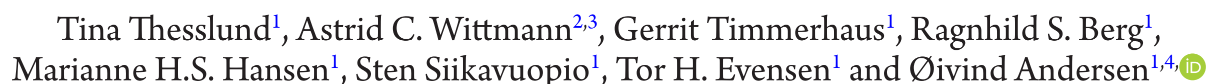
Journal of Crustacean Biology, 2024, **44**, ruae040
https://doi.org/10.1093/jcbiol/ruae040
Advance access publication 10 July 2024
Research Article

OXFORD

Temperature acclimation and response to acute thermal stress in the adults of the snow crab *Chionoecetes opilio* Fabricius, 1788 (Decapoda: Brachyura: Oregoniidae)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ABSTRACT

Ectothermic marine animals vary widely in their tolerance to temperature changes, and polar stenothermal species seem to have poor ability to compensate for a rise in water temperature. The sub-Arctic snow crab (*Chionoecetes opilio* Fabricius, 1788) lives in the northwestern Atlantic Ocean and northern Pacific Ocean at temperatures ranging from about −1.5 °C to 4 °C. Since the metabolic costs overtake caloric intake above 7 °C, the snow crab appears to be energetically restricted to cold water. We investigated thermal stress responses in adult male crabs exposed to a sudden temperature increase to 9.5 °C for 24 hr after four weeks of acclimation at 2.5 °C or 5.5 °C. Heart-rate loggers implanted in a limited number of crabs showed 60% increase in cardiac activity during the thermal stress. Surplus oxygen supply in all crabs was inferred by the low hemolymph lactate and unchanged glucose levels, but only the crabs acclimated at 5.5 °C were still active at the elevated temperature. Low heat shock and oxidative stress responses were suggested by the missing upregulation of the genes encoding four heat shock proteins (Hsp70a, Hsc71, Hsp90a2, Hsp60) and the antioxidative enzymes superoxide dismutase and catalase. The trend towards inverse temperature-dependent on the expression of the *hsp* genes may be related to increased protein damage at low temperatures, or possible trade-offs between costs and benefits of producing heat shock proteins at elevated temperature. Although adult snow crabs seem to be able to cope with short-term heat stress, the tolerance to chronic elevated temperatures should be further examined using a larger number of individuals.

KEY WORDS: climate change, Crustacea, heart rate, heat-shock proteins, oxidative stress, polar crustaceans, stenotherms

INTRODUCTION

Temperature has a strong impact on the biology of ectothermic animals by modifying their physiology, behaviour, and life history (Hofmann & Todgham, 2010; Hoffmann & Sgrò, 2011; Sunday *et al.*, 2014). There is increasing concern about the ability of ectothermic aquatic species to cope with climate change and how their geographic distribution and abundance will be affected (Perry *et al.*, 2005; Sunday *et al.*, 2012; Pinsky *et al.*, 2019; Jørgensen *et al.*, 2022). The annual temperatures in the Arctic Ocean range maximally between −1.86 °C and about 8 °C (Steele *et al.*, 2008), but this region is showing the most rapid change in temperature globally (Rantanen *et al.*, 2022). The chance of surviving global warming is largely dependent on the tolerance to short-term temperature changes and the ability to acclimate to warming environments (Somero, 2010). Increased temperature tolerance through acclimatization differs strongly among ectotherms and may largely govern the different thermal responses of eurytherms and stenotherms (Logan & Buckley, 2015). Studying thermal dependence of cardiac function in three closely related species of sculpin fishes, Franklin *et al.* (2013) found that the eurythermal shorthorn sculpin (*Myoxocephalus scorpius* Linnaeus, 1758) maximized cardiorespiratory performance over a wider temperature range (1–10 °C) than the narrow range (1-4 °C) in the endemic stenothermal Arctic sculpin (*M. scorpioides* Fabricius, 1780) and Arctic staghorn sculpin (*Gymnocanthus tricuspis* Reinhardt, 1830). Further, the eurythermal crab *Carcinus maenas* (Linnaeus, 1758) exhibited significantly higher critical thermal maximum than the

Received 5 March 2024; Accepted 18 June 2024
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stenothermal *Cancer pagurus* (Linnaeus, 1758) at acclimation temperatures of 8 °C and 22 °C (Cuculescu *et al.*, 1998).

The heat shock response and the production of heat shock proteins (HSP) are classical cellular responses to various stressful conditions, particularly thermal stress (Parsell & Lindquist, 1993; Feder & Hofmann, 1999). These molecular chaperones play a crucial role during stress by binding to and interacting with misfolded proteins (Frydman, 2001; Hartl & Hayer-Hartl, 2002). Despite the universality of the heat shock response, species inhabiting different thermal environments exhibit distinct responses to changing temperatures (Tomanek, 2010; Clark *et al.*, 2008). Different thermal responses in congeneric species were shown by the Arctic copepod *Calanus glacialis* (Jaschnov, 1955) exhibiting no upregulated *hsp* expression under thermal stress, in sharp contrast to the response in the temperate *C. finmarchicus* (Gunnerus, 1770) (Smolina *et al.*, 2015). The Antarctic krill *Euphausia superba* (Dana, 1850) and *E. crystallorophias* (Holt & Tattersall, 1906) differed slightly in thermotolerance and showed notable differences in *hsp70* expression at increased temperature (Cascella *et al.*, 2015). The heat shock response is lacking in most Antarctic fishes, and external stresses do not induce the production of Hsp70 due to a mutation in the gene promoter (Buckley *et al.*, 2004; Clark *et al.*, 2008).

