UNIVERSITÉ DU QUÉBEC À MONTRÉAL

SENSIBILITÉ DES POPULATIONS DE CREVETTE NORDIQUE AUX CHANGEMENTS GLOBAUX LE LONG DE LA CÔTE EST DU CANADA

THÈSE

PRÉSENTÉE

COMME EXIGENCE PARTIELLE

DU DOCTORAT EN BIOLOGIE

PAR

© ELLA GUSCELLI

JUIN 2023

REMERCIEMENTS

Pour commencer, je tiens à remercier mes directeurs Piero Calosi ainsi que Denis Chabot et ma directrice Fanny Noisette pour m'avoir fait confiance en me proposant de travailler sur un si beau projet qui m'a permis de découvrir des aspects de la science qui m'ont passionné au cours de ces cinq dernières années. Merci pour votre encadrement qui m'a permis de me développer scientifiquement, de me dépasser et de gagner encore plus en autonomie, tout en apprenant de vos expertises complémentaires. Fanny et Denis, je vous remercie très sincèrement pour votre écoute et soutien sans faille, pour vos exemples de leadership et de passions respectives. Merci d'avoir mené mon regard sur mes accomplissements et de m'avoir motivé lors des moments les moins faciles de ce doctorat, je vous en suis très reconnaissante.

Ensuite, je tiens à remercier Alison Derry et Fabrice Pernet pour avoir accepté de constituer mon jury pour cette dernière évaluation. Merci pour les commentaires bienveillants que vous avez adressez à mon travail et pour la belle discussion eue lors de ma soutenance. Si jamais le syndrome de l'imposteur se présente à nouveau je n'aurais qu'à lire vos rapports pour regagner confiance en moi. Je tiens également à remercier Pierre Pepin, Geneviève Parent, Diana Madeira, Souhir Marsit, Réjean Tremblay et François Vézina pour leurs évaluations constructives au cours de différentes étapes doctorales.

Merci à mes co-auteurs pour leurs commentaires qui m'ont permis de toujours m'améliorer, je tiens à remercier en particulier Pierre Blier et Diana Madeira qui ont été des collaborateurs en or. Merci d'avoir été mes guides dans l'univers cellulaire qui s'est ouvert à moi pendant ces études, j'avoue que je m'y serais perdue sans vous ! Diana, thank you for our time in Aveiro, which made the difference.

Merci à toutes les personnes qui ont fait partie des différentes équipes de travail et analyses, à l'IML et à l'UQAR : Jérôme, David, Véronique, mais surtout Tanya qui a partagé avec moi les moments qui m'ont créé, malgré les difficultés vécues, les plus beaux souvenirs de ce projet doctoral : les expériences à la salle des bassins.

De nombreuses personnes ont fait partie de ma « vie de laboratoire » au cours des dernières années et m'ont soutenue en m'offrant des bons conseils. Je tiens à les remercier toutes mais une mention spéciale va à Mathilde, Gloria, Tessa et Fanny. Merci Mathilde d'avoir partagé avec moi les débuts de cette aventure, j'aurais aimé collaborer avec toi plus longtemps. Gloria, merci de chercher toujours le côté positif là où c'est dur pour moi de le voir. Tessa, your visit was short but intense and led to a strong friendship, thank you for our crazy moments and our « Friday pizza » as well as for your support from close and far ! Fanny, merci d'avoir été à mon côté dès le début, de m'avoir écouté quand j'avais besoin de ventiler et d'avoir célébré nos succès parce qu' « on est capable ! ». Je peux t'assurer aujourd'hui que le tunnel n'est pas infini et que la lumière à la fin est hyper brillante.

Dans le cas de cette aventure doctorale « le meilleur pour la fin » prend tout son sens : merci Sarah d'être une amie si spéciale et d'avoir fait la différence pour cette dernière ligne droite de ce marathon appelé doctorat ! J'espère de tout mon cœur que notre amitié perdure dans le temps malgré la distance physique.

Merci à Léa et Claudia, mes grandes amies de Rimouski et Florence, pour votre amitié et joie de vivre contagieuse. J'aimerais vous avoir du même côté de l'océan.

Finalement, je souhaite remercier ma famille et celle de Simone pour les encouragements constants et le support durant cette aventure « à l'autre bout du monde ». Merci maman de m'inspirer au quotidien, pour tes bons conseils et tes colis de l'Italie qui me faisaient sentir plus proche de toi que je ne l'étais.

Un dernier mot pour Simone, mon co-pilote dans ce beau voyage qui est la vie. Merci d'avoir vécu cette aventure Rimouskoise à mes côtés. Ton soutien et ta patience incroyables ont fait toute la différence. Simplement, merci pour tout.

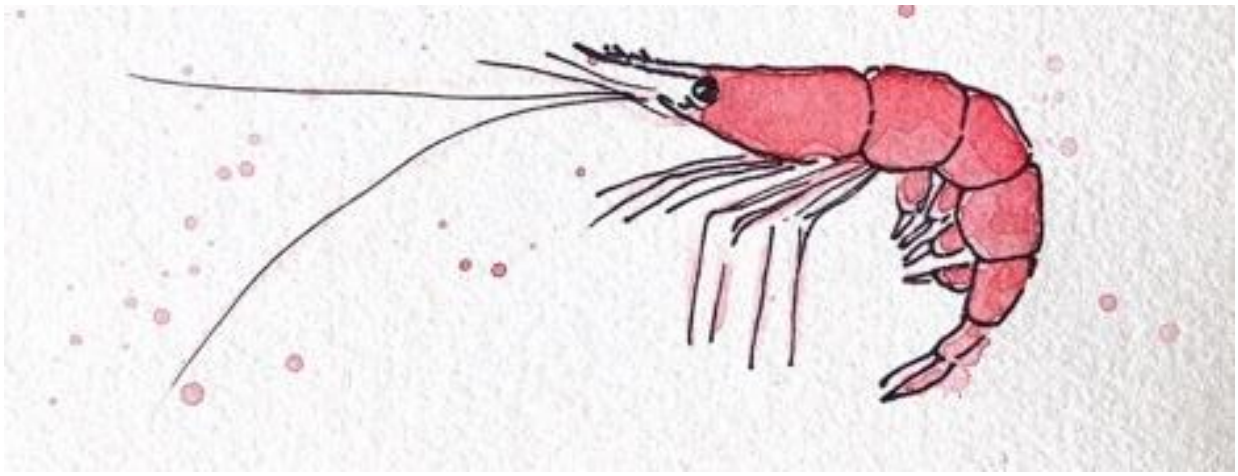
DÉDICACE

A Simone, che rende la mia vita speciale.

A mia madre, fonte di forza e ispirazione.

A mio fratello, che mi ha insegnato il significato di « resilienza ».

A me stessa, per essere arrivata fin qui.



© Ella Guscelli

AVANT-PROPOS

Ce projet de doctorat en biologie a été réalisé à l'Université du Québec à Rimouski (UQAR) et à l'Institut Maurice Lamontagne, Ministère des Pêches et Océans (IML-MPO), et s'intitule « Sensibilité des populations de crevette nordique aux changements globaux le long de la côte est du Canada ». Il visait à estimer la sensibilité de la crevette nordique, *Pandalus borealis*, une espèce d'importance écologique et commerciale au Canada, aux changements globaux cumulés à travers l'utilisation complémentaire de trois approches : multifactorielle, intégrative et macrophysiologique. Ce doctorat as été realisé sous la co-supervision du professeur Piero Calosi (UQAR), de la professeure Fanny Noisette (Institut des Sciences de la Mer, UQAR) et du chercheur Denis Chabot (IML-MPO), en collaboration avec le professeur Pierre Blier (UQAR) et la chercheuse Diana Madeira (ECOMARE-CESAM).

Ce projet de doctorat s'inscrit dans un projet de recherche interdisciplinaire financé par Ouranos et intitulé « Vulnérabilité des populations de crevette nordique aux changements climatiques et globaux le long de la côte est du Canada - de la ressource naturelle aux communautés côtières », mené dans le but de sensibiliser les principaux acteurs de la pêcherie aux défis apportés par les changements globaux. Les résultats de ce projet ont été publiés sous forme de rapport scientifique.

Le volet expérimental de ce projet était une combinaison de deux projets du MPO: le projet « Linking physiology to biogeography of Northern Shrimp to facilitate adaptation to climate change » financé par le programme stratégique de recherche et d'avis fondés sur l'écosystème (PSRAFE) du MPO, et le projet « Impact of warming on the metabolic rate, hypoxia tolerance and survival of commercial species in the Estuary and Gulf of St. Lawrence » financé par le programme des services d'adaptation aux changements climatiques en milieu aquatique (PSACCMA) du MPO.

La thèse présentée se compose d'une introduction générale rédigée en français, de deux chapitres écrits en anglais sous forme d'articles scientifiques, d'un troisième chapitre composé d'un article scentifique et d'analyses supplementaires rédigés en anglais et français respectivement, et d'une conclusion générale rédigée en français. Les trois articles scientifiques sont en cours de révison dans les journaux : *Conservation Physiology*, *Journal of Experimental Biology* et *Frontiers in*

Marine Science. Les diffèrents travaux de cette thèse on été présentés sous differents types de communications et discutés avec différents acteurs et actrices (Figure 1 et sections suivantes). Les données récoltées en expérience et en laboratoire seront publiées et disponibles sur la plateforme publique PANGEA. Le travail de recherche à l'origine de cette thèse a également permis l'établissement de nombreuses collaborations dont les résultats ont été présentés sous forme d'affiches, de présentations orale et d'articles scientifiques (Figure 1 et sections suivantes). Finalement, ce projet doctoral m'a permis d'enrichir mon portfolio de compétences et expériences (Figure 1).

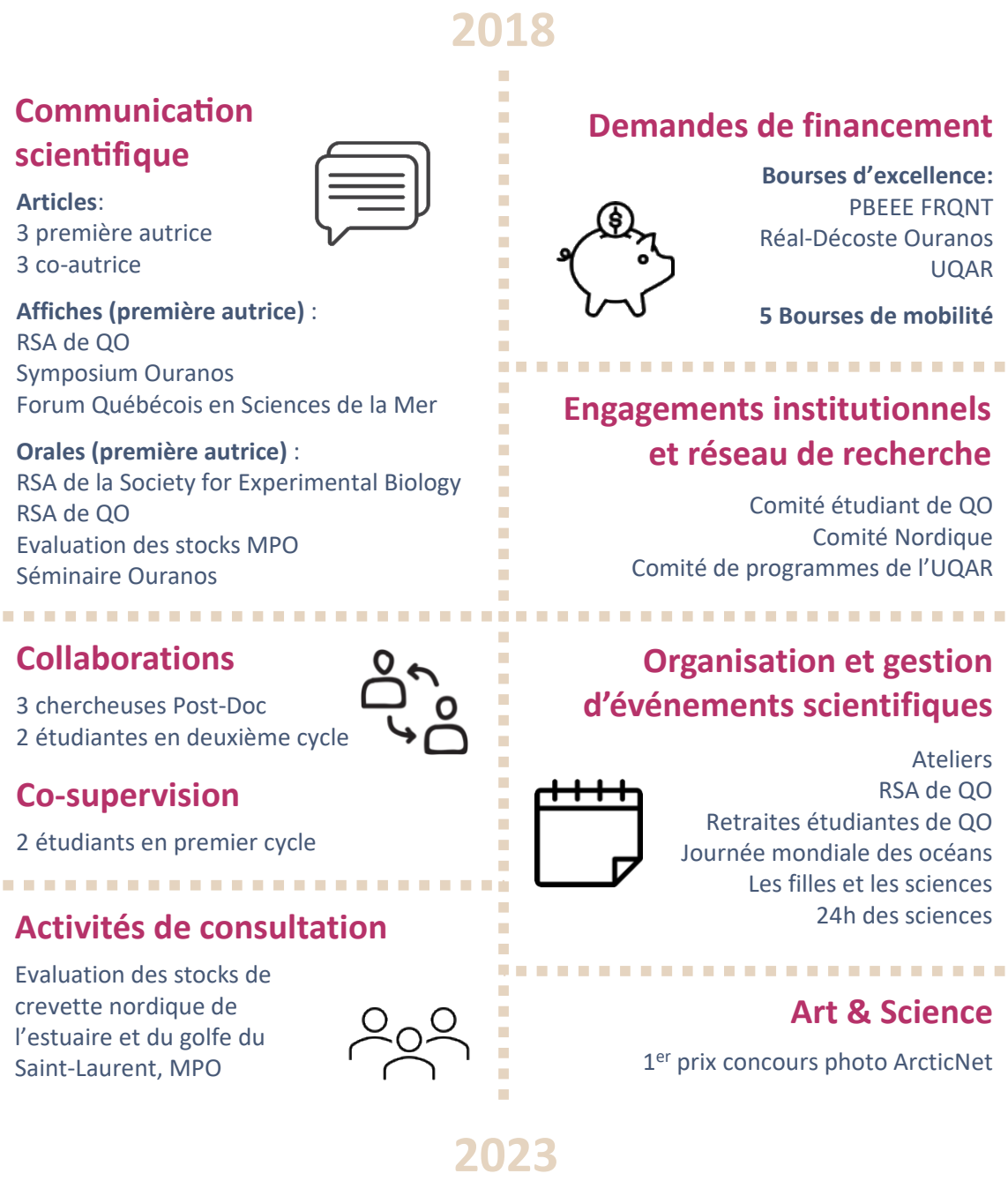


Figure 1 Représentation du portfolio de compétences et expériences acquises au cours de ce projet doctoral. Acronymes : FRQNT (Fonds de recherche du Québec – Nature et Technologies), MPO (Ministère des Pêches et Océans), PBEEE (Bourse d'excellence pour étudiants étrangers), QO (Québec Océan), RSA (Réunion Scientifique Annuelle), UQAR (Université du Québec à Rimouski).

Publications:

Guscelli, E., Chabot, D., Noisette, F., Blier, P., Chemel, M. & Calosi, P. Survival and aerobic capacity of the northern shrimp are threatened by exposure to combined ocean global change drivers. En révision chez *Conservation Physiology*.

Guscelli, E., Noisette, F., Chabot, D., Blier, P., Hansen, T., Cassista-Da Ros, M., Pepin, P., Skanes, K. & Calosi, P. Northern shrimp from multiple origins show similar sensitivity to global change drivers, but different cellular energetic capacity. En révision chez *Journal of Experimental Biology*.

Guscelli, E., Chabot, D., Vermandele, F., Madeira, D. & Calosi, P. All roads lead to Rome : Metabolomic reprogramming of the northern shrimp from different origins exposed to combined warming and acidification. En révision chez *Frontiers in Marine Science*.

Barria, A., Tai, T., **Guscelli, E.**, Cheung, W. & Calosi. P. Projecting the distribution of the Northern shrimp under ocean warming and acidification: integrating metabolic rates and survival from multiple populations. En révision chez *Scientific Reports*.

Leung, C., **Guscelli, E.**, Chabot, D., Bourret, A., Calosi, P. & Parent, G. (2023). The lack of genetic variation underlying thermal transcriptomic plasticity suggests limited adaptability of the northern shrimp, *Pandalus borealis*. Front. Ecol. Evol. 11, 166. https://doi.org/10.3389/fevo.2023.1125134

Chemel, M., Noisette, F., Chabot, D., **Guscelli, E.**, Leclerc, L. & Calosi, P. (2020). Good News — Bad News: Combined Ocean Change Drivers Decrease Survival but Have No Negative Impact on Nutritional Value and Organoleptic Quality of the Northern Shrimp. Front. Mar. Sci. 7, 1–13. https://doi.org/10.3389/fmars.2020.00611

Noisette, F., Alberio. M., Calosi, P., Barria, A., Boissonneault, M., Chemel, M., Grech, T., **Guscelli, E.** & Soubirou, M. (2021) Vulnérabilité des populations de crevette nordique (*Pandalus borealis*) aux changements climatiques et globaux le long de la côte Est du Canada : de la ressource naturelle aux communautés côtières, rapport final du projet # 550027, Ouranos, 83 pp

Communications orales :

Guscelli E, Chabot D, Noisette F, Blier P & Calosi P (2022). Does geographic origin matter? Whole-organism and cellular responses of the Northern shrimp to ocean warming and acidification. Réunion Scientifique Annuelle de la Society for Experimental Biology (5 – 8 juillet 2022, Montpellier, France)

Guscelli E, Chabot D, Noisette F & Calosi P (2022). Survie et performance physiologique de la crevette nordique dans le contexte de changements globaux combinés futurs. Réunion Scientifique Annuelle du regroupement stratégique Québec Océan (31 janvier – 3 février 2022, Rimouski, QC, Canada)

Guscelli E, Chabot D, Noisette F & Calosi P (2022). Réponses biologiques de la crevette nordique de l'estuaire du Saint-Laurent aux changements globaux cumulés. Evaluation des stocks de crevette nordique de l'estuaire et du golfe du Saint-Laurent, Ministère Pêche et Océans Canada (27 – 28 janvier 2022, Rimouski, QC, Canada)

Guscelli E (2020). Sensibilité des populations de crevette nordique aux changements globaux le long de la côte est du Canada. Séminaire Ouranos (7 Octobre 2020, Rimouski, QC, Canada)

Barria A, Boissonneault M, **Guscelli E**, Soubirou M, Chemel M, Alberio M, Brêthes J-C, Calosi P, Chabot D, Cheung W, Guimond L, Noisette F & Tai T (2020) Défis et adaptations des pêcheries autochtones aux effets des changements climatiques sur la crevette nordique dans l'Est du Québec. Forum sur les pêches mi'gmaques et malécites (13 – 14 Janvier 2020, Rivière-du-Loup, QC, Canada)

Alberio M, **Guscelli E**, Soubirou M, Barria A, Boissoneault M, Brêthes J-C, Calosi P, Chabot C, Chemel M, Cheung W, Guimond L, Noisette F, Small D & Tai T (2019) Défis et adaptations des pêcheries autochtones face aux effets des changements globaux sur la crevette nordique. Forum Québécois en Sciences de la Mer (11 – 13 Novembre 2019, Rimouski, QC, Canada)

Calosi P, Noisette F, Barria A, Boissonneault M, Chabot D, Chemel M, Cheung W, Gurney-Smith H, **Guscelli E**, Hansen T & Tai T (2019). Conséquences biologiques de l'acidification des océans dans l'estuaire et le golfe du Saint-Laurent. Forum Québécois en Sciences de la Mer (11 – 13 Novembre 2019, Rimouski, QC, Canada)

Noisette F, Alberio M, Brêthes JC, Calosi P, Chabot C, Chemel M, Dancette R, Guimond L, **Guscelli E** & Soubirou M (2018) Impacts des changements globaux sur la pêcherie de crevette nordique : de la ressource naturelle aux communautés côtières. Réunion annuelle du regroupement interinstitutionnel Ressources Aquatiques Québec (7 Novembre 2018, Québec, QC, Canada)

<u>Communications par affiche :</u>

Guscelli E, Chabot D, Noisette F, Blier P, Madeira D & Calosi P (2023). Sensibilité intraspécifique de la crevette nordique face au « trio mortel » dans l'Atlantique nord-ouest. Réunion Scientifique Annuelle du regroupement stratégique Québec Océan (6 février – 7 février 2023, Rivière-du-Loup, QC, Canada)

Guscelli E, Noisette F, Chabot D, Blier P & Calosi P (2022). Changements globaux : Qu'adviendra-t-il des crevettes nordiques ? Symposium Ouranos (1-2 décembre, Québec, QC, Canada) – Présentation d'une affiche scientifique

Guscelli E, Noisette F, Chabot D & Calosi P (2019) Face-à-face entre la crevette nordique et les changements globaux cumulés. Forum Québécois en Sciences de la Mer (11 – 13 Novembre 2019, Rimouski, QC, Canada) – Présentation d'une affiche scientifique

Guscelli E, Calosi P, Noisette F & Chabot D (2018) Sensibilité intraspécifique face aux

changements globaux : Le cas de la crevette nordique. Réunion Scientifique Annuelle du regroupement stratégique Québec Océan (5 – 6 Novembre 2018, Rivière-du-Loup, QC, Canada)

Barria A, Tai T, **Guscelli E**, Cheung W & Calosi P (2020). Integrating local adaptation and ontogenic shifts to improve projections for the distribution of the Northern shrimp *Pandalus borealis* along the Atlantic coast of Canada. Réunion Scientifique Annuelle du regroupement stratégique Québec Océan (9 – 10 Mars 2020, Château Mont-Sainte-Anne, QC, Canada)

Boissonneault M, **Guscelli E**, Madeira D & Calosi P (2019) Plasticité protéomique et cellulaire face aux changements globaux dans le contexte de l'estuaire du Saint-Laurent. Forum Québécois en Sciences de la Mer (11 – 13 Novembre 2019, Rimouski, QC, Canada)

TABLE DES MATIÈRES

LISTE DES FIGURES

Introduction

Chapitre 1

Chapitre 3

Conclusion

LISTE DES TABLEAUX

Introduction

Chapitre 1

Chapitre 2

Chapitre 3

Conclusion

LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

ACP	Analyse en Composantes Principales
ADP	Adenosine Diphosphate
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AS	Aerobic Scope
ATP	Adenosine Triphosphate
BSR	Biomasse du Stock Reproducteur
CESAM	Centre for Environmental and Marine Studies
CI	Confidence Interval
CO_2	Carbon dioxide / Dioxyde de carbone
CO_3^{2-}	Carbonate ions / Ions carbonate
COX	Cytochrome C Oxydase
CS	Citrate Synthase
CV	Coefficient de Variation
DEB	Dynamic Energy Budget / Dynamique du Budget Énergétique
DIC	Dissolved Inorganic Carbon
DO	Dissolved Oxygen
DRO	Dérivés Réactifs de l'Oxygène
EC	Esquiman Channel / Chenal Esquiman
ECOMARE	Laboratory for Innovation and Sustainability of Marine Biological Resources
EGSL	Estuary and Gulf of the St. Lawrence / Estuaire et Golfe du Saint-Laurent
ESS	Eastern Scotian Shelf / Est du plateau Néo-Écossais

ETS	Electron Transport System / Système de Transport des Électrons
$FADH_2$	Flavin adenine dinucleotide
FRQNT	Fonds de recherche du Québec – Nature et Technologies
GES	Gas à Effet de Serre
GIEC	Groupe Intergouvernemental sur l'Évolution du Climat
GSL	Gulf of St. Lawrence / Golfe du Saint-Laurent
H^+	Ions hydrogène
H_2CO_3	Acide carbonique
HCO_3^-	Bicarbonate ions / Ions bicarbonate
IC	Intervalle de Confiance
IML	Institut Maurice Lamontagne
ISMER	Institut des Sciences de la Mer
IPCC	Intergovernmental Panel on Climate Change
LDH	Lactate Dehydrogenase / Lactate Déshydrogénase
LOL	Limiting Oxygen Level
MLI	Maurice Lamontagne Institute
MMR	Maximum Metabolic Rate
$\dot{M}_{O_2}$	oxygen uptake / consommation d'oxygène
MPO	Ministère des Pêches et Océans du Canada
N_2	Nitrogen / Azote
NADH	Nicotinamide adenine dinucleotide
nGSL	nord du Golfe du Saint-Laurent
NNC	Northeast Newfoundland Coast / Nord-est de la côte de Terre-Neuve
O_2	Oxygen / Oxygène

$O_{2\ crit}$	Seuil d'oxygène critique
OA	Ocean Acidification
OCLTT	Oxygen and Capacity Limited Thermal Tolerance
OD	Oxygène Dissous
OW	Ocean Warming
PCA	Principal Component Analysis
***p*CO_2**	Carbon dioxide partial pressure / Pression partielle de dioxyde de carbone
PBEEE	Bourse d'Excellence pour Étudiants Étrangers
PERMANOVA	Permutational multivariate analysis of variance
Q_{10}	Temperature coefficient
QO	Québec Océan
RA	Registre Aérobie
RCP	Representative Concentration Pathway
ROS	Reactive Oxygen Species
RSA	Réunion Scientifique Annuelle
SD	Standard Deviation
SE	Standard Error
SLE	St. Lawrence Estuary / Estuaire du Saint-Laurent
SMR	Standard Metabolic Rate
TA	Total Alcalinity
TCA	Tricarboxylic Acid Cycle / Cycle de l'Acide Tricarboxylique
TMM	Taux Métabolique Maximum
TMS	Taux Métabolique Standard
UQAR	Université du Québec à Rimouski

MW	Wet Mass
WW	Window Width
ZPC	Zone de Pêche à la Crevette
2C	Low temperature and current pH treatment (2C: 2 °C, pH 7.75, normoxia)
2A	Low temperature and low pH treatment (2A: 2 °C, pH 7.40, normoxia)
2CH	Low temperature, current pH and low DO treatment (2CH: 2 °C, pH 7.75, hypoxia)
6C	Intermediate temperature and current pH treatment (6C: 6 °C, pH 7.75, normoxia)
6A	Intermediate temperature and low pH treatment (6A: 6 °C, pH 7.40, normoxia)
10C	Elevated temperature and current pH treatment (10C: 10 °C, pH 7.75, normoxia)
10A	Elevated temperature and low pH treatment (10A: 10 °C, pH 7.40, normoxia)
10AH	Elevated temperature, low pH and low DO treatment (10AH: 10 °C, pH 7.40, hypoxia)

GLOSSAIRE

Acclimatation : en français ce terme est utilisé indifféremment pour indiquer l'ajustement phénotypique de l'individu suite à la manipulation expérimentale d'un ou plusieurs paramètres environnementaux (en anglais *Acclimation*) et l'ajustement phénotypique de l'individu à la variation naturelle de multiples paramètres environnementaux *in situ* (en anglais *Acclimatization*).

Adaptation : sélection naturelle des génotypes à plus grande valeur reproductive d'une espèce ou d'une population suite à la variation naturelle de l'environnement.

Changements globaux : changements du climat attribuables à l'activité humaine et qui s'ajoutent à la variabilité naturelle du climat observée au cours de périodes comparables (Nations Unies, 1992).

Macrophysiologie : étude de la variation des traits physiologiques sur de grandes échelles géographiques, temporelles et phylogénétiques (Chown & Gaston, 2008).

***Optimum* environnemental** : conditions environnementales les plus favorables pour les organismes.

Performance : ensemble des capacités fonctionnelles exécutées d'un organisme.

Plasticité phénotypique : capacité d'un génotype à produire plusieurs phénotypes selon l'environnement expérimenté (West-Eberhard, 1989).

Potentiel adaptatif : capacité des espèces et des populations à s'adapter en réponse à des nouvelles conditions environnementales.

Registre aérobie : capacité du système cardiorespiratoire à fournir de l'oxygène pour toutes les activités au-dessus du maintien de base (Fry, 1947).

Résilience : capacité d'un organisme à revenir à un état initial après une perturbation.

Sensibilité : capacité d'un organisme à réagir, en termes de réponses physiologiques, aux variations environnementales.

Taux métabolique maximal : capacité maximale de transport de l'oxygène aux tissus en condition d'effort intense (aussi appelé AMR pour Active Metabolic Rate; Fry, 1971; Norin & Clark, 2016).

Taux métabolique standard : taux métabolique minimal nécessaire à la survie des organismes en condition de jeûne et de repos (Chabot et al., 2016a; Fry, 1971).

Tolérance : capacité d'un organisme à supporter les variations environnementales.

Vulnérabilité : propension ou prédisposition des espèces ou des populations à être affectées négativement par les changements globaux (IPCC, 2014). Elle dépend de la durée et de l'intensité de l'exposition des organismes, mais aussi de leur sensibilité, résilience et potentiel adaptatif (Huey et al., 2012; Pacifici et al., 2015).

RÉSUMÉ

Les changements globaux sont reconnus comme des vecteurs majeurs de la perte de biodiversité. L'ampleur de ces phénomènes sur les invertébrés marins demeure fortement sous-estimée. Même si les études individuelles sur les réponses physiologiques des invertébrés marins à certains facteurs des changements globaux se multiplient, il manque encore beaucoup de données sur les effets combinés pour comprendre l'impact sur les espèces. Les modèles développés pour définir la sensibilité des espèces se basent sur des études qui considèrent les espèces comme une seule unité homogène d'organismes dans leur distribution géographique. Ceci peut amener à une sur- ou sous-estimation des prédictions de l'abondance et de la distribution des espèces car plusieurs populations d'une même espèce peuvent être acclimatées ou adaptées à leur environnement local, ce qui peut influencer la direction et l'intensité de leurs réponses aux changements environnementaux et donc aux changements globaux.

L'identification des effets des changements globaux et de leurs conséquences sur le devenir des espèces nécessite donc l'utilisation d'une approche intégrative, rarement développée dans les études portant sur les conséquences biologiques des changements globaux. Ceci est vrai à l'échelle de comparaison de différentes populations au sein d'une même espèce, mais aussi à l'échelle individuelle lorsqu'on étudie les différents niveaux d'organisation biologique chez un organisme. L'utilisation complémentaire des approches multifactorielle (c.-à-d. combinant plusieurs variables environnementales), intégrative (c.-à-d. intégrant les réponses de plusieurs échelles d'organisation biologique) et macrophysiologique (c.-à-d. étudiant la variation des traits physiologiques à l'échelle géographique) est primordiale afin d'améliorer les capacités prédictives d'abondance et distribution des espèces. Ceci est particulièrement vrai pour les ectothermes marins d'intérêt économique et d'importance écologique tels que la crevette nordique, *Pandalus borealis*, dont la gestion des stocks dans l'est du Canada ne prend actuellement pas en compte les changements environnementaux.

Dans le but de définir la sensibilité de la crevette nordique aux phénomènes de réchauffement, d'acidification des océans et d'accentuation de l'hypoxie, les crevettes provenant de quatre origines géographiques différentes dans l'Atlantique nord-ouest, caractérisées par des environnements physico-chimiques contrastés, ont été exposées en laboratoire durant un mois à trois températures (2, 6 ou 10 °C) et deux niveaux de pH (7.75 ou 7.40). Ces traitements simulent les conditions environnementales du fond actuelles et futures (prévues pour 2100 dans le contexte des changements globaux) de l'habitat de cette espèce. De plus, les crevettes provenant de l'estuaire du Saint-Laurent ont été exposées à une teneur d'oxygène dissous de 35 % de saturation en air (représentative de conditions hypoxiques chroniques non létales) aux deux traitements extrêmes (c.-à-d. 2 °C/pH 7.75 et 10 °C/pH 7.40). Le taux de survie, les taux métaboliques de l'organisme entier, l'activité enzymatique et les profils métabolomiques du muscle de l'abdomen ont été mesurés et déterminés pour un sous-échantillon d'individus de chaque traitement afin d'intégrer

les réponses des individus aux différentes échelles organisationnelles : de l'organisme à la cellule. Ceci a permis de créer un lien entre le fonctionnement de l'organisme et les réponses cellulaires et de comparer les réponses entre les origines afin de définir la sensibilité de l'espèce.

Les résultats indiquent que la crevette nordique semble relativement bien tolérer les effets isolés du réchauffement, de l'acidification des océans et de l'hypoxie. Cependant, la survie et la capacité aérobie des crevettes sont fortement réduites en conditions combinées, prédites pour la fin du siècle. De plus, les différences intraspécifiques présentes au niveau cellulaire se traduisent en une performance aérobie comparable parmi les populations et une réponse aux facteurs des changements globaux comparable parmi les crevettes provenant de différentes origines. Enfin, bien que le métabolisme aérobie soit maintenu, le stress subi par les crevettes pourrait avoir induit l'activation de la réponse immunitaire.

En général, l'utilisation des approches multifactorielle, intégrative et macrophysiologique dans cette étude met en évidence la sensibilité élevée de cette espèce aux scénarios de changements globaux combinés futurs. En raison de certaines conditions environnementales régionales actuelles proches des limites de tolérance de cette espèce la pêche à la crevette pourrait être compromise d'ici la fin du siècle dans le système du Saint-Laurent. Certaines populations comme celle de l'estuaire du Saint-Laurent semblent déjà fonctionner près de la limite de leurs capacités, ce qui pourraient les rendre plus vulnérable à la prédation et la pêche dans des conditions environnementales futures. La sensibilité de la crevette nordique et ses conséquences sur les populations mise en évidence dans ce travail de thèse pourraient être intégrées aux mesures de gestion dans le but d'assurer la pérennité de la pêche à crevette nordique dans le nord-ouest de l'Atlantique dans le contexte des changements globaux rapides.

Mots-clés : multi-facteurs, intégration, macrophysiologie, changements globaux, métabolisme, gestion

ABSTRACT

Global changes are recognized as major drivers of biodiversity loss. The extent of these phenomenon on marine invertebrates remains greatly underestimated, despite the increasing number of individual studies on the physiological responses of marine invertebrates to some global changed drivers, we still lack data on combined effects to understand the impact on species. However, the models developed to define species' sensitivity are based on studies that consider species as a single homogeneous unit of organisms in their geographical distribution. This can lead to over- or under-estimation of species' abundance and distribution as multiple populations of the same species may be acclimatized or adapted to their local environment, which may influence the direction and the intensity of their responses to environmental changes and therefore to global changes.

Identifying the effects of global changes and their consequences on the future of species therefore requires the use of an integrative approach, rarely developed in studies focusing on the biological consequences of global changes. This is true, when comparing different populations within the same species, but also at the individual level when studying the different levels of biological organization. The complementary use of multi-driver (i.e. combining multiple environmental variables), integrative (i.e. integrating the responses from multiple levels of biological organization) and macrophysiological (i.e. studying the physiological traits' variation at the geographic scale) approaches is essential to improve the predictive capacities of species' abundance and distribution. This is particularly true for marine ectotherms of economic interest and ecological value such as the northern shrimp, *Pandalus borealis*, whose stock management in eastern Canada does not consider yet environmental changes.

To define the northern shrimp sensitivity to ocean warming, acidification and hypoxia increase, shrimp from four different geographic origins in the northwest Atlantic, characterized by contrasting physico-chemical conditions, were exposed in the laboratory for one month to three temperatures (2, 6 or 10 °C) and two pH levels (7.75 or 7.40). These treatments mimic the current and future bottom environmental conditions (predicted to occur by 2100 in the context of global changes) of this species' habitat. In addition, shrimp from the St. Lawrence Estuary were exposed to a dissolved oxygen level of 35 % air saturation (representative of chronic but non-lethal hypoxic conditions) at the two extreme treatments (i.e. 2 °C/pH 7.75 and 10 °C/pH 7.40). Survival rate, whole-organism metabolic rates, enzyme activity and metabolomic profiles of abdominal muscle were measured and determined for a subsample of individuals from each treatment to integrate the responses of individuals at different levels of organization: i.e. from the organism to the cell. This allowed us to create a link between organism functioning and cellular responses and to compare responses among origins in order to define the species' sensitivity.

Results show that northern shrimp can relatively well tolerate the isolated effects of ocean warming, acidification and hypoxia. However, shrimp survival and aerobic capacity are greatly reduced when exposed to the combined conditions. Moreover, the intraspecific differences existing at the cellular level translate into comparable aerobic performances among populations and into comparable responses to global change drivers among shrimp from different origins. Finally, despite the maintenance of the aerobic metabolism, the stress experienced by shrimp could have induced the enhancement of the immune response.

Overall, the use of multi-driver, integrative and macrophysiological approaches in this study highlights the high sensitivity of this species to future combined global change scenarios. Due to the particular current regional environmental conditions close to the tolerance limits of this species, the shrimp fishery could be compromised by the end of the century in the St. Lawrence system. Some populations, such as the one in the St. Lawrence Estuary, already seem to operate near the limit of their capacities, which could make them more vulnerable to predation and fishing under future environmental conditions. The sensitivity of the northern shrimp and its consequences on the populations highlighted in this thesis' work could be integrated into management measures aiming to ensure the sustainability of the northern shrimp fishery in the northwest Atlantic in the context of rapid global changes.

Key words : multi-driver, integration, macrophysiology, global changes, metabolism, management

INTRODUCTION

I. Perte de biodiversité et vulnérabilité spécifique dans l'Anthropocène

La perte de diversité biologique est l'un des problèmes environnementaux mondiaux les plus graves à l'heure actuelle (Maxwell et al., 2016). Des centaines d'espèces se sont éteintes au cours des 100 dernières années et des centaines d'autres sont menacées d'extinction chaque année (IUCN, 2020). Ce déclin de diversité spécifique, fonctionnelle et physiologique que l'on constate prend le nom de « sixième extinction de masse » (Barnosky et al., 2011; Ceballos et al., 2017). L'augmentation exponentielle de la population humaine et de ces activités est reconnue être la source principale de la perte de biodiversité, la surexploitation et la destruction d'habitat étant les causes principales des récentes extinctions (Sodhi et al., 2009). Cependant, les activités humaines sont aussi responsables de l'induction des changements globaux [c.-à-d. les changements du climat attribuables à l'activité humaine et qui s'ajoutent à la variabilité naturelle du climat observée au cours de périodes comparables – Nations Unies, 1992], qui représentent les moteurs de perte de biodiversité de plus en plus importants au cours du 21ème siècle (Scholes, 2010). Par conséquent, comprendre et prévoir les impacts des changements globaux sur les organismes, les populations, les espèces, les communautés et les écosystèmes est fondamental et représente un domaine de recherche très actif (Bellard et al., 2012; Cheung et al., 2009; Cooke et al., 2013). Dans ce contexte, bien que le déclin des espèces de vertébrés soit plus flagrant, il ne correspond pas au déclin le plus important en termes d'effectif d'individus. La disparition continue de nombreux invertébrés est majeure et a également des conséquences dramatiques sur la modification des écosystèmes (Wilson, 1987), de ce fait elle nécessite davantage d'attention (Eisenhauer et al., 2019).

Dans le contexte des changements globaux, lorsque les conditions environnementales dépassent les fluctuations naturelles, les organismes peuvent persister dans leur nouvel environnement s'ils possèdent la plasticité phénotypique [c.-à-d. la capacité d'un génotype à produire plusieurs phénotypes selon l'environnement expérimenté – West-Eberhard, 1989] nécessaire à l'acclimatation physiologique (Ghalambor et al., 2007). Les organismes peuvent même s'adapter aux nouvelles conditions environnementales grâce au succès des phénotypes et la sélection des génotypes à plus grande valeur reproductive (Davis & Shaw, 2001; Hoffmann & Sgró, 2011). S'ils ne possèdent pas ces capacités physiologiques, les organismes qui sont en mesure de le faire

peuvent migrer vers des conditions meilleures (Parmesan, 2006). Dans le cas contraire ils risquent l'extinction. La vulnérabilité des espèces aux changements globaux [c.-à-d. la propension ou la prédisposition des espèces à être affectées négativement – IPCC, 2014] dépend de la durée et de l'intensité de l'exposition des organismes, mais aussi de leur sensibilité, résilience et potentiel adaptatif (Figure 2) (Huey et al., 2012; Pacifici et al., 2015). La sensibilité des espèces dépend de la capacité des organismes à réagir, en termes de réponses physiologiques, aux variations environnementales. La tolérance d'un organisme, quant à elle est la capacité d'un organisme à supporter les variations environnementales sans que sa survie ne soit compromise (Figure 2). Finalement, la résilience et le potentiel adaptatif se définissent comme la capacité d'un organisme à revenir à un état initial après une perturbation et la capacité à s'adapter en réponse à des nouvelles conditions environnementales, respectivement.

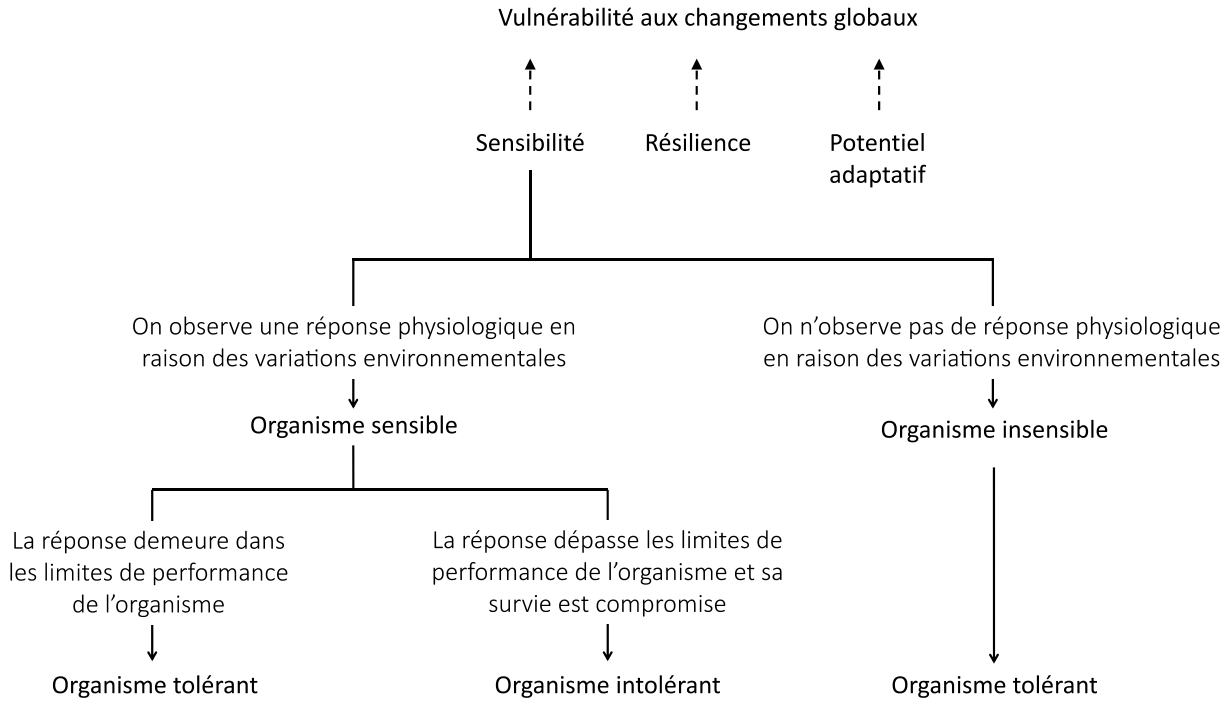


Figure 2 Représentation schématique des liens et dépendances entre les termes vulnérabilité, sensibilité, résilience et potentiel adaptatif, utilisés dans cette thèse.

Présentement, un nombre croissant d'études vise à déterminer la sensibilité des espèces face aux changements globaux. Cependant, les prédictions du devenir des espèces pourraient être mal estimées. Cette sur- ou sous-estimation est causée par l'étude d'une espèce comme une seule unité homogène alors qu'il existe des différences de sensibilité entre les individus d'une même population et les populations d'une même espèce. En effet, les individus appartenant à des espèces avec une large distribution géographique peuvent vivre dans différentes conditions environnementales et être acclimatées ou adaptées aux conditions locales dominantes (Bozinovic et al., 2011; De Wit & Palumbi, 2013; Gaston et al., 2009; Sanford & Kelly, 2011; Sork, 2018). L'acclimatation et l'adaptation influencent la biologie et la physiologie des organismes provenant de différents environnements et peuvent influencer la direction et l'intensité des leurs réponses aux facteurs des changements globaux (Calosi et al., 2017; Chen et al., 2013; Gaston & Spicer, 1998; Merilä et al., 2004). L'acclimatation et l'adaptation locale se révèlent avantageuses, car elles permettent de limiter les coûts liés à la plasticité en alignant les performances moyennes sur l'optimum environnemental [c.-à-d. les conditions environnementales les plus favorables pour les organismes] ce qui permet de maximiser le succès reproducteur (Kawecki & Ebert, 2004). Cependant, ce même avantage peut entrainer des coûts (Clarke, 2003) qui pourraient se révéler fatals pour les organismes exprimant déjà les limites de leurs capacités plastiques, d'autant plus dans le contexte des changements globaux rapides qui se produisent au sein d'une seule génération ou sur quelques générations (Alley et al., 2003). Ainsi, il devient nécessaire d'appliquer une vision intégrative des informations issues des réponses biologiques et physiologiques des populations pour comprendre la sensibilité des espèces aux changements globaux (Chown & Gaston, 2008; Gaston et al., 2009).

Actuellement, les études qui reconnaissent l'importance d'utiliser l'approche macrophysiologique, c.-à-d. combinant les réponses de plusieurs populations aux facteurs des changements globaux, sont encore rares (Alberto et al., 2013; Cummins et al., 2019; Rudin-Bitterli et al., 2020), particulièrement dans le contexte du milieu marin (Gaitán-Espitia et al., 2014; Sorte et al., 2011; Thor et al., 2018; Vargas et al., 2017). Par conséquent, nos capacités de prédictions biogéographiques (c.-à-d. distribution et abondance) sont réduites et ne prennent pas en considération l'effet des différents environnements sur la réponse physiologique des populations et des espèces aux conditions environnementales futures. Cependant, estimer correctement la

sensibilité des espèces, afin de prédire leur distribution et abondance dans les conditions environnementales futures, est urgent pour les espèces marines (Pinsky et al., 2019) et particulièrement pour celles à haute valeur écologique et commerciale, car la migration ou l'extinction de ces espèces peut avoir d'importantes retombées sur l'industrie de la pêche et sur la sécurité alimentaire des communautés qui en dépendent (Allison et al., 2009; Cheung et al., 2010).

II. Changements globaux en milieu marin

Depuis le début de la révolution industrielle, l'augmentation de l'activité anthropique a causé l'augmentation des concentrations atmosphériques des gaz à effet de serre (GES), dont le dioxyde de carbone (CO_2). Cette augmentation induit le réchauffement global qui affecte directement et indirectement l'environnement marin par l'augmentation des températures terrestres et océaniques de surface (IPCC, 2021). En effet, les océans jouent un rôle majeur dans la régulation des changements globaux et agissent en tant que tampon des effets des GES en absorbant, entre autres, l'excès de chaleur. Ceci se traduit par une augmentation de la température de l'eau, phénomène connu sous le nom de réchauffement des océans (IPCC, 2022; Mitchell, 1989). Durant la période 2011–2020, la température de l'eau moyenne, au niveau mondial, était de 0.88 [0.68 à 1.01] °C supérieure à la température moyenne observée de 1850 à 1900 (IPCC, 2021). Selon le scénario de forçage radiatif le plus pessimiste, mais aussi le plus réaliste (RCP8.5, maintenant identifié SSP5-8.5), du Groupe Intergouvernemental sur l'Évolution du Climat (GIEC, en anglais IPCC), le phénomène du réchauffement devrait s'aggraver d'ici la fin du siècle, jusqu'à atteindre une augmentation moyenne globale de la température de surface des océans de plus de 4 °C comparé à 1850-1900 (IPCC, 2021). À l'échelle globale, l'Arctique et l'Antarctique se réchauffent plus que les tropiques et le réchauffement est plus prononcé dans l'hémisphère nord (Figure 3).

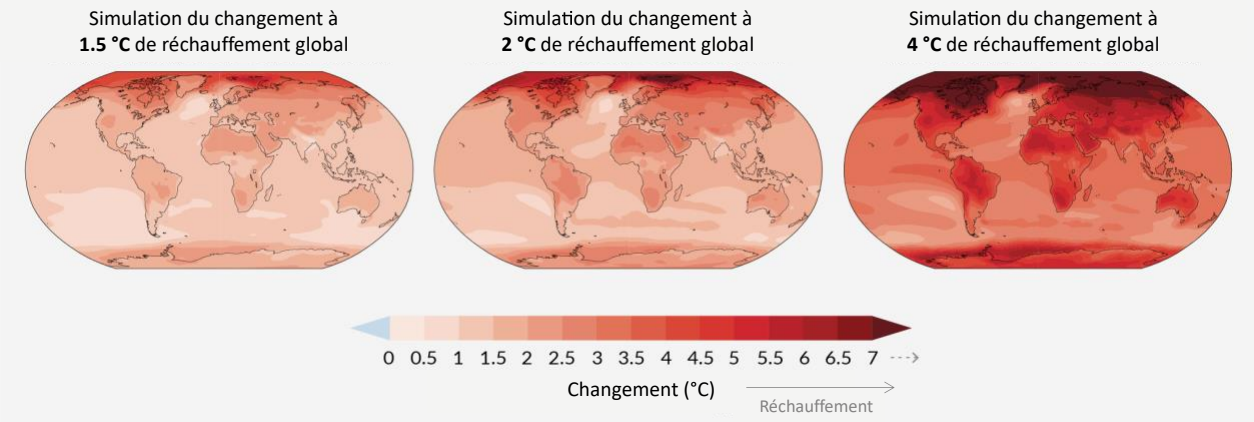


Figure 3 Simulation du changement de la température annuelle moyenne (°C) de surface mondiale sur 20 ans par rapport à 1850-1900 en fonction des différents niveaux de réchauffement global de 1,5 °C, 2 °C et 4 °C. Figure adaptée de IPCC (2021).

De plus, environ un tiers du CO_2 atmosphérique d'origine anthropique est absorbé par les océans (Sabine et al., 2004) et affecte directement l'environnement marin *via* la diminution du pH moyen mondial de l'eau de mer et la modification de la chimie des carbonates (Feely et al., 2009; IPCC, 2022). Ce phénomène est connu sous le nom d'acidification des océans et consiste en une diminution du pH marin résultant de l'altération des équilibres thermodynamiques du système des carbonates (Doney et al., 2009). En conditions d'acidification des océans, la formation d'acide carbonique (H_2CO_3), des ions bicarbonates (HCO_3^-) et des ions hydrogène (H^+) est favorisée, tandis que la concentration des ions carbonate (CO_3^{2-}) diminue (Figure 4). L'augmentation de la concentration des ions hydrogène induit la diminution du pH *via* la relation $pH = -\log_{10} [H^+]$. Depuis 1850, le pH de surface des océans a diminué de 0.1 unité pH (Caldeira & Wickett, 2003; Doney et al., 2009; IPCC, 2013) et le scénario RCP8.5 du GIEC prévoit une diminution globale de 0.036-0.042 unités pH sur la totalité des eaux de surface d'ici 2081-2100 par rapport à 2006-2015, en particulier aux latitudes les plus élevées (IPCC, 2022), car les eaux plus froides absorbent plus de CO_2 (AMAP, 2018; Doney et al., 2009).

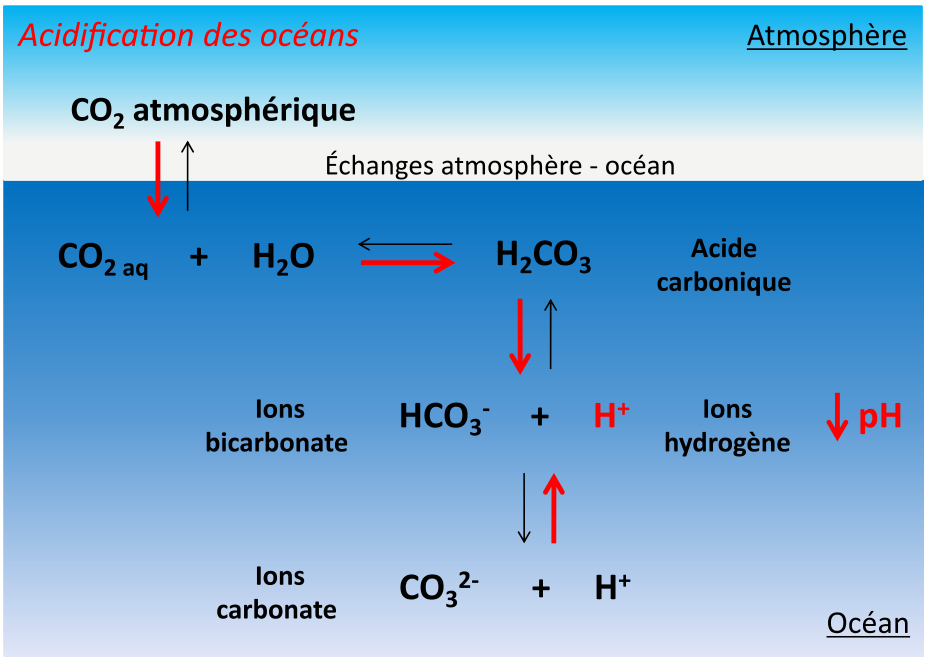


Figure 4 Représentation simplifiée de l'acidification des océans et de la chimie des carbonates. Les flèches rouges indiquent le déplacement de l'équilibre. Figure crée à partir de Gattuso & Hansson (2011).

Enfin, l'augmentation de la température des océans (plus marquée à la surface) est aussi responsable des phénomènes de stratification de la colonne d'eau, qui devraient aussi gagner en ampleur d'ici la fin du siècle (IPCC, 2022), et qui influencent la disponibilité en oxygène dissous (OD) des eaux profondes (Breitburg et al., 2018; Matear & Hirst, 2003; Rabalais et al., 2010). En effet, la stratification empêche les mélanges entre les eaux de surface riches en oxygène (O_2) et les eaux profondes, où le processus de respiration contribue à la diminution de la saturation en O_2. On définit ce phénomène sous le nom de désoxygénation des océans (Gruber, 2011; Keeling et al., 2010) et depuis 1960 on estime une perte de 1-2 % de l'O_2 dans les eaux entre 100 et 600 m de profondeur. D'ici 2081-2100 on prévoit une diminution de 3,2-3,7 % d'O_2 par rapport à 2006-2015 (scénario RCP8.5; IPCC, 2022). Par conséquent, les régions à faible saturation en O_2 augmentent en nombre, fréquence, durée et intensité et donnent lieu à des conditions hypoxiques ou anoxiques (Figure 5) (Breitburg et al., 2018; Matear & Hirst, 2003). L'hypoxie se définit comme une condition de faible teneur en OD à laquelle les processus physiologiques et écologiques des organismes sont altérés (2 mg O_2 L^{-1}, Diaz & Rosenberg, 2008). Cependant, il existe une grande variation entre les seuils de tolérances chez les espèces marines et la définition d'hypoxie peut varier en fonction des effets négatifs sur la survie, la physiologie et le comportement des

organismes, qui peuvent surgir à des concentrations en dessous de 2 mg O_2 L^{-1} (Diaz & Rosenberg, 1995; Vaquer-Sunyer & Duarte, 2008). Ces seuils sont également associés au type d'hypoxie : on parle d'hypoxie aigüe quand les organismes sont exposés pendant de courtes périodes et d'hypoxie chronique quand l'exposition est de longue durée. En milieu marin, les conditions d'hypoxie aigüe sont souvent liées à la désoxygénation des eaux côtières due au processus d'eutrophisation (Diaz, 2001; Diaz & Rosenberg, 2008), tandis que l'hypoxie chronique est liée aux eaux profondes et est due au phénomène d'isolement des masses d'eau expliqué plus haut.

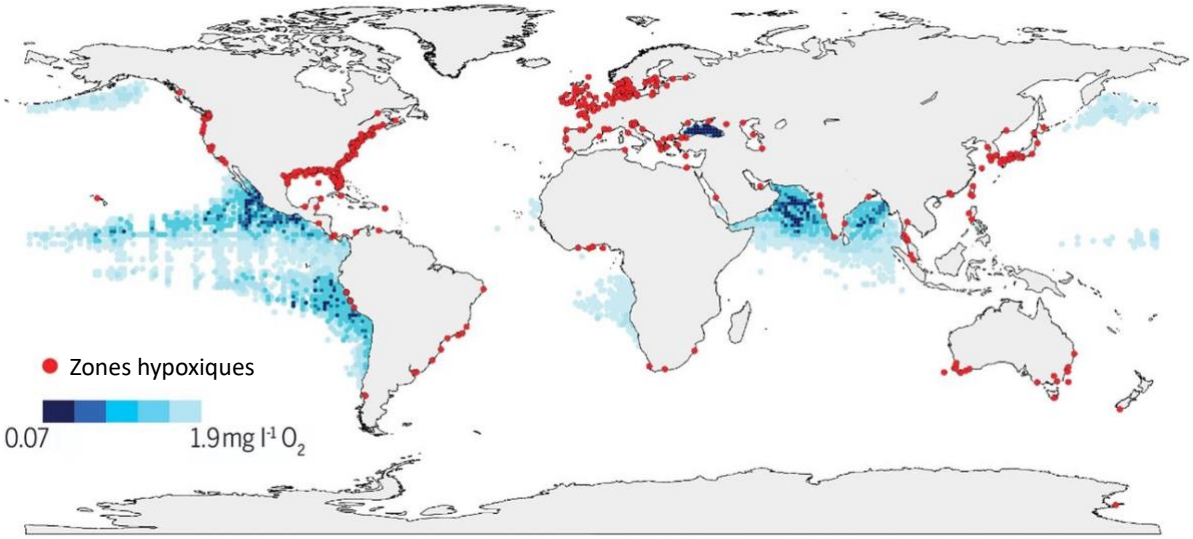


Figure 5 Carte géographique indiquant les zones à faible concentration d'oxygène et les zones hypoxiques côtières. Figure tirée de Breitburg et al. (2018).

Les phénomènes de réchauffement, acidification et désoxygénation des océans sont souvent liés et peuvent co-exister en milieu marin. Ensemble, ils sont identifiés comme le « trio mortel », car historiquement ils ont contribué aux cinq évènements d'extinction de masse et constituent aujourd'hui une menace majeure pour différentes espèces puisqu'ils ont, seuls ou combinés, des effets négatifs sur les écosystèmes marins (Bijma et al., 2013; Sampaio et al., 2021). Ils sont susceptibles d'influencer l'abondance et la distribution des espèces en impactant de nombreuses fonctions biochimiques, physiologiques et métaboliques chez les organismes marins (Fry, 1971; Melzner et al., 2009; Whiteley, 2011). Au niveau de l'organisme, ces facteurs influencent directement et indirectement le métabolisme, le développement, la croissance, la reproduction et les processus de calcification et dissolution des structures calcifiées ainsi que les réponses

comportementales (p.ex. consommation de nourriture et capacité d'éviter les prédateurs) (Chabot & Claireaux, 2008; Claireaux & Chabot, 2019; Hofmann et al., 2010; Kroeker et al., 2010; Levin et al., 2009; McMahon, 2001; Orr et al., 2005). De plus, lorsque ces facteurs co-existent, ils peuvent s'influencer l'un l'autre et influencer en co-occurrence la réponse des organismes (Kroeker et al., 2013; Pörtner & Farrel, 2008). Par exemple, le processus de respiration dans les eaux profondes donne lieu à des conditions hypoxiques et acides à cause de l'accumulation du CO_2 (Gobler & Baumann, 2016; Melzner et al., 2013). Ou encore, à des températures élevées, la solubilité de l'O_2 diminue, réduisant la disponibilité d'O_2 tandis que la demande en O_2 des organismes augmente avec le réchauffement (Verberk et al., 2018). Souvent les facteurs environnementaux s'influencent de manière non linéaire, mais plutôt en synergie ou antagonisme (Côté et al., 2016; Gunderson et al., 2016; Todgham & Stillman, 2013) rendant difficile la prédiction des effets combinés à partir de l'interprétation des effets simples. En conséquence, les études qui utilisent des scénarios multifactoriels auront un meilleur pouvoir prédictif des réponses des espèces aux facteurs des changements globaux, ce qui permet une meilleure estimation de la sensibilité des espèces et par conséquent de leur vulnérabilité. Cependant, les études portant sur l'interaction entre ces trois facteurs environnementaux sont très rares (Sampaio et al., 2021) bien que le réchauffement, l'acidification des océans et l'hypoxie soient considérés comme une menace majeure, particulièrement pour les ectothermes (Paaijmans et al., 2013). En effet, les ectothermes sont considérés être très sensibles en raison de leur physiologie et du fait qu'il s'agit d'organismes hétérotrophes aérobies, dont la température corporelle dépend largement de la température environnementale (Angilletta, 2009), et avec des capacités homéostatiques généralement assez limitées (Melzner et al., 2009; Whiteley, 2011). Il est donc primordial d'étudier comment leur performance [c.-à-d. l'ensemble des capacités fonctionnelles exécutées d'un organisme] varie en fonction des différents scénarios environnementaux futurs afin de déterminer leur sensibilité et vulnérabilité.

III. Performance sous contrainte du « trio mortel »

La performance des organismes ectothermes aérobies est souvent représentée par une courbe qui a la forme d'une cloche (Pörtner & Farrel, 2008; Schulte, 2015). D'après cette représentation, la performance est à son maximum (*Optimum*) lorsque la température est optimale et elle diminue (*Pejus*, *Pessimum*), en fonction de la tolérance des espèces, au fur et à mesure que les conditions environnementales deviennent défavorables : c-à-d. quand la température diminue ou augmente s'éloignant de l'*optimum* (Figure 6). La forme et l'étendue des courbes de performance varient en fonction des traits considérés (p.ex. survie, locomotion, croissance, développement et reproduction), des stades de vie des organismes et des facteurs environnementaux à l'étude.

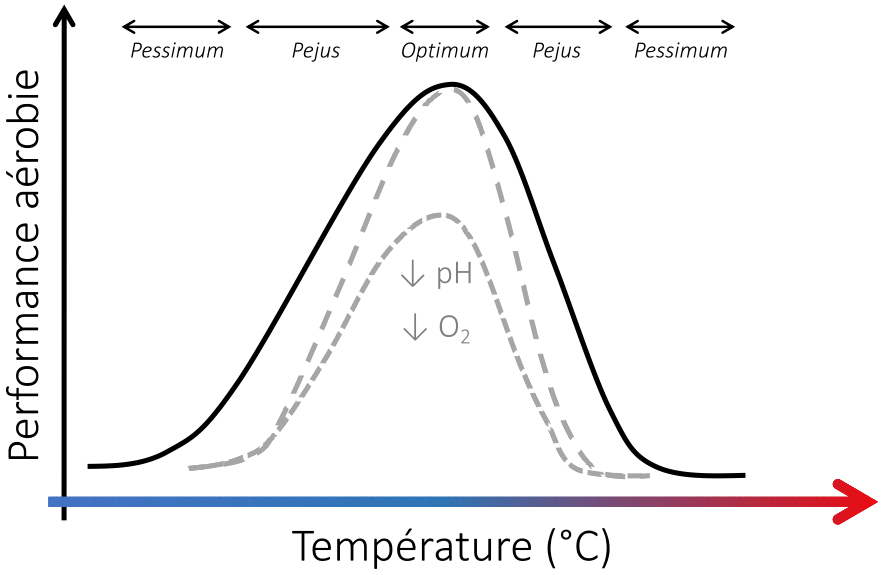


Figure 6 Représentation simplifiée d'une courbe de performance thermique d'un organisme ectotherme illustrant l'*optimum* et les limites inférieure et supérieure de la fenêtre thermique (ligne continue) ainsi que des exemples de possibles modifications de la courbe en fonction de la diminution du pH et de l'O_2 (lignes pointillées en gris). Figure modifiée à partir de Pörtner & Farrel (2008).

Il a été proposé, en principe par Fry (1947) et par la suite par Pörtner & Knust (2007), que la forme de cette courbe serait induite par les effets contrôlants de la température sur les taux métaboliques des organismes, qui définissent leurs coûts de vie et qui sont souvent estimés en mesurant leur taux de consommation d'O_2 ($\dot{M}_{O_2}$) (Fry, 1971). Le Taux Métabolique Standard (TMS – défini comme le taux métabolique minimal nécessaire à la survie des organismes en condition de jeûne et de

repos ; Chabot et al., 2016a; Fry, 1971) augmente linéairement ou exponentiellement avec l'augmentation de la température, tandis que le Taux Métabolique Maximal (TMM – défini comme la capacité maximale de transport de l'O_2 aux tissus en condition d'effort intense ; Fry, 1971; Norin & Clark, 2016) augmente uniquement à basse température et diminue à haute température (Figure 7). Par conséquent, le Registre Aérobie (RA = TMM-TMS – soit la capacité du système cardiorespiratoire à fournir de l'O_2 pour toutes les activités au-dessus du maintien ; Fry 1947), atteindrait son maximum à la température optimale et diminuerait en dehors de la plage de température optimale (Figure 7). D'après l'hypothèse de Pörtner & Knust (2007) intitulée « *Oxygen and Capacity Limited Thermal Tolerance* » (OCLTT), la diminution du RA serait due à l'altération de la capacité des mitochondries à utiliser l'O_2 à basse température et à l'altération de la capacité du système cardiorespiratoire à fournir de l'O_2 à haute température. Le RA est donc considéré comme un indicateur de performance aérobie de l'organisme entier puisque la plupart des processus se produisant chez un animal nécessitent de l'énergie et que la voie de production d'énergie la plus efficace nécessite de l'O_2 (Pörtner & Knust, 2007).

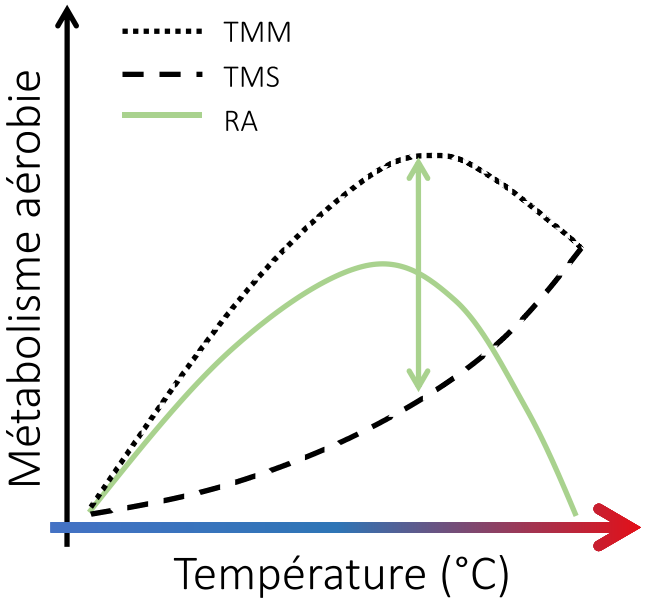


Figure 7 Représentation simplifiée de l'effet de l'augmentation de la température sur les taux métaboliques (Taux Métabolique Standard – TMS et Maximum – TMM) et par conséquent sur le Registre Aérobie (RA). Figure modifiée à partir de Lefevre (2016).

