Vol. 505: 177–191, 2014
doi: 10.3354/meps10767

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published May 28

Energetic adaptations to larval export within the brackish-water palaemonine shrimp, *Palaemonetes varians*

Andrew Oliphant*, Sven Thatje

Ocean and Earth Science, University of Southampton, European Way, Southampton SO14 3ZH, UK

ABSTRACT: Decapod crustaceans have repeatedly colonised brackish, freshwater, and terrestrial environments. Many decapods that inhabit brackish and freshwater habitats export larvae into estuarine and coastal areas where conditions for larval development may be better. In this study, we assessed the starvation resistance, biochemical composition and respiration rate during larval development of the brackish-water palaemonine shrimp, *Palaemonetes varians*, and the effects of temperature on these factors. Our results demonstrate that *P. varians* is highly resistant to starvation and may be considered facultative lecithotrophic in its first and second larval instars, and planktotrophic from its third instar. This high starvation resistance is associated with a relatively large size, high carbon content (~45%) and C:N ratio (~4.2), and visible yolk reserves at hatching. These energy reserves are interpreted as an adaptation to the exportation of larvae from peripheral adult environments into mid- and lower estuarine waters. Respiration rates varied with the moult cycle and were similar between fed and unfed larvae, suggesting that starved larvae do not suppress their metabolism as an energy-saving strategy. Despite higher respiration rates at higher temperatures, energy loss throughout development (estimated from respiration rates) increased with decreasing temperature, whilst larval growth and development rates increased with increasing temperature. High energy reserves at hatching, as within *P. varians*, is an important life history adaptation in the colonisation of brackish and fresh water, initially enabling the exportation of larvae from adult environments and eventually enabling lecithotrophy and direct development.

KEY WORDS: Elemental composition · Larval ecology · Planktotrophy · Facultative feeding · Evolutionary temperature adaptation

INTRODUCTION

The palaemonine shrimp, *Palaemonetes varians,* commonly known as the variable shrimp or ditch shrimp, is the most northerly and high latitude-distributed species of the *Palaemonetes* genus, inhabiting peripheral estuarine and brackish-water environments along western European coasts in the Northeast Atlantic (Dolmen 1997, Falciai 2001, Hindley 2001, González-Ortegón & Cuesta 2006 and references therein). The Palaemoninae are dominated by the genera *Macrobrachium*, *Palaemon*, and *Palaemonetes*, all of which have representatives in marine, brackish, and freshwater environments (De Grave et al. 2009, Vogt 2013). Among decapods, the evolutionary transition from marine (via brackish) to freshwater environments is associated with major evolutionary changes in reproduction and development (Anger 1995, 2001, Vogt 2013). Within estuarine and freshwater environments, decapods may exhibit behavioural and physiological traits that place them on a spectrum of life-cycle adaptation to the estuarine environment. At one extreme are decapods displaying behavioural and physiological traits that enable

*Corresponding author: andrew.oliphant@noc.soton.ac.uk

larvae to be retained within the estuarine environment, usually by larvae accumulating in bottom water layers in which transport is generally upstream (Sandifer 1975, Strathmann 1982, Anger 2001). At the other extreme there are decapods exhibiting traits that promote the exportation of larvae from freshwater and estuarine environments into coastal waters (Sandifer 1975, Strathmann 1982, Anger 2001). Most decapods show life cycles somewhere between these extremes. Decapod estuarine life-cycle adaptations are considered intermediates in the transition from marine to freshwater habitats and, as such, are of particular interest in studying the evolutionary processes of transition and adaptation to freshwater environments.

Marine decapods typically have long-lived feeding larval phases (though at high latitudes larval phases tend to be abbreviated and non-feeding; Thorson 1950, Anger 2001, Thatje et al. 2003), whilst freshwater decapods have abbreviated and non-feeding larval phases, and even direct development (Anger 2001, Vogt 2013). Non-feeding larvae require maternally derived, energy-rich yolk to sustain development, and consequently, freshwater decapods produce fewer, more energy-rich eggs and larvae than their marine counterparts (Urzúa et al. 2013, Vogt 2013). Brackish- and freshwater decapods, which export larvae to lower estuarine and coastal waters, produce eggs and larvae with intermediate energy reserves. Relatively high lipid content at hatching enables high starvation resistance during early larval stages, which are exported from adult habitats (Anger & Hayd 2009).

Within decapod larvae, carbon and nitrogen content are accurate proxies for lipid and protein, respectively (Anger & Harms 1990). Changes in the relative composition of these elements (C:N ratio) provide information about the relative utilisation of lipid and protein for energy metabolism (Anger & Harms 1990, Anger 2001). Inter-specific differences in the energy content of eggs along the gradient from marine to freshwater (and terrestrial) habitats have been highlighted in a comparison of carbon (lipid) content, C:N (lipid:protein) ratio and egg size within grapsoid crabs (see Anger 2001, p. 110). Egg size, lipid content, and lipid:protein ratio all increase along the gradient from marine to freshwater. Associated with this change in the energy content of eggs is a decrease in the number of larval instars during development, and an increase in the extent to which larvae may develop in the absences of food (Hubschman & Broad 1974, Anger 2001). Studies have assessed changes in lipid content and lipid:protein ratio during larval development for both planktotrophic and lecithotrophic marine decapods (e.g. Dawirs 1986, Anger 1996, 2001, Anger & Ismael 1997, Calcagno et al. 2003, Lovrich et al. 2003, Thatje et al. 2004, Anger & Hayd 2009, Weiss et al. 2009, Urzúa et al. 2013); however, only a few studies have investigated brackish and freshwater decapods (Torres et al. 2002, Anger et al. 2007, 2009, Anger & Hayd 2009, 2010, Urzúa et al. 2013). Similarly, there are only a few studies on the changes in lipid content and lipid:protein ratio during development of marine decapods at different temperatures (Dawirs et al. 1986, Anger 1987, Weiss et al. 2009) and, to our knowledge, there are no such studies on brackish and freshwater decapods.

Adult *P. varians* inhabit peripheral estuarine and coastal habitats such as brackish-water drainage channels, salt marshes, and coastal ponds and lagoons, which are often under tidal influence and regularly flooded (Gurney 1924, Lofts 1956). The export of *P. varians* larvae from such habitats has been observed (Gurney 1924), and zoea 1 and 2 larvae and juvenile *P. varians* have been sampled from the lower Ria de Aveiro, Portugal (Pereira et al. 2000). Further, early descriptions of *P. varians* larval development used specimens obtained from plankton samples, indicating the presence of larvae in estuarine and coastal waters (see Gurney 1942 and Fincham 1979 for references). Larval development within *P. varians* is feasible at salinities from 5 to 42, suggesting that development can occur entirely under estuarine conditions (Antonopoulou & Emson 1988). The ubiquitous distribution of this species around the UK has been attributed to the abundance of suitable habitat and large macrotidal hydrodynamics which aid dispersal (Dolmen et al. 2004). These data indicate export of larvae from peripheral adult habitats into estuarine and coastal waters and dispersal through these environments; however, no study has yet followed the life cycle of *P. varians* larvae in wild populations.

Temperature affects all aspects of biology (Clarke 2003) and is one of the most important environmental factors governing growth and development rates in decapods, and ectotherms in general (Anger 2001). Within studies assessing the effects of temperature on growth (both alone and in combination with salinity and nutrition), measures of growth are often limited to changes in total length, carapace length, or dry weight during development (Rothlisberg 1979, Criales & Anger 1986, Oliphant et al. 2013). *P. varians* is a strongly eurythermal species (Oliphant et al. 2011), and its larval development is successful

between 10 and 30°C (30°C was the highest temperature tested; Oliphant et al. 2013). For *P. varians* larvae, growth rate in terms of dry weight accumulation increased approximately linearly, and development rate increased in an exponential fashion between 15 and 25°C (Oliphant et al. 2013). Further, the number of larval instars was also affected by temperature; larvae developed through 4 instars more often at higher temperatures and 5 instars more often at low temperatures (Oliphant et al. 2013, Oliphant & Thatje 2013). The effect of temperature on larval growth and development is significant and may have carry-over effects into early juvenile life (Oliphant et al. 2013). A greater understanding of the effects of temperature on the biochemical composition of larvae during development and post-settlement is requisite.

Here, the effects of temperature and starvation on changes in dry weight, lipid and protein contents, and respiration rate during development were assessed for *P. varians* larvae. These measurements were made to assess the physiological adaptation of *P. varians* to its brackish water distribution, and how temperature may affect the larval ecology of this species. As few data are available for brackish and freshwater decapods (and especially carideans) concerning changes in elemental composition during development, this study will contribute fundamentally to our understanding of the energetic changes necessary for development in the evolutionary transition to freshwater.

