

Effects of acclimation temperature and exposure time on the scope for growth of the blackfoot Pāua (*Haliotis iris*)

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Handling Editor:

Lauren Nadler

Received: 7 July 2023

Accepted: 17 October 2023

Published: 13 November 2023

Cite this:

Nguyen TT et al. (2023)
Marine and Freshwater Research, **74**(17),
1465–1477.
doi:[10.1071/MF23131](https://doi.org/10.1071/MF23131)

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ABSTRACT

Context. Climate change and increased seawater temperatures can greatly affect physiological processes and growth of marine ectotherms, including the blackfoot haliotid, Pāua (*Haliotis iris*). Scope for growth (SFG) is a laboratory-derived measure of the energy available for growth but this has not been examined in Pāua. **Aims.** To measure SFG of seasonally collected Pāua and their haemolymph parameters at constant acclimation temperatures of 12, 15, 18 and 21 °C for 28 days. **Methods.** Energy available for growth was measured from kelp food and losses due to respiration and ammonia excretion calculated on Days 1, 14, 21 and 28 of acclimation. Haemolymph parameters were also measured. **Key results.** After 3 days of acclimation, SFG was highly variable. Following 2 weeks of acclimation, SFG was positive for all temperatures. Respiration and excretion energies depended on both acclimation temperature and time. Haemolymph parameters were affected by acclimation temperature. **Conclusion.** Pāua have limited ability to acclimate to a temperature 21 °C suggesting that they would not grow effectively at this temperature. **Implications.** This research suggests that adult Pāua can be adversely affected by increased seawater temperature, resulting from climate change and this could affect their future growth and distribution.

Keywords: acclimation capacity, biochemistry, energy, global warming, haemolymph, *Haliotis iris*, Pāua, physiology.

Introduction

Global warming is one of the greatest environmental challenges that affect marine organisms and ecosystems (Pachauri et al. 2014). Although increasing temperature is known to significantly affect the activity, physiology and energy balance of marine ectotherms (Bayne and Newell 1983; Cossins and Bowler 1987; Somero 2012), prolonged periods of energy imbalance could affect an organism's growth, reproduction and performance. Some marine molluscs, however, can adapt, acclimate or acclimatise to changing temperatures, which helps them maintain energy balance (Peck et al. 2014; Mardones et al. 2022). Those species with poor acclimation capacity may be unable to adjust to increased temperatures associated with climate change, global warming and marine heatwaves. Temperature can also affect the feeding rate, the amount of energy absorbed from food, rates of metabolism and ammonia excretion, functions that are used to determine the scope for the growth (SFG) (Hawkins 1995; Britz et al. 1997; Hooper et al. 2007). Within an organisms' usual temperature range, these rates usually rise with increasing temperature, whereas outside the optimum range, the rates can rapidly reduce (Gillooly et al. 2001; Hernández-Sandoval et al. 2018). SFG has been used to evaluate the available energy for growth and reproduction after fulfilling other necessary biological processes (Genoni and Pahl-Wostl 1991). It is regarded as a good predictor of actual growth in invertebrates and provides a rapid and quantitative assessment of the energy status of organisms (Beiras et al. 1994; Baillieul et al. 1996). Also, SFG has been used to indicate the ability of an organism to cope with future stress (Baillieul et al. 1996). The effects of temperature on SFG has been carried out in several marine molluscs including the juvenile scallops (*Argopecten purpuratus*; González et al. 2002), the blue mussel (*Mytilus edulis*; Widdows 1976; Mubiana and Blust 2007), the Red Sea mussel (*Brachidontes pharaonis*; Sarà et al. 2008) and the marine

scavenging gastropod (*Nassarius festivus*; Zhang *et al.* 2016). In addition, several studies have investigated the effects of temperature on the growth of abalone species, including South African abalone (*Haliotis midae*; Britz *et al.* 1997), blackfoot Pāua (*H. iris*; Searle *et al.* 2006) and disc abalone (*H. discus hannai*) in South Korea (Kong *et al.* 2017).

Increasing exposure temperature can also affect the haemolymph of marine ectotherms including haemocyanin, protein, sodium and potassium. Haemolymph protein includes haemocyanin, which is a major component, hormones, nutritional peptides and immune components (Fredrick and Ravichandran 2012). In molluscs, haemocyanin transports oxygen and assists in immune responses (Jaenicke *et al.* 2009). Elevating temperature can raise haemocyanin to transport more oxygen to the tissue, thus increasing haemolymph protein (Behrens *et al.* 2002; Giomi and Pörtner 2013). Lu *et al.* (2016) found that temperature affected both haemolymph sodium and potassium concentrations of the gazami crab (*Portunus trituberculatus*). By contrast, Einarson (1993) found that temperature did not affect haemolymph sodium and potassium concentrations in the amphipod (*Gammarus oceanicus*).

The abalone (*Haliotis iris*, hereafter Pāua) is one of three species of haliotid in New Zealand, contributing a large and critical cultural, recreational and economic resource (Prince 2005). They live both in intertidal and subtidal zones with abundant seaweed, their main food resource. Living in coastal zones, they are frequently exposed to daily and seasonal temperature changes. Like other haliotids, Pāua growth depends on size, food, temperature and environment (Stuart and Brown 1994; Steinarsson and Imsland 2003; Bansemer *et al.* 2016). However, there is no information on the effects of temperature on SFG of this species and associated changes to their haemolymph parameters including ionic concentration, haemocyanin and protein levels. Furthermore, in New Zealand, the current distribution of Pāua extends from Whangarei in the north to Stewart Island in the south, where the average maximum seawater temperatures in summer range between 14.3 and 21.4°C. Along the northern coastline, where average temperatures are ~19°C, populations are often stunted with adults reaching only 90–100-mm shell length, below the value of 125-mm shell length required for legal collection. Stunted populations can also occur elsewhere and arise due to a combination of genetic and environmental factors

(Naylor and Andrew 2004; Naylor *et al.* 2006; Venter *et al.* 2018). The aims of this study were to measure: (1) SFG of Pāua and their haemolymph parameters at constant acclimation temperatures of 12, 15, 18 and 21°C; and (2) determine the effects of 28 days of acclimation on various physiological parameters. The three lower temperatures selected for this experiment were in the range of sea surface temperature in Christchurch, Canterbury, New Zealand where Pāua were acclimated (Table 1), including 12°C presenting winter (June to August), 15°C representing spring and autumn (September to November and March to May) and 18°C representing summer (November to February). The acclimation to 21°C has been used in other studies to assess the potential response to increased temperature due to ocean warming as a result of climate change. The Pāua were collected from the field seasonally and thus were seasonally acclimated close to the new exposure acclimation temperature. The results from this study provide a basic framework for physiological research on Pāua and information about the potential physiological processes to predict the ability of this species to adapt to increased seawater temperatures associated with climate change.