La performance varie donc en fonction de la température et définit ainsi la fenêtre thermique de chaque organisme, considérée comme une plage située entre les limites inférieure et supérieure et à l'intérieur de laquelle se trouve la température optimale pour le rendement métabolique (Frederich & Pörtner, 2000). Cette relation entre la température et la performance a été montrée chez de nombreux invertébrés et poissons (p.ex. Chen et al., 2013; Ern et al., 2015; Lefevre et al., 2015), mais chez d'autres espèces le RA ne diminue pas à des températures plus élevées que la température optimale, questionnant l'applicabilité de la théorie OCLTT chez tous les ectothermes (Clark et al., 2013; Lefevre, 2016; Schulte, 2015). De plus, tel que mentionné plus haut, la performance des organismes varie aussi en fonction d'autres facteurs environnementaux, et dans le contexte des changements globaux en milieu marin présenté dans cette introduction il est intéressant de s'attarder sur l'influence de la diminution du pH et de l'OD sur les taux métaboliques des organismes et le conséquent impact sur le RA (Figure 6).

Une augmentation de la pression partielle de CO_2 (pCO_2) conjointe à une diminution du pH entraine une diminution du pH des liquides intra- et extracellulaires qui peut compromettre, à long terme, la survie, la reproduction et la croissance des organismes. Les mécanismes qui permettent aux organismes de répondre à l'acidification des liquides corporels (acidose) comprennent la régulation des liquides intra- et extracellulaires, le transport et l'échange d'ions et du CO_2 et la dépression métabolique temporaire (Fabry et al., 2008; Seibel & Walsh, 2003). En premier lieu les organismes cherchent à rétablir l'équilibre du pH interne à travers la régulation acido-basique. Ce processus étant énergivore il entraine une augmentation de la consommation d'O_2 (TMS) (Figure 8) (Whiteley, 2011). Si la compensation n'est pas atteinte sur une courte période, l'acidose peut mener à la dépression métabolique : réduisant les coûts de maintien (TMS), la synthèse de protéines et de glucose et induisant la production de l'énergie à travers la voie métabolique anaérobie (voir la section IV « Métabolisme énergétique »), les organismes peuvent alors tenter de surmonter des périodes d'acidose (Grieshaber et al., 1993; Guppy & Withers, 1999). Cependant, cette stratégie de dépression métabolique peut être soutenue seulement pendant de courtes périodes. De plus, en conditions d'acidose l'affinité de l'O_2 avec les pigments respiratoires diminue, réduisant la capacité de transport de l'O_2 aux tissus et aux mitochondries (Taylor & Whiteley, 1989), qui se traduit par une diminution du TMM (Rosa & Seibel, 2008). Par conséquent en conditions acidifiées le RA aurait tendance à diminuer (Figure 8). De nombreuses études visant à déterminer les effets de

l'acidification des océans sur la performance des ectothermes ont utilisé des niveaux de pCO_2 bien plus élevés que ceux prévus pour la fin du siècle et ont montré des effets négatifs liés aux faibles capacités régulatoires des ectothermes. Toutefois, les poissons, les céphalopodes et les crustacés seraient tolérants aux niveaux de pCO_2/pH prévus pour la fin du siècle en raison de leur système de transport des ions adapté à leur taux métabolique plus élevé (Lefevre, 2016; Melzner et al., 2009).

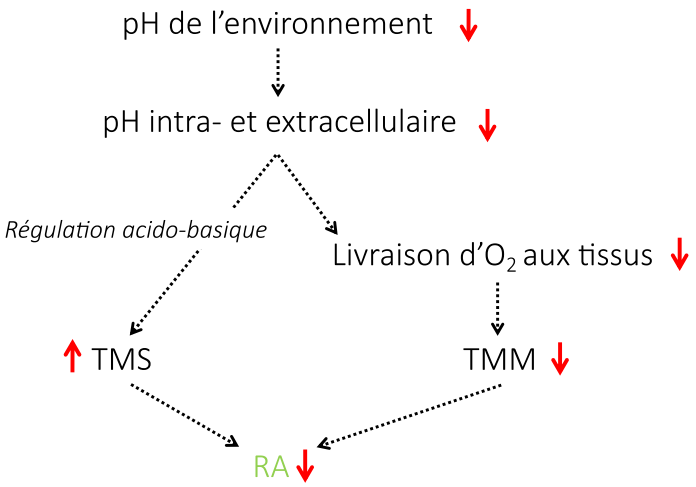


Figure 8 Représentation simplifiée de l'effet de la diminution du pH sur les taux métaboliques (Taux Métabolique Standard – TMS et Maximum – TMM) et par conséquent sur le Registre Aérobie (RA). Les flèches rouges indiquent la direction de la réponse. Figure modifiée à partir de Lefevre (2016).

En fin, face à la diminution de l'O_2 environnemental, les organismes mobiles auront tendance à se déplacer pour trouver des conditions plus favorables, tandis que les ectothermes avec de faibles capacités locomotrices ou sessiles, tenteront d'améliorer le transport de l'O_2 *via* l'augmentation du rythme cardiaque et ventilatoire et en modifiant l'affinité des pigments respiratoires pour l'O_2 (Childress & Seibel, 1998; Czyzyk-Krzeska, 1997; McMahon, 2001). Cependant, si le phénomène hypoxique est prolongé dans le temps ou si les organismes mobiles ne peuvent trouver d'eau mieux oxygénée, l'hypoxie aura tendance à limiter la performance des organismes en raison de la diminution de la quantité d'O_2 utilisable pour la production d'énergie *via* la voie aérobie (voir la section IV « Métabolisme énergétique ») (Fry, 1947, 1971). Pour cette raison, l'O_2 est considéré être une variable environnementale limitante et la relation entre les taux métaboliques et la diminution de l'O_2 environnemental est décrite par la courbe nommée « *Limiting Oxygen Level* »

(LOL) (Fry, 1971; Neill et al., 1994). D'après cette représentation (Figure 9), alors que le TMS est maintenu quand l'O_2 environnemental diminue afin de maintenir l'organisme en vie, le TMM diminue progressivement car la capacité de consommation d'O_2 diminue. Par conséquent, le RA diminue avec la désoxygénation jusqu'à possiblement rejoindre un seuil d'oxygène critique ($O_{2\,crit}$, Figure 9) au-delà duquel l'organisme n'est plus en mesure d'être actif et ne peut plus utiliser uniquement la voie aérobie pour produire l'énergie nécessaire à se maintenir en vie ; l'énergie doit alors aussi être produite via la voie anaérobie en combinaison éventuellement à la dépression métabolique (Claireaux & Chabot, 2019; Grieshaber et al., 1993).

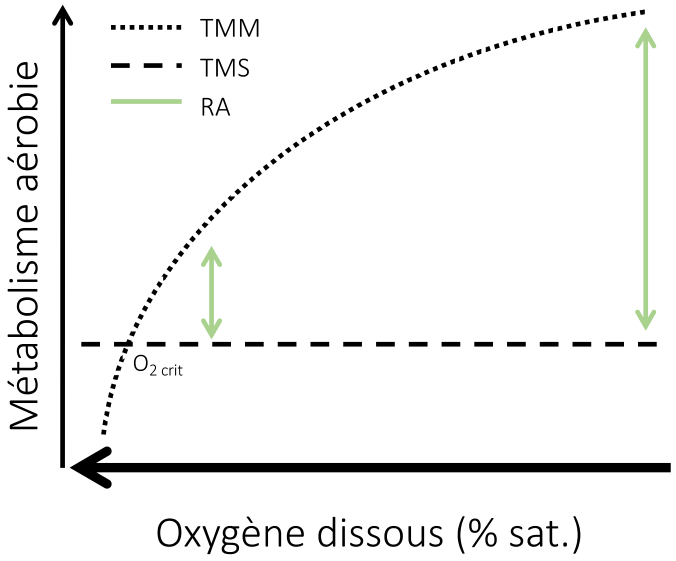


Figure 9 Représentation simplifiée de l'effet de la diminution de l'O2 sur les taux métaboliques (Taux Métabolique Standard – TMS et Maximum – TMM) et par conséquent sur le Registre Aérobie (RA). Figure modifiée à partir de Claireaux & Chabot (2016).

La diminution du RA en raison des effets de réchauffement, acidification des océans et hypoxie sur les taux métaboliques impacte le bilan énergétique des organismes et perturbe l'équilibre entre les fonctions physiologiques et les disponibilités énergétiques. En conditions environnementales favorables les organismes sont en mesure de produire suffisamment d'énergie pour que cette dernière soit répartie entre les différentes fonctions, principalement : le maintien, la digestion, la locomotion, la croissance et la reproduction (Figure 10). Cependant, si l'environnement change, affecté par les changements globaux (p.ex. la température augmente ou le pH diminue ; scénario « Facteur 1 » dans la Figure 10), les organismes devront faire face à l'augmentation des coûts

énergétiques de maintien et réparation. Avec un apport énergétique stable, ils disposeront donc de moins d'énergie allouable aux autres fonctions physiologiques (Figure 10). Dans un cas différent, (p.ex. l'OD diminue ; scénario « Facteur 2 » dans la Figure 10), les changements globaux pourraient négativement affecter la capacité des organismes à produire l'énergie. Comme dans le cas précédent, les fonctions physiologiques (autre que le maintien) seront réduites (Figure 10). Finalement, si les facteurs des changements globaux co-existent dans le même environnement, (p.ex. l'OD diminue sous effet de l'augmentation de la température ; scénario « Combinaison des facteurs » dans la Figure 10), les organismes disposeront de moins d'énergie qu'en conditions favorables et cette dernière sera allouée en plus grande partie aux fonctions de maintien et réparation, au détriment des autres fonctions physiologiques (Figure 10).

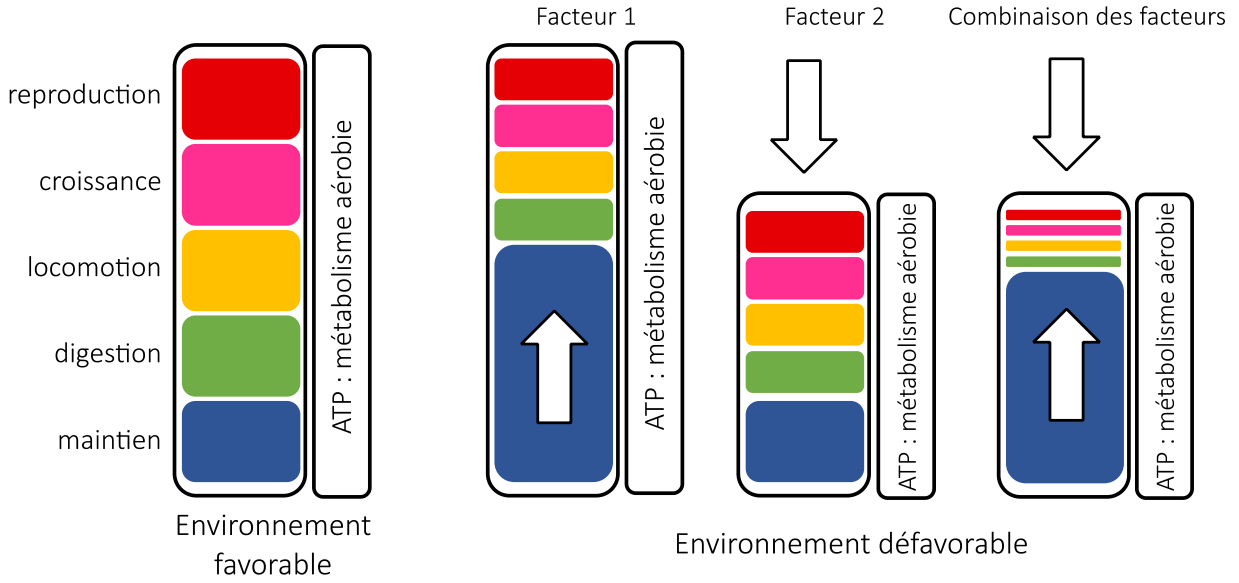


Figure 10 Variation du cadre bioénergétique en fonction de différentes conditions environnementales. Les flèches indiquent l'impact et la direction du changement de l'allocation de l'énergie induit par les effets des facteurs environnementaux. Dans un environnement favorable, le métabolisme aérobie fournit suffisamment d'énergie pour supporter les différentes fonctions physiologiques, représentées par les cases de couleurs différentes (à noter que la taille des cases ne reflète pas l'allocation énergétique réelle, mais est ici simplifiée par souci de clarté). Dans un environnement défavorable, les facteurs des changements globaux peuvent influencer la disponibilité d'énergie et son allocation. Figure modifiée à partir de Sokolova et al. (2012).

IV. Métabolisme énergétique

Le métabolisme énergétique joue un rôle central pour la survie et le fonctionnement des organismes ainsi que pour leur persistance dans des environnements en changement. L'énergie nécessaire aux organismes est produite à partir des glucides, lipides et protéines qui sont décomposés dans le cytosol des cellules et transportés dans la matrice des mitochondries. Les mitochondries sont des organites cellulaires, communément appelées « centrales énergétiques », car elles produisent la majeure partie de l'énergie nécessaire aux organismes, sous forme d'adénosine triphosphate (ATP). Les mitochondries possèdent une membrane externe, qui délimite le cytosol de l'espace intramembranaire, et une membrane interne, qui délimite l'espace intramembranaire de la matrice mitochondriale (Figure 11). Dans la matrice mitochondriale, les glucides décomposés sont oxydés en Acétyl-CoA qui entre ensuite dans le cycle de l'acide tricarboxylique (TCA, ou cycle de l'acide citrique ou cycle de Krebs (Figure 11). Son entrée est réglée par la citrate synthase (CS), un enzyme situé dans la matrice mitochondriale, qui catalyse la première réaction du cycle de Krebs, soit celle entre l'acétyl-CoA et l'oxaloacétate pour former le citrate. La fonction du cycle de Krebs est de former les molécules de NADH et $FADH_2$ nécessaires à la livraison des électrons au système de transport des électrons (ETS). L'ETS est considéré comme un marqueur de la demande énergétique et de la capacité de synthèse d'ATP, car il établit le gradient de protons nécessaire à la production d'ATP. Il se compose de quatre complexes enzymatiques (complexes I, II, III et IV, Figure 11) et deux transporteurs d'électrons, situés dans la membrane mitochondriale interne, qui assurent le transport des électrons et la réduction de l'O_2 en eau. C'est le complexe IV, la cytochrome C oxydase (COX), qui réduit l'O_2. Pendant les réactions d'oxydoréduction, des protons (H^+) sont transférés de la matrice vers l'espace intermembranaire et créent le gradient électrochimique qui permet la phosphorylation de l'adénosine diphosphate (ADP) par l'ATP synthase (Figure 11). Le processus permettant cette phosphorylation grâce à l'énergie libérée par l'oxydation de donneurs d'électrons par la chaîne respiratoire est nommé phosphorylation oxydative (Moyes & Schulte, 2008).

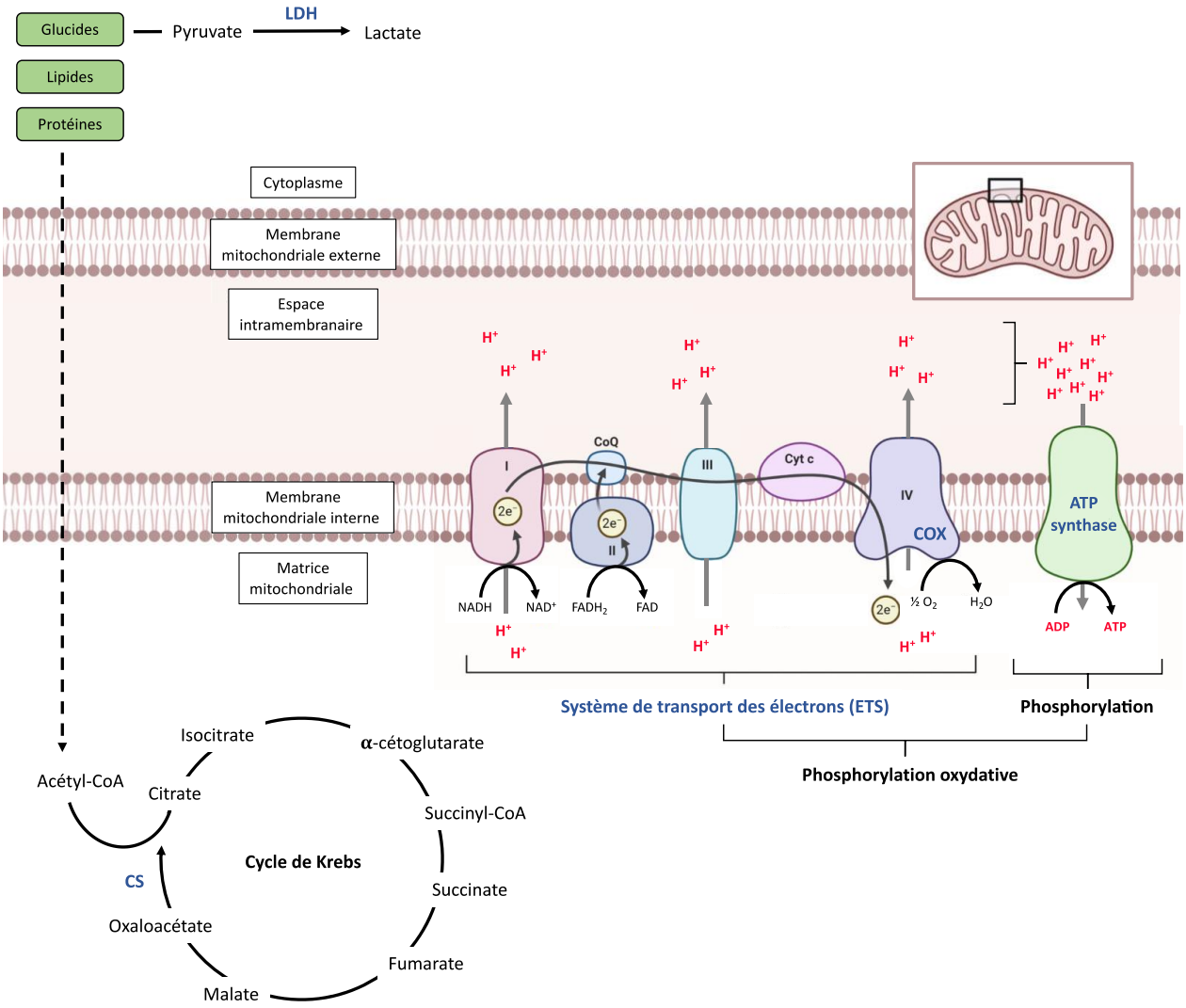


Figure 11 Représentation simplifiée de la mitochondrie et des voies métaboliques énergétiques, ainsi que du cycle de Krebs et la phosphorylation oxydative. Figure crée à partir de Moyes & Schulte, (2008) et d'une image crée avec BioRender.com

La phosphorylation oxydative a le rendement le plus élevé d'ATP par molécule métabolisée ; elle permet la production de 32 ATP à partir d'une molécule de glucose, qui en conditions environnementales favorables est l'une des sources principales de production d'énergie (Sokolova et al., 2012). Cependant, l'O_2 pour soutenir cette voie n'est parfois pas disponible. Une disponibilité réduite en O_2 peut résulter de l'hypoxie physiologique (fonctionnelle, par exemple due à la réduction du transport de l'O_2 aux tissus en conditions de réchauffement ou acidification des océans, tel que vu plus haut) ou l'hypoxie environnementale (due au phénomène de la désoxygénation). En conditions de faible disponibilité d'O_2 les organismes sont en mesure de produire l'ATP à travers

la fermentation lactique qui prévoit la réduction du pyruvate à lactate par action de la lactate déshydrogénase (LDH), enzyme qui se situe dans le cytosol (Figure 11). Cette voie métabolique anaérobie est cependant largement moins efficace en termes de production d'ATP (2 ATP par molécule de glucose) et entraîne une accumulation de déchets potentiellement toxiques. D'autres voies métaboliques existent afin de produire l'ATP en conditions anaérobie et leur utilisation varie en fonction des espèces et des conditions environnementales (p.ex. la voie de l'opine ; Gäde, 1983; Han et al., 2017). Cependant, elles demeurent toutes moins efficaces par rapport aux voies métaboliques aérobies, qui elles aussi peuvent varier en fonction des conditions environnementales. En effet, alors que les glucides et les lipides alimentent la voie aérobie en conditions environnementales favorables, les protéines et les acides aminés libres sont utilisés en conditions de carence énergétique (Sokolova et al., 2012). L'utilisation des différentes voies peut toutefois dépendre de la condition physiologique cellulaire des organismes, car à des températures élevées la synthèse des protéines et des lipides est compromise (Carter & Houlihan, 2001; Hochachka & Somero, 2002; Pörtner, 2002) et en conditions acidifiées la régulation osmo-ionique et le fonctionnement de certains enzymes sont perturbés (Fabry et al., 2008; Wheatly & Henry, 1992). Ainsi, les conditions environnementales influencent la physiologie cellulaire ayant des conséquences importantes sur la disponibilité d'énergie et le budget énergétique des organismes. La complexité des réponses étant élevée, il est primordial d'intégrer les réponses aux changements globaux à plusieurs niveaux d'organisation biologique afin de comprendre les mécanismes sous-jacents des réponses des organismes entiers et éviter la sur- ou sous- estimation de la sensibilité des espèces (Bartholomew, 1964; Harvey et al., 2014). Ce type d'approche intégrative est de plus en plus adoptée (Calosi et al., 2017; Noisette et al., 2021; Thor et al., 2022) mais rarement dans un contexte de changements globaux cumulés (cf. Flynn et al., 2015; Matoo et al., 2021).

V. La crevette nordique de l'Atlantique nord-ouest

Les crustacés représentent un groupe d'organismes idéal et intéressant à étudier dans le contexte des changements globaux. En tant qu'ectothermes ils sont sensibles au réchauffement et se révèlent très sensibles à l'hypoxie, mais tolérants à l'acidification des océans (Lefevre, 2016; Melzner et al., 2009; Sampaio et al., 2021; Vaquer-Sunyer & Duarte, 2008). Le déclin de leur abondance pourrait avoir des conséquences importantes sur l'économie à l'échelle mondiale (FAO, 2020). En effet, ils constituent une partie importante du régime alimentaire omnivore de la plupart des populations

humaines car ils représentent une source importante de protéines, lipides, vitamines et minéraux (Venugopal & Gopakumar, 2017). Compte tenu de la sensibilité et de la valeur commerciale des crustacés, l'organisme modèle sélectionné pour cette étude est la crevette nordique *Pandalus borealis*, Krøyer 1838. Ce choix a été fait aussi en raison de son déclin sans précèdent dû, en partie, aux conditions environnementales actuelles que l'on retrouve dans son aire de répartition dans l'Atlantique nord-ouest ainsi qu'en raison de son importance écologique et économique au Canada (voir les sections suivantes). L'étude de cette espèce permettra la mise en valeur des approches multifactorielle, intégrative et macrophysiologique précédemment introduites, dont l'utilisation est particulièrement intéressante pour la gestion des espèces commerciales dont le découpage des stocks n'est pas forcément calqué sur la réalité biologique des espèces qui possèdent une vaste distribution géographique.

Biologie, distribution et structure génétique

La crevette nordique (Figure 12) est une espèce d'eau froide (0 - 5 °C), bien que les adultes de cette espèce puissent vivre et se reproduire à des températures aussi élevées que 11,1 °C (Allen, 1959; Shumway et al., 1985). Il s'agit d'une espèce hermaphrodite de type protérandrique, c'est-à-dire que les individus atteignent d'abord la maturité sexuelle en tant que mâles et suite à une phase intersexuelle deviennent femelles (Berkeley, 1930). Les crevettes changent de sexe à l'âge de quatre/cinq ans et leur durée de vie moyenne est de six/sept ans (Bergström, 2000).

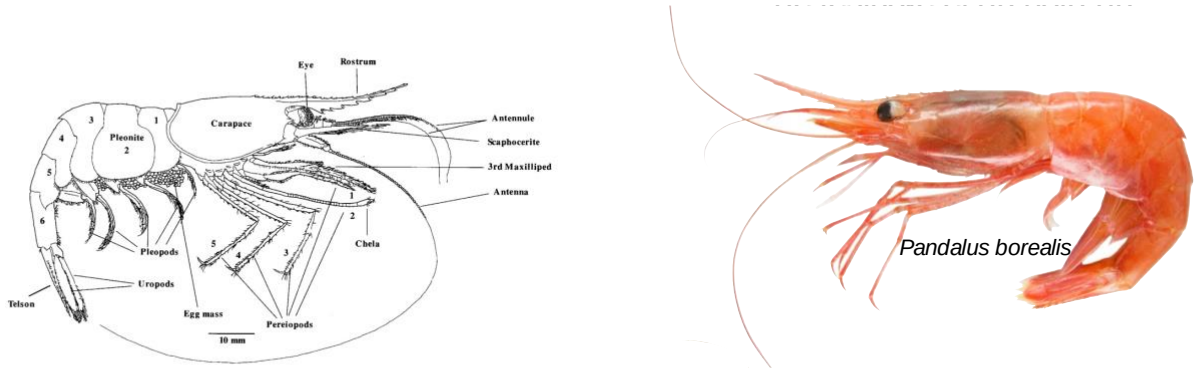


Figure 12 Illustration et photographie d'une crevette nordique, *Pandalus borealis*. Illustration tirée de Bergström (2000). Photographie de ©Claude Nozères.

Cette espèce a une large répartition circumpolaire et se retrouve en abondance dans les zones froides de l'océan Atlantique (Figure 13) (Shumway et al., 1985). Sa distribution artico-atlantique de l'ouest s'étend de la baie de Baffin au golfe du Maine et les conditions environnementales de son aire de répartition ne sont pas homogènes, notamment en termes de température (Bergström, 2000). La distribution varie selon la latitude, la saison et l'âge des crevettes, toutefois les adultes sont communément retrouvés entre 50 et 500 m de profondeur (Shumway et al., 1985), sur les fonds argileux ou sableux. Les différents stades ontogéniques de cette espèce à cycle bentho-pélagique vivent à différentes profondeurs de la colonne d'eau. En effet, les larves sont pélagiques jusqu'au stade de juvéniles, stade auquel les crevettes retournent vers le fond pour adopter une vie benthique (Shumway et al., 1985). Cependant, les femelles effectuent des migrations verticales en lien avec leur cycle de reproduction, qui est aussi marqué par les saisons : la reproduction a lieu à l'automne (septembre-octobre) et les femelles œuvées migrent vers des zones moins profondes où elles restent jusqu'à l'éclosion des larves au printemps suivant (avril-mai), pour après retourner dans les eaux profondes. Les crevettes des deux sexes migrent verticalement dans la colonne d'eau pour se nourrir, mais ne sont pas en mesure de migrer entre régions géographiques due à leur faible capacité locomotrice et en raison des barrières topographiques existantes entre les régions.

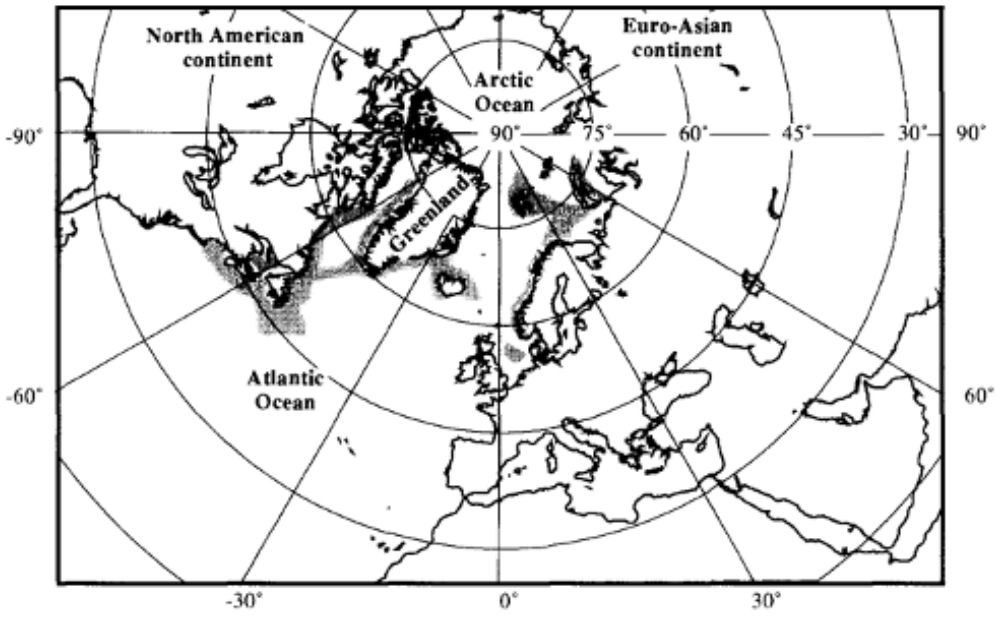


Figure 13 Carte géographique indiquant les zones de distribution de la crevette nordique *P. borealis*. Figure tirée de Bergström (2000).

La connexion entre régions étant limitée, la crevette nordique semble posséder une structure génétique le long de la côte est du Canada (Jorde et al., 2015). Une première suggestion de possible adaptation locale des larves aux différentes températures, et à la prolifération du phytoplancton, avait été faite sur la base de différences phénologiques et physiologiques entre crevettes de différentes origines géographiques (Koeller et al., 2009). Par la suite, cette suggestion a été reconfirmée en lumière des différentes réponses observées chez les larves de l'estuaire du Saint-Laurent (SLE de l'anglais *St. Lawrence Estuary*) comparé à deux autres origines géographiques dans l'Atlantique nord-ouest (Ouellet et al., 2017). Comme montré chez d'autres espèces (p.ex. Calosi et al., 2017), l'adaptation locale pourrait mener à des seuils de sensibilité différents aux changements globaux. Toutefois, l'adaptation locale de la crevette nordique aux différentes conditions environnementales de son aire de répartition dans l'Atlantique nord-ouest n'a actuellement pas été confirmée puisque la structure génétique de cette espèce n'a pas été définie à l'échelle régionale.

Écologie, exploitation et déclin

La crevette nordique est reconnue mondialement pour sa valeur économique et son importance écologique. Il s'agit d'une espèce clé de la chaine trophique en tant qu'espèce fourrage pour de nombreux poissons (p.ex. le sébaste, les flétans du Groenland et atlantique ainsi que la morue) dont certains à haute valeur commerciale (Parsons, 2005; Savenkoff et al., 2006). Elle représente également un des produits de la mer à haute valeur nutritive pour le régime alimentaire omnivore de la plupart des populations humaines, et fait l'objet d'une importante pêche commerciale dans l'Atlantique nord depuis plus que 50 ans (Holthuis, 1980; Hvingel et al., 2021). Il s'agit de la troisième espèce la plus lucrative de l'est du Canada, après le homard américain *Homarus americanus* (Milne-Edwards, 1837) et le crabe des neiges *Chionoecetes opilio* (Fabricius, 1788), et contribue à la subsistance des nombreuses communautés côtières, dont certains Peuples du Canada. Plus précisément, pour l'Atlantique, les débarquements s'élevaient à 164 270 t en 2010, au maximum de son exploitation, avec des valeurs au débarquement évalués à plus de 546 millions de dollars en 2015 (MPO, 2010, 2015). Après un maximum d'abondance enregistré entre 2004 et 2005 pour les régions de SLE et du golfe du Saint-Laurent (GSL) (Figure 14A), Terre-Neuve et Labrador (Figure 14B) et Nouvelle-Écosse (MPO, 2022a), l'abondance a commencé à décliner dans l'Atlantique nord-ouest, ce qui a mené à une progressive, mais importante diminution des

quotas de pêche au cours des dernières années (Bourdages et al., 2022; MPO, 2021a, 2022a). Cette diminution pourrait être due à des interactions biotiques (p.ex. augmentation de l'abondance du sébaste dans le système du Saint-Laurent) et/ou des changements physico-chimiques sous influence des changements globaux. En effet, le réchauffement des eaux, de surface et de fond, dans le golfe du Maine a eu un impact négatif sur le recrutement et l'établissement des crevettes, ce qui a mené à l'effondrement du stock et à l'établissement d'un moratoire maintenant en vigueur depuis dix ans (Richards & Hunter, 2021; Whitmore et al., 2013). Bien que les mécanismes à l'origine du déclin de la crevette ne soient pas entièrement compris, le réchauffement est fortement soupçonné d'être une cause importante du déclin observé. Pour ce, l'étude de la réponse physiologique des crevettes aux effets de l'augmentation de la température a gagné de plus en plus d'intérêt (Arnberg et al., 2013; Brillon et al., 2005; Chabot & Ouellet, 2005; Dupont-Prinet et al., 2013).

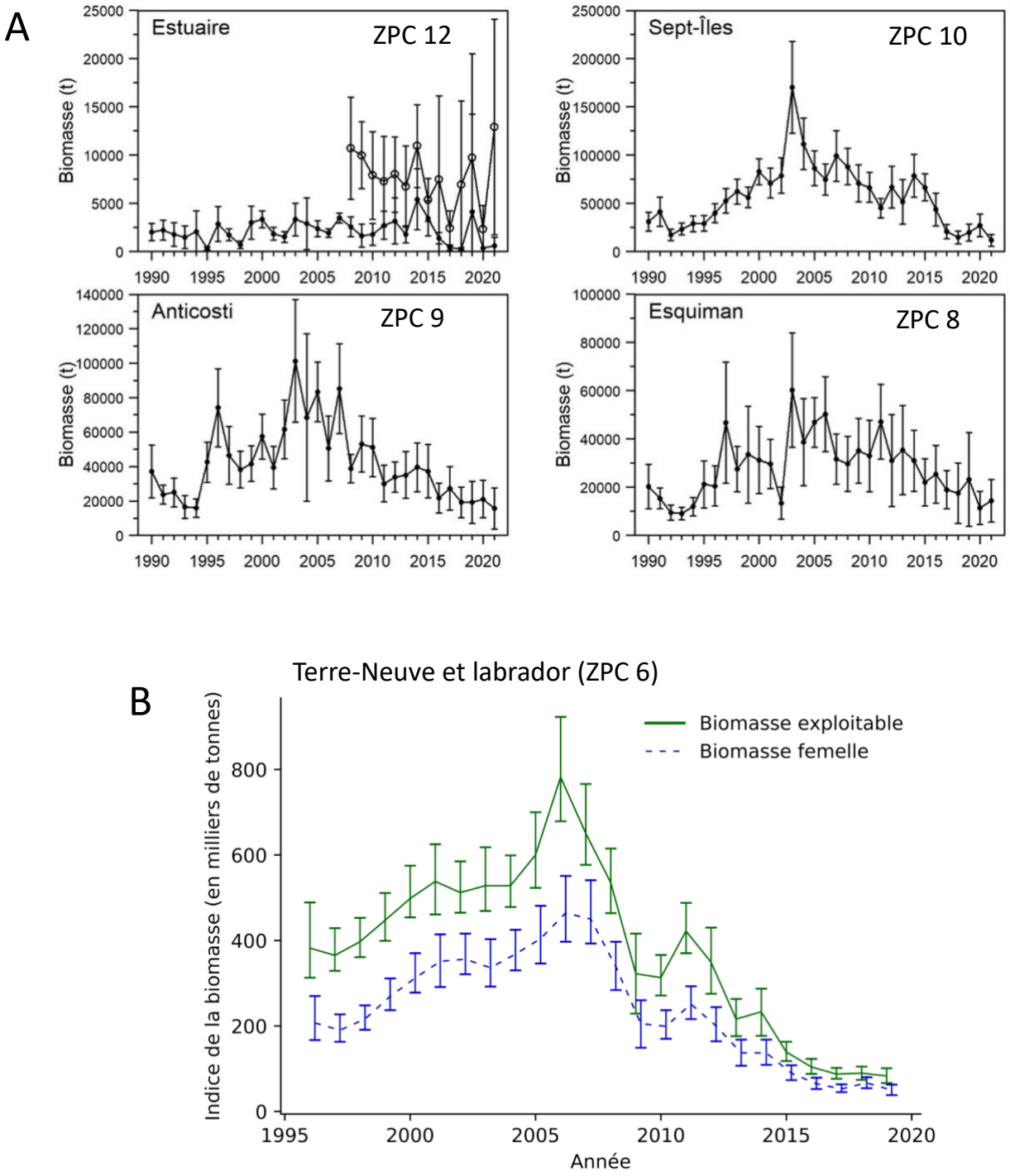


Figure 14 A) Indice de biomasse du relevé de recherche dans l'estuaire et le golfe du Saint-Laurent dans les zones de pêche (ZPC) 8, 9, 10 et 12 (Esquiman, Anticosti, Sept-Îles et Estuaire, respectivement). Pour Estuaire, les cercles ouverts représentent les résultats obtenus en incluant les strates peu profondes ajoutées en 2008. B) Indices de biomasse du stock exploitable (ligne pleine verte) et de la biomasse du stock reproducteur (BSR) femelle (ligne bleue tiretée) dans la ZPC 6 (Terre-Neuve et Labrador). Les barres d'erreur indiquent les intervalles de confiance à 95 %. Figures adaptées de MPO (2021a & 2022b).

Habitat

Dans le nord-ouest de l'Atlantique, l'habitat de la crevette nordique présente des conditions physico-chimiques contrastées à l'échelle régionale, en raison de la présence des masses d'eau froide du courant du Labrador (nord) et d'eau chaude du Gulf Stream (sud). La plus grande partie des crevettes se retrouvent habituellement à des profondeurs de 150 à 600 m, là où les températures varient entre 2 et 4 °C dans la zone extracôtière de Terre-Neuve et du Labrador et entre -1 et 6 °C à l'est du plateau néo-écossais (Cyr et al., 2022; MPO, 2022a). En revanche, les eaux profondes de SLE et du nord du GSL (nGSL) (150 m à 300 m) sont caractérisées par des températures plus chaudes (5-7 °C, et plus) (Figure 15) en raison du mélange des deux masses d'eau, qui rentre dans le chenal Laurentien par le détroit de Cabot (Bourdages et al., 2022; Galbraith et al., 2022). Dans les années 1930, le ratio de ces deux masses d'eau était plus riche en eau du courant du Labrador, tandis qu'aujourd'hui le mélange est plus chaud, plus salé et moins oxygéné à cause d'un pourcentage plus important d'arrivée d'eau du courant du Gulf Stream (Galbraith et al., 2022; Gilbert et al., 2007). L'augmentation des températures dans les eaux profondes de SLE et du nGSL est responsable de l'augmentation du processus de respiration. Ce dernier a à son tour contribué à la diminution de l'OD dans les zones hypoxiques dans SLE et nGSL (Gilbert et al., 2007). La combinaison de l'hypoxie et de la respiration des organismes sont responsables de la diminution du pH des eaux profondes : de 0,2 à 0,3 unité pH depuis 75 ans (Mucci et al., 2011) pour des valeurs actuelles se situant entre 7,4 et 7,7 unité pH (MPO, 2021b).

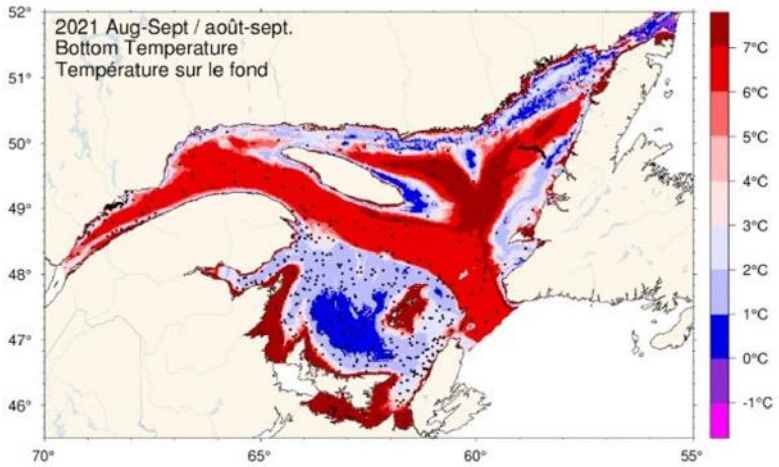


Figure 15 Température de l'eau de fond observée en août-septembre 2021 dans l'estuaire et le golfe du Saint-Laurent. L'échelle de couleur représente les différentes températures. Figure tirée de MPO (2022b).

Réponse physiologique au réchauffement, à l'acidification des océans et à l'hypoxie

Les expériences en laboratoire qui se sont penchées sur la détermination de la sensibilité de la crevette nordique aux facteurs des changements globaux se sont, jusqu'à maintenant, principalement concentrées sur les effets isolés de la température. Alors que les jeunes stades de vie montrent une croissance plus rapide lorsqu'ils sont exposés à des augmentations de température entre 2 et 8 °C (Brillon et al., 2005; Ouellet & Chabot, 2005; Stickney & Perkins, 1977), leur survie est plus faible, les mues sont anticipées, leur taille est plus petite et les réserves énergétiques sont réduites (Arnberg et al., 2013; Brillon et al., 2005; Daoud et al., 2010; Shumway et al., 1985; Wieland, 2004). Le réchauffement est aussi reconnu augmenter la consommation d'O_2 à la fois chez les larves (Arnberg et al., 2013; Chabot & Ouellet, 2005) et chez les adultes des deux sexes (Daoud et al., 2007; Dupont-Prinet et al., 2013; Hall, 2017). Les femelles adultes ont une croissance plus lente et se révèlent moins tolérantes lorsque la température environnementale augmente (Apollonio et al., 1986). Leur contenu en minéraux est plus élevé à des températures élevées, mais sans que la qualité organoleptique soit affectée (Chemel et al., 2020).

Présentement, les effets de l'acidification des océans chez la crevette nordique sont très peu connus. Chez les larves, la diminution de pH environnemental implique un ralentissement du temps de développement embryonnaire et larvaire (Arnberg et al., 2013; Bechmann et al., 2011) même si ni le temps de première éclosion, ni le succès et la durée de l'éclosion ne semblent être affectés par l'exposition des femelles ovigères à un pH de 7,6 (Arnberg et al., 2013). De même, le taux métabolique et le taux d'alimentation demeurent les mêmes en conditions acidifiées par rapport au contrôle (Arnberg et al., 2013). Chez les femelles de *P. borealis* l'exposition à un bas pH n'a globalement pas eu d'effet sur le contenu en minéraux ni sur la qualité organoleptique (Chemel et al., 2020). En revanche, l'exposition des mâles à la diminution du pH environnemental peut provoquer l'acidose extracellulaire, partiellement compensée à travers l'accumulation d'ions bicarbonates, et une légère diminution du taux métabolique (Hammer & Pedersen, 2013). Ces rares études suggèrent ainsi une tolérance de *P. borealis* à la diminution de pH environnemental. En revanche, l'exposition à un bas pH implique une augmentation significative du taux d'excrétion d'ammoniac ce qui indique une augmentation du catabolisme des protéines et des aminoacides. Il est possible aussi que l'ammoniac soit utilisée par les crevettes pour contribuer à la régulation

acido-basique, mais cela semble négligeable par rapport à l'accumulation d'ions bicarbonates, source première de compensation de l'acidose (Hammer & Pedersen, 2013).

Chez la crevette nordique, aucune étude a été menée sur l'effet de l'hypoxie sur les larves, cependant, chez les adultes, les femelles semblent être moins tolérantes par rapport aux mâles (Dupont-Prinet et al., 2013; Pillet, 2013; Pillet et al., 2016). En effet, le seuil critique de saturation d'O_2 des mâles est moins élevé que celui des femelles (9 % par rapport à 15,5 % sat.) à la température de 5 °C (Dupont-Prinet et al., 2013). Comme prévu, au-dessus du seuil critique, le TMS n'est pas affecté par les faibles niveaux d'OD (Dupont-Prinet et al., 2013; Hall, 2017) contrairement au TMM qui diminue significativement, réduisant le RA (Dupont-Prinet et al., 2013). Au niveau cellulaire l'exposition des organismes à l'hypoxie aigüe entraine peu de variations de l'activité des enzymes intervenant dans le métabolisme aérobie (Dupont-Prinet et al., 2013; Pillet, 2013). Seule l'activité spécifique de la CS est plus faible chez les femelles exposées à l'hypoxie aigüe et chronique (Dupont-Prinet et al., 2013; Pillet, 2013; Pillet et al., 2016). Contrairement aux attentes, l'exposition à l'hypoxie n'a pas augmenté l'activité des enzymes impliquées dans le métabolisme anaérobie ; en revanche, une baisse d'activité de la LDH a été mesurée chez les femelles exposées à l'hypoxie aigüe et chronique (Dupont-Prinet et al., 2013; Pillet, 2013; Pillet et al., 2016). De plus, le contenu en protéines, mesuré afin de standardiser les valeurs d'activité enzymatique dans le muscle et l'hépatopancréas, n'a pas subi de modifications significatives ni chez les femelles ni chez les mâles lors de l'exposition à l'hypoxie aigüe (Pillet, 2013). De même, le contenu en minéraux et la qualité organoleptique n'ont pas changé chez les femelles exposées à l'hypoxie chronique (Chemel et al., 2020). La crevette nordique semble être capable de supporter à long terme une diminution de la saturation d'oxygène jusqu'à 22% d'OD (Dupont-Prinet et al., 2013). En effet, dans le nGSL plus de 80 % de leur biomasse se trouve dans les zones à moins de 40 % sat. (Bourdages et al., 2022; Gilbert et al., 2007).

La plupart des études conduites en physiologie sur la crevette nordique examinent les effets d'une variable environnementale à la fois. Une seule étude s'est intéressée à l'effet combiné du réchauffement et de l'acidification des océans chez les larves, montrant un effet négatif de l'interaction de ces deux facteurs sur leur développement et le métabolisme (Arnberg et al., 2013). Des dix études s'intéressant à l'impact du réchauffement, de l'acidification des océans et de l'hypoxie sur les adultes de crevette nordique (Tableau 1), six se concentrent sur l'effet isolé de la

température. Seulement deux études prennent en considération l'effet isolé du pH/pCO_2 et cinq considèrent l'effet isolé de l'OD. Seules deux études sur dix se penchent sur l'effet combiné de la température et du pH et une seule autre sur l'effet combiné de la température et de l'OD. Une seule étude (issue de la collaboration avec ce projet de thèse) se penche sur l'effet combiné des trois facteurs environnementaux (Tableau 1). Les études multifactorielles conduites jusqu'à présent ont montré que la tolérance à l'hypoxie aigüe diminue avec l'augmentation de la température, et aussi, que chez les mâles et les femelles le seuil d'$O_{2\ crit}$ augmente (de 52 et 44 % respectivement) quand les organismes sont exposés à 8 °C par rapport au contrôle de 5 °C (Dupont-Prinet et al., 2013). En revanche, l'exposition de la crevette nordique à la combinaison d'hypoxie chronique et température élevée n'a pas eu d'effet significatif sur le taux métabolique ni sur l'endurance (représentée par le taux d'épuisement ; Hall, 2017). L'importance de conduire des expériences tenant compte de l'effet combiné de plusieurs paramètres environnementaux est soulignée chez les adultes lorsqu'elle implique une augmentation du taux de mortalité (Chemel et al., 2020; Dupont et al., 2014). Actuellement les effets combinés de l'augmentation de la température, la diminution du pH et la diminution de l'OD sur la réponse énergétique n'ont jamais été étudiés chez la crevette nordique, lacune à laquelle ce projet se propose de remédier.

Tableau 1 Résumé des réponses biologiques et physiologiques des adultes de crevette nordique *Pandalus borealis* face aux changements globaux isolés et combinés : réchauffement (T), acidification des océans (pH) et hypoxie (O_2). Les flèches indiquent la direction de la réponse, le symbole – indique qu'il n'y a pas de changement de réponse en fonction des conditions.

Références	Paramètre biologique ou physiologique	Facteurs environnementaux					
		T	pH	O_2	T x pH	T x O_2	T x pH x O_2
Brillon et al., 2005	Contenu énergétique	↓					
Daoud et al., 2007	Taux métabolique standard	↑					
Daoud et al., 2010	Période d'inter-mue	↓					
	Taux de croissance	↑					
Dupont-Prinet et al., 2013	Taux métabolique standard	↑		–			
	Taux métabolique maximal			↓			
	Registre aérobie			↓			
	Seuil d'oxygène critique	↑					
	Activité enzymatique			–			
Hammer & Pedersen, 2013	Taux métabolique standard		↓				
	Régulation acido-basique		–				
	Taux d'excrétion d'ammoniac		↑				
Pillet, 2013	Activité enzymatique			–			
Dupont et al., 2014	Survie				↓		
Pillet et al., 2016	Activité enzymatique			–			
Hall, 2017	Taux métabolique standard	↑		–		–	
Chemel et al., 2020	Contenu en minéraux	↑	–	–			
	Survie	–	–	–	↓		↓

VI. Objectifs de la thèse

L'objectif principal de cette thèse doctorale est de définir le niveau de sensibilité aux phénomènes de réchauffement, acidification des océans et hypoxie d'individus de la même espèce provenant de plusieurs origines géographiques différentes, caractérisées par des environnements physico-chimiques contrastés, en intégrant les réponses des individus aux différentes échelles d'organisation biologique (c.-à-d. de l'organisme à la cellule), dans le but de mieux comprendre le devenir de la crevette nordique sous contrainte des facteurs des changements globaux (température, pH et O_2) le long de la côte est canadienne (Figure 16).

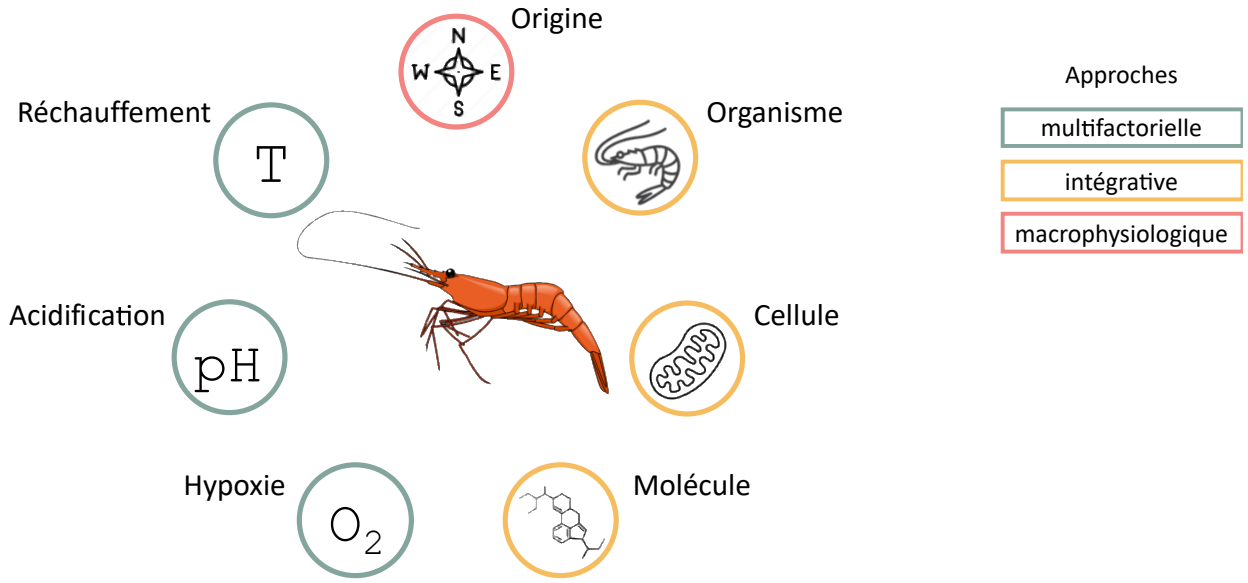


Figure 16 Représentation schématique de l'objectif général de cette thèse et des approches utilisées afin d'y répondre.

Pour cette étude, les femelles de crevette nordique ont été sélectionnées en raison du fait qu'elles sont la cible principale de la pêche, puisque leur taille est plus grande que celle des mâles, et parce qu'elles se sont révélées plus sensibles à l'hypoxie comparé aux mâles (Dupont-Prinet et al., 2013). Les femelles de crevette nordique seront identifiées comme « crevettes » pour la suite de ce document.

Pour répondre à l'objectif général de cette recherche, les approches multifactorielle, intégrative et macrophysiologique sont utilisées en co-occurrence dans cette thèse, qui se développe en 3 chapitres.

Le premier chapitre vise à caractériser les réponses biologiques et physiologiques, à plusieurs niveaux d'organisation biologique de crevettes provenant de SLE, exposées en laboratoire à la combinaison de plusieurs facteurs des changements globaux, soit l'augmentation de la température et la diminution du pH et de l'OD (Figure 17). Ce chapitre fait l'objet de l'analyse de la probabilité de survie et de la capacité aérobie des organismes entiers (traits métaboliques) intégrées avec la réponse cellulaire issue des analyses des activités enzymatiques (métabolisme aérobie et anaérobie). L'hypothèse générale de ce chapitre est que l'exposition à la combinaison des facteurs utilisés réduira de manière significative la probabilité de survie et la capacité aérobie (des crevettes survivantes et plus tolérantes), jusqu'à possiblement observer un passage du métabolisme aérobie

vers le métabolisme anaérobie. Ceci serait montré par l'augmentation du métabolisme de maintien et la diminution de la capacité du transport de l'oxygène au niveau de l'organisme entier, et par la diminution de l'activité des enzymes aérobie et l'augmentation de l'activité des enzymes anaérobie au niveau cellulaire.

Le chapitre suivant est dédié à l'évaluation du niveau de variation intraspécifique des réponses biologiques et physiologiques (à plusieurs niveaux d'organisation biologique) des crevettes provenant de quatre origines différentes, qui présentent différentes conditions physico-chimiques, et exposées à six scénarios combinant différentes conditions actuelles et prédites de température et pH (Figure 17). Ceci permettra de montrer si les différentes conditions environnementales en milieu naturel influencent la réponse des crevettes contribuant à la définition de la sensibilité de l'espèce. Les origines de collecte des crevettes sont situées dans le nord-ouest de l'Atlantique : estuaire du Saint-Laurent (SLE), chenal Esquiman (EC de l'anglais *Esquiman Channel*), est du plateau néo-écossais (ESS de l'anglais *Eastern Scotian Shelf*) et nord-est de la côte de Terre-Neuve (NNC de l'anglais *Northeast Newfoundland Coast*). Compte tenu des différences physico-chimiques existantes entre les différentes origines d'étude, notamment en termes de température, de la potentielle adaptation suggérée pour les larves de SLE et du GSL et de la sensibilité des adultes aux facteurs des changements globaux, tel que vu dans l'introduction de cette thèse, l'hypothèse générale de ce chapitre est que les taux de survie, la performance aérobie (traits métaboliques) et la capacité énergétique (activité enzymatique) diffèrent entre les crevettes des différentes origines. De plus, ces dernières possèderaient différentes habilités de réponse aux facteurs des changements globaux, ce qui les amènerait à montrer différents niveaux de sensibilité physiologique. En particulier, les crevettes de SLE et celles du EC seraient plus sensibles et vulnérables que les crevettes des autres origines car elles vivent déjà à des conditions similaires à celles attendues pour les autres origines à l'horizon 2100. En conclusion, afin de définir la sensibilité de l'espèce et sa vulnérabilité aux changements globaux, les réponses biologiques et physiologiques seront liées aux variables physico-chimiques mesurées à chaque origine géographique.

Le dernier chapitre, complémentaire aux précédents, vise à mettre en lumière les mécanismes sous-jacents des réponses biologiques et physiologiques des crevettes aux variables environnementales illustrées dans les chapitres 1 et 2, et à caractériser leur niveau de variation intraspécifique (Figure 17). Pour ce faire, les voies métaboliques impliquées dans les mécanismes de réponse ont été

déterminées, pour les crevettes de chaque origine, en utilisant l'approche métabolomique. L'hypothèse générale de ce chapitre est que les crevettes de différentes origines possèdent différents mécanismes de réponse et qu'on observe un passage du métabolisme aérobie vers le métabolisme anaérobie chez les crevettes exposées aux conditions les plus sévères. Dans ce cas spécifique on observerait une accumulation des métabolites intermédiaires du cycle de Krebs, en raison de son interruption, et une augmentation du lactate due au passage à la voie anaérobie pour la production de l'énergie.

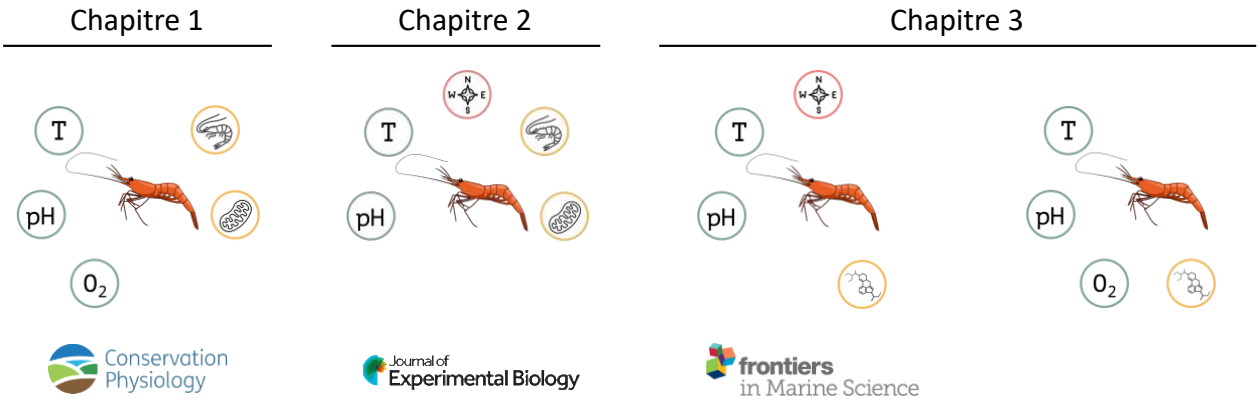


Figure 17 Représentation simplifiée des objectifs de chaque chapitre composant cette thèse et identification des journaux scientifiques choisis pour la publication des articles.

Cette thèse vise à montrer l'importance des approches multifactorielle, intégrative et macrophysiologique intégrant la mesure de plusieurs traits à différents niveaux d'organisation biologique d'organismes provenant de plusieurs origines géographiques pour mieux définir la sensibilité des espèces à multiples facteurs des changements globaux. Dans l'ensemble, elle a pour but de contribuer à une meilleure compréhension de la sensibilité de la crevette nordique aux facteurs des changements globaux considérés et prédits pour la fin du siècle et permettra d'explorer les conséquences sur la vulnérabilité de l'espèce.

CHAPITRE 1
LA SURVIE ET LA CAPACITÉ AÉROBIE DE LA CREVETTE NORDIQUE SONT MENACÉES PAR L'EXPOSITION AUX FACTEURS DES CHANGEMENTS GLOBAUX COMBINÉS

Ce premier article intitulé « *Survival and aerobic capacity of the northern shrimp are threatened by exposure to combined ocean global change drivers* » a été corédigé avec Denis Chabot (IML-MPO), Fanny Noisette (ISMER-UQAR), Pierre Blier (UQAR), Mathilde Chemel et Piero Calosi (UQAR). Sa version finale a été soumise pour révision par les pairs le 17 octobre 2022 dans la revue *Conservation Physiology* et as été acceptée avec révisions mineures le 24 Décembre 2022. Denis Chabot, Fanny Noisette et Piero Calosi ont fourni l'idée originale, conçu le plan expérimental général et fourni le support financier. Les crevettes, ainsi que les installations pour l'expérience, ont été fournies par Denis Chabot. Mathilde Chemel et moi-même avons mené à bien l'expérience, que j'ai complété en réalisant, avec le support de Tanya Hansen, le mesures de consommation d'oxygène. Les taux métaboliques ont été déterminés par Denis Chabot et moi-même. Sous la supervision de Véronique Desrosiers j'ai réalisé les analyses d'activité enzymatique dans le laboratoire de Pierre Blier. La rédaction, les analyses statistiques, les figures et les tableaux ont été réalisés par moi-même avec la contribution des co-auteurs.

Les différents résultats de cet article ont été présentés aux réunions annuelles du regroupement stratégique Québec Océan 2018, 2022 et 2023, à la réunion d'évaluation des stocks de crevette nordique de l'estuaire et du golfe du Saint-Laurent 2022 du MPO, au Symposium Ouranos 2022, au Forum sur les pêches mi'gmaques et malécites 2020 et au Forum Québecois en sciences de la mer 2019 (voir la liste des communications dans l'avant-propos de cette thèse).

1.1 RÉSUMÉ

Les changements environnementaux peuvent influencer le développement, la croissance, la taille, la distribution et l'abondance des espèces, et lorsqu'ils ont un impact négatif, ils peuvent potentiellement conduire au déclin d'une espèce et, ultimement, à son extinction locale. Par conséquent, l'évaluation de l'impact isolé et combiné des facteurs des changements globaux des océans est particulièrement pertinente pour les espèces d'importance écologique et économique qui garantissent la sécurité alimentaire et les revenus des communautés côtières. Cette étude visait à déterminer les réponses physiologiques de la crevette nordique *Pandalus borealis* à la combinaison du réchauffement, de l'acidification des océans et de l'hypoxie à plusieurs niveaux de son organisation biologique (c.-à-d. de l'organisme entier à la cellule), afin d'aider dans la prédiction du sort de cette espèce dans un océan en changement rapide. Pour ce faire, les crevettes ont été exposées pendant 30 jours à différentes combinaisons de température (2, 6 et 10 °C), de pH (7,75 et 7,40) et d'oxygène (100 et 35 % par rapport à la saturation de l'air), et leurs survie, performance aérobie et capacité énergétique cellulaire ont été caractérisées. Nos résultats montrent que les crevettes étaient dans l'ensemble tolérantes aux effets isolés du réchauffement, de l'acidification des océans et de l'hypoxie, mais lorsqu'elles étaient exposées aux facteurs combinés, leur survie et la performance aérobie de l'organisme entier diminuaient considérablement. Les facteurs isolés et combinés n'avaient dans l'ensemble aucun effet sur les activités enzymatiques, suggérant une faible capacité de réorganisation métabolique. Néanmoins, sous la combinaison des facteurs, nous avons observé un ajustement de la stœchiométrie des enzymes mitochondriales qui pourrait aider la cellule à maintenir son efficacité de production d'énergie. Dans l'ensemble, l'état physiologique de la crevette nordique est compromis par l'exposition à la combinaison des facteurs des changements globaux des océans, qui, combinés aux niveaux élevés de mortalité observés, soulignent le risque d'une extinction commerciale locale. Nos résultats seront utiles pour améliorer le cadre de modélisation qui projette l'abondance et la distribution futures de la crevette nordique, contribuant ainsi à la gestion et à la conservation des espèces en améliorant les évaluations des populations et des stocks commerciaux et les exercices de gestion.

Mots-clés : réchauffement des océans, acidification des océans, hypoxie, taux métaboliques, performance aérobie, activité enzymatique, *Pandalus borealis*, crustacés, fruits de mer, pêcheries.

1.2 SURVIVAL AND AEROBIC CAPACITY OF THE NORTHERN SHRIMP ARE THREATENED BY EXPOSURE TO COMBINED OCEAN GLOBAL CHANGE DRIVERS

Ella Guscelli[1,*], Denis Chabot[2], Fanny Noisette[3], Pierre U. Blier[1], Mathilde Chemel[1] and Piero Calosi[1]

[1]Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, QC G5L 3A1, Canada

[2]Institut Maurice-Lamontagne, Fisheries and Oceans Canada, 850 Rte de la Mer, Mont-Joli, QC G5H 3Z4, Canada

[3]Institut des sciences de la mer, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, QC G5L 3A1, Canada

*Corresponding author

1.3 ABSTRACT

Environmental changes can influence species development, growth, size, distribution, and abundance, and when having a negative impact they can potentially lead to a species' decline, and ultimately its local extinction. Consequently, evaluating the impact of isolated and combined ocean global change drivers is particularly relevant for ecologically and economically important species which guarantee food security and income for coastal communities. This study aimed to determine physiological responses of the northern shrimp *Pandalus borealis* to combined ocean warming (OW), acidification (OA) and hypoxia at multiple levels of its biological organization (i.e. from the whole-organism to the cell), to help in predicting the fate of this species in a rapidly changing ocean. To do so, shrimp were exposed for 30 days to different combinations of seawater temperature (2, 6 and 10 °C), pH (7.75 and 7.40) and oxygen (100 and 35 % relative to air saturation), and their survival, aerobic performance, and cellular energetic capacity were characterised. Our results show that shrimp were overall tolerant to the isolated effects of OW, OA and hypoxia, but when exposed to combined drivers their survival and whole-organism aerobic performance severely decreased. Isolated and combined drivers had overall no effect on enzymes activities, suggesting a low ability for metabolic reorganization. Nonetheless, under combined drivers, we observed an adjustment of the mitochondrial enzymes stoichiometry that might help the cell to maintain its energy production efficiency. Overall, the northern shrimp physiological status is compromised under combined ocean global change drivers, which together with the high mortality levels observed, point to the risk for a local commercial extinction. Our results will be useful to refine the modelling framework that projects the northern shrimp future abundance and distribution, thus contributing to the species management and conservation *via* improving population and commercial stock assessments and management exercises.

