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ORIGINAL ARTICLE

Substitution of microalgae by bioflocs as a food source for the brine shrimp *Artemia franciscana*

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Abstract

This research was done to investigate the application of halophilic bacteria in a biofloc production system to grow *Artemia franciscana* in the laboratory, in which part of a standard microalgae feeding regime was replaced by bioflocs. Bioflocs were produced according to a standard procedure, using rice bran and vinasse as carbon sources, a commercial probiotic product, and halophilic *Bacillus* sp., originating from Urmia Lake, Iran, as halophilic bacteria. Three successive laboratory culture tests were conducted at salinities 60 (experiments 1 and 3) and 120 g L^{-1} (experiment 2) for 21, 14 and 14 days, respectively. Five feeding regimes were examined; the control treatment feeding a standard regime of only the microalga *Dunaliella tertiolecta*, and four biofloc treatments: 90% and 75% replacement of microalgae by bioflocs, both without and with halophilic addition. Depending on the experiment, *Artemia* performance was assessed as growth, reproductive performance, fatty acid and proximate composition and/or digestive enzyme activity. Applying halophilic bacteria in biofloc systems had no significant effect on the measured parameters. We further conclude that *Artemia franciscana* can be cultured successfully using bioflocs as the main food source, with limited microalgae supplementation (10%–25%) with no significant effect on survival, length, proximate composition and levels of most fatty acids in *Artemia* biomass. A number of fecundity parameters were negatively affected by 75% substitution of microalgae by bioflocs. This research opens interesting perspectives to produce *Artemia* at a bigger scale, e.g., in tanks or pond conditions, where production of sufficient quantities of suitable microalgae species might be problematic.

KEYWORDS
Artemia franciscana, bioflocs, halophilic bacteria, microalgae

The authors confirm that they are not employed by the government of Iran or are not preparing articles in their 'personal capacity' (in other words, 'not as an official representative or on behalf of a sanctioned government'), and are employed at an academic or research institution where research or education is the primary function of the entity.

1 | INTRODUCTION

Biofloc technology (BFT) is a technique of enhancing water quality, biosecurity and productivity of aquaculture animals by reducing ammonium nitrogen and converting it into microbial protein, stabilizing the microbial community and decreasing the cost of production (De Schryver et al., 2008; Hari et al., 2004; Nootong et al., 2011).

So far, most research on bioflocs has been done within a freshwater and marine aquaculture environment for the rearing of aquatic animals including tilapia, penaeid shrimp etc., and very little work has been carried out on bioflocs within a halophilic context. Halophilic microorganisms comprise a heterogeneous group of microorganisms including Archaea, Bacteria and Eukarya, which play an important role in the aquatic food chain, nutrient recycling and energy flow within hypersaline environments (Bhattacharyya et al., 2015; Francis et al., 2005). In these environments, the brine shrimp *Artemia* occurs as the only metazoan organism, using these halophiles as part of its diet (Lopes-dos-Santos, De Troch, et al., 2019; Lopes-Dos-Santos, Groot, et al., 2019).

The brine shrimp *Artemia* is an important live food to satisfy the nutritional requirements of various aquatic animals in the larval stage (Bengtson et al., 1991; Sorgeloos et al., 1980; Sorgeloos et al., 2001). Almost all the *Artemia* offered on the world market is collected from natural salt lakes (Lavens & Sorgeloos, 2000), although *Artemia* cyst production in solar saltworks can be important to supply local aquaculture demands (Le et al., 2019). *Artemia* is typically cultured with microalgae (Thinh et al., 1999), but it is costly to grow *Artemia* exclusively on microalgae, either in tank culture systems (Lavens & Sorgeloos, 1991) or in ponds.

Some studies, both in laboratory tests and in pond trials, have reported that bioflocs as a food source can be applied successfully in *Artemia* production (Anh et al., 2009; Gao et al., 2017; Luo et al., 2017; Ronald et al., 2014; Sui et al., 2013; Toi et al., 2013; Wang et al., 2019). However, rarely the addition of halophilic bacteria to a biofloc production system as food for *Artemia* has been investigated (Gao et al., 2017). Improved water quality and enhanced *Artemia* production could be obtained using different carbon sources to stimulate biofloc production when adding halophilic bacteria and archaea to the *Artemia* culture system (Gao et al., 2017). Moreover, bioflocs as food resulted in higher growth of *Artemia* compared with a microalgae diet, producing *Artemia* containing higher levels of fatty acids and crude protein (Luo et al., 2017). In the majority of these studies, however, the evaluation of *Artemia* performance was limited to its growth and survival rate. Only a few studies on reproductive performance (Anh et al., 2009; Ronald et al., 2014; Sui et al., 2013) and proximate composition (Luo et al., 2017; Yao et al., 2018) of *Artemia*, grown on bioflocs, respectively, in ponds and in the laboratory, were carried out, and these showed better growth, higher biomass and cyst production, improved proximate composition and water quality.

In the present study, we therefore aimed to investigate the application of halophilic bacteria in a biofloc production system to grow *Artemia*, in which part of a standard microalgae feeding regime was replaced by bioflocs. This was done in order to come ultimately to a protocol for *Artemia* pond production in Iran, using bioflocs and halophilic bacteria. For this purpose, a number of successive laboratory tests were conducted, using rice bran and vinasse, being cheap and available by-products of the local agricultural and sugar-ethanol industry, as carbon sources; cost and availability are important factors to select carbon sources for biofloc systems (Wilén et al., 2000). As halophilic bacteria, we used a halophilic *Bacillus* sp., isolated from Urmia Lake, Iran. Additionally, a commercial probiotic product, which are live microorganisms providing health benefits for the host (FAO, 2002), consisting of *Bacillus subtilis* and *Bacillus coagulans* (Parsilact Company, Iran) was also used in the biofloc production system.

2 | MATERIALS AND METHODS

2.1 | Experimental design

The present study was done at the *Artemia* and Aquaculture Research Institute (AARI), Urmia University, Urmia, Iran. Three successive experiments were run. In the first experiment, *Artemia* were cultured in 1.5 L containers with a stocking density of 1 nauplius 2 ml^{-1} at salinity 60 $g\,L^{-1}$ for 21 days. The second experiment was carried out in 1.5 L containers with a stocking density of 1 nauplius 10 ml^{-1} for 14 days at 120 $g\,L^{-1}$ salinity, comparable to pond conditions. In the third experiment, *Artemia* were cultured in 5 L containers with a higher initial stocking density of 2 nauplii mL^{-1} for 14 days at salinity 60 $g\,L^{-1}$. All experiments and analyses were done in triplicate. In experiments 1 and 2, thirty couples of *Artemia* were isolated from each treatment (10 couples per replicate), once the riding stage had been attained and transferred to 50-ml Falcon tubes to study reproductive performance. Temperature and photoperiod were, respectively, kept at 28 ± 2°C (by aquarium heaters) and 12 L:12D during the experiments. All saline water used was prepared by mixing tap water with Persian Gulf sea salt (purchased from Soil, Water and Structure Company), measuring and adjusting salinity with a hand-held refractometer (Atago Company). Saline water was only filtered (0.22 μm) for algae culture.

