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ORIGINAL RESEARCH

The use of *Artemia* sp. conserved on larval performance of the pacific white shrimp *Penaeus vannamei*

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Abstract Brine shrimp is an essential food for marine shrimp larviculture due to their nutritional composition, but the effects of the use of a conserved product is not yet available in the literature. In view this, this study evaluated the use of fresh brine shrimp (F-BS) and conserved (C-BS) of *Artemia* sp. on the larval performance of *Penaeus vannamei*. Two trials, i.e., the first on a laboratorial scale (experimental units of 10 L) with four independent replications, and the second on a commercial (large-scale ponds of 25 m^3) in triplicate, were conducted to assess the effects of F-BS and C-BS on water quality, bacterial presence, and zootechnical performance of *P. vannamei*. The data were submitted to normality and homoscedasticity tests, when these assumptions were met, Student's t test was used. To data non-parametric was used Mann-Whitney U test. For all analyses, a significance level of 5 %. Water quality parameters were within the recommended levels for the specie and did not differ between treatments. Bacterial colonies in the water were higher when using F-BS compared to C-BS in both trials; in the shrimp, no significant different were found between the diets, but the number of bacterial colonies were higher in commercial scale trial when compared to laboratorial scale one. For water quality, bacterial concentration, larval development indexes and zootechnical performance, no significant different were observed between the brine shrimp forms at two trials. In conclusion, the use of C-BS promotes a similar performance in comparison to F-BS in the larval cultivation of *P. vannamei*.

Keywords Live feed . Brine shrimp . Aquafeed

Introduction

Aquaculture is the fastest food production industry growing in the world, reaching almost 88 million tons in 2020 (FAO 2022). Within this industry, although the crustaceans represent a relatively small volume of production (approximately 11.2 million tons), the shrimps farming represented 16.4 % of the value of global exports of aquatic products, adding USD 24.7 billion (FAO 2022). The success of the activity is due to the Pacific white shrimp *Penaeus vannamei* responsible to 51.7 % of the total crustaceans cultivated, with almost 5.8 million tons per year (FAO 2022).

One of the most important steps in shrimp production is the larval stage, where the supply of high-quality food is one of the determining factors for productive success (Silva et al. 2011). Some studies have reported that the absence of live feed in the diet of aquatic organisms can negatively impact the zootechnical performance of shrimp (Anh et al. 2011). Thus, to choose the appropriate diet, factors such as size, nutri-

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tional composition, and easily and availability in source/inputs must be considered (Lavens et al. 2000). As a result, several alternatives have been developed to improve the productive quality of shrimp, as well as reduce production costs (Silva et al. 2011).

On the herbivorous phase, diatoms are the main microalgae supplied as food for penaeid shrimp larvae (Jaime-Ceballos et al. 2006; Moraes et al. 2022). These microalgae are rich in essential amino acids and polyunsaturated fatty acids, mainly from the ω-3 family, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential for the growth and survival of shrimp (Ju et al. 2008; 2009). However, the use of microalgae can represent around 30-50% of the total cost of production in hatcheries (Richmond 2004; Tredici et al. 2009).

For the omnivorous phases, several zooplankton have been used as a food source, such as rotifers, nematodes, copepods, cladocerans, brine shrimp, among others (Lavens and Sorgeloos 1996). Commercially, brine shrimp are widely used from larvicultures. This is because these microcrustaceans contain a high level of crude protein, composed mainly of essential amino acids, polyunsaturated fatty acids, easy handling, mobile prey, and bioencapsulation (Léger et al. 1986, 1987), which favors an immunostimulating effect on the quality of shrimp post-larvae (Lavens et al. 2000).

However, the high demand for this live feed has caused inconvenience to the aquaculture sector, as its low supply has made the prices of its products skyrocket in the market (Lavens and Sorgeloos 2000). In hatcheries, even with the use of inert feed, the use of brine shrimp can reach up to 40 % of the total cost (Lavens et al. 2000). In addition, there are other factors that make the use of brine shrimp biomass and its derivatives less attractive. This biomass contains almost 90 % water and is rich in proteolytic enzymes, making it highly subject to decomposition. Therefore, adequate preservation methods are necessary to maintain the quality of this biomass (Sorgeloos et al. 2001). In recent years, efforts are being applied to the development of concentrated/preserved live foods in order to dispense with *on-site* live food production. The use of preserved live food reduces the staff and production area, without compromising product quality. Although studies on microalgae concentrates are already advanced, few reports have evaluated the conserved form of zooplankton (Oliveira et al. 2022; Sales et al. 2022). Furthermore, when handled improperly, brine shrimp can be a vector for infectious for some diseases in shrimp, such as necrotizing hepatopancreatitis bacteria (NHPB), infectious myonecrosis virus (IMNV) and white spot syndrome virus (WSSV) (Avila-Villa et al. 2011; Silva et al. 2015; Zhang et al. 2010). From this, the need arises to seek processes that are biologically safe and nutritionally adequate (Marques et al. 2006).