Key words: ocean warming, ocean acidification, hypoxia, metabolic rates, aerobic performance, enzyme activity, *Pandalus borealis*, crustacean, seafood, fisheries

1.4 INTRODUCTION

The global extinction of a species results from a series of local and regional extinction events, often announced by drastic declines in abundances (Dulvy et al., 2003; Pitcher, 2001), which in turn are the first alarm signals to improve the conservation and management of a species (Pitcher, 2001). Exploitation and habitat loss are currently the two major causes of local and regional extinctions in the ocean, however global changes are expected to gain in magnitude in the near future, therefore also representing a threat to global biodiversity (Dulvy et al., 2004). In fact, ocean global changes influence species' performance, survival, fertility, and ultimately their abundance and distribution (Parmesan, 2006), and may lead to their decline and eventually extinction (Calosi et al., 2019; Thomas et al., 2004). To anticipate mortality level, and thus prevent species extinction, there is an urgent need to acquire an in-depth understanding of an organism's biological and physiological response to global changes (Cooke et al., 2013; Wikelski & Cooke, 2006). However, the effects of global change drivers are highly dependent on the level of biological organisation considered (Harvey et al., 2014), with lower biological levels (e.g. cellular) defining the mechanisms underpinning the higher level of response (e.g. whole-organism), which are linked to the ecology of a species (Bartholomew, 1964; e.g. Calosi et al., 2017). Therefore, it is necessary to adopt an integrative approach to disentangle the complexity of biological systems, particularly within the context of rapid environmental changes we are undergoing, and investigate different levels of biological organization (Bartholomew, 1964; Harvey et al., 2014).

Marine species are often considered to be less vulnerable to extinction compared to terrestrial ones, as they usually possess broad geographic ranges, are highly fecund and have higher ability to evade capture (Pinsky et al., 2019; Roberts & Hawkins, 1999). However, local and regional extinction risks should not be underestimated, especially for species with paramount ecosystem engineer role and/or economic and food security value (Dulvy et al., 2003). Indeed, the loss of valuable species might impact the global seafood market size, which was valued at $159,311.9 million in 2019 and is predicted to increase by approximately 22 % by 2027 (Businesswire, 2020), as a result of increasing global human food consumption (FAO, 2018). In addition, seafood is an important source of various nutrients, such as proteins, lipids, vitamins and minerals, and contains unique fatty acids that have important health benefits (Hosomi et al., 2012). Moreover, costal zones host

more than half of the World's human population, who depend heavily on seafood to compensate for the loss in terrestrial resources threatened by climate change (Bindoff et al., 2019).

Species can avoid global decline and extinction *via* dispersing into more suitable habitats (Parmesan & Yohe, 2003). Many marine species already have shifted their geographic range in response to global changes (e.g. Perry et al., 2005). However, some species may not be able to do so, for example due to a lack of alternate suitable habitats available. Polar and subpolar species are especially at risk of decline when compared to species from other climatic regions, as their habitat is increasingly reduced with ongoing warming, and they are limited in how much they can shift northward or southward through migration (Wassmann et al., 2011). In addition, polar and subpolar species are thought to possess reduced homeostatic ability when compared to species at lower latitudes (Thor et al., 2018), thus being considered among the most vulnerable of marine taxa.

The northern shrimp *Pandalus borealis*, Krøyer 1838, is a cold-adapted species with a circumpolar distribution. It has a high ecological value, as it constitutes a large proportion of the diet of >25 cm redfish (*Sebastes* spp.), Greenland halibut (*Reinhardtius hippoglossoides*, Walbaum 1792) and Atlantic cod (*Gadus morhua*, Linnaeus 1758) (Bernier & Chabot, 2013; Brown-Vuillemin et al., 2022; Dawe et al., 2012; Ouellette-Plante et al., 2020), whilst it also has a high commercial value, in particular in eastern Canada (DFO, 2021a). As a cold-water species, the northern shrimp lives at temperatures ranging from 1 to 6 °C (Allen, 1959) and is sensitive to temperature increases at both larval and adult stages. In fact, temperature affects shrimp larval survival, development, growth and shrimp adult size (Arnberg et al., 2013; Brillon et al., 2005; Daoud et al., 2010; Ouellet & Chabot, 2005). Temperature is also known to affect metabolism of both larvae (Arnberg et al., 2013; Chabot & Ouellet, 2005) and adults (Daoud et al., 2007, 2010; Dupont-Prinet et al., 2013; Hall, 2017), as well as mineral content of female abdominal muscle (Chemel et al., 2020). The sensitivity of shrimp to temperature increases is of particular interest to investigate within the context of the ongoing phenomenon of ocean warming (OW) (IPCC, 2022). Nonetheless, ocean acidification (OA), a global change phenomenon consisting in the decrease in ocean pH and change in seawater carbonate chemistry (Feely et al., 2009; IPCC, 2022; Orr et al., 2005), is also known to affect the northern shrimp, increasing the duration of the larval development (Arnberg et al., 2013; Bechmann et al., 2011) and enhancing extracellular acidosis in adult males (Hammer & Pedersen, 2013). Interestingly, adult *P. borealis* show the ability to partially compensate

extracellular acidosis, suggesting males to be tolerant to increased pCO_2/decreased pH (hereafter decreased pH) (Hammer & Pedersen, 2013). Finally, another important (but highly understudied) global phenomenon is the progressive de-oxygenation of the World's oceans (IPCC, 2022). Within this context, male shrimp have been shown to be more tolerant than females when exposed to hypoxia (Dupont-Prinet et al., 2013; Hall, 2017). Nonetheless, severe hypoxia (35-22 % O_2 saturation relative to air, % O_2 sat. hereafter) decreases shrimp metabolic capacity (Dupont-Prinet et al., 2013) and affects metabolic pathways (Pillet et al., 2016).

Whilst our current understanding of the impacts of isolated ocean global change drivers (i.e. OW, OA and hypoxia) on the northern shrimp is relatively well-understood, only very few studies have attempted to shed light on their combined effects. For example, Dupont-Prinet et al. (2013) showed that the tolerance of *P. borealis* adults to hypoxia is reduced under increasing temperatures. In addition, survival rates decrease when shrimp are exposed to the combination of OW and OA, and decrease further when the effect of hypoxia is superimposed (Chemel et al., 2020; Dupont et al., 2014). Yet, no study has been conducted on the combined effects of these three major ocean global change drivers on shrimp physiological responses, despite the fact they can naturally co-occur and influence each other. For this reason, an ideal study system to test the combined effects of ocean global change drivers on northern shrimp physiological responses is the Estuary and Gulf of the St. Lawrence system (EGSL). In the EGSL, shrimp are most abundant in deep waters, between 150 and 300 m deep (Dupont-Prinet et al., 2013). Deep waters are characterised by temperatures that increased to 5-6 °C in the St. Lawrence Estuary (SLE) and to 6-7 °C in the Gulf (GSL) in 2020 (Galbraith et al., 2021), a warming of ~ 0.5–1.5 °C from the period 1991–2020 due to changes in water mass supply from the Labrador current and the Gulf Stream, which have decreased and increased respectively (Galbraith et al., 2021; Gilbert et al., 2007). Gulf Stream water masses are warmer and impoverished in oxygen, thus their proportional increase compared to the Labrador ones represents the major cause of increasing temperatures and decreasing dissolved oxygen (DO) levels in the EGSL (Gilbert et al., 2005, 2007; Jutras et al., 2020). In deep waters, shrimp experience average yearly values of ~30-40 % O_2 sat. in the GSL and ~18-25 % O_2 sat. in the SLE (Blais et al., 2019; Gilbert et al., 2005). Moreover, hypoxia and respiration of deep water organisms are responsible for deep water decrease in seawater pH (pH of 7.75, Mucci et al., 2011).

In this context, the aim of the present study was to determine, under laboratory conditions, the impact of isolated and combined ocean global change drivers on the survival probability, together with the aerobic capacity, of the northern shrimp in the EGSL. To do so, we exposed female shrimp to different combinations of seawater temperature, pH and oxygen for 30 days. Survival was determined daily over the exposure period, at the end of which we determined standard and maximum metabolic rates (SMR and MMR, respectively) using oxygen uptake ($\dot{M}_{O_2}$) as a proxy (Chabot et al., 2016a, 2016b; Norin & Clark, 2016). We then calculated shrimp aerobic scope (AS), the difference between MMR and SMR (Fry, 1971), and the temperature coefficient (Q_{10}), as proxy for metabolic rate sensitivity to temperature change (Schmidt-Nielsen, 1990). Finally, we measured the activity of aerobic and anaerobic enzymes in shrimp abdomen muscle tissue as proxy for shrimp cellular aerobic and anaerobic capacity. Based on our current understanding of global change single driver' effects on adult shrimp survival and metabolism, we hypothesise that the exposure to combined OW, OA and hypoxia will decrease survival probability and impact metabolic traits at the whole-organism level and on the aerobic energetic capacity at the cellular level.

1.5 MATERIAL AND METHODS

1.5.1 Specimen collection, transport and maintenance

Adult individuals of the northern shrimp *Pandalus borealis* were collected in May 2018 off the coast of Rimouski (QC, Canada, ~ 48° 35' N, 68° 35' W), and transported to the Maurice-Lamontagne Institute (MLI) of Fisheries and Oceans Canada (Mont-Joli, QC, Canada). Shrimp were maintained in rectangular rearing tanks (1700 L) for approximately eight weeks before the beginning of the experiment, to allow them to adjust to laboratory conditions. For more detailed information about the collection, transport and maintenance of shrimp, see Chemel et al. (2020).

1.5.2 Experimental design, setup, system monitoring and protocol

The experimental design and setup are identical to those used by Chemel et al. (2020). Briefly, to determine the effects of elevated seawater temperature, low pH and hypoxia, in isolation and combined, on the survival and whole-organism and cellular physiology of shrimp, we employed a collapsed experimental design (Figure 1.1) (see Boyd et al., 2018 for a definition and Britton et al., 2020 for a description). In more detail, we tested the combined effect of temperature and pH, and superimposed the effect of hypoxia to two of the "temperature x pH" treatments. Three levels of

seawater temperature were chosen: (i) 2 °C, for most shrimp populations of the north-west Atlantic, the bulk of the biomass is found at 0–4 °C (see for instance, Orr and Sullivan, 2013) and 2 °C was selected as a favourable temperature: an important proportion of the shrimp used for this study was found at 1–4 °C in 2008–2017 (DFO, 2022a); (ii) 6 °C, to account for the +4 °C scenario predicted globally (RCP 8.5 scenario, IPCC, 2014) and to represent recent (1990–2017) temperature for shrimp in the Gulf of St. Lawrence (GSL) (5–6 °C, DFO, 2022a); and (iii) 10 °C, representing conditions at the end of the century in the GSL (Lavoie et al., 2020). Two levels of pH were also selected, pH 7.75 based on the current conditions in the deep waters of the EGSL (Mucci et al., 2011, 2018) and pH 7.40, representing a pH decrease between -0.3 and -0.4 pH unit predicted to occur in the EGSL by the year 2100 (RCP 8.5 scenario, IPCC, 2014). Finally, we chose two levels of dissolved oxygen (DO), normoxia 100 % and hypoxia 35 % air saturation, a non-lethal (chronic) level of hypoxia commonly encountered by shrimp in the EGSL (Dupont-Prinet et al., 2013). Our collapsed experimental design consisted of a total of eight treatments with two replicate tanks *per* treatment, designated as follow: low temperature and current pH (2C: 2 °C, pH 7.75, normoxia), low temperature and low pH (2A: 2 °C, pH 7.40, normoxia), intermediate temperature and current pH (6C: 6 °C, pH 7.75, normoxia), intermediate temperature and low pH (6A: 6 °C, pH 7.40, normoxia), elevated temperature and current pH (10C: 10 °C, pH 7.75, normoxia), elevated temperature and low pH (10A: 10 °C, pH 7.40, normoxia), low temperature, current pH and low DO (2CH: 2 °C, pH 7.75, hypoxia) and elevated temperature, low pH and low DO (10AH: 10 °C, pH 7.40, hypoxia). Employing a collapsed experimental design allowed us to explore (i) the impact of elevated seawater temperature and low pH, in isolation and combined, on the traits investigated (*Design A*) and (ii) the isolated effect of low oxygen and its superimposed effect upon combined elevated seawater temperature and low pH (*Design B*, treatments: 2C, 2CH, 10A, 10AH).

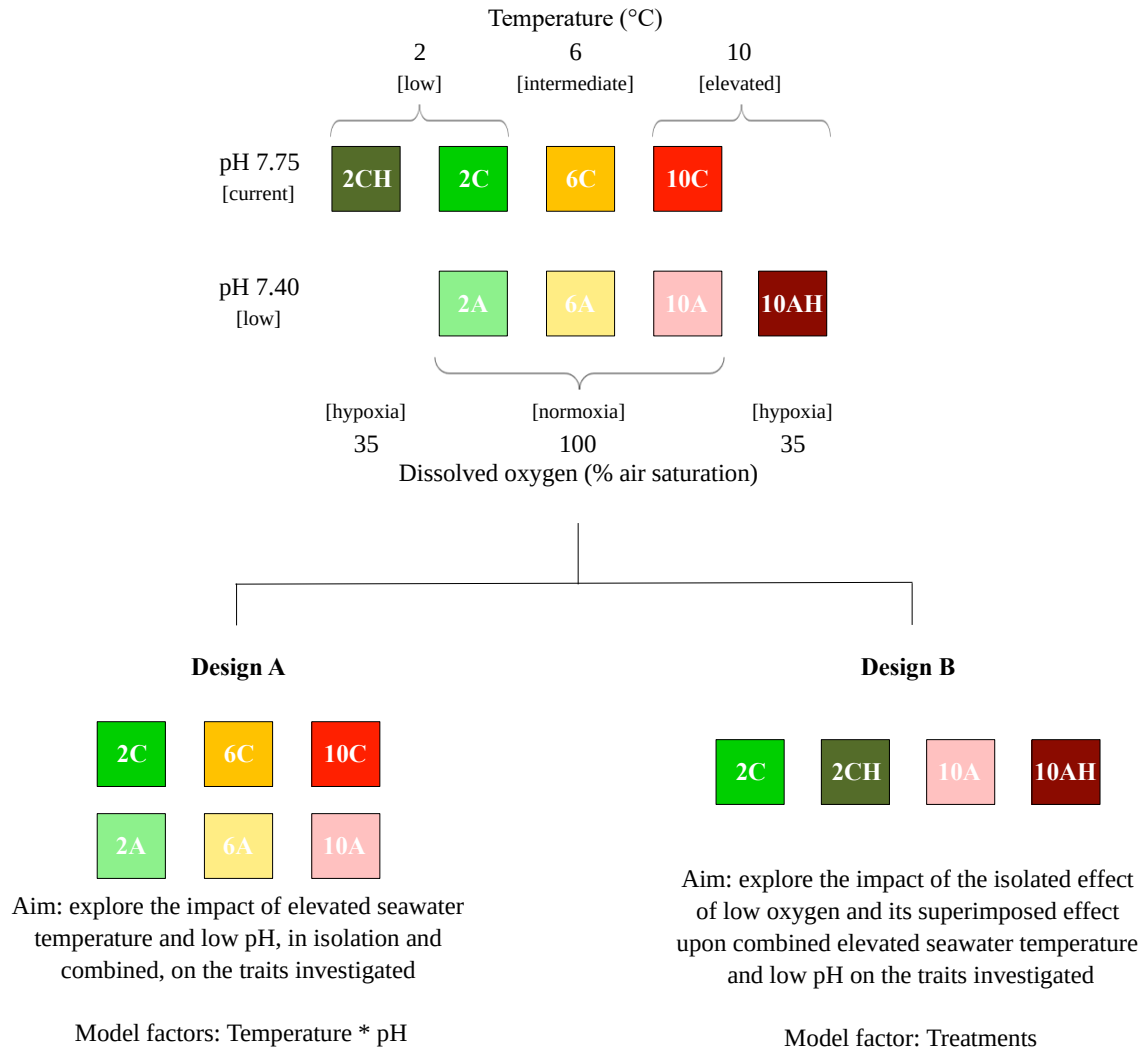


Figure 1.1 Schematic representation of the collapsed experimental design and of the two design (*Design A* and *B*) used to determine the effects of elevated seawater temperature, low pH and low oxygen, in isolation and combined, on the survival and physiology of female of the northern shrimp *Pandalus borealis* exposed over 30 d. Treatments correspond to: 2C **(**2 °C, pH 7.75, normoxia - green), 2A (2 °C, pH 7.40, normoxia - light green), 6C (6 °C, pH 7.75, normoxia - yellow), 6A (6 °C, pH 7.40, nomoxia - light yellow), 10C (10 °C, pH 7.75, nomoxia - red), 10A (10 °C, pH 7.40, nomoxia - light red), 2CH (2 °C, pH 7.75, hypoxia - dark green) and 10AH (10 °C, pH 7.40, hypoxia - dark red).

The regulation and monitoring of temperature, pH and oxygen in the experimental setup were identical as those described in Chemel et al. (2020). Briefly, the open-flow experimental tanks were supplied with sea water from two reservoirs at a constant flow rate of 3.5 L min^{-1} and equipped with a regulation system. The regulation system consisted of two independent feedback systems: one that allowed the regulation of temperature (1/16 DIN Micromega autotune PID Temperature, Omega Engineering inc., Norwalk, USA), and one that allowed the regulation of pH and oxygen (IKS Aquastar, Karlsbad, Germany). The feedback system (i) regulated the automatic mixing of cold and warm water to provide each tank with sea water at the set temperature and (ii) controlled the injection of pure gaseous CO_2 and N_2 into each tank's gas exchange column to maintain respectively pH and DO levels at target values. Environmental parameters were monitored daily with handheld multimeters in each tank throughout the duration of the experiment. Carbonate chemistry parameters were calculated weekly, based on pH and alkalinity measurements, using the R package seacarb (Gattuso et al., 2021). For more details about the instruments and techniques used see Chemel et al. (2020). Mean ± SD physico-chemical parameters for the duration of the 30-d experiment are summarized in Table 1.1.

Table 1.1 Summary (mean ± SD) of the physico-chemical parameters of the sea water measured and calculated (*) over the duration of the experiment (30 d) in different treatments: temperature (°C), pH (Total scale), dissolved oxygen (% sat.), salinity, total alkalinity (TA, µEq kg^{-1}), total dissolved carbon dioxide* (DIC, µmol kg^{-1}), carbon dioxide partial pressure* (pCO_2 µatm), bicarbonate concentration* ([HCO_3^-], µmol kg^{-1}) and carbonate concentration* ([CO_3^{2-}], µmol kg^{-1}), Ω calcite* and Ω aragonite*. Treatments correspond to 2C (2 °C, pH 7.75, normoxia), 2A (2 °C, pH 7.40, normoxia), 6C (6 °C, pH 7.75, normoxia), 6A (6 °C, pH 7.40, normoxia), 10C (10 °C, pH 7.75, normoxia), 10A (10 °C, pH 7.40, normoxia), 2CH (2 °C, pH 7.75, hypoxia) and 10AH (10 °C, pH 7.40, hypoxia).

Treatment	Temperature	pH_T	Oxygen	Salinity	TA	DIC *	pCO_2 *	[HCO_3^-] *	[CO_3^{2-}] *	Ω cal *	Ω ara *
2C	2.32 ± 0.10	7.76 ± 0.01	99.85 ± 0.19	32.96 ± 0.08	2071.87 ± 13.24	2065.88 ± 13.46	752.34 ± 19.79	1972.72 ± 12.98	49.56 ± 1.43	1.2 ± 0.03	0.75 ± 0.02
2A	2.35 ± 0.12	7.42 ± 0.02	99.75 ± 0.17	32.94 ± 0.08	2056.21 ± 13.07	2130.36 ± 15.09	1690.98 ± 55.31	2007.62 ± 13.65	24.29 ± 1.45	0.59 ± 0.03	0.37 ± 0.02
2CH	2.46 ± 0.11	7.74 ± 0.01	36.10 ± 1.90	32.97 ± 0.08	2067.63 ± 13.01	2065.61 ± 13.39	783.04 ± 17.39	1973.42 ± 12.83	47.09 ± 0.97	1.14 ± 0.02	0.71 ± 0.01
6C	6.02 ± 0.06	7.74 ± 0.01	98.08 ± 0.29	32.96 ± 0.07	2040.07 ± 13.33	2026.49 ± 13.74	789.28 ± 18.88	1933.12 ± 13.25	53.46 ± 1.13	1.29 ± 0.03	0.81 ± 0.02
6A	6.09 ± 0.07	7.43 ± 0.02	97.55 ± 0.15	33.07 ± 0.07	2067.63 ± 13.01	2124.2 ± 13.74	1694.84 ± 52.39	2009.68 ± 12.76	28.97 ± 1.61	0.70 ± 0.04	0.44 ± 0.02
10C	9.68 ± 0.10	7.76 ± 0.01	97.16 ± 0.20	33.11 ± 0.07	2056.21 ± 13.07	2025.04 ± 13.57	769.17 ± 21.95	1925.39 ± 13.24	65.39 ± 1.39	1.58 ± 0.03	1.00 ± 0.02
10A	9.61 ± 0.14	7.45 ± 0.02	97.26 ± 0.21	33.03 ± 0.07	2040.07 ± 13.33	2080.44 ± 15.4	1680.24 ± 68.89	1971.38 ± 14.32	34.34 ± 1.83	0.83 ± 0.04	0.52 ± 0.03
10AH	9.64 ± 0.12	7.43 ± 0.02	37.55 ± 2.04	33.18 ± 0.07	2071.87 ± 13.24	2118.49 ± 14.32	1768.75 ± 52.37	2007.71 ± 13.31	32.07 ± 1.49	0.77 ± 0.04	0.49 ± 0.02

For this experiment, we selected non-ovigerous females to avoid any potential confounding effect linked to differences in reproductive stages and the oxygen consumption of the egg mass and because they are the main target of the fishery and are more sensitive than males to ocean global change drivers such as hypoxia (Dupont-Prinet et al., 2013). Groups of approximately 70 females were randomly assigned to one of the 16 tanks of the experimental setup. Starting from the conditions prevailing during the stabulation period (4.5 °C, pH 7.90, normoxia, salinity 33), the experimental tanks were gradually adjusted over 4 d until treatment values for temperature, pH and oxygen were reached, whilst keeping salinity always close to 33. Shrimp were then exposed for a total of 30 d at each treatment. During the exposure period shrimp were fed *ad libitum* three times a week, with equal proportions of capelin (*Mallotus villosus*, Müller 1776) and shrimp (*Pandalus* spp.) and uneaten food was removed 24 h after each feeding period. We recorded the number of live shrimp daily, to determine survival rate throughout the experiment, and removed dead individuals and exuviae to prevent the proliferation of bacteria and ammonia accumulation. This allowed the maintenance of high water quality. Shrimp were fasted after 23 d of exposure to avoid energy demands related to digestion during the oxygen uptake measurement which started on day 28, when five individuals *per* tank (10 *per* treatment) were haphazardly selected and prepared for the measurement (see section 3.2 below for details). At the end of each oxygen uptake measurement, at day 30, individuals were removed from the respirometer, gently blotted with tissue paper, and weighed with a digital high-precision scale (Mf-300, A&D Company, Tokyo, Japan; ± 0.001 g) in order to determine wet mass (WM). Finally, to preserve the abdomen muscle tissue for further analyses, shrimp were rapidly dissected on ice, to prevent tissue degeneration. The procedure lasted less than a minute. The cephalothorax and the carapace were carefully removed using ceramic knife and plastic forceps. The abdomen muscle tissue, representing the largest proportion of body mass, was then sectioned in three equal parts (approximately 1 g each), placed in Eppendorf tubes and flash-frozen in liquid nitrogen, to instantly interrupt all biochemical reactions. Samples were stored at -80 °C for further analyses.

1.5.3 Metabolic traits and temperature coefficient

In order to determine the effect of combined ocean global change drivers on shrimp metabolic rates, individuals' oxygen uptake ($\dot{M}O_2$, in mg O_2 h^{-1}) was used as a proxy (Chabot et al., 2016a, 2016b; Fry, 1971; Norin and Clark, 2016) and measured *via* intermittent-flow respirometry in static

respirometers (Steffensen, 1989; Svendsen et al., 2016). Specifically, resting condition $\dot{M}_{O_2}$ was used as proxy for standard metabolic rate (SMR) (Chabot et al., 2016a; Fry, 1971) and post exercise $\dot{M}_{O_2}$ was used as proxy for maximum metabolic rate (MMR) (Fry, 1971; Norin & Clark, 2016). We then calculated the aerobic scope (AS = MMR – SMR) (Fry, 1947) as it provides a tangible estimate of the aerobic metabolic rate available to an animal above SMR. Additionally, the temperature coefficient (Q_{10}) was calculated for both SMR and MMR to assess the sensitivity of these traits to temperature variations (Schmidt-Nielsen, 1990).

1.5.3.1 Respirometry method and setup

Intermittent-flow respirometry consists of an alternating series of closed phases permitting the measurement of oxygen uptake, and open phases when water flow resumed to renew the water inside the respirometry chambers (hereafter respirometer). Respirometers used to determine oxygen uptake, were kept in two dedicated respirometry tanks, independent from the experimental tank, but identical in their setup (see section 2 above for details). Each respirometry tank was filled with sea water at the same temperature, pH and DO as that of the specific treatments shrimp were kept within the experimental system. Each respirometry tank hosted ten custom-built cylindrical glass respirometers (vol. ≈ 300 mL) and two submersible flush pumps (1048, Eheim, Stuttgart, Germany) with two PVC manifold that supplied clean and aerated seawater to five respirometers each. Each respirometer was equipped with a recirculation loop, consisting of a recirculating pump (AD20P-1230C, DollaTek, Hong Kong, China) and Tygon tubing, to ensure good mixing (Svendsen et al., 2016). Respirometers were flushed regularly (see section 3.2 below for details) and water overflow was evacuated above the water surface. During the closed phase and after a stabilisation period, DO was measured every second with fiber optic oxygen probes (OXYPro DP-PSt3, PreSens Precision Sensing GmbH) placed on the positive pressure side of the recirculation loop and coupled to an oxygen meter (Oxy-4 mini or Fibox 3, PreSens Precision Sensing GmbH, Regensburg, Germany). Data acquisition and flush activation was controlled by a data acquisition unit (DAQ-M, Loligo systems apS, Viborg, Denmark) associated to the AutoResp 2™ software (Loligo systems apS).

1.5.3.2 Oxygen uptake measurement

In order to measure shrimp oxygen uptake, specimens were first individually transferred to a flow-through tank (i.e. exhaustion tank) were seawater parameters matched those of the specific experimental treatment from which the shrimp was haphazardly selected. Here, individuals were chased to exhaustion, defined as the moment they were not able to flick their tail anymore. Time of exhaustion and the number of tail flicks were recorded. Immediately, shrimp were exposed for 1 min to air to further increase their oxygen debt (method modified from Roche et al., 2013), and then rapidly but carefully transferred into individual respirometers. During the first 2 h or so (long closed phase), the respirometers were not flushed to ensure that the fastest rate of DO decline did not occur during a flush phase. For the rest of the experiment (over 2 d) respirometers were flushed with experimental treatment seawater for 300 s and closed for 660 s to enable multiple $\dot{M}O_2$ measurements used to estimate SMR. The linear decline in DO observed during the last 480 s was used to calculate $\dot{M}O_2$ according to Steffensen (1989; equation 2) and Garcia and Gordon (1992; equation 8) for oxygen solubility. Background $\dot{M}O_2$ was measured before shrimp were introduced into the respirometers and after shrimp removal and a linear regression was fitted to the background respiration values. This regression of background respiration as a function of time for each shrimp was used to correct $\dot{M}O_2$.

1.5.3.3 Determination of SMR and MMR

Shrimp SMR and MMR were determined from the raw data acquired by the AutoResp 2™ software (DO as a function of time). To improve signal to noise ratio, raw data were first smoothed with a running average with window width varying from 15 to 75 depending on the amount of noise present in the data for a given shrimp. The low signal to noise ratio was caused by a relatively high ratio of water volume to shrimp mass (40.6 ± 5.7). The smooth signal was then split into 20-s blocks and the median DO of each block was retained. This further improved the signal to noise ratio (Chabot et al., 2021). Processed traces of DO over time were plotted and $\dot{M}O_2$ recalculated for all slopes for a given shrimp. These plots were examined to select an appropriate minimal value of r^2 below which slopes were deemed unusable (too noisy or not linear). For most shrimp, minimum r^2 was set to 0.95. In rare cases the minimum r^2 was decreased (to a minimum of 0.80) to include a higher number of slopes, which were judged to be linear despite their lower r^2. For each shrimp SMR was determined using the quantile method detailed in Chabot et al. (2016b), with

a q of 0.2, after excluding $\dot{M}O_2$ determinations obtained during the recovery and acclimation periods: 15 and 24 h for normoxia and hypoxia treatments, respectively, as visual examination of the plots of $\dot{M}O_2$ *vs.* time showed that shrimp required more time to pay their oxygen debt and acclimate to the respirometer under hypoxia conditions.

MMR was estimated as in Zhang et al. (2019). Oxygen uptake typically slows down rapidly when a fatigued animal is placed in a static respirometer and calculating regressions of DO over time using short time periods reduces the likelihood of underestimating MMR. The shortest reliable window width was estimated to be 3 min (Supplementary Material – Determination of the shortest reliable interval to estimate maximum metabolic rate (MMR)). Rolling regressions (i.e. regressions calculated over 3 min, each one starting 1 s later than the previous one) were calculated over the long closed phase and the steepest slope was taken to calculate MMR. A second estimate of MMR for each shrimp was the highest $\dot{M}O_2$ observed during the regular intermittent-flow cycles, due to spontaneous activity. MMR was the highest of the two values (Dupont-Prinet et al., 2013) and in the majority of cases, it was the post-chase value.

Of the 80 shrimp for which SMR and MMR were determined *via* respirometry, nine individuals were removed from the final dataset: six shrimp moulted and/or died in the respirometers and three others did not reach low stable $\dot{M}O_2$ levels confirming that they acclimated to the respirometer, or failed to increase $\dot{M}O_2$ following exercise.

1.5.3.4 Temperature coefficient calculation

The temperature coefficient (Q_{10}) of female shrimp was calculated for SMR and MMR as:

$$Q_{10} = (R_2 / R_1)^{10/(T2 - T1)}$$

Where R_1 and R_2 are the mean metabolic rates (mean SMR and mean MMR) at temperatures $T2 > T1$ (Schmidt-Nielsen, 1990).

1.5.4 Cellular aerobic and anaerobic capacity

We measured aerobic and anaerobic enzyme activity in shrimp abdomen muscle tissue as proxy for shrimp cellular energetic capacity. To relatively determine aerobic capacity, we measured the

citrate synthase (CS), the cytochrome C oxidase (COX) and the complexes I and III (electron transport system, ETS) activities, which are respectively a proxy for mitochondrial density (Moyes et al., 1997; Rabøl et al., 2006), and for aerobic metabolic capacity or maximum mitochondrial oxygen consumption rate (Marie et al., 2006; Schmidlin et al., 2015). Additionally, we measured the lactate dehydrogenase (LDH) activity, which is a proxy for anaerobic glycolytic capacity (Farhana & Lappin, 2021). We then calculated the ratio of (1) CS to ETS activities and (2) COX to ETS activities as any divergence between CS and ETS activity and COX to ETS activities expresses differences in mitochondrial morphology or structural and functional organization; (3) CS to LDH activities and (4) COX to LDH activities as any divergence between CS and LDH activity and COX to LDH activities should express differences in metabolic phenotype; and (5) CS to COX activities as any divergence between CS and COX activity suggest differences in mitochondrial morphology, indeed this ratio relates to the surface to volume ratio of mitochondria.

To measure enzyme activity, frozen abdomen muscle tissue samples were rapidly minced on ice with a razor blade (11-515 1-1/2 inch, Stanley, Towson, Maryland) and weighed with a high precision digital scale (Quintix X124-1S, Sartorius, Göttingen, Germany; ± 0.0001 g). Samples were then homogenised on ice using a sonicator (Polytron PT 1200, Kinematica AG, Luzern, Switzerland) with four volumes of phosphate buffered saline solution (PBS, pH 7.5) containing 0.1 % Triton X-100 and 1 mM methylene diamine tetra-acetic acid (EDTA). Post homogenization, samples were centrifuged (500 *g*) for 5 min at 4 °C (centrifuge 5810 R, Eppendorf, Hamburg, Germany). Finally, the supernatant was divided into aliquots for enzyme activity measurements. The CS, COX and LDH activities were measured according to Thibeault et al. (1997) and the ETS activity was measured according to Lannig et al. (2003). Total protein content was determined on homogenates using the bicinchoninic acid method (Smith et al., 1985) with BSA as standard. For each analysis, homogenates were diluted to obtain linear reaction slopes for a minimum of 5 min. The dilution factor was 5 for CS, COX and protein content analyses, 20 for ETS and 100 for LDH. Analyses were carried out at a constant temperature of 20 °C using a spectrophotometer (Cary 100 UV-Vis, Agilent, Petaling Jaya, Malaysia) and the proprietary software (CaryWin UV, 4.20). Total protein contents were measured using a UV/VIS microplate spectrophotometer (Perkin Elmer Envision, Foster City, CA, USA). All chemicals used for these essays were obtained from Sigma-Aldrich or Thermo Fisher Scientific. The enzymatic activities were measured and expressed as U

g^{-1} of wet tissue and U g^{-1} of protein for total and specific activity respectively. All analyses were performed in duplicate and if the two measurements were more than 10 % apart, the analysis was repeated. For seven shrimp the COX activity measurements remained more than 10 % apart, thus those shrimp were removed from the dataset.

1.5.5 Statistical analysis

In order to test for the effects of elevated temperature, low pH and low oxygen in isolation, and combined, on shrimp survival over 30 d, survival was expressed as survival probability: i.e. the probability of surviving at the end of the 30 d exposure period. Thus, we compared survival probability curves between treatments, using a Kaplan-Meier plot. At day 28, five shrimp *per* tank were removed from the experimental tank for respirometry measurements, thus, they were declared right censored. To compare survival probability curves between treatments, we first performed a log-rank test (survival package, Therneau and Lumley, 2015) to compare survival curves within treatments to determine if the two replicate tanks differed. As no significant difference between replicates was found (min p-value = 0.11), shrimp from replicate treatments were pooled to produce a Kaplan-Meier plot complemented by a log-rank test. Bonferroni corrected log-rank *post-hoc* tests were then computed to compare the survival curves of the different treatments.

Mixed effect models (lmerTest package, Kuznetsova et al., 2017; and lme4 package, Bates et al., 2015) were used to test the effect of ocean global change drivers on shrimp SMR, MMR, AS and enzyme activities and ratios. The effect of ocean warming and acidification, in isolation and combined, on the traits investigated was tested on treatments employed in *Design A*. Temperature and pH were set as crossed fixed factors and replicate tank as a random variable to account for the non-independence of shrimp in the same tank. Additionally, *Design B* was used to explore the isolated effect of low oxygen and its superimposed effect upon combined elevated seawater temperature and low pH on the traits investigated: treatments were set as fixed factors and replicate tank as a random variable. For both designs, SMR, MMR, AS and wet mass (WM) were $\log_{10}$ transformed to meet the assumption for linearity and $\log_{10}$ WM was set as covariate. In addition, as $\log_{10}$ WM had a significant effect on $\log_{10}$ SMR and $\log_{10}$ AS when analysed to test the effects of treatments, it was kept as covariate for all analyses. The effect of mass on SMR, MMR and AS between treatments was verified testing the homogeneity of the slopes which were overall

considered to be homogeneous between treatments. As replicate tank was never significant, the term "tank" was removed from the analysis, thus an ANCOVA was performed on SMR, MMR and AS and an ANOVA was performed on enzyme activity and ratios (lmTest package, Hothorn et al., 2015). Tukey HSD tests (Hothorn et al., 2008) were used to conduct *post-hoc* analyses when significant effects were evidenced. Normality of distribution assumptions were tested using the Shapiro-Wilk's test. COX data (in *Design A*), ETS data and CS:COX data were $\log_{10}$ transformed to meet the normality assumption. Homoscedasticity was verified using both the Brown-Forsythe's and Fligner-Killeen's tests, in all cases the variances were homogeneous with the exception for $\log_{10}$ CS:COX for which we performed a Welch's ANOVA followed by Games-Howell *post-hoc* tests to analyse the effect of hypoxia and its superimposed effect upon combined elevated seawater temperature and low pH on this trait (rstatix package, Kassambara, 2021). A visual analysis of the residuals was also performed to confirm the appropriateness of the models used. Pearson's correlations between total and specific enzymatic activity responses were verified for all enzymes. All enzymes measured in shrimp muscle tissue were significantly correlated (r between 0.80 and 0.97 depending on the enzymes, $p < 0.001$ for all analyses) so only specific activity are presented. All analyses were performed using the software R 3.6.3 version (R Core Team, 2020).

1.6 RESULTS

1.6.1 Survival

Survival probability curves for females of the northern shrimp *Pandalus borealis* exposed for 30 d to different combination of ocean global change drivers are presented in Figure 1.2. Differences in survival among different treatments were observed ($\chi^2(7, N = 1067) = 256$, $p < 0.0001$). In more details, the treatment combining the highest temperature, low pH and low DO (10AH) resulted in the fastest and greatest reduction in survival over the exposure period, which drastically dropped to approximately 40 %, being significantly different from all other treatments. At the elevated temperature and low pH treatment (10A) survival probability decreased less acutely when compared to the 10AH treatment, but was still significantly different from all other treatments. Finally, survival probabilities curves did not significantly differ among all other treatments tested, and their average survival was 88 % at the end of the exposure period.

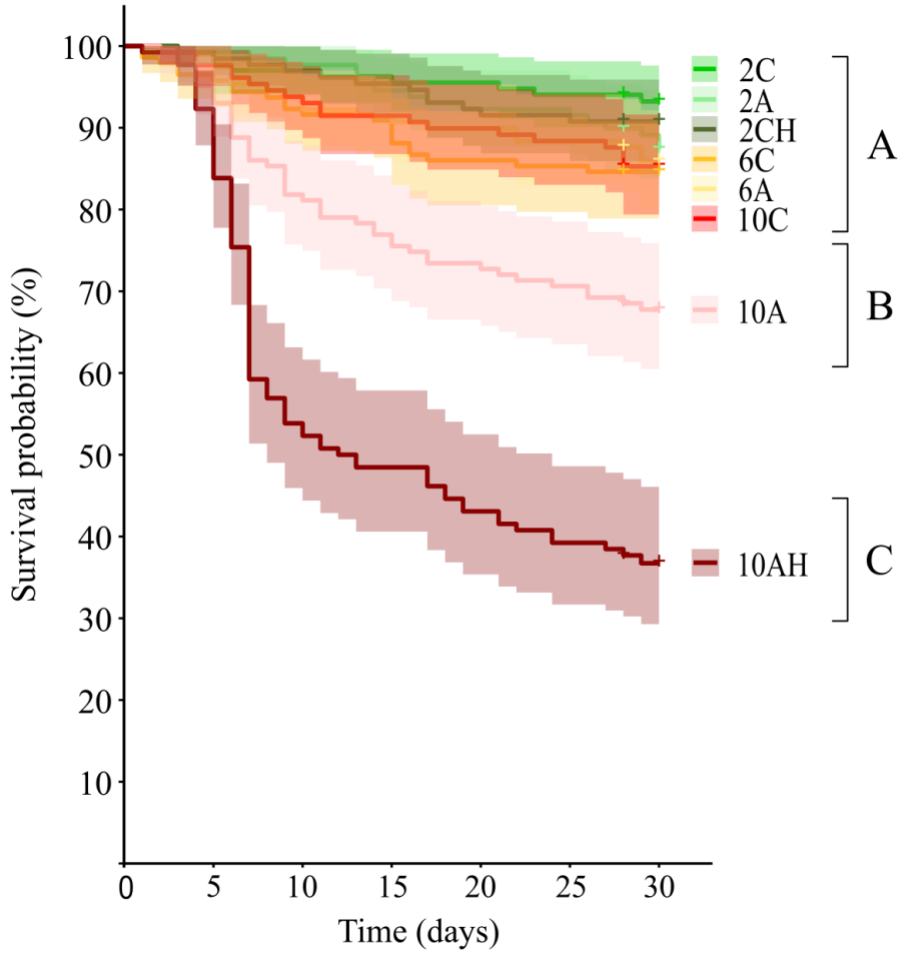


Figure 1.2 Kaplan-Meier plot of survival probability curves for female shrimp *P. borealis* exposed over 30 d to elevated temperature, low pH and low oxygen, in isolation and combined. Each curve represents the survival probability of a specific treatment, and the shaded areas represent its 95 % CI. Treatments correspond to 2C (green), 2A (light green), 6C (yellow), 6A (light yellow), 10C (red), 10A (light red), 2CH (dark green) and 10AH (dark red). Upper case letters identify significant differences ($p < 0.05$) among treatments.

1.6.2 Standard metabolic rate

Mean SMR measured in northern shrimp exposed to elevated temperature, low pH and low oxygen in isolation and combined are summarised in Table 1.2.

Design A: Mean SMR increased with increasing temperatures (Table 1.3, Figure 1.3), along a linear trend, significantly increasing from 0.37 to 0.65 mg O_2 h^{-1} at 2 and 10 °C, respectively ($p < 0.05$). In addition, no effect of pH in isolation or of the interaction between temperature and pH on this trait was found (Table 1.3).

Design B: Different treatments showed a significant difference in mean SMR (Table 1.3, Figure 1.3). In particular, mean SMR values measured at the 10 °C treatments were two times higher when compared to those at the 2 °C treatments. However, mean SMR between the normoxic and hypoxic treatment were comparable regardless of the temperature.

1.6.3 Maximum metabolic rate

Mean MMR measured in northern shrimp exposed to elevated temperature, low pH and low oxygen in isolation and combined are summarised in Table 1.2.

Design A: An increase in temperature led to an increase in mean MMR (Table 1.3, Figure 1.3). In more detail, mean MMR values measured at the two highest temperatures tested were significantly higher (34 and 42 %) than that reported at the lowest temperature condition, but comparable with each other. In addition, a significant decrease of 14 % in mean MMR was observed at low pH (Table 1.3, Figure 1.3). The interaction between temperature and pH was found to have no significant effect on mean MMR (Table 1.3).

Design B: Different treatments showed a significant difference in mean MMR (Table 1.3, Figure 1.3). Specifically, mean MMR at the low temperature, current pH and low DO treatment (2CH) was the lowest observed, followed by the one measured at the 10AH treatment, the one measured at low temperature and current pH (2C) treatment and finally, the one measured at the 10A treatment. Additionally, mean MMR values of shrimp exposed to the hypoxic treatments at both temperatures were significantly lower (55 and 41 %, at 2 and 10 °C respectively) when compared to mean MMR values for shrimp exposed to the normoxic treatments at both temperatures.

Table 1.2 Summary of mean (± SE) of morphological and physiological traits measured in females of the northern shrimp *Pandalus borealis* exposed over 30 d to elevated temperature, low pH and low oxygen, in isolation and combined. Here we provide *per* treatment: *Wet body mass* (WM); *Metabolic Traits* – Standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS); *Cellular energetic capacity* – specific activity of Citrate Synthase (CS), Cytochrome C Oxidase (COX), Electron Transport System (ETS), Lactate Dehydrogenase (LDH), and ratios for Citrate Synthase – Electron Transport System Ratio (CS:ETS), Cytochrome C Oxidase – Electron Transport System (COX:ETS), Citrate Synthase – Lactate Dehydrogenase Ratio (CS:LDH), Cytochrome C Oxidase – Lactate Dehydrogenase (COX:LDH) and Citrate Synthase – Cytochrome C Oxidase (CS:COX).

	Treatment	**2C**	**2A**	**2CH**	**6C**	**6A**	**10C**	**10A**	**10AH**
		n = 9	n = 9	n = 9	n = 9	n = 9	n = 8	n = 9	n = 9
	WM (g)	8.41 ± 0.22	9.98 ± 0.62	9.14 ± 0.50	9.33 ± 0.30	9.06 ± 0.39	9.08 ± 0.17	8.89 ± 0.39	10.16 ± 0.58
Metabolic	SMR (mg O_2 h^{-1})	0.38 ± 0.03	0.36 ± 0.02	0.32 ± 0.02	0.52 ± 0.02	0.56 ± 0.06	0.61 ± 0.03	0.70 ± 0.05	0.72 ± 0.05
	MMR (mg O_2 h^{-1})	1.66 ± 0.11	1.42 ± 0.15	0.75 ± 0.05	2.27 ± 0.17	1.86 ± 0.11	2.30 ± 0.21	2.09 ± 0.15	1.23 ± 0.05
	AS (mg O_2 h^{-1})	1.27 ± 0.10	1.06 ± 0.15	0.44 ± 0.05	1.75 ± 0.16	1.30 ± 0.10	1.69 ± 0.24	1.39 ± 0.15	0.51 ± 0.06
Cellular energetic capacity	CS (U g^{-1} proteins)	6.405 ± 0.45	5.397 ± 0.56	5.344 ± 0.71	6.667 ± 0.71	4.610 ± 0.68	6.098 ± 0.40	5.373 ± 0.78	5.262 ± 0.50
	COX (U g^{-1} proteins)	1.931 ± 0.15	1.810 ± 0.19	2.343 ± 0.40 (n=7)	2.327 ± 0.41 (n=8)	2.051 ± 0.27 (n=8)	2.445 ± 0.19 (n=7)	1.756 ± 0.21 (n=8)	2.758 ± 0.35 (n=8)
	ETS (U g^{-1} proteins)	33.132 ± 2.13	30.825 ± 3.46	33.511 ± 3.38	37.326 ± 4.86	30.976 ± 1.93	36.802 ± 1.86	32.239 ± 2.55	34.709 ± 2.07
	LDH (U g^{-1} proteins)	326.842 ± 30.28	365.748 ± 37.07	375.703 ± 38.54	337.778 ± 38.76	323.130 ± 45.02	389.768 ± 33.30	320.924 ± 26.80	398.635 ± 35.98
	CS:ETS	0.198 ± 0.02	0.183 ± 0.02	0.158 ± 0.02	0.188 ± 0.02	0.148 ± 0.02	0.167 ± 0.01	0.166 ± 0.02	0.158 ± 0.02
	COX:ETS	0.061 ± 0.01	0.060 ± 0.004	0.068 ± 0.01 (n=7)	0.059 ± 0.01 (n=8)	0.067 ± 0.01 (n=8)	0.067 ± 0.01 (n=7)	0.058 ± 0.01 (n=8)	0.080 ± 0.01 (n=8)
	CS:LDH	0.021 ± 0.002	0.015 ± 0.001	0.015 ± 0.003	0.020 ± 0.002	0.015 ± 0.002	0.016 ± 0.002	0.017 ± 0.002	0.014 ± 0.001
	COX:LDH	0.0061 ± 0.0004	0.0052 ± 0.0007	0.0064 ± 0.0011 (n=7)	0.0064 ± 0.006 (n=8)	0.0062 ± 0.0007 (n=8)	0.0063 ± 0.0006 (n=7)	0.0060 ± 0.0009 (n=8)	0.0079 ± 0013 (n=8)
	CS:COX	3.37 ± 0.17	3.29 ± 0.57	2.81 ± 0.26 (n=7)	3.35 ± 0.33 (n=8)	2.51 ± 0.29 (n=8)	2.51 ± 0.31 (n=7)	3.01 ± 0.60 (n=8)	1.97 ± 0.24 (n=8)

Table 1.3 Results for best-fitted model testing the effect of 30 d exposure to elevated temperature, low pH and low oxygen, in isolation and combined (*Design A* and *B*) on the metabolic traits and cellular energetic capacity of female of the northern shrimp *P. borealis*. Numbers in bold indicate significant *p*-values.

		Best-fitted model - Design A												Best-fitted model - Design B					
		Temperature			pH			Temperature * pH			Wet mass			Treatment			Wet mass		
		df	F	p	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
Metabolic traits	log_{10} SMR	2	44.135	**< 0.001**	1	0.286	0.595	2	2.415	0.101	1	3.261	0.077	log_{10} SMR	3	43.192	**< 0.001**	1	39.389	**< 0.001**
	log_{10} MMR	2	13.386	**< 0.001**	1	7.099	**0.011**	2	0.740	0.483	1	1.588	0.214	log_{10} MMR	3	65.621	**< 0.001**	1	0.140	0.711
	log_{10} AS	2	3.552	**0.037**	1	6.182	**0.017**	2	0.354	0.704	1	0.605	0.441	log_{10} AS	3	29.172	**< 0.001**	1	12.574	**0.001**
Cellular energetic capacity	CS	2	0.097	0.908	1	6.325	**0.015**	2	0.642	0.531				CS	3	0.749	0.531			
	log_{10} COX	2	0.488	0.617	1	3.276	0.077	2	0.740	0.483				COX	3	2.590	0.073			
	log_{10} ETS	2	0.521	0.598	1	3.540	0.066	2	0.052	0.950				log_{10} ETS	3	0.226	0.878			
	LDH	2	0.213	0.809	1	0.222	0.640	2	1.117	0.336				LDH	3	1.294	0.293			
	CS:ETS	2	1.106	0.339	1	1.624	0.209	2	0.596	0.555				CS:ETS	3	1.244	0.310			
	COX:ETS	2	0.100	0.905	1	0.011	0.918	2	0.011	0.918				COX:ETS	3	1.515	0.232			
	CS:LDH	2	0.386	0.682	1	7.181	**0.010**	2	1.751	0.185				CS:LDH	3	2.442	0.082			
	COX:LDH	2	0.495	0.613	1	0.812	0.373	2	0.156	0.856				COX:LDH	3	0.868	0.470			
	log_{10} CS:COX	2	1.431	0.250	1	1.057	0.310	2	1.223	0.304				log_{10} CS:COX	3	5.051	**0.014**			

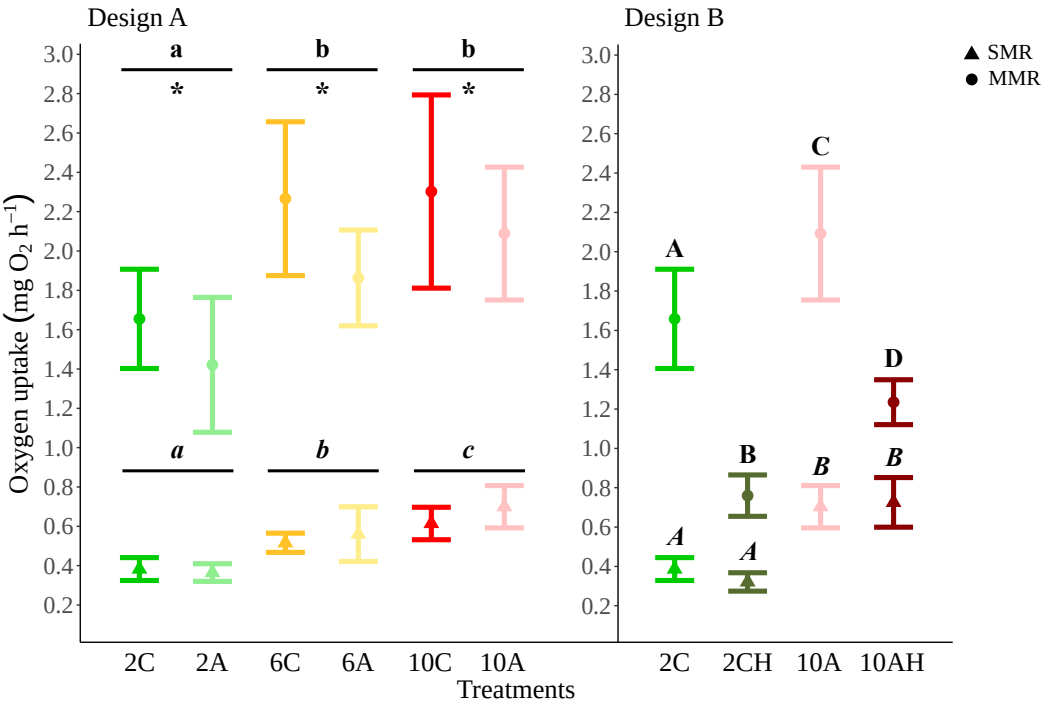


Figure 1.3 The effects of 30 d exposure to elevated temperature, low pH and low oxygen, in isolation and combined (*Design A* and *B*), on mean standard metabolic rate (SMR) and mean maximum metabolic rate (MMR) of female shrimp *P. borealis* are represented by triangles and dots, respectively. Treatments correspond to 2C (green), 2A (light green), 6C (yellow), 6A (light yellow), 10C (red), 10A (light red), 2CH (dark green) and 10AH (dark red). Triangles and dots represent the mean value and the associated error bars the 95 % CI. Lower case letters identify significant differences ($p < 0.05$) between temperature treatments independently of pH, whilst asterisks identify significant differences ($p < 0.05$) between pH treatments, combining all temperatures. Italic upper-case letters indicate significant differences ($p < 0.05$) among treatments for mean SMR and classical upper-case letters indicate significant differences ($p < 0.05$) among treatments for mean MMR.

1.6.4 Aerobic scope

Mean AS measured in northern shrimp exposed to elevated temperature, low pH and low oxygen in isolation and combined are summarised in Table 1.2.

Design A: Exposure to low seawater pH caused a significant decrease (20 %) in mean AS at all temperatures combined (Table 1.3, Figure 1.4). In addition, mean AS was significantly influenced by temperature (Table 1.3, Figure 1.4). In more detail, a significant 31 % increase in mean AS was observed between 2 and 6 °C, whilst the mean AS measured at 10 °C was found to be comparable to the mean AS obtained at the two lower temperatures tested. However, the interaction between temperature and pH did not exert a significant effect on mean AS (Table 1.3).

Design B: Different treatments showed a significant difference in mean AS (Table 1.3, Figure 1.4). Precisely, mean AS values measured in hypoxic treatments at both temperatures were significantly lower when compared to those measured in normoxic treatments at both temperatures (65 and 63 %, 2 and 10 °C respectively). In addition, the mean AS was comparable between the normoxic and hypoxic treatments.

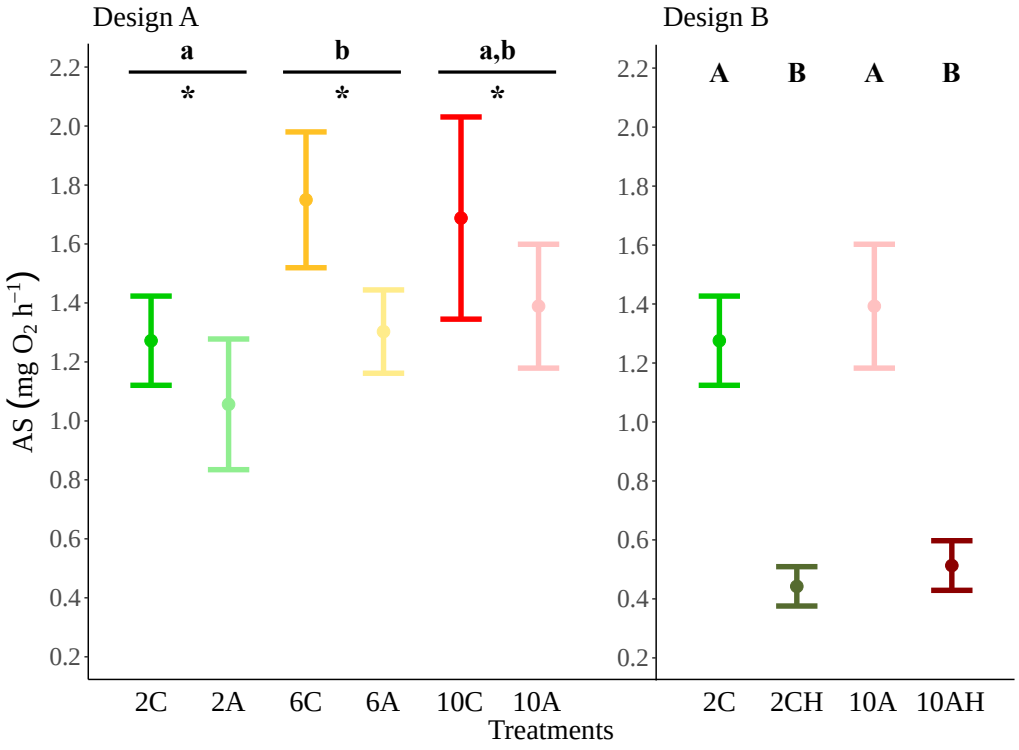


Figure 1.4 The effects of 30 d exposure to elevated temperature, low pH and low oxygen, in isolation and combined (*Design A* and *B*), on mean aerobic scope (AS) of female shrimp *P. borealis*. Treatments correspond to 2C (green), 2A (light green), 6C (yellow), 6A (light yellow), 10C (red), 10A (light red), 2CH (dark green) and 10AH (dark red). Dots represent the mean value and the associated error bars the 95 % CI. Lower case letters identify significant differences ($p < 0.05$) between temperature treatments independently of pH, whilst asterisks identify significant differences ($p < 0.05$) between pH treatments, combining all temperatures. Upper-case letters indicate significant differences ($p < 0.05$) among treatments.

1.6.5 Temperature coefficient

Design A only: Temperature coefficient (Q_{10}) calculated for SMR and MMR between 2 and 10 °C varied between 1.02 and 2.15 (Table 1.4). For both SMR and MMR, Q_{10} values were higher for the 2 to 6 °C interval, followed by the 2 to 10 °C interval and then by the 6 to 10 °C interval.

Table 1.4 Summary of temperature coefficients (Q_{10}) calculated for SMR and MMR of female shrimp *P. borealis* at current pH and experimental range of temperatures (2 –10 °C).

	Temperature range		
	2 - 6 °C	6 - 10 °C	2 - 10 °C
Q_{10} SMR	2.15	1.48	1.79
Q_{10} MMR	2.15	1.02	1.49

1.6.6 Enzymes activities and ratios

Mean specific enzymatic activities and their ratios measured in the abdominal muscle tissue of the northern shrimp exposed to elevated temperature, low pH and low oxygen in isolation and combined (*Design A* and *B*) are summarized in Table 1.2. The factors tested for both designs were found not to be significant on the mean activity of COX, ETS and LDH, nor on their ratios CS:ETS, COX:ETS and COX:LDH (Table 1.3). Exposure to low seawater pH (*Design A*) caused a significant decrease (20 %) in mean CS activity (Table 1.3), and 16 % in mean CS:LDH (Table 1.3) when combining all temperatures tested. Additionally, mean CS:COX (*Design B*) at the 2C treatment was the highest (3.37) when compared to all other treatments, and showed comparable means with the 2CH and 10A treatment (Table 1.3, Figure 1.5). Finally, the mean CS:COX values reported at 2CH and 10A treatment were shown to be also comparable to the mean at the 10AH treatment (Figure 1.5).

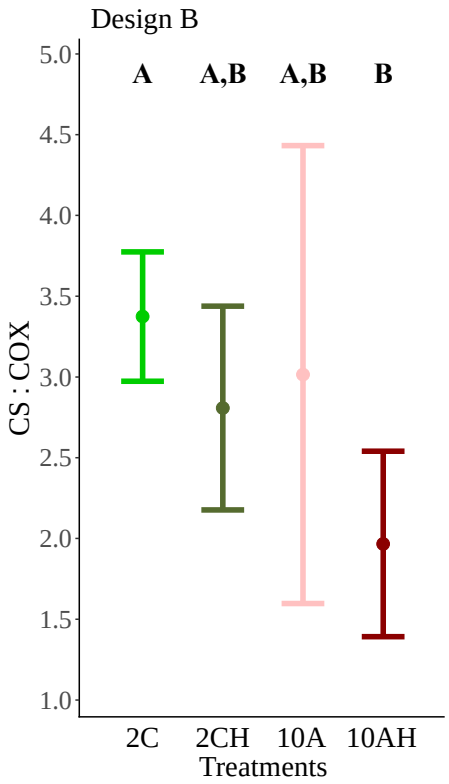


Figure 1.5 The effects of 30 d exposure to elevated temperature, low pH and low oxygen combined (*Design B*) on mean citrate synthase – cytochrome C oxidase ratio (CS:COX) specific activity in the muscle of female shrimp *P. borealis*. Treatments correspond to 2C (green), 2CH (dark green), 10A (light red) and 10AH (dark red). Dots represent the mean values and the associated error bars the 95 % CI. Upper case letters indicate significant differences ($p < 0.05$) among treatments.

1.7 DISCUSSION

Altogether, our results raise concerns about the future conservation of the northern shrimp *Pandalus borealis* in the Estuary and Gulf of St. Lawrence, from an ecological and socio-economic point of view. Ocean warming (OW), acidification (OA) and hypoxia in isolation are shown to control and limit shrimp aerobic performance. Despite the lack of interactive effect between temperature and pH in this study, combined ocean global change drivers severely affect the response of shrimp from the whole-organism to the cellular level. In particular, survival, metabolic rate and ultimately aerobic performance are severely reduced under combined ocean global change drivers. Nonetheless, shrimp cellular aerobic capacity, as expressed by enzymatic activity, is stable across all treatment combinations. Interestingly, in shrimp exposed to combined ocean global changes, we observe an adjustment of the mitochondrial phenotype, possibly a plastic response, to maintain cellular energetic capacity. In general, our study confirms the importance of conducting

multi-driver and multi-trait experiments when investigating the ability of marine organism to tolerate combined ocean global change drivers, underlining the importance of accounting for both controlling and limiting effects.

Most studies so far conducted on the responses of northern shrimp to changing environments have focused on temperature as the driver which defines the species' sensitivity. Shrimp have been shown to be sensitive to increases of this driver throughout their life cycle, with females showing to be more sensitive than males (Arnberg et al., 2013; Brillon et al., 2005; Daoud et al., 2007, 2010; Dupont-Prinet et al., 2013). As an expression of the positive relationship between temperature and metabolic rate (measured *via* oxygen consumption) Q_{10} values generally decrease with increasing temperatures. Thus, as expected, Q_{10} values for the northern shrimp in our study are greater between 2 and 6 °C than between 6 and 10 °C for both standard and maximum metabolic rates (SMR and MMR respectively). It suggests that female shrimp are more sensitive to increases in temperature from lower to intermediate temperatures: 2 to 6 °C in the present study, and 2 to 5 °C in Daoud et al. (2007). However, our results also show that shrimp can tolerate temperatures up to 6 °C at least, as their aerobic scope (AS) increased by 31 % between 2 and 6 °C, confirming that temperature acts as a controlling factor of *P. borealis* metabolic rate (Fry, 1971; Pörtner, 2010). The absence of an increase in AS between 6 and 10 °C likely shows that our highest temperature treatment is close to the upper critical temperature for shrimp (Allen, 1959; Brillon et al., 2005). However, the absence of a drop in survival at 10 °C after 30 days of exposure, when temperature is the only driver, supports the idea that shrimp can tolerate temperature increases close to those predicted to occur within the context of the ongoing OW (IPCC, 2022). This finding is in line with that by Allen (1959), who stated that adult *P. borealis* can live and reproduce at temperatures as high as 11.1 °C.

In addition, our study confirms that adult shrimp can tolerate OA conditions when it is the only driver to which they are exposed (Hammer & Pedersen, 2013). Indeed, OA has no effect on survival when in isolation after 30 days of exposure, and little effect on AS. In fact, AS decreases by 20 % from the high to the low pH treatment, driven by the marked decrease in MMR. The reason for shrimp' MMR decline at low pH is likely linked to the drop in extracellular pH, which decreases the affinity between respiratory pigments and oxygen, thus less oxygen is delivered to tissues (Pörtner et al., 2004; Whiteley, 2011).

Fry (1971) showed that hypoxia acts as a limiting factor for metabolism: organisms must decrease their oxygen uptake when environmental dissolved oxygen levels are too low. The northern shrimp is able to maintain its SMR when exposed to acute severe hypoxia (~16 % O_2 sat.; Dupont-Prinet et al., 2013) and to chronic hypoxia (35 % O_2 sat. for 30 d, this study). However, both our study and Dupont-Prinet and colleagues' show that adult shrimp MMR is severely reduced under hypoxic conditions. It decreases approximately by half, independently of temperature, due to the limiting effect that hypoxia exerts on MMR (Fry, 1971). By consequence, as SMR is maintained and MMR decreases, AS decreases by about 60 % from normoxic to hypoxic conditions when shrimp are exposed for longer periods than first shown by Dupont-Prinet et al. (2013). Finally, and not surprisingly, survival is not affected by hypoxia in isolation because we chose a chronic, non-lethal, level of dissolved oxygen (Dupont-Prinet et al., 2013).

While shrimp seem to tolerate temperatures and pH predicted to occur for the end of the century (IPCC, 2022; Lavoie et al., 2020) well when tested in isolation, the co-occurrence of these environmental changes can lead to drastically different impacts, confirming that multiple major drivers should always be studied together in order to determine the physiological responses of organisms to ocean changes. Indeed, shrimp survival is negatively affected by the combination of OW and OA (Dupont et al., 2014) and the superimposed effect of hypoxia (Chemel et al., 2020). In our study, survival is reduced by approximately 23 % when shrimp are exposed to the combination of elevated temperature and low pH, and by approximately 58 % when they are exposed to combined elevated temperature, low pH and low oxygen levels. Considering the hypoxia level we used (35 % O_2 sat.), our survival results can therefore be considered optimistic when compared to the predicted future conditions for 2100 for the EGSL. Indeed, average deep-water (300 m) oxygen level in 2020 was already as low as 15 % O_2 sat. in the SLE and less than 40 % O_2 sat. in the GSL (Blais et al., 2021b).