2.2 | Determination and identification of the halophilic bacteria

Brine water (340 $g\,L^{-1}$) samples were collected from Urmia Lake and transferred to the microbiological laboratory of AARI, Iran. Halophilic bacteria isolation was carried out based on Kashi et al. (2014). The results of phenotypic tests, as currently done at AARI, showed that the isolate consisted of gram-positive bacilli, rod-shaped, nonmotile with endospores, having nonpigmented colonies (cream or white), circular and opaque. Also, growth occurred in 1%–24% NaCl at 10–49°C. Moreover, results of the biochemical tests indicated that they were catalase and oxidase-positive, gelatin

hydrolysis-positive, indole-negative, cimon citrate-negative and H_2S-negative. Based on these phenotypic and biochemical tests, we identified the isolate from the brine sample as *Bacillus* sp., as had already been demonstrated by Kashi et al. (2014) in Urmia lake brine samples. Then, molecular characterization of the strain was done by amplification of 16S rDNA gene by PCR using primers SP6-16s (5′TTATTTAGGTGACACTATAGAATACTCAAGCTA TGC3′) and T7-16S (TAATACGACTCACTATAGGGGGGCGA ATTG GGCCC), and sequenced by T7 and SP6 primer at gcgenomics.com. The sequences were confirmed as *Bacillus* sp. by a similarity-based search using the web server https://blast.ncbi.nlm.nih.gov/Blast.cgi.

After identification, a single colony was streaked on marine broth at 37°C for 48 h. The bacteria were centrifuged at 2300 *g* for 20 min and washed twice with saline water. Finally, the bacteria were freeze-dried and kept at −20°C to be used later.

2.3 | Halophilic and probiotic bacteria culture

The bacterial strains, including the halophilic bacteria (*Bacillus sp.*) isolated from Urmia lake and the commercial probiotics *B. subtilis* and *B. coagulans*, were streaked on culture-medium (CM60) agar plates (Ventosa et al., 1990; Yuangao et al., 2015), containing yeast extract (10 $g L^{-1}$), peptone (7.5 $g L^{-1}$) and agar bacteriological grade (20 $g L^{-1}$), dissolved in filtered (0.2 μm) 60 $g L^{-1}$ saline water, and the pH of the medium was adjusted to 7.2–7.4. The commercial probiotics were used as they are part of the standard AARI biofloc production procedure. The plates were incubated for 2 days at 37°C. For each strain, a single colony was selected from the plates and incubated for 2 days on shakers (150 rpm, 28°C) in a liquid CM60 medium. Next, the bacterial suspension was centrifuged at 2300 *g* for 10 min. Then, the supernatant was decanted and the sediment was washed twice with filtrated autoclaved artificial saline water (FAASW). Finally, the bacteria were resuspended using FAASW and brought to the density of 10^7 CFU ml^{-1} according to the McFarland standard (Sutton, 2006).

2.4 | Biofloc production system

Firstly, four 25-L conical polyethylene containers containing 20 L of 60 ± 2 $g L^{-1}$ saline water (see 2.1) were used to set up a biofloc production system: two replicate containers to produce bioflocs without halophilic bacteria, two other containers with halophiles. Each container was aerated using a pipe connected to an aerator.

Rice bran (42% carbon) and vinasse (34% carbon, 62% dry matter) were used as carbon sources, at a ratio of 90% and 10% of carbon, respectively. Ammonium sulfate was used as a nitrogen source (C/N ratio of 15). Rice bran and vinasse were analysed using a CHN analyser (Elementar Company) and stored at 4°C. Daily quantities of rice bran and vinasse were calculated according to Avnimelech (1999), weighed and added to the containers. Probiotic and halophilic bacteria were cultured (see 2.3) and introduced to the containers at a density of 10^7 CFU ml^{-1} (Avnimelech, 2007) on day 1 and every 2 weeks. From 3 weeks onwards, 8 L of the contents of one replicate container of each type of bioflocs (with or without halophiles) was harvested alternately twice a week and centrifuged at 4000 *g* for 10 min (Sigma Centrifuge), and the sedimented fraction was resuspended with a small amount of supernatant and supplied to the *Artemia* culture vessels according to Table 1. Total suspended solids of the solution (TSS) were determined by centrifuging 50 ml of the solution (in duplicate) at 2300 *g* for 10 min, washing by ammonium formate (0.5 M) to remove salt (Zhu & Lee, 1997) and drying at 105°C for 4 h (APHA, 1981). A stock suspension with TSS of 180 $mg L^{-1}$ was used and stored at 4°C for 3–4 days.

2.5 | Algae culture

Microalgae *Dunaliella tertiolecta* (originating from a cultured stock from AARI, kept at 4°C) was cultured in 10 L containers containing 8 L saline water (35 $g L^{-1}$, see 2.1) under standard laboratory conditions at a temperature of 22–25°C. The Gillard (F/2) nutrient medium and vitamin solution were added at inoculation time at 1 and 0.5 $ml L^{-1}$, respectively. Strong bottom aeration and continuous light were provided for photosynthesis activity. The algae suspension was harvested after 8–10 days of culture and centrifuged at 3000 *g* for 6 min (Sigma Centrifuge). The concentration of the suspension was then checked by means of a haemocytometer and microscope, adjusted to a density of 18×10^6 cells ml^{-1} and stored at 4°C for 3–4 days. Multiple culture containers were maintained at the same time to guarantee a constant supply of microalgae throughout the consecutive experiments.

2.6 | *Artemia* cyst hatching and feeding regime

Locally produced *Artemia franciscana* cysts (originating from Great Salt Lake strain, Iran Artemia Company) were hatched according to standard procedures (Van Stappen, 1996): salinity 35 $g L^{-1}$, temperature 28 ± 1°C and under continuous illumination and aeration. Hatching cones were put in an aquarium having a submerged heater to keep the temperature at 28 ± 1°C. After 20 h hatching, *Artemia* nauplii were collected and transferred into the containers.

