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Research Article

Effect of addition of *Navicula* sp. on plankton composition and postlarvae growth of *Litopenaeus vannamei* reared in culture tanks with zero water exchange

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ABSTRACT. The aim of this study was to evaluate the effect of the addition of *Navicula* sp. on plankton composition and postlarvae growth of *Litopenaeus vannamei* reared in culture tanks with zero water exchange systems. Four treatments were considered: zero water exchange (ZWE); ZWE with the addition of feed (ZWE-F); ZWE with the addition of *Navicula* sp. (ZWE-N) and ZWE with the addition of feed and *Navicula* sp. (ZWE-FN), all in triplicate. Shrimp of 17.7 ± 0.02 mg were stocked at a density of 2500 shrimp m^{-3} and microalgae added on the 1st, 5th and 15th day at a density of $5x10^4$ cell mL^{-1}. The shrimp were fed a commercial feed composed by 42% crude protein four times a day except in the ZWE treatment. For data analysis we used Cochran, Shapiro-Wilk, ANOVA, Tukey and Student-t tests ($P < 0.05$). The most frequent genera were: *Anabaena, Arcella, Asplanchma, Bosmina, Brachionus, Cylindrotheca, Daphnia, Fragilaria, Hemiaulus, Keratella, Orthoseira, Oscillatoria, Phymatodocis, Rhabdonema, Skeletonema, Sckizothrix* and *Ulothrix*. Significant differences between treatments were observed for TAN, NO_2-N, alkalinity, final weight, weight gain, final biomass, biomass gain, feed conversion ratio, specific growth rate and survival. The ZWE-FN treatment showed better production parameters, indicating the benefits of the addition of *Navicula* sp. as a natural food source for *L. vannamei* postlarvae in zero water exchange systems.

Keywords: *Litopenaeus vannamei*, *Navicula*, phytoplankton, zooplankton, cyanobacteria, aquaculture.

Efecto de la adición de *Navicula* sp. sobre la composición del plancton y el crecimiento de postlarvas de *Litopenaeus vannamei* criadas en estanques de cultivo sin recambio de agua

RESUMEN. El objetivo de este estudio fue evaluar el efecto de la adición de *Navicula* sp. en la composición del plancton y el crecimiento de postlarvas de *Litopenaeus vannamei* en estanques de cultivo sin recambio de agua. Se realizaron cuatro tratamientos: sin recambio de agua (ZWE); ZWE con adición de ración alimenticia (ZWE-F); ZWE más la adición de la diatomea *Navicula* sp. (ZWE-N) y ZWE con adición de la ración alimenticia y adicionalmente *Navicula* sp. (ZWE-FN), todos ellos con tres repeticiones. Los camarones de 17,7 ± 0,02 mg fueron sembrados a una densidad de 2500 camarones m^{-3}, las microalgas fueron adicionadas el 1º, 5º y 15º día de cultivo a una densidad de $5x10^4$ cel mL^{-1}. Los camarones se alimentaron con una ración comercial con 42% de proteína cruda, cuatro veces al día. Para los análisis estadísticos se utilizaron las pruebas de Cochran, Shapiro Wilk, ANOVA, Tukey y t de Student ($P < 0,05$). Los géneros más frecuentes fueron: *Anabaena, Arcella, Asplanchma, Bosmina, Brachionus, Cylindrotheca, Daphnia, Fragilaria, Hemiaulus, Keratella, Orthoseira, Oscillatoria, Phymatodocis, Rhabdonema, Skeletonema, Sckizothrix* and *Ulothrix*. Se encontraron diferencias significativas entre los tratamientos para TAN, NO_2-N, alcalinidad, peso final, ganancia de peso, biomasa final, ganancia de biomasa, índice de conversión, tasa de crecimiento específico y supervivencia. El tratamiento ZWE-FN mostró los mejores parámetros de producción, resaltando los beneficios de la adición de *Navicul*a sp. como fuente de alimento natural para las postlarvas *L. vannamei*.

Palabras clave: *Litopenaeus vannamei*, *Navicula*, fitoplancton, zooplancton, cyanobacteria, acuicultura.

