Latin American Journal of Aquatic Research

E-ISSN: 0718-560X

lajar@ucv.cl

Pontificia Universidad Católica de Valparaíso

Chile

Mutti, Diana W.; Ballester, Eduardo L.C.; Martino, Ricardo C.; Wasielesky Jr., Wilson; Cavalli, Ronaldo O.

Feeding n - 3 HUFA enriched Artemia to the larvae of the pink shrimp Farfantepenaeus paulensis increases stress tolerance and subsequent growth

Latin American Journal of Aquatic Research, vol. 45, núm. 1, marzo, 2017, pp. 18-24

Pontificia Universidad Católica de Valparaíso

Valparaíso, Chile

Lat. Am. J. Aquat. Res., 45(1): 18-24, 2017
DOI: 10.3856/vol45-issue1-fulltext-2

Research Article

Feeding n-3 HUFA enriched *Artemia* to the larvae of the pink shrimp *Farfantepenaeus paulensis* increases stress tolerance and subsequent growth

Diana W. Mutti[1], Eduardo L.C. Ballester[2], Ricardo C. Martino[3] Wilson Wasielesky Jr.[1] & Ronaldo O. Cavalli[4]

[1]Universidade Federal do Rio Grande-FURG, Instituto de Oceanografia, Rio Grande, RS, Brazil
[2]Universidade Federal do Paraná-UFPR, Campus Palotina, Palotina, PR, Brazil
[3]Fundação Instituto de Pesca do Estado do Rio de Janeiro-FIPERJ
Unidade de Tecnologia do Pescado, Rio de Janeiro, RJ, Brazil
[4]Universidade Federal Rural de Pernambuco-UFRPE
Departamento de Pesca e Aquicultura, Recife, PE, Brazil
Corresponding author: Ronaldo Cavalli (ronaldocavalli@gmail.com)

ABSTRACT. There has been a considerable amount of work on the effects of *Artemia* enrichment on shrimp larvae. However, the effects on the subsequent growth performance have not been thoroughly investigated. From mysis I to eight day-old postlarvae (PL8), shrimp (*Farfantepenaeus paulensis*) were fed either newly hatched *Artemia* nauplii or *Artemia* enriched with an emulsion containing high levels of n-3 highly unsaturated fatty acids (n-3 HUFA). Upon reaching PL8, shrimp were exposed to salinity and ammonia stress tests. Growth performance was assessed by further rearing shrimp for 40 days. Shrimp fed the enriched *Artemia* (named EG) had higher tolerance to both salinity and ammonia than those fed newly hatched *Artemia* (named CG). Weight of PL from EG was also significantly higher than in CG. Higher levels of n-3 HUFA, especially 20:5 n-3 and 22:6 n-3, were found in the enriched *Artemia*. In the postlarvae, higher levels of n-6 and n-3 HUFA were observed in EG, while PL from CG presented higher levels of 18:3 n-3 and 18:4 n-3. At the end of the nursery rearing, no differences in survival were observed, but weight was higher in EG (1.08 g) than in CG (0.90 g). The supplementation of n-3 HUFA during larviculture increases the tolerance of *F. paulensis* postlarvae to salinity and ammonia, and positively affects growth performance after a 40 days long rearing period.

Keywords: *Farfantepenaeus paulensis***,** penaeids, *Artemia*, nutrition, HUFA, enrichment, tolerance to ammonia and salinity.

INTRODUCTION

It is well known that penaeid shrimp have a limited ability to perform *de novo* synthesis of highly unsaturated fatty acids (HUFA). This was first shown in *Marsupenaeus japonicus* (Kanazawa *et al.*, 1979a), *Penaeus monodon* and *Fenneropenaeus merguiensis* (Kanazawa *et al.*, 1979b). HUFA, particularly eicosapentaenoic acid (20:5 n-3; EPA) and docosahexaenoic acid (22:6 n-3; DHA), are vital in the maintenance of normal cell function; EPA and DHA are essential compounds required for cell membrane formation, osmoregulation, the synthesis of prostaglandin and they also appear to have an activating role in the immune system (Lèger & Sorgeloos, 1992). Therefore, diets for penaeids must contain sufficient supplies of these essential nutrients.

Production of penaeid shrimp postlarvae relies heavily on the brine shrimp *Artemia* as the main live food organism used in commercial hatcheries (Sorgeloos *et al.*, 1998; Wouters *et al.*, 2009; Cobo *et al.*, 2015). Although from a hatchery management standpoint the use of *Artemia* presents several advantages, the most important being easiness of storage and use (Lèger & Sorgeloos, 1992), levels of n-3 HUFA in newly hatched *Artemia* nauplii are either low or greatly variable depending on origin (Lavens & Sorgeloos, 1996; Figueiredo *et al.*, 2009). Since *Artemia* is a non-selective filter feeding organism, enhancement of n-3 HUFA levels is achieved through enrichment techniques, by which n-3 HUFA rich compounds are provided to *Artemia* and consequently increase their nutritional value (Sorgeloos *et al.*, 1998; Wouters *et al.*, 2009).

