UQAR
Université du Québec
à Rimouski

Étude de l'effet de toxines sur le comportement valvaire de bivalves

Thèse présentée

dans le cadre du programme de doctorat en Océanographie

en vue de l'obtention du grade de *Philosophiæ Doctor* (Ph.D)

PAR

Guillaume DURIER

Juin 2022

Composition du jury :

Fanny Noisette, présidente du jury, Université du Québec à Rimouski

Réjean Tremblay, directeur de recherche, Université du Québec à Rimouski

Luc Comeau, codirecteur de recherche, Centre du Golfe, Pêches et Océans Canada

David Drolet, examinateur externe, Institut Maurice Lamontagne, Pêches et Océans Canada

Dépôt initial le 24 Mars 2022 Dépôt final le 17 Juin 2022

REMERCIEMENTS

Je tiens tout d'abord à remercier mes directeurs de thèse, Réjean Tremblay et Luc Comeau. J'ai été vraiment chanceux de pouvoir travailler sur un projet aussi intéressant et aussi concret. Je me suis senti accompagné et soutenu tout au long de ce doctorat malgré la distance imposée par la pandémie pendant plus de la moitié de celui-ci. Chacun des échanges était toujours motivant pour la suite et m'ont donné envie de m'accrocher encore plus pour la suite.

Je remercie également Fanny Noisette d'avoir présidé mon jury de thèse et David Drolet d'avoir évalué ma thèse comme examinateur externe. Je vous remercie pour votre bienveillance et vos conseils tous très intéressants.

Je remercie mon comité de thèse, Richard St-Louis pour toutes les connaissances apportées en écotoxicologie et en chimie, et Damien Tran qui m'a apporté toutes les bases de la valvométrie et pour m'avoir permis d'accéder à ce doctorat.

Ce projet a été réalisé avec l'appui financier du gouvernement du Canada, this project was undertaken with the financial support of the Government of Canada. Je remercie donc Environnement et Changement Climatique Canada d'avoir permis à ce projet d'avoir vu le jour.

Je remercie Jean-Bruno Nadalini pour son énorme soutien au laboratoire, ta gentillesse, ton expérience, ta patience et tes connaissances ont été d'une aide indispensable pour ce doctorat et même au-delà. Travailler avec toi a été un grand plaisir !

Je remercie Michel Starr pour son expertise sur les algues toxiques, les échanges intéressants et pour son aide lors des activités plomberie au moment de monter les bassins à l'IML. Je remercie Sonia Michaud pour sa disponibilité et son expérience des tests Elisa.

Je remercie Romain Lavaud avec qui j'ai eu la chance de partager un des articles de ma thèse, rassembler deux parties du projet Alexandrium a été très intéressant.

Lors de ce doctorat j'ai eu la chance de pouvoir faire partie du regroupement Ressources Aquatique du Québec, je remercie donc le RAQ et Marine Holbach pour tout le soutien apporté, les réunions annuelles toujours attendues avec impatience, les nombreuses activités organisées pour les étudiants.

J'ai également eu la chance de faire partie d'une super équipe, le Laboratoire d'Écophysiologie et d'Aquaculture, avec qui les échanges ont toujours été enrichissant dans le travail ou en dehors.

Un grand merci à tout le personnel de l'ISMER, toujours présent, efficace et de bonne humeur!

Je remercie Samuel et Hermann pour leur aide et leur sérieux, l'encadrement de. Ces stages ont été très agréable intéressant.

Je remercie le O262, à coup sur le meilleur bureau de l'ISMER ! Toujours là pour des conseils, de l'aide, du soutien mais aussi plein d'excellents moment !!! Merci Efflam, Sarah, Antoine, Camille, Lola, Léo, Maria, Delphine, Anthony, Quentin, Élodie et j'en oublie !

Pendant ces un peu plus de trois ans et demi de doctorat j'ai eu l'occasion de rencontrer pleins de personnes formidables donc un grand merci aux amis du Québec, de France avec qui j'ai pu rester en contact malgré l'éloignement !

Merci à toute ma famille pour le gros soutien malgré la distance et le fait que je n'ai pas pu rentrer. Heureusement que les appels visio existent !
Je remercie aussi la famille Arseneault et Rioux d'avoir pris soin de moi !

Enfin merci Lisa Marie, ma plus belle rencontre québecoise !!! Merci ton soutien exceptionnel et sans faille, ton extrême patience, ta bonne humeur, tous ces moments parfaits passés ensemble ! La vie ici serait très différente sans toi !!!!

RÉSUMÉ

Les fermes aquacoles étant situées sur les côtes en milieu ouvert, elles peuvent être exposées à l'arrivée de toxines présentes dans l'eau de mer. Ces toxines peuvent entrainer la fermeture de la récolte des organismes cultivés et donc des pertes économiques pour les aquaculteurs. Une solution pour minimiser ces pertes économiques serait de détecter l'arrivée de ces toxines avant que les seuils critiques pour la fermeture de la récolte des bivalves ne soient atteints. Ainsi les aquaculteurs pourraient prendre les mesures adaptées pour continuer de commercialiser leur production comme la placer en vivier et éviter ces pertes économiques. Deux types de toxines ont été étudiées au cours de ce doctorat. La première était une substance synthétique, le dispersant d'hydrocarbures Corexit 1500, utilisé lors d'évènements tel que des marées noires dans le but de disperser les nappes d'hydrocarbures. La seconde toxine était d'origine naturelle et correspond à un groupe de composés toxiques produits par le dinoflagellé *Alexandrium catenella* agissant comme des toxines paralysantes (Paralytic Shellfish Toxins).

L'hypothèse de ce doctorat était que les changements de comportement valvaire des bivalves pourraient être utilisés pour détecter l'arrivée de ces toxines dans les fermes aquacoles. Pour observer et enregistrer le comportement des bivalves et ainsi détecter leurs changements de comportements, la valvométrie a été utilisée. Cette technique consiste à mesurer l'ouverture d'un bivalve grâce à un capteur et un aimant placé sur chacune des valves. Le capteur est relié au boitier du valvomètre permettant d'enregistrer l'écartement entre les valves à haute fréquence et ainsi enregistrer le comportement valvaire au cours du temps et en perturbant le comportement du bivalve le moins possible, celle-ci étant non-invasive. La valvométrie permet donc également d'observer d'éventuels changements de comportement. L'enregistrement de données à haute fréquence générant une très grande quantité de données, l'automatisation du traitement de celles-ci a été obligatoire. Dans ce but, un programme informatique a été développé pour compiler les données, les convertir de mV à une amplitude d'ouverture, produire des graphiques permettant de visualiser les comportements enregistrés et finalement de calculer des indicateurs comportementaux décrivant les comportements enregistrés pour les étudier.

Pour étudier la possibilité d'utiliser le comportement valvaire des bivalves pour la détection de toxines, la première étape a été de déterminer quelle espèce est la plus adaptée. Pour cela, la moule bleue *M. edulis* et le pétoncle géant *P. magellanicus* ont été comparés lors d'une exposition au Corexit 1500. Les résultats obtenus montrent que contrairement au pétoncle la moule était capable de s'isoler du milieu extérieur, ce qui lui donne une meilleure survie et offre un changement de comportement facile à détecter. Cette espèce est donc plus adaptée à la détection de toxines. L'étape suivant fut de déterminer l'effet des deux toxines étudiées sur le comportement valvaire. Dans le cas de la substance synthétique, Corexit 1500, lors d'une exposition il a été observé qu'au-dessus d'une concentration seuil la moule se ferme tant que la toxine est présente dans son environnement, révélant ainsi la présence de la toxine lors d'un suivi. Dans le cas d'une exposition à *A. catenella*, au contraire, il a été

observé que les moules restaient plus ouvertes que le comportement classique d'une moule révélant ainsi une paralysie occasionnée par la toxine. Cette paralysie a pu être observée dès 30 µg STXeq $100g^{-1}$ (STXeq comprenant la saxitoxine et ses analogues) lors d'une exposition à une eau naturelle contaminée par cette algue toxique. Lors d'une exposition régulière à *A. catenella*, une résistance pourrait être acquise par la moule. Pour écarter cette hypothèse, une comparaison entre deux populations de moules, l'une régulièrement intoxiquée et l'autre rarement, a été effectuée. Les résultats obtenus montrent que chez la moule cette résistance n'est pas développée. Un système de détection utilisant le comportement des moules pourrait donc être utilisé y compris en zone régulièrement exposée à *A. catenella*. Finalement, l'étude de la dépuration de la moule après intoxication par *A. catenella* a mis en évidence un fort allongement de la dépuration en absence de nutrition comme cela pourrait être le cas lors d'un isolement en vivier confortant l'intérêt de la détection précoce des toxines.

Les résultats obtenus valident la possibilité d'utiliser le comportement valvaire pour la détection précoce de toxines cela permettant de détecter l'arrivée des toxines suffisamment tôt pour laisser un délai aux aquaculteurs de prendre les mesures adaptées et commercialiser leur production.

Mots clés :

Mytilus edulis, *Placopecten magellanicus*, Dispersant d'hydrocarbures, Valvométrie, comportement valvaire, Saxitoxine, Toxine paralysante, Suivis biologiques

ABSTRACT

Aquaculture farms being situated on the coast in open environments, they are sometimes exposed to toxins present in sea water. These toxins can induce the harvesting closure of the cultivated organisms and economic losses for the producers. A solution to minimize the economic losses could be to detect these toxins before they reach the harvesting closure threshold. Thus, the producers could take the appropriates measures to continue the production commercialization and avoid the economical lost. Two toxin types were studied during this PhD. The first was a synthetic substance, the oil dispersant Corexit 1500, used during events like oil spill. The second toxin, of natural origin, is produced by the dinoflagellate *Alexandrium catenella* belong to a group of toxic compounds, acting as paralytic toxins (Paralytic Selfish Toxins).

The hypotheses of this PhD were that the gaping behaviour changes could be used to detect the toxin arrival on the aquaculture farms. To observe and record the bivalve behaviour and detect changes, valvometry was used. This technique consists in measure the spacing between bivalve valves with a sensor and a magnet glued on each valve. The sensor is linked to the valvometer to record the space between the valves with high frequency and record the gaping behaviour over time without perturbation of the bivalve behaviour, this method being non-invasive. Thus, valvometry also permits to observe behavioral changes. The high frequency recording generates a very high amount of data. Thus, the automatization of the data treatment is necessary. In this way, a computer program was developed to compilate data, convert from to mV to opening amplitude, produce graphs to visualize the recorded behaviours and finally describe the recorded behaviour to study them.

To study the possibility of using bivalve gaping behaviours for toxin detection, the first step was to determine the species the most adapted. In this objective, the response of the blue mussel *Mytilus edulis* and the giant scallop *Placopecten magellanicus* was compared during an exposure to Corexit 1500. The results showed that contrary to the scallop, the mussel was able to isolate itself from the outside environment, thus giving a better surviving capacity and offer a gaping behaviour change easily detected. Thus, this specie is the most adapted for toxin detection. The next step was to determine the impact of the two toxins on the gaping behaviour in mussels. During an exposure to Corexit 1500, a valve closure was observed when the concentration was higher than a threshold and the mussel stayed closed as long as the toxin was present, revealing the presence of the toxin during a monitoring. In contrast, an exposure to *A. catenella* resulted in mussels staying more opened than the classic mussel behaviour, indicating a paralysis induced by the toxin. This paralysis was observed at minimum concentration of 30 µg STXeq $100g^{-1}$ during an exposure to natural seawater contaminated by this toxic alga. During a long *A. catenella* exposure, a resistance could be

acquired by mussels. To rule out this hypothesis, a comparison of two mussel populations, one often intoxicated and the other rarely, was made. The obtained results show that the mussel doesn't develop this resistance. Thus, an early warning system using the mussel gaping behaviour could be used evens in area often exposed to *A. catenella*. Finally, the study of the mussel depuration after an *A. catenella* intoxication showed a long prolongation of the depuration in absence of feeding like could be observed if mussels are isolated in holding tanks, confirming the usefulness of the early detection of toxins.

The obtained results validate the possibility of using the gaping behaviour for the early detection of toxins, gallowing mussel farmers to detect toxin arrival early enough to give time to take appropriate measures and continue their product commercialization.

Key words :

Mytilus edulis, *Placopecten magellanicus*, Oil dispersant, Valvometry, Gaping behaviour, Saxitoxin, Paralytic toxin, Biomonitoring

TABLE DES MATIÈRES

REMERCIEMENTS vii

RÉSUMÉ ix

ABSTRACT xi

TABLE DES MATIÈRES xiii

LISTE DES TABLEAUX xvi

LISTE DES FIGURES xviii

LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES xxiii

INTRODUCTION GÉNÉRALE 1

1. SUIVIS ENVIRONNEMENTAUX 1

2. TOXINES 2

2.1 Corexit 2
2.2 Paralytic Shellfish Poisoning 4

3. ESPECES DE BIVALVE 14

3.1 Mytilus edulis Linnaeus 1958 14
3.2 Placopecten magellanicus 29

4. SYSTEMES DE SUIVI DES ALGUES TOXIQUES 32

4.1 Valvométrie 32
4.2 Autres systèmes de détections 38

5. HYPOTHESE ET OBJECTIFS 40

CHAPITRE 1 Sensibilité aux dispersants de pétrole : effet sur les mouvements valvaires de la moule bleue *Mytilus edulis* et sur le pétoncle géant *Placopecten magellanicus*, en conditions sub-arctique 43

1.1 RESUME EN FRANÇAIS DU PREMIER ARTICLE 43

1.2 SENSITIVITY TO OIL DISPERSANTS: EFFECTS ON THE VALVE MOVEMENTS OF THE BLUE MUSSEL *MYTILUS EDULIS* AND THE GIANT SCALLOP *PLACOPECTEN MAGELLANICUS*, IN SUB-ARCTIC CONDITIONS 45

1.2.1 Abstract 45
1.2.2 Introduction 46
1.2.3 Material and methods 48
1.2.4 Results: 55
1.2.5 Discussion 67
1.2.6 Conclusion 71
1.2.7 References 72

CHAPITRE 2 Utilisation de la valvométrie comme un outil d'alerte pour la présence de l'algue toxique *Alexandrium catenella* par *Mytilus edulis* 82

2.1 RESUME EN FRANÇAIS DU DEUXIEME ARTICLE 83

2.2 USE OF VALVOMETRY AS AN ALERT TOOL TO SIGNAL THE PRESENCE OF TOXIC ALGAE *ALEXANDRIUM CATENELLA* WITH *MYTILUS EDULIS* 84

2.2.1 Abstract: 84
2.2.2 Introduction: 85
2.2.3 Materials and Methods: 88
2.2.4 Results: 99
2.2.5 Discussion: 110
2.2.6 Conclusion 115
2.2.7 References 116

2.3 RESUME EN FRANÇAIS DU TROISIEME ARTICLE 125

2.4 IMPACT OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM CATENELLA* ON THE VALVE BEHAVIOUR OF BLUE MUSSELS (*MYTILUS EDULIS*): A COMPARISON BETWEEN TWO POPULATIONS WITH CONTRASTING HISTORIES OF PST EXPOSURE 126

2.4.1 Abstract: 126
2.4.2 Introduction 127
2.4.3 Materials and method 128
2.4.4 Results 131
2.4.5 Discussion 134
2.4.6 References 136

CHAPITRE 3 Effet du Dinoflagellé *Alexandrium catenella* sur le comportement et la physiologie de la moule bleue *mytilus edulis* 140

3.1 RESUME EN FRANÇAIS DU QUATRIEME ARTICLE 140

3.2 EFFECT OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM CATENELLA* ON THE BEHAVIOUR AND PHYSIOLOGY OF THE BLUE MUSSEL *MYTILUS EDULIS* 142

3.2.1 Abstract 142
3.2.2 Introduction 144
3.2.3 Material and Methods 147
3.2.4 Results 156
3.2.5 Discussion 163
3.2.6 References 171

CONCLUSION GÉNÉRALE 182

PERSPECTIVES 186

Système de détection précoce 186
Utilisation de la valvométrie pour la détection d'autres types de pollutions 188

LIMITES DE L'ETUDE 189

RÉFÉRENCES BIBLIOGRAPHIQUES 190

LISTE DES TABLEAUX

Tableau 1 : Pearson correlations between valve movement behaviour of *M. edulis* and *P. magellanicus* with oil dispersant concentration and the combined effect of oil dispersant concentration and 13C enriched microalgae exposure. NC: Number of closures. TDC: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average Valve Opening Amplitude. MC: Micro Closure. SC: Speed of Closure. In bold: significant correlations for $\alpha = 0.05$. 62

Tableau 1 : Correlation between PSTm content in mussel tissues at the end of the controlled laboratory experiment and the behavioural indicators measured in the controlled laboratory experiment before (T0) and during *A. catenella* exposition (T1), then and food addition (T2). Behavioural indicators included Number of closures (NC), Total Closure Duration (TCD), Average Closure Duration (ACD), Average Valve Open Amplitude (AVOA), Number of Micro Closures (MC). In bold: significant correlation at α=0.05, n= 19. ……..……102

Tableau 3 : Correlations between the *M. edulis* gaping behaviour and the variability of physical and chemical parameters measured in the natural seawater from the head tank. Number of closures (NC), Total Closure Duration (TCD), Average Valve Open Amplitude (AVOA) ……..…..……..106

Tableau 4 : Pearson correlation between the *M. edulis* gaping behaviour and the cells abundance (cells L^{-1}) of toxic algae in natural seawater measured in the head tank and PST concentration measured in field mussels (PSTm). Number of closures (NC), Total Closure Duration (TCD), Average Valve Open Amplitude (AVOA), Average Closure Duration (ACD), A.c: *Alexandrium catenella*, D.a: *Dinophysis acuminata*, P-N: *Pseudo-nitzschia* spp. In bold: significant correlation at α=0.05, n=19. ……..104

Tableau 5 : PERMANOVA results for the comparison of Prince Edward Island and St. Lawrence Estuary mussel behaviour for each behavioural indicator. ……132

Tableau 6 : Mean values (± SEM) of valvometry variables within three distinct phases. Week 1 corresponds to the exposure of mussels from the 'toxic' treatment to *A. catenella*. Week 2 constitutes the peak of toxicity in mussel tissues.

Weeks 3 to 8 saw a gradual decrease of toxicity as depuration happened. Letters in exponent indicate significance of the statistical test carried out within each phase on individual metrics for each variable ($p < 0.05$). …..154

LISTE DES FIGURES

Figure 1 : Dispersions d'une nappe d'hydrocarbure par un dispersant chimique, a : action chimique ; b : action biologique (IPIECA and IOGP, 2015) 4

Figure 2 : *Alexandrium catenella* (A) microscope électronique, barre = 5µm, 1'-6'': plaques (B) microscope optique (Kim et al., 2017) https://www.eoas.ubc.ca/research/phytoplankton/dinoflagellates/alexandrium/a_catenella.html (C) Schéma de la morphologie d'*A. catenella* 9

Figure 3 : Répartition géographique d'*A. catenella*. Chaque symbole représente un clade différent. Noir : toxique ; blanc : non toxique (Lilly et al., 2007) 11

Figure 4 : Structure générale de la saxitoxine (Solter And Beasley, 2013) 13

Figure 5 : Répartitions géographiques de *M. edulis* (Mathiesen et al., 2017) 15

Figure 6 : Valve de moule bleue (Eggermont, 2020) 19

Figure 7 : Morphologie interne de la moule bleue (Eggermont, 2020) 21

Figure 8 : Production mondiale de *M. edulis* par l'aquaculture (FAO, 2016) 27

Figure 9 : Boudins de moules en Gaspésie (Rejean Tremblay) 28

Figure 10 : Valves du pétoncle *Placopecten magellanicus*. DFO-MPO 30

Figure 11 : Déplacement des pétoncles par clapping (Gosling, 2015) 32

Figure 12 : Installation des moules équipées de valvomètres pour les experiences en laboratoire. 33

Figure 13 : Valvomètre et aimant sur les valves d'une moule. 34

Figure 14 : Définition des indicateurs comportementaux 36

Figure 15 : Experimental design (a), experimental timeline (b) 51

Figure 16 : Valve Opening Amplitude (VOA) of *M. edulis* (A, B, C, D) and *P. magellanicus* (E, F, G, H) in relation to exposure time to Corexit™ at different concentrations (0, 0.01, 0.05, 0.25 g L^{-1}). The dashed grey line signifies the time of Corexit™ addition, while the solid grey line signifies time of algae addition. 57

Figure 17 : Boxplot of the variability in behavioural parameters of *M. edulis* when exposed to each Corexit™ exposure concentration. NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average VOA. MC: Micro Closure. 59

Figure 18 : Boxplot of the variability in behavioural parameters of *P. magellanicus* when exposed to each Oil dispersant exposure concentration. NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average VOA. MC: Micro Closure. No data from the 0.25 g L^{-1} exposure were included in the analysis as this concentration was fatal for all individuals. 60

Figure 19 : Correlation circle derived from the Principal Component Analysis for *M. edulis*. NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average VOA MC: Micro Closure. T1: Corexit™ single. T2: Corexit™ with 13C marked algae.....63

Figure 20 : Cluster analysis grouping Corexit™ concentration with behavioural parameters of mussels using the SIMPROF method.....64

Figure 21 : Boxplots summarizing the 13C rate in mussels (a) and scallops (b) for each of the levels of Corexit™ concentration. Different letters indicate significant differences between groups ($p < 0.05$). 66

Figure 22 : Experimental design of the lab experiment (A) and of the natural seawater experiment (B). 1: seawater, 2: water intake, 3: water pump, 4: head tank, 5: CTD, 6: valvometers, 7: battery, 8: Tap, 9: sample, 10: flowmeter, 11: water sampled, 12: Elisa test, 13: cell count, 14: Jellet test.....90

Figure 23 : Physical and biological observations over the course of the 90-day natural seawater experiment. In panel a, the blue and black lines represent photoperiod and water level, respectively. The actograms depict the mean hourly VOA (%) of the group (n = 27 mussels) in relation to *A. catenella* abundance (panel b, black line) and PSP concentration in the mussel tissue (panel c, black line). The black rectangles represent the times windows studies, with a duration of 4 days for panel b and 6 days for panel c. 98

Figure 24 : Variation of the mussel's behavioural indicators for PSP concentration for laboratory experiment. NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average VOA. MC: Microclosure. C0: <20 µg STXeq 100g-1, C1: 20 to 50 µg STXeq $100g^{-1}$, C2: 50 to 80 µg STXeq $100g^{-1}$, C3: >80 µg STXeq $100g^{-1}$, T0: Acclimation and reference behaviour, T1: *A. catenella* exposition, T2: food addition.....100

Figure 25 : A) Correlation circle of Principal Component Analysis for the behavioural indicators (NC: Number of closures. TCD: Total Closure Duration. ACD:

Average Closure Duration. AVOA: Average VOA. MC: Microclosure) in the laboratory experiment during the period T1 (addition of *A. catenella*), in relation to PST concentration in mussel. B) Cluster analysis of behavioural indicators in relation to PST concentration in mussels during laboratory experiment. Red line: separation of concentration groups, C) Correlation circle of Principal Component Analysis for the behavioural indicators in the natural seawater experiment and PST in mussels, D) Cluster analysis for groupings of PST concentrations with behavioural indicators in natural seawater experiment by SIMPROF method, C0: <10 µg STXeq $100g^{-1}$, C1: 30 to 50 µg STXeq $100g^{-1}$, C2: 50 to 80 µg STXeq $100g^{-1}$, C3: >80 µg STXeq $100g^{-1}$. Red line: group 1, blue line, group 2..... 101

Figure 26 : Mussel gaping metrics and corresponding standard errors associated to running daily means (shaded areas) over the course of the 90-d experiment in natural seawater for A: NC - Number of Closures (Count day-1), B: TCD - Total Closure Duration (hour), C: ACD - Average Closure Duration (hour), and D: AVOA - Average Valve Open Amplitude (%). E: *A. catenella* abundance (cells $litre^{-1}$; solid line) in seawater and PST concentration in mussels from the shore (µg STXeq. $100g^{-1}$; shaded area). F: *D. acuminate* (cells $litre^{-1}$; solid line) and *Pseudo-nitzschia* spp. (cells $litre^{-1}$; dotted line) abundances in seawater. G: Temperature (°C; solid line), Salinity (dotted line) and Turbidity (broken line)..... 105

Figure 27 : Boxplot of the A) Average VOA, B) total closure duration and C) number of closures in function of the *A. catenella* abundance during the tested period of the time window of the fig 23b. A0: 0 cell L^{-1}, A1: 560 cell L^{-1} (SE=23.38), A2: 1190 cell L^{-1} (SE = 39.87), A3: 1680 cell L^{-1} (SE = 26.07).... 108

Figure 28 : Lomb and Scargle periodograms done on VOA data during the time windows of the fig 23c. A0: 3.71 µg STXeq $100g^{-1}$, A1: 49 µg STXeq $100g^{-1}$ (SE=4.1), A2: 72.16 µg STXeq $100g^{-1}$ (SE=3.8), A3: 104.48 µg STXeq $100g^{-1}$ (SE=3.72). 110

Figure 29 : Experimental design used during the experiment with 8 tanks for mussels of mixed origins (St. Lawrence estuary and Prince Edward Island). 130

Figure 30 : Mussel's behavioural indicators exposed to toxic algae for mussels from St. Lawrence estuary (SL) and Prince Edward Island (PEI). NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average Valve Opening Amlitude. MC: Microclosure. T0: Acclimation and reference behaviour, T1: *A. catenella* exposition, T2: food addition 133

Figure 31 : Sampling timeline of the experiment..... 150

Figure 32 : Paralytic shellfish toxin concentration (µg STXeq 100 g–1) in tissues of mussels exposed to A. catenella during the first week of the experiment (N ranges between 7 and 14 from week to week). The grey area indicates the period during which mussels were fed A. catenella. 157

Figure 33 : Condition index (a; mean ± SEM) and average individual shell growth (b; mean ± SEM; N = 20 in 'control' and 'starvation' treatments; N = 40 in 'toxic' treatment) of mussels in the 'control' (white), 'starvation' (grey), and 'toxic' (black) treatments. The crosshatched grey area indicates the period (one week) during which mussels in the 'starvation' and 'toxic' treatments were fed the control diet and *A. catenella*, respectively, before being starved. 158

Figure 34 : Standardized clearance rate (a) and mean oxygen consumption rate (b) through time of mussels in the 'control' (white circles), 'starvation' (grey circles), and 'toxic' (black circles) treatments. Clearance rate (CR) data are expressed for a standard shell length of 40 mm. Oxygen consumption rate (OCR) data are standardized to a standard tissue dry weight of 1 g. The grey areas indicate the period (one week) during which mussels in the 'starvation' and 'toxic' treatments were fed the control diet and *A. catenella*, respectively, before being starved. 159

Figure 35 : Valvometry variables in the 'control' (top row), 'starvation' (mid row), and 'toxic' (bottom row) treatments showing the opening amplitude as a percentage of the maximum aperture (a-c), the daily number of closures (d-f) and of micro-closures (g-i), and the average (j-l) and total (m-o) closure duration. For each variable (each column), the dotted line represents the average value of the 'control' treatment and the dashed line the average value for the row's treatment ('starvation' or 'toxic'). N = 10 for the 'control' treatment; N = 11 for the 'starvation' and the 'toxic' treatments. Data were lost between day 44-48 due to an issue following the retrieval of data on day 44. The dark grey areas indicate the period during which mussels in the 'toxic' treatment were fed *A. catenella*. The light grey areas indicate the period during which mussels in the 'control' and 'starvation' treatments were fed the control diet. 162

Figure 36 : Attachment strength (a) and number of byssus threads (b). Byssus attachment strength was measured on week 1 and 4 (N varied between 15 and 19 in 'control' and 'starvation' treatments and N = 33 in 'toxic' treatment). Number of threads were counted in week 4 (N = 7, N = 9, and N = 14 for the 'control', 'starvation, and 'toxic' treatments, respectively). 163

Figure 37 : Schéma de la détection de toxines grâce au comportement valvaire de bivalve. 185

Figure 38 : Étapes de la détection de toxines en milieu naturel 186
Figure 39 : Détection de toxine par la valvométrie HFNI..... 188

LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

ACD : Average Closure Duration

AVOA : Average Valve Opening Amplitude

CI : Condition Index

CR : Clearance Rate

HAB : Harmful Algae Bloom

HFNI : High Frequency – Non-Invasive

MC: Micro Closure

NC: Number of Closures

PSP : Paralytic Selfish Poisoning

PST : Paralytic Selfish Toxin

STXeq : Saxitoxine équivalent

TDC : Total Closure Duration

VOA : Valve Opening Amplitude

INTRODUCTION GÉNÉRALE

1. SUIVIS ENVIRONNEMENTAUX

La présence de toxines dans les eaux côtières, qu'elles soient naturelles, comme celles produites par des algues toxiques ou des bactéries, ou anthropiques comme des polluants chimiques, a rendue nécessaire la surveillance de ces côtes. En plus des dommages environnementaux, ces toxines peuvent impacter de nombreux domaines comme le tourisme en rendant certains lieux moins attractifs (Bechard, 2019) ou l'aquaculture (Matsuyama and Shumway, 2009). L'aquaculture est le secteur alimentaire ayant le développement le plus important au cours des dernières années (FAO, 2009). Celle-ci est une solution permettant de répondre à la forte demande en nourriture et au déclin des stocks naturels de poissons et invertébrés. Cependant l'aquaculture est très vulnérable aux conditions environnementales (FAO, 2012). De nombreux accidents d'empoisonnements liés à la présence de biotoxines marines ont été enregistrés dans le monde. Par exemple, 7 foyers d'intoxication ont été enregistrés en Norvège entre 1901 et 1992, provoquant la mort de 32 personnes (FAO, 2004). En 1946, 100 victimes dont 6 décès d'enfants ont été rapportés au Portugal, liés à la consommation de bivalves contaminés aux biotoxines du lagon Óbidos (Correira, 1946), 21 autres victimes ont ensuite été enregistrées en 1955 dans la même zone (Pinto and Silva, 1956). En 1989 les premiers cas d'intoxication par des biotoxines de types ASP (Amnesic Shellfish Poisoning) ont été documentés en Amérique du Nord (Quilliam and Wright, 1989) ainsi que des cas de AZP (Azaspiracid Poisoning) en 1996 en Europe (McMahon, 1996). Des cas d'intoxication à *Alexandrium fundyense* ont également été référencés à Terre-Neuve en 1982 après l'ingestion de moules contaminées (Schwinghamer et al., 1994). Pour éviter ce type d'incidents, de nombreux pays ont mis en place des systèmes de suivi et d'alerte visant à signaler la présence de toxines dans les eaux côtières. Pour les suivis environnementaux,

de plus en plus de biocapteurs sont utilisés pour comprendre la biologie des organismes (Curtis et al., 2000) et pourraient être utilisés pour la mise en place de système de détection précoce (Kramer and Foekema, 2001). Ces suivis utiliseraient des biocapteurs sur des espèces sentinelles pour la surveillance en milieu côtier, dont les fermes aquacoles (Andrewartha and Elliott, 2015; Comeau et al., 2019) et pourraient alerter de la présence d'une éventuelle pollution (Kramer and Botterweg, 1991). Les systèmes de détection précoce permettraient de déclencher des mesures pour identifier des polluants (Gruber et al., 1994) et protéger l'industrie aquacole. Les bivalves peuvent être utilisés pour le suivi de la qualité de l'eau (Burns and Smith, 1981; Goldberg et al., 1978; Martin, 1985; Phillips, 1980). Les bivalves présentent de nombreux avantages pour effectuer ces suivis, ils sont capables d'accumuler les contaminants présents dans l'eau, ils peuvent être déplacés facilement, tolèrent des environnements contaminés et informent sur la biodisponibilité des contaminants présents (Dame and Kenneth, 2011; Martin and Richardson, 1991).

2. TOXINES

2.1 Corexit

Les dispersants font partie de la réponse primaire en cas d'accidents pétroliers, ceux-ci sont considérés comme plus efficaces et peuvent être utilisés avec des conditions météorologiques plus difficiles que les méthodes consistant à brûler ou à contenir les nappes d'hydrocarbures (IPIECA and IOGP, 2015). Les dispersants peuvent être déployés à grande échelle avec un épandage par avion ou à plus petite échelle dans des zones précises en hélicoptère ou en bateau (Canevari et al., 2008).

L'utilisation de dispersants de pétrole a commencé après l'accident de l'Exxon Valdez sur les côtes de l'Alaska en 1989 (Wise and Wise, 2011) déversant 180 000 tonnes de pétrole brut suite à un échouement polluant 800 km de côtes (Cedre, 2015). Son utilisation s'est ensuite intensifiée lors de l'accident de la plateforme pétrolière Deepwater Horizon en 2010

dans le Golfe du Mexique (Wise and Wise, 2011) déversant entre 700 000 et 860 000m^3 de pétrole brut (Cedre, 2020). L'objectif du Corexit est de disperser les hydrocarbures sous forme de gouttelettes avant que les nappes de ces hydrocarbures ne viennent envahir les côtes (Fig. 1 ; Lindstrom et al., 2000). Son action est basée sur l'accélération du processus naturel de dispersion déclenché par l'addition d'un surfactant (LLC, 2016). Cette dispersion en petites gouttelettes permet également d'accélérer la dégradation des hydrocarbures par les microorganismes présents dans la colonne d'eau (IPIECA and IOGP, 2015). Les dispersants assurent donc une action chimique favorisant l'action biologique sur les hydrocarbures.

Certaines formes de Corexit contiennent un solvant permettant la pénétration du dispersant dans les nappes d'hydrocarbure lourd (George-Ares and Clark, 2000). Le Corexit 9500A, développé par Ecolab et utilisé lors de l'accident de Deepwater Horizon en 2010, possède une composition chimique permettant de minimiser l'impact des hydrocarbures sur les côtes (National Research Council, 1989), mais provoque un plus grand risque d'exposition des habitats subtidaux et des organismes benthiques (George-Ares and Clark, 2000; Schmidt, 2010). L'utilisation de dispersant de pétrole en cas d'accident ne peut se faire que si certaines conditions géographiques sont respectées. En effet, pour limiter l'impact de ces dispersants sur les écosystèmes, certains pays fixent une limite réglementaire de profondeur et d'éloignement des côtes pour autoriser leur utilisation (Merlin, 2014). La présence de zones protégées est également prise en compte. Leur utilisation est également contre-indiquée proche des zones de pêche ou d'aquaculture (Canevari et al., 2008).

L'évaluation de la toxicité du Corexit est complexe. Certains auteurs l'attribuent uniquement à sa concentration (Pace et al., 1995), d'autres font l'hypothèse que c'est la fixation du dispersant aux hydrocarbures qui augmente sa toxicité (Kenneth et al., 2015). La toxicité pourrait donc ne pas être liée directement au dispersant lui-même, mais il augmenterait la biodisponibilité des carbones aromatiques polycycliques présents dans les hydrocarbures et toxiques pour les organismes les absorbants (Nikolaou et al., 2009).

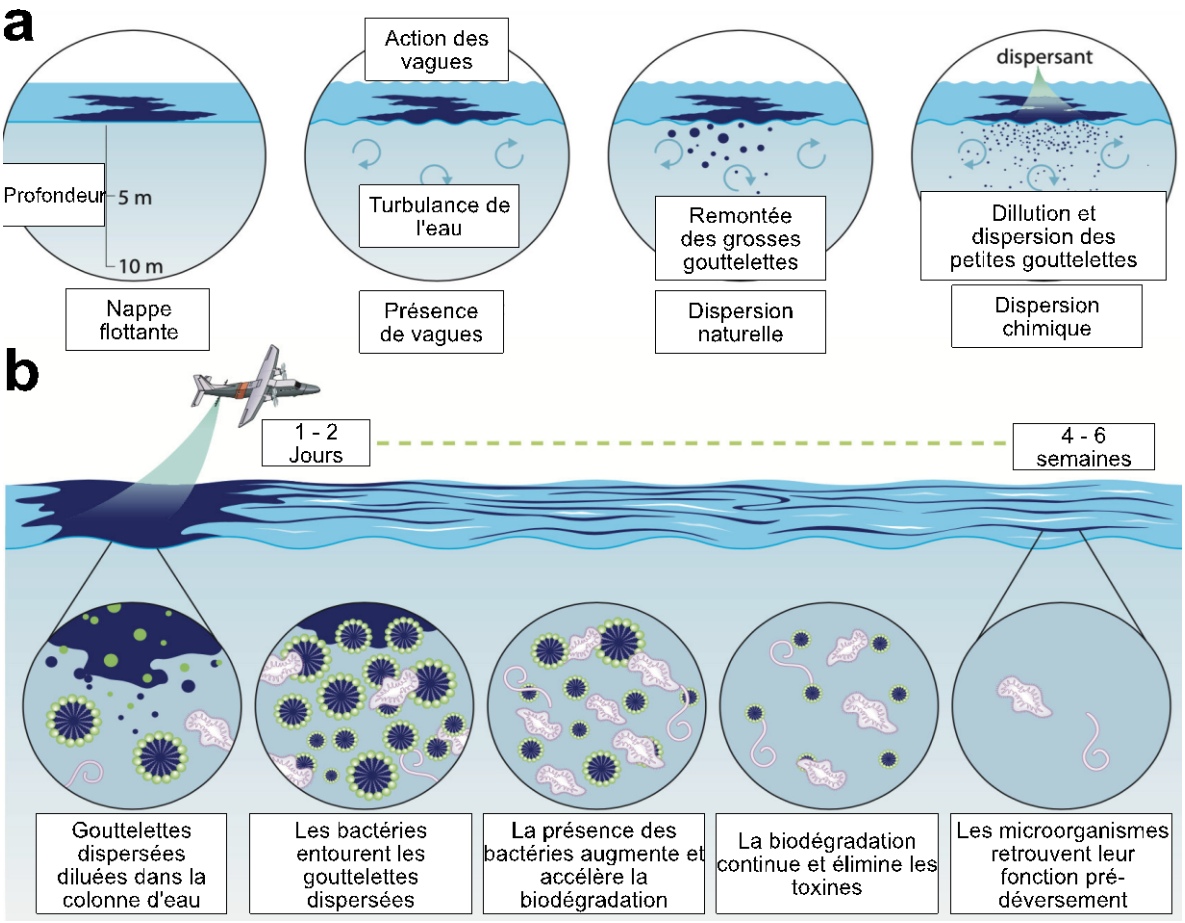

Figure 1 : Dispersions d'une nappe d'hydrocarbure par un dispersant chimique, a : action chimique ; b : action biologique (IPIECA and IOGP, 2015)

2.2 Paralytic Shellfish Poisoning

2.2.1 Harmful Algal Blooms

Lorsque certaines conditions environnementales, notamment de température et de salinité, sont réunies, il est possible d'observer un phénomène appelé marées rouges. Cela est lié à une efflorescence de phytoplancton, notamment des dinoflagellés dont certaines

espèces sont toxiques, pouvant colorer l'eau. Les efflorescences d'algues toxiques sont appelées « Harmful Algal Blooms » (HABs) (Anderson et al., 2011). Les HABs sont causés par environ 300 espèces, dont une trentaine, produisant des toxines pour l'humain (Hallegraeff, 1993) sur les 5000 espèces de phytoplanctons marins connues (Sournia et al., 1991). Celles-ci impactent l'environnement ainsi que la santé des consommateurs (Hallegraeff, 1993). Les dinoflagellés sont l'un des producteurs primaires les plus importants dans les écosystèmes marins (Sunda et al., 2006). Les efflorescences de microalgues sont en général un phénomène positif pour les espèces s'en nourrissant. Cependant lorsque ces algues sont toxiques, cette efflorescence peut avoir des effets néfastes sur l'environnement et devenir un danger pour la santé humaine par l'accumulation des différents niveaux trophiques (Evangelista et al., 2008; Hallegraeff, 1993). Certaines marées rouges de dinoflagellés et autres algues toxiques produisent de puissantes toxines pouvant causer la mort ou l'empoisonnement de bivalves ou de poissons. Ces toxines peuvent être des PSP (Paralytic Shellfish Poisoning), des DSP (Diarrhetic Shellfish Poisoning), des ASP (Amnesic Shellfish Poisoning), des NSP (Neurotoxic Shellfish Poisoning) ainsi que d'autres toxines encore indéterminées (Cembella, 2003; Turner and Tester, 1997). Les plus grands impacts arrivent lorsque le phytoplancton toxique est filtré par des mollusques qui accumulent les toxines jusqu'au seuil létal pour les consommateurs (Ahmed, 1991; Shumway, 1990).

Selon certains auteurs, les pertes économiques engendrées par ces HABs dans différents domaines dont les production maricoles, les types de ressources affectées et le nombre de toxines et espèces toxiques identifiés sont en hausse depuis les dernières années dans le monde (Anderson et al., 1989; Bates et al., 2020; Hallegraeff, 1993; Smayda, 1990). Il existe plusieurs explications à ce phénomène (Anderson, 1995), notamment la dispersion d'espèces par des mécanismes naturels tel que les courants marins ou les tempêtes (Anderson et al., 1982). Les activités humaines, peuvent également affecter les populations de dinoflagellés toxiques de plusieurs manières (Burkholder, 1998; Galimany et al., 2008a; Glibert et al., 2005; Hallegraeff, 1993) : l'enrichissement des côtes en nutriments par l'agriculture pouvant provoquet la prolifération des algues toxiques sur certains sites (Smayda, 1990, Davidson et al. 2014), l'augmentation de l'aquaculture enrichissant l'eau et

stimulant la croissance des algues (Gowen and Bradbury, 1987) et les transferts d'espèces cultivées qui introduisent de nouvelles espèces d'algues toxiques (Anderson et al., 1989). Le transport maritime et l'eau des ballasts utilisés par les bateaux participent au transport d'algues et de kystes et qui peuvent contaminer de nouvelles zones (Carlton and Scanlon, 1985; Hallegraeff and Bolch, 1992; Masó and Garcés, 2006). L'élimination des niveaux supérieurs des réseaux trophiques par l'industrialisation de la pêche déstabilise et provoque des modifications de ces réseaux trophiques (Frank et al., 2005), qui peuvent favoriser la croissance des espèces phytoplanctoniques responsables des HABs. Les changements climatiques jouent également un rôle dans l'augmentation des épisodes de HABs avec les changements de températures, de vent et l'insolation (Boivin-Rioux et al., 2021; Reid et al., 1990; Sellner et al., 2003). D'après Hallegraeff (2021), l'augmentation des épisodes de HABs perçue pourrait également être amplifiée à une intensification des suivis effectués.

Chez les végétaux, les interactions chimiques entre espèces peuvent entraîner une stimulation ou une inhibition de la croissance. Cette action est appelée l'allélopathie est également observée chez certaines espèces de microalgues marines (Chan et al., 1980; Maestrini and Robert, 1981), telles que les dinoflagellés du genre *Alexandrium spp.* sur d'autres espèces cultivées (Arzul et al., 1999). Le genre *Alexandrium spp.* peut produire différents types de composés toxiques comme des PSP, mais également des composés allopathiques dans le but de gagner un avantage compétitif sur d'autres espèces phytoplanctoniques (Borcier et al., 2017; Fistarol et al., 2004; Legrand et al., 2003). Il est communément assumé que les toxines sont produites pour se protéger des organismes brouteurs, cependant ces toxines empoisonnent principalement les prédateurs de ces brouteurs, ce qui peut faire douter certains auteurs de cette hypothèse (Turner et al., 1998).

Les bivalves filtreurs ou le zooplancton sont relativement tolérants aux PSP et peuvent accumuler cette toxine en consommant les microalgues de la colonne d'eau (Dame and Kenneth, 2011; Doucette et al., 2006). Les toxines peuvent donc être concentrées par les consommateurs secondaires et accumulées dans les réseaux trophiques (Burkholder, 1998; Hallegraeff, 1993). La propagation et la biomagnification (amplification de l'intoxication

dans les réseaux trophiques) de ces toxines le long des chaînes trophiques peuvent provoquer une nage erratique et la mort de poissons, la mort d'invertébrés ainsi que de mammifères, de tortues ou d'oiseaux marins (Anderson, 1995; Anderson and White, 1992; Coulson et al., 1968; Geraci et al., 1989; Martín-González et al., 2006; Samson, 2002; Sephton et al., 2007; Starr et al., 2017; Turner et al., 1998; Turner and Tester, 1997). Ces efflorescences provoquent aussi une réduction du taux de filtration, du taux de croissance et de l'indice de condition chez les bivalves (Bricelj et al., 1993; Landsberg, 2002). Cependant certains bivalves comme les moules peuvent utiliser les microalgues toxiques comme source de nourriture malgré leur toxicité (Bricelj et al., 1993).

Ces toxines contaminent la nourriture provenant de la mer et posent d'importants problèmes de santé publique (Smayda, 1997). Une mortalité accrue chez les poissons et l'accumulation des toxines chez les bivalves produits en mariculture pendant les périodes d'efflorescences ont des impacts majeurs sur la santé humaine tout en ayant des conséquences économiques (Shumway, 1990). En rendant les espèces commerciales temporairement interdites à la consommation, en provoquant une mortalité importante chez ces espèces et en ayant un impact sur le tourisme et les loisirs, les HABs impactent l'économie, principalement des régions côtières (Hoagland and Scatasta, 2006; Larkin and Adams, 2007). Les concentrations en algues toxiques peuvent être en dessous de celle provoquant des marées rouges, mais être suffisamment hautes pour obliger l'arrêt de la consommation des bivalves (Blasco et al., 2003) limitant les revenus des conchyliculteurs.

2.2.2 Alexandrium catenella

Les dinoflagellés marins du genre *Alexandrium* font partie des plus étudiés en raison de leur production de neurotoxines paralysantes (Anderson et al., 1998). *Alexandrium catenella* (Balech 1985) a récemment été révisé en regroupant les trois espèces *A. catenella*, *A. tamarense* et *A. fundyense* (Fraga et al., 2015; John et al., 2014; Prud'Homme Van Reine, 2017). Comme beaucoup d'espèces d'algues produisant des PSP, *A. catenella* possède un cycle de vie complexe comprenant une phase dormante sous forme de kystes dans les

sédiments et une phase végétative dans la colonne d'eau. Après la phase de dormance, les kystes vont germer pour retourner à la phase végétative et migrer vers la surface et potentiellement initier une nouvelle efflorescence lorsque l'environnement sera favorable (Therriault et al., 1985).

2.2.2.1 Morphologie

Les cellules d'*A. catenella* mesurent entre 25 et 50 µm de longueur et entre 26 et 48 µm de largeur et peuvent être solitaire ou par paires (Tomas, 1997). *A. catenella* possède une thèque résistante (Bérard-Therriault et al., 1999), composée d'une épithèque conique arrondie, une hypothèque de forme trapézoïdale et le côté gauche est souvent plus long que le côté droit (Fig. 2) (Tomas, 1997). Les cellules possèdent de nombreux chloroplastes (Bérard-Therriault et al., 1999) contenant de la chlorophylle de type a et c ainsi que des pigments additionnels (Tomas, 1997). En dehors des saisons favorables au développement de *A. catenella* celui-ci peut survivre sous forme de kyste de résistance pouvant passer plusieurs années dans les sédiments.

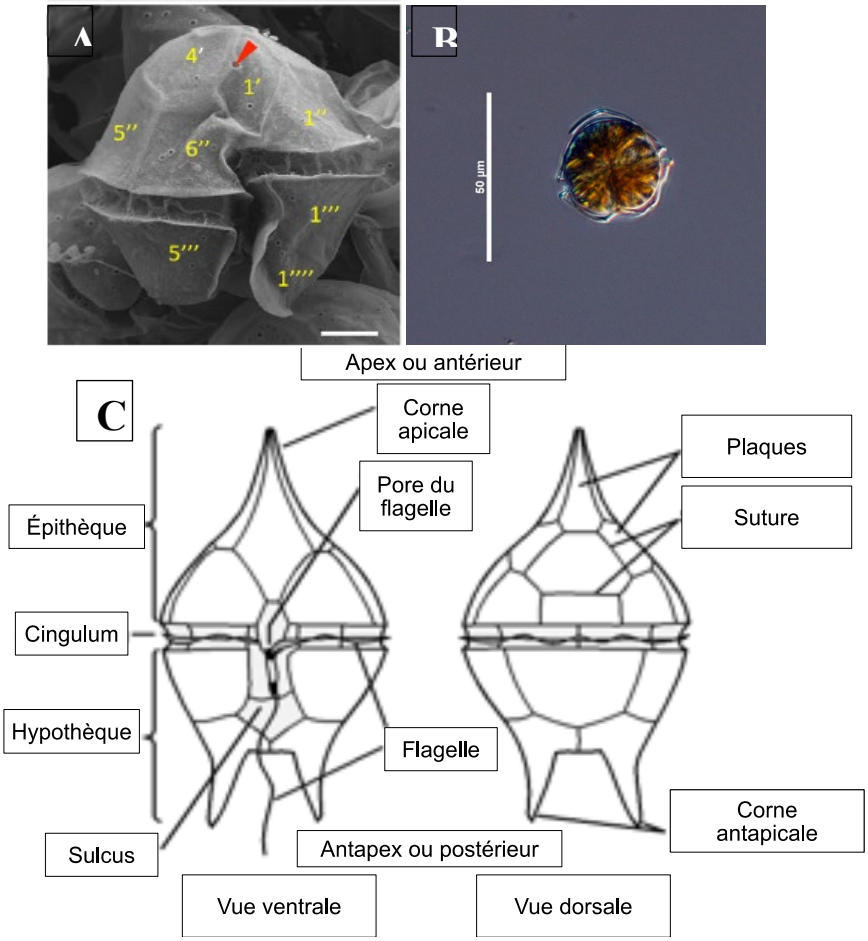

Figure 2 : *Alexandrium catenella* (A) microscope électronique, barre = 5µm, 1'-6'': plaques (B) microscope optique (Kim et al., 2017) https://www.eoas.ubc.ca/research/phytoplankton/dinoflagellates/alexandrium/a_catenella.html (C) Schéma de la morphologie d'*A. catenella*

2.2.2.2 Nutrition

Les modes de nutrition des microalgues sont liés à leur façon de se procurer le carbone nécessaire à leur survie et croissance. L'autotrophie est le mode de nutrition par lequel les microalgues assimilent le carbone inorganique sous la forme de CO_2 via la photosynthèse produisant l'énergie nécessaire à cette assimilation. En l'absence de lumière, l'énergie requise pour la croissance est fournie par l'oxydation du substrat organique, tel que le glucose, glycérol, acétate de sodium, etc., et l'on parle alors de chemo-hétérotrophie (Chojnacka and Marquez-Rocha, 2004). La mixotrophie est une variante de l'hétérotrophie

où le CO_2 et d'autres sources de carbones peuvent être assimilés simultanément ou alternativement (Ceron et al., 2006). Elle implique la cohabitation dans la cellule des métabolismes de respiration cellulaire et de photosynthèse qui peuvent opérer en parallèle ou en alternance. Ce mode de nutrition représente un avantage évolutif par sa versatilité aux sources de carbone permettant aux espèces mixotrophe de survivre à l'absence de CO_2 ou de dominer des populations autotrophes strictes en présence de carbone organique dissous (Burkholder et al., 2008).

De manière générale les dinoflagellés varient d'autotrophe à mixotrophe (Gaines, 1987; Kimor, 1981; Schnepf and Elbrächter, 1992) dans le cas des espèces photosynthétiques. Dans une proportion égale à celle des espèces photosynthétiques, certaines espèces de dinoflagellés sont non photosynthétiques et hétérotrophes strictes (Taylor, 1987). Dans le cas d'*Alexandrium spp.*, dinoflagellé autotrophe, il a été démontré que la température, les nutriments et la salinité vont impacter la photosynthèse et donc la production d'énergie ainsi que la croissance et/ou la toxicité (John and Flynn, 2000; McLeroy-Etheridge, 2002; Ogata et al., 1987; Parkhill and Cembella, 1999; Schofield et al., 1998; White, 1978). Une forte relation entre la température et la photosynthèse peut être observée. Au contraire la relation entre la salinité et la photosynthèse est beaucoup plus faible (Etheridge and Roesler, 2005). La lumière influence la distribution verticale l'efficacité du taux de photosynthèse, l'adaptation des pigments et les voies métaboliques (Taylor, 1987).

2.2.2.3 Zone de répartition spatiale et temporelle

Les dinoflagellés sont principalement estuariens et marins et seulement de 250 à 300 espèces ont été répertoriées en eau douce sur les 2000 espèces connues (Carty and Wujek, 2003; Graham and Wilcox, 2000). Le clade *d'A. catenella* se retrouve sur toute la côte est du Canada, mais aussi sur la côte ouest de l'Amérique du Nord ainsi qu'en Amérique du Sud (côté Atlantique et Pacifique), au nord de l'Europe, en Asie de l'Est et au sud-ouest de l'Afrique du Sud (Fig. 3) (Lilly et al., 2007).

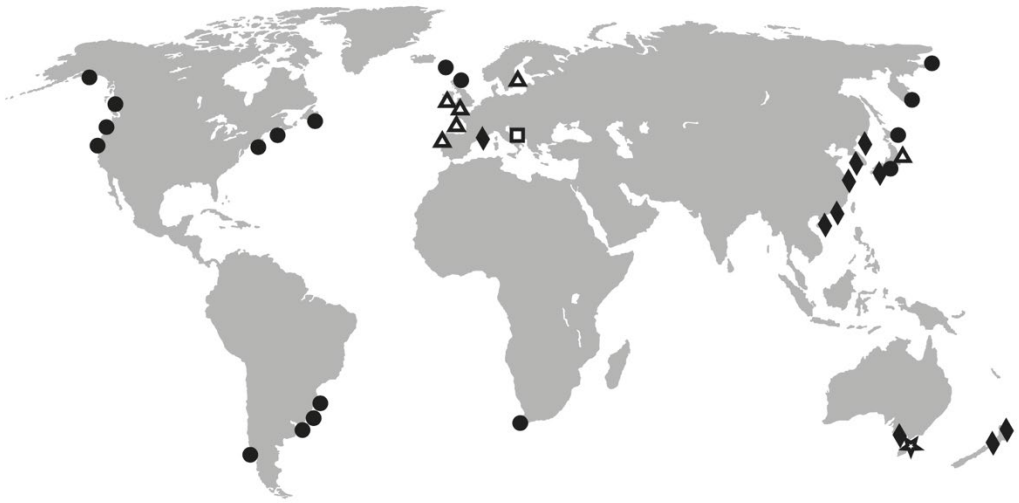

Figure 3 : Répartition géographique d'*A. catenella*. Chaque symbole représente un clade différent. Noir : toxique ; blanc : non toxique (Lilly et al., 2007)

Dans le cas de l'estuaire du Saint-Laurent, les premières cellules de *A. catenella* peuvent être observées au début du mois de mai, même si la concentration de celles-ci reste très faible. La concentration en toxine et l'abondance d'*A. catenella* commencent à augmenter au mois de juin avec un maximum observé entre la mi-juin et le début juillet. Selon les zones géographiques, le début du développement de ces microalgues est variable. Durant le reste de l'été, les concentrations en toxines ne diminuent que très peu et *A. catenella* demeure présent jusqu'au début de l'automne (Blasco et al., 2003). Les efflorescences du stade mobile d'*A. catenella* sont des phénomènes récurrents (Blasco et al., 2003) facilités par

la présence de kystes en dormance entre les périodes de développement actif (Cembella et al., 1988; Turgeon, 1990).

Le développement de *A. catenella* dans l'eau dépend de certains paramètres physico-chimiques et notamment la température et la salinité (Aguilera-Belmonte et al., 2013; Boivin-Rioux et al., 2021; Fauchot et al., 2005; Navarro et al., 2006; Starr et al., 2017). La température optimale pour le développement de *A. catenella* se situe entre 14°C (Boivin-Rioux et al., 2021) et 15°C (Aguilera-Belmonte et al., 2013), température présente uniquement en été dans l'estuaire du Saint-Laurent. La salinité joue également un rôle dans le développement de *A. catenella*, en effet celle-ci sera plus importante lorsque la salinité sera plus élevée (Aguilera-Belmonte et al., 2013).

2.2.2.4 Toxicité

Les PSP sont des neurotoxines produites par les populations de certains dinoflagellés marins et principalement par *Alexandrium spp.* (Landsberg, 2002). Le lien entre les PSP et *A. catenella* a été établi depuis longtemps (Medcof et al., 1947; Prakash, 1967). Cependant seule la phase mobile d'*A.catenella* est toxique (Blasco et al., 2003). Dans la région du Saint-Laurent, *A. catenella* serait la plus grande source de toxicité chez les bivalves (Anderson et al., 1994; Cembella et al., 1988; Therriault et al., 1985; Trites and Drinkwater, 1991). Les PSP comprennent la saxitoxine ainsi que beaucoup d'autres dérivés produits à différentes concentrations et dans différentes combinaisons selon les algues avec des niveaux de toxicité variable (Landsberg, 2002). Les dinoflagellés du genre *Alexandrium* produisent également des composés extracellulaires en plus des PSP dont la composition est encore inconnue, mais qui peuvent être toxiques pour les autres organismes (Arzul et al., 1999; Borcier et al., 2017; Lelong et al., 2011; Tillmann et al., 2007).

La toxine principale contenue dans les PSP (Paralytic selfish poisoning) est la saxitoxine avec ses analogues (Hégaret et al., 2007). Sur les animaux, celle-ci se fixe sur les canaux sodium voltage-dépendant de manière très spécifique, mais réversible. Ce blocage entraîne un arrêt de la production des potentiels d'action dans les nerfs et les muscles particulièrement chez les mammifères, les oiseaux et les poissons avec un potentiel toxique 100 fois supérieur au cyanure de sodium (Hégaret et al., 2007; James et al., 2010; Landsberg, 2002; Narahashi and Moore, 1968). Environ 50 analogues de la saxitoxine ont été identifiés avec une structure commune, la tétrahydropurine (Fig. 4) (Dell'Aversano et al., 2008; Wiese, 2010). Environ 18 composés peuvent être toxiques pour l'homme. Ces toxines sont solubles dans l'eau et sont résistantes à la température même à plus de 100°C (Kodama and Sato, 2008). Selon le pH acide ou basique, les groupes d'analogue peuvent subir des conversions pouvant augmenter la toxicité (Hall and Reichardt, 1984).

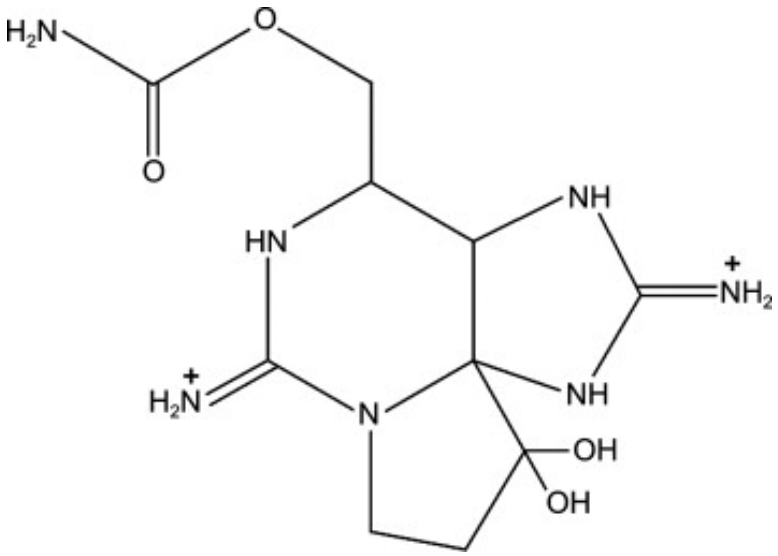

Figure 4 : Structure générale de la saxitoxine (Solter And Beasley, 2013)

Chez l'homme, les premiers symptômes après une intoxication légère sont des sensations de picotements ou d'engourdissement autour des lèvres puis autour du visage et du cou accompagnés par des picotements au bout des doigts et dans les pieds (James et al., 2010). En cas d'intoxication plus sévère, les symptômes seront plus importants avec des

maux de tête, des nausées, des troubles gastro-intestinaux et une paresthésie du visage. Une paralysie musculaire va entraîner d'importants troubles respiratoires pouvant rapidement entraîner la mort si aucune aide respiratoire n'est apportée (Etheridge, 2010; James et al., 2010). La dose létale pour l'homme est de 1 à 4 mg de saxitoxine, certains bivalves pouvant contenir plus de 100 µg STXeq/g lors de grandes efflorescences, cette dose peut être atteinte avec la consommation d'un faible nombre de ces bivalves (James et al., 2010). L'intoxication à la saxitoxine peut être traitée avec un lavage d'estomac et avec une respiration artificielle le temps que les symptômes disparaissent. Cependant cette intoxication ne laisse aucune séquelle (Hallegraeff, 1993).

3. ESPECES DE BIVALVE

3.1 Mytilus edulis Linnaeus 1958

3.1.1 Répartition

La moule bleue a une répartition cosmopolite (Bayne, 1976). Elle est donc retrouvée dans plusieurs régions du monde, notamment sur les côtes de l'Atlantique Nord. À l'ouest elle est présente sur les côtes des États-Unis et du Canada où elle cohabite avec *M. trossulus*. À l'est, elle se répartit entre le golfe de Gascogne en cohabitation avec *M. galloprovincialis* et le nord de la Norvège (Gaitán-Espitia et al., 2016). Une sous-espèce de *M. edulis* est également retrouvée sur les côtes Argentines. Certain considèrent qu'il s'agit de la même espèce (McDonald et al., 1991; Seed and Suchanek, 1992), cependant selon Dellatorre et al., (2007) il s'agirait de *M. edulis platensis* et selon De Moreno et al., (1980) *M. platensis*. La moule bleue a également été retrouvée dans l'océan Indien sur les côtes des îles Kerguelen (Bayne, 1976; Gaitán-Espitia et al., 2016). Elle tolère une grande gamme de températures allant de 0°C dans le Golfe du St Laurent (Comeau et al., 2008) à 22°C régulant leur aire de répartition et de distribution, en affectant la survie des larves et des adultes ainsi que les comportements de reproduction et de métamorphose (Bayne, 1976; Brenko and Calabrese,

1969; Gosling, 2015). L'augmentation des températures liée aux changements climatiques provoque donc une modification de l'aire de répartition de cette espèce (IPCC, 2014). En effet, celle-ci peut maintenant être observée jusqu'au nord du Groenland ainsi que dans l'archipel du Svalbard (Mathiesen et al., 2017). En effet, *M. edulis* est capable de supporter des températures négatives y compris en zone intertidale avec une LT50 (température à laquelle 50% des individus meurent) située autour de -12,8°C lors de l'exondation, atteinte de plus en plus rarement en Arctique (Thyrring et al., 2017). Au sud, la limite de l'aire de répartition coïncide à l'isotherme 27°C pour la température de surface (Stubbings, 1954). Cependant même si leur répartition s'étend jusqu'à cette isotherme, on observe un effet létal de la température sur les moules lorsque celle-ci est au-delà de 25°C (Tremblay et al., 1998) avec une diminution du taux de filtration au-delà de 22°C (Seed and Suchanek, 1992). La salinité va également jouer un rôle dans la distribution de la moule bleue. Celle-ci supporte une grande gamme de salinité (15 à 40 unités) (Brenko and Calabrese, 1969), cependant celles-ci seront plus faibles dans les estuaires. Cette espèce est donc moins présente dans ces zones au profit d'autres espèces comme *Mytilus trossulus* (Fig. 5) (Gardner and Thompson, 2001).

