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Environmentally mediated phenotypic links and performance in larvae of a marine invertebrate

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ABSTRACT: We studied the effects of environmental conditions experienced during embryonic and larval phases on development and larval survival of the marine shrimp *Palaemon serratus,* and examined how these conditions modified the relationship between larval and maternal phenotypes. Egg-carrying females were incubated at different temperatures (12 and 18°C), and freshly hatched larvae were exposed to a combination of temperatures (18 and 24°C), salinities (25 and 32 PSU) and food conditions (ad libitum vs. limited). Temperatures experienced by embryos had no significant effects on development, and only weak effects on survival, whereas environmental conditions experienced by larvae had strong effects on development — the duration of development was longer at lower temperatures and under food-limited conditions, and food limitation increased the number of larval instars necessary to reach the juvenile phase (especially at the highest temperature), perhaps reflecting a mismatch between increased metabolic demands and reduced energy supply. Links between larval and female phenotypes were evident: large females generally produced significantly larger larvae than smaller females. In larvae reared under food limitation, average development time and number of instars required to reach the juvenile phase were negatively correlated with average larval body mass at hatching. Thus, larval development is linked to initial larval body mass and female body size; however, these links can be modified by environmental conditions experienced by the larvae. In situations of high temperatures and food limitation, larger *P. serratus* females may play a more important role in the maintenance of populations, as they produce large offspring capable of ameliorating the effects of temperature and food limitation on development.

KEY WORDS: Acclimation · Body size · Developmental plasticity · Marine larvae · Maternal effects

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INTRODUCTION

There is a growing body of work suggesting that the patterns of survival in aquatic organisms depend on interactions between past and present environmental conditions, as well as on the different phenotypes that are expressed during the organisms' life cycle (Pechenik 2006, Giménez 2006, Marshall & Morgan 2011). This scenario is further complicated by phenotypic links, i.e. situations in which phenotypes expressed at early stages in life affect the phenotypes at advanced stages (sensu Podolsky & Moran 2006). For example, in species that develop through a larval phase, the initial larval body mass, developmental rates and developmental pathways followed during the larval phase are usually linked to the body mass of eggs and embryos, which reflect the quantity of reserves mothers allocate to eggs (e.g. Giménez 2006). Furthermore, the allocation of reserves to eggs is linked to maternal phenotypes (Bernardo 1996, Wade 1998, Giménez & Anger 2001, Marshall et al. 2008, Marshall & Morgan 2011).

We still know very little about how phenotypic links are modified by environmental conditions ex-

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perienced throughout the life cycle of an organism. Indeed, the conditions experienced at each stage of development (or by the parents) has the potential to modify existing phenotypic links (Allen & Marshall 2013). In these species, the environmental conditions experienced at the time of spawning, oviposition or embryogenesis may be controlled by behavioural choices made by mothers, but may also depend on stochastic environmental variability. Such conditions may affect developmental processes that occur during embryogenesis, and thus modify larval phenotypes. For instance, the osmoregulatory capacity of estuarine crab larvae has been linked to salinity conditions experienced during embryogenesis (Charmantier et al. 2002). Increases in larval osmoregulatory capacity as a consequence of the embryos being exposed to low salinities must be based on modifications in the larval osmoregulatory tissues that are developed during the late embryonic stages. This phenotypic response leads to increased survival at low salinities in these larvae (Giménez & Anger 2003), a phenomenon consistent with beneficial acclimation (Gomez-Diaz 1987, Wilson & Franklin 2002, Bownds et al. 2010). Phenotypic links between larval and post-metamorphic stages can also be modified by both the maternal and larval environments (Giménez 2010, Marshall & Morgan 2011). The collective effects of these links on development and survival can affect population dynamics (Beckerman et al. 2003, Plaistow & Benton 2009, Venturelli et al. 2010).

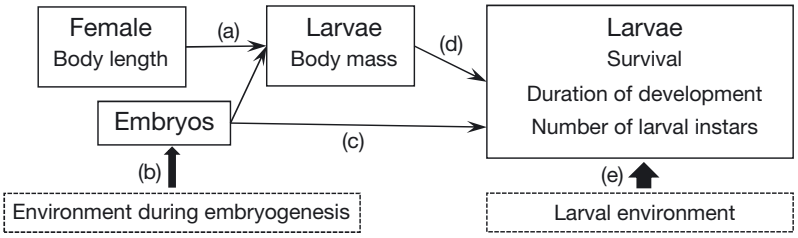

Fig. 1. *Palaemon serratus.* Summary of links between maternal and larval phenotypes and effects on larval survival and development: (a) maternal body length may affect embryonic and, subsequently, larval body size at hatching; (b) environmental conditions experienced during embryogenesis may affect embryonic physiological processes. Larval survival and development may respond to the combined effect of (c) the embryonic environment, (d) larval body mass at hatching, and (e) the environmental conditions experienced by larvae

Most knowledge regarding environmental effects on phenotypic links comes from studies focusing on single factors (e.g. temperature or nutrition). However, in nature, phenotypic links are likely modified by the interacting effects of several factors. This is most likely the case of marine coastal species, as coastal habitats are characterised by heterogeneity of salinity, temperature and food conditions produced by topography, climatic conditions and water circulation patterns (Mann & Lazier 2006). Many coastal species with complex life cycles develop through larval stages that are sensitive to these conditions (e.g. food supply: Olson & Olson 1989, Durant et al. 2005; salinity: Torres et al. 2011; temperature: Pörtner & Farrell 2008).