It is indeed interesting to compare the favourable *versus* combined scenario (elevated temperature, low pH and low DO treatment) to determine, from a conservation point of view, the magnitude of change in the physiological status of the northern shrimp under combined future global change drivers. Our results show that under the combined scenario SMR increases by approximately 90 % when compared to favourable conditions, meaning that maintenance costs will be almost two times higher and shrimp will need to eat almost twice as much just to meet their maintenance costs.

Additionally, MMR decreases by approximately 26 %, meaning that the rate at which oxygen can be transported to the mitochondria for energetically demanding activities will decrease by a third, having possible negative consequences, on organisms vagility, as well as on predatory and anti-predatory behaviour of shrimp. Consequently, AS decreases by approximately 60 %, thus, the framework available to meet the aerobic metabolic demand is reduced by more than half. Ultimately, the energy budget available to support physiological performance of shrimp, and ultimately fitness-related activities, is greatly reduced. This in turn, could have consequences on growth, body size and reproduction (Claireaux & Lefrançois, 2007; Fry, 1947; Pörtner & Farrel, 2008), and ultimately shrimp distribution and abundance (Cucco et al., 2012; Pörtner & Knust, 2007).

However, the level of tolerance of a species can differ depending on the organisational level considered (Harvey et al., 2014). In line with this, we show that exposure of female shrimp to isolated and combined ocean global change drivers induces little or no variation in aerobic and anaerobic enzyme activity, as measured in the abdomen muscle tissue. The specific activity of CS and the CS:LDH are only reduced by pH, both by approximately 20 %. The activity levels and ratios for all other enzymes remain stable, suggesting that the northern shrimp can partly maintain its metabolic apparatus even when exposed to multiple global ocean change drivers, and for longer periods than previously shown by Dupont-Prinet et al. (2013) and Pillet et al. (2016). Nonetheless, these results also suggest that shrimp possess low ability for metabolic reorganisation, as an increase in cellular aerobic capacity could have led to a compensation of the negative effects of combined drivers on the AS. Interestingly, exposure to the combined scenario (OW + OA + hypoxia) decreases the CS:COX by approximately 41 % compared to the favourable scenario, supporting the interpretation of a change in the mitochondrial phenotype and likely morphology. Specifically, CS is an enzyme located in the mitochondrial matrix, and its level of activity correlates to the mitochondrial volume (Moyes et al., 1997), whilst COX is an integral membrane enzyme and its activity correlates with the mitochondrial membrane surface. The phenotypic change could help to maintain aerobic efficiency, or capacity under combined ocean global change drivers, since COX is the last acceptor of electrons of the Electron Transport System responsible for the reduction of molecular oxygen. Higher COX over CS (and lower CS:COX) could therefore result in mitochondria with higher oxidative capacity relative to the reducing capacity (CS and

Krebs cycle pathway), which might accommodate elevated aerobic capacity at high temperatures and lower pH and oxygen levels. In other words, mitochondria with relatively more COX could partly maintain oxidative phosphorylation capacity despite the negative effect of combined drivers at the whole-organism level.

Our laboratory-based results show that under the combination of ocean global change drivers shrimp cellular aerobic capacity is stable, whilst whole-organism physiological performance decreases in individuals surviving exposure, in addition to survival being severely reduced. These findings are overall in line with our hypothesis, and confirm the importance of integrating the physiological responses of a species at different levels of biological organization to provide a more comprehensive estimate of a species' sensitivity (Harvey et al., 2014), and thus enhance (using a critical understanding of physiological systems) its conservation (Cooke et al., 2013; Wikelski & Cooke, 2006). In conclusion, our results raise concerns about the future health of northern shrimp and its conservation status in the ESGL, considering the predicted changes in temperature, pH and dissolved oxygen for the end of the century. Specifically, shrimp inhabiting the GSL are still most abundant at a depth of ~ 250 m, where pH is approximately 7.75 and where the temperatures they encounter went from ~5–6 to ~ 6–7 °C, from 2006 to 2021 (DFO, 2022a; Lavoie et al., 2020). In the same period and at the same depth, DO levels went from ~35 to ~30 % O_2 sat. (DFO, 2022a). At those depths, shrimp are likely to experience environmental conditions predicted to occur by the end of the century (IPCC, 2022) and tested in our study, or even worse as discussed above, supporting our concerns. Indeed, environmental changes are considered to have been responsible for part of the northern shrimp biomass loss in the southern regions of the northwest Atlantic during the last 15 y (e.g., Estuary and Gulf of St. Lawrence; DFO, 2020) as previously suggested for the shrimp stock collapse in the Gulf of Maine (Richards & Hunter, 2021). Our results suggest that shrimp abundance may decline to the point of possibly observing a local commercial extinction: for example as already observed in the Gulf of Maine and in the western North Sea (ICES, 2004, 2013). This would severely impact the local fishing industry, leading to a loss of economic revenue, as the northern shrimp is the third most lucrative species in Canada (DFO, 2021a). Shrimp in the GSL could avoid global decline and extinction by migrating into more suitable habitats. Adult shrimp are not able to emigrate to other regions, due to physical barriers characteristics of the topography of the EGSL, but have been shown to be able to migrate bathymetrically to lower

depths (< 150 m) in the SLE, where environmental conditions are more favourable (DFO, 2022a). Interestingly, the vertical distribution change observed in the SLE is possibly a response to a drop in dissolved oxygen levels in deeper waters, as recent oxygen levels are close to or even below the critical oxygen level obtained by Dupont-Prinet et al. (2013). Indeed, hypoxia seems to greatly influence shrimp distribution, as shrimp in the SLE, where deep waters are most severely hypoxic, occupy shallower waters than neighbouring GSL shrimp populations. In addition, shallower waters are colder and have higher pH (DFO, 2022a; Mucci et al., 2011, 2018). Colder temperatures combined with higher oxygen and pH levels represent more favourable conditions for the physiological status of the northern shrimp, but this strategy is only applicable if the sediment type and food availability match shrimp requirements. If so, migrating to shallower depths still brings some negative effects. In fact, the habitat might be limited in term of space, involving an increase in shrimp density which exposes them, for example, to higher fishing and predator pressures. Whilst shrimp fishing quotas have been reduced over recent years, potentially reducing pressure on these stocks, interestingly, the shrimp proportion in the diet of the redfish and the Greenland halibut has been maintained despite shrimp biomass decreased over the last decade (Brown-Vuillemin et al., 2022). For these reasons, shrimp of the SLE are to be considered at risk of local commercial extinction. Results like ours can be integrated in models to help in predicting species future size, abundance, and distribution (see for example Tai et al., 2021), which will provide valuable information to assist in the maintenance of the northern shrimp's health and conservation status in the ESGL.

1.8 STATEMENTS

Data availability statement

The original data generated in the course of the study and underlying this article will be available on PANGAEA® Data Publisher. A digital object identifier (DOI) will be assigned later.

Author contributions

DC, FN and PC conceived the general experimental design, and supported EG in finalizing the design and protocol used. DC provided the installations for the experimental setups and the live shrimp for the study, with additional equipment from PC. EG and MC carried out the experiment. EG carried out the oxygen uptake measurements and DC and EG did the related data analyses. PB

provided the installations for the enzymatic activity analyses, which were carried out by EG. EG completed the statistical analyses with the support for R coding from DC and FN, and discussions with DC, FN and PC. EG conducted the interpretation of the results with support from DC, FN, PB and PC. EG wrote the first draft of the manuscript with support from DC, FN and PC. All authors contributed to the final version of the manuscript.

Funding

This work was supported by: (i) an Ouranos grant [554023 to DC, FN, and PC]; (ii) a DFO Strategic Program for Ecosystem-Based Research and Advice grant and an Aquatic Climate Change Adaptation Services Program grant to DC; (iii) a FIR UQAR grant, a Canada Foundation for Innovation grant and a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant [RGPIN-2015-06500 and RGPIN-2020-05627 to PC]; (iv) a Canada Foundation for Innovation grant and a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant [RGPIN-05992 to PB]; (v) a MITACS-Ouranos Accelerate grant and a MITACS-Merinov Accelerate grant [grant number IT010005] to support EG and MC respectively; (vi) a Fonds de Recherche du Québec - Nature et Technologies (FRQNT) scholarship (PBEEE, 289597) to EG and (vii) a Réal-Décoste Ouranos scholarship (286109) to EG.

Acknowledgments

The authors are especially grateful to T. Hansen for her technical support during the experiment and the respirometry measurements at the Maurice Lamontagne Institute (DFO, Canada) and wish to thank V. Desrosiers, J. Gagnon and D. Picard, for technical support during the experiment at the Maurice Lamontagne Institute (DFO, Canada) and at the University of Québec in Rimouski (Canada). EG, MC, FN and PC are active members of the inter-institutional strategic research network Québec-Océan. PB is member of the inter-institutional strategic research network Quebec Center for Biodiversity Science (QCBS) and EG, MC, and PC were member of QCBS. FN, DC and PB are active member of the Ressources Aquatiques Québec inter-institutional strategic research network.

1.9 SUPPORTING INFORMATION

1.9.1 Determination of the shortest reliable interval to estimate maximum metabolic rate (MMR)

To reduce the risk of underestimating MMR, flushes were cancelled for each group of five respirometers hooked to the same flush pump until the slope of all five shrimp was visibly reduced. This long closed phase lasted 56.7 min ± 23.9 (mean and SD). DO declines rapidly during this phase and further, the rate of decline diminishes rapidly. Another way of reducing the risk of underestimating MMR is to use rolling regressions (Zhang et al., 2019). Contrary to sequential regressions, when one interval begins after the end of the previous interval, in rolling regressions each interval begins one point (1 s) after the beginning of the previous interval.

Using a too long interval to calculate the slope of DO decline over time could result in underestimating MMR. However, using a too short interval, especially with rolling regressions, could overestimate MMR if the DO decline is not perfectly smooth and is influenced by noise. The shortest reliable interval or window width (WW) was determined according to the methodology outlined by Zhang et al. (2019), with a few modifications. The objective is to use periods of DO declines and analyse them several times with sequential regressions, using a specific WW each time. The variability, either standard deviations (SDs) or coefficient of variations (CVs) of different WWs are then compared statistically.

In our study, the last 8.25 min of the long closed phase were used to determine the shortest reliable WW. The rate of DO decline changed slowly near the end of the long closed phases and it was possible to compare different WWs using these data, smoothed with a 15-point moving average to reduce noise without changing the slope of the signal (Chabot et al., 2021). For a given shrimp we calculated as many sequential regressions of DO over time as it was possible to fit into 8.25 min, investigating WWs from 0.5 to 4 min, in steps of 0.25 min. Thus, for example, 16 regressions were calculated for WW of 0.5 min and 2 regressions were calculated for WWs of 3–4 min. For each shrimp and WW, mean slope was calculated across all the regressions obtained, along with the SD and CV of the mean slopes, mean R^2 and the SD of the mean R^2. This was done for each of the 71 shrimp that were retained for statistical analyses. The final data set included 1065 observations (71

shrimp x 15 WWs) and the following variables: shrimp ID, WW, mean slope, slope SD, slope CV, mean R^2, R^2 SD.

Mean slopes were very similar for all WWs. However, SDs and CVs of the slopes, as well as mean R^2s and their SDs differed markedly. SDs were compared across WWs in R (R Core Team, 2020) using a mixed-effect model (package NLME, Pinheiro et al., 2020), where shrimp ID was set as random variable. SDs were log transformed to improve the behaviour of the residuals. *Post-hoc* comparisons consisted in paired-t-tests (i.e. repeated measures for each shrimp, 2 WWs at a time) of all other WWs against the longest WW, 4 min, which was considered as most reliable. The risk of type I error was maintained at 0.05 using the Holm method (pairewise_t_test function of package rstatix, Kassambara, 2021).

Mean SDs were significantly greater than the control (WW = 240 s) when WW was less than 180 s (3 min) (Figure S 1.6) and 3 min was taken to be the shortest reliable WW to run rolling regressions covering the entire long closed phase. This analysis was repeated with log-transformed CVs, and the shortest reliable WW was again 3 min.

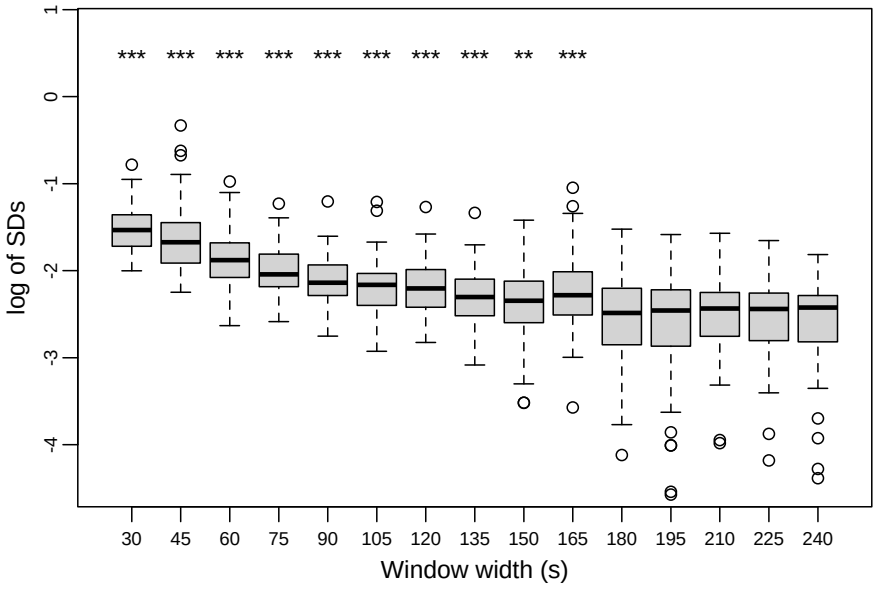


Figure S 1.6 Boxplot of SDs calculated on the slopes obtained for each window width of each shrimp. Asterisks (*, ** and *** correspond to $p < 0.05$, 0.01 and 0.001, respectively) show which WWs differ significantly from WW of 240 s.

Differences with the methodology of Zhang et al. (2019) were the choice of data type (end of long closed phases instead of blank respirometry cycles), their duration (8.25 instead of 20 min), the number of samples (71 instead of 5) and the use of a mixed-effects model considering that all WWs are analysed for each shrimp and are not independent. This also influenced *post-hoc* comparisons, which were made on the differences between pairs of WWs instead of on values of the two WWs directly, and the number of comparisons, which was reduced to comparisons of other WWs against a control WW (240 s or 4 min).

Even with log transformation, the assumptions of normality (Shapiro-Wilk test on the residuals of the model) and homoscedasticity (Levene's test using the median) were not met. However, as Anova-type analyses are robust against departures from these assumptions when sample sizes are equal (Quinn & Keough, 2002) this analysis was judged adequate to select minimum window width.

CHAPITRE 2

LES CREVETTES NORDIQUES DE DIFFÉRENTES ORIGINES MONTRENT UNE SENSIBILITÉ SIMILAIRE AUX FACTEURS DES CHANGEMENTS GLOBAUX, MAIS UNE CAPACITÉ ÉNERGÉTIQUE CELLULAIRE DIFFÉRENTE

Ce deuxième article intitulé « *Northern shrimp from multiple origins show similar sensitivity to global change drivers, but different cellular energetic capacity* » a été corédigé avec Fanny Noisette (ISMER-UQAR), Denis Chabot (IML-MPO), Pierre Blier (UQAR), Tanya Hansen (IML-MPO), Manon Cassista-Da Ros (MPO), Pierre Pepin (MPO), Katherine Skanes (MPO) et Piero Calosi (UQAR). Sa version finale a été soumise pour révision par les pairs le 8 décembre 2022 dans la revue *Journal of Experimental Biology* et as été acceptée avec révisions mineures le 13 Mars 2023. Denis Chabot, Fanny Noisette et Piero Calosi ont fourni l'idée originale et le support financier. Comme pour le chapitre 1, Denis Chabot as fourni les crevettes et les installations pour les expériences. Ces dernières et les mesures de consommation d'oxygène ont été réalisées par moi-même avec le support de Tanya Hansen. Les taux métaboliques ont été déterminés par Denis Chabot et moi-même. J'ai réalisé les analyses d'activité enzymatique dans le laboratoire de Pierre Blier. La rédaction, les analyses statistiques, les figures et les tableaux ont été réalisés par moi-même avec la contribution des co-auteurs.

Les différents résultats de cet article ont été présentés à la réunion annuelle du regroupement stratégique Québec Océan 2023, au Symposium Ouranos 2022 et au congrès international de la *Society for Experimental Biology* 2022 (voir la liste des communications dans l'avant-propos de cette thèse).

2.1 RÉSUMÉ

Les espèces avec une vaste distribution peuvent être exposées, à l'échelle régionale, à un large éventail de conditions environnementales, auxquelles elles peuvent s'acclimater ou s'adapter. Par conséquent, l'origine géographique d'un organisme peut influencer ses réponses aux changements environnementaux, et donc sa sensibilité aux facteurs combinés de changement global. Cette étude visait à déterminer les réponses physiologiques de la crevette nordique *Pandalus borealis*, à différents niveaux d'organisation biologique et provenant de quatre origines géographiques différentes, exposées à la température élevée et au bas pH afin de définir sa sensibilité aux futurs réchauffement et acidification des océans. Les crevettes collectées dans l'Atlantique nord-ouest ont été exposées pendant 30 jours aux combinaisons de trois températures (2, 6 ou 10 °C) et de deux niveaux de pH (7,75 ou 7,40). La survie, les taux métaboliques, la performance aérobie de l'organisme entier et la capacité énergétique cellulaire ont été évalués à la fin de l'exposition. Nos résultats montrent que la survie des crevettes est négativement affectée par une température supérieure à 6 °C et le bas pH, quelle que soit leur origine. De plus, les crevettes d'origines différentes montrent, dans l'ensemble, des performances similaires au niveau de l'organisme : le registre aérobie augmente avec l'augmentation de la température et diminue avec la diminution du pH. Enfin, la stabilité du métabolisme aérobie semble liée à des ajustements cellulaires propres à l'origine des crevettes. Nos résultats montrent que le niveau de variation intraspécifique diffère entre les niveaux d'organisation biologique : des capacités cellulaires différentes mènent à des performances similaires. Ainsi, la sensibilité de la crevette nordique au réchauffement et à l'acidification des océans est généralement comparable entre les origines. Néanmoins, la vulnérabilité des crevettes aux scénarios de changement global prévus pour 2100 pourrait être différente entre les origines en raison des différentes conditions environnementales régionales.

Mots-clés : Réchauffement des océans, acidification des océans, physiologie comparative, performance aérobie, *Pandalus borealis*, conservation.

2.2 NORTHERN SHRIMP FROM MULTIPLE ORIGINS SHOW SIMILAR SENSITIVITY TO GLOBAL CHANGE DRIVERS, BUT DIFFERENT CELLULAR ENERGETIC CAPACITY

Ella Guscelli[1,*], Fanny Noisette[2], Denis Chabot[3], Pierre U. Blier[1], Tanya Hansen[3], Manon Cassista-Da Ros[4], Pierre Pepin[5], Katherine R. Skanes[5] and Piero Calosi[1]

[1]Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada

[2]Institut des sciences de la mer, Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada

[3]Institut Maurice-Lamontagne, Fisheries and Oceans Canada, 850 Rte de la Mer, Mont-Joli, QC G5H 3Z4, Canada

[4]Fisheries and Oceans Canada, 176 Portland St, Halifax, NS B2Y 1J3, Canada

[5]Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada, 80 E White Hills Rd, St. John's, NL A1C 5X1, Canada

*Corresponding author

Summary statement

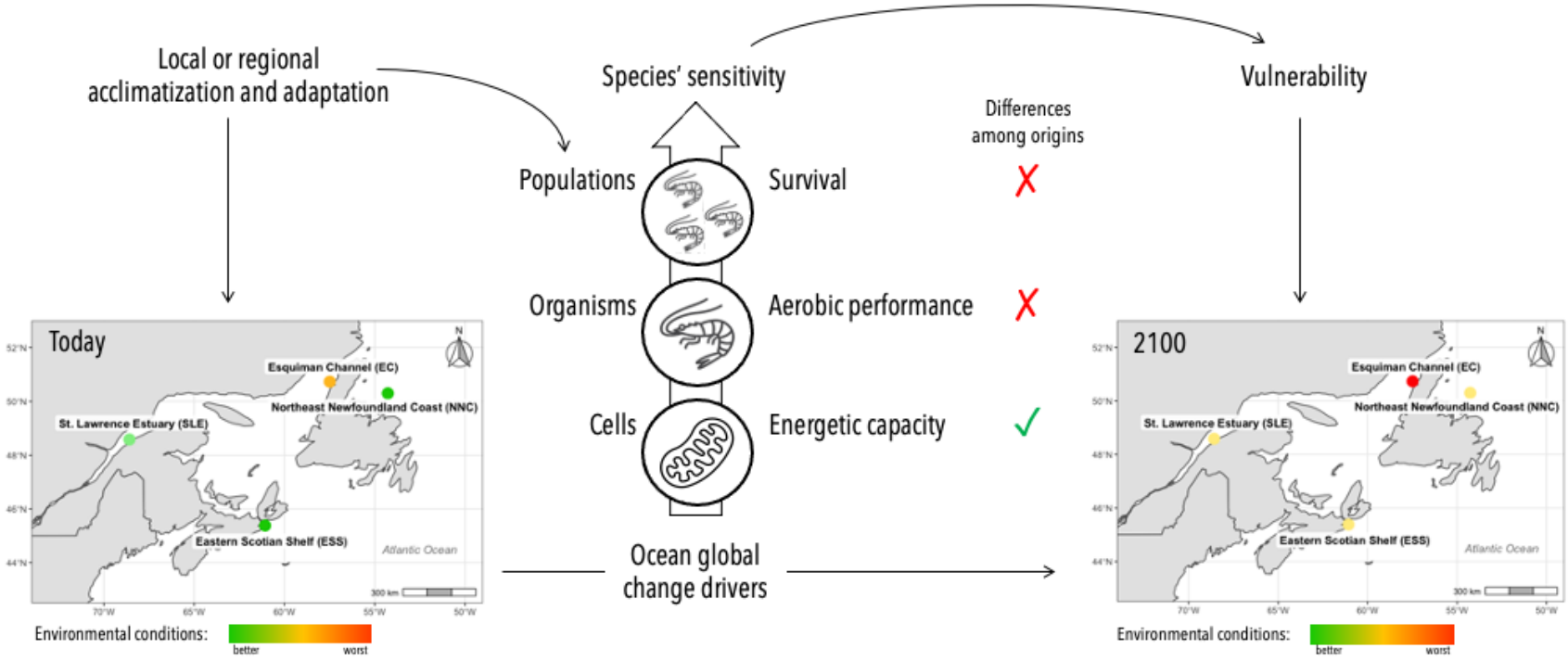
Pandalus borealis shrimp from different geographic origins, differing in their environmental regimes, are similarly sensitive, but differently vulnerable, to rapid ocean global change drivers.

2.3 ABSTRACT

Species with a wide distribution can experience regionally a wide range of environmental conditions, to which they can acclimatize or adapt. Consequently, the geographic origin of an organism can influence its responses to environmental changes, and therefore its sensitivity to combined global change drivers. This study aimed at determining the physiological responses of the northern shrimp *Pandalus borealis*, at different levels of biological organization and from four different geographic origins, exposed to elevated temperature and low pH to define its sensitivity to future ocean warming (OW) and acidification (OA). Shrimp sampled within the northwest Atlantic, were exposed for 30 days to combinations of three temperature (2, 6 or 10 °C) and two pH levels (7.75 or 7.40). Survival, metabolic rates, whole-organism aerobic performance and cellular energetic capacity were assessed at the end of the exposure. Our results show that shrimp survival was negatively affected by temperature above 6 °C and low pH, regardless of their origin. Additionally, shrimp from different origins show overall similar whole-organism performances: aerobic scope increasing with increasing temperature and decreasing with decreasing pH. Finally, the stability of aerobic metabolism appears to be related to cellular adjustments specific to shrimp origin. Our results show that the level of intraspecific variation differs among levels of biological organization: different cellular capacities lead to similar performances. Thus, the northern shrimp sensitivity to OW and OA is overall comparable among origins. Nonetheless, shrimp vulnerability to predicted global change scenarios for 2100 could differ among origins due to different regional environmental conditions.

Key words: Ocean warming, ocean acidification, comparative physiology, aerobic performance, *Pandalus borealis*, conservation

2.4 GRAPHICAL ABSTRACT



2.5 INTRODUCTION

Latitudinal gradients of environmental parameters promote large-scale physiological variation among species, populations from species with a wide distribution likely experiencing a broad range of various regional and local environmental conditions (Addo-Bediako et al., 2000; Lardies et al., 2014; Orr et al., 2005). This may lead to acclimatization or adaptation of organisms to prevalent environmental conditions but macrophysiological studies addressing this topic, at the population level, are scarce (Chown et al., 2004; Gaston et al., 2009). Local adaptation can be advantageous as it limits the costs linked to plasticity by aligning organisms' average performances to the environmental optimum, ultimately maximizing their fitness (Kawecki & Ebert, 2004). However, local adaptation can sometimes be disadvantageous as organisms can reach the limits of their plastic capacity, and thus be more sensitive to future rapid environmental changes (Calosi et al., 2016). Indeed, while inter-individual variation promotes allostasis to environmental changes, resistance to extinction risk and even range expansions in newly colonized environments (Forsman & Wennersten, 2016), low intraspecific phenotypic variation suggests a low ability for further adaptation based on the selection of existing phenotypes (Calosi et al., 2013, 2016; Sunday et al., 2014). Moreover, long-term acclimatization and adaptation can influence population responses to changes in global environmental conditions, such as increasing temperature and increasing pCO_2/decreasing pH (Bozinovic et al., 2011; Darnell & Darnell, 2018; Gaston et al., 2009; Hollarsmith et al., 2020; Lardies et al., 2014; Sorte et al., 2011). The variation in the physiological responses of populations to temperature changes is of particular interest within the context of ongoing ocean warming (OW) (IPCC, 2022). Acclimatization and adaptation have also been shown to shape the responses of multiple populations to future ocean acidification (OA) (Calosi et al., 2017; Pespeni et al., 2013; Thor et al., 2018; Vargas et al., 2017). We have a relatively solid understanding of the physiological variation among populations to OW and OA in isolation. However, very few studies have investigated the potential influence of acclimatization and adaptation on the physiological response of populations to these combined drivers, which will co-occur in the global ocean change (e.g., Leung et al., 2021; Rivest et al., 2017). Consequently, it is paramount to address the various responses among populations of a same species to combined OW and OA which are ultimately responsible for defining the overall sensitivity of a species: particularly for those species declining under global changes. As shown in the common periwinkle *Littorina littorea* (Linnaeus, 1758) (Calosi et al., 2017), populations closer to the edges of their

species' distribution range are often more sensitive to global changes compared to central populations (Kolzenburg, 2022). This can be due to the limited adaptive ability of marginal populations as a consequence of their lower genetic diversity, or to their lower population density, which is itself linked to living under less favourable environmental conditions (Kolzenburg, 2022).

Many species are already declining due to the negative effects of global change drivers. For example, as a consequence of the increase in seawater temperature, the Israeli population of the purple sea urchin *Paracentrotus lividus*, Lamarck 1816, collapsed in the early 2000's (Yeruham et al., 2015), and the blue mussel *Mytilus edulis*, Linnaeus 1758, recruitment has been declining every year in the northwest Atlantic over the last two decades (Petraitis & Dudgeon, 2020). Also in the northwest Atlantic in the past 15 years, the abundance of the northern shrimp *Pandalus borealis*, Krøyer 1838, fluctuated or remained stable in the northern fishing areas (DFO, 2021b), but drastically declined in the southern regions (e.g., Gulf of Maine, Estuary and Gulf of St. Lawrence, Newfoundland and Labrador, DFO, 2021c, 2022a; Hunter et al., 2021). These declines may also be related to increasing predator pressure and changes in phenology but are most likely linked to direct environmental changes, including OW (Richards & Hunter, 2021). For instance, over the last decades, both surface and bottom waters have warmed in the Gulf of Maine, negatively impacting recruitment and settlement of shrimp (Richards & Hunter, 2021). This appears to have led to the shrimp stock collapse in the Gulf of Maine that raised the urgent need of a fishery moratorium, established in 2013 (Whitmore et al., 2013).

Considering the unprecedented decline of shrimp stocks, its broad circumpolar distribution, and the contrasting regional environmental conditions it experiences, *P. borealis* is an ideal candidate species for examining the relevance of using intraspecific variation in the response to global change drivers to define a species' sensitivity. Being a cold-water species, *P. borealis'* thermal window of preference spans from 0 to 5 °C (Shumway et al., 1985) and OW has been shown to negatively impact both larvae and adults (Arnberg et al., 2013; Brillon et al., 2005; Chabot & Ouellet, 2005; Chemel et al., 2020; Daoud et al., 2007, 2010; Dupont-Prinet et al., 2013; Ouellet & Chabot, 2005). Shrimp larvae have also been shown to be sensitive to OA, whilst adults seem to better tolerate it (Arnberg et al., 2013; Bechmann et al., 2011; Chemel et al., 2020; Hammer & Pedersen, 2013). Nonetheless, the sensitivity of both larvae and adults to OW and OA may vary along the species distribution range under the influence of the potential local acclimatization or adaptation to

different regional environmental conditions throughout the north Atlantic (Koeller et al., 2009; Ouellet et al., 2017). Indeed, along the east coast of Canada and outside the St. Lawrence system shrimp are most abundant in deep waters characterized by ~ 0-3 °C and ~ 7.8-8 pH units, while in the Estuary and Gulf of St. Lawrence shrimp are most abundant at depths where temperatures are higher and pH levels are lower (~ 4-7 °C, ~ 7.6 pH units) (Bourdages et al., 2022; Cyr et al., 2022; DFO, 2021c, 2022c, 2022b). In this context, our study aimed at determining the level of intraspecific variation in physiological responses of female shrimp, the life stage targeted by the fishery, exposed to future OW and OA conditions at multiple levels of biological organization and from different geographic origins, to understand the potential contribution of acclimatization and local adaptation in defining the species' sensitivity. For each geographic origin, survival, whole-organism aerobic performance and cellular energetic capacity were estimated. Based on our current understanding on larval potential adaptation in the EGSL and adult sensitivity to ocean global change drivers, we hypothesise that shrimp from different geographic origins will show different levels of sensitivity at the whole-organism and cellular levels, when exposed to isolated and combined OW and OA conditions.

2.6 MATERIAL AND METHODS

2.6.1 Specimen collection, transport and maintenance

Female shrimp were collected between 2018 and 2019 from four different geographic origins within the northwest Atlantic: i.e. St. Lawrence Estuary (SLE, 48° 35' N, 68° 35' W; May 2018), Eastern Scotian Shelf (ESS, 45° 23' N, 61° 04' W; February 2019), Esquiman Channel (EC, 50° 44' N, 57°29' W; July 2019) and Northeast Newfoundland Coast (NNC, 50° 18' N, 54° 16' W; November 2019) (Figure 2.1). Specimens from SLE were fished with a rigid-frame trawl, those from EC and NNC were collected by fishermen with commercial shrimp trawls, whilst specimens from ESS were collected by fishing traps. After collection, shrimp were held in 750 L tanks filled with cold (2 – 3 °C), well oxygenated seawater and transported to the Maurice-Lamontagne Institute (MLI), Fisheries and Oceans Canada (Mont-Joli, Qc, Canada). Here, shrimp were kept approximately eight weeks in rectangular rearing tanks (1700 L) before the beginning of each experiment to reduce some of the potential physiological variability linked to differences in collection times, while maintaining long-term acclimatization or adaptation signals. Experiments were performed during August 2018 for shrimp from SLE and April, September and December

2019 for shrimp from ESS, EC and NCC, respectively. Maintenance of shrimp from all geographic origins were similar to those detailed for shrimp of SLE in Chemel et al. (2020). Average conditions during this period were 4.5 °C, pH 7.9 (total scale, pH_T), 100% O_2 saturation relative to air and salinity 32. Shrimp were fed *at libitum* three times a week with capelin (*Mallotus villosus*, Müller 1776) and shrimp (*Pandalus* spp.) and uneaten food was removed after 24 h to ensure high water quality levels.

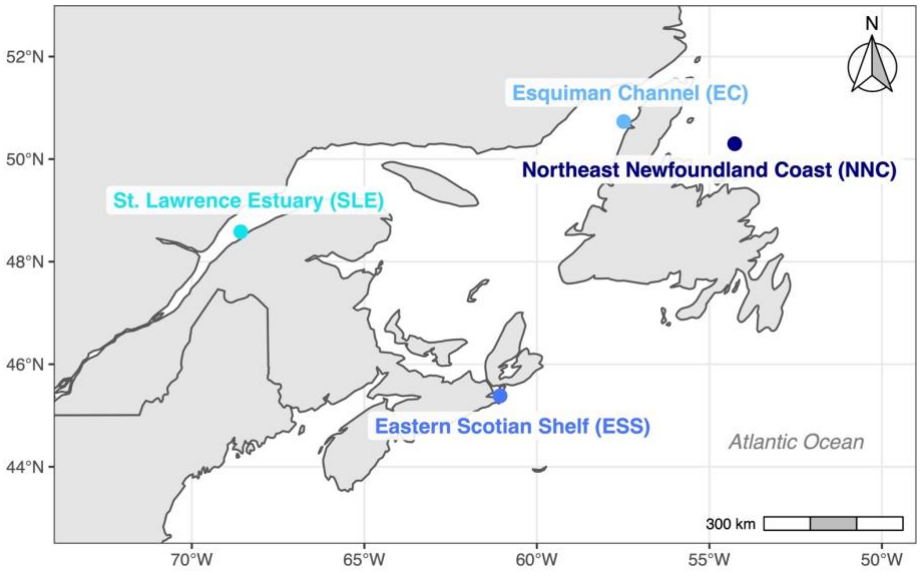


Figure 2.1 Map representing the collection sites of female northern shrimp *Pandalus borealis* in the northwest Atlantic. The four geographic origins are SLE (St. Lawrence Estuary - azure), EC (Esquiman Channel - light blue), ESS (Eastern Scotian Shelf - blue), and NNC (Northeast Newfoundland Coast - dark blue).

2.6.2 Experimental design, setup and system monitoring

To determine the effects of isolated and combined seawater temperature and pH on the survival and physiology of shrimp, we employed an orthogonal experimental design. Three levels of seawater temperature were chosen: (i) 2 °C, a favourable temperature for this species (Shumway et al., 1985), (ii) 6 °C, the recent (1990-2021) temperature of shrimp habitat in the Gulf of St. Lawrence (GSL) (5–7 °C, Bourdages et al., 2022) also corresponding to a +4 °C increase scenario predicted globally for the end of the century (RCP 8.5 scenario, IPCC, 2014) for other origins, such as ESS and NNC; and (iii) 10 °C, representing predicted conditions at the end of the century for shrimp of the GSL (Lavoie et al., 2020). Two levels of pH were selected: (i) pH 7.75, based on the current conditions of the deep waters of the EGSL (Mucci et al., 2011, 2018) and a -0.3/-0.4 pH

unit decrease scenario predicted to occur in the northwest Atlantic by the year 2100 (RCP 8.5 scenario, IPCC, 2014) and (ii) pH 7.40, representing predicted conditions at the end of the century in the EGSL bottom waters (RCP 8.5 scenario, IPCC, 2014).

Treatments were identified as: low temperature and current pH (2C: 2 °C, pH 7.75), low temperature and low pH (2A: 2 °C, pH 7.40), intermediate temperature and current pH (6C: 6 °C, pH 7.75), intermediate temperature and low pH (6A: 6 °C, pH 7.40), elevated temperature and current pH (10C: 10 °C, pH 7.75), elevated temperature and low pH (10A: 10 °C, pH 7.40), for a total of six treatments with two replicate tanks *per* treatment.

The regulation and monitoring of temperature and pH in the experimental setup was described in Chemel et al. (2020). Briefly, a 1/16 DIN Micromega autotune PID Temperature controller (Omega Engineering inc., Norwalk, USA) regulated the automatic mixing of cold and hot water to provide each tank with seawater at the set temperature, whereas an Aquastar (IKS ComputerSysteme GmbH, Karlsbad, Germany) controlled the injection of pure gaseous CO_2 into each tank's gas exchange column to maintain pH levels. Environmental parameters were monitored daily with handheld multimeters in each tank throughout the duration of each experiment. Carbonate chemistry parameters were calculated weekly, based on pH and alkalinity measurements, using the R package seacarb (Gattuso et al., 2021). Mean ± SD physico-chemical parameters for the duration of the 30-d experiment are summarized in Table S 2.1.

2.6.3 Experimental protocol

At the beginning of each experiment, approximately 60 non-ovigerous females were randomly assigned to one of the 12 tanks composing the experimental setup. The experimental tanks were gradually adjusted over 4 d until treatment values were reached, and shrimp were exposed for a total of 30 d. The number of live shrimp was recorded daily to determine survival rate. Over the exposure period shrimp were fed the same way as during their maintenance period and they were fasted at day 23 to avoid energy demands related to food ingestion (Specific Dynamic Action, SDA) during the following metabolic rate determination. The duration of the fasting period was judged sufficient because even at 2 °C, when digestion is slowest, oxygen uptake was elevated due to a combination of oxygen debt payment following chasing, handling stress and possible SDA for less than 24 h after introducing shrimp into the respirometers. On day 28 of exposure, five individuals *per* tank (10 *per* treatment) were haphazardly selected for metabolic rate determination. They were

transferred to an "exhaustion tank" where they were first chased to exhaustion and then exposed for 1 min to air to further increase their oxygen debt (method modified from Roche et al., 2013). They were then rapidly and carefully transferred into individual respirometers for the metabolic rate determination by measuring oxygen uptake ($\dot{M}O_2$, in mg O_2 h^{-1} g^{-1}) *via* intermittent-flow respirometry (Steffensen, 1989; Svendsen et al., 2016) (see the following section and Supporting Information 2.10.1 for details). At day 30 shrimp were removed from their respirometers, gently blotted with tissue paper, and weighed on a digital scale (Mf-300, A&D Company, Tokyo, Japan; ± 0.001 g) to determine wet mass (WM). Shrimp were then rapidly dissected on ice and the abdomen muscle was sectioned in three equal parts (approximately 1 g each) and flash-frozen in Eppendorf tubes in liquid nitrogen to instantly interrupt all biochemical reactions. Samples were then stored at -80 °C pending analyses.

2.6.4 Metabolic traits

To determine shrimp whole-organism responses to isolated and combined seawater temperature and pH, standard and maximum metabolic rate (SMR and MMR, respectively) were estimated individually from oxygen uptake measurements and used to calculate aerobic scope (AS = MMR - SMR) (Chabot et al., 2016a, 2016b; Fry, 1947, 1971; Norin & Clark, 2016). We report the detailed description of respirometry method and setup and the determination of metabolic traits in Supporting Information 2.10.1 and Table S 2.2 suggested by Killen et al. (2021).

2.6.5 Cellular energetic capacity

To determine the effect of isolated and combined seawater temperature and pH on the cellular energetic capacity of shrimp, we measured the activity of enzymes involved in the aerobic and anaerobic pathways of the abdominal tissue and calculated their ratio. Specifically, we measured citrate synthase (CS), cytochrome C oxidase (COX) and lactate dehydrogenase (LDH) enzyme activity as proxy for mitochondrial density (Moyes et al., 1997; Rabøl et al., 2006), for aerobic metabolic capacity (Marie et al., 2006) and for anaerobic metabolic capacity (Farhana & Lappin, 2021), respectively. We then calculated the ratio of CS to LDH activities, COX to LDH activities as expressions of metabolic phenotype and CS to COX activities as expression of mitochondrial morphology. Details of the protocol for sample preparation can be found in Supporting Information 2.10.2.

2.6.6 Statistical analyses

Mixed effect models (lmerTest package, Kuznetsova et al., 2017; and lme4 package, Bates et al., 2015) were used to test the effects of isolated and combined seawater temperature and pH, and geographic origin (fixed factors) on shrimp survival, metabolic traits and cellular energetic capacity, with replicate tank as a random variable. Survival rates were arcsin-square-root-transformed as required (Sokal & Rohlf, 1995). Mass-specific metabolic traits and wet mass (WM) were $\log_{10}$ transformed to meet the assumption of linearity. Log_{10} WM was used as covariate for analyses of mass-specific metabolic traits. Log_{10} WM had a significant effect on $\log_{10}$ SMR, $\log_{10}$ MMR and $\log_{10}$ AS. Considering that the random term "tank" was never found to be significant, it was removed from the analyses. Thus ANCOVAs were performed on $\log_{10}$ SMR, $\log_{10}$ MMR and $\log_{10}$ AS and ANOVAs were performed on enzyme activities and their ratios (lmTest package, Hothorn et al., 2015). Tukey HSD tests (Hothorn et al., 2008) were used to conduct *post-hoc* analyses when significant effects were found for main factors in absence of interactions. Alternatively, glht tests (multcomp package, Bretz et al., 2016) were used to conduct *post-hoc* analyses on selected comparisons when significant interactions were evidenced in order to increase the analysis' power and focus on comparisons relevant for our study. Specifically, we compared (i) means at different temperatures for the same origin and means of different origins at the same temperature when the interaction between temperature and origin was found to be significant; (ii) means at different temperatures for the same pH and means at the two pH levels at the same temperature when the interaction between temperature and pH was found to be significant, and (iii) means of different origins at the same pH and means at the two pH levels for the same origin when the interaction between origin and pH was found to be significant. *p*-values were adjusted for multiple comparisons using the Holm method. Normality of residuals was verified using the Shapiro-Wilk's test, whilst homoscedasticity was verified using the Brown-Forsythe's test. Most data met the assumptions of normality and homoscedasticity without transformation or following $\log_{10}$ transformation, except for $\log_{10}$ AS and CS. However, we considered our analysis to be tolerant to deviations from the assumptions of normality and heteroscedasticity given our level of replication and structure of the experimental design used (Sokal & Rohlf, 1995; Underwood, 1997). Additionally, a statistical (Hartig, 2020) and visual analysis of the residuals were performed to confirm the appropriateness of the models used.

Pearson's correlations between total and specific activity were calculated for all enzymes. Both measures of activity were significantly correlated for all enzymes measured in shrimp abdominal muscle for each origin (R between 0.56 and 0.98 depending on the enzyme and the origin, *p*-value < 0.001 for all analyses), so only specific activity data are presented.

All the statistical analyses were performed using the software R 3.6.3 version (R Core Team, 2020).

2.7 RESULTS

2.7.1 Survival

Mean survival rates decreased significantly at the highest temperature and at the low pH level tested (Table S 2.3). In detail, mean survival was significantly lower at 10 °C (approximately 77 %) than at 2 and 6 °C (approximately 92 % and 88 %, respectively), with the two colder temperatures being comparable to each other (Figure 2.2A). In addition, a significant decrease of approximately 5% in mean survival was observed at low pH (Figure 2.2B). Finally, shrimp from different origins showed comparable survival rates and none of the interactions were found to have a significant effect on survival (Table S 2.3).

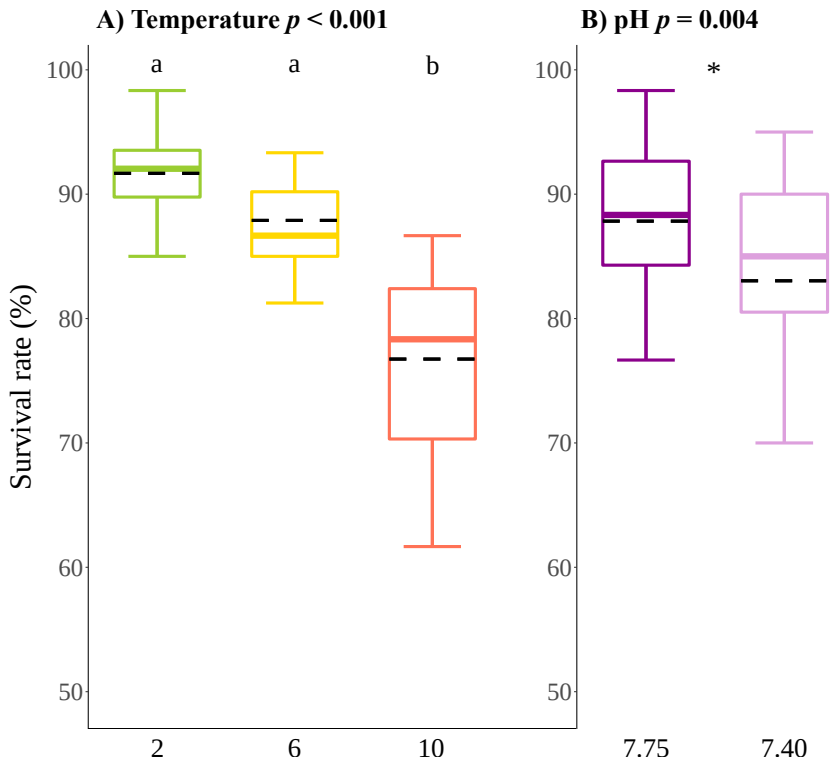


Figure 2.2 The effects of exposure to isolated and combined seawater temperature and pH over 30 d on mean survival rate (%) of female northern shrimp *P. borealis*. Solid lines represent the median and dashed lines represent the mean. A) Temperature treatments are: 2 (2 °C - yellow), 6 (6 °C - green) and 10 (10 °C - red). Lower case letters indicate the presence of a significant difference ($p < 0.05$) among temperatures treatments. B) pH treatments are: 7.75 (purple) and 7.40 (light purple). The asterisk indicates the presence of a significant difference ($p < 0.05$) between the two pH treatments. No significant differences among origins were found.

2.7.2 Metabolic traits

2.7.2.1 Standard metabolic rate (SMR)

Mean SMR increased differently with increasing temperature for shrimp from different origins, as indicated by the presence of a significant interaction between temperature and origin (Table S 2.3, Figure 2.3A). Specifically, for shrimp from all origins, mean SMR increased with increasing temperature along a linear trend, with the exception of shrimp from EC which showed an increase in mean SMR only between 2 and 6 °C (Figure 2.3A). In addition, mean SMR values of shrimp from different origins measured at the same temperature differed significantly at all temperatures tested (Figure 2.3A). Specifically, mean SMR of shrimp from SLE were significantly higher than those of NNC, and among the highest, at all temperatures tested (Figure 2.3A). Furthermore, the interaction between temperature and pH was found to be significant (Table S 2.3) Mean SMR increased with increasing temperature within the same pH treatment (average increase of approximately 84 % from 2 to 10 °C), at both pH levels tested (Figure 2.3B) and was comparable between the two pH levels tested at all temperatures (Figure 2.3B), as the multiple comparisons tests for mean SMR values between different pH levels (at the same temperature) failed to detect significant differences.

Additionally, mean SMR of shrimp from different origins measured at the same pH level differed significantly at the lowest pH tested only, as indicated by the presence of a significant interaction between origin and pH (Table S 2.3, Figure 2.3C). Specifically, mean SMR of shrimp from EC and NNC differed significantly from each other, being the highest (0.063 O_2 h^{-1} g^{-1}) and lowest (0.049 O_2 h^{-1} g^{-1}) respectively, whilst both being comparable to mean SMR of SLE and ESS shrimp (Figure 2.3C). Mean SMR of each origin was comparable between the two pH levels tested (Figure 2.3C), as the multiple comparisons tests for mean SMR values between different pH levels (for the same origin) failed to detect significant differences.

Finally, no effect of the interaction among temperature, pH and origin was found to be significant for this trait.

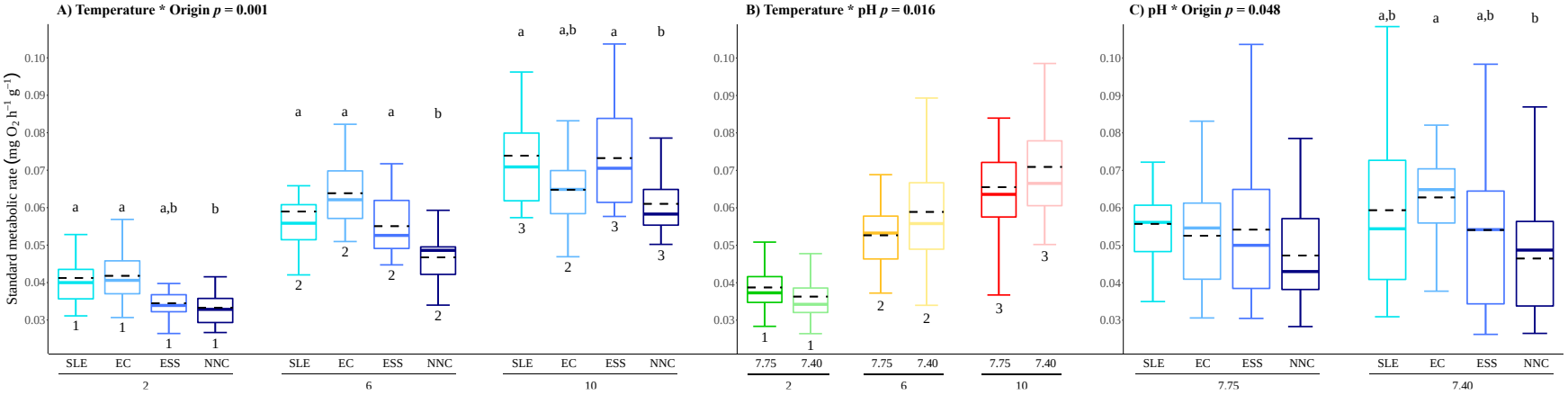


Figure 2.3 The effects of exposure to isolated and combined seawater temperature and pH over 30 d on mean standard metabolic rate (SMR) of female northern shrimp *P. borealis* from different origins. Solid lines represent the median and dashed lines represent the mean. A) Treatments are: temperatures (2, 6 and 10 °C) and origins: SLE (St. Lawrence Estuary - azure), EC (Esquiman Channel - light blue), ESS (Eastern Scotian Shelf - blue) and NNC (Northeast Newfoundland Coast - dark blue). Numbers indicate the presence of a significant difference ($p < 0.05$) among temperature treatments for the same origin and lower case letters indicate the presence of a significant difference ($p < 0.05$) among origins at the same temperature treatment. B) Treatments are: 2C (2 °C, pH 7.75 - green), 2A (2 °C, pH 7.40 - light green), 6C (6 °C, pH 7.75 - yellow), 6A (6 °C, pH 7.40 - light yellow), 10C (10 °C, pH 7.75 - red), 10A (10 °C, pH 7.40 - light red). Numbers indicate the presence of a significant difference ($p < 0.05$) among temperature treatments at the same pH treatment. C) Treatments are: pH (7.75 and 7.40) and origins (SLE, ESS, EC and NNC). Lower case letters indicate the presence of a significant difference ($p < 0.05$) among origins at the same pH treatment.

2.7.2.2 Maximum metabolic rate (MMR)

Mean MMR significantly increased with temperature along a linear trend, rising from 0.157 to 0.240 mg O_2 h^{-1} g^{-1} from 2 to 10 °C (Table S 2.3, Figure 2.4A). In addition, a significant decrease of approximately 12 % in mean MMR was observed at low pH (Table S 2.3, Figure 2.4B). Moreover, different origins differed in mean MMR (Table S 2.3, Figure 2.4C). Specifically, mean MMR of shrimp from SLE was the highest (0.213 mg O_2 h^{-1} g^{-1}) when compared to all other origins and significantly differed from mean MMR of shrimp from ESS (0.190 mg O_2 h^{-1} g^{-1}) which was the lowest (Figure 2.4C). In addition, mean MMRs reported for shrimp from EC and NNC were comparable to mean MMRs reported for shrimp from all other origins (Figure 2.4C).

Finally, none of the interactions had a significant effect on mean MMR (Table S 2.3).

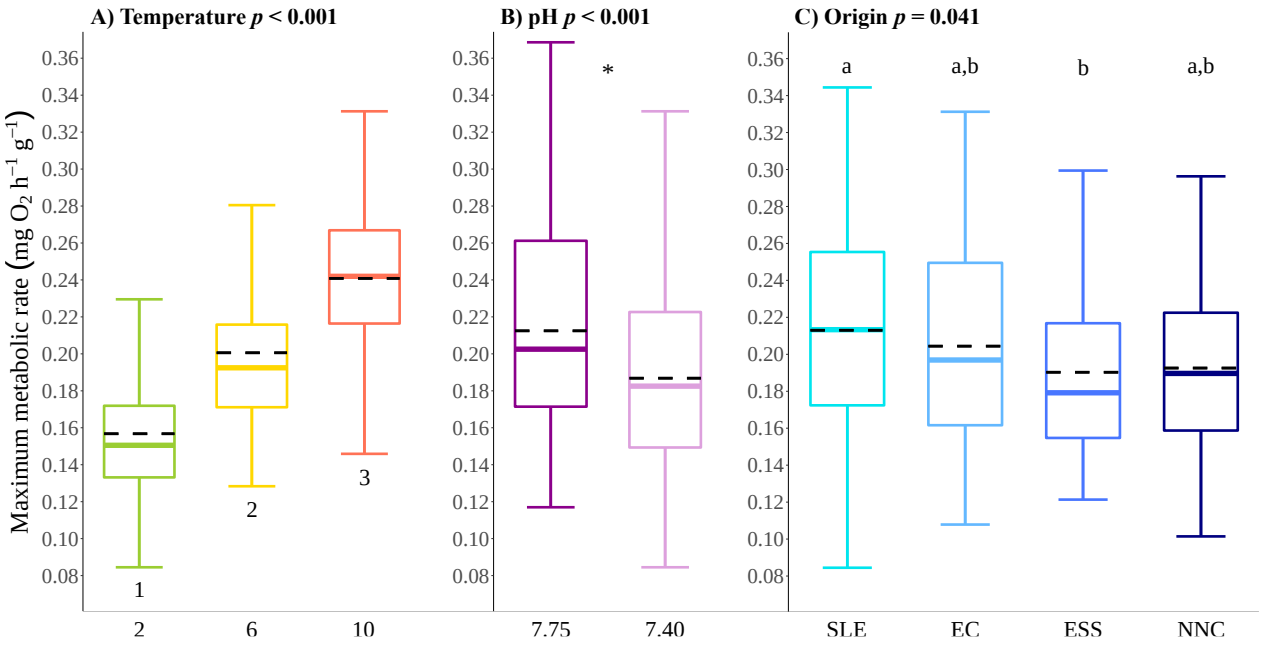


Figure 2.4 The effects exposure to isolated and combined seawater temperature and pH over 30 d on mean maximum metabolic rate (MMR) of female northern shrimp *P. borealis* from different origins. Solid lines represent the median and dashed lines represent the mean. A) Temperature treatments are: 2 (2 °C - yellow), 6 (6 °C - green) and 10 (10 °C - red). Numbers indicate the presence of a significant difference ($p < 0.05$) among temperature treatments. B) pH treatments are: 7.75 (purple) and 7.40 (light purple). The asterisk indicates the presence of a significant difference ($p < 0.05$) between the two pH treatments. C) Origins: SLE (azure), EC (light blue), ESS (blue) and NNC (dark blue). Lower case letters indicate the presence of a significant difference ($p < 0.05$) among origins.

2.7.2.3 Aerobic scope (AS)

Mean AS increased with increasing temperature differently for shrimp from different origins as indicated by the presence of a significant interaction between temperature and origin (Table S 2.3, Figure 2.5A). Specifically, for shrimp from EC and NNC, mean AS increased significantly with increasing temperature (increase of approximately 81 % and 43%, respectively, from 2 to 10 °C), whilst for shrimp from SLE and ESS mean AS was similar among all temperatures (Figure 2.5A). Moreover, mean AS of shrimp from different origins measured at the same temperature were comparable at each temperature tested (Figure 2.5A), as the multiple comparisons tests for mean AS values between different origins (at the same temperature) failed to detect significant differences.

Finally, exposure to low seawater pH caused a significant decrease of approximately 18 % in mean AS (Table S 2.3, Figure 2.5B) and the remaining interactions in our analyses were not significant (Table S 2.3).

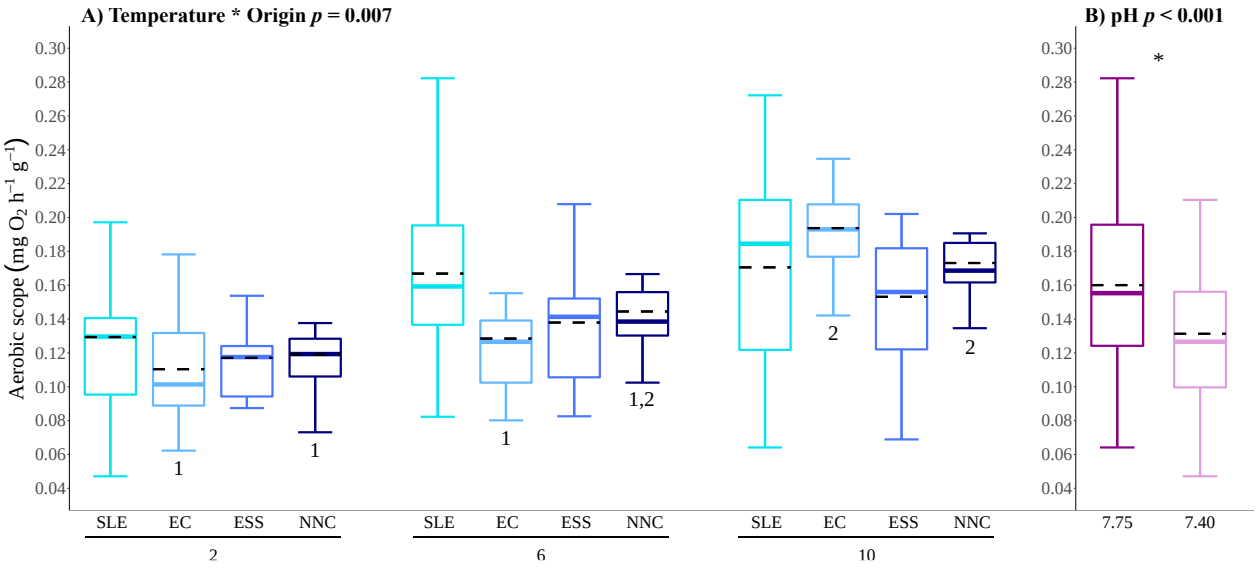


Figure 2.5 The effects exposure to isolated and combined seawater temperature and pH over 30 d on mean aerobic scope (AS) of females of the northern shrimp *P. borealis* from different origins. Solid lines represent the median and dashed lines represent the mean. A) Treatments are: temperatures (2, 6 and 10 °C) and origins: SLE (azure), EC (light blue), ESS (blue) and NNC (dark blue). Numbers indicate the presence of a significant difference ($p < 0.05$) among temperature treatments for the same origin. B) pH treatments are: 7.75 (purple) and 7.40 (light purple). The asterisk indicates the presence of a significant difference ($p < 0.05$) between the two pH treatments.

2.7.3 Cellular energetic capacity

Mean enzyme activities and their ratios differed significantly for shrimp from different origins (Table S 2.3, Figure 2.6). Specifically, mean CS, COX and LDH of shrimp from SLE were the lowest measured (5.9, 2.6 and 338.8 U g^{-1} of proteins respectively) when compared to those of shrimp from other origins (Figure 2.6A,B,C). In addition, shrimp from ESS and NNC showed the highest mean CS and COX, respectively, when compared to shrimp from all other origins, and about double the mean values of shrimp from SLE (Figure 2.6A,B). Furthermore, mean enzyme activities of shrimp from SLE always differed significantly from those of shrimp from ESS and NNC (Figure 2.6A,B,C).

Finally, shrimp from ESS showed the highest mean CS:LDH and CS:COX compared to those of shrimp from all other origins (Figure 2.6D,F) and shrimp from NNC showed the highest mean COX:LDH (6.37) compared to an average of 3.30 shown from shrimp from all other origins (Figure 2.6E).

All other terms in our analyses were found to be non-significant (Table S 2.3).

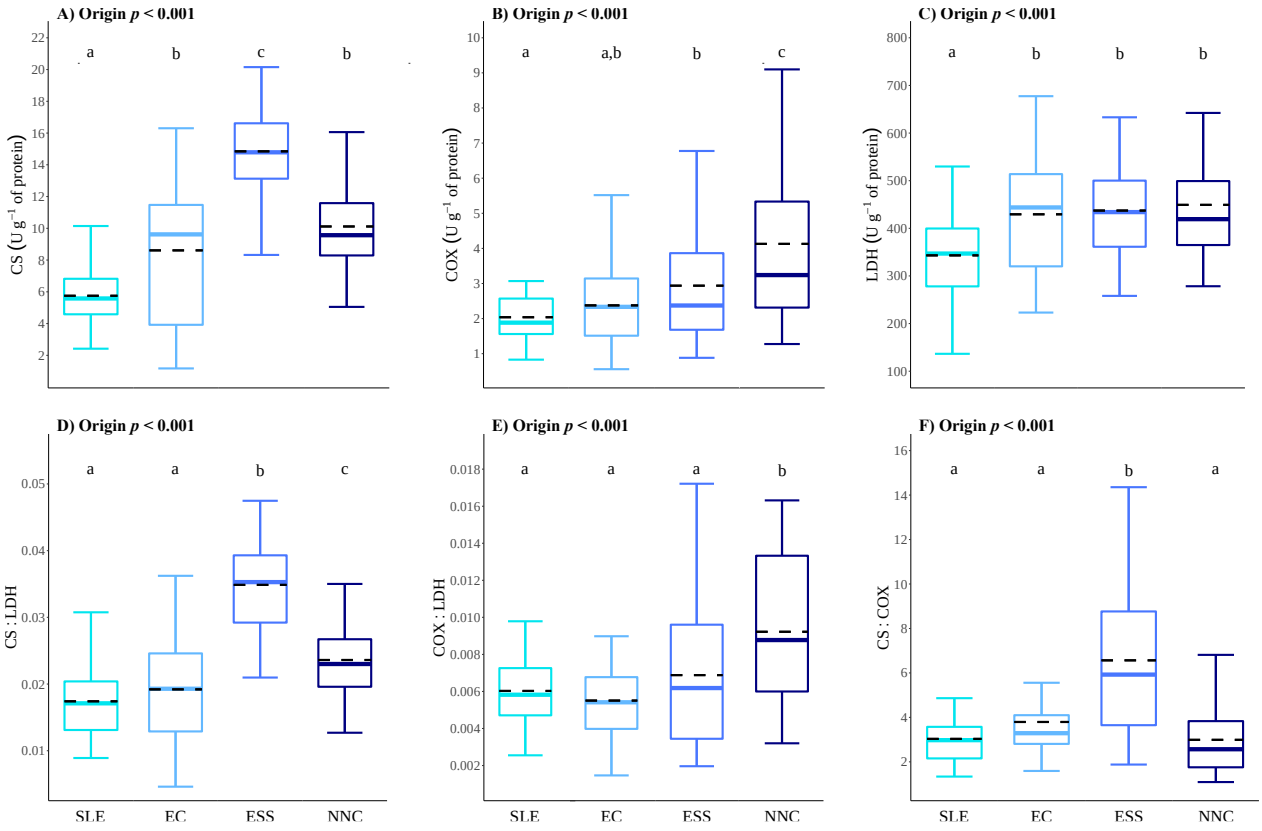


Figure 2.6 Specific enzyme activities and their ratios in the muscle of female northern shrimp *P. borealis* from different origins after 30 d exposure to isolated and combined seawater temperature and pH. A) citrate synthase (CS), B) cytochrome C oxidase (COX), C) lactate dehydrogenase (LDH), D) citrate synthase – lactate dehydrogenase ratio (CS:LDH), E) cytochrome C oxidase – lactate dehydrogenase ratio (COX:LDH) and F) citrate synthase – cytochrome C oxidase ratio (CS:COX). Solid lines represent the median and dashed lines represent the mean. Origins are: SLE (azure), EC (light blue), ESS (blue) and NNC (dark blue). Lower case letters indicate the presence of a significant difference ($p < 0.05$) among origins. No effects of temperature nor pH were found to be significant.

2.8 DISCUSSION

Altogether, our results show that *P. borealis* is overall relatively tolerant to the combined exposure to OW and OA conditions predicted to occur at the end of the century. Indeed, OW and OA are shown to reduce shrimp survival rates moderately under short exposure to laboratory conditions and increase their whole-organism aerobic performance. Interestingly, small differences in metabolic rates among origins do not appear to impact the overall aerobic scope of shrimp, which, along with survival rates, is comparable among shrimp from different origins. This suggests that northern shrimp sensitivity to OW and OA is highly comparable among individuals from different geographic origins close to the southern edge of its distribution. Moreover, isolated and combined OW and OA do not alter shrimp cellular enzyme activities, either because there is little plasticity associated to short-term changes in environmental conditions or because there is no need of compensation. However, it is noteworthy that shrimp cellular aerobic capacity (CS and COX activities) differs among origins, suggesting signs of regional long-term acclimatization or adaptation at the cellular level. This highlights the importance of testing multiple levels of biological hierarchy (Bartholomew, 1964; Harvey et al., 2014) whilst investigating the responses of organisms from different geographic origins (Gaston et al., 2009) when attempting to define species' sensitivity to combined global changes, to ensure we avoid over- or underestimating global change impacts.