In all experiments, feeding treatments were according to Table 2 and based on offering equal dry weight across treatments, according to Coutteau et al. (1990). First, the weight of 18×10^6 cells of algae was determined (1.98 ± 0.10 mg dry weight, $n = 3$) by centrifuging 50 ml of the algae stock suspension (density 18×10^6 cells ml^{-1}), washing with ammonium formate (0.5 M) (Zhu & Lee, 1997), drying it at 105°C for 4 h and weighing with a precision balance (Axis company) up to 1 mg. The volume of bioflocs needed at each culture day in T1-T4 was calculated based on the following formula (Tran Thi, 2016):

$$\text{Floc volume (L)} = (\text{mg required feed for individual } \textit{Artemia} \times \text{number of animals}) / \text{total suspended solids (TSS)} \left(\text{mg L}^{-1}\right) \times 1000$$

TABLE 1 Feeding schedule for culturing 100 *Artemia franciscana* individuals in laboratory conditions based on Coutteau et al. (1992) for microalgae and based on Tran Thi (2016) for bioflocs

Day	Coefficient of increase[a]	Food suspension (ml day^{-1})				
		Algae[b] (control)	Algae[b] (T1,T3)	Algae[b] (T2,T4)	Bioflocs[c] (T1,T3)	Bioflocs[c] (T2,T4)
1	–	0.80	0.08	0.20	8.20	6.90
2–4	1.90	1.60	0.16	0.40	15.50	13.10
5,6	1.50	2.50	0.25	0.60	23.20	19.60
7	1.40	3.50	0.35	0.90	32.50	27.50
8	1.20	4.20	0.42	1.05	39.10	33.00
9	1.60	6.80	0.68	1.70	62.40	52.80
10,11	1.10	8.00	0.80	2.00	68.70	58.10
12,13	1.40	10.00	1.00	2.50	96.20	81.40
14,15	1.20	12.00	1.20	3.00	115.40	97.60
16,17	1.10	14.00	1.40	3.50	127.00	107.40
18,19	1.20	17.00	1.70	4.20	152.40	128.90
20,21	1.10	20.00	2.00	5.00	167.60	141.80

[a]The quantity was adjusted based on the survival data after each count.

[b]Algae concentration in stock suspension was 18×10^6 cells ml^{-1}.

[c]TSS of the biofloc stock suspension was 180 mg L^{-1} (Crab et al., 2010).

TABLE 2 Feeding treatments used in all *Artemia franciscana* culture experiments

Treatments	Type of feeding regime Bioflocs + microalgae
T1	90% BF + 10% MA
T2	75% BF + 25% MA
T3	90% BF-H$^+$ + 10% MA
T4	75% BF-H$^+$ +25% MA
T5-Control	100% MA

Abbreviations: BF, Bioflocs produced using rice bran + vinasse + no halophiles; BF-H$^+$, Bioflocs produced using rice bran + vinasse + halophile; MA, Microalgae. Bioflocs replaced microalgae *Dunaliella tertiolecta* based on dry weight (see 2.6).

Uneaten flocs and wastes from the *Artemia* culture medium were siphoned through a filter screen (250 μm mesh size) every week. No water exchange was done during the experimental period except for the control (about 50% each week). Dechlorinated tap water was daily used to compensate for evaporation.

2.7 | Parameter analysis

2.7.1 | Abiotic parameters

In all experiments, the pH, temperature and dissolved oxygen (DO) were recorded daily in the morning using a multimeter (Multi 3630 IDS), and salinity was recorded twice a week by a handheld refractometer (Atago Company). In experiments 1 and 3, NO_2-N and NO_3-N were measured using an interface photometer (Palin-Test 7500) and NH_3-N was measured using a photometer (Nessler, Hack DR 2000). To analyse these parameters, 50 ml of water sample was weekly taken from each replicate and centrifuged at 2300 g for 5 min to remove suspended solids. Then, the measurement was done based on the standard protocol for each analysis. All parameters were analysed for the water in the *Artemia* culture vessels and in the biofloc production systems.

2.7.2 | Survival and growth performance of *Artemia*

Survival and growth were measured in experiments 1 and 3. During the first experiment, *Artemia* were counted on days 8, 11, 14, 17 and 21 (Triantaphyllidis et al., 1995) to determine survival. To determine total length, 10 animals from each replicate were randomly taken on days 8, 14 and 21 (Lavens & Sorgeloos, 1991). In the third experiment, given its bigger culture volume, survival was determined on days 7 and 14 by taking 5 subsamples of 100 ml from each replicate. In both experiments, *Artemia* were returned back to the culture

medium after counting. The total length in the third experiment was determined on days 7 and 14 by sampling 10 animals per replicate. The total length was drawn from the anterior head part to the end of the telson using a stereomicroscope (Stemi SV11, Zeiss Company) equipped with a phototube. Drawings were later digitized using a digitizer connected to a computer with a digitizer software. The percentage survival was calculated based on the following formula:

$$\text{Percentage survival } (\%) = (\text{number of } \textit{Artemia} \text{ at sampling} / \text{initial number of stocked } \textit{Artemia} - \text{number removed for length measurements}) \times 100$$

At the end of experiment 3, also total *Artemia* biomass was measured. Therefore, the surviving population of each replicate was collected through a 350 μm filter screen and washed with distilled water. Then, excess water was removed using paper tissue from the bottom of the filter, and the biomass was weighed by a precision balance (Axis Company, Poland) up to 1 mg. For the sake of comparison with literature data, biomass production was expressed per volume of culture medium.

2.7.3 | *Artemia* reproductive performance

As soon as *Artemia* had reached the riding stage, thirty couples from each treatment in experiments 1 and 2 were isolated and cultured per couple separately in a Falcon tube containing 40 ml saline water of 60 (experiment 1) and 120 (experiment 2) $g L^{-1}$, immersed in a water bath at a temperature of 28 ± 1°C. *Artemia* feeding was done based on Table 1. Every day, the Falcon tubes containing offspring were picked out. The *Artemia* male and female were transferred to a new Falcon tube containing 40 ml water of the same salinity, while the offspring in the previous tube was collected over a 150 μm sieve (Ø = 25 mm) and counted under a stereomicroscope. Reproduction was followed for 2 months.

Throughout the culture period, dead *Artemia* males were replaced by new ones. Tubes containing dead *Artemia* females were removed from the setup. For any treatment, the culture was stopped when more than 80% of female mortality occurred during the treatment. Reproductive parameters were assessed as follows: pre-productive period (days): period from hatching until first spawning; reproductive period (days): period from first to last spawning; spawning interval (days): period between two spawnings; brood size: number of nauplii or cysts per spawning; brood number: number of broods per female; fecundity: number of nauplii and cysts over the total reproductive period.

2.7.4 | Proximate and fatty acid analysis

Proximate analysis of *Artemia* from each replicate was carried out at the end of experiment 3. For proximate analysis of bioflocs and microalgae, a sample was taken at the end of the experiment and analysed in three technical replicates. To analyse bioflocs, samples (about 2 L) were harvested from each reactor and pooled two by two, and algae (about 8 L) were centrifuged at 3700 *g* for 10 min, then washed with ammonium formate (0.5 M) to remove salts (Zhu & Lee, 1997). The samples were dried in preweighed aluminium cups in an oven at 60°C until they reached a constant weight. Then, they were stored in a freezer at −20°C until proximate analysis.

The entire *Artemia* biomass, surviving at the end of the experiment in each replicate, after determining total biomass (see 2.7.2) was used for nutrient analysis. All samples were dried and preserved in a freezer until further analysis as explained earlier.

The protein content was calculated based on Lowry et al. (1951). For ash content, a known amount of dry sample was burnt in a muffle furnace at 550°C for 6 h. The crude lipid was determined by extracting the lipids with diethyl ether following the procedure of Christie (1993). Protein, lipid and ash contents were expressed as a percentage of the dry weight (% DW) of bioflocs, algae and *Artemia*. The total carbohydrate amount was calculated according to the following formula: carbohydrate (% DW) = 100 − (crude protein (% DW) + lipid (% DW) + ash (% DW)) (Manush et al., 2005). The fatty acid methyl ester (FAME) profile of *Artemia* biomass was analysed via a modified procedure by Lepage and Roy (1984).