MATERIALS AND METHODS

Adult *Palaemonetes varians* collection and maintenance

Larvae used in these experiments were bred in the laboratory under constant conditions using females collected from a wild population of *P. varians* from the Lymington salt marshes (Hampshire, UK). Male and female adult *P. varians* were collected via hand-netting from ditches at the salt marshes in November 2011. Shrimp were transferred (within 1 h) to the research aquarium at the National Oceanography Centre Southampton in sealed, 10 l buckets containing water from the point of collection. Shrimp were sexed via the presence (male) or absence (female) of the appendix masculine on the second pleopod pair, and placed in two 15 l aquaria containing 10 l of filtered (1 µm) seawater at 11°C (field temperature at time of collection) and a salinity of 32, with 15 females and 10 males per aquaria. These aquaria were placed in a temperature-controlled water bath set at 11°C and illuminated on a 8:16 h light:dark cycle (day length was 8 h 32 min at the time of collection). Shrimp were acclimated to these conditions for 4 d. After this period, the temperature was increased by 1°C d^{-1} until 15°C was achieved, and day length was increased by 2 h d^{-1} until 18:6 h light:dark was achieved. These conditions (15°C and 18:6 light:dark) were chosen to represent warm temperatures and long day length, and have previously been found to induce breeding in *P. varians* (Bouchon 1991a,b). At all times, shrimp were fed Tetra goldfish flakes 3 times per week to excess and water changes (>80%) were done 3 times per week. After extruding eggs, females were removed from the aquaria and placed individually in 1 l plastic buckets containing 850 ml of 15°C, 1 µm filtered, UV treated, 32 salinity seawater, which were then placed in an incubator set at 15°C. Feeding and water changes were as before.

Larval maintenance

Two parallel experiments were run. Expt 1: 'The effects of temperature on larval development', monitored larval development in terms of moulting frequency, number of larval instars during development, overall development time, and juvenile dry weight at settlement for groups of 'fed' and 'unfed' larvae at 3 temperatures. This experiment repeated the work of earlier papers to demonstrate the repeatability of the results; methods and results for Expt 1 are detailed in the Supplement at www.int-res.com/articles/suppl/m505p177_supp.pdf. Expt 2: 'The effects of temperature on elemental composition during larval development', monitored larval development from a more physiological standpoint; taking measurements of larval respiration rates, dry weight, and elemental composition throughout development, again for groups of 'fed' and 'unfed' larvae at 3 temperatures.

Expt 2: The effects of temperature on elemental composition during development

On hatching, actively swimming larvae were separated from 12 females using a plastic pipette and individually and haphazardly transferred to 100 ml plastic beakers containing ~80 ml (15°C, 32 salinity, 1 µm filtered, UV treated seawater). Eleven of the

females from which larvae were obtained were the same females as those used in Expt 1 (see Supplement); therefore, larvae used in both experiments were (for the most part) from the same broods. Larvae were divided between incubators set at 15, 20, and 25°C and 12:12 h (light:dark); larvae used for both Expts 1 and 2 were maintained in the same incubators. This temperature range reflected the temperature range recorded *in situ* within the environment of adult shrimp during summer months and across 3 yr (Oliphant 2014). Larvae were maintained under a 12:12 h light:dark cycle to enable direct comparison with previous studies using the same light regime (Oliphant et al. 2013, Oliphant & Thatje 2013), as this cycle is known to affect larval development. The first instar (zoea 1) of *P. varians* is facultative lecithotrophic: larvae have been observed to prey on and ingest *Artemia* sp. nauplii and gain weight relative to starved first instar larvae (A. Oliphant unpubl. data). As such, the first instar was not fed (following Oliphant et al. 2013, Oliphant & Thatje 2013). At each temperature, a portion of the larvae from each female was not fed (unfed category) and a portion was fed (fed category); feeding was from the start of the second instar (following Oliphant et al. 2013). During development, larval respiration rate measurements were made (see below). Subsequently, larvae used for respiration rate measurements were blotted on tissue paper and transferred to pre-weighed tin capsules, frozen at –80°C, and later freeze-dried for 24 h and then weighed for larval dry weight (DW, µg). Carbon and nitrogen composition were measured using a CHNS-O EA1108-elemental analyser (Carlo ERBA Instruments). Respiration rate, DW and elemental composition measurements were made daily during the initial 10 d of larval development, then every second day thereafter for 5 unfed larvae and 5 fed larvae at each temperature.

Respiration rate measurements

Larvae (5 fed and 5 unfed) were transferred individually to 2.8 ml plastic vials containing 15, 20, or 25°C (respective of temperature treatment), 32 salinity, 1 µm filtered, UV treated seawater. Vials were sealed underwater to ensure no air was trapped inside and then incubated in temperature-controlled incubators set at 15, 20, or 25°C (again, respective of temperature treatment). Vial volume was constant between temperature treatments (2.8 ml); therefore, the incubation period was varied between temperature treatments to account for lower respiration rates under cooler conditions. Vials were incubated for 4 h at 15°C, 3 h at 20°C, and 2.5 h at 25°C. Incubations were started at approximately 10:00 h. Five control vials containing no larva were run per temperature treatment and were subjected to the same procedure as experimental vials. At the end of the incubation period, the % O_2 level of water inside the vials was measured using a temperature-adjusted oxygen meter and microoptode (Microx TX 3, PreSens; accuracy ±0.4 % O_2 at 20.9 % O_2, ±0.05 % O_2 at 0.2 % O_2). These measurements were calibrated with fully aerated seawater that had been left to settle for 30 min (100 % O_2 saturation) and seawater deoxygenated by over-saturation with sodium sulphite anhydrous (0 % O_2 saturation). Calibration solutions were incubated at 15, 20, or 25°C prior to use. O_2 concentration of 100 % O_2-saturated seawater was calculated according to Benson & Krause (1984). The difference between control and experimental vials was used to calculate a value for O_2 consumption (µmol O_2). Using the incubation period and DW, a value for respiration rate (µmol O_2 h^{-1} $µg^{-1}$) was calculated for each larva.

Statistical analyses

Data were tested for normality of distribution and equality of variance using the Kolmogorov-Smirnov Test and Levene's Test, respectively. Box-Cox transformation was used to calculate the most likely successful power transformation for data. Where data were non-normally distributed and could not be successfully transformed to meet assumptions, non-parametric statistics were used.

At 15°C, differences in larval DW between fed and unfed larvae were analysed by non-parametric Kruskal-Wallis comparison, and the relationships between larval DW and larval age for both unfed and fed larvae were tested by Spearman's correlations. At 20 and 25°C, differences in larval DW between fed and unfed larvae were analysed by general linear model (GLM) ANOVA with post-hoc testing using the Sidak method, with larval age and unfed vs. fed as factors. The relationships between larval DW and larval age for fed and unfed larvae were assessed via linear regression analyses.

Larval DW data for individual larval instars across all temperatures were analysed by Kruskal-Wallis comparisons. Average daily growth per instar (both DW and carbon mass) data were analysed via GLM ANOVA; post-hoc testing (Sidak method) with temperature and larval instar number as factors.

Average daily growth increment data were transformed by log(n + constant). Respiration rate data for fed larvae were analysed by 1-way ANOVA and data for both fed and unfed larvae were analysed using GLM ANOVA; post-hoc testing (Sidak method) with larval age and fed vs. unfed as factors. Cumulative energy loss both within larval instars and throughout development were analysed using GLM ANOVA; post-hoc testing (Sidak method) with temperature and larval instar as factors. Carbon content data were analysed by non-parametric Kruskal-Wallis comparisons and the relationship between carbon content and larval age was assessed via Spearman's correlation. C:N data were analysed via 1-way ANOVA with Tukey's HSD post-hoc testing. All statistical analysis was done using Minitab v16 software and in accordance with Sokal & Rohlf (1995).

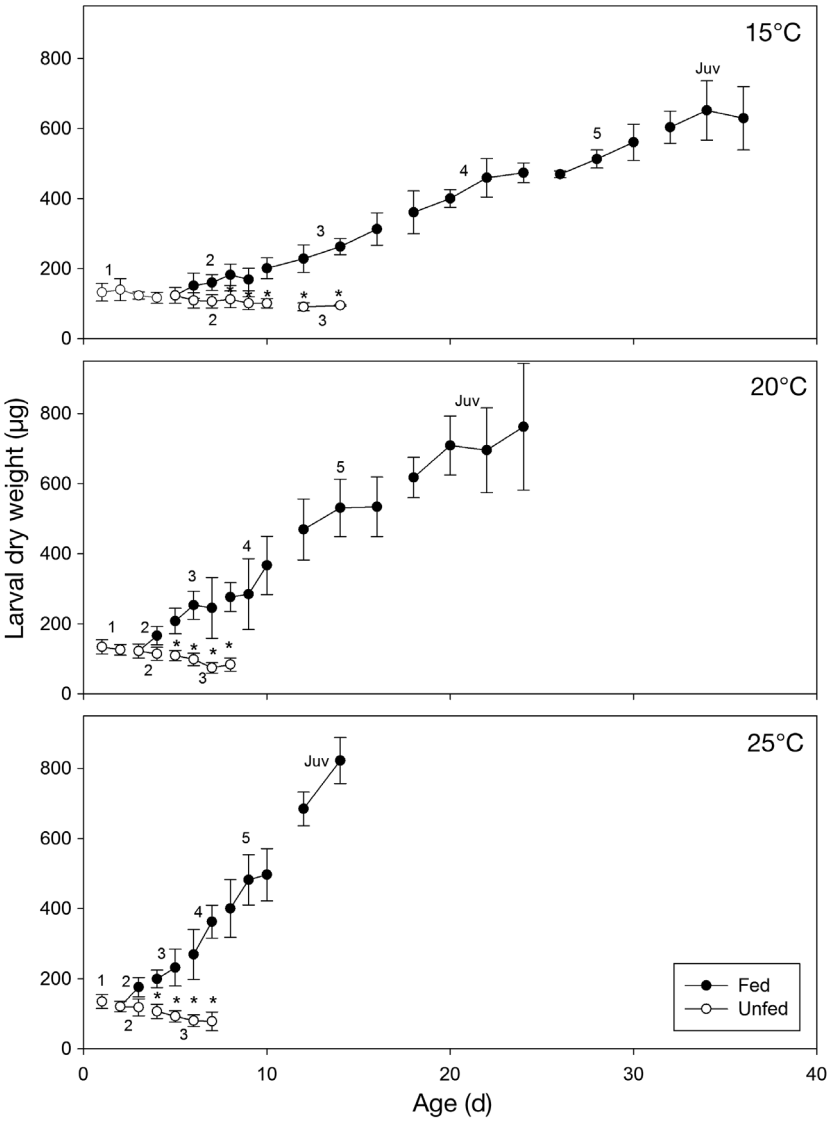

Fig. 1. *Palaemonetes varians* larval dry weight (mean ± SD) throughout development for fed and unfed larvae at 3 temperatures (15, 20, and 25°C). Data points within the same instar are joined by lines, instar number is indicated. Significant differences between fed and unfed larval dry weights are indicated by asterisks (*)

