

## 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*

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In marine benthic invertebrates with complex life cycles, recruitment success, juvenile survival, and growth may be affected by variation in both maternal factors and environmental conditions prevailing during preceding embryonic or larval development. In an estuarine crab, *Chasmagnathus granulata*, previous investigations have shown that initial larval biomass is positively correlated with the biomass of recently extruded eggs, and it depends also on the salinity experienced during embryogenesis. Biomass at hatching has consequences for the subsequent larval development which, in this species, comprises two alternative developmental pathways with four or five zoeal instars (short or long pathway) and a megalopa. Larvae hatching with a lower than average biomass tend to develop through the long pathway and metamorphose to megalopae with higher biomass. In the present study, we show experimentally that the long pathway produces also significantly larger juveniles (crab size measured as carapace width, biomass as dry mass, carbon and nitrogen contents). Compared with juveniles originating from the short pathway, those from the long pathway showed in successive instars longer moulting cycles and larger carapace width, but lower size increments at ecdysis. In consequence, differences in size or biomass of long pathway vs short pathway crabs tended to disappear in later instars (after stage V). Furthermore, we tested in juveniles the tolerance of starvation at three salinities (5‰, 15‰, 32‰). Tolerance of starvation was significantly higher in juveniles originating from the long pathway, indicating higher energy reserves. While salinity played only a minor role for survival, it exerted significant effects on the time of moulting to the second juvenile instar, regardless of the preceding developmental pathway. The biomass of first juveniles obtained from the short pathway showed a significant positive correlation with the biomass of the freshly hatched zoea I, but not in those from the long pathway. In conclusion, the fitness of juvenile *C. granulata* is linked with previous developmental processes and environmental conditions during the embryonic and larval phase. Hence, a better understanding and prediction of the recruitment success of marine benthic invertebrates with a complex life cycle may require more comprehensive life-history investigations.

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In the past two decades, growing evidence both from the field and laboratory has shown that processes affecting the survival and growth in a given life-history phase of

benthic invertebrates may affect the performance in subsequent phases. Since field studies suggest that the distribution of adult benthic populations reflect varia-

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bility in the rate of settlement (Connell 1985, Gaines and Roughgarden 1985, Karlson and Levitan 1990, Grosberg and Levitan 1992), benthic patterns should be influenced also by processes and factors affecting larval survival in the pelagic environment (e.g. larval transport, predation, planktonic food availability, Strathmann 1982, Gaines and Roughgarden 1987, Roughgarden et al. 1988, Morgan 1995). Integrative studies of coupled pelagic and benthic processes as interrelated determinants of the structure and dynamics of marine benthic invertebrate communities may thus lead to a new synthesis of population dynamic theory (Connolly and Roughgarden 1998).

In the field, variability in the quality of settling larvae and in the rates of survival and growth of juvenile barnacles (Jarrett and Pechenik 1997, Miron et al. 1999, Jarrett 2000) and crabs (Luppi et al. 2002a) suggests that environmental stress during larval development affects also postsettlement performance. Field studies reported high mortality rates during the first few days of benthic juvenile life (reviewed by Gosselin and Qian 1997, Hunt and Scheibling 1997). The juveniles are particularly sensitive to various types of physical stress (Etter 1989, Gosselin 1997) and vulnerable to predation and cannibalism (Fernández et al. 1993, Eggleston and Armstrong 1995, Gosselin 1997, Moksnes et al. 1997, 1998, Luppi et al. 2002b), which may be related also to previous larval experience. For instance, an experimentally enhanced duration of larval swimming led to reduced colony growth and fecundity in a bryozoan (Wendt 1998). Similarly, exposure to food limitation during the larval phase led to reduced growth and increased mortality rates of juvenile mussels transplanted to the field (Phillips 2002).

In addition to effects of larval experience, juvenile quality may be affected by processes taking place before larval hatching or even before spawning of gametes. For example, variation in egg size as well as the salinity experienced during embryogenesis were found to influence larval performance in various crustaceans and echinoderms (Laughlin and French 1989, George 1999, Qiu and Qian 1999, Charmantier et al. 2002, Giménez 2002, Giménez and Anger 2003). If larval performance affects the subsequent juvenile survival and growth, there should be a strong interdependence between life history traits and physiological processes occurring in different life-history phases. Collectively, effects of previous experience on growth and survival in advanced stages may be seen as late life-history effects on population dynamics (Beckerman et al. 2002). In the present study, we investigated experimentally if body size, survival, and growth of the juveniles of an estuarine grapsoid crab, *Chasmagnathus granulata* Dana 1851, are influenced by initial size and growth during the larval phase. Adult and juvenile populations of this species inhabit brackish salt marshes in southern Brazil, Uruguay

and Argentina (Boschi 1964, Spivak 1997). The early life history of this species is characterised by an export of its early larval stages to lower estuaries and/or coastal marine waters (Anger et al. 1994). The megalopa is eventually re-imported to upper estuarine areas where it invades the parental environment; juveniles are typically found inside and near the burrows of conspecific adults (Spivak et al. 1994, Luppi et al. 2002b).

