**Variability in hydrostatic pressure tolerance between *Palaemon* species: implications for insights into the colonisation of the deep sea**

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**ABSTRACT**

Experimental approaches to assess whether shallow-water benthic invertebrates can extend bathymetric ranges in response to changing climate have focused on the developing ecophysiological model *Palaemon varians*. However, *P. varians* may not be representative of other shallow-water shrimp species: this species inhabits the highly variable salt marsh environment and is eurythermal, euryhaline, and euryoxic. Inferences concerning the capacity of an ancestral species to directly colonise the deep sea have therefore been regarded with caution. We provide evidence that acute thermal and hyperbaric tolerance in the intertidal and subtidal shrimp *Palaemon serratus* is lower than in the salt marsh and brackish-water shrimp *P*. *varians*, suggesting that adaptation to differing habitats has resulted in differing physiological tolerance to acute stress conditions. Nonetheless, hyperbaric tolerance in *P*. *serratus* supports the proposition that the common ancestor of these species may have possessed the physiological capability to colonise bathyal depths. The consistent interaction between temperature and hydrostatic pressure tolerance in these species supports the suggestion that shallow-water species may have the capacity to deepen bathymetric distribution in response to ocean warming.

**Keywords:** Caridea; bathymetric distribution; biogeography; deep sea; evolution; physiology.

**1. Introduction**

The deep sea (depths greater than 200 m) covers more than 90% of the ocean surface area and hosts significant biodiversity (see Brown and Thatje 2014). However, multiple mass extinction events occurred in the deep sea during the past ~541 Myr (Bambach 2006), caused by dysoxia resulting from climate-driven shifts in ocean circulation (Horne 1999; Wilson 1999). Deep-sea environments were subsequently invaded from shallow-water, with biodiversity increasing through differentiation and adaptive radiation (see Brown and Thatje 2014). Extant high latitude shallow-water species are preadapted to deep-sea thermal conditions through their cold-stenothermal lifestyle (Smith and Thatje 2012), and high-latitude bathymetric thermal profiles are not expected to limit migrations to greater depth in these regions (Tyler et al. 2000). In contrast, the constant low temperatures which dominate the deep sea are thought to inhibit colonisation by warm- or temperate-adapted shallow-water species (see Brown and Thatje 2014). Consequently, it has recently been suggested that ocean warming may facilitate bathymetric range extension into the deep-sea by shallow-water species (Brown and Thatje 2015). However, whilst increasing ocean temperature may allow increases in the depth range of shallow-water species, these species must also tolerate greater hydrostatic pressure in the deep sea (Brown and Thatje 2014; 2015).

Although temperature demonstrates strong latitudinal and bathymetric gradients, typically decreasing towards the poles and with depth, the bathymetric hydrostatic pressure gradient is constant, increasing by 0.1 MPa with every 10 m increase in depth (Gage and Tyler 1991). Synergistic effects of low temperature and high hydrostatic pressure are proposed to physiologically limit the potential bathymetric distribution of shallow-water species at a physiological bottleneck between 2,000 and 3,000 m depth (Brown and Thatje 2011; 2014). Thermodynamic effects of both temperature and hydrostatic pressure affect rates of biological processes and biochemical equilibria, although in contrasting ways (see Brown and Thatje 2014). Low temperatures reduce energy in systems reducing reaction rates. In contrast, high hydrostatic pressure can increase or decrease reaction rates: reactions that result in system volume increase are retarded, but reactions that result in system volume decrease are facilitated (Somero 1992). Low temperature and high hydrostatic pressure also increase structural ordering and decrease molecular flexibility in lipids (Balny et al. 2002). Consequently, the fluidity and permeability of membranes is reduced, which limits the movement of molecules across lipid bilayers, impeding crucial membrane functions such as cell signaling (Hazel and Williams 1990; Somero 1992). Deep-sea taxa are functionally adapted to counteract the physiological effects of high hydrostatic pressure and low temperature (Somero 1992; Hazel 1995). Evidently, the effects of both high hydrostatic pressure and low temperature must be overcome for survival in the present deep sea.

