Comparative Biochemistry and Physiology, Part A 212 (2017) 72–80

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Behavioural, physiological and biochemical responses to aquatic hypoxia in the freshwater crayfish, *Paranephrops zealandicus*

Rebecca J. Broughton[a], Islay D. Marsden[a], Jonathan V. Hill[a], Chris N. Glover[b,c,*]

[a] Biological Sciences, University of Canterbury, Christchurch, New Zealand
[b] Faculty of Science and Technology and Athabasca River Basin Research Institute, Athabasca University, Athabasca, Alberta, Canada
[c] Department of Biological Sciences, University of Alberta, Edmonton, Canada

ARTICLE INFO

Keywords:
Acid-base regulation
Anaerobic metabolism
Crustacean
Decapod
Emersion
Eutrophication
Freshwater crayfish
Hypoxia
Metabolic rate
Respirometry

ABSTRACT

Hypoxia resulting from aquatic eutrophication threatens the population health of the New Zealand freshwater crayfish (koura), *Paranephrops zealandicus*. An integrated study, combining behavioural, physiological and biochemical approaches, was therefore conducted to characterise the tolerance of this species to hypoxia. When provided with a choice between water flows of high or low dissolved oxygen in short-term laboratory assays, crayfish did not preferentially inhabit waters of higher PO_2. However, when an aerial refuge was provided and dissolved oxygen was progressively decreased, crayfish emersed at a PO_2 of 0.56 ± 0.03 kPa, suggesting a relatively high tolerance to hypoxia. Closed-box respirometry delineated a P_{crit}, the point at which crayfish transition from oxyregulating to oxyconforming, of 6.0 kPa. Simultaneous measurement of heart rate showed no changes across the PO_2 range. In response to 6-h exposures to fixed dissolved oxygen levels (normoxia, 19.3 kPa; moderate hypoxia, 3.5 kPa; and severe hypoxia, 1.7 kPa), *P. zealandicus* showed a haemolymph PO_2 that declined with the magnitude of hypoxia, and while plasma pH declined in severe hypoxia, there were no changes in plasma PCO_2. Plasma glucose concentrations fell, and plasma lactate increased in both hypoxic groups. There were no changes in tissue glucose or lactate concentrations. These data indicate that *P. zealandicus* is relatively tolerant of hypoxia, and possesses biochemical and physiological mechanisms that facilitate survival during short-term exposures to acute hypoxia. If hypoxia is severe and/or prolonged, then this species is capable of escaping to aerial refugia.

1. Introduction

Hypoxia is an increasingly prevalent threat to the integrity of aquatic systems worldwide (Smith, 2003). This is particularly true for lowland streams impacted by agricultural nutrient run-off. The introduction of nitrogen and phosphorus into these waters promotes growth of aquatic plants and algae, leading to strong diel cycles of photosynthesis and respiration (McDowell et al., 2009). This can result in periods of extreme hypoxia, or even anoxia, in these water bodies. For example, in near-coastal streams of the Canterbury region of New Zealand, oxygen partial pressure (PO_2) values of 3 kPa have been recorded (Urbina and Glover, 2012), while some eutrophic lakes in this area undergo extensive periods of complete anoxia (Fisher, 2011). The presence of low dissolved oxygen, in an otherwise ideal habitat, is likely to impact the ecology of local fauna, such as the freshwater crayfish, *Paranephrops zealandicus*.

Known locally as koura, *P. zealandicus* is one of two endemic freshwater crayfish species found in New Zealand. While *P. zealandicus* is distributed mainly in the agriculturally-intensive regions of the eastern and southern South Island, *P. planifrons* has a more northern distribution (Hopkins, 1970). These fossorial species perform vital ecological roles as bioturbators and predators, and therefore have a significant influence over the habitat distribution of other invertebrates (Usio and Townsend, 2004). They also serve as an important food source, for both fish and human populations (Hicks, 1997; Kusabs and Quinn, 2009), and consequently they are of significant cultural value. Critically, this species is considered to be “at risk”, with populations predicted to decline (Grainger et al., 2014).

One driver for the predicted population decline is eutrophication of freshwaters. In the sister species, *P. planifrons*, it has been shown that crayfish populations are excluded from deoxygenated zones in eutrophic lakes of New Zealand's Central North Island region (Kusabs et al., 2015). In fact, although *P. planifrons* are found in tributaries of Lake Okaro, they are totally excluded from the highly eutrophic lake itself, likely owing to prolonged and extensive periods of deoxygenation (Kusabs et al., 2015). A similar distribution pattern, wherein crayfish

* Corresponding author at: Faculty of Science and Technology, Athabasca University, 1 University Drive, Athabasca, Alberta T9S 3A3, Canada.
E-mail address: cglover@athabascau.ca (C.N. Glover).

http://dx.doi.org/10.1016/j.cbpa.2017.07.013
Received 11 May 2017; Received in revised form 21 July 2017; Accepted 21 July 2017
Available online 27 July 2017

are present in the tributaries but excluded from the main water body, is observed for *P. zealandicus* in the highly eutrophic, and often anoxic, Lake Ellesmere/Te Waihora (Environment Canterbury, 2012). These observations suggest that dissolved oxygen has an important role in shaping crayfish distributions. This is further supported by laboratory studies, where it has been shown that a PO_2 of 0.77 mg L^{-1} (~1.6 kPa) causes 50% mortality over 48 h in *P. planifrons* (Landman et al., 2005), a value consistent with field observations of *P. planifrons* distribution. However, to date, little is known regarding the sensitivity of *P. zealandicus* to hypoxia.

In other freshwater crayfish species, a number of behavioural, physiological and biochemical mechanisms are enacted in response to low dissolved oxygen. For example, freshwater crayfish exposed to natural or experimental hypoxia may emerse to escape aquatic hypoxia (e.g. Taylor and Wheatly, 1980; McMahon and Wilkes, 1983). However, given the risk of desiccation and predation, leaving hypoxic water is likely to be a last resort (Taylor et al., 1987). Instead, a suite of physiological strategies and compensatory mechanisms may be employed to cope with hypoxia (Mauro and Thompson, 1984; Reiber, 1995; Morris and Callaghan, 1998; Reiber and McMahon, 1998; da Silva-Castiglioni et al., 2010). These include up-regulating oxygen delivery, and/or reducing oxygen demands (Reiber, 1995; Gorr et al., 2010). Cardiac responses to hypoxia include bradycardia, which is compensated by an increase in cardiac stroke volume. This allows optimal perfusion of the respiratory system and the tissues (Reiber and McMahon, 1998). As hypoxia persists, biochemical mechanisms may then be enacted. The most prominent of these is a switch to anaerobic metabolism, whereby energy is derived in the absence of oxygen, allowing for maintenance of biological functions, but at the cost of lactate build-up (Mauro and Thompson, 1984).

The tolerance of a given species to hypoxia will depend on the presence and magnitude of behavioural, physiological and biochemical coping strategies. One indicator of hypoxia tolerance is the lowest PO_2 at which the organism can maintain oxygen consumption (P_{crit}; Reiber, 1995). Similarly, at a biochemical level, measures of energetic substrate (e.g. glucose, arginine phosphate), anaerobic metabolism (e.g. lactate), and how a species deals with the acidosis associated with anaerobiosis (e.g. plasma pH), may all be indicative of effective strategies for hypoxia tolerance (Mauro and Thompson, 1984; Morris and Callaghan, 1998).

The current study integrated behavioural, physiological and biochemical measures to determine the tolerance of the freshwater crayfish *P. zealandicus* to hypoxia. Initial studies examined the ability of this species to seek aquatic and aerial refugia in response to aquatic hypoxia. Subsequently, physiological measures of respiratory (oxygen consumption rate, MO_2; P_{crit}) and cardiac (heart rate) responses to progressive hypoxia were determined via closed-box respirometry. Finally, crayfish were subjected to 6-h exposures to fixed PO_2 (normoxia, 21 kPa; moderate hypoxia, 4 kPa; severe hypoxia, 1 kPa) to determine the impact on haemolymph (PO_2, PCO_2, pH, glucose, lactate) and tissue (glucose, lactate) biochemistry. This study provided novel insight into the biological responses, and tolerance, of *P. zealandicus* to aquatic hypoxia, and as such will contribute to our understanding of the importance of eutrophication in freshwater crayfish ecology.

