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Effects of reduced salinity on the biochemical composition (lipid, protein) of zoea 1 decapod crustacean larvae

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Abstract

Effects of reduced salinities on dry weight (DW) and biochemical composition (total lipid and protein contents) of zoea 1 larvae were evaluated in four decapod crustacean species differing in salinity tolerance (*Cancer pagurus*, *Homarus gammarus*, *Carcinus maenas*, *Chasmagnathus granulata*). The larvae were exposed to two different reduced salinities (15‰ and 25‰ in *C. granulata*, 20‰ and 25‰ in the other species) for a long (ca. 50% of the zoea 1 moulting cycle) or a short period (16 h, starting at ca. 40% of the moulting cycle), while a control group was continually maintained in seawater (32‰).

In general, the increments in dry weight, lipid and protein content were lower at the reduced salinities than in the control groups. In the zoea 1 of *H. gammarus* (stenohaline) and *C. pagurus* (most probably also stenohaline), the lipid and protein contents varied greatly among treatments: larvae exposed to low salinities exhibited very low lipid and protein contents at the end of the experiments compared to the controls. In some cases, there were negative growth increments, i.e. the larvae had, after the experimental exposure, lower lipid and protein contents than at the beginning of the experiment. *C. maenas* (moderately euryhaline) showed a lower variation in protein and lipid content than the above species. The zoea 1 of *C. granulata* (fairly euryhaline) showed the lowest variability in dry weight, protein and lipid content. Since salinity tolerance (eury- v. stenohalinity) is associated with the osmoregulatory capacity, our results suggest a relationship between the capability for osmoregulation and the degree of change in the biochemical composition of larvae exposed to variable salinities.

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Besides larval growth of these species should be affected by natural reductions of salinity occurring in coastal areas at different time scales. These effects may be potentially important for population dynamics since they should influence the number and quality of larvae reaching metamorphosis.

Keywords: Crustacean; Larvae; Development; Fitness; Growth; Salinity

1. Introduction

Decapod crustaceans and other marine benthic invertebrates with complex life cycles develop through a planktonic larval and a benthic juvenile–adult phase. The larvae show dramatic growth and morphogenetic changes, which are affected by environmental conditions. Changes in the biochemical composition of decapod crustacean larvae were studied in several species (reviewed in Anger and Harms, 1990; Anger, 1991, 1998). They typically show a high percentage of protein (>30%), followed by lipids (<20%), chitin (<15%) and free carbohydrates (<5%). Variation is due to differential phylogenetic position and variation in environmental factors such as temperature (Dawirs and Dietrich, 1986; Anger, 1987), food availability (Anger and Dawirs, 1982; Dawirs, 1986, 1987; Harms et al., 1991, 1994), and salinity (Pfaff, 1997; Anger et al., 1998, 2000). Temperature, for instance, may affect the reserves accumulated during growth through simultaneous but not always equal changes in instantaneous growth rate and development time (Anger, 1998). Under conditions of severe nutritional stress, there is usually a preferential degradation of lipids, while internal lipid reserves may partially be invested to complement insufficient nutrients available for growth and morphogenesis under moderate malnutrition (Anger, 1998).

In coastal and estuarine environments, crustacean larvae face temporal and spatial variability in salinity, experiencing osmotic stress that may reduce growth and survival. This effect should vary depending on physiological adaptations such as the capability of osmoregulation in early developmental life-history stages (Charmantier, 1998). In species where the larvae are osmoconformer until metamorphosis (e.g. *Libinia marginata*, *Chionoecetes opilio*; Charmantier, 1998), only a narrow range of salinity variation is tolerated. Osmoregulating species, whose larvae show the adult type of osmoregulation already from the beginning of larval development (e.g. *Macrobrachium petersi*, *Palaemonetes argentinus*), are found in freshwater habitats or in environments with highly variable or extreme salinities; they have a wide range of salinity tolerance (Charmantier, 1998). A third pattern is found in species like *Penaeus japonicus*, *Cancer irroratus* (Charmantier, 1998), and *Homarus gammarus* (Charmantier et al., 2001), where the larvae are osmoconformers while the adults are osmoregulators.

The regulation of intracellular osmotic effectors affects the amino-acid metabolism (Schoffeniels and Gilles, 1970), and thus, also the protein composition under osmotic

stress. Extracellular osmoregulation, on the other hand, is associated with energy expenditure for active ion transport, involving the degradation of energy-rich compounds such as lipids. These mechanisms should result in biochemical changes in response to salinity variation. For decapod larvae, however, there exists only scarce information about variation in biochemical composition under osmotic stress. Pfaff (1997) observed, in the stenohaline larvae of a marine crab, *Hyas araneus*, that lipid accumulation was affected during development at a reduced salinity (20‰), while the protein content was not affected. In euryhaline *Armases miersii* larvae, which are capable of osmoregulation early in development (Charmantier et al., 1998), Anger et al. (1998) found in a wide range of salinities (e.g. 15–45‰ for zoea 1) a significant accumulation of biomass (measured as carbon content per individual).

According to these observations, we may expect that the lipid and protein contents of stenohaline and osmoconforming species should exhibit a stronger response to salinity changes than in euryhaline and osmoregulating species. In this study, we compared the biochemical composition of decapod crustacean larvae exposed to differential degrees and for different periods to osmotic stress.

2. Materials and methods

2.1. Model species

Cancer pagurus, *H. gammarus*, *Carcinus maenas* and *Chasmagnathus granulata* were chosen, because they are known or believed to differ in their tolerance of low salinities.

The crab *C. pagurus* and the lobster *H. gammarus* occur in the subtidal zones of the North Sea, where very little variability occurs in the salinity conditions. Adult *H. gammarus* show a moderate osmoregulatory capacity, while the first larval stage of this species is a stenohaline osmoconformer (Charmantier et al., 2001). Larvae of both species show considerable mortality at 20‰ (G. Torres, personal observation). *C. maenas* occurs in the intertidal and subtidal of the North Sea, but also in estuarine environments with low or variable salinities. The zoea 1 from a North Sea population can survive for several days at 15‰ but is not able to moult to the zoea 2 (Anger et al., 1998). *C. granulata* is an extremely euryhaline crab inhabiting estuaries and salt marshes of Argentina, Uruguay and southern Brazil. The zoea 1 is able to survive at 15‰ (Giménez, 2000); under this condition it is capable of hyper osmoregulation (Charmantier et al., 2002).