Thermal stress also increases the production of cellular reactive-oxygen species (ROS) and causes oxidative stress if not properly removed by enzymatic and non-enzymatic antioxidants (Lesser, 2006; Freire *et al.*, 2011; Tomanek, 2015). Acute treatments of animals adapted to stable environments generally produce significant oxidative stress responses, whilst animals adapted to variable conditions exhibit capacity to cope with temperature changes and mitigate oxidative stress (Ritchie & Friesen, 2022). Superoxide dismutase (SOD) and catalase (CAT) provide the first line of defence against oxidative damage and play key roles in oxidative stress also in crustaceans (Halliwell & Gutteridge, 2007; Walters *et al.*, 2016). Antarctic notothenioid fishes seem to have lost the ability to elevate enzymatic antioxidants in response to heat stress, and the increased oxidative damage in the heart ventricle of white-blooded icefishes may contribute to the reduced thermal tolerance (Mueller *et al.*, 2012; Enzor & Place, 2014).

The snow crab (*Chionoecetes opilio* Fabricius, 1788) is distributed in the northwestern Atlantic Ocean and northern Pacific Ocean and has rapidly expanded in both distribution and abundance in the Barents Sea since the first findings in 1996 (Alvsvåg *et al.*, 2009; Pavlov & Sundet, 2011; Sundet & Bakanev, 2014). This sub-Arctic crab supports one of the commercially most important global crab fisheries, but the Canadian catches have shown dramatic variations during the last decades (DFO, 2023), and the collapse in the eastern Bering Sea population to historical lows in 2021 were linked to a recent marine heatwave (Szuwalski *et al.*, 2023). The snow crab inhabits depths down to 400 m at temperatures ranging from about −1.5 °C to 4 °C (Dawe & Colbourne, 2002). Adult males consistently displayed behavioural thermoregulation in an experimental gradient from 1.0 °C to 5.5 °C with a thermal preference within 1.0–1.6 °C after 6 hr (Siikavuopio *et al.*, 2019). This stenothermal crab species seems to be physiologically constrained to cold water, as its energy budget becomes negative above 7 °C due to reduced caloric intake and rising metabolic costs (Foyle *et al.*, 1989). Hence, the snow crab could be particularly vulnerable to continued warming in the polar regions without the capacity for acclimation (Fedewa *et al.*, 2020). Although sudden heat shock may not occur under natural conditions, this experimental treatment has been widely used to investigate the effects of thermal stress on aquatic organisms to test their acclimation capacity (Qari, 2014; Lagerspetz & Vainio 2014; Tepolt & Somero, 2014). Here, we examined molecular and physiological responses in adult snow crab males to acute temperature increase to 9.5 °C after acclimation at 2.5 °C or 5.5 °C. Cardiac activity were monitored by implantable heart rate loggers, and thermal stress responses were examined by quantifying the gene expression of heat shock proteins and antioxidants. We targeted the heart and the hepatopancreas, which is a multifunctional organ that is known to respond to environmental stress also in snow crab (Power *et al.*, 2023).

MATERIALS AND METHODS

Animals and experimental setup

Adult male snow crabs were caught by commercial fishing vessel in Barents Sea at temperatures of 1.5–3 °C and transported to the Aquaculture Research Station in Tromsø, Norway (latitude ~70°N) 10 weeks before the trial. The crabs were held in tanks with shaded natural light, water temperature within 3.2–4.2 °C and fed in excess with herring, shrimp, squid, and blue mussel three times weekly. In total, 29 crabs with carapace width (CW) of 10.05 ± 1.33 (SD) cm were used in the experiments. All crabs had hard shells and were considered as terminal-=moulted adults, although the maturity stage was not morphometrically determined (Conan & Comeau, 1986).

All crabs were of good vitality according to the scoring system described (James *et al.*, 2019). Before the start of acclimation, five crabs were sacrificed for blood and tissue sampling. For the temperature experiment, 18 crabs were randomly divided into two tanks. In addition, three crabs were placed in each tank for later mounting of heart rate loggers. The tanks had a bottom area of 4 m^2 and 80 cm water depth and the crabs were acclimated to 2.5 °C or 5.5 °C for 4 weeks (Fig. 1). The water inlet in both tanks was then switched to heated 10 °C sea water without disturbing the animals, and the temperature stabilized at 9.5 °C (9.6 °C and 9.4 °C in the initially 2.5 °C and 5.5 °C tanks, respectively) after 2.5 hr. The crabs were exposed to the high temperature for 24 hr followed by a recovery period of 48 hr at 2.5 or 5.5 °C.

The trial was undertaken under natural light conditions and the crabs were fed as described above. Water temperature was logged two weeks before the start of acclimation throughout the whole experiment with temperature loggers (HOBO TidbiT v2 - UTBI-001, 120 Onset®; HOBO, Bourne, MA, USA). Water flow was set at ~15 1 min^{-1}, which kept the oxygen level above 90% saturation during the acclimation period and above 85% saturation during the temperature increase. Oxygen was measured using an optical dissolved Oxygen meter (YSI ProSolo Professional Series; YSI, Yellow Springs. OH, USA). The behaviour of the crabs was followed by frequent daily observations. The experiment was performed following the Norwegian legislation for usage of animals in research under the permission FOTS ID: 21/87946.

