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Using residual water from a marine shrimp farming BFT system. Part II: *Artemia franciscana* biomass production fed microalgae grown in reused BFT water

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Abstract

The residual water from intensive aquaculture production systems can be utilized in integrated multitrophic cultivations. In this work, *Artemia franciscana* received daily feedings of 10 mg L^{-1} of *Tetraselmis chuii*, *Nannochloropsis oculata* and *Chaetoceros muelleri*, which were grown using residual water from an intensive biofloc *Litopenaeus vannamei* cultivation system. The goal of this study was to verify which species provide the best zootechnical performance and best crustacean biomass production efficiency. After 12 days of cultivation, *A. franciscana* wet biomass was 815.64 ± 18.74, 650.81 ± 83.98 and 40.76 ± 4.08 mg L^{-1} with *C. muelleri*, *T. chuii* and *N. oculata* ($P < 0.05$), with significant differences in dried biomass as well. As for the microalgae cultivation in the alternative culture medium, *T. chuii* had higher dry biomass gain, requiring less culture volume to achieve 10 mg L^{-1} and become ready to feed Artemia. Thus, *T. chuii* was the most efficient in Artemia biomass production with 0.83 L compared to 1.54 L g art^{-1} in *C. muelleri*. *C. muelleri* is recommended for feeding *A. franciscana* for biomass production purposes. However, due to its better efficiency, *T. chuii* can be selected to be part of a multitrophic system.

Keywords: aquaculture, brine shrimp, biofloc, multitrophic

Introduction

Integrated multitrophic cultivation incorporates species with different trophic levels, allowing the transformation of solid or soluble nutrients into biomass (Soto 2009). The residual water from intensive aquaculture production systems has a potential to be used for the cultivation of halophyte plants (Webb, Quinta, Papadimitriou, Norman, Rigby, Thomas & Le Vay 2012), microalgae (Borges, Silva, Moreira & Soares 2005; Attasat, Wanichpongpan & Ruenglertpanyakul 2013), macroalgae and bivalve mollusc (Jones, Preston & Dennison 2002; Neori, Chopin, Troell, Buschmann, Kraemer, Halling, Shpigel & Yarish 2004).

Among these aquaculture systems, intensive peneid shrimp cultivation with bioflocs stands out (Crab, Avnimelech, Defoirdt, Bossier & Verstraete 2007; De Schryver, Crab, Defoirdt, Boon & Verstraete 2008). This technology is based on minimal water exchange, receiving a great amount of organic matter in the form of food leftovers, fertilizers and excretion, resulting in a nutrient-rich effluent with high eutrophication potential (Alonso-Rodriguez & Paez-Osuna 2003; Tacon & Forster 2003; Viadero, Cunningham, Semmens & Tierney 2005).

The residual water of these cultivations has been proposed as an alternative culture medium for microalgae biomass production (Borges *et al.* 2005; Chen, Pan, Hong & Lee 2012; Attasat *et al.* 2013), reducing costs generated by the chemical fertilizers usage (Coutteau 1996). Microalgae biomass has great biotechnological potential for bioremediation, carbon sequestration, nutraceuticals production (De La Noue & De Pauw 1988), biofuel production (Mata, Martins & Caetano 2010; Varfolomeev & Wasserman 2011; Krishna, Dev & Thankamani 2012), in addition to being an

indispensible food source for the cultivation of several aquatic organisms, mainly in peneid larviculture, shellfish and marine fish systems (Lavens & Sorgeloos 1996; Duerr, Molnar & Sato 1998).

The use of *Artemia* sp. in residual water treatment must also be considered, due to their high filtration capability (Basil, Nair & Thatheyus 1995). Artemia are non-selective filtrators and obligatory fagotrophics (Sorgeloos, Baezamesa, Bossuyt, Bruggeman, Dobbeleir, Versichele, Lavina & Bernardino 1980) feeding mainly on microalgae present in natural hypersaline environments, bacterial rich debris (Intriago & Jones 1993) or other microparticulate feed (Sorgeloos *et al.* 1980; Lavens & Sorgeloos 1996). These characteristics make the feeding of Artemia with solid residues from the biofloc system possible; however, by using this practice the effluent nutrients will not be recycled.