Ovigerous *C. pagurus* and *H. gammarus* were caught in subtidally deployed cage-traps, *C. maenas* were collected by hand from the rocky intertidal, all at Helgoland (North Sea). Adult *C. granulata* originated from Mar Chiquita lagoon, Argentina (37°33′ S, 57°20′ W); they were originally transported to Helgoland and subsequently reared under controlled conditions of temperature (21 °C), salinity (32‰) and photoperiod (12:12). When females laid eggs, they were isolated in individual aquaria and kept under the same conditions. Adult crabs were fed with isopods (*Idotea* sp.) ad libitum.

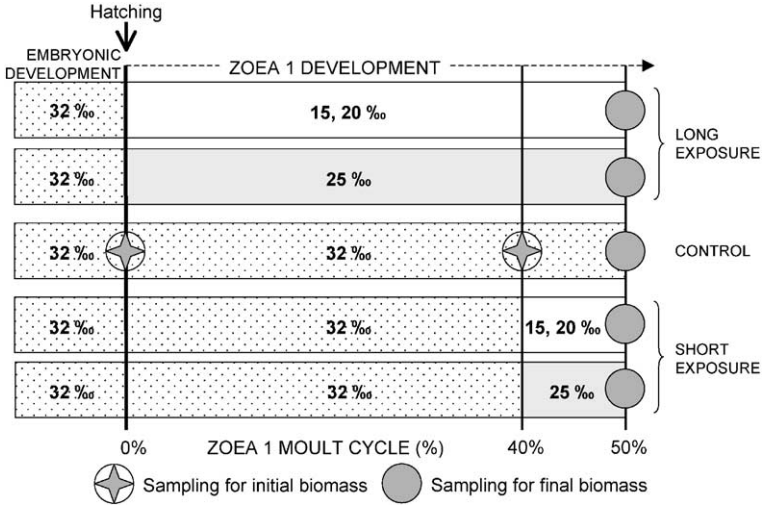

Fig. 1. Experimental design for maintenance of ovigerous females, larval rearing and sampling for measurement of dry weight, protein and lipid content.

2.2. Experimental design

After hatching in seawater (32 ‰), zoea 1 larvae of each species were divided in three groups: a control group was continually reared at 32 ‰; the other two groups were exposed to lower salinities (see Fig. 1). Among the latter, the larvae of *C. granulata* were exposed to 15 ‰ as the lowest salinity, because this species is known as the most euryhaline among the species studied here; all other species were exposed at most to 20 ‰. In addition, all species were exposed also to 25 ‰ salinity. These treatments represent conditions of severe and moderate hypo-osmotic stress, respectively. Each of these two groups was further subdivided in two treatments with different periods of exposure, referred to as "long exposure", LE (from hatching to the time when 50% of the zoea 1 moult-cycle duration had elapsed), and "short exposure", SE (from 40% to 50% of the moulting cycle) Table 1. While LE to reduced salinities included the postmoult and intermoult periods, SE included only a part of the intermoult period. Thus, differences in biomass after LE and SE to decreased salinities may not only reflect a different exposure time but also variation in salinity tolerance during the course of the moult cycle.

Average duration of the zoea 1 moulting cycle was 8 days in *C. pagurus*, 10 days in *H. gammarus*, and 6 days each in *C. granulata* and *C. maenas*. Samples of larvae were taken at hatching, i.e. at the beginning of the LE experiments, and again at 40% of the moult-cycle duration in seawater (32 ‰; this was also the beginning of the SE experiments); the last samples were taken at 50% of the moulting cycle (from all treatments). In all samples, larval dry mass (DW), lipid and protein contents were measured (see below) to quantify larval growth from hatching or from 40% (SE experiments) to 50% of the zoea 1 moult-cycle duration.

Table 1
Duration of development of zoea 1 at 32‰ (DD) and day of sampling after hatching for short and long exposure experiments

Species	DD (days)	Short exposure		Long exposure	
		40% DD initial 4:00 p.m.	50% DD final 8:00 a.m.	0% DD initial 8:00 a.m.	50% DD final 8:00 a.m.
C. pagurus	8	3	4	0	4
H. gammarus	10	4	5	0	5
C. maenas	6	2	3	0	3
C. granulata	6	2	3	0	3

2.3. Rearing

Larvae were reared in filtered water (Orion, mesh size: 1 μm). Water with reduced salinities (15‰, 20‰ and 25‰) was obtained by mixing seawater (32‰) with appropriate quantities of desalinated water. After preliminary tests, larvae of *C. granulata* (200 larvae/l) and *C. pagurus* (100 larvae/l) were mass-reared in 10-l glass bottles with gentle aeration. *H. gammarus* (50 larvae/l) were cultured in 5-l glass beakers with moderate aeration and *C. maenas* (40 larvae/l) in 0.3-l vials without aeration. The larvae were fed ad libitum with freshly hatched *Artemia* sp. Water was changed daily, dead larvae were removed, and new food was added.

2.4. Biochemical analyses

Samples for biochemical analyses were gently rinsed in distilled water for 10 s and dried on a filter paper. Then, they were transferred to an Eppendorf vial and frozen at −80 °C. These samples were left in a vacuum drier (Finn-Aqua Lyovac GT2E) for 48 h and their dry weight was determined in a Sartorius MC1 RC 210 S balance (precision: 0, 01 mg, capacity 210 g). Afterwards, they were homogenized by sonication (Branson, Sonifier, Cell Disruptor B 15) and each homogenate was divided in two aliquots to perform lipid and protein content determinations.

The protein content was determined following the Lowry method (Lowry et al., 1951), the lipid content with the sulphophosphovanillin method (kit: Merckotest 3321, Total lipids, Merck) following Zöllner and Kirsch (1962). Both methods were modified to perform measurements with microplates (Pfaff, 1997; Paschke, 1998). Spectrophotometric measurements were made in triplicate in a microplate spectrophotometer (Dynatech, MR 7000), using a 750-nm filter for measuring proteins and a 530-nm filter for lipids.