Materials and methods

Animal collection and acclimation

Pāua of both sexes (80–100 mm in shell length, 124.1 ± 19.9 g) were collected by snorkelling in Little Port Cooper (Lyttelton), Christchurch (S43°613' E172°816'). The average maximal shell length for this population between 2018 and 2020 was 112.4 mm. Pāua were collected for specific acclimation temperatures close to seasonal values from Lyttelton Harbour (Table 1). They were then transported in a chilly bin in aerated seawater to the aquarium at the University of Canterbury where they were tagged and held in recirculating natural seawater (salinity ~33‰) system for at least 3 days to reduce possible stress. Temperatures during transportation and in the aquarium were maintained similar at temperatures to the original ambient temperature where Pāua were caught (Table 1). After that, Pāua were transferred to control rooms with smaller recirculating seawater systems. Temperatures

Table 1. The average, minimum and maximum seasonal seawater temperature variation average in Lyttelton over the past 10 years (Sea Temperature Info 2022), the month of collection, the seawater temperature at the time of Pāua collection, and experimental acclimation temperature.

Season	Average seasonal temperature variation (°C)	Minimum and maximum seasonal temperature variation (°C)	Month of collection	Sea temperature in intertidal and subtidal zones at time of collection (°C)	Experimental acclimation temperature (°C)
Winter	9.5	6.0–12.0	June	10.5	12
Spring	11.9	8.2–17.2	April	12.7	15
Autumn	14.6	10.6–18.8	November	15.6	18
Summer	16.7	13.4–21.1	February	18.7	21

were raised $\sim 2^{\circ}\text{C day}^{-1}$ by adjusting constant temperature rooms until they reached the target water temperatures (i.e. 12, 15, 18 or 21°C). For each acclimation temperature, there were four tank replicates. Each replicate was a 60-L tank (with Pāua) connected to another 60-L tank (without Pāua) by a submersible pump (Moray 1300, 1100 L h^{-1} , China) to maintain nature sea water recirculation. Water from the upper tanks flowed through 50- μm polishing filter pads to trap particles in the water before going to the lower tanks. Air stones were put in the lower tanks to oxygenate the water. The light-dark cycle was 12–12 h. Pāua were fed seaweed (*Undaria pinnatifida* or *Macrocystis pyrifera*) at 15% body weight every other day. Salinity, dissolved oxygen, pH and ammonia concentration were monitored and maintained at $\sim 33\text{‰} \pm 2$, $95\% \pm 5$, 8.2 ± 4 and under 0.25 mg L^{-1} . Water was exchanged for 50% every second day. They were stocked at a rate of 8–10 Pāua per tank.

Experimental design

The experiment was designed to estimate the effects of acclimation at four constant temperatures (12, 15, 18 or 21°C) recorded at four different periods (Table 1) and acclimation times up to 28 days (measured on Days 1, 14, 21 and 28) on physiological responses (absorption efficiency, absorbed energy, respiration energy, ammonia excretion energy and SFG) of Pāua and their haemolymph parameters (haemocyanin, protein, sodium and potassium). A group of 32 Pāua was used for each temperature treatment (except for 18°C where 24 Pāua were used). Eight replicate Pāua were measured for each time interval within each temperature treatment. At 18°C , physiological responses were not measured for the Pāua acclimated on Day 1 because the time and facilities were limited.

Physiological measurements

Measurement of physiological responses using the energy balance equation and performance was evaluated in terms of scope for growth (SFG):

$$\text{SFG} = Ab - (R + U)$$

where Ab is the rate of energy absorbed from food obtained in experiments ($\text{J g}^{-1}\text{ DW h}^{-1}$), R is rate of energy lost to respiration obtained in experiments ($\text{J g}^{-1}\text{ DW h}^{-1}$), U is a rate of energy lost to ammonia excretion obtained in experiments ($\text{J g}^{-1}\text{ DW h}^{-1}$) (Vinberg 1960; Zhang *et al.* 2016).

Energy absorbed from food

Pāua were fasted for 36 h before the experiment and transferred separately into 4-L chambers with window nets for water exchange and fed with 12 g of fresh seaweed (*Undaria pinnatifida*), which had been cleaned of epiphytes and dried with paper towels. After 24 h, the unconsumed seaweed and faeces were collected separately. Absorption efficiency was evaluated following the method by Conover (1966). The percentage organic content of faeces collected from each of

the Pāua was measured. The faeces were collected by filtering water from a vacuum funnel onto 0.45- μm Whatman GF/C. The glass filter papers were then rinsed with distilled water to eliminate the salts. The material on the glass fibre filters was dried to constant weight for 48 h at 55°C , and then ashed at 450°C for 4.5 h to determine the ash-free dry weight of faeces. The unconsumed seaweed was also dried at 55°C for 48 h before ashing at 450°C for 4.5 h to determine the ash-free dry weight of the food.

Absorption efficiency (%) was calculated with the equation:

$$\text{Absorption efficiency} = \frac{(F - E)}{(1 - E) \times F} \times 100$$

where F is the fraction lost by ashing uneaten food, and E is the fraction lost by ashing the faeces.

Ab was calculated using the equation:

$$Ab = \text{rate of food consumption} \times \text{absorption efficiency} \times \text{calorific value}$$

where rate of food consumption was measured in grams; 1 g dry weight of *Undaria pinnatifida* was equivalent to 2.37 kcal (9916 J) (Lamare and Wing 2001). Calorific values Calories were converted into joules (1 cal = 4.184 J).

