The Journal of Experimental Biology 204, 967–977 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB2912

ECOPHYSIOLOGICAL ADAPTATION TO SALINITY THROUGHOUT A LIFE CYCLE: A REVIEW IN HOMARID LOBSTERS

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Accepted 1 December 2000; published on WWW 12 February 2001

Summary

Adaptations to salinity are reviewed throughout development in both species of the genus *Homarus*. Some populations of homarid lobsters are known to inhabit coastal and estuarine areas where salinity fluctuates. Salinity tolerance varies during development, with 50 % lethal salinities (LS_{50}) ranging from approximately 15–17 ‰ in larvae to approximately 12 ‰ in postlarvae and 10 ‰ in adults. Larval and adult lobsters can avoid low-salinity areas using behavioural strategies.

When exposed to low salinity, the capacity to osmoregulate varies with development. Embryos are osmoconformers and are osmotically protected by the egg membranes. Larvae are also osmoconformers, and the pattern of osmoregulation changes at metamorphosis to hyper-regulation, which is retained throughout the later stages up to the adult stage. Exposure to low salinity increases the activity of Na^+/K^+-ATPase in postlarvae and later stages. The level of osmoregulation evaluated through the osmoregulatory capacity (the difference between haemolymph and medium osmolalities) is negatively affected by low temperature (2 °C). The variations in haemolymph osmolality resulting from osmoconforming or partial osmoregulation are compensated by intracellular iso-osmotic regulation. Neuroendocrine control of osmoregulation appears in postlarvae and seems to involve the crustacean hyperglycaemic hormone. In adult lobsters, the gills appear to have a respiratory function only, and extracellular osmoregulation is effected by the epipodites, with the addition of the branchiostegites at low salinity. These organs are present at hatching. Transmission electron microscopy and immunolocalization of Na^+/K^+-ATPase reveal that the epipodites become functional in larvae and that the branchiostegites become functional in postlarvae. An integrated series of events links the appearance of osmoregulatory tissues, the increase in Na^+/K^+-ATPase activity, the occurrence in postlarvae of hyper-regulation at low salinity and the increase in salinity tolerance. Further ecological and physiological studies are proposed for a better understanding of the adaptive significance of the ontogeny of osmoregulation in lobsters.

Key words: salinity, adaptation, ontogeny, tolerance, osmoregulation, crustacean, lobster, *Homarus gammarus*, *Homarus americanus*.

Introduction

Homarid lobsters, like other aquatic organisms, are influenced during their entire life cycle by the composition of the water in which they live. Among numerous adaptations, the survival of individuals and the success of their populations are based on their ability to cope with the ambient salinity and its variations. In adult crustaceans, this is generally, but not exclusively, achieved through osmoregulation (for reviews, see Mantel and Farmer, 1983; Péqueux, 1995). Several studies conducted in diverse species have also demonstrated the adaptive importance of osmoregulation throughout development, since the adaptability of each developmental stage to salinity is one of the conditions for the successful establishment and/or maintenance of a species in a habitat (for a review, see Charmantier, 1998).

Because of the commercial importance of the lobsters *Homarus americanus* and *H. gammarus*, the literature related to their ecology and physiology is abundant. The objectives of this review will be to demonstrate (i) the adaptive significance of osmoregulation throughout the life of homarid lobsters and (ii) the physiological basis of osmoregulation during their development. The terminology used in this article will be based on previous reviews of the embryonic (Helluy and Beltz, 1991) and post-embryonic (Charmantier et al., 1991) development of homarids. The embryonic phase consists of a nauplius stage and a metanauplius stage, ending as a prelarva that emerges at hatch and moults into the first larval stage. Stage I is followed by two additional planktonic larval stages, II and III. The fourth postembryonic moult, or metamorphosis moult, results

in stage IV, which is the first postlarval stage and which tends to become benthic, as are the later stages. A series of juvenile stages then begins, corresponding to the different life history phases leading to adults (Lawton and Lavalli, 1995).

The term salinity will be used in its generally accepted form to mean salt concentration in grams per litre of water, denoted as ‰. When necessary, osmoconcentration (osmolality; in mosmol kg^{-1}) will also be used. A value of 3.4‰ is equivalent to 100 mosmol kg^{-1} (29.41 mosmol kg^{-1} per 1‰). Seawater salinity is approximately 34‰ (osmolality: 1000 mosmol kg^{-1}).

Ecological considerations

Before going further into physiological considerations, it is necessary to address the question of whether osmoregulation is relevant in homarid lobsters. A traditional approach is to consider them as stenohaline marine organisms limited to coastal and offshore habitats where salinities are typically over 25‰ (Dall, 1970). ‘Both homarid and palinurid lobsters occupy stable, fully marine habitats, are stenohaline, do not normally enter low salinity estuarines, and consequently hold little interest for the student of osmoregulation’ (Dall, 1980).

