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Salinity tolerance, ontogeny of osmoregulation and zootechnical improvement in the larval rearing of the Caledonian Blue Shrimp, *Litopenaeus stylirostris* (Decapoda, Penaeidae)

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Abstract:

The ontogeny of osmoregulation was investigated in *Litopenaeus stylirostris* by studying salinity tolerance and osmoregulatory capacity. Shrimp at different larval and postlarval stages were exposed to various salinities and survival was monitored for 24 h. Survival rates exceeded 80% at salinity over 25 ppt (750 mOsm.kg^{-1}) at all the stages. At salinities below to 25 ppt, salinity tolerance was higher in nauplii and zoeae than in mysis larvae. Postlarval stages were able to withstand lower salinities, e.g. 6.0 ppt (176 mOsm.kg^{-1}) at PL9 stage, but they were more sensitive than larvae to salinities over 35 ppt (1035 mOsm.kg^{-1}). Zoea and mysis larvae slightly hyper-regulated at all tested salinities. After metamorphosis, postlarvae progressively acquired the adult pattern of hyper-hypo-osmoregulation: At PL9, the estimated isosmotic salinity was 24.5 ppt (720 mOsm.kg^{-1}); below and over this salinity, animals hyper-regulated and hypo-regulated, respectively. Finally, we determine the effects of lowering salinity at different animal development stages. We conclude that seawater salinity (35 ppt) is optimum during larval stages; but for postlarval stages, lowering salinity at 27 ppt leads to a better growth in 19 days compared to those maintained at 35 ppt (1.07 mg vs 0.47 mg). These results are in agreement with penaeid natural life cycle during which larvae are released in oceanic water while juveniles live in coastal areas where salinity is more fluctuant.

Highlights

► Osmoregulation capacity in *L. stylirostris* is weak in the early life stage. ► Postlarvae progressively acquired the adult pattern of hyper-hypo-osmoregulation. ► At PL9, the estimated isosmotic salinity was 24.5 ppt (720 mOsm.kg^{-1}). ► A two-fold-growth in 19 days-old postlarvae was obtained in dilute seawater compared to seawater conditions.

Keywords: Penaeid ; *Litopenaeus stylirostris* ; Ontogeny ; Osmoregulation ; Growth

1. Introduction

Shrimp farming in New Caledonia is based upon the blue shrimp species, *Litopenaeus stylirostris,* which was imported for the first time in 1970's from Latin America (AQUACOP, 1979). The successful adaptation of the blue shrimp to local conditions and zootechnical improvements have permitted to rear the broodstock in captivity and to reach a production of 2500 metric tons of shrimps in 2005, which was equivalent to 57% of *L. stylirostris* world production (FAO, 2012). However, a 50% decline of the caledonian shrimp production has been recorded since 2009. This loss can be partly attributed to difficulties in producing juveniles in hatcheries, particularly to obtain nauplius during the warm season and unexplained mortalities at different larval rearing periods. At the same time, salinity water in the hatcheries can fluctuate from 20 to 40 ppt depending on the season. Today, a better understanding of key physiological functions of *L. stylirostris* may lead to improvements in technical management to sustain the production of juvenile blue shrimp in New Caledonia.

The ecology of penaeid shrimps has been widely described in the literature (Dakin, 1938; Linder and Anderson, 1954; Fujinaga, 1955; Dall et al., 1990; Bailey-Brock and Moss, 1992). Penaeid juveniles commonly inhabit estuaries with fluctuating environmental conditions while adults of these species migrate in oceanic deep water to spawn. After hatching, *Litopenaeus stylirostris* goes through different larval stages (Kitani, 1985): 6 nauplii, 3 zoeae and 3 mysis stages that live in a stable marine environment. But after the metamorphosis, postlarvae settle close to the coast in estuarine waters (Garcia and Le Restre, 1981). Temperature and salinity are among the abiotic factors that fluctuate in these shallow environments (Kinne, 1963; Kinne, 1964). For penaeid species like *P. merguiensis*, the influence of salinity can have a greater impact on survival than temperature in the early stages (Zacharia and Kakati, 2004).

Osmoregulatory mechanisms allow the adaptation of aquatic animals to salinity changes. But the capacity to osmoregulate differs among crustaceans and Charmantier (1998) has described three patterns of ontogeny of osmoregulation. A first group of species, generally marine and stenohaline, are osmoconformers all their lifetime: *Chionoecetes opilio* (Kalber, 1970), *Libilia emarginata* (Kalber, 1970), *Palunirus* species (Dall, 1974; Lucu et al., 2000) or *Hepatus ephiliticus* (Charmantier and Charmantier-Daures, 1995). A second group of animals, mostly euryhaline, have a strong ability to osmoregulate in their early life stages and a wide tolerance to salinity variations; mostly euryhaline, this pattern includes *Cladocera* species (Aladin and Potts, 1995), *Gammarus duebeni* (Morritt and Spicer, 1995) or *Macrobrachium petersi* (Read, 1984); in the third pattern, the capacity to osmoregulate shifts from weak to strong during the ontogeny. Several penaeid crustaceans belong to this last group including the penaeid shrimp *Peaneus japonicus* (Charmantier, 1986; Charmantier et al., 1988) and the osmoregulatory capabilities of this species during ontogenesis is known. Investigations in *L. stylirostris* have shown that juveniles and adults are hyper-hypo-osmoregulators (Lemaire et al., 2002; Wabete et al., 2006), but no studies were conducted to assess osmoregulation capacities during *L. stylirostris* larval and postlarval developments.

The aim of this study was to investigate the variations in salinity tolerance during the postembryonic development of *L. stylirostris*, to establish the relationship with the changes in the pattern of osmoregulation and to investigate the potential improvements in zootechnical parameters resulting from an adjustement of the larval rearing salinity.

2. Material and methods

2.1. Animals

Larvae and juveniles of the blue shrimp (*Litopenaeus stylirostris*) were supplied by the LEAD hatchery (Ifremer New Caledonia) from June 2009 to May 2010. Breeders were reared during 8 months in earthen ponds, and then transferred into the hatchery. In order to stimulate spawning, water temperature of 29°C, a 14:10 hrs day:night photoperiod and an enriched diet were used. Eyestalk ablation was also practiced on the females and they were artificially inseminated. Nauplii collected on the same day were stocked in a 2 cubic meter tank and mass reared according to Ifremer protocol (Pham, unpublished) : density of 180 larvae per liter, salinity of 35 ppt, temperature of 29°C and 14:10 hrs day:night photoperiod. Microparticles and *Artemia* nauplii were given *ad libitum*. Larvae were treated with antibiotics (2 ppm of erythromicyn) at day 3, 5, 7 and 9 without water renewal. After this period, 50% to 100% of the water was changed every day. Animals were kept in the hatchery for 10 days after becoming postlarvae. Rostral formulae (RF) was used to determine the postlarval stage: for instance, a RF of [3-0] meant that the numbers of spines over and under the rostrum were 3 and 0, respectively; postlarvae were designated with reference to the stage order (PL1, PL2, etc…) or to the number of days following metamorphosis (P1, P2, etc…). Correspondences between the postlarval stage, the number of days after metamorphosis and the rostral formulae are given in Table 1 (Goarant et al., 1998; Pham, unpublished data).

