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Modulation of thermal stress response by prostaglandins in gills of the blue mussel *Mytilus edulis*

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Abstract :

Mytilus edulis has experienced massive mortality events associated with thermal stress that have been documented in wild as well as cultivated organisms, since this species is traditionally farmed in intertidal areas that are exposed to temperature variations. The stress responses linked to changes in temperature are modulated by prostaglandins (PG), which induce apoptosis, synthesis of heat shock proteins and antioxidant enzymes, among others. PG are mainly synthesized from arachidonic (ARA), eicosapentaenoic (EPA) and docosahexaenoic (DHA), but PG-like compounds can be produced through non-enzymatic oxidation of fatty acids in response to increased reactive oxygen species (ROS). In this study, we applied acute (a single temperature increase of 13 °C, from 12 °C to 25 °C) or chronic (cyclical temperature increases and decreases of 13 °C for eight days) thermal stress to mussels fed a microalga mix of Nannochloropsis oculata and Pavlova lutheri. The response was quantified by measuring PG produced enzymatically by cyclooxygenase (COX) from ARA, EPA, and DHA (PGE2, PGE3, 13-HDHA, respectively), non-enzymatic PG (13,14-dihydro-15-keto-PGE2 PGF3α and 8-isoprostane). PGE2, PGE3, and 13-HDHA levels were higher in the gills of mussels exposed to acute stress compared to chronic stress while 3,14-dihydro-15-keto-PGE2 showed no significant differences as a result of chronic or acute stress. PGF3α had lower levels in mussels exposed to chronic stress compared to those under acute stress. In contrast, 8-isoprostane was significantly higher in mussels exposed to chronic stress compared to acute stress. Mussels exposed to acute stress showed an increase in hemocyte infiltration in the gills, while those exposed to chronic stress showed a decrease in gill filament width compared to mussels submitted to acute stress; both effects are associated with PG modulation. These findings suggest that acute and chronic stressors have distinct effects on lipid composition, prostaglandin levels, and gill filament morphology, highlighting potential adaptations or responses to different stress conditions. Understanding the mechanisms by which PG and PG-like compounds modulate the thermal stress response in mussels will contribute to the understanding of climate change impacts on bivalves and the possible adaptation processes involved.

Highlights

► Mussels exposed to acute and chronic heat produce enzymatic and oxidation eicosanoids. ► Increased hemocyte infiltration in gills in response to acute stress was found. ► Eicosanoids from ARA decreased after acute stress. ► Eicosanoids from DHA increased in gills after both acute and chronic stress.

Keywords : mussel, thermal stress, acute stress, chronic stress, fatty acids.

Introduction

The mussel *Mytilus edulis* inhabits temperate subtidal and intertidal waters around the world (Koehn, 1991; Spencer, 1994), and it is one of the most commonly farmed mussel species—mainly in northern Europe and the Atlantic coast of Canada—with a world production around 200,000 tons per year (FAO, 2023). On the east coast of Canada, *M. edulis* dominates shellfish production, with 22,000 tons and a total value of 39 million Canadian dollars (28 million USD) in 2021 (Statistics Canada, 2022); however, massive mortalities associated with temperature increases and the presence of marine heat waves

(Dobricic et al., 2020; Filbee-Dexter et al., 2020) during the warm months have caused the complete loss of some production stocks over the years and significant economic losses (Guyondet et al., 2015; Filgueira et al., 2016; Lupo et al., 2020; Guillou et al., 2023; Talevi et al., 2023).

Mussels facing thermal stress, which compromises the integrity and functionality of macromolecules, synthesize heat shock proteins (HSP) as a cytoprotective mechanism to repair and preserve the structure of macromolecules, in particular proteins that are especially labile (Feidantsis et al., 2020). Elevated temperatures also increase the metabolic rate and the production of reactive oxygen species (ROS). To combat ROS accumulated during thermal stress and prevent subsequent cell damage, marine invertebrates produce antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (Whitely and Mackenzie, 2016).

The stress response is modulated by eicosanoids, and specifically by prostaglandins (PG) (Furuyashiki and Narumiya, 2011; Balogh et al., 2013). PG modulates the response to thermal stress by inducing apoptosis (Zhang et al., 2015; Oksala et al., 2003) and the synthesis of HSP (Santoro et al., 1989; Collier et al., 2008) and antioxidant enzymes (Büyükgüzel et al., 2010; Vu and Acosta, 2014). Moreover, changes in metabolism and cellular processes in response to elevated temperatures cause changes in bivalves tissues, including alterations in digestive tubule morphology (Lardies et al., 2017), impairment of reproductive functions (Nash et al., 2019), and alterations of the gill filaments (Jones et al., 2021) including reduced thickness, which can negatively impact the ability to efficiently extract oxygen from the water (Zimmer and Perry, 2022). Since gills are the primary site for gas exchange in mussels, this thinning hinders their capacity to obtain enough oxygen for proper functioning and survival (Ragg, 2023).

In addition to gas exchange, gills also have mucosal cells that produce mucus with a cytoprotective role that is modulated by PG (De Petrocellis and Di Marzo, 1994; Knoop and Newberry, 2018; Lauriano et al., 2021). PG modulates inflammation and the immune response, which determine the organism's physiological response and survival when exposed to prolonged stress. PG is synthesized from several polyunsaturated fatty acids. PGE_2 is one of the most pro-inflammatory PG and is synthesized via phospholipase A2 (PLA_2) and cyclooxygenase (COX) from ARA, which is mainly found in phosphatidylinositol (Xu et al., 2022). However, non-enzymatic oxidation can also promote the production of several PG-like compounds that affect the stress response, particularly when there is an increase in ROS (Muri et al., 2020). Organisms under stress may decrease PG synthesis by reducing enzyme activity or synthesis, but non-enzymatic synthesis is not as easily regulated and depends on various factors, including substrate availability (ARA in cellular membranes) and ROS.