Corresponding editor: Fernando Vega

Previous studies have shown that feeding n-3 HUFA rich diets results in improved survival, growth and/or stress tolerance of several crustacean species (Tackaert *et al.*, 1989; Rees *et al.*, 1994; Cavalli *et al.*, 2000; Chim *et al.*, 2001; Martins *et al.*, 2006; Immanuel *et al.*, 2007; Sui *et al.*, 2007; Jinbo *et al.*, 2013). Nonetheless, few studies have considered the effects of the nutritional quality of larviculture diets on further shrimp performance. In this study, we assessed whether the subsequent growth of *F. paulensis* postlarvae during nursery rearing would be affected by previously feeding n-3 HUFA enriched *Artemia* during the larviculture period.

MATERIALS AND METHODS

Farfantepenaeus paulensis larvae were obtained from a routine larviculture at the Marine Aquaculture Center, Universidade Federal do Rio Grande, Brazil. Upon reaching mysis I, larvae were individually counted and transferred to triplicate 200 L larviculture tanks. Aeration was provided by four air stones per tank to maintain dissolved oxygen levels above 5.0 mg L^{-1}. Seawater was previously filtered by a 5 µm cartridge filter, and water renewal rate was 80% per day. Temperature was maintained at 26-27°C with submerged thermostatic heaters. Photoperiod regime was natural and the diurnal cycle lasted from sunrise at 06:20 h and sunset at 19:00 h over the course of the experiment. Shrimp were reared at a density of 55 larvae L^{-1}. After their transference to the tanks, larvae were fed solely *Artemia* according to the experimental design. For the control group (CG), larvae were fed newly hatched *Artemia* nauplii, whilst in the enriched group (EG) larvae were fed *Artemia* enriched with a commercial emulsion containing over 400 mg g^{-1} n-3 HUFA on a dry weight basis (Super SELCO®, INVE Aquaculture, USA).

Enrichment procedures were performed according to Sorgeloos *et al.* (1998) in 50 L cylindro-conical tanks. Density of *Artemia* during enrichment varied between 100-300 ind L^{-1}, temperature was kept between 25-28°C and strong aeration was provided to guarantee homogeneous distribution of the emulsion and dissolved oxygen levels above 5 mg L^{-1}. *Artemia* were enriched for 24 h with two doses of 300 mg of emulsion per liter added at 12 h intervals. After enrichment, *Artemia* were rinsed with water over a 120 µm sieve to remove any remaining emulsion.

Larvae were fed the *Artemia* diets twice a day (10:00 and 22:00 h). *Artemia* used for the night feeding were stored at 4°C. *Artemia* were offered at an initial density of 2 mL^{-1}, which increased gradually to 8 mL^{-1} at the end of the trial. Before daily water renewal, the number of remaining *Artemia* was estimated in each tank and increased as needed. These procedures were performed until PL8 (eight day-old postlarvae). At this point groups of PL8 from both groups were exposed to salinity and ammonia stress tests.

The salinity and ammonia stress tests were based on the static toxicity tests proposed by Greenberg *et al.* (1992). The tolerance to salinity and total ammonia were assessed separately in triplicate sets of plastic beakers containing 1 liter of water that were immersed in a water bath at 27-28°C. For the salinity stress test, thirty PL8 from each group were exposed for 24 h to a control (salinity 30 g L^{-1}) and to decreasing salinity levels (24, 18, 12, 6 and 0 g L^{-1}), while for the ammonia test batches of 30 PL8 from each group were transferred to the beakers and exposed during 24 h to increasing concentrations of total ammonia: 0 (control, no added ammonia), 15, 30, 45, 60 and 90 mg L^{-1}. Ammonia concentrations, which were based on previous studies with *F. paulensis* (Ostrensky & Wasielesky, 1995; Martins *et al.*, 2006), were added to the beakers immediately after stocking the postlarvae. During both stress tests, postlarvae were not fed and no water was exchanged. After 24 h postlarvae not responding to mechanical stimuli were considered dead and counted. Based on the mortality rates, the mean lethal salinity for 50% of the population (LS_{50}) and the mean lethal ammonia concentration for 50% of the population (LC_{50}) were estimated with the Trimmed Spearman Karber Method (Hamilton *et al.*, 1977).

