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Ontogeny of Osmoregulation and Salinity Tolerance in *Cancer irroratus;* Elements of Comparison with *C. borealis* (Crustacea, Decapoda)

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Abstract. Osmoregulation and salinity tolerance were studied in zoeae, megalopae, first crab stage (osmoregulation only), and adults of *Cancer irroratus,* and in zoeae and adults of *C. borealis.*

In *C. irroratus,* salinity tolerance was moderate in zoeae, decreased in late zoeae 5, was at a minimum in megalopae, and increased in adults. The lower and upper lethal salinities for 50% of the animals (48 h LS 50) at 15°C were about 13–17‰/42–50‰ in zoeae, 24‰/37‰ in megalopae, and 8.5‰/65‰ in adults.

In *C. borealis,* the corresponding values of LS 50s were 16–20‰/46–50‰ in zoeae and 12‰/65‰ in adults.

In both species, zoeae were hyper-osmoconformers; adults were isosmotic in high salinities and slightly hyper-regulators in low salinities. In *C. irroratus,* the change from larval to adult type of regulation occurred from megalopa (hyper-osmoconformer) to first crab stage (hyper-regulator in dilute media), *i.e.,* after the completion of metamorphosis.

Osmoregulation and salinity tolerance appear correlated and are modified at metamorphosis. These results are discussed with an emphasis on the effects of metamorphosis on osmoregulation of developing decapods.

Introduction

While osmoregulation has been extensively studied in adult crustaceans (review in Mantel and Farmer, 1983), relatively few data are available on larval or post-larval osmoregulation, in the following species: *Rhithropanopeus harrisii* (Kalber and Costlow, 1966), *Cardisoma guanhumi* (Kalber and Costlow, 1968), *Callinectes sapidus, Hepatus epheliticus, Libinia emarginata* (Kalber, 1970), *Sesarma reticulatum* (Foskett, 1977), *Clibanarius vittatus* (Young, 1979), *Callianassa jamaica* (Felder *et al.,* 1986), *Macrobrachium petersi* (Read, 1984), *Uca subcylindrica* (Rabalais and Cameron, 1985), *Homarus americanus,* and *Penaeus japonicus* (Charmantier, 1986; Charmantier *et al.,* 1984a, 1988).

For different reasons, including culture difficulties in late larval stages, osmoregulation was frequently studied in larval stages only, particularly in brachyuran crabs. However, in the four latter species, osmoregulation was studied throughout the post-embryonic development including post-metamorphic stages, thus osmoregulation in larvae, postlarvae, and adults could be compared. The ability to osmoregulate did not change along the development in some species. In other species, metamorphosis marked the appearance of the adult type of regulation; among brachyurans, this case has been so far documented in only one species, *U. subcylindrica,* the adults of which are strong hyper-hypo-regulators (Rabalais and Cameron, 1985).

Consequently, one of the objectives of this study was to determine whether comparable changes in osmoregulation at metamorphosis exist in brachyurans with lower abilities to osmoregulate, particularly in species of the genus *Cancer,* which slightly hyper-regulate in low salinities. Experiments designed to study the ontogeny of osmoregulation were performed on the most abundant *Cancer* species of the Canadian East coast, the rock crab *Cancer irroratus* Say 1817. Comparative data were also obtained from some developmental stages of a sympatric species, the Jonah crab *Cancer borealis* Stimpson, 1859.

Numerous studies have dealt with the tolerance to salinity of crustacean larvae and postlarvae, but the corre-

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lation between the salinity tolerance of different developmental stages and their corresponding osmoregulatory capabilities has only been experimentally investigated in a few species, *U. subcylindrica* (Rabalais and Cameron, 1985), *H. americanus* and *P. japonicus* (Charmantier *et al.*, 1988).

Thus, the second objective of this study was to determine the salinity tolerance of larval, postlarval, and adult stages of *C. irroratus* (and, for the purpose of comparison, of *C. borealis*), and to attempt to correlate their osmoregulatory abilities and their salinity tolerance. This is of particular interest in *Cancer* species, the larvae of which are submitted to different patterns of salinity changes: in *C. irroratus,* zoeae hatch offshore and, as development proceeds, late larvae and postlarvae are found nearer to shore (Sandifer, 1973).

In both species, the early post-embryonic development comprises one prezoea, five zoeal stages, one megalopal stage, and several early crab stages (Sastry, 1977a, b). Following a wide acceptance, zoeae are larval stages, and the following stages, beginning with the megalopa, are considered postlarvae (Felder *et al.*, 1985).

In *C. irroratus,* osmoregulation has been studied in adults and large juveniles (Thurberg *et al.*, 1973; Cantelmo *et al.*, 1975; Neufeld and Pritchard, 1979), and preliminary data on osmoregulation are also available in the early post-embryonic stages (Charmantier *et al.*, 1989).

Materials and Methods

Animals

Adult *C. irroratus* and *C. borealis* were caught by SCUBA diving in summer and autumn in Passamaquoddy Bay and transferred to the culture facility at the Biological Station, St Andrews, New Brunswick, Canada. They were kept in tanks supplied with running seawater ($S \cong 29$–$32‰$; $T \cong 12°C$) under natural photoperiod, and were fed cod, squid, and shrimp (*Pandalus borealis*). In September, two weeks before the experiments, four groups of crabs were selected: large and small *C. irroratus* [cephalothoracic width (CTW): 91 to 115 mm and 45 to 70 mm, respectively], and large and small *C. borealis* (CTW: 80–110 mm and 57–71 mm). Males and females were equally represented in each group. They were transferred to 250-l tanks with charcoal-filtrated recirculated seawater kept at 15°C. Only animals in molt stages C or Do (according to the nomenclature of Drach, 1939) were retained for survival and osmoregulatory experiments.

