Per offspring investment implications for crustacean larval development: evolutionary insights into endotrophy and abbreviated development

Andrew Oliphant*, Sven Thatje

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

ABSTRACT: At the inter-specific level, per offspring investment (POI) is associated with larval development mode and follows a macro-ecological trend within the marine environment; higher POI and its associated greater degree of endotrophy and abbreviated development is found in cooler, high latitude regions. Here, the implications of between-brood-variation in hatchling energy content (measured as carbon mass) on larval starvation resistance and developmental plasticity within the caridean shrimp *Palaemonetes varians* were assessed. Results demonstrate that greater POI provides increased endotrophy and the potential for abbreviated development. In the absence of food, *P. varians* larvae from broods of higher hatchling energy content developed to more advanced larval stages and survived for longer before succumbing to starvation. In the presence of food, *P. varians* larvae from broods of higher hatchling energy content developed through fewer larval instars, showed higher growth rates, and had shorter development times. Also, for larvae developing through the same number of larval instars, larvae from broods of higher hatchling energy content developed to greater juvenile dry weight. These data support the hypothesis that macro-ecological trends in development mode are driven by inter-specific variations in POI. At the intra-specific level, phenotypic plasticity allows for environmentally mediated variations in POI. Under differential selection pressures, this flexibility may, thus, eventually permit the evolution of the diverse and complex life cycles observed in the marine environment.

KEY WORDS: Carry-over effects · Evolution of lecithotrophy · Larval ecology · Palaemonidae · *Palaemonetes varians* · POI

INTRODUCTION

Per offspring investment (POI) — the quantity and quality of maternal resources allocated to a single offspring — is a trait with fundamental ecological and evolutionary implications (Vance 1973, Smith & Fretwell 1974, Christiansen & Fenchel 1979). POI may influence larval development, starvation resistance, larval ecology, planktonic longevity (for the dispersive propagules of marine invertebrates), and may have carry-over effects for juvenile and adult life (Fox 1994, Emlet & Høegh-Guldberg 1997, Ito 1997, Moran & Emlet 2001). Macro-ecological trends in

POI, and its related larval development modes at the inter-specific level, and geographic variation in POI at the intra-specific level, indicate past and continuing selection on this trait. The influence of changes in POI on larval development and their effect on later life stages is requisite if we are to understand how selective pressures act to change POI and development modes over evolutionary time scales.

At the inter-specific level, macro-ecological trends in POI (and its related development modes) are known for marine invertebrates (including marine arthropods); Danish ecologist Gunnar Thorson first observed higher POI in species from higher/polar

(cooler) latitudes relative to closely related species from lower/temperate (warmer) latitudes (Thorson 1936, 1950). Evidence also suggests an increase in POI with increasing depth within the oceans (Thorson 1961, 1964, King & Butler 1985, Pond et al. 1997). Based on observed trends in POI and knowledge of the relationship between POI and development mode, Thorson (1936, 1950) hypothesised that aplanktonic and planktonic non-feeding (lecithotrophic) larval development were more prevalent at high latitudes, whilst planktonic feeding (planktotrophic) larval development was more prevalent at lower latitudes. A recent meta-analysis of life-history data supports Thorson's hypotheses on the macroecological trends in development mode. Marshall et al. (2012) demonstrated relationships between development mode and latitude and, within development modes, between offspring size and latitude. Importantly, and despite Mileikovsky (1971) proposing 'Thorson's Rule', the concept proposed by Thorson should be considered as a general macro-ecological concept, not an absolute rule (e.g. Thatje et al. 2005).

Inter-specific level variation in POI among marine invertebrates (and marine arthropods) is related to development mode; high POI is associated with high nutritional independence (endotrophy/lecithotrophy) and abbreviated development, whilst low POI is associated with planktotrophy (Thorson 1936, 1950). In arthropods, abbreviated development consists of larvae that hatch in an advanced stage of development and proceed through relatively few larval instars before metamorphosing to juveniles, for example Antarctic shrimp (Thatje et al. 2003, 2004). High POI and abbreviated development are considered to be evolutionary adaptations to low or unpredictable food availability and/or the mis-match between short periods of primary production (resulting from extreme seasonality) and prolonged development (resulting from low temperatures) at high latitudes (Thorson 1950, Anger 2001, Thatje et al. 2003). High POI and abbreviated development are also known from brackish and freshwaters and are considered evolutionary adaptations to the limited food availability prevalent within these environments (Anger 2001).

Variation in POI is also known at the intra-specific level for marine invertebrates (including marine arthropods). Thorson observed that within a single species, populations from higher (cooler) latitudes exhibit greater POI than populations from lower (warmer) latitudes, reflecting the macro-ecological trend in POI at the inter-specific level (Thorson 1936). Mounting empirical evidence has demonstrated intra-specific variation in POI at both spatial and temporal scales; greater POI being observed under cooler conditions (often associated with higher latitude, greater water depth, or season; e.g. Thorson 1950, Barnes & Barnes 1965, Urzúa et al. 2012). Intra-specific variation in POI at spatial scales may indicate genetic divergence and local adaptation between populations, a result of selective pressures acting on POI within those populations (Lonsdale & Levinton 1985). Conversely, temporal variations in POI within a population demonstrate POI plasticity. Such plasticity is known at a range of scales, e.g. inter-annually, between seasons, and within a single breeding season (Boddeke 1982, Sheader 1983, 1996, Kattner et al. 1994, Oh & Hartnoll 2004, Urzúa & Anger 2012, Urzúa et al. 2012). POI plasticity is influenced by maternal size and age, food quantity and quality, population density, and temperature. Temperature-mediated shifts in POI may produce eggs which differ in size and dry weight (DW) (e.g. Blanckenhorn 2000, Fischer et al. 2003a,b, Liefting et al. 2010) and in relative yolk composition (e.g. water, carbohydrate, lipid, protein, and glycogen concentrations; Geister et al. 2008, Sloggett & Lorenz 2008, Liefting et al. 2010). Studies assessing the fitness implications of temperature-mediated POI have found that eggs with greater or intermediate POI are less prone to desiccation, more resistant to temperature shock, have greater hatching success, and give rise to larger, fitter juveniles across a range of thermal conditions (Blanckenhorn 2000, Fischer et al. 2003a,b, Liefting et al. 2010).

