EFFETS MULTI-OMIQUES SUR L'ONTOGENÈSE DE LA MOULE BLEUE *MYTILUS EDULIS* EXPOSÉE AU TRAFIC MARITIME

APPROCHE ÉCOPHYSIOLOGIQUE

Thèse présentée

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en vue de l'obtention du grade de *philosophiae doctor* (Ph.D.)

PAR

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Je dédie cette thèse à moi-même pour ma persévérance et ma résilience tout au long de cette épreuve.

« Il n'y a pas de chemins tracés pour la destinée. Ni autoroute, ni route secondaire, ni nationale, ni rien. On se met à marcher et puis en se retournant on observe ce que l'on croit être devenu derrière nous un sentier. On se dit : C'était ça notre chemin. »

Philippe Pollet-Villard

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AVANT-PROPOS

Ce projet de doctorat en océanographie, réalisé à l'Institut des Sciences de la Mer (ISMER) à l'Université du Québec à Rimouski (UQAR), intitulé « Effets multi-omiques sur l'ontogenèse de la moule bleue *Mytilus edulis* exposée au bruit du trafic maritime » vise à étudier l'impact du bruit anthropogénique et sa synergie avec la pollution chimique représentative du trafic maritime dans le cadre d'un environnement portuaire, sur le métabolisme d'un mollusque bivalve, la moule bleue *M. edulis*. Cette étude propose une approche multi-omique et écophysiologique innovante pour étudier l'impact d'un événement stressant sur l'ensemble du stade larvaire au niveau moléculaire. Ce doctorat a été réalisé sous la direction du professeur et chercheur Réjean Tremblay (UQAR-ISMER) et la co-direction du professeur et chercheur Frédéric Olivier (MNHN).

Ce projet de doctorat s'intègre au projet de recherche collaborative, interdisciplinaire et international AUDITIF (*Acoustic impact of ship traffic on mussels and scallops of the Gulf of Saint Lawrence - Impact acoustique du trafic maritime sur les moules et les pétoncles du golfe du Saint-Laurent*), dont font partie de nombreux chercheurs et chercheuses en France et au Québec. Il vise à améliorer nos connaissances sur les impacts potentiels de la combinaison des bruits sous-marins et des polluants causés par le trafic maritime sur les bivalves à haute valeur commerciale. En effet, les perturbations du cycle de vie des bivalves par les activités anthropiques peuvent avoir un impact écologique sur ces espèces essentielles pour le maintien de l'intégrité biologique des côtes, mais aussi sur les populations côtières humaines qui utilisent ces ressources pour leur développement économique. Les retombées de ce projet, y compris celles de ma thèse, contribueront à la gestion de l'aquaculture et des pêcheries de bivalves en favorisant le développement d'outils de gestion et de surveillance, tels que des cartes de vulnérabilité et une approche de modélisation permettant de prédire l'impact de l'augmentation de l'intensité du trafic maritime (nombre ou type de navires) sur

les bivalves cultivés/élevés. En parallèle des projets portant sur l'impact sonore sur différentes espèces in situ et in vivo, le projet comporte une approche innovante mêlant arts et sciences, à travers l'intégration d'artistes pendant les études de terrain couplées à des concerts spécifiques et des expositions de leurs expériences avec les scientifiques.

La thèse présentée se compose d'une introduction générale rédigée en français, de deux chapitres écrits en anglais sous forme d'articles scientifiques et d'une conclusion générale, rédigée en français et traduite en anglais, qui résume l'ensemble de la recherche et offre des perspectives d'études dans le domaine. Le premier article de thèse est en processus de soumission dans *Journal Experimental of Biology*, le deuxième est accepté avec révision mineure dans *Marine Ecology Progress Series*, le troisième sera prochainement soumis dans *Canadian Journal of Zoology*, le quatrième et le cinquième seront soumis dans *Aquatic Toxicology*. Les différents travaux de cette thèse ont été présentés sous différents types de communications et discutés avec différents acteurs.

Communications :

Veillard, D., Beauclercq, S., Olivier, F., Chauvaud, L., Genard, B., Marcotte, I., Tremblay, R. (2022). Impact of sound intensity related to maritime transport on the embryogenesis and metamorphosis of the blue mussel, *Mytilus edulis*. Aquaculture Canada and WAS North America. 15-16 août 2022, St. John's, Newfoundland, Canada. Présentation orale.

Veillard, D., Beauclercq, S., Olivier, F., Chauvaud, L., Genard, B., Marcotte, I., Tremblay, R. (2022). Impact of sound intensity related to maritime transport on the embryogenesis and metamorphosis of the blue mussel, *Mytilus edulis*. Réunion annuelle du projet AUDITIF à Montréal, novembre 2022. Présentation orale.

Veillard, D., Beauclercq, S., Olivier, F., Chauvaud, L., Genard, B., Marcotte, I., Tremblay, R. (2022). Impact de l'intensité sonore associée au transport maritime sur l'embryogenèse et la métamorphose de la moule bleue *Mytilus edulis*. Réunion annuelle du réseau Ressources Aquatiques Québec, Québec, 7-9 novembre 2022. Présentation orale.

Veillard, D., Beauclercq, S., Olivier, F., Chauvaud, L., Genard, B., Marcotte, I., Tremblay, R. (2022). Impact of sound intensity related to maritime transport on the embryogenesis and metamorphosis of the blue mussel *Mytilus edulis*. 4th International Symposium for Advances in Marine Mussel Research, Braunton, United Kingdom, 21-23 novembre 2022. Présentation orale.

Veillard, D., Beauclercq, S., Olivier, F., Chauvaud, L., Genard, B., Marcotte, I., Tremblay, R. (2023). Impact of sound intensity related to maritime transport on the embryogenesis and metamorphosis of the blue mussel *Mytilus edulis*. 115th National Shellfish Association (NSA), Annual Meeting Baltimore, Maryland, US, 27-30 mars 2023. Présentation orale.

Veillard, D., Beauclercq, S., Ghafari, N., Arnold, A.A., Genard, B., Sleno, L., Olivier, F., Choquet, A., Warschawski, D.E., Marcotte, I., Tremblay, R. (2024). Molecular evidence of shipping noise impact on the ontogeny of the blue mussel, *Mytilus edulis*. Aquaculture Association of Canada (AAC), Charlottetown, Île du Prince-Edouard, 16-19 juin 2024. Présentation orale.

Veillard, D., Beauclercq, S., Genard, B., Saint-Louis, R., Marcotte, I., Olivier, F., Tremblay, R. (2024). Preuve moléculaire de l'impact acoustique et chimique du trafic maritime sur l'ontogénie de la moule bleue *Mytilus edulis*. Réunion annuelle du réseau Ressources Aquatiques Québec, Québec, 4-6 novembre 2024. Présentation orale.

Participation à un projet Arts et Sciences dans le cadre du Projet AUDITIF. Travail avec des musiciens, en juillet 2022.

Entrevue radio « Quand la musique et la science se rencontrent ». Diffusé sur Même fréquence, Radio-Canada, le 14 février 2024. À écouter sur https://ici.radio-canada.ca/ohdio/premiere/emissions/meme-frequence/segments/rattrapage/478039/projet-auditif-ressources-aquatiques-quebec?isAutoPlay=1

Entrevue visuel sur « Quand la musique et la science se rencontrent » dans l'émission du téléjournal Est-du-Québec, le 16 février 2024. Disponible sur https://ici.radio-canada.ca

Vidéo de vulgarisation scientifique de mon projet de recherche avec le RAQ pour valoriser nos travaux, en juillet 2022. https://www.youtube.com/watch?v=8iel3KQwnpE

Vulgarisation scientifique orale pour des écoles Primaires, « Le son dans les océans », dans le cadre de la Semaine des Océans en Juin 2022.

Création d'un atelier de vulgarisation scientifique dans le cadre de la semaine des océans dans une école primaire au Bic, Bas St Laurent, en juin 2023.

Encadrement d'une étudiante à la maîtrise au laboratoire, Annabelle Blanchet, étudiante à la maîtrise en océanographie à l'UQAR-ISMER. Formation sur le projet, la mise en résine puis sur lame des larves de moules pour analyse en spectrométrie Raman.

Encadrement d'une stagiaire au laboratoire, Chloé Mordant, étudiante en 2ème année d'IUT de chimie à l'Université de Rennes II en France. Stagiaire pendant 3 mois (janvier à mars 2023) sur le traitement de mes données, développement d'un protocole pour la détection des métaux lourds et des hydrocarbures dans les larves de moule bleue.

Encadrement d'une étudiante au baccalauréat au laboratoire, Maureen Chapiteau, étudiante en 2ème année de baccalauréat en biologie à l'UQAR. Formation sur les prises de mesures pour la croissance larvaire des différents stades de moule bleue à l'aide du microscope numérique Keyence VHX-2000 Series.

RÉSUMÉ

Des recherches approfondies et récentes mettent en évidence les effets néfastes du bruit anthropique sur le biote marin, qui est désormais classé parmi les défis persistants en matière de conservation de la biodiversité aquatique. Le bruit anthropique induit des changements physiologiques qui affectent les mécanismes sous-jacents aux activités ou processus individuels, tels que le stress oxydatif, l'homéostasie énergétique, le métabolisme, la fonction immunitaire et la respiration, chez une variété d'espèces marines. En plus du bruit, la présence ponctuelle de polluants est également associée au trafic maritime. Compte tenu de la demande croissante du commerce maritime international, il est urgent d'améliorer notre connaissance de l'impact de la navigation sur les organismes marins afin de développer des stratégies d'atténuation efficaces. Les bivalves sont des organismes essentiels de l'écosystème intertidal où sont localisés les ports maritimes. Des changements physiologiques ont été signalés chez les invertébrés sessiles, tels que les bivalves, qui sont particulièrement sensibles aux sons et incapables de s'éloigner de manière significative du facteur de stress. Les études sur l'impact des bruits anthropiques sur les bivalves se sont principalement concentrées sur les stades adultes, et seules quelques expériences ont été menées sur les premiers stades larvaires. Celles-ci ont porté principalement sur le comportement, la croissance et les réponses de survie. Ces études ont montré que le bruit anthropique peut interférer avec les signaux acoustiques naturels utilisés par les organismes pour le recrutement et pourrait être (mal) interprété comme un signal de recrutement pour certains taxons.

Pour combler cette lacune, l'objectif général de cette thèse doctorale est d'étudier pour la première fois les effets du bruit d'un navire-cargo et sa synergie avec la pollution chimique sur le métabolisme des embryons et des stades larvaires compétents de la moule bleue *Mytilus edulis* à différents niveaux de stress, en utilisant différentes techniques d'analyse omiques. Les analyses ont permis d'explorer les réponses au stress primaire, secondaire et tertiaire lié au son, notamment i) le métabolisme oxydatif avec les prostaglandines, ii) le métabolisme énergétique incluant les lipides et iii) les performances larvaires par le biais de mesures via les coquilles.

Cette thèse est divisée en deux chapitres, qui dérivent de deux expériences réalisées au cours de deux étés dans des mésocosmes *Larvosonics*, créés pour émettre des sons à faible réverbération et résonance. Ces mésocosmes me permettent de mesurer les effets de l'impact de différents niveaux sonores du trafic maritime, en interactions avec d'autres conditions environnementales naturelles (trophiques) et anthropiques (pollution chimique), sur plusieurs stades de développement. Nous avons supposé que des fenêtres critiques de stress pourraient survenir au cours du développement larvaire, c'est pourquoi deux phases ontogénétiques ont été étudiées : l'embryogenèse (de l'embryon à la larve-D) et la métamorphose (des pédivéligères aux post-larves). L'embryogenèse et la métamorphose sont deux périodes critiques du développement modulant le succès d'établissement des nouvelles cohortes, et qui sont liées par des composantes communes de changements tissulaires importants supportés par les réserves énergétiques accumulées au stade précédent.

Le chapitre 1 a permis d'étudier les effets du bruit de la navigation pendant les stades larvaires cruciaux, l'embryogenèse et la métamorphose, sur le métabolome, le développement et la survie. Nous avons constaté que l'exposition au bruit des navires induit une inflammation due au stress, un déséquilibre métabolique ou un stress cellulaire résultant d'une demande énergétique accrue, conduisant à une perturbation de la glycolyse et à une augmentation de la réponse au stress oxydatif. Le bruit généré par un navire cargo a un impact direct sur le premier stade de développement des larves de moules. Plus tardivement, le bruit favorise un retard de métamorphose dans des habitats sous-optimaux avec des coûts métaboliques plus élevés, ce qui peut avoir un impact sur la durabilité de l'écosystème et de l'aquaculture, car les larves de moules compétentes luttent pour sélectionner des habitats de développement appropriés. Notre travail sur un taxon écologiquement et socio-économiquement important montre que le bruit anthropogénique peut affecter les capacités physiques des organismes. L'augmentation des coûts de la condition physique au début de la vie pourrait avoir un impact majeur sur la dynamique et la résilience de la population, avec des implications potentielles pour la structure et la fonction de la communauté.

Le chapitre 2 a permis d'évaluer la réponse des larves de moules exposées à des contaminants chimiques associés au trafic maritime. Ce chapitre se divise en trois articles. Dans la première partie, nous nous sommes concentrés sur l'embryogenèse, les premiers stades ontogéniques étant connus pour être les plus sensibles aux événements stressants et primordiaux pour le maintien de la structure de la communauté. Des embryons de moule bleue, *Mytilus edulis*, issus de deux pontes, ont été exposés jusqu'à la fin de l'embryogenèse à un cocktail de contaminants dont le profil est représentatif du trafic maritime. Nos résultats montrent une mortalité différentielle entre les cohortes larvaires, suggérant un investissement maternel plus faible dans la première cohorte larvaire. En outre, la survie des larves D, ayant un faible succès embryogénique, a montré une réponse métabolomique nettement différente de celle des larves D ayant un succès embryogénique élevé à une exposition similaire à un cocktail de contaminants. Les larves contaminées ont des besoins énergétiques plus importants et utilisaient des acides gras métabolisés par bêta-oxydation dans les mitochondries pour fournir cette énergie. Dans le second volet, les larves pédivéligères compétentes, issus de la première ponte, préalablement exposées au cocktail de polluants jusqu'à la fin de l'embryogenèse, ont été exposées à des perturbations acoustiques pendant la métamorphose. L'objectif était d'évaluer la réponse des larves compétentes lors de la métamorphose à la suite d'une contamination chimique en début de vie en synergie avec une exposition sonore représentative du trafic maritime. Cette étude montre que les effets de la pollution chimique précoce pendant l'embryogenèse ont un impact plus important sur les larves que l'exposition au bruit plus tard pendant la métamorphose. En effet, des effets latents de l'exposition chimique précoce persistent ayant des conséquences sur la taille à la métamorphose, le métabolisme énergétique et oxydatif et le profil lipidique des post-larves. Enfin, le dernier volet concerne la réponse des larves de *M. edulis* exposées à une pollution accidentelle au diesel, ainsi qu'au bruit de la navigation, pendant la phase de fixation et de métamorphose. L'objectif était d'évaluer la réponse des larves compétentes qui continuent à se métamorphoser en post-larves à la suite d'une pollution non contrôlée combinée à une exposition au bruit des cargos représentatifs du trafic maritime. Cette étude montre que les

post-larves, particulièrement exposées à des niveaux de bruit de navigation plus élevés, présentent un stress oxydatif significatif. Nous suggérons que la contamination pourrait affaiblir les larves et retarder leur métamorphose. Dans cette étude, nous suggérons l'existence d'une valeur seuil à partir de laquelle, après plus de 121 dB re 1µPa, les post-larves voient leur capacité de survie et leur succès de recrutement réduits. Les populations naturelles exposées à ces polluants ont probablement une résilience plus faible que celles associées aux ports, ce qui peut limiter leur capacité à recruter dans des conditions stressantes.

Les résultats de ce doctorat permettent de mieux comprendre les perturbations liées au bruit du trafic maritime sur le cycle de vie des bivalves marins. Nous pourrons ainsi émettre des recommandations pour améliorer la gestion de l'aquaculture et de la pêche pour ces espèces de bivalves pour l'ensemble du golfe du Saint-Laurent. La surveillance métabolomique des organismes côtiers et de leur réponse au stress couplée à des études acoustiques pourront alors être présentées comme une nouvelle approche pour aider à évaluer les impacts anthropologiques sur la biodiversité et l'aquaculture.

Mots clés : Anthropophonie, Trafic maritime, Réponses au stress, Multi-omique, Écophysiologie, Métabolomique, Métabolisme, *Mytilus edulis*, Bivalve, Embryogenèse, Métamorphose.

ABSTRACT

Extensive recent research has highlighted the damaging effects of anthropogenic noise on marine biota, which is now recognized as one of the persistent challenges to the conservation of aquatic biodiversity. Anthropogenic noise induces physiological changes that affect the mechanisms underlying individual activities or processes, such as oxidative stress, energy homeostasis, metabolism, immune function and respiration, in a wide range of marine species. In addition to noise, shipping is also associated with the occasional presence of pollutants. Given the growing demands of international maritime trade, there is an urgent need to improve our knowledge of the impacts of shipping on marine organisms in order to develop effective mitigation strategies. Bivalves are important organisms in the intertidal ecosystem where seaports are located. Physiological changes have been reported in sessile invertebrates such as bivalves, which are particularly sensitive to sound and unable to move significantly away from the stressor. Studies on the effects of anthropogenic noise on bivalve molluscs have mainly focused on adult stages, with few experiments conducted on early larval stages. These have focused on behavioral, growth, and survival responses. These studies have shown that anthropogenic noise can interfere with the natural acoustic signals used by organisms for recruitment and can be (mis)interpreted as a recruitment signal for certain taxa.

To fill this gap, the overall aim of this thesis was to investigate, for the first time, the effects of cargo ship noise and its synergy with chemical pollution on the metabolism of competent embryos and larval stages of the blue mussel *Mytilus edulis* at different stress levels using different omics analysis techniques. The analyses examined responses to primary, secondary, and tertiary sound-related stress, including i) oxidative metabolism with prostaglandins, ii) energy metabolism including lipids, and iii) larval performance via shell measurements.

This thesis is divided into two chapters, derived from two experiments carried out over two summers in *Larvosonics* mesocosms, created to emit low reverberation and resonance sounds. These mesocosms allowed me to measure the effects of different levels of marine traffic noise, in combination with other natural (trophic) and anthropogenic (chemical pollution) environmental conditions, on several developmental stages. We hypothesized that critical stress windows might occur during larval development, so two ontogenetic stages were studied: embryogenesis (from embryos to D-larva) and metamorphosis (from pediveliger to post-larva). Embryogenesis and metamorphosis are two critical developmental periods that modulate the establishment success of new cohorts and are linked by common components of major tissue changes supported by energy reserves accumulated in the previous stage.

Chapter 1 investigated the effects of shipping noise during the critical larval stages of embryogenesis and metamorphosis on the metabolome, development and survival. We found that exposure to shipping noise induces stress-induced inflammation, metabolic imbalance

or cellular stress resulting from increased energy demand, leading to disruption of glycolysis and increased oxidative stress response. Noise from a cargo ship has a direct effect on the first developmental stage of mussel larvae. Later, noise promoted delayed metamorphosis in suboptimal habitats with higher metabolic costs, which may affect ecosystem and aquaculture sustainability as competent mussel larvae struggle to select suitable developmental habitats. Our work on an ecologically and socio-economically important taxon shows that anthropogenic noise can affect the physical abilities of organisms. Increased fitness costs early in life could have a major impact on population dynamics and resilience, with potential implications for community structure and function.

Chapter 2 assessed the response of mussel larvae exposed to chemical contaminants associated with maritime traffic. This chapter is divided into three parts. In the first part, we focused on embryogenesis, as the earliest ontogenic stages are known to be the most sensitive to stressful events and crucial for maintaining community structure. Blue mussels, *Mytilus edulis*, embryos from two clutches were exposed until the end of embryogenesis to a cocktail of pollutants whose profile is representative of maritime traffic. Our results show a differential mortality between larval cohorts, suggesting a lower maternal investment in the first larval cohort. In addition, the survival of D larvae with low embryogenic success showed a markedly different metabolomic response than that of D larvae with high embryogenic success to similar exposure to a cocktail of contaminants. The contaminated larvae had higher energy requirements and used fatty acids metabolized by beta-oxidation in the mitochondria to provide this energy. In the second phase, competent pediveliger larvae from the first spawning period, previously exposed to the cocktail of pollutants until the end of embryogenesis, were exposed to acoustic perturbations during metamorphosis. The aim was to assess the response of competent larvae during metamorphosis to early life chemical pollution in synergy with noise exposure representative of maritime traffic. This study shows that the effects of early chemical pollution during embryogenesis have a greater impact on larvae than noise exposure later in metamorphosis. In fact, latent effects of early chemical exposure persist and influence size at metamorphosis, energy and oxidative metabolism, and lipid profiles of post-larvae. Finally, the last section is the response of *M. edulis* larvae exposed to accidental diesel pollution to shipping noise during their competent settlement and metamorphosis stage. The aim was to assess the response of competent larvae that continue to metamorphose into post-larvae following uncontrolled pollution combined with exposure to a cargo ship noise representative of maritime traffic. This study shows that post-larvae, particularly exposed to higher shipping noise levels, displayed significant oxidative stress, and we suggest that contamination could weaken larvae as they delay their metamorphosis. We suggest a threshold value from observations in this study whereby above 121 dB re 1 µPa post-larvae may have reduced recruitment success due to the higher energy demands resulting from the stress of noise exposure, and thus less ability to cope with other stressors they may also encounter during this critical period. Natural populations exposed to these pollutants probably have a lower resilience than those associated with ports, which may limit their ability to recruit under stressful conditions.

The results of this thesis will allow us to gain a better understanding of the disturbance caused by shipping noise on the life cycle of marine bivalves. This will allow us to make recommendations to improve the management of aquaculture and fisheries of these bivalve species throughout the Gulf of St. Lawrence. Metabolomic monitoring of coastal organisms and their response to stress, coupled with acoustic studies, could then be presented as a new approach to help assess anthropogenic impacts on biodiversity and aquaculture.

Keywords: Anthropophony, Maritime traffic, Stress response, Multi-omics, Ecophysiology, Metabolomics, Metabolism, *Mytilus edulis*, Bivalve, Embryogenesis, Metamorphosis.

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	Français, English
H-NMR	Résonance magnétique nucléaire du proton, Proton Nuclear Magnetic Resonance
AA	Acid Aminé, Amino Acid
AchE	Acétylcholinestérase, Acetylcholinesterase
ACN	Acétonitrile, Acetonitrile
ADP	Adénosine Diphosphate, Adenosine Diphosphate
AEC	Charge énergétique adénylique, Adenylate Energy Charge
ALA	Acide α-linolénique, α-linolenic acid
AMP	Adénosine Monophosphate, Adenosine Monophosphate
AMPK	Protéine kinase Adénosine Monophosphate, Protein kinase Adenosine Monophosphate
ARA	Arachidonic acid, Acide arachidonique
ARG	Arginine
ASO	Organe Sensoriel Abdominal, Abdominal Sense Organ
ATP	Adénosine triphosphate, Adenosine triphosphate
BABA	Acide β-aminobutyrique, β-aminobutyric acid
BAIBA	Acide β-aminoisobutyrique, β-aminoisobutyric acid
BCAAs	Acide aminé à chaîne ramifiée, Branched Chain Amino Acid
C	Contaminé, Contaminated
CAT	Catalase

Cd	Cadmium
CH_3Hg^+	Méthylmercure, Methylmercury
ClO^-	Anion hypochlorite
COX	Cyclooxygenase
CPT	Carnitine palmitoyltransférase, Carnitine palmitoyltransferase
Cu, Cu2+	Cuivre, Copper
cyPGs	Prostaglandines cyclopenténones, Cyclopentenone prostaglandins
D	Dissoconque, Dissoconch
dB	Decibel
DHA	Acide docosahexaénoïque, Docosahexaenoic acid
dpf	Jour après la fécondation, Day post-fecundation
EPA	Acide eicosapentaénoïque, Eicosapentaenoic acid
F1.6BP	D-fructose1,6-biphosphate
FAD	Flavine adénine dinucléotide, Flavin adenine dinucleotide
FAME	Ester méthylique d'acide gras, Fatty acid methyl ester
FAO	Organisation pour l'alimentation et l'agriculture, Food and Agriculture Organization
FA	Acide gras, Fatty Acid
FDR	Taux de découverte erronée, False Discovery Rate
G6P	Glucose-6-phosphate
GABA	Acide β-aminoisobutyrique, β-aminoisobutyric acid
GES	Bon statut environnemental, Good Environmental Status
GLA	Acide γ-linolénique, γ-linolenic acid

GLS	Glutaminase
GR	Glutathion réductase, Glutathione reductase
GSH, GSSG	Glutathion réduit, Reduced glutathione
GST	Glutathion-S-transférase, Glutathione-S-transferase
H_2O_2	Peroxyde d'hydrogène, Hydrogen peroxide
HDHA	Acides hydroxy docosahexaénoïques, Hydroxy docosahexaenoic acids
HEPE	Acide hydroxyicosapentaénoïque, Hydroxyicosapentaenoic acid
HETE	Acides hydroxyeicosatétraénoïques, Hydroxyeicosatetraenoic acid
Hg, Hg2+	Mercure, Mercury
HNO_3	Acide nitrique, Nitric acid
HPLC	Chromatographie en phase liquide à haute performance, High performance liquid chromatography
HSP	Protéine de choc thermique, Heat Shock Protein
IMO	Organisation Maritime Internationale, International Maritime Organization
LA	Acide linoléique, Linoleic acid
LC-HRMS/MS	Chromatographie liquide couplée à la spectrométrie de masse à haute résolution, Liquid chromatography coupled to high-resolution mass spectrometry
LOBE	Niveau d'apparition de l'effet biologique indésirable, Level of Onset of Biological Adverse Effect
LOX	Lipoxygénase, Lipoxygenase
LTs, LTB	Leucotriène, Leukotriene
LXs	Lipoxine, Lipoxine
MeOH	Méthanol, Methanol

mETC	Système de transport de la chaîne d'électrons mitochondriale, Mitochondrial electron chain transport system
MRM	Suivi de réactions multiples, Multiple reaction monitoring
mRNA	Acide ribonucléique messager, Messenger ribonucleic acid
MSEA	Analyse de l'enrichissement des ensembles de métabolites, Metabolite-set enrichment analysis
MSFD	Directive-cadre sur la stratégie pour le milieu marin, Marine Strategy Framework Directive
MTBE	Éther méthylique de tert-butyle, Methyl tert-butyl ether
NAD	Nicotinamide adénine dinucléotide (forme oxydée), Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adénine dinucléotide (forme réduite), Nicotinamide adenine dinucleotide (reduced form)
NADP	Nicotinamides adénine dinucléotide phosphate (forme oxydée), Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	Nicotinamides adénine dinucléotide phosphate (forme réduite), Nicotinamide adenine dinucleotide phosphate (reduced form)
NH3	Ammoniac, Ammonia
Non-C	Non-contaminé, Non-contaminated
O_2^-	Anion superoxyde, Superoxide anion
OH^-	Hydroxyle radical, Radical hydroxyle
OPLS-DA	Projection orthogonale structures latentes analyses discriminantes, Orthogonal projection latent structures discriminant analyses
ORA	Analyse de la surreprésentation, Over-representation analysis
oxoETE	Acide oxo-eicosatétraénoïque, Oxo-eicosatetraenoic acid
OXPHOS	Phosphorylation oxydative
PAHs	Polycyclic Aromatic Hydrocarbons or polyaromatic hydrocarbons

Pb, Pb2+	Plomb, Lead
PCA	Analyses en composantes principales, Principal component analyses
PDH	Pyruvate déshydrogénase, Pyruvate dehydrogenase
PG	Prostaglandines, Prostaglandins
PII	Prodissoconque II, Prodissoconch II
PSD	Densité spectrale de puissance, Power Spectral Density
PTFE	Polytétrafluoroéthylène, Polytetrafluoroethylene
PUFAs	Acide gras polyinsaturés, Polyunsaturated fatty acids
RMN	Résonance magnétique nucléaire, Nuclear Magnetic Resonance
ROS	Espèces réactives de l'oxygène, Reactive oxygen species
SE	Erreur standard, Standard error
SOD	Superoxydes dismutases, Superoxide dismutases
SPM	St-Pierre-et-Miquelon
TCA	Cycle de l'acide tricarboxylique, Krebs cycle, Citric acid cycle
TFE	2,2,2-trifluoroéthanol, 2,2,2-trifluoroethanol
TL	Perte de transmission, Transmission Lost
TMAH	Hydroxyde de tétraméthylammonium, Tetramethylammonium hydroxide
TXs, TXA	Thromboxanes
UNCLOS	Convention des Nations unies sur le droit de la mer, United Nations Convention on the Law of the Sea
UV	Ultra-violet
VIP	Importance variable dans la projection, Variable importance in the projection
WAF	Fraction de l'eau consommée, Water Accomodated Fraction

INTRODUCTION GÉNÉRALE

1. CONCEPTS DE « STRESS » : RÉPONSES BIOLOGIQUES

1.1 Qu'est-ce que le stress ?

Le stress au niveau physiologique est la réponse non spécifique d'un corps à toute demande qui lui est faite (Selye, 1973). En réponse à une exposition à divers éléments naturels (météorologiques, physiques, etc.) ou non naturels (c'est-à-dire anthropiques), les organismes exécutent certaines fonctions d'acclimatation afin de rétablir la normalité, celle-ci dépendant de l'activité spécifique qui a provoqué la condition. Un organisme peut répondre à ces changements environnementaux de deux façons : 1) en s'acclimatant, en modifiant sa physiologie et son comportement par le biais de la plasticité phénotypique ; ou 2) en s'adaptant par le biais de la sélection naturelle, qui entraîne la mort des individus les moins bien adaptés (Somero, 2010). La plasticité phénotypique est définie comme étant la capacité d'un génotype à exprimer plusieurs phénotypes en réponse à un changement environnemental (Schlichting & Smith, 2002). Les changements de taux métabolique, de croissance et les différences comportementales constituent les mécanismes de plasticité phénotypique les plus étudiés. C'est cette demande d'ajustement d'une fonction de l'organisme (épigénétique, biochimique, physiologique ou comportementale) qui est l'essence même du stress.

Par contraste, l'adaptation correspond à un processus évolutif génétique qui s'intègre à l'échelle de la population. Dans ce cas, les organismes ayant été « sélectionnés » possèdent le ou les gènes appropriés qui leur permettent de survivre (Sanford & Kelly, 2011). Des études ont démontré, par des déterminations biochimiques quantitatives, que certaines

réactions du corps sont totalement non spécifiques et communes à tous les types d'exposition, telles que le froid, la chaleur, les hormones, etc. (Selye, 1953, 1973). En outre, des réactions de stress concernent tous les organismes, même ceux sans système nerveux, ainsi que les plantes (Selye, 1973).

1.2 Le rôle du stress et des réponses biologiques

Certaines sources de stress requièrent des exigences physiologiques supplémentaires qui dépassent un niveau seuil que les mécanismes d'adaptation peuvent surmonter et qui peuvent par la suite entraîner des effets débilitants, voire délétères (Portz et al., 2006). Chez les poissons, ces effets peuvent se manifester par un système immunitaire affaibli, une diminution de la croissance, des performances de nage ou de la capacité de reproduction et la mort (Barton et al., 2002; Barton & Iwama, 1991). D'après Selye (1946), les réponses au stress se décomposent en plusieurs « phases » avec : 1) une réaction initiale dite d'alarme, puis 2) une phase d'acclimatation ou de résistance et enfin 3) une phase d'épuisement. Aujourd'hui, ce concept a évolué et les réponses au stress sont définies comme étant primaires, secondaires et dans certains cas tertiaires, comme l'illustre la Figure 1 chez les poissons téléostéens. Chez ces derniers, la réponse primaire au stress est rapide sous la forme d'une libération de catécholamines dans le système circulatoire (Randall & Perry, 1992) et d'une synthèse de cortisol en fonction de la température dès les premières minutes (Lankford et al., 2003; Wedemeyer et al., 1990). Le cortisol est généralement considéré comme « l'hormone du stress », car il favorise plusieurs mécanismes de réponse au stress secondaire et tertiaire. Ceux-ci incluent une altération du métabolisme énergétique, de l'osmorégulation ainsi que des fonctions immunitaires (Sundh et al., 2010). Le niveau secondaire représente une réponse systématique au stress facilitée par les hormones des réponses primaires. Une cascade de changements métaboliques et physiologiques est induite, qui entraîne une altération de la chimie du sang et des tissus, par exemple une augmentation de la glycémie et du lactate (Barton & Iwama, 1991). Cette réponse permet une meilleure mobilisation énergétique afin de répondre à des besoins métaboliques accrus (Portz et al., 2006). Ces modifications physiologiques sont pour la plupart réversibles et les organismes peuvent

revenir à leur état sain d'avant le stress. Les réponses au stress tertiaire qui y succèdent incluent un taux de croissance et une capacité métabolique réduits, des diminutions de la résistance aux maladies et de la capacité de reproduction, ainsi qu'une modification du comportement et de la capacité de survie (**Fig. 1**) (Barton & Iwama, 1991; Mommsen et al., 1999; Wedemeyer et al., 1990). L'ampleur des réponses tertiaires peut être directement liée à la gravité et à la durée du facteur de stress (Portz et al., 2006). Néanmoins, un stress chronique, c'est-à-dire un stress qui persiste sur une longue période, même à de faibles niveaux, peut altérer les performances en détournant des ressources énergétiques qui pourraient autrement être utilisées pour les activités de routine, la croissance, la fonction immunitaire et / ou la reproduction (**Fig. 1**) (Barton et al., 2002; Barton & Iwama, 1991). Par exemple, la mauvaise qualité de l'eau peut entraîner une redistribution de l'énergie des processus physiologiques secondaires (dits « non essentiels ») tels que la croissance et/ou la reproduction, vers les processus primaires (dits « essentiels »), tels que le métabolisme et/ou le système immunitaire (Portz et al., 2006). L'activation prolongée de la réponse au stress pourrait finalement conduire à des effets destructeurs au niveau tertiaire (Schreck & Tort, 2016). Certains de ces effets négatifs sont potentiellement irréversibles, comme l'indique la Figure 1, sont donc préoccupants.

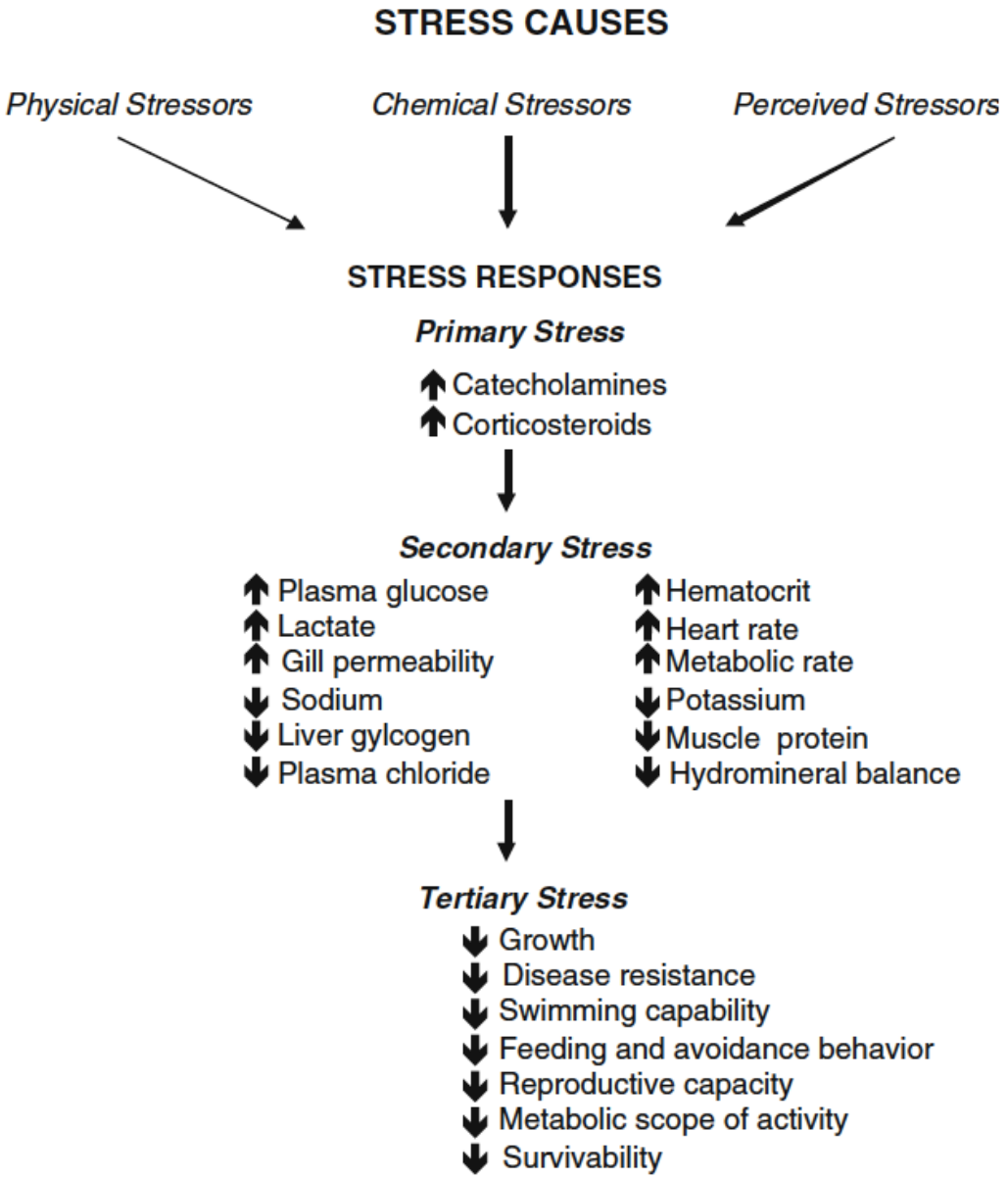


Figure 1. Réponses physiologiques aux facteurs de stress pendant la détention et le transport chez des poissons d'aquaculture (modifié de Barton et al., 2002).

1.3 Les facteurs de stress et leurs effets

Les organismes sont continuellement confrontés à une gamme de facteurs de stress et de variables environnementales qui agissent comme des agents d'acclimatation et/ou de sélection. Les facteurs de stress incluent ceux intrinsèques ou extrinsèques qui interpellent les individus et induisent à un ajustement comportemental (agressivité, niveau d'activité, etc.) ou physiologique (taux métabolique, profils hormonaux, etc.) pour y faire face (Schreck & Tort, 2016). Ceux-ci incluent des facteurs de stress abiotiques tels que la faible disponibilité d'oxygène ou les changements de température, mais aussi des facteurs de stress biotiques tels que la présence de prédateurs ou une concurrence accrue avec les congénères (Killen et al., 2013). Ces facteurs peuvent être caractérisés par leur fréquence et leur persistance dans le temps (aigus, séquentiels, épisodiques) (Sapolsky et al., 2000), comme le phénomène de migration chez certaines espèces.

Des organismes exposés à un facteur de stress modifient la priorité de certains comportements et fonctions physiologiques. Ainsi, les individus à jeun s'activent pour trouver de la nourriture et ceux qui sont exposés à un risque de prédation plus élevé sont plus susceptibles de se cacher ; les organismes endothermes exposés au froid augmentent généralement leur métabolisme pour accroître leur production de chaleur. Les facteurs de stress environnementaux semblent capables de révéler, masquer ou moduler la covariation des traits physiologiques et comportementaux en fonction de la sensibilité différentielle de ces phénotypes aux facteurs de stress (**Fig. 2**) (Killen et al., 2013). En effet, l'ampleur de la corrélation entre ces deux traits peut changer une fois que les organismes sont exposés à un facteur de stress, révélant ou amplifiant la relation (facteurs de stress légers à modérés, **Fig. 2 A, B**) ou masquant ou atténuant la relation (facteurs de stress plus graves, **Fig. 2 C-E**).

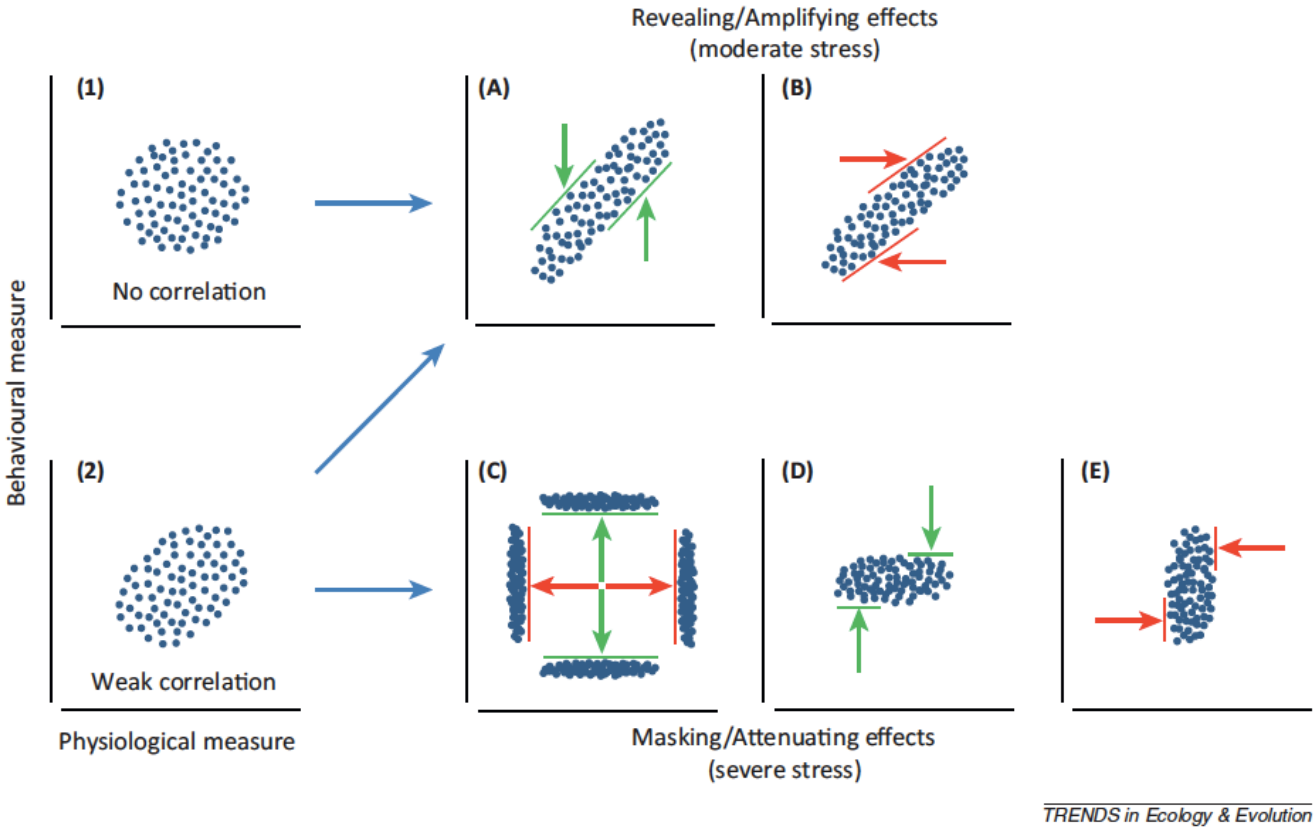


Figure 2. Représentations schématiques des effets révélateurs/amplificateurs et masquant/atténuant des facteurs de stress sur la relation entre des traits physiologiques et comportementaux spécifiques. Les facteurs de stress qui affectent le degré de variation comportementale et physiologique entre les individus sont illustrés par des flèches vertes et rouges, respectivement. Les panneaux à l'extrême gauche représentent les corrélations entre les traits physiologiques et comportementaux mesurés dans des conditions relativement peu stressées, dans lesquelles la corrélation est inexistante (**1**) ou faible (**2**) (Killen et al., 2013).

Les variations des traits comportementaux et physiologiques, souvent importantes et constantes entre les individus de la même espèce, ont des conséquences évidentes sur la forme physique et l'évolution des histoires de vie (Burton et al., 2011; Sih et al., 2004a). En outre, il existe des liens entre ces deux types de traits spécifiques qui sous-tendent un vaste éventail de réponses écologiques tels que la recherche de nourriture, les interactions compétitives, le choix du partenaire, les interactions prédateur-proie et la sélection de l'habitat (Biro & Stamps, 2010; Careau et al., 2008).

Pour la plupart des vertébrés, les réponses qui ont évolué pour faire face à de tels défis sont limitées par un seuil de détection, basé sur la pertinence et la capacité à répondre. En outre, la tolérance au stress peut dépendre de l'espèce, du stade biologique, du génome et du comportement (Barry et al., 1995; Barton, 2002; Barton & Iwama, 1991; Kjartansson et al., 1988; Woodward & Strange, 1987). Certaines espèces peuvent être anormalement sensibles au stress (ce qui a par exemple été observé chez des poissons rapides qui sautent et se retournent dans des réservoirs (Clearwater & Pankhurst, 1997). Ainsi, Mazeaud et al. (1977) montrent que bien que de nombreux poissons réussissent à survivre à une exposition aux facteurs de stress, ils meurent plus tard de maladies ou de dysfonctionnements osmotiques.

Les facteurs de stress qui remettent en question l'homéostasie, c'est-à-dire les processus physiologiques coordonnés qui maintiennent la plupart des états stables dans l'organisme, sont souvent considérés comme les besoins les plus urgents, sont les plus connus (Cannon, 1929; Cooper, 2008; Siegel, 2008). Lorsque la capacité d'un organisme à maintenir ses fonctions physiologiques (comme l'homéostasie) dans une plage de tolérance spécifique est dépassée, des réponses sont développées qui permettent à l'organisme de faire face en supprimant le facteur de stress ou en facilitant la coexistence avec lui (Antelman & Caggiula, 1980).

1.4 Plasticité phénotypique et effet dose

Les niveaux de plasticité phénotypique des animaux peuvent varier pendant certaines périodes nommées « fenêtres critiques » (**Fig. 3**) (Burggren & Mueller, 2015; Burggren & Reyna, 2011). En effet, au cours du développement, une espèce peut être plus sensible au stress et exprimer, après exposition, un niveau de plasticité phénotypique plus élevé (**Fig. 3A**). Par exemple, si un embryon est exposé à un facteur de stress environnemental, tel qu'un changement de température, avant ou après cette fenêtre critique, son phénotype ne changera pas (Mueller et al., 2015). Cependant, si un facteur de stress environnemental intervient pendant la fenêtre critique, alors le phénotype en développement est modifié (métabolisme, fréquence cardiaque ou encore survie), de manière transitoire ou permanente, ce qui indique

la présence d'une période sensible de vulnérabilité. Un autre exemple serait l'exposition à une hypoxie ou à une hyperoxie chronique tout au long du développement embryonnaire, qui entraîne des modifications des phénotypes morphologiques et respiratoires (Bavis, 2005; Bavis et al., 2013; Bavis & Mitchell, 2008). L'exposition à des agents de stress chimiques (perturbateurs endocriniens, éthanol et rétinoïdes) durant les fenêtres critiques peut également entraîner des changements négatifs dans la morphologie et la physiologie des poissons et des amphibiens (Ali et al., 2011; Ankley & Johnson, 2004; Degitz et al., 2000; Maack & Segner, 2004; Van Aerle et al., 2002). Dans certains cas, une fois le facteur de stress éliminé, le phénotype normal d'un animal est rétabli par des capacités d'autoréparation à un stade ultérieur (Burggren & Reyna, 2011). Cette plasticité phénotypique au cours du développement peut permettre la survie dans un environnement altéré, mais elle peut aussi avoir des conséquences potentielles à long terme, jusqu'à la transmission aux générations suivantes.

Actuellement, le concept unidirectionnel de la fenêtre critique (**Fig. 3A**) est le plus étudié. Néanmoins, cette approche explore les effets d'une seule intensité d'un agent stressant et son analyse pourrait être améliorée par l'utilisation de plusieurs niveaux d'intensités. En effet, une faible intensité peut ne produire aucun changement phénotypique, mais une intensité légèrement plus élevée pourrait révéler une modification de celui-ci (**Fig. 3B**). De plus, si l'intensité est encore plus élevée, la fenêtre critique pourrait se révéler plus large (**Fig. 3B**). Cependant, cette approche est limitée par la manière dont les modifications phénotypiques se produisent à l'intérieur d'une fenêtre critique (Mueller et al., 2015). Par conséquent, l'utilisation d'une fenêtre critique tridimensionnelle dans laquelle l'ampleur de l'effet, par exemple le changement phénotypique, varie en fonction du moment de l'exposition se produit et de l'intensité du facteur de stress appliqué est la plus représentative (**Fig. 3C**) (Burggren & Mueller, 2015).

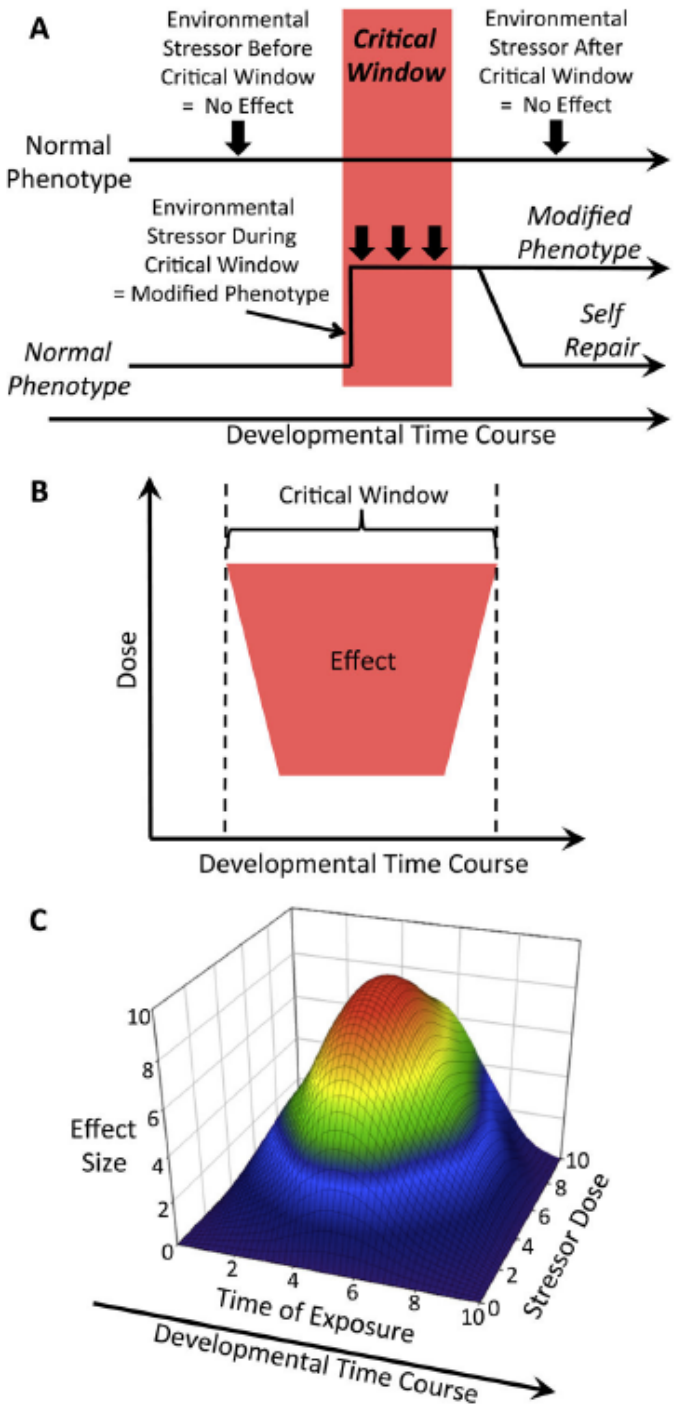


Figure 3. Représentation de différents types de fenêtre critique sous différents angles. (**A**) Fenêtre critique « unidimensionnelle », dans laquelle la modification phénotypique causée par un facteur de stress dépend du moment de l'exposition au facteur de stress au cours du développement. (**B**) Fenêtre critique « bidimensionnelle », dans laquelle une modification phénotypique dépend du niveau de développement et de l'intensité du facteur de stress. (**C**) Fenêtre critique « tridimensionnelle », dans laquelle l'ampleur du changement phénotypique dépend du niveau de développement, de l'intensité du facteur de stress et de la taille de l'individu (Müller et al., 2015).

2. EXPRESSION DU STRESS CHEZ LES MOLLUSQUES BIVALVES

Les organismes, lorsqu'ils sont mobiles, peuvent adopter des mécanismes d'acclimatation comportementale pour sélectionner des environnements permettant de minimiser leur ajustement interne (Hochachka & Somero, 2002). Toutefois, ce n'est pas le cas des mollusques bivalves. Les mollusques représentent 23 % de la diversité de la vie dans les océans et sont connus, en particulier les bivalves, pour être des espèces clés pour le développement, le fonctionnement et la durabilité des environnements côtiers (Smaal et al., 2019). Une des caractéristiques importantes de plusieurs espèces de bivalves est leur mode d'alimentation de type filtreur, qui leur permet de concentrer dans leurs tissus les éléments dissous et particulaires présents dans leur environnement. Avec des taux de filtration pouvant atteindre 10 litres par minute chez certaines espèces, les bivalves peuvent donc accumuler facilement les polluants et les pathogènes présents dans le milieu et sont donc très sensibles à la pollution anthropique. En raison de leur immobilité, les organismes sessiles comptent sur la plasticité physiologique (comme par exemple la régulation de l'expression génique) pour s'adapter aux agressions environnementales et coloniser rapidement des habitats fluctuants (Fabbri et al., 2008).

2.1 Le système immunitaire inné

Les bivalves disposent d'un système circulatoire de type semi-ouvert, comprenant un cœur et des vaisseaux par lesquels circule l'hémolymphe contenant des hémocytes. Ces cellules, qui sont des composants clés de la réponse immunitaire, jouent de multiples fonctions associées à des processus vitaux tels que la respiration, la digestion, le transport des nutriments, l'excrétion, les réactions de défense, la réparation de lésions et de la coquille (Mydlarz et al., 2006). Présents à la fois dans l'appareil circulatoire (vaisseaux et sinus hémolymphatiques) et dans les tissus et organes (glandes digestives, branchies, manteau, gonades), ils jouent des fonctions multiples associées à des processus vitaux tels que la respiration, la digestion, le transport des nutriments, l'excrétion, les réactions de défense, la

réparation de lésions et de la coquille (Samain, 2011). Le nombre d'hémocytes circulants peut varier et chuter considérablement lors d'une infection. Afin d'équilibrer ces fortes variations, les invertébrés, et en particulier les bivalves doivent renouveler sans cesse leurs hémocytes (Bassim et al., 2014a).

Les bivalves dépendent d'un mécanisme de défense non spécifique connu sous le nom de système immunitaire inné (Mydlarz et al., 2006). Il s'agit d'un mécanisme primitif qui déclenche en cascade des signaux moléculaires intracellulaires (Danilova, 2006). Ce système immunitaire sert de protection contre les organismes pathogènes (les protozoaires, les bactéries, les endoparasites, les mycoplasmes, les virus), les facteurs de stress environnementaux (les agressions xénobiotiques, les toxines algales lors de blooms) et d'autres stimuli immunomodulateurs (exposition à l'air, stress mécanique, températures élevées, conditions de salinité extrêmes) (Bassim et al., 2014a). Le mécanisme de protection chez les bivalves comprend deux actions complémentaires, reposant sur une réponse cellulaire (impliquant les hémocytes) et une réponse humorale (production d'anticorps) immédiate et de courte durée. Ces deux réponses peuvent agir de façon indépendante ou conjuguée (Brennan & Anderson, 2004; Hultmark, 2003). Certaines capacités immunocellulaires ont été caractérisées chez les larves de *M. edulis* (Dyrynda et al., 1995). Le système immunitaire inné des bivalves présente une grande variété d'effecteurs sélectifs, de récepteurs sensibles et de réseaux de régulation génétique synergique impliquant toutes les cellules de l'organisme (**Fig. 4**) (Barmo et al., 2013). Tout d'abord, le système de reconnaissance immunitaire (**Fig. 4a**) déclenche une multitude de voies de signalisation et de production d'effecteurs spécifiques (**Fig. 4b**), conduisant à la neutralisation des menaces par phagocytose (**Fig. 4c**). La phagocytose est considérée comme l'une des armes les plus efficaces pour éliminer des éléments étrangers, tels que des particules inorganiques, des organismes vivants étrangers (pathogènes ou non) et des cellules de l'hôte modifiées. Son rôle est non seulement défensif, mais aussi nutritif (Silverstein, 1995). Elle conduit généralement à la libération d'espèces réactives de l'oxygène (ROS, acronyme anglophone généralement utilisé) (*voir section suivante*) (**Fig. 4d**), qui participent également à l'élimination intracellulaire des particules phagocytées (chez *M. edulis* : Pipe, 1992; Winston

et al., 1996). Chez *M. edulis*, les hémocytes des moules adultes, mais aussi ceux des larves, sont capables de phagocytose (Dyrynda et al., 1995).

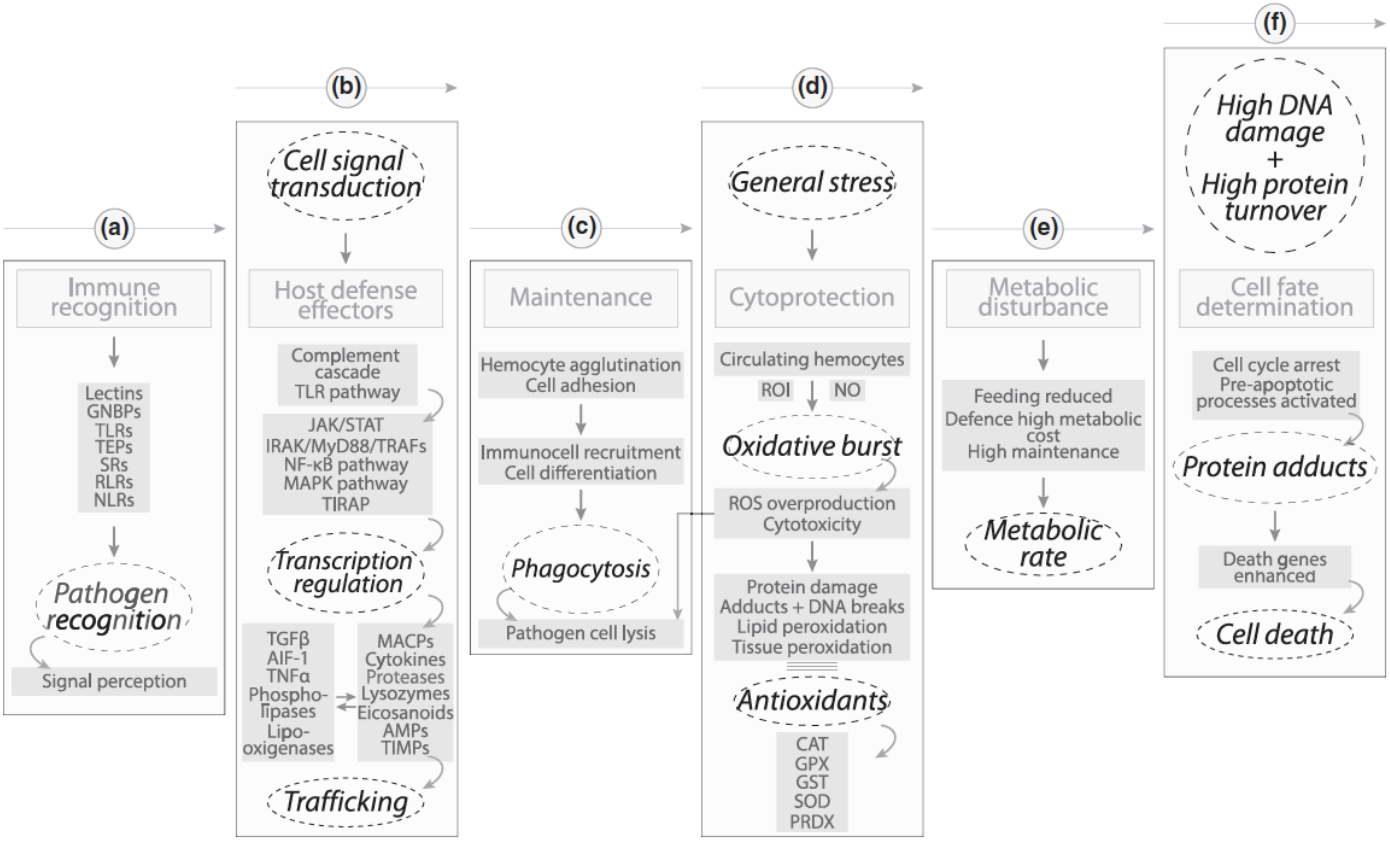


Figure 4. Déterminants moléculaires et physiologiques des mécanismes de défense cellulaire : (**a**) reconnaissance de la menace, (**b**) initiation de la défense, **(c**) distinction immunocellulaire, **(d**) activation immunocellulaire, (**e**) régulation métabolique et (**f**) accumulation des dommages cellulaires puis morts cellulaire. GNBP, TLR, TEP, SR, RLR, NLR : différents types de récepteur ; JAK, STAT, IRAK, MyD88, TRAF, MAPK : transducteurs de signal et activateurs de transcription ; MACP, TIMP, AMP : inhibiteurs ; TGFβ, TNFα, AIF-1 : facteurs de réponse (Bassim et al., 2014a).

2.2 Le stress oxydatif

L'homéostasie redox intracellulaire, une condition essentielle pour les organismes aérobies, dépend de l'équilibre entre la production d'oxydants et de leur élimination au fil du

temps (**Fig. 5**). Les métabolites/espèces réactives de l'oxygène (ROS) sont des molécules de courte durée contenant des électrons non appariés provenant de molécules d'oxygène partiellement réduit générées, transformées et éliminées dans divers processus cellulaires, comme le métabolisme, la prolifération, la différenciation, la régulation du système immunitaire et le remodelage vasculaire. Ce sont donc des dérivés du métabolisme qui agissent comme des acteurs importants de la signalisation cellulaire et de la régulation des métabolismes (Panieri & Santoro, 2016).

Les dérivés contenant de l'oxygène sont constitués de radicaux libres tels que l'anion superoxyde (O_2^-), le radical hydroxyle (OH^-), l'anion hypochlorite (ClO^-) et le peroxyde d'hydrogène (H_2O_2) (Torreilles et al., 1996). Les sources exogènes et endogènes de la production de ROS ont été largement décrites (Panieri & Santoro, 2016). Les plus pertinentes d'un point de vue biologique sont les nicotinamides adénine dinucléotide phosphate (NADPH) oxydases, liées à la membrane cellulaire. Ces enzymes catalysent la production d'O_2 ou de H_2O_2 en utilisant le NADPH comme réducteur (**Fig. 6**) (Lambeth, 2004) et le système de transport de la chaîne d'électrons mitochondriale (mETC), dans lequel les complexes I et II génèrent de l'O_2 à la suite d'une fuite d'électrons pendant la respiration mitochondriale en utilisant le nicotinamide adénine dinucléotide (forme réduite) (NADH) et le FADH (Kussmaul & Hirst, 2006; Quinlan et al., 2012). Les mitochondries sont les sources cellulaires majeures de ROS (**Fig. 4d, 7 et 8**). Le rôle majeur de la production endogène de ROS est une activité de régulation.

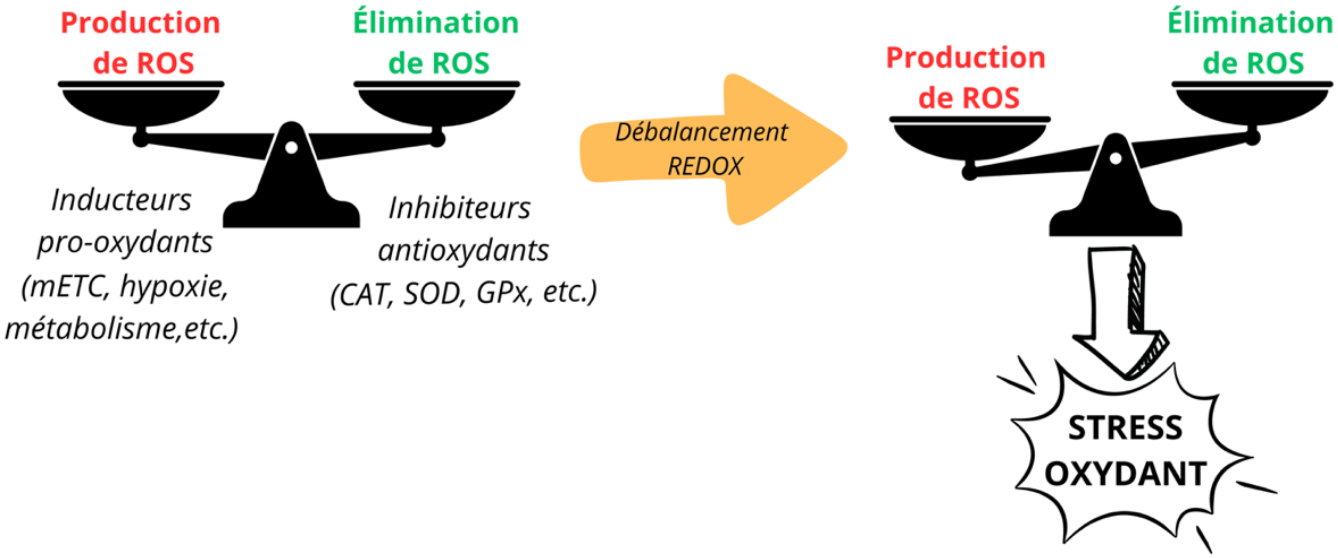


Figure 5. Sources et inhibiteurs de ROS dans le contrôle de l'homéostasie rédox dans les cellules. Modifiée de Panieri & Santoro 2016.

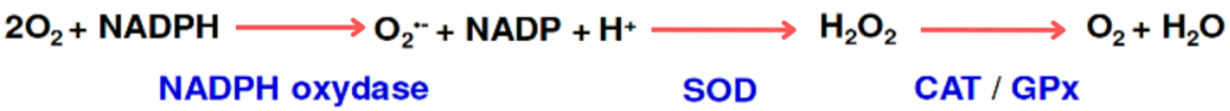


Figure 6. Réaction enzymatique du stress oxydant et des enzymes antioxydantes. Modifiée de Acosta (2010).

Harman (1956) a estimé que les mitochondries sont capables de convertir entre 1 et 3% de leur consommation totale d'oxygène en des dérivés d'oxygène toxiques hautement réactif. Ce stress oxydatif est donc souvent lié à des taux métaboliques élevés reflétant une augmentation du renouvellement mitochondrial de l'oxygène (**Fig. 4e et 8**). En condition de stress, la production de ROS s'accroît et ils peuvent, par oxydation en chaîne, altérer tous les substrats disponibles comme les constituants lipidiques, protéiques, l'ADN ou encore les polysaccharides des cellules par la création de radicaux. Les ROS modifient le potentiel redox cellulaire pour abroger la réponse hypoxique (Haddad, 2004). Ils peuvent interagir directement avec les molécules et changer leur conformation. Ils modifient ainsi les mécanismes de transduction du signal, comme la protéine kinase C (Dalton et al., 1999). Les variations du niveau de ces radicaux ont donc des effets importants sur les fonctions

cellulaires. En conséquence, les dommages oxydatifs favorisent la mort cellulaire (**Fig. 4f**) et peuvent limiter la survie d'un animal dans des conditions stressantes.