We hypothesize that in addition to reducing the enzymatic synthesis of PG (and probably increasing non-enzymatic synthesis), the availability of ARA for PG synthesis can be regulated under some circumstances by transference from PL to NL, which reduces ARA availability to ROS oxidation and prevents inflammation. One of these circumstances is chronic stress, which the organism may eventually adapt to, in contrast to punctual acute stress. While PGE_2 production during acute stress can decrease infection and modulate the stress response, a longer chronic stress during which PG continues to be produced can potentially be fatal (Navarro et al., 2019; Hundal et al., 2021; Bao et al., 2023; Lei et al., 2023). Previous observations in oysters and shrimp subjected to stress have shown that ARA levels decrease in PL and increase in NL (Aguilar et al., 2012; Duran-Encinas et al., 2018; Navarro et al., 2019). The ARA

accumulated in lipid droplets in the cytoplasm is not available for enzymatic or non-enzymatic PG production (Bermúdez et al., 2021).

To test this hypothesis, we compared mussels exposed to an acute (single temperature increase from 12°C to 25°C) or chronic (cyclical temperature increases of 13°C for eight days) thermal stress. PG synthetized by enzymatic and non-enzymatic pathways were analyzed together with fatty acids in PL and NL, and we performed histology on the gills to evaluate macrostructure modifications in the thermal stress responses.

Materials and methods

Mussel collection and acclimatization.

Adult blue mussels *Mytilus edulis* were collected from a farm culture in Prince Edward Island, Canada (46°25.963 N; 62°39.914 W), and were transported on ice to the UQAR-ISMER aquaculture station in Pointe-au-Père, Quebec, Canada (48°27 N, 68°32 W). Mussels were acclimatized to laboratory conditions for eight weeks at 12ºC. The acclimatization temperature corresponds to the mean water temperature in Prince Edward Island mussel farms during the months of October and November, when experiments were carried out. Salinity was maintained at a constant 28 ± 1.

Acute heat stress experiment.

Eighteen mussels previously acclimatized to laboratory conditions (average shell length 54.35 ± 0.8 mm) were randomly distributed in six 75 L fiberglass tanks at a density of three mussels per tank. Mussels were placed on a raised perforated platform in the top quarter of each tank, far from the direct effect of bubbling. Mussels were fed daily with a suspension of mixed microalgae, *Pavlova lutheri* and *Nannochloropsis occulata* (1:1 dry weight [DW]) for seven days at a ratio of 6% of mussel biomass DW and

maintained at a constant temperature of 12°C. On the final day of the experiment (day 8), mussels were exposed to an acute heat stress condition by transferring them from 12°C to 25ºC for one hour. We chose 25°C because it was the maximum temperature reached in some Prince Edward Island mussel farming sites during summer 2021, the year the experiment was performed (Smith and Ramsay, 2022). Even though there are few (e.g., Guyondet et al., 2015) reports of mortality at this temperature in eastern Canada, mortality has been observed for this species at 25°C in other areas around the world (Lupo et al., 2020). After acute heat stress, mussels were sampled, and samples were kept at -80°C until analysis. Simultaneously, another group of mussels was handled (transferred to another aquarium and back) in the same way but maintained at 12°C as a control. Water temperature was recorded every minute by a HOBO temperature data logger (Onset products), and it was maintained during all experiments. All experiments were performed in triplicate (n = 3).

Chronic heat stress experiment

A second experiment was carried out with the same experimental design and feeding as above, but with a different thermal treatment. In this case, mussels were subjected to the same acute heat stress as above, by transferring them from 12°C to 80 L aquaria at 25ºC for one hour, but mussels were then returned to the 12°C aquaria and the same stress repeated the next day. There was no mortality during the experiment. For the control, the same handling and transfer treatment was done but without thermal stress. This thermal treatment was repeated daily throughout the eight days of the experiment.

Sampling

At the end of both experiments, mussels were sacrificed and dissected on a cool surface. Shells were discarded and gills were rapid-frozen on dry ice then stored at -80°C. Experiments were conducted in compliance with Canadian regulations on animal care (Health of Animals Regulations, CRC, c. 296). To avoid degradation of unstable metabolites derived from fatty acids, tissues were lyophilized for 24 h before analysis. For histological analysis, whole soft tissues were fixed in Davidson Solution for 48 h and stored in 70% ethanol until histological processing (Howard et al., 2004).

Fatty acid methyl ester analysis

The analysis of fatty acids was performed on the gills of mussels subjected to acute and chronic stress and also on the microalgae mixture that was used as feed in both experiments. Lipids were extracted using chloroform–methanol (2:1) according to Folch et al. (1957). The neutral lipid (NL) fraction was eluted in 10 mL of chloroform–methanol (98:2) and 15 mL of methanol. Fatty acids were derivatized using a boron trifluoride–methanol solution (BF_3-methanol, 14% in methanol; Sigma-Aldrich; CAS:373-57-9) as described in Palacios et al. (2001). Samples were injected into a gas chromatography system coupled with a flame ionization detector (GC-FID, Agilent Technologies 6890N) equipped with a DB-23 capillary column (50% cyanopropyl)-methylpolysiloxane (Agilent) using helium as the carrier gas and a temperature ramp from 110 to 220°C at a rate of 3.5ºC/min. A splitless injector was set at 260ºC and the flame ionization detector (FID) was set at 280ºC. Fatty acids were identified by comparing retention times with those of commercial standards (Component FAME Mix 47885-U, Supelco; tricosanoic acid T6543, 99% purity, Sigma, St. Louis, MO, USA). Quantification was performed based on the area under the curve compared to the

internal standard (23:0). Data were analyzed using GC ChemStation Rev. A.10.02 (1757, Agilent Technologies, 2003) (Palacios et al., 2005).

