

## Nursery performance of the pink shrimp *Farfantepenaeus paulensis* (Crustacea: Decapoda: Penaeidae) postlarvae in different salinities

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**ABSTRACT.** Nursery performance, development, and RNA:DNA ratio were investigated in *Farfantepenaeus paulensis* (Pérez-Farfante, 1967) postlarvae acclimated from a salinity of 30‰ to higher (35‰) or lower (16, 22 and 29‰) salinities and reared for 20 days. Overall, higher final weight, yield and growth rate were observed at a salinity of 29‰. RNA:DNA ratio indicated reduced growth potential at a salinity of 35‰. Low salinities resulted in more developed individuals. Thus, early postlarval *F. paulensis* should not be stocked in salinities higher than that of the original hatchery, otherwise in lower salinities postlarvae should be older and/or have an extended nursery phase. Results may assist in the development of nursery rearing protocols for *F. paulensis*, an alternative species for aquaculture in subtropical areas.

**KEY WORDS.** Aquaculture; crustacean; development; growth.

Shrimp culture requires efficient use of resources in the various stages of the production chain. The nursery phase of shrimp culture is used to manage survival and growth of individuals between hatchery and final growout, and has been recognized as an important strategy to shorten pond production cycles as well as to regulate postlarval flow from hatcheries to farms (PERSYN & AUNGST 2006). Suitable rearing practices during the production cycle, for example, salinity control, may increase survival and growth because performance of euryhaline species, such as penaeid shrimp, may differ between life stages (MOURENTE & RODRÍGUEZ 1997, ANGER 2001).

Life cycles of most reared penaeid species include increased osmoregulatory capacity after penetrating into inshore brackish water areas at early postlarval stages (DALL *et al.* 1990). Though adapted to support salinity variations, optimal performance of early postlarvae may be achieved at definite salinity levels, which may vary among penaeid species (ZACHARIA & KAKATI 2004). Shrimp nurseries are often used to acclimatize postlarvae to pond water conditions, but depending on the age of the stocked individuals, pond salinity levels may not be optimal. RNA:DNA ratios are indicators of relative synthesis activity of growth-linked cells (BUCKLEY 1979, LEMMENS 1995). Thus, a condition index, such as the RNA:DNA ratio, combined with culture performance outputs could assist in understanding the optimal rearing conditions for early postlarval stages, which are marked by morphological, physiological and behavioral changes (BUCKLEY 1979, MOSS 1994a, LEMOS *et al.* 2002).

The pink shrimp *Farfantepenaeus paulensis* (Pérez-Farfante, 1967) is one of the main fishery resources of Southern Brazil

and is distributed along the Southwest Atlantic coast from 15 to 35°S (VALENTINI *et al.* 1991). As a subtropical species, tolerant to moderately low temperatures, *F. paulensis* has been recognized as appropriate for aquaculture with some commercial initiatives (VINATEA & ANDREATTA 1997, THOMPSON *et al.* 1999). The species life cycle consists of spawning in shelf areas followed by concomitant development and migration of larvae towards the coast (VALENTINI *et al.* 1991). Postlarvae with planktonic behavior are reported to penetrate inshore brackish nursery grounds after six molts from metamorphosis to develop as benthic juveniles prior to returning to open waters (D'INCAO 1991).

The present study investigated the consequences of salinity acclimation in commercial nursery practices and determined the effects on growth and survival of *F. paulensis* early postlarvae. Additionally, salinity effects on postlarval development and RNA:DNA ratios were also determined to assist in defining the most suitable culture conditions.

### MATERIAL AND METHODS

*Farfantepenaeus paulensis* postlarvae at age PL<sub>14</sub> (14 days after metamorphosis) were obtained during the summer from a public hatchery after spawning from wild broodstock. Postlarvae were initially stocked in four 5000 L tanks with continuous aeration, 30‰ (hatchery condition) salinity and at 24.7 ± 1.6°C until they reached adequate size for the experiment. Tanks included individual aeration, 50% volume water exchange every other day and shrimp were fed fresh hatched *Artemia* nauplii *ad libitum*. The experimental groups of

postlarvae were first acclimated to salinities higher or lower than the original hatchery condition (30‰), and then reared for 20 days under the same conditions to simulate the nursery phase in commercial farms.