Larvae of both species were obtained in spring and summer from some of the aforementioned crabs. After hatching, larvae were transferred to 40-l planktonkreisels (Hughes *et al.*, 1974) supplied with flow-through seawater at a salinity of 29–32‰ under natural photoperiod. The planktonkreisels, normally used for culturing lobster larvae, were modified for the culture of crab larvae. Seawater was filtered to 50 μm during the zoea development then suppressed; flow-rate was set at 2.5–3 l min^{-1} from zoeae 1 to early megalops, then at 1.–1.5 l min^{-1}; a 280 μm mesh screen was used around the overflow system. Water temperature was set at 15°C during the zoeal development, then at 19°C. Cephalothoracic length was about 0.56 mm in zoeae 1, 1.5 mm in zoeae 5, 2.2 mm in megalopae, and CTW was 2.3 mm in first crab stage (Charmantier-Daures and Charmantier, 1991). Crab larvae were fed three times a day with live *Artemia* nauplii. Larvae of *C. irroratus* were cultured to the second crab stage, and those of *C. borealis* to the third zoea. As each larval stage lasts several days, molting stages were obtained according to the time elapsed from the preceeding molt, and three groups of animals, postmolt stage A, stage C, and premolt stage D, were selected.

Preparation of media

Experimental media were prepared in compartmented 250-l tanks for adults and 0.5-l plastic containers for larvae and young crabs. Dilute media were prepared by adding tap water to seawater, and high salinity media were prepared by adding "Instant Ocean Synthetic Sea Salts" (Aquarium Systems, Inc.) to seawater. All experiments were conducted at 15°C. Salinities were expressed according to the osmotic pressure in mosm · kg^{-1}, and to the salt content in the medium in ‰. A value of 3.4‰ is equivalent to 100 mosm · kg^{-1}. Osmotic pressure was measured with an Advanced Instruments 31 LA or Wescor 5000 osmometer, and salinity on a YSI 33 salinometer.

Survival bioassays

Due to the small number of available animals, salinity tolerance in adults was evaluated only from the number of surviving and dead animals in media of different salinities. Adult crabs were progressively adapted from seawater to diluted or concentrated media by adding freshwater or Instant Ocean salts to the original medium; each change of 100 mosm · kg^{-1} in the salinity required about 24 h. Between two changes of salinity, they were kept for two days at constant salinity in each test medium, which differed from one another by increments of 100 mosm · kg^{-1} ($\cong 3.4‰$).

Acute static 48–96 h bioassays were conducted with zoeae and megalopae held in test media ranging from 100 mosm · kg^{-1} to seawater ($\cong$900–1000 mosm · kg^{-1}) and to 1600 mosm · kg^{-1}, and differing by increments of 100 mosm · kg^{-1}. Each bioassay was run on a group of 10 individuals and replicated. Animals were counted and dead animals removed at 0.5, 1, 3, 6, 12, 24, 36, 48, 72, 96 h according to the prescriptions of Sprague (1969) in toxicity studies. The criteria for death were total lack of

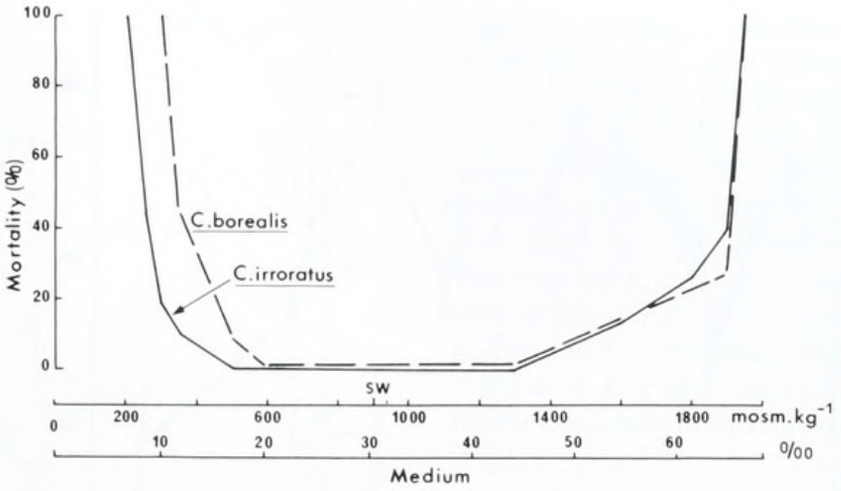

Figure 1. Salinity tolerance in adult *Cancer irroratus* and *C. borealis* at 15°C. Percent mortality of animals according to the salinity of the medium. Number of animals at the start of the experiments in seawater (SW): *C. irroratus:* 16 (to low salinity media) and 8 (to high salinity media); *C. borealis:* 19 and 7.

movement, immobility of appendages and heart, and lack of response after repeated touches with a probe. Median lethal salinities (LS 50) and 95% confidence intervals were calculated by techniques of probit analysis (Lichtfield and Wilcoxon, 1949; Finney, 1962) computerized on the Letcur program (Zitko, 1982; Lieberman, 1983). LS 50s were calculated at 24, 48, and 96 h. Survival bioassays were not run in first and second crab stages due to the small number of available animals.

Osmoregulation

The hemolymph was collected from adult crabs via a hypodermic needle inserted through the articulation membrane at the basis of the fourth or fifth pereiopods. At least seven days elapsed between hemolymph samples were taken from the same animal.