Analysis of PG by LC-MS/MS

For the analysis of PG by liquid chromatography with tandem mass spectrometry (LC-MS/MS), a 10 mg DW sample of each mussel's gill was placed in 2 mL homogenization mix tubes (VWR soft tissue homogenizing mix). Tissue was homogenized at 5800 rpm for three cycles of 20 seconds with a Precellys®24 homogenizer (Bertin Technologies, France) and a Cryolys cooling unit. We added 200 µL of 2,2,2 trifluoroethanol/extraction buffer (1/1, extraction buffer 50 mM ammonium formate at pH 3 with formic acid) to each sample. Samples were vortexed for 10 s, then 200 µL of acetonitrile/methanol (1/1) solution was added to each sample for protein crash. Samples were vortexed for 10 s then centrifuged for 5 min at 15000 RCF at 4°C, after which 250 µL of the supernatant was transferred to a 2 mL HPLC vial. We added 1 mL of extraction buffer (50 mM ammonium formate at pH3 with formic acid) containing 10 ng.mL^{-1} of internal standard (PGE_2-d_9) to the 2 mL HPLC vial for each sample. Samples (1 mL) were injected into an HPLC 1260 Infinity II (Agilent Technologies, USA) equipped with a mass spectrometer (65460B QTOF, Agilent Technologies, USA), and online-SPE was performed with a Security Guard phenyl-hexyl 4 × 2.0 mm cartridge coupled with an InfinityLab Poroshell HPH C-18, 2.1 × 100 mm 1.9 µm (Agilent Technologies, USA) column run at 40ºC. Data were processed with MassHunter Quantitative QTOF (Quant-my-Way) software from Agilent Technologies, USA (Le Faouder et al., 2013; Pisani et al., 2014).

8-isoprostane analysis

Pre-assay sample preparation was performed as described in Navarro et al. (2019). We placed 12 mg DW of each mussel's gills in a 1.5 mL tube (Eppendorf Tubes®) for the analysis of 8-isoprostane. Samples were homogenized on ice after adding 300 µL of homogenization buffer (0.1 M phosphate buffer, pH 7.4, containing 1 mL EDTA and 0.005% BHT) using a glass Potter-Elvehjem (VWR®) equipped with a glass pestle. Total 8-isoprostane (free + esterified) was measured by hydrolysis using 300 µL of 15% KOH (w/v). To precipitate proteins, 4 mL of ethanol was added to each sample. An additional purification step was performed to eliminate contaminants that could interfere with SPE cartridges (C-18, 6 mL, Cayman Chemical, USA, item no. 400020). Assay development was carried out as described in the 8-isoprostane ELISA Kit manual (Cayman Chemical, USA, item no. 516351) (Mabourt et al., 1987; Pradelles et al., 1990).

Histology

Samples were dehydrated in ethanol series of 70%, 80%, 90%, and 100% and xylene 100%, embedded in paraffin (Paraplast X-TRA, Medline Industries Inc., Tolleson, AZ), transversally cut into section of 4 µm, and then stained with hematoxylin-eosin (Howard and Smith, 1983). Tissue samples were examined using an Olympus BX41 microscope, and images were digitized using a Nikon Digital Sight DS-Ri1® camera connected to a computer. Gill photomicrographs were obtained using a 20X objective, and filament widths were analyzed with Image-Pro Premier software v9.0 (Media Cybernetics, Inc., Rockville, MD).

Statistical analysis

Assumptions of data normality and homoscedasticity were verified by visual histograms and by the Shapiro-Wilk and Levene tests, respectively, and a comparison of mean values from each treatment with its control was done by *t*-test. We used STATISTICA V6 software (StatSoft®, Inc., 1994–2004) for all tests, considering significance at $P \leq 0.05$ (Martín-Álvarez, 2014). PG data were standardized with their respective control values to compare concentrations resulting from acute stress vs. chronic stress, thus eliminating the handling effect. The comparison between groups (acute vs. chronic stress) was carried out using unpaired *t*-tests standardized with the appropriate control values.

Results

Gill fatty acids

The proportion of ARA in gill PL of mussels exposed to acute heat stress was significantly higher than that of mussels exposed to chronic stress (*P≤0.05*). There were no significant differences in NL ARA in mussels exposed to chronic or acute stress (Fig. 1). The proportion of EPA in PL was significantly higher in mussels exposed to acute stress compared to chronic stress (*P≤0.05*). No significant difference was found for NL EPA (Fig. 2). The proportion of DHA in PL was not significantly different in mussels exposed to acute or chronic stress, but it was significantly higher in NL of mussels exposed to chronic stress (Fig. 3).

PG by LC-MS/MS

PGE_2 produced from ARA through the action of COX was higher in mussels exposed to acute stress compared to those exposed to chronic stress (*P≤0.05*) (Fig. 4A). PGE_3 produced via COX from EPA was significantly higher in mussels exposed to acute

stress compared to those exposed to chronic stress (Fig. 4B). Gill concentrations of 13-HDHA produced from DHA by COX were lower in mussels subjected to chronic stress compared to mussels subjected to acute stress (Fig. 4C).

The metabolized product of PGE_2 13,14-dihydro-15-keto-PGE_2 was not significantly different in mussels exposed to chronic or acute stress (Fig. 5A). $PGF_{3\alpha}$ produced from EPA by ROS was higher in mussels exposed to acute compared to chronic stress (Fig. 5B).

Gill 8-isoprostane

A significantly higher concentration of 8-isoprostane was found in the gills of mussels exposed to chronic stress compared to mussels exposed to acute stress (Fig 5C).