Afin de se protéger des effets toxiques des ROS, les organismes aérobies ont développé un ensemble complexe de systèmes défensifs. Ces systèmes comprennent des enzymes antioxydantes endogènes qui limitent l'accumulation de ROS. Les plus connues sont (i) les superoxydes dismutases (SOD), qui convertissent le superoxyde (O_2^-) en H_2O_2 moins réactif (ii) la catalase (CAT) qui réduit le H_2O_2 en eau et en oxygène, et (iii) les glutathion peroxydases (GPx), qui éliminent le H_2O_2 en utilisant le pouvoir réducteur dérivé du glutathion (**Fig. 4d et 7**). Ainsi, pour prévenir un stress oxydatif excessif et favoriser la signalisation redox, les cellules ajustent stratégiquement plusieurs enzymes antioxydantes et utilisent largement leurs voies métaboliques pour fournir un apport adéquat de molécules antioxydantes (telles que le glutathion réduit (GSH) et le NADPH). Les glutathion S-transférases (GST) sont un groupe d'enzymes de détoxification multifonctionnelles qui neutralisent les effets toxiques des xénobiotiques en conjuguant du GSH à ces composés, produisant ainsi des substrats moins toxiques chez les invertébrés et les vertébrés (E. Büyükgüzel et al., 2010; Grant & Matsumura, 1989; Vontas et al., 2001) (**Fig. 4d et 8**). Elles jouent également un rôle important dans le système de détoxification des ROS formés par oxydation microsomale (Yu, 1999).

L'action la plus étudiée des ROS est la peroxydation lipidique, c'est-à-dire l'oxydation des membranes de phospholipides. Cette oxydation provoque des perturbations dans la structure de ces membranes, ce qui a pour conséquences : 1) une diminution de la fluidité des membranes et l'inactivation des récepteurs et enzymes situés à leur niveau (Snell & Mullock, 1987); 2) l'augmentation de la perméabilité des membranes, en particulier avec les ions calcium, ce qui conduit à la mort cellulaire (Gutteridge & Halliwell, 1990) et au niveau des mitochondries comme les lysosomes se traduit par 3) la lyse de ces organites et la libération d'enzymes. Ces enzymes catalysent ensuite les protéines, les acides nucléiques et les polysaccharides cellulaires (Horton et al., 1987; Pré, 1991; Snell & Mullock, 1987). Les sous-produits de peroxydation, c'est-à-dire les sous-produits des acides gras polyinsaturés

(PUFAs), présentent une double activité, pro- ou antioxydante, en fonction des conditions (cytotoxique/cytoprotecteur, pro-/anti-inflammatoire, pro-/anti-apoptotique) (Yadav et al., 2018).

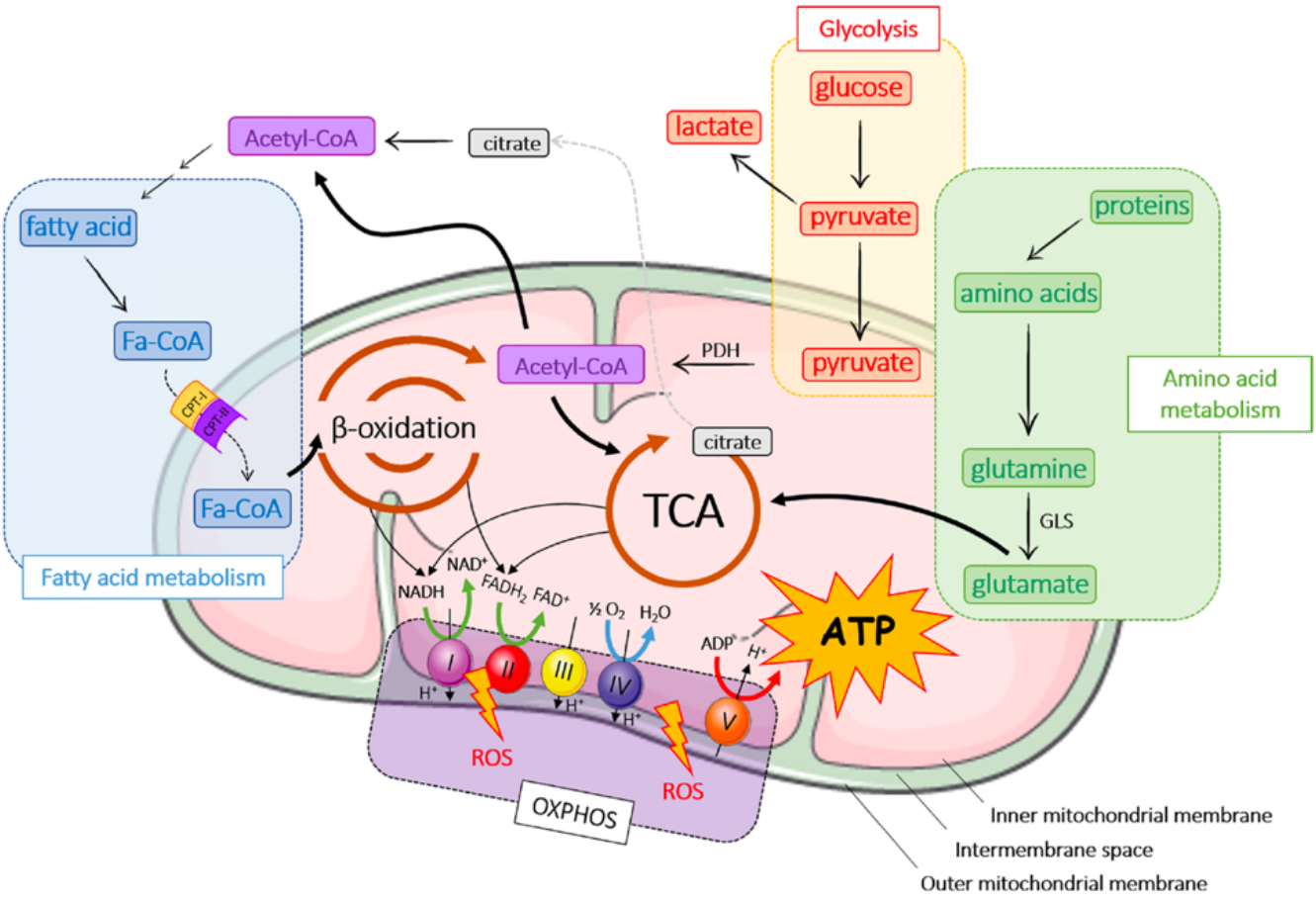


Figure 7. Métabolisme mitochondrial de Loussouarn et al. (2021). ADP, adénosine diphosphate; ATP, adénosine triphosphate; CPT, carnitine palmitoyltransférase; FADH2, flavine adénine dinucléotide; GLS, glutaminase; OXPHOS, phosphorylation oxydative; NADH, nicotinamide adénine dinucléotide hydrogénase; PDH, pyruvate déshydrogénase; ROS, espèces réactives de l'oxygène; TCA, cycle de l'acide tricarboxylique.

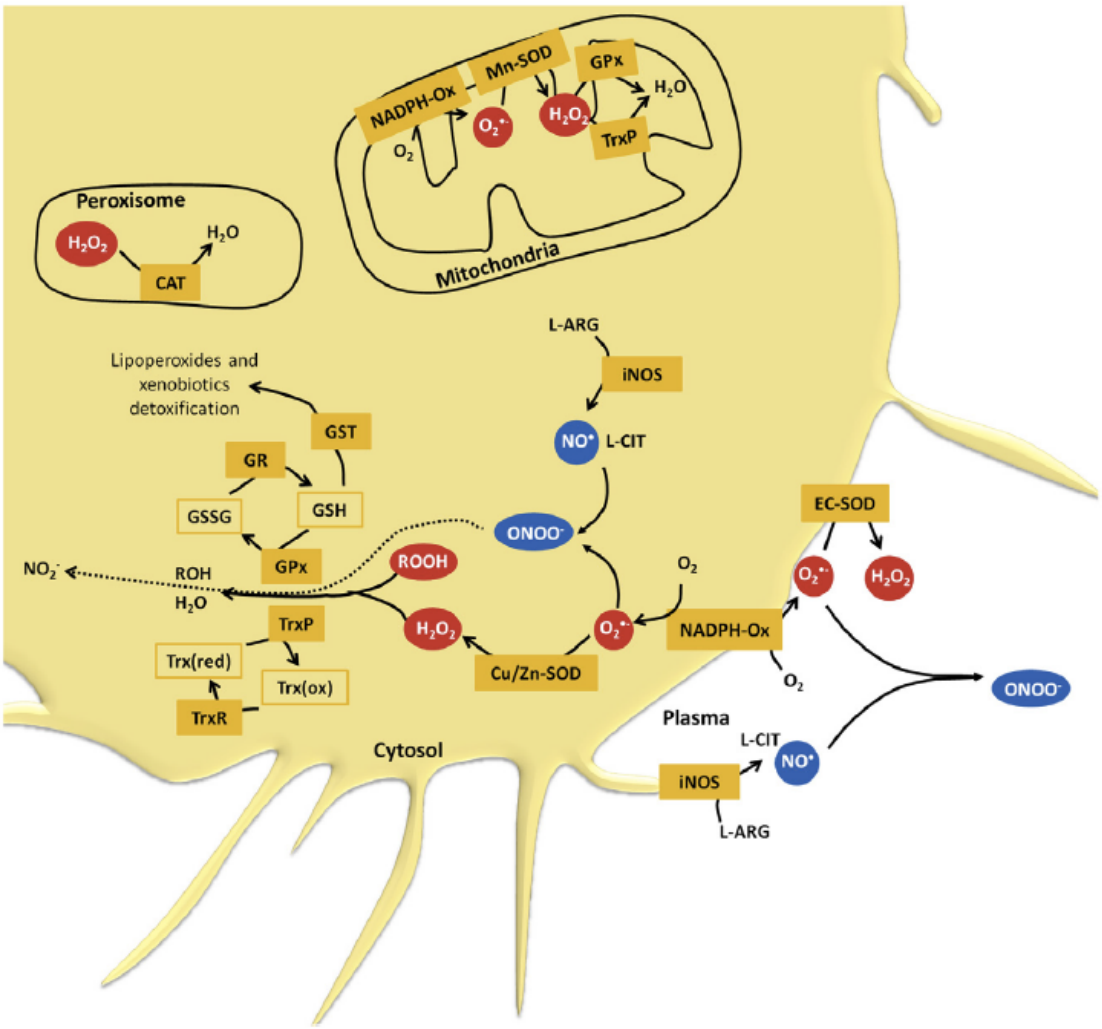


Figure 8. Voies antioxydantes enzymatiques impliquées dans la production et la désintoxication des espèces réactives de l'oxygène (ROS) (en rouge) et des espèces réactives de l'azote (en bleu) dans les hémocytes des bivalves, dans le compartiment intracellulaire (mitochondries, peroxysome, cytosol) et dans le compartiment extracellulaire (plasma) (Richard et al., 2016). GR : glutathion réductase ; GST : glutathion-S-transférase ; GSSG, GSH : glutathion oxydé / réduit, glutathion ; TrxP, TrxR : thiorédoxine peroxydase / réductase ; iNOS : oxyde nitrique synthase inductible ; ONOO- : peroxynitrite ; NO2- : nitrite ; L-ARG : L-Arginine ; L-CIT : L-Citrulline (Richard et al., 2016).

2.3 Modulation de la réponse au stress oxydatif

La réponse au stress est modulée par les eicosanoïdes (Basu, 2010; Furuyashiki & Narumiya, 2011; Stanley-Samuelson, 1994; Yadav et al., 2018). Les eicosanoïdes sont produits par oxydation enzymatique de l'acide arachidonique (ARA) ainsi que de deux autres

acides gras polyinsaturés (PUFAs) dérivés des phospholipides, l'acide eicosapentaénoïque (EPA) et l'acide docosahexaénoïque (DHA) (Stables & Gilroy, 2011; Stanley, 2006). Les eicosanoïdes sont principalement produits par deux enzymes : la cyclooxygénase (COX), qui donne les prostaglandines (PGs) et les thromboxanes (TXs), puis les produits de la lipoxygénase (LOX), qui sont les leucotriènes (LTs), les lipoxines (LXs) et les acides hydroxyeicosatétraénoïques (HETEs) (**Fig. 9**). Pratiquement tous les animaux biosynthétisent une large gamme d'eicosanoïdes, qui servent à un grand nombre d'actions moléculaires, physiologiques et écologiques (Büyükgüzel et al., 2002; Stanley, 2006). Les eicosanoïdes médient les réactions immunitaires cellulaires telles que la propagation et la migration des hémocytes ainsi que les mécanismes de phagocytose (nodulation, encapsulation, etc.) contre des agents pathogènes (Büyükgüzel et al., 2007; Dean et al., 2002; Durmuş et al., 2008; Figueiredo et al., 2008; Stanley & Shapiro, 2007). Ils modulent l'inflammation et la réponse immunitaire, qui détermine la réponse physiologique et la survie de l'organisme lorsqu'il est exposé à un stress prolongé. Chez les mammifères, les eicosanoïdes dérivées des acides gras oméga-6 (ω-6) sont plus pro-inflammatoires, vasoconstrictrices et prolifératives que celles dérivées des acides gras oméga-3 (ω-3) qui ont moins d'activité ou sont anti-inflammatoires, pro-résolvantes, vasodilatatrices et antiprolifératives (Ferreira et al., 2023; Serhan et al., 2015). Les PGs sont bien connus comme médiateurs de plusieurs réponses biologiques chez les organismes vertébrés et invertébrés (Stanley-Samuelson, 1987). Il est notamment connu que les PGs modulent la réponse au stress thermique en induisant l'apoptose et la synthèse d'HSP ainsi que d'enzymes antioxydantes (Balogh et al., 2013; Büyükgüzel et al., 2010; Duran-Encinas et al., 2024; Oksala et al., 2003; Santoro et al., 1989; Zhang et al., 2015). Les organismes soumis à un stress peuvent diminuer la synthèse des PGs en réduisant l'activité ou la synthèse enzymatique, mais la synthèse non-enzymatique n'est pas aussi facilement régulée et dépend de divers facteurs, notamment de la disponibilité du substrat (l'ARA dans les membranes cellulaires) et des ROS (Muri et al., 2020).

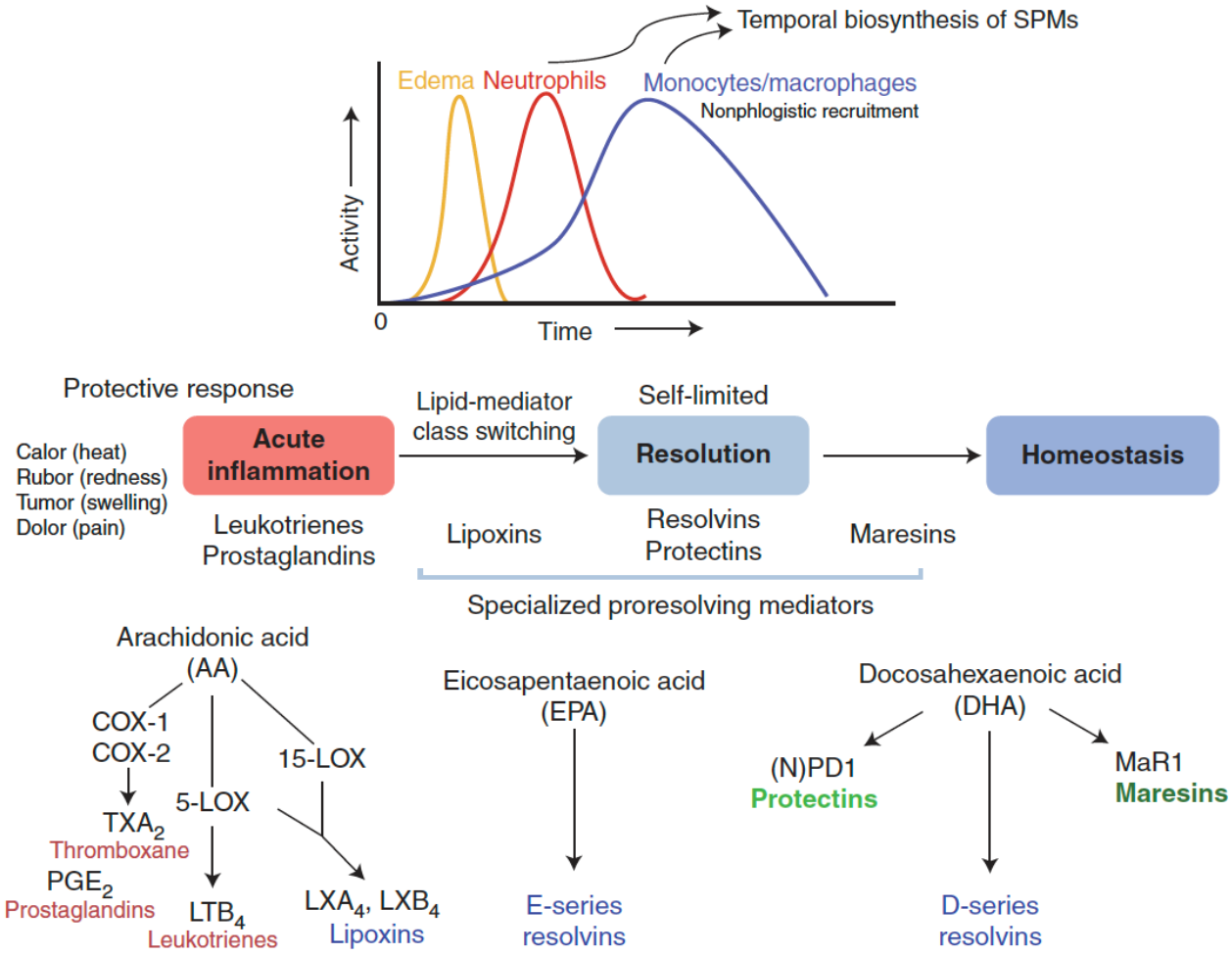


Figure 9. Biosynthèse des médiateurs lipidiques dans la résolution d'une inflammation aiguë (Serrhan et al., 2015)

2.4 Effets des ROS chez les mollusques

L'activité antioxydante des espèces marines varie selon les saisons en réponse aux fluctuations naturelles de température, de salinité, du taux d'oxygène et de la qualité de la nourriture disponible (Orbea et al., 2002; Viarengo et al., 1991). Les activités antioxydantes sont modulées par divers facteurs, tels que la respiration anaérobie qui diminue les activités enzymatiques et de la peroxydation lipidique, qui reviennent à un niveau normal lorsque l'oxygénation est rétablie (Viarengo et al., 1989).

Une étude de Samain et al. (2007) démontre que les individus de l'huître du Pacifique *Crassostrea gigas*, plus résistants à la mortalité estivale (combinaison de stress reproductif, pathologique et environnemental, associé à l'augmentation de la température de l'eau), synthétisaient de plus grandes quantités de protéines de choc thermique (HSP : *Heat Shock Protein*) (Lang et al., 2009). L'augmentation de cette synthèse d'HSP est nécessaire chez les huîtres exposées à des conditions intertidales, en raison de l'exposition à la température, à la dessiccation et à l'augmentation du métabolisme anaérobie (Zhang et al., 2012).

De leur côté, (Solé et al., 1994) montrent que la ponte de la moule *Mytilus galloprovincialis* stimule les activités antioxydantes en mars-avril, suivie d'une diminution progressive au printemps alors que la disponibilité en nourriture et la température augmentent. Enfin, l'âge sensibilise aux effets oxydants par la réduction des capacités antioxydantes, ce qui entraîne une augmentation des taux de peroxydation lipidique (Viarengo et al., 1991).

3. LE STRESS ENVIRONNEMENTAL, IMPACTS DES POLLUTIONS ANTHROPIQUES DANS LES MILIEUX CÔTIERS : CAS DU TRANSPORT MARITIME SUR LES BIVALVES FILTREURS.

Les organismes marins sont exposés non seulement aux facteurs de stress aigus ou chroniques d'origine environnementale (température, salinité, vitesse du vent, concentration de chlorophylle, prédation, etc.) et naturelle (par exemple, le phénomène El Niño), mais aussi aux effets cumulatifs des facteurs de stress anthropiques, notamment dans les milieux côtiers (Przeslawski et al., 2014). Au cours des dernières années, les pressions anthropiques sont devenues de plus en plus fortes dans les écosystèmes côtiers marins. Le trafic maritime, qui représente 80 % du commerce mondial de marchandises (Canton, 2021 ; CNUCED, 2016), est notamment l'une des plus grandes industries au monde. Afin de répondre à la demande croissante, de nouvelles routes commerciales et infrastructures de transport sont créées, facilitant le transport, reliant les lieux et concentrant les mouvements des navires (Laurance et al., 2009) vers des destinations portuaires communes. La forte concentration de navires qui en résulte exerce diverses pressions sur le milieu marin local. Les navires laissent après

leur passage des résidus chimiques de manière accidentelle (déversement) ou continue (fuite). Ces résidus contiennent généralement des hydrocarbures polyaromatiques (HAP), provenant de la combustion incomplète de la matière organique liée à l'activité anthropique dans les environnements portuaires (Baumard et al., 1998). En outre, la coque des navires est recouverte de produits antifouling, contenant de nombreux produits chimiques (par exemple le plomb et le cuivre) (Turner 2010). Aujourd'hui, certains de ces produits tels que le plomb sont interdits, mais certains persistent sur le marché. Ces métaux sont problématiques puisqu'ils ont tous un potentiel effet toxique. Cette pollution de l'eau nuit aux organismes et aux écosystèmes marins. Les invertébrés marins, tels que les bivalves, sont particulièrement exposés aux métaux lourds stockés dans les sédiments. Ceux-ci peuvent être facilement remobilisés dans l'eau par les courants, le dragage ou encore par bioturbation (Amiard et al., 1995; Galkus et al., 2012; Jupp et al., 2017; Kennish, 2002). Grâce à leur importante capacité de filtration (Baumard et al., 1998; Farrington et al., 2002), les bivalves peuvent les absorber et les accumuler (Fokina et al., 2013). Ils sont souvent utilisés comme espèces sentinelles pour le suivi écotoxicologique des écosystèmes marins (Beyer et al., 2017; His et al., 1999). Ce sont des organismes importants dans l'écosystème intertidal, présentant un fort intérêt socio-économique (aquaculture, pêcherie, écotourisme).

3.1 Impacts des contaminations chimiques associées aux transport maritime

Le trafic maritime est une source majeure de pollution chimique du milieu marin, notamment par l'introduction d'hydrocarbures polyaromatiques (HAP) issus des carburants et de métaux lourds associés aux anodes. La contamination par les métaux est une empreinte anthropique typique des zones côtières (Jupp et al. 2017, Hatje et al. 2018).

Le cuivre est un métal essentiel qui assure plusieurs activités enzymatiques essentielles, comme la cytochrome c oxydase, qui correspond à la dernière enzyme de la chaîne de transport des électrons (Deidda et al., 2021; Festa et al. 2011). Néanmoins, au-delà d'une certaine concentration, des effets délétères peuvent être observés, associés à la diminution de l'activité enzymatique au niveau des neurotransmetteurs, telle que l'acétylcholinestérase

(AchE) (Baatrup, 1991; Deidda et al., 2021). Hoare et al. (1995) ont ainsi démontré que l'ajout de cuivre au cours du stade embryonnaire de *Mytilus edulis* augmentait le taux de croissance des larves véligères et diminuait la survie des naissains due à des anomalies embryonnaires, démontrant ainsi la toxicité du cuivre à certaines concentrations. Le plomb (Pb) est également un neurotoxique, dont les effets ont été démontrés dans plusieurs études (Amiard et al., 1995; Marasinghe Wadige et al., 2014; Rault et al., 2013). L'exposition au plomb influence les réponses immunitaires, provoquant un stress oxydatif qui induit des dommages synaptiques et un dysfonctionnement des neurotransmetteurs, entraînant une neurotoxicité. Le mercure (Hg), notamment le méthylmercure (CH_3Hg^+, forme organique assimilable), est également très toxique et provoque des effets neurotoxiques démontrés sur plusieurs espèces, dont les bivalves (Baatrup et al. 1991; Deidda et al., 2021).

Les hydrocarbures (HAP) sont des composés xénobiotiques qui influencent des processus physiologiques essentiels et peuvent modifier la biologie de la reproduction d'une espèce, soit en retardant ou en inhibant la maturation gonadique (Frouin et al., 2007; Tapia-Morales et al., 2019). Par exemple, ils diminuent la viabilité des cellules reproductrices et, par conséquent, la qualité de la fécondation et le développement larvaire de *C. virginica* (Vignier et al., 2017). Plusieurs auteurs mentionnent qu'une exposition aux hydrocarbures provoque une immunosuppression chez les mollusques bivalves, avec certains gènes du système immunitaire régulés à la baisse (Fernley et al., 2000; Hannam et al., 2010; Tapia-Morales et al., 2019). La catalase (CAT) et le glutathion S-transférase (GST) sont des gènes liés à la réponse aux polluants, car ils sont induits ou inhibés par certains polluants du milieu marin (Bebianno et al., 2004; Jenny et al., 2016; Tapia-Morales et al., 2019; Vidal & Narbonne, 2000). La CAT est un gène de réponse au stress oxydatif (Blanchette et al., 2007). Le GST participe à la conjugaison du glutathion avec des composés xénobiotiques et à la défense contre les dommages oxydatifs de l'ADN et des lipides (Blanchette et al., 2007).

En outre, l'augmentation des températures peut influencer la distribution et le devenir des métaux dans les sédiments et l'eau de mer, ainsi que leur bioaccumulation dans les organismes marins. Par exemple, la température affecte la bioaccumulation des métaux en

améliorant la biodisponibilité (I. M. Sokolova & Lannig, 2008a) ou en augmentant ou en diminuant l'absorption animale par une modification de la ventilation et de l'activité alimentaire qui entraîne une augmentation de la demande d'énergie (Coppola et al., 2018; Nardi et al., 2018; Negri et al., 2013). Plusieurs travaux sur des bivalves marins dans le milieu naturel ont montré qu'il existe des corrélations entre la teneur en HAP et différentes activités enzymatiques telles que les enzymes liées au cytochrome P450, la NADPH-P450 réductase, la SOD, la CAT et le lysozyme (Fisher et al., 2003; Porte et al., 1991; Solé et al., 1994).

Les modifications physiologiques et morphologiques en réponse au stress chimique sont peu étudiées chez les stades larvaires d'invertébrés marins comparativement aux adultes, bien que quelques études existent (Geffard et al., 2003; Labarta et al., 2005; Pelletier et al., 2000; Toro et al., 2003; Vignier et al., 2015, 2016, 2017). Une étude récente, qui a consisté à exposer des géniteurs à du pétrole brut et à les dépurer rapidement, a démontré la bonne résilience des reproducteurs, mais également des effets transgénérationnels négatifs sur leurs larves. Les impacts observés sont liés à l'affaiblissement des réserves énergétiques des œufs ou au transfert maternel de contaminants (Schmutz et al., 2021). Les HAP s'accumulent dans les œufs, en raison de leur forte teneur en lipides, ce qui favorise leur transfert à la progéniture (Hansen et al., 2017; Knecht et al., 2017). De plus, leur potentiel de génotoxicité sur des embryons de bivalves a été démontré par une rupture des brins d'ADN, même à de faibles concentrations (Wessel et al., 2007).

3.2 Une source émergente de stress en milieu marin : l'anthropophonie

L'industrialisation a non seulement entraîné une pollution par les métaux, mais a également introduit une forme de pollution moins visible, mais tout aussi importante, à savoir la pollution sonore (Peng et al., 2015). Au cours des 50 dernières années, l'activité du trafic maritime n'a fait qu'augmenter et les pollutions chimiques qui lui sont associées deviennent omniprésentes (McDonald et al., 2006; Solan et al., 2016). L'augmentation est tellement rapide, que le bruit lié au trafic maritime double tous les 11,5 ans (Jalkanen et al., 2022). Aujourd'hui, les impacts potentiels du son, considéré actuellement comme un polluant

émergent, sur les organismes marins, est une préoccupation majeure. Selon l'Organisation mondiale de la santé, il s'agit de l'une des pollutions les plus répandues dans les environnements terrestres et aquatiques (World Health Organization, 2011).

Les paysages acoustiques sous-marins sont composés de sons d'origines différentes, incluant ceux de sources biologiques (biophonie) et non biologiques (géophonie et anthropophonie) (**Fig. 10**). La géophonie regroupe les sons produits par l'action physique des vagues, de la pluie sur la surface de l'eau, du vent, de l'activité volcanique, des tremblements de terre, etc. La biophonie correspond à tous les signaux produits naturellement, tels que les vocalisations des mammifères marins, poissons et crustacés (**Fig. 10A**). Ces signaux leur permettent de trouver un partenaire, de percevoir des prédateurs, de localiser des proies, de trouver des habitats appropriés ou encore pour les larves, de s'orienter et de s'installer, etc. (Duarte et al., 2021). Ce sont des signaux acoustiques essentiels pour de nombreuses activités et la survie des invertébrés benthiques. L'anthropophonie désigne l'ensemble des sons produits par les activités humaines tels que le trafic maritime, les sonars, les bruits d'aménagements portuaires ou d'installations d'éoliennes en mer (forage, battage de pieux, …), etc. (**Fig. 10C**).

Ces sons anthropiques sont caractérisés par différentes métriques, telles que l'intensité, la fréquence, la durée, la directivité et la temporalité (Weilgart, 2007). Le plus souvent, ces sons sont produits à des intensités élevées, couvrant une large gamme de fréquences (de 1 Hz à 200 kHz) et, en raison de l'excellente propagation du son sous l'eau, ils peuvent parcourir de très grandes distances depuis la source (Rogers & Cox, 1988) (**Fig. 10**). Les bruits anthropiques sont associés à plus de 90 % aux navires (Chauvaud et al., 2018; Duarte et al., 2021; McDonald et al., 2014; McDonald et al., 2006). Leur signature acoustique est principalement caractérisée par leur motorisation/méthode de propulsion (moteur diesel, pompe, 2 temps ou 4 temps, in-board, hors-bord, etc.), leur vitesse, leur fréquence et leur type, associé à leur taille et leur usage (commercial ou plaisance) (Erbe, 2002; Hildebrand, 2009; Richardson et al., 2013). Plus nombreux, ces navires sont aussi plus grands (Hildebrand, 2009; Stanley & Jeffs, 2016), ceux d'une longueur de plus de 100m émettent

des sons de haute intensité allant de 160 à 220 dB re 1 µPa 1 m à basse fréquence (de 5–500 Hz et parfois jusqu'à 10 kHz parfois), pendant de longues périodes (**Fig. 10**). Ces sons peuvent être entendus jusqu'à des centaines de kilomètres aux alentours (Arveson & Vendittis, 2000; Au & Hastings, 2008; Richardson et al., 2013; Rogers & Cox, 1988). Par conséquent, le son émis par les gros navires domine souvent le bruit sous-marin ambiant à basse fréquence dans de nombreux environnements marins (Greene & Moore, 1995; McDonald et al., 2006). De plus, les basses fréquences se propagent plus loin que les hautes fréquences (Hildebrand, 2009).

Les impacts potentiels de la pollution sonore sur la vie marine suscitent de grandes inquiétudes et il s'agit d'un domaine de recherche émergent en écologie marine (Chauvaud et al., 2018; National Research Council, 2003 ; Popper & Hawkins, 2016, 2019; Samson et al., 2016; Simpson et al., 2008; Weilgart, 2007). Malgré leur rôle central dans la plupart des réseaux trophiques marins et les services écosystémiques essentiels qu'ils fournissent (Smaal et al., 2019), les invertébrés marins (en particulier les mollusques) ont rarement été abordés dans les études sur les effets du bruit sous-marin et ils sont donc exclus des mesures réglementaires concernant les nuisances sonores (de Soto, 2016).

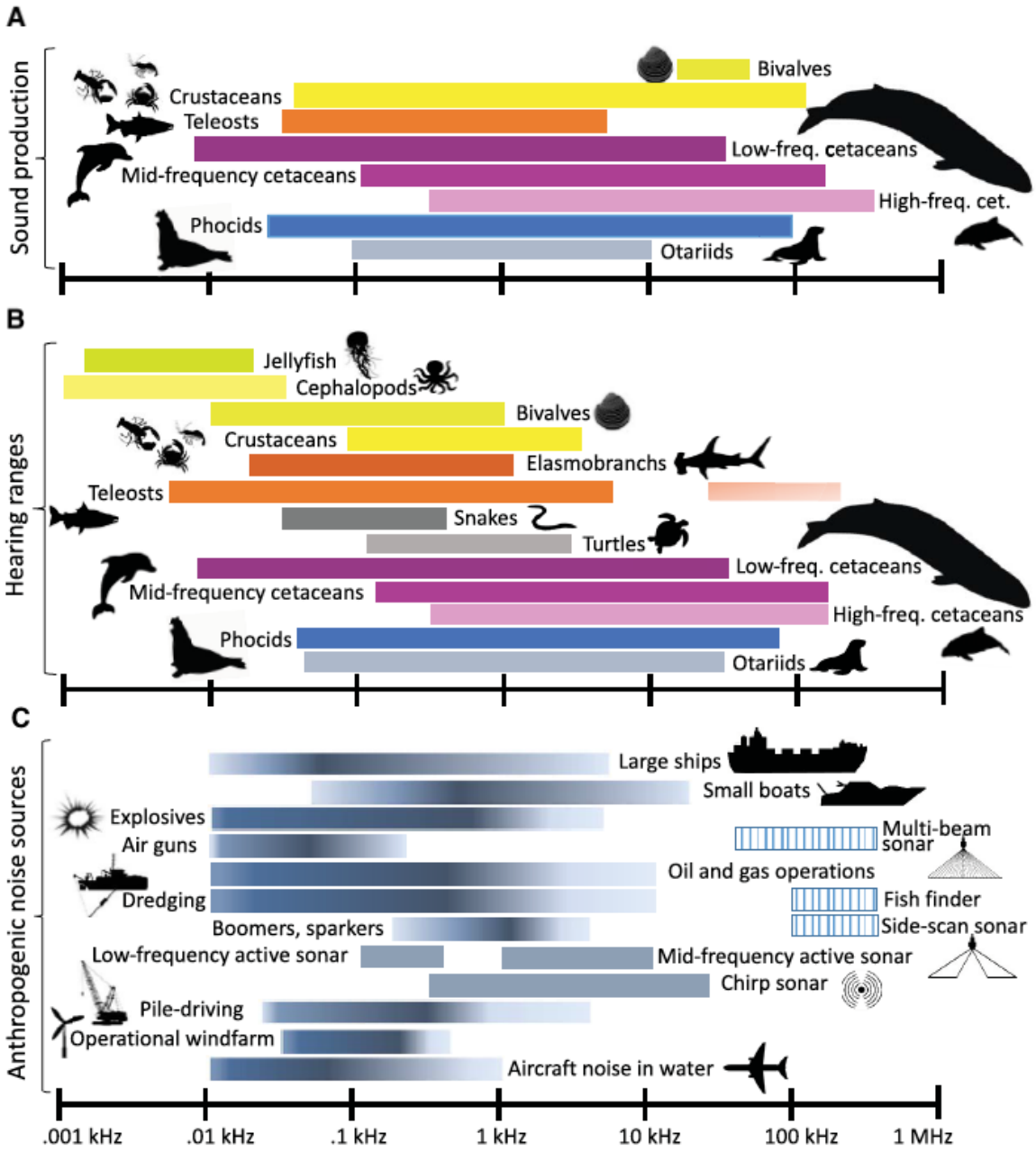


Figure 10. (**A**) Production sonore approximative, (**B**) gammes d'audition chez différents taxons marins et (**C**) Plage de fréquences dominante de la source sonore. Le dégradé de couleur correspond approximativement à la bande d'énergie dominante de chaque source. Les lignes pointillées représentent les sonars pour illustrer des sons à multifréquence (Duarte et al., 2021).

3.3 Impacts de l'anthropophonie chez les invertébrés marins

L'effet du bruit des navires peut augmenter ou diminuer certains paramètres chez différentes espèces d'invertébrés benthiques. Les études menées sur l'impact du son émis par les bateaux suggèrent que ces sons influencent significativement l'écologie de ces espèces. Chez les espèces à cycle bentho-pélagique, des effets positifs ont été démontrés sur la métamorphose (McDonald et al., 2014) ainsi que sur la fixation des larves (Jolivet et al., 2016; McDonald et al., 2014; Wilkens et al., 2012) (**Tab. 1**). Le son émis par les bateaux stimule ainsi les larves à se fixer plus rapidement (Wilkens et al., 2012), accélérant la métamorphose et améliorant la survie et la croissance larvaire (McDonald et al., 2014). L'augmentation de la fixation de larves exposées à des bruits de bateau a aussi été récemment démontrée par Jolivet et al. (2016) et Cervello et al. (2023). Le recrutement, phase cruciale dans le succès de l'intégration des larves au compartiment benthique (Broitman et al., 2008; Navarrete et al., 2005), implique plusieurs processus complexes agissant sur (1) le stade larvaire et (2) sa dispersion, (3) la fixation et (4) la vie benthique post-fixation (Pineda et al., 2009). À l'issue de cette période critique, le recrutement conditionne les variations d'abondance des populations d'espèces benthiques (Caley et al., 1996; Coe, 1956; Hughes, 1990). Le bruit des navires peut augmenter le recrutement des espèces d'invertébrés benthiques (Wilkens et al., 2012). De plus, Jolivet et al. (2016) démontrent que le bruit des navires couplé à un signal trophique de pic de picoplancton (*Nannochloropsis oculata*) agit en synergie et augmente le taux d'établissement de la moule bleue, par rapport à ces deux facteurs étudiés séparément. Ces résultats suggèrent que l'augmentation des bruits du trafic maritime devrait moduler le recrutement des moules.

Certaines études suggèrent que la fréquence des sons et leur nature impulsionnelle ou continue participeraient à l'identification des zones favorables à la colonisation de plusieurs espèces marines (Stanley et al., 2012, 2012). Dans un contexte où l'augmentation de l'anthropophonie modifie le paysage acoustique naturel, la réponse des organismes aux stimuli de leur environnement pourrait être impactée (Simpson et al., 2008, 2011). Les capacités auditives de la moule bleue ne sont pas connues, et encore moins celles des larves.

Néanmoins, la principale caractéristique sonore importante pour les invertébrés reste le mouvement des particules plutôt que le niveau de pression (Nedelec et al., 2016; Rogers et al., 2021; Solé et al., 2023). Des études sur la détection des sons par les invertébrés révèlent que les adultes de la moule bleue sont sensibles aux basses fréquences entre 5 et 400Hz avec une fréquence dominante d'environ 210 Hz (Chauvaud et al., 2018; Roberts et al., 2015). De plus, les moules possèdent des poils ou cils sensoriels, des statocystes ainsi qu'un organe sensoriel abdominal (ASO) (**Fig. 11**) capable de percevoir les changements de pressions liés aux ondes sonores (Chauvaud et al., 2018). Chez les larves véligères, le velum constitué de cils est rétractable et constitué de deux lobes ciliés. Au stade pédivéligère se développe un pied, caractérisé par un épithélium cilié, qui permet aux larves d'explorer le substrat afin d'identifier une surface adéquate pour la fixation. Ces cils sensoriels pourraient être impliqués dans la perception de stimuli mécaniques dans leur environnement. Ces perturbations peuvent être liées au déplacement de leurs proies ou à celui de prédateurs causant des écoulements hydrodynamiques ou des vibrations (Chauvaud et al., 2018). Toutefois, la perception des vibrations sonores par les larves véligères n'a pas encore été démontrée. Néanmoins, les stimuli vibratoires transmis et propagés à travers le substrat affectent les bivalves tels que *Macoma balthica*, *Scrobicularia plana* et *Cardium edule*, augmentant leur temps de fermeture de coquille, mécanisme généralement utilisé pour se protéger contre les prédateurs (Mosher, 1972). Une étude récente sur l'huître américaine démontre également que le bruit anthropique stimule la fermeture des valves, réduisant donc leur temps d'alimentation (Ledoux et al., 2023). D'autres études ont démontré des effets négatifs des bruits anthropiques sur le développement des embryons (Nedelec et al., 2014), le recrutement (Lecchini et al., 2018), la réponse immunitaire (Filiciotto et al., 2016), le taux métabolique et jusqu'à des lésions voire une surmortalité des larves (Ruiz-Ruiz et al., 2020).

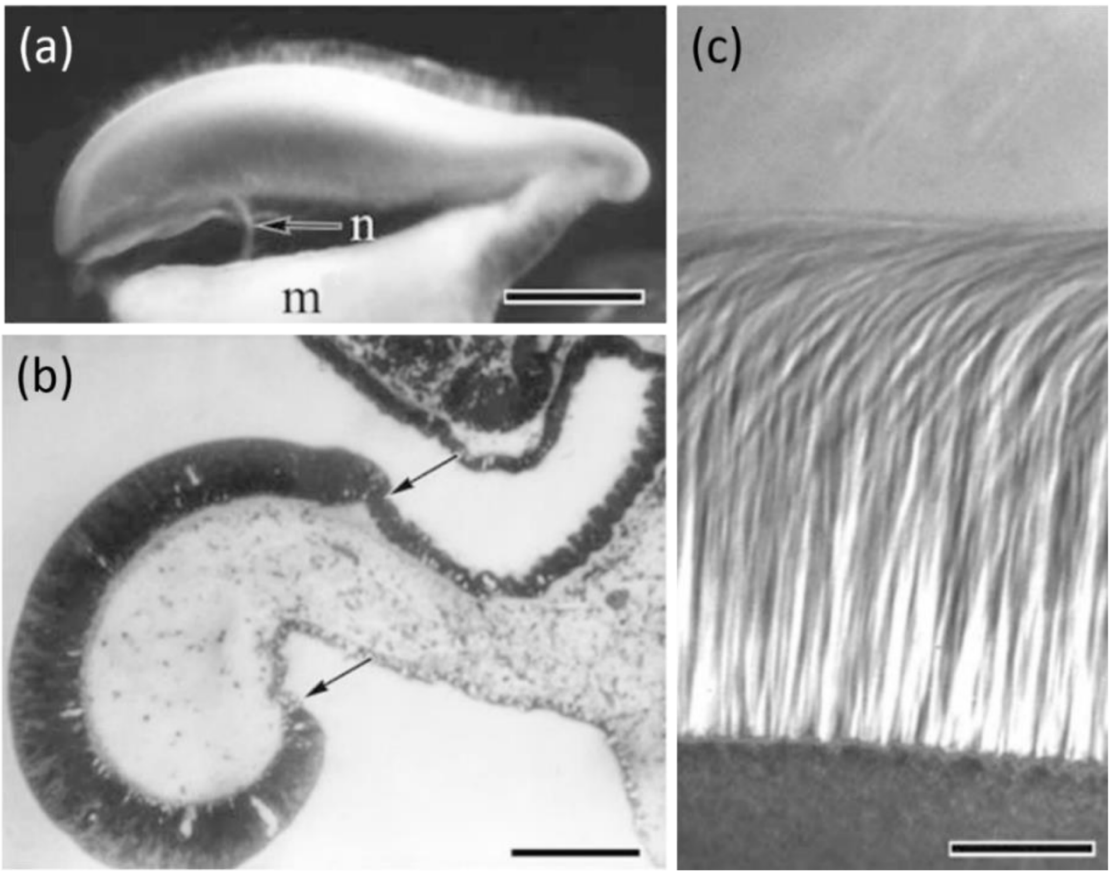


Figure 11. Organe sensoriel abdominal (ASO) de pétoncle japonais *Mizuchopecten jessoensis* observé en microscopie électronique à balayage (Solé, 2012). (**a**) ASO entier attaché à un morceau du manteau 'm'et au nerf sensitif 'n'; barre d'échelle = 1 mm. (**b**) Coupe transversale à travers l'ASO les flèches indiquent les limites de l'épithélium sensoriel ; barre d'échelle = 250µm. (**c**) Déformation des cils de l'ASO par un écoulement d'eau à une vitesse de 1.5 $m.s^{-1}$; barre d'échelle = 40 µm.

Table 1. Différentes catégories de réponse et leurs variables spécifiques associées, explorées dans différentes études de l'impact de l'anthropophonie chez les invertébrés marins (traduit de l'anglais, source Murchy et al., 2019).

Catégorie de réponse	Variables spécifiques de réponse
Anti-prédateur	Temps pour se cacher, temps pour se redresser, fréquence des changements de couleur, temps de nage, fréquence des bras levés, temps total de décision pour accepter ou rejeter la coque optimale
Taille du corps	Taille larvaire, longueur, largeur, masse sèche, hauteur de la coquille
État de santé	Diamètre du muscle adducteur, zone ovarienne, zone testiculaire, zone gonadique, stade gonadique
Développement	Pourcentage d'œufs qui n'ont pas réussi à se développer, pourcentage d'œufs non éclos
Énergie	Teneur en énergie, rapport oxygène / azote (O:N), teneur en ATP
Recherche alimentaire	Temps nécessaire pour trouver la source de nourriture, contenus stomacaux (proportion), consommation alimentaire moyenne mensuelle
Protéines de choc thermique (HSP)	Expression de la protéine de choc thermique 27, 60, 70 et 90, valeur de densité intégrée
Métaux lourds	Concentration de cadmium dans les branchies ou glande digestive
Quantité d'hémocytes	Hémocytes totaux, pourcentage d'hyalinocytes, pourcentage de semigranulocytes, pourcentage de granulocytes
Chimie de l'hémolymphe	Concentration de glucose, divers composants de l'hémolymphe (par exemple Cl, K, Na), pH de l'hémolymphe
Indice hépatopancréas	Indice hépatopancréas
Métamorphose	Pourcentage de réussite de la métamorphose
Phénoloxydase	Activité phénoloxydase, analyse densitométrique de la protéine phénoloxydase
Quantité de protéines	Nombre total de protéines et concentration, nombre de protéines d'hémolymphe, nombre de protéines cérébrales
Recrutement	Nombre de recrutement, proportion de larves installées, nombre de larves écloses
Taux de respiration	Absorption d'oxygène, fréquence respiratoire

4. ÉTUDE DES PREMIERS STADES DE VIE DES MOLLUSQUES

Il est généralement reconnu que les stades larvaires des invertébrés marins sont plus sensibles aux variations de température et de salinité que les stades juvéniles et adultes, comme démontré chez la moule bleue (Qiu et al., 2002; Rayssac et al., 2010), mais également chez d'autres taxons, tels les échinodermes (Przeslawski, 2005; Roller & Stickle, 1989, 1993). Il en résulte un retard de développement ainsi qu'une réduction de la croissance et du métabolisme probablement en raison du stress oxydatif, de la déstabilisation lysosomale et de l'augmentation de la peroxydation lipidique (Deschaseaux et al., 2011; Deschaseaux et al., 2010). De même, Przeslawski et al. (2008) et Byrne (2011) soulignent que les premiers stades de vie seraient plus vulnérables aux facteurs de stress associés au changement climatique et à l'acidification des océans, notamment en ce qui concerne les processus de calcification et la survie des individus (Kroeker et al., 2013). Selon ces auteurs, les premiers stades de vie des arthropodes et des cnidaires sont les phylums les plus résistants aux stress de température et de pH (et à la salinité, pour les arthropodes uniquement) (Arnberg et al., 2013; Egilsdottir et al., 2009; Pansch et al., 2012; Styf et al., 2013). Néanmoins, il est important de noter que les stades ultérieurs de la vie de certains arthropodes peuvent être vulnérables au stress environnemental. Par exemple, les larves de la balane subtropicale *Balanus trigonus,* qui se sont métamorphosées en cypris après une exposition aux eaux saumâtres chaudes, n'ont pas pu se fixer dans ces conditions (Thiyagarajan et al., 2003). À l'inverse, mollusques et échinodermes sont les plus vulnérables à l'ensemble des facteurs de stress (Byrne et al., 2011; Parker et al., 2010; Przeslawski et al., 2014). Néanmoins, certaines larves de gastéropodes de la famille des Littorinidae (*Bembicium nanum*) et des opisthobranches (*Dolabrifera brazieri*) possèdent des capacités de résistance surprenantes en réponse à des variations de pH (Davis et al., 2013), potentiellement en raison de leur adaptation à des environnements naturellement acides (tels que les sédiments acides) (Talmage & Gobler, 2011), ou en raison de la capacité tampon élevée (alcalinité totale très élevée) des eaux locales (Range et al., 2012).

4.1 L’ontogenèse de la moule bleue, *Mytilus edulis*

Mytilus edulis a un développement relativement rapide et peut se reproduire dès la première année (Duinker et al., 2008). Dans l'hémisphère Nord, la gamétogenèse débute à la fin de l'automne ou au début de l'hiver jusqu'à la maturité des gamètes à la fin de l'hiver ou au printemps. Elle aboutit ensuite à des pontes successives du printemps jusqu'au début de l'automne (Lowe et al., 1982). La qualité et la quantité de gamètes libérées par les moules peuvent varier en raison de multiples facteurs, notamment la disponibilité et la qualité de la nourriture (Seed & Suchanek, 1992). Son cycle de vie est bentho-planctonique (**Fig. 12**), incluant une phase pélagique puis benthique. Le passage du compartiment pélagique au compartiment benthique correspond à d'importants changements morphologiques et tissulaires chez les larves au cours de leur développement ainsi qu’à de fortes mortalités (**Fig. 13**) (Bishop et al., 2006; Cannuel et al., 2009; Veniot et al., 2003). Bien qu’il n’existe pas de mesure directe disponible en milieu naturel, il est suggéré que le taux de mortalité entre les larves pélagiques et les juvéniles benthiques excéderait 90 % (Morgan, 1995; Pedersen et al., 2008).

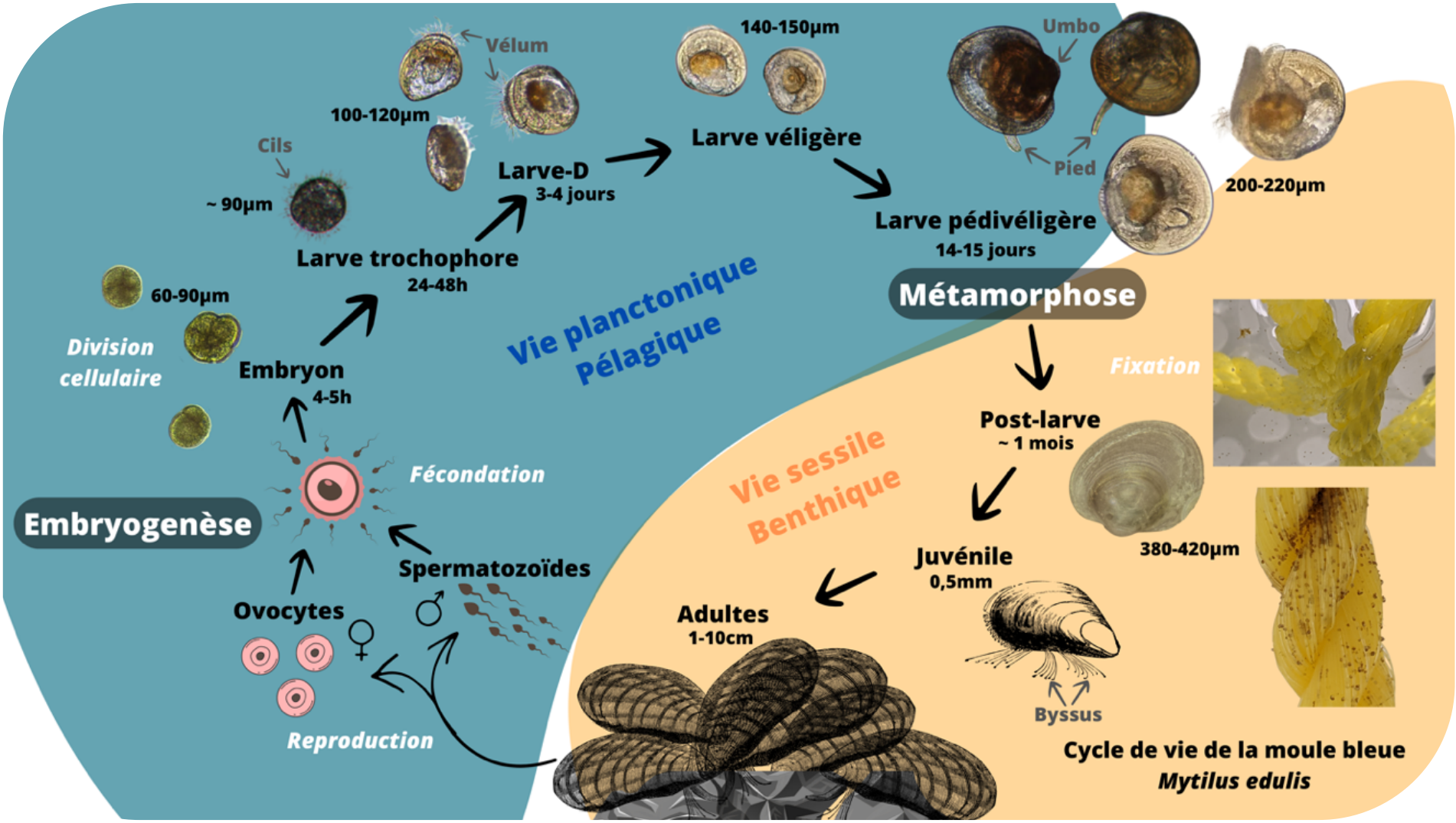


Figure 12. Cycle de développement de la moule bleue *Mytilus edulis* (inspiré de Toupoint et al., 2012).

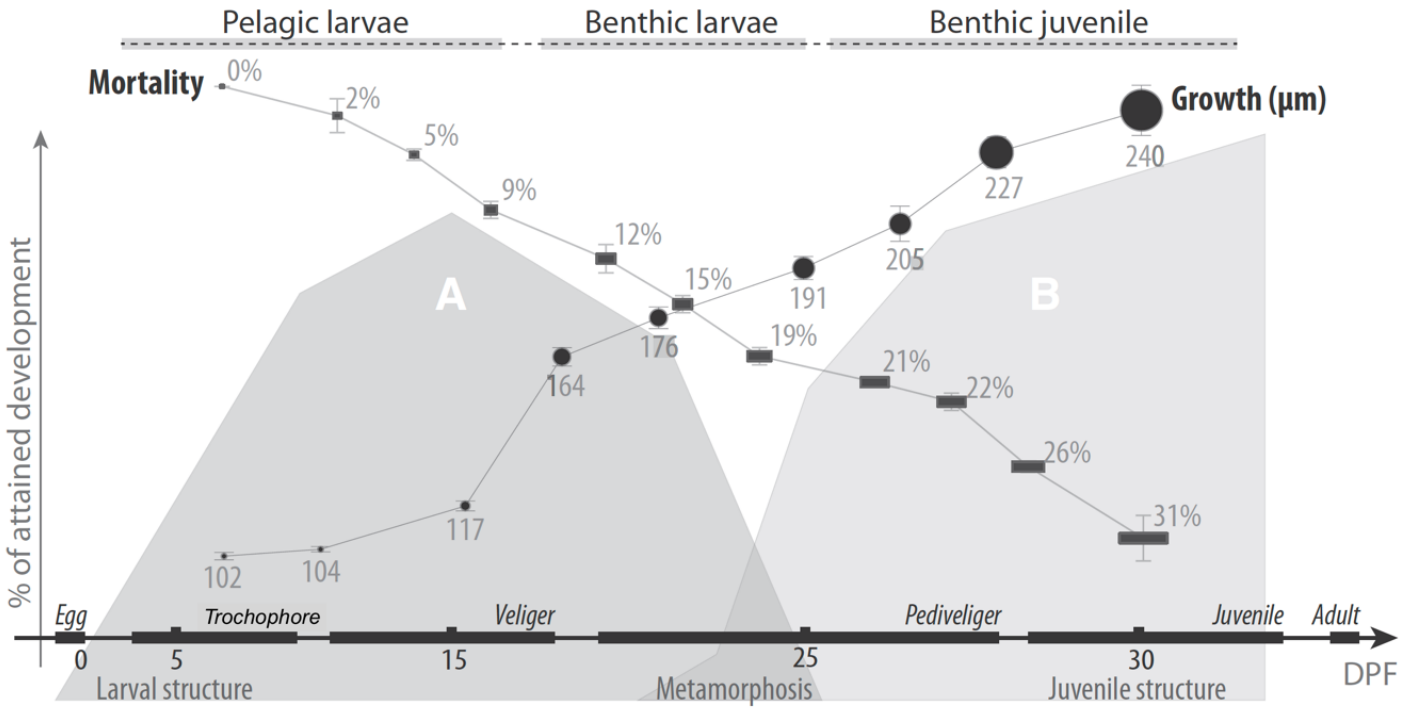


Figure 13. Cadres conceptuels et opérationnels du développement larvaire de *Mytilus edulis* par Bassim et al., (2014b). Les tracés de densité représentent les transitions majeures dans le remodelage des tissus et l'organogenèse, exprimées par les taux de pourcentage de développement larvaire (**A**) et post-larvaire (**B**). L'augmentation hypothétique du développement atteint (%) résume les pics singuliers (gris foncé ou gris clair) qui marquent la fin d'une phase de changement physiologique et le début d'une autre. Les pédivéligères post-métamorphiques (25 dpf) montrent la fin du développement des structures larvaires et le début de l'augmentation exponentielle de la structure juvénile/adulte.

4.2 Embryogenèse

Les moules ont un processus de fécondation externe qui dépend de la libération simultanée de spermatozoïdes et d'ovules dans la colonne d'eau (**Fig. 12**). Le sperme est libéré en continu tandis que les œufs sont relâchés par intermittence (Helm et al., 2004). Les œufs fécondés dans la colonne d'eau se divisent rapidement pour devenir une masse de cellules qui commencent à nager après environ 4-5 heures, suite à l'apparition de cils. C'est le stade d'embryon cilié (**Fig. 12 et 13a**). Au cours des 24 à 48 heures qui suivent, l'embryon se

transforme en une larve trochophore (longueur de la coquille : 60 à 80 µm). La larve secrète alors sa première coquille larvaire appelée la prodissoconque I, lors de sa transformation en larve D.

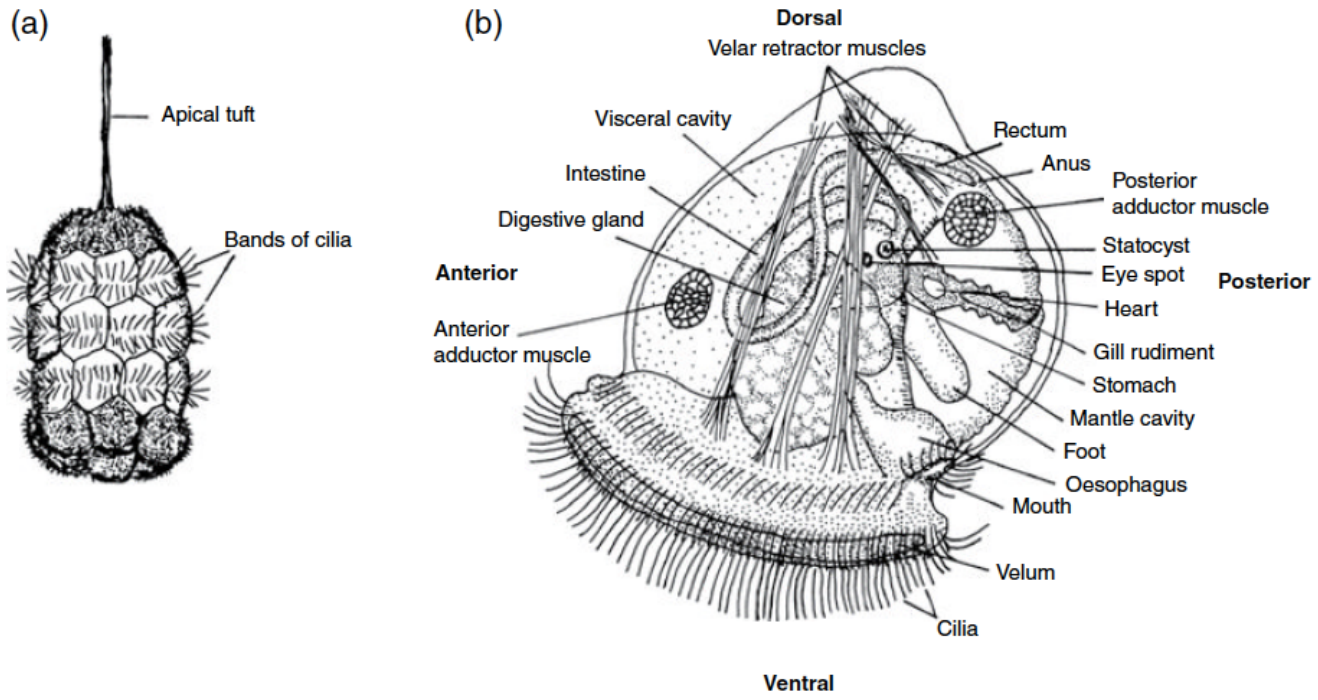


Figure 14. (**a**) Larve trochophore (× 400). (**b**) Larve véligère (prodissoconque II) de l'huître *Crassostrea virginica*, vue du côté gauche (× 380). Redessiné d'Eble et Scro (1996) puis Galstoff (1964) et Elston (1980).

4.3 Croissance véligère

Avec ces modifications structurelles, les larves évoluent en véligères dont la longueur varie entre 100 et 120 µm, taille à partir de laquelle débute la sécrétion d'une nouvelle coquille (prodissoconque II) (**Fig. 12 et 14b**). Cette coquille est sécrétée par le manteau et présente des lignes de croissance. Les larves véligères s'alimentent de petites particules (1–20 µm de diamètre) à l'aide des cils sur leur velum, qui leur permettent également de nager (**Fig. 14b**) (Strathmann et al., 1972; Bayne 2017). Chez *M. edulis*, ce stade dure de 2 à 3 semaines, selon la température et la disponibilité de la nourriture, jusqu'à ce que les larves

deviennent compétentes (longueur d'environ 250 µm) et aptes à se fixer grâce au développement d'un pied cilié extensible. Ce stade est appelé pédivéligère (**Fig. 15**).

Figure 15. Photo d'une larve pédivéligère de *Mytilus edulis* (Réjean Tremblay).

4.4 Métamorphose

En phase de compétence les larves ont la possibilité de se fixer sur un substrat et de se métamorphoser en post-larves puis en juvéniles (Seed & Suchanek, 1992). La métamorphose induit de nombreux changements morphologiques, avec une transition majeure dans le processus de captation des particules liée à la destruction du vélum et au développement des palpes labiaux et des filaments branchiaux (Bayne, 1971; Cannuel et al., 2009), ainsi que la sécrétion de la coquille dissoconche. Cette coquille se distingue par sa pigmentation, son orientation et sa composition minérale similaire à celles des coquilles des bivalves adultes (Bayne, 2017; Fuller & Lutz, 1988; Martel et al., 1995). La sécrétion de la dissoconche crée une ligne de démarcation avec la coquille prodissoconque II qui permet de déterminer la taille à la métamorphose et ainsi de servir de proxy du retard de métamorphose (Martel et al., 2014) (**Fig. 16**). En effet, la durée de la phase larvaire de la moule bleue *M. edulis* varie de deux à

quatre semaines et peut exceptionnellement atteindre plus de deux mois (Bayne, 1965 ; Lutz & Kennish, 1992), en fonction des conditions environnementales et des signaux chimiques/physiques et biologiques associés à la colonne d'eau ou au substrat. Les moules s'attachent par le byssus à une très grande variété de substrats dont ceux filamenteux, incluant des algues ou des cordes/collecteurs utilisées dans l'industrie mytilicole (Eyster & Pechenik, 1988; Petersen, 1984a, 1984b; Seed & Suchanek, 1992). Leur caractère sessile est assuré par la sécrétion constante de byssus (byssogenèse) qui constitue un processus énergétiquement coûteux, pouvant nécessiter entre 8 et 15 % du budget énergétique des moules (Babarro & Reiriz, 2010; Griffiths & King, 1979). En l'absence de substrat ou de conditions trophiques favorables, les larves restent dans la colonne d'eau, retardant temporairement leur fixation et leur métamorphose, attestées par une taille de coquille supérieure à 300 µm (Androuin et al., 2022; Bayne, 1965; Martel et al., 2014; Seed & Suchanek, 1992; Sprung, 1984; Toupoint et al., 2012). Des études ont montré que les larves pédivéligères sont capables de retarder leur métamorphose de 45 jours lorsque les conditions sont défavorables (Bayne, 1965; Bishop et al., 2006; Jolivet et al., 2016; Martel et al., 2014; Pechenik, 1990; Toupoint, et al., 2012a, 2012b).

La métamorphose chez la moule est un processus progressif avec une maturation complète des branchies quand les post-larves atteignent une taille aussi importante que 4 mm (Cannuel et al., 2009). Une fois métamorphosées, les post-larves et les juvéniles de la moule bleue ont la capacité de réaliser des migrations secondaires par sécrétion de filaments byssaux qui augmentent leur flottabilité (Forêt et al., 2018a; Lane et al., 1985) et leur permettent de regagner des habitats favorables à leur survie ou de fuir des conditions stressantes (Bayne 1964, Seed & Suchanek, 1992, Forêt et al., 2018b). Ces étapes successives sont également qualifiées de dérive bysso-pélagique et sont observées jusqu'à une longueur juvénile d'environ 2,5 mm (Forêt et al., 2018a; Lane et al., 1985). À partir de cette taille seuil, un juvénile est essentiellement benthique avec une mobilité limitée à la reptation par l'utilisation du pied qui autorise des redistributions à petite échelle d'espace, notamment pour former des colonies, ce que l'on qualifie de grégarisme (Seed & Suchanek, 1992). L'avantage de ces comportements grégaires est qu'une « moulière » offre une meilleure résistance aux forces

mécaniques de l'eau et à l'action des vagues en réduisant la surface exposée et en favorisant une meilleure fixation que celle fournie de manière individuelle (Seed & Suchanek, 1992).

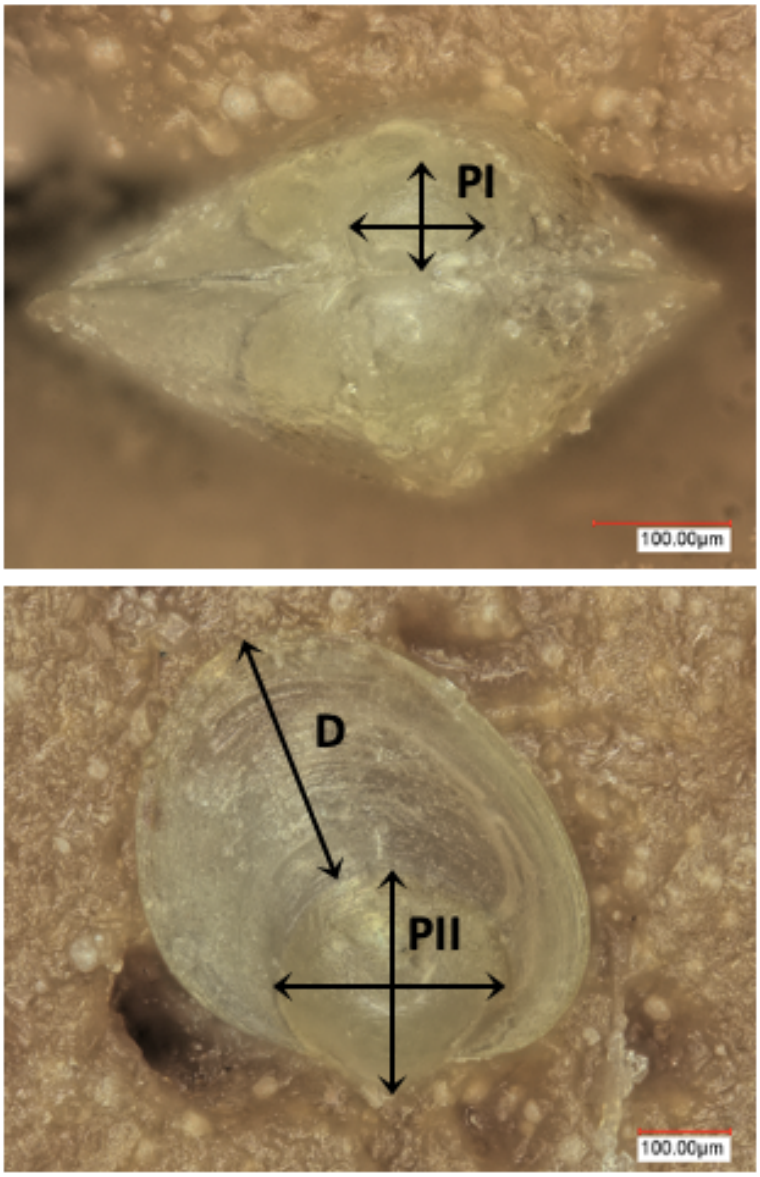


Figure 16. Photo d'une post-larve de *Mytilus edulis* avec PI : prodissoconque I, PII : prodissoconque II et D : dissoconque.

