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Original

Survival, development, and growth of *Penaeus vannamei* larvae fed on traditional and non-traditional diets shrimp larvae feeding

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ABSTRACT

Objetive. The effect of traditional diets (*Thalassiosira weissflogii* and *Artemia* nauplii) and two other alternatives (not traditional) based on microalgae with rotifers were evaluated on the survival, development and growth from nauplii V (NV) larvae until postlarvae (PL1) of *Penaeus vannamei*. **Materials and methods**. Sixteen replicates (experimental units) were applied for each of the three diets used. The larvae were placed in 12 L containers at 35 psu, 30°C and a density of 200larvae/L. At the beginning, every 24 h and the end of the experiment (PL1: day 8), samples of larvae were obtained to determine survival, development and growth (length and weight). A one-way ANOVA was applied to the data obtained. **Results**. Diet had no influence ($p>0.05$) on survival and weight. Survival ranged from 30.4% (alternative diet B) to 28.5% (traditional diet A). The lowest development, length and weight at PL1 was found with the traditional diet (6.71; 3.53 mm; 58.37 µg/organism) compared with the alternative diets supplied B and C (6.86-6.76; 3.79-3.82 mm; 60.7-65.0 µg/organism. **Conclusions.** Non-traditional alternative diet (B and C) composed of rotifers was the best diet for larval survival, development and growth.

Keywords: *Artemia*; Feeding behavior; rotifers; shrimp (*Source: DeCS*).

RESUMEN

Objetivo. Evaluar el efecto de dietas tradicionales (*Thalassiosira weissflogii* y nauplios de *Artemia*) y otras dos alternativas (no tradicionales) a base de microalgas y rotíferos sobre la supervivencia, el desarrollo y crecimiento de larvas nauplio V (NV) hasta el día de cambio a postlarvas (PL1) de camarón *Penaeus vannamei*. **Materiales y Métodos**. Se realizaron dieciséis réplicas (unidades experimentales) para cada una de las tres dietas utilizadas. Las larvas se colocaron en contenedores de 12 L a 35 ups, 30°C y una densidad de 200 larvas/L. La supervivencia, etapa del desarrollo y el crecimiento se determinaron al inicio, cada 24 h y al final del experimento (PL1: día 8). A los datos obtenidos se les aplicó un ANOVA de una vía. **Resultados**. La dieta no tuvo influencia ($p>0.05$) sobre

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supervivencia y el peso. La supervivencia osciló entre 30.4% (dieta alternativa B) y 28.5% (dieta tradicional A). El desarrollo, longitud y peso más bajo a PL1 fue encontrado con la dieta tradicional (6.71; 3.53 mm; 58.37 µg/organismo) en comparación con las dietas alternativas suministradas B y C (6.86-6.76; 3.79-3.82 mm; 60.7-65.0 µg/organismo). **Conclusiones**. La alimentación más adecuada para la supervivencia, desarrollo y crecimiento larval se obtuvieron con las dietas alternativas no tradicionales (B y C) compuestas de rotíferos.

Palabras clave: *Artemia*; conducta alimentaria; rotíferos; camarón (*Fuente: DeCS*).

INTRODUCTION

Penaeus vannamei shrimp culture in Latin America, and particularly in Mexico, is based on the use of postlarvae and juveniles produced under commercial laboratory conditions (1). In laboratories, the culture performance of penaeid shrimp larvae is related to controlled conditions, and adequate feeding, quantity and quality of food at each stage of development is essential for the success of the various morphological changes from the nauplius stage to postlarvae (2,3).

A great variety of commercial balanced diets exist as alternatives to substitute live food in shrimp larvae (4); however, because of its characteristics in its nutritional composition according to the species under culture, presentation, buoyancy, apprehension, digestibility, and assimilation efficiency, they have not been completely satisfactory when used as substitutes for live food (5). In addition, no favorable results have been obtained to solve the problem of the constant demand for live food, and therefore, larval production laboratories continue to require microalgae in the zoea stage, complemented with *Artemia* nauplii during the mysis stage of *P. vannamei* (6,7).

Diatoms of the genus *Thalassiosira* are frequently used as live food for shrimp larvae in northwestern Mexico (8) because of their size, cell density, growth rate, and biochemical composition (9).