Using the coastal shrimp *Palaemon serratus* as a model species, we studied the effects of multiple environmental factors on larval survival, as well as links between larval developmental time, the number of larval instars required to reach the first juvenile stage, initial larval body mass, and female body size. We considered the potentially interacting influences of temperature, salinity and food conditions experienced by larvae, as well as the temperature experienced by embryos.

We hypothesised that larval survival and development would be affected mainly by the larval environment, but that these effects would vary in relation to initial larval body mass and the temperature experienced during embryogenesis (Fig. 1). For instance, initial larval body mass can affect the number of instars required to reach the juvenile stage (cf. Giménez et al. 2004), but this pattern can be modified by food conditions experienced by larvae. This plastic response may be a general response to thermal, food or osmotic stress (Criales & Anger 1986), and its magnitude could depend on the type of stress experienced by the larvae. The importance of food limitation for larval survival and development could depend on temperature and salinity, because these environmental factors affect larval growth (e.g. salinity; Torres et al. 2011) and metabolic rates (e.g. temperature; Anger 2001).

Decapod crustacean embryos develop externally, and are only partially protected by egg membranes attached to the female pleopods; thus, embryos are exposed directly to the environment throughout their development. Exposure during embryogenesis to particular temperatures may result in either acclimation or chronic stress effects on larvae. Acclimation (or physiological stress) may be detected as increased (or decreased) survival, shorter (or longer) duration of development or a requirement for fewer (or more) larvae instars prior to the first juvenile stage.

MATERIALS AND METHODS

Study species

Palaemon serratus is a coastal estuarine shrimp distributed across west European estuaries and coastal zones (Smaldon 1993). These shrimps follow an ontogenetic migration whereby adults migrate from continental shelf waters to coastal areas to mate and reproduce; larval development takes place in these coastal waters. The larval development of *P. serratus* is characterised by 8 or 9 larval stages (Fincham 1983) which are likely to show developmentally plastic responses to the combination of maternal and larval phenotypes and environmental conditions.

Collection and maintenance of females

Ovigerous females with early-spawned eggs (i.e. eye not yet visible) were obtained in autumn 2010 from Amlwch Port (Isle of Anglesey, North Wales, UK) and were kept in the laboratory during embryonic development. Hatched larvae were reared at different combinations of temperature, salinity and food availability (see below). The response variables were percentage survival, number of larval instars, and mean duration of larval development.

A total of 40 ovigerous females covering a wide size range (carapace length; CL = 17 to 24 mm) were used to obtain larvae. CL of females was measured as the distance from the postorbital margin to the dorsal posterior end of carapace, using digital callipers (precision: 0.1 mm). In order to manipulate the temperature experienced by embryos, females with their embryos attached were maintained individually in separate aquaria (volume = 2 l), at 2 different temperatures (5 egg-carrying females at 12°C; 5 at 18°C). Females were randomly allocated to the different temperature treatments so as to avoid co-variation of temperature and female body size. The temperatures chosen represent the thermal conditions likely to be experienced in northern (e.g. North European Seas) and southern populations (e.g. Iberian Atlantic Coast) during the reproductive period. The average time of exposure of embryos varied according to the temperature (28 d at 18°C to 72 d at 12°C).

Embryos were not separated from the females: in decapod crustaceans, separation of embryos from the females can distort the hatching process and lead to unviable larvae (Giménez & Anger 2001). In nature, the temperature experienced by embryos of decapod crustaceans may be a consequence of maternal behavioural choices, and therefore considered as a maternal temperature condition. However, we call this factor 'embryonic temperature' since in our experiment the temperature operated directly on the embryos — unlike cases where temperature may affect the maternal allocation of reserves into eggs. In addition, this temperature may differ from the one experienced by mothers during oogenesis or after fertilization, but before spawning. This definition is also consistent with the previous use of the term (Giménez & Anger 2003).

Experimental work with larvae

Upon hatching, a group of larvae from each separate brood was used to calculate the average body mass of larvae produced by each female. Since there was only one brood per female, variations in the average larval body mass among broods is tantamount to variations among larvae produced by different females. Average larval body mass (expressed as dry weight) was calculated from 5 replicate samples per female; each replicate consisted of 12 larvae. Individuals were rinsed for a few seconds in distilled water in order to remove salts, then dried on filter paper, transferred to tin cartridges, freeze dried for 48 h (Edwards Supermodulyo 12k) and weighed on a microbalance (Mettler Toledo; precision: 1 µg).

