenance of *O. cf. siamensis* populations can be expected all along the coast of the Bay of Biscay.

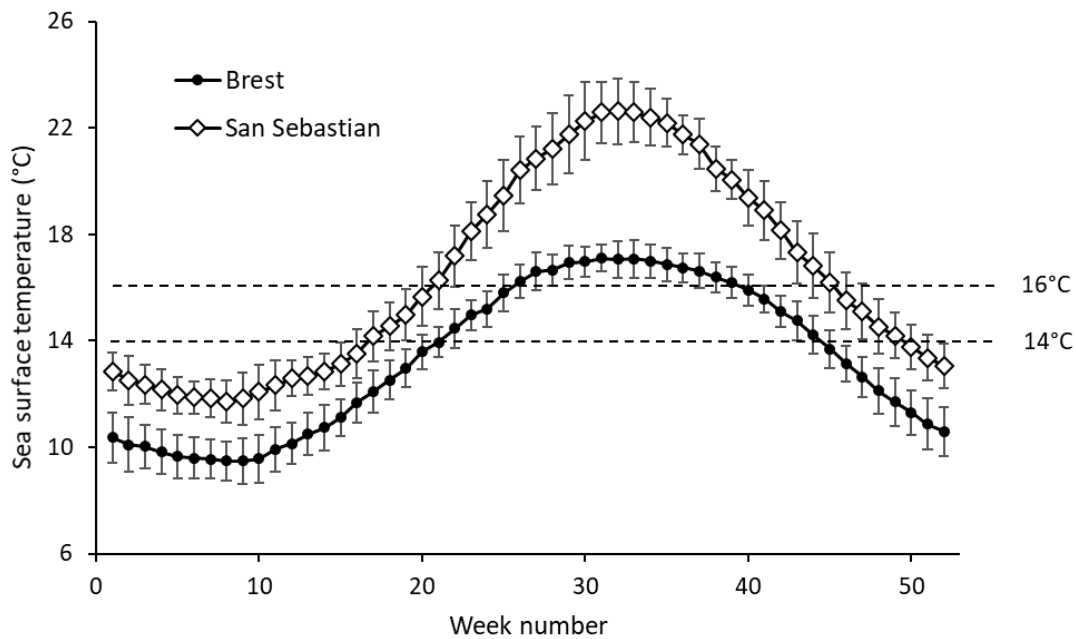


Figure 42: Weekly average of coastal SST in Brest and San Sebastian over the 2000 – 2019 period according to data provided by the SOMLIT and the Aquarium of San Sebastian, respectively.

In the Bay of Biscay, Drouet *et al.* (in prep.b) defined the southeastern part of the bay as the niche range of *O. cf. siamensis*, according to the localization of recurrent observations of *O. cf. siamensis* cells (David *et al.*, 2012; Drouet *et al.*, submitted). It has to be proven then if no population of *O. cf. siamensis* are able to grow and be maintained under higher latitudes than Biarritz (France), taking a better advantage of the range of the thermal niche. Other factors than temperature are controlling the dynamics of *O. cf. siamensis* blooms and could also be a reason why *O. cf. siamensis* blooms could not be observed under higher latitudes. For *O. cf. ovata*, the role of different environmental parameters was emphasized as controlling factors of bloom development. They included both abiotic (temperature, hydrodynamics, salinity, nutrients) and biotic (substratum) factors (Accoroni and Totti, 2016). Such a variety of

controlling factors are probably acting simultaneously during *O. cf. siamensis* development in the Bay of Biscay, but have still to be defined and characterized.

Along the coasts of the Iberian Peninsula, *O. cf. siamensis* have only been observed forming blooms in southeastern part of the Bay of Biscay (Drouet *et al.*, submitted; Laza-Martinez *et al.*, 2011) whereas *O. cf. ovata* has only been observed forming blooms in along the Spanish coast of the Mediterranean Sea (Mangialajo *et al.*, 2011; Vila *et al.*, 2016). However, both species have been detected in the western part of the Iberian Peninsula along the coast of Portugal albeit in relative low cell concentration (David *et al.*, 2013; Santos *et al.*, 2019). Further studies comparing differences of tolerance between *O. cf. siamensis* and *O. cf. ovata* to various controlling factors are required for better understanding such difference of niche repartition. The completion of the acclimation experiment described in the present study will give crucial additional information dealing with thermal tolerance of three strains of *O. cf. ovata* isolated in the Mediterranean Sea. It might show temperature as one of the main factors controlling the ecological niche of these species. Among other potential drivers, turbulence is one of the most interesting ones. Indeed, *O. cf. ovata* is known to be sensitive to high turbulence (Accoroni and Totti, 2016) when the presence of strong and continuous tides is one of the main differences between environmental conditions encountered in the Bay of Biscay and the Mediterranean Sea.

4.4. Toxicity and temperature

A large number of studies conducted on *Ostreopsis* sp. toxin production focused on the characterization of toxins produced by *O. cf. ovata* strains, especially concerning palytoxin, ovatoxin and other analogs (Ciminiello *et al.*, 2010, 2011, 2012), whereas little is known about toxin production in Atlantic and Mediterranean strains of *O. cf. siamensis*. In the present study, strains of *O. cf. siamensis* from the Bay of Biscay were here shown exhibiting toxic effects on *Artemia franciscana* nauplii. However, toxin characterization analysis through LC-MS/MS did not detect the presence of any known toxin commonly associated with *Ostreopsis* species. The same observations were made by Ciminiello *et al.* (2013) who did not observe the presence of either ovatoxins or ostreocins in Atlantic and Mediterranean strains of *O. cf. siamensis*. Only traces of palytoxin were detected ($< 1 \text{ fg.cell}^{-1}$), and no peak could be

associated with previously characterized palytoxin-like compounds (Ciminiello *et al.*, 2011). After observing no death in a mouse bioassay, they concluded in a total absence of toxicity in both Atlantic and Mediterranean strains *O. cf. siamensis*. Yet, the present study revealed the effective toxicity exhibited by *O. cf. siamensis* strains from the Bay of Biscay on *Artemia franciscana* nauplii. Different palytoxin-like compounds from those reported so far could thus be produced by these strains, and further toxin screening analyses would be required to characterize these allelopathic compounds.

The brine shrimp *Artemia* sp. has been widely used as a test organism for various types of ecotoxicological assays. Its use as a toxicity bioassay allowed for the development of easy, costless and sensitive tests to evaluate and compare toxicity among diverse organisms and compounds (Vanhaecke *et al.*, 1981). The *Artemia* bioassay has recently been re-designed and applied for *Ostreopsis* species by Pavaux *et al.* (2020b) in order to assess the variability of the toxic effect among Mediterranean strains throughout their life cycle and to monitor the evolution of toxicity throughout a bloom episode. This bioassay was also previously used by (Laza-Martinez *et al.*, 2011) to assess for the first time the toxicity of *O. cf. siamensis* strains isolated in the southeastern part of the Bay of Biscay. The use of the *Artemia franciscana* bioassay in the present study was aimed to determine the influence of temperature on toxic effect of various strains of *O. cf. siamensis* and *O. cf. ovata*. Although results concerning *O. cf. ovata* strains are not entirely collected yet, a clear relationship between toxicity and temperature was revealed in *O. cf. siamensis* strains from the Bay of Biscay. When grown under non-optimal temperature conditions, all strains of *O. cf. siamensis* showed a decrease in toxicity with decreasing temperature as a significant ranking was obtained for the LC₃₀ parameter following LC₃₀ 16°C > LC₃₀ 19°C > LC₃₀ 22°C. Conversely, the toxic effect on *Artemia franciscana* did not significantly increase after a change of temperature from 22°C to 25°C. Such trends are in accordance with variations of toxicity observed for *O. cf. ovata* as a function of temperature. Indeed, higher levels of toxicity of *O. cf. ovata* were reported at 25°C compared to 20°C (Faimali *et al.*, 2012; Pezzolesi *et al.*, 2012) and 30°C (Pezzolesi *et al.* 2012) using the *Artemia franciscana* bioassay, with a maximal growth rate measured at 25°C. Several strains *O. cf. ovata* from the Mediterranean Sea were also characterized by higher toxin content at optimal temperatures for growth compared to higher or lower levels of temperature (Pezzolesi *et al.*, 2012; Scalco *et al.*, 2012). A relationship between temperature and toxin production was also reported for other taxonomic groups, including both

dinoflagellates, such as *Alexandrium* sp. (Etheridge and Roesler, 2005; Lim *et al.*, 2006; Aguilera-Belmonte *et al.*, 2013; Laabir *et al.*, 2013) and *Prorocentrum* sp. (Accoroni *et al.*, 2018; Aquino-Cruz *et al.*, 2018), and diatoms such as *Pseudo-nitzschia* sp. (Lelong *et al.*, 2012; Thorel *et al.*, 2014). The relation between growth and toxicity is yet to be characterized in order to determine which of growth or temperature is the main driver of toxicity.

The values of LC₅₀ issued from *Artemia franciscana* mortality exposed to *O. cf. siamensis* and *O. cf. ovata* strains in the present study are in accordance with the ones found in previous studies conducted on *Ostreopsis* species (Pavaux *et al.*, 2020a, 2020b). At 22°C, the totality of strains exhibited LC_{50-48h} values inferior to 50 cells.mL⁻¹. All *O. cf. siamensis* strains except the ones from Saint-Jean-de-Luz exhibited LC_{50-48h} values inferior to 10 cells.mL⁻¹, whereas two *O. cf. ovata* strains (MCCV 98 and 99) exhibited LC_{50-48h} values inferior to 2 cells.mL⁻¹. Bioassays from the literature conducted on both *Artemia franciscana* and *salina* have been mostly performed on *O. cf. ovata* grown at temperatures ranging from 20°C to 25°C and usually exhibited LC_{50-48h} values ranging from 1 to 25 cells.mL⁻¹. However, values of LC_{50-24h} tend to be lower in the literature (< 1 – 100 cells.mL⁻¹) than in the present study (30 – 400 cells.mL⁻¹). Based on the *Artemia* bioassay, the overall allelopathic effect of *O. cf. siamensis* strains from the Bay of Biscay seems to be comparable to *O. cf. ovata* strains from Mediterranean Sea, even though the variation of toxicity between strains can sometimes be higher than between species.