However, lobster populations can be found from depths of approximately 700 m to intertidal and are subdivided into offshore and inshore populations (respectively offshore and inshore from the 50 km band of coastal waters) (Cooper and Uzmann, 1980). It has been known for some time that some of the latter, mainly *Homarus americanus*, can be found in estuarine and subtidal areas where they may be subjected to short-term exposure to varying salinities (Thomas, 1968; Thomas and White, 1969; Reynolds and Casterlin, 1985; Howell and Watson, 1991; Maynard, 1991; Jury et al., 1994a,b; Smith et al., 1998a,b; Watson et al., 1999; Moriyasu et al., 1999) that can occasionally lead to mortality after the spring run-off (Thomas and White, 1969). The estuarine populations have a high proportion of males (Munro and Therriault, 1983; Robichaud and Campbell, 1991; for reviews, see Jury et al., 1994b; Watson et al., 1999), which might result from differential movements of males and females in response to salinity and/or temperature gradients (Howell and Watson, 1991; Watson and Howell, 1991). Berried *Homarus americanus* females are known to migrate from deeper waters to shallow waters in spring (Morrissey, 1971; Munro and Therriault, 1983; Campbell, 1986, 1990). While these movements are probably linked to the search for the high water temperatures favourable for moulting, growth, mating, egg extrusion (Cooper and Uzmann, 1971, 1980; Aiken and Waddy, 1986) and rapid embryonic development (Campbell, 1986; Lawton and Lavalli, 1995; Talbot and Helluy, 1995), they result in the exposure of the females, late embryos and early larvae to potential variations in salinity. A recent detailed study conducted in Great Bay estuary, New Hampshire, USA, has shown that most movements of lobsters into the estuary occurred in spring, when salinities were over 15‰ (Watson et al., 1999). Comparable migrations of lobsters, particularly of females, have not been reported for *Homarus gammarus* (Smith et al., 1998a,b).

The settlement of postlarvae, of which little is known in the field (Lawton and Lavalli, 1995), seems to be based on environmental cues, including the presence or absence of a thermal gradient, which ‘could lead the postlarvae to warmer, shallower, inshore areas’ (Wahle and Steneck, 1991; Lawton and Lavalli, 1995). Field research is now concentrating on the identification of nursery areas where the settling postlarvae grow into juveniles before their dispersal. Of particular interest in recent years has been the finding of juvenile *Homarus americanus* (ranging from 3 to 42 mm cephalothorax length) in intertidal New England, from Maine to Connecticut (Krouse and Nutting, 1990a,b; Cowan, 1999), sometimes exposed to very low salinities down to 0‰ during the winter snow run-off (D. F. Cowan, cited in Lawton and Lavalli, 1995). Juvenile and adult lobsters have also been reported in intertidal zones in Maine, USA (J. S. Krouse, cited in Lawton and Lavalli, 1995) and in Prince Edward Island, Canada (Mackay, 1920).

Estuaries, at least the lower part of them, might also be possible sites for settlement of postlarvae, as demonstrated by the studies conducted in Narragansett Bay, Rhode Island, USA (Wahle, 1993), and in Great Bay, New Hampshire, USA (Watson et al., 1999). Recently, the occurrence of a few juveniles of *Homarus gammarus* (early benthic phase, cephalothorax length range 28–45 mm) has been reported in an intertidal habitat at Johnshaven, south of Aberdeen, Scotland, which receives ‘a substantial volume of freshwater inflow’ (Linnane et al., 2000).

Besides the benefits of higher water temperatures mentioned above, one hypothetical advantage of the estuarine and/or intertidal habitat for lobsters, especially for the young stages, might be the reduction in predation and competition in these environments, which might result both from the ready availability of hiding crevices (Wahle and Steneck, 1992; Linnane et al., 2000) and from varying salinity excluding strictly marine pelagic and demersal fish, which are among the most significant of their predators (for a review, see Ennis, 1995). *In situ* verification of these hypotheses would be of interest.

There is, therefore, mounting evidence that, in some of their inshore populations, homarid lobsters at all developmental stages, from embryos to adults, may be exposed to varying salinities. Additional data on this subject will certainly originate from further studies aimed at identifying settlement and nursery areas. Some of these studies should be conducted for *Homarus gammarus*, for which information is scarce compared with that available for the American species. It would also be profitable for ecologists and physiologists for salinities to be carefully and systematically monitored and recorded during these field observations.

Salinity tolerance

Even before comprehensive ecological studies had shown the potential exposure of lobsters to low salinity under natural

conditions, several authors had made similar observations. In addition, and from an economical point of view, which has frequently been important in lobster studies, 'heavy mortalities occur at times among lobsters that are held in large numbers by the industry... In general, adverse environmental conditions have been suspected' (McLeese, 1956). These observations led to different evaluations of the salinity tolerance of lobsters. Given that, in the latitudes where lobster populations are found, freshwater run-offs are a much more common occurrence in estuarine and subtidal conditions than seawater evaporation, these studies were aimed at investigating their tolerance to low salinities. Following preliminary investigations by Chaisson (1932), a comprehensive study was conducted by McLeese (1956) of the combined effects of temperature, salinity and oxygen levels on the survival of adult *Homarus americanus*. Lethal salinities for 50 % of the animals exposed for 48 h (48 h LS_{50}) varied according to the level of these variables during acclimation. For a combination of 5 °C, 30 ‰ and 6.4 mg O_2 l^{-1}, a lethal salinity as low as 6 ‰ was reported (see Table VI in McLeese, 1956). For combinations more representative of field conditions, LS_{50} varied from 8.0 to 11.7 ‰ (Table 1; see also Table XI in McLeese, 1956).

The tolerance to low salinity was lower in soft-shelled lobsters, i.e. shortly after moulting (see Table V in McLeese, 1956). Adults of the same species maintained at 15 °C were reported to survive for at least 3 days at 10 ‰ salinity (Jury et al., 1994a). In 1-year-old juveniles of *Homarus gammarus*, preliminary experiments showed survival with little or no mortality between 13.6 and 47.6 ‰ salinity (Charmantier et al., 1984c).