2.7.5 | Digestive enzymes assays

Artemia (500 mg WW, corresponding with 200–250 individuals) were collected, from each replicate at the end of experiment 3. The samples were first washed with distilled water, collected in 2 ml Eppendorf tubes and instantly transferred to a freezer (−80°C).

For enzyme extraction, the samples were defrosted at room temperature. Then, the samples were weighed in glass containers (10 ml) and homogenized in 50 mM Tris–HCl buffer at a ratio of 1:5 for 3 × 30 s using a homogenizer (Polytron PT 1300 D, Kinematica AG Company). The homogenates were then centrifuged (Sigma Company) at 10,000 *g* at 4°C for 20 min. Finally, the supernatant was divided into 0.5 ml Eppendorf tubes and stored at −80°C freezer until enzymatic assay (Chong et al., 2002).

Alpha-amylase activity was assayed according to Worthington (1991), using starch as substrate and lipase activity was determined spectrophotometrically (Biotek, Synergy HT) by hydrolysis of p-nitrophenyl myristate according to a modified method of Albro et al. (1985). Enzyme assay of total alkaline protease was done using azocasein as substrate followed by Garcia-Carreno & Haard (1993). The total soluble protein content was measured in diluted homogenates by the Bradford method (Bradford, 1976) using bovine serum albumin as a standard. All enzyme activity was calculated based on the total soluble protein and all assays were carried out in three biological replicates.

2.8 | Statistical analysis

Except for the abiotic parameters in the biofloc reactors (which were not statistically analysed) and the proximate composition of the food

sources (which were analysed by one-way ANOVA), all data were analysed first by two-way ANOVA to look for the effect of the microalgae replacement rate (10% and 25%) and the addition of halophilic bacteria, and the interaction of both factors. Then, data were analysed by one-way ANOVA to compare treatments with the control group, followed by the Tukey's test to compare the significant difference among the treatments (T1-T5) at $p < 0.05$ (SPSS Statistics, Version 16). All data are presented as mean ± standard deviation.

3 | RESULTS

3.1 | Abiotic parameters

Over the culture period in all experiments, the water temperature, dissolved oxygen and pH, respectively, ranged from 27 to 29°C, 4–6.5 mg L^{-1} and 8.3–8.4 in the biofloc reactors, and 26–27°C, 6.1–6.4 mg L^{-1}, and 8.4–8.6 in the *Artemia* culture media. The salinity was in a range of 58–63 g L^{-1} in the biofloc reactors, 60–67 g L^{-1} in the culture medium in experiments 1 and 3, and 120–130 g L^{-1} in experiment 2. The amount of NH_3-N, NO_3-N and NO_2-N in the biofloc reactors ranged from 2.5 to 4.5, 0.2 to 0.6 and 0.1 to 0.2 mg L^{-1} during all the experiments.

In experiment 1, results from two-way ANOVA showed that algae substitution rate had a significant effect on both NO_3-N and NO_2-N in the *Artemia* culture medium and halophilic bacteria only on NO_3-N (two-way ANOVA, $p < 0.05$). In experiment 3, there was a significant effect of algae substitution rate on NO_2-N, and moreover, a significant effect of interaction between both factors on NH_3-N. During the first experiment, the amount of NH_3-N in all treatments increased gradually and reached values above 1.5 mg L^{-1}, then decreased at the end of the experiment to 1.2 mg L^{-1} or less in the biofloc treatments, which was significantly lower than in the control treatment (one-way ANOVA, $p < 0.05$, Table 4). In experiment 3, as time went on, the amount of NH_3-N increased in all treatments and reached 10 mg L^{-1} in the control treatment, which was significantly higher than in the biofloc treatments (one-way ANOVA, $p < 0.05$). At the end of the experiments 1 and 3, NO_3-N in the control treatment was significantly higher than in the biofloc treatments (one-way ANOVA, $p < 0.05$). In experiments 1 and 3, the final amount of NO_2-N in the control treatment was significantly higher than in the biofloc treatments (one-way ANOVA, $p < 0.05$, Table 4).

3.2 | *Artemia* survival, growth and reproductive performance

The survival rate of all treatments was in the range 56.0%–75.0% (day 21, experiment 1), 47.0–58.0% (day 14, experiment 2) and 71.0%–82.6% (day 14, experiment 3); no significant difference was observed among biofloc treatments (two-way ANOVA, $p > 0.05$) and between treatments and the control treatment (one-way ANOVA, $p > 0.05$). At the end of experiment 1, the total length was in the range from 7.9 to 9.2 mm, without significant differences among the five treatments ($p > 0.05$, one-way ANOVA). Algae substitution had a significant effect on biomass production (two-way ANOVA, $p < 0.05$). Biofloc treatments with less bioflocs in their feeding regimes (T2 and T4) had significantly higher biomass than treatments with more bioflocs (T1 and T3) ($p < 0.05$, one-way ANOVA). As for experiment 3, the control treatment had significantly higher total length and biomass (7.4 mm, 5.5 g L^{-1} wet weight) than the biofloc treatments (5.4–5.9 mm, 2.1–2.7 g L^{-1} WW) ($p < 0.05$, one-way ANOVA).

Results from two-way ANOVA showed that algae substitution significantly affected all reproductive parameters of *Artemia* in both experiments 1 and 2 (with the exception of the pre-reproductive period in experiment 2). Halophilic bacteria addition, on the other hand, had a significant effect on spawning interval, brood number and fecundity in experiment 2. No significant effect whatsoever of the interaction of both factors on reproductive performance was observed ($p > 0.05$, Table 3). At lower salinity (experiment 1), the control treatment had significantly higher fecundity than all biofloc treatments, corresponding with its significantly higher brood size ($p < 0.05$, one-way ANOVA). However, the fecundity of the control at higher salinity (experiment 2) was only significantly higher than the treatments with the lowest algae substitution rate (T1, T3) and this corresponded with the differences observed for length of reproductive period and number of broods. Moreover, in both experiments, treatments fed less bioflocs had significantly higher fecundity than treatments fed more bioflocs, mostly due to a higher brood number, shorter spawning interval and longer reproductive period (Table 5). In addition, cyst production was only observed in the control treatment of experiment 1 (average brood size 85 ± 50 cysts).

3.3 | Proximate composition of *Artemia* biomass and food sources

The alga *D. tertiolecta* as food had significantly higher protein and lipid but lower ash content than the bioflocs (one-way ANOVA, $p < 0.05$, Table 6). In experiment 3, results from two-way ANOVA showed that algae substitution and the interaction of algae substitution and halophilic bacteria addition had a significant effect on ash content ($p < 0.05$, Table 3) in *Artemia* biomass. Protein, lipid and carbohydrate showed no significant difference among treatments (one-way ANOVA, $p > 0.05$). However, treatments with lower algae substitution (T1 and T3) had significantly higher ash content than treatments with higher algae substitution (T2 and T4), whereas the control treatment had a significantly lower ash content than all other treatments (one-way ANOVA, $p < 0.05$, Table 6).