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INTRODUCTION

Large quantities of formulated feed with high animal protein content can cause eutrophication in aquaculture systems, increasing the nutrient load in effluents (Tacon *et al*., 2002). Their use increases production costs (Audelo-Naranjo *et al*., 2012) and can result in an insufficient supply of some essential nutrients (Crab *et al*., 2007), thus becoming a limiting factor in intensive systems. To minimize or reduce this nutrient deficiency, organic and inorganic fertilizers can be added to the cultivation systems to promote growth of the microbial community, which is a food source (Brito *et al*., 2009a, 2009b; Asaduzzaman *et al*., 2010; Lara-Anguiano *et al*., 2013). Shrimp can feed on natural biota such as phytoplankton, zooplankton and bacteria present in culture systems (Otoshi *et al*., 2011). This biota can supply some of the shrimps' nutritional needs (Martínez-Cordova & Enríquez-Ocaña, 2007), and improve the activity of digestive enzymes (Xu *et al*., 2012).

In intensive farming systems with Pacific white shrimp (*Litopenaeus vannamei*), microalgae (through photosynthesis) and the other constituents of the microbial community can play an important role in recycling nutrients (Audelo-Naranjo *et al*., 2012; Sánchez *et al*., 2012) decreasing the anoxic zones in ponds and alleviating the nutrient load in wastewater (Martínez-Porchas *et al*., 2010), while providing a nutrition source for shrimp in semi-intensive (Otoshi *et al*., 2011) and intensive systems (Sánchez *et al*., 2012).

Depending on the species and culture conditions, benthic diatoms contain an average of 32 to 38% crude protein (Gordon *et al*., 2006). However, Khatoon *et al*. (2009) found that *Navicula* sp., grown in a Conway culture medium contains 494 g of crude protein, 259 g of lipids and 111 g of carbohydrates per kilogram of dry matter, and the profile of polyunsaturated fatty acids includes 82 g of EPA and 22 g of DHA for each kilogram of total fatty acids. Despite the importance of diatoms, little attention has been paid to them in zero water exchange systems, mainly due to the reduced availability of light and the predominance of heterotrophic bacteria.

In zero or minimal exchange systems, the main forms of nitrogen removal are photosynthetic and heterotrophic bacterial activities (Cohen *et al*., 2005; Becerra-Dorame *et al*., 2011). For this reason, in zero or minimal water exchange, it is necessary to know the components of the natural community and understand the role of each one in the entire ecosystem (Avnimelech, 2009; Crab *et al*., 2012).

In this respect, the aim of this study was to evaluate the effect of the addition of the benthic diatom *Navicula* sp. on the plankton composition and postlarvae growth of *Litopenaeus vannamei*, reared in culture tanks in zero water exchange.

MATERIALS AND METHODS

Experimental conditions

An indoor trial was conducted for 20 days at the Sustainable Mariculture Laboratory (LAMARSU) of the Fisheries and Aquaculture Department (DEPAq) of the Rural Federal University at Pernambuco (UFRPE), Recife, Brazil (08º01'00.16"S, 034º56'57.74"W). The experimental design was completely randomized with four treatments: zero water exchange (ZWE); ZWE with the addition of feed (ZWE-F); ZWE with the addition of *Navicula* sp. (ZWE-N) and ZWE with the addition of feed and *Navicula* sp. (ZWE-FN), all in triplicate.

Five days prior to stocking shrimp, water from a matrix tank (TAN 0.12 mg L^{-1}, NO_2-N 2.26 mg L^{-1}, alkalinity 100 mg $CaCO_3$ L^{-1} and settleable solids 27 mL L^{-1}) was mixed and equally distributed to fill twelve black-plastic tanks (50x35x23 cm) up to approximately 50% of the volume, completed with 50% sea water (with a salinity of 35 g L^{-1}, filtered and treated with a chlorine solution of 10 mg L^{-1}, then dechlorinated and aerated for 48 h).

Aeration was supplied with three airstones from a 2-HP blower. There was no water exchange during the experimental period, but dechlorinated freshwater was added to compensate for evaporation. The light intensity was kept at ~1000 lux using a fluorescent lamp with a 12-h light/dark photoperiod.

Shrimp stocking, feeding, and addition of organic carbon

Specific pathogen-free postlarvae (17.7 ± 0.02 mg) of *L. vannamei* were obtained from a commercial laboratory (Potiporã, Barra de Sirinhaém, PE, Brazil) and stocked at a density of 2500 shrimp m^{-3}. The postlarvae were fed four times a day (at 08:00, 11:00, 14:00 and 17:00 h), with a commercial shrimp feed with 42% crude protein (Aquavita Premiun, Guaraves, Paraíba, Brazil) based on Van Wyk table (1999), and adjusted daily according to estimated shrimp consumption, mortality rate and leftover feed. Molasses (40% organic carbon) were added once a day to establish a 12:1 C:N ratio in the experimental units throughout the culture period, assuming that 50% of the amount of feed is organic carbon and 1 kg of the 42% crude protein feed with 6.25%-N has 67.2 g of nitrogen, there is a need for 306.4 g organic carbon, or 766.1 g of molasses (Samocha *et al*., 2007; Avnimelech, 2009).