In *Chasmagnathus granulata*, initial larval size at hatching is correlated with the initial egg size at the onset of embryogenesis, and it is affected by the osmotic conditions experienced during embryonic development (Giménez and Anger 2001). Exposure of eggs to low salinities enhances subsequently the survival of the zoea I in brackish water due to an acclimatization process, but it may decrease survival at high salinity since it reduces the initial larval biomass (Charmantier et al. 2002, Giménez and Anger 2003).

The larval development of this species shows a variability of pathways: the third zoeal instar can either moult to a large zoea IVa stage or to a smaller zoea IVb. While the zoea IVa metamorphoses directly to the megalopa, the zoea IVb moults to a zoea V stage (Pestana and Ostrensky 1995). There are thus a short pathway with four and a long pathway with five zoeal stages. The frequency of larvae following the long pathway tends to increase in hatches with low initial larval biomass (Giménez and Torres 2002) as well as under nutritional or osmotic stress (Ostrensky et al. 1997, Giménez and Torres 2002, Giménez and Anger 2003). The megalopae originating from the long pathway show a higher biomass compared to those from the short pathway (Giménez and Torres 2002). The above-cited investigations considered only the links between embryos and larvae or between successive larval instars. However, differences in larval developmental pathways may also have consequences for juvenile fitness, reflected in differential body size, growth, and tolerance of environmental stress.

Hence, we studied if different larval pathways or variation in initial larval biomass are associated with variations in juvenile size, growth and survival. Specifically, we attempted to answer the following questions:

1) What are the consequences of following a developmental pathway with an additional zoeal instar for (a) the size and biomass of the first juvenile instar, (b) for growth and intermoult duration of the early juvenile stages, and (c) for tolerance of starvation and low salinities in newly metamorphosed crabs?

2) Is initial juvenile size affected by initial larval size regardless of the developmental pathway?

In addition, a synthesis of present and previous results allows us to propose a conceptual model of interrelationships between life-history and physiological traits of *C. granulata*, linking embryonic, larval and juvenile development with each other.

## Material and methods

### Experimental design and culture methods

Individual ovigerous females of *Chasmagnathus granulata* were maintained from egg laying to hatching of the zoea I (for ca 30 days) under controlled conditions of temperature (18°C), photoperiod (12:12h L:D), and salinity (15, 20, or 32‰). Immediately after hatching, samples of larvae taken from each egg mass were used to measure the initial larval biomass, others were reared at 20‰ during the zoea I stage (for 5–6 days), thereafter at 32‰ (from the beginning of the zoea II stage through metamorphosis to the first juvenile crab, in total another 30–40 days; for details of analytical and larval rearing techniques). Under these conditions, we obtained from each ovigerous female, in variable proportions, larvae that passed either through the short or the long developmental pathway. The zoeal instars were reared in 10 litre bottles with freshly hatched *Artemia* sp. nauplii given as food. Zoeal stages IV, V, megalopae, and juveniles were separated after moulting in order to obtain homogeneous cultures with respect to developmental stage and age within a given moulting cycle (Giménez and Torres 2002). Groups of 10 megalopae or juveniles metamorphosed from either zoea IV or V were reared separately in 500 ml bowls containing a piece of nylon gauze as a substrate.

### Measurements of biomass and body size

The biomass of freshly hatched zoea I larvae and of premoult (5 days old) first instar juvenile crabs (J-I) was measured as dry mass (W), carbon (C), and nitrogen (N) content in three to five replicate samples per hatch, each consisting of 40 zoeae or one juvenile, respectively. The samples were gently rinsed in distilled water, blotted on filter paper (fluff-free Kleenex for optical use), placed into preweighed tin cartridges, vacuum-dried for 48 h at <0.01 mbar in a Lyovac GT 2E (Leybold-Heraeus) apparatus, weighed to the nearest 0.1 µg on a Mettler UM3 microbalance, and stored in a freezer until determinations of the C and N contents were carried out with a Fisons (Carlo Erba Science) Model 1108 Elemental Analyzer. In juvenile crabs, body size was measured also as carapace width (CW; Luppi et al. 2002a) using the exuviae shed at the moult to the second instar. Statistical analyses followed Zar (1996) and Quinn and Keough (2002). Heterogeneity of variance was evaluated with Cochran test and normality was checked by inspection of the residuals.

### Consequences of different developmental pathways

Biomass (W, C, N) of first-stage juveniles (J-I) was determined in crabs obtained from 7 different hatches

and from both the short and long pathway; likewise, CW was measured in materials from nine hatches. The number of individuals measured (total = 116 ind) varied among hatches depending on the number of juveniles obtained and on the proportions of individuals passing through the short or long pathway. Comparisons of biomass or CW of crabs from short vs long pathway from the same hatch were made with paired t-tests. We also checked if, for a given developmental pathway, carapace width and biomass of J-I crabs instars were correlated.