Zoeae, megalopae, and young crabs were quickly dried on filter paper and immersed in mineral oil to avoid evaporation and desiccation. The hemolymph was then sampled with a glass micropipette inserted in the heart.

Osmotic pressure of hemolymph was measured on an Advanced Instruments 31 LA or Wescor 5000 osmometer (adults) or on a Kalber-Clifton micro-osmometer, with reference to the osmotic pressure of the medium (young stages). Student *t* tests were used for statistical comparisons.

Results

Salinity tolerance

The ability of *C. irroratus* and *C. borealis* to tolerate low and high salinities varied with post-embryonic development.

Adults of *C. irroratus* survived without mortality in media ranging from 500 mosm · kg^{-1} to 1300 mosm · kg^{-1} (≅17‰ to 44‰). The LS 50 s were about 250 and 1900 mosm · kg^{-1} (≅8.5 and 65‰). In adult *C. borealis,* no mortality was observed between 600 and 1300 mosm · kg^{-1} (≅20.4 and 44‰), and LS 50s were about 350 and 1900 mosm · kg^{-1} (12 and 65‰) (Fig. 1). No difference in salinity tolerance was detected between large and small crabs of either species.

In larvae and postlarvae of *C. irroratus* the 48 h LS 50 in low salinity media varied around 450 ± 60 mosm · kg^{-1} (≅15 ± 2‰) in zoeal stages 1 to 4 and early 5, then increased from the end of stage zoea 5 through early megalopae (≅600 mosm · kg^{-1}, 20‰) to a highly significant maximum value (corresponding to a minimum tolerance) of 700 mosm · kg^{-1} (≅24‰) in intermolt megalopae. The 24 h and 96 h LS 50s were, respectively, generally lower and higher than the 48 h value but followed the same pattern of variation. Maximum LS 50s at 24, 48, and 96 h occurred in megalopae with respective values of 520, 700, and 820 mosm · kg^{-1} (18, 24, and 28‰), differing significantly from one another.

In high salinity media, the 48 h LS 50 of *C. irroratus* young stages varied around 1350 ± 120 mosm · kg^{-1} (≅46 ± 4‰) in zoeal stages 1 to 5, then decreased in early megalopae (≅1240 mosm · kg^{-1}, ≅42‰) to a highly significant minimum value of 1100 mosm · kg^{-1} (≅37‰) in intermolt megalopae. The 24 h and 96 h LS 50s were respectively higher and lower than the 48 h value; they followed the same pattern of variation, decreasing to minima of 1150 mosm · kg^{-1} (24 h: 39‰) and 1000 mosm · kg^{-1} (96 h: 34‰) in megalopae (Fig. 2).

In zoeae of *C. borealis,* the 48 h LS 50 varied around 530 ± 60 mosm · kg^{-1} (≅18 ± 2‰) in low salinities. The 24 h and 96 h LS 50s were markedly lower and higher than the 48 h LS 50. The differences between 96 h and 24 h LS 50s were more important than in *C. irroratus:* in