Oliphant et al. (2013) identified temperature-mediated developmental plasticity for *Palaemonetes varians* larvae from temperate European waters (Hampshire, UK). For *P. varians*, 'normal' larval development proceeds through 5 larval instars (Fincham 1979). Oliphant et al. (2013) identified a 4-instar development pathway which omitted the final decapodid instar and which was temperature-dependent, occurring more often under warmer conditions. Observations for the estuarine crab *Neohelice* (*Chasmagnathus*) *granulata*, which also develops through either of 2 development pathways (Pestana & Ostrensky 1995), suggest that developmental plasticity is also influenced by hatchling DW (Giménez & Torres 2002). Larval instar number plasticity is an important trait among arthropod as it allows growth and development to a weight/development threshold under poor growth and development conditions (Nijhout 1994, Esperk et al. 2007, Kingsolver 2007, Etilé & Despland 2008). Variations in larval instar number are known to influence development time,

growth rate, and juvenile/post-metamorphosis DW and, consequently, are of ecological and potentially evolutionary importance (Kingsolver 2007, Etilé & Despland 2008, Oliphant et al. 2013).

Here, we use between-brood-variation in hatchling (zoea 1) energy content to assess the implications of POI for larval starvation resistance, instar number, growth rate, and development time, and juvenile DW within the caridean shrimp *Palaemonetes varians*. In accordance with Marshall & Keough (2007), we consider POI to be the energy content of a propagule once it has become independent of maternal nutritional investment. According to this definition, the energy content of a freely spawned egg is the appropriate measure of POI but the energy content of a direct developing snail egg before the embryo has ingested nurse eggs is not (adapted from Marshall & Keough 2007). We use the energy content of hatchling larvae as an approximation of POI as conditions during embryonic development can influence energy content at hatching (Wehrtmann & Lopez 2003). As proxies for energy content, propagule size/volume and propagule DW are routinely used in the literature. Given the inherent inaccuracies with the use of propagule volume (Moran & McAlister 2009), here, we use propagule carbon mass (μg ind.$^{-1}$) as an accurate proxy for lipid content and, thus, energy content (following Anger & Nair 1979), a more accurate measure than has been used previously in similar studies. The implications of POI for larval development and potential carry-over effects for early juvenile life are essential if we are to understand the selection pressures which shape POI.

MATERIALS AND METHODS

Collection and maintenance of ovigerous *Palaemonetes varians*

Ovigerous *Palaemonetes varians* were collected by hand-netting from ditches on Oxey Marsh salt-marsh (Lymington, Hampshire, UK) on 24 May, 12 and 26 June, 2012. Ovigerous shrimp were transferred (within 1 h) to the aquarium of the National Oceanography Centre Southampton (Hampshire, UK); here, brood embryonic development was assessed and staged according to Müller et al. (2004). Ovigerous *P. varians* with stage VII and VIII embryos were selected and isolated in 1 l plastic beakers filled with ~850 ml of 15°C (approx. field temperature at time of collections), salinity 32, 1 μm-filtered seawater and transferred to temperature-controlled incubators set

at 15°C and 12 h light:12 h dark. Ovigerous females were checked daily (at ~09:00 h) for hatched larvae. Between collection and larval hatching, ovigerous females were maintained at 15°C, salinity 32, 12 h light:12 h dark), and fed Tetra Goldfish flakes 3 times per week to excess. Water changes (>70%; 15°C, salinity 32, 1 μm-filtered seawater) were done every second day. Only larvae which hatched within ~10 d of collection dates were used (mean ± SD = 5.2 ± 3.8 d).

Larval maintenance and brood average carbon mass calculation

On hatching, actively swimming larvae were separated from 37 ovigerous *Palaemonetes varians* (using a pipette) and isolated individually in 100 ml plastic beakers filled with ~80 ml of 15°C, salinity 32, 1 μm-filtered and UV-treated seawater. To calculate a brood average DW (μg ind.$^{-1}$) and carbon mass (μg ind.$^{-1}$), a sample of 15 newly hatched larvae per brood (i.e. 15 larvae from each of the 37 ovigerous *P. varians*) were blotted on tissue paper, transferred individually to pre-weighed Elemental Microanalyser tin capsules and frozen at −80°C. They were later freeze-dried (for 24 h) and weighed for DW. Carbon and nitrogen (C and N) composition of n = 5 samples (from each of the 37 broods) was measured.

For each brood, n = 24 larvae were transferred to temperature-controlled incubators set at 15, 20, and 25°C, 12 h light:12 h dark (i.e. a total of n = 72 larvae per brood separated across 3 temperatures). This temperature range is within the tolerable range for viable *Palaemonetes varians* larval development (Oliphant et al. 2013). Water changes (100%; 15, 20, and 25°C, salinity 32, 1 μm-filtered and UV-treated seawater) were done daily at 20 and 25°C and every second day at 15°C. Of n = 24 larvae for each brood at each temperature, n = 12 larvae were fed and n = 12 were not fed (non-fed category). Zoea 1 of *P. varians* is facultative lecithotrophic (A. Oliphant unpubl. data), allowing it to follow an export strategy to estuarine and coastal waters where conditions for larval development may be more favourable (Anger 2001 and references therein). Consequently, larvae from both categories (fed and non-fed) were not fed during zoea 1 (mean ± SD duration: 15°C = 4.0 ± 0.5 d, 20°C = 2.3 ± 0.5 d, 25°C = 2.1 ± 0.3 d) (after Oliphant et al. 2013). On moulting to zoea 2, larvae within the fed group only were fed freshly hatched *Artemia* sp. nauplii to excess. Therefore, fed versus non-fed treatments were done only after larvae attained zoea 2.