3.4 | Fatty acid composition of *Artemia* biomass

In experiment 3, results from two-way ANOVA revealed that dietary algae substitution had a significant effect on fatty acids contents of

TABLE 3 Two-way ANOVA for effect of algae substitution and halophilic addition on *Artemia franciscana* performance, biomass and proximate omposition in different experiments

Parameters	Experiment	Algae substitution (AS)	Halophilic bacteria addition (HbA)	AS*HbA interaction
Biomass	3	0.004		
Pre-reproductive period	1	<0.001		
Reproductive period	1	<0.001		
	2	<0.001		
Female life span	1	<0.001		
	2	<0.001		
Spawning interval	1	0.002		
	2	0.017	0.011	
Average brood size	1	<0.001		
	2	0.034		
Brood number	1	<0.001		
	2	<0.001	0.015	
Fecundity	1	<0.001		
	2	<0.001	0.018	
Ash	3	<0.001		0.038

Note: Only significant effects are reported ($p < 0.05$).

TABLE 4 Abiotic parameters in the *Artemia franciscana* culture water with different feeding regimes in experiments 1 and 3 (mean ± SD; $n = 3$) at the end of the experiment (21 and 14 days, respectively)

Parameters (mg L^{-1})	Experiments	Treatments				
		T1	T2	T3	T4	T5
NH_3-N	1	0.88 ± 0.09^{b}	0.84 ± 0.14^{b}	0.79 ± 0.09^{b}	1.20 ± 0.35^{ab}	1.53 ± 0.02^{a}
	3	4.70 ± 0.50^{b}	3.01 ± 0.56^{c}	3.82 ± 0.75^{bc}	4.63 ± 0.56^{b}	10.14 ± 0.31^{a}
NO_3-N	1	1.53 ± 0.03^{c}	1.76 ± 0.13^{c}	1.83 ± 0.07^{bc}	1.86 ± 0.03^{b}	3.16 ± 0.16^{a}
	3	1.50 ± 0.17^{b}	1.30 ± 0.30^{b}	1.50 ± 0.03^{b}	1.47 ± 0.13^{b}	3.24 ± 0.03^{a}
NO_2-N	1	0.30 ± 0.00^{c}	0.39 ± 0.02^{b}	0.30 ± 0.00^{c}	0.36 ± 0.01^{b}	0.54 ± 0.02^{a}
	3	0.42 ± 0.00^{b}	0.21 ± 0.09^{c}	0.39 ± 0.03^{bc}	0.32 ± 0.05^{bc}	0.73 ± 0.12^{a}

Note: T1 (90% BF + 10% MA); T2 (75% BF + 25%MA); T3 (90% BF-H^++10%MA); T4 (75% BF-H^++25%MA); T5 (100% MA). Different superscripts represent significant differences among treatments in each row (one-way ANOVA, $p < 0.05$).

Artemia; however, halophilic bacteria addition had a significant effect only on C16:1n-7 content ($p < 0.05$). A significant effect of interaction between both factors was observed only in C16, C20:2n-6 and sum SFA. Our results from one-way ANOVA indicated that treatments fed more bioflocs showed significantly higher ARA and EPA than the other biofloc treatments and the control (T5). Treatments fed more bioflocs also showed higher DHA contents than the other biofloc treatments and the control, although the difference was not significant ($p > 0.05$). The control treatment showed significantly higher levels of linolenic acid (ALA) than all biofloc treatments, which showed, however, no significant difference among them (one-way ANOVA, Table 7). A significantly higher level of linoleic acid (LA) was observed in *Artemia* fed more bioflocs with halophilic addition (T3) than the *Artemia* fed less bioflocs without halophilic addition (T2) and the one fed microalgae (T5). Our results revealed that treatments fed more bioflocs (T1 and T3) had significantly higher levels of C18:1n-9 than in the treatments with less bioflocs (Table 7). Also, *Artemia* fed more bioflocs without halophilic addition (T1) indicated a significantly higher level of C18:1n-7 than the one fed less bioflocs with halophilic addition (T4) but had no significant difference with the other treatments. Regarding SFA, the control treatment showed a significantly higher value than the treatments fed less bioflocs but had no significant difference with the *Artemia* fed more bioflocs. Our results also showed significantly a higher level of MUFA in treatments fed more bioflocs than in the treatments fed less bioflocs and the control. Moreover, *Artemia* fed more bioflocs without halophilic addition showed significantly higher PUFA than the one fed less bioflocs with halophilic addition. No significant difference was

TABLE 5 Reproductive performance of *Artemia franciscana* under the different feeding regimes and at salinities 60 and 120 g L^{-1} (experiments 1 and 2) (mean ± SD, $n = 30$)

Parameters	Experiment	T1	T2	T3	T4	T5
Pre-reproductive period (days)	1	24 ± 1^{a}	22 ± 1^{b}	24 ± 0^{a}	23 ± 1^{b}	19 ± 1^{c}
	2	15 ± 2^{ab}	15 ± 1^{b}	15 ± 1^{b}	15 ± 1^{b}	16 ± 1^{a}
Reproductive period (days)	1	24 ± 16^{b}	52 ± 18^{a}	17 ± 17^{b}	48 ± 20^{a}	39 ± 27^{ab}
	2	15 ± 5^{b}	39 ± 17^{a}	13 ± 6^{b}	40 ± 16^{a}	29 ± 19^{a}
Female lifespan (days)	1	32 ± 14^{bc}	58 ± 15^{a}	28 ± 15^{c}	59 ± 18^{a}	46 ± 28^{ab}
	2	34 ± 6^{b}	58 ± 16^{a}	34 ± 7^{b}	60 ± 14^{a}	43 ± 20^{b}
Spawning interval (days)	1	5 ± 4^{a}	3 ± 1^{b}	5 ± 4^{a}	3 ± 1^{b}	3 ± 1^{b}
	2	4 ± 2^{ab}	3 ± 1^{b}	5 ± 3^{a}	4 ± 1^{ab}	3 ± 1^{b}
Average brood size (nauplii/female)	1	29 ± 14^{b}	41 ± 13^{b}	29 ± 14^{b}	38 ± 16^{b}	128 ± 46^{a}
	2	29 ± 13^{b}	38 ± 17^{b}	30 ± 18^{b}	35 ± 15^{b}	64 ± 23^{a}
Brood number	1	5 ± 5^{b}	12 ± 6^{a}	4 ± 4^{b}	12 ± 5^{a}	8 ± 5^{a}
	2	4 ± 2^{c}	12 ± 5^{a}	3 ± 1^{c}	10 ± 4^{ab}	7 ± 4^{b}
Fecundity (total nauplii/female)	1	154 ± 107^{c}	492 ± 271^{b}	122 ± 101^{c}	438 ± 189^{b}	989 ± 803^{a}
	2	125 ± 63^{b}	446 ± 199^{a}	103 ± 53^{b}	356 ± 138^{a}	474 ± 365^{a}

Note: T1 (90% BF + 10% MA); T2 (75% BF + 25%MA); T3 (90% BF-H^{+}+10%MA); T4 (75% BF-H^{+}+25%MA); T5 (100% MA). Different superscripts represent significant differences among treatments in each row (one-way ANOVA, $p < 0.05$).