PL8 remaining in the larviculture tanks were divided into groups of 300 shrimp and further reared for 40 days. The enrichment group (EG) had three replicated groups, while the control had four replicates. Shrimp were initially reared indoors for 10 days and were gradually acclimated to the salinity 15 using the methodology proposed by Tsuzuki *et al.* (2000). Upon reaching PL18, they were transferred to PVC-coated polyester cages (bottom area of 1 m^2, mesh size of 1.5 mm) placed outdoors in an estuarine inlet of Patos Lagoon (32º03'55"S, 52º12'30"W), southern Brazil. During both the indoor and outdoor rearing periods and irrespective of experimental treatment, management and feeding procedures were similar among all shrimp groups. Shrimp were fed *ad libitum* once a day in the late afternoon with a commercial nursery diet (CR2 Camaronina, Purina®, Brazil). At least 30 shrimp from each cage were randomly sampled for individual weight measurements on days 25 and 40. At the end of the trial shrimp remaining in each cage were hand counted to determine survival.

Temperature (mercury thermometer, precision ±0.5°C), salinity (Atago® optical refractometer, Japan, ±1.0 g L^{-1}), pH (Digital pHmeter HandyLab 2BNC

Schott, Germany) and dissolved oxygen (Digital oximeter Handylab OXI/SET, Schott, Germany) were recorded daily. Every morning, samples were collected to estimate the concentrations of ammonia (UNESCO, 1983) and nitrite (Bendschneider & Robinson, 1952).

Samples of newly hatched *Artemia* nauplii, enriched *Artemia* and postlarvae were washed with distilled water and frozen (-20°C) until analysis. After defrosting, samples were again washed and prepared for analysis. Crude lipids were extracted with a mixture of chloroform/methanol (2:1, v/v) as described by Folch *et al.* (1957). Fatty acids of the *Artemia* and shrimp were prepared from crude lipid by saponification with KOH (50%). Fatty acid methyl esters (FAME) were prepared by esterification with boron-trifluoride in methanol (7%) according to Metcalfe & Schmitz (1961). FAME were separated by gas-liquid chromatography on Shimadzu GC-15A (Shimadzu Corporation, Japan) equipped with a flame ionization detector (FID) and fitted with a fused silica capillary column Omegawax 320×30×0.32 mm (Supelco Inc., USA). Hydrogen was used as a carrier gas with a flow rate of 4.0 mL min^{-1}. Injector and detector temperatures were programmed to 240 and 250°C, respectively. Column temperature was programmed to be isothermal (205°C). FAME were identified by means of reference standard n-3 PUFA, menhaden fish oil (4-7085-U; Supelco Inc., USA) and quantified with a Shimadzu C-R4A integrator model (Shimadzu Corporation, Japan).

Significant differences ($P < 0.05$) of survival and final weight at the end of the larviculture and nursery rearing periods were determined using the *t*-test. Prior to the analysis of survival, data were arcsine-square root transformed. All tests were conducted after the confirmation of homogeneity of variances (Lévene's test) and normality distribution of data (Kolmogorov-Smirnov's test). Differences on the ammonia (LC_{50}) and salinity (LS_{50}) stress tests were graphically determined through the comparison of 95% confidence intervals (Greenberg *et al.*, 1992).

RESULTS

Mean values of temperature, salinity, dissolved oxygen, pH ammonia and nitrite during the larviculture period were 27.0°C, 32 g L^{-1}, 7.7 mg L^{-1}, 8.0 mg L^{-1}, 0.24 mg TAN L^{-1} and 0.01 mg $N\text{-}NO_2.L^{-1}$, respectively. No significant differences in any of these parameters were detected between EG and CG. Final weight of PL8 was significantly higher in EG (13 ± 7 mg) than in CG (10 ± 4 mg). The LS_{50} values and their 95% confidence intervals for EG and CG were 18.93 g L^{-1} (18.25-19.57) and 20.53 g L^{-1} (19.74-21.26), respectively. Graphical comparison indicates significant differences between groups (Fig. 1). Similarly, mean lethal concentrations of total ammonia (LC_{50}) were also significantly different (Fig. 1).

Water temperature and salinity during nursery rearing in the cages were 28.5°C (range 24-32°C) and 14.7 g L^{-1} (range 13-17), respectively. After the end of the nursery rearing, mean (±SD) survival of shrimp were 82.5% (±14.8) and 71.5% (±21.5) for EG and CG, respectively, and no significant differences were detected. Differences in weight were highly significant ($P = 0.00002$) after 15 days of cage rearing (Fig. 2). EG shrimp weighed 0.176 g (±0.03), while those from CG weighed 0.109 g (±0.02). After 40 days, significant differences ($P = 0.0001$) in shrimp weight persisted, with mean final weights of 1.082 g (±0.118) for EG and 0.896 g (±0.128) for CG, respectively (Fig. 2).

