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To David Towle,
wonderful memories of collaboration,
of writing together and friendship.

OSMOREGULATION IN THE PENAEID SHRIMP *LITOPENAEUS STYLIROSTRIS* : ONTOGENY AND LOCALIZATION OF TRANSPORTERS

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Introduction

Adult peneid shrimps which live in costal lagoons migrate in oceanic deep water to spawn (Fujinaga, 1955; Mistakidis, 1969; Dall et al., 1990) and larvae develop in a stable marine environment. After metamorphosis, postlarvae settle close to the coast in estuarine waters (Garcia and Le Restre, 1981). Among the abiotic factors that can fluctuate in these shallow environments, salinity plays an important role (Kinne, 1964). Osmoregulatory mechanisms allow the adaptation of aquatic animals to salinity changes. Adults of *L. stylirostris* are hyper-hypo-osmoregulators (Wabete et al., 2006) but no study has been conducted to assess the larval and postlarval osmoregulatory capabilities of this species.

The aims of this study were :

1) to investigate the salinity tolerance during the postembryonic development of *L. stylirostris*;

2) to establish the relationship between the changes in osmoregulatory capacities and the occurrence of Na+/K+-ATPase in the branchial cavity in the early life stages;

3) to assess and localize NKA, co-transporter NKCC1 and CFTR in gills, epipodites and branchiostegite at two salinities (12 and 35 ppt).

Materials and methods

Salinity tolerance and osmoregulatory capacity :

Nauplii, zoea 3, mysis 2, PL1 (P2), PL4 (P8) and PL9 (P25) were transferred from 35 ppt into six to eight salinities in three replicates at 29°C. No food was given during the experiment.

Dead animals were counted after 24 h for salinity tolerance. For osmoregulatory capacity, hemolymph osmolality was measured on 10 to 30 nanoliter of sample with a nano-osmometer (OTAGO, New Zealand).

Immunohistochemistry :

Sections were incubated with the following primary antibodies diluted in PBS-SM 0.5%:

- a rabbit antibody NKA alpha- H300sc-28800, from Santa Cruz Biotechnology, Inc – USA;
- a goat antibody NKCC1C14 - sc-21547) fromSanta Cruz Biotechnology, Inc - USA ;
- a mouse monoclonal antibody CFTR from R&D Systems, USA.

Secondary fluorescent antibodies were TRITC, Alexa 488 and Alexa 633. Observations and photographs were effected on a Leica TCS SPE DM2500 microscope in association with a Carl Zeiss Axio Vision 4 software.

