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Resilience of a harvested gastropod, *Turbo militaris*, to marine heatwaves

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ABSTRACT

Marine heatwaves (MHW) are predicted to occur with increased frequency, duration and intensity in a changing climate, with pervasive ecological and socioeconomic consequences. While there is a growing understanding of the ecological impacts of warming and marine heatwaves, much less is known about how they influence the underlying physiology and health of species, and the nutritional properties of tissue. We evaluated the effects of different heatwave scenarios and ocean warming on the nutritional properties and immune health of the harvested gastropod *Turbo militaris*. Neither heatwave scenarios nor elevated temperatures had significant impacts on morphometrics, proximate composition or inorganic content of *T. militaris*. However, an increased moisture content and non-significant trends, such as elevated amount of lipids, and an increased number of hemocytes in the blood of *T. militaris* in the heatwave treatments were suggestive of mild stress. Overall, our study suggests that *T. militaris* is resilient to marine heatwaves and warming, although delayed, additive or synergistic stress responses cannot be ruled out. Understanding the possible effects of ocean warming and heatwaves on fisheries species could improve management actions to avoid species impacts, socioeconomic losses and negative effects to ecosystem service provision in a changing climate.

1. Introduction

Climatic changes in ocean temperatures may have broad implications for coastal marine ecosystems (Doney et al., 2011). The average sea surface temperature (SST) has increased over recent decades and is predicted to continue rising in the future (IPCC et al., 2014). In conjunction with global warming, extreme weather events such as marine heatwaves are likely to occur more frequently and with increased duration and intensity (Hobday et al., 2018; Oliver, 2019; Smale et al., 2019). Marine heatwaves are defined as anomalously warm, discrete and prolonged ocean events in a particular location, with a duration of five days or longer and temperatures warmer than the long-term average for any given place (Hobday et al., 2016).

Marine heatwaves can affect coastal ecosystems at different spatial, as well as temporal scales, impacting the physiology and survivorship of marine organisms (Garrabou et al., 2009; Smale et al., 2019), subsequently resulting in changes to species distributions and community structure (Mouthon and Daufresne, 2006; Wernberg et al., 2013). Many temperate reefs globally have experienced long-term increases in water temperatures and are also being increasingly subjected to extreme events such as heatwaves (Oliver et al., 2018b). This phenomenon can severely impact sensitive sessile foundation species (e.g. kelp) (Ruthrof et al., 2018; Wernberg et al., 2016), as well as slow-moving benthic species, such as gastropods, that often cannot avoid unfavourable conditions (Pearce et al., 2011). The impacts of marine heatwaves on species and their interactions can also have socioeconomic implications when affecting the persistence and physiology of species that support fisheries (Frolicher and Laufkotter, 2018; Smale et al., 2019).

Important fisheries taxa including gastropods are a great source of high quality protein, essential micronutrients, trace elements, as well as vital fatty acids (Periyasamy et al., 2011). Ocean warming and acidification, however, can negatively affect the nutritional quality of seafood, causing a reduction in protein, lipid and carbohydrate content, as well as essential omega-3 fatty acids and vital mineral elements (Ab Lah et al., 2017b, 2018a; Lemasson et al., 2019; Tate et al., 2017; Valles-Regino et al., 2015; but see Coleman et al., 2019). While knowledge of the impacts of ocean warming and acidification on the nutritional properties of marine gastropods has increased substantially in the past

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decade (see above references), the effects of heatwave events on the physiology and survivorship of key fished species has received less attention. Notably, Gaines and Denny (1993) highlight that environmental extremes have the potential to impact ecosystems and species more severely than slower long-term changes. Hence, for a full picture, research needs to focus on the effects of long-term gradual trends, as well as on extreme events (Jentsch et al., 2007; Wernberg et al., 2013).

Here, we tested the impacts of marine heatwaves and other warming scenarios on the immune health and nutritional properties of the abundant and harvested gastropod, *Turbo militaris*. The effects of elevated temperatures on the health and nutritional properties of *T. militaris* have been studied by Ab Lah et al. (2018b), who increased temperatures from 22 °C to 25 °C (held for 38 d), and found detrimental impacts on the fatty acid composition of the flesh. The current study builds on this work by examining the resilience of *T. militaris* to an extreme heatwave, with and without prior exposure to sub-lethal temperatures to evaluate the influence of preconditioning on the gastropod. *T. militaris*, a large Australian turbinid, grows up to 110 mm (Rowling et al., 2010) and reaches densities of up to 30 individuals per 4 m^2 in shallow areas of rocky reefs on the east coast of Australia (Cooling and Smith, 2015). Its distributional range coincides with coastal areas that are predicted to experience faster than average ocean warming (Ab Lah et al., 2017a) and, concomitant, more frequent and intense ocean heatwaves (Frolicher et al., 2018). *T. militaris* grazes on macroalgae and influences the structure and function of temperate reefs (Cooling and Smith, 2015), which may be exacerbated under future scenarios of warming (Miranda et al., 2019). The species is also subject to indigenous and recreational harvest, as well as small scale commercial fisheries (Rowling et al., 2010) because of its high-quality nutritional properties, including the presence of omega-3 fatty acids that are essential to human well-being (Ab Lah et al., 2017b). Predicting how ocean warming and heatwaves alter the resilience of fisheries species can contribute to adaptive management plans to avoid species and economic loss and negative impacts to ecosystem service provision in changing climates (Garrabou et al., 2009; Zarnetske et al., 2012).