The fatty acid profiles of newly hatched *Artemia* nauplii and enriched *Artemia* presented significant differences (Table 1). Although no significant differences were observed for saturated, monounsaturated and n-6 PUFA, the levels of EPA and DHA, and consequently total concentration of n-3 HUFA, were significantly higher ($P = 0.000012$) in the enriched *Artemia* than in newly hatched nauplii. As a result the n-3/n-6 relation was significantly higher ($P = 0.000001$) in the enriched *Artemia*. In contrast, newly hatched nauplii had higher levels of linolenic (18:3 n-3) and stearidonic (18:4 n-3) acids.

Overall, fatty acid profile of *F. paulensis* postlarvae (Table 2) reflected the composition of their prey. Postlarvae fed enriched *Artemia* (EG) presented higher levels of n-6 PUFA and n-3 HUFA than postlarvae fed newly hatched nauplii (CG). Concentrations of arachidonic acid (ARA; 20:4n-6), EPA and DHA were higher in EG, while postlarvae fed newly hatched *Artemia* had significantly higher levels of linolenic and stearidonic acids.

DISCUSSION

As expected, the fatty acid composition of *Artemia* was influenced by enrichment, which, in turn, affected the composition of the larvae that fed upon them. Accordingly, higher n-3 HUFA levels, particularly EPA and DHA, were found in the tissues of postlarvae fed enriched *Artemia* in comparison to those fed newly hatched *Artemia*. Postlarvae fed enriched *Artemia* also presented higher weight at the end of the larviculture period. Although this corresponds well with previous findings demonstrating the positive effect of dietary n-3 HUFA in the enhancement of larval growth and development (Lèger & Sorgeloos, 1992; Rees *et al.*,

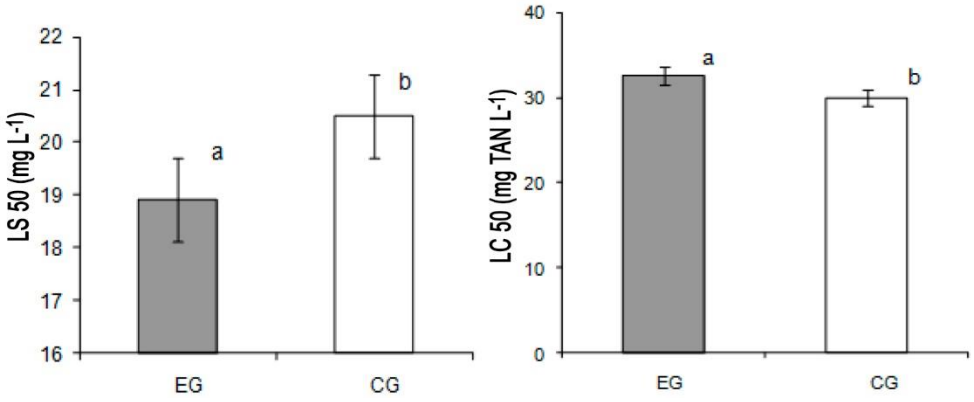

Figure 1. Mean (±95% confidence interval) lethal salinities (LS_{50} - left) and lethal concentrations of total ammonia (LC_{50} - right) for 50% of the population of *Farfantepenaeus paulensis* postlarvae (PL8) fed *Artemia* enriched with a commercial emulsion containing over 400 mg g^{-1} of n-3 highly unsaturated fatty acids (EG) or newly hatched *Artemia* (CG). Superscript letters indicate significant differences.

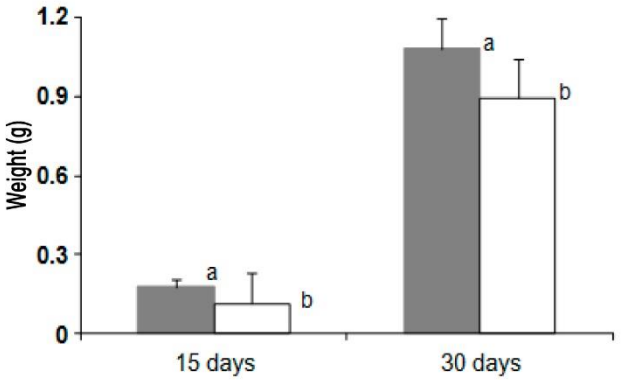

Figure 2. Mean (±SD) weight (g) of the pink shrimp *Farfantepenaeus paulensis* reared for 30 days in cages and that were originally fed either *Artemia* enriched with a commercial emulsion containing over 400 mg g^{-1} n-3 highly unsaturated fatty acids (dark) or newly hatched *Artemia* nauplii (light) during the larviculture period. Superscript letters indicate significant differences.