Overall, the northern shrimp is more sensitive to OW than to OA, confirming that temperature is a major controlling factor in ectotherms at the whole-organism level (Fry, 1971). Indeed, shrimp SMR increases only with increasing temperatures. OW also leads to an increase of approximately 53 % in shrimp MMR, whilst pH alone causes a more moderate decrease of this trait (~ 12 %). An increase in SMR means that more energy is required for maintenance (Brett & Groves, 1979; Chabot et al., 2016b), but shrimp appear to be able to support the increased energy demand as their capacity to transport oxygen to the tissues (MMR) also increases. Shrimp AS increases with OW, and moderately decreases with OA. This confirms previous findings that shrimp can tolerate temperature increases, as well as the pH decreases, predicted to occur by the year 2100 in the northwest Atlantic (Chemel et al., 2020; Lavoie et al., 2020), as long as the increased metabolic demand is fuelled by an adequate prey supply.

Interestingly, our results show the presence of differences among shrimp from different origins, considered as indicators of low level of intraspecific variation, for both SMR and MMR. Specifically, maintenance and repair costs of shrimp from SLE are among the highest compared to those from other origins, and are always higher than those of shrimp from NNC. In fish, individuals with a higher SMR tend to have a higher MMR and often show a greater AS (Metcalfe et al., 2016). We suggest that the differences observed in maintenance costs and oxygen transport capacity among shrimp from different origins, captured by SMR and MMR variations, respectively, do not impact the whole-organism performance of individuals that survived, as shrimp from different origins show comparable AS at each temperature tested and within the duration of exposure used in this study. Nonetheless, energy allocation and partitioning could differ among shrimp from different origins based on their different needs. However, further studies on energy allocation and partitioning, for example using modelling approaches (such as Dynamic Energy Budget models), are needed to unravel further potential differences among origins.

Altogether, similar whole-organism aerobic performances among shrimp origins suggest that sensitivity of shrimp to predicted OW and OA is comparable among origins. In contrast, other marine species showed geographically specific metabolic responses to these drivers, showing an increased response to OW and OA (Calosi et al., 2017; Lardies et al., 2014; Thor et al., 2018; Vargas et al., 2017). At the cellular level, OW and OA do not affect the activity of enzymes involved in energy metabolism, suggesting either low plasticity of metabolic apparatus or no requirement for compensation. The cellular energetic capacity, however, varies among shrimp from different origins, showing a high level of intraspecific variation and supporting the idea that long-term acclimatization or adaptation to regional environmental conditions has occurred at the cellular level. Indeed, shrimp from ESS and NNC have the greatest aerobic capacity compared to shrimp from other origins, as their CS and COX activities and their ratios to LDH are the highest, respectively. In fish, higher mitochondrial densities (i.e. higher CS activity) have been associated to cold acclimation (Battersby & Moyes, 1998; Lannig et al., 2003; Lucassen et al., 2003), supporting the suggestion that *P. borealis* could be regionally acclimatized or adapted to differing temperatures. Differences in metabolic enzyme activities among populations as a consequence of acclimatization or adaptation have also been shown in other marine species inhabiting different environmental regimes and latitudes (Liu et al., 2013; Rodríguez et al., 2019; Sokolova & Pörtner,

2001). Moreover, ESS and NNC enzyme activities always differ from those of shrimp from SLE, which can be considered to show the worst general physiological condition as they show the lowest aerobic and anaerobic enzyme activities, and the highest MMR. This suggests that shrimp from SLE might already operate close to the limit of their aerobic capacity, which can be detrimental. Indeed, it can promote oxidative stress and leaves little room for increasing aerobic capacity meaning faster and higher mobilization of anaerobic glycolysis and metabolic fatigue. Conversely, shrimp from ESS have the highest CS to COX ratio, suggesting that mitochondria have a greater reducing capacity since CS is an enzyme located in the mitochondrial matrix that catalyses the reaction at the entry to the Krebs cycle pathway promoting the aerobic metabolism (Wiegand & Remington, 1986).

Altogether, our study demonstrates that shrimp from different origins show different cellular aerobic and anaerobic capacities, while their whole-organism performance and survival rates are comparable. Additionally, OW and OA have a moderate effect on survival rate, as observed at the highest temperature and at the lowest pH tested, after 30 d of exposure. However, we should consider that over longer exposure periods *in situ*, and under exposure to additional co-occurrent global change drivers, like decreasing dissolved oxygen, mortality levels might be significantly higher (> 60 %, Chemel et al., 2020). We confirm that shrimp are relatively tolerant to OW and OA, as suggested before (Chemel et al., 2020; Dupont et al., 2014). Despite our results showing the sensitivity to OW and OA of shrimp from different origins is comparable, their vulnerability to environmental conditions predicted for the end of the century could still differ due to the environmental regime they are currently exposed to. In fact, shrimp experience contrasting regional environmental conditions in the northwest Atlantic: in some regions they already experience temperatures and pH levels close to those predicted to occur locally by the year 2100 (Lavoie et al., 2020). Recently, mean temperatures in EC increased from ~5–6 to ~ 6–7 °C at ~ 250 m deep, where shrimp are most abundant (DFO, 2022a). Across the ESS, bottom temperatures range from -1 to 6 ºC year-round (DFO, 2022c) and in the last decade they have been observed to be at least 3 °C in spring (May-June), and increasing annually since 2017 (DFO, 2022c). During the same period, NNC average bottom temperatures have ranged from 0.2 to 3.4 °C following decadal cycles, with a general warming from the 1980s to 2020 (Cyr et al., 2022). In the SLE, waters are most severely hypoxic (18–26% sat. between 1990 and 2008, Gilbert et al. 2005) and dissolved oxygen

(DO) levels of deep waters decreased even further in recent years (down to 14–16 % O_2 sat., Blais et al., 2021; Jutras et al., 2020), reaching levels close to the hypoxia tolerance level of shrimp (16 % O_2 sat. for females at 5 °C, Dupont-Prinet et al., 2013). The decrease in DO likely caused the recent shift to shallower (< 150 m), colder (2–3 °C) and better oxygenated (50–60 % O_2 sat.) waters observed for shrimp from the SLE (Bourdages et al., 2022, Figure 18). Hypoxia has also been shown to compromise shrimp physiology, and strongly reduce their survival when combined with OW and OA (Chemel et al., 2020), thus, DO needs to be taken into account when trying to define their vulnerability among origins. The NNC near-bottom DO ranges from 60 to 100 %, and is generally above 80%, whilst the overall ESS DO is above 60 % and has remained relatively stable since 2014. However, the northern parts of the ESS are influenced by the lower DO levels observed in the Laurentian Channel, and show a decrease to 40 to 50 % O_2 sat. (DFO, 2021d). Hypoxia is more severe in the EC, where shrimp experience DO levels of ~ 30 % O_2 sat. at 6.5 °C (DFO, 2022a). Unlike shrimp from SLE, EC shrimp have not moved to shallower depths, either because the conditions in deep water were not extreme enough or there is not enough habitat with appropriate sediments available at shallower depths. Similarly, seawater pH differs among regions. The most recent surveys in the SLE show bottom pH varying between 7.4 and 7.6, these values being a little lower when compared to those from the EC (DFO, 2021d). Furthermore, in the ESS and the NNC bottom water pH is higher and ranges from 7.8 to above 8 (DFO, 2021d, 2022b). Considering that shrimp from EC already experience the highest temperatures and the lowest DO levels compared to shrimp from other origins, based on recent findings their survival at the end of the century could be reduced to as low as 40 % (Chemel et al., 2020). This will likely have important consequences on shrimp abundance and hence on the fisheries, suggesting risks of local commercial extinction.

In conclusion, our study shows that the level of intraspecific variation can differ among biological compartments, highlighting the importance of measuring organisms' physiological responses at multiple levels of biological organization to avoid over or under-estimate species' sensitivity to future complex environmental conditions (Calosi et al., 2017). In addition, the high level of intraspecific variation reported for cellular responses underlines the importance of conducting multi-population studies instead of considering species as a single homogeneous unit (Gaston et al., 2009). This study also confirms that using a macrophysiological-macroecological approach

when attempting to define species' sensitivity to combined global changes is paramount, to avoid over- or underestimation, particularly for ectotherms of ecological, commercial and socio-economic values, such as the northern shrimp. Finally, our results indicate that despite the comparable responses of shrimp from the four distinctive origins, current differences in environmental conditions at different locations will yield different environmental conditions at the end of the century, this having in turns important implications for management and conservation of *P. borealis* and its stocks.

2.9 STATEMENTS

Data availability statement

The data that support the findings of this study will be available on PANGAEA® Data Publisher, under OA-ICC data compilation. A digital object identifier (DOI) will be assigned later.

Author contributions

DC, FN and PC conceived the general experimental design, and supported EG in finalizing the design and protocol used. DC, FN and PC obtained the necessary funding to support the research action associated with this work. DC provided the installations for the experimental setups and the live shrimp from the different geographic origins for the study. EG carried out the experiments and the oxygen uptake measurements supported by TH. EG carried out the oxygen uptake data analyses supported by DC. PB provided the installations for the enzymatic activity analyses, which were carried out by EG. EG completed the statistical analyses supported by DC, FN and PC. EG conducted the interpretation of the results and wrote the manuscript. All authors contributed to the final version of the manuscript.

Funding

This work was supported by: (i) an Ouranos grant [554023 to FN, DC and PC]; (ii) a DFO Strategic Program for Ecosystem-Based Research and Advice grant and an Aquatic Climate Change Adaptation Services Program grant to DC; (iii) a FIR UQAR grant, a Canada Foundation for Innovation grant and a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant [RGPIN-2015-06500 and RGPIN-2020-05627 to PC]; (iv) an NSERC Discovery grant [RGPIN-05992 to PB]. EG was supported by a MITACS-Ouranos Accelerate grant, a Fonds

de Recherche du Québec - Nature et Technologies (FRQNT) scholarship (PBEEE, 289597) and a Réal-Décoste Ouranos scholarship (286109).

Acknowledgments

The authors wish to thank J. Gagnon, D. Picard and V. Desrosiers for technical support during the experiment at the DFO Maurice Lamontagne Institute (Mont-Joli, QC, Canada) and during measurements at the University of Québec in Rimouski (Canada). The authors wish to acknowledge the Indigenous Peoples and the history of the traditional territories on which our work was conducted. EG, FN and PC are members of the inter-institutional strategic research network Québec-Océan. FN, DC and PB are members of the Ressources Aquatiques Québec inter-institutional strategic research network. PB is a member of the inter-institutional strategic research network Quebec Center for Biodiversity Science (QCBS).

Competing interests

No competing interests declared.

2.10 SUPPORTING INFORMATION

2.10.1 Respirometry method and setup and determination of metabolic traits

Ten custom-built cylindrical glass respirometers (vol. ≈ 300 mL) were kept in each of the two dedicated respirometry tanks to determine shrimp oxygen uptake ($\dot{M}_{O_2}$). Temperature and pH conditions during measurements of oxygen uptake were identical to the experimental tank setup. Each respirometer was supplied with clean and aerated seawater with a flush pump and equipped with a recirculation loop to keep water well mixed inside the respirometer. Dissolved oxygen (DO) was measured every second with fiber optic oxygen probes (OXYPro DP-PSt3, PreSens Precision Sensing GmbH) coupled to an oxygen meter (Oxy-4 mini or Fibox 3, PreSens Precision Sensing GmbH, Regensburg, Germany). Data acquisition and flush activation was controlled by a data acquisition unit (DAQ-M, Loligo systems apS, Viborg, Denmark) connected to the AutoResp 2™ software (Loligo systems apS). Each respirometry experiment included two parts.

The first part was designed to estimate maximum metabolic rate (MMR). Shrimp were chased until fatigued and then introduced into respirometers (see main text). Because $\dot{M}_{O_2}$ can decline rapidly

when a shrimp is placed inside a respirometer after chasing (Zhang et al., 2019), the flush pump was maintained in "OFF mode" during the first 2 h or so (long closed phase) and MMR was estimated using a modified version of the method described by Zhang et al. (2019). Briefly, the shortest reliable window width (WW) was determined for shrimp from SLE and EC using the last 8.25 min of the long closed phase. For each shrimp and WW (all possible WWs from 0.5 to 4 minutes, in increment of 0.25 minutes), the mean slope and its SD were calculated across all the regressions obtained (e.g., 16 regressions for WW = 0.5 min, down to 2 regressions for WW = 4 min). SDs were log transformed and compared across WWs in R (R Core Team, 2020) using a mixed-effect model (package NLME, Pinheiro et al., 2020), where shrimp ID was set as random variable. *Post-hoc* comparisons consisted of paired-t-tests (i.e. repeated measures for each shrimp, 2 WWs at a time) of all other WWs against the longest WW (4 min), considered to be the most reliable. *p*-values were adjusted for multiple comparisons using the Holm method (pairewise_t_test function of package rstatix, Kassambara, 2021). As mean SDs were significantly greater than the control (WW = 4 min) when WW was less than three minutes, that duration was taken to be the shortest reliable WW. This was done separately for the SLE and EC shrimp, with the same result. Considering that measuring $\dot{M}_{O_2}$ for shrimp from ESS and NNC involved the same respirometry setup and female shrimp of similar size, the three minute WW was used for all origins. For each shrimp, rolling regressions of DO as a function of time (i.e. regressions calculated over 3 min, each one starting 1 s later than the previous one) were calculated over the entire long closed phase and the steepest slope was taken to calculate MMR. The highest $\dot{M}_{O_2}$ observed during the period of regular intermittent-flow cycles (see below) was used as a second estimate of MMR. The highest of the two values was retained as MMR (Dupont-Prinet et al., 2013).

The second part of each respirometry experiment was designed to estimate the standard metabolic rate (SMR). After it was clear that oxygen uptake had slowed in the respirometers, the long closed phase was ended and regular cycles were initiated for the next 48 h: respirometers were flushed with experimental treatment seawater for five minutes and closed for 11 minutes. During flushes, water overflow was evacuated above the water surface. The linear decline in DO observed during the last eight minutes of the closed period was used to calculate $\dot{M}_{O_2}$ according to Steffensen (1989; equation 2) and Garcia and Gordon (1992; equation 8) for oxygen solubility.

Background $\dot{M}O_2$ was measured before shrimp were introduced into the respirometers and after shrimp removal and a linear regression was fitted to the background respiration values as a function of time, to correct $\dot{M}O_2$ according to Rosewarne et al. (2016).

Before calculating $\dot{M}O_2$ (background or otherwise), the raw data acquired by the AutoResp 2™ software (DO as a function of time) were first smoothed with a running average using a WW of 15 seconds (for the determination of the shortest reliable WW and for the rolling regressions to estimate MMR) or varying from 15 to 75 seconds for each shrimp to improve signal-to-noise ratio (Chabot et al., 2021). For the regular cycles, data from the trace were also separated into 20-s blocks and the median DO of each block was retained, another way to improve signal-to-noise ratio (Chabot et al., 2021).

Processed traces of DO over time were used to calculate and plot the slopes of DO decline over time for each shrimp. We then selected an appropriate minimal value of r^2 (min $r^2 = 0.95$ for most shrimp, min $r^2 = 0.80$ for a few shrimp) from the examination of the plots to exclude slopes based on non-linear DO declines. For each shrimp SMR was determined using the quantile method detailed in Chabot et al. (2016), with a q of 0.2, after excluding $\dot{M}O_2$ determinations obtained during the recovery and acclimation period (15h) and with a r^2 lower than the minimal value.

Of the 240 shrimp for which SMR and MMR were determined *via* respirometry, 24 individuals were removed from the final dataset: ten shrimp moulted and/or died in the respirometers and 14 others did not reach low stable $\dot{M}O_2$ levels to confirm that they acclimated to the respirometer, or failed to increase $\dot{M}O_2$ following exercise.

2.10.2 Cellular aerobic and anaerobic enzyme activities

To measure enzyme activities, frozen abdominal tissue samples were first rapidly minced and homogenized on ice with four volumes of phosphate buffered saline solution (PBS, pH 7.5) containing 0.1% Triton X-100 and 1 mM methylene diamine tetra-acetic acid (EDTA). They were then centrifuged (5 min, 4 °C, 500 *g*) and the supernatant was divided into aliquots for enzyme activity measurement. All enzyme activity analyses were carried out according to Thibeault et al. (1997) at a constant temperature of 20 °C using a spectrophotometer (Cary 100 UV-Vis, Agilent, Petaling Jaya, Malaysia) and the proprietary software (CaryWin UV, 4.20). Total protein content was measured on homogenates using the bicinchoninic acid method (Smith et al., 1985) and a UV/VIS microplate spectrophotometer (Perkin Elmer Envision, Foster City, CA, USA). Total and

specific enzymatic activities were measured and expressed as U g^{-1} of wet tissue and U g^{-1} of protein, respectively. All analyses were performed in duplicate and if the two measurements were more than 10 % apart, the analysis was repeated.

Four shrimp died in the respirometers after $\dot{M}_{O_2}$ determination due to a logistic problem and were thus removed from the enzyme dataset. Additionally, for 7 shrimp the COX activity measurements remained more than 10 % apart, thus those shrimp were also excluded from the COX dataset.

2.10.3 Tables

Table S 2.1 Summary (mean ± SD) of the physico-chemical parameters of the sea water measured and calculated (*) during our laboratory experiment in different treatments and for shrimp from different geographic origins: temperature (°C), pH (Total scale), dissolved oxygen (% sat.), salinity, total alkalinity (TA, µEq kg^{-1}), total dissolved carbon dioxide* (DIC, µmol kg^{-1}), carbon dioxide partial pressure* (pCO_2 µatm), bicarbonate concentration* ([HCO_3^-], µmol kg^{-1}) and carbonate concentration* ([CO_3^{2-}], µmol kg^{-1}), Ω calcite* and Ω aragonite*. Treatments correspond to: 2C (2 °C, pH 7.75), 2A (2 °C, pH 7.40), 6C (6 °C, pH 7.75), 6A (6 °C, pH 7.40), 10C (10 °C, pH 7.75), 10A (10 °C, pH 7.40). Origins: St. Lawrence Estuary (SLE), Esquiman Channel (EC), Easter Scotian Shelf (ESS) and Northeast Newfoundland Coast (NNC).

Origin	Treatment	Temperature	pH_T	Oxygen	Salinity	TA	DIC *	pCO_2 *	[HCO_3^-] *	[CO_3^{2-}] *	Ω cal *	Ω ara *
SLE	2C	2.32 ± 0.10	7.76 ± 0.01	99.85 ± 0.19	32.96 ± 0.08	2071.87 ± 13.24	2065.88 ± 13.46	752.34 ± 19.79	1972.72 ± 12.98	49.56 ± 1.43	1.20 ± 0.03	0.75 ± 0.02
SLE	2A	2.35 ± 0.12	7.42 ± 0.02	99.75 ± 0.17	32.94 ± 0.08	2056.21 ± 13.07	2130.36 ± 15.09	1690.98 ± 55.31	2007.62 ± 13.65	24.29 ± 1.45	0.59 ± 0.03	0.37 ± 0.02
SLE	6C	6.02 ± 0.06	7.74 ± 0.01	98.08 ± 0.29	32.96 ± 0.07	2040.07 ± 13.33	2026.49 ± 13.74	789.28 ± 18.88	1933.12 ± 13.25	53.46 ± 1.13	1.29 ± 0.03	0.81 ± 0.02
SLE	6A	6.09 ± 0.07	7.43 ± 0.02	97.55 ± 0.15	33.07 ± 0.07	2067.63 ± 13.01	2124.2 ± 13.74	1694.84 ± 52.39	2009.68 ± 12.76	28.97 ± 1.61	0.70 ± 0.04	0.44 ± 0.02
SLE	10C	9.68 ± 0.10	7.76 ± 0.01	97.16 ± 0.20	33.11 ± 0.07	2056.21 ± 13.07	2025.04 ± 13.57	769.17 ± 21.95	1925.39 ± 13.24	65.39 ± 1.39	1.58 ± 0.03	1.00 ± 0.02
SLE	10A	9.61 ± 0.14	7.45 ± 0.02	97.26 ± 0.21	33.03 ± 0.07	2040.07 ± 13.33	2080.44 ± 15.40	1680.24 ± 68.89	1971.38 ± 14.32	34.34 ± 1.83	0.83 ± 0.04	0.52 ± 0.03
EC	2C	2.14 ± 0.06	7.73 ± 0.01	98.92 ± 0.16	33.16 ± 0.04	2122.96 ± 2.03	2123.99 ± 3.53	831.68 ± 22.55	2028.11 ± 3.66	47.41 ± 1.45	1.14 ± 0.04	0.72 ± 0.02
EC	2A	2.13 ± 0.06	7.43 ± 0.02	99.72 ± 0.14	32.99 ± 0.04	2134.95 ± 1.83	2209.32 ± 5.44	1702.84 ± 54.05	2084.48 ± 3.62	25.23 ± 1.16	0.61 ± 0.03	0.38 ± 0.02
EC	6C	5.96 ± 0.04	7.72 ± 0.01	96.99 ± 0.29	33.08 ± 0.05	2128.55 ± 1.95	2118.46 ± 3.45	872.19 ± 29.74	2020.16 ± 3.56	54.18 ± 1.46	1.31 ± 0.04	0.82 ± 0.02
EC	6A	5.97 ± 0.04	7.42 ± 0.02	98.04 ± 0.10	33.17 ± 0.04	2122.96 ± 2.03	2186.57 ± 5.48	1820.94 ± 59.08	2065.84 ± 4.30	28.55 ± 1.58	0.69 ± 0.04	0.43 ± 0.02
EC	10C	9.66 ± 0.17	7.74 ± 0.01	96.22 ± 0.17	33.04 ± 0.04	2134.95 ± 1.83	2108.03 ± 2.29	834.39 ± 15.38	2006.69 ± 2.45	64.11 ± 1.02	1.55 ± 0.02	0.98 ± 0.02
EC	10A	9.38 ± 0.18	7.44 ± 0.02	96.44 ± 0.25	33.10 ± 0.04	2128.55 ± 1.95	2174.32 ± 5.07	1773.29 ± 58.19	2061.39 ± 4.08	33.57 ± 1.36	0.81 ± 0.03	0.51 ± 0.02
ESS	2C	2.07 ± 0.05	7.73 ± 0.01	99.31 ± 0.14	32.78 ± 0.04	2158.93 ± 1.31	2160.63 ± 2.28	832.11 ± 13.39	2065.02 ± 2.21	46.94 ± 0.71	1.14 ± 0.02	0.71 ± 0.01
ESS	2A	2.11 ± 0.05	7.41 ± 0.02	99.82 ± 0.13	32.84 ± 0.04	2164.04 ± 0.49	2247.76 ± 4.45	1840.08 ± 52.72	2115.78 ± 2.75	24.13 ± 1.36	0.58 ± 0.03	0.37 ± 0.02
ESS	6C	5.95 ± 0.05	7.72 ± 0.01	98.18 ± 0.17	33.14 ± 0.04	2162.59 ± 0.93	2153.3 ± 2.28	881.7 ± 20.32	2054.78 ± 2.24	53.89 ± 0.99	1.30 ± 0.02	0.82 ± 0.02
ESS	6A	5.92 ± 0.04	7.43 ± 0.01	98.08 ± 0.12	32.98 ± 0.05	2158.93 ± 1.31	2220.6 ± 4.20	1778.52 ± 45.62	2101.81 ± 2.96	28.55 ± 0.98	0.69 ± 0.02	0.43 ± 0.01
ESS	10C	9.44 ± 0.18	7.73 ± 0.01	96.97 ± 0.19	33.53 ± 0.05	2164.04 ± 0.49	2139.69 ± 1.75	868.78 ± 14.69	2037.42 ± 1.98	63.30 ± 0.92	1.52 ± 0.02	0.96 ± 0.01
ESS	10A	9.44 ± 0.18	7.42 ± 0.01	97.72 ± 0.18	33.39 ± 0.05	2162.59 ± 0.93	2212.42 ± 3.73	1847.6 ± 51.85	2097.39 ± 2.65	32.59 ± 0.94	0.78 ± 0.02	0.50 ± 0.01
NNC	2C	2.08 ± 0.06	7.76 ± 0.01	101.36 ± 0.12	31.75 ± 0.03	2137.99 ± 0.92	2134.16 ± 2.21	766.98 ± 13.85	2040.01 ± 2.34	48.98 ± 0.99	1.19 ± 0.02	0.74 ± 0.02
NNC	2A	2.10 ± 0.06	7.43 ± 0.01	101.50 ± 0.06	31.77 ± 0.03	2140.38 ± 0.92	2217.44 ± 3.87	1715.74 ± 45.73	2092.16 ± 2.23	24.1 ± 1.03	0.59 ± 0.02	0.37 ± 0.02
NNC	6C	5.91 ± 0.04	7.74 ± 0.01	99.36 ± 0.20	31.86 ± 0.03	2139.71 ± 0.95	2127.28 ± 2.01	825.06 ± 21.72	2030.58 ± 1.81	54.55 ± 0.84	1.32 ± 0.02	0.83 ± 0.01
NNC	6A	5.82 ± 0.08	7.46 ± 0.02	99.49 ± 0.10	31.83 ± 0.03	2137.99 ± 0.92	2191.68 ± 4.60	1649.26 ± 51.12	2076.32 ± 3.81	30.83 ± 1.74	0.75 ± 0.04	0.47 ± 0.03
NNC	10C	9.35 ± 0.18	7.73 ± 0.01	97.76 ± 0.17	31.98 ± 0.01	2140.38 ± 0.92	2118.53 ± 2.52	867.02 ± 25.06	2018.2 ± 2.57	61.07 ± 1.13	1.48 ± 0.03	0.94 ± 0.02
NNC	10A	9.47 ± 0.15	7.44 ± 0.01	98.15 ± 0.16	31.95 ± 0.02	2139.71 ± 0.95	2186.36 ± 3.36	1763.2 ± 48.41	2074.45 ± 2.45	32.62 ± 1.06	0.79 ± 0.03	0.50 ± 0.02

Table S 2.2 Checklist of 53 essential criteria for the reporting of methods for aquatic intermittent-flow respirometry (Killen et al., 2021). Level of background respiration (n° 32), total number of slopes measured and used to derive metabolic rate (n° 37) and the proportion of data removed due to being outliers below r-squared threshold (n° 40) were calculated for shrimp from SLE only.

Number	Criterion and Category	Response	Value (where required)	Units
	EQUIPMENT, MATERIALS, AND SETUP			
1	Body mass of animals at time of respirometry	Wet mass was measured immediately after respirometry.		
2	Volume of empty respirometers	Including recirculation loop.	≈ 378 ± 19.9	mL
3	How chamber mixing was achieved	With a recirculation loop, consisting of a recirculating pump (AD20P-1230C, DollaTek, Hong Kong, China) and gas tight Tygon ® tubing.		
4	Ratio of net respirometer volume (plus any associated tubing in mixing circuit) to animal body mass		40.6 ± 6.8	
5	Material of tubing used in any mixing circuit	Gas tight Tygon ® tubing.		
6	Volume of tubing in any mixing circuit		4.3	mL
7	Confirm volume of tubing in any mixing circuit was included in calculations of oxygen uptake	Yes.		
8	Material of respirometer (e.g. glass, acrylic, etc.)	Glass.		
9	Type of oxygen probe and data recording	Fiber optic oxygen probes (OXYPro DP-PSt3, PreSens Precision Sensing GmbH) coupled to an oxygen meter (Oxy-4 mini or Fibox 3, PreSens Precision Sensing GmbH, Regensburg, Germany).		
10	Sampling frequency of water dissolved oxygen		1	Measurement s^{-1}
11	Describe placement of oxygen probe (in mixing circuit or directly in chamber)	The oxygen probe was placed on the positive pressure side of the recirculation loop.		
12	Flow rate during flushing and recirculation, or confirm that chamber returned to normoxia during flushing	Chamber returned to normoxia during flushing.		
13	Timing of flush/closed cycles	Respirometers were flushed with experimental treatment seawater for 300 s and closed for 660 s.		
14	Wait (delay) time excluded from closed measurement cycles		180	s
15	Frequency and method of probe calibration (for both 0 and 100% calibrations)	Probes were calibrated before the beginning of each respirometry measurement. For the 0 % calibration we prepared a solution of sodium sulphite and boric acid in freshwater. Probes were calibrated in the solution at the experimental temperature once the probe signal was stable. For the 100% calibration, probes were calibrated in constantly aerated seawater in the experimental tank at the same temperature as for the 0% calibration.		
16	State whether software temperature compensation was used during recording of water oxygen concentration	No, a constant temperature was assumed, but these tanks were known to have stable temperature (± 0.1 °C).		
	MEASUREMENT CONDITIONS			
17	Temperature during respirometry	According to the treatment of measurement.	2, 6 or 10	°C
18	How temperature was controlled	With a feedback system (1/16 DIN Micromega autotune PID Temperature, Omega Engineering inc., Norwalk, USA) that regulated		

		the automatic mixing of cold and hot water to provide each tank with sea water at the set temperature.		
19	Photoperiod during respirometry	24h dark (respirometry tanks were completely surrounded by a thick opaque curtain).		
20	If (and how) ambient water bath was cleaned and aerated during measurement of oxygen uptake (e.g. filtration, periodic or continuous water changes)	We used water flow-through tanks supplied with sea water from two reservoirs at a constant flow rate of 3.5 L min^{-1} and equipped with a submersible pump (1048, Eheim, Stuttgart, Germany) that allowed water mixing.		
21	Total volume of ambient water bath and any associated reservoirs	Tank water volume = 240 L and reservoirs volume = 750 L each for cold and warm water.		
22	Minimum water oxygen dissolved oxygen reached during closed phases	At the end of the long closed phase used to determine MMR, DO had declined to 67.98 ± 10.29 % sat (mean ± SD), whereas DO reached 93.93 ± 2.87 % sat at the end of the cycles used to determine SMR.		
23	State whether chambers were visually shielded from external disturbance	Yes.		
24	How many animals were measured during a given respirometry trial (i.e. how many animals were in the same water bath)		10	
25	If multiple animals were measured simultaneously, state whether they were able to see each other during measurements	Ten respirometers were side by side in a tank, in darkness, but it is still possible that there was enough light in daytime for shrimp to see neighbouring respirometers.		
26	Duration of animal fasting before placement in respirometer	5 days fast was selected to ensure that the digestive tract was empty even at 2 °C. Fasting period was kept constant at the other temperatures.	5	days
27	Duration of all trials combined (number of days to measure all animals in the study)	Respirometry trials for shrimp from SLE started on the 6th of august 2018 and respirometry trials for shrimp from NNC ended on the 30th December 2019. For each origin respirometry trials lasted a total of 11 d.		
28	Acclimation time to the laboratory (or time since capture for field studies) before respirometry measurements	Approximately 8 weeks before the beginning of the experiment that itself lasted 30 days.		
	BACKGROUND RESPIRATION			
29	State whether background microbial respiration was measured and accounted for, and if so, method used (e.g. parallel measures with empty respirometry chamber, measurements before and after for all chambers while empty, both)	Background $\dot{M}O_2$ was measured and a linear regression was fitted to the background respiration values to estimate the evolution of background respiration with time. This regression of background respiration as a function of time for each shrimp was used to correct $\dot{M}O_2$. It should be noted that background respiration was in fact a spurious increase in DO caused by changes in pressure in the respirometers caused by the recirculation pumps; these changes were repeatable and treated as if they were background respiration, i.e., background slope were subtracted from respiration slopes, even though they were of the opposite sign.		
30	State if background respiration was measured at beginning and/or end, state how many slopes and for what duration	Background $\dot{M}O_2$ was measured before shrimp were introduced into the respirometers and after shrimp removal for a minimum of 4 h and 15 slopes each.		
31	State how changes in background respiration were modelled over time (e.g. linear, exponential, parallel measures)	Linear regression, with slopes close to zero in the vast majority of cases. The slope was always close to zero, indicating that background respiration was negligible and that most of the detected change in DO during each cycle was caused by pressure changes.		
32	Level of background respiration (e.g. as a percentage of SMR)	13.93 ± 8.97 (mean ± SD).		

33	Method and frequency of system cleaning (e.g. system bleached between each trial, UV lamp)	The system was cleaned with freshwater before the beginning of each respirometry trial. Additionally, each respirometer was gently rubbed with a brush.		
	STANDARD OR ROUTINE METABOLIC RATE			
34	Acclimation time after transfer to chamber, or alternatively, time to reach beginning of metabolic rate measurements after introduction to chamber	Time to reach beginning of metabolic rate measurements after introduction to chamber.	15	h
35	Time period, within a trial, over which oxygen uptake was measured (e.g. number of hours)		48	h
36	Value taken as SMR/RMR (e.g. quantile, mean of lowest 10 percent, mean of all values)	Quantile method with a q of 0.2.		
37	Total number of slopes measured and used to derive metabolic rate (e.g. how much data were used to calculate quantiles)	113 ± 7 (mean ± SD).		
38	Whether any time periods were removed from calculations of SMR/RMR (e.g. data during acclimation, periods of high activity [e.g. daytime])	Recovery and acclimation periods of 15h.		
39	r^2 threshold for slopes used for SMR/RMR (or mean)	For most shrimp, minimum r2 was set to 0.95. In rare cases the minimum r2 was decreased to a minimum of 0.80.		
40	Proportion of data removed due to being outliers below r-squared threshold	Number of slopes removed during SMR 2.75 ± 3.44 (mean ± SD).		
	MAXIMUM METABOLIC RATE			
41	When MMR was measured in relation to SMR (i.e. before or after)	MMR was measured before SMR.		
42	Method used (e.g. critical swimming speed respirometry, swim to exhaustion in swim tunnel, or chase to exhaustion)	Individuals were chased to exhaustion, defined as the moment they were not able to flick their tail anymore.		
43	Value taken as MMR (e.g. the highest rate of oxygen uptake value after transfer, average of highest values)	We retained the steepest slope over the long closed phase following introduction of each shrimp in the respirometer, or the highest $\dot{M}O_2$ observed during the regular intermittent-flow cycles, due to spontaneous activity. MMR was the highest of the two values and in the majority of cases, it was the post-chase value.		
44	If MMR measured post-exhaustion, length of activity challenge or chase (e.g. 2 min, until exhaustion, etc.)	Chased until no longer responsive to pinching of tail. Note that they were then exposed to air for 1 additional minute.	3.43	min
45	If MMR measured post-exhaustion, state whether further air-exposure was added after exercise	Yes, immediately after being chased to exhaustion, shrimp were exposed for 1 min to air to further increase their oxygen debt.		
46	If MMR measured post-exhaustion, time until transfer to chamber after exhaustion or time to start of oxygen uptake recording	After the 1 min air exposure, shrimp were rapidly but carefully transferred into individual respirometers.		
47	Duration of slopes used to calculate MMR (e.g. 1 min, 5 min, etc.)	This duration was obtained after a comparison of the variability when a test 8.25 period of data for each shrimp was analysed at all possible durations between 0.5 and 4 min, in steps of 0.25 min. This was done using our own R scripts.	3	min
48	Slope estimation method for MMR (e.g. rolling regression, sequential discrete time frames)	Rolling regression.		
49	How absolute aerobic scope and/or factorial aerobic scope is calculated (i.e. using raw SMR and MMR, allometrically mass-	Mass-specific MMR – Mass-specific SMR.		

	adjusted SMR and MMR, or allometrically mass-adjusting aerobic scope itself)			
	DATA HANDLING AND STATISTICS			
50	Sample size		260	indiv.
51	How oxygen uptake rates were calculated (software or script, equation, units, etc.)	Our own scripts to smooth raw data, plot and calculate individual slopes, correct for blank respiration and calculate $\dot{M}_{O_2}$. fishMO2 R package for calculation of SMR. Our own scripts to calculate MMR with rolling regressions.		
52	Confirm that volume (mass) of animal was subtracted from respirometer volume when calculating oxygen uptake rates	Yes.		
53	State whether analyses accounted for variation in body mass and describe any allometric mass-corrections or adjustments	Yes, we used mass specific metabolic traits, which were still related to shrimp mass. For this reason, shrimp mass was used as a covariable in our statistical analyses.		

Table S 2.3 Results for the best-fitted model tests carried out to determine the effect of temperature, pH and geographic origin and their interactions on survival, metabolic traits and cellular energetic capacity of female northern shrimp exposed over 30 d. Metabolic traits and cellular energetic capacity proxies are: standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope (AS), wet mass (WM), citrate synthase (CS), cytochrome C oxidase (COX), lactate dehydrogenase (LDH).

Survival				
		df	F	p
	Temperature	**2**	**34.995**	**< 0.001**
	pH	**1**	**10.047**	**0.004**
	Origin	3	0.749	0.533
	T * pH	2	0.910	0.416
	T * Origin	6	0.527	0.782
	pH * Origin	3	0.336	0.799
	T * pH * Origin	6	1.797	0.142

Metabolic traits				
		df	F	p
log_{10} SMR	**log_{10} WM**	**1**	**8.595**	**0.003**
	Temperature	**2**	**258.152**	**< 0.001**
	pH	1	3.771	0.053
	Origin	**3**	**16.624**	**< 0.001**
	T * pH	**2**	**4.227**	**0.016**
	T * Origin	**6**	**3.809**	**0.001**
	pH * Origin	**3**	**2.681**	**0.048**
	T * pH * Origin	6	1.380	0.224
log_{10} MMR	**log_{10} WM**	**1**	**8.453**	**0.004**
	Temperature	**2**	**102.453**	**< 0.001**
	pH	**1**	**23.789**	**< 0.001**
	Origin	**3**	**2.810**	**0.041**
	T * pH	2	0.671	0.512
	T * Origin	6	1.687	0.126
	pH * Origin	3	0.436	0.727
	T * pH * Origin	6	0.859	0.526
log_{10} AS	**log_{10} WM**	**1**	**4.725**	**0.030**
	Temperature	**2**	**33.918**	**< 0.001**
	pH	**1**	**27.545**	**< 0.001**
	Origin	3	1.506	0.214
	T * pH	2	0.040	0.668
	T * Origin	**6**	**3.048**	**0.007**
	pH * Origin	3	0.417	0.741
	T * pH * Origin	6	0.939	0.468

Cellular energetic capacity				
		df	F	p
CS	Temperature	2	0.126	0.881
	pH	1	0.0003	0.987
	Origin	**3**	**87.020**	**< 0.001**
	T * pH	2	0.016	0.984
	T * Origin	6	0.456	0.840
	pH * Origin	3	1.194	0.313
	T * pH * Origin	6	0.72	0.634
log_{10} COX	Temperature	2	2.521	0.0833
	pH	1	0.012	0.914
	Origin	**3**	**13.740**	**< 0.001**
	T * pH	2	1.211	0.300
	T * Origin	6	0.412	0.871
	pH * Origin	3	0.980	0.403
	T * pH * Origin	6	0.187	0.98
log_{10} LDH	Temperature	2	0.457	0.633
	pH	1	0.162	0.688
	Origin	**3**	**11.598**	**< 0.001**
	T * pH	2	0.560	0.572
	T * Origin	6	1.904	0.082
	pH * Origin	3	0.347	0.791
	T * pH * Origin	6	0.636	0.701
CS:LDH	Temperature	2	0.385	0.681
	pH	1	0.052	0.819
	Origin	**3**	**77.354**	**< 0.001**
	T * pH	2	0.572	0.565
	T * Origin	6	1.498	0.181
	pH * Origin	3	1.109	0.347
	T * pH * Origin	6	0.642	0.696
log_{10} COX:LDH	Temperature	2	3.035	0.050
	pH	1	0.022	0.883
	Origin	**3**	**9.278**	**< 0.001**
	T * pH	2	0.530	0.590
	T * Origin	6	0.371	0.896
	pH * Origin	3	0.750	0.523
	T * pH * Origin	6	0.322	0.925
log_{10} CS:COX	Temperature	2	2.243	0.109
	pH	1	0.379	0.539
	Origin	**3**	**28.278**	**< 0.001**
	T * pH	2	1.021	0.362
	T * Origin	6	0.481	0.822
	pH * Origin	3	0.314	0.815
	T * pH * Origin	6	0.080	0.998

CHAPITRE 3
REPROGRAMMATION MÉTABOLOMIQUE DES CREVETTES NORDIQUES DE DIFFÉRENTES ORIGINES EXPOSÉES AUX FACTEURS DES CHANGEMENTS GLOBAUX COMBINÉS

Ce chapitre se compose du troisième article intitulé « *All roads lead to Rome : Metabolomics reprogramming of the northern shrimp exposed to global changes leads to a comparable physiological status* » et d'une section d'analyses supplémentaires intitulée « Analyse des profils métabolomiques de la crevette nordique de l'estuaire du Saint-Laurent exposée à l'hypoxie et au ″trio mortel″ ». L'article a été corédigé avec Denis Chabot (IML-MPO), Fanny Vermandele (UQAR), Diana Madeira (ECOMARE-CESAM) et Piero Calosi (UQAR). Sa version finale a été publiée dans la revue *Frontiers in Marine Science* le 23 mai 2023 (DOI : 10.3389/fmars.2023.1170451). Les échantillons issus des expériences présentées dans le chapitre 2 ont été préparés par moi-même pour l'analyse menée par Les laboratoires Iso-BioKem Inc. La rédaction, les analyses statistiques, les figures et les tableaux ont été réalisés par moi-même avec la contribution des co-auteurs.

La section d'analyses supplémentaires présente les résultats des analyses métabolomiques menées par Les laboratoires Iso-BioKem Inc. sur les échantillons issus d'une partie de l'expérience présentée dans le chapitre 1. Sa rédaction, les analyses statistiques, les figures et le tableau ont été réalisés par moi-même.

Les résultats de l'article ont été présentés à la réunion annuelle du regroupement stratégique Québec Océan 2023 et au Symposium Ouranos 2022 (voir la liste des communications dans l'avant-propos de cette thèse).

3.1 RÉSUMÉ

Les impacts des changements globaux en milieu marin sur les espèces ont toujours été étudiés au niveau de l'organisme entier. Cependant, acquérir une compréhension approfondie des réponses métaboliques cellulaires des organismes est primordiale afin de mieux définir leur sensibilité aux changements environnementaux. Ceci est particulièrement pertinent pour les espèces qui vivent dans des conditions environnementales très différentes dans leur aire de répartition, car l'acclimatation ou l'adaptation locale peuvent influencer leurs réponses aux changements globaux en milieu marin. Nous avons cherché à dévoiler les mécanismes cellulaires qui sous-tendent la sensibilité à la combinaison du réchauffement et à l'acidification des océans chez la crevette nordique, *Pandalus borealis*, provenant de quatre origines géographiques différentes qui présentent des conditions environnementales distinctes dans l'Atlantique nord-ouest : c.-à-d. Estuaire du Saint-Laurent (SLE), est du plateau néo-écossais (ESS), chenal Esquiman (EC) et nord-est de la côte de Terre-Neuve (NNC). Nous avons caractérisé les profils métabolomiques du muscle des crevettes exposées à trois températures (2, 6 ou 10 °C) et deux niveaux de pH (7,75 ou 7,40). Dans l'ensemble, les profils métabolomiques des crevettes ont été modulés par une interaction significative entre la température, le pH et l'origine. La température a induit la majeure partie de la reprogrammation métabolomique, confirmant que *P. borealis* est plus sensible au réchauffement qu'à l'acidification des océans. Des différences dans les profils métabolomiques entre les origines ont également été observées, les interactions température*pH affectant uniquement les crevettes de SLE et ESS, le pH affectant uniquement les crevettes de SLE et la température affectant les crevettes de toutes les origines. La température a eu un impact sur les voies métaboliques liées au cycle de l'acide tricarboxylique (TCA) et au métabolisme des acides aminés, entraînant principalement une accumulation des intermédiaires du TCA et de la tyrosine. Les interactions température*pH et le pH isolé ont affecté seulement le métabolisme des acides aminés, entraînant une accumulation des acides aminés à faible pH. Cependant, le ratio ATP:ADP est resté constant à travers toutes les conditions et chez les crevettes de toutes les origines, ce qui suggère que leur état énergétique n'est pas affecté par le réchauffement et l'acidification des océans. Tout de même, l'accumulation des intermédiaires du TCA et de la tyrosine suggère un possible déclanchement des réponses immunitaires dans les conditions futures de réchauffement et d'acidification des océans. Nos résultats suggèrent que les crevettes de SLE sont plus sensibles au niveau moléculaire, comparé aux autres, aux futures conditions environnementales complexes. Ceci souligne l'importance d'étudier les mécanismes de réponses aux facteurs combinés intraspécifiquement lorsqu'on essaie de définir la sensibilité des espèces aux changements globaux en milieu marin.

Mots-clés : métabolomique (-OMIQUES), macrophysiologie, physiologie de la conservation, crustacés, *Pandalus borealis*, voies métaboliques, stress, immunité

3.2 ALL ROADS LEAD TO ROME: METABOLOMICS REPROGRAMMING OF THE NORTHERN SHRIMP EXPOSED TO GLOBAL CHANGES LEADS TO A COMPARABLE PHYSIOLOGICAL STATUS

Ella Guscelli[1*], Denis Chabot[2], Fanny Vermandele[1], Diana Madeira[3†] and Piero Calosi[1†]

[1] Marine Ecological and Evolutionary Physiology Laboratory, Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski, Rimouski, QC, Canada

[2] Institut Maurice-Lamontagne, Fisheries and Oceans Canada, Mont-Joli, QC, Canada

[3] ECOMARE-Laboratory for Innovation and Sustainability of Marine Biological Resources, CESAM-Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Aveiro, Portugal

*****Corresponding author

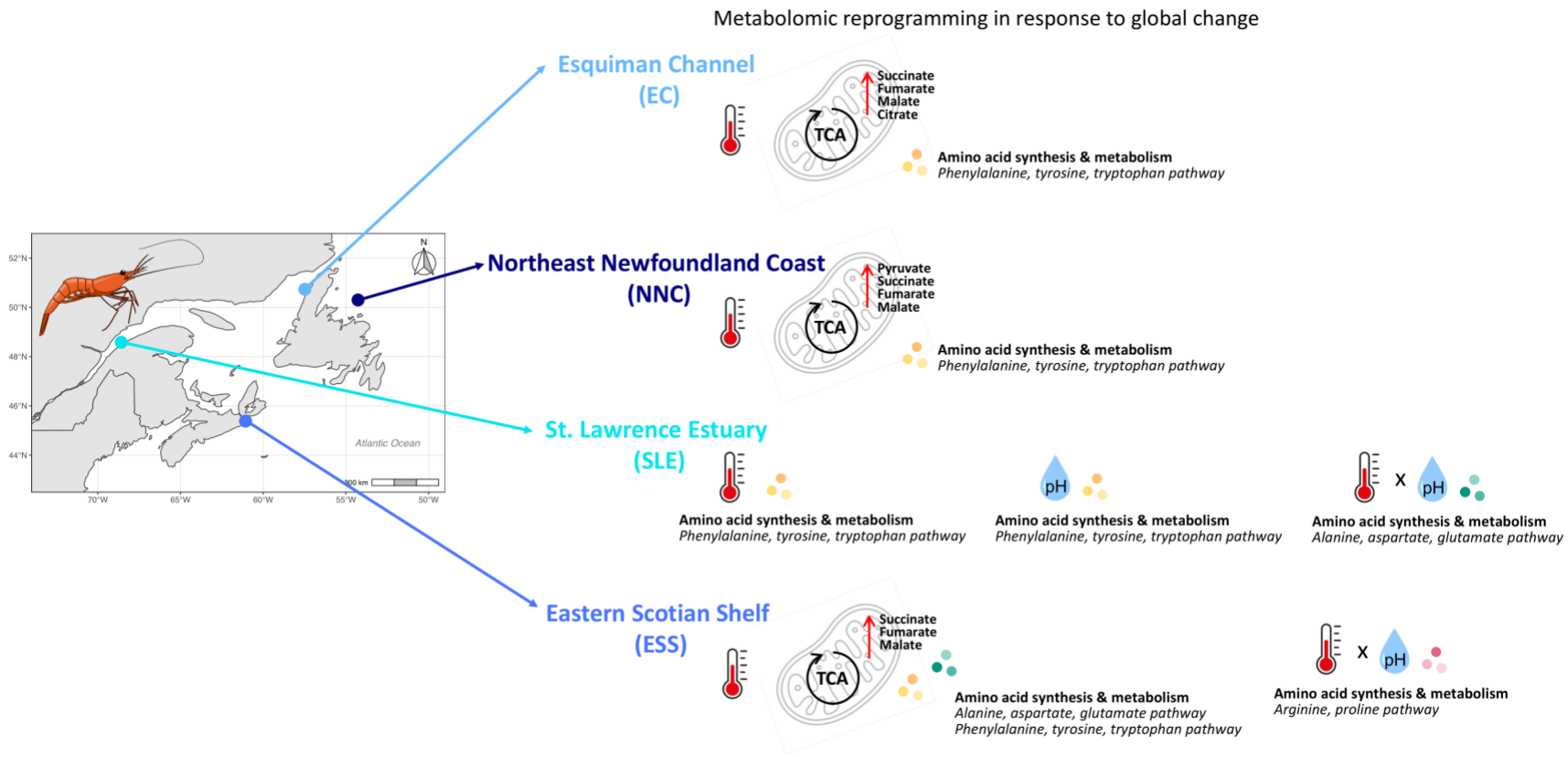
†These authors contributed equally to this work and share last authorship

3.3 ABSTRACT

Impacts of global ocean changes on species have historically been investigated at the whole-organism level. However, acquiring an in-depth understanding of the organisms' cellular metabolic responses is paramount to better define their sensitivity to environmental challenges. This is particularly relevant for species that experience highly different environmental conditions across their distribution range as local acclimatization or adaptation can influence their responses to rapid global ocean changes. We aimed at shedding light on the cellular mechanisms underpinning the sensitivity to combined ocean warming (OW) and acidification (OA) in the northern shrimp Pandalus borealis, from four different geographic origins defined by distinctive environmental regimes in the northwest Atlantic: i.e. St. Lawrence Estuary (SLE), Eastern Scotian Shelf (ESS), Esquiman Channel (EC) and Northeast Newfoundland Coast (NNC). We characterized targeted metabolomics profiles of the muscle of shrimp exposed to three temperatures (2, 6 or 10 °C) and two pH levels (7.75 or 7.40). Overall, shrimp metabolomics profiles were modulated by a significant interaction between temperature, pH and origin. Temperature drove most of the metabolomics reprogramming, confirming that P. borealis is more sensitive to OW than OA. Inter-origin differences in metabolomics profiles were also observed, with temperature*pH interactions impacting only shrimp from SLE and ESS, pH affecting only shrimp from SLE and temperature impacting shrimp from all origins. Temperature influenced metabolomics pathways related to the tricarboxylic acid cycle (TCA) and amino acid metabolism, resulting mainly in an accumulation of TCA intermediates and tyrosine. Temperature*pH and pH in isolation only affected amino acid metabolism, leading to amino acids accumulation under low pH. However, the ratio of ATP : ADP remained constant across conditions in shrimp from all origins suggesting that their energetic status is not affected by OW and OA. Still, the accumulation of TCA intermediates and tyrosine suggests the possible enhancement of immune responses under future OW and OA conditions. Our findings suggest that shrimp from SLE are more sensitive at the molecular level, compared to others, to future complex environmental conditions. This underlines the importance of investigating intraspecific variation in mechanisms of responses to combined drivers when trying to define species' sensitivity to global ocean changes.

Key words: metabolomics (OMICS), macrophysiology, conservation physiology, crustacean, *Pandalus borealis*, pathways, stress, immunity

3.4 GRAPHICAL ABSTRACT



3.5 INTRODUCTION

The rising concentration of anthropogenically emitted carbon dioxide (CO_2) in the atmosphere directly affects the marine environment *via* the decrease of global mean seawater pH and change in carbonate chemistry (ocean acidification, OA) (Caldeira & Wickett, 2003; IPCC, 2022). Indirectly, the increase of greenhouse gasses leads to rising seawater temperatures, a phenomenon known as ocean warming (OW) (IPCC, 2022). According to the most pessimistic, but also most realistic, scenario of the Intergovernmental Panel on Climate Change (IPCC, 2022), both OA and OW are predicted to worsen by the end of the century (+4 °C and -0.3 pH units mean globally) posing a major threat to marine biodiversity, particularly to calcifying organisms and ectotherms (Lefevre, 2016; Orr et al., 2005; Paaijmans et al., 2013). Survival, metabolism, growth, reproduction and behaviour are some of the traits affected by OW and OA, as observed at the whole organism level: which happen to be the most studied traits in global change biology to define species' sensitivity to global changes. However, to better understand the mechanisms underlying the responses to environmental changes at higher levels of biological organization (from whole organism to species, communities and ecosystems), it is necessary to elucidate and integrate the responses at lower levels of biological organization, i.e. cellular and molecular levels (Bartholomew, 1964; Harvey et al., 2014). In this context, the use of state-of-the-art molecular tools, such as -omics techniques, are of great interest as they allow the determination of which molecules and ultimately which cellular pathways play an important role in the response of organisms exposed to different climate change scenarios (Cappello, 2020; Ebner, 2021; Jones et al., 2013; Pinu et al., 2019). In particular, metabolites are the end products of cellular regulatory processes that involve multiple interactions among genes, transcripts, proteins and the environment. Therefore, metabolites are considered to be representative of cellular, organs, and organism phenotypes (Fiehn, 2002). Recently, the use of metabolomics analyses, i.e. the identification and quantification of targeted or all metabolites of a biological system (Bundy et al., 2009), has increased in studies investigating the responses of organisms to environmental changes (Aru et al., 2017; Li et al., 2020a; Li et al., 2020b; Mayor et al., 2015; Williams et al., 2009; Wu et al., 2017), and specifically to global change drivers such as OW, OA and ocean deoxygenation, in isolation or combined (e.g. Ellis et al., 2014; Huo et al., 2019; Wei et al., 2015; Zhang et al., 2017). Indeed, in multiple cases, global change drivers have been shown to induce a shift in metabolic pathways, phenomenon known as metabolic reprogramming, and can affect the energy metabolism

of organisms (Dong et al., 2022; Lannig et al., 2010; Liu et al., 2020; Noisette et al., 2021; Thor et al., 2022). Elevated levels of succinate, fumarate and malate suggest a disruption of the tricarboxylic acid cycle (TCA) that, together with elevated levels of lactate, support the hypothesis of a shift from aerobic to anaerobic metabolism (Clark et al., 2017; Huo et al., 2019; Noisette et al., 2021; Wei et al., 2015; Williams et al., 2009). However, metabolism not only changes in response to the effect of ocean global drivers, but it is also shown to vary among populations of the same species. For example, populations of the common periwinkle *Littorina littorea* in the northeast Atlantic appeared metabolically adapted to the regional environmental conditions they experience. It was shown by differences in their metabolic rates and metabolomics profiles that influenced their whole-organism response to OA (Calosi et al., 2017). Differences in metabolic rates among populations, and in their response to OW and OA, have been shown in a number of marine species (Di Santo, 2015; Thor et al., 2018; Vargas et al., 2017). Therefore, multi-population studies need to be conducted to accurately and reliably determine species' sensitivity to future ocean conditions, to help identifying the mechanisms underpinning known whole-organism physiological responses. This is especially true for species with a wide range of distribution and of ecological and commercial relevance. A good example is the northern shrimp *Pandalus borealis*. This species is widely distributed in the north Atlantic (Bergström, 2000), where it is a major prey for many fish and marine mammal predators of these ecosystems and supports important fisheries (Dawe et al., 2012; Hammill & Stenson, 2000; Jónsdóttir et al., 2012). In particular, it is the third most lucrative marine fishery in the Canadian waters of the northwest Atlantic (DFO, 2021a). The northern shrimp has been shown to be sensitive to both OW and OA (Arnberg et al., 2013; Bechmann et al., 2011; Brillon et al., 2005; Chabot & Ouellet, 2005; Chemel et al., 2020; Daoud et al., 2007; Hammer & Pedersen, 2013). In addition, it appears to be potentially long-term acclimatized or adapted to regional environmental conditions throughout the north Atlantic (Koeller et al., 2009; Ouellet et al., 2017). Indeed, average bottom waters, where shrimp are most abundant, are characterized by higher temperatures and lower pH levels (~6-7 °C, ~7.6 pH units) in the Gulf of St. Lawrence compared to the deep waters outside the St. Lawrence system (~0-3 °C, ~7.8-8 pH units) (Bourdages et al., 2022; Cyr et al., 2022; DFO, 2021d, 2022b, 2022c). Our study aimed at shedding light on the cellular mechanisms underpinning the physiological responses of the northern shrimp from different geographic origins with distinctive environmental conditions, under combined OW and OA scenarios predicted to occur at the end of the century, to characterize

the intraspecific variation in metabolic pathways across conditions. To do so, we exposed female shrimp from four different geographic origins within the northwest Atlantic (i.e. St. Lawrence Estuary, Eastern Scotian Shelf, Esquiman Channel and Northeast Newfoundland Coast) to different combinations of OW and OA, and we identified key metabolites associated to the aerobic and anaerobic metabolism to perform targeted metabolomics analyses. Based on our current knowledge, we hypothesize that shrimp from different origins will show different metabolomics profiles under exposure to combined OW and OA, but all possibly showing a shift from aerobic to anaerobic metabolism.

3.6 MATERIAL AND METHODS

3.6.1 Ethical statement

All procedures complied with Canadian legislation for animal experimentation as the Canadian Council for Animal Care does not require projects using crustaceans to be approved by an Animal Care Committee, thus an ethics approval was not required for this study.

3.6.2 Shrimp collection and experimental design

The analyses of key targeted metabolites associated to the aerobic and anaerobic metabolism was carried out on abdominal muscle samples of female shrimp *P. borealis* previously exposed to isolated and combined OW and OA scenarios (Guscelli et al. *under review*). Female shrimp were selected because they are the main target of the fishery, they have a greater role in defining population demography and are more sensitive compared to males to ocean global change drivers (Dupont-Prinet et al., 2013). Moreover, we selected only non-ovigerous females to avoid any potential confounding effect linked to differences in reproductive stages and the oxygen consumption of the egg mass. Briefly, shrimp were collected from four different geographic origins within the northwest Atlantic: i.e. St. Lawrence Estuary (SLE, 48° 35’ N, 68° 35’ W; May 2018), Eastern Scotian Shelf (ESS, 45° 23’ N, 61° 04’ W; February 2019), Esquiman Channel (EC, 50° 44’ N, 57°29’ W; July 2019) and Northeast Newfoundland Coast (NNC, 50° 18’ N, 54° 16’ W; November 2019) with shrimp trawls or traps. At depths where shrimp were sampled temperature and pH conditions are stable year-round, but subjected to slow inter-annual changes. Shrimp were then transported to the Maurice-Lamontagne Institute (MLI), Fisheries and Oceans Canada (Mont-Joli, Qc, Canada) where they were maintained in rearing tanks (1700 L) for approximately eight

weeks before the beginning of the experiment. Average conditions during this period were 4.5 °C, pH 7.9, 100 % oxygen saturation relative to air and salinity 32. Shrimp were then exposed for 30 d to one of six treatments (with two replicate tanks per treatment) combining temperatures and pH levels representing the favourable, actual, or future scenario in the northwest Atlantic. In detail, 2 °C was chosen as favourable temperature for this species (Shumway et al., 1985), while 6 °C represents the recent temperature of shrimp habitat in the Gulf of St. Lawrence (GSL) (Bourdages et al., 2022) and a +4 °C predicted globally for the end of the century (RCP 8.5 scenario, IPCC, 2014) for other origins, such as ESS and NNC, and 10 °C corresponds to the +4 °C increase for GSL shrimp (IPCC, 2014). A pH of 7.75 was selected based on the current conditions of the deep waters of the Estuary and Gulf of St. Lawrence (Mucci et al., 2011, 2018) and approximatively the -0.3 pH units decrease scenario predicted to occur globally by the year 2100 (RCP 8.5 scenario, IPCC, 2014) for other origins, such as ESS and NNC, while the pH of 7.40 was chosen to correspond to the -0.3 pH units drop for GLS shrimp (IPCC, 2014). Treatments were identified as low temperature and current pH (2C: 2 °C, pH 7.75), low temperature and low pH (2A: 2 °C, pH 7.40), intermediate temperature and current pH (6C: 6 °C, pH 7.75), intermediate temperature and low pH (6A: 6 °C, pH 7.40), elevated temperature and current pH (10C: 10 °C, pH 7.75), elevated temperature and low pH (10A: 10 °C, pH 7.40). At the end of the exposure period, we measured shrimp oxygen uptake as a proxy for metabolic rates *via* intermittent-flow respirometry on five individual *per* tank (10 *per* treatment) (results are presented in Guscelli et al. *under review*). At the end of the respirometry trials we rapidly dissected shrimp on ice and the abdomen muscle was carefully placed in microtubes (Eppendorf, Germany) and flash-frozen in liquid nitrogen to instantly interrupt all biochemical reactions. This procedure was conducted rigorously and rapidly, lasting less than a minute, in order to minimize the negative effects of handling and standardise them (if any) across all treatment and origin combinations. Samples were then stored at -80 °C pending analyses.

Shrimp that moulted and/or died during respirometry measurements were considered as unstable samples for metabolomics analysis and they were thus removed from the dataset.

Maintenance of shrimp from all geographic origins and monitoring of temperature and pH in the experimental setup were similar to those detailed for shrimp of SLE in Chemel et al. (2020). For details on the physico-chemical parameters for the duration of the 30-d experiment see Guscelli et al. (*under review*).

3.6.3 Metabolite extraction and quantification

Metabolite extraction was carried out by Les laboratoires Iso-BioKem Inc. following the method established in their laboratory. Briefly, samples were first freeze-dried for 24h and then divided in two centrifugal PP tubes (1.5 mL) to carry out the extraction for positive and negative analyses separately. Each part was then weighted, to later normalize metabolite concentration, and homogenized at 6000 rpm for 30 s at –4 °C (Precellys 24 with cryolis cooling unit, Bertin corporation, Montigny-le-Bretonneux, France). Once the extraction solution was added, samples were vortexed for 10 s and centrifuged at 30130 $\times g$ for 5 min at 5 °C to collect and transfer 250 µL of supernatant in an amber HPLC vial with insert (Wheaton, NJ, USA) to analyse the fresh extract. Finally, 225 µL of the supernatant were injected in a high-performance liquid chromatography system (HPLC 1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) coupled to a mass spectrometer (6420 Triple Quad, Agilent Technologies) to detect targeted metabolites. The absolute quantification of these metabolites (in ng mL^{-1}) was assessed with the MassHunter QQQ quantitative (Quant-my-Way) software (Agilent Technologies) using a calibration curve previously created using Phenylalanine-d8 and Fumarate-d4 standards (Cambridge Isotope Laboratories, Tewksbury, MA, USA).

3.6.4 Data pre-processing

Raw data matrices were normalized by wet weight and final concentration values were expressed as ng metabolite mg^{-1} wet weight. As LC-MS metabolomics datasets can contain a high amount of missing values (Wei et al., 2018), the matrices were first imported in the web tool MetImp 1.2 (https://metabolomics.cc.hawaii.edu/software/MetImp/, accessed in September 2021) for missing data imputation. As our missing data were due to limit of quantification (LOQ) of the technique, we followed the protocol proposed by Wei et al. (2018) for the 'Missing not at random (MNAR)' type of missing values. Briefly, we used the QRILC algorithm (Quantile Regression Imputation of Left-Censored Data) with 40% group wise missing filtering to input missing values (seed 1234). We decided to increase the threshold to 40 % missing values in order to keep more metabolites for the statistical analyses and only rejected 14 metabolites out of 48 identified. Finally, the database was imported into Metaboanalyst 5.0 (http://www.metaboanalyst.ca/, accessed in October 2021) to be log-transformed, to reduce lack of normality and heteroscedasticity in the data, and auto-scaled, to adjust for differences in fold-changes between metabolites.

3.6.5 Data analysis

Unsupervised principal components analysis (PCA) was applied to explore data structure (FactoMineR package, Husson et al., 2010) and one outlier was removed from the complete dataset. Then, to test the isolated and combined effects of elevated seawater temperature, low pH and geographic origin on metabolomics profiles of shrimp muscle tissue, we performed a three-way Permutational Multivariate Analysis of Variance (PERMANOVA). The PERMANOVA was applied to the data resemblance matrix (based on Euclidean distance) and was run using type III sums of squares and permutation of residuals under a reduced model (9999 permutations) using the PERMANOVA + add-on in Primer-E v6 (Anderson et al., 2008).

Multivariate and univariate analyses were performed to identify metabolites that discriminate between different temperatures and pH levels, within each geographic origin. Four PCAs were carried out to explore data structure, one *per* geographic origin. Additionally, to test for the effect of temperature, pH and their interaction within each geographic origin, a two-way analysis of variance (ANOVA) with a False Discovery Rate (FDR) cut-off set at 0.05 for significance was carried out for each metabolite on the database (specmine package, Costa et al., 2017). As commonly done when a high number of comparisons exists, the FDR was applied to correct p-values (separately for each origin and each factor) for 34 tests. When one of these tests was significant according to FDR, Tukey *post-hoc* tests were used to identify significant differences using the HSD.test or the glht function for isolated or interactive effects respectively (Bretz et al., 2016; Hothorn et al., 2008). Analyses were performed using the software R 4.1.1 version (R Core Team, 2020). Finally, significant metabolites identified by the ANOVAs were then used for pathway analysis to assess the most relevant metabolic pathways involved in the response of shrimp from different geographic origins to temperature increases and pH decreases. To do so, the chosen metabolites were imputed in the "Pathway Analysis" tool of the MetaboAnalyst software. As there are no libraries available for shrimp, we chose the pathway library of a model organism within the same phylum (Arthropoda), namely *Drosophila melanogaster* (fruit fly). Pathway analysis was carried out based on functional enrichment, assessed using hypergeometric tests for over-representation analysis ($p < 0.05$), and pathway topology analysis, implemented using the relative betweenness centrality (> 0.1). Pathways were considered relevant according to either over-representation $p < 0.05$ or pathway topology analysis with impact > 0.1 and most relevant according to the combination of both.