TABLE 6 Proximate composition of food sources and *Artemia franciscana* biomass, % dry weight (mean ± SD; $n = 3$)

Food sources	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)
Algae	41.5 ± 0.5^{a}	11.0 ± 1.1^{a}	6.3 ± 0.4^{b}	41.2 ± 2.0
Bioflocs (BF)	24.1 ± 3.6^{b}	4.4 ± 1.0^{b}	32.2 ± 3.9^{a}	39.4 ± 8.0
Bioflocs (BF-H^{+})	25.8 ± 5.6^{b}	4.8 ± 1.1^{b}	32.5 ± 1.7^{a}	36.9 ± 6.2
Artemia				
T1	52.7 ± 6.0	12.0 ± 2.9	15.9 ± 0.4^{a}	19.3 ± 7.6
T2	52.6 ± 5.2	10.2 ± 0.6	12.8 ± 0.6^{b}	24.4 ± 4.1
T3	53.8 ± 3.2	12.8 ± 4.8	15.1 ± 0.2^{a}	18.3 ± 2.4
T4	53.6 ± 3.5	12.7 ± 2.5	13.3 ± 0.5^{b}	20.5 ± 4.8
T5	51.0 ± 3.6	13.0 ± 2.0	9.5 ± 0.4^{c}	26.4 ± 5.7

Note: T1 (90% BF + 10% MA); T2 (75% BF + 25%MA); T3 (90% BF-H^{+}+10%MA); T4 (75% BF-H^{+}+25%MA); T5 (100% MA). Different superscripts represent significant differences among treatments in each column (one-way ANOVA, $p < 0.05$).

Abbreviations: BF, Bioflocs without halophilic bacteria; BF-H^{+}, Bioflocs with halophilic bacteria.

observed among treatments regarding n-3 levels. However, a significantly higher level of n-6 was observed in treatments fed more bioflocs than the one fed less bioflocs without halophilic addition and the control.

3.5 | Digestive enzyme assay of *Artemia*

At the end of experiment 3, the treatment with higher algae substitution and without halophilic addition (T1) showed significantly higher amylase activity compared with the control treatment, with intermediate values for the other treatments. Alkaline protease and lipase activities showed no significant difference between biofloc treatments and the control treatment (one-way ANOVA, Figure 1[a–c]).

4 | DISCUSSION

Previous studies have shown that bacterial growth stimulated by carbohydrate supplementation not only improves water quality but also increases the production of target aquaculture animals (Avnimelech, 1999; Nootong et al., 2011). Moreover, the use of bioflocs as part of the diet (Toi et al., 2013) and of halophilic bacteria as monodiets for *Artemia* (Lopes-dos-Santos, De Troch, et al., 2019; Lopes-Dos-Santos, Groot, et al., 2019) has been reported. The objective of the current study was to study the potential of halophilic bacteria in biofloc systems as the source of food for *Artemia*. Our results showed, overall, that incorporating halophilic bacteria in the biofloc system had no significant effect on *Artemia* performance and proximate composition compared with the treatments fed bioflocs

TABLE 7 Essential fatty acids contents (mg g^{-1} dry weight) of *Artemia franciscana* biomass under different feeding regimes (mean ± SD; $n = 3$) at the end of experiment 3

Fatty acids (mg g^{-1} DW)	Treatments				
	T1	T2	T3	T4	T5
C14	0.78 ± 0.23	0.48 ± 0.06	0.52 ± 0.11	0.61 ± 0.26	0.99 ± 0.63
C14:1n-5	0.58 ± 0.18	0.48 ± 0.04	0.44 ± 0.37	0.64 ± 0.60	0.82 ± 0.38
C16	19.40 ± 1.47^{ab}	14.84 ± 0.72^{c}	16.73 ± 0.83^{bc}	16.62 ± 1.29^{bc}	20.40 ± 1.68^{a}
C16:1n-7	4.04 ± 0.61^{a}	2.26 ± 0.13^{bc}	2.98 ± 0.51^{ab}	2.20 ± 0.23^{bc}	1.80 ± 0.31^{c}
C18	12.47 ± 0.41^{a}	9.14 ± 0.59^{b}	11.07 ± 1.87^{ab}	9.07 ± 0.88^{b}	12.48 ± 1.65^{a}
C18:1n-9	38.16 ± 0.73^{a}	26.34 ± 0.32^{b}	34.74 ± 2.47^{a}	26.31 ± 3.82^{b}	27.06 ± 1.26^{b}
C18:1n-7	17.72 ± 1.35^{a}	13.50 ± 1.06^{ab}	16.13 ± 1.14^{ab}	12.18 ± 1.32^{b}	14.22 ± 2.81^{ab}
18:2n-6; cis (LA)	31.92 ± 3.96^{ab}	23.21 ± 1.90^{b}	32.19 ± 1.98^{a}	23.89 ± 4.24^{abc}	23.53 ± 2.84^{bc}
18:3n-3 (ALA)	4.82 ± 0.66^{b}	5.25 ± 0.45^{b}	4.24 ± 0.34^{b}	4.50 ± 1.01^{b}	9.20 ± 1.56^{a}
C20	0.27 ± 0.07	0.26 ± 0.09	0.21 ± 0.06	0.37 ± 0.16	0.52 ± 0.19
C20:1n-9	0.48 ± 0.28^{ab}	0.33 ± 0.10^{ab}	0.21 ± 0.05^{b}	0.46 ± 0.11^{ab}	0.74 ± 0.15^{a}
C20:2n-6	0.61 ± 0.05	0.54 ± 0.07	0.56 ± 0.13	0.86 ± 0.17	1.04 ± 0.39
20:4n-6 (ARA)	1.71 ± 0.31^{a}	1.06 ± 0.08^{b}	1.75 ± 0.22^{a}	0.90 ± 0.18^{b}	0.26 ± 0.20^{c}
C20:3n-3	1.20 ± 0.60	0.81 ± 0.16	0.93 ± 0.22	0.63 ± 0.38	0.64 ± 0.46
20:5n-3 (EPA)	4.94 ± 0.41^{a}	2.83 ± 0.22^{b}	4.73 ± 0.57^{a}	2.29 ± 0.60^{b}	0.61 ± 0.23^{c}
C22:0	0.46 ± 0.07	0.30 ± 0.06	0.51 ± 0.33	0.44 ± 0.22	0.40 ± 0.20
22:6n-3 (DHA)	1.00 ± 0.26	0.52 ± 0.11	0.83 ± 0.26	0.56 ± 0.28	0.48 ± 0.34
∑SFA	33.38 ± 2.23^{ab}	25.01 ± 1.26^{c}	29.06 ± 2.98^{abc}	27.13 ± 2.55^{bc}	34.80 ± 3.69^{a}
∑MUFA	61.13 ± 1.32^{a}	42.92 ± 1.23^{b}	54.50 ± 3.63^{a}	41.80 ± 4.34^{b}	44.64 ± 3.51^{b}
∑PUFAs	45.02 ± 4.53^{a}	33.41 ± 1.48^{ab}	44.31 ± 2.82^{ab}	33.01 ± 6.16^{b}	35.14 ± 4.97^{ab}
∑n-3	10.76 ± 1.15	8.60 ± 0.64	9.80 ± 0.55	7.35 ± 1.73	10.29 ± 1.82
∑n-6	34.26 ± 3.72^{a}	24.81 ± 1.84^{b}	34.50 ± 25.65^{a}	25.65 ± 4.52^{ab}	24.84 ± 3.40^{b}
n-3/n-6	0.31 ± 0.03^{b}	0.35 ± 0.05^{ab}	0.28 ± 0.01^{b}	0.28 ± 0.02^{b}	0.4 ± 0.04^{a}
DHA/EPA	0.20 ± 0.05^{b}	0.19 ± 0.05^{b}	0.17 ± 0.03^{b}	0.25 ± 0.14^{b}	0.72 ± 0.25^{a}
EPA/ARA	5.15 ± 1.27^{ab}	5.61 ± 1.38^{a}	5.93 ± 1.02^{a}	4.75 ± 2.15^{ab}	1.50 ± 0.50^{b}