Salinity tolerance varies with the developmental stage. Embryos in eggs carried by female *Homarus americanus* died within 2 h of exposure to 17 ‰ but tolerated 24 ‰ for at least 12 h (Charmantier and Aiken, 1987). Larvae appear to be less tolerant than adults to low salinity. In *Homarus gammarus* and *Homarus americanus*, respectively, Gompel and Legendre (1927) and Templeman (1936) found that the larval period could progress to metamorphosis and stage IV at 15–17.5 °C only at salinities above 17 ‰. Sastry and Vargo (1977) observed that larvae of *Homarus americanus* developed to stage V in salinities above 20 ‰ at 15 °C and 15 ‰ at 20 °C (Table 1). In the same species, at 20 °C, 48 h LS_{50} ranged from 14 to 18 ‰ in larvae, was maximal at metamorphosis and decreased to approximately 12 ‰ in postlarvae; 48 h LS_{50} was approximately 10 ‰ in 1-year-old juveniles (Charmantier et al., 1988; Table 1). Moulting increased LS_{50} at all experimented stages, in general by approximately 1 ‰.

An overview of these results, which is possible in *Homarus americanus*, shows that the tolerance to low salinity, evaluated through reverse variations in 48 h LS_{50} at 20 °C, is moderate and tends to decrease during the larval phase (stages I–III: approximately 14–17 ‰), is minimal at metamorphosis (approximately 18 ‰) and increases sharply in the immediate

Table 1. *Values of lethal salinity for different developmental stages of homarid lobsters following or at different conditions of acclimation*

Species	Stage	Conditions	Lethal salinity	Reference
Homarus americanus	Adult	T: 5, 15, 20, 25, 30 °C S: 20, 25, 30 ‰ [O_2]: 2.9, 4.3, 6.4 mg l^{-1}	48 h LS_{50}: 8.0–11.7 ‰ (increase at moult)	1
Homarus americanus	I–IV	T: 15–17.5 °C	Development possible at S>17 ‰ Time to stage IV unaffected at 21–32 ‰ Survival: 83 % at 31–32 ‰ 63 % at 21–22 ‰ <10 % at 17<S<20 ‰	2
Homarus americanus	I–V	T: 15 °C T: 20 °C	Development to stage V possible at: S>20 ‰ S>15 ‰	3
Homarus americanus	I II III Metamorphosis (late III, early IV) IV V 1-year-old juveniles	T: 20 °C	48 h LS_{50}: 14.2 ‰ 14.9 ‰ 16.6 ‰ 17.5–18.0 ‰ 12.9 ‰ 11.6 ‰ 10.0 ‰ (increase at moult)	4
Homarus gammarus	I–IV	T: 15–17.5 °C	Development possible at S>17 ‰	5

[1]McLeese, 1956; [2]Templeman, 1936; [3]Sastry and Vargo, 1977; [4]Charmantier et al., 1988; [5]Gompel and Legendre, 1927.
S, salinity; T, temperature; 48 h LS_{50}, lethal salinity for 50 % of the animals exposed for 48 h.

postlarval stages (stages IV, V: approximately 12‰) and more slowly thereafter (juveniles, adults: approximately 10‰). The tolerance to low salinity is negatively affected by moulting throughout the life cycle of lobsters.

Behavioural response to variations in salinity

When lobsters are confronted with salinity variations in their environment, and if they are to survive them, they can theoretically react in two ways, either behaviourally by trying to avoid them or physiologically through processes of osmotic regulation (see below). In *Homarus americanus*, Scarratt and Raine (1967) demonstrated experimentally that, given the choice between sea water (31.7‰) and a dilute medium (21.4‰), newly hatched stage I larvae were able to detect, and then to avoid, the lower salinity. If, as is probable, this reaction extends throughout the larval period, larvae could avoid low surface salinities resulting, for instance, from rainfall. However, the poor swimming ability of larvae probably does not permit them to avoid large areas of reduced salinity.

In adults of the same species, behavioural data related to the avoidance of low salinity have been gathered experimentally (Jury et al., 1994b). They indicate that adult lobsters are capable of detecting changes in salinity comparable with those found during natural fluctuations in coastal bays and estuaries (between 20–25‰ and 10–15‰). When exposed to sufficiently low salinity, they attempt to avoid it. Jury et al. (1994b) reported that adult American lobsters moved out of their shelters when salinity in the shelter was below 12‰. This value is only marginally higher than the LS_{50} for this species (McLeese, 1956). Females appear to be more sensitive to reductions in salinity, a fact that the authors relate to the higher proportion of male lobsters in estuaries (for a review, see Jury et al., 1994b). According to these authors, the avoidance of low salinity exhibited by lobsters may influence their seasonal movements in estuaries, particularly the avoidance of the upper estuary in spring, followed by a migration into the estuary during spring or early summer (Munro and Therriault, 1983; Howell and Watson, 1991; Watson and Howell, 1991; Maynard, 1991; Robichaud and Campbell, 1991; Watson et al., 1999). In addition, Jury et al. (1994a) have reported high rates of energy consumption linked to osmoregulation in adult lobsters, and they suggest that behavioural strategies to avoid low salinity would be more adaptive than extended periods of ion pumping.

Osmotic regulation

When environmental salinity decreases and behavioural avoidance of low salinity is impossible, the survival of lobsters within the tolerance limits of each of their stages indicates that physiological mechanisms are involved. They can use extracellular osmoregulation, which minimizes osmolality variations in the haemolymph, and/or intracellular iso-osmotic regulation, which equilibrates the intra- and extracellular osmolalities and therefore prevents massive movements of water across the cell membranes and maintains an almost constant cell volume. Both mechanisms have now been demonstrated to exist in homarid lobsters, and the available information is reviewed below.