In view of this, the use of conserved brine shrimp has been evaluated to increase the quality/shelf life of this biomass. The technique is of great commercial interest since the product may be stored at room temperature, reducing energy costs when compared to other preservation methods, such as, freezing or freeze-drying (Abelin et al. 1991). Moreover, the conservation of brine shrimp can dispense an exclusive production sector, which can still be a vector of pathogens (Babu et al. 2014; Silva et al. 2015; Chanratchakool 2016; Han et al. 2016). Therefore, the present study aimed to compare the use of fresh and conserved *Artemia* sp. nauplii in the larval culture of *Penaeus vannamei*.

Materials and methods

Study area

Experiments were conducted in a commercial shrimp larvae farm (Celm – Aquaculture S/A), located at the city of Aracati, state of Ceará, Brazil (04° 35' 04.5" S 037° 38' 37.6" W).

Biological material

Penaeus vannamei larvae

The larvae were obtained from *P. vannamei* breeders that are kept at the same farm. Zoea larvae were stored in 25 m³ circular fiber tanks, under an average temperature of 29 °C, salinity of 35 PSU, dissolved oxygen above 4 mg L^{-1}, 8 pH, and a natural photoperiod.

Culture of the microalga *Chaetoceros muelleri*

Cultures of *C. muelleri* were conducted in 20 L sterile bags using filtered (5 µm) and treated (chlorinated and then dechlorinated) seawater (salinity of 35 PSU) with addition of the medium f/2 (Guillard 1975) and Complex B vitamins. The cultures were kept in a room with a controlled temperature of 22 ± 1 °C, constant aeration, and artificial lighting by fluorescent lamps in 24 h of light exposure.

During full cycle, the concentration of *C. muelleri* in the experimental units was mainted at 60×10^3 cells mL^{-1}, being checked daily and added according to the residual cells verified in the count using a Neubauer hemacytometer and an optical microscope (400× magnification).

Fresh Brine shrimp (F-BS)

The cysts of *Artemia* sp. were kindly donated by the Bioartêmia® (Grossos, Rio Grande do Norte, Brazil) and incubated for 24 hours under artificial lighting and turbulent aeration system, keeping the cysts in suspension. After then, brine shrimp nauplii were collected using a 75 µm mesh. The biomass was washed in running freshwater to remove cyst residues and finally stored in Beakers flask in a cold chamber for later use in experimental tests.

Conserved Brine shrimp (C-BS)

Conserved Brine shrimp nauplii were also kindly donated by the Bioartêmia®. The cysts of *Artemia* sp. were hatched by the mentioned company and submitted to a process of sanitization, sterilization and conservation at pH 4.0, some conversation detail cannot be described in the manuscript due to intellectual property production reason (patent under revision).

Experimental design and culture management

Experiment I – laboratorial scale

The first experiment was carried out in an experimental controlled room on the farm. Two treatments (F-BS and C-BS) with four independent replications for each condition were evaluated in the larval performance of *P. vannamei*. Larvae in stage Zoea 3 were stored in 10 L tanks of working volume at a density of 60 larvae L^{-1}. The larvae were kept in the tanks until they reached the stage of Mysis 1 and ended with 5 days after arrival at the stage of post-larvae 2. The main water quality parameters were maintained at the same levels previously described (i.e., temperature of 29 °C, salinity of 35 PSU, dissolved oxygen above 4 mg L^{-1}, 8 pH, and a natural photoperiod). Solids and nitrogen compound levels were controlled by siphoning and partial water changes (30% of the total volume every day), respectively.

As mentioned before, the microalga *C. muelleri* was maintained at 60×10^3 cells mL^{-1} throughout the cycle, then the larvae were fed twelve times a day with an interval of two hours, being offered exclusively the two forms of brine shrimp (i.e., F-BS and C-BS). Furthermore, the supply of Brine shrimp varied as the larvae changed stages: Zoea 3: 0; Misis 1: 10; Misis 2: 15; Misis 3: 30 and post-larvae 1-2: 30 nauplii per shrimp larvae.

Experiment II – Large-scale validation

The same experiment was conducted in outdoor large-scale tanks (25 m^3) in triplicate. These tanks are used by the farm for the commercial production of *P. vannamei* larvae. Larvae were stocked at a density of 180 post-larva L^{-1} in Zoea stage, and when they reached Misis 1, feeding with brine shrimp nauplii began. The cultures were also maintained until reaching the stage of post-larvae 2. In this experiment, the animals were also fed every two hours, however only two times a day the brine shrimp forms were added (one time in the morning and another in the afternoon). In the other feedings, commercial feed with ≥ 50% of crude protein were used. Furthermore, *C. muelleri* concentration was maintained at 60×10^3 cells mL^{-1}.

The water quality parameters were kept within the ideal for the development of the species, carrying out water exchanges. In addition, the tanks were submitted to a natural photoperiod, i.e.,12 h of light, under an average irradiance of 1500 µmol photons m^{-2} s^{-1}.

Analyses

Bacterial analysis

Samples of 1 mL of the cultivation water of the first, third, and fifth days, of both experiments, were collected and diluted (10^{-1} and 10^{-2}) in sterile aqueous saline solution.

In parallel, two shrimp larvae were also collected at the same time. The biological material was stored in a 2 mL sterile microtube and centrifuged at 14000 rpm. The supernatant was discarded and the precipitate was also diluted twice.