Shrimp performance parameters

Shrimp weight was monitored at the end of the experiment, when biomass gain, specific growth rate (SGR), mean final weight, weekly growth, feed conversion ratio (FCR), survival and yield were determined based on the following equations: Biomass gain (g) = final biomass (g) – initial biomass (g); SGR (% day^{-1}) = 100 x [ln final weight (g) – ln initial weight (g)]/time (days); Final weight (g) = final biomass (g)/survival; Weekly growth (g $week^{-1}$) = biomass gain (g)/times (weeks) of culture; FCR = feed supplied (dry weight)/biomass gain; Survival (%) = (number of individuals at the end of the evaluation period/initial number of individuals stocked) x 100; Yield (kg m^{-3}) = final biomass (kg)/volume of experimental unit (m^3).

Diatom addition

The benthic diatoms (*Navicula* sp.) were obtained from LAMARSU-DEPAq-UFRPE and cultured in a Conway medium (Walne, 1966) containing $FeCl_3.6H_2O$ 1.30 g L^{-1}; $MnCl_2.4H_2O$ 0.36 g L^{-1}; H_3BO_3 33.6 g L^{-1}; EDTA 45.0 g L^{-1}; $NaH_2PO_4.2H_2O$ 20.0 g L^{-1}; $NaNO_3$ 100.0 g L^{-1}; $ZnCl_2$ 1.1 g L^{-1}; $CoCl_2.6H_2O$ 1.0 g L^{-1}; $(NH_4)6M_{O7}O_{24}.4H_2O$ 0.45 g L^{-1}; CuSO4.5H2O 1.0 g L^{-1}; $Na_2SiO_3.5H_2O$ 2.0 g L^{-1}; vitamins B_{12} 0.1 g L^{-1} and B_1 1.0 g L^{-1}, which was used in a 1.0 mL L^{-1} solution, maintained in water with 30 g L^{-1} salinity, pH 7.9, temperature 25 ± 1°C and the light intensity was kept at ~2000 lux using a fluorescent lamp with a 12-h light/dark photoperiod. Diatoms were added on 1st, 5th, 10th and 15th days of cultivation in the (ZWE-N) and (ZWE-FN) tanks at a concentration of $5x10^4$ mL^{-1}, corresponding to an addition of approximately 400 mL of microalgae to the tanks.

Water quality monitoring

Dissolved oxygen and temperature were monitored with a DO meter (YSI model 55, Yellow Springs, Ohio, USA), twice a day (08.00 and 16.00 h). Salinity (YSI 30 model 30/50, Yellow Springs, Ohio, USA), pH (pH meter YSI model 100, Yellow Springs, Ohio, USA), total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N) and alkalinity ($CaCO_3$) were monitored every five days using a spectrophotometer (ALFAKIT-AT10P, Brazil) and a compact alkalinity kit (ALFAKIT, Brazil), respectively.

Phytoplankton, Zooplankton and Cyanobacteria monitoring

Vertical water sampling was performed at the start and end of the experiment using plastic bottles of 500 mL for phytoplankton, zooplankton and cyanobacteria collection. The water was filtered through a cylindrical-conical net (mesh: 15 µm for phytoplankton and cyanobacteria, 50 µm for zooplankton) to 10 mL, to obtain a 50-fold concentration. The phytoplankton, zooplankton and cyanobacteria were fixed with formalin (4%), buffered with borax (1%) and stored in 10-mL plastic recipients. A Sedgewick-Rafter chamber and stereomicroscope with magnification of 800x were used for identification and quantification of the phytoplankton, zooplankton and cyanobacteria samples, respectively (Pereira-Neto *et al*., 2008).

The phytoplankton and cyanobacteria were identified following Hoek *et al*. (1995) and Stanford (1999), and concentrations were estimated following Pereira-Neto *et al*. (2008) and expressed as cells mL^{-1}. The zooplankton was identified following Bradford-Grieve *et al*. (1999) and concentrations were estimated following APHA (2005) and expressed as ind mL^{-1}.