5. OBJECTIFS DE LA RECHERCHE

5.1 Objectifs spécifiques et hypothèses

Contrairement aux mammifères marins et aux poissons, il y a encore peu de recherches consacrées aux impacts sonores du transport maritime sur les invertébrés marins. L'objectif principal de ce projet est de documenter pour la première fois la réponse métabolomique des stades larvaires d'un mollusque bivalve, aux stress associés au trafic maritime dans le golfe du Saint-Laurent, soit les pollutions sonore et chimique. Bien que la sensibilité et la nature critique des premiers stades de vie des bivalves soient reconnues, les données sont rares. Le modèle d'étude choisi est la moule bleue *Mytilus edulis*.

L'approche métabolomique permettra d'identifier les mécanismes impactés par ces différents niveaux de stress (immunitaire, oxydatif, métabolique, énergétique) et la capacité des individus à mettre en place des mécanismes d'acclimatation. En effet, les différents métabolismes moléculaires, biochimiques et physiologiques sont potentiellement modulés par le son ou par les vibrations générées par celui-ci (**Fig. 17**). Les informations obtenues au niveau moléculaire seront comparées et corrélées à des informations métaboliques, physiologiques et comportementales complémentaires obtenues à plus grande échelle aux différents stades ontogéniques. Les données obtenues permettront d'intégrer les trois niveaux de stress comme vu précédemment dans la Figure 1. Tout d'abord, la réponse primaire au stress est analysée, impliquant les prostaglandines et les produits d'oxydation des acides gras, responsables de processus adaptatifs tels que l'inflammation. Ensuite, la réponse au stress secondaire est caractérisée par le métabolisme général (métabolismes des acides aminés et de l'énergie) et les réserves énergétiques avec la caractérisation des profils lipidiques. Enfin, le stress tertiaire sera mesuré via des données physiologiques telles que la croissance des larves aux différents stades de développement (Bayne, 1965; Martel et al., 1995) et la survie des larves (Nalepa & Schloesser, 1992).

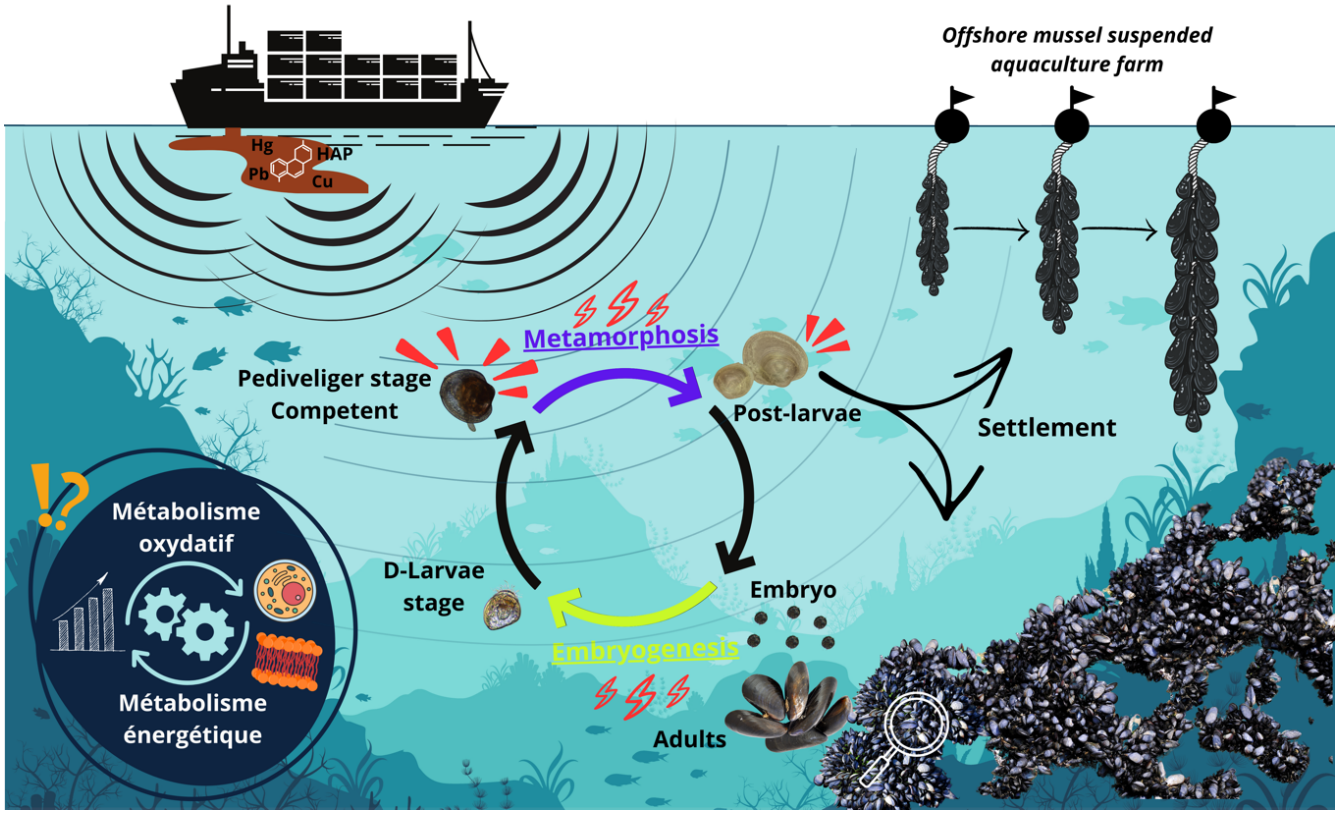


Figure 17. Schéma conceptuel de l'étude

Ce projet sera ainsi développé en deux chapitres, chacun traitant d'une expérimentation différente. Le premier chapitre est constitué de deux articles scientifiques axés sur la pollution sonore, tandis que le deuxième chapitre est structuré autour de trois articles scientifiques axés sur l'impact acoustique et chimique du trafic maritime, comme décrit ci-dessous (**Fig. 18**).

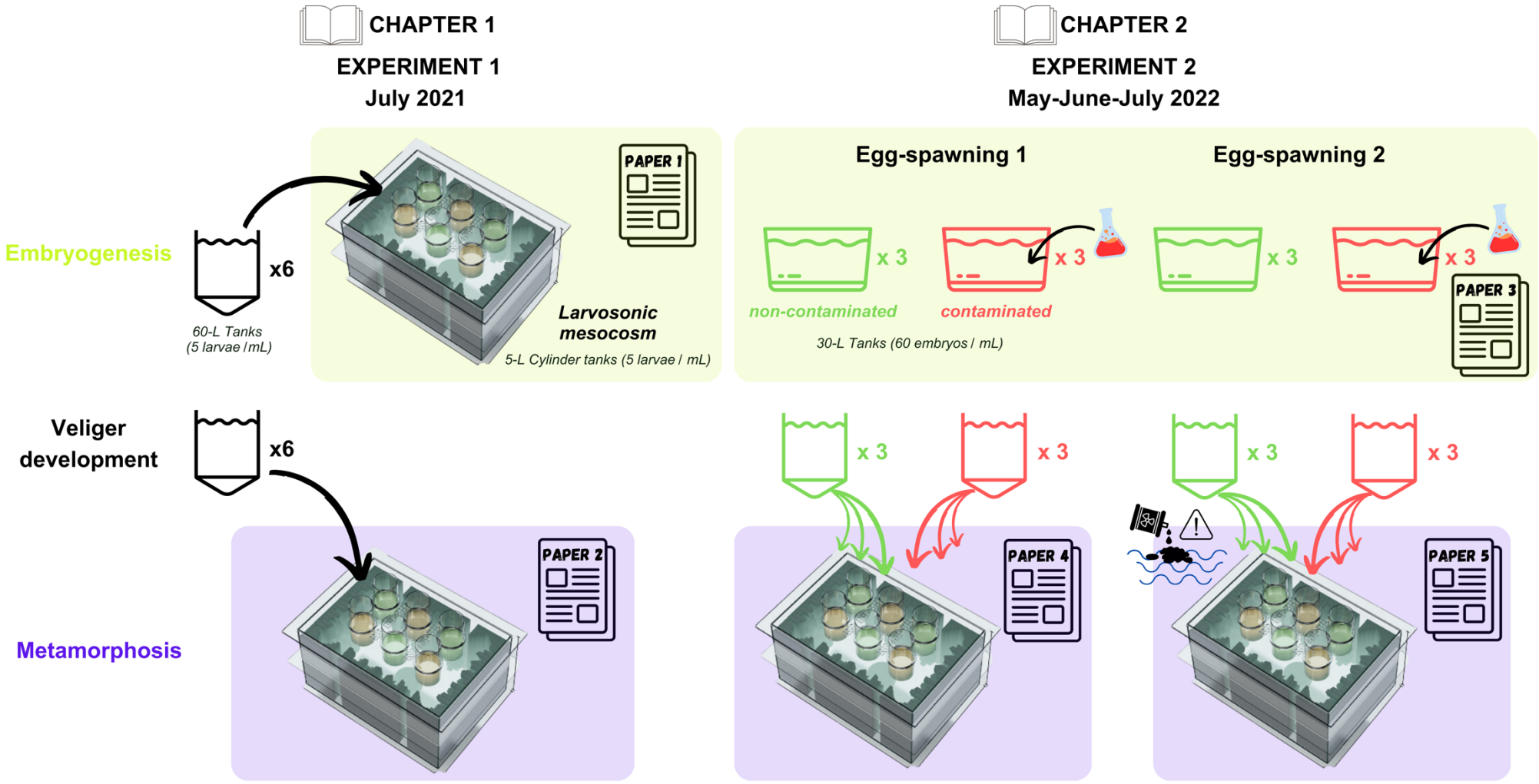


Figure 18. Schéma de l'articulation des chapitres et des articles dans la thèse.

5.2 Chapitre 1 - Impact de la pollution sonore sur les premiers stades larvaires de la moule bleue *Mytilus edulis* au niveau de leur métabolisme

Dans ce premier chapitre, l'objectif est d'étudier les effets du bruit de la navigation pendant les stades larvaires cruciaux que sont l'embryogenèse (de l'embryon aux larves véligères de forme D, larve-D) et la métamorphose (pédivéligère pélagique aux post-larves benthiques), sur le métabolome, le développement et la survie de la moule bleue, *M. edulis* (**Fig. 19**). Une perturbation acoustique a été appliquée lors de chacune de ces périodes de transition critique pour les larves (voir Annexe I). L'exposition sonore correspond au passage d'un navire cargo dont l'émission sonore augmente et diminue respectivement lors de l'arrivée et du départ de celui-ci (voir Annexe II). Les larves de moules sont exposées à des intensités sonores différentes liées à la distance de la source (navire cargo). Il existe des preuves que le bruit anthropique peut avoir des effets néfastes sur le comportement et la physiologie de nombreuses espèces, mais il existe peu d'études montrant comment la condition physiologique peut être directement affectée. À cet égard, nous émettons l'hypothèse qu'une fenêtre critique de stress pourrait survenir au cours de ces deux stades larvaires. De plus, nous supposons que le métabolome, incluant le métabolisme énergétique et les réponses hormonales, est affecté par une exposition sonore et peut influencer la condition physique individuelle. Enfin, nous suggérons l'existence d'une relation effet-dose selon l'intensité sonore à laquelle les larves sont exposées. Les coûts liés au maintien de l'homéostasie au début de la vie exercent une influence fondamentale sur la dynamique et la résilience des populations, avec des implications potentielles sur la structure et le fonctionnement des communautés.

Cette analyse a été divisée selon les trois axes, avec d'une part, une exploration des prostaglandines et du métabolisme oxydatif (stress primaire) caractérisée par une analyse en spectroscopie LC-HRMS ciblée (He et al., 2012), d'autre part l'exploration du métabolisme énergétique (stress secondaire) et enfin des informations sur la croissance et la survie des larves (stress tertiaire).

Ce chapitre a été divisé en deux articles scientifiques décrivant chacun un stade larvaire crucial. Le premier article se concentre sur l'embryogenèse et le second sur la métamorphose.

5.2.1 Article 1 - Embryogenèse de la moule bleue (*Mytilus edulis*) étudiée par une approche métabolomique ciblée pendant une exposition au bruit des navires

5.2.2 Article 2 - Preuve moléculaire de l'impact du bruit des navires sur la moule bleue, une espèce clé pour la durabilité des environnements marins côtiers

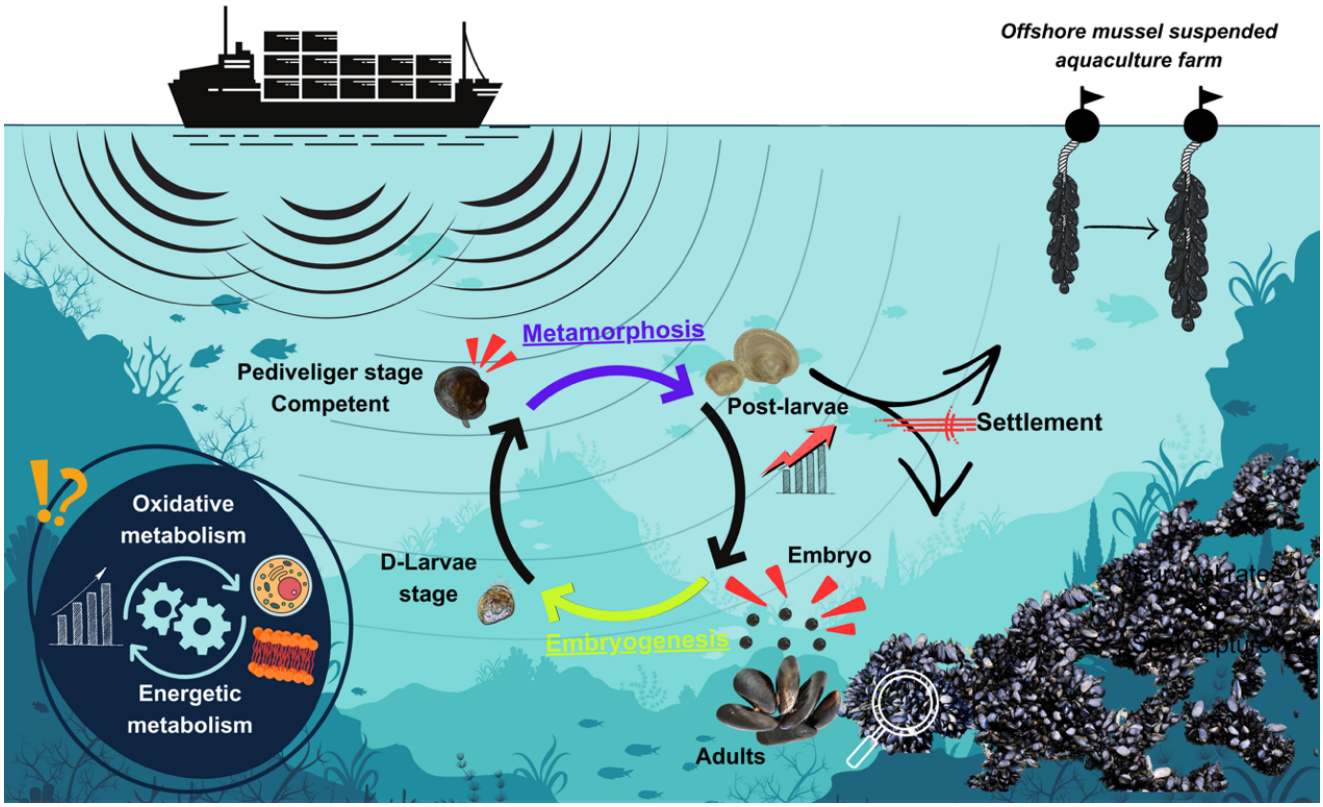


Figure 19. Schéma conceptuel du chapitre 1 sur les effets du bruit de la navigation pendant les stades larvaires cruciaux, l'embryogenèse (de l'embryon aux larves véligères de forme D, larve-D) et la métamorphose (pédivéligère pélagique aux post-larves benthiques).

5.3 Chapitre 2 - Impact acoustique et chimique du trafic maritime sur l'ontogenèse de la moule bleue *Mytilus edulis*

Dans ce deuxième chapitre, l'objectif général est d'évaluer la réponse des larves de moules exposées à une contamination chimique et sonore représentative du trafic maritime au cours d'une saison de ponte pendant un été (**Fig. 20**). Un nombre croissant d'études ont examiné les impacts acoustiques du trafic maritime sur les invertébrés, mais peu d'entre elles se sont penchées sur l'interaction de multiples facteurs de stress pour se rapprocher d'un contexte écologique réel. En effet, les navires délaissent après leur passage des résidus chimiques de manière accidentelle (déversement) ou continue (fuite). Différents types de polluants chimiques peuvent être retrouvés tels que les hydrocarbures polyaromatiques (HAP), des métaux lourds, du cuivre, du plomb, du mercure, etc. La contamination par les métaux est une empreinte anthropique typique dans les zones côtières (Hatje et al., 2018). Ces métaux sont problématiques puisqu'ils ont tous le potentiel de provoquer des effets toxiques. Les métaux ont tendance à s'accumuler dans les organismes marins via des processus tels que la bioaccumulation et la bioamplification, qu'ils soient essentiels ou non (Fokina et al., 2013). Le bruit peut interagir avec les contaminants chimiques en perturbant le comportement des organismes et avoir des effets complexes. Des études récentes sur des invertébrés ont démontré que le bruit amplifiait les effets néfastes des métaux lourds exposés à du cadmium.

Afin de répondre à cet objectif, ce chapitre est divisé en trois articles scientifiques décrivant la contamination chimique et l'interaction avec une exposition sonore au cours de l'ontogenèse de la moule bleue *M. edulis*.

5.3.1 Article 3 - Effet d'une pollution chimique représentative d'un port industriel sur le succès embryonnaire de la moule bleue, *Mytilus edulis* : une approche métabolomique

Dans l'article 3, le premier de ce chapitre, nous avons évalué la réponse des larves de moules exposées à des contaminants chimiques associés au trafic maritime. Nous nous

sommes concentrés sur l'embryogenèse, les premiers stades ontogéniques étant connus pour être les plus sensibles aux événements stressants et primordiaux pour le maintien de la structure de la communauté. Des embryons de moule bleue, *Mytilus edulis*, issus de deux pontes après maturation naturelle des gonades, ont été exposés à un cocktail de contaminants représentatifs d'un environnement portuaire jusqu'à la fin de l'embryogenèse, c'est-à-dire jusqu'au stade de larve véligère D. Les moules effectuent des événements de ponte successifs du printemps jusqu'au début de l'automne (Lowe et al., 1982). La qualité et la quantité de gamètes libérés par les moules peuvent varier en raison de multiples facteurs, notamment la disponibilité et la qualité de la nourriture (Seed & Suchanek, 1992). Il est notamment reconnu que la première ponte chez les moules bleues est de meilleure qualité que les suivantes au cours de l'été (Lowe et al., 1982). Cette expérience a ainsi été répétée à deux reprises au cours de l'été et la qualité des pontes est liée au succès de l'embryogenèse. Nous suggérons que l'effet de ponte montrera des variations importantes et cohérentes sur le succès d'embryogenèse en interaction avec la contamination chimique. Nous pourrons ainsi observer des changements dans les traits comportementaux (niveau d'activité) et physiologiques (taux métabolique, profils hormonaux) entre les individus d'une même espèce, et cette variation pourra avoir des conséquences évidentes sur la forme physique et l'évolution des cycles de vie (fitness) (Burton et al., 2011; Sih et al., 2004a).

5.3.2 Article 4 - Effet multi-omique de l'exposition aux contaminants pendant l'embryogenèse sur la métamorphose de la moule bleue *Mytilus edulis* exposée au bruit du trafic maritime

Dans l'article 4, le second de ce chapitre, une approche écophysiologique utilisant des outils métabolomiques et lipidomiques a été appliquée pour évaluer l'effet latent de l'exposition aux contaminants pendant l'embryogenèse sur le processus de métamorphose des larves de moules, *Mytilus edulis*, exposées au bruit des cargos pendant le développement de leur véligère. Afin d'y parvenir, des œufs nouvellement fécondés ont été exposés à un cocktail de polluants imitant l'environnement portuaire à faible dose jusqu'à la fin de l'embryogenèse, puis la pollution de l'eau de mer a été stoppée et les larves de moules ont été

exposées à des perturbations acoustiques de différentes intensités jusqu'à leur métamorphose. Nous suggérons que l'exposition à la pollution pendant l'embryogenèse amplifie les effets acoustiques du bruit de la navigation pendant la métamorphose des larves de moules en jeunes juvéniles. Cette étude se concentre sur l'effet latent de la contamination de l'embryon (premier stade larvaire) sur la métamorphose (dernier stade larvaire) des larves exposées à un stress acoustique au cours de leur développement. Plus précisément, nous émettons l'hypothèse qu'un effet dose-réponse sera mis en évidence par les réponses métabolomiques et lipidiques, avec une augmentation des besoins énergétiques et une diminution des taux de survie et de métamorphose.

5.3.3 Article 5 - Comment l'exposition au bruit de la navigation peut-elle amplifier les effets d'une contamination chimique accidentelle sur le recrutement de la moule bleue *Mytilus edulis* ?

Ce dernier article traite de l'impact de la pollution sonore et de la manière dont elle interfère avec une pollution chimique accidentelle représentative de la navigation. Des larves de *Mytilus edulis* exposées à une pollution accidentelle au diesel sont soumises à des perturbations acoustiques pendant leur phase de fixation et de métamorphose. L'objectif est d'évaluer la réponse des larves compétentes métamorphosées en post-larves après une contamination incontrôlée combinée à une exposition sonore représentative du trafic maritime. Le bruit anthropogénique peut affecter le comportement des larves de bivalves pendant la métamorphose (Cervello et al. 2023, Veillard et al. *in press*, voir Chapitre 1, Article 2). Nous supposons que l'augmentation du comportement de fermeture des post-larves liée au bruit de la navigation pourrait interférer avec le processus de bioaccumulation ou de dépuration du contaminant et/ou avec d'autres mécanismes physiologiques. Nous utilisons une approche métabolomique ciblée pour étudier la réponse au stress, les changements dans la performance et le comportement des larves en relation avec les altérations du métabolisme, considérant que le bruit est un indice possible pour l'installation des larves.

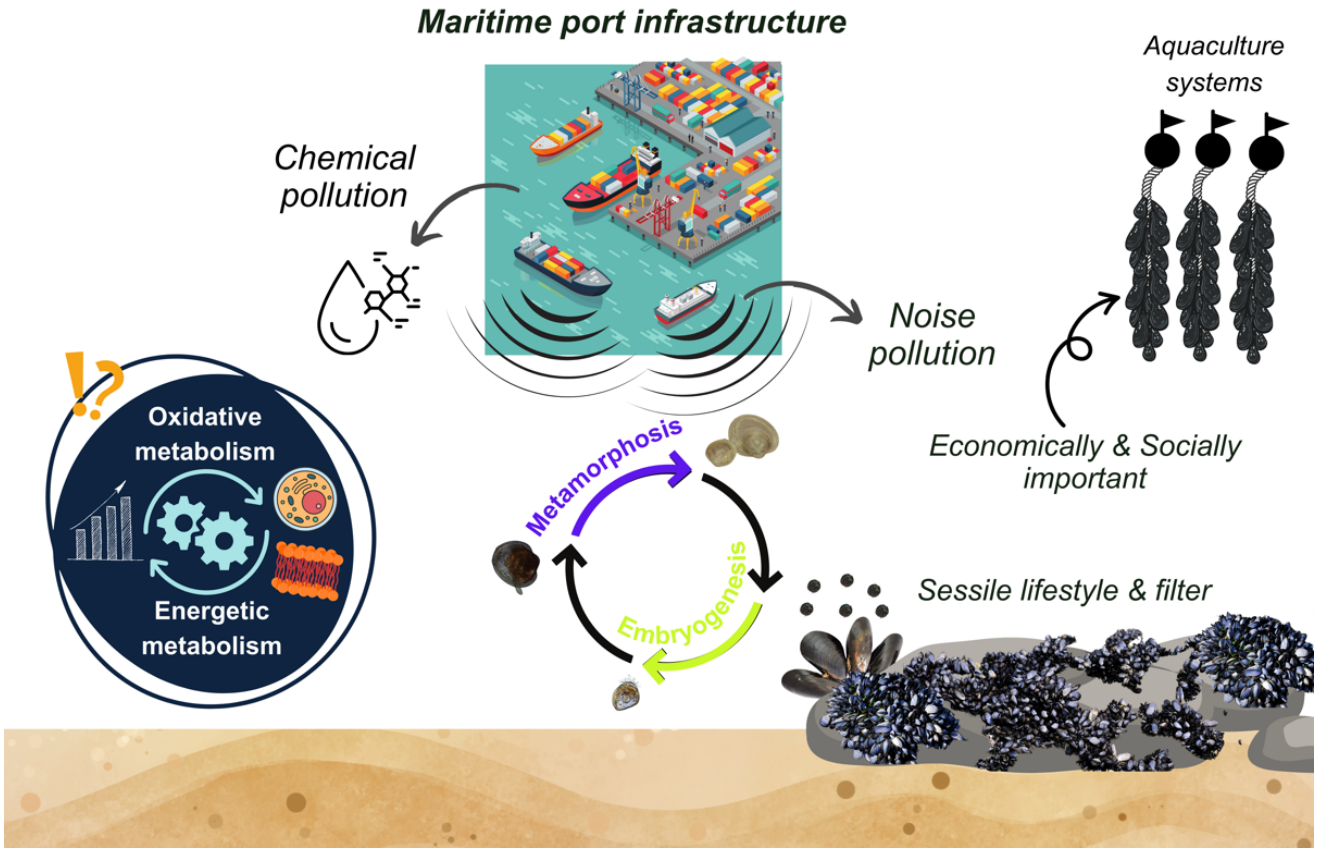
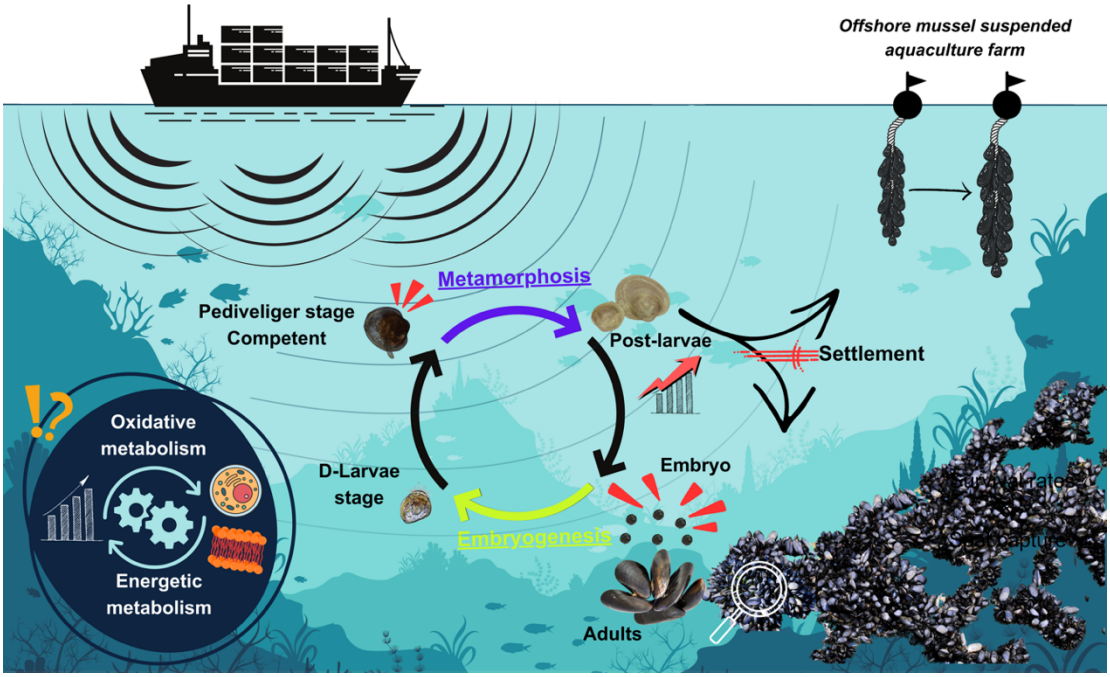


Figure 20. Schéma conceptuel du chapitre 2 sur les impacts acoustique et chimique du trafic maritime sur l'ontogenèse de la moule bleue *M. edulis*.

CHAPITRE 1

IMPACT DE LA POLLUTION SONORE SUR LES PREMIERS STADES LARVAIRES DE LA MOULE BLEUE *MYTILUS EDULIS* AU NIVEAU DE LEUR MÉTABOLISME



ARTICLE 1
EMBRYOGENÈSE DE LA MOULE BLEUE (*MYTILUS EDULIS*) ÉTUDIÉE PAR UNE APPROCHE MÉTABOLOMIQUE CIBLÉE PENDANT UNE EXPOSITION AU BRUIT DES NAVIRES

1.1 RÉSUMÉ EN FRANÇAIS DU PREMIER ARTICLE

Le bruit anthropique des navires dans les océans ne cesse d'augmenter, avec un doublement prévu tous les 11,5 ans, suscitant une inquiétude croissante au niveau mondial quant à ses effets potentiels sur la faune et la flore. Dans cette étude, nous avons évalué, au moyen d'analyses métabolomiques, les effets du bruit de la navigation pendant le stade précoce et crucial de l'embryogenèse sur le développement et la survie d'une espèce-clé de l'écosystème benthique marin, la moule bleue *Mytilus edulis*. Nous avons constaté que l'exposition au bruit des navires induit une inflammation due au stress, un déséquilibre métabolique ou un stress cellulaire résultant d'une demande énergétique accrue, conduisant à une perturbation de la glycolyse et à une augmentation de la réponse au stress oxydatif. Le bruit généré par un navire cargo a donc un impact direct sur le premier stade de développement des larves de moules. Notre travail sur un taxon écologiquement et socio-économiquement important montre que le bruit anthropogénique peut affecter la condition physique des individus. L'augmentation des coûts de la condition physique au début de la vie pourrait avoir un impact majeur sur la dynamique et la résilience de la population de moules, avec des implications potentielles pour la structure et la fonction de l'écosystème.

Mots-clés : embryogenèse, exposition sonore, navire-cargo, métabolomique, prostaglandines, réponses au stress, fenêtre critique.

Cet article, intitulé « *Embryogenesis of blue mussel (Mytilus edulis) studied by targeted metabolomic approach during shipping noise exposure* », est soumis dans la revue *Journal of Experimental Biology*. En tant que premier auteur, j'ai contribué à l'essentiel de la recherche sur l'état de la question, au développement des larves de moules, aux recueillement des données biologiques dans des mescosmes larvaires, aux mesures de performance larvaire, ainsi qu'à l'analyse par spectrométrie de masse. Stéphane Beauclercq a contribué aux analyses statistiques. Bertrand Genard a développé les méthodes liées aux analyses par spectrométrie de masse pour la quantification des acides aminés, des métabolites liés au métabolisme énergétique et des produits d'oxydation des acides gras polyinsaturés. Elena Palacios a contribué à l'interprétation des métabolites et des produits d'oxydation des acides gras polyinsaturés par son expertise dans le domaine. Frédéric Olivier a conçu les mésocosmes larvaires. Réjean Tremblay a supervisé les expériences de laboratoire et les analyses larvaires, ainsi que l'administration du projet et l'acquisition des fonds. Frédéric Olivier, Laurent Chauvaud, Isabelle Marcotte, et Réjean Tremblay ont conçu et supervisé l'étude. Tous les auteurs ont contribué à la rédaction du manuscrit et ont approuvé l'article final. Cet article a fait l'objet de plusieurs communications orales lors de congrès. Une version abrégée de cet article a été présentée au congrès *World Aquaculture Society (WAS), Aquaculture Association of Canada (AAC) et Newfoundland Aquaculture Industry Association (NAIA)* à St. John's (Newfoundland, Canada) en août 2022, à la conférence annuelle de *Ressources Aquatique Québec* en novembre 2022 à Québec, au *4th International Symposium for Advances in Marine Mussel Research (AMMR4)* en Angleterre en novembre 2022 ainsi qu'au *115th NSA Annual Meeting* à Baltimore dans le Maryland en mars 2023.

EMBRYOGENESIS OF BLUE MUSSEL (*MYTILUS EDULIS)* STUDIED BY A TARGETED METABOLOMIC APPROACH DURING SHIPPING NOISE EXPOSURE

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1.2 ABSTRACT

Anthropogenic ocean noise from shipping is steadily increasing, with a predicted doubling every 11.5 years, raising to growing global concern about its potential effects on wildlife. There is evidence that anthropogenic noise can affect the behavior and physiology of many species, but few examples of experiments that show how fitness can be affected. Here, we used metabolomics analyses to investigate the effects of shipping noise during the crucial early life stage of embryogenesis on a key ecosystem reporter species in the marine benthic system, the blue mussel *Mytilus edulis*. We found that exposure to shipping noise induces stress-induced inflammation, a metabolic imbalance or cellular stress as a result of increased energy demand, leading to disruption of glycolysis and increased oxidative stress response. The noise generated by a cargo ship has a direct impact on the first developmental stage of mussel larvae. Our work on an ecologically and socio-economically important taxon shows that anthropogenic noise can affect individual fitness. Increasing fitness costs early in life could have a major impact on population dynamics and resilience, with potential implications for community structure and function.

Keywords: Embryogenesis, Noise exposure, Cargo-ship, Metabolomics, Prostaglandins, Stress responses, Critical window.

1.3 INTRODUCTION

The recruitment of marine bivalve species is highly variable and unpredictable, and an importantly larval supply does not necessarily guarantee recruitment success (Hunt & Scheibling, 1996; Toupoint et al., 2012; St-Onge et al., 2015; Correia-Martins et al., 2022). Most marine bivalve species display a bentho-pelagic life cycle with recruitment corresponding to the transition from pelagic to benthic life (Thorson, 1950). The larval stages, which promote dispersal over large spatial, are sensitive to environmental conditions (Qiu et al., 2002; Rayssac et al., 2010) and to predation, with survival rates estimated to be less than 10% (Mileikovsky, 1971; Thorson, 1946, 1950). Gosselin and Qian (1997) have shown that the early life stages of benthic marine invertebrates represent a critical phase that regulates subsequent adult population size. In commercial bivalve species, such as mussels, oysters, scallops, and clams, there has been considerable research interest in their reproductive strategy based on high fecundity, small eggs, and external fertilization. To our knowledge, no data are available on the success of embryogenesis in marine bivalves under field conditions, but experimental studies have shown that survival from eggs to the first shell larval stage (D-larvae) is regularly less than 30% (Whyte et al., 1991; Soudant et al., 1996; Pernet et al., 2003a).

Marine mussels are abundant in both intertidal and subtidal habitats (Gosling, 1992) and provide important ecological services through a filter-feeding foraging strategy that minimizes eutrophication through their ability to construct self-growing mussel reefs, thereby increasing species richness and abundance, and constituting an important prey for invertebrate predators (*e.g.* polychaetes, sea stars, dog whelks and crabs), seabirds (*e.g.* eiders), sea otters, walruses and seals (Borthagaray & Carranza, 2007; Drapeau et al., 2006; Kautsky, 1982). Marine mussels are also widely used as bioindicators for monitoring coastal water pollution due to their biological and ecological characteristics (sessile species, mussel beds facilitate sampling, medium-sized ideal for chemical analysis, robust in laboratory conditions, and pumping large volumes of water for pollutant accumulation) (Beyer et al., 2017). Widely distributed around the world in temperate coastal habitats (Gosling, 1992),

marine mussels are commonly subjected to anthropogenic noise. Today, there is evidence of the impact of anthropogenic noise on benthic biodiversity (see reviews by Hildebrand, 2009; de Soto, 2016; Hawkins & Popper, 2017; Sordello et al., 2020; Duarte et al., 2021; Wale et al., 2021; Solé et al., 2023), and noise is now recognized as a major global pollutant. The most prevalent anthropogenic noise in aquatic habitats derives from maritime transport, which is steadily increasing, with global shipping noise emissions predicted to double every 11.5 years (Jalkanen et al., 2022). Sounds emitted by ships generate low-frequency noise (5 to 10000 Hz) that can reach 190 dB 1µPa@1m, propagate over long distances, and can directly damage exposed marine organisms, but also affect them indirectly by masking the detection of biologically relevant signals (Hawkins & Popper, 2017; Duarte et al., 2021; Solé et al., 2023). This is especially true for sedentary or sessile animals such as mussels that have limited or no ability to move away from sound sources.

Several studies have examined the effects of increasing noise levels on bivalves, most referring to the adult stage (Peng et al., 2016; Gigot et al., 2023a, b; Ledoux et al., 2023; Olivier et al., 2023), some concerning larval and post-larval stages (Gigot et al. 2023a, b; Olivier et al., 2023), and very few during embryogenesis. During this first larval stage, anthropogenic sound exposure resulted in delayed hatching and development of crustaceans eggs, and impaired embryonic development or significantly increased larval abnormalities and mortality rates in crustaceans, bivalves, and gastropods (Stanley et al., 2010; Aguilar de Soto et al., 2013; Nedelec et al., 2014). Nedelec et al. (2014) showed negative effects on sea hare *Stylocheilus striatus* embryos exposed to boat noise, while Aguilar de Soto et al. (2013) found a negative effect of exposure to high levels of seismic airgun noise on *Pecten novaezelandiae* embryos. Solé et al. (2023) suggested that the nature and level of response of embryos to noise maybe species-specific. However, to our knowledge, no study has been published using a metabolomic approach to investigate key metabolic pathways during the development of embryos exposed to shipping noise. Metabolomics as an emerging discipline provides a complex picture of an organism's phenotype, since metabolite concentrations are sensitive to environmental changes and provide information about what is happening at both the metabolic and physiological levels (Dumas et al., 2020; Li et al., 2020), providing a

"physiological snapshot" of a living cell, tissue, or organism at a given time. Metabolomics has gradually been applied to an increasing number of studies investigating the responses of organisms to external stimuli or pathogenic challenges (Huo et al., 2019; Li et al., 2020).

In the current study, we used a targeted metabolomic approach to investigate the stress response associated with the embryogenesis phase (embryo to D-larvae stage), considered to be particularly sensitive in the blue mussel, *Mytilus edulis*. We exposed them to different levels of sound sequences corresponding to the passage of a cargo ship, including increasing and decreasing sound level sequences mimicking an arrival and a departure, respectively. We hypothesize that a critical window of sensitivity to stress may occur during embryogenesis through changes in energy, oxidative and prostaglandin pathways that affect the fitness of D-larvae. We also hypothesize that there would be a dose-response relationship between noise level during exposure and its effect on larvae and this was assessed as primary (prostaglandins and oxidative metabolism), secondary (energy metabolism), and tertiary (shell larval growth and survival) stress.

1.4 MATERIALS AND METHODS

1.4.1 Experimental set-up

Blue mussels *M. edulis* embryos were exposed to contrasting sound treatments including a control (ambient sound) and three increasing levels (low, medium, and high; *see below*) of a sound sequence previously recorded and described in Veillard et al. (*in press*, see Chapter 1, Article 2). Briefly, the spectral composition, sound level, and recurrence of shipping noise in the port of Saint-Pierre-et-Miquelon (France) were evaluated from November 2020 to April 2021 using an Aural-M2 (Multi-Electronique Inc., Rimouski, QC, Canada) underwater acoustic recorder (sampling frequency of 32 kHz, 16-bit resolution) equipped with a HTI-96-MIN hydrophone (High Tech Inc., Long Beach, MS, USA) with a sensitivity of -165 dB re V/µPa and set at a gain of 22 dB. After this evaluation, the noise from a 120m long roll-on/roll-off cargo ship ('Nohlan Ava'), built in 2000, was selected as

it was the largest contributor to the noise emission from maritime traffic in the study area. The sound sequence was selected and processed using Audacity® software to create an 80-minute soundtrack consisting of the sound of the cargo ship's arrival (11 min), a 39-minute pause (simulating port handling), its departure (8.5 min), and a new pause of 21.5 min (Veillard et al. *in press*, see Chapter 1, Article 2), all of which were looped during the experiment.

We used *Larvosonics* mesocosms as a "bain-marie" to maintain different tanks (cylinders) under similar acoustic conditions and designed them to emit sounds with low reverberation and resonance (Olivier et al., 2023a). Four mesocosms were available for this study, each of them consisting of six independent circular 5L cylinders (6 replication units) where embryos (~25 000 per cylinder) were exposed to the sound loop continuously for 4 days until the larval population reached the D-shaped larval stage. Temperature (18.1 ± 0.1°C), salinity (23.7 ± 0.4 PSU), photoperiod (15h of light and 9h of night), and light intensity (97.1 ± 1.7 lux) were monitored during the experiments. Seawater was UV-treated and ultrafiltered (0.1 µm), and the larval survival rate was measured by optical microscopy. During this first development stage, each *Larvosonic* mesocosm tank was exposed to four different maximum sound levels: low (121 ± 4 dB re 1µPa), medium (127 ± 2 dB re 1µPa), high (151 ± 2 dB re 1µPa), and ambient sound as control (116 ± 1 dB re 1µPa) (Veillard et al. *in press*, see Chapter 1, Article 2). At the end of the sound exposure experiment, D-larvae from each cylinder were sieved through a 20 µm mesh and divided into two subsamples, flash-frozen in liquid nitrogen, lyophilized, and stored at -80°C until extraction for targeted metabolomics studies. One subsample was used for the evaluation of oxidative metabolism, mainly prostaglandin and oxidative pathways, and the other for energy metabolism.

1.4.2 Larval production

Blue mussel (*Mytilus edulis*) embryos were obtained as described in Rayssac et al. (2010). Briefly, 30 adult mussels (50-60 mm) from Saint-Peters Bay in Prince Edward Island (46.42948N, -62.66030W, Canada) were individually induced to spawn by a 10°C thermal

shock. All eggs and spermatozoa were mixed and a ratio of 10 spermatozoa per egg was used for the fertilization in UV-treated and ultrafiltered (1 µm) seawater at 18°C. The eggs were further washed with UV ultrafiltered seawater and maintained at 18°C. Embryogenesis success was calculated from the number of larvae at the D-shaped larval stage (with a prodissoconch II) compared to the number of initial eggs used for fertilization (only the prodissoconch I). The size of the D-shaped larval stage was measured by the maximum width (Martel et al., 2014b).

1.4.3 Quantification of amino acids, energy-related metabolites, and polyunsaturated fatty acid oxidation products

1.4.3.1 Amino acids and energetic metabolism

Approximately 3.5 mg of freeze-dried (~ 1500 individuals) D-shaped larvae were microground and homogenized (Precellys 24; Bertin Corporation) at 4,000 rpm for three cycles of 10 s before extraction with a 1:1 mixture of 2,2,2-trifluoroethanol (TFE) and water, according to a method proposed by Agilent and described in Veillard et al. (*in press,* see Chapter 1, Article 2). Briefly, metabolites were separated and quantified using an HPLC 1260 Infinity II instrument coupled to a 6420 Triple Quad mass spectrometer (Agilent Technologies) in positive and negative ionization modes (Huang et al., 2018; Linsmayer et al. 2020). Data were processed using MassHunter Quantitative QQQ (Quant-my-Way) software from Agilent Technologies. Because two samples presented poor peak shapes, a large change in retention time and were often too concentrated to be accurately quantified, they were removed from the statistical analysis.

1.4.3.2 Polyunsaturated fatty acids oxidation products from oxidative metabolism

Approximately 3.4 mg of freeze-dried (~ 1500 individuals) D-shaped larvae were microground and homogenized (Precellys 24; Bertin Corporation) to homogenize the sample at 5,800 rpm for three cycles of 20 s before extraction with TFE/ammonium formate buffer pH 3 and protein precipitation by addition of acetonitrile/methanol as described in Veillard

et al. (*in press,* see Chapter 1, Article 2). Briefly, samples were injected (1 mL) into the HPLC 1260 Infinity II system (Agilent Technologies) equipped with an InfinityLab Poroshell HPH C-18, 2.1 × 100 mm 1.9 µm columns (Agilent Technologies) followed by a solid-phase extraction column at 40°C (Phenyl-Hexyl 4 × 2.0 mm, Phenomenex) and coupled to a 6470B Q-TOF mass spectrometer (Agilent Technologies). Data were processed using MassHunter Quantitative Q-TOF (Quant-my-Way) software from Agilent Technologies.

1.4.4 Statistical analyses

1.4.4.1 Metabolite enrichment analysis

To identify the most significantly affected metabolic pathways, the discriminant metabolites were analyzed by metabolite set enrichment analysis (MSEA) (Xia & Wishart, 2010), implemented in Metaboanalyst, a freely available web-based metabolomics analysis suite (MetaboAnalyst 5.0*, http://www.metaboanalyst.ca*) (Pang et al., 2021). The metabolite enrichment analysis of each set of metabolites was performed using over representation analysis with the provided "pathway-associated metabolite sets", which contain 99 metabolite sets based on normal human metabolic pathways. The over-representation analysis was implemented using the hypergeometric test to evaluate whether a particular metabolite set was more highly represented than expected by chance within the given compound list. A cutoff was chosen at a False Discovery Rate (FDR) controlled P-value of 0.01 to retain the most significantly enriched metabolic pathways.

1.4.4.2 Heatmap

The average of normalized concentration in percent (0 to 100) for the heatmap was generated by dividing the data by the maximum concentration found for an amino acid. Note that the data were normalized by metabolites. For example, for "Histidine" if the highest value between the 4 treatments (control, low, medium, and high) was 2 500 $ng.mg^{-1}$, the concentration was normalized from this value to be transformed in percentages.

1.4.4.3 Permanova

D-larvae length and embryogenesis success were analyzed with PRIMER7 software, using analyses of variance with 9999 permutations (Legendre & Legendre, 2012) of residuals under a reduced model (PERMANOVA). The permutational method is a randomization that allows removing distributional assumptions such as normality and can be applied to very small samples (Legendre & Legendre, 2012). Similarity matrices based on ratios were produced using the Bray-Curtis distance. A one-way PERMANOVA was used to test the effect of sound intensities (4 levels: control, low, medium, and high) on D-shaped larval size and embryogenesis success. When differences were significant (p-perm ≤0.05 strong evidence, p-perm≤0.07 weak evidence) (Muff et al., 2022), pairwise multiple comparison tests were used to determine which groups were significantly different. Distance-based tests for homogeneity of multivariate dispersion (PERMDISP) were performed to assess the variability across conditions.

1.5 RESULTS

1.5.1 Tertiary stress exploration: growth and survival rates

We first explored the effect of ship noise exposure on blue mussel larvae during embryogenesis (2 days). Our results are displayed in Table 1 and reveal that the embryogenesis success was unaltered, with mean values of ~ 24% (P-perm=0,6587, df=3, Pseudo-F=0,5306; Permdisp: F=0,4113 and, P-perm=0,7682), nor was the mean size at the D-shaped larval stage, with a mean value of 110.2 ± 0.5 µm (P-perm=0,1846, df=3, Pseudo-F=1,7633) (**Tab. 2**). The dispersion of the data between treatments was also homogeneous (F=1,6198, df=3, P-perm=0,1646).

Table 2. Mean size of D-shaped larvae (in µm) and embryogenesis success (in percent) ± standard error of larvae exposed to increasing levels of sound (low, medium, and high).

Condition	Control	Low	Medium	High	Mean
Mean size (µm)	109.1 ± 0,4	109.7 ± 0,4	111.0 ± 0,3	111.1 ± 0,3	110.2 ± 0,5
Embryogenesis success	24% ± 1%	23% ± 2%	24% ± 1%	24% ± 2%	24% ± 0%

1.5.2 Secondary stress exploration: energetic metabolism

Using a targeted LC-MS/MS approach, we then investigated changes in the amino acid and energy metabolism profile of the D-larvae as a function of noise levels, based on the absolute quantification. As shown in Table 3, 18 metabolites out of the 48 measured were significantly affected by the noise treatments. These 18 metabolites, presented in Table 3, are involved in aerobic energy metabolism (5, *i.e.* including the glycolysis and the Krebs cycle), anaerobic energy metabolism (1), energy transfer (2), as well as essential (7) and non-essential amino acid metabolism (4). Interestingly, the ratio of Branched-Chain Amino Acids (BCAAs), *i.e.*, the sum of valine, leucine, and isoleucine, decreased in D-Larvae exposed to the medium- and high-noise treatments compared to the control and low level (**Tab. 3**). To improve the visualization of the data, Figure 21A presents a heatmap of the amino acids and energy-related metabolites according to sound intensities. In all cases, the concentrations in the D-larvae decreased as a function of increasing noise intensity, being lowest under medium and high sound levels as compared to the low sound intensity and the control (P-perm=0.0190, df=3, Pseudo-F=3.0339; Permdisp: F=2,4322, P-perm=0,3060) (**Fig. 21A**). An exception can, however, be observed for the α-aminobutyric acid, for which concentrations were higher in D-larvae exposed to all ship noise intensities as compared to the control (**Fig. 21A**).

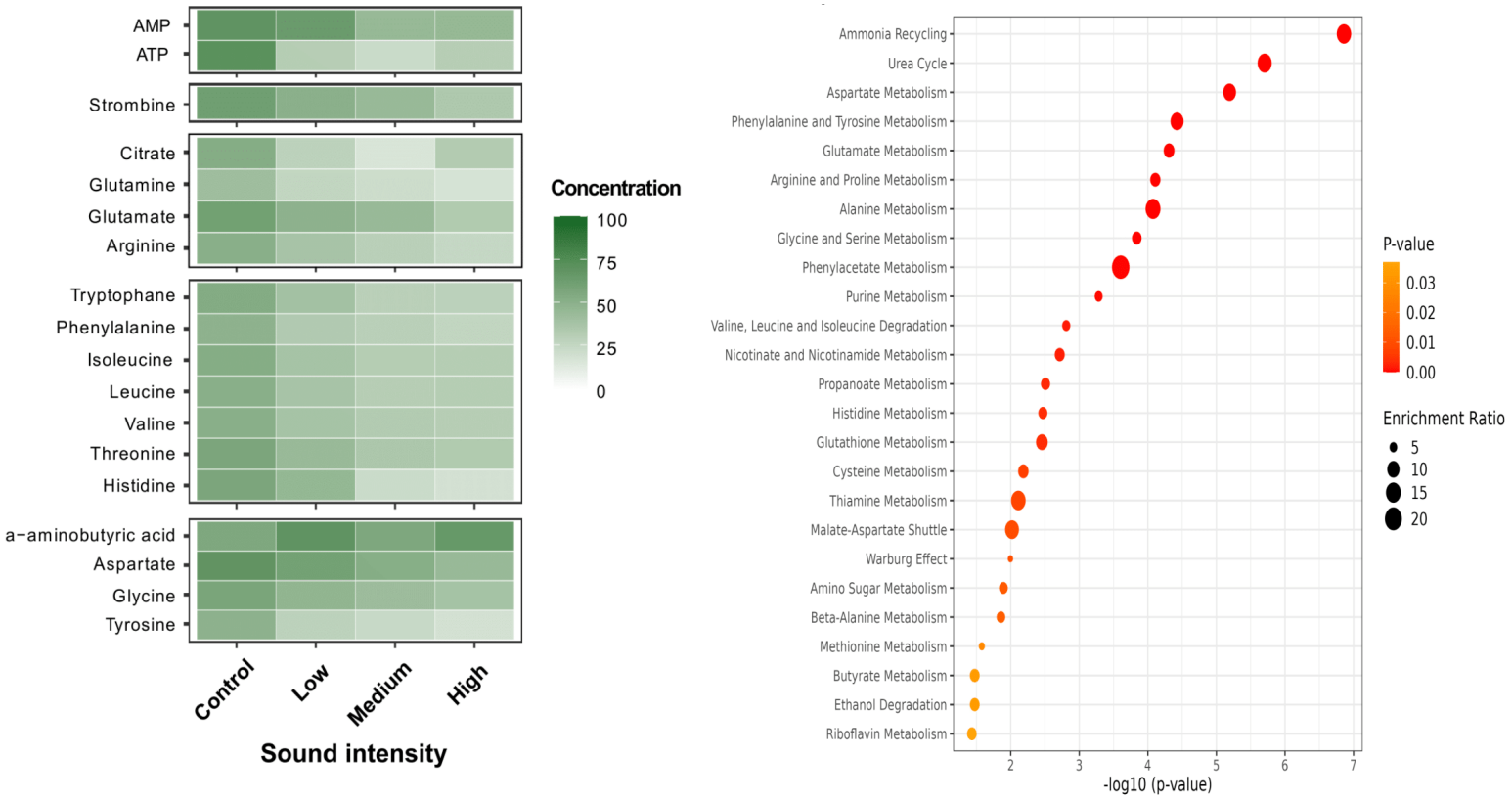

Figure 21. (A) Heat map of amino acids and energy-related metabolites presenting the average of the normalized concentration (in percent, 0 to 100) according to metabolic pathways and sound intensities (control, low, medium, and high). **(B)** Metabolite Set Enrichment Analysis performed on the list of metabolites obtained from the targeted metabolomics. Bubble sizes represent the fold enrichment of the metabolic pathways identified and red color gradient corresponds to the P-value corrected for multiple testing by FDR.

The impact on biochemical pathways of embryos exposed to noise during their development in D-larvae was determined by metabolite set enrichment analysis (**Fig. 21B**) based on the discriminant metabolites identified. This analysis connects the metabolites for which the concentration significantly increased (P-value<0.03) under noise treatment, to 25 biochemical pathways. Most of these pathways correspond to amino acid recycling through transamination (aspartate metabolism) and oxidative deamination (glutamate metabolism), leading to detoxification processes (urea cycle, ammonia recycling). Then, the pathways related to energy metabolism or synthesis of carbohydrates or lipids, as well as those for oxidative metabolism (glutathione metabolism), were enriched in D-larvae which developed from embryos exposed to shipping noise (Phe and Tyr metabolism, Arg and Pro metabolism, Ala metabolism, Gly and Ser metabolism, purine metabolism, Val, Leu and Ile degradation).

The results are further summarized in Figure 22, in a diagram of the cellular metabolism from glycolysis to the Krebs cycle as a function of ship noise. The energy metabolism is clearly triggered by sound treatments, revealing metabolic imbalance or cellular stress compared to the control larvae (**Fig. 22**). The high glucose-6-phosphate/ D-fructose1,6-biphosphate ratio (G6P/F1.6BP) in the organisms exposed to sound reveals an accumulation of G6P. On the contrary, the F1.6BP/pyruvate ratio strongly decreased under sound treatments, indicating a slowdown in its conversion into pyruvate (**Tab. 4**). In the Krebs cycle, the trend observed in the citrate/pyruvate, citrate/cis-aconitate and citrate/succinate ratios, as well as in the malate/citrate ratio (by inversion), was a strong decrease in the citrate concentration in response to the sound level. Figure 22 also shows an increase in the ATP/ADP and ATP/AMP ratios as well as in the Adenylate Energy Charge (AEC) index (equation 1) under the three sound treatments as compared to the control. The opposite trend was observed for the NADPH/NADP ratio.

$$AEC = ([ATP] + 0.5[ADP]/[ATP]+ [ADP]+ [AMP]) \qquad \textit{equation 1}$$

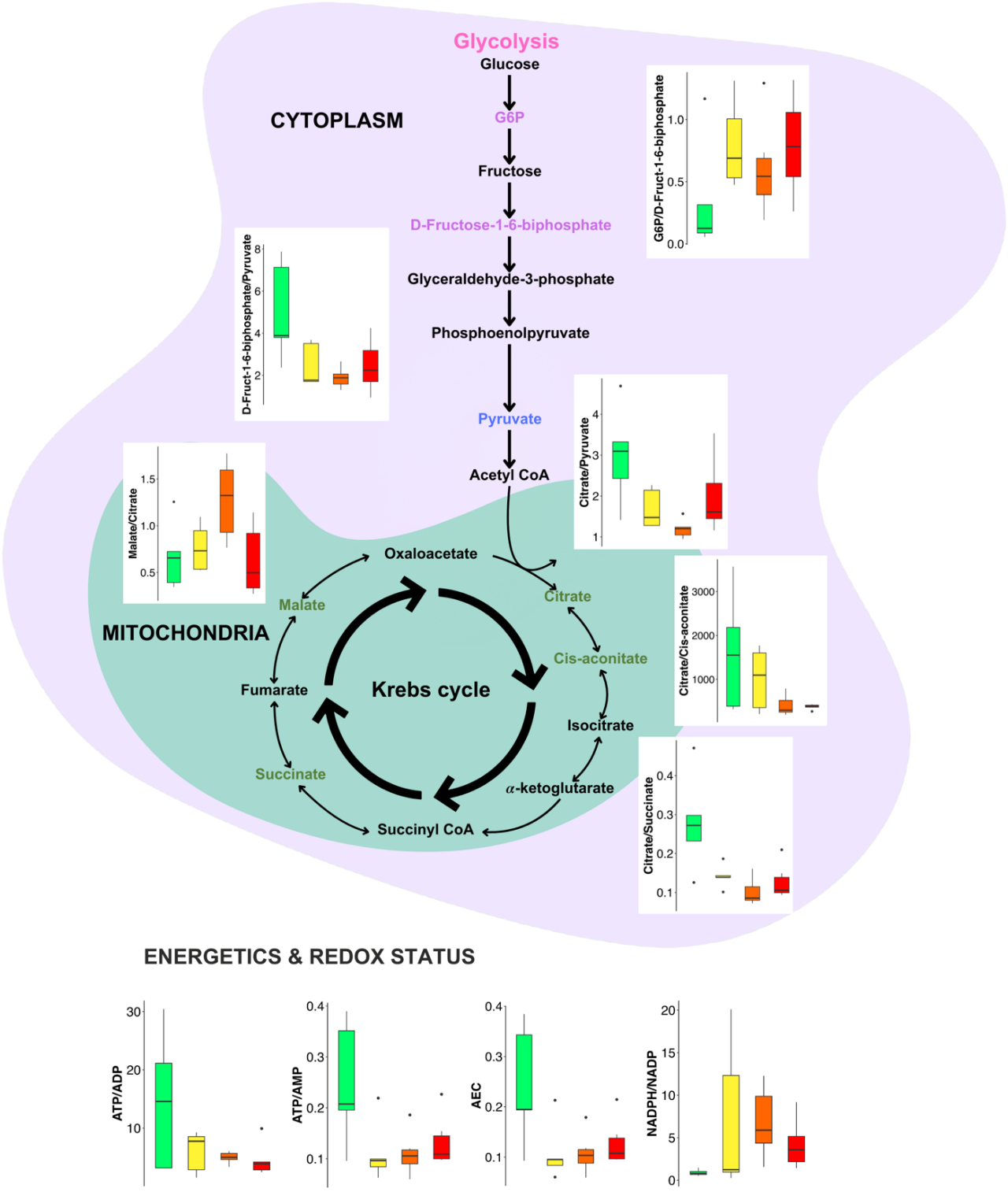

Figure 22. Synthetic diagram of the cellular metabolism including the Krebs cycle and glycolysis with the metabolic contribution to energy metabolism according to sound intensity, expressed as a ratio in ng/mg ± SE. In the boxplots, green refers to ambient noise control (n=5), yellow to low (max~121 re 1µPa) (n=5), orange to medium (max~127 re 1µPa) (n=6), and red to high (max~151 dB re 1 µPa) (n=6) shipping noise. Black dots are outliers.

Table 3. Mean concentrations of metabolites related to energetic metabolism in ng/mg ± SE and the associated statistics resulting from a one-factor permanova. The significant ones are in bold.

Metabolic pathways	Metabolites	Control	Low	Medium	High	P-perm	Df	Pseudo-F
Energetic	ATP	291 ± 47 a	128 ± 29 ab	91 ± 19 b	125 ± 57 b	**0.0277**	3	3.5018
	AMP	1236 ± 109 ac	1153 ± 21 a	799 ± 37 b	810 ± 202 bc	**0.0102**	3	4.742
	ADP	38 ± 16	29 ± 8	19 ± 4	33 ± 14	0.907	3	0.34664
	NADP	161 ± 48	77 ± 22	36 ± 9	33 ± 11	0.1204	3	2.0375
	NADPH	133 ± 45	202 ± 69	193 ± 42	115 ± 38	0.4906	3	0.91085
	NAD	1 ± 1	0 ± 0	1 ± 1	NA		NA	
	NADH	42 ± 11	46 ± 13	40.6 ± 16	97.0 ± 53	0.6366	3	0.71097
	FAD	7 ± 3	3 ± 1	1 ± 0	1 ± 0	0.1189	3	1.7886
Glycolysis	Glucose	1660 ± 1002	981 ± 108	706 ± 98	1300 ± 525	0.2217	3	1.3895
	Glucose-6-phosphate	107 ± 51	186 ± 51	77 ± 19	166 ± 72	0.3186	3	1.2112
	D-Fructose-1.6-biphosphate trisodium	439 ± 86 a	219 ± 33 ab	137 ± 22 b	222 ± 75 ab	**0.0347**	3	3.1256
	Phosphoenol pyruvate	60 ± 11	23 ± 7	17 ± 5	18 ± 8	0.0711	3	2.1215
	Pyruvate	97 ± 21	93 ± 11	71 ± 5	83 ± 12	0.4499	3	0.91807
Krebs	Acetyl-Coa	10 ± 3	5 ± 2	4 ± 1	7 ± 3	0.6266	3	0.69847
	Citrate	284 ± 72 a	151 ± 17 a	86 ± 12 b	177 ± 61 a	**0.0189**	3	3.691
	Cis-Aconitate	0 ± 0	0 ± 0	0 ± 0	1 ± 0	0.6713	3	0.63815
	Aketoglutarate	32 ± 9	20 ± 5	19 ± 5	40 ± 13	0.8029	3	0.49173
	Succinate	974 ± 66	1086 ± 120	872 ± 72	1621 ± 698	0.6821	3	0.74547
	Fumarate	26 ± 9	8 ± 3	11 ± 3	10 ± 3	0.573	3	0.73507
	Malate	176 ± 56	110 ± 11	102 ± 8	86 ± 16	0.2488	3	1.4826
	Oxaloacetate	277 ± 214	78 ± 28	96 ± 47	125 ± 61	0.9958	3	0.12447
Aerobic	Arginine	5265 ± 1318 a	3944 ± 98 a	3073 ± 120 b	2710 ± 220 b	**0.0008**	3	7.4371
	Glutamate	9436 ± 1578 a	7502 ± 240 **a**	6711 ± 268 **a**	5177 ± 444 **b**	**0.0016**	3	7.7346
	Glutamine	1280 ± 463 a	806 ± 16 a	651 ± 24 b	528 ± 63 b	**0.0009**	3	6.0676
Anaerobic	Lactate	930 ± 389	772 ± 126	607 ± 95	990 ± 357	0.4874	3	0.89482
	Strombine	9814 ± 1609 ac	7858 ± 218 a	6974 ± 290 c	5456 ± 439 b	**0.0006**	3	8.2967
	Octopine	1 ± 0	0 ± 0	1 ± 0	0 ± 0	0.6894	3	0.65521
Essential amino acids	Histidine	2406 ± 464 a	192 ± 100 a	919 ± 52 b	799 ± 52 b	**0.0001**	3	44.914
	Threonine	1001 ± 188 a	762 ± 40 a	608 ± 41 b	589 ± 38 b	**0.0035**	3	6.7457
	Valine	484 ± 115 a	357 ± 11 a	305 ± 16 b	287 ± 13 b	**0.0039**	3	6.9919
	Leucine	465 ± 113 a	341 ± 11 a	280 ± 17 b	288 ± 14 b	**0.0086**	3	5.0958
	Isoleucine	222 ± 51 a	160 ± 6 a	136 ± 8 b	133 ± 7 b	**0.0071**	3	5.8716
	Phenylalanine	534 ± 144 **a**	363 ± 12 ac	322 ± 19 bc	284 ± 21 b	**0.0069**	3	5.7115
	Tryptophane	162 ± 37 a	121 ± 7 a	89 ± 5 b	88 ± 8 b	**0.0016**	3	8.431
	Methionine	239 ± 62 a	154 ± 4 b	123 ± 6 c	96 ± 18 c	**0.0021**	3	2.6935
	Lysine	874 ± 163	724 ± 63	561 ± 40	977 ± 390	0.4375	3	0.97941
Non-essential amino acids	Tyrosine	831 ± 230 a	480 ± 33 ac	400 ± 37 bc	262 ± 56 b	**0.0168**	3	1.8722
	Glycine	19824 ± 3709 a	16143 ± 697 a	14367 ± 619 ab	13176 ± 907 b	**0.0405**	3	3.3228
	Aspartate	12369 ± 1479 a	10803 ± 351 a	9366 ± 387 b	7898 ± 565 b	**0.0014**	3	8.1477
	α-aminobutyric acid	6178 ± 1326 a	7784 ± 509 b	6343 ± 398 b	7433 ± 525 b	**0.0033**	3	6.2768
	Proline	358 ± 104 **a**	269 ± 13 a	216 ± 13 **b**	219 ± 10 **b**	**0.0113**	3	4.6083
	Hydroxyproline	21 ± 3	27 ± 3	24 ±3	29 ± 5	0.2287	3	1.5339
	Cystine	18 ± 5	19 ± 5	19 ± 4	13 ± 3	0.8941	3	0.35057
	Alanine	2128 ± 493	1752 ± 45	1560 ± 105	1574 ± 119	0.5053	3	0.83139
	Serine	2008 ± 469	1479 ± 35	1384 ± 71	1424 ± 61	0.5482	3	0.76739
	α-Aminoadipic acid	80 ± 17	50 ± 5	48 ± 4	36 ± 8	0.19	3	1.2894
	β-aminoisobutyric	803 ± 467	384 ± 21	384 ± 21	411 ± 26	0.2525	3	1.4601
	Betaine	134330 ± 31403	102344 ± 7288	89853 ± 4609	82539 ± 7773	0.1149	3	2.1583

Table 4. Average metabolic ratios of energetic metabolism in ng/mg ± SE and the associated statistics resulting from a one-factor permanova. The significant results (at $\alpha \leq 0.07$) are in bold.