Respiration energy

Respiration energy was measured ~ 1 h after the feeding experiment had been completed. Filtered seawater (0.45 μm) was aerated for at least 1 h to achieve 100% oxygen saturation. Pāua were transferred individually to closed-box cylindrical acrylic respirometers. They were left for 30 min in the respirometers containing saturated filtered seawater to allow them to settle before measurements were taken. The small submersible water pumps (model-04301, 378 L h^{-1} , Taiwan) were set up within the respirometers to ensure constant mixing of seawater. An oxygen electrode (FireSting, Pyroscience, Germany) was housed in a rubber bung inserted into a hole in the lid of each respirometer, connected to a Powerlab 4ST device (ADInstruments, Australia) to record data by using LabChart 7 software (ver. 7.3.8, ADInstruments, see <https://www.adinstruments.com/support/downloads/windows/labchart-0>). Before the respirometers were closed, all of the air bubbles were eliminated. Two respirometers were placed in 30-L flow through a water bath to maintain temperatures within $\pm 0.2^{\circ}\text{C}$ of the required setpoint. The dissolved oxygen in the respirometers was kept above 75% to prevent hypoxia from influencing metabolic rates (Harris *et al.* 1999). The amount of oxygen consumed by each Pāua was calculated as MO_2 ($\mu\text{g O}_2\text{ g}^{-1}\text{ h}^{-1}$), and $1\text{ }\mu\text{g O}_2\text{ g}^{-1}\text{ h}^{-1}$ was equivalent to the energy loss of $0.01398\text{ J g}^{-1}\text{ h}^{-1}$ (Elliot and Davison 1975).

$$\text{MO}_2 = \frac{(a \times \Delta\text{PO}_2 \times V) \times 32}{(w \times t)}$$

where a is the oxygen capacitance of water ($\mu\text{mol L}^{-1} \text{ mm}^{-1} \text{ Hg}$) (temperature and salinity dependent), ΔPO_2 is the change in oxygen partial pressure (mm Hg) over a set period time (t , h), V is the volume of the respirometer (L) (accounting for the volume of the Pāua), and w is the weight of the Pāua (g).

Ammonia excretion energy

Ammonia excretion energy was determined parallel in individual respiration chambers. Water samples of ~ 5 mL were collected at the beginning and the end of the period. They were then stored at -80°C until analysis. Ammonia excretion energy was determined using the salicylate method developed by the University of Canterbury. The samples were analysed using a Unicam 8625 UV/Vis spectrophotometer at a wavelength of 650 nm. The ammonia excretion ($\mu\text{g NH}_4\text{-N g}^{-1} \text{ h}^{-1}$) energy of the sample was calculated using:

$$\text{Ammonia excretion} = \frac{AC \times V}{w \times t}$$

where AC is ammonia concentration ($\mu\text{g g}^{-1} \text{ h}^{-1}$), V is the volume of the respirometer (L) (accounting for the volume of the Pāua), and w is the weight of the Pāua (g). $1 \mu\text{g NH}_4\text{-N g}^{-1} \text{ h}^{-1}$ was equivalent to the energy loss of $0.02485 \text{ J g}^{-1} \text{ h}^{-1}$ (Elliot and Davison 1975).

Oxygen and nitrogen ratio

For determination of the O:N ratio, oxygen consumption and ammonia excretion values were converted into their atomic equivalents (Hawkins et al. 2002):

$$\text{O:N ratio} = \frac{\text{MO}_2 \div 16}{AC \div 14}$$

The O:N ratio between 3 and 16 indicates that metabolism is mainly based on proteins. A ratio from 16 to 60 indicates a mixture of protein and lipid substrate (Mayzaud and Conover 1988).

Haemocyanin concentration and haemolymph protein

After measuring the physiological responses, blood samples from Pāua acclimated at 12, 15 and 21°C were collected to measure haemocyanin, haemolymph protein, haemolymph sodium and haemolymph potassium. Approximately 1 mL of haemolymph sample was drawn from the pedal sinus using a 1- or 2-mL syringe and 23-gauge needle. The haemolymph samples were dispensed into 1.5-mL Eppendorf centrifuge tubes, mixed by inversion and centrifuged at 960g for 15 min at 4°C to remove blood cells. The supernatant was transferred into fresh tubes to store frozen at -80°C until analysis. The frozen samples were thawed, mixed and re-centrifuged at 960g for 2 min at 4°C prior to analysis. Pāua were then euthanased by placing them in the freezer before the tissue

and shell were separated and dried to constant weight at 45°C for at least 2 days to calculate the soft dried tissue weight (DW).

The haemocyanin samples were diluted 51-fold in aerated buffer EDTA 10 mmol L^{-1} , pH = 8.8–9, in 1-cm² polystyrene cuvettes. Haemocyanin level was then determined from the absorbance at 346 nm using the Unicam 8625 UV/Vis spectrophotometer with a D2 lamp. Haemocyanin concentrations (Hcy) were calculated using a practical extinction coefficient determined from copper analysis ($E_{\text{mM Hcy}, 1 \text{ cm}} = 22.84$ for millimolar Hcy measured in a 1-cm cuvette) (Behrens et al. 2002; Ragg and Taylor 2006):

$$\text{Hcy} = \text{absorbance @ } 346 \text{ nm} \times 51 \div 22.84$$

Protein concentrations were also estimated by a similar procedure of Hcy from the absorbance at 280 nm. The haemolymph protein samples were diluted in the same buffer of haemocyanin with a dilution factor of 102 in 1-mL quartz micro cuvettes. Protein concentrations were expressed as grams per litre of equivalent bovine serum albumen (BSA) solution, using the extinction coefficient ($E_{1\%, 1 \text{ cm}} = 6.67$ for 1% solutions, i.e. 1 g/100 mL, measured in a 1-cm cuvette) (Sterman and Foster 1956; Wang and Annunziata 2007).

$$\text{Protein concentration} = \text{absorbance @ } 280 \text{ nm} \times 51 \times 10 \div 6.67$$

Ionic concentrations

Haemolymph sodium (Na^+) and potassium (K^+) were determined using a classic flame photometer (model 410, Sherwood, UK). For the sodium measurements, 100 μL of haemolymph was diluted 500 times, whereas the haemolymph was diluted by a dilution factor of 60 for the potassium measurements. The flame photometer for Na^+ was calibrated using solutions of 0.25, 0.50, 0.75, 1.00 and 1.25 mM. For K^+ , the flame photometer was calibrated using solutions of 0.05, 0.10, 0.15, 0.20 and 0.25 mM. These standards were used to determine calibration curves, and subsequent calibration equations, which were applied to calculate the ionic results.