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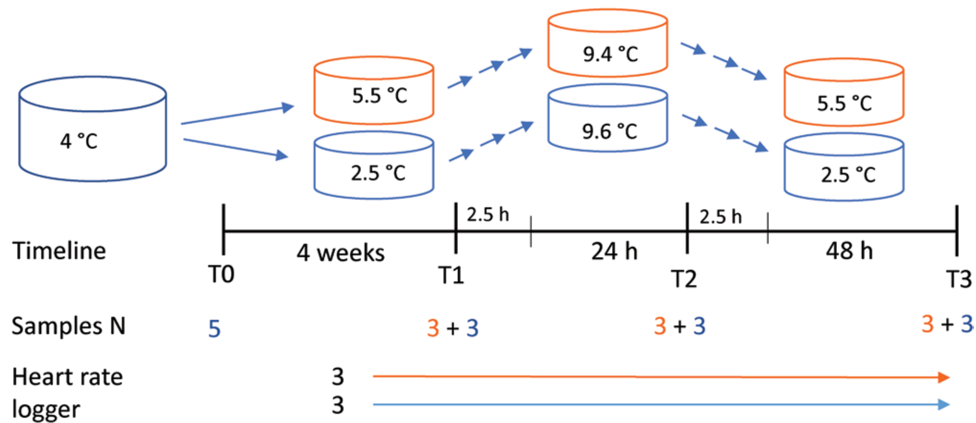

Figure 1. Experimental setup for studying responses in adult snow crab males to acute temperature increase after four weeks acclimation at either 2.5 °C or 5.5 °C. Sampling points T0-T3 are indicated together with the period of heart rate recordings.

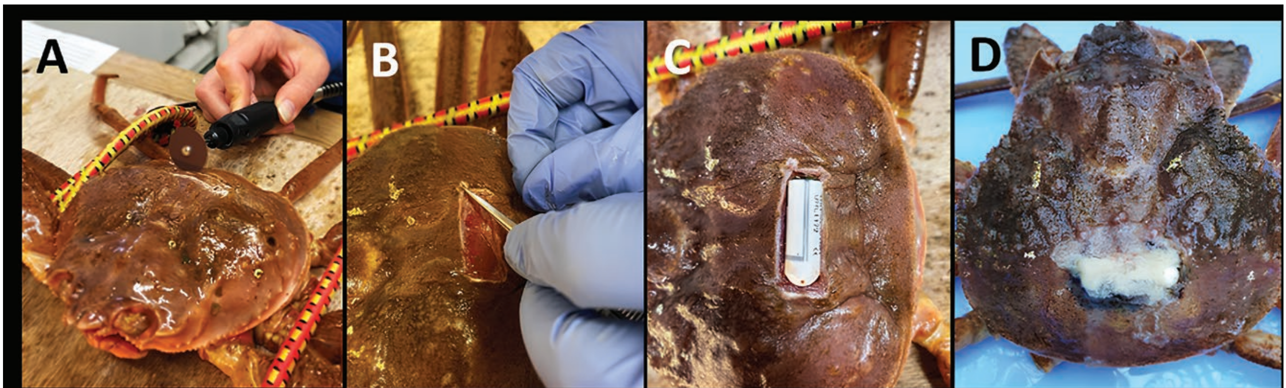

Figure 2. Mounting heart rate logger on snow crab. Carapace abraded above the heart of sedated crab (**A**), carapace removed from the underlying dermis (**B**), logger placed in the abraded area of the carapace with electrodes in contact with the tissue (**C**), logger secured with wax and sealed with quick glue (**D**).

Heart-rate recordings

Heart-rate loggers (DST micro-HRT; Star-Oddi, Garðabær, Iceland) were mounted on three crabs from each tank 17 d prior to the temperature stress as described by McGaw *et al.* (2018). Briefly, the crabs were exposed to clove oil for 10 min for sedation before immobilization on a table with rubber strips over legs on both sides (Fig. 2). The loggers were installed directly above the heart, which was easily identified in posterior central part of the carapace. A rectangular slot of the size of the logger was cut into the carapace using a Dremel® rotary tool (Dremel, Racine, WI, USA). The carapace piece was gently removed from the underlying tissue and dermis carefully loosened around the edges of the opening creating a hammock-like bed where the logger was placed. The logger was stabilized with surgical wax and sealed with cyanoacrylate quick glue.

Tissue samplings

The hepatopancreas and heart were sampled before (T0) and after (T1) four weeks acclimation at either 2.5 °C or 5.5 °C, after 24 hr exposure at high temperature (T2), and finally after recovery for 48 hr (T3). Three crabs were taken from each group at each timepoint, except at T0 where in total five crabs were sampled (Fig. 1). The crabs were sacrificed by an incision of the thoracic ganglion, and tissues from hepatopancreas and heart were secured in RNAlater and stored at –20 °C for qPCR analyses. Hemolymph was drawn with syringe and needle from the first joint from coxa at the fourth walking leg at sampling points T1–T3 and stored at –20 °C. The hemolymph samples were centrifugated to remove cells, and the supernatant was analysed for glucose and lactate concentrations in a Horiba ABX Pentra C400 clinical chemistry analyser (Horiba, Kyoto, Japan).

Gene search, mRNA extraction, cDNA synthesis, and qPCR.

We identified snow crab *hsp* genes by screening genomic sequences at NCBI and transcriptomic sequences in Andersen *et al.* (2022); *hsp90a1* (KAG0729994.1), *hsp90a2* (KAG0729995.1), *hsp70a* (not annotated in JACEEZ010011952), *hsc71* (KAG0710796), *hsp70c4* (KAG0728470.1), *hsp71a* (KAG0727865.1), *hsp71b* (KAG0727866.1), *hsp60* (KAG0711365.1), *cat* (KAG0722355.1), lactate dehydrogenase (*ldh*; JACEEZ010010775.1), and hemocyanin C-subunit (*hcyC*; KAG0724994.1) were identified at NCBI, while *sod* and elongation factor 1α (*ef1α*) were described by Hall *et al.* (2020) (Supplementary material Table S1).