2. Materials and methods

2.1. Experimental set up

To test the hypotheses that ocean warming and marine heatwaves affect the growth, physiology and tissue quality of *T. militaris*, we set up a mesocosm experiment using twenty independent 230 L round outdoor mesocosms (80 cm diameter x 45 cm high) at the National Marine Science Centre (NMSC), Coffs Harbour, Australia (30°16′02.9928″S, 153°08′14.2224″E). The experiment involved four temperature treatments ($n = 5$ replicate mesocosms per treatment), of which two were maintained at constant seawater temperatures: the ambient (“C21” 21.1 ± 0.4 °C [mean temperature ± SD]) and predicted future (“C23” 23.0 ± 0.4 °C) mean temperature, while a heatwave was implemented in the remaining two treatments. To simulate a marine heatwave, after 21 d at 21.0 ± 0.3 °C, the seawater temperature was gradually increased to 26.2 ± 1.2 °C over two days, and held for 21 d; then decreased to 21.4 ± 0.4 °C for the remaining 12 d post-recovery (“nvHW”). The variable heatwave treatment (“vHW”) followed a similar set up, however, it involved three short (2–3 d in duration) heat spikes (22.7 ± 0.9 °C) before initiating a heatwave of the same intensity and duration as that used in the nvHW treatment group (see Fig. 1). The heatwave set up mirrored the temperature profile observed during a major heatwave in 2011 in the shallow waters of Western Australia (Wernberg et al., 2013), a region which is climatologically and ecologically similar to the Coffs Harbour region (Ab Lah et al., 2017a). The degree-days (average daily temperature during warming - daily C21 temperature) were adapted from Coleman et al. (2019).

The mesocosms were supplied with sea water from the adjacent ocean, which was filtered at 50 μm prior to entering each mesocosm at 2.5 L/min. Water temperatures for each treatment were controlled using heater-chiller units (Aquahort Ltd., Omana Beach, New Zealand) and each mesocosm was enriched with air from large airstones. Water pH, temperature and salinity (in ppm) were measured daily with a Hach HQ40d multi probe calibrated with high precision buffers.

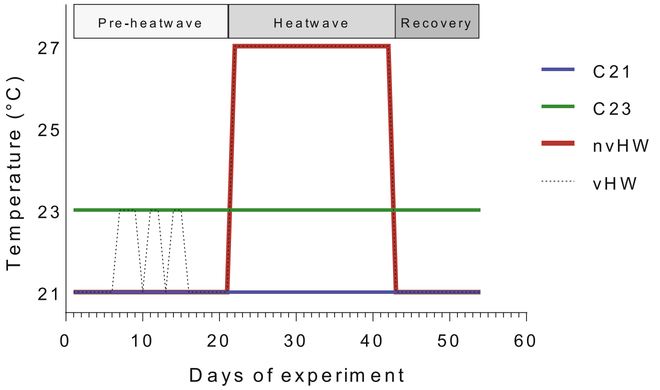

Fig. 1. Schematic diagram of the four temperature treatment profiles, which are based on a field record from the Western Australian marine heatwave in 2011 (Wernberg et al., 2013). Variation in lines indicate the magnitude and timing of temperature changes. C21: 21 °C control; C23: 23 °C warming control; nvHW: non-variable heatwave; vHW: variable heatwave.

T. militaris ($n = 60$; mean [± SD] diameter = 68.7 ± 2.9 mm) were hand collected from a shallow subtidal reef (30°06′31.0″S, 153°12′26.0″E) in August 2018 (ocean temperatures ranging from 19.6 °C to 21.9 °C) and placed in mesocosms at C21 temperature for 1 week for acclimation. Three gastropods were added to each of the 20 mesocosms. Gastropods were acclimated in mesocosms before the temperature trials began. Gastropods were fed freshly collected *Sargassum* spp. to satiation (ca. 20 g every few days) throughout the experiment to minimise effects associated with food limitation.

2.2. Sample processing and morphometrics

The length (apex – aperture) of each gastropod was measured with Vernier callipers to the nearest 0.01 mm before the experiment, post-heatwave (after 42 d), and post-recovery (after 54 d). Growth was defined as the change in shell length (final length - initial length). The fresh weight (FW; i.e. body weight plus shell weight) of *T. militaris* was determined using a top pan balance (Ohaus Navigator, Switzerland) with a precision of ± 0.01 g. Post-heatwave, as well as post-recovery, one *T. militaris* per tank was randomly selected for further analyses, generating a total of five independent replicate gastropods per treatment. Each of these gastropods was de-shelled utilising a bench vice and hemolymph was collected by piercing the mantle tissue, taking care not to disrupt the digestive material, and then inverting the gastropods in a petri dish for 1 – 2 min to collect the pooling hemolymph with a 1 mL sterile syringe. Gastropods were further dissected as described in Ab Lah et al. (2018b). The wet tissue excluding the operculum (TW) was weighed using an analytical balance with a precision of ± 0.0001 g (Mettler Toledo) and shell weight (SW) was calculated as FW - TW. The condition index (TW/SW × 100) and the meat yield (TW/FW × 100) were calculated adapting methods outlined by Vasconcelos et al. (2008) and Okumuş and Stirling (1998), respectively. Following Ab Lah et al. (2017b), muscle tissue from each foot was divided into four subsamples. These samples were then either snap frozen in liquid nitrogen and stored at −80 °C prior to lipid analysis or transferred to −20 °C until further processing for proximate and elemental analysis. Note: All biochemical and hemolymph parameters were only sampled post-heatwave due to no significant differences post-heatwave (see Supplementary Fig. 1 for more details).