2.5. Statistical analyses

The statistical analyses were performed following Zar (1996). All data were analysed with a two-factor ANOVA plus a control treatment. The two factors considered were salinity (two levels: 15‰ or 20‰, 25‰) and exposure time (two levels: short and long); the control treatment was the group reared at 32‰. Comparisons between different levels or factors, after finding significant differences in the ANOVA, were performed with the

Student–Newman–Keuls test (SNK). Comparisons with the control treatment (larvae at 32‰) were done using planned comparisons. The critical level (α) to reject the null hypothesis was fixed at 0.05. Previous to performing ANOVA, normality (normal plots) and variance homogeneity (Cochran test) were checked. In all cases the variances were homogeneous and all distributions were normal. Due to variable availability, the number of replicates was 10 for *C. pagurus*, *H. gammarus* and *C. granulata* and 5 for *C. maenas*.

3. Results

3.1. C. pagurus

Dry weight (DW) and biochemical composition (lipid and protein content) of the zoea 1 larvae were affected by reduced salinities. For DW, also the interaction with exposure time was significant (Table 2).

In general, final DW was lower at reduced salinities, both after short and long exposure (SE, LE; Fig. 2a). In LE experiments, DW was significantly lower at 20‰ than at 25‰ (Fig. 2a). During SE larvae lost significant amounts of DW at 20‰ but not at 25‰. During LE, DW increased significantly at all salinities, but the increment was smaller at the reduced salinities (Fig. 2b).

The effect of salinity on the lipid content depended on exposure time. After SE, the lipid content of *C. pagurus* larvae did not vary among treatments and the control group. After LE in contrast, the larval lipid content was significantly lower at 20‰ and 25‰ than in the control. Significant lipid losses were registered after both SE and LE to 20‰, and also after LE to 25‰ (Fig. 2d).

Protein content decreased in both SE and LE experiments at the lowest salinity (20‰) (Fig. 2e). After SE to 25‰, the protein content was significantly higher than in the control group. Compared with the initial value, the protein content increased significantly in all SE experiments (Fig. 2f). After LE to 20‰, the protein content

Table 2
C. pagurus: Two-factor ANOVA to evaluate differences in the biomass (dry weight, lipids and proteins), among different salinities (20‰ and 25‰) and exposure times

Variable	Factor	dff	MSf	dfe	MSe	*F*	*p*
Dry weight	S	1	105.5	41	1.51	69.7	<0.001
	E	1	130.5	41	1.51	86.2	<0.001
	S×E	1	50.0	41	1.51	33.0	<0.001
Lipid	S	1	0.104	41	0.016	6.470	<0.05
	E	1	0.347	41	0.016	21.5	<0.001
	S×E	1	0.001	41	0.016	0.044	0.834
Protein	S	1	37.9	41	0.544	69.7	<0.001
	E	1	65.7	41	0.544	120.7	<0.001
	S×E	1	2.15	41	0.544	3.95	0.053

Symbols: S: salinity; E: exposure time; dff, MSf, dfe, MSe: degrees of freedom and mean square of factor and error, respectively.

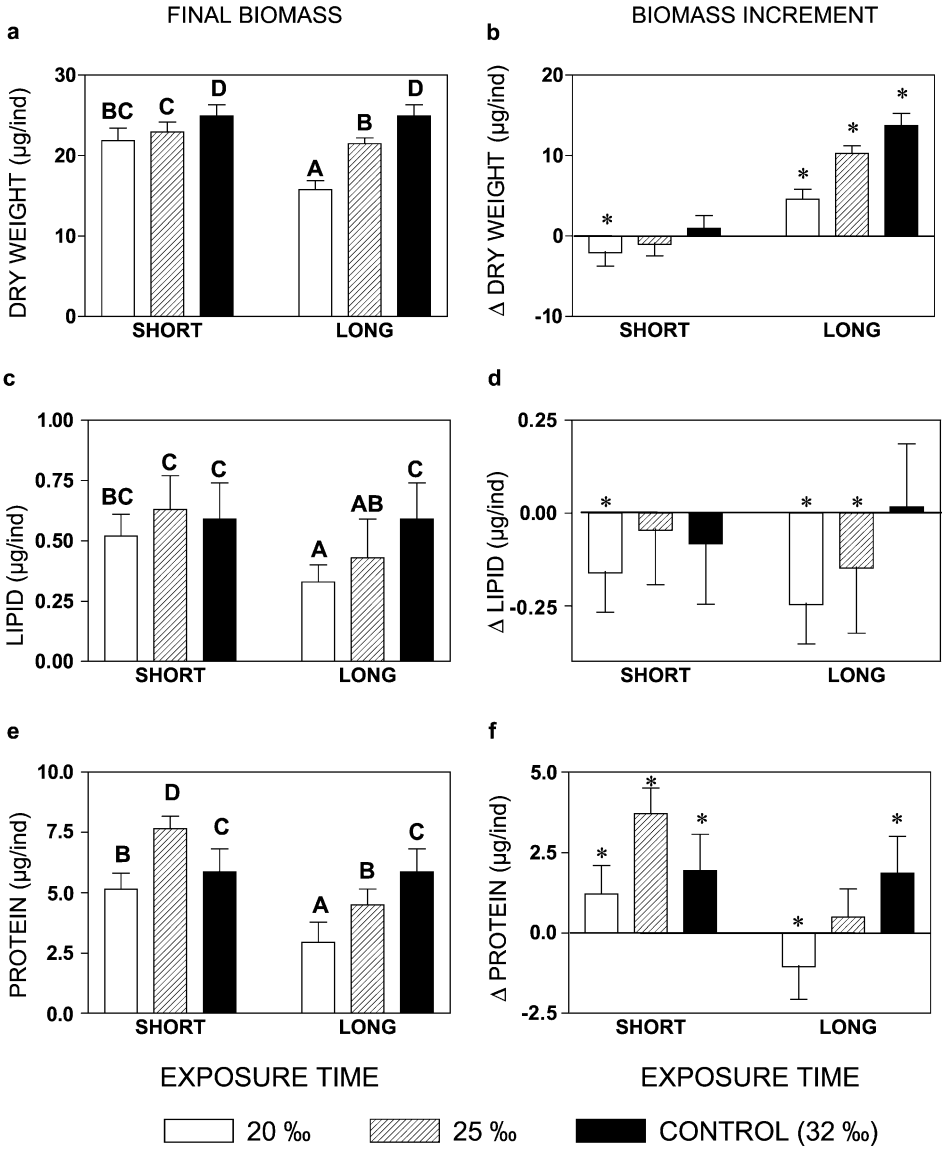

Fig. 2. *C. pagurus*. Larval biomass (dry weight, lipid, protein content per individual) after exposure to different salinities; left column of graphs: final biomass after short or long exposure (SE, LE; see Materials and methods); right column of graphs: increments or decrements in biomass compared with the values at the beginning of the experiments. Different letters or asterisks show significant differences after SNK test (comparison between treatments; left) and planned comparisons (comparison between initial and final values; right).

was lowest (Fig. 2e), due to significant losses since hatching (Fig. 2f). The final protein content after LE to 25‰ was lower than in the control group, without showing a significant increase during the time of the experiment (Fig. 2f).