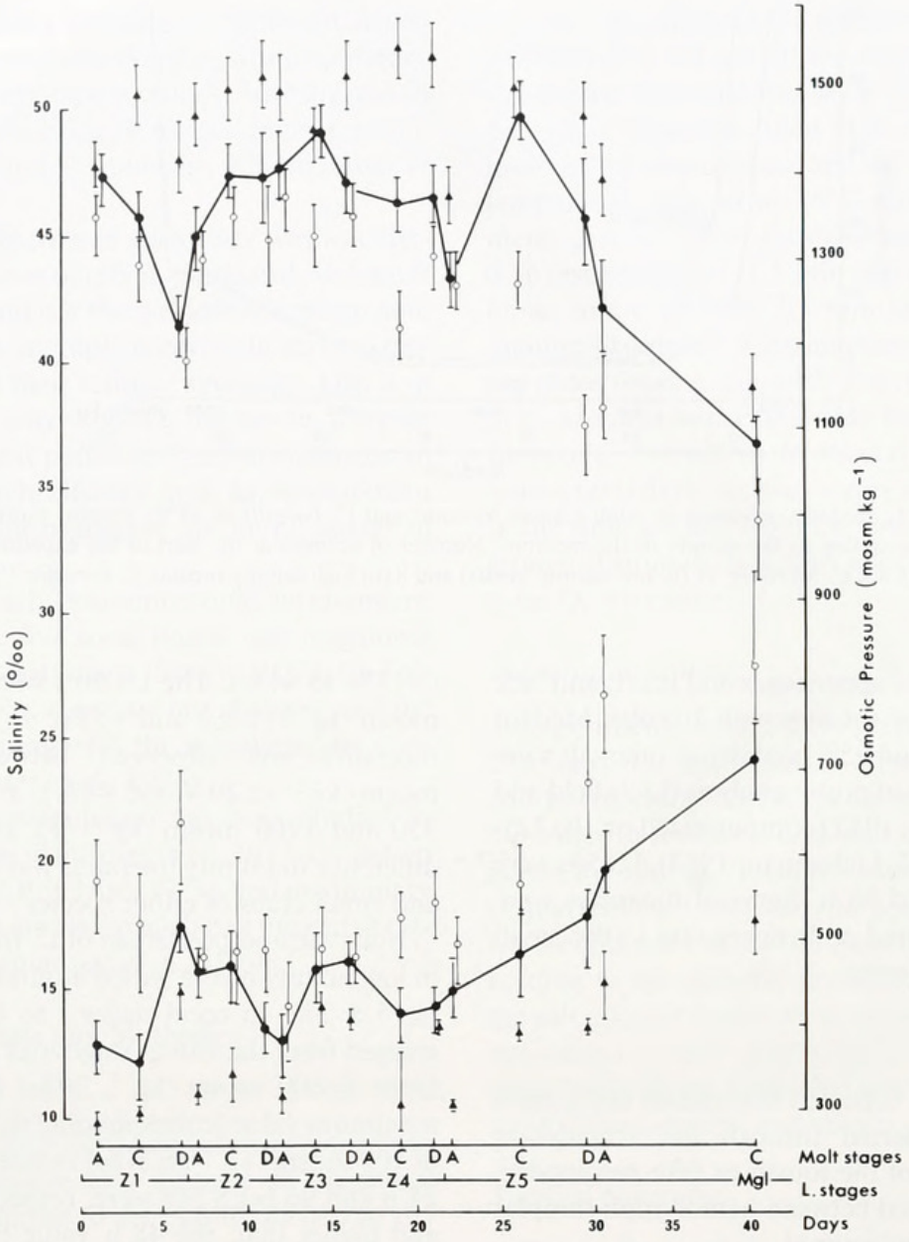

Figure 2. Salinity tolerance in zoeae 1–5 and megalopae of *Cancer irroratus* at 15°C. Variations in LS 50 in ‰ and mosm · kg^{-1} according to larval and molt stages and to days of development, in high and low salinities (upper and lower traces). Each point represents the mean value of at least two determinations from 10 animals, with 95% confidence interval. Closed triangles: 24 h LS 50. Closed circles: 48 h LS 50; open circles: 96 h LS 50.

some instances (early zoea 1 and zoea 2), the 24 LS 50 was about 300 mosm · kg^{-1} (10‰), the 96 h LS 50 reaching about 740 mosm · kg^{-1} (25‰). In high salinities, the 48 h LS 50 varied around 1420 ± 60 mosm · kg^{-1} (≅48 ± 2‰); 24 h and 96 h LS 50s were respectively higher and lower (Fig. 3).

Osmoregulation

Adaptation time. The time of adaptation after a change in the environmental salinity was evaluated in stage C zoeae 1 and 5 and in adults of *C. irroratus*. After a rapid transfer from seawater at 920 mosm · kg^{-1} (31‰) to a dilute medium of 500 mosm · kg^{-1} (17‰), the hemolymph osmotic pressure stabilized within 1 to 2 h in zoeae 1 and 5. In adults transferred from seawater to a dilute medium of 677 mosm · kg^{-1} (23‰), the corresponding time was 24 h (Fig. 4). The time of osmotic adaptation to concentrated media was not tested; in other species, it was shorter than the time of adaptation to dilute media (Charmantier *et al.*, 1988). In all subsequent experiments and in both species, we kept the young stages 6–24 h and the adults 3–4 days in each medium before sampling.

Osmoregulation. Adults of *C. irroratus* and *C. borealis* were almost osmoconformers in high salinities and

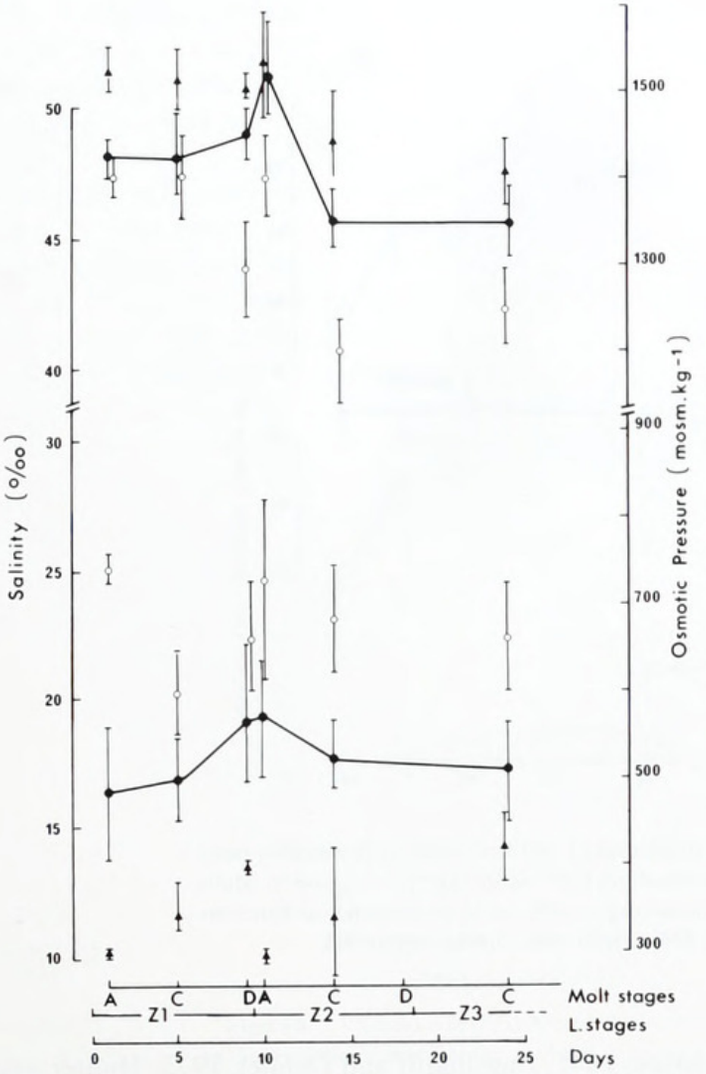

Figure 3. Salinity tolerance in zoeae 1–3 of *Cancer borealis* at 15°C. Variations in LS 50 in ‰ and mosm · kg^{-1} according to larval and molt stages and to days of development, in high and low salinities (upper and lower traces). Each point represents the mean value of two determinations from 10 animals, with 95% confidence interval. Closed triangles: 24 h LS 50; closed circles: 48 h LS 50; open circles: 96 h LS 50.