Contact interaction between *Ostreopsis* sp. cells and target organisms may be required for harmful effect. Ternon *et al.* (2018), through bioassay and co-culture experiments, observed that ovatoxins and other metabolites produced by *O. cf. ovata* would only affect the co-occurring diatom *Licmophora paradoxa* in a case of a close encountering, suggesting that the most part of allelochemical compounds would remain adsorbed onto the cell or the mucus produced by *Ostreopsis* cells. Similarly, both Pezolesi *et al.* (2012) and Faimali *et al.* (2012) observed a substantial higher toxic effect of *O. cf. ovata* live cells compared to filtered cultures devoid of cells using the *Artemia franciscana* bioassay. The active role of the mucus on the toxicity of *O. cf. ovata* was confirmed by Giussani *et al.* (2015) who observed higher mortality of *Artamia salina* when exposed to direct contact with *O. cf. ovata* cells or growth medium containing mucus and devoid of cells compared to growth medium devoid of both cells and mucus. The totality of toxins measured was found in cell pellets and in the mucilaginous matrix, but no toxin was found in the growth medium alone. Interestingly, this

central role of mucus in allelopathic effects of *Ostreopsis* species may add an indirect influence of temperature on the toxicity level. Indeed, optimal growth temperatures tend to favor high level of toxicity per cell as well as the production of carbohydrates composing the mucilaginous matrix (Vidyarathna and Granéli, 2012). Thus, temperature could play both directly, on toxin synthesis in the cell, and indirectly, on the quantity of mucilaginous matrix produced extracellularly that seems to concentrate toxins in the extracellular medium.

4.5. Risk repartition

Although toxin analyses did not observe the production of known toxic compounds in *O. cf. siamensis* strains, a potential risk can still be envisaged as allelopathic effects were observed on *Artemia franciscana*. The present study showed that lowest toxicity values exhibited in strains from the Bay of Biscay were associated with lowest growth rates and sub-optimal growth temperatures. Thus, low temperatures are lowering toxic risks through the decrease in both cell abundance and toxicity per cell. In the end, the risk associated with *O. cf. siamensis* in the Bay of Biscay may be considered as relatively low in comparison to the risk associated with *O. cf. ovata* in Mediterranean Sea where higher amount of potent toxins are found (Pavaux *et al.*, 2020a) and very high abundances can be reached. For now, this potential risk would be mostly localized in the southeastern part of the bay where SST are favoring both growth and toxic effect in established *O. cf. siamensis* populations, i.e. the southwestern coast of Portugal and the French and Spanish Basque Country coasts, but not in the coldest areas such as the northwestern part of the peninsula along Galician coasts. However, as pointed out in Drouet *et al.* (submitted), even if microscopy analyses of epiphytic samples do not reveal the observation of cells, it does not necessarily indicate the absence of the species. Water circulation could be responsible of cell transfer from well-established populations to less suitable areas, but sub-optimal environmental conditions would prevent large development in newly colonized areas. The use of molecular tools based on eDNA detection for rare species monitoring will allow for a better estimation of the distribution of *O. cf. siamensis* across the coasts of the Iberian Peninsula, as it has been done along coasts of the Bay of Biscay.

Climate change, and more particularly ocean warming, are affecting the Bay of Biscay. Indeed, a non-homogenous long-term warming trend of the waters of both the NE Atlantic Ocean and the Bay of Biscay have been observed (González-Pola, 2005; deCastro *et al.*, 2009;

Costoya *et al.*, 2015). Due to this global ocean warming trend, distribution and phenology of harmful microalgae are expected to be affected (Fu *et al.*, 2012; McLeod *et al.*, 2012; Paerl and Paul, 2012), notably benthic species such as *Ostreopsis* sp. (Tester *et al.*, 2020). Thus, *O. cf. siamensis* could potentially be observed in warming areas of the Bay of Biscay, especially the parts approaching the optimal growth temperature revealed in the present study.

In areas where optimal temperatures will be reached for a sufficient amount of time, the relative high growth rates of *O. cf. siamensis* populations associated with optimal temperatures would promote high cell abundance and, in association with the increase of toxicity corresponding to optimal growth temperatures, would increase the risk associated with the presence of *O. cf. siamensis* populations. In order to evaluate the potential risk evolution in these areas, a monitoring of the presence of *O. cf. siamensis* should be undertaken.

5. Conclusion

Temperature is a key factor controlling phytoplankton development. In this study, the effect of temperature on growth, toxicity and stress indicators was assessed for several strains of *O. cf. siamensis* from the Bay of Biscay, NE Atlantic, and partially achieved in three strains of *O. cf. ovata* from NW Mediterranean Sea. The acclimation to increasing and decreasing temperatures of a relatively large number of strains allowed the determination of the temperature niche representative of strains and species in the area of the study site. Temperatures around 22°C were characterized as optimal for *O. cf. siamensis* growth whereas active growth was observed between 16°C and 25°C. Among strategies of physiological adaptations to various temperature conditions, the role of non-assimilatory fluxes of N-uptake were observed for *O. cf. siamensis* and modifications of carotenoid and photosynthetic pigments were suspected. All the strains used in the acclimation experiment exhibited allelopathic effects on *Artemia franciscana* although previously described toxins associated with *Ostreopsis* species were not identified in these strains. A decrease in toxicity was observed at the lowest temperatures for all the strains tested and a highest toxicity values were obtained at temperatures exhibiting highest growth rates. This work suggests that, in a context of climate change, warming of waters of the Bay of Biscay could favor the growth and the increase in toxicity in *O. cf. siamensis* populations already established along the coasts of

the bay. More investigations are required, however, for forecasting changes in *O. cf. siamensis* distribution and associated risk in the Bay of Biscay, in response to environmental forcing. As strong clonal variability was noted in the present study, other processes such as sexual reproduction, cyst germination or even mutation can be at play for selecting particular phenotypes in the field, on a long-term perspective.

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Authors' contribution

K.D., C.J., R.S. and R.L. designed and the experimental protocol. A.L-Z. and S.S. sampled and isolated *O. cf. siamensis* strains from Spanish sites. K.D., C.J., E.G. and P.M. conducted the acclimation experiment. M.B. conducted the lipid analyses. D.R. and F.H. conducted the toxin profile analyses. S.S. coordinated the pigment analyses. K.D. and C.J. analyzed the results and prepared the figures and wrote the manuscript. All authors reviewed and accepted the final version of the manuscript.

Discussion générale et perspectives

Au cours de ces travaux de thèse de doctorat, j'ai eu l'occasion d'étudier différents aspects relatifs à l'effet de la température sur l'écologie de deux espèces de dinoflagellés benthiques toxiques du genre *Ostreopsis*. Un des intérêts majeurs de ces travaux était de coupler à la fois étude de terrain et expériences de laboratoire pour mieux comprendre les mécanismes de réponse de cette microalgue à la température, pour à terme tenter de prédire son comportement vis-à-vis des changements climatiques. Les trois chapitres détaillés dans cette thèse, axés respectivement sur la phénologie pluriannuelle, la distribution régionale et l'écophysiologie d'*Ostreopsis* spp., ont en commun de viser à déterminer l'effet de la température sur leur développement. L'originalité de cette thèse réside dans la combinaison de ces trois aspects de l'écologie d'*Ostreopsis* spp. en lien avec la température. Cette approche pluridisciplinaire permet de donner des nouveaux éléments pour répondre aux questions concernant le devenir des HABs benthiques dans le futur, et principalement ceux formés par *Ostreopsis* spp. dans un contexte de changements globaux.

L'ensemble des résultats obtenus répondant aux trois questions principales de cette thèse (cf. objectifs) sont ici discutés et mis en relation dans un contexte plus général afin d'ouvrir sur des perspectives de recherche permettant l'amélioration des connaissances concernant l'écologie d'*Ostreopsis* spp..

La température est-elle l'unique facteur écologique pouvant expliquer la phénologie et la répartition d'*Ostreopsis* spp. en Méditerranée nord-occidentale et dans le Golfe de Gascogne ?

Parmi les nombreux facteurs environnementaux influençant le développement des microalgues, et notamment des espèces benthiques, la température est sans doute le facteur physico-chimique le plus étudié et le plus surveillé. Le fait que les efflorescences de phytoplancton se développent de façon saisonnière permet d'attester du lien existant entre ce paramètre et le développement d'espèces formant des HABs (Hallegraeff *et al.*, 1995). Pourtant, ce lien reste encore relativement difficile à mettre en évidence, ou du moins à quantifier. Pour l'étudier en détails, les chercheurs peuvent faire appel à des approches de terrain, principalement basées sur la mise en place de suivi à long terme d'efflorescences *in situ*, incluant l'analyse des variations pluriannuelle des paramètres environnementaux pour

expliquer les traits phénologiques des blooms (comme la temporalité et l'intensité) et basé sur les abondances observées à chaque date de prélèvement. Un autre moyen correspond à une approche *in vitro*, en milieu contrôlé au laboratoire, sur des souches monoclonales isolées à partir d'échantillons de terrain. Les expérimentations de laboratoire permettent d'observer, entre autres, la réponse cellulaire (adaptations physiologiques, réponses au stress) à un paramètre environnemental d'intérêt. Ces deux méthodes ont pourtant chacune leurs propres atouts et limites. Dans le cas des suivis multiparamétriques *in situ*, il est relativement complexe de mettre en évidence les effets cumulés des nombreux facteurs environnementaux sur un phénomène naturel tel qu'une efflorescence. Concernant les approches de laboratoire mono-paramétriques, la transposition dans un contexte naturel de l'interprétation des résultats, compte tenu de la complexité des interactions biotiques et abiotiques auxquelles l'espèce est potentiellement soumise dans son écosystème, est également complexe.

Dans le cas d'*Ostreopsis* spp., l'interaction entre l'ensemble des paramètres environnementaux sur la phénologie des blooms semble tellement complexe que dans la majorité des études basées sur des suivi d'efflorescence, la variation de l'abondance des cellules phytoplanctoniques n'est jamais totalement corrélée avec un paramètre environnemental mesuré, mais plutôt associée à un regroupement de valeurs favorables d'un ensemble de facteurs. Par exemple, (Accoroni *et al.*, 2015a), grâce à des observations réalisées dans le cadre d'un suivi à long terme effectué entre 2007 et 2012, ont développé un modèle conceptuel expliquant que la dynamique des blooms d'*O. cf. ovata* dans la partie nord de la Mer Adriatique (Méditerranée) est régit par la combinaison de températures adéquates pour la germination des kystes, de conditions hydrodynamiques calmes et d'un ratio azote/phosphore spécifique des nutriments disponibles. Les travaux de (Cohu *et al.*, 2011), qui ont analysé les deux premières années de la série temporelle issue du monitoring réalisé à Monaco repris dans cette thèse, n'ont pas observé de relation directe entre les paramètres environnementaux, dont la température, avec l'abondance cellulaire. Par contre, la forte variabilité des traits phénologiques des blooms entre 2007 et 2008 leur ont permis d'observer une potentielle relation entre les températures de surface au printemps et la période d'initiation de l'efflorescence. Dans notre étude et en analysant cette fois-ci une série temporelle constituée de 13 années d'observation, la corrélation entre précocité du bloom et températures de surface au printemps a été confirmée (cf. Chapitre 1). Cette analyse pluriannuelle a mis en évidence que plus l'accumulation d'anomalies positives de

