Université du Québec
à Rimouski

EFFETS COMBINÉS DES FACTEURS DES CHANGEMENTS GLOBAUX SUR LA RÉPONSE CELLULAIRE AUX STRESS ET SUR LA PLASTICITÉ DU PROTÉOME DE LA CREVETTE NORDIQUE (*PANDALUS BOREALIS*) DE L'ESTUAIRE DU SAINT-LAURENT

Mémoire présenté

dans le cadre du programme de maîtrise en biologie

en vue de l'obtention du grade de maître ès sciences

Par

© Maude Boissonneault

Décembre 2022

Composition du jury :

Souhir Marsit, présidente du jury, Université du Québec à Rimouski

Piero Calosi, directeur de recherche, Université du Québec à Rimouski

Diana Madeira, codirectrice de recherche, Université d'Aveiro

Réjean Tremblay, examinateur externe, Université du Québec à Rimouski, Institut des Sciences de la mer (ISMER)

Dépôt initial le 14 août 2022 Dépôt final le 22 décembre 2022

À ma mère, qui par sa génétique et son temps m’a transmis le sens de l’humour et la ténacité nécessaire pour me rendre au bout de mes ambitions.

REMERCIEMENTS

Le chemin parcouru durant mes années à l'UQAR a été parsemé d'une multitude de moments mémorables, parfois merveilleux, parfois éprouvants, mais surtout de rencontres formidables. D'abord, je tiens à remercier sincèrement mon directeur Piero Calosi, qui en plus de m'enseigner avec passion, a su m'épauler dans les instants de doute et m'offrir tant d'opportunités incroyables. Merci également à ma co-directrice Diana Madeira, qui m'a grandement aidé dans l'atteinte de mes objectifs et qui m'a même accueilli dans sa famille et fait découvrir les merveilles du Portugal.

Je tiens à remercier Réjean Tremblay et Souhir Marsit d'avoir accepté de faire partie du jury d'évaluation de ce mémoire. Vos commentaires me permettront sans aucun doute de me développer encore plus en tant que scientifique. Merci aussi à Pierre Blier, pour ses commentaires et son temps lors de la présentation de ma proposition de recherche.

Un merci tout spécial à l'équipe crevette : Ella Guscelli et Mathilde Chemel. Ce fut un honneur de pouvoir participer à vos côtés au développement de ce projet d'envergure. Sachez que je suis hautement reconnaissante pour tout le travail que vous avez fait et dont j'ai pu bénéficier. Vos compétences et votre talent m'ont inspiré à me dépasser. Un grand merci également à Joana Fernandes (Obrigada, Joana!), qui a été d'une aide inespérée dans la réalisation de ce projet, ainsi qu'une amie rassurante tout au long de mon passage à Aveiro.

Merci à Marina Soubirou et Fanny Noisette de m'avoir permis de contextualiser mon apport à la science dans le cadre de nombreux échanges avec les acteurs du domaine des pêcheries. Les apprentissages que j'y ai fait ont dépassé largement mes attentes.

Un énorme merci à Valentine Loiseau, pour qui je manque de mot afin de décrire l'impact que tu as eu sur moi et mon projet. Ton support indéfectible par ta présence et ton amitié m'a permis de passer à travers de nombreux défis. Je suis convaincue que tu iras loin.

Merci à Fanny Vermandele de m'avoir épaulé, et de m'avoir présenté un exemple de ténacité et de compétences hors de l'ordinaire. Merci également à tous mes autres collègues de laboratoire : André, Élodie, Tessa, Giuseppe, Nicolas, Frankie, Camille, Nathaniel ainsi que les autres qui ont traversé le A-011.

Merci à Aura Barria et Gloria Massamba N'Siala qui ont su m'offrir mon premier défi en laboratoire lors de l'expérimentation de l'été 2018. Vous m'avez certainement inspiré à poursuivre dans cette branche.

Je tiens à remercier Béatrice, qui me suit dans mes épopées depuis maintenant plus de 10 ans. Malgré la distance qui nous sépare, ton amitié m'a très certainement aidé à passer au travers de cette épreuve. Merci aussi à Daphnée, Catherine et Carlu. Cassiopée passera à la postérité dans cet ouvrage.

Un merci tout spécial aux Frisés. Laura et Raphaël, vous faites partie de ma famille. Marie-Ève, Antony, Aurélien, Gaëlle et Éric, je me compte extrêmement chanceuse d'avoir partagé autant de moments incroyables avec vous. Merci de m'avoir supporté et fait rire aux larmes toutes ces fois.

Merci à mes parents et à mon frère pour leur encouragement qui m'ont poussé à poursuivre mes ambitions. Merci de croire en moi et de m'avoir donné les outils pour me dépasser. Surtout, malgré les 550 km qui nous sépare, merci de rester aussi près de moi. Un merci tout spécial à Eva, pour qui j'essaie de mettre mon grain de sable à rendre l'avenir meilleur.

Puis finalement, merci à Maxence, qui en plus de croire en moi de manière indéfectible, rend ma vie plus belle jour après jour.

Ce projet n'aurait pu être réalisé sans le support et/ou le financement des organismes suivants : l'Université du Québec à Rimouski, le Ministère Pêche et Océans Canada, Mitacs, Ouranos, Québec Océan, le Centre des Sciences de la biodiversité du Québec et Réseau Québec Maritime.

RÉSUMÉ

Les changements globaux induisent des altérations significatives des propriétés physico-chimiques des écosystèmes, conduisant les organismes à présenter des réponses physiologiques diverses à leur environnement dynamique. Comme les organismes sont naturellement exposés à plusieurs facteurs environnementaux changeant simultanément, une approche multifactorielle est essentielle pour évaluer les réponses des organismes. Dans le cas de l'estuaire du Saint-Laurent (ELS), une exposition des organismes au réchauffement des océans (RO), à l'acidification des océans (AO) et à l'hypoxie est attendue. Or, ce projet vise à évaluer les effets combinés du RO, de l'AO et de l'hypoxie sur la réponse cellulaire au stress (RCS) de la crevette nordique *Pandalus borealis*. Les crevettes ont été exposées à différents niveau de température (2, 6 et 10 °C), pH (7.75 et 7.40) et saturation en oxygène (100 et 35 % sat.) de façon isolée et combinée pendant 30 jours. La RCS du muscle abdominal d'individus survivants a été mesurée afin d'obtenir une image détaillée des effets des facteurs environnementaux sur la capacité plastique du système de défense antioxydant, de chaperon protéique, de contrôle de qualité des protéines et du protéome global grâce à l'analyse de biomarqueurs (i.e. CAT, SOD, GST, LPO, TAC, Hsp70 et Ub) et à une approche protéomique. Il était attendu que les crevettes présentent un ajustement important de leur phénotype relatif à la RCS et à la plasticité protéomique. Également, peu de variations inter-individuelles dans les mécanismes du maintien de l'homéostasie étaient attendus, considérant la nature sténotherme de *P. borealis*. Il s'est avéré que la RCS était suffisante pour contrer les impacts du stress oxydant dans tous les scénarios, mais que la combinaison des facteurs a eu un impact négatif sur la réponse antioxydante. Un total de 592 protéines ont été identifiées, dont neuf démontraient des variations en abondance entre les traitements. Les processus liés à l'adhésion cellulaire, à la transcription et à la dynamique du cytosquelette ont été affectés par l'exposition aux facteurs. Ainsi, la réponse limitée de *P. borealis* pourrait être le résultat d'une absence de capacité plastique, ou encore d'une tolérance naturelle élevée obtenue à partir de phénotypes diversifiés dans la population de l'ESL.

Mots clés : Écophysiologie, Plasticité phénotypique, Réponses métaboliques, Multistresseurs environnementaux, Sténothermes, Mécanismes cellulaire, Réponse cellulaire aux stress, Protéomique, Pandalus borealis, Crevette nordique

ABSTRACT

Global change induces significant alterations of the physicochemical properties of ecosystems, leading organisms to exhibit diverse physiological responses to their often dynamic environment. As organisms are naturally exposed to several environmental factors changing simultaneously, the use of a multifactorial approach is essential to determine organisms' physiological responses. The St. Lawrence Estuary (SLE) is no exception, with ocean warming (OW), ocean acidification (OA) and hypoxia affecting organisms. Within this context, this project aims at assessing the combined effects of OW, OA and hypoxia on the cellular stress response (CSR) of the northern shrimp *Pandalus borealis*. We exposed shrimp to different levels of temperature (2, 6 and 10 °C), pH (7.75 and 7.40) and oxygen saturation (100 and 35 % sat.) in isolation and combined for 30 days. We measured the CSR of the abdominal muscle to obtain a detailed picture of the effects of the drivers on the plastic capacity of the antioxidant, the protein chaperones and the protein quality control system by analyzing biomarkers (i.e. CAT, SOD, GST, LPO, TAC, Hsp70 and Ub) coupled with a proteomic approach, in order to assess the plasticity of *P. borealis*' proteome in the face of global change. We expected that the tolerance of *P. borealis* would depend on CSR and chaperones mechanisms to counter synergistic effect of the drivers, with biomarkers being over-expressed in treatments representing future environmental conditions. Differentially abundant proteins (DAPs) related to processes such as energetic metabolism, immune system, cytoskeletal dynamic or apoptosis were expected in individuals exposed to all drivers, due to the stenotherm nature of *P. borealis*. The CSR was sufficient to counter oxidative stress impacts under all scenarios, but the combination of drivers negatively impacted it. We identified a total of 592 proteins, including nine DAPs. Processes related to cell adhesion, transcription and cytoskeletal dynamic were impacted by the exposure to global change drivers. The limited response observed from the identification of DAPs and the high inter-individual variation is either the result of an absence of plastic capacity or the result of a high natural tolerance obtained from diversified genotypes across the SLE population.

Keywords: Ecophysiology, Phenotypic plasticity, Metabolic responses, Environmental multistressors, Stenotherms, Cellular mechanisms, Cellular stress response, Proteomics, Pandalus borealis, Northern shrimp

TABLE DES MATIÈRES

LISTE DES TABLEAUX

LISTE DES FIGURES

LISTE DES ACRONYMES

AO	Acidification des océans
CAT	Catalase
CG	Changements globaux
CSR	*Cellular stress response*
CV	*Coefficient of variation*
DAPs	*Differentially abundant proteins*
DO	*Dissolved oxygen*
ESL	Estuaire du St-Laurent
FDR	*False discovery rate*
GIEC	Groupe d'experts intergouvernemental sur l'évolution du climat
GST	*Glutathione-S-Transferase*
IPCC	*Intergovernmental Panel on Climate Change*
Hsp70	*Heat Shock Proteins 70kDa*
LPO	*Lipid peroxidation*
OA	*Ocean acidification*
OW	*Ocean warming*
RCP	*Representative concentration pathway*
RO	Réchauffement des océans
ROS	*Reactive oxygen species*
SLE	*St. Lawrence Estuary*
SOD	*Superoxide dismutase*
TAC	*Total antioxidant capacity*
Ub	*Ubiquitins*

INTRODUCTION GÉNÉRALE

La biodiversité se mesure dans le temps et l'espace à l'échelle des écosystèmes, des communautés, des espèces ou encore des gènes, ainsi que par les interactions entre chacun de ces niveaux d'organisation. Ces diverses formes du vivant sont toutes en relation directe avec leur environnement, et se développent généralement sous un ensemble de conditions environnementales spécifiques. Ainsi, la capacité d'adaptation du vivant face aux paramètres dynamiques de son environnement est une caractéristique clé de l'évolution. Ce processus demande toutefois du temps, s'étalant généralement sur de nombreuses générations. Le temps est donc aujourd'hui la variable qui dirige les efforts de recherche dans de nombreuse études en écophysiologie. Effectivement, puisque les changements environnementaux actuels ne se déroulent plus sur des échelles de temps vastes, mais bien sur quelques décennies, l'acclimatation et la plasticité représentent des moyens privilégiés de défenses primaires à court terme. Certes, considérant que le processus d'adaptation demande plus de temps, celui-ci pourrait ne pas se produire avant que les espèces ne soit confrontée à des conditions défavorables. Celles-ci doivent donc modifier leur phénotype afin de perdurer sous des conditions environnementales changeantes, ainsi que des phénomènes climatiques extrêmes variants en récurrence et en intensité. C'est ainsi que l'on s'intéresse à savoir : les organismes seraient-ils capables de s'acclimater à des modifications majeures de leur environnement ?

Or, les organismes doivent dès maintenant faire face aux défis issus de l'exposition aux changements globaux (CG). La croissance démographique et l'augmentation des activités industrielles sont en majeur partie responsable des variations environnementales actuelles au sein des écosystèmes planétaires (IPCC, 2021). Plus spécifiquement, l'augmentation croissante de la concentration atmosphérique en dioxyde de carbone (CO_2) a incité le Groupe d'experts intergouvernemental sur l'évolution du climat (GIEC) à émettre des projections concernant son évolution, réunis sous le couvert de scénarios nommés *Representative Concentration Pathway* (RCP; IPCC, 2021). Chaque scénario représente l'issu en termes de changements environnementaux d'un volume d'émission de gaz à effet de serre. Dans le cadre d'étude écophysiologique, il est cependant pertinent de considérer le RCP le plus pessimiste, soit le RCP

8.5, afin d'assurer une certaine magnitude des réponses physiologiques des organismes et ainsi être en mesure d'observer les processus d'acclimatation de même que leurs issus. De plus, le travail de modélisation de Tjiputra et al. (2014) a permis de démontrer que d'ici 2100, plus de 40 % de la surface des océans aura un taux de croissance de pCO_2 plus rapide que celui de l'atmosphère, ce qui implique que de nombreux écosystèmes tel que l'Atlantique Nord feront face à une acidification océanique potentiellement plus importante qu'initialement estimée par le GIEC.

1 Impacts des changements globaux sur les océans

Bien qu'observable au sein de tous les écosystèmes, les océans semblent particulièrement touchés par les changements environnementaux d'origine anthropique. Effectivement, plusieurs écosystèmes marins sont affectés par un ou plusieurs facteurs, comme le réchauffement (RO) et l'acidification des océans (AO), l'hypoxie, la pollution, les changements de salinité, la perturbation de la lumière, la disponibilité des nutriments ou encore les polluants (Halpern et al., 2015). Toutefois, trois de ces facteurs semblent être déterminants dans une grande portion des écosystèmes marins tel que les zones côtières : le RO, l'AO et l'hypoxie. Selon le RCP 8.5, le GIEC projette pour 2100 une augmentation d'environ 4 °C de la température océanique ainsi qu'une AO à l'échelle globale d'en moyenne de 0.3 unité de pH (IPCC, 2021). L'étendue, l'intensité et la fréquence des événements hypoxiques sera également amené à s'accroître sous les CG dans les écosystèmes marins (IPCC, 2021). Ces modèles de progression des facteurs des CG sont cependant émis à l'échelle globale, et ne considère pas que les impacts des CG à l'échelle régionale pourraient s'avérer plus ou moins importants.

2 Aspect multifactoriel des changements globaux

Ainsi, la plupart des organismes marins sont déjà confronté à des modifications majeures de leur environnement. Or, considérant l'aspect multifactoriel des CG, il est fondamental d'estimer les réponses physiologiques des organismes à plusieurs facteurs en combinaison. En effet, la plupart des écosystèmes (97.7 %) sont affectés par plusieurs facteurs simultanément, ce qui complexifie grandement l'étude des réponses à différentes conditions environnementales chez les organismes marins en créant une interaction entre les facteurs (Côté et al., 2016; Halpern et al., 2015). En plus de l'additivité des réponses qui est souvent attendue dans les études impliquant deux facteurs ou plus, les facteurs de CG combinés peuvent générer l'émergence d'effets non linéaires non additifs,

tel que des synergismes et antagonismes (Figure 1), conduisant ainsi à des réponses auparavant non documentées à tous les niveaux d'organisation biologique (Côté et al., 2016; Crain et al., 2008; Folt et al., 1999; Piggott et al., 2015). Cependant, la nature des interactions entre plusieurs facteurs peut également varier selon les niveaux d'organisation écologique, de niveau trophique, l'appariement ainsi que l'intensité et la durée de chacun des facteurs (Carrier-Belleau et al., 2021; Crain et al., 2008; Piggott et al., 2015). En considérant l'accumulation de facteurs de stress anthropiques observables dans les écosystèmes (Halpern et al., 2019), les synergies et les antagonismes pourraient être encore plus fréquents que supposé (Carrier-Belleau et al., 2021; Crain et al., 2008; Piggott et al., 2015). Effectivement, il semble de moins en moins pertinent de distinguer leurs impacts physiologiques individuels au profit d'étudier leurs effets combinés, notamment au niveau cellulaire, bien que seules quelques études aient abordé les trois facteurs de façon globale et conjointe (Sampaio et al., 2021). La nature multiforme des changements globaux est donc un élément essentiel à prendre en compte dans les études écophysiologiques (Piggott et al., 2015), en plus que dans le domaine de la conservation et dans les mesures politiques fondées sur la science (Bednaršek et al., 2021; Boyd et al., 2018; Côté et al., 2016).

3 Contexte de l'estuaire du Saint-Laurent

Ces considérations s'étendent également aux estuaires marins, qui n'échappent pas à cette tendance, étant en plus caractérisés par des paramètres abiotiques naturellement dynamiques (Day et al., 2012). Dans ces conditions, les organismes doivent faire preuve de résilience physiologique en s'acclimatant ou en s'adaptant aux conditions environnementales en constante évolution, qu'elles soient d'origine naturelle ou anthropique (IPCC, 2021).

L'estuaire du Saint-Laurent (ESL) en est un bon exemple. Il se caractérise par des conditions particulières des eaux de fond, dues à la variabilité inter-décennale des proportions d'eau du courant du Labrador et d'eau centrale de l'Atlantique Nord (*Gulf Stream*) qui alimentent l'estuaire par le chenal Laurentien (Gilbert et al., 2005). Au cours des dernières décennies, les conditions environnementales ont été modulées par un afflux important d'eaux chaudes et pauvres en oxygène en provenance de l'Atlantique Nord (Gilbert, 2004), et on s'attend à ce qu'elles s'aggravent à l'avenir dans le contexte des CG (IPCC, 2021). Actuellement, la couche d'eau profonde de l'ESL est caractérisée par une température de 5-6 °C (DFO, 2020b), un pH de 7.6-7.75 (DFO, 2017) et une saturation en oxygène dissous (DO sat.) inférieure à 20 % (DFO, 2020c), cette dernière valeurs

étant près de la limite de viabilité pour la plupart des organismes vivants (Diaz & Rosenberg, 1995; Vaquer-Sunyer & Duarte, 2008). Considérant les projections du GIEC pour 2100 (RCP 8.5), la température des eaux de fond de l'ESL devrait atteindre 10 °C et un pH de 7.40, et présenter des conditions presque anoxiques, affectant physiologiquement la plupart des organismes.

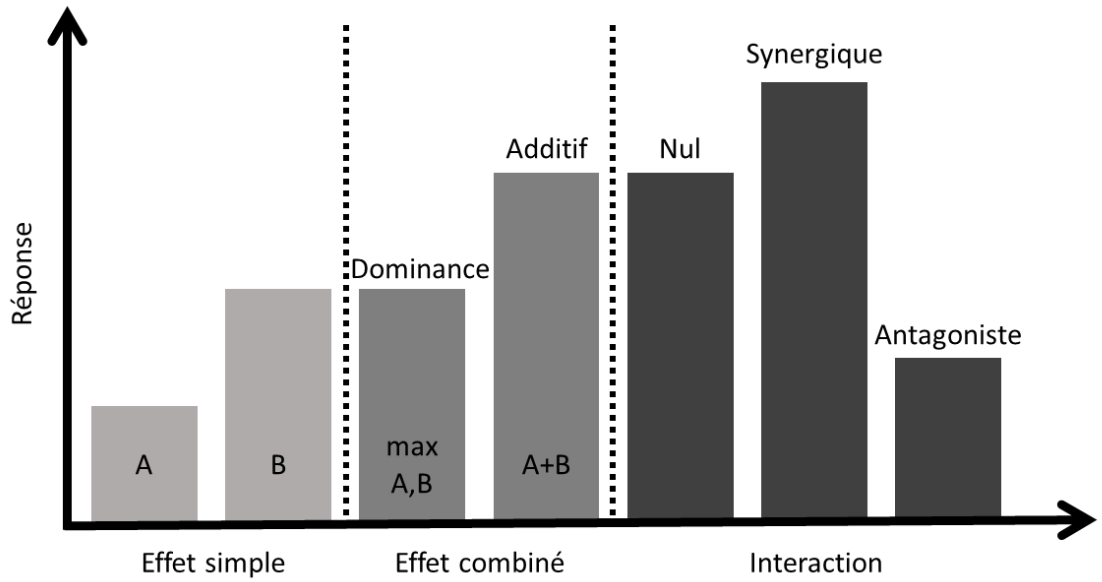

Figure 1. Modèle définissant les concepts de synergies et d'antagonisme écologiques entre des facteurs de stress multiples. Les stresseurs isolés A et B provoquent une réponse physiologique différente. Lorsque combinés, les stresseurs peuvent engendrer des effets linéaires (sans interaction) ou des effets d'interaction. Une dominance s'exerce lorsque la réponse physiologique à la combinaison de facteur est équivalente à la réponse du facteur provoquant la plus grande réponse. Un effet additif correspond à la somme de la réponse des facteurs initiaux. Les facteurs peuvent également interagir par synergie ou antagonisme. Le modèle nul, calculé selon un effet additif, permet de déterminer un seuil entre les types d'interaction. Celle-ci est donc synergique dans le cas d'une réponse plus importante que le modèle nul, alors qu'elle est antagoniste lorsque la réponse est inférieure au modèle nul. (Adapté de Côté et al., 2016 et de Piggott et al., 2015)

La plasticité sera alors primordiale pour les organismes afin d'assurer leur pérennité dans cet environnement changeant. Ainsi, l'ESL a été identifié comme un système d'étude idéal afin de s'interroger sur les impacts du réchauffement et de l'AO en plus de l'hypoxie sur les organismes, étant donné l'acclimatation (et possiblement l'adaptation) de ceux-ci à long terme aux conditions océanographiques existantes (Galbraith et al., 2021; Lavoie et al., 2020, 2021).

4 Impacts des changements globaux sur la physiologie des ectothermes marins

Plusieurs études ont démontré que le RO et l'AO, ainsi que l'hypoxie, ont des impacts physiologiques négatifs significatifs sur une vaste gamme d'organismes, mais plus spécifiquement sur les espèces d'ectothermes marins. D'abord, il est à noter que les ectothermes sont généralement sensibles aux changements de température, ne régulant pas métaboliquement leur température corporelle (Angilletta, 2009; Cossins & Bowler, 1987; Kinne, 1970; Newell, 1979; Peck et al., 2004). Dans ce contexte, l'ensemble des travaux produits à ce jour démontrent que l'élévation de la température affecte un large spectre de mécanismes et processus, tels que les processus enzymatiques accélérant les taux métaboliques ainsi que les taux de développement et croissance dans la plage de la courbe optimale (Figure 2; Schulte, 2015). D'autres processus et mécanismes biologiques ont également été observés chez certains ectothermes en réponse à l'exposition à une élévation de température. Par exemple, chez le saumon de l'Atlantique *Salmo salar* (Linnaeus, 1758), on a observé une régulation à la hausse des gènes de la réponse au choc thermique, du mécanisme d'apoptose et du système de défense immunitaire, et une régulation à la baisse des gènes du processus métabolique, de la protéolyse et du stress oxydatif (Beemelmanns et al., 2021). Chez la moule bleue *Mytilus edulis* (Linnaeus, 1758), Matoo et al. (2021) ont observé une modification du taux métabolique, des réserves énergétiques, des profils de métabolites et de l'activité des enzymes. D'autres processus de réponse au stress cellulaire tels que la réponse au choc thermique, l'ubiquitine, la réparation de l'ADN et les mécanismes d'apoptose ont été exprimés à la hausse chez le homard américain *Homarus americanus* (Milne-Edwards, 1837) à partir de larves exposées à des températures représentant les conditions de réchauffement océanique prévue pour 2100 selon le RCP 8.5 (IPCC, 2021; Lopez-Anido et al., 2021).

Cependant, certains organismes ectothermes sont également sténothermes, ce qui signifie qu'ils sont adaptés à un milieu sujet à de faibles variations de température (Prosser & Brown, 1973). De ce fait, les organismes ectothermes et sténothermes d'eau froide sont encore plus touchés par l'augmentation de la température, étant biochimiquement et physiologiquement adaptés à des conditions de basse température très stables (e.g. Peck et al., 2002; Pörtner et al., 2007).

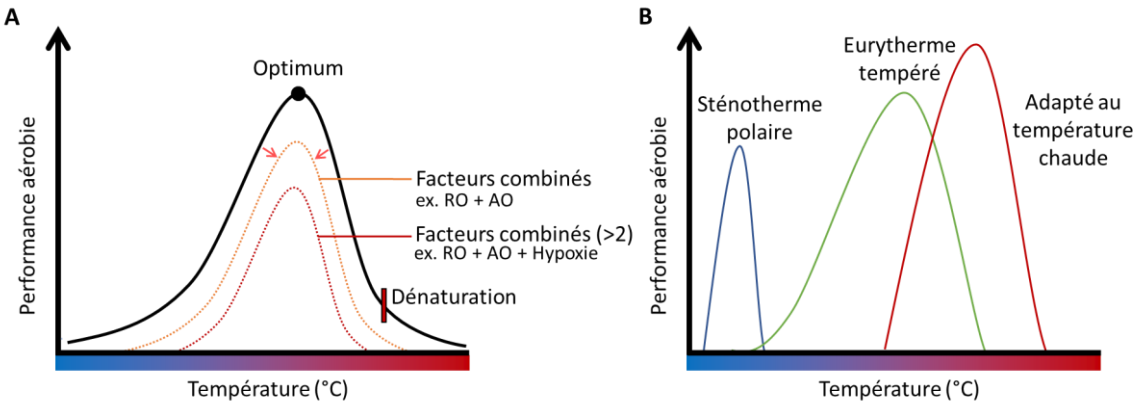

Figure 2. Schéma conceptuel de l'effet de la température et de l'accumulation de facteurs de changements globaux sur la performance aérobique des organismes aquatiques. **A)** La fenêtre de préférence thermique présente l'optimum de performance aérobique et la température de dénaturation (ex. des protéines), représentant le point limite de la tolérance physiologique. L'acclimatation aux conditions environnementales changeantes (ex. acclimatation saisonnière) permet l'ajustement de la fenêtre de performance aérobie selon les mécanismes de protection et la capacité plastique des organismes. L'accumulation de facteurs des changements globaux réduit la fenêtre de performance aérobie, selon l'étendue et l'intensité des facteurs, ainsi que leurs interactions. **B)** La fenêtre de performance aérobie des espèces varie généralement selon le dynamisme de leur environnement et leur tolérance physiologique. (Figure adaptée de Kroeker et al., 2017 et Pörtner & Farrell, 2008)

Des impacts physiologiques sont également signalés lorsque les organismes sont exposés aux conditions d'AO. En effet, les changements de pH/pCO_2 a un impact direct sur les processus physiologiques, tels que la sensibilité des enzymes (Dixon, 1953; Stillman & Paganini, 2015), l'équilibre acide-base (Small et al., 2010; Whiteley, 2011) et la vitesse des réactions biochimiques (Guppy & Withers, 1999), en perturbant le gradient de protons cellulaire, et donc l'homéostasie cellulaire (Stillman & Paganini, 2015). Des études ont déjà signalé les effets de l'AO sur les ectothermes marins, allant d'une augmentation du taux métabolique soutenue par une augmentation des mécanismes de production d'énergie (par exemple, la phosphorylation oxydative, le transport ionique), à une augmentation des mécanismes cellulaires liés à la formation des ribosomes, à l'adhésion cellulaire et au processus apoptotique, comme cela a été observé chez le copépode *Pseudocalanus acuspes* (Giesbrecht, 1881) (De Wit et al., 2016) et les larves de l'huître du Pacifique *Crassostrea gigas* (Thunberg, 1793) (Dineshram et al., 2021). Dans ces cas, une partie importante du budget énergétique semble être allouée au maintien du niveau de production d'énergie, ce qui peut limiter la capacité de défense en limitant les réserves énergétiques et les voies

de réponse immunitaire, ainsi que le développement et la croissance (De Wit et al., 2016; Dineshram et al., 2021; Freitas et al., 2016).

L'hypoxie affecte principalement le métabolisme cellulaire, entraînant un passage du métabolisme aérobie au métabolisme anaérobie, ce qui réduit sensiblement la production d'énergie (Michiels, 2004; J. G. Richards, 2009). Des études antérieures ont montré qu'une diminution de la saturation en oxygène dissous dans l'environnement a des effets physiologiques importants sur les ectothermes, comme chez le crabe *Neohelice granulate* (Dana, 1851) et la littorine *Littorina littorea* (Linnaeus, 1758), où l'on a observé une modulation de la réponse antioxydante par des cycles d'hypoxie et de ré-oxygénation (Geihs et al., 2016; Pannunzio & Storey, 1998). Dans les premiers stades de vie du saumon chinook *Oncorhynchus tshawytscha* (Walbaum, 1792), l'hypoxie induit une augmentation de la tolérance thermique et del'hypoxique aiguë, tout en diminuant la survie et la croissance (Del Rio et al., 2019). Ces études suggèrent que, bien que les organismes semblent répondre de manière appropriée à l'hypoxie, il semble qu'ils ne soient pas capables de dépenser le même niveau d'énergie dans d'autres fonctions telles que le développement ou la reproduction. Toutefois, à notre connaissance, l'interaction entre ces trois variables environnementales issues des CG n'a pas été prise en compte expérimentalement dans le passé.

5 Problématique et modèle d'étude

Ce raisonnement m'a mené à m'interroger sur les réponses physiologiques des organismes locaux. En effet, **la tolérance des sténothermes marins d'eaux froides face aux facteurs des changements globaux combinés dans le contexte de l'ESL** n'a toujours pas été exploré**.** Afin d'y répondre, un crustacé a été sélectionné en raison de l'importance socio-économique de ce groupe au sein des pêcheries et des communautés côtières (FAO, 2020; MPO, 2018). De plus, des études récentes suggèrent que les crustacés marins vivent dans une niche thermique étroite et proche de leur capacité thermique maximale, ce qui les rend d'autant plus sensibles aux modifications des conditions environnementales et ce dans une variété d'habitats (i.e. côtier, estuarien, intertidal) (Azra et al., 2018, 2020, 2022; Pinsky et al., 2019; Syafaat et al., 2021; Zhu et al., 2019). Cependant, leur sensibilité à l'AO semble moins importante que pour d'autres groupes (e.g. corail, mollusques, échinodermes) (Kroeker et al., 2013), ce qui pourrait complexifier leur réponse aux changements globaux.

À l’échelle mondiale, les crustacés ont une contribution économique, sociale et culturelle significative dans de nombreuses régions (FAO, 2020). Elles jouent un rôle clé dans la sécurité alimentaire, notamment dans les régions éloignées moins connectées aux centres de production (FAO, 2012), ainsi que pour la santé humaine, car ils fournissent des éléments alimentaires essentiels, tels que les acides gras essentiels oméga-3 à longue chaîne (Ackman, 2007), les protéines, la vitamine D et B, le sélénium, l'iode et le potassium (FAO, 2016). La pêche des crustacés est l’une des plus lucratives, considérant que mondialement ils représentent 9 % de la biomasse d’organismes sur le marché, mais compte pour 22 % de la valeur globale des pêcheries tous organismes confondus (FAO, 2020). L'augmentation constante de ce secteur socio-économique massif au fil des ans a généré d'importantes questions concernant sa durabilité (FAO, 2020).

Au Canada, cette pêche représente une ressource économique importante pour plusieurs communautés côtières autochtone et non autochtone, particulièrement le long des rives Nord et Sud de l'estuaire et du golfe du Saint-Laurent (MPO, 2018). Elle représente une pêche émergente dans l'Arctique canadien où les ressources alimentaires sont susceptibles de changer rapidement au cours des prochaines décennies considérant l’évolution des conditions environnementales et elle contribue également à la sécurité alimentaire de l'ensemble des communautés autochtones qui s’y trouve (Bennett et al., 2018; Kourantidou et al., 2021). Dans l’Est du Canada, les trois espèces commerciales les plus importantes (en termes de revenue) sont le crabe des neiges *Chionoecetes opilio* (Fabricius, 1788), le homard américain et la crevette nordique *Pandalus borealis* (Krøyer, 1838). Cependant, les stocks de crevette nordique connaissent déjà un certain niveau de déclin au niveau mondial (ASMFC, 2018; DFO, 2018; ICES, 2019). Il est donc pertinent d’accorder un intérêt particulier à cette espèce, pour qui les changements globaux pourraient engendrer de graves débalancements écologiques et socio-économique.

Ainsi, la crevette nordique *P. borealis*, issue de l’ESL, a été choisie comme espèce d'étude modèle. *Pandalus borealis* a une distribution circumboréale discontinue (Shumway et al., 1985) et est couramment observée dans la région de l'Atlantique Nord-Est (MAPAQ, 2015; Pillet, 2013). Dans l’ESL, la crevette nordique se retrouve généralement à une profondeur de 150 à 350 m où le taux d'oxygène est souvent compris entre 50 % et moins de 20 % sat. (MAPAQ, 2015; Pillet, 2013). Elle y est naturellement exposée à un pH de 7.75 (Mucci et al., 2011) et à une température comprise

entre 4 et 6 °C (Bourdages et al., 2017), alors que la plupart des populations atlantiques sont exposées à des températures avoisinant les 2 °C (e.g. Hardie et al., 2015; Orr & Sullivan, 2013). La crevette nordique de l'ESL est principalement prédatée par le sébaste (*Sebastes spp.*), qui semble jouer un rôle important dans le déclin de la biomasse des crevettes de cet écosystème (Brown-Vuillemin et al., 2022; DFO, 2020a, 2021).

Un changement important dans la structure et la dynamique de la population pourrait potentiellement réduire la diversité génétique et l'abondance (Pearson et al., 2009), compromettant ainsi le potentiel évolutif des crevettes nordiques. Dans ce cas, si l'espèce fait preuve d'un manque de variation phénotypique (élément essentiel de la sélection naturelle), et de plasticité physiologique (le système principal permettant aux organismes de faire face aux impacts négatifs des changements globaux préalablement à toute évolution), la viabilité des populations pourrait être encore plus menacée par les scénarios de CG (Seebacher et al., 2015). Cependant, les études réalisées à ce jour suggèrent que *P. borealis* semble être relativement tolérant au RO (Apollonio et al., 1986a; Shumway et al., 1985; Squires, 1990), à l'AO (Hammer & Pedersen, 2013) et à l'hypoxie (Dupont-Prinet et al., 2013) lorsqu'il est exposé individuellement à chaque facteur environnemental. La réponse de *P. borealis* aux CG est susceptible de varier selon le stade du cycle biologique (Arnberg et al., 2018; Daoud et al., 2007) ou le sexe (Dupont-Prinet et al., 2013), ainsi que selon la durée et l'intensité de l'exposition et la combinaison des facteurs. Cependant, les réponses physiologiques des crevettes exposées au RO, à l'AO et à l'hypoxie en combinaison au niveau cellulaire doivent encore être testées. Dans ce cas, si l'espèce démontre un manque de plasticité physiologique, la viabilité des populations pourrait être en danger dans le cadre de scénarios de changements globaux (Seebacher et al., 2015).