Ratio		Control	Low	Medium	High	P-perm	Df	Pseudo-F
	BCAAs	1171 ± 279 a	859 ± 25 a	721 ± 40 b	709 ± 33 b	**0.0004**	3	3.811
Glucolysis	G6P/D-Fruct-1-6-biphos	0 ± 0 a	1 ± 0 b	0.6 ± 0 b	0.8 ± 0 b	**0.0296**	3	2.8077
	D-Fruct-1-6-biphos/Pyruvate	5 ± 1 a	2 ± 1 b	2 ± 0 b	2 ± 1 b	**0.0222**	3	3.594
Krebs	Citrate/Cis-aconitate	1602 ± 605 a	1007 ± 316 ab	401 ± 96 ab	376 ± 24 b	**0.0559**	3	2.6563
	Malate/Citrate	0.7 ± 0 a	0.8 ± 0 a	1.3 ± 0 b	0.6 ± 0 a	**0.0471**	3	2.9314
	Succinate/Citrate	4 ± 1 a	7 ± 1 b	11 ± 1 b	9 ± 1 b	**0.0022**	3	6.756
	Citrate/Succinate	0.3 ± 0 a	0.1 ± 0 b	0.1 ± 0 b	0.1 ± 0 b	**0.0015**	3	6.756
	Citrate/Pyruvate	3 ± 1 a	2 ± 0 b	1.2 ± 0 b	2 ± 0 a	**0.0074**	3	5.2073
	Pyruvate/Citrate	0.4 ± 0 a	0.6 ± 0 b	0.9 ± 0 b	0.6 ± 0 a	**0.0075**	3	5.2073
Energetic	AEC	0.2 ± 0 a	0.1 ± 0 ab	0.1 ± 0 b	0.1 ± 0 ab	**0.0406**	3	3.0431
	ATP/AMP	0.3 ± 0 a	0.1 ± 0 ab	0.1 ± 0 b	0.1 ± 0 ab	**0.0425**	3	3.0344
	NADP/NADPH	1 ± 0 a	1 ± 1 ab	0.2 ± 0 b	0.4 ± 0 b	**0.0171**	3	3.2761
	NADPH/NADP	1 ± 0 a	7 ± 4 ab	7 ± 2 b	4 ± 1 b	**0.0153**	3	3.2761
	ATP/ADP	14 ± 5	6 ± 1	5 ± 0	5 ± 1	0.1388	3	1.795

1.5.3 Primary stress exploration: oxidative metabolism and prostaglandin chain

We investigated the effect of the noise level on the D-larvae using targeted LC-MS/MS. Of the 46 prostaglandins and oxidative metabolites analyzed, 11 were found to be significantly affected by noise exposure (**Tab. 5**). As revealed by the statistical analysis, these molecules include three cyclopentenone prostaglandins, one eicosapentaenoic acid (EPA) oxylipin prostaglandin, one α-linolenic (ALA) and one γ-linolenic acid (GLA) oxylipins, two oxylipins isomers of hydroxy-docosahexaenoic acid (HDHA), three linoleic acid derivatives, and one from arachidonic acid (PGD2, 13,14-dihydro-15-keto-PGD2, 15-keto-PGF2α, 11-HEPE, 9(S)-HOTrE, 5-HETE, 13-HODE, 13-oxoODE, (±)9(10)-EpOME, 16-HDHA and, 22-HDHA).

The results are displayed through a heatmap of the prostaglandins and metabolites of oxidative metabolism as a function of sound treatment in Figure 23A. The D-larvae submitted to the highest sound levels show higher concentrations of these metabolites compared to the other treatments (P-perm=0,0034, df=3, Pseudo-F=3,2744 and Permdisp: F=5,2219, P-perm=0.0285) (**Fig. 23A**). Exceptions can be observed for 22-HDHA, where oxylipin

isomers concentrations were lower in the high sound treatment (**Fig. 23A, Tab. 5**), and for 11-HEPE and 9(S)-HOTrE, for which levels are the highest in the control (**Fig. 23A, Tab. 5**).

According to the synthetic diagram in Figure 23B, which shows the origin of these prostaglandins and derivatives, the compounds that presented an "inverse" response (*i.e.,* a lower concentration in the shipping noise treatments compared to the control) mainly originate from the same pathway involving omega-3 biosynthesis ($\boldsymbol{\alpha}$-linolenic acid, ALA), which are mainly anti-inflammatory. Finally, embryos exposed to medium noise until they developed into D-larvae showed a decrease in products derived from the omega-6 synthesis pathway (linoleic acid, LA) compared to the control (**Fig. 23B**).

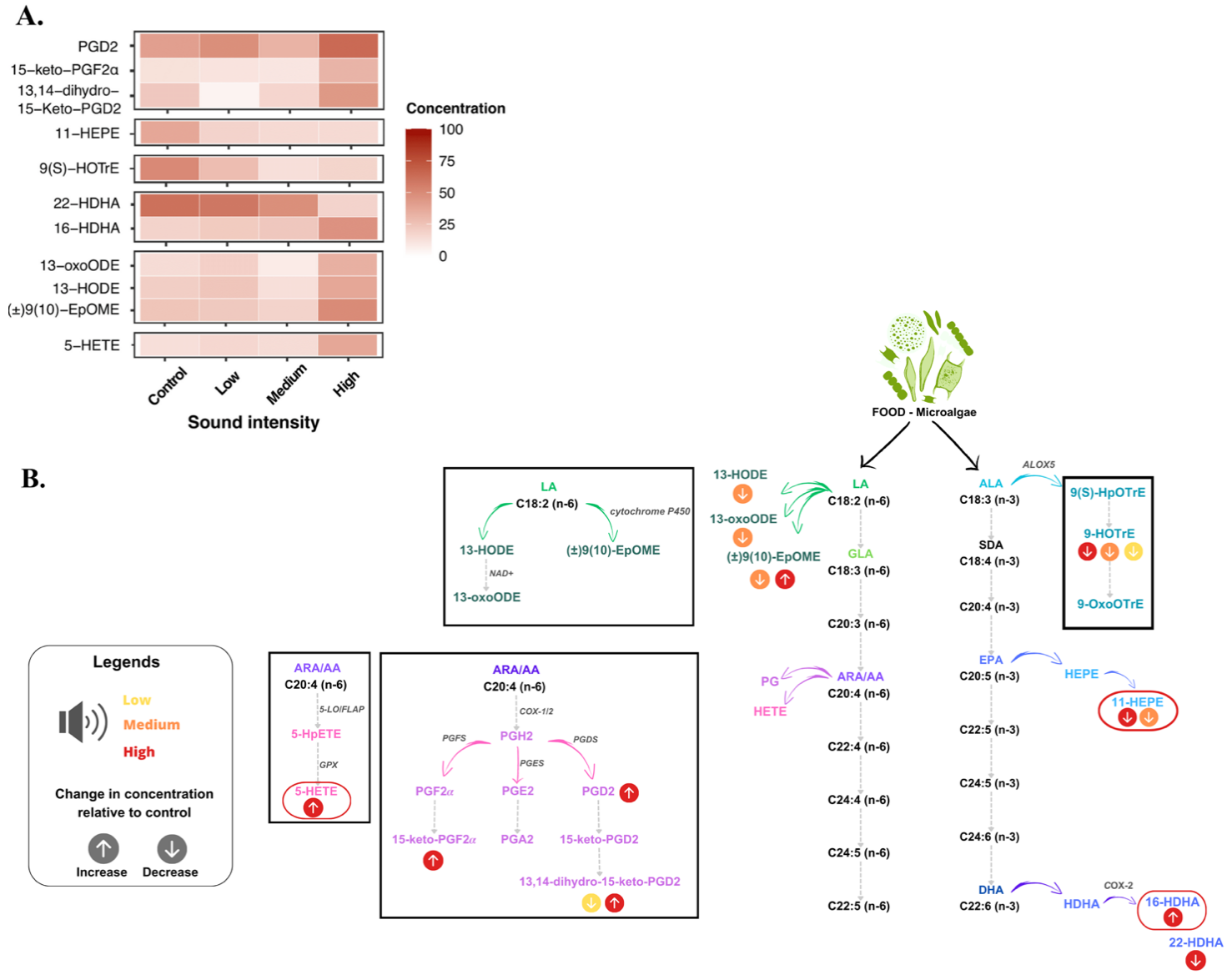

Figure 23. (**A**) Heat map of prostaglandins and metabolites of oxidative metabolism with changes in the average of the normalized concentration (in percent, 0 to 100) by metabolic pathways and sound intensities (control, low, medium, and high). (**B**) Synthetic diagram of the biosynthesis of fatty acids (ω6 and ω3) and eicosanoids linked to the impact of sound exposure (2 days) at different intensities during the embryogenesis.

Table 5. Mean concentrations of metabolites of interest in the oxidative metabolism in ng/mg ± SE and the associated statistics resulting from a one-factor permanova.

Metabolites	Control	Low	Medium	High
Cyclopentenone prostaglandin				
PGE2	1189 ± 296	991 ± 64	757 ± 90	1135 ± 367
PGD2	361 ± 44 **ab**	424 ± 69 **ab**	290 ± 50 **a**	565 ± 92 **b**
PGA2	92 ± 22	129 ± 43	106 ± 27	136 ± 48
PGJ2	795 ± 152	658 ± 68	585 ± 54	808 ± 288
15-deoxy-d12,14-PGJ2	1248 ± 463	1900 ± 607	1240 ± 192	2092 ± 829
11b_PGF2a	187 ± 27	224 ± 46	142 ± 12	294 ± 110
15-keto-PGE2	267 ± 53	287 ± 71	144 ± 17	366 ± 188
15-keto-PGF2α	1069 ± 456 **ab**	952 ± 189 **a**	889 ± 46 **a**	2725 ± 1180 **b**
13,14-dihydro-15-Keto-PGE2	396 ± 78	400 ± 75	231 ± 24	541 ± 191
13,14-dihydro-15-Keto-PGD2	621 ± 205 **abc**	123 ± 53 **a**	456 ± 98 **b**	1179 ± 303 **c**
13,14-dihydro-15-Keto-PGF2α	812 ± 348	767 ± 181	628 ± 124	300 ± 89
EPA oxylipin				
PGE3	462 ± 137	774 ± 245	448 ± 71	829 ± 392
PGF3α	516 ± 172	380 ± 43	276 ± 48	350 ± 92
8-HEPE	18402 ± 4867	12622 ± 1700	11642 ± 894	12475 ± 484
9-HEPE	696 ± 145	639 ± 103	407 ± 67	796 ± 265
11-HEPE	44780 ± 15919 **a**	21437 ± 3000 **a**	17971 ± 1364 **b**	18039 ± 1045 **b**
18-HEPE	1535 ± 440	1691 ± 331	908 ± 128	1454 ± 380
12-HEPE	188 ± 49	185 ± 36	107 ± 20	241 ± 107
15-HEPE	2512 ± 709	1562 ± 275	1619 ± 119	1982 ± 241
ALA-GLA-oxylipin				
9(S)-HOTrE	831 ± 182 **a**	454 ± 165 **b**	218 ± 25 **c**	285 ± 35 **bc**
13(S)-HOTrE	2882 ± 655	2136 ± 230	1835 ± 119	2456 ± 337
13(S)-HOTrE(g)	851 ± 230	523 ± 96	511 ± 92	732 ± 97
Arachidonic-oxylipin				
11-HETE	2118 ± 696	1288 ± 116	1040 ± 45	1141 ± 67
8-HETE	1291 ± 367	980 ± 87	876 ± 29	924 ± 41
9-HETE	87 ± 24	116 ± 27	83 ± 17	140 ± 37
5-HETE	118 ± 40 **a**	148 ± 57 **ab**	128 ± 7 **a**	337 ± 114 **b**
15-oxoHETE	218 ± 54	209 ± 42	128 ± 20	280 ± 122
Linoleic acid				
(±)12(13)-DiOME	105 ± 20	133 ± 45	103 ± 12	257 ± 103
(±)9(10)-DiHOME	134 ± 19	175 ± 45	106 ± 10	209 ± 87
13-oxoODE	190 ± 29 **a**	269 ± 63 **a**	116 ± 13 **b**	451 ± 186 **a**
9-oxoODE	863 ± 136	1069 ± 220	584 ± 45	1388 ± 480
13-HODE	929 ± 80 **a**	1094 ± 202 **a**	601 ± 66 **b**	1723 ± 590 **a**
9-HODE	1114 ± 182	1047 ± 146	733 ± 57	1857 ± 552
(±)12(13)-EpOME	992 ± 217	838 ± 107	640 ± 46	891 ± 280
(±)9(10)-EpOME	1338 ± 255 **abc**	1251 ± 101 **a**	996 ± 52 **b**	2694 ± 770 **c**
HDHA		0	0	0
22-HDHA	345 ± 67 **a**	330 ± 53 **a**	273 ± 45 **a**	101 ± 25 **b**
20-HDHA	793 ± 187	708 ± 169	513 ± 89	1063 ± 273
17-HDHA	3097 ± 799	2331 ± 296	2412 ± 275	4100 ± 1162
16-HDHA	870 ± 223 **a**	1047 ± 70 **a**	1093 ± 125 **a**	2208 ± 553 **b**
13-HDHA	20327 ± 8643	7766 ± 1307	7721 ± 833	7083 ± 775
14-HDHA	583 ± 111	544 ± 46	407 ± 67	912 ± 390
10-HDHA	2273 ± 924	831 ± 182	1330 ± 108	1811 ± 311
11-HDHA	108 ± 25	161 ± 64	78 ± 21	178 ± 61
7-HDHA	374 ± 98	268 ± 66	228 ± 42	423 ± 100
8-HDHA	4246 ± 1058	4891 ± 696	3999 ± 206	8056 ± 2576
4-HDHA	830 ± 181	768 ± 143	515 ± 79	894 ± 275

1.6 DISCUSSION

1.6.1 Characterization of the D-larvae metabolome in the control group

We investigated the metabolic response of blue mussels from the end of embryogenesis to the D-larval stage (4 days), which is a stage that is considered particularly sensitive to stress in this species. We consider targeted metabolomics to be a tool to find chemical signatures that reflect specific cell activities, in this case during mussel embryogenesis to D-larval development. To do so, we analyzed the pathways related to energy metabolism, oxidative stress and prostaglandin production associated with the immune system. At this stage, the organisms are microscopic, so targeted metabolomics was performed on whole individuals, providing an excellent snapshot of what is happening in the organism at a given time (Alfaro & Young, 2018). From the energy and amino acid metabolite profiling, nine metabolites represented 92.2% of the concentration of all measured molecules, with betaine being the main one (61.2%), followed by glycine (9.2%), aspartate (5.8%), strombine (4.5%), glutamate (4.3%), a-aminobutyric acid (2.8%), arginine (2.3%), histidine (1.1%), and sarcosine (1%). These metabolites are mainly amino acids and their derivatives, which are known to be involved in energy metabolism, osmotic regulation, immune function, and cell-cell communication (Cappello et al., 2018). In adult bivalves, the adenylate energy charge index (AEC) ranges from 0 to 1 and indicates energetic stress when close to 0 (Moal et al., 1989; Myrand et al., 2007). With values below 0.5, D-larvae appear stressed, but as this index has never been used for larvae, their high metabolic activities associated with intensive tissue production could explain the observed general values below 0.5.

In a second step, the prostaglandins and other biologically active derivatives of polyunsaturated fatty acids, the oxylipin family, were quantified for the first time in the blue mussels' D-shaped larvae. Prostaglandins are known as mediators of many biological responses in vertebrate and invertebrate organisms (Stanley-Samuelson, 1987). They are not preformed mediators and their biosynthesis is from a common precursor, such as arachidonic acid, which generally occurs *de novo* after chemical, immunological or mechanical

stimulation. Some prostaglandins, such as cyPGs, are rapidly metabolized by enzymatic reactions to promote the oxidation of polyunsaturated fatty acids (PUFAs) from phospholipids, which are particularly involved in inflammatory processes (Lee et al., 2021). The highest concentrations of oxylipins observed were related to omega-3 (ω-3) with 11-HEPE (from eicosapentaenoic acid, EPA, C20:5 ω-3) around 45 $ng.mg^{-1}$, 8-HEPE ~ 18 $ng.mg^{-1}$, 15-HEPE ~ 2.5 $ng.mg^{-1}$, 13(S)-HOTrE (from a-linolenic acid, ALA, C18:3 ω-3) ~ 3 $ng.mg^{-1}$, 8-HDHA (from docosahexaenoic acid, DHA, C22:6 ω-3) ~ 4.3 $ng.mg^{-1}$, 10-HDHA ~ 2.3 $ng.mg^{-1}$, 13-HDHA ~ 20 $ng.mg^{-1}$, and 17-HDHA ~ 3.1 $ng.mg^{-1}$. Only one measured oxylipin derivative was related to omega-6 (ω-6) with 11-HETE (from arachidonic acid, ARA, C20:4 ω-6) around 2.2 $ng.mg^{-1}$. All the others were below 1.5 $ng.mg^{-1}$. In mammals, oxylipins derived from ω-6 fatty acids have a pro-inflammatory, vasoconstrictive, and proliferative effect compared to those derived from ω-3 fatty acids (Lee et al., 2021). Balseiro et al. (2013) showed that the genes related to immune capacity were expressed during mussel development, as early as the trochophore stage. At this stage, genes expressions were mostly related to maternal origin, but stimulation induced the expression of immune-related genes, which increased after the veliger stage in preparation for larval settlement.

1.6.2 Impact of noise pollution on the metabolome of D-shaped larvae at the end of embryogenesis

1.6.2.1 First reaction of metabolism via polyunsaturated fatty acid oxidation products and evidence of apoptotic process

Oxylipins from the oxidation of ARA, DHA, and LA were found in larvae exposed to the loudest sound condition compared to the control. Inactivated prostaglandins from ARA, 15-keto-PGF2α and 13,14-dihydro-15-keto-PGD2α may indicate a higher oxidation of arachidonic acid to the proinflammatory mediators PGF2α and PGD2, suggesting higher oxidative stress in D-shaped larvae exposed to high noise (Basu, 2007). In addition, HETE metabolites seem to be primarily proinflammatory with a biological activity in the defense

role (chemotactic role in leukocytes) (Gabbs et al., 2015). The response to noise appears to trigger the activation of enzymatic pathways via lipoxygenase (LOX), via 5-lipoxygenase (5-LOX), via 15-lipoxygenase (15-LOX), via cyclooxygenase (COX), or via autoxidation (**Tab. 6**). 16-HDHA and 22-HDHA were significant in the analysis suggesting differential activation of enzymes that produce resolvins, protectins, and maresins, which affect, among other things, the resolution of inflammation (Ferreira et al., 2022). Like HDHA, 11-HEPE also produces resolvins, which play a role in the resolution of inflammation.

Table 6. Synthesis of the different oxylipin activation pathways (Ferreira et al., 2023; Gabbs et al., 2015; Geng et al., 2024; He et al., 2012; Loomba et al., 2015; McGurk et al., 2022; Saleh et al., 2021).

ω -3	ALA	9(S)-HOTrE	5-LOX
	EPA	11-HEPE	LOX, Autoxidation
	DHA	16- HDHA	LOX, Autoxidation
		22- HDHA	CYP-Hydroxylases
ω-6	ARA	5-HETE	5-LOX
		PGD2	COX
		13,14-dihydro-15-keto-PGD2α	COX
		15-keto-PGF2α	COX
	LA	13-oxoODE	15-LOX
		13-HODE	LOX
		(±)9(10)-EpOME	CYP-Epoxygenase

The greater activity of these oxylipins at high sound exposure could have two explanations related to tissue remodeling: Larval development involves mechanical remodeling of shape that affects their behavior, which requires control mechanisms involved

in tissue distribution and organ system differentiation (Ackerman et al., 1994; Bassim et al., 2014b) (**Fig. 21A, B**). This remodeling in embryos is characterized by fast cell division leading to their segmentation (Cao et al., 2004; Bassim et al., 2014b; Kniazkina & Dyachuk, 2022). We did not observe any variation in the shell size of D-larvae with noise exposure, but internal tissue development was not explored. However, the concentration of 9(S)-HOTrE was lower in sound-exposed organisms, suggesting that the synthesis of HOTrE from ALA may be reduced, as observed in human T cells from older individuals. This mechanism is a possible age-related immune dysregulation known as immunosenescence (Rodriguez et al., 2021; Gerichten et al., 2022a; 2022b). The other hypothesis to explain the greater activity of oxylipins in D-larvae under high shipping noise could be a potential tissue degradation, which can be summarized by the increased catalytic activity of destruction enzymes, *i.e.* apoptosis processes by phagocytosis (Dyrynda et al., 1995; Estévez-Calvar et al., 2013) or necrosis (uncontrolled cell death). Although these processes release reactive oxygen metabolites (ROS), we found no increase in larval mortality, and embryogenesis success in noise-stressed larvae remained the same as in controls. Neither of these two hypotheses seems to be clearly supported by the results obtained, so sampling throughout embryogenesis and perhaps a few days after hatching may be necessary to determine whether tissue development in D-larvae is accelerated or impaired by shipping noise. Overall, cargo shipping noise appears to modulate the production of lipid mediators from PUFA, possibly due to differences in ROS activity, leading to possible differences in the inflammatory process between control and noise-exposed D-shaped larvae (Veillard et al. *in press*, see Chapter 1, Article 2).

1.6.2.2 Increased energy demand and its complications

The decrease in the ATP/ADP and ATP/AMP ratios observed in D-larvae exposed to shipping noise could be interpreted as an increased energy demand of the cells, as ADP is converted to ATP and AMP can activate AMPK and other metabolic pathways to increase energy production in respond to metabolic demands. This can occur during intense cellular activity or in response to various cellular stresses. In addition, the AEC index was three times

lower in D-larvae exposed to shipping noise compared to the control. All these indicators suggest a higher metabolic demand for D-larvae exposed to all shipping noise treatments. Studies have shown that anaerobic metabolism is involved not only when oxygen uptake is blocked (natural anaerobiosis) but also when the energy requirements cannot be met by the respiratory capacity alone (de Zwaan et al., 1983a). Although glycogen is the major anaerobic energy source, its fermentation in most organisms does not lead to lactate accumulation, as observed here in this study, but rather to other anaerobic end products, such as succinate, alanine, and strombine, which have been previously identified as fermentation end products in invertebrates (de Zwaan et al., 1980; Malanga & Aiello, 1972; Stokes & Awapara, 1968). Here, alanine and strombine formation were important in the control group, but succinate was higher in D-larvae from shipping noise treatments. Strombine has been shown to be a product of glycolytic function during recovery, and not an end product of anaerobic metabolism in oysters (de Zwaan et al., 1983b; Fields et al., 1980; Georgoulis et al., 2022). The increased concentration of this metabolite in the control could be related to glycolytic energy production to meet overall tissue energy requirements of recovery after embryogenesis. A modified scheme of anaerobic metabolism appears to occur between the exposure conditions. The two usual end products of anaerobic metabolism (succinate and alanine) could be formed from different precursors (Collicutt & Hochachka, 1977; de Zwaan et al., 1982). In fact, the metabolism of glucose and aspartate is closely linked, with most of the glucose carbon being converted to alanine, while the aspartate carbon appears as succinate.

1.6.2.3 Disruption of glycolysis

A high G6P/F1,6BP ratio in the shipping noise treatments suggests an accumulation of G6P relative to F1,6BP, which could indicate a decrease in downstream glycolytic activity, perhaps due to inhibition of enzymatic or saturation of downstream pathways. In addition, the F1,6BP/pyruvate ratio was low, indicating a decrease in glycolysis activity, with a reduced conversion of F1,6BP to pyruvate, in contrast to the control. A low F1,6BP/pyruvate ratio may indicate defects in glycolysis, such as an enzyme deficiency or metabolic

dysfunction, leading to the accumulation of F1,6BP or slowing of its conversion to pyruvate. In addition, the high concentration of glutamate in the control group is more likely to play an important role as an intermediate amino group carrier in the transfer of amino groups from aspartate to alanine (Hochachka & Mustafa, 1972; Hochachka et al., 1973; de Zwaan et al., 1982). This relationship was supported by the enrichment analysis which highlighted pathways corresponding to amino acid recycling through transamination (aspartate metabolism) and oxidative deamination (glutamate metabolism). The mechanisms of detoxification of the ammonium ion, via the urea cycle and ammonia recycling produced by transamination or deamination are the main pathways (de Zwaan et al., 1982). Indeed, transamination or deamination leads to the production of ammonia (NH3), which is toxic for the organism, especially for the central nervous system (Cappello et al., 2018). It seems here that the cells recycle the excess of amino acid (which they cannot store) for the synthesis of another amino acid that can be used for the synthesis of carbohydrates (AA glucoformers), or for the synthesis of fatty acids (ketogenic AA). Concentrations of these amino acids were lower in D-larvae exposed to shipping noise treatments compared to the control, indicating a higher demand for amino acid catabolism, *i.e.* a greater energy demand caused by noise-induced stress. BCAAs (branched-chain amino acids: sum of valine, leucine, and isoleucine) are essential metabolites that provide energy and, as proteinogenic amino acids, act as precursors for the biosynthesis of new molecules and cells (role in regulating of protein turnover processes) (Calder, 2006), and this supports the hypothesis with a lower concentration of BCAAs in noise conditions. In fact, in the muscle, valine, leucine and isoleucine are used to produce ATP via the Krebs cycle by synthesizing ketone bodies such as acetoacetate and acetyl-CoA or succinyl-CoA (through glycolysis) (Dumas et al., 2020; Martínez-Reyes & Chandel, 2020; Neinast et al., 2019; I. M. Sokolova et al., 2012). This increased metabolic activity may lead to the generation of ROS as by-products of mitochondrial electron transport (Mullarky & Cantley, 2015; Trevisan & Mello, 2024), which may promote the development of defenses against oxidative stress, as indicated by changes in glutamate and glutamine levels, which reflect changes in the activity of the glutathione cycle (Lu, 2013).

1.6.2.4 Protection against oxidative stress

A high NADPH/NADP ratio in larval batches from shipping noise treatments suggests that their cells have an increased ability to reduce other molecules, which is essential for many anabolic reactions, including the synthesis of lipids, nucleotides, and other organic compounds (Trevisan & Mello, 2024). NADPH is also essential for the regeneration of glutathione, an important antioxidant, and for the detoxification of free radicals and reactive oxygen species (Imlay & Linn, 1988; Trevisan & Mello, 2024). Therefore, a high NADPH/NADP+ ratio may indicate an increased ability to neutralize oxidative stress. The citric acid cycle (TCA, Krebs cycle), citrate concentration (important intermediate) was lower under shipping noise conditions: A decrease in the citrate/pyruvate ratio may indicate a metabolic imbalance or cellular stress due to increased citrate production (Martínez-Reyes & Chandel, 2020; Sokolova et al., 2012). During embryogenesis, larvae undergo dramatic molecular and functional changes, including shell biogenesis and neurogenesis, which are supported by egg lipid reserves. Early larval stages represent a critical point in development, when the blueprint for calcification and neurodevelopment is established (Miglioli et al., 2021) . Studies have demonstrated links between oxidative stress, metabolic costs, and immunity during early life stages of marine bivalves (Estévez-Calvar et al., 2013). In particular, the establishment of a balanced cellular redox system to scavenge ROS produced in the oocyte before and during fertilization (Diaz de Cerio et al., 2013).

1.6.3 Potential ecological consequences including latent effects

Ship noise intensity treatments corresponded to larval exposures at a distance from a passing ship ranging from ~20m up to ~2000m (Veillard et al. *in press*, see Chapter 1, Article 2). The actual noise exposure that larvae are subjected to is determined by both the local conditions that enhance or reduce sound transmission (Hildebrand, 2009), such as the hydrodynamics (instantaneous currents and/or turbulence or residual circulation), and factors that control the spatial patterns of larval dispersal (Bayne, 1964). Nonetheless, this study demonstrates that the acoustic imprint in the immediate vicinity (< 2 km) of a cargo ship

could significantly and negatively affect the energetic metabolism of larvae during their transformation to D-larvae. However, changes in metabolomic patterns associated with shipping noise exposure did not induce either increased mortality or changes in D-larval size. Embryogenesis is a short critical phase (< 4 days) in the larval development of the blue mussel, so sites with high larval retention potential, such as bays or estuaries with intense shipping traffic (in or near ports), may represent the most extreme noise exposure sites. In addition, stress occurring during early development can manifest itself later in development and significantly affect the performance (fitness) of individuals (Beckerman et al., 2002). If the cells are damaged during this period, there is a risk that the development, growth, survival, and reproduction of the organism will be impaired later, during larval, juvenile, or adult stages (Burruel et al., 2014). Here, we did not observe morphological changes related to vessels noise exposure, but a clear and significant cellular stress affected D-larvae. Podolsky and Moran (2006) described potential scenarios for carryover effects of experiences during one life-history stage on performance and selection at subsequent stages. They demonstrated three possible scenarios: one in which carryover effects simply persist over time (persistence), another in which they are amplified in subsequent stages (amplification), and another in which they are mitigated (or even reversed) by compensatory effects (compensation). In our study, the metabolic pathways identified involved responses to, and compensation for, a stressor, in this case, noise pollution. In nature, however, the stressors for marine invertebrates from coastal habitats could be multiple, especially in the context of climate change. The potential delayed effects of shipping noise stress during embryogenesis at the larval, juvenile and adult stages need to be studied, specifically under conditions of multiple stressors, in order to better understand the influence of anthropogenic, induced environmental change at the population level.

1.7 ACKNOWLEDGEMENTS

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ARTICLE 2
PREUVE MOLÉCULAIRE DE L'IMPACT DU BRUIT DES NAVIRES SUR LA MOULE BLEUE, UNE ESPÈCE CLÉ POUR LA DURABILITÉ DES ENVIRONNEMENTS MARINS CÔTIERS

2.1 RÉSUMÉ EN FRANÇAIS DU DEUXIÈME ARTICLE

Le bruit océanique d'origine anthropique dû à la navigation a augmenté jusqu'à 12 dB re 1 µPa dans certaines régions au cours des cinq dernières décennies, mettant en danger les organismes marins. Si l'impact du bruit sur les mammifères marins est bien documenté, ses effets sur les mollusques, qui ont une importance économique et écologique considérable, restent largement inconnus. Afin d'étudier les conséquences du bruit sur le métabolisme des mollusques au cours des premières étapes cruciales de leur vie, des larves de moules ont été exposées au bruit des navires en laboratoire jusqu'au stade post-larvaire et leur métabolome a été analysé. Des analyses multivariées du métabolome ont montré que le bruit de la navigation induit une inflammation liée au stress, une demande énergétique accrue, un renouvellement plus important des protéines et une activité perturbée du système nerveux. Par conséquent, le bruit a favorisé une métamorphose retardée dans des habitats sous-optimaux avec des coûts métaboliques plus élevés, ce qui peut avoir un impact sur la durabilité de l'écosystème et de l'aquaculture, car les larves de moules compétentes luttent pour sélectionner des habitats de développement appropriés. Si aucune mesure n'est prise pour limiter le bruit sous-marin, de tels impacts pourraient perturber les structures des populations et la biodiversité marine.

Mots-clés : Pollution sonore, *Mytilus edulis*, Post-larves, Métabolisme, Métabolomique.

Cet article, intitulé « *Molecular evidence of shipping noise impact on the blue mussel, a key species for the sustainability of coastal marine environments* », est accepté avec des révisions mineures par les éditeurs de la revue *Marine Ecology Progress Series*. Stéphane

Beauclercq et moi-même, en tant que premier auteur, nous avons contribué à l'essentiel de la recherche sur l'état de la question, sur la rédaction du manuscrit et la réalisation des analyses chimiométriques et fonctionnelles. J'ai plus particulièrement élevé les larves de moules, effectué les mesures de performance post-larvaire et contribué à l'analyse ciblée par spectrométrie de masse. Stéphane Beauclercq a conçu et réalisé les expériences métabolomiques basées sur la RMN. Nathan Ghafari et Bertrand Genard ont réalisé les expériences de spectrométrie de masse non ciblées et ciblées, respectivement. Lekha Sleno a supervisé les expériences de spectrométrie de masse non ciblée. Alexandre A. Arnold a apporté son soutien technique à la spectroscopie RMN. Anne Choquet a contribué à la discussion sur les lois et règlements. Stéphane Beauclercq, Frédéric Olivier, Dror E. Warschawski, Isabelle Marcotte, et Réjean Tremblay ont conçu et supervisé l'étude. Tous les auteurs ont contribué à la rédaction du manuscrit et ont approuvé l'article final. À cet article a fait l'objet de plusieurs communications orales lors de congrès. Une version abrégée de cet article a été présentée au congrès *World Aquaculture Society (WAS), Aquaculture Association of Canada (AAC) et Newfoundland Aquaculture Industry Association (NAIA)* à St John's (Newfoundland, Canada) en Août 2022, à la conférence annuelle de *Ressources Aquatique Québec* en novembre 2022 à Québec, au *4th International Symposium for Advances in Marine Mussel Research (AMMR4)* en Angleterre en novembre 2022 ainsi qu'au *115th NSA Annual Meeting* à Baltimore dans le Maryland en mars 2023.

MOLECULAR EVIDENCE OF SHIPPING NOISE IMPACT ON THE BLUE MUSSEL, A KEY SPECIES FOR THE SUSTAINABILITY OF COASTAL MARINE ENVIRONMENTS

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2.2 ABSTRACT

The global anthropogenic oceanic noise due to shipping has doubled in just 11.5 years, putting marine organisms at risk. While the impact of noise on marine mammals is well documented, its effect on molluscs, which hold immense economic and ecological importance, remain largely unknown. To investigate the consequences of noise on mollusc metabolism during crucial early life stages, blue mussel larvae were exposed to shipping noise in a laboratory setting until the post-larval stage and their metabolome was analysed. Multivariate analyses of the metabolome showed that shipping noise induces stress-related inflammation with increased energy demand, higher protein turnover, and disrupted nervous system activity. Consequently, noise promoted a delayed metamorphosis in suboptimal habitats with greater metabolic costs, which may impact ecosystem and aquaculture sustainability as competent mussel larvae struggle to select suitable development habitats. Without action to limit underwater noise, such impacts could disrupt population structures and marine biodiversity.

Keywords : Noise pollution, *Mytilus edulis*, Post-larvae, Metabolism, Metabolomics**.**

2.3 INTRODUCTION

Over the last 50 years, shipping noise increased the ocean ambient noise in the Northeast Pacific ocean by 3-12 decibels referenced to a pressure of 1 microPascal (dB re 1 µPa) and may double globally even every 11.5 years, essentially due to the rising number of commercial vessels (Andrew et al., 2002; Frisk, 2012; McDonald et al., 2006). In 2017, during the Conference of the Parties of the "Convention on the Conservation of Migratory Species*"* (United Nations) (UNEP/CMS/Resolution 12.14, Adverse Impacts of Anthropogenic Noise on Cetaceans and Other Migratory Species, 2017), anthropogenic marine noise – primarily associated with shipping (90% ; Chauvaud et al., 2018) – has been recognized as an emerging and least understood source of pollution, representing 'a potential threat to marine species conservation and welfare'. Boats are known to generate low frequency noise (5–500 Hz up to 10,000 Hz) that can reach 190 dB re 1µPa at 1m and propagate over long distances, affecting marine organisms by a variety of effects ranging from physical damage to masking relevant biological signals (Duarte et al., 2021; Hawkins & Popper, 2017). For decades, there has been strong evidence of the negative impact of noise on marine mammals and fish, both of which possess important auditory senses. These include behavioral, habitat, and physiological changes leading to an increase in mortality in the most extreme cases (Duarte et al., 2021). The effects on mollusks are still mostly unknown despite studies showing their ability to produce and perceive sounds through their statocysts (balance sensory receptor) and abdominal sense organs (Solé et al., 2023).

Mollusks represent 23 % of the life diversity in oceans and among them, bivalves are especially known to be key species for the development, functioning, and sustainability of coastal environments (Smaal et al., 2019). Exposure of mollusks to anthropogenic noise could have a dramatic impact on the entire marine ecosystem and therefore must to be assessed. The auditory sensing range of adult mollusks is not fully known; nevertheless the adult blue mussels (*Mytilus edulis*) have been shown to respond by valve closure to noise between 5 and 410 Hz (Chauvaud et al., 2018; Roberts et al., 2015). Anthropogenic noise impact in bivalves includes oxygen consumption changes (Wale et al., 2019), increased

biochemical stress (Vazzana et al., 2016), metabolism adjustment (Peng et al., 2016), reduced growth and development (de Soto et al., 2013), modification of the recruitment process (Cervello et al., 2023; Jolivet et al., 2016) and even increase mortality (Day et al., 2017). From a behavioral perspective, anthropogenic noise could disrupt feeding and feeding rates (Spiga et al., 2016), due to a reduction in valve gape opening (Day et al., 2017; Ledoux et al., 2023). However, very few data are available on early life or larval stages of bivalves, which may be more sensitive (Rayssac et al., 2010) and are critical stages as pediveliger larvae use environmental stimuli to settle in an optimal habitat and undertake their metamorphosis (Hadfield & Paul, 2001; Jolivet et al., 2016; Lillis et al., 2013; Schmidlin et al., 2024). If settlement conditions are unsuitable, pediveliger larvae can prolong their pelagic dispersal life and delay their metamorphosis for several weeks (Martel et al., 2014; Pechenik, 1990). Moreover, anthropogenic sound, like vessel noise, increases mussel settlement (Jolivet et al., 2016; Wilkens et al., 2012). Metamorphosis represents a pivotal life-changing event for numerous molluscan species, marking the transition of free-swimming pelagic larvae to attached (sessile) benthic post-larvae (Joyce & Vogeler, 2018). At the pediveliger stage, mussel larvae attain “metamorphic competence”, initiating their transformative process with irreversible morphological and physiological changes (Hadfield, 2000). These changes include the development of a foot-like structure, loss of the velum, gill development, and secretion of the juvenile shell. This metamorphic transition relies heavily on environmental cues to guide the larvae in selecting an optimal habitat for settlement and subsequent development (Hadfield & Paul, 2001) **(Fig. 24)**.

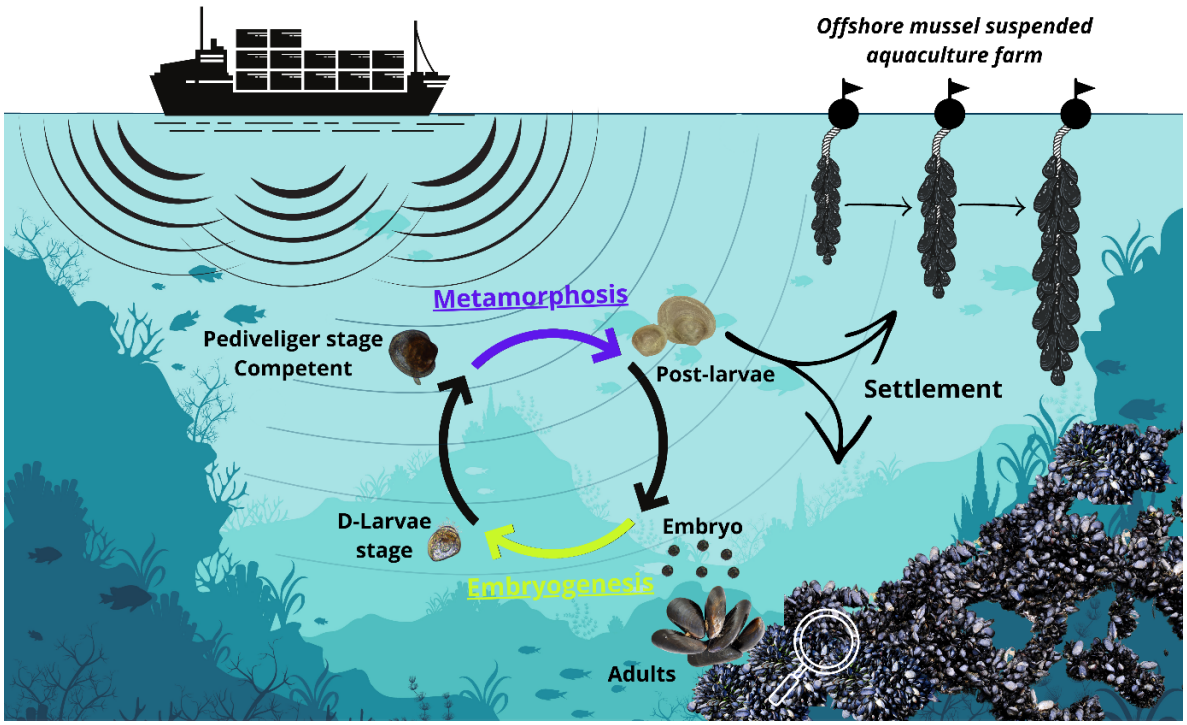

Figure 24. Diagram illustrating blue mussels' development in an environment perturbed by shipping noise.

Among bivalves, the blue mussel holds immense economic and ecological importance with a worldwide aquaculture production surpassing wild catches, reaching almost 170,000 t annually, for a revenue exceeding 300 million US$, according to the Food and Agriculture Organization (FAO, 2019). Mussel farming is a source of sustainable proteins and omega-3 fatty acids (FAs). Additionally, it plays a crucial role in ecology by promoting biodiversity. Indeed, *Mytilus* is a filter-feeding organism that limits eutrophication by consuming microalgae, which enhances the luminosity for benthic algae and increases the production of other benthic organisms (Borthagaray & Carranza, 2007). The mussel sessility and coastal lifestyle make them particularly exposed to repeated or long-lasting anthropogenic noise. Indeed, their habitat often colocalizes with zones of intense ship traffic as is the case in the Gulf of St. Lawrence where 98% of the Canadian mussels are produced. Canada being the first producer in the Americas and third-largest worldwide (Statistics-Canada, 2019). With

up to 60,000 ships traversing the Gulf on a monthly basis (Pelo & Wootton, 2004), effective management and prevention strategies are imperative.

The United Nations Convention on the Law of the Sea (UNCLOS) establishes a general obligation for member states to protect and preserve the marine environment in 1982 (Article 192, The Union Nations 1982). To address noise pollution from ships, both national and international legal frameworks are evolving. The European Union's Marine Strategy Framework Directive, specifically Descriptor 11, aims to prevent adverse effects of energy introduction, including underwater noise (Directive 2008/56/EC, European Union 2008). The International Maritime Organization (IMO) has also taken steps; in 2023, the Marine Environment Protection Committee approved revised guidelines for reducing underwater radiated noise from shipping, effective from October 1, 2023 (International Maritime Organization, Revised Guidelines for the Reduction of Underwater Radiated Noise from Shipping to Address Adverse Impacts on Marine Life, MEPC.1/Circ.906, 22 August 2023., 2023). These guidelines build upon previous ones and encourage member states to apply them in collaboration with relevant stakeholders. However, thresholds specifying the acceptability of sound levels are lacking for the enforcement of those guidelines.

In this study, the response of mussel larvae to cargo-ship sounds at different intensities recorded in the Gulf of St. Lawrence was evaluated. To archive this, acoustic perturbations were applied during larval metamorphosis and the subsequent stress on the post-larvae's metabolome, *i.e.*, the ensemble of all the small molecules found in an organism, examined along three axes. The first axis focused on lipidic mediators, such as prostaglandins and oxylipins. These molecules are responsible for processes such as inflammation (Lee et al., 2021)**.** A targeted lipidomics approach was used to analyze the. This approach screened for 11 prostaglandins and 38 oxylipins. These oxylipins derived from the eicosapentaenoic, α/γ-linolenic, arachidonic, linoleic, and docosahexaenoic acids. The analysis was performed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS/MS). Secondly, the polar metabolites involved in the general metabolism (mainly organic acids, nucleosides, and nucleotides) were characterized by a combination of

untargeted nuclear magnetic resonance (NMR) spectroscopy and LC-HRMS/MS-based metabolomics. This approach was enriched by an extensive targeted study of the amino acid (21 compounds) and energy (27 compounds) metabolism by LC-HRMS/MS. Finally, the third axis studied the changes in larval performance and behavior in relation to metabolism alterations, considering that noise is a possible cue for larval settlement. This combined approach based on complementary analytical methods provided new insights in the metabolic pathways impacted by this emerging pollutant in bivalves and its implication for the ecosystems and mussel aquaculture sustainability.

2.4 MATERIALS AND METHODS

2.4.1 Shipping noise sampling

The noise from a 120 m long roll-on/roll-off cargo ship built in 2000 (Nohlan Ava), leaving the most intense and distinguishable noise fingerprint in St-Pierre-et-Miquelon's harbor (France), was recorded between November 2020 and April 2021 using an Aural-M2 (Multi-Electronique Inc., Rimouski, QC, Canada) underwater acoustic recorder (sampling frequency of 32 kHz, 16 bits resolution) equipped with a HTI-96-MIN hydrophone (High Tech Inc., Long Beach, MS, USA) with a sensitivity of −165 dB re V/µPa and set with a gain of 22 dB. The sampling frequency (32 kHz) allowed to capture the entire acoustic signature of the vessel as the maximum of energy is below 10 kHz. The sound sequence was selected with Audacity® software to create an 80 min soundtrack composed of the sound of the cargo ship arrival (11 min), a 39 min break (simulating handling in harbor), its departure (8.5 min), and a new break of 21.5 min (10.5281/zenodo.10910123). The loudest vessel in the archipelago was selected and looped to standardize exposure to anthropogenic noise, as this approach avoided the complexities of replicating the variable acoustic landscape (*e.g.,* day-night cycles, weekly recreational peaks, and weather-driven fluctuations). The equivalent sound levels, expressed in sound pressure level RMS, are detailed in **Table 7**.

Table 7. Table of equivalent sound levels in sound pressure level RMS (SPL_{RMS}) measured.

Sound pressure level (SPL) RMS measured (dB re 1µPa)				
Conditions	**Arrival**	**Departure**	**Mean**	**SE**
Control	117.0	115.8	116.4	0.8
Low	124.3	118.2	121.3	4.3
Medium	128.8	125.3	127.1	2.5
High	152.6	149.2	150.9	2.4

2.4.2 Mussel larval culture and sound exposure

Blue mussel (*Mytilus edulis*) larvae rearing was performed as described in Rayssac et al. (2010) (**Fig. 25**). Briefly, 50 mm adult mussels from St. Peters Bay in Prince Edward Island (46.42948N, -62.66030W, Canada) were individually induced to spawn by 10°C thermal shock. A ratio of 10 spermatozoids per egg was used for the fertilization in UV ultra-filtered (1 µm) seawater at 18°C. Eggs were further washed with UV ultra-filtered seawater and maintained at 18°C. The obtained embryos were divided into 6 tanks of 60 L at a density of 5 larvae ml^{-1} maintained at 18°C until over 50% of the population was at the pediveliger stage (competent larvae) at 17 d post-fecundation (dpf) *i.e.,* eyed larvae, foot development and substrate exploration. Then, the six batches were randomly distributed in the 24 cylinders of 5 L in the four Larvosonic mesocosms tanks. Larvosonic is an innovative tank system composed of six circular culture cylinders of 5 L (unit of replication with 25,000 larvae for each cylinder) designed for the exposure of organisms to sounds which limits classical reverberation and resonance problems (Cervello et al., 2023; Olivier et al., 2023). Temperature (18.1 ± 0.1°C), salinity (23.7 ± 0.4 PSU), photoperiod (15h of light and 9h of night) and light intensity (9.0 ± 0.2 40 FC; 97.1 ± 1.7 400 Lux) were monitored during the experiments. During rearing and noise exposure experiments, larvae were fed every two days 60 cells.μl^{-1} of a mixed suspension of microalgae containing *Pavlova lutheri* (CCMP459), *Tisochrysis lutea* (CCMP1324), *Chaetoceros muelleri* (CCMP1317), *Tetraselmis suecica* (CCMP904), and *Nannochloropsis oculata* (CCMP525) at a ratio of 1:1:1:1:1 equivalent

biomass (dry weight). Collectors (constituted from two 30 cm polypropylene ropes) were introduced in the cylinders to allow the settlement of the larvae and the exposition to the recorded shipping sound started. During the metamorphosis stage, pediveliger (17 dpf) to post-larvae (25 dpf) were exposed in each *Larvosonic* tank to a different sound treatment for nine days (**Fig. 25**): low (121 ± 4 dB re 1µPa), medium (127 ± 2 dB re 1µPa), high (151 ± 2 dB re 1µPa), and silence playback with ambient room noise (control; 116 ± 1 dB re 1µPa) (**Tab. 7**). These sound levels are maximum emission level and related to a distance from the source (cargo ship), with a greater sound intensity the closer the source is and vice versa, thus corresponding to 18.5 m (high sound exposure), 735 m (medium), 1.8 km (low) from the source and compared to ambient lab conditions (control). This distance was calculated according to the sound propagation loss also called transmission loss (TL) formula taking the base of $15\log_{10}$ (**Fig. 26**). Every two days, collectors were carefully removed from each cylinder, hung up in the air to avoid juvenile detachment and the cylinders cleaned with Virkon VKS10 disinfectant (LANXESS Deutschland GmbH, Cologne, Germany) before water changing with UV ultrafiltered seawater and monitoring of the larval health, growth and survival rate by optical microscopy.

At the end of the sound exposure experiment, collectors were gently rinsed with sprayers on a 100 µm mesh sieve to retrieved post-larvae from the 24 cylinders (6 replicates per sound treatments). Settled growing post-larvae on the cylinder walls were carefully brushed and pooled with the post-larvae from the collectors to estimate the total wet biomass of recruits. Immediately after collection, larval pools were split into three sub-samples, *i.e.*, one for targeted metabolomics, and two for the untargeted metabolomics experiments presented in this article (if the quantity of post-larvae recovered allowed it), flash-frozen in liquid nitrogen, freeze-dried, and stored at −80°C until extraction for metabolomics.

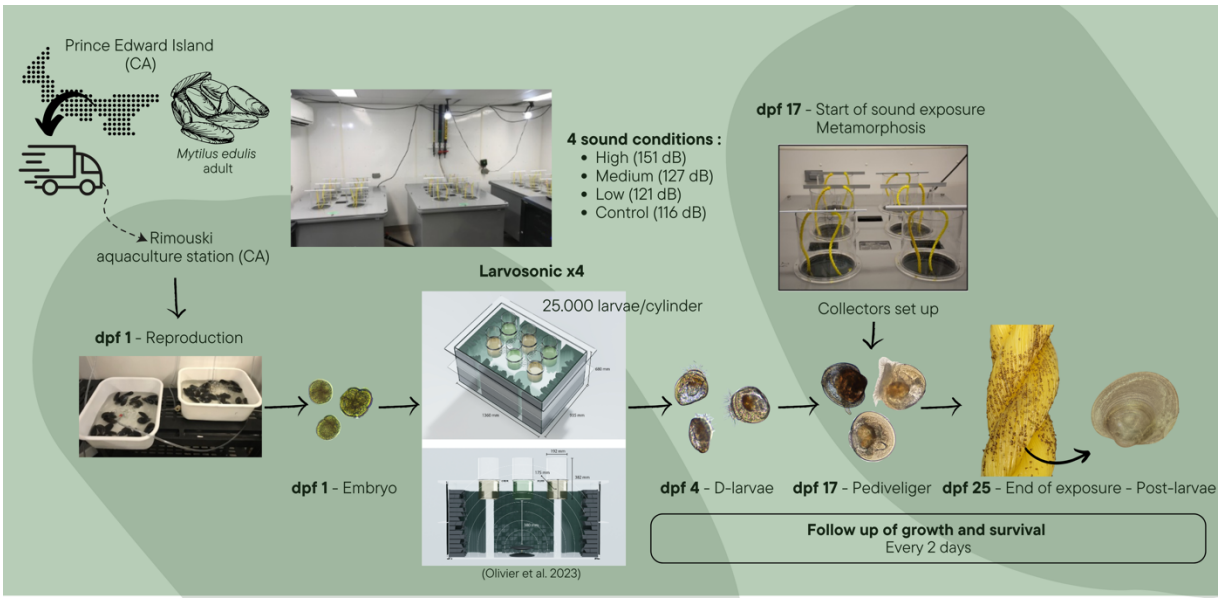

Figure 25. Experimental procedure developed for the exposition of blue mussel larvae during metamorphosis to shipping noise. dpf: day post-fecondation.

$$TL = 15\log_{10}(x)$$

$$170 - 151 = 15\log_{10}(x)$$

$$19 = 15\log_{10}(x)$$

$$\frac{19}{15} = 10^{x}$$

$$x = 10^{\frac{19}{15}}$$

$$x = 18.47\ m$$

Figure 26. Calculation of the estimation of the transmission loss (TL) of the different sound intensities to evaluate the exposure distance (x) from the source (cargo-ship). Examples of calculation for high sound exposure at 151 dB. Based on the original in situ sound recordings, we hypothesised a vessel noise source level of 170 dB re 1 µPa.

2.4.3 Growth and metamorphosis success

Larval growth was measured throughout the experiment using a Keyence VHX-2000 Series digital microscope with VH-Z100UR objectives (Osaka, Japan, 1 µm resolution and HDR). The size at metamorphosis was determined by the larval shell (prodissoconch II, PII) morphology and morphometry (Bayne, 1965; Martel et al., 1995). Furthermore, the metamorphosis success was calculated by the ratio of total biomass of post-larvae recovered from the collectors over the number of initial larvae put in each cylinder and the larval growth after metamorphosis by the measure of the larval shell (dissoconch, D) on post-larvae (Martel et al., 2014).

2.4.4 Untargeted metabolomics (^{1}H-NMR, LC-HRMS/MS)

The freeze-dried post-larvae pools (≈3.4 mg or ≈500 post-larvae) were crushed with a micro-pestle in microtubes before metabolite extraction using an adaptation of Folch's method (1 methanol : 1 chloroform : 1.4 water) including a 10 min. sonication on ice steps. Extracts were centrifugated for 10 min at 10,000 × g 4°C to separate the polar fraction. The extractions were repeated three more times and the polar extracts pooled before solvent evaporation in a SpeedVac (Thermo Fisher Scientific) at room temperature.

2.4.4.1 Proton nuclear magnetic resonance (^{1}H-NMR)

The larval extracts were reconstituted in 500 µL of NMR buffer (0.2 M pH 7.4 potassium phosphate buffer in 99.9% deuterium oxide with 0.13 mM 3-trimethylsilylpropionic acid) and transferred to standard 5mm NMR tubes. The ^{1}H-NMR spectra from the post-larvae were obtained with a Bruker Avance III spectrometer, operating at 600 MHz, with a broad-band BBFO probe. The spectra were further processed, bucketed and integrated using NMR ProcFlow tools (Jacob et al., 2017) and the identification of the metabolites was performed using Chenomx software and was further confirmed by 2D ^{1}H-NMR COSY and TOCSY.

2.4.4.2 Liquid chromatography in tandem with high resolution mass spectrometry (LC-HRMS/MS)

After NMR acquisition, the samples were recovered and analysed by LC-HRMS/MS using a Shimadzu Nexera UHPLC coupled to a quadrupole time-of-flight system (TripleTOF® 5600+, Sciex, Concord, ON, Canada), equipped with a Duospray ion source operated in positive and negative electrospray mode. Metabolite separation was conducted on a mixed-mode reverse-phase column using gradient elution with mobile phases of water and acetonitrile (CAN), both containing 0.1% formic acid. Feature annotation for putative metabolite identification was performed using MS/MS spectral matching with NIST2017 MS/MS spectral library, Sciex "All in one" accurate mass metabolite library, and an in-house spectral library from standard metabolites run under identical MS/MS conditions. Features with a library score ≥85 and an accurate mass measurement for protonated or deprotonated precursors <10 ppm were kept for further processing. Peak integration was verified for each putatively identified metabolite using Sciex OS-Q.

2.4.5 Quantification of amino acids, energy metabolism-related metabolites and polyunsaturated fatty acids oxidation products

Around 3.5 mg of freeze-dried post-larvae (≈ 500 post-larvae) were micro-ground and homogenized with a 2.8 mm ceramic bead at 4,000 rpm for three cycles of 10 s (Precellys 24; Bertin corporation). Analyte extraction for the quantification of amino acids, energy metabolism-related metabolites and oxylipins were performed on the same sample with an extraction process in two steps.

The first one allows extracting the oxylipins. One millilitre of a 5/1 (v/v) mix of methyl tert-butyl ether (MTBE) and 2,2,2-trifluoroethanol (TFE) was added to the homogenizing tubes containing the sample. The samples were vortexed for 30 s and centrifuged for 1 min at 2,460 g. This extraction was performed three times, and supernatants (750 µL) were pooled in 15 mL falcon tubes. For the second and third extraction cycles, 750 µL of 5/1 (v/v) MTBE/TFE was added to the samples. The supernatant was washed three times with 250 µL

of LC-MS grade water. The aqueous layer was collected and pooled with supernatant obtained during the second step of extraction protocol. The organic phase was dried using a vacuum concentrator (SpeedVac, Savant SPD2010, ThermoScientific) for 2 h without heating. Dried samples were reconstituted with 250 µL of a 1/1/1/1 (v/v/v/v) mix of TFE/ACN/MeOH/50 mM ammonium formate at pH 3 (ACN, Acetonitrile; MeOH, methanol). The samples were vortexed for 30 s and centrifuged for 1 min at 2,460 g. Then 250 µL of the supernatant was transferred to 2 mL HPLC vial containing one millilitre of 50 mM ammonium formate buffer at pH 3 with 10 $ng.mL^{-1}$ of internal standard PGE_2-d_9. The sample was analysed directly after the extraction process.

During the second step, amino acids and energy metabolism-related metabolites were extracted. One milliliter of a 1/1 (v/v) mix of TFE/LC-MS grade water was added to the homogenizing tubes, vortexed for 30 s and centrifuged for 1 min at 2,460 g. Three extraction cycle was performed, and supernatants (750 µL, except for the third extraction 1 mL) were pooled in 15 mL falcon tube, containing the aqueous layer obtained during oxylipins extraction and 4 mL of ACN. For the second and third extraction cycle, just 750µL of 1/1 (v/v) TFE/ LC-MS grade water was added to the samples. The sample was dried with a SpeedVac overnight without heating. The sample reconstitution was performed by adding 50 µL of LC-MS grade water following by the addition of 200 µL of LC-MS grade ACN. One hundred microliters of each reconstituted sample was transferred to two vials: one vial was for positive analysis and the other for the negative analysis. Compounds valine-d_8 (0.1 $\mu g.mL^{-1}$) and pyruvate-d_3 (1 $\mu g.mL^{-1}$) were used as internal standard to quantify the compounds in positive and negative ion modes, respectively.

Oxylipin analysis was performed with an HPLC 1260 Infinity II device coupled to a 6546 QTOF (Agilent Technologies) in negative ionization mode equipped with an Agilent 1290 Infinity Flexible Cube. Oxylipins extracts were separated using an InfinityLab Poroshell HPH C-18, 2.1 x 100 mm 1.9 µm (Agilent) and a Gemini C6-Phenyl guard column as on-line SPE (4 x 2.0 mm, SecurityGuard, Phenomenex) with a column temperature maintained at 40°C.

Metabolites were separated and quantified in multiple reaction monitoring (MRM) mode using an HPLC 1260 Infinity II device coupled to a 6420 Triple Quad mass spectrometer (Agilent Technologies) in positive and negative ionization mode. Ten microliters of the sample was injected, and the chromatography separation was performed with a InfinityLab Poroshell 120 HILIC-Z, 2.7 µm. 10 x 2.1 mm column.

Data were processed with MassHunter Quantitative QQQ (Quant-my-Way) software from Agilent Technologies. Two samples in the oxylipins analysis had bad peak shape and a large change in retention time. These two samples were removed from the analysis.

2.4.6 Statistical analyses

2.4.6.1 Multivariate statistics (OPLS-DA)

The metabolomics data were analysed by orthogonal projection latent structures discriminant analyses (OPLS-DA) using SIMCA 17 software (Sartorius). All data were scaled to units of variance. The minimum number of features needed for optimal classification of the Control and High samples in the OPLS-DA models was determined by iteratively excluding the variables with low regression coefficients and wide confidence intervals derived from "jack-knifing" combined with low variable importance in the projection (VIP) until maximum improvement of the quality of the models. The model quality was evaluated after 7-fold cross validation by cumulative R^2Y (goodness of fit), cumulative Q^2 (goodness of prediction), CV-ANOVA (test of cross-validated predictive residuals), and by fitting principal component analyses (PCA) which are an indicator of OPLS-DA model reliability (Eriksson et al., 2008). The contribution of each predictor in the model was evaluated through the variable score contribution, *i.e.*, the differences, in scaled units, for all the terms in the model, between the outlying and the normal observation, multiplied by the absolute value of the normalized weight. Metabolites included in the model with a VIP > 1 were considered as important. The effect of the intermediate noise levels (low

and medium) on the larval metabolome was predicted from the OPLS-DA models adjusted for the control and high conditions.

To identify the most relevant metabolites across the untargeted data sets (^{1}H-NMR, LC-HRMS/MS), an alternative model was fitted by multiblock OPLS-DA applied and structured in two blocks. In practice, a different block was assigned to the variables of each data set, and each block was scaled to avoid the domination of one block over the others. Thereafter, OPLS-DA was performed on the two blocks iteratively, excluding the least relevant variables from the model as described above.

The sets of features included in the OPLS-DA were further tested for their ability to explain the variations related to noise when modelled using principal component analysis, an unsupervised multivariate method (**Fig. 27**) (Worley & Powers, 2016).

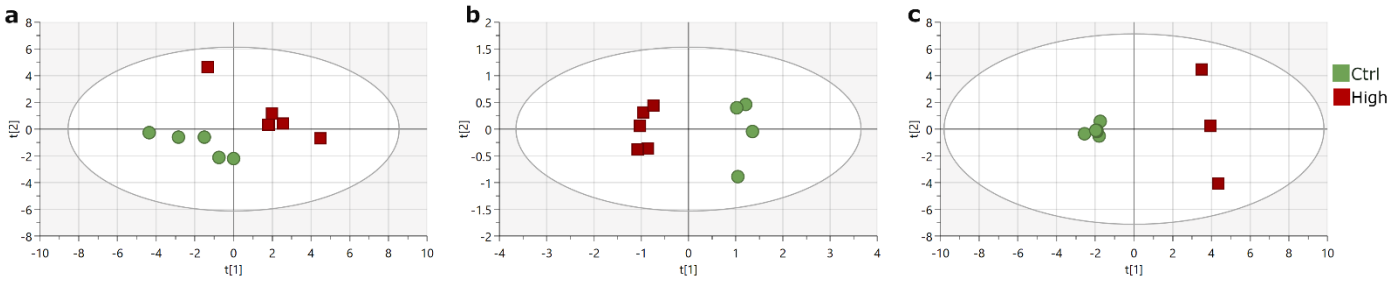

Figure 27. Principal component analysis (PCA) adjusted to the set of metabolites selected by multivariate OPLS-DA screening showing that supervised OPLS-DA as well as non-supervised PCA methods are able to cluster the pools of larvae in function of the intensity of the noise. Therefore, the set of metabolites included in the models are able to explain the variations related to the sound treatments. Green circles: control, red boxes: high shipping noise. (**a)** PCA fitted to the set of metabolites included in the OPLS-DA adjusted to the targeted prostaglandin, oxidant metabolism data. The PCA is composed of four components with the first two one explaining 79% of the variability in the metabolite set. **(b)** PCA fitted to the set of metabolites included in the OPLS-DA adjusted to the untargeted approach data. The PCA is composed of two components with the first one explaining 62% of the variability in the metabolite set. (**c)** PCA fitted to the set of metabolites included in the OPLS-DA adjusted to the targeted amino-acids, energy metabolism data. The PCA is composed of two components explaining 65% of the variability in the metabolite set.

2.4.6.2 Metabolite enrichment analysis

To identify the most significantly affected metabolic pathways, the discriminant metabolites (untargeted multiblock OPLS-DA and targeted OPLS-DA amino acid and energy metabolism) were analysed by metabolite-set enrichment analysis (MSEA) (Xia & Wishart, 2010), implemented in Metaboanalyst, a freely available web-based metabolomics analysis suite (MetaboAnalyst 5.0, *http://www.metaboanalyst.ca*) (Pang et al., 2021). The metabolite enrichment analysis of each set of metabolites was performed using over-representation analysis with the "pathway-associated metabolite sets" provided, containing 99 metabolite sets based on normal human metabolic pathways. The over-representation analysis (ORA) was implemented using the hypergeometric test to evaluate whether a particular metabolite set was more highly represented than expected by chance within the given compound list. A cutoff was chosen at a P-value controlled for False Discovery Rate (FDR) of 0.01 to retain the most significantly enriched metabolic pathways.

2.4.6.3 Permanova

The growth and metamorphosis success data were analysed on PRIMER7 software, using analyses of variance with 9999 permutations of residuals under a reduced model (PERMANOVA). Permutational method is a randomization which allows us to get rid of the distributional assumptions as normality and can be applied to very small samples. Similarities matrices based on ratios were produced using the Bray-Curtis distance. A one-way Permanova tested the effect of sound levels (control, low, medium, and high) on growth (size at metamorphosis and post-metamorphosis growth) and metamorphosis success. When differences were significant (p-perm ≤ 0.05), multiple comparison pairwise tests were used to determine which groups significantly differed. Distance-based tests for homogeneity of multivariate dispersion (PERMDISP) were carried out to evaluate the homogeneity of ratios in each condition.

2.5 RESULTS

Spectrograms **(Fig. 28a)** highlighted sustained low-frequency energy during arrival, while energy diminished more rapidly during departure. Power spectral density (PSD) analysis of noise from the *Nohlan Ava*, a 120 m roll-on/roll-off cargo ship, revealed distinct acoustic profiles during its arrival (11 min) and departure (8.5 min) phases **(Fig. 28b)**. The ship's noise exhibited slightly higher energy across most frequencies during arrival. The departure phase showed reduced and more uniform sound intensity. PSD comparisons across exposure conditions (high, medium, low, and control) demonstrated significantly elevated sound intensities in high-exposure conditions **(Fig. 28b)**, with medium and low exposures showing intermediate levels, and the control the lowest.

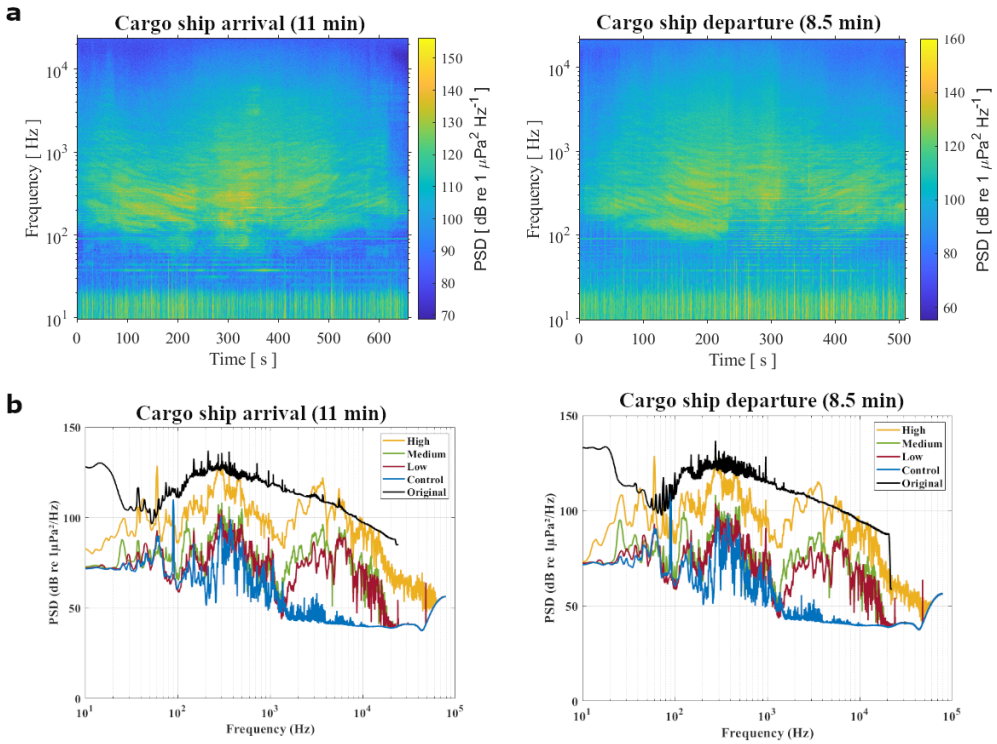

Figure 28. **(a)** Spectrograms of the cargo ship original *in situ* sound during the arrival (left) and the departure (right). **(b)** Power spectral density (PSD) analysis of the cargo ship original sound recorded *in situ* and at the center of the middle cylinder below the water surface, for all different experimental conditions and the control, during the arrival and the departure (window length: 1 second, window type: Hann).