Larval mortality and development, assessed by morphological changes and moulting (following Fincham 1979; larval nomenclature followed Oliphant et al. 2013) were monitored daily (between ~09:00 h and 11:00 h). On moulting to the juvenile stage, animals were blotted on tissue paper, transferred individually to pre-weighed tin capsules and frozen at −80°C; these samples were then freeze-dried and weighed for DW.
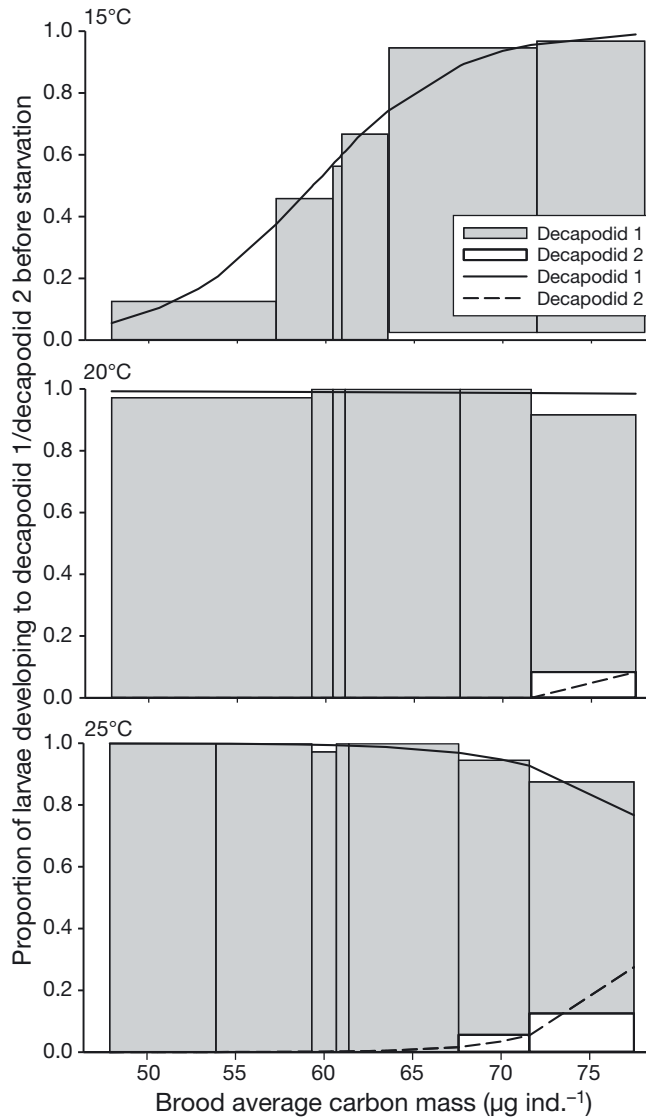

Fig. 1. *Palaemonetes varians.* Logistic regressions of the proportion of larvae developing to decapodid 1 and decapodid 2 before succumbing to starvation against brood average carbon mass (µg ind.$^{-1}$) at 15, 20, and 25°C. Bars indicate proportions data which were divided into intervals for graphical purposes only. Bar widths may vary between temperatures as the intervals were chosen so that each bar represented a similar n. Best-fit-lines for logistic regressions are shown

Statistical analysis

For non-fed larvae, logistic regression was used to analyse the relationship between brood average carbon mass (µg ind.$^{-1}$) and the proportion of larvae developing to decapodid 1 or decapodid 2 before starvation. Similarly, for fed larvae, logistic regression was used to analyse the relationship between brood average carbon mass and the proportion of larvae developing through 4 instars. To analyse the effects of brood average carbon mass on starvation resistance (days), analysis of covariance (ANCOVA) was used, with brood average carbon mass as a continuous predictor and temperature as a factor. The model was fitted using the generalised least squares (GLS) method and was weighted by a variance structure which accounted for heterogeneity within data. ANCOVA was also used to assess the effect of brood average carbon mass on larval development time (days), juvenile DW (µg ind.$^{-1}$), and larval growth rate (µg ind.$^{-1}$ d^{-1}). Brood average carbon mass was a continuous predictor and temperature and instar number (4 and 5 instars) were factors. Again, models were fitted using the GLS method and were weighted by variance structures which accounted for heterogeneity within data. Post hoc testing was done using the least squares mean method for factor combinations (temperature and instar number) and adjusted (Tukey method) for multiple comparisons. Statistical analysis was carried out using Minitab 16 for logistic regression; and Ri386 3.0.1 (R Development Core Team 2013) for ANCOVA and post hoc testing, using packages 'nlme' (Pinheiro et al. 2013) and 'lsmeans' (Lenth 2013) in accordance with Sokal & Rohlf (1995), Underwood (1997), and Zuur et al. (2009).

RESULTS

Larval starvation resistance

The stage to which larvae developed before succumbing to starvation was affected by brood average carbon mass. At 15°C, the proportion of larvae developing to decapodid 1 before succumbing to starvation significantly increased with increasing carbon mass (p < 0.001, $G = 87.70$; Fig. 1). At 25°C, the proportion of larvae developing to decapodid 1 before succumbing to starvation significantly decreased with increasing carbon mass (p = 0.001, $G = 13.90$), whilst the proportion of larvae developing to decapodid 2 before succumbing to starvation significantly
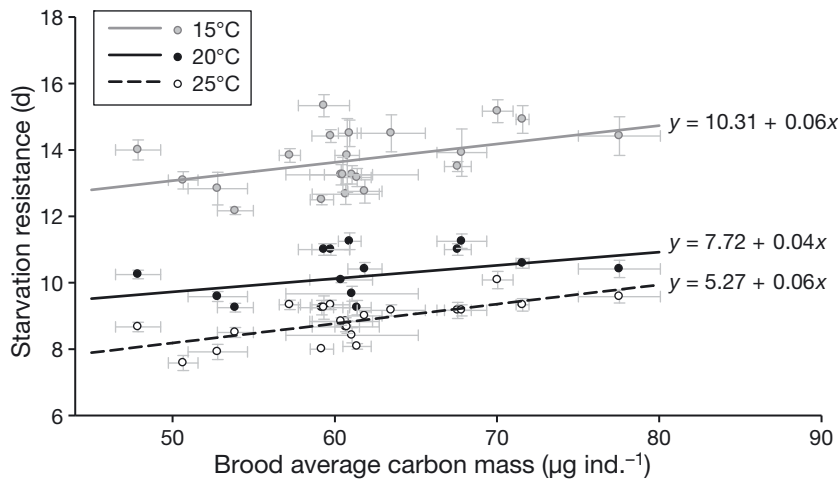
Fig. 2. *Palaemonetes varians*. Starvation resistance against brood average carbon mass at 15, 20, and 25°C. For clarity, data are presented as means ± SE, although statistical analysis was done on raw data. Best-fit-lines (fitted by generalised least squares method) and their equations are shown

increased with increasing carbon mass (p = 0.002, G = 18.21; Fig. 1). At 20°C, neither the proportion of larvae developing to decapodid 1 (p = 0.801, G = 0.06) nor decapodid 2 (p = 0.998, G = 5.63) before succumbing to starvation was significantly related to carbon mass.

Brood average carbon mass significantly affected starvation resistance; larvae from broods of greater brood average carbon mass were more resistant to starvation (Fig. 2, Table 1). Temperature also affected starvation resistance as starvation resistance increased with decreasing temperature (Fig. 2, Table 1). There was, however, no significant interaction between brood average carbon mass and temperature (Table 1). As the interaction between carbon mass and temperature was non-significant, no post hoc testing was done.

