**Phospholipid fatty acids are correlated with critical thermal tolerance but not with critical pressure tolerance in the shallow-water shrimp *Palaemon varians* during sustained exposure to low temperature**

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**ABSTRACT**

Some extant deep-sea shrimp are known to be descended from shallow-water ancestors that adapted to environmental conditions (constant low temperature and high hydrostatic pressure) in the deep sea. During acclimation to low temperature and high hydrostatic pressure representative of the deep-sea, critical thermal tolerance decreases and critical pressure tolerance increases in the shallow-water shrimp *Palaemon varians*. It has been suggested that these shifts may depend in part on adjustments to phospholipid fatty acid composition and/or metabolic adjustments. Here, we present evidence that metabolic rate does not change during sustained exposure to low temperature (5°C) in the shallow-water shrimp *Palaemon varians*, and that metabolic rate and acute environmental tolerances are not correlated during sustained exposure to low temperature, suggesting that standard metabolic rate does not affect acute environmental tolerances. In contrast, we present evidence that phospholipid fatty acid composition does shift during sustained exposure to low temperature. Desaturation of fatty acids during sustained exposure to low temperature supports the suggestion that cell lipid bilayer homeoviscous modifications are important in low temperature acclimation. Shifts in several individual phospholipid fatty acids during sustained low temperature exposure are correlated with critical thermal tolerance. Exploring the greater complexity apparent in the responses of these phospholipid fatty acids to sustained low temperature exposures suggests a potential homeostatic impact moderating adverse impacts on nervous system function. However, shifts in phospholipid fatty acids are not correlated with critical pressure tolerance during exposure to low temperature, suggesting that shifts in critical pressure tolerance are related to modifications other than cell lipid bilayer composition.

**Key words:** adaptation; Caridea; deep sea; ecology; physiology, shallow water

**1. Introduction**

Some extant deep-sea faunas have evolved from ancestors that inhabited shallow water (see Brown & Thatje, 2014). However, the physiological effects of the deep-sea environment are a significant challenge to shallow-water species colonising the deep sea (Brown & Thatje, 2014). Elucidating physiological adaptations allowing shallow-water taxa to colonise diverse deep-sea environments and radiate to generate high biodiversity is of fundamental importance to predicting future shifts in bathymetric ranges in response to changing climate and the consequent impacts on deep-sea biodiversity (Brown & Thatje, 2015; Morris et al., 2015a). Recent studies exploring the effects of deep-sea conditions on shallow-water organisms have typically focused on hyperbaric impacts. However, organisms colonising the deep sea must also tolerate constant low temperatures.

Hydrostatic pressure and temperature both impact membrane functioning through effects on the fluidity of cell membranes (Hazel & Williams, 1990; Hazel, 1995). Adjustments to the phospholipid fatty acid composition of cell membranes (homeoviscous acclimation) are essential to continuing cell membrane function: phospholipid fatty acid composition affects cell membrane fluidity, governing the permeability of the membrane and cell membrane protein mobility and function (Guschina & Harwood, 2006). Indeed, higher lipid levels with an increased proportion of unsaturated fatty acids are evident in deep-sea taxa (Hazel & Williams, 1990). Similarly, shallow-water organisms increase the proportion of unsaturated fatty acids in cell membranes to maintain membrane fluidity at increased pressure or decreased temperature (Guschina & Harwood, 2006; Hazel & Williams, 1990; Hazel, 1995). However, modifications to membrane composition may be more complex than simply elevating the unsaturated fatty acid component of cell membranes; shifts in membrane composition may also be driven by the specific function of individual phospholipid fatty acids. For example, unexpected increases in individual saturated phospholipid fatty acids and decreases in individual unsaturated phospholipid fatty acids during hyperbaric acclimation in the shallow-water shrimp *Palaemon varians* appear focused on mitigating adverse hyperbaric impacts on nervous system function (Brown et al. 2019). Exploring shifts in phospholipid fatty acid composition during sustained exposure to low temperature may reveal both mechanisms through which low temperature impairs membrane function and mechanisms through which adverse impacts are mitigated.