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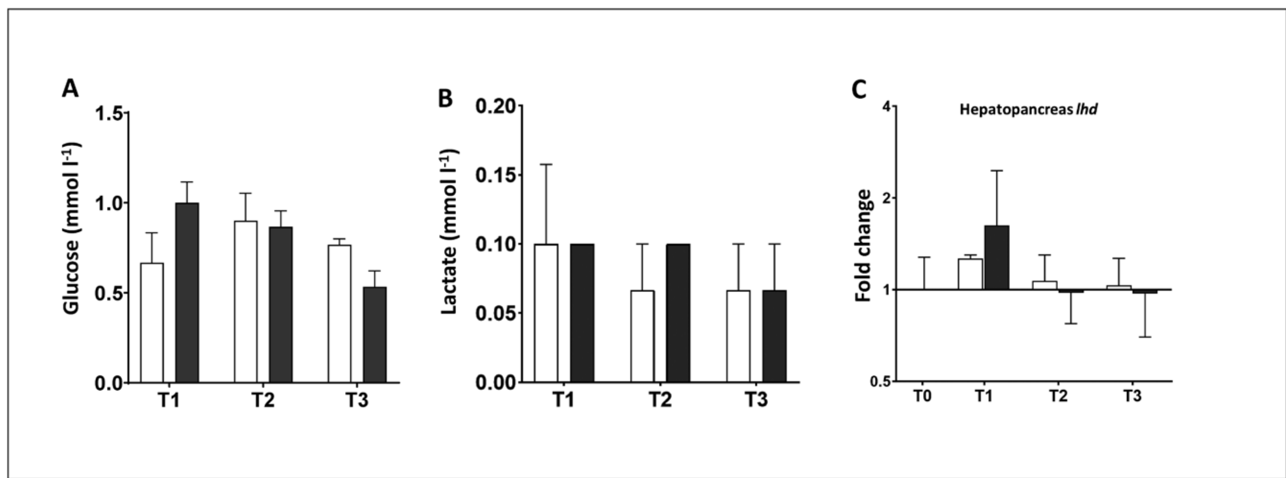

Figure 3. Hemolymph glucose (**A**) and lactate concentrations (**B**) and hepatopancreatic lactate dehydrogenase (*ldh*) expression in snow crab acclimated to 2.5 °C (white bars) or 5.5 °C (black bars) for four weeks (T1) before exposure to 9.5 °C for 24 hr (T2) followed by recovery for 48 hr (T3) (**C**). *Ldh* expression levels are presented as fold change relative to the initial expression levels at 4 °C (T0) set to 1. Y-axis is transformed to log2 scale.

Total RNA was isolated from hepatopancreas and heart using AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, Hilden, Germany). DNase treatment was performed using the TURBO DNAfree kit (Ambion, Austin, TX, USA) according to manufacturer's protocol. RNAs were quantified by NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the quality was further assessed using Agilent® 2100 Bioanalyzer™ RNA 6000 Nano kit (Agilent Technology, Santa Clara, CA, USA). Samples had RIN values above 8.0. cDNA synthesis was performed from 200 ng RNA input using the High-Capacity RNA-to-cDNA kit (Thermo Fisher Scientific) according to a synthesis protocol of 25 °C for 10 min, followed by 37 °C for 60 min, and then 5 min at 85 °C. Specific primers were designed using Primer Express 3 software (Life Technologies, Carlsbad, CA, USA) for amplification of eight target genes and the reference gene (Supplementary material Table S1) with sequences available from snow crab transcriptomes (Hall *et al.*, 2020; Andersen *et al.*, 2022). The amplification efficiency of each primer pair was calculated using eight two-fold serial dilutions of a cDNA mix from heart and hepatopancreas organs according to the equation: $E = 10^{-1}$/slope (Pfaffl, 2001). All primer pairs gave single distinctive melting peaks demonstrating that no primer dimers and unspecific amplification products were present. The qPCR products were cleaned and verified by sequencing. Absence of genomic DNA was verified by conducted qPCR in the absence of reverse transcriptase on three randomly selected RNA samples, and positive controls contained a cDNA mix from the two tissues.

The qPCR was run in duplicates in 384 well plates using QuantStudio 5 (Thermo Fisher Scientific) using the following recommended parameters: standard-run mode with 40 cycles at 50 °C for 2 min, 95 °C for 10 min and 60 °C for 1 min. Following by the melt curve stage at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Each well contained Power SYBR Green PCR Master Mix (Thermo Fisher Scientific), 300 nM final concentration of each primer, 7 µl diluted cDNA (1:40) and nuclease free water (Ambion) to a final reaction volume of 20 µl. All data were collected by the Quant Studio Design & Analysis Software (Thermo Fisher Scientific) and exported to Microsoft Excel for further analyses. Relative expression levels of the genes examined was calculated using the method of Pfaffl (2001) and normalized against the reference gene.

Statistical analyses

Glucose, lactate, and qPCR results were analysed by two-way ANOVAs and pairwise t-tests to test for groupwise differences. For these calculations, ΔΔCT values qPCR were used. Tests were calculated in R (version 4.2.2, https://cran.r-project.org) and with the functions aov() and TukeyHSD(). Results of these tests were marked in the figures by lower case letters. Groups which did not share a letter were significantly different ($P < 0.05$). All data are shown as average and SD in the figures.