2.2. Preparation of media

Tests to salinity exposure are commonly used to evaluate the shrimp larval quality predicting further performances of growth and fitness in grow out ponds (AQUACOP et al., 1991; Tackaert et al., 1989; Samocha et al., 1998; reviewed by Palacios and Racotta, 2007). Media at different salinities were prepared from 5 µm-sterilized seawater by adding sterilized and demineralized freshwater or marine salt (Sera, Germany). The media were prepared and stored over one week at most in a dark room at 24 °C. Each medium was checked with an osmometer (WESCOR Vapro 5520, USA) for osmolality expressed in $mOsm.kg^{-1}$. Osmolality standard solutions of 290 and 1000 $mOsm.kg^{-1}$ (WESCOR, USA) were used to calibrate the osmometer. Conversion in salinity was done with 1 ppt equivalent to 29.41 $mOsm.kg^{-1}$. Different salinities were tested depending on the larval stages: at nauplius (10; 14; 19; 22; 24; 27; 31; 36.5; 42; 46 ppt); at zoea 2 (19; 20; 22; 25; 26; 31; 33; 36.5; 42; 46 ppt); at mysis 2 (19; 20; 22; 25; 30; 36.5; 42; 46 ppt); at PL1 (17; 18,5; 19; 22; 24; 25.5; 30.5; 35; 42; 46 ppt); at PL4 (14; 16; 18; 21; 26; 30.5; 36.5; 42; 46 ppt); at PL9 (0; 5; 6; 12; 18; 25; 30.5; 38; 41; 46 ppt).

2.3. Salinity tolerance

Animals were collected at the same time from larval rearing tanks at different stages: nauplii, zoea 2, mysis 2, PL1 (P2), PL4 (P8) and PL9 (P25). Eight to ten salinities were tested at each stage in three replicates: ten animals were transferred directly in 30 ml conical centrifuge tubes containing the experimental media. For PL9 stage, only 5 animals were placed in 100 ml containers in 6 replicates. Control animals were kept in seawater salinities (35 to 38 ppt). All the containers were kept in a thermoregulated chamber at larval rearing temperature (29°C). No food was given during the experiment.

Dead animals were counted every hour during the first 6 hrs and then at 24 hrs after immersion. Animals were considered as dead when no heartbeat and lack of movement after stimulation were observed. Lethal salinities for 50% of animals at 6 hrs (6 h LS50) and at 24 hrs (24 h LS50) were estimated.

Statistical comparisons were performed between survival rate at each stage according to salinity at 6 hrs and 24 hrs separately. One-way analysis of variance was used to detect the differences in survival rates after data arcsin(√) transformation with Statview sofware. Student's *t*-tests were used to resolve differences among treatment means with $\alpha = 0.05$.

2.4. Osmoregulation

In order to determine the hemolymph osmolality at different stages and different salinities, the animals were stocked in the same way as in the tolerance test. Five to six salinities (from 6 to 45 ppt) were used for larvae and postlarvae at 29°C.

A minimum adaptation time of 4 hrs was allowed after direct transfer of the animals into the considered salinity prior to the measurements of hemolymph osmolality. Before sampling hemolymph, animals were dried with filter paper and immersed into mineral oil to avoid evaporation. Hemolymph was obtained by inserting a micropipette into the animal heart. Hemolymph osmotic pressure was obtained by measuring the freezing point depression with a nanoliter osmometer (Otago osmometers, New Zealand) requiring 10 to 30 nanoliter of sample. Measurements were conducted at least on three animals for each salinity and each stage.

For each stage and salinity tested, the osmoregulatory capacity (OC), defined as the difference between the hemolymph osmolality and the medium osmolality, was calculated.

2.5. Larval and postlarval rearing experiments

Four successive experiments were conducted to determine salinity impact on larval and postlarval rearing. Nauplius (Nii) hatched on the same day were gathered together in a ten-liter bucket and the number of larvae was estimated with five samples of one ml. Each tank of 80 to 150 liters was stocked at a density of 167 to180 nauplii per liter. The larval rearing was conducted as described above. Temperature, salinity, survival and growth were controlled every day. Survival was estimated by counting the number of larvae in 100 ml in three to five replicates. From Nii to PL1, a development index (DI) was calculated by using the stage index according to Villegas and Kanazawa (1980); a rank was attributed to each larval stage (0 to Nii, 1 to Zoea 1…till 7 to PL1) weighted by the percentage of animal at each stage in a day. After PL1, growth was estimated with the percentage of each rostral formula in a 30 PL sample and/or the individual mean dry weight in 100 individuals for each replicate. A preliminary experiment was done to test the effect of a direct transfer from seawater to six salinities (20, 25, 28, 31, 35 and 39 ppt with no replicate) during the larval phase, from nauplius to mysis for 8 days.

The second trial was conducted during 15 days to determine if postlarval survival can be affected by salinity variation during larval phase. The experiment started at nauplius stage with the three salinities which have obtained the best results in the first experiment (28, 30 and 35 ppt); these salinities tested in duplicates were maintained until day 9 and then, a 50% renewal water was operated at D9, D11, D13 and D14 with seawater (35 ppt) till PL3-4.

The third and fourth experiments were assessed to test the effect of different salinities in postlarval period. The third one started with two salinities (30 and 35 ppt) in twelve replicates from D0 (Nii) to D9 (mysis-PL). Due to high mortality in tanks at 30 ppt, only 35 ppt treatment was kept after D9 and the twelve tanks were shared in four groups with renewal water at different salinities in triplicates between D9 and D20: 24 (treatment 35-24), 27 (treatment 35-27), 30 (treatment 35-30) or 35 ppt (control treatment).

The fourth experiment was conducted for a whole larval rearing period (32 days). From D0 to D9, 6 tanks were filled with seawater at 35 ppt. At D9, the salinity of 3 of the tanks was gradually lowered to 27 ppt (treatment 35-27) while the 3 other tanks were maintained at 35 ppt (treatment 35-35). At D19, the animals of each tank were harvested and transferred to another tank for 12 more days at a lower density of 20 PL/liter. The PL from treatment 35-35 were divided up for the last period of the experiment to 3 tanks at a salinity of 27 ppt (treatment 35-35-27) and 3 at 35 ppt (treatment 35-35-35). The PL from treatment 27-27 were also transferred but salinity was maintained at 27 ppt (treatment 27-27-27).

Statistical comparisons were performed by using oneway analysis of variance to detect the differences in survival rates after data arcsin($\sqrt{}$) transformation and by non parametric analysis of variance (Kruskal-Wallis ANOVA) for osmoregulatory capacity with XLS Stat 2011 sofware. Probability limit of $p \leq 0.05$ was considered as significant.

3. Results

3.1. Salinity tolerance

Salinity tolerance varied according to development stages (Fig.1). Nauplii survival was higher than 80% for salinities > 26.7 ppt (785 mOsm.kg^{-1}) after 6 and 24 hrs. At 19.3 ppt (569 mOsm.kg^{-1}), significant mortality was observed after 6 hrs and only 30% survived after 24 hrs (Fig. 1A). At 42 and 46 ppt, no or low mortality was observed at this larval stage but higher activity was noticed in these conditions.