2. Materials and methods

2.1. Animals

Adult male *P. zealandicus* were obtained from a commercial supplier, and held in a recirculating freshwater aquarium system at the School of Biological Sciences, University of Canterbury. Crayfish were maintained at 15 ± 2 °C under a 12 h:12 h photoperiod for at least a week prior to experimentation, in vigorously aerated 500-L tanks provided with plastic tubing and artificial foliage for shelter. Animals were fed twice weekly on Nutrafin fish pellets but were fasted for 2 days prior to experimentation. Only intermoult animals were used for experiments, and animals with damaged chelipeds or walking legs were excluded. All animal procedures were approved by the University of Canterbury Animal Ethics Committee.

2.2. Behaviour: aquatic PO_2 preference

The ability of *P. zealandicus* to choose between two water flows differing in PO_2 was assessed in a hypoxic choice chamber, similar to that used previously for fish (Cook et al., 2011). The Perspex chamber (100 cm long × 50 cm wide × 30 cm deep) was gravity-fed with aquarium water from two header tanks, with flow rate controlled by taps. The PO_2 of each half of the behavioural arena was independently regulated by bubbling nitrogen gas and/or air, and monitored via a dissolved oxygen probe (Hach). Water flowed from each header tank into the anterior of the chamber, where PO_2 was adjusted, and then subsequently through honeycomb diffusers (plastic straws stacked in a Perspex chamber). These flows were separated by a Perspex partition. Thereafter, the water entered the behavioural arena (50 cm long × 50 cm wide × 30 cm deep) in two separate rectilinear flows that differed in their PO_2. There was no partition in the arena, allowing crayfish placed here to move freely between the two streams of water. Preliminary experiments using dyes showed that a flow rate of 5.9 L min^{-1} ensured minimal mixing of water flows through the arena. Water exited the chamber through posteriorly-located outflow valves, and was not recycled to avoid the presence of chemical cues influencing behavioural responses. Floating polystyrene blocks were fitted into the top of the anterior chamber to reduce gas exchange between the water and air interface, allowing PO_2 to be maintained. A video camera was placed on a tripod over the behavioural arena, which streamed video into a laptop computer running Microsoft Movie Maker for continuous recording of behaviour.

Crayfish were acclimated to the choice chamber 12 h prior to testing. During this period, water flow was reduced (~3 L min^{-1}), and air bubblers were placed in the anterior of each side of the chamber to ensure normoxia. Previous studies in fish have shown that hypoxia avoidance responses can depend on the absolute and relative differences in the chosen PO_2 pairs (Herbert et al., 2011). Therefore, several paired PO_2 combinations were tested (in kPa): 21 (normoxia) vs. 12, 21 vs. 8, 21 vs. 4, 12 vs. 8, 12 vs. 4 and 8 vs. 4. Each individual crayfish (n = 8; mean mass ± standard error of the mean = 39 ± 3 g) was exposed to all 6 protocols in one day. The protocol order was randomised and no crayfish was exposed in the same order. Furthermore, the side of the chamber that received the highest PO_2 was also randomly varied.

For each experimental PO_2, preliminary trials were performed to determine the rate of nitrogen gas bubbling of the header tanks required to reach the target PO_2 values. At the start of the experiment, and then 30 min following initiation of the trial, PO_2 values were checked with a dissolved oxygen probe (Hach), and gas flows adjusted as necessary. To initiate the trial, the normoxic water flow and aeration of the chambers ceased, and flows from the header tanks were started. Each paired PO_2 combination was tested for 2 h (with equilibration of the choice chamber water to target PO_2 for 1 h, followed by a 1 h testing period). Cook et al. (2013) found that a 1 h testing period was long enough for fish to show behavioural preferences, but sufficiently short that they did not acclimate to hypoxia. The percentage of time that a crayfish (or more than half of the body) spent on each side of the chamber was recorded over the last 60 min of the trial. After 2 h, the water PO_2 levels were again measured, the tank was flushed with normoxic water, and the next tested PO_2 combination was initiated. All 6 PO_2 pairs were tested in a single crayfish in a single day.

2.3. Behaviour: emergence

Crayfish (n = 21; mean mass = 39 ± 3, with no significant

difference in mass between groups) were assessed for the emersion behaviour in response to hypoxic water using a custom-built Perspex chamber (40 cm long × 15 cm wide × 30 cm deep). The chamber was shaded with black plastic on the sides, and plastic also covered one corner of the chamber, serving as a shelter. At the opposite end of the chamber from the sheltered corner, a Perspex ramp led up to an aerially-exposed platform. A video camera was connected to a computer, and was attached to a tripod placed over the tank.

To determine responses to a progressively-declining water PO_2, individual crayfish ($n = 8$) were first acclimated for 12 h to the chamber, with aeration provided by a bubbler. After acclimation, the aeration was changed to a nitrogen gas flow, increasing by 2 L per minute every 5 min. As the rate of nitrogen gas flow increased, the gas tank and the gas itself cooled, resulting in a decrease in water temperature from an initial value of 15 °C to 10 °C. Water PO_2 was monitored every minute using an oxygen probe which was left in the chamber for the entirety of the experiment. This recorded an exponential decay in chamber PO_2 ($y = 25e^{-0.19x}$), such that PO_2 values of 3.3, 0.9 and 0.5 kPa were achieved at approximately 10, 30 and 60 min, from a starting normoxic value of 21 kPa at time 0. Crayfish were monitored through the live feed from the video camera, and were undisturbed for the length of the experiment (~60 min). The water PO_2, and time at which the crayfish initiated emergence, were recorded. Emergence was defined as the movement of the crayfish completely out of the water for > 4 min.

To further explore emergence responses to hypoxia, individual crayfish ($n = 5$) were transferred directly into the emergence chamber where water PO_2 level was maintained at 0.44 kPa (the approximate average PO_2 of emergence in the progressively declining hypoxia experiment). The time at which they emerged from this extreme hypoxia was recorded. A separate group of individual crayfish ($n = 8$) were subjected to a similar protocol, where water PO_2 was maintained at the same dissolved oxygen level as that achieved during the progressive hypoxia experiment after 30 min (1.0 kPa). Again, the time at which crayfish emerged was recorded.

2.4. Physiology: oxygen consumption rate (MO_2) and heart rate

The effect of progressively-decreasing dissolved oxygen on *P. zealandicus* MO_2 was tested via closed-box respirometry. Concurrently, some of these animals were also fitted with heart rate sensors. Individual crayfish (mean mass = 31 ± 1, $n = 26$ for MO_2, $n = 17$ for heart rate), were placed in Perspex respirometers (volume 450 mL), contained within a temperature controlled water bath (15 °C), 12 h prior to any measurements being performed. A continuous flow of aerated aquarium water was flushed through the chambers over this period.

To monitor heart rate, a non-invasive infrared sensor was placed in a rubber ring that had been glued the carapace of the crayfish at the rear of the cephalothorax, directly above the heart, at least 24 h prior to introduction to the respirometry chamber. This sensor measures conformational changes in the heart which alters the intensity of light being received by a photodetector (Depledge and Andersen, 1990). Changes in light intensity were converted to changes in voltage which were fed into a PowerLab 4/25 unit (ADInstruments), via a custom-made signal conditioning box. Cardiac pulses were visually identified and recorded using Lab Chart 7 (ADInstruments). The heart rate sensors were fed through the lids of the respirometers via an opening, and then sealed with a rubber bung and glue. Heart rate was continuously measured for the duration of the exposure.

To commence MO_2 measurement, the respirometers were sealed, allowing the animals to gradually deplete the oxygen in the chamber. Thereafter, water PO_2 was sampled every 20 min, by extracting a 1-mL water sample from sampling ports using a gas-tight syringe. Removed water was replaced by opening the valve of a sample reservoir attached to the respirometer. To determine water PO_2, the water samples were injected into a MC100 oxygen microcell (Strathkelvin Instruments) which contained an IL1302 oxygen electrode. Attached to the electrode was a 781 oxygen meter (Strathkelvin Instruments) from which water PO_2 was recorded. The oxygen meter was calibrated (using air-saturated freshwater and correcting for atmospheric pressure) and zeroed (using a solution of 0.01 M of sodium tetraborate + sodium sulfite) before every experiment. The following equation was used to calculate MO_2:

$$MO_2(\mu\text{mol g}^{-1}\text{h}^{-1}) = \frac{\Delta PO_2 \times C \times V}{W \times t} \quad (1)$$

where $\triangle PO_2$ is the change in oxygen partial pressure (kPa), C is the oxygen capacitance of water (μmol L^{-1} kPa^{-1}), V is the volume of the respirometer (L), W is the crayfish mass (g), and t is the time over which MO_2 was calculated (h). Crayfish were removed from the respirometers once PO_2 fell below 1.3 kPa, or after 6 h, whichever occurred first. Measurements of MO_2 from a blank respirometer were used to determine microbial contributions to oxygen consumption, which were undetectable over the course of the experiments.