seawater, and their regulation was slightly hyper-osmotic in dilute media (Fig. 5). No difference of hyper-regulation was detected between large and small adults in *C. borealis*. In *C. irroratus* large crabs were significantly stronger regulators than small crabs (hemolymph-medium differences of 71 ± 7 and 55 ± 13 mosm · kg^{-1} respectively, $P < 0.005$, in a 500 mosm · kg^{-1}; 17‰, medium). The ability to hyper-regulate in dilute media was significantly higher in *C. irroratus* than in *C. borealis* (in large crabs, hemolymph-medium differences of 71 ± 7 and 37 ± 5 mosm · kg^{-1}, $P < 0.001$, in a 500 mosm · kg^{-1}, 17‰, medium).

Zoeae 1 to 5 of *C. irroratus* in molting stage C hyper-osmoconformed at almost all tested salinities, *i.e.*, their hemolymph osmotic pressure varied as a function of external osmotic pressure but remained above external by about 15–50 mosm · kg^{-1}; at the lowest tested salinity (300 mosm · kg^{-1}), zoeae were isosmotic. In some zoeal stages, regulation in dilute media was slightly more hyper-osmotic in premolt (zoeae 1,4 at 500 mosm · kg^{-1}: $P < 0.05$) and less hyper-osmotic in post-molt (zoeae 2, 3, 4 at 500 and 900 mosm · kg^{-1}: $P < 0.01$). Megalopae were also hyper-osmoconformers. The pattern of osmoregulation seemed to change after the completion of metamorphosis. First crab stages were almost osmoconformers in seawater and their regulation was slightly hyper-osmotic in a dilute medium of 500 mosm · kg^{-1}, 17‰ (hemolymph-medium difference of 55 ± 16 mosm · kg^{-1}) (Fig. 6).

Zoeae 1 to 3 of *C. borealis* had the same pattern of hyperosmoconforming regulation as zoeae of *C. irroratus* (Fig. 7).

Discussion

Salinity tolerance

In *Cancer irroratus*, the interval of tolerable salinities tends to decrease at the end of the larval development and is minimum in megalopae. At this stage, the 96 h LS 50s are about 28‰ and 34‰, which means that megalopae are almost restricted to seawater. In adults, approximate 48 h LS 50s are 8.5‰ and 65‰. The wide euryhalinity demonstrated by adults could be partly related to the relatively short time of exposure to the different media and to the progressive adaptation to changing salinities. Long-term exposure to extreme salinities could yield more restrictive results.

These results are in agreement with previous data. Sastry (1970) found that at 15°C, only 5.5% of megalopae of *C. irroratus* molted to the first crab stage in a medium of 15‰, while this molt was successful in a higher percentage of megalopae in media ranging from 20 to 35‰, with a maximum rate of 76% in a medium of 30‰. Complete development from zoea 1 to first crab stage was found possible at 15°C between 20 and 35‰ (Sastry and McCarthy, 1973) or 25 and 35‰, but survival exceeded 50% only in 30–35‰. (Johns, 1981). In adults, McCluskey (1975, cited in Bigford, 1979) found survival was possible for three days at 5–8°C in salinities ranging from 10 to 20‰ (the upper limit was not tested).

Compared to the larvae of *C. irroratus*, zoeae of *C. borealis* were less tolerant to prolonged exposure to low salinities. In *C. borealis*, Sastry and McCarthy (1973) found that complete larval development was only possible in a medium of 30‰ at 20°C. These and our results demonstrate that *C. borealis* is more stenohaline than *C. irroratus*, and, in particular, less tolerant to low salinities during the larval development and in adults.

Adaptation time

In *C. irroratus*, the time of osmotic equilibration in a dilute medium is about 1 to 2 h in larvae and 24 h in