3.2. H. gammarus

The effect of reduced salinities on DW and biochemical composition depended on the duration of exposure (Table 3).

After SE, the lowest DW was found at 25‰, with only slight differences among treatments (Fig. 3a). Significant decrements in DW occurred at all salinities (Fig. 3b). After LE, the larvae showed the lowest DW at 20‰ while those at 25‰ did not differ significantly from the control (Fig. 3a). LE to 20‰ caused significant DW losses, while significant increments were found at higher salinities (Fig. 3b).

The lipid content showed a similar pattern as DW. After SE to 20‰ and in the control group, the lipid contents were similar, but significantly higher values occurred at 25‰ (Fig. 3c). After long exposure to 20‰, the larvae showed a significantly lower lipid content than in the other treatments (Fig. 3c). Regardless of exposure time, there was an accumulation of lipids at 25‰ and in the control group, while 20‰ allowed for a significant lipid accumulation only during SE (Fig. 3d).

The protein content was generally lower after exposure to reduced salinities than in the control group. After SE to 25‰, the protein content did not differ from the control group, while significantly lower values occurred at 20‰ (Fig. 3e). Significant increments, compared with the initial protein content, were found after SE to 25‰ and in the control group, while an insignificant increase occurred at 20‰ (Fig. 3f). A stronger effect of salinity was found after LE to reduced salinities, with significant differences among all treatments (Fig. 3e). After LE to 20‰, the larvae lost significant amounts of protein, while a significant accumulation occurred in the control group (Fig. 3f).

3.3. C. maenas

Salinity affected the individual DW and lipid, but not the protein content (Table 4, Fig. 4a,c). Exposure time had a significant effect only in DW (Table 4).

Larvae in the control group had a significantly higher DW than those exposed for a short period to 20‰ or 25‰ (Fig. 4a). In these SE experiments, slight but significant loss

Table 3
H. gammarus: Two-factor ANOVA to evaluate differences in the biomass (dry weight, lipids and proteins), among different salinities (20‰ and 25‰) and exposure times

Variable	Factor	dff	MSf	dfe	MSe	*F*	*p*
Dry weight	S	1	6.3E+05	42	21,259	29.4	<0.001
	E	1	4.5E+05	42	21,259	20.9	<0.001
	S×E	1	1.5E+06	42	21,259	69.6	<0.001
Lipid	S	1	6.5E+04	42	855	76.2	<0.001
	E	1	6.0E+04	42	855	69.9	<0.001
	S×E	1	2.0E+05	42	855	23.2	<0.001
Protein	S	1	4.5E+05	42	9215	48.6	<0.001
	E	1	6.0E+05	42	9215	64.9	<0.001
	S×E	1	5.0E+04	42	9215	5.41	<0.05

For symbols see Table 2.