2.5. Biochemistry: exposure

Freshwater crayfish (mean mass = 43 ± 1 with no significant differences in mass between experimental treatments; $n = 6$–8) were subjected to closed-box respirometry, under a set-up and acclimation protocol that were identical to those described above. However, at the start of the experiment the water in the respirometers was replaced with aquarium water from one of three reservoirs: normoxic (21 kPa), moderately hypoxic (4 kPa) or severely hypoxic (1 kPa), achieved by air or nitrogen gas bubbling. Every 20 min the respirometers were opened, a water sample taken to check PO_2, and the chamber was flushed with fresh water at the target dissolved oxygen level, before the respirometer was resealed. This continued for 6 h, after which crayfish were removed and haemolymph was collected from the base of a walking leg using a gas-tight syringe for analysis of PO_2, PCO_2, pH, lactate and glucose. The crayfish were then immediately anaesthetised by placing them on ice, and euthanised by severing the ventral ganglion. The tail muscle was dissected for analysis of lactate and glucose, snap-frozen, and stored at − 80 °C.

2.6. Biochemistry: analysis

Collected haemolymph (~1–2 mL) was split between three 1 mL tubes. One tube was immediately used to determine haemolymph PO_2, which was assessed using a dissolved oxygen probe and oxygen meter as described above for water PO_2. The two remaining tubes were centrifuged at 5000 × *g* for 20–30 s. The centrifuged plasma was aspirated into two new tubes. One plasma sample was snap-frozen in liquid nitrogen, before being stored at − 80 °C. Once thawed these samples were assayed for glucose and lactate, as described below for tissue supernatants. The second sample was used for pH and PCO_2 analysis, with pH measured directly using a microprobe (MI-414-4; ADInstruments), which was connected to a Powerlab system with Lab Chart 7. The pH probe was calibrated using buffer solutions of pH 7 and 10, and was left in the sample until the reading stabilised (~2 min). A 10 μL plasma subsample was used to determine total CO_2 using a Corning CO_2 Analyser, which was calibrated before every measurement using 0.1 M sodium bicarbonate. PCO_2 was calculated via rearrangement of the Henderson-Hasselbalch equation:

$$PCO_2(\text{kPa}) = \frac{CCO_2}{\alpha CO_2(1 + 10^{(pH-pK_1)}(1 + 10^{pH-pK_2}))} \quad (2)$$

where CCO_2 is total carbon dioxide (mmol L^{-1}); αCO_2 is the solubility coefficient of CO_2 (0.419 mmol L^{-1} kPa^{-1}; Titulaer, 1995); pH is the plasma pH, pK_1 is the dissociation constant for reaction of carbonic acid to form bicarbonate and a proton (6.165; Titulaer, 1995); and pK_2 is the dissociation constant for the reaction of bicarbonate to form carbonate

and a proton (9.604; Titulaer, 1995).

Samples of tail muscle (~0.5 g) were thawed, had 0.5 mL of 70% perchloric acid added, before being homogenised using an Omni Bead Ruptor system. The homogenised sample was then centrifuged for 15 min at 14,000 × *g*. The supernatants from these samples were then utilised for assays.

Muscle and plasma glucose was quantified using the hexokinase/glucose-6-phosphate dehydrogenase enzymatic method (Sigma diagnostic kit GAHK-20), while lactate was quantified using an enzymatic kit (L-Lactate, Megazyme), both according to manufacturer's instructions. Because of plasma clotting and loss of some muscle samples, final n values for biochemical analyses ranged from 3 to 6.

3. Statistical analysis

For statistical analysis of data arising from the behavioural choice study, a *Z*-test was performed for each tested hypoxia pair to determine whether the percentage of time spent occupying the side of the chamber with higher dissolved oxygen differed significantly from 50% (i.e. expected percentage if there was no preference). The mean MO_2 and heart rate for each individual crayfish was calculated over 0.67-kPa PO_2 intervals, before being subjected to statistical analysis. Both sets of data failed tests for normality (Shapiro-Wilk) or equal variance (Levene's), and did not meet these assumptions even following transformation. Consequently, these data were subjected to a non-parametric Kruskal-Wallis ANOVA to determine differences in MO_2 and heart rate relative to the starting (i.e. normoxic) PO_2. Where differences were noted, a post-hoc Dunn's test was used to identify the groups that were significantly different. A P_{crit} was calculated using the "brokenstick" routine in the R statistical package. All other statistical analyses were performed in SigmaPlot (Ver. 11, Systat). Haemolymph and plasma parameters (PO_2, PCO_2, pH, and glucose), as well as tissue lactate and glucose, were subjected to one-way ANOVA, followed by Tukey's post-hoc tests. Plasma lactate data failed initial tests of normality and equality of variance, and were therefore log-transformed prior to being assessed via one-way ANOVA. All data are reported as the mean ± SEM, with significance tested at an alpha level of 0.05.

4. Results

Freshwater crayfish did not preferentially avoid hypoxic waters when placed in a chamber with freedom of movement to choose between two PO_2 flows. In all of the tested PO_2 combinations the observed percentage of time spent in the higher PO_2 side of the chamber did not differ significantly from the predicted 50%. Preferences for the higher PO_2 side ranged from 23 ± 15% for the 12 vs. 8 kPa test ($p = 0.17$), to 72 ± 15% for the 8 vs. 4 kPa test ($p = 0.26$; data not shown). There were also no significant differences in response between the different tested PO_2 pairs (one-way ANOVA, $p = 0.175$). This analysis was performed after an initial statistical assessment showed that there was no preference for a particular side of the chamber, allowing experiments where the same paired PO_2 choices were tested to be combined, irrespective of whether the higher PO_2 was on the left or right side of the chamber.

In tests where freshwater crayfish were offered an aerial refuge to escape a progressively increasing aquatic hypoxia, animals emerged at a PO_2 of 0.56 ± 0.03 kPa. Crayfish exposed in the same chamber but to a fixed PO_2 of 1.0 kPa emerged after 38 ± 4 min, and when the PO_2 was fixed at 0.44 kPa, emergence onto the aerial platform occurred at 22 ± 4 min.

Closed-box respirometry showed that at PO_2 levels of 6.0 kPa and above, *P. zealandicus* were able to maintain a relatively constant MO_2 (Fig. 1A). However, as PO_2 decreased, oxyregulation was lost, and crayfish MO_2 declined with declining PO_2. This resulted in significantly reduced MO_2 values in crayfish exposed to water PO_2 values of 2.7 kPa and below, relative to the normoxic control (21 kPa; Kruskal-Wallis ANOVA, $p < 0.001$). Statistical analysis showed that the transition point between oxyregulation and oxyconforming (P_{crit}) occurred at 6.0 kPa. In contrast, there were no significant changes in heart rate with PO_2 (Fig. 1B).

Freshwater crayfish subjected to 6-h exposures under fixed PO_2 conditions (normoxia, 19.3 ± 0.1 kPa; moderate hypoxia, 3.5 ± 0.1 kPa; severe hypoxia, 1.7 ± 0.1 kPa) displayed significant differences in haemolymph PO_2 (one-way ANOVA, $p < 0.001$). Both moderate and severe hypoxia resulted in haemolymph values (2.3 ± 0.1 and 1.5 ± 0.1 kPa, respectively), lower than those observed in normoxia (4.3 ± 0.4 kPa; Fig. 2). In contrast, a 6-h exposure caused no significant change in plasma pH between the normoxic control (7.73 ± 0.25) and moderate (8.24 ± 0.15) hypoxia groups (Fig. 3A). However, under conditions of severe hypoxia plasma pH fell to 6.19 ± 0.37, a value significantly lower than both other groups (one-way ANOVA, $p = 0.002$). In *P. zealandicus* that had experienced severe hypoxia a plasma PCO_2 of 2.0 ± 0.5 kPa was recorded (Fig. 3B), a value significantly greater than that displayed by crayfish under moderate hypoxia (0.07 ± 0.03 kPa), but neither of these values differed significantly from the normoxic control (0.7 ± 0.2 kPa; one-way ANOVA, overall $p = 0.023$).