RESULTS AND DISCUSSION

Haemolymph glucose levels and hemocyanin expression

Hyperglycaemia by mobilization of the hepatopancreatic glycogen stores is a typical response of crustaceans to physical and chemical environmental changes, including thermal stress (Lorenzon, 2005; Lu *et al.*, 2016; Vasudevan & Rajendran, 2021). The fed snow crabs, however, showed no significant changes in haemolymph glucose concentrations during the experiment (Fig. 3A). Surplus energy stores and aerobic metabolism were suggested by the low haemolymph lactate concentrations (Fig. 3B) and the low expression of the key glycolytic enzyme Ldh in both temperature groups (Fig. 3C). The large individual variation in *ldh* expression at 5.5 °C (T1) is due by the high levels measured in a likely adolescent crab (CW9.1 cm), whereas a smaller crab (CW 8.5 cm) did not differ from the other 2.5 °C-acclimated crabs during heat stress (T2).

The snow crab hemocyanin comprises hexameric and dodecameric forms of various subunits similar to other crab species, such as red king crab (*Paralithodes camtschaticus,* Tilesius, 1815), blue crab (*Callinectes sapidus* Rathbun, 1896) and European green crab (*Carcinus aestuarii* Nardo, 1847)

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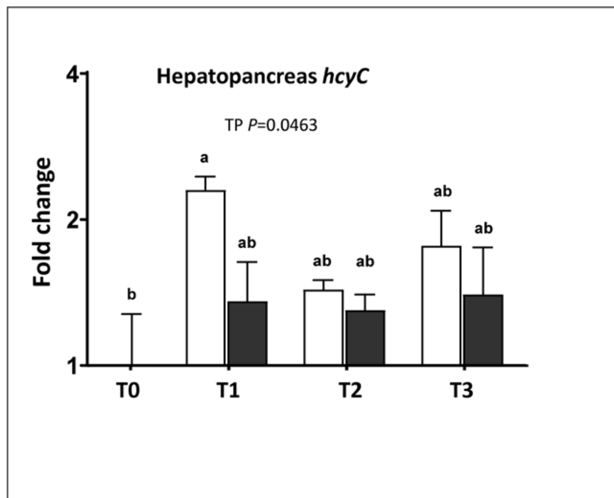

Figure 4. Relative expression of hemocyanin C (*hcyC*) in hepatopancreas of snow crab acclimated to 2.5 °C (white bars) or 5.5 °C (black bars) for four weeks (T1) before exposure to 9.5 °C for 24 hr (T2) followed by recovery for 48 hr (T3). Values are presented as fold change relative to the initial expression levels at 4 °C (T0) set to 1. Y-axis is transformed to log2 scale. Two-way ANOVA *P*-value is indicated on top of the figure for the two factors time point (TP). Different letters indicate statistically significant differences according to pairwise t-tests ($P < 0.05$).

(Mangum *et al.*, 1991; Dainese *et al.*, 1998; Molon *et al.*, 2000; Bouazza, 2009). We quantified the hepatopancreatic expression levels of the hemocyanin *hcyC* subunit, which showed no significant changes, except for the upregulated expression in crabs after four weeks at 2.5 °C (Fig. 4). High hemocyanin expression and haemolymph concentrations in the Antarctic amphipod *Eusirus cf. giganteus* (Andres, Lörz & Brandt, 2002) and the Antarctic octopus *Pareledone charcoti* (Joubin, 1905) were suggested as a key strategy for cold adaptation to compensate for the increased hemocyanin binding affinity and reduced oxygen unloading at lower temperatures (Giomi & Pörtner, 2013; Oellermann *et al.*, 2015; Greco *et al.*, 2021). Whereas *P. charcoti* and the green crab (*Carcinus maenas*) benefits from a thermally sensitive hemocyanin that extends oxygen supply at warmer temperatures (Giomi & Pörtner, 2013; Oellermann *et al.*, 2015), the hemocyanin in the stenothermal Antarctic octopus *Megaleledone setebos* (Robson, 1932) showed high oxygen affinity irresponsive to temperature, implying poor oxygen unloading and limited temperature tolerance (Zielinski *et al.*, 2001). Similarly, the respiratory system of the snow crab appears to be designed to meet the low metabolic needs at 0–3 °C and is probably incapable of supplying sufficient oxygen in adult crabs at higher temperatures (Foyle *et al.*, 1989; Siikavuopio *et al.*, 2017). The temperature tolerance may be higher in juvenile snow crabs, and higher growth indices were reported in crabs reared at 5 °C then at 1 °C and 3 °C, but also at 8 °C (Yamamoto *et al.*, 2015).

Cardiac and locomotor activities

While cardiac output is influenced by changes in both heart rate and stroke volume, heart rate measurements alone provide important information on metabolism and responses to environmental fluctuations, such as temperature changes (Stillman, 2004; McLean & Todgham, 2015; McGaw *et al.*, 2018). Six snow crabs were mounted with heart-rate loggers, but good quality recordings were unfortunately obtained from only two 5.5 °C-acclimated crabs and a single 2.5 °C-acclimated crab. The three crabs showed similar heart rates prior to the acute temperature increase (mean 27.5 beats per minute, bpm) and during recovery (mean 27.9 bpm) (Fig. 5). The heart rate increased about 60% (mean 43.8 bpm) during the heat exposure in all three crabs, but the influence of temperature acclimation should be examined in a larger number of animals. The cardiac function in American lobsters (*Homarus americanus* H. Milne Edwards, 1837) was improved at the warm extreme of the temperature range with significantly lower heart rates after acclimation to 20 °C compared to 4 °C (Camacho *et al.*, 2006). Acclimation also affects the behavioural activity in crustaceans (Lagerspetz & Vainio, 2006), such as blue swimmer crab (*Portunus pelagicus* Linnaeus, 1758) wherein instar displayed increased locomotor activity and thermal tolerance when acclimated at higher temperature (Azra *et al.*, 2018). We observed that the 5.5 °C-acclimated crabs were still moving, whereas all the cold-water crabs became inactive when exposed to elevated temperature, but had resumed activity after 24-hr heat stress prior to recovery at 2.5 °C.