Statistical analysis

The R statistical program was used for all analyses (ver. 2021.09.0, R Foundation for Statistical Computing, Vienna, Austria, see <https://www.r-project.org/>). Two-way ANOVA was used to compare SFG, physiological responses and haemolymph parameters of Pāua between different acclimation temperatures and exposure times. The assumptions of normality and homogeneity of variances were tested for all variables by viewing quantile-quantile normality plots and histograms of the model residuals. If the main effects (acclimation temperature and exposure time) were significant

($P \leq 0.05$), then Tukey's Honest Significant Difference (HSD) *post hoc* test was applied to detect significant differences between treatment means. When a significant interaction between main effects was observed, plots were used to interpret the interaction effects.

Results

The overall, the survival rate in the experiments was 99.99%. Pāua appeared healthy, showing normal clamping and other behaviours.

Absorption efficiency

Acclimation temperature and exposure time affected absorption efficiency (Fig. 1a). Values were generally above 70%, with the exception being for Pāua 3 days after they had been collected from the field in summer (February). There was significant variation at the start of the acclimation period when the absorption efficiency in the 15°C acclimation being remarkably high (96.4%) and that for the 21°C acclimation was very low (53.4%). From day 14 onwards, there were not many differences in the absorption efficiency of Pāua at different acclimation temperatures. Both acclimation

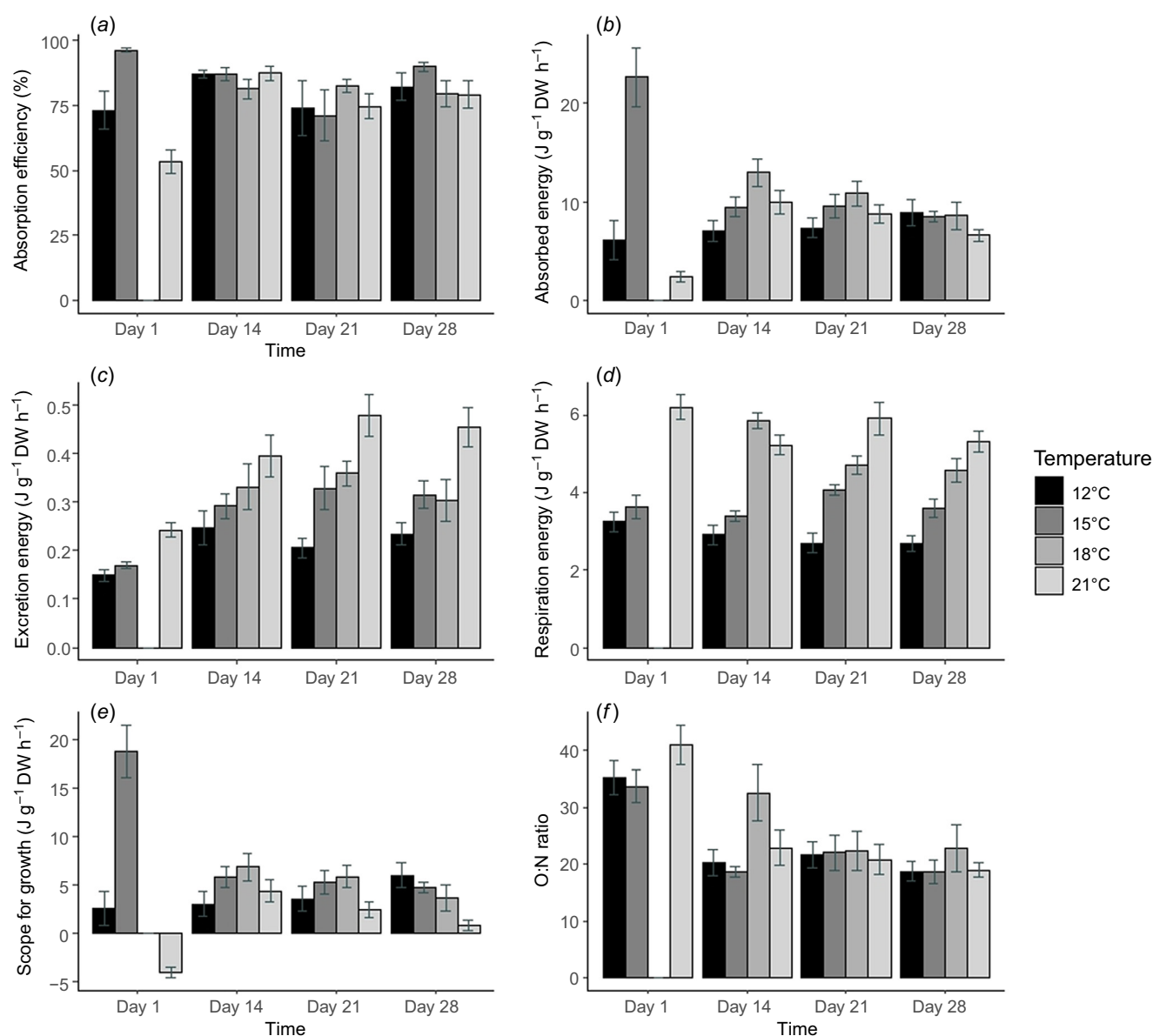


Fig. 1. Mean physiological responses and SFG of Pāua acclimated at 12, 15, 18 and 21°C, measured multiple times over an acclimation period of 28 days for (a) absorption efficiencies, (b) absorbed energy, (c) excretion energy, (d) respiration energy, (e) scope for growth, and (f) N:O ratio. Bars depict the mean and error bars are $\pm$ s.e. from eight replicates per treatment.

temperature and exposure time affected absorption efficiency, and there was a significant interaction between these factors ($P < 0.001$, Table 2).

Energy absorbed from food

The energy absorbed from food ($\text{J g}^{-1} \text{DW h}^{-1}$) varied widely on Day 1, with values in the 15°C acclimation 3.7 and 9.6 times higher than that for the 12 and 21°C acclimation treatments respectively. After 2 weeks of acclimation, the absorbed energy was more stable (Fig. 1b). The energy absorption response depended on both acclimation temperature and exposure time, and there was a significant interaction effect between the two factors ($P < 0.001$, Table 2).