In zoea stage, survival was close to 80% from 21.8 to 45.7 ppt (641 to 1345 mOsm.kg^{-1}) after 24 hrs. But at 20.2 ppt (595 mOsm.kg^{-1}), only 13% of the zoea 3 were alive at 6 hrs and all were dead at 24 hrs (Fig. 1B). Significant mortalities were also observed at salinities above 42 ppt with a loss between 30 and 40% after 24 hrs. Salinity tolerance in zoea 2 was similar to zoea 3 (not illustrated).

In mysis 2, survival rate exceeded 80% after 24 hrs at salinities > 26.3 ppt (775 mOsm.kg^{-1}). At 24.5 ppt (723 mOsm.kg^{-1}), survival declined and only 53% of the specimen were alive after 24 hrs. At 21.8 ppt (641 mOsm.kg^{-1}), all the mysis were dead within 1 hour (Fig. 1C). After 24 hours at 46 ppt, low but significant mortality was noticed compared to 35 ppt.

In PL1 (P2), survival was over 80% at salinities > 25.5 ppt (750 mOsm.kg^{-1}). At 21.6 ppt (636 mOsm.kg^{-1}), only 10% and 3% of animals survived after 6 hrs and 24 hrs, respectively. At 17.1 ppt (503 mOsm.kg^{-1}), total mortality occurred within 1 hr (Fig. 1D). No significant difference in survival rate was observed at salinities above 42 ppt compared to control treatment but a hyperactivity and abnormal behaviour were detected.

At PL4, survival was close to 100% after 6 hrs from 20.8 to 45.7 ppt (612 and 1345 mOsm.kg^{-1}). This high survival rate was maintained after 24 hrs except at 45.7 ppt (1345 mOsm.kg^{-1}) where survival was 63%. Only 43% of the animals survived at 18.1 ppt (532 mOsm.kg^{-1}) after 6 hrs and 24 hrs (Fig. 1E). Survival rates were only lower than 60% after 24 hrs at 46 ppt in PL4.

At PL9, animals could stand osmolarity variations from 6 to 45 ppt (175 to 1350 mOsm.kg^{-1}); 93% of shrimp were still alive one day after immersion at the lowest salinity. In media more concentrated than seawater, 30% of mortality was observed after 24 hrs (Fig. 1F). At salinity above 41 ppt, low but significant mortality was recorded after 24 hrs and even after 6 hrs for the PL9. In all cases, animals showed abnormal behaviour, particularly hyperactivity.

Values of lethal salinities (LS50) at 24 hrs are given in Fig. 2. LS50 was 22 ppt in nauplii. In zoea stages, the LS50 were 22 and 21 ppt. The highest LS50 was recorded in mysis stages with a value of 25 ppt. LS50 were 23 ppt, 19 ppt 17 ppt and 3 ppt in PL1, PL2, PL4 and PL9, respectively.

3.2. Osmoregulation

Variations of hemolymph osmolality vs external osmolality and salinity are illustrated in Fig. 3A; the corresponding osmoregulatory capacities (OC) are given in Fig. 3B.

Larvae, zoeae and mysis slightly hyper-osmoregulated over the range of tolerable salinities from 668 to 1347 $mOsm.kg^{-1}$ (Fig. 3A). OCs ranged from 0 to 150 $mOsm.kg^{-1}$. The OC values were significantly lower at high salinities in mysis stages. A progressive change in pattern of osmoregulation was observed following metamorphosis, in postlarvae. PL1 slighty hyper-hypo-osmoregulated. Hyper-OCs were below 50 $mOsm.kg^{-1}$, with hypo-OCs ranging from -10 to -100 $mOsm.kg^{-1}$ (Fig. 3B). At 971 $mOsm.kg^{-1}$, OC was close to zero. The capacity to hyper- and hypo-osmoregulate increased slightly in PL4 (hyper-OC, 50-100 $mOsm.kg^{-1}$; hypo-OC, -100 to -200 $mOsm.kg^{-1}$) with a shift between 680 and 851 $mOsm.kg^{-1}$. In PL9, the OC was amplified; at this stage, hyper-OC was 200 $mOsm.kg^{-1}$and up to 380 $mOsm.kg^{-1}$ at the lowest salinity (6 ppt) where no mortality was observed. A similar trend was noted for hypo-OC that reached –580 $mOsm.kg^{-1}$ at the highest tested salinity of 46 ppt. All the OC were significantly different at this stage except at 363 and 530 $mOsm.kg^{-1}$. The isosmotic point was determined from the intersection between the isosmotic line and the osmoregulation curve in postlarval stages, and it was 720 $mOsm.kg^{-1}$ (24.5 ppt) at PL9 (Fig. 3A).

3.3. Larval and postlarval rearing experiments

In the first experiment (Fig. 4), survival rates in nauplius stages were above 80% at 20 and 25 ppt only the first day. Then they quickly decreased and at D3, total mortality was observed at these two lowest salinities. At salinities of 28 ppt or above, the survival rate was above 70% at D7 and DI around 4 was measured in all media.

In the second experiment (Table 2), larval survival rates at 28 ppt, 30 ppt and 35 ppt were 56.2%, 64.4% and 80.8%, respectively after 15 days. However, development was better in the lower salinity media with 20% of PL4 and 80% PL3 while all the animals were still in PL3 in the other treatments.

In the third experiment (Fig.5), the evolution of the survival rates was similar for the 30 ppt and 35 ppt treatments until D4, i.e. at D3, there were 87 and 90%, respectively. From D5, survival rate decreased in the 30 ppt treatment and was only 53% at D7 while it was still above 85% in the 35 ppt treatment. Growth was significantly lower in dilute seawater (DI=2.1$\pm$0.15) than in seawater (DI=2.8$\pm$0.28) only at D3; this difference was wider but not statistically different at D7 since DI were 3.80$\pm$1.22 and 5.18$\pm$0.98 for 30 ppt and 35 ppt, respectively. Mortalities were observed in 10 of the 12 replicates at 30 ppt so, all the tanks of this treatment were eliminated. At D9, water renewal at different salinities was conducted until D19, and the best survival rate (84%) was obtained in the 35-35 treatment, while the lowest one (73%) was observed for the 35-24 treatment (table 3). Final mean weights were between 0.47 mg (35-30 treatment) and 0.60 mg (35-24 treatment) and the percentage of PL4 was above 78% for all the treatments. No significant difference was observed for any of these parameters among the different treatments (α=0.05).