Table 1. Effects of brood average carbon mass (C_{mass}) and temperature (Temp), and their interaction on starvation resistance within *Palaemonetes varians*. ANCOVA analysis, with brood average carbon mass as a continuous predictor and temperature as a factor. Models fitted using the generalised least squares method and weighted by variance structures which accounted for heterogeneity within data

Factor	df	F	p
C_{mass}	1	56.67	<0.0001
Temp	2	1121.40	<0.0001
$C_{mass} \times$ Temp	2	1.11	0.3299
Error	690		

Larval developmental plasticity

The number of larval instars through which larvae developed was significantly affected by brood average carbon mass. At all temperatures, the proportion of larvae developing to juvenile through 4 instars significantly increased with increasing brood average carbon mass (p < 0.001 in all cases; 15°C, G = 18.54; 20°C, G = 30.13; 25°C, G = 24.52; Fig. 3).

Larval development time, juvenile DW, and larval growth rate

Larval development time was affected by brood average carbon mass, temperature, and instar number during development: larval development time decreased with increasing brood average carbon mass, with increasing temperature, and was reduced for larvae developing through 4 instars (Figs. 4A & 5A, Table 2). There were significant interactions between carbon mass and temperature, between temperature and instar number, and between carbon mass, temperature, and instar number (Figs. 4A & 5A, Table 2). Post hoc testing indicated that the linear relationship between carbon mass and larval development time for larvae developing through 4 instars at 15°C was significantly different from that for larvae developing through 4 and 5 instars at both 20 and 25°C (p < 0.05 in all cases). The linear relationship was not significantly different from that at 15°C for larvae developing through 5 instars (p = 0.106), indicating that the slopes of their best fit-lines were not different. All other linear relationships were also statistically indistinct, indicating that the slopes of their best fit-lines were not different.

Juvenile DW was also affected by brood average carbon mass, temperature, and instar number during development: juvenile DW increased with brood average carbon mass, generally increased with temperature, and was greater for development through 5 instars (Figs. 4B & 5B, Table 2). There were significant interactions between carbon mass and instar number, temperature and instar number, and between carbon mass, temperature, and instar number (Figs. 4B & 5B, Table 2). Post hoc testing indicated that the linear relationship between carbon mass and juvenile DW for larvae developing
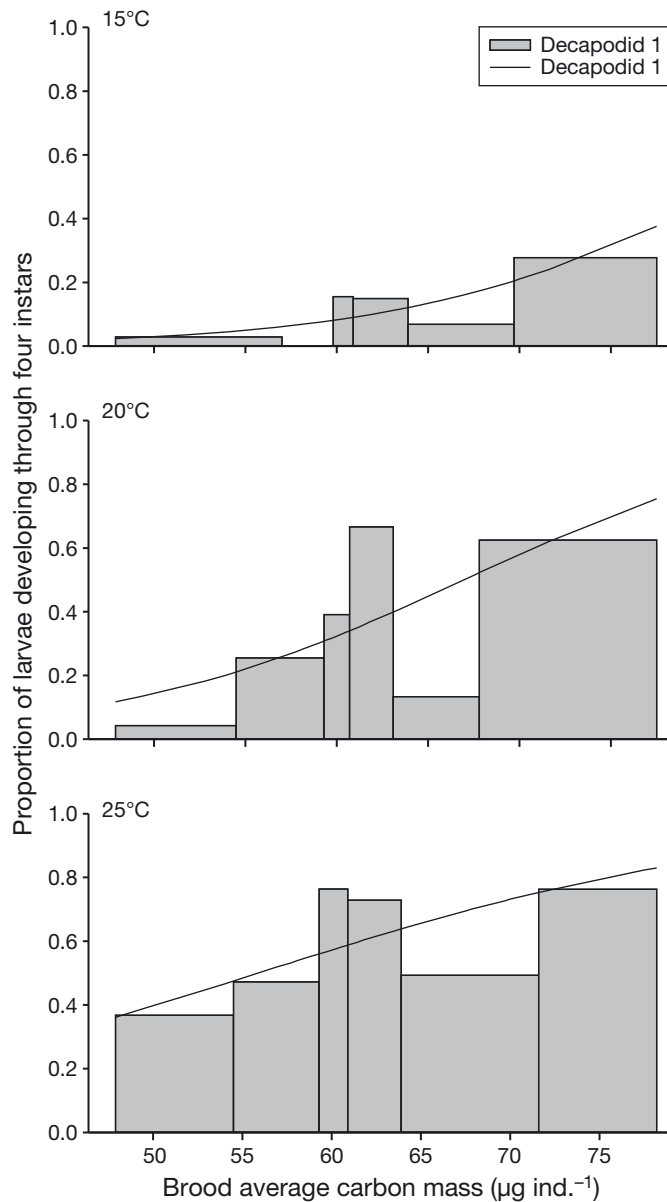
Fig. 3. *Palaemonetes varians.* Logistic regressions of the proportion of larvae developing through 4 instars against brood average carbon mass (µg ind.$^{-1}$) at 15, 20, and 25°C. Bars indicate proportions data which were divided into intervals for graphical purposes only. Bar widths may vary between temperatures as the intervals were chosen so that each bar represented a similar n. Best-fit-lines for logistic regressions are shown
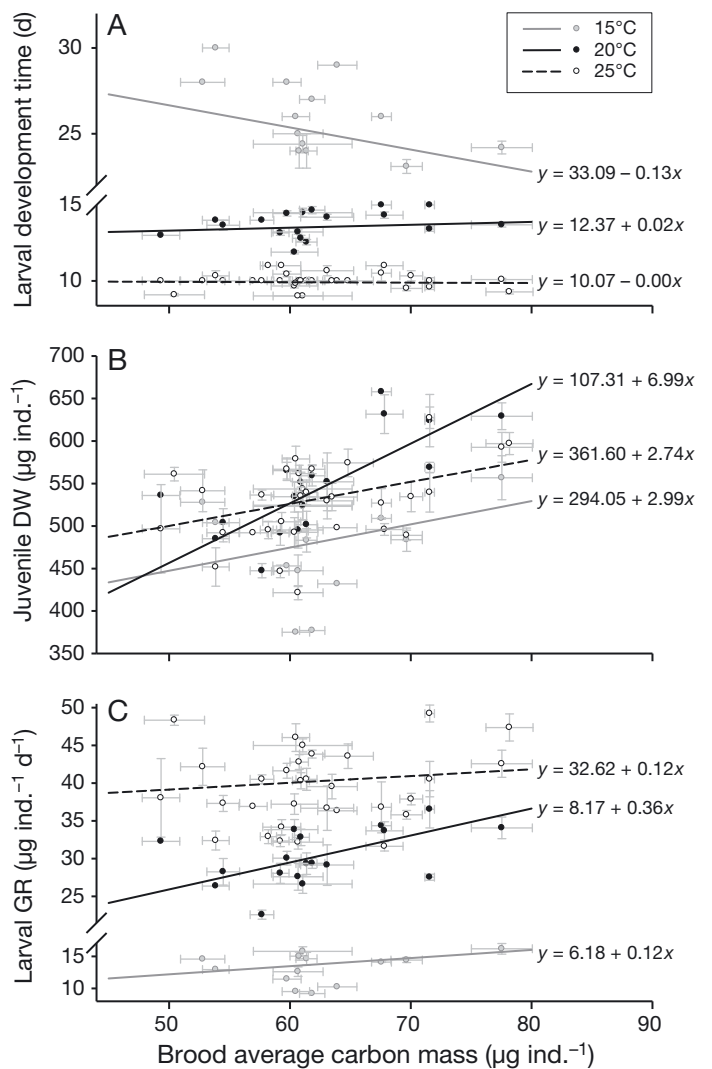