RESULTS

Effect of starvation and of temperature on larval DW during development

For fed larvae, larval DW increased throughout development (i.e. with increasing larval age), whilst for unfed larvae, larval DW decreased until mortality ensued, at all temperatures (Fig. 1). These relationships appeared approximately linear and were analysed as such. For unfed larvae, negative linear relationships were evident between larval age and DW at all temperatures (at 15°C, Spearman's correlation: $p < 0.001$ and at 20 and 25°C, linear regressions: $F = 48.10$, $p < 0.001$ and $F = 38.22$, $p < 0.001$, respectively; see Table 1 for fitted parameters and correlation coefficients); indicating that larval DW decreased significantly with larval age (Fig. 1). For fed larvae, positive linear relationships were evident between larval age and DW at all temperatures (at 15°C, Spearman's correlation: $p < 0.001$ and at 20 and 25°C, linear regressions: $F = 347.09$, $p < 0.001$ and $F = 517.51$, $p < 0.001$, respectively; see Table 1 for fitted parameters and correlation coefficients); indicating that larval DW increased significantly with larval age (Fig. 1).

At 15°C, larval DW was different between unfed and fed larvae from Day 8 ($p = 0.027$; K-W) onwards; larval DW being greater for fed larvae than unfed larvae. Larval DW differed between unfed and fed larvae from Day 5 ($p = 0.0023$; GLM ANOVA) onwards at 20°C and Day 4 ($p = 0.002$; GLM ANOVA) onwards at 25°C; again with fed larvae having greater DW. At these temperatures, the onset of these differences corresponded to the moulting of both fed and unfed

Table 1. *Palaemonetes varians*. Fitted parameters (*a*, *b*) and correlation coefficients (r) for linear regressions ($y = a + bx$) describing the relationship between larval age and larval dry weight (DW) for fed and unfed larvae at 3 temperatures (15, 20, and 25°C). At 15°C, r was determined by Spearman's correlation, and *a* and *b* calculated by pair-wise slopes. At 20°C and for fed larvae, linear regression was done on transformed (λ = 0.44) data

Temp.	Unfed			Fed		
(°C)	*a*	*b*	r	*a*	*b*	r
15	109.00	−0.11	−0.637	38.20	17.40	0.973
20	144.46	−8.19	−0.748	10.93	0.75	0.914
25	143.77	−9.82	−0.733	−54.20	59.93	0.958

larvae to the third larval instar (Fig. 1). The initial DW of larvae of each instar were not different between temperatures; however, average daily growth per instar, in terms of dry weight, was affected by temperature ($F = 10.93$, $p < 0.001$), instar number ($F = 30.59$, $p < 0.001$) and the interaction between temperature and instar number ($F = 4.04$, $p = 0.001$) (Fig. 2A). Within the second larval instar, average daily growth rate per instar increased from 5.45 ± 2.58 µg DW d^{-1} at 15°C to 55.4 ± 15.66 µg DW d^{-1} at 25°C ($T = 4.52$, $p = 0.0032$). Similarly, within the fourth larval instar, average daily growth rate per instar increased from 18.80 ± 6.25 µg DW d^{-1} at 15°C to 79.75 ± 34.95 µg DW d^{-1} at 25°C ($T = 4.39$, $p = 0.0048$) (Fig. 2A).

Average daily growth per instar in terms of carbon mass was similarly affected by temperature ($F = 17.53$, $p < 0.001$), larval instar number ($F = 37.99$, $p < 0.001$) and the interaction between these factors ($F = 5.01$, $p < 0.001$) (Fig. 2B). Again, average daily growth per instar increased significantly within the second larval instar ($T = 5.15$, $p = 0.0004$) from 2.69 ± 1.03 µg C d^{-1} at 15°C to 26.94 ± 5.91 µg C d^{-1} at 25°C. Also, within the fourth larval instar, average daily growth per instar increased from 8.23 ± 2.62 µg C d^{-1} at 15°C to 31.92 ± 9.04 µg C d^{-1} at 25°C ($T = 5.03$, $p = 0.0005$).

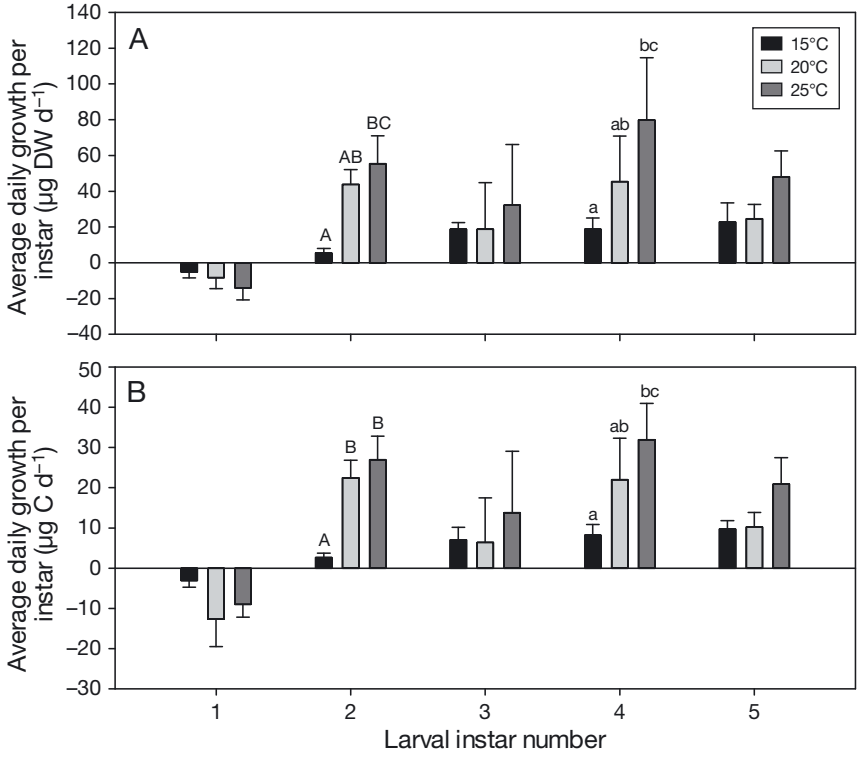

Fig. 2. *Palaemonetes varians* average daily growth (mean ± SD) per instar in terms of (A) dry weight and (B) carbon mass at 3 temperatures (15, 20, and 25°C). Letters indicate differences between temperatures within instars

Effect of temperature on respiration rate

At 15°C, respiration rates varied with the moult cycle, generally being higher in post-moult larvae and decreasing through the inter-moult period, especially for post-hatching first instar and post-moult second and third instar larvae (Fig. 3). Although 1-way ANOVA indicated a significant effect of larval age on respiration rates for fed larvae ($F = 2.50$, $p = 0.001$), variations in respiration rates associated with the moulting cycle were not significant. Respiration rates of fed and unfed larvae were similar with high post-moult respiration rates, which decreased during the inter-moult period. No differences were found between fed and unfed larvae (GLM ANOVA: $F = 0.42$, $p = 0.517$). Similarly, respiration rates of fed and unfed larvae were not different at 20°C (GLM ANOVA: $F = 0.859$, $p = 0.859$) and 25°C (GLM ANOVA: $F = 0.04$, $p = 0.852$). At these temperatures, respiration rates varied within larval instars, generally decreasing through the inter-moult period; however, these changes were subtle (Fig. 3). At 20°C, there was no effect of larval age on respiration rate (1-way ANOVA: $F = 0.98$, $p = 0.484$), whilst at 25°C there was ($F = 2.94$, $p = 0.005$). Again, there were no differences in respiration rates associated with the moult cycle. At all