The fourth experiment (Table 4) started with 6 tanks at 35 ppt until D9. The mean survival rate at D9 was 85% with a DI of 5. At D19, 35-27 treatment and control treatment (35-35)

showed 56.6% and 47.6% survival, respectively. The percentage of PL4 and the mean dry weight were also higher in the dilute seawater but the differences were not significant at this stage. In the last period of the experiment, the mean survival rates were respectively 97.2%, 89.4% and 84.2% for the 35-27-27, 35-35-27 and 35-35-35 treatments, with no significant difference. On the other hand, significant differences in growth were obtained between the animals maintained at 27 ppt from D9 (35-27-27 treatment) and the 35-35-35 treatment with 1.07 mg and 0.43 mg, respectively (Fig. 6). The difference were also significant in development stage between the two treatments with 52% of PL5 in dilute seawater while only 11% of PL5 or more was observed in the control group. The 35-35-27 treatment has obtained intermediate results but none of them was significantly different from the two others treatments.

4. Discussion

Tests to salinity exposure are commonly used to evaluate the shrimp larval quality predicting further performances of growth and fitness in grow out ponds (AQUACOP et al., 1991; Tackaert et al., 1989; Samocha et al., 1998; Racotta et al., 2003; Palacios and Racotta, 2007). Under the present experimental conditions (starvation for 24 hrs), a salinity shock is a very stressful test for penaeid at early stages but it permitted to determine the critical periods in the early development of *L. stylirostris*. In this study, tolerance to an acute salinity change varied with the developmental stage, and from nauplius to PL1 the salinity tolerance decreases. An increase of 2 ppt between 17 and 25 ppt was applied to detect the differences of sensitivity among the stages. The LS50 at 24hrs in nauplii, zoeae, mysis and PL1 were 23 ppt, 22 ppt, 25 ppt and 23 ppt, respectively. In other decapod crustaceans as *Armases miersii* (Anger, 1996), *P. japonicus* (Charmantier et al., 1988), *Carcinus maenas* (Cieluch et al., 2004) or *Cangron cangron* (Cieluch et al., 2005), a lower salinity tolerance was also reported for stages close to metamorphosis. The lower salinity tolerance of mysis compared with nauplius or zoea stages could be explained by a higher energy expenditure with the shift from a herbivorous feeding regime to a carnivorous one, some anatomical changes and the occurrence of incomplete osmoregulatory functions as we observed. A nutritional hypothesis could also explain a higher resistance in nauplius compared to others larval stages. During the early development, a larva has important energy requirements supplied by the yolk at nauplius stage; but in zoea and mysis stages, food supply is necessary and starvation for 24 hrs could be an additional stress.

In postlarvae older than PL1, tolerance to lower salinity range was observed while resistance to high salinity slightly decreased. A study of the salinity preference of four *Penaeus* species -including *L. stylirostris* has shown that postlarvae were more attracted by low salinity waters (Mair, 1980). Juveniles of some *Penaeus* species were found to tolerate lower salinities than adults (Dall, 1981). An improvement in salinity tolerance throughout postlarval development from 2 day-old postlarvae (5% of survival after 2 hrs at 20 ppt) to 20 day-old postlarvae of *Litopenaeus vannamei* (90% of survival after 2 hrs at 0 ppt) has also been reported by Aquacop et al. (1991).

Similar data on the ontogeny of osmoregulation in young stages are also available in another penaeid species *P. japonicus* (Charmantier et al., 1988). In this species, mysis were also found to be the most sensitive stages to salinity variations, and hyper-hypo-osmoregulation was gradually established after metamorphosis. Through transmission electronic microscopy observations, Bouaricha et al. (1994) have shown that osmoregulatory organs were progressively implicated in ionic regulation during postembryonic development of this species: the branchiostegites and pleurae at zoea 2 then, the epipodites in postlarval stages.

L. stylirostris is a slight hyper-osmoregulator close to osmoconformity in the larval stages at any tested salinity. After metamorphosis, the osmoregulation pattern changes progressively towards hyper-hypo-osmoregulation. Compared to previous results in adults (Wabete et al., 2006), this change is achieved at PL9 (25 day old postlarvae at 29°C). At salinities ranging from 6 to 45 ppt, PL9 can maintain the hemolymph osmolality values between 550 and 800 $mOsm.kg^{-1}$ and the isosmotic point is 720 $mOsm.kg^{-1}$. These values are slightly lower than those obtained in the same species by Lemaire et al. (2002) and Wabete et al. (2006) on 10 g subadults at 28°C and 40 g adults at 26.7°C with an isosmotic point at 735 $mOsm.kg^{-1}$ and 756 $mOsm.kg^{-1}$, respectively. However, these results are in agreement with the previously observed increase of haemolymph osmotic pressure with the body weight in the same species (Lignot et al., 1999b). Isosmotic points were evaluated in *Palaemon pacificus*, *Exopalaemon orientalis*, *Metapenaeus joyneri* and *Penaeus semisulcatus* with values of 648, 653, 649 and 412 $mOsm.kg^{-1}$ respectively (Dissanayake and Ishimatsu, 2011) at 22°C. And the authors have also shown that the two penaeids were less tolerant to low salinity of 10 ppt after 2 days compared with the two palaemonids. With a higher isosmotic point than the previous species, the blue shrimp juveniles were able to tolerate 5 ppt for one day in our experiment. In other trials, it was noticed that the same species juveniles could stand salinity as low as 3 ppt for 5 days (Pham, not published) at the optimum temperature of 28°C.

L. stylirostris may be associated to the third pattern of osmoregulation capacity as defined by Charmantier (1998). In this group, the early postembryonic stages are osmoconformers or they slighty osmoregulate, Thereafter, a shift occurs at the metamorphic larva-juvenile transition and the animals become progressively hyper-hypo-osmoregulators as the adults do. Studies conducted on other decapods have revealed similar ontogeny of osmoregulation in *Homarus americanus* (Charmantier et al., 1984; 1988; 2009), *Penaeus japonicus* (Charmantier, 1986; Charmantier et al., 1988), *Homarus gammarus* (Thuet et al., 1988), *Cancer irroratus* (Charmantier and Charmantier-Daures, 1991), *Carcinus maenas* (Cieluch et al., 2004) and *Crangon crangon* (Cieluch et al., 2005), despite different types of habitat or migration. However, in the crab *C. maenas*, osmocomforming zoea stages can tolerate salinity as low as 15ppt; this ability may be an ecological adaptation as the eggs hatch in the same habitat as the one occupied by adults and characterized by low and fluctuant salinities (Cieluch et al., 2004). In penaeid shrimps, adults migrate to oceanic water to spawn, where salinity conditions are stable and low salinity tolerance is not necessary. After metamorphosis, the progressive shift to a juvenile/adult type of osmoregulation allows the postlarvae to enter estuaries where salinity is more variable than in open sea.