A second group of freshly hatched larvae from each female were used for a series of experiments carried out under controlled conditions of temperature, salinity, food and photoperiod (12:12 h light: dark), in automatic and fully programmable incubators. These experiments involved the individual rearing of larvae assigned to each of the combination of temperatures (18 and 24°C), salinities (25 and 32 PSU) and food conditions (access to food limited to 4 h d^{-1} vs. unlimited access to food) with 10 larvae per treatment combination. Therefore, the experiment consisted of 80 larvae per female (10 × 2 × 2 × 2) resulting in a total of 800 larvae (80 × 10 females). Larvae were reared in individual containers (50 ml) with water initially at the same temperature as for embryonic development, and then were distributed to incubators according to the experimental temperature treatments. This resulted in a gradual change to experimental temperatures (18 and 24°C) over a period of 24 h, which allowed time for the larvae to adapt to the new temperatures (Richard 1978).

The temperatures chosen corresponded to those in which larval development has been completed successfully (Kelly et al. 2012); the salinities used covered

a range that is likely to be experienced by larvae in coastal areas. Food treatments simulated the expected temporal pattern of daily access to prey as a consequence of plankton patchiness and larval vertical migration (see Sulkin et al. 1998); i.e. larvae migrate vertically through fields of low prey densities over a prolonged time period, but for restricted periods may encounter patches of high food density, usually located in surface waters. Field observations suggest that larvae remain at the surface for a brief period of time (4 to 6 h) (Abelló & Guerao 1999, dos Santos et al. 2008). This natural pattern was mimicked in our experiments by exposing larvae to food for a period of 4 h d^{-1} (cf. Giménez & Anger 2005); a control group was allowed unlimited (24 h) access to prey.

Salinity was determined through a calibrated multi-field meter (WTW Cond 315i; ± 0.1). Water at salinity 25 PSU was prepared every day, and obtained by mixing filtered seawater with distilled water. Full salinity seawater (32 PSU) was directly obtained through the seawater filtration system (1 µm and UV-filtered seawater). Food consisted of freshly hatched *Artemia* sp. nauplii that were added ad libitum (density ca. 10 nauplii ml^{-1}). At daily intervals, larvae were checked for mortality or moulting, and freshly hatched food was added and removed with the culture water after a period of 4 h (food-limited condition) or 24 h (unlimited access to prey). All experiments were run until all larvae had either died or moulted to the second juvenile stage.

Juvenile stages were identified from morphological features, i.e. well developed and functional pleopods and pereiopods, and a change in individual behaviour from free-swimming to bottom-dwelling. The duration of larval exposure to the different conditions varied according to the treatments, ranging from 18 d (at 24°C, 25 PSU and unlimited access to prey) to 88 d (at 18°C, 32 PSU and limited food availability).

Data analysis

Effects on percentage survival were analysed by between-within-subject ANOVA (Searle et al. 1992), considering larval temperature, salinity and food conditions as within-subject factors, and temperature experienced by embryos as a between-subject factor. In this case, each brood was considered to be the replicate unit within each embryonic temperature, since the proportion of survivors is obtained as a single value per brood. This justified that the temperature experienced by embryos was considered a between-subject factor. The larval temperature, food and salinity were considered within-subject (repeated measures) factors because we obtained one value of survival for each of these conditions, for each of the broods. We tested normality through a Shapiro-Wilk's test, and also through inspection of the distribution of residuals (Q-Q plot of residuals); variance heterogeneity was evaluated through a Cochran's *C*-test, and by checking any structure in the residual plot against the predicted values. For survival, variances were homogeneous and inspection of residuals did not give evidence of deviations from the normal distribution.

For duration of development and number of instars, analyses were run following a linear model and Type I error, which is appropriate for nested/hierarchical designs since no components of variation are left out (Anderson et al. 2008, p. 71). The design contained the following factors: (1) embryonic temperature (fixed factor), (2) female of origin (random factor) nested within embryonic temperature, (3) larval salinity (fixed factor), (4) larval temperature (fixed factor), and (5) larval food (fixed factor). These analysis were restricted to larvae that hatched from 8 females (instead of 10), because larvae from 2 females (one per embryonic temperature) showed very low survival rates at some treatment combinations, and thus did not provide replication to test the hypotheses regarding duration of development or number of juvenile stages. Test construction followed Anderson et al. (2008); details are provided in Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m502p185_supp.pdf. In this case, the assumption of variance homogeneity was not met and residuals were not normally distributed. Logarithmic transformation resulted in normal distribution of residuals for both variables but variances were still heterogeneous. ANOVAs were performed on both untransformed and transformed data, in addition to analysis of variance based on permutations (PERMANOVA: Anderson et al. 2008): these 3 approaches gave the same results in terms of significance; thus, we report results based on PERMANOVA. For duration of development, heterogeneity in variance occurred mainly in response to temperature, rather than food and salinity. We are confident that temperature affected the average duration of development because the ranges of variation did not overlap (i.e. all larvae reared at 24°C developed faster than any larvae reared at 18°C; see Fig. 3). In the case of the number of instars, larger variances were found under limited access to prey (LA) compared to treatments under unlimited access to prey (PA), and the ranges of variation overlapped (LA: 7 to

19 instars; PA: 6 to 10; see Fig. 4). However, an inspection of the frequency distributions (see Fig. S1 in the Supplement) suggested that food limitation resulted in a displacement of the central tendencies apart from the change in variance. Therefore, the significant effects found through ANOVA and post-hoc tests (SNK test) must have reflected differences in average number of instars, apart from differences in variances.