On the other hand, together with the costs and the great demand for *Artemia* cysts routinely used in the larviculture of penaeid shrimp, there could be a deficit in the supply of cyst requirements by commercial laboratories for postlarvae production, resulting in a problem in the feeding of *Artemia* cysts to shrimp larvae. Therefore, a reliable alternative live food urgently needed for shrimp larvae is the use of copepods (10,11) and rotifers (12,13) as a substitute for *Artemia* nauplii.

Rotifers are widely used in the feeding of shrimp and fish larvae because of their high nutritional value and small size, in addition to the fact that their constant natural movement in the water stimulates the larvae's predatory behavior (14,15). Furthermore, it has been suggested that *Brachionus plicatilis* and *Brachionus rotundiformis* species are potential prey for the initial stages of shrimp larvae, particularly the mysis stage of *P. vannamei* (12,16), as well as fish alevin (14), for which mass production techniques have been described (13,14). However, for any cultured aquatic species of commercial importance, there are physicochemical maintenance factors of interest such as salinity, temperature, among others, as well as the type of food and its availability (quality and quantity), which may alter the development and survival of aquatic organisms (3).

While new effective alternatives must be proposed to reduce costs in the production of live food that can replace the use of *Artemia* nauplii (e.g. rotifers) (13), it is also important to determine the influence that these diets have on their survival, as well as on the development and growth of cultured larvae.

Some studies have analyzed the replacement of *Artemia* nauplii by the rotifer *B. plicatilis* in white shrimp larvae (2,16), however, the use of the rotifer *B. rotundiformis* (size less than 200 µm) as food in *P. vannamei* larvae has not been explored, particularly during the zoea stages, when they begin to ingest exogenous food, and therefore, require food according to the size of their mouths. Moreover, studies of the supply of this rotifer in comparison with *Artemia* nauplii (traditional diet) on the growth and development of white shrimp larvae are still unknown. Therefore, in this work, the growth, development, and survival of white shrimp *P. vannamei* larvae fed with different live diets: microalgae, *Artemia* nauplii, and rotifers were analyzed.

MATERIALS AND METHODS

Culture of microalgae, rotifers, and *Artemia nauplii*. *Thalassiosira weissflogii* was the microalgae species used in this work, while, as regards to live prey, the rotifer *Brachionus rotundiformis* and *Artemia franciscana* nauplii were used.

The cultures of *T. weissflogii* were grown in circular transparent plastic containers with a useful volume of 16 L and a maximum capacity of 20 L (dimensions: radius = 13.5 cm and height = 35 cm). The seawater used was filtered down to 1 µm and disinfected with 1 mL/L of 5% commercial sodium hypochlorite for at least 24 h. The microalgae were cultured in the containers. The microalgae were grown on Guillard's f medium, with constant aeration (filtered to 10 µm), illumination of 250-260 µmol/m^2/s, salinity of 35 ups, temperature of 22 to 25°C, pH of 7.0-10.0 (9). Culture density was determined by direct counts under the microscope with the aid of a 0.1-mm-deep Neubauer chamber (Optik Labor).

Rotifers were cultured according to a previous study (13); these were grown in circular transparent plastic vessels with a useful volume of 12 L and a maximum capacity of 17 L (dimensions: radius = 13.5 cm and height = 30 cm) of seawater filtered to 1 µm, with air filtered to 10 µm (higher than 4 mg/L), salinity of 34-36 ups, temperature of 28±1.5°C maintained with thermo-regulated heaters (FINNEX, HMO-50) and without illumination to avoid increases in pH to 8.0±1.0 caused by the photosynthetic activity of the microalgae used as food (2.50±0.29x105 cells/mL). This food was supplied in three daily rations, with a total volume of approximately 3 L, which replaced the harvested volume (25%) of the rotifer containers used to feed the shrimp larvae during the experiment. Under these conditions, a biomass of 154.35±10.46 rotifers/mL was harvested daily, which was estimated by direct stereoscopic counts with the aid of a Sedgwick-Rafter chamber of 1 mL capacity.

To obtain *Artemia* nauplii, the cysts were first hydrated with water for 30 min and then decapsulated by using sodium hypochlorite. Once decapsulated, the cysts were transferred to clear-walled conical containers with a usable volume of 15 L of seawater (35 ups) for hatching. The seawater was filtered to 1 µm and the temperature was maintained at 28°C by using a heater and thermoregulator (FINNEX, HMO-50). In addition, the vessel was kept under intense bubbling from the bottom in order to keep the cysts suspended (17). After 18 h of incubation of the cysts, the nauplii were harvested and immediately inactivated with fresh water at 60°C, then stored at -20°C for later use as food, with a time of no more than 72 h (3).

