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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* are 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.

1. Introduction

The deep sea (depths > 200 m) covers > 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 2000 and 3000 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

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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 ($\leq$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 12 h:12 h light:dark photoperiod. Temperature was adjusted to 10 °C at a rate of 1 °C h^{-1}. The shrimp were fed with Tetra Goldfish flakes three 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 (CT_{max}) and critical pressure maximum (CP_{max}) 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). CT_{max} and CP_{max} 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 CT_{max} and CP_{max} treatments.

During CT_{max} 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).

CP_{max} treatment temperatures (5, 10, 15 °C) were selected to provide a range matching some of Oliphant et al.'s (2011) *P. varians* CP_{max} treatment temperatures (5, 10 °C) and without significant temperature effects (15 °C), determined based on CT_{max} 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 min 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 CT_{max} behaviour was determined during the 30 s around each 1 °C increment (following New et al., 2014). For CP_{max} 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