2.5.1 Post-larval prostaglandins and fatty acid oxidation products

The stress induced by the noise treatment could promote the oxidation of polyunsaturated fatty acids (PUFAs) from phospholipids notably involved in inflammatory processes. Accordingly, the cyclopentenone prostaglandins (cyPGs) derived from arachidonic acid, as well as oxylipins were quantified in the blue mussels' post-larvae (**Tab. 8**). An orthogonal partial least squares discriminant analysis model (OPLS-DA) (**Fig. 29, Tab. 9**) was fitted to identify the compounds from the oxidative metabolism that were differentially regulated between ambient (control, n=5) and high noise (~151 dB re 1 µPa, n=4) exposure. This model ($R^2Y = 0.97$, $Q^2 = 0.95$, CV-ANOVA = 0.0016), composed of one predictive and one orthogonal component, was able to explain the variability induced by the sound treatment (**Fig. 29a**) and contained 14 compounds (**Fig. 29b**). The larval pools exposed to low noise (~121 dB re 1µPa, n=6) were also correctly discriminated from those from the high sound treatment. The intermediate level (~127 dB re 1µPa, n=5) induced a similar response to the high sound treatment.

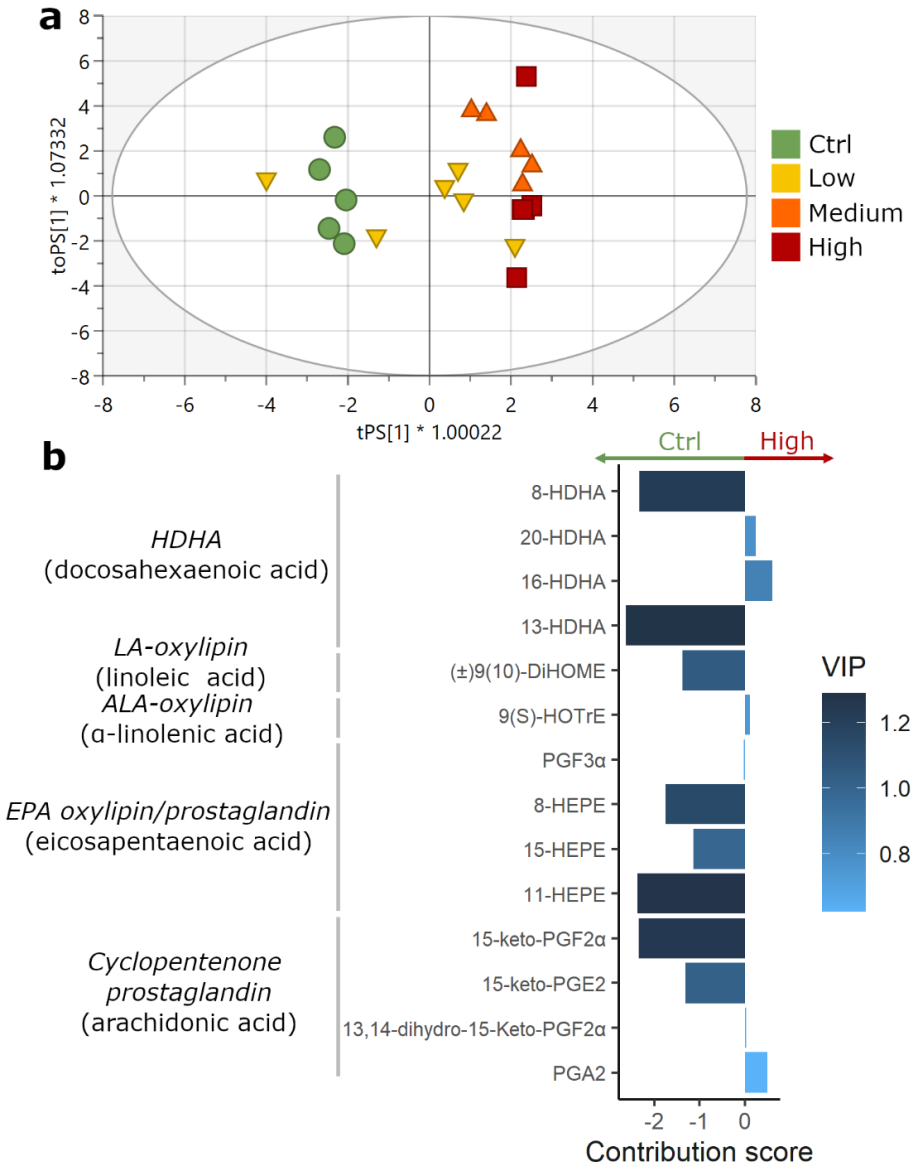

Figure 29. Impact of the shipping noise on blue mussel post-larvae's prostaglandins and fatty acid oxidative metabolism. (**a)** OPLS-DA scores plot based upon the fitted model ($R^2Y = 0.97$, $Q^2 = 0.95$, CV-ANOVA = 0.0016) of targeted lipid oxidation products quantification as a function of shipping noise levels. Green circles refer to ambient noise control (n=5), yellow inversed triangles to low (~121 re 1µPa) (n=6), orange triangles to medium (~127 re 1µPa) (n=5), and red boxes to high (~151 dB re 1 µPa) (n=4) shipping noise. (**b)** Contribution and importance in the model (VIP) of the compounds identified by the OPLS-DA-based screening to explain the impact of shipping noise upon post-larval mussel metabolome.

Table 8. Targeted LC-MS-based quantification (mean ± standard error ng/g) of prostaglandins and unsaturated fatty oxidation products. The significance of the differences in concentrations of the metabolites between noise levels were tested by Permanova (P-value < 0.05). Significant differences of concentration are indicated by letters.

	Control	Low	Medium	High
Cyclopentenone prostaglandin				
PGE2	**375 ± 116**	**246 ± 31**	**279 ± 27**	**225 ± 36**
PGD2	212 ± 57	188 ± 15	155 ± 24	234 ± 63
PGA2	**27 ± 4 b**	**22 ± 2 b**	**52 ± 5 a**	**36 ± 6 ab**
PGJ2	194 ± 24	169 ± 24	169 ± 8	150 ± 19
15-deoxy-d12,14-PGJ2	237 ± 29	260 ± 68	281 ± 50	156 ± 14
11b_PGF2a	37 ± 5	28 ± 3	39 ± 7	24 ± 4
15-keto-PGE2	20 ± 3	20 ± 3	17 ± 4	12 ± 2
15-keto-PGF2α	**286 ± 36 a**	**244 ± 27 a**	**228 ± 33 a**	**88 ± 14 b**
13,14-dihydro-15-Keto-PGE2	86 ± 11	75 ± 12	65 ± 7	80 ± 14
13,14-dihydro-15-Keto-PGD2	465 ± 133	350 ± 47	287 ± 25	335 ± 42
13,14-dihydro-15-Keto-PGF2α	79 ± 9	78 ± 14	113 ± 18	83 ± 18
EPA oxylipin				
PGE3	**49 ± 4**	**56 ± 10**	**45 ± 12**	**30 ± 8**
PGF3α	66 ± 19	65 ± 8	61 ± 11	61 ± 10
5-HEPE	315 ± 19	325 ± 30	384 ± 55	290 ± 29
8-HEPE	**2111 ± 100**	**1746 ± 166**	**1883 ± 181**	**1571 ± 130**
9-HEPE	**353 ± 104 a**	**155 ± 26 ab**	**150 ± 27 ab**	**100 ± 14 b**
11-HEPE	**1275 ± 82 a**	**830 ± 133 b**	**719 ± 51 b**	**575 ± 100 b**
18-HEPE	3182 ± 492	3669 ± 348	4604 ± 428	3032 ± 416
12-HEPE	362 ± 96	223 ± 25	213 ± 9	227 ± 28
15-HEPE	**661 ± 76**	**557 ± 36**	**529 ± 50**	**466 ± 50**
ALA-GLA-oxylipin				
9(S)-HOTrE	**270 ± 41 b**	**301 ± 47 b**	**499 ± 43 a**	**302 ± 44 b**
13(S)-HOTrE	91 ± 10	83 ± 15	67 ± 11	64 ± 16
13(S)-HOTrE(g)	54 ± 8	52 ± 8	63 ± 6	49 ± 11
Arachidonic-oxylipin				
11-HETE	425 ± 24	338 ± 54	403 ± 29	314 ± 44
12-HETE	211 ± 29	165 ± 26	175 ± 10	160 ± 21
15-HETE	77 ± 21	85 ± 12	66 ± 15	49 ± 12
8-HETE	562 ± 37	464 ± 63	535 ± 61	459 ± 40
9-HETE	106 ± 15	98 ± 13	97 ± 10	75 ± 10
5-HETE	68 ± 3	62 ± 4	69 ± 6	61 ± 7
15-oxoHETE	348 ± 110	223 ± 25	209 ± 9	225 ± 29
Linoleic acid				
(±)12(13)-DiOME	14 ± 1	11 ± 2	14 ± 3	11 ± 4
(±)9(10)-DiHOME	11 ± 1	9 ± 2	10 ± 2	7 ± 1
13-oxoODE	108 ± 9	103 ± 11	110 ± 13	96 ± 12
9-oxoODE	191 ± 10	174 ± 27	166 ± 29	165 ± 23
13-HODE	155 ± 18	152 ± 18	183 ± 15	147 ± 27
9-HODE	435 ± 169	123 ± 25	186 ± 84	103 ± 21
(±)12(13)-EpOME	61 ± 21	23 ± 5	24 ± 5	49 ± 25
(±)9(10)-EpOME	458 ± 64	358 ± 52	449 ± 114	352 ± 75
HDHA				
22-HDHA	16 ± 4	17 ± 3	17 ± 4	20 ± 5
20-HDHA	**305 ± 73**	**381 ± 31**	**507 ± 22**	**401 ± 97**
17-HDHA	1071 ± 126	908 ± 93	896 ± 69	731 ± 96
16-HDHA	**177 ± 29**	**194 ± 45**	**242 ± 17**	**269 ± 66**
13-HDHA	**648 ± 36 a**	**307 ± 74 b**	**224 ± 29 b**	**212 ± 45 b**
14-HDHA	359 ± 71	298 ± 39	309 ± 21	313 ± 43
10-HDHA	282 ± 33	290 ± 44	321 ± 34	270 ± 31
11-HDHA	217 ± 103	111 ± 23	111 ± 25	69 ± 9
7-HDHA	72 ± 3	73 ± 9	72 ± 5	58 ± 5
8-HDHA	**759 ± 25**	**582 ± 136**	**601 ± 70**	**403 ± 58**
4-HDHA	57 ± 4	47 ± 6	49 ± 6	39 ± 8

Table 9. Contribution and importance of the variables in the projection (VIP) of the metabolites included in the OPLS-DA model (R^2Y=0.97, Q^2=0.95, CV-ANOVA=0.0016) for the targeted LC-MS-based characterization of prostaglandins and unsaturated fatty acids (**Fig. 29**).

Compound	Contribution	VIP
(±)9(10)-DiHOME	-1.37425	1.05501
11-HEPE	-2.36357	1.27140
13,14-dihydro-15-Keto-PGF2α	0.02000	0.68116
13-HDHA	-2.62205	1.28919
15-HEPE	-1.13632	0.99026
15-keto-PGE2	-1.31368	1.02460
15-keto-PGF2α	-2.34545	1.25178
16-HDHA	0.60088	0.85104
20-HDHA	0.23909	0.78353
8-HDHA	-2.32204	1.22525
8-HEPE	-1.75619	1.14781
9(S)-HOTrE	0.09783	0.75118
PGA2	0.489607	0.62523
PGF3α	-0.02746	0.65959

As shown in Figure 29, the OPLS-DA reveals that five classes of lipid mediators were affected by noise, namely (1) the cyPGs; (2) the omega-3 eicosapentaenoic acid (EPA)-derived oxylipin; (3) the α-linolenic acid (ALA)-derived oxylipin; (4) the linoleic acid (LA)-derived oxylipin; and (5) hydroxy docosahexaenoic acids (HDHAs). CyPGs are derived from C20-unsaturated FAs with a cyclopentane ring, formed from arachidonic acid (C20:4 ω-6) or eicosatetraenoic acid (C20:4 ω-3) through cyclooxygenase. They are rapidly metabolized by enzymatic reactions to inactive forms. The OPLS-DA show the presence of two active cyPGs, *i.e.*, PGA2 more concentrated in mussels exposed to noise – possibly indicating inflammation or oxidative stress (Lee et al., 2010) – and PGF3α which were more prominent in control samples. Inactivated prostaglandin 15-keto-PGF2α and 13,14-dihydro-15-keto-PGF2α were also detected in mussels' larvae exposed to medium-to-high noise. The inactivation product of PGE2, *i.e.* 15-keto-PGE2 and 15-deoxy-Δ12,14-prostaglandin J2,

were also more concentrated in the controls, indicating the termination of proinflammatory reactions (Kim et al., 2021). Oxylipins – resulting from the oxidation of eicosapentaenoic acid (EPA, C20:5 ω-3), *i.e.*, α-linolenic acid (C18:3 ω-3) and linoleic acid (C18:2 ω-6) – were generally higher in controls. Finally, four HDHAs, which derive from docosahexaenoic acid (DHA, C22:6 ω-3), were retained in the OPLS-DA.

2.5.2 Post-larval polar metabolites

The response to stress could also impact general metabolism as amino acid synthesis or catabolism in relation with energy metabolism, the synthesis/degradation of DNA, RNA and involved multiple classes of molecules. Therefore, untargeted metabolomics, a technique facilitating the simultaneous exploration of multiple molecules in a biological sample employing proton nuclear magnetic resonance (^{1}H-NMR) and LC-HRMS/MS, was implemented. This approach enabled the relative quantification and identification of 133 metabolites (67 and 84 by ^{1}H-NMR and LC-HRMS/MS, respectively) in post-larvae exposed to noise. Notably, only 18 compounds were found to be common to both instrumental methods, which had similar individual classification performances (**Fig. 31, 32**). A multiblock OPLS-DA with an explicative ability (R^2Y) of 1.00 and a predictive ability (Q^2) of 0.98 for a CV-ANOVA of 0.0010 was fitted to reveal the alterations induced to the larval metabolome by exposure to the ship noise at high levels (n=5) (**Fig. 30a, b, Tab. 10**). This model, composed of one predictive and one orthogonal component, included 26 metabolites (11 detected by ^{1}H-NMR, 13 by LC-HRMS/MS, and 2 by both methods). It was able to discriminate the larvae under control condition and those exposed to all the noise levels, thus confirming that the metabolites included in the model are effectively correlated to the noise level. The discriminant metabolites included 15 amino acids, 4 nucleosides and derivatives, 2 cholines, and 5 other molecules, including one prostaglandin.

This untargeted exploration of the impact of noise on post-larvae's metabolome was completed by a systematic characterization of the amino acid and energy metabolism, based on the absolute quantification of a set of 48 metabolites (**Tab. 11**). These metabolites were

subjected to a multivariate screening, as performed with the untargeted dataset, to identify the molecules with concentrations affected by an exposition to the high shipping noise level. Twenty metabolites were conserved in the OPLS-DA model composed of one predictive and three orthogonal components (R^2Y_{cum} = 1.00, Q^2_{cum} = 0.99, CV-ANOVA = 0.0245) (**Fig. 30c, Tab. 12**). Five of these metabolites (all of which were amino acids) were also conserved in the untargeted model (glutamate, glutamine, glycine, leucine and methionine; **Fig. 30a**).

The impact on biochemical pathways of the mussel post-larvae exposure to noise during metamorphosis was determined by Metabolite Set Enrichment Analysis (**Fig. 30d**) based on the combination of the discriminant metabolites identified in the untargeted and targeted approaches. This analysis highlighted 17 pathways significantly enriched in metabolites (P-value FDR <0.01). Among them, pathways related to amino acids, including the urea cycle, glutamate and alanine metabolism, were the most extensively impacted. They were followed by pathways connected to energy metabolism, such as the transfer of acetyl groups into mitochondria, the Warburg effect (anaerobic glycolysis), pyruvate metabolism, gluconeogenesis, as well as β-oxidation of very long chain FAs. Finally, the oxidative stress response via glutathione metabolism was also affected.

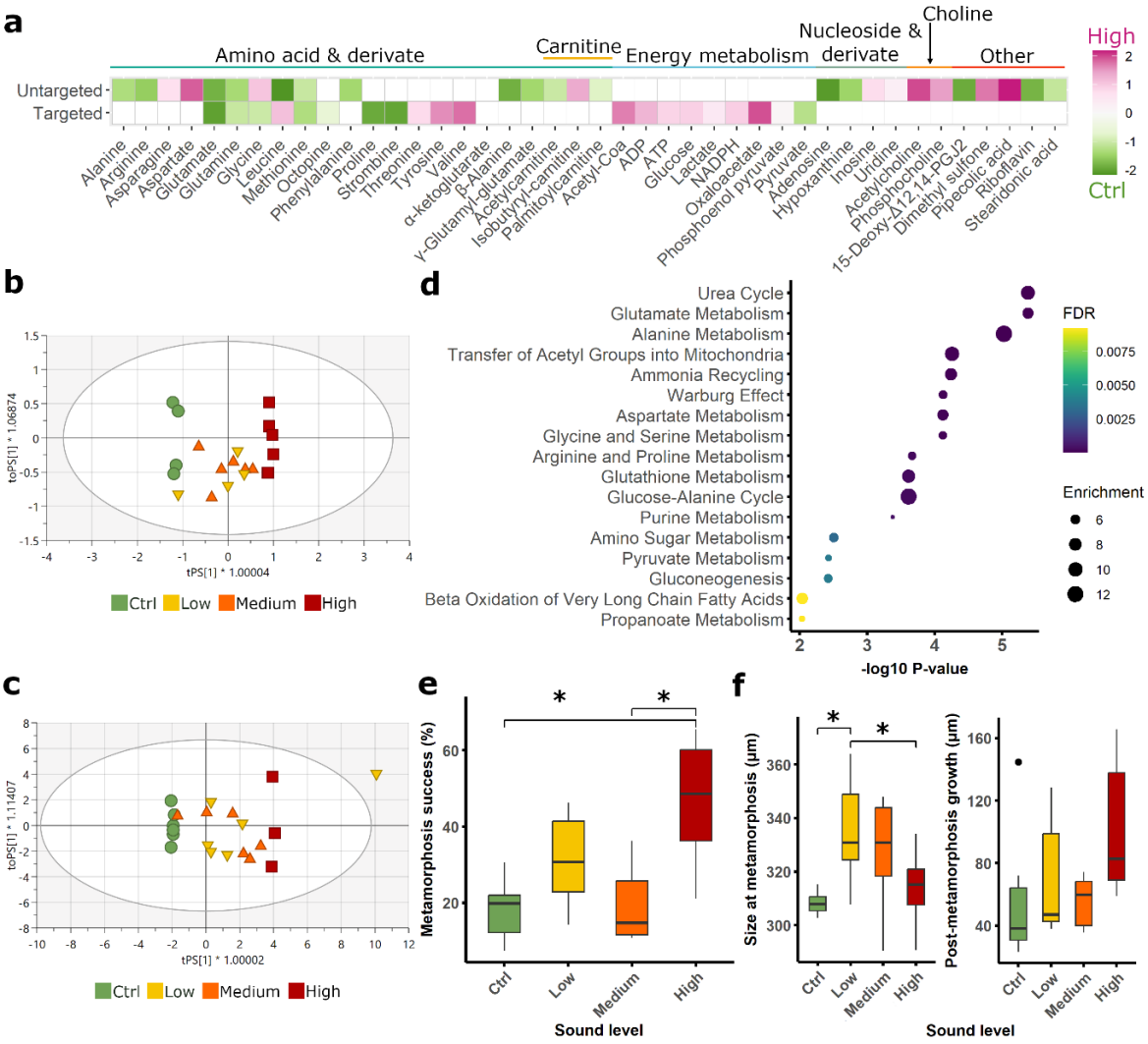

Figure 30. Impact of the shipping noise on the blue mussel post-larvae's amino acids, energy and oxidative metabolism. **(a)** Contribution of the metabolites identified by the multiblock OPLS-DA-based screening of the untargeted ^{1}H-NMR and MS data, as well as of the targeted LC-MS data. (**b)** Multi-block OPLS-DA scores plot based upon the fitted model ($R^2Y = 1.00$, $Q^2 = 0.98$, CV-ANOVA = 0.0010) on untargeted ^{1}H-NMR and MS data. And (**c)** OPLS-DA scores plot based upon the fitted model ($R^2Y_{cum} = 1.00$, $Q^2_{cum} = 0.99$, CV-ANOVA = 0.0245) on the targeted LC-MS amino acid and energy metabolism data. Green circles refer to ambient noise control (n=4), yellow inversed triangles to low (~121 re 1µPa) (n=6), orange triangles to medium (~127 re 1µPa) (n=5), and red boxes to high (~151 re 1 µPa) (n=4) shipping noise. (**d)** Metabolite Set Enrichment Analysis performed using the list of metabolites obtained from the untargeted and targeted metabolomics OPLS-DA models. The bubble sizes represent the fold enrichment of the metabolic pathways identified and the blue colour gradient corresponds to the P-value corrected for the False Discovery Rate. (**e)** Boxplot (minimum, first quartile, median, third quartile, maximum) representing the percentage of metamorphosis success of the larvae exposed to different noise levels (*P-value <0.05). (**f)** boxplot (minimum, first quartile, median, third quartile, maximum) representing the size of the larvae at the metamorphosis stage (PII) and their post-metamorphosis growth (D) (*P-value <0.05, outliers are represented by dots).

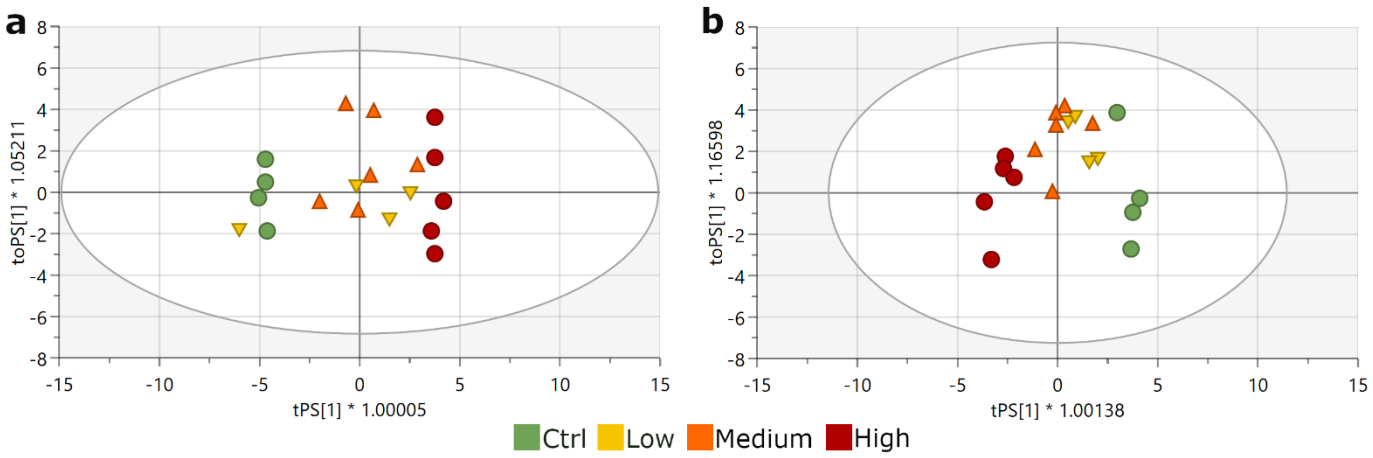

Figure 31. (a) Parsimonious OPLS-DA scores plot based upon the fitted model (R^2Y=1, Q^2=0.99, CV-ANOVA= 0.0005, 36 features) on the untargeted NMR data. Green circles: control, yellow inversed triangles: low, orange triangles: medium, and red boxes: high shipping noise. **(b)** Parsimonious OPLS-DA scores plot based upon the fitted model (R^2Y=0.98, Q^2=0.90, CV-ANOVA=0.0275, 28 features) on the untargeted LC-HRMS data. Green circles: control, yellow inversed triangles: low, orange triangles: medium, and red boxes: high shipping noise.

^{1}H-NMR: 67 metabolites detected

Acetate
Acetoacetate
Acetone
Acetylcarnitine
Acetylcholine
Alanine
Alloisoleucine
alpha Aminobutyrate
AMP
Arginine
Asparagine
Aspartate
beta-Alanine
Betaine
Carnitine
Choline
Citrate
Creatine
Cysteine
Dimethyl sulfone
Dimethylglycine
Ethanol
Formate
Fumarate
Glucose
Glucose-6-phosphate
Glutamate
Glutamine
Glycine
Guanidoacetate
Homarine
Hypotaurine
IMP
Inosine
Isoleucine
Isopropanol
Ketoleucine
Lactate
Leucine
Lysine
Malonate
Maltose
Mannitol
Methionine
myo-Inositol
Mytilitol
Ornithine
Phenylalanine
Phosphocholine
Phosphocreatine
Phosphoethanolamine
Proline
Propionate
Pyruvate
Sarcosine
Serine
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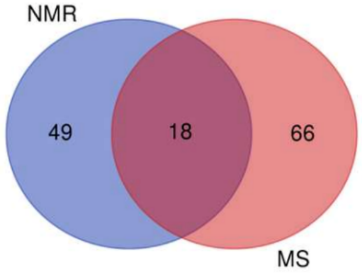
Uracil
Uridine
Valine
Xylitol



LC-MSMS (ESI + & -): 84 metabolites annotated

(2R)-3-Hydroxyisovaleroylcarnitine
.alpha.-L-Glu-L-Tyr
1,11-Undecanedicarboxylic acid
1,2-Benzenedicarboxylic acid*
1,3-Dicyclohexylurea
15-Deoxy-.DELTA.12,14-prostaglandin J2
1-Aminocyclopropanecarboxylic acid
1-Myristoyl-sn-glycero-3-phosphocholine
2-Phenylacetamide
3-Cyclohexyl-1,1-dimethylurea
3-Indoleacrylic acid
4,5.alpha.-Dihydronorethisterone
4-Ethoxybenzoic acid*
4-Nitrophenol*
4-Picoline
5-Isoprostaglandin-F2.alpha.-VI
5'-S-Methyl-5'-thioadenosine
7-Methylguanine
Acetylcarnitine
Adenosine
ADP
Alanine
AMP
Aspartate
Azelate
Benzanilide*
Benzophenone*
Carnitine
Citrate
Cysteine-glutathione disulfide
Diethyl phthalate*
Diethylene glycol*
Diethylene glycol monoethyl ether*
Ethyl 3-hydroxybenzoate*
Glycerophosphocholine
Guanine
Guanosine
Guanosine monophosphate
Hexaethylene glycol*
Hexanoyl-L-carnitine
Hexapropylene glycol*
Hypoxanthine
Indole-6-carboxaldehyde
Inosine
Inosinic acid
Isobutyryl-L-carnitine
Isophorone*
L-.gamma.-Glutamyl-L-glutamic acid
Lactate
Leucine
Malate
Methionine
Myristoleate
Myristoyl-L-carnitine
Nicotinate
Ophtalmic acid
Palmitoylcarnitine
Pantothenate
Pentapropylene glycol*
Phenylalanine
Phosphocholine
Phthalic anhydride*
Pipecolate
Propionylcarnitine
p-tert-Butylcatechol
Pyroglutamate
Riboflavin
S-(5'-Adenosyl)-L-homocysteine
Sebaceate
Stachydrine
Stearidonate
Taurine
Tetradecanedioate
Tetraethylene glycol*
Thymoquinone
Tri(3-chloropropyl) phosphate*
Tributylamine*
Trigonelline
Tryptophan
Tyrosine
Uracil
Uridine
Urocanate
Xanthurenate

Figure 32. List of the metabolites detected by ^{1}H-NMR and detected and annotated by LC-MSMS in the untargeted metabolomics analysis. *Compounds excluded from the analysis for suspicion to be contaminant based on data in the Human Metabolome Database (*https://hmdb.ca*)

Table 10. Contribution and importance of the variables in the projection (VIP) of the metabolites included in the multi-block OPLS-DA model (R^2Y=1.00, Q^2=0.98, CV-ANOVA=0.0010) for the untargeted LC-MS (green) and NMR (purple) -based metabolomics (**Fig. 30**).

Metabolite (chemical shift)	Contribution	VIP
15-Deoxy-Δ12,14-prostaglandin J2	-1.91282	1.2142
Acetylcarnitine	-1.03669	0.8516
Acetylcholine (3.227)	1.98841	1.0722
Adenosine	-2.07545	1.2191
Alanine (3.769)	-1.41936	0.9114
Alanine (3.780)	-1.22053	0.8513
Arginine (3.775)	-1.54783	0.9528
Asparagine (2.851)	0.714892	0.6348
Aspartate	2.14305	1.2373
Aspartate (3.888)	1.62194	1.0120
β-Alanine (2.564)	-1.99612	1.0699
β-Alanine (3.170)	-1.77388	1.0225
β-Alanine (3.182)	-1.8582	1.0411
β-Alanine (3.191)	-1.8117	1.0286
Dimethyl sulfone (3.151)	1.72833	0.9958
γ-Glutamyl-glutamic acid	-1.42953	1.0990
Glutamate (3.753)	-1.85861	1.0375
Glutamate (3.759)	-1.9704	1.0664
Glutamate (3.772)	-1.68471	0.9953
Glutamine (3.765)	-1.61215	0.9787
Glutamine (3.786)	-1.15392	0.8363
Glycine (3.563)	1.04622	0.8467
Hypoxanthine	-1.47309	1.1620
Inosine (4.436)	0.761747	0.7055
Isobutyryl-carnitine	1.35022	0.9760
Leucine	-2.14092	1.2338
Methionine	-1.66535	1.1038
Methionine (2.648)	-0.45398	0.6326
Palmitoylcarnitine	-0.79373	0.8246
Phenylalanine	-1.41777	1.0852
Phosphocholine (3.220)	1.46923	0.9566
Pipecolic acid	2.17632	1.2433
Riboflavin	-1.81308	1.1789
Stearidonic acid	-1.10188	0.9089
Unknown 1 (3.747)	-1.29557	0.8690
Unknown 2 (1.884)	1.88602	1.0524
Unknown 3 (1.520)	-0.704	0.6964
Uridine	0.312618	0.8998

Table 11. Targeted LC-MS-based quantification of amino acids and energy metabolism metabolites. The significance of the differences in concentrations of the metabolites between noise levels were tested by Permanova (P-value < 0.05). Significant differences of concentration are indicated by letters.

Metabolic pathways	Metabolites	Control	Low	Medium	High
Aerobic (mitochondria - Krebs and Urea cycle)	Aketoglutarate	1 ± 0	3 ± 1	4 ± 2	3 ± 0
	Cis-aconitate	0 ± 0	0 ± 0,03	0 ± 0	0 ± 0
	Citrate	19 ± 2	31 ± 6	29 ± 3	114 ± 60
	Succinate	260 ± 37	227 ± 61	278 ± 24	443 ± 95
	Fumarate	18 ± 2	27 ± 9	25 ± 5	24 ± 10
	Malate	283 ± 40	368 ± 104	347 ± 75	636 ± 218
	Oxaloacetate	**17 ± 5 b**	**48 ± 10 ab**	**24 ± 7 ab**	**41 ± 24 a**
	Glutamate	2765 ± 94	1710 ± 167	2099 ± 345	3933 ± 1416
	Glutamine	1282 ± 50	1203 ± 36	1180 ± 66	1015 ± 315
	Arginine	1397 ± 81	1229 ± 148	1310 ± 66	1297 ± 397
Anaerobic (lactic fermentation - Glycolysis)	Strombine	2793 ± 96	1741 ± 168,6	2141 ± 347,1	3862 ± 1345,3
	Octopine	144 ± 18	137 ± 38,3	117 ± 14,2	105 ± 14,9
	Lactate	105 ± 6	126 ± 28,7	129 ± 10,5	126 ± 24,3
Molecule and Coenzymes related to energy transfer	AMP	292 ± 27	194 ± 45	229 ± 36	1175 ± 662
	ADP	3 ± 1	30 ± 28	10 ± 4	5 ± 3
	ATP	14 ± 3	17 ± 3	17 ± 2	31 ± 19
	NAD	26 ± 3	15 ± 8	12 ± 6	31 ± 25
	NADH	11 ± 3	7 ± 2	10 ± 3	15 ± 11
	NADP	5 ± 1	9 ± 4	7 ± 2	4 ± 3
	NADPH	47 ± 10	49 ± 8	59 ± 8	160 ± 102
	FAD	0 ± 0	0 ± 0	0 ± 0	0 ± 1
	Glucose	115 ± 5	137 ± 24	121 ± 6	129 ± 10
	Glucose-6-phosphate	96 ± 17	105 ± 29	124 ± 12	1080 ± 717
	Pyruvate	40 ± 2	23 ± 5	34 ± 6	20 ± 8
	phosphoenol pyruvate	2 ± 0	3 ± 0,9	2 ± 0	2 ± 1
	Acetyl-Coa	**1 ± 0 b**	**1 ± 0 ab**	**1 ± 0 ab**	**2 ± 1 a**
	D-Fructose-1.6-biphosphate trisodium	24 ± 2	24 ± 3	41 ± 7	24 ± 12
Essential amino acids	**Histidine**	**441 ± 10 a**	**305 ± 22 ab**	**241 ± 17 b**	**1168 ± 514 ab**
	Methionine	**32 ± 2 a**	**26 ± 2 ab**	**23 ± 1 b**	**24 ± 4 ab**
	Threonine	470 ± 26	452 ± 9	465 ± 17	582 ± 164
	Tryptophan	79 ± 4	82 ± 5	68 ± 4	74 ± 10
	Phenylalanine	443 ± 20	456 ± 25	432 ± 28	480 ± 90
	Valine	427 ± 20	459 ± 19	466 ± 37	545 ± 67
	Leucine	384 ± 21	413 ± 13	405 ± 28	546 ± 198
	Isoleucine	145 ± 7	146 ± 4	139 ± 8	159 ± 24
	Lysine	123 ± 7	119 ± 17	103 ± 7	115 ± 38
Non-essential amino acids	**Proline**	**766 ± 60 a**	**485 ± 15 b**	**526 ± 70 b**	**330 ± 90 b**
	Hydroxyproline	40 ± 3	39 ± 3	41 ± 4	52 ± 15
	Cystine	3 ± 1	6 ± 1	6 ± 1	89 ± 60
	Tyrosine	**559 ± 22 b**	**546 ± 14 b**	**596 ± 32 ab**	**731 ± 142 a**
	Alanine	3561 ± 181	2889 ± 190	2812 ± 137	3299 ± 680
	Serine	1001 ± 56	920 ± 42	1016 ± 29	1037 ± 341
	Glycine	8634 ± 363	7579 ± 849	7490 ± 671	7118 ± 1665
	Aspartate	1832 ± 85	1607 ± 201	1769 ± 163	1845 ± 602
	a-aminobutyric acid	2581 ± 250	1630 ± 118	2006 ± 206	4176 ± 1285
	a-Aminoadipic acid	61 ± 5	35 ± 4	42 ± 4	185 ± 85
	b-aminoisobutyric	189 ± 15	164 ± 25	165 ± 13	158 ± 24
	Betaine	13287 ± 986	15319 ± 1355	13972 ± 1123	16407 ± 10385
Ratio	Glycine/Succinate	37 ± 6	40 ± 11	25 ± 4	22 ± 3
	AEC	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	BCAAs	956 ± 47	1018 ± 33	1010 ± 73	1250 ± 288
	$NADH/NAD^+$	1 ± 0	1 ± 1	1 ± 1	8 ± 6

Table 12. Contribution and importance of the variables in the projection (VIP) of the metabolites included in the OPLS-DA model (R^2Y_{cum}=1.00, Q^2_{cum}=0.99, CV-ANOVA=0.0245) for the targeted LC-MS-based characterisation of amino acids and energy metabolism (**Fig. 30**)

Metabolite	Contribution	VIP
Acetyl-Coa	1.68002	1.08603
ADP	1.23701	1.01615
Aketoglutarate	2.64558	1.25994
ATP	0.869137	0.938782
Glucose	0.913422	0.957866
Glutamate	-2.01153	1.13567
Glutamine	-1.06618	0.928844
Glycine	-1.01697	0.956994
Lactate	0.465301	0.861751
Leucine	1.10796	0.939639
Methionine	-1.30631	0.990473
NADPH	0.937975	0.966666
Octopine	-0.622776	0.871872
Oxaloacetate	1.95708	1.08207
Phosphoenol pyruvate	0.109994	0.733108
Proline	-1.91747	1.09706
Pyruvate	-1.22877	1.0199
Strombine	-1.97715	1.12948
Threonine	0.779409	0.80869
Tyrosine	1.59111	1.0074
Valine	1.78067	1.06564

2.5.3 Post-larval performance

Post-larval performances were evaluated using a Keyence VHX-2000 Series digital microscope. Those observations showed that the success of metamorphosis increased significantly with the sound treatment (+21% High *vs.* Control; P-value = 0.01) in mussels sampled on collectors **(Fig. 30e)**. Furthermore, the size at metamorphosis (PII measurements) (**Fig. 30f**) was significantly impacted by the noise (+18 µm Low *vs.* Control; P-value = 0.02,

−17 µm Low *vs.* High; P-value = 0.04) while the post-metamorphosis growth (D) did not differ significantly between the sound treatments (**Fig. 30f**).

2.6 DISCUSSION

2.6.1 Shipping noise treatment impact on post-larval metabolism

Noise modified the life cycle of mussel larvae by stimulating their settlement and modulating their metabolome. Those alterations may result from a direct effect of the sound or different levels of metamorphosis development, including tissue morphology, induced by the sound treatment.

2.6.1.1 Modification of polyunsaturated fatty acid oxidation patterns indicated an inflammatory response.

The responses to noise-induced stress triggered a modification of the lipid mediators' production (oxylipins and prostaglandins) by oxidation of PUFAs through direct interaction with reactive oxygen species (ROS), produced during oxidative stress, or ROS-activated enzymatic pathways, *i.e.*, cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450. Therefore, noise could induce, directly or indirectly through the regulation of the metamorphosis process, a modification of lipid mediators' patterns known to play essential roles in inflammation, reproduction, regulation of ion flux, thermoregulation, and activation of the immune response in aquatic invertebrate (Canesi et al., 2002; Di Costanzo et al., 2019; Gagné et al., 2007). Several oxylipins and prostaglandins were retained in the OPLS-DA which could suggest a modification of the pro-inflammatory / pro-resolving balance, although their role in stress and inflammation in aquatic organisms is not well understood (Gabbs et al., 2015).

Besides these physiological processes, oxylipins and CyPGs levels may also affect the larval development, especially during the metamorphosis. Indeed, metamorphosis is associated with the development of juvenile-specific structures including the loss of the

velum and the development of the gills (Yang et al., 2013). During this transition, the innate immune system is solicited for the resorption and reorganization of larval tissues through the ROS regulated process (Redza-Dutordoir & Averill-Bates, 2016) of apoptosis and ROS-generating phagocytosis which may impact the lipid oxidation patterns of the post-larvae (Davidson & Swalla, 2002; Wang et al., 2023; Winston et al., 1996). Interestingly, CyPGs and oxylipins may also be involved in marine invertebrates settlement (Knight et al., 1999), suggesting that elevated ship noise levels could trigger this action on larvae, with the potential impact on the ecosystems as well as aquaculture.

Overall, noise-induced stress appeared to modulate the production of lipid mediators from PUFAs possibly leading to different inflammatory profiles between control and noise-exposed post-larvae. However, it was not clear whether its impact was direct or linked to different ontogenic developments at metamorphosis.

2.6.1.2 Increased energy requirement and protein turnover are coupled with disrupted nervous system activity.

Gluconeogenesis and energy production is increased in post-larvae exposed to noise. Amino acids and their derivatives, comprising 22 out of 42 compounds affected by noise in both untargeted and targeted metabolomes, showed significant changes, akin to the response observed in adult mussels when exposed to pollutants, leading to stress and reduced feeding rates (Wale et al., 2019) due to a reduction in valve gape opening (Day et al., 2017; Ledoux et al., 2023). In response to food restriction, mussels increased gluconeogenesis to produce glucose from oxidizable amino acids, which helps maintain intracellular osmolarity (Moyes et al., 1990). Consequently, lower levels of alanine, arginine, glutamate, glutamine, methionine, phenylalanine, and proline in noise-exposed post-larvae suggest higher oxidation rates to compensate for reduced feeding (Spiga et al., 2016) and greater energy demands caused by noise-related stress and the extension of the prospecting pelagic phase (Gigot et al., 2023b; Vazzana et al., 2016).

Additionally, lipid β-oxidation may be heightened in noise-exposed post-larvae, indicated by lower levels of certain acylcarnitines, such as acetylcarnitine and palmitoylcarnitine, suggesting increased transport of FAs into mitochondria for breakdown, generating glucose and energy (Longo et al., 2016). This elevated gluconeogenesis rate led to higher glucose levels and greater depletion of glycolysis products (pyruvate, phosphoenolpyruvate), accompanied by increased levels of ATP, ADP, and Krebs cycle intermediates (acetyl-CoA, oxaloacetate, α-ketoglutarate), reflecting intensified aerobic energy production and amino acids turnover. The aerobic energy production is complemented by the transformation via the anaerobic glycolysis of glucose into lactate, which is higher in post-larvae exposed to noise. However, the level of octopine (a derivative of arginine and alanine) and strombine [N-(carboxymethyl)-D-alanine] involved in maintaining the redox balance under anaerobic conditions and anaerobic respiration end-products, respectively, were higher in the controls suggesting higher energy demands. These findings indicate lower metamorphosis development in the control group, as evidenced by morphometric data, correlating with metabolic activity, specifically the NADH/NAD+ ratio. The elevated ratio observed in the high sound condition may suggest an increased reliance on anaerobic metabolism, potentially related to the demands of gill development and/or the stress induced by sound exposure.

Protein and cellular turnover increased in post-larvae exposed to noise. In post-larvae exposed to noise, five proteogenic amino acids (asparagine, aspartate, threonine, tyrosine, valine) were found at higher levels. Threonine, tyrosine as well as aspartate and asparagine may be precursors for the biosynthesis of new proteins in response to the stress-induced formation of ROS provoking protein damage or the increased protein demand for the remodelling occurring during the metamorphosis. The branched-chain amino acid valine is essential for the immune system by providing energy and being a precursor for the biosynthesis of protective molecules (Calder, 2006), but it also plays an important role in the regulation of protein turnover processes as observed in *Mytilus galloprovincialis* challenged by *Vibrio anguillarum* (Ji et al., 2013). The post-larvae immune system was likely to be triggered by the response to stress, involving prostaglandins and oxylipins (Canesi et al.,

2002). The exposure of mussel larvae to noise, or the regulation of their metamorphosis by noise, may thus have an impact on their protein turnover, in combination with a higher energy level due to increased gluconeogenesis, which could lead to a tendency to grow faster after metamorphosis (**Fig. 30f**).

Action on nervous system activity. The level of the neurotransmitter acetylcholine – which plays an important role in the cholinergic system involved in the digestion, control of the heartbeat and movement – is dependent on its rate of inactivation via hydrolysis into choline by acetylcholinesterase (AchE). The activity of this enzyme is considered the most effective biomarker in assessing neurological changes caused by xenobiotics (Fontes et al., 2022). The higher level of acetylcholine (decreased hydrolysis by AchE) in post-larvae exposed to noise, which is consistent with the decrease of AchE activity after exposition to pollutants such as cocaine (Fontes et al., 2022) and the typical decreased enzyme activity in mussels exposed to environmental stress (Ricciardi et al., 2006), could have a stimulatory effect. The formation of ROS might be the cause of the decreased hydrolysis by AchE similarly to what has been observed during the exposure of mussels to waste water (Gagné et al., 2011). Acetylcholine is also an inhibitor of some ctenidial lateral cilia that reduce water pumping rate in *Mytilus edulis* (Jones & Richards, 1993), in good agreement with the reduced respiration and feeding rates observed during noise exposition (Wale et al., 2019). Additionally, this neurotransmitter induces both settlement behaviour and metamorphosis development in bivalves (Bayne, 2017; Beiras & Widdows, 1995). Its higher level in post-larvae exposed to noise could therefore explain the increase settlement level and faster metamorphosis development.

2.6.2 Noise induces settlement in bad habitats and in an altered physiological condition.

Competent veliger mussel larvae can delay their metamorphosis and remain adrift up to a size of 400 µm − almost 100% increase from the 230 µm observed for the most precocious ones − due to potential absence of a suitable environment required for stimulating settlement, thus forcing larvae to continue planktonic growth (Martel et al., 2014). At an

average size of 300 µm, the larvae were already in a delayed metamorphosis condition, undoubtedly linked to the absence of turbulence in the Larvosonic systems to limit noise perturbations (Olivier et al., 2023). Indeed, settlement success is positively correlated with centrifugal and advective flow velocity and turbulence (Pernet et al., 2003b). As highlighted by the metabolomic characterization, the post-larvae exposed to high sound level have higher energy expenditures than the others, which potentially results in a decrease in energy reserves and therefore activates the oxidative energy pathways. Those larvae would thus consume considerably more energy daily than the others (Bayne, 1965; Gigot et al., 2023b), which will result in them becoming "desperate larvae" faster (Knight-Jones, 1951; Toonen & Pawlik, 1994), *i.e.*, forced to settle without any positive settlement cue, or even on unfavourable environment, at a smaller size. Alternatively, a second hypothesis could be that there existed a disparity in the metamorphosis stage of the larvae between the control and high sound exposure groups that the latter group may exhibit a more advanced metamorphic state compared to the former (Cannuel et al., 2009).

In summary, marine traffic noise delays metamorphosis − leading to post-larval size differences − while increasing its success in a suboptimal habitat. The post-larvae that settled and underwent metamorphosis in this suboptimal habitat show increased metabolomic demands and an altered prostaglandin profile, indicating stress and inflammatory responses, which may reduce their chances of survival. However, these results need to be enriched by long-term studies.

Perspectives for sessile bivalve aquaculture, ecosystems, and regulations. Anthropogenic noise, associated to maritime traffic, modulates larval metamorphosis dynamics and recruitment processes, potentially altering larval selectivity and decreasing survival rates to adulthood, with potential cascading effect on population dynamics and marine biodiversity (Gigot et al., 2023b; Pechenik, 2006). Those perturbations may, additionally, carry socio-economic implications, particularly for aquaculture systems reliant on spat capture (Kamermans & Capelle, 2019). Our innovative approach coupling metabolomic monitoring of *Mytilus edulis* larvae stress responses and acoustic studies

provide the first metabolic dose-response data about the impact of shipping noise on sessile bivalve development. These results could be used by regulators and decision-makers. Indeed, no binding regulations currently require the reduction of manmade underwater noise. There are, nevertheless, some regulations and recommendations to help maritime professionals reduce noise pollution, but with limited legal status, *i.e.*, the International Maritime Organization Guidelines (Maruf & Chang, 2023). Those initiatives are based on thresholds − defined by the noise level at which individual animals start to have adverse effects – as it is the case for the 'level of Onset of Biological Adverse Effect (LOBE)' proposed by the European Union (European Marine Strategy Framework, Recommendations from Technical Group on Underwater Noise for EU Threshold Values for Continuous Noise., 2022). The definition of thresholds for a variety of organisms is crucial for the implementation of precautionary management and mitigation measures – such as the reduction vessel speed (Lajaunie et al., 2023), the modulation of ship traffic and speed in reproduction zone during spawning season, or the promotion of ship design that reduces the propeller, machinery, and flow noises (Arveson & Vendittis, 2000) − to limit the impacts on the biodiversity, and the management of fisheries and aquaculture.

2.7 CONCLUSION

This study highlights that marine traffic noise disrupts the metamorphosis and settlement of *Mytilus edulis* larvae, resulting in size differences and increased stress-related metabolomic expenditures in suboptimal habitats. Those findings emphasize the significant impact of anthropogenic noise on larval development, with potential negative effects on bivalve aquaculture, ecosystem health, and marine biodiversity.

2.8 ACKNOWLEDGEMENTS

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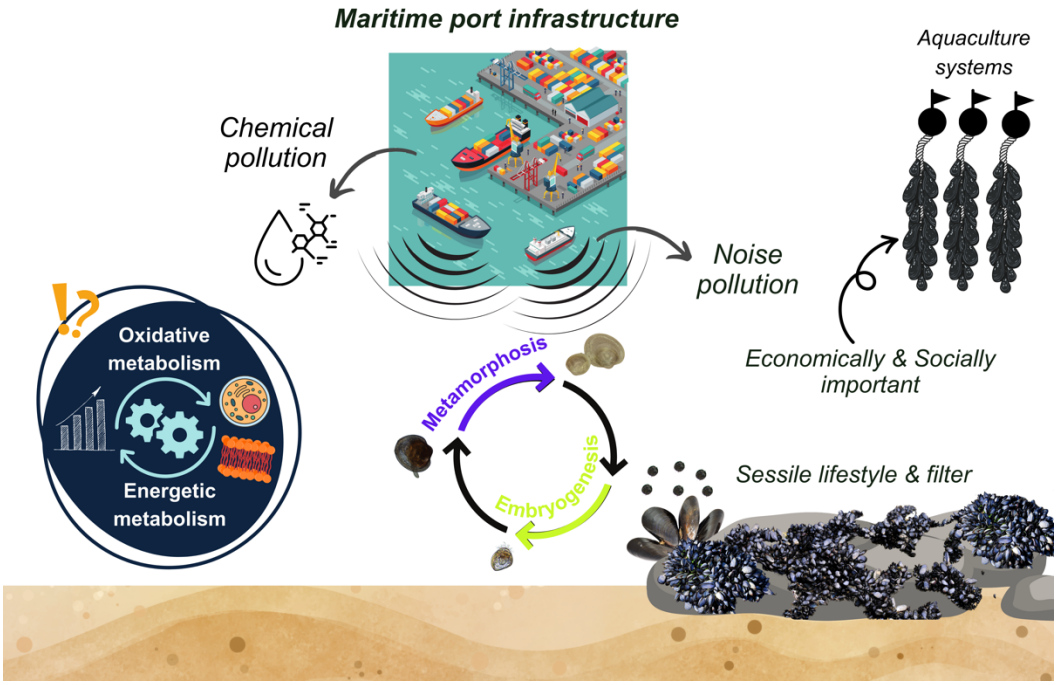
2.9 DATA AVAILABILITY

The untargeted metabolomic datasets generated and analysed during the current study are available in the MetaboLights repository hosted by the EMBL-EBI (Haug et al., 2020), http://www.ebi.ac.uk/metabolights/MTBLS5678. All the quantitative data extracted from the targeted metabolomics are provided as supplementary data. The soundtrack used during the experiment was deposited under the DOI 10.5281/zenodo.10910123 in the Zenodo repository (https://zenodo.org) hosted by the European Organization for Nuclear Research (CERN).

CHAPITRE 2

IMPACT ACOUSTIQUE ET CHIMIQUE DU TRAFIC MARITIME SUR L'ONTOGENÈSE DE LA MOULE BLEUE *MYTILUS EDULIS*



ARTICLE 3
EFFET D'UNE POLLUTION CHIMIQUE REPRÉSENTATIVE D'UN PORT INDUSTRIEL SUR LE SUCCÈS EMBRYONNAIRE DE LA MOULE BLEUE, *MYTILUS EDULIS* : UNE APPROCHE MÉTABOLOMIQUE

3.1 RÉSUMÉ EN FRANÇAIS DU TROISIÈME ARTICLE

Compte tenu de la demande croissante du commerce maritime international, il est urgent d'améliorer notre connaissance de l'impact de la navigation sur les organismes marins afin de développer des stratégies d'atténuation efficaces. Les bivalves sont des organismes importants dans l'écosystème intertidal où se trouvent les ports maritimes. Dans ce travail, nous avons évalué la réponse des larves de moules exposées à des contaminants chimiques associés au trafic maritime. Nous nous sommes concentrés sur l'embryogenèse, les premiers stades ontogéniques étant connus pour être les plus sensibles aux événements stressants et primordiaux pour le maintien de la structure de la communauté. Ainsi, des embryons de moule bleue, *Mytilus edulis*, issus de deux pontes après maturation naturelle des gonades, ont été exposés à un cocktail de contaminants représentatif d'un environnement portuaire jusqu'à la fin de l'embryogenèse. Nos résultats montrent une réponse métabolomique différente des larves D à la contamination en fonction du succès embryogénique. Les larves contaminées présentaient des besoins énergétiques plus importants et semblaient utiliser avec succès une variété de mécanismes impliquant la biosynthèse d'antioxydants et la restructuration du métabolisme énergétique pour atténuer les effets toxiques sur les cellules et les tissus en développement. Ces travaux démontrent la vulnérabilité des larves de moules dans un environnement exposé à un trafic maritime important et soulignent la nécessité d'une réglementation.

Mots-clés : métabolomique, écotoxicologie, développement larvaire, embryogenèse, physiologie des bivalves/mollusques.

Cet article, intitulé « *Effect of chemical pollution representative of an industrial port on the embryonic success of the blue mussel, Mytilus edulis: a metabolomic approach* », est soumis dans la revue *Canadian Journal of Zoology*. En tant que premier auteur, j'ai contribué à l'essentiel de la recherche sur l'état de la question, au développement des larves de moules, aux mesures de performance post-larvaire, à l'exécution des analyses chimiométriques et fonctionnelles ainsi qu'à l'analyse par spectrométrie de masse. Stéphane Beauclercq a contribué aux analyses fonctionnelles. Bertrand Genard a supervisé les expériences de spectrométrie de masse. Richard Saint-Louis a apporté un soutien technique à l'analyse chimique. Mathieu Bianic a quantifié les contaminants retrouvés dans les échantillons de larves. Frédéric Olivier, Laurent Chauvaud, Isabelle Marcotte, et Réjean Tremblay ont conçu et supervisé l'étude. Tous les auteurs ont contribué à la rédaction du manuscrit et ont approuvé l'article final. Une version abrégée de cet article a été présentée à la conférence annuelle de *Ressources Aquatique Québec* en 2024 à Québec.

EFFECT OF CHEMICAL POLLUTION REPRESENTATIVE OF AN INDUSTRIAL PORT ON THE EMBRYONIC SUCCESS OF THE BLUE MUSSEL, *MYTILUS EDULIS*: A METABOLOMIC APPROACH

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3.2 ABSTRACT

With the growing demand for international maritime trade, there is an urgent need to improve our knowledge of the impact of shipping on marine organisms to develop effective mitigation strategies. Bivalves are important organisms in intertidal ecosystems where maritime harbors are situated. In this work, we evaluated the response of mussel larvae exposed to chemical contaminants associated with maritime traffic. We focused on embryogenesis since the first ontogenic stages are sensitive to stressful events of primary importance for the maintenance of community structure. Thus, embryos of the blue mussel, *Mytilus edulis*, from two spawning events following natural gonad maturation, were exposed to a cocktail of contaminants representative of a port environment until the end of embryogenesis. Our results show a different metabolomic response of D-larvae to contamination depending on the embryogenic success. Contaminated larvae had greater energy requirements and appeared to successfully employ a variety of mechanisms involving antioxidant biosynthesis and energy metabolism restructuring to mitigate toxic effects on cells and developing tissues. This work demonstrates the vulnerability of mussel larvae in an environment exposed to high maritime traffic and emphasizes the need for regulations.

Keywords: metabolomics, ecotoxicology, larval development, embryogenesis, bivalve/mollusc physiology.

3.3 INTRODUCTION

Maritime traffic is one of the world's largest industries, carrying 80% of the global merchandise trade (Canton, 2021; UNCTAD, 2016). This growing sector creates trade routes and transportation infrastructure in response to globalization, and includes an aging fleet responsible for water pollution that can harm marine organisms and ecosystems (Pirotta et al., 2019). These shipping routes facilitate transportation, connect locations, and concentrate vessel movements toward common port destinations (Laurance et al., 2009). The resulting high concentration of vessels exerts various pressures on the local marine environment, such as the loss or discharge of solid wastes, the emission of continuous and impulsive underwater noise and vibration, the release of oil and other pollutants (*e.g.* antifouling paints, anodes), and the introduction of invasive species through ballast water and hull fouling (Bellas et al., 2008; Kasiotis & Emmanouil, 2015; Liubartseva et al., 2015; Wilcock et al., 2014). Moreover, polyaromatic hydrocarbons (PAHs) are also present, originating from the incomplete combustion of organic matter linked to anthropogenic activity in harbor environments (Baumard et al., 1998). As shipping impacts are expected to rise alongside growing demand for international maritime trade (Pirotta et al., 2019), there is an urgent need to deepen our understanding of the effects of pollution on marine organisms in order to develop effective mitigation strategies.

Bivalves are ideal sentinel species for use in ecotoxicological monitoring (Beyer et al., 2017; His et al., 1999), due to their important filtration capacity, sessile nature and robustness (Baumard et al., 1998; Farrington et al., 2002). They are therefore frequently used to determine the contaminants that can accumulate in a marine ecosystem (His et al. 1999). Early life stages of marine invertebrates are particularly suitable for marine environmental bioassays due to their high sensitivity to pollutants (Beiras et al., 2003; Bellas et al., 2008; Geffard et al., 2003; His et al., 1999). Indeed, chemicals can affect morphogenetic processes such as gastulation, and tissue interactions such as induction, growth, and degeneration or cell death, which are inherent parts of embryonic development (Liu et al., 2024; Weis, 2014).

The objective of this work was to assess the response of the first larval development stages of the blue mussel (*Mytilus edulis)* when exposed to chemical contaminants representative of maritime traffic. For that purpose, we used here a chemical cocktail representative of elements and concentrations measured and quantified in adult mussels from the St-Pierre et Miquelon harbor (France). Since the first ontogenic stages are known to be the most sensitive to stressors (Rayssac et al., 2010; Schmutz et al., 2021) and crucial for maintaining community structure, we focused on embryogenesis. Specifically, embryos from two spawning events, following natural gonad maturation, were exposed in the laboratory to a contaminant mixture simulating conditions along a shipping lane through to the end of the embryogenesis stage. We hypothesized that chemical pollution may have different effects depending on egg maturation (early *vs.* late in the spawning season), on both physical (embryogenesis success and larval length) and physiological traits, as estimated by metabolomic analyses (Sih et al., 2004). Through a novel metabolomics approach, we aim to understand mechanisms involved in mussel responses to chemical pollution. We focused on the metabolites related to energy (Bayona et al., 2022) and prostaglandin pathways (Duran-Encinas et al., 2024), since prostaglandins modulate stress responses by inducing apoptosis, synthesizing heat shock proteins to protect macromolecular structures, and producing antioxidant enzymes (Collier et al., 2008; Vu & Acosta, 2014; Zhang et al., 2015).

3.4 MATERIALS AND METHODS

3.4.1 Mussel larval culture and experimental set-up

Adult *Mytilus edulis* (total lengths 50-60 mm) were collected in St. Peters Bay in Prince Edward Island (46.42948N, -62.66030W, Canada) on May 27 (beginning of spawning season) and 3 July 2022 (end of spawning season). In this area, mussels spawn from around mid-May to mid-July (Filgueira et al., 2015; Toupoint et al., 2012b). Upon arrival, 40 mussels were individually induced to spawn by 10°C thermal shock, as described in Rayssac et al. (2010). A ratio of 10 spermatozoids per egg was used for the fertilization in UV ultra-filtered (1µm) seawater at 18°C. Fertilized eggs were further washed with UV ultra-filtered seawater

and maintained at 18.0 ± 0.2°C, with a salinity of 23.1 ± 0.1 PSU, under a photoperiod of 15h of light (light intensity of 97.1 ± 1.7 lux), and 9h of dark, and exposed to chemical pollution representative of harbor conditions, as described below. Embryos from each spawning period were subdivided in six tanks (25,000 embryos per tanks), *i.e.*, three for the control (without chemical pollution) and three others with the addition of chemical pollutants. At the end of embryogenesis when over 75 to 80% of the population was at the D-shaped larval stage (4 days), experiments were stopped. D-larvae from each tank were sieved on a 20 µm mesh and subsamples were collected to estimate the embryogenesis success (number of D-larvae X 100 / numbers of fertilized eggs) and D-larval size (maximum width, Martel et al., 2014). The remaining larvae were split into three sub-samples, all flash-frozen in liquid nitrogen, lyophilized, and stored at -80°C until extraction for analysis. One subsample was used to explore the oxidative metabolism, mainly prostaglandin pathways (primary stress), another one for the energy metabolism (secondary stress), and the third one to determine the contaminants.

3.4.2 Chemical challenge conditions

The level of chemical pollution in the harbor of Saint-Pierre at Saint-Pierre-et-Miquelon (SPM) (France) was evaluated in October 2020, before the challenge experiments, using soft tissues of 16 adult mussels (50-60 mm) collected at subtidal level (46°46'44.90"N, -56°10'38.28"W). Contaminants were identified and quantified in the samples by untargeted GS-MS analysis (**Tab. 13**). The data indicated that the main chemical products bioaccumulated in the mussels were copper, lead, mercury and hydrocarbons. The concentration used in the challenge experiments on embryos were similar to the mean concentrations of these elements measured in the tissues of adult mussels.

The metal ion solutions were made from ICP-MS 1 000 $mg.L^{-1}$ standard solutions. For copper and lead, the respective solutions were diluted in 0.45 µm filtered seawater in order to obtain final concentrations of 4 $\mu g \cdot L^{-1}$ (Cu^{2+}) and 23 $\mu g \cdot L^{-1}$ (Pb^{2+}). The mercury standard solution was diluted by 10 in nanopure water acidified with high purity nitric acid (HNO_3)

(±2% final), then diluted again in seawater to obtain a final Hg^{2+} concentration of 0.05 µg·L^{-1}. For the hydrocarbon solution, 10 mL of diesel was mixed with 200 mL of filtered seawater (0.45 µm) in a 500 mL separatory funnel for at least 2h at room temperature and only the aqueous phase saturated with hydrocarbons was preserved (soluble part of diesel). Finally, this aqueous phase was diluted in seawater to obtain a final concentration of 0.01 µL·L^{-1}.

Table 13. Average concentration of various contamination in mussel tissues from the harbor of Saint-Pierre (France).

Metals	Average concentrations (mg/kg)
Arsenic (As)	NA
Cadium (Cd)	5.9 ± 3
Chromium (Cr)	1.3 ± 1
Copper (Cu)	6.2 ± 2
Nickel (Ni)	1.4 ± 0
Mercury (Hg)	0.1 ± 0
Lead (Pb)	21.2 ± 5
Zinc (Zn)	87.5 ± 36

3.4.3 Determination of contaminants in larval samples

Around 1 mg of freeze-dried D-larvae were used for the dosage of metals and hydrocarbons based on a method described in Lemos et al., (2021). Briefly after 30 s of ultra-freeze micro-grinding and homogenization (Cryolys-Precellys 24; Bertin corporation), alkaline digestion in 200 µL of tetramethylammonium hydroxide (TMAH, 25 % aqueous) was carried out at 60°C for 1 h with constant stirring. Then, the solution was acidified with nitric acid (HNO_3) 67-70%. Metals and hydrocarbons were then extracted and separated by adding 300 µL of hexane-toluene, repeated twice. Inorganic compounds, such as Cu, Pb and Hg ions, were obtained from the aqueous phase while organic compounds, such as hydrocarbons (including polycyclic aromatic hydrocarbons (PAHs) and alkanes), were collected from the organic phase. The two phases were subsequently treated independently. The aqueous phase containing the metal ions was filtered through a 45-µm

polytetrafluoroethylene (PTFE) Teflon filter then analyzed by GF-AAS 240Z atomic absorption spectrometer equipped with a GTA 120 graphite tube atomizer (Agilent Technologies). For lead measurements, a solution of palladium and magnesium was used (Agilent technologies, Pre-Mixed GF AAS: 750 $\mu g \cdot mL^{-1}$ Pd and 500 $\mu g \cdot mL^{-1}$ Mg in 2 % $HN0_3$). The organic phase was purified with 100 mg of magnesium sulfate (Sigma-Aldrich) and 50 mg of Florisil. The hydrocarbon content was then analyzed by injecting 200 µL of samples in a 8890 Gas Chromatograph (GC) System (Agilent Technologies) equipped with a Rxi-5ms capillary column, (30 m in length, 0.53 mm of internal diameter, 0.25 µm thickness (Restek, USA).

3.4.4 Quantification of amino acids and energetic metabolism

To extract amino acids and metabolites linked to energy metabolism, the ultra-freeze-dried D-larvae were micro-ground and homogenized with a 2.8 mm ceramic beads 3 times for 10 s (Cryolis-Precellys 24; Bertin corporation). Then, 500 µL of 1/1 (v/v) mix of TFE/LC-MS grade water was added in the 3 mL Agilent Captiva EMR–Lipid solid phase extraction cartridge (part number 5190-1003 – Agilent Technologies) and 750µL of a 1/1 (v/v) mix of TFE/LC-MS grade water was added to the homogenizing tubes, vortexed for 30 s and centrifuged for 1 min at 2,460 g. Three extraction cycles (500 µL, with 1 mL of acetonitrile (ACN) for the last one) were performed using a Captiva EMR-Lipid cartridge, and filtrates were pooled in a 15-mL falcon tube. The cartridge was then rinsed with 3 mL of a 1/1 (v/v) mix of TFE/ LC-MS grade water and drained under high vacuum for 30 s. The sample in the falcon tube was dried with a SpeedVac overnight without heating. Sample were reconstituted by adding 50 µL of LC-MS grade water followed by 200 µL of LC-MS grade ACN. A total of 100 µL of each reconstituted sample was transferred into two vials: one for positive-ion and the other for negative-ion analysis. Deuterated compounds valine-d_8 (10 µg/mL) and pyruvate-d_3 (10 µg/mL) were used as internal standards for quantification in positive- and negative-ion modes, respectively. Metabolites were separated and quantified in multiple reaction monitoring mode using an HPLC 1260 Infinity II device coupled to a 6470B Triple Quad mass spectrometer (Agilent Technologies) in positive and negative-ionization modes.

Precisely 10 µL of the sample was injected, and the chromatography separation was performed with a InfinityLab Poroshell 120 HILIC-Z (2.7 µm 10 × 2.1 mm) column.