Finally, linear models were used to test the effects of isolated and combined seawater temperature and pH, and geographic origin (fixed factors) on shrimp ATP:ADP ratio, which was calculated and analysed *a posteriori* to verify the cellular energetic status.

3.7 RESULTS

3.7.1 Whole metabolome

The overall PCAs, applied to explore the general data structure by factor (geographic origin, temperature, pH and temperature*pH treatments), did not show clear group separation (Figure S 3.6).

However, temperature and pH significantly and differently modulated the metabolomic profiles of shrimp from the four geographic origins as indicated by the presence of a significant interaction among origin, temperature and pH (Table 3.1).

Table 3.1 Summary of the results of the PERMANOVA test employed to investigate the effects of origin, temperature and pH and their interactions on the northern shrimp muscle metabolomics profiles. The analysis was based on a Euclidean distance matrix, 9999 permutations and type III sums of squares. Numbers in bold indicate significant *p*-values.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Origin (O)	3	881.92	293.97	11.74	**0.0001**	9863
Temperature (T)	2	395.17	197.58	7.89	**0.0001**	9885
pH	1	76.45	76.45	3.05	**0.0002**	9905
O*T	6	294.49	49.08	1.96	**0.0001**	9795
O*pH	3	91.91	30.64	1.22	0.1239	9861
T*pH	2	72.89	36.44	1.46	**0.0475**	9887
O*T*pH	6	241.27	40.21	1.61	**0.0003**	9831
Residuals	196	4907.80	25.04			
Total	219	6957				

3.7.2 Origins

For each origin, the PCAs did not show a clear group separation as some overlapping was found among temperature*pH treatments (Figure 3.1). However, some differences in metabolomics profiles of shrimp exposed to current and low pH levels were shown for shrimp from the SLE (Figure 3.1), while some differences between the lowest and highest temperatures were shown for the metabolomics profiles of shrimp from the three other origins (Figure 3.1). Furthermore, for all

geographic origins, between seven and 12 metabolites were found to change significantly among temperature, pH and temperature*pH treatments (Table S 3.3). Three metabolites of shrimp from the SLE and one metabolite of shrimp from ESS differed among temperature*pH treatments as indicated by the presence of a significant interaction between temperature and pH (Table 3.2). However, no effect of this interaction was found to be significant on metabolomic profiles of shrimp from EC and NNC (Table 3.2). Additionally, temperature alone was found to significantly affect the metabolomic profile of shrimp from all geographic origins whilst pH in isolation was found to significantly affect the metabolomic profile of shrimp from SLE (Table 3.2).

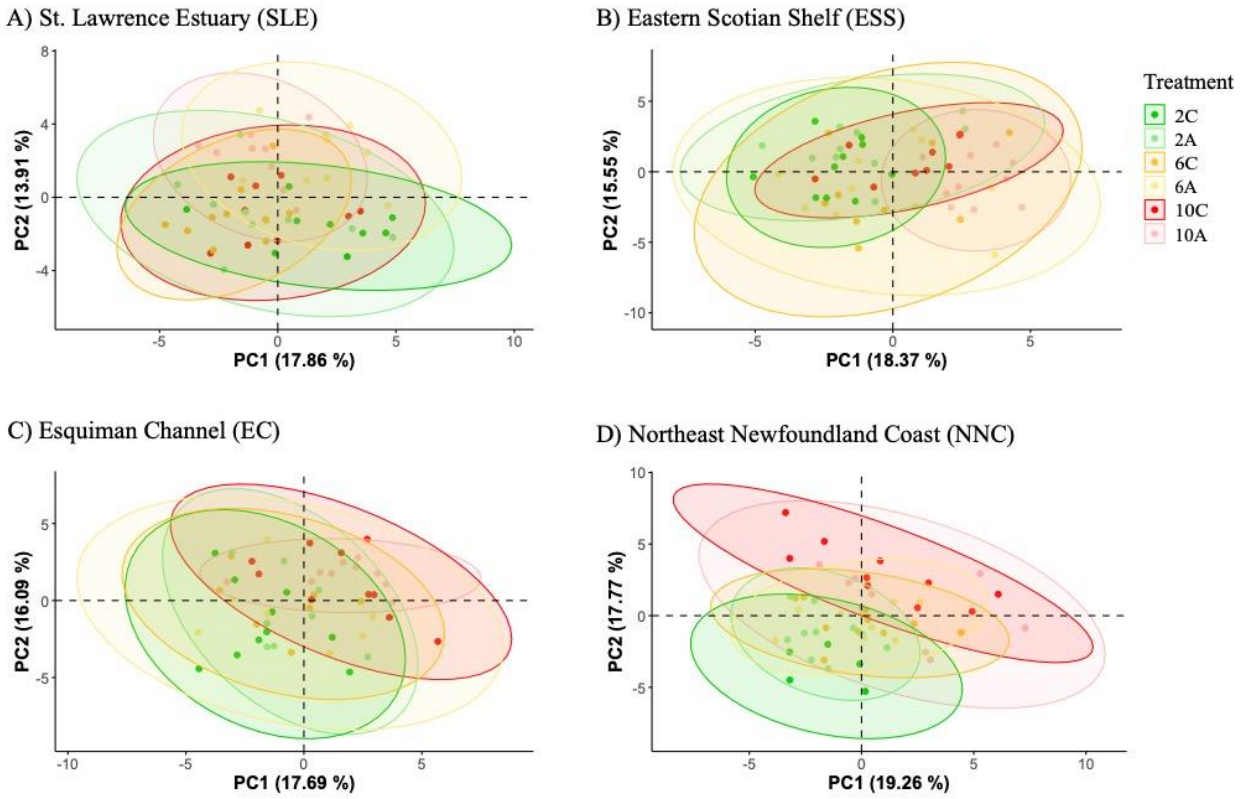


Figure 3.1 PCA 2D score plot with 95 % confidence ellipse representing the variation in metabolomics profiles in the northern shrimp *Pandalus borealis* according to temperature*pH treatments: 2C (2 °C, pH 7.75), 2A (2 °C, pH 7.40), 6C (6 °C, pH 7.75), 6A (6 °C, pH 7.40), 10C (10 °C, pH 7.75) and 10A (10 °C, pH 7.40) for A) St. Lawrence Estuary (SLE), B) Eastern Scotian Shelf (ESS), C) Esquiman Channel (EC) and D) Northeast Newfoundland Coast (NNC).

Table 3.2 Summary of the results of the ANOVA tests used to investigate the effects of temperature and pH and their interaction on metabolites quantified in the northern shrimp muscle samples. Origins: St. Lawrence Estuary (SLE), Eastern Scotian Shelf (ESS), Esquiman Channel (EC) and Northeast Newfoundland Coast (NNC). Only significant metabolites are included in the table. Numbers in bold indicate significant *p*-values.

		Temperature			pH			Temperature*pH		
Origin	**Metabolite**	**F value**	**p value**	**FDR**	**F value**	**p value**	**FDR**	**F value**	**p value**	**FDR**
SLE	Betaine	0.339	0.714	0.905	4.996	0.030	0.136	8.512	0.001	**0.011**
SLE	Tyrosine	10.305	0.000	**0.003**	21.395	< 0.001	**0.001**	0.368	0.694	0.787
SLE	Hydroxyproline	0.553	0.579	0.905	15.241	0.000	**0.005**	0.124	0.884	0.911
SLE	Serine	11.841	< 0.001	**0.002**	0.618	0.436	0.549	4.851	0.012	0.101
SLE	Aspartate	0.333	0.719	0.905	5.084	0.029	0.136	10.503	0.000	**0.005**
SLE	Lysine	3.086	0.054	0.236	14.176	0.000	**0.005**	2.653	0.080	0.241
SLE	Pyruvate	0.420	0.659	0.905	0.684	0.412	0.539	6.450	0.003	**0.037**
ESS	Tryptophan	19.920	< 0.001	**0.000**	1.773	0.189	0.611	1.204	0.308	0.552
ESS	Tyrosine	7.507	0.001	**0.010**	2.929	0.093	0.452	2.107	0.132	0.552
ESS	Hydroxyproline	0.076	0.927	0.927	0.147	0.703	0.886	7.929	0.001	**0.034**
ESS	Threonine	6.629	0.003	**0.011**	0.008	0.931	0.931	0.008	0.304	0.552
ESS	Serine	15.735	< 0.001	**< 0.001**	4.339	0.042	0.287	2.245	0.116	0.552
ESS	α Aminoadipic acid	5.532	0.007	**0.023**	9.040	0.004	0.069	0.251	0.779	0.855
ESS	Aspartate	10.194	0.000	**0.002**	0.950	0.334	0.757	2.603	0.084	0.474
ESS	Cystine	4.617	0.014	**0.044**	0.669	0.417	0.757	2.674	0.078	0.474
ESS	Glucose	6.519	0.003	**0.011**	0.019	0.892	0.920	0.737	0.483	0.632
ESS	Succinate	6.850	0.002	**0.011**	5.881	0.019	0.213	1.441	0.246	0.552
ESS	Fumarate	7.397	0.001	**0.010**	0.325	0.571	0.809	0.747	0.479	0.632
ESS	Malate	7.036	0.002	**0.011**	0.485	0.490	0.774	1.643	0.203	0.552
EC	Tyrosine	12.535	< 0.001	**0.001**	3.785	0.058	0.492	2.297	0.112	0.615
EC	β Aminoisobutyric	7.273	0.002	**0.007**	0.149	0.702	0.822	0.967	0.388	0.791
EC	α Aminobutyric acid	20.310	< 0.001	**< 0.001**	0.253	0.618	0.822	1.909	0.160	0.615
EC	Threonine	9.664	0.000	**0.003**	1.900	0.175	0.609	0.210	0.811	0.869
EC	Serine	20.890	< 0.001	**< 0.001**	2.632	0.112	0.516	0.253	0.778	0.869
EC	α Aminoadipic acid	4.724	0.014	**0.042**	1.047	0.311	0.688	1.826	0.173	0.615
EC	Succinate	7.181	0.002	**0.007**	4.832	0.033	0.374	0.277	0.759	0.869
EC	Fumarate	8.581	0.001	**0.003**	0.271	0.605	0.822	0.946	0.396	0.791
EC	Malate	8.705	0.001	**0.003**	0.540	0.466	0.822	0.236	0.791	0.869
EC	ADP	9.377	0.000	**0.003**	0.215	0.645	0.822	5.116	0.010	0.168
EC	Citrate	6.502	0.003	**0.011**	1.673	0.202	0.625	0.572	0.569	0.869
NNC	Phenylalanine	50.640	< 0.001	**< 0.001**	0.589	0.447	0.805	0.125	0.883	0.968
NNC	Isoleucine	6.171	0.004	**0.016**	0.447	0.507	0.805	0.397	0.675	0.900
NNC	Tyrosine	13.309	< 0.001	**0.000**	0.881	0.353	0.805	0.641	0.531	0.838
NNC	α Aminobutyric acid	5.213	0.009	**0.030**	1.817	0.184	0.805	1.163	0.321	0.761
NNC	α Aminoadipic acid	9.510	0.000	**0.002**	0.609	0.439	0.805	0.308	0.736	0.900
NNC	Histidine	5.089	0.010	**0.031**	0.026	0.873	0.885	0.032	0.969	0.991
NNC	Cystine	12.279	< 0.001	**0.000**	0.231	0.633	0.828	3.667	0.033	0.280
NNC	Pyruvate	8.259	0.001	**0.004**	0.113	0.738	0.839	0.928	0.402	0.765
NNC	Succinate	13.599	< 0.001	**0.000**	0.572	0.453	0.805	3.891	0.027	0.280
NNC	Fumarate	19.579	< 0.001	**< 0.001**	0.330	0.568	0.805	5.248	0.009	0.280
NNC	Malate	20.494	< 0.001	**< 0.001**	0.135	0.715	0.839	4.071	0.023	0.280

3.7.2.1 SLE

i) The concentration of three metabolites differed significantly among temperature*pH treatments as indicated by the presence of a significant interaction between temperature and pH (Table 3.2). Pathway analysis showed that these metabolites were involved in five relevant pathways, including: amino acid and pyruvate metabolism and arginine biosynthesis (Figure 3.2A). The most relevant pathway was the alanine, aspartate and glutamate metabolism (Figure 3.2A). In this pathway, aspartate mean concentration increased between current and low pH at the intermediate temperature (Figure 3.2A). Despite the fact that significant effects were evidenced by the ANOVA on pyruvate, multiple comparisons tests between treatments failed to detect significant differences among groups.

ii) The concentration of three metabolites differed significantly between pH treatments (Table 3.2), and pathway analysis showed that these metabolites were involved in six relevant pathways, including: amino acid biosynthesis and metabolism, aminoacyl-tRNA biosynthesis, ubiquinone and other terpenoid-quinone biosynthesis and biotin metabolism (Figure 3.2B). The most relevant pathway was the phenylalanine, tyrosine and tryptophan biosynthesis (Figure 3.2B). In this pathway, tyrosine mean concentration increased at low pH (Figure 3.2B).

iii) The concentration of two metabolites differed significantly among temperature treatments (Table 3.2), and the pathway analysis showed that these metabolites were involved in four relevant pathways, including: amino acid biosynthesis and metabolism and ubiquinone and other terpenoid-quinone biosynthesis (Figure 3.2C). The most relevant pathway was the phenylalanine, tyrosine and tryptophan biosynthesis (Figure 3.2C). Within this pathway, tyrosine mean concentration increased with increasing temperature being the highest and similar at the two highest temperature treatments (Figure 3.2C).

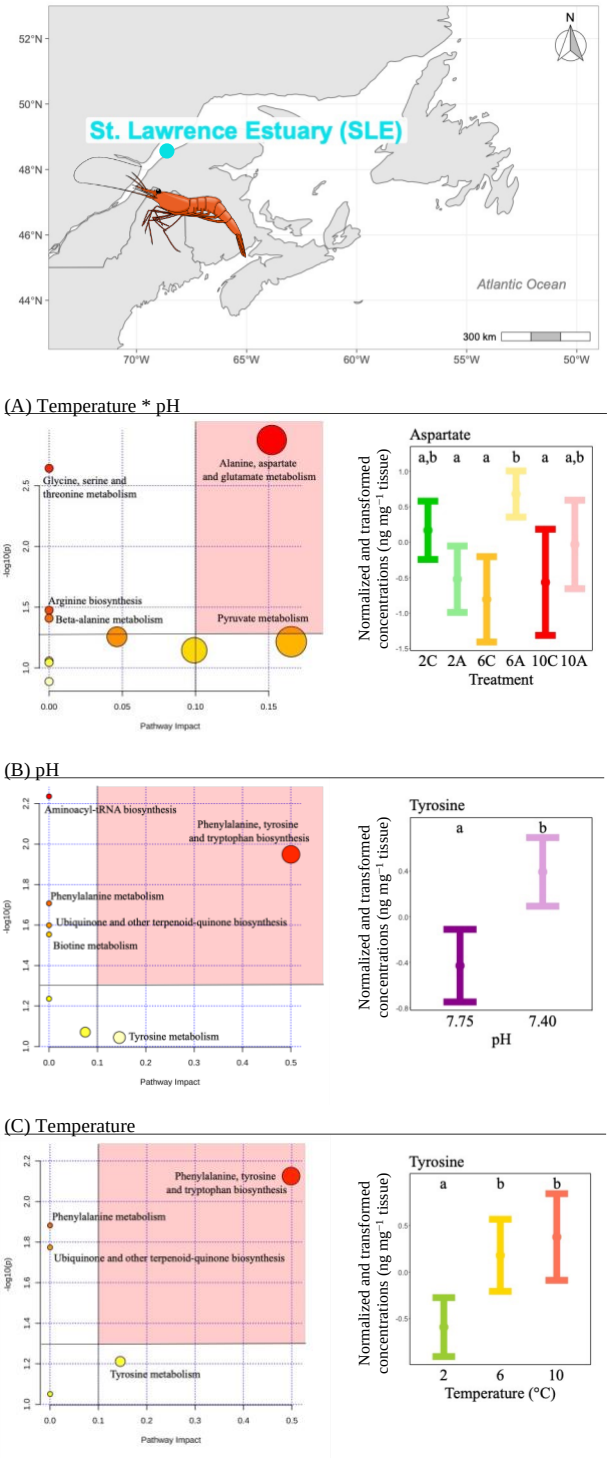


Figure 3.2 Metabolomics differences due to isolated and combined effects of seawater temperature and pH in the northern shrimp *P. borealis* from the St. Lawrence Estuary (SLE). In more detail, at the top of the figure, we show the map representing the collection site and at the left results for the pathway analysis of metabolic changes carried out with the statistically significant metabolites (ANOVA $p < 0.05$) due to (A) the combined effect of seawater temperature and pH, (B) the isolated effect of seawater pH and (C) the isolated effect of seawater temperature. Pathways within the

red area were considered the most relevant as they have $p < 0.05$ and impact > 0.1. On the right, (A) mean and 95 % CI for aspartate concentration for each temperature*pH treatments: 2C (green, N=9), 2A (light green, N=10), 6C (yellow, N=10), 6A (light yellow, N=9), 10C (red, N=9) and 10A (light red, N=9); (B) mean and 95 % CI for tyrosine concentration for each pH treatment: 7.75 (purple, N=28) and 7.40 (light purple, N=28), combining all temperatures; (C) mean and 95 % CI for tyrosine concentration for each temperature treatment: 2 (green, N=19), 6 (yellow, N=19) and 10 °C (red, N=18), combining all pH levels. Lower case letters identify significant differences ($p < 0.05$) among treatments.

3.7.2.2 ESS

i) Only the concentration of hydroxyproline differed significantly among temperature*pH treatments as indicated by the presence of a significant interaction between temperature and pH (Table 3.2), and the pathway analysis showed that this metabolite was involved in the arginine and proline metabolism, despite the fact that MetaboAnalyst was unable to produce a pathway figure due to the input metabolite list being only one metabolite. Moreover, multiple comparisons tests between treatments failed to detect significant differences in hydroxyproline concentrations among groups.

ii) The concentration of 12 metabolites differed significantly among temperature treatments (Table 3.2), and the pathway analysis showed that these metabolites were involved in eight relevant pathways, including: citrate cycle, amino acid biosynthesis and metabolism, aminoacyl-tRNA biosynthesis and pyruvate metabolism (Figure 3.3). Among these, four were the most relevant pathways: the citrate cycle, the alanine, aspartate and glutamate metabolism, the phenylalanine, tyrosine and tryptophan biosynthesis and the tyrosine metabolism (Figure 3.3). Within these most relevant pathways, malate, fumarate and succinate mean concentrations increased with increasing temperature being the highest and similar at the two highest temperature treatments (Figure 3.3). Conversely, tyrosine mean concentrations measured at the two lowest temperatures tested were similar but lower than mean concentration at the highest temperature condition (Figure 3.3). Similarly, aspartate mean concentration significantly increased between 6 and 10 °C and the mean concentration measured at the lowest temperature was comparable to the one measured at the two highest temperatures tested (Figure 3.3).

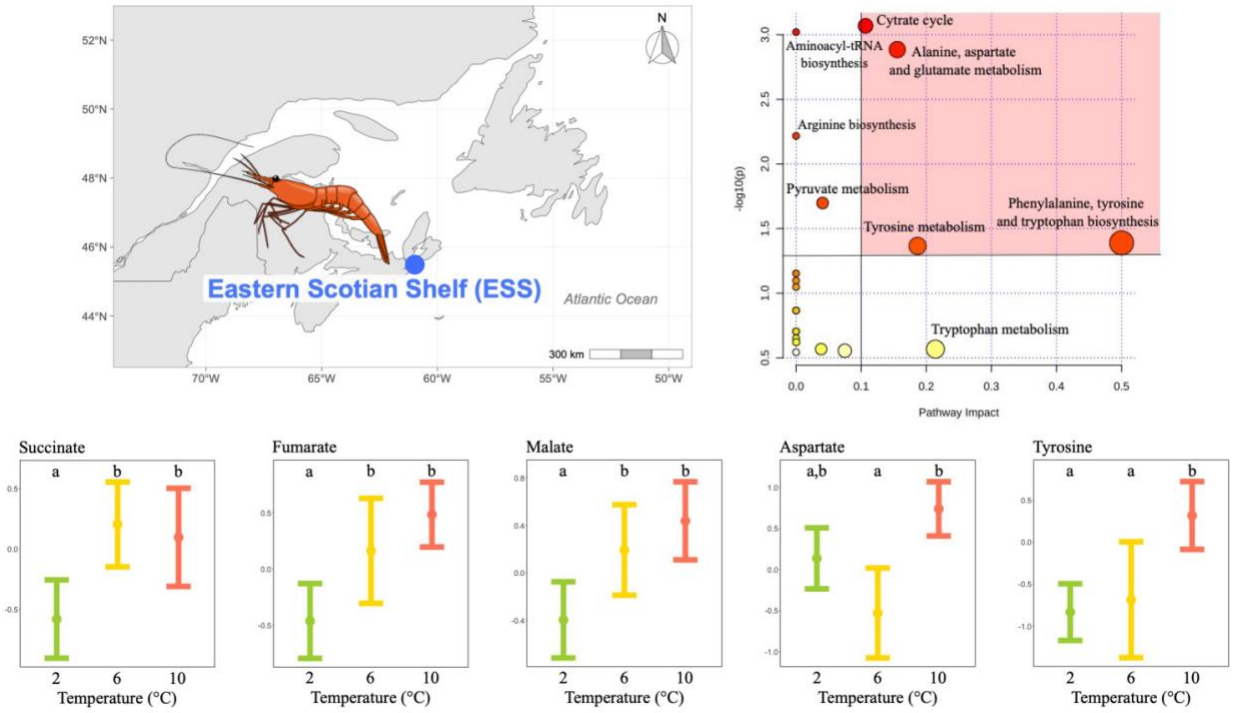


Figure 3.3 Metabolomics differences among temperatures in the northern shrimp *P. borealis* from the Eastern Scotian Shelf (ESS). In more detail, at the top left of the figure we show the map representing the collection site and at the top right of the figure we show the pathway analysis of metabolic changes carried out with the statistically significant metabolites (ANOVA $p < 0.05$). Pathways within the red area were considered the most relevant as they have $p < 0.05$ and impact > 0.1. At the bottom of the figure we show the mean concentration and 95 % CI for succinate, fumarate, malate, aspartate and tyrosine for each temperature treatment: 2 (green, N=20), 6 (yellow, N=19) and 10 °C (red, N=19), combining all pH levels. Lower case letters identify significant differences ($p < 0.05$) among treatments.

3.7.2.3 EC

The concentration of 11 metabolites differed significantly among temperature treatments (Table 3.2), and the pathway analysis showed that these metabolites were involved in six relevant pathways, including: citrate cycle, amino acid biosynthesis and metabolism, pyruvate metabolism and glyoxylate and dicarboxylate metabolism (Figure 3.4). Among these, three were the most relevant pathways: the citrate cycle, the phenylalanine, tyrosine and tryptophan biosynthesis and the tyrosine metabolism (Figure 3.4). Within these pathways, malate, fumarate, succinate, citrate and tyrosine mean concentrations all increased with increasing temperature. In more detail, malate, fumarate and citrate mean concentrations measured at the two lowest temperatures tested were similar and the lowest compared to the mean reported at the highest temperature condition (Figure 3.4). Differently, tyrosine mean concentrations measured at the two highest temperatures were significantly higher than that reported at the lowest temperature condition, but comparable to each

other (Figure 3.4). Finally, succinate mean concentration measured at the highest and lowest temperatures were significantly different from each other, and both comparable to the mean measured at the intermediate temperature (Figure 3.4).

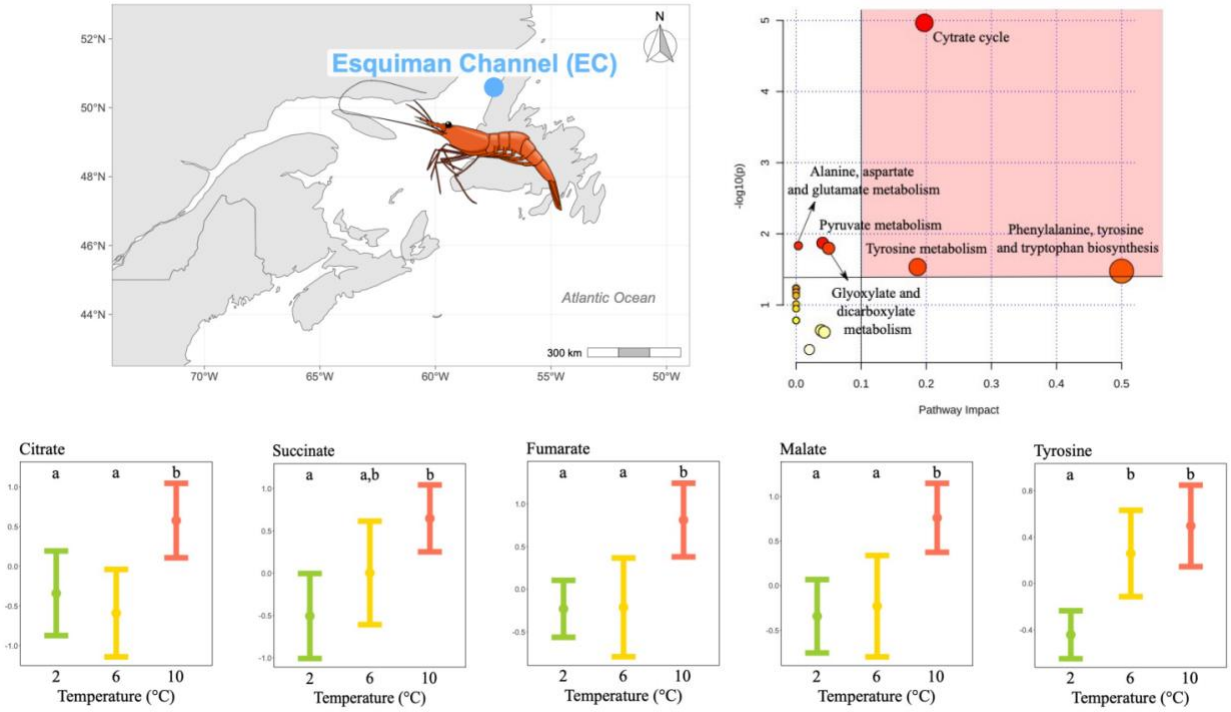


Figure 3.4 Metabolomics differences among temperatures in the northern shrimp *P. borealis* from the Esquiman Channel (EC). In more detail, at the top left of the figure we show the map representing the collection site and at the top right of the figure we show the pathway analysis of metabolic changes carried out with the statistically significant metabolites (ANOVA $p < 0.05$). Pathways within the red area were considered the most relevant as they have $p < 0.05$ and impact > 0.1. At the bottom of the figure we show the mean concentration and 95 % CI for citrate, succinate, fumarate, malate and tyrosine for each temperature treatment: 2 (green, N=18), 6 (yellow, N=14) and 10 °C (red, N=20), combining all pH levels. Lower case letters identify significant differences ($p < 0.05$) among treatments.

3.7.2.4 NNC

The concentration of 11 metabolites differed significantly among temperature treatments (Table 3.2), and the pathway analysis showed that these metabolites were involved in nine relevant pathways, including: citrate cycle, amino acid biosynthesis and metabolism, aminoacyl-tRNA biosynthesis and pyruvate metabolism (Figure 3.5). Among these, five were the most relevant pathways: the citrate cycle, the phenylalanine, tyrosine and tryptophan biosynthesis, the tyrosine metabolism, phenylalanine metabolism and the pyruvate metabolism (Figure 3.5). In these pathways, metabolites such as malate, fumarate, succinate, pyruvate, phenylalanine and tyrosine

mean concentrations increased with increasing temperature. In more detail, succinate and pyruvate mean concentration were similar at the two lowest temperatures tested, but lower than the mean concentration at the highest temperature condition (Figure 3.5), whilst mean concentration for all other metabolites significantly differed among all temperatures tested (Figure 3.5).

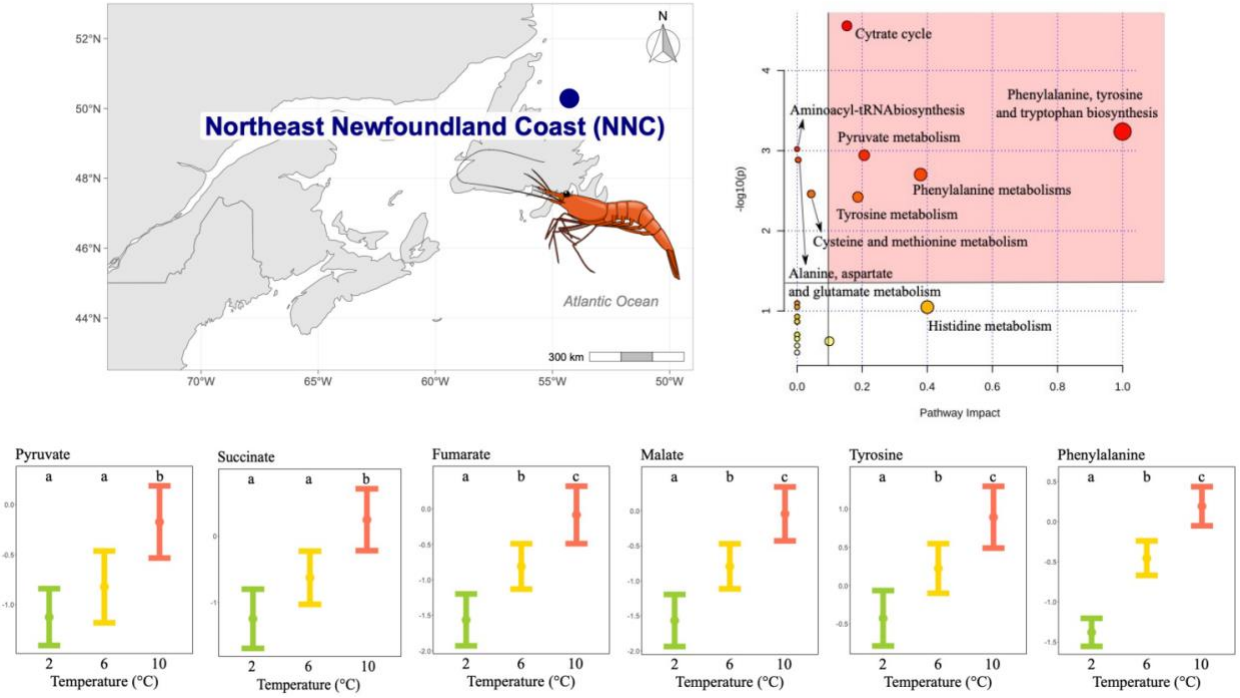


Figure 3.5 Metabolomics differences among temperatures in the northern shrimp *P. borealis* from the Northeast Newfoundland Coast (NNC). In more detail, at the top left of the figure we show the map representing the collection site and at the top right of the figure we show the pathway analysis of metabolic changes carried out with the statistically significant metabolites (ANOVA $p < 0.05$). Pathways within the red area were considered the most relevant as they have $p < 0.05$ and impact > 0.1. At the bottom of the figure we show the mean concentration and 95 % CI for pyruvate, succinate, fumarate, malate, tyrosine and phenylalanine for each temperature treatment: 2 (green, N=15), 6 (yellow, N=20) and 10 °C (red, N=19), combining all pH levels. Lower case letters identify significant differences ($p < 0.05$) among treatments.

3.7.3 ATP:ADP ratio

No significant effect of temperature, pH and their interaction was found on the ATP : ADP ratio of shrimp from any origin (minimum *p*-value = 0.6339).

3.8 DISCUSSION

Here, we show that the ecologically and economically important northern shrimp undergoes metabolomics reprogramming under future global change scenarios, shrimp from different origin

responding differently to the combined exposure to temperature and pH. This said, temperature drives most of the responses, followed by a modest effect of the interaction between temperature and pH and almost no effect of pH. These results confirm that the northern shrimp is more sensitive to OW than to OA, similarly to what has been reported for other marine organisms (e.g. Matoo et al., 2021; Noisette et al., 2016; Schalkhausser et al., 2014). Additionally, inter-origin differences are observed, as combined effects of temperature and pH are only detected in SLE and ESS shrimp, and the isolated effect of pH is only evident in SLE shrimp. Despite these inter-origin differences, the metabolomics changes observed are comparable and all related to the regulation of the aerobic energy production pathway and amino acid metabolism, with environmental drivers mostly impacting metabolites of the tricarboxylic acid cycle (TCA). While these metabolites are intrinsically linked to energy metabolism, recent studies emphasize that TCA intermediates participate in a wide range of cellular processes (Choi et al., 2021; Martínez-Reyes & Chandel, 2020). Based on our previous results on *P. borealis* physiological responses to global change drivers (Guscelli et al. *under review*) and the present study, we propose that modulation of TCA intermediates and amino acids may be an important mechanism regulating immune and stress responses of shrimp under global change scenarios.

Overall, the northern shrimp metabolomics profiles are mainly affected by temperature. Indeed, only metabolites concentrations of shrimp from SLE changed under exposure to low pH or its combination with temperature, suggesting that shrimp from SLE are more sensitive to OA than shrimp from other origins, at the molecular level. These results are coherent with the results obtained previously at higher levels of biological organization, which confirm that overall adults of *P. borealis* are more sensitive to OW than OA (Chemel et al., 2020; Guscelli et al. *under review;* Hammer and Pedersen, 2013). Interestingly, the SLE shrimp response to OW and OA is the most divergent when compared to those of shrimp from all other origins. In fact, in shrimp from SLE, the alanine, aspartate and glutamate metabolism pathway is affected but the TCA pathway is not. These results suggest that the responses of shrimp from SLE differ from the ones of shrimp from other origins at multiple levels of biological organization: metabolic, cellular and whole-organism. Indeed, we previously reported that shrimp from SLE possess the worst general physiological condition as they show the highest maximum metabolic rate associated to the lowest cellular energetic capacity (Guscelli et al. *under review*). Moreover, the differences in metabolomics

profiles of shrimp from different origins exposed to combined global change drivers, support the previous suggestion of a long-term acclimatization or adaptation of this species to different regional environmental conditions (Guscelli et al. *under review*). Although we recognize that inter-origin differences could be influenced by the potential effect of seasonality on the physiological responses of shrimp to global change drivers, its influence was minimized to a very marginal effect by ensuring similar (approximately eight weeks) and stable pre-exposure laboratory conditions and by selecting only non-ovigerous females. Variation in the metabolome among populations as sign of local adaptation has been shown in other marine species (e.g. Calosi et al., 2017; Vohsen et al., 2019). However, to our knowledge this is the first study that provides evidence of an inter-origin metabolomics reprogramming under combined OW and OA.

Interestingly, our results show an increase in concentration of TCA intermediates in shrimp from ESS, EC and NNC exposed to increasing temperature. Specifically, we show an increase of succinate, fumarate and malate concentrations in shrimp from these three origins and an increase in citrate and pyruvate concentrations in shrimp from EC and NNC, respectively, with increasing temperature. Usually, the accumulation of TCA metabolites suggests a disruption of the TCA cycle and a consequent shift from aerobic to anaerobic metabolism, as previously shown in the saltwater clam *Laternula elliptica* exposed to acute warming and in the olive flounder *Paralichthys olivaceus* exposed to low salinity (Clark et al., 2017; Wu et al., 2017). In the olive flounder, the accumulation of lactate under low salinity exposure further supports the shift to anaerobic metabolism. However, in our study lactate concentrations are under the limit of detection, which do not allow us to confirm that shrimp shift their energy production to anaerobic metabolism, limiting our further discussion of this hypothesis. Moreover, previous results on the cellular energetic capacity of the northern shrimp exposed to combined OW and OA showed that activities of enzymes catalysing reactions of the oxidative phosphorylation pathway remained stable and that shrimp aerobic scope increased with increasing temperature between 2 and 10 °C suggesting that shrimp can tolerate this range of temperatures and sustain an aerobic metabolism (Guscelli et al. *under review*). In the present study AMP, ADP and ATP levels remained stable and comparable among shrimp from different origins in all treatments, further suggesting that shrimp can maintain aerobic respiration even under exposure to high temperatures. This led us to *a posteriori* investigate if temperature, pH and/or origin affect the ATP:ADP ratio as it is a relevant indicator of energy consumption and changes in

cellular energy status (Metallo & Vander Heiden, 2013; Tantama et al., 2013; Yuan et al., 2013). Higher levels of ATP:ADP ratio indicate that organisms possess enough energy to sustain oxidative metabolism while lower levels of ATP:ADP ratio indicates that glycolysis needs to be enhanced. In our study, ATP:ADP ratio is not affected by global change drivers and does not differ among origins, further suggesting that shrimp can maintain their energy status and possibly sustain aerobic metabolism, confirming that the northern shrimp can tolerate temperatures up to 10 °C and low pH conditions, but survival is reduced under these conditions (Guscelli et al. *under review*).

The release of TCA metabolites by mitochondria has been recently linked to the regulation of cell fate and function as they are also involved in controlling chromatin modifications, DNA methylation ultimately affecting protein functions (Martínez-Reyes & Chandel, 2020). TCA metabolites, in particular succinate, have been shown to exert signalling functions and to regulate immune and inflammatory responses, regulating tissue damage (Choi et al., 2021; Mills & O'Neill, 2014). Succinate is a substrate for reactive oxygen species (ROS) production as it provides electrons to the respiratory chain, thus its accumulation can promotes the production of ROS linked to oxidative stress and immune response (Li et al., 2020a; Lushchak, 2011; Murphy, 2009; Zhang et al., 2020). However, ROS production was not measured here, and we are unable to validate whether the accumulation of TCA metabolites in the abdominal muscle is linked to oxidative stress in the northern shrimp exposed to OW and OA. Additionally, the antioxidant defence response previously measured on *P. borealis* exposed to low dissolved oxygen levels is unclear (Dupont-Prinet et al., 2013; Pillet et al., 2016). However, the increase in tyrosine concentration in shrimp from all origins under elevated temperature and low pH further supports the suggestion of the enhancement of the immune response. Indeed, metabolites derived from the phenylalanine, tyrosine and tryptophan biosynthesis and degradation play key roles in plants and animals' immune responses (Parthasarathy et al., 2018). Recently, the regulation of phenylalanine, tyrosine and tryptophan biosynthesis and the tyrosine accumulation in the thick shell mussels *Mytilus coruscus* exposed to OA have been suggested to potentially be linked to the immune response (Shang et al., 2022), although this needs to be investigated in the northern shrimp. We therefore suggest future studies to explore the effect of global ocean changes on *P. borealis* oxidative stress (for example measuring ROS production and antioxidant enzymes activity) and immune response. To do so, multiple tissues should be examined to acquire a more complete understanding of the northern

shrimp response to OW and OA, especially hepatopancreas that is considered as the most sensitive tissue to oxidative stress (Ruppert et al., 2004). Nonetheless, as the northern shrimp low response of antioxidant enzymes (Dupont-Prinet et al., 2013; Pillet et al., 2016) could be due to the investigation of such response over short exposure, we suggest developing experiments employing longer exposure periods, as in the present study. Finally, to further acquire information on the potential immune response of shrimp, it would be interesting to measure the itaconate concentration as it is a key immune-responsive metabolite, stemming from diversion of TCA cycle flux, that has been linked to disease in mussels (Li et al., 2020a). Confirming that global change drivers induce oxidative stress in the northern shrimp and enhance their immune response could lead to the re-evaluation of shrimp sensitivity and vulnerability to OW and OA. Indeed, although our results do not support a switch to anaerobiosis, the accumulation of TCA intermediates in shrimp exposed to the highest temperature and the potential enhancement of an immune response confirm that 10 °C is a temperature close to the northern shrimp thermal tolerance limit (Guscelli et al. *under review*). This is also confirmed by the increase in free amino acid levels with increasing temperature, which can be indicative of altered protein synthesis/breakdown, possibly to use amino acids as energy sources to feed the TCA cycle. Additionally, amino acids have been increasingly recognised for their function in the immune response and tissue repair of crustaceans and other marine animals, suggesting their importance in coping with stress (Herrera et al., 2019; Huang et al., 2020).

In conclusion, our study confirms that *P. borealis* is overall tolerant to OW and OA conditions predicted to occur by 2100 as the cellular energetic status of shrimp from all origins is maintained. However, OW exposure, and in minor proportion OA, induce the accumulation of metabolites capable of regulating cell physiology and enhancing immune responses, suggesting signs of stress under OW and OA exposure in shrimp from all origins. Our results highlight the importance of investigating cellular mechanisms underpinning whole organism's physiological responses, to better estimate species' sensitivity to future complex environmental conditions (Bartholomew, 1964; Harvey et al., 2014). Finally, the inter-origin differences shown in the metabolic response of shrimp exposed to OW and OA, and the intraspecific similitudes shown in form of the potential enhancement of immune response, highlights the importance of conducting macrophysiological studies to define species' sensitivity to future environmental conditions (Chown & Gaston, 2008).

3.9 STATEMENTS

Data availability

The dataset generated for this study will be available in the online repository PANGEA. Further inquiries can be directed to the corresponding author.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

DC provided the installations for the experimental setups and the live shrimp for the study and EG carried out the experiments, the sample collection and the sample preparation for analysis. PC provided the fundings to support the sample analysis at Les laboratoires Iso-BioKem Inc. EG prepared the dataset and conducted the bioinformatics analyses with support from DM and FV, and discussions with DM and PC. EG conducted the interpretation of the results and prepared figures and tables. EG wrote the manuscript and all authors contributed to its final version. All authors contributed to the article and approved the submitted version.

Funding

Our work has been funded by: (i) an OURANOS grant (554023) to DC and PC; (ii) a DFO Strategic Program for Ecosystem-Based Research and Advice grant and an Aquatic Climate Change Adaptation Services Program grant to DC; (iii) a FIR UQAR grant, a MITACS-Ouranos Accelerate grant, a Canada Foundation for Innovation (CFI) grant and a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grants (RGPIN-2015-06500 and RGPIN-2020-05627) to PC; (iv) a Fundação para a Ciência e Tecnologia (Portugal) through a Scientific Employment Stimulus researcher contract granted to DM (CEECIND/01250/2018) and the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/ 2020); (v) a MITACS-Ouranos Accelerate grant to support EG; (vi) a Fonds de Recherche du Québec - Nature et Technologies (FRQNT) scholarship (Doctoral PBEEE, 289597) and a Réal- Decoste Ouranos

scholarship (286109) to EG; and (vii) a FRQNT scholarship (Doctoral, 274427) and a Vanier Canada Graduate Scholarships (433956) to FV.

Acknowledgments

The authors are especially grateful to Bertrand Genard, Judith Savoie, Anthony Schmutz and Mathieu Millour from Les laboratoires Iso-BioKem Inc. EG, FV and PC are members of the inter-institutional strategic research network Québec-Océan. DC is member of the inter-institutional strategic research network Ressources Aquatiques Québec.

3.10 SUPPORTING INFORMATION

3.10.1 Figure

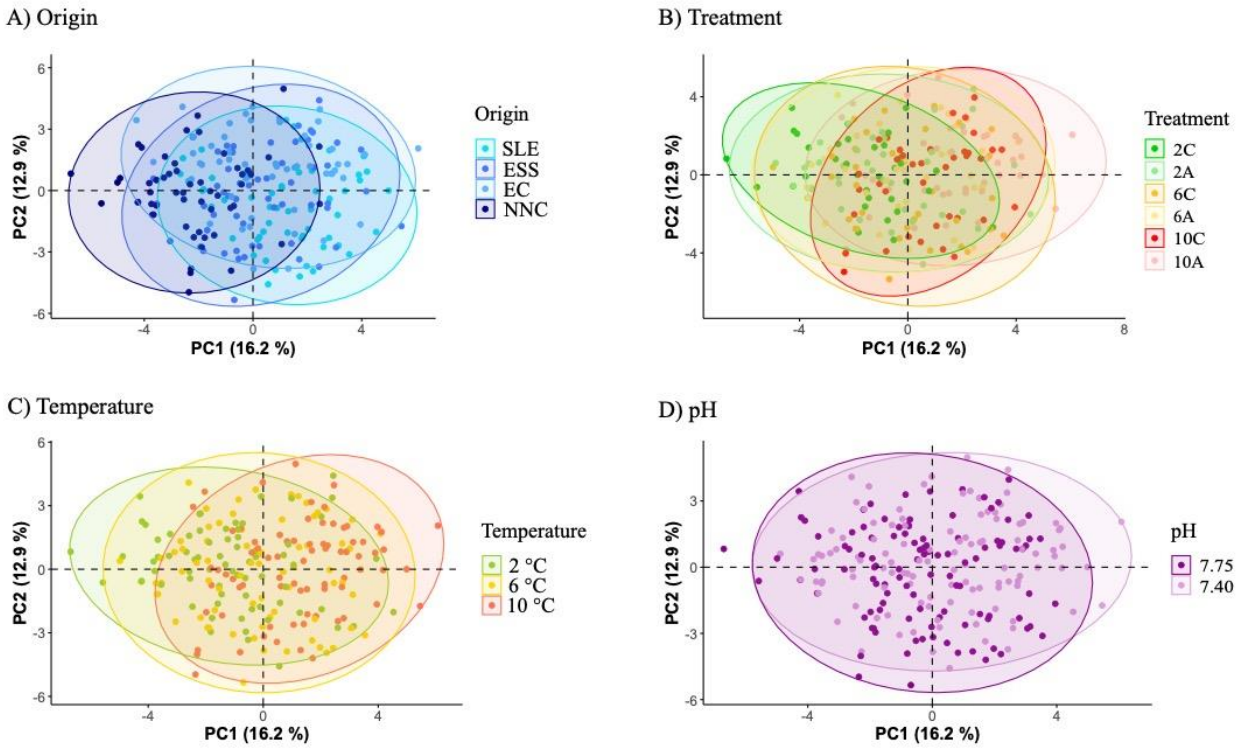


Figure S 3.6 PCA 2D score plot with 95 % confidence ellipse representing the variation in metabolites profile in the northern shrimp according to A) origins: St. Lawrence Estuary (SLE), Eastern Scotian Shelf (ESS), Esquiman Channel (EC) and Northeast Newfoundland Coast (NNC); B) temperature*pH treatments: 2C (2 °C, pH 7.75), 2A (2 °C, pH 7.40), 6C (6 °C, pH 7.75), 6A (6 °C, pH 7.40), 10C (10 °C, pH 7.75) and 10A (10 °C, pH 7.40); C) temperature: 2, 6 and 10 °C and D) pH: 7.75 and 7.40.

3.10.2 Table

Table S 3.3 Summary of the results of the ANOVA tests used to investigate the effects of temperature and pH on metabolites quantified in the northern shrimp muscle samples. Origins: St. Lawrence Estuary (SLE), Eastern Scotian Shelf (ESS), Esquiman Channel (EC) and Northeast Newfoundland Coast (NNC). Numbers in bold indicate significant *p*-values.

		Temperature			**pH**			**Temperature*pH**		
Origin	**Metabolite**	**F value**	**p value**	**FDR**	**F value**	**p value**	**FDR**	**F value**	**p value**	**FDR**
SLE	Phenylalanine	2.974	0.060	0.236	1.063	0.307	0.475	1.620	0.208	0.354
SLE	Tryptophan	3.413	0.041	0.233	2.059	0.158	0.357	1.465	0.241	0.390
SLE	Betaine	0.339	0.714	0.905	4.996	0.030	0.136	8.512	0.001	**0.011**
SLE	Isoleucine	0.133	0.876	0.921	5.256	0.026	0.136	0.422	0.658	0.787
SLE	Tyrosine	10.305	0.000	**0.003**	21.395	< 0.001	**0.001**	0.368	0.694	0.787
SLE	Valine	0.205	0.816	0.905	1.844	0.181	0.384	0.020	0.980	0.980
SLE	β Aminoisobutyric	0.284	0.754	0.905	4.640	0.036	0.136	1.727	0.188	0.337
SLE	α Aminobutyric acid	1.056	0.355	0.711	0.568	0.454	0.552	0.592	0.557	0.728
SLE	Hydroxyproline	0.553	0.579	0.905	15.241	0.000	**0.005**	0.124	0.884	0.911
SLE	Threonine	0.113	0.894	0.921	0.415	0.523	0.613	1.004	0.374	0.530
SLE	Glutamine	0.193	0.825	0.905	3.064	0.086	0.225	2.210	0.120	0.273
SLE	Glycine	0.035	0.966	0.966	4.899	0.031	0.136	4.110	0.022	0.126
SLE	Serine	11.841	< 0.001	**0.002**	0.618	0.436	0.549	4.851	0.012	0.101
SLE	α Aminoadipic acid	2.808	0.070	0.236	3.704	0.060	0.185	1.250	0.295	0.456
SLE	AMP	0.847	0.435	0.778	0.032	0.859	0.913	0.246	0.783	0.859
SLE	Glutamate	1.322	0.276	0.656	4.813	0.033	0.136	3.186	0.050	0.226
SLE	FAD	2.097	0.134	0.349	2.392	0.128	0.312	2.448	0.097	0.241
SLE	Aspartate	0.333	0.719	0.905	5.084	0.029	0.136	10.503	0.000	**0.005**
SLE	Histidine	0.607	0.549	0.905	3.277	0.076	0.216	2.421	0.099	0.241
SLE	NAD	1.149	0.325	0.691	0.897	0.348	0.503	3.057	0.056	0.226
SLE	Lysine	3.086	0.054	0.236	14.176	0.000	**0.005**	2.653	0.080	0.241
SLE	Cystine	4.159	0.021	0.185	1.396	0.243	0.446	0.402	0.671	0.787
SLE	Pyruvate	0.420	0.659	0.905	0.684	0.412	0.539	6.450	0.003	**0.037**
SLE	Glucose	0.995	0.377	0.712	1.186	0.281	0.475	1.935	0.155	0.310
SLE	NADH	0.218	0.805	0.905	1.358	0.249	0.446	2.955	0.061	0.226
SLE	α Ketoglutaric acid	1.272	0.289	0.656	0.006	0.937	0.965	0.160	0.853	0.906
SLE	Succinate	4.135	0.022	0.185	1.448	0.235	0.446	2.560	0.087	0.241
SLE	Fumarate	2.632	0.082	0.236	0.781	0.381	0.518	1.791	0.177	0.335
SLE	Malate	3.405	0.041	0.233	1.118	0.295	0.475	2.862	0.067	0.226
SLE	ADP	0.271	0.764	0.905	0.331	0.568	0.643	1.128	0.332	0.491
SLE	ATP	2.612	0.083	0.236	0.001	0.980	0.980	2.060	0.138	0.294
SLE	Glucose 6 phosphate	0.329	0.721	0.905	3.827	0.056	0.185	4.183	0.021	0.126
SLE	Citrate	2.800	0.070	0.236	0.067	0.797	0.874	0.374	0.690	0.787
SLE	Phosphoenyl pyruvate	0.519	0.598	0.905	0.872	0.355	0.503	0.798	0.456	0.620
ESS	Phenylalanine	1.595	0.213	0.301	1.373	0.247	0.699	2.938	0.062	0.474
ESS	Tryptophan	19.920	< 0.001	< **0.001**	1.773	0.189	0.611	1.204	0.308	0.552
ESS	Betaine	1.022	0.367	0.430	0.641	0.427	0.757	1.069	0.351	0.596
ESS	Isoleucine	0.181	0.835	0.887	0.034	0.854	0.920	1.728	0.188	0.552
ESS	Tyrosine	7.507	0.001	**0.010**	2.929	0.093	0.452	2.107	0.132	0.552
ESS	Valine	1.333	0.273	0.331	0.412	0.524	0.774	0.319	0.729	0.826
ESS	β Aminoisobutyric	3.653	0.033	0.080	0.229	0.635	0.863	1.012	0.371	0.598
ESS	α Aminobutyric acid	2.621	0.082	0.165	0.184	0.670	0.876	0.369	0.693	0.813
ESS	Hydroxyproline	0.076	0.927	0.927	0.147	0.703	0.886	7.929	0.001	**0.034**
ESS	Threonine	6.629	0.003	**0.011**	0.008	0.931	0.931	0.008	0.304	0.552
ESS	Glutamine	3.652	0.033	0.080	0.078	0.781	0.915	0.606	0.550	0.667
ESS	Glycine	1.470	0.239	0.306	0.592	0.445	0.757	1.240	0.298	0.552

ESS	Serine	15.735	< 0.001	< **0.001**	4.339	0.042	0.287	2.245	0.116	0.552
ESS	α Aminoadipic acid	5.532	0.007	**0.023**	9.040	0.004	0.069	0.251	0.779	0.855
ESS	AMP	3.202	0.049	0.111	1.967	0.167	0.611	0.218	0.805	0.855
ESS	Glutamate	4.175	0.021	0.059	4.483	0.039	0.287	1.711	0.191	0.552
ESS	FAD	0.116	0.891	0.917	1.059	0.308	0.757	0.966	0.387	0.598
ESS	Aspartate	10.194	0.000	**0.002**	0.950	0.334	0.757	2.603	0.084	0.474
ESS	Histidine	1.455	0.243	0.306	1.704	0.198	0.611	3.070	0.055	0.474
ESS	NAD	1.910	0.158	0.256	0.018	0.893	0.920	0.856	0.431	0.610
ESS	Lysine	1.755	0.183	0.270	0.099	0.754	0.915	0.890	0.417	0.610
ESS	Cystine	4.617	0.014	**0.044**	0.669	0.417	0.757	2.674	0.078	0.474
ESS	Pyruvate	1.841	0.169	0.261	0.665	0.419	0.757	4.996	0.010	0.176
ESS	Glucose	6.519	0.003	**0.011**	0.019	0.892	0.920	0.737	0.483	0.632
ESS	NADH	2.385	0.102	0.183	10.035	0.003	0.069	0.122	0.885	0.912
ESS	α Ketoglutaric acid	1.470	0.239	0.306	0.424	0.518	0.774	1.245	0.296	0.552
ESS	Succinate	6.850	0.002	**0.011**	5.881	0.019	0.213	1.441	0.246	0.552
ESS	Fumarate	7.397	0.001	**0.010**	0.325	0.571	0.809	0.747	0.479	0.632
ESS	Malate	7.036	0.002	**0.011**	0.485	0.490	0.774	1.643	0.203	0.552
ESS	ADP	2.041	0.140	0.238	0.026	0.872	0.920	1.272	0.289	0.552
ESS	ATP	0.918	0.406	0.460	0.986	0.325	0.757	0.623	0.540	0.667
ESS	Glucose 6 phosphate	3.031	0.057	0.121	0.754	0.389	0.757	1.474	0.238	0.552
ESS	Citrate	2.392	0.101	0.183	2.278	0.137	0.583	1.382	0.260	0.552
ESS	Phosphoenyl pyruvate	0.806	0.452	0.496	3.898	0.054	0.304	0.058	0.944	0.944
EC	Phenylalanine	2.418	0.100	0.201	0.031	0.862	0.945	1.945	0.155	0.615
EC	Tryptophan	0.070	0.933	0.961	0.996	0.324	0.688	2.405	0.102	0.615
EC	Betaine	0.081	0.922	0.961	0.226	0.636	0.822	0.277	0.759	0.869
EC	Isoleucine	0.027	0.974	0.974	1.179	0.283	0.688	0.871	0.425	0.804
EC	Tyrosine	12.535	< 0.001	**0.001**	3.785	0.058	0.492	2.297	0.112	0.615
EC	Valine	0.580	0.564	0.767	0.008	0.929	0.987	1.432	0.249	0.657
EC	β Aminoisobutyric	7.273	0.002	**0.007**	0.149	0.702	0.822	0.967	0.388	0.791
EC	α Aminobutyric acid	20.310	< 0.001	< **0.001**	0.253	0.618	0.822	1.909	0.160	0.615
EC	Hydroxyproline	0.629	0.538	0.767	1.862	0.179	0.609	0.231	0.795	0.869
EC	Threonine	9.664	0.000	**0.003**	1.900	0.175	0.609	0.210	0.811	0.869
EC	Glutamine	0.598	0.554	0.767	0.635	0.430	0.822	1.381	0.262	0.657
EC	Glycine	0.191	0.827	0.937	0.152	0.698	0.822	0.732	0.487	0.869
EC	Serine	20.890	< 0.001	< **0.001**	2.632	0.112	0.516	0.253	0.778	0.869
EC	α Aminoadipic acid	4.724	0.014	**0.042**	1.047	0.311	0.688	1.826	0.173	0.615
EC	AMP	0.370	0.693	0.872	0.619	0.436	0.822	1.863	0.167	0.615
EC	Glutamate	0.110	0.896	0.961	1.008	0.321	0.688	1.623	0.208	0.644
EC	FAD	1.702	0.194	0.346	2.551	0.117	0.516	1.272	0.290	0.657
EC	Aspartate	1.265	0.292	0.473	2.489	0.121	0.516	1.891	0.163	0.615
EC	Histidine	2.631	0.083	0.188	0.001	0.981	0.993	0.279	0.758	0.869
EC	NAD	0.522	0.597	0.780	0.341	0.562	0.822	0.137	0.873	0.899
EC	Lysine	0.321	0.727	0.883	0.298	0.588	0.822	7.117	0.002	0.069
EC	Cystine	4.149	0.022	0.062	0.237	0.629	0.822	0.334	0.718	0.869
EC	Pyruvate	2.312	0.110	0.209	1.326	0.255	0.688	0.202	0.818	0.869
EC	Glucose	0.238	0.789	0.925	0.000	0.993	0.993	0.104	0.901	0.901
EC	NADH	0.769	0.469	0.725	8.347	0.006	0.200	0.306	0.738	0.869
EC	α Ketoglutaric acid	2.525	0.091	0.194	0.056	0.813	0.922	1.339	0.272	0.657
EC	Succinate	7.181	0.002	**0.007**	4.832	0.033	0.374	0.277	0.759	0.869
EC	Fumarate	8.581	0.001	**0.003**	0.271	0.605	0.822	0.946	0.396	0.791
EC	Malate	8.705	0.001	**0.003**	0.540	0.466	0.822	0.236	0.791	0.869
EC	ADP	9.377	0.000	**0.003**	0.215	0.645	0.822	5.116	0.010	0.168
EC	ATP	1.304	0.281	0.473	5.461	0.024	0.374	1.776	0.181	0.615
EC	Glucose 6 phosphate	3.474	0.039	0.103	0.192	0.664	0.822	0.491	0.615	0.869
EC	Citrate	6.502	0.003	**0.011**	1.673	0.202	0.625	0.572	0.569	0.869
EC	Phosphoenyl pyruvate	3.264	0.047	0.115	2.722	0.106	0.516	0.634	0.535	0.869

NNC	Phenylalanine	50.640	< 0.001	< **0.001**	0.589	0.447	0.805	0.125	0.883	0.968
NNC	Tryptophan	1.570	0.219	0.388	2.867	0.097	0.805	0.951	0.394	0.765
NNC	Betaine	0.221	0.803	0.853	0.884	0.352	0.805	2.772	0.073	0.352
NNC	Isoleucine	6.171	0.004	**0.016**	0.447	0.507	0.805	0.397	0.675	0.900
NNC	Tyrosine	13.309	< 0.001	**0.000**	0.881	0.353	0.805	0.641	0.531	0.838
NNC	Valine	1.524	0.228	0.388	0.021	0.885	0.885	0.010	0.991	0.991
NNC	β Aminoisobutyric	1.120	0.335	0.474	2.628	0.112	0.805	0.889	0.418	0.765
NNC	α Aminobutyric acid	5.213	0.009	**0.030**	1.817	0.184	0.805	1.163	0.321	0.761
NNC	Hydroxyproline	1.317	0.277	0.410	0.345	0.560	0.805	0.152	0.859	0.968
NNC	Threonine	3.255	0.047	0.124	3.847	0.056	0.805	0.302	0.741	0.900
NNC	Glutamine	0.639	0.532	0.696	4.700	0.035	0.805	0.071	0.931	0.989
NNC	Glycine	2.512	0.092	0.223	0.075	0.785	0.839	0.620	0.542	0.838
NNC	Serine	1.698	0.194	0.388	0.710	0.404	0.805	1.565	0.220	0.711
NNC	α Aminoadipic acid	9.510	0.000	**0.002**	0.609	0.439	0.805	0.308	0.736	0.900
NNC	AMP	1.449	0.245	0.396	0.554	0.460	0.805	0.798	0.456	0.775
NNC	Glutamate	0.232	0.794	0.853	1.194	0.280	0.805	0.256	0.775	0.909
NNC	FAD	0.448	0.642	0.779	1.194	0.280	0.805	2.128	0.130	0.492
NNC	Aspartate	0.396	0.675	0.792	2.030	0.161	0.805	1.445	0.246	0.711
NNC	Histidine	5.089	0.010	**0.031**	0.026	0.873	0.885	0.032	0.969	0.991
NNC	NAD	0.880	0.421	0.573	1.088	0.302	0.805	3.330	0.044	0.301
NNC	Lysine	2.410	0.101	0.228	0.881	0.353	0.805	0.572	0.568	0.840
NNC	Cystine	12.279	< 0.001	**0.000**	0.231	0.633	0.828	3.667	0.033	0.280
NNC	Pyruvate	8.259	0.001	**0.004**	0.113	0.738	0.839	0.928	0.402	0.765
NNC	Glucose	4.213	0.021	0.058	0.475	0.494	0.805	0.513	0.602	0.853
NNC	NADH	1.388	0.260	0.401	0.440	0.510	0.805	1.202	0.310	0.761
NNC	α Ketoglutaric acid	0.065	0.937	0.937	0.260	0.612	0.828	0.312	0.734	0.900
NNC	Succinate	13.599	< 0.001	**0.000**	0.572	0.453	0.805	3.891	0.027	0.280
NNC	Fumarate	19.579	< 0.001	< **0.001**	0.330	0.568	0.805	5.248	0.009	0.280
NNC	Malate	20.494	< 0.001	< **0.001**	0.135	0.715	0.839	4.071	0.023	0.280
NNC	ADP	1.557	0.221	0.388	0.164	0.687	0.839	2.277	0.114	0.483
NNC	ATP	0.465	0.631	0.779	0.086	0.770	0.839	1.423	0.251	0.711
NNC	Glucose 6 phosphate	0.150	0.862	0.888	0.072	0.789	0.839	1.117	0.336	0.761
NNC	Citrate	0.346	0.709	0.804	0.392	0.534	0.805	0.865	0.428	0.765
NNC	Phosphoenyl pyruvate	2.244	0.117	0.249	0.554	0.460	0.805	2.877	0.066	0.352

3.11 ANALYSE DES PROFILS MÉTABOLOMIQUES DE LA CREVETTE NORDIQUE DE L'ESTUAIRE DU SAINT-LAURENT EXPOSÉE À L'HYPOXIE ET AU « TRIO MORTEL »

3.11.1 Mise en contexte

L'activité humaine, notamment l'ajout d'éléments nutritifs en zone côtière et l'eutrophisation qui en découle, a grandement augmenté le nombre d'écosystèmes marins qui subissent des épisodes hypoxiques (faibles valeurs d'oxygène dissous, OD), ainsi que la durée et l'intensité de ces épisodes (Diaz et al., 2019). Dans le contexte des changements globaux, le réchauffement des eaux va empirer la situation, puisque la solubilité de l'oxygène (O_2) diminue lorsque la température de l'eau augmente (Breitburg et al., 2018; IPCC, 2022). Cette baisse de l'OD constitue une menace majeure pour les organismes utilisant la respiration aérobie car en conditions hypoxiques leurs capacités à faire correspondre les approvisionnements et les demandes énergétiques sont limitées. Ainsi, la disponibilité énergétique est réduite en conditions hypoxiques, ce qui entraine des conséquences négatives sur les fonctions physiologiques (p.ex. croissance et reproduction) et la survie des organismes (Chabot & Claireaux, 2008; Gewin, 2010; McMahon, 2001). Au niveau cellulaire, le manque d'O_2 limite la production de l'énergie *via* la phosphorylation oxydative jusqu'à que, dans des conditions particulières, les voies métaboliques anaérobies constituent la seule source de production de l'ATP (Burnett & Stickle, 2011; Gäde, 1983). Des changements de profils métabolomiques (c.-à-d. changements de voies et/ou de substrats) ont été observés chez plusieurs espèces exposées à l'hypoxie (p.ex. Lardon et al., 2013; Venter et al., 2018; Zhang et al., 2017). Cependant, l'hypoxie est rarement étudiée dans un cadre expérimental multifactoriel, ce qui permet difficilement de prendre en considération l'ampleur des impacts de ce phénomène dans un contexte de changement global (cf. Huo et al., 2019; Lu et al., 2016).

La sensibilité des organismes à l'hypoxie dépend, entre autres, du niveau d'OD considéré. À cet égard, des niveaux d'OD aussi faibles que 35 % sat. représentent pour cette espèce des conditions hypoxiques chroniques non léthales (Dupont-Prinet et al., 2013). En effet, le RA diminue en conditions hypoxiques mais est encore 40 % de celui en normoxie à 22 % sat. (Dupont-Prinet et al., 2013). En revanche, des niveaux d'OD de 9 et 15,5 % sat. représentent pour cette espèce les limites de tolérance à 5 °C pour les mâle et les femelles, respectivement (Dupont-Prinet et al., 2013). Cependant, si ce facteur est combiné à d'autres facteurs environnementaux la tolérance à l'hypoxie peut varier. Par exemple, la tolérance à l'hypoxie des crevettes diminue à des

températures plus élevées (Dupont-Prinet et al., 2013) et leur taux de survie est réduit à 40 % quand les crevettes sont exposées à la combinaison des facteurs du « trio mortel » (voir le chapitre 1 de cette thèse). Le chapitre 1 a aussi montré les impacts négatifs du « trio mortel » aux niveaux de l'organisme entier et cellulaire chez les crevettes plus tolérantes. Brièvement, le RA est réduit à 40 % en raison de l'augmentation du SMR et de la diminution du MMR et on observe un ajustement phénotypique et morphologique des mitochondries.