Respiration energy

The energy required for respiration ($\text{J g}^{-1} \text{DW h}^{-1}$) increased with higher temperatures and changed over exposure time. Pāua acclimated to 21°C consumed the highest amount of oxygen at $6.22 \pm 0.90 \text{ J g}^{-1} \text{DW h}^{-1}$, whereas that of 12°C was lowest, at $2.77 \pm 0.67 \text{ J g}^{-1} \text{DW h}^{-1}$ (Fig. 1d). The values accounted for the highest proportion of energy absorbed, increasing with rising acclimation temperatures of ~36, ~40, ~49 and ~69% at 12, 15, 18 and 21°C respectively. The respiration energy was significantly affected by acclimation temperature and exposure time ($P < 0.001$, Table 2).

Ammonia excretion energy

The ammonia excretion energy ($\text{J g}^{-1} \text{DW h}^{-1}$) of Pāua increased from Days 1 to 14 before remaining fairly stable until Day 28 (Fig. 2c). During 4 weeks of acclimation, both

temperature ($P < 0.001$, Table 2) and acclimation time ($P < 0.001$) influenced the ammonia excretion energy significantly, but there was no interaction between them ($P < 0.203$). However, these values only accounted for a small amount of the absorbed energy, ranging from 2.9 to 5.6%.

Scope for growth

The SFG ($\text{J g}^{-1} \text{DW h}^{-1}$) of Pāua varied for the different acclimation temperatures and exposure times (Fig. 1e). On Day 1 after collection from the field in spring, the SFG in the 15°C acclimation was very high ($18.76 \text{ J g}^{-1} \text{DW h}^{-1}$) and Day 1 values for those collected in summer for the 21°C was negative ($-4.10 \text{ J g}^{-1} \text{DW h}^{-1}$). After a 2-week acclimation period, SFG became more stable and the positive SFG values were obtained for Pāua at all acclimation temperatures. The SFG of Pāua acclimated at 21°C was lowest on Day 28. For the 18°C acclimation, SFG was highest on Day 14 and reduced until Day 28. By contrast, the values for 12°C increased during the acclimation period and became highest in all groups on Day 28. The SFG of Pāua acclimated at 15°C remained almost unchanged after 2 weeks' acclimation. During the 4 weeks, the SFG of Pāua was significantly affected by an interaction between acclimation temperature and exposure time ($P < 0.001$, Table 2). The significant interaction was due to considerable variation on Day 1.

O:N ratio

After the 2-week acclimation period, the ratio ranged from 18.7 to 32.5, with the highest value at 18°C. This ratio was generally similar between 21 and 28 days (Fig. 1f). Time but not acclimation temperature significantly affected the

Table 2. Results of two-way ANOVA performed to investigate the effects of 4 week acclimation temperatures and acclimation time for SFG components, O:N ratio and haemolymph characteristics of Pāua.

Variable	Temperature effect			Time effect			Interaction		
	d.f.	F-value	P	d.f.	F-value	P	d.f.	F-value	P
Absorption efficiency	3	4.97	0.003**	3	4.26	0.007**	8	4.64	<0.001***
Ab	3	14.01	<0.001***	3	2.28	0.84	8	11.03	<0.001***
R	3	84.29	<0.001***	3	2.67	0.05*	8	1.56	0.14
U	3	24.59	<0.001***	3	16.42	<0.001***	8	1.41	0.20
SFG	3	20.64	<0.001***	3	1.51	0.22	8	11.59	<0.001***
O:N ratio	3	0.76	0.52	3	24.34	<0.001***	8	1.05	0.401
Haemocyanin	2	8.37	<0.001***	3	1.54	0.21	6	0.90	0.50
Haemolymph protein	2	8.95	<0.001***	3	0.39	0.76	6	0.69	0.66
Haemolymph sodium	2	36.60	<0.001***	3	1.47	0.23	6	1.26	0.28
Haemolymph potassium	2	2.95	0.06	3	1.39	0.25	6	0.33	0.92

SFG equation: $\text{SFG} = \text{Ab} - (\text{R} + \text{U})$, where Ab is the rate of energy absorbed from food obtained in experiments ($\text{J g}^{-1} \text{DW h}^{-1}$), R is the rate of energy lost to respiration obtained in experiments ($\text{J g}^{-1} \text{DW h}^{-1}$), U is the rate of energy lost to ammonia excretion ($\text{J g}^{-1} \text{DW h}^{-1}$). Values shown in bold and with an asterisk indicate that they are statistically significant (*, $P \leq 0.05$; **, $P < 0.01$; and ***, $P < 0.001$).