3.4.5 Quantification of polyunsaturated fatty acids oxidation products

Oxylipins linked to oxidative metabolism were extracted from ultra-freeze-dried D-larvae micro-ground and homogenized with a 2.8 mm ceramic bead three times for 10 s (Cryolis- 24; Bertin corporation). A total of 200 µL of a 1/1 (v/v) mixture of 2,2,2-trifluoroethanol (TFE) and extraction buffer (ammonium formate buffer at pH 3) was added to the homogenizing tubes containing the sample. Samples were vortexed for 10 s and centrifuged for 1 min at 2,460 g. Then, 200 µL of a 1/1 (v/v) mixture of ACN and methanol (MEOH) was added to the homogenizing tubes containing the sample. Samples were vortexed for 10 s and centrifuged for 5 min at 2,460 g, 4°C. Precisely 250 µL of the supernatant was transferred to a 2-mL HPLC vial containing 1 mL of 50 mM ammonium formate buffer at pH 3 with 10 ng/mL of internal standard PGE_2-d_9 (Prostaglandin E2-d9). Each sample was analyzed directly after the extraction process with an HPLC 1260 Infinity II device coupled to a 6470B Triple Quad mass spectrometer (Agilent Technologies) using on-line SPE method allowing sample cleaning and oxylipin draping from 1 ml of samples. Oxylipin extracts were separated using a Gemini C6-Phenyl 100Å, 2 x150 mm 5 µm (Phenomenex) and a Gemini C6-Phenyl guard column as on-line SPE (4 × 2.0 mm, SecurityGuard, Phenomenex) with column temperature maintained at 40°C.

3.4.6 Statistical analyses

Multivariate statistics to test the impact of the presence of contaminants on metabolomic data were carried out on each spawning event with Metaboanalyst, a freely available web-based metabolomics analysis suite (MetaboAnalyst 5.0, http://www.metaboanalyst.ca) (Pang et al., 2021) to produce heatmaps, and with R studio to produce Principal Component Analyses (PCA). Data were centered and transformed. To identify the most significantly affected metabolic pathways, discriminant metabolites square-

root were analyzed by metabolite-set enrichment analysis (MSEA) (Xia & Wishart, 2010), implemented in Metaboanalyst. The metabolite enrichment analysis of each set of metabolites was performed using over-representation analysis with the "pathway-associated metabolite sets" provided, containing 99 metabolite sets based on normal human metabolic pathways. The ORA (over-representation analysis) was implemented using the hypergeometric test to evaluate whether a particular metabolite set was more highly represented than expected by chance within the given compound list. One-way univariate PERMANOVA was used to test the differences between contaminant treatment for each spawning event and between spawning events on the total length of D-larvae and embryogenesis success by using PRIMER7 software. The permutational method is a randomization that allows us to eliminate the distributional assumptions as normality and can be applied to very small samples (Legendre & Legendre, 2012). Similarities matrices based on ratios were produced using the Bray-Curtis distance. When differences were significant (p-perm ≤ 0.05), multiple comparison pairwise tests were used to determine which groups significantly differed. Distance-based tests for homogeneity of multivariate dispersion (PERMDISP) were carried out to evaluate the homogeneity of ratios in each condition.

3.5 RESULTS

3.5.1 Quality of egg-quality in relation to chemical contamination

We first investigated the effect of chemical contamination on embryogenesis, and the interaction of this egg-quality. For this purpose, we used a cocktail of contaminants (**Tab. 14**) representative of the chemical pollution in the port of Saint-Pierre at (SPM, France) in the Gulf of St. Lawrence.

Table 14. Contaminant cocktail representative of Saint-Pierre's harbor pollution and used for egg exposure.

Contaminant	Concentration ($\mu g \cdot L^{-1}$)
Copper	4
Lead	23
Mercury	0.05
Alcane	0.01

The results reported in Table 15 show that the control fertilized eggs from the spawning at the end-May (spawning 1) had a significantly lower (54%) embryogenesis success as compared to the spawning in early July (spawning 2) (Df = 1, Pseudo-F = 6.57, P-perm = 0.05). These results suggest that spawn 1 could be classified as "poor quality" and the second as "better quality". When exposed to contaminants, the embryogenesis success was still low (21%) for spawning 1, although it was 7% higher than the control and close to being significantly different (**Tab. 15**). As for spawning 2, exposure to contaminants did not affect the embryogenesis success with a mean of 67% ± 11%. Contamination did not impact on the total length of D-larvae from either spawning events, although larvae from the May spawning were 14 µm longer than those from the July spawning (**Tab. 15**). Table 15 shows that all contaminants were present in the controls, and that the accumulation in the D-larvae from exposed embryos was related to the spawning period. For the spawn 1 D-larvae accumulated only lead and alkanes, while copper concentration was higher than the control (14 $ng.g^{-1}$ *vs.* 3.5 $ng.g^{-1}$ in the exposed larvae).

Table 15. Mean level of embryogenic success, D-larvae length and concentration of chemical pollutants found in D-larvae after exposure in ng/g ± standard error (SE). Bold indicates p-values near or below 0.05.

	Spawning 1				Spawning 2			
	Control	Exposed	P-perm	Pseudo-F	Control	Exposed	P-perm	Pseudo-F
Embryogenesis success	13% ± 1	21% ± 3	**0.0622**	6.1431	67% ± 11		0.296	1.4127
D-larvae size	110.1µm ± 2.3		0.2776	1.6293	95.8µm ± 2.4		0.1925	2.3741
All pollutants			**0.0004**	7.9139			**0.0047**	5.9803
Copper	13.9 ± 3.2	3.5 ± 0.9	**0.0008**	5.6271	1.2 ± 0.1	2.4 ± 0.2	**0.001**	12.228
Lead	1.4 ± 0.6	60.0 ± 13.5	**0.0016**	6.4782	0.9 ± 0.9	17.5 ± 6.3	**0.0209**	4.1443
Mercury	0.0011 ± 0.000095	0.0012 ± 0.000087	0.2859	1.2161	0.0006 ± 0.000042	0.0016 ± 0.00018	**0.0004**	25.372
Alkane	0.3 ± 0.1	0.5 ± 0.1	**0.0731**	2.334	0.1 ± 0.0	0.3 ± 0.1	**0.0584**	3.1438
PAH	0.5 ± 0.1	0.5 ± 0.1	0.8512	0.079934	0.1 ± 0.0	0.1 ± 0.1	0.1119	2.5921

Only D-larvae from spawning 2 accumulated mercury in their tissues, and the lead content increased by 20-fold; similarly, in the first spawn, lead levels increased 43-fold. No PAHs were accumulated in D-larvae from both egg laying with levels similar to the control; however, PAH levels were higher in spawning 1. Alkane concentration was quantified to follow the chemical contamination (*i.e.,* chemical fingerprint), as the contamination was via diesel and not via the addition of individual PAHs.

3.5.2 Metabolic pathways impacted by chemical contamination

3.5.2.1 Metabolic pathways

As a next step, we evaluated the metabolic pathways affected by the contamination in the D-larvae. The results displayed in Figure 33 show the amino acids and energy metabolism related metabolites. Surprisingly, despite the differences revealed in Table 15, the targeted metabolic profile (*i.e.*, all metabolites) related to energy metabolism was similar between larvae from the two spawning events (Df = 1, Pseudo-F = 1.08, P-perm = 0.33) and between larvae exposed to the contaminant cocktail and the control (Df = 1, Pseudo-F = 1.19, P-perm = 0.30) (**Fig. 33a, b**). However, 'metabolite by metabolite' analyses revealed differences between the contaminated and the unexposed larvae, with higher and significant differences

in metabolites in spawning 2 (**Tab. 16**). In fact, metabolite concentrations were always lower in contaminated larvae than in the controls for both spawning events. To further investigate these differences, an enrichment analysis was performed only on this second spawning event. This analysis highlighted differences in 25 metabolic pathways involved in or affected by contamination, particularly for the transfer of acetyl groups into mitochondria, the Citric Acid Cycle (TCA or Krebs cycle), gluconeogenesis, glycolysis, glucose-alanine cycle, and glycine and serine metabolism (**Fig. 34**). In addition, the enrichment analysis in Figure 35 revealed an alteration of glycolysis as well as the Krebs cycle when the ratio of related metabolites was examined.

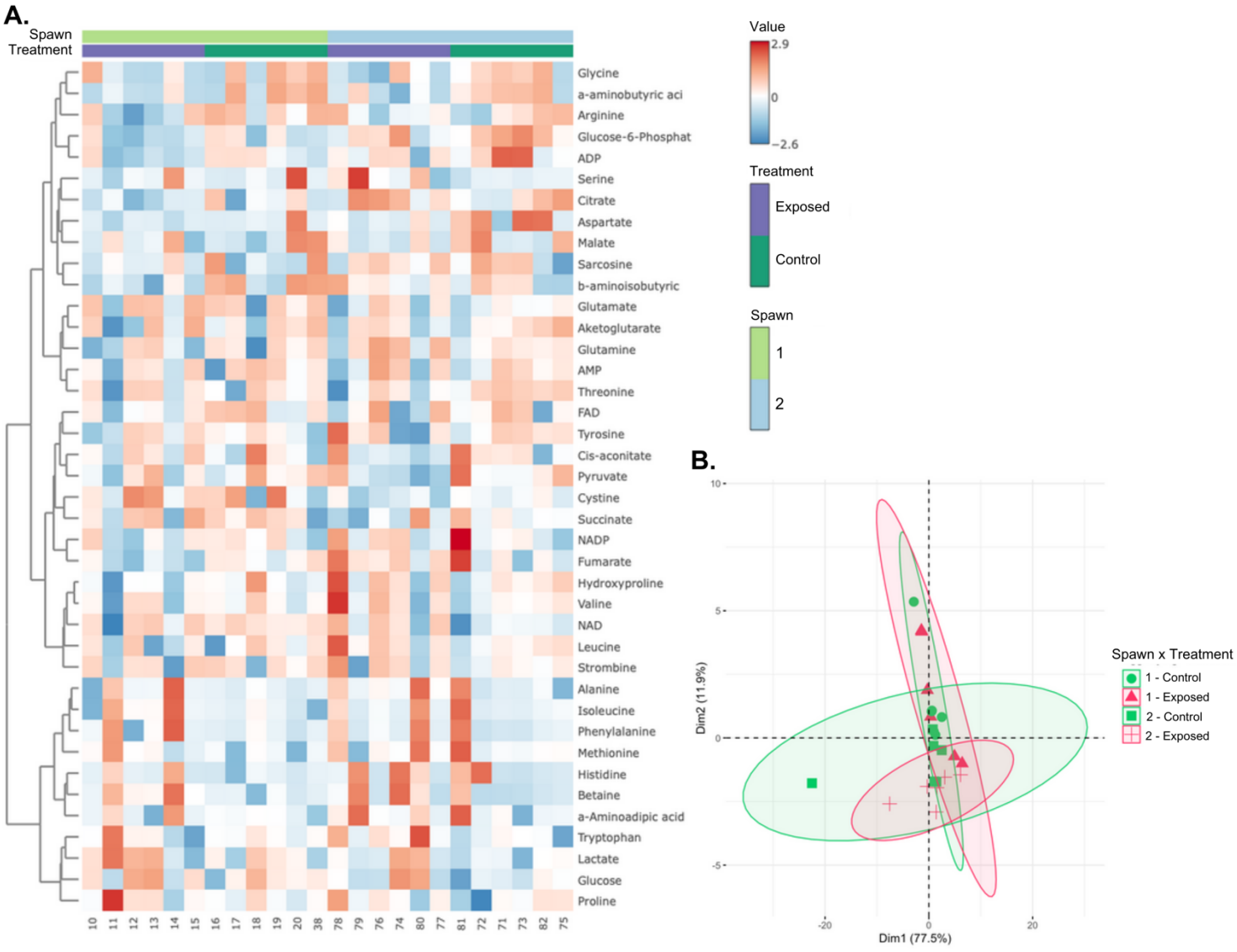

Figure 33. (A) Heatmap of amino acids and energy metabolism-related metabolites in relation to spawning and pollutant exposure (exposed or control). **(B)** PCA plot based on the amino acids and energy metabolism data. Green circles refer to the control of spawning 1 (n=6), pink triangles to the effect of contamination for spawning 1 (n=6), green boxes to the control of spawning 2 (n=6), and pink crosses to the effect of contamination for spawning 2 (n=6).

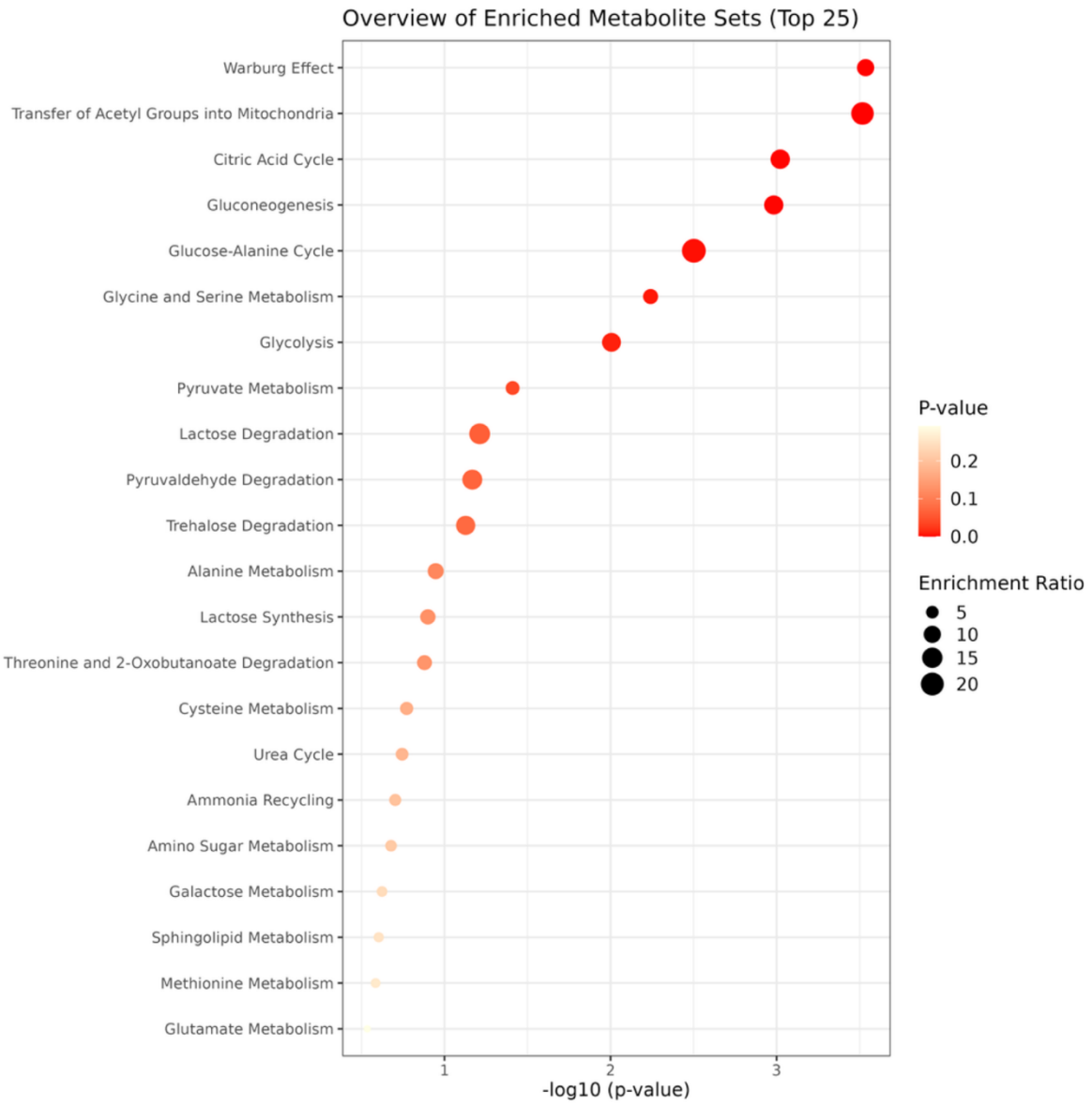

Figure 34. Metabolite Set Enrichment Analysis performed on the list of significant metabolites for spawning 2. Bubble sizes represent the fold enrichment of the metabolic pathways identified, and the red colour gradient corresponds to the P-value corrected for multiple testing by FDR.

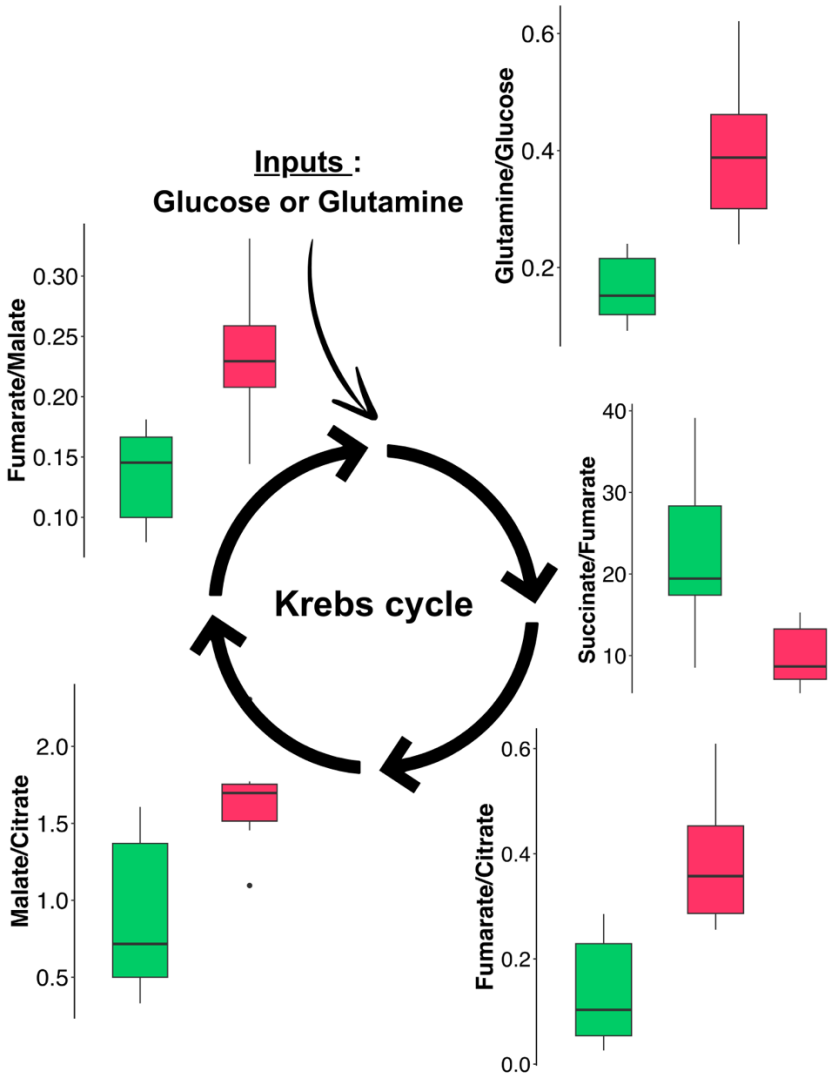

Figure 35. Synthetic diagram of the Krebs cycle and inputs of the glucose and glutamine metabolism, with metabolic contribution following contamination conditions for spawning 2, expressed as a ratio in ng/mg ± SE. In the boxplots, green refers to the control (n=6) and pink to the contaminant-exposed D-larvae (n=6). Abbreviations: ADP= adenosine diphosphate, AMP= adenosine monophosphate, FAD= flavin adenine dinucleotide, and NAD= nicotinamide adenine dinucleotide**.**

Table 16. Targeted LC-MS-based quantification (mean ± standard error ng/mg) of amino acids and energy metabolism metabolites. The significance of the differences in concentrations of the metabolites between noise levels were tested by PERMANOVA. Bold indicate p-values near or below 0.05. AEC: Adenylate Energy Charge, BCAAs: Branched Chain Amino Acid.

		Spawning 1				Spawning 2			
		Control	Exposed	P-perm	Pseudo-F	Control	Exposed	P-perm	Pseudo-F
	All metabolites			0.412	1.0165			0.6355	0.58512
Glycolysis	Glucose	471.5 ± 58.2	506.3 ± 129.6	0.5125	0.81532	377.3 ± 102.2	163.6 ± 38.8	**0.0216**	5.3316
	Glucose-6-Phosphate	2.5 ± 0.5	1.6 ± 0.5	0.2084	1.5554	8.4 ± 3.2	5.6 ± 2.3	0.5203	0.76669
	Fructose-1,6-biphosphate	NQ	1.1 ± 0.3			NQ	NQ	NA	NA
	phosphoenol pyruvate	NQ	NQ	NA	NA	NQ	NQ	NA	NA
	Pyruvate	33.3 ± 5.5	26.7 ± 6.4	0.3884	0.88975	38.2 ± 7.6	19.5 ± 6.2	**0.0351**	4.5382
Anaerobic	Lactate	325.1 ± 34.6	367,2 ± 94.2	0.4651	0.86887	251.0 ± 62.6	118.4 ± 26.9	**0.0266**	5.2498
	Octopine	NQ	NQ	NA	NA	NQ	NQ	NA	NA
	Strombine	2.9 ± 0.2	2.4 ± 0.4	0.2726	1.4685	3.7 ± 1.6	2.8 ± 0.6	0.9787	0.14279
Krebs cycle	Acetyl-Coa	NQ	NQ	NA	NA	NQ	NQ	NA	NA
	Citrate	81.1 ± 47.5	33.9 ± 14.7	0.8408	0.41079	22.8 ± 6.1	8.7 ± 2.4	**0.0561**	3.4349
	Cis-aconitate	0.1 ± 0.0	0.1 ± 0.0	0.6927	0.32953	0.1 ± 0.0	0.1 ± 0.0	**0.0501**	3.8209
	Aketoglutarate	7.1 ± 1.2	7.2 ± 1.6	0.8047	0.2013	8.7 ± 2.3	6.5 ± 1.9	0.5562	0.72581
	Succinyl CoA	NQ	NQ	NA	NA	NQ	NQ	NA	NA
	Succinate	60.9 ± 9.2	50.2 ± 11.0	0.4243	0.83283	39.1 ± 7.6	33.3 ± 14.3	0.1787	1.8544
	Fumarate	2.0 ± 0.3	1.2 ± 0.3	**0.0925**	3.0472	2.7 ± 1.2	3.2 ± 0.8	0.2809	1.1913
	Malate	12.8 ± 1.4	12.7 ± 2.5	0.6663	0.34979	18.1 ± 7.0	14.2 ± 4.0	0.9348	0.078838
	Oxaloacetate	NQ	NQ	NA	NA	NQ	NQ	NA	NA
BCAAs	Valine	36.7 ± 3.1	32.3 ± 6.0	0.3976	0.91299	63.6 ± 26.6	45.8 ± 10.0	0.9678	0.14731
	Leucine	38.4 ± 3.5	35.7 ± 7.0	0.4606	0.6711	62.4 ± 25.1	46.6 ± 10.7	0.968	0.11971
	Isoleucine	17.8 ±1.7	16.3 ± 3.2	0.4573	0.75048	27.6 ± 10.6	18.1 ± 3.8	0.8376	0.41852
Amino acids	Aspartate	1096.7 ± 63.6	881.0 ± 164.4	0.3219	1.6479	1523.2 ± 563.0	1044.2 ±228.7	0.8484	0.34934
	Glutamate	748.8 ± 42.3	653.3 ± 118.9	0.4321	1.0689	860.4 ± 348.2	637.2 ± 138.5	0.9618	0.14402
	Glutamine	44.2 ± 3.1	38.6 ± 6.8	0.4137	0.9325	66.2 ± 28.3	62.6 ± 12.6	0.6257	0.48627
	Arginine	11.1 ± 3.0	10.2 ± 3.7	0.5248	0.68059	10.9 ± 2.4	6.8 ± 1.5	0.1317	2.4025
Energy transfer	ATP	0.8 ± 0.2	0.8 ± 0.1	0.8269	0.2318	NQ	NQ	NA	NA
	ADP	2.1 ± 0.4	1.0 ± 0.5	**0.0617**	3.3223	6.6 ± 2.4	5.6 ± 3.4	0.5944	0.5566
	AMP	610.6 ± 48.2	434.5 ± 80.2	0.1276	2.3419	855.9 ± 328.9	674.1 ± 179.4	0.9187	0.17756
	FAD	6.0 ± 0.9	4.0 ± 0.7	0.1913	1.69	12.5 ± 6.0	8.5 ± 2.4	0.9662	0.13229
	NAD	6.1 ± 0.3	5.6 ± 1.1	0.4642	0.89886	6.8 ± 3.1	5.9 ± 1.3	0.8198	0.40971
	NADH	NQ	NQ	NA	NA	NQ	NQ	NA	NA
	NADP	0.8 ± 0.2	0.7 ± 0.2	0.406	0.82416	1.4 ± 0.8	1.0 ± 0.3	0.9134	0.16297
	NADPH	NQ	NQ	NA	NA	NQ	NQ	NA	NA
	Lysine	NQ	NQ	NA	NA	NQ	NQ	NA	NA
	Tryptophan	21.1 ± 1.5	17.1 ± 3.1	0.3063	1.5168	33.2 ± 12.9	22.9 ± 5.0	0.9036	0.26626
	Tyrosine	27.9 ± 2.6	31.5 ± 5.7	0.5595	0.42625	51.8 ± 16.1	42.2 ± 11.1	0.86	0.19312
	Phenylalanine	20.6 ± 1.9	19.6 ± 3.9	0.4933	0.6565	28.1 ± 10.3	22.4 ± 5.2	0.9671	0.084457
	Histidine	155.5 ± 11.5	116.7 ± 22.2	0.1956	2.1959	178.6 ± 67.4	99.0 ± 19.2	0.2949	1.1175
	Proline	31.4 ± 3.1	28.8 ± 5.5	0.4634	0.67771	52.3 ± 18.0	35.9 ± 7.0	0.8001	0.413
	Threonine	54.4 ± 5.4	51.1 ± 10.0	0.4832	0.66614	128.6 ± 55.2	57.3 ± 13.0	**0.0989**	1.8073
	Methionine	16.0 ± 1.2	14.7 ± 2.8	0.3991	0.77842	27.5 ± 10.1	20.2 ± 4.4	0.9197	0.18888
	Sarcosine	13.4 ± 2.4	13.8 ± 3.2	0.5779	0.51969	12.8 ± 3.4	6.1 ± 1.1	**0.0443**	3.6207
	Glycine	932.0 ± 62.8	820.1 ± 149.6	0.4183	0.97394	1290.5 ± 473.3	988.9 ± 203.5	0.958	0.11271
	Cystine	6.1 ± 1.1	4.4 ± 0.8	0.2542	1.3656	26.1 ± 1.8	15.4 ± 4.1	**0.0345**	3.905
	Alanine	154.2 ± 11.1	129.6 ± 23.6	0.3687	1.2231	233.5 ± 80.7	205.0 ± 38.9	0.9536	0.095724
	Serine	114.2 ± 10.4	108.5 ± 20.7	0.5238	0.62809	218.2 ± 91.0	138.8 ± 30.0	0.8879	0.3564
	Hydroxyproline	3.2 ± 0.5	2.3 ± 0.4	0.2773	1.475	5.5 ± 2.1	3.9 ± 0.8	0.8935	0.23591
	a-aminobutyric acid	10.4 ± 0.9	9.0 ± 1.7	0.4231	0.9425	14.3 ± 6.0	10.1 ± 2.2	0.95	0.16118
	b-aminoisobutyric	10.3 ± 1.7	10.7 ± 2.6	0.5447	0.58165	9.7 ± 2.7	4.3 ± 0.8	**0.0344**	4.4598
	a-Aminoadipic acid	18.1 ± 1.0	11.3 ± 2.0	**0.0105**	4.5387	30.0 ± 12.6	14.8 ± 3.2	0.3932	1.1001
	Betaine	2119.1 ± 188.0	2023.4 ± 429.8	0.4793	0.77797	1924.7 ± 671.9	1163.8 ± 284.0	0.4284	0.96851
Ratio	AEC	0	0	NA	NA	0	0	NA	NA
	BCAAs	92.9 ± 8.2	84.4 ± 16.1	0.4399	0.77117	153.7 ± 62.3	110.5 ± 24.5	0.9525	0.1582
	Glutamine/Glucose	0.1 ± 0.0	0.1 ± 0.0	0.4338	0.69018	0.2 ± 0.0	0.4 ± 0.1	**0.0054**	13.822
	Citrate/Cis-aconitate	711.5 ± 137.8	340.6 ± 130.1	**0.0563**	3.9483	173.1 ± 45.0	119.4 ± 15.8	0.3501	0.966
	Succinate/Fumarate	34.0 ± 6.5	42.6 ± 6.8	0.3614	0.86336	22.4 ± 4.4	9.7 ± 1.8	**0.0165**	6.0917
	Fumarate/Malate	0.2 ± 0.0	0.1 ± 0.0	0.1568	2.2041	0.1 ± 0.0	0.2 ± 0.0	**0.0117**	8.3074
	Malate/Fumarate	7.7 ± 2.0	11.0 ± 1.7	0.1487	2.2614	8.2 ± 1.2	4.6 ± 0.5	**0.0118**	8.4662
	Fumarate/Citrate	0.1 ± 0.0	0.1 ± 0.0	0.9856	0.020069	0.1 ± 0.1	0.4 ± 0.1	**0.0126**	7.2757
	Malate/Citrate	0.4 ± 0.1	0.6 ± 0.2	0.4022	0.84677	0.9 ± 0.2	1.7 ± 0.2	**0.0267**	6.3757
	Glycine/Succinate	16.5 ± 1.8	17.2 ± 1.3	0.7547	0.22533	29.9 ± 4.2	39.2 ± 5.5	0.2305	1.5472
	NADH/NAD	NA	NA	NA	NA	NA	NA	NA	NA

3.5.2.2 Oxidative pathways

Figure 34 presents the prostaglandins and metabolites associated with oxidative metabolism. In contrast to the metabolic pathways, oxylipin profiles differed in D-larvae between spawning events (Df = 1, Pseudo-F = 9.7056, P-perm = 0.0002). However, no differences were associated with exposure to the chemical cocktail (Df = 1, Pseudo-F = 1.9161, P-perm = 0.1193), and there was no significant interaction between the spawning and the contaminants (Df = 1, Pseudo-F = 1.1567, P-perm = 0.3016) (**Fig. 36a**). The total sum of oxylipins quantified in the larvae was two times higher in spawning 2 than in spawning 1 (**Tab. 17**). Metabolite by metabolite analyses show that the two spawning events had three common oxylipin variations related to contamination: the 12-HEPE (hydroxyicosapentaenoic acid), 5-oxoETE (5-oxo-eicosatetraenoic acid) and 20-HDHA (20-hydroxy-docosahexaenoic acid) had significantly higher values in spawn 1 (**Fig. 36a, b**). In the case of oxidative metabolism, the enrichment analysis could not be performed due to a lack of information in metabolic pathway databases.

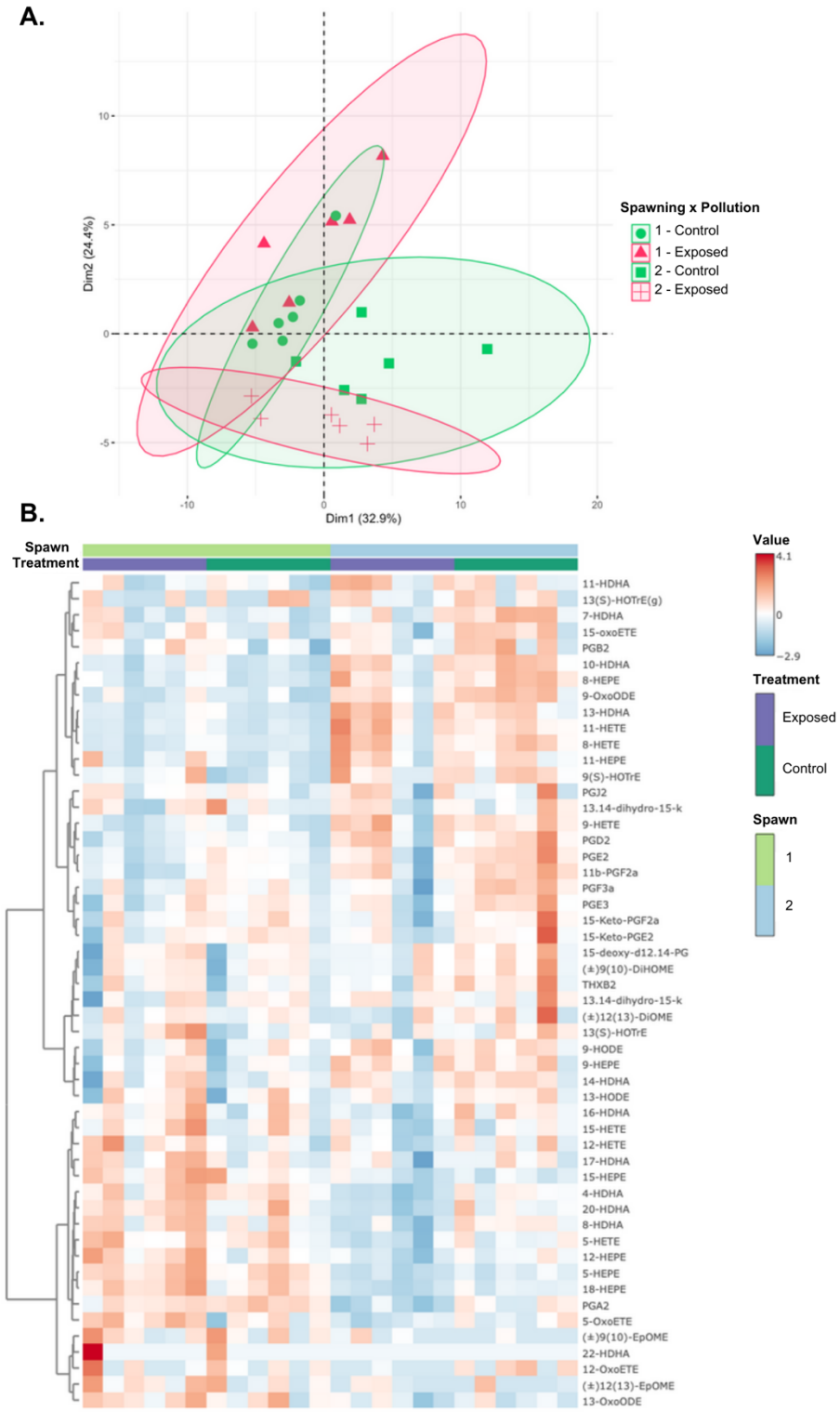

Figure 36. (A) PCA plot based on the prostaglandins and metabolites related to oxidative metabolism. Green circles refer to the control (n=6), and pink triangles to the exposed larvae (n=6) from spawn 1, and green squares refer to the control (n = 5) and pink cross to the exposed larvae (n=6) from spawn 2. **(B)** Heatmap of prostaglandins and metabolites related to oxidative metabolism in relation to spawning and contaminant exposure.

Table 17. Targeted LC-MS-based quantification (mean ± standard error ng/g) of prostaglandins and unsaturated fatty oxidation products in relation to spawning event and chemical exposure of blue mussel D-larvae. The significance of the differences in concentrations of the metabolites between noise levels were tested by PERMANOVA. Bold indicates p-values near or below 0.05.

	Spawn 1				Spawn 2			
	Control	Exposed	P-perm	Pseudo-F	Control	Exposed	P-perm	Pseudo-F
All metabolites			0.1533	1.8111			0.2177	1.3337
Total sum	40191.5 ± 5963.8	55806.4 ± 13780.1	0.5058	0.67012	109514.6 ± 12145.4	110759.3 ± 32464.9	0.4728	0.88924
Cyclopentenone prostaglandin								
PGE2	1082.4 ± 129.0	785.5 ± 106.6	0.1337	2.5222	1979.6 ± 347.7	1079.7 ± 250.6	**0.0299**	2.6904
15-keto-PGE2	71.0 ± 7.6	55.2 ± 11.1	0.2917	1.2604	96.8 ± 30.2	50.3 ± 8.9	**0.0705**	2.8889
PGD2	279.8 ± 32.5	208.6 ± 30.8	0.1691	1.9607	554.8 ± 122.7	470.1 ± 99.8	0.8538	0.38086
13,14-dihydro-15-Keto-PGD2	77.1 ± 11.9	71.4 ± 20.6	0.7693	0.69412	108.7 ± 30.5	90.2 ± 10.5	0.977	0.15072
PGA2	41.4 ± 6.3	29.4 ± 5.0	0.2003	1.5743	18.2 ± 4.9	2.1 ± 1.6	**0.0093**	4.1937
PGJ2	12.3 ± 1.8	15.3 ± 2.1	0.4312	0.73331	17.6 ± 7.4	18.0 ± 4.6	0.2567	1.2374
15-deoxy-d12,14-PGJ2	64.9 ± 14.3	95.3 ± 21.1	0.4624	0.72793	124.1 ± 28.8	77.4 ± 17.5	0.1586	2.0386
11b_PGF2α	14615.6 ± 1929.1	9016.9 ± 1879.5	**0.0759**	3.4234	30868.0 ± 4854.4	19000.9 ± 4927.9	0.1341	1.5225
15-keto-PGF2α	166.2 ± 19.3	147.0 ± 26.0	0.4764	0.60472	220.5 ± 57.4	107.1 ± 22.3	**0.0186**	3.4762
13,14-dihydro-15-Keto-PGF2α	258.9 ± 94.8	222.3 ± 57.2	0.6692	0.36968	246.8 ± 61.6	217. 0 ± 47.2	0.8843	0.17837
PGB2	48.8 ± 11.0	74.2 ± 12.8	0.2038	1.4093	104.8 ± 22.7	61.7 ± 7.0	0.1546	2.1872
THXB2	39.2 ± 6.5	53.6 ± 9.7	0.497	0.71426	78.8 ± 16.5	49.4 ± 7.3	0.1258	2.2399
EPA oxylipin								
PGE3	364.7 ± 29.7	297.3 ± 70.2	0.2471	1.6574	528.8 ± 79.5	297.8 ± 59.1	**0.0303**	3.7418
PGF3α	882.5 ± 106.1	795.7 ± 163.3	0.4615	0.58844	1489.2 ± 169.7	650.7 ± 130.0	**0.0021**	5.0903
5-HEPE	789.2 ± 143.4	1109.3 ± 204.4	0.2272	1.5591	263.4 ± 38.3	138.7 ± 21.3	**0.0248**	6.3843
8-HEPE	3956.0 ± 928.5	5330.5 ± 1061.9	0.3697	0.90183	21452.7 ± 3762.2	14403.8 ± 5349.3	0.1647	1.8939
9-HEPE	627.9 ± 150.2	889.8 ± 239.1	0.6088	0.38889	1465.9 ± 323.1	1561.2 ± 364.6	0.8166	0.17139
11-HEPE	5572.6 ± 1609.8	21517.0 ± 9874.3	0.2093	1.6847	22421.9 ± 4989.8	34809.7 ± 12945.2	0.5077	0.77096
12-HEPE	361.6 ± 65.8	768.2 ± 156.2	**0.0366**	4.8114	324.9 ± 44.7	166.1 ± 39.6	**0.0278**	4.1399
15-HEPE	679.9 ± 207.8	988.7 ± 201.0	0.1947	1.7895	290.7 ± 43.3	285.5 ± 67.3	0.7935	0.22145
18-HEPE	923.7 ± 219.8	1583.4 ± 333.2	0.1225	2.3462	369.2 ± 61.8	150.6 ± 25.7	**0.007**	9.9492
ALA-GLA-oxylipin								
9(S)-HOTrE	63.2 ± 10.0	121.4 ± 26.2	**0.0154**	6.5693	155.7 ± 20.6	146.8 ± 32.5	0.6229	0.29556
13(S)-HOTrE	62.1 ± 11.2	124.1 ± 31.7	0.1155	2.5436	105.5 ± 19.1	67.8 ± 12.7	0.1482	2.2102
13(S)-HOTrE(g)	12.9 ± 6.3	5.4 ± 3.4	0.7405	0.2812	10.1 ± 3.9	6.5 ± 3.3	0.3187	0.99871
Arachidonic-oxylipin								
5-HETE	113.5 ± 24.5	156.9 ± 34.8	0.3882	0.75559	75.9 ± 14.6	31.8 ± 9.5	**0.0492**	2.3302
8-HETE	1472.2 ± 271.0	1400.5 ± 295.7	0.8113	0.085166	5937.6 ± 1200.1	7322.6 ± 2400.5	0.5272	0.6667
9-HETE	250.0 ± 46.5	231.3 ± 43.1	0.8857	0.060367	581.6 ± 78.9	612.8 ± 156.5	0.5476	0.78695
11-HETE	952.4 ± 164.4	1259.0 ± 290.5	0.4969	0.5772	3944.6 ± 603.0	7344.2 ± 2460.5	0.1486	1.7707
12-HETE	79.6 ± 10.3	150.4 ± 28.7	**0.042**	4.7019	114.9 ± 19.6	81.6 ± 16.7	0.213	1.6217
15-HETE	226.8 ± 44.9	322.9 ± 60.4	0.1989	1.7238	224.2 ± 34.6	169.2 ± 32.5	0.2784	1.2152
5-OxoETE	25.4 ± 6.7	55.6 ± 8.1	**0.0234**	3.2633	23.3 ± 5.0	11.4 ± 2.0	**0.054**	3.6539
12-OxoETE	9.9 ± 5.0	20.9 ± 17.1	0.6077	0.44909	14.2 ± 5.3	0.6 ± 0.6	**0.0587**	3.8904
15-oxoETE	42.5 ± 8.6	65.8 ± 12.2	0.1678	1.9326	90.0 ± 14.1	50.8 ± 10.8	0.1328	1.953
Linoleic acid								
9-HODE	568.8 ± 114.2	658.6 ± 145.1	0.9123	0.095897	881.2 ± 94.0	842.0 ± 177.7	0.719	0.52212
13-HODE	501.2 ± 141.4	681.8 ± 159.0	0.6224	0.35857	640.1 ± 78.7	470.0 ± 70.8	0.1247	2.5338
9-oxoODE	107.8 ± 17.9	137.1 ± 27.1	0.4735	0.58438	347.1 ± 43.9	230.1 ± 31.1	0.1211	2.7525
13-oxoODE	26.8 ± 9.9	38.7 ± 8.2	0.2747	1.2903	12.0 ± 3.8	10.7 ± 3.7	0.8439	0.50256
(±)9(10)-DiHOME	5.1 ± 1.2	6.2 ± 1.4	0.5754	0.12009	9.4 ± 2.2	5.9 ± 1.1	0.173	2.0476
(±)12(13)-DiOME	28.3 ± 2.8	31.7 ± 3.9	0.556	0.37197	42.9 ± 10.6	25.1 ± 4.7	0.1375	2.3623
(±)9(10)-EpOME	25.8 ± 16.3	27.1 ± 18.4	0.9868	0.24781	0.8 ± 0.8	4.9 ± 4.1	0.3483	1.0444
(±)12(13)-EpOME	24.4 ± 17.5	46.6 ± 23.5	0.6173	0.78194	11.5 ± 11.4	5.2 ± 3.2	0.6577	0.45018
HDHA								
4-HDHA	440.1 ± 38.6	520.9 ± 86.3	0.5227	0.50355	357.7 ± 31.1	160.6 ± 18.4	**0.0021**	21.96
7-HDHA	103.2 ± 11.4	147.6 ± 28.0	0.2479	1.4398	257.1 ± 35.0	155.1 ± 26.8	**0.0564**	4.3603
8-HDHA	203.9 ± 29.9	295.0 ± 36.2	0.1087	3.3316	235.4 ± 18.0	120.4 ± 22.4	**0.0054**	11.602
10-HDHA	228.2 ± 37.6	296.0 ± 54.8	0.3896	0.83601	788.9 ± 113.6	656.5 ± 125.2	0.4729	0.61202
11-HDHA	344.6 ± 58.8	356.4 ± 68.4	0.9958	0.0046532	442.5 ± 71.5	640.7 ± 115.4	0.248	1.3602
13-HDHA	1968.1 ± 685.8	2544.0 ± 696.7	0.5209	0.68921	9462.3 ± 2255.5	16677.0 ± 4581.1	0.2526	1.362
14-HDHA	359.0 ± 62.5	535.0 ± 143.0	0.2995	1.1935	673.2 ± 86.2	556.4 ± 101.3	0.362	0.68667
16-HDHA	125.8 ± 26.4	167.1 ± 26.6	0.2162	1.6167	158.7 ± 23.4	82.2 ± 13.7	**0.0122**	6.2194
17-HDHA	486.9 ± 66.3	770.9 ± 118.2	**0.0919**	3.3496	525.9 ± 76.7	431.9 ± 88.3	0.622	0.66667
20-HDHA	416.0 ± 102.4	573.3 ± 56.3	**0.0802**	3.7328	365.1 ± 75.9	150.5 ± 17.0	**0.0021**	11.193
22-HDHA	1.4 ± 1.4	3.7 ± 3.7	0.8329	0.098337	0	0	NA	NA

3.6 DISCUSSION

3.6.1 Interaction between egg quality and contaminant uptake

In this present work, we exposed the embryos of the blue mussel obtained from two spawning events to contaminants representative of a harbor environment, until the end of embryogenesis. Based on the control treatment results, there was a 54% difference in the embryogenesis success between the early (Spawn 1) and late (Spawn 2) spawning events, which occurred in May and July, respectively. The partially synchronized spawning strategy of the mussels could explain such results by inducing the production of several contrasting larval pools within a single reproductive cycle (Barber et al., 2005; Lowe et al., 1982; Myrand et al., 2000). This synchronous release of only a fraction of the gonadal contents is repeated until the gonads are completely exhausted, redeveloped or resorbed (Cardoso et al., 2009). This reproductive process constitutes a valid strategy to ensure optimal environmental conditions for the survival of some larval cohorts, *i.e.,* optimal surface water temperature, favorable trophic conditions, relative absence of predators or reduced competition for various important resources, which increases recruitment success (Cledon et al., 2004; Seed & Suchanek, 1992; St-Onge et al., 2015).

In the bivalve *Mya arenaria* – which also has a partially synchronized spawning strategy - St-Onge et al. (2015) showed through a parental genetic linkage approach that the first larval cohort produced in the season was the most successful. Our results show an opposite trend, as the embryogenic success was much lower in the early spawning event (13% in the control treatment) than in the late-July spawning event (67%), suggesting a weak recruitment success for the May spawning event. Mussel embryos lack the organs to feed, their metabolism and development must thus be supported by maternal transfer of energy and essential elements until the larvae can feed following the development of the velum at the D-larvae stage (Bayne et al., 1975). Therefore, our results may indicate lower maternal investment in the first larval cohort, which could be investigated in the future using lipid

profiles, as an indicator of egg quality (Pernet et al., 2003a) and potential maternal investment.

The embryos of the blue mussel obtained from two spawning events were exposed to a contaminant mixture representative of a commercial port environment, until the end of embryogenesis. This cocktail included copper, lead and mercury ions, as well as alkanes and PAHs. Our results indicate the influence of egg quality on contaminant uptake, as D larvae from the "good quality" spawning event (spawn 2) have higher concentrations of most contaminants. That was not the case for PAHs, perhaps because we used low concentrations during exposure compared to other previous studies. The low levels of PAHs found in D larvae (0.1-0.5 $ng \cdot g^{-1}$) in the present study are well below the level of 0.3 µg g-1 (dry weight) at which biological effects (abnormalities) have been observed in *Crassostrea gigas* larvae (Geffard et al., 2003) or at which adult mussels are no longer able to transform and accumulate PAHs (Baumard et al., 1998).

While lead concentrations increased in the D-larvae of the early spawning event (spawn 1) under contaminant exposure, those of copper concentrations surprisingly decreased. We hypothesize that copper ions present in the chemical cocktail could promote the development of embryos into D-larvae, as it is an essential element for this process (Weng et al., 2019). These results seem to support the hypothesis of a lack of maternal investment that could have been compensated by an increased copper consumption to achieve larval development.

3.6.2 Metabolomic impact of the contaminants

3.6.2.1 Energy metabolism

We investigated the impact of the contamination on the energy metabolism of the D-larvae using reporter metabolites involved in the Krebs cycle, as well as lipids, which are the major source of energy during embryonic and larval development in many bivalve species (Sánchez-Lazo & Martínez-Pita, 2012). Our results showed that mussel larvae from the late spawning event have a high energy demand when exposed to chemical stress, as indicated

by the alteration of metabolite concentrations and ratios related to glycolysis and the Krebs cycle. In response, the larvae, unable to feed themselves, rely on their maternal reserves and metabolize them through beta-oxidation to meet the increased cellular demand, and those from the late spawning event showed lower citrate, malate and succinate reserves. In addition, our results highlight the pathways of the acetyl-groups transfer to mitochondria and a down-modulation of citrate and cis-aconitate - two citric acid cycle intermediates. A change in the citric acid cycle may be consistent with the use of alternative substrates for energy production, such as amino acids.

Under normal physiological states, the levels of TCA cycle intermediates are nearly constant. When intermediates are used as biosynthetic precursors, they are normally replenished in a dynamic equilibrium via anaplerotic responses (*i.e.*, glutamine and ketoglutarate) (Owen et al., 2002; Sweetlove et al., 2010). Thus, a decrease in the amount of TCA cycle intermediates can be considered as an indicator of increased pathway flux, ATP demand, utilization for secondary metabolism, and/or decreased mitochondrial function (Sweetlove et al., 2010). Environmental stressors can lead to an increased demand for ATP to support essential maintenance costs (*e.g.* cellular defense mechanisms, detoxification) and ensure organism survival (Sokolova et al., 2012). Bivalve embryos contain high levels of maternally derived fatty acid (FA) desaturase mRNA to alter the degree of FA saturation during early development, allowing them to perform specific FA conversions (Balseiro et al., 2013; da Costa et al., 2015; Ferreira et al., 2022; Liu et al., 2014). When energy demand is high, FAs are rapidly metabolized by beta-oxidation in the mitochondria and finally enter the TCA cycle under optimal conditions to provide ATP for a variety of essential cellular processes (Walter & Söling, 1976). In fact, in response to stress, organisms are able to switch between different metabolic processes involved in energy acquisition and conversion (Sokolova et al., 2012), which explains the altered metabolic states of exposed larvae, indicating they are coping with increased energy requirements.

3.6.2.2 Indicators of oxidative stress

After chemical exposure, D-larvae displayed a 2- to 2.5-fold decrease of the levels of some biologically active lipid mediators, *i.e.* cyclopentenone prostaglandins (cyPGs), PGA2, PGE2, PGF2α, PGE3, PGF3α, and their metabolites, indicating a possible metabolic imbalance.

The increase in FA pools and the highest energy demand may induce oxidative stress through the generation of reactive oxygen species (ROS) and stimulate inflammation, which may be related to mechanisms of immunotoxicity (Young et al., 2023). Several contaminants present in our cocktail have been reported to affect the immune system of *Mytilus spp.* (Duchemin et al., 2008; Prato & Biandolino, 2007; Renault, 2015). Generation of ROS/oxidative stress is a well-recognized mechanism of metal toxicity in aquatic organisms (Sevcikova et al., 2011; Valavanidis et al., 2006). Xie et al. (2024) showed that mercury caused a disruption in lipid metabolism with abnormal increases in leukotriene D4 and prostaglandin E2 (PGE2). To restore homeostasis, the cytochrome P450 pathway was activated to produce anti-inflammatory substances.

CyPGs are essential regulators of inflammation, cell proliferation, apoptosis, angiogenesis, cell migration, and stem cell activity (Lee et al., 2021). In addition, oxylipins biosynthesized from docosahexaenoic acid (DHA), hydroxy docosahexaenoic acid (HDHA), were detected at lower concentrations in larvae exposed to contaminants in this study. This suggests differential activation of enzymes that produce resolvins, protectins, and maresins, which influence inflammatory resolution (Ferreira et al., 2022). Like HDHA, HEPE from eicosapentaenoic acid (EPA) also produces resolvins, which play a role in the resolution of inflammation. However, the levels of many of these metabolites were lower in the contaminant-exposed D larvae than in controls, indicating a potential metabolic dysregulation. Nevertheless, this perturbation appeared to trigger the restoration of baseline metabolism during development to overcome the potential deleterious effects of sublethal treatments. Larvae appeared to successfully employ a variety of mechanisms involving the

biosynthesis of antioxidants and a restructuring of energy-related metabolism to mitigate the toxic effects on the cells and developing tissues. In addition, three oxylipins are common to the different spawning (12-HEPE, 5-oxoETE and 20-HDHA) and may be involved in embryonic morphogenesis in mollusks, including innate immune responses, inflammation, cell proliferation and differentiation, and apoptosis.

3.6.2.3 Neurotoxicity

Following copper exposure, Young et al. (2023) showed that glutamine and glutamate biosynthesis are disrupted in mussel embryos, suggesting Cu-induced neurotoxicity. In addition, differential regulation of glutamine and glutamate metabolism as a neurotoxic response to Hg, Zn, and Cd has also been demonstrated in mussels and clams (Wu & Wang, 2010; Liu et al., 2011a, b; Deidda et al., 2021). Cadium and/or copper exposure also affects the expression of glutamine synthetase mRNA and/or glutamate dehydrogenase activity in mussels and fish (Casanova et al., 2013; Venkata & Radhakrishnaiah, 2013). In our study, no changes in the concentrations of glutamine and glutamate concentrations were observed, suggesting that the Cu concentration used in this experiment did not induce neurotoxicity. However, a disruption of β-aminoisobutyric acid (GABA) has been identified, suggesting that excessive levels of Mn, Hg, and Pb could induce neurotoxicity through disruption of the glutamine/glutamate-GABA cycle, a common mechanism of metal toxicity (Fitsanakis & Aschner, 2005; Sidoryk-Wegrzynowicz & Aschner, 2013). Pb also affects the GABAergic system in a dose-dependent and temporally variable manner throughout embryogenesis, as demonstrated by Wirbisky et al. (2014) in fish embryos. In this study, Pb was concentrated weakly at near trace levels in D-larvae and did not appear to interfere with this mechanism.

3.7 CONCLUSION

Mussel embryos during their embryonic development to the D-larval stage exposed to chemical contaminants found in abundance in a commercial harbor environment, exhibited several metabolic changes. These alterations were related to the metabolism of several amino

acids (threonine, sarcosine, cystine, β-aminobutyric acid [BABA]), glycolysis and the citric acid cycle (glucose, pyruvate, lactate, fumarate, citrate and cis-aconitate), as well as oxylipins involved in the immune system and oxidative stress defense (prostaglandins, HEPE, HETE and HDHA). To our knowledge, these results are the first to highlight a disruption of these metabolic pathways in *M. edulis* embryos in response to a contamination profile representative of a commercial port. This response was not uniform for all embryos and related to the spawning event (May vs. July), which had very different levels of embryogenic success, as highlighted by the PCA (**Fig. 33B, 36A**). Embryogenesis is a critical period in the early larval life cycle, supported by the maternal transfer of energy and essential elements to the eggs. The very low embryogenic success obtained for the first spawning event suggests low energy reserves and/or low maternal transfer of essential elements and/or other factors such as existing contamination in eggs or sperm or both. Surprisingly, exposed D-larvae from the first spawn had a higher embryogenic success than the non-exposed ones. We suggest that the copper in the chemical cocktail could promote the development of embryos into D-larvae, as Cu is an essential element for their development (Weng et al., 2019). Surviving exposed D-larvae from the first spawning with very low embryogenic success showed fewer metabolic differences from the control when compared to the second spawning event which had a much higher embryogenic success. Differences in energetic metabolites and amino acids were observed only for a-aminoadipic acid and citrate/cis-aconitate ratio, with lower values in contaminated D-larvae. For prostaglandin and oxylipin metabolites, contaminated D-larvae had lower values for 11b-PGF2α, 12-HEPE and 9(S)-HOTrE. We hypothesize that lower energetic and oxidative responses of the first larval batch exposed to chemical contaminants could be the result of higher selective mortality during embryogenesis compared to the late spawning (79% mortality versus 33%).

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ARTICLE 4
EFFETS MULTI-OMIQUE DE L'EXPOSITION AUX CONTAMINANTS PENDANT L'EMBRYOGENÈSE SUR LA MÉTAMORPHOSE DE LA MOULE BLEUE *MYTILUS EDULIS* EXPOSÉE AU BRUIT DU TRAFIC MARITIME

4.1 RÉSUMÉ EN FRANÇAIS DU QUATRIÈME ARTICLE

Le bruit sous-marin d'origine anthropique est désormais considéré comme l'une des « menaces émergentes et l'un des défis permanents en matière de conservation de la biodiversité ». Des changements physiologiques ont été recensés chez les invertébrés sessiles, tels que les bivalves. En raison de leur sensibilité au son, de leur incapacité à s'éloigner de la source sonore ainsi que leurs intérêts économiques et sociaux considérables, les bivalves font l'objet d'une attention particulière. Un nombre croissant d'études ont examiné les impacts acoustiques du trafic maritime sur les invertébrés, mais peu d'entre elles se sont penchées sur l'interaction de multiples facteurs de stress pour se rapprocher d'un contexte écologique réel. Le bruit peut interagir avec les contaminants chimiques en perturbant le comportement des organismes et avoir des effets complexes. Des récentes études sur des invertébrés ont démontré que le bruit amplifiait les effets néfastes des métaux lourds exposés à du cadmium. Dans un contexte portuaire du golfe du Saint-Laurent, la réponse aux stress des post-larves de moules, *Mytilus edulis*, a été évaluée à l'aide d'une approche écophysiologique-écotoxicologique. Afin d'y parvenir, des larves pédivéligères compétentes, préalablement exposées à un cocktail de polluants reproduisant l'environnement maritime d'un port jusqu'à la fin de l'embryogenèse, ont été exposées à des perturbations acoustiques pendant la métamorphose. L'objectif était d'évaluer la réponse des larves compétentes (c'est-à-dire pendant la métamorphose) à la suite d'une contamination chimique en début de vie et d'une interaction avec une exposition sonore représentative du trafic maritime. Cette étude montre que les effets de la pollution chimique précoce pendant l'embryogenèse ont un impact plus important sur les larves que l'exposition au bruit plus tard pendant la métamorphose. En effet, des effets latents de l'exposition chimique précoce

persistent ayant des conséquences sur la taille à la métamorphose, le métabolisme énergétique et oxydatif et le profil lipidique des post-larves.

Mots-clés : Anthropophonie, métabolomique, écotoxicologie, physiologie des bivalves/mollusques, effets latents.

Cet article, intitulé « *Multi-omics effects of contaminant exposure during embryogenesis on metamorphosis of blue mussel Mytilus edulis submitted to maritime traffic noise* », sera soumis dans la revue *Aquatic Toxicology*. En tant que premier auteur, j'ai contribué à l'essentiel de la recherche sur l'état de la question, au développement des larves de moules, aux mesures de performance post-larvaire, à l'exécution des analyses chimiométriques et fonctionnelles ainsi qu'à l'analyse par spectrométrie de masse. Stéphane Beauclercq a contribué aux analyses fonctionnelles. Bertrand Genard a supervisé les expériences de spectrométrie de masse. Richard Saint-Louis a apporté un soutien technique à l'analyse chimique. Frédéric Olivier, Laurent Chauvaud, Isabelle Marcotte, et Réjean Tremblay ont conçu et supervisé l'étude. Tous les auteurs ont contribué à la rédaction du manuscrit et ont approuvé l'article final. Cet article a été présenté à la conférence annuelle de *Ressources Aquatique Québec* en 2024 à Québec.

MULTI-OMICS EFFECTS OF CONTAMINANT EXPOSURE DURING EMBRYOGENESIS ON METAMORPHOSIS OF BLUE MUSSEL *MYTILUS EDULIS* SUBMITTED TO MARITIME TRAFFIC NOISE

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4.1 ABSTRACT

Anthropogenic underwater noise is now considered one of the 'emerging threats and ongoing challenges to biodiversity conservation'. Physiological changes have been observed in sessile invertebrates such as bivalves. Bivalve mollusks are of particular interest because of their sensitivity to sound, their inability to move away from the sound source, and their considerable economic and social importance. A growing number of studies have examined the acoustic effects of shipping on invertebrates, but few have considered the interaction of multiple stressors to approximate a real ecological context. Noise can interact with chemical contaminants to disrupt the behavior of organisms and have complex effects. Recent studies on invertebrates have shown that noise amplifies the harmful effects of heavy metals such as cadmium. In a harbor environment in the Gulf of St. Lawrence, the stress response of post-larval mussels, *Mytilus edulis*, was studied using an ecophysiological-ecotoxicological approach. To achieve this, competent pediveliger larvae, previously exposed to a cocktail of pollutants simulating the maritime environment of a port until the end of embryogenesis, were exposed to acoustic perturbations during metamorphosis. The aim was to assess the response of competent larvae (*i.e.* during metamorphosis) following early life chemical contamination and interaction with noise exposure representative of maritime traffic. This study shows that the effects of early chemical pollution during embryogenesis have a greater impact on larvae than noise exposure later during metamorphosis. In fact, latent effects of early chemical exposure persist, with consequences for size at metamorphosis, energy and oxidative metabolism, and lipid profile of post-larvae.

Keywords: Anthropophony, metabolomics, ecotoxicology, bivalve/mollusc physiology, latent effects.

4.2 INTRODUCTION

Extensive research has highlighted the harmful effects of anthropogenic noise on marine biota, which is now categorized among persistent conservation challenges for aquatic biodiversity (Reid et al., 2019). Anthropogenic noise induces physiological changes that affect the mechanisms underlying individual activities or processes such as oxidative stress, energy homeostasis, metabolism, immune function, and respiration in a variety of marine species (El-Dairi et al., 2024; Tu et al., 2021; Wale et al., 2019). Physiological changes have been reported in sessile invertebrates, such as bivalves (Solé et al. 2023), which are of particular importance due to their sensitivity to sound and their inability to move significant distances away from the stressor. Furthermore, bivalves support one of the most economically and socially important sources of livelihood in the world (FAO, 2024) and are recognized to support important ecosystem services through their large filtration capacity, which reduces eutrophication (Smaal et al., 2019). Studies on the impact of anthropogenic noises on bivalves have mostly focused on adult stages (Solé et al., 2023), and only a few experiments have been conducted on the first larval stage and mostly focusing on behavior, growth and survival responses (Cervello et al., 2023; Gigot et al., 2024; Gigot et al., 2023a, b ; Jolivet et al., 2016; Stocks et al., 2012; Wilkens et al., 2012). These studies have shown that anthropogenic noise can interfere with the natural acoustic cues used by organisms for recruitment and could be (mis)interpreted as a cue to settle for some taxa (Williams et al., 2024); Veillard et al. *in press*, see Chapter 1, Article 2).

Anthropogenic noise has several sources, but one of the most common is the noise associated with maritime traffic. To support this traffic, humans have built ports on all the world's coast. For example, the French metropolitan coastline has 473 maritime ports (Mission Plaisance, 2015). Seaports have been suggested to be considered as giant mesocosms, where marine species from this novel and unique environment are exposed to new abiotic stressors, such as pollutant accumulation and noise pollution (Touchard et al., 2023). In fact, one of the most common species in European ports, known as the 'dock mussel' due to its strong association with port habitats, was found to be a genetic admixture of

Mytilus edulis and the Mediterranean lineage of *Mytilus galloprovincialis*, and may be adapted to the port environment (Simon et al., 2020). The impact of pollutants from ports on marine organisms has been extensively studied (Airoldi & Bulleri, 2011; Breitwieser et al., 2018; Chura et al., 2021; Rivero et al., 2013), especially on mussels, which are recognized as sentinel organisms for coastal pollution monitoring (Beyer et al., 2017). However, the combined effects of chemical and noise pollution are poorly known. Exposure to noisy environments could potentially impair an organism's ability to cope with contaminant stress responses, ultimately leading to a compromise between physiological functions, such as the immune system and detoxification mechanisms. A recent study highlighted that the noise-induced stress response enhances the adverse effects of heavy metals on sea slugs (Tu et al., 2024). Other studies also showed that anthropogenic underwater noise exacerbated the adverse effects of cadmium (Cd) on metabolic processes in Blood Clam *Tegillarca granosa* (Shi et al., 2019). Following the combination of vessel noise and Cd exposure, oysters showed a decrease in daily valve activity, which was associated with a slowdown in lipid metabolism and growth (Charifi et al., 2018). This reduction of metabolism caused by anthropogenic underwater noise could be interpreted as an adaptive mechanism adopted by marine organisms in response to different environmental stressors (Pörtner, 2012). The lack of integrative studies is a major challenge to understanding or mitigating the actual impacts of shipping (Popper et al., 2020; Thomsen & Popper, 2024).

In this study, an ecophysiological approach using metabolomic and lipidomic tools was applied to evaluate the latent effect of contaminant exposure during embryogenesis on the metamorphosis process of mussel larvae, *Mytilus edulis*, exposed to cargo ship noise during their veliger development. To achieve this objective, newly fertilized eggs were exposed to a cocktail of pollutants mimicking a low intensity seaport environment until the end of embryogenesis, then seawater pollution was stopped, and mussel larvae were exposed to acoustic perturbations of different intensities until their metamorphosis. With this approach, we tested the main hypothesis that exposure to pollution during embryogenesis amplifies the acoustic effects of shipping noise during the metamorphosis of mussel larvae into young juveniles. This stress-on-stress study focused on the latent effect of embryo contamination

(first larval stage) on metamorphosis (last larval stage) of larvae exposed to acoustic stress during their development. Specifically, we hypothesized that a dose-response effect would be indicated by metabolomic and lipid responses, with increased energy requirements and decreased survival and metamorphosis rates.

4.3 MATERIALS AND METHODS

4.3.1 Mussel larval rearing and experimental set-up

Blue mussel (*Mytilus edulis*) larval rearing was performed as described in Rayssac et al. (2010). Briefly, adult mussels from St. Peters Bay in Prince Edward Island (46.42948N, -62.66030W, Canada) were individually induced to spawn by 10°C thermal shock. A ratio of 10 spermatozoids per egg was used for the fertilization in UV ultrafiltered (1 µm) seawater at 18°C. Fertilized eggs were further washed with UV ultrafiltered seawater and exposed or not to chemical pollution, representative of the pollution level in a harbor as described in Veillard et al. (*submitted,* see Chapter 1, Article 1). Embryos were exposed in three replicates 30-L tanks per treatment (exposed or not to chemical pollutants at a concentration of 60 embryos.mL^{-1}) until the end of embryogenesis (4 days) determined by the presence of D-shaped larvae (**Fig. 37**). At the end of the exposure, the obtained D-larvae from each tank were sieved on 20 µm mesh and transferred to a replicate 60-L cylindrical tank (5 larvae.mL^{-1}) for the complete veliger larval development at similar temperature (18°C). Every two days, larvae were sieved on 35 µm mesh, cylinder tanks were cleaned with Virkon VKS10 disinfectant (LANXESS Deutschland GmbH, Cologne, Germany) and refilled with UV ultrafiltered (1 µm) seawater. Larvae were fed with 60 cells.µL^{-1} of a mixed microalgal suspension containing *Pavlova lutheri* (CCMP459), *Tisochrysis lutea* (CCMP1324), *Chaetoceros muelleri* (CCMP1317), *Tetraselmis suecica* (CCMP904), and *Nannochloropsis oculata* (CCMP525) in a ratio of 1:1:1:1:1 equivalent biomass (dry weight).

