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A Red Shrimp, *Farfantepenaeus brasiliensis* (Latreille, 1817), Larvae Feeding Regime Based on Live Food

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Abstract:

Red shrimp, *Farfantepenaeus brasiliensis*, larvae's response to different concentrations of live foods (diatoms *Chaetoceros gracilis*: 20–100 × 10^3 cells/mL; flagellate *Tetraselmis chuii*: 2–10 × 10^3 cells/mL and *Artemia nauplii* [NA]: 1–5 NA/mL) was investigated in three experiments. Experimental assessments were based on four variables: survival rate, weight gain, development index (DI), and resistance to salinity stress. A combination of *C. gracilis* 80 × 10^3 cells/mL, *T. chuii* 2 × 10^3 cells/mL, and *Artemia* 4 NA/mL provided the best experimental response. Specifically, *F. brasiliensis* larvae fed with the above-mentioned optimal concentrations of microalgae and *Artemia* grew faster and reached the postlarval stage in less time (168 h) than larvae in other feeding regimes evaluated. The effect of *C. gracilis* concentration on larval growth, survival, and the DI demonstrated that microalgae-based foods could be a highly productive alternative to more traditional aquaculture feeding regimes.

The spatial distribution of Caribbean red shrimp, *Farfantepenaeus brasiliensis*, extends from North Carolina and the Florida Keys in the north to the Río Grande do Sul, Brazil in the south, and encompasses the southern Gulf of Mexico (including Yucatan), the West Indies, Bermuda, and the Caribbean coast of Central and South America (Perez Farfante and Kensley 1997). *F. brasiliensis* has been recognized as an excellent candidate species for aquaculture (Anguiano 1999) because populations remain abundant in the wild, growth rates under culture conditions are relatively good, and maximum adult size is large comparable to other penaeid species.

Although this species could be effectively farmed, efficient larval rearing production techniques need to be developed. Penaeid larvae inhabit coastal waters where they prey on a variety of phytoplankton and zooplankton. Under laboratory conditions, cultivation of such plankton communities has proven unreliable and impractical. Hence, a limited array of food organisms, mainly selected strains of microalgae and *Artemia* NA, has provided the basis for indoor shrimp larvae production; although, nematodes and ciliates have also been exploited with moderate success (Leger and Sorgeloos 1992).

Because shrimp larvae begin exogenous feeder by ingesting phytoplankton (Jones et al. 1997), several algae species were cultured for penaeid larvae culture. It included diatoms (e.g., *Chaetoceros* spp.), which are among the best food for early shrimp larvae stages, as well as other species e.g. flagellates such as *Tetraselmis* spp. to enhance the nutritional profile and to provide an adequate size range between $8\text{-}12 \times 7\text{-}10\mu$ exclusive of the setae (Simon 1978; Brown 1991). Consequently, the use of two algal strains as a food source, generally a diatom and a flagellate, has been a popular option (Leal et al., 1985; Aquacop 1983; Gallardo et al. 1995). Although *Tetraselmis* spp. alone were successfully used for penaeid larvae (Aujero et al. 1983; Souza and Kelly 2000), a mixture of algae gained popularity due to the fact that a single algae could not fulfill all larval-stage lipid requirements. Lipids provide endogenous energy reserves during the embryonic and larval periods in decapods crustaceans (Kattner et al., 1994), because they possess little or no ability to biosynthesize n-3 highly unsaturated fatty acids, HUFA's (Teshima 1997). Importantly for aquaculture, the composition of both *C. gracilis* and *T. chuii* contain these HUFA's (Brown et al. 1997). *Artemia,* probably the most frequently-used food organism in aquaculture because of its high nutritional value (Sorgeloos 1980) and good fit with the raptorial habit of shrimp larvae, offers another viable source of HUFA's (Emmerson 1980, 1984; Yufera et al. 1984; Lovett and Felder 1989; Rodriguez et al. 1994).

The purpose of the present study was to determine the optimal feeding regime for *F. brasiliensis* larvae by testing the impact of various concentrations of *C. gracilis*, *T. chuii*, and *Artemia* NA on survival, growth and development index. The quality index was also calculated based on the resistance to salinity stress of the postlarvae harvested from the different treatments.