Statistical analysis

A parametric one-way ANOVA was used to analyze production parameters, after confirming homocedasticity (Cochran $P < 0.05$) and normality (Shapiro-Wilk $P < 0.05$). The Student's t-test ($P < 0.05$) was used in the analysis of FCR. Water quality parameters, phytoplankton, zooplankton and cyanobacteria density were analyzed by performing repeated measures ANOVA. Data analyses were performed using ASSISTAT Version 7.7 (Assistat Analytical Software, Campina Grande, Paraiba, Brazil).

RESULTS

The mean values of dissolved oxygen, temperature, pH and salinity determined in the four treatments were not significantly different ($P > 0.05$) (Table 1). However, significant differences ($P < 0.05$) were detected for TAN, NO_2-N and alkalinity (Table 1).

The phytoplankton population was composed of 35 genera at the start of the experiment and 28 genera at the end. The most frequent genera were *Fragilaria*, *Orthoseira*, *Rhabdonema* and *Skeletonema* at the start and *Cylindrotheca*, *Hemiaulus*, *Skeletonema*, *Phymatodocis* and *Ulothrix* at the end (Table 2). The zooplankton population was composed of seven genera at the start and 13 at the end. The most frequent genera were *Daphnia* and *Brachionus* at the start, and *Arcella*, *Bosmina*, *Daphnia*, *Asplanchma*, *Brachionus* and *Keratella* at the end (Table 3). The cyanobacteria were composed of 13 genera at the start and 11 at the end. The most frequent genera were *Anabaena*, *Oscillatoria* and *Sckizothrix* at the start and at the end (Table 4). However, no significant differences ($P > 0.05$) were detected for phytoplankton, zooplankton and cyanobacteria density.

Table 1. Water quality parameters during the culture (20 days) of *Litopenaeus vannamei* postlarvae reared in zero water exchange, with and without the addition of feed and/or diatoms. [1]The data correspond to the mean ± standard deviation. Mean values in same row with different superscript differ significantly ($P < 0.05$). Results from repeated measures ANOVA and Tukey test; Zero water exchange (ZWE); ZWE with the addition of feed (ZWE-F); ZWE with the addition of *Navicula* sp. (ZWE-N) and ZWE with the addition of feed and *Navicula* sp. (ZWE-FN); dissolved oxygen (DO), total ammonia nitrogen (TAN) and nitrite-nitrogen (NO_2–N).

Parameters / Treatments[1]	Salinity (ppt)	Temperature (ºC)	DO (mg L^{-1})	pH	TAN (mg L^{-1})	NO_2–N (mg L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})
ZWE	27.0 ± 0.10a	25.0 ± 0.10a	6.6 ± 0.03a	7.4 ± 0.13a	0.10 ± 0.09b	2.71 ± 0.15a	96.7 ± 8.10b
ZWE-F	27.0 ± 0.06a	25.0 ± 0.10a	6.2 ± 0.07a	7.4 ± 0.06a	0.32 ± 0.04b	2.56 ± 0.22a	87.3 ± 7.60b
ZWE-N	27.0 ± 0.07a	25.0 ± 0.12a	6.5 ± 0.04a	7.4 ± 0.05a	0.40 ± 0.02b	2.76 ± 0.05a	99.3 ± 4.41ab
ZWE-FN	26.9 ± 0.01a	24.5 ± 3.15a	6.1 ± 0.12a	7.4 ± 0.08a	1.07 ± 0.21a	1.52 ± 0.26b	131.3 ± 9.75a

The shrimp survival rates were all above 87% during the 20-day experimental period in ZWE-FN and ZWE-F. However in ZWE and ZWE-N the survival rates were below 50%. The shrimp FCR in ZWE-FN was significantly lower ($P < 0.05$) than the ZWE-F. Shrimp performance parameters (final weight, final biomass, weight gain, biomass gain and SGR in the ZWE-FN were significantly higher ($P < 0.05$) than in the other treatments (Table 5).

DISCUSSION

The water quality parameters of dissolved oxygen, pH, salinity and TAN were within the ranges suggested by Van Wyk & Scarpa (1999) for marine shrimp. However, temperature and NO_2-N for all treatments and alkalinity, with the exception of ZWE-FN, were different than that recommended. The water temperature was lower in all treatments, yet presented no influence on growth and feed consumption, because growth and FCA rates were good.

The NO_2-N levels found in this study did not cause great problems when salinity was between 20-35 g L^{-1} (Wasielesky *et al*., 2006) However, Cohen *et al.* (2005), studying a zero water exchange system, observed an exponential increase in NO_2-N levels during the growth period, causing shrimp mortality. The ZWE-FN had the highest concentration of TAN among the treatments. A zero water exchange system can have sudden changes of TAN and NO_2-N and accumulate NO_3-N, due to variations in the microbial biomass during the culture period (Cohen *et al*., 2005), even with a higher C:N ratio (15-20:1) (Gao *et al*., 2012).