At 20 days post-fertilization (dpf), when more than 50% of the larval population was at the pediveliger stage (eyed larvae, foot development and substrate exploration), larvae

from each larval rearing tank were sieved on 100 µm mesh and transferred to cylinder tanks (5-L, 5 larvae.mL^{-1}). Collectors (consisting of two 30 cm polypropylene ropes) were placed in each cylinder tank to allow the settlement and metamorphosis of the larvae. Each cylinder tank was distributed in one of the four *Larvosonic* systems described by Cervello et al. (2023) (**Fig. 37**). *Larvosonic* is an innovative system designed to expose small aquatic organisms to noise, and is composed of six independent circular cylinders of 5-L, which limits classical reverberation and resonance problems (Olivier et al. 2023). Thus, each *Larvosonic* system contained a total of three-cylinder tank per *Larvosonic* with larvae having undergone chemical exposure and three without chemical exposure (**Fig. 37**). Each *Larvosonic* system was used to emit different sound treatments: low (121 ± 4 dB re 1µPa), medium (127 ± 2 dB re 1µPa), and high (151 ± 2 dB re 1µPa) shipping noise treatments and ambient sound with no emission considered as a control (116 ± 1 dB re 1µPa) (Veillard et al. *in press*, see Chapter 1, Article 2). Shipping noise treatment levels were related to distances from a source (cargo ship) corresponding to 18.5 m (high sound exposure), 735 m (medium), 1.8 km (low) as measured in the field (Byrro-Gauthier et al, in press). Every two days, collectors were gently removed, swimming larvae were sieved on 100 µm mesh, cylinder tanks were cleaned and refilled with water, and collectors and free-swimming larvae were replaced.

Throughout the experiments, from fertilization to post-larval stage, temperature (18.0 ± 0.2°C), salinity (23.1 ± 0.1 PSU), photoperiod (15h of light and 9h of dark) with light intensity (9.0 ± 0.2 40 FC; 97.1 ± 1.7 400 lux) were controlled and monitored in each tank (Hanna multiparameter probe HI98194 and Hobo ONSET UA-0002-64). At 39 dpf (20 days after the start of sound emission), each cylinder tank was sampled. Collectors from each cylinder tank were gently rinsed with sprayers on a 100 µm mesh sieve to obtain settled post-larval samples. In addition, settled, growing post-larvae on the cylinder walls were carefully brushed off and pooled with the post-larvae from the collectors. Immediately after collection, the total wet biomass of post-larvae for each cylinder tank (24 samples) was determined, divided into four subsamples (each one weighed), flash-frozen in liquid nitrogen, lyophilized, and stored at -80°C until analysis. One subsample was used for morphological measurements,

one for metabolomic and lipidomic analysis, another for oxylipin analysis, and the last one for the chemical quantification.

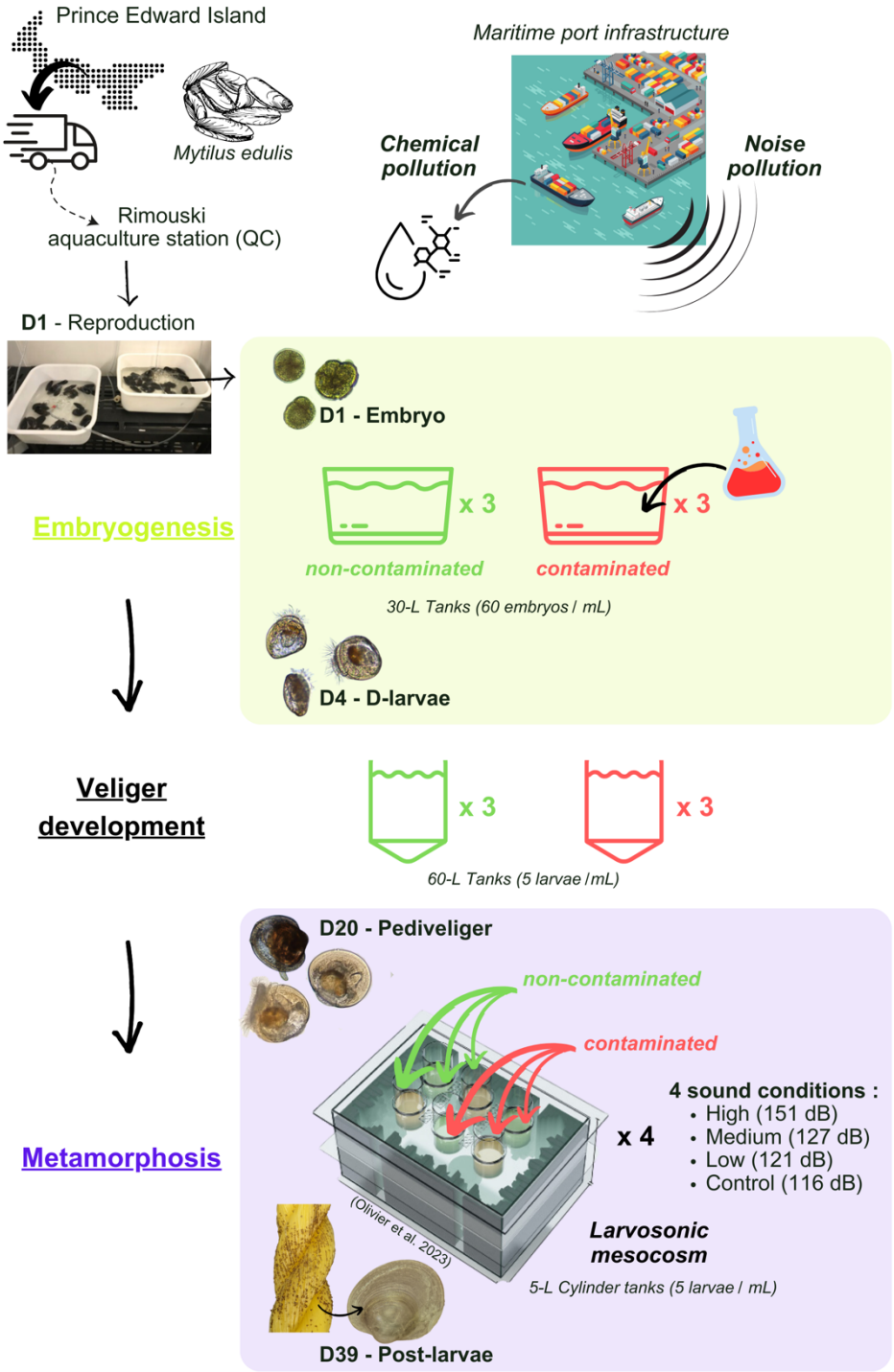

Figure 37. Experimental procedure developed for the exposure of larvae during metamorphosis to shipping noise with an early chemical exposure during embryogenesis.

4.3.2 Chemical exposure

Four chemical pollutants were used for exposure during embryogenesis: copper, lead, mercury and a hydrocarbon (diesel), as previously described in Veillard et al. (*submitted*, see Chapter 2, Article 3). Briefly, this mixture was composed to mimic the level of chemical pollution in the mussels sampled in the seaport of Saint-Pierre in Saint-Pierre-et-Miquelon (France), a low-intensity industrial marine infrastructure. Copper (Cu^{2+}), lead (Pb^{2+}) and mercury (Hg^{2+}) solutions were prepared from standard ICP-MS solutions to obtain final concentrations of 4, 23 and 0.05 µg L^{-1}, respectively. For the hydrocarbon solution, only the aqueous phase (soluble part of diesel, *i.e.,* saturated with hydrocarbons) was preserved and diluted to obtain a final concentration of 0.01 µg L^{-1}.

4.3.2.1 Determination of contaminants in post-larval samples

Freeze-dried larvae were used to measure the accumulation of metals and hydrocarbons based on a method described in Lemos et al. (2021), as previously described in Veillard et al. (*submitted*, see Chapter 2, Article 3). Briefly after micro-grinding and homogenization, tissues were alkaline digested in 200 µL of tetramethylammonium hydroxide (TMAH, 25 % aqueous) and heated to 60°C for one hour with constant stirring. Then, the solution was acidified by adding nitric acid (HNO_3) 67-70% and metals and hydrocarbons extracted and separated by twice the addition of 300 µL of hexane-toluene. Inorganic compounds (Cu, Pb, Hg) were found in the aqueous phase and organic compounds (polycyclic aromatic hydrocarbons (PAHs) and alkanes) in the organic phase. The aqueous phase was filtered through a 45µm polytetrafluoroethylene (PTFE) Teflon filter and metal composition analyzed by GF-AAS 240Z AA (Agilent Technologies) equipped with a GTA 120 Graphite Tube Atomizer (Agilent Technologies). For lead measurements, a solution of palladium and magnesium was used (Agilent technologies, Pre-Mixed GF AAS: 750 µg mL^{-1} Pd and 500 µg mL^{-1} Mg in 2 % $HN0_3$). Mercury and lead concentrations accumulated in embryos were lower than the detection threshold. The organic phase was purified with the use of 100 mg of magnesium sulfate and 50 mg of Florisil (Sigma-Aldrich). Samples (200 µL) containing

hydrocarbons were injected in an 8890 Gas Chromatograph (GC) System (Agilent Technologies) equipped with a Rxi-5ms capillary column, L 30 m, DI 0.53 mm, 0.25 µm (Restek, USA).

4.3.3 Morphometric measures and metamorphosis success

Measures of the prodissoconch II (PII) shell was used as an indicator of the size at metamorphosis and determined using a Keyence VHX-2000 Series digital microscope with VH-Z100UR objectives (Osaka, Japan, 1 µm resolution and HDR). The total post-larvae size less the PII size was used to estimate the post-metamorphic growth (D) obtained in the cylinder tanks. The metamorphosis success was calculated as the ratio of total post-larvae recovered in each cylinder to the number of initial pediveliger larvae introduce in each cylinder at 20 dpf. The total number of post-larvae was calculated by the multiplication of the number of post-larvae in the subsamples with the total wet biomass of post-larvae and subdivided by the wet mass of subsamples collected for morphometric measures.

4.3.4 Omics characterization

Freeze-dried larvae were micro-ground and homogenized with a 2.8 mm ceramic bead at 4,000 rpm for three cycles of 10 s (Precellys 24; Bertin corporation). Analyte extraction for the quantification of free amino acids, energy-related metabolites and fatty acid methyl ester (FAME) was performed on the same sample using a two-step extraction procedure with the same Captiva EMR-Lipid cartridge. The first elution was performed to extract metabolites and the second elution to extract lipids. Free amino acids and energy metabolism-related metabolites were analyzed together and fatty acids separately. Analyte extraction for the quantification of oxylipins was performed in another subsample.

4.3.4.1 Amino acids and energetic metabolism

500 µL of 1/1 (v/v) mix of TFE/LC-MS grade water was added in the 3 mL Agilent Captiva EMR–Lipid solid phase extraction cartridge (part number 5190-1003 – Agilent

Technologies) and 750µL of a 1/1 (v/v) mix of TFE/LC-MS grade water was added to the homogenizing tubes, vortexed for 30 s and centrifuged for 1 min at 2,460 g. Three extraction cycles (500µL, with 1 mL of ACN for the last one) were performed, and the filtrates were pooled in a 15 mL falcon tube. Then, the cartridge was rinsing with 3 mL of 1/1 (v/v) mix of TFE/ LC-MS grade water and drained using a high vacuum for 30 seconds. Samples (in the falcon tube) were dried with SpeedVac overnight without heating. Samples were reconstituted by adding 50 µL of LC-MS grade water followed by 200 µL of LC-MS grade ACN. One hundred microliters of each reconstituted sample was transferred to two vials: one vial was for positive analysis and the other for the negative analysis. Compounds valine-d_8 (10 µg/mL) and pyruvate-d_3 (10 µg/mL) were used as internal standard to quantify the compounds in positive and negative ion modes, respectively.

Metabolites were separated and quantified in multiple reaction monitoring (MRM) mode using an HPLC 1260 Infinity II device coupled to a 6420 Triple Quad mass spectrometer (Agilent Technologies) in positive and negative ionization mode. Ten microliters of the sample was injected, and the chromatography separation was performed with a InfinityLab Poroshell 120 HILIC-Z, 2.7 µm 10 x 2.1 mm column.

4.3.4.2 Fatty acid methyl ester (FAME)

For the lipidomic extraction, the homogenizing tubes containing the sample from metabolite extraction were taken and 1 mL of 2,2,2-trifluoroethanol (TFE) at 100% was added. Samples were vortexed for 30 s and centrifuged for 1 min at 2,460 g. The supernatants (1 mL) were pooled in 15 mL falcon tubes. Then, 500 µL of 5/1 (v/v) mix of methyl tert-butyl ether (MTBE) and TFE was added to the homogenizing tubes, vortexed for 30 s and centrifuged for 1 min at 2,460 g. This extraction was performed three times, and supernatants (500 µL) were pooled in 15 mL falcon tubes. The cartridge of the Captiva EMR-Lipid was rinsed with 3 mL of 5/1 (v/v) mix of MTBE/TFE and drained by attaching a vacuum to the manifold to obtain a flux of 1 drop each 3 to 5 s. The filtrate was collected in a 15 mL falcon tube. Samples were dried with a SpeedVac (SPD2010, Savant) overnight without heating.

Samples were reconstituted by adding 250 µL of 3/1 (v/v) mix of MTBE / Methanol HPLC grade (MeOH) and 50µL of each reconstituted sample was transferred in 8 mL transesterification vials following by addition of 800µL of toluene and 3 mL of 12% sulphuric acid methanol solution. Vials were heated at 90°C for 1 hour in a dry bath under a fume-hood and vortexed each 15 min. When the vials were cooled to room temperature, 3 mL of H2O nano-filtered water and 800 µL of hexane were added in the transesterification vials, vortexed and centrifuged for 10 min at 2,750 g. The supernatant was transferred into 1.5 mL GC vial and dried with a SpeedVac (SPD2010, Savant). Lipids were separated and quantified using a GC-FID 8890 (G3540A, Agilent Technologies). Two microliters of the sample was injected, and the chromatography separation was performed with a DB-23, 120 m (2 x 60 m), 250 µm x 0.15 µm (Agilent Technologies).

4.3.4.3 Polyunsaturated fatty acids oxidation products, oxidative metabolism

As for metabolites, to extract oxylipins linked to oxidative metabolism, the freeze-dried D-larvae were first micro-ground and homogenized with a 2.8 mm ceramic bead at 4,000 rpm for three cycles of 10 s (Precellys 24; Bertin corporation). Then, 200 µL of a 1/1 (v/v) mixture of 2,2,2-trifluoroethanol (TFE) and extraction buffer (ammonium formate buffer at pH 3) was added to the homogenizing tubes containing the sample. Samples were vortexed for 10 s and centrifuged for 1 min at 2,460 g. Then, 200 µL of a 1/1 (v/v) mixture of ACN and methanol (MEOH) was added to the homogenizing tubes containing the sample. Samples were vortexed for 10 s and centrifuged for 5 min at 2,460 g, 4°C. 250 µL of the supernatant was transferred to 2 mL HPLC vial containing 1 mL of 50 mM ammonium formate buffer at pH 3 with 10 ng/mL of internal standard PGE_2-d_9. Sample was analyzed directly after the extraction process, with an HPLC 1260 Infinity II device coupled to a 6470B Triple Quad mass spectrometer (Agilent Technologies) using on-line SPE method allowing sample cleaning and oxylipin separation from 1 ml of samples. Oxylipins extracts were separated using a Gemini C6-Phenyl 100Å, 2 x150 mm 5 µm (Phenomenex) and a Gemini C6-Phenyl

guard column as on-line SPE (4 × 2.0 mm, SecurityGuard, Phenomenex) with the column temperature maintained at 40°C.

4.3.5 Statistical analyses

One-way univariate PERMANOVA was used to test for significant effects of chemical contamination during embryogenesis (2 treatments) and noise conditions (4 treatments) on metamorphosis success, shell size, post-settlement growth and the concentration of each pollutant in larval tissues (copper, lead, mercury, alkane and PAH). Furthermore, multivariate PERMANOVA was used on the energy metabolism, oxidative metabolism, lipid and pollutant profiles to determine general pattern differentiation between noise conditions. All PERMANOVAs were realized using PRIMER7 software. The permutational method is a randomization that allows us to remove the distributional assumption of normality and can be applied to very small samples (Legendre & Legendre, 2012). Similarity matrices based on ratios were produced using the Bray-Curtis distance. When differences were significant (p-perm ≤0.07), pairwise multiple comparison tests were used to determine which groups were significantly different. Distance-based tests for homogeneity of multivariate dispersion (PERMDISP) were carried out to assess the homogeneity of the ratios in each condition.

Multivariate statistical analyses to test, metabolite by metabolite, the effect of chemical contamination during embryogenesis (2 treatments) and noise conditions (4 treatments) on omics data (metabolism, oxidative and fatty acids) obtained from post-larvae were performed using the vegan package from R studio, and graphical visualizations such as principal component analysis (PCA) were produced using the factoextra package. Similarity matrices based on ratios (using the Bray-Curtis distance) were generated using the metaMDS function, which is considered the most robust unconstrained ordination method in community ecology (Minchin, 1987).

4.4 RESULTS

No differences in metamorphic success were observed between treatments (chemical exposure during embryogenesis and noise emission during metamorphosis, **Tab. 18**) with an overall mean of 60 ± 6% (**Tab. 19**). Differences related to chemical exposure during embryogenesis were observed in size at metamorphosis, metabolomic and lipidomic profiles and the chemical content of post-larvae (**Tab. 18**). A significant effect of shipping noise emission was observed only for the post-larval size, and an interaction between both treatments was observed for the oxidative metabolite profile (**Tab. 18**). Post-larvae exposed to chemical pollution during embryogenesis, showed a general contaminant profile different from post-larvae obtained from non-contaminated embryos (**Tab. 18**). However, this difference was only related to the persistent accumulation of lead, which was maintained throughout larval development (**Tab. 18 and 19**). Copper was also accumulated in post-larvae, but at levels similar to those in embryos not exposed to chemical pollutants, suggesting a potential biological function (**Tab. 19**). Only traces of PAHs and alkanes were accumulated in post-larvae (**Tab. 19**), suggesting their depuration during larval development. Surprisingly, the persistent accumulation of lead had no effect on metamorphic success but, was associated with a 23 % increase in metamorphic size (PII) and a 76% increase in post-metamorphic growth (D) (**Tab. 19**). Post-metamorphic growth (D) was also affected by noise emission, with more than two times higher growth in post-larvae exposed to high shipping noise levels compared to the control and low noise levels. Medium levels of vessel noise showed intermediate levels of post-metamorphic growth.

Table 18. Multivariate analysis summary. Bold indicates p-values near or below 0.07.

		Df	Pseudo-F	P-perm
Metamorphic success	Sound	3	1.6898	0.1704
	Pollution	1	0.12345	0.907
	SoundxPollution	3	1,4042	0,2519
Metamorphic size (PII)	Sound	3	0.67818	0.576
	Pollution	1	0.25847	0.6106
	SoundxPollution	3	1,164	0,3518
Post-metamorphic growth (D)	Sound	3	0.63403	0.6752
	Pollution	1	7.1344	**0.0087**
	SoundxPollution	3	1,2953	0,2833
Energy metabolism profile	Sound	3	1,8216	0,1599
	Pollution	1	0,56943	0,5145
	SoundxPollution	3	0,50324	0,7327
Oxidative metabolism profile	Sound	3	1,1621	0,3279
	Pollution	1	5,4761	**0,0013**
	SoundxPollution	3	1,9169	0,0823
Lipid profile	Sound	3	2,4125	**0,0589**
	Pollution	1	2,7154	**0,0768**
	SoundxPollution	3	0,42324	0,8751
All pollutants	Sound	3	2,4526	**0,019**
	Pollution	1	1,5382	0,2048
	SoundxPollution	3	0,46451	0,8847
Copper contamination	Sound	3	1,2504	0,2745
	Pollution	1	0,72005	0,5476
	SoundxPollution	3	0,42761	0,9279
Lead contamination	Sound	3	1,1185	0,3624
	Pollution	1	0,30517	0,7417
	SoundxPollution	3	1,1361	0,3512
Alkane contamination	Sound	3	3,6283	**0,0041**
	Pollution	1	1,5943	0,1692
	SoundxPollution	3	1,1382	0,3339
PAH contamination	Sound	3	0,91759	0,4929
	Pollution	1	0,43106	0,7039
	SoundxPollution	3	0,21156	0,9683

Table 19. Mean level of metamorphic success, larval size at metamorphosis, post-larval growth and concentration of chemical pollutants found in post-larvae submitted to different noise treatments in ng/g ± SE. Bold letters indicate p-values near or below 0.07.

	Non-contaminated	Contaminated	Control	Low	Medium	High
Metamorphosis success	60 ± 6%					
Metamorphosis size (PII)	231.7 ± 8 μm	286.9 ± 7 μm				
Post-metamorphosis growth (D)	67.2 ± 16 μm	117.8 ± 15 μm	67.9 ± 24.0 **a**	73.9 ± 13.4 **a**	98.8 ± 26.8 **ab**	148.3 ± 21.3 **b**
Copper contamination	8.7 ± 3.7 ng.g^{-1}					
Lead contamination	2.1 ± 0.8 ng.g^{-1}	8.3 ± 1.5 ng.g^{-1}				
Alkane contamination	0.3 ± 0.1 ng.g^{-1}					
PAH contamination	0.4 ± 0.1 ng.g^{-1}					

In terms of energy metabolites and free amino acid profiles, latent effects of contamination during embryogenesis were clearly observed in post-larval stages (**Fig. 38A**). Of the 24 energetic metabolites measured, 17 show significant changes related to chemical exposure during embryogenesis, with higher levels in post-larvae from contaminated embryos for 14 metabolites. A similar pattern was observed for free amino acids, with 13 of the 17 amino acids measured showing significant changes. Higher levels in post-larvae from contaminated embryos were observed for 12 amino acids. Lower levels were observed for lactate, glucose, pyruvate, sarcosine metabolites and b-aminoisobutyric acid (BAIBA) (**Tab. 20**).

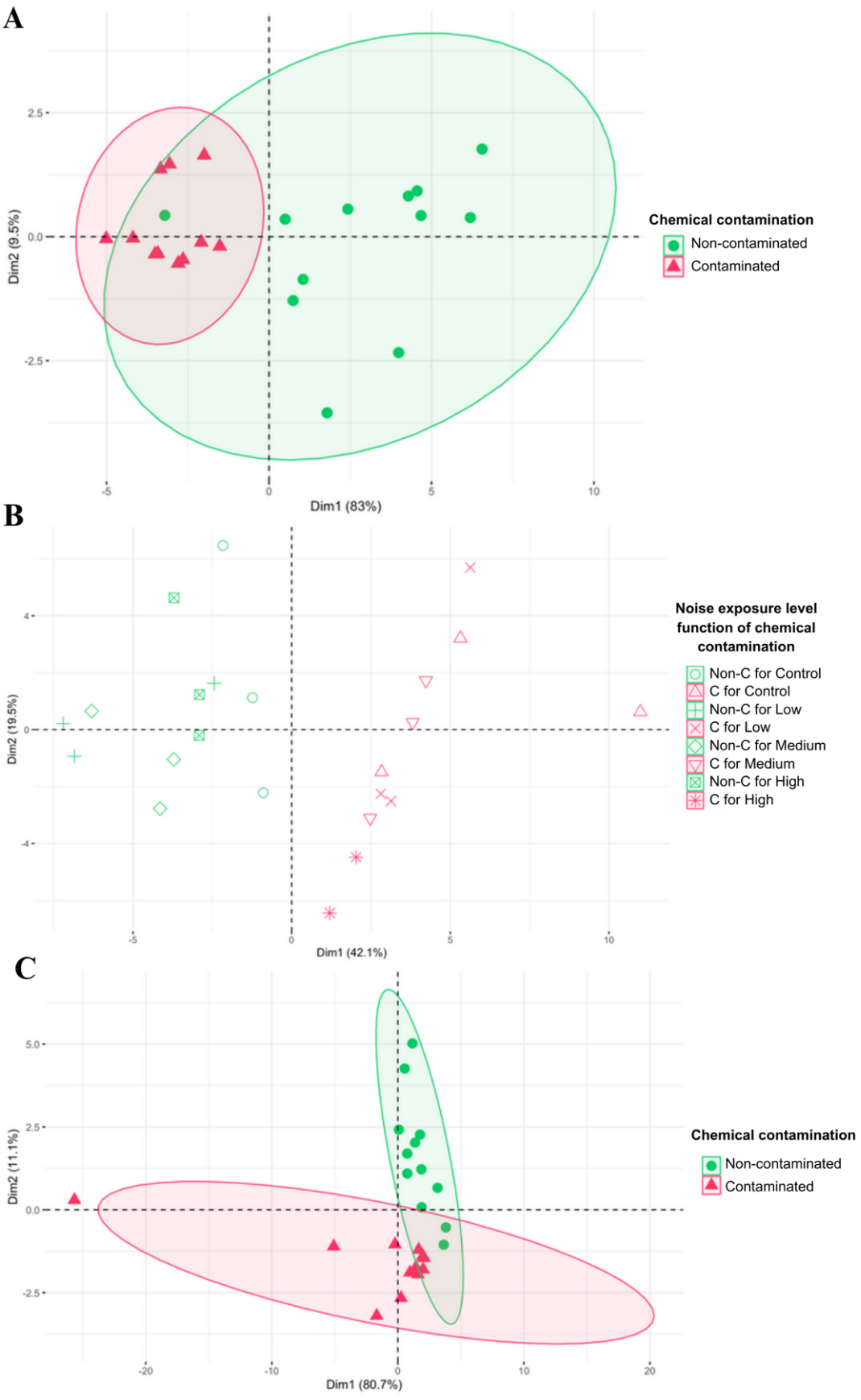
A
Dim2 (9.5%)
Dim1 (83%)
Chemical contamination
Non-contaminated
Contaminated
B
Dim2 (19.5%)
Dim1 (42.1%)
Noise exposure level function of chemical contamination
Non-C for Control
C for Control
Non-C for Low
C for Low
Non-C for Medium
C for Medium
Non-C for High
C for High
C
Dim2 (11.1%)
Dim1 (80.7%)
Chemical contamination
Non-contaminated
Contaminated

Figure 38. (A) PCA plot based on the free amino acid and energy metabolites data. Green circles refer to the non-exposed (n=12) and pink box triangles to the exposed treatment (n=12). (**B)** PCA plot based on prostaglandins and oxidative metabolites based on the interaction of chemical contamination during embryogenesis and noise exposure during metamorphosis. Green circles refer to the non-contaminated control larvae (n=3), red triangles refer to the contaminated control larvae (n=3), green plus to the non-contaminated larvae exposed to low noise treatment (n=3), red cross to the contaminated larvae exposed to low noise treatment (n=3), green diamond to the non-contaminated larvae exposed to medium noise treatment (n=3), red inverse triangle contaminated larvae exposed to medium noise treatment (n=3), green crossed square to non-contaminated larvae exposed to high noise treatment (n=3), and red asterisk to the contaminated larvae exposed to high noise treatment (n=3). (**C)** PCA plot based on the fatty acids profile of post-larvae based on the chemical contamination during embryogenesis. Green circles refer to the non-contaminated (n=6) and pink box triangles to the contaminated treatments (n=6). "Non-C": non-contaminated and "C": contaminated.

Table 20. Targeted LC-MS-based quantification (mean ± standard error ng/mg) of amino acids and metabolites of energy metabolism for post-larvae after the sound exposure level and/or depending on early chemical contamination. The significance of the differences in concentrations of the metabolites between contaminated and control was tested by PERMANOVA using R. Bold indicates p-values near or below 0.07 and the asterisks correspond to: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1.

		Non-contaminated	Contaminated	P-value	
Glycolysis	**Glucose**	223.1 ± 31.8	96.3 ± 36.8	0.0069	**
	Glucose-6-phosphate	3.1 ± 0.8	15.6 ± 5.7	0.0003	***
	Fructose-1,6-biphosphate	ND	ND	NA	
	Phosphoenol pyruvate	ND	ND	NA	
	Pyruvate	19.9 ± 2.7	8.3 ± 2.9	0.0032	**
Anaerobic	**Lactate**	151.9 ± 22.3	57.2 ± 20.6	0.0057	**
	Octopine	ND	ND	NA	
	Strombine	2.2 ± 0.4	14.0 ± 6.1	0.0006	***
Krebs cycle	**Acetyl-CoA**	0.2 ± 0.1	1.0 ± 0.5	0.0026	**
	Citrate	6.6 ± 1.2	7.1 ± 2.1	0.9777	
	Cis-aconitate	0.1 ± 0.0	0.1 ± 0.0	0.9451	
	Aketoglutarate	7.3 ± 1.1	11.4 ± 4.0	0.9952	
	Succinyl CoA	ND	ND	NA	
	Succinate	25.8 ± 2.8	49.7 ± 17.2	0.2568	
	Fumarate	1.2 ± 0.2	7.6 ± 3.0	0.0001	***
	Malate	5.7 ± 1.0	32.7 ± 10.7	0.0001	***
	Oxaloacetate	ND	ND	NA	
BCAAs	**Valine**	41.7 ± 6.6	166.0 ± 62.6	0.0001	***
	Leucine	39.5 ± 5.7	108.7 ± 32.5	0.0038	**
	Isoleucine	20.5 ± 3.1	58.2 ± 17.7	0.0017	**
Central metabolism	**Aspartate**	252.4 ± 38.4	762.0 ± 299.1	0.0095	**
	Glutamate	430.3 ± 66.2	925.9 ± 346.3	0.3862	
	Glutamine	117.6 ± 22.3	436.9 ± 173.2	0.0021	**
	Arginine	6.2 ± 1.2	134.7 ± 56.3	0.0005	***
Energy transfer	ATP	ND	ND	NA	
	ADP	0.8 ± 0.3	5.3 ± 2.5	0.0006	***
	AMP	84.3 ± 16.7	429.0 ± 170.8	0.0002	***
	FAD	2.3 ± 0.4	9.4 ± 4.5	0.0302	
	NAD	3.7 ± 0.7	17.9 ± 7.2	0.0080	**
	NADH	ND	ND	NA	
	NADP	0.2 ± 0.1	0.7 ± 0.1	0.0145	
	NADPH	ND	ND	NA	
Amino acids	Lysine	ND	ND	NA	
	Tryptophan	17.1 ± 2.6	59.5 ± 20.1	0.0003	***
	Tyrosine	28.5 ± 4.1	123.4 ± 50.5	0.0001	***
	Phenylalanine	21.6 ± 2.9	64.1 ± 23.6	0.0056	**
	Histidine	42.4 ± 8.0	229.2 ± 92.3	0.0003	***
	Proline	159.4 ± 27.7	290.2 ± 99.3	0.5706	
	Threonine	33.9 ± 5.1	125.2 ± 50.2	0.0008	***
	Methionine	6.4 ±1.1	21.6 ± 8.5	0.0045	**
	Sarcosine	6.6 ± 1.0	2.6 ± 1.1	0.0030	**
	Glycine	811.8 ± 126.6	1803.6 ± 665.4	0.3187	
	Cystine	3.1 ± 0.4	17.2 ± 9.1	0.0004	***
	Alanine	137.6 ±23.1	523.3 ± 217.9	0.0010	***
	Serine	76.3 ±11.4	280.9 ± 113.6	0.0009	***
	Hydroxyproline	2.8 ± 0.4	9.8 ± 4.2	0.0095	**
	a-aminobutyric acid	8.3 ± 1.4	17.2 ± 5.9	0.3274	
	b-aminoisobutyric acid	5.0 ± 0.8	1.7 ± 0.7	0.0028	**
	a-aminoadipic acid	9.9 ± 1.5	55.1 ± 25.3	0.0006	***
	Betaine	520.8 ± 66.8	1007.2 ± 381.6	0.2269	
Ratio	AEC		NA		
	BCAAs	101.6 ± 15.3	332.9 ± 111.6	0.0009	***
	Glutamine/Glucose	0.5 ± 0.1	5.7 ± 1.3	0,0001	***

The total sum of oxylipins analyzed and quantified in the post-larvae varied differently in relation to shipping noise emission in post-larvae from the non-contaminated embryos compared to post-larvae from the contaminated embryos, as demonstrated by the significant interaction between both factors (**Tab. 18**). However, the PCA plot (**Fig. 38B**) suggests a clear trend with different oxylipin profiles between post-larvae obtained from contaminated compared to non-contaminated embryos. Table 21 shows higher values for the majority of prostaglandins and oxidative metabolites in the post-larvae from contaminated embryos in the different noise treatments, except for the high sound intensity, which explains the interaction value in Tables 18 and 22. The oxylipins that underlie the difference between post-larvae from non-contaminated and contaminated embryos are mainly oxylipins biosynthesized from docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), *i.e.,* hydroxydocosahexaenoic acid (HDHA) and hydroxyeicosatetraenoic acid (HETE), respectively.

Table 21. Targeted LC-MS-based quantification (mean ± standard error ng/g) of prostaglandins and unsaturated fatty acid oxidation products for the post-larvae by noise exposure level and as a function of early chemical contamination. The significance of the difference in the concentrations of the metabolites between noise levels or contamination was tested by PERMANOVA. Bold indicates p-values near or below 0.07.

Sound intensity	**Control**		**Low**		**Medium**		**High**	
Early chemical contamination	Non-contaminated	Contaminated	Non-contaminated	Contaminated	Non-contaminated	Contaminated	Non-contaminated	Contaminated
Total sum	96506.7 ± 6925.7	259251.8 ± 56411.4	46003.7 ± 21713.2	223541.8 ± 50770.7	31266.4 ± 3056.2	189772.2 ± 23737.9	74114.0 ± 9243.6	106237.7 ± 18572.5
Cyclopentenone prostaglandin								
PGE2	326.8 ± 59.7	1452.2 ± 821.5	207.9 ± 49.1	691.2 ± 61.7	233.2 ± 18.9	450.0 ± 69.2	371.7 ± 46.1	636.1 ± 7.5
15-keto-PGE2	45.8 ± 11.2	75.7 ± 11.1	57.1 ± 10.3	62.9 ± 5.5	70.0 ± 16.3	55.8 ± 4.3	56.4 ± 3.5	64.2 ± 5.0
PGD2	115.4 ± 29.6	183.1 ± 55.0	79.9 ± 12.7	152.3 ± 10.8	88.0 ± 13.0	100.4 ± 17.0	113.6 ± 37.3	127.6 ± 1.2
13,14-dihydro-15-Keto-PGD2	39.2 ± 10.0	13.0 ± 1.5	63.8 ± 8.7	13.6 ± 1.1	55.5 ± 10.2	15.4 ± 3.3	47.1 ± 3.5	12.1 ± 4.0
PGA2	15.1 ± 3.5	3.5 ± 1.4	**12.9 ± 2.8**	**4.2 ± 2.0**	**21.2 ± 11.4**	**7.6 ± 3.5**	**9.7 ± 2.5**	**4.3 ± 0.5**
PGJ2	5.6 ± 2.0	4.3 ± 0.6	1.5 ± 1.5	3.1 ± 0.7	3.9 ± 0.5	3.2 ± 1.0	1.9 ± 0.9	3.3 ± 1.1
15-deoxy-d12,14-PGJ2	64.4 ± 13.5	14.3 ± 3.5	117.3 ± 25.3	22.1 ± 4.7	104.4 ± 18.8	18.8 ± 2.4	83.9 ± 7.2	16.0 ± 2.7
11b_PGF2α	905.7 ± 178.4	6651.4 ± 3413.0	609.6 ± 132.9	3978.6 ± 305.0	745.8 ± 114.1	2030.1 ± 502.7	823.1 ± 56.4	3232.5 ± 292.3
15-keto-PGF2α	183.9 ± 57.4	299.6 ± 104.6	105.3 ± 46.6	241.9 ± 39.8	113.0 ± 23.0	202.2 ± 20.6	191.7 ± 38.3	188.2 ± 20.2
13,14-dihydro-15-Keto-PGF2α	65.3 ± 16.2	89.1 ± 27.4	41.5 ± 11.6	83.0 ± 8.0	48.9 ± 11.6	100.8 ± 20.8	48.3 ± 10.6	61.9 ± 11.4
PGB2	189.1 ± 71.2	148.9 ± 6.7	**109.6 ± 30.3**	**193.7 ± 35.7**	**173.2 ± 46.5**	**284.2 ± 18.2**	191.0 ± 22.5	177.6 ± 20.6
THXB2	33.1 ± 8.6	11.5 ± 0.1	59.6 ± 10.9	14.4 ± 1.1	56.0 ± 13.7	12.3 ± 1.6	49.3 ± 5.6	12.0 ± 2.0
EPA oxylipin								
PGE3	84.7 ± 14.5	134.1 ± 32.8	72.8 ± 3.1	123.7 ± 6.6	68.9 ± 6.0	100.9 ± 20.4	81.6 ± 5.0	95.6 ± 7.5
PGF3α	157.5 ± 50.7	243.2 ± 86.8	142.0 ± 19.6	190.9 ± 26.3	126.7 ± 7.3	147.9 ± 19.6	152.8 ± 15.2	148.2 ± 12.5
5-HEPE	823.2 ± 181.3	519.3 ± 52.6	720.4 ± 8.5	471.3 ± 91.8	**555.3 ± 34.1**	**554.8 ± 74.8**	**827.6 ± 140.6**	**288.4 ± 65.0**
8-HEPE	4489.4 ± 938.2	32076.0 ± 8989.6	2460.5 ± 1094.8	25810.1 ± 1040.2	1341.9 ± 454.7	27027.4 ± 5311.2	3781.2 ± 849.2	15364.9 ± 2093.5
9-HEPE	2744.4 ± 167.2	2777.5 ± 410.7	**1344.4 ± 136.4**	**2553.9 ± 323.6**	**1136.1 ± 37.4**	**3194.9 ± 266.5**	**1539.3 ± 60.2**	**2259.2 ± 749.6**
11-HEPE	14245.0 ± 6981.6	33322.4 ± 7724.1	**7914.6 ± 6168.2**	**27182.7 ± 10474.4**	**2447.4 ± 261,7**	**23619.7 ± 9607.4**	6304.7 ± 2160.7	7407.0 ± 1224.3
12-HEPE	33406.0 ± 4291.7	96448.2 ± 39271.4	9364.0 ± 6833.0	89722.5 ± 21483.3	7352.4 ± 3364.6	60171.9 ± 10698.1	31363.0 ± 9206.6	38228.1 ± 5965.7
15-HEPE	6743.0 ± 1197.7	7723.0 ± 521.6	2574.2 ± 772.4	7352.8 ± 1927.9	1605.9 ± 219.6	9177.9 ± 1100.7	3618.9 ± 559.0	4657.2 ± 1573.9
18-HEPE	1470.8 ± 263.2	1093.9 ± 264.1	1161.8 ± 92.4	754.8 ± 165.2	953.7 ± 169.3	994.9 ± 266.7	1430.4 ± 307.5	601.8 ± 172.7
ALA-GLA-oxylipin								
9(S)-HOTrE	80.1 ± 24.6	73.5 ± 4.0	**73.8 ± 4.8**	**85.5 ± 22.4**	**74.8 ± 4.9**	**73.1 ± 13.5**	71.7 ± 11.4	64.5 ± 13.1
13(S)-HOTrE	120.0 ± 42.9	54.5 ± 23.0	**176.0 ± 50.0**	**140.3 ± 52.7**	**95.2 ± 27.3**	**141.1 ± 25.5**	137.6 ± 31.4	116.9 ± 13.0
13(S)-HOTrE(g)	67.4 ± 7.3	58.6 ± 5.5	55.0 ± 32.5	65.2 ± 11.7	51.5 ± 14.1	64.8 ± 3.4	76.3 ± 21.8	55.9 ± 7.9
Arachidonic-oxylipin								
5-HETE	220.8 ± 26.5	116.1 ± 25.1	199.6 ± 32.1	143.6 ± 33.7	**200.7 ± 45.8**	**130.9 ± 18.8**	**242.8 ± 22.5**	**64.6 ± 9.4**
8-HETE	1408.5 ± 380.3	9903.2 ± 2136.0	916.4 ± 294.5	7537.2 ± 406.1	787.4 ± 143.9	6186.2 ± 224.7	1501.0 ± 218.2	4600.9 ± 601.2
9-HETE	877.5 ± 131.9	999.4 ± 107.0	**396.6 ± 37.9**	**843.9 ± 156.7**	**531.8 ± 113.5**	**1316.0 ± 65.4**	676.4 ± 78.9	728.8 ± 69.5
11-HETE	1934.4 ± 729.8	6575.8 ± 1296.9	**1334.1 ± 798.2**	**5571.1 ± 1810.5**	**1115.5 ± 143.5**	**4725.4 ± 1974.3**	1366.5 ± 299.7	2023.7 ± 198.7
12-HETE	2502.7 ± 575.0	7273.9 ± 2124.1	911.7 ± 334.3	6759.4 ± 1362.3	1177.6 ± 382.3	4393.2 ± 391.1	1859.8 ± 320.9	3832.8 ± 471.3
15-HETE	678.1 ± 208.7	523.0 ± 98.2	555.1 ± 74.5	558.0 ± 130.6	606.4 ± 113.7	596.5 ± 134.9	657.1 ± 23.2	286.9 ± 47.4
5-OxoETE	65.1 ± 23.3	39.0 ± 5.4	85.0 ± 10.1	42.7 ± 11.4	81.1 ± 13.0	40.6 ± 10.2	87.1 ± 14.7	27.1 ± 7.9
12-OxoETE	8.6 ± 1.5	1.1 ± 0.0	**18.3 ± 9.7**	**4.0 ± 0.6**	**8.9 ± 4.5**	**5.5 ± 1.6**	4.7 ± 2.8	2.4 ± 2.0
15-oxoETE	326.8 ± 66.1	243.8 ± 12.0	193.5 ± 44.3	244.9 ± 62.7	187.8 ± 29.4	234.3 ± 28.1	364.6 ± 45.9	174.9 ± 18.1
Linoleic acid								
9-HODE	383.1 ± 120.9	285.6 ± 37.7	754.3 ± 114.6	378.6 ± 61.5	852.5 ± 160.4	316.0 ± 66.4	767.8 ± 71.5	312.0 ± 37.8
13-HODE	469.1 ± 196.4	233.1 ± 21.3	717.3 ± 113.2	333.7 ± 61.5	843.8 ± 39.6	253.8 ± 75.3	784.3 ± 58.7	243.2 ± 41.5
9-oxoODE	419.6 ± 80.0	546.6 ± 231.0	292.7 ± 73.3	408.8 ± 63.8	330.9 ± 87.4	304.5 ± 50.7	349.3 ± 74.3	317.5 ± 6.9
13-oxoODE	254.1 ± 43.6	630.0 ± 172.8	150.4 ± 33.4	456.5 ± 83.4	275.0 ± 73.7	391.1 ± 68.4	266.8 ± 39.0	539.0 ± 62.4
(±)9(10)-DiHOME	7.9 ± 2.5	1.6 ± 0.3	12.7 ± 3.1	2.6 ± 0.4	10.3 ± 2.3	2.1 ± 0.2	9.0 ± 0.1	2.3 ± 0.4
(±)12(13)-DiOME	39.0 ± 14.3	7.7 ± 1.2	**65.1 ± 16.3**	**11.4 ± 2.0**	**50.1 ± 8.6**	**10.4 ± 1.7**	**41.8 ± 3.2**	**10.9 ± 2.0**
(±)9(10)-EpOME	152.0 ± 50.3	67.0 ± 7.7	190.6 ± 50.6	86.9 ± 20.7	148.8 ± 25.1	47.2 ± 5.3	124.6 ± 12.1	47.6 ± 18.5
(±)12(13)-EpOME	119.2 ± 26.0	51.7 ± 10.0	**147.4 ± 28.5**	**107.2 ± 36.2**	**113.7 ± 51.1**	**61.5 ± 5.1**	105.4 ± 13,8	57.1 ± 4.6
HDHA								
4-HDHA	340.6 ± 74.4	323.8 ± 104.7	333.2 ± 21.1	310.8 ± 67.5	273.3 ± 41.9	293.6 ± 17.1	373.5 ± 66.3	180.2 ± 59.1
7-HDHA	174.7 ± 35.4	213.6 ± 7.8	147.4 ± 48.3	212.8 ± 20.3	122.2 ± 30.6	167.9 ± 19.0	175.4 ± 57.1	121.6 ± 18.3
8-HDHA	288.4 ± 32.9	247.1 ± 42.7	**314.7 ± 25.0**	**274.9 ± 48.0**	239.6 ± 40.5	226.6 ± 29.3	**275.1 ± 41.0**	**147.7 ± 32.0**
10-HDHA	505.7 ± 61.2	1016.4 ± 228.4	327.8 ± 64.7	745.9 ± 89.3	217.0 ± 36.4	695.9 ± 14.7	545.1 ± 35.7	440.3 ± 60.1
11-HDHA	595.6 ± 87.8	547.9 ± 117.5	483.2 ± 123.0	492.0 ± 121.5	307.6 ± 63.8	430.2 ± 64.5	661.1 ± 121.6	347.5 ± 82.0
13-HDHA	2148.3 ± 659.1	14614.9 ± 6599.9	3549.1 ± 2819.8	11087.4 ± 3854.1	1243.3 ± 156.9	12184.2 ± 5384.8	1921.2 ± 186.3	2828.5 ± 585.3
14-HDHA	1919.8 ± 518.1	5804.6 ± 1103.5	1170.0 ± 441.7	5282.6 ± 1253.9	1113.4 ± 165.5	3945.6 ± 947.0	1461.5 ± 151.0	2867.1 ± 581.0
16-HDHA	8750.6 ± 3255.0	14852.5 ± 949.2	2541.5 ± 927.2	13825.6 ± 6158.9	1164.5 ± 467.1	16458.6 ± 2549.7	3759.2 ± 719.3	7257.6 ± 3254.5
17-HDHA	4955.7 ± 1304.2	10296.4 ± 1934.7	2171.6 ± 698.6	7567.8 ± 2324.3	1371.9 ± 92.8	7482.1 ± 1950.1	3813.8 ± 806.4	4761.8 ± 2172.5
20-HDHA	540.1 ± 80.2	365.3 ± 54.6	499.1 ± 51.3	336.7 ± 82.7	368.4 ± 62.7	322.2 ± 30.6	548.5 ± 84.3	189.8 ± 43.8

Table 22. Pairwise multiple comparison tests summary of prostaglandins and unsaturated fatty acid oxidation products for the post-larvae after the sound exposure level and depending on early chemical contamination.

Difference in oxylipin profiles between uncontaminated and contaminated larvae for each sound treatment

	t	P-perm
within level "Control"	2.3795	0.0227
within level "Low"	2.8068	0.0227
within level "Medium"	4.7782	0.0017
within level "High"	1.7108	0.0908

Comparison of non-contaminated larvae according to the different sound conditions

	t	P-perm
"Control" vs "Medium"	3.251	0.0073
"Medium" vs "High"	2.3677	0.0264

Comparison of contaminated larvae in response to different sound conditions

	t	P-perm
"Control" vs "High"	2.3039	0.044
"Low" vs "High"	2.2789	0.0466
"Medium" vs "High"	1.982	0.0707

All quantified fatty acids measured in post-larvae showed no variation in relation to noise treatments, but varied in relation to the embryo origin (**Tab. 18**, **Fig. 38C**). Of the 16 fatty acids measured, 10 showed higher values in post-larvae from contaminated embryos, and only the C15:1n-5 showed no variation (**Tab. 23**). Post-larvae from contaminated embryos showed at least 60% higher levels of essential fatty acids consisting of eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, ARA).

Table 23. Mean level of the fatty acid and lipid profile for the post-larvae after the sound exposure level and/or depending on the early chemical contamination in ng/g ± SE. The significance of the differences in concentrations of the fatty acid between contamination was tested by PERMANOVA using R. Bold indicate p-values near or below 0.07 and the asterisks correspond to: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1.

	Non-contaminated	Contaminated	P-value	
Total sum (ng/mg)	6787.9 ± 2546.5	6292.7 ± 1995.7		
C14:0	0.9 ± 0.1	1.2 ± 0.2	0.0057	**
C15:0	ND			
C15:1n-5	3.0 ± 0.4	1.8 ± 0.2	0.0975	.
C16:0	18.6 ± 1.0	22.9 ± 0.5	0.0037	**
C16:1n-7	5.4 ± 1.1	14.0 ± 0.9	0.0001	***
C17:0	ND			
C18:0	28.2 ± 2.3	12.4 ± 1.1	0.0001	***
C18:1n-9	4.2 ± 0.6	8.8 ± 0.3	0.0001	***
C18:1n-7	1.6 ± 0.2	2.8 ± 0.1	0.0001	***
C18:2n-6	1.2 ± 0.2	1.6 ± 0.0	0.0001	***
C18:3n-6	ND			
C18:3n-3	ND			
C20:0	5.6 ± 0.6	2.1 ± 0.2	0.0002	***
C20:1n-9	1.6 ± 0.1	2.7 ± 0.1	0.0001	***
C20:2n-6	ND			
C20:3n-6	ND			
C20:4n-6	1.7 ± 0.2	2.8 ± 0.1	0.0001	***
C20:5n-3	5.7 ± 0.9	12.0 ± 0.4	0.0001	***
C22:0	5.7 ± 0.6	2.2 ± 0.2	0.0001	***
C22:1n-9	13.8 ± 1.4	4.3 ± 0.7	0.0001	***
C22:4n-6	ND			
C22:5n-6	ND			
C24:0	6.2 ± 0.7	2.3 ± 0.2	0.0002	***
C22:5n-3	ND			
C22:6n-3	2.7 ± 0.6	4.5 ± 0.2	0.0001	***

4.5 DISCUSSION

The main hypothesis that exposure to contamination during embryogenesis amplifies the acoustic impact of shipping noise during the metamorphosis of mussel larvae to young juveniles cannot be confirmed for the metamorphosis success, as no differences were observed between all treatments. However, the hypothesis was confirmed for post-metamorphic growth, as a positive effect of pollution during embryogenesis was observed on post-larval growth with an increase of shipping noise intensity. No interaction between the two factors was observed, but post-metamorphic growth was almost two times higher in individuals exposed to contamination at embryo stage and more than two times higher in post-larvae exposed to more intense shipping noise. This positive latent effect by stimulation of post-larval growth by embryo contamination is surprising and does not seem to be related to chemical accumulation in the post-larvae. In fact, the levels of alkanes and PAHs in post-larvae were below the detection limit, suggesting depuration during their larval growth. Mussels are known to exhibit a rapid depuration rate during the first days after exposure to hydrocarbons (Fossato & Canzonier, 1976; Schmutz et al., 2021). The similar levels of copper in post-larvae from the two contamination treatments during embryogenesis suggest that the latent positive effect observed in post-larval growth cannot be related to Cu. The accumulation of Cu in all post-larvae, probably related to phytoplankton feeding (Coale, 1991), could reflect the essential role of this element in the development of the first bivalves stage (Weng et al., 2019). Only the lead content differentiates the post-larvae from both contamination treatments, with almost 4 times higher values in individuals from contaminated embryos. This accumulation 39 days after their exposure at the embryo stage may reflect the general inability of mussels to actively excrete heavy metals (Depledge & Rainbow, 1990). However, it would be surprising that the very low level observed in post-larvae (8.3 $ng.g^{-1}$) would affect their growth. In a polluted area close to mining operation, mussels were shown to accumulate lead up to 3,000 $\mu g.g^{-1}$ (Ansari et al., 2004).

We suggest that the latent positive effect of the embryo-stage contamination on post-larval growth could be the result of a potential differential selective mortality between the two treatments. Fertilized eggs came from the same spawners and spawning events induced in the laboratory. The only difference was the exposure or not to the contaminant cocktail. Embryogenesis success was different between the two treatments with 13% for the control and 21% for the contamination exposure (Veillard et al. *submitted*, see Chapter 1, Article 1). The authors suggested that the low overall embryogenic success obtained was related to poor egg quality. The higher embryogenic success of the contaminated embryos exposed to contaminants could be from the presence of Cu in the cocktail promoting their development of embryos into D-larvae, as Cu is an essential element for their development (Weng et al., 2019). Thus, the survivors from the contaminated treatment could have been selected for their higher metabolic level, as reflected by the higher concentrations of energetic metabolites observed at post-larval stage. This is in accordance with the significantly larger size at metamorphosis measured for this group, which could be related to higher larval growth (Jablonski & Lutz, 1983) and their higher post-metamorphic growth. The greater relative accumulation of trophic fatty acid markers associated with phytoplankton (16:1n7 and 20:5n3 for diatoms, 18:1n9 for prymnesiophytes and 22:6n3 for dinoflagellates; (Parrish, 2013) suggests a higher ingestion rate for post-larvae from the contaminated treatment, associated with their higher growth. This potential selective pressure on embryos from chemical contamination mimicking the levels measured in an industrial seaport does not seem surprising. It is now recognized that this anthropogenic environment can lead to the emergence of new port-adapted lineages through the coupling between local adaptation clines and intrinsic isolation (Touchard et al., 2023).

The absence of an effect of shipping noise exposure on metamorphosis success is in contrast to a previous study performed in the same system under similar conditions (Veillard et al. *in press*, see Chapter 1, Article 2). The only difference between the studies was the duration of sound exposure, which was extended from 9 to 20 days during the settlement and metamorphosis of mussels from the pediveliger to post-larval stage in the current study. This longer duration confirms the hypothesis suggested in the previous study (Veillard et al. *in*

press, see Chapter 1, Article 2) that shipping noise emission appears to stimulate post-metamorphic growth. The higher post-metamorphic growth could be related to a more rapid settlement dynamics, but as no difference in size at metamorphosis was observed, this hypothesis does not seem adequate. One hypothesis could be related to tissue maturation during metamorphosis, in particular a more rapid development of gills in mussels exposed to shipping noise. Post-larval gill development is a long maturation process, but once developed, gills greatly increase the ingestive capacity of mussels compared to the larval velum (Cannuel et al., 2009). This hypothesis could also be supported by the higher relative accumulation of trophic fatty acid markers associated with phytoplankton feeding (Parrish 2013), as discussed above. Another explanation could be a positive effect of shipping noise on valve-opening activity, as the valve opening could be related to filtration and feeding (Newell et al., 2001). However, with the absence of change in metabolite content from anaerobic metabolism (lactate, octopine and strombine) associated with shell closure (de Zwaan et al., 1983a) in relation to shipping noise, this hypothesis seems not to be confirmed. Oxidative metabolism also showed higher activities, especially for oxylipin biosynthesis from DHA and EPA (HDHA and HETE metabolites) in relation to shipping noise, but not to higher noise intensity. Thus, shipping noise appeared to modulate the production of lipid mediators from PUFAs, possibly leading to different inflammatory profiles between control and noise-exposed post-larvae, as already observed by Veillard et al. (*in press*, see Chapter 1, Article 2). However, it was not clear whether this was the result of a direct effect or the effect of a more rapid metamorphic development, as is suggested by the more pronounced post-larval growth observed in shipping noise-exposed post-larvae.

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ARTICLE 5
COMMENT L'EXPOSITION AU BRUIT DE LA NAVIGATION PEUT-ELLE AMPLIFIER LES EFFETS D'UNE CONTAMINATION CHIMIQUE ACCIDENTELLE SUR LE RECRUTEMENT DE LA MOULE *MYTILUS EDULIS* ?

5.1 RÉSUMÉ EN FRANÇAIS DU CINQUIÈME ARTICLE

Un nombre croissant d'études examinent les effets acoustiques du trafic maritime sur les invertébrés, mais peu d'entre elles se sont penchées sur l'interaction de plusieurs facteurs de stress dans un contexte maritime réel. Le bruit peut interagir avec les contaminants chimiques et perturber le comportement des organismes. Des études récentes montrent que le bruit renforce les effets nocifs des métaux lourds tels que le cadmium. La réponse au stress des moules post-larvaires (*Mytilus edulis*) dans un environnement portuaire du golfe du Saint-Laurent a été étudiée en utilisant une double approche écophysiologique et écotoxicologique. Pour ce faire, des larves de *M. edulis* soumises à une pollution accidentelle au diesel ont été exposées à un bruit de navigation pendant leur phase de recrutement et de métamorphose. L'objectif est alors d'évaluer la réponse des larves compétentes, qui se métamorphosent ensuite en post-larves, après une contamination incontrôlée à un déversement de diesel combinée à une exposition au bruit d'un navire cargo représentatif du trafic maritime. Nous avons utilisé une approche métabolomique ciblée pour étudier la réponse au stress, les changements dans la performance et le comportement des larves en relation avec les altérations du métabolisme, considérant que le bruit des cargos pourrait être un signal potentiel pour la fixation larvaire. Les post-larves, particulièrement exposées à des niveaux de bruit de navigation plus élevés, ont montré un stress oxydatif significatif. Nous suggérons ainsi que la contamination par un écoulement de diesel pourrait affaiblir les larves via un retard de métamorphose. Dans le cas d'émissions sonores fortes, le taux de succès de métamorphose des post-larves est environ deux fois plus faible que dans des conditions sonores faibles. En effet, l'intensité sonore élevée a augmenté la demande d'énergie, ce qui a

conduit à une bioaccumulation accrue des polluants, et par cascade, à une augmentation du stress oxydatif aboutissant à une dynamique de métamorphose ralentie. Dans cette étude, nous suggérons la valeur seuil 121 dB re 1µPa au-delà de laquelle la survie et succès de recrutement des post-larves est réduit. Les populations naturelles exposées à ces polluants ont ainsi probablement une résilience moindre que celles associées aux ports, ce qui peut limiter leur capacité à recruter dans des conditions stressantes.

Mots-clés : Anthropophonie, métabolomique, écotoxicologie, physiologie des bivalves/mollusques, effets latents.

Cet article, intitulé « *How shipping noise exposure can amplify the effects of an accidental chemical contamination on mussel recruitment Mytilus edulis* », sera soumis dans la revue *Aquatic Toxicology*. En tant que premier auteur, j'ai contribué à l'essentiel de la recherche sur l'état de la question, au développement des larves de moules, aux mesures de performance post-larvaire, à l'exécution des analyses chimiométriques et fonctionnelles ainsi qu'à l'analyse par spectrométrie de masse. Stéphane Beauclercq a contribué aux analyses fonctionnelles. Bertrand Genard a supervisé les expériences de spectrométrie de masse. Richard Saint-Louis a apporté un soutien technique à l'analyse chimique. Frédéric Olivier, Laurent Chauvaud, Isabelle Marcotte, et Réjean Tremblay ont conçu et supervisé l'étude. Tous les auteurs ont contribué à la rédaction du manuscrit et ont approuvé l'article final. Une version abrégée de cet article a été présentée à la conférence annuelle de *Ressources Aquatique Québec* en 2024 à Québec.

HOW SHIPPING NOISE EXPOSURE CAN AMPLIFY THE EFFECTS OF AN ACCIDENTAL CHEMICAL CONTAMINATION ON MUSSEL RECRUITMENT *MYTILUS EDULIS*.

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5.2 ABSTRACT

A growing number of studies are examining the acoustic effects of shipping on invertebrates, but few have examined the interaction of multiple stressors in a real ecological context. Noise can interact with chemical contaminants to disrupt the behavior of organisms. Recent studies show that noise enhances the harmful effects of heavy metals such as cadmium. The stress response of post-larval mussels (*Mytilus edulis*) in a harbor environment in the Gulf of St. Lawrence was studied using an ecophysiological and ecotoxicological approach. To achieve this, *M. edulis* larvae exposed to an accidental diesel pollution were exposed to a shipping noise during their competent stage to settle and metamorphosis. The objective was to assess the response of competent larvae to further metamorphose into post-larvae after an uncontrolled contamination combined with a cargo ship noise exposure representative of maritime traffic. We used a targeted metabolomic approach to investigate the stress response, the changes in larval performance and behavior in relation to metabolic alterations, considering that cargo noise could be a potential cue for larval settlement. Post-larvae, particularly those exposed to higher shipping noise levels, displayed significant oxidative stress, and we suggest that contamination could weaken larvae as they delay their metamorphosis. With high levels of shipping noise, the metamorphic success is around half of the low noise emission conditions. In fact, high sound intensity increased the energy demand, which led to higher bioaccumulation of pollutants, resulting in oxidative stress and, ultimately, lower metamorphic success. We suggest a SPLrms threshold value of 121 dB re 1µPa after which more post-larvae reduced their ability to survive. Other populations exposed to these pollutants probably have a lower resilience than those associated with ports, which may limit their ability to recruit under stressful conditions.

Keywords: Anthropophony, metabolomics, ecotoxicology, bivalve/mollusc physiology, latent effects.

5.3 INTRODUCTION

The increasing industrialization of the world's oceans has led to light, noise, and chemical pollution, as well as facilitated biological invasions, and a range of cumulative and additive impacts (McCormick et al., 2018). Marine systems and their associated species are under increasing pressure through habitat loss and degradation, ecological trap formation, and mortality (Komyakova et al., 2022). The ecological impact of anthropogenic activities on the marine environment has become one of the most important issues in the field of ecology (Crain et al., 2008). The majority of marine infrastructure is located in or near ports and marinas - areas that naturally have high levels of shipping activity (Bugnot et al., 2021; Sardain et al., 2019), but also where marine organisms reside. Shipping is now largely recognized as a major source of pollution, and that includes noise the sound pollution considered as one of the 11 descriptors of the Marine Strategy Framework Directive (MSFD) to achieve a Good Environmental Status (GES). Furthermore, ports and harbors are commonly associated with a range of heavy metal industries and refineries (Chatzinikolaou et al., 2018; Yu et al., 2017). In the last few decades, human activities have generated not only chemical pollution, but also an increasing amount of anthropogenic noise in both the open ocean and coastal areas (Peng et al., 2015). Anthropogenic underwater noise – primarily associated with shipping (Chauvaud et al., 2018) (90 %) – is now categorized among 'emerging threats and persistent conservation challenges for freshwater biodiversity' (Reid et al., 2019). Global shipping noise emissions have doubled every 11.5 years (Jalkanen et al., 2022).

Noise and chemical pollution associated with harbors, marinas and some independent marine structures (*e.g.* oil rigs) have the potential to weaken the resilience of local ecosystems via alterations of animal behavior, reduced fitness and reproductive output, increased mortality and erosion of biodiversity (Cones et al., 2024; Duarte et al., 2021; Komyakova et al., 2022). Although several studies have examined the chemical impacts of shipping on invertebrates (McKenzie et al., 2012), and more recently, the acoustic impacts (Solé et al., 2023). Few have examined the interaction of multiple stressors/pressures

(synergistic/antagonistic interactions) to approximate a real ecological context (Popper et al., 2020; Thomsen & Popper, 2024). To our knowledge, only Shi et al. (2019) and Tu et al. (2024) highlighted that the stress response induced by anthropogenic noise amplifies the adverse effects of heavy metals, mutagenicity or neurotoxicity. When adult oysters were exposed to both shipping noise and Cd, their daily valve activity decreased, as did lipid metabolism and growth (Charifi et al., 2018).

Mytilus edulis is a bivalve mollusk of great economic interest, often used as a bioindicator of the health of the marine environment (Smaal et al., 2019). The present study investigates the effect of noise pollution and how it interferes with an accidental chemical pollution from shipping. Based on the previous work (Cervello et al. 2023, Veillard et al. *in press*, see Chapter 1, Article 2) showing how anthropogenic noise modifies the behavior of bivalve larvae during metamorphosis, we hypothesized that the increased closure behavior of post-larvae associated with shipping noise could interfere with the bioaccumulation or depuration process of the contaminant and/or other physiological mechanisms. To achieve this, *Mytilus edulis* larvae exposed to an accidental diesel pollution were exposed to a shipping noise during their competent stage to settle and metamorphosize. The objective was to assess the response of competent larvae that further metamorphose into post-larvae after an uncontrolled contamination combined with a cargo ship noise exposure representative of maritime traffic. We used a targeted metabolomic approach to investigate the stress response and the changes in larval performance and behavior in relation to metabolism alterations, considering that cargo noise could be a potential cue for larval settlement.

5.4 MATERIALS AND METHODS

5.4.1 Mussel larval culture and experimental set-up

Mussel larvae were produced as described in Rayssac et al. (2010) with adults originating from the St. Peters' Bay in Prince Edward Island (46.42948N, -62.66030W, Canada) individually induced to spawn by 10°C thermal shock. Fertilization was realized

with a ratio of 10 spermatozoids per egg and embryogenesis obtained after 4 days in three flat-bottom 30-L tanks at 18°C in UV ultra-filtered seawater (1 µm) and maintained at 18°C. Each tank of D-larvae was, furthermore, sieved through a 20 µm mesh then transferred in a conic 60-L cylinder tank (5 larvae mL^{-1}) to achieve the veliger larval development at similar temperature (18°C) and UV ultra-filtered seawater. Every two days, larvae were sieved through a 35 µm mesh, tanks were cleaned with Virkon VKS10 disinfectant (LANXESS Deutschland GmbH, Cologne, Germany) then refilled with UV ultrafiltered seawater and larvae. The diet of the larvae was composed of a mixed suspension of microalgae containing *Pavlova lutheri* (CCMP459), *Tisochrysis lutea* (CCMP1324), *Chaetoceros muelleri* (CCMP1317), *Tetraselmis suecica* (CCMP904), and *Nannochloropsis oculata* (CCMP525) at a ratio of 1:1:1:1:1 equivalent biomass (dry weight) and a density of 60 cells μL^{-1}.

At the pediveliger stage (17 days post-fertilization, dpf), estimated by the presence of eyed larvae, foot development and a substratum exploration over 50% of the larval batch, larvae from each larval rearing tank were sieved through a 100 µm mesh then transferred in four cylinders tanks (5-L, 5 larvae mL^{-1}) containing settlers collectors (constituted from two 30 cm polypropylene ropes). Each cylinder tank was distributed in one of the four *Larvosonic* systems (Olivier et al. 2023; Cervello et al. 2023), to obtain four acoustic conditions. In a single *Larvosonic*, no sound was emitted and the sound level corresponded to the ambient room condition (Control: SPL_{rms}=116 ± 1 dB re 1µPa), while a shipping noise sequence was emitted at three increasing levels (Low: 121 ± 4 dB re 1µPa, Medium: 127 ± 2 dB re 1µPa, High: 151 ± 2 dB re 1µPa) (Veillard et al. *in press,* see Chapter 1, Article 2 and Chapter 2, Article 4). Based on transmission loss calculation (Byrro-Gauthier et al, in press) such levels correspond to a distance from the shipping noise source of either 18 (high), 735 (medium) or 1800 m (low). Every two days, the collectors were gently removed, swimming larvae were sieved through a 100 µm mesh, the cylinder tank was cleaned, and the larvae were fed as previously described. From fertilization to the post-larval stage, temperature (18.0 ± 0.2°C), salinity (23.1 ± 0.1 PSU), photoperiod (15h of light and 9h of the night) and light intensity (9.0 ± 0.2 40 FC; 97.1 ± 1.7 400 Lux) were controlled with dedicated probes (Hanna multiparameters HI98194 probe and Hobo ONSET UA-0002-64). At 37 dpf (20 days after

the start of sound emission), collectors from each cylinder tank and individuals settled on the cylinder were gently rinsed over a 100 µm mesh to collect all post-larvae. Immediately after collection, total wet biomass of post-larvae was determined for each cylinder tanks (24 samples), split into four sub-samples (each one weighted), flash-frozen in liquid nitrogen, freeze-dried, and stored at −80°C until analysis. One subsample was used for morphological measures, one for metabolomics and lipidomic analysis, another for the oxylipins analysis and the last one for the chemical quantification.