Obtaining experimental organisms. The *P. vannamei* shrimp larvae used in this study were from the same batch that was in nauplius stage IV. These larvae were donated by the commercial production laboratory "FITMAR" located in southern Sinaloa, Mexico (22°54'28''N and 106°05'44''W), so the larvae had to be transported to the experimental facilities of the Faculty of Marine Sciences in Mazatlan, Sinaloa, Mexico. Transportation took approximately 2 h and the larvae were transported in transparent plastic bags filled with sea water at oxygen saturation and at a temperature of 28-29°C. Once in the experimental facilities, the larvae were transferred to a 450 L plastic tank filled with seawater (35 ups) filtered to 1 µm at a temperature of 30°C and constant aeration.

Experimental design. The experiment began when 100% of the larvae reached nauplius stage V. The effect of three diets was evaluated: one traditional (*Thalassiosira weissflogii* and *Artemia* nauplii) and two non-traditional (microalgae and rotifer *B. rotundiformis*). Diets A and B consisted of supplying only microalgae in the zoea stage, and later for mysis with a combination of microalgae and *Artemia* nauplii (traditional diet A) and microalgae and rotifers (non-traditional diet B) according to the amounts indicated in a previous study (3), while the non-traditional diet (C), involved the combined supply of microalgae and *B. rotundiformis* from zoea I. It should be noted that the diets used were designed based on the organic weights of both microalgae and *Artemia* nauplii and rotifers (Table 1). The experiments were completed when the treatments (diets) recorded more than 50% of the shrimp at the PL1 stage. Each treatment (diet) was replicated 16 times, with a total of 48 experimental units (aquaria). For this, circular plastic containers with transparent walls (dimensions: radius = 13.5 cm and height = 30 cm) with a useful volume of 12 L of seawater and constant aeration, both filtered to 1 µm were used. Salinity in the cultures was 35 ups. Initial density was 200 larvae/L. Larvae in each experimental unit were fed their respective diet on a daily ration. Temperature was maintained at 30°C by using a heater and a thermoregulator (FINNEX, HMO-50).

Table 1. Diets used for feeding *P. vannamei* larvae: microalgae and *Artemia* nauplii from mysis (A), microalgae and rotifers (B), microalgae and rotifers from zoea I to mysis III (C), and average values (standard deviation) of organic weight PO of the organisms used as food in the diets.

Staging	Microalgae	A	B	C
	Cells /mL	Nauplii/ larvae	Rotifers/ larvae	Rotifers/ larvae
Zoea I	10000			10
Zoea II	12000			30
Zoea III	15000			90
Mysis I	5000	30	270	270
Mysis II	5000	40	360	360
Mysis III	5000	50	450	450
			PO	
	Microalgae	(pg/cell) 274.26 (33.39)	(µg/ organism)	
	Rotifer		0.32 (0.58)	
	Nauplii		2.85 (0.68)	

Every day and before each feeding of the diets, a water replacement of 30% was performed in the treatments, as well as the elimination of the bio-deposits (feces and excess food) in the experimental units by siphoning the bottom. Subsequently, the volume of the treatments was adjusted to 12 L, making the replenishment with seawater filtered to 1 µm and set at the same temperature as the cultures.

Survival (S) of larvae in the treatments was assessed every 24 hours by counting live organisms in 500 mL samples. Surviving organisms were calculated by using the equation: $S=(Nf/Ni)\times100$, where the percentage of survival is indicated, Nf represents the number of larvae remaining, and Ni is the initial number of organisms (15).

Additionally, every 24 hours, the developmental index (DI) was assessed with samples of 25-30 larvae from each treatment checked *in vivo* under a stereoscope (LEICA, ZOOM-2000) to determine the stage of development. Subsequently, larvae were returned to the corresponding treatments to reduce sampling mortality. The DI was calculated by using the equation: $DI=(\sum ini)/N$, where i is the value assigned to each larval developmental stage (nauplius V=0; zoea I-III: 1-3; mysis I-III: 4-6; PL1 = 7), ni is the number of larvae of stage i, and N is the total number of organisms analyzed in the sample (3).