The samples were seeded in Petri dishes containing Thiosulfate Citrate Bile Sucrose Agar (TCBS) medium and incubated in an oven at 35 °C for 24 h. After that, the growth responses of sucrose positive colonies (yellow colonies = YC) and sucrose negative colonies (green colonies = GC) were evaluated. The total count of the sum of colonies, related to their respective dilution, was expressed in CFU mL^{-1} for both samples – water and shrimp.

Water quality monitoring

Temperature (°C) was taken twice a day using an alcohol thermometer. Salinity (PSU), using a refractometer. The pH, nitrogen-nitrite ($N\text{-}NO_2$, mg L^{-1}), total ammonia nitrogen ($N\text{-}NH_3$ + $N\text{-}NH_4^+$, mg L^{-1}), and unionized ammonia (NH_3, mg L^{-1}) were measured once a day, using colorimetric kits.

Nutritional and growth indexes

Nutritional assessment of larvae

Ten larvae from each experimental unit on the first (initial), third (middle), and fifth day (end) were collected and analyzed under a binocular optical microscope in terms of: (1) larval stage, (2) average length (mm) and (3) total length of larvae (mm). In addition, a table with a score from 0 to 2 was constructed to assess the nutritional status of the larvae (Table 1).

Zootechnical performance

The larval development index was calculated according to Villegas and Kanazawa (1979): ID = Σi/n, where "i" is the absolute value for each larval stage (Misis 1 = 4; Misis 2 = 5; Misis 3 = 6; post-larvae 1 = 7 and post-larvae 2 = 8) and "n" is the number of larvae in the sample. The data were presented in initial (day 1), middle (day 3) and end (day 5).

Survival was estimated using the equation: Survival (%) = (Nf/Ni) * 100, where ''Nf'' is the number of individuals estimated at the end of the experiment and ''Ni'' is the number of individuals stocked at the beginning of the experiment. Length data were used to calculate the specific growth rate (SGR, $\%.day^{-1}$) = [(Ln final length – Ln initial length) / days] * 100.

Statistical analysis

The data were submitted to normality (Shapiro-Wilk) and homoscedasticity (Levene's) tests, when these assumptions were met, Student's *t* test was used. The data that did not fit the assumptions were submitted to the non-parametric test, Mann-Whitney U test. For all analyses, a significance level of 5% was used and were conducted using Statistica® v.12 software.

Results

Water quality

No significant differences were found in water quality parameters between the use of F-BS and C-BS diets

Table 1 Nutritional assessment of *Penaeus vannamei* larvae

Index	Status	Score
Filling the digestive tube	Empty	0
	Partially full	1
	Full	2
Gut filling to body ratio	75 %	0
	50 %	1
	25 %	2
Presence of lipids	Low	0
	Mean	1
	High	2
Chromatophore expansion	Very expanded	0
	Expanded	1
	None or little expanded	2
Setation	Absent	0
	Present	1
	Very present	2

Table 2 Water quality parameters of *Penaeus vannamei* larvae cultured in laboratorial and commercial scales feed with fresh and conserved Brine shrimp.

Parameter	Treatment	
	F-BS	C-BS
Experiment I – Laboratorial scale		
Temperature (morning) (°C)	26.41 ± 0.49	26.44 ± 0.48
Temperature (afternoon) (°C)	28.44 ± 0.44	28.47 ± 0.46
Salinity (PSU)	34.50 ± 0.89	34.50 ± 0.89
pH	8.34 ± 0.35	8.22 ± 0.31
Total ammonia nitrogen (mg L^{-1})	1.16 ± 1.32	0.83 ± 1.12
Toxic ammonia (mg L^{-1})	0.17 ± 0.21	0.09 ± 0.09
Nitrogen-nitrite (mg L^{-1})	0.97 ± 0.38	0.84 ± 0.36
Experiment II – Commercial scale		
Temperature (morning) (°C)	28.14 ± 0.38	28.17 ± 0.24
Temperature (afternoon) (°C)	29.01 ± 0.56	29.11 ± 0.49
Salinity (PSU)	35.00 ± 0.58	34.93 ± 1.17
pH	8.21 ± 0.27	8.14 ± 0.24
Total ammonia (mg L^{-1})	2.64 ± 0.94	2.43 ± 1.02
Toxic ammonia (mg L^{-1})	0.35 ± 0.21	0.28 ± 0.22
Nitrite (mg L^{-1})	0.57 ± 0.19	0.5 ± 0.25

for both experiments (Table 2). The higher temperature oscillations were found in experiment I (26 - 28 ºC) than in experiment II (28 - 29 °C). In both experiments, levels of nitrogen compounds (toxic ammonia, total ammonia and nitrogen-nitrite) remained within the ideal range for larval cultivation of *P. vannamei*.

Bacterial analysis

In the experiment I, a qualitative difference the presence of bacteria between the treatments, both in the water and in the animals, was observed (Fig. 1). Shrimp fed with F-BS diets showed sucrose-negative colonies (Fig. 1a and 1c), although in low concentration. Furthermore, while the C-BS diets showed a decrease in the concentration of colonies in the water throughout the culture (Fig. 1b and 1d), the analyzes carried out with the shrimp showed an increase in the concentration of *Vibrio* spp. throughout the cultivation (Fig. 1c and 1d). In a similar way to the laboratory scale experiment, in the validation, the F-BS treatment showed sucrose-negative colonies, also within the recommended levels for the larviculture of the species. On the third day of cultivation, there was a peak in the C-BS treatment, both in the water and in the animals, followed by a drop on day 5.