6 Techniques utilisées

Afin d'évaluer la tolérance de *P. borealis* face aux facteurs combinés des CG, je me suis intéressée à l'étude des variations phénotypiques cellulaires, en suivant l'énoncé de Bartholomew (1964), qui stipule que l'évaluation des mécanismes subjacents à une réponse physiologique à un niveau de complexité donné (e.g. l'organisme en entier) peut être retrouvé à des niveaux de complexité plus bas (e.g. au niveau cellulaire). Ainsi, afin d'expliquer les impacts sur le patron de survie sous le RO, l'AO et l'hypoxie individuels et combinés connus de la littérature (e.g. Arnberg et al., 2013, 2018; Chemel et al., 2020; Dupont-Prinet et al., 2013; Hammer & Pedersen, 2013), nous avons

investigué les réponses cellulaires de *P. borealis* en intégrant les techniques d'évaluation de la réponse cellulaire aux stress (RCS) par biomarqueurs du stress oxydant, des protéines chaperonnes et du contrôle de qualité protéiques, couplé à une approche protéomique. Nous définissons ici les biomarqueurs comme : " presque toute mesure reflétant une interaction entre un système biologique et un danger potentiel, qui peut être chimique, physique ou biologique et pour lequel la réponse mesurée peut être fonctionnelle et physiologique, biochimique au niveau cellulaire, ou une interaction moléculaire " (WHO & IPCS, 1993). Ces mesures ont été effectuées sur les individus tolérants (c.-à-d. les survivants à un mois d'exposition au conditions expérimentales) ayant survécu à l'expérience, ce qui représente environ 20 % des individus initiaux pour tous les traitements, hormis les traitements correspondant à la température et au pH attendue pour 2100 en condition normoxique (~25 %) et hypoxique (~50 %) (Chemel et al., 2020).

6.1 Biomarqueurs de la réponse cellulaire au stress

La production et l'accumulation d'espèces réactives d'oxygène « *Reactive oxygen species* » (ROS) est une composante importante de la réponse cellulaire des organismes marins exposés à des modifications de leur environnement (Lesser, 2006). Les principales ROS trouvées couramment dans la cellule sont le peroxyde d'hydrogène (H_2O_2) et les radicaux libres, tels que ($O_2^{\cdot-}$, $HO^{\cdot}$). Lorsque retrouvées en trop grande quantité, les ROS peuvent oxyder une variété de biomolécules nécessaires à l'intégrité cellulaire, tel que l'ADN ou l'ARN, les lipides membranaires, les acides aminées formant les protéines, etc. (Somero et al., 2017). Ce processus, également appelé stress oxydant, est cytotoxique pour la cellule. La capacité de détoxification de la cellule repose sur les enzymes antioxydantes, dont les fonctions consistent à catalyser la réaction d'une molécule cytotoxique en une molécule moins nocive. Ce processus vise à réduire la concentration en ROS, afin qu'ils retrouvent leur fonction initiale dans la signalisation et l'homéostasie cellulaire (Ighodaro & Akinloye, 2018). Pour ce faire, la cellule présente différentes lignes de défense d'enzymes antioxydantes. La première, est composée par des enzymes qui suppriment et empêchent la formation de radicaux libres et autres ROS dans la cellule, comme la catalase (CAT) et la superoxyde dismutase (SOD) (Ighodaro & Akinloye, 2018). La catalase catalyse la réaction de décomposition du peroxyde d'hydrogène (H_2O_2) en eau et en oxygène, tandis que la SOD catalyse la dismutation du radical superoxyde (O_2^-) en oxygène moléculaire (O_2) et H_2O_2. Dans la deuxième ligne de défense, les antioxydants tels que la glutathion S-transférase (GST) piègent les radicaux

actifs, inhibent et interrompent par le même processus l'initiation et la propagation des réactions cellulaires dommageables (Ighodaro & Akinloye, 2018). La GST est principalement connue pour son objectif de détoxification, avec son rôle dans la catalyse de la conjugaison de la forme réduite du glutathion (GSH) aux substrats xénobiotiques. Une fois que les dommages causés par les radicaux libres se sont produits, les antioxydants de la troisième ligne de défense, tels que les polymérases, les glycosylases et les nucléases du système enzymatique de réparation de l'ADN, réparent les dommages causés aux biomolécules et à la membrane cellulaire et dégradent les protéines endommagées (Ighodaro & Akinloye, 2018). La quatrième ligne de défense utilisent sous la forme de mécanisme d'adaptation la signalisation des radicaux libres pour induire et diriger les antioxydants là où leurs actions sont nécessaires (Ighodaro & Akinloye, 2018; Niki, 1993). La mesure de la capacité antioxydante totale « *Total antioxidant capacity* » (TAC) permet de déterminer la capacité antioxydante cumulative d'origine non-enzymatique (e.g. vitamines), incluant l'effet des interactions synergiques entre les composantes (Serafini & Del Rio, 2004). La mesure de peroxydation des lipides permet d'évaluer l'oxydation des lipides insaturés cellulaires par les ROS, ou en d'autres termes, de mesurer les dommages effectués par le stress oxydant sur la membrane cellulaire (Figure 3).

De nombreuses autres études ont rapporté l'augmentation générale du stress oxydatif chez les crustacés marins d'eau froide exposés à des changements environnementaux, cependant peu d'entre elles démontrent que ces mécanismes sont suffisants (e.g. Hernroth et al., 2012). Parmi les stress cytotoxiques, les stress protéotoxiques ont également un rôle majeur dans la capacité d'un organisme à faire face aux changements environnementaux. En effet, la dénaturation des protéines est un processus crucial par lequel l'intégrité des fonctions cellulaires est menacée (Hochachka & Somero, 2002). Les perturbations thermiques sont connues pour être les principaux perturbateurs de la structure des protéines. Suivant la thermodynamique, une élévation ou une baisse significative de la température affecte la structure tridimensionnelle des protéines en affaiblissant les liaisons moléculaires au sein même de la protéine (Somero et al., 2017). Les régions hydrophobes normalement contenu au centre des protéines se retrouvent alors en contact avec l'eau de la cellule, créant des interactions eau-protéine déstabilisant les protéines (Somero et al., 2017). Cette modification de la conformation de la protéine, en plus de provoquer la perte de ses fonctions, peut initier une chaîne de réactions en provoquant l'agrégation des protéines, ce qui endommage la membrane cellulaire et peut générer certaines maladies (Somero et al., 2017). L'un des principaux

mécanismes cellulaires employés pour répondre à une perte de protéostasie suite à des stress environnementaux est la réponse au choc thermique, par la production de protéines chaperonnes « *Heat shock proteins* » (Hsp) (Somero et al., 2017). Présent chez tous les taxons, ce mécanisme semble être l'une des adaptations les plus essentielles des organismes aux changements environnementaux. Le rôle des Hsp dans l'acclimatation a été bien étudié dans le passé (e.g. Berger & Emlet, 2007; González et al., 2016; Oksala et al., 2014).

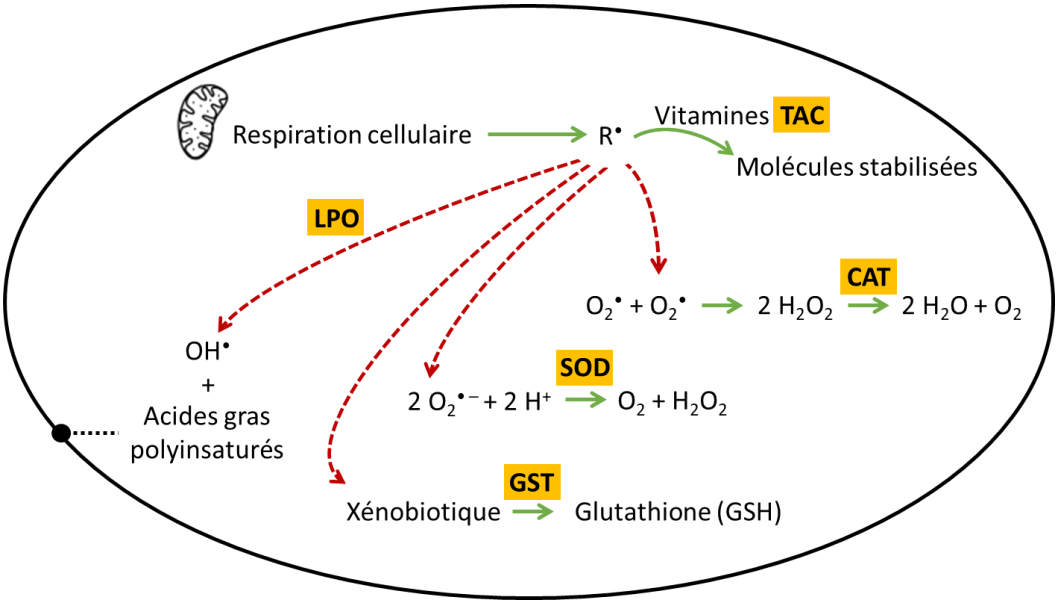

Figure 3. Représentation schématique de l'activité des molécules antioxydantes (i.e. Catalase ; CAT, Superoxyde dismutase ; SOD, Glutathione-S-Transférase ; GST et *Total antioxidant* capacity ; TAC) et de l'impact de la peroxydation des lipides (LPO) sur les acides gras polyinsaturés de la membrane cellulaire au sein de la cellule, suite à la production d'espèces réactives d'oxygènes (ROS ; R˙) par la respiration cellulaire mitochondriale. Les flèches vertes représentent les processus chimiques subjacents à la transformation des composés chimiques, alors que les flèches rouges indiquent les différents types de ROS ciblés par les mécanismes antioxydants et les ROS ayant un impact sur l'intégrité de la membrane cellulaire.

Les chaperons sont nécessaires pour limiter la dénaturation des protéines au sein de la cellule, ainsi que pour dégrader celles dont les fonctions ont été altérées. Les Hsp, telles que les Hsp70, sont des protéines chaperonnes qui aident au repliement des protéines dénaturées de manière réversible afin de les ramener à leur conformation native, limitant ainsi la perte des fonctions cellulaires et le risque associé aux agrégations de protéines (Somero et al., 2017). Les ubiquitines (Ub) sont des chaperonnes qui agissent comme marqueurs des protéines irréversiblement dénaturées, permettant leur élimination définitive (Hershko & Ciechanover, 1998). L'analyse de ces protéines

chaperonnes a permis de mesurer la plasticité phénotypique relative à l'expression des molécules limitants les dommages protéiques chez *P. borealis* exposé aux CG.

6.2 Approche protéomique

Les protéines sont des composantes essentielles de tout organisme vivant. Elles représentent approximativement 60% du poids sec d'une cellule chez les mammifères (Feijó Delgado et al., 2013). Elles assurent de nombreuses fonctions spécifiques : c'est-à-dire structurelles, de transport, de régulation, de signalisation, hormonales, de réception, sensorielles, motrices, de défense, de stockage, et enzymatiques (Lodish & Darnell, 1995). La fonction de chaque protéine est déterminée par leur séquence unique d'acides aminés et la structure tridimensionnelle de la molécule (Berg et al., 2002). Cependant, les protéines subissent généralement une re-conformation structurelle réversible tout en accomplissant leur fonction biologique, créant ainsi des isomères (Alberts et al., 2002). Elles sont identifiées par leur masse, qui dépend de la longueur de leur chaîne d'acides aminés (Cooper, 2000) et de leur charge (Karpievitch et al., 2010). Ainsi, l'ensemble de toutes les protéines contenues dans un organisme exprimé par le génome est appelé le protéome (Ali-Khan et al., 2002). La spécificité des ensembles de séquences génétiques permet l'identification et la classification de tous les organismes vivants (Ali-Khan et al., 2002). Elle nous fournit des informations précieuses sur la structure, ainsi que sur la fonction et l'expression du protéome codé (Ali-Khan et al., 2002). Cependant, les modifications post-traductionnelles ou les changements induits par l'environnement sont également responsables des variations dans l'abondance des protéines fonctionnelles dans un organisme (Karpievitch et al., 2010). Le protéome est alors directement lié au phénotype dans un laps de temps spécifique. Par conséquent, la protéomique est une approche plus ciblée que la transcriptomique ou la génomique pour évaluer la plasticité des unités fonctionnelles (Diz et al., 2012).

Le protéome peut être considérablement modifié sur de courtes périodes de temps (Ali-Khan et al., 2002). Les protéines peuvent se trouver en concentration stable dans la cellule mais peuvent également être sous-exprimées ou surexprimées lorsque cela est nécessaire pour assurer des fonctions homéostatiques, la réparation ou la stabilité des membranes cellulaires (Kültz, 2005). Ce processus dynamique permet aux organismes d'exprimer divers phénotypes en réponse à des circonstances environnementales spécifiques (Tomanek, 2011). Les variations de la capacité de plasticité du protéome entre espèces, populations ou individus détermineront alors leur capacité à

faire face aux fluctuations de leur environnement. La plasticité désigne la capacité d'un organisme à modifier son phénotype en réponse à des altérations de l'environnement, ce qui peut également être observé au niveau cellulaire (Skipper et al., 2010) ainsi qu'à une échelle temporelle au sein des individus (Murren et al., 2015). Ainsi, les organismes ajustent leur protéome pour faire face aux stress environnementaux (Tomanek, 2011). Par exemple, un stress thermique aigu peut déséquilibrer les fonctions cellulaires, entraînant éventuellement un dysfonctionnement, une dénaturation ou une agrégation des protéines et un stress oxydatif (Fields et al., 2016). Cette perte d'homéostasie menace l'intégrité de la cellule et de l'organisme tout entier (Kültz, 2005). Par conséquent, la plasticité du protéome peut permettre aux organismes de corriger ces déséquilibres. Cela peut se faire par la production d'enzymes participant à l'élimination des espèces réactives de l'oxygène (ROS), par l'augmentation de la présence de protéines chaperonnes, ou encore de protéines structurelles pour assurer la dynamique du cytosquelette, réguler l'équilibre acido-basique, etc. afin de protéger l'intégrité de la cellule et de l'organisme (Artigaud et al., 2015; Chang et al., 2016a; Fields et al., 2012, 2016, 2016; Jiang et al., 2009; D. Madeira, Araújo, et al., 2016; D. Madeira et al., 2017; Sokolov et al., 2019; Timmins-Schiffman et al., 2014; Tomanek, 2011; Tomanek et al., 2011; Wei et al., 2015). Des variations du protéome sont observées en réponse à des stimuli environnementaux biotiques et abiotiques (Ali-Khan et al., 2002). Ces stimuli incluent la température (Fields et al., 2012, 2016; D. Madeira et al., 2017; Schwerin et al., 2009), l'acidification (Chang et al., 2016b; J. Mukherjee et al., 2013; Timmins-Schiffman et al., 2014), l'hypercapnie (Tomanek, 2011), l'hypoxie (Artigaud et al., 2015; J. Mukherjee et al., 2013), les polluants (Galland et al., 2013), l'osmolarité, les infections ou les symbioses (Tomanek, 2011; Tomanek et al., 2011). La caractérisation détaillée des changements protéiques, utilisés comme biomarqueurs, nous permettra d'approfondir notre compréhension des variations phénotypiques et de participer au développement des ressources -omiques, qui restent encore excessivement rares pour les organismes marins, ayant surtout été employées et développées sur des espèces modèles (i.e. la souris domestique *Mus musculus* (Linnaeus, 1758), la drosophile *Drosophila melanogaster* (Meigen, 1830) et le poisson zèbre *Danio rerio* (Hamilton, 1822; Edison et al. 2016).

7 Objectifs spécifiques et hypothèses

Afin d'évaluer la tolérance d'un sténotherme marin d'intérêt commercial face aux changements globaux, l'objectif général de l'étude est de caractériser en détail la plasticité phénotypique chez

les adultes de *P. borealis* grâce à l'analyse de la réponse cellulaire au stress ainsi que des variations du protéome, lorsqu'exposé au RO, à l'AO ainsi qu'à l'hypoxie. Pour y répondre, l'étude a été divisé en deux sous-objectifs sous le couvert de deux expériences, rapportés individuellement aux chapitres 1 et 2. D'abord, le premier sous-objectif vise à étudier la capacité plastique de la réponse cellulaire au stress chez *P. borealis* confronté aux facteurs combinés des changements globaux en observant les mécanismes cellulaires, tels que la réponse des antioxydants et des protéines chaperonnes. Ensuite, le deuxième sous-objectif vise à caractériser la plasticité des profils protéomiques ainsi que la variation inter-individuelle de *P. borealis* face au réchauffement et à l'acidification de l'océan, ainsi qu'à l'hypoxie.

J'ai donc émis l'hypothèse que **la tolérance de *P. borealis* à la combinaison des facteurs environnementaux testés ici dépend de la capacité cellulaire à induire fortement les mécanismes antioxydants, les protéines chaperonnes et le système de contrôle de la qualité des protéines pour contrer l'effet négatif des conditions extrêmes créées par une interaction synergique entre les facteurs**. Je m'attends à ce que la réponse cellulaire des individus survivants de *P. borealis* exposés aux facteurs de changements globaux repose sur ces mécanismes, en diminuant l'abondance de protéines de certaines fonctions (e.g. stockage, hormonal) et ainsi prioriser ces processus tout au long de l'exposition. Aussi, je m'attends à ce que les dommages cellulaires chez *P. borealis* soient observables dans les conditions combinées de température élevée, d'acidification et d'hypoxie, car la plasticité cellulaire ne sera pas en mesure de compenser dans ce scénario.

Il est également attendu que *P. borealis* doit ajuster significativement l'abondance de protéines issues de mécanismes de maintien des fonctions homéostatiques afin de tolérer les conditions environnementales auxquelles les crevettes seront exposées. Les processus de métabolisme énergétique, de réponse au stress cellulaire, de défense immunitaire, du cytosquelette, de l'apoptose et des protéines liées aux chaperons devraient présenter les changements les plus importants dans les conditions de température élevée, d'acidification et d'hypoxie.

Finalement, **je prévoie une modulation de l'intensité de la variation inter-individuelle selon le degré d'exposition aux facteurs**, considérant que les individus tolérants aux conditions plus extrêmes devraient être ceux qui présentent le plus de variations phénotypiques leur permettant de développer les mécanismes et la plasticité nécessaires au maintien de l'homéostasie et des fonctions

cellulaires. Cependant, la nature sténotherme de *P. borealis* pourrait faire en sorte de limiter considérablement ses capacités d'ajustement du phénotype, malgré certaines différences entre les traitements.

CHAPITRE 1

EFFETS COMBINÉS DU RÉCHAUFFEMENT ET DE L'ACIDIFICATION DES OCÉANS ET DE L'HYPOXIE SUR LA RÉPONSE CELLULAIRE AUX STRESS CHEZ LA CREVETTE NORDIQUE *PANDALUS BOREALIS*

RESUME EN FRANÇAIS DU PREMIER ARTICLE

Les changements globaux induisent des altérations significatives des propriétés physico-chimiques des écosystèmes qui peuvent conduire les organismes à présenter des réponses physiologiques diverses à leur environnement dynamique. Comme les organismes sont naturellement exposés à plusieurs facteurs environnementaux changeant simultanément, l'utilisation d'une approche multifactorielle considérant les interactions entre les stresseurs est essentielle pour déterminer les réponses physiologiques des organismes. Dans le cas de l'estuaire du Saint-Laurent (ELS), une exposition des organismes au réchauffement des océans (RO), à l'acidification des océans (AO) et à l'hypoxie est attendue. Dans ce contexte, ce projet vise à évaluer les effets combinés du RO, de l'AO et de l'hypoxie sur la réponse cellulaire au stress (RCS) de la crevette nordique *Pandalus borealis*, une espèce de grande importance écologique et économique dans l'Est du Canada. Nous avons donc exposé les crevettes à différents niveaux de températures (2, 6 et 10 °C), pH (7.75 et 7.40) et de saturation en oxygène (100 et 35 % sat.) de façon isolée et combinée pendant 30 jours. Nous avons ensuite mesuré la RCS du muscle abdominal des individus survivants afin d'obtenir une image détaillée des effets des facteurs des changements globaux sur la capacité plastique du système de défense des cellules par les antioxydants, les chaperons protéiques et le système de contrôle de la qualité protéique grâce à l'analyse de biomarqueurs (i.e. CAT, SOD, GST, LPO, TAC, Hsp70 et Ub). La réponse antioxydante s'est avérée être principalement dirigée par l'activité de la CAT, celle-ci étant plus élevée chez les crevettes exposées au RO et à l'AO combinés, avec la température comme effet principal. La capacité antioxydante semblait être suffisante pour contrer les impacts du stress oxydant dans tous les scénarios. Cependant, les chaperons protéiques et le système de contrôle de qualité protéique ont diminué chez les crevettes exposées à l'hypoxie. Ainsi, l'ensemble de nos résultats semblent suggérer que la combinaison des facteurs de changements globaux altère la capacité de la RCS de *P. borealis*, mettant à risque les crevettes du SLE sous les scénarios environnementaux futurs.

THE COMBINED EFFECTS OF OCEAN WARMING, OCEAN ACIDIFICATION AND HYPOXIA ON THE CELLULAR STRESS RESPONSE OF THE NORTHERN SHRIMP *PANDALUS BOREALIS*

Maude Boissonneault[1], Ella Guscelli[1], Joana Fernandes[2], Diana Madeira[2] and Piero Calosi[1]

[1] Département de biologie, chimie et géographie, Université du Québec à Rimouski, Rimouski, Québec, Canada
[2] Centre for Environmental and Marine Studies, University of Aveiro, Aveiro, Portugal

1 Abstract

Global change induces significant alterations of the physicochemical properties of ecosystems that can lead organisms to exhibit diverse physiological responses to their dynamic environment. As organisms are naturally exposed to several environmental factors changing simultaneously, the use of a multifactorial approach considering the interactions among stressors is essential to determine organisms' physiological responses. In the specific case of the St. Lawrence Estuary (SLE), exposure of organisms to ocean warming (OR), ocean acidification (OA) and hypoxia is expected to superimpose. Within this context, this project aims at assessing the combined effects of OW, OA and hypoxia on the cellular stress response (CSR) of the northern shrimp *Pandalus borealis*, a species of high ecological and economic importance in Eastern Canada. First, we exposed shrimp to different levels of temperatures (2, 6 and 10 °C), pH (7.75 and 7.40) and oxygen saturation (100 % and 35 % sat.) in isolation and combined for 30 days. We then measured the CSR of the abdominal muscle of surviving individuals to obtain a detailed picture of the effects of the global change drivers on the plastic capacity of the antioxidant, protein chaperones and protein quality control defense system of cells by analyzing a number of biomarkers: CAT, SOD, GST, LPO, TAC, Hsp70 and Ub. The antioxidant response was found to be led mostly by CAT activity, this being higher in shrimp exposed to OW and OA combined, with temperature as the leading effect. The antioxidant capacity seemed to be sufficient to counter oxidative stress impacts under all scenarios. However, protein chaperones and protein quality control system decreased in shrimp exposed to hypoxia. Thus, our results suggest that the combination of global change drivers seems to impair the CSR capacity of *P. borealis*, making shrimp from the SLE to be at great risk under future environmental scenarios.

2 Introduction

Estuaries are naturally dynamic ecosystems, characterised by wide variations in several abiotic parameters (Day et al., 2012). In the context of ongoing global change, the rapidity of changes in the environment is unprecedented, making estuaries challenging environments for living organisms (Elliott & Whitfield, 2011). Under such conditions, organisms that show physiological resilience by acclimation or adaptation to the constantly will be the ones to persist (IPCC, 2021).

Ectotherms do not metabolically regulate their body temperature, and are therefore extremely susceptible to temperature changes (Angilletta Jr., 2009; Cossins & Bowler, 1987; Kinne, 1970; Newell, 1979; Peck et al., 2004). Increases in temperature are known to accelerate metabolic rates, enzymatic processes and development rates in the range of the thermal performance curve (Schulte, 2015). The impacts of ocean warming (OW) on marine ectotherms were previously reported in studies and vary over a broad spectrum, going for example from the up-regulation of genes from the heat shock response, apoptosis mechanism and immune defense system to the down-regulation of metabolic processes, proteolysis and oxidative stress genes. For instance, Zhou et al. (2010) observed that the exposure to acute elevated temperature induced antioxidant and heat shock responses in the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931). Also, cellular stress responses (CSR) such as heat shock response, ubiquitin-dependant proteolysis, DNA repair and apoptosis mechanisms were enhanced in American lobster *Homarus americanus* larvae exposed to OW (Lopez-Anido et al., 2021). Cold-water stenotherms are even more impacted by temperature increases, being biochemically and physiologically adapted to very stable low temperatures (Hochachka & Somero, 2002; Peck et al., 2002; Peck & Conway, 2000; Pörtner et al., 2007; Somero et al., 2017).

Physiological impacts are also reported when organisms are exposed to ocean acidification (OA) conditions, another important driver to consider in estuarine studies. OA has direct impacts on physiological processes, such as enzymes conformation (Dixon, 1953; Stillman & Paganini, 2015), acid-base balance (Melzner et al., 2009) and biochemical reaction rates (Guppy & Withers, 1999), by disrupting the cellular proton gradient, and thus cellular homeostasis (Stillman & Paganini, 2015). Studies previously reported impacts of OA on marine ectotherms, ranging from an increase in metabolic rate supported by an increase in energy production mechanisms (e.g. oxidative phosphorylation, ion transport), to an increase in cellular mechanisms related to ribosome

formation, cell adhesion and apoptotic process, as observed in copepod *Pseudocalanus acuspes* (Giesbrecht, 1881) and larvae of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) (De Wit et al., 2016; Dineshram et al., 2021). Wang et al. (2009) observed in the *L. vannamei* an increase in ROS production and DNA damages, an up-regulation of gene expression of antioxidant defense as well as variations in calcium ions in response to an exposure to acute pH stress (acid and alkaline). In those cases, an important part of the energy budget seems to be allocated toward the maintenance of the energy production level, which can limit defense capacity by limiting energy reserves and immune response pathways, as well as development and growth (De Wit et al., 2016; Dineshram et al., 2021; Freitas et al., 2016).

Widespread in estuaries, hypoxia affects the cellular metabolism, leading to a switch from aerobic to anaerobic metabolism, which in turns reduces energy production (Michiels, 2004). A decrease in environmental dissolved oxygen saturation has been demonstrated in past studies to induce important physiological impacts on ectotherms, such as in the crab *Neohelice granulate* (Dana, 1851) and in the gastropod *Littorina littorea* (Linnaeus, 1758), where a modulation of the antioxidant response through hypoxia and re-oxygenation cycles was observed (Geihs et al., 2016; Pannunzio & Storey, 1998). In the early life stages of Chinook salmon *Oncorhynchus tshawytscha* (Walbaum, 1792), hypoxia induced an increase in acute thermal and hypoxia tolerance, while decreasing survival and growth (Del Rio et al., 2019). These studies suggest that although organisms appear to be able to deal with hypoxia, it seems that they are not able to spend the same level of energy in other functions such as development or reproduction.

However, combinations of factors often create interactions that have even greater physiological impacts on organisms. Depending on the duration and intensity of exposure to each factor, organisms exhibit complex responses that cannot be detected when exposed to single factors (e.g. Godbold & Solan, 2013; Swiney et al., 2017). It increasingly appears less and less relevant to distinguish their individual physiological impacts and investigate their combined effects, particularly at the cellular level, although only few studies addressed the three drivers comprehensively together (Sampaio et al., 2021). Furthermore, OW, OA and hypoxia have been shown to have, both individual and combined, significant negative physiological impacts on a vast range of organisms (Götze et al., 2020; Khan et al., 2020; Sampaio et al., 2021). All considered, it is therefore fundamental to study multiple factors in combination as in nature organisms are

exposed to the variation of several parameters simultaneously (Côté et al., 2016; Halpern et al., 2015). In fact, the vast majority of ecosystems (97.7 %) are affected by multiple drivers simultaneously, complexifying greatly the study of stress responses in marine organisms by creating interaction among the drivers (Côté et al., 2016; Halpern et al., 2015). Indeed, beside additivity of responses that is often expected with studies involving two or more factors, combined global change drivers can generate the emergence of synergistic and antagonistic responses, that one may have not expect based on responses to single drivers' (Côté et al., 2016; Crain et al., 2008). The nature of interactions among multiple drivers can also vary at different levels of ecological organization, trophic level and drivers pairing (Carrier-Belleau et al., 2021; Crain et al., 2008; Piggott et al., 2015). Considering that in nature the accumulation of anthropogenic stressors is increasing (Halpern et al., 2019), synergies and antagonisms may be even more common that hypothesized in the past (Carrier-Belleau et al., 2021; Crain et al., 2008; Piggott et al., 2015). It is then paramount to account for combined drivers, specifically in stenotherms, as growing evidence suggest that as they live in narrower thermal niche and closer to their maximal thermal capacity, they are likely among the most sensitive to environmental changes (Azra et al., 2018, 2020, 2022; Pinsky et al., 2019; Syafaat et al., 2021; Zhu et al., 2019). Thus, stenotherms represent an important group of species having significant ecological and socio-economical purposes, in addition to exhibit sensitivity to environmental modifications (e.g. Serpetti et al., 2017).

The aim of this chapter is to assess the plastic capacity of the CSR in a marine cold-water stenotherm facing combined global change drivers by investigating cell cytoprotective mechanisms, such as the antioxidant and the protein chaperones heat shock response, as well as ubiquitin-dependent proteolysis and level of oxidative damages. To do so, the northern shrimp *Pandalus borealis* (Krøyer, 1838) was selected as an ideal study species, as it possesses a discontinuous circumboreal distribution (Shumway et al., 1985) and is commonly observed in the North-East Atlantic region (MAPAQ, 2015; Pillet, 2013). It is also one of the three most commercially valuable fished species in Quebec and Eastern Canada, and the most abundant shrimp species in this region (MAPAQ, 2015), its fishery representing an important economic resource for several coastal communities including indigenous and non-indigenous one along the North and Southern shores of the St-Lawrence estuary and gulf (MPO, 2018). In addition, the population *P. borealis* found in the St. Lawrence Estuary (SLE) was identified as an ideal study

system given the long-term acclimatisation (or even adaptation) to existing and rapidly changing oceanographic conditions found in this habitat (Galbraith et al., 2021; Lavoie et al., 2020, 2021).

The SLE is characterized by specific bottom waters conditions, due to interdecadal variability in proportions of Labrador Current Water (LCW) and North Atlantic Central Water (NACW) supplying the estuary through the Laurentian Channel (Gilbert et al., 2005). In the past decades, the environmental conditions were modulated by a major influx of warm oxygen-poor waters from the NACW (Gilbert, 2004), and are expected to worsen in the future in the context of global change (IPCC, 2021). OW is expected to induce an increase of 4 °C by 2100 (RCP 8.5; IPCC et al., 2021) of the bottom water temperature, currently at 5-6 °C (DFO, 2020b), while a decrease of 0.35 pH units is projected due to OA (RCP 8.5; IPCC et al., 2021), bringing the pH from 7.6-7.75 to a pH of at least 7.40 (DFO, 2017). Finally, the hypoxic conditions of the SLE, with a DO sat. below 20 % (DFO, 2020c) close to the limit of sustainability for living organisms (Diaz and Rosenberg 1995; Rosenberg 1992), is expected to decrease even more in the upcoming decades (RCP 8.5; IPCC et al., 2021). Plasticity is then paramount for organisms to ensure their perennity in this changing environment.

Studies to date suggest that *P. borealis* seems to be relatively tolerant to warming (Apollonio et al., 1986a; Shumway et al., 1985; Squires, 1990), acidification (Hammer & Pedersen, 2013) and hypoxia (Dupont-Prinet et al., 2013) when exposed separately to each type of environmental driver. *Pandalus borealis*' response to global change is likely to vary by life-history stage (Daoud et al., 2007) or sex (Dupont-Prinet et al., 2013), as well as by the duration and intensity of exposure and the combination of drivers. Chemel et al. (2020) found that survival was significantly lower in shrimp exposed to OW and OA, and even lower in shrimp exposed to OW, OA and hypoxia. Arnberg et al. (2013) found that in larvae a decrease in pH increased development time, but only at low temperature. They found that OW should exert a greater effect that OA on larval shrimp development, having observed a lower hatching success in eggs under elevated temperature. However, they later found that the combination of OW and OA had negative impacts on survival, development and growth in larval stages, and that these effects were stage-dependent. However, the impact of multi-stressor on *P. borealis* is yet to be studied and the physiological responses of *P. borealis* shrimp exposed to combined global change drivers at the cellular level currently unknown.

Specifically, here we intended to assess **(Obj. 1)** the extent of cytotoxic stress and protein damages in shrimp exposed to a combination of global change drivers. We hypothesize that the tolerance of *P. borealis* to the combination of drivers depends on an important cellular capacity to induce mainly antioxidant and protein chaperones mechanisms to counter the negative effect of the extreme conditions created by a synergistic interaction among the drivers. We expect that the cellular damages in *P. borealis* will be observable at the highest temperature, low pH/elevated pCO_2 and hypoxic conditions, as cellular plasticity will not be able to compensate in this situation. We also aimed to evaluate **(Obj. 2)** the cellular plastic capacity in regard of antioxidant, protein chaperones mechanisms and protein quality control system, testing the hypothesis that *P. borealis'* cellular response to global change drivers rely on the phenotype's adjustment ability, even though this shrimp is expected to show limited variations in used mechanisms due to their stenotherm nature, mainly under elevated temperature, low pH/elevated pCO_2 and hypoxic conditions in combination.

3 Material and methods

The tissue samples used for the investigation of the combined effects of OW, OA and hypoxia on the plastic capacity of the northern shrimp's cellular stress response (CSR) were obtained from a previous study (details published in Chemel et al. 2020 and Guscelli et al., *in prep*). Here we provide a brief account of the experimental design, set up and specimens' collection carried out and describe the biomarkers assays we used for the determination of the antioxidant defense, protein chaperones and protein quality control system of *Pandalus borealis* exposed to elevated temperature, low pH/elevated pCO_2 and hypoxic conditions.

3.1 Specimens' collection, transport and maintenance under laboratory conditions

Non-ovigerous females of northern shrimp *Pandalus borealis* with a carapace length of 25.45 ± 0.14 mm (mean ± SE) were selected as they represent the main target of this fishery because of their larger size (ASMFC, 2019), and they are known to be more sensitive to hypoxia when compared to males (Dupont-Prinet et al., 2013; Pillet, 2013). They were fished in the SLE off Rimouski (Quebec, Canada; ∼58◦36′N, 68◦35′W) by scientific trawling at 120-150 m depth using hauls of ∼16 min at an average speed of 2.5 knots, with a rigid frame trawl of the Maurice Lamontagne Institute (MLI, Department of Fisheries and Oceans Canada, Mont-Joli, QC, Canada) from the 22-m CCGS Leim research vessel. The collection took place in between May 20th and 30th 2018, then the captured individuals were held in isolated tanks (750 L Xacticts^{tm}) filled with cold (2 and 3 °C) and oxygenated water and transported by truck to MLI within 4-10 h of capture. They were then transferred to rectangular basins (1700 L) supplied with natural sea water. Salt (Common salt without additives, K+S Windsor Salt, Pointe-Claire, QC, Canada) was added to sea water to increase the salinity from 28 to ∼32. Shrimps were maintained at a temperature of 4.5 °C, salinity of 32, 100 % O_2 sat. and pH of 7.9 (total scale, pH_T), where they remained for eight weeks before the exposure to the different treatments. Shrimp were fed *ad libitum* with equal proportion of frozen capelin *Mallotus villosus* (Müller, 1776) and shrimp (*Pandalus* spp.) three times a week in order to simulate their natural diet (Wienberg,

1980). Dead individuals, exuviae and excess food were removed daily to limit bacteria proliferation and ammonia accumulation to ensure high quality water.

3.2 Experimental design

To explore the combined effects of OW, OA and hypoxia on the plasticity of the CSR of the northern shrimp, two experimental designs were established, as was previously done in Chemel et al. (2020) and Guscelli et al. (*in prep*):

Experimental design A was employed to investigate the impact of OW and OA in isolation and combined on the CSR of the northern shrimp. In more detail, individuals were exposed to three temperatures levels (2, 6 and 10 °C) and two pH levels (7.75, 7.40), in a six treatments fully orthogonal experimental design. The temperature treatments were chosen according to the preferred temperature of the specie (2 °C; Orr & Sullivan, 2013, Siferd, 2015), the current temperature to which the SLE shrimp are exposed (6 °C; DFO, 2018), as well as the expected scenario for 2100 of a +4 °C increase (RCP 8.5; IPCC et al., 2021). The pH treatments were selected to depict current (7.75; Mucci et al., 2011, 2017) and the expected bottom water pH of the SLE (7.40) for the end of the century, considering a decrease of -0.35 pH unit (RCP 8.5; IPCC et al., 2021). This design comprises six experimental treatments.