températures de surface entre les mois de mars et de juin était forte, plus l'efflorescence d'*O. cf. ovata* à Monaco avait lieu tôt dans la saison. D'autre part, cette analyse pluriannuelle a permis de mettre en évidence une gamme de températures idéales favorisant la croissance cellulaire *in situ* et d'affiner celle déjà observée par (Cohu *et al.*, 2011). Si ceux-ci estimaient une possible formation d'efflorescence entre 18°C et 30°C, les présents travaux ont permis de préciser que les taux de croissance maximum dans cette zone ont été atteints pour des températures variant entre 21°C et 25°C en fonction des années. Ces observations *in situ* semblent corroborer les résultats obtenus en laboratoire par (Scalco *et al.*, 2012) qui ont observé les taux de croissance les plus élevés entre 22°C et 26°C chez des souches d'*O. cf. ovata* provenant du Golfe de Naples (Italie). L'analyse multivariée réalisée dans cette étude, bien que prometteuse, n'a cependant pas permis de mettre en relation les variations d'abondance cellulaire d'*O. cf. ovata* observées avec les différents paramètres environnementaux suivis. Ceci peut être dû à l'absence d'autres paramètres physico-chimiques et biotiques clés pouvant impacter le développement d'*O. cf. siamensis*, ainsi qu'à la très forte variabilité inter-sites et interannuelle du développement de ces efflorescences. Le lien entre l'accumulation d'anomalies positives de températures au printemps et la précocité des efflorescences, bien que fortement probable, doit être réévalué et confirmé dans un contexte environnemental plus ample et sur la base de suivi temporels plus complets. En effet, s'il peut sembler clair que la température joue un rôle prépondérant dans le développement des espèces d'*Ostreopsis* et de leurs efflorescences, notamment dans leur saisonnalité, ce paramètre seul n'explique pas les différents traits phénologiques de ces blooms dans leur globalité.

D'autres études ont montré à quel point les blooms d'*Ostreopsis* spp. pouvaient être affectés par d'autres facteurs, qu'ils soient biotiques et abiotiques. En effet, l'article de revue élaboré par (Accoroni and Totti, 2016) recense les différentes études observant l'influence des paramètres environnementaux sur le développement d'*Ostreopsis* spp. à travers le monde. La salinité (Mangialajo *et al.*, 2008; Carnicer *et al.*, 2015), les nutriments (Cohu *et al.*, 2011; Accoroni *et al.*, 2015a) l'hydrodynamisme (Vila *et al.*, 2001; Totti *et al.*, 2010) ainsi que le type de substrat (Totti *et al.*, 2010; Blanfuné *et al.*, 2015) semblent impacter le développement des blooms d'*Ostreopsis* spp. mais de manière contrastée et parfois de manière opposée. D'un autre côté, l'influence de la température, bien que non linéaire, semble, elle, être relativement similaire entre régions géographiquement proches.

La distribution géographique des microalgues, tout comme le développement d'efflorescence, est, elle aussi, déterminée par les facteurs environnementaux favorisant la croissance cellulaire. David *et al.* (2012) ont par exemple étudié la distribution d'*O. cf. siamensis* le long des côtes Atlantiques de la péninsule Ibérique et ont suggéré qu'*O. cf. siamensis* y serait observable à condition que la température de l'eau soit supérieure à 19.5°C pendant une durée d'au moins 3 mois, une condition qui ne se vérifie pour le moment que dans la partie sud-est du Golfe de Gascogne et au sud du Portugal.

Notre étude de la distribution d'*Ostreopsis* spp. en Atlantique, focalisée dans le Golfe de Gascogne, zone à fort gradient thermique à la fois latitudinal et longitudinal, a eu pour but d'élargir la zone d'étude échantillonnée par David *et al.* (2012) et de déterminer la distribution actuelle d'*Ostreopsis* spp.. A notre connaissance, le développement d'efflorescences d'*O. cf. siamensis* dans cette région a uniquement lieu dans le sud-est du Golfe où les températures sont les plus élevées, et pourrait donc potentiellement servir de zone témoin de l'expansion d'*O. cf. siamensis* dans le futur en relation avec le réchauffement des eaux. La campagne d'échantillonnage d'ADN environnemental ainsi que les analyses de connectivité entre les zones échantillonnées nous ont permis de suggérer la présence de cellules d'*O. cf. siamensis*, en très faible abondance non détectable par échantillonnage benthique classique, jusqu'au nord du Golfe, à l'entrée de Manche. Or, les relevés de températures côtières de la rade de Brest, où des traces d'ADN environnemental appartenant à cette espèce ont été détectées, attestent la présence de températures supérieures à 16°C pendant de nombreuses semaines entre la période estivale et automnale. Pourtant, lors de notre campagne d'échantillonnage, aucune cellule intacte n'a pu être observée visuellement dans cette zone et dans cette période précise. Ceci suggère que la température, bien que primordiale pour le développement d'*Ostreopsis* spp. et des autres microalgues et influençant une partie de leur phénologie et de leur distribution, ne peut pas à elle seule expliquer la présence de l'espèce et le développement d'efflorescence. Ce concept est particulièrement important à envisager dans le Golfe de Gascogne, qui est une zone caractérisée par des habitats très contrastés en fonction de la latitude. Dans cette zone, les variations des paramètres environnementaux comme la température et la diversité de substrat sur lequel l'espèce peut se développer, qu'il soit biotique ou abiotique, sont relativement fortes entre la partie sud et nord. Cette différence d'habitat global pourrait être la raison de l'absence de développement d'*O. cf. siamensis* au nord de Biarritz. En effet, d'après nos observations de terrain, les eaux

relativement chaudes de la région du Pays Basque se sont avérées favorables au développement d'*O. cf. siamensis* vis-à-vis de sa niche thermique, mais aussi d'autres critères d'habitat tels que des substrats rocheux et colonisés par une large diversité de macroalgues ramifiées. Aux latitudes plus hautes, ces conditions environnementales font rapidement place aux zones sableuses des Landes, puis aux zones vaseuses des pertuis Charentais, où l'habitat semble peu propice au développement d'*Ostreopsis* spp. Dans la partie nord du Golfe, des habitats caractérisés par des substrats rocheux et abrités ont été retrouvés, mais la diversité des communautés macroalgales présentes ne correspondaient pas à celle trouvée dans la partie sud.

De manière générale, pour observer un développement relativement conséquent d'*O. cf. siamensis* ou d'autres microalgues, le taux de croissance de la population doit excéder le taux de mortalité, dû à des pertes de biomasses telles que par prédation, advection ou lyses parasitaires et virales. Nous suggérons donc que pour observer un développement significatif de cellules d'*O. cf. siamensis* dans le Golfe de Gascogne, une température favorable est un paramètre nécessaire mais pas suffisant pour permettre une production de biomasse excédant nettement les pertes. Mais la présence combinée de substrats adaptés, d'hydrodynamisme modéré et de températures favorables (entre 16°C et 25°C) pendant une période assez longue pourrait le permettre chez des populations d'*O. cf. siamensis* déjà implantées. Dans les zones où les conditions environnementales seraient moins favorables, comme dans la partie nord du Golfe de Gascogne, des cellules d'*O. cf. siamensis* pourraient survivre et se diviser, sans toutefois mener à un fort développement d'efflorescence. Ceci justifierait leur détection via des outils moléculaires, adaptés à la détection d'organismes rares, et non pas par prélèvement benthique classique.

Selon nos résultats issus de la campagne d'échantillonnage et couplés à notre approche de modélisation Lagrangienne, la limite nord de distribution actuelle d'*O. cf. siamensis* sur les côtes Européennes de l'Atlantique Nord-Est se trouverait à Plouguerneau, dans la partie Nord-Ouest du Finistère (Bretagne) en début de Manche. Il est pour le moment impossible d'affirmer si cette limite établie n'est pas en réalité située plus au Nord, étant donné qu'aucun échantillonnage ni qu'aucune analyse de connectivité spatiale visant à étudier la dispersion de cellules d'*Ostreopsis* spp. n'ont été réalisés dans cette zone. On ne peut exclure l'hypothèse que des cellules résistant aux conditions défavorables puissent être régulièrement advectées vers la Manche ou encore plus au Nord, comme nous l'avons suggéré

pour les côtes du Golfe de Gascogne. Des cellules pourraient en effet probablement survivre, même dans des environnements défavorables hors de leur niche écologique, notamment sous forme de kystes, et y attendre l'apparition de conditions favorables à leur développement. D'après les critères listés ci-dessus, les conditions environnementales semblent de moins en moins propices au développement d'efflorescence d'*Ostreopsis* spp. à mesure que les habitats se trouvent dans des hautes latitudes. Ceci limite la probabilité d'observer *O. cf. siamensis* dans des eaux telles que celles de la Mer Celtique, de la Manche ou de la Mer du Nord, du moins de nos jours.

Aucune trace d'*Ostreopsis cf. ovata* n'a été détectée dans le Golfe de Gascogne lors de notre étude. Cette espèce a cependant été observée au Sud du Portugal et surtout en Mer Méditerranée où elle forme des efflorescences denses récurrentes. Au niveau des côtes atlantiques, (Santos *et al.*, 2019) ont observé la présence simultanée d'*O. cf. ovata* et d'*O. cf. siamensis* dans la baie de Lagos, au sud du Portugal, mais seul *O. cf. siamensis* a été observé plus au nord dans la baie de Lisbonne, et également dans le sud-est du Golfe de Gascogne (Laza-Martinez *et al.*, 2011; David *et al.*, 2013). Au vu de la différence de répartition de ces deux espèces autour de la péninsule Ibérique et des conditions environnementales variant de long des côtes, *O. cf. ovata* semblerait mieux se développer sous des températures chaudes et dans des zones encore plus protégées de l'hydrodynamisme et des marées. Ainsi, les températures plus basses couplées à l'hydrodynamisme intense des côtes ouest portugaises agiraient en tant que barrière à l'expansion d'*O. cf. ovata* en limitant sa distribution au sud du Portugal (cf. Fig. 10 (Santos *et al.*, 2019)). Une barrière environnementale doit probablement exister au niveau de Portugal pour *O. cf. ovata* car aucune trace d'ADN environnemental lié de cette espèce n'a été détectée dans le Golfe de Gascogne lors de notre campagne d'échantillonnage. A l'inverse, les facteurs environnementaux limitant la croissance d'*O. cf. siamensis* en Méditerranée restent à être définis. Les températures élevées atteintes en été ne sembleraient pas en être la cause car, dans ce cas présent, des efflorescences auraient lieu plus tôt, comme par exemple au printemps.

Les capacités d'acclimatation et d'adaptation physiologiques aux variations de température expliquent-elles la distribution des espèces d'*Ostreopsis* ? Quels mécanismes physiologiques ces espèces mettent-elles en place pour s'adapter au changement de température ?