1. Material and Methods

1.1. Source of Larvae

F. brasiliensis larvae used in each experiment were obtained from a single spawning of naturally inseminated females harvested from the wild. They were captured at a depth of 42 to 67 m near Isla Contoy, Quintana Roo, Mexico. Females were unilaterally eyestalk ablated to accelerate gonad maturation. Eggs were collected and placed in a small tank to obtain the nauplii that were harvested from the hatching tank at stage $N_{1\text{-}2}$.

1.2. Breeding Conditions of the Larvae

Food components were collected from two sources. Algae were cultured in a Guillard medium and given to the larvae during the growth culture's stationary phase. Nauplii (NA) were obtained from cysts of *Artemia franciscana* collected from Great Salt Lake Brine shrimp (Prime *Artemia* Incorporatzio, Utah, U.S.A).

The experiments to determine the optimum concentrations of *C. gracilis* (Experiment 1) and *T. chuii* (Experiment 2) involved monitoring shrimp larvae from the Protozoea$_1$ stage (Pz$_1$) through the postlarvae$_1$ (PL$_1$) stage. Experimental tanks were initially stocked to a density of 100 NA/L.

The experiment to determine the optimum concentration of *Artemia* NA (Experiment 3) involved monitoring larvae from the mysis I (M$_1$) stage through the PL$_1$ stage. This experiment involved stocking 10L conical bottom fiberglass tanks with 4L seawater containing an initial larval density of 50 mysis per liter (volumetric count). Survival rates were estimated using 1L round bottom glass flasks kept in a thermostatically controlled water bath (28 ± 0.5 C) and aerated using a glass rod with 2-4 bubbles/second. Flasks were filled with 500 mL of seawater.

Natural seawater used in the experiments was filtered at 5 μm and treated with EDTA (10 mg/L) recycled through a 1200-L/h capacity power filter (Fluval 403 Hagen, Phyladelphia, Pensilvania, U.S.A) with UV sterilization, was maintained in a 1100L tank for 5 hours prior to use. Temperature, salinity (ppt), oxygen (mg/L), and pH were measured and recorded twice a day. 50% of the water was exchanged in the tanks everyday between M$_1$ and PL$_1$ stages and no antibiotic was added.

Experiment 1

To determine the optimum concentration of diatoms, *F. brasiliensis* larvae were fed five different concentrations (20 x 10^3, 40 x 10^3, 60 x 10^3, 80 x 10^3 and 100 x 10^3 cells/mL) with three replicates for each. During Pz$_2$-Pz$_3$ stages, the concentration of flagellates, T. *chuii* was maintained at a constant level (5 x 10^3 cells/mL). Then, during the period from Pz$_3$ to PL$_1$, the concentration was adjusted to 7 x 10^3 cells/mL and maintained at that level. The flagellate concentrations followed experimental levels used previously for pink shrimp, *F. duorarum* larvae. The addition of *Artemia* NA began after the onset of the M$_1$ stage and continued to PL$_1$ stage (Lopez 1998).

Experiment 2

To determine the optimum concentration of flagellates, larvae were fed five concentrations of *T. chuii* (2 x10^3, 4 x10^3, 6 x10^3, 8 x10^3 and 10 x10^3 cells/mL), each with three replicates. The results obtained in the Experiment 1 were taken into consideration and the *C. gracilis* concentration that provided the best results (80 x 10^3 cells/mL) was used in all treatments from the Pz$_1$ stage to the PL$_1$ stage. *Artemia* NA counts were adjusted accordingly (López 1998).

Experiment 3

To determine the optimum concentration of *Artemia* NA, larvae were fed five different concentrations (1, 2, 3, 4, and 5 NA/mL) with three replicates each. Pz$_3$ stage larvae were placed in tanks at an exact density of 50 per L. Again, diatoms were provided at a concentration of 80 x10^3 cells/mL, following the results achieved in experiment 1; where as, flagellate concentration was modified based on results obtained in experiment 2 and was provided at a density of 2 x 10^{3}cells/mL. Finally, when larvae molted to the M$_1$ stage, *Artemia* NA were added.

