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Ontogeny of salinity tolerance and hyper-osmoregulation by embryos of the intertidal crabs *Hemigrapsus edwardsii* and *Hemigrapsus crenulatus* (Decapoda, Grapsidae): Survival of acute hyposaline exposure

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Abstract

The adults of *Hemigrapsus edwardsii* and *Hemigrapsus crenulatus* are euryhaline crabs and strong hyper-osmoregulators. Their embryos are carried externally attached to the abdominal pleopods of female crabs, where they are exposed to temporal and spatial changes in salinity associated with their intertidal and estuarine habitats. Although embryos lack the branchial and excretory organs responsible for adult osmoregulation, post-gastrula embryos were highly tolerant of exposure to hypo-osmotic sea water. Detached eggs (embryos + envelopes), of both species, at all developmental stages between gastrulation and hatching, exhibited 80–100% survival for periods up to 96 h in sea water (osmolality, 1050 mmol kg^{-1}) and in dilutions to 50%, 10%, and 1%. Cleavage stages were less tolerant of dilution; *H. edwardsii*, <50% survived 24 h in 10% sea water; *H. crenulatus* <50% survived 6 h in 10% sea water. Post-gastrulation stages strongly hyper-osmoregulated but cleavage stages were hyper-osmoconformers (maintaining internal osmolality ≈ 150 mmol kg^{-1} above external). Osmoregulatory capacity was reduced just prior hatching, particularly in *H. crenulatus*, although salinity tolerance remained high. Gastrulation therefore marks a critical stage in the ontogeny of osmoregulation and salinity tolerance. Total Na^+/K^+-ATPase activity increased greatly during embryogenesis of *H. crenulatus* (undetectable in blastulae; gastrulae 0.31 ± 0.05 pmol P_i $embryo^{-1}$ min^{-1}; pre-hatching 16.4 ± 1.0 pmol P_i $embryo^{-1}$ min^{-1}). Na^+/K^+-ATPase activity increased in embryos exposed to dilute sea water for 24 h implicating regulation of this transporter in a short-term acclimation response.

Keywords: Crustacea; *Hemigrapsus*; Crab; Embryo; Salinity tolerance; Osmoregulation; Development; Na^+/K^+-ATPase

1. Introduction

Survival of adult crustaceans in osmotically labile littoral and estuarine habitats has been extensively studied and is typically associated with efficient regulation of the volume, and often the osmolarity, of the body fluids (reviews: Mantel and Farmer, 1983; Pequeux, 1995). These functions depend on specialised branchial epithelia and excretory organs. It is therefore of interest to consider mechanisms of salinity tolerance in larval and, particularly, embryonic stages before the appearance, or full differentiation, of these organs.

The purple rock crab, *Hemigrapsus edwardsii*, and the hairy-handed crab, *Hemigrapsus crenulatus* are mid- to high-shore crabs endemic to New Zealand. They occasionally co-occur, but *H. edwardsii* is more prevalent on protected un-silted boulder beaches, whereas *H. crenulatus* commonly burrows in sand or mud beneath stones, particularly in estuarine habitats (Morton and Miller, 1968; McLay, 1988). Ovigerous females of both species brood their embryos attached ventrally to the abdominal pleopods. In this position, they are exposed to variations in salinity due to rain, freshwater runoff and tides throughout development, from extrusion as zygotes to hatching zoea larvae. The adults of both species occur in proximity to fresh water (Jones, 1976; McLay, 1988) and both are very

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euryhaline, tolerating salinities <3–45 psu for several weeks, strongly hyper-osmoregulating in dilute sea water and weakly hypo-osmoregulating at the higher salinities (Phillips, 1968; Hicks, 1973; Jackson, 1976; Jones, 1976; H.H. Taylor, unpublished observations).

In general, animals may survive salinity variations by a combination of: 1) avoidance behaviours, 2) tolerance of internal change (osmoconformity), and 3) physiological compensation (osmotic, ionic, volume regulation). For embryos of euryhaline crabs, avoidance would require a protective response on the part of the brooding females. Although it has been reported that ovigerous *H. edwardsii* migrate to the lower shore, where salinities are presumably higher (Williams, 1969), avoidance of dilution does not appear to be the main mechanism employed by this crab. In fact, early ovigerous females of *H. edwardsii* occur in tidal pools adjacent to freshwater streams with salinities <5 psu. Ovigerous *H. crenulatus* may be collected from estuarine channels in which river water and sea water admix (H.H. Taylor and D. Seneviratna, unpublished observations). It is therefore important to determine the salinity tolerance of developing embryos, and the physiological bases of such tolerance.