Estimates of growth were made for 40 crabs from each developmental pathway. Juveniles were reared for two months in individual containers, from instars J-II to J-VII with seawater and fed ad libitum with *Artemia* sp. Water and food was changed every day. After moulting, the exuvia of each individual was measured and the day of moult registered to calculate the cumulative duration of development from the moult to J-II. We tested with Student t-test, if there were significant differences in carapace width or duration of development between developmental pathways at the J-I instar, i.e just before starting the experiment. The effects of the instar and developmental pathway on carapace width and duration of development (from J-II) were evaluated with within-between-subject two-way ANOVA using instar as the within-subject factor and developmental pathway as the between-subject factor. For carapace width the factor instar had five levels (J-II to J-VI). The J-VII instar was not included since by the end of the experiments not all individuals moulted to J-VIII so that we had fewer measurements of carapace width of the J-VII. We tested for significant differences in size at J-VII using t-test with separate variance estimation. The cumulative duration of development was measured from J-II to subsequent stages until J-VII so that the factor instar of the ANOVA included five levels. For carapace width and duration of development, variances were heterogeneous in some stages even after several data transformations. Thus, we followed Underwood (1981) and adjusted the critical value to reject  $H_0$  in the ANOVA to 0.005 (instead of 0.05) for carapace width and to 0.01 for duration of development. These values are lower than the lowest p-value found for variance heterogeneity in each variable. For instance the lowest p-value of the test of heterogeneity of variance for carapace width was 0.0061 which is larger than 0.005.

In order to compare growth rates between developmental pathways, we constructed growth curves for each individual that reached at least J-VI (35 ind for short pathway and 32 ind for long pathway). In each growth curve, carapace width (A) was a linear function ( $A = a + b \times Ct$ ) of the cumulative duration of development (Ct) starting from J-II as  $Ct = 0$ . Growth during the juvenile stages may be described as a linear relationship (Luppi et al. 2002a). Since both A and Ct are random variables we

used the reduced major axis (SMA in Legendre and Legendre 1998, RMA in Quinn and Keough 2002) regression to estimate the slope (b) of the linear growth equation. The significance of differences in mean growth rates (= mean slopes) between the developmental pathways was evaluated by t-test.

Starvation tolerance of crabs was quantified as time of survival (days after metamorphosis) in juveniles from two different hatches maintained at three salinities. In each experiment, juveniles from each developmental pathway were maintained individually in beakers without food at 5, 15 and 32‰ until all individuals died. Effects of differential developmental pathways and salinities on survival time were evaluated with separate two-way ANOVAs.

### Relationships between initial biomass of larvae and juveniles

Since we found a significant effect of the developmental pathway on biomass of first-stage juvenile crabs, the initial biomass of juveniles and larvae could be linked in a model using information from previous reports (discussion; additional data from Giménez and Anger 2001, 2003, Giménez and Torres 2002). Furthermore, we studied for each developmental pathway the correlation between biomass (W, C, N) measured in premoult J-I and in freshly hatched zoea I larvae, using data from 17 different hatches for the short pathway and 9 hatches for the long pathway.

## Results

### Consequences of differential developmental pathways

In larvae following the short developmental pathway, metamorphosis to the first juvenile instar took place, on average, 41.3 days after hatching, while the long pathway took 46.9 days. The biomass of first-instar juveniles (J-I) from the short pathway ranged from 330.7 to 498.0 µg dry mass (W), which contained 140.3 to 185.4 µg carbon (C) and 32.04 to 33.02 µg nitrogen (N). In juveniles from the long pathway, it ranged from 485.2 to 622.8 µg W, 186.6 to 219.8 µg C, and 34.51 to 46.42 µg N. Carapace width (CW) was 1.21 to 1.34 mm in short pathway and 1.29 to 1.42 mm in long pathway crabs. All differences in mean biomass and body size (amounting to 27% in W, 25% in C, 29% in N content, and 8.6% in CW, Fig. 1) were statistically significant, with consistently lower values found in juvenile crabs obtained from larvae following the short pathway as compared to those from the long pathway (W:  $t = 5.64$ ,