Ainsi, cette analyse vise à dévoiler les mécanismes cellulaires qui sous-tendent les réponses physiologiques de la crevette nordique de l'estuaire du Saint-Laurent (SLE) exposée à l'hypoxie et à la combinaison de cette dernière avec le réchauffement et l'acidification des océans. Pour ce faire, comme dans l'article scientifique « *All roads lead to Rome : Metabolomics reprogramming of the northern shrimp exposed to global changes leads to a comparable physiological status* » faisant partie de ce chapitre, des analyses métabolomiques ciblées ont été réalisées sur des échantillons de muscle de crevette préservés à la fin de l'expérience décrite dans le chapitre 1 de cette thèse. En considération des résultats obtenus aux niveaux de l'organisme entier et cellulaire à la suite de l'exposition des crevettes à l'hypoxie (chapitre 1), on s'attend à ce que les profils métabolomiques soient comparables entre les traitements normoxiques et hypoxiques et que la production de l'énergie continue à être assurée par la voie métabolique aérobie. En revanche, l'exposition à la combinaison des facteurs du « trio mortel », comparé au scénario favorable, induirait un changement dans les profils métabolomiques, tel que récemment montré chez la coquille Saint-Jacques (*Pecten maximus*, Linnaeus, 1758) (Götze et al., 2020), indiquant un passage du métabolisme aérobie au métabolisme anaérobie.

3.11.2 Méthodes

Les échantillons de muscle des crevettes exposées aux traitements appartenant au « Design B » (précédemment décrit dans le chapitre 1 et illustré dans la Figure 3.7) ont été obtenus et traités comme décrit dans l'article scientifique « *All roads lead to Rome : Metabolomics reprogramming of the northern shrimp exposed to global changes leads to a comparable physiological status* » faisant partie de ce chapitre. Brièvement, une fois les mesures de respirométrie terminées, les crevettes ont été rapidement disséquées sur de la glace et le muscle de l'abdomen a été congelé dans l'azote liquide et conservé à -80 °C en attendant le début des analyses. L'extraction et la

quantification des métabolites ainsi que le prétraitement des données étaient identiques à ceux décrits précédemment. L'analyse des données prévoyait une analyse en composantes principales (ACP) non supervisée, appliquée pour explorer la structure des données. Pour tester l'effet des traitements sur les profils métabolomiques du muscle de crevette, une PERMANOVA a été réalisée en utilisant Primer-E v6 (Anderson et al., 2008) tel que décrit précédemment dans ce chapitre. Une ANOVA avec un seuil de taux de fausses découvertes (FDR) fixé à 0,05, a été réalisée pour chaque métabolite afin d'identifier les métabolites qui diffèrent entre les traitements. Les tests de Tukey ont été utilisés pour effectuer des analyses *post-hoc* lorsque l'effet du traitement avait été mis en évidence. Enfin, les métabolites dont la concentration différait significativement entre les traitements (identifiés par les ANOVAs) ont été utilisés pour l'analyse des voies métaboliques afin d'identifier les voies les plus impliquées dans la réponse des crevettes de SLE à l'hypoxie et à la combinaison du réchauffement, de l'acidification des océans et de l'hypoxie. L'analyse a été conduite telle que décrite dans les méthodes de l'article scientifique « *All roads lead to Rome : Metabolomics reprogramming of the northern shrimp exposed to global changes leads to a comparable physiological status* » faisant partie de ce chapitre. En conclusion, un modèle linéaire a été utilisé pour tester l'effet du traitement sur le ratio ATP:ADP des crevettes, calculé et analysé pour vérifier l'état énergétique cellulaire des individus (Metallo & Vander Heiden, 2013; Tantama et al., 2013; Yuan et al., 2013).

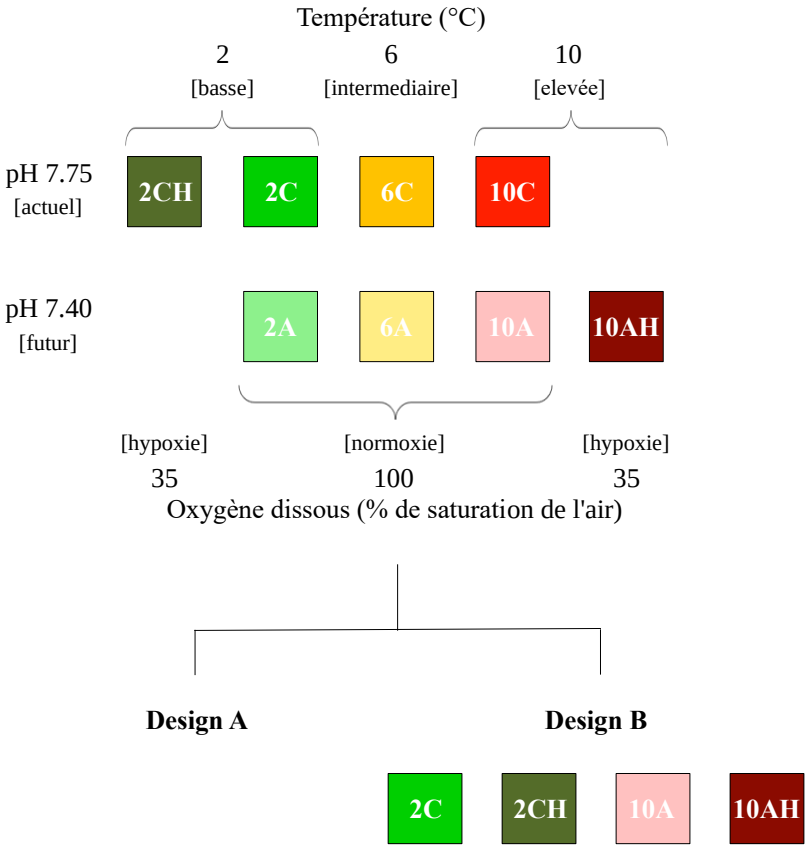


Figure 3.7 Représentation schématique du plan expérimental utilisé dans le chapitre 1 et dans cette analyse (« Design B ») afin de déterminer les effets de l'hypoxie et de la combinaison des facteurs du « trio mortel » sur les profils métabolomiques de la crevette nordique de l'estuaire du Saint-Laurent exposée pendant 30 jours. Les traitements du « Design B » correspondent à : 2C (2 °C, pH 7,75, normoxie - vert), 2CH (2 °C, pH 7,75, hypoxie - vert foncé), 10A (10 °C, pH 7,40, nomoxie - rouge clair) et 10AH (10 °C, pH 7,40, hypoxie - rouge foncé).

3.11.3 Résultats

Les différents groupes (traitements) n'ont pas été clairement séparés, tel que montré par l'ACP appliquée pour explorer la structure des données. Cependant, l'ACP a permis de mettre en évidence quelques différences entre les profils métabolomiques des crevettes exposées aux deux températures à l'étude (2 et 10 °C). De plus, quelques différences ont été mises en évidence entre les traitements normoxique et hypoxique à la température la plus élevée (Figure 3.8). Par ailleurs, les profils métabolomiques des crevettes sont significativement différents entre les traitements (Tableau 3.4). De plus, la concentration de sept des 34 métabolites quantifiés était significativement différente entre les traitements (Tableau 3.5). L'analyse des voies métaboliques a montré que les voies les plus pertinentes (combinaison de surreprésentation $p < 0,05$ et impact des voies $> 0,1$),

dans lesquelles ces métabolites étaient impliqués, étaient : (i) le cycle de l'acide citrique, (ii) la biosynthèse de la phénylalanine, de la tyrosine et du tryptophane et (iii) le métabolisme de la tyrosine (Figure 3.9). En détail, les concentrations de la tyrosine, du succinate, du fumarate et du malate étaient comparables entre les traitements normoxiques et hypoxiques aux deux températures testées, et la concentration moyenne de ces métabolites était toujours plus élevée chez les crevettes exposées aux traitements combinés (10A et 10AH) par rapport à celle des crevettes exposées au traitement favorable (2C) (Figure 3.9). Le ratio ATP:ADP était comparable entre tous les traitements ($F_{3,32}$ = 1.057, *p*-value = 0.381).

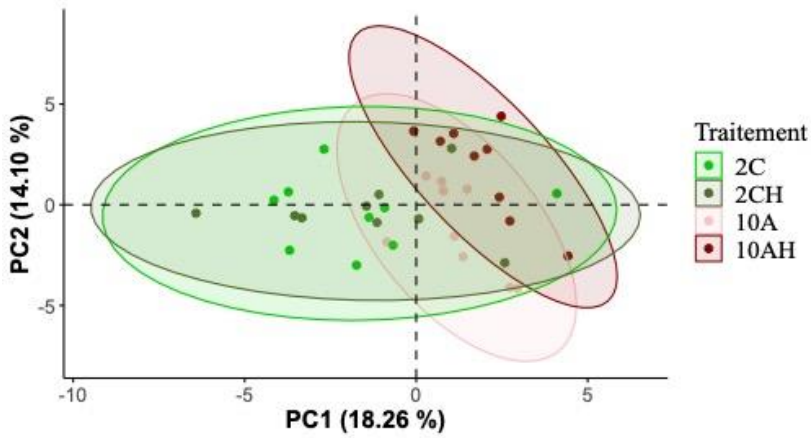


Figure 3.8 Représentation graphique en deux dimensions de l'ACP avec les intervalles de confiance à 95 % représentant la variation des profils métabolomiques de la crevette nordique de l'estuaire du Saint-Laurent en fonction des traitements : 2C (vert), 2CH (vert foncé), 10A (rouge clair) et 10AH (rouge foncé).

Tableau 3.4 Résumé des résultats du test PERMANOVA utilisé pour examiner l'effet des traitements du « Design B » sur les profils métabolomiques du muscle de la crevette nordique de l'estuaire du Saint-Laurent.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Traitement	3	240.57	80.19	2.70	**0.0001**	9858
Résidus	32	949.43	29.67			
Total	35	1190				

Tableau 3.5 Résumé des résultats du test ANOVA utilisé pour identifier les métabolites qui diffèrent entre les traitements du « Design B » dans le muscle de la crevette nordique de l'estuaire du Saint-Laurent. Seuls les métabolites dont la concentration change de manière significative entre les traitements (sept des 34 métabolites quantifiés) sont inclus dans le tableau.

Métabolites	**p value**	**FDR**
Tyrosine	< 0.001	**< 0.001**
Serine	< 0.001	**< 0.001**
Glucose 6 phosphate	< 0.001	**< 0.001**
Succinate	< 0.001	**0.004**
Malate	0.002	**0.015**
NADH	0.008	**0.043**
Fumarate	0.009	**0.043**

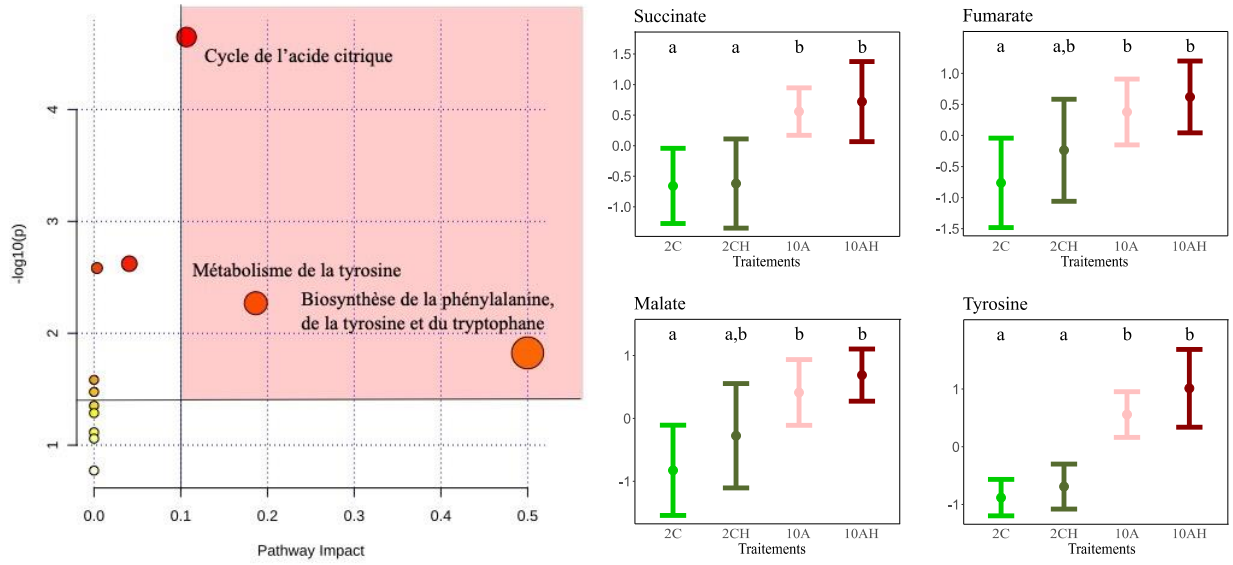


Figure 3.9 Différences du métabolome de la crevette nordique de l'estuaire du Saint-Laurent (SLE) exposée à différent traitements du « Design B ». En détail, le panneau de gauche de la figure montre les résultats de l'analyse des voies métaboliques effectuée avec les métabolites dont la concentration diffère statistiquement entre les traitements (ANOVA $p < 0,05$). Les voies dans la zone rouge sont identifiées comme les plus impliquées dans la réponse des crevettes de SLE ($p < 0,05$ et impact > 0,1). Le panneau de droite de la figure, montre la moyenne et l'IC à 95% de la tyrosine, du succinate, du malate et du fumarate selon les différents traitements. Les lettres minuscules identifient les différences significatives ($p < 0,05$) entre les traitements. Les couleurs identifient les traitements : 2C (vert), 2CH (vert foncé), 10A (rouge clair) et 10AH (rouge foncé).

3.11.4 Discussion

Le peu de différences observées dans le métabolome de la crevette nordique aux différents traitements confirme la tolérance de cette espèce à l'hypoxie (Dupont-Prinet et al., 2013 et voir le chapitre 1 de cette thèse) et montre que les crevettes de SLE subissent une reprogrammation métabolique lorsque exposées à des conditions environnementales mimant le scénario futur de changements globaux cumulés.

Nos résultats montrent que l'exposition des crevettes à l'hypoxie pendant 30 jours n'a pas d'effets sur le métabolisme énergétique des crevettes plus tolérantes, contrairement à ce qui est observé chez d'autres espèces exposées à ce facteur de changement global (Lardon et al., 2013; Zhang et al., 2017). En effet, l'exposition à des conditions hypoxiques peut induire des changements de concentrations des métabolites associés au cycle de l'acide citrique et des ratios ATP:ADP (Izral et al., 2018; Lardon et al., 2013). Cependant, les profils métabolomiques de la crevette nordique et leur ratio ATP:ADP sont comparables entre les traitements normoxiques et hypoxiques (2C *vs* 2CH et 10A *vs* 10AH). Ceci est cohérent avec les résultats obtenus aux niveaux d'organisation biologique supérieurs qui montrent que bien que l'exposition à hypoxie réduit la performance aérobie des crevettes d'environ 60 % leur capacité aérobie cellulaire est maintenue (chapitre 1). Néanmoins, les différences observées entre la crevette nordique et les autres espèces pourraient être liées à l'intensité du « stress ». En milieu naturel, la crevette nordique est exposée à des niveaux d'OD autour de ceux utilisés dans cette étude (35 % sat.; Bourdages et al., 2022; Dupont-Prinet et al., 2013). Il est donc possible qu'on observe des effets sur le métabolisme énergétique à des niveaux d'OD plus faibles, cette hypothèse est à valider dans le futur.

En revanche, les résultats montrent des différences entre les profils métabolomiques des crevettes plus tolérantes exposées aux conditions combinées (10AH) comparé à ceux des crevettes exposées aux conditions favorables (2C). En effet, les facteurs du « trio mortel » ont un impact sur les métabolites du cycle de l'acide citrique et le métabolisme des acides aminés. Cette même réponse a récemment été observée chez la coquille Saint-Jacques exposée à la combinaison du réchauffement, de l'acidification des océans et de l'hypoxie (Götze et al., 2020). Chez cette dernière, tout comme chez la crevette nordique, on observe une augmentation des concentrations de succinate et des acides aminés. Bien que ces métabolites soient intrinsèquement liés au

métabolisme énergétique aérobie, chez la crevette nordique ce dernier semble être maintenu, tel que montré par les ratios ATP:ADP qui sont comparables entre les traitements. En revanche, ces métabolites participent à de nombreux processus cellulaires (Martínez-Reyes & Chandel, 2020) et leur accumulation (succinate, fumarate, malate et tyrosine) en conditions combinées (10AH) suggère le déclenchement d'une réponse immunitaire (Choi et al., 2021; Huang et al., 2020). Ceci a précédemment été suggéré pour la moule *Mytilus coruscus* (Valenciennes, 1858) exposées à l'acidification des océans (Shang et al., 2022) et pour les crevettes provenant des différentes origines exposées au réchauffement et à l'acidification des océans (voir l'article scientifique « *All roads lead to Rome : Metabolomics reprogramming of the northern shrimp exposed to global changes leads to a comparable physiological status* »). Ces résultats seront intégrés aux résultats obtenus aux niveaux d'organisation biologique supérieurs et discutés afin de définir la sensibilité de la crevette nordique aux changements globaux cumulés futurs dans la section de conclusion de cette thèse.

CONCLUSION

Les changements globaux de l'océan sont responsables de la perte de biodiversité marine. Il est ainsi primordial de définir la vulnérabilité des espèces aux facteurs des changements globaux, en particulier les changements affectant les paramètres physico-chimiques des habitats, dans le but d'implanter les diverses stratégies et mesures de gestion et conservation. Pour ce faire, il est essentiel de ne pas sur- ou sous-estimer la sensibilité des espèces aux conditions environnementales futures. Ceci est particulièrement important pour les espèces d'intérêt écologique et commercial, pour lesquelles les changements de distribution et abondance pourraient avoir des impacts négatifs sur l'équilibre de l'écosystème et la pêcherie.

L'objectif principal de cette thèse était de définir le niveau de sensibilité aux phénomènes de réchauffement, acidification des océans et hypoxie d'individus de la même espèce provenant de plusieurs origines géographiques différentes, caractérisées par des environnements physico-chimiques contrastés, en intégrant les réponses des individus aux différentes échelles d'organisation biologique : de l'organisme à la cellule. Pour répondre à cet objectif, la crevette nordique *P. borealis* a été choisie comme organisme modèle dans le but de mieux comprendre ses réponses sous la contrainte de multiples facteurs des changements globaux le long de la côte est du Canada.

À travers l'utilisation complémentaire de trois approches (multifactorielle, intégrative et macrophysiologique) ce travail de thèse a permis d'estimer les niveaux de sensibilité de la crevette nordique aux conditions environnementales complexes, actuelles et futures, dans le nord-ouest de l'Atlantique. Considérant que chaque chapitre comprend une discussion approfondie des résultats spécifiques qui y sont présentés, cette conclusion générale se propose de (i) mettre en lumière l'importance d'utiliser de manière complémentaire les approches multifactorielle, intégrative et macrophysiologique pour bien définir la sensibilité de la crevette nordique aux changements globaux, (ii) estimer sa vulnérabilité aux scénarios futurs, (iii) évaluer les conséquences du déclin de cette espèce sur sa pêcherie, tout en offrant des perspectives de recherche en continuité de ce sujet.

Les termes sensibilité, tolérance, vulnérabilité, potentiel adaptatif, résilience et plasticité phénotypique, déjà introduits dans l'introduction de cette thèse, seront utilisés dans cette conclusion générale. Leurs définitions se trouvent dans le glossaire.

I. Sensibilité de la crevette nordique aux changements globaux cumulés

Importance des approches multifactorielle, intégrative et macrophysiologique pour éviter de sur- et sous-estimer la sensibilité des espèces

Les résultats obtenus dans le cadre de ce projet doctoral mettent en évidence que l'utilisation complémentaire des approches multifactorielle, intégrative et macrophysiologique permet d'obtenir une meilleure estimation de la sensibilité des espèces aux changements globaux (Figure 18).

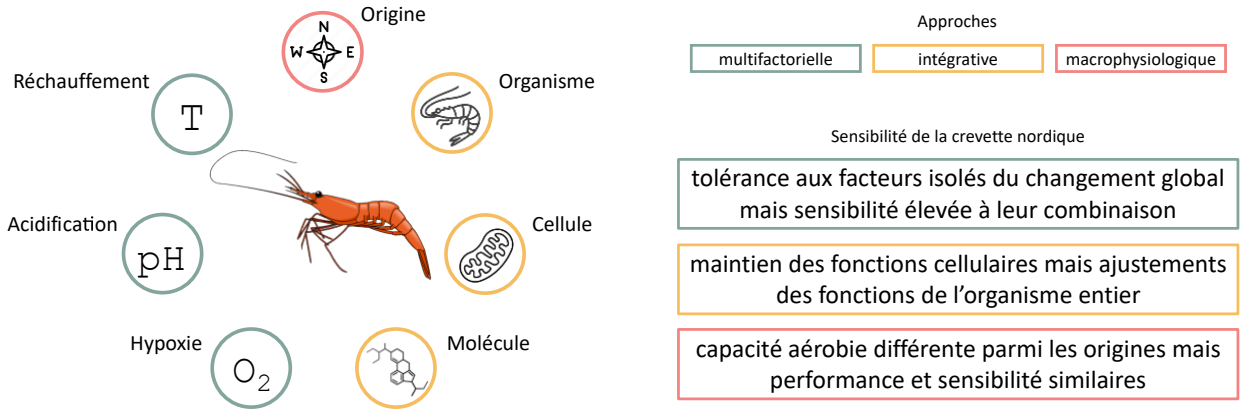


Figure 18 Représentation schématique des approches utilisées pour répondre à l'objectif général de cette thèse et des principaux résultats obtenus grâce à leur utilisation complémentaire.

L'utilisation de l'approche multifactorielle a montré que la direction et l'intensité des réponses des crevettes nordiques varient en fonction du facteur environnemental à l'étude (p.ex. la performance aérobie augmente avec l'augmentation des températures mais diminue avec la diminution du pH et de l'oxygène dissous (OD)) et que la sensibilité est facteur-dépendante : les crevettes sont plus sensibles aux augmentations de température qu'aux diminutions de pH et d'OD en isolation (Tableau 2). Ainsi, bien que les interactions ne soient généralement pas significatives, les résultats issus de ce projet de thèse montrent l'importance de considérer plusieurs facteurs environnementaux combinés pour définir la sensibilité des espèces dans la mesure où ces facteurs

peuvent co-exister en milieu naturel et varier simultanément dans le contexte des changements globaux. L'utilisation de cette approche a permis de montrer que la sensibilité de la crevette nordique à la combinaison des facteurs aurait été sous-estimée si la prédiction des effets combinés avait été faite à partir de l'interprétation des effets simples. En effet, alors que la survie des crevettes diminue d'environ 14,5 et 5,5 % par effet isolé de la température et du pH respectivement, elle diminue d'environ 23 % lorsque les crevettes sont exposées à la combinaison du réchauffement et acidification des océans (traitement 10A) et d'environ 58 % lorsqu'elles sont exposées au « trio mortel » (traitement 10AH). Les facteurs des changements globaux semblent s'influencer de manière synergique (c.-à-d. que leur effet combiné est supérieur à la somme des effets isolés), montrant ainsi que les études multifactorielles ont un meilleur pouvoir prédictif des réponses des espèces aux changements globaux, et par conséquent permettent une meilleure estimation de leur sensibilité aux conditions environnementales futures (Côté et al., 2016; Todgham & Stillman, 2013).

Tableau 2 Résumé simplifié des réponses biologiques et physiologiques des femelles de crevette nordique *Pandalus borealis* de plusieurs origines dans l'Atlantique nord-ouest (SLE : Estuaire du Saint-Laurent) face aux facteurs isolés de changement global : réchauffement (T), acidification des océans (pH) et hypoxie (O_2). Les flèches indiquent la direction de la réponse, le symbole – indique que la réponse est similaire entre toutes les conditions.

Origine	Facteur environnemental	Survie	Performance aérobie	Capacité cellulaire	Voies métaboliques	État énergétique cellulaire
Toutes	T	↓	↑	–	↑	–
Toutes	pH	↓	↓	–	–	–
SLE	O_2	–	↓	–	–	–

L'utilisation de l'approche intégrative a montré que la sensibilité des crevettes nordiques varie en fonction du niveau organisationnel à l'étude, les crevettes étant en général plus sensibles aux niveaux d'organisation supérieurs (Tableau 2). De plus, l'approche intégrative permet d'ouvrir une réflexion sur le possible lien entre l'ajustement phénotypique et morphologique des mitochondries, le stress oxydatif et la réponse immunitaire lorsque les crevettes sont exposées au « trio mortel » (Figure 19). En effet, la diminution du ratio citrate synthase – cytochrome C oxydase (CS:COX) en condition combinée suggère que chez les crevettes survivantes les mitochondries sont plus petites et ont une capacité oxydative plus élevée par rapport à leur capacité réductive. Ceci leur permettrait de maintenir la capacité et l'efficacité cellulaire élevées et ainsi faire face à une perte

de fonctionnalité du système de transport des électrons (ETS). En revanche cela pourrait augmenter la concentration des dérivés réactifs de l'oxygène (DRO) qui agissent comme molécules signaux du stress oxydatif (Schieber & Chandel, 2014). Un des principaux DRO, l'anion superoxyde, se forme pendant le transfert des électrons à travers l'ETS lorsque l'O_2 se lie à un électron libre (Murphy, 2009). Par conséquent, les potentiels stress oxydatif et réponse immunitaire résultants de l'exposition des crevettes, pendant 30 jours, au « trio mortel » pourraient être dus à l'accumulation de succinate qui fournit les électrons à la chaine respiratoire (Zhang et al., 2020). Ces résultats montrent que la complexité des réponses est très élevée et justifient l'utilisation de l'approche intégrative pour une estimation améliorée de la sensibilité des espèces à travers la compréhension des mécanismes sous-jacents des réponses des organismes entiers (Bartholomew, 1964; Harvey et al., 2014).

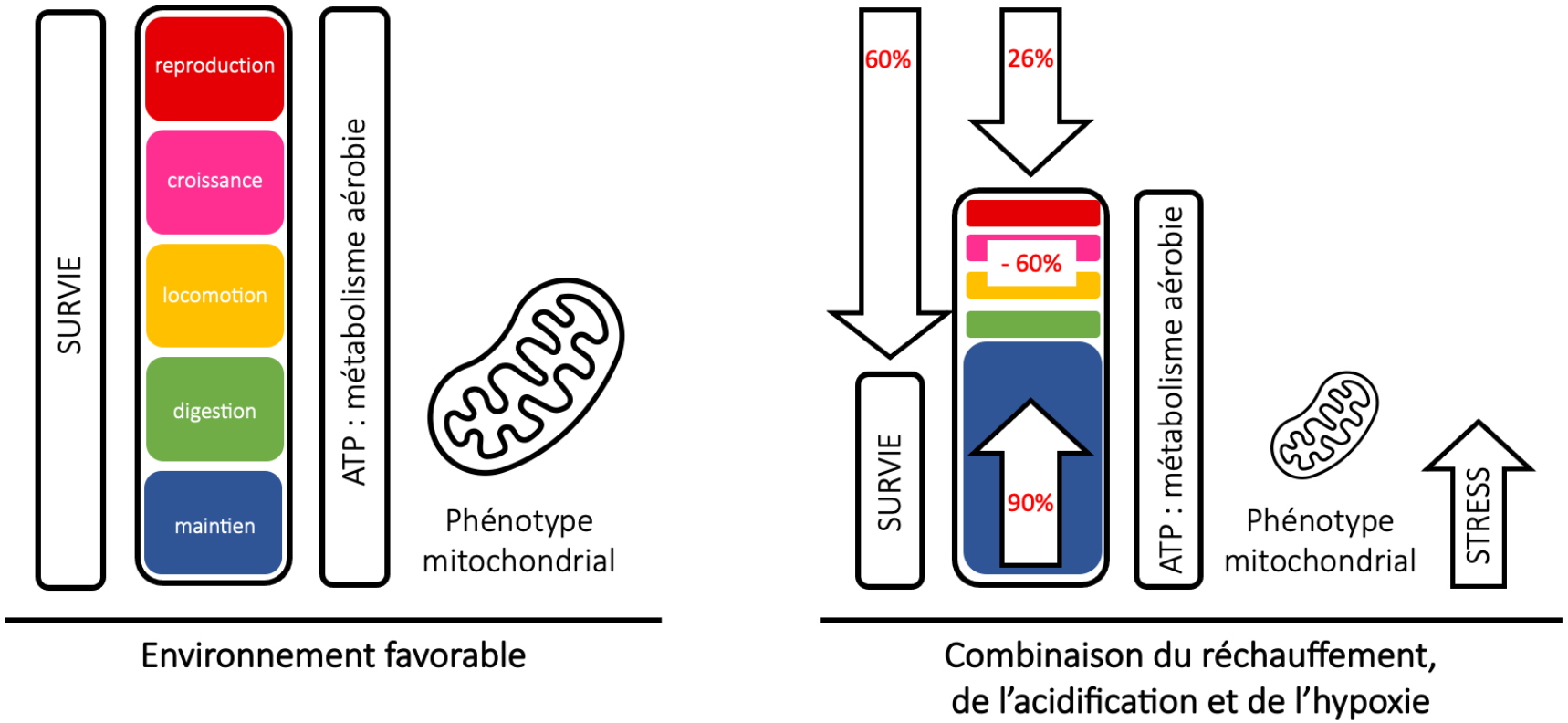


Figure 19 Représentation simplifiée de la réponse biologique et physiologique des femelles de crevette nordique *Pandalus borealis* de l'estuaire du Saint-Laurent en fonction de différentes conditions environnementales : favorable (traitement 2C) et combinée (traitement 10AH). Les flèches indiquent l'impact et la direction du changement induit par les effets des facteurs environnementaux sur la survie, la performance physiologique (différentes fonctions physiologiques représentées par les cases de couleurs différentes ; la taille des cases ne reflète pas l'allocation énergétique réelle, mais est ici simplifiée par souci de clarté), le métabolisme aérobie, le phénotype mitochondrial et le stress.

Finalement, l'utilisation de l'approche macrophysiologique a montré l'existence de différences physiologiques entre les crevettes de différentes origines, particulièrement en termes de capacités cellulaires et de profils métabolomiques. Ces différences semblaient être le fruit d'une acclimatation locale, tel que confirmé par l'absence d'effets génétiques sur l'expression des gènes en réponse au réchauffement (Leung et al., 2023). Cependant, cette dernière étude (issue de la collaboration avec ce projet de thèse) a aussi confirmé l'existence d'une structure de populations en identifiant des différences génétiques significatives entre les origines (Leung et al., 2023). Néanmoins, les différences physiologiques observées au niveau cellulaire entre les populations provenant des origines sélectionnées en début de ce travail de thèse se traduisent en une performance aérobie comparable parmi les populations et vers une réponse similaire de ces populations aux scenarios environnementaux testés : reprogrammation métabolomique, performance aérobie et taux de survie. Par conséquent, la sensibilité des crevettes nordiques aux facteurs des changements globaux est comparable parmi les origines, dans les limites des valeurs utilisées dans ce projet et de durée d'exposition. Néanmoins, l'utilisation de l'approche macrophysiologique en combinaison avec celle intégrative a permis de suggérer que les crevettes de l'estuaire du Saint-Laurent (SLE) seraient déjà proches de la limite de leur capacité aérobie en plus d'être plus sensibles au niveau métabolomique. Par rapport aux crevettes des autres origines, les crevettes de SLE seraient donc possiblement plus vulnérables aux facteurs biotiques et à la pêche en plus de devoir faire face aux changements environnementaux prédits pour la fin du siècle.

Tolérance aux facteurs des changements globaux isolés et sensibilité élevée au « trio mortel »

La combinaison des trois approches a permis de définir précisément la sensibilité de la crevette nordique aux futurs scénarios de changement global dans l'Atlantique nord-ouest grâce à l'intégration des réponses physiologiques, pour chaque niveau d'organisation biologique, des crevettes de quatre origines géographiques exposées, de façon isolée ou combinée, aux facteurs des changements globaux.

La crevette nordique, indépendamment de son origine géographique, se montre relativement tolérante au réchauffement, à l'acidification des océans et à l'hypoxie lorsque l'effet de ces phénomènes est étudié de façon isolée. En effet, les réponses physiologiques observées (performance aérobie, capacité et état énergétique cellulaire) sont comparables entre les traitements

ou demeurent dans les limites de tolérance de cette espèce. Néanmoins, indépendamment de son origine et du niveau organisationnel à l'étude, la crevette nordique est plus sensible aux augmentations de la température par rapport aux autres facteurs, dans les limites des valeurs utilisées dans ce projet et de durée d'exposition (Tableau 2). En effet, pour la plupart des origines, le registre aérobie (RA) des crevettes est comparable entre 6 et 10 °C et la survie diminue lorsque les crevettes sont exposées à la température la plus élevée, ce qui suggère qu'à 10 °C la crevette nordique se trouve proche de sa limite de tolérance thermique. Ces résultats montrent que la réponse de la crevette nordique à l'augmentation de la température ne suit pas une courbe en cloche telle que prédite par la théorie OCLTT (Pörtner & Knust, 2007). Comme chez d'autres espèces d'ectothermes marins, la crevette nordique maintient ou continue d'augmenter son RA jusqu'à une température critique à laquelle la survie des individus est compromise (Figure 20) (Clark et al., 2013; Lefevre, 2016; Schulte, 2015).

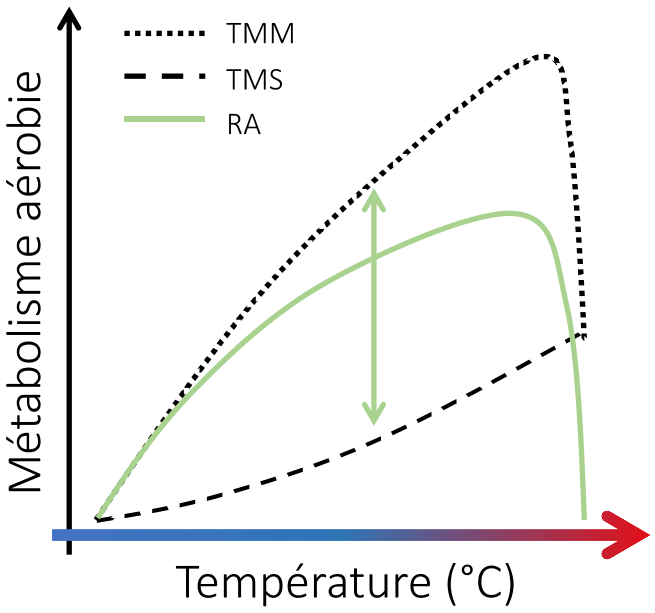


Figure 20 Représentation simplifiée de l'effet de l'augmentation de la température sur les taux métaboliques (Taux Métabolique Standard – TMS et Maximum – TMM) et par conséquent sur le Registre Aérobie (RA). Figure modifiée à partir de Lefevre (2016).

Malgré la relative tolérance de la crevette nordique au réchauffement, à l'acidification des océans et à l'hypoxie, sa sensibilité pourrait se définir extrême lorsque ces phénomènes co-existent (Figure 19). L'exposition au « trio mortel » réduit la survie des crevettes à environ 40 %. Chez les crevettes plus tolérantes, cette même exposition double les coûts de maintien et réduit d'environ 60 % la performance aérobie (Figure 19). Dans les mêmes conditions, la capacité et l'état énergétique cellulaire sont maintenus, potentiellement grâce à la modification phénotypique et morphologique des mitochondries. Ces ajustements permettent la production de l'énergie *via* le métabolisme aérobie mais semblent aussi être impliqués dans les potentielles réponses immunitaires et au stress (Figure 19). Ceci pourrait être couteux et avoir davantage une influence sur l'allocation de l'énergie.

Perspective : Pour compléter les résultats obtenus dans le cadre de ce projet doctoral, il serait pertinent de poursuivre les recherches en approfondissant les connaissances sur la production et l'allocation de l'énergie chez la crevette nordique et voir comment celles-ci varient dans les scénarios futurs. Ceci pourrait être fait en utilisant des modèles basés sur la théorie de la dynamique du budget énergétique (DEB) sans oublier de considérer les différences entre les origines. En effet, la taille des crevettes présente une tendance à la baisse au cours de la série chronologique 1990-2021 et varie entre les origines, les plus petites étant observées dans le chenal Esquiman (Bourdages et al., 2022). Ceci pourrait être le résultat d'un apport énergétique différent basé sur des régimes alimentaires différents en milieu naturel et/ou d'une différente répartition de l'énergie qui reflète l'effet des facteurs de changent global sur les fonctions physiologiques (p.ex. l'effet de la température sur la croissance, Daoud et al., 2010; Koeller et al., 2007). Seules des recherches futures pourront répondre à ces questions et améliorer davantage l'estimation de la sensibilité de la crevette nordique.

II. Vulnérabilité de la crevette nordique aux changements globaux cumulés

Comme mentionné précédemment, la sensibilité de la crevette nordique aux facteurs des changements globaux est comparable parmi les crevettes des différentes origines. Cependant, leur vulnérabilité pourrait différer en raison de possibles différences entre leurs potentiels adaptatifs. Tel que vu dans l'introduction de cette thèse, les organismes peuvent persister dans un nouvel environnement en ajustant leur physiologie à travers la plasticité phénotypique (acclimatation) ou via la sélection des génotypes associés à des phénotypes qui sont meilleurs pour faire face aux

changements (adaptation) (Ghalambor et al., 2007; Hoffmann & Sgró, 2011). Les résultats de ce projet doctoral ont montré que la crevette nordique possède, au niveau de l'organisme entier et indépendamment de l'origine des crevettes, la plasticité nécessaire pour « encaisser le coût » des effets négatifs des facteurs des changements globaux, mais seulement lorsque ces deniers sont isolés et dans les limites des valeurs utilisées dans ce projet et de durée d'exposition. Cette plasticité pourrait permettre l'acclimatation des crevettes aux conditions futures de réchauffement et d'acidification des océans en impliquant néanmoins des coûts liés à la réallocation de l'énergie disponible. L'acclimatation pourrait permettre de maintenir la survie des crevettes élevée malgré les effets négatifs du réchauffement et de l'acidification des océans et favoriser leur adaptation rapide si l'espèce possède un niveau élevé de variation génétique à travers sa distribution (Ghalambor et al., 2007), tout en tenant compte que le cycle de vie de la crevette nordique est long. En effet, la variation génétique implique qu'une espèce possède une plus grande capacité à subir une adaptation supplémentaire via la sélection naturelle et est généralement associée à une plus grande variation phénotypique (Fisher, 1930; Hansen, 2006). Dans ce contexte, la mesure de la variation phénotypique d'un trait peut fournir un aperçu du potentiel adaptatif d'une espèce ou de plusieurs populations (Sunday et al., 2011). Elle pourrait ainsi être utilisée pour mieux estimer la vulnérabilité de la crevette nordique aux scénarios futurs et définir si la vulnérabilité diffère entre les crevettes de différentes origines.

La variation phénotypique peut être mesurée à travers le calcul du coefficient de variation (CV) qui montre l'ampleur de la dispersion des valeurs individuelles par rapport à la moyenne de la population ou de l'espèce (Abdi, 2010). Ceci correspond au ratio entre la déviation standard (σ) et la moyenne (μ) d'un trait particulier :

$$CV = \frac{\sigma}{\mu}$$

Un CV qui augmente ou reste stable entre des conditions favorables (traitement 2C dans le cas présent) et combinées (traitements 10A et 10AH dans le cas présent), pourrait indiquer un certaine capacité adaptative basée sur une quantité raisonnable de variation génétique. Si le CV diminue, on pourrait déduire qu'il reste peu de place pour l'adaptation. De manière similaire, si les CV diffèrent parmi les origines, une différence de capacité adaptative entre les populations de crevettes

pourrait être alors suggérée. Ainsi, les CV ont été calculés pour les traits physiologiques de l'organisme entier qui montrent de la plasticité phénotypique (TMS, TMM et RA) pour les trois scénarios d'intérêt adaptatif (traitements 2C, 10A et 10AH) et pour toutes les origines (Tableau 3).

Tableau 3 Valeurs des CV calculées pour le taux métabolique standard (TMS), le taux métabolique maximal (TMM) et le registre aérobie (RA) des crevettes des quatre origines exposées aux conditions environnementales favorables et combinées. Traitements : 2C (2 °C, pH 7.75), 10A (10 °C, pH 7.40) et 10AH (10 °C, pH 7.40, 35 % sat. d'O_2). Origines : estuaire du Saint-Laurent (SLE), chenal Esquiman (EC), est du plateau Néo-Écossais (ESS) et nord-est de la côte de Terre-Neuve (NNC).

	TMS			TMM			RA		
Origines	2C	10A	10AH	2C	10A	10AH	2C	10A	10AH
SLE	0.17	0.21	0.17	0.20	0.16	0.21	0.25	0.26	0.46
ESS	0.11	0.21		0.20	0.15		0.27	0.29	
EC	0.11	0.11		0.21	0.13		0.28	0.19	
NNC	0.16	0.19		0.18	0.11		0.22	0.14	

Bien que l'interprétation des résultats présentés dans le Tableau 3 ne se base pas sur une analyse statistique (n = 1 pour chaque condition), aucune tendance ne semble exister. Les valeurs de CV calculées se ressemblent à travers les traitements et les origines pour les trois traits considérés. Basé sur cette métrique, le potentiel adaptatif de la crevette nordique ne semble pas être compromis par l'effet des facteurs des changements globaux et semble comparable entre les crevettes de différentes origines. Cette hypothèse est soutenue par des résultats récents qui suggèrent un potentiel adaptatif limité de la plasticité de la transcriptomique en réponse aux augmentations de la température, indépendamment des origines des crevettes (Leung et al., 2023). Cependant, l'analyse basée sur plusieurs individus, traits et origines, pourrait mener à des conclusions différentes. Ainsi, l'estimation du potentiel adaptatif de la crevette nordique requiert des études plus approfondies car ce potentiel dépend non seulement de la variation génétique mais aussi d'autres facteurs comme par exemple le stress environnemental (Charmantier & Garant, 2005).

Alors que la sensibilité et le potentiel adaptatif de la crevette nordique aux facteurs des changements globaux sont comparables parmi les crevettes des différentes origines, la vulnérabilité diffère en raison des conditions environnementales contrastées auxquelles les crevettes sont actuellement exposées dans leur milieu (Figure 21). En effet, les crevettes du chenal Esquiman (EC) sont actuellement sujettes à des conditions de température et pH qui correspondent aux conditions physico-chimiques prévues pour la fin du siècle pour les autres origines. Ainsi, la population du

EC serait d'ici 2100 exposée aux conditions environnementales similaires à celles utilisées dans le scénario combiné (traitement 10AH) (Figure 21). Les crevettes de cette région sont alors considérées comme étant plus vulnérables aux changements environnementaux prévus d'ici la fin du siècle, et seraient potentiellement à risque d'extinction commerciale. Les crevettes de SLE pourraient faire face au même risque d'extinction commerciale d'ici la fin du siècle : leur sensibilité moléculaire est la plus élevée de toutes les populations et elles semblent déjà fonctionner près de la limite de leurs capacités dans les conditions environnementales actuelles. Ceci pourrait être lié au fait qu'elles ont subi une sélection majeure puisque cette population possède la plus basse diversité génétique (Leung et al., 2023).

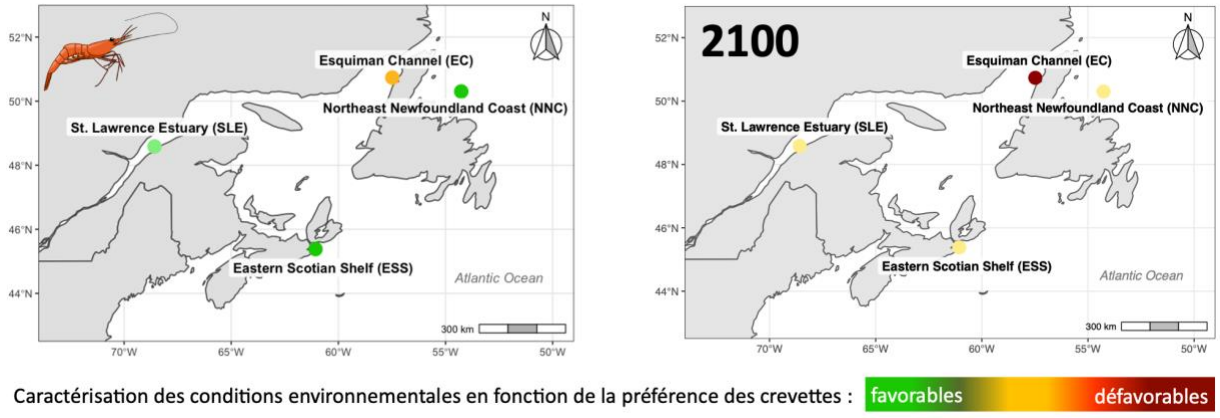


Figure 21 Cartes représentant les lieux de collecte (origines) des crevettes dans l'Atlantique nord-ouest et les conditions environnementales respectives actuelles (gauche) et prédites pour la fin du siècle (droite) en fonction de la préférence des crevettes.

Perspective : Cette étude s'est focalisée sur les réponses physiologiques des femelles adultes en raison de leur plus grande taille (qui les rend la cible principale de la pêche), et sensibilité en comparaison aux mâles. Cependant il est reconnu dans la littérature que les jeunes stades de vie sont généralement les plus sensibles aux effets des changements environnementaux. Ceci étant vrai aussi chez la crevette nordique (Arnberg et al., 2013; Brillon et al., 2005; Chabot & Ouellet, 2005), les recherches futures pourraient contribuer à l'estimation de la vulnérabilité de cette espèce en étudiant la résilience des jeunes stades de vie aux évènements extrêmes de réchauffement régional tel que les vagues de chaleur marines qui touchent principalement les eaux de surface dans lesquelles vivent les larves de crevette (IPCC, 2021).

III. Déclin prévu et conséquences pour la pêcherie de l'espèce

Les connaissances acquises au cours de ce projet sur la sensibilité et la vulnérabilité des populations de crevette nordique face aux changements globaux cumulés dans l'Atlantique nord-ouest ne laissent pas présager un futur propice pour l'exploitation commerciale de l'espèce dans l'estuaire et dans le golfe du Saint-Laurent (EGSL). En plus de sa vulnérabilité aux changements globaux, la crevette nordique doit se confronter à la récente, inattendue et massive augmentation du sébaste, *Sebastes* sp. (Cuvier, 1829) (Figure 22), qui par sa prédation accélère le déclin de la crevette nordique dans le système Saint-Laurent (MPO, 2022c; Parsons, 2005). Trois fortes cohortes (2011 – 2013) ont contribué à l'augmentation de la biomasse de ce prédateur dans le golfe du Saint-Laurent (GSL) estimée à 3.2 millions de tonnes en 2021 (Figure 22) (MPO, 2022c).

Sebastes sp.

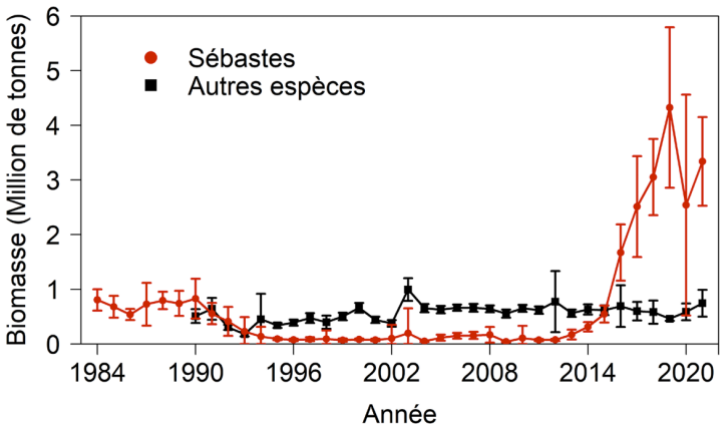


Figure 22 Photographie d'un sébaste (*Sebastes* sp.) et graphique de la variation de la biomasse minimale chalutable (millions de tonnes avec des intervalles de confiance à 95%) de sébastes (en rouge) et de toutes les autres espèces (en noir) capturés dans le relevé du MPO dans l'unité 1 (GSL) de 1984 à 2021. Figure tirée de MPO (2022c). Photographie de ©Claude Nozères.

La crevette nordique représente une partie importante du régime alimentaire du sébaste. En effet, le régime alimentaire des poissons de taille moyenne et grande (20 – 30 cm) est composé d'un minimum de 50 % de crevettes (toutes les espèces confondues) et la proportion de crevette nordique est restée constante dans le temps malgré la récente diminution de l'abondance de cette espèce (Brown-Vuillemin et al., 2022). Actuellement, les sébastes de la cohorte 2011 ont une taille modale de 24 cm, et les plus grands sébastes se distribuent principalement dans les chenaux profonds du GSL, incluant le chenal Esquiman (Bourdages et al., 2022). Ainsi, la diminution de l'abondance de la crevette nordique prédite pour la fin du siècle en raison des effets des changements globaux

sur la physiologie des crevettes pourrait être d'autant plus marquée pour les crevettes du EC, par rapport au reste du GSL et en général de l'Atlantique nord-ouest, à cause de la pression de la prédation par les sébastes (Brown-Vuillemin et al., 2022). Ceci pourrait influencer la distribution future et la dynamique de cette espèce pour laquelle on observe déjà une réduction de distribution à la limite sud de son aire de répartition à cause des effets des changements globaux (Barria et al., en révision; Richards & Hunter, 2021; Wilson et al., 2020).

Bien que la pérennité de l'espèce dans l'est du Canada ne semble pas en péril d'ici la fin du siècle, l'ensemble de ces réflexions soulève de fortes inquiétudes par rapport au futur de sa pêcherie dans l'EGLS. La pêche commerciale de la crevette nordique génère d'importants revenus financiers (MPO, 2015), ainsi l'annonce d'une réduction drastique des quotas ou d'un moratoire dans l'EGSL aurait des conséquences importantes pour les communautés côtières qui en dépendent et sur l'économie canadienne. Bien que la pêche de la crevette nordique ne soit pas en péril d'ici 2100 au large de la Nouvelle-Écosse, de Terre-Neuve et du Labrador, les effets des changements globaux sur ces populations ne sont pas à sous-estimer. Dans ce contexte, considérant que les qualités nutritionnelle et organoleptique de la crevette nordique ne sont pas affectées par les facteurs des changements globaux, il est tout à fait possible d'envisager une adaptation de la pêcherie afin de miser sur la qualité du produit plutôt que sur la quantité de crevettes pêchées pour continuer à générer des revenus (Chemel et al., 2020; Noisette et al., 2022).

Les résultats de cette thèse mettent en évidence la nécessité de prendre en considération les paramètres de sensibilité et de vulnérabilité des espèces et de les inclure dans les mesures de gestion qui se basent, actuellement et en général, seulement sur l'approche monospécifique (MPO, 2011). Ces résultats pourraient permettre aux gestionnaires de l'exploitation et de la conservation de la crevette nordique d'anticiper les changements de distribution et d'abondance dans le nord-ouest de l'Atlantique résultant des changements globaux dans le but d'implanter les diverses stratégies et mesures de gestion, migrant vers une approche écosystémique, afin d'assurer la pérennité de la pêcherie.

Perspective : Finalement, dans le but d'améliorer les stratégies et mesures de gestion, les interactions biologiques proie-prédateur devraient être prises en considération. Ainsi, les futures recherches pourraient se pencher sur l'étude de la relation crevette-sébaste en cherchant à

comprendre l'impact de l'activité de prédation sur la sensibilité et sur les bilans énergétiques de la crevette et comment les changements globaux influencent la relation entre les deux espèces (Allan et al., 2017; Domenici et al., 2019).

IV. Contributions et retombées

Les travaux de recherche menés dans le cadre de ce projet doctoral contribuent à l'estimation de la sensibilité et vulnérabilité de la crevette nordique, *P. borealis*, aux conditions environnementales futures à travers l'étude des effets des facteurs des changements globaux sur la physiologie de cette espèce. L'utilisation complémentaire des trois approches a permis d'estimer la sensibilité de la crevette nordique aux changements globaux et d'identifier les populations plus vulnérables aux scénarios prédits pour la fin du siècle. Ces travaux de recherche soulignent l'importance d'utiliser et intégrer ces approches, particulièrement pour les espèces d'intérêt commercial. Ainsi, les résultats issus de ce projet doctoral pourront être utiles pour adapter les stratégies de gestion actuelles au contexte des changements globaux rapides.

RÉFÉRENCES BIBLIOGRAPHIQUES

Abdi, H., 2010. Coefficient of Variation. Encycl. Res. Des. 1. https://doi.org/10.1007/978-3-642-04898-2_177

Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. Proc. R. Soc. B Biol. Sci. 267, 739–745. https://doi.org/10.1098/rspb.2000.1065

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O., 2013. Potential for evolutionary responses to climate change - evidence from tree populations. Glob. Chang. Biol. 19, 1645–1661. https://doi.org/10.1111/gcb.12181

Allan, B.J.M., Domenici, P., Watson, S.A., Munday, P.L., McCormick, M.I., 2017. Warming has a greater effect than elevated CO2 on predator–prey interactions in coral reef fish. Proc. R. Soc. B Biol. Sci. 284, 1–9. https://doi.org/10.1098/rspb.2017.0784

Allen, J.A., 1959. On the biology of *Pandalus borealis* krøyer, with reference to a population off the northumberland coast. J. Mar. Biol. Assoc. United Kingdom 38, 189–220. https://doi.org/10.1017/S002531540001568X

Alley, R.B., Marotzke, J., Nordhaus, W.D., Overpeck, J.T., Peteet, D.M., Pietke, R.A., Pierrehumbert, R.T., Rhines, P.B., Stocker, T.F., Tattey, L.D., Wallace, J.M., 2003. Abrupt climate change. Science. 299, 2005–2010. https://doi.org/10.1126/science.1081056

Allison, E.H., Perry, A.L., Badjeck, M.C., Neil Adger, W., Brown, K., Conway, D., Halls, A.S., Pilling, G.M., Reynolds, J.D., Andrew, N.L., Dulvy, N.K., 2009. Vulnerability of national economies to the impacts of climate change on fisheries. Fish Fish. 10, 173–196. https://doi.org/10.1111/j.1467-2979.2008.00310.x

AMAP, 2018. Arctic Monitoring and Assessment Programme: Biological responses to ocean acidification, Arctic Monitoring and Assessment Programme (AMAP).

Anderson, M., Gorley, R.N., Clarke, R.K., 2008. Permanova+ for primer: Guide to software and statisticl methods. Primer-E Limited.

Angilletta, M., 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University press, Oxford 289.

Apollonio, S., Stevenson, D.K., Dunton, E.E., 1986. Effects of temperature on the biology of the northern shrimp, *Pandalus borealis*, in the Gulf of Maine.

Arnberg, M., Calosi, P., Spicer, J.I., Tandberg, A.H.S., Nilsen, M., Westerlund, S., Bechmann, R.K., 2013. Elevated temperature elicits greater effects than decreased pH on the development, feeding and metabolism of northern shrimp (*Pandalus borealis*) larvae. Mar. Biol. 160, 2037–2048. https://doi.org/10.1007/s00227-012-2072-9

Aru, V., Engelsen, S.B., Savorani, F., Culurgioni, J., Sarais, G., Atzori, G., Cabiddu, S., Marincola, F.C., 2017. The effect of season on the metabolic profile of the European clam *Ruditapes decussatus* as studied by 1H-NMR spectroscopy. Metabolites 7, 1–14. https://doi.org/10.3390/metabo7030036

Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., Marshall, C., McGuire, J.L., Lindsey, E.L., Maguire, K.C., Mersey, B., Ferrer, E.A., 2011. Has the Earth's sixth mass extinction already arrived? Nature 471, 51–57. https://doi.org/10.1038/nature09678

Bartholomew, G.A., 1964. The roles of physiology and behavior in the maintenance of homeostasis in the desert environment. Symp. Soc. Exp. Biol 18, 7–29.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67(1), 1–48.

Battersby, B.J., Moyes, C.D., 1998. Influence of acclimation temperature on mitochondrial DNA, RNA, and enzymes in skeletal muscle. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 275, 905–912. https://doi.org/10.1152/ajpregu.1998.275.3.r905

Bechmann, R.K., Taban, I.C., Westerlund, S., Godal, B.F., Arnberg, M., Vingen, S., Ingvarsdottir, A., Baussant, T., 2011. Effects of ocean acidification on early life stages of shrimp (*Pandalus borealis*) and mussel (*Mytilus edulis*). J. Toxicol. Environ. Heal. - Part A 74, 424–438. https://doi.org/10.1080/15287394.2011.550460

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., Courchamp, F., 2012. Impacts of climate change on the future of biodiversity. Ecol. Lett. 15, 365–377. https://doi.org/10.1111/j.1461-0248.2011.01736.x

Bergström, B.I., 2000. The biology of *Pandalus*. Adv. Mar. Biol. 38, 55–245.

Berkeley, A.A., 1930. The post-embryonic development of the common pandalids of British Columbia. Contrib. to Can. Biol. Fish. 6, 79-163.

Bernier, B., Chabot, D., 2013. Évaluation de l'état du stock de flétan du Groenland (*Reinhardtius hippoglossoides*) du golfe du Saint-Laurent (4RST) en 2010 et description de son régime alimentaire. Secrétariat Can. Consult. Sci. du MPO, Doc. Rech. 2012/140 viii + 85.

Bijma, J., Pörtner, H.O., Yesson, C., Rogers, A.D., 2013. Climate change and the oceans - What does the future hold? Mar. Pollut. Bull. 74, 495–505. https://doi.org/10.1016/j.marpolbul.2013.07.022

Bindoff, N.L., Cheung, W.W., Kairo, J.G., Arístegui, J., Guinder, V.A., Hallberg, R., Hilmi, N.J.M., Jiao, N., Karim, M.S., Levin, L., O'Donoghue, S., Purca Cuicapusa, S.R., Rinkevich, B., Suga, T., Tagliabue, A., Williamson, P., 2019. Changing ocean, marine ecosystems, and dependent communities. IPCC Spec. Rep. Ocean Cryosph. a Chang. Clim. 477–587.

Blais, M., Galbraith, P.S., Plourde, S., Devine, L., Lehoux, C., 2021a. Chemical and biological

oceanographic conditions in the estuary and gulf of St. Lawrence during 2019. DFO Can. Sci. Advis. Sec. Res. Doc. 2021/002 iv + 66 p.

Blais, M., Galbraith, P.S., Plourde, S., Devred, E., Clay, S., Lehoux, C., Devine, L., 2021b. Chemical and biological oceanographic conditions in the Estuary and Gulf of St. Lawrence during 2020. DFO Can. Sci. Advis. Sec. Res. Doc. 2021/060, iv + 67 p.

Blais, M., Galbraith, P.S., Plourde, S., Scarratt, M., Devine, L., Lehoux, C., 2019. Chemical and biological oceanographic conditions in the estuary and gulf of St. Lawrence during 2017. DFO Can. Sci. Advis. Sec. Res. Doc. 2019/009 iv + 56 pp.

Bourdages, H., Roux, M.-J., Marquis, M.-C., Galbraith, P., Isabel, L., 2022. Assessment of northern shrimp stocks in the Estuary and Gulf of St. Lawrence in 2021: commercial fishery and research survey data. DFO Can. Sci. Advis. Sec. Res. Doc. 2022/027 xiv + 195 p.

Boyd, P.W., Collins, S., Dupont, S., Fabricius, K., Gattuso, J.P., Havenhand, J., Hutchins, D.A., Riebesell, U., Rintoul, M.S., Vichi, M., Biswas, H., Ciotti, A., Gao, K., Gehlen, M., Hurd, C.L., Kurihara, H., McGraw, C.M., Navarro, J.M., Nilsson, G.E., Passow, U., Pörtner, H.O., 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change—A review. Glob. Chang. Biol. 24, 2239–2261. https://doi.org/10.1111/gcb.14102

Bozinovic, F., Calosi, P., Spicer, J.I., 2011. Physiological correlates of geographic range in animals. Annu. Rev. Ecol. Evol. Syst. 42, 155–179. https://doi.org/10.1146/annurev-ecolsys-102710-145055

Breitburg, D., Levin, L.A., Oschlies, A., Grégoire, M., Chavez, F.P., Conley, D.J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G.S., Limburg, K.E., Montes, I., Naqvi, S.W.A., Pitcher, G.C., Rabalais, N.N., Roman, M.R., Rose, K.A., Seibel, B.A., Telszewski, M., Yasuhara, M., Zhang, J., 2018. Declining oxygen in the global ocean and coastal waters. Science. 359. https://doi.org/10.1126/science.aam7240

Brett, J.R., Groves, T.D.D., 1979. Physiological energetics. Fish Physiol. 8(6), 1-786.

Bretz, F., Hothorn, T., Westfall, P., 2016. Multiple comparisons using R., Chapman and Hall/CRC. https://doi.org/10.1201/9781420010909-f

Brillon, S., Lambert, Y., Dodson, J., 2005. Egg survival, embryonic development, and larval characteristics of northern shrimp (*Pandalus borealis*) females subject to different temperature and feeding conditions. Mar. Biol. 147, 895–911. https://doi.org/10.1007/s00227-005-1633-6

Britton, D., Schmid, M., Noisette, F., Havenhand, J.N., Paine, E.R., McGraw, C.M., Revill, A.T., Virtue, P., Nichols, P.D., Mundy, C.N., Hurd, C.L., 2020. Adjustments in fatty acid composition is a mechanism that can explain resilience to marine heatwaves and future ocean conditions in the habitat-forming seaweed *Phyllospora comosa* (Labillardière) C.Agardh. Glob. Chang. Biol. 26, 3512–3524. https://doi.org/10.1111/gcb.15052

Brown-Vuillemin, S., Chabot, D., Nozères, C., Sirois, P., Robert, D., 2022. Diet composition of redfish (*Sebastes* sp.) during periods of population collapse and massive resurgence in the Gulf of St. Lawrence. Front. Mar. Sci. 1–21. https://doi.org/10.3389/fmars.2022.963039

Bundy, J.G., Davey, M.P., Viant, M.R., 2009. Environmental metabolomics: A critical review and future perspectives. Metabolomics 5, 3–21. https://doi.org/10.1007/s11306-008-0152-0

Burnett, L.E., Stickle, W.B., 2011. Physiological responses to hypoxia, in: Coastal Hypoxia: Consequences for Living Resources and Ecosystems. pp. 101–114. https://doi.org/10.1029/ce058p0101

Businesswire, 2020. Global Seafood Market (2020 to 2027) - by Type, and Application - ResearchAndMarkets.com. Retrieved from Businesswire: https://www.businesswire.com/news/ home/20200908005793/en/Global-Seafood-Market-2020-to-2027---by-Type-and-Application--- ResearchAndMar.

Caldeira, K., Wickett, M.E., 2003. Anthropogenic carbon and ocean pH. Nature 425, 365.

Calosi, P., De Wit, P., Thor, P., Dupont, S., 2016. Will life find a way? Evolution of marine species under global change. Evol. Appl. 9, 1035–1042. https://doi.org/10.1111/eva.12418

Calosi, P., Melatunan, S., Turner, L.M., Artioli, Y., Davidson, R.L., Byrne, J.J., Viant, M.R., Widdicombe, S., Rundle, S.D., 2017. Regional adaptation defines sensitivity to future ocean acidification. Nat. Commun. 8(1), 1–10. https://doi.org/10.1038/NCOMMS13994

Calosi, P., Putnam, H.M., Twitchett, R.J., Vermandele, F., 2019. Marine metazoan modern mass extinction: Improving predictions by integrating fossil, modern, and physiological data. Ann. Rev. Mar. Sci. 11, 369–390. https://doi.org/10.1146/annurev-marine-010318-095106

Calosi, P., Turner, L.M., Hawkins, M., Bertolini, C., Nightingale, G., Truebano, M., Spicer, J.I., 2013. Multiple physiological responses to multiple environmental challenges: An individual approach. Integr. Comp. Biol. 53, 660–670. https://doi.org/10.1093/icb/ict041

Cappello, T., 2020. NMR-based metabolomics of aquatic organisms. EMagRes 9, 81–100. https://doi.org/10.1002/9780470034590.emrstm1604

Carter, C.G., Houlihan, D.F., 2001. Protein Synthesis. Fish Physiol. 31–75.