Feeding

Larvae were fed every 12 hours at 08:00 and 20:00 following the feeding regime established by Gallardo et al. (1995) for *L. setiferus*. During the experiments, *C. gracilis, T. chuii*, and *Artemia* NA concentrations were adjusted to stick on following schedule (Seafdec 1984):

$$AV = \frac{TV\,(WCC - RCC)}{FCC - RCC}$$

where AV is volume to add; TV is tank volume; WCC is desired cell concentration; RCC is residual cell concentration; and FCC is food cell concentration.

The larval population's development stage in each tank was assessed using a DI (Villegas and Kanazawa 1979). DI, also called metamorphosis rate, was calculated based on the daily observation of 5 larval stages randomly selected from each experimental 10 L tank using the ranking formula:

$$DI = \frac{(\sum A)}{N}$$

where A is the absolute value given per number of larvae of each sub-stage examined, and N is the total number of larvae in each sample. Absolute values assigned for each substages were protozoea$_1$ =1; protozoea $_2$=2; protozoea$_3$=3; mysis$_1$=4; mysis $_2$=5, mysis$_3$= 6; postlarvae$_1$=7.

Growth

Growth was determined by using the total length measurements from 5 randomly selected larvae from each experimental 10-L tank. Measurements of Pz$_1$ stage larvae were made from the tip of the cephalothorax to the end of the telson, including the spines. Pz$_2$ and Pz$_3$ stage larvae were measured from the front of the rostrum to the end of the telson, excluding the spines. For the mysis stage, the larvae were measured from the anterior edge of the cephalothorax to the end of the telson. Once measurements had been recorded, the larvae were dried at 60 C and weighed in pools consisting of 5 larvae grouped by tank and stage. The daily growth coefficient (DGC) was used to estimate the weight gain (Bureau et al. 2000), assuming linear weight increase over the short period of larval development.

$$DGC = \left(\frac{\sqrt[3]{W_f} - \sqrt[3]{W_i}}{t(days)}\right) 100$$

where Wf is the final dry weight; Wi is initial dry weight; and t is time in days to reach postlarval stage 1.

Survival (S)

Survival was calculated and expressed in percentage based on the final number of larvae in the 1L flasks.

Quality Index (QI)

Three groups of thirty postlarvae (PL$_2$) organisms were randomly selected from each of the treatments and placed in 2-L glass flasks with previously filtered, sterilized, and aerated seawater. To reduce stress, postlarvae were kept in these containers for a minimum of 1 h with constant aeration at 28 $\pm$ 1 C. Then, an osmotic shock was achieved by the addition of distilled water so that salinity decreased from 35 to 25 ppt. Postlarvae were counted and the Quality

index (QI) was calculated as the percentage of organisms that survived this shock. QI was calculated for each treatment.

<u>Statistical Analysis</u>

A one-way ANOVA was performed on the growth (DGC, AGR), development index (DI), and quality index (QI) data to detect significant differences among treatments in each experiment. Where significant differences were detected, a test of Tuckey for means comparison was applied (Zar 1996). Data expressed as percentages (survival) were transformed to arcsine before ANOVA analysis taking into account a significance level of 0.05.

2. Results

<u>Experiment 1</u>

Larval weight and length gain are presented Table 1 after feeding with different diatom concentrations. For DGC, the larvae fed 20 x 10^3 cells/mL had significantly lower values when compared to all other treatments. There was also a clear trend in which DGC increased with the *C. gracilis* concentration up to 80 x 10^3 cells/mL. Highest absolute growth rate (AGR) were reached in larvae fed 80 and 100 x 10^3 cells/mL ($P < 0.05$, Table 1). In relation to DI, after 24 h, there were already significant differences among treatments. The 20 x 10^3 cells/mL treatment produced a low DI (Table 2) as compared with other treatments. After 96 h, larvae fed 80 x 10^3 and 100 x 10^3 cells/mL reached the M_l stage. After 168 h, these two treatments led to postlarval stage. In comparison, only 53% of larvae fed 60 x 10^3 cells/mL reached the PL_1 stage at the same time. After 10 days (240 h) the larvae fed 20 x 10^3 cells/mL (Table 2) remained at the M_2 stage indicating a significant delay in development after 216 h. Survival rates of larvae significantly increased with *C. gracilis* concentration. The significant lowest survival was observed with the larvae fed the lowest cell concentration (20 x10^3 cells/mL; Table 1). Postlarvae tested through salinity shock showed significantly higher resistance when fed 60 x 10^3 cells/mL while organisms fed 20 x 10^3 cells/mL showed lowest resistance (Table 1). Postlarvae fed 100 x 10^3 cells/mL show no difference as compared with those fed 80 x 10^3 cells/mL.