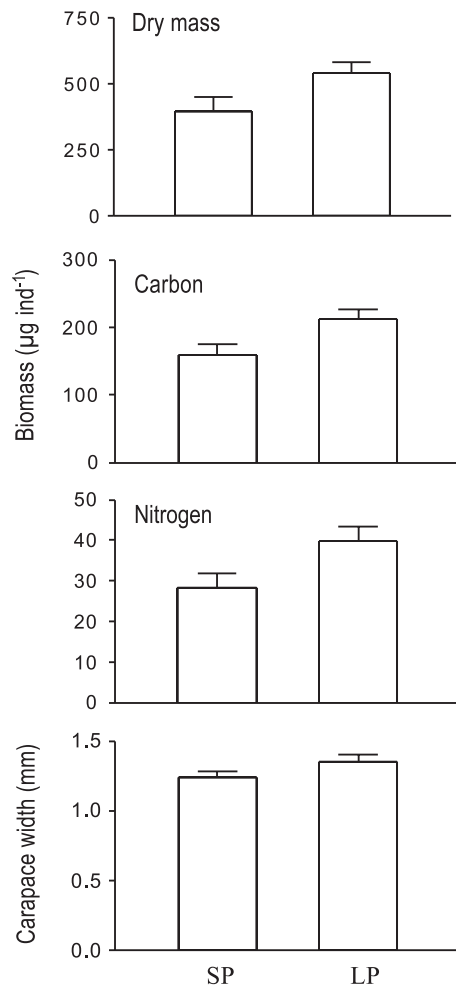


Fig. 1. Consequences of following either a short or a long larval developmental pathway (short pathway and long pathway respectively) for mean biomass (dry mass, carbon and nitrogen content) and size (carapace width) of first juvenile instars. Standard deviations showing variability among hatches are given as bars.

$p < 0.01$ ; C:  $t = 6.65$   $p < 10^{-3}$ ; N:  $t = 7.74$ ,  $p < 10^{-3}$ ; CW:  $t = 7.23$ ,  $p < 10^{-4}$ ).

In freshly metamorphosed juveniles from the short pathway, CW correlated with juvenile biomass (measured as W:  $r = 0.65$ ,  $p < 0.05$ ; C:  $r = 0.56$ ,  $p < 0.05$ ; N:  $r = 0.65$ ,  $p < 0.05$ ). However, mean CW of juveniles from the long pathway did not show a significant correlation with the biomass ( $r < 0.40$ ,  $p > 0.10$  for W, C, and N).

Differences observed between the two developmental pathways in average juvenile CW persisted throughout the first seven crab instars (Fig. 2a, c). T-tests performed in the J-I stage, i.e. before starting of the experiment, showed significant differences in CW (mean size of short pathway crabs: 1.25 mm vs 1.41 mm in long pathway individuals;  $t = -11.4$ ,  $p < 10^{-6}$ ,  $n = 40$ ). An ANOVA conducted for CW in instars J-II to J-VI detected a

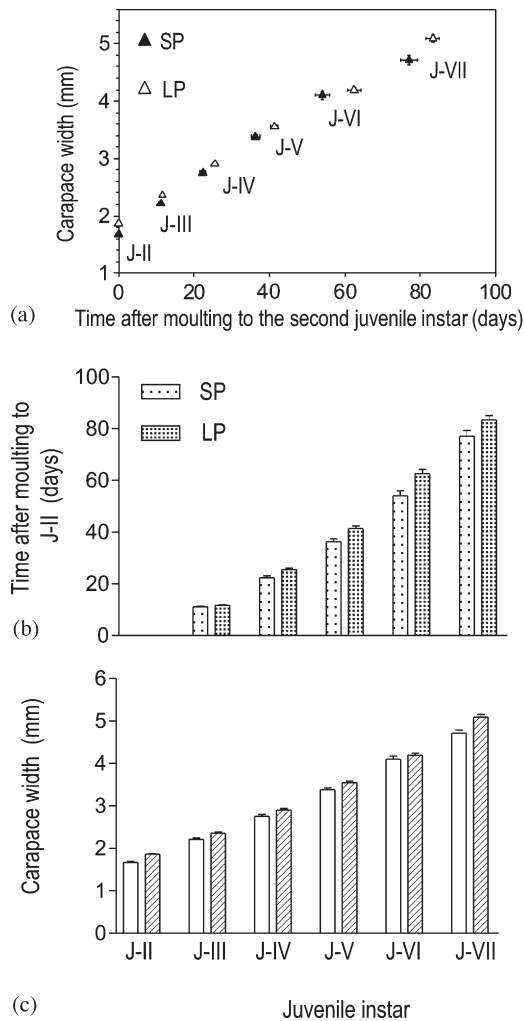


Fig. 2. Consequences of following different larval developmental pathways for juvenile growth measured as changes in mean carapace width and cumulative duration of development counting from the second instar: (a) changes in carapace width through time; (b) cumulative time after moulting to J-II; (c) changes in carapace width from J-II to J-VII. Symbols as in Fig. 1. Error bars denote standard errors.

significant main effect of the developmental pathway but no significant interaction with the instar number

(Table 1). A t-test comparing CW in the J-VII detected only a marginally significant effect of the pathway ( $t = 1.83$ ,  $p = 0.07$ ,  $n_{SP} = 28$ ,  $n_{LP} = 19$ ). The percentage size increments between successive instars ranged from 18 to 32%, with slightly higher values in crabs from the short pathway.

Mean duration of development from the first to the second instar did not differ significantly between developmental pathways (8.08 vs 8.04 days for short pathway and long pathway, respectively;  $t = 0.18$ ;  $p \geq 0.05$ ). However, in later instars, the crabs from the long pathway showed a longer duration of development (Fig. 2a, b). The difference in moult-cycle duration increased from  $< 1$  day in J-II to 5.3 in J-IV, and to 11.8 days in J-V, remaining constant (ca 8.0 days) in the J-VI and VII instars. ANOVA detected a significant interaction between the effects of pathway and instar number on instar duration (Table 1), because the difference was significant for J-IV and subsequent instars but not for J-III.