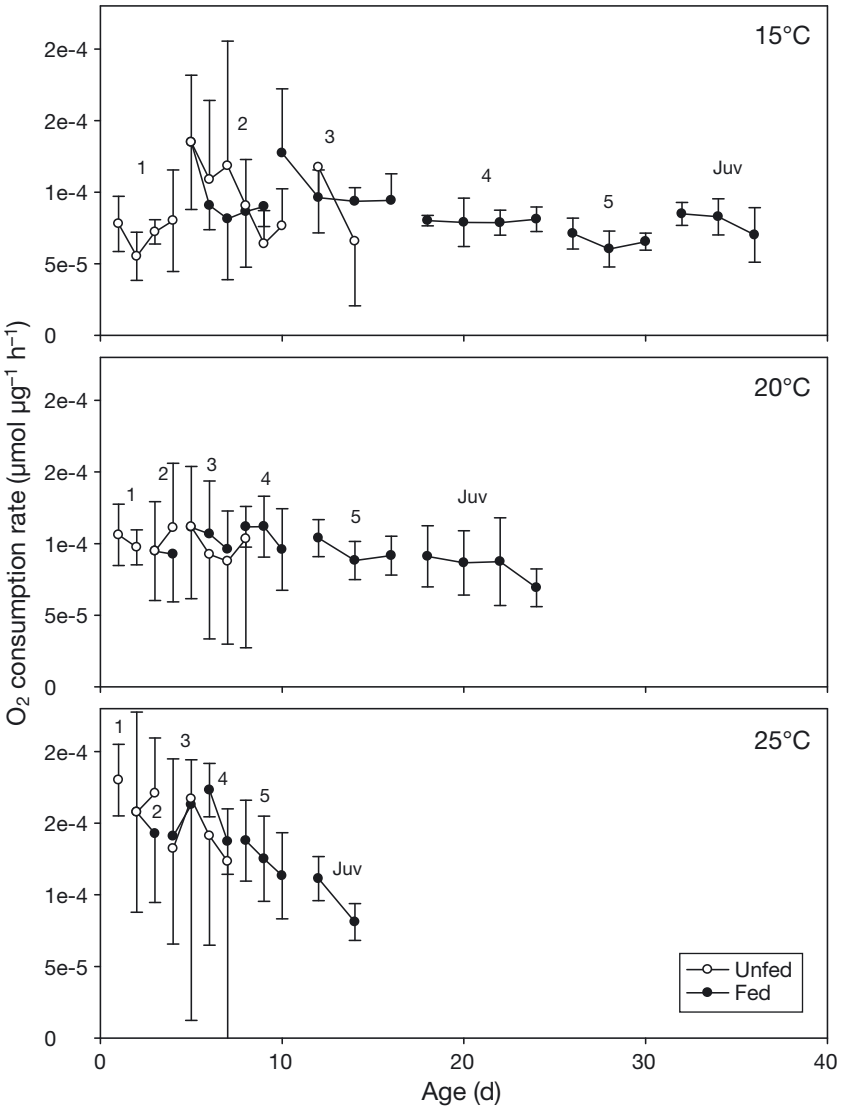

Fig. 3. *Palaemonetes varians* respiration rates (means ± SD) throughout development for fed and unfed larvae at 3 temperatures (15, 20, and 25°C). Data points within the same instar are joined by lines, and instar number is indicated

temperatures, the standard deviations of data for unfed larvae were greater than those for fed larvae.

Respiration rate data (µmol O_2 h^{-1}) were converted to energy loss data (J h^{-1}) according to Gnaiger (1983) (1 µmol O_2 h^{-1} = 0.450 J h^{-1}). These values were then used to estimate energy loss per day and then added to give an estimate of energy loss within individual larval instars and throughout development (Fig. 4A,B). Cumulative energy loss within individual larval instar was affected by temperature (GLM ANOVA: $F = 46.71$, $p < 0.001$), generally being greater for larvae developing at 15°C. Larval instar ($F = 137.29$, $p < 0.001$) and the interaction between temperature and instar ($F = 12.73$, $p < 0.001$) affected cumulative energy loss within individual larval instars. For example, during the second, third, and fourth instar, cumulative energy loss within these instars was significantly greater at 15°C than at 20 and 25°C, which were not distinct from one another (Fig. 4A). For the fifth larval instar, cumulative energy loss during this instar was greatest at 20°C and significantly higher than at 25°C (Fig. 4A). Cumulative energy loss throughout development was influenced by development temperature (GLM ANOVA: $F = 44.18$, $p < 0.001$), being greatest at 15°C within all larval instars. Advancing larval instar also affected energy loss ($F = 244.83$, $p < 0.001$) but there was no interaction, indicating that the trend was the same in all larval instars (Fig. 4B).

Influence of temperature on elemental composition

Carbon content was approximately 45% at hatching, whilst nitrogen content was approximately 11%. The effects of temperature on DW, carbon mass (C, µg), nitrogen mass (N, µg), and carbon:nitrogen ratio (C:N) are shown in Table 2. Growth rates (final zoea DW divided by initial hatchling DW × 100) were highest at 20°C (409.84 ± 112.89%) and lowest at 25°C (369.71 ± 31.68%), and was 394.85 ± 56.56% at 15°C. Growth factor F_G (carbon content of final zoea divided by carbon content of freshly hatched zoea) was lowest at 20°C (3.46 ± 1.06), highest at 15°C (3.89 ± 0.49), and 3.49 ± 0.42 at 25°C.

Carbon content (% DW) appeared to decrease with increasing development at all temperatures. Spearman's correlation indicated negative relationships between % DW and larval age at 15°C ($p = 0.009$), 20°C ($p < 0.001$) and 25°C ($p < 0.001$) (Fig. 5; see Table 3 for fitted parameters and correlation coefficients). At all temperatures, % DW appeared to decrease within the first instar, which was not fed, and then increase during the second instar, corresponding to the onset of larvae being fed (Fig. 5). Nonparametric testing did not support these observations

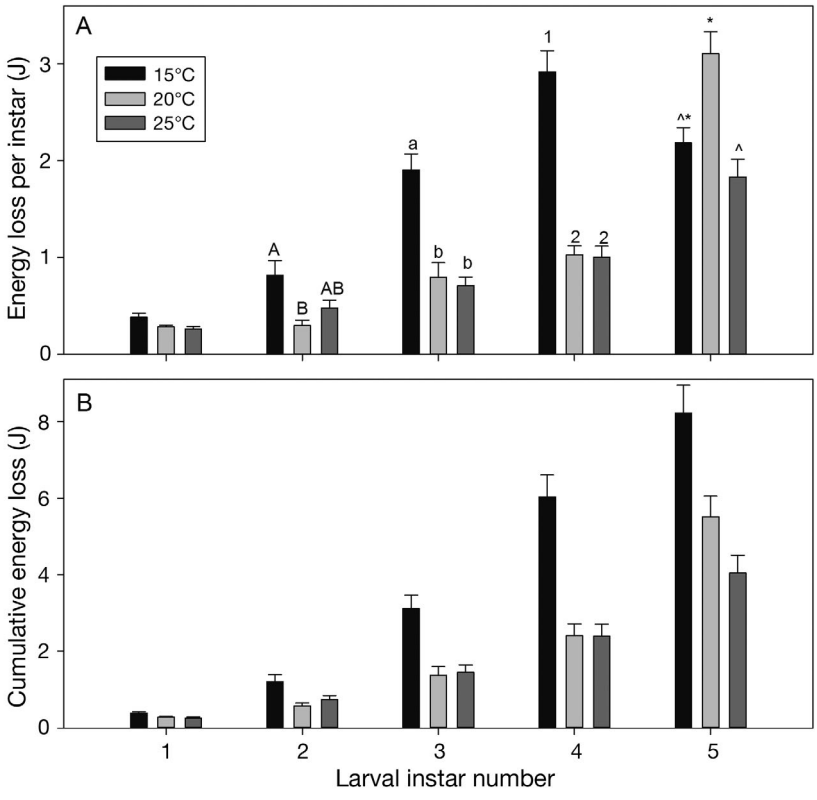

Fig. 4. *Palaemonetes varians*. (A) Energy loss within individual instars throughout development for fed larvae, and (B) cumulative energy loss throughout development for fed larvae. Data are means ± SD. Differences between temperatures within instars are shown by letters, numbers, or symbols

statistically. Similarly, C:N ratios appeared to decrease within the first instar and increase within the second instar at all temperatures (Fig. 5); these changes were supported statistically. At 15°C, C:N changed significantly during development ($F = 15.39$, $p < 0.001$). Post-hoc Tukey tests indicated that the C:N ratio decreased during the first instar from 4.23 ± 0.17 on Day 1 to 3.73 ± 0.23 on Day 4 (Fig. 5). The C:N ratio then increased from 4.13 ± 0.23 on Day 5 to 4.55 ± 0.14 on Day 8 during the second instar. C:N ratio decreased between Day 8 and Day 10 (4.40 ± 0.08), and from Day 16 until the end of the experiment (Day 36).

At both 20 and 25°C, larval C:N ratio changed significantly during development ($F = 8.66$, $p < 0.001$ and $F = 6.00$, $p < 0.001$, respectively), following similar patterns to those observed at 15°C. Post-hoc testing indicated that the C:N ratio decreased during the first instar at 20°C, from 4.42 ± 0.27 on Day 1 to 3.87 ± 0.26 on Day 2. C:N ratios increased during the second instar at 20°C, from 3.96 ± 0.28 on Day 3 to 4.36 ± 0.13 on Day 4 (Fig. 5). Larval C:N ratio then decreased from Day 12 onwards. At 25°C, the decrease in C:N ratio through the first instar, and the increase during the second instar, were not significant. The C:N ratio did, however, decrease significantly from Day 10 onwards (Fig. 5).

DISCUSSION

The general decrease in both carbon content and C:N ratio during larval development of *Palaemonetes varians* indicates the utilisation of stored lipid and the construction of muscle (Anger & Hayd 2009, Weiss et al. 2009). Changes in the relative elemental composition of *P. varians* larvae during development are not influenced by temperature; however, the timing and rate of changes are. We also demonstrate the impressive starvation resistance of *P. varians* larvae, which is associated with a high maternal energy investment in offspring and can be considered as an important evolutionary adaptation in the ecology of this species.

