OSMOREGULATION IN THE PACIFIC BLUE SHRIMP *LITOPENAEUS STYLIROSTRIS* IN NEW CALEDONIA : FIRST ATTEMPTS TO ELUCIDATE THE MOLECULAR BASES OF SALINITY ACCLIMATION AND ION TRANSPORT.

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Introduction

Shrimp farming represents the second export business in New Caledonia. However, this industry is facing difficulties since 2006, in part because of a dramatic decrease in the production of post-larvae. The origin of these problems is multifactorial, involving environmental parameters, among which salinity and temperature, two of the most important abiotic factors affecting the growth and survival of aquatic organisms. In New Caledonia, the water is pumped from the lagoon, sometimes at the mouth of rivers or directly from mangrove swamps. The salinity may thus suddenly drop following rainstorms, which occur most frequently during the warm season. However, the effects of salinity changes on the tolerance, survival, and growth of *Litopenaeus stylirostris* remain poorly understood and little is known about ion transport in this shrimp species.

This study was therefore aimed at characterizing and examining in the Pacific blue shrimp the expression of mRNA encoding the catalytic α -subunit of Na+/K+-ATPase (or NKA), a transmembrane protein playing an important role in epithelial cells of most animals, not only to sustain intracellular homeostasis, but also to provide a driving force for many transport systems in a variety of osmoregulatory epithelia.

Materials and methods

Shrimp were grown at the IFREMER laboratory (New Caledonia). Total RNA was extracted and reverse-transcribed to single-stranded cDNA as previously described (Labreuche *et al.*, 2010). Blast analysis of the partial cDNA amplified by PCR revealing high sequence homologies with NKA sequences of other peneids, a new set of primers was designed to obtain the full-length cDNA of the NKA gene in *L. stylirostris*.

Analysis of NKA mRNA expression was performed by real-time quantitative PCR (qPCR) on an ABI 7300 system, following the procedure described in Labreuche *et al.* (2010). The relative mRNA expression levels were determined using the two standard curve methodology (QuantiTect® SYBR Green PCR Handbook) and the elongation factor 1-alpha gene (EF1, acc.n°AY117542.1) was used as the internal reference (normaliser) mRNA.

For immunohistochemistry, shrimp tissue sections were incubated with a rabbit primary antibody NKA alpha- H300sc-28800 (Santa Cruz Biotechnology, Inc. USA) and with a secondary fluorescent antibody TRITC, diluted in PBS-SM 0.5%.