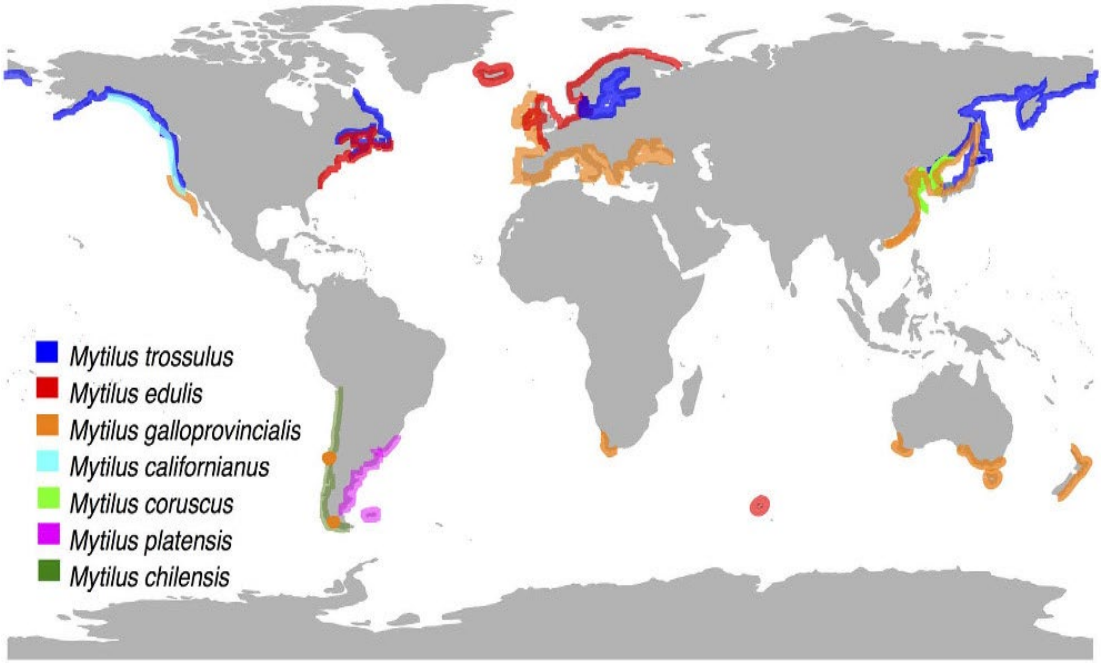

Figure 5 : Répartitions géographiques de *M. edulis* (Mathiesen et al., 2017)

Les moules bleues sont souvent regroupées en populations très denses. On les retrouve dans des habitats variés comme les zones sédimentaires ou les zones rocheuses, en milieu exposé ou non, dans les régions boréales ou tempérées (Bayne, 1976). Cette espèce localisée dans les zones intertidales et subtidales peut être dominante en zone subarctique (Mathiesen et al., 2017). La répartition verticale des moules est contrôlée par l'énergie dépensée pour résister à l'exondation (Baird and Drinnan, 1957) ainsi que par l'effet des vagues (Gosling, 2015). La disponibilité de la nourriture joue également un rôle sur la distribution verticale, les moules les plus hautes et donc ayant un temps d'exondation le plus long, auront une période de nutrition plus courte et seront plus petites que les moules situées plus bas (Bayne, 1976). D'après Baird (1966), au-delà de 56% du temps passé hors de l'eau, l'énergie dépensée et le manque de nourriture rendent la survie des moules impossible.

3.1.2 Nutrition

Les bivalves filtreurs contrôlent la production primaire et les flux de matière organique du pélagos vers le benthos dans de nombreux écosystèmes peu profonds. Ils jouent donc un rôle majeur dans le fonctionnement des écosystèmes côtiers (Bayne et al., 1993; Cloern, 1982; Norén et al., 1999). Les moules bleues sont capables de filtrer et de consommer une large gamme de taille de phytoplancton, notamment les diatomées, les dinoflagellés ainsi que les bactéries, de la matière détritique et le microzooplancton (Trottet et al., 2008). Cependant, même si les moules sont capables de consommer une grande diversité de particules, une sélection est effectuée sur la taille et les caractéristiques chimiques (Jones et al., 2020; Newell, 1989; Ward and Targett, 1989). Il existe des divergences selon les auteurs entre les groupes phytoplanctoniques sélectionnés. Certains auteurs ont démontré que les moules sélectionnent préférentiellement les dinoflagellés par rapport aux diatomées avec une dominance de diatomées benthiques pour celles consommées (Sidari et al., 1998). Au contraire, d'autres auteurs ont démontré que les diatomées sont majoritairement présentes dans le régime alimentaire de la moule bleue (Newell, 1989). D'après Sidari et al. (1998),

ces divergences pourraient s'expliquer par des variations de la composition des communautés phytoplanctoniques consommées par les moules sur les sites des différentes études. La moule bleue est capable de filtrer du picophytoplancton de 0.2 à 2 µm avec une efficacité de 20% et du seston de 1 à 4 µm avec une efficacité allant de 27 à 60% (Rosa et al., 2018) environ 80% des particules de taille supérieure à 2 µm et 100% de celles supérieures à 4 µm sont retenues (Møhlenberg and Riisgård, 1978; Sonier et al., 2020).

Le taux de filtration, volume d'eau filtré par les branchies par unité de temps (Gosling, 2015), varie au cours du temps. Ces variations sont principalement liées à la nourriture présente dans l'eau. En effet, la présence de celle-ci sous forme de phytoplancton ou de détritus stimule et maintien la filtration des moules (Wilson and Seed, 1974). De plus, la quantité et la qualité de cette nourriture vont également influer sur ce taux de filtration (Bricelj, 1991). Lorsque les moules sont en contact avec des algues, leurs valves et leurs siphons réagissent rapidement en s'ouvrant. À l'inverse, lorsque la concentration algale dans l'eau passe en dessous du seuil de 800 cellules ml^{-1} les moules diminuent l'ouverture de leurs valves et de leurs siphons jusqu'à les fermer complètement lorsque cette concentration en algue est trop faible (Riisgård, 1991). D'après Riisgård and Kamermans (2001), ce seuil de fermeture correspondrait à un changement de type de nutrition, les moules passant de suspensivore à déposivore pour leur survie. Deux écoles s'opposent sur la régulation de la filtration de la moule bleue (Gosling, 2015; Maire et al., 2007). D'un côté certains auteurs suggèrent que la filtration est régulée de manière autonome en étant maximale au-dessus d'un certain seuil de concentration algal (Clausen and Riisgård, 1996; Dolmer, 2000; Riisgård, 2001). De l'autre côté, certains auteurs suggèrent que le taux de filtration est contrôlé par des processus physiologiques comme la sélection de nourriture, la variation du taux d'alimentation, du processus de digestion et de l'absorption des nutriments. Cela permet de maximiser l'apport d'énergie en fonction de la nourriture disponible et des besoins de l'organisme (Bayne, 1998; Bayne et al., 1993; Prins et al., 1994).

3.1.3 Morphologie

3.1.3.1 Valves/muscles

La moule bleue possède deux valves de taille égale et de formes proches du triangle et de couleur variant entre le noir et le bleu (Figure 6a) (Eggermont et al., 2020; Gosling, 2015). La connexion entre les deux valves est effectuée par un ligament élastique au niveau de l'umbo (Figure 6b) (Eggermont et al., 2020; Gosling, 2015). Ces valves sont principalement composées de carbonate de calcium (Addadi et al., 2006) et plus particulièrement de calcite sur la première couche et d'aragonite sur la couche nacrée ainsi que sur les perles formées (Fitzer et al., 2015). Cette matière minérale compose 95% des valves. Les 5% restants sont composés d'une matrice organique (Addadi et al., 2006; Currey, 1999). Les valves de la moule bleue sont utiles pour deux fonctions principales, la première est la protection contre les prédateurs (Gosling, 2015), la seconde est de servir de structure ou de squelette sur lequel sont fixés les muscles (Eggermont et al., 2020; Gosling, 2015). La croissance des valves des bivalves est faite par ajout de matière minérale provenant du bord du manteau. Celui-ci épaissit les valves par dépôt. Elle est activée par des précurseurs de la minéralisation sécrétée par les cellules épithéliales du manteau et réalisée à partir des ions minéraux ainsi que des composants organiques (Marin and Luquet, 2005). Le calcium nécessaire à la croissance est obtenu par la nourriture ou pris dans l'eau de mer. Le processus de croissance des valves représente une part importante du budget énergétique des bivalves (Hawkins et al., 1992). En zone subtidale les moules bleues peuvent atteindre une taille de 100 à 130 mm. Dans le haut de la zone intertidale, les moules n'atteindront que des tailles de 20 à 30 mm en raison de l'impossibilité de se nourrir en période d'exondation (Seed, 1976).

L'ouverture des valves est régulée grâce aux muscles adducteurs (Fig. 6b et 6c). Leur contraction entraîne la fermeture des valves et leur relâchement entraîne l'ouverture (Gosling, 2015). Le muscle adducteur postérieur est plus développé que le muscle antérieur, les deux étant composés de fibres musculaires parallèles (Eggermont et al., 2020). Pour ces

deux muscles, une partie des fibres musculaires sont lentes et l'autre partie des fibres sont rapides (Gosling, 1992).

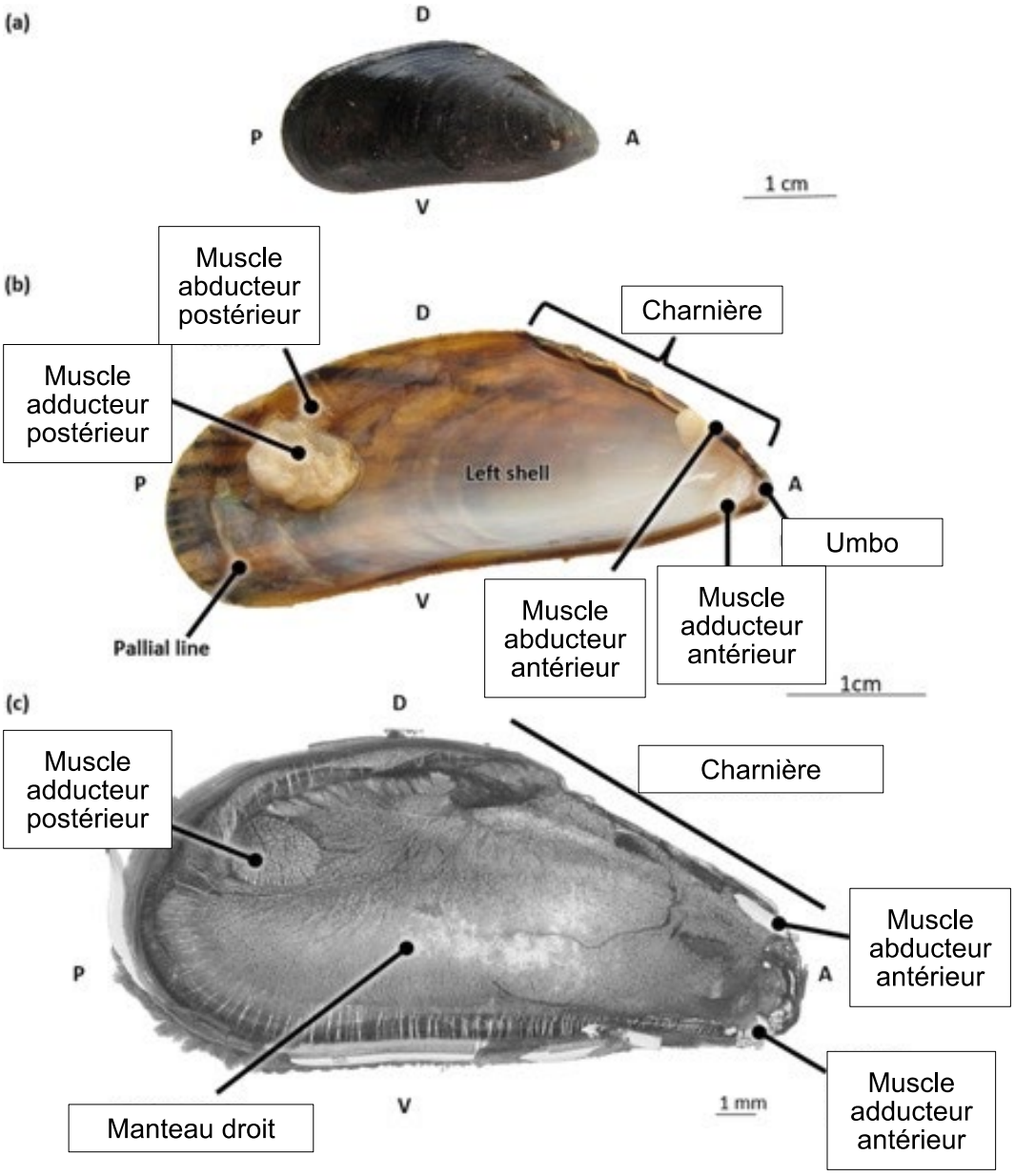

Figure 6 : Valve de moule bleue (Eggermont, 2020)

3.1.3.2 Manteau

Chez les bivalves, le manteau est composé de deux lobes de tissus enfermant l'animal à l'intérieur de la coquille avec une cavité entre les organes et le manteau (Seed, 1971). Le manteau est constitué de tissus conjonctifs avec des vaisseaux d'hémolymphe, des nerfs ainsi que des muscles très développés le long des marges du manteau. Les cils sur la surface intérieure du manteau permettent de conduire les particules aux branchies et rejeter les particules les plus grosses sous forme de pseudofécès (Gosling, 2015). La partie interne de la coquille sur laquelle est fixé le bord du manteau est la ligne palléale. Le manteau recouvre la cavité palléale, remplie d'eau de mer, elle est constituée de la chambre infra branchiale située du côté ventral des branchies et de la chambre supra branchiale située du côté dorsal des branchies (Fig. 7a) (Eggermont et al., 2020). Les marges du manteau sont composées de 3 couches. La couche extérieure participe à la sécrétion de la coquille (Bubel, 1973), la couche intermédiaire à une fonction de capteur sensitif et la couche interne contrôle musculairement les flux d'eau dans le manteau. La cavité palléale sépare le manteau de la coquille à l'exception des zones d'attache des muscles. La couche intermédiaire du manteau contient des cellules tactiles et des chémorécepteurs. Ces cellules jouent donc un rôle important dans la détection des prédateurs et leur évitement. Dans cette couche, le manteau possède également des cellules sensibles aux changements d'intensité lumineuse (Gosling, 2015). La moule bleue fait circuler l'eau pour la filtration et la respiration dans la cavité du manteau par les siphons inhalant et exhalant (Bayne, 1976; Gosling, 2003). Ces siphons sont formés par la fusion du manteau entre ces deux siphons (Gosling, 1992). Le siphon inhalant s'étend en continu le long de la partie ventrale au bord de la coquille, le siphon exhalant est plus petit et est de forme conique (Fig. 7b) (Bayne, 1976; Gosling, 2003).

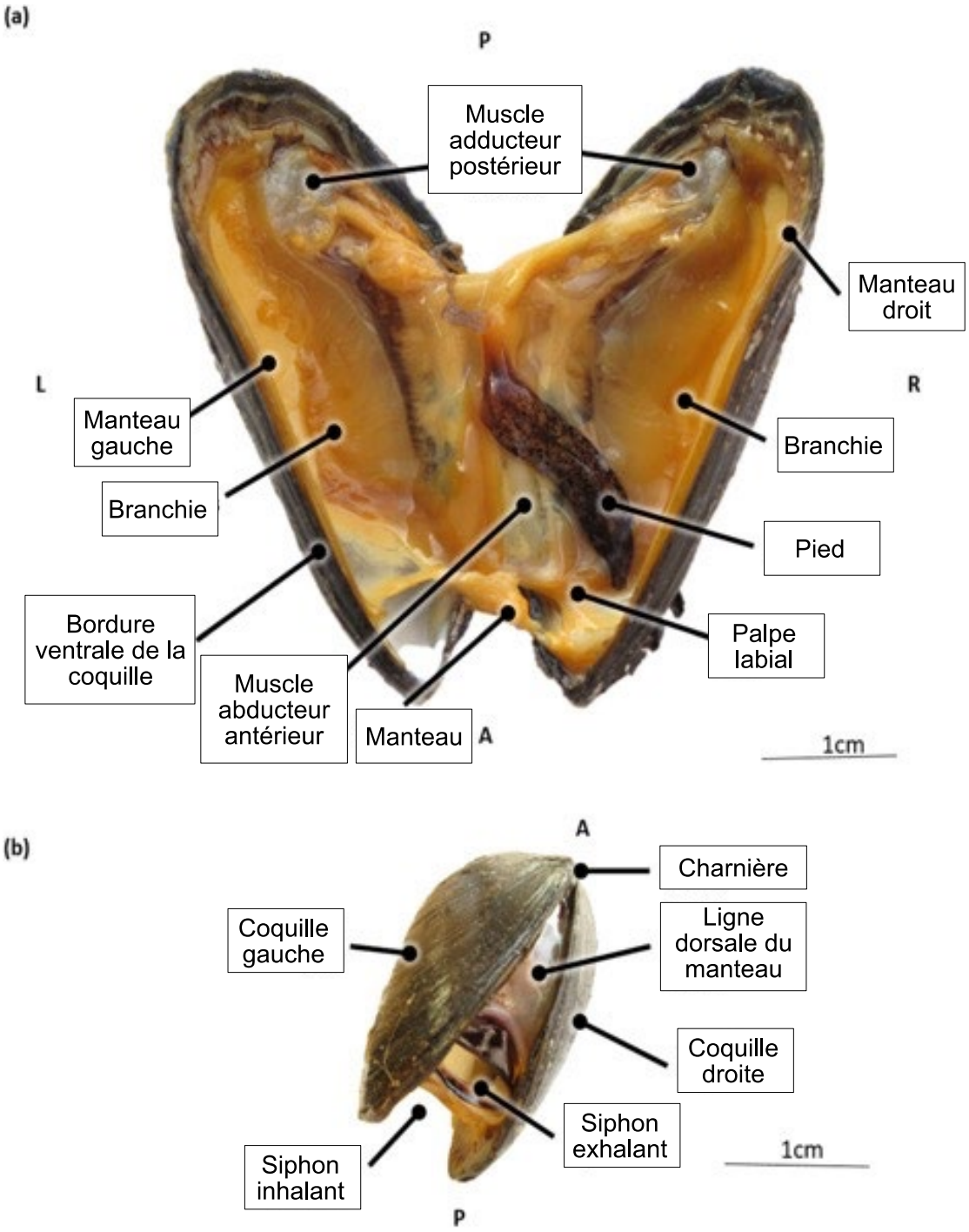

Figure 7 : Morphologie interne de la moule bleue (Eggermont, 2020)

3.1.3.3 Branchies

Les branchies ont pour rôles principaux la respiration et la nutrition. Les cils présents sur ces branchies servent de pompe pour l'eau de mer ainsi que de transport et de tri des particules contenu dans l'eau de mer (Morton, 1983). Les moules font partie du groupe des lamellibranches possédant des branchies formées par deux structures suspendues le long de la marge dorsale du manteau. Les branchies épousent la courbe des valves tout en exposant un maximum de surface au flux d'eau de mer entrant par le siphon inhalant. Les branchies forment un W composé d'un squelette riche en collagène à partir duquel partent des filaments en forme de V (Gosling, 2015). La surface des branchies influence la quantité d'eau pompée ainsi que la capacité de filtration (Foster-Smith, 1975; Meyhöfer, 1985; Møhlenberg and Riisgård, 1979; Vahl, 1973). Cette surface varie entre les populations de moules selon la quantité de sédiments présents dans l'eau. Ainsi une moule vivant dans un environnement peu chargé en sédiments a des branchies plus grandes qu'une moule vivant dans un environnement avec une concentration en sédiments plus forte (Essink et al., 1989; Theisen, 1982). Plusieurs types de cils sont présents sur les branchies, les cils latéraux sont placés sur le côté des filaments des branchies. Ils sont responsables du mouvement d'eau dans la cavité du manteau et dans les branchies jusque dans la chambre exhalant puis dans le siphon exhalant (Gosling, 2015). Les cils frontaux sont actifs en permanence dans le but de maintenir un courant d'eau dans les filaments branchiaux (Jørgensen, 1974). Les cils situés entre les cils latéraux et frontaux ont une forme de plume et ont pour rôle de séparer les particules en suspension de l'eau et de les transporter vers les cils frontaux (Gosling, 2015). La taille des branchies permet la rétention efficace de particules supérieures à 4µm de diamètre. En dessous de cette taille, la moule peut retenir les particules avec une efficacité plus faible (Strohmeier et al., 2012; Vahl, 1972). Pour une particule de 1µm de diamètre, l'efficacité de rétention des moules ne sera plus que de 14 à 64% (Strohmeier et al., 2012). Cette efficacité de capture peut varier grâce à un ajustement des espaces entre les cils (Cranford and Gordon, 1992) et par ajustement des mouvements des cils latéraux frontaux (Dral, 1967).

3.1.4 Physiologie

3.1.4.1 Réaction aux stress

3.1.4.1.1 Réactions comportementales

Les moules étant des organismes semi-sessiles, celles-ci ne peuvent pas fuir lorsqu'elles sont exposées à un stress. Leur réponse est donc souvent caractérisée par une grande augmentation des mouvements valvaires et notamment la fermeture de leurs valves (Borcherding, 2006) et par la production de mucus (Shumway et al., 1987). Une réduction du taux de filtration est souvent observée lors d'exposition à des toxines chimiques (Howell et al., 1984), a des phycotoxines sécrétées par des algues toxiques (Binzer et al., 2018; Nielsen et al., 2020; Widdows et al., 1979), en présence de prédateurs (Dzierżyńska-Białończyk et al., 2019), de perturbation abiotique tel que des changements de température, de salinité ou encore en présence de fort courants (Davenport and Manley, 1978; Newell et al., 2001) et lorsque la concentration en nourriture est en dessous d'un certain seuil (Newell et al., 2001; Pascoe et al., 2009; Riisgård et al., 2011). Dans le cas de la survie face à des prédateurs, la moule va également augmenter sa résistance à l'arrachement en renforçant son byssus (Kobak et al., 2010; Naddafi and Rudstam, 2013), son agrégation (Naddafi and Rudstam, 2013) ainsi que la résistance de sa coquille (Czarnołçski et al., 2006; Naddafi and Rudstam, 2014). En présence de prédateurs les moules vont également se fermer pour empêcher des substances pouvant révéler leur présence d'être excrétées dans l'eau environnante (Robson et al., 2007, 2010 ; Antoł et al., 2018)). En présence de forts courants, les moules gardent leurs valves ouvertes pour leurs besoins respiratoires, mais leur taux de filtration diminue (Newell et al., 2001). Lors d'une concentration trop faible en particule nutritive dans l'eau (entre 2000 et 6000 particules), les moules se ferment (Campbell and Newell, 1998; Newell et al., 2001). D'autres auteurs comme ont observé un seuil plus faible avec une diminution de la filtration à des concentrations en phytoplancton inférieure à 1500 cellules par ml (Riisgard and Randløv, 1981).

L’exposition aux algues toxique provoque différentes réactions chez les moules. La première réaction des moules face aux microalgues produisant du PSP est la fermeture des valves (Tran et al., 2010) pour les isoler du milieu extérieur (Mafra et al., 2010; Pousse et al., 2018). Dans le cas d’une exposition aux DSP, le taux de filtration diminue également même si celui-ci n’affecte pas la consommation d’oxygène par les moules (Nielsen et al., 2020). Ces réactions aux algues toxiques ne sont pas permanentes, et le taux de filtration revient à la normale avec une baisse importante des concentrations d’algues toxiques (Widdows et al., 1979). La provenance géographique des moules peut jouer un rôle sur l’effet des toxines et deux moules provenant de deux environnements différents pourront avoir des intensités de réponses différentes (Shumway et al., 1987).

3.1.4.1.2 Réaction cellulaire et physiologique

Les toxines, chimiques ou biologiques, absorbées par les moules ont également des impacts au niveau cellulaire (Wikfors and Smolowitz, 1993) en provoquant des lésions (Galimany et al., 2008a; Pearce et al., 2005), des dysfonctionnements cellulaires (Hégaret and Wikfors, 2005), affecter la nutrition (Bianchi et al., 2019; Nielsen et al., 2020), affecter leur taux de respiration (MacQuarrie and Bricelj, 2008; Nielsen et al., 2020), altérer la structure de l’ADN et la transcription des gènes (Mat et al., 2013) et diminuer l’énergie disponible à la croissance (Basti et al., 2016; Li et al., 2002; Nielsen and Strømgren, 1991). Le bivalve peut également être affecté avec une perte de masse indiquant une détérioration de son état de santé (Delaporte et al., 2006; Hendriks et al., 2003). L’exposition aux PSP entraîne également une perte de l’homéostasie de la glande digestive (Galimany et al., 2008b). Les réactions inflammatoires sont également présentes lors d’intoxication avec des toxines chimiques (Farley, 1988; George and Viarengo, 1985; Sunila, 1984; Viarengo, 1985) et une réponse immunitaire est observée lors d’exposition au PSP par la migration importante des hémocytes dans l’estomac et les intestins pour protéger les tissus des toxines (Galimany et al., 2008a). En effet les toxines s’accumulent dans les viscères des moules (Bricelj et al.,

1990; Bricelj and Shumway, 1998; Kwong et al., 2006) en causant des dommages dans la glande digestive (Widdows et al., 1979). L'efficacité d'accumulation des toxines dans les tissus est reliée à la probabilité de contact avec les parois du système digestif (Moroño et al., 2001). Les hémocytes encapsulent les cellules toxiques dans le canal alimentaire et forment des agrégats hémocytes-algue pour éliminer ces cellules de la moule et limiter le contact avec d'autres tissus (Galimany et al., 2008a). La migration des hémocytes depuis le système vasculaire semi-ouvert jusque dans l'estomac ou l'intestin a été observée chez des moules exposées ; cette réponse est décrite comme étant un système de défense chez les bivalves (Stauber, 1950). Lors de la réponse inflammatoire, les hémocytes sont aussi retrouvés dans le tissu conjonctif entre les follicules des gonades altérant le cycle de reproduction des bivalves (Gosling, 2003) et provoquant un arrêt de la reproduction (Basti et al., 2018; Granmo et al., 1988). Certains auteurs ont observé une diminution de l'immunité chez les moules exposées aux PSP (Hégaret and Wikfors, 2005; Lassudrie et al., 2016, 2015; Ordás et al., 2007). Cependant une réponse immunitaire plutôt qu'une immunosuppression chez la moule exposée aux PSP a également été observé par Galimany et al., (2008a) et suggère que les moules perçoivent les algues toxiques comme des pathogènes et cherchent donc à les éliminer. Les moules peuvent donc reconnaitre les algues toxiques (Bushek et al., 2002; Galimany et al., 2008a). Après une exposition aux PSP les muscles adducteurs des moules se paralysent et deviennent incapable de fermer les valves (Galimany et al., 2008b; Hégaret et al., 2007). Cette paralysie est liée au blocage des canaux sodium voltage dépendants par les PSP arrêtant la production de potentiels d'action faisant fonctionner les neurones (Bricelj et al., 2005; Hégaret et al., 2007; James et al., 2010). Les moules accumulent les toxines telles que les PSP dans leurs tissus, notamment dans les muscles, le manteau, le pied ainsi que dans les branchies (Bricelj et al., 1990; Bricelj and Shumway, 1998; Denardou-Queneherve et al., 1999; Pousse et al., 2018). Cependant, les moules sont capables d'éliminer rapidement les toxines par excrétion (Nielsen et al., 2016). Ce temps d'excrétion varie selon les études allant de 72h (Novaczek et al., 1992) à une élimination beaucoup plus longue entre 2 et 4 mois (Duinker and Greig, 2007; Sephton et al., 2007).

3.1.5 Dépuration

La moule bleue est considérée comme un organisme à détoxification rapide pouvant excréter des toxines sur une durée de l'ordre d'une semaine (Nielsen et al., 2016), contrairement à d'autres bivalves comme *Placopecten magellanicus* qui a une durée de détoxification beaucoup plus longue allant du mois à l'année (Bricelj and Shumway, 1998). Plusieurs facteurs influencent la vitesse de dépuration des toxines ; ainsi l'âge, la taille (Duinker and Greig, 2007) et la disponibilité de la nourriture peut influencer l'excrétion des toxines (Marcaillou et al., 2010; Svensson, 2003). La dépuration peut être effectuée par différents mécanismes. Les toxines peuvent être simplement éliminées, subir une épimérisation (changement de configuration de la molécule) ou être transformées par réduction ou par hydrolyse (Bricelj and Shumway, 1998). L'épimérisation va intervenir à court terme et sert de système de désintoxication précoce (Bricelj et al., 1991). La biotransformation va agir plus lentement que l'épimérisation (Bricelj and Shumway, 1998) et est causée par des réducteurs naturels (Oshima, 1995). Les toxines absorbées peuvent également être transformées en acides gras pour être dépurées (Rossignoli et al., 2011). Selon (Nielsen et al., 2016), la fin de la dépuration a lieu avec l'excrétion des toxines plutôt que par métabolisation.

3.1.6 Aquaculture

Dans le monde, la production de moules par l'aquaculture est en hausse. Elle est vue comme une solution potentielle pour subvenir à la demande croissance de nourriture provenant de la mer (Costa-Pierce, 2002). Avant 1960, elle était inférieure à̀ 100 kT par an, depuis 2000 celle-ci approche les 200 kT par an pour *M. edulis* (Fig. 8 ; FAO, 2016). Les principaux producteurs de *M. edulis* sont les pays de l'Atlantique Nord, donc l'Amérique du Nord et l'Europe de l'Ouest (FAO, 2016). Trois espèces de moules sont cultivées en Amérique du Nord ; *M. edulis* à l'est et *M. trossulus* et *M. galloprovincialis* à l'ouest (Hilbish et al., 2000). La production canadienne de *M. edulis* était d'environ 26 000 tonnes en 2019

(Statistiques Canada, 2020) et était assurée à environ 80% par l'Île du Prince Édouard (Chopin, 2015). L'aquaculture des bivalves est différente de celle des poissons ou des crustacés, celle-ci exploitant directement le phytoplancton présent naturellement dans l'eau sans apports de nourritures artificiels (Crawford et al., 2003).

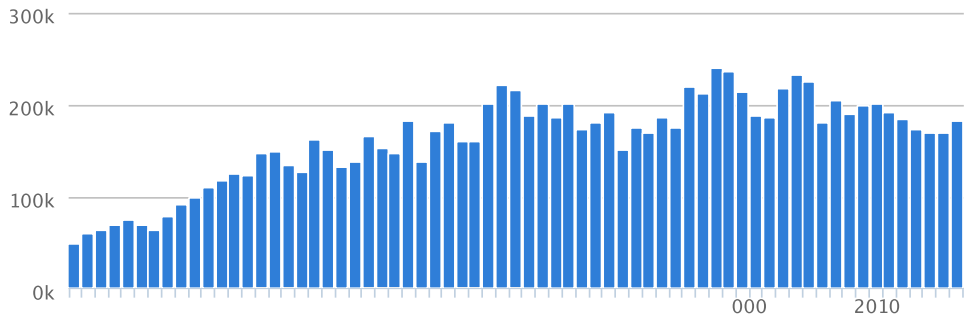

Figure 8 : Production mondiale de *M. edulis* par l'aquaculture (FAO, 2016)

Au Canada les moules cultivées sont produites avec des cultures suspendues. Les moules sont placées dans des boudins fixés sur des filières mytilicoles (Fig. 9 ; Drapeau et al., 2006). Ces filières mesurent environ 200m avec des boudins de 3m de longueur, séparés de 60 à 90cm. En situation normale les lignes flottent à la surface de l'eau et, avant que l'eau gèle des poids sont placés pour faire descendre les lignes (Scarratt, 2000). Les filières permettent également une exploitation optimale de la colonne d'eau. Elles peuvent être installées dans des baies avec une nourriture et une température propice à la culture des moules (Garen et al., 2004).


Figure 9 : Boudins de moules en Gaspésie (Rejean Tremblay)

L'une des difficultés rencontrées par les fermes aquicoles est l'arrivée de polluants ou d'efflorescence d'algue conduisant à l'intoxication des espèces cultivées et notamment des bivalves filtreurs qui vont accumuler ces toxines (Starr et al., 2017) chimiques comme le Corexit ou biologiques comme les HABs. Les HABs sont l'un des problèmes les plus sérieux pour l'aquaculture dans le monde (Hallegraeff, 1993; Shumway, 1990). Dans les sites concernés par les efflorescences de HABs, les bivalves cultivés sont affectés par ces derniers pouvant conduire à une longue fermeture de la commercialisation de ces bivalves provoquant une grande perte économique pour les aquaculteurs (Hoagland et al., 2002). Un des plus grands évènements d'intoxication humaine liée aux HABs au Canada est survenu en 1987 avec plus de 100 personnes intoxiquées et 3 tuées après avoir consommé des moules contaminées à l'île du Prince Édouard (Bates et al., 1998). Pour lutter contre les efflorescences de PSP, des systèmes de suivi ont été étudiés dans le but d'éviter que cela se reproduise.

3.2 Placopecten magellanicus

3.2.1 Répartition

Le pétoncle géant *Placopecten magellanicus* (Gmelin 1791) est présent sur les côtes nord-ouest de l'Atlantique, du Golfe du Saint-Laurent au nord (Squires, 1962) jusqu'à Cape Hatteras en Caroline du Nord pour la limite sud (Posgay, 1957).

P. magellanicus est une espèce subtidale vivant à une profondeur située entre 10 et 100m sur des fonds de type vaseux, sableux ou encore rocheux (Gosling, 2015) à l'interface eau-sédiment (Shumway et al., 1987).

3.2.2 Morphologie

3.2.2.1 Valves, muscles et manteau

P. magellanicus possède deux valves d'une taille pouvant varier de 200 à 230 mm au maximum (Gosling, 2015). Des anneaux concentriques apparaissent annuellement sur la surface de la valve gauche (Naidu, 1969; Posgay, 1962; Stevenson and Dickie, 1954) pouvant être utilisée pour des études sclérochronologiques et pour déterminer l'âge des pétoncles étudiés. Chez les pétoncles, comme tous les bivalves avec deux valves différentes (Fig. 10), un seul muscle adducteur central permet le mouvement des valves (Gosling, 2015).

Les pétoncles possèdent des yeux sur le manteau dont le nombre peut varier de 50 à 100 selon les espèces. Ces yeux sont plus développés chez les pétoncles que chez les autres espèces de bivalves. Chacun de ces yeux est composé d'une cornée, d'un cristallin et d'une rétine produisant des images avec peu de contraste (Colicchia et al., 2009). Le manteau ainsi

que les palpes labiaux et les lèvres complètent les branchies dans la rétention des particules (Foster-Smith, 1975; Kellogg, 1915; Shumway et al., 1985; Theisen, 1982).

Figure 10 : Valves du pétoncle *Placopecten magellanicus*. DFO-MPO

3.2.2.2 Branchies

P. magellanicus est un filtreur utilisant la matière organique particulaire comme source de nourriture (Shumway et al., 1987). Chez les pétoncles, les branchies sont l'organe principal permettant de contrôler le volume d'ingestion des particules (Beninger et al., 1992) et de sélection de ces particules (Atkins, 1937). Le flux d'eau entrant est parallèle aux filaments branchiaux en arrivant dorsalement puis est dévié vers les filaments principaux. Ce flux d'eau est créé par les surfaces ciliées des filaments et par les cils des autres filaments (Jørgensen, 1981). Les particules sont ensuite incorporées à un mucus (Beninger et al., 1992).

3.2.3 Filtration

Le pétoncle *Placopecten magellanicus* possède un régime alimentaire opportuniste de filtreur pouvant ingérer un grand spectre d'organismes pélagiques, benthiques ou de matière détritique allant de 10 à 350 µm (Shumway et al., 1987). Quatre mécanismes permettent au pétoncle de réguler l'arrivée de particules dans la région buccale. Le premier est l'utilisation des cils pour évacuer les particules, la deuxième est l'arrêt du flux d'eau généré par les cils, le troisième est le décollement des plis dorsaux pour bloque l'arrivée des particules et le quatrième est un arrêt momentané de l'arrivée de particules par le filament principal (Jørgensen et al., 1990). Les pétoncles peuvent réduire leur taux de filtration en diminuant l'efficacité de rétention des branchies évitant de filtrer une partie du volume d'eau de mer inhalé Famme et al., 1986; (Famme and Kofoed, 1983; Wildish et al., 1987) ou en réduisant l'efficacité de capture des cils par une compression des branchies (Jørgensen et al., 1990).

3.2.4 Physiologie

3.2.4.1 Réaction aux stress

Lors d'un stress, deux types de comportements peuvent être observés chez les pétoncles, la fermeture des valves ou la fuite. Lors d'un stress tel que des exondations, la présence de prédateurs, des conditions environnementales défavorables ou encore la présence de toxine, les pétoncles ferment leurs valves (Gosling, 2015; Shumway et al., 1987). Le deuxième mécanisme utilisé est la fuite par clapping (Fig. 11) correspondant à une fermeture rapide des valves (Cheng and DeMont, 1996; Guderley and Tremblay, 2013). Avec un ajustement des bords du manteau, le pétoncle peut produire un jet d'eau lui permettant de se déplacer latéralement et verticalement avec des mouvements saccadés et lui permettant une rotation. En présence d'un prédateur, différents types de mouvements peuvent être observés. Le

pétoncle peut expulser de l'eau du bord de sa coquille et le propulser en arrière ou faire varier ce jet pour effectuer un saut lui permettant de se retourner (Cox, 1957; Gosling, 2015; Shumway et al., 1987; Thompson et al., 1980). En général, la plupart des espèces utilisent des mouvements rapides sur de courtes distances (<5m). Cette stratégie est courante pour les réactions de fuite, mais n'est pas efficace pour des mouvements sur de longues distances (Brand, 2006). Les pétoncles peuvent également utiliser l'enfouissement pour fuir (Gosling, 2015).

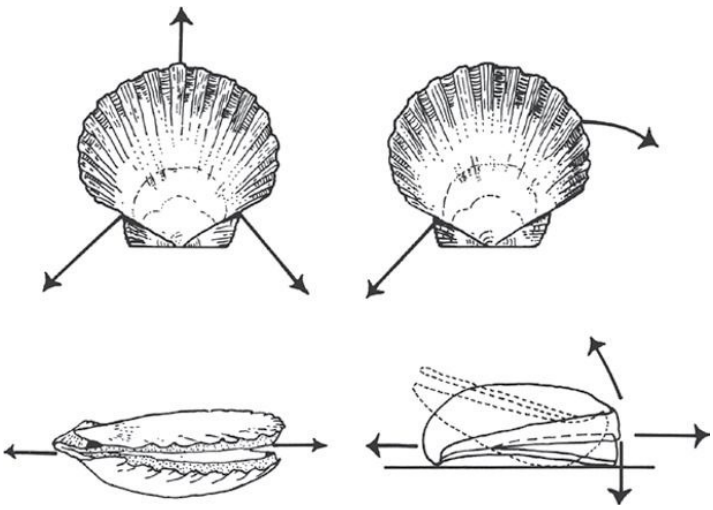

Figure 11 : Déplacement des pétoncles par clapping (Gosling, 2015)

4. SYSTEMES DE SUIVI DES ALGUES TOXIQUES

4.1 Valvométrie

4.1.1 Principe général

La valvométrie est une technique permettant d'enregistrer le comportement d'ouverture et fermeture de bivalve à haute fréquence et de manière non invasive pour éviter les modifications du comportement (Andrade et al., 2016; Tran et al., 2011, 2003). Le

comportement valvaire peut être relié à de nombreux processus physiologiques ; en effet la nutrition, la respiration et l'excrétion induisent des mouvements de valves pouvant être enregistrés (García-March et al., 2008; Sow et al., 2011; Tran et al., 2011). Lorsqu'un stress intervient, le comportement valvaire des bivalves peut être modifié et mettre en évidence cette perturbation (Comeau et al., 2019; Fournier et al., 2004; Sow et al., 2011; Tran et al., 2015, 2010, 2003), particulièrement lorsque le comportement de plusieurs de ces bivalves est modifié simultanément.

Figure 12 : Installation des moules équipées de valvomètres pour les experiences en laboratoire.

4.1.2 Utilisation

L'ouverture valvaire est mesurée à l'aide d'une électrode et d'un aimant collés chacun sur une valve du bivalve (Fig 13). Le valvomètre enregistre les variations d'ouverture valvaire en utilisant l'effet de Hall, dans lequel l'intensité du champs magnétique détecté par

l'électrode varie en fonction de la distance de l'aimant (Nagai et al., 2006). Les valeurs mesurées sont enregistrées sur une carte mémoire avant d'être ensuite analysées. L'enregistrement est généralement réalisé à une fréquence de 10 données chaque seconde, cette haute fréquence génère un nombre de données important, difficile à traiter, cependant elle donne l'avantage d'offrir une résolution permettant l'observation d'évènements comportementaux très brefs tel que des microfermetures. Cette résolution peut être diminuée à une donnée par seconde pour alléger le jeu de données lorsque les évènements recherchés ne nécessitent pas une résolution aussi fine.

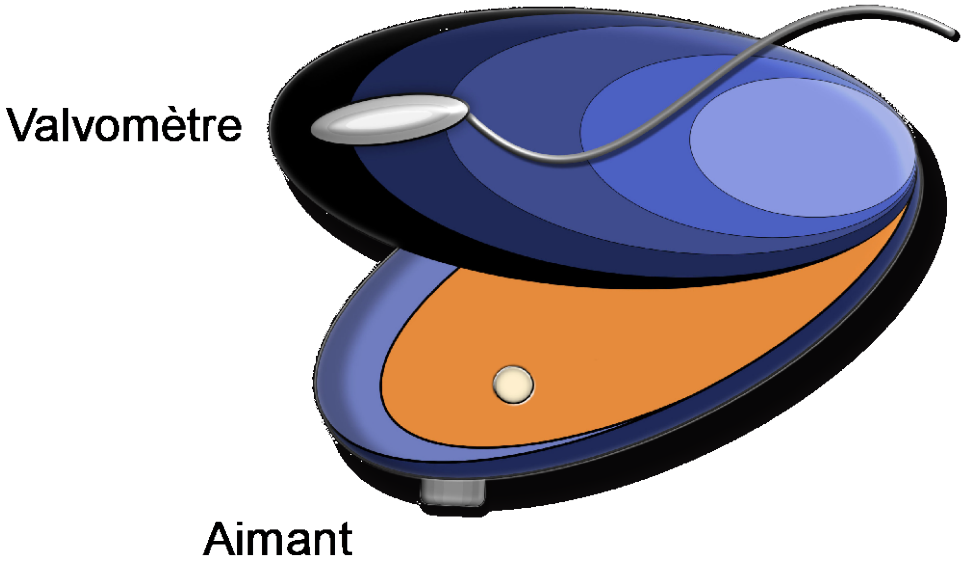

Figure 13 : Valvomètre et aimant sur les valves d'une moule.

4.1.3 Traitement des données

La valvométrie générant des jeux de données très volumineux, le traitement devient extrêmement long, voire impossible, manuellement. L'automatisation de ce traitement de données devient donc nécessaire, ce que nous avons pu faire grâce au développement d'un programme informatique avec le logiciel R (R Core Team, 2020). Les exigences pour le

développement de ce programme informatique étaient d'être capable d'aller chercher les données au bon endroit sur les ficher de valvométrie et de combiner les données des différents fichiers en un seul pour pouvoir réaliser un traitement simultané. Il devait aussi être capable de trouver les informations (dates, heures, nombre de données) de manière autonome ainsi que réaliser les calibrations pour convertir les données enregistrées par les valvomètres en millivolt en millimètre d'ouverture. Finalement ce programme devait être capable de donner les graphes de comportement de chacun des individus et calculer les différents indicateurs comportementaux automatiquement. Ces indicateurs sont le nombre de fermetures des valves, l'amplitude moyenne d'ouverture, la durée totale de fermeture, la durée moyenne de fermeture ainsi que le nombre de microfermetures.

Nombre de fermetures :

Nombre de fois que le bivalve suivi est ouvert à moins de 10% de sa VOA (Valve Opening Amplitude, l'amplitude d'ouverture du bivalve). Le seuil de 10% de la VOA a été choisi en raison de la distribution des fréquences d'ouverture. En effet, d'après Comeau et al. (2018), la gamme de VOA entre 0 et 10% suit une fréquence d'ouverture différente des autres indiquant que cette dernière correspond à un autre type de comportement. Une entrée et une sortie de cette zone correspondent à une fermeture (Fig. 14A)

Durée de fermeture :

Deux indicateurs sont calculés à partir de la durée de fermeture (Fig. 14B) :

- **La durée totale de fermeture** correspond à l'addition de la durée de chaque fermeture sur la période étudiée.
- **La durée moyenne de fermeture** correspond à la durée moyenne des fermetures sur la période étudiée

VOA moyenne :

Correspond à l'ouverture moyenne du bivalve sur la période étudiée (Fig. 14C).

Microfermetures :

Une microfermeture est comptée lorsqu'une diminution supérieure à 3% de la VOA sur une durée de 0,1 seconde est détectée. Si une autre diminution de plus de 3% en 0,1 seconde suit directement la précédente, elle sera comptée comme une seconde microfermeture (Fig. 14D) (Comeau et al., 2019). Le seuil de 3% a été choisi en raison de la sensibilité de la technique, pour éviter le bruit de fond. Cette précision a été mesurée par Luc Comeau (données non publiées) par des mesures répétées sur des cales de tailles connues.

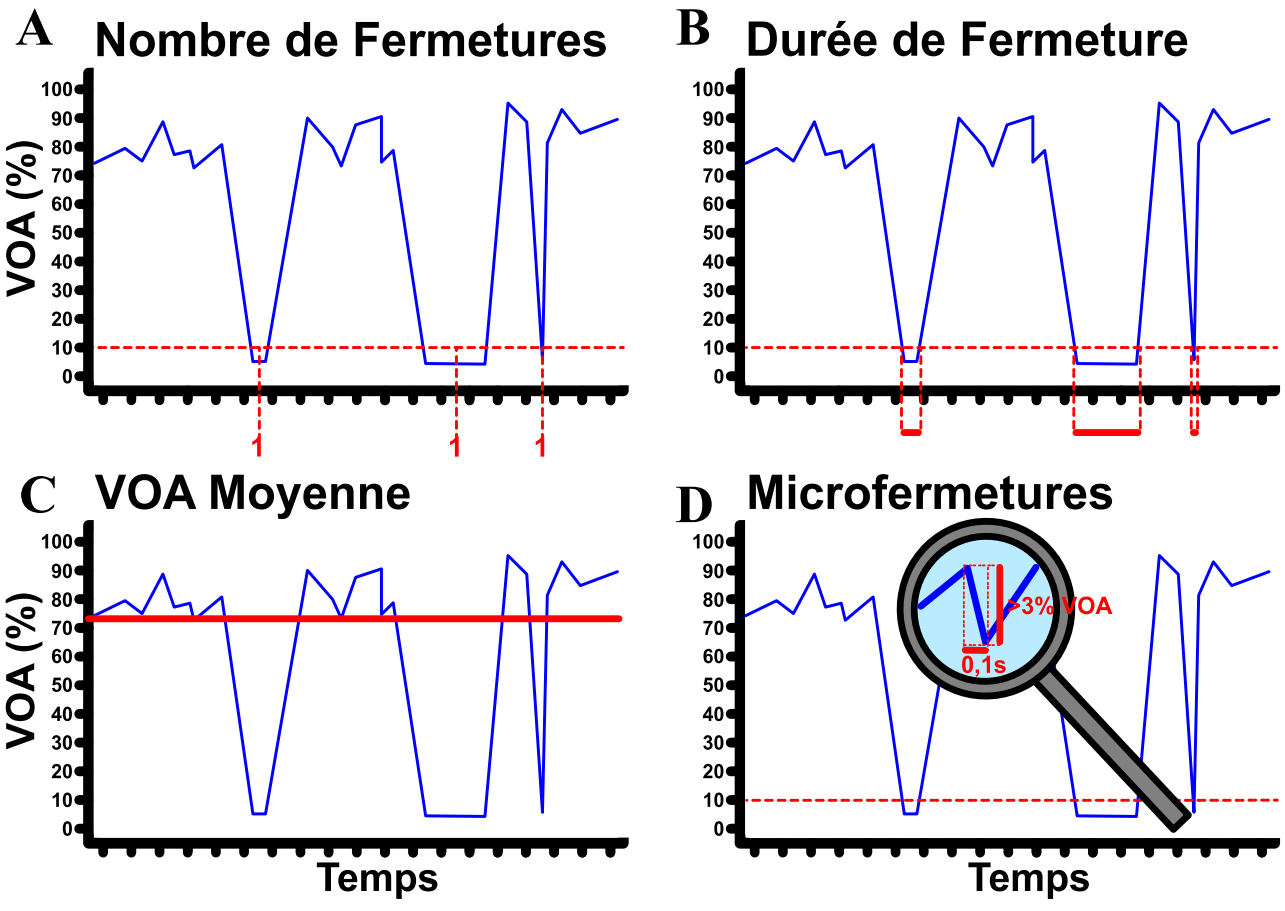

Figure 14 : Définition des indicateurs comportementaux

Le programme a été écrit de manière qu'il soit applicable à toutes les expériences de valvométrie. Pour faciliter son utilisation, les modifications à apporter pour une expérience particulière sont regroupées sous forme de paramètres dans les premières lignes de ce programme. Cela permet l'utilisation facile de ce programme sans avoir à comprendre la construction entière de ce programme.

4.1.4 Intérêt de la valvométrie

Le suivi du comportement valvaire des bivalves et donc de leur activité pourrait être un outil intéressant pour les suivis environnementaux dans de nombreux domaines comme la surveillance des fermes aquacoles, la détection de polluants dans l'eau ou la détection d'efflorescences d'algues toxiques (Andrade et al., 2016). En effet, il a été observé en laboratoire que des huîtres *Crassostrea gigas*, maintenant appelées *Magallana gigas* (Bayne et al., 2017) avaient un comportement valvaire différent selon si celles-ci étaient exposées au dinoflagellé toxique *Alexandrium minutum* ou aux dinoflagellés *Heterocapsa triquetra* et *Isochrysis galbana*. Les huîtres exposées aux algues toxiques présentaient rapidement une durée d'ouverture plus longue, une amplitude d'ouverture plus faible et une augmentation du nombre de microfermetures (Haberkorn et al., 2011; Mat et al., 2013; Tran et al., 2010). Chez la moule *Mytilus galloprovincialis*, une augmentation du nombre de microfermetures a été observée lors d'une exposition au dinoflagellé *A. minutum* (Comeau et al., 2019). Les rythmes biologiques des bivalves peuvent également être suivis grâce à la valvométrie. Cela a notamment permit d'observer des modifications chez les huîtres en présence d'algues toxiques (Tran et al., 2015). Un changement de comportement a également été observé chez certains bivalves en présence de polluants. Chez la moule d'eau douce *Corbicula flumineato*, un changement de comportement valvaire a été observé lorsque celles-ci sont exposées à des métaux (cadmium, cuivre, uranium et mercure inorganique) (Tran et al., 2003).

La valvométrie est donc un outil pour le suivi du comportement valvaire des bivalves capable de détecter les variations de ces comportements permettant de les relier à des changements dans les écosystèmes. Il pourrait donc être envisageable d'utiliser cet outil pour la surveillance de l'état de ces écosystèmes et des fermes aquacoles si les résultats obtenus en laboratoire se vérifient en conditions naturelles.

4.2 Autres systèmes de détections

De la même manière que la valvométrie, dans le but de détecter la présence de toxines dans l'eau à court terme, de nombreuses solutions ont été étudiées (Bates et al. 2020). Une première étude concerne une méthode moléculaire permettant de détecter l'ADN ribosomal chez les espèces de *Pseudo-nitzschia* (Bates et al., 2018, 2019), les kystes de *A. catenella* dans les sédiments (Erdner et al., 2010) ainsi que les cellules du genre *Alexandrium* spp. dans l'eau (Hatfield et al., 2019). Cette méthode donne de bons résultats pour la recherche rapide des algues toxiques en comparaison des comptages microscopiques (Hatfield et al., 2019), mais offre peu de prédictibilité. La détection d'espèce toxique et de phycotoxines *in situ* par cytométrie en flux portable (Görögcs et al., 2018) est également étudiée pour quantifier les cellules présentes dans les masses d'eau. De nombreux systèmes s'appuyant sur ce principe sont actuellement développés (Lombard et al. 2019). Cependant, le déclenchement de ces appareils ne peut se faire que pour un nombre d'espèces limité, il est donc possible que certaines ne soient pas détectées (Reynolds et al. 2010). Certaines cellules peuvent également être trop petites pour être détectées (Lombard et al. 2019). Deux techniques permettent la détection aérienne d'algues toxiques. La télédétection par avion (Sathyendranath et al., 1997) permet de détecter la signature optique de *Pseudo nitzschia*. Les images satellites permettent d'analyser la couleur de l'eau par radiométrie (Bernard, 2021). Cet outil permet de surveiller de grandes surfaces, mais ne permet pas de discriminer les souches toxiques des souches non-toxiques. Il existe également des difficultés pour différencier les groupes d'espèce toxiques, les interférences avec les sédiments en eau côtière et pour les faibles abondances en algues toxiques, celles-ci pouvant causer des perturbations a faible abondance (Page et al., 2004). Il est donc nécessaire d'utiliser des mesures *in situ* en complément (Sathyendranath et al.,

1997). Une autre méthode est la Phase Adsorption Toxin Tracking : SPATT (MacKenzie et al. 2004), qui permet d'absorber les toxines dans l'eau sans transformer ces toxines (Zendong et al., 2015). Ce dispositif peut suivre de manière spatiale et temporelle la quantité de toxines dans l'eau. Cependant, la durée d'exposition des dispositifs SPATT est très discutée en raison de la limite de saturation, l'efficacité d'absorption est variable selon les toxines et les conditions environnementales et les niveaux de toxines relevés sont difficiles à convertir en concentration de toxine (Roué et al., 2018). Ces technologies présentent des solutions intéressantes pour la détection de toxines dans l'eau de mer, mais sont peu ou pas prédictives de l'arrivée des blooms d'algues toxiques. Un travail récent de Boivin-Rioux et al. (2021) démontre que *A. catenella* répond principalement aux variations de températures et salinité et qu'il est donc possible de prédire l'arrivée des blooms selon les modèles météorologiques reconnus fiables sur une base de 48 heures. Le suivi du comportement valvaire présente une alternative à ces techniques permettant d'apporter d'autres avantages. Les bivalves ayant des temps de réaction rapides, ils permettent de détecter de faibles concentrations en toxine à court terme et pourraient offrir une période de prédictibilité plus longue (Comeau et al., 2019). De plus les techniques présentées précédemment permettent de détecter principalement des phycotoxines alors que les le comportement valvaire des bivalves va varier en réponse à de nombreux types de toxines. Cela pourrait permettre de détecter la présence de contaminants non suspectée dans les masses d'eau. Une fois installée, l'utilisation des valvomètres ne nécessite que peu de manutention, ce qui présente également un avantage face à des techniques demandant des analyses de laboratoire régulières.

Au Canada, l'Agence canadienne d'Inspection des Aliments (ACIA) a mis en place un programme de surveillance des biotoxines dans le but de protéger les consommateurs d'une éventuelle intoxication lors de la consommation de mollusque. Cette surveillance s'effectue sur des mollusques prélevés a une fréquence dépendant des risques d'intoxication de ces derniers. Si un secteur dépasse les concentrations de 80 µg 100g-1 pour les PSP, 20 µg g-1 pour les ASP, 0,20 µg g-1 ou 0,20 µg g-1 pour les pecténotoxine, l'ACIA recommandera au Ministère Pêches et Océan de le fermer pour la récolte des bivalves. Le secteur pourra être réouvert lorsque trois échantillons en dessous de ces seuils auront été prélevés sur une période

de 14 jours (Gouvernement du Canada, 2018). La valvométrie pourrait de faciliter cette surveillance en permettant la surveillance continue des secteurs sensibles et ainsi informer les exploitants de fermes conchylicoles ou le public récoltant des bivalves sauvages. De plus, la valvométrie permettant de détecter les différents types de toxines, celle-ci offrirait une surveillance plus complète des différents risques d'intoxication des consommateurs.

Jusqu'à récemment, les concentrations en PSP dans les bivalves étaient mesurées grâce aux tests sur des souris. La durée entre l'injection des tissus contaminés et le décès de l'animal permettait de connaître leur concentration en toxine (AOAC, 1990). Actuellement, les dosages des PST peuvent être effectués par HPLC (Chromatographie en phase liquide à haute performance), permettant ainsi d'éviter l'usage de souris (Negri et Jones, 1995 ; Negri & Llewellyn, 1998 ; Rourke et al., 2008). Le test Elisa (Enzyme-Linked ImmunoSorbent Assay), basé sur l'utilisation d'anticorps, est également utilisé pour la détection des PST (Usleber et al., 1991).

5. HYPOTHESE ET OBJECTIFS

Lors de ce doctorat, l'objectif principal a été d'identifier des comportements spécifiques potentiellement exploitables pour un système de détection précoce permettant une surveillance des écosystèmes côtiers, dont les zones de production aquacole.

Dans un premier temps il a été nécessaire de déterminer l'espèce de bivalve la plus adaptée entre une espèce résistante et une espèce sensible aux toxines. Le deuxième sous objectif était de mettre en évidence un comportement valvaire caractéristique de la présence de substance synthétique (Corexit 1500) et de toxine biologique (PSP) chez *Mytilus edulis*. Le sous objectif suivant était de déterminer la sensibilité des moules à ces deux types de toxine pour identifier le seuil de sensibilité du système de détection précoce et donc sa capacité à détecter les toxines avant l'atteinte de contamination entraînant la fermeture de la récolte des bivalves. Le développement d'une résistance des moules aux PSP a également

été étudié dans le but d'adapter le système de détection précoce si nécessaire. Le dernier sous objectif était d'observer les changements de comportements valvaires au cours de la dépuration après une intoxication par des algues toxiques.

Pour répondre à ces objectifs, cette thèse se découpe en 3 chapitres. Le premier chapitre consiste à identifier l'effet d'une substance synthétique, le Corexit 1500, un dispersant d'hydrocarbure, sur le comportement de deux espèces de bivalves. Ce chapitre permet également d'identifier quelle espèce est la plus propice pour servir d'espèce sentinelle dans le cadre d'un système de détection précoce. Ce chapitre a fait l'objet d'un article intitulé "Sensitivity to oil dispersants: Effects on the valve movements of the blue mussel *Mytilus edulis* and the giant scallop *Placopecten magellanicus*, in sub-arctic conditions" publié dans la revue scientifique Aquatic Toxicology.

Le chapitre 2 est partagé en deux parties. Lors de la première partie de ce chapitre, l'effet de l'algue toxique *Alexandrium catenella* a été testé sur la moule *Mytilus edulis* en laboratoire et en conditions naturelles dans le but d'identifier un comportement valvaire caractéristique à la présence de cette algue toxique. La sensibilité de la valvométrie pour la détection des algues toxiques a également pu être étudiée afin de valider le potentiel de cet outil comme système de détection précoce. La deuxième partie de ce chapitre a permis d'identifier une éventuelle résistance de la moule lors d'une exposition répétée à *A. catenella*. La première partie de ce chapitre a fait l'objet d'un article intitulé « Use of valvometry as an alert tool to signal the presence of toxic algae *Alexandrium catenella* by *Mytilus edulis* » prochainement soumis. La deuxième partie de ce chapitre a été rédigée sous la forme d'un article court intitulé « Impact of regular exposition to the toxic algae, *Alexandrium catenella* on the valve behaviour of blue mussels (*Mytilus sp*) » prochainement soumis.

L'objectif principal du chapitre 3 consistait à étudier le comportement valvaire des moules lors de la dépuration après une intoxication par *A. catenella*. L'article de ce chapitre ayant été corédigé avec Romain Lavaud dans le cadre du projet sur l'étude de l'effet d'*A. catenella* sur la physiologie et le comportement de *M. edulis*, ce chapitre traite également de l'impact des PSP sur la croissance et l'indice de condition des moules, leur taux de respiration

ainsi que sur le développement et la résistance de leur byssus. Ce chapitre a fait l'objet d'un article intitulé « Effects of the toxic dinoflagellate *Alexandrium catenella* on the behaviour and physiology of the blue mussel *Mytilus edulis* » publié dans le revue scientifique Harmful Algae.

***

CHAPITRE 1
SENSIBILITE AUX DISPERSANTS DE PETROLE : EFFET SUR LES MOUVEMENTS VALVAIRES DE LA MOULE BLEUE *MYTILUS EDULIS* ET SUR LE PETONCLE GEANT *PLACOPECTEN MAGELLANICUS*, EN CONDITIONS SUB-ARCTIQUE

Ce premier article, intitulé "*Sensitivity to oil dispersants: Effects on the valve movements of the blue mussel Mytilus edulis and the giant scallop Placopecten magellanicus, in sub-arctic conditions*" a été corédigé par moi-même ainsi que par Jean-Bruno Nadalini, Richard Saint-Louis, Bertrand Genard, Luc Comeau et Réjean Tremblay. Cet article a été accepté le 27 février 2021 et publié le 3 mars 2021 dans la revue *Aquatic Toxicology*. En tant que premier auteur, ma contribution à cet article a été principalement le traitement et l'analyse des données et la rédaction de l'article. Jean-Bruno Nadalini a réalisé les expériences, Bertrand Genard a analysé les taux de ^{13}C dans les tissus des bivalves et Richard Saint-Louis, Luc Comeau et Réjean Tremblay ont fourni l'idée originale et la problématique. Tous les auteurs ont participé à la révision de l'article. Cet article a été présenté au congrès international Physiomar, à Nelson en Nouvelle Zélande en 2021.

1.1 RESUME EN FRANÇAIS DU PREMIER ARTICLE

En réponse aux accidents pétroliers en mer, des dispersants d'hydrocarbures chimiques sont utilisés pour limiter l'impact sur les zones littorales proches. Cependant, certaines preuves suggèrent que ces dispersants pourraient être toxiques pour les organismes aquatiques. La moule bleue (*Mytilus edulis*) et le pétoncle géant (*Placopecten magellanicus*) ont été exposés à différentes concentrations de dispersant d'hydrocarbures et leurs réponses comportementales ont été suivies en utilisant la valvométrie à haute fréquence (10Hz). La

réponse du comportement valvaire montre des fermetures rapides lorsque les dispersant est ajouté aux bassins expérimentaux. Pour la plus haute concentration, les moules restent fermées sur toute la période d'exposition. Le pétoncle géant montre un comportement de fuite (clapping) avant de mourir, suggérant la toxicité du dispersant. Les relations entre les différents indicateurs comportementaux et la concentration en dispersant ont été observées pour les deux espèces avec des tendances différentes. Les pétoncles montrent une corrélation positive entre les comportements valvaires et les dispersants de pétrole alors que chez les moules on observe un seuil au-delà duquel survient une longue fermeture caractéristique. Cette étude souligne les différentes réponses comportementales spécifiques aux traits biologiques des bivalves : une fermeture continue pour une espèce intertidale, *M. edulis*, attachée à son substrat et un comportement de fuite pour l'espèce subtidale semi mobile, *P. magellanicus*. D'après ces observations, il apparait que la valvométrie pourrait être utilisée comme outil pour les suivis environnementaux en utilisant *Mytilus edulis*, celle-ci étant plus adaptée à ces suivis.