Fig. 4. *Palaemonetes varians.* (A) Larval development time, (B) juvenile dry weight (DW), and (C) larval growth rate (GR) against brood average carbon mass (µg ind.$^{-1}$) for larvae developing through 4 instars at 15, 20, and 25°C. For clarity, data are presented as means ± SE, although statistical analyses were done on raw data. Best-fit-lines (fitted by generalised least squares method) and their equations are shown

through 4 instars at 20°C was significantly different from that for larvae developing through 4 instars at 15 and 25°C and 5 instars at 20°C (p < 0.005 in all cases), and 5 instars at 25°C (p < 0.05). All other linear relationships were also statistically indistinct, indicating that the slopes of their best fit-lines were not different.

Larval growth rate was also affected by brood average carbon mass, temperature, and instar number during development: larval growth rate increased with increasing brood average carbon mass, increased with temperature, and was greater for development through 5 instars (Figs. 4C & 5C, Table 2). There were significant interactions between carbon mass and instar number, and temperature and instar number (Figs 4C & 5C, Table 2). As the interaction between carbon mass, temperature, and instar number was non-significant for larval growth rate (Table 2), no post hoc testing was done.
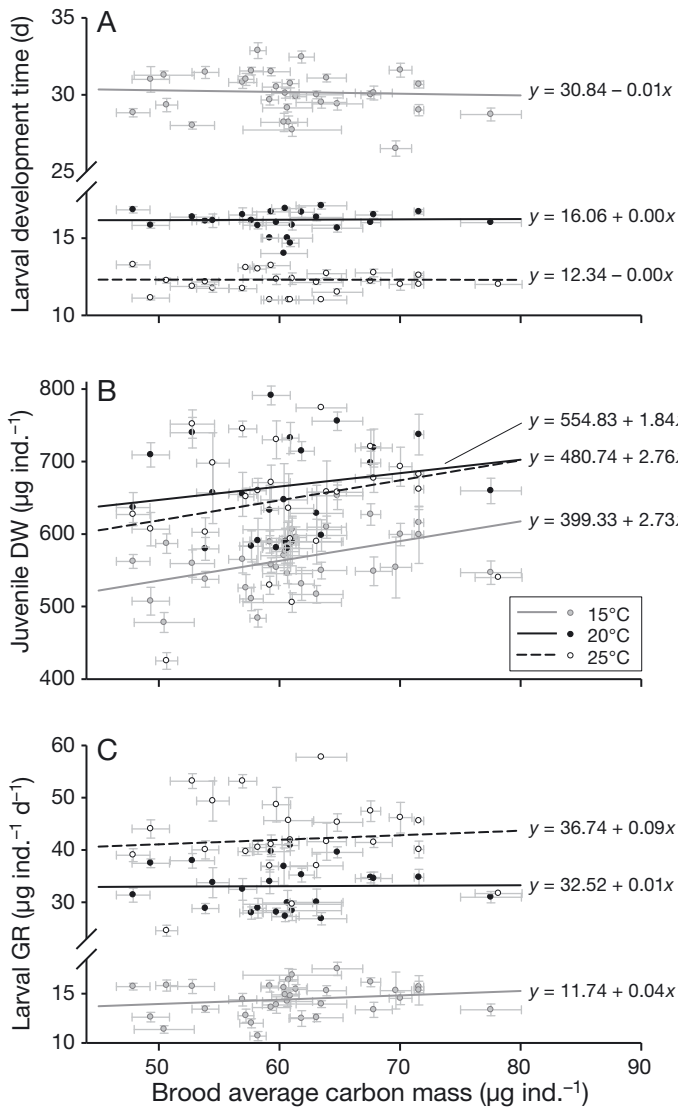
Fig. 5. *Palaemonetes varians.* (A) Larval development time, (B) juvenile dry weight (DW), and (C) larval growth rate (GR) against brood average carbon mass (µg ind.$^{-1}$) for larvae developing through 5 instars at 15, 20, and 25°C. For clarity, data are presented as means ± SE, although statistical analyses were done on raw data. Best-fit-lines (fitted by generalised least squares method) and their equations are shown

Table 2. Effects of brood average carbon mass (C_{mass}), temperature (Temp), and instar number (Instar), and their interactions on larval development time, juvenile dry weight, and larval growth rate within *Palaemonetes varians.* ANCOVA analysis, with brood average carbon mass as a continuous predictor and temperature and instar number (4 and 5 instars) as factors. Models fitted using the generalised least squares method and weighted by variance structures which accounted for heterogeneity within data

Factor	df	F	p
Larval development time			
C_{mass}	1	794.70	<0.0001
Temp	2	20215.85	<0.0001
Instar	1	1758.55	<0.0001
C_{mass} × Temp	2	7.52	0.0006
C_{mass} × Instar	1	1.49	0.2225
Temp × Instar	2	45.16	<0.0001
C_{mass} × Temp × Instar	2	3.78	0.0231
Error	1036		
Juvenile dry weight			
C_{mass}	1	23.77	<0.0001
Temp	2	41.31	<0.0001
Instar	1	444.71	<0.0001
C_{mass} × Temp	2	1.79	0.1669
C_{mass} × Instar	1	7.38	0.0067
Temp × Instar	2	4.02	0.0182
C_{mass} × Temp × Instar	2	4.38	0.0128
Error	1036		
Larval growth rate			
C_{mass}	1	34.564	<0.0001
Temp	2	3370.120	<0.0001
Instar	1	13.955	0.0002
C_{mass} × Temp	2	0.713	0.4905
C_{mass} × Instar	1	11.020	0.0009
Temp × Instar	2	3.379	0.0345
C_{mass} × Temp × Instar	2	2.945	0.0530
Error	1036		

DISCUSSION

At the inter-specific level among marine arthropods, high POI is related to endotrophy and abbreviated development and is associated with environments in which food production is limited or unpredictable (Thorson 1950, Anger 2001). Results presented here demonstrate the advantage of greater POI under a range of temperatures and the implications of POI for larval development and starvation resistance at the intra-specific level.