In conditions close to larval rearing productions, *L. stylirostris* larvae have shown best survival rates and reliable results at salinities of 35 ppt compared to lower salinity media. In the same species, Marty-Ordonez (1972) has shown that breeders had a preference for high salinities around 35-38 ppt and best survival rates at first postlarval stage were also obtained at seawater salinity. In a review on penaeid biology, Rothlisberg (1999) concluded that oceanic salinities were necessary to obtain good growth and survival of *P. merguiensis*. This result was confirmed by Zacharia and Kakati (2004) who observed that a salinity range of 30-35 ppt was optimum for larval development of this species. Several works about other penaeid species such as *P. kerathurus* (Klaoudatos, 1978), *P. plebejus*, *M. macleayi* (Preston, 1985), *M. ensis* (Chu and So, 1987), *P. penicillatus*, *M. affinis*, *P. monodon* (Parado-Estepa et al., 1993) or *P. stylifera* (Nisa and Ahmed, 2000) have confirmed that a salinity above 30 ppt was the best for larval development. This disability to tolerate dilute seawater before metamorphosis is linked to the osmoconformity of the regulation pattern at this development stage and which can upset nutritional processes. For instance, larvae of different crab species exposed to salinity reduction have shown lower food assimilation; moreover, biochemical composition of the osmoconformer *Cancer paragus* was subject to higher variations than the hyper-regulator *C. granulata* when they were transferred in dilute seawater (Torres et al., 2002).

On the other hand, larval rearing in dilute seawater after PL1 has shown consistent improvement in growth compared to animals maintained at 35 ppt during all the post-embryonic development. Without reaching the isosmotic point estimated at 24-25 ppt, a two-fold growth was obtained only by decreasing salinity at 27 ppt from the 9th day of larval rearing. These results are in agreement with those observed in *Fenneropenaeus paulensis* with a better growth at 25 ppt compared to 34 ppt (Lemos et al., 2001). In contrast with larval stages, the same authors suggested a lower food assimilation after metamorphosis in seawater salinity to explain the growth reduction as well as an increase in metabolic demand which would result in lipid catabolism. For other species such as *L. vannamei*, *P. setiferus*, or *P. schmitti*, a lower salinity, between 15 and 25 ppt, also seems to be optimal for their growth (Castille and Lawrence, 1981; Boyd, 1989). And Dall (1981) even noticed that juveniles of some *Penaeus* species were found to tolerate lower salinities than adults.

With temperature, salinity is the most important environmental factor which affect the aquatic species metabolic rate. Animals reared in salinity conditions close to their isosmotic point can take advantage of the situation by saving energy, devoted to ionic regulation, for growth. Oxygen consumption and ammonia excretion are often used as indicators to estimate the energy metabolism in crustaceans and Zang et al. (2009) have shown that in *L. vannamei* juveniles, their values were lower at 5 ppt compared with 25 ppt while O/N ratio was higher. On the other hand, in the adult chinese mitten crab *Eriocheir sinensis*, oxygen consumption increases but the ammonia excretion decreases with salinity and the food consumption is not affected by salinity (Normant et al., 2012). This difference of effect on bioenergetic parameters can be explained by higher tolerance to salinity variation in the brachyuran crabs compared to penaeids. Optimum and adaptation to salinity conditions depend on both species and considered developmental stage.

5. Conclusion

By optimizing media salinity, shrimp can stand up to a greater variation of other abiotic factors such as temperature (Kumlu et al., 2000, Zacharia and Kakati, 2004) or oxygen concentration (Rosa et al., 1997). Based on the results presented here in larval rearing of *L. stylirostris* in New Caledonia, it is now recommended to maintain seawater salinity from nauplius to PL1 and then, to progressively lower salinity by renewing with desalted water. These results and the subsequent procedures are in accordance with the biological cycle of penaeids in their natural environment. Spawning occurs in offshore seawater where larvae are not exposed to salinity variations. The time for juveniles to reach the coastal and estuarine zones depends on several external parameters such as oceanic circulation, light or temperature, but also on intrinsic factors such as growth rate or behavior of larvae (Calderon-Aguilera et al., 2003). Before reaching the coastal nursery areas, shrimps must have acquired a high capacity to osmoregulate to withstand salinity variations.

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Tables

Table 1 : *Litopenaeus stylirostris.* Relation between rostral formulae, postlarval stage and age reared at 29°C.

Rostral formulae	**Postlarval stage Stage number (PLn)**	**Age of postlarvae Number of day after metamorphosis (Pn) at 29 °C**
1/0	PL1	P1 to P3
2/0	PL2 and PL3	P4 to P7
3/0	PL4 and PL5	P8 to P12
4/0 and 4/1	PL 6	P13 to P14
5/0, 5/1 and 5/2	PL 7	P15 to P18
6/0, 6/1 and 6/2	PL 8	P19 to P22
7/2 to 9/4	PL 9	> P22

Table 2 : *Litopenaeus stylirostris.* Survival rate and percentage of PL3/PL4 stage according to larval rearing salinity for each replicate.

Treatment (ppt)	**28**		**30**		**35**	
Replicate number	1	2	1	2	1	2
Survival rate (%)	66.9	45.6	67.9	61	85.5	76.2
% [PL3 / PL4]	80/20	80/20	100/0	100/0	100/0	100/0

Table 3 : *Litopenaeus stylirostris.* Survival rate (mean± SD), percentage of PL3/PL4 stage and mean dry weight (mean± SD) according to postlarval rearing salinity.

Treatment	**35-24**	**35-27**	**35-30**	**35-35**
Number of replicates	3	3	3	3
Survival rate at D19 (%)	73.1 ± 1.9	77.3 ± 4.7	79.8 ± 13.2	84.1 ± 6.4
% [PL3 / PL4]	6/94	22/78	12/88	22/78
Mean dry weight at D19 (mg)	0.60 ± 0.03	0.59 ± 0.04	0.47 ± 0.21	0.62 ± 0.07

Table 4 : *Litopenaeus stylirostris.* Survival rate (mean ± SD), percentage of PL3/PL4 stage (mean ± SD) and dry weight (mean ± SD) according to postlarval rearing salinity.

Treatment	**35-27**	**35-35**
Number of replicates	4	4
Survival rate at D19 (%)	56.6 ± 21.4	47.6 ± 17.7
Percentage of PL4 (%)	72.5 ± 5.1	45.7 ± 19.3
Mean dry weight at D19 (mg)	0.24 ± 0.05	0.20 ± 0.06

Figures

Figure 1 : *Litopenaeus stylisrostris*. Survival rates at 6 and 24 hrs according to the media osmolality and the developmental stages (A-F). Values are means ± SD (n=3). Different letters indicate significant differences between survival ($p < 0.05$) at 24 hrs.