1.2 SENSITIVITY TO OIL DISPERSANTS: EFFECTS ON THE VALVE MOVEMENTS OF THE BLUE MUSSEL *MYTILUS EDULIS* AND THE GIANT SCALLOP *PLACOPECTEN MAGELLANICUS*, IN SUB-ARCTIC CONDITIONS

Guillaume Durier[a], Jean-Bruno Nadalini[a,b], Richard Saint-Louis[b], Bertrand Genard[c], Luc A. Comeau[d], Réjean Tremblay[a]

[a] Institut des Sciences de la Mer, Université du Québec à Rimouski, Rimouski, Québec, Canada

[b] Département de biologie, chimie et géographie, Université du Québec à Rimouski, Rimouski Québec, Canada

[c] Les laboratoires Iso-BioKem Inc., 367, rue Gratien-Gélinas, Rimouski, Québec, Canada

[d] Fisheries and Oceans Canada, Gulf Region, Moncton, New Brunswick, Canada

1.2.1 Abstract

In response to accidental oil spills at sea, chemical oil dispersants are utilized to limit negative impacts on nearby littoral zones. However, current evidence suggests that such dispersants may be toxic to aquatic organisms. Blue mussels (*Mytilus edulis*) and giant scallops (*Placopecten magellanicus*) were exposed to different environmentally relevant concentrations of oil dispersant and their behavioural responses were closely monitored using high frequency (10Hz) valvometry. Behavioural valve responses included rapid closures when oil dispersant was added to the experimental tanks. At higher concentrations, the mussels remained closed throughout the exposure period. The giant scallop displayed escape behaviours (clapping) prior to mortality, suggesting toxicity of the oil dispersant. Relationships between different behavioural indicators and oil dispersant concentrations were observed for both species, but with different trends. While scallops demonstrated positive correlations between gaping behaviours and dispersant concentration, mussels exhibited a concentration threshold beyond which the gaping behaviour was characteristic of

longer closure periods. This study highlights behavioural response differences consistent with bivalve-specific biological traits: the continuous valve closure of an intertidal species, *M. edulis*, firmly attached to the substrate, and the escapement behaviours of a semi-mobile subtidal species, *P. magellanicus*. From these observations, it appears that valvometry could be used as a tool for environmental assessments.

1.2.2 Introduction

Interest in the development of oil dispersants began after the Exxon Valdez accident in Alaska in 1989, and has since intensified following the Deepwater Horizon oil rig catastrophe in the Gulf of Mexico in 2010 (Wise and Wise, 2011). The objective of dispersants is the disruption and dispersion of oil slicks, and the resulting oil droplets, before they reach the coast (Lindstrom *et al*., 2000). The principle is based on the acceleration of natural dispersive processes triggered by the addition of surfactant molecules (LLC, 2016). Some products also contain solvents for a better penetration of the surfactant, thus promoting the dispersion of diverse types of oils (George-Ares and Clark, 2000). Corexit™ 9500A, a type of dispersant developed by Ecolab, was used during the Deepwater Horizon incident in 2010. This dispersant's chemical composition can minimize direct impacts of oil-spills on coastlines (National Research Council, 1989), but exposes the associated subtidal habitats and their benthic organisms more directly (George-Ares and Clark, 2000; Schmidt, 2010).
The toxicity of oil dispersants is complex. Toxicity can be mainly attributable to dispersant concentration (Pace *et al*. 1995), but Kenneth *et al.* (2015) postulate that the fixation of oil dispersant to the oil present in the water column increases said toxicity. Thus, in both cases, the toxicity to organisms seems not related to the oil dispersant itself, but rather in its association to the enhanced bioavailability of toxic hydrocarbons (e.g. polycyclic aromatic carbons) present in oil. Conversely, recent studies have demonstrated direct impact of dispersants on benthic organisms. For example, decreased feeding rates have been observed for Eastern oysters (*Crassostrea virginica*) subjected to an oil dispersant (Jasperse *et al*., 2018). Others studies have revealed toxic accumulation in bivalves exposed to oil dispersants and, surprisingly, higher toxicity was observed in blue mussels (*Mytilus edulis*) exposed to

lower dispersant concentration (Scarlett *et al.*, 2005, Berthod and Saint-Louis, personal communication). The authors suggest that this relationship could be related to mussel gaping behaviour, specifically that longer shell closures at higher concentrations might protect mussels, as they inhabit intertidal habitats and are well adapted to complete valve closure for several hours when exposed to air. This response could be different for other bivalve species. For example, scallops in subtidal habitats have their valves nearly fully open most of the time (Tran *et al.*, 2016), and for that reason, may be affected more severely by dissolved chemical pollutants from oil and oil dispersants.

Changes in behaviour from chemical stress, such as a decrease in swimming speed or avoidance of contaminated sediments, can be considered a biological behavioural endpoint in marine organisms. In bivalves, contaminants in seawater can trigger sudden or erratic valve movements, and these endpoints can be quantified by valvometry. Valvometry is a non-invasive technique that monitors fine valve movements in terms of closures and openings from sensors fixed to the shells (Andrade *et al*., 2016). Several previous studies have validated this approach when reporting behavioural responses of bivalves to various environmental stressors (Comeau et al., 2019; Starr et al., 2017; Tran et al., 2015, 2010, 2003). In this study, we relied on the unique capabilities of valvometry to monitor, in real-time, the gaping behaviours of two bivalve species exposed to an oil dispersant, at lethal and sub-lethal concentrations. The development and the use of new tools is necessary to improve environmental assessments. The present study highlights the potential application of valvometry in assessing environmental stress on fauna. We selected two species from two distinct habitats. The blue mussel is an epibenthic, intertidal species with the ability to close its valves for long periods of time in order to resist desiccation during emersion, whereas the giant scallop (*Placopecten magellanicus*) is an epibenthic subtidal species that does not exhibit the same valve closure abilities, never being exposed to emersion. Cold seawater conditions (4°C) were chosen despite of a bivalve filtration rate sensible to the seawater temperature (Kittner and Riisgård, 2005), to simulate the Gulf of Saint-Lawrence (eastern Canada), which exhibits these temperatures 8 months of the year. There is a current paucity of ecotoxicological and behavioural information for these species at these particular

temperature conditions (Comeau *et al*., 2012). Herein, we tested the hypothesis that these above noted biological traits (valve gaping behaviour and filtration rate) were more sensitive to oil dispersant in scallops as compared to mussels. We predicted that mussels would respond to the presence of the oil dispersant by keeping their valves continuously closed, thus isolating themselves from the toxic environmental additive.

1.2.3 Material and methods

1.2.3.1 Oil dispersant

For this study, the oil dispersant used was Corexit™ 9500A manufactured by Corexit™ Environmental Solution LLC (Texas, USA). Of note, this type of Corexit™ contains an oleophilic solvent. This solvent offers a better penetration of the surfactants in heavy oil slicks, thus increasing the dispersion efficiency (LLC, 2016). Place *et al*. (2016) performed a quantitative chemical analysis of surfactants in Corexit™ 9500A; in their sample, the anionic surfactant bis-(2-ethylhexyl)sulfosuccinate (DOSS) represented 18% of the formulation and non-ionic surfactants Span 80 (4.4%), Tween 80 (18%) and Tween 85 (4.6%) complete the remaining fractions of surfactants in the formulation. Because the chemical formulation is viscous, the exposure concentrations in this study were calculated on the basis of mass of Corexit™ (g L^{-1}) added to experimental tanks. The Corexit^{TM} being soluble in sea water (Nalco Environmental Solutions, 2014), the homogenisation in the tanks was driven by seawater movements, which was induced by the air addition without co-solvent. To verify the homogeneity of the Corexit^{TM} concentration, phytoplankton cells were added in tanks and different parts of the tanks were sampled to count cells. Since the count results were similar in the different areas of the tanks, it can be reasonably assumed that Corexit^{TM} was also homogeneously distributed in the tanks.

1.2.3.2 Studied bivalves

Adult blue mussels were obtained from mussel aquaculture leases (46°25.963 N; 62°39.914 W) in Prince Edward Island, Canada (Tremblay *et al.*, 2011; Moreau *et al.*, 2005). Adult giant scallops were collected from a shellfish aquaculture lease (48°39.658 N; 65°64.289 W), located in Gaspé Bay, Québec, Canada. For each species, shell size was of commercial range and shell length variability was minimized (mussels: 56.46 ± 3.05 mm; scallops: 82.23 ± 2.51 mm; means ± SE) in order to compare among individuals of the same cohort.

1.2.3.3 Experimental design

a) *BIVALVE ACCLIMATATION*

One hundred individuals from each species were transported on ice to the UQAR-ISMER marine laboratory in Pointe-au-Père, Qc, Canada. Bivalves were maintained in four flow-through thermo-regulated tanks of 200 L each at 4°C, a natural early or late winter temperature, for four weeks, in order to acclimate the bivalves prior to the oil dispersant exposure experiment. Since the mussels originated from Prince Edward Island under sub-arctic conditions, their metabolism was already acclimated to such cold conditions. Their clearance rates show a significant correlation with temperature between 0 to 12ºC, with values over 4 L hr^{-1} in winter conditions. Salinity was 28 ± 1 ppt and did not fluctuate during all experiments. Bivalves were fed three times each week with a suspension of mixed microalgae *Tisochrysis lutea* (CCMP 1324)*, Chaetoceros neogracilis* (CCMP 1317)*, Pavlova lutheri* (CCMP 459) and *Nannochloropsis occulata* (CCMP 525) at a rate of 30 000 cells ind^{-1} d^{-1}. Strains were obtained from the Center for Culture of Marine Phytoplankton (CCMP), Bigelow Laboratory for Ocean Sciences (West Boothbay Harbor, ME, USA) and

cultivated with the middle of culture F/2 without silica except for *C. neogracilis*, which was cultivated with the F/2 with silica (Guillard, 1975).

b) *EXPOSURE TO OIL DISPERSANT*

Eight mussels and eight scallops were distributed among eight static tanks of 75 L (Figure 15a), with one mussel and one scallop per tank. Conical tanks were used with low aeration in the bottom to maintain oxygenation and minimize perturbations of bivalve behaviour. Bivalves were placed in the top quarter of the tank on a raised perforated platform, far from the direct effect of bubbling. Two tanks were used for each of the four oil dispersant concentrations tested (0, 0.01, 0.05 and 0.25 g L^{-1}). The Lower concentration (0.01 g L^{-1}) correspond to the first index of the GESAMP (Group of Expert on Scientific Aspect of Marine Environmental Protection) classification. The higher concentration correspond to the Lower Observed Effect Concentration for Scarlett et al., (2005). For the control tanks without oil dispersant, a corresponding volume of seawater was added to simulate the addition of oil dispersant. A cooling system was added in each tank to maintain temperatures near 4°C (3.86 ± 0.7°C), corresponding to early or late winter conditions in the Gulf of St Lawrence's coastal areas.

c) *VALVE OPENING MONITORING*

Valve opening was measured by wiring the bivalves to a non-invasive valvometry system, as described in Nagai *et al.* (2006). A detailed description of the operating principle and sensor attachment is provided in Clements *et al.* (2020) and Comeau *et al.* (2019). A Hall element and a small magnet (4.8 mm diameter, 0.8 mm height; 0.1 g) were glued on the right and left valves using a cyanoacrylate super glue, which polymerized rapidly. Once attached to the valves, the magnetic field (flux density) between the Hall sensor and magnet is a function of the gap between the two valves. This field was recorded in the form of output voltage by dynamic strain recording devices (DC-204R, Tokyo Sokki Kenkyujo Co., Japan). For each individual bivalve, the signal was recorded at a frequency of 10 Hz to allow the calculation of various indicators and to detect subtle changes in gaping behaviour.

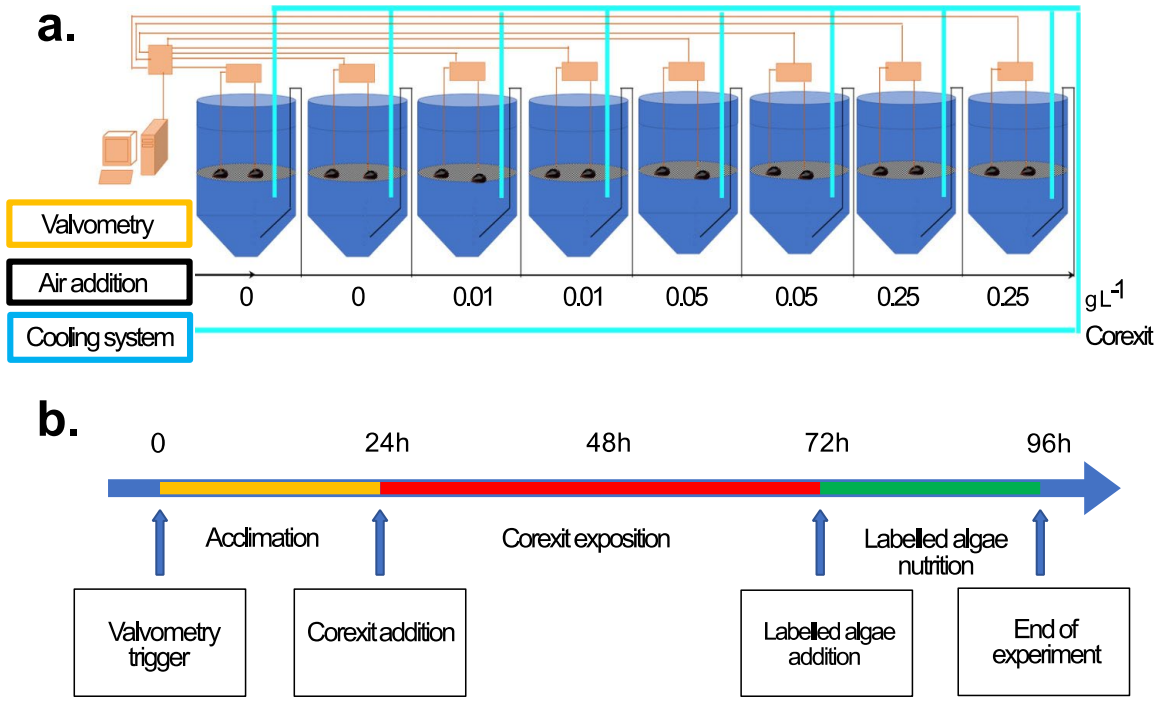

Figure 15 : Experimental design (a), experimental timeline (b)

d) *EXPERIMENT CHRONOLOGY*

The bivalves were acclimated for 24 hours in filtered seawater at 1 µm without oil dispersant, and baseline gaping behaviour was measured for each individual. Following this 24-h acclimation phase, the different oil dispersant concentrations and seawater for the control were added with the objective to obtain the target concentrations (0, 0.01, 0.05 and 0.25 g L^{-1}) in the tanks and bivalve behaviour was monitored for 48 hours. 24 hours following the addition of the oil dispersant, 120 000 cells ml^{-1} of *Pavlova lutheri* enriched with ^{13}C (Leblanc *et al*., 2012) was added to each tank, including the control (Figure 15b). These ^{13}C-labelled *P. lutheri* cells were used to validate food ingestion by the bivalves, as described below. Ammonia concentration in each tank was monitored daily with a Lamotte Smart2 colorimeter (LaMotte, Maryland, USA). Valvometry recording devices were stopped 96 hours after the beginning of the experiment, at which point a voltage-shell opening relationship was derived by sequentially inserting a series of spacers of known sizes between the two valves, thus yielding for each individual, an equation for converting raw voltage recordings of mV, to millimetres of valve opening or gape. To maximize the number of replicates, the entire experiment was repeated seven times, yielding a total of 56 mussels and 56 scallops (n = 14 mussels and 14 scallops per treatment).

e) *LABELLED ALGAE*

Each individual was dissected to assess the quantity of ^{13}C incorporated in their digestive gland. The ^{13}C measurements were acquired with a continuous-flow Isotope Radio Mass Spectrometer (CF-IRMS, Deltaplus XP mass spectrometer coupled to an elemental analysis COSTECH, Thermoscientific) on the previously lyophilised digestive gland.

The ^{13}C enrichment in percent of the nominal carbon mass in the mussels and scallops was measured in %atom^{13}C, the conversion between this unit and the usual delta notation was made with the following formula (Larsson *et al*., 2015):

$$\%atom^{13}C = 100 \times \frac{\left(\frac{\delta^{13}C}{1000} + 1\right) \times \left(\frac{^{13}C}{^{12}C}\right)_{VPDB}}{1 + \left(\frac{\delta^{13}C}{1000} + 1\right) \times \left(\frac{^{13}C}{^{12}C}\right)_{VPDB}}$$

Typically, under natural conditions, the ^{13}C ratio is comprised of between 1 and 1.1 %atom^{13}C. The enriched algae used exhibited values of 99 %atom^{13}C. If bivalves ingested food particles after being exposed to the oil dispersant, their ^{13}C ratio was greater than 1.1 %atom^{13}C, whereas bivalves inhibiting food ingestion through valve closure exhibited values similar to natural conditions. As feeding rate is related to bivalve size, the calculated ^{13}C ratios were normalized with the mass of each individual.

1.2.3.4 Valvometry and data treatment

CSV files were extracted from the valvometry hardware and were analysed using R (version 3.6.1) with an automated procedure made necessary by the large amount of data generated. The data management maintained all available information (date, hours, number of measurements), and using the following formula coupled with valve gape in millimetres, Valve Opening Amplitude (VOA) was calculated:

VOA (%) = ((*opening* (mm) – *min* (mm)) / (*max* (mm) – *min* (mm)) x 100

The maximum and the minimum were calculated from the duration of the experiment to be able to compare the VOA variation of the different experimental periods. VOA was used to determine valve closure (VOA values below 10%), micro closure (3% of closure in one second as in Comeau *et al*. (2019)) and calculation of indicators characterizing the gaping behaviour of each bivalve (number of closures, total duration of closure, average closure duration and the number of micro closures). The pulse response to oil dispersant for each individual was measured graphically. Using the R software function “Locator”, each point on the graph was obtained and used to determine the exact time and duration of the closure after the addition of the oil dispersant. Closure speed, representing the response time,

expressed in percent closed per second were compared between treatments and species. Each graph was visually observed to identify death (mussels: n=3, scallops: n=12) or valvometry error (magnet or sensor lost – mussels: n=6, scallops: n=8, power cut). Oil dispersant and microalgae addition were programmed to be identified directly on the graph to facilitate observation of events related to these experimental periods.

1.2.3.5 Statistical analysis

Correlations between oil dispersant concentration in the tanks and each bivalve behavioural indicator (number of closures, average VOA, total duration of closure, average closure duration, number of micro closures and response time) were obtained using R (version 3.6.1; R Core Team, 2019). Non-parametric Pearson correlations were used, as data were not normally distributed according to the Shapiro-Wilk test. A multivariate analysis using Principal Component Analysis (PCA; package ade4; Dray and Dufour, 2007) was used to visualize which behavioural indicator variables contributed most to the effect of oil dispersant concentration variation. The behavioural indicators selected were analysed by multivariate Permutational analyses of variance (PERMANOVA – package vegan; Oksanen *et al.*, 2019) on Euclidean distance. If significant, *a posteriori* comparisons were completed using a PERMANOVA pairwise test to identify differences between oil dispersant concentrations that induced change in the gaping behaviour of *M. edulis* and *P. magellanicus* (Martinez Arbizu, 2019). PERMANOVA was preferred to ANOVA, as it can be used on the non-normal data obtained in this experiment. For dead animals, only the response time has been tested following treatment addition. Finally, a cluster analysis by permutation using SIMPROF was completed on the behavioural indicators identified by the PCA to define groups of oil dispersant concentrations showing similar impact on the gaping behaviour of mussels and scallops.

To compare the enriched algae ingested by the bivalves exposed to different oil dispersant concentrations, PERMANOVA was used, as data showed heteroscedasticity through a

Shapiro-Wilk test. This was followed by a PERMANOVA pairwise test to determine differences between concentrations.

1.2.4 Results:

1.2.4.1 Behaviour monitoring

VOA in relation to exposure time of different oil dispersant concentrations of a representative individual is presented in Figure 16. The dotted line corresponds to the oil dispersant and the solid line to the ^{13}C enriched microalgae pulses in the tanks. For the control tanks without oil dispersant, no behavioural changes were observed, and these graphs were used as reference to reveal behaviour events linked to oil dispersant exposure in the experimental bivalves. Furthermore, for each bivalve, the period before the addition of the oil dispersant was also used as a reference.

Significant VOA drops without full closure were observed in mussels immediately after the addition of a low quantity of dispersant into the tank (0.01 g L^{-1}). Following the addition of ^{13}C enriched microalgae, VOA returned to a reference level recorded before the oil dispersant addition. At higher oil dispersant concentrations (0.05 g L^{-1}), an important first valve closure of mussels was observed after the addition of the oil dispersant, followed by several opening and closure movements. Contrary to behaviour observed when mussels were exposed to 0.01 g L^{-1} of oil dispersant, VOA remained below the reference level after addition of ^{13}C microalgae. At the highest oil dispersant concentration (0.25 g L^{-1}), similar large initial valve closures were recorded after addition of the oil dispersant, but mussels remained fully closed until the end of the experiment, with occasional brief re-openings. Thus, in *M. edulis*, results exhibit a gradual increase of major behavioural events with increasing oil dispersant concentration.

Scallops displayed specific behaviours also characterized by an important first valve closure when 0.01 g L^{-1} of oil dispersant was added, followed by VOA measurements similar

to a reference level after only a few hours. At an oil dispersant concentration of 0.05 g L^{-1}, the closure duration was noticeably extended, followed by large openings with few gaping movements. No mortality was observed at this concentration. At the highest dispersant concentration of 0.25 g L^{-1}, similar rapid and extended closures were observed, followed by very fast gaping movements of large amplitude (between 0 and 98% VOA), ultimately resulting in death of all scallops after some hours. With the same concentration no death was observed with mussels. Similar to the behaviour of mussels, oil dispersant exposure induced major changes in the gaping behaviour of scallops. Unlike mussels, however, the highest dispersant concentration was fatal for scallops.

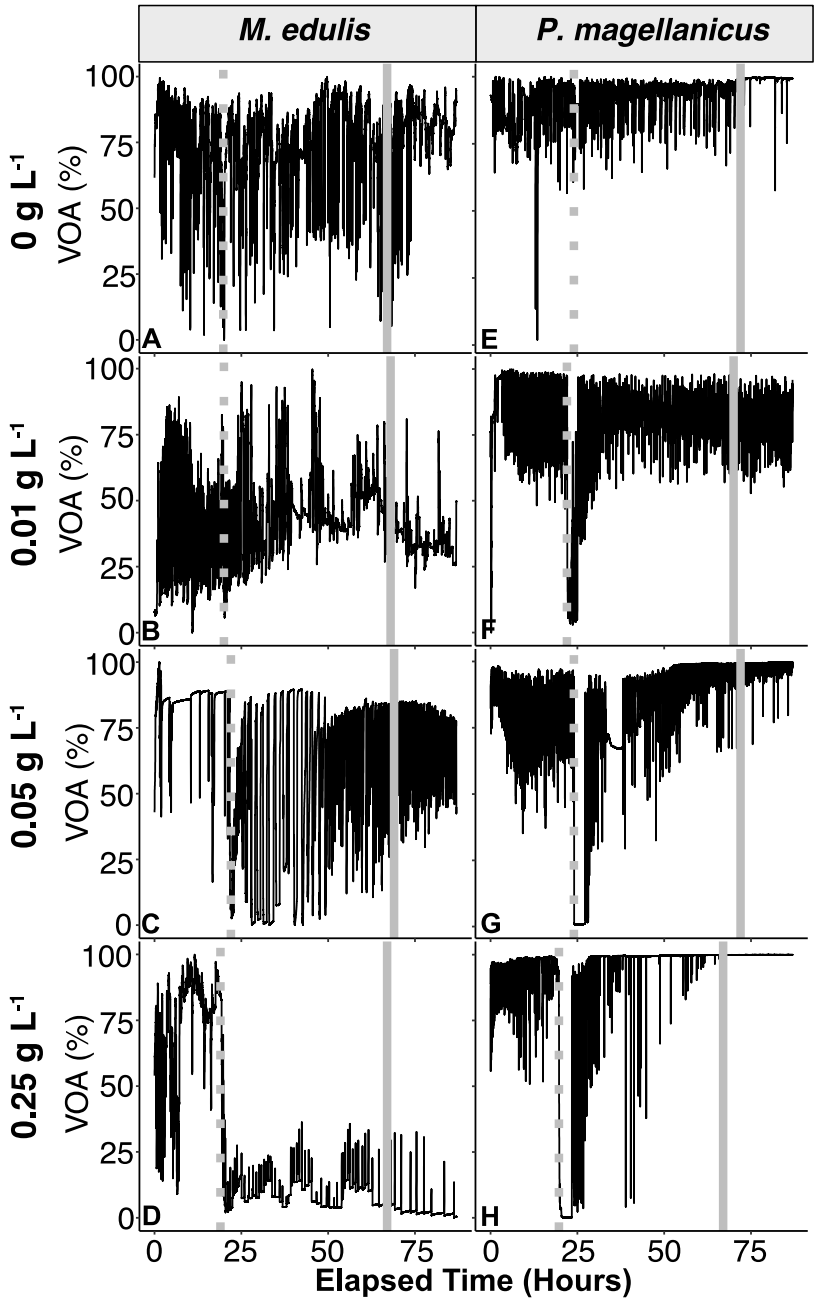

Figure 16 : Valve Opening Amplitude (VOA) of *M. edulis* (A, B, C, D) and *P. magellanicus* (E, F, G, H) in relation to exposure time to Corexit™ at different concentrations (0, 0.01, 0.05, 0.25 g L^{-1}). The dashed grey line signifies the time of Corexit™ addition, while the solid grey line signifies time of algae addition.

1.2.4.2 Behavioural indicators correlation

Behavioural indicators of mussels and scallops (number of closures, VOA, total duration of closure, average closure duration and the number of micro closures) following exposure to oil dispersant and ^{13}C microalgae are presented in Figures 17 and 18, respectively. For mussels, positive correlations were observed for the majority of behavioural indicators (number of closures, total closure duration and average closure duration) with oil dispersant concentration, while correlations with VOA were negative. For these four behavioural indicators, the correlations remained significant after the ^{13}C microalgae addition, but with a smaller rho value compared to correlations during the non-feeding phase (oil dispersant only). In the case of micro closures, no correlations were observed with oil dispersant concentrations alone, but negative correlations appeared when ^{13}C microalgae were added 24-h after the oil dispersant. Response time showed no correlation with oil dispersant concentration, with or without the addition of ^{13}C microalgae. Scallops displayed no significant correlations between any of the behavioural indicators and oil dispersant concentrations, regardless of the presence of ^{13}C microalgae.

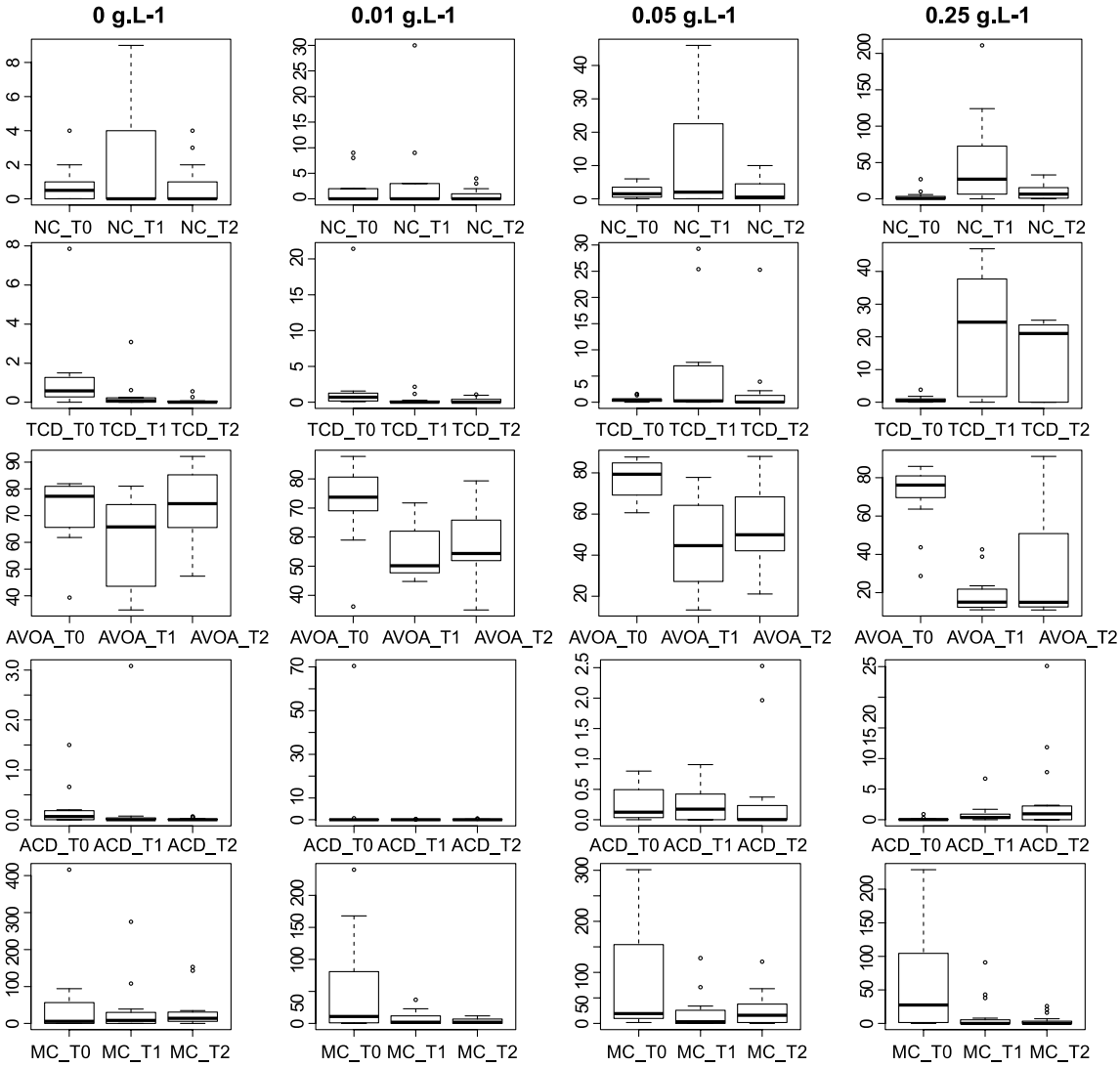

Figure 17 : Boxplot of the variability in behavioural parameters of *M. edulis* when exposed to each Corexit™ exposure concentration. NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average VOA. MC: Micro Closure.

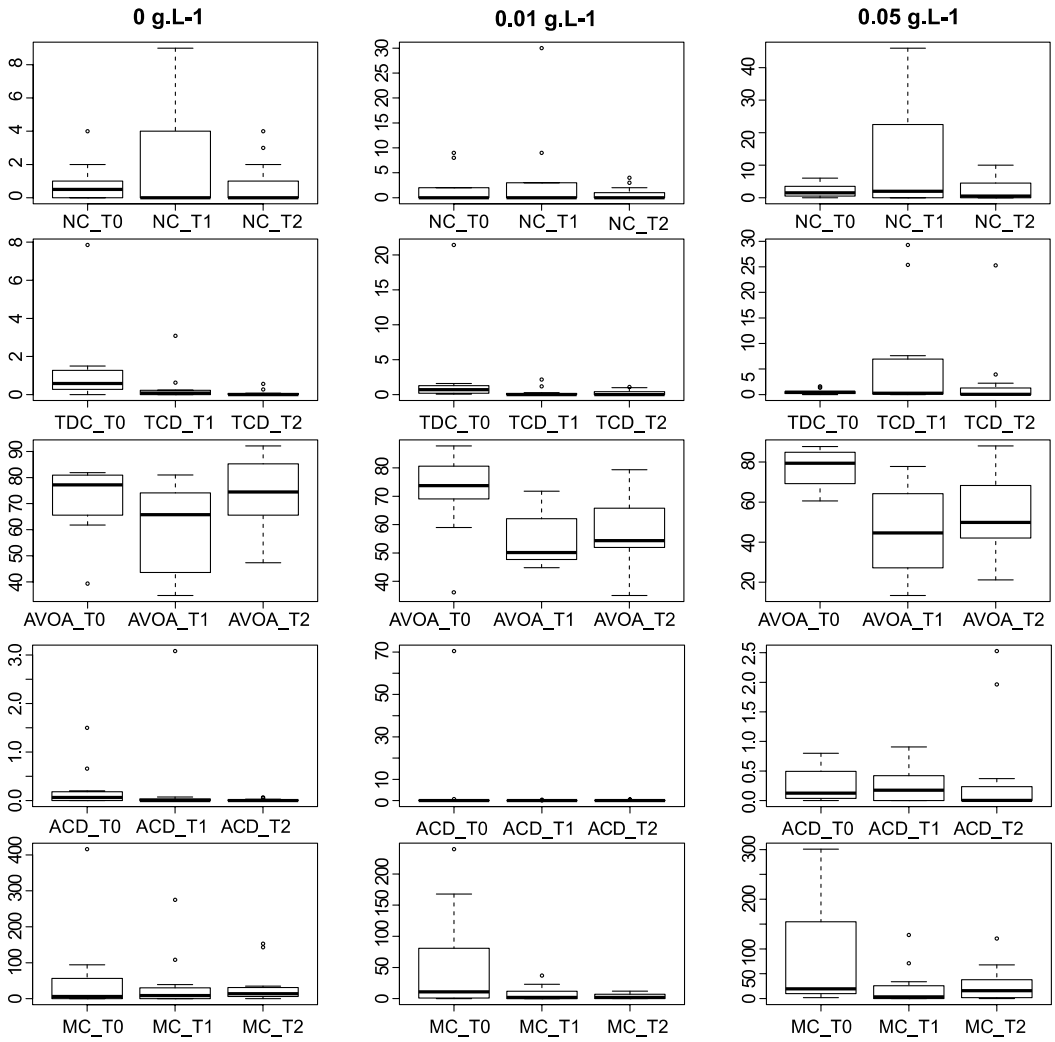

Figure 18 : Boxplot of the variability in behavioural parameters of *P. magellanicus* when exposed to each Oil dispersant exposure concentration. NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average VOA. MC: Micro Closure. No data from the 0.25 g L^{-1} exposure were included in the analysis as this concentration was fatal for all individuals.

1.2.4.3 Selection of behavioural indicators

As mussels displayed various responses when exposed to different oil dispersant concentrations, their behavioural indicators were analysed with a PCA to identify which indicator(s) contributed more substantially to the differences observed. A correlation circle is depicted around all behavioural indicators (Figure 19) for the oil dispersant alone (T1) or combined with ^{13}C microalgae (T2). For each indicator on the correlation circle, the position of T1 and T2 were close, suggesting that they covary, and can therefore be studied together. The oil dispersant concentration was near the horizontal axis of the correlation circle. For each behavioural indicator, the closer its position was to oil dispersant concentration and the correlation circle, the more it contributed to the correlation. The two average closure durations showed weak contributions, given their close position to the vertical axis. Similar outcomes were observed for micro closures, as their positions were relatively far from the correlation circle and thus from a correlational association to oil dispersant concentrations. By contrast, strong positive correlations with oil dispersant concentrations were observed for the number of closures and the total closure duration. The position of the VOAs falling at the opposite side of the oil dispersant concentrations, along the horizontal axis and in close proximity to the circle, indicated negative and strong relationships with oil dispersant concentrations. Overall, these results corroborate those from Table 1, except for the average closure duration. As all Pearson's correlations were non-significant for scallops, no PCA was applied for this species.

Tableau 1 : Pearson correlations between valve movement behaviour of *M. edulis* and *P. magellanicus* with oil dispersant exposure concentration and the combined effect of oil dispersant concentration and 13C enriched microalgae exposure. NC: Number of closures. TDC: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average Valve Opening Amplitude. MC: Micro Closure. SC: Speed of Closure. In bold: significant correlations for $\alpha = 0.05$.

	M. edulis				*P. magellanicus*			
	Corexit™		Corexit™+algae		Corexit™		Corexit™+algae	
	rho	P-value	rho	P-value	rho	P-value	rho	P-value
NC	**0.577**	**<0.0001**	**0.498**	**0.0001**	0.224	0.252	0.129	0.512
TCD	**0.621**	**<0.0001**	**0.507**	**0.0001**	0.244	0.210	0.225	0.249
ACD	**0.562**	**<0.0001**	**0.493**	**<0.0001**	0.257	0.186	0.307	0.111
AVOA	**-0.714**	**<0.0001**	**-0.617**	**<0.0001**	-0.067	0.733	0.095	0.628
MC	-0.207	0.135	**-0.379**	**0.005**	-0.104	0.6	0.158	0.421
SC	-0.024	0.887	-	-	0.251	0.173	-	-

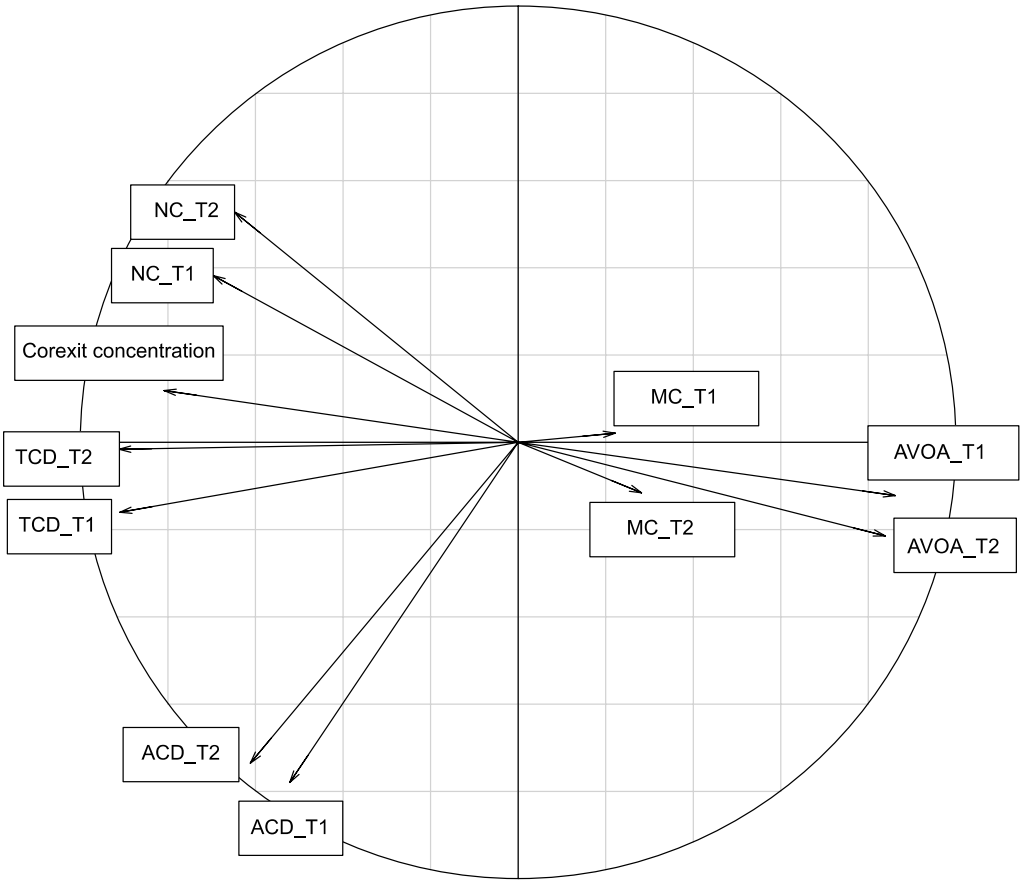

Figure 19 : Correlation circle derived from the Principal Component Analysis for *M. edulis*. NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average VOA MC: Micro Closure. T1: Corexit™ single. T2: Corexit™ with 13C marked algae

1.2.4.4 Behavioural impacts of different oil dispersant concentrations

A multivariate PERMANOVA analysis integrating the three behavioural indicators demonstrated an effect of oil dispersant addition on mussels (DF = 1 and 51, Pseudo-*F* = 27.645, $P_{(MC)}$ = <0.0001). The PERMANOVA pairwise comparison test indicated differences between oil dispersant concentration 0 to 0.05 g L^{-1} and 0.25 g L^{-1}. The cluster analysis and dendrogram following the SIMPROF procedure (Figure 20) indicated the presence of two distinct clusters: one group for concentration below 0.25 g L^{-1} and the other for the higher concentration of 0.25 g L^{-1}. The univariate PERMANOVA on mussel behaviour indicated correlation with the level of oil dispersant concentration, closure number, total closure duration and average VOA. We observed an effect of oil dispersant exposure on the increase of the number of closures and the total closure duration, as well as a decrease of the average VOA at the highest concentration (0.25 g L^{-1}) of oil dispersant. The values for the closure number at oil dispersant exposure of 0.05 g L^{-1} were in the group with the low oil dispersant concentrations.

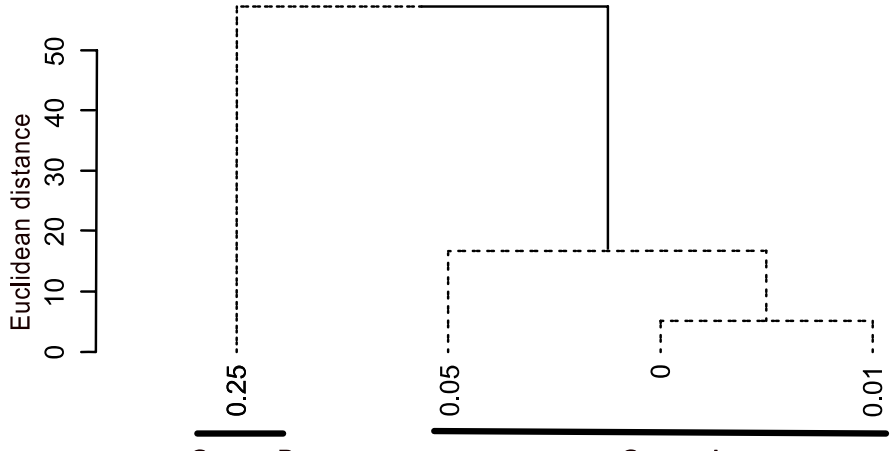

Figure 20 : Cluster analysis grouping Corexit™ concentration with behavioural parameters of mussels using the SIMPROF method.

1.2.4.5 Enriched algae

The ^{13}C algae was used to evaluate the potential ingestion of food particles following oil dispersant exposure. Results demonstrated a significant difference in amounts of ^{13}C absorbed in mussels exposed to different oil dispersant concentrations (PERMANOVA: DF = 1 and 47, Pseudo-*F* = 5.5947, $P_{(MC)}$ = 0.0186). We detected ^{13}C enrichment only in the mussels subjected to the control treatment (Figure 21a), which was an indication of algae ingestion. The ^{13}C rates in mussels exposed to all oil dispersant concentrations indicated no ingestion of enriched algae. ^{13}C enrichment in scallops was marginally non-significant (PERMANOVA: DF = 1 and 23, Pseudo-*F* = 1.9546, $P_{(MC)}$ = 0.0885). While mean values were near the natural observed levels, it seems that some individuals in the control effectively ingested enriched algae, as their ^{13}C values were noticeably higher than natural levels.

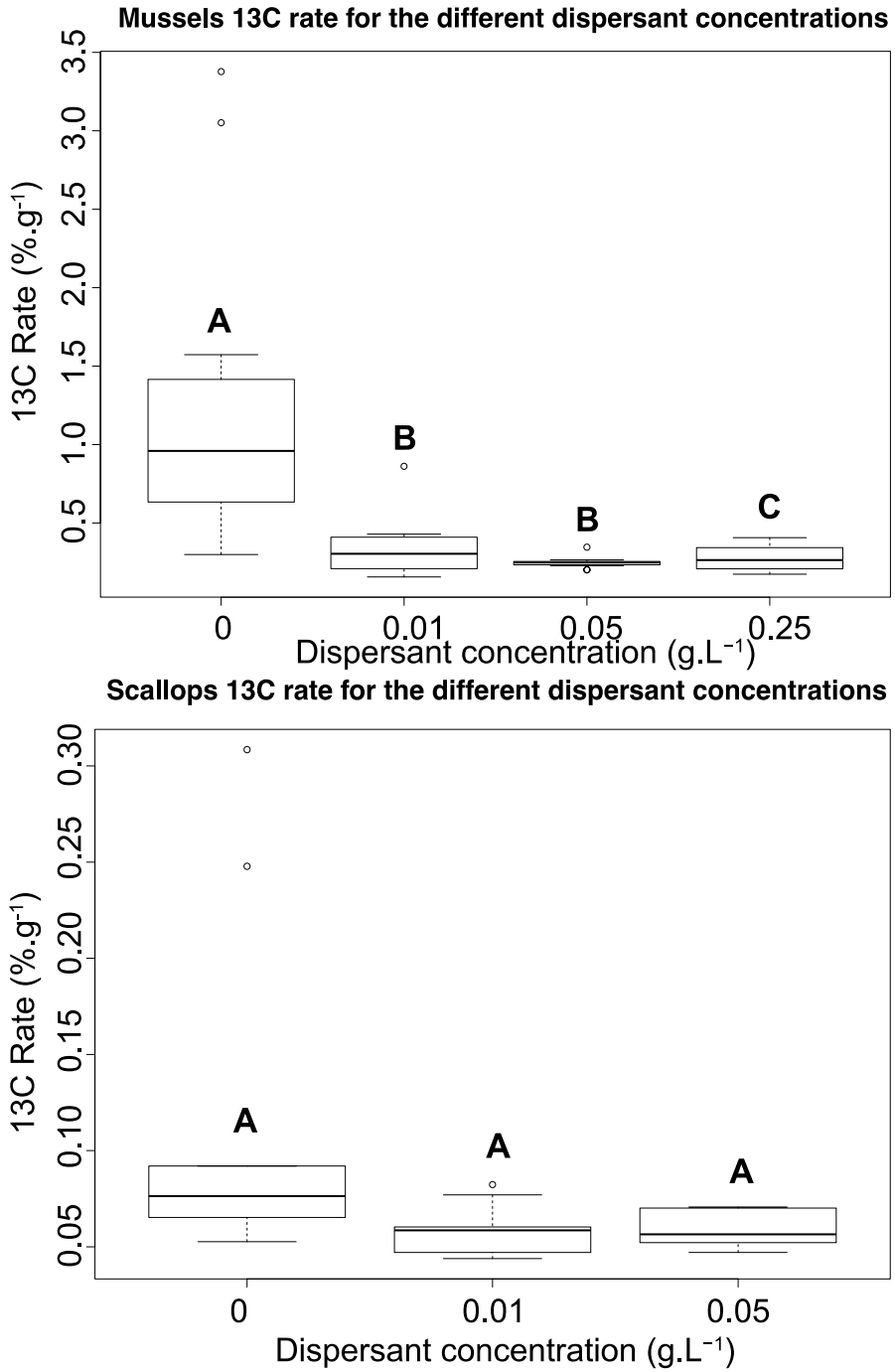

Figure 21 : Boxplots summarizing the 13C rate in mussels (a) and scallops (b) for each of the levels of Corexit™ concentration. Different letters indicate significant differences between groups ($p < 0.05$).

1.2.5 Discussion

1.2.5.1 Behaviour change

For both investigated species, *M. edulis* and *P. magellanicus*, the sudden addition of the oil dispersant (Corexit™) into the experimental environment immediately induced a series of gaping movements (e.g., closures). This behavioural response was observed for all individuals, indicating the perturbing nature of the oil dispersant on bivalves. External experimental manipulations or water movement associated to the addition of oil dispersant into the experimental tanks cannot be used to explain these responses, as water addition in the control tanks induced no gaping movements. Recovery speed from initial gaping varied between species and oil dispersant concentrations. In bivalves, valves closure behaviours are viewed as a defence mechanism in response to stressful conditions (Kramer and Botterweg, 1991). Therefore, it could be concluded that stress levels increased alongside oil dispersant concentrations, as the highest concentration (0.25 g L^{-1}) induced persistent closures in mussels, and frequent gaping movements of very large amplitudes in scallops. Such atypical behaviours in scallops are generally associated with escape responses triggered by stressful events and predators (Cheng and DeMont, 1996; Guderley and Tremblay, 2013), given that they can swiftly relocate by repeatedly clapping their valves. Unfortunately, the scallops had no escape possibilities in our experimental tanks, which led to the mortality of all individuals over the 72-h 0.25 g L^{-1} oil dispersant concentration exposure period. In contrast to mussels, which are adapted to emersion in intertidal habitats, the scallop *P. magellanicus* is a subtidal species and lacks the ability to maintain shell closure due to their morphology with spacing between the valves. Overall, these behaviours suggest a substantial toxic effect of oil dispersant, namely at concentration > 0.25 g L^{-1} over a timescale of hours. By maintaining valve closure, mussels isolated themselves from the contaminated environment, thus presumably limiting the accumulation of the toxin in their tissues. Nonetheless, mussels partially opened their valves when exposed to intermediate concentrations of oil dispersant,

a behaviour which could explain the mussels toxicity results, higher with an exposition to lower oil dispersant concentration than higher reported in Scarlett *et al.* (2005).

1.2.5.2 Behavioural indicators

Correlations between oil dispersant concentration and behavioural indicators were generally significant in mussels, except for micro closures and response time. In the presence of the oil dispersant concentration, mussels closed their valves over time spans proportional to oil dispersant concentration, with an increasing time spent closed with increasing concentration. During these extended closure periods, mussels frequently reopened their valves, with an amplitude intensity directly proportional to oil dispersant concentration; reopening amplitudes were relatively small at the highest oil dispersant concentration. We suggest that such re-openings allowed a periodic "testing" of their environment and water quality. These frequent re-openings concurrently increased the frequency of closures. It was, however, the total closure duration (sum of all closure durations over the oil dispersant concentration exposure phase) that was the most correlated with oil dispersant concentration. This behaviour was most frequent at the highest oil dispersant concentration, suggesting a gradual isolation of mussels from their environment as oil dispersant concentration increased. A similar trend was observed with the VOA of mussels. This particular indicator inherently excludes the frequent valve movements, with its computation yielding instead a coarse indication of opening state. In the final phase of the experiment, the addition of labelled microalgae curtailed all correlations with behaviour indicators. The weaker, but still significant, correlations could be related to the presence of food stimulating filtration processes, and thereby confounding the oil dispersant concentration effect. However, actual ingestion of the microalgae was minimal as ^{13}C levels in mussel tissues were statistically similar to natural ^{13}C levels; it therefore appears that the lack of ingestion was due the presence of oil dispersant, regardless of concentration. Hence, we suggest that while food

can stimulate the opening of mussels in the presence of an oil dispersant, physiological processes are negatively impacted.

With respect to scallops, we offer three interpretations for the lack of correlation between the valve behaviour indicators and oil dispersant concentration. Firstly, the outcome may be related to the regulation of the scallop's filtration capacity by the thickness at the edge of the mantle, as described in Tran *et al*. (2020). More precisely, the mantle edge regulates water flow in the paleal cavity and the subsequent food uptake in the scallop. This self-regulating process can occur with minimal valve movement, such that our valvometry approach may have been inadequate for detecting responses, with the exception of the notable closure event that immediately followed the addition of oil dispersant. Filming the mantle edge movements in the presence of oil dispersant or other dispersants could potentially reveal finer scale behavioural responses. Secondly, it is possible that this particular scallop species reacts only to sudden, abrupt changes in their environment, such as in our experiment when oil dispersant was first applied to our tank water. Finally, the lack of correlation between oil dispersant and valve behaviour indicators in scallops may be attributable to our experimental design containing only three levels of oil dispersant concentrations, and the mortality of all individuals at the highest concentration. The reason for this mortality event is unclear. ^{13}C enrichments in scallop tissues indicated no food ingestion for all treatments, including the control.

Another investigated behaviour for both species was the response time to the closure immediately following oil dispersant addition into the tanks, including the highest concentration. This response was not correlated to oil dispersant concentration, suggesting it is modulated by other factors. It varied between treatments, with increasing oil dispersant concentration seemingly lowering the relevance of this indicator.

The effects of oil dispersant concentration on the number of closures, the total closure duration, and the average closure duration are hereby discussed for mussels only, as no correlations were observed in scallops. It was found that only the highest Corexit™ concentration (0.25 g L^{-1}) induced a significant effect on these indicators for mussels,

comparatively to controls. This outcome suggests the existence of a threshold concentration (between 0.05-0.25 g L^{-1}), which possibly explains why mussels were reportedly contaminated at low (< 0.2 g L^{-1}) oil dispersant concentrations (Scarlett *et al.* 2005). In our study, the behavioural shift at high oil dispersant concentration was manifested through reduced VOA, increased closure frequency, and augmented total closure duration. These changes in behaviour curtailed feeding processes as indicated by a lack of ^{13}C enrichment in the digestive glands of mussels exposed to oil dispersant and labelled microalgae. Clearance rate is defined as the volume of water cleared of suspended particles per unit time (l h^{-1}). In optimal environmental conditions, the mussel's clearance rate is generally maximal, allowing food ingestion in the digestive gland (Thorin *et al*., 1998; Riisgård *et al*., 2011). Under stressful conditions, however, mussels first respond by closing their valves (Borcherding, 2006), thus isolating themselves from the external environment (Rajagopal *et al*., 1997). A reduction in clearance rate is generally observed during a stressful event, such as an exposure to chemical toxins (Howell *et al*., 1984), toxic algae (Widdows *et al*., 1979; Binzer *et al.*, 2018; Nielsen *et al.*, 2020), predators (Dzieryńska-Białończyk et al., 2019), abiotic disturbances (Davenport and Manley, 1978), and food concentrations below starvation levels (Newell *et al*., 2001; Pascoe *et al*., 2009; Riisgård *et al*., 2011). In this study, the lack of ^{13}C enrichment in the digestive glands of mussels is indicative of feeding cessation. In contrast with Eastern oysters (*Crassostrea virginica*) (Comeau *et al*., 2012), mussels display multiple opening behaviours, even at low temperatures. Low temperatures decreased food filtration and ingestion rate, but these rates were far from zero for mussels and scallops (Cranford and Hill, 1999; Cusson *et al*., 2005).

This study showed that frequent gaping measurements on mussels and scallops allows the detection of changes in water quality. It is therefore possible to imagine valvometry as a tool in future environmental assessments, especially considering emerging technical advancements in regard to real-time monitoring (Andrewartha and Elliott, 2015; Borcherding, 2006; Kramer and Foekema, 2001). Mussels seem particularly suitable for such monitoring, since it appears their persistent valve closure allows them to survive lethal contaminant concentrations while concomitantly signalling an abnormal behaviour.

1.2.6 Conclusion

Our application of valvometry to monitor valve movements of *M. edulis* and *P. magellanicus* enabled the detection of behavioural responses related to the presence of an oil dispersant. When oil dispersant was added, the two species showed rapid and pronounced valve closures. This prompt closure reaction seems to be a defence mechanism to isolate themselves from a contaminated environment. However, the response time could not be used as an indicator of oil dispersant concentration. After this initial closure, the mussels survived, mostly remaining closed and limiting their exposure to oil dispersant; the integrated closure duration or avoidance behaviour was directly proportional to the oil dispersant concentration in the water. Scallops showed a different response, one dominated by substantial clapping movements, presumably as an attempt to escape the toxic environment, which was impossible under the experimental conditions, thus resulting in the death of all individuals in the highest concentration of dispersant. Overall, the behavioural reaction of the two species, coupled with the high mortality observed in scallops, suggests a significant toxic impact of this compound on bivalves. As some of the behavioural indicators were correlated to the oil dispersant concentration, their integration in ecotoxicological monitoring programs may be useful for detecting the presence of the toxins and their potential ecological impacts.

Author contribution

Study design: Richard Saint-Louis, Réjean Tremblay, Jean-Bruno Nadalini; laboratory work: Jean-Bruno Nadalini, Bertrand Genard; data treatment: Guillaume Durier, Jean-Bruno Nadalini; interpretation: Guillaume Durier, Réjean Tremblay, Richard Saint-Louis, Luc Comeau; manuscript writing: Guillaume Durier

Funding

This project was undertaken with the financial support of the Government of Canada, specifically by Environment and Climate Change Canada provided by RT's (FDE-CA-2017i002). Fisheries and Oceans Canada provided in-kind assistance.

Acknowledgment

The authors thank the scientific group Ressources Aquatique du Québec for their implication in this study. This is also a contribution of the scientific group EcotoQ. The authors are also grateful to Luke Poirier and Jeff Clements for reviewing a draft version of the paper.

1.2.7 References

Andrade, H., Massabuau, J.-C., Cochrane, S., Ciret, P., Tran, D., Sow, M., Camus, L., 2016. High Frequency Non-invasive (HFNI) Bio-Sensors as a potential tool for marine monitoring and assessments. Frontier in Marine Science 3, 1–10. https://doi.org/10.3389/fmars.2016.00187

Andrewartha, S., Elliott, N., 2015. Aquaculture sentinels: Smart-farming with biosensor equipped stock. Journal of Aquaculture Research and Developpment. 07, 1–4. https://doi.org/10.4172/2155-9546.1000393

Binzer, S.B., Lundgreen, R.B.C., Berge, T., Hansen, P.J., Vismann, B., 2018. The blue mussel *Mytilus edulis* is vulnerable to the toxic dinoflagellate *Karlodinium armiger*—*Adult* filtration is inhibited and several life stages killed. PLoS One 13, 1–16. https://doi.org/10.1371/journal.pone.0199306

Borcherding, J., 2006. Ten years of practical experience with the *dreissena*-monitor, a biological early warning system for continuous water quality monitoring. Hydrobiologia 556, 417–426. https://doi.org/10.1007/s10750-005-1203-4

Cheng, J.Y., DeMont, M.E., 1996. Jet-propelled swimming in scallops: Swimming mechanics and ontogenic scaling. Canadian Jounal Zoology 74, 1734–1748. https://doi.org/10.1139/z96-192

Clements, J.C., Poirier, L.A., Pérez, F.F., Comeau, L.A., Babarro, J.M.F., 2020. Behavioural responses to predators in Mediterranean mussels (*Mytilus galloprovincialis*) are unaffected by elevated pCO2. Marine Environmental Research. 161. https://doi.org/10.1016/j.marenvres.2020.105148

Comeau, L.A., Babarro, J.M.F., Riobó, P., Scarratt, M., Starr, M., Tremblay, R., 2019. PSP-producing dinoflagellate *Alexandrium minutum* induces valve microclosures in the mussel *Mytilus galloprovincialis*. Aquaculture 500, 407–413. https://doi.org/10.1016/j.aquaculture.2018.10.025

Comeau, L.A., Mayrand, É., Mallet, A., 2012. Winter quiescence and spring awakening of the Eastern oyster *Crassostrea virginica* at its northernmost distribution limit. Marine Biology. 159, 2269–2279. https://doi.org/10.1007/s00227-012-2012-8

Cranford, P.J., Hill, P.S., 1999. Seasonal variation in food utilization by the suspension-feeding bivalve molluscs *Mytilus edulis* and *Placopecten magellanicus*. Marine Ecology Progress Series. 190, 223–239. https://doi.org/10.3354/meps190223

Cusson, M., Tremblay, R., Daigle, G., Roussy, M., 2005. Modeling the depuration potential of blue mussels (*Mytilus* spp.) in response to thermal shock. Aquaculture 250, 183–193. https://doi.org/10.1016/j.aquaculture.2005.03.045

Davenport, J., Manley, A., 1978. The detection of heightened sea-water cooper concentration by mussels *Mytilus edulis*. Journal of Marine Biology. 58, 843–850.

Dray, S., Dufour, A.B., 2007. The ade4 package: Implementing the duality diagram for ecologists. Journal of Statistical Software. 22, 1–20. https://doi.org/10.18637/jss.v022.i04

Dzierżyńska-Białończyk, A., Jermacz, Ł., Zielska, J., Kobak, J., 2019. What scares a mussel? Changes in valve movement pattern as an immediate response of a byssate bivalve to biotic factors. Hydrobiologia 841, 65–77. https://doi.org/10.1007/s10750-019-04007-0

George-Ares, A., Clark, J.R., 2000. Aquatic toxicity of two Corexit® dispersants. Chemosphere 40, 897–906. https://doi.org/10.1016/S0045-6535(99)00498-1

Guderley, H.E., Tremblay, I., 2013. Escape responses by jet propulsion in scallops. Canadian Journal of Zoology. 91, 420-430 dx.doi.org/10.1139/cjz-2013-0004

Guillard, R. R. (1975). Culture of phytoplankton for feeding marine invertebrates. In Culture of marine invertebrate animals, Smith, W.L., Chanley, M.A(pp. 29-60). Plenum Press, New York

Howell, R., Grant, A.M., Maccoy, N.E.J., 1984. Effect of treatment with reserpine on the change in filtration rate of *Mytilus edulis* subjected to dissolved copper. Marine. Pollution. Bulletin. 15, 436–439. https://doi.org/10.1016/0025-326X(84)90142-5

Jasperse, L., Levin, M., Tsantiris, K., Smolowitz, R., Perkins, C., Ward, J.E., De Guise, S.,

2018. Comparative toxicity of Corexit® 9500, oil, and a Corexit®/oil mixture on the eastern oyster, *Crassostrea virginica* (Gmelin). Aquatic. Toxicology. 203, 10–18. https://doi.org/10.1016/j.aquatox.2018.07.015

Kenneth, L., Michel, B., Bing, C., Julia, F., Peter, H., Stella, S., Albert, V., 2015. Expert panel report on the behaviour and environmental impacts of crude oil released into aqueous environments. Royal Society of Canada. Ottawa, ON, ISBN: 978-1-928140.02.03

Kittner, C., Riisgård, H.U., 2005. Effect of temperature on filtration rate in the mussel *Mytilus edulis*: No evidence for temperature compensation. Marine Ecology Progress Series. 305, 147–152. https://doi.org/10.3354/meps305147

Kramer, K.J.M., Botterweg, J., 1991. Aquatic biological early warning systems: an overview. Jeffrey D.W., Madden B. 95-126 Academic Press

Kramer, K.J.M., Foekema, E.M., 2001. The "Mussel monitor®" as biological early warning system, in: Butterworth, F.M., Gunatilaka, A., Gonsebatt, M.E. (Eds.), Biomonitors and Biomarkers as Indicators of Environmental Change 2: A Handbook. pp. 59–87 Springer, Boston, MA.