Most studies assessing the implications of POI for larval development and carry-over effects have manipulated POI either directly, by removing yolk (Emlet & Høegh-Guldberg 1997) or by 'twinning' 2- and 4-cell embryos (Sinervo & McEdward 1988, Hart 1995), or indirectly, by maternal effects on POI; e.g. temperature-mediated POI plasticity (e.g. Blanckenhorn 2000, Fischer et al. 2003a, Bownds et al. 2010, Liefting et al. 2010). Such studies have found that POI influences size at metamorphosis, post-metamorphosis growth, and juvenile survivorship (Sinervo & McEdward 1988, Hart 1995, Emlet & Høegh-Guldberg 1997). Knowledge on the influence of POI on larval developmental plasticity is poor. Poorer still is our knowledge of the interaction between POI and environmental factors and their combined effect on larval developmental plasticity. Here,

we show that between-brood variations in hatchling energy content (measured as carbon mass) significantly affects larval starvation resistance, instar number, growth rate, and development time, and has potential implications for juvenile and adult performance through differences in initial juvenile DW.

In the absence of food, *Palaemonetes varians* larvae from broods of higher hatchling energy content developed to more advanced larval stages and survived for longer before succumbing to starvation, indicating a higher degree of nutritional independence (endotrophy). Within the caridean shrimp *Crangon crangon*, greater POI is known to increase starvation resistance and provide an earlier point of saturation for secondary lecithotrophy (Paschke et al. 2004). Similarly, for the butterfly *Bicyclus anynana*, greater POI increases starvation resistance, and general survivorship (Fischer et al. 2003a).

In the presence of food, *Palaemonetes varians* larvae from broods of higher hatchling energy content developed through fewer larval instars, showed higher growth rates, and had shorter development times. Further, for those larvae developing through the same number of larval instars, larvae from broods of higher hatchling energy content developed to greater juvenile DW. The significant interactions between hatchling energy content and instar number for both juvenile DW and larval growth rate, coupled with the greater slopes of the linear relationships for larvae developing through 4 instars, indicates a stronger relationship between hatchling energy content and larval traits for those larvae developing through 4 instars. Similarly, the slope of the linear relationship between hatchling energy content and larval development time was greater for larvae developing through 4 instars at 15°C. With increasing hatchling energy content, larvae are better able to develop through fewer instars, though the effects of hatchling energy content on larval traits may be greater than if larvae were to develop through 5 instars. Development through 5 instars appears to buffer larval traits against initial energy reserves as larval development time, juvenile DW, and larval growth rate are less strongly coupled with hatchling energy content.

Evidence suggests that greater POI allows development of *Crangon crangon* and *Neohelice* (*Chasmagnathus*) *granulata* larvae through fewer instars (see Anger 2001, Giménez & Torres 2002, Giménez 2006). Importantly, at all temperatures, juveniles that developed through 4 instars had significantly lower DW than those that developed through 5 instars. Thus, larger hatchlings (with greater energy content)

generally metamorphosed at a smaller size after a shorter development time which consisted of fewer larval instars. This trend is similar to that at the interspecific level for North American shrimp species in the *Palaemonetes* genus (Table 3; after Hubschman & Broad 1974). These *Palaemonetes* sp. produce larger hatchlings, metamorphose at a smaller juvenile size, have a shorter pelagic period, and develop through fewer larval instars. Although this trend in life histories occurs across a salinity gradient, the ecological basis for this trend is analogous with that of latitudinal trends in life histories; planktonic food availability becomes more limited with increasing latitude and with decreasing marine connectivity (Anger 2001, Anger & Hayd 2009, 2010).

At the intra-specific level, variation in larval instar number is a common plastic trait among arthropods and is usually associated with the addition of larval instars which are thought to extend larval development, enabling larvae to achieve a development and size threshold under poor growth and development conditions (Nijhout 1994, Kingsolver 2007, Etilé & Despland 2008; for reviews see Knowlton 1974, Esperk et al. 2007). Additional larval instars may enable development to, or even surpassing this threshold; however, this comes at the disadvantage of longer development period and the fitness implications associated with such (Kingsolver 2007, Oliphant et al. 2013). The ability to develop and to tolerate unfavourable growth and development conditions without extending larval development (through additional instars) would appear advantageous. Development through relatively fewer larval instars under more unfavourable conditions is associated with greater POI and may be selected for in environments in which unfavourable growth and development conditions prevail, i.e. in cooler high latitude or deep-sea environments where food availability for plankotrophic larvae is limited or unpredictable. The results of this study, and those of other works, demonstrate the importance of POI in enabling development without extending larval development through the addition of larval instars. Thus, macro-ecological patterns in larval development are likely primarily influenced by POI and secondarily influenced by developmental plasticity and larval experience.

Our results at the intra-specific level emulate observations at the inter-specific level and demonstrate that greater POI provides increased endotrophy and the potential for abbreviated development. A greater degree of endotrophy can be assumed to be advantageous in environments of limited or unpredictable

Table 3. Size (mean, range, or mean ± SD) at hatching and metamorphosis, and larval period and larval instar number for species of *Palaemonetes* from differing environments. *P. kadiakensis* is highlighted (grey); this species does not fit the general trend and is considered a recent colonist of the freshwater environment (Hubschman & Broad 1974). z1: zoea 1

Species	Reference(s)	Environment	Trophy	Hatchling size (mm)	Juvenile size (mm)	Larval period (d)	No. of instars
P. vulgaris	Broad (1957)	Marine/brackish	Fully planktotrophic	2.3	6.3	15–>30	7–11
P. pugio	Broad (1957)	Marine/brackish	Planktotrophic (z1 facultative lecithotrophic)	2.6	6.2	15–>40	7–11
P. atrinubes	Bray (1976)	Marine/brackish	Planktotrophic (facultative lecithotrophy unknown)	2.9–3.1	7.0–8.1	20–44	7–10
P. intermedius	Hubschman & Broad (1974)	Marine/brackish	Planktotrophic (facultative lecithotrophy unknown)	3.5	7	13	6–8
P. varians	Fincham (1979)	Marine/brackish	Planktotrophic (z1 facultative lecithotrophic)	3.8 (3.5–4.1)	6.4 (6.0–7.0)	–	5
	Oliphant et al. (2013)			–	–	7–66	3–7
	Present study			–	–	9–36	4–6
P. kadiakensis	Broad & Hubschman (1963)	Freshwater	Planktotrophic (facultative lecithotrophy unknown)	4.4	7.5	16–30	5–8
P. paludosus	Dobkin (1963)	Freshwater	Lecithotrophic (facultative lecithotrophy unknown)	3.8	4.5	5–10	3
P. cummingi	Dobkin (1971)	Freshwater	Lecithotrophic (facultative lecithotrophy unknown)	4.8	5.5	9	3
P. australis	Bray (1976)	Freshwater	Lecithotrophic (facultative lecithotrophy unknown)	5.0 (4.4–5.6)	5.2 (4.5–5.8)	4–17	3
P. zariquieyi	Guerao (1993)	Freshwater	Fully lecithotrophic	3.9–4.03	4.2–4.6	4.5–6	3
P. ivonicus	Magalhães (1986)	Freshwater	Fully lecithotrophic	4.55 ± 0.14	5.27 ± 0.32	4–6	3
P. mercedaes	Magalhães (1988)	Freshwater	Fully lecithotrophic	4.68 ± 0.03	4.92 ± 0.24	5	1