Significant changes in plasma glucose and lactate were also observed following a 6-h exposure to moderate or severe hypoxia. Plasma glucose decreased significantly in both hypoxia groups to 59–62% of the normoxic control (one-way ANOVA, $p = 0.006$), but there were no differences between the two hypoxic groups (Fig. 4A). However, plasma lactate increased significantly as a function of exposure PO_2, with values in *P. zealandicus* exposed to extreme hypoxia 46-fold higher than those of normoxic animals (Fig. 4B; one-way ANOVA, $p < 0.001$). There were no significant changes in either tail muscle glucose (one-way ANOVA, $p = 0.051$) or lactate (one-way ANOVA, $p = 0.370$; data not shown).

5. Discussion

5.1. Behaviour

The ability to detect an environmental stressor and initiate an appropriate behavioural response that minimises exposure and/or impact, is the first line of defence for mobile animals. In the current study, however, *P. zealandicus* did not display an avoidance behaviour when offered the choice between two flows of water with distinct PO_2 values. This lack of preference persisted independent of the absolute or relative PO_2 values of the paired waters. We are aware of only one other study in which the PO_2 preference of freshwater crayfish has been examined. Bierbower and Cooper (2010), used a Y-maze and showed that *Procambarus clarkii* did not avoid hypoxia when given the choice between a water flow bubbled with nitrogen, and an aerated flow. This outcome corresponds to the findings of the current study.

While there was no response of crayfish to hypoxia when offered an aquatic refuge, when exposed to a progressively declining PO_2, *P. zealandicus* was shown to emerge from the water onto an aerial platform. Emergence into the air is a commonly observed phenomenon in hypoxia-exposed freshwater crayfish. For example, in *Cherax destructor* aerial exposure following severe aquatic hypoxia was associated with an initial increase in oxygen uptake, and an overall improvement in metabolic status (Morris and Callaghan, 1998). However, the relief offered by aerial exposure will depend on species-specific adaptations for aerial gas exchange, including changes in gill structures that minimise collapse and maintain surface area (van Aardt, 1990), and a haemocyanin responsive to modifications that enhance oxygen binding affinity (Taylor and Wheatly, 1981). Knowledge of the effectiveness of aerial gas exchange in *P. zealandicus* would be required to determine the success of emersion as an escape from aquatic hypoxia.

In freshwater crayfish, the PO_2 at which emergence occurs varies between species. For example, both *Austropotamobius pallipes* (5.3 kPa)

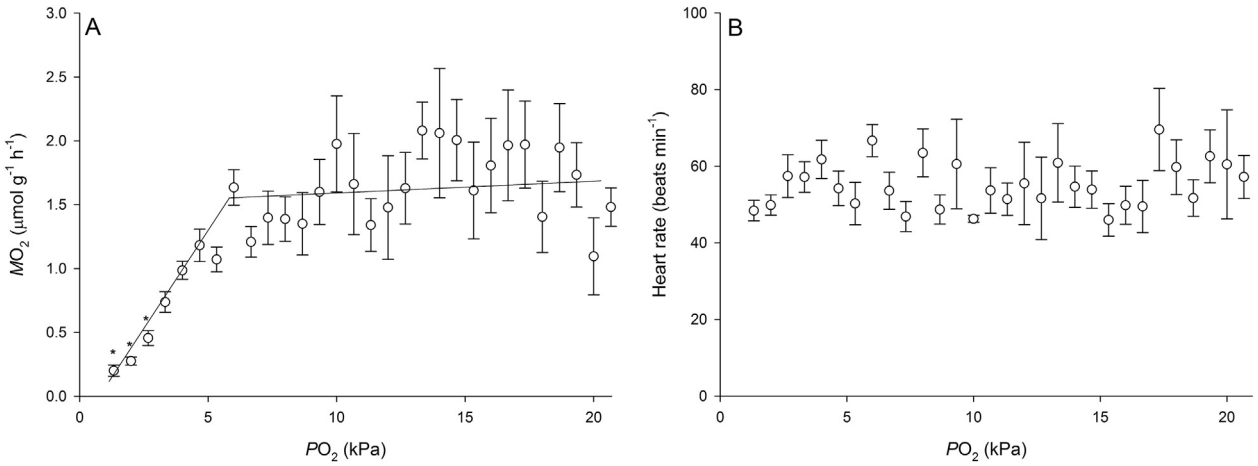

Fig. 1. *P. zealandicus* MO_2 (A) or heart rate (B) as a function of water PO_2, determined via closed-box respirometry. Plotted values represent the means (± SEM) of 5–26 individuals at each time-point. The intersection of the solid lines in Panel A represents the P_{crit}, as determined by the "brokenstick" routine in the R statistical package. Significant differences (*) between MO_2 values and the normoxic control (21 kPa), were determined via Kruskal-Wallis ANOVA, followed by Dunn's post-hoc test, at an alpha level of 0.05.

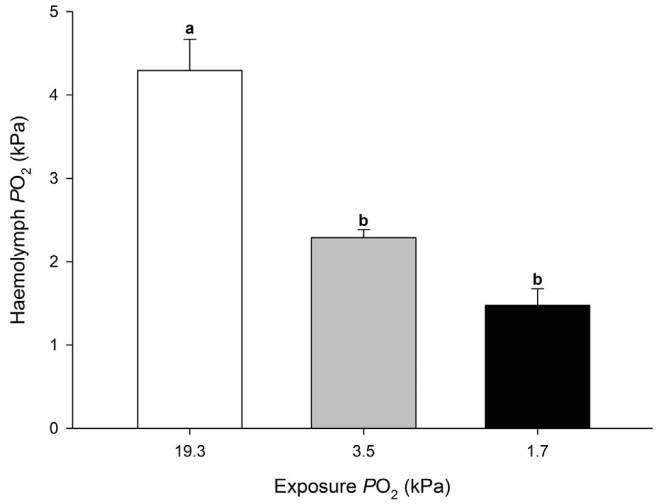

Fig. 2. Haemolymph PO_2 in *P. zealandicus* following a 6 h exposure to normoxia (19.3 kPa), moderate hypoxia (3.5 kPa), or severe hypoxia (1.7 kPa). Plotted points represent means (± SEM) of 3 (moderate hypoxia) or 6 (normoxia and severe hypoxia) replicates. Bars sharing letters are not significantly different, as determined via one-way ANOVA, followed by Tukey's post-hoc test, at an alpha level of 0.05.

and *Orconectes rusticus* (4.0 kPa) emerge at PO_2 values close to their P_{crit} (Taylor and Wheatly, 1980; McMahon and Wilkes, 1983). In the current study, *P. zealandicus* was shown to voluntarily leave hypoxic water only when PO_2 fell below 0.7 mm Hg, well below its P_{crit} of 6.0 kPa. As temperatures were similar in all studies (a drop from 15 to 10 °C in the current study (see Methods), 13 °C for *A. pallipes*, 15 °C for *O. rusticus*), it is likely that the relatively low emersion PO_2 of *P. zealandicus* relates to the severity and length of exposure to hypoxia. In the current investigation, water PO_2 levels decreased exponentially over the course of an hour. In a previous study, Titulaer (1995) showed that *P. zealandicus* exposed to a progressive hypoxia decline over 84 h emersed at a PO_2 of 6.0 kPa, equivalent to its P_{crit}. The more rapid PO_2 decline in the current study (from normoxia to extreme hypoxia in ~1 h) may have resulted in a delayed build-up of potentially toxic metabolites, such as lactate. In fish and other studied crustaceans that exhibit emersion, this behaviour is associated with the accumulation of anaerobic metabolites, which start to impair physiological and biochemical function (Albert and Ellington, 1985; Sloman et al., 2008; Urbina and Glover, 2012). This hypothesis is supported by the fixed hypoxia emersion experiments. When *P. zealandicus* were subjected to severe (1.0 kPa) and extreme (0.4 kPa) hypoxia they emerged from the water faster (38 and 22 min, respectively) than they did under progressive hypoxia (54 min), indicating that a threshold of perturbation had been reached more rapidly.