Results

cDNA cloning and sequencing

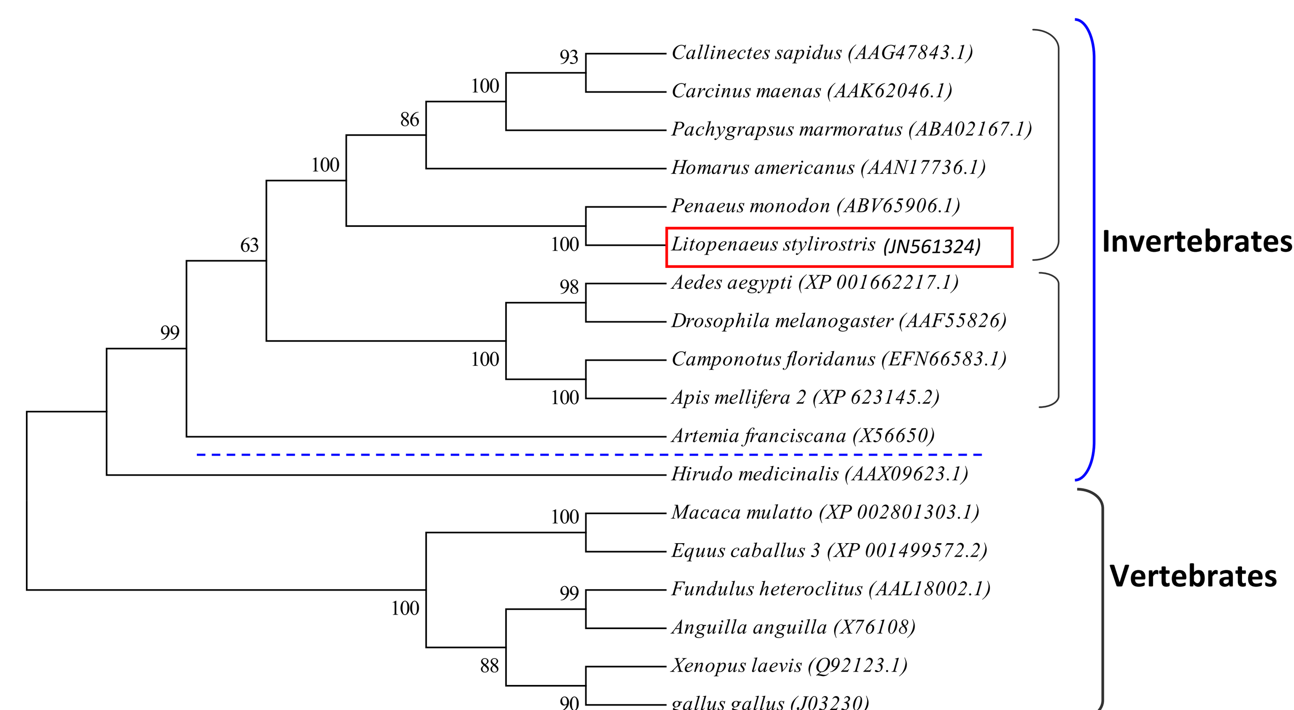
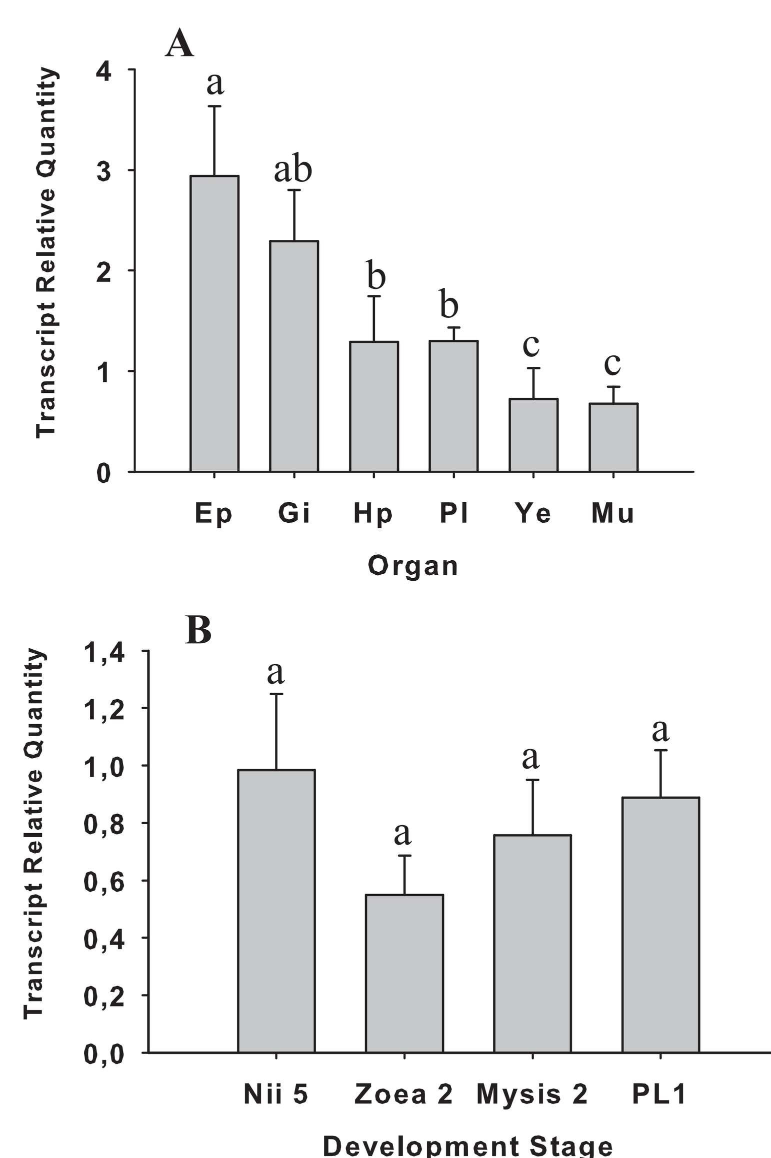