Gill histology

After the acute heat stress treatment, we observed that mussel gills filaments were slightly swollen and had hemocyte infiltration (Fig. 6B) compared to non-stressed mussels (Fig. 6A); however, this difference was not significant ($P \leq 0.05$). There was a significant decrease in the width of gill filaments in mussels exposed to chronic stress (Fig. 7) compared to acute stress ($P \leq 0.05$).

Discussion

Gills are one of the most delicate tissues in aquatic animals. Gill epithelium is thin and highly vascularized to facilitate gas exchange during respiration, making this organ highly susceptible to ambient stressors (Foyle et al., 2020). Depending on the stressor, gill branchial epithelium thickness can increase or decrease (Chen et al., 2019; Islam et al., 2022; Rogers et al., 2023; Wang et al., 2023). The gills are in direct contact with the

aquatic environment, making them more sensitive to heat stress than are other organs (Chen et al., 2021). Because gills are exposed to ambient conditions, allowing pathogens to infiltrate the organism, they have a highly responsive cellular defense. Hemocyte infiltration among the gill's filaments, in the intestinal epithelia, and gonad of mussels has been observed in response to environmental disturbances, to the presence of pathogenic bacteria (Matozzo et al., 2018) and microplastics (Lu et al., 2018; Vasanthi et al., 2021; Iheanacho et al., 2023), and to the combined effects of thermal stress and hypoxic conditions (Bosch-Belmar et al., 2022). We observed hemocytes infiltrating the gill tissue of mussels submitted to acute stress (Fig. 6B), indicating inflammation (Monteiro et al., 2008; Trancart et al., 2020). We did not quantify mucus, but it has also been reported to increase in fish in response to chronic exposure to copper and other metal contamination (Monteiro et al., 2008; Vosloo et al., 2012; Carvalho et al., 2020). Fish can also remodel gill morphology in response to unfavourable conditions like high temperatures (Wu et al., 2017; Chen et al., 2019). Chen et al. (2021) found that the gills increased in size as a result of inflammation of the epithelial cells of *Sander lucioperca* in response to elevated temperatures (48 h). Inflammation of the gills serves as protection by preventing the entry of pathogens into deeper tissues, but increased inflammation has the disadvantage of reducing the gas exchange capacity (Chen et al., 2021), probably affecting osmoregulation (Graves and Dietz, 1982; Uchida and Kaneko, 1996) and particle uptake in filter-feeding bivalves (Beninger et al., 1993).

In our experiment, mussels exposed to chronic heat stress showed significant thinning of gill filaments compared to acute stress (Fig. 7). Gill filament thinning in mussels exposed to prolonged heat stress has been reported before (Yang et al., 2021). Gill filaments are essential structures in bivalves, responsible for vital functions such as

respiration, feeding, and waste removal (Muznebin et al., 2023). As temperatures rise, oxygen levels decrease; this, in addition to thinner gills, might produce respiratory distress in mussels that could be struggling to meet their oxygen demands. This could be one physiological reason for summer mortality in mollusks.

It is well known that PGE_2 increases inflammation and triggers a general immune response, while PGE_3 down-regulates inflammation (Whitehouse et al., 1997; Ricciotti and FitzGerald, 2011). Different PG are also secreted in response to stressors other than pathogens, such as reproduction, increased temperature, and contamination (Cubero-Leon et al., 2010; El Mohajer et al., 2022; Shen et al., 2023). However, organisms can survive and adapt if the stress is not too strong or too long. Some might even learn to anticipate stress if it is periodical, as has been observed in oysters submitted to tidal rhythms (Tran et al. 2020), and repetitive stressors are even needed in some species of oysters to stimulate maturation and induce spawning (Hurtado et al., 2009). Here, we observed an increase of PGE_2, PGE_3, and 13-HDHA (synthesized enzymatically from ARA, EPA, and DHA, respectively) in response to acute thermal stress while there was significant reduction in all three during chronic stress, supporting our hypothesis that PG enzymatic production decreases as an acclimation process of mussels to repeated stress (Fig. 4). The PG produced from DHA are resolvins, such as 13-HDHA (Itoh et al., 2016; Perry et al., 2022), which have been described as down-regulators of an even greater inflammation response after PGE_3 (Marchand et al., 2023). These molecules could have a more important effect on modulating inflammation in mussels, which have a higher proportion of DHA in gills (around 25%) than EPA (15%) or ARA (7%). In contrast, in *Crassostrea gigas,* which has more ARA and EPA and much less DHA (Seguineau et al., 2005; Hurtado et al., 2009), PG derived from ARA and EPA might have a stronger modulating effect in response to stress (Duran-Encinas et al., 2018).

These PG are produced enzymatically by the same enzyme, COX-2 (among others), during stress and thus compete. However, several PG-like compounds also affect the stress response and are produced by non-enzymatic oxidation in response to an increase in ROS (Trostchansky et al., 2019). Here, the results were not so clear-cut for non-enzymatically produced PG: we noted no significant differences for 13,14-dihydro-15-keto-PGE_2 (from ARA), but we did observe a significant decrease in gill $PGF_{3\alpha}$ (from EPA) and a significant increase in 8-isoprastane in mussels that were subjected to chronic stress. While an organism subjected to stress can decrease PG production by reducing COX activation or synthesis, production via ROS is more chaotic since it depends on substrate availability, i.e., the concentration of ARA and other polyunsaturated fatty acids in cellular membranes that are exposed to ROS, and the quantity of ROS that has not been scavenged by antioxidants, among other things (Araújo et al., 2021; Bao et al., 2023). Uncontrolled production of PG-like compounds can lead to negative physiological effects in the organism and, ultimately, death. Continuous stress can increase the production of PG-like compounds that can decrease survival in shrimp (Navarro et al., 2019). A metabolomic analysis of PG could shed light on the mechanisms involved in the response of acclimation to repetitive stress in mussels.