Larsson, C., Ahlstrøm, Ø., Junghans, P., Jensen, R.B., Blache, D., Tauson, A.H., 2015. The oral [13C]bicarbonate technique for measurement of short-term energy expenditure of sled dogs and their physiological response to diets with different fat: Carbohydrate ratios. Journal of Nutrition. Science. 4, 1–10. https://doi.org/10.1017/jns.2015.23

Leblanc, A., Arnold, A.A., Genard, B., Nadalini, J., Heine, M.S., Marcotte, I., Tremblay, R., Sleno, L., 2012. Determination of isotopic labeling of proteins by precursor ion scanning liquid chromatography / tandem mass spectrometry of derivatized amino acids applied to nuclear magnetic resonance studies. RCMS 26, 1165–1174. https://doi.org/10.1002/rcm.6204

Lindstrom, J.E., Braddock, J.F., Fiocco, R.J., Lewis, A., Burns, P.N., Becher, H., Burns, P.N., Becher, H., The, I.N., Wise, J.P.J., Wise, J.P.J., Tran, D., Ciutat, A., Mat, A., Massabuau, J.C., Hégaret, H., Lambert, C., Le Goic, N., Soudant, P., Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., Blasco, D., Levasseur, M., Bonneau, E., Gelinas, R., Packard, T.T., Comeau, L.A., Babarro, J.M.F., Riobó, P., Scarratt, M., Starr, M., Tremblay, R., Davenport, J., Smith, R.J.J.W., Packer, M., Galimany, E., Sunila, I., Hégaret, H., Ramón, M., Wikfors, G.H., Kudela, R.M., Berdalet, E., Bernard, S., Burford, M., Fernand, L., Lu, S., Roy, S., Tester, P., Usup, G., Magnien, R., Anderson, D.M., Cembella, A.D., Chinain, M., Hallegraeff, G.M., Reguera, B., Zingone, A., Enevoldsen, H., Hégaret, H., Wikfors, G.H., Soudant, P., Lambert, C., Shumway, S.E., Bérard, J.B., Lassus, P., Lauzon-Guay, J.S., Hamilton, D.J., Barbeau, M.A., Mathiesen, S.S., Thyrring, J., Hemmer-Hansen, J., Berge, J., Sukhotin, A., Leopold, P., Bekaert, M., Sejr, M.K., Nielsen, E.E., Tran, D., Ciret, P., Ciutat, A., Durrieu, G., Massabuau, J.C., Oceanographic, W.H., Hole, W., 2000. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant Corexit 9500. Aquaculture 10, 702–711. https://doi.org/10.3989/scimar.2003.67n3261

LLC, N.E.S., 2016. Oil Spill Dispersants [WWW Document]. URL https://www.nalcoenvironmentalsolutionsllc.com/wp-content/uploads/NES-LLC-COREXIT.pdf

MacDonald, B.A., Thompson, R.J., 1986. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus* - III. Physiological ecology, the gametogenic cycle and scope for growth. Marine. Biology. 93, 37–48. https://doi.org/10.1007/BF00428653

Martinez Arbizu, P., 2019. Pairwiseadonis: Pairwise multilevel comparison using adonis. R Packag. version 0.3.

Moreau, A.V., Tremblay, R., Bourget, E., 2005. Distribution of *Mytilus edulis* and *M. trossulus* on the Gaspé coast in spatial scale relation to spatial scale Journal of Shellfish Research 24, 545–551.

Nagai, K., Honjo, T., Go, J., Yamashita, H., Seok Jin Oh, 2006. Detecting the shellfish killer *Heterocapsa circularisquama* (Dinophyceae) by measuring bivalve valve activity with a Hall element sensor. Aquaculture 255, 395–401. https://doi.org/10.1016/j.aquaculture.2005.12.018

Nalco Environmental Solutions, 2014. Safety Data Sheet Corexit ® Ec9500a 1–11.

National Research Council (US). Committee on Effectiveness of Oil Spill Dispersants. (1989). Using oil spill dispersants on the sea. National Academy Press.

Newell, C.R., Wildish, D.J., MacDonald, B.A., 2001. The effects of velocity and seston concentration on the exhalant siphon area, valve gape and filtration rate of the mussel *Mytilus edulis*. Journal of Experimental Marine Biology and Ecology. 262, 91–111. https://doi.org/10.1016/S0022-0981(01)00285-4

Nielsen, P., Krock, B., Hansen, P.J., Vismann, B., 2020. Effects of the DSP-toxic dinoflagellate *Dinophysis acuta* on clearance and respiration rate of the blue mussel, *Mytilus edulis*. PLoS One 15, 1–19. https://doi.org/10.1371/journal.pone.0230176

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Maintainer, H.W., 2019. Community Ecology Package: ordination, diversity and dissimilarities. Vegan Community Ecology Package R package version 2, 1–297.

Pace, C.B., Clark, J.R., Bragin, G.E., 1995. Comparing crude oil toxicity under standard and environmentally realistic exposure. Oil Spill Conference. 1995, 1003–1004.

Pascoe, P., Parry, H., Hawkins, S.A.J., 2009. Observations on the measurement and interpretation of clearance rate variations in suspension-feeding bivalve shellfish. Aquatic Biology 6, 181–190.

Place, B. J., Perkins, M. J., Sinclair, E., Barsamian, A. L., Blakemore, P. R., & Field, J. A., 2016. Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. Deep Sea Research Part II: Topical Studies in Oceanography, 129, 273-281.

Rajagopal, S., Van Der Velde, G., Jenner, H.A., 1997. Shell valve movement response of

dark false mussel, *Mytilopsis leucophaeta*, to chlorination. Water Research. 31, 3187–3190. https://doi.org/10.1016/S0043-1354(97)00163-2

Riisgård, H.U., Egede, P.P., Barreiro Saavedra, I., 2011. Feeding behaviour of the mussel, *Mytilus edulis* : new observations, with a minireview of current knowledge. Journal of Marine Biology. 2011, 1–13. https://doi.org/10.1155/2011/312459

Scarlett, A., Galloway, T.S., Canty, M., Smith, E.L., Nilsson, J., Rowland, S.J., 2005. Comparative toxicity of two oil dispersants, superdispersant-25 and corexit 9527, to a range of coastal species. Environmental. Toxicology. and Chemistry. 24, 1219–1227. https://doi.org/10.1897/04-334R.1

Schmidt, C. W. (2010). Between the devil and the deep blue sea: dispersants in the Gulf of Mexico. Environmental Health Perspectives 118, 8 https://doi.org/10.1289/ehp.118-a338

Starr, M., Lair, S., Michaud, S., Scarratt, M., Quilliam, M., Lefaivre, D., Robert, M., Wotherspoon, A., Michaud, R., Ménard, N., Sauvé, G., Lessard, S., Béland, P., Measures, L., 2017. Multispecies mass mortality of marine fauna linked to a toxic dinoflagellate bloom. PLoS One 12, 1–18. https://doi.org/10.1371/journal.pone.0176299

Thorin, S., Bourdages, H., Vincent, B., 1998. Study of siphon activity in *Mya arenaria* (L.) in the intertidal zone by means of an underwater video camera. Journal. of Experimental. Marine. Biology. and Ecology. 224, 205–224. https://doi.org/10.1016/S0022-

0981(97)00196-2

Tran, D., Andrade, H., Durier, G., Ciret, P., Leopold, P., Sow, M., Ballantine, C., Camus, L., Berge, J., Perrigault, M., 2020. Growth and behaviour of blue mussel, a re-emerging polar resident, follow a strong annual rhythm shaped by the extreme high Arctic light regime. Royal Society Open Science. 7, 0–5.

Tran, D., Ciret, P., Ciutat, A., Durrieu, G., Massabuau, J.C., 2003. Estimation of potential and limits of bivalve closure response to detect contaminants: Application to cadmium. Environmental Toxicology Chemistry. 22, 914–920. https://doi.org/10.1897/1551-5028(2003)022<0914:EOPALO>2.0.CO;2

Tran, D., Ciutat, A., Mat, A., Massabuau, J.C., Hégaret, H., Lambert, C., Le Goic, N., Soudant, P., 2015. The toxic dinoflagellate *Alexandrium minutum* disrupts daily rhythmic activities at gene transcription, physiological and behavioral levels in the oyster *Crassostrea gigas*. Aquatic Toxicology 158, 41–49. https://doi.org/10.1016/j.aquatox.2014.10.023

Tran, D., Haberkorn, H., Soudant, P., Ciret, P., Massabuau, J.C., 2010. Behavioral responses of *Crassostrea gigas* exposed to the harmful algae *Alexandrium minutum*. Aquaculture 298, 338–345. https://doi.org/10.1016/j.aquaculture.2009.10.030

Tran, D., Sow, M., Camus, L., Ciret, P., Berge, J., Massabuau, J.C., 2016. In the darkness of the polar night, scallops keep on a steady rhythm. Scientific Report 6, 1–9. https://doi.org/10.1038/srep32435

Tremblay, R., Landry, T., Leblanc, N., Pernet, F., Barkhouse, C., Sévigny, J., 2011. Physiological and biochemical indicators of mussel seed quality in relation to temperatures Aquatic Living Ressources282, 273–282.

Widdows, J., Moore, M. N., Lowe, D. M., & Salkeld, P. N. (1979). Some effects of a dinoflagellate bloom (*Gyrodinium aureolum*) on the mussel, *Mytilus edulis*. Journal of the Marine Biological Association of the United Kingdom, 59(2), 522-524.

Wise, J., Wise, J.P., 2011. A review of the toxicity of chemical dispersants. Review. Environmental Health 26, 281–300. https://doi.org/10.1515/REVEH.2011.035

CHAPITRE 2
UTILISATION DE LA VALVOMETRIE COMME UN OUTIL D'ALERTE POUR LA PRESENCE DE L'ALGUE TOXIQUE *ALEXANDRIUM CATENELLA* PAR *MYTILUS EDULIS*

Le deuxième chapitre a donné lieu à deux articles. Le premier, intitulé « Use of valvometry as an alert tool to signal the presence of toxic algae *Alexandrium catenella* by *Mytilus edulis* » a été corédigé par moi-même ainsi que par Jean-Bruno Nadalini, Luc Comeau, Michel Starr, Sonia Michaud, Damien Tran, Richard St-Louis, José Babarro, Jeff Clements, Réjean Tremblay. Cet article va être soumis dans les jours suivant le dépôt initial de la thèse dans la revue *Aquaculture*. En tant que premier auteur, ma contribution à cet article a été la réalisation des expériences, le traitement et l'analyse des données, leur interprétation et la rédaction de l'article. Jean-Bruno Nadalini et Sonia Michaud ont également participé aux expériences, Luc Comeau, Michel Starr, José Babarro, Damien Tran et Réjean Tremblay ont participé à l'interprétation des données. Tous les auteurs ont participé à la révision de l'article. Cet article a été présenté sous forme d'affiche au Canadian Ecotoxicity Workshop à Québec et sous forme de présentation aux réunions annuelles des Ressources Aquatiques du Québec 2020 et 2021. Le second, intitulé « Impact of the toxic dinoflagellate *Alexandrium catenella* on the valve behaviour of blue mussels (*Mytilus edulis*): A comparison between two populations with contrasting histories of PST exposure » a été corédigé avec Luc Comeau, José Babarro, Michel Starr, Jeff Clements, Réjean Tremblay. Cet article va également être soumis dans les jours suivants le dépôt initial de la thèse dans la revue *Aquaculture Report.* En tant que premier auteur, ma contribution à cet article a été la réalisation des expériences, le traitement et l'analyse des données, leur interprétation et la rédaction de l'article. Réjean Tremblay a aidé à l'interprétation des données. Tous les auteurs ont participé à la révision de l'article.

2.1 RESUME EN FRANÇAIS DU DEUXIEME ARTICLE

La valvométrie est une méthode non-invasive utilisée pour suivre en continue le comportement valvaire de bivalves à haute fréquence. Dans des études précédentes au laboratoire, la valvométrie a révélé une sensibilité comportementale des bivalves en présence de microalgues toxiques en eau de mer. Cependant, l'application de la valvométrie comme un système de détection précoce pour détecter des efflorescences naturelles de microalgues toxiques et leur toxicité résultante sur les bivalves reste largement inexplorée. Dans cette étude, la valvométrie a été utilisée pour caractériser des changements dans le comportement valvaire de la moule bleue (*Mytilus edulis*) durant une exposition graduelle au dinoflagellé toxique *Alexandrium catenella*, produisant des Paralytic Shellfish Toxins (PST). Premièrement des expériences en laboratoire ont été réalisées pour identifier un comportement valvaire spécifique, puis ces réponses ont été validées en eau de mer naturelle dans une seconde expérience. En conditions de laboratoire ou naturelles, les moules exposées à *A. catenella* ont eu tendance à rester ouverte (bâillement) plus longtemps que les moules non-exposées. Ce changement dans le comportement valvaire a été observé à des concentrations en PST inferieures à 30 µg STXeq 100 g^{-1} dans les tissus de moules. Cela suggère que l'augmentation de l'ouverture des moules est reliée à une paralysie temporaire des muscles adducteurs des moules provoqué par les algues toxiques, comme ce mécanisme a déjà été relevé chez d'autres espèces de bivalves. De plus, il a été observé que les rythmes biologiques du comportement valvaire liés aux rythmes tidaux et journaliers ont été modifiés lorsque les moules ont été intoxiquées par les PST. En conclusion, l'effet des algues toxiques sur le comportement valvaire des moules a révélé que la valvométrie peut être utilisée comme un outil de détection précoce pour la présence du dinoflagellé toxique *A. catenella* dans l'environnement avant que les moules n'atteignent le seuil règlementaire (80 µg STXeq 100 g^{-1}) pour la récolte des bivalves.

2.2 USE OF VALVOMETRY AS AN ALERT TOOL TO SIGNAL THE PRESENCE OF TOXIC ALGAE *ALEXANDRIUM CATENELLA* WITH *MYTILUS EDULIS*

Guillaume Durier[a],*, Jean-Bruno Nadalini[a], Luc A. Comeau[b], Michel Starr[c], Sonia Michaud[c], Damien Tran[d], Richard St-Louis[e], José M.F. Babarro[f], Jeff C. Clements[b], Réjean Tremblay[a]

[a]Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada

[b]Fisheries and Oceans Canada, Gulf Region Center, Moncton, NB, Canada

[c]Fisheries and Oceans Canada, Quebec Region, Institut Maurice Lamontagne, Mont-Joli, QC, Canada

[d]University of Bordeaux, and CNRS, EPOC, UMR 5805, F-33120 Arcachon, France

[e]Département de biologie, chimie et géographie, Université du Québec à Rimouski, Rimouski, Québec, Canada

[e]Instituto de Investigaciones Marinas-CSIC, Vigo, Spain

Keywords: Behavioural ecology; Biomonitoring; Environmental stress; Harmful algal blooms (HABs); Paralytic shellfish poisoning (PSP)

2.2.1 Abstract:

Valvometry is a non-invasive technique used to continuously monitor gaping behaviour of bivalves at high frequency. In previous laboratory studies, valvometry has

revealed a behavioural sensitivity of bivalves to the presence of toxic microalgae in seawater. However, the application of valvometry as an early-warning system for detecting natural occurrences of toxic microalgae and their resulting toxicity in bivalves remains largely unexplored. In this study, valvometry was used to characterize changes in blue mussels (*Mytilus edulis*) gaping behaviour during gradual exposure to the toxic dinoflagellate, *Alexandrium catenella*, which produces paralytic shellfish toxins (PST). Laboratory experiments were first performed to identify specific gaping behaviour changes and these responses were subsequently validated in natural seawater conditions in a second experiment. Under both laboratory and natural seawater conditions, mussels exposed to *A. catenella* tended to remain open (yawning) longer than non-exposed mussels. This change in gaping behaviour was observed at PST concentration as low as 30 µg STXeq 100 g–1 of mussel tissue. We suggest that increased opening is likely related to temporary muscular paralysis induced by toxic algae, as this mechanism has been previously reported in other bivalve species. Furthermore, we observed that biological rhythms of valve behavior related to tidal and daily rhythms were modified when mussels were intoxicated by PSP. In conclusion, the effects of toxic algae on mussel gaping behaviour reveals that valvometry could be used as an early-warning tool for the presence of toxic *Alexandrium sp*. in the environment prior to mussels reaching the regulatory threshold (80 µg STXeq 100 g^{-1}) for harvest interdiction.

2.2.2 Introduction:

Toxic algae are present in most aquatic ecosystems (Roelke, 2007), and represent around 300 species among the 5,000 species of marine phytoplankton known (Bates, 2020; Hallegraeff, 1993; McKenzie et al., 2021). Under certain environmental conditions, these algae can proliferate, causing toxic algae blooms known as “Harmful Algal Blooms” (HABs) (Anderson et al., 2011) that can negatively impact aquaculture activities through mass mortalities and/or harvest interruption to avoid human intoxication (Granéli and Turner, 2006). For example, in Nova Scotia, Canada, mass mortalities have been observed in salmon farming during an *Alexandrium catenella* bloom (Cembella et al., 2002). The dinoflagellate *A. catenella* can also be consumed by filter-feeding organisms such as bivalves before being

bioaccumulated in higher trophic levels (Starr et al., 2017). In contrast to finfish, many bivalve species can safely ingest toxic algae, leading to toxin bioaccumulation in associated food webs. Harvesting bivalves for human consumption is interrupted during HABs until the concentration of toxins in bivalve tissues is reduced to levels safe for human consumption (Bricelj et al., 1990; Galimany et al., 2008). Given the continual growth of bivalve aquaculture worldwide (FAO, 2016), ways of detecting HABs prior to bivalve intoxication could aid in mitigating impacts to shellfish aquaculture industry.

One tool that has the potential to serve as an early-warning system for environmental stressors, including HABs, is bivalve valvometry (Andrade et al., 2016; Clements and Comeau, 2019). Valvometry is a non-invasive, high-frequency monitoring systems of the opening and closing behaviour of bivalves (Andrade et al., 2016). Herein, individual's valve movements are measured with a small electrode sticked on one valve and a magnet on the other one. These electrodes are connected to a dynamic strain recorder, which translates the magnetic flux between the sensor and magnet into a voltage value proportional to the length of this opening. Thus, as bivalves open and close their valves, these movements can be empirically measured through changes in voltage readings produced by valvometry systems. Since bivalves typically respond to stressful events by modifying their gaping behaviour to avoid stressful conditions, behavioural indicators can be identified using high frequency valvometry and related to the presence and intensity of specific environmental stressors. As such, monitoring gaping behaviour through valvometry can be used to both detect and characterize the impact of environmental perturbations (Comeau et al., 2019; Tran et al., 2015, 2010, 2003).

Among bivalve species cultured worldwide, mussels are some of the most economically important. Mussel culture has continuously increased through time, with global *Mytilus edulis* production more than doubling from ≈100 KT in 1960 to >200 KT in 2000 (FAO, 2016). The main producers for the North American market are located on the east coast of Canada and exclusively culture blue mussels, *Mytilus edulis* (FAO, 2016). Naturally, these mussels form dense populations in temperate and boreal regions, generally residing on

hard substrates of exposed or unexposed shores (Bayne, 1976). This species is located in the intertidal and subtidal zones and can be dominant, particularly in subarctic regions (Mathiesen et al., 2017). To feed, blue mussels filter and consume phytoplankton including diatoms and dinoflagellates, as well as some heterotrophic flagellates and bacteria (Trottet et al., 2008). From a valvometry research perspective, blue mussels (as well as other mussel species) are among the most well studied and can thus serve as a model species for contemporary valvometry research (e.g., Clements et al., 2021; Riisgård et al., 2006; Robson et al., 2007, 2010). Furthermore, related mussel species are known to respond to toxic algae by exhibiting increased "microclosures" (Comeau et al. 2019). Moreover, *M. edulis* has the capacity to accumulate more toxins than other sensitive species (Bricelj et al., 1990; Lassus et al., 1999). As such, blue mussels are a prime candidate for exploring the use of valvometry as an early-warning tool for detecting HABs before they become problematic, and notably to aquaculture and shellfish farming industries.

In eastern Canada where blue mussels are cultured, the dinoflagellate *Alexandrium catenella* is known to be involved in some HAB events, particularly in the St. Lawrence Estuary (Larocque and Cembella, 1990; Therriault et al., 1985). Globally, *A. catenella* is present in Atlantic and Pacific America, Northern Europe, South-East Asia and South-West Africa coasts (Lilly et al.,2007). The St. Lawrence Estuary strain of *A. catenella* who served in this study has a size between 25 to 50 µm length and 26 to 48 µm wide (Bérard-Therriault et al., 1999) and produces a potent mixture of toxins like saxitoxin and numerous derivatives, called Paralytic Shellfish Toxins (PST), which are associated with Paralytic Shellfish Poisoning (PSP) syndrome in humans (Hégaret et al., 2007). These toxins attach specifically but reversibly to the voltage-dependant sodium channels of animals, stopping the action potential production in the nervous system and muscles (Hégaret et al., 2007; James et al., 2010). In the case of bivalves, adductor muscle paralysis can be observed along with digestive system damage and reduced hemocytes following exposure (Galimany et al., 2008).

Given the importance of blue mussels to the regional economy of eastern Canada, coupled with the importance of developing early-warning tools for HABs, the aim of this

study was to explore the potential for *M. edulis* valve gaping to serve as an early-warning biomonitoring tool for HABs using high frequency valvometry. Herein, we sought to identify gaping behaviours and rhythmicity characteristic of *A. catenella* presence, and to determine the ability of valvometry to detect the presence *A. catenella* before blooms occur, in both laboratory and natural seawater settings. We hypothesized that specific gaping behaviour changes would be detected in the presence of PST using high frequency valvometry monitoring systems.

2.2.3 Materials and Methods:

2.2.3.1 Experiment 1: Laboratory Experiment

In November 2018, two-year-old cultured *M. edulis* (average shell length, 65.65 ± 2.33 mm n = 24) were collected from a mussel lease in St. Peter's Bay, Prince Edward Island, Canada (46º 26' 30.7" N, 62º 44' 51.3" W), characterised by a pure *M. edulis* population (Tremblay et al., 2011). The animals were immediately transported on ice to the Station Aquicole de Pointe-au-Père (Institut des Sciences de la Mer, Rimouski, Canada) and acclimated to laboratory conditions for 30 days in two 300 L maintenance tanks with 1 µm filtered seawater (continuously aerated with a salinity ≈ 28, and a natural photoperiod). During acclimation, the temperature was increased by 1°C day^{-1} until the desired experimental temperature was reached (described below). Mussels were continuously fed with live non-toxic algae of *Tisochrysis lutea* CCMP 1324, *Chaetoceros muelleri* CCMP 1316, and *Pavlova lutheri* CCMP 1325 (ratio 1:1:1 respectively) at a rate of 60,000 cells L^{-1} per 50 mussels repartited in the two 300 L tanks. Algae were batch-cultured in f/2 medium (with Si for the diatom *C. muelleri*) (Guillard, 1975) at 18°C, under continuous illumination, in 20 L carbons supplied continuously with CO^2 to maintain a pH of ≈ 8 and a photosynthetic active radiation of 100 µmol quanta m^{-2} s^{-1}. Cell counts were determined with a Multisizer 4e Beckman Coulter counter with a 50-µm pore orifice.

For the laboratory experiment, the strain of *A. catenella* 'AT6' from the St. Lawrence Estuary isolated at the Maurice Lamontagne Institute (Department of Fisheries and Oceans; DFO) during a 2008 severe red tide event was used (Starr et al., 2017). Cells of *A. catenella* were cultured as already described for other species and harvested in late exponential growth and distributed in the mussel tanks during the experiment, according to the necessary concentrations detailed in 2.2. to obtain target toxicity. Previous cultures of *A. catenella* showed toxin cell quotas ranging between 3 and 60 pg STXeq $cell^{-1}$ (Nadalini, J.-B. and Tremblay, R., unpublished data). Assuming the lowest toxin content, production was set to exceed the regulatory threshold for shellfish harvesting closure of 80 µg STXeq 100 g–1.

The experiment was conducted over three days at 18°C, an intermediate temperature between the optima for mussels (20°C; Almada-Villela et al., 1982) and 14°C for *A. catenella* (Boivin-Rioux et al., 2021). Following the 30-day acclimatation period (as described above), 24 mussels were connected to valvometry monitoring systems described in Nagai et al. (2006) and Comeau (2014) (see Section 2.5 below for details). During the experiment, mussels were equally distributed in six tanks (4 mussels $tank^{-1}$) containing 100-L of 1 µm filtered seawater (Fig. 22a). Each mussel was analysed individually for behaviour and PST concentration accumulated in tissues.

Gaping behaviour of the mussels was continuously monitored using valvometry (see Section 2.5 below) for 24-hours prior to exposure to toxic algae to obtain baseline data. After this reference period, cells of *A. catenella* were supplied to each tank at the following concentrations: 0 (control), 1,330, 2,670, 4,000, 5,330 and 10,666 cell L-1. Twenty-four hours later, a second pulse of non-toxic microalgae naturally consumed by the mussels, (*Tetraselmis suecica*), was added in the tanks (5,000 cells mL-1) after a water change and maintained for one day with a peristaltic pump to eliminate potential gaping behaviour changes induced by food supply variation. Gaping behaviour was continuously measured during the exposure of both *A. catenella* and *T. suecica*.

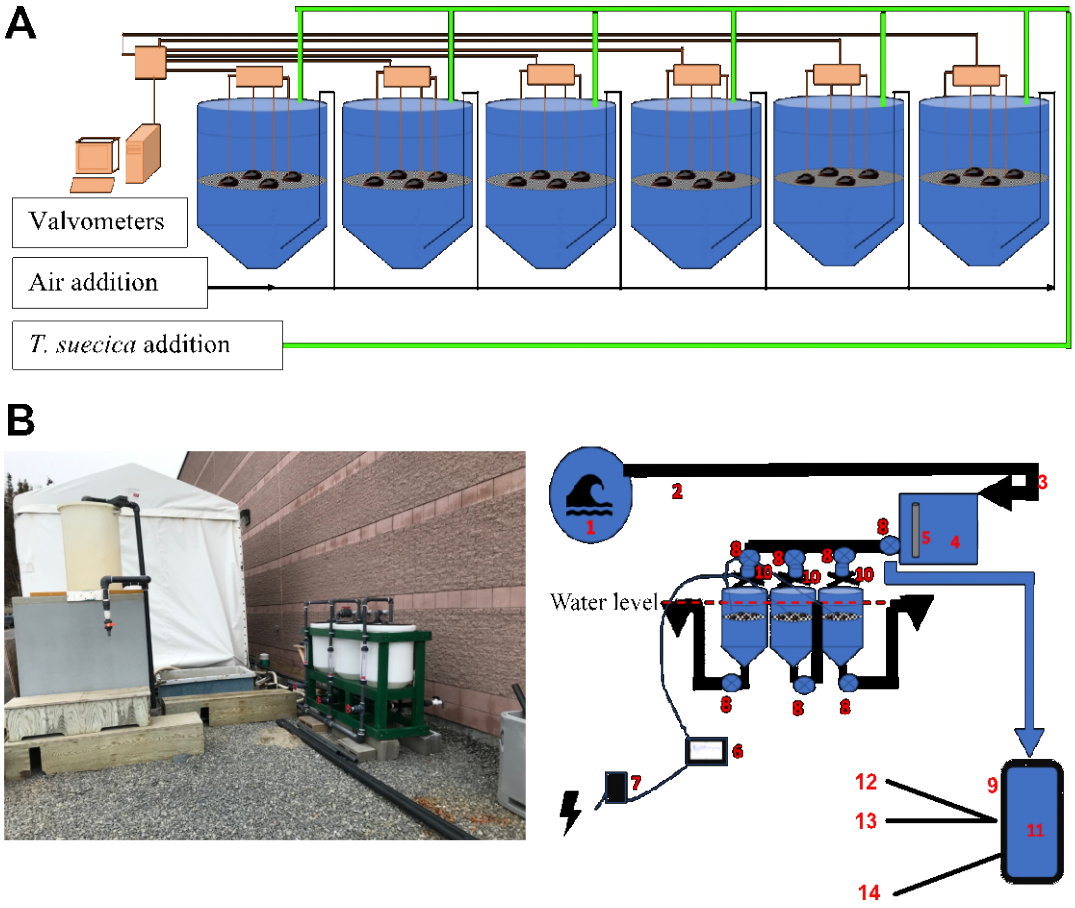

Figure 22 : Experimental design of the lab experiment (A) and of the natural seawater experiment (B). 1: seawater, 2: water intake, 3: water pump, 4: head tank, 5: CTD, 6: valvometers, 7: battery, 8: Tap, 9: sample, 10: flowmeter, 11: water sampled, 12: Elisa test, 13: cell count, 14: Jellet test

2.2.3.2 Experiment 2: Natural Seawater Experiment

In addition to the controlled laboratory experiment, a 90-day *in situ* experiment was conducted with unfiltered natural seawater at the Maurice- Lamontagne Institute (DFO, Mont-Joli, Québec, Canada) from June to September 2019 – a period in which *A. catenella* blooms are known to occur in the St. Lawrence Estuary (Boivin-Rioux et al., 2021; Parkhill and Cembella, 1999). The natural seawater experiment was performed with *M. edulis* collected in a mussel lease in St Peter's Bay, Prince Edward Island in 2019. To expose mussels to natural seawater and meteorological conditions, this natural seawater experiment was carried out in outdoor tanks supplied with unfiltered raw seawater from the adjacent St. Lawrence Estuary. The raw seawater pumped at the surface (1-10m, depending of the tide) was directed in a 150 L header tank and distributed by gravity to three experimental conical 100 L tanks containing mussels at the top of each tank (Fig. 22b). The seawater was supplied with a flow rate of 15 L min-1, replacing the total volume of the header tank every 3 minutes. Homogenous seawater mixing in the experimental tanks was achieved by using a grid at the surface of each tank. Mixing was validated by dispersion of food colourants and use of GoPro cameras in each tank (GoPro HERO4 Silver +LCD), demonstrating uniform repartition and eventual elimination of coloured water. A calibrated CTD probe was installed in the header tank to monitor temperature, salinity and turbidity every 15 minutes. A total of 36 mussels were equipped for valvometry and distributed equally among the experimental tanks at the same depth (n = 12 mussels experimental per tank), where they were allowed to acclimate to the natural seawater conditions for one week prior to experimentation; valvometry was not recorded during this acclimation period. The vertical flow of water and consistent depth of the mussels ensured that secondary assimilation through the absorption of pseudofeces and feces (Sonier et al., 2020) was negligible.

The presence and concentration of *A. catenella* in the header tank were monitored daily during low tide via detection tests and microscope identification. Two detection tests (1. Jellet from Scotia Rapid Testing, NS, Canada; and 2. Algal Toxin Enzyme-Linked Immunosorbent Assay ELISA Plate Kits from Eurofins Abraxis, PA, USA) were used to detect *A. catenella*

in 48 L seawater samples filtered to retain the 20-100 µm fraction, which was concentrated in a 200 mL solution. The qualitative Scotia Rapid Testing LTD test (SRT test) was use dayly and indirectly detects *A. catenella* via the presence of saxitoxin and neosaxitoxin antibodies (Jellett et al., 2002), with a manufacturer indicated saxitoxin detection limit between 0.02 and 0.1 µg mL^{-1}. For the first 50 days, a 50 mL subsample obtained from the 200 mL sample mentioned above was concentrated and re-suspended in 5 mL of 1 µm filtered seawater and a subsample of 500 µL was used for the test. The following day 50, however, we adjusted the protocol to ensure that we were not losing sensitivity because of the re-suspension by recuperating the material filtered directly with a spatula to avoid dilution and thus increasing the detection limit of the SRT test. The rest of the protocol remained unchanged, and the toxin extraction was realized with acetic acid before being buffered and the test applied. Another subsample from the 200 mL fraction described above was used for the ELISA assay and was filtered on 11 µm nylon filter (Millipore Sigma) and subsequently frozen. The saxitoxins and the analogues potentially present on the nylon filter was extracted with a known volume of 5 or 10 mL of 0.1 M acetic acid (Dell'Aversano et al., 2005) depending of the difficulty of resuspending the material. Extracts were submitted to ultrasonic treatment for 5 minutes to favor cell disruption followed by centrifugation and the filtrate submitted to ELISA assay following the manufacturer protocol. Briefly, the saxitoxin present in the sample, and a saxitoxin-enzyme conjugate, each compete for rabbit saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by an anti-rabbit antibody immobilized on a microtiter plate (Abraxis LLC, Warminster, PA, USA). In contrast to the SRT test, the detection limit of the ELISA assay is much lower at 0.015 ng mL^{-1}. This assay recognizes saxitoxin and its derivatives with varying degrees; the cross reactivities are 100% for Saxitoxin (STX), 29% for Decarbamoyl STX, 23% for GTX 2 & 3 and GTX-5B, 13% for Lyngbyatoxin, but drop to 2.0 % for Sulfo GTX 1 & 2, 1.4% for Decarbamoyl GTX 2 & 3, 0.6% Decarbamoyl Neo STX, and for the powerful Neosaxitoxin and GTX 1 & 4, this decreases to less than 1.3 and 0.2 % respectively.

In addition to seawater samples for detecting PST, separate samples of unfiltered seawater (200 mL) coming from the header tank were also preserved daily in acidic Lugol's

solution for microscope cell counts to quantify the concentration of *A. catenella*. The daily microscopic count of potential toxic algae (cells L-1 of *A. catenella*, *Dinophysis acuminata* and *Pseudo-nitzschia* spp.) were estimated using the standard Utermöhl method (Andersen and Throndsen, 2004).

2.2.3.3 PST Detection in Mussel Tissues

The concentration of PST in the tissues of experimental mussels, as well as in wild mussels, sampled from the shore of the St. Lawrence Estuary (adjacent to our natural seawater setup to estimate natural contamination levels) was determined using the Algal Toxin ELISA Plate Kits as described above. The test was conducted on each mussel individually, by crushing and homogenizing whole mussel tissues in acetic acid with a ratio of 1 mL of acid acetic for 1 g of mussel's tissue. In addition, PST content was regularly measured in PST concentrations in the two mussel populations (estuarine shoreline three times every week and experimental tanks at the end of the experience) sampled the same day showed no significant difference (Student test: df = 24,94, T-value = 0.055, p-value = 0.956). Same PST level was measured in wild mussels tissue than monitored.

2.2.3.4 Valvometry

To measure gaping behaviour in each experiment, mussels were equipped with a Hall element sensor (HW-300a, Asahi Kasei, Japan; 0.5 g) and a magnet (4.8 mm diameter × 0.8 mm height; 0.2 g) glued with Solarez® UV epoxy resin (Wahoo International, CA, USA), which polymerizes rapidly under UV light. Electrodes were connected to 4-channel dynamic strain recorders (DC-204 R, Tokyo Sokki Kenkyujo Co., Japan) equipped with memory cards for data storage, which converts the magnetic flux between the sensor and magnet into a voltage value. To avoid pseudo replication, each of the four mussels linked to the same valvometer were placed in different tanks, except for the natural seawater experiment in which two mussels from the same valvometer needed to be placed in the same tank (i.e., there were only three experimental tanks). Electrodes and magnets were fixed on mussels' valves

with an initial target reading of ≈-80,000 µV when the bivalves were closed. Recording was set to a frequency of 10 measurements sec-1 to obtain high resolution behavioural data. Data were retrieved from the memory cards at the end of the experiments as .csv files and were subsequently processed using R statistical software version 1.3.1073 (R Core Team 2020). Herein, Valve Opening Amplitude (VOA) was computed for each individual after conversion of µV data to mm by the use of calibration curves estimated with known size spacers. VOA was calculated as:

$$VOA = [(opening-min) / (max-min)] \times 100$$

where VOA is the valve opening amplitude in %, opening is the measured valve opening in mm at a given period in time, and max and min are, respectively, the maximum and minimum opening (in mm) values observed over the entire observation period. VOA was then used to compute various behavioural indicators, including 1. the number of closures; 2. total closure duration; 3. average closure duration; 4. the average VOA and 5. the number of microclosures (as defined by Comeau et al., 2019). A mussel was considered closed when VOA was <10% of max. A microclosure was counted when a 3% reduction of VOA within 0.1 sec was observed. In the laboratory experiment, the behavioural indicators were calculated for 3 periods: before and after the *A. catenella* exposure, and during the following exposure of *T. suecica*. In the natural seawater experiment, the behavioural indicators were calculated for each day. All raw data were handled and processed (i.e., µV values converted to mm values, and computations of behavioural indicators) in R (R Core Team, 2020).

2.2.3.5 Statistical Analysis

a) *EXPERIMENT 1. LABORATORY EXPERIMENT*

Statistical analyses were performed using R (R Core Team, 2020) with a significance threshold of $p < 0.05$. The impact of mussel tissue PST concentration on gaping behaviour indicators (number of closures, total duration of closures, average closures duration and number of microclosures; n = 18 mussels) was evaluated with non-parametric Pearson

correlations (base package), as Shapiro-Wilk test indicated non-normal distribution of the data. Correlations were applied for the period of *A. catenella* exposure (T1) and food addition (T2) with the PST concentration to avoid tank effect and use the real PST quantity affecting the mussel behaviour. Principal Component Analysis (PCA; package ade4; Dray and Dufour, 2007) was also used to determine whether the T1 gaping behaviour indicators were influenced by PST concentration in mussels. Then, the mean of each gaping behaviour indicator measured at different PST concentration ranges (C0: <10 µg STXeq 100g-1, n = 3, C1: 20 to 50 µg STXeq 100g-1, n = 4, C2: 50 to 80 µg STXeq 100g-1, n = 4, C3: >80 µg STXeq 100g-1, n = 7) were compared with a univariate one-way permutation analyses of variance (PERMANOVA – package vegan; (Oksanen et al., 2019)), using *A. catenella* concentration as factor (n=2). With the non-normal distribution of the databases, this approach is more accurate than parametric analysis. The similarity matrices were calculated based on Euclidean distances as recommended by Anderson et al., (2008) for univariate analyses. A posteriori PERMANOVA pairwise tests (Martinez Arbizu, 2019) were applied to determine the concentration at which gaping behaviour changes were induced. Finally, a cluster analysis with permutations (SIMPROF) was applied to validate *A. catanella* concentrations showing a similar effect on gaping behaviour.

b) *EXPERIMENT 2: NATURAL SEAWATER EXPERIMENT*

For the natural seawater experiment, Pearson correlations were applied between the four gaping behaviours and the daily measures of PST and the cell abundance of toxic algae (*A. catenella*, *Pseudo-nitzschia* spp. and *Dinophysis acuminata*) in the header tank. In parallel, the gaping behaviour indicators were also correlated with a daily mean (90 days) of physicochemical parameters obtained by the CTD probe (temperature, salinity, fluorescence, and turbidity). With the same data set, a PCA was used to determine which gaping behaviour indicators were most linked to PST concentration in seawater (PSTw). These gaping behaviour indicators were selected for PERMANOVA analyses. Based on the daily estimations of PST concentration in field mussels (PSTm), behavioural data were grouped into four PSTm concentrations (C0: <10 µg STXeq $100g^{-1}$, C1: 30 to 50 µg STXeq $100g^{-1}$,

C2: 50 to 80 µg STXeq $100g^{-1}$, C3: >80 µg STXeq $100g^{-1}$) and compared by univariate one-way PERMANOVA (n = 6-15 mussels for each concentration group), followed by a posteriori pairwise test (Martinez Arbizu, 2019) when significant. Finally, a cluster analysis with permutations (SIMPROF) was applied to validate PSTm groups with similar effects on gaping behaviour.

2.2.3.6 Chronobiological analysis

The mussel gaping behaviour was represented with actogram (fig.23b and c). Each column of the actogram represents 2 days of VOA. The second day of a column correspond at the first day of the next column. The colours correspond to 10 VOA range detailed in the fig 23, indicating the mussel opening

To detect potential differences in the rhythmicity (tidal and daily) of mussels gaping behaviour in relation to PST concentration (Between 0 and 118.21 µg STXeq $100g^{-1}$), the gaping behaviour recording was divided in hours and the population (n = 27 mussels) average VOA was calculated for each hour. Chronobiological analyses were performed using TSA Serial Cosinor 6.3 software (http://www.euruestech.net/mainnuk.php) on time windows presented in the fig. 23 with a duration selected to have enough of cycle occurrence to detect them but with a duration adapted to follow the speed of the abundance or PSP concentrations variations. Several steps were required to validate a significant rhythm (Gouthiere et al., 2005; Payton et al., 2017). First, the quality of the data set was assessed by controlling for the absence of randomness using the autocorrelation diagram. Second, the absence of a stationary phenomenon was assessed by using a partial autocorrelation function (PACF) calculation. Third, the recorded data were tested for periodicities in the tidal (12.4 ± 3.1 h) and daily range (24 ± 4 h) by the spectral method of the Lomb and Scargle periodogram (Lomb, 1976; Scargle, 1982). Fourth, the rhythmicity was then validated and modeled with the cosinor model. For a given period, the model is written as $Y(t) = A\cos(\pi t/\tau + \phi) + M + \varepsilon(t)$ where Y (t) is an observation of the mean VOA at time t, A is the amplitude, ϕ is the

acrophase, τ is the period, M is the mesor and ε is the relative error. Two key tests validated the calculated model and the existence of a rhythm: the elliptic test had to be rejected, and the probability for the null amplitude hypothesis had to be < 0.05. A chronobiometric parameter was calculated; the percent rhythm (PR, %) is the percentage of cyclic behavior explained by the model. For a set of data, several significant periodicities could occur. To identify significant secondary periodicities, we reinjected the previously calculated residues of the Cosinor model to remove the trend related to the first statistical period and then repeated the entire procedure.

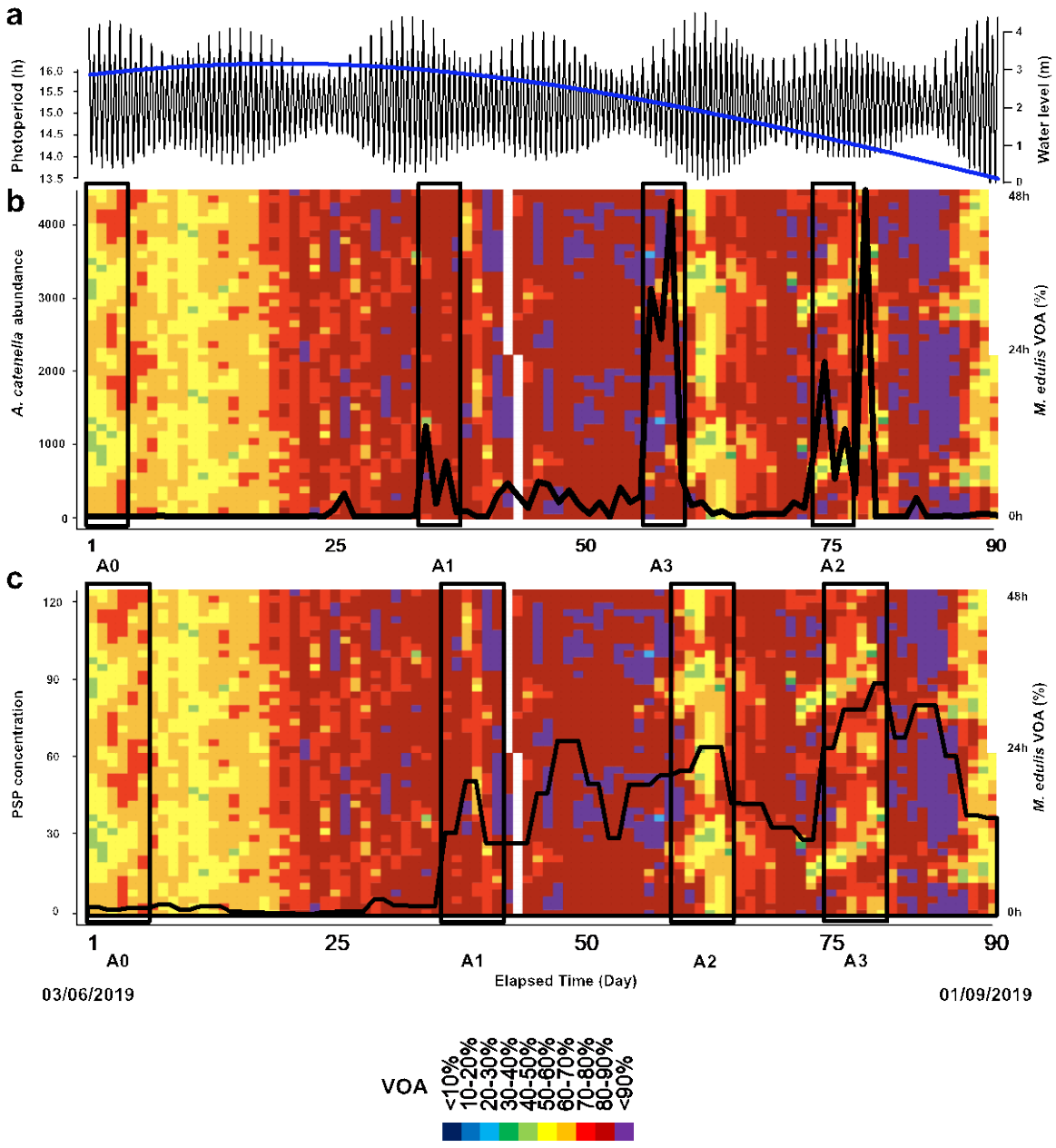

Figure 23 : Physical and biological observations over the course of the 90-day natural seawater experiment. In panel a, the blue and black lines represent photoperiod and water level, respectively. The actograms depict the mean hourly VOA (%) of the group (n = 27 mussels) in relation to *A. catenella* abundance (panel b, black line) and PSP concentration in the mussel tissue (panel c, black line). The black rectangles represent the times windows studies, with a duration of 4 days for panel b and 6 days for panel c.

2.2.4 Results:

2.2.4.1 Experiment 1: Laboratory experiment

The variations of the behavioural indicators during the controlled laboratory experiment are presented in the Fig. 24. Significant negative correlations between gaping behaviour indicators and PST concentration during the *A. catenella* exposure period were evident for total closure duration and the average closure duration with an increasing of the opening (Table 2). For the period following *A. catenella* exposure, no significant correlations were detected (Table 2). Principal component analysis confirmed that PST concentration was negatively correlated with total closure duration and average closure duration (Fig. 25a). Herein, a weak correlation between microclosures and PST concentration was also evident. As such, total closure duration, average closure duration, and microclosures were selected as behavioural indicators for the univariate PERMANOVA analyses. In the univariate PERMANOVA, significant differences between the PST concentrations were observed for total closure duration (DF= 3 and 14, Pseudo-F= 10.18, P(MC)= 0.0027) and average closure duration (DF= 3 and 14, Pseudo-F= 4.16, P(MC)= 0.0242), but not Microclosures ((DF= 3 and 14, Pseudo-F= 2.09, P(MC)= 0.1082)). Significant differences between PST concentrations were also present when the three behavioural indicators were analysed together with a multivariate PERMANOVA (DF= 3 and 14, Pseudo-F= 10.119, P(MC)= 0.003). PERMANOVA pairwise tests showed significant differences between the higher concentration (C3) of PST accumulated in mussels and the other lower concentrations: C0, C1, C2.

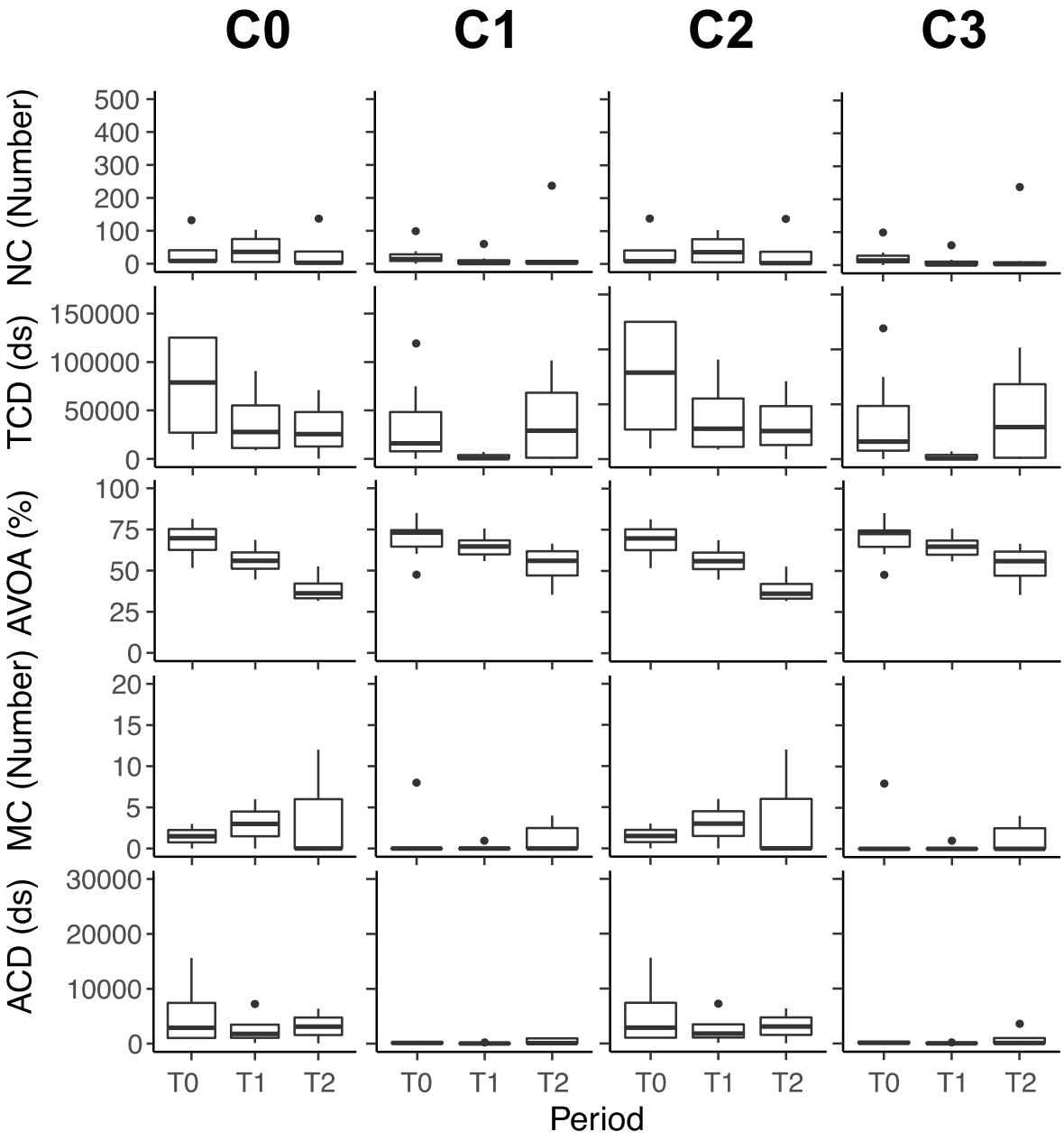

Figure 24 : Variation of the mussel's behavioural indicators for PSP concentration for laboratory experiment. NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average VOA. MC: Microclosure. C0: <20 µg STXeq 100g-1, C1: 20 to 50 µg STXeq $100g^{-1}$, C2: 50 to 80 µg STXeq $100g^{-1}$, C3: >80 µg STXeq $100g^{-1}$, T0: Acclimation and reference behaviour, T1: *A. catenella* exposition, T2: food addition

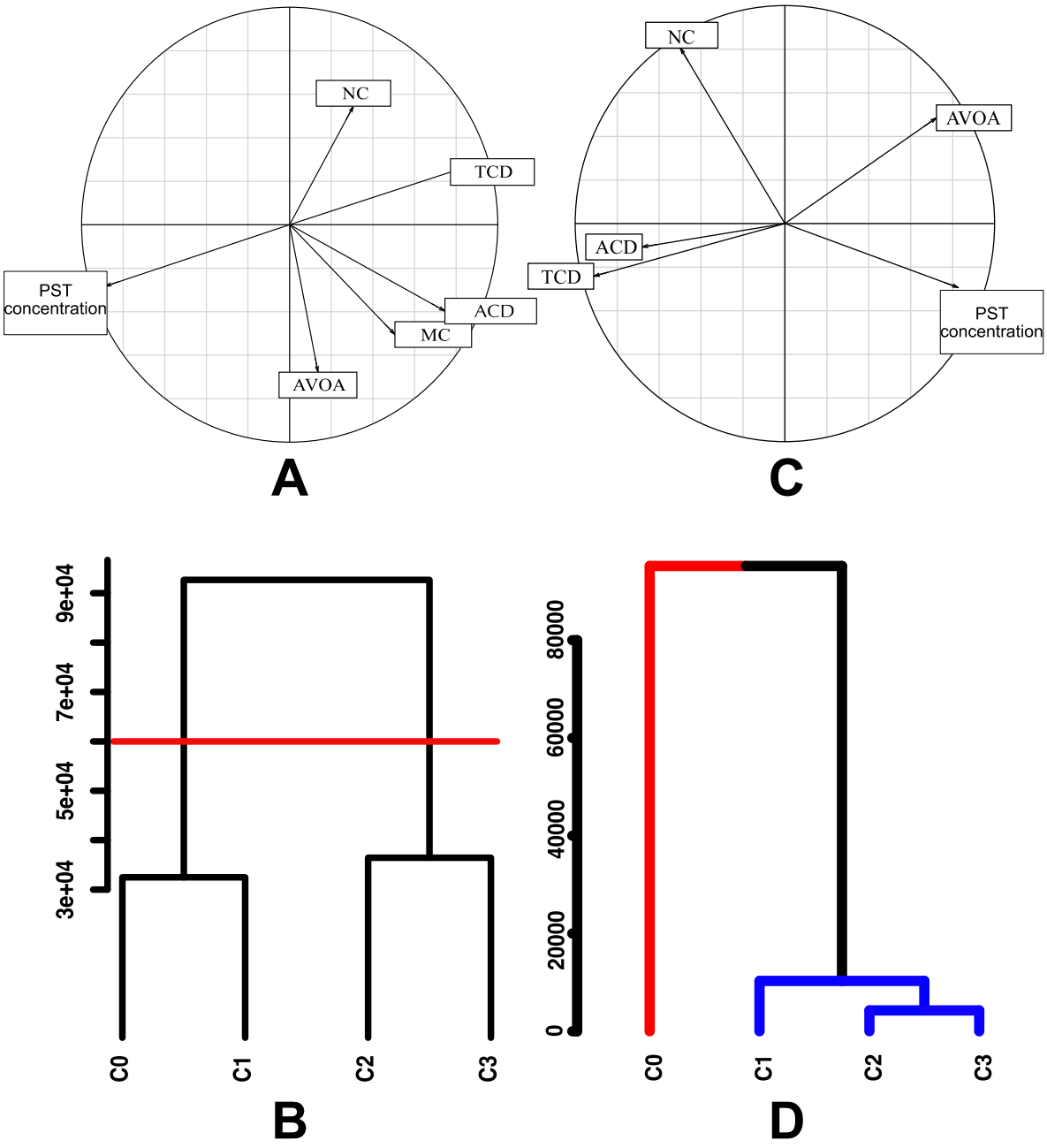

Figure 25 : A) Correlation circle of Principal Component Analysis for the behavioural indicators (NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average VOA. MC: Microclosure) in the laboratory experiment during the period T1 (addition of *A. catenella*), in relation to PST concentration in mussel. B) Cluster analysis of behavioural indicators in relation to PST concentration in mussels during laboratory experiment. Red line: separation of concentration groups, C) Correlation circle of Principal Component Analysis for the behavioural indicators in the natural seawater experiment and PST in mussels, D) Cluster analysis for groupings of PST concentrations with behavioural indicators in natural seawater experiment by SIMPROF method, C0: <10 µg STXeq $100g^{-1}$, C1: 30 to 50 µg STXeq $100g^{-1}$, C2: 50 to 80 µg STXeq $100g^{-1}$, C3: >80 µg STXeq $100g^{-1}$. Red line: group 1, blue line, group 2.

SIMPROF did not detect any sufficient difference in Euclidean distance to establish clusters between PST accumulation for the valve gaping indicators. As such, clusters were established without using SIMPROF but with the selection of the longest distance between two knots. Valve gaping indicators showed different clustering patterns in relation to PST accumulation (Fig. 25b). The red line represents a separation between the concentration groups established from these two clusters. This line was traced at the higher distance between two knots of the cluster representing a group with the concentration C0 and C1 and a second group with the concentrations C2 and C3. Thus, mussel behaviour was impacted only when PST accumulation was over values measured in C1.

Table 2: Correlation between PSTm content in mussel tissues at the end of the controlled laboratory experiment and the behavioural indicators measured in the controlled laboratory experiment before (T0) and during *A. catenella* exposition (T1), then and food addition (T2). Behavioural indicators included number of closures (NC), Total Closure Duration (TCD), Average Closure Duration (ACD), Average Valve Open Amplitude (AVOA), Number of Micro Closures (MC). In bold: significant correlation at 0.05, n = 19.

	Laboratory experiment			
	T1		***T2***	
	Rho	***p-v***	**Rho**	***p-v***
NC	-0.394	0.105	0.007	0.764
TCD	**-0.711**	**0.001**	-0.045	0.858
ACD	**-0.559**	**0.015**	0.099	0.693
AVOA	0.063	0.803	0.274	0.269
MC	-0.399	0.099	-0.238	0.339

2.2.4.2 Experiment 2: Natural Seawater experiment

For the natural seawater experiment, all data (i.e., valve gaping indicators, *A. catenella* concentrations, and seawater PST [PSTw]) are presented daily in Fig. 26. The most important valve gaping changes were observed when *A. catenella* was initially detected in the seawater (Fig. 26a-d), which corresponds to the onset of PSTm accumulation in the tissues of field mussels. Prior to day 25, PSTm concentration and *A. catenella* abundance were negligible (Fig. 26e).

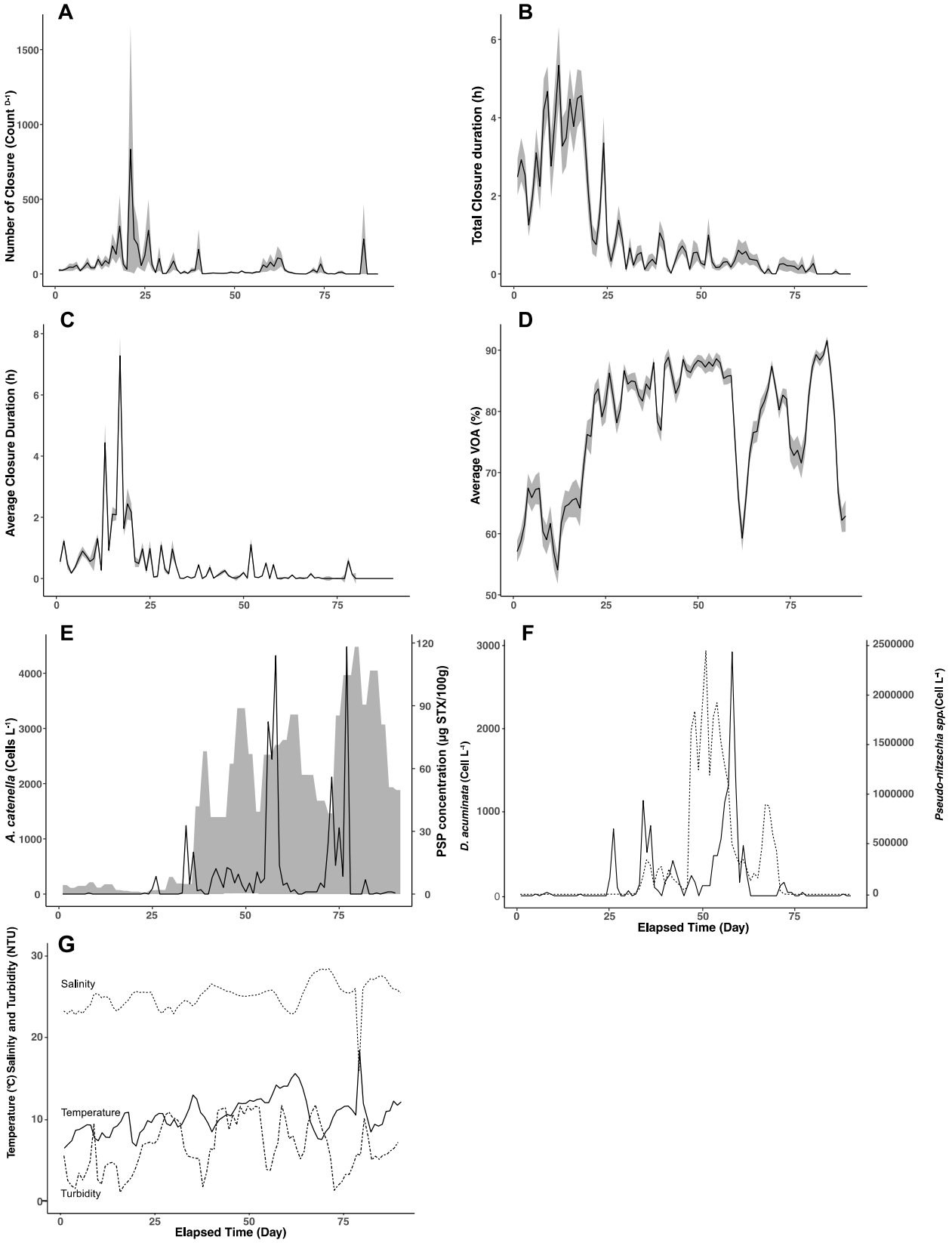
A
Number of Closure (Count D-1)
B
Total Closure duration (h)
C
Average Closure Duration (h)
D
Average VOA (%)
E
A. catenella (Cells L-1)
PSP concentration (µg STX/100g)
F
D. acuminata (Cell L-1)
Pseudo-nitzschia spp.(Cell L-1)
Elapsed Time (Day)
G
Temperature (°C) Salinity and Turbidity (NTU)
Salinity
Temperature
Turbidity
Elapsed Time (Day)

Figure 26 : Mussel gaping metrics and corresponding standard errors associated to running daily means (shaded areas) over the course of the 90-d experiment in natural seawater for A: NC - Number of Closures (Count day-1), B: TCD - Total Closure Duration (hour), C: ACD - Average Closure Duration (hour), and D: AVOA - Average Valve Open Amplitude (%). E: *A. catenella* abundance (cells litre^{-1}; solid line) in seawater and PST concentration in mussels from the shore (µg STXeq. 100g^{-1}; shaded area). F: *D. acuminate* (cells litre^{-1}; solid line) and *Pseudo-nitzschia* spp. (cells litre^{-1}; dotted line) abundances in seawater. G: Temperature (°C; solid line), Salinity (dotted line) and Turbidity (broken line).

The correlation between the behavioural indicators and the physico-chemical parameters (Fig. 26f) measured in the head tank are presented in Table 3. No microclosures were detected over the entire experiment. Average closure duration was not correlated with any of the three physicochemical parameters tested, while the number of closures was negatively correlated with turbidity. Salinity and temperature were negatively correlated with total closure duration and positively correlated with average valve open Amplitude, indicating that mussels opened more when temperature and salinity were high. Of the physicochemical parameters measured, salinity and the temperature were most strongly correlated with mussel gaping behaviour. The correlation test between the PST concentration in seawater and the physicochemical parameters show a positive correlation for the temperature (Rho = 0.397, p-v = 0.014) but not for the salinity and the turbidity (respectively Rho = -0.197, p-v = 0.241 and Rho = 0.035, p-v = 0.834) indicating a link between the *A. catenella* presence and the seawater temperature.