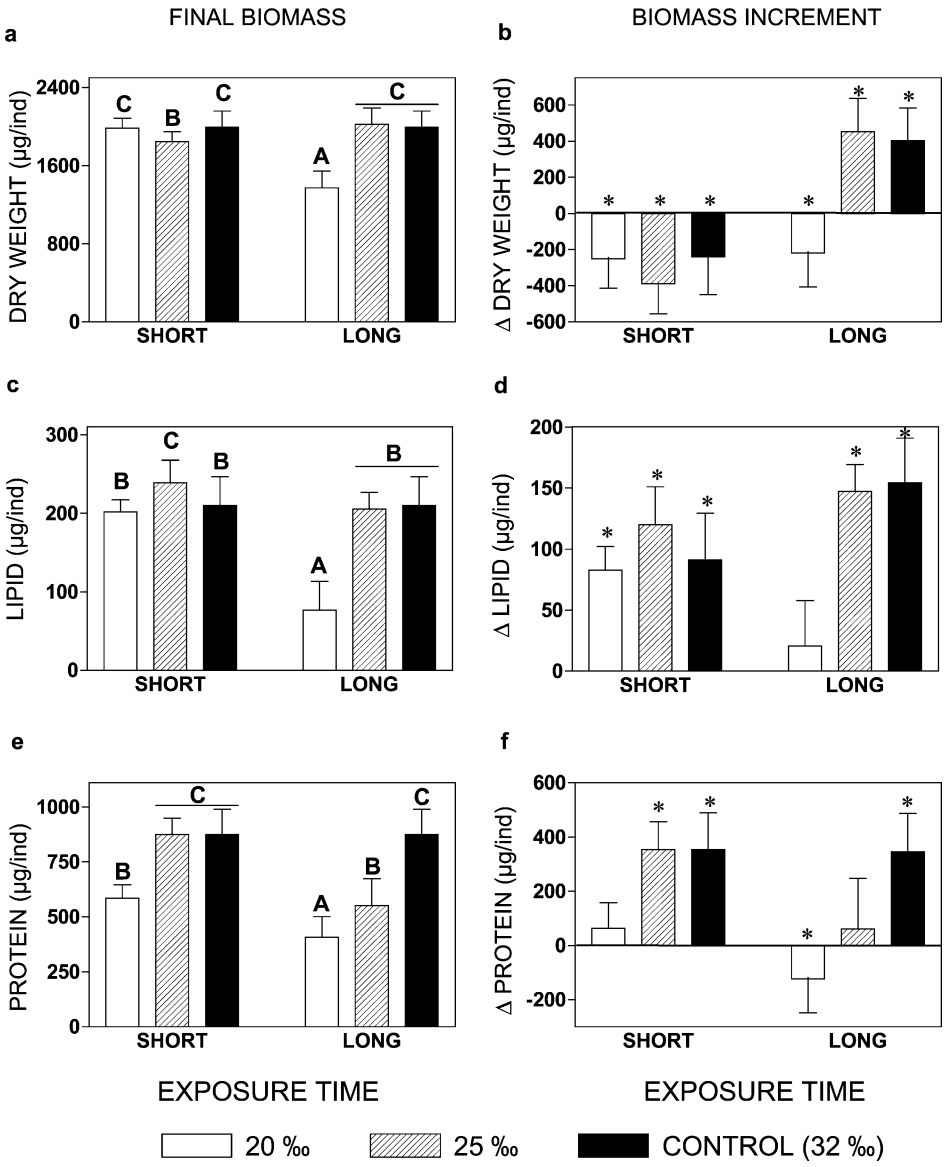

Fig. 3. *H. gammarus*. Differences of biomass between different salinities and treatments. For symbols see Fig. 2.

of DW was found only at 25 ‰ (Fig. 4b). After LE, there were no significant differences in final DW among treatments (Fig. 4a), consistently with a significant increase since hatching (Fig. 4b).

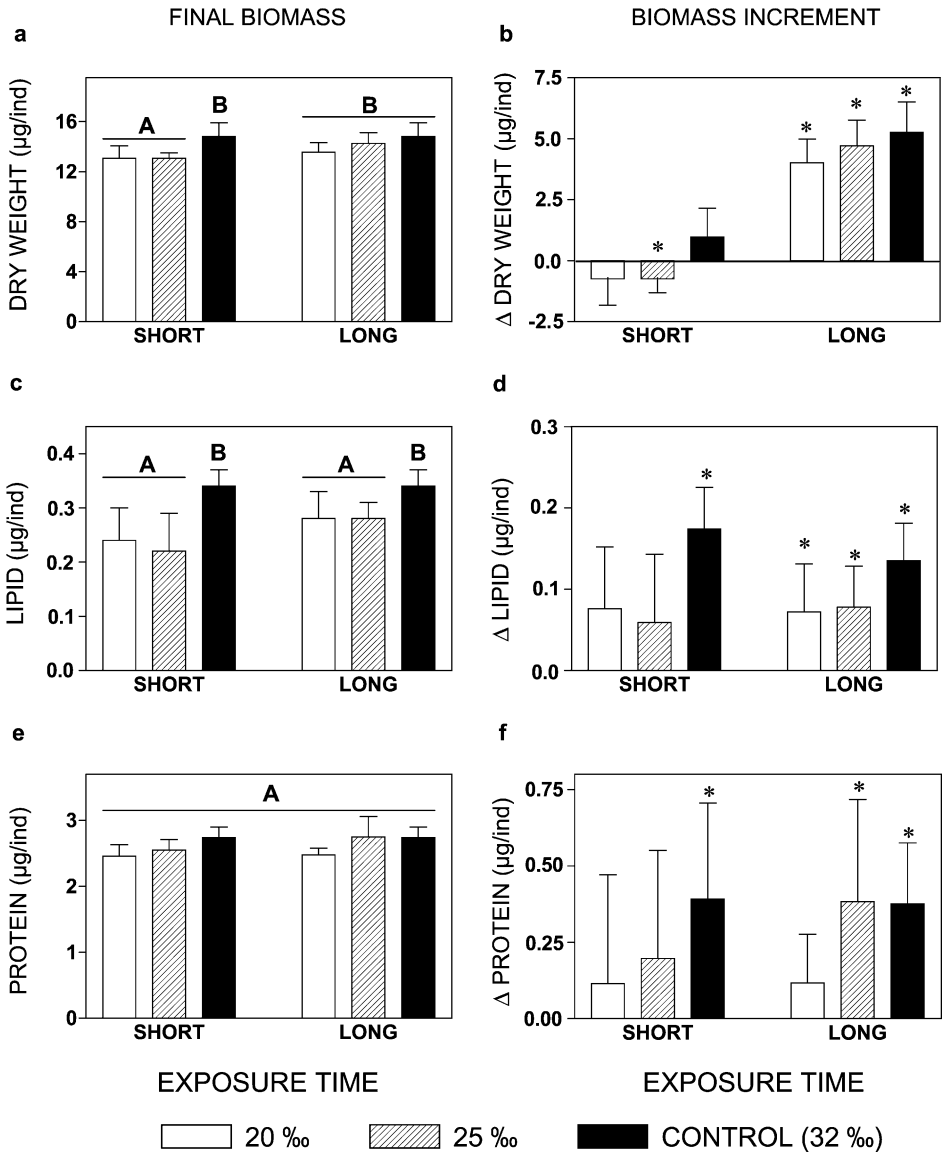

Fig. 4. *C. maenas*. Differences of biomass between different salinities and treatments. For symbols see Fig. 2.

The lipid content was significantly affected by salinity, but not by exposure time: after SE or LE to 20 ‰ or 25 ‰, the larvae showed lower values than in the control (Fig. 4c). There was a significant accumulation of lipids in all treatments except after SE to 20 ‰ or 25 ‰ (Fig. 4d).

Table 4
C. maenas: Two-factor ANOVA to evaluate differences in the biomass (dry weight, lipids and proteins), among different salinities (20‰ and 25‰) and exposure times

Variable	Factor	dff	MSf	dfe	MSe	*F*	*p*
Dry weight	S	1	0.613	20	0.750	0.816	0.377
	E	1	3.403	20	0.750	4.536	<0.05
	S×E	1	0.613	20	0.750	0.816	0.377
Lipid	S	1	1E-04	20	0.0027	0.053	0.820
	E	1	0.011	20	0.0027	4.001	0.059
	S×E	1	6E-04	20	0.0027	0.219	0.644
Protein	S	1	0.152	20	0.037	4.059	0.058
	E	1	0.060	20	0.037	1.620	0.218
	S×E	1	0.041	20	0.037	1.104	0.306

For symbols see Table 2.

The final protein content was not affected by salinity (Fig. 4e); significant accumulation of protein was found in the control group and after LE to 25‰ (Fig. 4f).