One-hundred and fifty postlarvae at age PL<sub>18</sub> ( $3.57 \pm 0.61$  mg/ind wet weight) were stocked in 200-L circular plastic tanks with 50 L of water at a density of 3 ind/L. Treatments were assigned in triplicate tanks for the four test salinities of 16, 22, 29 and 35‰. Shrimp groups were acclimated in such a way that, starting from the original stocking salinity of 30‰, all experimental units reached the respective test salinity at the same time (CHARMANTIER *et al.* 1988, LEMOS *et al.* 2001). Salinity was gradually raised to 35‰ by adding filtered (1 µm) sea water (35‰) at a rate of 1‰/h. Similarly, freshwater was added at 1‰/h to decrease at a rate of 3 L/h for 29‰ treatment and at 2.18 L/h for eight hours for the 22‰ treatment. To lower to a salinity of 16‰, the same procedure as described for the 22‰ treatment was followed, after which an eight hours interval with no salinity shift was used, followed by subsequent inputs of freshwater at 4.25 L/h for six hours. Tank volumes were then filled to 50 L and after 24 hours two groups of 20 individuals per replicate tank were sampled to determine initial weight, stage of development, and physiological condition by nucleic acid analysis. For RNA and DNA analysis, twenty individuals were pooled per replicate and immediately frozen in liquid nitrogen at -180°C. Initial experimental density corresponded to 388 ind/m<sup>2</sup>. Postlarvae groups were reared for 20 days at the test salinities, at a temperature of  $24.7 \pm 1.6^\circ\text{C}$  and a daily 30% water volume renewal. Feeding consisted of fresh hatched *Artemia* nauplii at a concentration of five nauplii/mL until day 8 and seven nauplii/mL until day 20. Food concentration was checked and corrected by adding newly hatched nauplii twice a day at 08:00 and 18:00 h. At the end of the nursery period, shrimp were harvested, sampled for nucleic acid analysis and all remaining shrimp were individually weighed to determine mean final weight. For the initial and final weight determinations, postlarvae were gently rinsed with distilled water, dried on filter paper and weighed using an analytical balance (Sartorius BL 210S, Bradford, MA, USA) to the nearest 0.0001 g. The stage of development was assessed in five and ten individuals per replicate at the beginning and at the end of the experiment, respectively. Developmental stage was determined according to the number of molts including the metamorphosis from Mysis III to Postlarva I indicated by the number of rostral teeth and setae of the sixth segment (LEMONS & PHAN 2001) and expressed in roman numerals.

RNA and DNA were separately extracted in triplicate samples from each replicate tank. Postlarvae were analyzed in pools of five individuals for samples taken from the beginning and the end of the nursery period (days 0 and 20, respectively). Frozen samples were weighed (50-100 mg) in microtubes free of RNase and DNase and homogenized with a Teflon pestle. All reagents were free of RNase and DNase, and all containers and

equipment were sterilized. Samples for RNA determination were homogenized (room temperature) in 750 µL Trizol LS reagent (Invitrogen, São Paulo, Brazil), a monophasic solution of phenol and guanidine isothiocyanate, for five minutes. This reagent does not destroy the integrity of the RNA during homogenization while disrupting tissue and dissolving cell components (CHOMCZYNSKI & SACCHI 1987). RNA was separated by adding 200 µL chloroform, shaking vigorously, and left standing for 15 minutes. The treated samples were centrifuged at 12 000 x g at 5°C for 15 minutes (CHOMCZYNSKI 1993). The aqueous phase containing the RNA was transferred to a new tube and the RNA was precipitated with 500 µL isopropyl alcohol. After 10 minutes, the RNA samples were centrifuged at 12 000 x g and 5°C for 10 minutes. The pellets containing RNA were treated with 1 ml 75% ethanol and centrifuged at 7 500 x g and 5°C for five minutes to eliminate any residual isopropyl alcohol that might interfere with the analyses. The ethanol was then discarded and the pellets were air-dried. The RNA was dissolved in 100 µL distilled water and incubated at 55°C for 10 minutes.