<u>Experiment 2</u>

The concentration of *C. gracilis* (Table 1) that provided the best results in Experiment 1 (80 x 10^3 cells/mL) was maintained during the second experiment. In this experiment, neither DGC nor AGR were affected by changing the concentration of *T. chuii* (Table 3). After 168 h, more than 50% of the larvae from all treatments had reached postlarvae stage, regardless of cell concentrations ($P > 0.05$ Table 4). These findings were similar to those of the first experiment with larvae fed 80 x 10^3 cell/mL or more cells/mL of *C. gracilis*. The larvae's resistance to salinity shock also remained unaffected by variation in *T. chuii* concentration (Table 3). Survival rates of the larvae fed flagellate at different cell concentrations differed in a range between 68.7 $\pm$ 1.5 and 89.4 $\pm$ 0.8% ($P > 0.05$ Table 3).

<u>Experiment 3</u>

At a constant cell concentration for both microalgae (80 x 10^3 cells/mL for *C. gracilis* and 2 x 10^3 cells/mL for *T. chuii*), the best DGC values (31.4 $\pm$ 0.4μg live weight day^{-1}) ($P < 0.05$) were achieved with larvae fed 4 *Artemia* NA/mL (Table 5). However, AGR values were not significantly different (P > 0.05) between all *Artemia* NA concentrations tested (Table 5).

DI values did not vary significantly with an increase in *Artemia* NA concentration and remained equal for all treatments. Larvae from all replicates reached the postlarval stage within 72 h from the M_1 stage (Table 6). There were no significant differences in survival rates and tolerance to salinity shock among the various treatments (Table 5). The postlarvae's resistance to salinity shock (QI) for all treatments showed values between 94.5 ± 0.1 and $98.4 \pm 0.7\%$ survival ($P > 0.05$, Table 5).

3. Discussion

The best cell concentration for each kind of live food were selected using the combination of maximum survival percentage, DGC, and DI values as criteria, generating a feeding schedule for *F. brasiliensis* larvae (Table 7). The ultimate test of suitable nutrition was the production of somatic tissues and consequent larval development, which can be summarized by this statement, "larvae that grow well, survive well" (Jones et al. 1997). The concentration of *C. gracilis* that resulted in the highest *F. brasiliensis* larvae weight gain was 80 x 10^3 cells/mL. It also provided the best conditions for survival, AGR, and DI, which improved with the availability of diatoms. As would be expected at low levels of inclusions, at the low cell concentration (20 x 10^3 cells/mL of *C. gracilis*), larvae remained at the Pz_1 stage for an extra day and refrained from feeding. Subsequently, they remained in the Pz_1 to Pz_2 stage for an abnormally long period or faced mortality during the next change from the Pz_3 to M_1 stage. These low growth rates can be explained by the inability of *C. gracilis*, at low concentrations, to provide essential nutrients at the start of larval development. The use of salinity shock is a good measure of the fitness of larvae and is often utilized as a measure of larval quality. In the present experiment, the QI data indicated that larvae fed 40 to 60 x 10^3 cells/mL of *C. gracilis* (Table 1) had the best resistance to salinity change. However, DGC and DI values were significantly lower when compared with the feed containing 80 x 10^3 cells/mL.

Similar results were reported in *Penaeus monodon* postlarvae fed n-3-HUFA enriched *Artemia* NA (Rees et al. 1994). Shrimp that achieved the highest growth rate were less resistant than small individuals to the salinity test. It appears that supplying postlarvae with a diet high in HUFA's may affect the balance among some physiological functions and growth-related activities (e.g. molting, cellular proliferation). As with *P. monodon*, an excess of dietary lipid in *F. brasiliensis* postlarvae fed high a concentration of both *C. gracilis* (80 x 10^3 and 100 x 10^3 cells/mL) and *T. chuii* (5 x 10^3 to 7 x 10^3 cells/mL) could affect the balance between growth and other physiological functions. Both microalgae contained around 13% lipid, but eicosapenthanoic acid (EPA), docohexaenoic acid (DHA), and arachidonic acid (AA) concentrations in HUFA's for diatoms were 3%, 20%, and 2% and for flagellates were 4%, 3%, and 1% respectively (Brown et al. 1997).