3.4. *C. granulata*

In *C. granulata*, 15‰ instead of 20‰ was used as the lowest salinity to test for effects of severe hypo-osmotic stress (see Materials and methods). Individual DW and lipid content were in this species affected by salinity; however, there was no effect on the protein content (Table 5).

The effect of salinity on DW was independent of exposure time (Table 5). Larvae exposed to 15‰ showed a significantly lower DW than those at 25‰ (Fig. 5a). After SE, there was no significant loss in DW, and after LE increased significantly at all salinities (Fig. 5b).

The effect of salinity on the lipid content depended on the exposure time (Table 5). After SE to 15‰ or 25‰ the lipid content was significantly lower than in the control

Table 5
C. granulata: Two-factor ANOVA to evaluate differences in the biomass (dry weight, lipids and proteins), among different salinities (20‰ and 25‰) and exposure times

Variable	Factor	Dff	MSf	dfe	MSe	*F*	*p*
Dry weight	S	1	5.446	43	1.282	4.250	<0.05
	E	1	2.319	43	1.282	1.810	0.186
	S×E	1	0.749	43	1.282	0.585	0.449
Lipid	S	1	0.121	43	0.011	11.354	<0.01
	E	1	0.015	43	0.011	1.379	0.247
	S×E	1	0.078	43	0.011	7.356	<0.05
Protein	S	1	0.039	43	0.335	0.115	0.736
	E	1	0.217	43	0.335	0.647	0.426
	S×E	1	0.112	43	0.335	0.334	0.566

For symbols see Table 2.

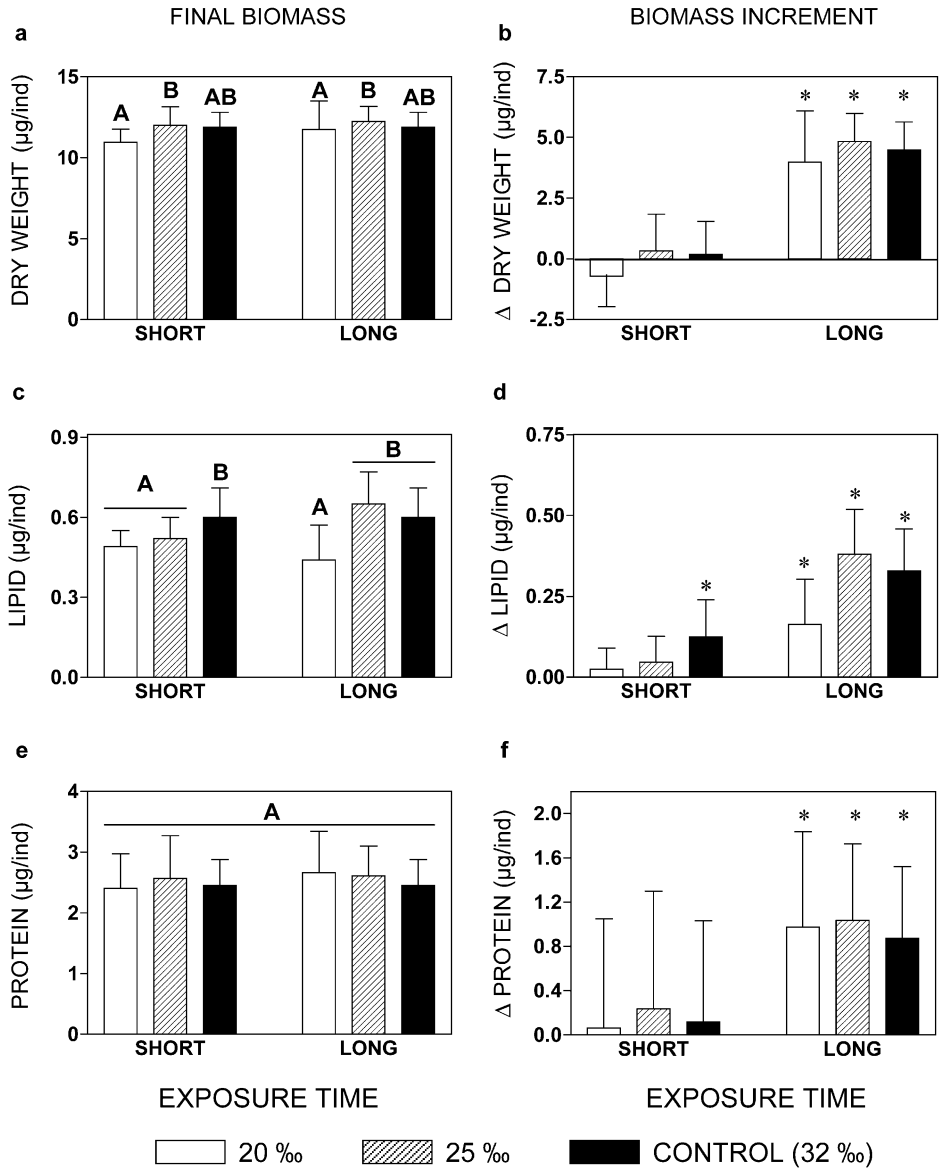

Fig. 5. *C. granulata*. Differences of biomass between different salinities and treatments. For symbols see Fig. 2.

group (Fig. 5c). After LE, the lowest lipid content was found at 15‰, while no significant differences were detected between the 25‰ treatment and the control group (Fig. 5c). Significant increments in lipid were found in the control group of the SE experiment and at all salinities after LE (Fig. 5d).