Linear growth curves (CW as a function of time elapsed since moulting to the second juvenile instar) were highly significant ( $r^2$  ranging from 0.871 to 0.999 for the short pathway, from 0.924 to 0.9997 for long pathway). The average daily growth rate (i.e. the slope of the growth curve in mm/day) varied from 0.024 to 0.072 for the short pathway and from 0.026 to 0.057 for long pathway. The mean parameters of all individual growth curves led to the following linear equations describing body size (CW) as a function of the cumulative duration of development (Ct):  $CW = 1.71 + 0.044 \times Ct$  ( $n = 35$ ) for short pathway, and  $CW = 1.90 + 0.039 \times Ct$  ( $n = 32$ ) for long pathway, with significantly higher average growth rate for juveniles from the short pathway ( $t = 2.27$ ,  $p < 0.025$ ).

The tolerance of starvation (quantified as mean time of survival of juvenile crabs in absence of food) ranged from 17.9 to 25.8 days. In both hatches tested, the survival time of crabs from the long pathway was significantly longer than in those from the short pathway (Table 2, Fig. 3a). Juveniles from the short pathway showed also a slightly higher proportion of individuals dying after  $< 10$  days of starvation (Fig. 3b); in both hatches, this occurred more frequently at 5 and 15‰ as

Table 1. ANOVA to evaluate the effect of developmental pathway and stage on carapace width and duration of development of early juvenile stages. Symbols: MSf, MSe mean square of factor and error, dff, dfe degrees of freedom of factor and error. Significant values ( $p < 0.005$  in carapace width,  $p < 0.01$  in duration of development) are in bold.

Factor	dff	MSf	dfe	MSe	F	P
Carapace width						
Pathway (P)	1	2.178	71	0.228	9.57	<b>0.0028</b>
Instar (I)	4	64.227	284	0.051	1254	<b><math>&lt; 10^{-6}</math></b>
P $\times$ I	4	0.035	284	0.051	0.693	0.598
Duration of development						
Pathway (P)	1	1916	67	174.1	11.00	<b>0.009</b>
Instar (I)	4	51614	268	34.8	1484.10	<b><math>&lt; 10^{-5}</math></b>
P $\times$ I	4	162	268	34.8	4.66	<b>0.002</b>



Table 2. ANOVAs to evaluate the effect of developmental pathway and salinity on survival of first juvenile instars metamorphosed from larvae that followed different developmental pathways. Symbols as in Table 1. Significant values ( $p < 0.05$ ) are in bold.

	MSf	Dff	Mse	dfe	F	p
Hatch 1						
Pathway	350.4	1	56.52	54	6.20	<b>0.015</b>
Salinity	170.4	2	56.52	54	3.01	0.057
P × S	41.87	2	56.52	54	0.74	0.480
Hatch 2						
Pathway	268.47	1	38.43	45	6.99	<b>0.011</b>
Salinity	81.37	2	38.43	45	2.11	0.130
P × S	125.61	2	38.43	45	3.27	<b>0.047</b>

compared to 32‰. The survival time in full-strength seawater (32‰) was slightly longer than in reduced salinities, but the differences were only in one hatch statistically significant (Table 2).

During the starvation experiment, several J-I crabs moulted once, a few even twice. The proportion of individuals that moulted to the J-II instar is shown in Table 3. In both hatches, this proportion was higher at 32‰ than at lower salinities. A log-linear analysis including all variables of Table 4 confirmed a significant

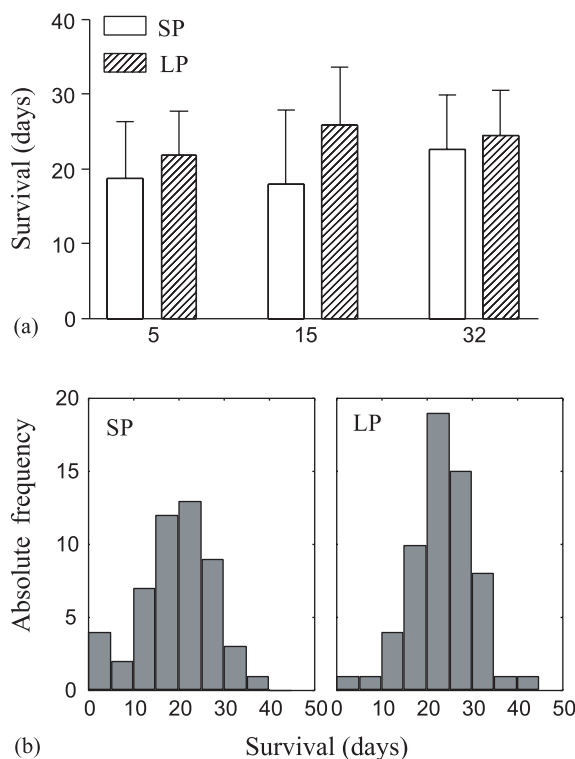


Fig. 3. (a) Consequences of following different developmental pathways for tolerance to starvation, measured as the time of survival since metamorphosis to the first juvenile instar. Error bars denote standard deviations. (b) Distribution of survival time for juveniles after following different larval developmental pathways. Symbols as in Fig. 1.

effect of the salinity factor on this proportion ( $\chi^2 = 7.51$ ,  $p < 0.05$ ). We measured also the size (CW) of J-I that moulted to the J-II (short pathway:  $1.227 \pm 0.075$  mm,  $n = 21$ ; long pathway:  $1.342 \pm 0.072$  mm,  $n = 18$ ). In spite of having starved, these juveniles showed an increase in size at moulting (CW of J-II:  $1.47 \pm 0.098$  mm,  $n = 4$ ).