2.3. Hemolymph parameters

For the total hemocyte counts (THC), hemolymph was prepared following the method described by Dang et al. (2012). Briefly, immediately after collection, 100 μL of hemolymph was fixed in 50 μL of 5% formalin, inverted to mix, and then stored at 4 °C for further assessment. A 50 μL aliquot of fixed hemolymph was pipetted onto a Neubauer improved bright-line hemocytometer (depth 0.100 mm and 0.0025 mm^2 area) and observed under a phase contrast microscope. Using the six medium-sized grid squares, duplicate counts were performed in both chambers of the hemocytometer and the number of cells per mL was calculated then averaged for each replicate gastropod.

To determine phagocytosis rates, zymosan A from *Saccharomyces cerevisiae* was prepared as a 0.25% solution in 4% congo red in sulfate-free seawater (SFSW) according to Dang et al. (2012) and Hooper et al. (2011). The prepared zymosan solution (0.0025 g) was autoclaved, washed twice, and diluted to a concentration of approximately 1.0×10^8 per mL before storing for a maximum of 1 week in a refrigerator. Immediately after collection, an aliquot of fresh hemolymph was added to the prepared zymosan solution (15:1 v/v) in an Eppendorf tube (Sigma Aldrich). After gentle inversion, samples were incubated for approximately 15 min in a dark cool box. After the incubation period, the samples were inverted and 100 μL of the solution (hemolymph containing zymosan) was pipetted onto a clean glass slide and incubated for an additional 15 min in the dark on the glass slide. Excess hemolymph was removed by gently tipping the glass slide. The slides were fixed with 10% formalin for 20 min, rinsed with sterile filtered seawater and then covered with a cover slip before viewing under a compound microscope (40× magnification) in three distinct fields of view (right, centre and left). In the three fields of view, a minimum of 40 cells were scored for the presence or absence of zymosan particles inside the cells. Cells with zymozan particles only adhered to the outside of the cells were scored as negative, following Hooper et al. (2011). Mean percentage of phagocytic cells (with zymosan particles inside) was calculated from the three fields of view.

2.4. Proximate composition

Moisture and ash content were obtained following methods described by Ab Lah et al. (2018b). Briefly, one subsample of foot tissue from each gastropod was weighed and dried in an oven at 60 °C for up to 48 h. Moisture content was calculated based on the percentage weight loss after drying to a constant weight. Each foot tissue sample was then transferred to a muffle furnace (Barnstead thermolyne 30400 furnace) at 550 °C for 4 h to obtain the ash content.

Lipids were extracted from another subsample of wet foot tissue from each gastropod, which was soaked in 1:2 chloroform:methanol for 1.5 h, then dried under high-purity nitrogen gas (Valles-Regino et al., 2015). The extract was weighed and lipids were calculated as mg per g sample weight. For protein analysis, a further subsample of wet foot tissue from each gastropod was digested in 10 mL of 1M NaOH until the tissue was completely dissolved, and total nitrogen was determined using the Kjeldahl method (Nongmaithem et al., 2018), according to standard protocols, with a conversion factor of 6.25 to convert total nitrogen to crude proteins (AOAC, 1995).

2.5. Fatty acids

Fatty acid methyl esters (FAMEs), derivatised from the lipid extracts using boron trifluoride (BF_3) in methanol, were used for fatty acid analysis. For each gastropod, FAMEs were prepared as outlined in Ab Lah et al. (2017b) and analysed using Flame Ionisation Detection (FID) gas chromatography (GC, Agilent 6890N, Santa Clara, CA, USA). Briefly, the GC oven temperature was programmed with 100 °C, held for 5 min and then increased at a rate of 5 °C/min until the final temperature (240 °C) was reached. One microliter of the extract containing the FAMEs was injected with a split ratio of 200:1 and a column flow of 1 mL/min. FAMEs were identified by comparison of peak retention times to reference standard FAMEs test mix (Sigma) and percent composition relative to all peaks was calculated for each FAME using ChemStation (Agilent). Unidentified fatty acid peaks were analysed using GC/mass spectrometry (Agilent 5973 Mass Selective Detector) and matched to a mass spectral library (NIST14.L).

2.6. Elemental analysis

For elemental analysis, a subsample of foot tissue from each gastropod was digested in 1:3 nitric acid:hydrochloric acid subjected to hot-block at 120 °C for 1 h (Hot-Block; Environmental Express, South Carolina, USA), following standard acid digestion procedure according to APHA (Rice et al., 2017). Elemental concentrations were analysed on an inductively coupled plasma mass spectrometer (NexION 300 D series) with an ESI SC-FAST Auto Sampler (PerkinElmer, Massachusetts, USA).

2.7. Statistical analysis

To test the effect of heatwaves and ocean warming on the health and nutritional quality of *T. militaris*, data were analysed using permutational multivariate analyses of variance (PERMANOVA; Anderson, 2001) based on Euclidian distance similarity matrices in PRIMER v. 7 + PERMANOVA. Pairwise post-hoc tests were used to identify patterns among treatments. PERMANOVAs for proximate composition, fatty acid profiles, elemental analyses and cellular immunity were based on one factor: treatment (fixed; C21, C23, vHW, nvHW); whereas PERMANOVAs for morphometrics and moisture were based on three factors: treatment (fixed; C21, C23, vHW, nvHW), replicate mesocosms (random, nested within treatment) and time (fixed; post-heatwave and post-recovery). Multivariate PERMANOVAs were used to statistically analyse fatty acid profiles and elemental composition of the flesh. Fatty acid data were standardised to the total, whereas the data was normalised for elemental analyses (macro- and microelements and trace elements were analysed separately). Univariate PERMANOVAs were used to test the temperature treatment on the percent of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), omega-3 (n-3), omega-6 (n-6) fatty acids, as well as the n-3/n-6 ratio. Fatty acid profiles were visualised using multidimensional scaling (MDS) ordination based on bootstrap average analysis.