Biochemical composition, metabolism, and larval ecology

P. varians larvae are highly resistant to starvation, surviving for prolonged periods in the absence of food. Despite starvation, larval development may proceed to the third (results presented here) and even fourth larval instar (Oliphant & Thatje 2013); thus, *P. varians* can be considered facultative lecithotrophic in its first and second larval instars and planktotrophic in its third larval instar. The extent to which larvae developed was temperature dependant, with a greater proportion of larvae developing to the third larval stage at higher temperatures without food (see Expt 1 in the Supplement at www.int-res.com/articles/suppl/m505p177_supp.pdf, also see Oliphant & Thatje 2013). This level of starvation resistance is greater than that observed for the North American *Palaemonetes* species, *P. vulgaris* and *P. pugio*: the former can survive for ~5 d and is unable to moult whilst the latter can survive for ~10 d and can moult up to only the second larval instar (Broad

Table 2. *Palaemonetes varians*. Changes in larval biomass and elemental composition throughout development at 15, 20, and 25°C for larvae fed *Artemia* sp. nauplii. Dry weight (DW), carbon and nitrogen mass (C, N), and C:N ratios are shown; n = 5 replicate analyses

Temp. (°C)	In-star	Day	DW (µg) Mean	DW (µg) SD	C (µg) Mean	C (µg) SD	N (µg) Mean	N (µg) SD	C:N Mean	C:N SD
15	1	1	132.800	25.332	58.045	11.019	13.678	2.105	4.225	0.171
	1	2	140.200	31.076	63.597	17.303	15.041	2.841	4.179	0.366
	1	3	123.600	10.164	53.079	6.394	13.608	1.196	3.892	0.157
	1	4	117.200	15.928	48.724	7.615	13.042	1.561	3.727	0.229
	2	5	147.200	26.310	62.359	13.731	15.028	2.786	4.126	0.225
	2	6	151.800	36.044	63.895	15.822	14.869	3.243	4.276	0.143
	2	7	160.400	22.876	70.156	14.566	16.057	2.910	4.354	0.138
	2	8	182.600	30.729	80.498	14.122	17.698	3.086	4.548	0.140
	2	9	169.000	32.031	73.100	13.053	16.639	3.141	4.401	0.080
	3	10	201.200	29.550	85.622	9.689	20.064	2.441	4.272	0.122
	3	12	228.600	39.336	100.805	19.676	18.848	11.166	4.271	0.100
	3	14	263.000	23.152	112.250	9.503	26.158	2.107	4.291	0.123
	3	16	313.200	46.370	127.827	26.565	29.141	6.465	4.403	0.156
	4	18	360.800	60.998	157.076	28.325	35.830	6.028	4.377	0.077
	4	20	400.400	25.314	174.544	11.684	39.966	3.096	4.370	0.066
	4	22	459.400	54.875	201.149	23.570	47.035	5.535	4.277	0.062
	4	24	473.600	28.343	206.458	12.836	49.006	3.342	4.215	0.093
	5	26	469.600	9.607	191.836	20.306	46.921	4.195	4.085	0.140
	5	28	513.000	25.797	221.719	16.192	55.000	2.816	4.029	0.122
	5	30	560.800	51.582	230.702	26.323	59.233	6.375	3.893	0.067
	J	32	603.800	46.165	251.487	21.190	64.589	4.473	3.891	0.075
	J	34	652.000	84.637	272.904	36.077	71.237	9.613	3.832	0.029
	J	36	629.400	90.544	256.827	41.308	66.799	10.269	3.841	0.052
20	1	1	134.000	20.211	66.351	9.133	14.960	1.572	4.424	0.265
	1	2	125.600	15.307	53.654	9.431	13.802	1.567	3.867	0.256
	2	3	122.000	20.075	48.350	6.859	14.112	1.882	3.955	0.275
	2	4	165.800	26.148	70.816	11.220	15.725	2.434	4.356	0.134
	3	5	207.800	36.396	89.394	14.314	20.517	3.415	4.362	0.083
	3	6	253.200	40.158	111.725	19.513	26.324	4.890	4.255	0.143
	3	7	245.200	86.952	102.228	34.944	23.673	7.194	4.261	0.251
	4	8	276.200	41.197	117.115	19.625	28.359	5.285	4.141	0.099
	4	9	284.400	101.434	121.440	45.374	29.705	11.370	4.096	0.153
	4	10	367.000	83.295	161.056	37.174	39.417	9.171	4.086	0.132
	5	12	469.200	86.975	201.003	41.102	49.311	10.174	4.077	0.120
	5	14	531.000	82.265	228.948	34.733	57.091	8.881	4.012	0.070
	5	16	534.000	85.194	222.578	32.354	57.582	7.908	3.863	0.081
	5	18	617.400	57.413	262.310	26.285	67.607	5.945	3.877	0.081
	J	20	708.800	84.200	278.910	30.792	74.231	8.149	3.757	0.060
	J	22	695.600	120.877	277.810	50.590	76.116	11.630	3.640	0.130
	J	24	762.400	180.542	280.437	53.319	73.210	16.961	3.860	0.178
25	1	1	134.600	19.844	61.357	10.458	14.696	1.872	4.158	0.196
	2	2	120.600	14.775	52.369	8.861	13.255	1.342	3.931	0.290
	2	3	176.000	27.331	61.334	35.750	17.588	2.335	4.352	0.172
	3	4	199.400	24.966	86.082	10.402	20.226	2.547	4.262	0.195
	3	5	231.600	52.524	99.864	23.000	23.569	5.889	4.258	0.177
	4	6	269.250	71.309	90.921	57.175	27.528	7.282	4.129	0.185
	4	7	362.600	46.645	152.558	29.123	36.792	7.329	4.152	0.062
	5	8	400.400	82.773	170.498	36.202	41.703	8.994	4.093	0.111
	5	9	482.200	71.709	206.257	26.166	51.466	7.717	4.018	0.112
	5	10	496.800	74.764	212.325	28.183	52.537	6.723	4.040	0.084
	J	12	684.600	48.169	282.935	19.720	73.855	5.507	3.833	0.055
	J	14	822.600	65.401	330.550	26.062	87.784	8.258	3.770	0.056

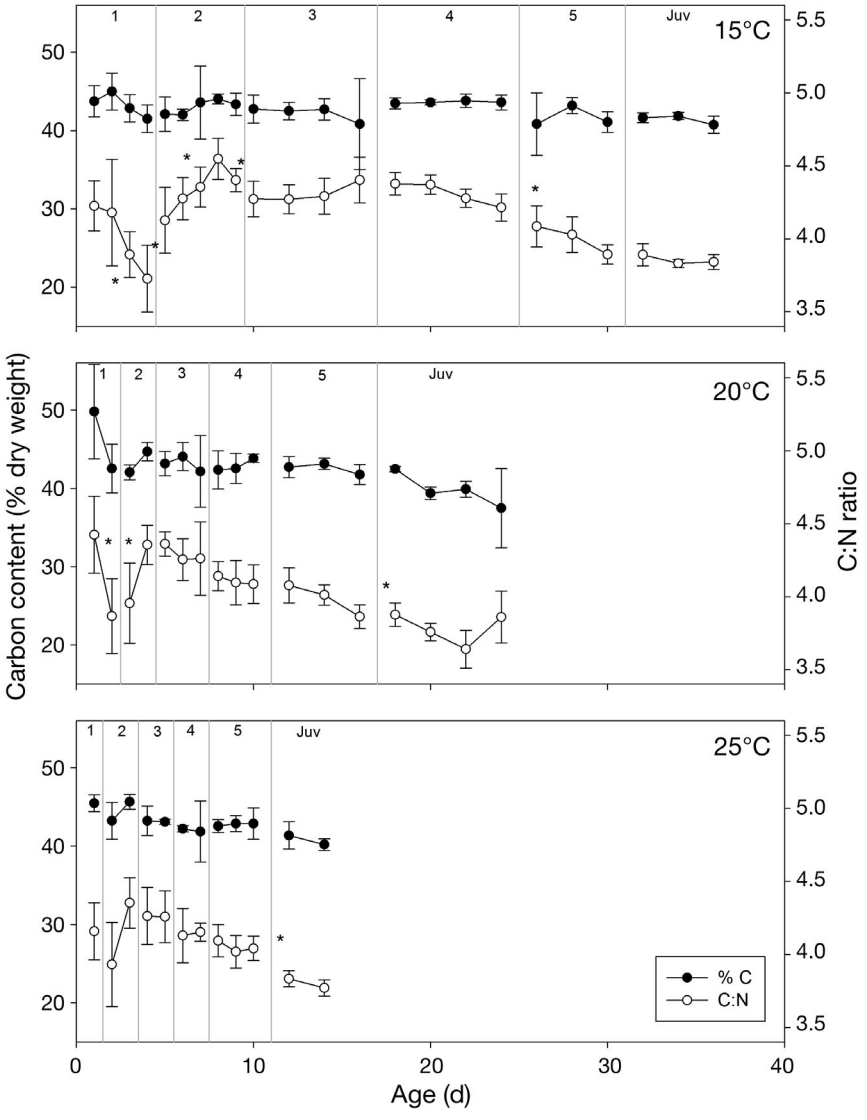

Fig. 5. *Palaemonetes varians* carbon content (% DW) and C:N ratio throughout development (instar numbers indicated) for fed larvae at 3 temperatures (15, 20, and 25°C). Data are presented as means ± SD. Significant changes in C:N ratios are indicated by asterisks (*)

Table 3. *Palaemonetes varians*. Fitted parameters (*a*, *b*) and correlation coefficients (r) for linear regressions ($y = a + bx$) describing the relationship between larval age and carbon content (% DW) at 3 temperatures (15, 20, and 25°C). At 15°C, r was determined by Spearman's correlation, and *a* and *b* calculated by pair-wise slopes

Temp. (°C)	*a*	*b*	r
15	42.244	−0.040	−0.245
20	40.707	−0.210	−0.544
25	34.024	−0.301	−0.593

1957a,b). *P. varians*' starvation resistance is more comparable with that of the palaemonine shrimp *Macrobrachium amazonicum*, which is lecithotrophic in its first instar, facultative lecithotrophic in its second instar, planktotrophic in its third larval instar, and can survive in the absence of food for ~12 d and moult to its third larval instar (Anger & Hayd 2009).