Examining temperature and hydrostatic pressure tolerance in shallow-water fauna may reveal constraints to range extension imposed by these factors and deliver insight into the mechanism of deep-sea colonisation (see Brown and Thatje 2014). Tolerance of low temperature and high hydrostatic pressure by shallow-water animals has previously been assessed to determine differences in physiological responses among species. The shallow-water shrimp *Palaemon varians* (recently identified as the senior synonym of *Palaemonetes varians*; De Grave and Ashelby 2013) has emerged as a model taxon for hyperbaric and thermal stress physiology, and ecotoxicology: studies have examined behavioural, respiratory, and molecular responses to acute and sustained temperature, hydrostatic pressure, and toxic metal exposures (Cottin et al. 2010; Oliphant et al. 2011; Cottin et al. 2012; Ravaux et al. 2012; Smith et al. 2013; New et al. 2014; Morris et al. 2015a; Morris et al. 2015b; Morris et al. 2015c; Brown et al. 2017a). *P*. *varians* has a close phylogenetic relationship to both hydrothermal vent shrimp species (Tokuda et al. 2006; Li et al. 2011), which constitute a key component of many active hydrothermal vent communities (Lunina and Vereshchaka, 2014), and non-vent deep-sea shrimp species (Tokuda et al. 2006; Li et al. 2011), which are important constituents of inactive vent and wider deep-sea communities (Boschen et al. 2015; Boschen et al. 2016). *P. varians* tolerates a wide range of temperature (0 °C to 36 °C) but also a wide range of hydrostatic pressure (≤21 MPa ≈ 2100 m depth), despite inhabiting shallow-water (0-10 m depth) (Cottin et al. 2010; Cottin et al. 2012; Oliphant et al. 2011; Ravaux et al. 2012; Smith et al. 2013; New et al. 2014; Morris et al. 2015a; Morris et al. 2015b; Morris et al. 2015c). Subsequently, it has been hypothesised that these tolerances may reflect an ancestral species’ physiological capability to colonise bathyal depths (Cottin et al. 2012; New et al. 2014). However, *P. varians* may not be representative of other shallow-water shrimp species: this species inhabits brackish waters with acutely variable temperature, salinity, and oxygen concentration, from Northern Europe to the coasts of Morocco and in the Mediterranean Sea (Hayward and Ryland 1995; Barnes 1994). The environmental variability may have contributed to the evolution of eurythermality, euryhalinity, and euryoxicity in *P*. *varians* (González-Ortegón et al. 2013) and it has been hypothesised that adaptation to a highly variable environment may also contribute to eurybaricity (Brown et al. 2017a). Species that inhabit less variable marine waters are typically less tolerant to variation in environmental conditions (González-Ortegón et al. 2013) and may, therefore, be less tolerant to hydrostatic pressure than suggested by studies on *P*. *varians*. Different hydrostatic pressure tolerances in shallow-water marine species may have implications for understanding both ecological and evolutionary processes. Consequently, the aim of this study was to assess acute hyperbaric tolerance and its interaction with temperature in *Palaemon serratus,* which inhabits a less variable shallow-water marine environment (intertidal and subtidal waters down to 40 m; Hayward and Ryland 1995), and determine whether this contrasts with acute hyperbaric tolerance and its interaction with temperature in *P. varians*.