To determine the effect of the diets on the growth of the organisms, first, two samples were taken from the larvae at the nauplius V stage, one of 15 and the other of 1000 individuals to measure the total length (TL) and the initial organic weight (OW), respectively. Also, at the end of the experiment, 12-15 larvae from each treatment were sampled to evaluate the final TL. TL measurements were performed according to the recommendations of previous studies (6,7). Regarding the final OW, 400 organisms were randomly selected from each treatment and were concentrated on 25 mm diameter Whatman GF-C glass fiber filters. The initial values of TL and OW in V nauplii were 0.92±0.01 mm and 4.46±0.48 µg/larva, respectively. Additionally, the individual OW of microalgae, rotifers, and *Artemia* nauplii were estimated according to previous studies (2,18); for this purpose, they were concentrated separately in 47 mm (microalgae) and 25 mm (rest of the organisms) Whatman GF-C glass fiber filters.

Statistical analysis. To verify that the data obtained in relation to survival (transformed to arcsine), development index, length, and larval weight had a normal distribution and that their variances were homogeneous, the normality test (Lillieford) and homoscedasticity test (Bartlett) were applied. Subsequently, a one-way analysis of variance (ANOVA) was applied. When ANOVA showed significant differences, Tukey's multiple comparisons tests were performed to detect these differences. In all cases, a significance level (α) of 0.05 was used (19).

RESULTS

Survival rates of more than 25% were obtained with the diets tested. In general, the survival percentages of larvae during the whole experimental period were higher when fed with microalgae in the zoea stage and supplemented with rotifers or *Artemia* nauplii from mysis I (Figure 1). The highest percentage of survival at the end of the experiment was obtained with alternative diet B (30.4%), followed by traditional diet A (28.5%), and later, with non-traditional diet C (microalgae and rotifers from zoea I), however, these observed differences were not statistically significant ($p>0.05$) among the evaluated diets, which revealed that the survival of white shrimp larvae at the end of the experiment (PL1) did not depend on the food supplied (Table 2).

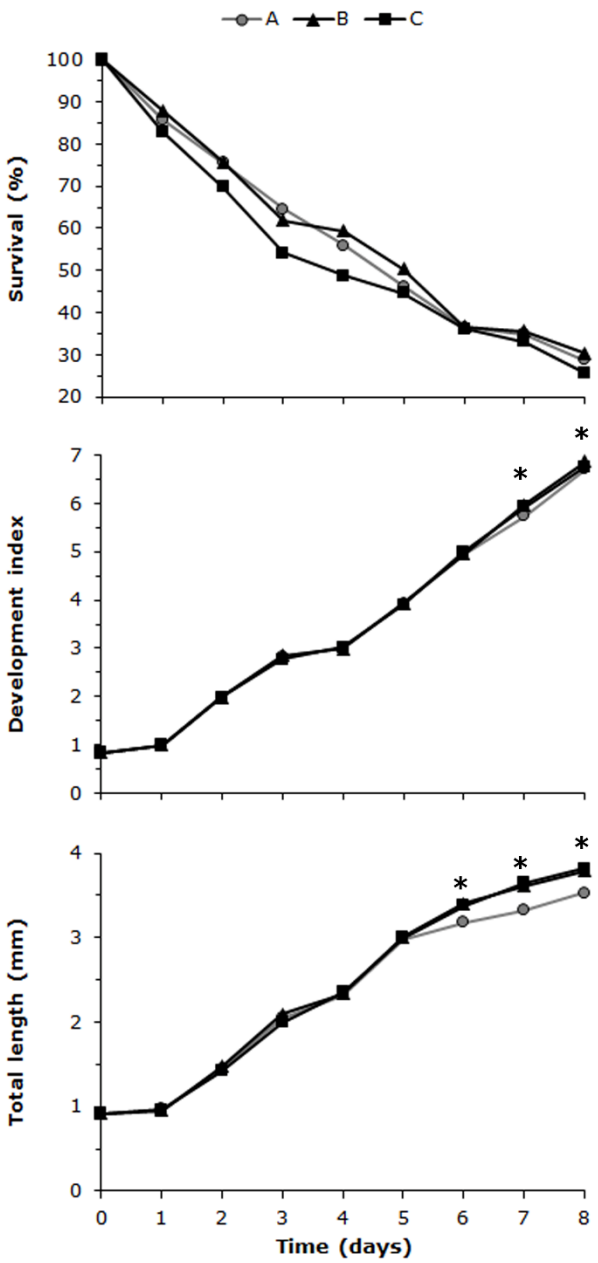

Figure 1. Survival (%), index of development (DI) and average total length (TL in mm) of *P. vannamei* larvae fed on microalgae and (A) *Artemia* from mysis, (B) rotifers, from mysis I and (C) rotifers from zoea I. The symbol (*) indicates statistical differences.