Osmoregulation

The osmolality and ionic composition of the haemolymph of lobsters in sea water have been investigated previously (for reviews, see Charmantier et al., 1984a,c). Preliminary data on the osmoregulation of *Homarus americanus* were reported by Cole (1940, 1941), McLeese (1956) and Burger (1957). A thorough study showed that adults of this species were almost iso-osmotic (hyper-osmoconformers) in sea water and were slight hyper-regulators in dilute media (Dall, 1970). Similar results have been reported in juveniles of the same species (Charmantier et al., 1984a) and of *Homarus gammarus* (Charmantier et al., 1984c). In both species, and for media osmolalities ranging from 400 to 600 mosmol kg^{-1} (13.5–20.5‰), juvenile and adult lobsters maintain an osmoregulatory capacity (the difference between the osmolalities of the haemolymph and the medium) of approximately 100–125 mosmol kg^{-1}. Comparable values were recorded in a more recent study of osmoregulation in adult *Homarus americanus* (Jury et al., 1994a). No difference was found between the haemolymph osmolalities of males and females at low salinity. In both sexes, exposure to low salinity caused an increase in the rate of oxygen consumption, heart rate and scaphognathite beating rate. However, at 10‰ salinity, females appeared to use more energy (evaluated through oxygen consumption) than males to maintain the same osmotic balance. The authors conclude that the energetic demands of osmoregulation may determine, in part, the distribution and/or movements of lobsters in estuarine habitats and the domination of male lobsters among the populations of some estuaries.

The ability of lobsters to osmoregulate is affected by temperature (G. Charmantier, M. Charmantier-Daures and D. E. Aiken, unpublished data). Following 10–15 days of culture at temperatures of 2, 11, 20 and 25 °C, late juveniles (25–30 mm cephalothorax length) of *Homarus americanus* in intermoult stage C exhibited large differences in their ability to hyper-regulate at low salinities. At temperatures of 20 °C and 25 °C, the osmoregulatory capacity was maximal (93±13 mosmol kg^{-1} at 20 °C and 89±14 mosmol kg^{-1} at 25 °C in 500 mosmol kg^{-1}, 17‰ salinity). In contrast, lower temperatures of 11 °C and especially 2 °C resulted in significantly lower osmoregulatory capacities of, respectively, 54±8 and 16±3 mosmol kg^{-1} (Fig. 1). There is therefore no clear relationship between low salinity tolerance (McLeese, 1956) and high osmoregulatory capacity at 25 °C. The main enzymes of metabolism generally have a thermal optimum close to the mean temperature of the habitat (Schoffeniels and Dandrifosse, 1994), e.g. 12 °C for lactate dehydrogenase in *Homarus gammarus* (Trausch, 1976). However, the optimum temperature for the activity of the key osmoregulatory enzyme

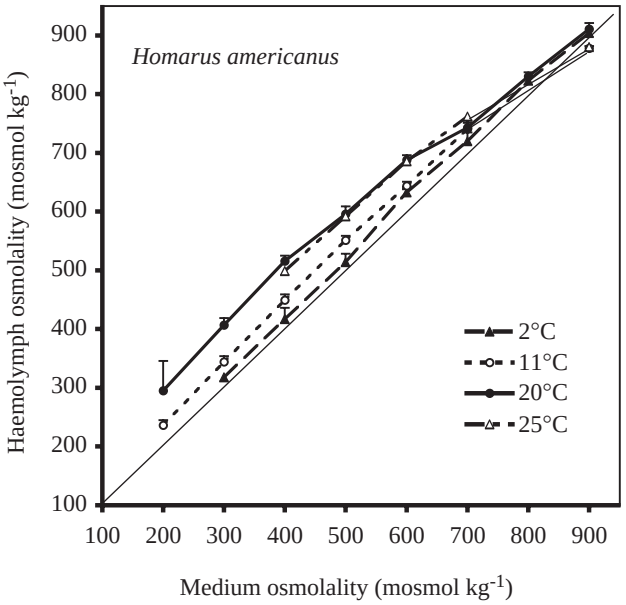

Fig. 1. Variations in the haemolymph osmolality of late juvenile *Homarus americanus* as a function of that of the medium following 10–15 days of exposure to different temperatures. Values are means + S.D. from measurements on 7–10 animals (from G. Charmantier, M. Charmantier-Daures and D. E. Aiken, unpublished data). The iso-osmotic line is drawn.