Tableau 3 : Correlations between the *M. edulis* gaping behaviour and the variability of physical and chemical parameters measured in the natural seawater from the head tank. Number of closures (NC), Total Closure Duration (TCD), Average Valve Open Amplitude (AVOA), n = 90

	NC		***TCD***		***AVOA***		***ACD***	
	Rho	***p-v***	**Rho**	***p-v***	**Rho**	***p-v***	**Rho**	***p-v***
Temperature	-0.122	0.254	**-0.443**	**0.000013**	**0.251**	**0.017**	-0.023	0.8481
Salinity	-0.144	0.176	**-0.377**	**0.00026**	**0.376**	**0.0002**	-0.0211	0.8629
Turbidity	**-0.335**	**0.04**	-0.265	0.108	0.208	0.209	-0.284	0.082

PST concentration measured in the field (PSTm) mussels was strongly correlated with all behavioural indicators. Herein, PSTm was negatively correlated with the number of closures and total closure duration, and positively correlated with average valve open amplitude and average closure duration. These correlations were stronger than those obtained with algae abundances (Table 4). The correlation between experimental mussel gaping indicators with *A. catenella*, *Dinophysis acuminata*, *Pseudo-nitzschia* spp., and the PST measured from the field mussels are presented in the Table 4. In the case of *D. acuminata*, a weak negative correlation was observed with total closure duration and a weak positive correlation with the average valve open amplitude (Fig. 26g), suggesting that mussels react little in presence of *D. acuminata*. The abundance of *Pseudo-nitzschia* spp. was positively correlated with average valve open amplitude and negatively correlated with total closure duration. Only total closure duration was negatively correlated with *A. catenella* abundance. The correlation between *A. catenella* and the behavioural indicators was lower than the correlation observed with *Pseudo-nitzschia* spp., suggesting a stronger reaction to *Pseudo-nitzschia* compared to *A. catenella* abundance. A similar trend was observed for *D. acuminata* and *Pseudo-nitzschia*, but the correlation was lower (respectively Rho = -0.218 and -0.218 for total closure duration and Rho = 0.195 and 0.282 for average VOA) than the

correlations between the behavioural indicators and *A. catenella*. No correlation between the behavioural indicators and the PST measured in the seawater with the Elisa test were noted.

Table: 4 Pearson correlation between the *M. edulis* gaping behaviour and the cells abundance (cells L^{-1}) of toxic algae in natural seawater measured in the head tank and PST concentration measured in field mussels (PSTm). Number of closures (NC), Total Closure Duration (TCD), Average Valve Open Amplitude (AVOA), Average Closure Duration (ACD), A.c: *Alexandrium catenella*, D.a: *Dinophysis acuminata*, P-N: *Pseudo-nitzschia* spp. In bold: significant correlation at 0.05, n=90

	NC		***TCD***		***AVOA***		***ACD***	
	Rho	***p-v***	**Rho**	***p-v***	**Rho**	***p-v***	**Rho**	***p-v***
PSP_m	**-0.526**	**0.00069**	**-0.675**	**0.0000033**	**0.429**	**0.0071**	**0.151**	**0.018**
A.c	-0.109	0.308	**-0.218**	**0.039**	0.195	0.066	-0.115	0.342
D.a	-0.0307	0.774	**-0.218**	**0.039**	**0.282**	**0.007**	-0.124	0.243
P-N	-0.202	0.057	**-0.280**	**0.007**	**0.415**	**5.2e-05**	-0.178	0.093

The boxplot of the fig. 27 shows the average VOA, the total closure duration, and the number of closures in function of the *A. catenella* abundance during the time windows shown on fig. 23b. Average VOA and total closure duration varied among the different *A. catenella* concentration. PERMANOVA analysis (respectively DF= 3 and 380, Pseudo-F= 166,39, P(MC)= 1e-04 and DF= 3 and 380, Pseudo-F= 226,29, P(MC)= 1e-04) and pairwise tests indicated A0 was effectively lower than A1 and A2 for the two indicators and A3 was significantly higher A1 and A2 also for the two indicators. No significant difference was observed for the number of closures.

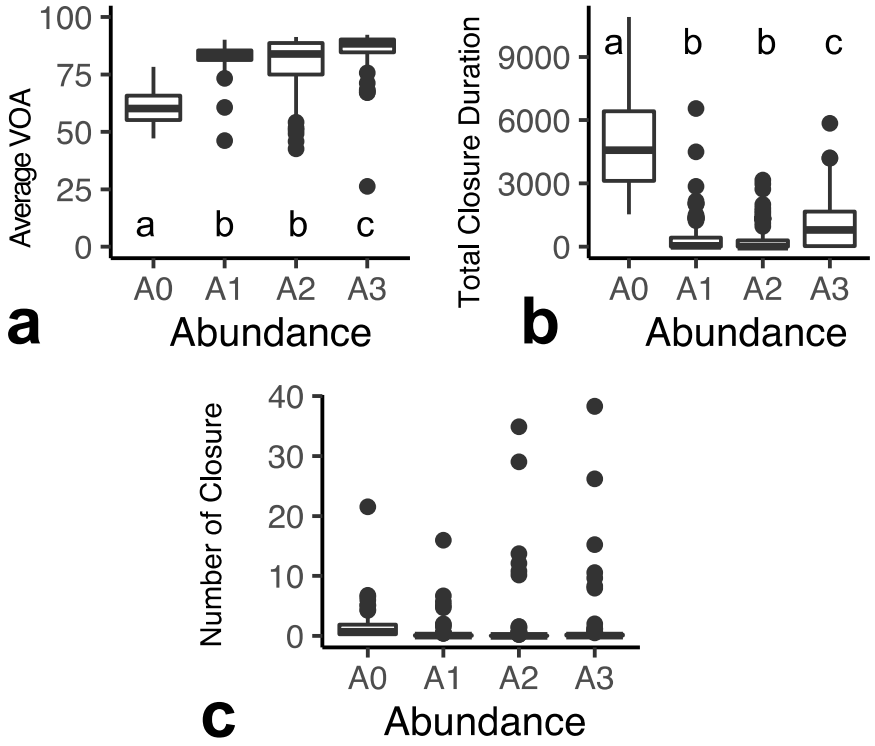

Figure 27 : Boxplot of the A) Average VOA, B) total closure duration and C) number of closures in function of the *A. catenella* abundance during the tested period of the time window of the fig 23b. A0: 0 cell L^{-1}, A1: 560 cell L^{-1} (SE=23.38), A2: 1190 cell L^{-1} (SE = 39.87), A3: 1680 cell L^{-1} (SE = 26.07).

In the natural sea water experiment, total closure duration and number of closures were negatively correlated with PST concentration in the littoral mussels (Fig. 25c). Average closure duration and average valve open amplitude were not enough strongly correlated with mussel PST concentration, as closure duration was too distant from the correlation circle, and valve amplitude was in a different direction (Fig. 25c). Thus, only the total closure duration and the number of Closure were selected to be tested with the univariate PERMANOVA analysis.

PERMANOVA revealed the presence of significant differences between the different PST concentrations for the number of closures (DF= 3 and 34, Pseudo-F=4.634, P(MC)=0.0086) and total closure duration (DF= 3 and 34, Pseudo-F= 13.67, P(MC)= 1e-04).

The pairwise test for number of closures indicated very significant effects of PST, with an increasing of closure, in the C2 (50 to 80 µg STXeq 100g-1, n = 9) and C3 (>80 µg STXeq 100g-1, n = 8) concentration range, with significant results for C1 (30 to 50 µg STXeq 100g-1, n = 6), relative to C0. For total closure duration, no significant difference was observed between C0 (<10 µg STXeq 100g-1, n = 15) and C1 or between C1 and C2; however, all other contrasts were significant. Additionally, multivariate PERMANOVA revealed a significant effect of mussel PST accumulation when these two behavioural indicators were treated simultaneously (DF=3 and 4, pseudo-F=13.67; P(MC)=0.0004). A pairwise test of this analysis indicated an effect of PST in concentration ranges >C1.

Contrary to the laboratory experiment, a SIMPROF test was possible for natural seawater experiment and showed two distinct groups (Fig. 25d). One group included only the C0 mussels, while the other group included C1, C2, and C3 mussels. Herein, C2 and C3 concentration ranges had the most similar effects on gaping behaviour, with C1 showing a slightly lower similarity to C2 and C3.

In figure 23, the actograms show the population-scale VOA time series alongside the water-level and photoperiod (Fig. 23a), *A. catenella* abundance (Fig. 23b) and PST concentration in mussel tissue (Fig. 23c). Firstly, the presence of tidal and daily rhythms of VOA was not detected by chronobiological analysis with the use of 4-days windows chosen to analyse rhythmicity of mussel gaping behaviour in relation to *A. catenella* abundance. However, for the PSP concentration, the 6-day windows used to compare the rhythmicity of mussels with the Lomb and Scargle periodogram showed a significant daily period of 23.8 h (Fig. 28a) for the concentration A0 (3.72 µg STXeq $100g^{-1}$ ± 0.84), and the rhythm was validated by the Cosinor model (p = 0.001) with a percent rhythm of 63.53%. After residues injection, the Lomb and Scargle periodogram showed a second significant period in the tidal range equal to 12.9 h, and the rhythm was validated by the Cosinor model (p = 0.028) with a percent rhythm of 45.6%. For mussels contaminated with the PSP concentration A1 (49 µg STXeq $100g^{-1}$ ± 16.85), no significant period was detected with the Lomb and Scargle periodogram. For the concentrations A2 (72.16 µg STXeq $100g^{-1}$ ± 14.47) and A3 (104.46

µg STXeq $100g^{-1}$ ± 13.84), the Lomb and Scargle detected respectively a 12.3 h and 12.5 h period. However, no rhythms were detected by the Cosinor model for the mussels intoxicated by *A. catenella*.

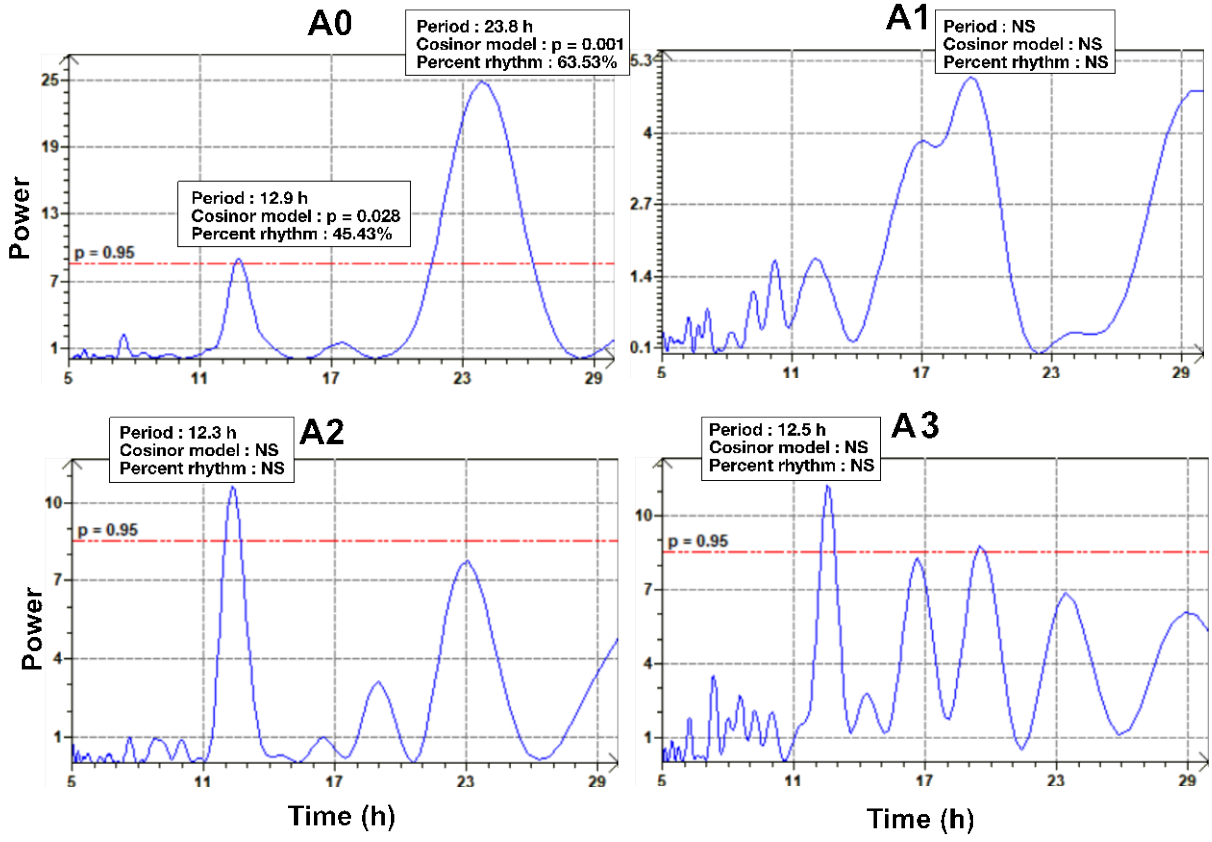

Figure 28 : Lomb and Scargle periodograms done on VOA data during the time windows of the fig 23c. A0: 3.71 µg STXeq $100g^{-1}$, A1: 49 µg STXeq $100g^{-1}$ (SE=4.1), A2: 72.16 µg STXeq $100g^{-1}$ (SE=3.8), A3: 104.48 µg STXeq $100g^{-1}$ (SE=3.72).

2.2.5 Discussion:

In both the laboratory and natural seawater experiments, behaviour indicators could be used to indicate the presence of toxic algae, like *A. catenella*. Particularly, the number of closures, total closure duration and average valve open amplitude were adequate indicators of toxic algae presence indicating an increasing of opening. Results of the laboratory experiment indicated that total closure duration and average closure duration were negatively

correlated with PST accumulated in mussels, indicating that mussels remained open longer as *A. catenella* accumulation increased. Likewise, a similar trend was observed in the natural seawater experiment, as the number of closures and total closure duration decreased, and as average valve open amplitude increased, with increasing PST accumulation in mussels (Table 4). These results confirm a longer period of valve opening when PSP concentration in mussel tissue is higher, and when PST contamination in mussels increases as compared to when *A. catenella* is absent.

Changes in mussels' gaping behaviour indicates a real impact of the algae *A. catenella* that is observable by monitoring valvometry. In the context of stressful conditions as chemical toxin or predator presence, mussels close their valves to be isolated from the external environment (Clements et al., 2021; Durier et al., 2021; Rajagopal et al., 1997). As such, a decrease in average valve opening amplitude and an increase in total closure duration and the number of closures, should be anticipated under stressful conditions. However, the results of our study reveal the inverse phenomenon when these animals are exposed to *A. catenella*. This response has been observed in other bivalve species exposed to toxic algae as well. For example, Tran et al. (2010) and Haberkorn et al. (2011) reported similar results for the oyster, *Crassostrea (Magallana) gigas*, exposed to PST-producing dinoflagellate, *Alexandrium minutum*. Bricelj et al. (2005) also observed increased in valve opening of soft-shell clams, *Mya arenaria*, exposed to PST from toxic dinoflagellates, *Alexandrium* spp.. The increased opening under the stress of toxic algae could be explained by a paralysis of the adductor muscle induced by PSP toxins, as demonstrated for multiple species of bivalves including *M. edulis* (Galimany et al., 2008) , *Crassostrea virginica* and *C. gigas* exposed to *A. catenella* and/or *A. fundyense* (Hégaret et al., 2007; Tran et al., 2010). Since mussels accumulate PSP toxins in the muscle, mantle, foot, and gills (Bricelj and Shumway, 1998), the aforementioned mechanism seems probable. Mechanistically, voltage-gated sodium channels can be blocked by the toxin, thus limiting action potential in the muscle and the nervous system (Hégaret et al., 2007).

The correlations between behavioural indicators and physico-chemical seawater parameters suggest that mussels may be sensitive to these parameters. Indeed, both temperature (Clements et al., 2021, 2018) and salinity are reported to affect valve gaping behaviour in bivalves but with more of closures contrary to our results who shown less of closure. However, *A. catenella* development in the Lower St. Lawrence Estuary is also associated with these physico-chemical parameters, in particular salinity and water temperature (Aguilera-Belmonte et al., 2013; Boivin-Rioux et al., 2021; Fauchot et al., 2005; Navarro et al., 2006; Starr et al., 2017). The optimal temperature for the development of this algae is ≈14°C (Boivin-Rioux et al., 2021), a temperature only present during summer in the St. Lawrence Estuary. Salinity also regulates *A. catenella* development, with growth increasing at higher salinity (Aguilera-Belmonte et al., 2013). In the St. Lawrence Estuary, salinity increases after the spring when snow melt stops, and precipitation decreases. Thus, the correlation between these physico-chemical parameters and the variation of the behavioural indicators could be, at least partly, explained by the ecological link between temperature, salinity, and *A. catenella* development in the Lower St-Lawrence Estuary. This idea is further supported by the correlation between seawater temperature and *A. catenella* abundance observed in the study, as well as correlations between behavioural indicators and PST accumulation in the laboratory experiment when temperature and salinity were stable.

Correlations between behavioural indicators and the abundance of the multiple algae species (*A. catenella*, *D. acuminata* and *Pseudo-nitzschia* spp., the two last don't producing PST) were evident in this study. Correlations were the strongest for *Pseudo-nitzschia* spp., suggesting that mussel gaping behaviour may be more strongly linked to *Pseudo-nitzschia* spp. than *A. catenella*. Despite the low correlations detected with the comparison of the mussel gaping behaviour with the *A. catenella* abundance during the 90 days of the experience, it was possible do identify differences between the gaping behaviour with the use of time windows corresponding at the different *A. catenella* abundances. The bioaccumulation of the toxins produced by *A. catenella* in mussels (Starr et al., 2017) and their subsequent detoxification have a half-life time between 5 days (Nielsen et al., 2016) and 20 days (Marcaillou et al., 2010), corresponding to the increased period of *A. catenella*

influence on mussel gaping behaviour observed in this study. This could explain the higher correlation coefficient for the relationship between gaping behaviour and PSP concentration in the mussels' tissue, as compared to the relationship between gaping behaviour and *A. catenella* in the seawater. This result was also observed with seawater PST concentration measured with the ELISA test. Thus, using PSP toxin concentration in mussel tissues makes it possible to determine the period where mussels and their gaping behaviour were affected. The domoic acid presence were not tested in this study, however, in St Lawrence Estuary the domoic acid is below the regulatory action level (20 µg g^{-1} shellfish tissue ; Bates, 2020). This indicating *the Pseudo-nitzschia* specie detected could be a non-toxic specie considering the abundance measured.

In the laboratory experiment, correlations between the behavioural indicators and the toxin concentration were weaker after *A. catenella* exposure (T2) than during *A. catenella* exposure (T1). This difference could be explained by the mussel detoxification process, and by mussels feeding on non-toxic alga (*T. suecica*) during T2. Previous studies indicate that mussel detoxification rates are high during the first days following intoxication (Blanco et al., 1997) and increase in mussels fed non-toxic algae (Marcaillou et al., 2010). Thus, a depuration period in the presence of non-toxic food can be expected to decrease the toxin concentration in the mussels' tissue and, thus, the potential for paralysis and effects on gaping behaviour.

Based on the analysis of behavioural indicators selected from PCA, is it possible to identify the PST concentration in mussel tissues whereby gaping behaviour is affected. Identifying this PST concentration allows for the suggestion of a detection threshold for the use of valvometry as an early warning system in detecting toxic algae in the environment. In the laboratory experiment, behavioural change was detected above a PST tissue concentration exceeding the regulatory harvest closure limit (>80 µg STXeq $100g^{-1}$). In the natural seawater experiment, behavioural change was detected with a lower PST concentration (>30 µg STXeq $100g^{-1}$). The difference between these detection thresholds could be explained by the duration of exposure to *A. catenella*, being longer in the natural

seawater experiment (1 day in laboratory conditions and between 3 to 21 days in natural environment conditions). The SIMPROF test revealed gradual change in mussel gaping behaviour in relation to PST accumulated in mussels until C2 concentration, as effects at C2 and C3 were similar. Given the detection of the threshold >30 µg STXeq $100g^{-1}$ under field conditions, valvometry may be considered as an early-warning system against toxic blooms. Under the field conditions of 2019, it provided a warning signal 10 days prior to PST levels reaching the regulatory harvest closure limit. The use of actogram could also early indicate the presence of *A. catenella*. Indeed, on the actogram of the fig. 23, it was possible to visually observe a behavioural change in mussels before their contamination at the detection threshold.

In this study, two techniques were used to detect PST in mussel tissues – valvometry and ELISA assays. Likewise, two techniques were used to detect PST in seawater (SRT test and ELISA assays). The use of these three techniques (valvometry, SRT and ELISA) allowed us to compare these methods as early-warning systems for toxic *A. catenella* presence. Considering a detection threshold of >30 µg STXeq $100g^{-1}$ in mussel tissues as observed in the natural seawater experiment (estimated with ELISA), valvometry is less sensitive than the SRT (0.2 µg STXeq $100g^{-1}$) and ELISA assay (0.0015 µg STXeq $100g^{-1}$). However, valvometry offered other advantages that the other two methods did not. Firstly, valvometry and ELISA are applied directly to mussels and consequently are sensitive to the depuration period, during which time a SRT test may not detect any toxic algae in seawater. Secondly, the use of valvometry is easier than both other tests, as once the mussels are equipped with the valvometer, no additional manipulations are required. The automation of data collection and signal processing should ultimately allow the detection of PST without much human intervention. Finally, the frequency of measurements provided by valvometry far exceeds that of both SRT and ELISA, increasing the temporal resolution at which toxic algae can be detected.

Generally, mussel species follow a tidal rhythm synchronized by the tides cycle and a daily rhythm linked to the day-night succession (Gnyubkin, 2010). Without *A. catenella*, the

mussels monitored during this study followed these rhythms. However, when the mussels were intoxicated by *A. catenella* and accumulated PSP, these two rhythms became non-significant. These results were in agreement with the previous studies on oysters *Crassostrea gigas* in the case of intoxication by *A. minutum* for the daily rhythm (Tran et al., 2015) and for the tidal rhythm (Mat et al. 2016). Thus, *A. catenella* impacted the biological rhythms of the mussels during a PSP intoxication, probably by the paralysis observed indicating that mussel becomes unable to follow the rhythm. This effect on rhythms could impact the metabolism and the physiology (Tran et al 2015; Mat et al. 2016).

2.2.6 Conclusion

We evaluated the potential use of the valvometry to detect changes in *M. edulis* gaping behaviour during an exposure to the toxic dinoflagellate *A. catenella* to determine the efficacy of valvometry to serve as an early warning system. Our results suggest that valvometry is adequate for detecting paralysis induced by PSP toxins produced by *A. catenella*, as the reduction of valve closure duration (i.e., increased valve opening) were indicative of toxic *A. catenella* presence. Herein, mussel gaping behaviour was rapidly impacted at the onset of PST accumulation. The fast reactivity of the mussels in presence of PST and the capacity of valvometry to detect these changes suggests that this technology can be used on this bivalve species to alert the presence of *A. catenella* blooms before they become problematic. As such, valvometry systems such as the HFNI valvometry biosensor linked to the GPRS (General Packet Radio Service ; Andrade et al., 2016), which wirelessly transmit real-time data, could be used as early warning systems by shellfish growers to quickly detect the presence of PST producing algae and take appropriate measures to minimize negative impacts to their operations.

Acknowledgments:

The authors thank the scientific group Ressources Aquatiques du Québec for their implication in this study and the help offered by the IML technicians.

Funding

This project was undertaken with the financial support of the Government of Canada, specifically by Environment and Climate Change Canada provided by RT's (FDE-CA-2017i002) and by the Ressources Aquatiques Québec research network (Fonds de Recherche du Québec – Nature et Technologies, no. 2014-RS-171172). Fisheries and Oceans Canada provided in-kind assistance that was motivated by the Galway Statement on Atlantic Ocean Cooperation.

2.2.7 References

Aguilera-Belmonte, A., Inostroza, I., Carrillo, K.S., Franco, J.M., Riobó, P., Gómez, P.I., 2013. The combined effect of salinity and temperature on the growth and toxin content of four Chilean strains of *Alexandrium catenella* (Whedon and Kofoid) Balech 1985 (Dinophyceae) isolated from an outbreak occurring in southern Chile in 2009. Harmful Algae 23, 55–59. https://doi.org/10.1016/j.hal.2012.12.006

Almada-Villela, P.C., Davenport, J., Gruffydd, L.D., 1982. The effects of temperature on the shell growth of young *Mytilus edulis* L. Journal. of Experimental. Marine. Biology. and Ecology. 59, 275–288. https://doi.org/10.1016/0022-0981(82)90121-6

Andersen, P., Throndsen, J., 2004. Estimating cell numbers. In: Manual on Harmful Marine Microalgae. Hallegraeff G.M., Anderson, Cembella A.D. pp. 99–129. UNESCO Publishing, Paris, ISBN 92-3-103948-2

Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., 2011. Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. Annual Review of Marine Science 4, 143–176. https://doi.org/10.1146/annurev-marine-120308-081121

Anderson, M., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA + for PRIMER user manual 1, 1:218.

Andrade, H., Massabuau, J.-C., Cochrane, S., Ciret, P., Tran, D., Sow, M., Camus, L., 2016. High frequency non-invasive (HFNI) bio-sensors as a potential tool for marine monitoring and assessments. Frontier in Marine Science 3, 1–10. https://doi.org/10.3389/fmars.2016.00187

Bates, S.S., Beach, Daniel G.; Comeau, Luc. A.; Haigh, Nicola; Lewis, Nancy I.; Locke, Andrea; Martin, Jennifer L.; McCarron, Pearse; McKenzie, Cynthia H.; Michel, Christine; Miles, Christopher O.; Poulin, Michel; Quilliam, Michael A.; Rourke, Wade A.; Scarratt, Michael G.; Starr, Michel; Wells, Terri; Fisheries and Oceans Canada 2020. Marine harmful algal blooms and phycotoxins of concern to Canada. Canadian Technical Report of Fisheries and Aquatic Science. 3384, 322p

Bayne, B.L., 1976. Marine mussels: their ecology and physiology. Cambridge University Press, Cambridge. 506p.

Bérard-Therriault, L., Poulin, M., & Bossé, L. (1999). Guide d'identification du phytoplancton marin de l'estuaire et du golfe du Saint-Laurent: incluant également certains protozoaires. Canadian Speial Publication of Fisheries and Aquatic Science 128, 387p

Blanco, J., Morono, A., Franco, J., Reyero, M.I., 1997. PSP detoxification kinetics in the mussel *Mytilus galloprovincialis*. One- and two-compartment models and the effect of some environmental variables. Marine. Ecology. Progress. Series. 158, 165–175. https://doi.org/10.3354/meps158165

Boivin-Rioux, A., Starr, M., Chassé, J., Scarratt, M., Perrie, W., Long, Z., 2021. predicting the effects of climate change on the occurrence of the toxic dinoflagellate *Alexandrium catenella* along canada's east coast. Frontier in Marine Science 7, 1–20. https://doi.org/10.3389/fmars.2020.608021

Bricelj, V.M., Connell, L., Konoki, K., MacQuarrie, S.P., Scheuer, T., Catterall, W.A., Trainer, V.L., 2005. Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434, 763–767. https://doi.org/10.1038/nature03415

Bricelj, V.M., Lee, J.H., Cembella, A.D., Anderson, D.M., 1990. Uptake kinetics of paralytic shellfish toxins from the dinoflagellate *Alexandrium fundyense* in the mussel *Mytilus edulis*. Marine Ecology Progress Series. 63, 177–188.

Bricelj, V. M., & Shumway, S. E. (1998). Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. Reviews in Fisheries Science 6(4), 315-383.

Cembella, A.D., Quilliam, M.A., Lewis, N.I., Bauder, A.G., Aversano, C.D., Thomas, K., Jellett, J., Cusack, R.R., 2002. The toxigenic marine dinoflagellate *Alexandrium tamarense* as the probable cause of mortality of caged salmon in Nova Scotia. Harmful Algae 1, 313–325.

Clements, J.C., Comeau, L.A., 2019. Use of high-frequency noninvasive electromagnetic biosensors to detect ocean acidification effects on shellfish behavior. Journal of Shellfish Research. 38, 811–818. https://doi.org/10.2983/035.038.0330

Clements, J.C., Hicks, C., Tremblay, R., Comeau, L.A., 2018. Elevated seawater temperature, not pCO2, negatively affects post-spawning adult mussels (*Mytilus edulis*) under food limitation. Conservation Physiology. 6, 1–14. https://doi.org/10.1093/conphys/cox078

Clements, J. C., Ramesh, K., Nysveen, J., Dupont, S., & Jutfelt, F. 2021. Animal size and sea water temperature, but not pH, influence a repeatable startle response behaviour in a wide-ranging marine mollusc. Animal Behaviour, 173, 191-205.

Comeau, L.A., 2014. Spring awakening temperature and survival of sediment-covered eastern oysters *Crassostrea virginica*. Aquaculture 430, 188–194. https://doi.org/10.1016/j.aquaculture.2014.04.009

Comeau, L.A., Babarro, J.M.F., Riobó, P., Scarratt, M., Starr, M., Tremblay, R., 2019. PSP-producing dinoflagellate *Alexandrium minutum* induces valve microclosures in the mussel Mytilus galloprovincialis. Aquaculture 500, 407–413. https://doi.org/10.1016/j.aquaculture.2018.10.025

Dell'Aversano, C., Hess, P., Quilliam, M.A., 2005. Hydrophilic interaction liquid chromatography-mass spectrometry for the analysis of paralytic shellfish poisoning (PSP) toxins. Journal of Chromatography A. 1081, 190–201. https://doi.org/10.1016/j.chroma.2005.05.056

Dray, S., Dufour, A.B., 2007. The ade4 package: Implementing the duality diagram for ecologists. Journal of Statistical Software. 22, 1–20. https://doi.org/10.18637/jss.v022.i04

Durier, G., Nadalini, J.B., Saint-Louis, R., Genard, B., Comeau, L.A., Tremblay, R., 2021. Sensitivity to oil dispersants: Effects on the valve movements of the blue mussel *Mytilus edulis* and the giant scallop *Placopecten magellanicus*, in sub-arctic conditions. Aquatic Toxicology. 234, 105797. https://doi.org/10.1016/j.aquatox.2021.105797

FAO, 2016. la situation pêches et de l'aquaculture 2016., Contribuer à la sécurité alimentaire et à la nutrition de tous. Rome, 224p https://doi.org/10.1136/qshc.2005.015107

Fauchot, J., Levasseur, M., Roy, S., Gagnon, R., Weise, A.M., 2005. Environmental factors controlling *Alexandrium tamarense* (dinophyceae) growth rate during a red tide event in the St. Lawrence Estuary (Canada). Journal. of Phycology. 41, 263–272. https://doi.org/10.1111/j.1529-8817.2005.03092.x

Galimany, E., Sunila, I., Hégaret, H., Ramón, M., Wikfors, G.H., 2008. Experimental exposure of the blue mussel (*Mytilus edulis*, L.) to the toxic dinoflagellate *Alexandrium fundyense*: Histopathology, immune responses, and recovery. Harmful Algae 7, 702–711. https://doi.org/10.1016/j.hal.2008.02.006

Gnyubkin, V.F., 2010. The circadian rhythms of valve movements in the mussel *Mytilus galloprovincialis*. Russian Journal of Marine Biology. 36, 419–428. https://doi.org/10.1134/S1063074010060039

Gouthiere, L., Mauvieux, B., Davenne, D., Waterhouse, J., 2005. Complementary methodology in the analysis of rhythmic data, using examples from a complex situation, the rhythmicity of temperature in night shift workers. Biological Rhythm Research. 36, 177–193.

Granéli, E. (2006). Ecology of harmful algae (Vol. 189, p. 406). J. T. Turner (Ed.). Berlin: Springer.

Guillard, R. R. (1975). Culture of phytoplankton for feeding marine invertebrates. In Culture of marine invertebrate animals (pp. 29-60). Smith, W.L. Chanley. M.H, Plenum Press New York.

Haberkorn, H., Tran, D., Massabuau, J.C., Ciret, P., Savar, V., Soudant, P., 2011. Relationship between valve activity, microalgae concentration in the water and toxin accumulation in the digestive gland of the Pacific oyster *Crassostrea gigas* exposed to *Alexandrium minutum*. Marine Pollution Bulletin. 62, 1191–1197. https://doi.org/10.1016/j.marpolbul.2011.03.034

Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. Physiological Reviews. 32, 79–99.

Hégaret, H., Wikfors, G.H., Soudant, P., Lambert, C., Shumway, S.E., Bérard, J.B., Lassus, P., 2007. Toxic dinoflagellates (*Alexandrium fundyense* and *A. catenella*) have

minimal apparent effects on oyster hemocytes. Marine Biology. 152, 441–447. https://doi.org/10.1007/s00227-007-0703-3

James, K.J., Carey, B., O'Halloran, J., Van Pelt, F.N.A.M., Škrabáková, Z., 2010. Shellfish toxicity: Human health implications of marine algal toxins. Epidemiology Infection. 138, 927–940. https://doi.org/10.1017/S0950268810000853

Jellett, J.F., Roberts, R.L., Laycock, M. V., Quilliam, M.A., Barrett, R.E., 2002. Detection of paralytic shellfish poisoning (PSP) toxins in shellfish tissue using MIST AlertTM, a new rapid test, in parallel with the regulatory AOAC® mouse bioassay. Toxicon 40, 1407–1425. https://doi.org/10.1016/S0041-0101(02)00153-8

Larocque, R., Cembella, A.D., 1990. Ecological parameters associated with the seasonal occurrence of *Alexandrium* spp. and consequent shellfish toxicity in the lower St. Lawrence Estuary(eastern Canada). In Granéli, E.P, Sundstöm, B. Edler, L. Anderson; D.M. (eds), Toxic Marine Phytoplankton, Elsevier, Ney York, pp. 368-373.

Lassus, P., Bardouil, M., Beliaeff, B., Masselin, P., Naviner, M., Truquet, P., 1999. Effect of a continuous supply of the toxic dinoflagellate *Alexandrium minutum* Balim on the feeding behavior of the Pacific oyster (*Crassostrea gigas* Thunberg). Journal. of Shellfish Research. 18, 211_216.

Lilly, E.L., Halanych, K.M., Anderson, D.M., 2007. Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). Journal. of Phycology. 43, 1329–1338. https://doi.org/10.1111/j.1529-8817.2007.00420.x

Lomb, N. R. (1976). Least-squares frequency analysis of unequally spaced data. Astrophysics and Space science, 39(2), 447-462.

Marcaillou, C., Haure, J., Mondeguer, F., Courcoux, A., Dupuy, B., Pénisson, C., 2010. Effect of food supply on the detoxification in the blue mussel, *Mytilus edulis*, contaminated by diarrhetic shellfish toxins. Aquatic. Living Resources. 23, 255–266. https://doi.org/10.1051/alr/2010026

Martinez Arbizu, P., 2019. Pairwiseadonis: Pairwise multilevel comparison using adonis. R Packag. version 0.3.

Mat, A.M., Perrigault, M., Massabuau, J.-C., Tran, D., 2016. Role and expression of cry1 in the adductor muscle of the oyster *Crassostrea gigas* during daily and tidal valve activity rhythms. Chronobiology International. 33, 949–963.

Mathiesen, S. S., Thyrring, J., Hemmer-Hansen, J., Berge, J., Sukhotin, A., Leopold, P., ... & Nielsen, E. E. (2017). Genetic diversity and connectivity within *Mytilus spp*. in the subarctic and Arctic. Evolutionary Applications, 10(1), 39-55.

McKenzie, C.H., Bates, S.S., Martin, J.L., Haigh, N., Howland, K.L., Lewis, N.I., Locke, A., Peña, A., Poulin, M., Rochon, A., Rourke, W.A., Scarratt, M.G., Starr, M., Wells, T., 2021. Three decades of Canadian marine harmful algal events: Phytoplankton and phycotoxins of concern to human and ecosystem health. Harmful Algae 102. https://doi.org/10.1016/j.hal.2020.101852

Nagai, K., Honjo, T., Go, J., Yamashita, H., Seok Jin Oh, 2006. Detecting the shellfish killer *Heterocapsa circularisquama* (Dinophyceae) by measuring bivalve valve activity with a Hall element sensor. Aquaculture 255, 395–401. https://doi.org/10.1016/j.aquaculture.2005.12.018

Navarro, J.M., Muñoz, M.G., Contreras, A.M., 2006. Temperature as a factor regulating growth and toxin content in the dinoflagellate *Alexandrium catenella*. Harmful Algae 5, 762–769. https://doi.org/10.1016/j.hal.2006.04.001

Nielsen, L.T., Hansen, P.J., Krock, B., Vismann, B., 2016. Accumulation, transformation and breakdown of DSP toxins from the toxic dinoflagellate *Dinophysis acuta* in blue mussels, *Mytilus edulis*. Toxicon 117, 84–93. https://doi.org/10.1016/j.toxicon.2016.03.021

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs,

E., Maintainer, H.W., 2019. Community Ecology Package: ordination, diversity and dissimilarities. vegan Community Ecology Package. 2, 1–297.

Parkhill, J.P., Cembella, A.D., 1999. Effects of salinity, light and inorganic nitrogen on growth and toxigenicity of the marine dinoflagellate *Alexandrium tamarense* from northeastern Canada. Journal. Plankton Research. 21, 939–955. https://doi.org/10.1093/plankt/21.5.939

Payton, L., Sow, M., Massabuau, J.-C., Ciret, P., Tran, D., 2017. How annual course of photoperiod shapes seasonal behavior of diploid and triploid oysters, *Crassostrea gigas*. PLoS One 12, e0185918.

R Core Team, 2020. R.

Rajagopal, S., Van Der Velde, G., Jenner, H.A., 1997. Shell valve movement response of dark false mussel, *Mytilopsis leucophaeta*, to chlorination. Water Research 31, 3187–3190. https://doi.org/10.1016/S0043-1354(97)00163-2

Riisgård, H.U., Lassen, J., Kittner, C., 2006. Valve-gape response times in mussels (*Mytilus edulis*) - Effects of laboratory preceding-feeding conditions and in situ tidally induced variation in phytoplankton biomass. Journal of Shellfish Research. 25, 901–911. https://doi.org/10.2983/0730-8000(2006)25[901:VRTIMM]2.0.CO;2

Robson, A., Wilson, R., De Leaniz, C.G., 2007. Mussels flexing their muscles: A new method for quantifying bivalve behaviour. Marine Biology. 151, 1195–1204. https://doi.org/10.1007/s00227-006-0566-z

Robson, A. A., Garcia De Leaniz, C., Wilson, R. P., & Halsey, L. G. (2010). Behavioural adaptations of mussels to varying levels of food availability and predation risk. Journal of Molluscan Studies, 76(4), 348-353.

Roelke, D.L., 2007. Ecology of Harmful Algae, Eos, Transactions American Geophysical Union. https://doi.org/10.1029/2007eo300006

Scargle, J. D. (1982). Studies in astronomical time series analysis. II-Statistical aspects of spectral analysis of unevenly spaced data. The Astrophysical Journal, 263, 835-853.

Sonier, R., Comeau, L.A., Tremblay, R., Olivier, F., Meziane, T., Genard, B., 2020. *Mytilus edulis* and *Styela clava* assimilate picophytoplankton carbon through feces and pseudofeces ingestion. Aquaculture 531, 735868. https://doi.org/10.1016/.aquaculture.2020.735868

Starr, M., Lair, S., Michaud, S., Scarratt, M., Quilliam, M., Lefaivre, D., Robert, M., Wotherspoon, A., Michaud, R., Ménard, N., Sauvé, G., Lessard, S., Béland, P., Measures, L., 2017. Multispecies mass mortality of marine fauna linked to a toxic dinoflagellate bloom. PLoS One 12, 1–18. https://doi.org/10.1371/journal.pone.0176299

Therriault, J.C., Painchaud, J., Levasseur, M., 1985. Factors controlling the occurrence of *Protogonyaulax tamarensis* and shellfish toxicity in the St. Lawrence Estuary: freshwater runoff and the stability of the water column. Toxic Dinoflagate. Elsevier, New York 41–51.

Tran, D., Ciret, P., Ciutat, A., Durrieu, G., Massabuau, J.C., 2003. Estimation of potential and limits of bivalve closure response to detect contaminants: Application to cadmium. Environmental Toxicology and Chemistry. 22, 914–920. https://doi.org/10.1897/1551-5028(2003)022<0914:EOPALO>2.0.CO;2

Tran, D., Ciutat, A., Mat, A., Massabuau, J.C., Hégaret, H., Lambert, C., Le Goic, N., Soudant, P., 2015a. The toxic dinoflagellate Alexandrium minutum disrupts daily rhythmic activities at gene transcription, physiological and behavioral levels in the oyster *Crassostrea gigas*. Aquatic Toxicology. 158, 41–49. https://doi.org/10.1016/j.aquatox.2014.10.023

Tran, D., Haberkorn, H., Soudant, P., Ciret, P., Massabuau, J.C., 2010. Behavioral responses of *Crassostrea gigas* exposed to the harmful algae *Alexandrium minutum*. Aquaculture 298, 338–345. https://doi.org/10.1016/j.aquaculture.2009.10.030

Tremblay, R., Landry, T., Leblanc, N., Pernet, F., Barkhouse, C., Sévigny, J., 2011. Physiological and biochemical indicators of mussel seed quality in relation to temperatures. Aquatic Living Resources. 282, 273–282.

Trottet, A., Roy, S., Tamigneaux, E., Lovejoy, C., Tremblay, R., 2008. Impact of suspended mussels (*Mytilus edulis* L.) on plankton communities in a Magdalen Islands lagoon (Québec, Canada): A mesocosm approach. Journal of Experimental Marine Biology and Ecology. 365, 103–115. https://doi.org/10.1016/j.jembe.2008.08.001

2.3 RESUME EN FRANÇAIS DU TROISIEME ARTICLE

Par leur situation, les fermes aquacoles peuvent être exposées aux efflorescences d'algues toxique. Les Paralytic Sellfish Toxines (PST) produites par le dinoflagellé *Alexandrium catenella* causent de nombreuses fermetures de la récolte des bivalves, ces espèces filtrantes, qui accumulent les toxines dangereuses pour les humains. Pour limiter les pertes économiques, la détection des PST avant que leur concentration ne dépasse le seuil critique pour la récolte des bivalves peut être intéressante. La détection d'algues toxiques est possible grâce à l'utilisation d'un système de détection précoce basé sur le comportement valvaire de la moule bleue *Mytilus edulis*. Cependant, certaines études récentes ont révélé la présence d'une résistance chez des population de mye régulièrement exposées aux PST. Si cette résistance est observée sur le comportement de la moule bleue, cette espèce pourrait ne pas être appropriée pour être utilisée comme espèce sentinelle. Dans cette étude, le comportement valvaire de deux populations de moules avec une passé d'exposition à des évènements de PST différents (régulièrement ou jamais exposées à *A. catenella* produisant ces PST) ont été comparées lors d'une exposition expérimentale à *A. catenella*. Nous avons observé que les moules des deux populations montraient le même comportement valvaire lors d'une exposition à *A. catenella*. Donc nos résultats confirment la possibilité d'utiliser *Mytilus sp.* comme sentinelle sans discrimination de l'origine pour la détection de PST dans les fermes aquacoles.

2.4 IMPACT OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM CATENELLA* ON THE VALVE BEHAVIOUR OF BLUE MUSSELS (*MYTILUS EDULIS*): A COMPARISON BETWEEN TWO POPULATIONS WITH CONTRASTING HISTORIES OF PST EXPOSURE

Guillaume Durier[a],*, Luc A. Comeau[b], José M.F. Babarro[c], Michel Starr[d], Jeff C. Clements[b], Réjean Tremblay[a]

[a]Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada

[b]Fisheries and Oceans Canada, Gulf Region Center, Moncton, NB, Canada

[c]Instituto de Investigaciones Marinas-CSIC, Vigo, Spain

[d]Institut Maurice-Lamontagne, Pêches et Océans Canada, Région du Québec, Mont-Joli, Québec, Canada

Keywords: Early warning system; Biotoxins; Harmful algal blooms (HABs); Paralytic shellfish poisoning (PSP), Valvometry, PSP resistance.

2.4.1 Abstract:

By their coastal situation, shellfish aquaculture farms can be exposed to harmful algal blooms. The Paralytic Shellfish Toxins (PST) produced by the dinoflagellate *Alexandrium catenella* cause regular closure of bivalves harvesting, as these filter species accumulate toxins that are poisonous for human. To minimize these economic losses, detection of PST before reaching the critical threshold for the harvesting closure will be a valuable solution. Toxic algae detection is possible with the use of an early warning system based on the valve-gaping behaviour of blue mussel *Mytilus edulis*. However, some studies observed presence of toxin resistance in clam populations when they are regularly exposed to PST. If the resistance is observed on blue mussel behaviour, this species could be inappropriate as a sentinel candidate. In this study the gaping behaviour of two mussel populations with contrasting long-term histories of PST events (regularly or not previously exposed to the PST

producer *A. catenella*) have been compared during experimental exposure to *A. catenella*. We observed that mussels from both populations showed similar gaping behaviour pattern when exposed to *A. catenella*. Thus, our results confirm the possibility to use *Mytilus sp.* without origin discrimination as sentinel for the early detection of PST in the aquaculture farms.

2.4.2 Introduction

PST (Paralytic Shellfish Toxin) are toxins produced by the dinoflagellate *Alexandrium catenella* (Anderson et al., 1998). Found along the eastern coast of North America (Lilly et al., 2007) during the summer (Blasco et al., 2003) in relation to salinity and temperature variability, the main physico-chemical parameters regulating the presence of this species (Aguilera-Belmonte et al., 2013; Boivin-Rioux et al., 2021; Fauchot et al., 2005; Navarro et al., 2006; Starr et al., 2017). PSTs are responsible for important economic losses in aquaculture, particularly for shellfish culture, as these species accumulate dinoflagellate and their neurotoxins by their filtration-feeding process posing a severe human health risk when they are consumed (Matsuyama and Shumway, 2009). Thus, during outbreaks of PST, shellfish commercialization is closed until depuration process lower toxicity below the authorized limit which require several weeks or months. In order to minimize the economic losses due to PST, development of early warning systems of toxic algae is needed (Kramer and Foekema, 2001). Recent studies suggest that deploying *in situ* valvometry sensors on sentinel species may provide an early warning of harmful algal blooms (Andrewartha and Elliott, 2015; Comeau et al., 2019). Bivalves, particularly the blue mussels, *Mytilus sp*. are interesting as sentinel as their valve-gaping behaviour is very sensitive to harmful algae, by their facility to be transferred between area and their resistance to contaminated environment (Dame and Kenneth, 2011; Martin and Richardson, 1991).

A. catenella exposure induced gaping behaviour changes in mussels, particularly with longer valve opening (yawning) suggesting partial and temporary muscle paralysis (Durier

et al., submitted article). As reported, this behavioural indicator is detected at low HAB concentration, up to two weeks before toxin accumulation in mussels induce harvesting closure. However, studies on soft-shell clams, *Mya arenaria*, revealed the development of PST resistance (Bricelj et al., 2005, 2004; MacQuarrie and Bricelj, 2008) translated into a diminution of paralysis researched for the development of an early warning system using valve-gaping behaviour. Thus we need to know if mussels could develop this kind of resistance that could be problematic for the sensitivity of the system. To validate potential use of *Mytilus sp*. for early warning system development based on valvometry technology, we compared gaping behaviour of mussels originating from two different area: one never exposed to *A. catenella* in 20 years and one area exposed yearly to important *A. catenella* blooms. The objective was to determine whether gaping response to an *A. catenella* exposure varied among mussels with different histories of exposure to test the hypothesis of an absence of PST adaptive behaviour in blue mussels.

2.4.3 Materials and method

2.4.3.1 Animal collection

To compare the gaping behaviour of mussels never exposed to *A. catenella*, we used two-years-old blue mussels *M. edulis* (63.45±2.79 mm SE) from a mussel lease in St. Peter's Bay, Prince Edward Island, Canada (46º 26' 30.7" N, 62º 44' 51.3" W) in October 2021. Over 20 the last years, this HAB species has never been documented in this area (Bates, 2020). The mussels were transported on ice to the Station Aquicole from Pointe-au-Père (Institut des Sciences de la Mer, Rimouski, Canada). Mussels (61.27±3.60 mm SE) regularly exposed to *A. catanella* were collected in October 2021 in the St Lawrence Estuary at Métis bay (48° 40' 49.10" N, 68° 1 '58.20" W). These mussels are exposed annually to *A. catenella* in summer between June to September (Boivin-Rioux et al., 2021; Parkhill and Cembella, 1999). The mussels from each origin were acclimated during 4 weeks to laboratory condition and placed in 300 L maintenance tanks filled with 1-µm filtered seawater continuously aerated, a salinity ≈ 28 and a natural photoperiod. Mussels were daily fed with live

Tisochrysis lutea CCMP 1324, *Chaetoceros muelleri* CCMP 1316, and *Pavlova lutheri* CCMP 1325 (1:1:1) at a rate of 60 000 cells L^{-1} $mussel^{-1}$. Algae were batch-cultured in f/2 medium (with Si for the diatom *C. muelleri*) (Guillard, 1975) at 18°C, under continuous illumination, in 20 L carbons supplied continuously with CO_2 to maintain a pH of ≈ 8 and a light intensity of 100 µm m^{-2} s^{-1}. Cell counts were determined with a Multisizer 4e Beckman Coulter counter with a 50-µm pore orifice.

The *A. catenella* strain used during the experiment was the "AT6" isolated from the St. Lawrence Estuary at the Maurice Lamontagne Institute (Department of Fisheries and Oceans; DFO) during a red tide event that occurred in 2008. The toxin concentration of this strain was measured and showed values between 3 to 60 pg STXeq cell–1. *A. catenella* was cultured in f/2 medium without Si under continuous illumination with a photosynthetic active radiation of 100 µmol quanta m^{-2} s^{-1}, a temperature of 18°C and CO2 addition to maintain a pH of 8.

2.4.3.2 Experimental design

After the acclimation period, 36 mussels were connected to the valvometry monitoring system (Comeau et al., 2019; Nagai et al., 2006) before the beginning of the experiment. Monitored mussels were distributed in 6 conic tanks containing each 6 mussels (3 mussels from St. Lawrence Estuary and 3 others from Prince Edward Island) filled with 75L of 1µm filtered seawater (Fig. 29). The experiment was conducted over 5 days and mussels were maintained at 18°C, intermediate between the optima of both species, i.e 20°C for mussels (Almada-Villela et al., 1982) and 14°C for *A. catenella* (Boivin-Rioux et al., 2021). The first 24h (T0 period) were used to obtain a baseline gaping behaviour without algae addition. After this 24h period, *A. catenella* was added to expose mussels to 10 000 cells L^{-1} during 42h (T1 period), the aeration system maintained the cells suspended in the water column. Then, water from tanks was renewed and mussels were exposed to *Tetraselmis suecica* (5,000 cell mL^{-1}), a non-toxic algae, during 24h (T2 period). The gaping behaviour was continuously monitored from the T0 to teh T2 period.

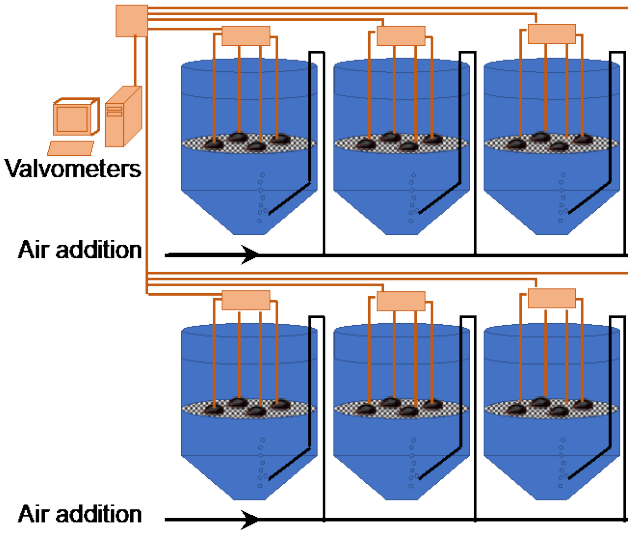

Figure 29 : Experimental design used during the experiment with 8 tanks for mussels of mixed origins (St. Lawrence estuary and Prince Edward Island).

2.4.3.3 Valvometry

To record the mussel gaping behaviour, Hall element sensor (HW-300a, Asahi Kasei, Japan; 0.5 g) were glued on a mussel valve and a magnet was glued on the other valve. using the Solarez® UV epoxy resin (Wahoo International, CA, USA). This resin polymerizes rapidly under UV light. The sensors were connected to 4-channel dynamic strain recorders (DC-204 R, Tokyo Sokki Kenkyujo Co., Japan) equipped with memory cards. The magnetic flux between the sensor and the magnet was converted in voltage values recorded in the memory card. A target of ≈ -80,000 µV were used for the fixation of the sensor and the magnet on the valves of the mussels. We used 10 measures sec^{-1} to obtain high resolution of mussel gaping behaviour. Data were treated with R statistical software version 1.4.1717 (R Core Team, 2021) and converted from µV to Valve Opening Amplitude (VOA) calculated as:

$$VOA = [(opening-min) / (max-min)] \times 100$$

VOA is the valve opening amplitude in % at a given time. Max and min corresponded respectively to the maximum and the minimum opening in µV measured during the experiment. Five behavioural indicators were calculated from the VOA, the number of closures, the total closure duration, the average VOA, the average closure duration and the number of micro-closures (Comeau et al., 2019). Mussels were considered as closed when VOA was <10% of max. The micro-closures were defined by a 3% reduction of VOA within 0.1 sec. These different indicators were compared for mussels of both population for each experimental period (T0, T1 and T2).

2.4.3.4 Statistical analysis

All the statistical analysis were realized with R software using univariate two-way permutation analyses of variance (PERMANOVA – package vegan; (Oksanen et al., 2019)), because of the non-normal distribution of the database. If a significant effect was detected, a posteriori PERMANOVA pairwise test was used (Martinez Arbizu, 2019). Periods (T0, T1, T2) and mussel population (St. Lawrence Estuary and Prince Edward Island) were the factors compared to observe potential difference for each of the 5 behavioural indicators tested.

2.4.4 Results

2.4.4.1 Behaviour indicators variation

For each behaviour indicator, no interaction between experimental period (T0, T1 and T2) and mussel population was observed (Table 5). The absence of interaction between the two parameters studied indicate the possibility to analyze these parameters independently. Effect of mussel population was non-significant for all behaviour indicators measured with the exception of the average VOA (Table 5) showing higher values for mussels from St. Lawrence Estuary regularly exposed to toxic cells compared to the PEI population. Some

other differences were observed before, during and after *A. catenella* exposure, for the number of closure and total closure duration (Fig. 30). Number of closures and total closure duration showed similar trend with an increase of values at T1 during the *A. catenella* exposure with a decreasing of the opening.

Table 5: PERMANOVA results for the comparison of Prince Edward Island and St. Lawrence Estuary mussel behaviour for each behavioural indicator.

Indicator	Factor	DF	Pseudo-F	p-v
Number of Closure	Mussel population	1	3.366	0.07
	Period	**2**	**10.632**	**0.0001**
	Interaction	2	0.960	0.403
Total Closure Duration	Mussel population	1	0.225	0.637
	Period	**2**	**16.958**	**0.0001**
	Interaction	2	0.285	0.754
Average VOA	Mussel population	**1**	**19.49**	**0.0001**
	Period	**2**	**16.726**	**0.0001**
	Interaction	2	1.208	0.303
Microclosure	Mussel population	1	0.041	0.949
	Period	2	0.969	0.457
	Interaction	2	0.464	0.808
Average Closure Duration	Mussel population	1	0.809	0.55
	Period	2	1.505	0.123
	Interaction	2	0.546	0.824

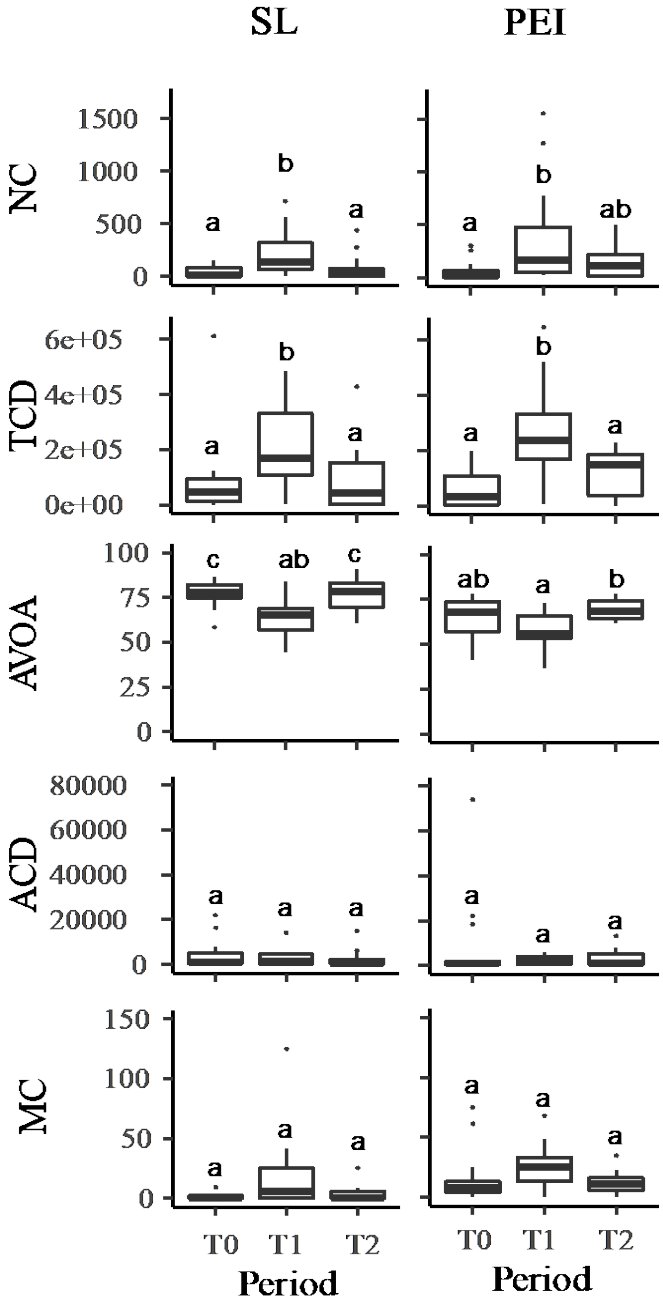

Figure 30 : Mussel's behavioural indicators exposed to toxic algae for mussels from St. Lawrence estuary (SL) and Prince Edward Island (PEI). NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average Valve Opening Amlitude. MC: Microclosure. T0: Acclimation and reference behaviour, T1: *A. catenella* exposition, T2: food addition

2.4.5 Discussion

Mussels of both origins showed similar changes of their gaping behaviour during the exposure to *A. catenella* suggesting an impact of *A. catenella*. Indeed, changes were observed for the total closure duration, the number of closure and the average closure duration during the exposition of the mussels to *A. catenella*. During the exposition period (T1), similar patterns for both mussel populations were detected for all gaping behaviour indicators. This pattern is different than Durier et al. (submitted article), with an increasing of closures. This difference could be explained by the quantity of *A. catenella* added in the tanks, higher than the concentration of this study.

We detected no differences between the two mussel populations for all behavioural indicators with the exception of the average VOA. This indicate that the mussel gaping behaviour remains essentially the same despite of a difference of the intoxication history. In the case of the average VOA, the differences between the two populations were present before the *A. catenella* exposure suggesting different behaviour patterns are related to other parameters than PST intoxication. Nevertheless, when mussels were exposed to toxic algae, both populations showed a decrease of their VOA. This difference between both populations in respect to the average VOA prior to *A. catenella* exposure could be at least partially explained by potential shell morphology differences due to their habitat (suspension culture for Prince Edward Island mussels compared to intertidal coastal shore for St. Lawrence mussels). Tidal habitats expose mussels to higher hydrodynamic shear stress stimulating mussels to develop thicker shell (Babarro and Carrington, 2013), which may increase the average VOA. Moreover, Durier et al. (submitted article) demonstrated that the mussel gaping behaviour change provoked by the *A. catenella* exposure were mainly related to the number of closures, the total closure duration, and the average closure duration.

Thus, we validated the hypothesis of an absence of adaptive gaping behaviour during exposure of mussels to *A. catenella*. We know that soft-shell clams *M. arenaria* regularly exposed to toxic algae showed adaptation to the presence of *A. catenella* toxins (Bricelj et

al., 2005, 2004; MacQuarrie and Bricelj, 2008). However, these authors have not tested impact on gaping behaviour of soft-shell clam exposed to *A. catenella* with less of paralysis when often intoxicated. The valvometry tool used in this study showed the power of this tools to detect change in mussel behavior in presence of *A. catenella* bloom. Furthermore, absence of gaping behaviour differences between mussels from the St. Lawrence Estuary, exposed to *A. catenella* annually, with those from the Prince Edward Island rarely or not previously exposed, suggest that mussels from different origin can be used to detect presence of this toxic algae. This result is encouraging to the possibility of developing an early warning system detecting HAB events before harvest closure threshold of cultured bivalves is reached.

Acknowledgments:

We thank the ISMER technical staff, namely Nathalie Gauthier, for support given for the microalgae production.

Funding

This research was supported by the Ressources Aquatiques Québec research network (Fonds de Recherche du Québec – Nature et Technologies, no. 2014-RS-171172), Fisheries and Oceans Canada provided in-kind assistance that was motivated by the Galway Statement on Atlantic Ocean Cooperation and by the Natural Sciences and Engineering Research Council of Canada through Discovery Grants awarded to RT (no. 2020-05176)

2.4.6 References

Aguilera-Belmonte, A., Inostroza, I., Carrillo, K.S., Franco, J.M., Riobó, P., Gómez, P.I., 2013. The combined effect of salinity and temperature on the growth and toxin content of four Chilean strains of *Alexandrium catenella* (Whedon and Kofoid) Balech 1985 (Dinophyceae) isolated from an outbreak occurring in southern Chile in 2009. Harmful Algae 23, 55–59. https://doi.org/10.1016/j.hal.2012.12.006

Almada-Villela, P.C., Davenport, J., Gruffydd, L.D., 1982. The effects of temperature on the shell growth of young *Mytilus edulis* L. Journal of Experimental Marine Biology and Ecology. 59, 275–288. https://doi.org/10.1016/0022-0981(82)90121-6

Anderson, D. M. , Cembella, A. and Hallegraeff, G. M. 1998: Physiological Ecology of Harmful Algal Blooms , Ecological Sciences, 41 Anderson, D. M. , Cembella, A. and Hallegraeff, G. M. Heidelberg, Springer-Verlag.

Andrewartha, S., Elliott, N., 2015. Aquaculture Sentinels: Smart-farming with Biosensor Equipped Stock. Journal Aquaculture Research Development. 07, 1–4. https://doi.org/10.4172/2155-9546.1000393

Babarro, J.M.F., Carrington, E., 2013. Attachment strength of the mussel *Mytilus galloprovincialis*: Effect of habitat and body size. Journal of Experimental Marine. Biology. and Ecology. 443, 188–196. https://doi.org/10.1016/j.jembe.2013.02.035

Bates, S.S., Beach, Daniel G.; Comeau, Luc. A.; Haigh, Nicola; Lewis, Nancy I.; Locke, Andrea; Martin, Jennifer L.; McCarron, Pearse; McKenzie, Cynthia H.; Michel, Christine; Miles, Christopher O.; Poulin, Michel; Quilliam, Michael A.; Rourke, Wade A.; Scarratt, Michael G.; Starr, Michel; Wells, Terri; Fisheries and Oceans Canada 2020. Marine harmful algal blooms and phycotoxins of concern to Canada. Canadian Technical Report of Fisheries and Aquatic Science. 3384, 322p

Blasco, D., Levasseur, M., Bonneau, E., Gelinas, R., & Packard, T. T. (2003). Patterns of paralytic shellfish toxicity in the St. Lawrence region in relationship with the abundance and distribution of *Alexandrium tamarense*. Scientia Marina, 67(3), 261-278.