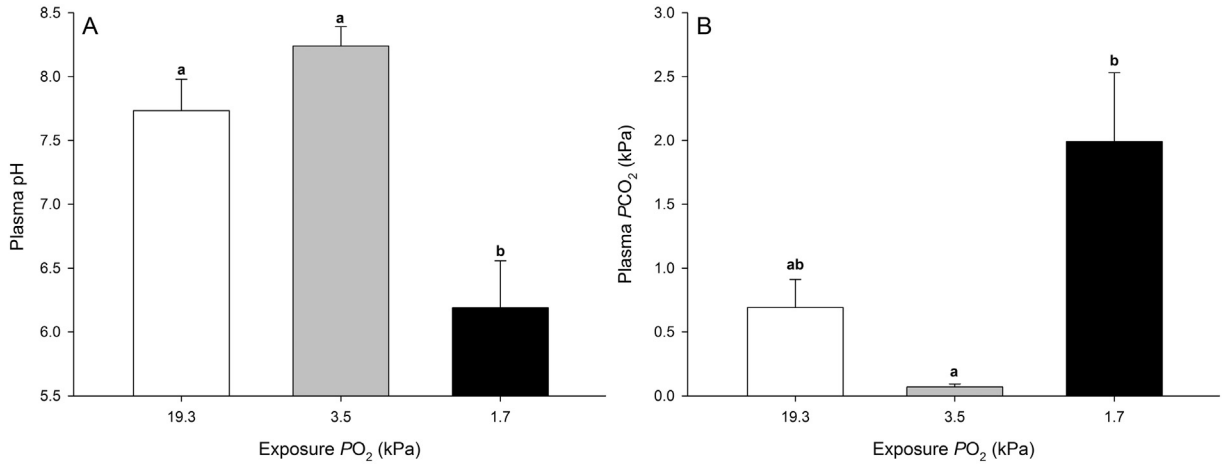

Fig. 3. Plasma pH (A) and PCO_2 (B) in *P. zealandicus* following a 6 h exposure to normoxia (19.3 kPa), moderate hypoxia (3.5 kPa), or severe hypoxia (1.7 kPa). Plotted points represent means (± SEM) of 3 (moderate hypoxia) or 6 (normoxia and severe hypoxia) replicates. Bars sharing letters are not significantly different, as determined via one-way ANOVA, followed by Tukey's post-hoc test, at an alpha level of 0.05.

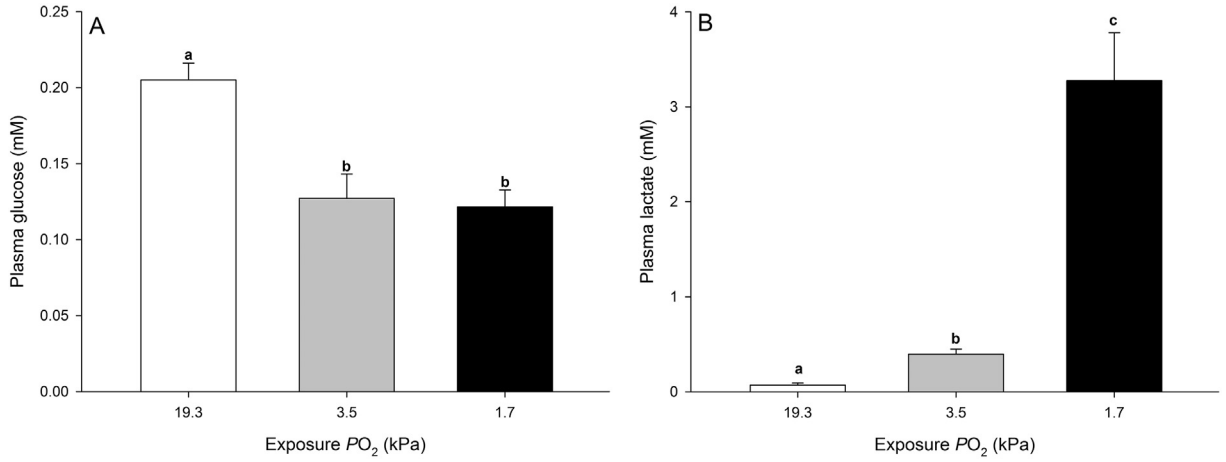

Fig. 4. Plasma glucose (A) and lactate (B) in *P. zealandicus* following a 6 h exposure to normoxia (19.3 kPa), moderate hypoxia (3.5 kPa), or severe hypoxia (1.7 kPa)·Plotted points represent means (± SEM) of 3 (severe hypoxia glucose, normoxia lactate), 4 (normoxia glucose, severe hypoxia lactate), 5 (moderate hypoxia lactate), or 6 (moderate hypoxia glucose) replicates. Bars sharing letters are not significantly different, as determined via one-way ANOVA, followed by Tukey's post-hoc test, at an alpha level of 0.05.

However, the experimental regime alone does not explain the pattern of "delayed" emersion. In emergence experiments by Wheatly and Taylor (1979) and Taylor et al. (1973), where emersion occurred at oxygen levels close to the P_{crit}, the reductions in water PO_2 were also rapid (~45 min), similar to those in the current study. Consequently, if there are delays in the build-up of metabolites in *P. zealandicus* with time, a hypothesis that requires investigation, it may reflect a higher tolerance to hypoxia than in species such as *A. pallipes* and *O. rusticus*. Importantly, as hypoxia tolerance dictates avoidance behaviour in fish (e.g. Burleson et al., 2001), if a similar relationship between tolerance and avoidance exists in freshwater crayfish, then this may also explain both the relative lack of choice in the avoidance studies, and its high threshold for emersion.

Temperature is also clearly an important factor influencing emergence. In the current study the rapid flow of nitrogen gas required to reduce chamber PO_2 chilled the nitrogen tank, and resulted in a cooling of the water over time (from 15 to 10 °C). Wheatly and Taylor (1979), in a study on the crab *Carcinus maenas* and using similar rates of hypoxia induction to the current work, showed that emergence was strongly affected by water temperature, with cooler water leading to an enhanced capacity to endure hypoxia. However, even at very low temperatures (e.g., 7 °C), the PO_2 of emergence did not drop below 2.7 kPa, suggesting that the low emergence PO_2 of *P. zealandicus* (0.56 kPa) is, at least in part, related to an enhanced behavioural hypoxia tolerance.

5.2. Physiology

While behavioural responses suggested that *P. zealandicus* is more tolerant to hypoxic waters relative to other freshwater crayfish, MO_2 data did not support this. The average MO_2 for *P. zealandicus* under normoxic conditions in the current study was 1.48 ± 0.15 μmol g^{-1} h^{-1}, within the range reported for other freshwater crayfish (0.67 to 2.33 μmol g^{-1} h^{-1}; see Table 1).

Typically, aquatic animals, including crayfish, attempt to maintain MO_2 as water PO_2 declines (Reiber, 1995; McMahon, 2001). They are able to do so down to a critical oxygen tension (P_{crit}), after which MO_2 is no longer maintained, and thus oxygen supply is compromised. The calculated P_{crit} for *P. zealandicus* was 6.0 kPa, a value supported by the fall in haemolymph PO_2 following a 6-h exposure to water PO_2 below this threshold (Fig. 2). As P_{crit} represents the point where the animal needs to engage mechanisms for reducing oxygen consumption, and/or enact mechanisms for increasing energy supply through anaerobic pathways, this metric is often used as a measure of hypoxia tolerance (Reiber, 1995). The calculated P_{crit} for *P. zealandicus* suggests this species is of similar hypoxia tolerance to other freshwater crayfish species. For example, *A. pallipes* maintains MO_2 down to a PO_2 of 5.3 kPa, and *P. clarkii* down to 6.0 kPa (Taylor and Wheatly, 1980; Reiber and McMahon, 1998). Consequently, the physiological evidence counters the behavioural data that suggested *P. zealandicus* is a comparatively hypoxia tolerant species.

It is, however, important to note that the MO_2 data in the current study exhibited high variability. In fact, while most *P. zealandicus* individuals exhibited standard oxyregulatory characteristics (maintained MO_2 followed by decline with decreasing PO_2), some individuals oxyconformed (MO_2 matching declining PO_2 across tested PO_2 range; data not shown). Studies have shown that oxyregulatory behaviour can vary between individuals of the same species (Duke and Ultsch, 1990), while the degree to which individuals within a species either oxyregulate or oxyconform is dependent on factors such as salinity, moult cycle, stress, body size, developmental stage and temperature (Pörtner and Grieshaber, 1993). In the current study, these variables were constant, so the source of the variation in individual MO_2 is not known.

Heart rate did not vary as a function of water PO_2 in *P. zealandicus*.

Table 1
Summary of literature MO_2 values in freshwater crayfish.