Gene expression of heat shock proteins and antioxidants

We identified five *hsp70* genes in the snow-crab genome, and the deduced heat shock proteins possessed conserved Hsp70 signatures (Karlin & Brocchieri, 1998; Rensing & Maier, 1994) (Supplementary material Fig. S2). Although functional differences between the Hsp70 members have been predicted in many taxa, including crustaceans, the inducible and cognate Hsp70 types may not be recognized only by differences in sequence motifs, number of introns and presence of heat shock elements in the promoter region (Drozdova *et al.*, 2019). We herein designed specific qPCR primers against *hsp70a* and *hsc71* in addition to *hsp90a2* and *hsp60*.

Acute exposure of adult snow crabs to 9.5 °C did not invoke any up-regulated expression of the four *hsp* genes in the hepatopancreas and heart (Fig. 6). In fact, the *hsp* genes tended to be downregulated at elevated temperatures, but only *hsp60* showed significant changes. Similarly, adult red king crab had lower expression of four *hsp* genes examined in the hepatopancreas at 10 ºC than at 4 ºC (Andersen *et al.*, 2022). Our results contrast with the strong heat shock response induced by acute thermal stress reported in various crustaceans (Li, 2017), such as the deep-sea crab *Chaceon affinis* (A. Milne-Edwards & Bouvier, 1894) displaying stronger temperature-induced upregulation of *hsp70* expression at 10 MPa than at 0.1 MPa (Mestre *et al.*, 2015). The constitutive or upregulated *hsp* expression in snow and red king crabs at low temperatures resembles the permanent constitutive expression patterns in Antarctic notothenioid fishes and may be a compensatory mechanism for coping with elevated protein damage at low temperatures (Privalov 1990; Tsai *et al.*, 2002; Place *et al.*, 2004; Clark & Peck, 2009). On the other hand, the energy budget in the snow crab becomes negative above 7 °C (Foyle *et al.*, 1989), and trade-offs between costs and benefits of producing heat shock proteins may occur at elevated temperatures (Sokolova *et al.*, 2012; Logan & Buckley, 2015).

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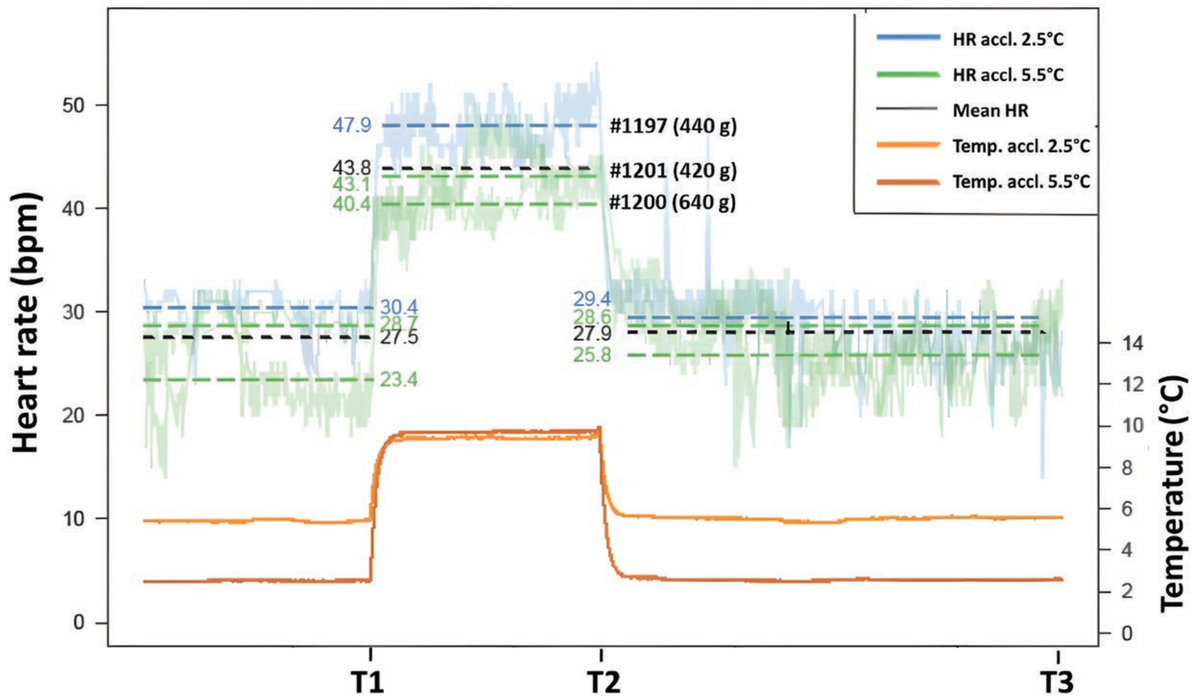

Figure 5. Heart rates recorded in three snow crabs acclimated to 2.5 °C or 5.5 °C before 24-hr exposure to 9.5 °C followed by recovery for 48 hr. Dashed blue and green lines show the mean heart rate for each of the three individuals with ID number and initial body weight indicated, and the black line gives the overall mean heart rate. The mean values were separated for the three phases of the experiment. The orange lines indicate the measured temperature by the installed loggers.