**2. Materials and Methods**

***2.1 Sampling and maintenance***

Adult specimens of *Palaemon serratus* were collected from Calshot, Hampshire, UK (50°80.9 N, 1°31.8 W) between 20th March and 20th May 2015. Sampling was performed using hand-held nets in shallow-water (<1 m water depth) during low tide. *P. serratus* were placed inside 10 l buckets containing seawater from the point of collection. The shrimp were transported to the National Oceanography Centre Southampton, UK (NOCS) and were introduced to a recirculating seawater aquarium system set at the water temperature of the sampling location (Supplementary Material Fig. S1) and with 12h:12h light:dark photoperiod. Temperature was adjusted to 10 °C at a rate of 1 °C h-1. The shrimp were fed with Tetra Goldfish flakesthree times per week *ad libitum*, and were maintained for between 3 and 4 weeks before experimental treatments. The shrimp used in experiments were male and ranged in total length between 45 mm and 60 mm.

Prior to experimental treatments, the shrimp were transferred to 10 l tanks filled with filtered seawater (1-μm filtered; salinity 32.7), which were located in water baths. Following Oliphant et al. (2011), temperature was initially set at maintenance temperature (10 °C), and acclimated stepwise at a rate of 1 °C h-1 to the desired experimental temperature, which was maintained for a period of 3 days prior to experimental exposures. The shrimp were not fed during these 3 days to reduce potential variability in responses during experimental exposures due to differences in digestive state (Thatje and Robinson 2011).

***2.2 Critical thermal and pressure maxima***

Critical thermal maximum (CTmax) and critical pressure maximum (CPmax) treatments are commonly used indices of thermal and baric tolerance, and are determined based on indicative behaviours (see below) (e.g. Oliphant et al. 2011; Ravaux et al. 2012; González-Ortegón et al. 2013; New et al. 2014; see also Angilletta 2009). CTmax and CPmax treatments used the IPOCAMP hyperbaric system (Shillito et al. 2006) following established protocols (Oliphant et al. 2011; New et al., 2014). A temperature/hydrostatic pressure data logger (SP2T4000, NKE instrumentation) was used to record temperature and hydrostatic pressure during CTmax and CPmax treatments.

During CTmax treatments, the IPOCAMP system was maintained at 0.1 MPa (surface pressure) at all times. In each of three replicates, performed on consecutive days, ten shrimp were placed inside the PVC cage within the IPOCAMP (see Shillito et al. 2006). Following 1 h acclimation and recovery, the temperature within the system was increased at a constant rate by 0.29 °C min-1 from 10 °C to 35 °C (following New et al. 2014). The shrimp were removed from the IPOCAMP immediately following experimental treatments and preserved at -80 °C for subsequent measurement and sex determination. Animals were defrosted and total length was measured (from the tip of the rostrum to the posterior margin of the tail) (mm) using Vernier calipers. Animals were sexed by inspecting the shape of the appendix internal of the first pleopod and establishing the presence or absence of an appendix masculina on the second pleopod (Forster 1951), using an optic microscope (LEICA MZ 16).

CPmax treatment temperatures (5, 10, 15 °C) were selected to provide a range matching some of Oliphant et al.’s (2011) *P*. *varians* CPmax treatment temperatures (5, 10 °C) and without significant temperature effects (15 °C), determined based on CTmax treatment analysis). In each of three replicates at each temperature, performed on consecutive days, ten shrimp were placed inside the PVC cage within the IPOCAMP. Following 1 h acclimation and recovery, the hydrostatic pressure within the system was increased stepwise by 1 MPa (0.1 MPa = 10 m water depth) every 5 minutes to 30 MPa, and then decreased to 0.1 MPa in the same stepwise manner (following Oliphant et al. 2011). Following experimental treatments the shrimp were preserved at -80 °C for subsequent measurement and sex determination as described previously.