Links between larval body mass at hatching, number of stages and total duration of development were evaluated by ANCOVA and linear regression, with average larval body mass per brood as a continuous predictor, and temperature, salinity and food conditions as categorical predictors. In this model, the response variables were the average duration of development (or number of instars required to reach the juvenile stage) exhibited by individuals that hatched from each brood and cultured under the different combinations of temperature, food and salinity (the categorical predictors). The ANCOVA was carried out through generalized least squares modelling (GLS) with the VarIdent function to incorporate heterogeneity in residual variation into the model. This analysis was performed in the 'R' environment (R Development Core Team 2008) using the 'nlme' package (Pinheiro et al. 2013). Model selection followed the backward procedure based on maximum likelihood (Zuur et al. 2009), and the final model was presented using restricted maximum likelihood (REML). The application of model validation showed that the underlying statistical assumptions were not violated.

Links between larval body mass and maternal size were also evaluated by ANCOVA. In this model, we included the average dry mass of larvae produced by each female as a response variable, maternal body size as a continuous predictor and the temperature experienced by embryos as a categorical predictor; we thus tested the hypothesis that maternal temperature would affect the rate of use of reserves during embryogenesis. For body mass at hatching, data met the assumptions of normal distribution and constant variance.

RESULTS

Effects of embryonic and larval environments on survival and development

Average survival rates varied between 45 and 70%; however, survival varied among larvae originating from different females, ranging from 0 to 100%. Survival was generally affected by 3-way interactions involving embryonic and larval temperatures, larval food and larval salinity (Table S3 in the Supplement). Embryonic temperature appeared to modify the effects of food limitation and larval temperature on survival (Fig. 2a): when embryos were reared at 12°C, survival did not vary significantly among food–temperature treatment combinations, and generally remained in the range of 50 to 60%. However, when embryos where reared at 18°C, survival depended on the interaction between temperature and food, with the lowest survival in larvae reared at 18°C under food limitation (40%), compared with other treatments (50 to 60%).

Embryonic temperature also appeared to modify the effect of larval temperature and salinity on survival (Fig. 2b). When the larval-rearing salinity was 25 PSU, larval survival was high and varied little (range = 65 to 72%). However, embryonic temperature affected survival in those larvae reared in seawater: under this condition, the lowest survival was observed when embryos were reared at 12°C and larvae were reared at 24°C (49%), and when both embryos and larvae were reared at 18°C (46%).

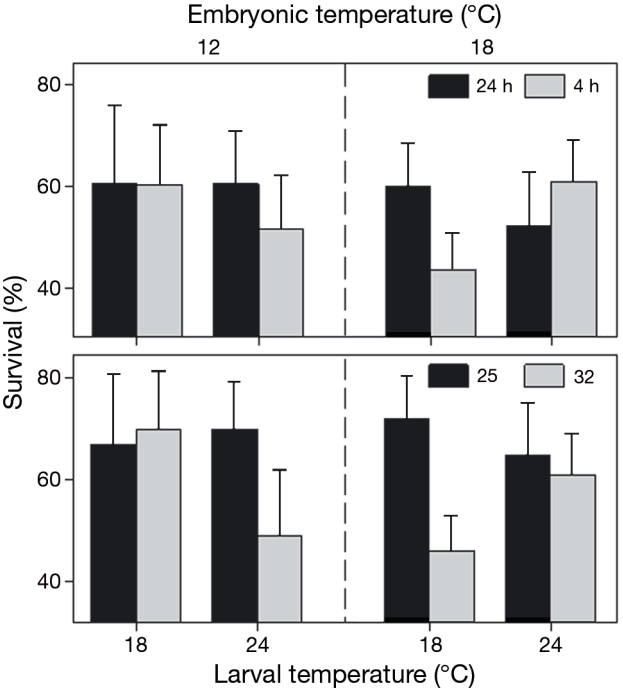

Fig. 2. *Palaemon serratus.* Survival of larvae to the first juvenile stage in response to the interacting effects of embryonic temperature (12 and 18°C) and conditions experienced by larvae (salinity: 25 and 32; temperature: 18 and 24°C; daily access to prey: unlimited access [feeding 24 h] vs. limited access [4 h d^{-1}]). Error bars = standard deviation based on survival of larvae originating from different females