Compared with other species, *F. brasiliensis* larvae showed an optimum *C. gracilis* cell concentration higher than that reported for *F. duorarum* (50 x 10^3 cells/mL) (Durruty 1993) and lower than those reported for *F. paulensis* (Alfonso and Silva 1992). The optimum cell concentration (80 x 10^3 cells/mL) reported here was twice that reported for L. *setiferus* (Gallardo et al. 1995) and *L. schmitti* (Alfonso and Gelabert 1986). Present results suggest that if concentrations, used by these authors, were applied to *F. brasiliensis* larvae, poorer results should be expected. *F. brasiliensis* larvae were raised successfully with two microalgal species and monitoring showed an adequate nutrient supply, both in quality and quantity, during a complete larval period.

The absence of statistical differences observed in responses for survival, DGC, AGR, and DI measured at varying concentrations of *T. chuii* indicated that no improvement could be reached with an increase in cell concentration. Therefore, the lowest cell concentration (2 x10^3 cells/mL)

could be used as the flagellate's optimum in the feeding schedule of *F. brasiliensis* larvae. In this study, QI improved 100% when *T. chuii* was included in the larvae regime with a cell concentration of at least 2 x 10^3 cells/mL. Similar results were achieved with *L. setiferus* larvae (Gallardo et al. 1995).

The nutritional value of *T. chuii* for shrimp larvae has been a topic of discussion in the literature. Several researchers reported that *T. chuii* could be eliminated from the feeding regime of shrimp larvae (Kuban et al. 1985; Holloway and Hopkins 1987). However, others concluded that diatoms had a positive and significant impact on larvae nutrition only when complemented by flagellates in an appropriate feeding schedule (Quinitio and Villegas 1982; Alfonso et al. 1988). The optimum *T. chuii* cell concentration (2 x 10^3 cells/mL) for *F. brasiliensis* was higher than that previously reported for *L. setiferus* (1 x 10^3 cells/mL) (Gallardo et al. 1995), and lower than those reported for *F. aztecus*, at 50 x 10^3 cells/mL, (Kuban et al. 1985), for *P. indicus*, at 25 x 10^3 cells/mL (Kumlu and Jones 1993), for *F. duorarum*, at 5-7 x 10^3 cells/mL (Durruty 1993), and for *L. stylirostris*, at 5-10 x 10^3 cells/mL (Ottogalli 1991).

Artemia NA results produced the best DGC at a concentration of 4 NA/mL, but AGR and DI were not statistically different. Inclusion of *Artemia* NA under controlled rearing conditions fit the natural feeding habits of the later larval stages. Feeding habits of shrimp vary during larval development as a result of several metamorphic changes. Filter-feeding habit is well pronounced during the protozoea stages, however it is replaced by a raptorial feeding habit during mysis stages in *L. setiferus* larvae (Lovett and Felder 1989) whereas *P. monodon* larvae it was observed since protozoea stages (Kumarly et al. 1989). The shift from filter to raptorial feeding produces a distinct energy benefit during the M_1-M_2 larval stage (Jones et al. 1997).

In summary, under the reported condition *F. brasiliensis* larvae reached a maximum weight of around 200 μg and high algae densities resulting in reduce weights. However, length increase followed the gradient of algae concentrations. Therefore, food should include at least 80 x 10^3 cells/mL of *C. gracilis*. Lower cell concentrations of this microalgae affected survival, growth, and development of larvae significantly showing *F. brasiliensis*'s strong dependence on it. A combination of *C. gracilis* and *T. chuii* was optimum for larval development when *T. chuii* was maintained at the lowest tested concentration (2 x 10^3 cells/mL). *Artemia* at a density of 4 NA/mL can effectively satisfy animal protein requirements during the mysis stages.