The behaviour of individual shrimp was determined using established protocols and criteria (Ravaux et al. 2003; Shillito et al. 2006; Oliphant et al. 2011; New et al. 2014; Morris et al. 2015c). For CTmax, behaviour was determined during the 30 s around each 1 °C increment (following New et al. 2014). For CPmax, behaviour was determined during the final 30 s at each hydrostatic pressure increment (following Oliphant et al. 2011). Four behaviour categories were used following previous studies (following Ravaux et al. 2003; Shillito et al. 2006; Oliphant et al. 2011; New et al. 2014; Morris et al. 2015c):

* Active movement (AM) – the shrimp was observed to swim or walk a distance greater than the length of its own body in less than 30 s.
* Movement – the shrimp was not observed to swim or walk a distance greater than the length of its own body in less than 30 s, but was observed to move antennae, maxillae, scaphognathites, pereopoda or pleopoda.
* Motionless – the shrimp was not observed to move.
* Loss of equilibrium (LoE) – the shrimp was observed in either a ‘sideways’ or ‘upside-down’ position for more than 2 s.

Whilst LoE could occur with another behaviour, AM, Movement, and Motionless were mutually exclusive behaviours. LoE was interpreted as an indicator of impaired function, and AM was interpreted as an indicator of stress (following Ravaux et al. 2003; Shillito et al. 2006; Oliphant et al. 2011; New et al. 2014; Morris et al. 2015c).

CTmax and CPmax were determined as the temperature or hydrostatic pressure at which 50% of the shrimp experienced LoE (following Ravaux et al. 2003; Shillito et al. 2006; Oliphant et al. 2011; New et al. 2014). Probit analysis was used to model LoE and deliver values for CTmax and CPmax: , where *X* is the exposure temperature or hydrostatic pressure and *Y* is the proportion of individuals demonstrating LoE (following New et al. 2014). Multiple pairwise comparisons of hydrostatic pressure treatments were used to identify which treatment temperatures resulted in significantly different LoE responses, with the family-wise error rate maintained using the Holm–Bonferroni correction.

The effect of temperature on AM was analysed using one-way analysis of variance (ANOVA) (following New et al. 2014). Arcsine-square-root transformation was necessary to achieve homogeneity of variance because AM data were proportional. The effect of hydrostatic pressure on AM and the influence of temperature on that effect were analysed using two-way ANOVA. Temperatures or hydrostatic pressures resulting in significant differences were determined using the post hoc multiple pairwise comparisons Holm-Sidak test.

***2.3 Respiratory response to hydrostatic pressure at different temperatures***

Respiratory responses to environmental challenges are an established measure reflecting environmental tolerance and bioenergetics (Sokolova et al. 2012). Following Oliphant et al. (2011), individual shrimp were isolated in 55 ml plastic vials filled with filtered seawater (1µm filtered; salinity 32.7) at experimental temperature (5, 10, 15 °C). Each vial was put inside a freshwater-filled hydrostatic pressure vessel (see Mestre et al. 2009) at the experimental temperature. The hydrostatic pressure vessel was pressurised to experimental hydrostatic pressure (0.1, 5, 10, 15, 20, 25, 30 MPa) and placed in a temperature-controlled incubator: pressurisation took ≤10 s. To reduce the potential for hypoxic effects on the respiratory capacity of the shrimp, exposure durations were adjusted so that oxygen concentrations within vials remained above 50% oxygen saturation throughout the experimental treatment. Isolation periods were reduced with increasing temperature, from 40 min at 5 °C to 30 min at 10 °C and 20 min at 15 °C, to compensate for thermal effects on metabolism. Hydrostatic pressure vessels were depressurised instantaneously at the end of the isolation period, and the vial was removed. A temperature-adjusted oxygen meter and micro-optode (Microx TX 3, PreSens, Regensburg, Germany; accuracy ±0.4% O2 at 20.9% O2, ±0.05% O2 at 0.2% O2) were used to measure seawater oxygen concentration inside the vial. The shrimp was removed from the vial following determination of oxygen concentration and preserved at -80 °C for subsequent total wet mass determination (mg), measurement, and sex determination. To determine mass, the shrimp were defrosted, blotted to remove excess water, and weighed (mg). Measurement and sex determination were performed as described previously. Five biological replicates were run at each combination of temperature and hydrostatic pressure. Three replicate blank vials, containing no animals, were run at each temperature/hydrostatic pressure combination. Oxygen consumption was calculated by subtracting the final oxygen concentration in experimental vials from the final oxygen concentration in blank vials (Thatje et al. 2010). The oxygen concentration of 100% oxygen-saturated seawater was calculated according Benson and Krause (1984).