Experimental design B was used in order to explore the combined effect of OW and OA (here defined as the current and future environmental horizons) together with hypoxia on the CSR. Two treatments of hypoxia (35 % O_2 sat.) were overlay to two experimental conditions from design A, (i) the most favorable environmental conditions (2 °C, pH 7.75) and (ii) the expected conditions for 2100 taking into account OW and OA scenarios (10 °C, pH 7.40), according to the oxygen saturation commonly encountered by *P. borealis* in the St. Lawrence Estuary and Gulf, while still being over its lethal threshold (Dupont-Prinet et al., 2013). This design comprises four experimental treatments.

A total of eight experimental treatments were used, and labeled as: (2C) low temperature and current pH (2 °C, pH 7.75, 100 % O_2 sat.), (2A) low temperature and low pH (2 °C, pH 7.40, 100 % O_2 sat.), (6C) intermediate temperature and current pH (6 °C, pH 7.75, 100 % O_2 sat.), (6A) intermediate temperature and low pH (6 °C, pH 7.40, 100 % O_2 sat.),

(10C) elevated temperature and current pH (10 °C, pH 7.75, 100 % O_2 sat.), (10A) elevated temperature and low pH (10 °C, pH 7.40, 100 % O_2 sat.), (2CH) low temperature, current pH and low O_2 (2 °C, pH 7.75, 35 % O_2 sat.) and (10AH) elevated temperature, low pH and low O_2 (10 °C, pH 7.40, 35 % O_2 sat.).

3.3 Experimental set up, protocol and system monitoring

Two reservoirs (750 L) provided sea water at a constant flow rate of 3.5 L min^{-1} to 16 large insulated and re-circulated tanks (240 L, one duplicate *per* treatment) holding all shrimp. Each reservoir was maintained at constant temperature of approximately 0 and 10 °C, respectively, by mean of thermal pumps (Gell'Air, Mont-Joli, Canada). A controller (1/16 DIN Micromega autotune PID Temperature, Omega Engineering inc., Norwalk, CT, USA) and two mixing valves (sv3109, Omega Engineering inc.) in each tank allowed to obtain the set temperature by mixing the appropriate proportion of cold and warm water (total 3.5 L min^{-1}). To ensure the continuous monitoring and regulation of temperature and pH, two feedback systems (1/16 DIN Micromega autotune PID Temperature, Omega Engineering inc. and IKS Aquastar, respectively) were couple to a temperature probe (HRSTD-3-100-A-240-E, Omega Engineering Inc., Norwolk, CT, USA) and a pH probe (1001 modules, IKS Aquastar, Karlsbad, Germany) in each tank. Moreover, a feedback system to monitor and adjust the O_2 sat. coupled with O_2 probes (1008 module, IKS Aquastar), by regulating the proportion of pure gaseous CO_2 and N_2 into gas exchange column, were operative in each tank for hypoxic treatments. To limit the formation of physical and chemical gradient, each tank was equipped with a submersible pump (1048, Eheim, Stuttgart, Germany) allowing the water mixing. To minimize O_2 sat. fluctuations in hypoxic treatment tanks, the pump were connected to the top of the gas exchange column, increasing flow rate through it.

Each tanks held approximatively 70 non-ovigerous females randomly assigned. The environmental conditions were gradually adjusted from the acclimation conditions (4.5 °C, pH 7.9 and 100 % sat.) to treatments values over 4 d. Temperature was increased or decreased by 1.5 °C *per* day and pH was decreased by 0.15 pH unit *per* day. O_2 sat. was decreased by daily increments from 100 to 70 %, to 50 %, to 40 % and to 35 %. Once the

experimental conditions have been reached, shrimp were exposed for 30 days. Dead individuals, exuviae and excess food were removed daily as described previously.

Following the exposure period of 28-d, five shrimp *per* tank were randomly selected for subsequent analyses. Each individual was put into respirometers chambers at the same environmental conditions as their respective treatment during 48 h in order to measure their standard and maximum metabolic rate. These results are presented in Guscelli et al. (*in prep*). Each individual was rapidly blotted with a paper towel, weighed on a precision scale (Mf-300, AandD Company, Tokyo, Japan; ± 0.001 g precision) and sacrificed by removing the abdomen muscle with a ceramic knife. The samples were flash frozen in liquid nitrogen, then kept at -80 °C until biomarkers analyses were carried out.

3.4 Biomarkers' analyses

3.4.1 Proteins extraction and total proteins quantification

Samples of 200 mg of shrimp abdomen muscle tissue (n = 80) were homogenized (Homogenizer Omni TH, Bebensee, Germany) in 1 mL of 1X PBS (pH 7.4) + 0.03 % Triton X-100 buffer to ensure the proteins solubilization, while PBS stabilizes enzymes without altering them (Ugwu & Apte, 2004). They were then centrifuged (Sorval Legend Micro 21R, ThermoFisher Scientific®, Osterode am Harz, Germany) for 15 min at 4 °C at 10 000 g. The supernatant containing the proteins from the samples was transferred to a new microtubes and frozen at -20 °C since the manipulation was conducted in a short time after.

Total protein was quantified following the Bradford methodology (Bradford, 1976) for 96-well microplates. Briefly, 150 µL of each sample, previously diluted in 1X PBS buffer + 0.03 % Triton X-100 to 1:500, was added to each well (in duplicates). Subsequently, 150 µL of Bradford reagent (Comassie Blue G-250, methanol, phosphoric acid, distilled water) were added to each well and absorbance was read at 595 nm in Synergy™ HTX Multi-Mode Microplate BioTek® reader. An eight-point calibration curve was built using BSA (Bovine Serum Albumin, Sigma-Aldrich, USA) as standards (0 - 25 µg mL^{-1}). Total protein measurements were used to normalize all biomarker levels (Heat shock protein 70 kDa –

Hsp70, Total ubiquitin - Ub, antioxidant enzymes: Catalase - CAT, Glutathione-S-transferase - GST, Superoxide dismutase - SOD, non-enzymatic antioxidant agents: Total antioxidant capacity – TAC and oxidative damage products: Lipid peroxidation – LPO).

3.4.2 Antioxidant biomarkers

3.4.2.1 Catalase activity

The Catalase (CAT) (EC 1.11.1.6) assay was adapted from Johansson and Borg (1988) and Madeira et al. (2019). As a positive control, the activity of a standard bovine CAT solution of 1523.6 U^{-1} was used. The calibration curve was done with formaldehyde standards (0-75 µM), and CAT activity was calculated considering that one unit of CAT is the amount that will cause the formation of 1.0 nmol of formaldehyde per minute at 25 °C at 530 nm using a microplate reader (BIO-RAD, Benchmark, USA).

3.4.2.2 Glutathione-S-transferase activity

The glutathione-S-Transferase (GST) (EC 2.5.1.18) activity assay was adapted from Habig et al. (1974) and Madeira et al. (2019). The substrate used was 1-Chloro-2,4-dinitrobenzene (CDNB), and the absorbance was read at 340 nm using a microplate reader (BIO-RAD, Benchmark, USA), and the GST activity was calculated using a molar extinction coefficient of 0.00503 µM (adapted for 96 well microplates) following the equations:

1) GST Abs340/min = (Abs_{340} final read – Abs_{340} initial read) / reaction time (min)

2) GST specific activity = (GST Abs_{340}/min x 0.2 mL) / (0.00503 µM × 0.02 mL)

3.4.2.3 Superoxide dismutase inhibition

The superoxide dismutase (SOD) activity assay was adapted from Sun et al. (1988), using nitroblue tetrazolium (NBT) and xanthine oxidase (XOD). The absorbance was read at 560 nm using a microplate reader (BIO-RAD, Benchmark, USA), and the % inhibition was calculated using the equations:

1) SOD Abs_{560}/min = (Abs_{560} final read – Abs_{560} initial read) / reaction time (min)

2) SOD % inhibition = ((Abs_{560}/min negative control – Abs_{560}/min sample) / (Abs_{560}/min negative control)) × 100

3.4.2.4 Total antioxidant capacity

The Total Antioxydant Capacity (TAC) determination was adapted from Miller et al. (1993) and Kambayashi et al. (2009), using the trolox equivalent antioxidant capacity assay. The method is based on the reduction of ABTS (2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) in $ABTS^{\bullet+}$ by the antioxidants in each sample. Coupled to a chromogenic reagent that produces a blue-green coloration with a maximum absorbance at 405 nm. The absorbance values of antioxidants were compared to a Trolox (water-soluble vitamin E analog) standard curve (0 to 0.330 mM) and were proportional to the sample's total reductive capacity. In brief, 10 µL of each sample were placed inside the microplate wells (duplicates). After that, 10 µL of myoglobin 90 µM and 150 µL of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) 600 µM were added. The reaction was started by adding 40 µL of hydrogen peroxide 500 µM (0.0017 %). Microplates were incubated for 5 min at room temperature and absorbance was read at 405 nm in a microplate reader (BIO-RAD, Benchmark, USA).

3.4.3 Oxidative damage products (Lipid peroxidation)

The lipid peroxidation (LPO) assay using thiobarbituric acid reactive substances (TBARS) protocol was adapted from Vinagre et al. (2014). Briefly, 5 µL of each sample were added to 45 µL of 50 mM PBS buffer. Then, 12.5 µL of SDS 8.1 %, 93.5 µL of TCA 20 %, 93.5 µL of TBA 1 % and 50.5 µL of Milli-Q grade pure water were added to the Eppendorfs before being vortexed for 30 s. The lids were punctured with a needle before the Eppendorf were incubated in boiling water for 10 min. The samples were then cooled on ice, before adding 62.5 µL of Milli-Q grade pure water. They were centrifuged for 5 min at 10 000 g (Sorval Legend Micro 21R, ThermoFisher Scientific®, Osterode am Harz, Germany). Then, 150 µL of the supernatant were put in a 96-well microplate well, and the absorbance was read at 530 nm using a microplate reader (BIO-RAD, Benchmark, USA). A 12 points standard curve of malondialdehyde (MDA; 0-0.3 µM) was used to quantify the lipid peroxides.

3.4.4 Heat Shock Protein 70 and Total ubiquitin quantification

Heat Shock Protein 70 (Hsp70) and Total Ubiquitin (Ub) were quantified by indirect Enzyme-linked immunosorbent assay (ELISA) with 96-well microplates following the method described in (D. Madeira, Mendonça, et al., 2014). Samples were diluted to 1:50 for Hsp70 and 1:10 for Ub in PBS buffer to avoid interference and to maximize the signal-to-noise ratio in the assays. For both ELISAs, 50 µL of each sample, in two replicates, were added in microplates and incubated overnight at 4 °C. The microplates were washed (3X) in PBS 0.05 % Tween-20 before 200 µL of a blocking solution of 1 % BSA in PBS (Bovine Serum Albumin, Sigma-Aldrich, USA) was added to the wells. The microplates were incubated for another 90 min at 37 °C and washed as previously. Afterwards, 50 µL of the primary antibody (Hsp70: Mouse monoclonal Hsp70/Hsc70 Antibody, OriGene, USA; Ub: Mouse monoclonal Ubi-1, #ab7254, Abcam, UK), diluted to 2 µg mL^{-1} for Hsp70 and 1.5 µg mL^{-1} for Ub, in a 1 % solution of BSA, were added to the microplates before being incubated for another 90 min at 37 °C and washed. Then, 50 µL of the secondary antibody (Hsp70 and Ub: Anti-Mouse IgG, Fab specific, Alkaline Phosphatase antibody produced in goat, Sigma- Aldrich, USA), diluted to 2.5 µg mL^{-1} for Hsp70 and 2 µg mL^{-1} for Ub in 1 % BSA, were added to the wells. The microplates were incubated again at 37 °C for 90 min and washed. Finally, 100 µL of substrate-alkaline phosphatase buffer (SIGMA FAST™ p-Nitrophenyl Phosphate Tablets, Sigma-Aldrich, USA) were added to the wells before the final incubation of 10-30 min at 37 °C. Finally, 50 µL of stop solution (3 M NaOH) was added to each well before reading the absorbance in a microplate reader at 405 nm (BIO-RAD, Benchmark, USA). Calibration curves were produced using serial dilutions to obtain a range from 0 to 1 µg mL^{-1} for the Hsp70 analysis (Hsp-70 active protein, #AR03018PU-N, OriGene, USA) and purified ubiquitin (UbpBio, E-1100, USA) with a range from 0 to 2 µg mL^{-1} for Ub analysis.

3.5 Statistical analyses

In order to investigate the combined effects of OW, OA and hypoxia on the plasticity of the northern shrimp' CSR, statistical tests were applied to detect significant differences between treatments for each biomarker in both designs. Biomarkers data were analyzed by

mean of ANOVA tests using *car* (Fox & Weisberg, 2019) and *lmer* packages (Kuznetsova et al., 2017) in R 4.1.2 (R core team, 2021), for which a best-fit model exercise was carried out in order to uncover the model that explains the greatest amount of variation using the fewest possible independent variables: 1) the simple linear model, 2) the mixed model with the tank as a random effect, and 3) another mixed model with tank nested in treatment. The terms *Temperature* and *pH* in design A and *Horizons* (i.e. current *Temperature* and *pH* horizon, future *Temperature* and *pH* horizon) and O_2 *levels* in design B were used as fixed factors (Table 1). A small-sample corrected Akaike information criterion (AICc) was then used to choose the most parsimonious model which for all the biomarker were linear models, except for GST for which the best model was the mixed model with the tank as random effect in both designs (*nlme* package, Pinheiro et al., 2021; Table 1). Tukey's multiple-comparison tests were then carried out to assess which treatments were significantly different from each other for each biomarker. Normality of distribution was verified using the Shapiro-Wilk's test and homoscedasticity of variances by mean of Levene's test, in addition to verify the independence of residuals visually (Table 1). Logarithmic, square root and cube root transformations were employed according to the need for each model to ensure data fitted the assumptions (Table 1). Then, a bootstrap method was used to calculate a 95 % confidence interval in order to test for the significance of the model which were not fulfilling the normality assumptions. Outliers were assessed graphically (box-whiskers plot, coefficient 1.5 for outliers and extremes) and kept throughout the analyses.

3.6 Integrated biomarker response

All biomarkers described previously were combined into a numeric value or stress index known as "integrated biomarker response version 2 (IBRv2)" to acquire a deeper understanding of the general cellular stress responses to global change drivers. This index was calculated in R (R Core Team, 2021) using the IBRtools package (Resende & Pereira, 2021) with the method described by Sanchez et al. (2013). This method adapted from Beliaeff and Burgeot (2002) allows the integration of up- and down- regulated biomarkers simultaneously. The index was calculated for each combination of global change drivers. For each individual biomarker, the standardized mean was compared to a reference value,

here corresponding to the preferential environmental parameters for *P. borealis* (2 °C, pH 7.75, 100 % sat., Mucci et al., 2011, 2017; Orr & Sullivan, 2013; Siferd, 2015). The calculations were made as described in Caliani et al. (2021). The results were then summarized using a radarplot, where an area up to 0 represents biomarker induction while an area down to 0 represents biomarker inhibition.

Table 1. Corrected Akaike information criterion (AICc), ΔAICc, transformation applied, normality (Norm.) and homoscedasticity p-value for the multiple ANOVA models generated for each biomarker (Catalase [CAT], Total antioxidant capacity [TAC], Superoxide dismutase [SOD], Glutathione-S-Transferase [GST], Lipid peroxidation [LPO], Heat shock proteins 70 kDa [Hsp70] and Total ubiquitins [Ub]) used to characterize the cellular stress response of the northern shrimp *Pandalus borealis* exposed to combination of Temperature and pH in design A and Horizons (i.e. current Temperature and pH horizon, future Temperature and pH horizon) and O_2 levels in design B (see methods). The values in bold show the best models selected. Data transformation (Trans.) were either Log_{10}, square-root (sqrt) or cube-root (cbrt). Bootstrap method was used for biomarkers who were not reaching assumptions.

Variable	Experimental design	Model	AICc	ΔAICc	Transf.	Norm.	Homoscedasticity
CAT	A	**Temperature * pH**	**-391.4**	**0**	**-**	**0.227**	**0.5662**
		Temperature * pH * (1|Tank)	-314.8	76.6			
		Temperature * pH * (1|Tank/Treatment)	-310.7	80.7			
	B	**O_2 levels * Horizons**	**-264.1**	**0**	**Sqrt**	**0.6780**	**0.624**
		O_2 levels * Horizons * (1|Tank)	-223.7	40.4			
		O_2 levels * Horizons * (1|Tank/Treatment)	-220.8	43.3			
TAC	A	**Temperature * pH**	**-1248**	**0**	**Sqrt**	**0.290**	**0.276**
		Temperature * pH * (1|Tank)	-1100	148			
		Temperature * pH * (1|Tank/Treatment)	-1097	151			
	B	**O_2 levels * Horizons**	**-844.4**	**0**	**-**	**0.512**	**0.00052**
		O_2 levels * Horizons * (1|Tank)	-743	101.4			
		O_2 levels * Horizons * (1|Tank/Treatment)	-740.1	104.3			
SOD	A	**Temperature * pH**	**187.2**	**0**	**Log_{10}**	**0.228**	**0.206**
		Temperature * pH * (1|Tank)	192.3	5.1			
		Temperature * pH * (1|Tank/Treatment)	195	7.8			
	B	**O_2 levels * Horizons**	**134.2**	**0**	**-**	**0.863**	**0.491**
		O_2 levels * Horizons * (1|Tank)	137.8	3.6			
		O_2 levels * Horizons * (1|Tank/Treatment)	140.7	6.5			
GST	A	Temperature * pH	613.5	48.8			
		Temperature * pH * (1|Tank)	**564.7**	**0**	**-**	**0.576**	**0.095**
		Temperature * pH * (1|Tank/Treatment)	567.5	2.8			
	B	O_2 levels * Horizons	415.6	24.6			
		O_2 levels * Horizons * (1|Tank)	**391**	**0**	**-**	**0.415**	**0.409**

		O_2 levels * Horizons * (1|Tank/Treatment)	394	3			
LPO	A	**Temperature * pH**	**-746.1**	**0**	**Sqrt**	**0.128**	**0.707**
		Temperature * pH * (1|Tank)	-647.7	98.4			
		Temperature * pH * (1|Tank/Treatment)	-644.9	101.2			
	B	**O_2 levels * Horizons**	**-481.4**	**0**	**Sqrt**	**0.289**	**0.563**
		O_2 levels * Horizons * (1|Tank)	-416.6	64.8			
		O_2 levels * Horizons * (1|Tank/Treatment)	-413.7	67.7			
Hsp70	A	**Temperature * pH**	**-1213**	**0**	**Bootstrap**		
		Temperature * pH * (1|Tank)	-1075	138			
		Temperature * pH * (1|Tank/Treatment)	-1072	141			
	B	**O_2 levels * Horizons**	**-1046**	**0**	**Bootstrap**		
		O_2 levels * Horizons * (1|Tank)	-924	122			
		O_2 levels * Horizons * (1|Tank/Treatment)	-921.1	124.9			
Ub	A	**Temperature * pH**	**-1857**	**0**	**Bootstrap**		
		Temperature * pH * (1|Tank)	-1648	209			
		Temperature * pH * (1|Tank/Treatment)	-1646	211			
	B	**O_2 levels * Horizons**	**-1213**	**0**	**-**	**0.111**	**0.0910**
		O_2 levels * Horizons * (1|Tank)	-1075	138			
		O_2 levels * Horizons * (1|Tank/Treatment)	-1072	141			

4 Results

4.1 Antioxidants biomarkers analyses

Results for the investigation of the effect of OW, OA and hypoxia on each antioxidants biomarker are reported in Table 2 and Figure 4. In general, they presented different responses following the exposure to global change drivers in isolation and combined.

Design A - Exposure to low pH caused a significant increase in mean CAT activity at 2 °C, whilst no significant change in mean CAT activity was detected under low pH at 6 and 10 °C (Table 2, Figure 4a), whilst at current pH, there was a gradual increase in mean CAT activity between 2, 6 and 10 °C (Table 2, Figure 4a), as indicated by the presence of a significant interaction between the terms *Temperature* and *pH* for ($F_{2,54}$ = 7.467, p-value = 0.001; Table 2, Figure 4a). Mean TAC was also differently affected by temperature at the different pH tested, as indicated by the presence of a significant interaction between *Temperature* and *pH* ($F_{2,54}$ = 3.994, p-value = 0.024; Table 2, Figure 4b). In more detail, exposure to current pH caused a significant increase in mean TAC from 6 to 10 °C, but no change at 2 °C (Table 2, Figure 4b). Differently, no effect of temperature was reported at low pH (Table 2). Also mean GST and SOD activity were affected by a significant interaction between *Temperature* and *pH* ($F_{2,54}$ = 3.980, p-value = 0.024, $F_{2,54}$ = 3.284, p-value = 0.045, respectively; Table 2). However, here no difference was detected by the post-hoc test. There was no effect of *Temperature* and *pH* on mean LPO (Table 2).

Design B - Mean CAT activity changed significantly according to different *O_2 level* when shrimp were exposed to different *Environmental* horizon, as indicated by the presence of a significant interaction between these two terms ($F_{1,36}$ = 10.657, p-value = 0.002; Table 2, Figure 4e). In more detail, exposure to low O_2 conditions did not exert a significant effect on mean CAT activity under both current and future environmental horizons, whilst at control O_2 conditions the exposure to future environmental horizon caused a significant increase on mean CAT from current environmental horizon (Table 2, Figure 4e). There was no effect of low O_2 on any other antioxidant biomarkers (i.e. TAC, SOD, GST, LPO; Table 2).

4.2 Protein chaperones and protein quality control system biomarkers analyses

Protein chaperones biomarkers also presented different responses following the exposure to global change drivers in isolation and combined.

Design A - Both mean Hsp70 and Ub decreased as a consequence of the exposure to low pH (p-value = 0.001 and p-value = 0.015, respectively; Table 2, Figure 4c and Figure **4**d), with no significant effect of temperature or the interaction between *Temperature* and *pH* (minimum p-value = 0.202; Table 2, Figure 4c and Figure **4**d).

Design B - An increase in mean Ub was observed under the exposure to low O_2 conditions independently of the environmental horizon (Table 2, Figure 4f), with only the terms *O_2* being significant (Table 2, Figure 4f). There was no effect of environmental horizons and low O_2 on mean Hsp70 (Table 2).

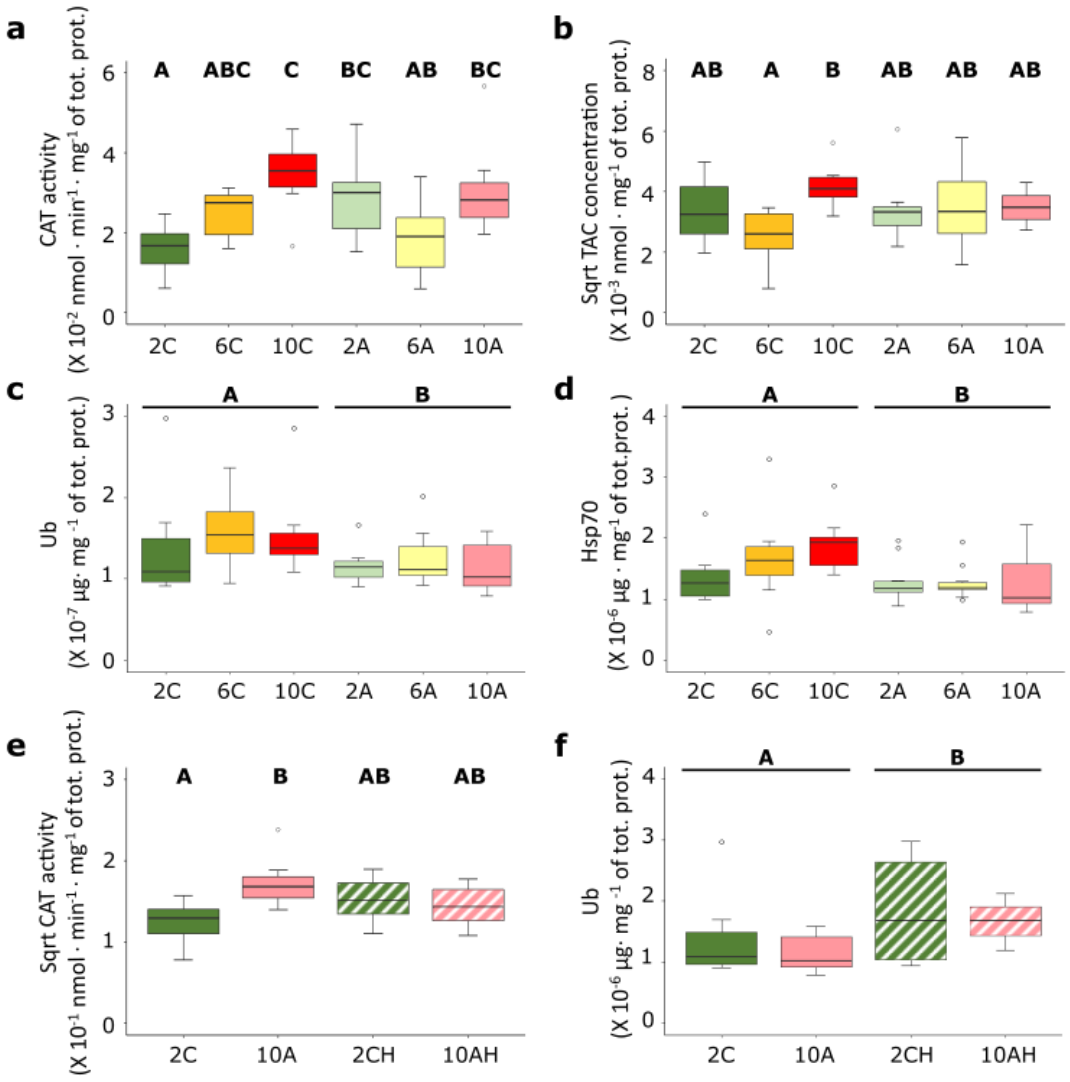

Figure 4. Effect of exposure of adult non-ovigerous females of the northern shrimp *Pandalus borealis* to **a-d)** combined high temperature and low pH (isolated and combined) (n=60) and **e-f)** future environmental horizon (high temperature and low pH) and low O_2 (n=40), on **a)** catalase (CAT) activity (nmol min^{-1} mg total $protein^{-1}$), **b)** Sqrt total antioxidant capacity (TAC) concentration (nmol mg total $protein^{-1}$), **c)** total ubiquitins (Ub) concentration (µg mg total $protein^{-1}$), **d)** heat shock proteins 70 kDa (Hsp70) (µg mg total $protein^{-1}$), **e)** Sqrt CAT activity (nmol min^{-1} mg total $protein^{-1}$) and **f)** Ub concentration (µg mg total $protein^{-1}$). Each box plot corresponds to a treatment for each design (A and B), identified as following **Code Treatment – Temperature/pH/% O_2 sat.**: (2C - 2 °C/7.75), (2A - 2 °C/7.40), (6C - 6 °C/7.75), (6A - 6 °C/7.40), (10C - 10 °C/7.75), (10A – 10 °C/7.40), (2CH - 2 °C/7.75/35 % sat.) and (10AH - 10 °C/7.40/35 % sat). Box plots represent treatment delimited by lower and upper quartile. Plain lines indicate the median, bars represent data outside the 25^{th}-75^{th} percentiles range while empty circles indicate extreme values exceeding the 95 % for each treatment. Capital letters indicate differences between treatments following a Tukey's post hoc.

Table 2. Summary of the analysis of variance (ANOVA) of catalase (CAT), glutathione-s-transferase (GST), superoxide dismutase (SOD), lipid peroxidation (LPO), Total antioxidant capacity (TAC), Heat shock proteins 70 kDa (Hsp70) and Total ubiquitins (Ub) from the effects of exposure of adult non-ovigerous females of the northern shrimp *P. borealis* to **a)** combined high temperature and low pH (isolated and combined) (n = 60) and **b)** future environmental horizon (high temperature and low pH) and low O_2 (n = 40). Significant values are reported in bold. For both Hsp70 and Ub in design A and Hsp70 in design B, p-value were obtained with a bootstrap method.

Biomarkers		Model	Design A				Design B		
			df	F	p-value		df	F	p-value
Antioxidants	CAT	***Temperature***	**2**	**9.378**	**< 0.001**	***Horizon***	**1**	**5.663**	**0.023**
		pH	1	0.124	0.726	*O_2 level*	1	0.0003	0.986
		Temperature*pH	**2**	**7.467**	**0.001**	***Horizon* O_2 level***	**1**	**10.657**	**0.002**
		Residuals	54			*Residuals*	36		
	TAC	***Temperature***	**2**	**3.788**	**0.029**	*Horizon*	1	-	0.684
		pH	1	0.125	0.725	*O_2 level*	1	-	0.165
		Temperature*pH	**2**	**3.994**	**0.024**	*Horizon* O_2 level*	1	-	0.615
		Residuals	54			*Residuals*	36	-	
	GST	*Temperature*	2/54	1.931	0.155	*Horizon*	1/36	1.491	0.230
		pH	1/54	0.005	0.943	*O2 level*	1/36	0.055	0.817
		Temperature*pH	**2/54**	**3.980**	**0.024**	*Horizon* O2 level*	1/36	3.329	0.076
	SOD	*Temperature*	2	0.206	0.815	*Horizon*	1	0.589	0.449
		pH	1	1.286	0.262	*O_2 level*	1	0.282	0.599
		Temperature*pH	**2**	**3.284**	**0.045**	*Horizon* O_2 level*	1	2.022	0.165
		Residuals	54			*Residuals*	32		
	LPO	*Temperature*	2	1.068	0.351	*Horizon*	1	0.625	0.434
		pH	1	1.750	0.192	*O_2 level*	1	0.949	0.337
		*Temperature*pH*	2	1.034	0.363	*Horizon* O_2 level*	1	0.027	0.871
		Residuals	54			*Residuals*	36		
Proteins chaperones	Hsp70	*Temperature*	2	-	0.206	*Horizon*	1	-	0.549
		pH	**1**	-	**0.001**	*O_2 level*	1	-	0.067
		*Temperature*pH*	2	-	0.202	*Horizon* O_2 level*	1	-	0.232
		Residuals	54	-		*Residuals*	36	-	

Proteins control	**quality**	Ub	*Temperature*	2	-	0.546	*Horizon*	1	1.045	0.314
			pH	**1**	-	**0.015**	*O_2 level*	1	7.718	**0.009**
			*Temperature*pH*	2	-	0.801	*Horizon* O_2 level*	1	0.006	0.941
			Residuals	54	-		*Residuals*	36		

4.3 Integration of biomarkers responses

The cellular stress response (CSR) of *P. borealis* exposed to OW, OA and hypoxia is reported in Table 3 and Figure 5. Patterns of CSR, both regarding antioxidant (Figure 5a) and protein quality control (Figure 5b) biomarkers, showed substantially different patterns and IBRv2 values (Table 3). In more detail:

Design A - Higher values of IBRv2 were found in the 2 °C low pH treatment compared to the other treatments, with other treatments presenting comparable IBRv2 values (Table 3, Figure 5a). CAT appeared to be the biomarker having the greatest effect on the CSR (Table 3, Figure 5a).

Design B - The highest IBRv2 value was found at 2 °C current pH and low O_2 treatment, followed by that measured at 10 °C low pH (Table 3, Figure 5b). The 10 °C low pH and low O_2 treatment presented the IBRv2 value that was the closest to the 2 °C current pH reference treatment (Table 3, Figure 5b). No biomarker appeared to exert a significantly greater effect, when compared to other biomarkers, on the CSR when shrimp were exposed to the different environmental horizons and the low O_2 conditions (Table 3, Figure 5b).

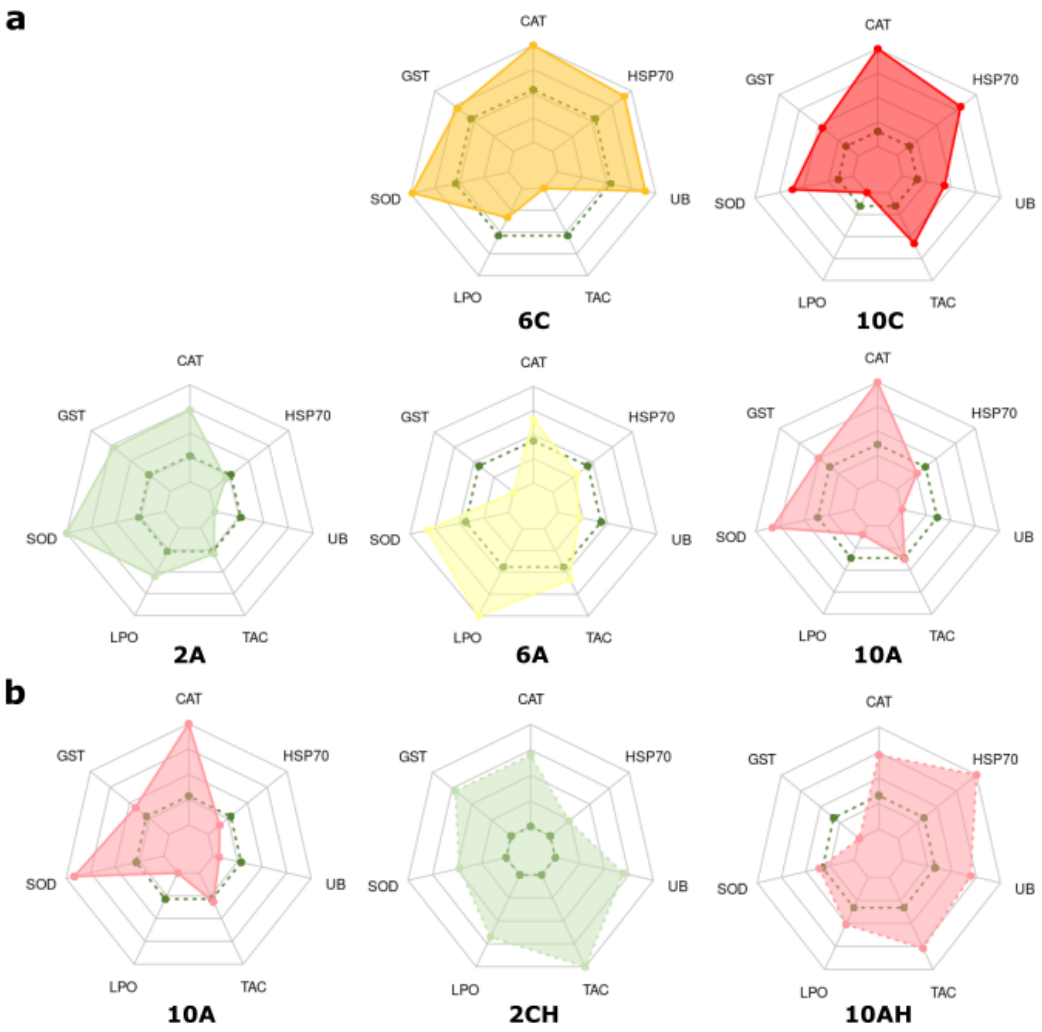

Figure 5. Representation of the integrated biomarker responses (IBRv2) of catalase (CAT), glutathione-s-transferase (GST), superoxide dismutase (SOD), lipid peroxidation (LPO), total antioxidant capacity (TAC), total ubiquitins (Ub) and heat shock proteins 70 kDa (Hsp70) depicting the effect of a 30-d exposure of females *Pandalus borealis* (n=80) to **a)** elevated temperature and low pH (isolated and combined), and **b)** future environmental horizon (elevated temperature and low pH) and hypoxia. Each radar plot corresponds to a treatment for each design (A and B), identified as following by Code Treatment – Temperature/pH/% O_2 sat.: (2C - 2 °C/7.75), (2A - 2 °C/7.40), (6C - 6 °C/7.75), (6A - 6 °C/7.40), (10C - 10 °C/7.75), (10A – 10 °C/7.40), (2CH - 2 °C/7.75/35 % sat.) and (10AH - 10 °C/7.40/35 % sat).