Acknowledgments

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Table 1. *Daily growth coefficient (DGC), absolute growth rate (AGR), survival percentage and quality index (QI) of* Farfantepenaeus brasiliensis *larvae fed with five different concentrations of* Chaetoceros gracilis *(Experiment 1). Mean± SE based on three replicates. Different letters in each row show significant differences (P < 0.05).*

V	Treatments (cell/mL)				
	20×10^3	40×10^3	60×10^3	80×10^3	100×10^3
DGC (µg/d)	35.7 ± 3.4 [d]	43.9 ± 2.9 [bc]	45.5 ± 1.2 [bc]	53.1 ± 0.5 [a]	50.7 ± 1.0 [ab]
AGR (µm/d)	475.6 ± 0.0 [b]	516.1 ± 26.9 [b]	595.6 ± 16.4 [b]	702.1 ± 23.2 [a]	717.5 ± 45.1 [a]
Survival (%)	33.8 ± 6.8 [b]	52.5 ± 10.6 [ab]	70.9 ± 4.6 [ab]	81.5 ± 16 [ab]	84.5 ± 9 [ab]
QI (%)	53.3 ± 11.7 [b]	85.5 ± 9.5 [ab]	98.9 ± 1 [a]	52.8 ± 18 [b]	39.9 ± 20 [b]

Table 2. *Development index (DI) for* Farfantepenaeus brasiliensis *larvae fed with five different cell concentrations of the diatom* Chaetoceros gracilis *(Experiment 1). Mean± SE based on three replicates. Different letters in the same row indicate significant differences (P < 0.05).*

Hours	Treatments (cell/mL)				
	20×10^3	40×10^3	60×10^3	80×10^3	100×10^3
0	1 ± 0 [a]	1 ± 0 [a]	1 ± 0 [a]	1 ± 0 [a]	1 ± 0 [a]
24	1.1 ± 0.1 [a]	1.9 ± 0.1 [b]	2 ± 0 [b]	2 ± 0 [b]	2 ± 0 [b]
48	2 ± 0 [a]	2 ± 0 [a]	2.3 ± 0.12 [b]	2.6 ± 0.1 [c]	2.9 ± 0.1 [c]
72	2.4 ± 0.13 [a]	2.7 ± 0.1 [a]	3 ± 0 [b]	3 ± 0 [b]	3 ± 0 [b]
96	2.8 ± 0.14 [a]	3 ± 0 [a]	3.7 ± 0.125 [b]	4 ± 0 [c]	4 ± 0 [c]
120	2.9 ± 0.1 [a]	3.7 ± 0.2 [b]	4.5 ± 0.3 [c]	4.9 ± 0.1 [d]	5 ± 0 [d]
144	3.1 ± 0.1 [a]	4.5 ± 0.2 [b]	5.3 ± 0.2 [c]	6 ± 0 [d]	6 ± 0 [d]
168	3.9 ± 0.1 [a]	5.3 ± 0.2 [b]	6.53 ± 0.1 [c]	6.9 ± 0.1 [d]	7 ± 0 [d]
192	4.9 ± 0.2 [a]	6.3 ± 0.3 [b]	7 ± 0 [c]		
216	5.4 ± 2.3 [a]	7.0 ± 0 [b]			

Table 3. *Daily growth coefficient (DGC), absolute growth rate (AGR), survival percentage and quality index (QI)* Farfantepenaeus brasiliensis *larvae fed with five different concentrations of* Tetraselmis chuii *(Experiment 2). Mean± SE based on three replicates. Different letters in each row show significant differences (P < 0.05).*

V	Treatments (cell/mL)				
	2×10^3	4×10^3	6×10^3	8×10^3	10×10^3
DGC (µg/d)	45.5 ± 3.5^a	38.05 ± 4.3^a	41.2 ± 0.5^a	40.5 ± 1.2^a	39.5 ± 0.9^a
AGR (µm/d)	707.0 ± 5.8^a	702.0 ± 42.5^a	735.0 ± 5.2^a	692.0 ± 19.1^a	728.0 ± 12.0^a
Survival (%)	78.5 ± 14^a	68.7 ± 1.5^a	78.7 ± 0.01^a	73.8 ± 1.5^a	89.4 ± 0.8^a
QI (%)	100 ± 0^a	99.4 ± 1.5^a	99.7 ± 0.6^a	98.4 ± 1.4^a	99.9 ± 0.2^a