DNA was quantified after adaptation of a plasmid purification system (Marlingen Bioscience Inc., Ijamsville, MD, USA). Samples (50-100 mg fresh weight) were homogenized in 500 µL DEPC (diethylpyrocarbonate) water with a Teflon pestle. Two hundred and fifty µL of cell suspension buffer (50 mM Tris-HCL, pH 8, 10 mM EDTA) containing RNase (20 mg/mL) were added followed by 250 µL of cell lysis solution (200 mM NaOH, 1% SDS w/v). The volume was gently mixed and incubated at room temperature for five minutes before the addition of 350 µL of neutralizing buffer (acetate and guanidine hydrochloride), gently mixed and centrifuged at 12 000 x g and 5°C for 10 minutes. Supernatant was transferred into a specific 2-mL plastic tube with DNA filter and centrifuged at 12 000 x g and 5°C for one minute. The volume filtrated was discarded and the filter washed with 700 µL of cleaning buffer (NaCl, EDTA and Tris-HCL, pH 8.0) following centrifugation at 12 000 x g and 5°C for one minute. Residues of cleaning buffer were eliminated by centrifugation. The filter containing DNA was attached to a clean tube and TE buffer was added at 65°C and incubated for one minute. The solution was centrifuged at 12 000 x g for two minutes, DNA was separated from filter, and 425 µL DEPC water was added.

Absorbance readings at 260 nm were used for quantitative determination of nucleic acids. One unit of optical density (OD) corresponded to approximately 40 µg/mL of RNA and 50 µg/mL of DNA. The ratio between the readings at 260:280 nm provided an estimate of the purity of the nucleic acids (CHOMCZYNSKI 1993). Most of the RNA measured in this way is considered to belong to the ribosomal pool (CLEMMESSEN 1989). RNA:DNA ratios were calculated as an indicator of relative synthesis activity of growth-linked cells.

Data from the salinity treatments were compared by a one-way ANOVA followed by a Student-Newman-Keuls multi-comparison test after checking for normality and homogene-

ity of variance. When data did not meet these assumptions, a Kruskal-Wallis rank analysis was applied followed by Dunn's multiple comparison test. Pearson's correlation analysis was applied to correlate between culture performance features and RNA:DNA ratios. Differences were considered significant at  $p < 0.05$  (ZAR 1984).

## RESULTS

The gradual acclimation of PL<sub>18</sub> postlarvae to the experimental salinities before the culture trial produced important effects upon postlarval growth, development and RNA:DNA ratios. Though not statistically significant ( $p > 0.05$ ) individuals acclimated to lower salinities presented higher initial weight (Tab. I).

On the other hand, a significant negative correlation ( $p < 0.05$ ) was found between initial weight and acclimation salinity (data not shown). Salinity affected postlarval development as a lower number of individuals at stage PL V-VI were observed at salinities of 16 and 22‰ than at 29 and 35‰ (Fig. 1). Moreover, individuals at advanced developmental stages (PL IX-X) were absent at salinities of 35‰ and few at 29‰, whereas at 16 and 22‰ they constituted an important percentage of the population. Acclimation procedure to stocking salinity also produced significant effects upon postlarval physiological condition as denoted by the decreasing trend of RNA:DNA ratios with increased salinity (Fig. 2). Accordingly, RNA:DNA ratios were significantly correlated to initial weight ( $R^2 = 0.69$ ,  $p = 0.005$ ,  $n = 9$ ), appearing to be an indicator of the growth potential.