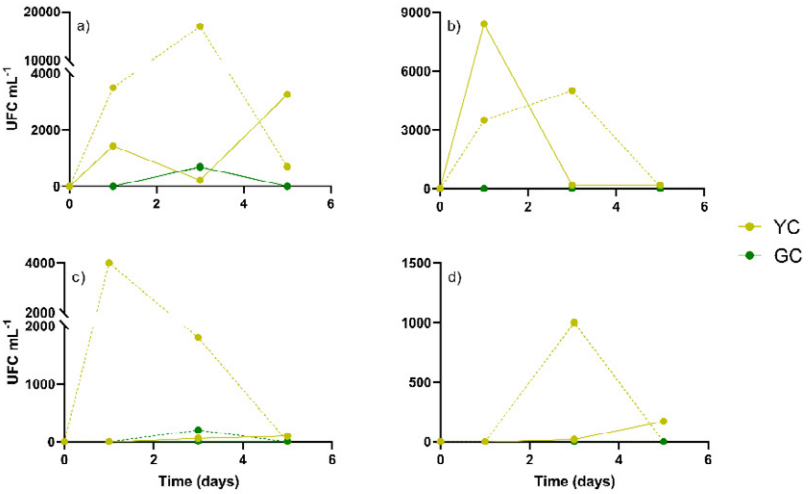

Fig. 1 Bacterial analysis of water (A and B) and shrimp (C and D) of *Penaeus vannamei* larvae feed with fresh (A and C) and conserved (B and D) Brine shrimp from laboratorial (solid lines) and commercial (dashed lines) scales trials. YC – yellow colonies; GC – green colonies.

Quality assessment of larvae

The larval quality scores of *P. vannamei* fed F-BS and C-BS were listed in Table 3. The larvae developed adequately in both treatments and showed no significant difference.

Larval development index

For the two evaluations, i.e., tests in laboratory and commercial scales, no significant differences were found in the larval development index (Table 4). Moreover, in the middle of the evaluation slightly lower values were observed in relation to the laboratory scale (experiment I) compared to the commercial scale (experiment II). This can be explained by a delay in the metamorphosis of larvae from commercial tanks, which resulted in lower rates. However, at the end of the experiment, all evaluated larvae were in the same phase in both experiments.

Zootechnical performance

No significant differences were found between final length, specific growth rate and survival for shrimp fed F-BS and C-BS diets, either in the laboratory or commercial scale experiments (Table 5). Interestingly, on the commercial scale, higher survival values were observed with the use of both forms of brine shrimp, compared to values obtained on the laboratory scale.

Discussion

In order to have a satisfactory development of *P. vannamei* larvae, it is important to keep the levels of the main parameters within the ideal range for the species (Nunes 2001). As nursery systems are constantly aerated there are few problems with dissolved oxygen (Cohen et al. 2001). Temperature is one of the most important factors in hatchery, because it is the main physical parameter related to metabolic activity and growth (Wyk 2004). Therefore, both experiments were suitable for the species (25 to 32 °C) (Boyd and Clay 2002). The pH levels were approximately 8, also at adequate levels for shrimp development (6.5 to 9) (Lopes et al. 2001; Boyd 2001). In both experiments, ammonia was within the recommended range, below 3 mg L^{-1} (Barbieri and Ostrenky-Neto 2001). This can be a result of daily water changes and microalgae action in the absorption of nitrogen compounds (Oliveira et al. 2021). Another highlight is the total ammonia of the experiment I compared to experiment II, where the first was fed exclusively with brine shrimp, while

Table 3 Quality assessment scores of *Penaeus vannamei* larvae from the experiment feed with fresh and conserved Brine shrimp from laboratorial and commercial scale

Parameter	Score					
	F-BS			C-BS		
Laboratorial scale	Initial	Middle	End	Initial	Middle	End
Filling the digestive tube	2	2	1.8	1.9	1.3	1.9
Gut filling to body ratio	2	2	2	2	2	2
Presence of lipids	2	2	2	2	2	2
Chromatophore expansion	2	2	2	2	2	2
Setation	2	2	2	2	2	2
Commercial scale						
Filling the digestive tube	1.71	1.79	1.14	1.29	1.29	1.57
Gut filling to body ratio	1.64	1.93	1.79	1.93	1.57	1.93
Presence of lipids	2	1.79	1.79	1.86	1.57	1.79
Chromatophore expansion	2	1.93	2	1.93	1.79	2
Setation	2	1.93	1.93	2	2	1.93

Score attributed according to Table 1.

Table 4 Larval development index of *Penaeus vannamei* larvae from the experiment feed with fresh and conserved Brine shrimp from laboratorial and commercial scale trials.