Although essential membrane function may be maintained at low temperature and/or high hydrostatic pressure by maintaining membrane fluidity, membrane unsaturation can increase transmembrane leakage (Hazel & Williams 1990; Hazel 1995). Proton leakage across the inner mitochondrial membrane may increase oxygen demand and contribute to decreased critical thermal tolerance with low temperature acclimation (Pörtner 2001). However, metabolic costs associated with acclimation to low temperature may shift temperature envelopes too. Acute thermal tolerance appears limited through inability to meet increased mitochondrial oxygen demand imposed by elevated homeostatic effort in response to the effects of temperature with respiratory capacity delivered through ventilation and circulation (e.g. Frederich & Pörtner, 2000). Thus, increased metabolic effort focused on acclimation to low temperature likely reduces acute thermal tolerance by decreasing the temperature at which oxygen demand outstrips the capacity of ventilation and circulation to supply oxygen. Mismatch between oxygen supply and demand appears similarly fundamental to hyperbaric limitation (e.g. Munro et al. 2015; Brown et al., 2017b). Exploring correlations between oxygen consumption and reported critical thermal tolerance or critical hyperbaric tolerance during sustained low temperature exposure may therefore reveal the contribution of metabolic costs associated with acclimation to low temperature to acute temperature and hydrostatic pressure tolerance. Similarly, exploring correlations between phospholipid fatty acids and critical thermal tolerance or critical hyperbaric tolerance may reveal the contribution of homeoviscous acclimation to acute thermal and hyperbaric tolerance. These approaches are contingent on selection of suitable study taxa.

The shallow-water species *Palaemon* *varians* (the senior synonym of *Palaemonetes varians*; De Grave & Ashelby, 2013) has been developed as a model for thermal, hyperbaric, and ecotoxicological stress physiology. Experiments have assessed responses (survival, behavioural, respiratory, and molecular) to acute and sustained exposures to temperature, hydrostatic pressure, and toxic metals (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; Brown & Hauton, 2018; Pallareti et al. 2018; Brown et al., 2019; Mestre et al., 2019). *P*. *varians*’ tolerates sustained exposure to hydrostatic pressure equivalent upper bathyal depths (10 MPa ≈ 1000 m depth) (New et al., 2014; Cottin et al., 2012; Morris et al., 2015a) and *P*. *varians* is therefore an appropriate model for exploring the effects of exposure to deep-sea environmental conditions. Further, extant deep-sea shrimp putatively evolved from shallow-water shrimp that colonised the deep-sea following dysoxic mass extinction events (Sun et al., 2018). *P*. *varians* therefore also appears an appropriate species to compare with deep-sea shrimp (e.g. Gonzalez-Rey et al. 2006, 2007; Smith et al., 2013; Brown et al. 2017a; Mestre et al., 2019).

Studies exploring the physiological effects of low temperature and/or high hydrostatic pressure in *P*. *varians* have focused on exploring hypotheses relating to the colonisation of the deep continental margins and/or deep-sea hydrothermal vent environments by shallow-water fauna (i.e. 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; Brown & Hauton, 2018; Brown et al., 2019; Mestre et al., 2019). Oliphant et al. (2011) demonstrated that *P*. *varians* tolerates sustained exposure to lower temperatures than are representative of deep continental margins (i.e. 0°C) and subsequent studies therefore focused on exploring thermal tolerance in the context of hydrothermal vent colonisation, assessing critical thermal maximum (CTmax) rather than critical thermal minimum (CTmin). The effect of acclimation to low temperature on CTmax has been assessed (New et al., 2014), thus CTmax data rather than CTmin data are available to explore associations with metabolic rate and phospholipid fatty acid composition during sustained exposure to low temperature. Critical hyperbaric tolerance (CPmax) has been assessed at a range of temperatures (Oliphant et al., 2011) and the effect of acclimation to low temperature on CPmax has been explored (New et al., 2014), thus CPmax data are also available to explore associations with metabolic rate and phospholipid fatty acid composition during sustained exposure to low temperature.

Increases in hyperbaric tolerance and decreases in thermal tolerance have been reported in *P*. *varians* in response to high hydrostatic pressure and may be driven by homeoviscous acclimation (New et al., 2014; Brown et al., 2019). Similarly, acclimation to low temperature increases hyperbaric tolerance and decreases thermal tolerance in *P*. *varians* (New et al., 2014). However, concurrent shifts in cell membrane lipid bilayer composition during sustained exposure to low temperature acclimation have not yet been explored in this species. Whether shifts in lipid composition are similar during exposure to high hydrostatic pressure and low temperature is therefore uncertain. Shifts in metabolic rate during sustained exposure to low temperature have not yet been explored in *P*. *varians* either. Consequently, the aim of this study is to resolve uncertainty relating to low temperature homeoviscous and metabolic acclimation by exploring cell membrane lipid bilayer composition and metabolic rate in *P*. *varians* during sustained exposure to low temperature.