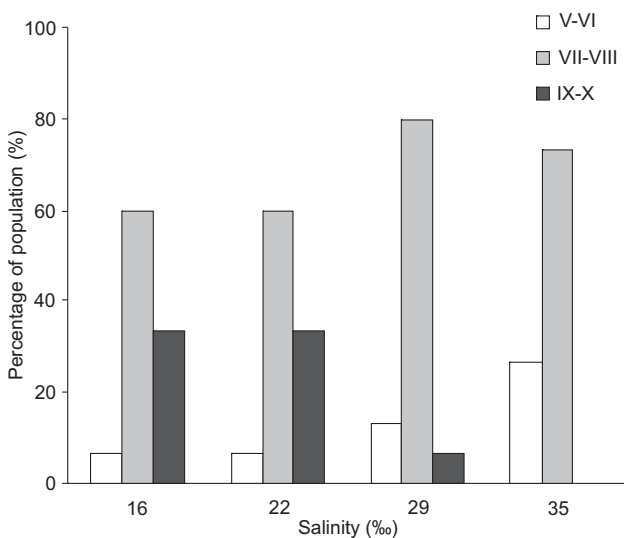


Figure 1. Stage of development (number of molts including metamorphosis, in roman numerals) of *F. paulensis* postlarvae (PL<sub>18</sub>) after acclimation from a salinity of 30‰ to different test salinities (16, 22, 29, and 35‰) at  $24.7 \pm 1.6^\circ\text{C}$  and 388 ind/m<sup>2</sup>.

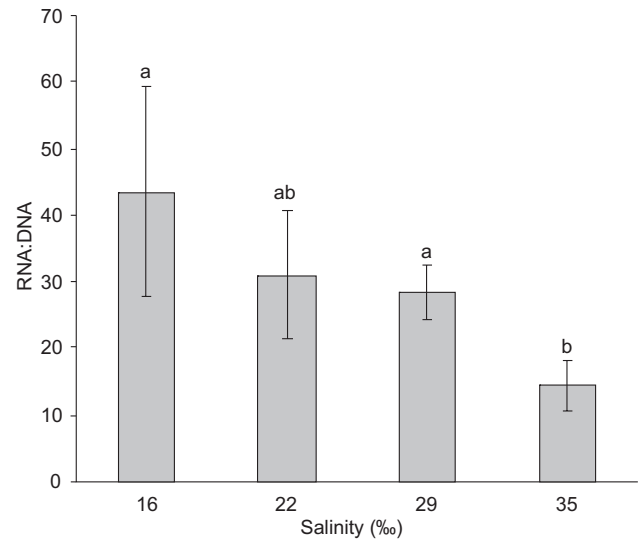


Figure 2. RNA:DNA ratios of *F. paulensis* postlarvae (PL<sub>18</sub>) after acclimation from a salinity of 30‰ to different test salinities (16, 22, 29, and 35‰) at  $24.7 \pm 1.6^\circ\text{C}$  and 388 ind/m<sup>2</sup>. Vertical bars indicate standard deviations. Different superscript letters denote significant differences ( $p < 0.05$ ).

After the 20-day rearing trial, salinity affected postlarval development and performance to various degrees. Salinity effects upon development were less marked compared to observations at the beginning of the culture trial (Fig. 3). The highest percentage of individuals older than PL XXVI (30%) was at

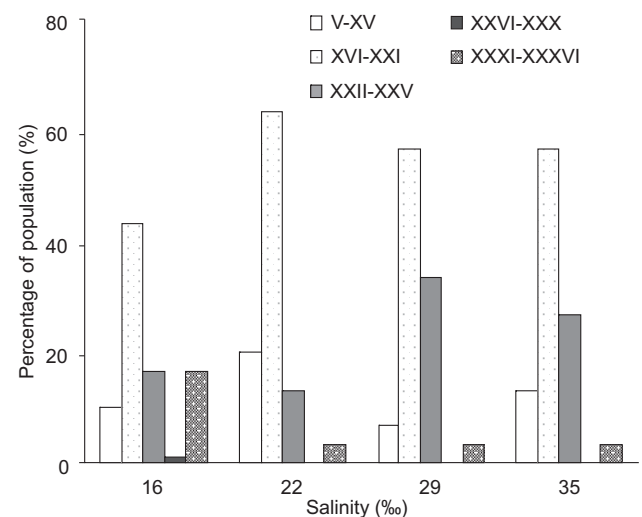


Figure 3. Stage of development (number of molts including metamorphosis, in roman numerals) of *F. paulensis* postlarvae (PL<sub>38</sub>) after 20 days of culture in different test salinities (16, 22, 29, and 35‰) at  $24.7 \pm 1.6^\circ\text{C}$ , and 388 ind/m<sup>2</sup>.