Chez les microalgues, les changements de température induisent un stress traduit par un déséquilibre de l'homéostasie et l'activation des voies de stress cellulaire face auxquels les cellules doivent s'acclimater (Borowitzka, 2018). Cette acclimatation se fait par la régulation de certains processus physiologiques, la plupart associés à la photosynthèse, afin de restaurer l'homéostasie et de permettre à la cellule de poursuivre sa croissance. Pour cela, la cellule peut utiliser différents mécanismes comme la production de pigments photoprotecteurs (Lopez *et al.*, 2019), la production de certaines classes de lipides dans les membranes photosynthétiques (Mizusawa *et al.*, 2009) ou la modification des flux d'azotes au sein de la cellule (Glibert *et al.*, 2016). La régulation de ces processus aurait pour but principal de ralentir la machinerie photosynthétique, permettant à la cellule de s'acclimater aux nouvelles conditions environnementales. Lorsque ces conditions sont trop contraignantes et provoquent un stress trop important et non gérable par la cellule, comme par exemple une exposition prolongée à de faibles températures, celle-ci peut potentiellement entrer en quiescence afin de former des kystes temporaires résistants aux conditions non favorables (Bravo *et al.*, 2012). Les expériences de culture en laboratoire effectuées sur *Ostreopsis* spp. confirment le rôle clé que joue la température sur le développement de ce genre de microalgue (Laabir *et al.*, 2011; Pistocchi *et al.*, 2011; Pezzolesi *et al.*, 2012; Tawong *et al.*, 2015; Aquino-Cruz and Okolodkov, 2016; Carnicer *et al.*, 2016). L'analyse de l'évolution de la croissance cellulaire en fonction de la température selon une gamme définie permet alors de définir la niche thermique théorique d'une espèce, et d'observer les températures limites (minimale et maximale) et optimales de croissance (Bernard and Rémond, 2012). Cependant, même si cette niche observée en laboratoire reflète normalement les préférences thermiques de l'espèce dans son milieu naturel, l'absence d'interaction avec les autres paramètres retrouvés *in situ* pourrait induire un biais dans la réponse cellulaire vis-à-vis de la température et ne pas correspondre au comportement qui aurait lieu en milieu naturel.

Notre expérience d'acclimatation thermique menée sur les différentes souches d'*O. cf. siamensis* isolées dans le sud-est du Golfe de Gascogne (H1, H8, H10, Z1, Z7 et Z8) nous a permis de définir leur niche thermique théorique, et par conséquent d'identifier les températures favorables à leur croissance. En effet, nous avons observé que les différentes souches étaient capables de pousser activement entre 16°C et 25°C suite à des acclimatations successives, avec des taux de croissances maximums obtenus à 22°C, mais qu'elles n'étaient plus capables de se développer à 14°C et 28°C. En effet, l'absence d'accumulation de ROS intracellulaire, l'absence de variations significative des valeurs de F_v/F_m ainsi que l'observation de taux de croissance successifs stables indiquent que les souches ont réussi à s'acclimater à ces températures. Cependant, nous ne pouvons malheureusement pas encore nous prononcer sur les variations des profils pigmentaires et lipidiques des cellules en réponse à l'acclimatation aux températures. Ces analyses, actuellement en cours, pourraient nous permettre d'observer ces régulations physiologiques jouant un rôle dans le rééquilibrage de l'homéostasie chez *O. cf. siamensis* et *O. cf. ovata* et de caractériser les profils pigmentaires et lipidiques indicateurs de stress thermique. Des résultats préliminaires effectués sur certaines souches montrent la présence de pigments caroténoïdes photoprotecteurs qui sont la péridinine, la dinoxanthine et la diadinoxanthine. Comme observé par (Kuczynska *et al.*, 2015) chez des diatomées, le stress causé par de fortes températures pourrait induire la conversion de la diadinoxanthine en dinoxanthine (diatoxanthine chez les diatomées), renforçant l'effet photoprotecteur, ainsi qu'une augmentation du ratio caroténoïdes/pigments chlorophylliens.

En se basant sur ces observations expérimentales et en ne prenant en compte que la température, les zones du Golfe de Gascogne où la température serait supérieure à 16°C pendant une certaine période pourraient potentiellement permettre le développement d'*O. cf. siamensis*. Dans la zone du Pays Basque franco-espagnol (de Zierbena à Saint-Jean-de-Luz) où ces souches d'*O. cf. siamensis* ont été isolées, des efflorescences sont régulièrement observées entre la fin de l'été et le début de l'automne (août-septembre), où la température de l'eau correspond à leurs températures optimales de croissance selon la niche thermique définie en laboratoire (entre 20°C et 24°C). Pendant les périodes hivernales et printanières, la température peut descendre sous le seuil des 14°C pendant de nombreuses semaines (cf. Figure 8 Chapitre 3), représentant un environnement défavorable au développement cellulaire. Pour survivre et pouvoir se redévelopper de nouveau l'été suivant avec le retour

des conditions favorable, il apparaît indispensable aux cellules des populations d'*O. cf. siamensis* de passer ces périodes sous forme de kystes de résistance, issus de la reproduction sexuée (Bravo *et al.*, 2012), et non pas seulement sous forme de kystes temporaires, qui ne seraient potentiellement pas capable de survivre autant de temps. (Lopez *et al.*, 2019) ont ainsi montré que les facteurs environnementaux, et principalement la température, contrôlaient le cycle de dormance chez le dinoflagellé *Pyrodinium bahamense* et que ce cycle était responsable de la périodicité de ses efflorescences dans la Baie de Tampa, en Floride. En effet, les kystes de dinoflagellés auraient besoin d'une période obligatoire de dormance pour pouvoir germer au printemps suivant (Binder and Anderson, 2004). Accoroni *et al.* (2014) ont également observé que la germination des kystes d'*O. cf. ovata* était induite sous l'effet de certaines températures. On peut alors supposer que des mécanismes similaires contrôleraient les efflorescences d'*O. cf. siamensis* dans le sud-est du Golfe de Gascogne. Concernant les populations d'*O. cf. siamensis* supposément installées au nord du Golfe et dont la présence n'a été observée qu'à travers la détection d'ADN environnemental, la température optimale de croissance modélisée de 22.45°C n'est jamais atteinte et la fenêtre temporelle de températures qui pourraient permettre la croissance est plus restreinte que pour les populations du sud (14 semaines en moyenne au-dessus de 16°C à Brest contre 25 semaines à San Sebastian). Ainsi, pendant une période de 3-4 mois, les cellules d'*O. cf. siamensis* pourraient donc survivre dans les zones au nord du Golfe grâce à la présence de températures incluses dans leur niche thermique (supérieures à 16°C), mais en exhibant un taux de croissance relativement faible. Cependant, ces températures n'étant pas non plus optimales à la croissance de cette espèce, les populations d'*O. cf. siamensis* présentes dans ces zones ne sont donc pas capables de former des efflorescences comme celles observées au sud du Golfe. Seules de petites quantités de cellules pourraient être observées dans ces zones, expliquant par conséquent l'absence de cellules observées au cours de notre campagne.

Le reste de l'année et notamment l'hiver, lorsque les températures diminuent et ne sont plus favorables à la croissance cellulaire, le devenir des cellules d'*Ostreopsis* spp. reste encore à éclaircir. En effet, lors de notre expérience de laboratoire, nous n'avons pas observé de formation de kystes temporaires d'*O. cf. siamensis* lorsque ces souches étaient exposées aux faibles températures (14°C). De plus, notre expérience de laboratoire n'avait pas pour objectif d'observer la production de kystes de résistance chez ces souches, d'où l'absence de croisement entre souches différentes, une étape essentielle pour induire la formation de

kystes des espèces hétérothalliques de dinoflagellés. Cependant, la formation de kystes de résistance permettant la saisonnalité des efflorescences chez les dinoflagellés est un phénomène relativement connu (Anderson *et al.*, 1984; Kremp and Heiskanen, 1999), et également observé chez des souches d'*O. cf. ovata* de Méditerranée (Bravo *et al.*, 2012; Accoroni *et al.*, 2014). La formation de kystes dormants par les populations d'*O. cf. siamensis* pourrait donc être une hypothèse expliquant comment *Ostreopsis* spp. pourrait survivre aux conditions hivernales et ainsi coloniser durablement les côtes du Golfe de Gascogne. Concernant les populations d'*O. cf. siamensis* implantées dans le sud du Golfe, la formation de kyste de résistance survivant aux périodes hivernales apparaît comme l'hypothèse la plus plausible. Pour les populations implantées dans le nord du Golfe, une hypothèse alternative à la formation de kystes et qui pourrait expliquer la présence annuelle de cette espèce dans cette région serait l'advection de cellules provenant de la partie sud du Golfe où les efflorescences se développent. En effet, notre étude a pu démontrer la connexion physique entre les différentes zones du Golfe de Gascogne et estimer que des cellules provenant des côtes Basques pouvaient être advectées d'une année sur l'autre jusqu'à l'entrée de la Manche par les masses (cf. Chapitre 2), permettant ainsi le renouvellement des populations colonisant ces zones chaque année. Ces deux hypothèses ne sont pas incompatibles l'une avec l'autre et pourraient expliquer ensemble la distribution d'*O. cf. siamensis* observée dans le Golfe de Gascogne.

Le réchauffement des eaux associé aux changements climatiques peut-il impacter la phénologie et la distribution des espèces d'*Ostreopsis* en Europe ?

L'Atlantique nord-est et la Méditerranée sont deux régions exposées aux changements climatiques, et notamment à l'augmentation des températures des eaux de surface (deCastro *et al.*, 2009; Costoya *et al.*, 2015; Pastor *et al.*, 2020), où les microalgues toxiques sont un problème récurrent pour la santé publique. Une des principales préoccupations de la communauté scientifique étudiant les HABs est de savoir si dans le futur, ces phénomènes vont s'intensifier et s'étendre dans des zones encore peu touchées aujourd'hui (Wells *et al.*, 2015; Berdalet *et al.*, 2017b; Gobler, 2020). Dans cette thèse, le modèle *Ostreopsis* spp. a été étudié en Méditerranée nord-occidentale et dans le Golfe de Gascogne dans l'objectif de comprendre comment une microalgue toxique benthique répondrait aux changements

environnementaux, et principalement à l'augmentation des températures des océans. Un premier constat relativement logique mais non moins important de ces travaux est qu'aujourd'hui, les efflorescences d'*O. cf. siamensis* et d'*O. cf. ovata* apparaissent dans des zones où la température favorable à leur croissance est présente. En effet, nous avons pu confirmer que la niche thermique théorique d'*O. cf. siamensis* observée dans notre étude de laboratoire (cf. Chapitre 2 et 3) reflétait les températures observées lors des blooms du sud-est du Golfe de Gascogne (David *et al.*, 2012), et que la niche thermique réelle d'*O. cf. ovata* observée pendant notre monitoring à Monaco (cf. Chapitre 1) correspondait à la niche thermique théorique de souches d'*O. cf. ovata* isolées dans le Golfe de Naples observée par (Scalco *et al.*, 2012). Ceci confirme bien le rôle clé de la température dans la phénologie des blooms d'*Ostreopsis* spp. ainsi que dans sa répartition.