	Treatment	
	F-BS	C-BS
Laboratorial scale		
Initial	4.10 ± 0.32	4.30 ± 0.48
Middle	6.40 ± 0.52	6.50 ± 0.53
End	8.00 ± 0.00	8.00 ± 0.00
Commecial scale		
Initial	4.79 ± 0.43	4.86 ± 0.36
Middle	5.86 ± 0.36	5.79 ± 0.43
End	8.00 ± 0.00	8.00 ± 0.00

Table 5 Zootechnical performance of *Penaeus vannamei* larvae feed using fresh (F-BS) and conserved (C-BS) Brine shrimp *Artemia* sp.

Parameter	Treatment	
	F-BS	C-BS
Laboratorial scale		
Initial length (mm)	3.44 ± 0.41	3.66 ± 0.35
Final length (mm)	5.14 ± 0.16	5.18 ± 0.18
SGR (% day^{-1})	8.11 ± 0,35	7.04 ± 0,75
Survival (%)	81.46 ± 7.18	83.67 ± 3.47
Comercial scale		
Initial length (mm)	3.57 ± 0.43	3.46 ± 0.41
Final length (mm)	5.87 ± 0.32	5.50 ± 0.39
SGR (% day^{-1})	9.76 ± 1.53	9.28 ± 2.34
Survival (%)	95.93 ± 3.93	84.09 ± 10.27

the other had the use of inert feed, which can be justified by the greater leaching of dry diets, mainly due to low attractability (Velasco et al. 1999). Nitrite also remained at acceptable levels, below 1 mg. L^{-1} (Nunes et al. 2005). These results contributed to a better growth performance in the experiments.

Furthermore, *Vibrio* spp. are the main gram-negative bacteria causing infection and disease in *P. vannamei* culture (Brock and Lightner 1990). Among them, the ones that cause the most damage in shrimp hatchery are: *V. harveyi*, *V. alginolyticus*, *V. fluvialis*, while *V. penaeicida* and *V. parahaemolyticus* occur

more frequently in the fattening phase. (Vieira et al. 2000; Gomez-Gil et al. 2004). Some studies report significant mortalities due to vibriosis in marine shrimp hatcheries (Lightner 1993). According to Verdonck et al. (1994), the brine shrimp are contaminated with bacteria > 10^7 CFU g^{-1}, mostly being *Vibrio* spp. In the present study, the reduction in bacterial concentration can be justified by the use of microalgae (Lavens and Sorgeloos 2000), since both treatments had reduced concentrations at the end of the experiment. The microalgaes have bactericidal activity (Olsen et al. 2000). Considering this, in commercial marine shrimp hatcheries, brine shrimp are offered frozen (Wilkenfeld et al. 2009), as a way to avoid competition for food, facilitate the capture of live food by shrimp and maintain the safety of brine shrimp. However, Interaminense et al. (2014) highlighted that although freezing for 48 h reduced *Vibrio* spp. counts, the concentration remains > 10^7 CFU g^{-1}. In the present study, a lower concentration (close to 10^3 CFU mL^{-1}) was found when the conserved diet was used, the reduction of this microbial load is due to processes used in sanitizing, sterilizing and reducing the pH of conserved biomass.

In addition, there are several indicators to assess the quality of marine shrimp larvae (Knoll 2010). Among them, larval stage, presence of lipids, chromatophore expansion, setation (rostrum deformity), the filling of the digestive tube and body to gut ratio, highlight for the last two. Larval development is influenced both by the abundance and quality of food offered, as well as by abiotic factors (Zacharia and Kakati 2004). Absence of food in the tubule can indicate illness, poor-nutrition, poor quality feed or stress (Morales-Covarrubias 2004). It is worth noting that healthy larvae feed continuously. In view of this, in the experiment I the larvae were well fed in both treatments throughout the experiment, only on the third day of the experiment the animals fed with conserved brine shrimp showed a balanced number of individuals with partially filled and filled tubes, this may indicate underfeeding in the middle of experiment I, however corrected at the end. In the experiment II, at the beginning and a half, the larvae of the C-BS treatment had 70% of the individuals partially full and at the end the larvae of the F-BS treatment were 90% partially full. In general, these results imply that the larvae were well fed, either with the F-BS or C-BS diets.

Another important factor, the gut to body ratio is one of the indicators of the nutritional status of the larvae, being visualized in the sixth abdominal segment (Knoll 2010). Ideally, the gut takes up only 25% of the body, which is commonly referred to as a 3:1 ratio (muscle: gut). Lower rates than these arise when the larvae are not eating well, either because of the type of food, form or state of conservation. In the experiment I, the treatments were in all phases with 25%. However, in the experiment II in the middle of the evaluation, the individuals fed with preserves were balanced in 50 and 25% of gut filling to body ratio, it is possible that the transition of the ration granulometry may have interfered, even so, in the end all had adequate conditions.

As seen above that the diets promoted the quality of the larvae, this also resulted in excellent results in the zoothenic performance of *Penaeus vannamei*. Both treatments in these experiments showed a post-larvae (PL 2) length higher than that found by Lima (2007) 4.68 ± 0.47 mm. In experiment I (fed only with brine shrimp and microalgae) the mean was 5.16 ± 0.17 mm and the second experiment (feed, brine shrimp and microalgae) 5.68 ± 0.35 mm, also demonstrating the importance of inert food when combined with live food to meet the nutritional requirements of shrimp. Additionally, as well the growth, the experiments showed high survival rates, more than 80 %. Thus, the results indicate that replacing fresh to conserved *Artemia sp.* does not change the productive performance.