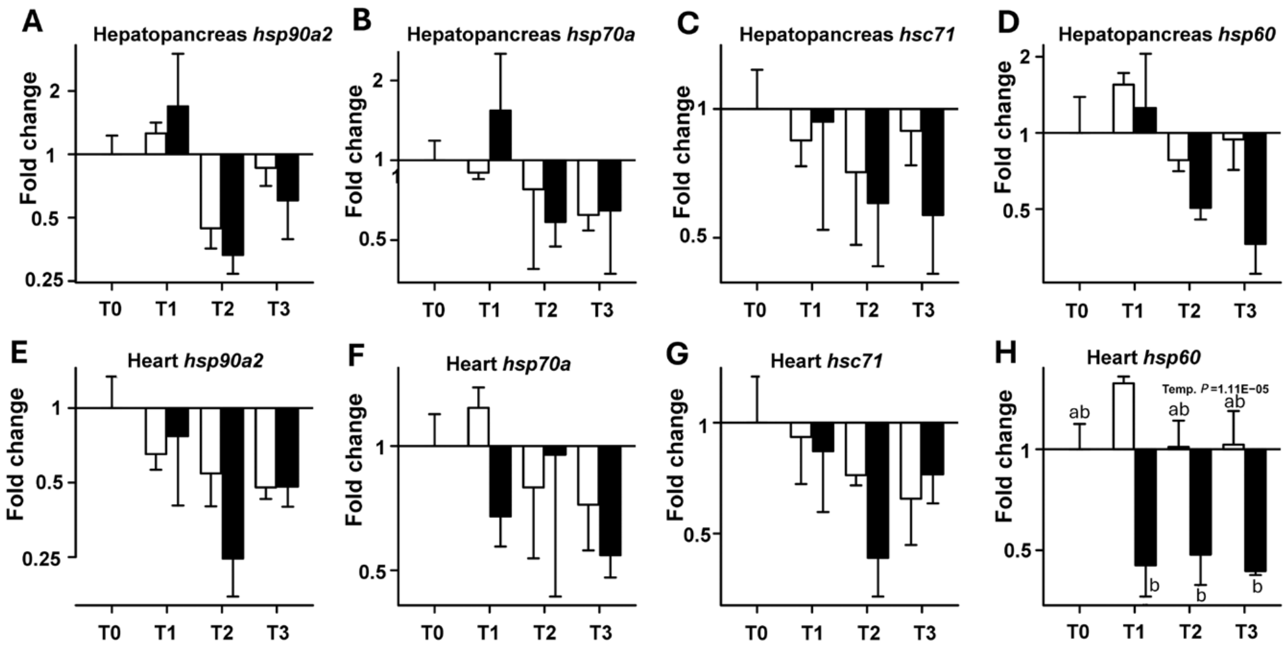

Figure 6. Relative expression levels of heat shock proteins *hsp90a2* (**A**, **E**), *hsp70a* (**B**, **F**), *hsc71* (**C**, **G**) and *hsp60* (**D**, **H**) in hepatopancreas (**A**–**D**) and heart (**E**–**H**) of snow crab. The crabs were acclimated to 2.5 °C (white bars) or 5.5 °C (black bars) for four weeks (T1) before exposure to 9.5 °C for 24 hr (T2) followed by recovery for 48 hr (T3). Expression levels are presented as fold change relative to the initial expression levels at 4 °C (T0) set to 1. Y-axis is transformed to log2 scale. Two-way ANOVA *P*-values are indicated on top of the figures for the two factors time point (TP) and temperature treatment (Temp). Different letters indicate statistically significant differences according to pairwise t-tests ($P < 0.05$).

We examined the oxidative stress response by quantifying the gene expression levels of the antioxidants SOD and CAT, which were shown to reflect the corresponding enzyme activities in Antarctic fishes (Mueller *et al.*, 2012). The acute temperature increase did not elicit any significant changes in *sod* and *cat* mRNA levels but tended to be upregulated in the hepatopancreas