Non-enzymatic PG production could decrease if ARA and other polyunsaturated fatty acids are incorporated from the phospholipid cell membrane into lipid droplets in the cytoplasm. We did find that the proportion of ARA decreased in the PL of chronically stressed mussels (Fig. 1A); however, no significant differences were found for ARA in the NL fraction, contrary to what we expected. Modulation of the fatty acid membrane can occur relatively quickly—within a matter of hours to days—since the lipid composition of the cell membrane adjusts in response to temperature fluctuations (Ibarz

et al., 2005). However, more long-term adaptations can also take place over weeks to months while the organism experiences prolonged exposure to different temperatures (Pernet et al., 2008; Portilla, 2016; Fernandes et al., 2021) due to readjustments of the physical state of the membrane required for maintaining membrane activities (Farkas et al., 2001). Such adjustments likely vary among organisms: species, stage of culture, diet, and temperature are some factors that can modify these changes (Bendiksen et al., 2002). The transference of ARA from PL to NL involves the action of some enzymes like phospholipases, which release ARA from membrane phospholipids. Free ARA can then be re-esterified into NL by a process facilitated by acyltransferases (Tallima, 2021). The transference of ARA to different lipid pools could be crucial for the dynamic regulation of cellular signaling pathways linked to the ARA-derived lipid mediators that play pivotal roles in inflammation, immune responses, and vascular function (Guijas et al., 2019). ARA availability can influence the production of these lipid mediators. LN can act as a reservoir, ensuring a steady supply of ARA for various physiological processes (Missaglia et al., 2019). Despite the potential importance of this mechanism for lipid signaling, we did not observe the transference of ARA from PL to NL after thermal stress. The total (NL + PL) loss of ARA in the gills of mussels exposed to chronic temperature stress might be due to a selective decreased incorporation of ARA from microalgae. This could be analyzed using isotopes in future studies.

Conclusions

This study provides insight into the modulation of PG in response to thermal stress in *M. edulis*. While there was a strong increase in the production of the pro-inflammatory prostaglandin PGE_2 during acute thermal stress that was linked to the activation of cytoprotective mechanisms, such as inflammation and immune response, the levels of

these decreased during chronic stress. PGE_3 and 13-HDHA levels also increased under acute stress and decreased under chronic stress, but the increase was at least 10-fold smaller and the decrease during chronic stress was less marked than for PGE_2. These compounds are involved in the regulation of inflammation, suggesting a potential shift towards down-regulating inflammation to avoid excessive stress-induced tissue damage. ARA decreased during chronic stress in PL, but—in contrast to what we expected—it also decreased in NL. The presence of hemocytes in mussel gills in response to acute stress and the alteration of gill filament thickness due to chronic thermal stress are both significant responses that provide insight into the adaptive mechanisms of aquatic organisms facing environmental thermic challenges. While rapid activation of the immune system through increased hemocyte presence demonstrates the immediate nature of the response as an essential first-line defense against acute stressors, changes in gill filament thickness occur under chronic thermal stress exposure, revealing the organism's ability to adapt to persistent stressors. These adaptations may involve restructuring gill tissues to optimize respiratory function, maintain electrolyte balance, or minimize energy expenditure. Understanding the response of PG and other lipid compounds to biological effects in mussels, particularly when comparing acute and chronic thermal stress, can contribute to our understanding of mollusk acclimation and adaption to their environment. Mussel loss during culture could be reduced by adapting the diet in anticipation of short stressors such as handling or temperature changes, or to long-term climate changes.

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Conflicts of interest

The authors declare no conflicting interest.

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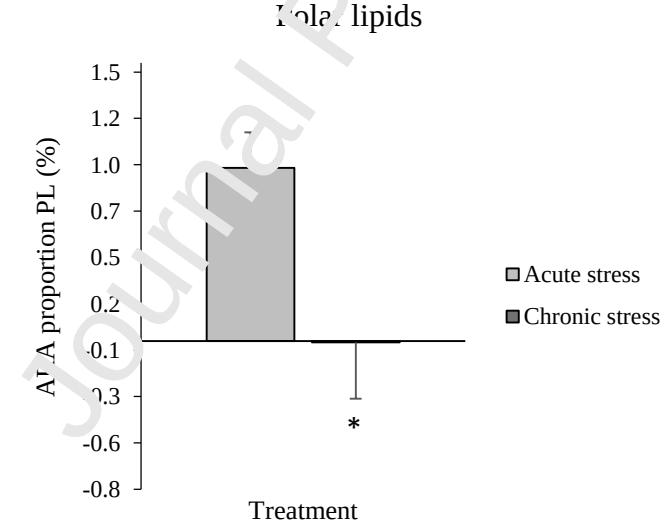

B

Neutral lipids

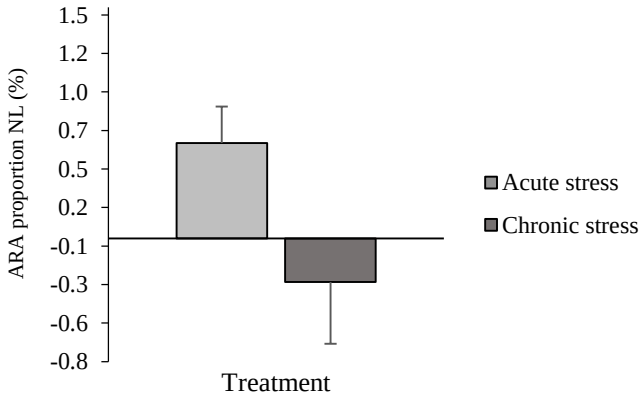

Fig. 1. Normalized proportions of ARA (% of fatty acids) in the gills of *M. edulis* mussels exposed to acute or chronic heat stress quantified by GC-FID (mean ± standard error) in A) polar lipids and B) neutral lipids. The asterisk indicates a significant difference ($P \leq 0.05$) as determined by *t*-tests between acute or chronic stress and their corresponding controls.