**2. Materials and Methods**

**2.1 Capture and maintenance of *Palaemon varians***

*Palaemon varians* were sampled, transferred, and maintained as described by New et al. (2014). In brief, ~600 adult *P*. *varians* were hand-netted in Lymington salt marshes (UK) in October, 2011 and were transported to the National Oceanography Centre Southampton (NOCS) in 10 l buckets containing water from the point of collection. Shrimp were transferred to 10 l aquaria containing 15°C and 32.7 salinity seawater at the NOCS, and acclimated to 15°C with 12:12 photoperiod for 30 days. Aeration was provided using air pumps and bubble stones. Subsequently, the temperature was reduced to 5°C. Throughout maintenance, shrimp were fed three times per week with Tetra Goldfish flakes to excess, with water changes conducted on days following feeding (>50%; 15°C, 32.7 salinity, 1 µm-, UV-filtered seawater). To explore the effects of increasing acclimation time on metabolic rate and phospholipid fatty acid composition, oxygen consumption and phospholipid fatty acid composition were determined after 30 days acclimation to 15°C, and then 2, 10, 23, 51, and 79 days after temperature was reduced to 5°C.

**2.2 Effect of low temperature acclimation on metabolic rate**

To minimise potential variability in respiration rate resulting from differences in digestive state, shrimp were starved for 3 days before oxygen consumption rates were measured. An adaptation of existing protocols described by Thatje et al. (2010) and Brown et al. (2017a) was used to determine oxygen consumption rates. In brief, 5 individuals were transferred to 33 ml plastic vials filled with water from maintenance conditions. Vials were closed underwater to ensure the absence of air bubbles and incubated for 20 minutes (15°C) or 40 minutes (5°C). Oxygen concentration within the vial remained above 50% oxygen saturation, minimising potential for metabolic influences of hypoxia in *P*. *varians* (Nielsen & Hagerman, 1998; Peruzza et al. 2018). To control for microbial respiration within the seawater, 5 control vials containing only seawater were isolated for each treatment using an identical procedure.

Following the isolation period, each vial was inverted three times to ensure homogeneity of seawater oxygen within the vial. The lid of the vial was removed and an oxygen micro-optode connected to a PreSens Microx TX3 array, calibrated according to the manufacturer’s instructions was used to measure seawater oxygen saturation. Subsequently, the shrimp was removed from the vial, blotted gently on tissue paper, weighed, transferred to a 1.5 ml centrifuge tube, and flash frozen in liquid nitrogen. Samples were flash frozen within 10 minutes of departure from experimental condition and were stored at -80°C for subsequent biochemical analysis. The difference between the oxygen saturation in the treatment vials and the oxygen saturation in the control vials was used to calculate molar oxygen consumption (MO2, µmol O2 mg-1 h-1), employing an established equation to calculate oxygen concentration in air-saturated seawater (Benson & Krause, 1984).

**2.2.1 Statistical analysis**

Statistically significant differences in oxygen consumption were assessed by one-way ANOVA with time as the fixed factor (α = 0.05), and significant differences were explored using Holm-Sidak post-hoc comparisons. Associations between mean metabolic rate and critical thermal and hyperbaric tolerances reported by New et al. (2014) were assessed using Pearson’s Product Moment Correlation Coefficient. Specifically, mean metabolic rates at sampling points were compared with CTmax or CPmax reported at sampling points (New et al. 2014). For clarity, New et al. (2014) established CTmax by exposing shrimp to constant increase in temperature (0.26°C min-1), assessing behaviour for the 15 seconds before and after each 1°C increment, and analysing the temperature at which 50% of shrimp demonstrated loss of function by modelling the loss of equilibrium response using probit analysis assuming a logistic distribution. Similarly, New et al. 2014 established CPmax by exposing shrimp to stepwise increase in pressure (1 MPa 5 min-1), assessing behaviour for the final 30 seconds at each pressure increment, and analysing the pressure at which 50% of shrimp demonstrated loss of function by modelling the loss of equilibrium response using probit analysis assuming a logistic distribution.

**2.3 Phospholipid fatty acid composition**

Phospholipid fatty acid composition was determined using the protocol described by Brown et al. (2019). In brief, 1.5 ml centrifuge tubes containing individuals (n = 3 per treatment, selected at random) were removed from -80°C storage and shrimp were rapidly separated into head and abdomen sections with single clean cuts made vertically from the dorsal extension of the carapace. Lower magnitude responses to high hydrostatic pressure have been reported for the head of *P*. *varians* than for its abdomen (Morris et al., 2015c), therefore the abdomen sections were selected for analysis in this study (following Brown et al. 2019). An IKA T10 Basic Ultra-Turrax homogenizer was used to homogenise individual abdomen sections were in chloroform:methanol (2:1, v/v). Total lipid was extracted following Folch et al. (1957). Aqueous potassium chloride (0.88%, w/v) was added to samples, which were thoroughly vortexed and then centrifuged for 5 min at 1500 rpm to establish a biphasic system. Non-lipid substances were removed via pipetting and discarding the upper phase. The lower phase (lipid in chloroform) was filtered into pre-weighed vials through a Whatman No.1 filter paper, pre-washed with chloroform:methanol 2:1 v/v. An N-EVAP system (Organomation) was used to dry samples under N2 gas. Samples were then placed in a desiccator for 1 hour and total lipid mass was established by weighing. Samples were redissolved in chloroform to a final concentration of 1 mg ml-1 and then stored at -20˚C.