Inter-specific differences in starvation resistance among these palaemonine shrimp are likely a reflection of the extent to which these species are adapted to brackish and freshwater environments. *P. vulgaris* and *P. pugio* both inhabit estuarine environments but *P. vulgaris* occurs in deeper, more saline waters, whilst *P. pugio* occurs on mud flats and saltmarsh creeks (Jenner 1955). Adult salinity tolerances within these species reflect this distribution, as *P. pugio* is more tolerant of lower salinities than *P. vulgaris*; larval salinity tolerances are similar, however (Knowlton & Kirby 1984, Knowlton & Schoen 1984). *P. varians* inhabits more peripheral brackish-water habitats whilst *M. amazonicum* inhabits brackish and fresh waters as adults, but the larvae of this species require salinities of 6 to 35 to develop (Moreira & McNamara 1986). Starvation resistance within early larval stages of brackish and fresh water palaemonine (and decapods in general) is considered an evolutionary adaptation to the export of larvae by river- and tidal-flow from adult environments into estuarine and coastal marine waters, where conditions for larval development may be more favourable than those in the adult environment (Hovel & Morgan 1997, Anger 2001, Anger & Hayd 2009, Vogt 2013). The differing levels of starvation resistance among these palaemonine shrimp reflect the extent to which adults inhabit peripheral brackish and freshwater habitats and, consequently, the period of time taken for larvae to reach estuarine and coastal marine waters.

During the period of export from the adult environment, larvae may encounter low food availability and, consequently, high maternal energy investment per offspring is selected for, enabling starvation

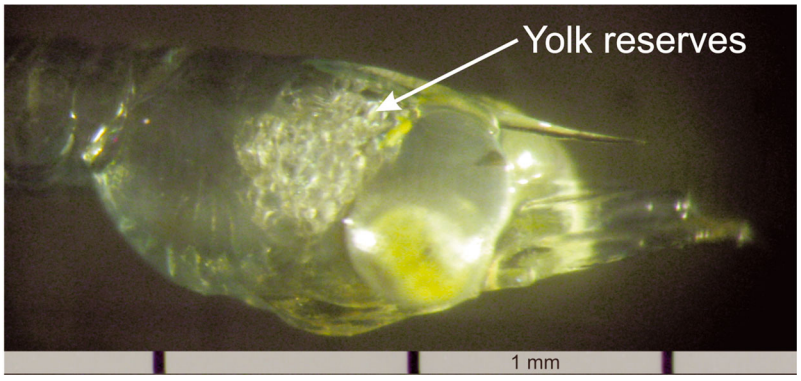

Fig. 6. *Palaemonetes varians*. Photograph of a first instar (zoea 1) larva (lateral view of cephalothorax) showing yolk reserves at hatching

resistance in early-stage larvae. Like *M. amazonicum* and the lecithotrophic palaemonid *Palaemonetes zarqueiyi*, *P. varians* larvae hatch with visible maternally derived yolk reserves (Fig. 6) (Anger & Hayd 2009, Urzúa et al. 2013). Although *P. varians* larvae are similar in starvation resistance to *M. amazonicum*, the relative dry weight, carbon content and C:N ratio differ considerably between these species. Relative to *M. amazonicum*, *P. varians* has higher hatchling DW (~62 µg < ~120 µg, respectively), higher carbon mass (~33 µg < ~54 µg, respectively), but lower carbon content (as % DW) (~54% > ~45%, respectively), and C:N ratio (~5.5 > ~4.2, respectively). *P. varians*, despite having lower carbon content and C:N ratio, has greater DW and carbon mass than *M. amazonicum*, which may be reflected in the more abbreviated development within *P. varians* (4 to 5 larval instars compared with 9 for *M. amazonicum*). Hubschman & Broad (1974) identified a continuum of *Palaemonetes* species occupying increasingly freshwater environments and which demonstrate increasingly abbreviated development. *P. varians* has moderately abbreviated development relative to both *P. pugio* and *P. vulgaris* (7 to 11 instars) which both hatch at a smaller size than *P. varians* (Broad 1957a,b, Hubschman & Broad 1974).

Limited data are available on elemental composition changes during larval development for caridean shrimp (Anger & Harms 1990, Thatje et al. 2004, Anger & Hayd 2009, 2010, Anger et al. 2009, Urzúa et al. 2013). Generally, during larval development, the carbon content of planktotrophic larval decapods increases through successive larval stages, indicating the storage of lipids assimilated during larval feeding (Anger 2001, p. 194). Here, the experimental design meant that carbon content and C:N ratio changes during development were more complex than in previous studies. During the first instar (which was not fed), carbon content and C:N ratios decreased, indicating that maternally derived lipid reserves were preferentially utilised for energy metabolism (Anger 1998, 2001). At the onset of feeding, carbon content and C:N ratio initially increased during the second instar, evidencing an accumulation of lipid reserves. As for brachyuran crabs with feeding larvae, carbon content reported for larval development increases with advancing development, indicating the accumulation of lipid reserves: e.g. *Neohelice granulata*, *Hyas araneus*, *Carcinus maenas*, *Liocarcinus holsatus*, *Cancer setosus* and the anomuran crab, *Pagurus bernhardus* (Dawirs et al. 1986, Anger 1989, 2001, Anger et al. 1989, Harms 1990, Anger & Ismael 1997, Weiss et al. 2009). After the second larval instar, development through subsequent larval instars marked a gradual decrease in carbon content and C:N ratios. This utilisation of lipid reserves during development is consistent with results reported for the larvae development of *M. amazonicum* (Anger & Hayd 2009). This trend suggests the use of lipids and the formation of muscle structure (Anger & Hayd 2009, Weiss et al. 2009).

Typically, both carbon content and C:N ratio show short-term cyclical changes during development which correspond to the moult cycle (Dawirs et al. 1986, Anger 1989, 2001, Anger et al. 1989, Harms 1990, Anger & Ismael 1997, Weiss et al. 2009). In post-moult larvae, carbon content is generally lower as a result of water and mineral uptake after moulting. Similarly, post-moult C:N ratios generally increase to a maximum in the inter-moult and then decrease through the pre-moult (Dawirs et al. 1986, Anger 1989, 2001, Anger et al. 1989, Harms 1990, Anger & Ismael 1997, Weiss et al. 2009). Generally, these cyclical patterns in carbon content and C:N ratio are not evident in the results reported here for *P. varians*. This may point at the relatively low resolution within this study: although monitored daily, rapid development meant much of these cycles were missed.

Temperature effects on growth and elemental composition during larval development

The results of Expt 1 (see Supplement) indicate that the effects of temperature on the larval development of *P. varians* in terms of development time,

growth rate, juvenile dry weight, and developmental plasticity are consistent with results of previous studies (Oliphant et al. 2013, Oliphant & Thatje 2013). The implications of larval instar plasticity for post-settlement juvenile traits are poorly reported for decapods (Giménez et al. 2004, Oliphant et al. 2013), but may have significant ecological and evolutionary implications (Kingsolver 2007, Etilé & Despland 2008, Oliphant et al. 2013).

Few data are available on the effects of temperature on elemental composition and, thus, the utilisation of lipid and protein during development at different temperatures (e.g. Dawirs et al. 1986, Anger 1987, Weiss et al. 2009). For *P. varians*, the initial larval dry weights, carbon and nitrogen contents, and C:N ratios of post-moult larvae at each stage were not different between temperatures, suggesting that growth, development, and the moulting cycle were not de-coupled by temperature. However, the results of Expt 1 (see Supplement) and those of previous studies demonstrate that temperature mediated developmental plasticity is driven by the decoupling of development from the moult cycle (Oliphant et al. 2013, Oliphant & Thatje 2013). Given the methods used in Expt 2, it was not possible to separate larvae developing through different larval instars and observe how growth rates and elemental composition may change with developmental plasticity. The results of Expt 2 do, however, demonstrate a significant effect of temperature on growth rates: average daily growth rates, both in terms of dry weight and carbon mass, increased with temperature. The second larval instar, which was the first to be fed, appeared particularly important in the assimilation of lipid resources which were subsequently utilised throughout development. Within the second instar, average daily growth rates for dry weight and carbon mass increased significantly with temperature (Fig. 2). Increasing temperature has been found to increase the rate of carbon content growth, and thus lipid assimilation, within zoeal stages of the brachyuran crab *Carcinus maenas* (Dawirs et al. 1986) and early larval stages of the brachyuran crab *Cancer setosus* (Weiss et al. 2009). Interestingly, although growth rates were higher at 22°C than at 20°C for *C. setosus*, larvae did not reach the juvenile stage at 22°C (Weiss et al. 2009), indicating that higher growth rates do not necessarily imply better conditions for development. This was consistent with the fact that carbon content remained generally stable whilst C:N ratio increased within *C. setosus*, indicating degradation of protein as an energy source, resulting from high metabolism, and indicative of suboptimal conditions (Weiss et al. 2009). In the present study, carbon content and C:N ratio decreased throughout development at all temperatures. For *C. setosus*, larvae developing at 16°C showed a decreasing carbon content and C:N ratio and failed to develop fully to the juvenile stage; given the higher utilisation of lipid reserves, this temperature was interpreted as suboptimal for growth (Weiss et al. 2009). For *P. varians*, larval development was successful at all temperatures tested here, and suggests that the decreasing carbon content and C:N ratio is not deleterious to development (Fincham 1979, Oliphant et al. 2013, Oliphant & Thatje 2013). A similar decrease in carbon content within *M. amazonicum* was thought to indicate a 'programmed' degradation of maternal resources (Anger & Hayd 2009).