From that, currently, brine shrimp is an indispensable item in commercial shrimp larviculture. This can be justified by results found by Samocha et al. (1999), when they reduced the number of *Artemia sp.* nauplii, they also reduced the growth of *P. vannamei*. Although other live foods have advanced as alternatives for fish and shrimp larviculture (e. g. rotifer and copepod) (Lavens and Sorgeloos 1996), so far none of these can meet the logistical needs and production practices to replace *Artemia spp.* on a global scale. As a result, brine shrimp production continues to be under strong pressure due to high demand (Bengtson et al. 2018).

Thus, the main factor for the fluctuation of the price of artemia is the seasonal harvest and consequently the low supply of cysts (Lavens and Sorgeloos 2000). The present research observes as promising the processing of conserved brine shrimp, as an alternative to marketing strategies, control of brine shrimp stock, product safety, improving logistics in larvicultures (possible cost reduction compared to frozen brine shrimp method, smaller space for production and storage in a natural ambience) and biosecurity in larviculture.

Conclusion

The results of the present study suggest that the use of conserved *Artemia* sp. (C-BS) did not negatively impact the overall performance of *Penaeus vannamei* larviculture, in terms of water quality, zootechnical performance, and bacterial concentration. Future studies should evaluate the shelf life, enzymatic activity, and economic viability of using C-BS *vs.* F-BS.

Ethical approval It is not required to get an "Approval of Animal use Protocol" when using invertebrates as experimental animal in Brazil.

Authors' contributions GFGJ Investigation, Formal analysis, Data curation, Writing - Original Draft. RFBS: Resources, Formal analysis, Writing - Review snd Editing. CYBO: Formal analysis, Data curation, Writing - Original Draft. ARAS: Data curation, Writing - Review and Editing. EPS: Formal Analysis, Writing - Review and Editing. RSB: Supervision, Resources, Writing - Review and Editing. AOG: Supervision, Resources, Writing - Review and Editing.

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Competing interests The authors have no competing interests to declare.

References

Abelin P, Tackaert W, Sorgeloos, P (1991) Ensiled Artemia biomass: a promising and practical feed for penaeid shrimp postlarvae, *in*: Lavens, P. et al. (Ed.) Larvi '91. Short communications and abstracts of contributions presented at the international Symposium on Fish and Crustacean Larviculture. Gent, Belgium, August 27-30, 1991. EAS Special Publication 15:125-127. https://www.vliz.be/imisdocs/publications/ocrd/135220.pdf. Accessed on 28 July 2022

Anh NTN, Wille M, Van Hoa N, Sorgeloos P (2011) Formulated feeds containing fresh or dried Artêmia as food supplement for larval rearing of Black Tiger shrimp, *Penaeus monodon*. J Appl Aquac 23(3):256-270. https://doi.org/10.1080/10454438.2011.601254

Avila-Villa LA, Martínez-Porchas M, Gollas-Galván T, López-Elías JA, Mercado L, Murguia-López A, Hernández-López J (2011) Evaluation of different microalgae species and Artemia (*Artemia franciscana*) as possible vectors of necrotizing hepatopancreatitis bacteria. Aquaculture 318(3-4):273-276

Babu MM, Citarasu T, Selvaraj T, Punitha SMJ, Albindhas S (2014) Isolation and identification of pathogenic *Enterococcus* sp. from Artemia population from Puthalam saltpan of Kanyakumari District, India. J Aquac Tropics 29(3/4):97

Barbieri RC, Ostrensky-Neto A (2001) Camarões marinhos: Reprodução, Maturação e Larvicultura. Viçosa: Aprenda Fácil ed. 255

Bengtson DA, Léger P, Sorgeloos P (2018) Use of artemia as a food source for aquaculture. In: Artemia biology (pp. 255-286). CRC Press

Boyd C (2001) Water composition and shrimp pond management. ABCC Magazine 17-19

Boyd C, Clay J (2002) Evaluation of belize aquaculture ltd: A superintensive shrimp aquaculture system. FAO. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.463.8794&rep=rep1&type=pdf. Accessed on 28 July 2022

Brock JA, Lightner DV (1990) Diseases of crustacea. Diseases caused by microorganisms. In: Diseases of marine animals, 245-349

Chanratchakool P (2016) Good aquaculture and biosecurity practices in shrimp farming. Paper presented at the first international technical Seminar/workshop on Early Mortality Syndrome (EMS) and Acute Hepatopancreatic Necrosis Syndrome (AHPNS): government, scientist and farmer responses (under the FAO Technical Cooperation Programme [TCP/INT/3502]). Panama City, Florida, USA, June 22–24, 2015. FAO Fish and Aquac Report

Cohen JM, Samocha TM, Gandy RL, Jones ER, Fox JM, Lawrence AL (2001) The culture of *Litopenaeus vannamei* under bio-secured and zero exchange conditions. Aquaculture 1:130

FAO (2022) The state of world fisheries and aquaculture 2022. Towards blue transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