Table I. Nursery performance of *F. paulensis* postlarvae (PL<sub>18</sub>- PL<sub>38</sub>) acclimated and reared in clear water of four different salinities (16, 22, 29, and 35‰) at 24.7 ± 1.6°C and 388 ind/m<sup>2</sup>. Results expressed as means (SD). Different superscript letters in the same row indicate significant differences (p < 0.05).

	16‰	22‰	29‰	35‰
Survival (%)	78.90 (9.50)	96.7 (2.90)	92.2 (10.70)	94.40 (5.60)
Initial weight (mg)	6.10 (0.70)	5.9 (0.50)	5.5 (1.00)	4.80 (0.70)
Final weight (mg)	116.80 <sup>a</sup> (6.40)	97.4 <sup>b</sup> (5.70)	125.5 <sup>a</sup> (14.80)	91.00 <sup>b</sup> (15.00)
Yield (mg/L)	120.50 (25.80)	131.2 (11.30)	164.1 (46.70)	121.20 (23.50)
Total harvest (g)	6.02 (1.29)	6.56 (0.56)	8.2 (2.34)	6.06 (1.17)
Growth (mg/day)	5.50 <sup>ab</sup> (0.40)	4.6 <sup>a</sup> (0.30)	6.0 <sup>b</sup> (0.70)	4.30 <sup>a</sup> (0.70)
Biomass gain (mg)	110.70 <sup>a</sup> (7.00)	91.5 <sup>b</sup> (5.60)	119.9 <sup>a</sup> (13.80)	86.20 <sup>b</sup> (14.30)

16‰ salinity. At 29‰, there was a higher amount of individuals at PL XXII-XXV compared to the other salinity levels. Increased number of less developed postlarvae (PL V-XV) was found at 22 and 35‰ salinities.

Though not significant (p > 0.05), survival was higher between the salinities of 22 and 35‰ compared to 16‰, with mean values of over 90% and of 78.9%, respectively (Tab. I). In contrast, individual final weight was significantly higher (p < 0.05) at salinities of 16 and 29‰ with mean values up to 37% higher than means at 22 and 35‰. As products of survival rates and final weight, yield (mg/L) and total harvest (g) are closely related parameters, and presented means of up to 26% higher at 29‰ salinity when compared to the other test salinities, followed by much lower values at 22‰. Postlarval growth rates (mg/day) were significantly higher at 29‰ salinity (p < 0.05). In contrast, reduced growth was observed at salinities of 22 and 35‰. Biomass gain (mg) followed the trend of final weight results with significant higher values at salinities of 16 and 29‰ than at 22 and 35‰. In spite of elevated initial and final weights, growth rates and biomass gain, postlarvae reared at 16‰ salinity did not achieve high yield due to reduced survival compared to the other salinities. Overall, the best performance was observed at the salinity of 29‰ and resulted in higher weight gain, growth rates, and yield.

RNA:DNA ratios varied according to salinity and corroborated with most of the culture performance data. Though not statistically significant (p > 0.05), higher and lower RNA:DNA values were found at salinities of 29 and 22‰, respectively, and RNA:DNA ratio at 16‰ was greater than at 35‰ (Fig. 4).

Performance features such as growth and total harvest were positively correlated (p < 0.05) with RNA:DNA ratios (Tab. II). These relationships confirmed the RNA:DNA ratio as a biochemical indicator of growth potential in postlarval *F. paulensis*.