Temperature effects on respiration rate

The respiration rates of both fed and unfed larvae were not statistically different, and varied cyclically with the moult cycle. Although respiration rate data of sufficiently high resolution to allow the observation of variation associated with the moult cycles is rare, available data indicate a consistent pattern: respiration rates are highest post-moult, decrease through the inter-moult period and may increase again pre-moult (Anger & Jacobi 1985, Jacobi & Anger 1985b, Anger et al. 1989, 1990, Carvalho & Phan 1998, Anger 2001). High respiration rates at pre- and post-moult may be related to energy-demanding 'reconstruction processes', whilst low inter-moult levels correspond to a phase of little structural change and high mass accumulation (Anger 2001). Results presented here are consistent with this pattern. Unfed and fed larvae showed the same pattern, indicating that respiration rate is not down-regulated in response to unfavourable conditions and that the moult cycle progresses as usual, being comparable to fed larvae. This is consistent with the results of Expt 1 (see Supplement): larvae continue to develop and moult when starved. It appears, therefore, that *P. varians* larvae do not show energy-saving traits, in terms of metabolic rate and development, in response to starvation.

Respiration rates generally increased with temperature, and this has been demonstrated for adult *P. varians* (Oliphant et al. 2011) and other decapod larvae (Moreira et al. 1980, Jacobi & Anger 1985a, Ismael et al. 1998). Importantly, cumulative energy losses were highest at the lower temperature. Despite lower respiration rates, the longer develop-

ment time resulted in overall higher energy loss. This indicates that development is more constrained and less efficient for *P. varians* at lower temperatures and could be a reason for lower growth rates at lower temperatures. The negative effect of low temperature on *P. varians* development is consistent with the ancestry of palaemonid shrimp, which are thought to have evolved in tropical regions and which are generally distributed in tropical and temperate regions; of all *Palaemonetes* species, *P. varians* occurs at the highest latitudes. (De Grave et al. 2009, Ashelby et al. 2012, Anger 2013, OBIS 2013). The lesser extent of development within unfed larvae at 15°C relative to development at higher temperatures may be due to the influence of greater energy loss coupled with finite maternally derived energy resources. More energy may be utilised for basal metabolic costs, leaving less energy available for development at this lower temperature; consequently, larval death from starvation occurs at a less advanced developmental stage relative to higher temperatures. Combined, greater energy loss and lower growth and development rates throughout development may yield more larval instars at lower temperatures.

CONCLUSIONS

Low temperatures appear to constrain growth and development rates. This may reflect the warm-water ancestry of *Palaemonetes* spp. and may explain why development through fewer larval instars is more prevalent at higher temperatures. *P. varians* is highly resistant to starvation and may be considered facultative lecithotrophic in its first and second larval instars and planktotrophic from its third instar. This high starvation resistance is enabled by relatively high lipid content at hatching and the degradation of this energy resource throughout development. Such high maternal investment and high starvation resistance is an adaptation to the exporting of larvae from peripheral adult environments into mid- and lower estuarine and coastal environments where larval development takes place. Increasing maternal investment and the consequent decrease in fecundity are important life-history adaptations in the colonisation of freshwater and terrestrial environments among decapods.

Acknowledgements. The authors thank Shir Akbari for his instruction and technical assistance during elemental analysis. Matteo Ichino, James Morris and Alastair Brown helped with adult shrimp maintenance. A.O. was supported by a PhD studentship from the University of Southampton.

LITERATURE CITED

Anger K (1987) Energetics of spider crab *Hyas araneus* megalopa in relation to temperature and the moult cycle. Mar Ecol Prog Ser 36:115–122

Anger K (1989) Growth and exuvial loss during larval and early juvenile development of the hermit crab *Pagurus bernhardus* reared in the laboratory. Mar Biol 103: 503–511

Anger K (1995) The conquest of freshwater and land by marine crabs: adaptations in the life-history patterns and larval bioenergetics. J Exp Mar Biol Ecol 193:119–145

Anger K (1996) Physiological and biochemical changes during lecithotrophic larval development and early juvenile growth in the northern stone crab, *Lithodes maja* (Decapoda: Anomura). Mar Biol 126:283–296

Anger K (1998) Patterns of growth and chemical composition in decapod crustacean larvae. Invertebr Reprod Dev 33:159–176

Anger K (2001) The biology of decapod crustacean larvae. AA Balkema, Lisse

Anger K (2013) Neotropical *Macrobrachium* (Caridea: Palaemonidae): on the biology, origin, and radiation of freshwater-invading shrimp. J Crustac Biol 33:151–183

Anger K, Harms J (1990) Elemental (CHN) and proximate biochemical composition of decapod crustacean larvae. Comp Biochem Physiol B Biochem Mol Biol 97:69–80

Anger K, Hayd L (2009) From lecithotrophy to planktotrophy: ontogeny of larval feeding in the Amazon River prawn *Macrobrachium amazonicum.* Aquat Biol 7:19–30

Anger K, Hayd L (2010) Feeding and growth in early larval shrimp *Macrobrachium amazonicum* from the Pantanal, southwestern Brazil. Aquat Biol 9:251–261

Anger K, Ismael D (1997) Growth and elemental composition (C, N, H) in larvae and early juveniles of a South American salt marsh crab, *Chasmagnathus granulata* (Decapoda: Grapsidae). Mangroves Salt Marshes 1: 219–227

Anger K, Jacobi CC (1985) Respiration and growth of *Hyas araneus* L. larvae (Decapoda: Majidae) from hatching to metamorphosis. J Exp Mar Biol Ecol 88:257–270

Anger K, Harms J, Montú M, Bakker C (1989) Growth and respiration during the larval development of a tropical spider crab, *Libinia ferreirae* (Decapoda: Majidae). Mar Ecol Prog Ser 54:43–50

Anger K, Montú M, de Bakker C (1990) Energy partitioning during larval development of the hermit crab *Pagurus bernhardus* reared in the laboratory. J Exp Mar Biol Ecol 141:119–129

Anger K, Torres G, Nettelmann U (2007) Adaptive traits in ecology, reproduction and early life history of *Sesarma meridies*, an endemic stream crab from Jamaica. Mar Freshw Res 58:743–755

Anger K, Hayd L, Knott J, Nettelmann U (2009) Patterns of larval growth and chemical composition in the Amazon River prawn, *Macrobrachium amazonicum.* Aquaculture 287:341–348

Antonopoulou E, Emson R (1988) The combined effects of temperature and salinity on survival, moulting and metamorphosis of the larval stages of three species of palaemonid prawns. In: Ryland JS, Tyler PA (eds) Reproduction, genetics and distributions of marine organisms: Proc 23rd Eur Mar Biol Symp, Swansea, 5–9 September 1988. Olsen & Olsen, Fredensborg, p 339–348

Ashelby CW, Page TJ, De Grave S, Hughes JM, Johnson ML

(2012) Regional scale speciation reveals multiple invasions of freshwater in Palaemoninae (Decapoda). Zool Scr 41:293–306

Benson BB, Krause D (1984) The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. Limnol Oceanogr 29:620–632

Bouchon D (1991a) Biological clock in seasonal reproductive cycle in the ditch shrimp *Palaemonetes varians* Leach. I. Photoperiodic time measurement. J Exp Mar Biol Ecol 146:1–12

Bouchon D (1991b) Biological clock in seasonal reproductive cycle in the ditch shrimp *Palaemonetes varians* Leach. II. Ovarian state-dependent responses to non-diel light-dark cycles. J Exp Mar Biol Ecol 146:13–26

Broad AC (1957a) Larval development of *Palaemonetes pugio* Holthuis. Biol Bull 112:144–161

Broad AC (1957b) The relationship between diet and larval development of *Palaemonetes.* Biol Bull 112:162–170

Calcagno JA, Thatje S, Anger K, Lovrich GA, Kaffenberger A (2003) Changes in biomass and chemical composition during lecithotrophic larval development of the southern stone crab *Paralomis granulosa*. Mar Ecol Prog Ser 257: 189–196

Carvalho PSM, Phan VN (1998) Oxygen consumption and ammonia excretion during the moulting cycle in the shrimp *Xiphopenaeus kroyeri.* Comp Biochem Physiol A Mol Integr Physiol 119:839–844