Ceballos, G., Ehrlich, P.R., Dirzo, R., 2017. Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proc. Natl. Acad. Sci. 114, E6089–E6096. https://doi.org/10.1073/pnas.1704949114

Chabot, D., Claireaux, G., 2008. Environmental hypoxia as a metabolic constraint on fish: The case of Atlantic cod, *Gadus morhua*. Mar. Pollut. Bull. 57, 287–294. https://doi.org/10.1016/j.marpolbul.2008.04.001

Chabot, D., McKenzie, D.J., Craig, J.F., 2016a. Metabolic rate in fishes: Definitions, methods and significance for conservation physiology. J. Fish Biol. 88, 1–9.

https://doi.org/10.1111/jfb.12873

Chabot, D., Ouellet, P., 2005. Rearing *Pandalus borealis* larvae in the laboratory : II. Routine oxygen consumption, maximum oxygen consumption and metabolic scope at three temperatures. Mar. Biol. 147, 881–894. https://doi.org/10.1007/s00227-005-1626-5

Chabot, D., Steffensen, J.F., Farrell, A.P., 2016b. The determination of standard metabolic rate in fishes. J. Fish Biol. 88, 81–121. https://doi.org/10.1111/jfb.12845

Chabot, D., Zhang, Y., Farrell, A.P., 2021. Valid oxygen uptake measurements: using high r^2 values with good intentions can bias upward the determination of standard metabolic rate. J. Fish Biol. https://doi.org/10.1111/jfb.14650

Charmantier, A., Garant, D., 2005. Environmental quality and evolutionary potential: Lessons from wild populations. Proc. R. Soc. B Biol. Sci. 272, 1415–1425. https://doi.org/10.1098/rspb.2005.3117

Chemel, M., Noisette, F., Chabot, D., Guscelli, E., Leclerc, L., Calosi, P., 2020. Good News — Bad News: Combined Ocean Change Drivers Decrease Survival but Have No Negative Impact on Nutritional Value and Organoleptic Quality of the Northern Shrimp. Front. Mar. Sci. 7, 1–13. https://doi.org/10.3389/fmars.2020.00611

Chen, Z., Anttila, K., Wu, J., Whitney, C.K., Hinch, S.G., Farrell, A.P., 2013. Optimum and maximum temperatures of sockeye salmon (*Oncorhynchus nerka*) populations hatched at different temperatures. Can. J. Zool. 91, 265–274. https://doi.org/10.1139/cjz-2012-0300

Cheung, W.W.L., Lam, V.W.Y., Sarmiento, J.L., Kearney, K., Watson, R., Pauly, D., 2009. Projecting global marine biodiversity impacts under climate change scenarios. Fish Fish. 10, 235–251. https://doi.org/10.1111/j.1467-2979.2008.00315.x

Cheung, W.W.L., Lam, V.W.Y., Sarmiento, J.L., Kearney, K., Watson, R., Zeller, D., Pauly, D., 2010. Large-scale redistribution of maximum fisheries catch potential in the global ocean under climate change. Glob. Chang. Biol. 16, 24–35. https://doi.org/10.1111/j.1365-2486.2009.01995.x

Childress, J.J., Seibel, B.A., 1998. Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. J. Exp. Biol. 201, 1223–1232. https://doi.org/10.1039/c7cc09389f

Choi, I., Son, H., Baek, J.H., 2021. Tricarboxylic acid (TCA) cycle intermediates: Regulators of immune responses. Life 11, 1–19. https://doi.org/10.3390/life11010069

Chown, S.L., Gaston, K.J., 2008. Macrophysiology for a changing world. Proc. R. Soc. B Biol. Sci. 275, 1469–1478. https://doi.org/10.1098/rspb.2008.0137

Chown, S.L., Gaston, K.J., Robinson, D., 2004. Macrophysiology: Large-scale patterns in physiological traits and their ecological implications. Funct. Ecol. 18, 159–167. https://doi.org/10.1111/j.0269-8463.2004.00825.x

Claireaux, G., Chabot, D., 2016. Responses by fishes to environmental hypoxia: Integration through Fry's concept of aerobic metabolic scope. J. Fish Biol. 88, 232–251. https://doi.org/10.1111/jfb.12833

Claireaux, G., Chabot, D., 2019. The significance of ocean deoxygenation for the physiology of marine organisms, in: Laffoley, D., and Baxter, J.M., (Eds.), Ocean Deoxygenation: Everyone's Problem. Causes, Impacts, Consequences and Solutions. IUCN, Gland, Switzerland. pp. 461–484. https://doi.org/10.2305/iucn.ch.2019.13.en

Claireaux, G., Lefrançois, C., 2007. Linking environmental variability and fish performance: Integration through the concept of scope for activity. Philos. Trans. R. Soc. B Biol. Sci. 362, 2031–2041. https://doi.org/10.1098/rstb.2007.2099

Clark, M.S., Sommer, U., Sihra, J.K., Thorne, M.A.S., Morley, S.A., King, M., Viant, M.R., Peck, L.S., 2017. Biodiversity in marine invertebrate responses to acute warming revealed by a comparative multi-omics approach. Glob. Chang. Biol. 23, 318–330. https://doi.org/10.1111/gcb.13357

Clark, T.D., Sandblom, E., Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. J. Exp. Biol. 216, 2771–2782. https://doi.org/10.1242/jeb.084251

Clarke, A., 2003. Costs and consequences of evolutionary temperature adaptation. Trends Ecol. Evol. 18, 573–581. https://doi.org/10.1016/j.tree.2003.08.007

Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance, 2007. https://doi.org/10.1126/science.1135471

Cooke, S.J., Sack, L., Franklin, C.E., Farrell, A.P., Beardall, J., Wikelski, M., Chown, S.L., 2013. What is conservation physiology? Perspectives on an increasingly integrated and essential science. Conserv. Physiol. 1, 1–23. https://doi.org/10.1093/conphys/cot001

Costa, C., Afonso, T., Cardoso, S., Maraschin, M., Rocha, M., 2017. Specmine: an R package for metabolomics and spectral data analysis and mining., BOOK OF ABSTRACTS OF.

Côté, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their importance in conservation. Proc. R. Soc. B Biol. Sci. 283. https://doi.org/10.1098/rspb.2015.2592

Cucco, A., Sinerchia, M., Lefrançois, C., Magni, P., Ghezzo, M., Umgiesser, G., Perilli, A., Domenici, P., 2012. A metabolic scope based model of fish response to environmental changes. Ecol. Modell. 237–238, 132–141. https://doi.org/10.1016/j.ecolmodel.2012.04.019

Cummins, D., Kennington, W.J., Rudin-Bitterli, T., Mitchell, N.J., 2019. A genome-wide search for local adaptation in a terrestrial-breeding frog reveals vulnerability to climate change. Glob. Chang. Biol. 25, 3151–3162. https://doi.org/10.1111/gcb.14703

Cyr, F., Snook, S., Bishop, C., Galbraith, P.S., Chen, N., Han, G., 2022. Physical Oceanographic

Conditions on the Newfoundland and Labrador Shelf during 2021. Can. Sci. Advis. Secr. Res. Doc. 2022/040.

Czyzyk-Krzeska, M.F., 1997. Molecular aspects of oxygen sensing in physiological adaptation to hypoxia. Respir. Physiol. 110, 99–111. https://doi.org/10.1016/S0034-5687(97)00076-5

Daoud, D., Chabot, D., Audet, C., Lambert, Y., 2007. Temperature induced variation in oxygen consumption of juvenile and adult stages of the northern shrimp, *Pandalus borealis*. J. Exp. Mar. Bio. Ecol. 347, 30–40. https://doi.org/10.1016/j.jembe.2007.02.013

Daoud, D., Lambert, Y., Audet, C., Chabot, D., 2010. Size and temperature-dependent variations in intermolt duration and size increment at molt of Northern Shrimp, *Pandalus borealis*. Mar. Biol. 157, 2655–2666. https://doi.org/10.1007/s00227-010-1526-1

Darnell, M.Z., Darnell, K.M., 2018. Geographic variation in thermal tolerance and morphology in a fiddler crab sister-species pair. Mar. Biol. 165, 1–12. https://doi.org/10.1007/s00227-017-3282-y

Davis, M.B., Shaw, R.G., 2001. Range shifts and adaptive responses to quaternary climate change. Science. 292, 673–679. https://doi.org/10.1126/science.292.5517.673

Dawe, E.G., Koen-Alonso, M., Chabot, D., Stansbury, D., Mullowney, D., 2012. Trophic interactions between key predatory fishes and crustaceans: Comparison of two Northwest Atlantic systems during a period of ecosystem change. Mar. Ecol. Prog. Ser. 469, 233–248. https://doi.org/10.3354/meps10136

De Wit, P., Palumbi, S.R., 2013. Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. Mol. Ecol. 22, 2884–2897. https://doi.org/10.1111/mec.12081

DFO, 2020. Assessment of Northern Shrimp stocks in the Estuary and Gulf of St. Lawrence in 2019. Can. Sci. Advis. Secr. Sci. Advis. Rep. 2020/010.

DFO, 2021a. 2020 Value of Atlantic & Pacific coast commerical landings, by province. Available online at: https://www.dfo-mpo.gc.ca/stats/commercial/land-debarq/sea-maritimes/s2020pv-eng.htm?

DFO, 2021b. Assessment of Northern Shrimp (*Pandalus borealis*) and Striped Shrimp (*Pandalus montagui*) in the Eastern and Western Assessment Zones. Can. Sci. Advis. Secr. Sci. Advis. Rep. 2021/014.

DFO, 2021c. An assessment of Northern shrimp (*Pandalus borealis*) in shrimp fishing areas 4 – 6 in 2019. Can. Sci. Advis. Secr. Sci. Advis. Rep. 2021/010.

DFO, 2021d. Oceanographic conditions in the Atlantic zone in 2020. Can. Sci. Advis. Secr. Sci. Advis. Rep. 2021/026.

DFO, 2022a. Assessment of Northern Shrimp stocks in the Estuary and Gulf of St. Lawrence in

2021. Can. Sci. Advis. Secr. Sci. Advis. Rep. 2022/006.

DFO, 2022b. Oceanographic conditions in the Atlantic zone in 2021. Can. Sci. Advis. Secr. Sci. Advis. Rep. 2022/025.

DFO, 2022c. 2021 Assessment of Northern Shrimp on the Eastern Scotian Shelf (SFAS 13–15). Can. Sci. Advis. Secr. Sci. Advis. Rep. 2022/033.

Di Santo, V., 2015. Ocean acidification exacerbates the impacts of global warming on embryonic little skate, *Leucoraja erinacea* (Mitchill). J. Exp. Mar. Bio. Ecol. 463, 72–78. https://doi.org/10.1016/j.jembe.2014.11.006

Diaz, R.J., 2001. Overview of hypoxia around the world. J. Environ. Qual. 30, 275. https://doi.org/10.2134/jeq2001.302275x

Diaz, R.J., Rosenberg, R., 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr. Mar. Biol. an Annu. Rev. Vol. 33 245–303.

Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science. 321, 926–929. https://doi.org/10.1126/science.1156401

Diaz, R.J., Rosenberg, R., Sturdivant, K., 2019. Chapter 2.5 Hypoxia in estuaries and semi-enclosed seas., in: Laffoley, D., and Baxter, J.M., (Eds.), Ocean Deoxygenation: Everyone's Problem. pp. 85–102.

Domenici, P., Allan, B.J.M., Lefrançois, C., McCormick, M.I., 2019. The effect of climate change on the escape kinematics and performance of fishes: Implications for future predator-prey interactions. Conserv. Physiol. 7, 1–22. https://doi.org/10.1093/conphys/coz078

Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: The other CO2 problem. Ann. Rev. Mar. Sci. 1, 169–192. https://doi.org/10.1146/annurev.marine.010908.163834

Dong, X., Yang, Z., Liu, Z., Wang, X., Yu, H., Peng, C., Hou, X., Lu, W., Xing, Q., Hu, J., Huang, X., Bao, Z., 2022. Metabonomic analysis provides new insights into the response of Zhikong scallop (*Chlamys farreri*) to heat stress by improving energy metabolism and antioxidant capacity. Antioxidants 11. https://doi.org/10.3390/antiox11061084

Dulvy, N.K., Ellis, J.R., Goodwin, N.B., Grant, A., Reynolds, J.D., Jennings, S., 2004. Methods of assessing extinction risk in marine fishes. Fish Fish. 5, 255–276. https://doi.org/10.1111/j.1467-2679.2004.00158.x

Dulvy, N.K., Sadovy, Y., Reynolds, J.D., 2003. Extinction vulnerability in marine populations. Fish Fish. 4, 25–64. https://doi.org/10.1046/j.1467-2979.2003.00105.x

Dupont-Prinet, A., Pillet, M., Chabot, D., Hansen, T., Tremblay, R., Audet, C., 2013. Northern shrimp (*Pandalus borealis*) oxygen consumption and metabolic enzyme activities are severely

constrained by hypoxia in the Estuary and Gulf of St. Lawrence. J. Exp. Mar. Bio. Ecol. 448, 298–307. https://doi.org/10.1016/j.jembe.2013.07.019

Dupont, S., Hall, E., Calosi, P., Lundve, B., 2014. First evidence of altered sensory quality in a shellfish exposed to decreased pH relevant to ocean acidification. J. Shellfish Res. 33, 857–861. https://doi.org/10.2983/035.033.0320

Ebner, J.N., 2021. Trends in the application of "omics" to ecotoxicology and stress ecology. Genes (Basel). 12(10), 1481. https://doi.org/10.1007/978-94-007-2072-5

Eisenhauer, N., Bonn, A., A. Guerra, C., 2019. Recognizing the quiet extinction of invertebrates. Nat. Commun. 10, 1–3. https://doi.org/10.1038/s41467-018-07916-1

Ellis, R.P., Spicer, J.I., Byrne, J.J., Sommer, U., Viant, M.R., White, D.A., Widdicombe, S., 2014. 1H NMR metabolomics reveals contrasting response by male and female mussels exposed to reduced seawater pH, increased temperature, and a pathogen. Environ. Sci. Technol. 48, 7044–7052. https://doi.org/10.1021/es501601w

Ern, R., Huong, D.T.T., Phuong, N.T., Madsen, P.T., Wang, T., Bayley, M., 2015. Some like it hot: Thermal tolerance and oxygen supply capacity in two eurythermal crustaceans. Sci. Rep. 5, 1–11. https://doi.org/10.1038/srep10743

Fabry, V.J., Seibel, B. a, Feely, R. a, Orr, J.C., 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J. Mar. Sci. 65, 414. https://doi.org/10.1093/icesjms/fsn048

FAO, 2018. La situation mondiale des pêches et de l'aquaculture 2018. Atteindre les objectifs de développement durable. Rome FAO.

FAO, 2020. The state of world fisheries and aquaculture 2020. https://doi.org/10.4060/ca9229en

Farhana, A., Lappin, S.L., 2021. Biochemistry, lactate dehydrogenase. StatPearls [Internet]. StatPearls Publ.

Feely, R.A., Doney, S.C., Cooley, S.R., 2009. Ocean acidification. Ocean Acidif. Strateg. Res. Monit. Plan 22, 1–7.

Fiehn, O., 2002. Metabolomics – the link between genotypes and phenotypes. Plant Mol. Biol. 48, 155–171.

Fisher, R., 1930. The genetical theory of natural selection. Oxford, UK: Clarendon Press.

Flynn, E.E., Bjelde, B.E., Miller, N.A., Todgham, A.E., 2015. Ocean acidification exerts negative effects during warming conditions in a developing Antarctic fish. Conserv. Physiol. 3, 1–16. https://doi.org/10.1093/conphys/cov033

Forsman, A., Wennersten, L., 2016. Inter-individual variation promotes ecological success of populations and species: evidence from experimental and comparative studies. Ecography

(Cop.). 39, 630–648. https://doi.org/10.1111/ecog.01357

Frederich, M., Pörtner, H.O., 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, R1531–R1538.

Fry, F.E.J., 1947. Effects of the environment on animal activity. Pub. Ontario Fish. Lab. No. 68. U. Toronto Stud. Biol. Ser. 55, 1-52.

Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish. Fish Physiol. 1-98.

Gäde, G., 1983. Energy metabolism of arthropods and mollusks during environmental and functional anaerobiosis. J. Exp. Zool. 228, 415–429. https://doi.org/10.1002/jez.1402280304

Gaitán-Espitia, J.D., Bacigalupe, L.D., Opitz, T., Lagos, N.A., Timmermann, T., Lardies, M.A., 2014. Geographic variation in thermal physiological performance of the intertidal crab *Petrolisthes violaceus* along a latitudinal gradient. J. Exp. Biol. 217, 4379–4386. https://doi.org/10.1242/jeb.108217

Galbraith, P.S., Chassé, J., Dumas, J., Shaw, J.-L., Caverhill, C., Lefaivre, D., Lafleur, C., 2022. Conditions océanographiques physiques dans le golfe du Saint-Laurent en 2021. Secr. can. des Avis sci. du MPO. Doc. rech. 2022/034 iv + 85 p.

Galbraith, P.S., Chassé, J., Shaw, J.-L., Dumas, J., Caverhill, C., Lefaivre, D., Lafleur, C., 2021. Physical Oceanographic Conditions in the Gulf of St. Lawrence during 2020. DFO Can. Sci. Advis. Sec. Res. Doc. 2021/045. iv + 81 p.

Garcia, H.E., Gordon, L.I., 1992. Oxygen solubility in seawater: Better fitting equations. Limnol. Oceanogr. 37, 1307–1312. https://doi.org/10.4319/lo.1992.37.6.1307

Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., Clusella-Trullas, S., Ghalambor, C.K., Konarzewski, M., Peck, L.S., Porter, W.P., Pörtner, H.O., Rezende, E.L., Schulte, P.M., Spicer, J.I., Stillman, J.H., Terblanche, J.S., van Kleunen, M., 2009. Macrophysiology: A Conceptual Reunification. Am. Nat. 174, 595–612. https://doi.org/10.1086/605982

Gaston, K.J., Spicer, J.I., 1998. Do upper thermal tolerances differ in geographically separated populations of the beachflea *Orchestia gammarellus* (Crustacea: Amphipoda)? J. Exp. Mar. Bio. Ecol. 229, 265–276. https://doi.org/10.1016/S0022-0981(98)00057-4

Gattuso, J.-P., Epitalon, J.-M., Lavigne, H., Orr, J., 2021. seacarb: seawater carbonate chemistry. R package version 3.3.0. http://CRAN.R-project.org/package=seacarb.

Gattuso, J.P., Hansson, L., 2011. Ocean acidification, 2011th ed. Oxford university press.

Gewin, V., 2010. Dead in the water. Nature 812–814. https://doi.org/10.1016/S0262-4079(17)31028-X

Ghalambor, C.K., McKay, J.K., Carroll, S.P., Reznick, D.N., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21, 394–407. https://doi.org/10.1111/j.1365-2435.2007.01283.x

Gilbert, D., Chabot, D., Archambault, P., Hébert, S., 2007. Appauvrissement en oxygène dans les eaux profondes du Saint-Laurent marin - Causes possibles et impacts écologiques. Nat. Can. 131, 67–75.

Gilbert, D., Sundby, B., Gobeil, C., Mucci, A., Tremblay, G.H., 2005. A seventy-two-year record of diminishing deep-water oxygen in the St. Lawrence estuary: The northwest Atlantic connection. Limnol. Oceanogr. 50, 1654–1666. https://doi.org/10.4319/lo.2005.50.5.1654

Gobler, C.J., Baumann, H., 2016. Hypoxia and acidification in ocean ecosystems: Coupled dynamics and effects on marine life. Biol. Lett. 12. https://doi.org/10.1098/rsbl.2015.0976

Götze, S., Bock, C., Eymann, C., Lannig, G., Steffen, J.B.M., Pörtner, H.O., 2020. Single and combined effects of the "Deadly trio" hypoxia, hypercapnia and warming on the cellular metabolism of the great scallop *Pecten maximus*. Comp. Biochem. Physiol. Part - B Biochem. Mol. Biol. 243–244. https://doi.org/10.1016/j.cbpb.2020.110438

Grieshaber, M.K., Hardewig, I., Kreutzer, U., Pörtner, H.O., 1993. Physiological and metabolic responses to hypoxia in invertebrates. Rev. Physiol. Biochem. Pharmacol. 125, 43–147.

Gruber, N., 2011. Warming up, turning sour, losing breath: Ocean biogeochemistry under global change. Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 369, 1980–1996. https://doi.org/10.1098/rsta.2011.0003

Gunderson, A.R., Armstrong, E.J., Stillman, J.H., 2016. Multiple stressors in a changing world: The need for an improved perspective on physiological responses to the dynamic marine environment. Ann. Rev. Mar. Sci. 8, 357–378. https://doi.org/10.1146/annurev-marine-122414-033953

Guppy, M., Withers, P., 1999. Metabolic depression in animals: Physiological perspectives and biochemical generalizations. Biol. Rev. 74, 1–40. https://doi.org/10.1111/j.1469-185X.1999.tb00180.x

Hall, E., 2017. The vulnerability of different populations of the commercially-important shrimp *Pandalus borealis* to environmental stress. (Doctoral dissertation, University of Plymouth).

Hammer, K.M., Pedersen, S.A., 2013. Deep-water prawn *Pandalus borealis* displays a relatively high pH regulatory capacity in response to CO_2-induced acidosis. Mar. Ecol. Prog. Ser. 492, 139–151. https://doi.org/10.3354/meps10476

Hammill, M.O., Stenson, G.B., 2000. Estimated prey consumption by harp seals (*Phoca groenlandica*), hooded seals (*Cystophora cristata*), grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) in Atlantic Canada. J. Northwest Atl. Fish. Sci. 26, 1–23. https://doi.org/10.2960/J.v26.a1

Han, G., Zhang, S., Dong, Y., 2017. Anaerobic metabolism and thermal tolerance: The importance of opine pathways on survival of a gastropod after cardiac dysfunction. Integr. Zool. 12, 361–370. https://doi.org/10.1111/1749-4877.12229

Hansen, T.E., 2006. The evolution of genetic architecture. Annu. Rev. Ecol. Evol. Syst. 37, 123–157. https://doi.org/10.1146/annurev.ecolsys.37.091305.110224

Hartig, F., 2020. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.3, 3.

Harvey, B.P., Al-Janabi, B., Broszeit, S., Cioffi, R., Kumar, A., Aranguren-Gassis, M., Bailey, A., Green, L., Gsottbauer, C.M., Hall, E.F., Lechler, M., Mancuso, F.P., Pereira, C.O., Ricevuto, E., Schram, J.B., Stapp, L.S., Stenberg, S., Santa Rosa, L.T., 2014. Evolution of marine organisms under climate change at different levels of biological organisation. Water 6, 3545–3574. https://doi.org/10.3390/w6113545

Herrera, M., Mancera, J.M., Costas, B., 2019. The use of dietary additives in fish stress mitigation: Comparative endocrine and physiological responses. Front. Endocrinol. (Lausanne). 10, 1–22. https://doi.org/10.3389/fendo.2019.00447

Hochachka, P.W., Somero, G.N., 2002. Biochemical adaptation: mechanism and process in physiological evolution., Oxford university press.

Hoffmann, A.A., Sgró, C.M., 2011. Climate change and evolutionary adaptation. Nature 470, 479–485. https://doi.org/10.1038/nature09670

Hofmann, G.E., Barry, J.P., Edmunds, P.J., Gates, R.D., Hutchins, D.A., Klinger, T., Sewell, M.A., 2010. The effect of Ocean acidification on calcifying organisms in marine ecosystems: An organism-to-ecosystem perspective. Annu. Rev. Ecol. Evol. Syst. 41, 127–147. https://doi.org/10.1146/annurev.ecolsys.110308.120227

Hollarsmith, J.A., Buschmann, A.H., Camus, C., Grosholz, E.D., 2020. Varying reproductive success under ocean warming and acidification across giant kelp (*Macrocystis pyrifera*) populations. J. Exp. Mar. Bio. Ecol. 522, 151247. https://doi.org/10.1016/j.jembe.2019.151247

Holthuis, L.B., 1980. FAO species catalogue. Volume 1-Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries (No. 125)., FAO Fisheries Synopsis.

Hosomi, R., Yoshida, M., Fukunaga, K., 2012. Seafood consumption and components for health. Glob. J. Health Sci. 4, 72–86. https://doi.org/10.5539/gjhs.v4n3p72

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical J. 50(3), 346-363.

Hothorn, T., Zeileis, A., Farebrother, R.W., Cummins, C., Millo, G., Mitchell, D., Zeileis, M.A., 2015. Package 'lmtest'. Testing linear regression models. https//cran. R-project. Org/web/packages/lmtest/lmtest. Pdf. Accessed, 6.

Huang, Z., Aweya, J.J., Zhu, C., Tran, N.T., Hong, Y., Li, S., Yao, D., Zhang, Y., 2020. Modulation of crustacean innate immune response by amino acids and their metabolites: Inferences from other species. Front. Immunol. 11, 1–15. https://doi.org/10.3389/fimmu.2020.574721

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M., Williams, S.E., 2012. Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. Philos. Trans. R. Soc. B Biol. Sci. 367, 1665–1679. https://doi.org/10.1098/rstb.2012.0005

Hunter, M., Atwood, R., Miller, A., Wilcox, S., Drew, K., Leaning Colson, D., 2021. Northern Shrimp Stock Assessment Update 2021. Atl. States Mar. Fish. Comm. Purs. to Natl. Ocean. Atmos. Adm. Award No. NA20NMF4740012 iv + 33 p.

Huo, D., Sun, L., Zhang, L., Ru, X., Liu, S., Yang, H., 2019. Metabolome responses of the sea cucumber *Apostichopus japonicus* to multiple environmental stresses: Heat and hypoxia. Mar. Pollut. Bull. 138, 407–420. https://doi.org/10.1016/j.marpolbul.2018.11.063

Husson, F., Le, S., Pages, J., 2010. Exploratory Multivariate Analysis by Example Using R, Chapman and Hall.

Hvingel, C., Sainte-Marie, B., Kruse, G.H., 2021. Cold-water shellfish as harvestable resources and important ecosystem players. ICES J. Mar. Sci. 78, 479–490. https://doi.org/10.1093/icesjms/fsab005

ICES, 2004. Report of the *Pandalus* Assessment working group, ICES CM 2005/ACFM: 05. 27 October—5 November 2004. Copenhagen, Denmark. 74 pp.

ICES, 2013. NAFO/ICES *Pandalus* Assessment Group Meeting, 12–19 September 2013, NAFO SCS Doc. 13/19 Serial No. N6235. ICES CM 2013/ACOM: 14. 70 pp.

IPCC, 2013. Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Cambridge Univ. Press. Cambridge, United Kingdom New York, NY, USA. https://doi.org/10.1260/095830507781076194

IPCC, 2014. Summary for Policy Makers, Climate Change 2014: Impacts, Adaptation and Vulnerability - Contributions of the Working Group II to the Fifth Assessment Report. https://doi.org/10.1016/j.renene.2009.11.012

IPCC, 2021. Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 3−32, doi:10.1017/9781009157896.001. https://doi.org/10.1017/CBO9781139177245.003

IPCC, 2022. The Ocean and Cryosphere in a Changing Climate, The Ocean and Cryosphere in a Changing Climate. https://doi.org/10.1017/9781009157964

IUCN, 2020. IUCN Red List 2017-2020 Report 2–40.

Izral, N.M., Brua, R.B., Culp, J.M., Yates, A.G., 2018. Developing metabolomics-based bioassessment: crayfish metabolome sensitivity to food and dissolved oxygen stress. Environ. Sci. Pollut. Res. 25, 36184–36193. https://doi.org/10.1007/s11356-018-3518-5

Jones, O.A.H., Maguire, M.L., Griffin, J.L., Dias, D.A., Spurgeon, D.J., Svendsen, C., 2013. Metabolomics and its use in ecology. Austral Ecol. 38, 713–720. https://doi.org/10.1111/aec.12019

Jónsdóttir, I.G., Björnsson, H., Skúladóttir, U., 2012. Predation by Atlantic cod *Gadus morhua* on northern shrimp *Pandalus borealis* in inshore and offshore areas of Iceland. Mar. Ecol. Prog. Ser. 469, 223–232. https://doi.org/10.3354/meps09977

Jorde, P.E., Søvik, G., Westgaard, J.I., Albretsen, J., André, C., Hvingel, C., Johansen, T., Sandvik, A.D., Kingsley, M., Jørstad, K.E., 2015. Genetically distinct populations of northern shrimp, *Pandalus borealis*, in the North Atlantic: Adaptation to different temperatures as an isolation factor. Mol. Ecol. 24, 1742–1757. https://doi.org/10.1111/mec.13158

Jutras, M., Dufour, C.O., Mucci, A., Cyr, F., Gilbert, D., 2020. Temporal changes in the causes of the observed oxygen decline in the St. Lawrence Estuary. J. Geophys. Res. Ocean. 125, 1–20. https://doi.org/10.1029/2020JC016577

Kassambara, A., 2021. Package 'rstatix'. Pipe-Friendly Framework for Basic Statistical Tests. https://CRAN.R-project.org/package=rstatix.

Kawecki, T.J., Ebert, D., 2004. Conceptual issues in local adaptation. Ecol. Lett. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Keeling, R.F., Körtzinger, A., Gruber, N., 2010. Ocean deoxygenation in a warming world. Ann. Rev. Mar. Sci. 2, 199–229. https://doi.org/10.1146/annurev.marine.010908.163855

Killen, S.S., Christensen, E.A.F., Cortese, D., Závorka, L., Norin, T., Cotgrove, L., Crespel, A., Munson, A., Nati, J.J.H., Papatheodoulou, M., McKenzie, D.J., 2021. Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. J. Exp. Biol. 224(18), jeb242522. https://doi.org/10.1242/jeb.242522

Koeller, P., Fuentes-Yaco, C., Platt, T., Sathyendranath, S., Richards, A., Ouellet, P., Orr, D., Skúladóttir, U., Wieland, K., Savard, L., Aschan, M., 2009. Basin-scale coherence in phenology of shrimps and phytoplankton in the North Atlantic Ocean. Science. 324, 791–793. https://doi.org/10.1126/science.1170987

Koeller, P.A., Fuentes-Yaco, C., Platt, T., 2007. Decreasing shrimp (*Pandalus borealis*) sizes off Newfoundland and Labrador - Environment or fishing? Fish. Oceanogr. 16, 105–115. https://doi.org/10.1111/j.1365-2419.2006.00403.x

Kolzenburg, R., 2022. The direct influence of climate change on marginal populations: a review. Aquat. Sci. 84, 1–20. https://doi.org/10.1007/s00027-022-00856-5

Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.P., 2013. Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. Glob. Chang. Biol. 19, 1884–1896. https://doi.org/10.1111/gcb.12179

Kroeker, K.J., Kordas, R.L., Crim, R.N., Singh, G.G., 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol. Lett. 13, 1419–1434. https://doi.org/10.1111/j.1461-0248.2010.01518.x

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest Package: Tests in Linear Mixed Effects Models . J. Stat. Softw. 82. https://doi.org/10.18637/jss.v082.i13

Lannig, G., Eckerle, L.G., Serendero, I., Sartoris, F.J., Fischer, T., Knust, R., Johansen, T., Pörtner, H.O., 2003. Temperature adaptation in eurythermal cod (*Gadus morhua*): A comparison of mitochondrial enzyme capacities in boreal and Arctic populations. Mar. Biol. 142, 589–599. https://doi.org/10.1007/s00227-002-0967-6

Lannig, G., Eilers, S., Pörtner, H.O., Sokolova, I.M., Bock, C., 2010. Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas* - Changes in metabolic pathways and thermal response. Mar. Drugs 8, 2318–2339. https://doi.org/10.3390/md8082318

Lardies, M.A., Arias, M.B., Poupin, M.J., Manríquez, P.H., Torres, R., Vargas, C.A., Navarro, J.M., Lagos, N.A., 2014. Differential response to ocean acidification in physiological traits of *Concholepas concholepas* populations. J. Sea Res. 90, 127–134. https://doi.org/10.1016/j.seares.2014.03.010

Lardon, I., Eyckmans, M., Vu, T.N., Laukens, K., De Boeck, G., Dommisse, R., 2013. 1H-NMR study of the metabolome of a moderately hypoxia-tolerant fish, the common carp (*Cyprinus carpio*). Metabolomics 9, 1216–1227. https://doi.org/10.1007/s11306-013-0540-y

Lavoie, D., Lambert, N., Rousseau, S., Dumas, J., Chassé, J., Long, Z., Perrie, W., Starr, M., Brickman, D., Azetsu-Scott, K., 2020. Projections of future physical and biogeochemical conditions in the Gulf of St. Lawrence, on the Scotian Shelf and in the Gulf of Maine. Can. Tech. Rep. Hydrogr. Ocean Sci. 334, xiii + 102 p.

Lefevre, S., 2016. Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO_2 and their interaction. Conserv. Physiol. 4, 1–31. https://doi.org/10.1093/conphys/cow009

Lefevre, S., Watson, S.A., Munday, P.L., Nilsson, G.E., 2015. Will jumping snails prevail? Influence of near-future CO_2, temperature and hypoxia on respiratory performance in the tropical conch *Gibberulus gibberulus gibbosus*. J. Exp. Biol. 218, 2991–3001. https://doi.org/10.1242/jeb.120717

Leung, C., Guscelli, E., Chabot, D., Bourret, A., Calosi, P., Parent, G.J., 2023. The lack of genetic variation underlying thermal transcriptomic plasticity suggests limited adaptability of the Northern shrimp, *Pandalus borealis*. Front. Ecol. Evol. 11, 166. https://doi.org/10.3389/fevo.2023.1125134

Leung, J.Y.S., Russell, B.D., Coleman, M.A., Kelaher, B.P., Connell, S.D., 2021. Long-term thermal acclimation drives adaptive physiological adjustments of a marine gastropod to reduce sensitivity to climate change. Sci. Total Environ. 771, 145208. https://doi.org/10.1016/j.scitotenv.2021.145208

Levin, L.A., Ekau, W., Gooday, A.J., Jorissen, F., Middelburg, J.J., Naqvi, S.W.A., Neira, C., Rabalais, N.N., Zhang, J., 2009. Effects of natural and human-induced hypoxia on coastal benthos. Biogeosciences 6, 2063–2098. https://doi.org/10.1029/2012JC008317

Li, S., Alfaro, A.C., Nguyen, T. V., Young, T., Lulijwa, R., 2020a. An integrated omics approach to investigate summer mortality of New Zealand Greenshell™ mussels. Metabolomics 16, 1–16. https://doi.org/10.1007/s11306-020-01722-x

Li, Y., Yin, W., Zhan, Y., Jia, Y., Cui, D., Zhang, W., Chang, Y., 2020b. Comparative metabolome analysis provides new insights into increased larval mortality under seawater acidification in the sea urchin *Strongylocentrotus intermedius*. Sci. Total Environ. 747, 141206. https://doi.org/10.1016/j.scitotenv.2020.141206

Liu, Z., Zhang, Y., Zhou, Z., Zong, Y., Zheng, Y., Liu, C., Kong, N., Gao, Q., Wang, L., Song, L., 2020. Metabolomic and transcriptomic profiling reveals the alteration of energy metabolism in oyster larvae during initial shell formation and under experimental ocean acidification. Sci. Rep. 10, 1–11. https://doi.org/10.1038/s41598-020-62963-3

Liu, Z.M., Wang, G.Z., Wu, L.S., Zeng, Z.S., Chen, X.L., 2013. Seasonal change in mitochondrial function and metabolic enzyme activity of different populations of the mud crab, *Scylla paramamosain*, from China. Aquaculture 376–379, 68–75. https://doi.org/10.1016/j.aquaculture.2012.11.007

Lu, J., Shi, Y., Wang, S., Chen, H., Cai, S., Feng, J., 2016. NMR-based metabolomic analysis of *Haliotis diversicolor* exposed to thermal and hypoxic stresses. Sci. Total Environ. 545–546, 280–288. https://doi.org/10.1016/j.scitotenv.2015.12.071

Lucassen, M., Schmidt, A., Eckerle, L.G., Pörtner, H.O., 2003. Mitochondrial proliferation in the permanent vs. temporary cold: Enzyme activities and mRNA levels in Antarctic and temperate zoarcid fish. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 285, 1410–1420. https://doi.org/10.1152/ajpregu.00111.2003

Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30. https://doi.org/10.1016/j.aquatox.2010.10.006

Marie, B., Genard, B., Rees, J.F., Zal, F., 2006. Effect of ambient oxygen concentration on activities of enzymatic antioxidant defences and aerobic metabolism in the hydrothermal vent worm, *Paralvinella grasslei*. Mar. Biol. 150, 273–284. https://doi.org/10.1007/s00227-006-0338-9

Martínez-Reyes, I., Chandel, N.S., 2020. Mitochondrial TCA cycle metabolites control physiology and disease. Nat. Commun. 11, 1–11. https://doi.org/10.1038/s41467-019-13668-3

Matear, R.J., Hirst, A.C., 2003. Long-term changes in dissolved oxygen concentrations in the ocean caused by protracted global warming. Global Biogeochem. Cycles 17. https://doi.org/10.1029/2002gb001997

Matoo, O.B., Lannig, G., Bock, C., Sokolova, I.M., 2021. Temperature but not ocean acidification affects energy metabolism and enzyme activities in the blue mussel, *Mytilus edulis*. Ecol. Evol. 11, 3366–3379. https://doi.org/10.1002/ece3.7289

Maxwell, S., Fuller, R., Brooks, T., Watson, J., 2016. The ravages of guns, nets and bulldozers. Nature 536, 143–145.

Mayor, D.J., Sommer, U., Cook, K.B., Viant, M.R., 2015. The metabolic response of marine copepods to environmental warming and ocean acidification in the absence of food. Sci. Rep. 5, 1–12. https://doi.org/10.1038/srep13690

McMahon, B.R., 2001. Respiratory and circulatory compensation to hypoxia in crustaceans. Respir. Physiol. 128, 349–364. https://doi.org/10.1016/S0034-5687(01)00311-5

Melzner, F., Gutowska, M. a., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., Bleich, M., Pörtner, H.-O., 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences Discuss. 6, 4693–4738. https://doi.org/10.5194/bgd-6-4693-2009

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M.A., Bange, H.W., Hansen, H.P., Körtzinger, A., 2013. Future ocean acidification will be amplified by hypoxia in coastal habitats. Mar. Biol. 160, 1875–1888. https://doi.org/10.1007/s00227-012-1954-1

Merilä, J., Söderman, F., O'Hara, R., Räsänen, K., Laurila, A., 2004. Local adaptation and genetics of acid-stress tolerance in the moor frog, *Rana arvalis*. Conserv. Genet. 5, 513–527. https://doi.org/10.1023/B:COGE.0000041026.71104.0a

Metallo, C.M., Vander Heiden, M.G., 2013. Understanding metabolic regulation and its influence on cell physiology. Mol. Cell 49, 388–398. https://doi.org/10.1016/j.molcel.2013.01.018

Metcalfe, N.B., Van Leeuwen, T.E., Killen, S.S., 2016. Does individual variation in metabolic phenotype predict fish behaviour and performance? J. Fish Biol. 88, 298–321. https://doi.org/10.1111/jfb.12699

Mills, E., O'Neill, L.A.J., 2014. Succinate: A metabolic signal in inflammation. Trends Cell Biol. 24, 313–320. https://doi.org/10.1016/j.tcb.2013.11.008

Mitchell, J.F.B., 1989. The "greenhouse" effect and climate change. Rev. Geophys. 27, 115–139. https://doi.org/10.1088/0034-4885/65/1/201

Moyes, C.D., Mathieu-Costello, O.A., Tsuchiya, N., Filburn, C., Hansford, R.G., 1997. Mitochondrial biogenesis during cellular differentiation. Am. J. Physiol. Physiol. 272: C1345–C1351.

Moyes, C.D., Schulte, P.M., 2008. Principles of Animal Physiology. Pearson Education Inc., London.

MPO, 2010. Valeur de la pêche commericales des cotes Atlantique et Pacifique, par province - 2010. Dispon. en ligne https//www.dfo-mpo.gc.ca/stats/commercial/land-debarq/sea-maritimes/s2010pq-eng.htm.

MPO, 2011. Points de référence conformes à l'approche de précaution pour la crevette nordique de l'estuaire et du golfe du Saint-Laurent. Secrétariat Can. Consult. Sci. du MPO. Avis Sci. 2011/062.

MPO, 2015. Valeur de la pêche commericales des cotes Atlantique et Pacifique, par province - 2015. Dispon. en ligne https//www.dfo-mpo.gc.ca/stats/commercial/land-debarq/sea-maritimes/s2015pv-eng.htm.

MPO, 2021a. Évaluation de la crevette nordique (*Pandalus borealis*) dans les zones de pêche de la crevette 4 à 6 en 2019. Secrétariat Can. Consult. Sci. du MPO. Avis Sci. 2021/010.

MPO, 2021b. Conditions océanographiques dans la zone Atlantique en 2020. Secrétariat Can. Consult. Sci. du MPO. Avis Sci. 2021/026.

MPO, 2022a. Évaluation de la crevette nordique de l'est du plateau néo-écossais en 2021 (ZPC 13 à 15). Secrétariat Can. Consult. Sci. du MPO. Avis Sci. 2022/033.

MPO, 2022b. Évaluation des stocks de crevette nordique de l'estuaire et du golfe du Saint-Laurent en 2021. Secrétariat Can. Consult. Sci. du MPO. Avis Sci. 2022/006.

MPO, 2022c. Évaluation des stocks de Sébastes (*Sebastes mentella* et *Sebastes fasciatus*) des Unités 1 et 2 en 2021. Secrétariat Can. Consult. Sci. du MPO. Avis Sci. 2022/039.

Mucci, A., Levasseur, M., Gratton, Y., Martias, C., Scarratt, M., Gilbert, D., Tremblay, J.É., Ferreyra, G., Lansard, B., 2018. Tidally induced variations of ph at the head of the laurentian channel. Can. J. Fish. Aquat. Sci. 75, 1128–1141. https://doi.org/10.1139/cjfas-2017-0007

Mucci, A., Starr, M., Gilbert, D., Sundby, B., 2011. Acidification of Lower St. Lawrence estuary bottom waters. Atmos. - Ocean 49, 206–218. https://doi.org/10.1080/07055900.2011.599265

Murphy, M.P., 2009. How mitochondria produce reactive oxygen species. Biochem. J. 417, 1–13. https://doi.org/10.1042/BJ20081386

Nations Unies, 1992. Convention cadre des Nations Unies sur les changements climatiques, Rapport FCCC/INFORMAL/84, GE.05- 62221 (F) 180705. Rome, Italie.

Neill, W.H., Miller, J.M., Van Der Veer, H.W., WINEMIllER, K.O., 1994. Ecophysiology of marine fish recruitment: a conceptual framework for understanding interannual variability. Netherlands J. Sea Res. 32, 135–152.

Noisette, F., Alberio, M., Calosi, P., Barria, A., Boissonneault, M., Grech, T., Guscelli, E.,

Soubirou, M., 2022. Vulnérabilité des populations de crevette nordique (*Pandalus borealis*) aux changements climatiques et globaux le long de la côte Est du Canada : de la ressource naturelle aux communautés côtières. Rapport final du projet # 550027, Ouranos, 83pp.

Noisette, F., Bordeyne, F., Davoult, D., Martin, S., 2016. Assessing the physiological responses of the gastropod *Crepidula fornicata* to predicted ocean acidification and warming. Limnol. Oceanogr. 61, 430–444. https://doi.org/10.1002/lno.10225

Noisette, F., Calosi, P., Madeira, D., Chemel, M., Menu-Courey, K., Piedalue, S., Gurney-Smith, H., Daoud, D., Azetsu-Scott, K., 2021. Tolerant larvae and sensitive juveniles: Integrating metabolomics and whole-organism responses to define life-stage specific sensitivity to ocean acidification in the American lobster. Metabolites 11. https://doi.org/10.3390/metabo11090584

Norin, T., Clark, T.D., 2016. Measurement and relevance of maximum metabolic rate in fishes. J. Fish Biol. 88, 122–151. https://doi.org/10.1111/jfb.12796

Orr, D.C., Sullivan, D.J., 2013. The february 2013 assessment of northern shrimp (*Pandalus borealis*) off Labrador and Northeastern Newfoundland. DFO Can. Sci. Advis. Secr. Res. Doc. 2013/055. 144.

Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F. et al., 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437(7059), 681–686.

Ouellet, P., Chabot, D., 2005. Rearing *Pandalus borealis* (Krøyer) larvae in the laboratory : I. Development and growth at three temperatures. Mar. Biol. 147, 869–880. https://doi.org/10.1007/s00227-005-1625-6

Ouellet, P., Chabot, D., Calosi, P., Orr, D., Galbraith, P.S., 2017. Regional variations in early life stages response to a temperature gradient in the northern shrimp *Pandalus borealis* and vulnerability of the populations to ocean warming. J. Exp. Mar. Bio. Ecol. 497, 50–60. https://doi.org/10.1016/j.jembe.2017.09.007

Ouellette-Plante, J., Chabot, D., Nozères, C., Bourdages, H., 2020. Diets of demersal fish from the CCGS Teleost ecosystemic surveys in the estuary and northern Gulf of St. Lawrence, August 2015-2017. Can. Tech. Rep. Fish. Aquat. Sci. v +121 p.

Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C., Thomas, M.B., 2013. Temperature variation makes ectotherms more sensitive to climate change. Glob. Chang. Biol. 19, 2373–2380. https://doi.org/10.1111/gcb.12240

Pacifici, M., Foden, W.B., Visconti, P., Watson, J.E.M., Butchart, S.H.M., Kovacs, K.M., Scheers, B.R., Hole, D.G., Martin, T.G., Akcakaya, H.R., Corlett, R.T., Huntley, B., Bickford, D., Carr, J.A., Homann, A.A., Midgley, G.F., Pearce-Kelly, P., Pearson, R.G., Williams, S.E., Willis, S.G., Young, B., Rondinini, C., 2015. Assessing species vulnerability to climate change. Nat. Clim. Chang. 5, 215-225.

Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. 37, 637–669. https://doi.org/10.2307/annurev.ecolsys.37.091305.30000024

Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421, 37.

Parsons, D.G., 2005. Predators of northern shrimp, *Pandalus borealis* (Pandalidae), throughout the North Atlantic. Mar. Biol. Res. 1, 48–58. https://doi.org/10.1080/17451000510018944

Parthasarathy, A., Cross, P.J., Dobson, R.C.J., Adams, L.E., Savka, M.A., Hudson, A.O., 2018. A Three-Ring circus: Metabolism of the three proteogenic aromatic amino acids and their role in the health of plants and animals. Front. Mol. Biosci. 5, 1–30. https://doi.org/10.3389/fmolb.2018.00029

Perry, a L., Low, P.J., Ellis, J.R., Reynolds, J.D., 2005. Climate change and distribution shifts in marine fishes. Science. 308, 1912–1915. https://doi.org/10.1126/science.1111322

Pespeni, M.H., Chan, F., Menge, B.A., Palumbi, S.R., 2013. Signs of adaptation to local pH conditions across an environmental mosaic in the california current ecosystem. Integr. Comp. Biol. 53, 857–870. https://doi.org/10.1093/icb/ict094

Petraitis, P.S., Dudgeon, S.R., 2020. Declines over the last two decades of five intertidal invertebrate species in the western North Atlantic. Commun. Biol. 3, 1–7. https://doi.org/10.1038/s42003-020-01326-0

Pillet, M., 2013. Détermination des contraintes métaboliques en fonction du niveau d'hypoxie chez des espèces commercialement exploitées dans le Saint-Laurent mécanismes biochimiques et génomiques. ProQuest Diss. Theses 194.

Pillet, M., Dupont-Prinet, A., Chabot, D., Tremblay, R., Audet, C., 2016. Effects of exposure to hypoxia on metabolic pathways in northern shrimp (*Pandalus borealis*) and Greenland halibut (*Reinhardtius hippoglossoides*). J. Exp. Mar. Bio. Ecol. 483, 88–96. https://doi.org/10.1016/j.jembe.2016.07.002

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2020. Nlme: Linear and Nonlinear Mixed Effects Models. R Packag. version 3.1-148, <URL https//CRAN.R-project.org/package=nlme>.

Pinsky, M.L., Eikeset, A.M., McCauley, D.J., Payne, J.L., Sunday, J.M., 2019. Greater vulnerability to warming of marine versus terrestrial ectotherms. Nature 569, 108–111. https://doi.org/10.1038/s41586-019-1132-4

Pinu, F.R., Beale, D.J., Paten, A.M., Kouremenos, K., Swarup, S., Schirra, H.J., Wishart, D., 2019. Systems biology and multi-omics integration: Viewpoints from the metabolomics research community. Metabolites 9, 1–31. https://doi.org/10.3390/metabo9040076

Pitcher, T.J., 2001. Fisheries managed to rebuild ecosystems? Reconstructing the past to salvage the future. Ecol. Appl. 11, 601–617. https://doi.org/10.1890/1051-

0761(2001)011[0601:FMTRER]2.0.CO;2

Pörtner, H.O., Knust, R., 2007. Constraints and trade-offs in climate dependent adaptation: Energy budgets and growth in a latitudinal cline. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 146, S205–S206. https://doi.org/10.1016/j.cbpa.2007.01.457

Pörtner, H.O., 2002. Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 132, 739–761. https://doi.org/10.1016/S1095-6433(02)00045-4

Pörtner, H.O., 2010. Oxygen- And capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213, 881–893. https://doi.org/10.1242/jeb.037523

Pörtner, H.O., Farrel, A.P., 2008. Physiology and climate change. Science (80-.). 322(5902), 690–692.

Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science. 315, 95–97.

Pörtner, H.O., Langenbuch, M., Reipschläger, A., 2004. Biological impact of elevated ocean CO_2 concentrations: Lessons from animal physiology and earth history. J. Oceanogr. 60, 705–718. https://doi.org/10.1007/s10872-004-5763-0

Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge university press.

R Core Team, 2020. R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL https//www.R-project.org/.

Rabalais, N.N., Díaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D., Zhang, J., 2010. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7, 585–619. https://doi.org/10.5194/bg-7-585-2010

Rabøl, R., Boushel, R., Dela, F., 2006. Mitochondrial oxidative function and type 2 diabetes. Appl. Physiol. Nutr. Metab. 31(6), 675–683.

Richards, R.A., Hunter, M., 2021. Northern shrimp *Pandalus borealis* population collapse linked to climate-driven shifts in predator distribution. PLoS One 16, 1–26. https://doi.org/10.1371/journal.pone.0253914

Rivest, E.B., Chen, C.S., Fan, T.Y., Li, H.H., Hofmann, G.E., 2017. Lipid consumption in coral larvae differs among sites: A consideration of environmental history in a global ocean change scenario. Proc. R. Soc. B Biol. Sci. 284(1853), 20162825. https://doi.org/10.1098/rspb.2016.2825

Roberts, C.M., Hawkins, J.P., 1999. Extinction risk in the sea. Trends Ecol. Evol. 14, 241–246.

https://doi.org/10.1016/S0169-5347(98)01584-5

Roche, D.G., Binning, S.A., Bosiger, Y., Johansen, J.L., Rummer, J.L., 2013. Finding the best estimates of metabolic rates in a coral reef fish. J. Exp. Biol. 216, 2103–2110. https://doi.org/10.1242/jeb.082925

Rodríguez, E., Dégletagne, C., Hagen, T.M., Abele, D., Blier, P.U., 2019. Mitochondrial traits previously associated with species maximum lifespan do not correlate with longevity across populations of the Bivalve *Arctica islandica*. Front. Physiol. 10, 1–9. https://doi.org/10.3389/fphys.2019.00946

Rosa, R., Seibel, B.A., 2008. Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proc. Natl. Acad. Sci. U. S. A. 105, 20776–20780. https://doi.org/10.1073/pnas.0806886105

Rudin-Bitterli, T.S., Evans, J.P., Mitchell, N.J., 2020. Geographic variation in adult and embryonic desiccation tolerance in a terrestrial-breeding frog. Evolution (N. Y). 74, 1186–1199. https://doi.org/10.1111/evo.13973

Ruppert, E.E., Fox, R.S., Barnes, R.D., 2004. Invertebrate zoology: a functional evolutionary approach, Brooks/Cole Publishing Company.

Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., Wanninkhof, R., Wong, C.S., Wallace, D.W.R., Tilbrook, B., Millero, F.J., Peng, T.H., Kozyr, A., Ono, T., Rios, A.F., 2004. The oceanic sink for anthropogenic CO_2. Science. 305, 367–371. https://doi.org/10.1126/science.1097403

Sampaio, E., Santos, C., Rosa, I.C., Ferreira, V., Pörtner, H.O., Duarte, C.M., Levin, L.A., Rosa, R., 2021. Impacts of hypoxic events surpass those of future ocean warming and acidification. Nat. Ecol. Evol. 5, 311–321. https://doi.org/10.1038/s41559-020-01370-3

Sanford, E., Kelly, M.W., 2011. Local adaptation in marine invertebrates. Ann. Rev. Mar. Sci. 3, 509–535. https://doi.org/10.1146/annurev-marine-120709-142756

Savenkoff, C., Savard, L., Morin, B., Chabot, D., 2006. Main prey and predators of northern shrimp (*Pandalus borealis*) in the northern Gulf of St. Lawrence during the mid-1980s, mid-1990s, and early 2000s. Can. Tech. Rep. Fish. Aquat. Sci. 2639 v+28 pp.

Schalkhausser, B., Bock, C., Pörtner, H.O., Lannig, G., 2014. Escape performance of temperate king scallop, *Pecten maximus* under ocean warming and acidification. Mar. Biol. 161, 2819–2829. https://doi.org/10.1007/s00227-014-2548-x

Schieber, M., Chandel, N.S., 2014. ROS function in redox signaling and oxidative stress. Curr. Biol. 24, R453–R462. https://doi.org/10.1016/j.cub.2014.03.034

Schmidlin, L., von Fumetti, S., Nagel, P., 2015. Temperature effects on the feeding and electron transport system (ETS) activity of *Gammarus fossarum*. Aquat. Ecol. 49, 71–80. https://doi.org/10.1007/s10452-015-9505-8

Schmidt-Nielsen, K., 1990. Animal Physiology: Adaptation and Environment., Cambridge University Press.

Scholes, B., 2010. Biodiversity Scenarios: Projections of 21st century change in biodiversity and associated ecosystem services., Secretariat of the Convention on Biological Diversity.

Schulte, P.M., 2015. The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. J. Exp. Biol. 218, 1856–1866. https://doi.org/10.1242/jeb.118851

Seibel, B.A., Walsh, P.J., 2003. Biological impacts of deep-sea carbon dioxide injection inferred from indices of physiological performance. J. Exp. Biol. 206, 641–650. https://doi.org/10.1242/jeb.00141

Shang, Y., Wang, X., Shi, Y., Huang, W., Sokolova, I., Chang, X., Chen, D., Wei, S., Khan, F.U., Hu, M., Wang, Y., 2022. Ocean acidification affects the bioenergetics of marine mussels as revealed by high-coverage quantitative metabolomics. Sci. Total Environ. 858, 160090. https://doi.org/10.1016/j.scitotenv.2022.160090

Shumway, S.E., Perkins, H.C., Schick, D.F., Stickney, A.P., 1985. Synopsis of biological data on the pink shrimp, *Pandalus borealis* Krøyer, 1838.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85. https://doi.org/10.1016/0003-2697(85)90442-7

Sodhi, N.S., Brook, B.W., Bradshaw, C.J., 2009. Causes and consequences of species extinctions. Princet. Guid. to Ecol. 1, 514-520.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research., 3rd ed. New York.

Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar. Environ. Res. 79, 1–15. https://doi.org/10.1016/j.marenvres.2012.04.003

Sokolova, I.M., Pörtner, H.O., 2001. Temperature effects on key metabolic enzymes in *Littorina saxatilis* and *L. Obtusata* from different latitudes and shore levels. Mar. Biol. 139, 113–126. https://doi.org/10.1007/s002270100557

Sork, V.L., 2018. Genomic studies of local adaptation in natural plant populations. J. Hered. 109, 3–15. https://doi.org/10.1093/jhered/esx091

Sorte, C.J.B., Jones, S.J., Miller, L.P., 2011. Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. J. Exp. Mar. Bio. Ecol. 400, 209–217. https://doi.org/10.1016/j.jembe.2011.02.009

Steffensen, J.F., 1989. Some errors in respirometry of aquatic breathers: How to avoid and correct for them. Fish Physiol. Biochem. 6, 49–59. https://doi.org/10.1007/BF02995809

Stickney, A., Perkins, H., 1977. Environmental physiology of commercial shrimp, *Pandalus borealis*. Proj. 3-202-R Complet. Report, Febr. 1, 1974 to January 31, 1977. Dept Mar Resour, W Boothbay Harb. Maine.

Sunday, J.M., Calosi, P., Dupont, S., Munday, P.L., Stillman, J.H., Reusch, T.B.H., 2014. Evolution in an acidifying ocean. Trends Ecol. Evol. 29, 117–125. https://doi.org/10.1016/j.tree.2013.11.001

Sunday, J.M., Crim, R.N., Harley, C.D.G., Hart, M.W., 2011. Quantifying rates of evolutionary adaptation in response to ocean acidification. PLoS One 6, 1–8. https://doi.org/10.1371/journal.pone.0022881

Svendsen, M.B.S., Bushnell, P.G., Steffensen, J.F., 2016. Design and setup of intermittent-flow respirometry system for aquatic organisms. J. Fish Biol. 88, 26–50. https://doi.org/10.1111/jfb.12797

Tai, T.C., Calosi, P., Gurney-Smith, H.J., Cheung, W.W.L., 2021. Modelling ocean acidification effects with life stage-specific responses alters spatiotemporal patterns of catch and revenues of American lobster, *Homarus americanus*. Sci. Rep. 11, 1–14. https://doi.org/10.1038/s41598-021-02253-8

Tantama, M., Martínez-François, J.R., Mongeon, R., Yellen, G., 2013. Imaging energy status in live cells with a fluorescent biosensor of the intracellular ATP-to-ADP ratio. Nat. Commun. 4, 2550. https://doi.org/10.1038/ncomms3550

Taylor, E.W., Whiteley, N.M., 1989. Oxygen transport and acid-base balance in the haemolymph of the lobster, *Homarus Gammarus*, during aerial exposure and resubmersion. J. Exp. Biol. 144, 417–436. https://doi.org/10.1242/jeb.144.1.417

Therneau, T.M., Lumley, T., 2015. Package 'survival'. R Top Doc, 128(10), 28-33. URL https//github.com/therneau/survival.

Thibeault, M., Blier, P.U., Guderley., H., 1997. Seasonal variation of muscle metabolic organization in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 16(2), 139–155.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., De Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., Van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., Williams, S.E., 2004. Letter to nature: Extinction risk from climate change. Nature 427, 145–148.

Thor, P., Bailey, A., Dupont, S., Calosi, P., Søreide, J.E., De Wit, P., Guscelli, E., Loubet-Sartrou, L., Deichmann, I.M., Candee, M.M., Svensen, C., King, A.L., Bellerby, R.G.J., 2018. Contrasting physiological responses to future ocean acidification among Arctic copepod populations. Glob. Chang. Biol. 24(1), e365–e377. https://doi.org/10.1111/gcb.13870

Thor, P., Vermandele, F., Bailey, A., Guscelli, E., Loubet-Sartrou, L., Dupont, S., Calosi, P., 2022. Ocean acidification causes fundamental changes in the cellular metabolism of the Arctic copepod *Calanus glacialis* as detected by metabolomic analysis. Sci. Rep. 12, 22223. https://doi.org/10.1038/s41598-022-26480-9

Todgham, A.E., Stillman, J.H., 2013. Physiological responses to shifts in multiple environmental stressors: Relevance in a changing world. Integr. Comp. Biol. 53, 539–544. https://doi.org/10.1093/icb/ict086

Underwood, A.J., 1997. Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge.

Vaquer-Sunyer, R., Duarte, C.M., 2008. Thresholds of hypoxia for marine biodiversity. Proc. Natl. Acad. Sci. U. S. A. 105, 15452–15457. https://doi.org/10.1073/pnas.0803833105

Vargas, C.A., Lagos, N.A., Lardies, M.A., Duarte, C., Manríquez, P.H., Aguilera, V.M., Broitman, B., Widdicombe, S., Dupont, S., 2017. Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. Nat. Ecol. Evol. 1(4), 1-7. https://doi.org/10.1038/s41559-017-0084

Venter, L., Loots, D.T., Mienie, L.J., Jansen van Rensburg, P.J., Mason, S., Vosloo, A., Lindeque, J.Z., 2018. Uncovering the metabolic response of abalone (*Haliotis midae*) to environmental hypoxia through metabolomics. Metabolomics 14, 1–12. https://doi.org/10.1007/s11306-018-1346-8

Venugopal, V., Gopakumar, K., 2017. Shellfish: Nutritive value, health benefits, and consumer safety. Compr. Rev. Food Sci. Food Saf. 16, 1219–1242. https://doi.org/10.1111/1541-4337.12312

Verberk, W.C.E.P., Leuven, R.S.E.W., van der Velde, G., Gabel, F., 2018. Thermal limits in native and alien freshwater peracarid Crustacea: The role of habitat use and oxygen limitation. Funct. Ecol. 32, 926–936. https://doi.org/10.1111/1365-2435.13050

Vohsen, S.A., Fisher, C.R., Baums, I.B., 2019. Metabolomic richness and fingerprints of deep-sea coral species and populations. Metabolomics 15, 1–13. https://doi.org/10.1007/s11306-019-1500-y

Wassmann, P., Duarte, C.M., Agustí, S., Sejr, M.K., 2011. Footprints of climate change in the Arctic marine ecosystem. Glob. Chang. Biol. 17, 1235–1249. https://doi.org/10.1111/j.1365-2486.2010.02311.x

Wei, L., Wang, Q., Ning, X., Mu, C., Wang, C., Cao, R., Wu, H., Cong, M., Li, F., Ji, C., Zhao, J., 2015. Combined metabolome and proteome analysis of the mantle tissue from Pacific oyster *Crassostrea gigas* exposed to elevated pCO_2. Comp. Biochem. Physiol. - Part D Genomics Proteomics 13, 16–23. https://doi.org/10.1016/j.cbd.2014.12.001

Wei, R., Wang, J., Su, M., Jia, E., Chen, S., Chen, T., Ni, Y., 2018. Missing value imputation approach for mass spectrometry-based metabolomics data. Sci. Rep. 8, 1–10.

https://doi.org/10.1038/s41598-017-19120-0

West-Eberhard, M.J., 1989. Phenotypic plasticity and the origin of diversity. Annu. Rev. Ecol. Syst. 20, 249–278.

Wheatly, M.G., Henry, R.P., 1992. Extracellular and intracellular acid-base regulation in crustaceans. J. Exp. Zool. 263, 127–142.

Whiteley, N.M., 2011. Physiological and ecological responses of crustaceans to ocean acidification. Mar. Ecol. Prog. Ser. 430, 257–271. https://doi.org/10.3354/meps09185

Whitmore, K., Richards, A., Carloni, J., Hunter, M., Hawk, M., Drew, K., 2013. Assessment report for Gulf of Maine Northern shrimp.

Wiegand, G., Remington, S.J., 1986. Citrate synthase: structure, control, and mechanism. Annu. Rev. Biophys. Biophys. Chem. 15(1), 97–117.

Wieland, K., 2004. Length at sex transition in northern shrimp (*Pandalus borealis*) off West Greenland in relation to changes in temperature and stock size. Fish. Res. 69, 49–56. https://doi.org/10.1016/j.fishres.2004.04.003

Wikelski, M., Cooke, S.J., 2006. Conservation physiology. Trends Ecol. Evol. 21, 38–46. https://doi.org/10.1016/j.tree.2005.10.018

Williams, T.D., Wu, H., Santos, E.M., Ball, J., Katsiadaki, I., Brown, M.M., Baker, P., Ortega, F., Falciani, F., Craft, J.A., Tyler, C.R., Chipman, J.K., Viant, M.R., 2009. Hepatic transcriptomic and metabolomic responses in the Stickleback (*Gasterosteus aculeatus*) exposed to ethinyl-estradiol. Environ. Sci. Technol. 43(16), 6341–6348.

Wilson, E.O., 1987. The little things that run the world (the importance and conservation of invertebrates). Conserv. Biol. 344-346.

Wilson, T.J.B., Cooley, S.R., Tai, T.C., Cheung, W.W.L., Tyedmers, P.H., 2020. Potential socioeconomic impacts from ocean acidification and climate change effects on Atlantic Canadian fisheries. PLoS One 15, 1–30. https://doi.org/10.1371/journal.pone.0226544

Wu, H., Liu, J., Lu, Z., Xu, L., Ji, C., Wang, Q., Zhao, J., 2017. Metabolite and gene expression responses in juvenile flounder *Paralichthys olivaceus* exposed to reduced salinities. Fish Shellfish Immunol. 63, 417–423. https://doi.org/10.1016/j.fsi.2017.02.042

Yeruham, E., Rilov, G., Shpigel, M., Abelson, A., 2015. Collapse of the echinoid *Paracentrotus lividus* populations in the Eastern Mediterranean - Result of climate change? Sci. Rep. 5, 1–6. https://doi.org/10.1038/srep13479

Yuan, H.X., Xiong, Y., Guan, K.L., 2013. Nutrient sensing, metabolism, and cell growth control. Mol. Cell 49, 379–387. https://doi.org/10.1016/j.molcel.2013.01.019

Zhang, Y., Gilbert, M.J.H., Farrell, A.P., 2019. Finding the peak of dynamic oxygen uptake during

fatiguing exercise in fish. J. Exp. Biol. 222. https://doi.org/10.1242/jeb.196568

Zhang, Y., Wu, H., Wei, L., Xie, Z., Guan, B., 2017. Effects of hypoxia in the gills of the Manila clam *Ruditapes philippinarum* using NMR-based metabolomics. Mar. Pollut. Bull. 114, 84–89. https://doi.org/10.1016/j.marpolbul.2016.08.066

Zhang, Y., Zhang, M., Zhu, W., Yu, J., Wang, Q., Zhang, J., Cui, Y., Pan, X., Gao, X., Sun, H., 2020. Succinate accumulation induces mitochondrial reactive oxygen species generation and promotes status epilepticus in the kainic acid rat model. Redox Biol. 28, 101365. https://doi.org/10.1016/j.redox.2019.101365