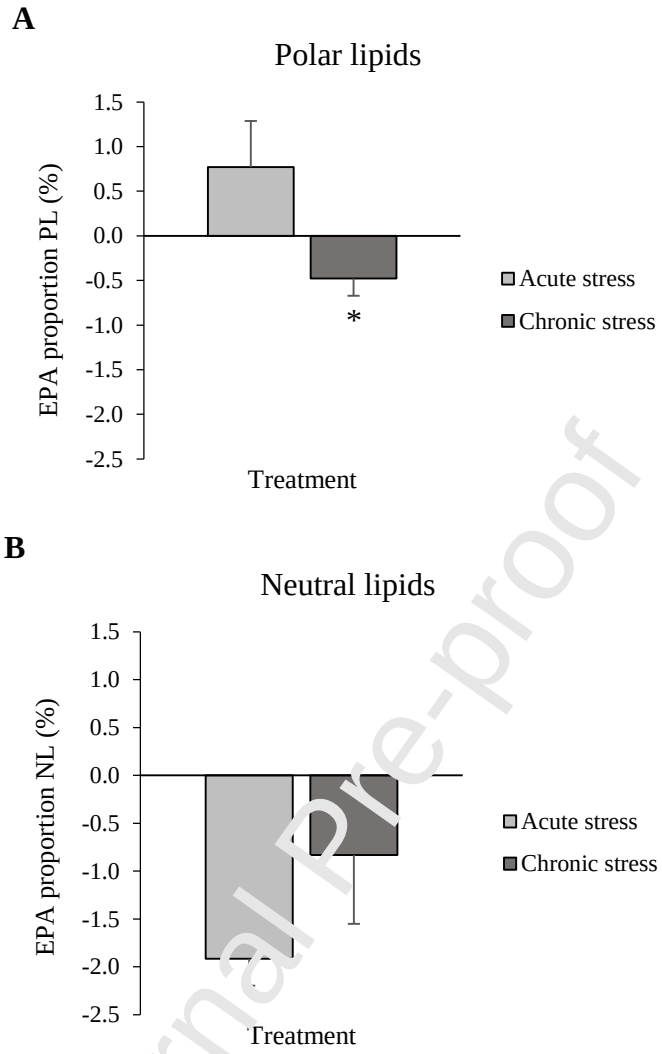

Fig. 2. Normalized proportions of EPA (% of fatty acids) in the gills of *M. edulis* mussels exposed to acute or chronic heat stress quantified by GC-FID (mean ± standard error) in A) polar lipids and B) neutral lipids. The asterisk indicates a significant difference ($P \leq 0.05$) as determined by *t*-tests between acute or chronic stress and their corresponding controls.

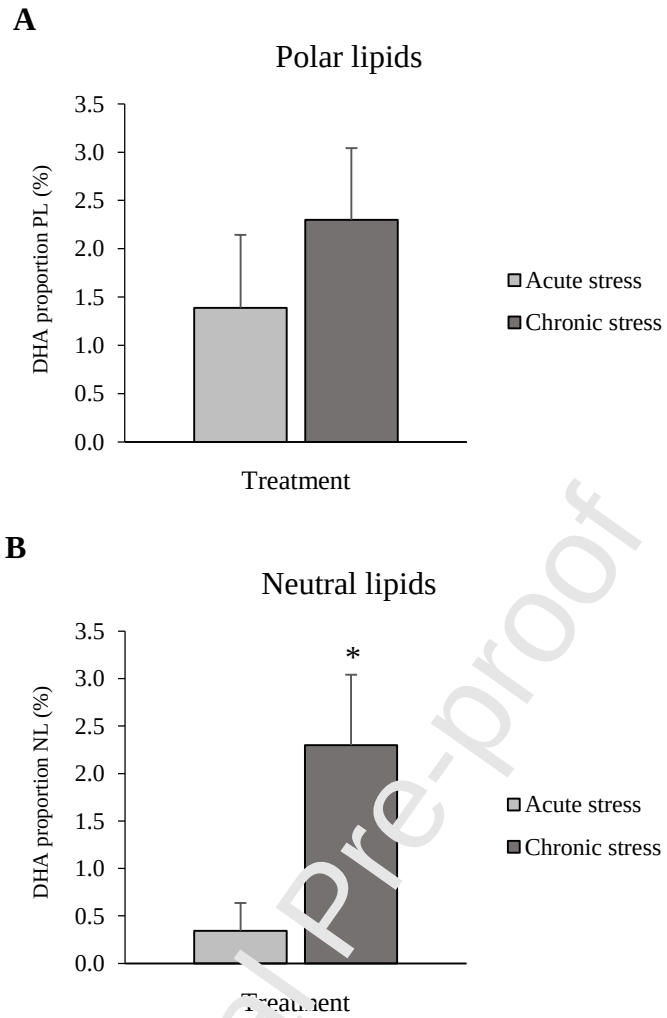

Fig. 3. Normalized proportions of DHA (% of fatty acids) in the gills of *M. edulis* mussels exposed to acute or chronic heat stress quantified by GC-FID (mean ± standard error) in A) polar lipids and B) neutral lipids. The asterisk indicates a significant difference ($P \leq 0.05$) as determined by *t*-tests between acute or chronic stress and their corresponding controls.