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Results

Salinity tolerance and osmoregulatory capacity

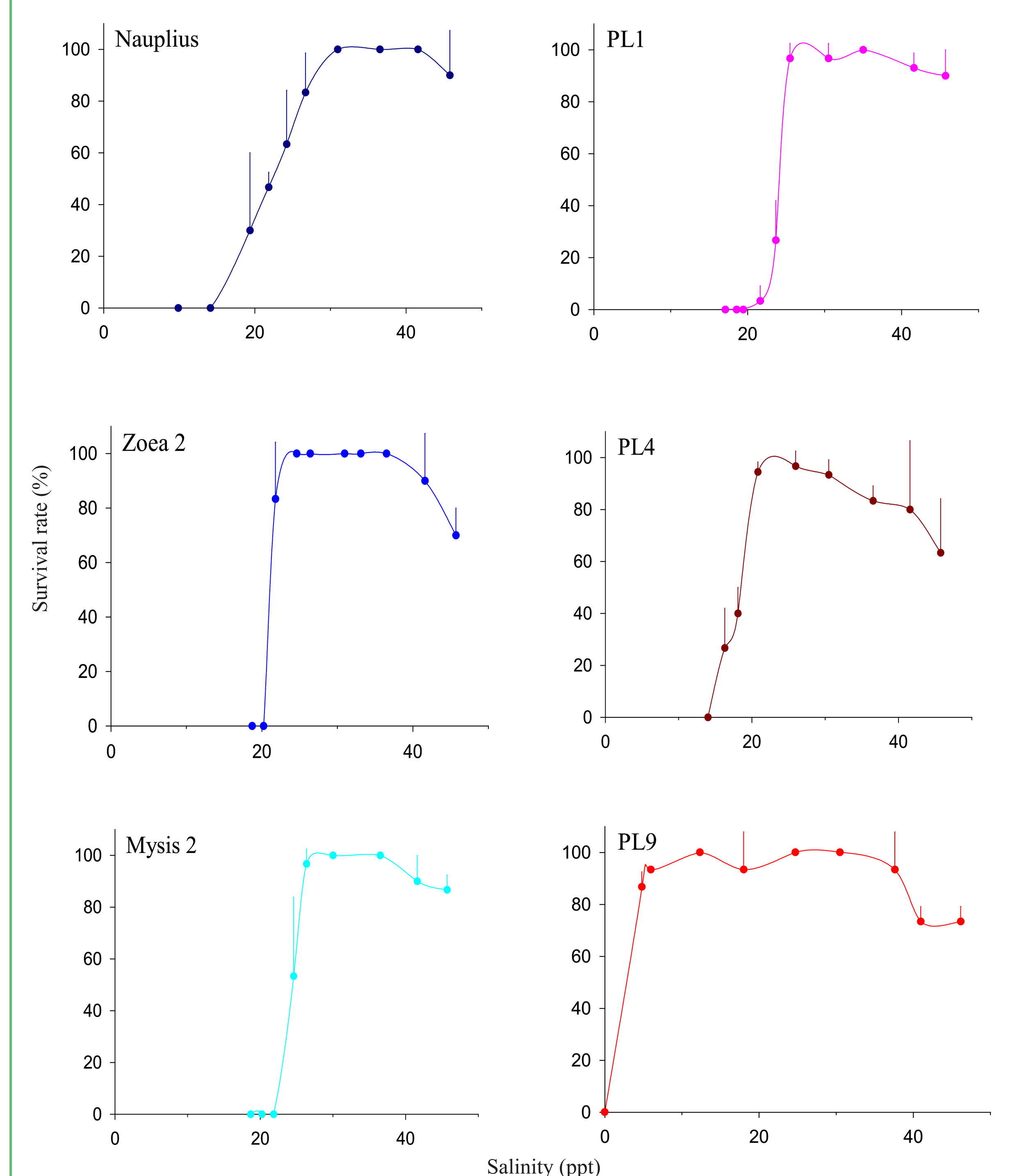

Figure 1: Survival rate at 24 hrs according to salinity and developmental stages. Values are means ± SD (n=3).

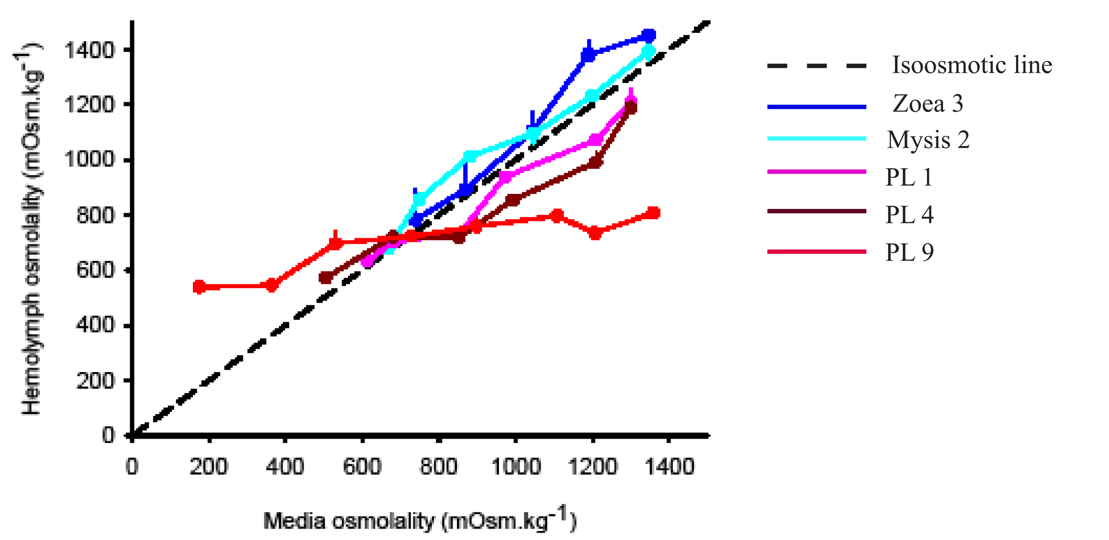

Figure 2 : Hemolymph osmolality in relation to the osmolality of the external medium. Values are means ± SD (n=3 to 10).

Survival rates exceeded 80% at salinity over 25 after 24 hours in all stages. At salinities below 25, salinity tolerance was higher in nauplii and zoeae than in mysis. Postlarvae were able to withstand lower salinities, e.g. 6 at PL9, but they were more sensitive than larvae to salinities over 35.
Zoea and mysis slightly hyper-regulated at all tested salinities. After metamorphosis, postlarvae progressively acquired an hyper-hypo-osmoregulation type.