Table 3. Index values of the integrated biomarker responses (IBRv2) of catalase (CAT), glutathione-s-transferase (GST), superoxide dismutase (SOD), lipid peroxidation (LPO), Total antioxidant capacity (TAC), Heat shock proteins 70 kDa (Hsp70) and Total ubiquitins (Ub) depicting the effect of a 30-d exposure of females *Pandalus borealis* (n=80) to **a)** elevated temperature and low pH (isolated and combined) and **b)** future environmental horizon (elevated temperature and low pH) and hypoxia. Each treatment for both design (A and B) are identified as following **Code Treatment – Temperature/pH/% O_2 sat.**: (2C - 2 °C/7.75), (2A - 2 °C/7.40), (6C - 6 °C/7.75), (6A - 6 °C/7.40), (10C - 10 °C/7.75), (10A – 10 °C/7.40), (2CH - 2 °C/7.75/35 % sat.) and (10AH - 10 °C/7.40/35 % sat). The highest index value among biomarkers for each treatment and the highest IBRv2 value among treatment for each design are reported in bold.

Treatment		CAT	GST	SOD	LPO	TAC	Ub	Hsp70	IBRv2 value
a	2C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2A	2.00	1.88	**3.09**	1.20	0.11	-1.13	-0.28	**8.28**
	6C	**1.49**	0.56	1.43	-0.67	-1.76	1.15	1.20	5.92
	6A	0.58	-1.13	1.00	**1.46**	0.38	-0.59	-0.36	4.55
	10C	**2.62**	0.93	1.44	-0.47	1.32	0.86	2.01	6.80
	10A	**2.13**	0.48	1.56	-0.89	0.05	-1.21	-0.36	5.11
b	2C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2CH	1.48	1.50	1.01	1.43	**2.12**	1.47	0.50	**7.54**
	10A	**2.42**	0.48	2.14	-0.97	0.11	-0.76	-0.48	6.13
	10AH	1.09	-0.90	0.09	0.50	1.21	1.00	**1.84**	3.79

5 Discussion

We show that the cellular stress response (CSR) through antioxidants and protein quality control mechanisms of the abdominal muscle of adult female of the northern shrimp *Pandalus borealis* individuals exposed to global change scenarios is negatively affected by the chronic exposure to OW, OA and hypoxia. In more detail, OA tends to overwhelm the CSR of both the antioxidants and protein chaperon pathways. Differently, we show that OW affects mostly the antioxidants response of the northern shrimp female, whilst hypoxia mainly affects the protein quality control system. Furthermore, we show that the complexity of the CSR is expressed throughout various pathways and appears to show a high degree of inter-individual variation, as biomarkers do not respond in a single clear pattern through treatments and that their important variation among individuals reduce the potential to find significant differences. Our discussion focuses on the CSR to global change of the tolerant individuals that survived the experimental exposure. Measurements were performed on tolerant individuals (i.e., survivors of one month of exposure to the experimental conditions) that survived the experiment, which represents approximately 20% of the initial individuals for all treatments, excluding treatments corresponding to the temperature and pH expected for 2100 under normoxic (~25%) and hypoxic (~50%) conditions (Chemel et al., 2020). The aim is to provide a better understanding of the stress response mechanisms under acclimation to future environmental conditions within the context of ecology.

5.1 Antioxidant response to global change drivers

Increase in environmental temperature in invertebrates is known to induce an increase in metabolic rates (Schulte, 2015; Vernberg & Vernberg, 1969), and therefore an increase in aerobic respiration within mitochondria, where the majority of ROS are produced as by-products (Finkel & Holbrook, 2000). However, certain doubts remain to whether a higher aerobic respiration truly modify *in vivo* ROS levels, inducing higher level of oxidative stress thereby an antioxidant response (Salin et al., 2015). Thus, the complexity of the response we report for specimens exposed to OW in combination with another driver, such as OA, is as we expected. We find that the significant interactions between OW and OA lead to a moderate antioxidant response in shrimp, as only CAT and TAC among all studied biomarkers increase. Thus, OW seems to be preponderant in the modulation of antioxidants, including both CAT activity and TAC, when compared to OA. In the case of CAT activity, the pH effect is temperature specific as we see that the positive effect of OA

is only observable at low temperature. The absence of oxidative damage to the cell membrane confirms by stable LPO levels establish the cellular capacity of tolerant individuals to manage the oxidative stress level they were exposed to. This suggests that the level of oxidative stress caused by OW was moderate, as the individuals presented a moderate response accordingly. Knowing that CAT is a first line defense antioxidant, this enzyme acts rapidly to neutralize potentially harmful molecules (H_2O_2) that could develop into free radicals or even free radicals that could induce production of other radicals (Ighodaro & Akinloye, 2018). Being a global indicator of non-enzymatic antioxidant activity, TAC is also an important component of the antioxidant response (Kumar, 2012). It appeared that *P. borealis* demonstrated sufficient plasticity in antioxidant response by increasing CAT activity and TAC to counter the oxidative stress generated by OW in isolation. This result suggests that the tolerant individuals might overcome oxidative stress with a moderate antioxidant response. Our results generally agree with those reported by previous studies, which reported variation in antioxidant response with organisms exposed to OW, but also reported a complexification of the response with an exposure to combined drivers. For instance, the common prawn *Palaemon serratus* (Pennant, 1777) and the rockpool shrimp *Palaemon elegans* (Rathke, 1836) presented an increase in CAT activity with moderate elevation from control, but also a decrease in CAT activity at extreme temperature conditions under an acute thermal challenge (Vinagre et al., 2014). An increase in TAC was also observed when exposed to elevated temperature in marine gastropods (Leung et al., 2019) and in the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) (Duan et al., 2018). Although, an alternating increase and decrease in antioxidant response was also observed in coastal fishes (Madeira et al., 2013). Finally, when exposed to a combination of OW and OA, copepods demonstrated a reduced antioxidant capacity (Vehmaa et al., 2013), while the mussel *Mytilus coruscus* (Gould, 1861) (Khan et al., 2021) and the commercial top shell *Trochus niloticus* (Linnaeus, 1767) (Zhang et al., 2021) presented significant decrease in CAT activity. In the case of the tropical peppermint shrimp *Lysmata lipkei* (Okuno & Fiedler, 2010) subjected to simulated OW, an increase in antioxidant enzyme activity (particularly CAT) and LPO was observed in the muscle (Madeira et al., 2019). Moreover, increases in Hsp70 and Ub were also detected in the gills (but not in the muscle) of *L. lipkei* shrimp exposed to increased temperature (Madeira et al 2018).These results added to ours suggest that the increase in temperature is an important inducer of oxidative stress in crustaceans and other

ectotherms, but that the addition or the increase of intensity and duration of exposure to drivers might cause a decrease in the antioxidant response.

Considering the current level of dissolved oxygen in the SLE system being already less than 20 % in the bottom water layer (>150 m; DFO, 2020c), the impact of hypoxia on marine invertebrates is an important factor to include in our evaluation of the combined impacts of global change. Nevertheless, we report no difference in the antioxidant response among normoxic and hypoxic conditions (35 % O_2 sat.), indicating that the exposed individuals do not develop oxidative stress nor a response to differing O_2 level. The SLE northern shrimp long-termed acclimation to high level of hypoxia could explain the absence of an antioxidant response, confirming that this species is especially tolerant to hypoxia. Dupont-Prinet et al. (2013) revealed that the critical O_2 threshold of this species is lower than 23 % DO sat. In addition, specific adaptations to low level of O_2 are common among marine zooplankton and fish species living in oxygen minimum zones, having evolved in low O_2 habitats (Roman et al., 2019). These results highlight the great tolerance of *P. borealis* to hypoxic conditions relatively to oxidative stress, but do not always reflect the conclusion of precedent studies in other shrimp species. For instance, in the *L. vannamei*, hypoxia seems to generally increase the antioxidant response (defined as increases in mean CAT and SOD) in the hepatopancreas and the muscle (Parrilla-Taylor & Zenteno-Savín 2011). However, for the same specie, Estrada-Cárdenas et al. (2021) observed no difference in mean CAT activity in the hepatopancreas and the gills, despite the hepatopancreas normally showing higher antioxidant defense activity than other organs (Ruppert et al., 2004).

Although a heterogenous antioxidant response was observed in *P. borealis'* muscle, with an increase in SOD but no response from CAT at 22 % sat. (Dupont-Prinet et al., 2013). While these results might be comparable, we have to take into account that in addition to being tissue-specific in the study by Parrilla-Taylor & Zenteno-Savín (2011) and Estrada-Cárdenas et al. (2021), the antioxidant response was also analyzed through exposure to short-term hypoxic conditions while our study exposed the shrimp to chronic hypoxic conditions. Although, it seems that an antioxidant response can normally be induced in hypoxia-resistant species as part of the mechanism preparing the organism to the aerobic metabolism boost following the return to normoxic conditions (Hermes-Lima & Zenteno-Savín, 2002; Lushchak et al., 2001; Moreira et al., 2016; Pannunzio &

Storey, 1998; Parrilla-Taylor & Zenteno-Savín, 2011). This fact would then explain the variation among acute and chronic hypoxia exposure studies.

5.2 Protein quality control system response to global change drivers

Environmental challenges often lead to proteotoxic damages for which chaperones proteins, such as Hsp70, are produced to maintain cellular homeostasis, by either re-folding damaged proteins, stabilising denatured proteins, or triaging damaged proteins for degradation in the proteasome (Somero et al., 2017). When proteins are irreversibly damaged, ubiquitin tags these proteins so that they can be sent for degradation in the proteasome, thus functioning as a marker of enhanced proteolysis in the cell (Somero et al., 2017). Unexpectedly, our results show that exposure to a chronic thermal stress of + 4 °C and + 8 °C from optimal temperature in *P. borealis* is not sufficient to trigger an increase in the chaperone Hsp70, nor in ubiquitin, suggesting that OW does not extensively increase protein damage in *P. borealis* muscle. This suggests that protein functions will be preserved under future warming scenarios alone. However, under combined global change drivers' scenarios, a loss of homeostasis leading to mortality could still be caused by the cumulative or interactive effect of another environmental drivers with OW.

Our results do not seem to agree with the extant literature, which generally report increases in chaperones molecules following an exposure to acute or chronic thermal stress in marine species. However, diversification of molecular response do exists among species in response to OW (Clark et al., 2017). For example, Clark et al. (2008) demonstrated that cold-water molluscs up-regulated their Hsp70 gene expression under a temperature increase of + 6–8°C for the Antarctic soft-shell clam *Laternula elliptica* (P. P. King, 1832) and + 8–10°C for the limpet *Nacella concinna* (Strebel, 1908) after a 2-h shock treatment. Madeira et al. (2016) also observed in gilt-head bream *Sparus aurata* (Linnaeus, 1758) larvae an increase of both Hsp70 and Ub to a 7-d exposure to an increase in temperature within an ecologically realistic range. In contrast, in Brokordt et al. (2015), mature and spawned Peruvian scallop *Argopecten purpuratus* (Lamarck, 1819) individuals had a reduced capacity to increase Hsp70 mRNA levels following a 6 hours exposure to an acute thermal stress (18 to 24 °C in 24h), while Hsp70 activity did not differ from the control. Mohamad et al. (2018) demonstrated that after 30-d at 35 °C, a significant increase in Hsp70 relative expression level was observable in the hepatopancreas of giant freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879). Our results might differ from the literature because of a long-term acclimation to higher

temperatures than the species' optimal. Indeed, the *P. borealis* from the SLE is already exposed to temperatures of 6 °C, while other populations are commonly found in 8 °C water and sporadically in waters characterised by temperatures up to 12 °C (Apollonio et al., 1986b; Daoud et al., 2007; DFO, 2020b; Dupont-Prinet et al., 2013; Koeller, 2000; Shumway et al., 1985).

Interestingly, the protein quality control response does not follow the same pattern when shrimp were exposed to OA when compared to shrimp exposed to OW. Indeed, Hsp70 and Ub abundance decreased in response to OA at all temperature levels. On the contrary, the absence of a protein quality control response under OW indicates that elevated temperature does not impose extensive protein damage. The decrease of Hsp70 and Ub under OA implies that *P. borealis* presents an impaired CSR under acidification scenarios, which could lead to develop complications in the event of being exposed to additional environmental challenges. Our results are in agreement with previous studies, reporting that exposure to OA seems to increase the sensitivity of marine invertebrates to other stressors. For example, a study on *L. vannamei* confirmed that the exposure to a OA and hypoxia altered the transcriptome stress response with a decrease in the ubiquitin/proteasomal degradation pathway and the chaperones response, when compared to single factor response (Johnson et al., 2015). Madeira et al. (2014) demonstrated that exposure to acidified water and elevated temperature, the marbled rock crab *Pachygrapsus marmoratus* (Fabricius, 1787) did not exhibit a strong HSP production. In intertidal limpet *Cellana toreuma* (Reeve, 1854) sensitivity of the heat-shock response increased synergistically under exposure to combined OA and thermal stress (Wang et al., 2018). Finally, sea urchin larvae shown a lesser Hsp70 gene expression, so response ability, to thermal stress when previously acclimated to elevated CO_2 conditions (O'Donnell et al., 2009). The chaperones responses analyses in most studies seems to explain this result by the fact that Hsp70 synthesis, maintenance and Hsp70 genes up-regulation are highly energy-consuming mechanisms, and that a significant part of the energy budget is directed toward cellular homeostasis functions rather than to defense mechanisms (i.e. immune system, antioxidant and chaperones response) or growth and reproduction processes (Feder & Hofmann, 1999; Somero et al., 2017; Tomanek & Sanford, 2003; J. Wang et al., 2018).

In contrast to the homogeneous response of Hsp70 chaperone and Ub to OA among invertebrates, exposure to hypoxia only resulted in an increase in Ub, not Hsp70. This means that the decrease in environmental oxygen greatly contributed to cause irreversible damages to proteins, suggesting

that hypoxia is a major factor to consider in environmental studies, as the maintenance of proteins is essential to ensure cellular functionality (Somero et al., 2017). To the extent of our knowledge, no study reports on the impact of hypoxia on the Ub activity in marine invertebrates. Although, Johnson et al. (2015) observed a down-regulation of the ubiquitin-proteasomal degradation pathway under hypercapnic hypoxia. This result is not in accordance with our results, but the implication of hypercapnia and the fact that they observed transcriptomic response (mRNA levels) while we refer to protein quantity makes the comparison trivial.

5.3 Cellular stress response complexity expressed through various pathways

The CSR in its whole is expressed throughout various pathways, creating complex mechanisms that vary according to the environmental challenges the organisms are exposed to, as reported here. It fulfills four main functions, interrelated to ensure the maintenance of homeostasis and cellular functions. Indeed, the CSR is used to restore and defend the integrity of macromolecular systems, modulate energy metabolism in order to reallocate energy to the CSR system by limiting the support to other housekeeping cell functions, to regulate the cellular proliferation to ensure growth arrest when necessary and to program apoptosis if repair capacities are exceeded following extensive damages from exposure to stressors (Kültz, 2020a, 2020b; Somero, 2020). All of these functions must be performed simultaneously in order to adequately protect the organism from changing environmental conditions. However, it has been observed that CSR is not necessarily stressor-specific, since the cell stress level is based on the level of macromolecular damages, and not according to the factor creating such damages (Kültz, 2005).

Although we here deconstructed the impact on antioxidant, protein chaperones an Ub response to each driver (i.e. OW, OA and hypoxia), it is paramount to consider their combined effects. Indeed, this is paramount for environmental management to target most impactful stressors in order to direct protection effort where the need is more pressing. Among the various pathways and mechanisms of the CSR that may be triggered to respond to environmental challenges, our results demonstrated that the antioxidant response was mostly led by CAT accompanied by the modulation of the protein quality control system, including Hsp70 and Ub. Considering that CAT is a first line defense antioxidant enzyme (Ighodaro & Akinloye, 2018) and that beside TAC no other antioxidant response molecules (e.g. SOD or GST) were found significantly induced. This suggests that the surviving individuals were not under a great oxidative stress. The importance of the protein quality

control system in the shrimp's muscle CSR suggests that the protein damages might be the reason for the loss of functions leading to the mortality of individuals as seen in Chemel et al. (2020). Their role as protein restorers (Hsp70) and as destruction of irreversibly damaged protein (Ub) being clearly limited by OA, and the importance of irreversible protein damages with hypoxia suggest that the cellular integrity of *P. borealis* would be limited in future environmental conditions. These findings are in agreement with those by Madeira et al. (2016), who observed that CAT, Hsp70 and Ub activity were the most correlated biomarkers to mortality in *S. aurata* larvae under OW among the other biomarkers studied (i.e. GST, SOD, LPO, protein carbonylation). This validates the importance of CAT and protein chaperones response in CSR analysis.

In general, surviving *P. borealis* females seemed to maintain cellular homeostasis in OW conditions, but far less when exposed to a combination of drivers. The plastic capacity to acclimate to different environmental challenges is beneficial for the species but does not seem sufficient to counter the expected future environmental conditions of the SLE. Indeed, the addition of OA seems to overwhelm the CSR capacity. Hypoxia seems to act similarly to OA, being an aggravating factor that prevents cellular homeostatic regulation. Our results provide a mechanistic underpinning to the mortality patterns reported for P. borealis exposed to combined global change drivers (Chemel et al., 2020; Guscelli et al., *in prep*), stating that the individuals exposed to the highest level of OW and to OA, in normoxic (10A) and hypoxic conditions (10AH), were the ones most impacted.

6 Conclusion

Although our results regarding the antioxidant and protein quality control responses in *P. borealis* muscle are indicative of the mechanisms underlying CSR in females, further knowledge of acclimation processes on cold water stenotherms is needed. Indeed, in addition to being species specific, numerous studies have demonstrated the tissue (e.g. Estrada-Cárdenas et al., 2021; Parrilla-Taylor & Zenteno-Savín, 2011), life stage (e.g. Brokordt et al., 2015), sex (e.g. Dupont-Prinet et al., 2013) and even individual specificity of this type of molecular responses. Broader studies could be considered. However, the physiological results obtained here demonstrate the sensitivity of tolerant individuals of adult females *P. borealis* to future environmental conditions. Although the impact of an increase in bottom temperature up to 10 °C does not appear to be problematic in terms of oxidative stress and protein damage, the combination of ocean warming with ocean acidification and hypoxia appears to disrupt the CSR plastic capacity of the species. The socio-economic value of this species will most likely change in the coming decades, at the same time that its ecological functions will be greatly limited within the St. Lawrence estuary ecosystem.

CHAPITRE 2

EFFETS COMBINÉS DU RÉCHAUFFEMENT ET DE L'ACIDIFICATION DES OCÉANS ET DE L'HYPOXIE SUR LA PLASTICITÉ DU PROTÉOME DE LA CREVETTE NORDIQUE *PANDALUS BOREALIS*

RESUME EN FRANÇAIS DU DEUXIEME ARTICLE

Les changements globaux et climatiques induisent des altérations significatives des propriétés physico-chimiques des écosystèmes qui peuvent conduire les organismes à présenter des réponses physiologiques diverses à leur environnement changeant. Comme les organismes sont naturellement exposés à plusieurs facteurs environnementaux changeant simultanément, une approche multifactorielle est essentielle pour évaluer les réponses des organismes. Dans le cas de l'estuaire du Saint-Laurent (ELS), une exposition des organismes au réchauffement des océans (RO), à l'acidification des océans (AO) et à l'hypoxie est attendue. En utilisant ce système comme modèle d'étude, ce projet vise à évaluer les effets combinés du RO, de l'AO et de l'hypoxie sur la plasticité du protéome de la crevette nordique *Pandalus borealis*, une espèce de grande importance écologique et économique dans l'Est du Canada. Nous avons donc exposé les crevettes à différents niveaux de température (2, 6 et 10 °C), pH (7.75 et 7.40) et de saturation en oxygène (100 % et 35 % sat.) de façon isolée et combinée pendant 30 jours. L'utilisation de techniques de protéomique nous a permis d'identifier sur les individus survivants un total de 592 protéines, sur lesquelles seules neuf ont révélé des différences significatives. L'AO a provoqué l'augmentation d'une protéine impliquée dans l'adhésion cellulaire, et une diminution des protéines impliquées dans la régulation de la transcription et à la dynamique du cytosquelette. L'hypoxie a provoqué l'augmentation d'un facteur de modification de la chromatine et la diminution des protéines impliquées dans la dynamique du cytosquelette. Le RO n'a pas semblé exercer une variation dans le protéome de *P. borealis*. Ainsi, la plasticité limitée du protéome pourrait être le résultat d'une absence de plasticité phénotypique, ou encore d'une tolérance naturelle élevée obtenue à partir de phénotypes diversifiés dans la population de l'ESL. L'utilisation d'une approche protéomique a permis d'ouvrir une fenêtre sur la sensibilité d'une espèce socio-économiquement importante aux changements environnementaux en cours, dans un système estuarien déjà complexe, régi par l'interaction entre ses composantes.

THE COMBINED EFFECTS OF OCEAN WARMING, OCEAN ACIDIFICATION AND HYPOXIA ON THE PROTEOME PLASTICITY OF THE NORTHERN SHRIMP *PANDALUS BOREALIS*

Maude Boissonneault[1], Ella Guscelli[1], Diana Madeira[2] and Piero Calosi[1]

[1] Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski, Rimouski, Québec, Canada
[2] ECOMARE, Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Aveiro, Portugal

1 Abstract

Global and climate changes induce significant alterations in physicochemical properties of ecosystems that can lead organisms to exhibit diverse physiological responses to their changing environment. As organisms are naturally exposed to several environmental factors changing simultaneously, a multifactorial approach is essential to assess organism responses. In the case of the St. Lawrence Estuary (SLE), exposure of organisms to ocean warming (OR), ocean acidification (OA) and hypoxia is expected. Using this system as a case study, this study aims at assessing the combined effects of OW, OA and hypoxia on the proteome plasticity of the northern shrimp *Pandalus borealis*, a species of high ecological and economic importance in Eastern Canada. First, we exposed adult female shrimp to different levels of temperature (2, 6 and 10 °C), pH (7.75 and 7.40) and oxygen saturation (100 % and 35 % sat.) in isolation and combined for 30 days. The use of SWATH mass-spectrometry based proteomics techniques on surviving individuals allowed us to identify a total of 592 proteins, among which nine were revealed to be differentially abundant between global change scenarios. OA caused the increase of a protein related to cell adhesion, and a decrease of proteins related to chromatin modification and cytoskeletal dynamic. Hypoxia triggered the increase of chromatin modification factor and the decrease of proteins related to cytoskeletal dynamic. OW when faced alone did not exert a significant effect on the proteome of *P. borealis*. In general, the limited phenotypic plasticity of the northern shrimp observed makes it at risk from future environmental changes, considering that in addition to the progression of OW, OA and hypoxia, the frequency or intensity of extreme events are expected to increase. The use of a proteomic approach allowed to open a window onto the sensitivity of a socio-economically important specie to ongoing environmental changes, in an already complex estuarine system driven by the dynamic interaction between different environmental drivers.

2 Introduction

The commercial exploitation of marine species has been historically an important industrial sector for the revenue that it generates, and it has also contributed to food security and socio-economic development of many communities around the World (FAO, 2020). However, global change projections pose a significant threat to species of commercial interest, in addition to causing severe ecological imbalances (IPCC, 2021). For instance, overfishing, pollution (e.g. plastics, mercury, etc.), ocean warming (OW) and ocean acidification (OA) are known to negatively affect a vast range of species, making fisheries at risk under future expected global change scenarios (Issifu et al., 2022).

Different global change drivers will modify the oceanographic conditions of marine environments, with OW, OA and ocean deoxygenation (or hypoxia) expected to be among the most impactful drivers (IPCC, 2021). Thus, commercially exploited species are expected to exhibit physiological modifications in response to those environmental changes, leading to possible changes in body size, growth, development, and ultimately mortality rates (Calosi et al., 2019; Cheung et al., 2013; Poloczanska et al., 2016), having important consequences on fisheries by inducing changes in distribution, a decrease in abundance and a reduction in body size and reproduction output (FAO et al., 2018). For instance, Cheung et al. (2013) modelling work revealed that a reduction in body size from 14 to 24 % in fishes is expected from the 2000 to 2050 under high-emission scenario (comparable to RCP 8.5). Fernandes et al. (2017) modelling work showed patterns of response on demersal fishes and shellfish biomass projections. Indeed, they observed in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) that the impacts of OA was limited in comparison with OW, while an increase in biomass was observed at low pCO_2 (but not at higher emissions) (Fernandes et al., 2017). Also, OW caused an increase in biomass in Atlantic cod *Gadus morhua* (Linnaeus, 1758), but the combination of OW and OA exerted negatives impacts (Fernandes et al., 2017).

The tolerance and resilience of these marine commercial species to environmental changes is therefore essential to ensure the sustainability of resources, as well as the maintenance of ecosystems integrity. Global fisheries predominantly target fishes and crustaceans, with marine catches representing 84.4 million tonnes in 2018, including 71.9 million tonnes of finfishes and 6.0 million tonnes of crustaceans (FAO, 2020). Capture capacity could decline sharply in the coming

decades, specifically in the environments most affected by global change drivers (FAO et al., 2018).

The St. Lawrence Estuary (SLE) is not immune to these projections. The bottom temperature of the SLE is currently characterized by a temperature of 5-6 °C (DFO, 2020b), a pH of 7.6-7.75 (DFO, 2017) and a dissolved oxygen saturation (DO sat.) below 20 % (DFO, 2020c), which is close to the limit of sustainability for most living organisms (Diaz & Rosenberg, 1995; Vaquer-Sunyer & Duarte, 2008). By 2100, brought by the increase of atmospheric CO_2 from the demographic growth and industrial activities, global change are expected to globally lead to an increase in ocean's temperature and an increase in pCO_2/decrease in pH (hereafter decrease in pH) of 4 °C and 0.3 pH unit, respectively: according to Representative Concentration Pathway RCP 8.5 (IPCC, 2014). The temperature of SLE's bottom waters is therefore projected to reach 10 °C and a pH of 7.40, as well as present severely hypoxic (to almost anoxic) conditions. This environmental scenario is expected to affect the physiology of most organisms.

To assess the impacts of these changes on marine organisms according to future predictions, state-of-the art proteomics technology may be a usefool tool. The environmental sensitivity of the proteome can give us insights about physiological processes and the role of phenotypic plasticity in changing environments, ultimately contributing to understand the ecology and evolution of species (Diz et al., 2012; Silvestre et al., 2012). The proteome is closely linked to the cellular and organismal phenotype (as proteins are the functional units of the cell, they give more information on the phenotype when compared to genome or transcriptome) (Diz et al., 2012; Silvestre et al., 2012). Proteome signatures can be modified considerably over short periods of time (Ali-Khan et al., 2002), leading to new cellular phenotypes under new environmental conditions, potentially improving the performance/fitness of the organism (Silvestre et al., 2012). Thus, proteins can be found in stable concentrations in the cell but can be under- or over-abundant when needed to ensure homeostatic functions, cells repair or protein stability (Kültz, 2005). This process allows cells to express various cellular phenotypes in response to specific environmental circumstances (Tomanek, 2011). The variations within the capacity of proteome plasticity between species, populations or individuals will then determine their ability to face fluctuations in their environment. For instance, acute heat stress can unbalance cellular functions, possibly leading to protein dysfunction, denaturation or aggregation and oxidative stress (Fields et al., 2016). This loss of

homeostasis threatens the integrity of the cell and the whole organism (Kültz, 2005). Therefore, the plasticity of the proteome may allow organisms to buffer or even correct these imbalances. An organism can in fact not only produce enzymes that help eliminating reactive oxygen species (ROS), but also increase of the production of chaperone proteins, enhance proteolysis, or produce structural proteins to ensure the cytoskeletal dynamics, as well as regulate proteins involved in acid-base balance, etc. (e.g. Artigaud et al., 2015; Chang et al., 2016a; Timmins-Schiffman et al., 2014).

Proteomics studies within the context of global change have already been carried out in various marine organisms including the great scallop *Pecten maximus* (Linnaeus, 1758) (Artigaud et al., 2015), the brine shrimp *Artemia sinica* (Cai, 1989) (Chang et al., 2016b) or the Pacific oysters *Crassostrea gigas* (Thunberg, 1793) (Timmins-Schiffman et al., 2014). Proteomic responses to OW usually involve the enhancement of epigenetic gene regulation and cytoskeletal remodeling (Fields et al., 2012), whereas under OA organisms usually involve the regulation of the antioxidant response, carbohydrate metabolism and transcription pathways (Timmins-Schiffman et al., 2014). Regarding hypoxia, proteins associated with protein modifications, signaling, energy metabolism and cytoskeleton were observed to change (Artigaud et al., 2015). However, studies on the proteomic response of these three drivers combined on commercial species are still scarce.

As there is a pressing need to evaluate the tolerance of commercial species under future environmental scenarios , the aim of this study is to characterize in detail the proteome signatures of a commercial marine ectotherm exposed to combined global change drivers: OW, OA and hypoxia. To do so, the northern shrimp *Pandalus borealis* (Krøyer, 1838) from the SLE, was selected as an ideal study system. First, a substantial decline in the overall stock biomass has been reported in many populations from North America to Europe (e.g. Carruthers et al., 2019; Knutsen et al., 2015; R. A. Richards & Hunter, 2021), making *P. borealis* an important species to monitor. Indeed, in Norway, the stock dropped from a stable average since the mid-1990s to an historical low around 2015 (Knutsen et al., 2015). The same goes for the Gulf of Maine's population, which suffered a massive decline in 2012 following a heatwave event (Richards & Hunter, 2021), and Newfoundland and Labrador waters where the decline started from 2006 (Carruthers et al., 2019). In the SLE case, if the species demonstrates a lack of physiological plasticity, the viability of populations may be at risk under global change scenarios (Seebacher et al., 2015). It is also one of

the three most exploited crustacean in Quebec, and along Eastern Canada, and the most abundant shrimp in this region (MAPAQ, 2015). This fishery represents an important economic resource for several coastal communities, including the First Nations on the shore of the St. Lawrence (MPO, 2018). *Pandalus borealis* has a discontinuous circumboreal distribution (Shumway et al., 1985) and it is commonly observed in the North-East Atlantic region (MAPAQ, 2015; Pillet, 2013). In the SLE, the northern shrimp is found at depth from 150 to 350 m (MAPAQ, 2015; Pillet, 2013) where the oxygen level is often between 50 to less than 20 % sat. (Pillet, 2013).

According to previous studies, larvae and adults of *P. borealis* present diverse responses when exposed to global change drivers. Ouellet et al. (2017) found in larvae from the SLE significant mortality over 12 °C, and that the SLE population could be closer to their upper thermal tolerance limit than population from the St. Lawrence Gulf or from the Labrador–Newfoundland Shelf. Arnberg et al. (2013) found that in larvae a decrease in pH increased development time, but only at low temperature. They found that OW exerts a greater effect that OA on larval shrimp development, having observed a lower hatching success in eggs under elevated temperature. However, they later found that the combination of OW and OA had negative impacts on survival, development and growth in larval stages, and that these effects were stage-dependent. Hammer & Pedersen (2013) observed that adults are tolerant to acidification, having efficient mechanisms to counteract CO_2-induced acidosis. *P. borealis* was also found tolerant to hypoxia, with females presenting a higher critical oxygen threshold than males. However, under the exposure to severe low oxygen conditions (22 % sat.), they observed a significantly reduced maximum metabolic rate, suggesting that shrimp capacity to respond to metabolic demands could decrease in hypoxic conditions (Dupont-Prinet et al., 2013). Finally, Chemel et al. (2020) found that survival was significantly lower in shrimp exposed to OW and OA, and even lower in shrimp exposed to OW, OA and hypoxia.

However, the impact of combined global change drivers on *P. borealis* cellular functions has not been studied yet. Consequently, we aimed to characterise **(Obj 1.)** changes in the proteome under different combinations of OW, OA and hypoxia. We hypothesized that energy metabolism processes, cellular stress response, immune defense, cytoskeletal, apoptosis and chaperone related proteins would show increased abundance under the most severe scenario of future global change conditions, reflecting the tolerance of *P. borealis* against global change drivers. We also intended

to evaluate the **(Obj. 2)** inter-individual variations of the proteome across different environmental scenarios. We hypothesized that this variation should be limited considering the stenotherm nature of *P. borealis*, but that modulation across treatments should be observable with a higher inter-individual variation in most extreme environmental scenarios. Previous studies suggest that extreme scenarios may either unleash or decrease variation (Madeira et al., 2021; Salinas et al., 2019) so we expect that under the combined scenario (elevated temperature, low pH and hypoxic conditions) shrimp should present either the highest or lowest inter-individual variation.

3 Material and methods

The tissue samples used for the investigation of the combined effects of OW, OA and hypoxia on the plastic capacity of the northern shrimp's cellular stress response (CSR) were obtained from previous studies (Chemel et al. 2020, Guscelli et al., *in prep.*). Here we provide a brief account of the specimens' collection and experiment carried out by Guscelli et al. (*in prep.*) and describe in detail the proteomics analyses carried out on these samples.

3.1 Specimens' collection, transport and maintenance under laboratory conditions

Non-ovigerous females of northern shrimp (carapace length of 25.45 ± 0.14 mm, mean ± SE) were selected for our study as they represent the main target of the northern shrimp fishery because of their larger size (ASMFC, 2019), and because they are known to be more sensitive when compared to males to hypoxia (Dupont-Prinet et al., 2013; Pillet, 2013). They were fished in the SLE off Rimouski (Quebec, Canada; ∼58◦36′N, 68◦35′W) by scientific trawling at 120-150 m depth using hauls of ∼16 min at an average speed of 2.5 knots, with a rigid frame trawl of the Maurice Lamontagne Institute (MLI) of the Department of Fisheries and Oceans Canada (Mont-Joli, QC, Canada) from the 22-m CCGS Leim research vessel. The collection took place in between May 20th and 30th in 2018, then the captured indiv. were held in isolated tanks (750 L Xacticts^{tm}) filled with cold (2 and 3 °C) and oxygenated water and transported by truck to MLI within 4-10 h of capture. They were then transferred to rectangular basins (1700 L) supplied with natural sea water enriched with salt (Common salt without additives, K+S Windsor Salt, Pointe-Claire, QC, Canada) in order to increase the salinity from 28 to ∼32: to mimic saline conditions shrimp experience in the SLE. Shrimps were maintained at a temperature of 4.5 °C, salinity 32, 100 % O_2 sat. and pH of 7.9 (total scale, pH_T), where they remained for eight weeks before the experimental exposure to the different treatments. Shrimp were fed *ad libitum* with equal proportion of frozen capelin *Mallotus villosus* (Müller, 1776) and shrimp (*Pandalus* spp.) three times a week, in order to simulate their natural diet (Wienberg, 1980). Dead individuals, exuviae and excess food were removed daily to limit bacteria proliferation and ammonia accumulation to ensure high quality water.

3.2 Experimental design

To explore the combined effects of OW, OA and hypoxia on the plasticity of the CSR of the northern shrimp, two experimental designs were established, as was previously done in Chemel et al. (2020) and Guscelli et al. (*in prep*):

Experimental design A was employed to investigate the impact of OW and OA in isolation and combined on the CSR of the northern shrimp. In more detail, individuals were exposed to three temperatures levels (2, 6 and 10 °C) and two pH levels (7.75, 7.40), in a six treatments fully orthogonal experimental design. The temperature treatments were chosen according to the preferred temperature of the specie (2 °C, Orr & Sullivan, 2013; Siferd, 2015), the current temperature to which the SLE shrimp are exposed (6 °C, DFO, 2018) as well as the expected scenario for 2100 of a +4 °C increase (RCP 8.5, IPCC et al., 2021). The pH treatments were selected to depict current (7.75, Mucci et al., 2011, 2017) and expected bottom water pH of the SLE (7.40) for the end of the century, considering a decrease of -0.35 pH unit (RCP 8.5, IPCC et al., 2021).