Dans le Golfe de Gascogne, les températures optimales de croissance d'*O. cf. siamensis* ne sont pour l'instant qu'observées dans la partie sud-est. Compte tenu du réchauffement graduel des eaux du Golfe, estimé entre 0.22°C et 0.45°C par décennie au cours des 40 dernières années (deCastro *et al.*, 2009; Costoya *et al.*, 2015), il est probable que dans le futur les efflorescences d'*O. cf. siamensis* s'intensifient dans la partie sud du Golfe et que l'espèce se développe de plus en plus vers le nord, sachant que la colonisation de cette zone est régulièrement assurée par l'advection de cellules du sud du golfe (cf. Chapitre 2). Ceci correspondrait à l'hypothèse émise par (Tester *et al.*, 2020) stipulant que les HABs benthiques des zones tempérées auraient tendance, avec le réchauffement climatique, à s'étendre vers de plus hautes latitudes. Des phénomènes similaires d'expansion de zone de distribution ont pu être mis en évidence chez d'autres espèces de dinoflagellés toxiques comme par exemple *Cochlodinium* spp. (Kudela and Gobler, 2012; Griffith *et al.*, 2019). Cette expansion des efflorescences d'*O. cf. siamensis* vers le nord du Golfe de Gascogne pourrait également être facilitée par un potentiel changement des communautés macroalgales favorisant le développement des cellules épiphytiques, pouvant être stimulé par une augmentation de la température. En effet, (Díez *et al.*, 2012) ont observé une augmentation des macroalgues de type corallines, connues pour potentiellement favoriser le développement d'*Ostreopsis* (Vila *et al.*, 2001; Cohu *et al.*, 2013), ainsi qu'une forte diminution des macroalgues de type laminaire le long des côtes est de la Mer Cantabrique (sud-est du Golfe de Gascogne) entre 1991 et 2008 et ont lié ces changements à l'augmentation des températures de l'eau. L'augmentation des températures dans cette zone pourrait donc favoriser l'apparition

d'habitats favorables à l'apparition d'efflorescences causées par *O. cf. siamensis*. Un des moyens potentiels de vérifier ce processus d'expansion de l'aire de répartition d'*O. cf. siamensis* serait de mettre en place un suivi de la présence et de développement de cellules dans certaines zones stratégiques du Golfe de Gascogne. Ces zones sentinelles, déterminée au cours de notre étude (cf. Chapitre 2), pourraient être annuellement étudiées à travers des prélèvements benthiques et observations microscopiques couplées aux techniques moléculaires de détection de l'ADN environnemental. En effet, la zone où des efflorescences sont régulièrement observées (San Sebastian / Hondaribia / Hendaye, zone D cf. Chapitre 2) ainsi que les zones fortement interconnectées et faisant le lien entre partie sud et nord du Golfe (Les Sables d'Olonne, zone H ; Concarneau, zone K) constitueraient des zones clés à surveiller pouvant témoigner de l'expansion d'*O. cf. siamensis*.

O. cf. ovata est largement répandue sur une grande partie des côtes de Méditerranée nord-occidentale (Mangialajo *et al.*, 2011) et des efflorescences y sont régulièrement observées. Dans cette région, les températures estivales dépassent souvent les températures optimales à la croissance d'*O. cf. ovata*, et pourraient même parfois impacter négativement les blooms. En effet, il semblerait que des températures trop importantes (au-delà de 30°C) pourraient limiter la croissance d'*O. cf. ovata* (Pistocchi *et al.*, 2011; Scalco *et al.*, 2012) et ainsi expliquer pourquoi les abondances maximales lors des blooms ne sont que rarement associées aux températures maximales (cf. Chapitre 1 ; (Cohu *et al.*, 2011; Mangialajo *et al.*, 2011). Par conséquent, en dépassant les températures maximales de croissance, l'augmentation de la température pourrait impacter la phénologie des blooms d'*O. cf. ovata* en produisant un décalage dans la fenêtre temporelle de développement de leurs efflorescences. On assisterait alors à des épisodes apparaissant plus précocement au cours de l'année, potentiellement avant l'été (cf. Chapitre 1) et disparaissant également plus tôt.

Le risque toxique lié à la présence d'*Ostreopsis* spp. pourrait également évoluer avec l'augmentation des températures. A ce jour, aucune toxine connue associée à celles produites par *Ostreopsis* spp. n'a été détectée chez des souches d'*O. cf. siamensis* provenant des côtes Atlantiques Européennes (Ciminiello *et al.*, 2013), et ce même au cours de notre étude (cf. Chapitre 3). D'autres analyses chimiques effectuées par le laboratoire PHYC de l'Ifremer Nantes sur 17 autres souches d'*O. cf. siamensis* issues de 5 sites différents (D. Réveillon, comm. pers) ont donné le même résultat négatif, sans présence avérée de toxine connue appartenant à la famille des palytoxines et autres analogues.

Cependant, l'utilisation de bio-tests sur *Artemia franciscana* confirme la toxicité et la production de composés allélopathiques par ces souches (cf. Chapitre 3) déjà observées auparavant (Laza-Martinez *et al.*, 2011). L'effet nuisible sur l'homme resterait par contre encore à confirmer. Un signalement récent datant du 13 septembre 2020 et recueilli par l'office du tourisme d'Hendaye (Pays Basque) pourrait être associé à un potentiel premier cas d'intoxication lié à *O. cf. siamensis* dans le Golfe de Gascogne (Ifremer LER-AR comm. pers, note en cours de rédaction). Ce signalement regroupe plusieurs témoignages décrivant des symptômes d'irritation des voies aériennes et de la peau ainsi que des maux de têtes chez des personnes ayant passé une journée sur une plage d'Hendaye, après une période estivale où les eaux côtières sont restées très calmes et ont dépassé les 26°C. L'absence d'anomalie concernant les analyses de qualité de l'eau par les autorités sanitaires ainsi que l'observation de la présence de cellules d'*Ostreopsis* spp. dans l'eau et sur des macroalgues dans un prélèvement effectué peu après ce signalement renforce l'idée que cet épisode nuisible sur l'homme pourrait être lié à la présence d'*O. cf. siamensis*. Ceci constituerait le premier témoignage de l'effet nuisible d'une efflorescence d'*O. cf. siamensis* sur l'homme dans le Golfe de Gascogne et également en Europe.

Au cours de notre expérience d'acclimatation, nous avons en effet observé un effet allélopathique des souches d'*O. cf. siamensis* plus important à 22°C et 25°C qu'à 19°C et 16°C, indiquant que la toxicité de ces souches variait avec la température. Ainsi, aujourd'hui dans le Golfe de Gascogne, le risque associé à *O. cf. siamensis* semble être localisé dans la partie sud-est (Pays Basque) dû aux températures favorisant la croissance et la toxicité des souches isolées dans cette zone, même si des études complémentaires sont nécessaires à l'évaluation de son potentiel réel de toxicité sur l'homme. A l'heure actuelle, les faibles températures retrouvées au nord du Golfe de Gascogne semblent indiquer une absence de risque toxique étant donné la relative faible croissance et toxicité observés chez les souches d'*O. cf. siamensis* cultivées à ces faibles températures. Cependant, l'augmentation des températures associée aux changements climatiques pourrait néanmoins constituer un potentiel facteur d'augmentation des risques de toxicité dans ces régions soumises au réchauffement des eaux côtières.

Chez *O. cf. ovata* en Méditerranée, le lien entre température et toxicité reste encore à démontrer même si certaines études (Faimali *et al.*, 2012; Pezolesi *et al.*, 2012) ont observé une variation de la toxicité en fonction de la température. Nos résultats prochainement issus

de l'expérience d'acclimatation thermique portant sur les souches d'*O. cf. ovata* de Méditerranée associés aux bio-tests *Artemia* à des températures variables pourraient nous permettre de vérifier ce lien et d'en apprendre plus sur l'évolution du risque toxique lié à *O. cf. ovata* exposé au réchauffement des eaux de Méditerranée.

Le devenir des microalgues benthiques toxiques face aux changements climatiques est d'autant plus délicat à estimer que les réponses évolutives et adaptatives des espèces face à ces changements sont relativement peu connues. En effet, afin de prédire la future distribution ou phénologie d'une espèce en relation avec des facteurs environnementaux changeants, il faudrait, en plus d'avoir déterminé les liens entre l'ensemble de ces facteurs et le développement de l'espèce, prendre en compte l'évolution, la sélection et l'adaptation au cours du temps de l'espèce concernée. Par exemple, l'étude de laboratoire menée sur près de 4 ans par (Flores-Moya *et al.*, 2012) a montré que l'acclimatation et l'adaptation à long terme de deux souches du dinoflagellé toxique *Alexandrium minutum* à des conditions simulant le changement climatique leur permettrait d'observer un taux de croissance plus important dans ces conditions futures. Différents processus agissent également sur l'évolution génétique des populations et permettent à celles-ci de s'adapter aux changements environnementaux. La reproduction sexuée permet la recombinaison génétique et donc de nouvelles combinaisons d'allèles, et la division clonale permet la diffusion des génotypes les mieux adaptés de la population. Si la variabilité génétique entre des souches issues d'une même efflorescence d'*Ostreopsis* spp. a déjà été observée (Pin *et al.*, 2001), à notre connaissance, aucune étude de génétique de population n'a été menée sur des espèces d'*Ostreopsis* pour étudier l'alternance de populations au cours d'un seul et même épisode d'efflorescence. Des études conduites sur d'autres dinoflagellés toxiques pourraient ouvrir la voie et inspirer cette nouvelle recherche. A titre d'exemple, (Dia *et al.*, 2014) ont observé une forte diversité génotypique et génétique ainsi que de rapides variations de la diversité génétique au cours d'une même efflorescence d'*Alexandrium minutum*, ce qui pourrait potentiellement influencer l'adaptation des populations face à l'évolution des conditions environnementales en permettant la sélection des clones génotypiquement mieux adaptés.