Figure 1: Phylogenetic analysis of NKA acid sequences. A Neighbor-Joining tree was generated using the MEGA 5 software. Bootstrap values from 1000 replications are presented on the branch points. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. GenBank accession numbers are indicated between parenthesis.

A RT-PCR approach allowed us to amplify a 3036-bp cDNA encoding a putative 1011-aa protein. No evidence of additional isoforms or alternative splicing products was observed. Structural analysis of the deduced protein led to a prediction of 10 transmembrane domains, in agreement with previously suggested topologies (Kaplan, 2002), and revealed conserved regions (such as the trademark P-type ATPase motif D364KTGTLT370) possibly involved in ATP binding and hydrolysis. Very close percentage identities were found with NKA aa sequences of other arthropods such as *Penaeus monodon* (99%) or *Homarus americanus* (93%).

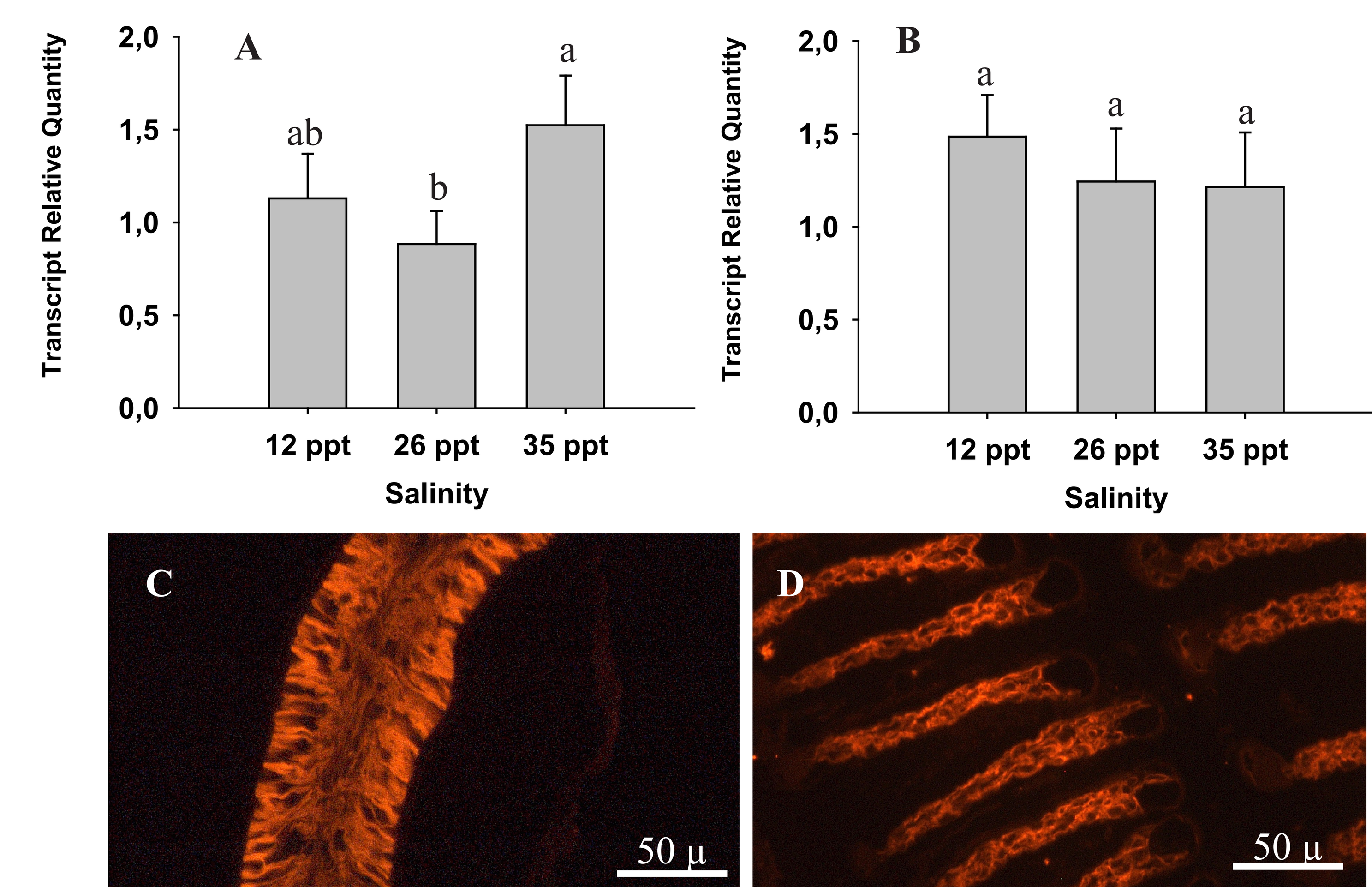
Spatio-temporal NKA mRNAexpression



Figures 2: Quantitative PCR analysis of NKA mRNA transcript abundance (A) in various organs and (B) during the embryonic development of *L. stylirostris* (from Nauplius to Postlarvae 1). Ep: Epipodite; Gi: Gills; Hp: Hepatopancreas; Pl: Pleopods; Ye: Eyestalk; Mu: Muscle; Nii: Nauplius; PL: Postlarvae.

NKA transcripts could be detected by qPCR in all tested tissues, although their expression levels were 2-3 orders higher in epipodites and gills than in other organs. Expression of mRNA was also evidenced during post-embryonic development (from nauplii to postlarvae) but showed higher levels at the first larval stages compared to zoea stage. However, no significant difference was noticed between the different larval stages.

Salinity impact on NKA mRNAexpression