1994; Cavalli *et al.*, 2000; Palacios *et al.*, 2004; Immanuel *et al.*, 2007), it disagrees with studies where no effect of n-3 HUFA enriched *Artemia* were observed in the size of *P. monodon* (Rees *et al.*, 1994) and *F. paulensis* postlarvae (Pontes & Andreatta, 2003) or where a negative relationship between dietary n-3 HUFA levels and postlarval length was observed (Martins *et al.*, 2006). Differences between these studies may be due to various causes, including variations in experimental design, species, feed management, composition of the emulsions, postlarval size and age, or duration of the experimental period.

One practical advantage of producing larger postlarvae is that they are knowingly more stress tolerant (Charmantier *et al.*, 1988; Dall *et al.*, 1990; Samocha *et al.*, 1998; Tsuzuki *et al.*, 2000). In other words, larger and older postlarvae are better prepared not only to deal with the stress involved in the transference to growout units, but also to cope with possible variations in environmental variables such as low salinity levels (Samocha *et al.*, 1998; Tsuzuki *et al.*, 2000).

A positive effect of the provision of dietary n-3 HUFA to crustaceans is that it often results in higher stress tolerance (Tackaert *et al.*, 1989; Dhert *et al.*, 1992; Rees *et al.*, 1994; Cavalli *et al.*, 2000; Chim *et al.*, 2001; Palacios *et al.*, 2004; Immanuel *et al.*, 2007; Sui *et al.*, 2007). It is therefore recognized that penaeids fed n-3 HUFA deficient diets generally express a low tolerance to stress (Chim *et al.*, 2001; Palacios & Racotta, 2007). In the present study, comparison of the salinity (LS_{50}) and ammonia tolerance (LC_{50}) of 8 day-old postlarvae fed enriched or newly hatched *Artemia* indicated pronounced differences. The estimated LS_{50} values for salinity were consistently higher for postlarvae fed enriched *Artemia*. Similarly, a higher tolerance to total ammonia was observed in postlarvae fed enriched *Artemia*. All this confirms the superior ability of the animals fed n-3 HUFA enriched *Artemia* to cope with adverse environmental conditions. Similar to the present findings, Tackaert *et al.* (1989) and Immanuel *et al.* (2007) verified that *P. monodon* postlarvae fed n-3 HUFA enriched *Artemia* had higher survival when exposed to a range of salinity levels in comparison to postlarvae fed unenriched *Artemia*, while Chim *et al.* (2001) demonstrated that feeding higher n-3 HUFA levels increased the cellular membrane

Table 1. Mean values (± SE) of the main fatty acids (% area) in Artemia enriched with a commercial emulsion containing over 400 mg g-1 of n-3 highly unsaturated fatty acids (EG) and newly hatched Artemia nauplii (CG). [1]n-6 PUFA ≥ 18:2 n-6, [2]n-3 HUFA ≥ 20:3 n-3. EG: enriched group, CG: control group. For each fatty acid, values with different superscripts were significantly different ($P < 0.05$).