3. Results

3.1. Health of T. militaris

3.1.1. Survivorship and morphometrics

After 42 days of the experiment, all *T. militaris* remained alive and visibly healthy. Behavioural observations during daily temperature checks always found the gastropods inside the water. Overall, changes in fresh weight (1.1 ± 2.1 g) and shell length (1.2 ± 0.7 mm), as well as meat yield and condition indices of *T. militaris* did not differ significantly among treatments or replicate mesocosms (Table 1). However, there was a significant difference between growth post-heatwave and post-recovery (Table 1), with no net growth during the recovery phase.

3.1.2. Hemolymph parameters

The mean number of total hemocytes per mL for all treatments was $10.6 \times 10^5 \pm 7.9 \times 10^5$. The mean proportion of phagocytic cells was 28.9 ± 10.0% across all treatments. The cellular immune parameters were not significantly influenced by elevated temperatures or heatwaves (PERMANOVA, $p > 0.05$ for all factors; Table 1), although a trend was observed for the total hemocyte count, with higher numbers

Table 1
Summary of PERMANOVA results for the multivariate (x^a) and univariate analyses. For the morphometrics and moisture, a three factor nested design was used (treatment; fixed, mesocosm; random and nested in treatment, and time (post-heatwave vs. post-recovery); fixed). The interaction terms were also tested but were not significant in any case ($p < 0.05$). For the remaining biochemical composition, inorganic parameters, as well as cellular immunity of *Turbo militaris,* one factor PERMANOVA was used to analyse differences between the four treatments and controls, for snails collected after the heatwave. Significant *p*-values in bold.

	Treatment		Mesocosm (Treatment)		Time	
	Pseudo *F*	*p*-value	Pseudo *F*	*p*-value	Pseudo *F*	*p*-value
Morphometrics						
Growth	1.27	0.31	0.81	0.67	144.18	**0.0001**
Change in fresh weight	1.62	0.24	0.69	0.79	0.003	0.95
Condition index	1.79	0.19	0.56	0.83	1.70	0.22
Meat yield	1.64	0.22	0.54	0.85	1.66	0.21
Proximate composition						
Moisture	3.49	**0.04**	3.08	0.19	5.91	**0.02**
Ash	1.03	0.41	–	–	–	–
Protein	1.88	0.17	–	–	–	–
Lipids	1.89	0.18	–	–	–	–
Fatty acids						
Total^a	0.69	0.77	–	–	–	–
SFAs	1.41	0.27	–	–	–	–
MUFAs	0.49	0.70	–	–	–	–
PUFAs	1.07	0.40	–	–	–	–
n-3	1.03	0.40	–	–	–	–
n-6	0.43	0.73	–	–	–	–
n-3:n-6 ratio	0.45	0.72	–	–	–	–
Inorganic content						
Macroelements^a	0.98	0.42	–	–	–	–
Microelements^a	0.52	0.97	–	–	–	–
Trace elements^a	0.69	0.88	–	–	–	–
Cellular immunity						
Total hemocyte numbers	1.73	0.19	–	–	–	–
Phagocytosis	1.30	0.31	–	–	–	–

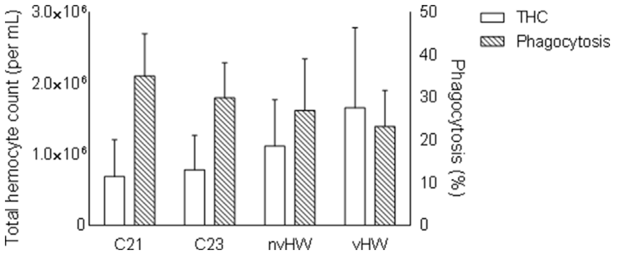

Fig. 2. The effects of heatwaves and ocean warming on the immune parameters total hemocyte count (THC) per mL and percent phagocytosis of *Turbo militaris*. C21: 21 °C control; C23: 23 °C warming control; nvHW: non-variable heatwave; vHW: variable heatwave. Error bars are standard error of mean.

in the variable heatwave treatment compared to both controls (Fig. 2).

3.2. Flesh nutritional quality

3.2.1. Proximate analysis

The average moisture content in the flesh was significantly different between heatwave treatments and controls, both post-heatwave and post-recovery (Fig. 3, Table 1, no interaction between treatment and time, $p = 0.3$). Furthermore, there was a significant difference between moisture content post-heatwave when compared to post-recovery (Table 1), with slightly elevated numbers after the 12-d recovery period. Proximate analysis revealed that the wet tissue of *T. militaris* contained 23.5 ± 1.9% of crude protein, 5.2 ± 2.9% of ash, and 0.9 ± 0.5% of lipids. None of these parameters differed significantly between treatments (Table 1).

3.2.2. Fatty acids

The fatty acid composition of the lipid extracts were dominated by SFAs and PUFAs, with palmitic acid (C16:0), arachidonic acid (C20:4 n-6) and margaric acid (C17:0) being the most abundant (Table 2). Oleic acid (C18:1) was the most abundant MUFA (Table 2). The omega-3 fatty acids in the gastropod tissue consisted of α-linolenic acid, eicosatrienoic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). The omega-3 to omega-6 fatty acid ratio was < 1 for all treatments (Table 2). The overall fatty acid composition and all other fatty acid parameters did not differ significantly among treatments and no trends were observed (Table 1).