5.4.2 Determination of contaminants in larval samples

Due to the failure of the water supply system and the subsequent use of a diesel-powered pump from the beginning of embryogenesis until sampling at the post-larval stage, potential chemical contamination of larvae during the veliger and post-larval stages was expected. Therefore, the presence of contaminants in post-larvae was tested on freeze-dried larvae to test for the presence of metals and hydrocarbons based on a method described in Lemos et al. (2021), as previously described in Veillard et al. (*submitted*, see Chapter 2, Article 3). Briefly after micro-grinding and homogenization, tissues underwent alkaline digestion in 200 µL of tetramethylammonium hydroxide (TMAH, 25 % aqueous), heated to 60°C for one hour with constant stirring. Then, the solution was acidified by adding nitric acid (HNO3) 67-70%. The metals and hydrocarbons were extracted and separated by adding 300 µL of hexane-toluene, repeated twice. Inorganic compounds such as metals (Cu, Pb, Hg) were found in the aqueous phase and organic compounds such as hydrocarbons (polycyclic aromatic hydrocarbons (PAHs) and alkanes) are present in the organic phase. The aqueous phase was filtered through a 45µm polytetrafluoroethylene (PTFE) Teflon filter then analyzed by GF-AAS 240Z AA (Agilent Technologies) equipped with a GTA 120 Graphite Tube Atomizer (Agilent Technologies). For lead measurements, a solution of palladium and magnesium was used (Agilent technologies, Pre-Mixed GF AAS: 750 µg. mL-1 Pd and 500 µg. mL-1 Mg in 2 % HN03). Organic phase was purified by 100mg of magnesium sulfate from Sigma-Aldrich and 50 mg of Florisil, then injected (200µL) in the 8890 Gas

Chromatograph (GC) System (Agilent Technologies) equipped with a Rxi-5ms capillary column, L 30 m, DI 0.53 mm, 0.25 µm (Restek, USA).

5.4.3 Quantification of amino acids, energy metabolism-related metabolites and fatty acid

Freeze-dried post-larvae were micro-ground and homogenized with a 2.8 mm ceramic bead at 4,000 rpm for three cycles of 10 s (Precellys 24; Bertin corporation). Analyte extraction for the quantification of amino acids, energy metabolism-related metabolites and fatty acid methyl ester (FAME) were performed on the same sample with a two steps extraction process using the same captiva as already described in Veillard et al. (*in submission*, see Chapter 2, Article 4). The first elution process was performed to extract metabolites and the second elution for lipid extraction. Free amino acids and energy metabolism-related metabolites were analyzed together and fatty acids separately. Analyte extraction for the quantification of oxylipins was performed in another subsample.

Briefly, 500 µL of 1/1 (v/v) mix of TFE/LC-MS grade water was added in the 3 mL Agilent Captiva EMR–Lipid solid phase extraction cartridge (part number 5190-1003 – Agilent Technologies) and 750µL of a 1/1 (v/v) mix of TFE/LC-MS grade water was added to the homogenizing tubes, vortexed for 30 s and centrifuged for 1 min at 2,460 g. Three extraction cycles (500µL, with 1 mL of ACN for the last one) were performed and the filtrates were pooled in a 15 mL falcon tube. Then, the cartridge was rinsing with 3 mL of 1/1 (v/v) mix of TFE/ LC-MS grade water and drained using a high vacuum for 30 seconds. Samples were dried with a SpeedVac overnight without heating. Sample were reconstituted by adding 50 µL of LC-MS grade water followed by 200 µL of LC-MS grade ACN. One hundred microliters of each reconstituted sample was transferred to two vials: one vial was for positive analysis and the other for the negative analysis. Compounds valine-d_8 (10 µg/mL) and pyruvate-d_3 (10 µg/mL) were used as an internal standard to quantify the compounds in positive and negative ion modes, respectively. Metabolites were separated and quantified in multiple reaction monitoring (MRM) mode using an HPLC 1260 Infinity II device coupled to a 6420 Triple Quad mass spectrometer (Agilent Technologies) in positive and negative

ionization mode. Ten microliters of the sample was injected, and the chromatography separation was performed with a InfinityLab Poroshell 120 HILIC-Z, 2.7 µm 10 x 2.1 mm column.

For fatty acids extraction, 1mL of 2,2,2-trifluoroethanol (TFE) at 100% was added to the homogenizing tubes containing the sample from the metabolite extraction. Samples were vortexed for 30 s and centrifuged for 1 min at 2,460 g. The supernatants (1000 µL) were pooled in 15 mL falcon tubes. Then, 500 µL of 5/1 (v/v) mix of methyl tert-butyl ether (MTBE) and TFE was added to the homogenizing tubes, vortexed for 30 s and centrifuged for 1 min at 2,460 g. This extraction was performed three times, and supernatants (500 µL) were pooled in 15 mL falcon tubes. The cartridge of the Captiva EMR-Lipid was rinsed with 3 mL of 5/1 (v/v) mix of MTBE/TFE and drained by attaching a vacuum to the manifold to obtain a flux of 1 drop each 3 to 5 s. The filtrate was collected in a 15 mL falcon tube. Samples were dried with a SpeedVac (SPD2010, Savant) overnight without heating. Sample were reconstituted by adding 250 µL of 3/1 (v/v) mix of MTBE / Methanol HPLC grade (MeOH) and 50 µL of each reconstituted sample was transferred in 8 mL transesterification vials following by addition of 800 µL of toluene and 3 mL of 12% sulfuric acid methanol solution. Vials were heated at 90°C for 1 hour in a dry bath under a fume-hood and vortexed each 15 min. When the vials were cooled to room temperature, 3 mL of H_2O nano-filtered water and 800 µL of hexane was added in the transesterification vials, vortexed and centrifuged for 10 min at 2,460 g. The supernatant was transferred into 1.5 mL GC vial and dried with a SpeedVac (SPD2010, Savant). Lipids were separated and quantified using an GC-FID 8890 (G3540A, Agilent Technologies). Two microliters of the sample was injected, and the chromatography separation was performed with a InfinityLab Poroshell 120 HILIC-Z (2.7 µm 10 × 2.1 mm) column.

5.4.4 Polyunsaturated fatty acids oxidation products, oxidative metabolism

Oxylipins linked to oxidative metabolism were extracted on freeze-dried post-larvae micro-ground and homogenized with a 2.8 mm ceramic bead at 4,000 rpm for three cycles

of 10 s (Precellys 24; Bertin corporation). A total of 200 µL of a 1/1 (v/v) mixture of 2,2,2-trifluoroethanol (TFE) and extraction buffer (ammonium formate buffer at pH 3) was added to the homogenizing tubes containing the sample. Samples were vortexed for 10 s and centrifuged for 1 min at 2,460 g. Then, 200 µL of a 1/1 (v/v) mixture of ACN and methanol (MEOH) was added to the homogenizing tubes containing the sample. Samples were vortexed for 10 s and centrifuged for 5 min at 2,460 g, 4°C. Precisely 250 µL of the supernatant was transferred to a 2-mL HPLC vial containing 1 mL of 50 mM ammonium formate buffer at pH 3 with 10 ng/mL of internal standard PGE_2-d_9. (Prostaglandin E2-d9). Sample was analyzed directly after the extraction process, with an HPLC 1260 Infinity II device coupled to a 6470B Triple Quad mass spectrometer (Agilent Technologies) using on-line SPE method allowing samples cleaning and oxylipin separation from 1 ml of samples. Oxylipins extracts were separated using a Gemini C6-Phenyl 100Å, 2 x150 mm 5 µm (Phenomenex) and a Gemini C6-Phenyl guard column as on-line SPE (4 × 2.0 mm, SecurityGuard, Phenomenex) with column temperature maintained at 40°C.

5.4.5 Statistical analyses

One-way univariate PERMANOVA was used to test for significant effects of noise conditions (4 treatments) on metamorphic success, shell size, post-settlement growth and the concentration of each pollutant in larval tissues (copper, lead, mercury, alkane and PAH). Furthermore, multi-variate PERMANOVA was used on the energy metabolism, oxidative metabolism, lipid and pollutant profiles to determine general pattern differentiation between noise conditions. All PERMANOVAs were realized using PRIMER7 software. The permutational method is a randomization that allows us to remove the distributional assumption of normality and can be applied to very small samples (Legendre & Legendre, 2012). Similarity matrices based on ratios were produced using the Bray-Curtis distance. When differences were significant (p-perm ≤0.07), pairwise multiple comparison tests were used to determine which groups were significantly different. Distance-based tests for homogeneity of multivariate dispersion (PERMDISP) were carried out to assess the homogeneity of the ratios in each condition.

Multivariate statistical analyses to test, ‘metabolite by metabolite’, the effect of noise conditions (4 treatments) on omics data (metabolism, oxidative and fatty acids) obtained from post-larvae were performed using the vegan package from R studio, and graphical visualizations such as principal component analysis (PCA) were produced using the factoextra package. Similarity matrices based on ratios (using the Bray-Curtis distance) were generated using the metaMDS function, which is considered the most robust unconstrained ordination method in community ecology (Minchin, 1987).

5.5 RESULTS

Metamorphic success (**Tab. 24**) was influenced by vessel noise, with values about half in the Medium and High treatments compared to those in the Low treatment, and intermediate values for the control treatment (**Tab. 25**). Neither size at metamorphosis nor post-metamorphic growth was affected by shipping noise in larvae exposed to pollution throughout larval development. All contaminants were present in the post-larvae (copper, lead, alkane and PAH), but only alkane and PAH showed variation between shipping noise conditions (**Tab. 24**) with higher values of alkane in medium and high noise treatments, and the lower and null values of PAH in the high noise treatment.

Table 24. Statistical analysis summary. Bold indicates p-values near or below 0.06.

	Df	Pseudo-F	P-perm
Metamorphic success	3	2.907	**0.0577**
Metamorphic size (PII)	3	0.97169	0.4537
Post-metamorphic growth (D)	3	1.784	0.1884
Energy metabolism profile	3	1.3783	0.2797
Oxidative metabolism profile	3	3.6243	**0.0114**
Lipid profile	3	7.7784	**0.0066**
All pollutants	3	2.3025	**0.0495**
Copper contamination	3	1.0099	0.473
Lead contamination	3	1.4356	0.3046
Alkane contamination	3	3.1046	**0.0157**
PAH contamination	3	3.5151	**0.0468**

Table 25. Mean level of metamorphic success, larval size at metamorphosis, post-larval growth and concentration of chemical pollutants found in post-larvae in ng/g ± SE. Letters in bold indicates p-values near or below 0.07.

	Control	Low	Medium	High
Metamorphosis success	42 ± 6% **ab**	50 ± 8% **a**	22 ± 7% **b**	29 ± 4% **b**
Metamorphosis size (PII)	283.6 ± 5 μm			
Post-metamorphosis growth (D)	170.5 ± 30 μm			
Copper contamination	9.8 ± 2.8			
Lead contamination	1.1 ± 0.8			
Alkane contamination	0.3 ± 0.2 **a**	0.5 ± 0.1 **a**	7.9 ± 4.9 **b**	5.4 ± 0.9 **b**
PAH contamination	0.1 ± 0.0 **ab**	0.4 ± 0.2 **a**	0.1 ± 0.1 **ab**	0.0 ± 0.0 **b**

While the general profile of energetic metabolites and free amino acids in post-larvae mussels did not differ between sound treatments (**Tab. 24 and 26**), those of fatty acids (Medium) and oxidative metabolism (oxylipin concentrations, high treatment) were clearly different (**Tab. 24**). Statistical analysis revealed a large number of oxylipins that differed between treatments (**Tab. 27**). Most are oxylipins biosynthesized from linoleic acid, an essential fatty acid (18:2n-6, LA), with a clear pattern identified of higher values in the Medium noise treatments compared to control and intermediate values in Low and High noise conditions. In addition, the statistical analysis revealed some arachidonic acid-derived oxylipins (ARA) (*i.e.* cyclopentenone prostaglandins (cyPGs) and hydroxyeicosatetraenoic acids (HETE)), also identified at higher concentrations in sound condition (especially in Medium and High conditions) compared to the control. All these oxylipins are mainly derived from the same pathway of omega-6 synthesis. A large number of HEPE (hydroxy-eicosapentaenoic acid) from eicosapentaenoic acid (EPA) was also identified, with even higher levels at high sound exposure. Only 16-HDHA from docosahexaenoic acid (22:6n-3, DHA) differed according to sound intensity with higher concentrations at high sound exposure. The latter two are derived from omega-3 synthesis pathways.

For fatty acids, we observed a generally lower proportion of almost all fatty acids in the medium sound exposure condition, and many are essential fatty acids such as EPA (20:1n-9), ARA (20:4n-6), and LA (18:2n-6) (**Tab. 28**). These variations resulted in a much lower total amount of fatty acids in the post-larvae that were exposed to the medium intensity sound. However, several saturated fatty acids such as lignoceric acid (24:0), docosanoic acid (22:0), eicosanoic/arachidic acid (20:0), and stearic acid (18:0) were found in higher proportions in the medium intensity treatment compared to the other conditions.

Table 26. Targeted LC-MS-based quantification (mean ± standard error ng/mg) of amino acids and energy metabolism metabolites for the post-larvae.

		Overall average
Glycolysis	Glucose	57.1 ± 5.5
	Glucose-6-phosphate	5.2 ± 1.0
	Fructose-1,6-biphosphate	ND
	Phosphoenol pyruvate	ND
	Pyruvate	5.3 ± 0.6
Anaerobic	Lactate	39.2 ± 4.4
	Octopine	0.3 ± 0.1
	Strombine	2.8 ± 0.4
Krebs cycle	Acetyl-CoA	0.5 ± 0.1
	Citrate	4.7 ± 0.6
	Cis-aconitate	0.1 ± 0.0
	Aketoglutarate	2.1 ± 0.3
	Succinyl CoA	ND
	Succinate	35.7 ± 6.1
	Fumarate	4.9 ± 0.9
	Malate	23.7 ± 4.5
	Oxaloacetate	ND
BCAAs	Valine	60.1 ± 8.5
	Leucine	54.3 ± 8.1
	Isoleucine	23.0 ± 3.2
Central metabolism	Aspartate	260.2 ± 36.1
	Glutamate	338.2 ± 47.7
	Glutamine	182.3 ± 24.3
	Arginine	11.4 ± 1.9
Energy transfer	ATP	4.2 ± 1.1
	ADP	9.1 ± 2.1
	AMP	31.4 ± 3.7
	FAD	1.6 ± 0.2
	NAD	12.1 ± 1.3
	NADH	ND
	NADP	0.5 ± 0.1
	NADPH	ND
Amino acids	Lysine	1.8 ± 0.2
	Tryptophan	27.7 ± 4.0
	Tyrosine	45.8 ± 6.3
	Phenylalanine	20.6 ± 2.8
	Histidine	40.5 ± 4.1
	Proline	120.8 ± 14.8
	Threonine	40.4 ± 4.9
	Methionine	11.1 ± 1.6
	Sarcosine	1.6 ± 0.2
	Glycine	863.5 ± 99.2
	Cystine	0.6 ± 0.1
	Alanine	201.7 ± 24.3
	Serine	98.2 ± 12.2
	Hydroxyproline	2.9 ± 0.4
	a-aminobutyric acid	7.2 ± 0.9
	b-aminoisobutyric acid	0.7 ± 0.1
	a-aminoadipic acid	8.0 ± 1.2
	Betaine	371.4 ± 41.2
Ratio	AEC	0.2 ± 0.0
	BCAAs	137.3 ± 19.6
	Glutamine/Glucose	3.3 ± 0.4

Table 27. Targeted LC-MS-based quantification (mean ± SE ng/g) of prostaglandins and unsaturated fatty oxidation products for the post-larvae. Bold indicates p-values near or below 0.07 and the letters corresponds to the post-hoc test between the treatments.

Sound intensity				
Early chemical contamination	Control	Low	Medium	High
Total sum	6904.9 ± 952.5	14440.5 ± 3905.2	7581.0 ± 380.1	19892.5 ± 6338.0
Cyclopentenone prostaglandin				
PGE2	42.0 ± 16.8	83.9 ± 50.7	56.5 ± 27.8	98.7 ± 42.2
15-keto-PGE2	6.1 ± 0.9 **a**	6.6 ± 2.6 **ab**	9.9 ± 2.5 **b**	9.6 ± 2.8 **ab**
PGD2	14.7 ± 3.8	25.9 ± 10.7	22.1 ± 4.2	34.5 ± 15.1
13,14-dihydro-15-Keto-PGD2	9.9 ± 0.3 **a**	11.2 ± 1.3 **ab**	20.8 ± 5.5 **b**	16.0 ± 2.0 **ab**
PGA2	0.9 ± 0.4 **a**	2.5 ± 2.3 **ab**	4.5 ± 2.0 **b**	2.4 ± 2.1 **ab**
PGJ2	0.4 ± 0.1 **a**	0.1 ± 0.1 **ab**	0.7 ± 0.4 **b**	0.4 ± 0.4 **ab**
15-deoxy-d12,14-PGJ2	19.3 ± 1.2 **a**	21.4 ± 2.6 **ab**	40.1 ± 12.4 **b**	29.4 ± 3.4 **ab**
11b_PGF2α	93.2 ± 37.7	209.4 ± 119.9	150.9 ± 79.9	306.5 ± 176.4
15-keto-PGF2α	7.8 ± 0.0	16.8 ± 8.7	10.7 ± 2.2	14.8 ± 2.1
13,14-dihydro-15-Keto-PGF2α	5.3 ± 1.4	3.8 ± 0.9	8.5 ± 2.5	13.6 ± 6.2
PGB2	11.9 ± 2.1	10.2 ± 0.0	17.3 ± 6.7	30.9 ± 6.1
THXB2	9.8 ± 0.6 **a**	11.3 ± 0.7 **ab**	20.5 ± 6.4 **b**	15.0 ± 1.6 **ab**
EPA oxylipin				
PGE3	18.7 ± 2.5 **a**	23.8 ± 12.0 **ab**	18.3 ± 0.4 **a**	34.8 ± 3.5 **b**
PGF3α	15.3 ± 6.4 **a**	24.1 ± 0.5 **ab**	24.0 ± 0.3 **ab**	42.4 ± 13.1 **b**
5-HEPE	139.2 ± 15.4 **a**	159.0 ± 0.7 **ab**	144.0 ± 34.8 **b**	166.9 ± 12.6 **ab**
8-HEPE	2001.8 ± 679.2	3062.0 ± 1538.8	1278.0 ± 322.4	3961.0 ± 2103.8
9-HEPE	281.9 ± 69.6 **a**	255.3 ± 22.8 **ab**	418.3 ± 105.6 **b**	548.7 ± 217.5 **ab**
11-HEPE	217.9 ± 9.4 **ab**	307.0 ± 68.9 **ab**	192.6 ± 44.3 **a**	597.3 ± 288.7 **b**
12-HEPE	864.1 ± 108.6 **a**	6021.5 ± 1429.2 **bc**	1498.4 ± 186.5 **ab**	7530.4 ± 1316.6 **c**
15-HEPE	322.4 ± 32.1	406.9 ± 58.9	385.6 ± 70.6	508.9 ± 72.7
18-HEPE	188.3 ± 7.9	241.8 ± 32.9	186.6 ± 34.2	269.7 ± 33.7
ALA-GLA-oxylipin				
9(S)-HOTrE	12.9 ± 1.0 **a**	17.4 ± 0.2 **ab**	22.3 ± 3.9 **b**	23.3 ± 3.6 **ab**
13(S)-HOTrE	27.9 ± 6.7	36.0 ± 11.5	40.1 ± 9.1	55.0 ± 10.9
13(S)-HOTrE(g)	13.3 ± 5.6 **a**	9.3 ± 3.4 **ab**	6.0 ± 4.0 **b**	23.8 ± 2.5 **a**
Arachidonic-oxylipin				
5-HETE	28.9 ± 4.4 **a**	30.4 ± 9.9 **ab**	21.7 ± 8.0 **b**	25.6 ± 2.2 **ab**
8-HETE	498.3 ± 149.4	724.5 ± 260.7	421.2 ± 112.9	1267.7 ± 676.6
9-HETE	61.0 ± 16.2 **a**	97.7 ± 33.2 **ab**	117.4 ± 19.8 **b**	143.5 ± 45.2 **ab**
11-HETE	78.4 ± 5.9	103.7 ± 47.4	67.0 ± 22.9	169.3 ± 78.9
12-HETE	88.1 ± 7.5 **a**	221.4 ± 36.0 **ab**	151.7 ± 31.1 **ab**	328.5 ± 71.2 **b**
15-HETE	43.5 ± 1.2 **a**	63.3 ± 1.3 **ab**	71.7 ± 17.7 **b**	91.5 ± 14.1 **ab**
5-OxoETE	5.5 ±1.2	8.4 ± 2.3	3.2 ± 0.7	7.0 ± 3.0
12-OxoETE	3.6 ± 0.8 **a**	0.1 ± 0.1 **b**	4.1 ± 1.0 **a**	0.8 ± 0.4 **ab**
15-oxoETE	6.2 ± 0.8	14.7 ± 4.0	9.1 ± 3.5	19.0 ± 10.8
Linoleic acid				
9-HODE	89.4 ± 15.2 **a**	97.8 ± 76.5 **ab**	203.9 ± 68.4 **b**	223.7 ± 43.9 **ab**
13-HODE	95.8 ± 5.8 **a**	135.0 ± 0.0 **ab**	207.3 ± 70.0 **b**	186.9 ± 20.0 **ab**
9-oxoODE	35.4 ± 18.3 **a**	59.4 ± 7.6 **ab**	70.6 ± 16.7 **b**	74.0 ± 12.6 **ab**
13-oxoODE	9.3 ± 2.6 **a**	33.5 ± 4.8 **ab**	31.2 ± 6.5 **b**	47.3 ± 27.9 **ab**
(±)9(10)-DiHOME	1.7 ± 0.1 **a**	2.1 ± 0.2 **ab**	4.3 ± 1.4 **b**	3.1 ± 0.4 **ab**
(±)12(13)-DiOME	8.1 ± 1.0 **a**	9.6 ± 0.6 **ab**	18.6 ± 5.8 **b**	15.6 ± 2.7 **ab**
(±)9(10)-EpOME	27.0 ± 3.9 **a**	22.7 ± 2.0 **ab**	43.2 ± 14.8 **b**	26.2 ± 2.0 **ab**
(±)12(13)-EpOME	14.7 ± 2.9 **a**	19.7 ± 2.9 **ab**	28.6 ± 11.8 **b**	23.0 ± 9.3 **ab**
HDHA				
4-HDHA	91.7 ± 2.3	121.6 ± 23.5	96.0 ± 26.7	137.6 ± 28.0
7-HDHA	54.6 ± 1.1	58.2 ± 8.3	64.1 ± 8.4	76.7 ± 20.6
8-HDHA	79.6 ± 19.8	86.8 ± 21.5	74.7 ± 14.2	131.7 ± 32.2
10-HDHA	200.0 ± 10.1	221.8 ± 34.6	188.2 ± 18.9	280.4 ± 72.5
11-HDHA	93.2 ± 5.5	120.5 ± 18.2	86.4 ± 4.0	139.3 ± 64.0
13-HDHA	226.9 ± 54.8	228.2 ± 29.1	177.8 ± 24.2	697.7 ± 411.9
14-HDHA	266.0 ± 15.2	428.0 ± 56.3	241.3 ± 61.0	556.2 ± 254.2
16-HDHA	58.6 ± 5.2 **ab**	72.6 ± 8.6 **ab**	50.9 ± 13.4 **a**	91.4 ± 17.2 **b**
17-HDHA	327.8 ± 10.2	366.1 ± 29.3	435.6 ± 151.5	659.6 ± 204.7
20-HDHA	86.8 ± 11.4	125.4 ± 4.1	114.5 ± 33.1	135.2 ± 21.9

Table 28. Mean level of fatty acids for the post-larvae in ng/mg ± SE. Bold indicates p-values near or below 0.07 and the letters corresponds to the post-hoc test between the treatments.

	Control	Low	Medium	High
Total sum (ng/mg)	5732.1 ± 1822.5	5661.1 ± 2463.8	3666.8 ± 1537.6	7391.9 ± 1361.5
C14:0	2.1 ± 0.4 **a**	1.8 ± 0.4 **a**	0.6 ± 0.3 **b**	1.2 ± 0.1 **ab**
C15:0			ND	
C15:1n-5	1.1 ± 0.1 **a**	1.2 ± 0.4 **a**	2.3 ± 0.3 **b**	1.6 ± 0.3 **ab**
C16:0	23.4 ± 0.1	23.5 ± 0.4	**20.8 ± 0.5***	23.5 ± 0.6
C16:1n-7	18.8 ± 0.8 **a**	17.0 ± 1.5 **a**	9.3 ± 1.6 **b**	14.9 ± 0.7 **ab**
C17:0			ND	
C18:0	11.0 ± 0.7 **a**	13.0 ± 2.0 **a**	22.2 ± 2.9 **b**	14.4 ± 1.8 **ab**
C18:1n-9	8.2 ± 0.1	8.2 ± 0.1	**5.8 ± 0.5***	8.0 ± 0.6
C18:1n-7	2.9 ± 0.1	2.8 ± 0.1	**2.2 ± 0.2***	2.8 ± 0.1
C18:2n-6	1.5 ± 0.0	1.6 ± 0.1	**1.1 ± 0.1***	1.5 ± 0.1
C18:3n-6			ND	
C18:3n-3			ND	
C20:0	1.5 ± 0.1	1.9 ± 0.2	**4.3 ± 0.3***	2.1 ± 0.5
C20:1n-9	2.3 ± 0.1 **a**	2.2 ± 0.1 **ab**	1.9 ± 0.1 **b**	2.2 ± 0.2 **ab**
C20:2n-6			ND	
C20:3n-6			ND	
C20:4n-6	2.0 ± 0.1	1.9 ± 0.1	**1.0 ± 0.5***	1.9 ± 0.1
C20:5n-3	10.0 ± 0.2	9.9 ± 0.3	**7.1 ± 0.7 ***	9.5 ± 0.6
C22:0	1.5 ± 0.1	2.0 ± 0.2	**4.3 ± 0.3***	2.1 ± 0.5
C22:1n-9	2.5 ± 0.1 **a**	2.6 ± 0.7 **a**	6.4 ± 2.1 **b**	3.7 ± 0.9 **ab**
C22:4n-6			ND	
C22:5n-6	1.3 ± 0.2	1.0 ± 0.5	0.9 ± 0.4	1.3 ± 0.2
C24:0	1.5 ± 0.1	2.2 ± 0.3	**4.6 ± 0.3 ***	2.2 ± 0.5
C22:5n-3			ND	
C22:6n-3	6.8 ± 0.2	6.4 ± 0.2	**5.0 ± 0.7***	6.2 ± 0.3

5.6 DISCUSSION

Unfortunately, the use of a diesel-pump resulted in the accumulation of pollutants during the larval development, as observed at the pediveliger stages. Surprisingly, the level of contamination was not similar in post-larvae submitted to contrasting shipping noise levels

and ambient room soundscape. This is particularly true for alkane concentrations which were over 10 times higher in Medium and High noise levels than the control and the low noise level treatments (< 0.5 ng g^{-1}). Alkanes are derived from the soluble fraction of diesel (Water Accomodated Fraction - WAF), which is considered as the most toxic fraction (Lari et al., 2016; Wells et al., 1995), especially to marine invertebrates (Eickhoff, 2020). This result highlights some evidence of a detoxification process, as post-larvae contain only trace amounts of polycyclic aromatic hydrocarbons (PAHs). Also, the metamorphic success of contaminated larvae was 2-fold lower in Medium and High shipping noise than other treatments. Contrary to previous results of Veillard et al. (*in press*, see Chapter 1, Article 2) showing an increase of metamorphosis under similar levels of shipping noise emission for non-contaminated larvae.

The same authors demonstrated an increase in the energy metabolism due to a higher energy demand under similar sound exposure (> 121 dB re 1µPa) which could explain the accumulation of contaminants in those post-larvae exposed to higher sound intensities. These larvae must therefore have a higher food intake in order to cover their energy requirements (Sokolova, 2021; Sokolova & Lannig, 2008). In addition, the analyses show an important concentration of copper in the post-larvae. Copper is an essential metal in photosynthetic phytoplankton species (Coale, 1991), and this copper bioaccumulation could reflect the phytoplankton feeding by larvae and post-larvae. Contrary to Veillard et al. (*in press*, see Chapter 1, Article 2) where the post-larvae were sampled sooner (9 days following presence of pediveliger) than in the present (20 days), the energetic metabolite profile was not influenced by the exposure of shipping noise whatever the levels. We thus suggest that a longer noise exposure could stabilize the energetic status by acclimation processes and/or the general contamination phase could impact the response in the other direction compared to the previous study.

In the post-larval stage, mussels develop gills that maximize food intake (Cannuel & Beninger, 2006) and greater energy intake. Increased energy demands could induce oxidative stress through reactive oxygen species (ROS) generation and stimulate inflammation, which

may be associated with mechanisms of immunotoxicity (Young et al., 2023). In fact, oxylipins from ARA may indicate a higher oxidation of arachidonic acid to the proinflammatory mediators PGJ2 and PGD2, suggesting higher oxidative stress (Basu, 2007). In particular, post-larvae exposed to higher levels of shipping noise, showed significant oxidative stress and we suggest that pollution may weaken larvae by delaying their metamorphosis. To support this result, most of the oxylipins biosynthesized are from the linoleic acid (LA, 18:2n-6), recognized as ω-6 fatty acid and the most prominent biological fatty acid with a pro-inflammatory response (Yadav et al., 2018). An imbalance between the amounts of ω6 and ω3 fatty acids, with higher amounts of ω6 than ω3, may promote the pathogenesis of many human diseases (cardiovascular, inflammatory disease, cancer, etc.), whereas elevated levels of ω3 FA exert suppressive effects (Roland et al., 2004). At Medium and High intensity levels of shipping noise, metamorphic success was approximately half of that under low noise emission conditions. The low and high intensity levels showed an intermediate profile between the control and medium noise exposure conditions. Nevertheless, the larvae exposed to Low noise emission level showed a better metamorphic success, which means that a high sound intensity decreased the success, probably due to a higher bioaccumulation of the pollutants and, finally, the resulting oxidative stress. We suggest that low sound levels were used as a threshold here in this study, where exposure to more than 121 dB re 1µPa, the post-larvae reduced their ability to survive and recruitment success.

Anthropogenic noise and chemical pollution, associated with maritime traffic, a situation typically found in a port (Bagočius & Narščius, 2022; Galkus et al., 2012; Jupp et al., 2017; Komyakova et al., 2022), could thus increase larval selectivity and decrease survival rates to adulthood, with potential cascading effects on population dynamics and marine biodiversity (Pechenik, 2006). In this context, native populations exposed to these two sources of pollution would be less resistant than those associated with ports, limiting their recruitment dynamics in such stressful environments.

5.7 ACKNOWLEDGEMENTS

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CONCLUSION GÉNÉRALE - PERSPECTIVES

Les travaux réalisés au cours de cette thèse nous permettent de mieux comprendre les perturbations liées au bruit du trafic maritime sur le cycle de vie des bivalves marins. Ces études démontrent la vulnérabilité des larves de moules dans un environnement exposé à un trafic maritime important et soulignent la nécessité d'une réglementation. Dans cette dernière partie, j'aborderai des questions plus larges en proposant des perspectives de recherches sur la base des travaux effectués durant cette thèse.

5.8 PÉRIODES CRITIQUES PENDANT LES PREMIERS STADES DE VIE LARVAIRE *DE M. EDULIS*

Cette thèse met en évidence deux périodes critiques pendant les premiers stades de vie larvaires chez un taxon écologiquement et socio-économiquement important. Le bruit généré par un navire cargo a un impact direct sur l'embryogenèse et la métamorphose des larves de moules bleues *Mytilus edulis*. Au cours de l'embryogenèse (Chapitre 1, article 1), nous avons constaté que l'exposition au bruit des navires induit une inflammation due au stress, un déséquilibre métabolique ou un stress cellulaire résultant d'une demande énergétique accrue, conduisant à une perturbation de la glycolyse et à une augmentation de la réponse au stress oxydatif. Pendant la métamorphose (Chapitre 1, article 2), les mêmes effets sont observés, ainsi qu'un renouvellement plus important des protéines et une perturbation de l'activité du système nerveux, comme en témoignent les analyses complémentaires multivariées du métabolome.

5.8.1 L'embryogenèse

L'embryogenèse semble être une période plus sensible au bruit anthropique du trafic maritime, avec une augmentation des coûts énergétiques et du stress oxydatif pour cette

espèce. Dans un contexte de désynchronisation entre le cycle larvaire des moules et la production primaire (Toupoint et al., 2012 ; Androuin et al., 2022), lié à la théorie du « match/mismatch » (Cushing, 1990), le milieu ne serait pas en mesure d'apporter suffisamment de nourriture en fin d'embryogenèse, et les chances de survie des larves-D pourraient diminuer en raison d'un développement antérieur impacté et d'une taille commerciale réduite (Leal et al., 2018). De plus, la fragilisation en début de vie de ces larves pourrait avoir un impact majeur sur la dynamique et la résilience de la population, avec des implications potentielles pour la structure et la fonction de la communauté. Des études sur d'autres invertébrés marins ont démontré que le bruit anthropique pendant l'embryogenèse peut impacter l'aptitude individuelle (Nedelec et al., 2014 ; Ruiz-Ruiz et al., 2020). De même, dans ces études, les organismes sont endommagés par le son (Filiciotto et al., 2016; Lecchini et al., 2018; Nedelec et al., 2014; Ruiz-Ruiz et al., 2020). Dans notre étude, les larves sont affectées et perturbées par le son, mais elles n'ont pas présenté de déformation cellulaire. ***Il serait intéressant d'aller vérifier les organes sensoriels de ces larves grâce à des coupes histologiques des cils des statocystes*** (**Fig. 39**) et de l'ASO, comme l'ont réalisé Solé et al., (2018) sur les céphalopodes ou plus récemment Kellner et al. (*données non publiées*) chez les coquilles Saint-Jacques.

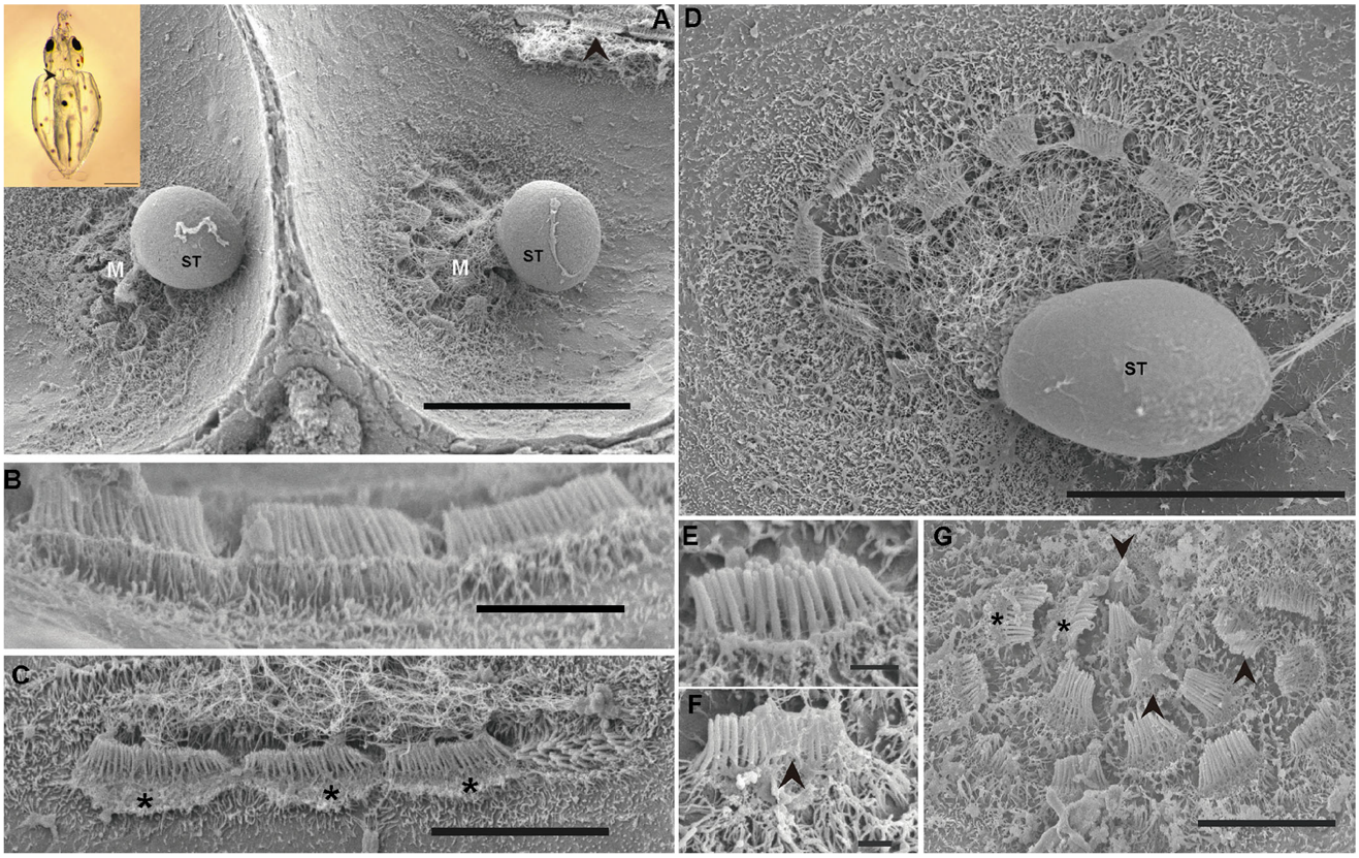

Figure 39. Morphologie d'un statocyste interne chez les œufs de céphalopode *Illex coindetii* tiré de Sole et al., (2018). La flèche indique la position du statocyste sur la position postéro-ventrale de la tête du céphalopode. Encadré en Éclosions témoins (**A, B, D, E**), euthanasiées immédiatement (**F**) et 24 h après l'exposition au son (**C, G**). Les cavités des statocystes ont été ouvertes transversalement. Chaque cavité de statocyste montre le statolithe (ST) attaché à la *macula statica princeps* (M). Les groupes kinocilliaires des cellules ciliées sont disposés en anneaux presque concentriques autour d'un centre. (**B**) Système de *crista*. Dans ce cas, seule une rangée de cellules ciliées sensorielles est visible. (**C**) Sur une éclosion exposée, la *crista* présente le pôle apical des cellules ciliées partiellement extrudé (astérisques). (**E**) Détail d'une cellule ciliée avec ici aspect sain du faisceau du groupe kinociliaire. (**F**) La cellule ciliée montre que les kinocils ont fusionné immédiatement après l'exposition au son (pointe de flèche). (**G**) Certaines cellules ciliées présentent le pôle apical extrudé de l'épithélium sensoriel (astérisques noirs).

L'embryon de moule est un stade de vie sensible aux changements de leur milieu. Afin d'obtenir une vue globale sur la réponse des larves de bivalves marins aux pressions anthropiques, il est donc nécessaire de s'approcher au plus près des conditions naturelles. Dans un contexte de changement climatique, l'augmentation de la température des eaux à

venir entraînera un développement bactérien plus important (Vezzulli et al., 2010). Par leur activité de filtration, les bivalves concentrent les bactéries, souvent de manière transitoire. Néanmoins, les infections bactériennes sont courantes chez les larves de bivalves et peuvent entraîner une mortalité importante, notamment dans les écloseries. Ces mortalités sont généralement liées aux bactéries du genre *Vibrio* et l'émergence de Vibrio pathogènes a été associée aux changements climatiques et anthropiques (Vezzulli et al., 2013). ***Je suggère qu'un stress induit par une exposition sonore durant les premiers stades de vie fragilise les bivalves et favorise le développement ultérieur de pathogènes.*** Il serait donc intéressant de définir quelles souches de *Vibrio* sont pathogènes pour la moule en étudiant la survie et la croissance des larves de moule bleue *M. edulis*, puis de valider un effet additif, voire de synergie, entre le stress sonore et l'infection. Les larves pourraient être affaiblies par une pression sonore pendant l'embryogenèse, puis soumises à un *Vibrio* pathogène. Il s'agirait alors d'analyser leur état physiologique face à ces facteurs en utilisant notre approche écophysiologique via l'analyse de leur métabolome.

5.8.2 La métamorphose

Pendant la métamorphose, les résultats montrent que les sons anthropiques augmentent les processus physiologiques associés aux taux de colonisation et de croissance, comme on a pu le constater précédemment chez d'autres espèces d'invertébrés (Stanley et al., 2014 ; Wilkens et al., 2012). Au début de cette thèse, nous suggérions que les effets « positifs » observés sur la fixation et le recrutement larvaire ne pouvaient être bénéfiques qu'à court terme. Dans le chapitre 1, nous avons montré que le bruit avait modifié la dynamique des larves de moules en stimulant leur installation ainsi qu'en altérant leur métabolome. Néanmoins, il n'était pas clair si ces altérations résultaient d'un effet direct du bruit ou d'un niveau différent de développement pendant la métamorphose, en particulier le développement plus rapide des branchies chez les moules exposées au bruit de la navigation. Le développement des branchies post-larvaires est un long processus de maturation, mais une fois développées, les branchies augmentent considérablement la capacité d'ingestion des moules par rapport au vélum utilisé chez les larves (Cannuel et al., 2009). Dans le chapitre

2, nous avons donc prolongé l'exposition sonore de 9 à 20 jours pendant la fixation et la métamorphose. Cette expérience a permis de confirmer l'hypothèse selon laquelle les émissions sonores des navires semblent stimuler la croissance post-métamorphose. Des coupes histologiques des branchies chez ces post-larves soumises aux différents traitements sonores permettraient de valider cette hypothèse. Enfin, ces résultats amènent à penser que le son pourrait être utilisé dans le processus de captage des larves et avoir un intérêt socio-économique au niveau de l'aquaculture.

5.9 PERSPECTIVES POUR L'AQUACULTURE DES BIVALVES SESSILES

La modulation sonore de la dynamique de métamorphose des larves ainsi que des processus de recrutement pourrait avoir des implications socio-économiques, en particulier pour les systèmes d'aquaculture qui dépendent du captage de naissain (Kamermans & Capelle, 2019). Notre approche innovante, qui allie le suivi métabolomique des réponses au stress des larves de *Mytilus edulis* et les études acoustiques, fournit les premières données métaboliques dose-réponse concernant l'impact du bruit de la navigation sur le développement des bivalves sessiles. Notre étude montre que dans les mésocosmes Larvosonics, qui ne constituent pas des habitats optimaux pour les larves en raison de l'absence de turbulences (pas de bullage) pour ne pas ajouter d'autres perturbations sonores (Olivier et al., 2023), le bruit de cargo a retardé la métamorphose dans ces habitats sous-optimaux, avec des coûts métaboliques plus élevés. Dans des conditions naturelles, le succès de l'installation est positivement corrélé à la vitesse des flux centrifuges et advectifs et à la turbulence (Pernet et al., 2003b). Néanmoins, dans un contexte de captage, le bruit anthropique associé au trafic maritime pourrait aider les larves de moules compétentes à sélectionner des habitats de développement appropriés et ainsi réduire les taux de mortalité. De telles méthodes pourraient avoir un impact significatif sur la durabilité de l'écosystème et de l'aquaculture. En effet, nous pourrions utiliser le bruit pour diriger les larves dans des zones propices à leur développement. Il serait néanmoins intéressant de décomposer les fréquences sonores de ces bruits de navire cargo afin d'obtenir un audiogramme précis des fréquences qui pourrait être utilisé. Grâce à notre nouvelle approche métabolomique, il serait

également possible de déterminer les impacts de celle-ci. Ces résultats pourraient par la suite être utilisés par les autorités et les gestionnaires.

5.10 NOTION ACTUELLE SUR LES RÉGLEMENTATIONS CONCERNANT LA POLLUTION SONORE

Actuellement, aucune réglementation contraignante n'exige la réduction du bruit sous-marin d'origine humaine. Face à l'intensification du trafic maritime, il est essentiel de réfléchir à la manière de gérer les impacts sonores de la navigation et de les prévenir. Comme indiqué précédemment, si aucune mesure n'est prise pour limiter le bruit sous-marin pendant les périodes d'embryogenèse, des impacts à long terme pourraient perturber la structure des populations et la biodiversité marine. La prise en compte de la pollution sonore des navires étant récente, le cadre réglementaire se met progressivement en place, tant au niveau national qu'international. Il existe des réglementations et des recommandations pour aider les professionnels de la mer à réduire la pollution sonore, mais celles-ci ont un statut juridique limité.

i) La directive-cadre « Stratégie pour le milieu marin » (DCSMM) (*https://eur-lex.europa.eu/eli/dir/2008/56/oj*) est un texte législatif européen qui vise à établir et à maintenir un « bon état écologique » (BEE) des eaux européennes d'ici à 2020. Le bruit anthropique y est défini comme l'un des indicateurs du BEE : « l'introduction d'énergie, y compris le bruit sous-marin, se fait à des niveaux qui n'ont pas d'effets néfastes sur le milieu marin ».

ii) L'Organisation maritime internationale (OMI) a également pris des mesures pour prévenir la pollution sonore (Maruf & Chang, 2023). En 2023, le Comité de protection du milieu marin (MEPC) a approuvé des directives visant à réduire le bruit sous-marin associé à la navigation, afin de remédier aux effets néfastes sur la vie marine (MEPC.1/Circ.906), notamment en ce qui concerne la conception des navires, susceptibles de minimiser le bruit introduit dans l'environnement.

iii) Aux États-Unis, le National Environmental Policy Act (NEPA) interdit depuis longtemps toute activité humaine, y compris les bruits anthropiques, susceptible de harceler les mammifères marins (Richardson et al., 1995).

La majorité de ces textes se sont focalisés sur les incidences liées aux mammifères marins, comme dans le cas de la NEPA et des réglementations de l'OMI, et n'ont pas pris en compte les effets plus complexes, tels que les réactions de stress induites par le bruit chez d'autres espèces, comme les poissons et les invertébrés. Ces effets sont pourtant bien documentés chez les poissons, notamment, et de plus en plus, chez les invertébrés (Solé et al., 2023). L'attention actuelle dans la législation et la recherche qui leur est portée est moindre par rapport à l'importance de ces espèces dans les écosystèmes (Popper et al., 2020; Thomsen & Popper, 2024).

5.11 ACTIONS DE MITIGATION

Notre étude pourrait contribuer à renforcer la réflexion des gestionnaires et favoriser la prise de décision en matière de prévention des nuisances sonores des navires. En effet, la réduction de la vitesse, par exemple, semble résoudre ou du moins atténuer de nombreuses nuisances sonores du transport maritime tels que les collisions, les émissions de gaz et la diffusion de bruit sous-marin (Sèbe et al., 2022). Une combinaison de limitations de vitesse et de mesures relatives à la conception des navires eux-mêmes serait utile avec un engagement des États, même s'ils ne sont pas réalisables à court terme en raison de la nécessité de modifier les flottes. Il est important d'adopter des mesures réglementaires le plus tôt possible afin qu'elles puissent être mises en œuvre avec succès.

Différentes actions de mitigation sur les navires cargo sont déjà en cours d'investigation pour réduire, voire éliminer le bruit sous-marin. Cela passe notamment par la modification des pales d'hélice, l'insonorisation des moteurs ou encore par l'essai de peintures antifouling insonorisantes (Cruz et al., 2022; Virto et al., 2022). Il serait également intéressant de créer des corridors de navigation afin de préserver les zones sensibles des nuisances sonores pendant certaines périodes, comme pendant les périodes de reproduction. Dans notre étude,

nous n'avons étudié qu'un son de navire-cargo représentatif d'une zone d'étude, mais il serait intéressant d'intégrer un cocktail de sons maintenant que nous avons défini l'impact d'un son unique afin de voir les effets multiples en milieu naturel, notamment en étudiant les bruits émis par les navires de plaisance et touristiques équipés de moteurs hors-bord, les motos des mers et les ferrys (Duarte et al., 2021 ; Cruz et al., 2022). L'impact des ferrys n'est pas à négliger au vu de la demande croissante de croisières en mer et de la conception de paquebots de plus en plus grands et puissants (Breton, 2021). De plus, ces navires longent très souvent les côtes et font des arrêts réguliers dans des zones côtières de plus en plus exposées.

En Europe, une proposition de modification de la directive 2005/35/CE relative à la pollution causée par les navires et à l'introduction de sanctions, notamment pénales, en cas d'infractions de pollution est en cours de négociation au sein des institutions européennes (*https://eur-lex.europa.eu/eli/dir/2008/56/oj*). Des valeurs seuils, c'est-à-dire des valeurs de référence, devront être prises en compte dans les études d'impact acoustique, notamment celles requises par la directive-cadre « Stratégie pour le milieu marin » (Directive 2008/56/EC). En ce qui concerne les seuils de réaction comportementale, il n'y a pas actuellement de consensus scientifique. Enfin, dans un contexte de changement climatique et de fonte des glaces, de nouvelles routes maritimes s'ouvrent dans l'Arctique, donnant accès à un plus grand nombre de navires dans des zones où les sons anthropiques sont moindres pour la faune et la flore locale. Des stratégies ainsi que des valeurs seuils sont à mettre en place afin de préserver ces écosystèmes fragiles.

5.12 DÉFINITION DE VALEURS SEUILS

Un nombre croissant d'études examinent les effets acoustiques du trafic maritime sur les invertébrés, mais peu d'entre elles se sont penchées sur l'interaction de plusieurs facteurs de stress dans un contexte maritime réel. Le bruit peut interagir avec les contaminants chimiques et perturber le comportement des organismes. Dans le chapitre 2, article 5, nous suggérons une valeur seuil de 121 dB re 1 µPa, au-delà de laquelle la survie et la réussite du recrutement des post-larves sont réduites après une contamination à un déversement

accidentel de diesel combiné à une exposition au bruit d'un navire cargo représentatif du trafic maritime. Dans notre cas d'étude, l'intensité sonore élevée a augmenté la demande d'énergie, ce qui a induit une bioaccumulation accrue des polluants et, par cascade, à une augmentation du stress oxydatif et finalement à une dynamique de métamorphose ralentie. Ces résultats amènent à penser que les populations naturelles exposées à ces polluants ont ainsi probablement une résilience moindre que celles associées aux ports, ce qui peut limiter leur capacité à recruter dans des conditions stressantes. Il est donc primordial d'examiner l'interaction de multiples facteurs de stress/pressions (interactions synergiques/antagonistes) pour se rapprocher d'un contexte écologique réel (Popper et al., 2020; Thomsen & Popper, 2024).

À notre connaissance, la plupart des études réalisées ont été menées dans des conditions expérimentales et peu d'entre elles ont été réalisées *in situ* (Solé et al., 2023). Combiner les approches *in situ* et expérimentale (mésocosmes) est la meilleure stratégie pour étudier l'effet de facteurs multiples dans l'environnement naturel et valider par la suite des mécanismes spécifiques observés. Notre manque actuel de données *in situ* nous limite à l'établissement de niveaux seuils de pollution sonore pour les espèces d'invertébrés. Par conséquent, il existe un besoin urgent d'expériences *in situ* pour fournir des résultats écologiquement réalistes, complétant les résultats obtenus en laboratoire (Hawkins & Popper, 2017; Merchant et al., 2022; Popper et al., 2020; Solé et al., 2023).

5.13 RÉPONSE SPÉCIFIQUE À CHAQUE ESPÈCE

Une récente étude *in situ* dans le cadre du projet AUDITIF démontre que les bruits anthropiques affectent la diversité et l'homogénéité des communautés, avec des impacts se répercutant au niveau des espèces, en inhibant ou stimulant le recrutement d'espèces tolérantes à ce stress (Byrro-Gauthier et al., *soumis*). Cette première étude sur la dynamique de recrutement des communautés benthiques a permis d'établir un niveau seuil pour les invertébrés (< 140 dB re 1µPa2.s). Ce n'est pas la première fois que des réponses contrastées sont observées chez différentes espèces d'invertébrés (Gigot et al. 2023). Il est donc

primordial d'intégrer un plus grand nombre d'espèces afin d'étudier les changements dans la composition des espèces et caractériser les effets contrastés en fonction du bruit anthropique lié au trafic maritime. Notre étude a montré que la moule bleue, une espèce résistante à de nombreux changements (stress thermique et salinité) (Thyrring et al., 2015, 2019), est impactée par le bruit anthropique ainsi que de plus en plus d'invertébrés, comme en témoignent le nombre croissant d'études dans ce domaine (Solé et al., 2023). De plus, les impacts sur les premiers stades ont des répercussions sur le développement ultérieur des organismes.

5.14 EFFET PONTE, MATERNEL ET TRANSGÉNÉRATIONNEL

Le bruit et la pollution chimique associés aux ports ont le potentiel d'affaiblir la résilience des écosystèmes locaux via des altérations du comportement animal, une réduction de la condition physique et du rendement reproductif, une augmentation de la mortalité et une érosion de la biodiversité (Cones et al., 2024; Duarte et al., 2021; Komyakova et al., 2022). Le chapitre 2 présente des effets latents de l'exposition chimique précoce des larves, avec des conséquences sur la taille à la métamorphose, le métabolisme énergétique et oxydatif et le profil lipidique des post-larves. En effet, l'exposition à de multiples facteurs de stress chez les jeunes stades peut avoir des effets latents plus tard chez les jeunes juvéniles (Lagarde et al., 2018; Martel et al., 2014).

La récente étude de Gigot et al. (2024) montre un effet transgénérationnel du bruit anthropogénique chez la coquille Saint-Jacques *Pecten maximus*. L'exposition des parents aurait modifié la sensibilité au son de la descendance. Les adultes montrent une stratégie maternelle qui prioriserait la qualité plus que la quantité des œufs dans un environnement stressant (Parker et al., 2012). Les larves produites présentent également une stratégie adoptée par les adultes pour augmenter les chances de survie dans des conditions stressantes avec notamment un taux de croissance plus rapide (Gigot et al., 2024). Néanmoins, il semblerait que l'exposition parentale du son ne confère un avantage aux larves que lorsque celles-ci sont soumises à un son anthropique. En effet, l'acquisition d'une résistance n'est

bénéfique qu'en présence du stress et un coût (désavantage) en absence de ce stress dû à un niveau d'énergie réparti dans les mécanismes de résistance de l'organisme et encore plus dans les fonctions basales (Levinton et al., 2003).

La plasticité transgénérationnelle correspond à l'effet de l'environnement vécu par les parents affectant les traits phénotypiques de la progéniture (Munday et al., 2013). Elle peut être définie comme une transmission parentale de facteurs autres que les séquences d'ADN agissant sur le phénotype de la progéniture. Cette transmission se produit par un transfert maternel et/ou paternel non génétique de constituants cytoplasmiques, tel que les nutriments et les hormones. Elle comprend également des modifications épigénétiques transmissibles d'une génération à une autre et qui pourront influencer la façon dont les gènes sont exprimés chez la progéniture (Penney et al., 2021).

Dans notre étude, les effets de la pollution chimique précoce pendant l'embryogenèse ont un impact plus important sur les larves qu'une exposition au bruit plus tardive pendant la métamorphose. Notre étude suggère que les populations naturelles exposées à ces deux sources de pollution seraient moins résistantes que celles associées aux ports. En conséquence, leur dynamique de recrutement serait limitée dans ces environnements stressants. Cette potentielle pression sélective sur les embryons d'une contamination chimique mimant les niveaux mesurés dans un port maritime industriel ne semble pas surprenante. Il est maintenant reconnu que cet environnement anthropique peut conduire à l'émergence de nouvelles lignées adaptées aux conditions portuaires par des mécanismes d'adaptation locale et isolement intrinsèque (Touchard et al., 2023). Ainsi, les prochaines recherches devraient s'attacher à comprendre si l'interaction ou l'association de ces facteurs pourrait générer des effets latents altérant le recrutement ou si les effets se contrebalancent et diminuent à des stades ultérieurs (Podolsky & Moran, 2006; Sal Moyano et al., 2024).

Les facteurs de stress combinés poussent les organismes vers leurs seuils de tolérance (Barrett et al., 2022) et le degré de tolérance de ces stress chez les espèces de *Mytilus* reste encore inconnu. Cependant, il est possible de prévoir les zones à risque, c'est-à-dire les zones avec une forte affluence du trafic maritime, via des analyses géographiques et la création de

cartes permettant de visualiser les corridors de circulation. En mettant en corrélation ces informations, nous pourrons visualiser les populations les plus exposées, comme réalisées dans le projet ATLAS (https://soundscape-atlas.uqar.ca) (**Fig. 40**). Puis, en comparant ces populations avec celles dites « *pristine* », nous pourrons identifier des marqueurs moléculaires et épigénétiques liés à ces stress. L'acclimatation et la prévisibilité du stress sont des facteurs clés de la résilience cellulaire (Clark et al., 2021; Thyrring et al., 2019).

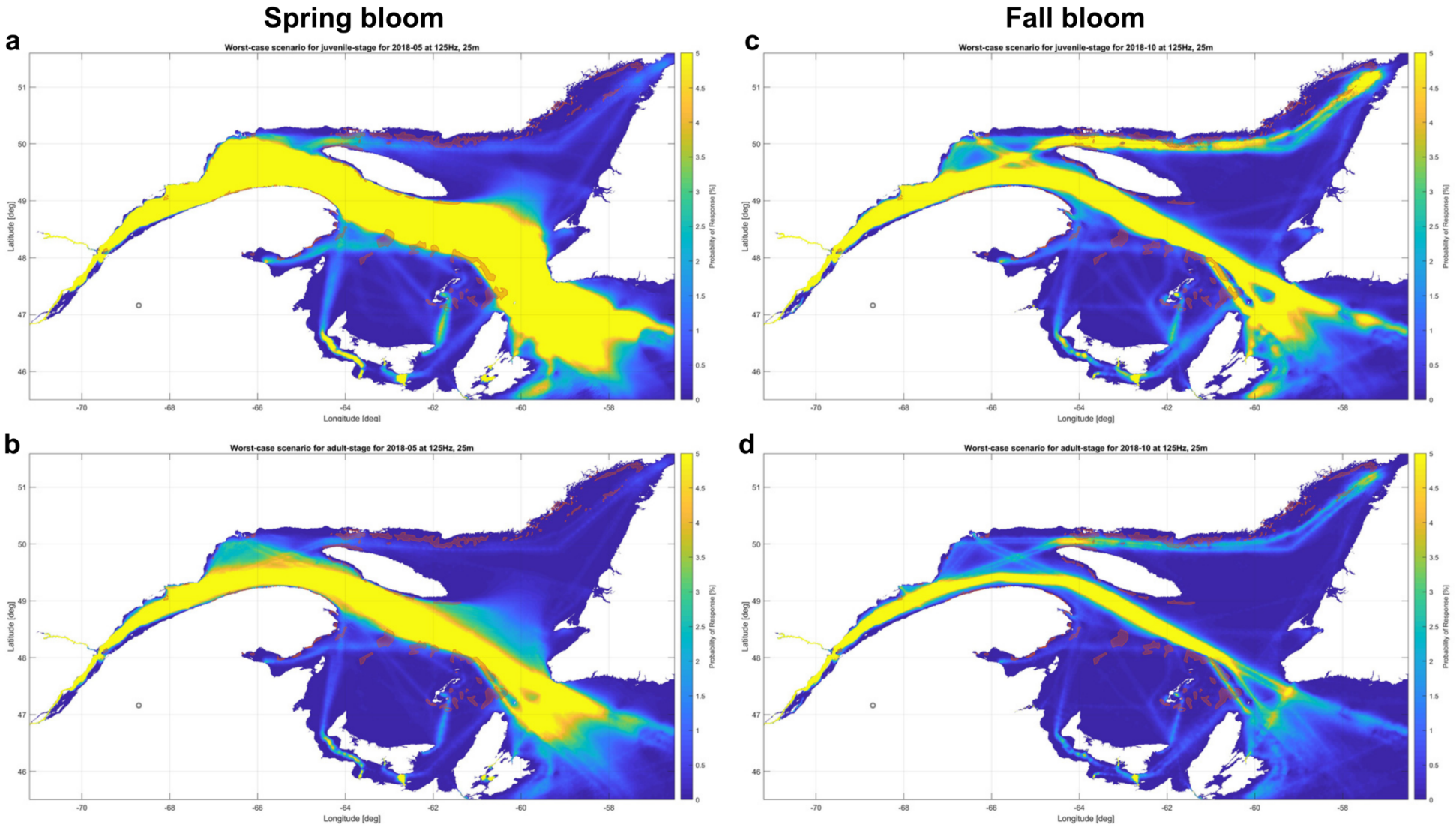

Figure 40. Exemple de carte réalisée dans le cadre du projet AUDITIF représentant le pourcentage du temps (échelle de couleur à droite) où le bruit de navigation dépasse le seuil sonore entraînant des réponses valvaires chez des juvéniles (**a, c**) et des adultes (**b, d**) de *Placopecten magellanicus* à 25m de profondeur pour les efflorescences printanières et automnales dans le Golfe du Saint-Laurent. (Poitevin et al., *en soumission*).

ANNEXES

ANNEXE I - CONFIGURATION DES BASSINS EXPÉRIMENTAUX – SYSTÈME *LARVOSONIC*

Les expérimentations s'appuient sur le système *Larvosonic*, conçu et testé en France en janvier 2019, financé par le programme de recherche IMPAIC, et reproduit à la Station Aquicole de l'ISMER/UQAR. Ces mésocosmes permettent d'évaluer l'influence du bruit sur les stades larvaires ou sur les adultes de manière individuelle, minimisant ainsi tout biais lié à la réverbération des ondes sonores contre les parois des réservoirs et à la résonance de la cuve. En effet, ces mésocosmes de 800L (**Fig. 41** ; dimensions externes : longueur x largeur x hauteur = 1360 x 935 x 680 mm, 10 mm d'épaisseur) comprennent des panneaux de diffusion acoustique en polystyrène expansé (Vicoustic © ; http://www.vicoustic.com) fixés sur des plaques de plexiglas supplémentaires installées sur les parois internes. Le réservoir principal est rempli complètement de sorte que le couvercle scellé soit en contact direct avec l'eau de mer afin d'éviter toute interface air-eau (à l'exception de quelques microbulles) qui est connue pour altérer la propagation du son (Rogers et al., 2016 ; Olivier et al., 2023). Lors des expériences, les bassins sont entièrement remplis d'eau de sorte que le couvercle est directement en contact avec l'eau de mer pour éviter toute interface air-eau connue pour altérer la propagation du son (Rogers et al., 2016). Au centre de ce mésocosme se trouve une plaque amovible sur laquelle est fixé un haut-parleur sous-marin, Clark Synthesis AQ339 Diluvio™ (https://clarksynthesis.com/aq339/). Un processeur matriciel numérique Yamaha MTX3, couplé à un amplificateur Powersoft Otto 1204 DSP à 8 canaux (8 x 150 W) est utilisé pour permettre des émissions sonores simultanées de différents types et niveaux par unité Larvosonic. Les fichiers audios sont lus via un Denon CD/Media Player DN300Z (https://www.denonpro.com/index.php/products/view3/dn-300z) et les réglages sont effectués via un processeur matriciel MTX-MRX Editor V4.0.0 (https://usa.yamaha.com/products/proaudio/processors/mtx/downloads.html#product-tabs).

Quatre bassins sont disponibles pour le projet, chacun d'entre eux comprenant 6 cylindres circulaires en plexiglas (dimensions internes : diamètre = 192 mm, hauteur totale = 382 mm) de 5L où les organismes sont exposés à différentes conditions expliquées dans

les designs expérimentaux pour diverses combinaisons de tests, tels que les interactions entre le bruit et les polluants. Les cylindres sont remplis à moitié (hauteur d'eau = 175 mm) avec de l'eau de mer filtrée ainsi d'un mélange de nourriture. Les larves seront nourries avec un mélange de microalgues de *Pavlova lutheri, Tisochrysis lutea, Chaetoceros muelleri, Tetraselmis suecica, Nannochloropsis oculata* à une concentration cellulaire de 60 000 cellules/mL. Tous les bassins seront échantillonnés (croissance et survie) tous les 2 jours dans le cadre des procédures de routine pour vérifier que les larves se développent sainement. Un grand nombre de larves est nécessaire pour effectuer les analyses métabolomiques et lipidiques. Le volume total dans le cylindre de 5 L nous permet de maintenir 40 000 individus au stade véligère et 10 000 au stade post-larvaire (concentration larvaire < 1 larve mL-1 pour éviter tout effet dépendant de la densité) au cours de l'expérience. La température, la salinité, la photopériode et l'intensité lumineuse sont également contrôlées pendant les expériences.

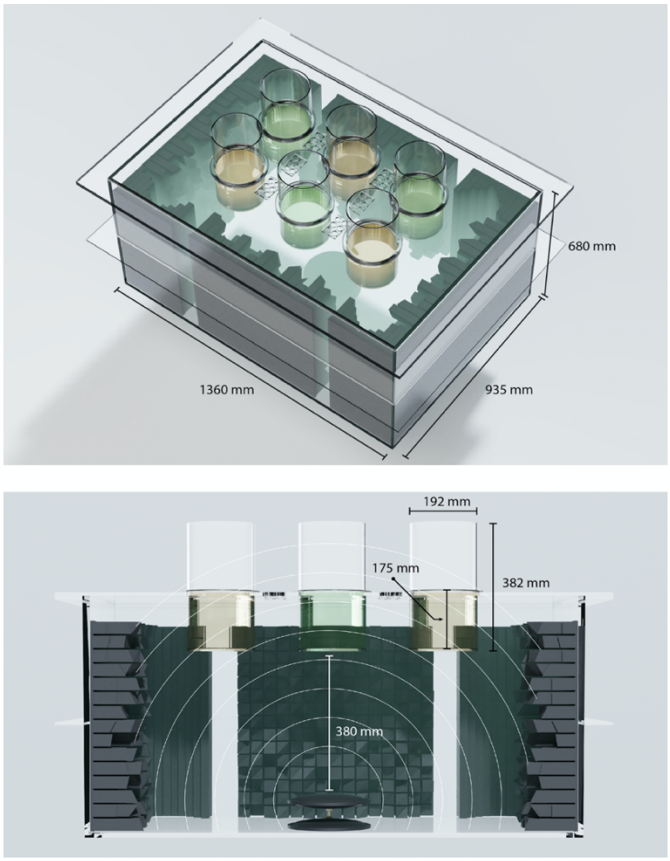

Figure 41. Schéma du système *Larvosonic* (Olivier et al. 2023).

ANNEXE II - ÉCHANTILLONNAGE DE LA SÉQUENCE SONORE DU BRUIT DE NAVIRE-CARGO

Des enregistrements sonores ont été réalisés dans le port de St-Pierre-et-Miquelon (France) de novembre 2020 à avril 2021 à l'aide d'un hydrophone (Multi-Electronique Inc., Rimouski, QC, Canada) enregistreur acoustique sous-marin (fréquence d'échantillonnage de 32 kHz, résolution de 16 bits) équipé d'un hydrophone HTI-96-MIN (High Tech Inc., Long Beach, MS, USA) d'une sensibilité de -165 dB re V/µPa et réglé avec un gain de 22 dB. Les enregistrements ont montré que le bruit d'un cargo roulier de 120 m de long, construit en 2000 (Nohlan Ava), était le navire qui laissait une empreinte sonore la plus intense et la plus distincte dans le port de St-Pierre-et-Miquelon (France). La séquence sonore a été sélectionnée et traitée avec le logiciel Audacity® pour créer une bande sonore de 80 min composée du son de l'arrivée du cargo (11 min), d'une pause de 39 min (simulant la manutention au port), de son départ (8,5 min) et d'une nouvelle pause de 21,5 min. Cette bande sonore (10.5281/zenodo.10910123) a été répétée en boucle tout au long des expériences. La densité spectrale de puissance (DSP) et les spectrogrammes du son original du cargo enregistré in situ, ainsi que la DSP dans le cylindre de culture, sont présentés dans l'article 2 du Chapitre 1. Les niveaux sonores équivalents, exprimés en niveau de pression acoustique RMS.

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