Gomez-Gil B, Soto-Rodriguez S, García-Gasca A, Roque A, Vazquez-Juarez R, Thompson FL, Swings J (2004) Molecular identification of *Vibrio harveyi*-related isolates associated with diseased aquatic organisms. Microbiology 150(6):1769-1777. https://doi.org/10.1099/mic.0.26797-0

Guillard RR (1975) Culture of phytoplankton for feeding marine invertebrates. In culture of marine invertebrate animals (pp. 29-60). Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-8714-9_3

Interaminense, JA, Calazans NF, Valle BC, Vogeley JL, Peixoto S, Soares R, Lima-Filho JV (2014) *Vibrio* spp. control at brine shrimp, Artemia, hatching and enrichment. J World Aquac Soc 45(1):65-74. https://doi.org/10.1111/jwas.12096

Jaime-Ceballos BJ, Hernández-Llamas A, Garcia-Galano T, Villarreal H (2006) Substitution of *Chaetoceros muelleri* by *Spirulina platensis* meal in diets for *Litopenaeus schmitti* larvae. Aquaculture 260(1-4):215-220. https://doi.org/10.1016/j.aquaculture.2006.06.002

Ju ZY, Forster I, Conquest L, Dominy W, Kuo WC, Horgen FD (2008) Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. Aquacult Res 39(2):118-133. https://doi.org/10.1111/j.1365-2109.2007.01856.x

Ju ZY, Forster IP, Dominy WG (2009) Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). Aquaculture 292(3-4):237-243. https://doi.org/10.1016/j.