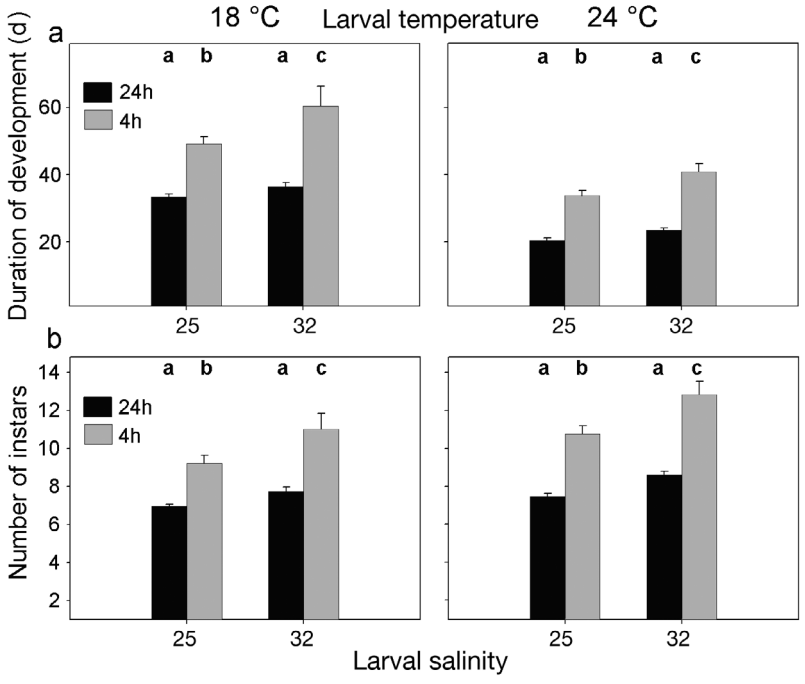

Fig. 3. *Palaemon serratus.* Duration of larval development and number of larval instars required to reach the juvenile stage in response to the interacting effects of salinity (25 and 32), temperature (18 and 24°C) and access to prey (unlimited access [feeding 24 h] vs. limited access [4 h d^{-1}]). Error bars = standard deviations based on the average among larvae originating from different females. Letters above bars = significant differences ($p < 0.05$) among larval condition exposures

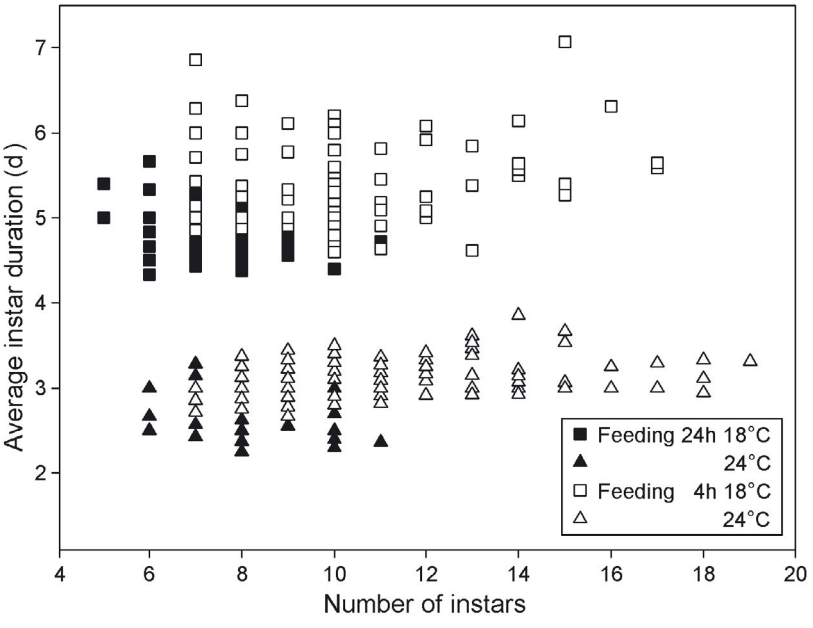

Fig. 4. *Palaemon serratus.* Instar duration versus number of larval instars required to reach the first juvenile stage in larvae reared under 2 feeding regimes (unlimited food access [24 h] vs. limited access [4 h d^{-1}]) and temperatures (18 and 24°C). Each square and triangle represents an individual larva

The temperature experienced by embryos did not have any significant effects on the duration of development or the number of instars followed by larvae. However, both response variables were significantly affected by the interaction between larval temperature, salinity and food, and this effect varied among larvae produced by different females (significant 4-way interactions: temperature × salinity × food × female; Tables S4 & S5 in the Supplement). Variations in developmental responses among larvae from different females were linked to larval body mass at hatching and female size, and are reported in the next section.

For larvae from all females, longer development times occurred at lower temperatures and under food limitation (Fig. 3a). A low temperature increased the duration of development mainly through increments in intermoult duration. Food limitation affected the duration of development through an increase in the number of instars followed by larvae (Fig. 4) and in the average instar duration (Fig. 5). The effect of salinity on the duration of development was weak but significant: the duration was longer by increments in the number of stages in larvae reared in full salinity sea water (32 PSU) compared to those larvae at 25 PSU (Fig. 3b).

Larvae developed through a variable number of instars (range = 6 to 22; Fig. 4) depending mainly on food availability and temperature. Irrespective of the female of origin, the number of instars was significantly higher under food limitation — especially under high temperature and high salinity conditions (Fig. 3b; significant interaction term temperature × food × salinity; Table S5 in the Supplement).

Links between larval development, mass at hatching and maternal body size

Average survival and development as well as average larval body mass at hatching varied among larvae originating from different females. In larvae reared under food-limitation conditions (but not in those reared under ad libitum food), the average

duration of development and the average number of instars followed by larvae were both negatively correlated with average larval mass at hatching (Figs. 6 & 7, Tables 1 & 2; ANCOVA: significant interaction dry mass × larval food). These patterns were consistent at all salinity–temperature combinations tested. These results suggest that, on average, small larval size at hatching results in a longer duration of development and in more larval instars required to reach the juvenile stage if larvae experience food limitation.