Khatoon *et al*. (2009) observed higher TAN and NO_2-N concentrations in the control than in the groups treated with the addition of diatoms during the culture of *Penaeus monodon*. Sanchez *et al*. (2012) observed significant differences in concentrations of NO_2-N in tanks with and without the addition of diatoms in cultivation of *L. vannamei*. However, Godoy *et al*. (2012), when comparing tanks receiving bioflocs, tanks with addition of diatoms and mixed tanks (bioflocs and diatoms), noted significant differences in water quality variables. The diatoms can probably absorb part of the nutrients provided in autotrophic microbial-based-systems, but in heterotrophic microbial-based-systems the accumulation of particles reduces the penetration of light, which in turn likely reduces the nutrient absorption rates of the diatoms. Castillo-Soriano *et al*. (2013) showed that hetero-trophic and nitrifying bacteria are the main factors responsible for the transformation of TAN and NO_2-N in heterotrophic microbial-based-systems.

Levels of $CaCO_3$ less than or equal to 100 mg L^{-1} and pH under 7 for long periods can affect the performance of shrimp in zero water exchange systems (Furtado *et al*., 2011). The alkalinity levels in the ZWE-FN was at the recommended level, which probably contributed to the better growth of shrimp. The higher alkalinity may be related to phytoplankton production since microalgae take in CO_2 from the water column during photosynthesis, leading to $CO_2 + H_2O = HCO_3^- + H^+$, thus making more bicarbonate ions available in the water column (Van Wyk & Scarpa, 1999; Becerra-Dórame *et al*., 2011).

Cyanobacteria were the most abundant organisms, followed by Heterokontophyta and Chlorophyta. However, *Microcystis* and *Merismopedia* (Cyano-bacteria) were not observed in the ZWE-N and ZWE-FN treatments, at the end of the experiment. The data in the literature on the quantity and composition of phytoplankton in shrimp farming systems are extremely variable. Maia *et al.* (2011, 2013), studying intensive culture systems in Brazil, reported densities above 400,000 cells mL^{-1}. These amounts may vary according to the fertilization regime and environmental conditions (temperature and salinity), which can

Table 2. Phytoplankton composition during the culture (20 days) of *Litopenaeus vannamei* postlarvae reared in zero water exchange, with and without the addition of feed and/or diatoms. [1]The data correspond to the mean. Mean values in same row with different superscript differ significantly ($P < 0.05$). Results from repeated measures ANOVA; Zero water exchange (ZWE); ZWE with the addition of feed (ZWE-F); ZWE with the addition of *Navicula* sp. (ZWE-N) and ZWE with the addition of feed and *Navicula* sp. (ZWE-FN).