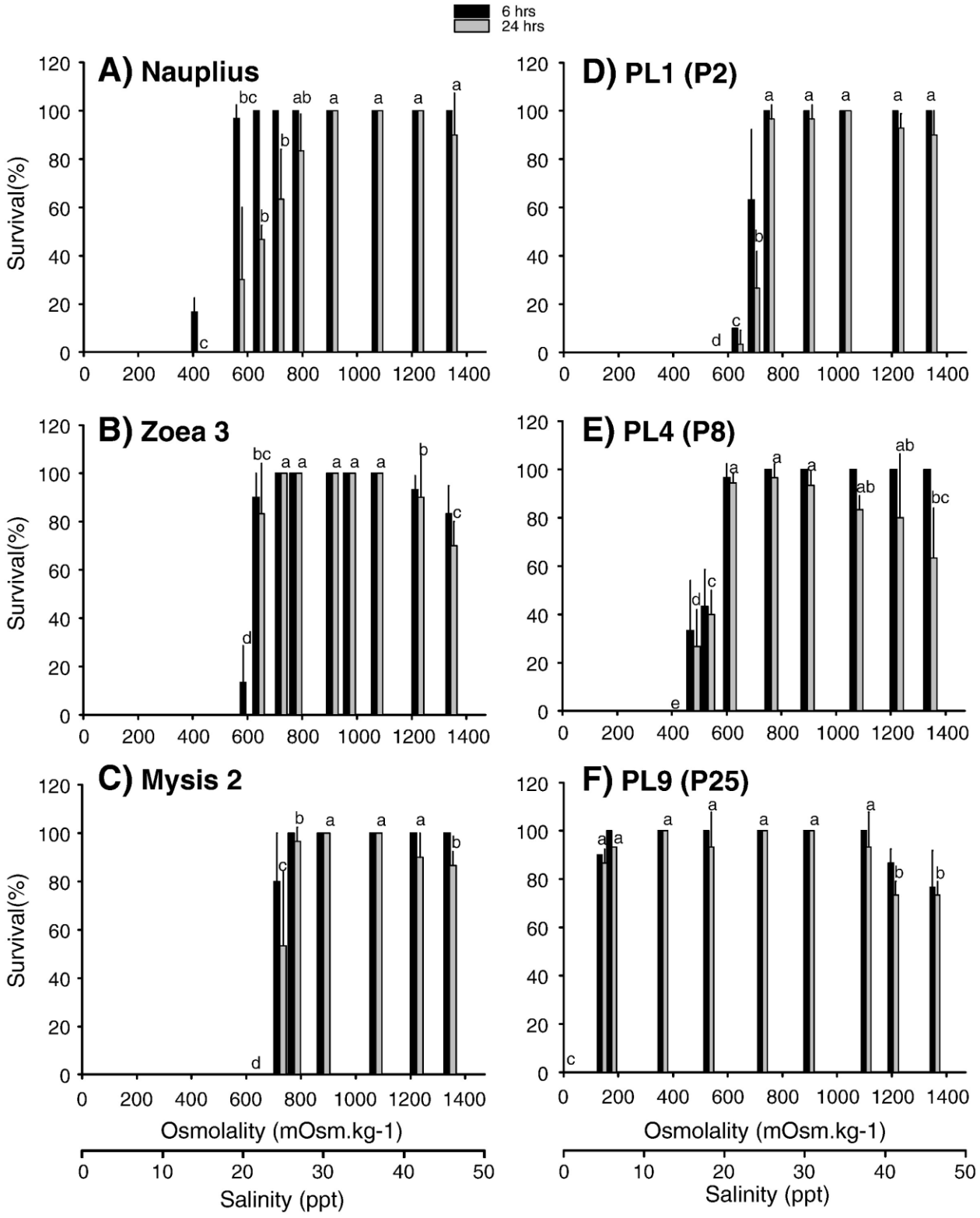


Figure 2 : *Litopenaeus stylisrostris*. Lethal salinity for 50% of animals at 24 hrs according to the development stage. Nii: nauplii, Z: zoea, M:mysis, PL: post-larvae.

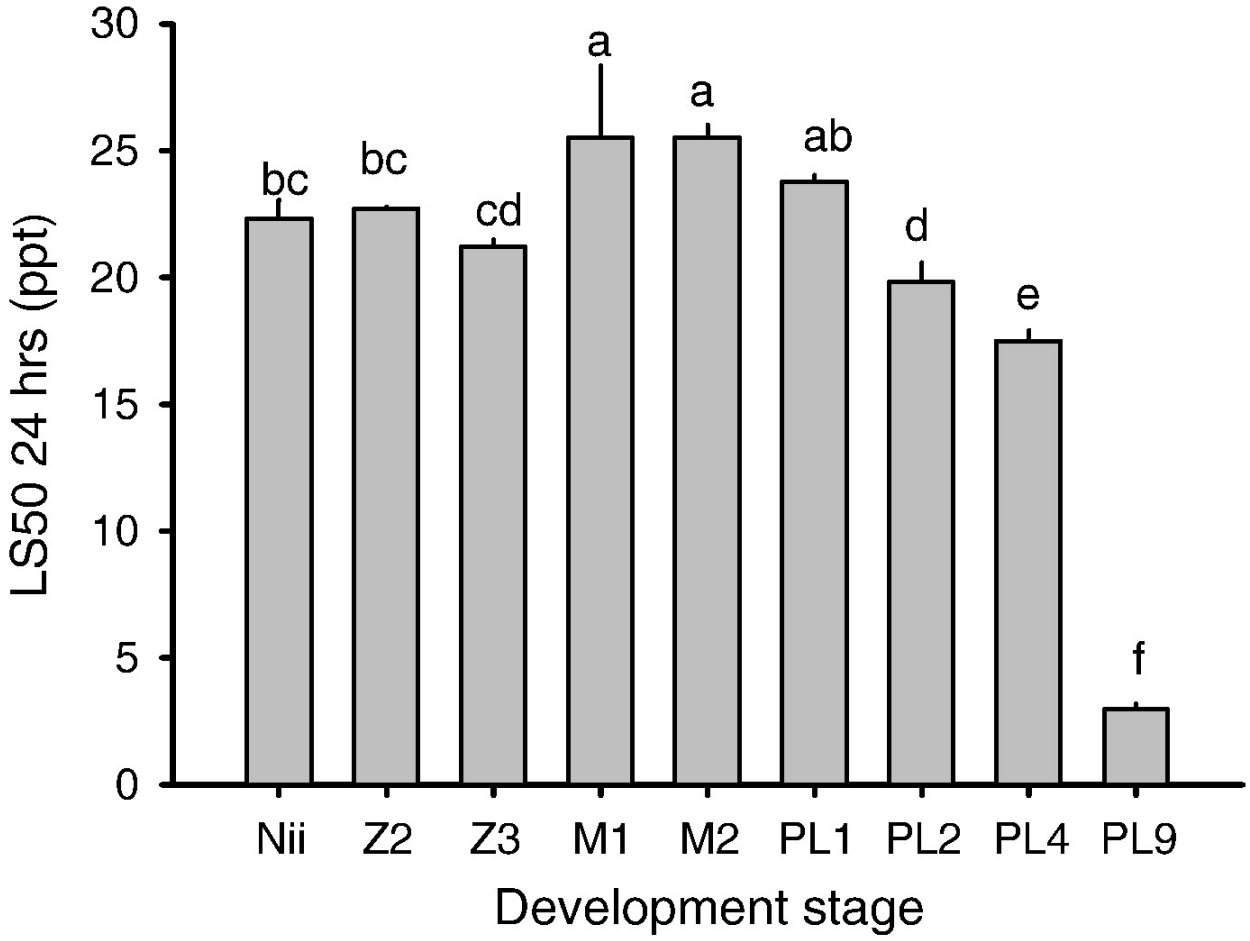


Figure 3 : *Litopenaeus stylisrostris*. A: Variations of hemolymph osmolality in selected stages of development in relation to the osmolality of the external medium; diagonal dashed line: isosmotic line. Values are means ± SD (n=3 to 10). B: Variations in osmoregulatory capacity at different stages of development in relation to the osmolality of the external medium. Values are means ± SD (n=3 to 10). Different letters indicate significant differences between osmoregulatory capacity ($p < 0.05$) for each stage.