Thin layer chromatography was used to purify phospholipids. 1 mg aliquots of total lipid were run on 2 mm, 20×20 cm silica gel plates in a hexane:diethyl ether:acetic acid (80:20:2 v/v/v) solvent system. Plates were sprayed with 2’,7’dichloro-fluorescein in methanol (0.1%, w/v) to allow visualisation of lipids under ultraviolet light. Phospholipids were scraped off plates into vials in which trans-methylation reactions were established following Christie (1982). Scrapings were dissolved in 1% sulphuric acid in methanol and toluene (1:2 v/v) and incubated at 50 ˚C for 16 hours. Subsequently, equal volumes of deionised milli-Q water and hexane:diethyl ether (1:1 v/v) were added and samples were thoroughly mixed by vortexing. Centrifuging for 2 min at 1500 rpm established a biphasic system. The upper phase was removed into a vial and aqueous sodium bicarbonate (2% w/v) was added. Thorough mixing was ensured by vortexing. Centrifuging for 2 min at 1500 rpm established a biphasic system. After removal into vials, the upper solvent fraction was dried under N2 gas. This purified phospholipid fraction was redissolved in ~0.1 ml hexane. The solution was run through a TRACE2000, Thermo Electron, Gas Chromatograph (Thermo Scientific, UK) equipped with a Restek Stabilwax column (0.32 mm i.d. x 30 m) with hydrogen used as the carrier gas (Pond et al., 2014).

**2.3.1 Statistical analysis**

Phospholipid fatty acid data were expressed as percent composition of total fatty acids. Consequently, phospholipid fatty acid data were proportional and were arcsine-square-root transformed. The relationship between duration of exposure to 5°C and percent composition of total fatty acids was explored by linear regression (α = 0.05) to test predictions based on the concept of homeoviscous acclimation. Where there was no significant linear relationship between duration of exposure to 5°C and percent composition of total fatty acids, differences among durations were assessed by one-way ANOVA with duration of exposure to 5°C as the fixed factor (α = 0.05). Significant differences were explored using Holm-Sidak post-hoc comparisons. Associations between phospholipid fatty acid composition and environmental tolerances – critical thermal maximum and critical pressure maximum – reported by New et al. (2014) were assessed using Pearson’s Product Moment Correlation Coefficient. Specifically, different components of phospholipid fatty acid composition at sampling points were compared with CTmax or CPmax reported by New et al. (2014) at sampling points.

**3. Results**

**3.1 Effect of low temperature acclimation on metabolic rate**

Oxygen consumption decreased significantly with the decrease from 15°C to 5°C, but was not affected by duration of exposure to low temperature (F5,24 = 24.650, p < 0.001) (Fig. 1). Critical temperature tolerance was not correlated with oxygen consumption when 15°C data were included (p = 0.468, r2 = 0.186), or when 15°C data were excluded (p = 0.102, r2 = 0.493) (Fig. 2). Critical pressure tolerance was correlated with oxygen consumption when 15°C data were included (p = 0.002, r2 = 0.937), but not when 15°C data were excluded (p = 0.216, r2 = 0.449) (Fig. 2).

**3.2 Phospholipid fatty acid composition**

Neither total saturated fatty acids nor total unsaturated fatty acids were affected by duration of exposure to low temperature (Fig. 3). In contrast, total monounsaturated fatty acids increased with duration of exposure to low temperature, and total polyunsaturated fatty acids decreased with duration of exposure to low temperature. However, neither total monounsaturated fatty acids or total polyunsaturated fatty acids correlated with CTmax or CPmax reported by New et al. (2014) (Fig. 4). ANOVA indicated significant differences in percentage composition values between acclimation time points for total unsaturated fatty acids (Fig. 3).

Compositional changes in individual phospholipid fatty acid species, in response to low temperature acclimation, were more complex than saturation category. Of the 20 phospholipid fatty acids analysed here for *Palaemon varians*, the percentage composition of six showed significant linear relationships with low temperature acclimation duration (Fig. 3). For five of these phospholipid fatty acids, significant correlations were observed between percentage composition values and CTmax (Fig. 4).