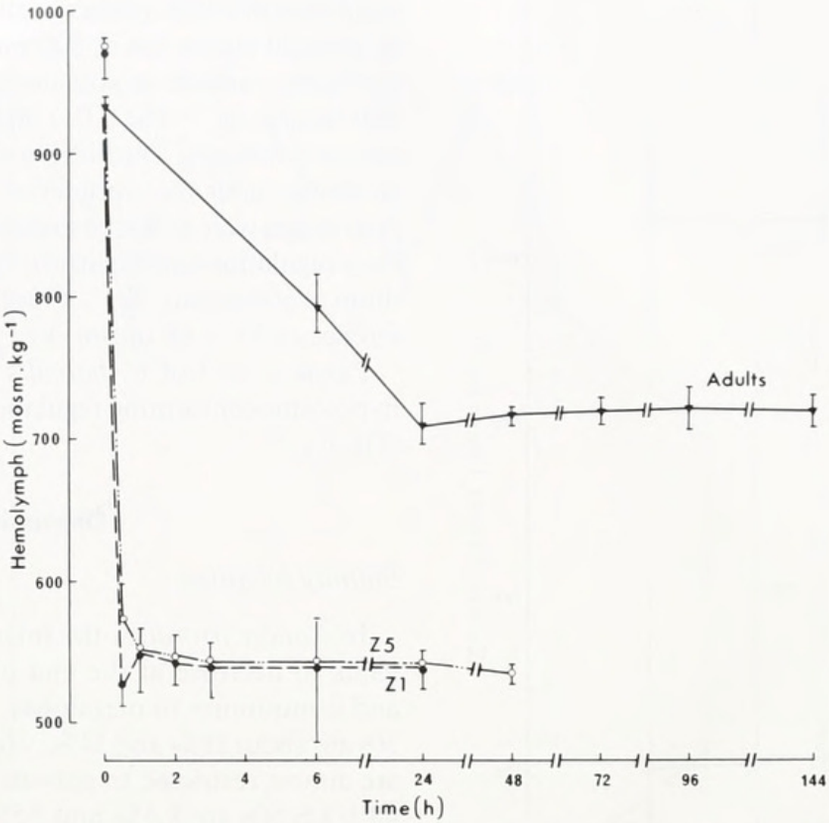

Figure 4. Change in hemolymph osmotic pressure in stages zoeae 1 and 5 of *Cancer irroratus* after rapid transfer from seawater (920 mosm · kg^{-1}, 31‰) to a dilute medium (500 mosm · kg^{-1}, 17‰), and in adults of *C. irroratus* after rapid transfer from seawater to 677 mosm · kg^{-1}, 23‰, at 15°C. Each point represents the mean value of determinations from 3 to 5 zoeae or 5 adults, with 95% confidence interval.

adults. Adaptation time is thus size-dependent, which could be related to differences in the volumes of water and ion exchanges and to differences in tegument permeability between development stages. These times of adaptation are similar to those of the corresponding stages of other species (see Charmantier *et al.*, 1988).

Osmoregulation

In *C. irroratus* and *C. borealis*, adults osmoconform in high salinities and seawater and slightly hyper-regulate in dilute media. The ability to hyper-regulate is higher in *C. irroratus*.

A similar pattern of osmoregulation has been described in *C. irroratus* and other species of *Cancer*, but the ability to hyper-regulate varies with the species, although other factors such as size and temperature can affect this parameter. In media of approximately 500 mosm · kg^{-1}, 17‰, at temperatures of ≅15–20°C, the difference between the osmotic pressures of hemolymph and medium expressed in mosm · kg^{-1} is about 15 in *C. antennarius* (Jones, 1941), 30–40 in *C. borealis* (this study), 50 in *C. pagurus* (Wanson *et al.*, 1983), 50–120 in *C. irroratus* (Thurberg *et al.*, 1973; Cantelmo *et al.*, 1975; Neufeld and Pritchard, 1979; this study), 150–250 in *C. magister* (Jones, 1941; Engelhardt and Dehnel, 1973; Hunter and Rudy, 1975).

Zoeae of *C. irroratus* hyper-osmoconform in all tested salinities. Most decapod larvae that have been studied

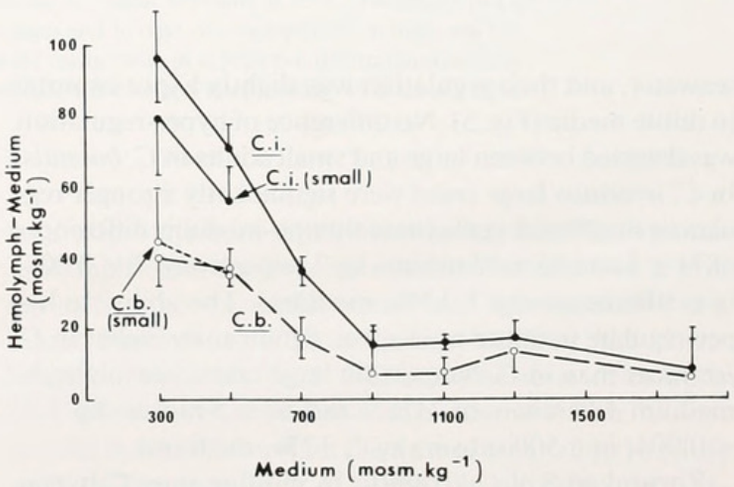

Figure 5. Variations in the difference between the osmotic pressures of hemolymph and medium according to the osmotic pressure of the medium in large and small adults of *Cancer irroratus* and *C. borealis* at 15°C. Each point represents the mean value of determinations from 7 to 10 animals (exception in lowest salinity: 4–6 animals) with 95% confidence interval.