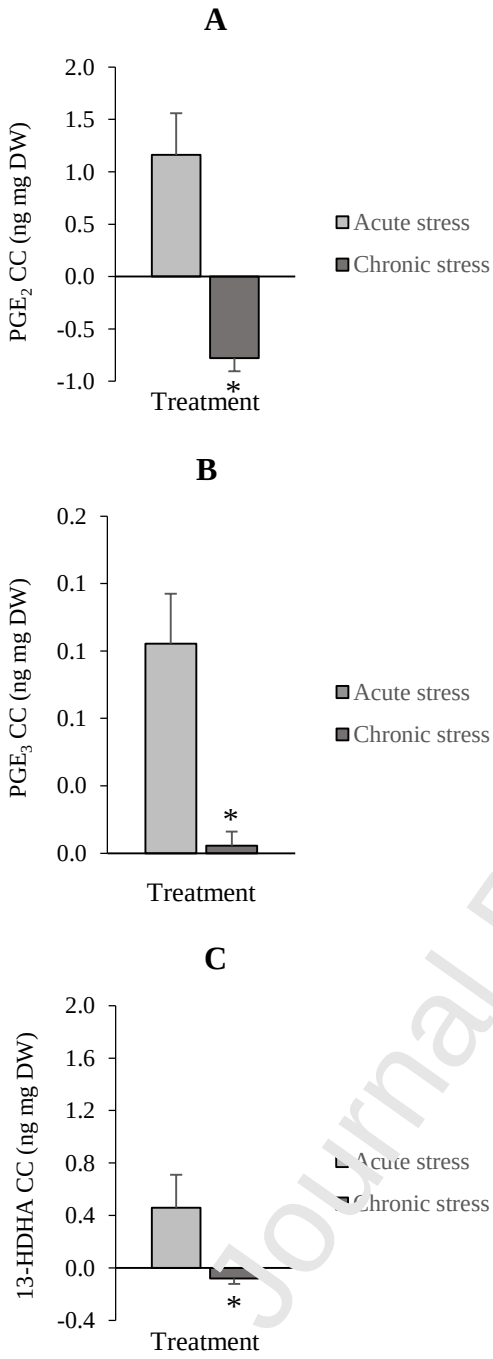

Fig. 4. Normalized concentrations of ARA-derived PGE_2 (A), EPA-derived PGE_3 (B), and DHA-derived 13-HDHA metabolites by the action of COX in the gills of *M. edulis* mussels exposed to acute or chronic heat stress measured by LC-MS/MS (mean ± standard error). The asterisks indicate significant differences ($P \leq 0.05$) as determined by *t*-tests between acute or chronic stress and their corresponding controls.

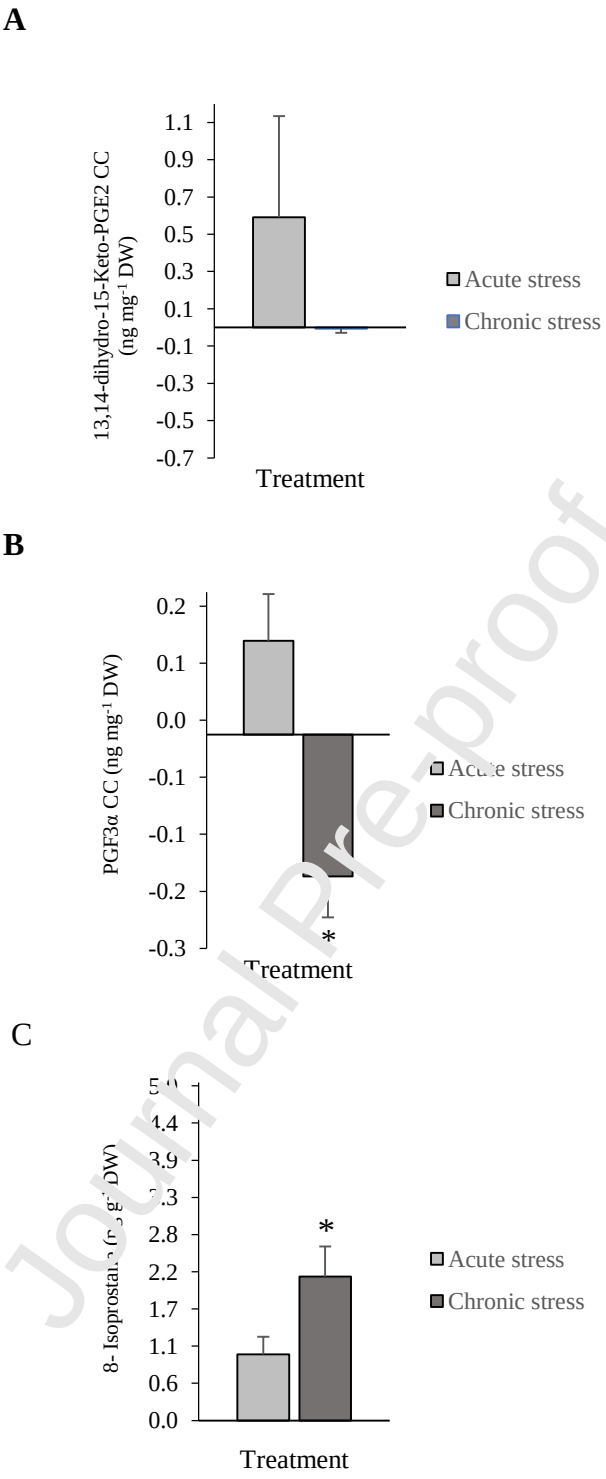

Fig. 5. Normalized concentrations of ARA-derived 13,14-dihydro-15-keto-PGE_2 (A) and EPA-derived $PGF_{3\alpha}$ (B), by the action of ROS measured by LC-MS/MS and 8-isoprostane (C) measured using an enzymatic kit in gills of *M. edulis* mussels exposed to acute or chronic heat stress (mean ± standard error). The asterisks indicate significant

differences ($P \leq 0.05$) as determined by *t*-tests between acute or chronic stress and their corresponding controls.