Species	MO_2 (μmol g^{-1} h^{-1})	Temperature (°C)	Fossorial species?	Reference
Austropotamobius pallipes	0.67	15	No	Wheatly and Taylor, 1981
Orconectes rusticus	2.33	15	No	Wilkes and McMahon, 1982
Astacus leptodactylus	0.83	13	No	Massabuau and Burtin, 1984
Astacopsis franklinii	2.05	15	No	Swain et al., 1987
Parastacoides tasmanicus	1.91	15	Yes	Swain et al., 1987
Cherax destructor	1.44	20	Yes	Morris et al., 2005
Paranephrops zealandicus	1.48	15	Yes	Current study

This is somewhat surprising as bradycardia is the most common cardiac response to hypoxia in freshwater crayfish (e.g. Wheatly and Taylor, 1981; Reiber and McMahon, 1998). Bradycardia is especially prominent at PO_2 values below the animals P_{crit}, where it is postulated that there is insufficient oxygen to meet the aerobic demands of the circulatory pump (Airriess and McMahon, 1994). However, this decrease in heart rate is usually accompanied by an increase in stroke volume, which acts to maintain, or even increase, cardiac output (Wheatly and Taylor, 1981; Reiber, 1995; Reiber and McMahon, 1998; McMahon, 2001). These cardiovascular changes promote an increased blood flow to the branchial surface, potentially increasing oxygen loading, while slowing perfusion to the tissues, which increases diffusion time, and thus oxygen extraction (McMahon, 2001).

Although bradycardia is the most common response to hypoxia in crustaceans, it is not universally displayed. For example, *P. clarkii* exhibit an increased heart rate in response to hypoxia (Bierbower and Cooper, 2010), while a tachycardia is also seen in the grass shrimp, *Palaemonetes pugio* (Harper and Reiber, 1999). As in the present study, a maintenance of heart rate has also been observed in the freshwater crayfish *O. rusticus* when subjected to 144 h of hypoxia (Wilkes and McMahon, 1982). This lack of change was suggested to reflect a lack of hypoxia stress (Wilkes and McMahon, 1982). Similarly, Mendonca and Gamperl (2010) concluded that bradycardia was an indicator of hypoxia sensitivity in fish. Thus animals, such as *P. zealandicus*, which lack alterations in heart rate may be considered more tolerant of hypoxia. However, it is important to note that heart rate alone may not reflect cardiac changes in response to hypoxia. For example, changes in cardiac output, through an increase in stroke volume with minimal change in heart rate, have been shown in hypoxic crustaceans (McMahon, 2001).

5.3. Biochemistry

Disturbances in haemolymph acid-base status can impair normal enzymatic function and regulation of metabolic processes. Exposure of *P. zealandicus* to severe (1.7 kPa), but not moderate (3.4 kPa), hypoxia resulted in a plasma acidosis (Fig. 3A). The extent of acid-base disturbance is dependent on the severity of hypoxia. For example, acidosis is usually seen only in hypoxia-stressed animals that have lost the ability to buffer against this decrease in pH (Taylor and Spicer, 1991). This suggests that exposure of *P. zealandicus* to moderate hypoxia (3.4 kPa, below this species P_{crit}), is insufficient to disturb plasma biochemistry. Corroborating the results of the current study, Mauro and Thompson (1984) also showed a drop in plasma pH in *P. clarkii* exposed to hypoxic waters with a PO_2 of 2.0 kPa. While acidosis is often attributed to a build-up of CO_2 due to a compromised gas exchange capacity (Lutz and Storey, 1997), there were no significant changes in PCO_2 in the plasma of *P. zealandicus* exposed to hypoxia in the current study. Instead, increased plasma lactate was the likely driver of the acidosis observed in *P. zealandicus* exposed to severe hypoxia.

An elevation in plasma lactate is a commonly observed response to hypoxia in freshwater crayfish (e.g. Morris and Callaghan, 1998; da Silva-Castiglioni et al., 2010). These increases are seen below P_{crit} and are an indication of anaerobic metabolism. In the current study, plasma lactate increased under both moderate and severe hypoxia, but only in the latter was plasma pH impacted. This suggests that *P. zealandicus* possesses sufficient plasma buffering capacity to withstand lactate production resulting from 6-h of exposure to a PO_2 of 3.5 kPa. Furthermore, it is known that low concentrations of lactate can enhance the affinity of haemocyanin for oxygen (Morris et al., 1986), and may therefore play an important role in increasing oxygen supply and thus increasing tolerance to hypoxia (Reiber, 1995). However, after 6 h of exposure to 1.7 kPa, lactate concentrations started to impact acid-base homeostasis. This is likely to be the driver for the emersion behaviour observed under extreme hypoxia (Albert and Ellington, 1985; Sloman et al., 2008).

In response to hypoxia, *P. zealandicus* displays a hypoglycaemia. This differs to the standard response in decapod crustaceans, with shrimp (Racotta et al., 2002), crab (Zou et al., 1996), lobster (Ocampo et al., 2003) and crayfish (da Silva-Castiglioni et al., 2010), all showing hypoxia-induced hyperglycaemia. This increase in plasma glucose is thought to prepare the animals for the impending high substrate demands of anaerobic glycolysis (Zou et al., 1996; da Silva-Castiglioni et al., 2010). While uncommon, others have reported deviations from this standard hyperglycaemia response to hypoxia. For example, an initial hyperglycaemia was followed by a decline in haemolymph glucose in the hypoxia-exposed freshwater crayfish *Parastacus defossus* (da Silva-Castiglioni et al., 2010). Differences in plasma glucose dynamics are likely related to variations in exposure conditions and substrate handling between species (Bonvillain et al., 2012). These studies highlight that the temporal pattern of plasma glucose is fluid, and spot sampling after 6 h of exposure, as in the current study, may be insufficient to attain a reliable perspective of plasma glucose dynamics.

Hyperglycaemia, the conserved response to hypoxia, is often associated with a decrease in tissue glycogen stores (van Aardt, 1988; Zou et al., 1996). Unfortunately, glycogen concentrations were not measured in the current study. However, there were no changes in tissue glucose observed in *P. zealandicus*, even after 6 h of extreme hypoxia. As such, the data generated from the current study could be interpreted as a rapid mobilisation of glycogen to glucose, followed by an equally rapid conversion of glucose to lactate via anaerobic metabolism. Supporting this concept, Muusze et al. (1998) also suggested the observed hypoxia-induced hypoglycaemia in the cichlid fish, *Astronotus ocellatus*, indicated substrate mobilisation was unable to keep up with glycolysis. However, a decline in tissue glycogen would be required to confirm this hypothesis. Alternatively, it has also been suggested that decreases in haemolymph glucose in animals exposed to moderate hypoxia may be a consequence of glycogen resynthesis (Bonvillain et al., 2012), although the utility of such a response in a hypoxic animal is unclear.

It is also important to note that tissue arginine phosphate was not measured in the current study. Crustacean muscle contains high concentrations of this phosphagen, which acts as both an energy substrate and an ATP buffer during periods of hypoxia (Ellington, 2001). For example, after 8 h of exposure to a moderate hypoxia (~4.5 kPa), muscle arginine phosphate concentrations were significantly reduced in the freshwater crayfish, *Parastacus brasiliensis* (da Silva-Castiglioni et al., 2010). In the more hypoxia-tolerant sister species (*P. defossus*), the initial muscle arginine phosphate concentration was higher, however, after 8 h of hypoxia exposure there was no change in this parameter (da Silva-Castiglioni et al., 2010). This suggests that arginine phosphate metabolism is species-dependent and may be reflective of hypoxia tolerance, making this an interesting avenue for future investigation in *P. zealandicus*.

6. Conclusions and environmental context

Overall, *P. zealandicus* is relatively tolerant to aquatic hypoxia, and employs mechanisms generally similar to other freshwater crayfish species that enable it to withstand low dissolved oxygen. Although oxyregulation fails at a PO_2 of 6.0 kPa, there are no changes in heart rate even when PO_2 falls to values around 1.3 kPa. Acid-base regulation also persists in this species, only failing under a prolonged 6-h exposure to 1.7 kPa, where the decline in plasma pH appears to be driven by a significant increase in plasma lactate. This species does not display a behavioural preference for waters of higher PO_2 under acute assays, but will emerse when PO_2 falls to levels of around 0.5 kPa. Emersion occurs more rapidly the more intense the hypoxia, suggesting that the accumulation of anaerobic metabolites drives this phenomenon. However, by analogy with other species (Morris et al., 1986), it is likely that at PO_2 values between the P_{crit} and the PO_2 of emersion, low concentrations of lactate facilitate improved oxygen delivery through interactions with haemocyanin, thus prolonging tolerance. Emerging out of the

water increases both risk of desiccation and predation and is therefore often only used as a last resort when water conditions become unbearable. Because *P. zealandicus* emerged at a much lower PO_2 than other freshwater crustaceans, this suggests a comprehensive compensatory ability of the physiological and biochemical responses, which allow the animal to remain in hypoxic waters.