Division/Genera	Initial culture	Final culture			
		ZWE	ZWE-F	ZWE-N	ZWE-FN
Dinophyta (cells mL^{-1})	1.37	5.38a	6.15a	2.69a	3.46a
Gymnodinium	0.23	1.54	3.08	1.92	1.15
Peridinium	0.21	3.08	0.77	0.77	1.15
Scrippsiella	0.93	0.77	2.31	0.00	1.15
Heterokontophyta (cells mL^{-1})	1828.95	3546.74a	3218.17a	3514.92a	3683.04a
Biddulphia	0.06	0.00	0.00	0.00	0.00
Characiopsis	0.03	0.00	0.00	0.00	0.00
Chloridella	9.31	1.92	5.77	3.46	1.92
Cocconeis	0.18	0.00	0.00	0.00	0.00
Coscinodiscus	0.08	0.00	0.38	0.38	0.38
Cyclotela	0.08	1.54	13.47	1.15	0.00
Cylindrotheca	26.82	1412.74	1994.46	1495.85	1488.92
Cymbella	0.93	0.00	0.38	0.00	0.38
Diatoma	14.00	28.09	61.17	47.32	50.40
Diploneis	0.00	0.00	0.00	0.00	0.00
Fragilaria	555.17	0.00	7.31	21.54	38.47
Hemiaulus	49.58	663.67	150.70	736.77	939.13
Navicula	101.89	17.31	13.85	116.19	36.55
Ophiocytium	0.03	0.00	0.00	0.00	0.00
Orthoseira	192.79	14.11	31.55	0.00	0.00
Rhabdonema	454.45	0.00	1.92	0.00	0.00
Skeletonema	421.57	1402.36	934.90	1090.72	1121.88
Synedra	1.78	5.00	2.30	1.54	5.00
Tetracyclus	0.13	0.00	0.00	0.00	0.00
Thalassiosira	0.03	0.00	0.00	0.00	0.00
Triceratium	0.03	0.00	0.00	0.00	0.00
Chlorophyta (cells mL^{-1})	1310.67	2726.22a	2873.73a	1896.35a	1572.98a
Actinastrum	0.06	0.00	0.00	0.00	0.00
Botryococcus	14.98	106.19	35.39	13.85	30.78
Characium	0.03	0.00	0.00	0.00	0.00
Haematococcus	0.71	1.15	1.15	0.77	1.15
Koliella	0.00	6.16	2.31	146.58	8.85
Micrasterias	0.00	0.38	0.00	0.77	0.00
Mychonastes	344.77	747.92	706.37	0.00	173.13
Phymathodocis	92.30	1064.17	860.80	1029.55	634.81
Planctonema	291.58	126.96	459.76	288.55	173.13
Spirogyra	145.02	121.96	73.10	9.62	71.37
Spirotaenia	2.49	0.00	0.00	0.00	0.00
Tetradesmus	0.00	0.00	0.77	0.00	0.00
Ulothrix	418.73	551.32	734.07	548.63	479.76
Euglenophyta (cells mL^{-1})	3.05	3.08a	6.93a	1.92a	2.31a
Euglena	0.79	0.77	0.77	0.00	0.77
Trachelomonas	2.26	2.31	6.16	1.92	1.54
Total phytoplankton (cells mL^{-1})	3.144	6.281a	6.104a	5.415a	5.261a

Table 3. Zooplankton composition during the culture (20 days) of *Litopenaeus vannamei* postlarvae reared in zero water exchange, with and without the addition of feed and/or diatoms. [1]The data correspond to the mean. Mean values in same row with different superscript differ significantly ($P < 0.05$). Results from repeated measures ANOVA; Zero water exchange (ZWE); ZWE with the addition of feed (ZWE-F); ZWE with the addition of *Navicula* sp. (ZWE-N) and ZWE with the addition of feed and *Navicula* sp. (ZWE-FN).

Division/ Genera	Initial culture	Final culture			
		ZWE	ZWE-F	ZWE-N	ZWE-FN
Protozoa (ind mL^{-1})	0.30	0.31a	0.41a	0.31a	0.55a
Arcella sp.	0.22	0.25	0.21	0.21	0.43
Leprotintinnus sp.	0.08	0.05	0.20	0.10	0.13
Cladocera (ind mL^{-1})	0.43	0.89a	0.97a	1.16a	1.07a
Bosmina sp.	0.09	0.39	0.40	0.47	0.53
Daphnia sp.	0.35	0.50	0.58	0.69	0.54
Cirripedia (ind mL^{-1})	0.00	0.16a	0.25a	0.10a	0.28a
Nauplios	0.00	0.16	0.25	0.10	0.28
Copepoda (ind mL^{-1})	0.13	0.27a	0.51a	0.96a	0.13a
Clausocalanus sp.	0.00	0.10	0.11	0.39	0.00
Euterpina sp.	0.13	0.12	0.17	0.26	0.11
Harpaticoida sp	0.00	0.04	0.23	0.31	0.02
Rotifers (ind mL^{-1})	0.62	1.54a	1.08a	1.51a	1.62a
Asplanchna sp.	0.06	0.39	0.31	0.44	0.63
Brachionus sp.	0.56	0.43	0.27	0.45	0.43
Euchlanis sp.	0.00	0.08	0.14	0.04	0.09
Filinia sp.	0.00	0.23	0.10	0.32	0.11
Keratella sp.	0.00	0.40	0.26	0.27	0.35
Total zooplankton (ind mL^{-1})	1.48	3.16a	3.21a	4.05a	3.65a

Table 4. Cyanobacteria composition during the culture (20 days) of *Litopenaeus vannamei* postlarvae reared in zero water exchange, with and without the addition of feed and/or diatoms. [1]The data correspond to the mean. Mean values in same row with different superscript differ significantly ($P < 0.05$). Results from repeated measures ANOVA; Zero water exchange (ZWE); ZWE with the addition of feed (ZWE-F); ZWE with the addition of *Navicula* sp. (ZWE-N) and ZWE with the addition of feed and *Navicula* sp. (ZWE-FN).