Boivin-Rioux, A., Starr, M., Chassé, J., Scarratt, M., Perrie, W., Long, Z., 2021. Predicting the effects of climate change on the occurrence of the toxic dinoflagellate *Alexandrium catenella* along Canada's East Coast. Frontier in Marine Science. 7, 1–20. https://doi.org/10.3389/fmars.2020.608021

Bricelj, V.M., Connell, L., Konoki, K., MacQuarrie, S.P., Scheuer, T., Catterall, W.A., Trainer, V.L., 2005. Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434, 763–767. https://doi.org/10.1038/nature03415

Bricelj, V.M., MacQuarrie, S.P., Twarog, B.M., Trainer, V.L., 2004. Characterization of sensitivity to PSP toxins in North American populations of the softshell clam *Mya arenaria*. Harmful Algae 2002, 172–174.

Bricelj, V. M., & Shumway, S. E. (1998). Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. Reviews in Fisheries Science, 6(4), 315-383.

Comeau, L.A., Babarro, J.M.F., Riobó, P., Scarratt, M., Starr, M., Tremblay, R., 2019. PSP-producing dinoflagellate *Alexandrium minutum* induces valve microclosures in the mussel *Mytilus galloprovincialis*. Aquaculture 500, 407–413. https://doi.org/10.1016/j.aquaculture.2018.10.025

Dame, R.F., Kenneth, M.J., 2011. Ecology of marine bivalves: an ecosystem approach. Kennish M.J. Taylor & Francis. 284p. DOI 10.1201/b11220

Fauchot, J., Levasseur, M., Roy, S., Gagnon, R., Weise, A.M., 2005. Environmental factors controlling *Alexandrium tamarense* (dinophyceae) growth rate during a red tide event in the St. Lawrence Estuary (Canada). Journal. of Phycology. 41, 263–272. https://doi.org/10.1111/j.1529-8817.2005.03092.x

Kramer, K.J.M., Foekema, E.M., 2001. The “Musselmonitor®” as biological early warning system. In: Butterworth, F.M., Gunatilaka, A., Gonsebatt, M.E. (Eds.), Biomonitors and Biomarkers as Indicators of Environmental Change 2: A Handbook. 59–87.

Lilly, E.L., Halanych, K.M., Anderson, D.M., 2007. Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). Journal of Phycology. 43, 1329–1338. https://doi.org/10.1111/j.1529-8817.2007.00420.x

MacQuarrie, S.P., Bricelj, V.M., 2008. Behavioral and physiological responses to PSP toxins in *Mya arenaria* populations in relation to previous exposure to red tides. Marine Ecology Progress. Series. 366, 59–74. https://doi.org/10.3354/meps07538

Martin, M., Richardson, B.J., 1991. Long term contaminant biomonitoring: views from southern and northern hemisphere perspectives. Marine Pollution Bulletin 22, 533–537.

Martinez Arbizu, P., 2019. Pairwiseadonis: Pairwise multilevel comparison using adonis. R Packag. version 0.3.

Matsuyama, Y., Shumway, S., 2009. Impacts of harmful algal blooms on shellfisheries aquaculture. In New Technologies in Aquaculture (pp. 580-609). Woodhead Publishing

Nagai, K., Honjo, T., Go, J., Yamashita, H., Seok Jin Oh, 2006. Detecting the shellfish killer *Heterocapsa circularisquama* (Dinophyceae) by measuring bivalve valve activity with a Hall element sensor. Aquaculture 255, 395–401. https://doi.org/10.1016/j.aquaculture.2005.12.018

Navarro, J.M., Muñoz, M.G., Contreras, A.M., 2006. Temperature as a factor regulating growth and toxin content in the dinoflagellate *Alexandrium catenella*. Harmful Algae 5, 762–769. https://doi.org/10.1016/j.hal.2006.04.001

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O’hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs,

E., Maintainer, H.W., 2019. Community ecology package: ordination, diversity and dissimilarities. vegan Community Ecology Package. 2, 1–297.

Parkhill, J.P., Cembella, A.D., 1999. Effects of salinity, light and inorganic nitrogen on growth and toxigenicity of the marine dinoflagellate *Alexandrium tamarense* from northeastern Canada. Journal of Plankton Research. 21, 939–955. https://doi.org/10.1093/plankt/21.5.939

Starr, M., Lair, S., Michaud, S., Scarratt, M., Quilliam, M., Lefaivre, D., Robert, M., Wotherspoon, A., Michaud, R., Ménard, N., Sauvé, G., Lessard, S., Béland, P., Measures, L., 2017. Multispecies mass mortality of marine fauna linked to a toxic dinoflagellate bloom. PLoS One 12, 1–18. https://doi.org/10.1371/journal.pone.0176299

CHAPITRE 3
EFFET DU DINOFLAGELLE *ALEXANDRIUM CATENELLA* SUR LE COMPORTEMENT ET LA PHYSIOLOGIE DE LA MOULE BLEUE *MYTILUS EDULIS*

Le quatrième article, intitulé "*Effect of the toxic dinoflagellate* Alexandrium catenella *on the behaviour and physiology of the blue mussel* Mytilus edulis", a été corédigé par Romain Lavaud, moi-même, Jean-Bruno Nadalini, Ramón Filgueira, Luc Comeau, Jose Babarro, Sonia Michaud, Michael Scarratt et Réjean Tremblay. Cet article a été accepté le 24 aout 2021 par la revue *Harmful Algae*. En tant que deuxième auteur, ma contribution à cet article a été la mise en place de la valvométrie, le traitement et l'analyse des données liées à la valvométrie ainsi que les tests Elisa et leurs analyses. Romain Lavaud a effectué l'analyse des données issues des autres parties des expériences ainsi que la rédaction de l'article. Jean-Bruno Nadalini a réalisé les expériences d'indice de condition, de taux physiologique, de croissance, de consommation d'oxygène et de résistance de byssus. Sonia Michaud a participé à l'interprétation des tests ELISA. Ramón Filgueira, Luc Comeau, Jose Babarro, Michael Scarratt et Réjean Tremblay ont fourni l'idée originale et participé à l'interprétation des résultats. Tous les auteurs ont participé à la révision de l'article.

3.1 RESUME EN FRANÇAIS DU QUATRIEME ARTICLE

Les effets des algues toxiques sur la physiologie des bivalves sont complexes et implique à la fois une réponse physiologique et comportementale. Étudier ces réponses est essentiel pour mieux comprendre, décrire et prédire leur impact sur l'aquaculture des bivalves et les risques sur la santé humaine. Dans cette étude nous avons enregistré pendant deux mois la réponse physiologique de la moule bleue *Mytilus edulis* provenant de l'est du Canada à

une exposition d'une semaine aux toxines paralysantes produites par le dinoflagellé *Alexandrium catenella*, isolé de l'estuaire du St Laurent au Canada. Les moules du traitement « contrôle » ont été nourries en continue avec un régime non toxique et les moules avec le traitement « stabulation » ont été nourries avec le même régime non toxique pendant une semaine avant de ne plus être nourries pendant sept semaines. Les moules du traitement « toxique » ont reçu *A. catenella* pendant une semaine avant de ne plus être nourries jusqu'à la fin de l'expérience. Durant cette expérience de deux mois nous avons suivi la croissance des coquilles et des tissus, la capacité de filtration, le taux de respiration, la force d'attachement des byssus, le comportement valvaire et le contenu des tissus en toxine. Les moules nourries avec le dinoflagellé toxique ont accumulé une moyenne de 51.6 µg STXeq 100 g^{-1} après une exposition d'une semaine. Après sept semaines de dépuration, la moitié des individus ont montré un niveau de toxine autour de 18 µg STXeq 100 g^{-1}. L'indice de condition des moules exposées au traitement « toxique » décroit rapidement par rapport au début en comparaison des moules ayant reçu les algues non toxiques pendant une semaine avant le traitement de stabulation. Le taux de consommation d'oxygène croit avec le traitement « toxique » avant de se niveler avec celui des moules du traitement « stabulation ». L'amplitude d'ouverture valvaire était plus faible pour le traitement « toxique » pendant et après l'exposition. La durée moyenne de fermeture était plus haute juste après l'exposition, pendant le pic d'intoxication des tissus. Aucun changement significatif dans la force des fils des byssus n'a été observé au cours du temps dans chacun des traitements mais une force plus faible a été nécessaire pour détacher les moules des traitements « toxiques » et « stabulation ».

3.2 EFFECT OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM CATENELLA* ON THE BEHAVIOUR AND PHYSIOLOGY OF THE BLUE MUSSEL *MYTILUS EDULIS*

ROMAIN LAVAUD [1,2,3*], GUILLAUME DURIER [1], JEAN-BRUNO NADALINI [1,4], RAMON FILGUEIRA [2], LUC A. COMEAU [5], JOSE M.F. BABARRO [6], SONIA MICHAUD [4], MICHAEL SCARRATT [4], AND REJEAN TREMBLAY [1]

[1] Institut des Sciences de la Mer de Rimouski, Université du Québec à Rimouski, Rimouski, QC, Canada

[2] Marine Program Affairs, Dalhousie University, Halifax, NS, Canada

[3] School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA, United States – *Current address*

[4] Fisheries and Oceans Canada, Quebec Region, Institut Maurice Lamontagne, Mont-Joli, QC, Canada

[5] Fisheries and Oceans Canada, Gulf Region Center, Moncton, NB, Canada

[6] Instituto de Investigaciones Marinas IIM-CSIC, Vigo, Spain

Publié dans Harmful Algae en Aout 2021

doi.org/10.1016/j.hal.2021.102097

3.2.1 Abstract

The effects of harmful algae on bivalve physiology are complex and involve both physiological and behavioural responses. Studying those responses is essential to better describe and predict their impact on shellfish aquaculture and health risk for humans. In this study we recorded for two months the physiological response of the blue mussel *Mytilus edulis* from Eastern Canada to a one-week exposure to a paralytic shellfish poisoning producing dinoflagellate strain of *Alexandrium catenella*, isolated from the St

Lawrence estuary, Canada. Mussels in a 'control' treatment were fed continuously with a non-toxic diet, while mussels in a 'starvation' treatment were fed the same non-toxic diet the first week and subsequently starved for seven weeks. Mussels in a 'toxic' treatment received *A. catenella* for one week before being starved until the end of the experiment. Over a two-month experiment we monitored shell and tissue growth, filtration capacity, respiration rate, byssal attachment strength, valve opening behaviour, and toxin content in tissues. Mussels fed normally on the toxic dinoflagellate and accumulated an average of 51.6 µg STXeq 100 g^{-1} after one week of exposure. After seven weeks of depuration, about half of the specimen showed levels around 18 µg STXeq 100 g^{-1}. The condition index of exposed mussels ('toxic' treatment) decreased rapidly from the start as compared to mussels that received a one-week non-toxic diet ('starvation' treatment). Oxygen consumption rates increased in the 'toxic' treatment before leveling out with that of mussels from the 'starvation' treatment. Valve opening amplitude was lower in the 'toxic' treatment during and following the exposure. Average valve closure duration was higher right after the exposure, during the peak of mussel tissue intoxication. No significant change in byssal thread strength was observed through time in each treatment but less force was required to detach mussels from the 'toxic' and 'starvation' treatments. The number of byssus threads produced by mussels exposed to the toxic dinoflagellate was also lower than in the control group. These results represent advancements in our understanding of the impacts of harmful algae on bivalves and contribute to the development of mitigation measures necessary to both the safety of consumers and the sustainability of aquaculture operations.

Keywords

Paralytic shellfish poisoning; Harmful algal bloom; Clearance rate; Respiration rate; Valvometry; Byssus strength; Toxin depuration; Saxitoxin.

3.2.2 Introduction

Bivalve aquaculture relies on the natural environment for the growth of farmed animals, including naturally occurring phytoplankton as main food source. Some phytoplankton species, however, produce toxins or bioactive compounds that can be harmful to bivalves (Lassudrie et al., 2020) or to humans through consumption of contaminated shellfish (Maso and Garcés, 2006), particularly during harmful algal bloom (HAB) events. The increase in reports and spread of HABs in recent decades has been linked to ocean warming, eutrophication, and human activities (Basti et al., 2018). HABs are considered a major threat to marine coastal areas due to the wide range of impacts they have on the ecology of coastal marine ecosystems, either through a sheer increase of their biomass or through the production of potent toxins and bioactive compounds (Burkholder, 1998). In Eastern Canada, which accounts for almost 80% of the country's aquaculture shellfish activities (Statistics Canada, 2018), saxitoxin and its derivatives produced by the dinoflagellate *Alexandrium catenella* (formerly known under different names, see John et al., 2014) are responsible for paralytic shellfish poisoning and have been known to cause intoxication and death since 1936 (Gibbard and Naubert, 1948). Shellfish monitoring programs for paralytic shellfish toxins (PSTs) have emerged (Blasco et al., 2003) and demonstrated their utility to reduce the risks posed by the Canadian strains of this dinoflagellate species, which show particularly high toxic effects at various trophic levels (Starr et al., 2017). While the impacts of HABs on aquaculture production remain uncertain, rising concerns for the future of shellfish production emerge (Collins et al., 2020).

The effects of PSTs on human health through seafood consumption have long been described and documented and are now well understood (Gibbard and Naubert, 1948; Schantz, 1970; Shumway, 1990; Vilariño et al., 2018; Guillotreau et al., 2021). However, evidence that HABs can also be harmful to grazers, including bivalves, has emerged since then (reviewed in Bricelj and Shumway, 1998; Manfrin et al., 2012; Lassudrie et al., 2020). Short-term effects may include reduction in filtration and clearance rates (Mafra

Jr. et al., 2010; Pousse et al., 2018), shell valve closing (Tran et al., 2010; Comeau et al., 2019), increase in toxin concentration in tissues (Bricelj et al., 1990; Pousse et al., 2018), and inhibition of neuroenzymatic activity (Basti et al., 2016). Long-term exposure (>few days) has been showed to cause tissue lesions (Galimany et al., 2008) and to affect feeding activity (Bianchi et al., 2019; Nielsen et al., 2020), respiration rates (MacQuarrie and Bricelj, 2008; Nielsen et al., 2020), immunity (Hégaret and Wikfors, 2005; Galimany et al., 2008; Lassudrie et al., 2015, 2016), metabolic pathways (Fabioux et al., 2015), reproduction (Basti et al., 2018), gene transcription and alteration of DNA structure (Mat et al., 2013), predator escape response (Hégaret et al., 2012), and energy budgets (Nielsen and Strømgren, 1991; Li et al., 2002; Basti et al., 2016).

The blue mussel, *Mytilus edulis*, in addition to being the most important species for the Canadian shellfish aquaculture industry (Statistics Canada, 2018), has long been considered as a sentinel species for monitoring of coastal water quality (Goldberg, 1975; Beyer et al., 2017). While most research has focused on blue mussel ecotoxicology and pollution monitoring (e.g. Jones et al., 2001; Beaudry et al., 2015; Lacroix et al., 2017), important work has been carried out on PST accumulation (Svensson, 2003; Nielsen et al., 2016) and depuration (Svensson and Förlin, 2004; Duinker et al., 2007; Jaime et al., 2007 ; Marcaillou et al., 2010; Nielsen et al., 2016). The accumulation of toxins by blue mussels through microalgae ingestion depends on many factors, for example the time of the year (Martin and Richard, 1996), history of exposure (Shumway and Cucci, 1987), latitudinal position (Bricelj and Shumway, 1998), their position in the intertidal or subtidal zone (Desbiens et al., 1990), among others. These factors are sources of variability in the intoxication levels, but a laboratory experiment demonstrated that blue mussels reached maximum (saturating) levels of toxin concentration around 4.5 10^4 µg STXeq 100 g^{-1} within 2 weeks of exposure (Bricelj et al., 1990). Blue mussels have been classified as 'rapid' detoxifiers, eliminating toxins primarily through excretion within weeks (Nielsen et al., 2016), as opposed to 'slow' detoxifiers such as the giant scallop, *Placopecten magellanicus*, which takes months to years to detoxify (Bricelj and Shumway, 1998). Age, size (Duinker et al., 2007), and food availability (Svensson, 2003;

Marcaillou et al., 2010), among other factors have been identified as sources of variability in toxin elimination. Some studies on *Crassostrea gigas* also showed that the detoxification time of oysters feeding on nontoxic algae after an exposure to toxic algae was significantly reduced (Lassus et al., 2000; Guéguen et al., 2008). An increased gut evacuation rate and an acceleration of metabolism were suggested as mechanisms to explain this pattern (Bricelj and Shumway, 1998). Pousse et al. (2019) therefore noted that to quantify the true cost of toxin depuration, detoxification experiments should include non-toxic feeding and starvation treatments.

This comprehensive body of literature contributes to our understanding of toxin dynamics in bivalves and how farmed species impacted by HABs may constitute a risk for human health. But in a rapidly changing ocean, and particularly in coastal areas subject to acute changes caused by human activities, there is a need for the integration of this knowledge into predictive tools that allow managers and decision-makers to make timely informed decisions, before negative outcomes are unavoidable. In this way, numerical models could play a key role in forecasting and preventing health risks linked to HABs. The development of mechanistic models capable of integrating the physiological response of organisms and their energetic state has been proved to accurately predict toxin dynamics in bivalves (Guéguen et al., 2010; Pousse et al., 2019). Ultimately, such models constitute a step forward in the capacity to predict and manage HAB impacts on shellfish; however extensive data are required for their calibration and validation. For instance, sub-lethal effects of PST accumulation in tissues might impact the bioenergetics of intoxicated bivalves, even at low PST concentrations (Pousse et al., 2019). To expand our knowledge on the effects of *A. catenella* on *M. edulis* we conducted an experiment in which we monitored a large set of variables including toxin concentration in tissues, filtration and respiration rates, shell growth, valve movements, and byssus attachment properties of mussels fed toxic algae for one week. Measurements were carried out during the exposure to *A. catenella* and during the seven following weeks of detoxification to assess the physiological and behavioural response of mussels to this acute exposure. The data collected in this comprehensive and integrated study should

help manage the health risks associated with paralytic shellfish poisoning from blue mussel aquaculture in Canada.

3.2.3 Material and Methods

3.2.3.1 Mussels and algae

On 23 September 2019, 680 mussels, *Mytilus edulis*, (35.05 ± 7.81 mm, 4.62 ± 3.27 g total wet weight, n = 100) were removed from long lines of an aquaculture farm in Malpeque Bay, Prince Edward Island (Canada) and transported to the Université du Québec à Rimouski's aquaculture research facility (Pointe-au-Père, Québec, Canada). Upon arrival mussels were scrubbed, rinsed with UV treated filtered (1 µm) sea water, placed in eight 100-L sea water open flow tanks (85 mussels per tank) and acclimated for 25 days. During acclimation mussels were fed a diet composed of *Tisochrysis lutea* CCMP 1324, *Chaetoceros muelleri* CCMP 1316, and *Pavlova lutheri* CCMP 1325 (1:1:1) at a rate of 2.1 10^7 cells ind^{-1} d^{-1} (equivalent to 1% of their dry weight). These three algal strains were obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA), Maine, USA and were produced in autoclaved 20-L bottles with F/2-Si, daylight 24/0 (D/N) at 20 °C, and a light intensity of 100 µm m^{-2} s^{-1}. In the first ten days of acclimation, temperature was progressively increased at a rate of ~1 °C d^{-1} until all tanks reached a temperature of 17 °C. After acclimation, and prior to the start of the experiment 20 mussels were sampled for initial length and weight measurements and twelve mussels per treatments were wired with valvometers for valve opening measurements (Nagai et al., 2006; Comeau et al., 2012). A total of 22 mussels died during this acclimation period and 11 mussels died during the remainder of the experiment.

3.2.3.2 Experimental treatments

The experiment lasted eight weeks and consisted of three feeding treatments: 'control', 'starvation', and 'toxic'. Mussels were kept in 100-L static replicate tanks containing 85 individuals each. Half of the water volume was renewed twice a week and one complete water renewal was carried out every week. In the 'control' treatment (two replicate tanks), mussels were fed the same non-toxic algal mix as during the acclimation period at a rate of 4.79 10^8 cells ind^{-1} d^{-1} (equivalent to 5% of their dry weight). Three days per week, following the water renewal, fresh algae were added to a holding tank and continuously delivered to the mussels via peristaltic pumps. In the 'starvation' treatment (two replicate tanks), mussels were fed the non-toxic algal mix as in the control treatment for the first week only and then kept without any food supply for the remaining duration of the experiment. In the 'toxic' treatment (four replicate tanks), mussels were fed *Alexandrium catenella* during the first week of the experiment (see below the feeding regime) and then kept without any food for the remaining duration of the experiment. UV treated filtered sea water (1 µm) was delivered to tanks under starvation in lieu of algae to maintain a similar experimental setup between treatments. Such a design, with no feeding after the exposure, allows the study of potential energetic effects of depuration as well as the determination of depuration rates unimpacted by the metabolic activity engendered by feeding (Pousse et al., 2019).

A. catenella cultures originate from isolates from the St. Lawrence Estuary, Atlantic Canada (clone formerly named AT6 for *A. tamarense*, renamed AC6) obtained during a bloom monitored in 2008 (Starr et al., 2017). Algal production started during the acclimation period under the same conditions as for the non-toxic diet (see section 2.1). Previous cultures of *A. catenella* grown at the UQAR's aquaculture research station reached toxin cell quotas ranging between 3 and 60 pg STXeq $cell^{-1}$ (Nadalini, J.-B. and Tremblay, R., unpublished data). Assuming the lowest toxin content, production was set to exceed the regulatory threshold for shellfish harvesting closure of 80 µg STXeq 100 g^{-1}. Thereby, during the first seven days of the experiment mussels in the 'toxic'

treatment were fed *A. catenella* daily via peristaltic pumps at an average concentration of 2.02 10^5 cells L^{-1} in each of the four replicate tanks (equivalent to 1.16 10^5 cells ind^{-1} d^{-1} or 1% of their dry weight).

3.2.3.3 Experimental variables

a) *SHELL LENGTH, TISSUE MASS, AND CONDITION INDEX*

A timeline for the collection of experimental data in the different treatments is provided in Figure 31. Upon arrival to the laboratory, 100 individuals were randomly selected for determination of their shell length, shell height and whole wet mass (tissue + shell). During the experiment, 20 individuals per treatment were randomly sampled on a weekly basis to measure shell length, shell height, tissue wet mass and dry mass (48 h at 70 °C). Mussel collection was balanced to maintain similar densities in each replicate tank. Condition index (CI, in g mm^{-3}) was calculated as the ratio of tissue dry weight (DW_{tissue}, g) over volume (V, mm^3). Since only shell length and height were measured, we used an allometric relationship obtained by Alunno-Bruscia et al. (2001) to estimate shell width based on length (length: width = 2.3) in order to calculate shell volume (length × height × width). Due to a suspected bias in the sampling of larger individuals in the first weeks of the experiment we decided to monitor shell growth individually from week 5 until the end of the experiment on week 8. Ten mussels per tank (total N = 80) were then tagged and their shell length measured every week using a digital caliper (Mitutoyo 500-196-30 AOS absolute; precision of 0.01 mm).

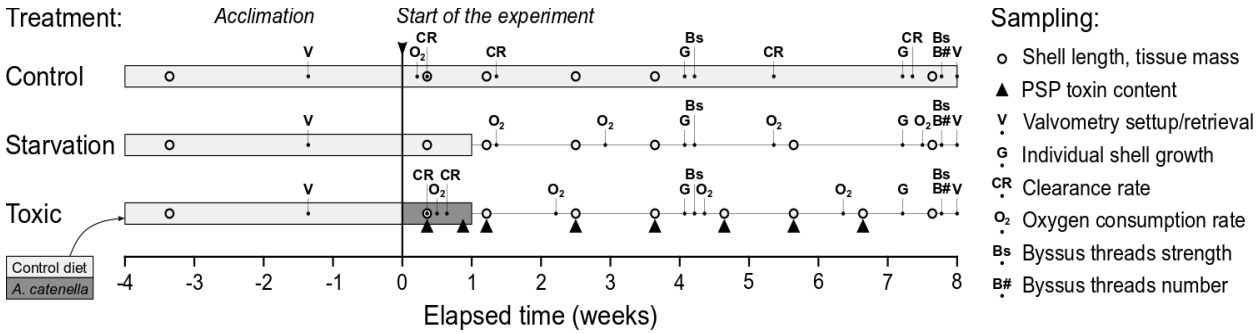

Figure 31 : Sampling timeline of the experiment.

b) *TISSUE TOXIN CONTENT*

In the 'toxic' treatment 15 additional individuals were randomly sampled each week (3-4 per 'toxic' treatment tank), their tissue was dissected and frozen at – 20 °C until toxin quantification. During the exposure period the first week, sampling was split up to better track toxin accumulation and 8 individuals were collected on day 3 and 7. The concentration of saxitoxin and some of its derivatives was quantified individually in total body tissues using saxitoxin (PSP) ELISA plate kits (Eurofins Abraxis), following the method described in Starr et al. (2017). PST concentration was expressed in µg STX equivalent per 100 g of tissue (µg STXeq 100 g^{-1}). The detection limit of the ELISA kit for Saxitoxin is 0.015 ng mL^{-1}, or about 3.0 ± 1 µg STXeq 100 g^{-1}. Toxin cross-reactivities are as followed: Saxitoxin (STX) 100%, Decarbamoyl STX 29%, $GTX_{2,3}$ 23%, $GTX\text{-}_{5B}$ 23%, Lyngbyatoxin 13%, Sulfo $GTX_{1,2}$ 2.0%, Decarbamoyl $GTX_{2,3}$ 1.4%, Neosaxitoxin (NEO) 1.3%, Decarbamoyl NEO STX 0.6%, and $GTX_{1,4} < 0.2\%$. The toxin depuration rate was calculated as daily % of decrease from the maximum toxin concentration in tissues.

c) *CLEARANCE RATE*

Individual clearance rate (CR_i, L h^{-1} ind^{-1}), defined as the volume of water completely cleared of suspended particles per unit of time, was calculated using a static system

(Widdows, 1985). The CR_i of mussels from the 'toxic' treatment was measured twice during the week of exposure to *A. catenella* (day 3 and 5) and in week 1, 5, and 7 for the 'control' treatment, with different individuals each time. Only *T. lutea* algae were used during these measurements to remove any variability in CR_i due to the type of algae fed in each treatment, as mussels may reduce their clearance rate by up to 48% in presence of a closely related toxic algae, *A. fundyense* (Bricelj et al., 1990). This allows the identification of potential effects of the exposure to toxic algae on the physical capacity of mussel to filtrate. Individual mussels were placed in 1-L beakers filled with 0.8 L of UV treated filtered sea water (1 µm) and left undisturbed for about 60 min. Algal culture of *Tisochrysis lutea* was then added to each beaker to obtain an initial concentration of 1 10^5 cell mL^{-1}. Algal concentration was measured every 15 min for an hour with a particle size analyzer (Multisizer 4e Beckman Coulter counter, 50-µm pore orifice). Algal sedimentation was reduced by gentle aeration of the water with air stones. Beakers containing empty mussel shells were used as controls. CR_i was calculated using the formula (Cranford et al., 2016):

$$CR_i = [\ln(s) - ln(s')] \times V \times 60 \quad (1)$$

where s is the slope of the linear regression between algal concentration (cell min^{-1}) and time (min^{-1}), s' the slope for the control beaker, V is the beaker volume (0.8 L), and the multiplication by 60 allows for the conversion from min to h. This generalization of Coughlan's (1969) method is used to integrate the values of intermediate sampling points and only linear regressions of concentration over time with an $r^2 > 0.90$ are included in the calculation of CR_i. In order to correct for variations in individual size, clearance rates were standardized to a 40-mm mussel using the formula:

$$CR_{std} = CR_i \left(\frac{L_{std}}{L_i}\right)^b \quad (2)$$

where CR_{std} is the standardized clearance rate for a mussel of standard shell length L_{std} (i.e., 40 mm), CR_i is the measured clearance rate for the mussel of shell length L_i, and b is the allometric coefficient (2.19; Jones et al., 1992).

Additional data on cell ingestion were derived from calculations linking PST concentration in *A. catenella* cells and in mussel tissues in each tank. These data complement the CR data obtained by feeding mussels with non-toxic *Tisochrysis lutea*. Knowing the toxicity and quantity of *A. catenella* cells fed to mussels in the 'toxic' treatment, we calculated the number of cells ingested according to the following formula:

$$IR = \frac{\sum_i \frac{PST_{mussel_i} DW_i}{100}}{PST_{Alex} Nt} \quad (3)$$

where IR is the ingestion rate (cell ind^{-1} d^{-1}), PST_{mussel_i} the individual concentration of PST in mussel tissues (µg STXeq 100 g^{-1}), DW_i the individual dry weight of mussel tissues (g), PST_{Alex} the concentration of PST per cell of *A. catenella* (cell quota; µg STXeq cell^{-1}), N the number of mussels per tank (85), and t the time of exposure (7 d). This formula was applied to each of the four replicate tanks of the 'toxic' treatment and then averaged. Equal detoxification rates between the four tanks were assumed.

d) *OXYGEN CONSUMPTION RATE*

Individual oxygen consumption rate (OCR_i, mg h^{-1} ind^{-1}) were performed using a Q-Box AQUA Aquatic Respirometry Package with intermittent closed respirometry, in which measurements obtained by an optical DO probe were made sequentially, without removing the animal from the chamber (Qubit System Inc, Kingston, On, Canada). Mussels were placed in 0.275-L chambers filled with UV treated filtered (1 µm) sea water and left undisturbed for about 60 min. The volume of the whole system external to the chamber (tubing, pump and 3-way valve) was 0.75-L. Every week, the OCR_i of six

mussels from either the 'starvation' or the 'toxic' treatments were measured, giving a measure every other week for each treatment. The individual volume (V_i) of mussels was calculated before the measurement. Before each analysis, a blank was performed by measuring the oxygen concentration in a chamber with an empty shell for 1h30. The automated system conducted repeated measures of the decrease of oxygen concentration in closed chambers over 15-min intervals, alternating with 15-min periods of chamber flushing for a total duration of 17 h. OCR_{obs} was calculated using the formula:

$$OCR_{obs} = \frac{(\Delta[O_2]_i - \Delta[O_2]_c)(V - V_i)}{t} \quad (4)$$

where $\Delta[O_2]_i$ is the mean difference in oxygen concentration between the beginning and the end of the measurement period t (i.e., 15 min), $\Delta[O_2]_c$ is the difference in oxygen concentration during the flushing period and serves as a control, V is the chamber volume (0.275 L), and V_i the mussel volume (L). In order to correct for variations in individual size, oxygen consumption rates were standardized to a 1-g dry weight mussel using the formula from Smaal et al. (1997):

$$OCR_{std} = OCR_{obs}\left(\frac{W_{std}}{W_{obs}}\right)^b \quad (5)$$

where OCR_{std} is the standardized oxygen consumption rate for a mussel of standard dry weight W_{std} (i.e., 1 g), OCR_{obs} is the measured oxygen consumption rate for the mussel of dry weight W_{obs}, and b is the allometric coefficient (0.73; Smaal et al., 1997).

e) *VALVE OPENING*

Ten days before the start of the experiment, twelve mussels per treatment (six per tank in 'control' and 'starvation', three per tank in 'toxic') were equipped with a valvometry system previously described in Nagai et al. (2006), Basti et al. (2009), and

Comeau et al. (2012) to monitor valve opening. For each mussel, a coated Hall element sensor (HW-300a, Asahi Kasei Corp, Chiyoda-ku, Tokyo, Japan; 0.5 g) was glued to one valve at the maximum distance from the hinge. Then a small magnet (4.8 mm diameter × 0.8 mm height; 0.1 g) was glued to the other valve, directly below the Hall sensor. The magnetic field between the sensor and the magnet, expressed as output voltage (μV), is a function of the gap between the two valves. Valve movements were recorded at a frequency of 100 ms over the eight weeks of the experiment using dynamic strain recording devices (DC 204R, Tokyo Sokki Kenkyujo Co., Shinagawa-ku, Tokyo, Japan). At the end of the experiment, the adductor muscle was cut and small calibration wedges (1–6 mm) were inserted between the valves at the opposite point from the hinge to derive a voltage versus gap calibration curve ($r^2 > 0.90$) for each individual mussel. With these calibration curves output voltage measurements were converted into valve opening data. Data were then treated in R (R core team, 2020) to express valve opening amplitude (in % of max aperture), mean and total closure time (s), the number of full closures, and the number of micro-closures. Micro-closures were defined as a 3% (or greater) reduction in valve opening over a short 100 ms time span. Results from four individuals could not be used as one mussel from the 'starvation' treatment died and the magnets on one and two mussels from the 'toxic' and 'control' treatments, respectively, detached during the experiment.

f) *BYSSUS ATTACHMENT*

Ten mussels per tank were tagged and isolated in a basket to measure the strength of byssus attachment. A tensometer (Dillon Quantrol GTX force gauge) connected to custom-made forceps was used to measure the strength (N) required to pull each mussel. Care was taken to avoid disturbing neighbouring mussels when dislodging one individual. Repeated measurements on the same individuals were carried out in the fourth and eighth weeks of the experiment on wet byssus threads. An additional 15 mussels per treatment were glued on an L-shaped glass support at the beginning of the experiment to

visually determine the number of byssal threads produced by mussels in each treatment during week 8.

3.2.3.4 Statistical analysis

All analyses were conducted in RStudio 1.3.1073 (R Core Team, 2020) with a significance threshold of $\alpha < 0.05$. Assumptions of residual normality and homoscedasticity were assessed visually using Q–Q plots and residual-fitted plots, respectively, after logarithm or squared root transformation when required. We compared means of variables under the three experimental treatments using one-way ANOVAs followed by Tukey's range tests. In case assumptions for these tests were not met, Kruskal-Wallis and pairwise Wilcoxon tests were conducted instead. A permutational multivariate analysis of variance (PERMANOVA) was used to analyze micro-closure data as they are subject to zero-inflation. A repeated measures ANOVA was carried out on byssus strength data as measurements were conducted on the same individuals. Results are reported as means ± standard error of the mean (SEM).

3.2.4 Results

3.2.4.1 Toxin content in tissues and depuration rate

A relatively low cell quota (i.e., toxicity) of 2.1 pg STXeq cell^{-1} was measured for our culture of *Alexandrium catenella* cultures. The uptake of PSTs by mussels reached a peak in the second week of the experiment, following seven days of exposure to *A. catenella*. Although the maximum concentration measured in mussel tissue, 99.5 µg STXeq 100 g^{-1}, was well above the regulatory threshold for fishery closure, the average concentration was 51.6 µg STXeq 100 g^{-1} (N = 13; figure 32) and therefore remained below the threshold. PST concentration then dropped in the third week and stabilized at an average level below 20 µg STXeq 100 g^{-1} until the end of the experiment. High inter-individual variability was observed, with some mussels able to eliminate the toxin in a few days and exhibit concentrations similar to the initial level, while other individuals remained intoxicated with concentrations around 40 µg STXeq 100 g^{-1} at the end of the study. The depuration rate of toxins accumulated during the initial week of exposure was 1.4% d^{-1} over the remaining 7 weeks. The sharpest decline in toxin concentration occurred in week 2 at a rate of 8.2% d^{-1}.

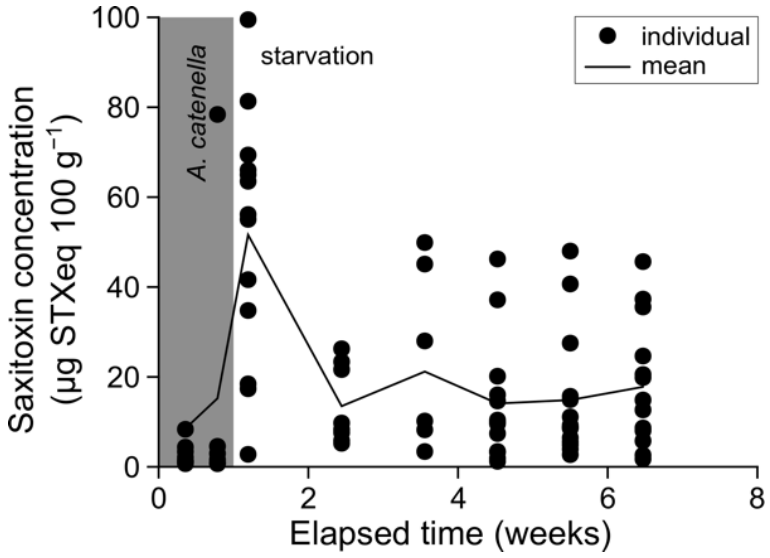

Figure 32 : Paralytic shellfish toxin concentration (µg STXeq 100 g–1) in tissues of mussels exposed to A. catenella during the first week of the experiment (N ranges between 7 and 14 from week to week). The grey area indicates the period during which mussels were fed A. catenella.

3.2.4.2 Condition index and shell growth

The condition index (CI) of mussels in the 'toxic' treatment, which were fed *A. catenella* during the first week, fell precipitously over the first two weeks and was thereafter stable until the end of the experiment (Figure 3a). In comparison, mussels from the 'starvation' treatment, which were fed the non-toxic algal mix during the first week, experienced a delayed and more gradual decrease in CI (Figure 33a). The CI of mussels in the 'control' treatment remained high and stable through the duration of the experiment (Figure 33a). Individual growth monitored from week 5 to week 8 indicated no difference between individuals from the 'toxic' treatment and those from the 'starvation' treatment, with close to no shell growth, while mussels from the 'control' treatment averaged 0.06 mm d^{-1} (Figure 33b).

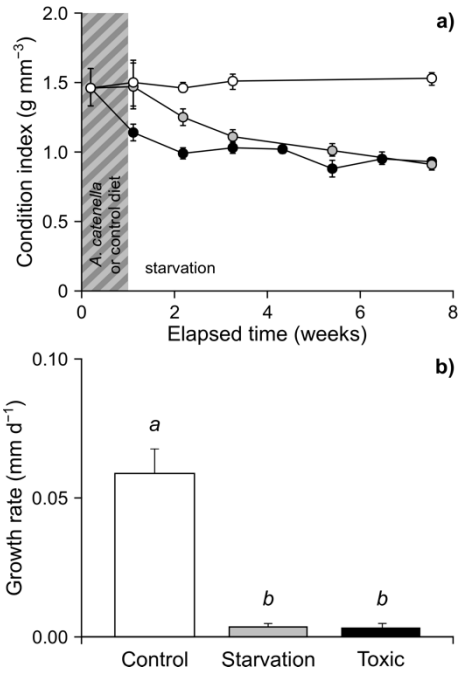

Figure 33 Physiological rates (a; mean ± SEM) and average individual shell growth (b; mean ± SEM; N = 20 in 'control' and 'starvation' treatments; N = 40 in 'toxic' treatment) of mussels in the 'control' (white), 'starvation' (grey), and 'toxic' (black) treatments. The crosshatched grey area indicates the period (one week) during which mussels in the 'starvation' and 'toxic' treatments were fed the control diet and *A. catenella*, respectively, before being starved.

3.2.3 Physiological rates

Measurement of clearance rate (CR) in the 'toxic' treatment was carried out mostly to check that filtration capacity of mussels was not impaired by *A. catenella*. No measurements were carried out during the starvation period of this treatment. Mussels did maintain a CR close to the initial value during the exposure to the toxic algae, reaching 3.66 L h^{-1} at the end of the feeding period (Figure 4a). Mussels from the 'control' treatment exhibited a fairly stable CR around 3.00 L h^{-1} (Figure 34a). Additional calculations of individual ingestion rates (IR) in each tank based on the formula in eq. (3) revealed that mussels ingested on average 6.9 10^4 cell ind^{-1} d^{-1} during the week of exposure. Compared to the number of *A. catenella* cells provided through a peristaltic pump (1.16 10^5 cells ind^{-1} d^{-1}), this value shows that mussels in the 'toxic' treatment ingested most of the microalgae delivered.

The mean oxygen consumption rate (OCR) for mussels in the 'toxic' treatment remained around 0.90 mg O_2 g^{-1} h^{-1} during the first five weeks and then increased towards the end to reach a maximum of 1.20 mg O_2 g^{-1} h^{-1} in the seventh week of the

experiment. After a two-fold decrease between the first and second weeks, the OCR of starved mussels increased and reached a maximum at 1.65 mg O_2 g^{-1} h^{-1} in the last week. The OCR of continuously fed mussels in the 'control' treatment was measured at 0.62 mg O_2 g^{-1} h^{-1} during the first week (Figure 34b).

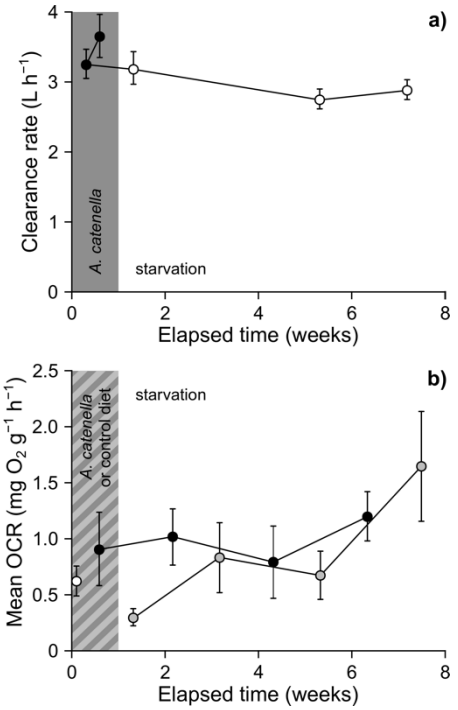

Figure 34 : Standardized clearance rate (a) and mean oxygen consumption rate (b) through time of mussels in the 'control' (white circles), 'starvation' (grey circles), and 'toxic' (black circles) treatments. Clearance rate (CR) data are expressed for a standard shell length of 40 mm. Oxygen consumption rate (OCR) data are standardized to a standard tissue dry weight of 1 g. The grey areas indicate the period (one week) during which mussels in the 'starvation' and 'toxic' treatments were fed the control diet and *A. catenella*, respectively, before being starved.

3.2.4.4 Valvometry

Five variables were retained in the temporal study of mussel behaviour through valvometry. The experimental timeline was analyzed in three segments based on the PST dynamics in mussels from the 'toxic' treatment: week 1, corresponding to the exposure period to *A. catenella*; week 2, corresponding to the maximum toxicity measured in

mussel tissues (Figure 32); and weeks 3 to 8, corresponding to the PST depuration period. The average valve opening amplitude was significantly lower in mussels from the 'toxic' treatment during weeks 1 and 2 in comparison to the other two treatments ($p < 0.001$; Table 1; Figure 35a-c). From week 3 to 8, mussels from the 'starvation' and 'toxic' treatments showed lower opening amplitudes than mussels from the 'control' treatment. No significant differences in the number of closures were observed between treatments at any time, although exposed mussels seemed to close more than starved and control mussels in week 2 and especially in week 1 (Table 1; Figure 35d-f). In terms of micro-closures, defined as rapid (≥ 3% over 100 ms) reductions in valve opening, only results obtained in week 1 showed significant differences among treatments ($p < 0.05$; Table 1; Figure 35g-i): mussels from the 'toxic' treatment had more micro-closures than those from the 'starvation' treatment, but they did not differ significantly from mussels in the 'control' treatment ($p = 0.3159$). No significant differences among treatments were found in the average ($p = 0.3752$; Figure 5j-l) and total closure duration in week 1 ($p = 0.9258$; Figure 5m-o). In week 2, mussels from the 'toxic' treatment closed for longer periods of time as compared to the other two treatments ($p < 0.01$; Table 1). From week 3 to 8, individuals from the 'toxic' and 'starvation' treatments showed longer average and total closure durations than individuals from the 'control' treatment (Table 1; Figure 35j-o).

Table 6. Mean values (± SEM) of valvometry variables within three distinct phases. Week 1 corresponds to the exposure of mussels from the 'toxic' treatment to *A. catenella*. Week 2 constitutes the peak of toxicity in mussel tissues. Weeks 3 to 8 saw a gradual decrease of toxicity as depuration happened. Letters in exponent indicate significance of the statistical test carried out within each phase on individual metrics for each variable ($p < 0.05$).

Treatment	Amplitude (%)	Closures (# d^{-1})	Micro-closures (# d^{-1})	Average Closure Duration (h)	Total Closure Duration (h d^{-1})
			Week 1 - Exposure		
control	59.3 (± 1.8) a	40.1 (± 12.4)	2.5 (± 1.0) ab	0.4 (± 0.1)	6.3 (± 0.7)
starvation	63.2 (± 1.8) a	58.2 (± 15.9)	1.0 (± 0.3) a	0.9 (± 0.2)	8.5 (± 0.8)
toxic	45.5 (± 1.6) b	114.2 (± 26.5)	4.6 (± 1.2) b	0.7 (± 0.2)	8.8 (± 0.8)
			Week 2 - Max toxicity		
control	72.3 (± 1.3) a	117.1 (± 34.6)	0.2 (± 0.1)	0.1 (± 0.0) a	2.9 (± 0.4) a
starvation	66.7 (± 2.1) a	140.9 (± 30.2)	1.1 (± 0.2)	0.2 (± 0.0) a	6.3 (± 0.6) ab
toxic	40.7 (± 1.9) b	177.4 (± 73.6)	2.1 (± 0.6)	1.3 (± 0.4) b	9.7 (± 0.9) b
			Week 3–8 - Depuration		
control	70.7 (± 1.4) a	69.4 (± 15.7)	0.4 (± 0.1)	0.1 (± 0.0) a	1.2 (± 0.2) a
starvation	51.2 (± 1.3) b	151.8 (± 28.5)	0.5 (± 0.1)	1.8 (± 0.2) b	8.0 (± 0.4) b
toxic	42.8 (± 1.1) b	146.9 (± 39.8)	1.1 (± 0.2)	2.4 (± 0.3) b	9.1 (± 0.4) b

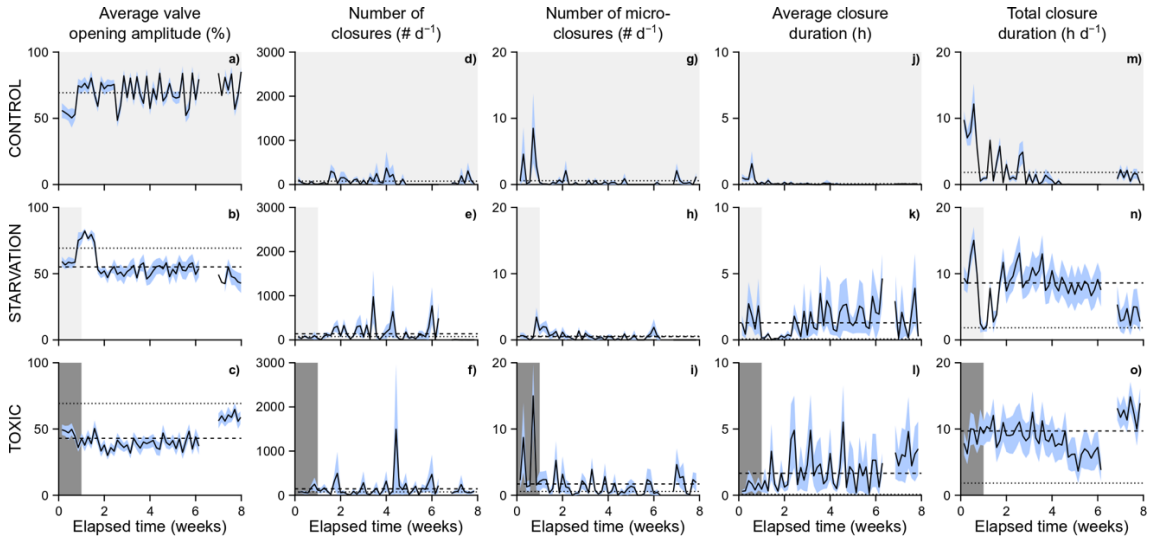

Figure 35 : Valvometry variables in the 'control' (top row), 'starvation' (mid row), and 'toxic' (bottom row) treatments showing the opening amplitude as a percentage of the maximum aperture (a-c), the daily number of closures (d-f) and of micro-closures (g-i), and the average (j-l) and total (m-o) closure duration. For each variable (each column), the dotted line represents the average value of the 'control' treatment and the dashed line the average value for the row's treatment ('starvation' or 'toxic'). N = 10 for the 'control' treatment; N = 11 for the 'starvation' and the 'toxic' treatments. Data were lost between day 44-48 due to an issue following the retrieval of data on day 44. The dark grey areas indicate the period during which mussels in the 'toxic' treatment were fed *A. catenella*. The light grey areas indicate the period during which mussels in the 'control' and 'starvation' treatments were fed the control diet.

3.2.4.5 Byssus attachment

The required strength to detach starved mussels and those exposed to *A. catenella* averaged 1.30 ± 0.20 N and 1.41 ± 0.14 N, respectively, about two times less than the strength required to dislodge mussels from the control group: 3.17 ± 0.33 N (Figure 36a). No significant differences were observed between measurements conducted at the end of

the first week and during the fourth week of the experiment. Mussels from the 'control' treatment had on average significantly more byssus threads than mussels from the 'toxic' treatment. Starved mussels stood in between, not significantly different from the two other treatments (Figure 36b).

3.2.5 Discussion

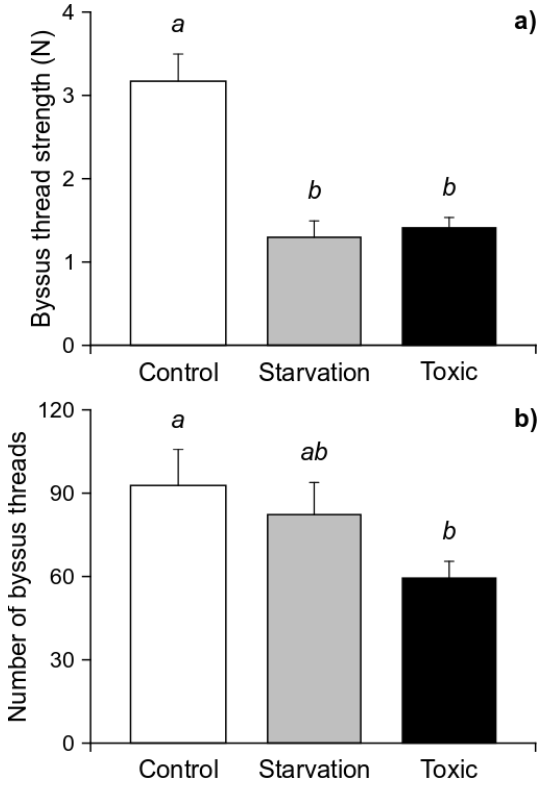

Figure 36 : Attachment strength (a) and number of byssus threads (b). Byssus attachment strength was measured on week 1 and 4 (N varied between 15 and 19 in 'control' and 'starvation' treatments and N = 33 in 'toxic' treatment). Number of threads were counted in week 4 (N = 7, N = 9, and N = 14 for the 'control', 'starvation, and 'toxic' treatments, respectively).

The response of bivalves to HABs involves both physiological and behavioural mechanisms. A better description of these effects can help manage the risks that HABs

pose to shellfish aquaculture and human health. In this experiment, we recorded the physiological and behavioural responses of blue mussels to the exposure to a St-Lawrence strain of *Alexandrium catenella*, a toxic dinoflagellate responsible for PST in many coastal areas in Northwest Atlantic waters but also worldwide. Despite the low toxicity of *A. catenella* in this study, blue mussels exposed for one week to the toxic algae accumulated significant amounts of PSTs, which remained in tissues for >2 months despite a decrease in concentration over time. Exposure of mussels to toxic algae resulted in physiological and behavioural changes including increased respiration rates, reduced valve opening amplitude and increased valve closure duration at the peak of toxicity. Most importantly, the ingestion of *A. catenella* caused a prolonged intoxication that can be of concern for aquaculture production in a natural environment where the presence of toxic algae cannot be controlled.

Toxin cell quota of *Alexandrium catenella* (formerly *A. tamarense*) from NW Atlantic waters ranges from undetectable to 79 pg STXeq cell^{-1} (Bianchi et al., 2019), meaning that the isolates used in our study were of low toxicity (2.1 pg STXeq cell^{-1}). This could explain why, on average, toxin concentration in mussel tissues did not reach the regulatory threshold for fishery harvesting closure. The maximum toxin content observed in mussel tissues neared 100 µg STXeq 100 g^{-1} and intoxication level remained above pre-exposure levels even after two months of depuration (maximum level near 40 µg STXeq 100 g^{-1}). The measured detoxification rate of 1.4% d^{-1} is very similar to lab observations ranging from 0.7 to 1.8% d^{-1} made by Sephton et al. (2007) on *M. edulis* from the Bay of Fundy (Canada) exposed to *A. fundyense* (1.2 to 2.4% d^{-1} in field samples). As noted by Sephton et al. (2007), these values are lower than the average estimate of 10.2% d^{-1} for mussel species (Bricelj and Shumway, 1998). The level of intoxication in our experiment likely accounted for part of that difference. Nevertheless, when looking at the depuration rate in the week following the maximum intoxication level (week two), the toxin elimination rate reached 8.2% d^{-1}, which is comparable to the compilation of observations by Bricelj and Shumway (1998). Previous reports on the dynamics of PST depuration in the blue mussel are therefore not unanimous. While some

studies pointed towards rapid and total toxin elimination within 72 hours (Novaczek et al., 1992), other studies indicated prolonged intoxication lasting from 2 to 4 months (Duinker et al., 2007; Sephton et al., 2007). The differences observed between these studies in the detoxification dynamics may be linked to the process and the speed of elimination of various toxic derivatives produced by *A. catenella* (Lassus et al., 1993; Bricelj et al., 1990; Blanco et al., 2003). Digestive activity was not correlated with faster detoxification rates in previous studies (Svensson, 2003; Duinker et al., 2007). However, the absence of feeding and the subsequent decreasing of metabolic rate to standard (or basal) level during starvation (Bayne, 1973) likely slowed down the processes of transformation and elimination of saxitoxin and its derivatives, which could explain the observed prolonged intoxication in this study. Another apparent driver is inter-individual variability as one third of sampled mussels in the last week of the experiment had a concentration of toxins <10 µg STXeq 100 g^{-1}. Such inter-individual variability could be caused by different energetic status at the start of the study or phenotypic differences in toxin accumulation and depuration rate (Pousse et al., 2018). Further investigations looking at the relationship between toxin accumulation and valve opening behaviour on the same individuals could help answer these questions. Additional measurements of assimilation efficiency (digestibility), known to be adversely affected by exposure to toxic algae (Navarro and Contreras, 2010), may also help understand the observed inter-individual intoxication variability and the high difference in previously reported toxin uptake efficiency, which have been showed to vary between 20% (Lassus and Berthome, 1987) and 79% (Bricelj et al., 1990).

Mussels in the 'toxic' treatment showed a particularly rapid decrease in CI, even compared to mussels in the 'starvation' treatment, which were kept unfed after the first week as well (Figure 3a). While impacts of PST on mussel physiology are relatively well documented, few studies have reported effects on energy budgets and tissue mass. The relationship between physiological performances and energy budgets represents, however, an important link for decision making as energy budget descriptors (scope for growth, dynamic energy models) integrate the physiological response to environmental

pressures and can be particularly valuable for the quantification of sub-lethal effects (e.g., Muller et al., 2010; Sarà et al., 2011; Pousse et al., 2019). Indirect measures of dry weight in juvenile mussel *Perna viridis* exposed to *A. catenella* exhibited similar mass increase as in a control group, although the scope for growth of adults in the same study was found to be lower when fed the toxic algae (Li et al., 2002). In a study on the effect of *Alexandrium minutum* on Pacific oysters *Crassostrea gigas*, Pousse et al. (2018) showed that oysters starved after being fed the toxic algae lost weight faster than unexposed starved oysters. Our results clearly match these observations and could be interpreted through three hypotheses: 1) decreased ingestion rates which would reduce the energy input to the metabolism, 2) additional energetic costs due to the repair of cellular and tissue damage caused by PSTs or by their metabolization, which could tap into the organism's reserves, or 3) a combination of both.

First, a reduction in filtration activity by mussels provided with *A. catenella* may have caused the bivalves to use their reserves more rapidly (first hypothesis). Although a reduction in the amplitude of valve opening, observed during the week of exposure to the toxic algae (Figure 35c), may suggest that ingestion of toxic algae was reduced (Newell et al., 2001; Maire et al., 2007), three observations point towards the opposite conclusion: 1) the level of toxin concentration in mussel tissues is evidence of an active ingestion of toxic algae (Figure 32); 2) mussels in the 'toxic' condition did not close their valves for a longer duration than mussels in other treatments (Figure 35, Table 1); and 3) the high CR measurements in mussels exposed to *A. catenella* during the first week together with the IR calculations seem to indicate that exposed mussels were ingesting toxic algae. Note that CR measurements were carried out with non-toxic algae, which limits our interpretations. Similarly, the positive shift (peak at 3.66 L h^{-1}) compared to the control, when switching from toxic to non-toxic algae, appears too small to suggest possible avoidance of the former. While bivalves do ingest PST producing microalgae (Nagai et al., 2006; Basti et al., 2018), these observations go against numerous reports of a reduction of filtration under exposure to toxic microalgae (Mafra Jr et al., 2010; Pousse et al., 2018; Bianchi et al., 2019; Nielsen et al., 2020). Different algal species and mussels

from different locations may explain this result. Bricelj et al. (1990) also suggested that mussels with prior history of exposure to PSTs were less likely to reduce their filtration rates in future exposures and suggested that *A. catenella* could represent a valuable food source for the bivalve. However, this is unlikely as mussels used in this study came from a region (Prince Edward Island) without recent history of PST intoxication events (McKenzie et al., 2020). The filtration activity of mussels exposed to toxic algae may also have been impacted by a repetition of micro-closures of shell valves, as first reported by Tran et al. (2010), who suggested that this behaviour may play a role in mitigating the effects of toxic algae on bivalves. While our results indicate no difference in valve micro-closures (Figure 35i), two high peaks in the number of micro-closures measured in mussels from the 'control' treatment during the first week of the experiment may be responsible for the non-significance of the comparison. The link between valvometry and CR measurements also remains complex and debated (Newell et al., 2001). Despite previous studies indicating a correlation between the closure reaction time of Manila clams and *Heterocapsa circularisquama* concentrations (Basti et al., 2009), the absence of clear results indicating a definite reduction in ingestion limits our conclusions and more detailed experiments including CR with toxic algae should therefore be conducted. Accordingly, the first hypothesis may not be fully validated.

The loss of mass in mussel tissues observed in the days following the week of exposure to *A. catenella* may also result from cellular damage and/or toxin elimination (second hypothesis). Widdows et al. (1979) described marked damages in digestive cells of *M. edulis* fed with the toxic dinoflagellate *Gyrodinium aureolum*. Galimany et al. (2008) observed a reduction in the size, number, and complexity of hemocytes, the immune cells involved in the response to chemical or cellular stimuli as part of defense mechanisms in bivalves. The repair of these damaged tissues is likely to add costs to the energy budget of exposed mussels. The observed increase in oxygen consumption rates during the exposure of mussels to *A. catenella* (Figure 34b) supports this hypothesis. In fact, bivalves have been known to increase their oxygen consumption rate when exposed to contaminants (Rao and Khan, 2000), pathogens (Flye-Sainte-Marie et al., 2007), and

parasites (Chambon et al., 2007), which trigger defense mechanisms that increase metabolic activity and therefore oxygen consumption. Although one study indicated that *M. edulis* did not modify its oxygen consumption rate after a 1 h-exposure to *A. catenella* (Marsdsen and Shumway, 1993), it is possible that a prolonged exposure (seven days) to the toxic microalgae caused an elevation in respiration rates of the mussels in our study. Some studies cited above also reported a rapid recovery in oxygen consumption rates after exposure to toxic microalgae (Widdows et al., 1979; Galimany et al., 2008; Marsden and Shumway, 1993), which seems to be the case in the present work as indicated by similar oxygen consumption rates between the 'toxic' and the 'starvation' treatments past the third week of experiment. Galimany et al. (2008) also described an increase in the migration (diapedesis) of hemocytes carrying the toxin in lipofuchsin granules (ceroidosis) across the stomach and intestine epithelia into the alimentary canal. The energetic costs of the repair of damaged cells and the elimination of the toxin as a direct consequence of exposure to *A. catenella* may, therefore, be valid explanations of the rapid decrease in CI of intoxicated mussels. However, we cannot at this point determine nor quantify the respective role of these two processes. These additional energy expenses following PST accumulation by mussels may have tempered with other biological functions such as growth or the production of byssus. Mussels from the 'toxic' and 'starvation' treatments showed almost no shell growth (Figure 32b). However, we observed a reduction in the number of threads by individuals from the 'toxic' treatment (Figure 6b). Previous findings from the literature are contradictory on this matter as some authors reported an inhibition of byssus production in *M. edulis* exposed to *Protogonyaulax tamarensis* (an epitype of *A. catenella*, Shumway et al., 1987). Other studies observed no change in *Perna canaliculus* exposed to *A. tamarense*, despite elevated PST content in tissues (Marsden and Shumway, 1993). Clearly, more research is needed to assert the impact on byssus production. This second hypothesis of additional energy costs to cope with the intoxication, therefore, appears more likely to explain the reduction in CI early on in exposed mussels, although long-term consequences on their energy budget remain uncertain.

This study is one of few looking at potential effects following an acute exposure to toxic algae in an integrative way. Despite the incomplete depuration of some mussels after seven weeks of detoxification, there is no clear evidence that *M. edulis* remained metabolically impacted by the early exposure to *A. catenella*. Past the early differences in CI reduction and valve behaviour between mussels from the 'toxic' and 'starvation' treatments, organisms from both groups followed the same rate of mass loss and showed similar activity (Figure 33a; Figure 35). Similarly, the late increase in oxygen consumption rate in these two treatments might be attributed to the loss of mass through the experiment in starved mussels. Moreover, the force required to detach mussels from their byssus anchorage did not seem to vary because of the exposure to *A. catenella*, as equal strength of byssal threads was observed between mussels from these two treatments (Figure 6a). A fast response in the elimination of the toxin involving the mobilisation of energy reserves reduced the level of intoxication by 65% in one week after the peak in week 2 likely mitigated the negative effects of PST. In fact, *M. edulis* is recognized as a fast detoxifier (Bricelj and Shumway, 1998). Another means of mitigation, though, is the pre- and post-ingestive selection of non-toxic algal cells by the bivalves, as it has been evidenced in several studies (e.g., Shumway and Cucci, 1987; Pousse et al., 2019). However, although mussels in our study were fed with either toxic or non-toxic diets, no pseudofaeces were observed. Moreover, a previous experiment conducted in our laboratory indicated no apparent pre-ingestive selection by *M. edulis* between *A. catenella* and *Heterocapsa triquetra* fed at similar concentrations as in the present study (Tremblay et al., 2018).

While the effects of PST on human health have been well documented, a significant lack of knowledge is yet to be filled to fully understand their impacts on shellfish, the main vectors of such intoxication. Moreover, with an increasing number of reports of HAB worldwide, working toward a better understanding of such impacts is of importance for aquaculture production (Collins et al., 2020). In this study, we showed that the physiological changes linked to toxin accumulation and depuration can momentarily be associated with behavioural perturbations. Immediate response of blue mussels to the

exposure to *Alexandrium catenella* involved complex physiological and behavioural changes including rapid mass loss, increased respiration rates, longer valve closure durations, and a reduction in valve opening amplitude. In the weeks following the exposure, no clear differences were observed in comparison to non-exposed mussels. These results represent advancements in our understanding of the impacts of HAB on bivalves and may contribute to the development of predicting tools to help track the depuration of PST in *M. edulis* (Rosland et al., 2013; Pousse et al., 2019). They should also provide valuable data and knowledge to managers and help design mitigation measures necessary to both the safety of consumers and the sustainability of aquaculture operations.