Experimental design B was used in order to explore the impacts of the effect of current and end-of-the-century OW and OA scenarios in combination (here defined as the "current environmental horizons" and "future environmental horizons") together with hypoxia on the CSR. Two treatments of hypoxia (35 % O_2 sat.) were added to the most favorable environmental conditions (2 °C, pH 7.75) and to the expected conditions for 2100 taking into account OW and OA scenarios (10 °C, pH 7.40), considering the range of oxygen saturation conditions commonly encountered by *P. borealis* in the St. Lawrence Estuary and Gulf, while still being over its lethal threshold (Dupont-Prinet et al., 2013).

A total of eight treatments (with two replicates *per* treatments) were created simultaneously in the experimental system (see section "Experimental set-up, protocol and system monitoring") combining both *experimental design A* and *B*. The different treatments were labeled as low temperature and current pH (2C: 2 °C, pH 7.75, 100 % O_2 sat.), low temperature and low pH (2A: 2 °C, pH 7.40, 100 % O_2 sat.), intermediate temperature and current pH (6C: 6 °C, pH 7.75, 100 % O_2 sat.), intermediate temperature and low pH (6A: 6 °C, pH 7.40, 100 % O_2 sat.), elevated temperature and current pH (10C: 10 °C, pH 7.75,

100 % O_2 sat.), elevated temperature and low pH (10A: 10 °C, pH 7.40, 100 % O_2 sat.), low temperature, current pH and low O_2 (2CH: 2 °C, pH 7.75, 35 % O_2 sat.) and elevated temperature, low pH and low O_2 (10AH: 10 °C, pH 7.40, 35 % O_2 sat.).

3.3 Experimental set up, protocol and system monitoring

Two large reservoirs (750 L) provided sea water at a constant flow rate of 3.5 L min^{-1} to 16 large insulated and re-circulated tanks (240 L, one duplicate *per* treatment) holding all shrimp. Each reservoir was maintained at constant temperature of approximately 0 and 10 °C, respectively, by mean of a thermopump (Gell'Air, Mont-Joli, Canada). A controller (1/16 DIN Micromega autotune PID Temperature, Omega Engineering inc., Norwalk, CT, USA) and two mixing valves (sv3109, Omega Engineering inc., Norwolk, CT, United States) in each tank allowed to obtain the set temperature by mixing the appropriate proportion of cold and warm water: total 3.5 L min^{-1}. In order to ensure the continuous monitoring and regulation of temperature and pH, two feedback systems were used: a temperature system (1/16 DIN Micromega autotune PID Temperature, Omega Engineering inc., Norwolk, CT, USA) coupled with a temperature probe (HRSTD-3-100-A-240-E, Omega Engineering Inc.), and a pH system (Aquastar IKS, Karlsbad, Germany) coupled with a pH probe (1001 modules, Aquastar IKS). Moreover, O_2 sat. was monitored and adjusted *via* a feedback system (Aquastar IKS) and coupled with O_2 probes (1008 module, Aquastar IKS, Norwolk, CT, USA), by regulating the proportion of pure gaseous CO_2 and N_2 into gas exchange column, were operative in each tank for hypoxic treatments. To limit the formation of physical and chemical gradient, each tank was equipped with a submersible pump (1048, Eheim, Stuttgart, Germany) allowing the water mixing. To minimize O_2 sat. fluctuations in hypoxic treatment tanks, the pump were connected to the top of the gas exchange column, increasing flow rate through it.

Each tanks held approximatively 70 non-ovigerous females randomly assigned. The environmental conditions were gradually adjusted from the acclimation conditions (4.5 °C, pH 7.9 and 100 % sat.) to treatments values over 4 days. Temperature was increased or decreased by 1.5 °C *per* day and pH was decreased by 0.15 pH unit *per* day. O_2 sat. was decreased by daily increments from 100 to 70 %, 50 %, 40 % and 35 %. Once the

experimental conditions have been reached, shrimp were exposed for 30-d. Dead indiv. exuviae and excess food were removed daily as described previously.

Following the exposure period of 28-d, five shrimp *per* tank were randomly selected for subsequent analyses. Each individual was put into respirometers chambers at the same environmental conditions as their respective treatment during 48 h in order to measure their standard and maximum metabolic rate. Results are presented in Guscelli et al. (*in prep*). Each individual was rapidly blotted with a paper towel, weighed on a precision scale (Mf-300, AandD Company, Tokyo, Japan; ± 0.001 g precision) and sacrificed by removing the abdomen muscle with a ceramic knife. The samples were flash frozen in liquid nitrogen, then kept at -80 °C until biomarkers analyses were carried out.

3.4 Proteomic analysis

3.4.1 Sample preparation

Shrimp abdominal muscle samples (~100 mg; analytical scale RADWAG AS 220.R", Random, Poland) were homogenized (Omni TH, Bebensee, Germany) in 2 mL of Laemmli buffer (60 mM Tris-HCl, pH 6.8, 2 % SDS - sodium dodecyl sulfate) at medium velocity during 10 s. Then, an analog ultrasonic cell disruptor (Sonifier 250, Branson) was used in three cycles of 5 s plus 10 s resting on ice (output control 1.5). Crude homogenates (1 mL) were then centrifuged with a microcentrifuge (Sorval Legend Micro 21R, ThermoFisher Scientific®, Osterode am Harz, Germany) at 4 °C for 15 min at 10 000 × g and the supernatant was collected and preserved at -80 °C until proteomic analyses. Protein extracts (1 mL) were then diluted with 1 mL of 1× Laemmli Sample Buffer and sonicated using a cup-horn (VibraCell VCX 750W, Sonics®, Newtown, Connecticut, United States) for about 2 min, 40 % amplitude, and pulses of 1 sec ON/OFF. An aliquot of 50 µL was further diluted with 50 µL of 2× Laemmli Sample Buffer and used for total protein concentration measurement and LC-MS/MS experiments. The total protein concentration was measured in each sample using a protein assay kit (Pierce 660 nm, Thermo Scientific™, Waltham, Massachusetts, United States). For data-dependent acquisition (DDA) experiments, replicates from different temperatures and shrimp populations were pooled into twelve different samples before sample processing. Although in this

experiment, only shrimp from SLE were used, various populations collected for other experiments (Guscelli et al., *in prep*) were used to construct a comprehensive ion-library and proteome database for the species. For data-independent acquisition (DIA), each sample was processed individually. Protein content from each sample/pool (about 50 µg) was separated by SDS-PAGE (Fig. 1) for about 15 min at 110 V: (Short-GeLC approach and stained with Coomassie Brilliant Blue G-250). For both DDA and DIA experiments, each lane was divided into three gel pieces for further individual processing. After the destaining step, gel bands were incubated overnight with trypsin for protein digestion and peptides were extracted from the gel using three solutions containing different percentages of acetonitrile (30, 50, and 98 %) with 1 % formic acid. In this step, the three fractions were put together in the case of individual samples (DIA experiments) and kept separate in the case of pooled samples (DDA experiments). The organic solvent was evaporated using a vacuum-concentrator and peptides were resuspended in 30 µL (for DIA experiments) and 25 µL (for DDA experiments) of 2 % acetonitrile and 0.1 % formic acid. Each sample was sonicated using a cup-horn (Ultrasonic processor, 750W) for about 2 min, 20 % amplitude, and pulses of 1 sec ON/OFF. Samples were then centrifuged at 14,100 × g, for about 5 min and the supernatant was collected into LC vials. Ten microliters of each sample were analyzed by LC-MS/MS, either for DIA or DDA experiments.

3.4.2 LC-MS methodology

Samples were analyzed on a NanoLC™ 425 System (Eksigent) coupled to a Triple TOF™ 6600 mass spectrometer (Sciex) and the ionization source (ESI DuoSpray™ Source). The chromatographic separation was performed on a Triart C18 Capillary Column 1/32" (12 nm, S-3µm, 150 x 0.3 mm, YMC) and using a Triart C18 Capillary Guard Column (0.5 × 5 mm, 3 µm, 12nm, YMC) at 50°C. The flow rate was set to 5 µL min^{-1} and mobile phases A and B were 5% DMSO plus 0.1 % formic acid in water and 5 % DMSO plus 0.1 % formic acid in acetonitrile, respectively. The LC program was performed as follows: 5 – 30 % of B (0 - 50 min), 30 – 98 % of B (50 – 52 min), 98 % of B (52-54 min), 98 – 5 % of B (54 – 56 min), and 5 % of B (56 – 65 min). The ionization source was operated in the positive mode set to an ion spray voltage of 5500 V, 25 psi for nebulizer gas 1 (GS1), 10 psi for nebulizer gas 2 (GS2), 25 psi for the curtain gas (CUR), and source temperature

(TEM) at 100 °C. For DDA experiments, the mass spectrometer was set to scanning full spectra (m/z 350-2250) for 250 ms, followed by up to 100 MS/MS scans (m/z 100 – 1500). Candidate ions with a charge state between +1 and +5 and counts above a minimum threshold of 10 counts per second were isolated for fragmentation and one MS/MS spectrum was collected before adding those ions to the exclusion list for 15 sec (mass spectrometer operated by Analyst® TF 1.8.1, Sciex®). The rolling collision was used with a collision energy spread of 5. For SWATH experiments, the mass spectrometer was operated in a looped product ion mode and specifically tuned to a set of 42 overlapping windows, covering the precursor mass range of 350-1400 m/z. A 50 ms survey scan (3502250 m/z) was acquired at the beginning of each cycle, and SWATH-MS/MS spectra were collected from 100-2250 m/z for 50 ms resulting in a cycle time of 2.2 sec.

3.5 Data analysis

3.5.1 Ion-Library construction (DDA information)

A specific ion-library of the precursor masses and fragment ions was created by combining all files from the DDA experiments in one protein identification search using the ProteinPilot™ software (v5.0, Sciex). The paragon method parameters were the following: searched against the Decapoda database (NCBI; 342 438 protein sequences), cysteine alkylation by acrylamide, digestion by trypsin, and gel-based ID. An independent False Discovery Rate (FDR) analysis, using the target-decoy approach provided by Protein Pilot™, was used to assess the quality of identifications.

3.5.2 Relative quantification of proteins (SWATH-MS)

SWATH data processing was performed using SWATH™ processing plug-in for PeakViewTM (v2.0.01, Sciex®). Protein relative quantification was performed in all samples using the information from the protein identification search. Quantification results were obtained for peptides with less than 1 % of FDR and by the sum of up to five fragments/peptide. Each peptide was normalized for the total sum of areas for the respective sample. Protein relative quantities were obtained by the sum of the normalized values for up to 15 peptides/protein.

3.5.3 Main biological functions retrieving

To ensure the availability of gene ontology terms for each differentially expressed proteins, we found the corresponding proteins from NCBI database to Uniprot by fast alignment (FASTA) sequences analyses with basic local alignment search tool (BLAST) tool. From there, biological functions were retrieved manually from the literature, as seen in Table 6.

3.6 Statistical analyses

3.6.1 Outlier samples identification

In order to identify outlier samples, a correlation analysis between samples protein abundance was performed using the MetaboAnalyst (https://www.metaboanalyst.ca/) platform. Considering that the analysis showed most samples being highly correlated between them, only two samples were removed from the dataset before proceeding with other analyses.

3.6.2 Differential abundance of proteins

In order to investigate the combined effects of OW, OA and hypoxia on the plasticity of the northern shrimp's proteome from SLE, statistical tests were applied to detect significant differences between treatments for each protein in both designs. Proteomic data were analyzed using R software version 4.1.2 (R Core Team, 2021). Differentially abundant proteins (DAPs) were assessed using protein-wise linear models combined with empirical Bayes statistics with the *DEP* package (Zhang et al., 2018), based on the *limma* algorithm. The dataset was filtered for proteins that were identified in eight out of ten replicates of at least one condition, limiting missing values. Imputation of missing values was performed using left censored-imputation method considering a non-random distribution (NMAR), taking random draws from a Gaussian distribution centered around a minimal value (MinProb function, $q = 0.01$). Proteins with adjusted p-values below $\alpha = 0.05$ with a $\log_2$ fold change (lfc) beyond 1.0 were considered as differentially abundant, following a differential enrichment test based on protein-wise linear models and empirical Bayes statistics. All pairwise comparisons were tested, but only the ones of interest ecologically

were kept for further investigation (namely 2C vs. 2A, 6C vs. 6A, 10C vs. 10A, 2C vs. 6C, 2C vs. 10C, 6C vs. 10C, 2A vs. 6A, 2A vs. 10A and 6A vs. 10A for *design A* and 2C vs. 2CH, 10A vs. 10AH, 2C vs. 10A, 2CH vs. 10AH for *design B*; pairwise comparisons with no ecological interest will not be discussed here namely 2C vs. 6A, 2C vs. 10A, 6C vs. 2A, 6C vs. 10A, 10C vs. 2A, 10C vs. 6A for *design A* and 2C vs. 10AH and 2CH vs. 10A for *design B*). All plots were generated using the *DEP* package (Zhang et al., 2018).

3.6.3 Inter-individual variation

In order to evaluate the inter-individual variation, the abundance (mean ± SD) were used to calculate the coefficient of variation (% CV) of each differentially abundant proteins (DAPs) in each treatment.

$$(1)\ \%\ CV = \text{mean} / \text{standard deviation} * 100$$

An ANOVA test using car package(Fox & Weisberg, 2019) in R 4.1.2 (R core team, 2021) was used to detect significant differences among treatments.

4 Results

Overall, 808 proteins were identified from the *Decapoda* NCBI database for all conditions after performing the quality filter, considering 5 % FDR. From those, 592 proteins were identified through the proteomic analysis of 80 shrimp abdominal muscle samples from six treatments of temperature and pH in design A, and two additional treatments of low O_2 level hyper-impose on current and future temperature and pH environmental horizons in design B.

4.1 DAPs

The quantitative proteomic analysis revealed only six differentially abundant proteins (DAPs) in design A and 3 DAPs in design B (Table 4 and **Table 5**, Figure 6 andFigure **7**), corresponding respectively to 1% and 0.5% of the total identified proteins. These proteins are involved in various functions, related to growth and reproduction, organization of the extracellular matrix, cell migration, modification of chromatin structure, regulation of muscle contraction, cytoskeletal dynamic and mRNA splicing (Table 6). However, no protein related to the cellular stress response through antioxidants and protein quality control mechanisms of the abdominal muscle of adult female of the northern shrimp *Pandalus borealis* were found to be differentially abundant (Chapter 1).

Design A - The proteomic analysis showed that in all comparisons, four DAPs were up-regulated and two were down-regulated from current pH to low pH treatments (Table 5

and Table **6**,

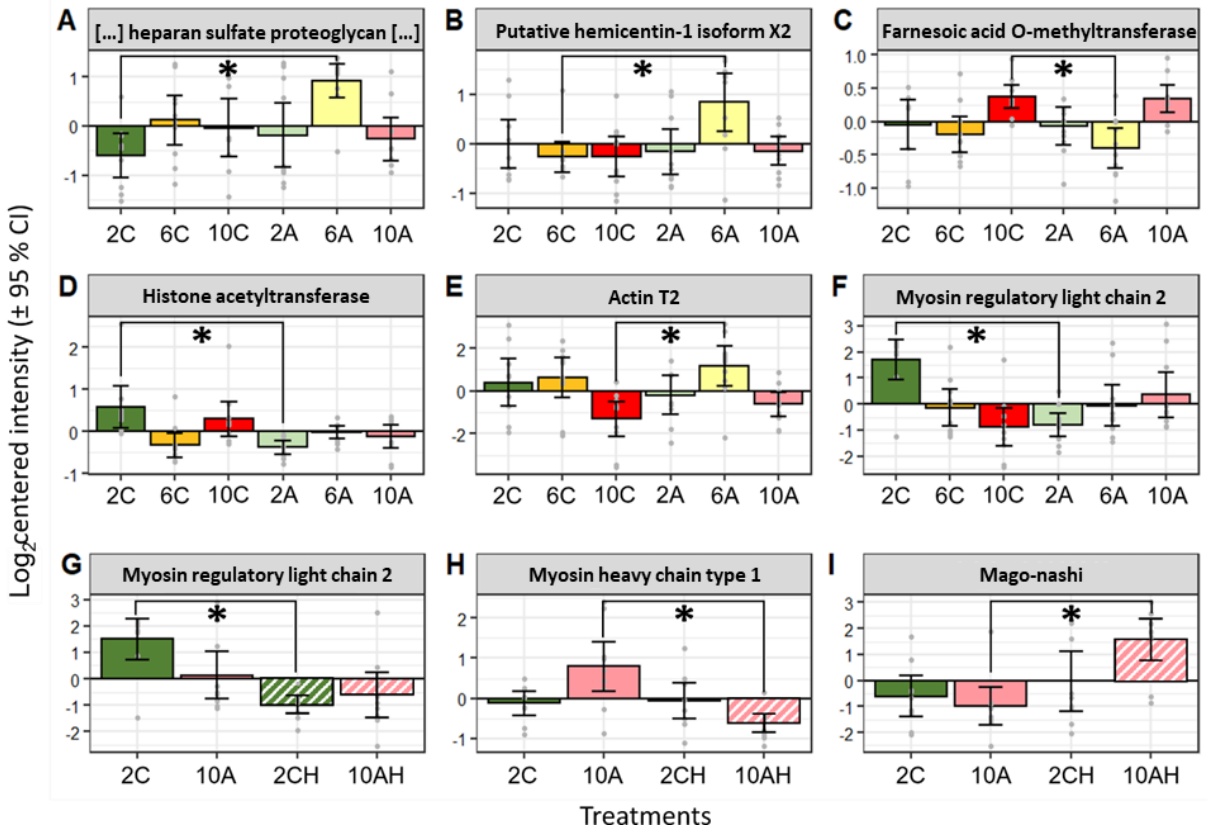

Figure **8**). Each DAPs revealed one significant difference among all comparisons of treatments (Table 5 and Table **6**, Figure 8). Among those, three comparisons were found to be of ecological interest. At low temperature (2 °C) treatments, histone acetyltransferase was down-regulated, while myosin regulatory light chain 2 protein was up-regulated from current to low pH. At intermediate temperature (6 °C) treatments, only the putative hemicentin-1 isoform X2 was up-regulated from current to low pH (Table 5 and Table **6**, Figure 8). No DAPs were detected in relation to OW, or to the combination of OW and OA.

Design B - The statistical analysis revealed that one DAP, myosin regulatory light chain 2, was down-regulated in the muscle of shrimp exposed to the current environmental horizon under hypoxia (Table 5 and Table **6**, Figure 8). The two other DAPs demonstrated opposite responses, myosin heavy chain type 1 being down-regulated (Table 5 and Table **6**, Figure 8) and Mago-nashi protein being up-regulated (Table 5 and Table **6**, Figure 8) in the muscle of shrimp exposed to the future environmental horizon under hypoxia.

Table 4. List of the differentially abundant proteins (DAPs) across design A (temperature * pH) and B (O_2 level * environmental horizon) identified using NCBI [left-hand side of the table where we provide the: reference sequence (*RefSeq*), protein description (*Description*), source specie (*Specie*: *Penaeus japonicus*, *Homarus americanus*, *Scylla olivacea*) and percentage peptide count (% $_{pep.}$, > 95 % confidence)] and their corresponding protein in UniProt found by FASTA sequences analyses with BLAST tool [right-hand side of the table, where we provide the: matching percentage (% $_{ID}$), *Accession number*, protein description (*Description*), source species (*Species*): *Penaeus vannamei, Portunus trituberculatus*].

	NCBI identification				**UniProt identification**		
	RefSeq	**Description**	**Species**	**% pep.**	**% ID**	**Accession number**	**Description**	**Specie**
Design A	XP_042864615.1	Uncharacterized protein LOC122248571 isoform X4	*P. japonicus*	1	93.5	A0A3R7PCP7	Farnesoic acid O-methyltransferase	*P. vannamei*
	XP_042238894.1	Titin-like	*H. americanus*	3	70.2	A0A3R7MML6	Putative hemicentin-1 isoform X2	*P. vannamei*
	XP_042203061.1	Basement membrane-specific heparan sulfate proteoglycan core protein-like	*H. americanus*	8	61.7	A0A3R7SUV3	Putative basement membrane-specific heparan sulfate proteoglycan core protein isoform X11	*P. vannamei*
	XP_042213001.1	Flightin-like isoform X2	*H. americanus*	8	48.4	A0A423TSE5	Histone acetyltransferase	*P. vannamei*
	JAI67558.1	Hypothetical protein PF13405.2	*S. olivacea*	2	97.2	A0A5B7DK96	Myosin regulatory light chain 2	*P. trituberculatus*
	XP_042203404.1	Actin-like isoform X4	*H. americanus*	14	95.0	A0A2H4V3G1	Actin T2	*P. vannamei*
Design B	XP_042893735.1	Protein mago nashi homolog	*P. japonicus*	1	100.0	A0A423U3V8	Mago-nashi	*P. vannamei*
	JAI67558.1	Hypothetical protein PF13405.2	*S. olivacea*	2	97.2	A0A5B7DK96	Myosin regulatory light chain 2	*P. trituberculatus*
	XP_042212115.1	Myosin heavy chain, muscle-like isoform X1	*H. americanus*	17	89.3	A0A423TG84	Myosin heavy chain type 1	*P. vannamei*

Table 5. List of the differentially abundant proteins (DAPs) identified in the muscle of *Pandalus borealis* via mass-spectrometry based proteomics analysis. In the table we provide for design A – temperature * pH (six DAPs) and B – O_2 level * environmental horizon (three DAPs) the: DAPs *Accession number*, adjusted p-value (*p.adj.*, FDR = 0.05) and average Log_2 fold-changes (*Lfc*) obtained from each pairwise comparison. Significant values are reported in bold.

			Pairwise comparisons														
	Accession number (Uniprot)		**2C *vs.* 2A**	**6C *vs.* 6A**	**10C *vs.* 10A**	**2C *vs.* 6C**	**2C *vs.* 10C**	**6C *vs.* 10C**	**2A *vs.* 6A**	**2A *vs.* 10A**	**6A *vs.* 10A**	**2C *vs.* 6A**	**2C *vs.* 10A**	**6C *vs.* 2A**	**6C *vs.* 10A**	**10C *vs.* 2A**	**10C *vs.* 6A**
Design A	A0A3R7PCP7	*Lfc*	-0.019	0.202	-0.037	-0.151	0.419	-0.571	0.334	0.401	0.736	-0.353	0.383	-0.133	-0.534	-0.438	**0.773**
		p.adj	0.984	0.955	0.997	1.000	1.000	0.319	0.508	0.955	0.136	0.798	0.933	0.972	1.000	1.000	**0.037**
	A0A3R7MML6	*Lfc*	-0.147	**-1.110**	0.113	-0.255	-0.249	-0.006	-0.998	0.011	-0.987	0.851	-0.136	-0.108	-0.119	0.101	-1.100
		p.adj	0.976	**0.010**	0.996	1.000	1.000	0.986	0.071	0.998	0.187	0.361	0.991	0.981	1.000	1.000	0.073
	A0A3R7SUV3	*Lfc*	0.422	-0.803	-0.232	0.724	0.569	0.154	-1.110	-0.084	-1.190	**1.530**	0.338	0.302	0.386	-0.148	-0.957
		p.adj	0.937	0.525	0.994	0.996	1.000	0.979	0.071	0.998	0.179	**0.005**	0.984	0.964	1.000	1.000	0.408
	A0A423TSE5	*Lfc*	**-0.962**	-0.310	-0.425	-0.913	-0.284	-0.629	-0.359	0.253	-0.105	-0.603	-0.708	0.049	-0.205	-0.678	0.319
		p.adj	**0.005**	0.910	0.950	0.300	1.000	0.319	0.518	0.993	0.956	0.403	0.857	0.983	1.000	1.000	0.936
	A0A5B7DK96	*Lfc*	**-2.490**	-0.068	1.240	-1.840	-2.580	0.743	-0.724	1.150	0.425	-1.770	-1.340	0.656	-0.493	0.086	-0.811
		p.adj	**0.000**	0.988	0.810	0.628	1.000	0.899	0.552	0.915	0.932	0.083	0.857	0.924	1.000	1.000	0.907
	A0A2H4V3G1	*Lfc*	-0.589	-0.550	0.696	0.207	-1.710	1.920	-1.350	-0.429	-1.770	0.757	-1.020	0.796	1.220	1.130	**-2.470**
		p.adj	0.955	0.964	0.988	1.000	1.000	0.319	0.408	0.996	0.225	0.923	0.957	0.924	1.000	1.000	**0.037**

			2C vs. 2CH	10A vs. 10AH	2C vs. 10A	2CH vs. 10AH	2C vs. 10AH	2CH vs. 10A
Design B	A0A423U3V8	*Lfc*	-0.572	**-2.530**	-0.372	1.590	2.160	-0.943
		p.adj	0.999	**0.020**	1.000	0.339	0.161	1.000
	A0A5B7DK96	*Lfc*	**2.480**	0.747	-1.360	0.367	-2.110	1.110
		p.adj	**0.005**	0.887	1.000	0.895	0.134	1.000
	A0A423TG84	*Lfc*	-0.061	**1.400**	0.917	-0.548	-0.487	0.856
		p.adj	1.000	**0.011**	1.000	0.564	0.889	1.000

Table 6. List of the differentially abundant proteins (DAPs) identified in the muscle of *Pandalus borealis* via mass-spectrometry based proteomics analysis for design A (temperature * pH) and B (O_2 level * environmental horizon) and their main biological functions retrieved from the literature.

	Accession number (Uniprot)	Regulation	Description	Biological functions
Design A	A0A3R7PCP7	↓ (10C vs. 6A)	Farnesoic acid O-methyltransferase	Catalyzes the conversion of farnesoic acid (FA) to methylfarnesoate (MF) in the final step of MF synthesis (similar to insect juvenile hormone III). Implication in growth and reproduction, including molt cycle and ovarian development. (Duan et al., 2014; Li et al., 2006; Qian & Liu, 2019)
	A0A3R7MML6	↑ (6C vs. 6A)	Putative hemicentin-1 isoform X2	Organisation and structural component of the extracellular matrix (anchors mechanosensory neurons to the epidermis, organizes hemidesmosomes, etc.). Ensure cell contact and cell adhesion. (Vogel & Hedgecock, 2001; Welcker et al., 2021)
	A0A3R7SUV3	↑ (2C vs. 6A)	Putative basement membrane-specific heparan sulfate proteoglycan core protein isoform X11	Provide matrix for cell migration. Play a role in coagulation, host defense, wound repair, etc. (Sarrazin et al., 2011)
	A0A423TSE5	↓ (2C vs. 2A)	Histone acetyltransferase	Modify chromatin structure, increases DNA accessibility and promotes gene expression. (Mukherjee et al., 2012)
	A0A5B7DK96	↑ (2C vs. 2A)	Myosin regulatory light chain 2	Responsible for myosin-linked regulation of muscle contraction. (Ochiai & Ozawa, 2020)
	A0A2H4V3G1	↑ (10C vs. 6A)	Actin T2	Contractile component in myofibrils of muscle cells and the cytoskeletal component of non-muscle cells. (Mounier et al., 1992)

Design B	A0A423U3V8	↑ (10A vs. 10AH)	Mago-nashi	Participate in mRNA splicing in the exon junction complex. (Choudhury et al., 2016)
	A0A5B7DK96	↓ (2C vs. 2CH)	Myosin regulatory light chain 2	Responsible for myosin-linked regulation of muscle contraction. (Ochiai & Ozawa, 2020)
	A0A423TG84	↓ (10A vs. 10AH)	Myosin heavy chain type 1	Motor protein housing the myosin ATPase and nucleotide binding site. (LaFramboise et al., 2000; Zhang et al., 2019a)

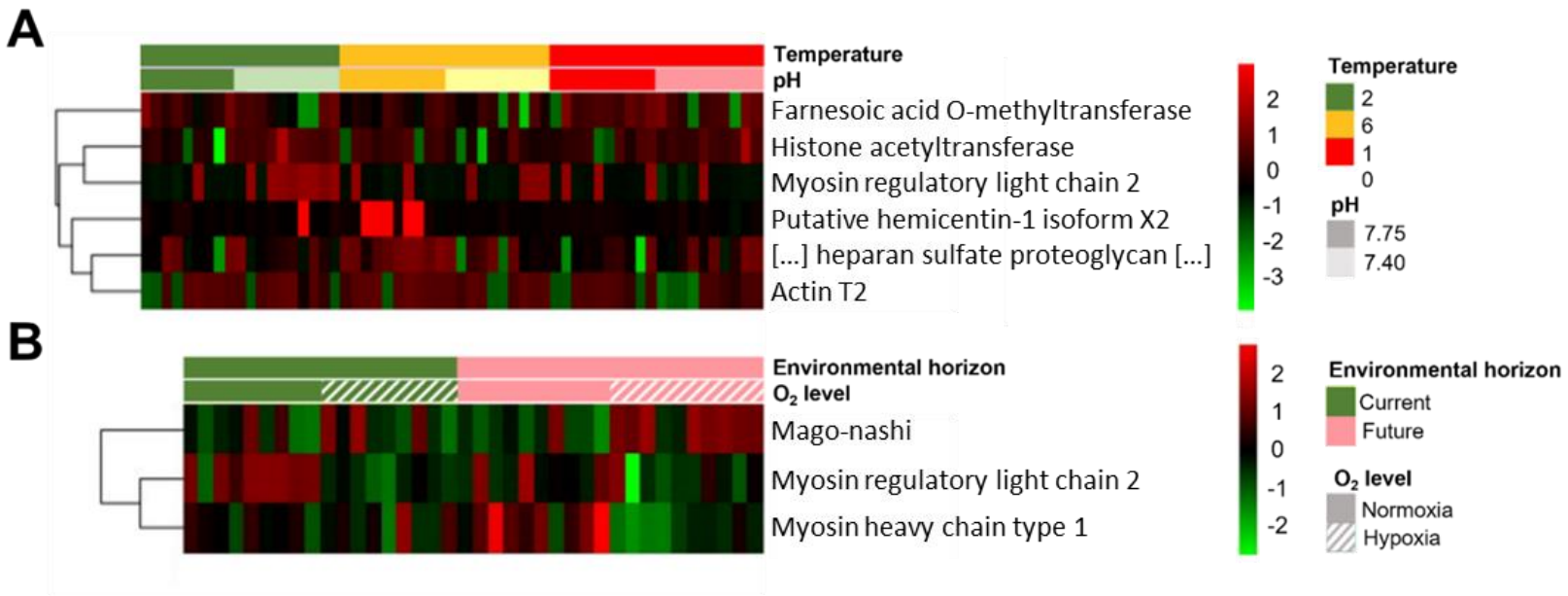

Figure 6. Heatmap of the clustered data from Pearson correlation coefficient matrix in which each cell represents the relative muscle protein quantification among treatments, describing the effect of a 30 d exposure of females *Pandalus borealis* to **a)** elevated temperature and low pH (isolated and combined) (n = 59) and **b)** future environmental horizon (high temperature and low pH) and hypoxia (n = 38). The color scale ranges from red (higher than the mean) to green (lower than the mean). Columns represent the different treatments, while rows represent the differentially abundant proteins (DAPs).

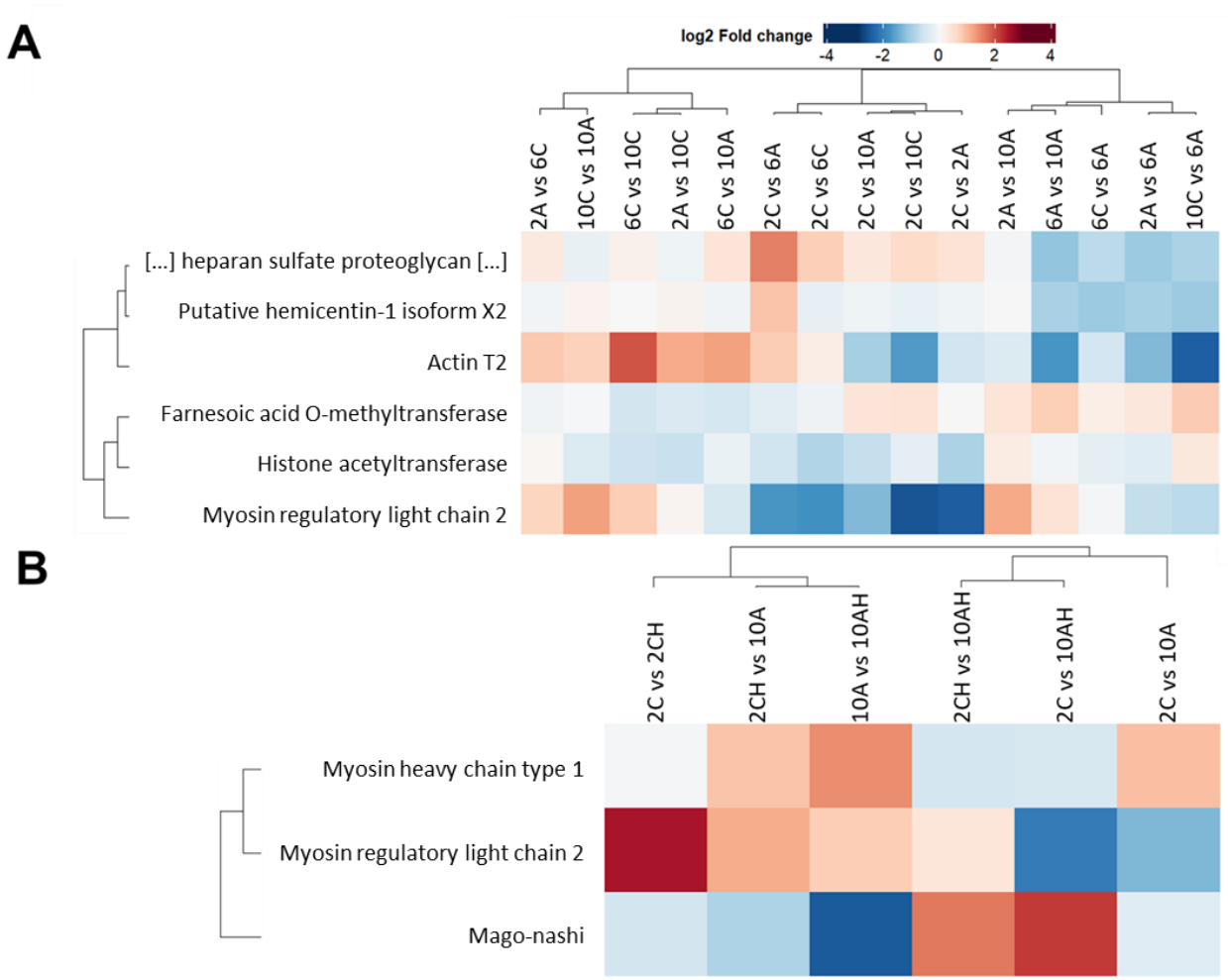

Figure 7. Heatmap representation of the clustered data using Pearson correlation coefficient matrix in which each cell represents the Log_2 fold-change values of each pairwise comparison of muscle protein abundance among treatments, describing the effect of a 30 d exposure of females *Pandalus borealis* (n = 80) to **a)** elevated temperature and low pH (isolated and combined) and **b)** future environmental horizon (elevated temperature and low pH) and hypoxia. Each treatment for both design (A and B) are identified as following **Code Treatment – Temperature/pH/% O_2 sat.**: (2C - 2 °C/7.75), (2A - 2 °C/7.40), (6C – 6 °C/7.75), (6A – 6 °C/7.40), (10C – 10 °C/7.75), (10A – 10 °C/7.40), (2CH – 2 °C/7.75/35 % sat.) and (10AH – 10 °C/7.40/35 % sat.). The color scale ranges from red (Log_2 fold-change increase) to blue (Log_2 fold-change decrease). Columns represent the different pairwise comparisons, while rows represent the differentially abundant proteins (DAPs).