Wang (2003) described a multitrophic system comprised of intensive marine shrimp production tanks, microalgae, shellfish or Artemia. However, the functionality of integrated systems is complex, due to the requirements of the organisms, the influence of water quality, and separation strategies (Neori *et al.* 2004). To improve a system's viability, the cultivated species selection must be made very carefully, to achieve the best productivity and production efficiency (Barrington, Chopin & Robinson 2009).

In this experiment, Artemia were fed with the microalgae species *Nannochloropsis oculata*, *Tetratselmis chuii* and *Chaetoceros muelleri*, produced utilizing residual water from intensive *Litopenaeus vannamei* biofloc cultivation as a culture medium. The goal of the study was to determine which feed (microalgae) provides the best zootechnical performance and presents the best Artemia biomass production efficiency as part of an integrated multitrophic system.

Material and methods

Experimental design

For Artemia cultivation, nine translucid plastic (PET) units were utilized as experimental units with 3 L of working volume. Photoperiods of 12 h of light and 12 h of dark at 2000 LUX, at a height of 2 m were employed. Aeration was conducted by pressurized atmospheric air injection. The cultures were maintained with 35 g L^{-1} salinity and an immersion bath at 26 ± 1°C controlled by a digital thermostat and a 500 W heater.

The experiment was divided into three treatments according to the microalgae species provided as feed for the Artemia; TCH – *Tetraselmis chuii*, NOC – *Nannochloropsis oculata* and CMU – *Chaetoceros muelleri*. Every day, 10 mg L^{-1} of microalgal biomass were provided to each Artemia experimental unit. The *Artemia franciscana* cysts from Great Salt Lake, UT, USA (INVE Aquaculture, Belgium) were hydrated, decapsulated and harvested according to Lavens & Sorgeloos (1996). The nauplii were then transferred to the experimental units at a density of 1.4 nauplii mL^{-1}.

Microalgae production

The microalgae inoculum was cultivated in a sterile f/2 culture medium (Guillard 1975) with 80 mg L^{-1} sodium silicate added for *C. muelleri*. The cultivation conditions were: 35 g L^{-1} salinity, 22 ± 0.5°C temperature, a 24-hour light photoperiod with photon flux of 150 μmol m^{-2} s^{-1}, and mixing by atmospheric air injection with a flow of 0.4 L min^{-1} for each flask.

The microalgae inocula were then transferred to glass cylindrical containers with a 4.5 L working volume. The culture medium was residual water from an intensive biofloc shrimp production system tank. The *C. muelleri* cultures also received 80 mg L^{-1} of sodium silicate.

Every 24 h, samples were collected to verify the cultures turbidity (NTU) using a turbidimeter at a 860 nm wavelength. These values were used to calculate the required volume to feed Artemia in a 10 mg L^{-1} microalgae concentration. For these calculations, a calibration curve of Turbidity (NTU) versus Biomass (mg L^{-1}) was used (Zhu & Lee 1997), with R^2 = 0.982; 0.964; 0.951 for *T. chuii*, *N. oculata* and *C. muelleri* respectively.

Collection and residual water disinfection

Residual water was collected from an intensive marine shrimp (*L. vannamei*) biofloc system, undertaken in a circular fibreglass tank (8 m in diameter, 50 m^2 bottom area, 48 m^3 volume). The tank was populated with 18–19 g of animals at an average density of 1.5 kg m^{-2}. The tank water was not renovated for 3 months and the total suspended solids (TSS) were maintained between 400 and 600 mg L^{-1} using a conical cylindrical decanter. After water collection, the residual water was submitted to solids sedimentation for 15 min

and supernatant double filtration (through a 1 μm cartridge) for the removal of suspended solids. The water was then chlorinated with 20 ppm NaClO for 1.5 h and then neutralized with 25 ppm of sodium tiosulphate solution.