Fatty acid	EG	CG	Fatty acid	EG	CG
14:0	2.44 (±1.47)	4.67 (± 6.13)	20:3n-6	0.22 (± 0.15)	0.34 (± 0.23)
15:0	0.56 (± 0.21)[b]	0.98 (± 0.28)[a]	20:4n-6	1.13 (± 0.16)[a]	0.70 (± 0.18)[b]
16:0	10.65 (± 2.93)	9.06 (± 2.02)	16:3n-3	0.53 (± 0.10)	1.50 (± 1.26)
17:0	1.26 (±1.44)	1.39 (± 1.09)	18:3n-3	15.76 (± 6.28)[b]	31.32 (± 1.67)[a]
18:0	3.98 (± 1.26)	3.27 (± 1.45)	18:4n-3	2.67 (± 1.24)[b]	6.79 (± 2.15)[a]
20:0	0.41 (± 0.17)[a]	0.12 (± 0.07)[b]	20:3n-3	0.43 (± 0.12)	0.43 (± 0.41)
22:0	0.26 (± 0.15)[a]	0.02 (± 0.05)[b]	20:4n-3	0.60 (± 0.08)	0.55 (± 0.32)
14:1	1.33 (± 0.66)[a]	0.47 (± 0.55)[b]	20:5n-3	6.66 (± 0.88)[a]	1.62 (± 0.22)[b]
16:1n-7	4.08 (± 0.93)	3.32 (± 0.41)	22:5n-3	0.86 (± 0.31)[a]	0.02 (± 0.04)[b]
18:1n-9	18.57 (± 4.42)	13.62 (± 5.77)	22:6n-3	5.52 (± 2.27)[a]	0.08 (± 0.11)[b]
18:1n-7	3.32 (± 2.82)	4.48 (± 1.82)	∑ Saturated	19.56 (± 5.36)	19.52 (± 3.87)
20:1n- 7+9+11	1.25 (± 1.02)	0.75 (± 0.25)	∑ Mono-unsaturated	29.70 (± 3.75)	22.78 (± 6.78)
22:1	0.68 (± 0.60)[a]	0.02 (± 0.84)[b]			
18:2n-6	5.53 (± 0.43)	5.39 (± 1.25)	n-6 PUFA[1]	8.61 (± 0.89)	7.47 (± 1.62)
18:3n-6	0.38 (± 0.16)[b]	0.71 (± 0.20)[a]	n-3 HUFA[2]	14.07 (± 2.88)[a]	2.71 (± 0.88)[b]
18:4n-6	0.52 (± 0.11)[a]	0.17 (± 0.20)[b]	DHA/EPA	0.84 (± 0.35)[a]	0.05 (± 0.06)[b]
20:2n-6	0.18 (± 0.12)	0.16 (± 0.13)	n-3/n-6	1.63 (± 0.26)[a]	0.36 (± 0.07)[b]

Table 2. Main fatty acids (% area) in *Farfantepenaeus paulensis* postlarvae fed *Artemia* enriched with a commercial emulsion containing over 400 mg g^{-1} n-3 highly unsaturated fatty acids (EG) and newly hatched *Artemia* nauplii (CG). [1]∑ (n-6) ≥ 18:2 n-6. [2]∑ (n-3) ≥ 20:3 n-3.

Fatty acid	EG	CG	Fatty acid	EG	CG
14:0	9.78	7.10	20:2 n-6	0.35	0.0
15:0	0.58	0.0	20:3 n-6	0.31	0.0
16:0	12.67	10.22	20:4 n-6	1.53	0.0
17:0	0.76	1.3	16:3 n-3	0.55	0.0
18:0	3.86	3.78	18:3 n-3	12.80	31.25
20:0	0.21	0.0	18:4 n-3	1.90	5.74
22:0	0.25	0.0	20:3 n-3	0.22	0.0
14:1	1.25	0.0	20:4 n-3	0.56	0.0
16:1 n-7	4.03	3.41	20:5 n-3	7.84	3.67
16:1 n-9	0.0	0.0	22:5 n-3	0.72	0.0
18:1 n-9	11.32	14.12	22:6 n-3	3.57	1.51
18:1 n-7	3.83	5.21	∑ Saturated	28.11	22.40
20:1 n- 7+9+11	1.29	1.26	∑ Monounsaturated	22.15	24.00
22:1	0.43	0.0	∑ (n-6) PUFA[1]	12.09	9.03
18:2 n-6	8.77	7.56	∑ (n-3) HUFA[2]	12.91	5.18
18:3 n-6	0.23	0.0	DHA/EPA	4.96	0.41
18:4 n-6	0.33	1.47	∑ (n-3)/∑ (n-6)	1.07	0.57

resistance of *Litopenaeus stylirostris* which increased the tolerance to abrupt salinity changes. The beneficial effects of n-3 HUFA on tolerance to salinity stress may also be related to changes in the arrangement of fatty acids in the gill membrane and the consequent increase in shrimp osmosis capability (Palacios *et al.*, 2004). Furthermore, shrimp fed n-3 HUFA rich diets have larger gill area (Palacios *et al.*, 2004), which concurs with the more ramified gill structure reported in n-3 HUFA fed *P. monodon* postlarvae (Rees *et al.*, 1994).

Although stress tests have been commonly used as a tool to compare the quality of crustacean larvae and juveniles in terms of tolerance to a number of physical and chemical parameters (Tackaert *et al.*, 1989; Samocha

et al., 1998; Cavalli *et al.*, 2000; Chim *et al.*, 2001; Álvarez *et al.*, 2004; Palacios & Racotta, 2004; Martins *et al.*, 2006; Immanuel *et al.*, 2007; Sui *et al.*, 2007), to date few studies have attempted to correlate the results of stress tests with the posterior growth performance of shrimp. In this regard, Bauman & Jamandre (1990) reported that batches of *P. monodon* postlarvae that responded positively to a salinity stress test presented a comparatively superior growout performance in ponds. Álvarez *et al.* (2004) found a correlation between stress testing and the survival of *Litopenaeus vannamei* postlarvae during simulated stocking into growout units. These authors, however, were unable to correlate the results of the stress test to growth or survival during a two-month long growout period. Palacios & Racotta (2007) hypothesized that survival of postlarvae to salinity stress tests would be a short-termed phenomenon and thus not necessarily an adequate predictive criterion for longer performance. Results from the present study, however, indicate a possible relationship between survival in the stress tests (for both salinity and ammonia) and posterior growth of shrimp during nursery.