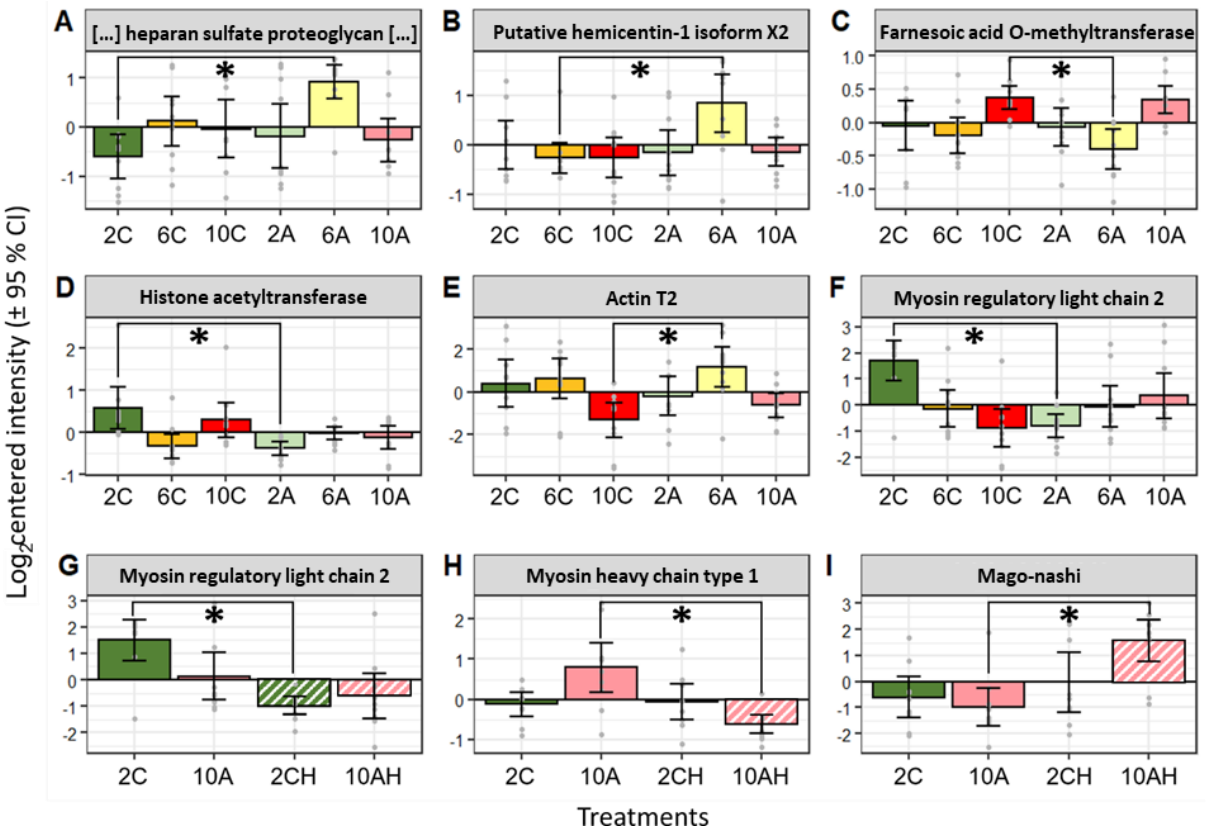

Figure 8. The effect of exposure of adult non-ovigerous females of the northern shrimp *Pandalus borealis* to global change scenarios of **a-f)** combined high temperature and low pH (isolated and combined) (n = 59) and **g-i)** future environmental horizon (high temperature and low pH) and low O_2 (n = 38), on each of the differentially abundant proteins (DAPs) found in the muscle of analyzed shrimp. Each box plot corresponds to a treatment within design A or B, identified as following: **Code Treatment – Temperature/pH/% O_2 sat.**: (2C – 2 °C/7.75), (2A – 2 °C/7.40), (6C – 6 °C/7.75), (6A – 6 °C/7.40), (10C – 10 °C/7.75), (10A – 10 °C/7.40), (2CH – 2 °C/7.75/35 % sat.) and (10AH – 10 °C/7.40/35 % sat). Box plots represent the mean for each treatment, bars represent data outside the standard deviation range while grey dots indicate all replicates for each treatment. Asterisks indicate differences between the two treatments joined by the black line.

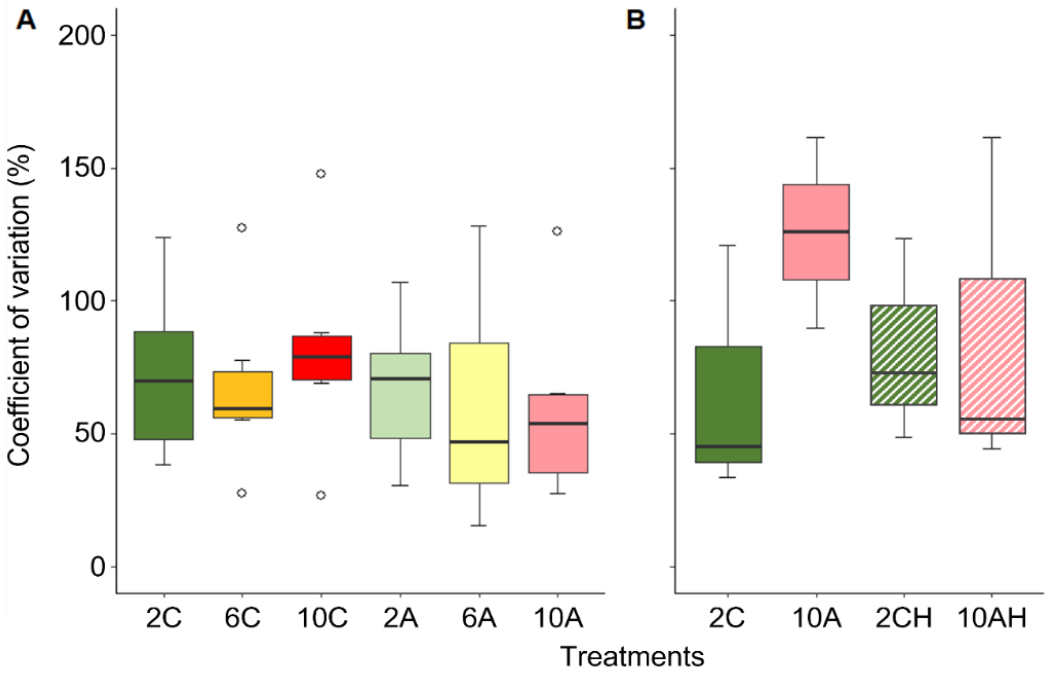

Figure 9. The effect of exposure of adult non-ovigerous females of the northern shrimp *Pandalus borealis* to global change conditions on the inter-individual variation in differentially abundant protein levels (% coefficient of variation): **a)** combined high temperature and low pH (isolated and combined) (n = 59) and **b)** future environmental horizon (high temperature and low pH) and low O_2 (n = 38). Each box plot corresponds to a treatment for each design (A and B), identified as following **Code Treatment – Temperature/pH/% O_2 sat.**: (2C – 2 °C/7.75), (2A – 2 °C/7.40), (6C – 6 °C/7.75), (6A – 6 °C/7.40), (10C – 10 °C/7.75), (10A – 10 °C/7.40), (2CH – 2 °C/7.75/35 % sat.) and (10AH – 10 °C/7.40/35 % sat). Box plots represent treatment delimited by lower and upper quartile. Plain lines indicate the median, bars represent data outside the 25^{th}-75^{th} percentiles range while empty circles indicate extreme values exceeding the 95 % for each treatment. No significant difference in mean CV was observed among treatments.

4.2 Inter-individual variation

Design A – The overall mean coefficient of variation (% CV) for DAPs was 68.2 ± 33.8 (mean ± SD), ranging from a minimum of 59.9 ± 36.1 for treatment 10A and 91.5 ± 39.1 for treatment 10C (Figure 9a). There were no significant differences in mean % CV among the three temperature levels ($F_{2, 30} = 0.321$, $p = 0.728$), the two pH levels ($F_{1, 30} = 1.399$, $p = 0.246$), or their interaction ($F_{2, 30} = 0.160$, $p = 0.853$).

Design B – The overall mean % CV for DAPs was 90.3 ± 46.7, ranging from 66.5 ± 47.4 for treatment 2C to 125.9 ± 35.8 for treatment 10A (Figure 9b). However, there were still no significant

differences in mean % CV between the two O_2 levels ($F_{1, 8} = 0.118$, $p = 0.740$), the two environmental horizons ($F_{1, 8} = 1.326$, $p = 0.283$), or their interaction ($F_{1, 8} = 1.549$, $p = 0.249$).

5 Discussion

In this study, we show that the impact of the exposure to OW and OA on the proteome of the muscle of adult females *Pandalus borealis* is mostly affecting proteins having functions in cell adhesion, chromatin modifications and cytoskeletal mechanisms, while the exposure to hypoxia in isolation and together with the combination of OW and OA mostly affect cytoskeletal and mRNA splicing proteins. In addition, we find that the *P. borealis* proteome shows a great deal of inter-individual variation at all environmental conditions. Our discussion focuses on the plasticity of the proteome under future environmental conditions, with particular emphasis on the few differentially abundant proteins under global change drivers and concluding on the consequences of plasticity limitation on the ecological and socio-economical (i.e. fisheries) level.

5.1 Proteome response to OW, OA and hypoxia

5.1.1 Global muscle proteomic response of *Pandalus borealis* to OW, OA and hypoxia

Tolerance of organisms under on-going global change depends on their ability to undergo physiological modification to ensure cellular homeostasis, and thus the maintenance of cell mechanisms, structure and functions (Somero et al., 2017). The proteome being a dynamic component of an organism's cell, it is expected that proteins will be differentially abundant under different environmental scenarios (Kültz, 2005; Tomanek, 2011). However, we find that the global muscle proteomic response of *P. borealis* is rather limited, with only 1 % of the proteome being differentially abundant under OW and OA scenarios, and 0.5 % under hypoxia and future environmental horizon scenarios. This on one hand can suggest that *P. borealis* is able to largely maintain the integrity of most of its proteome, as well as cell functions under an elevation of temperature, low pH and hypoxic conditions. But on the other hand, it may suggests that the proteome has little ability to buffer environmental changes, which could explain in previous studies of *P. borealis* exposed to global change drivers (Chemel et al., 2020). Altogether, future environmental conditions projected for 2100 (IPCC, 2021) are not expected to have significant impact on *P. borealis'* proteome, however it could

be expected that extreme events such as heat waves combined with other environmental factors might cause further major mortality events, considering the limited phenotypic plasticity. Interestingly, our results are generally not in accordance with previous proteomic studies on decapod shrimps, as most of them present a greater proteome plasticity upon exposure to environmental changes or anthropic modifications in aquaculture context. For example, the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) showed 15.8 % differentially abundant proteins (DAPs) on total identified proteins when exposed to low salinity conditions (Xu et al., 2017), and 3.4 % under cold stress (from 28 °C to 13 °C; Fan et al., 2019). In the fleshy prawn *Fenneropenaeus chinensis* (Osbeck, 1765), exposure to OA (pH 6.5) led to the regulation of 4.9 % of the total identified proteome (He et al., 2022), while in the rockpool shrimp *Palaemon elegans* (Rathke, 1836), seasonal change led to the regulation of 10.6 % of the detected muscle proteome (Madeira et al., 2020). It is important to notice though that these are tropical and temperate species, in contrast with the northern shrimp which as a circumboreal distribution. Indeed, *P. borealis'* proteome shows a minimal degree of alteration following exposure to OW, OA and hypoxia, appearing to have a proteome plasticity that is between ~ 4 and 21 times smaller when compared to that of other shrimp species challenged by unfavourable environmental conditions. This, however, may imply that very few changes in energy and resource utilisation is necessary to maintain homeostasis and cellular functions in this species (Kültz, 2005; Tomanek, 2011), but also highlight its limited ability for plastic responses that can effectively buffer global change drivers, hence lead to important mortality across the different levels of exposure to environmental changes, as observed by Chemel et al. (2020). The implications of both hypotheses are widely different, but we tend to incline toward the fact that *P. borealis* lacks in proteome phenotypical plasticity, based on stenotherm known limited homeostatic abilities (Peck, 2002) and the mortality levels observed in previous studies (Chemel et al., 2020). This could represent a major threat for the northern shrimp with ongoing global change and when facing (ever more frequent and intense) extreme climatic events (Oliver et al., 2018).

5.1.2 Impacts of OA on cell adhesion, chromatin modification and cytoskeleton

Despite the global proteomic response being subtle in *P. borealis*, we observed that OA tends to induce modifications of few biological functions: cell adhesion, chromatin modification and cytoskeletal dynamics. In fact, we report of only few proteins being differentially abundant when shrimp are exposed to OA: i.e. the farnesoic acid O-methyltransferase, putative basement membrane-specific heparan sulfate proteoglycan core protein isoform X11, putative hemicentin-1 isoform X2, histone acetyltransferase and myosin regulatory light chain 2 proteins. Here, we will be discussing only the three latter proteins, since only them were differentially abundant in pairwise comparisons of ecological interest (namely 2C vs. 2A, 6C vs. 6A, 10C vs. 10A, 2C vs. 6C, 2C vs. 10C, 6C vs. 10C, 2A vs. 6A, 2A vs. 10A and 6A vs. 10A for *design A* and 2C vs. 2CH, 10A vs. 10AH, 2C vs. 10A, 2CH vs. 10AH for *design B*; pairwise comparisons with no ecological interest will not be discussed here namely 2C vs. 6A, 2C vs. 10A, 6C vs. 2A, 6C vs. 10A, 10C vs. 2A, 10C vs. 6A for *design A* and 2C vs. 10AH and 2CH vs. 10A for *design B*).

First, hemicentin are dynamic components of the extracellular matrix, participating in the organization and stability of membranes, cell adhesion and migration (Vogel et al., 2006; Vogel & Hedgecock, 2001; Welcker et al., 2021). Secreted among others by skeletal muscles, they form fine tracks at specific sites as they associate with cell surface or, for example, create junctions necessary to maintain organs attached to the body wall (Vogel et al., 2006; Vogel & Hedgecock, 2001). They also play an important role in cell division during cleavage furrow maturation (Xu et al., 2013). In *P. borealis*, we find that hemicentin proteins were up-regulated after the exposure to OA at intermediate temperature. This suggests that OA induces an increase in cell-matrix adhesion, which plays an important role in many biological processes, such as cell motility, proliferation, differentiation, survival and communication (Zhao et al., 2020). This result is in accordance with studies previously held in marine invertebrates. For example, in Pacific oyster *Crassostrea gigas* (Thunberg, 1793), both Timmins-Schiffman et al. (2014) and Dineshram et al. (2021) found an up-regulation in hemicentin genes expression when exposed to OA. Also, the up-regulation of genes related

to cell adhesion were found in the mussel *Mytilus coruscus* (Gould, 1861) (Zhao et al., 2020) and the Japanese pearl oyster *Pinctada fucata* (A. Gould, 1850) (Li et al., 2016) under OA conditions. This suggests that OA somehow alters cell contact, but the absence of other DAPs in this regard potentially indicates that *P. borealis* is able to adjust its phenotype only limitedly or that one single protein (i.e. hemicentin) is sufficient to conserve organismal functions. Considering that past studies on *P. borealis* tend to confirm the tolerance of *P. borealis* to OA as a single factor (Arnberg et al., 2013; Chemel et al., 2020; Hammer & Pedersen, 2013), we conclude that the impact of OA could be buffered by the limited phenotypical plasticity. However, the combination of drivers in the context of global change could still overcome this process and induce some cellular dysfunction.

Histone acetyltransferases (HATs) were also differentially abundant under OA. HATs proteins are epigenetic enzymes that acetylate histone proteins resulting in the dispersion of the chromatin structure (Mukherjee et al., 2012). This process then makes DNA accessible for transcriptional factors (Sun et al., 2015). They are involved in many processes such as nucleosome assemble, DNA damage repair and transcription regulation (Gadhia et al., 2004). In insect, HATs are known to have a role in transcriptional reprogramming during metamorphosis, but also in the response to wounding and infection (Mukherjee et al., 2012). Therefore, HATs are key regulators of gene expression (Mukherjee et al., 2012). Here, we find that *P. borealis* presented down-regulated HATs proteins when exposed to OA. This result may suggest that OA reduces gene transcription processes in *P. borealis*. It has been hypothesized that HATs could act as metabolic sensors by converting metabolic changes into stable patterns of gene expression, considering that histones are consumers of key metabolites (Katada et al., 2012). Along this line of thinking, Padilla-Gamiño et al. (2013) found a down-regulation of histone encoding genes in the larvae of the purple sea urchins *Strongylocentrotus purpuratus* (Stimpson, 1857) exposed to combined OW and OA. They hypothesize that larvae undergone metabolic depression, leading to an increase in their energy demand, hence limiting the production of key metabolites necessary for HATs to remodel chromatin (Kamemura et al., 2012; Katada et al., 2012; Padilla-Gamiño et al., 2013). However, in the case of *P. borealis*, the absence of other clues implying a change in metabolic

processes seems to indicate that the down-regulation of HATs might confirm the metabolic stability of the shrimp under OA conditions despite a potential decrease in transcriptional performances. In contrast, Lee et al. (2020) found an up-regulation of histone acetylation in the generation following a parental exposure of the rotifer *Brachionus koreanus* (Hwang, Dahms, Park & Lee, 2013) to OA. They hypothesize that the increase in oxidative stress leads to an increase in HATs, hence an up-regulation in DNA repair genes (Lee et al., 2020). Despite differences with our findings, this illustrates the epigenetic nature of HATs, considering that they can alter chromatin structure and thus have a regulation role in gene expression. Thus, further studies should assess whether HATs can function as an epigenetic mechanism contributing to plasticity across generations in organisms exposed to global change drivers.

Finally, myosin regulatory light chain proteins were also differentially abundant under OA. They are a subunit of the myosin molecule providing mechanical stability to the myosin head proteins, which makes them important components of the cytoskeleton (Kamm & Stull, 2011). They are responsible for the regulation of the muscle contraction, as well as cell movement and intracellular transport and trafficking by regulating the actin-myosin interaction (Cooper, 2000; Kamm & Stull, 2011; Titus, 2018; Wei et al., 2015). Here, we find that OA induces a down-regulation of myosin light chain in *P. borealis*. This result suggests that OA induces disturbance in cytoskeleton, by potentially impairing motility and organisation in the cytoskeleton, as also suggested in *C. gigas* exposed to elevated pCO_2 (Wei et al., 2015). Considering that we sampled abdominal muscle, this may suggest a decrease in locomotory activity (Gracey et al., 2001). Our results are not in accordance with previous studies, in which myosin up-regulation was observed at different time scale of exposure. In brine shrimp *Artemia sinica* (Cai, 1989), it only took one day of exposure to induce a significant enrichment of the protein (Chang et al., 2016b), while in *C. gigas*, the up-regulation was significant at 28-d (Wei et al., 2015). Finally, in blue rockfish *Sebastes mystinus* (Jordan & Gilbert, 1881), there was an up-regulation in contractile genes 24 h after the exposure, as well as two weeks after (Cline et al., 2020). In contrast with their finding, *P. borealis* does not seem to increase the production of muscle contractile genes nor isoforms

more adapted to OA conditions, as suggested in *S. mystinus* under high pCO_2 and hypoxia (Cline et al., 2020). This could indicate that in addition to having disrupted cytoskeleton, the locomotory ability of *P. borealis* could be negatively affected. Their vertical migration for feeding, the ability to escape predators or sub-optimal environmental conditions could be impaired, which could have important implications for this species survival (Li et al., 2018) and ecological interactions. Nonetheless, such hypothesis could be further tested trough locomotory performance trials.

5.1.3 Impacts of hypoxia on cytoskeleton and mRNA splicing processes in the muscle of *P. borealis*

The SLE is characterized by specific bottom waters conditions, due to interdecadal variability in proportions of Labrador Current Water (LCW) and North Atlantic Central Water (NACW) supplying the estuary through the Laurentian Channel (Gilbert et al., 2005). In the past decades, the environmental conditions were modulated by a major influx of warm oxygen-poor waters from the NACW (Gilbert, 2004), and are expected to worsen in the future in the context of global change (IPCC, 2021). Low dissolved oxygen is a factor of great importance, considering that in addition to O_2 playing a fundamental role in the biogeochemical cycling of carbon, nitrogen and others, it is also essential for all aerobic life (Keeling et al., 2010). The proteomic response of aquatic ectotherm in response to hypoxia is diverse, with changes related to protein modifications, signaling, energy metabolism, cytoskeleton, O_2 transport or carbohydrate metabolic process (Artigaud et al., 2015; Chen et al., 2013; Huo et al., 2019).

Here, we find that myosin regulatory light chain 2, myosin heavy chain type 1 and mago nashi proteins were differentially abundant in individuals exposed to hypoxia. Indeed, myosin light chain, as described previously, and heavy chain proteins were differentially abundant in the muscle of *P. borealis* shrimp. Myosin heavy chains is a motor protein that plays a role on cytoskeletal dynamics by being the main component of the contractile muscle (LaFramboise et al., 2000; Zhang et al., 2019b). Here, we find that both myosin types were down-regulated in individuals exposed to hypoxia at current environmental horizons. Considering that cytoskeletal dynamics is energetically costly, the decrease of myosin protein

abundance could represent an important re-direction of energy resources toward other energy-demanding processes during a time of environmental challenge caused by hypoxia, as described in studies by Gracey et al. (2001) and Ton et al. (2003). However, it also suggests that locomotory activity could be inhibited (Gracey et al., 2001), reinforcing that hypoxia could have consequences on shrimp mobility. Our results are generally in accordance with previous studies. Indeed, the longjaw mudsucker *Gillichthys mirabilis* (Cooper, 1864) and the zebrafish *Danio rerio* (Hamilton, 1822) showed down-regulated genes encoding for both contractile proteins under hypoxia (Gracey et al., 2001; Ton et al., 2003). In contrast, Chen et al. (2013) found that genes coding for contractile proteins were up-regulated in *D. rerio* exposed to hypoxia, which they linked to a change in metabolism considering that myosin is specific to fast glycolytic pathways. Overall, these findings suggest that in addition to OA, hypoxia could also have an important impact on the locomotory ability of *P. borealis*. However, the decrease in the expression of contractile proteins in *P. borealis* does not seem to be due to a metabolic change, considering that no changes occurred in the proteins involved in energy production and consumption.

Mago-nashi proteins, principally characterized in the fruit fly *Drosophilia sp.*, are part of a four-protein exon junction complex (Choudhury et al., 2016; Kataoka, 2001). It mainly participates in pre-mRNA splicing, an important transcriptional process (Choudhury et al., 2016; Kataoka, 2001). RNA processing proteins are part of the most important functional category contributing to the cellular stress response (Kültz, 2020b). In human cells, oxidative stress seem to lead to mis-splicing which can change alternative splicing mechanisms, generating aberrant splice variants leading to diseases (Disher & Skandalis, 2007). However, mRNA splicing also contributes to cellular adjustment to hypoxic conditions in cancer (Kanopka, 2017). (Kanopka, 2017). Although our model system is different, a few insights derive from such studies in other systems. Here, we find that mRNA splicing protein is up-regulated at future environmental horizons under hypoxia. This suggests that the mechanism under splicing is an important mechanism in acclimation. Kawabe & Yokoyama (2011) found in the pacific oyster *C. gigas* that hypoxia induce the transcription of heat shock proteins isoforms with alternative splicing mechanisms, leading to a more efficient cellular

stress response to environmental stress. Huang et al. (2016) found in the same specie under the combination of OW, change in salinity and air exposure, that a complex alternative splicing system seems important for adaptation to local environment. To our knowledge, few studies involving mRNA splicing results have been carried out on marine invertebrates exposed to environmental changes. However, our results are not in accordance with Huo et al. (2019), who found a down-regulation of proteins involved in RNA splicing in Japanese sea cucumber *Apostichopus japonicus* (Selenka, 1867) exposed to OW and hypoxia. This suggests that the over-abundance of mRNA splicing proteins might be a key mechanism in the acclimatization of shrimp to hypoxia.

5.2 Inter-individual variation in proteomic response

Inter-individual variation is the substrate for natural selection to act upon, in order to enable rapid evolution and realignment of a population phenotypic mean to fit the new environmental conditions (Calosi, Turner, et al., 2013; Falconer & Mackay, 2009), thus promoting the ecological success of populations and species under ongoing environmental changes (Forsman & Wennersten, 2016). A higher level of phenotypic variation ensures a limitation in population size fluctuations and an increase in distribution range, and makes populations less prone to extinction (Forsman & Wennersten, 2016). We find that inter-individual variation in protein expression in *P. borealis* exposed to OW, OA and hypoxia, was comparable among treatments and no significant difference among treatments could be detect. Although only DAPs were tested and further studies on other proteins may lead to different results, considering that a change in mean is not necessarily related to a change in variation. Extreme stress is thought to induce changes in phenotypic variation leading to either an increase in trait variation (driving evolutionary transitions in novel environments) or a decrease in trait variation (possibly associated with physiological canalization and homeostasis maintenance under stress) (Madeira et al., 2021; Salinas et al., 2019). The outcome might depend on the extremeness of the environment or on the historical environmental variability of the habitat; or how well-adapted is the species to local conditions. This said inter-individual variation was overall really high. This result is in

agreement with Madeira et al. (2021), who found an overall high % CV (ranging from 87 to 111 %) in protein abundance in the marine ragworm *Hediste diversicolor* (O.F. Müller, 1776), and Madeira et al. (2019) who also found a high degree of variation in proteins related to the cellular stress response in many different taxa in the marine environment (ranging from 35 to 94 %). Our results are also in agreement with Forsman & Wennersten (2016) whom concluded that a higher phenotypic variation is usually observed in experimental settings at more stressful conditions. Also, Tanner et al. (2022) demonstrated that overall variability is less constrained at the protein level than at the transcript level, as opposed to what was previously expected. This high biological variation in the proteome (which is closely linked to phenotype) might have a positive impact on the ability of *P. borealis* to adapt to environmental changes. This vast range of variability in proteomic response could indicate that a portion of the SLE's *P. borealis* population could possess the physiological ability to thrive even under expected global change, while another portion would not be able to maintain homeostasis and cell functions under those conditions. These results are not in accordance with previous studies, in which inter-individual variation was much more moderate. For example, Madeira et al. (2020) found a maximum of 8.3 % variation in protein expression in temperate intertidal decapod shrimps under natural environmental fluctuations. *Pandalus borealis* could therefore be able to tolerate environmental changes predicted to occur by the end of the century, which would ensure sustainability in St. Lawrence fisheries.

6 Conclusion

Although the conclusions of our study tend towards the fact that females of *P. borealis* surviving exposure to global change drivers seem surprisingly tolerant to the impact of global change drivers at the proteome level, there is still a strong need to rule out that this is not in fact a lack of phenotypic plasticity. Indeed, the implications of the two hypotheses are diametrically opposed. On the one hand, the hypothesis stipulating that a portion of the SLE northern shrimp population does not require phenotypic plasticity in order to resist the environmental changes predicted for 2100 (RCP 8.5) would mean that the population would be able to persist, in addition to continuing to fulfill the ecosystem functions attributed to them. The fisheries could therefore potentially persist, but at a smaller scale, taking into account the decrease in biomass due to the loss of individuals with a non-tolerant phenotype (i.e. 12 % of mortality rate in all treatments, except 32 % in elevated temperature with low pH, and 63 % in elevated temperature with low pH and hypoxia; Chemel et al., 2020). On the other hand, the lack of phenotypic plasticity could also greatly impact the SLE northern shrimp population considering that although some individuals survived the 30 days of experimentation in all treatments, prolonged exposure or exposure to extreme events could result in a substantial decrease in biomass. This could have a significant impact on the ecosystem functions performed by the species, but also on the fisheries that depend on the sustainability of the SLE population. However, to obtain a more comprehensive view, there is also an important need to study the other phases of the shrimp life cycle, considering that the larval phases often do not show the same phenotypic response as the adults. Here, we showed that proteome changes in the muscle of *P. borealis* are particularly limited. While OA tend to modulate cell adhesion, chromatin modification and cytoskeleton dynamics, hypoxia modulates mostly cytoskeleton dynamics and RNA splicing processes. The important magnitude of inter-individual variation seems to be the cause of the remarkable performance in terms of tolerance of the species. However, the ability to cope with extreme environmental conditions is still to be explored. Although *P. borealis* is tolerant of the conditions forecast for 2100, it must also be tolerant of the extreme events that will certainly

occur in the future (Schlegel et al., 2021), potentially severely impacting the sustainability fisheries through the decades.

CONCLUSION GÉNÉRALE

Cette étude a permis d'identifier les mécanismes subjacents à la tolérance des sténothermes marins faces aux facteur isolés et combinés des changements globaux dans le contexte de l'estuaire du Saint-Laurent (ESL). Cela a été réalisé en étudiant la plasticité de la réponse cellulaire au stress et du protéome de la crevette nordique *Pandalus borealis* face aux effets combinés du réchauffement (RO) et de l'acidification des océans (AO), ainsi qu'à l'hypoxie.

D'abord, il s'est avéré que la tolérance des individus ayant survécu face aux conditions environnementales représentant les projections pour 2100 selon le RCP 8.5 (IPCC, 2021) ne repose pas entièrement sur la capacité cellulaire à induire des mécanismes antioxydants, des protéines chaperonnes et des protéines de contrôle de qualité protéiques, que ce soit lors d'une exposition aux facteurs isolés ou en combinaison. Effectivement, seul les défenses antioxydantes dite de première ligne, soit la catalase et les molécules antioxydantes non-enzymatiques, ont été induite de manière significative en réponse à l'augmentation de température. L'exposition à l'AO a pour sa part induit une diminution de cette même réponse antioxydante. Ainsi, la combinaison de facteurs semblent diminuer la capacité plastique de la réponse antioxydante de la crevette nordique. Il en va de même pour les protéines chaperonnes et les protéines de contrôle de qualité, dont la concentration a diminué dans les traitements exposés à l'acidification océanique, mais a considérablement augmenté sous les conditions hypoxiques. Ainsi, la plasticité de la réponse cellulaire au stress par ces mécanismes semble encore une fois être très limitée, induisant certains dommages protéiques irréparables induite par l'hypoxie. L'effet du pH semble être antagoniste à celui de la température en ce qui a trait à la réponse antioxydante, alors que l'hypoxie semble entraîner des conséquences plus importantes que l'exposition aux conditions environnementales futures (température élevée et bas pH) sur la réponse des protéines chaperonnes et des protéines de contrôle de qualité. Ces résultats témoignent donc la présence des effets

d'interactions entre les facteurs qui sont plus complexe que préalablement hypothétisé. Les résultats suggèrent également que la crevette nordique pourrait diminuer la portion du budget énergétique allouée au maintien des processus de la réponse cellulaire aux stress, au profit d'autres processus physiologiques tel que le développement, la mue ou la reproduction. L'absence de réponse des protéines chaperonnes lors de l'exposition au réchauffement et à l'acidification océanique semble également indiquer que l'énergie normalement allouée pour ces mécanismes a été redirigé vers d'autres processus nécessaire dans le maintien de l'homéostasie cellulaire. L'absence de patron clair de réponse cellulaire aux stress est possiblement liée à la grande variabilité inter-individuelle de la capacité plastique chez *P. borealis*, considérant qu'il est possible que les individus priorisent différents mécanismes dans le maintien de l'homéostasie. Finalement, l'exposition aux facteurs combinés des changements globaux ne semble pas avoir provoqué de dommage significatif à la membrane cellulaire chez la crevette nordique, malgré la capacité plastique très limitée de la réponse antioxydante. Ce résultat devrait cependant être ré-évalué dans le cadre d'une exposition à plus long terme.

Globalement, le protéome de *P. borealis* a présenté une plasticité très limitée face aux facteurs des changements globaux : entre 4 et 21 fois moins qu'observé chez d'autres crevette décapode lors d'étude comparables (Fan et al., 2019; He et al., 2022; Xu et al., 2017). Malgré cela, certains mécanismes ont été impactés par l'AO, incluant les processus d'adhésion cellulaire, de modification de la chromatine et de dynamique du cytosquelette, alors que l'hypoxie a impacté les processus de dynamique du cytosquelette et de l'épissage de l'ARNm. Aucune protéine relative à la réponse cellulaire au stress par antioxydants et par protéines issues des mécanismes de contrôle de la qualité des protéines, à l'ajustement du métabolisme ou encore à la défense immunitaire n'a été différemment exprimées en réponse aux facteurs des changements globaux. La variation inter-individuelle était comparable entre les différents degrés d'exposition aux facteurs. Il semblerait qu'aucun mécanisme précis ait été ciblé afin de répondre aux effets des conditions environnementales futures, puisque seul une exposition à l'hypoxie et non aux conditions environnementales futures ont entraîné une variation dans l'abondance de certaines protéines.

En contraste, la variation inter-individuelle du protéome est particulièrement importante, quoique comparable entre chacun des traitements. Selon les résultats obtenus, ces conclusions peuvent donc être expliquées de deux façons : par une tolérance naturelle aux conditions environnementales changeantes par une absence de besoin en modifications phénotypiques ou encore par une incapacité à induire des modifications phénotypiques. Effectivement, il serait possible que la grande variation inter-individuelle observée dans l'analyse protéomique témoigne de l'importante variété génotypique présente au sein de la population de crevette nordique de l'ESL des individus (Sévigny et al., 2000). Considérant que les organismes sont susceptibles d'entraîner des changements phénotypiques plus rapidement lors d'événements environnementaux extrêmes que lors d'événements étendus sur de longues périodes à l'échelle populationnelle (Logan & Cox, 2020), il est plausible que les individus de *P. borealis* de l'estuaire ait préalablement acquis un phénotype résistant aux conditions environnementales tel que projetées pour 2100, par processus épigénétique ou par adaptation sélective opportuniste, aussi appelé exaptation (Gould & Vrba, 1982). Ainsi, les individus ne portant pas les phénotypes résistants pourraient être morts lors de l'expérimentation. En effet, les variations inter-individuelles favorisent la performance des populations, en permettant à une portion d'individus d'être phénotypiquement plus tolérants aux changements environnementaux alors qu'une autre portion pourrait ne pas l'être (Forsman & Wennersten, 2016). Cependant, ces individus doivent représenter une fraction suffisante de la population initiale, afin d'assurer le succès reproductif et ainsi faire évoluer l'ensemble de la population sous le concept de sauvetage évolutif (Carlson et al., 2014).

Il serait également possible que la plasticité très limitée de *P. borealis* provienne d'une incapacité à ajuster son phénotype lorsqu'exposé à des conditions environnementales changeantes. Étant un sténotherme, la crevette nordique pourrait posséder des habiletés d'homéostasie limitée (Peck, 2002) et ainsi ne pas avoir les capacités physiologiques nécessaires pour ajuster son phénotype et ultimement exprimer une réponse cellulaire aux stress. Cela signifierait qu'au-delà une exposition de 30 jours, la mortalité pourrait continuer de s'accroître, passant le seuil limite de la capacité homéostatique.

Si les projections environnementales du GIEC (IPCC, 2014) pour 2100 selon le RCP 8.5 s'avèrent vraies, dans les deux cas, il est plutôt plausible que trop peu d'individus présente le phénotype requis afin de survivre sous ces conditions. Cependant, bien que ce scénario soit souvent qualifié de *business-as-usual*, il serait juste de spécifier que celui-ci est maintenant considéré comme moins probable que lors de son développement et de sa publication entre 2008 et 2011, considérant qu'en réalité l'augmentation d'émissions de gaz à effet de serre devrait être moins importante que prévue initialement selon ce scénario (Hausfather & Peters, 2020; Ritchie & Dowlatabadi, 2017). Une pression environnementale moins fortes nécessiterait un phénotype moins adapté, et provoquerait une moins grande mortalité chez *P. borealis*, tel qu'observé par Chemel et al. (2020). Ainsi, si la crevette nordique de l'ESL venait à perdurer malgré des conditions environnementales affectant son homéostasie et le fonctionnement cellulaire, il serait plus plausible que ce soit à cause du sauvetage évolutif grâce à la variabilité phénotypique que par ajustement du phénotype aux conditions d'exposition.