Note: T1 (90% BF + 10% MA); T2 (75% BF + 25%MA); T3 (90% BF-H^{+}+10%MA); T4 (75% BF-H^{+}+25%MA); T5 (100% MA). Different superscripts represent significant differences among treatments in each row (one-way ANOVA, $p < 0.05$).Linoleic acid (LA; 18:2n-6; cis); Linolenic acid (ALA; 18:3n-3); Arachidonic acid (ARA); Eicosapentaenoic acid (EPA); Docosahexaenoic acid (DHA); Saturated fatty acids (SFA); Mono-unsaturated fatty acids (MUFA); Polyunsaturated fatty acids (PUFA).

without halophilic addition and the control treatment fed only microalgae. In general, *Artemia* fed on biofloc exhibited almost similar survival, proximate composition and digestive enzyme activities as the control.

Our results revealed almost similar biomass but higher survival than values reported previously in laboratory conditions (respectively, 15%–50% and 1.5–8.0 g L^{-1} WW; Toi et al., 2013; Gao et al., 2017; Yao et al., 2018); different results may also be related to differences in for example feeding regime and type of biofloc culture (in-situ or ex-situ relative to the *Artemia* culture).

Our results showed that the inclusion of halophilic bacteria in the biofloc system had no effect on reproductive parameters of *Artemia* except for a reduced spawning interval, brood number and fecundity at salinity 120 g L^{-1}. However, algae substitution had a significant effect on reproductive performance at both salinities. At lower salinity, *Artemia* fed only algae showed significantly higher fecundity and average brood size than the biofloc treatments. However, at higher salinity, 25% algae substitution resulted in similar reproductive performance to the control treatment, but reproductive parameters decreased when fed only 10% algae. Our fecundity results from *Artemia* fed 75% bioflocs were within a similar range of previous studies who reported 300–600 offspring individuals per female fed algae (Sellami et al., 2021; Velasco et al., 2016) and bioflocs (Tran Thi, 2016) in laboratory conditions. On the other hand, the average brood size in our biofloc treatments was lower than in previous studies (60–120 nauplii per female; Browne & Wanigasekera, 2000; Tran Thi, 2016; Velasco et al., 2016). The difference in quality and quantity of available food, abiotic culture conditions, duration of observation period and *Artemia* species may contribute to differences in reproductive performance in *Artemia*, observed in different studies (Fábregas et al., 1998).