Artemia cultures monitoring

The treatments were evaluated daily to verify the relative survival and estimated population count for each unit. To do so, 5 × 10 mL samples from each experimental unit were counted and the average was calculated. The sampling and counts were performed by turning off the aeration, homogenizing and suctioning the water with a 10 mL volume glass tube. Every 3 days, 20 individuals were collected and fixed in a 5% lugol solution to verify the total body length and instar stage identification (Schrehardt 1987). The total body length was considered to be the length from the top of the head to the end of the abdomen for nauplii and adults, measured with a metric scale attached to a stereoscopic magnifying glass.

Every 6 days, the individual body weight (μg artemia^{-1}) and dried biomass (mg L^{-1}) were determined by sample filtration in glass fibre filters (porosity 1.6 μm) and dried at 50°C until constant weight. For the analysis, 20 mL was sampled on day 0, 500 mL on day 6 and 2.5 L (remaining volume) on day 12. Before filtration, the Artemia were washed in freshwater and filtered in 100 μm screens to lixiviate salt and residual solids. It was then possible to calculate individual weight gain (IWG) (μg artemia day^{-1}).

On the day 12, the filters were moistened and previously weighed using a digital four decimal scale. The Artemia were then weighed, to establish their wet biomass.

Each day, the Artemia were concentrated and washed to remove residual solids, using 100 μm screens on days 0–5 and 350 μm screens from days 6 to 12. During this procedure, all of the water from each experimental unit was renovated to maintain low concentrations of nitrogenous compounds, phosphorus and remove residual microalgae and faeces.

Statistical analysis

The Levene Test was used to verify the homocedasticity and the Shapiro-Wilk Test to verify the data normality. Data were submitted to ANOVA and a Tukey Test to determine the significant differences, both with a $P < 0.05$ significance index. The survival values were previously transformed to arc-sine ($y^{0.5}$) but presented as a percentage. STATISTICA 7.0 software was used to apply the tests.

Results and discussion

The survival rate did not present significant differences ($P > 0.05$) between treatments until the sixth cultivation day. After the seventh day, survival was smaller in NOC ($P < 0.05$), gradually decreasing and reaching minimal values of 20 ± 5.77% on the twelfth day (Fig. 1). Seixas, Coutinho, Ferreira & Otero (2009) evaluated the Artemia growth and survival fed with *Rhodomonas lens*, *Tetraselmis suecica*, *Isochrysis galbana* and *Nannochloropsis gaditana* for 8 days and verified similar results, with 18 ± 3% survival in *N. gaditana* fed treatment and between 69% to 88% with the other species.

The Artemia fed with *N. oculata* presented the lowest results for all the parameters analysed (Table 1) ($P < 0.05$). The results of TCH and CMU were different regarding dry and wet biomass only

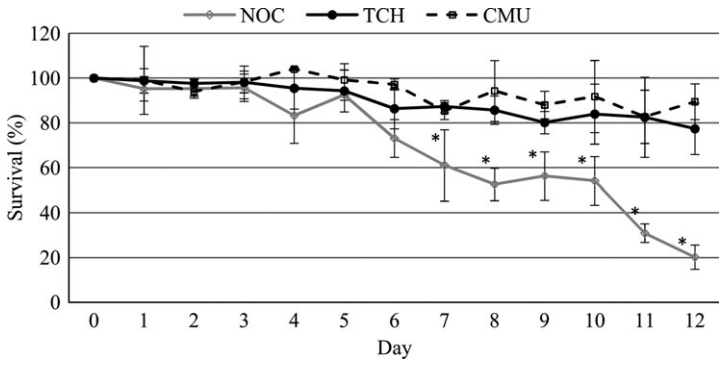

Figure 1 *Artemia franciscana* survival (%) fed with *N. oculata* (NOC), *T. chuii* (TCH), *C. muelleri* (CMU) during 12-day cultivation. *indicates significant differences ($P < 0.05$) verified by ANOVA and Tukey.