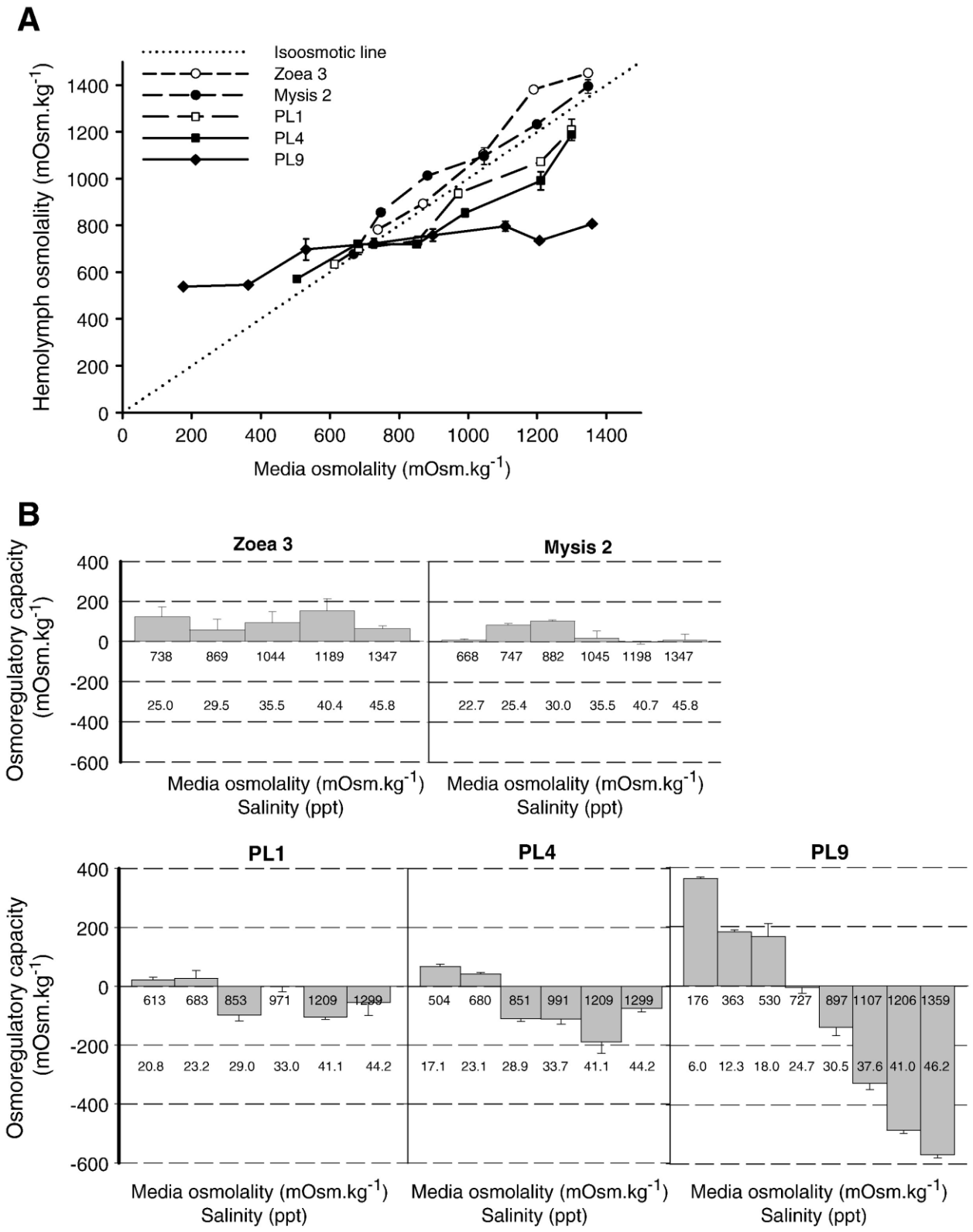


Figure 4 : *Litopenaeus stylisrostris*. Evolution of survival rate according to the rearing day (D) after direct transfer of nauplii from 39 ppt to different experimental salinities.

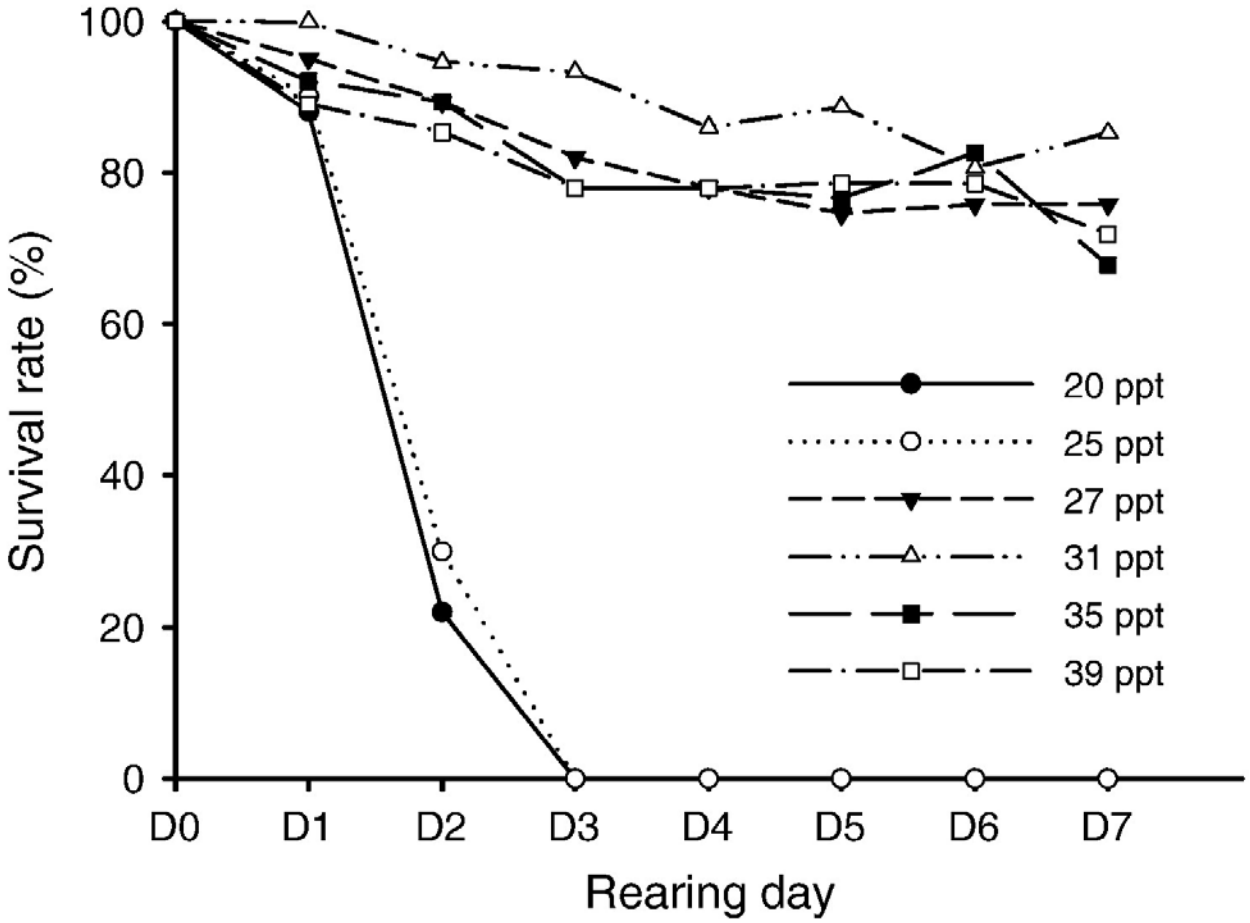


Figure 5 : *Litopenaeus stylisrostris*. Survival rate (S%)and the development index (DI) according to two rearing salinities (30 and 35 ppt) during the larval phase. * indicates significant differences between rearing salinities.