aquaculture.2009.04.040
Knoll RC (2010) Quality assessment system of marine shrimp post-larvae *Litopenaeus vannamei* (Boone 1931). Dissertation (Master's) - Federal University of Santa Catarina, Agricultural Sciences Center, Postgraduate Program in Aquaculture, Florianópolis. 50. https://repositorio.ufsc.br/bitstream/handle/123456789/94316/281001.pdf?sequence=1&isAllowed=y. Accessed on 28 July 2022
Lavens P, Sorgeloos P (1996) Manual on the production and use of live food for aquaculture (No. 361). Food and Agriculture Organization (FAO)
Lavens P, Sorgeloos P (2000) The history, present status and prospects of the availability of Artemia cysts for aquaculture. Aquaculture 181(3-4):397-403. https://doi.org/10.1016/S0044-8486(99)00233-1
Lavens P, Thongrod S, Sorgeloos P (2000) Larval prawn feeds and the dietary importance of Artemia. In: freshwater prawn culture: The farming of *Macrobrachium Rosenbergii* Author: Michael B. New with Wagner Cotroni Valenti, Blackwell Science, Oxford (UK), 2000, 91-111
Léger P, Bengtson DA., Simpson KL, Sorgeloos P (1986) The use and nutritional value of Artemia as a food source. Ocean Mar Biol
Léger P, Naessens-Foucquaert E, Sorgeloos P (1987) International study on Artemia. XXXV. Techniques to manipulate the fatty acid profile in Artemia nauplii and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M.). Artemia research and its applications 3:411-424. https://www.vliz.be/imisdocs/publications/ocrd/133992.pdf. Accessed on 28 July 2022
Lightner DV (1993) Diseases of penaeid shrimp, p. 393-486 in McVey, J.P. (ed.), Handbook of Mariculture. I - Crustacean Aquaculture, CRC Press, 2nd edition Boca Ratón, 1993
Lima CM (2007) Survival and larval growth of the marine shrimp *Litopenaeus vannamei* fed on the benthic copepod *Tisbe biminiensis.* Dissertation (Master's). Federal University of Pernambuco, Postgraduate program in animal biology, Recife, 2007. https://repositorio.ufpe.br/handle/123456789/788. Accessed on 28 July 2022
Lopes JM, Silva LVF, Baldisserotto B (2001) Survival and growth of silver catfish larvae exposed to different water pH. Aquac Int 9(1):73-80. https://doi.org/10.1023/A:1012512211898
Marques A, Thanh TH, Sorgeloos P, Bossier P (2006) Use of microalgae and bacteria to enhance protection of gnotobiotic Artemia against different pathogens. Aquaculture 258(1-4):116-126. https://doi.org/10.1016/j.aquaculture.2006.04.021
Morales-Covarrubias, MS (2004) Enfermedades del camarón: detección mediante análisis en fresco e histopatología (No. Sirsi) (i9789682471124)
Moraes LBS, Santos RFB, Gonçalves Junior GF, Mota GCP, Dantas DMDM, Bezerra RS, Gálvez AO (2022) Microalgae for feeding of penaeid shrimp larvae: an overview. Aquac Int 1-19. https://doi.org/10.1007/s10499-022-00857-z
Nunes AJP (2001) Panorama de cultivo de camarões marinhos no Brasil. Revista Brasileira de Agropecuária 1:40-41
Nunes AJP, Gesteira TCV, Oliveira GG, Lima RC, Miranda PTC, Madrid RM (2005) Princípios para boas práticas de manejo na engorda de camarão marinho no Estado do Ceará. Instituto de Ciências do Mar (Labomar/UFC). Programa de Zoneamento Ecológico Econômico (ZEE) do Estado do Ceará, Fortaleza, Ceará. 2005. 109 p
Oliveira CYB, Oliveira CDL, Prasad R, Ong HC, Araujo ES, Shabnam N, Gálvez AO (2021) A multidisciplinary review of *Tetradesmus obliquus*: a microalga suitable for large-scale biomass production and emerging environmental applications. Rev Aquac 13(3):1594-1618. https://doi.org/10.1111/raq.12536
Oliveira CYB, Jacob A, Nader C, Oliveira CDL, Matos ÂP, Araújo ES, Shabnam N, Ashok B, Gálvez AO (2022) An overview on microalgae as renewable resources for meeting sustainable development goals. J Environ Manage 320:115897. https://doi.org/10.1016/j.jenvman.2022.115897
Olsen AI, Olsen Y, Attramadal Y, Christie K, Birkbeck TH, Skjermo J, Vadstein O (2000) Effects of short term feeding of microalgae on the bacterial flora associated with juvenile *Artemia franciscana*. Aquaculture 190(1-2):11-25. https://doi.org/10.1016/S0044-8486(00)00396-3
Richmond A (2004) Biological principles of mass cultivation. In: Richmond A (ed) Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Science, Oxford 125–177
Samocha TM, Matsumoto T, Jones ER and Torano M (1999) Use of artificial diets to reduce Artemia nauplii requirements for production of *Litopenaeus vannamei* postlarvae. Isr J Aquac–Bamidgeh 51(4):157-168
Sales R, Lopes RG, Derner RB, Tsuzuki MY (2022) Concentrated microalgal biomass as a substitute for fresh microalgae produced on site at hatcheries. Aquac Res 53:5771-5786. https://doi.org/10.1111/are.16072
Silva AS, Brito LO, de Melo Costa W, Vasconcelos RFL, Galvez AO (2011) Utilização de *Brachionus plicatilis* (Müller, 1786) na alimentação de larvas do camarão marinho *Litopenaeus vannamei* (Boone 1931). Arquivos de Ciências do Mar 44(3). https://doi.org/10.32360/acmar.v44i3.152. Accessed on 28 July 2022
Silva SMBC, Lavander HD, de Santana Luna MM, Gálvez AO, Coimbra MRM (2015) *Artemia franciscana* as a vector for infectious myonecrosis virus (IMNV) to *Litopenaeus vannamei* juvenile. J Invertebr Pathol 126:1-5. https://doi.org/10.1016/j.jip.2015.02.001
Sorgeloos P, Dhert P, Candreva P (2001) Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture 200(1-2):147-159. https://doi.org/10.1016/S0044-8486(01)00698-6
Tredici MR, Biondi N, Ponis E, Rodolfi L, Zittelli GC (2009) Advances in microalgal culture for aquaculture feed and other uses. In: Burnell G, Allan G (eds) New technologies in aquaculture: Improving production efficiency, quality and environmental management. FL: Woodhead Publishing, Boca Raton, pp 610–676. https://doi.org/10.1533/9781845696474.3.610
Velasco M, Lawrence AL, Castille FL (1999) Effect of variations in daily feeding frequency and ration size on growth of shrimp, *Litopenaeus vannamei* (Boone), in zero-water exchange culture tanks. Aquaculture 179(1-4):141-148. https://doi.org/10.1016/S0044-8486(99)00158-1
Villegas CT, Kanazawa A (1979) Relationship between diet composition and growth rate of the zoeal and mysis stages of *Penaeus japonicus* Bate. Fish Res J Philippines 4(2):32-40. http://hdl.handle.net/10862/1090
Vieira RHSDF, Gesteira TCV, Marques LC, Martins PCC, Monteiro CM, Carvalho RDL (2000) *Vibrio spp.* and its bearing on marine shrimp larviculture. Arquivo de Ciências do Mar. Fortaleza 33(1-2):107-112. 2000. https://repositorio.ufc.br/handle/riufc/54489. Accessed on 28 July 2022
Verdonck L, Swings J, Kersters K, Dehasque M, Sorgeloos P, Leger P (1994) Variability of the microbial environment of Rotifer *Bra-*

chionus plicatilis and *Artemia* production systems. J World Aquac Soc 25(1):55-59. https://doi.org/10.1111/j.1749-7345.1994.tb00804.x

Wilkenfeld JS, Fox JM, Lawrence AL (2009) Consumption of frozen and live Artemia by protozoea of *Penaeus setiferus*. J World Maric Soc 12(2):250–259. doi:10.1111/j.1749-7345.1981.tb00300.x

Wyk PV (2004) Nutrition and feeding of *Litopenaeus vannamei* in culture system. In: Farming marine shrimp in recirculating freshwater systems. Harbor Branch Oceanographic Institution, Florida. p 220

Zacharia S, Kakati VS (2004) Optimal salinity and temperature for early developmental stages of *Penaeus merguiensis* De man. Aquaculture 232(1-4):373-382. https://doi.org/10.1016/S0044-8486(03)00538-6

Zhang JS, Dong SL, Dong YW, Tian XL, Cao YC, Li ZJ, Yan DC (2010) Assessment of the role of brine shrimp Artemia in white spot syndrome virus (WSSV) transmission. Vet Res Communic 34(1):25-32. https://doi.org/10.1007/s11259-009-9329-x