Table 4. *Development index (DI) values of* Farfantepenaeus brasiliensis *larvae fed with five different cell concentrations of the flagellate* Tetraselmis chuii *(Experiment 2). Mean± SE based on three replicates. Different letters in the same row indicate significant differences (P < 0.05).*

Hours	Treatments (cell/mL)				
	2×10^3	4×10^3	6×10^3	8×10^3	10×10^3
0	1 ± 0^a	1 ± 0^a	1 ± 0^a	1 ± 0^a	1 ± 0^a
24	1.9 ± 0.1^a	1.5 ± 0.26^a	1.5 ± 0.1^a	1.5 ± 0.2^a	1.4 ± 0.2^a
48	2 ± 0^a	2 ± 0^a	2 ± 0^a	2 ± 0^a	2 ± 0^a
72	3 ± 0^a	3 ± 0^a	3 ± 0^a	3 ± 0^a	3 ± 0^a
96	4 ± 0^a	3.7 ± 0.26^a	4 ± 0^a	4 ± 0^a	4 ± 0^a
120	4.7 ± 0.1^a	4.5 ± 0.33^a	4.8 ± 0^a	4.9 ± 0.13^a	5 ± 0^a
144	5.8 ± 0^a	5.7 ± 0.24^a	5.7 ± 0.24^a	5.7 ± 0.24^a	6 ± 0^a
168	6.7 ± 0.13^a	6.7 ± 0.26^a	6.9 ± 0.13^a	6.6 ± 0.30^a	7 ± 0^a

Table 5. *Daily growth coefficient (DGC), absolute growth rate (AGR), survival percentage and quality index (QI) for* Farfantepenaeus brasiliensis *larvae fed with five different concentrations of* Artemia franciscana *(Experiment 3). Mean± SE based on three replicates. Different letters in each row show significant differences (P < 0.05).*

V	Treatments (NA/mL)				
	1	2	3	4	5
DGC (µg/d)	25.5 ± 1.8 [ab]	19.8 ± 1.4 [b]	26.6 ± 0.8 [ab]	31.4 ± 0.4 [a]	23.4 ± 3.3 [b]
AGR (µm/d)	690.0 ± 58 [a]	735.0 ± 20 [a]	776.0 ± 34 [a]	742.0 ± 33 [a]	788.0 ± 13 [a]
Survival (%)	91 ± 4 [a]	96.6 ± 2.8 [a]	93.8 ± 1.2 [a]	98.3 ± 2.9 [a]	65.4 ± 1.4 [a]
QI (%)	96.2 ± 1.6 [a]	97.2 ± 1.5 [a]	97.2 ± 1.5 [a]	94.5 ± 0.1 [a]	98.4 ± 0.7 [a]

DI values did not vary significantly with an increase in *Artemia* NA concentration and remained equal for all treatments. Larvae from all replicates reached the postlarval stage within 72 h from the M_1 stage (Table 6). There were no significant differences in survival rates and tolerance to salinity shock among the various treatments (Table 5). The postlarvae's resistance to salinity shock (QI) for all treatments showed values between 94.5 ± 0.1 and 98.4 ± 0.7% survival ($P > 0.05$, Table 5).

Table 6. *Development index (DI) for the larvae of* Farfantepenaeus brasiliensis *fed with five different concentrations of the* Artemia *nauplii (Experiment 3). Mean± SE based on three replicates. Different letters in the same row indicate significant differences (P < 0.05).*

Hours	Treatments (NA/mL)				
	1	2	3	4	5
0	4 ± 0 [a]	4 ± 0 [a]	4 ± 0 [a]	4 ± 0 [a]	4 ± 0 [a]
24	5 ± 0 [a]	5 ± 0 [a]	5 ± 0 [a]	5 ± 0 [a]	5 ± 0 [a]
48	6 ± 0 [a]	6 ± 0 [a]	6 ± 0 [a]	5.7 ± 0.33 [a]	6 ± 0 [a]
72	7 ± 0 [a]	7 ± 0 [a]	7 ± 0 [a]	7 ± 0 [a]	7 ± 0 [a]