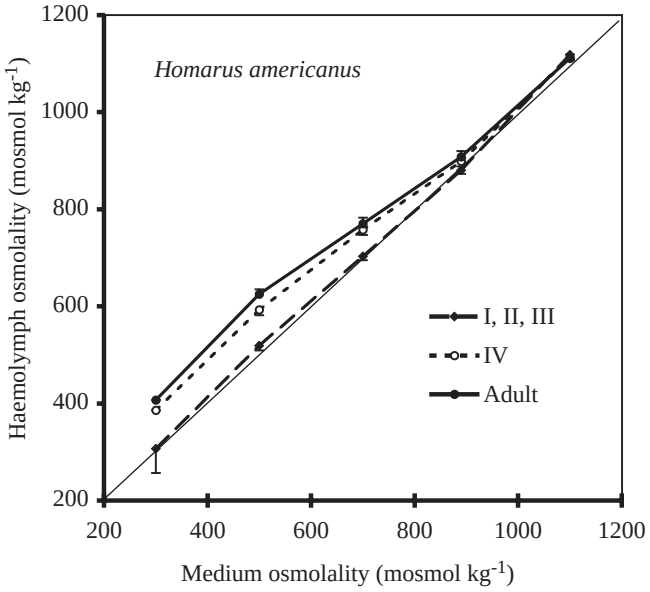

Fig. 2. Variations in the haemolymph osmolality of larval (stages I, II and III), postlarval (stage IV) and adult *Homarus americanus* as a function of that of the medium at 20 °C. Values are means + S.D. from 5–20 individuals. In larvae, data are shown from stage II only. At each salinity, data from stages I, II and III are not significantly different (after Dall, 1970; Charmantier et al., 1988; G. Charmantier, M. Charmantier-Daures and D. E. Aiken, unpublished data). The iso-osmotic line is drawn.

Na^+/K^+-ATPase (see below) is close to 37 °C (Thuet et al., 1988; Lucu and Devescovi, 1999), a fact that may help to explain the increase in osmoregulatory capacity with temperature up to 20–25 °C.

Osmoregulation in lobsters is apparently achieved through the hyper-regulation of Na^+ and, to a lesser extent, Cl^-. K^+ is hyporegulated at high salinities, and Mg^{2+} is strongly hyporegulated at all salinities (Charmantier et al., 1984c).

Experimental eyestalk ablation and reimplantation have shown that the former operation removes the ability to hyper-regulate at low salinity. Na^+ levels and osmotic regulation are neuroendocrinologically controlled in juvenile and adult *Homarus americanus* (Charmantier et al., 1984a; Charmantier-Daures et al., 1994). This control is probably mediated through an isoform of the neuropeptide crustacean hyperglycaemic hormone (CHH) synthesized in the eyestalk neuroendocrine centres and released through the sinus glands (Charmantier-Daures et al., 1994).

The pattern of osmoregulation changes during the early development of lobsters. Embryos are unable to osmoregulate (Charmantier and Aiken, 1987). When experimentally directly exposed to the external medium after removal of the embryonic egg membranes, they react as osmoconformers. If exposed to a dilute medium, 24‰ for instance, their internal osmolality drops within 1 h, and they do not survive for more than 3 h. In contrast, embryos maintained in intact eggs retain a higher osmolality (by approximately 100 mosmol kg^{-1} at 24‰) and survive longer exposures to low salinity, e.g. 12 h at the same salinity. At low salinity, the osmotic water intake is limited, because the egg volume does not increase greatly (G. Charmantier, unpublished data), and the diffusional loss of ions is reduced. Both these properties might originate from the impermeability of the egg membranes or, for the former, from the limit to egg volume increase provided by the membranes. The structure of the lobster egg membranes has been described (for reviews, see Aiken and Waddy, 1980; Talbot and Helluy, 1995), but their permeability has yet to be studied in detail. It has been suggetsed that the permeability increases in lobster eggs before hatching which, given their high internal osmolality, would result in osmotic intake of water and subsequent rupture of the membranes (Pandian, 1970a,b). In summary, the egg membranes protect lobster embryos osmotically against variations in the external salinity. This can be considered as an adaptation to fluctuating salinities in coastal and estuarine areas (Charmantier and Aiken, 1987).

Immediately after hatching, prelarvae of *Homarus americanus* are also osmoconformers (Charmantier and Aiken, 1987), as are the three successive larval stages, I, II and III (Charmantier et al., 1988). Their time of osmotic adaptation to a lower salinity is short, close to 1 h. These planktonic stages therefore osmotically closely follow the changes in the medium salinity, after rainfall for instance. This has led to the hypothesis, as will be seen below, of intracellular iso-osmotic regulation in the larval stages. Following metamorphosis, the adult pattern of osmoregulation is acquired in stage IV postlarvae, which are iso-osmotic in sea water and are slight hyper-regulators at low salinity. At 17‰ (500 mosmol kg^{-1}),

for instance, their osmoregulatory capacity is approximately 80 mosmol kg^{-1} (Charmantier at al., 1988) (Fig. 2). It increases in the following stages to the juvenile and adult values of 100–125 mosmol kg^{-1}. The same pattern of changes in osmotic regulation throughout post-embryonic development, and very similar values of osmolality, have been found in *Homarus gammarus* (Thuet et al., 1988).

The ontogeny of osmoregulation found in both *Homarus* species relates them to the third pattern recognized in crustaceans (Charmantier, 1998) in which metamorphosis marks the appearance of the adult type of osmoregulation. The change in the osmoregulatory ability of postlarvae provides them with an increased density at low salinity since their extracellular medium is more concentrated than the external medium. As noted by Foskett (1977), this consequence of hyperosmotic regulation is a positive adaptation for the planktonic-to-benthic transitional stages IV and V since their increased density helps them to seek and stay on the bottom. The changes in osmoregulation at metamorphosis appear to originate from changes in Na^+ regulation, Na^+ levels being iso-ionic in stage III and slightly hyperionic in stage IV. Concomitantly, water content drops from 82 % in stage III to 71.5 % in stage IV (Charmantier et al., 1984b).