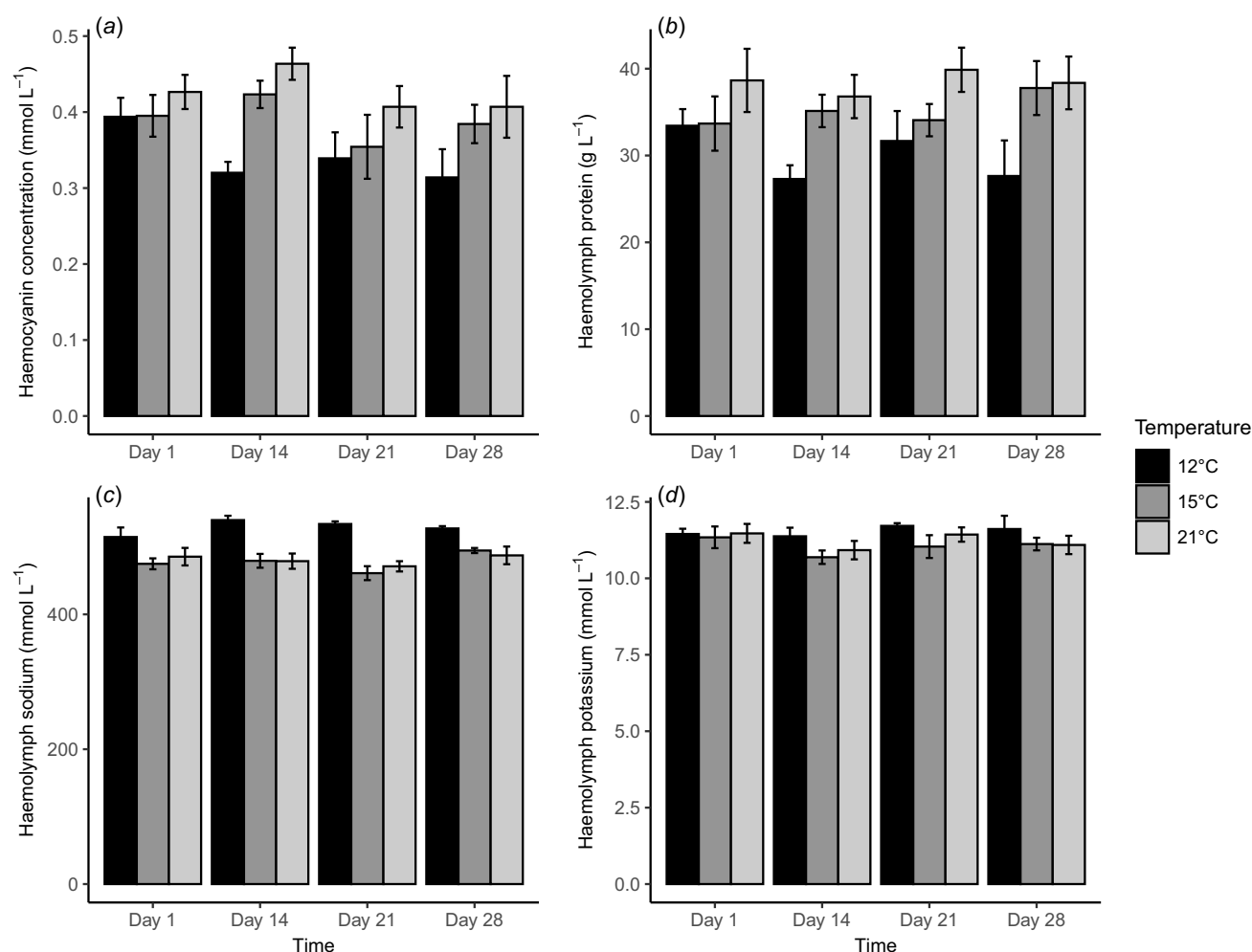


Fig. 2. Haemolymph responses of Pāua acclimated at 12, 15 and 21°C, measured multiple times over an acclimation period of 28 days for (a) haemocyanin concentration, (b) haemolymph protein, (c) haemolymph sodium and (d) haemolymph potassium. Bars depict the mean and error bars are $\pm$ s.e. from eight replicates per treatment.

O:N ratio ($P < 0.001$, Table 2). The values on Day 1 were significantly higher than those for Days 14, 21 and 28 (Tukey's HSD test, $P < 0.001$). All other groups were similar (Tukey's HSD test, $P > 0.05$).

Haemocyanin concentration and haemolymph protein

Acclimation temperature and exposure time had similar effects on haemocyanin concentration (mmol L^{-1}) and haemolymph protein (g L^{-1}). They all increased with rising acclimation temperatures, with the highest at 21°C and the lowest at 12°C (Fig. 2a, b). They were affected significantly by acclimation temperature ($P < 0.001$, Table 2) but not by exposure time ($P > 0.05$). Also, there was no interaction between the two factors. Haemocyanin concentration at 12°C was significantly lower than 21°C (Tukey's HSD test, $P < 0.001$). By contrast, haemolymph protein at 12°C was significantly lower than at 15 and 21°C (Tukey's HSD test, $P < 0.05$).

Haemolymph sodium and potassium

Both haemolymph sodium (mmol L^{-1}) and potassium (mmol L^{-1}) were highest at 12°C (Fig. 2c, d). Temperature had a significant effect on the haemolymph sodium ($P < 0.001$, Table 1), but acclimation time did not ($P > 0.001$). There was no interaction between the two factors. The levels for Pāua acclimated at 12°C were significantly higher than at other temperatures (Tukey's HSD test, $P < 0.001$). However, neither temperature nor time affected haemolymph potassium ($P > 0.05$, Table 2).

Discussion

The energy relationships of New Zealand Pāua were highly variable for individuals collected from the field and acclimated to four constant temperatures between 12 and 21°C for 28 days. Like other molluscs living in the shallow coastal

environment, Pāua are exposed to daily, tidal and seasonal temperature variations and these most likely affect their growth and reproduction. Other variables likely to affect these changes include the availability and quality of food, space requirements and water quality including oxygen levels. Temperature, however, remains a key factor directly and indirectly affecting growth in haliotids (González et al. 2002; Vosloo et al. 2013; Hooper et al. 2014; Kang et al. 2019).

Seasonal physiological responses of field-collected Pāua

It is well known that the physiology of molluscs can show adaptations to seasonal changes in their environment and these results in seasonal differences in reproduction, fecundity and growth (Vilchis et al. 2005; Shin et al. 2020; Oliveira et al. 2021). Such seasonal adjustments have been shown in metabolism, oxygen uptake, ammonia excretion and digestive enzymes (Bayne and Newell 1983). In such studies, the main factors thought to affect these changes include fluctuating or constant exposure temperature, high temperatures or food availability or quality. In the present study, seasonally collected Pāua maintained for 1 day at a constant temperature close to their field temperature had variable feeding, respiration and excretion rates. Pāua collected in spring had the highest energy intake and those collected in summer had the highest energy loss due to respiration. Calculations for the SFG of these Pāua were low for winter collected individuals, highest for spring collected individuals and negative for summer collected individuals. Negative values for SFG have been recorded for other haliotids in other studies (Vosloo and Vosloo 2010). For Pāua these values most likely reflect the field conditions where, during spring, high food availability and temperature conditions close to 15°C are most likely optimal for metabolism, gonad development and spawning. High temperatures over summer are consistent with higher oxygen demands and limited food intake most likely resulting in impaired SFG. If summer collected individuals are not in good physiological health then they may not adapt quickly to laboratory conditions that are two degrees above the seawater temperature at the time of collection.

Absorbed energy and absorption efficiency

For field collected haliotids, absorption efficiencies usually exceed 50% depending on food types (Peck et al. 1987) but in laboratory experiments or in aquaculture, where a single favoured algal food is supplied, the expectation is that absorption efficiency would be higher (Bansemer et al. 2016). In the present study after 2 weeks of acclimation, absorption efficiencies for Pāua were similar to other studies. For example, values for the European abalone *H. tuberculata* were between 78 and 81% (Peck et al. 1987), for the green abalone, *H. fulgens* 84.9% (Farías et al. 2003) and for Thai abalone *H. asinina*,

22–42 mm in length, the average absorption efficiency was 81.04% (Ganmanee et al. 2010).