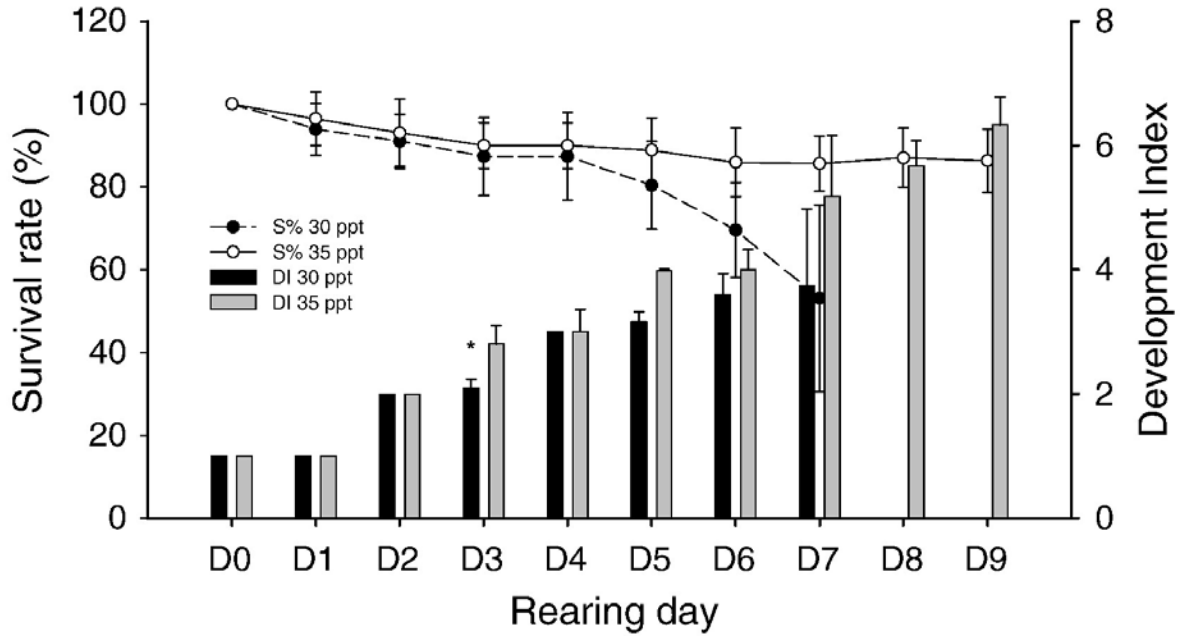
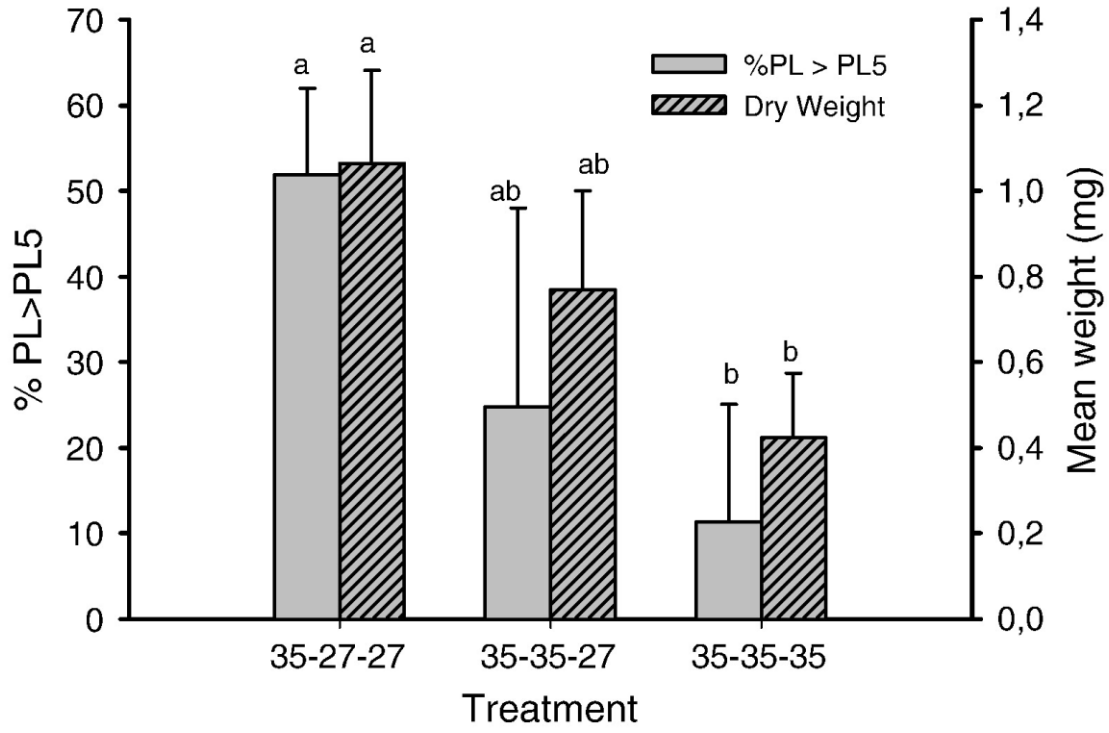


Figure 6 : *Litopenaeus stylisrostris*. Percent of postlarvae at PL5 stage or beyond and mean dry weight of postlarvae at the end of the larval rearing (32 days) after exposure to different culture conditions. 35-27-27: day 0 to 9 at 35 ppt, day 9 to 32 at 27 ppt; 35-35-27: day 0 to 19 at 35 ppt, day 19 to 32 at 27 ppt; 35-35-35: day 0 to 32 at 35 ppt. Bars sharing different letters are significantly different ($p < 0,05$).



List of symbols and abbreviations

6 h LS50 : Lethal salinities for 50% of animals at 6 hours
24 h LS50 : Lethal salinities for 50% of animals at 24 hours
OC : Osmoregulatory Capacity
PBS : Phosphate-Buffered Saline
PLi : Postlarvae on stage i
Pj : j day-old postlarvae after metamorphosis
ppt : part per thousand
RF : Rostral Formulae