During acclimation to low temperature, percentage composition values for the saturated fatty acid, pentadecanoic acid (15:0), and the unsaturated fatty acids, hexadecatrienoic acid (16:3) and hexadecatetraenoic acid (16:4n-1) decreased (Fig. 3). The percentage composition values for these species were positively correlated with CTmax values reported by New et al. (2014), so that greater proportions of these phospholipid species were associated with higher CTmax values (Fig. 4). In contrast, percentage composition values for the saturated fatty acid eicosanoic acid (20:0) and the unsaturated fatty acids eicosenoic acid (20:1n-9) and octadecoic acid (18:1n-9) increased during low temperature acclimation (Fig. 3). For eicosanoic acid (20:0) and eicosenoic acid (20:1n-9) percentage composition values were negatively correlated with CTmax values, so that lesser proportions of these phospholipid species were associated with higher CTmax values (Fig. 4). However, no correlation was evident for octadecoic acid (18:1n-9) (Fig. 4). None of the phospholipid species for which a linear relationship was demonstrated between percentage composition and low temperature acclimation were correlated with CPmax (New et al. 2014) (Fig. 4).

For several phospholipid fatty acids no linear relationship was evident between percentage composition values and duration of low temperature acclimation. However, ANOVA indicated significant differences in percentage composition values between acclimation time points for the unsaturated fatty acid species: 18:1n-7; 20:4n-6; 20:5n-3; 22:6n-3; 24:1n-9 (Fig. 3). ANOVA also indicated that there were differences among exposure durations in both 18:2n-6 and 22:0, but Holm-Sidak post-hoc comparisons did not support this.

**4. Discussion**

Species adapted to extreme low temperature and high hydrostatic pressure typically display homeoviscous adaptations in the cell membrane bilayer, maintaining membrane fluidity and thus function under these conditions (Hazel and Williams, 1990). Consequently, homeoviscous shifts have been proposed as a mechanism of acclimation to low temperature and high hydrostatic pressure (Hazel and Williams, 1990). In low temperature and high hydrostatic pressure conditions cell lipid bilayers typically shift the proportion of saturated fatty acids downward and the proportion of mono- and poly-unsaturated fatty acids upward.

Small but statistically significant trends in phospholipid fatty acid composition occur in *P. varians* during acclimation to 5°C, but these trends were inconsistent with the simple prediction based on the homeoviscous acclimation hypothesis that the proportion of saturated fatty acids in cell membranes decreases in response to low temperature whilst the proportion of unsaturated fatty acids increases. There was no trend in total saturated phospholipid fatty acids or total unsaturated phospholipid fatty acids. In contrast, there was a decreasing trend in total monounsaturated phospholipid fatty acids and an increasing trend in total polyunsaturated phospholipid fatty acids. Increasing unsaturation of fatty acids achieved by decreasing monounsaturated fatty acids and increasing polyunsaturated fatty acids may result in increased membrane fluidity (Hazel & Williams, 1990; Hazel, 1995). However, neither total monounsaturated phospholipid fatty acid nor total polyunsaturated fatty acid were correlated with critical tolerances.

Greater complexity exists among shifts in individual saturated and unsaturated fatty acids during low temperature acclimation. 15:0 (pentadecanoic acid) decreased during acclimation to low temperature, whereas 20:0 (eicosanoic acid) increased. Both 18:1n-9 (octadecenoic acid) and 20:1n-9 (eicosenoic acid) increased during acclimation to low temperature, whilst both 16:3 (hexadecatrienoic acid) and 16:4n-1 (hexadecatetraenoic acid) decreased. Critical thermal tolerance (CTmax) during exposure to low temperature, reported by New et al. (2014), was correlated with each of these phospholipid fatty acids, with the exception of octadecenoic acid (18:1n-9). These correlations suggest that the reason for contrasting trends may be related to the specific functions of individual phospholipid fatty acids. There were also significant differences which were not part of a constant trend: 18:1n-7 (octadecenoic acid), 20:4n-6 (eicosatetraenoic), 20:5n-3 (eicosapentaenoic acid), 22:6n-3 (docosahexaenoic acid), and 24:1n-9 (tetracosenoic acid) all varied during exposure to low temperature. These shifts in fatty acid composition may relate to individual phospholipid fatty acid function too and suggest that responses to low temperature are more complex than simple trends in the proportion of individual fatty acids in cell membranes.

The increase in eicosanoic acid (20:0) during exposure to low temperature may promote activation of toll-like receptors (Milanski et al. 2009), which influence production of cytokines and thus the broad inflammatory response to environmental insult (De Nardo 2015). Shifts in other fatty acids may be more specific responses to challenge. The decrease in pentadecanoic acid (15:0) during acclimation to low temperature may relate to impaired membrane protein function at low temperature, such as Na+ K+ ATPase (Kong et al. 2012). Na+ K+ ATPase is important in maintaining Ca2+ homeostasis in crustaceans (Freire et al. 2008) and maintains membrane potential necessary to establish action potential in cells modulating cardiac function (Lodish et al. 2003). Pentadecanoic acid promotes Ca2+ sequestration by components of the endoplasmic reticulum (Rys-Sikora 1998). Thus, the decrease in pentadecanoic acid may mitigate the effect of impaired Na+ K+ ATPase function.