ACKNOWLEDGEMENTS

This study was partially funded by the Brazilian Council for Scientific and Technological Development (CNPq) through a study grant to the first author. E.L.C. Ballester, W. Wasielesky Jr. and R.O. Cavalli are research fellows of this agency.

REFERENCES

Álvarez, A.L., I.S. Racotta, O. Arjona & E. Palacios. 2004. Salinity stress test as a predictor of survival during growout in Pacific white shrimp. Aquaculture, 237: 237-249.

Bauman, R.H. & D.R. Jamandre. 1990. A practical method for determining quality of *Penaeus monodon* Fabricius fry for stocking in grow-out ponds. In: M.B. New, H. Saram & T. Singh (eds.). Proceedings of the Aquatech Conference, technical and economic aspects of shrimp farming. Aquatech, Kuala Lumpur, pp. 124-137.

Bendschneider, K. & R.J. Robinson. 1952. A new spectrophotometric method for the determination of nitrite in seawater. J. Mar. Res., 11: 87-96.

Cavalli, R.O., E.V. Berghe, P. Lavens, N.T.T. Thuy, M. Wille & P. Sorgeloos. 2000. Ammonia toxicity as a criterion for the evaluation of larval quality in the prawn *Macrobrachium rosenbergii*. Comp. Biochem. Physiol., 125: 333-343.

Charmantier, G., M. Charmantier-Daures, N. Bouraricha, P. Thuet, D.E. Aiken & J.P. Trilles. 1988. Ontogeny of osmoregulation and salinity tolerance in two decapod crustaceans: *Homarus americanus* and *Penaeus japonicus*. Biol. Bull., 175: 102-110.

Chim, L., P. Lemaire, M. Delaporte, G.L Moullac, R. Galois & J.L.M. Martin. 2001. Could a diet enriched with n-3 highly unsaturated fatty acids be considered a promising way to enhance the immune defences and the resistance of *Penaeid* prawns to environmental stress? Aquacult. Res., 32: 91-94.

Cobo, M.L., R. Wouters, M. Wille, S. Sonnenholzner & P. Sorgeloos. 2015. Evaluation of frozen umbrella-stage *Artemia* as first animal live food for *Litopenaeus vannamei* (Boone) larvae. Aquacult. Res., 46: 2166-2173.

Dall, W., B.J. Hill, P.C. Rothlisberg & D.J. Sharples. 1990. Behavioural responses to the environment. In: H.S. Blaxter & A.J. Southward (eds.). The biology of the Penaeidae. Academic Press, London, pp. 333-355.

Dhert, P., P. Lavens & P. Sorgeloos. 1992. Stress evaluation: a tool for quality control of hatchery-produced shrimp and fish fry. Aquacult. Europe, 17: 6-10.

Figueiredo, J., R. Van Woesik & J. Lin. 2009. *Artemia franciscana* enrichment model - How to keep them small, rich and alive. Aquaculture, 294: 212-220.

Folch, J., M. Lees & G.H.S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 266: 497-509.

Greenberg, A.E., L.S. Clesceri & A.D Eaton. 1992. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, 1100 pp.

Hamilton, M.A., R.S. Russo & R.V. Thurston. 1977. Trimmed Spearman Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol., 11: 714-719.

Immanuel, G., T. Citarasu, V. Sivaram, M.M. Babu & A. Palavesam. 2007. Delivery of HUFA, probionts and biomedicine through bioencapsulated *Artemi*a as a means to enhance the growth and survival and reduce the pathogenesity in shrimp *Penaeus monodon* postlarvae. Aquacult. Int., 15: 137-152.

Jinbo, T., S. Dan, M. Nakaya, M. Ashidate & K. Hamasaki. 2013. Effects of n-3 highly unsaturated fatty acid content in *Artemia* on survival and development of laboratory-reared horsehair crab *Erimacrus isenbeckii* larvae. Fish. Sci., 79: 459-467.

Kanazawa, A., S. Teshima & K. Ono. 1979a. Relationship between essential fatty acid requirements of aquatic animals and capacity of bioconversion of linolenic

acid to highly unsaturated fatty acid. Comp. Biochem. Physiol. B, 63: 295-298.