Conclusions générales

La température est un facteur primordial pour le développement des espèces responsables de HABs. Les travaux effectués au cours de cette thèse ont permis de mettre en évidence comment la température pouvait influencer la physiologie, la distribution ainsi que la phénologie des efflorescences des espèces du dinoflagellé benthique toxique *Ostreopsis* spp. présentes en Méditerranée nord-occidentale et dans le Golfe de Gascogne :

- Notre étude, basée sur les observations recueillies au cours de la plus longue série temporelle associée aux efflorescences d'*Ostreopsis* spp. connue à ce jour, a montré que les anomalies de températures printanières des eaux de surface permettaient d'expliquer une partie de la phénologie des blooms saisonniers d'*O. cf. ovata* à Monaco, en suggérant que l'accumulation d'anomalies thermiques positives au printemps engendrerait une apparition précoce du bloom estival d'*O. cf. ovata*.
- La campagne d'échantillonnage menée sur les côtes Atlantiques françaises et espagnoles basée sur l'utilisation d'outils moléculaires pour la détection d'ADN environnemental et couplée à une approche de modélisation Lagrangienne a permis de révéler la présence d'*O. cf. siamensis* dans la quasi totalité du Golfe de Gascogne. Cette étude a ainsi confirmé la formation d'efflorescences de cette espèce dans le sud du Golfe et a également, pour la première fois, mis en évidence la colonisation des côtes de la partie nord du Golfe jusqu'à l'entrée de la Manche par *O. cf. siamensis*. Il reste cependant à déterminer si cette colonisation se déroule de manière annuelle suite à l'advection de cellules provenant du sud, ou si des populations stables d'*O. cf. siamensis* demeurent présentes dans la zone tout au long de l'année. Cette incertitude dérive de l'absence d'information sur le potentiel de survie d'*O. cf. siamensis* pendant l'hiver à des températures inférieures à 16°C. La colonisation dans le nord du golfe se traduit par une présence rare de l'espèce, détectable seulement par approche moléculaire, et par l'absence d'efflorescence. Ceci semblerait être dû aux habitats et températures peu favorables restreignant sa fenêtre de développement et limitant sa croissance.

- L'étude de l'acclimatation thermique de différentes souches d'*Ostreopsis* spp. menée en laboratoire a permis de définir la niche thermique théorique de 6 souches d'*O. cf. siamensis* isolées dans le sud-est du Golfe de Gascogne en observant leur température limites et optimales de croissance suite à une acclimatation lente à un gradient de températures, mais aussi d'évaluer l'évolution de leur toxicité en fonction du changement de température. La température optimale de croissance de ces souches a ainsi été estimée à 22.45°C, tandis que les températures limites de croissances ont été observées à 16°C et 25°C. Le bio-test *Artemia* a également révélé que ces souches étaient plus toxiques à 22°C et 25°C qu'à 16°C et 19°C.

Perspectives

Face aux changements climatiques et à l'augmentation des températures des océans, la phénologie et la distribution des phénomènes d'efflorescences algales toxiques associées notamment aux espèces d'*Ostreopsis* risquent d'être affectés dans les eaux européennes. Il est donc nécessaire de continuer à étudier les réponses écologiques et physiologiques de ces espèces vis-à-vis des facteurs environnementaux afin de pouvoir potentiellement prédire l'apparition de ces épisodes et de prévenir les conséquences sanitaires, économiques et écologiques associées.

La mise en place de suivis *in situ* multiparamétriques est une solution pour à la fois évaluer l'expansion et comprendre l'écologie d'un tel modèle à long terme en relation avec l'ensemble de paramètres biotiques et abiotiques de l'écosystème. En effet, l'influence du changement des facteurs environnementaux ne peut se faire qu'à travers l'étude de séries temporelles assez longues pour constater de potentiels effets. Ainsi, le suivi d'efflorescences établi chaque saison estivale depuis 2007 à Monaco doit être poursuivi afin de continuer à récolter des données biologiques et environnementales précieuses et nécessaires pour de futures analyses écologiques et renforcer les connaissances locales. Le suivi local et à fine échelle temporelle de l'ensemble des paramètres abiotiques mais aussi biotiques, comme l'ensemble des communautés macro/microalgales, bactériennes et zooplanctoniques serait

également nécessaire pour comprendre les fortes variabilités spatiales et temporelles de la phénologie de ces efflorescences.

La détermination et le suivi de zones sentinelles serait également nécessaire pour monitorer l'expansion d'*Ostreopsis* spp. dans les zones soumises au réchauffement des eaux. Les zones dans lesquelles des efflorescences sont régulièrement observées (San Sebastian / Hondaribia / Hendaye) ainsi que les zones fortement interconnectées et faisant le lien entre partie sud et nord et Golfe (Les Sables d'Olonne et Concarneau) constitueraient des zones clés à surveiller pouvant témoigner de l'expansion d'*O. cf. siamensis* dans le Golfe de Gascogne. Afin de vérifier nos hypothèses concernant l'expansion de l'aire de répartition populations d'*O. cf. siamensis* du sud-est du Golfe de Gascogne vers le nord, il serait nécessaire de mettre en place un suivi multiparamétrique à long terme basé à la fois sur une approche de biologie moléculaire visant à confirmer la présence même rare de l'espèce via la détection d'ADN environnemental, et sur une approche d'observation en microscopie afin d'obtenir des informations d'abondance cellulaire de l'espèce. Ces suivis pourraient également permettre de détecter la présence de l'espèce durant l'hiver et de confirmer ou non la formation de kystes de résistances en présence de températures non favorables à la croissance cellulaire.

Enfin, concernant l'écologie d'*Ostreopsis*, il est nécessaire d'évaluer, comment les variations journalières de certains paramètres peuvent impacter la physiologie des cellules. En effet, notre expérience d'acclimatation thermique, bien que nécessaire à la détermination des températures favorables à la croissance d'*Ostreopsis* spp., ne reflète pas entièrement les conditions de températures rencontrées *in situ*. En milieu naturel, les paramètres environnementaux fluctuent constamment et les cellules sont exposées à des variations journalières très fortes. Ceci est notamment vérifié pour les populations d'*O. cf. siamensis* du Golfe de Gascogne qui, vivant dans des zones fortement affectées par les marées, sont soumises à des variations journalières d'irradiance, de salinité et de température importantes. Une expérience visant à évaluer les chocs de température, d'irradiance ou d'hydrodynamisme permettrait de comprendre encore plus en détail les mécanismes adaptatifs de cette espèce face aux stress environnementaux.

L'autre préoccupation associée au potentiel essor des efflorescences de microalgues toxiques le long des côtes européennes est l'ensemble des impacts sanitaires, économiques et écologiques liés à la toxicité des espèces nuisibles se développant massivement. Il est donc ainsi nécessaire d'évaluer la toxicité des espèces d'*Ostreopsis* présentes en Europe face à la

variation de température ainsi que la variabilité inter-souches. Nous avons pu observer un effet allélopathique de toutes les souches d'*O. cf. siamensis* testées lors de notre expérience d'acclimatation à travers l'utilisation du bio-test *Artemia*, mais la caractérisation des toxines produites par les souches d'*O. cf. siamensis* d'Atlantique n'a pas pu être encore réalisée. Une fois ces composés allélopathiques et/ou toxiques caractérisés, le potentiel de toxicité ainsi que l'impact sanitaire de cette espèce pourront être estimés. Cette recherche pourrait conduire à l'identification de nouvelles substances nuisibles pour l'homme puisque les toxines connues à ce jour n'ont pas été identifiées dans les souches testées. L'étude de la toxicité de ces espèces pourrait également viser à interpréter le rôle des facteurs environnementaux ainsi que les mécanismes biologiques impliqués dans la production de toxines, étant donné que des variations de la toxicité des souches ont été observées en fonction de la température et de la phase de croissance.

Enfin, les résultats d'analyses encore en attente issus de l'expérience d'acclimatation devraient permettre de définir la niche thermique et d'évaluer la toxicité de 3 autres souches d'*O. cf. siamensis* du Golfe de Gascogne ainsi que celle de 3 souches d'*O. cf. ovata* provenant de Méditerranée nord-occidentale (Villefranche-sur-Mer et Monaco), mais également d'en apprendre plus sur les mécanismes écophysiologiques impliqués dans le processus d'acclimatation, notamment à travers la variation des profils pigmentaires et lipidiques. Ces analyses pourraient permettre de comparer la biologie et les mécanismes adaptatifs des espèces du genre *Ostreopsis*, contribuant ainsi à expliquer les raisons du partitionnement de leur distribution.