The energy obtained by grazers from their algal food depends upon its availability, its nutritional value, the feeding rate and the ability of the animal to digest it (Barkai and Griffiths 1987). All of these variables showed variability in the field and linked directly or indirectly with temperature change. The grazing rate of molluscs generally increases with exposure temperature up to a certain limit that is regarded as the optimal temperature. It then declines as in *H. midae* (Britz et al. 1997). The optimum temperature differs amongst haliotid species depending on species and habitat characteristics. In summer, for *H. tuberculata*, the amount of energy absorbed from food was greater than *H. fulgens* but the opposite was found in winter. In the present study, after 2 weeks of acclimation to constant temperatures absorbed energy increased with exposure temperature up to 18°C but decreased in the 21°C acclimation. Following a further 2 weeks of acclimation, the values were broadly similar for all acclimation temperatures. Over the 28-day time period, Pāua acclimated to 12°C had the most stable energy uptake generally increasing over time.

Respiration and ammonia excretion energies

Consistent with numerous other studies on molluscs, the energy consumed in respiration and ammonia excretion energies of Pāua increased markedly with acclimation temperature (McBride et al. 2001; Kang et al. 2019) and also varied over time. The two factors acted independently and values in the current study were generally higher than those from other haliotids. These differences may be due to body size or the exposure temperatures investigated. For *H. tuberculata*, Lopez and Tyler (2006) found that energy lost due to respiration and ammonia excretion increased greatly with the temperature from 15 to 22°C. They also found that respiration energy depended on size, accounting for 31% of the energy intake for the 10–20-mm length group, and 50.7% for the 50–60-mm group. By contrast, ammonia excretion comprised 0.9% of energy intake for the small up to 3.5% for the larger group.

Barkai and Griffiths (1988) reported that less than 1% of the energy uptake of the South African haliotid (*H. midae*) was lost by ammonia excretion and ~32% of energy was spent on respiration. Similarly, for Thai abalone *H. asinina* between 22 and 42 mm, more than 30% of the energy obtained was used for metabolism and from 0.8 to 1.8% spent for ammonia excretion (Ganmanee et al. 2010). In the present study, energy lost by respiration was 36% of intake for animals kept at 12°C, and this increased to over 40% at the higher temperatures of 15 and 18°C. At the highest temperature (21°C) energy lost through respiration was 69%, indicating that this temperature is well above optimal for this species.

For adults of the South African *H. midae*, Vosloo and Vosloo (2010), acclimation of cold-acclimated individuals to 16, 19

and 22°C for 1 month decreased both their specific oxygen consumption and nitrogen excretion at higher temperatures. This ability to adapt was not the same response recorded for oxygen uptake in Pāua acclimated between 12 and 21°C over a period of 28 days. By contrast, the oxygen uptake of Pāua acclimated to 21°C remained high throughout the acclimation period, confirming the limited ability to adapt to this higher temperature regime.

The O:N ratio is useful in determining the energy sources used in the metabolism of marine ectotherms. In the present study the O:N ratio for field-collected Pāua was between 33 and 41, values similar to *H. fulgens*, with an average value of 21.6 and 47.0 for large individuals and smaller individuals respectively (Farías *et al.* 2003). For Pāua, values decreased after 2 weeks of acclimation then remained at levels close to 20 for the following 14 days. Generally, the O:N ratio of Pāua was lower than other haliotids. The O:N ratio of *H. discus hannai* juveniles was higher than *H. iris*, ranging from 32.1 ± 1.4 to 38.1 ± 1.8 , with a mean of 34.3 ± 0.3 (González *et al.* 2010). These findings suggest that, in the field, Pāua use both proteins and lipids to produce energy and that during the stable 28-day acclimation period, there was a shift towards utilisation of protein.

Scope for growth

Scope for growth (SFG), the difference between energy gains and losses and the energy available for shell, somatic and reproductive growth, has been used as a predictor of actual growth in many molluscs including bivalves (Widdows and Johnson 1988) but the relationship is less well known for haliotids. For Pāua both acclimation temperature and exposure time had interactive effects on the SFG.

Although Pāua acclimated at 12, 15, 18 and 21°C all had a positive SFG following 2 weeks of acclimation to constant temperatures, the value decreased considerably following 28 days of acclimation at 21°C. This is most likely due to the increased demands of oxygen to maintain metabolism at this temperature (Guppy and Withers 1999; Shao *et al.* 2015). In the 21°C acclimation, there was a steady decline over the acclimation period indicating that this acclimation temperature is above the optimal level. The positive values for SFG for the 12, 15 and 18°C acclimations suggest these haliotids could grow and mature at these lower seawater temperatures in New Zealand.

SFG values recorded previously for abalone are both higher and lower than those recorded here for Pāua (Ganmanee *et al.* 2010; González *et al.* 2010). Some of the differences can be explained by exposure temperature and others on body size; for example for *H. asinina*, feeding on an artificial diet at 28°C, the SFG increased with size for three size classes between 22- and 42-mm length (approximately 2–25 g) giving SFG estimates between 2.9 and 5.7 J g⁻¹ DW h⁻¹. SFG dependence on size was also found in juvenile green abalone *H. fulgens*, ranging from 99.8 to 173.7 J abalone⁻¹ day⁻¹ for

abalone between 10 and 50 mm at 16°C, using a diet formulation (Farías *et al.* 2003). Also, the SFG of juvenile European abalone *H. tuberculata* was 33.9 cal g⁻¹ DW day⁻¹ (~5.91 J g⁻¹ DW h⁻¹) at 15°C when fed on *Ulva lactuca* (Peck *et al.* 1987) was a little lower than was found in the current research. The effects of temperature on the SFG have also been investigated in some other species including other molluscs and crustaceans (Yurista 1999; Navarro *et al.* 2002). These results were consistent with those for Pāua, where, within the optimal temperature range, SFG reached the highest values.

Haemolymph properties

Haemolymph is the circulatory fluid in molluscs transporting oxygen, nutrients, hormones, proteins and immune cells. It contains haemocyanin that accounts for a large proportion of haemolymph proteins (Fredrick and Ravichandran 2012). Both haemolymph haemocyanin and protein levels of Pāua showed a similar response after 14 days of acclimation, with levels increasing with higher acclimation temperatures from 12 to 21°C. The elevation of haemocyanin levels is consistent with the ability of Pāua to maintain high levels of oxygen uptake at temperatures above 20°C. This ability, however, may not be present in juveniles as Wells *et al.* (1998) reported that aerobic metabolism is limited above 20°C owing to a limitation of their ability for oxygen storage in haemocyanin. These results contrast with research on the marsh isopod *Saduria entomon* where the haemocyanin concentration remained almost unchanged with increasing temperatures (Borecka *et al.* 2016).