Most investigations into the ontogeny of salinity tolerance and osmoregulation in crustaceans have focused on the changes that occur during postembryonic larval development and during metamorphosis to post-larvae or juveniles. In a synthetic review of these studies, Charmantier (1998) identified three major ontogenetic patterns. The first group, exemplified by the shore crab *Libinia emarginata*, osmoconform as adults and either osmoconform, or weakly osmoregulate as larvae. In the second group (e.g. *Homarus americanus*, *Cancer irroratus*), the adults osmoregulate but larvae osmoconform (or hyper-osmoconform i.e., their body fluids follow the isosmotic line but at a slight elevation relative to the medium) and only establish the adult pattern at metamorphosis (Charmantier et al., 1988, 2001; Charmantier and Charmantier-Daures, 1991).

A third group of crustaceans, develops the capacity for osmoregulation during embryogenesis (Charmantier and Charmantier-Daures, 2001). In non-decapods this ability is frequently linked with the appearance of specific embryonic structures not present in adults, e.g. the dorsal, dorsolateral and nuchal organs of Amphipoda, Isopoda, and Branchiopoda (Strömberg, 1972; Meschenmoser, 1989; Aladin and Potts, 1995; Morritt and Spicer, 1995, 1996). Freshwater crayfish (*Astacus leptodactylus*) develop directly and juveniles hatch with a hyper-/iso-osmoregulatory pattern similar to that of adults. However, this capacity develops only shortly before hatching with the appearance of the gills. The tolerance of hyposaline water by earlier crayfish embryos is thought to depend on their protection by impermeable egg membranes (Susanto and Charmantier, 2000, 2001). Hatching zoea of the euryhaline thalassinid ghost shrimp *Callianassa jamaicense* lack gills but are also capable of hyper-osmoregulation. Putative osmoregulatory cells in the pre-larval branchiostegites are revealed by silver staining and their appearance coincides with increased embryonic Na^+/K^+-ATPase activity (Felder et al., 1986).

Embryos of intertidal and estuarine brachyuran crabs may be exposed to widely varying salinities but their osmoregulatory capacities have not been investigated and no osmoregulatory structures have been reported. Bas and Spivak (2000) noted that embryos of two estuarine grapsid crabs *Chasmagnathus granulatus* and *Cyrtograpsus angulatus* acquired tolerance to salinities ranging from 3 to 44 psu two or three days after extrusion and attributed this to a decrease in the permeability of the egg membranes at this time. There is little quantitative information on the permeability of crustacean egg envelopes but measurements of embryonic respiration (Taylor and Leelapiyanart, 2001), and changes in the volume (Wear, 1974) and ion content (Pandian, 1970) of decapodan eggs imply a moderate permeability to gases, water and salts. In long term studies, the crabs *H. crenulatus*, *H. edwardsii*, and *Macrophthalmus hirtipes* completed embryonic development and hatched normally in sea water diluted to 18 psu but failed to do so at lower salinities (Clark, 1987). However, as noted, eggs of the two *Hemigrapsus* species experience much greater external dilution for periods of many hours and it is important to investigate the potential effects of such exposure on embryonic homeostasis.

The objective of this study was to examine the tolerance of embryos of *H. edwardsii* and *H. crenulatus* at different developmental stages to acute changes in salinity. We also determined whether these embryos osmoconformed or maintained internal osmolality different from the external osmolality over a range of sea water dilutions. A relationship between Na^+/K^+-ATPase activity and differences in the osmoregulatory capacity of crustacean adults, larvae and embryos is well-established (Mantel and Farmer, 1983; Felder et al., 1986; Bouaricha et al., 1991; Pequeux, 1995). Thus changes in the activities of this transporter in *H. crenulatus* embryos during development, and following acute hyposaline exposure, were also investigated.

2. Methods

2.1. Animals

Ovigerous crabs were collected on the east coast of South Island, New Zealand during their spawning periods. *H. edwardsii* Hilgendorf, 1882 (according to McLay and Schubart, 2004, the correct name is *H. sexdentatus* (H. Milne Edwards, 1837)) were collected intertidally from Glenafric Beach, Waipara between mid April and late July, and *H. crenulatus* Milne Edwards, 1837 from the Avon-Heathcote Estuary between August and January. They were transported to the Department of Zoology, University of Canterbury and were maintained in a recirculating seawater