Mass-specific oxygen consumption data were analysed using two-way ANOVA with temperature and hydrostatic pressure as factors. Significant differences were explored using the post-hoc Holm-Sidak multiple comparisons test.

**3. Results**

***3.1 Critical thermal and pressure maxima***

Observed Loss of Equilibrium data were not significantly different from LoE models (Goodness-of-fit Deviance *P* > 0.05; Table 1).

LoE and Active Movement depended on temperature during CTmax treatments (LoE probit analysis *p* < 0.001; AM one-way ANOVA *F*25,52 = 15.375, *p* < 0.001). LoE rose from 0% at 20 °C to 100% at 25 °C (Fig. 1): CTmax was 22.3 °C (Table 1). AM decreased with increasing temperature and was significantly lower at temperature ≥19 °C than at 10 °C. AM decreased from 100% at 10 °C to 56% ± 29 at 19 °C and to 0% at 30 °C.

LoE and AM depended on hydrostatic pressure during CPmax experiments with the effect of hydrostatic pressure on LoE and AM dependent on temperature, i.e. there was a significant interaction effect (LoE probit analysis *p* < 0.001; AM two-way ANOVA *F*60,186 = 6.907, *p* < 0.001). At 15 °C, LoE remained constant at 0% from 0.1 MPa to 8 MPa before increasing to 100% at 20 MPa and remaining constant to 30 MPa (Fig. 2). At 10 °C, LoE occurred at lower hydrostatic pressure (*p* < 0.001) and the gradient of the modelled LoE response was significantly greater (*p* < 0.001) than at 15 °C: LoE remained constant at 0% from 0.1 MPa to 7 MPa before increasing to 100% at 13 MPa and remaining constant to 30 MPa (Fig. 2). Similarly, at 5 °C LoE occurred at significantly lower hydrostatic pressure (*p* < 0.001) than at 10 °C, although the gradient of the modelled LoE response did not differ significantly from the gradient at 10 °C (*p* = 0.171): LoE remained constant at 0% from 0.1 MPa to 1 MPa before increasing to 100% at 9 MPa and remaining constant to 30 MPa (Fig. 2). CPmax decreased with decreasing temperature: CPmax was 14.1 at 15 °C, 10.1 at 10 °C, and 5.9 MPa at 5 °C (Table 1). At 15 °C and 10 °C, AM decreased with increasing hydrostatic pressure and was significantly lower at hydrostatic pressure ≥12 MPa than at 0.1 MPa. In contrast, AM did not differ significantly as hydrostatic pressure increased at 5 °C, although there was no AM at hydrostatic pressure ≥8 MPa. However, AM at 0.1 MPa depended on temperature: AM was significantly greater at both 15 °C and 10 °C than at 5 °C.

***3.2 Respiratory response to hydrostatic pressure at different temperatures***

The effect of hydrostatic pressure on respiration rate depended on temperature (*F*12,84 = 5.693, *p* < 0.001) (Fig. 3). At 15 °C, MO2 at 5 MPa was significantly lower than at 0.1 MPa, but MO2 at 10 and 15 MPa did not differ significantly from 0.1 MPa. At hydrostatic pressure ≥20 MPa MO2 was significantly lower than at 0.1 MPa. At 10 °C and 5 °C, MO2 did not differ significantly between 0.1 and 5 MPa but was significantly greater at 10 MPa than at 0.1 MPa. At 10 °C MO2 was significantly lower at hydrostatic pressure ≥15 MPa than at 0.1 MPa. In contrast, at 5 °C MO2 at 15 MPa remained significantly higher than at 0.1 MPa and was significantly lower than 0.1 MPa at hydrostatic pressure ≥20 MPa.