The morphological changes in the development of nauplius V larvae up to PL1 with the non-traditional diets B and C (composed of rotifers) were better. On the eighth day, the treatment on diet B recorded the highest DI value (6.86), followed by an DI of 6.76 on diet C. Although the lowest DI value was found on the traditional diet A with *Artemia* nauplii. The comparison of the progression of the DI during the days of culture with the different traditional and non-traditional diets is shown in figure 1. The increase recorded in the DI during the first three days was similar in all the diets evaluated; however, from days four and six, the increase in the DI was higher on the non-traditional diet C, followed by the traditional diet A and the non-traditional diet B. During the period from day 7 until the end of the experiment, the DI was higher in the alternative non-traditional diets than in the traditional diet.

Table 2. Survival average and (standard deviation) final values, DI development index of *Penaeus vannamei* larvae fed on microalgae and (A) *Artemia* nauplii from mysis I, (B) rotifers, from mysis I and (C) rotifers, from zoea I.

Diet	Survival (%)	DI	Larval Stage
A	28.50 (10.78)[a]	6.71 (0.12)[a]	PL1
B	30.36 (10.04)[a]	6.86 (0.11)[b]	PL1
C	25.70 (11.14)[a]	6.76 (0.06)[ab]	PL1

Different letters indicate significant differences (one-way ANOVA).

The results of the statistical tests between the DI treatments indicated that on the eighth day of culture (PL1), there were significant differences between diets A and B, although when compared with treatment C (Table 2), there were no differences in larval development ($p>0.05$). With these DI results, it is evident that *B. rotundiformis* as a food from mysis had better results than *Artemia* nauplii, in addition to the fact that the anticipated use in zoea presents the same results as the traditional diet.

At the end of the experiment, all treatments had organisms at the PL1 stage and it was found that the average obtained from the traditional feeding regime (A) for larvae, yielded a daily rate of increase in length of 0. 33 mm/day, which is equivalent to a total of 3.53±0.21 mm during the eight days of culture, values that are about 7 and 8% lower than the 0.36 mm/day values for diets B and C (3.79±0.14 and 3.82±0.09 mm, respectively), both based on *B. rotundiformis* rotifers. The increase in length was similar between diets, although from the fifth day on, growth differed, being lower in treatment A, as opposed to B and C (Figure 1).

The results of the statistical tests with the average values obtained for the eighth day (PL1) and TL in the organisms fed with microalgae and *Artemia* nauplii (A) yielded significant differences in the larval sizes ($p<0.05$) between the treatments that received B and C feeding (Table 3). With these TL results, as well as the DI, it was confirmed that the use of *B. rotundiformis* as food for *P. vannamei* larvae yielded better results than *Artemia* nauplii.

Table 3. Final average values (and standard deviation) of total length (TL), organic weight (OW), growth rate (GR) and larval stage of *Penaeus vannamei* fed on microalgae and (A) *Artemia* nauplii from mysis I, (B) rotifers, from mysis I and (C) rotifers from zoea I.

Diet	TL	OW	GR		Larval Stage
	(mm)	(µg/ organism)	(mm/ day)	(µg/ day)	
A	3.53(0.21)[a]	58.37(19.09)[a]	0.33	6.74	PL1
B	3.79(0.14)[b]	60.70(19.78)[a]	0.36	7.03	PL1
C	3.82(0.09)[b]	64.95(18.02)[a]	0.36	7.56	PL1

Different letters indicate significant differences (one-way ANOVA).

As with survival, the average values of the final organic weights of the larvae were not statistically different between diets ($p>0.05$), however, the highest value in weight (64.95 µg) and the highest growth rate (7.56 µg/day) occurred in organisms cultured with *B. rotundiformis* from zoea I on diet C (Table 3).