The relatively high tolerance of *P. zealandicus* to hypoxia may relate to its burrowing behaviour. This species actively burrows into silty stream and lake beds (Hopkins, 1970), environments that tend to be oxygen-limited. Burrowing crustaceans are known to be more tolerant of hypoxia than non-burrowing species (e.g. Holman and Hand, 2009), and exhibit adaptations to respiratory pigments that facilitate oxygen binding at lower PO_2 values than in non-burrowers (McMahon, 2001). Importantly, this high tolerance may be of ecological significance. This species shares habitat preferences with species such as brown trout (Shave et al., 1994). These fish are predators of *P. zealandicus* and thus can directly impact *P. zealandicus* populations, but their presence may also exert indirect negative impacts through impaired feeding, growth and survival (Stein and Magnuson, 1976; Usio and Townsend, 2000). As *P. zealandicus* displays a higher tolerance to low PO_2 than brown trout (Landman et al., 2005), then the ability to withstand hypoxia may be an important mechanism for limiting negative interspecific interactions.

The data in the current study do, however, suggest that hypoxic waters may be a factor contributing towards the decline of *P. zealandicus*. Oxygen partial pressures in some waters within the geographical range of this species have been measured to fall below levels where significant biochemical and behavioural disturbances to *P. zealandicus* may arise (Fisher, 2011). Importantly, as *P. zealandicus* are principally nocturnal (Usio and Townsend, 2000), their active period coincides with aquatic plant and algal respiration, which may exacerbate hypoxia and impair feeding and reproductive activities. Furthermore, given the co-occurrence of hypoxia with enhanced levels of nutrients, it is possible that the presence of dissolved nutrients such as ammonia and nitrite may have compounding effects.

Acknowledgements

The authors are grateful to the excellent technical assistance provided by Alan Woods and Nick Etheridge. RJB was supported by a scholarship provided by the Ngāi Tahu Research Centre at the University of Canterbury and CNG is supported by a Campus Alberta Innovates Program Research Chair. Funding sources provided only financial support and had no other involvement in the study. The authors declare no conflicts of interest.

References

Airriess, C.N., McMahon, B.R., 1994. Cardiovascular adaptations enhance tolerance of environmental hypoxia in the crab *Cancer magister*. J. Exp. Biol. 190, 23–41.
Albert, J.L., Ellington, W.R., 1985. Patterns of energy metabolism in the stone crab, *Menippe mercenaria*, during severe hypoxia and subsequent recovery. J. Exp. Zool. 234, 175–183.
Bierbower, S.M., Cooper, R.L., 2010. The effects of acute carbon dioxide on behavior and physiology in *Procambarus clarkii*. J. Exp. Zool. A 313, 484–497.
Bonvillain, C.P., Rutherford, D.A., Kelso, W.E., Green, C.C., 2012. Physiological biomarkers of hypoxic stress in red swamp crayfish *Procambarus clarkii* from field and laboratory experiments. Comp. Biochem. Physiol. A 163, 15–21.
Burleson, M., Wilhelm, D., Smatresk, N., 2001. The influence of fish size on the avoidance of hypoxia and oxygen selection by largemouth bass. J. Fish Biol. 59, 1336–1349.
Cook, D.G., Wells, R.M.G., Herbert, N.A., 2011. Anaemia adjusts the aerobic physiology of snapper (*Pagrus auratus*) and modulates hypoxia avoidance behaviour during oxygen choice presentations. J. Exp. Biol. 214, 2927–2934.
Cook, D.G., Iftikar, F.I., Baker, D.W., Hickey, A.J., Herbert, N.A., 2013. Low-O_2 acclimation shifts the hypoxia avoidance behaviour of snapper (*Pagrus auratus*) with only subtle changes in aerobic and anaerobic function. J. Exp. Biol. 216, 369–378.
Depledge, M.H., Andersen, B.B., 1990. A computer-aided physiological monitoring system for continuous, long-term recording of cardiac activity in selected invertebrates. Comp. Biochem. Physiol. A 96, 473–477.
Duke, J.T., Ultsch, G.R., 1990. Metabolic oxygen regulation and conformity during submergence in the salamanders *Siren lacertina*, *Amphiuma means*, and *Amphiuma tridactylum*, and a comparison with other giant salamanders. Oecologia 84, 16–23.
Ellington, W.R., 2001. Evolution and physiological roles of phosphagen systems. Annu. Rev. Physiol. 63, 289–325.
Environment Canterbury, 2012. A Review of Critical Lake Opening Periods, Habitats and Locations for Fish in the Te Waihora/Lake Ellesmere Catchment. Report No. R12/122. (Christchurch, New Zealand).
Fisher, K., 2011. Diurnal Variation in Te Waihora Water Quality. Waterways Centre for Freshwater Management, Christchurch, New Zealand.
Gorr, T.A., Wichmann, D., Hu, J., Hermes-Lima, M., Welker, A.F., Terwilliger, N., Wren, J.F., Viney, M., Morris, S., Nilsson, G.E., Deten, A., Soliz, J., Gassmann, M., 2010. Hypoxia tolerance in animals: biology and application. Physiol. Biochem. Zool. 83, 733–752.
Grainger, N., Collier, K., Hitchmough, J.R., Harding, J.S., Smith, B.J., Sutherland, D.L., 2014. Conservation Status of New Zealand Freshwater Invertebrates, 2013. Department of Conservation, Wellington, New Zealand.
Harper, S.L., Reiber, C.L., 1999. Influence of hypoxia on cardiac functions in the grass shrimp (*Palaemonetes pugio* Holthuis). Comp. Biochem. Physiol. A 124, 569–573.
Herbert, N.A., Skjaeraasen, J.E., Nilsen, T., Salvanes, A.G.V., Steffensen, J.F., 2011. The hypoxia avoidance behaviour of juvenile Atlantic cod (*Gadus morhua* L.) depends on the provision and pressure level of an O_2 refuge. Mar. Biol. 158, 737–746.
Hicks, B.J., 1997. Food webs in forest and pasture streams in the Waikato region, New Zealand: a study based on analyses of stable isotopes of carbon and nitrogen, and fish gut contents. N. Z. J. Mar. Freshw. Res. 31, 651–664.
Holman, J.D., Hand, S.C., 2009. Metabolic depression is delayed and mitochondrial impairment averted during prolonged anoxia in the ghost shrimp, *Lepidophthalmus louisianensis* (Schmitt, 1935). J. Exp. Mar. Biol. Ecol. 376, 85–93.
Hopkins, C., 1970. Systematics of the New Zealand freshwater crayfish *Paranephrops* (Crustacea: Decapoda: Parastacidae). N. Z. J. Mar. Freshw. Res. 4, 278–291.
Kusabs, I.A., Quinn, J.M., 2009. Use of a traditional Maori harvesting method, the tau koura, for monitoring koura (freshwater crayfish, *Paranephrops planifrons*) in Lake Rotoiti, North Island, New Zealand. N. Z. J. Mar. Freshw. Res. 43, 713–722.
Kusabs, I.A., Quinn, J.M., Hamilton, D.P., 2015. Effects of benthic substrate, nutrient enrichment and predatory fish on freshwater crayfish (koura, *Paranephrops planifrons*) population characteristics in seven Te Arawa (Rotorua) lakes, North Island, New Zealand. Mar. Freshw. Res. 66, 631–643.
Landman, M.J., van den Heuvel, M.R., Ling, N., 2005. Relative sensitivities of common freshwater fish and invertebrates to acute hypoxia. N. Z. J. Mar. Freshw. Res. 39, 1061–1067.
Lutz, P.L., Storey, K.B., 1997. Adaptations to variations in oxygen tension by vertebrates and invertebrates. In: Dantzler, W.H. (Ed.), Handbook of Physiology. Oxford University Press, Oxford, pp. 1479–1522.
Massabuau, J., Burtin, B., 1984. Regulation of oxygen consumption in the crayfish *Astacus leptodactylus* at different levels of oxygenation: role of peripheral O_2 chemoreception. J. Comp. Physiol. B. 155, 43–49.
Mauro, N.A., Thompson, C., 1984. Hypoxia adaptation in the crayfish *Procambarus clarkii*. Comp. Biochem. Physiol. A 79, 73–75.
McDowell, R.W., Larned, S.T., Houlbroke, D.J., 2009. Nitrogen and phosphorus in New Zealand streams and rivers: control and impact of eutrophication and the influence of land management. N. Z. J. Mar. Freshw. Res. 43, 985–995.
McMahon, B.R., 2001. Respiratory and circulatory compensation to hypoxia in crustaceans. Respir. Physiol. 128, 349–364.
McMahon, B.R., Wilkes, P.R.H., 1983. Emergence responses and aerial ventilation in normoxic and hypoxic crayfish *Orconectes rusticus*. Physiol. Zool. 56, 133–141.
Mendonca, P.C., Gamperl, A.K., 2010. The effects of acute changes in temperature and oxygen availability on cardiac performance in winter flounder (*Pseudopleuronectes americanus*). Comp. Biochem. Physiol. A 155, 245–252.
Morris, S., Callaghan, J., 1998. The emersion response of the Australian yabby *Cherax destructor* to environmental hypoxia and the respiratory and metabolic responses to consequent air-breathing. J. Comp. Physiol. B. 168, 389–398.
Morris, S., Tyler-Jones, R., Bridges, C.R., Taylor, E.W., 1986. The regulation of haemocyanin oxygen affinity during emersion of the crayfish *Austropotamobius pallipes*. II. An investigation of *in vivo* changes in oxygen affinity. J. Exp. Biol. 121, 327–337.
Morris, S., van Aardt, W.J., Ahern, M.D., 2005. The effect of lead on the metabolic and energetic status of the Yabby, *Cherax destructor*, during environmental hypoxia. Aquat. Toxicol. 75, 16–31.
Muusze, B., Marcon, J., van den Thillart, G., Almeida-Val, V., 1998. Hypoxia tolerance of Amazon fish: respirometry and energy metabolism of the cichlid *Astronotus ocellatus*. Comp. Biochem. Physiol. A 120, 151–156.
Ocampo, L., Patiño, D., Ramírez, C., 2003. Effect of temperature on hemolymph lactate and glucose concentrations in spiny lobster *Panulirus interruptus* during progressive hypoxia. J. Exp. Mar. Biol. Ecol. 296, 71–77.
Pörtner, H.O., Grieshaber, M.K., 1993. Critical PO_2(s) in oxyconforming and oxyregulating animals: gas exchange, metabolic rate and the mode of energy production. In: Bicudo, J.E.P.W. (Ed.), The Vertebrate Gas Transport Cascade: Adaptations to Environment and Mode of Life. CRC Press, Boca Raton, FL, USA.
Racotta, I.S., Palacios, E., Mendez, L., 2002. Metabolic responses to short and long-term exposure to hypoxia in white shrimp (*Penaeus vannamei*). Mar. Freshw. Behav. Physiol. 35, 269–275.
Reiber, C.L., 1995. Physiological adaptations of crayfish to the hypoxic environment. Integr. Comp. Biol. 35, 1–11.
Reiber, C.L., McMahon, B.R., 1998. The effects of progressive hypoxia on the crustacean cardiovascular system: a comparison of the freshwater crayfish (*Procambarus clarkii*), and the lobster (*Homarus americanus*). J. Comp. Physiol. B. 168, 168–176.
Shave, C.R., Townsend, C.R., Crowl, T.A., 1994. Antipredator behaviours of a freshwater crayfish (*Paranephrops zealandicus*) to a native and introduced predator. N. Z. J. Ecol. 18, 1–10.
da Silva-Castiglioni, D., Oliveira, G.T., Buckup, L., 2010. Metabolic responses of