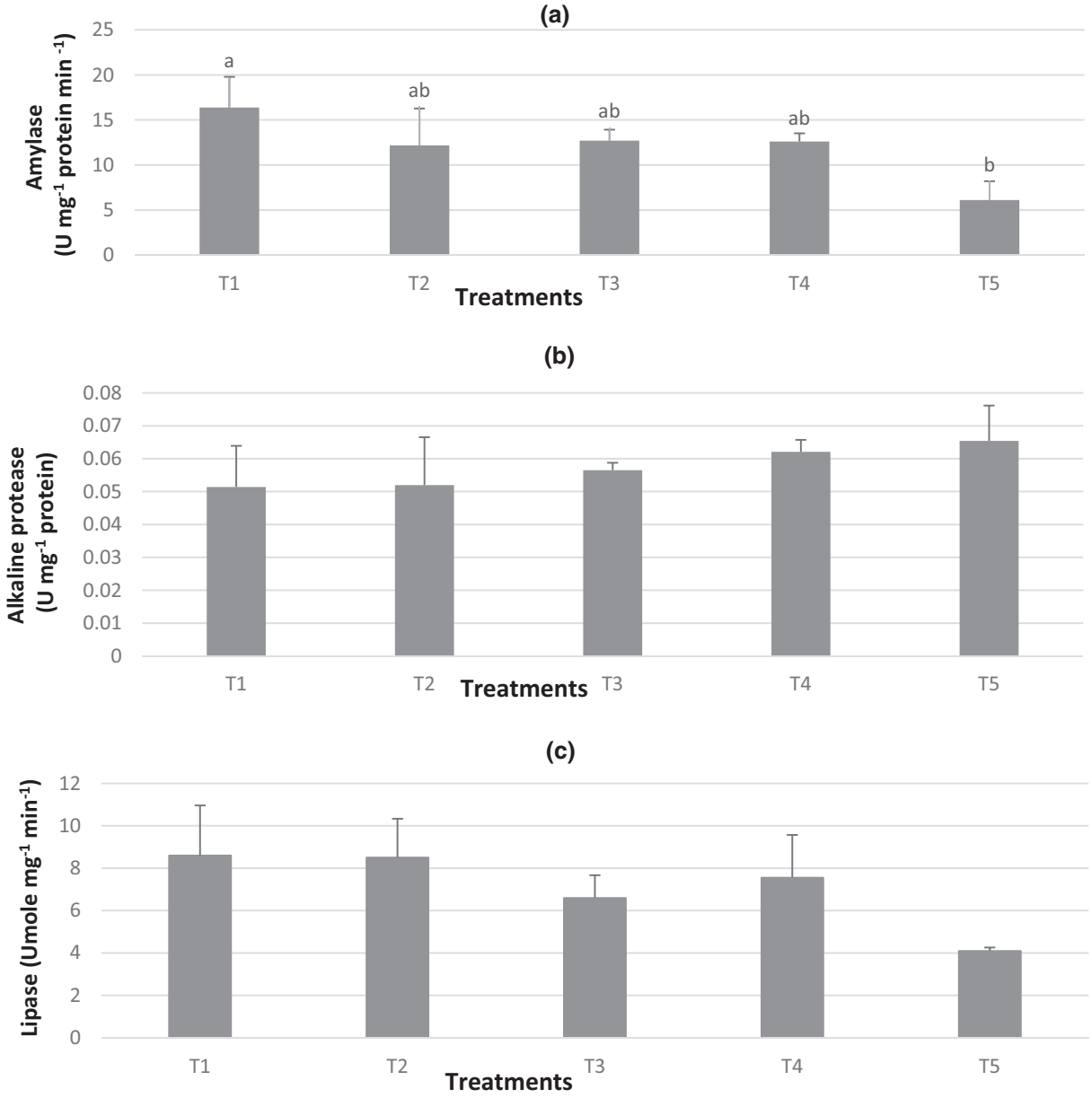

FIGURE 1 (a–c): Digestive enzyme activity (a: Amylase; b: Alkaline protease; c: Lipase) of *Artemia franciscana* biomass cultured under different feeding regimes, at the end of experiment 3 (mean ± SD; n = 3). T1 (90% BF + 10% MA); T2 (75% BF + 25%MA); T3 (90% BF-H^++10%MA); T4 (75% BF-H^++25%MA); T5 (100% MA). Different letters indicate significant differences among treatments (one-way ANOVA, $p < 0.05$).

In the present study, no cysts were produced by *Artemia* fed on bioflocs at both salinities, which is in agreement with the absence of cyst production at salinity 60 and 80 g L^{-1}, observed by Browne and Wanigasekera (2000) and Velasco et al. (2016). Contrary to what might be expected, it seems that the *Artemia* in our study even at the higher salinity were apparently not sufficiently stressed for cyst production. The relatively stable physical and chemical conditions in our culture set-up may have maintained *Artemia* in the ovoviviparous stage (Camargo et al., 2004). However, switching of reproductive mode in the natural environment can be expected depending on the environmental conditions (Lenz & Dana, 1987).

Also in hypersaline pond conditions, supplementing microalgae with bioflocs resulted in better growth, faster maturation and higher fecundity in *Artemia* (Anh et al., 2009; Masumbuko, 2014; Sui et al., 2013). Biofloc characteristics and in-situ or ex-situ production conditions, including carbon to nitrogen ratio and type of carbon source used (Nam, 2012), the rate of suspended solids (Ebeling et al., 2006), biofloc size (Ekasari et al., 2014) and age (Kuhn et al., 2017), microbial composition (Van Wyk, 2006; Wei et al., 2016) and protein level (Li et al., 2018), all are factors influencing the quality of bioflocs as food source and thus can result in differences in our study compared with previous studies.

Digestive enzyme activity is one of the most important indicators to estimate the nutritional value and availability of aquatic animal diets, and it reflects the digestive and metabolic capacity of the organisms (Anand et al., 2014). In this study, no significant difference was observed in the specific activity of the digestive enzymes alkaline protease and lipase across treatments, in contrast with previous studies reporting bioflocs as digestive enzyme enhancers in aquatic animals, such as in carp *Cyprinus carpio* and tiger shrimp *Penaeus monodon* (Anand et al., 2014; Najdegerami et al., 2016). However, substituting algae by bioflocs at the highest rate used, in combination with the addition of halophilic bacteria, resulted in significantly higher amylase activity in *Artemia* as compared to animals fed only algae. So the higher growth and biomass production in the latter group, as compared to the animals fed bioflocs, cannot be linked with higher enzyme production. No literature information is available related to digestive enzyme activity in *Artemia*, so more research is needed to expand our knowledge on the effect of feeding *Artemia* with different types of food on this parameter.

As bioflocs can be used as a food source for aquatic animals (Crab et al., 2012), their nutritional value is of importance. In this study, no significant difference was observed in the proximate composition of bioflocs with or without halophiles, and values of the different nutrients were in the range reported by previous studies (Hargreaves, 2013; Luo et al., 2017). Algae substitution rate and halophilic addition had no significant effect on the protein and lipid composition of *Artemia*. Although the bioflocs produced in our study contained significantly less protein and lipid than the microalgae used, this was not reflected in the proximate composition of the *Artemia* fed under different feeding regimes. In our study, protein, lipid and ash content of *Artemia* fed on bioflocs was in the range of previous studies, which reported 45%–58% protein and 9%–19% lipid for *Artemia* fed with bioflocs (Anh et al., 2009), aquaculture effluent (Gharibi et al., 2021) and microalgae (Turcihan et al., 2021), with the ash content in our study being in the range of the first two studies (11%–22%).

Dietary fatty acids are important for reproduction in aquatic animals because they improve the fecundity, egg hatching and ovary development, and play an important role in embryogenesis (Wouters et al., 2001). The fatty acid profile in adult *Artemia* is partially influenced by the composition of the diet (Dhont & Lavens, 1996). In the present study, the amounts of fatty acids in cultured *Artemia* were affected by the diet, as treatments fed more bioflocs had significantly higher levels of C18:1n-9, ARA, EPA, MUFA and total n-6 PUFA. Our results on EPA (except from the control treatment), DHA, ARA and ALA were in the range of values reported in *Artemia* fed on microalgae as a main food supplemented by bioflocs and in *Artemia* fed on aquaculture effluent (Toi et al., 2013; Gharibi et al., 2021), whereas LA and DHA were, respectively, higher and lower than ranges reported in the literature (respectively, 14–17 and 6–8 mg g^{-1} DW (Toi et al., 2013; Turcihan et al., 2021). These differences may be due to the different types of food offered to *Artemia*, including the strains of microalgae, quality and quantity of produced bioflocs, and the ratio of microalgae and of bioflocs in the diet.

In conclusion, our results indicate that applying halophilic bacteria in bioflocs as a food source for *Artemia* had almost no effect on the measured parameters. Although the present results still need to be validated in pond production conditions, this research indicates that bioflocs could improve water quality and provide a suitable environment for *Artemia* culture. In addition, bioflocs as a food source may contribute to *Artemia* performance when there is low algae supply, resulting in similar proximate composition of the biomass and digestive enzyme activity as an algae diet. However, a sufficient amount of algae seems needed in order to obtain high *Artemia* fecundity.

Overall, our results open up new perspectives for alternative protocols for pond production, in which the focus may rely more on bioflocs as a source of food for the *Artemia* population. Therefore, it is recommended to perform biofloc feeding experiments in ponds, both using ex-situ and in-situ biofloc systems, not only analysing the proximate composition and fatty acids but also other nutritional compounds such as amino acids and vitamins of the bioflocs and the *Artemia* fed with them. Also, the microbial composition of the bioflocs and *Artemia* should be analysed and digestive enzyme activity of *Artemia* of different age classes. It is also recommended to determine floc size at different stages of floc production. Finally, *Artemia* biomass is widely used for feeding shrimp, juvenile sturgeon fish and aquarium fish and has an important role in the early sexual maturation of shrimp. Therefore, it could be interesting to study the effects of *Artemia* produced in biofloc systems as a food source on reproductive performance and progeny of fish and shellfish.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

All relevant data are available from the authors upon request.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

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