Genera	Initial culture	Final culture			
		ZWE	ZWE-F	ZWE-N	ZWE-FN
Anabaena	25.47	153.51	185.83	48.48	122.35
Aphanocapsa	529.54	937.98	2300.71	575.18	2004.85
Dactylococcopsis	17.05	25.01	19.24	33.86	21.55
Gloeothece	2.76	0.00	0.00	0.00	0.00
Merismopedia	28.27	0.00	19.24	0.00	0.00
Microcystis	6.41	134.66	192.37	0.00	0.00
Oscillatoria	542.28	5454.80	4969.61	5251.62	4816.10
Plectonema	38.12	22.89	114.46	34.34	57.23
Pseudanabaena	59.96	205.06	155.05	120.04	175.05
Schizothrix	838.57	3123.27	2369.96	3758.08	1599.72
Spirulina	18.52	90.03	74.64	20.39	143.50
Radiocystis	0.00	0.00	0.00	0.00	9.62
Synechocystis	0.45	0.00	0.00	0.00	0.00
Total Cyanobacteria (cells mL^{-1})	2.107a	10.147a	10.401a	9.841a	8.949a

Table 5. Shrimp production parameters during the culture (20 days) of *Litopenaeus vannamei* postlarvae reared in zero water exchange, with and without feed and/or diatoms. [1]The data correspond to the mean of three replicates ± standard deviation. Mean values in same row with different superscript differ significantly ($P < 0.05$). Results from one-way ANOVA, Tukey test and Student's t-test. Zero water exchange (ZWE); ZWE with the addition of feed (ZWE-F); ZWE with the addition of *Navicula* sp. (ZWE-N) and ZWE with the addition of feed and *Navicula* sp. (ZWE-FN); SGR (% day^{-1}) = 100 x [ln final weight (g) - ln initial weight (g)] / time, and FCR: amount of feed consumed / biomass.

Parameters/ Treatments	Final weigth (mg)	Final biomass (mg)	Weight gain (mg)	Biomass gain (mg)	SGR (% day^{-1})	Survival (%)	FCR
ZWE	242 ± 31.2b	10056 ± 1297c	224 ± 31.2b	8286 ± 1297c	13.05 ± 0.65b	41.5 ± 0.70b	-
ZWE-F	272 ± 7.5b	23693 ± 658b	254 ± 7.5b	21923 ± 658b	13.66 ± 0.13b	87.0 ± 13.0a	1.2 ± 0.11a
ZWE-N	256 ± 31.5b	11278 ± 1386c	238 ± 31.5b	9508 ± 1386c	13.34 ± 0.61b	44.0 ± 2.82b	-
ZWE-FN	348 ± 41.5a	33440 ± 3992a	330 ± 41.5a	31670 ± 3992a	14.87 ± 0.61a	96.0 ± 1.41a	0.9 ± 0.22b

favor undesirable blooms of Pyrrophyta and Cyanobacteria (Campos *et al*., 2007). Ray *et al*. (2010) and Becerra-Dórame *et al*. (2012) found a predominance of cyanobacteria in relation to other plankton groups, in zero water exchange systems. The prevalence of cyanobacteria in shrimp culture is probably related to the accumulation of phosphorus and eutrophication of the culture environment, as documented by Emerenciano *et al*. (2011), who found an increase in the concentration of phosphorous in systems with zero water exchange.

In zero water exchange systems, zooplankton can be part of the microbial aggregate (Ray *et al*., 2010), however, factors such as the addition of feed and *Navicula* sp. appear not to influence the development of zooplankton, because its composition was very similar in all treatments. The higher Rotifera density observed, in comparison with other zooplankton groups, is probably related to the adaptation of these organisms to higher levels of nutrients and solids. Casé *et al*. (2008) found a higher rotifer density with increased availability of organic matter in shrimp ponds. Similar results were reported in zero water exchange systems by Anand *et al*. (2013) and Campos *et al*. (2009). Other zooplankton groups, such as Copepoda, Cladocera and Protozoa were found in biofloc systems (Anand *et al*., 2013; Emerenciano *et al*., 2013).

Shrimp prefer diatoms over other microalgae groups (Jú et *al*., 2008, 2009). Even in intensive culture systems, the microbial community may play an important role in nutrient cycling (Sánchez *et al*., 2012) providing important nutritional compounds, such as essential amino acids and highly unsaturated fatty acids that are essential to shrimp survival and growth (Jú *et al*., 2008, 2009; Khatoo *et al*., 2009). Increased natural productivity can cause a positive productive response in the shrimp postlarvae (Becerra-Dórame *et al*., 2011). According to Porchas-Cornejo *et al*. (2012) shrimp in the enhanced ponds consumed 68% natural foods and 32% formulated feed, while shrimp in unenhanced ponds consumed 42% natural foods and 58% formulated feed.