Experimental eyestalk ablation/reimplantation has demonstrated that Na^+ concentration is regulated from stage IV, as in adults, by eyestalk neuroendocrine factors. Such factors are not present or efficient in stage III, and osmoregulation in stage III is not affected by implantation of stage IV eyestalks (Charmantier et al., 1984b). Histological, ultrastructural and immunocytochemical studies conducted in *Homarus gammarus* have revealed that the eyestalk neuroendocrine system is present, and that CHH is localized in some of its neuroendocrine cells, during the embryonic metanauplius stage (Rotllant et al., 1995) and in the larval and postlarval stages (Rotllant et al., 1993). But the sinus gland, a neurohaemal site, is functional only from larval stage I (Rotllant et al., 1994, 1995). Taken together, these results suggest that the neuroendocrine system appears to be functional before the appearance at metamorphosis of the ability to hyper-regulate. It is therefore probable that metamorphosis marks the appearance of functional osmoregulatory effector organs. This issue will be addressed below.

The moult cycle has been shown to affect osmotic regulation in young postembryonic stages of *Homarus americanus* (Charmantier et al., 1988). In premoult larval stages I–III, the haemolymph osmolality increases in all media, which might favour the uptake of water at ecdysis. Postmoult postlarval stages IV and V demonstrate a reduced ability to hyper-regulate compared with intermoult stage C animals, perhaps following the water intake at moult. Correlations can be established in early larval and postlarval stages between osmoregulation and salinity tolerance (Charmantier et al., 1988). Osmoconforming larvae have a comparatively low salinity tolerance, which improves in hyper-regulating postlarvae (Table 1).

Enzymes such as carbonic anhydrase and Na^+/K^+-ATPase are known to be implicated in ion transport. A study conducted in *Homarus gammarus* revealed that carbonic anhydrase activity was higher in the gills and epipodites in stage IV than in stage III. In addition, in all organs tested (gills, epipodites, cephalothorax, abdomen and pleopods) and compared with sea water, a 5 h exposure of individuals to 17 ‰ salinity induced no change in Na^+/K^+-ATPase activity in stage III, but this exposure was followed by a highly significant increase in stage IV, probably as a result of an activation process (Thuet et al., 1988). These increases in enzymatic activity are probably one of the physiological bases of the changes in patterns of ionic and osmotic regulation at metamorphosis.

Ionic and water exchanges, and ionic transport, occur at the level of structures separating the body of the animal from the external medium. Several specialized structures have been thought and/or shown to participate in osmoregulation in crustaceans, particularly the gut, the excretory organs and the gills (for reviews, see Mantel and Farmer, 1983; Taylor and Taylor, 1992; Péqueux, 1995; Ahearn et al., 1999). The digestive system of lobsters has been described in great detail (for a review, see Factor, 1995) as has its ontogeny (Factor, 1981). Ion and water uptake have been demonstrated in the intestine, the posterior midgut caecum and the hindgut (Mykles, 1980; Conklin, 1995). While the role of the two former organs in water uptake at moult in adults has been clearly demonstrated (Mykles, 1980, 1981), their function in osmoregulation is unproved, and no functional data are available during the early developmental phase.

The excretory organs of lobsters, the two antennal glands, have been the subject of few studies, and it must be emphasized that no detailed information on their ontogeny is available. Urine is essentially iso-osmotic to the haemolymph in *Homarus americanus*, especially at low salinity (Dall, 1970), which excludes any significant role of the excretory organs in osmoregulation. They may be involved in the regulation of Mg^{2+} levels (Robertson, 1949) which, as already noted, are strongly hypo-regulated in *Homarus gammarus* (Charmantier et al., 1984c). In larvae of *Homarus americanus*, Mg^{2+} was found to be excreted at a lower rate than in adults (Newton and Potts, 1993), suggesting that the excretory organs are not fully functional since their development may still be incomplete (Waite, 1899).

The gills are the site of several functions in lobsters, e.g. gas exchange, acid–base regulation and nitrogen excretion (McMahon, 1995), and, since there is ample evidence that they are an important site for ion exchange in many crustaceans (for reviews, see Taylor and Taylor, 1992; Péqueux, 1995), it has been suggested that the gills are the site of osmoregulation in homarid lobsters (McMahon, 1995). For these reasons, their perfusion by haemolymph (for a review, see Martin and Hose, 1995) and their ventilation (for a review, see McMahon, 1995) have been studied in detail in lobsters.

However, although the organization of the gills is known in lobsters (McLaughlin, 1983), their histology and ultrastructure have been described only recently. A study combining

confocal laser scanning microscopy with a fluorescent vital stain for mitochondria (DASPMI) and electron microscopy has been performed to locate ion-transporting tissues in the branchial chamber of adult *Homarus gammarus* (Haond et al., 1998). Such tissues, composed of ionocytes (including typical features such as apical microvilli, basolateral infoldings and numerous mitochondria), have been found on the inner side of the branchiostegites and over the entire surface of the epipodites, but no evidence of osmoregulatory structures has been observed in the gills.

In homarid lobsters, osmoregulation is probably achieved mostly by the branchiostegites and the epipodites, with the gills devoted to gas exchange (Haond et al., 1998). These results have been confirmed in the same species by immunocytochemistry and immunogold electron microscopy, using an antibody raised against Na^+/K^+-ATPase (Lignot et al., 1999). In large juveniles held in sea water, the enzyme was present only in the epipodite epithelium. Exposure to low salinity (22‰) was followed by increased immunostaining in the epipodites and additional staining along the inner-side epithelium of the branchiostegites.