### Relationship between initial biomass of juveniles and first-stage larvae

The initial biomass of first-instar juveniles (measured in terms of W, C or N) obtained from the short pathway, but not that of long pathway individuals, was significantly correlated with the biomass measured at larval hatching (Fig. 4). In the N content of short pathway crabs, however, this relationship was only marginally significant.

### Discussion

Our results show that variation in the developmental pathways and initial biomass of *Chasmagnathus granulata* larvae has significant consequences for postsettlement life. Juvenile life history traits such as body size at metamorphosis as well as in later crab instars are related to previous larval characters such as size at hatching and the developmental pathway. In addition, a synthesis of present and previous data shows us how life-history traits and physiological characters in different phases of the life cycle are connected throughout development. Those delayed life history effects may even influence also the population dynamics of *C. granulata*, especially when different larval cohorts experience different environmental conditions (Beckerman et al. 2002). In *C. granulata*, this situation is likely to occur, as its embryos, larvae, juveniles, and adults may be spread over heterogeneous estuarine and coastal marine habitats (Giménez 2003).

Table 3. Number of juveniles that moulted to J-II under starvation at different salinities in relation to the total number of juveniles (either 10 or 7 given as the denominators), and relative frequency (Rf) at different salinities and developmental pathways. The crabs are from two hatches and either the short or the long developmental pathway.

	5‰	15‰	32‰	Rf
Hatch 1				
short pathway	1/10	1/10	3/10	0.16
long pathway	5/10	2/10	8/10	0.50
Rf	0.30	0.15	0.55	
Hatch 2				
short pathway	5/7	5/7	6/7	0.76
long pathway	0/10	2/10	4/10	0.20
Rf	0.29	0.41	0.59	

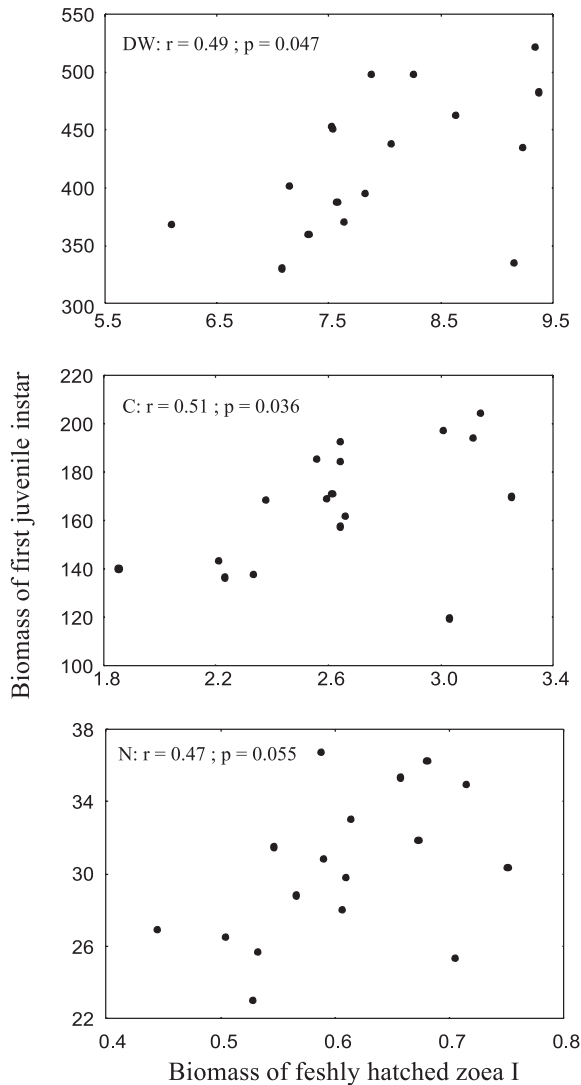


Fig. 4. Relationships between juvenile and initial larval biomass, measured as dry mass (DW) carbon (C) and nitrogen (N) content after following the short larval developmental pathway.