1 Limites de la recherche et pistes à explorer

Bien que l'analyse des principales composantes de la réponses antioxydantes et des protéines chaperonnes ont permis d'établir un portrait global de la réponse cellulaire aux stress chez *P. borealis*, il serait pertinent dans le futur de mettre ces résultats en relation avec des marqueurs enzymatiques issus du métabolisme énergétique : p.ex. citrate synthase, cytochrome oxydase et chaîne de transport d'électrons. Sachant que les invertébrés utilisent généralement la réduction et la dépression métabolique (Calosi, Rastrick, et al., 2013; Reipschläger & Pörtner, 1996; Turner et al., 2015) et les mécanismes de protection aux stress afin de limiter les effets des chocs environnementaux à court ou moyen terme (Kültz, 2005), il serait intéressant de savoir si à long terme, *P. borealis* possède le métabolisme énergétique requis pour supporter les modifications phénotypiques lors d'une exposition aux facteurs des changements globaux.

Il est aussi important de considérer les limites de l'analyse protéomique. Comme les protéines ne peuvent être amplifiées à l'instar des nucléotides en génomique (Sidoli et al., 2017), il est

plus difficile de détecter les protéines qui s'y retrouvent en faible abondance, mais qui ont malgré tout une valeur biologique importante (Righetti & Boschetti, 2013). Le manque de génome de référence chez les organismes marins rend également l'identification des protéines plus complexe et imprécise (Chandramouli, 2016). Il serait donc fort intéressant de coupler l'approche protéomique à de la génomique, de la transcriptomique ou encore de la métabolomique, et ainsi procéder avec une approche multi-omique. Cela permettrait d'identifier les autres voies de régulation empruntées par *P. borealis* dans la réponse cellulaire aux stress (Layton & Bradbury, 2022). Une approche multi-omique permettrait également de cibler la source de l'importante variation phénotypique du protéome, qui pourrait être dû à une forte variété de génotype au sein de la population ou encore certains processus épigénétiques (Subramanian et al., 2020).

Dans l'étude de la réponse des crustacés face aux changements globaux, il est également indispensable d'inclure les réponses des différents stades du cycle de vie et des sexes puisque ceux-ci comportent des caractéristiques physiologiques différentes. De plus, il est primordial de considérer la variété des habitats utilisés durant le développement, les larves étant pélagique alors que les stades juvéniles et adultes sont plutôt benthiques.

Finalement, une étude effectuée sur une échelle de temps plus grande et comportant des échantillonnages à plusieurs moments de l'exposition permettrait de comprendre l'évolution de la réponse au stress chez *P. borealis* et observer et comparer les mécanismes de protection des organismes tolérants et ceux plus sensibles aux conditions environnementales.

2 Portée de l'étude

Cette étude a permis de démontrer l'importance d'inclure des espèces dites sensibles dans l'effort de recherche global afin de les cibler plus efficacement dans les politiques de conservation. Effectivement, comme la majorité des études sont effectuées sur des organismes modèles, il est difficile d'appliquer les conclusions de ces dernières sur des organismes ne possédant pas les mêmes capacités physiologiques. Ces espèces sensibles, en plus de remplir des fonctions écosystémiques importantes, sont parfois la cible d'importantes

pêcheries. Ainsi, leur importance écologique et socio-économique justifie le besoin d'amélioration des outils de modélisation, principalement dans le cadre des changements globaux. L'analyse protéomique de *P. borealis* a également aidé au développement des ressources de type -omique chez les organismes marins, qui restent encore plutôt rares à ce jour. Effectivement, cette étude présente, pour la première fois chez la crevette nordique, des données de protéomique dans un contexte écophysiologique.

RÉFÉRENCES

Ackman, R. (2007). Fatty Acids in Fish and Shellfish. In C. Kuang Chow (Ed.), *Fatty Acids in Foods and their Health Implications,Third Edition* (Vol. 20073230, pp. 155–185). CRC Press. https://doi.org/10.1201/9781420006902.ch8

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). *Molecular biology of the cell* (4th ed). Garland Science.

Ali-Khan, N., Zuo, X., & Speicher, D. W. (2002). Overview of Proteome Analysis. *Current Protocols in Protein Science*, *30*(1). https://doi.org/10.1002/0471140864.ps2201s30

Angilletta Jr., M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198570875.001.1

Apollonio, S., Stevenson, D., & Dunton, E. (1986a). Effects of Temperature on the Biology of the Northern Shrimp, *Pandalus borealis*, in the Gulf of Maine. *NOAA Tech. Rep. NMFS*, *42*.

Apollonio, S., Stevenson, D. K., & Dunton, Jr., Earl E. (1986b). *Effects of temperature on the biology of the northern shrimp, Pandalus borealis, in the Gulf of Maine* (noaa:23116). https://repository.library.noaa.gov/view/noaa/23116

Arnberg, M., Calosi, P., Spicer, J., Bamber, S., Westerlund, S., Vingen, S., Baussant, T., Bechmann, R., & Dupont, S. (2018). Effects of oil and global environmental drivers on two keystone marine invertebrates. *Scientific Reports*, *8*. https://doi.org/10.1038/s41598-018-35623-w

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. *Marine Biology*, *160*(8), 2037–2048. https://doi.org/10.1007/s00227-012-2072-9

Artigaud, S., Lacroix, C., Richard, J., Flye-Sainte-Marie, J., Bargelloni, L., & Pichereau, V. (2015). Proteomic responses to hypoxia at different temperatures in the great scallop (*Pecten maximus*). *PeerJ*, *3*, e871. https://doi.org/10.7717/peerj.871

ASMFC. (2019). *Data Update for Gulf of Maine Northern Shrimp*. http://www.asmfc.org/uploads/file/5dee9a69NShrimpAssessmentUpdateReport_2019.pdf

Azra, M. N., Aaqillah-Amr, M. A., Ikhwanuddin, M., Ma, H., Waiho, K., Ostrensky, A., Tavares, C. P. dos S., & Abol-Munafi, A. B. (2020). Effects of climate-induced

water temperature changes on the life history of brachyuran crabs. *Reviews in Aquaculture*, *12*(2), 1211–1216. https://doi.org/10.1111/raq.12380

Azra, M. N., Chen, J.-C., Ikhwanuddin, M., & Abol-Munafi, A. B. (2018). Thermal tolerance and locomotor activity of blue swimmer crab *Portunus pelagicus* instar reared at different temperatures. *Journal of Thermal Biology*, *74*, 234–240. https://doi.org/10.1016/j.jtherbio.2018.04.002

Azra, M. N., Noor, M. I. M., Eales, J., Sung, Y. Y., & Ghaffar, M. A. (2022). What evidence exists for the impact of climate change on the physiology and behaviour of important aquaculture marine crustacean species in Asia? A systematic map protocol. *Environmental Evidence*, *11*(1), 9. https://doi.org/10.1186/s13750-022-00263-1

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

Bednaršek, N., Calosi, P., Feely, R. A., Ambrose, R., Byrne, M., Chan, K. Y. K., Dupont, S., Padilla-Gamiño, J. L., Spicer, J. I., Kessouri, F., Roethler, M., Sutula, M., & Weisberg, S. B. (2021). Synthesis of Thresholds of Ocean Acidification Impacts on Echinoderms. *Frontiers in Marine Science*, *8*, 602601. https://doi.org/10.3389/fmars.2021.602601

Beemelmanns, A., Zanuzzo, F. S., Xue, X., Sandrelli, R. M., Rise, M. L., & Gamperl, A. K. (2021). The transcriptomic responses of Atlantic salmon (*Salmo salar*) to high temperature stress alone, and in combination with moderate hypoxia. *BMC Genomics*, *22*(1), 261. https://doi.org/10.1186/s12864-021-07464-x

Bennett, N. J., Kaplan-Hallam, M., Augustine, G., Ban, N., Belhabib, D., Brueckner-Irwin, I., Charles, A., Couture, J., Eger, S., Fanning, L., Foley, P., Goodfellow, A. M., Greba, L., Gregr, E., Hall, D., Harper, S., Maloney, B., McIsaac, J., Ou, W., … Bailey, M. (2018). Coastal and Indigenous community access to marine resources and the ocean: A policy imperative for Canada. *Marine Policy*, *87*, 186–193. https://doi.org/10.1016/j.marpol.2017.10.023

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *Chapter 3: Protein Structure and Function. In Biochemistry* (5th ed). W.H. Freeman.

Berger, M. S., & Emlet, R. B. (2007). Heat-shock response of the upper intertidal barnacle *Balanus glandula*: Thermal stress and acclimation. *The Biological Bulletin*, *212*(3), 232–241. https://doi.org/10.2307/25066605

Bourdages, H., Marquis, M.-C., Nozères, C., & Ouellette-Plante, J. (2017). *Assessment of northern shrimp stocks in the Estuary and Gulf of St. Lawrence in 2017: Data from the research survey*. 71.

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., … Pörtner, H.-O. (2018). Experimental strategies to assess the biological

ramifications of multiple drivers of global ocean change-A review. *Global Change Biology*, *24*(6), 2239–2261. https://doi.org/10.1111/gcb.14102

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Brokordt, K., Pérez, H., Herrera, C., & Gallardo, A. (2015). Reproduction reduces HSP70 expression capacity in *Argopecten purpuratus* scallops subject to hypoxia and heat stress. *Aquatic Biology*, *23*(3), 265–274. https://doi.org/10.3354/ab00626

Brown-Vuillemin, S., Chabot, D., Nozères, C., Tremblay, R., 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. *Frontiers in Marine Science*, *9*. https://www.frontiersin.org/articles/10.3389/fmars.2022.963039

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. *Annual Review of Marine Science*, *11*(1), 369–390. https://doi.org/10.1146/annurev-marine-010318-095106

Calosi, P., Rastrick, S. P. S., Lombardi, C., de Guzman, H. J., Davidson, L., Jahnke, M., Giangrande, A., Hardege, J. D., Schulze, A., Spicer, J. I., & Gambi, M.-C. (2013). Adaptation and acclimatization to ocean acidification in marine ectotherms: An *in situ* transplant experiment with polychaetes at a shallow CO_2 vent system. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1627), 20120444. https://doi.org/10.1098/rstb.2012.0444

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. *Integrative and Comparative Biology*, *53*(4), 660–670. https://doi.org/10.1093/icb/ict041

Carlson, S. M., Cunningham, C. J., & Westley, P. A. H. (2014). Evolutionary rescue in a changing world. *Trends in Ecology & Evolution*, *29*(9), 521–530. https://doi.org/10.1016/j.tree.2014.06.005

Carrier-Belleau, C., Drolet, D., McKindsey, C. W., & Archambault, P. (2021). Environmental stressors, complex interactions and marine benthic communities' responses. *Scientific Reports*, *11*(1), Article 1. https://doi.org/10.1038/s41598-021-83533-1

Carruthers, E. H., Parlee, C. E., Keenan, R., & Foley, P. (2019). Onshore benefits from fishing: Tracking value from the northern shrimp fishery to communities in Newfoundland and Labrador. *Marine Policy*, *103*, 130–137. https://doi.org/10.1016/j.marpol.2019.02.034

Chandramouli, K. (2016). Marine Proteomics: Challenges and Opportunities. *Journal of Data Mining in Genomics & Proteomics*, *07*(02). https://doi.org/10.4172/2153-0602.1000e122

Chang, X., Zheng, C., Wang, Y., Meng, C., Xie, X., & Liu, H. (2016a). Differential protein expression using proteomics from a crustacean brine shrimp (*Artemia sinica*) under CO_2-driven seawater acidification. *Fish & Shellfish Immunology*, *58*, 669–677. https://doi.org/10.1016/j.fsi.2016.10.008

Chang, X., Zheng, C., Wang, Y., Meng, C., Xie, X., & Liu, H. (2016b). Differential protein expression using proteomics from a crustacean brine shrimp (*Artemia sinica*) under CO_2-driven seawater acidification. *Fish & Shellfish Immunology*, *58*, 669–677. https://doi.org/10.1016/j.fsi.2016.10.008

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. *Frontiers in Marine Science*, *7*, 611. https://doi.org/10.3389/fmars.2020.00611

Chen, K., Cole, R. B., & Rees, B. B. (2013). Hypoxia-induced changes in the zebrafish (*Danio rerio*) skeletal muscle proteome. *Journal of Proteomics*, *78*, 477–485. https://doi.org/10.1016/j.jprot.2012.10.017

Cheung, W. W. L., Sarmiento, J. L., Dunne, J., Frölicher, T. L., Lam, V. W. Y., Deng Palomares, M. L., Watson, R., & Pauly, D. (2013). Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. *Nature Climate Change*, *3*(3), Article 3. https://doi.org/10.1038/nclimate1691

Choudhury, S. R., Singh, A. K., McLeod, T., Blanchette, M., Jang, B., Badenhorst, P., Kanhere, A., & Brogna, S. (2016). Exon junction complex proteins bind nascent transcripts independently of pre-mRNA splicing in *Drosophila melanogaster*. *ELife*, *5*, e19881. https://doi.org/10.7554/eLife.19881

Clark, M. S., Fraser, K. P. P., & Peck, L. S. (2008). Antarctic marine molluscs do have an HSP70 heat shock response. *Cell Stress and Chaperones*, *13*(1), 39–49. https://doi.org/10.1007/s12192-008-0014-8

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. *Global Change Biology*, *23*(1), 318–330. https://doi.org/10.1111/gcb.13357

Cline, A. J., Hamilton, S. L., & Logan, C. A. (2020). Effects of multiple climate change stressors on gene expression in blue rockfish (*Sebastes mystinus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *239*, 110580. https://doi.org/10.1016/j.cbpa.2019.110580

Cooper, G. M. (2000). *The cell: A molecular approach* (2. ed). ASM Press [u.a.].

Cossins, A. R., & Bowler, K. (1987). *Temperature Biology of Animals*. Springer Netherlands. https://doi.org/10.1007/978-94-009-3127-5

Côté, I. M., Darling, E. S., & Brown, C. J. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1824), 20152592. https://doi.org/10.1098/rspb.2015.2592

Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, *11*(12), 1304–1315. https://doi.org/10.1111/j.1461-0248.2008.01253.x

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*. *Journal of Experimental Marine Biology and Ecology*, *347*(1–2), 30–40. https://doi.org/10.1016/j.jembe.2007.02.013

Day, J. W., Kemp, W. M., Yáñez-Arancibia, A., & Crump, B. C. (2012). *Estuarine Ecology*. Wiley. https://books.google.ca/books?id=xvuHTzOwZ9AC

De Wit, P., Dupont, S., & Thor, P. (2016). Selection on oxidative phosphorylation and ribosomal structure as a multigenerational response to ocean acidification in the common copepod *Pseudocalanus acuspes*. *Evolutionary Applications*, *9*(9), 1112–1123. https://doi.org/10.1111/eva.12335

Del Rio, A. M., Davis, B. E., Fangue, N. A., & Todgham, A. E. (2019). Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. *Conservation Physiology*, *7*(1), coy078. https://doi.org/10.1093/conphys/coy078

DFO. (2017). *Bottom water pH in the Estuary and Gulf of St.Lawrence*. https://gisp.dfo-mpo.gc.ca/arcgis/rest/services/FGP/Teleost_Fond_pH_2017/MapServer

DFO. (2020a). *Assessment of Northern Shrimp stocks in the Estuary and Gulf of St. Lawrence in 2019.* (No. 2020/010). DFO Can. Sci. Advis. Sec. Sci. Advis. Rep.

DFO. (2020b). *Bottom water temperature and salinity in the Estuary and Gulf of St.Lawrence*. https://gisp.dfo-mpo.gc.ca/arcgis/rest/services/FGP/Teleost_BottomTemperatureSalinityFond/MapServer

DFO. (2020c). *Deep water dissolved oxygen in the Estuary and Gulf of St.Lawrence—Map of deep water dissolved oxygen in the Estuary and Gulf of St.Lawrence*. https://gisp.dfo-mpo.gc.ca/arcgis/rest/services/FGP/Deep_water_dissolved_oxygen_Teleost/MapServer

DFO. (2021). *Update of stock status indicator for Northern Shrimp in the Estuary and Gulf of St. Lawrence* (No. 2021/015). DFO Can. Sci. Advis. Sec. Sci. Resp.

Diaz, R., & Rosenberg, R. (1995). Marine benthic hypoxia: A review of its ecological effects and the behavioural response of benthic macrofauna. *Oceanography and Marine Biology. An Annual Review [Oceanogr. Mar. Biol. Annu. Rev.]*, *33*, 245–303.

Dineshram, R., Xiao, S., Ko, G. W. K., Li, J., Smrithi, K., Thiyagarajan, V., Zhang, Y., & Yu, Z. (2021). Ocean Acidification Triggers Cell Signaling, Suppress Immune and Calcification in the Pacific Oyster Larvae. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.782583

Disher, K., & Skandalis, A. (2007). Evidence of the modulation of mRNA splicing fidelity in humans by oxidative stress and p53. *Genome / National Research Council Canada = Génome / Conseil National de Recherches Canada*, *50*, 946–953. https://doi.org/10.1139/g07-074

Dixon, M. (1953). The effect of pH on the affinities of enzymes for substrates and inhibitors. *Biochemical Journal*, *55*(1), 161–170. https://doi.org/10.1042/bj0550161

Diz, A. P., Martínez-Fernández, M., & Rolán-Alvarez, E. (2012). Proteomics in evolutionary ecology: Linking the genotype with the phenotype: Proteomics in evolutionary ecology. *Molecular Ecology*, *21*(5), 1060–1080. https://doi.org/10.1111/j.1365-294X.2011.05426.x

Duan, Y., Liu, P., Li, J., Wang, Y., Li, J., & Chen, P. (2014). A farnesoic acid O-methyltransferase (FAMeT) from *Exopalaemon carinicauda* is responsive to *Vibrio anguillarum* and WSSV challenge. *Cell Stress and Chaperones*, *19*(3), 367–377. https://doi.org/10.1007/s12192-013-0464-5

Duan, Y., Wang, Y., Zhang, J., & Xiong, D. (2018). Elevated temperature disrupts the mucosal structure and induces an immune response in the intestine of whiteleg shrimp *Litopenaeus vannamei* (Boone, 1931) (Decapoda: Dendrobranchiata: Penaeidae). *Journal of Crustacean Biology*, *38*(5), 635–640. https://doi.org/10.1093/jcbiol/ruy055

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. *Journal of Experimental Marine Biology and Ecology*, *448*, 298–307. https://doi.org/10.1016/j.jembe.2013.07.019

Edison, A., Hall, R., Junot, C., Karp, P., Kurland, I., Mistrik, R., Reed, L., Saito, K., Salek, R., Steinbeck, C., Sumner, L., & Viant, M. (2016). The Time Is Right to Focus on Model Organism Metabolomes. *Metabolites*, *6*(1), 8. https://doi.org/10.3390/metabo6010008

Elliott, M., & Whitfield, A. K. (2011). Challenging paradigms in estuarine ecology and management. *Estuarine, Coastal and Shelf Science*, *94*(4), 306–314. https://doi.org/10.1016/j.ecss.2011.06.016

Estrada-Cárdenas, P., Cruz-Moreno, D. G., González-Ruiz, R., Peregrino-Uriarte, A. B., Leyva-Carrillo, L., Camacho-Jiménez, L., Quintero-Reyes, I., & Yepiz-Plascencia, G. (2021). Combined hypoxia and high temperature affect differentially the response of antioxidant enzymes, glutathione and hydrogen peroxide in the white shrimp *Litopenaeus vannamei*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *254*, 110909. https://doi.org/10.1016/j.cbpa.2021.110909

Falconer, D. S., & Mackay, T. (2009). *Introduction to quantitative genetics* (4. ed., [16. print.]). Pearson, Prentice Hall.

Fan, L., Wang, L., & Wang, Z. (2019). Proteomic characterization of the hepatopancreas in the Pacific white shrimp *Litopenaeus vannamei* under cold stress: Revealing the organism homeostasis mechanism. *Fish & Shellfish Immunology*, *92*, 438–449. https://doi.org/10.1016/j.fsi.2019.06.037

FAO. (2012). *The state of world fisheries and aquaculture 2012.* Food and Agriculture Organization of the United Nations ; Eurospan [distributor.

FAO. (2016). *The state of world fisheries and aquaculture 2016: Contributing to food security and nutrition for all.*

FAO. (2020). *The State of World Fisheries and Aquaculture 2020*. FAO. https://doi.org/10.4060/ca9229en

FAO, Barange, M., Bahri, T., Beveridge, M. C. M., Cochrane, K. L., Funge Smith, S., & Poulain, F. (2018). *Impacts of climate change on fisheries and aquaculture: Synthesis of current knowledge, adaptation and mitigation options*.

Feder, M. E., & Hofmann, G. E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and Ecological Physiology. *Annual Review of Physiology*, *61*(1), 243–282. https://doi.org/10.1146/annurev.physiol.61.1.243

Feijó Delgado, F., Cermak, N., Hecht, V. C., Son, S., Li, Y., Knudsen, S. M., Olcum, S., Higgins, J. M., Chen, J., Grover, W. H., & Manalis, S. R. (2013). Intracellular Water Exchange for Measuring the Dry Mass, Water Mass and Changes in Chemical Composition of Living Cells. *PLOS ONE*, *8*(7), e67590. https://doi.org/10.1371/journal.pone.0067590

Fernandes, J. A., Papathanasopoulou, E., Hattam, C., Queirós, A. M., Cheung, W. W. W. L., Yool, A., Artioli, Y., Pope, E. C., Flynn, K. J., Merino, G., Calosi, P., Beaumont, N., Austen, M. C., Widdicombe, S., & Barange, M. (2017). Estimating the ecological, economic and social impacts of ocean acidification and warming on UK fisheries. *Fish and Fisheries*, *18*(3), 389–411. https://doi.org/10.1111/faf.12183

Fields, P. A., Burmester, E. M., Cox, K. M., & Karch, K. R. (2016). Rapid proteomic responses to a near-lethal heat stress in the salt marsh mussel *Geukensia demissa*. *The Journal of Experimental Biology*, *219*(17), 2673–2686. https://doi.org/10.1242/jeb.141176

Fields, P. A., Zuzow, M. J., & Tomanek, L. (2012). Proteomic responses of blue mussel (*Mytilus*) congeners to temperature acclimation. *Journal of Experimental Biology*, *215*(7), 1106–1116. https://doi.org/10.1242/jeb.062273

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, *408*(6809), 239–247. https://doi.org/10.1038/35041687

Folt, C. L., Chen, C. Y., Moore, M. V., & Burnaford, J. (1999). Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, *44*(3part2), 864–877. https://doi.org/10.4319/lo.1999.44.3_part_2.0864

Forsman, A., & Wennersten, L. (2016). Inter-individual variation promotes ecological success of populations and species: Evidence from experimental and comparative studies. *Ecography*, *39*(7), 630–648. https://doi.org/10.1111/ecog.01357

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression* (Third). Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Freitas, R., Pires, A., Moreira, A., Wrona, F. J., Figueira, E., & Soares, A. M. V. M. (2016). Biochemical alterations induced in *Hediste diversicolor* under seawater acidification conditions. *Marine Environmental Research*, *117*, 75–84. https://doi.org/10.1016/j.marenvres.2016.04.003

Gadhia, S., Shrimp, J. H., Meier, J. L., McGee, J. E., & Dahlin, J. L. (2004). Histone Acetyltransferase Assays in Drug and Chemical Probe Discovery. In S. Markossian, A. Grossman, K. Brimacombe, M. Arkin, D. Auld, C. Austin, J. Baell, T. D. Y. Chung, N. P. Coussens, J. L. Dahlin, V. Devanarayan, T. L. Foley, M. Glicksman, J. V. Haas, M. D. Hall, S. Hoare, J. Inglese, P. W. Iversen, S. C. Kales, … X. Xu (Eds.), *Assay Guidance Manual*. Eli Lilly & Company and the National Center for Advancing Translational Sciences. http://www.ncbi.nlm.nih.gov/books/NBK442298/

Galbraith, P. S., Canadian Science Advisory Secretariat, Canada, Department of Fisheries and Oceans, & Qu??bec Region. (2021). *Physical oceanographic conditions in the Gulf of St. Lawrence during 2020*. https://epe.lac-bac.gc.ca/100/201/301/weekly_acquisitions_list-ef/2021/21-28/https@publications.gc.ca/collections/collection_2021/mpo-dfo/fs70-5/Fs70-5-2021-045-eng.pdf

Galland, C., Dupuy, C., Capitaine, C., Auffret, M., Quiniou, L., Laroche, J., & Pichereau, V. (2013). Comparisons of liver proteomes in the European flounder *Platichthys flesus* from three contrasted estuaries. *Journal of Sea Research*, *75*, 135–141. https://doi.org/10.1016/j.seares.2012.05.009

Geihs, M. A., Vargas, M. A., Maciel, F. E., Vakkuri, O., Meyer-Rochow, V. B., Allodi, S., & Nery, L. E. M. (2016). Effects of hypoxia and reoxygenation on the antioxidant defense system of the locomotor muscle of the crab *Neohelice granulata* (Decapoda, Varunidae). *Journal of Comparative Physiology B*, *186*(5), 569–579. https://doi.org/10.1007/s00360-016-0976-2

Gilbert, D. (2004). *Propagation of temperature signals from the northwest Atlantic continental shelf edge into the Laurentian Channel* (ICES CM 2004/N:07; p. 12).

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. *Limnology and Oceanography*, *50*(5), 1654–1666. https://doi.org/10.4319/lo.2005.50.5.1654

Godbold, J. A., & Solan, M. (2013). Long-term effects of warming and ocean acidification are modified by seasonal variation in species responses and environmental conditions. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *368*(1627), 20130186–20130186. PubMed. https://doi.org/10.1098/rstb.2013.0186

González, K., Gaitán-Espitia, J., Font, A., Cárdenas, C. A., & González-Aravena, M. (2016). Expression pattern of heat shock proteins during acute thermal stress in the Antarctic sea urchin, *Sterechinus neumayeri*. *Revista Chilena de Historia Natural*, *89*(1), 2. https://doi.org/10.1186/s40693-016-0052-z

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*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *243–244*, 110438. https://doi.org/10.1016/j.cbpb.2020.110438

Gould, S. J., & Vrba, E. S. (1982). Exaptation—A Missing Term in the Science of Form. *Paleobiology*, *8*(1), 4–15. https://doi.org/10.1017/S0094837300004310

Gracey, A. Y., Troll, J. V., & Somero, G. N. (2001). Hypoxia-induced gene expression profiling in the euryoxic fish *Gillichthys mirabilis*. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(4), 1993–1998.

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

Halpern, B. S., Frazier, M., Afflerbach, J., Lowndes, J. S., Micheli, F., O'Hara, C., Scarborough, C., & Selkoe, K. A. (2019). Recent pace of change in human impact on the world's ocean. *Scientific Reports*, *9*(1), Article 1. https://doi.org/10.1038/s41598-019-47201-9

Halpern, B. S., Frazier, M., Potapenko, J., Casey, K. S., Koenig, K., Longo, C., Lowndes, J. S., Rockwood, R. C., Selig, E. R., Selkoe, K. A., & Walbridge, S. (2015). Spatial and temporal changes in cumulative human impacts on the world's ocean. *Nature Communications*, *6*(1), 7615. https://doi.org/10.1038/ncomms8615

Hammer, K., & Pedersen, S. (2013). Deep-water prawn *Pandalus borealis* displays a relatively high pH regulatory capacity in response to CO_2-induced acidosis. *Marine Ecology Progress Series*, *492*, 139–151. https://doi.org/10.3354/meps10476

Hardie, D., Covey, M., Nickerson, K., & King, M. (2015). *Scotian shelf shrimp 2014-2015* (p. 44). Canadian Science Advisory Secretariat Research Document.

Hausfather, Z., & Peters, G. P. (2020). Emissions—The "business as usual" story is misleading. *Nature*, *577*(7792), 618–620. https://doi.org/10.1038/d41586-020-00177-3

He, Y., Wang, Q., Li, J., & Li, Z. (2022). Comparative proteomic profiling in Chinese shrimp *Fenneropenaeus chinensis* under low pH stress. *Fish & Shellfish Immunology*, *120*, 526–535. https://doi.org/10.1016/j.fsi.2021.12.032

Hermes-Lima, M., & Zenteno-Savín, T. (2002). Animal response to drastic changes in oxygen availability and physiological oxidative stress. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *133*(4), 537–556. https://doi.org/10.1016/S1532-0456(02)00080-7

Hershko, A., & Ciechanover, A. (1998). The ubiquitin system. *Annual Review of Biochemistry*, *67*(1), 425–479. https://doi.org/10.1146/annurev.biochem.67.1.425

Hochachka, P. W., & Somero, G. N. (2002). *Biochemical Adaptation Mechanism and Process in Physiological Evolution*.

Huang, B., Zhang, L., Tang, X., Zhang, G., & Li, L. (2016). Genome-Wide Analysis of Alternative Splicing Provides Insights into Stress Adaptation of the Pacific Oyster. *Marine Biotechnology*, *18*(5), 598–609. https://doi.org/10.1007/s10126-016-9720-x

Huo, D., Sun, L., Zhang, L., Ru, X., Liu, S., Yang, X., & Yang, H. (2019). Global-warming-caused changes of temperature and oxygen alter the proteomic profile of sea cucumber *Apostichopus japonicus*. *Journal of Proteomics*, *193*, 27–43. https://doi.org/10.1016/j.jprot.2018.12.020

ICES. (2019). *Northern shrimp (Pandalus borealis) in divisions 3.a and 4.a East (Skagerrak and Kattegat and northern North Sea in the Norwegian Deep)*. https://doi.org/10.17895/ICES.ADVICE.4892

Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, *54*(4), 287–293. https://doi.org/10.1016/j.ajme.2017.09.001

IPCC. (2014). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]* (p. 151).

IPCC. (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Issifu, I., Alava, J. J., Lam, V. W. Y., & Sumaila, U. R. (2022). Impact of Ocean Warming, Overfishing and Mercury on European Fisheries: A Risk Assessment and Policy

Solution Framework. *Frontiers in Marine Science*, *8*. https://www.frontiersin.org/articles/10.3389/fmars.2021.770805

Jiang, H., Li, F., Xie, Y., Huang, B., Zhang, J., Zhang, J., Zhang, C., Li, S., & Xiang, J. (2009). Comparative proteomic profiles of the hepatopancreas in *Fenneropenaeus chinensis* response to hypoxic stress. *Proteomics*, *9*(12), 3353–3367. https://doi.org/10.1002/pmic.200800518

Johnson, J. G., Paul, M. R., Kniffin, C. D., Anderson, P. E., Burnett, L. E., & Burnett, K. G. (2015). High CO_2 alters the hypoxia response of the Pacific whiteleg shrimp (*Litopenaeus vannamei*) transcriptome including known and novel hemocyanin isoforms. *Physiological Genomics*, *47*(11), 548–558. https://doi.org/10.1152/physiolgenomics.00031.2015

Kambayashi, Y., Binh, N. T., W. Asakura, H., Hibino, Y., Hitomi, Y., Nakamura, H., & Ogino, K. (2009). Efficient Assay for Total Antioxidant Capacity in Human Plasma Using a 96-Well Microplate. *Journal of Clinical Biochemistry and Nutrition*, *44*(1), 46–51. https://doi.org/10.3164/jcbn.08-162

Kamemura, K., Ogawa, M., Ohkubo, S., Ohtsuka, Y., Shitara, Y., Komiya, T., Maeda, S., Ito, A., & Yoshida, M. (2012). Depression of mitochondrial metabolism by downregulation of cytoplasmic deacetylase, HDAC6. *FEBS Letters*, *586*(9), 1379–1383. https://doi.org/10.1016/j.febslet.2012.03.060

Kamm, K. E., & Stull, J. T. (2011). Signaling to Myosin Regulatory Light Chain in Sarcomeres *. *Journal of Biological Chemistry*, *286*(12), 9941–9947. https://doi.org/10.1074/jbc.R110.198697

Kanopka, A. (2017). Cell survival: Interplay between hypoxia and pre-mRNA splicing. *Experimental Cell Research*, *356*(2), 187–191. https://doi.org/10.1016/j.yexcr.2017.03.018

Karpievitch, Y. V., Polpitiya, A. D., Anderson, G. A., Smith, R. D., & Dabney, A. R. (2010). Liquid chromatography mass spectrometry-based proteomics: Biological and technological aspects. *The Annals of Applied Statistics*, *4*(4), 1797–1823. https://doi.org/10.1214/10-AOAS341

Katada, S., Imhof, A., & Sassone-Corsi, P. (2012). Connecting Threads: Epigenetics and Metabolism. *Cell*, *148*(1–2), 24–28. https://doi.org/10.1016/j.cell.2012.01.001

Kataoka, N. (2001). Magoh, a human homolog of *Drosophila* mago nashi protein, is a component of the splicing-dependent exon-exon junction complex. *The EMBO Journal*, *20*(22), 6424–6433. https://doi.org/10.1093/emboj/20.22.6424

Kawabe, S., & Yokoyama, Y. (2011). Novel isoforms of heat shock transcription factor 1 are induced by hypoxia in the Pacific oyster *Crassostrea gigas*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *315A*(7), 394–407. https://doi.org/10.1002/jez.685

Keeling, R. F., Körtzinger, A., & Gruber, N. (2010). Ocean Deoxygenation in a Warming World. *Annual Review of Marine Science*, *2*(1), 199–229. https://doi.org/10.1146/annurev.marine.010908.163855

Khan, F. U., Chen, H., Gu, H., Wang, T., Dupont, S., Kong, H., Shang, Y., Wang, X., Lu, W., Hu, M., & Wang, Y. (2021). Antioxidant responses of the mussel *Mytilus coruscus* co-exposed to ocean acidification, hypoxia and warming. *Marine Pollution Bulletin*, *162*, 111869. https://doi.org/10.1016/j.marpolbul.2020.111869

Khan, F. U., Hu, M., Kong, H., Shang, Y., Wang, T., Wang, X., Xu, R., Lu, W., & Wang, Y. (2020). Ocean acidification, hypoxia and warming impair digestive parameters of marine mussels. *Chemosphere*, *256*, 127096. https://doi.org/10.1016/j.chemosphere.2020.127096

Kinne, O. (1970). *Marine ecology: A comprehensive, integrated treatise on life in oceans and coastal waters*. Wiley-Interscience.

Knutsen, H., Jorde, P. E., Blanco Gonzalez, E., Eigaard, O. R., Pereyra, R. T., Sannæs, H., Dahl, M., André, C., & Søvik, G. (2015). Does population genetic structure support present management regulations of the northern shrimp (*Pandalus borealis*) in Skagerrak and the North Sea? *ICES Journal of Marine Science*, *72*(3), 863–871. https://doi.org/10.1093/icesjms/fsu204

Koeller, P. A. (2000). Relative Importance of Abiotic and Biotic Factors to the Management of the Northern Shrimp (*Pandalus borealis*) Fishery on the Scotian Shelf (updated 4 April 2001). *Journal of Northwest Atlantic Fishery Science*, *27*.