Our results illustrate the beneficial effects of a bacterial and *Navicula* sp. consortium on growth of shrimp postlarvae in a zero water exchange system. Similar results indicating the beneficial effects of diatoms were observed by Moss & Pruder (1995) with the use of pennate and centric diatoms, which improved growth of *L. vannamei* in intensive systems; Otoshi *et al*. (2011) with higher growth percentages (22-390%) in tanks with high concentrations of diatoms, especially of the genera *Navicula* sp. in a semi-intensive system and Khatoon *et al*. (2009) which found a significantly higher growth rate of *P. monodon* (postlarvae) shrimp reared in tanks containing substrate coated with *Amphora*, *Navicula* and *Cymbella*. The final weights (242-348 mg) at 20 days were higher than those found by Becerra-Dórame *et al*. (2011), in autotrophic (72 mg) and heterotrophic (93 mg) microbial-based-systems at 28 days, and Kim *et al*. (2014) in heterotrophic (132 mg) microbial-based-systems at 14 days with *L. vannamei* postlarvae, this demonstrates high natural productivity in the experimental tanks in our study. The SGR (14.9% day^{-1}) in ZWE-FN were significantly higher as compared to Becerra-Dórame *et al*. (2011) in autotrophic (5.6% day^{-1}) and heterotrophic (6.2% day^{-1}) microbial-based-systems. This is similar to that observed by Banerjee *et al*. (2010), who found a significantly higher SGR (~15% day^{-1}) for shrimp *P. monodon* (postlarvae) reared with additional *Bacillus pumilus* and periphytic microalgae.

The survival rate was highest in ZWE-F (87%) and ZWE-FN (96%) indicating that shrimp of this species need commercial feed for their survival and growth. Becerra-Dórame *et al*. (2011) (76%) and Kim *et al*. (2014) (91.5%) found higher survival rates in heterotrophic microbial-based-systems. Khatoon *et al*.

(2009) found that the use of diatoms increased the survival rate and growth of postlarvae, because the biochemical composition of the shrimp raised in tanks with substrates coated with mixed diatoms had significantly higher protein, lipids, PUFA, and EPA and DHA content than those reared in control tanks.

The lower FCR (0.99) in ZEW-FN showed that *Navicula* sp. are a significant food source for postlarvae shrimp. Sánchez *et al*. (2012) reported that microalgae present in the culture system significantly improved weight gain and FCR of shrimp, thus potentially reducing the feed cost associated with shrimp production. Lower FCR in a zero water exchange system was also observed by Silva *et al*. (2009) (0.8-1.2), Becerra-Dórame *et al*. (2011) (0.65-0.69) and Becerra-Dórame *et al*. (2012) (0.54-0.61).

According to Otoshi *et al*. (2011) and Kent *et al*. (2011), *L. vannamei* has a good ability to utilize the microbial community present in aquaculture systems as a food source. Xu *et al*. (2012) showed that the accumulation of microorganisms in the form of flocs substantially contributes to nourishment of the shrimp. However, the availability of these microbial aggregates alone is not enough for the satisfactory growth of shrimp. Similar results were observed by Emerenciano *et al*. (2007, 2011).

In intensive systems a beneficial microbial community should be developed and sustained (Ray *et al*., 2010). But it is difficult to maintain high densities of diatoms in bioflocs systems, because of competition with bacteria for nutrients, reduction in light and higher levels of suspended matter (Godoy *et al*., 2012). The addition of *Navicula* sp. appears to boost the postlarval growth of *L. vannamei* in zero water exchange systems. Nevertheless, the data obtained in the ZWE-F and ZWE-FN treatments showed that even with plentiful natural food, shrimp of this species need commercial feed for their survival and growth, but the presence of benthic diatoms appears to increase the efficiency of the use of the commercial feed in systems with zero water exchange, because the FCR was significantly lower in the ZWE-FN than in the ZWE-F treatment.

Thus, the addition of the benthic diatom *Navicula* sp. increased the growth of postlarvae *L. vannamei* and improved the FCR in a zero water exchange system. These diatoms provide a significant natural food source for shrimp in their early stage. However, further studies related to the density and frequency of adding *Navicula* sp., or other diatoms are needed to improve control over cyanobacteria and increase the shrimp growth rate in zero water exchange systems.

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