DISCUSSION

Despite possible attempts to substitute live food by an equally effective, convenient and reliable inert diet, nowadays, microalgae and macroinvertebrates still play a crucial and important role in survival and metamorphosis when used as a food source in the early larval stages of penaeid shrimp (4), which can influence positively or negatively the growth field of cultured organisms depending on the type and size of the selected food (20). This coincided with this study because the zoea and mysis larvae adequately utilized the rotifers provided in diets B and C. Also, the use of rotifers and other zooplanktonic organisms in considerable amounts are beneficial for the production of fish and shrimp larvae, not only in growth but also in survival, both in laboratory conditions and in commercial farms (11,14,15).

In addition, when *B. rotundiformis* was used to fed to white shrimp larvae, it was found that it can be used as live food at an early stage (zoea I), which had not been reported by other authors, and (zoea III and mysis I) for *P. vannamei*, when *Artemia* nauplii and the rotifer *B. plicatilis* were used for feeding purposes as larger prey compared to the sizes of *B. rotundiformis* (2,16).

This study found that alternative or non-traditional diets (B and C) were as efficient as the traditional diet (A) in terms of survival; however, in the development index and larval growth, the best results were obtained using the B diet. This could be due to the fact that, although the organic composition of the diets was equivalent, the proximal composition was different between diets. In this sense, it has been recorded that *Artemia* nauplii have 58.20% protein, 19.27% lipids, and 3.69% carbohydrates, while the rotifer *B. rotundiformis* have 45.73-61.37% protein, 21.04-24.12% lipids, and 10.36-22.72% carbohydrates (21,22).

Although the survival rates were low, probably as a consequence of the daily water changes to which the larvae were subjected, the results differ from those reported previously by other authors (23,24,25), where *Artemia nauplii* are mentioned as the main alternative and the best live food for the successful growth and survival of peneid larvae. However, studies on *P. vannamei* (15,16) showed that other macroinvertebrates such as rotifers may be better alternatives than *Artemia* to increase survival and development of shrimp larvae. Although the effect of food was not different on survival in the three diets tested for this study, it is known that the time it takes for the penaeids to pass to each larval stage and the growth of the shrimp has some influence.

The results of this study show that diets based on *B. rotundiformis* rotifers used to verify the efficiency of alternative non-traditional diets (B and C) compared to *Artemia* nauplii (A) produced the best results in the development index. The facts indicate that, during the culture of zoea stages of this species, these do not feed exclusively on phytoplankton (microalgae) as live food, but also on other zooplanktonic organisms according to their mouth size and

the development of other specialized feeding structures (26,27). A study conducted with *P. vannamei* larvae (16) found that the rate of development decreases when they are fed on *Artemia* nauplii compared to when *B. plicatilis* is used as food. This could be attributed to the various nutritional components in the diets, which are necessary to reach these stages according to their changing feeding habits during larval development (2).

Some researchers have also pointed out that feeding has a significant influence on the length of *P. vannamei* during the first life stages (26,28) and on the biomass of juveniles of the same species (15,25). The incidence of food and diets on the growth of the early life stages of this shrimp species confers an important role on the feeding factor, which cannot be ignored when culturing aquatic organisms (27). In this study, the major growth was achieved with the rotifer-based alternative diets, since the length and weight were considerably lower when fed on *Artemia* nauplii-based diets. Another report reveals improved growth in length and weight of *P. vannamei* larvae (mysis I) and postlarvae (1 and 6) when fed diets (nematode-based) other than *Artemia* nauplii (24), which corroborates the influence of the feeding factor and type of food on the growth, development, and survival of white shrimp larvae, as well as other marine organisms.

Therefore, it is essential to continue with the studies on the search and use of new organisms as food sources, among them, other species of rotifers and their most appropriate quantity to feed shrimp larvae, resulting in a better growth, survival and development when supplied at least in an advanced stage to zoea III, as suggested by a study where *B. plicatilis* was used to feed shrimp larvae (16). In addition, in recent years new techniques have been developed for the massive production of rotifers (13,14,29), their easy capture and digestion allow a better utilization by zoea and mysis larvae of penaeid shrimp and other fish larvae.

In conclusion, it was determined that shrimp larvae fed with rotifers in non-traditional diets (B and C) recorded a similar performance compared to the traditional diet based on *Artemia* nauplii (A), with advantages in development and growth, suggesting the implementation of new feeding alternatives based on *B. rotundiformis* rotifers in culture for commercial companies producing *P. vannamei* shrimp larvae.

Conflict of interest

The authors of this article declare that we have no conflicts of interest.

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