Measurement of Na^+/K^+-ATPase activity, and of short-circuit current and conductance in a micro-Ussing chamber, have also recently confirmed the involvement of the epipodites in active ion transport in adult *Homarus gammarus* (Lucu and Devescovi, 1999). A high Na^+/K^+-ATPase activity was found in the epipodites and branchiostegites of the same species (Flik and Haond, 2000). We propose, therefore, that, in sea water, the limited amount of ions lost by the osmoconforming lobsters through diffusion is compensated through ion pumping by the epipodites. At low salinity, resulting in a higher ion loss, active uptake of ions, at least of Na^+, would be effected by the branchiostegites in addition to the epipodites, resulting in a slight hyper-ionic and hyperosmotic regulation.

In addition, the ontogeny of the osmoregulatory structures of the branchial chamber has been examined in *Homarus gammarus*. At no stage have osmoregulatory structures been found in the gills. The epipodites and branchiostegites display a few poorly differentiated (few microvilli and mitochondria, few and short infoldings) ionocytes in stage I. The differentiation of these cells increases during the larval phase, particularly after metamorphosis (C. Haond and G. Charmantier, unpublished data). Na^+/K^+-ATPase immunostaining reveals that the enzyme is also present only on the epipodites in stage I, even after exposure to low salinity. Its additional location on the inner side of the branchiostegites occurs after metamorphosis in stage IV (J. H. Lignot and G. Charmantier, unpublished data). We propose, therefore, that a clear correlation exists between (i) the differentiation of ionocytes and the location of Na^+/K^+-ATPase along first the epipodites and then the branchiostegites, and (ii) the appearance of the ability to hyperosmoregulate in stage IV.

Intracellular iso-osmotic regulation

Even if the increase in salinity tolerance can be related to the ability to hyper-regulate in stage IV, it must be noted that stage I–III larvae, although osmoconformers, are able to tolerate salinities down to 15–17‰. In addition, postlarval, juvenile and adult lobsters possess only a limited ability to hyper-regulate. Thus, when salinity decreases, the change is either totally (in larvae) or partially (in postlarvae and later stages) reflected in the haemolymph osmolality. Cells are consequently submitted to changes in the osmolality of their extracellular medium, and the regulation of their volume requires iso-osmotic intracellular regulation, as in adult crustaceans (for reviews, see Gilles and Péqueux, 1983; Kirschner, 1979). A recent study has confirmed this hypothesis through the titration of intracellular free amino acids in different developmental stages of *Homarus gammarus* (Haond et al., 1999). Compared with their level in animals held in sea water, exposure to low salinity (22‰) induced a decrease in intracellular free amino acid concentration of 46% in stages I–III, of only 29% in stage IV postlarvae and of 20% in juveniles. The main amino acids involved were glycine, proline and alanine. Thus, a decrease in salinity is compensated either through iso-osmotic intracellular regulation alone in larvae or, starting in postlarvae, through the combined effects of iso-osmotic intracellular regulation and extracellular osmoregulation.

Conclusions and directions for future work

During embryonic development, homarid lobster embryos are osmoconformers. They are osmotically protected by the egg membranes, which limit water influx and ion loss at low salinity. Following hatching, the larvae (stages I–III) are osmoconformers, and their comparatively limited salinity tolerance appears to be based only on iso-osmotic intracellular regulation. An integrated series of events then occurs that marks the transition from the larval to the postlarval phase (Fig. 3). Anatomical changes (increased differentiation of the osmoregulatory epithelia on the epipodites, and particularly on the branchiostegites, possible changes in the eyestalk neuroendocrine system) lead to physiological modifications (increased Na^+/K^+-ATPase concentration and activity, appearance of the neuroendocrine control of osmoregulation, both linked to the occurrence of the ability to hyper-regulate at low salinity) that increase salinity tolerance. These anatomical and ecophysiological events are coordinated, and they occur and/or are completed at the metamorphic transition from stage III to stage IV. Metamorphosis is, therefore, a major event during the postembryonic development of homarid lobsters, with anatomical, physiological and behavioural changes contributing to the adaptation to the ecological shift from a planktonic to a benthic habitat (Charmantier et al., 1991).

The pattern of hyper-regulation acquired at metamorphosis remains unchanged in juveniles and adults. The osmoregulatory capacity of approximately 80 mosmol kg^{-1} in stage IV increases to 100–125 mosmol kg^{-1} in 1-year-old juveniles and is stable during the later phases, except at the time of moults and following changes in temperature. During