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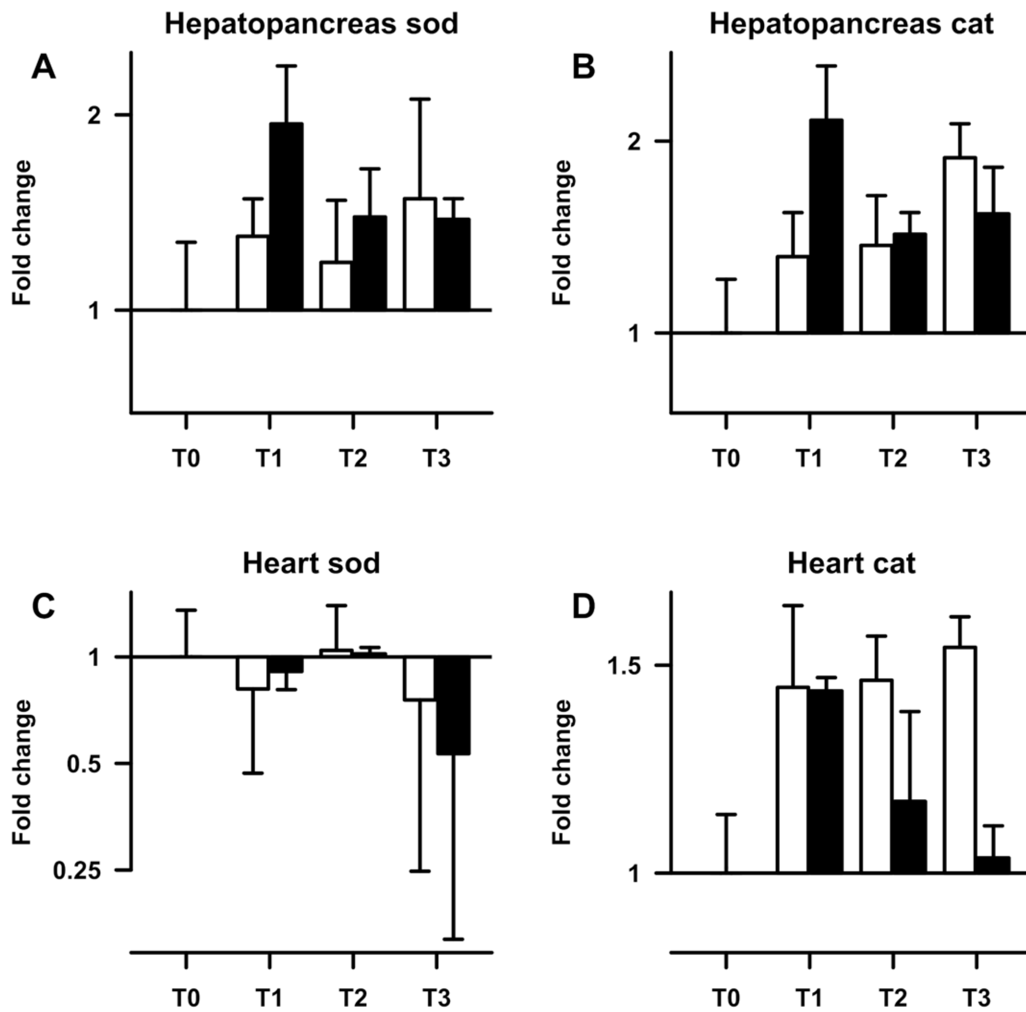

Figure 7. Relative expression levels of superoxidase dismutase (*sod*; **A**, **C**) and catalase (*cat*; **B**, **D**) in hepatopancreas (**A**, **B**) and heart (**C**, **D**) of snow crabs acclimated to 2.5 °C (white bars) or 5.5 °C (black bars) for four weeks (T1) before exposure to 9.5 °C for 24 hr (T2) followed by recovery for 48 hr (T3). Expression levels are presented as fold change relative to the initial expression levels at 4 °C (T0) set to 1. Y-axis is transformed to log2 scale.

after four weeks at 5.5 °C (Fig. 7). In comparison, SOD and CAT activities were strongly induced by temperature stress in the hepatopancreas and gills of Cape River (*Potamonautes perlatus* H. Milne Edwards, 1837) and mud (*Scylla paramamosain* Estampador, 1950) crabs (Walters *et al.*, 2016; Liu *et al.*, 2018). In contrast, Antarctic notothenioid fishes showed no changes in SOD and CAT activities exposed to elevated temperatures (Mueller *et al.*, 2012; Enzor & Place, 2014; Machado *et al.*, 2014). Surprisingly, the cellular oxidative damage decreased in the emerald rockcod (*Trematomus bernacchii* Boulenger, 1902) after long-term acclimation suggesting adequate basal antioxidants levels to compensate for the induced cellular damage (Enzor & Place, 2014). We propose that the reservoir of the antioxidants, mainly carotenoids and vitamin E, in the snow-crab shell (Lage-Yusty *et al.*, 2011) could be mobilized during oxidative stress to reduce cellular damage.

According to related studies of the heat response in polar animals, we decided to examine the responses in the snow crabs after 24 hr heat exposure. Acute heat-stressed (0 – > 6 °C) Antarctic icefish showed significantly upregulated liver transcripts only after 12 hr with maximal response after 24 hr, and downregulated genes were measured only after 48 hr (Thorne *et al.*, 2010). In the copepod *Calanus finmarchicus*, the 11 genes quantified using qPCR were all significantly upregulated after six days at elevated temperatures, while none were differentially expressed after 4 hr (Smolina *et al.*, 2015). Moreover, the comparison between the short-term and long-term heat exposure revealed 212 differently expressed transcript in the copepod. We therefore did not expect any immediate changes in gene expression in the heat-exposed snow crabs, while the thermal response to chronic temperature stress should be further examined in a larger number of animals.

CONCLUSIONS

Despite large individual variations in the small experimental groups, this study suggests that the heat shock and oxidative stress responses are low, or are lacking, in the cold-stenothermal snow crab. Any temperature-induced expression of the additional *hsp70* genes in the snow-crab genome should, however, not be excluded. Although the cardiac activity was strongly increased at elevated temperature, the resulting higher metabolic costs may

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not be met by the extra oxygen supply and food intake needed for surviving longer periods of time than experienced in this study.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. qPCR analysis of the snow-crab genes listed together with gene ID, primer sequences, amplification efficiency, and correlation coefficient

S2 Figure. Sequence alignment of the five Hsp70 family members identified in the snow crab genome. Conserved Hsp70 signatures and the C-terminal motif E-E-V-D typical for the cytosolic Hsp70s are boxed.

ACKNOWLEDGEMENTS

The authors greatly recognize the excellent care of the snow crabs by Hugo Tøllefsen at Tromsø Aquaculture Research Station. We thank the anonymous reviewers for their careful reading and insightful comments and suggestions that lead to improve the quality of this manuscript. The study received funding from The Norwegian Research Council.

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