Kanazawa, A., S. Teshima, K. Ono & K. Chalayodeja. 1979b. Biosynthesis of fatty acids from acetate in the prawn, *Penaeus monodon* and *Penaeus merguiensis*. Mem. Fac. Fish. Kagoshima Univ., 28: 21-26.

Lavens, P. & P. Sorgeloos. 1996. Manual on the production and use of live food for aquaculture. FAO, Rome, 295 pp.

Lèger, P. & P. Sorgeloos. 1992. Optimized feeding regimes in shrimp hatcheries. In: A.W. Fast & J.L. Lester (eds.). Marine shrimp culture: principles and practices. Elsevier, Amsterdam, pp. 225-244.

Martins, T.G., R.O. Cavalli, R.C. Martino, C.E.M. Rezende & W. Wasielesky Jr. 2006. Larviculture output and stress tolerance of *Farfantepenaeus paulensis* postlarvae fed *Artemia* containing different fatty acids. Aquaculture, 252: 525-533.

Metcalfe, A.P. & A.A. Schmitz. 1961. The rapid preparation of fatty acids for gas chromatographic analysis. Anal. Chem., 33: 363-364.

Ostrensky, A. & W. Wasielesky. 1995. Acute toxicity of ammonia to various life stages of the Sao Paulo shrimp, *Penaeus paulensis* (Pérez-Farfante, 1967). Aquaculture, 132: 339-347.

Palacios, E. & I.S. Racotta. 2007. Salinity stress test and its relation to future performance and different physiological responses in shrimp postlarvae. Aquaculture, 268: 123-135.

Palacios, E., A. Bonilla, A. Pérez, I.S. Racotta & R. Civera. 2004. Influence of highly unsaturated fatty acids on the responses of white shrimp (*Litopenaeus vannamei*) postlarvae to low salinity. J. Exp. Mar. Biol. Ecol., 299: 201-215.

Pontes, C.S. & E.R. Andreatta. 2003. Efeito da oferta de náuplios de *Artemia franciscana* enriquecidos com ácidos graxos poliinsaturados sobre o desenvolvimento de pós-larvas do camarão marinho *Farfantepenaeus paulensis*. Rev. Bras. Zootec., 32: 1544-1550.

Received: 25 April 2016; Accepted: 28 August 2016

Rees, J.F., K. Curé, S. Piyatiratitivorakul, P. Sorgeloos & P. Menasveta. 1994. Highly unsaturated fatty acid requirement of *Penaeus monodon* postlarvae: an experimental approach based on *Artemia* enrichment. Aquaculture, 122: 193-207.

Samocha, T.M., H. Guajardo, A.L. Lawrence, F.L. Castille, M. Speed, D.A. McKee & K.I. Page. 1998. A simple stress test for *Penaeus vannamei* post-larvae. Aquaculture, 165: 233-242.

Sorgeloos P., P. Coutteau, P. Dhert, G. Merchie & P. Lavens. 1998. Use of brine shrimp, *Artemia* spp., in larval crustacean nutrition: a review. Rev. Fish. Sci., 6: 55-68.

Sui, L., M. Wille, Y. Cheng & P. Sorgeloos. 2007. The effect of dietary n-3 HUFA levels and DHA/EPA ratios on growth, survival and osmotic stress tolerance of Chinese mitten crab *Eriocheir sinensis* larvae. Aquaculture, 273: 139-150.

Tackaert, W., P. Abelin, P. Lèger & P. Sorgeloos. 1989. Stress resistance as a criterium to evaluate quality of postlarval shrimp reared under different feeding procedures. In: Brazilian Shrimp Farmers Association-ABCC (eds.). Proceedings of the 3rd Brazilian Symposium on Shrimp Culture. ABCC, João Pessoa, Brazil, pp. 393-403.

Tsuzuki, M.Y., R.O. Cavalli & A. Bianchini. 2000. The effects of temperature, age and acclimation to salinity on the survival of *Farfantepenaeus paulensis* postlarvae. J. World Aquacult. Soc., 31: 459-468.

United Nations Educational, Scientific and Cultural Organization (UNESCO). 1983. Chemical methods for use in marine environmental monitoring. Intergovernmental Oceanographic Commission, Manual and Guides, 12: 53 pp.

Wouters R., M.L. Cobo, J. Dhont & M. Wille. 2009. Developments in feed formulations, feeding practices and culture techniques for marine shrimp larvae. In: C.L. Browdy & D.E. Jory (eds.). The rising tide. Proceedings of the Special Session on Sustainable Shrimp Farming, Aquaculture 2009. The World Aquaculture Society, Baton Rouge, pp. 79-91.