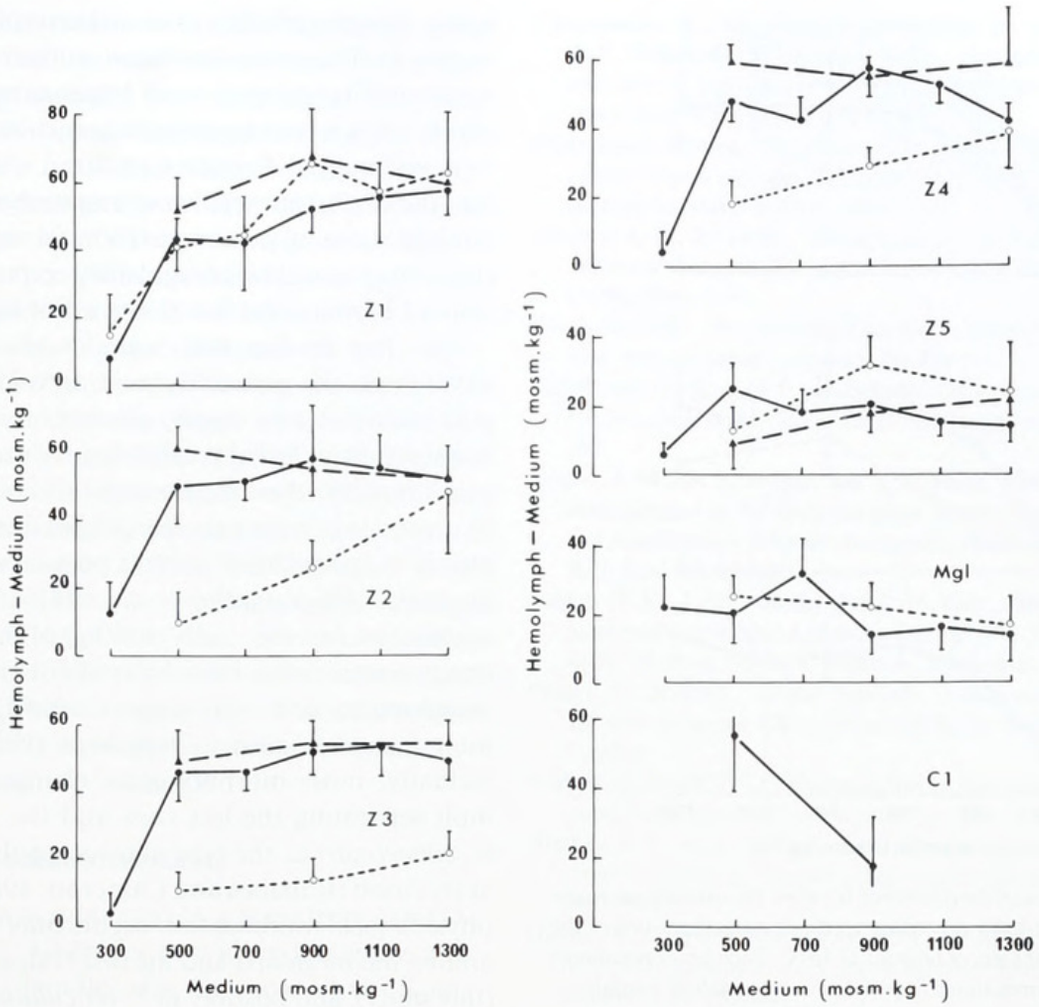

Figure 6. Variations in the difference between the osmotic pressures of hemolymph and medium according to the osmotic pressure of the medium in zoeae 1–5, megalopae, and first crab stage of *Cancer irroratus* at 15°C. Each point represents the mean value of determinations from 9–15 animals (5–10 animals in extreme salinities) with 95% confidence interval. ○-----○: post-molt; ● —— ●: stage C; ▲ – – ▲: premolt.

hyper-osmoconform in salinities that they normally encounter in their environment (Charmantier *et al.,* 1988). This could be considered an adaptation of small organisms to planktonic or pelagic life. The slight positive difference in osmotic pressure between hemolymph and medium maintains an osmotic influx of water, which in turn favors the turgescence of the body and particularly of the extended appendages and exopodites involved in the buoyancy of the larvae. In a few zoeal stages of *C. irroratus,* the osmotic pressure of hemolymph is affected by the molting stage, increasing in premolt and decreasing in postmolt but much less regularly than in other species like *Rhithropanopeus harrisii* (Kalber and Costlow, 1966), *Cardisoma guanhumi* (Kalber and Costlow, 1968), *Homarus americanus* and *Penaeus japonicus* (Charmantier *et al.,* 1988). Like zoeae, megalopae of *C. irroratus* hyper-osmoconform in all media. First crab stages osmoconform in seawater, but they hyper-regulate in a dilute medium. Thus, the adult type of osmoregulation seems to be acquired at the first crab stage. However, the ability to hyper-regulate, evaluated by the difference between the osmotic pressures of hemolymph and medium in a dilute medium, increases with size in adults: in a medium of 500 mosm · kg^{-1}, 17‰, this difference was 55 ± 16 mosm · kg^{-1} in first crab stage, 55 ± 13 mosm · kg^{-1} in small adults, and 71 ± 7 mosm · kg^{-1} in large adults. As in *C. irroratus,* zoeae of *C. borealis* hyper-osmoconform, while adults have a slight hyper-isoregulation. In a preliminary study, Brown and Terwilliger (1989) found that megalopae and first crabs of *C. magister* were weaker osmoregulators than adults, after 8 h exposure to dilute media. A comparison with our results is difficult due to the lack of numerical data. Additionally, it is possible that the short time of exposure did not allow for complete osmotic equilibration in adults.

In a recent study, we reviewed the evolution of osmoregulatory abilities that have been described for the post-embryonic development of decapod crustaceans (Charmantier *et al.,* 1988). Most decapod larvae that have been studied, including zoeae of *C. irroratus* and *C. bo-*

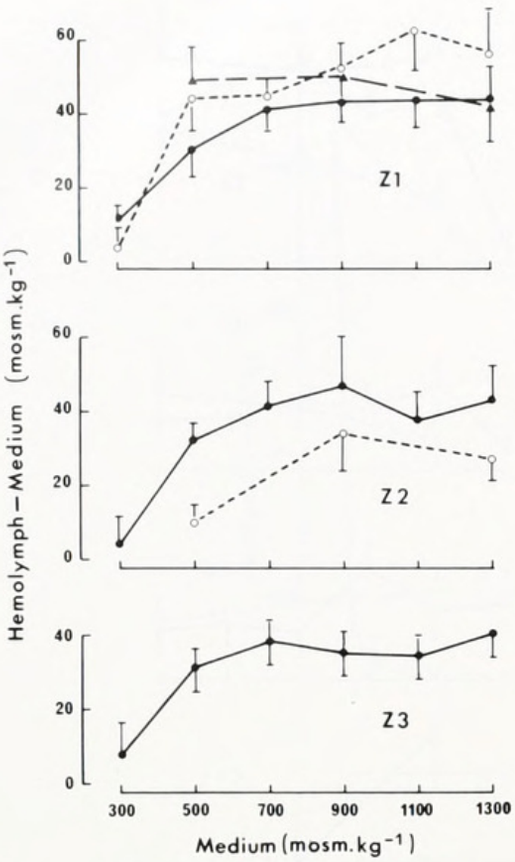

Figure 7. Variations in the difference between the osmotic pressures of hemolymph and medium according to the osmotic pressure of the medium in zoeae 1–3 of *Cancer borealis* at 15°C. Each point represents the mean value of determinations from 10 animals with 95% confidence interval. ○-----○: post-molt; ● —— ●: stage C; ▲ – – ▲: premolt.

