

## ABSTRACT

Aspects of osmoregulation such as salinity tolerance, osmoregulatory capacity, the location of transporting epithelia, and the expression of the enzyme  $\text{Na}^+/\text{K}^+$ -ATPase were investigated during the ontogeny of three euryhaline decapod crustacean species from the North Sea: in the green crab, *Carcinus maenas*, in the Chinese mitten crab, *Eriocheir sinensis*, and in the brown shrimp, *Crangon crangon*.

Hemolymph osmolality was measured in laboratory-reared developmental stages that were exposed to a wide range of salinities, and osmoregulatory capacity was calculated in relation to the osmolality of the external medium. Salinity tolerance was determined by survival rates. With the exception of the slightly hyper-regulating zoea I, zoeal development in *C. maenas* was stenohaline. The ability to hyper-regulate appeared after the first metamorphosis, in the megalopa, and increased in subsequent juvenile crab stages. In *E. sinensis*, hyper-regulation was strong at hatching, decreased in later stages and reappeared in the megalopa. The strong hyper-hypo-regulating capability of adult mitten crabs was established in the first juvenile instar. In *C. crangon*, an ability to hyper/iso-regulate was present at hatching and remained in zoeal stages and decapodids. The hyper-hypo-regulating ability of adults was established at the transition from the decapodid to the first juvenile stage.

The expression of the  $\text{Na}^+/\text{K}^+$ -ATPase and ion-transporting cells were located by means of immunofluorescence microscopy and transmission electron microscopy, respectively. During the zoeal development of *C. maenas*, organs of the branchial chamber did not possess ionocytes or positive immunoreactivity. In the megalopa,  $\text{Na}^+/\text{K}^+$ -ATPase was located in ionocytes of the posterior gills, but was not detectable in the anterior gills. This remained the case in subsequent crab I juveniles and adults. In *E. sinensis*, positive immunolabelling of  $\text{Na}^+/\text{K}^+$ -ATPase was noted in the branchiostegites of the zoeal stages I and II, but not in the last zoeal stage V. In the megalopa and the first juvenile crab,  $\text{Na}^+/\text{K}^+$ -ATPase was located in the most posterior gills, whereas the anterior gills lacked immunolabelling of the enzyme. In the zoeal stages I and VI of *C. crangon*, specific immunoreactivity of the  $\text{Na}^+/\text{K}^+$ -ATPase was observed in the epithelia lining the branchiostegites and the pleura. In subsequent decapodids and juveniles, immunolabeled  $\text{Na}^+/\text{K}^+$ -ATPase remained located in ionocytes in the branchiostegite epithelium, but it disappeared from the pleurae and appeared in the epipodites. In larger juveniles of *C. crangon*, the shaft of the gills showed specific immunoreactivity.

Regardless of species, newly hatched zoeal stages showed an adaptation to low and/or varying salinities. The osmoregulatory capabilities were closely related to the development of ion-transporting cells, and with the expression of the  $\text{Na}^+/\text{K}^+$ -ATPase. In all three species, metamorphosis to the first juvenile instar marked the appearance of the adult pattern of osmoregulation.