Figures 3 : Quantitative PCR analysis of NKA mRNA abundance in individual epipodite (A) and gill (B) preparations from 7g *L. stylirostris* following transfer from 35ppt to 27 and 12 ppt. Expression values (means ± standard error) are presented as relative abundance in relation to EF1 gene (N=6 to 9 measurements). Different lower-case letters indicate significant difference between treatments. NKA immunofluorescence in epipodite (C) and gills (D) in 25g *L. stylirostris*.

Finally, we measured the abundance of NKA transcripts following salinity changes. Obtained results showed that salinity transfer of shrimp from 35 to 27 ppt was accompanied at 6 h post-transfer by a significant decrease of NKA mRNA levels in the epipodites (P<0,05), while no significant difference occurred after low salinity (12 ppt) transfer. In the gills, NKA mRNA levels increased following transfer at 12 ppt although the results were not statistically different. At 1 and 6 d post-transfer, mRNA levels remained stable in both tested tissues (P > 0.05).

Immunofluorescence data support the results previously obtained by qPCR analysis. Immunoreactive staining appeared to be stronger in epipodites when compared to the gills and was evenly distributed, whereas immunofluorescence was limited to central parts in gills.

Conclusions

This study allowed us to characterize for the first time the gene encoding the NKA in *L. stylirostris* and to evaluate its spatio temporal expression. All the data show that NKA activity is mainly located in the osmoregulatory organs i.e. epipodites ans gills; the former seems to be specialized in ionic regulation while the latter is also devoted to respiration. In short term control, modification of the NKA enzymatic kinetic and/or protein translocation between the cell membrane and storage compartments and/or activation of other transporters could modulate the ionic activity. More molecular studies associated to NKA specific activity measurements will be conducted to determine if de novo synthesis of enzyme occurs in *L. stylirostris.*

References

Labreuche Y, Veloso A, de la Vega E, Gross PS, Chapman RW, Browdy CL, Warr GW. (2010). Dev Comp Immunol; 34(11):1209-18.

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