3.2.3. Elemental composition

The most abundant macroelements in the gastropod flesh were sulphur (S), potassium (K) and sodium (Na), and the most abundant microelements were silicon (Si), iron (Fe), zinc (Zn), and copper (Cu) (Table 3). Additionally, *T. militaris* had high concentrations of the trace element arsenic (As) and considerate amounts of nickel (Ni), chromium (Cr), manganese (Mn), selenium (Se), and lead (Pb) (Table 3). Elemental composition of *T. militaris* was not significantly impacted by heatwaves (PERMANOVA, $p > 0.05$ for all comparisons among treatments and mesocosms; Table 1).

4. Discussion

Ocean warming is a major driver for the increased frequency and intensity of marine heatwaves (Oliver, 2019), with pervasive consequences for ecosystems and important fisheries species (Smale et al., 2019). Although elevated ocean temperatures can affect the immune health (Dang et al., 2012; Hooper et al., 2014) and nutritional quality of gastropods (Ab Lah et al., 2018b; Tate et al., 2017; Valles-Regino et al., 2015), the effects of heatwaves are not yet fully understood. Here, we demonstrated that neither heatwaves nor higher temperatures had a significant effect on morphometrics, proximate composition (except for moisture content) or inorganic content of the large gastropod *T. militaris*. There were, however, trends suggesting signs of heat stress, including elevated amount of lipids, and an increased number of hemocytes in the blood of *T. militaris*. Furthermore, the moisture content of *T. militaris* was significantly elevated in the heatwave treatments relative to the controls post-heatwave and this difference persisted post-recovery. Thus, while this gastropod is relatively resilient to immediate effects of warming or heatwaves, we cannot rule out longer term or cumulative impacts.

The overall immune health of *T. militaris* showed signs of heat stress with an elevated number of hemocytes. Hemocytes play a vital role in cell immunity (Hooper et al., 2007) and they can be useful in identifying sublethal responses to environmental stressors (Li et al., 2015). An increase in hemocyte count can indicate proliferation or movement of the cells out of the tissue and into circulating hemolymph (Pipe and Coles, 1995) to facilitate a rapid response to any invasion by foreign particles and organisms. The predominant cellular defence mechanism involving hemocytes is phagocytosis (Pipe and Coles, 1995), however, this parameter was not impacted by temperature in this study, indicating that capacity of *T. militaris* to defend against microbial infection was not impacted by the experimental setup investigated here, including a range of ocean temperatures and time of exposure. Similar to the current study, an elevated hemocyte count at higher temperatures has been found in other marine gastropods, such as abalone (*Haliotis rubra*, Dang et al., 2012; *Haliotis laevigata* x *H. rubra*, Hooper et al., 2014). Interestingly, Li et al. (2015) found no immune response for the pearl oyster (*Pinctada fucata*) at 25 °C (+6 °C compared to control), but detected a significant increase of hemocyte numbers after short-term (3 d) exposure at 31 °C (+12 °C compared to control).

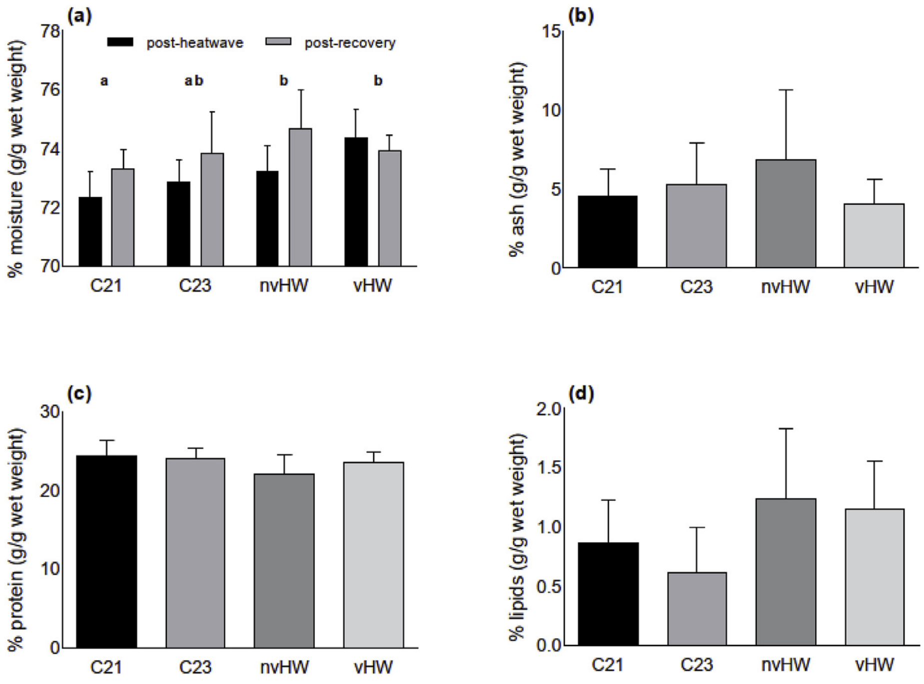

Fig. 3. The effects of heatwaves and ocean warming on the proximate composition of *Turbo militaris* as a percentage of the wet weight for a) moisture, b) ash, c) protein and d) lipid. Different letters indicate statistically different groups in pair-wise post-hoc tests for moisture (factor: treatment, $p < 0.05$). C21: 21 °C control; C23: 23 °C warming control; nvHW: non-variable heatwave; vHW: variable heatwave. Error bars are standard error of mean.

Table 2
Fatty acid composition in the foot tissue of *Turbo militaris* after exposure to different temperature treatments. Mean percentage ± standard deviation of all fatty acids.