NKA localization during development

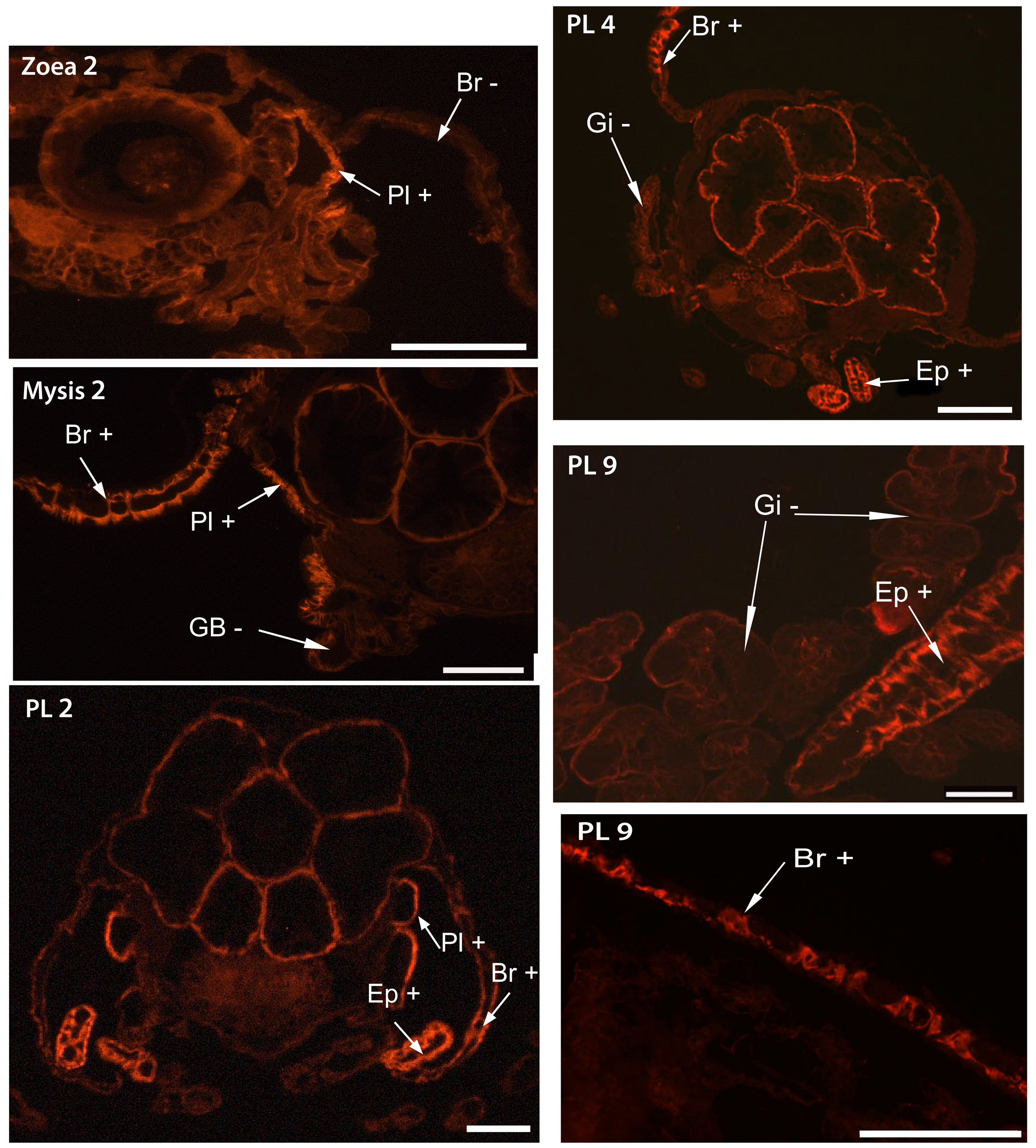

Figure 3 : Immunolocalization of Na+,K+-ATPase in organs of the branchial cavity. Bars = 100 µm.
Br : branchiostegite; GB : gill bud; Ep : epipodite; Gi : gills; Pl : pleurea.

Immunolocalization of NKA showed a sequential involvement of the different branchial organs: first, pleurae in zoeae, then branchiostegites in mysis and finally the epipodites in postlarval stages. No immunoreactivity was observed in the developing gills at any stage during ontogenesis.