Table 1 Averages ± SD of individual dry weight (IDW), daily individual weight gain (IWG), dried (DB) and wet biomass (WB) of *A. franciscana* fed with *N. oculata* (NOC), *T. chuii* (TCH), *C. muelleri* (CMU) on cultivation days 0, 6, and 12

	Day	NOC	TCH	CMU
IDW (μg artemia^{-1})	0	2.69	2.69	2.69
	6	5.38 ± 0.51^{b}	25.91 ± 2.26^{a}	24.26 ± 2.36^{a}
	12	21.65 ± 3.61^{b}	37.86 ± 3.35^{a}	44.85 ± 4.41^{a}
IWG (μg day^{-1})	[0, 12]	1.58 ± 0.30^{b}	2.93 ± 0.28^{a}	3.51 ± 0.37^{a}
DB (mg L^{-1})	0	11.31	11.31	11.31
	6	5.47 ± 0.23^{b}	31.13 ± 0.95^{a}	32.93 ± 2.39^{a}
	12	6.00 ± 1.22^{c}	40.69 ± 3.63^{b}	55.81 ± 1.08^{a}
WB (mg L^{-1})	12	40.76 ± 4.08^{c}	650.81 ± 83.98^{b}	815.64 ± 18.74^{a}

Averages in the same line followed by different superscript letters shown significant differences ($P < 0.05$) verified by ANOVA and Tukey tests.

on the twelfth day, when the *C. muelleri* fed individuals showed better performance ($P < 0.05$).

In TCH and CMU treatments, a majority of L13 individuals were found in the instar development stage. According to Schrehardt (1987), these are named Postlarvae stage 1. In NOC, the individual majority was at stage L7, characterized as Post-Metanauplii 2, with the presence of Post-Metanauplii 1 (L6) and Post-Metanauplii 10 (L10) (Fig. 2).

The difference between the development levels directly influenced the total body length of Artemia. The average total body length of Artemia fed with NOC was 2.5 ± 0.3 mm ($P < 0.05$) on the twelfth day, while the average for those raised on TCH and CMU was close to 4 mm ($P > 0.05$) (Fig. 2). The latter average is similar to that found by Naegel (1999) when feeding with *Chaetoceros* sp. for 11 days. Seixas *et al.* (2009) also found total body length of 4.3 mm in Artemia fed with *Tetraselmis suecica* for 8 days and 1.5 mm with *Nannochloropsis gaditana*.

In the observations (days 3, 6, 9 and 12), the Artemia from all treatments had digestive tracts filled with microalgae, however, individuals in the

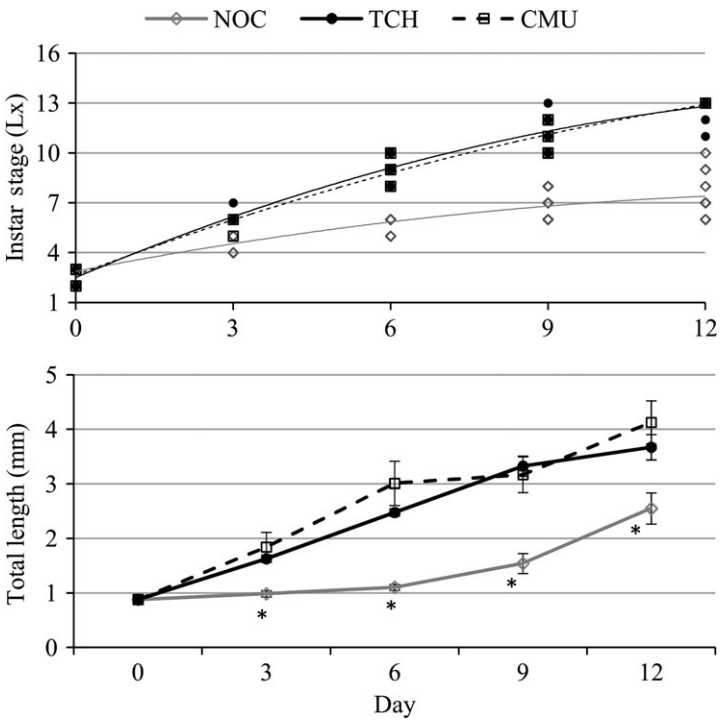

Figure 2 Development stage (Instar) and Artemia total body length submitted to NOC (◊), TCH (●) and CMU (□) treatments during 12 days of cultivation. *indicates significant difference ($P < 0.05$) verified through ANOVA and Tukey tests.