Fatty acid	Trivial name	Treatment			
		C21 (21 °C)	C23 (23 °C)	nvHW (21/27 °C)	vHW (21/23/27 °C)
Saturated fatty acids (SFAs)					
C14:0	Myristic	1.10 ± 0.22	1.08 ± 0.11	0.91 ± 0.59	1.27 ± 0.39
C15:0	Pentadecanoic	2.97 ± 0.45	3.10 ± 0.60	2.81 ± 0.84	3.08 ± 0.47
C16:0	Palmitic	16.53 ± 0.84	17.48 ± 0.76	17.16 ± 1.11	17.12 ± 1.48
C17:0	Margaric	12.38 ± 1.83	12.75 ± 2.89	10.07 ± 3.71	11.73 ± 2.03
C18:0	Stearic	6.03 ± 0.98	6.00 ± 2.47	8.16 ± 3.71	6.51 ± 2.21
C24:0	Lignoceric	7.95 ± 0.67	8.18 ± 0.55	8.24 ± 0.50	8.14 ± 1.15
Total SFA		46.95 ± 1.51	48.59 ± 1.17	47.34 ± 0.88	47.86 ± 1.65
Monounsaturated fatty acids (MUFAs)					
C16:1	Palmitoleic	3.83 ± 0.38	3.79 ± 0.35	3.67 ± 0.79	3.94 ± 0.46
C18:1	Oleic	6.70 ± 0.59	6.44 ± 0.24	6.64 ± 0.59	7.17 ± 1.33
C20:1	Gadoleic	1.54 ± 1.36	1.46 ± 1.44	1.11 ± 1.28	1.93 ± 1.41
C22:1	Erucic	2.64 ± 0.96	2.30 ± 0.37	2.51 ± 1.24	2.28 ± 1.08
Total MUFA		14.72 ± 1.92	13.99 ± 1.28	13.93 ± 1.83	15.32 ± 3.02
Polyunsaturated fatty acids (PUFAs)					
C18:2 (n-6)	Linolenic	2.43 ± 0.44	2.34 ± 0.30	2.33 ± 0.39	2.16 ± 0.23
C18:3 (n-3)	α-Linolenic	2.07 ± 0.83	2.08 ± 0.85	2.58 ± 0.77	1.61 ± 1.21
C20:3 (n-3)	Eicosatrienoic	0.55 ± 0.93	0.15 ± 0.09	0.67 ± 0.84	0.65 ± 1.23
C20:4 (n-6)	Arachidonic (ARA)	15.41 ± 0.82	15.09 ± 0.62	15.19 ± 1.20	15.02 ± 1.47
C20:5 (n-3)	Eicosapentaenoic (EPA)	3.40 ± 1.93	4.00 ± 0.60	3.04 ± 1.75	3.44 ± 2.04
C22:2 (n-6)	Docosadienoic	5.03 ± 0.82	5.09 ± 0.44	5.41 ± 0.72	5.20 ± 0.86
C22:4 (n-6)	Docosatetraenoic	1.49 ± 0.93	0.95 ± 0.24	0.95 ± 0.54	1.01 ± 0.24
C22:5 (n-3)	Docosapentaenoic (DPA)	7.66 ± 1.04	7.56 ± 1.21	8.27 ± 1.75	7.42 ± 1.11
C22:6 (n-3)	Docosahexaenoic (DHA)	0.30 ± 0.39	0.14 ± 0.09	0.29 ± 0.41	0.32 ± 0.50
Total n-3		13.97 ± 0.68	13.94 ± 1.39	14.85 ± 1.33	13.44 ± 1.57
Total n-6		24.36 ± 1.25	23.47 ± 0.86	23.89 ± 1.51	23.38 ± 2.20
Total n-3: n-6		0.58 ± 0.05	0.59 ± 0.06	0.63 ± 0.09	0.58 ± 0.08
Total PUFA		38.34 ± 0.99	37.42 ± 1.83	38.73 ± 1.24	36.83 ± 2.86

Stress-related immune responses in molluscs may be dependent on a number of factors, such as temperature tolerance (Liang et al., 2014; Yao and Somero, 2012), difference in hemocyte sub-population proportions (Donaghy and Volety, 2011; Li et al., 2015), and duration and severity of stress application (e.g. Chen et al., 2007).

Increase in moisture content may be caused involuntarily through changes in other proximate components or elemental composition (Tate et al., 2017). In the case of *T. militaris*, however, it was most likely caused by an active uptake of body water. Pincebourde et al. (2008) suggest that sea stars might confuse an increase in body temperature with desiccation stress and actively increase their body water content. Similar observations were made for the marine gastropod *Nerita atramentosa*, suggesting that this gastropod is capable of behaviourally adjusting its body water content in anticipation of possible dehydration (Chapperon et al., 2013). The even further increased moisture content post-recovery may suggest that the gastropods subjected to heatwave

Table 3
Elemental compositions in the foot tissue of *Turbo militaris* after exposure to different temperature treatments. Mean ± standard deviation for each element as fraction of kg fresh weight.