**4. Discussion**

Critical thermal and pressure maxima, and respiratory responses to temperature and hydrostatic pressure, were assessed in *Palaemon serratus* to constrain the thermal and baric limits of this species. CTmax was 22.3 °C in *P. serratus* acclimated to 10 °C for 3 weeks. Specimens of *P. serratus* were caught between April and May 2015 when the average daily environmental temperature ranged between 7.8 and 13.3 °C, thus the acclimation temperature was similar to the environmental temperature of April and May and is likely to represent *in situ* CTmax at this time. Remarkably, *P. serratus* acclimated to 10 °C were capable of tolerating at least acute exposure to the highest environmental temperature experienced by this population annually (21.4 °C). Acclimation influences capacity to tolerate high temperatures: CTmax is higher in warm-acclimated and summer-captured specimens in *P*. *varians* (Ravaux et al. 2012) and decreases during acclimation to low temperature (New et al. 2014). Thus it is likely that CTmax in summer-captured or warm-acclimated *P*. *serratus* in this population will be greater than in 10 °C acclimated individuals. Indeed, the CTmax of *P*. *serratus* collected in tidal pools on the Portuguese west coast at 20 °C and acclimated at 20 °C for 7 days was 33.0 °C (Vinagre et al. 2013). Whilst acclimation temperature and acclimation duration are likely to have a significant role in establishing differences in CTmax among populations of *P*. *serratus*, local adaptation may also contribute to shifts in thermal tolerance. Populations of *P*. *serratus* inhabiting tidal pools in the rocky shore on the Portuguese west coast are likely to experience greater variability in environmental temperature than the population examined in this study which inhabits an intertidal and subtidal sand and mud habitat, and adaptation to that greater environmental variability may contribute to the greater CTmax reported for the Portuguese population.

It is likely that acclimation temperature and duration contribute to interspecific differences in CTmax too. Thermal tolerance in warm acclimated populations of *P*. *serratus* and *P*. *varians* appears similar: the CTmax of *P*. *serratus* and *P*. *varians* differ little following sustained 20 °C acclimation (respectively 33 °C and 36 °C) (Ravaux et al. 2012; Vinagre et al. 2013), but differences in CTmax are much greater between 10 °C acclimated *P*. *serratus* (22.3 °C) and *P*. *varians* (31 °C; Oliphant et al. 2011). However, the difference in CTmax between cool acclimated *P*. *serratus* and *P*. *varians* and the similarity between CTmax in warm acclimated *P*. *serratus* and *P*. *varians* together suggest that the significant difference between these species relates to capacity to respond to acute environmental shifts rather than to capacity to acclimate *per se*. This difference is likely to result from adaptation to contrasting habitats: the examined *P. serratus* population inhabits the intertidal and subtidal zone, where temperature ranges between 5 °C in winter and 21 °C in summer and the maximum variation is only 2 °C in a 24 h period (bramblemet database), whereas *P. varians* inhabits salt marshes and brackish ponds and lagoons where the temperature ranges e.g. from 0 °C in winter to 33 °C in summer and can vary by >5 °C in less than a 12 h period (own unpublished data). Whether differences in capacity to respond to acute environmental shifts persist between populations of *P*. *varians* and populations of *P*. *serratus* inhabiting tidal pools in rocky shores is uncertain and requires further exploration.