### Effects of larval characters on juvenile life

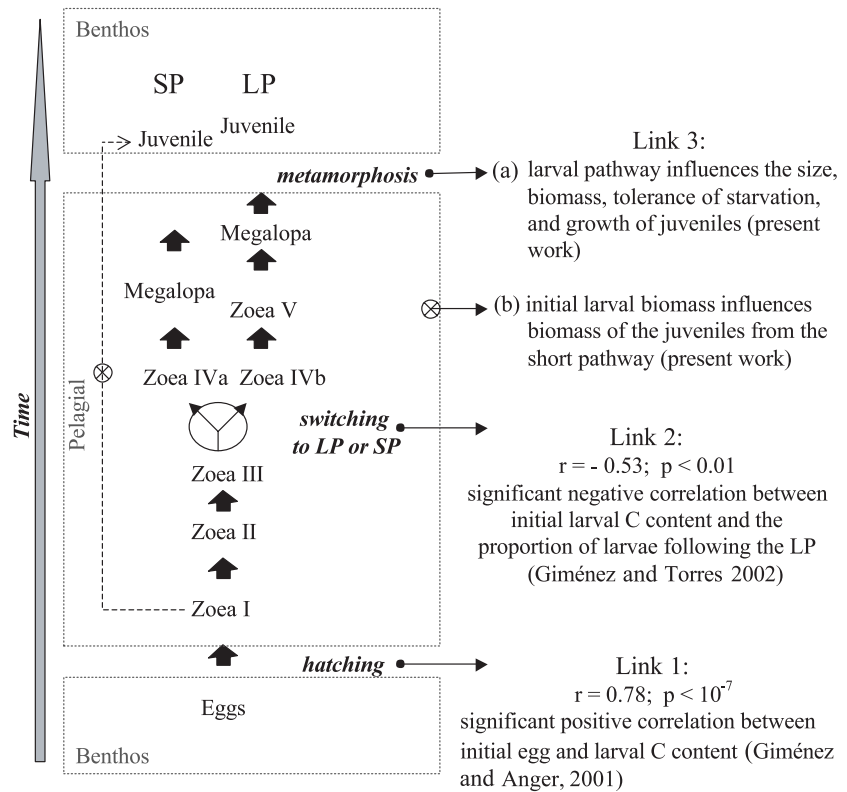
The switch to a given developmental pathway, most likely occurring during the third zoeal stage, codetermines the initial size and biomass of the first juvenile instar. The difference in carapace width (CW) between crabs from the two developmental pathways was much smaller than the difference in biomass, implying that the initial juvenile energy reserves may differ greatly even when the difference in body size is small. Within a given developmental pattern, the correlation between body size and biomass was significant only for juveniles from the short but not from the long pathway. This could be due to the fact that we were not able to measure the size and biomass in the same individual, but used mean values for

each hatch for our correlations. On the other hand, size may not be a reliable measure of biomass within a developmental pathway. Likewise, we observed an increase in CW of juveniles which were able to moult in complete absence of food to the second instar. The average size of the J-I instar observed in this study (1.2–1.4 mm) was in the range of values obtained by Gebauer et al. (1999) at 18°C (1.3 mm) and by Rieger and Nakagawa (1995) at 20°C (1.26 mm), but smaller than those obtained by Luppi et al. (2002a) from field-caught megalopae reared at 24°C (1.61 mm). These differences indicate that the larval environment influences the size of the first juvenile.

Under ad libitum feeding conditions, very few juveniles died during our experiments. Differences in size found in J-I crabs persisted throughout the subsequent instars II–VI. However, a statistically insignificant difference in the J–VII suggests that juveniles from the short pathway grew slightly faster than those from the long pathway, so that they gradually caught up with the latter, which were significantly larger at metamorphosis and in several subsequent instars. This is reflected also in differential percentage size increments which were slightly higher in juveniles from the short than those from the long pathway. The moult-cycle duration in crabs from the short and long pathway was not significantly different in the first three juvenile instars. Later, however, those from the short pathway showed shorter average instar durations. Growth curves describing both the size increments and moult-cycle durations in successive juvenile instars showed typical patterns that are known also from many other crustaceans, namely a successively decreasing size increment and an increasing duration of development (Hartnoll 2001). Also these curves indicated a faster, i.e. compensatory growth in short pathway compared to crabs from the long pathway.

Under simulated environmental stress, juveniles from the long pathway tolerated starvation for a longer time than those from the short pathway. In the field, several factors may affect crabs in a size-selective manner: habitats based on these factors are “offspring size-sensitive” (Begon et al. 1996) and may therefore select individuals on a size basis. Sub-optimal food conditions, for instance, may lead to differential survival in juvenile crabs, as larger crabs show a higher capability of pursuing and capturing prey. Also mortality due to intercohort cannibalism, which is probably a structuring force in *C. granulata* populations (Luppi et al. 2001, 2002b), may vary with differential size at settlement. In both cases, offspring fitness should increase with offspring body size (Stearns 1992), implying that crabs from the long pathway should initially have a higher fitness than individuals from the short pathway settled at the same time, and their larger size should reduce cannibalism by conspecific adults and earlier settled juveniles. However, such favourable effects may disappear in later

Fig. 5. Schematic model showing the links among life history characters and physiological processes occurring during the embryonic larval and juvenile phases, that lead to variation in size, biomass, growth, and survival of juveniles. Symbols: SP = short pathway, LP = long pathway.