## DISCUSSION

The inclusion of the nursery phase between the hatchery and final growout is generally considered to be an important management strategy and may provide hatcheries or farms with some economic advantages (PERSIN & AUNGST 2006). Be-

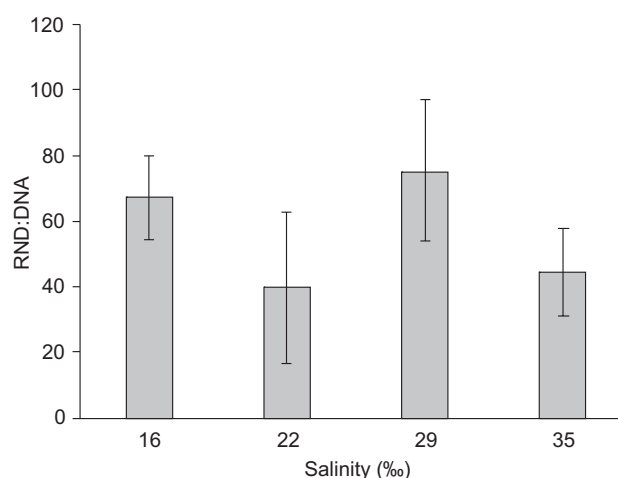


Figure 4. RNA:DNA ratios of *F. paulensis* postlarvae (PL<sub>38</sub>) after 20 days of culture in different test salinities (16, 22, 29, and 35‰) at 24.7 ± 1.6°C and 388 ind/m<sup>2</sup>. Vertical bars indicate standard deviations. No significant differences were detected (p > 0.05). n = 11.

Table II. Correlation of growth rates and total harvest with RNA:DNA ratios of *F. paulensis* postlarvae (PL<sub>38</sub>) after 20 days of culture in four different salinities (16, 22, 29 and 35‰). Correlations were considered significant at p < 0.05.

	R <sup>2</sup>	P	n
Growth (mg/day)	0.54	0.010	11
Total harvest (g)	0.47	0.019	11

sides acclimating postlarvae to pond water conditions, nurseries also regulate the flow of postlarvae from hatcheries to farms. At this phase, individuals are expected to grow and get fit to cope with the fluctuating environmental conditions of the growout ponds (HIRONO 1983). Similar to managing seedstocks, performance of postlarvae in nursery facilities can be economically critical and requires proper management to maintain survival and health of individuals for the growout phase. Efficient

postlarvae harvest depends on adequate culture conditions and survival during larval and postlarval stages, since they may be associated with better growth in the remaining developmental stages (CASTILLE *et al.* 1993).

Defining culture conditions for euryhaline marine species is essential for the development of culture protocols (ZACHARIA & KAKATI 2004); this is also the case with *F. paulensis*. Optimal environmental conditions for growth are species-specific and may vary according to life history and stage of development (ANGER 2001). As in the life cycle of most penaeid shrimp species, *F. paulensis* includes migration as early postlarvae into inshore brackish nursery grounds, with a concomitant increment in the ability to support salinity variation (CHARMANTIER *et al.* 1988). Nursery rearing conditions should ideally agree with the species environmental requirements and regulatory capacity, and salinity should be the primary parameter. Postlarvae of *F. paulensis* exposed to a very high stocking density at  $24.7 \pm 1.6^\circ\text{C}$  were able to grow and survive properly in salinities between 22 and 35‰, while the overall best performance was achieved at 29‰, as denoted by significant higher final weight, growth rate, and biomass gain. The density used in the present study (338 ind/m<sup>2</sup>) was higher compared to previous reports for penaeid species (28 to 200 ind/m<sup>2</sup>) demonstrating the ability of *F. paulensis* to deal with high density stocking conditions (STURMER *et al.* 1992).

Moreover, postlarvae in nurseries are often acclimated to the pond water salinity before stocking to avoid sudden osmotic stress (SAMOCHA *et al.* 1993). Postlarvae are transferred from hatchery salinity of 30-35‰ to usually lower, but sometimes higher, salinities. The transfer from 30‰ salinity to a nursery at 35‰ was not beneficial for the performance of *F. paulensis*, as denoted by reduced weight gain after acclimation and low growth rate after 20 days of culture. Similar results have also been observed in other penaeid shrimps (CHEN *et al.* 1992, ROSAS *et al.* 1999). Better performance at lower salinities may be related to ontogenetic decreases in the isosmotic point in the early postlarvae, and juvenile penaeids compared to larvae or older juveniles (DALL 1981, CHARMANTIER *et al.* 1988). At 25°C, PL<sub>18</sub> corresponded to V to VII molts after the metamorphosis from mysis (LEMO & PHAN 2001) and coincided with the *F. paulensis* stage observed reaching estuarine areas (D'INCAO 1991). In the natural environment, individuals at this age should be able to cope with salinity fluctuations during the growout period in estuaries. Large salinity shifts during the nursery phase and hyper- and hypo-osmotic regulation in *F. paulensis* would therefore require low energy expenditure, especially through protein deamination (LEMO *et al.* 2001), at salinities close to postlarval isosmotic point, which has been reported to be below 30‰ for juveniles (WASIELESKY 1999). The present results coincided with the trends in growth rates of older *F. paulensis* postlarvae (PL<sub>27</sub> to PL<sub>40</sub>) at similar salinities (LEMO *et al.* 2001).