Increase in oleic acid (18:1n-9) and eicosenoic acid (20:1n-9) during exposure to low temperature may also be related to adverse effects on nervous function. Oleic acid can be elongated to eicosenoic acid which can then be elongated to tetracosenoic acid (24:1n-9) (Tvrzicka et al., 2011). During nerve biosynthesis, tetracosanoic acid is incorporated in the myelin sheath of nerve fibres (Stoffel & Bosio 1997) and tetracosenoic acid increased significantly between 9 days and 23 days exposure to low temperature. Similarly, the significant decrease in eicosapentaenoic acid (20:5n-3) between 2 days and 9 days exposure to low temperature and the significant increase in docosahexaenoic acid (22:6n-3) at the same time, may be related to nerve biosynthesis. Eicosapentaenoic acid can be elongated through intermediates to form docosahexaenoic acid, which is a significant constituent of neuronal plasma membranes that maximises neural impulse speed and energy efficiency (Valentine & Valentine, 2012). The synthesis of both eicosapentaenoic acid and docosahexaenoic acid is modulated by temperature and hydrostatic pressure (Valentine & Valentine, 2004).

Homeostatic focus on mitigating the adverse impacts of temperature on neurofunction may also explain response in eicosatetraenoic acid (20:4n-6). Eicosatetraenoic acid decreased significantly between 2 days and 9 days exposure to low temperature, remaining at a similar level after 23 days exposure, before increasing by 51 days exposure. Eicosatetraenoic acid is the precursor to eicosanoids (e.g. prostaglandins, thromboxanes, and leukotrienes) which are involved in regulating cell growth and the nervous system (Needleman et al., 1988, Calder, 2015). Eicosatetraenoic acid influences cell signalling, interacting with voltage- and ligand-gated ion channel functions (Antollini and Barrantes, 2016). For example, eicosatetraenoic acid synthesis is linked to NMDA receptor activity (Dumuis et al., 1988). NMDA receptors mediate excitatory neurotransmission (Dingledine et al., 1999) and the release of eicosatetraenoic acid potentiates NMDA receptor currents (Miller et al., 1992). Transcription for NMDA receptors is reported to decrease in crustaceans in response to reduced temperature (Stillman and Tagmount, 2009) and NMDA receptor regulation is thought to be involved in limitation of physiological tolerance to low temperature (Brown and Thatje, 2018).

Specific functions of hexadecatrienoic and hexadecatetraenoic acids are unclear. Despite this, the decrease in hexadecatetraenoic acid in *P*. *varians* in response to low temperature is particularly interesting, contrasting with the reported increase in hexadecatetraenoic acid in *P*. *varians* in response to high hydrostatic pressure (Brown et al., 2019). Indeed, few fatty acids shifted both during acclimation to high hydrostatic pressure (Brown et al., 2019) and during acclimation to low temperature. Nonetheless, detailed consideration of the specific roles of the individual phospholipid fatty acids indicates a potential homeostatic focus on moderating adverse impacts on nervous system function in response to high hydrostatic pressure (Brown et al., 2019) or low temperature. However, whilst CTmax was correlated with phospholipid acids during low temperature acclimation, critical pressure tolerance (CPmax) reported by New et al. (2014) was not, suggesting that the dominant mechanism through which high hydrostatic pressure and low temperature affect neurofunction may differ.

Sustained remodelling of membrane composition at low temperature also suggests that membrane composition remodelling at high hydrostatic pressure may be greater than previously reported. Studies examining the effect of acclimation to high hydrostatic pressure on phospholipid fatty acid composition have been of relatively short duration (< 10 days) and have not explored the impact of nutrition (e.g., Brown et al., 2017b; Brown et al., 2019) due to the inability to introduce food into hyperbaric aquaria (but see Shillito et al., 2020). Remodelling membrane phospholipid fatty acids at high pressure may occur over longer timescales than those examined or may depend on assimilation of dietary phospholipids. Studies on the effects of sustained hyperbaric exposure on phospholipid fatty acid composition and on nutritive effects during hyperbaric exposures are required to resolve outstanding uncertainties regarding acclimation to high hydrostatic pressure. Shifts in tolerance to both high hydrostatic pressure and low temperature may also relate to other acclimation mechanisms. For example, despite relatively constant metabolic rate during sustained exposure to low temperature suggesting that metabolic costs do not change significantly, elevated mitochondrial densities and adjustments in mitochondrial functional properties during acclimation to low temperature may affect critical thermal tolerance (Pörtner, 2010) or critical hyperbaric tolerance (Brown & Thatje, 2015). Such responses may not be detected in standard metabolic rate measured here and thus remain uncertainties that require exploration.