Hydrostatic pressure tolerance was also significantly lower in *P*. *serratus* than in *P*. *varians*, but the effect of temperature on hydrostatic pressure tolerance was consistent among species: CPmax decreased with decreasing temperature (Figs. 2 and 4). The effects of high hydrostatic pressure and low temperature on biological systems are similar (see Brown and Thatje 2014), consequently low temperature increases the effects of high hydrostatic pressure in shallow-water marine invertebrates (e.g. Oliphant et al. 2011; Cottin et al. 2012; New et al. 2014; Morris et al. 2015a; Morris et al. 2015b). The lipid bilayers of biological membranes are pressure-sensitive (Somero 1992). High hydrostatic pressure increases structural order and decreases flexibility in lipids, carbohydrates, and proteins similarly to low temperature (Balny et al. 2002). Consequently, increasing hydrostatic pressure by 100 MPa has equivalent effects to decreasing temperature by 13–21 °C, depending on the composition of the membrane (Somero 1992). Decreased membrane fluidity adversely affects membrane processes such as neurotransmission, limiting systemic functioning (Siebenaller and Garrett 2002). Deep-sea species maintain membrane fluidity at low temperature and high hydrostatic pressure by accumulating higher levels of lipid, increased proportion of unsaturated fatty acids, and higher sterol or protein concentrations (Somero 1992). Similar, adjustments occur during homeoviscous acclimation in shallow-water fauna (Somero 1992; Hazel 1995; Winter and Dzwolak 2005). Such acclimatory shifts may result in increasing hydrostatic pressure tolerance, but this process is slow relative to acute experimental hydrostatic pressure exposures typically employed (see New et al. 2014). *P. varians*’ greater capacity to tolerate acute increases in hydrostatic pressure than the examined *P. serratus* population may therefore result from adaptive influences on physiology imposed by their contrasting habitats (Brown et al. 2017a). *P*. *varians* is significantly more tolerant of environmental (thermal, haline, oxic) challenge than caridean shrimp inhabiting fully marine or freshwater environments (González-Ortegón et al. 2013). Such adaptations may have preadapted *P*. *varians* to tolerate stressors that impose similar molecular challenges. For example, euryhalinity in crustaceans typically depends on osmoregulation rather than osmoconfomation and therefore tolerance of acute haline changes depends on maintenance of membrane processes (Hauton 2016). Membrane adaptations that provide high osmoregulatory capacity may deliver cross-tolerance to hydrostatic pressure exposure by mitigating hyperbaric effects on membrane processes (Brown and Thatje 2014; Brown et al. 2017a). Consequently, sustained hydrostatic pressure acclimation in *P*. *serratus* will be required to determine whether absolute hyperbaric tolerances differ between species.

Although behavioural responses in *P*. *serratus* demonstrate thermal effects on hyperbaric tolerance that are consistent with observations in *P*. *varians* (Oliphant et al. 2011), thermal effects on metabolic responses to hyperbaric exposures differ between these species. Significant increases in metabolic rate at high hydrostatic pressure likely represent increased homeostatic effort required to mitigate the hyperbaric effects on e.g. membrane function (Brown et al. 2017a,b). Increasing mitochondrial oxygen demand is typically not matched by increased respiratory capacity provided by increased ventilation and circulation (Frederich and Pörtner 2000). Mitochondrial respiration shifts from aerobic to anaerobic where mitochondrial oxygen demand exceeds respiratory capacity (Somero 2005). Ventilation and circulation may be limited by interference with neurotransmission (Brown et al. 2017a,b), or by the aerobic capacity of mitochondria at low temperatures or high hydrostatic pressures (Sommer and Pörtner 2002). Subsequent significant decreases in metabolic rate in response to hydrostatic pressure are proposed to represent impaired aerobic metabolism (Brown and Thatje 2011; Brown et al. 2017b). Whilst vital metabolic processes can be maintained beyond the critical threshold, non-essential processes such as reproduction, growth, feeding, and active movement are reduced (Peck et al. 2008; Sokolova et al. 2012). Decreases in active movement with increasing thermal and baric challenge in *P*. *serratus* (Figs. 1 and 2) may reflect this narrowing focus on essential maintenance activity. The strikingly different trends in active movement with increasing temperature and hydrostatic pressure in *P*. *varians* may reflect an escape behavioural adaptation that mitigates the effects of acute environmental changes in the highly variable salt marsh habitat *P*. *varians* occupies. Differences in the effect of hydrostatic pressure on metabolic rate suggest that metabolism may be approaching the limit of metabolic scope at 15 °C. The lack of significant increase in metabolic rate at elevated hydrostatic pressure at 15 °C contrasts with significant increases in metabolic rate at 10 °C and 5 °C. However, the hydrostatic pressure at which metabolic rate significantly decreases is not correlated with temperature in *P*. *serratus* (Fig. 3). The delayed onset of critical hyperbaric effects on metabolism at 5 °C and 15 °C relative to 10 °C may be due to cross-tolerance delivered by acclimation responses to temperature shifts away from the 10 °C maintenance temperature. Testing this hypothesis will require experiments exploring the effects of sustained thermal acclimation on metabolic responses.