realis, are hyper-osmoconformers or weak regulators, an exception being the larvae of *Macrobrachium petersi* (Read, 1984), which are confronted with very low salinities in their natural environment and which can efficiently regulate the osmotic concentration of their hemolymph. During or after the larval phase, different patterns of ontogeny of osmoregulation have been described, which we proposed to separate into three groups (Charmantier *et al.,* 1988). In one group of species, osmoregulation varies little with developmental stage; the adults of these species are often weak regulators or osmoconformers, like *Hepatus epheliticus* and *Libinia emarginata* (Kalber, 1970). In *M. petersi* (Read, 1984), which lives in variable salinities due to its migration, the adult type of regulation is established as early as the first larval stage. In a third group of species, which includes *Uca subcylindrica* (Rabalais and Cameron, 1985), *Homarus americanus, Penaeus japonicus* (Charmantier *et al.,* 1988), and *Cancer irroratus* (this study), metamorphosis marks the appearance of the adult type of regulation. In other species that do not still fit in those three categories, in which "no clear trend toward development of adult osmoregulatory patterns toward the end of larval life" was found (Foskett, 1977), this could be due to the lack of information about the osmoregulatory capacity of early post-metamorphic stages. For example, in *Sesarma reticulatum* studied by Foskett, 1977, zoeae and megalopae were hyper-osmoconformers and adults were hyper-hyporegulators, but early crab stages were not studied. Foskett stated: ". . . even by late megalops the adult osmoregulatory response is still not attained. Examination of osmoregulation in early juvenile crab stages may reveal osmoregulatory responses that are transitional between the larval and adult forms."

The few studies that were conducted in decapods throughout the post-embryonic development, including post-metamorphic stages, confirm this statement. The transition from larval to adult type of osmoregulation may occur rapidly when metamorphosis itself is sudden, as in *H. americanus,* or may be progressive when metamorphosis is spread over several post-larval stages as in *P. japonicus* (Charmantier *et al.,* 1988). There is a lack of agreement on the exact timing of metamorphosis in brachyurans: it has been located either at the molt from megalopa to first crab stage (Costlow, 1968), or at the molt from last zoea to megalopa (Felder *et al.,* 1985). Actually, most morphological changes result from the molt separating the last zoea and the megalopa and, in *U. subcylindrica,* the type of osmoregulation also changes at this molt (Rabalais and Cameron, 1985). However, this physiological modification occurs only after the molt separating the megalopa and the first crab stage in *C. irroratus* (this study), and possibly in *S. reticulatum* (Foskett, 1977). Additionally, as stated by Felder *et al.,* (1985), "in almost all the Decapoda, some ontogenic changes in locomotion, feeding, and habitat coincide with early postlarval growth." Thus, in our opinion, metamorphosis in brachyurans requires two molts to be completed and the megalopa, while clearly postlarval, is a transitional stage between the larvae and the postmetamorphic stages starting with the first crab stage.

In summary, studies conducted on the species of the third group of decapods cited above demonstrate that the completion of metamorphosis yields a change to the adult type of osmoregulation. We propose the hypothesis that, in most species in which larvae are hyper-osmoconformers or weak regulators and adults are efficient osmoregulators, the transition from the larval type to the adult type of osmoregulation occurs at metamorphosis. More generally, metamorphosis can be considered a combination of morphological, ecological, behavioral, and physiological changes (Costlow, 1968; Charmantier *et al.,* 1984b).

Relation between osmoregulation and salinity tolerance

In *C. irroratus* and *C. borealis,* zoeae are weak regulators and their salinity tolerance is comparatively moderate or low. In megalopae of *C. irroratus,* salinity tolerance is minimum just before the pattern of osmoregula-

tion changes. Under natural conditions, developing brachyurans are known to settle on the bottom during the megalopal stage, usually near the shores (Sandifer, 1973), *i.e.*, in an environment subjected to possible variations of salinity. The limited salinity tolerance of megalopae could cause high rates of mortality, which have been noted in different species of *Cancer*, at least under culture conditions (Charmantier-Daures and Charmantier, 1991). Unsufficient number of available animals prevented us from determining the salinity tolerance of early crab stages, in which the pattern of osmoregulation has changed. We may suppose that their salinity tolerance has correlatively increased. In adults of both species, the ability to osmoregulate is higher and so are their salinity tolerances. Compared to *C. borealis*, adult *C. irroratus* are stronger hyper-regulators in dilute media and are more tolerant to low salinity. Thus, as previously noted in *Macrobrachium petersi* (Read, 1984), *Uca subcylindrica* (Rabalais and Cameron, 1985), *Homarus americanus* and *Penaeus japonicus* (Charmantier *et al.*, 1988), there is a strong correlation between increased ability to osmoregulate and improved salinity tolerance.

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