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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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Valentine, R.C., Valentine, D.L., 2012. *Neurons and the DHA principle*. CRC Press, Boca Raton.**FIGURES AND FIGURE LEGENDS**

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**Fig. 1** Metabolic rate of *Palaemon varians* at 15°C before exposure to low temperature (5°C), and at 5°C during exposure to low temperature (mean ± SD, n = 5).

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**Fig. 2** Relationship between mean oxygen consumption and critical temperature tolerance (CTmax) or critical pressure tolerance (CPmax) in *Palaemon varians*. CTmax was not correlated with oxygen consumption when 15°C data were included (p = 0.468, r2 = 0.186), or when 15°C data were excluded (p = 0.102, r2 = 0.493). CPmax was correlated with oxygen consumption when 15°C data were included (p = 0.002, r2 = 0.937), but not when 15°C data were excluded (p = 0.216, r2 = 0.449).

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**Fig. 3** Phospholipid fatty acid composition of *Palaemon varians* at 15°C before exposure to low temperature (5°C), and at 5°C during exposure to low temperature.Bars and error bars represent mean ±SD; n = 3. Phospholipid fatty acids in bold demonstrated either significant linear relationship with duration of exposure to low temperature (indicated by regression line) or significant differences among durations (indicated by letters: data that do not share a common letter are significantly different).

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**Fig. 4** Relationship between phospholipid fatty acid composition and (**A**) critical temperature tolerance (CTmax) or (**B**) critical pressure tolerance (CPmax). Phospholipid fatty acids in bold demonstrated significant correlation with critical environmental threshold (indicated by line of best fit).