Average larval body mass at hatching was positively correlated with female body length (Fig. 8). This relationship was not affected by the temperature experienced during embryogenesis nor by its interaction with female length (Table 3). Females with a CL < 20 mm produced larvae in the range of 65 to 90 µg dry mass, while larger females (CL = 21 to 24 mm) produced larvae ranging from 80 to 110 µg.

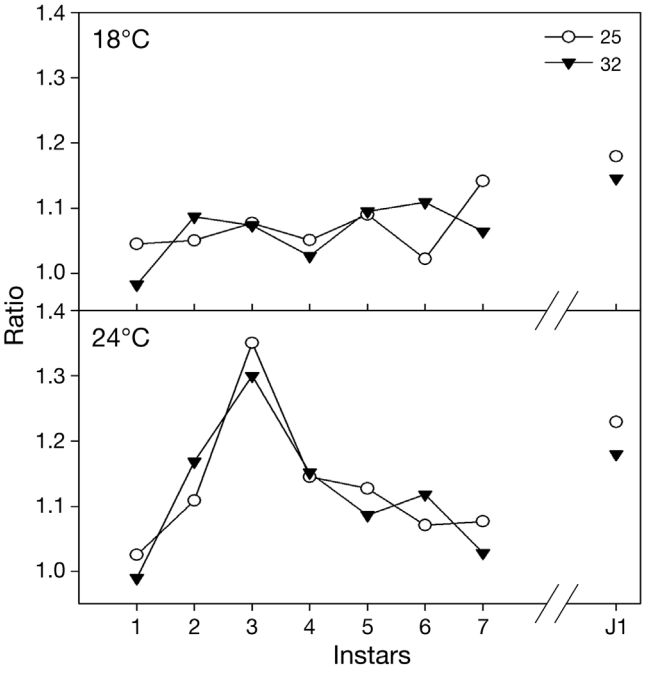

Fig. 5. *Palaemon serratus.* Ratios between the instar duration under unlimited and limited access to food (24 vs. 4 h d^{-1}) at different larval temperatures (18 and 24°C) and salinities (25 and 32). Ratios were calculated as $R = t_L / t_P$, where t is the average instar duration under either limited (L) or permanent (P) access to food. A ratio of 1 means the durations under both limited and permanent access were the same

DISCUSSION

One of the main gaps in our understanding of how organisms respond to variable environmental conditions comes from the limited information about how phenotypic links operate on individual performance and survival under different environmental contexts. In this study, we found that links between mother and offspring phenotypes are complex and context-dependent. Survival was affected by environmental conditions experienced during embryogenesis and larval development. In most treatment combinations, survival ranged between 60 and 70%. An exception occurred when both the larval and embryonic temperatures were 18°C, and larvae were reared in full salinity seawater (32 PSU) or under food limitation. This is in contrast to similar studies where exposure of embryos or mothers to a particular environment resulted in increased larval performance in the same environment (embryonic acclimation: Giménez & Anger 2003; maternal effects: Burgess & Marshall 2011). A second exception occurred when embryonic temperature was 12°C and larval temperature was 24°C, but only for larvae reared under full salinity seawater (survival = 49%).

The duration of larval development and the number of instars followed by

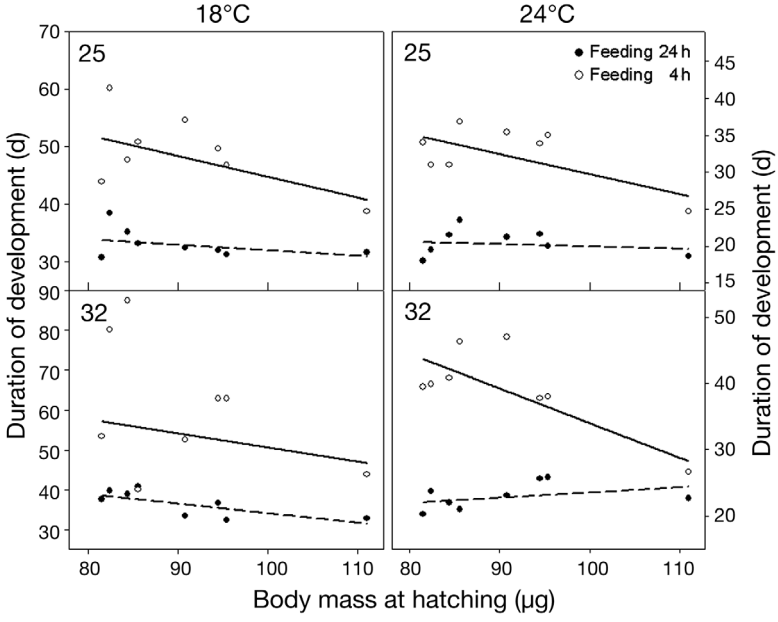

Fig. 6. *Palaemon serratus.* Relationship between larval body mass at hatching and duration of development in individuals reared at different temperatures (18 and 24°C), salinities (25 and 32) and food conditions (feeding 24 vs. 4 h d^{-1}). Each point represents the average body mass and the average duration of development obtained from larvae originating from the same female; data are from 8 females (= 8 points per panel). Statistical results are given in Table 1