food supply, and low temperatures when larval development is prolonged relative to food availability. POI plasticity, a widespread trait among taxa, therefore, has significant ecological implications and can confidently be assumed to drive evolutionary trajectories.

Our data support the hypothesis that the evolutionary transition towards endotrophy and abbreviated development is driven by increasing POI which provides greater energy reserves and, therefore, increasing nutritional independence (endotrophy). Such energy reserves may allow and/or promote more rapid development, especially when food is scarce or patchy, or when feeding is difficult (i.e. during periods of dark for visual predators). For some species, high POI allows development within a larval stage or a sequence of larval stages without feeding; such larvae often retain functioning feeding structures and feed in the presence of food, so called facultative lecithotrophy (Anger 2001). The varying degrees of facultative lecithotrophy are considered intermediates between planktotrophy and full lecithotrophy. The evolution of full lecithotrophy leads to the redundancy of feeding structures. Fully lecithotrophic development is often obligatory lecithotrophic because larvae are unable to process external food, a result of under-developed feeding structures and low activity of several key digestive enzymes (Saborowski et al. 2006). The evolutionary transition towards complete endotrophy (full lecithotrophy) is likely a result of increasing POI and its influence on endotrophy and abbreviated development; however, the conclusion of increasing POI (and its influence on endotrophy and abbreviated development) is direct development, evident in peracarids and freshwater and terrestrial decapods (Anger 1995).

Temperature-mediated POI plasticity is hypothesised to be an emergent property of the physiochemical nature of organisms, resulting from the differing effect of temperature on the processes of differentiation (egg production) and growth (yolk production) (Van der Have & de Jong 1996). High POI is advantageous, especially under conditions of limited/unpredictable food avail-

ability and low growth potential. Macro-ecological trends in development mode are governed by POI at the inter-specific level. Phenotypic plasticity at the intra-specific level allows for environmentally mediated variation in POI, which, under differential selection pressures, may eventually permit the evolution of the diverse and complex life cycles observed in the marine environment.

LITERATURE CITED

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

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

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, Nair KKC (1979) Laboratory experiments on the larval development of *Hyas araneus* (Decapoda, Majidae). Helgol Wiss Meeresunters 32:36–54

Barnes H, Barnes M (1965) Egg size, nauplius size, and their variation with local, geographical, and specific factors in some common cirripedes. J Anim Ecol 34:391–402

Blanckenhorn WU (2000) Temperature effects on egg size and their fitness consequences in the yellow dung fly *Scathophaga stercoraria.* Evol Ecol 14:627–643

Boddeke R (1982) The occurrence of 'winter' and 'summer' eggs in the brown shrimp (*Crangon crangon*) and the impact on recruitment. Neth J Sea Res 16:151–162

Bownds C, Wilson R, Marshall DJ (2010) Why do colder mothers produce large eggs? An optimality approach. J Exp Biol 213:3796–3801

Bray DM (1976) Larval development of two Western Australian shrimps, *Palaemonetes australis* Dakin and *Palaemonetes atrinubes* Bray (Decapoda, Palaemonidae), reared in the laboratory. Rec West Aust Mus 4:145–162

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

Broad AC, Hubschman JH (1963) The larval development of *Palaemonetes kadiakensis* M.J. Rathbun in the laboratory. Trans Am Microsc Soc 82:185–196

Christiansen FB, Fenchel TM (1979) Evolution of marine invertebrate reproductive patterns. Theor Popul Biol 16: 267–282

Dobkin S (1963) The larval development of *Palaemonetes paludosus* (Gibbes, 1850) (Decadopa, Palaemonidae), reared in the laboratory 1). Crustaceana 6:41–61

Dobkin S (1971) The larval development of *Palaemonetes cummingi* Chace, 1954 (Decapoda, Palaemonidae) reared in the laboratory. Crustaceana 20:285–297

Emlet RB, Høegh-Guldberg O (1997) Effects of egg size on postlarval performance: experimental evidence from a sea urchin. Evolution 51:141–152

Esperk T, Tammaru T, Nylin S (2007) Intraspecific variability in number of larval instars in insects. J Econ Entomol 100:627–645

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

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

Fischer K, Bot ANM, Brakefield PM, Zwaan B (2003a) Fitness consequences of temperature-mediated egg size plasticity in a butterfly. Funct Ecol 17:803–810

Fischer K, Brakefield PM, Zwaan BJ (2003b) Plasticity in butterfly egg size: Why larger offspring at lower temperatures? Ecology 84:3138–3147

Fox CW (1994) The influence of egg size on offspring performance in the seed beetle, *Callosobruchus maculatus.* Oikos 71:321–325

Geister TL, Lorenz MW, Meyering-Vos M, Hoffmann KH, Fischer K (2008) Effects of temperature on reproductive output, egg provisioning, juvenile hormone and vitellogenin titres in the butterfly *Bicyclus anynana.* J Insect Physiol 54:1253–1260

Giménez L (2006) Phenotypic links in complex life cycles: conclusions from studies with decapod crustaceans. Integr Comp Biol 46:615–622

Giménez L, Torres G (2002) Larval growth in the estuarine crab *Chasmagnathus granulata*: the importance of salinity experienced during embryonic development, and the initial larval biomass. Mar Biol 141:877–885

Guerao G (1993) The larval development of a fresh-water prawn, *Palaemonetes zariquieyi* Sollaud, 1939 (Decapoda, Palaemonidae), reared in the laboratory. Crustaceana 64:226–241

Hart MW (1995) What are the costs of small egg size for a marine invertebrate with feeding planktonic larvae? Am Nat 146:415–426