Chemical element	Treatment				
	C21 (21 °C)	C23 (23 °C)	nvHW (21/27 °C)	vHW (21/23/27 °C)	All
Macroelements (g/kg)					
Ca	1.03 ± 0.92	0.55 ± 0.28	0.79 ± 0.32	0.69 ± 0.50	0.76 ± 0.54
Mg	0.65 ± 0.14	0.68 ± 0.08	0.69 ± 0.08	0.66 ± 0.07	0.67 ± 0.09
K	3.70 ± 0.39	3.75 ± 0.25	3.54 ± 0.30	3.91 ± 0.35	3.73 ± 0.34
Na	3.67 ± 1.13	3.85 ± 0.56	3.97 ± 0.64	3.93 ± 0.76	3.87 ± 0.78
S	9.83 ± 0.93	9.58 ± 0.86	9.29 ± 0.82	9.96 ± 0.58	9.70 ± 0.80
P	1.52 ± 0.09	1.45 ± 0.08	1.40 ± 0.10	1.57 ± 0.17	1.48 ± 0.13
Microelements (mg/kg)					
Cu	7.48 ± 1.98	8.31 ± 5.64	8.66 ± 8.62	6.43 ± 2.98	7.70 ± 5.14
Zn	15.82 ± 8.26	13.84 ± 1.13	13.86 ± 1.24	16.47 ± 3.52	15.05 ± 4.51
Fe	31.78 ± 17.01	36.05 ± 20.14	29.67 ± 17.15	21.28 ± 9.39	29.42 ± 16.00
Al	2.68 ± 1.41	2.64 ± 1.16	2.38 ± 1.06	3.06 ± 1.22	2.96 ± 1.16
B	0.69 ± 0.20	0.57 ± 0.41	0.91 ± 0.22	0.69 ± 0.42	0.72 ± 0.33
Si	72.76 ± 11.02	68.60 ± 13.74	64.60 ± 4.94	68.28 ± 23.15	68.65 ± 14.06
Trace elements (mg/kg)					
Ag	0.04 ± 0.05	0.06 ± 0.09	0.03 ± 0.04	0.07 ± 0.05	0.05 ± 0.04
As	6.43 ± 1.45	7.67 ± 2.45	8.23 ± 3.47	7.28 ± 1.80	7.39 ± 2.36
Pb	0.19 ± 0.19	0.18 ± 0.10	0.15 ± 0.12	0.20 ± 0.14	0.18 ± 0.14
Cd	0.04 ± 0.01	0.08 ± 0.07	0.05 ± 0.03	0.03 ± 0.01	0.05 ± 0.02
Cr	0.34 ± 0.18	0.34 ± 0.12	0.25 ± 0.11	0.23 ± 0.08	0.29 ± 0.14
Mn	0.30 ± 0.09	0.33 ± 0.18	0.26 ± 0.11	0.29 ± 0.09	0.29 ± 0.12
Ni	0.40 ± 0.36	0.55 ± 0.25	0.36 ± 0.17	0.53 ± 0.16	0.45 ± 0.24
Se	0.28 ± 0.09	0.30 ± 0.16	0.24 ± 0.08	0.25 ± 0.06	0.27 ± 0.09
Hg	0.01 ± 0.003	0.01 ± 0.003	0.008 ± 0.003	0.01 ± 0.01	0.01 ± 0.005
V	0.09 ± 0.10	0.07 ± 0.03	0.07 ± 0.08	0.04 ± 0.02	0.07 ± 0.07
Co	0.02 ± 0.01	0.03 ± 0.005	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
Mo	0.06 ± 0.02	0.11 ± 0.06	0.07 ± 0.04	0.08 ± 0.05	0.08 ± 0.05
Ba	0.04 ± 0.03	0.03 ± 0.02	0.04 ± 0.02	0.06 ± 0.05	0.04 ± 0.03

conditions have not yet adjusted to ambient temperature conditions. Moisture content is linked to the seafood appeal of molluscs, where a higher moisture content indicates an increased juiciness of the flesh (Lemasson et al., 2019). However, the concurrent decrease in the proportion of other tissue compounds such as proteins could also mean lower nutritional return for the same amount of food consumed (Tate et al., 2017).

Proteins are important components of the physiological temperature stress response, with the expression of heat-shock proteins being common in molluscs (Denny et al., 2011). The results of this study indicate that protein content of *T. militaris* in the heatwave treatments was similar to the controls. Notably, total protein was considered as a whole; hence, any impacts of heat on structural and storage proteins due to altered catabolism might have masked the immediate up-regulation of heat-shock and other stress-related proteins. Similar to the current study, observations for *T. militaris* revealed no temperature effect on the protein content (Ab Lah et al., 2018b). In contrast, experiments on the marine predatory gastropod *Dicathais orbita* resulted in a significant reduction in protein content with a 3 °C temperature increase (Tate et al., 2017). The different protein content responses of gastropods to temperature suggest that the future impacts of marine heatwaves and ocean warming are likely to vary among species.