Kourantidou, M., Hoagland, P., Dale, A., & Bailey, M. (2021). Equitable Allocations in Northern Fisheries: Bridging the Divide for Labrador Inuit. *Frontiers in Marine Science*, *8*, 590213. https://doi.org/10.3389/fmars.2021.590213

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. *Global Change Biology*, *19*(6), 1884–1896. https://doi.org/10.1111/gcb.12179

Kroeker, K. J., Kordas, R. L., & Harley, C. D. G. (2017). Embracing interactions in ocean acidification research: Confronting multiple stressor scenarios and context dependence. *Biology Letters*, *13*(3), 20160802. https://doi.org/10.1098/rsbl.2016.0802

Kültz, D. (2005). Molecular and evolutionary basis of the cellular stress response. *Annual Review of Physiology*, *67*(1), 225–257. https://doi.org/10.1146/annurev.physiol.67.040403.103635

Kültz, D. (2020a). Defining biological stress and stress responses based on principles of physics. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *333*(6), 350–358. https://doi.org/10.1002/jez.2340

Kültz, D. (2020b). Evolution of cellular stress response mechanisms. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *333*(6), 359–378. https://doi.org/10.1002/jez.2347

Kumar, S. (2012). Assay Guided Comparison for Enzymatic and Non-Enzymatic Antioxidant Activities with Special Reference to Medicinal Plants. In M. A. El-Missiry (Ed.), *Antioxidant Enzyme*. InTech. https://doi.org/10.5772/50782

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

LaFramboise, W. A., Griffis, B., Bonner, P., Warren, W., Scalise, D., Guthrie, R. D., & Cooper, R. L. (2000). Muscle type-specific myosin isoforms in crustacean muscles. *The Journal of Experimental Zoology*, *286*(1), 36–48. https://doi.org/10.1002/(SICI)1097-010X(20000101)286:1<36::AID-JEZ4>3.0.CO;2-G

Lavoie, D., Canada, & Department of Fisheries and Oceans. (2020). *Projections of future physical and biogeochemical conditions in the Gulf of St. Lawrence, on the Scotian Shelf and in the Gulf of Maine*. https://epe.lac-bac.gc.ca/100/201/301/weekly_acquisitions_list-ef/2020/20-47/publications.gc.ca/collections/collection_2020/mpo-dfo/Fs97-18-334-eng.pdf

Lavoie, D., Lambert, N., Starr, M., Chassé, J., Riche, O., Le Clainche, Y., Azetsu-Scott, K., Béjaoui, B., Christian, J. R., & Gilbert, D. (2021). The Gulf of St. Lawrence Biogeochemical Model: A Modelling Tool for Fisheries and Ocean Management. *Frontiers in Marine Science*, *8*, 732269. https://doi.org/10.3389/fmars.2021.732269

Layton, K. K. S., & Bradbury, I. R. (2022). Harnessing the power of multi-omics data for predicting climate change response. *Journal of Animal Ecology*, *91*(6), 1064–1072. https://doi.org/10.1111/1365-2656.13619

Lee, Y. H., Kang, H.-M., Kim, M.-S., Lee, J.-S., Wang, M., Hagiwara, A., Jeong, C.-B., & Lee, J.-S. (2020). Multigenerational Mitigating Effects of Ocean Acidification on *In Vivo* Endpoints, Antioxidant Defense, DNA Damage Response, and Epigenetic Modification in an Asexual Monogonont Rotifer. *Environmental Science & Technology*, *54*(13), 7858–7869. https://doi.org/10.1021/acs.est.0c01438

Lesser, M. P. (2006). Oxidative stress in marine environments: Biochemistry and physiological ecology. *Annual Review of Physiology*, *68*(1), 253–278. https://doi.org/10.1146/annurev.physiol.68.040104.110001

Leung, J. Y. S., Russell, B. D., & Connell, S. D. (2019). Adaptive Responses of Marine Gastropods to Heatwaves. *One Earth*, *1*(3), 374–381. https://doi.org/10.1016/j.oneear.2019.10.025

Li, D.-X., Du, X.-J., Zhao, X.-F., & Wang, J.-X. (2006). Cloning and expression analysis of an o-methyltransferase (OMT) gene from Chinese shrimp, *Fenneropenaeus*

chinensis. *Fish & Shellfish Immunology*, *21*(3), 284–292. https://doi.org/10.1016/j.fsi.2005.12.005

Li, J., Li, W., Zhang, X., & He, P. (2018). Physiological and behavioral responses of different modes of locomotion in the whiteleg shrimp *Litopenaeus vannamei* (Boone, 1931) (Caridea: Penaeidae). *Journal of Crustacean Biology*, *38*(1), 79–90. https://doi.org/10.1093/jcbiol/rux107

Li, S., Huang, J., Liu, C., Liu, Y., Zheng, G., Xie, L., & Zhang, R. (2016). Interactive Effects of Seawater Acidification and Elevated Temperature on the Transcriptome and Biomineralization in the Pearl Oyster *Pinctada fucata*. *Environmental Science & Technology*, *50*(3), 1157–1165. https://doi.org/10.1021/acs.est.5b05107

Lodish, H. F., & Darnell, J. E. (Eds.). (1995). *Molecular cell biology* (3rd ed). Scientific American Books : Distributed by W.H. Freeman and Co.

Logan, M. L., & Cox, C. L. (2020). Genetic Constraints, Transcriptome Plasticity, and the Evolutionary Response to Climate Change. *Frontiers in Genetics*, *11*. https://www.frontiersin.org/articles/10.3389/fgene.2020.538226

Lopez-Anido, R. N., Harrington, A. M., & Hamlin, H. J. (2021). Coping with stress in a warming Gulf: The postlarval American lobster's cellular stress response under future warming scenarios. *Cell Stress and Chaperones*, *26*(4), 721–734. https://doi.org/10.1007/s12192-021-01217-1

Lushchak, V. I., Lushchak, L. P., Mota, A. A., & Hermes-Lima, M. (2001). Oxidative stress and antioxidant defenses in goldfish *Carassius auratus* during anoxia and reoxygenation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *280*(1), R100–R107. https://doi.org/10.1152/ajpregu.2001.280.1.R100

Madeira, C., Mendonça, V., Leal, M. C., Diniz, M. S., Cabral, H. N., Flores, A. A. V., & Vinagre, C. (2019). Present and future invasion perspectives of an alien shrimp in South Atlantic coastal waters: An experimental assessment of functional biomarkers and thermal tolerance. *Biological Invasions*, *21*(5), 1567–1584. https://doi.org/10.1007/s10530-019-01921-1

Madeira, D., Araújo, J. E., Madeira, C., Mendonça, V., Vitorino, R., Vinagre, C., & Diniz, M. S. (2020). Seasonal proteome variation in intertidal shrimps under a natural setting: Connecting molecular networks with environmental fluctuations. *Science of The Total Environment*, *703*, 134957. https://doi.org/10.1016/j.scitotenv.2019.134957

Madeira, D., Araújo, J. E., Vitorino, R., Capelo, J. L., Vinagre, C., & Diniz, M. S. (2016). Ocean warming alters cellular metabolism and induces mortality in fish early life stages: A proteomic approach. *Environmental Research*, *148*, 164–176. https://doi.org/10.1016/j.envres.2016.03.030

Madeira, D., Araújo, J. E., Vitorino, R., Costa, P. M., Capelo, J. L., Vinagre, C., & Diniz, M. S. (2017). Molecular Plasticity under Ocean Warming: Proteomics and Fitness Data Provides Clues for a Better Understanding of the Thermal Tolerance in Fish. *Frontiers in Physiology*, *8*, 825. https://doi.org/10.3389/fphys.2017.00825

Madeira, D., Costa, P. M., Vinagre, C., & Diniz, M. S. (2016). When warming hits harder: Survival, cellular stress and thermal limits of *Sparus aurata* larvae under global change. *Marine Biology*, *163*(4), 91. https://doi.org/10.1007/s00227-016-2856-4

Madeira, D., Fernandes, J. F., Jerónimo, D., Ricardo, F., Santos, A., Domingues, M. R., & Calado, R. (2021). Calcium homeostasis and stable fatty acid composition underpin heatwave tolerance of the keystone polychaete *Hediste diversicolor*. *Environmental Research*, *195*, 110885. https://doi.org/10.1016/j.envres.2021.110885

Madeira, D., Mendonça, V., Dias, M., Roma, J., Costa, P. M., Diniz, M. S., & Vinagre, C. (2014). Physiological and biochemical thermal stress response of the intertidal rock goby *Gobius paganellus*. *Ecological Indicators*, *46*, 232–239. https://doi.org/10.1016/j.ecolind.2014.06.029

Madeira, D., Mendonça, V., Madeira, C., Gaiteiro, C., Vinagre, C., & Diniz, M. S. (2019). Molecular assessment of wild populations in the marine realm: Importance of taxonomic, seasonal and habitat patterns in environmental monitoring. *Science of The Total Environment*, *654*, 250–263. https://doi.org/10.1016/j.scitotenv.2018.11.064

Madeira, D., Narciso, L., Cabral, H. N., Vinagre, C., & Diniz, M. S. (2013). Influence of temperature in thermal and oxidative stress responses in estuarine fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *166*(2), 237–243. https://doi.org/10.1016/j.cbpa.2013.06.008

Madeira, D., Narciso, L., Diniz, M. S., & Vinagre, C. (2014). Synergy of environmental variables alters the thermal window and heat shock response: An experimental test with the crab *Pachygrapsus marmoratus*. *Marine Environmental Research*, *98*, 21–28. https://doi.org/10.1016/j.marenvres.2014.03.011

MAPAQ. (2015). *Monographie de l'industrie de la crevette nordique au Québec / rédaction et coordination [...]* (Collections de BAnQ). https://numerique.banq.qc.ca/patrimoine/details/52327/2500524

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*. *Ecology and Evolution*, *11*(7), 3366–3379. https://doi.org/10.1002/ece3.7289

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?* Biodiversity and Ecosystem Function: Marine. https://doi.org/10.5194/bgd-6-4693-2009

Michiels, C. (2004). Physiological and Pathological Responses to Hypoxia. *The American Journal of Pathology*, *164*(6), 1875–1882. https://doi.org/10.1016/S0002-9440(10)63747-9

Mohamad, A., Arshad, A., Sung, Y. Y., & Jasmani, S. (2018). Effect of thermal stress on Hsp70 gene expression and female reproductive performance of giant freshwater prawn, *Macrobrachium rosenbergii*. *Aquaculture Research*, *49*(1), 135–150. https://doi.org/10.1111/are.13442

Moreira, D. C., Venancio, L. P. R., Sabino, M. A. C. T., & Hermes-Lima, M. (2016). How widespread is preparation for oxidative stress in the animal kingdom? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *200*, 64–78. https://doi.org/10.1016/j.cbpa.2016.01.023

Mounier, N., Gouy, M., Mouchiroud, D., & Prudhomme, J. C. (1992). Insect muscle actins differ distinctly from invertebrate and vertebrate cytoplasmic actins. *Journal of Molecular Evolution*, *34*(5), 406–415. https://doi.org/10.1007/BF00162997

MPO. (2018). *Plans de gestion intégrée des pêches: Crevette nordique – Zones 8, 9, 10 et 12 (L'estuaire et le golfe du Saint-Laurent)*. https://www.dfo-mpo.gc.ca/fisheries-peches/ifmp-gmp/shrimp-crevette/shrimp-crevette-2018-fra.html

Mucci, A., Levasseur, M., Gratton, Y., Martias, C., Scarratt, M., Gilbert, D., Tremblay, J.-E., Ferreyra, G., & Lansard, B. (2017). Tidally induced variations of pH at the head of the Laurentian Channel. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*. 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. *Atmosphere-Ocean*, *49*(3), 206–218. https://doi.org/10.1080/07055900.2011.599265

Mukherjee, J., Wong, K. K. W., Chandramouli, K. H., Qian, P.-Y., Leung, P. T. Y., Wu, R. S. S., & Thiyagarajan, V. (2013). Proteomic response of marine invertebrate larvae to ocean acidification and hypoxia during metamorphosis and calcification. *Journal of Experimental Biology*, *216*(24), 4580–4589. https://doi.org/10.1242/jeb.094516

Mukherjee, K., Fischer, R., & Vilcinskas, A. (2012). Histone acetylation mediates epigenetic regulation of transcriptional reprogramming in insects during metamorphosis, wounding and infection. *Frontiers in Zoology*, *9*(1), 25. https://doi.org/10.1186/1742-9994-9-25

Murren, C. J., Auld, J. R., Callahan, H., Ghalambor, C. K., Handelsman, C. A., Heskel, M. A., Kingsolver, J. G., Maclean, H. J., Masel, J., Maughan, H., Pfennig, D. W., Relyea, R. A., Seiter, S., Snell-Rood, E., Steiner, U. K., & Schlichting, C. D. (2015). Constraints on the evolution of phenotypic plasticity: Limits and costs of phenotype and plasticity. *Heredity*, *115*(4), 293–301. https://doi.org/10.1038/hdy.2015.8

Newell, R. C. (1979). *Biology of intertidal animals*. Marine Ecological Surveys.

Niki, E. (1993). Antioxidant Defenses In Eukariotic Cells: An Overview. In G. Poli, E. Albano, & M. U. Dianzani (Eds.), *Free Radicals: From Basic Science to Medicine* (pp. 365–373). Birkhäuser Basel. https://doi.org/10.1007/978-3-0348-9116-5_31

Ochiai, Y., & Ozawa, H. (2020). Biochemical and physicochemical characteristics of the major muscle proteins from fish and shellfish. *Fisheries Science*, *86*(5), 729–740. https://doi.org/10.1007/s12562-020-01444-y

O'Donnell, M. J., Hammond, L. M., & Hofmann, G. E. (2009). Predicted impact of ocean acidification on a marine invertebrate: Elevated CO2 alters response to thermal stress in sea urchin larvae. *Marine Biology*, *156*(3), 439–446. https://doi.org/10.1007/s00227-008-1097-6

Oksala, N. K. J., Ekmekçi, F. G., Özsoy, E., Kirankaya, Ş., Kokkola, T., Emecen, G., Lappalainen, J., Kaarniranta, K., & Atalay, M. (2014). Natural thermal adaptation increases heat shock protein levels and decreases oxidative stress. *Redox Biology*, *3*, 25–28. https://doi.org/10.1016/j.redox.2014.10.003

Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., Benthuysen, J. A., Feng, M., Sen Gupta, A., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Straub, S. C., & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature Communications*, *9*(1), Article 1. https://doi.org/10.1038/s41467-018-03732-9

Orr, D., & Sullivan, D. (2013). *The February 2013 assessment of northern shrimp (Pandalus borealis) off Labrador and northeastern Newfoundland* (p. 144). Canadian Science Advisory Secretariat Research Document.

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. *Journal of Experimental Marine Biology and Ecology*, *497*, 50–60. https://doi.org/10.1016/j.jembe.2017.09.007

Padilla-Gamiño, J. L., Kelly, M. W., Evans, T. G., & Hofmann, G. E. (2013). Temperature and CO_2 additively regulate physiology, morphology and genomic responses of larval sea urchins, *Strongylocentrotus purpuratus*. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1759), 20130155. https://doi.org/10.1098/rspb.2013.0155

Pannunzio, T. M., & Storey, K. B. (1998). Antioxidant defenses and lipid peroxidation during anoxia stress and aerobic recovery in the marine gastropod *Littorina littorea*. *Journal of Experimental Marine Biology and Ecology*, *221*(2), 277–292. https://doi.org/10.1016/S0022-0981(97)00132-9

Parrilla-Taylor, D. P., & Zenteno-Savín, T. (2011). Antioxidant enzyme activities in Pacific white shrimp (*Litopenaeus vannamei*) in response to environmental hypoxia and reoxygenation. *Aquaculture*, *318*(3–4), 379–383. https://doi.org/10.1016/j.aquaculture.2011.05.015

Pearson, G. A., Lago-Leston, A., & Mota, C. (2009). Frayed at the edges: Selective pressure and adaptive response to abiotic stressors are mismatched in low diversity edge populations. *Journal of Ecology*, *97*(3), 450–462. https://doi.org/10.1111/j.1365-2745.2009.01481.x

Peck, L. S. (2002). Ecophysiology of Antarctic marine ectotherms: Limits to life. In W. E. Arntz & A. Clarke (Eds.), *Ecological Studies in the Antarctic Sea Ice Zone: Results of EASIZ Midterm Symposium* (pp. 221–230). Springer. https://doi.org/10.1007/978-3-642-59419-9_29

Peck, L. S., & Conway, L. Z. (2000). The myth of metabolic cold adaptation: Oxygen consumption in stenothermal Antarctic bivalves. *Geological Society, London, Special Publications*, *177*(1), 441–450. https://doi.org/10.1144/GSL.SP.2000.177.01.29

Peck, L. S., Pörtner, H. O., & Hardewig, I. (2002). Metabolic Demand, Oxygen Supply, and Critical Temperatures in the Antarctic Bivalve *Laternula elliptica*. *Physiological and Biochemical Zoology*, *75*(2), 123–133. https://doi.org/10.1086/340990

Peck, L. S., Webb, K. E., & Bailey, D. M. (2004). Extreme sensitivity of biological function to temperature in Antarctic marine species. *Functional Ecology*, *18*(5), 625–630. https://doi.org/10.1111/j.0269-8463.2004.00903.x

Piggott, J. J., Townsend, C. R., & Matthaei, C. D. (2015). Reconceptualizing synergism and antagonism among multiple stressors. *Ecology and Evolution*, *5*(7), 1538–1547. https://doi.org/10.1002/ece3.1465

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*. Université du Québec à Rimouski.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2021). *nlme: Linear and Nonlinear Mixed Effects Models*. 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*(7754), 108–111. https://doi.org/10.1038/s41586-019-1132-4

Poloczanska, E. S., Burrows, M. T., Brown, C. J., García Molinos, J., Halpern, B. S., Hoegh-Guldberg, O., Kappel, C. V., Moore, P. J., Richardson, A. J., Schoeman, D. S., & Sydeman, W. J. (2016). Responses of Marine Organisms to Climate Change across Oceans. *Frontiers in Marine Science*, *3*. https://www.frontiersin.org/articles/10.3389/fmars.2016.00062

Pörtner, H. O., & Farrell, A. P. (2008). Physiology and Climate Change. *Science*, *322*(5902), 690–692. https://doi.org/10.1126/science.1163156

Pörtner, H. O., Peck, L., & Somero, G. (2007). Thermal limits and adaptation in marine Antarctic ectotherms: An integrative view. *Philosophical Transactions of the Royal*

Society B: Biological Sciences, *362*(1488), 2233–2258. https://doi.org/10.1098/rstb.2006.1947

Prosser, C. L., & Brown, F. A. (1973). *Comparative Animal Physiology*. Saunders. https://books.google.ca/books?id=4MNqAAAAMAAJ

Qian, Z., & Liu, X. (2019). Elucidation of the role of farnesoic acid O-methyltransferase (FAMeT) in the giant freshwater prawn, *Macrobrachium rosenbergii*: Possible functional correlation with ecdysteroid signaling. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *232*, 1–12. https://doi.org/10.1016/j.cbpa.2019.03.003

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Reipschläger, A., & Pörtner, H. O. (1996). Metabolic depression during environmental stress: The role of extracellular versus intracellular pH in *Sipunculus nudus*. *Journal of Experimental Biology*, *199*(8), 1801–1807. https://doi.org/10.1242/jeb.199.8.1801

Resende, A. C., & Pereira, D. M. C. (2021). *IBRtools: Indexes for biomonitoring assessment and graphic creation*.

Richards, J. G. (2009). Chapter 10 Metabolic and Molecular Responses of Fish to Hypoxia. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology* (Vol. 27, pp. 443–485). Academic Press. https://doi.org/10.1016/S1546-5098(08)00010-1

Richards, R. A., & Hunter, M. (2021). Northern shrimp *Pandalus borealis* population collapse linked to climate-driven shifts in predator distribution. *PLOS ONE*, *16*(7), e0253914. https://doi.org/10.1371/journal.pone.0253914

Righetti, P. G., & Boschetti, E. (2013). Introducing Low-Abundance Species in Proteome Analysis. In *Low-Abundance Proteome Discovery* (pp. 1–11). Elsevier. https://doi.org/10.1016/B978-0-12-401734-4.00001-4

Ritchie, J., & Dowlatabadi, H. (2017). The 1000 GtC coal question: Are cases of vastly expanded future coal combustion still plausible? *Energy Economics*, *65*, 16–31. https://doi.org/10.1016/j.eneco.2017.04.015

Roman, M. R., Brandt, S. B., Houde, E. D., & Pierson, J. J. (2019). Interactive Effects of Hypoxia and Temperature on Coastal Pelagic Zooplankton and Fish. *Frontiers in Marine Science*, *6*. https://doi.org/10.3389/fmars.2019.00139

Ruppert, E. E., Fox, R. S., & Barnes, R. D. (2004). *Invertebrate Zoology: A Functional Evolutionary Approach*. Thomson-Brooks/Cole. https://books.google.ca/books?id=A3opAQAAMAAJ

Salin, K., Auer, S. K., Rudolf, A. M., Anderson, G. J., Cairns, A. G., Mullen, W., Hartley, R. C., Selman, C., & Metcalfe, N. B. (2015). Individuals with higher metabolic rates have lower levels of reactive oxygen species *in vivo*. *Biology Letters*, *11*(9), 20150538. https://doi.org/10.1098/rsbl.2015.0538

Salinas, S., Irvine, S. E., Schertzing, C. L., Golden, S. Q., & Munch, S. B. (2019). Trait variation in extreme thermal environments under constant and fluctuating temperatures. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1768), 20180177. https://doi.org/10.1098/rstb.2018.0177

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. *Nature Ecology & Evolution*, *5*(3), 311–321. https://doi.org/10.1038/s41559-020-01370-3

Sarrazin, S., Lamanna, W. C., & Esko, J. D. (2011). Heparan Sulfate Proteoglycans. *Cold Spring Harbor Perspectives in Biology*, *3*(7), a004952–a004952. https://doi.org/10.1101/cshperspect.a004952

Schlegel, R. W., Oliver, E. C. J., & Chen, K. (2021). Drivers of Marine Heatwaves in the Northwest Atlantic: The Role of Air–Sea Interaction During Onset and Decline. *Frontiers in Marine Science*, *8*. https://www.frontiersin.org/articles/10.3389/fmars.2021.627970

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, *218*(12), 1856–1866. https://doi.org/10.1242/jeb.118851

Schwerin, S., Zeis, B., Lamkemeyer, T., Paul, R. J., Koch, M., Madlung, J., Fladerer, C., & Pirow, R. (2009). Acclimatory responses of the *Daphnia pulex* proteome to environmental changes. II. Chronic exposure to different temperatures (10 and 20°C) mainly affects protein metabolism. *BMC Physiology*, *9*(1), 8. https://doi.org/10.1186/1472-6793-9-8

Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, *5*(1), 61–66. https://doi.org/10.1038/nclimate2457

Serafini, M., & Del Rio, D. (2004). Understanding the association between dietary antioxidants, redox status and disease: Is the Total Antioxidant Capacity the right tool? *Redox Report*, *9*(3), 145–152. https://doi.org/10.1179/135100004225004814

Serpetti, N., Baudron, A. R., Burrows, M. T., Payne, B. L., Helaouët, P., Fernandes, P. G., & Heymans, J. J. (2017). Impact of ocean warming on sustainable fisheries management informs the Ecosystem Approach to Fisheries. *Scientific Reports*, *7*(1), Article 1. https://doi.org/10.1038/s41598-017-13220-7

Sévigny, J.-M., Savard, L., & Parsons, D. (2000). Genetic Characterization of the Northern Shrimp, *Pandalus borealis* in the Northwest Atlantic Using Electrophoresis of Enzymes. *Journal of Northwest Atlantic Fishery Science*, *27*, 161–175. https://doi.org/10.2960/J.v27.a15

Shumway, S., Perkins, H. C., Schick, D. F., Stickney, A. P., & FI. (1985). Synopsis of biological data on the pink shrimp, *Pandalus borealis* Krøyer, 1838. NOAA Technical Report NMFS 30. *XF2006254177*, *144*.

Sidoli, S., Kulej, K., & Garcia, B. A. (2017). Why proteomics is not the new genomics and the future of mass spectrometry in cell biology. *Journal of Cell Biology*, *216*(1), 21–24. https://doi.org/10.1083/jcb.201612010

Siferd, T. D. (2015). *2015 Assessment of Northern Shrimp (Pandalus borealis) and Striped Shrimp (Pandalus montagui) in the Eastern and Western Assessment Zones (SFAs Nunavut, Nunavik and Davis Strait)* (p. 70). Canadian Science Advisory Secretariat Research Document.

Silvestre, F., Gillardin, V., & Dorts, J. (2012). Proteomics to Assess the Role of Phenotypic Plasticity in Aquatic Organisms Exposed to Pollution and Global Warming. *Integrative and Comparative Biology*, *52*(5), 681–694. https://doi.org/10.1093/icb/ics087

Skipper, M., Weiss, U., & Gray, N. (2010). Plasticity. *Nature*, *465*(7299), 703–703. https://doi.org/10.1038/465703a

Small, D., Calosi, P., White, D., Spicer, J., & Widdicombe, S. (2010). Impact of medium-term exposure to CO_2 enriched seawater on the physiological functions of the velvet swimming crab *Necora puber*. *Aquatic Biology*, *10*(1), 11–21. https://doi.org/10.3354/ab00266

Sokolov, E. P., Markert, S., Hinzke, T., Hirschfeld, C., Becher, D., Ponsuksili, S., & Sokolova, I. M. (2019). Effects of hypoxia-reoxygenation stress on mitochondrial proteome and bioenergetics of the hypoxia-tolerant marine bivalve *Crassostrea gigas*. *Journal of Proteomics*, *194*, 99–111. https://doi.org/10.1016/j.jprot.2018.12.009

Somero, G. N. (2020). The cellular stress response and temperature: Function, regulation, and evolution. *Journal of Experimental Zoology. Part A, Ecological and Integrative Physiology*, *333*(6), 379–397. https://doi.org/10.1002/jez.2344

Somero, G. N., Lockwood, B. L., & Tomanek, L. (2017). *Biochemical adaptation: Response to environmental challenges, from life's origins to the Anthropocene*. Sinauer Associates, Inc. Publishers.

Squires, H. J. (1990). *Decapod crustacea of the Atlantic coast of Canada*. Dept. of Fisheries and Oceans.

Stillman, J. H., & Paganini, A. W. (2015). Biochemical adaptation to ocean acidification. *Journal of Experimental Biology*, *218*(12), 1946–1955. https://doi.org/10.1242/jeb.115584

Subramanian, I., Verma, S., Kumar, S., Jere, A., & Anamika, K. (2020). Multi-omics Data Integration, Interpretation, and Its Application. *Bioinformatics and Biology Insights*, *14*, 1177932219899051. https://doi.org/10.1177/1177932219899051

Sun, X.-J., Man, N., Tan, Y., Nimer, S. D., & Wang, L. (2015). The Role of Histone Acetyltransferases in Normal and Malignant Hematopoiesis. *Frontiers in Oncology*, *5*. https://doi.org/10.3389/fonc.2015.00108

Swiney, K. M., Long, W. C., & Foy, R. J. (2017). Decreased pH and increased temperatures affect young-of-the-year red king crab (*Paralithodes camtschaticus*). *ICES Journal of Marine Science*, *74*(4), 1191–1200. https://doi.org/10.1093/icesjms/fsw251

Syafaat, M. N., Azra, M. N., Mohamad, F., Che-Ismail, C. Z., Amin-Safwan, A., Asmat-Ullah, M., Syahnon, M., Ghazali, A., Abol-Munafi, A. B., Ma, H., & Ikhwanuddin, M. (2021). Thermal Tolerance and Physiological Changes in Mud Crab, *Scylla paramamosain* Crablet at Different Water Temperatures. *Animals*, *11*(4). https://doi.org/10.3390/ani11041146

Tanner, R., Gleason, L., & Dowd, W. (2022). Environment-Driven Shifts in Inter-Individual Variation and Phenotypic Integration within Subnetworks of the Mussel Transcriptome and Proteome. *Molecular Ecology*, *31*. https://doi.org/10.1111/mec.16452

Timmins-Schiffman, E., Coffey, W. D., Hua, W., Nunn, B. L., Dickinson, G. H., & Roberts, S. B. (2014). *Shotgun proteomics reveals physiological response to ocean acidification in Crassostrea gigas*. 18.

Titus, M. A. (2018). Myosin-Driven Intracellular Transport. *Cold Spring Harbor Perspectives in Biology*, *10*(3), a021972. https://doi.org/10.1101/cshperspect.a021972

Tjiputra, J., Olsen, A., Bopp, L., Lenton, A., Pfeil, B., Roy, T., Segschneider, J., Totterdell, I., & Heinze, C. (2014). Long-term surface pCO_2 trends from observations and models. *Tellus B*, *66*, 23083. https://doi.org/10.3402/tellusb.v66.23083

Tomanek, L. (2011). Environmental Proteomics: Changes in the Proteome of Marine Organisms in Response to Environmental Stress, Pollutants, Infection, Symbiosis, and Development. *Annual Review of Marine Science*, *3*(1), 373–399. https://doi.org/10.1146/annurev-marine-120709-142729

Tomanek, L., & Sanford, E. (2003). Heat-Shock Protein 70 (Hsp70) as a Biochemical Stress Indicator: An Experimental Field Test in Two Congeneric Intertidal Gastropods (Genus: *Tegula*). *The Biological Bulletin*, *205*(3), 276–284. https://doi.org/10.2307/1543291

Tomanek, L., Zuzow, M. J., Ivanina, A. V., Beniash, E., & Sokolova, I. M. (2011). Proteomic response to elevated pCO_2 level in eastern oysters, *Crassostrea virginica*: Evidence for oxidative stress. *Journal of Experimental Biology*, *214*(11), 1836–1844. https://doi.org/10.1242/jeb.055475

Ton, C., Stamatiou, D., & Liew, C.-C. (2003). Gene expression profile of zebrafish exposed to hypoxia during development. *Physiological Genomics*, *13*(2), 97–106. https://doi.org/10.1152/physiolgenomics.00128.2002

Turner, L. M., Ricevuto, E., Gallucci, A. M., Gambi, M.-C., & Calosi, P. (2015). Energy metabolism and cellular homeostasis trade-offs provide the basis for a new type of sensitivity to ocean acidification in a marine polychaete at a high CO_2 vent: Adenylate and phosphagen energy pools vs. carbonic anhydrase. *Journal of Experimental Biology*, jeb.117705. https://doi.org/10.1242/jeb.117705

Ugwu, S., & Apte, S. (2004). The effect of buffers on protein conformational stability. *Pharmaceutical Technology*, 86–108.

Vaquer-Sunyer, R., & Duarte, C. M. (2008). Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences*, *105*(40), 15452–15457. https://doi.org/10.1073/pnas.0803833105

Vehmaa, A., Hogfors, H., Gorokhova, E., Brutemark, A., Holmborn, T., & Engström-Öst, J. (2013). Projected marine climate change: Effects on copepod oxidative status and reproduction. *Ecology and Evolution*, *3*(13), 4548–4557. https://doi.org/10.1002/ece3.839

Vernberg, F. J., & Vernberg, W. B. (1969). Thermal Influence on Invertebrate Respiration. *Chesapeake Science*, *10*(3/4), 234. https://doi.org/10.2307/1350460

Vinagre, C., Madeira, D., Mendonça, V., Dias, M., Roma, J., & Diniz, M. S. (2014). Effect of temperature in multiple biomarkers of oxidative stress in coastal shrimp. *Journal of Thermal Biology*, *41*, 38–42. https://doi.org/10.1016/j.jtherbio.2014.02.005

Vogel, B. E., & Hedgecock, E. M. (2001). Hemicentin, a conserved extracellular member of the immunoglobulin superfamily, organizes epithelial and other cell attachments into oriented line-shaped junctions. *Development*, *128*(6), 883–894. https://doi.org/10.1242/dev.128.6.883

Vogel, B. E., Muriel, J. M., Dong, C., & Xu, X. (2006). Hemicentins: What have we learned from worms? *Cell Research*, *16*(11), Article 11. https://doi.org/10.1038/sj.cr.7310100

Wang, J., Russell, B. D., Ding, M.-W., & Dong, Y.-W. (2018). Ocean acidification increases the sensitivity of and variability in physiological responses of an intertidal limpet to thermal stress. *Biogeosciences*, *15*(9), 2803–2817. https://doi.org/10.5194/bg-15-2803-2018

Wang, W.-N., Zhou, J., Wang, P., Tian, T.-T., Zheng, Y., Liu, Y., Mai, W., & Wang, A.-L. (2009). Oxidative stress, DNA damage and antioxidant enzyme gene expression in the Pacific white shrimp, *Litopenaeus vannamei* when exposed to acute pH stress. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *150*(4), 428–435. https://doi.org/10.1016/j.cbpc.2009.06.010

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. *Comparative*

Biochemistry and Physiology Part D: Genomics and Proteomics, *13*, 16–23. https://doi.org/10.1016/j.cbd.2014.12.001

Welcker, D., Stein, C., Feitosa, N. M., Armistead, J., Zhang, J.-L., Lütke, S., Kleinridders, A., Brüning, J. C., Eming, S. A., Sengle, G., Niehoff, A., Bloch, W., & Hammerschmidt, M. (2021). Hemicentin-1 is an essential extracellular matrix component of the dermal–epidermal and myotendinous junctions. *Scientific Reports*, *11*(1), 17926. https://doi.org/10.1038/s41598-021-96824-4

Whiteley, N. M. (2011). Physiological and ecological responses of crustaceans to ocean acidification. *Marine Ecology Progress Series*, *430*, 257–271. https://doi.org/10.3354/meps09185

WHO, & IPCS. (1993). *Biomarkers and risk assessment: Concepts and principles / published under the joint sponsorship of the United Nations environment Programme, the International Labour Organisation, and the World Health Organization* (p. Summary and recommendations in French and Spanish). World Health Organization.

Wienberg, R. (1980). *On the food and feeding habits of Pandalus borealis KRØYER 1838*. *3*, 123–137.

Xu, C., Li, E., Liu, Y., Wang, X., Qin, J. G., & Chen, L. (2017). Comparative proteome analysis of the hepatopancreas from the Pacific white shrimp *Litopenaeus vannamei* under long-term low salinity stress. *Journal of Proteomics*, *162*, 1–10. https://doi.org/10.1016/j.jprot.2017.04.013

Xu, X., Xu, M., Zhou, X., Jones, O. B., Moharomd, E., Pan, Y., Yan, G., Anthony, D. D., & Isaacs, W. B. (2013). Specific structure and unique function define the hemicentin. *Cell & Bioscience*, *3*(1), 27. https://doi.org/10.1186/2045-3701-3-27

Zhang, T., Qu, Y., Zhang, Q., Tang, J., Cao, R., Dong, Z., Wang, Q., & Zhao, J. (2021). Risks to the stability of coral reefs in the South China Sea: An integrated biomarker approach to assess the physiological responses of *Trochus niloticus* to ocean acidification and warming. *Science of The Total Environment*, *782*, 146876. https://doi.org/10.1016/j.scitotenv.2021.146876

Zhang, X., Smits, A., van Tilburg, G., Ovaa, H., Huber, W., & Vermeulen, M. (2018). *Proteome-wide identification of ubiquitin interactions using UbIA-MS*. Nature Protocols. https://bioconductor.org/packages/DEP/

Zhang, X., Yuan, J., Zhang, X., Liu, C., Li, F., & Xiang, J. (2019a). Genome-Wide Identification and Expression Profiles of Myosin Genes in the Pacific White Shrimp, *Litopenaeus vannamei*. *Frontiers in Physiology*, *10*, 610. https://doi.org/10.3389/fphys.2019.00610

Zhang, X., Yuan, J., Zhang, X., Liu, C., Li, F., & Xiang, J. (2019b). Genome-Wide Identification and Expression Profiles of Myosin Genes in the Pacific White Shrimp,

Litopenaeus vannamei. *Frontiers in Physiology*, *10*, 610. https://doi.org/10.3389/fphys.2019.00610

Zhao, X., Han, Y., Chen, B., Xia, B., Qu, K., & Liu, G. (2020). CO_2-driven ocean acidification weakens mussel shell defense capacity and induces global molecular compensatory responses. *Chemosphere*, *243*, 125415. https://doi.org/10.1016/j.chemosphere.2019.125415

Zhou, J., Wang, L., Xin, Y., Wang, W.-N., He, W.-Y., Wang, A.-L., & Liu, Y. (2010). Effect of temperature on antioxidant enzyme gene expression and stress protein response in white shrimp, *Litopenaeus vannamei*. *Journal of Thermal Biology*, *35*(6), 284–289. https://doi.org/10.1016/j.jtherbio.2010.06.004

Zhu, G., Liu, Z., Yang, Y., Wang, Z., Yang, W., & Xu, L. (2019). Thermal and saline tolerance of Antarctic krill *Euphausia superba* under controlled in-situ aquarium conditions. *Journal of Oceanology and Limnology*, *37*(3), 1080–1089. https://doi.org/10.1007/s00343-019-8002-7