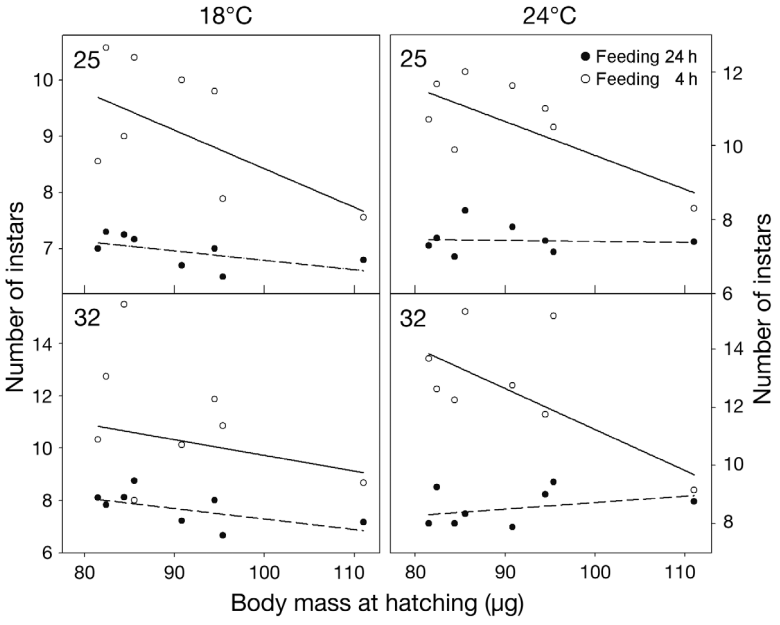

Fig. 7. *Palaemon serratus.* Relationship between larval body mass at hatching and the number of larval instars required to reach the first juvenile stage in individuals reared under different temperatures (18 and 24°C), salinities (25 and 32) and food conditions (feeding 24 h vs. 4 h d^{-1}). Each point represents the average body mass and the average number of instars obtained from larvae originating from the same female; data are from 8 females (= 8 points per panel). Statistical results are given in Table 2

larvae responded to larval temperature, food and salinity, but the magnitude of this effect varied among larvae hatched from different females. Under food-limitation, among-female variation in larval development was related to average larval body mass at hatching, which in turn was correlated to female body size. Collectively, these results highlight the complexity of the interactions between maternal and larval phenotypes and environmental conditions, which lead to changes in larval survival and development. Furthermore, these relationships are occasionally puzzling; for instance, some are not significant even under resource limitation (Marshall & Bolton 2007, Rollinson & Hutchings 2010).

Relationships between development, larval body mass and maternal size

Two main factors affected development: (1) the direct effects of larval environment, through thermal-dependent effects of food conditions; and (2) effects of larval body mass at hatching, which are linked to female body mass. We expected to observe reduced duration of development at higher temperatures (Anger 2001). Under food or osmotic stress, larvae responded by developing in additional stages — as was expected based on previous work (Knowlton 1974, Fincham, 1983, Anger 2001). Larval developmental plasticity, i.e. development through additional instars, is considered an adaptive response in which maintenance is prioritised at the expense of growth or morphogenesis. Food limitation reduces growth rates, and osmotic stress is known to reduce growth in stenohaline decapod crustacean larvae (Torres et al. 2011). In our experiment, larvae under stress moulted, but development progressed at a slower rate through a pathway characterised by additional larval instars. In contrast, under unlimited food conditions, the resources available for growth and morphogenesis were high, and larvae followed a developmental pathway characterised by only a few stages.

One of the main results with respect to developmental plasticity was that a high temperature increased the effect of food limitation on the number of

Table 1. *Palaemon serratus.* ANCOVA results evaluating the responses of the duration of development to larval body mass at hatching (DW) in individuals reared under different temperatures (T), salinities (‰) and food conditions (F)

Factor	df	*F*	p
Initial larval biomass (DW)	1	10.660	$<10^{-3}$
Larval salinity (‰)	1	4.775	0.03
Food (F)	1	173.207	$<10^{-4}$
Larval temperature (T)	1	403.031	$<10^{-4}$
DW × F	1	4.624	0.03
DW × T	1	6.350	0.01
Error	57		

Table 2. *Palaemon serratus.* ANCOVA results evaluating the responses of the number of instars followed by larvae to the larval body mass at hatching (DW) in individuals reared under different temperatures (T), salinities (‰) and food conditions (F)

Factor	df	*F*	p
Initial larval biomass (DW)	1	5.66	0.02
Larval salinity (‰)	1	26.668	$<10^{-4}$
Food (F)	1	160.349	$<10^{-4}$
Larval temperature (T)	1	22.384	$<10^{-4}$
DW × F	1	8.579	0.01
F × T	1	4.387	0.04
Error	57		