Total blood protein has been applied as a health status indicator in marine invertebrates (Paschke *et al.* 2010) and when haemocyanin levels increase due to increased temperatures, haemolymph proteins are also expected to increase. Studies on the effects of heat stress on juvenile haliotids (hybrid *Haliotis laevis* × *Haliotis rubra*) found that haemolymph protein was not significantly affected by increasing temperature from 16 to 26°C after 1 week of acclimation (Hooper *et al.* 2014). These results suggest that protein regulation in haliotids may differ from other invertebrates.

For Pāua, haemolymph sodium was affected by temperature with the highest values at the lowest acclimation temperature of 12°C. However, haemolymph potassium was similar for Pāua acclimated between 12 and 21°C. Day *et al.* (2010) found that both haemolymph sodium and potassium of an Australian haliotid increased as exposure temperature decreased. In contrast, in another study on farmed Australian haliotids (hybrid *Haliotis laevis* × *H. rubra*), Hooper *et al.* (2014), found that haemolymph protein and haemolymph sodium were unaffected by increasing temperature from 16 to 26°C after 1 week of acclimation. The results of that study are similar to the results on the oyster drill (*Thais haenastonzia*) where Hildreth and Stickle (1980) reported

that temperature did not affect haemolymph sodium and potassium of this species.

Ecological context

Like all molluscs, Pāua show seasonal adaptations in their physiology as demonstrated by their patterns of growth and reproduction (Hooker et al. 1997). This is consistent with other haliotids, including those in aquaculture that also show adaptations to multiple stressors including hypoxia, air exposure and elevated temperatures (Rogers-Bennett et al. 2010; Ragg and Watts 2015). Previous research on oxygen uptake in haliotids suggests that the long and short-term responses to elevated temperatures are both species and location specific (Hooper et al. 2014). It also depends on the exposure temperature and whether this is within its normal environmental range.

The laboratory acclimation responses of Pāua are consistent with their seasonal responses and geographical distribution. Because Pāua cannot significantly reduce their energy expenditure due to respiration with increasing temperature they are therefore vulnerable to long periods of seawater temperatures above 18°C. This could induce an energy imbalance at high temperatures, in which the metabolic energy expenses of Pāua exceed their energy gain. In turn, this could affect their growth and reproduction. As a result, Pāua will be likely vulnerable to increasing global warming and marine heatwaves (Morash and Alter 2016; Markle and Kozak 2018).

Some haliotid species appear better able than others to adapt to changing environmental temperatures (Kang et al. 2019). For example, Alter et al. (2017) found that *H. laevigata* and *H. laevigata* × *H. rubra* hybrids have thermal acclimation capacity, but *H. rubra* do not. In other studies, Barkai and Griffiths (1987), investigated *H. midae* in South Africa and found that the respiration rate of abalone in the field was highly affected by temperature and did not have an acclimatory adjustment of its metabolism, which rose significantly during the summer. However, studies on the same haliotid suggested this species reduced the specific oxygen consumption and nitrogen excretion as temperature increased (Vosloo and Vosloo 2010). In a later study, Vosloo et al. (2013) suggest that 19°C is the temperature acclimation limit for winter-acclimated *H. midae* and that above this temperature energy is reallocated to survival mechanisms instead of growth. It follows then that adult Pāua show similar thermal acclimatory responses to juvenile *H. midae* from South Africa.

The inability of Pāua to reduce energy expenditure at seawater temperatures of 21°C suggests that, in the event of future seawater warming, there is likely to be a southern shift in the distributions to more favourable temperatures. Throughout New Zealand, however, Pāua growth differs with seawater temperatures and on small scales with higher growth on promontories than within bays (Naylor and Andrew 2004; Haist 2018). In addition, ocean warming and marine

heatwaves are major threats to marine biodiversity, including effects on primary productivity and kelp forest, which provide food for Pāua (Arafeh-Dalmau et al. 2019; Rogers-Bennett and Catton 2019; Thomsen et al. 2019; Rogers-Bennett et al. 2021). Thus, there may be direct and indirect effects of climate change. The present study highlighted the likely inability of adult Pāua to survive long-term exposure to increased seawater temperatures ~21°C and this vulnerability confirms research by Aalto et al. (2020), which has suggested that, in haliotids, adults are the life stage that is most sensitive to environmental stress. In the current study, if Pāua return back to lower temperatures after a short exposure to 21°C, they may get to recover quickly. This is because they are acclimatised at lower temperature. Therefore, in aquaculture systems for Pāua, adults most likely should be managed below 21°C. Also, using high quality foods or varied food sources may help Pāua to survive and grow at elevated temperatures.

Pāua in this study were maintained under the same laboratory conditions with high water quality and enough food. However, space may have been limited in the experimental tanks. Handling could also have resulted in variations of in the physiology of Pāua in this study. Future studies should explore these factors in combination with temperature effects affecting the SFG of Pāua.

In conclusion, the findings from the present study suggest that in the future, if warming seawater temperatures due to climate change result in exceeding 21°C for a long period, this could act as a severe stress to adult Pāua. The results confirm that energy relationships are affected by acclimation temperature and time. At 21°C, energy expenditure exceeded energy uptake suggesting that there would be insufficient energy for growth. Because growth is affected by other factors, then similar studies need to be undertaken using combinations of stressors including reduced oxygen levels and salinity. It would also be useful to examine the responses of juvenile Pāua living in low intertidal habitats with more variable temperature extremes and different food characteristics.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of interest. The authors declare that they have no conflicts of interest.

Declaration of funding. This project was supported by Brian Mason Trust Grant. The project is part of a PhD thesis, funded by a Manaaki New Zealand Scholarship awarded to Thuy Thi Nguyen under the supervision of John Pirker, Islay Marsden and William Davison. The funding bodies and project partners had no role in the design, data collection, analysis or interpretation of data.

Acknowledgements. The authors are thankful for the field support of Renny Bishop, for the technical laboratory support of Johnathan Hill and Jan McKenzie.

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