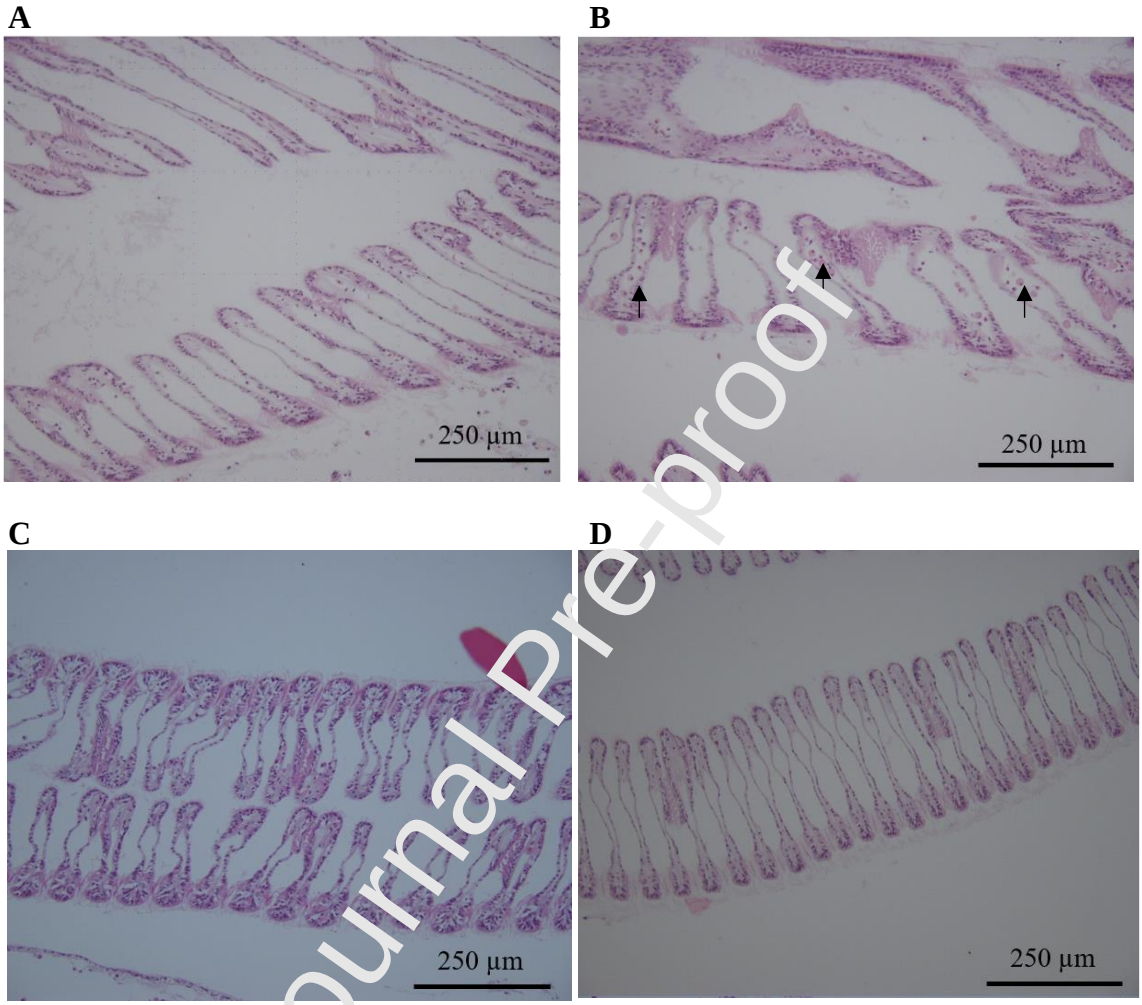

Fig. 6. Gill filaments of *M. edulis* mussels exposed to acute B) or chronic D) heat stress and their respective controls (A and C, respectively). Arrows indicate the presence of hemocytes. Scale bar is 250 µm.

A

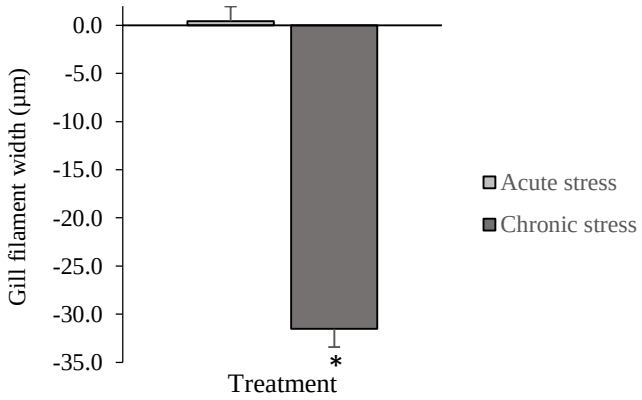

Fig. 7. Normalized mean width of gill filaments (µm) of *M. edulis* mussels exposed to acute and chronic heat stress (mean ± standard error). The asterisk indicates a significant differences ($P \leq 0.05$) as determined by *t*-test between acute or chronic stress subtracting their corresponding controls.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Declaration of interests

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

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Highlights

1. Mussels exposed to acute and chronic heat produce enzymatic and oxidation eicosanoids.
2. Increased hemocyte infiltration in gills in response to acute stress was found.
3. Eicosanoids from ARA decreased after acute stress.
4. Eicosanoids from DHA increased in gills after both acute and chronic stress.