Acute thermal and hyperbaric tolerance in the examined intertidal and subtidal shrimp *P*. *serratus* population is narrower than in the salt marsh and brackish-water shrimp *P*. *varians*, suggesting that adaptation to differing habitats has resulted in differing physiological tolerance to acute stress conditions. Nonetheless, these data support the proposition that the common ancestor of these species may have possessed the physiological capability to colonise bathyal depths (Cottin et al. 2012; New et al. 2014). Further, the consistent interaction between temperature and hydrostatic pressure tolerance in these species supports the suggestion that shallow-water species may have the capacity to deepen bathymetric distribution in response to ocean warming (Brown and Thatje 2015).

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**Ethical approval**

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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**Figures and Figure legends**

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**Fig. 1** Temperature tolerance of *Palaemon serratus* acclimated to 10 °C. Open and closed circles represent Active Movement and Loss of Equilibrium, respectively (mean + SD for clarity; n = 3). The solid line and dashed lines represent LoE and 95% confidence intervals modelled using probit analysis, assuming a logistic distribution.

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**Fig. 2** Pressure tolerance of *Palaemon serratus* acclimated to different temperatures. Open and closed circles represent Active Movement and Loss of Equilibrium, respectively (mean + SD for clarity; n = 3). The solid line and dashed lines represent LoE and 95% confidence intervals modelled using probit analysis, assuming a logistic distribution.



**Fig. 3** Respiratory response to pressure in *Palaemon serratus* acclimated to different temperatures. Closed circles represent molar oxygen consumption (MO2) (mean ± SD; n = 5). Significant differences within a temperature are indicated by letters: data that do not share a common letter are significantly different.



**Fig. 4** The effect of temperature on pressure tolerance in *Palaemon serratus* and *Palaemon varians*. Critical pressure maxima for *P*. *varians* were recalculated from Oliphant et al.’s (2011) raw data employing the statistical methods employed for *P.* *serratus*. Vertical lines indicate critical thermal maxima: dotted line *P*. *serratus*; dashed line *P*. *varians* (from Oliphant et al. 2011).

**Tables**

**Table 1** Critical Temperature maximum (CTmax) and Critical Pressure maximum (CPmax) Loss of Equilibrium (LoE) model parameters, with values for critical thresholds (CTmax or CPmax). Models were derived using probit analysis of LoE data, assuming a logistic distribution: , where is the exposure temperature or pressure and is the proportion of individuals demonstrating LoE (following New et al. 2014).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Experiment** | **Treatment** | **a** | **b** | **Goodness of fit****(Deviance *P*)** | **CTmax (°C) or****CPmax (MPa)** |
| CTmax |  | -23.4306 | 1.04913 | 0.422 | 22.3 |
| CPmax | 15 °C | -8.2036 | 0.05804 | 0.228 | 14.1 |
| 10 °C | -14.7685 | 0.14574 | 1 | 10.1 |
| 5 °C | -6.8660 | 0.11638 | 1 | 5.9 |