Further evidence that *T. militaris* showed little or no signs of acute heat stress was also apparent in the lipid content and fatty acid composition of *T. militaris*, as there were no significant differences found between controls and heatwave treatments. Nonetheless, a trend was observed in the amounts of lipids in the tissue of gastropods, which were higher in heatwave treatments relative to the controls. This trend suggests that all fatty acids were affected equally by the heatwave (as analyses were based on percentages), or it may have caused an increase in other lipophilic components in the extracts. Variation within heatwave treatments appeared higher than within the controls (see Supplementary Fig. 2), indicating that individual stress-response may become more variable with magnitude of stress; however, this needs further investigation. Rais et al. (2010) also did not find a change in fatty acid composition in multiple intertidal gastropods under elevated temperatures, suggesting that vital membrane fluidity can be maintained through other processes such as changes in the surrounding microhabitat. However, Ab Lah et al. (2018b) observed a change in fatty acid composition with increasing temperature in *T. militaris*, which might indicate that the composition depends on other factors, such as feeding during the experiment. Notably, the gastropods in the current study had constant access to fresh algae, meaning they did not need to invest much energy into feeding excursions (Morais, 2003), whereas the *T. militaris* in the study by Ab Lah et al. (2018b) were just grazing microalgae and diatom growth on the mesocosm walls without additional food supplied.The basic composition of fatty acids appears to be dependent on such non-species-specific factors (e.g. food quality, Morais, 2003), as the composition of *T. militaris* studied here and *T. militaris* from the same area tested in a previous study by Ab Lah et al. (2018b) showed differences. Gastropods from the current study had higher levels of SFAs (+20%), MUFAs (+30%), and n-6 fatty acids (+30%) when compared to the previous study and palmitoleic acid and margaric acid were approximately four times as high. However, the observed variations might not only be influenced by nutrition but also by the fact that the experiments were carried out during different seasons (Austral spring in Ab Lah et al., 2018b and Austral winter in the current study). Other studies have shown that fatty acid composition, as well as proximate composition can undergo seasonal variations in molluscs (e.g. Ab Lah et al., 2018a; Irisarri et al., 2015; Okumuş and Stirling, 1998), suggesting that endogenous factors such as lipid mobilisation (e.g. linked to the reproductive cycle, Ab Lah et al., 2018a), oxidative stress processes (Filho et al., 2001), and immune parameters (Duchemin et al., 2007) may play a role. Seasonal variations might also partially explain the observed non-significant increase of lipids, which was in contrast to previous studies on marine molluscs, which found that higher temperatures reduced the lipid content in the flesh (*T. militaris*, Ab Lah et al., 2018b; *Magallana gigas* & *Ostrea edulis*, Lemasson et al., 2019; *D. orbita*, Tate et al., 2017; *D. orbita*, Valles-Regino et al., 2015). Lemasson et al. (2019) suggest that a reduction in oyster lipid content after exposure to heat might indicate that lipids are the primary source of energetic reserves at higher temperatures. However, similar to the present study, Clements et al. (2018) observed a non-significant trend of increased lipids at higher temperatures (+7 °C over 30 – 90 d) for mussels (*Mytilus edulis*), arguing that even though other reserves such as carbohydrates were depleted during thermal stress, lipid reserves were not utilised. The authors conclude that there might be a certain threshold of energy depletion required to initiate the activation of lipid reserves, which is species-specific and probably dependent on a number of other factors that need further investigation.

Elemental composition of *T. militaris* was not significantly impacted by the heatwave. Irrespective of treatment, sulphur (S), potassium (K), and sodium (Na) were the most abundant macroelements in the gastropod tissue, which is consistent with previous nutritional studies on *T. militaris* (Ab Lah et al., 2017b). The gastropods had considerable amounts of microelements essential to human health, including iron, zinc, and copper (Fraga, 2005), whereas the concentration of trace elements possibly toxic to humans, such as lead (Pb), cadmium (Cd), and mercury (Hg), were all under their maximum levels for safe seafood consumption (FSANZ, 2016). In contrast, total arsenic (As) was high, which was also found in other marine gastropods (Ab Lah et al., 2017b, 2018b; Phillips and Depledge, 1986; Tate et al., 2017). However, the component toxic to humans is the inorganic arsenic (Edmonds and Francesconi, 1993), which comprises a very small portion (ca. 1%) of the total arsenic in marine invertebrates (Borak and Hosgood, 2007).

The observed trends in *T. militaris* triggered by heatwaves for proximate composition, as well as hemocyte count, suggest that the gastropods may have experienced mild stress. Notably, heatwaves might trigger different responses than a long stretch of slightly elevated temperatures. More severe and possibly delayed stress effects can become apparent long after a stressor occurs (Tate et al., 2017; Walter et al., 2013), with potentially significant implications for the survival, reproduction, physiology, and nutritional quality of *T. militaris*. Additionally, the gastropods of this study were adults and environmental stressors have been shown to affect earlier and later life history phases differently (Byrne and Przeslawski, 2013; Davis et al., 2013; Przeslawski et al., 2015), suggesting that heatwaves might have a different effect on juveniles or embryos. Furthermore, whilst heatwaves alone may not have major impacts on adult *T. militaris*, if they occur at biologically vulnerable times (e.g. spawning; Li et al., 2007), in synergy with other stressors (Smale et al., 2019; Valles-Regino et al., 2015) or repetitively, the consequences for the health and nutritional quality of *T. militaris* could be much more pronounced. Repetitive sub-lethal exposure to temperature stress has been shown to both increase tolerance to future stress through acclimatisation (Ainsworth et al., 2016; Walter et al., 2013) or decrease stress resistance through additive effects (Pansch et al., 2018). The significance of results for moisture content and the trends observed for hemocyte count, suggest that preconditioning may negatively affect *T. militaris*, making the gastropods more susceptible to stress. However, if heatwaves occur more frequently as predicted (Oliver et al., 2018a), there is a possibility that species such as *T. militaris* develop acclimatisation by genetic adaption over time, making future generations more resilient (Hoffmann and Sgro, 2011).

Overall, the immunological and nutritional responses of *T. militaris* suggest that this gastropod experienced weak signs of heat stress but the adults appear to be relatively resilient to marine heatwaves. Hence, we predict that the seafood quality, harvest output, as well as ecological key role of *T. militaris* will unlikely change in a future of warming oceans and more frequent and intense heatwaves. Nevertheless, long-term *in situ* studies, which take into account an array of "natural" factors such as food quality and availability, as well as habitat complexity, community structure, tidal influence (Olabarria et al., 2016) and life history (Byrne and Przeslawski, 2013) may further elucidate possible stress effects that predicted marine heatwaves may have on *T. militaris*.

Conflicts of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.104769.

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