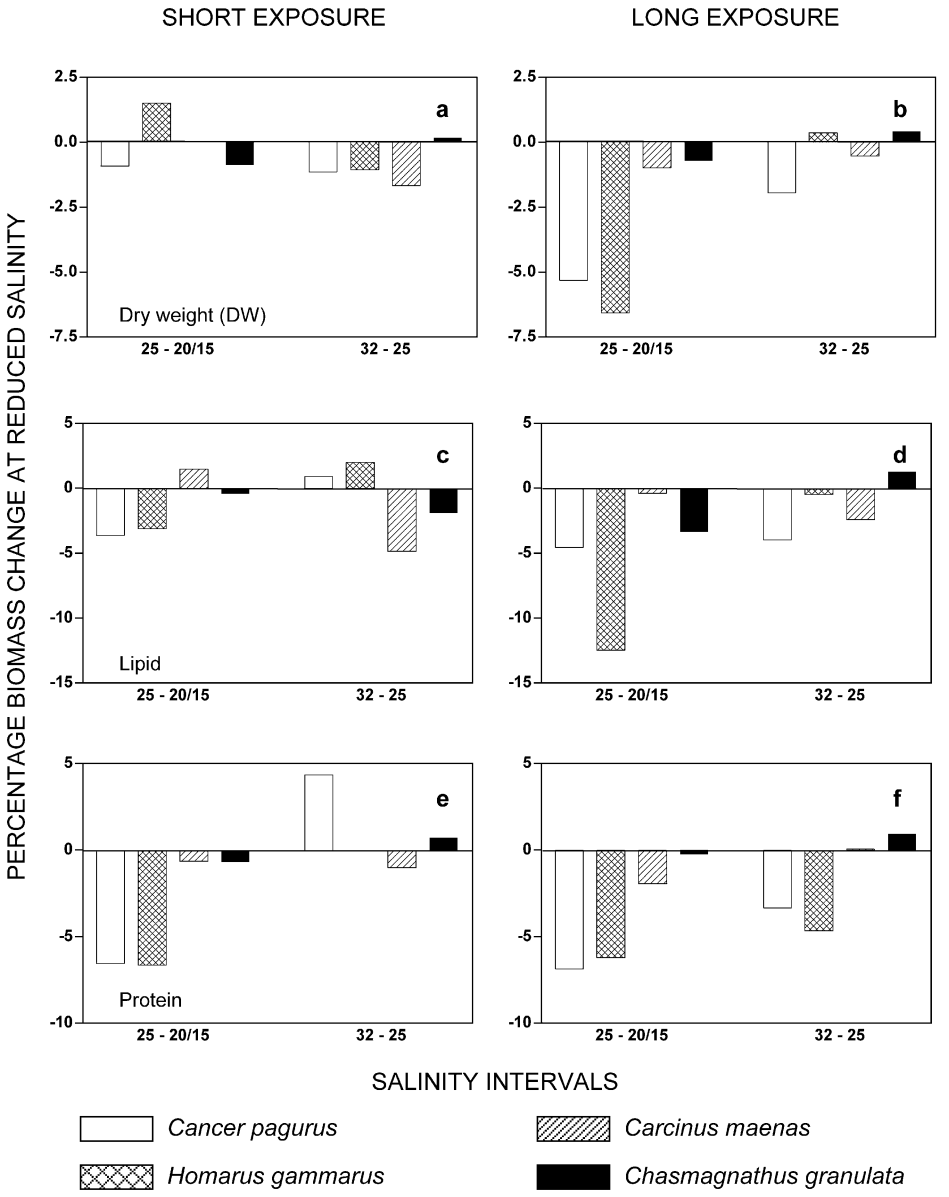

Fig. 6. Biomass changes in the zoea 1 of four decapod species exposed to different salinities expressed as a percentage of biomass (reference base: biomass at the higher salinity) per ‰ of salinity reduction.

The protein content was not affected by salinity (Fig. 5e). Significant accumulation of protein was detected in all groups after long exposure, but not after short exposure (Fig. 5f).

3.4.1. Sensitivity to reduced salinity: interspecific comparisons

Fig. 6 shows the biomass-specific effect of reduced salinity for each species; it is calculated as:

$$(B_L - B_H) \cdot 100/B_H \cdot (S_H - S_L)$$

where B_H is individual biomass (DW, lipid or protein content) measured at the end of the experiment at the higher salinity (S_H) and B_L is the biomass at the lower salinity (S_L) for an interval of salinities (S_H–S_L). The dimension is a percentage change of biomass (reference base: biomass at the higher salinity) per ‰ of salinity reduction.

After SE, there were no a clear patterns of biomass specific-change. *C. pagurus* and *H. gammarus* showed generally the largest variations of biomass, with losses exceeding 6% per ‰ of salinity reduction (Fig. 6).

After LE, biomass-specific change was larger than in the SE (Fig. 6). The largest variations were again found in *C. pagurus* and *H. gammarus*, especially in the interval 20–25 ‰. An exception occurred in the interval 25–32 ‰ where *H. gammarus* exhibited only small variations in DW and lipid. *C. maenas* and *C. granulata* showed consistently small biomass changes in all intervals.

4. Discussion

The effect of reduced salinities on the biochemical composition (lipid and protein content, in μg/ind) of early decapod crustacean larvae depends on the species, the extent of salinity reduction, and the time of exposure. After short exposure (SE, 10% of zoea 1 moult-cycle duration) there was no clear pattern of change in dry weight or biochemical composition. The effect, if statistically significant at all, was generally small. Clearer patterns appeared after long exposure (LE, 50% of zoea 1 moult-cycle duration). This may be a consequence not only of longer exposure time but also due to an interaction with the moulting cycle. In LE experiments, the larvae were exposed to reduced salinities from postmoult (stage A of Drach's classification system; see Drach, 1939) through intermoult (stage C); in SE experiments, by contrast, the exposure occurred exclusively during the stage C of the moulting cycle. During postmoult and early intermoult, a strong accumulation of biomass occurs in decapod crustacean larvae (Anger, 1991; Anger and Ismael, 1997; Anger et al., 1998). Significantly reduced rates of growth and survival have been detected when decapod crustacean larvae were exposed during the initial stages of the moulting cycle to osmotic or nutritional stress (Anger and Dawirs, 1982; Dawirs, 1986; Anger, 1987; Anger et al., 1998; Giménez, 2000).