Clarke A (2003) Costs and consequences of evolutionary temperature adaptation. Trends Ecol Evol 18:573–581

Criales MM, Anger K (1986) Experimental studies on the larval development of the shrimps *Crangon crangon* and *C. allamanni.* Helgol Meeresunters 40:241–265

Dawirs RR (1986) Influence of limited food supply on growth and elemental composition (C, N, H) of *Carcinus maenas* (Decapoda) larvae, reared in the laboratory. Mar Ecol Prog Ser 31:301–308

Dawirs RR, Püschel C, Schorn F (1986) Temperature and growth in *Carcinus maenas* L. (Decapoda: Portunidae) larvae reared in the laboratory from hatching through metamorphosis. J Exp Mar Biol Ecol 100:47–74

De Grave S, Pentcheff ND, Ahyong ST, Chan TY and others (2009) A classification of living and fossil genera of decapod crustaceans. Raffles Bull Zool 21:1–109

Dolmen D (1997) *Palaemonetes varians* (Leach) (Crustacea, Decapoda, Natantia) in Norway. Sarsia 82:19–21

Dolmen D, Hindley JD, Kleiven E (2004) Distribution of *Palaemonetes varians* (Leach) (Crustacea, Decapoda) in relation to biotope and other caridean shrimps in brackish waters of southern Norway and southwestern Sweden. Sarsia 89:8–21

Etilé E, Despland E (2008) Developmental variation in the forest tent caterpillar: life history consequences of a threshold size for pupation. Oikos 117:135–143

Falciai L (2001) Occurrence of *Palaemonetes varians* (Leach, 1814) (Decapoda, Palaemonidae) in a brackish pond in Algeria. Crustaceana 74:697–701

Fincham AA (1979) Larval development of British prawns and shrimps (Crustacea: Decapoda: Natantia). 2. *Palaemonetes* (*Palaemonetes*) *varians* (Leach, 1814) and morphological variation. Bull Br Mus (Nat Hist) Zool 35: 163–182

Giménez L, Anger K, Torres G (2004) Linking life history traits in successive phases of a complex life cycle: effects of larval biomass on early juvenile development in an estuarine crab, *Chasmagnathus granulata.* Oikos 104: 570–580

Gnaiger E (1983) Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In: Gnaiger E, Forstner H (eds) Polarographic oxygen sensors. Springer-Verlag, Berlin, p 337–345

González-Ortegón E, Cuesta JA (2006) An illustrated key to species of *Palaemon* and *Palaemonetes* (Crustacea: Decapoda: Caridea) from European waters, including the alien species *Palaemon macrodactylus.* J Mar Biol Assoc UK 86:93–102

Gurney R (1924) The larval development of some British prawns (Palaemonidae). I. *Palaemonetes varians*. Proc Zool Soc Lond 94:297–328

Gurney R (1942) Larvae of decapod Crustacea. HR Engelmann (J Cramer) & Wheldon & Wesley, London

Harms J (1990) Accumulation and loss of biomass in *Liocarcinus holsatus* larvae during growth and exuviation. Mar Biol 104:183–190

Hindley J (2001) The ecology and dynamics of the brackish water prawn, *Palaemonetes varians* (Leach) and its interrelationship with the common goby, *Pomatoschistus microps* (Krøyer) in artificial coastal lagoons of the Ribble Estuary, Lancashire. PhD thesis, University of Lancaster, Edge Hill

Hovel KA, Morgan SG (1997) Planktivory as a selective force for reproductive synchrony and larval migration. Mar Ecol Prog Ser 157:79–95

Hubschman JH, Broad AC (1974) The larval development of *Palaemonetes intermedius* Holthuis, 1949 (Decapod, Palaemonidae) reared in the laboratory. Crustaceana 26: 89–103

Ismael D, Anger K, Moreira GS (1998) Influence of temperature on larval survival, development, and respiration in *Chasmagnathus granulata* (Crustacea, Decapoda). Helgol Meeresunters 51:463–475

Jacobi CC, Anger K (1985a) Effects of temperature on respiration of larval stages of *Hyas araneus* and *H. coarctatus* (Decapoda, Majidae). Mar Ecol Prog Ser 26:181–186

Jacobi CC, Anger K (1985b) Growth and respiration during the larval development of *Hyas coarctatus* (Decapoda: Majidae). Mar Biol 87:173–180

Jenner CE (1955) A field character for distinguishing *Palaemonetes vulgaris* from *Palaemonetes pugio.* Biol Bull 109:360

Kingsolver JG (2007) Variation in growth and instar number in field and laboratory *Manduca sexta.* Proc Biol Sci 274: 977–981

Knowlton RE, Kirby DF (1984) Salinity tolerance and sodium balance in the prawn *Palaemonetes pugio* holthuis, in relation to other *Palaemonetes* spp. Comp Biochem Physiol Part A Physiol 77:425–430

Knowlton RE, Schoen RH (1984) Salinity tolerance and sodium balance in the prawn *Palaemonetes vulgaris* (say) compared with *P. pugio.* Comp Biochem Physiol Part A Physiol 79:519–524

Lofts B (1956) The effects of salinity changes on the respiratory rate of the prawn *Palaemonetes varians* (Leach). J Exp Biol 33:730–736

Lovrich GA, Thatje S, Calcagno JA, Anger K, Kaffenberger A (2003) Changes in biomass and chemical composition during lecithotrophic larval development of the southern king crab, *Lithodes santolla* (Molina). J Exp Mar Biol Ecol 288:65–79

Moreira GS, McNamara JC (1986) The effect of salinity on

the upper thermal limits of survival and metamorphosis during larval development in *Macrobrachium amazonicum* (Heller) (Decapoda, Palaemonidae). Crustaceana 50:231–238

Moreira GS, McNamara JC, Moreira PS, Weinrich M (1980) Temperature and salinity effects on the respiratory metabolism of the first zoeal stage of *Macrobrachium holthuisi* Genofre & Lobão (Decapoda: Palaemonidae). J Exp Mar Biol Ecol 47:141–148

OBIS (2013) Ocean biogeographic information system. www.iobis.org (Accessed on 10 September 2013)

Oliphant A (2014) Decapod crustacean larval developmental plasticity and the evolution of lecithotrophy and abbreviated development. PhD thesis, University of Southampton

Oliphant A, Thatje S (2013) Per offspring investment implications for crustacean larval development: evolutionary insights into endotrophy and abbreviated development. Mar Ecol Prog Ser 493:207–217

Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of the shallow-water caridean shrimp *Palaemonetes varians* across its thermal tolerance window. J Exp Biol 214:1109–1117

Oliphant A, Hauton C, Thatje S (2013) The implications of temperature-mediated plasticity in larval instar number for development within a marine invertebrate, the shrimp *Palaemonetes varians*. PLoS ONE 8:e75785

Pereira F, Pereira R, Queiroga H (2000) Flux of decapod larvae and juveniles at a station in the lower Canal de Mire (Ria de Aveiro, Portugal) during one lunar month. Invertebr Reprod Dev 38:183–206

Rothlisberg PC (1979) Combined effects of temperature and salinity on the survival and growth of the larvae of *Pandalus jordani* (Decapoda: Pandalidae). Mar Biol 54:125–134

Sandifer PA (1975) The role of pelagic larvae in recruitment to populations of adult decapod crustaceans in the York River estuary and adjacent lower Chesapeake Bay, Virginia. Estuar Coast Mar Sci 3:269–279

Sokal R, Rohlf F (1995) Biometry. Freeman & Company, New York, NY

Strathmann RR (1982) Selection for retention or export of larvae in estuaries. In: Kennedy VS (ed) Estuarine comparisons. Academic Press, New York, NY, p 521–536

Thatje S, Schnack-Schiel S, Arntz WE (2003) Developmental trade-offs in Subantarctic meroplankton communities and the enigma of the low decapod diversity in high southern latitudes. Mar Ecol Prog Ser 260:195–207

Thatje S, Lovrich GA, Torres G, Hagen W, Anger K (2004) Changes in biomass, lipid, fatty acid and elemental composition during the abbreviated larval development of the subantarctic shrimp *Campylonotus vagans*. J Exp Mar Biol Ecol 301:159–174

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biol Rev Camb Philos Soc 25:1–45

Torres G, Giménez L, Anger K (2002) Effects of reduced salinity on the biochemical composition (lipid, protein) of zoea 1 decapod crustacean larvae. J Exp Mar Biol Ecol 277:43–60

Urzúa Á, Guerao G, Cuesta JA, Rotllant G, Estévez A, Anger K (2013) The bioenergetic fuel for non-feeding larval development in an endemic palaemonid shrimp from the Iberian Peninsula, *Palaemonetes zariquieyi*. Mar Freshw Behav Physiol 46:381–397

Vogt G (2013) Abbreviation of larval development and extension of brood care as key features of the evolution of freshwater Decapoda. Biol Rev Camb Philos Soc 88: 81–116

Weiss M, Heilmayer O, Brey T, Thatje S (2009) Influence of temperature on the zoeal development and elemental composition of the cancrid crab, *Cancer setosus* Molina, 1782 from Pacific South America. J Exp Mar Biol Ecol 376:48–54

Editorial responsibility: Steven Morgan, Bodega Bay, California, USA

Submitted: November 12, 2013; Accepted: February 18, 2014
Proofs received from author(s): May 20, 2014