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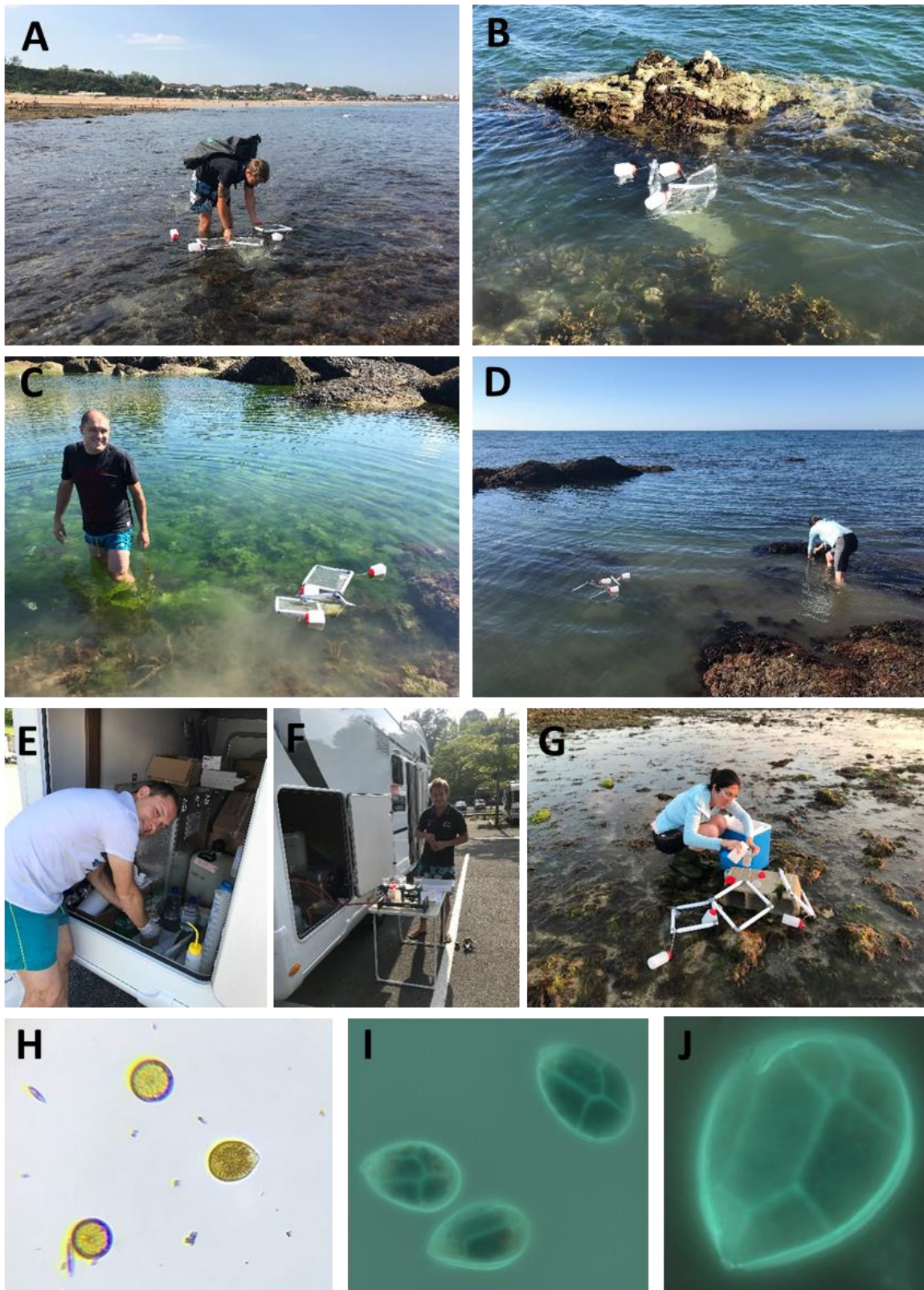
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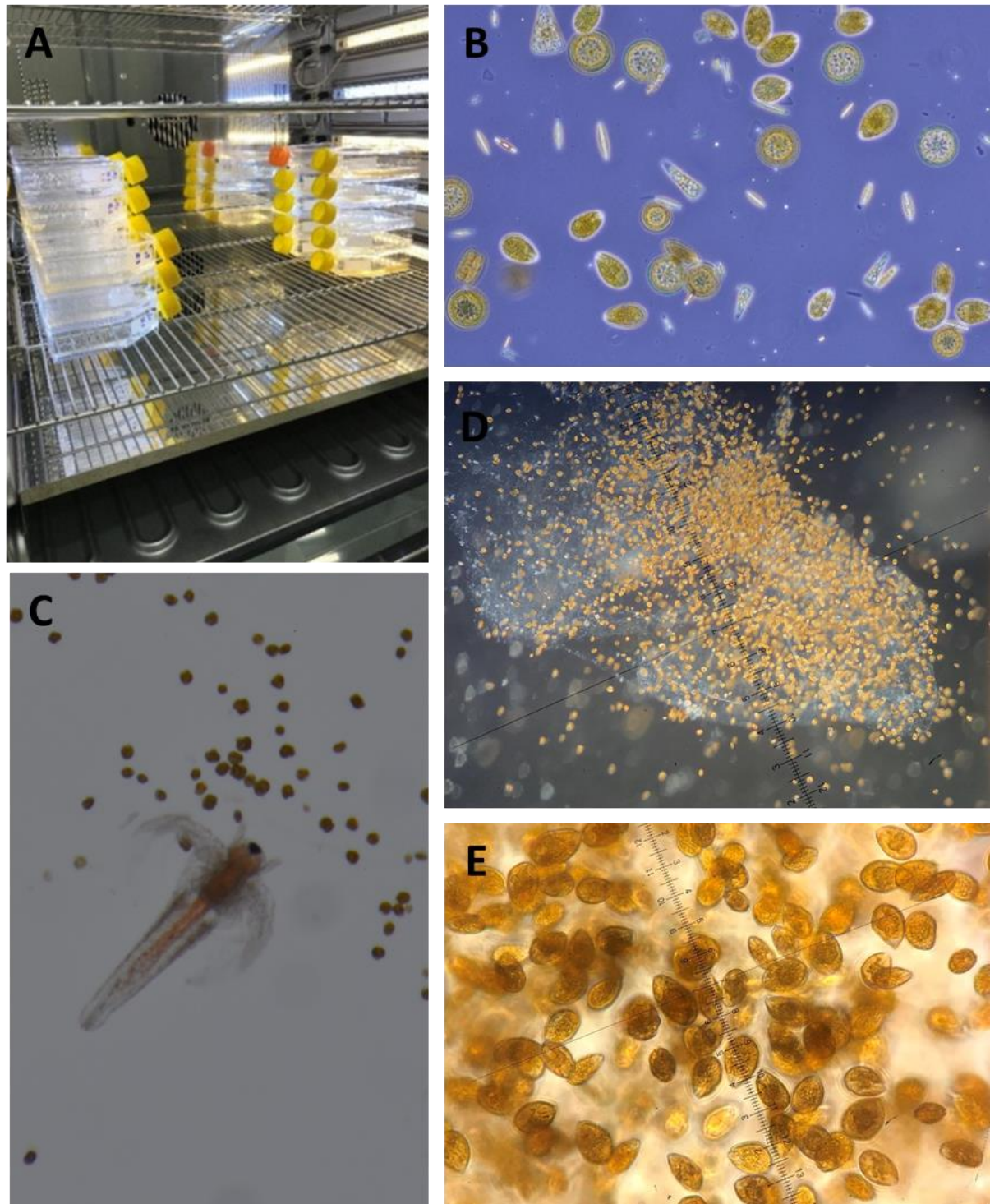
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Annexes




Annexe 1 : Images de la campagne d'échantillonnage de 2018. (A – G) Prélèvements benthiques, pose et récupération des substrats artificiels, filtration des échantillons d'ADN environnemental. (H) Première observation de cellules d'*Ostreopsis* cf. *siamensis* durant la campagne, à St-Jean-de-Luz. Observations au microscope (I) x200 et (J) x400 de cellules d'*O.* cf. *siamensis* marquées au Solophényl Flavine provenant d'échantillons benthiques de St-Jean-de-Luz.



Annexe 2 : (A) Cultures d'*O. cf. ovata* en flasques en incubateur. (B) Observation au microscope (x100) ce cellules d'*O. cf. ovata* et de diatomées à partir d'un échantillon naturel provenant de la crique de Rochambeau (Villefranche-sur-Mer). (C) Nauplii d'*Artemia franciscana* en présence de cellules d'*O. cf. ovata*. (D, E) Observations au microscope de cultures d'*O. cf. ovata* avec matrice mucilanigeuse isolées à Villefranche-sur-Mer.



Mediterranean long-term study shows correlation between *Ostreopsis cf. ovata* bloom timing and spring temperature

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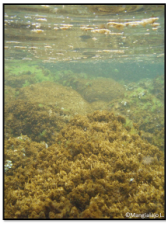
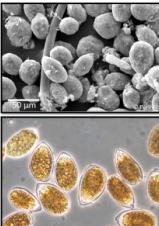
Introduction

Global warming has been suggested to influence *Ostreopsis cf. ovata* expansion from tropical areas to temperate waters, such as the Mediterranean Sea, but little is known about the role of temperature on the phenology of *Ostreopsis* blooms.

Benthic toxic dinoflagellate *Ostreopsis cf. ovata* events are responsible for ecological, sanitary and economic issues in different temperate areas and need better understanding.



Indeed, *Ostreopsis cf. ovata* produces toxins (ovatoxins, palytoxin analogs), and when suitable environmental conditions are gathered, a massive development of cells can occur leading to a toxic outbreak.

Predicting the distribution and the phenology of *Ostreopsis* in European coastal waters is a crucial challenge, especially in a context of global warming.

Ostreopsis monitoring in Monaco, NW Mediterranean Sea

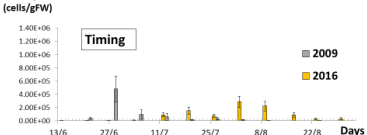
- From 2007 to 2017: 5 sites sampled once a week during the bloom period, from June to end August.
- Benthic abundance estimations: Cell abundance were quantified counting cells attached to macroalgae (cells per gram of fresh weight).

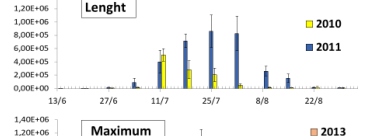
Blooms phenology

- Highly variable phenological features**
Throughout years, blooms differ from each other in timing, length and maximum abundance.
- Optimum growth temperature**
Variations of net growth rate estimated during the phases of bloom development were analyzed as a function of Sea Surface Temperature (SST). Highest growth rates were not associated with the maximal temperature records (27.5°C) but were estimated when temperature ranged between 21°C and 25°C. This suggests a more complex role of temperature in bloom dynamics than a simple facilitation factor for algal growth, at least in temperate areas.

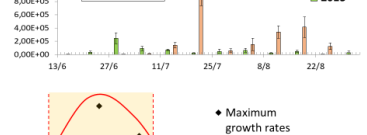
Timing



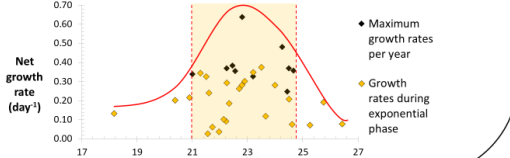
Length



Maximum abundance



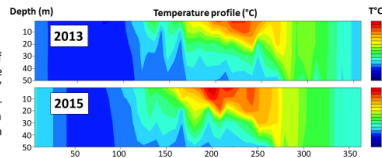
Net growth rate (day⁻¹) vs Temperature (°C)



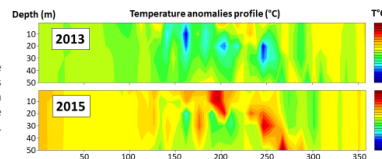
Spring temperature : Warm and cold years

- Annual temperature profiles**
All over the 11 years of survey, temperature were recorded at "Point B" station (Villefranche-sur-Mer, 12 km West from Monaco) each week, from surface to 50 m.
- Annual temperature anomalies profiles**
SST anomalies were calculated for each week as differences between temperature values and the mean SST value over the 11 years of survey.
- Cumulative sum of SST anomalies**
SST anomalies were accumulated starting from the beginning of the year in order to quantify the accumulation of positive and negative heat anomalies, separating years with warmer springs from years with colder springs.

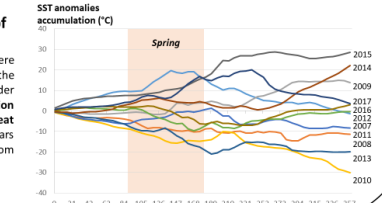
Temperature profile (°C)



Temperature anomalies profile (°C)



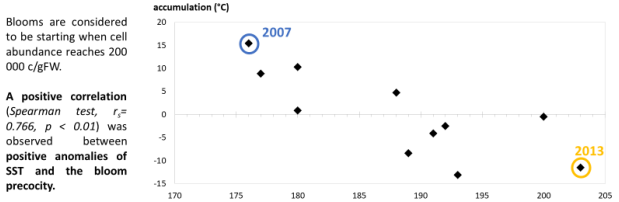
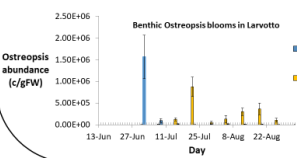
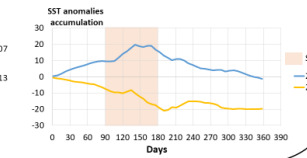
SST anomalies accumulation (°C)



The warmer the spring, the earlier the bloom

Blooms are considered to be starting when cell abundance reaches 200 000 c/gFW.