Acknowledgements

The Galway Statement on Atlantic Ocean Cooperation motivated this collaborative project. This project was undertaken with the financial support of the Government of Canada, specifically by Environment and Climate Change Canada (FDE-CA-2017i002) and Fisheries and Oceans Canada. In kind support including travel and salary contribution was provided by the Spanish Instituto de Investigaciones Marinas in Vigo (IIM-CSIC) and Dalhousie University (NSERC Discovery Grant).

Declaration of competing interest

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

Credit author statement

Romain Lavaud: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization.

Jean-Bruno Nadalini: Conceptualization, Methodology, Formal analysis, Writing - review & editing.

Guillaume Durier: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization.

Ramón Filgueira: Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision.

Luc Comeau: Conceptualization, Methodology, Investigation, Writing - review & editing.

Jose M.F. Babarro: Conceptualization, Methodology, Investigation, Writing - review & editing.

Sonia Michaud: Methodology, Formal analysis, Resources, Writing - review & editing.

Michael Scarratt: Methodology, Formal analysis, Resources.

Réjean Tremblay: Conceptualization, Methodology, Investigation, Resources, Supervision, Project administration, Funding acquisition.

3.2.6 References

Alunno-Bruscia, M., Bourget, E. and Fréchette, M., 2001. Shell allometry and length-mass-density relationship for *Mytilus edulis* in an experimental food-regulated situation. Marine Ecology Progress Series. 219, 177–188P.

Basti, L., Nagai, K., Shimasaki, Y., Oshima, Y., Honjo, T. and Segawa, S., 2009. Effects of the toxic dinoflagellate *Heterocapsa circularisquama* on the valve movement behaviour of the Manila clam *Ruditapes philippinarum*. Aquaculture. 291(1-2), 41-47.

Basti, L., Nagai, S., Watanabe, S., Oda, T. and Tanaka, Y., 2016. Neuroenzymatic activity and physiological energetics in Manila clam, *Ruditapes philippinarum*, during short-term sublethal exposure to harmful alga, *Heterocapsa circularisquama*. Aquatic Toxicology, 176, 76-87.

Basti, L., Hégaret, H. and Shumway, S.E., 2018. Harmful algal blooms and shellfish. In: John Wiley & Sons, Ltd., Harmful Algal Blooms: A Compendium Desk Reference. Hoboken, NJ, USA,135–190.

Bayne, B., 1973. Aspects of the metabolism of *Mytilus edulis* during starvation. Netherlands Journal of Sea Research. 7, 399–410.

Beaudry, A., Lacaze, E., Jobin-Piché, A., Masson, S., Auffret, M., Brousseau, P. and Fournier, M., 2015. Ecotoxicological evaluation of the immunocompetence of two bivalve species (*Mya arenaria* and *Mytilus edulis*) in the Saguenay Fjord including a salinity gradient. Journal of Xenobiotics. 5(2),.5769.

Beyer, J., Green, N.W., Brooks, S., Allan, I.J., Ruus, A., Gomes, T., Bråte, I.L.N. and Schøyen, M., 2017. Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: a review. Marine Environmental Research. 130, 338–365.

Bianchi, V.A., Langeloh, H., Tillmann, U., Krock, B., Müller, A., Bickmeyer, U. and Abele, D., 2019. Separate and combined effects of neurotoxic and lytic compounds of *Alexandrium* strains on *Mytilus edulis* feeding activity and hemocyte function. Fish & Shellfish Immunology. 84, 414–422.

Blanco, J., Reyero, M.I. and Franco, J., 2003. Kinetics of accumulation and transformation of paralytic shellfish toxins in the blue mussel *Mytilus galloprovincialis*. Toxicon, 42(7). 777–784.

Blasco, D., Levasseur, M., Bonneau, E., Gelinas, R. and Packard, T.T., 2003. Patterns of paralytic shellfish toxicity in the St. Lawrence region in relationship with the abundance and distribution of *Alexandrium tamarense*. Scientia Marina, 67(3). 261–278.

Bricelj, V.M., Lee, J.H., Cembella, A.D. and Anderson, D.M., 1990. Uptake kinetics of paralytic shellfish toxins from the dinoflagellate *Alexandrium fundyense* in the mussel *Mytilus edulis*. Marine Ecology Progress Series. 63. 177–188.

Bricelj, V.M. and Shumway, S.E., 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. Reviews in Fisheries Science. 6(4). 315–383.

Burkholder, J.M., 1998. Implications of harmful microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries. Ecological Applications. 8(1). S37–S62.

Chambon, C., Legeay, A., Durrieu, G., Gonzalez, P., Ciret, P. and Massabuau, J.C., 2007. Influence of the parasite worm *Polydora* sp. on the behaviour of the oyster *Crassostrea gigas*: a study of the respiratory impact and associated oxidative stress. Marine Biology. 152(2), 329–338.

Collins, C., Bresnan, E., Brown, L., Falconer, L., Guilder, J., Jones, L., Kennerley, A., Malham, S., Murray, A. and Stanley, M., 2020. Impacts of climate change on aquaculture. MCCIP Science Review 2020. 482–520.

Comeau, L.A., Mayrand, É. and Mallet, A., 2012. Winter quiescence and spring awakening of the eastern oyster *Crassostrea virginica* at its northernmost distribution limit. Marine Biology 159, 2269–2279.

Comeau, L.A., Babarro, J.M., Riobó, P., Scarratt, M., Starr, M. and Tremblay, R., 2019. PSP-producing dinoflagellate *Alexandrium minutum* induces valve microclosures in the mussel *Mytilus galloprovincialis*. Aquaculture. 500, 407–413.

Cranford, P.J., Strohmeier, T., Filgueira, R. and Strand, Ø., 2016. Potential methodological influences on the determination of particle retention efficiency by suspension feeders: *Mytilus edulis* and *Ciona intestinalis*. Aquatic Biology 25. 61–73.

Davidson, K., Gowen, R.J., Harrison, P.J., Fleming, L.E., Hoagland, P., Moschonas, G, 2014. Anthropogenic nutrients and harmful algae in coastal waters. Journal of Environmental Management, 46. 206-216.

Desbiens, M., Coulombe, F., Gaudreault, J., Cembella, A.D. and Larocque, R., 1990. PSP toxicity of wild and cultured blue mussels induced by *Alexandrium excavatum* in Gaspé Bay (Canada): Implications for aquaculture. In: Graneli, E., Sundstrom, B., Edler, L., Anderson, D.M. (Eds.), Toxic marine phytoplankton. Elsevier, NY, USA. 459–462.

Duinker, A., Bergslien, M., Strand, Ø., Olseng, C.D. and Svardal, A., 2007. The effect of size and age on depuration rates of diarrhetic shellfish toxins (DST) in mussels (*Mytilus edulis* L.). Harmful Algae, 6(2). 288–300.

Fabioux, C., Sulistiyani, Y., Haberkorn, H., Hégaret, H., Amzil, Z. and Soudant, P., 2015. Exposure to toxic *Alexandrium minutum* activates the detoxifying and antioxidant systems in gills of the oyster *Crassostrea gigas*. Harmful Algae. 48, 55–62.

Flye-Sainte-Marie, J., Pouvreau, S., Paillard, C. and Jean, F., 2007. Impact of Brown Ring Disease on the energy budget of the Manila clam *Ruditapes philippinarum*. Journal of Experimental Marine Biology and Ecology, 349(2). 378–389.

Galimany, E., Sunila, I., Hégaret, H., Ramón, M. and Wikfors, G.H., 2008. Experimental exposure of the blue mussel (*Mytilus edulis*, L.) to the toxic dinoflagellate *Alexandrium fundyense*: Histopathology, immune responses, and recovery. Harmful Algae. 7(5), 702–711.

Gibbard, J. and Naubert, J., 1948. Paralytic shellfish poisoning on the Canadian Atlantic coast. American Journal of Public Health and the Nations Health, 38(4), pp.550–553.

Goldberg, E.D., 1975. The mussel watch-a first step in global marine monitoring. Marine Pollution Bulletin. 6, 111.

Guéguen, M., Bardouil, M., Baron, R., Lassus, P., Truquet, P., Massardier, J. and Amzil, Z., 2008. Detoxification of Pacific oyster *Crassostrea gigas* fed on diets of *Skeletonema*

costatum with and without silt, following PSP contamination by *Alexandrium minutum*. Aquatic Living Resources. 21(1), 13–20.

Guéguen, M., Baron, R., Duinker, A., Aasen, J. and Naustvoll, L.J., 2010. Modelling the environmental variable influences on the detoxification kinetics on mussels *Mytilus edulis* containing lipophilic toxins. IFAC Proceedings Volumes. 43(6), 508–512.

Guillotreau, P., Le Bihan, V., Morineau, B. and Pardo, S., 2021. The vulnerability of shellfish farmers to HAB events: An optimal matching analysis of closure decrees. Harmful Algae, 101, p.101968.

Hégaret, H. and Wikfors, G.H., 2005. Effects of natural and field-simulated blooms of the dinoflagellate *Prorocentrum minimum* upon hemocytes of eastern oysters, *Crassostrea virginica*, from two different populations. Harmful Algae. 4(2), 201-209.

Hégaret, H., Brokordt, K.B., Gaymer, C.F., Lohrmann, K.B., García, C. and Varela, D., 2012. Effects of the toxic dinoflagellate *Alexandrium catenella* on histopathogical and escape responses of the Northern scallop *Argopecten purpuratus*. Harmful Algae. 18, 74–83.

Jaime, E., Gerdts, G. and Luckas, B., 2007. In vitro transformation of PSP toxins by different shellfish tissues. Harmful Algae, 6(3). 308–316.

John, U., Litaker, W., Montresor, M., Murray, S., Brosnahan, M.L. and Anderson, D.M., 2014. (2302) Proposal to reject the name *Gonyaulax catenella* (*Alexandrium catenella*) (Dinophyceae). Taxon. 63(4), 932.

Jones, H.D., Richards, O.G. and Southern, T.A., 1992. Gill dimensions, water pumping rate and body size in the mussel *Mytilus edulis* L. Journal of Experimental Marine Biology and Ecology, 155(2), pp.213–237.

Jones, S.H., Chase, M., Sowles, J., Hennigar, P., Landry, N., Wells, P.G., Harding, G.C.H., Krahforst, C. and Brun, G.L., 2001. Monitoring for toxic contaminants in *Mytilus edulis* from New Hampshire and the Gulf of Maine. Journal of Shellfish Research. 20(3), 1203–1214.

Lacroix, C., Duvieilbourg, E., Guillou, N., Guyomarch, J., Bassoulet, C., Moraga, D., Chapalain, G. and Auffret, M., 2017. Seasonal monitoring of blue mussel (*Mytilus* spp.) populations in a harbor area: A focus on responses to environmental factors and chronic contamination. Marine environmental research. 129, 24–35.

Lassudrie, M., Soudant, P., Nicolas, J.L., Fabioux, C., Lambert, C., Miner, P., Le Grand, J., Petton, B. and Hégaret, H., 2015. Interaction between toxic dinoflagellate *Alexandrium catenella* exposure and disease associated with herpesvirus OsHV-1 µVar in Pacific oyster spat *Crassostrea gigas*. Harmful Algae. 45, 53–61.

Lassudrie, M., Soudant, P., Nicolas, J.L., Miner, P., Le Grand, J., Lambert, C., Le Goïc, N., Hégaret, H. and Fabioux, C., 2016. Exposure to the toxic dinoflagellate *Alexandrium catenella* modulates juvenile oyster *Crassostrea gigas* hemocyte variables subjected to different biotic treatments. Fish & Shellfish Immunology. 51, 104–115.

Lassudrie, M., Hégaret, H., Wikfors, G.H. and da Silva, P.M., 2020. Effects of marine harmful algal blooms on bivalve cellular immunity and infectious diseases: A review. Developmental & Comparative Immunology. 108, 103660.

Lassus, P. and Berthome, J.P., 1987. Toxicité des moules : étude de la décontamination PSP et DSP in vitro et in situ. Ifremer report DER0-86.13-MR, DRV-86.01-SR, 37.

Lassus, P., Ledoux, M., Bardouil, M. and Bohec, M., 1993. Influence of initial toxicity and extraction procedure on paralytic toxin changes in the mussel. Toxicon, 31(3), pp.237–242.

Lassus, P., Bardouil, M., Masselin, P., Naviner, M. and Truquet, P., 2000. Comparative efficiencies of different non-toxic microalgal diets in detoxification of PSP-contaminated oysters (*Crassostrea gigas* Thunberg). Journal of Natural Toxins. 9(1), 1–12.

Li, S.C., Wang, W.X. and Hsieh, D.P., 2002. Effects of toxic dinoflagellate *Alexandrium tamarense* on the energy budgets and growth of two marine bivalves. Marine environmental research. 53(2), 145–160.

MacQuarrie, S.P. and Bricelj, V.M., 2008. Behavioral and physiological responses to PSP toxins in *Mya arenaria* populations in relation to previous exposure to red tides. Marine Ecology Progress Series. 366, 59–74.

Mafra Jr, L.L., Bricelj, V.M., Ouellette, C. and Bates, S.S., 2010. Feeding mechanics as the basis for differential uptake of the neurotoxin domoic acid by oysters, *Crassostrea virginica*, and mussels, *Mytilus edulis*. Aquatic Toxicology. 97(2), 160–171.

Maire, O., Amouroux, J.M., Duchêne, J.C. and Grémare, A., 2007. Relationship between filtration activity and food availability in the Mediterranean mussel *Mytilus galloprovincialis*. Marine Biology. 152(6), 1293–1307.

Manfrin, C., De Moro, G., Torboli, V., Venier, P., Pallavicini, A. and Gerdol, M., 2012. Physiological and molecular responses of bivalves to toxic dinoflagellates. Invertebrate Survival Journal. 9(2), 184–199.

Marcaillou, C., Haure, J., Mondeguer, F., Courcoux, A., Dupuy, B. and Pénisson, C., 2010. Effect of food supply on the detoxification in the blue mussel, *Mytilus edulis*, contaminated by diarrhetic shellfish toxins. Aquatic Living Resources. 23(3), 255–266.

Marsden, I.D. and Shumway, S.E., 1993. The effect of a toxic dinoflagellate (*Alexandrium tamarense*) on the oxygen uptake of juvenile filter-feeding bivalve molluscs. Comparative Biochemistry and Physiology Part A: Physiology. 106(4), 769–773.

Martin, J.L. and Richard, D., 1996. Shellfish toxicity from the Bay of Fundy, eastern Canada: 50 years in retrospect. In: Yasumoto, T., Oshima, Y., Fukuyo, Y. (Eds.), Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO. 3–6.

Maso, M. and Garcés, E., 2006. Harmful microalgae blooms (HAB); problematic and treatments that induce them. Marine Pollution Bulletin. 53(10–12), 620–630.

Mat, A.M., Haberkorn, H., Bourdineaud, J.P., Massabuau, J.C. and Tran, D., 2013. Genetic and genotoxic impacts in the oyster *Crassostrea gigas* exposed to the harmful alga *Alexandrium minutum*. Aquatic Toxicology. 140, 458–465.

McKenzie, C.H., Bates, S.S., Martin, J.L., Haigh, N., Howland, K.L., Lewis, N.I., Locke, A., Peña, A., Poulin, M., Rochon, A. and Rourke, W.A., 2020. Three decades of Canadian marine harmful algal events: Phytoplankton and phycotoxins of concern to human and ecosystem health. Harmful Algae. 102, 101852.

Muller, E.B., Nisbet, R.M. and Berkley, H.A., 2010. Sublethal toxicant effects with dynamic energy budget theory: model formulation. Ecotoxicology 19(1), 48-60.

Navarro, J.M. and Contreras, A.M., 2010. An integrative response by *Mytilus chilensis* to the toxic dinoflagellate *Alexandrium catenella*. Marine Biology. 157(9), 1967–1974.

Nagai, K., Honjo, T., Go, J., Yamashita, H. and Oh, S.J., 2006. Detecting the shellfish killer *Heterocapsa circularisquama* (Dinophyceae) by measuring bivalve valve activity with a Hall element sensor. Aquaculture,.255(1–4), 395–401.

Newell, C.R., Wildish, D.J. and MacDonald, B.A., 2001. The effects of velocity and seston concentration on the exhalant siphon area, valve gape and filtration rate of the mussel *Mytilus edulis*. Journal of Experimental Marine Biology and Ecology. 262(1), 91–111.

Nielsen, M.V. and Strømgren, T., 1991. Shell growth response of mussels (*Mytilus edulis*) exposed to toxic microalgae. Marine Biology. 108(2), 263–267.

Nielsen, L.T., Hansen, P.J., Krock, B. and Vismann, B., 2016. Accumulation, transformation and breakdown of DSP toxins from the toxic dinoflagellate *Dinophysis acuta* in blue mussels, *Mytilus edulis*. Toxicon. 117, 84–93.

Nielsen, P., Krock, B., Hansen, P.J. and Vismann, B., 2020. Effects of the DSP-toxic dinoflagellate *Dinophysis acuta* on clearance and respiration rate of the blue mussel, *Mytilus edulis*. Plos One. 15(3), e0230176.

Novaczek, I., Madhyastha, M.S. and Ablett, R.F., 1992. Depuration of domoic acid from live blue mussels (*Mytilus edulis*). Canadian Journal of Fisheries and Aquatic Sciences. 49, 312–318.

Pousse, É., Flye-Sainte-Marie, J., Alunno-Bruscia, M., Hégaret, H. and Jean, F., 2018. Sources of paralytic shellfish toxin accumulation variability in the Pacific oyster *Crassostrea gigas*. Toxicon. 144, 14–22.

Pousse, É., Flye-Sainte-Marie, J., Alunno-Bruscia, M., Hégaret, H., Rannou, É., Pecquerie, L., Marques, G.M., Thomas, Y., Castrec, J., Fabioux, C. and Long, M., 2019. Modelling paralytic shellfish toxins (PST) accumulation in *Crassostrea gigas* by using Dynamic Energy Budgets (DEB). Journal of Sea Research. 143, 152–164.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.

Rao, D.P. and Khan, M.A.Q., 2000. Zebra mussels: enhancement of copper toxicity by high temperature and its relationship with respiration and metabolism. Water Environment Research. 72(2), 175–178.

Rosland, R., Alunno-Bruscia, M., Strand, Ø, Strohmeier, T., 2013. A DEB based analysis of growth and toxin elimination processes in mussels (*Mytilus edulis*) exposed to Diarrhetic Shellfish Toxins (DST). Poster presentation at DEB 2013 - third DEB symposium, 23–26 April 2013, Texel, Netherlands. Available at: https://archimer.ifremer.fr/doc/00148/25936/24001.pdf.

Sarà, G., Kearney, M. and Helmuth, B., 2011. Combining heat-transfer and energy budget models to predict thermal stress in Mediterranean intertidal mussels. Chemistry and Ecology. 27(2), 135-145.

Schantz, E.J., 1970. Algal toxins. In: Properties and Products of Algae. Zajic J.E., 83–96. Springer, Boston, MA, USA

Sephton, D.H., Haya, K., Martin, J.L., LeGresley, M.M. and Page, F.H., 2007. Paralytic shellfish toxins in zooplankton, mussels, lobsters and caged Atlantic salmon, *Salmo salar*, during a bloom of *Alexandrium fundyense* off Grand Manan Island, in the Bay of Fundy. Harmful Algae. 6(5), 745–758.

Shumway, S.E., 1990. A review of the effects of algal blooms on shellfish and aquaculture. Journal of the World Aquaculture Society. 21(2), 65–104.

Shumway, S.E. and Cucci, T.L., 1987. The effects of the toxic dinoflagellate *Protogonyaulax tamarensis* on the feeding and behaviour of bivalve molluscs. Aquatic toxicology. 10(1), 9–27.

Shumway, S.E., Pierce, F.C. and Knowlton, K., 1987. The effect of *Protogonyaulax Tamarensis* on byssus production in *Mytilus edulis* l., *Modiolus modiolus* linnaeus, 1758 and *Geukensia demissa* dillwyn. Comparative Biochemistry and Physiology Part A: Physiology. 87(4), 1021–1023.

Smaal, A.C., Vonck, A.P.M.A. and Bakker, M., 1997. Seasonal variation in physiological energetics of *Mytilus edulis* and *Cerastoderma edule* of different size classes. Journal of the Marine Biological Association of the United Kingdom. 77(3), 817–838.

Starr, M., Lair, S., Michaud, S., Scarratt, M., Quilliam, M., Lefaivre, D., Robert, M., Wotherspoon, A., Michaud, R., Ménard, N. and Sauvé, G., 2017. Multispecies mass mortality of marine fauna linked to a toxic dinoflagellate bloom. PloS One, 12(5), e0176299.

Statistics Canada, 2018. Table 32-10-0107-01 Aquaculture, production and value. Available at: https://www.dfo-mpo.gc.ca/stats/aqua/aqua18-eng.htm.

Svensson, S., 2003. Depuration of Okadaic acid (Diarrhetic Shellfish Toxin) in mussels, *Mytilus edulis* (Linnaeus), feeding on different quantities of nontoxic algae. Aquaculture, 218(1–4), 277–291.

Svensson, S. and Förlin, L., 2004. Analysis of the importance of lipid breakdown for elimination of okadaic acid (diarrhetic shellfish toxin) in mussels, *Mytilus edulis*: results from a field study and a laboratory experiment. Aquatic Toxicology. 66(4), 405–418.

Tran, D., Haberkorn, H., Soudant, P., Ciret, P. and Massabuau, J.C., 2010. Behavioral responses of *Crassostrea gigas* exposed to the harmful algae *Alexandrium minutum*. Aquaculture. 298(3–4), 338–345.

Tremblay, R., Nadalini, J.-B., Comeau, L., Marcotte, I., Filgueira, R., Starr, M., Scarratt, M., Lavaud, R. Durier, G., Grenier, O., Warschawski, D, Hégaret, H., Tran, D. Wikfors, G., Babarro, J., Galimany, E. 2018. Comportement valvométrique de la moule bleue en présence d'algues toxiques *Alexandrium tamarense*. Annual meeting of the research network Ressources Aquatic Québec. 7–8 November 2018, Québec city, Canada.

Vilariño, N., Louzao, M.C., Abal, P., Cagide, E., Carrera, C., Vieytes, M.R. and Botana, L.M., 2018. Human poisoning from marine toxins: unknowns for optimal consumer protection. Toxins. 10(8), 324.

Widdows, J. (1985). The effects of fluctuating and abrupt changes in salinity on the performance of *Mytilus edulis*. In: Gray J.S., Christiansen M.E. (Eds.), Marine biology of polar regions and effects of stress on marine organisms. Wiley, Chichester. 555–566.

Widdows, J., Moore, M.N., Lowe, D.M. and Salkeld, P.N., 1979. Some effects of a dinoflagellate bloom (*Gyrodinium aureolum*) on the mussel, *Mytilus edulis*. Journal of the Marine Biological Association of the United Kingdom, 59(2). 522–524.

CONCLUSION GÉNÉRALE

L'aquaculture marine étant un secteur ayant un très grand développement sur les dernières années (FAO, 2009), l'impact des contraintes auxquelles elle fait face est de plus en plus fort. L'une de ces contraintes est l'exposition à des toxines biologiques (Díaz et al., 2019) ou chimiques. Dans le but de protéger les consommateurs des produits issus de l'aquaculture ainsi que la santé économique des fermes aquacoles, il est donc important d'être capable d'alerter les aquaculteurs de l'arrivée de ces toxines pour permettre de prendre les mesures adaptées (Kramer and Botterweg, 1991). Pour cela il est intéressant d'utiliser des espèces sentinelles comme des bivalves pour assurer cette veille (Andrewartha and Elliott, 2015; Comeau et al., 2019). Ces derniers étant capables d'accumuler les contaminants, de survivre dans des environnements contaminés et faciles à manipuler, ils sont en effet très adaptés pour cette utilisation (Dame and Kenneth, 2011; Martin and Richardson, 1991). Il existe cependant de grandes différences morphologiques et comportementales chez les bivalves, ce groupe étant le deuxième plus diversifié chez les mollusques (Gosling, 2015). Les espèces de bivalves vivent dans des environnements différents et ont donc des traits pouvant différer d'une espèce à l'autre. Par exemple la moule est une espèce intertidale qui doit d'adapter à l'exondation en étant capable de se fermer de manière étanche. Quant à lui, le pétoncle qui est une espèce subtidale n'a pas cette contrainte. Les bivalves réagiront donc différemment aux toxines auxquelles ils sont exposés. Identifier l'espèce de bivalve la plus adaptée à une surveillance de la présence de toxine présente un intérêt pour l'aquaculture. De la même manière, il existe une grande diversité de polluants pouvant avoir des effets variables sur les bivalves. Les toxines pouvant impacter les fermes aquicoles sont les toxines produites par des algues toxiques (Anderson et al., 1989; Hallegraeff, 1993; Smayda, 1997) et les toxines provenant des activités humaines. Il est donc nécessaire d'identifier les effets de ces différents types de toxines sur les bivalves pour identifier les changements permettant de détecter de manière précoce l'arrivée de ces toxines dans les zones d'aquaculture. Le

comportement valvaire des bivalves est un paramètre pouvant être utilisé pour ces suivis, car il peut être enregistré de manière non invasive et à haute fréquence (Andrade et al., 2016). De plus, il est connu que lors de l'apparition d'un stress, le comportement valvaire des bivalves change, révélant ce stress (Comeau et al., 2019; Fournier et al., 2004; Sow et al., 2011; Tran et al., 2015, 2010, 2003). Enfin il est également intéressant de connaître l'impact général des toxines sur la physiologie des bivalves afin de mieux comprendre leur mode d'action notamment lors de la dépuration dans le but d'aider les aquaculteurs à réagir lors de l'intoxication de leurs bivalves.

Au cours de cette étude il a donc été question dans le chapitre 1 d'observer les différences de comportement entre la moule bleue et le pétoncle géant, l'une étant intertidale et l'autre subtidale et d'identifier la réaction de ces deux espèces à une exposition à une substance synthétique, celle-ci étant un dispersant de pétrole. Le chapitre 2 a quant à lui été consacré à l'étude de l'impact du dinoflagellé toxique, *Alexandrium catenella* produisant des PST sur le comportement valvaire de la moule bleue. Cette étude a ensuite été complétée par la recherche d'une éventuelle résistance aux toxines lors d'une intoxication régulière. Finalement le chapitre 3 a été consacré à l'étude de l'effet *d'A. catenella* sur la physiologie de la moule bleue et de l'évolution de ces effets lors de la dépuration des moules contaminées par la toxine produite par ce dinoflagellé.

Lors de ce doctorat, le premier objectif était de mettre en place un système de détection précoce de toxine. La première étape a été d'identifier quelle espèce de bivalve est la plus adaptée pour la détection des toxines. Il a été démontré que la moule, espèce intertidale capable de s'isoler du milieu extérieur, est plus adaptée à une utilisation pour un système de détection précoce que le pétoncle, subtidal et incapable de s'isoler du milieu extérieur. L'étape suivante a été de comparer deux types de toxines dans le but d'identifier les différents types de réactions comportementales à ces toxines et donc identifier des indicateurs comportementaux décrivant les changements de comportements valvaires. Il a été observé qu'une substance synthétique comme le Corexit 1500 provoque des fermetures des valves du bivalve pour s'isoler de la toxine alors qu'une toxine naturelle comme les PSP produite par

A. catenella vont provoquer des paralysies du muscle adducteur induisant une plus longue ouverture des valves. Les mesures de taux de filtration effectués dans le chapitre 3 indiquent que les moules consomment *A. catenella* de la même manière que les algues non toxiques et vont donc absorber les PST en même temps qu'elles se nourrissent. Au contraire, le Corexit étant libre dans l'eau de mer, il sera plus facile pour la moule de ne pas l'absorber en se fermant. De plus, les corrélations entre la concentration en Corexit et les indicateurs comportementaux suggèrent que la présence de nourriture stimule le processus de filtration. Cela provoque donc une légère ouverture des moules en présence de Corexit sauf pour les concentrations les plus fortes, mais cela peut également suggérer que la moule ayant une préférence pour les dinoflagellés (Bougrier et al., 1997; Cucci et al., 1985), aura tendance à facilement consommer *A. catenella* malgré sa toxicité.

L'objectif suivant était de déterminer le délai dont pourrait disposer un aquaculteur entre la détection des toxines par le système de détection précoce et le moment ou le seuil d'interdiction de récolte des bivalves lié aux toxines. Ce délai a pu être mesuré en conditions naturelles pour coller au maximum à la réalité et a été estimé à 10 jours. Un aquaculteur disposerait donc de 10 jours pour prendre des mesures. Ce délai étant conséquent, il est donc possible de conclure que le système de détection précoce utilisant la moule bleue comme espèce sentinelle peut être efficace et peut donc être développé pour une utilisation par les aquaculteurs. Chez la Mye commune, il a été démontré qu'une résistance pouvait être développée (Bricelj et al., 2005). Il a donc été nécessaire de vérifier si cette résistance pouvait être développée chez la moule. Les résultats obtenus démontrent qu'aucune différence n'a pu être observée entre une population de moule souvent exposée ou rarement exposée au PSP rendant le système de détection précoce utilisable, peu importe la fréquence d'exposition aux toxines. La dépuration des toxines par les moules a également été étudiée au cours de ce doctorat. Cela a permis de mettre en évidence la nécessité d'avoir un apport de nourriture pour les moules pour que cette dépuration s'effectue. En effet, dans le cadre de notre expérience, les moules étaient toujours toxiques 7 semaines après l'arrêt de la nutrition avec des algues toxiques, indiquant que la dépuration est ralentie par l'absence de nourriture. L'apport d'énergie étant réduit durant cette période sans nourriture, la moule n'aurait pas

l'énergie nécessaire pour la réparation cellulaire et l'évacuation des toxines apportées par la consommation d'*A. catenella*. Étudier l'effet de *A. catenella* sur la physiologie des moules peut permettre de mieux appréhender les difficultés liées à cette algue toxique pour l'aquaculture. Ce doctorat a donc permis de démontrer la possibilité de mettre en place d'un système de détection précoce utilisant le comportement valvaire la moule bleue. Ce système serait certainement plus couteux que d'autres lors de l'achat, cependant sa durabilité pourrait le rendre intéressant, celui-ci ne générant peu de frais de fonctionnement un fois acheté. Le développement d'un logiciel donnant une alerte en cas de présence d'une toxine permettrait également la nécessité de ressource humaine traitant les données. Le processus de détection en fonction de la toxine est résumé dans la fig. 37. Pour le développement de ce système de détection, il sera nécessaire d'optimiser le traitement des données pour que celui-ci soit plus performant. Il sera également intéressant de développer l'utilisation de valvomètres fonctionnant avec le réseau GPRS pour permettre de suivre plusieurs sites simultanément.

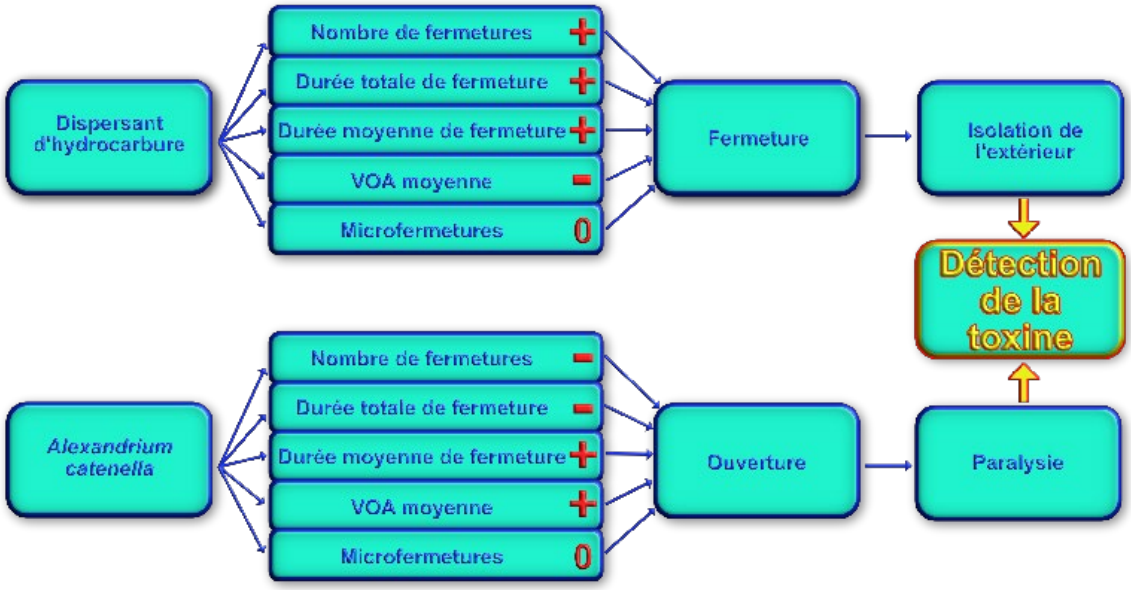

Figure 37 : Schéma de la détection de toxines grâce au comportement valvaire de bivalve.

PERSPECTIVES

Système de détection précoce

Lors de ce doctorat, il a été démontré que les moules bleues peuvent être utilisées comme une espèce sentinelle efficace pour la détection de l'arrivée d'algues toxiques comme *A. catenella* ou de substances synthétiques comme le dispersant de pétrole Corexit. La prochaine étape dans le développement de ce système serait son utilisation en condition de détection de toxine. Le système de détection serait déployé dans une zone pouvant être exposée à une toxine et suivre le comportement des moules tout en suivant la concentration de ces toxines à l'aveugle, c'est-à-dire sans connaître leur concentration en temps réel mais à posteriori donc une fois qu'un changement de comportement valvaire est détecté. Cela validerait sa capacité à avertir de la présence de toxine en condition réelle et évaluerait le risque de manque ou de fausse détection pour le producteur. L'étape suivante serait la mise

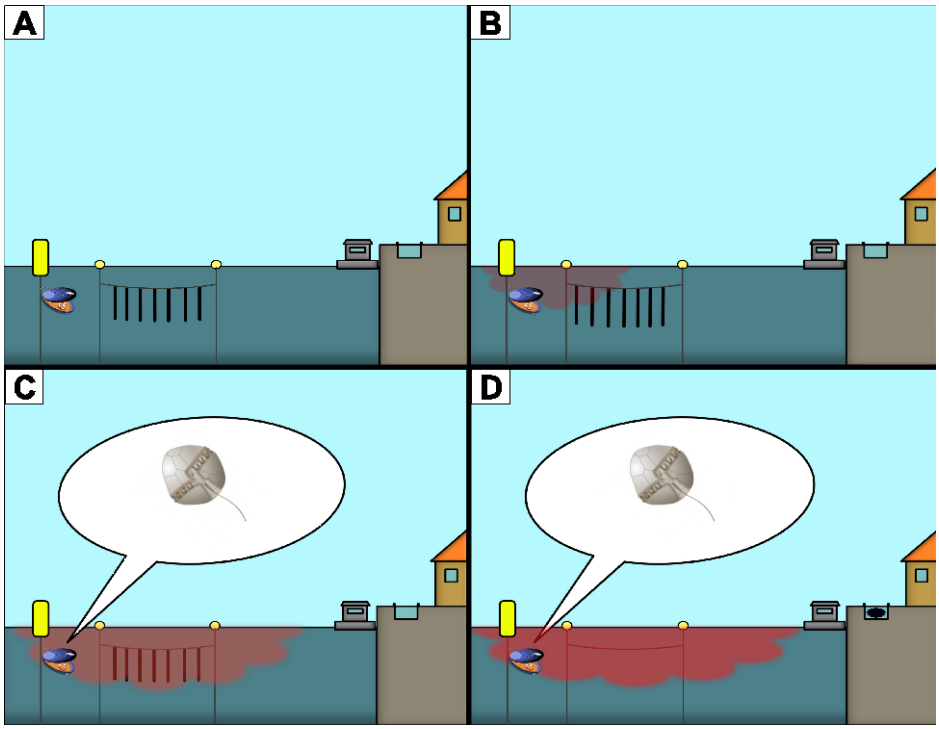

Figure 38 : Étapes de la détection de toxines en milieu naturel

en place de ce système de détection précoce dans les conditions d'utilisation par les aquaculteurs.

La Fig. 38 représente les différentes étapes de la détection d'une toxine, dans ce cas une efflorescence de *A. catenella*, en milieu naturel. Lors de l'étape A, le dispositif composé des moules sentinelles et des valvomètres est déployé dans une ferme aquacole. La surveillance peut démarrer grâce au suivi des indicateurs comportementaux identifiés lors de ce doctorat, révélant les changements de comportements liés à la présence de toxines. L'étape B correspond à l'apparition d'un début d'efflorescence de l'algue toxique *A. catenella* dans l'eau de la ferme aquacole surveillée par le dispositif. À cette étape la concentration en *A. catenella* est encore en dessous du seuil de détection et en dessous du seuil de fermeture de la récolte des bivalves. Il n'y a donc pas de danger pour les consommateurs. Lors de l'étape C, le seuil de détection de *A. catenella* est atteint. La paralysie des moules liée aux toxines produites par cette algue a été détectée grâce au suivi des indicateurs comportementaux. Cependant, le seuil de fermeture de la récolte des bivalves n'est pas encore atteint, les moules ne présentent pas encore de danger. Les aquaculteurs étant avertis, ces derniers disposent donc d'un certain temps (environ 10 jours) pour prendre les mesures adaptées. Lorsque l'étape D est atteinte, avec une concentration en *A. catenella* dépassant le seuil de fermeture de la récolte des bivalves, les moules sont déjà sorties de l'eau et peuvent donc continuer à être commercialisées sans attendre leur dépuration. Les mesures à prendre pourraient être adaptées aux possibilités du producteur (délais de récolte, entreposage…). En effet celui-ci pourrait ne récupérer que le stock nécessaire pour couvrir ses besoin le temps que les toxines soient éliminées des moules restées sur les filières.

Le système de détection précoce pourrait être amélioré grâce au développement de la valvométrie HFNI (fig. 39) (Andrade et al., 2016) sans fil et relié au réseau GPRS. Cette technologie permet l'envoi de données en temps réel sans limites de distance. Cela rendrait possible d'effectuer le suivi de plusieurs fermes aquacoles dans une très grande zone géographique en centralisant le traitement des données à un seul endroit.

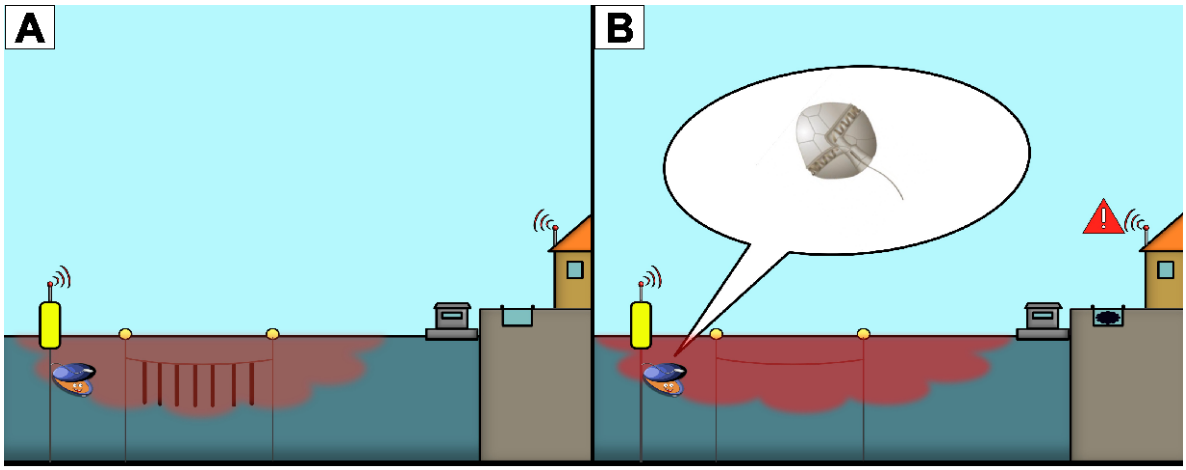

Figure 39 : Détection de toxine par la valvométrie HFNI

Utilisation de la valvométrie pour la détection d'autres types de pollutions

En plus de l'utilisation pour la détection de toxines synthétiques ou biologiques, la valvométrie pourrait être utilisée pour la détection d'autre type de polluants. En effet, lors d'un projet parallèle à ce doctorat, l'exposition de moules bleue au son de bateau a été testée (Byrro Gauthier, communication personnelle). Lors de ce test, les moules ont été exposées au son du Nolhan Ava, cargo ravitaillant l'archipel de St Pierre et Miquelon. Les sons utilisés correspondent à une boucle comprenant 11 minutes de son de bateau, 39 minutes sans son, 8 minutes et 30 secondes de son de bateau puis 21 minutes et 30 secondes sans le son pour un total de 1h 20 min d'exposition au son de cargo par cycle. Les résultats préliminaires semblent indiquer une hausse importante des mouvements valvaires lorsque le son est fort, limitant même la fermeture de la moule lors des changements d'eau journaliers effectués. Cela semble indiquer une perturbation du comportement valvaire des moules par le son. L'observation de ces perturbations liées à une pollution sonore montre la possibilité d'utiliser la valvométrie pour l'étude de l'effet d'autres types de polluants que ceux étudiés lors de ce doctorat. Dans le cas d'une exposition à deux toxines simultanément ayant des effets antagonistes sur le comportement valvaire des bivalves, il serait probable que l'une des deux toxines prenne le dessus sur l'autre et serait détecté. La fermeture liée à une exposition à une

substance synthétique étant complète et rapide, la toxine provoquant l'ouverture ne pourrait pas provoquer de comportement intermédiaire.

LIMITES DE L'ETUDE

La valvométrie génère un nombre de données très important en raison de la fréquence d'enregistrement très élevée, celle-ci étant de 10 données par seconde. Ainsi, près de 85 millions de données ont été enregistrées pour chacune des moules lors de l'expérience en milieu naturel réalisée pour le chapitre 2. Ce volume de donnée rend impossible le traitement manuel des données. Il a donc été nécessaire de développer un programme sur le logiciel R (R Core Team, 2021) permettant l'automatisation des données. Les fichiers sortant des valvomètres étant trop importants pour être mis en forme avant de les traiter sur R, il a fallu adapter le programme pour le rendre autonome sur toute la chaîne de traitement des données. Il est également nécessaire de passer par ce programme pour la visualisation des données. Cependant, lors de l'analyse d'un trop grand jeu de données, la limite du logiciel R peut rapidement être atteinte en ce qui concerne le volume de données possible à traiter. Cela impose donc de séparer le jeu de données en sous-parties traitées séparément. Le temps de traitement des données est donc beaucoup plus long. Pour améliorer cela, il serait donc intéressant d'utiliser un logiciel possédant une plus grande capacité de calcule et étant capable de gérer un très grand volume de données. Il pourrait également être intéressant de réaliser un partenariat avec un spécialiste de la gestion de grands jeux de données dans le but d'optimiser le programme informatique actuellement réalisé sur le logiciel R et ainsi permettre d'augmenter les capacités de traitement de ces grands jeux de données par ce programme informatique.

RÉFÉRENCES BIBLIOGRAPHIQUES

Addadi, L., Joester, D., Nudelman, F., & Weiner, S. (2006). Mollusk shell formation: a source of new concepts for understanding biomineralization processes. Chemistry–A European Journal, 12(4), 980-987.

Aguilera-Belmonte, A., Inostroza, I., Carrillo, K.S., Franco, J.M., Riobó, P., Gómez, P.I., 2013. The combined effect of salinity and temperature on the growth and toxin content of four Chilean strains of *Alexandrium catenella* (Whedon and Kofoid) Balech 1985 (Dinophyceae) isolated from an outbreak occurring in southern Chile in 2009. Harmful Algae 23, 55–59. https://doi.org/10.1016/j.hal.2012.12.006

Ahmed, F.E., 1991. Seafood Safety, 432 pp.

Anderson, D. M. (1995). Toxic red tides and harmful algal blooms: A practical challenge in coastal oceanography. Reviews of Geophysics. 33(S2), 1189-1200.

Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., 2011. Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. Annual. Review Marine Science. 4, 143–176. https://doi.org/10.1146/annurev-marine-120308-081121

Hallegraeff, G. M., Anderson, D. M., Cembella, A. D., & Enevoldsen, H. O. 2004. Manual on harmful marine microalgae. 793p Unesco. Paris

Anderson, D.M., Kulis, D.M., Cosper, E.M., 1989. Immunofluorescent detection of the brown tide organism, *Aureococcus anophagefferens*. In: Novel Phytoplankton Blooms. Cosper, E.M., Bricelj V.M., Carpenter E.J. Springer. 213–228.

Anderson, D.M., Kulis, D.M., Doucette, G.J., Gallagher, J.C., Balech, E., 1994.

Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. Marine Biology 120, 467–478. https://doi.org/10.1007/BF00680222

Anderson, D.M., Kulis, D.M., Orphanos, J.A., Ceurvels, A.R., 1982. Distribution of the toxic dinoflagellate G*onyaulax tamarensis* in the Southern New England Region. "Estuarine, Coastal Shell Science. 14, 447–458. https://doi.org/10.1016/S0272-7714(82)80014-0

Anderson, D.M., White, A.W., 1992. Marine biotoxins at the top of the food chain. Oceanus 35, 55–61.

Andrade, H., Massabuau, J.-C., Cochrane, S., Ciret, P., Tran, D., Sow, M., Camus, L., 2016. High Frequency Non-invasive (HFNI) bio-Sensors as a potential tool for marine monitoring and assessments. Frontier in Marine Science. 3, 1–10. https://doi.org/10.3389/fmars.2016.00187

Andrewartha, S., Elliott, N., 2015. Aquaculture sentinels: smart-farming with biosensor equipped stock. Journal of Aquaculture Research. Devevelopment. 07, 1–4. https://doi.org/10.4172/2155-9546.1000393

Antoł, A., Kierat, J., Czarnoleski, M., 2018. Sedentary prey facing an acute predation risk: testing the hypothesis of inducible metabolite emission suppression in zebra mussels, *Dreissena polymorpha*. Hydrobiologia 810, 109–117.

AOAC 1990 - Paralytic Shellfish Poison. Biological Method Final Action In AOAC Official Methods of Analysis. Hellrich, K. (Ed.). Virginia; Association of Official Analytical Chemists: 88] -882.

Arzul, G., Seguel, M., Guzman, L., Erard-Le Denn, E., 1999. Comparison of allelopathic properties in three toxic *Alexandrium* species. Journal of Experimental Marine Biology and Ecology. 232, 285–295. https://doi.org/10.1016/S0022-0981(98)00120-8

Atkins, D. (1937). Memoirs: On the Ciliary Mechanisms and Interrelationships of

Lamellibranchs: Part III: Types of Lamellibranch Gills and their Food Currents. Journal of Cell Science. 2(315), 375-421.

Baird, R.H., 1966. Factors affecting growth and condition of mussels (Mytilus edulis L.). Great Britain. Ministry of Agriculture, Fisheries and Food. Fisheries Investigations, series 2 ; 25, no. 2 HMSO

Baird, R. H., & Drinnan, R. E. (1957). The ratio of shell to meat in Mytilus as a function of tidal exposure to air. Journal Consdu Conseil Permanent International pour l'Exploration de la Mer, 22, 329-336.

Basti, L., Hégaret, H., Shumway, S.E., 2018. Harmful algal blooms and shellfish. Harmful Algal Bloom. A Compendium Desk Reference. Shumway, S.E., Burholder, J.M., Morton S.L. John Wiley Sons, Ltd. Hoboken, NJ, USA 135–190.

Basti, L., Nagai, S., Watanabe, S., Oda, T., Tanaka, Y., 2016. Neuroenzymatic activity and physiological energetics in Manila clam, R*uditapes philippinarum*, during short-term sublethal exposure to harmful alga, *Heterocapsa circularisquama*. Aquatic Toxicology. 176, 76–87. https://doi.org/10.1016/j.aquatox.2016.04.011

Bates, S.S., N. Lundholm, K.A. Hubbard, M. Montresor, and C.P. Leaw. 2019. Toxic and harmful marine diatoms. In Diatoms: fundamentals and applications [Volume 1 in the series: Diatoms: Biology & Applications]. Edited by R. Gordon and J. Seckbach. Wiley-Scrivener Beverly, MA, USA, pp. 389–434.

Bates, S.S., K.A. Hubbard, N. Lundholm, M. Montresor, and C.P. Leaw. 2018. *Pseudo-nitzschia*, *Nitzschia*, and domoic acid: new research since 2011. Harmful Algae 79: 3–43.

Bates, S.S., Beach, Daniel G.; Comeau, Luc. A.; Haigh, Nicola; Lewis, Nancy I.; Locke, Andrea; Martin, Jennifer L.; McCarron, Pearse; McKenzie, Cynthia H.; Michel, Christine; Miles, Christopher O.; Poulin, Michel; Quilliam, Michael A.; Rourke, Wade

A.; Scarratt, Michael G.; Starr, Michel; Wells, Terri; Fisheries and Oceans Canada 2020. Marine harmful algal blooms and phycotoxins of concern to Canada. Canadian Technical Report of Fisheries and Aquatic Science. 3384, 322p

Bates, S. S., Garrison, D. L., & Horner, R. A. (1998). Bloom dynamics and physiology of domoic-acid-producing *Pseudo-nitzschia* species. Nato asi series Gecological Sciences, 41, 267-292.

Bayne, B.L., 1998. The physiology of suspension feeding by bivalve molluscs: An introduction to the Plymouth "TROPHEE" workshop. Journal of Experimental Marine Biology and Ecology. 219, 1–19. https://doi.org/10.1016/S0022-0981(97)00172-X

Bayne, B.L., 1976. Marine mussels: their ecology and physiology. Bayne, B.L. Cambridge University. 506p ISBN 9780521112888

Bayne, B.L., Ahrens, M., Allen, S.K., D'Auriac, M.A., Backeljau, T., Beninger, P., Bohn, R., Boudry, P., Davis, J., Green, T., Guo, X., Hedgecock, D., Ibarra, A., Kingsley-Smith, P., Krause, M., Langdon, C., Lapègue, S., Li, C., Manahan, D., Mann, R., Perez-Paralle, L., Powell, E.N., Rawson, P.D., Speiser, D., Sanchez, J.L., Shumway, S., Wang, H., 2017. The proposed dropping of the genus *Crassostrea* for all pacific cupped oysters and its replacement by a new genus *Magallana*: A dissenting view. Journal of Shellfish Research. 36, 545–547. https://doi.org/10.2983/035.036.0301

Bayne, B. L., Iglesias, J. I. P., Hawkins, A. J. S., Navarro, E., Heral, M., & Deslous-Paoli, J. M. (1993). Feeding behaviour of the mussel, *Mytilus edulis*: responses to variations in quantity and organic content of the seston. Journal of the Marine Biological Association of the United Kingdom, 73(4), 813-829.

Bechard, A., 2019. Red tide at morning, tourists take warning? County-level economic effects of HABS on tourism dependent sectors. Harmful Algae 85, 101689. https://doi.org/10.1016/j.hal.2019.101689

Beninger, P. G., Ward, J. E., MacDonald, B. A., & Thompson, R. J. (1992). Gill function and

particle transport in Placopecten magellanicus (Mollusca: Bivalvia) as revealed using video endoscopy. Marine Biology, 114(2), 281-288.

Bérard-Therriault, L., Poulin, M., & Bossé, L. (1999). Guide d'identification du phytoplancton marin de l'estuaire et du golfe du Saint-Laurent: incluant également certains protozoaires. Canadian Speial Publication of Fisheries and Aquatic Science 128, 387p

Bernard, S., Kudela, R. M., Robertson Lain, L., & Pitcher, G. (2021). Observation of Harmful Algal Blooms with Ocean Colour Radiometry. (eds. Bernard, S., Kudela, R., Robertson Lain, L. and Pitcher, G.C.). Dartmouth, NS, Canada, International Ocean-Colour Coordinating Group (IOCCG), 165pp. (Reports of the International Ocean-Colour Coordinating Group, No. 20). DOI: http://dx.doi.org/10.25607/OBP-1042

Bianchi, V.A., Langeloh, H., Tillmann, U., Krock, B., Müller, A., Bickmeyer, U., Abele, D., 2019. Separate and combined effects of neurotoxic and lytic compounds of *Alexandrium* strains on *Mytilus edulis* feeding activity and hemocyte function. Fish and Shellfish Immunology. 84, 414–422. https://doi.org/10.1016/j.fsi.2018.10.024

Binzer, S.B., Lundgreen, R.B.C., Berge, T., Hansen, P.J., Vismann, B., 2018. The blue mussel *Mytilus edulis* is vulnerable to the toxic dinoflagellate *Karlodinium armiger*—Adult filtration is inhibited and several life stages killed. PLoS One 13, 1–16. https://doi.org/10.1371/journal.pone.0199306

Blasco, D., Levasseur, M., Bonneau, E., Gelinas, R., Packard, T.T., 2003. Patterns of paralytic shellfish toxicity in the St. Lawrence region in relationship with the abundance and distribution of *Alexandrium tamarense*. Sciencia. Marina. 67, 261–278. https://doi.org/10.3989/scimar.2003.67n3261

Boivin-Rioux, A., Starr, M., Chassé, J., Scarratt, M., Perrie, W., Long, Z., 2021. Predicting the Effects of Climate Change on the Occurrence of the Toxic Dinoflagellate *Alexandrium* catenella Along Canada's East Coast. Frontier. Marine. Science 7, 1–20.

https://doi.org/10.3389/fmars.2020.608021

Borcherding, J., 2006. Ten Years of Practical Experience with the Dreissena-Monitor, a Biological Early Warning System for Continuous Water Quality Monitoring. Hydrobiologia 556, 417–426. https://doi.org/10.1007/s10750-005-1203-4

Borcier, E., Morvezen, R., Boudry, P., Miner, P., Charrier, G., Laroche, J., Hegaret, H., 2017. Effects of bioactive extracellular compounds and paralytic shellfish toxins produced by *Alexandrium minutum* on growth and behaviour of juvenile great scallops *Pecten maximus*. Aquatic Toxicology. 184, 142–154. https://doi.org/10.1016/j.aquatox.2017.01.009

Bougrier, S., Hawkins, A.J.S., Héral, M., 1997. Preingestive selection of different microalgal mixtures in *Crassostrea gigas* and *Mytilus edulis*, analysed by flow cytometry. Aquaculture 150, 123–134. https://doi.org/10.1016/S0044-8486(96)01457-3

Brand, A. R. (2016). Scallop ecology: distributions and behaviour. In Developments in Aquaculture and Fisheries Science, Shumway, S.E., Parson, G.J. (Vol. 40, pp. 469-533). Elsevier.

Brenko, M.H., Calabrese, A., 1969. The combined effects of salinity and temperature on larvae of the mussel *Mytilus edulis*. Marine Biology. 4, 224–226. https://doi.org/10.1007/BF00393897

Bricelj, V.M., 1991. Physiology: energy acquisition and utilization. Scallops Biology. Ecology. Aquaculture. 305–346.

Bricelj, V.M., Connell, L., Konoki, K., MacQuarrie, S.P., Scheuer, T., Catterall, W.A., Trainer, V.L., 2005. Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434, 763–767. https://doi.org/10.1038/nature03415

Bricelj, V.M., Greene, M., Lee, J.H., Cembella, A., 1993. Growth of the blue mussel *Mytilus edulis* on toxic *Alexandrium fundyense* and effects of gut passage on dinoflagellate cells,

in: Toxic Phytoplankton Blooms in the Sea, TJ Smayda, Y. Shimuzu (Eds.). 371–376. Elsevier, Amsterdam.

Bricelj, V.M., Lee, J.H., Cembella, A.D., 1991. Influence of dinoflagellate cell toxicity on uptake and loss of paralytic shellfish toxins in the northern quahog *Mercenaria mercenaria*. Marine Ecology. Progress Series. 74, 33–46. https://doi.org/10.3354/meps074033

Bricelj, V.M., Lee, J.H., Cembella, A.D., Anderson, D.M., 1990. Uptake kinetics of paralytic shellfish toxins from the dinoflagellate *Alexandrium fundyense* in the mussel *Mytilus edulis*. Marine Ecology Progress Series. 63, 177–188.

Bricelj, V. M., & Shumway, S. E. (1998). Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. Reviews in Fisheries Science. 6(4), 315-383.

Bubel, A., 1973. An electron-microscope study of periostracum formation in some marine bivalves. II. The cells lining the periostracal groove. Marine Biology. 20, 222–234. https://doi.org/10.1007/BF00348988

Burkholder, J. M. (1998). Implications of harmful microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries. Ecological Applications. 8(1), S37-S62.

Burkholder, J.M., Glibert, P.M., Skelton, H.M., 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae. 8, 77–93.

Burns, K. A., & Smith, J. L. (1981). Biological monitoring of ambient water quality: the case for using bivalves as sentinel organisms for monitoring petroleum pollution in coastal waters. Estuarine, Coastal and Shelf Science. 13(4), 433-443.

Bushek, D., Ford, S. E., & Chintala, M. M. (2002). Comparison of in vitro-cultured and wild-type *Perkinsus marinus*. III. Fecal elimination and its role in transmission. Diseases of

Aquatic Organisms, 51(3), 217-225.

Campbell, D.E., Newell, C.R., 1998. MUSMOD©, a production model for bottom culture of the blue mussel, *Mytilus edulis* L. Journal of Experimental Marine Biology and Ecology. 219, 171–203.

Canevari, J., Lindblom, G., Becker, K., Bowes, S., Brown, H., Cashion, B., Chen, A., Clark, J., Demarco, G., Fiocoo, B., Et, A., 2008. ExxonMobil Oil Spill Dispersant Guidelines.

Carlton, J.T., Scanlon, J.A., 1985. Progression and dispersal of an introduced alga: *Codium fragile* ssp. *tomentosoides* (Chlorophyta) on the Atlantic coast of North America. Botanica Marina. 28, 155-165

Carty, S. U. S. A. N., & Wujek, D. E. (2003). A new species of *Peridinium* and new records of dinoflagellates and silica-scaled chrysophytes from Belize. Caribbean Journal of Science, 39(1), 136-139.

Cedre, 2020. Deepwater horizon. Petrol Economy. 77, 98.

Cedre, 2015. Exxon Valdez. Operator Risk Management. 33, 48–63. https://doi.org/10.1002/9781119202745.ch5

Cembella, A.D., 2003. Chemical ecology of eukaryotic microalgae in marine ecosystems. Phycologia 42, 420–447.

Cembella, A.D., Taylor, F.J.R., Therriault, J.-C., 1988. Cladistic analysis of electrophoretic variants within the toxic dinoflagellate genus *Protogonyaulax*.

Ceron, G.M.C., Camacho, F.G., Mirón, A.S., Sevilla, J.M., Chisti, Y., Grima, E.M., 2006. Mixotrophic production of marine microalga *Phaeodactylum tricornutum* on various carbon sources. Journal Microbiology Biotechnology. 16, 689–694.

Chan, A.T., Andersen, R.J., Le Blanc, M.J., Harrison, P.J., 1980. Algal plating as a tool for investigating allelopathy among marine microalgae. Marine. Biology. 59, 7–13.

Cheng, J. Y., & DeMont, M. (1996). Jet-propelled swimming in scallops: swimming mechanics and ontogenic scaling. Canadian Journal of Zoology. 74(9), 1734-1748.

Chojnacka, K., Marquez-Rocha, F.-J., 2004. Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. Biotechnology 3, 21–34.

Chopin, T., 2015. Marine aquaculture in canada: well-established monocultures of finfish and shellfish and an emerging integrated multi-trophic aquaculture (IMTA) approach including seaweeds, other invertebrates, and microbial communities. Fisheries. 40, 28–31. https://doi.org/10.1080/03632415.2014.986571

Clausen, I., Riisgård, H.U., 1996. Growth, filtration and respiration in the mussel *Mytilus edulis*: No evidence for physiological regulation of the filter-pump to nutritional needs. Marine Ecology Progress Series. 141, 37–45. https://doi.org/10.3354/meps141037

Clements, J.C., Poirier, L.A., Pérez, F.F., Comeau, L.A., Babarro, J.M.F., 2020. Behavioural responses to predators in Mediterranean mussels (*Mytilus galloprovincialis*) are unaffected by elevated pCO2. Marine Environmental Research. 161. https://doi.org/10.1016/j.marenvres.2020.105148

Cloern, J.E., 1982. Does the enthos control phytoplankton. Transport 9, 191–202.

Colicchia, G., Waltner, C., Hopf, M., & Wiesner, H. (2009). The scallop's eye—a concave mirror in the context of biology. Physics Education. 44(2), 175.

Comeau, L.A., Babarro, J.M.F., Riobó, P., Scarratt, M., Starr, M., Tremblay, R., 2019. PSP-producing dinoflagellate *Alexandrium minutum* induces valve microclosures in the mussel *Mytilus galloprovincialis*. Aquaculture 500, 407–413. https://doi.org/10.1016/j.aquaculture.2018.10.025

Comeau, L. A., Babarro, J. M., Longa, A., & Padin, X. A. (2018). Valve-gaping behavior of raft-cultivated mussels in the Ría de Arousa, Spain. Aquaculture Reports, 9, 68-73.

Comeau, L.A., Mayrand, É., Mallet, A., 2012. Winter quiescence and spring awakening of

the Eastern oyster *Crassostrea virginica* at its northernmost distribution limit. Marine. Biology. 159, 2269–2279. https://doi.org/10.1007/s00227-012-2012-8

Comeau, L. A., Pernet, F., Tremblay, R., Bates, S. S., & LeBlanc, A. (2008). Comparison of eastern oyster (*Crassostrea virginica*) and blue mussel (*Mytilus edulis*) filtration rates at low temperatures. Canadian Technical Report of Fisheries and Aquatic Sciences, 2810, 1-17.

Correira, F.D.A.S., 1946. Um caso raro de intoxicação alimentar colectiva. Boletim do Instituto Superior de Higiene Dr. Ricardo Jorge, 3 (1946), pp. 216-221

Costa-Pierce, B. A. 2002. Ecology as the paradigm for the future of aquaculture. In: Ecological Aquaculture: The Evolution of the Blue Revolution. Costa-Pierce, B. Blackwell Science, Oxford, UK, 339-372.

Coulson, J. C., Potts, G. R., Deans, I. R., & Fraser, S. M. 1968. Exceptional mortality of shags and other seabirds caused by paralytic shellfish poison. British Birds, 61(38), 1-404.

Cox, I.H., 1957. The Scallop: Studies of a shell and its influences on humankind. London:" Shell" Transport and Trading Company. Cox, I.H., Limited, London, 135p.

Cranford, P.J., Gordon, D.C., 1992. The influence of dilute clay suspensions on sea scallop (*Placopecten magellanicus*) feeding activity and tissue growth. Netherlands Journal. Sea Research. 30, 107–120. https://doi.org/10.1016/0077-7579(92)90050-O

Cranford, P.J., Hill, P.S., 1999. Seasonal variation in food utilization by the suspension-feeding bivalve molluscs *Mytilus edulis* and *Placopecten magellanicus*. Marine. Ecology Progress Series. 190, 223–239. https://doi.org/10.3354/meps190223

Crawford, C.M., Macleod, C.K.A., Mitchell, I.M., 2003. Effects of shellfish farming on the benthic environment. Aquaculture 224, 117–140. https://doi.org/10.1016/S0044-8486(03)00210-2

Cucci, T.L., Shumway, S.E., Newell, R.C., Selvin, R., Guillard, R.R.L., Yentsch, C.M., 1985. Flow cytometry: a new method for characterization of differential ingestion, digestion and egestion by suspension feeders. Marine Ecology Progress Series. 24, 201–204.