Localization of NKA, NKCC1 and CFTR in juveniles

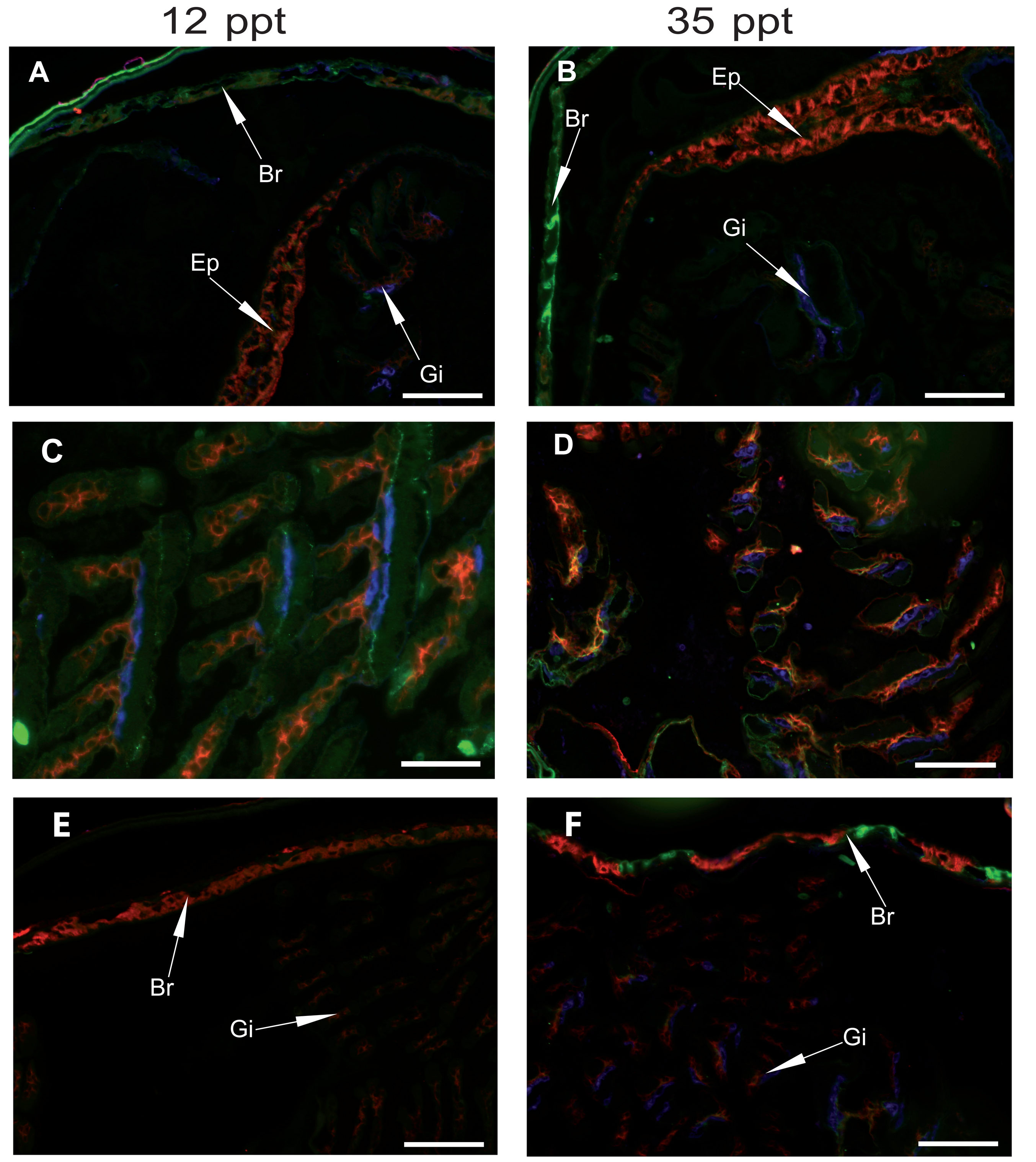

Figure 4 : Immunolocalization of NKA, NKCC1 and CFTR in juveniles branchial cavity (BC) at two salinities. Bars = 50 µm.
A: front side of the BC at 12 ppt; B: front side of the BC at 35 ppt; C: gills in back side in the BC at 12 ppt; D: gills back side in the BC at 35 ppt; E: back side of the BC at 12 ppt; F: back side of the BC at 35 ppt.
Br : branchiostegite; Ep : epipodite; Gi : gills.

In early juveniles (0,05g), NKA was present at 12 ppt and 35 ppt in all organs. CFTR was mostly localized in the branchiostegites at 35 ppt (fig. B and F). NKCC1 was detected in the gills at both salinities, but in cells different from those with NKA (fig. C and D).

Conclusions

In *L. stylirostris,* larvae are stenohaline and hyperosmoconformer while late postlarvae are euryhaline and hyper-hyporegulator. The acquisition of the adult pattern after metamorphosis, side *L. stylirostris* with the third pattern of osmoregulation as defined by Charmantier (1988). These results are in accordance with the biological cycle of penaeids in their natural environment.
Pleurea and branchiostegite are the only osmocompetent tissues in the branchial cavity which are present in larvae. Epipodites and gills appear after metamorphosis and seem to acquire a major role in osmoregulation in early juveniles.
Ionocytes are localized in epipodites and branchiostegite while in the gills, a cellular complex may be involved in their osmoregulatory function.

During the development of *Litopenaeus stylirostris*, a correlation has been shown between the ontogeny of the osmoregulatory tissues, the occurrence of the hyper-hypo adult pattern of osmoregulation and the ecological requirements of the species for its migration between sea and coastal lagoons.

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