A positive correlation (Spearman test, $r_s = 0.766$, $p < 0.01$) was observed between positive anomalies of SST and the bloom precocity.

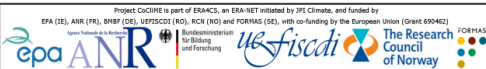
Conclusions

The relation between SST and net growth rate of the cells during bloom events is one of the main question concerning *Ostreopsis* bloom phenology in Mediterranean Sea. Here, we show that the optimum growth temperature is located between 21°C and 25°C, and not at the highest temperatures recorded like some are suggesting.

For the first time in the study of *Ostreopsis*, we show a direct correlation between temperature and its phenology, indicating that blooms seem to occur earlier in the season when spring is warmer. This suggests that bloom phenology could be influenced by temperature both before and during the event :

- During warmer springs, the higher accumulation of heat could induce an earlier germination among resting cells, therefore leading to an earlier bloom event.
- The key role played by temperature on blooms could be linked with physiological mechanisms happening before the beginning of the bloom.

Considering the global increase of SST in a context of climate change, those relations between bloom phenology and SST should be of great interest for modelers in order to better understand the dynamics of the bloom and to predict future harmful events.



Annexe 3 : Poster présenté lors de la 18^{ème} conférence ICHA en 2018 à Nantes.

Impact of sea temperature on the distribution and phenology of the toxic benthic dinoflagellate genus *Ostreopsis* in the Mediterranean Sea and the Atlantic

Abstract:

Harmful algal blooms (HABs) associated with the toxic benthic dinoflagellate *Ostreopsis* spp. are increasing around the world and responsible for sanitary, economic and ecological issues. The increasing number of reports of this dinoflagellate in recent decades suggests that climate change, and more particularly ocean warming, may be partly responsible for its expansion, especially in temperate zones. However, this link between warming waters and the development of *Ostreopsis* spp. blooms is difficult to prove and data concerning distribution areas or cellular responses to thermal variations are still lacking. This PhD thesis work provides new elements to answer this issue by addressing three main themes: 1) the phenology of blooms, 2) the distribution of species along a latitudinal gradient and 3) physiological adaptations to a temperature gradient. These questions were addressed by keeping the effect of temperature on the ecology of *Ostreopsis* spp. as the main thread and link between the three questions asked. This work was carried out using a multidisciplinary approach including statistical analyses of datasets, genetic approaches based on environmental DNA, as well as ecophysiology and ecotoxicology approaches. The data used were acquired both from samples collected in the field and from experiments in a controlled environment in the laboratory.

To meet the first objective, we analyzed the time series resulting from the ongoing monitoring of *O. cf. ovata* summer blooms in Monaco (NW Mediterranean Sea) set up since 2007 in order to determine the relationship between blooms phenology and environmental parameters. Although no environmental factor measured was able to fully explain the phenology of blooms, a strong positive correlation was found between positive sea surface temperature anomalies during spring and the timing of blooms, suggesting that the warmer the spring, the earlier the blooms of *O. cf. ovata* in Monaco.

The second question of the thesis was addressed by analyzing new samples collected as part of a sampling campaign conducted along the coast of the Bay of Biscay in August-September 2018. This campaign aimed to establish the current distribution of *Ostreopsis* spp. in this area exhibiting a strong South-North thermal gradient, in order to initiate the analysis of a possible future expansion due to ocean warming. The processed results combined a classic benthic sampling approach, the use of artificial substrates for the collection of environmental DNA analyzed through molecular tools (rt-qPCR) as well as a Lagrangian modeling approach aiming at establishing the connectivity between sampling areas. We showed that only *O. cf. siamensis* was present in the Bay and that it could be found in certain areas up until the entrance to the English Channel, although blooms of *O. cf. siamensis* were only observed in the southeastern part of the Bay. This study allowed us to identify sentinel zones along the Bay of Biscay where new long-term monitoring could be set up in order to observe the potential expansion of *O. cf. siamensis* towards the North of the bay.

Finally, the response of *Ostreopsis* spp. in relation with varying temperatures was studied in terms of growth capacity, physiological adaptations and toxicity in a thermal acclimation experiment. Different monoclonal strains were compared, including strains of *O. cf. siamensis* isolated in the Bay of Biscay during our 2018 campaign, as well as strains of *O. cf. ovata* from the northwestern Mediterranean Sea, corresponding to the two areas studied in this thesis. The first results of this experiment indicate that the strains of *O. cf. siamensis* are able to acclimate and grow at temperatures between 16 °C and 25 °C, with an optimum growth temperature estimated around 22 °C. In addition, the strains of *O. cf. siamensis* exhibited a higher level of toxicity at 22°C and 25°C than at 16°C and 19°C, indicating an effect of temperature on the toxicity of *Ostreopsis* spp..

This work demonstrates the influence of temperature on the phenology, distribution and physiology of *Ostreopsis* spp. in European waters. It also suggests that, in a context of climate change and increasing water temperatures, the risk associated with the expansion and toxicity of *Ostreopsis* spp. must be followed. The study of these species along the Mediterranean and Atlantic coasts is essential to forestall future health and ecological issues associated with the expansion of this genus of harmful benthic dinoflagellate.

Keywords: *Ostreopsis* spp.; Dinoflagellates; Harmful Algal Blooms; Coastal ecology; Phenology; Biogeography; Environmental DNA; Climate Change; Ocean warming; Thermal niche; Ecophysiology; Ecotoxicology; Coastal risk management

Impact de la température sur la biogéographie et la phénologie des dinoflagellés benthiques toxiques du genre *Ostreopsis* en Méditerranée et en Atlantique

Résumé :

Les efflorescences algales nuisibles (HABs) associées aux dinoflagellés benthiques toxiques du genre *Ostreopsis* sont des phénomènes observés de plus en plus fréquemment à travers le monde et sont responsables de problèmes sanitaires, économiques et écologiques. L'augmentation du nombre d'observations de ce dinoflagellé depuis les dernières décennies laisse penser que les changements climatiques, et plus particulièrement le réchauffement des océans, seraient en partie responsable de son expansion, notamment dans les zones tempérées. Cependant, ce lien entre réchauffement des eaux et développement d'efflorescences d'*Ostreopsis* est difficile à prouver et certaines données concernant les aires de répartition ou la réponse des cellules aux variations thermiques sont encore manquantes. Ces travaux de thèse ont fourni des nouveaux éléments pour répondre à cette problématique en abordant trois thématiques principales : 1) la phénologie des efflorescences, 2) la distribution d'espèce le long d'un gradient latitudinal et 3) les adaptations physiologiques à un gradient de température. Ces questions ont été abordées en gardant l'effet de la température sur l'écologie des espèces étudiées comme fil conducteur principal et lien entre les trois questions posées. Les travaux ont été menés avec une approche multidisciplinaire incluant des analyses statistiques de jeux de données, des approches génétiques basées sur l'ADN environnemental, ainsi que des approches d'écophysiologie et d'écotoxicologie. Les données utilisées ont été acquises à la fois à partir de prélèvements de terrain et d'expériences en milieu contrôlé au laboratoire.

Pour répondre au premier objectif, nous avons analysé la série temporelle issue du suivi d'efflorescences d'*O. cf. ovata* à Monaco, mis en place depuis 2007 et toujours poursuivi à ce jour, afin de mettre en relation la phénologie des efflorescences avec les paramètres environnementaux. Bien que, d'après nos analyses, aucun facteur physico-chimique mesuré ne soit en mesure d'expliquer la phénologie des efflorescences, une forte corrélation positive entre l'accumulation d'anomalies positives des températures de surface au printemps et la date de début des efflorescences a été mise en évidence, suggérant que plus les températures printanières seraient élevées, plus les efflorescences d'*O. cf. ovata* à Monaco seraient précoces.

La deuxième question de la thèse a été abordée en analysant de nouveaux échantillons collectés dans le cadre d'une campagne d'échantillonnage conduite le long des côtes du Golfe de Gascogne en août-septembre 2018. Cette campagne avait pour objectif d'établir l'aire de répartition actuelle d'*Ostreopsis* spp. dans cette zone à fort gradient thermique sud-nord, afin de poser les bases pour l'analyse d'une possible future expansion en cas de réchauffement des eaux. Les résultats traités ont combiné une approche classique d'échantillonnage benthique, l'utilisation de substrats artificiels pour la collection d'ADN environnemental analysés via des outils moléculaires (rt-qPCR) ainsi qu'une approche de modélisation Lagrangienne visant à établir la connectivité physique entre différents sites de prélèvement. Nous avons pu montrer que seule l'espèce *O. cf. siamensis* était présente dans le Golfe, et que celle-ci pouvait être retrouvée jusqu'à l'entrée de La Manche, bien que les populations formant des efflorescences n'aient été observées que dans la partie sud-est du Golfe. Cette étude nous a permis d'identifier des zones sentinelles le long du Golfe de Gascogne où il serait intéressant de mettre en place de nouveaux suivis à long terme afin d'observer la potentielle expansion vers le nord d'*O. cf. siamensis*.

La réponse d'*Ostreopsis* spp. face à des changements de température a été étudiée en termes de capacité de croissance, d'adaptations physiologiques et de toxicité, au cours d'une expérience d'acclimatation thermique. Différentes souches monoclonales ont été comparées, dont des souches d'*O. cf. siamensis* isolées dans le Golfe de Gascogne au cours de notre campagne de 2018, ainsi que sur des souches d'*O. cf. ovata* provenant de Méditerranée nord-occidentale, les deux aires d'études de cette thèse. Les premiers résultats de cette expérience indiquent que les souches d'*O. cf. siamensis* sont capables de s'acclimater et de croître à des températures comprises entre 16°C et 25°C, avec un optimum de température obtenu aux alentours de 22°C. De plus, les souches d'*O. cf. siamensis* testées ont exhibé une toxicité plus forte à 22°C et 25°C qu'à 16°C et 19°C, indiquant un effet de la température sur la toxicité d'*Ostreopsis* spp..

Ces travaux démontrent l'influence de la température sur la phénologie, la distribution et la physiologie d'*Ostreopsis* spp. dans les eaux européennes. Ils suggèrent également que, dans un contexte de changements climatiques et d'élévation de la température des eaux de surface, le risque associé à l'expansion et à la toxicité des espèces d'*Ostreopsis* spp. doit être suivi. Continuer à étudier ces espèces le long des côtes méditerranéennes et atlantiques est essentiel pour appréhender de futurs risques sanitaires et écologiques associés à l'expansion de ce genre de dinoflagellé benthique nuisible.

Mots-clés : *Ostreopsis* spp. ; Dinoflagellés ; Efflorescences algales nuisibles ; Ecologie côtière ; Phénologie ; Biogéographie ; ADN environnemental ; Changements climatiques ; Réchauffement des océans ; Niche thermique ; Ecophysiologie ; Ecotoxicologie ; Management des risques côtiers