instars due to faster growth in crabs from the short pathway. Furthermore, the advantage of the long pathway vs short pathway may be offset by risks associated with a prolonged planktonic period and delayed metamorphosis in the former pathway. Although salinity varies considerably in estuarine environments, this factor seemed to play only a minor role for the survival and growth of early juvenile *C. granulata*. This is in contrast to another study (Rieger and Nakagawa 1995), where low salinity (5‰) was observed to cause reduced survival. Variability in survival at low salinities does not depend significantly on crab size or biomass, similar as observed in the larvae of this species (Giménez and Anger 2003). Habitats differing only in salinity may thus be “offspring size insensitive” (Begon et al. 1996), i.e. not favouring a particular juvenile size over another.

Therefore, the consequences of following a particular developmental pathway for later performance may strongly depend on habitat characteristics. If food availability is high in the juvenile environment, the developmental pathway may be less important than in food-limited habitats. In the latter, long pathway individuals with higher biomass should have an advantage over those from the short pathway. Consequences for adult size and reproduction may occur also, if size at the

onset of reproduction is a fixed character of a species; this should be studied in future experiments.

In addition, our present results combined with those from previous reports show that there is a variety of effects of larval experience on juvenile condition. Environmental factors prevailing during the pelagic larval phase, in combination with developmental and other life-history traits, determine the initial size and distribution of benthic invertebrates due to combined effects on (1) the time of metamorphosis (Pechenik et al. 1998, 2001a, Gebauer et al. 2002), (2) size at metamorphosis (Hart and Strathmann 1994, Gebauer et al. 1999, Meidel et al. 1999), or (3) subsequent juvenile growth (Pechenik et al. 1993, 2001b, 2002, Qian and Pechenik 1998). If delayed metamorphosis, smaller initial juvenile size, and reduced growth co-occur as a common response to a single stress factor, differences in size should tend to increase in later benthic stages. On the other hand, effects exerted only on size at or time of metamorphosis, but not on subsequent growth, should only propagate initially present differences in size throughout later development. In *C. granulata*, the absence of natural cues tends to delay metamorphosis and to reduce size at settlement, but differences in size may disappear as early as in the J-III instar (Gebauer et al. 1999), implying a



limited propagation of initial size differences. On the other hand, food limitation and salinity stress are responsible for a high proportion of larvae following the long pathway (Ostrensky, et al. 1997, Giménez and Anger 2003), leading to delayed metamorphosis, but also to an enhanced size at metamorphosis and to higher tolerance of starvation. Hence, there is a partial compensation of differential presettlement effects. Similarly, food limitation stimulated metamorphosis in a gastropod, *Crepidula fornicata*, but caused also reduced juvenile growth (Pechenik et al. 1996a, b). Thus, within a certain environmental context, the relationships between physiological processes and life-history traits determine whether initial differences among recently settled cohorts or individuals are enhanced, propagated, or compensated in subsequent developmental stages.

### Linking the life history and physiological characters from embryonic larval and juvenile phases

Summarizing present and previous observations from an estuarine crab, *C. granulata*, we may say that there are different kinds of interdependencies between life history and physiological traits within a complex life cycle (Fig. 5). Various effects of alternative developmental pathways on juvenile size, biomass, growth, and survival under stress conditions link juvenile and larval life-history traits with each other and with physiological processes occurring even before hatching. The biomass at larval hatching depends not only on the initial egg biomass, but also on the energy consumption during embryogenesis which depends also on salinity (Giménez and Anger 2001). Thus, interactions between metabolic processes and environmental conditions define a first link between the embryonic and the larval phase of the life cycle (Fig. 5).

As another link, initially smaller larvae tend to pass more frequently through the long pathway (Giménez and Torres 2002). The long pathway, as a response to low initial biomass, implies priority for growth over morphogenesis during the development through an additional zoeal stage (Knowlton 1974, Anger 2001). In consequence, such larvae tend to metamorphose to larger megalopae and reverse the initial differences in size. On the other hand, if an initially small larva develops through the short pathway, it will metamorphose to a particularly small megalopa, so that an initial size difference is propagated through to advanced stages (Giménez and Torres 2002). The reversal or propagation, respectively, of variability in early larval body size represents a further link between successive phases of the life cycle (Fig. 5: link 3a and b respectively).

Juvenile size or biomass distribution in a benthic population may thus, in part, be a side effect of environmental and maternal effects on metabolic and

hormonal systems that control the processes of moulting and morphogenesis. Initial differences in juvenile size may persist through several subsequent stages, and they may lead to differential survival under nutritional or other kinds of stress. Thus, individual responses to different environments are limited by physiological mechanisms which are related to developmental processes (Ricklefs and Wikelski 2002). While a response may be adaptive in a particular phase of a complex life cycle, its late effects in subsequent phases are not necessarily beneficial for individual fitness. The understanding of causes of fluctuation in populations with complex life cycles should thus increasingly require the consideration of changes in individual condition as consequences not only of present but also of previous events.

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