NOC treatment had slow development and low survival rates.

It has been observed that larger microalgae provide better growth results in Artemia (Seixas *et al.* 2009). Even with particle filtration capacity ranging from 1 to 50 μm (D'Agostino 1980), other studies showed that intervals from 7 to 28 μm are preferred, with an optimum at 16 μm (Fernandez 2001). The *T. chuii* species is, on average, 12 μm long and 8 μm wide, and *C. muelleri* 5.33 by 3.15 μm (Ohse, Derner, Ozório, Braga, Cunha, lamarca & dos Santos 2008). The poor performance of *N. oculata* may be related to its lower digestibility, its smaller size, 2 μm or its spherical shaped cell (Ohse *et al.* 2008). These results were also found by Seixas *et al.* (2009) using *N. gaditana* and described by Dhont & Lavens (1996) with *Chlorella* sp. and *Stichococcus* sp.

During the 12-day cultivation, about 1.49 L of *T. chuii*, 1.75 L of *N. oculata* and 3.5 L of *C. muelleri* were supplied to each experimental unit. These data are equivalent to volumes 0.83 ± 0.11, 15.67 ± 1.65 and 1.54 ± 0.03 L of culture for each gram of Artemia produced. These results show *T. chuii* to be twice efficient in Artemia biomass production than *C. muelleri*.

The microalgae cultures used for Artemia feeding had final dried biomass of 271 ± 32 mg L^{-1} for *T. chuii*, 225 ± 14 mg L^{-1} for *N. oculata* and 111 ± 17 mg L^{-1} for *C. muelleri*. The higher final biomass explains the lower volume of *T. chuii* culture necessary for the daily 10 mg L^{-1} microalgae supply during the experiment. The difference in biomass production from these three microalgae species when grown in residual water from biofloc cultivation systems was also verified by Magnotti, Lopes, Derner & Vinatea (2015). These authors cultivated these three microalgae species for 10 days, with the same type of residual water and obtained final dried biomass of 702 ± 120 mg L^{-1}, 589 ± 89 mg L^{-1} and 170 ± 30 mg L^{-1} de *T. chuii*, *N. oculata* and *C. Muelleri*, respectively, without addition of sodium silicate for the diatom cultivation. Due to the small biomass gain, Magnotti *et al.* (2015) do not recommend *C. muelleri* for biofloc residual water-based cultivations, at least without the addition of silicate to the culture medium, and further studies are needed.

During the experiment, *C. muelleri* biomass production was also low, even with silicate addition. Therefore, this lower biomass gain may be associated to other residual water characteristics, not only the silicate privation as discussed by Magnotti *et al.* (2015).

For choosing the microalgae species to be used in a multitrophic system, all the production parameters must be considered (Barrington *et al.* 2009). The best results in the zootechnical indices achieved by Artemia fed with *C. muelleri* are minimized when the large microalgae culture volume is considered.

Production process efficiency is extremely important when choosing the microalgae species in this kind of system. The better efficiency, the less residual water is needed, producing higher Artemia biomass at a lower production cost. These costs are related to tank volume, physical structure, manpower and electrical energy expenses, which can make the process unfeasible.

The Artemia biomass produced can also be used as a feed for the shrimp cultivated in the residual water supplier tanks. The purpose of this practice would be to supplement the feeding, lowering the use of industrialized feed and thus reducing financial and environmental costs. To elucidate this hypothesis, more studies are needed about the centesimal microalgae and Artemia biomass composition grown in the residual water. Only with these results, would it be possible to justify the choice of microalgae species and introduce them in a multitrophic system, integrating *L. vannamei* intensive biofloc cultivation, microalgae biomass production and *Artemia franciscana* biomass production for feeding shrimp.

Conclusion

Artemia presented the highest dried and wet biomass gain when fed with *C. muelleri*. However, *T. chuii* had a higher final biomass when cultivated in residual water, with only 0.83 L needed to produce 1 g of Artemia, compared with 1.54 L for the *C. muelleri* culture.

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