Currey, J. D. (1999). The design of mineralised hard tissues for their mechanical functions. Journal of Experimental Biology. 202(23), 3285-3294.

Curtis, T.M., Williamson, R., Depledge, M.H., 2000. Simultaneous, long-term monitoring of valve and cardiac activity in the blue mussel *Mytilus edulis* exposed to copper. Marine Biology. 136, 837–846. https://doi.org/10.1007/s002270000297

Cusson, M., Tremblay, R., Daigle, G., Roussy, M., 2005. Modeling the depuration potential of blue mussels (*Mytilus spp.*) in response to thermal shock. Aquaculture 250, 183–193. https://doi.org/10.1016/j.aquaculture.2005.03.045

Surówka, K. (2006). Cross-habitat differences in crush resistance and growth pattern of zebra mussels (*Dreissena polymorpha*): effects of calcium availability and predator pressure. Archiv fur. Hydrobiologie, 165(2), 191-208.

Dame, R.F., Kenneth, M.J., 2011. Ecology of marine bivalves: an ecosystem approach. Kennish M.J. Taylor & Francis. 284p. DOI 10.1201/b11220

Davenport, J., Manley, A., 1978. The detection of heightened sea-water cooper concentration by mussels *Mytilus edulis*. Journal of Marine Biology. 58, 843–850.

De Moreno, J.E.A., Pollero, R.J., Moreno, V.J., Brenner, R.R., 1980. Lipids and fatty acids of the mussel (*Mytilus platensis d'Orbigny*) from South Atlantic waters. Journal. of Experimental. Marine Biology and Ecology. 48, 263–276.

Delaporte, M., Soudant, P., Lambert, C., Moal, J., Pouvreau, S., Samain, J.F., 2006. Impact of food availability on energy storage and defense related hemocyte parameters of the Pacific oyster *Crassostrea gigas* during an experimental reproductive cycle. Aquaculture 254, 571–582. https://doi.org/10.1016/j.aquaculture.2005.10.006

Dell'Aversano, C., Walter, J. A., Burton, I. W., Stirling, D. J., Fattorusso, E., & Quilliam, M. A. (2008). Isolation and structure elucidation of new and unusual saxitoxin analogues from mussels. Journal of natural products. 71(9), 1518-1523.

Dellatorre, F.G., Pascual, M.S., Barón, P.J., 2007. Feeding physiology of the Argentine mussel *Mytilus edulis platensis* (d'Orbigny, 1846): Does it feed faster in suspended culture systems? Aquaculture. International. 15, 415–424. https://doi.org/10.1007/s10499-007-9105-2

Denardou-Queneherve, A., Grzebyk, D., Pouchus, Y.F., Sauviat, M.P., Alliot, E., Biard, J.F., Berland, B., Verbist, J.F., 1999. Toxicity of French strains of the dinoflagellate *Prorocentrum minimum* experimental and natural contaminations of mussels. Toxicon 37, 1711–1719. https://doi.org/10.1016/S0041-0101(99)00113-0

Díaz, P.A., Álvarez, G., Varela, D., Pérez-Santos, I., Díaz, M., Molinet, C., Seguel, M., Aguilera-Belmonte, A., Guzmán, L., Uribe, E., Rengel, J., Hernández, C., Segura, C., Figueroa, R.I., 2019. Impacts of harmful algal blooms on the aquaculture industry: Chile as a case study. Perspectives Phycology. 6, 39–50. https://doi.org/10.1127/pip/2019/0081

Dolmer, P., 2000. Algal concentration profiles above mussel beds. Journal Sea Research. 43, 113–119. https://doi.org/10.1016/S1385-1101(00)00005-8

Doucette, G.J., Cembella, A.D., Martin, J.L., Michaud, J., Cole, T.V.N., Rolland, R.M., 2006. Paralytic shellfish poisoning (PSP) toxins in North Atlantic right whales *Eubalaena glacialis* and their zooplankton prey in the Bay of Fundy, Canada. Marine. Ecology Progess Series. 306, 303–313.

Dral, A.D.G., 1967. The movements of the latero-frontal cilia and the mechanism of particle retention in the mussel. Netherlands Journal of Sea Research. 3, 391–422. https://doi.org/10.1016/0077-7579(67)90012-9

Drapeau, A., Comeau, L.A., Landry, T., Stryhn, H., Davidson, J., 2006. Association between

longline design and mussel productivity in Prince Edward Island, Canada. Aquaculture 261, 879–889. https://doi.org/10.1016/j.aquaculture.2006.07.045

Dray, S., Dufour, A.B., 2007. The ade4 package: Implementing the duality diagram for ecologists. Journal of Statistical Software. 22, 1–20. https://doi.org/10.18637/jss.v022.i04

Duinker, P. N., & Greig, L. A. (2007). Scenario analysis in environmental impact assessment: Improving explorations of the future. Environmental Impact Assessment Review, 27(3), 206-219.

Dzierżyńska-Białończyk, A., Jermacz, Ł., Zielska, J., Kobak, J., 2019. What scares a mussel? Changes in valve movement pattern as an immediate response of a byssate bivalve to biotic factors. Hydrobiologia 841, 65–77. https://doi.org/10.1007/s10750-019-04007-0

Eggermont, M., Cornillie, P., Dierick, M., Adriaens, D., Nevejan, N., Bossier, P., Van den Broeck, W., Sorgeloos, P., Defoirdt, T., Declercq, A.M., 2020. The blue mussel inside: 3D visualization and description of the vascular-related anatomy of *Mytilus edulis* to unravel hemolymph extraction. Scientific Report. 10, 1–16. https://doi.org/10.1038/s41598-020-62933-9

Erdner, D.L., L. Percy, B. Keafer, J. Lewis, and D.M. Anderson. 2010. A quantitative real-time PCR assay for the identification and enumeration of *Alexandrium* cysts in marine sediments. Deep- Sea Research II 57: 279–287

Essink, K., Tydeman, P., De Koning, F., & Kleef, H. L. (1989). On the adaptation of the mussel *Mytilus edulis* L. to different environmental suspended matter concentrations. Proceedings of the 21st EMBS Gdansk, 14–19 September 1986, 41-51

Etheridge, S.M., 2010. Paralytic shellfish poisoning: Seafood safety and human health perspectives. Toxicon 56, 108–122. https://doi.org/10.1016/j.toxicon.2009.12.013

Etheridge, S.M., Roesler, C.S., 2005. Effects of temperature, irradiance, and salinity on

photosynthesis, growth rates, total toxicity, and toxin composition for *Alexandrium fundyense* isolates from the Gulf of Maine and Bay of Fundy. Deep-sea. Research. Part II Topical Studies in Oceanography 52(19-21), 2491–2500. https://doi.org/10.1016/j.dsr2.2005.06.026

Evangelista, V., Barsanti, L., Frassanito, A.M., Passarelli, V., Gualtieri, P., 2008. Algal toxins: nature, occurrence, effect and detection. Evangelista, V., Barsanti, L., Frassanito, A.M., Passarelli, V., Gualtieri, P., 397p, Springer Science & Business Media.

Famme, P., Kofoed, L.H., 1983. Shunt water flow through the mantle cavity in *Mytilus edulis* L. and its influence on particle retention. Marine Biology Letter. 4(4), 207-218

FAO, 2016. La situation mondiale des pêches et de l'aquaculture 2016., 227p Rome ISBN: 978-92-5-209185-1

FAO, 2012. World Fisheries and Aquaculture. 209p ISBN: 9789251072257

FAO, 2009. Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria 240.

FAO, 2004. Marine biotoxins : food and nutrition paper 80. Food Agric. Organ. Rome. ISBN 95-5-105129-1

Farley, C.A., 1988. A computerized coding system for organs, tissues, lesions, and parasites of bivalve mollusks and its application in pollution monitoring with *Mytilus edulis*. Marine Environmental Research. 24, 243–249.

Fauchot, J., Levasseur, M., Roy, S., Gagnon, R., Weise, A.M., 2005. Environmental factors controlling *Alexandrium tamarense* (dinophyceae) growth rate during a red tide event in the St. Lawrence Estuary (Canada). Journal of Phycology. 41, 263–272. https://doi.org/10.1111/j.1529-8817.2005.03092.x

Fistarol, G.O., Legrand, C., Selander, E., Hummert, C., Stolte, W., Granéli, E., 2004.

Allelopathy in *Alexandrium* spp.: Effect on a natural plankton community and on algal monocultures. Aquatic Microbial Ecology 35, 45–56. https://doi.org/10.3354/ame035045

Fitzer, S. C., Zhu, W., Tanner, K. E., Phoenix, V. R., Kamenos, N. A., & Cusack, M. (2015). Ocean acidification alters the material properties of *Mytilus edulis* shells. Journal of the Royal Society Interface, 12(103), 20141227.

Foster-Smith, R.L., 1975. The effect of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). Journal of Experimental Marine Biology and Ecology. 17, 1–22. https://doi.org/10.1016/0022-0981(75)90075-1

Fournier, E., Tran, D., Denison, F., Massabuau, J.-C., Garnier-Laplace, J., 2004. Valve closure response to uranium exposure for a freshwater bivalve (*Corbicula fluminea)*: Quantification of the influence of pH. Environmental Toxicolology and Chemistry. 23.

Fraga, S., Sampedro, N., Larsen, J., Moestrup, Ø., Calado, A.J., 2015. Arguments against the proposal 2302 by John & al. To reject the name G*onyaulax catenella* (A*lexandrium catenella*). Taxon 64, 634–635. https://doi.org/10.12705/643.15

Frank, K. T., Petrie, B., Choi, J. S., & Leggett, W. C. (2005). Trophic cascades in a formerly cod-dominated ecosystem. Science, 308(5728), 1621-1623.

Gaines, G., and M. Elbrachter. 1987. Heterotrophic nutrition,. Taylor, Zn E J. R., The biology of dinoflagellates. 224-268. Blackwell.

Gaitán-Espitia, J.D., Quintero-Galvis, J.F., Mesas, A., D'Elía, G., 2016. Mitogenomics of southern hemisphere blue mussels (Bivalvia: *Pteriomorphia*): Insights into the evolutionary characteristics of the *Mytilus edulis* complex. Scientific Report. 6, 1–10. https://doi.org/10.1038/srep26853

Galimany, E., Sunila, I., Hégaret, H., Ramón, M., Wikfors, G.H., 2008a. Pathology and

immune response of the blue mussel (*Mytilus edulis* L.) after an exposure to the harmful dinoflagellate *Prorocentrum minimum*. Harmful Algae 7, 630–638. https://doi.org/10.1016/j.hal.2008.01.001

Galimany, E., Sunila, I., Hégaret, H., Ramón, M., Wikfors, G.H., 2008b. Experimental exposure of the blue mussel (*Mytilus edulis*, L.) to the toxic dinoflagellate *Alexandrium fundyense*: Histopathology, immune responses, and recovery. Harmful Algae 7, 702–711. https://doi.org/10.1016/j.hal.2008.02.006

García-March, J.R., Sanchís Solsona, M.Á., García-Carrascosa, A.M., 2008. Shell gaping behaviour of *Pinna nobilis* L., 1758: Circadian and circalunar rhythms revealed by in situ monitoring. Marine Biology. 153, 689–698. https://doi.org/10.1007/s00227-007-0842-6

Gardner, J.P.A., Thompson, R.J., 2001. The effects of coastal and estuarine conditions on the physiology and survivorship of the mussels *Mytilus edulis*, *M. trossulus* and their hybrids. Journal. of Experimental. Marine Biology and Ecology. 265, 119–140. https://doi.org/10.1016/S0022-0981(01)00328-8

Garen, P., Robert, S., Bougrier, S., 2004. Comparison of growth of mussel, *Mytilus edulis*, on longline, pole and bottom culture sites in the Pertuis Breton, France. Aquaculture 232, 511–524. https://doi.org/10.1016/S0044-8486(03)00535-0

George-Ares, A., Clark, J.R., 2000. Aquatic toxicity of two Corexit® dispersants. Chemosphere 40, 897–906. https://doi.org/10.1016/S0045-6535(99)00498-1

George, S.G., Viarengo, A., 1985. A model for heavy metal homeostasis and detoxication in mussel

Marine Pollution Physiology: Recent Advances (1985), pp. 125-143

Geraci, J. R., Anderson, D. M., Timperi, R. J., St. Aubin, D. J., Early, G. A., Prescott, J. H., & Mayo, C. A. (1989). Humpback whales (*Megaptera novaeangliae*) fatally poisoned

by dinoflagellate toxin. Canadian Journal of Fisheries and Aquatic Sciences, 46(11), 1895-1898.

Glibert, P.M., Seitzinger, S., Heil, C.A., Burkholder, J.M., Parrow, M.W., Codispoti, L.A., Kelly, V., 2005. The role of eutrophication in the global proliferation of harmful algal blooms. Oceanography 18, 198–209. https://doi.org/10.5670/oceanog.2005.54

Goldberg, E. D., Bowen, V. T., Farrington, J. W., Harvey, G., Martin, J. H., Parker, P. L., ... & Gamble, E. (1978). The mussel watch. Environmental conservation, 5(2), 101-125.

Göröcs, Z., M. Tamamitsu, V. Bianco, P. Wolf, S. Roy, K. Shindo, K. Yanny, Y. Wu, H.C. Koydemir, Y. Rivenson, and A. Ozcan. 2018. A deep learning-enabled portable imaging flow cytometer for cost-effective, high-throughput, and label-free analysis of natural water samples. Light: Science & Application 7: 66.

Gosling, E. (2015). Marine bivalve molluscs. John Wiley & Sons. 536p Blackwell, ISBN: 978-0-470-67494-9

Gosling, E. (2003). Bivalve Molluscs: Biology. Ecology and Culture. Blackwell Publishing, Oxford. 443p DOI:10.1002/9780470995532

Gosling, E., 1992. The mussel mytilus: ecology, physiology, genetics and culture. In: Development in Aquaculture and Fishery Science volume 25, Gosling, 169p, Elsevier

Gouvernement du Canada, A. canadienne d'inspection des aliments, 2018. Manuel du Programme canadien de contrôle de la salubrité des mollusques [WWW Document]. URL https://inspection.canada.ca/exigences-et-documents-d-orientation-relatives-a-c/poisson/programme-canadien-de-controle-de-la-salubrite-des/fra/1527251566006/1527251566942?chap=0#s4c3 (accessed 6.15.22).

Gowen, R.J., Bradbury, N.B., 1987. The ecological impact of salmonid farming in coastal waters: a review. Oceanography and Marine Biology. 25, 563–575.

Graham, L. E., & Wilcox, L. W. (2000). Dinoflagellates. Algae. Upper Saddle River (NJ):

Prentice-Hall, 198-231.

Granmo, Å., Havenhand, J., Magnusson, K., Svane, I., 1988. Effects of the planktonic flagellate *Chrysochromulina polylepis* Manton et Park on fertilization and early development of the ascidian *Ciona intestinalis* (L.) and the blue mussel *Mytilus edulis* L. Journal of Experimental Marine Biology and Ecology. 124, 65–71. https://doi.org/10.1016/0022-0981(88)90206-7

Gruber, D., Frago, C. H., & Rasnake, W. J. (1994). Automated biomonitors—first line of defense. Journal of Aquatic Ecosystem Health, 3(2), 87-92.

Guderley, H.E., Tremblay, I., 2013. Escape responses by jet propulsion in scallops. Canadian Journal of Zoology. 91, 420-430 dx.doi.org/10.1139/cjz-2013-0004

Guillard, R. R. (1975). Culture of phytoplankton for feeding marine invertebrates. In: Culture of marine invertebrate animals (29-60). Smith, W.L. Chanley. M.H, Plenum Press New York.

Haberkorn, H., Tran, D., Massabuau, J.C., Ciret, P., Savar, V., Soudant, P., 2011. Relationship between valve activity, microalgae concentration in the water and toxin accumulation in the digestive gland of the Pacific oyster *Crassostrea gigas* exposed to *Alexandrium minutum*. Marine Pollution Bulletin. 62, 1191–1197. https://doi.org/10.1016/j.marpolbul.2011.03.034

Hall, S., Reichardt, P.B., 1984. Cryptic paralytic shellfish toxins.In: ACS Symposium Series, Ragelis E.P. 262, 113-123, American Chemical Society

Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. Physiology Review. 32, 79–99.

Hallegraeff, G.M., Bolch, C.J., 1992. Transport of diatom and dinoflagellate resting spores in ships' ballast water: implications for plankton biogeography and aquaculture. Journal

of Plankton Research. 14, 1067–1084

Hallegraeff, G. M., Anderson, D. M., Belin, C., Bottein, M. Y. D., Bresnan, E., Chinain, M., Enevoldsen, H., Iwataki, M., Karlson, B., McKenzie, C.H., Sunesen, I., Pitcher, G.C., Provoost, P., Richardson, A., Schweibold, L., Tester, P.A., Trainer, V.L., Yñiguez, A.T., Zingone, A. (2021). Perceived global increase in algal blooms is attributable to intensified monitoring and emerging bloom impacts. Communications Earth & Environment, 2(1), 1-10.

Hatfield, R.G., T. Bean, A.D. Turner, D.N. Lees, J. Lowther, A. Lewis, and C. Baker-Austin. 2019. Development of a TaqMan qPCR assay for detection of *Alexandrium* spp and application to harmful algal bloom monitoring. Toxicon: X 2: 100011.

Hawkins, A. J. S., Bayne, B. L., & Gosling, E. (1992). Physiological interrelations, and the regulation of production [in *Mytilus*]. Developments in aquaculture and fisheries science, 25, 171-222

Hégaret, H., Wikfors, G.H., 2005. Effects of natural and field-simulated blooms of the dinoflagellate *Prorocentrum minimum* upon hemocytes of eastern oysters, *Crassostrea virginica*, from two different populations. Harmful Algae 4, 201–209. https://doi.org/10.1016/j.hal.2003.12.005

Hégaret, H., Wikfors, G.H., Soudant, P., Lambert, C., Shumway, S.E., Bérard, J.B., Lassus, P., 2007. Toxic dinoflagellates (*Alexandrium fundyense* and *A. catenella*) have minimal apparent effects on oyster hemocytes. Marine Biology. 152, 441–447. https://doi.org/10.1007/s00227-007-0703-3

Hendriks, I.E., Van Duren, L.A., Herman, P.M.J., 2003. Effect of dietary polyunsaturated fatty acids on reproductive output and larval growth of bivalves. Journal of Experimental Marine Biology and Ecology. 296, 199–213. https://doi.org/10.1016/S0022-0981(03)00323-X

Hilbish, T.J., Mullinax, A., Dolven, S.I., Meyer, A., Koehn, R.K., Rawson, P.D., 2000.

Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): Routes and timing of transequatorial migration. Marine Biology. 136, 69–77. https://doi.org/10.1007/s002270050010

Hoagland, P., Anderson, D.M., Kaoru, Y., White, A.W., 2002. The economic effects of harmful algal blooms in the United States: Estimates, assessment issues, and information needs. Estuaries 25, 819–837. https://doi.org/10.1007/BF02804908

Hoagland, P., Scatasta, S., 2006. The economic effects of harmful algal blooms. In: Ecology of Harmful Algae. Graneli E., Turner, J.T., Springer, 391–402.

Howell, R., Grant, A.M., Maccoy, N.E.J., 1984. Effect of treatment with reserpine on the change in filtration rate of *Mytilus edulis* subjected to dissolved copper. Marine Pollution Bulletin. 15, 436–439. https://doi.org/10.1016/0025-326X(84)90142-5

IPCC, 2014. Climate Change 2014: Mitigation of Climate Change Working groupe III. Cambridge University. Press.

IPIECA, IOGP, 2015. Dispersants : surface application. IPIECA/IOGP Oil Spill Response JIP 1–70.

James, K.J., Carey, B., O'Halloran, J., Van Pelt, F.N.A.M., Škrabáková, Z., 2010. Shellfish toxicity: Human health implications of marine algal toxins. Epidemiology Infectiology. 138, 927–940. https://doi.org/10.1017/S0950268810000853

Jasperse, L., Levin, M., Tsantiris, K., Smolowitz, R., Perkins, C., Ward, J.E., De Guise, S., 2018. Comparative toxicity of Corexit® 9500, oil, and a Corexit®/oil mixture on the eastern oyster, *Crassostrea virginica* (Gmelin). Aquatic Toxicology. 203, 10–18. https://doi.org/10.1016/j.aquatox.2018.07.015

John, E. H., & Flynn, K. J. (2000). Growth dynamics and toxicity of *Alexandrium fundyense* (Dinophyceae): The effect of changing N [ratio] P supply ratios on internal toxin and nutrient levels. European Journal of Phycology, 35(1), 11-23.

John, U., Litaker, R.W., Montresor, M., Murray, S., Brosnahan, M.L., Anderson, D.M., 2014. Formal revision of the *Alexandrium tamarense* species complex (dinophyceae) taxonomy: The introduction of five species with emphasis on molecular-based (rDNA) classification. Protist 165, 779–804. https://doi.org/10.1016/j.protis.2014.10.001

Jones, J., Allam, B., Espinosa, E.P., 2020. Particle selection in suspension-feeding bivalves: Does one model fit all? Biology Bulletin. 238, 41–53.

Jørgensen, C.B., 1981. A hydromechanical principle for particle retention in *Mytilus edulis* and other ciliary suspension feeders. Marine Biology. 61, 277–282. https://doi.org/10.1007/BF00401566

Jørgensen, C.B., 1974. On gill function in the mussel *Mytilus edulis* L. Ophelia 13, 187–232. https://doi.org/10.1080/00785326.1974.10430595

Jørgensen, C.B., Larsen, P.S., Riisgård, H.U., 1990. Effects of temperature on the mussel pump. Marine Ecology Progress Series. 64, 89–97.

Kellogg, J.L., 1915. Ciliary mechanisms of lamellibranchs with descriptions of anatomy. Journal of Morphology. 26, 625–701.

Kenneth, L., Michel, B., Bing, C., Julia, F., Peter, H., Stella, S., Albert, V., 2015. Expert panel report on the behaviour and environmental impacts of crude oil released into aqueous environments. Royal Society of Canada.

Kimor, B., 1981. Seasonal and bathymetric distribution of thecate and nonthecate dinoflagellates off La Jolla, California. CalCOFI Invest. Rep 22, 126–134.

Kittner, C., Riisgård, H.U., 2005. Effect of temperature on filtration rate in the mussel *Mytilus edulis*: No evidence for temperature compensation. Marine Ecology Progress Series. 305, 147–152. https://doi.org/10.3354/meps305147

Kobak, J., Kakareko, T., Poznańska, M., 2010. Changes in attachment strength and aggregation of zebra mussel, *Dreissena polymorpha* in the presence of potential fish

predators of various species and size. Hydrobiologia 644, 195–206.

Kodama, M., & Sato, S. (2008). Metabolism of paralytic shellfish toxins incorporated into bivalves. In Seafood and Freshwater Toxins. Botana, L.M. 183-194. CRC Press.

Kramer, K. J., & Botterweg, J. (1991). Aquatic biological early warning systems: an overview. In: Bioindicators and environmental management, Jeffrey, D.W., Madden, B. 95-126. Academic Press

Kramer, K.J.M., Foekema, E.M., 2001. The "Musselmonitor®" as biological early warning system. In: Butterworth, F.M., Gunatilaka, A., Gonsebatt, M.E. (Eds.), Biomonitors and Biomarkers as Indicators of Environmental Change 2: A Handbook. 59–87.

Kwong, R.W.M., Wang, W.X., Lam, P.K.S., Yu, P.K.N., 2006. The uptake, distribution and elimination of paralytic shellfish toxins in mussels and fish exposed to toxic dinoflagellates. Aquatic Toxicology. 80, 82–91. https://doi.org/10.1016/j.aquatox.2006.07.016

Landsberg, J. H. (2002). The effects of harmful algal blooms on aquatic organisms. Reviews in fisheries science, 10(2), 113-390.

Larkin, S. L., & Adams, C. M. (2007). Harmful algal blooms and coastal business: economic consequences in Florida. Society and Natural Resources, 20(9), 849-859.

Larsson, C., Ahlstrøm, Ø., Junghans, P., Jensen, R.B., Blache, D., Tauson, A.H., 2015. The oral [13C]bicarbonate technique for measurement of short-term energy expenditure of sled dogs and their physiological response to diets with different fat: Carbohydrate ratios. Journal of Nutrition. Science. 4, 1–10. https://doi.org/10.1017/jns.2015.23

Lassudrie, M., Soudant, P., Nicolas, J.L., Fabioux, C., Lambert, C., Miner, P., Le Grand, J., Petton, B., Hégaret, H., 2015. Interaction between toxic dinoflagellate *Alexandrium catenella* exposure and disease associated with herpesvirus OsHV-1µVar in Pacific oyster spat *Crassostrea gigas.* Harmful Algae 45, 53–61.

https://doi.org/10.1016/j.hal.2015.04.007

Lassudrie, M., Soudant, P., Nicolas, J.L., Miner, P., Le Grand, J., Lambert, C., Le Goïc, N., Hégaret, H., Fabioux, C., 2016. Exposure to the toxic dinoflagellate *Alexandrium catenella* modulates juvenile oyster *Crassostrea gigas* hemocyte variables subjected to different biotic conditions. Fish and Shellfish Immunology. 51, 104–115. https://doi.org/10.1016/j.fsi.2016.02.017

Leblanc, A., Arnold, A.A., Genard, B., Nadalini, J., Heine, M.S., Marcotte, I., Tremblay, R., Sleno, L., 2012. Determination of isotopic labeling of proteins by precursor ion scanning liquid chromatography / tandem mass spectrometry of derivatized amino acids applied to nuclear magnetic resonance studies. RCMS 26, 1165–1174. https://doi.org/10.1002/rcm.6204

Legrand, C., Rengefors, K., Fistarol, G.O., Graneli, E., 2003. Allelopathy in phytoplankton-biochemical, ecological and evolutionary aspects. Phycologia 42, 406–419.

Lelong, A., Haberkorn, H., Le Goïc, N., Hégaret, H., Soudant, P., 2011. A New Insight into Allelopathic Effects of *Alexandrium minutum* on Photosynthesis and Respiration of the Diatom *Chaetoceros neogracile* Revealed by Photosynthetic-performance Analysis and Flow Cytometry. Microbial Ecology. 62, 919–930. https://doi.org/10.1007/s00248-011-9889-5

Li, S.C., Wang, W.X., Hsieh, D.P.H., 2002. Effects of toxic dinoflagellate *Alexandrium tamarense* on the energy budgets and growth of two marine bivalves. Marine Environmental Research. 53, 145–160. https://doi.org/10.1016/S0141-1136(01)00117-9

Lilly, E.L., Halanych, K.M., Anderson, D.M., 2007. Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). Journal of Phycology. 43, 1329–1338. https://doi.org/10.1111/j.1529-8817.2007.00420.x

Lindstrom, J.E., Braddock, J.F., Fiocco, R.J., Lewis, A., Burns, P.N., Becher, H., Burns, P.N.,

Becher, H., The, I.N., Wise, J.P.J., Wise, J.P.J., Tran, D., Ciutat, A., Mat, A., Massabuau, J.C., Hégaret, H., Lambert, C., Le Goic, N., Soudant, P., Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., Blasco, D., Levasseur, M., Bonneau, E., Gelinas, R., Packard, T.T., Comeau, L.A., Babarro, J.M.F., Riobó, P., Scarratt, M., Starr, M., Tremblay, R., Davenport, J., Smith, R.J.J.W., Packer, M., Galimany, E., Sunila, I., Hégaret, H., Ramón, M., Wikfors, G.H., Kudela, R.M., Berdalet, E., Bernard, S., Burford, M., Fernand, L., Lu, S., Roy, S., Tester, P., Usup, G., Magnien, R., Anderson, D.M., Cembella, A.D., Chinain, M., Hallegraeff, G.M., Reguera, B., Zingone, A., Enevoldsen, H., Hégaret, H., Wikfors, G.H., Soudant, P., Lambert, C., Shumway, S.E., Bérard, J.B., Lassus, P., Lauzon-Guay, J.S., Hamilton, D.J., Barbeau, M.A., Mathiesen, S.S., Thyrring, J., Hemmer-Hansen, J., Berge, J., Sukhotin, A., Leopold, P., Bekaert, M., Sejr, M.K., Nielsen, E.E., Tran, D., Ciret, P., Ciutat, A., Durrieu, G., Massabuau, J.C., Oceanographic, W.H., Hole, W., 2000. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant Corexit 9500. Aquaculture 10, 702–711. https://doi.org/10.3989/scimar.2003.67n3261

LLC, N.E.S., 2016. Oil Spill Dispersants [WWW Document]. URL https://www.nalcoenvironmentalsolutionsllc.com/wp-content/uploads/NES-LLC-COREXIT.pdf

Lombard, F., E. Boss, A.M. Waite, M. Vogt, J. Uitz, L. Stemmann, H.M. Sosik, J. Schulz, J.-B. Romagnan, M. Picheral, J. Pearlman, M.D. Ohman, B. Niehoff, K.O. Möller, P. Miloslavich, A. Lara-Lpez, R. Kudela, R.M. Lopes, R. Kiko, L. Karp-Boss, J.S. Jaffe, M.H. Iversen, J.-O. Irisson, K. Fennel, H. Hauss, L. Guidi, G. Gorsky, S.L.C. Giering, P. Gaube, S. Gallager, G. Dubelaar, R.K. Cowen, F. Carlotti, C. Briseño-Avena, L. Berline, K. Benoit-Bird, N. Bax, S. Batten, S.D. Ayata, L.F. Artigas, and W. Appeltans. 2019. Globally consistent quantitative observations of planktonic ecosystems. Frontier in Marine Sciences 6: 196

MacKenzie, L.; Beuzenberg, V.; Holland, P.; McNabb, P.; Selwood, A. Solid phase adsorption toxin tracking (SPATT): A new monitoring tool that simulates the biotoxin

contamination of filter feeding bivalves. Toxicon 2004, 44, 901–918.

MacQuarrie, S.P., Bricelj, V.M., 2008. Behavioral and physiological responses to PSP toxins in *Mya arenaria* populations in relation to previous exposure to red tides. Marine Ecology Progress Series. 366, 59–74. https://doi.org/10.3354/meps07538

Maestrini, S. Y., & Robert, J. M. (1981). Rendements d'utilisation des sels nutritifs et variations de l'état des cellules de trois diatomées de claires à huitres de Vendée. Oceanologica acta, 4(1), 13-21.

Mafra, L.L., Bricelj, V.M., Fennel, K., 2010. Domoic acid uptake and elimination kinetics in oysters and mussels in relation to body size and anatomical distribution of toxin. Aquatic Toxicology. 100, 17–29. https://doi.org/10.1016/j.aquatox.2010.07.002

Maire, O., Amouroux, J.M., Duchêne, J.C., Grémare, A., 2007. Relationship between filtration activity and food availability in the Mediterranean mussel *Mytilus galloprovincialis*. Marine Biology. 152, 1293–1307. https://doi.org/10.1007/s00227-007-0778-x

Marcaillou, C., Haure, J., Mondeguer, F., Courcoux, A., Dupuy, B., Pénisson, C., 2010. Effect of food supply on the detoxification in the blue mussel, *Mytilus edulis*, contaminated by diarrhetic shellfish toxins. Aquatic Living Resource 23, 255–266. https://doi.org/10.1051/alr/2010026

Marin, F., & Luquet, G. (2005). Molluscan biomineralization: The proteinaceous shell constituents of *Pinna nobilis* L. Materials Science and Engineering: C, 25(2), 105-111.

Martín-González, A., Díaz, S., Borniquel, S., Gallego, A., Gutiérrez, J.C., 2006. Cytotoxicity and bioaccumulation of heavy metals by ciliated protozoa isolated from urban wastewater treatment plants. Research in Microbiology 157, 108–118.

Martin, M., 1985. State mussel watch: toxics surveillance in California. Marine Pollution Bulletin. 16, 140–146.

Martin, M., Richardson, B.J., 1991. Long term contaminant biomonitoring: views from southern and northern hemisphere perspectives. Marine Pollution Bulletin. 22, 533–537.

Martinez Arbizu, P., 2019. Pairwiseadonis: Pairwise multilevel comparison using adonis. R Packag. version 0.3.

Masó, M., Garcés, E., 2006. Harmful microalgae blooms (HAB); problematic and conditions that induce them. Marine Pollution Bulletin 53, 620–630. https://doi.org/10.1016/j.marpolbul.2006.08.006

Mat, A.M., Hakerkorn, H., Bourdineaud, J.-P., Massabuau, J.-C., Tran, D., 2013. Genetic and genotoxic impacts in the oyster *Crassostrea gigas* exposed to the harmful alga *Alexandrium minutum.* Aquatic Toxicology. 140–141, 458–461.

Mathiesen, S. S., Thyrring, J., Hemmer-Hansen, J., Berge, J., Sukhotin, A., Leopold, P., ... & Nielsen, E. E. (2017). Genetic diversity and connectivity within *Mytilus* spp. in the subarctic and Arctic. Evolutionary Applications, 10(1), 39-55.

Matsuyama, Y., Shumway, S., 2009. Impacts of harmful algal blooms on shellfisheries aquaculture. New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management. 580-609. Woodhead Publishing Limited. https://doi.org/10.1533/9781845696474.3.580

McDonald, J.H., Seed, R., Koehn, R.K., 1991. Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Marine Biology. 111, 323–333.

McLeroy-Etheridge, S., 2002. Ecophysiology and optical detection of harmful algal blooms. 169p. University of Connecticut.

McMahon, T., 1996. Winter toxicity of unknown aetiology in mussels. Harmful Algae News 14, 2.

Medcof, J. C., Leim, A. H., Needler, A. B., Needler, A. W. H., Gibbard, J., & Naubert, J.

(1947). Paralytic shellfish poisoning on the Canadian Atlantic coastal. Bulletin of Fishery Resource. Broad. Canada, 75, 1-32.

Merlin, F.X., 2014. Traitement aux dispersants des nappes de pétrole en mer Traitement par voie aérienne et par bateau. Guide Opérationnel. Brest : Cedre, 59p.

Berglund, A., & Lagercrantz, U. (1983). Genetic differentiation in populations of two *Palaemon* prawn species at the Atlantic east coast: does gene flow prevent local adaptation?. Marine Biology, 77(1), 49-57.

Møhlenberg, F., Riisgård, H.U., 1979. Filtration rate, using a new indirect technique, in thirteen species of suspension-feeding bivalves. Marine Biology. 54, 143–147. https://doi.org/10.1007/BF00386593

Møhlenberg, F., Riisgård, H.U., 1978. Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia 17, 239–246.

Moreau, V., Tremblay, R., & Bourget, E. (2005). Distribution of Mytilus edulis and M. trossulus on the Gaspe coast in relation to spatial scale. Journal of Shellfish Research, 24(2), 545-551.

Moroño, A., Franco, J., Miranda, M., Reyero, M.I., Blanco, J., 2001. The effect of mussel size, temperature, seston volume, food quality and volume-specific toxin concentration on the uptake rate of PSP toxins by mussels (*Mytilus galloprovincialis* Lmk). Journal. of Experimental Marine Biology and Ecology. 257, 117–132. https://doi.org/10.1016/S0022-0981(00)00336-1

Morton, B., 1983. Feeding and Digestion in Bivalvia, in: The Mollusca: Physiology, Part 2. Wilbur, K.M., Saleudon, A.S.M. 65–147. Academic Press, London

Naddafi, R., Rudstam, L.G., 2014. Predation on invasive zebra mussel, *Dreissena polymorpha*, by pumpkinseed sunfish, rusty crayfish, and round goby. Hydrobiologia 721, 107–115.

Naddafi, R., Rudstam, L.G., 2013. Predator-induced behavioural defences in two competitive invasive species: the zebra mussel and the quagga mussel. Animal Behavior. 86, 1275–1284.

Nagai, K., Honjo, T., Go, J., Yamashita, H., Seok Jin Oh, 2006. Detecting the shellfish killer *Heterocapsa circularisquama* (*Dinophyceae*) by measuring bivalve valve activity with a Hall element sensor. Aquaculture 255, 395–401. https://doi.org/10.1016/j.aquaculture.2005.12.018

Naidu, K. S. (1969). Growth, reproduction, and unicellular endosymbiotic alga in the giant scallop, *Placopecten magellanicus* (Gmelin), in Port au Port Bay, Newfoundland (Doctoral dissertation, Memorial University of Newfoundland).

Nalco Environmental Solutions, 2014. Safety Data Sheet Corexit ® Ec9500a 1–11.

Narahashi, T., & Moore, J. W. (1968). Neuroactive agents and nerve membrane conductances. The Journal of general physiology, 51(5), 93.

National Research Council (US). Committee on Effectiveness of Oil Spill Dispersants. (1989). Using oil spill dispersants on the sea. National Academy Press.

Navarro, J.M., Muñoz, M.G., Contreras, A.M., 2006. Temperature as a factor regulating growth and toxin content in the dinoflagellate *Alexandrium catenella*. Harmful Algae 5, 762–769. https://doi.org/10.1016/j.hal.2006.04.001

Negri, A. P., & Jones, G. J. (1995). Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinali*s by the freshwater mussel *Alathyria condola*. Toxicon, 33(5), 667-678.

Negri, A., & Llewellyn, L. (1998). Comparative analyses by HPLC and the sodium channel and saxiphilin 3H-saxitoxin receptor assays for paralytic shellfish toxins in crustaceans and molluscs from tropical North West Australia. Toxicon, 36(2), 283-298

Newell, C.R., Wildish, D.J., MacDonald, B.A., 2001. The effects of velocity and seston

concentration on the exhalant siphon area, valve gape and filtration rate of the mussel *Mytilus edulis*. Journal of Experimental Marine Biology and Ecology. 262, 91–111. https://doi.org/10.1016/S0022-0981(01)00285-4

Newell, R. I. (1989). Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (north and mid-atlantic): blue mussel (No. 4). The Center. Biological Report. 82, 11-102.

Nielsen, L.T., Hansen, P.J., Krock, B., Vismann, B., 2016. Accumulation, transformation and breakdown of DSP toxins from the toxic dinoflagellate *Dinophysis acuta* in blue mussels, *Mytilus edulis*. Toxicon 117, 84–93. https://doi.org/10.1016/j.toxicon.2016.03.021

Nielsen, P., Krock, B., Hansen, P.J., Vismann, B., 2020. Effects of the DSP-toxic dinoflagellate *Dinophysis acuta* on clearance and respiration rate of the blue mussel, *Mytilus edulis*. PLoS One 15, 1–19. https://doi.org/10.1371/journal.pone.0230176

Nielsen, M. V., Strømgren, T., 1991. Shell growth response of mussels (*Mytilus edulis*) exposed to toxic microalgae. Marine Biology. 108, 263–267.

Nikolaou, A., Kostopoulou, M., Petsas, A., Vagi, M., Lofrano, G., & Meric, S. (2009). Levels and toxicity of polycyclic aromatic hydrocarbons in marine sediments. TrAC Trends in Analytical Chemistry, 28(6), 653-664

Norén, F., Haamer, J., Lindahl, O., 1999. Changes in the plankton community passing a *Mytilus edulis* mussel bed. Marine Ecology Progress Series. 191, 187–194. https://doi.org/10.3354/meps191187

Novaczek, I., Madhyastha, M. S., Ablett, R. F., Donald, A., Johnson, G., Nijjar, M. S., & Sims, D. E. (1992). Depuration of domoic acid from live blue mussels (*Mytilus edulis*). Canadian Journal of Fisheries and Aquatic Sciences, 49(2), 312-318

Ogata, T., Ishimaru, T., Kodama, M., 1987. Effect of water temperature and light intensity

on growth rate and toxicity change in *Protogonyaulax tamarensis*. Marine Biology. 95, 217–220.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Maintainer, H.W., 2019. Community Ecology Package: ordination, diversity and dissimilarities. Vegan Community Ecology Package. 2, 1–297.

Botelho, G. G., Golin, M., Bufalo, A. C., Morais, R. N., Dalsenter, P. R., & Martino-Andrade, A. J. (2009). Reproductive effects of di (2-ethylhexyl) phthalate in immature male rats and its relation to cholesterol, testosterone, and thyroxin levels. Archives of Environmental Contamination and toxicology, 57(4), 777-784.

Oshima, Y. (1995). Chemical and enzymatic transformation of paralytic shellfish toxins in marine organisms. Harmful marine algal blooms., 475-480.

Pace, C.B., Clark, J.R., Bragin, G.E., 1995. Comparing crude oil toxicity under standard and environmentally realistic exposure. Oil Spill Conference. 1995, 1003–1004.

Page, F.H., J.L. Martin, A. Hanke, and M.M. LeGresley. 2004. The relationship of Alexandrium fundyense to the temporal and spatial pattern in phytoplankton community structure within the Bay of Fundy, eastern Canada. Harmful algae 2002. Edited by K.A. Steidinger, J.H. Landsberg, C.R. Tomas, and G.A. Vargo. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and the Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 92–94

Parkhill, J.P., Cembella, A.D., 1999. Effects of salinity, light and inorganic nitrogen on growth and toxigenicity of the marine dinoflagellate *Alexandrium tamarense* from northeastern Canada. Journal of Plankton Research. 21, 939–955. https://doi.org/10.1093/plankt/21.5.939

Pascoe, P., Parry, H., Hawkins, S.A.J., 2009. Observations on the measurement and interpretation of clearance rate variations in suspension-feeding bivalve shellfish.

Aquatic Biology. 6, 181–190.

Pearce, I., Handlinger, J.H., Hallegraeff, G.M., 2005. Histopathology in Pacific oyster (*Crassostrea gigas*) spat caused by the dinoflagellate *Prorocentrum rhathymum*. Harmful Algae 4, 61–74. https://doi.org/10.1016/j.hal.2003.11.002

Phillips, D.J.H., 1980. Quantitative aquatic biological indicators: their use to monitor trace metal and organochlorine pollution. 488p Applied Science Publishers, Barking, England

Pinto, J.S., Silva, E.S., 1956. The toxicity of *Cardium edule* L. and its possible relation to the dinoflagellate *Prorocentrum micans*. Notas e Estud. Instuto Biologia Marítim. 12, 21.

Place, B. J., Perkins, M. J., Sinclair, E., Barsamian, A. L., Blakemore, P. R., & Field, J. A. (2016). Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. Deep Sea Research Part II: Topical Studies in Oceanography, 129, 273-281.

Posgay, J.A., 1962. Maximum yield per recruit of sea scallops, in: ICNAF Annual Meeting Series. 1016, 20

Posgay, J.A., 1957. The range of the sea scallop. *Nautilus* (Philadelphia). 71, 55–57.

Pousse, É., Flye-Sainte-Marie, J., Alunno-Bruscia, M., Hégaret, H., Jean, F., 2018. Sources of paralytic shellfish toxin accumulation variability in the Pacific oyster *Crassostrea gigas*. Toxicon 144, 14–22. https://doi.org/10.1016/j.toxicon.2017.12.050

Prakash, A., 1967. Growth and Toxicity of a Marine Dinoflagellate, *Gonyaulax tamarensis* . Journal of Fishery Research. Board Canada 24, 1589–1606. https://doi.org/10.1139/f67-131

Prins, T.C., Dankers, N., Smaal, A.C., 1994. Seasonal variation in the filtration rates of a semi-natural mussel bed in relation to seston composition 1. Journal of Experimental. Marine Biology and Ecology. 176, 69–86. https://doi.org/10.1016/0022-0981(94)90198-8

Prud'Homme Van Reine, W.F., 2017. Report of the nomenclature committee for algae: 15. Taxon 66, 191–192. https://doi.org/10.12705/661.16

Quilliam, M. A., & Wright, J. L. (1989). The amnesic shellfish poisoning mystery. Analytical chemistry, 61(18), 1053A-1060A.

Rajagopal, S., Van Der Velde, G., Jenner, H.A., 1997. Shell valve movement response of dark false mussel, *Mytilopsis leucophaeta*, to chlorination. Water Research. 31, 3187–3190. https://doi.org/10.1016/S0043-1354(97)00163-2

Reid, P.C., Lancelot, C., Gieskes, W.W.C., Hagmeier, E., Weichart, G., 1990. Phytoplankton of the North Sea and its dynamics: A review. Netherlands, Journal of Sea Research. 26, 295–331. https://doi.org/10.1016/0077-7579(90)90094-W

Reynolds, R., Stramski, D., Wright, V., and Woźniak, S. (2010). Measurements and characterization of particle size distributions in coastal waters. Journal of Geophysical Research in Oceans 115:C08024. doi: 10.1029/2009JC005930

Riisgård, H.U., 2001. Physiological regulation versus autonomous filtration in filter feeding bivalves: starting points for progress. Ophelia. 54, 193–209.

Riisgård, H.U., 1991. Filtration rate in the blue mussel *Mytilus edulis* Linneaus, 1758: dependence on algal concentration. Journal of Shellfish Research. 10, 29–35.

Riisgård, H.U., Egede, P.P., Barreiro Saavedra, I., 2011. Feeding Behaviour of the Mussel, *Mytilus edulis* : New Observations, with a Minireview of Current Knowledge . Journal of Marine Biology. 2011, 1–13. https://doi.org/10.1155/2011/312459

Riisgård, H.U., Kamermans, P., 2001. Switching between deposit and suspension feeding in coastal zoobenthos. In: Ecological Comparisons of Sedimentary Shores. Reise, K., Springer. 73–101.

Riisgard, H.U., Randløv, A., 1981. Energy budgest, growth and filtration rates in *Mytilus edulis* at different algal concentrations. Marine Biology. 61, 227–234.

Robson, A., Wilson, R., de Leaniz, C.G., 2007. Mussels flexing their muscles: a new method for quantifying bivalve behaviour. Marine Biology. 151, 1195–1204.

Robson, A. A., Garcia De Leaniz, C., Wilson, R. P., & Halsey, L. G. (2010). Behavioural adaptations of mussels to varying levels of food availability and predation risk. Journal of Molluscan Studies, 76(4), 348-353.

Rosa, M., Ward, J.E., Shumway, S.E., 2018. Selective capture and ingestion of particles by suspension-feeding bivalve molluscs: a review. Journal of Shellfish Research. 37, 727–746.

Rossignoli, A.E., Fernández, D., Regueiro, J., Mariño, C., Blanco, J., 2011. Esterification of okadaic acid in the mussel *Mytilus galloprovincialis*. Toxicon 57, 712–720. https://doi.org/10.1016/j.toxicon.2011.02.003

Roué, M., H.T. Darius, and M. Chinain. 2018. Solid Phase Adsorption Toxin Tracking (SPATT) technology for the monitoring of aquatic toxins: a review. Toxins 10: 167.

Rourke WA, Murphy CJ, Pitcher G, de Riet van JM, Burns BG, Thomas KM, Quilliam MA. Rapid postcolumn methodology for determination of paralytic shellfish toxins in shellfish tissue. Journal of AOAC International 2008; 91: 589–597. pmid:18567305

Samson, J.C., 2002. Lethal and sublethal effects of the toxic dinoflagellate, Alexandrium fundyense on three species of larval fish. Rutgers The State University of New Jersey-Newark. 140p. ISBN 978-0-493-57502-5

Sathyendranath, S., D.V. Subba Rao, Z. Chen, V. Stuart, T. Platt, G.L. Bugden, W. Jones, and P. Vass. 1997. Aircraft remote sensing of toxic phytoplankton blooms: a case study from Cardigan River, Prince Edward Island. Canadian Journal Remote Sensing 23:15–23.

Scarlett, A., Galloway, T.S., Canty, M., Smith, E.L., Nilsson, J., Rowland, S.J., 2005. Comparative toxicity of two oil dispersants, superdispersant-25 and corexit 9527, to a

range of coastal species. Environmental Toxicology and Chemistry. 24, 1219–1227. https://doi.org/10.1897/04-334R.1

Scarratt, D.J., 2000. Development of a mussel industry in eastern Canada. Bulletin of Aquaculture Association Canada 100, 37–40.

Schmidt, C. W. (2010). Between the devil and the deep blue sea: dispersants in the Gulf of Mexico. Environmental Health Perspectives 118, 8 https://doi.org/10.1289/ehp.118-a338

Schnepf, E., & Elbrächter, M. (1992). Nutritional strategies in dinoflagellates: a review with emphasis on cell biological aspects. European journal of protistology, 28(1), 3-24.

Schofield, O., Grzymski, J., Moline, M.M.A., Jovine, R.V.M., 1998. Impact of temperature acclimation on photosynthesis in the toxic red-tide dinoflagellate *Alexandrium fundyense* (Ca28). Journal of Plankton Research. 20, 1241–1258. https://doi.org/10.1093/plankt/20.7.1241

Schwinghamer, P., Hawryluk, M., Powell, C., MacKenzie, C.H., 1994. Resuspended hypnozygotes of *Alexandrium fundyense* associated with winter occurrence of PSP in inshore Newfoundland waters. Aquaculture 122, 171–179. https://doi.org/10.1016/0044-8486(94)90508-8

Seed, R., & Bayne, B. L. (1976). Ecology. Marine mussels: their ecology and physiology. Ed. Bayne BL. 528p. Cambridge University Press, Cambridge.

Seed, R. (1971). A physiological and biochemical approach to the taxonomy of *Mytilus edulis* L. and *M. galloprovincialis* Lmk. from SW England. Cahiers de biologie marine, 12(3), 291-322.

Seed, R., & Suchanek, T. H. (1992). Population and community ecology of *Mytilus*. The mussel *Mytilus*: ecology, physiology, genetics and culture, 25, 87-170

Sellner, K. G., Doucette, G. J., & Kirkpatrick, G. J. (2003). Harmful algal blooms: causes, impacts and detection. Journal of Industrial Microbiology and Biotechnology, 30(7), 383-406.

Sephton, D.H., Haya, K., Martin, J.L., LeGresley, M.M., Page, F.H., 2007. Paralytic shellfish toxins in zooplankton, mussels, lobsters and caged Atlantic salmon, *Salmo salar*, during a bloom of *Alexandrium fundyense* off Grand Manan Island, in the Bay of Fundy. Harmful Algae 6, 745–758. https://doi.org/10.1016/j.hal.2007.03.002

Shumway, S.E., 1990. A Review of the Effects of Algal Blooms on Shellfish and Aquaculture. Journal of World Aquaculture Society. 21, 65–104. https://doi.org/10.1111/j.1749-7345.1990.tb00529.x

Shumway, S.E., Cucci, T.L., Newell, R.C., Yentsch, C.M., 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. Journal of Experimental Marine Biology and Ecology 91, 77–92. https://doi.org/10.1016/0022-0981(85)90222-9

Shumway, S.E., Selvin, R., Schick, D.F., 1987. Food resources related to habitat in the scallop *Placopecten magellicanus* (Gmelin, 1791) a qualitative study. Journal of Shellfish Research. 6, 89–95.

Sidari, L., Nichetto, P., Cok, S., Sosa, S., Tubaro, A., Honsell, G., Della Loggia, R., 1998. Phytoplankton selection by mussels, and diarrhetic shellfish poisoning. Marine Biology. 131, 103–111. https://doi.org/10.1007/s002270050301

Smayda, T.J., 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanographie. 42, 1137–1153. https://doi.org/10.4319/lo.1997.42.5_part_2.1137

Smayda, T. J. (1990). " Novel and nuisance phytoplankton blooms in the sea" Evidence for a global epidemic. Toxic marine phytoplankton, 29-40.

Sonier, R., Comeau, L.A., Tremblay, R., Olivier, F., Meziane, T., Genard, B., 2020. *Mytilus*

edulis and *Styela clava* assimilate picophytoplankton carbon through feces and pseudofeces ingestion *Mytilus edulis* and *Styela clava* assimilate picophytoplankton carbon through feces and pseudofeces ingestion. Aquaculture 531, 735868. https://doi.org/10.1016/j.aquaculture.2020.735868

Sournia, A., Chrdtiennot-Dinet, M.-J., Ricard, M., 1991. Marine phytoplankton: how many species in the world ocean? Journal of Plankton Research. 13, 1093–1099.

Sow, M., Durrieu, G., Briollais, L., Ciret, P., & Massabuau, J. C. (2011). Water quality assessment by means of HFNI valvometry and high-frequency data modeling. Environmental monitoring and assessment, 182(1), 155-170.

Squires, H.J., 1962. Giant scallops in Newfoundland coastal waters. Fisheries Research Board of Canada. Ricker, W.E., Carter, N.M., 94-135, Quenn's printer and controller of stationery, Ottawa

Starr, M., Lair, S., Michaud, S., Scarratt, M., Quilliam, M., Lefaivre, D., Robert, M., Wotherspoon, A., Michaud, R., Ménard, N., Sauvé, G., Lessard, S., Béland, P., Measures, L., 2017. Multispecies mass mortality of marine fauna linked to a toxic dinoflagellate bloom. PLoS One 12, 1–18. https://doi.org/10.1371/journal.pone.0176299

Statistics Canada, 2020. Tableau 32-10-0107-01 La production et la valeur de l'aquaculture [WWW Document]. https://doi.org/https://doi.org/10.25318/3210010701-fra

Stauber, L.A., 1950. The problem of physiological species with special reference to oysters and oyster drills. Ecology 31, 109–118.

Stevenson, J.A., Dickie, L.M., 1954. Annual Growth Rings and Rate of Growth of the Giant Scallop, *Placopecten magellanicus* (Gmelin) in the Digby Area of the Bay of Fundy . Journal of Fishery Research Board Canada 11, 660–671. https://doi.org/10.1139/f54-042

Strohmeier, T., Strand, Ø., Alunno-Bruscia, M., Duinker, A., Cranford, P.J., 2012. Variability in particle retention efficiency by the mussel *Mytilus edulis*. Journal. of Experimental. Marine Biology and Ecology. 412, 96–102. https://doi.org/10.1016/j.jembe.2011.11.006

Stubbings, H.G., 1954. The biology of the common mussel in relation to fouling problems. Research 7, 222–229.

Sunda, W.G., Graneli, E., Gobler, C.J., 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. Journal of Phycology. 42, 963–974. https://doi.org/10.1111/j.1529-8817.2006.00261.x

Sunila, I., 1984. Copper-and cadmium-induced histological changes in the mantle of *Mytilus edulis* L.(Bivalvia). Limnologica 15, 523–527.

Svensson, S., 2003. Depuration of Okadaic acid (Diarrhetic Shellfish Toxin) in mussels, *Mytilus edulis* (Linnaeus), feeding on different quantities of nontoxic algae. Aquaculture 218, 277–291.

Taylor, F.J.R., 1987. General group characteristics, special features, short history of dinoflagellate study. Biology of Dinoflagelates.

Theisen, B.F., 1982. Variation in size of gills, labial palps, and adductor muscle in *Mytilus edulis* L. (bivalvia) from danish waters. Ophelia 21, 49–63. https://doi.org/10.1080/00785236.1982.10426576

Therriault, J.C., Painchaud, J., Levasseur, M., 1985. Factors controlling the occurrence of *Protogonyaulax tamarensis* and shellfish toxicity in the St. Lawrence Estuary: freshwater runoff and the stability of the water column. In: Toxic Dinoflagelate. 41–51. Elsevier, New York

Thompson, R.J., Livingstone, D.R., de Zwaan, A., 1980. Physiological and biochemical aspects of the valve snap and valve closure responses in the giant scallop *Placopecten*

magellanicus - I. Physiology. Journal of Comparative Physiology. 137, 97–104. https://doi.org/10.1007/BF00689207

Thorin, S., Bourdages, H., Vincent, B., 1998. Study of siphon activity in *Mya arenaria* (L.) in the intertidal zone by means of an underwater video camera. Journal. of Experimental Marine Biology and Ecology. 224, 205–224. https://doi.org/10.1016/S0022-0981(97)00196-2

Thyrring, J., Blicher, M.E., Sørensen, J.G., Wegeberg, S., Sejr, M.K., 2017. Rising air temperatures will increase intertidal mussel abundance in the Arctic. Marine Ecology Progess Series. 584, 91–104. https://doi.org/10.3354/meps12369

Tillmann, U., John, U., Cembella, A., 2007. On the allelochemical potency of the marine dinoflagellate A*lexandrium ostenfeldii* against heterotrophic and autotrophic protists. Journal of Plankton Research. 29, 527–543. https://doi.org/10.1093/plankt/fbm034

Tomas, C.R., 1997. Identifying Marine Phytoplankton. Tomas, C.R., 858p. Academic Press

Tran, D., Andrade, H., Durier, G., Ciret, P., Leopold, P., Sow, M., Ballantine, C., Camus, L., Berge, J., Perrigault, M., 2020. Growth and behaviour of blue mussels , a re-emerging polar resident , follow a strong annual rhythm shaped by the extreme high Arctic light regime. Royal Society Open Science. 7, 0–5.

Tran, D., Ciret, P., Ciutat, A., Durrieu, G., Massabuau, J.C., 2003. Estimation of potential and limits of bivalve closure response to detect contaminants: Application to cadmium. Environmental. Toxicology and Chemistry. 22, 914–920. https://doi.org/10.1897/1551-5028(2003)022<0914:EOPALO>2.0.CO;2

Tran, D., Ciutat, A., Mat, A., Massabuau, J.C., Hégaret, H., Lambert, C., Le Goic, N., Soudant, P., 2015. The toxic dinoflagellate *Alexandrium minutum* disrupts daily rhythmic activities at gene transcription, physiological and behavioral levels in the oyster *Crassostrea gigas*. Aquatic Toxicology. 158, 41–49. https://doi.org/10.1016/j.aquatox.2014.10.023

Tran, D., Haberkorn, H., Soudant, P., Ciret, P., Massabuau, J.C., 2010. Behavioral responses of *Crassostrea gigas* exposed to the harmful algae *Alexandrium minutum*. Aquaculture 298, 338–345. https://doi.org/10.1016/j.aquaculture.2009.10.030

Tran, D., Nadau, A., Durrieu, G., Ciret, P., Parisot, J.-P., Massabuau, J.-C., 2011. Field chronobiology of a Molluscan bivalve: How the moon and the sun cycles interact to drive oyster activity rhythms. Chronobiology International. 28.

Tran, D., Sow, M., Camus, L., Ciret, P., Berge, J., Massabuau, J.C., 2016. In the darkness of the polar night, scallops keep on a steady rhythm. Scientific Report. 6, 1–9. https://doi.org/10.1038/srep32435

Tremblay, R., Landry, T., Leblanc, N., Pernet, F., Barkhouse, C., & Sévigny, J. M. (2011). Physiological and biochemical indicators of mussel seed quality in relation to temperatures. Aquatic Living Resources, 24(3), 273-282.

Tremblay, R., Myrand, B., Sevigny, J.M., 1998. Genetic characterization of wild and suspension-cultured blue mussels (*Mytilus edulis* Linneaus, 1758) in the Magdalen Islands (southern Gulf of St Lawrence, Canada). Journal of Shellfish Research. 17, 1191–1202.

Trites, R. W., & Drinkwater, K. F. (1991). The possible role of the surface circulation in the spread of PSP in the western Gulf of St. Lawrence in 1988. Canadian special publication of fisheries and aquatic sciences/Publication speciale canadienne des sciences halieutiques et aquatiques. 1991. In: The Gulf of St .Lawrence: small ocean or big estuary? Canadian Special Publication of Fishery and Aquatic Sciences, Therriault J. C. 153-160

Trottet, A., Roy, S., Tamigneaux, E., Lovejoy, C., Tremblay, R., 2008. Impact of suspended mussels (*Mytilus edulis* L.) on plankton communities in a Magdalen Islands lagoon (Québec, Canada): A mesocosm approach. Journal of Experimental Marine Biology and Ecology 365, 103–115. https://doi.org/10.1016/j.jembe.2008.08.001

Turgeon, J., 1990. Spatial distribution of resting cysts of *Alexandrium* spp. in sediments of the lower St. Lawrence estuary and the Gaspé coast (eastern Canada). Toxic Marine Phytoplankton. 52, (3)

Turner, J.T., Tester, P.A., 1997. Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. Limnology. Oceanography 42, 1203–1213. https://doi.org/10.4319/lo.1997.42.5_part_2.1203

Turner, J. T., Tester, P. A., & Hansen, P. J. (1998). Interactions between toxic marine phytoplankton and metazoan and protistan grazers. Nato Asi Series G Ecological Sciencs, 41, 453-474

Usleber, E., Schneider, E., & Terplan, G. (1991). Direct enzyme immunoassay in microtitration plate and test strip format for the detection of saxitoxin in shellfish. Letters in applied microbiology, 13(6), 275-277.

Vahl, O. (1973). Pumping and oxygen cunsumption rates of *Mytilus edulis* L. of different sizes. Ophelia, 12(1-2), 45-52

Vahl, O., 1972. Efficiency of particle retention in *Mytilus edulis* L. Ophelia 10, 17–25. https://doi.org/10.1080/00785326.1972.10430098

Viarengo, A., 1985. Biochemical effects of trace metals. Marine Pollution Bulletin. 16, 153–158. https://doi.org/10.1016/0025-326X(85)90006-2

Ward, J.E., Targett, N.M., 1989. Influence of marine microalgal metabolites on the feeding behavior of the blue mussel *Mytilus edulis*. Marine Biology. 101, 313–321.

White, A.W., 1978. Salinity effects on growth and toxin content of *Gonyaulax excavata*, a marine dinoflagellate causing paralytic shellfish poisoning. Journal of Phycology. 14, 475–479.

Widdows, J., Moore, M. N., Lowe, D. M., & Salkeld, P. N. (1979). Some effects of a dinoflagellate bloom (*Gyrodinium aureolum*) on the mussel, *Mytilus edulis*. Journal of

the Marine Biological Association of the United Kingdom, 59(2), 522-524.

Wiese, M., D'agostino, P. M., Mihali, T. K., Moffitt, M. C., & Neilan, B. A. (2010). Neurotoxic alkaloids: saxitoxin and its analogs. Marine drugs, 8(7), 2185-2211.

Wikfors, G.H., Smolowitz, R., 1993. Detrimental effects of a *Prorocentrum* isolate upon hard clams and bay scallops in laboratory feeding studies, in: Shimizu, T.J.S. a. Y. (Ed.), Toxic Phytoplankton Blooms in the Sea.

Wildish, D.J., Kristmanson, D.D., Hoar, R.L., DeCoste, A.M., McCormick, S.D., White, A.W., 1987. Giant scallop feeding and growth responses to flow. Journal. of Experimental. Marine. Biology. and Ecology. 113, 207–220. https://doi.org/10.1016/0022-0981(87)90101-8

Wilson, J.H., Seed, R., 1974. Laboratory Experiments on Pumping and Filtration in'*Mytilus edulis* L. using Suspensions of Colloidal Graphite.

Wise, J., Wise, J.P., 2011. A review of the toxicity of chemical dispersants. Review. Environmental Health 26, 281–300. https://doi.org/10.1515/REVEH.2011.035

Zendong, Z., P. McCarron, C. Herrenknecht, M. Sibat, Z. Amzil, R.B. Cole, and P. Hess. 2015. High resolution mass spectrometry for quantitative analysis and untargeted screening of algal toxins in mussels and passive samplers. Journal of Chromatography A 1416: 10–21