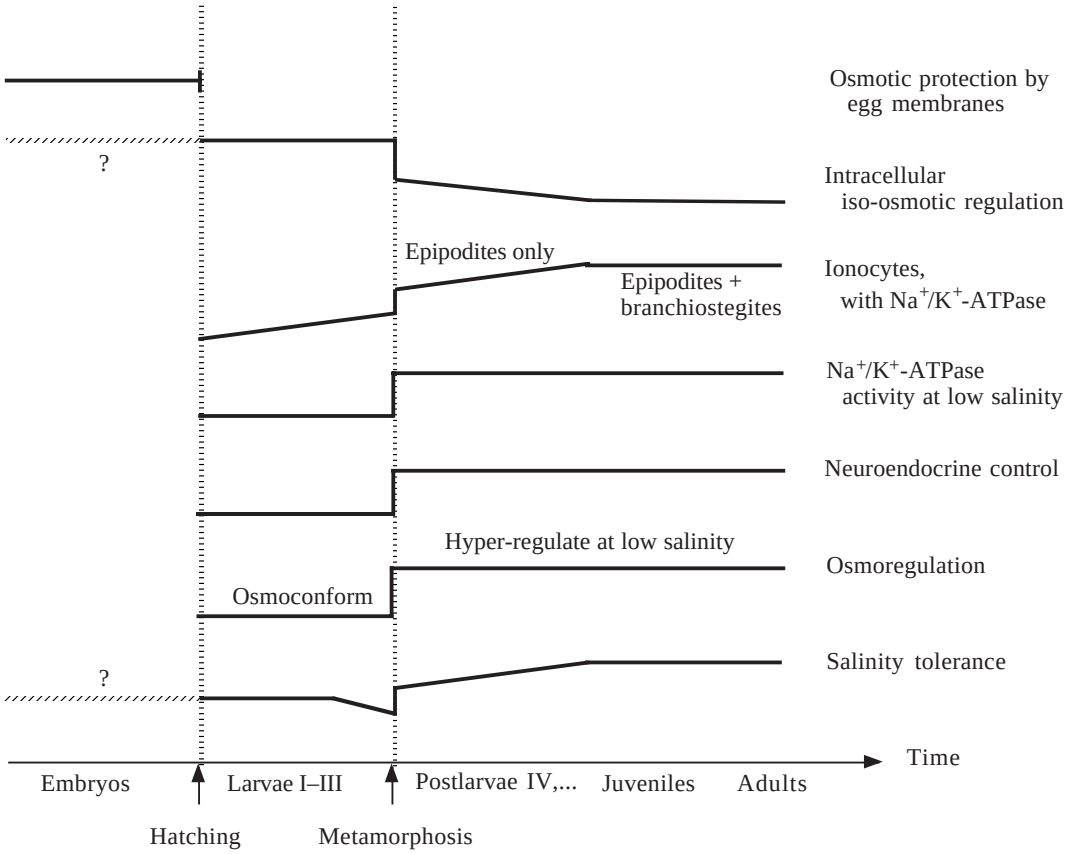

Fig. 3. Summary of the changes in and relationships among the anatomical and physiological events and salinity tolerance throughout the development of homarid lobsters. The diagram should be read from top to bottom.

their entire postembryonic development, homarid lobsters are able to tolerate salinities down to 15–17‰ (in larvae) and to approximately 10–12‰ (in postlarvae, juveniles and adults). This ability represents one of the adaptations that permits some populations of lobsters to spend their entire life cycle in habitats in which salinity varies, such as in coastal and estuarine waters.

Future research should be aimed at ecological and physiological studies. Studies initiated in the 1990s concerning the location and migration of the estuarine and coastal populations should be continued. Other areas of varying salinities, such as coastal marshes and lagoons, should be investigated as possible nursery areas. Such studies should be extended to both sides of the Atlantic Ocean, in North America, where they are well under way, and also along European coasts for which scant data are available. During these investigations, as much attention should be paid to salinity as is usually the case for temperature and oxygen concentration. Measurements, and if possible monitoring and recording, of salinity will reveal the lower levels of salinity actually tolerated in the field by the different developmental stages of lobsters. Since the transition from stage III to stage IV corresponds to the phase of minimum salinity tolerance, investigations should also be aimed at determining the areas where metamorphosis occurs and the local physico-chemical conditions.

Several aspects of the anatomy of the effector organs are worth investigating. The excretory organs, besides their main excretory function, seem to be only partially involved in the osmoregulation of lobsters. However, further work on their structure and functions is needed in adults, and their ontogeny remains to be described. Since the epipodites are one of the major osmoregulatory sites, their physiological functions should be investigated, possibly after isolation and perfusion. Similar experiments on the branchiostegites should also be conducted, perhaps taking advantage of their extended and relatively large surface area to use Ussing chambers, as already been achieved for the epipodites (Lucu and Devescovi, 1999).

Isolated epipodites and/or branchiostegites could be used to study the effects of pollutants on water and ion exchanges. They should also permit further investigations of the neuroendocrine control of osmoregulation. Determining and isolating the implicated neurohormone(s) should permit the topic of the onset of neuroendocrine control to be examined using techniques such as immunocytochemistry and *in situ* hybridization. Whether CHH is also involved in the control of osmoregulation starting in postlarvae should be investigated. Since the organs of the branchial chambers are already present in stage I larvae, their ontogeny in embryos should be investigated. Another important area for future research concerns the enzymes involved in osmoregulation, mainly Na^+/K^+-ATPase, but also other enzymes such as carbonic anhydrase and other ATPases. The application of molecular techniques should lead to the determination of the sequence of

these enzymes and of their coding genes, at each developmental stage. These results would be relevant to systematic studies. Is the Na^+/K^+-ATPase sequence as conserved in lobsters as it generally is in other animals? Does it vary between *Homarus americanus* and *Homarus gammarus*? Another goal of this study would be to detect the possible occurrence of molecular shifts during development, similar to those demonstrated for haemocyanin (Terwilliger and Brown, 1993; Brown and Terwilliger, 1998; Terwilliger, 1998). Also worth studying would be the regulation of gene expression at different phases of development and under different environmental conditions, and its onset during embryonic or postembryonic development. In conclusion, the integrated set of events mentioned above should be investigated further at all possible, ecological, anatomical, physiological and molecular levels.

Part of this review was presented at the 'Physiology of Decapod Crustacea: A Tribute to Jean-Paul Truchot' session of the Society of Experimental Biology 2000 Meeting in Exeter. G.C. thanks the organizers for their invitation and the Company of Biologists for funding. The authors also thank Dr Klaus Anger for useful exchanges.

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