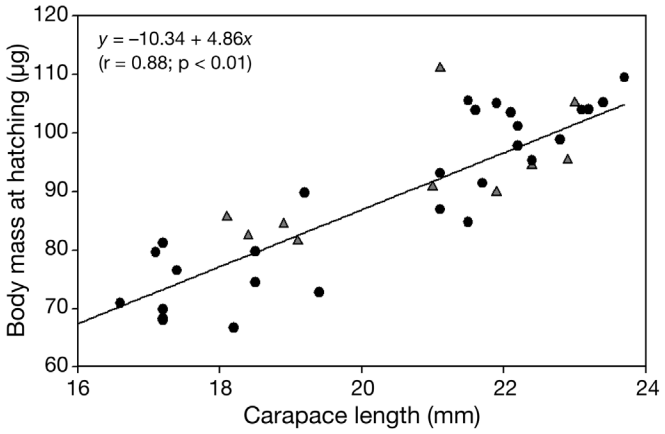

Fig. 8. *Palaemon serratus.* Relationship between female size (carapace length) and larval body mass at hatching (dry weight). Triangles represent those ovigerous females used for experimental work with larvae

instars followed by larvae, which is most likely due to a mismatch between supply and demand. This effect was observed irrespective of the female of origin. It therefore reflects variations in environmental conditions experienced by larvae hatched from the same female. First, under food limitation (reduced supply), the requirements for development through short larval pathways may not have been met. Second, metabolic demands (which are higher at 24°C than at 18°C) would have contributed to the mismatch; at higher temperatures, metabolic rates (demands) would increase (Kooijman 2009) but the food supply (access to prey) would have been low. Third, high temperatures also increased developmental rates, and would have reduced the cumulative growth rate per stage. These mechanisms usually lead to a smaller body size (cf. Yampolsky & Scheiner 1996). Thus, mismatches between supply and demand would have resulted in low growth rates per instar, for which larvae compensated by reducing the rate of morphogenesis (Knowlton 1974).

A significant part of the variation in responses among larvae from different females was explained through phenotypic links. Links between female body size, larval mass at hatching and larval development also reflect the metabolic mismatches explained above. Either larger female size or larger larval body size at hatching appeared to mitigate the effects of food limitation on development. Negative correlations between larval body mass at hatching and the duration of larval development have also been found in a decapod crustacean, where larger females do not produce larger larvae (Giménez & Anger 2001). We therefore assume that female size operates on larval development through its link with body size at hatching. Larger larvae exhibit higher feeding rates (Kiørboe 2008), accumulating more reserves at early stages. Therefore, these larvae require a lower energy supply to follow the shortest larval pathways. Differences in body mass are usually carried over to subsequent stages (Pechenik et al. 1998, Giménez et al. 2004). Thus, a larger size at hatching would mitigate the effect of food limitation on the growth of subsequent stages. This is consistent with the pattern found for an estuarine crab *Neohelice granulata* (Giménez & Anger 2003): higher initial body size at hatching reduced the proportion of larvae that followed a long developmental pathway, especially when they experienced osmotic stress.

Table 3. *Palaemon serratus.* ANCOVA results evaluating the responses of larval body mass at hatching to female body length (L) and temperature experienced during embryogenesis (T)

Factor	df	SS	*F*	p
L	1	1157.63	24.1605	$<10^{-4}$
T	1	200.87	4.1922	0.06
L × T	1	43.94	0.9170	0.35
Error	13	622.89		

Implications for populations

Thermal and food-dependent links between maternal body size and larval development can help us understand how *Palaemon serratus* populations respond to the coastal environment. It has been hypothesised that relationships between female and offspring size result from selective forces (Parker & Begon 1986, Allen et al. 2008) or physiological constraints (Sakai & Harada 2001). A key selective process experienced by *P. serratus* larvae is the heterogeneous and variable coastal environment in terms of the magnitude, timing and duration of peaks of planktonic production. Offspring from large females could have a selective advantage if larvae are released at times of low food production, or transported to areas characterised by low food availability. Longer development in larvae hatched from small females may result in lower larval or post-settlement survival through indirect effects of growth on predation (Giménez 2010, Harrison et al. 2011).

In addition, phenotypic links may have important implications for how populations respond to climate change. If projected changes in the marine environ-

ment result in reduced food availability and increased temperatures (Richardson 2008), large females could play a more important role in the maintenance of populations, leading to an increase in selective pressures for either higher growth rates or delayed size at reproduction.

Using *Palaemon serratus* as a model species, this work has shown that phenotypic links involving mothers and larval stages can be modified by environmental conditions experienced by larvae. Our results suggest that environmental conditions may operate on these links by modifying the balance of metabolic supply and demand, which may in turn trigger adaptive developmental responses. In addition, phenotypic links have important implications for how populations may adapt to a changing climate. Similar patterns could occur in other ectothermic species, because phenotypic plasticity could involve thermal-dependent physiological/developmental mechanisms. To uncover these mechanisms, it will be necessary to carry out multi-factorial experiments that consider the cascading effects of successive phenotypic responses — information that is important for understanding how organisms respond to variable environments. Our analysis suggests that much would be gained from studies that evaluate these relationships in the light of the physiological and metabolic processes that occur in parallel with development.

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