Since organisms may respond to the environment at the molecular level, the RNA:DNA ratio has been considered a valu-

able condition index related to instantaneous growth and growth potential (BUCKLEY 1979, CLEMMESSEN 1989, MOSS 1994a, LEMMENS 1995). The concept is based on the fact that the amount of DNA per cell is relatively constant while RNA varies with the physiological status, the requirement for protein synthesis and growth (BUCKLEY *et al.* 1999). Thus, the RNA:DNA ratio becomes an indicator of the relative synthesis activity of growth-linked cells. Growth and total harvest of postlarval *F. paulensis* were adequately explained by RNA:DNA ratios after the 20-days nursery culture. Detrimental effects of acclimation to higher salinity (35‰) were also detected by RNA:DNA ratios and coincided with reduced growth both after acclimation and in the nursery period. After the 20-days experimental period, higher RNA:DNA ratios were observed at 29‰, coinciding with the salinity of the best shrimp performance. However, the absence of significant differences in RNA:DNA ratios of PL<sub>38</sub> among the test salinities could be attributed to data variability and possible acclimation of some individuals to long term conditions contrasting with the rapid response of the nucleic acids ratio to previous salinity acclimation (MOSS 1994b). Elevated RNA:DNA ratios further indicate higher growth potential at the salinity of 29‰ that may also be related to isosmotic point at similar level in early postlarvae. In the course of development, older postlarvae may show reduced isosmotic point, as reported for several penaeid species including *F. paulensis* (CHARMANTIER *et al.* 1988, DALL *et al.* 1990, WASIELESKY 1999).

Developmental effects were also detected after postlarvae acclimation to the experimental salinities. Higher percentages of advanced stages were related to low salinities (16 and 22‰) after the transfer from 30‰ salinity. This effect was less pronounced after 20 days of experimental rearing when the postlarval stage was higher than thirty molts, and was mainly seen at 16‰ salinity. Differences in developmental stage between postlarvae (denoted by the number of molts after metamorphosis) may be related to a variation in the intermolt period and molting frequency at different salinities (GUERIN & STICKLE 1997). Decreased salinities are reported to increase molting frequency in juvenile *Penaeus chinensis* although it may be further modulated by culture temperature (CHEN *et al.* 1996). Moreover, lower survival of postlarvae acclimated to 16‰ may be accompanied by selection for resistant and more developed individuals as indicated by the higher initial weights compared to the other treatments. Conversely, higher occurrence of more developed individuals, combined with high survival after acclimation, may have contributed to elevated yields at salinities of 22‰.

The present study has shown that nursery output may be affected by stocking salinity. Though recommended for proper acclimation (CHARMANTIER *et al.* 1988), the rates of salinity shift may not have been sufficient for optimal acclimation of young postlarvae (PL<sub>18</sub>) compared to older counterparts at ages over PL<sub>27</sub> (LEMO *et al.* 2001). Accordingly, *F. paulensis* early postlarvae (PL<sub>18</sub> or younger) should not be stocked in salinities higher than that of the hatcheries. If postlarvae are to be accli-

mated for further stocking in low salinity pond waters, nursery acclimation should consider older individuals possibly combined with a longer period of salinity shift and acclimation. Further studies are required to determine the optimal salinity level for the nursery rearing of *F. paulensis*, as well as the effects of postlarval age and stage of development upon the definition of suitable culture conditions for best performance.

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