Parastacus defossus and *Parastacus brasiliensis* (Crustacea, Decapoda, Parastacidae) to hypoxia. Comp. Biochem. Physiol. A 156, 436–444.
Sloman, K.A., Mandic, M., Todgham, A.E., Fangue, N.A., Subrt, P., Richards, J.G., 2008. The response of the tidepool sculpin, *Oligocottus maculosus*, to hypoxia in laboratory, mesocosm and field environments. Comp. Biochem. Physiol. A 149, 284–292.
Smith, V.H., 2003. Eutrophication of freshwater and coastal marine ecosystems: a global problem. Environ. Sci. Pollut. Res. 10, 126–139.
Stein, R.A., Magnuson, J.J., 1976. Behavioural response of crayfish to a fish predator. Ecology 57, 751–761.
Swain, R., Marker, P.F., Richardson, A.M.M., 1987. Respiratory responses to hypoxia in stream-dwelling (*Astacopsis franklinii*) and burrowing (*Parastacoides tasmanicus*) parastacid crayfish. Comp. Biochem. Physiol. A 87, 813–817.
Taylor, A.C., Spicer, J.I., 1991. Acid-base disturbances in the hemolymph of the prawns, *Palaemon elegans* (Rathke) and *P. serratus* (Pennant) (Crustacea, Decapoda) during exposure to hypoxia. Comp. Biochem. Physiol. A 98, 445–452.
Taylor, E.W., Wheatly, M.G., 1980. Ventilation, heart rate and respiratory gas exchange in the crayfish *Austropotamobius pallipes* (Lereboullet) submerged in normoxic water and following 3 h exposure in air at 15 °C. J. Comp. Physiol. B. 13, 67–78.
Taylor, E.W., Wheatly, M.G., 1981. The effect of long-term aerial exposure on heart rate, ventilation, respiratory gas exchange and acid-base status in the crayfish *Austropotamobius pallipes*. J. Exp. Biol. 92, 109–124.
Taylor, E.W., Butler, P.J., Sherlock, P.J., 1973. The respiratory and cardiovascular changes associated with the emersion response of *Carcinus maenas* (L.) during environmental hypoxia, at three different temperatures. J. Comp. Physiol. 86, 95–115.
Taylor, E.W., Tyler-Jones, R., Wheatly, M.G., 1987. The effects of aerial exposure on the distribution of body water and ions in the freshwater crayfish *Austropotamobius pallipes* (Lereboullet). J. Exp. Biol. 128, 307–322.
Titulaer, W.A., 1995. Respiration Physiology and the Gill Structure of the New Zealand Freshwater Crayfish *Paranephrops zealandicus* (White 1847) (Decapoda: Parastacidae). Unpublished Ph.D. thesis University of Canterbury, Christchurch, New Zealand.
Urbina, M.A., Glover, C.N., 2012. Should I stay or should I go? Physiological, metabolic and biochemical consequences of voluntary emersion upon aquatic hypoxia in the scaleless fish *Galaxias maculatus*. J. Comp. Physiol. B. 182, 1057–1067.
Usio, N., Townsend, C.R., 2000. Distribution of the New Zealand crayfish *Paranephrops zealandicus* in relation to stream physico-chemistry, predatory fish, and invertebrate prey. N. Z. J. Mar. Freshw. Res. 34, 557–567.
Usio, N., Townsend, C.R., 2004. Roles of crayfish: consequences of predation and bioturbation for stream invertebrates. Ecology 85, 807–822.
van Aardt, W.J., 1988. Lactate metabolism and glucose patterns in the river crab, *Potamonautes warreni* Calman, during anoxia and subsequent recovery. Comp. Biochem. Physiol. A 91, 299–304.
van Aardt, W.J., 1990. Aerial and aquatic respiration in the river crab *Potamonautes warreni* Calman with notes on gill structure. South. Afr. J. Aquat. Sci. 16, 16–36.
Wheatly, M.G., Taylor, E.W., 1979. Oxygen levels, acid-base status and heart rate during emersion of the shore crab *Carcinus maenas* (L.) into air. J. Comp. Physiol. B. 132, 305–311.
Wheatly, M.G., Taylor, E.W., 1981. The effect of progressive hypoxia on heart rate, ventilation, respiratory gas exchange and acid-base status in the crayfish *Austropotamobius pallipes*. J. Exp. Biol. 92, 125–141.
Wilkes, P.R.H., McMahon, B.R., 1982. Effect of maintained hypoxic exposure on the crayfish *Orconectes rusticus*: I. Ventilatory, acid-base and cardiovascular adjustments. J. Exp. Biol. 98, 119–137.
Zou, E., Du, N., Lai, W., 1996. The effects of severe hypoxia on lactate and glucose concentrations in the blood of the Chinese freshwater crab *Eriocheir sinensis* (Crustacea: Decapoda). Comp. Biochem. Physiol. A 114, 105–109.