**TABLES**

**Table 1**

Statistical analysis of phospholipid fatty acid during exposure to low temperature association with critical temperature maximum (CTmax) and critical pressure maximum (CPmax). Linear regression was used to identify individual components of phospholipid fatty acid with a linear relationship with duration of exposure to low temperature. Where no linear relationship was identified, ANOVA was used to explore variation in individual components of phospholipid fatty acid during exposure to low temperature. Subsequently, associations between individual components of phospholipid fatty acid and critical temperature or pressure maxima were assessed using Pearson’s Product Moment Correlation Coefficient. Significant relationship, variation, or correlation, is highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Phospholipid fatty acid | Linear regression | ANOVA | Correlation with | |
|  |  |  | CTmax | CPmax |
| Total saturated | *F*1,16 = 0.036, *p* = 0.851, *r*2 < 0.001 | *F*5,12 = 2.914, *p* = 0.060 | *p* = 0.474, *r*2 = 0.032 | *p* = 0.338, *r*2 = 0.058 |
| Total unsaturated | *F*1,16 = 1.528, *p* = 0.234, *r*2 = 0.030 | ***F*5,12 = 4.039, *p* = 0.022** | *p* = 0.829, *r*2 = 0.003 | *p* = 0.537, *r*2 = 0.024 |
| Total monounsaturated | ***F*1,16 = 10.237, *p* = 0.006, *r*2 = 0.352** |  | *p* = 0.318, *r*2 = 0.063 | *p* = 0.642, *r*2 = 0.014 |
| Total polyunsaturated | ***F*1,16 = 5.803, *p* = 0.028, *r*2 = 0.220** |  | *p* = 0.233, *r*2 = 0.088 | *p* = 0.872, *r*2 = 0.002 |
| Doceanoic (12:0) | *F*1,16 = 0.969, *p* = 0.340, *r*2 < 0.001 | *F*5,12 = 1.634, *p* = 0.225 | *p* = 0.116, *r*2 = 0.147 | *p* = 0.112, *r*2 = 0.150 |
| Tetradecanoic (14:0) | *F*1,16 = 1.232, *p* = 0.283, *r*2 = 0.014 | *F*5,12 = 1.571, *p* = 0.241 | *p* = 0.915, *r*2 = 0.001 | *p* = 0.742, *r*2 = 0.007 |
| Pentadecanoic (15:0) | ***F*1,16 = 12.615, *p* = 0.003, *r*2 = 0.406** |  | ***p* =** **0.016, *r*2 = 0.310** | *p* = 0.241, *r*2 = 0.085 |
| Hexadecanoic (16:0) | *F*1,16 = 0.395, *p* = 0.539, *r*2 < 0.001 | *F*5,12 = 2.229, *p* = 0.118 | *p* = 0.375, *r*2 = 0.049 | *p* = 0.898, *r*2 = 0.001 |
| Hexadecenoic (16:1n-7) | *F*1,16 = 1.887, *p* = 0.188, *r*2 = 0.050 | *F*5,12 = 1.224, *p* = 0.356 | *p* = 0.321, *r*2 = 0.062 | *p* = 0.683, *r*2 = 0.011 |
| Hexadecatrienoic (16:2) | *F*1,16 = 3.012, *p* = 0.102, *r*2 = 0.106 | *F*5,12 = 1.161, *p* = 0.383 | *p* = 0.186, *r*2 = 0.106 | *p* = 0.493, *r*2 = 0.030 |
| Hexadecatetraenoic (16:3) | ***F*1,16 = 21.483, *p* < 0.001, *r*2 = 0.546** |  | ***p* =** **0.006, *r*2 = 0.388** | *p* = 0.388, *r*2 = 0.047 |
| Hexadecatetraenoic (16:4n-1) | ***F*1,16 = 32.481, *p* < 0.001, *r*2 = 0.649** |  | ***p* = 0.001, *r*2 = 0.534** | *p* = 0.124, *r*2 = 0.141 |
| Octadecanoic (18:0) | *F*1,16 = 0.983, *p* = 0.336, *r*2 < 0.001 | *F*5,12 = 2.695, *p* = 0.074 | ***p* = 0.008, *r*2 = 0.366** | *p* = 0.112, *r*2 = 0.151 |
| Octadecenoic (18:1n-9) | ***F*1,16 = 9.071, *p* = 0.008, *r*2 = 0.322** |  | *p* = 0.307, *r*2 = 0.065 | *p* = 0.209, *r*2 = 0.097 |
| Octadecenoic (18:1n-7) | *F*1,16 = 1.874, *p* = 0.190, *r*2 = 0.049 | ***F*5,12 = 7.594, *p* = 0.002** | *p* = 0.947, *r*2 < 0.001 | *p* = 0.607, *r*2 = 0.017 |
| Octadecadienoic (18:2n-6) | *F*1,16 = 0.466, *p* = 0.505, *r*2 < 0.001 | ***F*5,12 = 4.349, *p* = 0.017** | *p* = 0.444, *r*2 = 0.037 | *p* = 0.194, *r*2 = 0.103 |
| Eicosanoic (20:0) | ***F*1,16 = 20.669, *p* < 0.001, *r*2 = 0.536** |  | ***p* = 0.032, *r*2 = 0.253** | *p* = 0.092, *r*2 = 0.166 |
| Eicosenoic (20:1n-9) | ***F*1,16 = 7.455, *p* = 0.015, *r*2 = 0.275** |  | ***p* = 0.033, *r*2 = 0.255** | *p* = 0.984, *r*2 < 0.001 |
| Eicosatetraenoic (20:4n-6) | *F*1,16 = 0.018, *p* = 0.895, *r*2 < 0.001 | ***F*5,12 = 7.770, *p* = 0.002** | *p* = 0.363, *r*2 = 0.019 | *p* = 0.581, *r*2 = 0.020 |
| Eicosapentaenoic (20:5n-3) | *F*1,16 = 1.800, *p* = 0.198, *r*2 = 0.050 | ***F*5,12 = 8.255, *p* = 0.001** | *p* = 0.039, *r*2 = 0.239 | *p* = 0.986, *r*2 < 0.001 |
| Docosanoic (22:0) | *F*1,16 = 2.729, *p* = 0.118, *r*2 = 0.092 | ***F*5,12 = 3.119, *p* = 0.049** | *p* = 0.473, *r*2 = 0.033 | *p* = 0.278, *r*2 = 0.073 |
| Docosapentaenoic (22:5n-3) | *F*1,16 = 0.089, *p* = 0.769, *r*2 < 0.001 | *F*5,12 = 2.003, *p* = 0.146 | *p* = 0.554, *r*2 = 0.023 | *p* = 0.396, *r*2 = 0.045 |
| Docosahexaenoic (22:6n-3) | *F*1,16 = 0.063, *p* = 0.805, *r*2 < 0.001 | ***F*5,12 = 6.681, *p* = 0.003** | *p* = 0.097, *r*2 = 0.162 | *p* = 0.299, *r*2 = 0.067 |
| Tetracosenoic (24:1n-9) | *F*1,16 = 1.310, *p* = 0.269, *r*2 = 0.018 | ***F*5,12 = 3.568, *p* = 0.033** | *p* = 0.169, *r*2 = 0.115 | *p* = 0.231, *r*2 = 0.089 |