After LE, stenohaline osmoconformers (in our experiments, larvae of *C. pagurus* and *H. gammarus*) showed a stronger response in their DW, lipid and protein content than euryhaline osmoregulators (*C. maenas*, *C. granulata*). Zoea 1 larvae of *C. pagurus* showed great variations in DW, lipid and protein content, and there were important biomass losses at low salinities (Fig. 2). Likewise, the osmoconforming zoea 1 of *H. gammarus* (see Charmantier et al., 2001) showed lower DW, lipid and protein contents at reduced salinities. This response was weaker than in *C. pagurus*, but stronger than in the

zoea 1 of *C. maenas*, where low salinities caused DW and lipid losses but no effects in the protein content (Fig. 4). Calculations of biomass-specific changes of growth in the zoea 1 of a stenohaline osmoconforming spider crab, *H. araneus*, reared at 20‰, 25‰ and 32‰ (data from Pfaff, 1997; Table 6) show a higher sensitivity of this species than in either *C. maenas* or *C. granulata*, similarly as in *H. gammarus*. The hyper-regulating zoea 1 of *C. granulata* (see Charmantier et al., 2002), in contrast, showed a biomass increase during all experiments, indicating that its sensitivity to reduced salinity was low. The increment in DW and lipid was smaller compared with the control, while the protein content did not change (Fig. 5). Unlike *C. maenas*, the zoea 1 of *C. granulata* is able to complete its first larval stage at a salinity as low as 10‰ (Giménez, 2000).

Effects of salinity on larval biomass may have occurred either due to metabolic disadjustments induced by osmotic stress or due to physiological strategies to face such stress. Osmotic stress may cause for instance, functional or structural damage at the protein, cell or tissue levels, critical variations in the metabolic rate, or disharmonizing effects on mechanisms of organismic integration (Kinne, 1971). Physiological strategies, on the other hand, consist of isosmotic intracellular ion regulation (IIR) and anisosmotic extracellular osmoregulation (AER) (for review see Schoffeniels and Gilles, 1970; Péqueux, 1995). In IIR, which occurs in both osmoconformers and osmoregulators, the amino-acid metabolism is involved. In AER, which occurs exclusively in osmoregulating species, a passive mechanism (Donnan effect) and an active mechanism (active transport of ions) are involved (Schoffeniels and Gilles, 1970; Péqueux, 1995). In *C. pagurus* larvae, which presumably are osmoconformers, the biochemical composition may have been affected primarily by disturbance of the water and mineral balance and of the amino-acid metabolism. Under osmotic stress, conversion efficiencies might have decreased below a level where metabolic maintenance required the utilization of lipid and/or protein reserves, explaining negative growth. In the osmoconforming zoea 1 of *H. araneus*, low salinity affected significantly the lipid but not the protein content (Pfaff, 1997). Protein losses in *C. pagurus* may reflect a yet higher sensitivity to osmotic stress. The disturbance

Table 6
H. araneus: Dry weight, lipid and protein content, increments between zoea 1 at hatching and after 4 days at three salinities (20‰, 25‰ and 32‰) (source: Pfaff, 1997), and biomass specific changes per ‰ of salinity variation (BSC)

Time salinity	Day 0 32‰	32‰ (control)	Day 4 25‰	20‰
Dry weight				
µg/ind	73.33	136.29	116.39	105.28
ΔDry weight		62.96	42.06	31.95
BSC (%)			1.99	2.08
Protein				
µg/ind	3.82	9.71	8.15	6.40
Δ proteins		5.89	4.33	2.58
BSC (%)			2.30	4.29
Lipid				
µg/ind	5.21	7.13	7.00	6.08
ΔLipids		1.92	1.79	0.87
BSC (%)			0.26	2.63

of the mineral balance appears to be weaker in the osmoconforming zoea 1 of *H. gammarus*, where no negative growth occurred. In *C. granulata*, the zoea 1 is capable of extracellular osmoregulation (Charmantier et al., 2002). This process may have prevented major effects on biochemical composition. The energy necessary for this process must have originated primarily from the catabolism of lipids, making protein degradation unnecessary. The same explanation should apply to the strongly osmoregulating zoea of *A. miersii*, whose carbon content was shown to vary only slightly in a very wide range of salinities (15–45 ‰; Anger et al., 2000), and in those of *C. maenas*, where salinity affected DW and the lipid content but not the protein fraction of biomass. The fact that the zoea 1 of this species is able to survive for several days at 15 ‰ but not to moult to the zoea 2 (Anger et al., 1998) indicates a limited euryhalinity, and some osmoregulatory capacity. The zoea 1 of *C. maenas* may thus be a weak osmoregulator, intermediate in strength between *C. granulata* and *H. gammarus*.

Effects of salinity have potentially important implications for individual fitness and, in consequence, for population dynamics. Our results suggest that the fitness of osmoconforming larvae may be negatively affected even by moderately reduced salinities, as biomass losses make them more vulnerable to food stress and predation. Additionally, stressed larvae usually take longer to pass through subsequent larval stages. Dawirs (1986) suggested that delayed development under nutritional stress is a consequence of a prolonged feeding time necessary to meet enhanced metabolic demands or to replenish lost reserves; similar effects may occur under salinity stress. The individual fitness of osmoregulating larvae, by contrast, should remain rather constant under moderately reduced salinities. In estuaries or coastal waters, influenced by river discharge, detrimental effects of salinity may occur. Fluctuations in salinity, due to effects of tidal cycles or wind stress on river discharge, should particularly affect *C. pagurus* and *H. gammarus*, since our laboratory experiments showed that they are sensible to reductions in salinity even in a short exposure time (16 h). In addition, heavy rainfalls may drop salinity in coastal waters affecting larval survival, as suggested for polychaetes and barnacles (Qiu and Qian, 1997, 1999). If rainfalls drop salinity during some days, a detrimental effect of osmotic stress on growth of *C. maenas* and *C. granulata* should be expected. In particular, for species such as *C. granulata* whose larvae are released in coastal lagoons, detrimental effects of low salinity on growth may be very important if sand bars obstruct the lagoon inlet (see Giménez, submitted for discussion). Finally, interannual changes in climatic conditions may change salinity and thus lead to interannual variability in larval survival and growth.

In summary, our results suggest a relationship between the osmoregulatory capacity of decapod larvae and the response of dry weight, lipid, and protein to reduced salinity. In osmoconforming species like *H. araneus*, *H. gammarus*, and, most probably in *C. pagurus*, the sensitivity to salinity changes tended to be high, in some cases leading to negative growth. In osmoregulators (*A. miersii*, *C. granulata*, and possibly *C. maenas*) growth remained always positive. The response was mostly stronger when the larvae were exposed to hyposaline stress for a few days instead of several hours only. Future studies with species whose osmoregulatory capacity is already known should further analyse the relationships between osmoregulatory capacity, larval sensitivity to salinity changes (eury- or stenohalinity), and changes in feeding, growth, and biochemical composition.

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