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

Ito K (1997) Egg-size and -number variations related to maternal size and age, and the relationship between egg size and larval characteristics in an annual marine gastropod, *Haloa japonica* (Opisthobranchia; Cephalaspidea). Mar Ecol Prog Ser 152:187–195

Kattner G, Wehrtmann IS, Merck T (1994) Interannual variations of lipids and fatty acids during larval development of *Crangon* spp. in the German Bight, North Sea. Comp Biochem Physiol B Biochem Mol Biol 107:103–110

King MG, Butler AJ (1985) Relationship of life-history patterns to depth in deep-water caridean shrimps (Crustacea: Natantia). Mar Biol 86:129–138

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

Knowlton RE (1974) Larval development processes and controlling factors in decapod Crustacea, with emphasis on Caridea. Thalassia Jugosl 10:138–168

Lenth R, R Development Core Team (2013) lsmeans: Least-squares means. http://cran.r-project.org/web/packages/lsmeans/

Liefting M, Weerenbeck M, van Dooremalen C, Ellers J (2010) Temperature-induced plasticity in egg size and resistance of eggs to temperature stress in a soil arthropod. Funct Ecol 24:1291–1298

Lonsdale DJ, Levinton JS (1985) Latitudinal differentiation in embryonnic duration, egg size, and newborn survival in a harpacticoid copopod. Biol Bull (Woods Hole) 168: 419–431

Magalhães C (1986) The larval development of palaemonid shrimps from the Amazon Region reared in the laboratory. IV. Abbreviated development of *Palaemonetes ivonicus* Holthuis, 1950 (Crustacea: Decapoda). Amazoniana 10:63–78

Magalhães C (1988) The larval development of palaemonid shrimps from the Amazon Region reared in the laboratory. III. Extremely abbreviated development of *Palaemonetes (Palaemonetes) mercedae* Pereira, 1986 (Crustacea, Decapoda). Stud Neotrop Fauna Environ 23:1–8

Marshall D, Keough MJ (2007) The evolutionary ecology of offspring size in marine invertebrates. Adv Mar Biol 53: 1–60

Marshall D, Krug PJ, Kupriyanova EK, Byrne M, Emlet RB (2012) The biogeography of marine invertebrate life histories. Annu Rev Ecol Evol Syst 43:97–114

Mileikovsky SA (1971) Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Mar Biol 10: 193–213

Moran AL, Emlet RB (2001) Offspring size and performance in variable environments: field studies on a marine snail. Ecology 82:1597–1612

Moran AL, McAlister LR (2009) Egg size as a life history character of marine invertebrates: Is it all it's cracked up to be? Biol Bull (Woods Hole) 216:226–242

Müller Y, Ammar D, Nazari E (2004) Embryonic development of four species of palaemonid prawns (Crustacea, Decapoda): pre-naupliar, naupliar and post-naupliar periods. Rev Bras Zool 21:27–32

Nijhout HF (1994) Insect hormones. Princeton University Press, Princeton, NJ

Oh CW, Hartnoll RG (2004) Reproductive biology of the common shrimp *Crangon crangon* (Decapoda: Crangonidae) in the central Irish Sea. Mar Biol 144:303–316

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

Paschke K, Gebauer P, Buchholz F, Anger K (2004) Seasonal variation in starvation resistance of early larval North Sea shrimp *Crangon crangon* (Decapoda: Crangonidae). Mar Ecol Prog Ser 279:183–191

Pestana D, Ostrensky A (1995) Occurrence of an alternative pathway in the larval development of the crab *Chasmagnathus granulata* Dana, 1851 under laboratory conditions. Hydrobiologia 306:33–40

Pinheiro J, Bates D, DebRoy S, Sarkar D, and R Development Core Team (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-111 http://cran.r-project.org/web/packages/nlme/index.html

Pond D, Dixon D, Sargent J (1997) Wax-ester reserves facilitate dispersal of hydrothermal vent shrimps. Mar Ecol Prog Ser 146:289–290

R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, www.R-project.org

Saborowski R, Thatje S, Calcagno JA, Lovrich GA, Anger K (2006) Digestive enzymes in the ontogentic stages of the southern king crab, *Lithodes santolla.* Mar Biol 149: 865–873

Sheader M (1983) The reproductive biology and ecology of *Gammarus duebeni* (Crustacea: Amphipoda) in southern England. J Mar Biol Assoc UK 63:517–540

Sheader M (1996) Factors influencing egg size in the gammarid amphipod *Gammarus insensibilis.* Mar Biol 124: 519–526

Sinervo B, McEdward LR (1988) Developmental consequences of an evolutionary change in egg size: an experimental test. Evolution 42:885–899

Sloggett JJ, Lorenz MW (2008) Egg composition and reproductive investment in aphidophagous ladybird beetles (Coccinellidae: Coccinellini): egg development and interspecific variation. Physiol Entomol 33:200–208

Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. Am Nat 108:499–506

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

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, Anger K (2004) Egg production, hatching rates, and abbreviated larval development of *Campylonotus vagans* Bate, 1888 (Crustacea: Decapoda: Caridea), in subantarctic waters. J Exp Mar Biol Ecol 301: 15–27

Thatje S, Hillenbrand CD, Larter R (2005) On the origin of Antarctic marine benthic community structure. Trends Ecol Evol 20:534–540

Thorson G (1936) The larval development, growth, and metabolism of Arctic marine bottom invertebrates compared with those of other seas. Medd Gronl 100:1–155

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

Thorson G (1961) Length of pelagic larval life in marine bottom invertebrates as related to larval transport by ocean currents. In: Sears M (ed) Oceanography, Book 67. American Association for the Advancement of Science, Washington, DC, p 455–474

Thorson G (1964) Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. Ophelia 1:167–208

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge

Urzúa Á, Anger K (2012) Seasonal variations in larval biomass and biochemical composition of brown shrimp, *Crangon crangon* (Decapoda, Caridea), at hatching. Helgol Mar Res 67:267–277

Urzúa Á, Paschke K, Gebauer P, Anger K (2012) Seasonal and interannual variations in size, biomass and chemical composition of the eggs of the North Sea shrimp, *Crangon crangon* (Decapoda: Caridea). Mar Biol 159:583–599

Van der Have TM, de Jong G (1996) Adult size in ectotherms: temperature effects on growth and differentiation. J Therm Biol 183:329–340

Vance RR (1973) On reproductive strategies in marine benthic invertebrates. Am Nat 107:339–352

Wehrtmann IS, Lopez GA (2003) Effects of temperature on the embryonic development and hatchling size of *Betaeus emarginatus* (Decapoda: Caridea: Alpheidae). J Nat Hist 37:2165–2178

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer Verlag, New York, NY