Received: 2 August 2022 | Revised: 24 October 2022 | Accepted: 30 October 2022

DOI: 10.1002/aff2.89

AQUACULTURE, FISH and FISHERIES Open Access WILEY

ORIGINAL ARTICLE

# Biofloc and green water condition improves reproductive traits and fatty acid composition of *Artemia franciscana* cultured under limited algal conditions

**Erick O. Ogello** | **Nicholas O. Outa** | **Bramwel O. Mukaburu** | **Mavindu Muthoka**

Department of Animal and Fisheries Sciences, Maseno University, Maseno, Kenya

**Correspondence**
Erick Ogello, Department of Animal and Fisheries Sciences, Maseno University, P.O. Box Private Bag, Maseno, Kenya.
Email: erick.ogello@gmail.com

**Funding information**
Western Indian Ocean Marine Science Association, Grant/Award Number: MASMA/OP/2020/02

## Abstract

This study investigated the effect of biofloc and green water conditions on the survival, growth, reproductive traits, and fatty acid composition of the brine shrimp *Artemia franciscana*. *Artemia* was cultured in glass jars using three replicated treatments, that is, Control ($7.0 \times 10^6$ cells $ml^{-1}$ of freshly cultured *Tetraselmis tetrathele*), green water technology—GWT (control + 0.5 g/L of chicken manure extract [CME]) and biofloc technology—BFT (GWT + molasses). *Artemia* cysts were hatched and batch cultured in a 1-L glass jar containing 500 ml of natural seawater, stocked at 2 nauplii/ml. In the first 2 days, *Artemia* was fed with fresh *T. tetrathele* for conditioning, after which each treatment was applied daily until the end of the experiment. *Artemia* cultures were maintained at 28.0 ± 1°C using an electrically heated water bath system with constant aeration and light (2000 lux) for 30 days. There was higher *Artemia* survival in BFT (91.3 ± 3.2%) cultures than GWT (78.1 ± 2.9%) and Control (66.8 ± 1.3%). Female pre-reproductive period (days) was longer (25 ± 0.5) in control than in GWT (21.8 ± 0.7) and BFT (19.6 ± 0.6), while reproductive period (days) was longer in BFT (20.9 ± 2.6) than GWT (16.4 ± 2.3) and Control (12.8 ± 1.9). Total broods per female per day were higher in BFT (4.3 ± 0.3) than in GWT (3.5 ± 0.2) and Control (2.5 ± 0.3). BFT enhanced the ovoviviparous reproduction cycle with higher total offspring per female (73.5 ± 6.1) than GWT (66.2 ± 7.9) and Control (42.6 ± 12.1). BFT-cultured *Artemia* had higher concentrations of myristic acid, oleic acid, palmitic acid, linoleic acid, and arachidic acid. Better *Artemia* reproductive and nutritive traits in BFT conditions could have been enhanced by the combination of nutritious biofloc and algal materials. BFT and CME promise a major leap towards developing a nutritionally rich diet for *Artemia*.

KEYWORDS
*Artemia franciscana*, biofloc technology, carbon-nitrogen ratio, chicken manure extract (CME), green water technology

## 1 | INTRODUCTION

The brine shrimp *Artemia* is a crustacean that typically colonizes in extremely saline aquatic environments, such as coastal lagoons, inland lakes, and saltworks all over the world (Persoone & Sorgeloos, 1980; Turcihan et al., 2021). *Artemia* is a highly nutritious live food resource with tremendous value in aquaculture as the first diet for fish larvae (Sorgeloos, 1980). *Artemia* provides digestive enzymes for fish larvae, whose rudimentary digestive tract cannot digest inert diets (Sorgeloos & Tackaert, 1991). The nutrition value of *Artemia* can be improved through encapsulation techniques to attain the required levels needed by cultured animals (Fernández, 2001; Dhont & Sorgeloos, 2002; Sorgeloos et al., 2001). Due to these characteristics, *Artemia* has facilitated the expansion of global aquaculture production of finfish and crustaceans.

To enhance the supply of *Artemia* cysts in global hatcheries, *Artemia* has been integrated into various saltworks (Van Stappen et al., 2020). In Kenya, *Artemia* was inoculated in Malindi area about 4 decades ago (Rasowo & Radull, 1986). However, optimization of the *Artemia* biotope for local aquaculture initiatives is yet to take place due to a lack of sufficient data on reproductive characteristics and nutritive value of the local *Artemia* biotope, to inform mass production procedures. *Artemia* is adapted to produce dormant cysts, which can remain viable over a long period with good hatching efficiency when good conditions return. *Artemia* cysts can be used as direct food for larvae with the help of the 'cyst decapsulation' technique (to remove the thick outer shell of the cyst) to facilitate ingestion and allow for its digestion (Van Stappen, 1996). Despite the economic and biological significance of *Artemia* in aquaculture, a regular supply of *Artemia* nauplii is stressful to many hatcheries, primarily due to the high cost of producing microalgal paste, which is the preferred *Artemia* diet. Therefore, studies are needed to develop low-cost but nutritious diets as a relevant priority for aquaculture.

*Artemia* is an obligatory nonselective particle filter feeder (Dhont & Sorgeloos, 2002). Feed reported to date include bioflocs (i.e., bacteria, protozoa, organic detritus, and other particulate matter of biological origin) and green water (i.e., nonfilamentous algae cells) (Eryalcin, 2018; Van Stappen, 1996). Newly hatched *Artemia* do not feed because their mouth and anus are not fully developed. A few hours after hatching, *Artemia* molts into the second larval stage (instar II), which can filter small food particles from 1 to 50 $\mu$m, including bacteria. The nauplii grow and progress through 15 molts before reaching adulthood in about 8 days. Due to filter-feeding behaviour, *Artemia* is useful in salt works as they feed on algae particles, hence eliminating salt contamination, leading to first-class salt quality production (Faruque et al., 2010).

Biofloc condition yields huge densities of algal cells (dinoflagellates and diatoms), fungi, ciliates, flagellates, rotifers, copepods, nematodes, metazoans, and heterotrophic bacteria cells such as *Pseudomonas and Aeromonas* family that conglomerate together under optimum carbon/nitrogen (C/N) ratio and adequate aeration (Avnimelech, 2003; Manan et al., 2016). Bacteria, and by extension the microbial loop is known to play important roles as recycling pathways for C and N in food webs (Azam et al., 1983). Bioflocs have good nutrition including fatty acids, which are essentially transferred to *Artemia* when feeding on them. Usually, an optimum C/N ratio ensures the immobilization of inorganic nitrogen into huge bacterial proteins (biomass) and restores good water quality by removing toxic ammonia (Azim & Little, 2008). This study explores the feasibility of promoting the simultaneous growth of highly dense bacterial biomass and *Artemia* in the same culture facilities, where *Artemia* feeds directly on the microbial flora.

Green water technology encourages the growth of high *Tetraselmis* sp. algal densities (Toi et al., 2013). The algal density is promoted by chicken manure, which contains nutrients, especially nitrogen which is useful for algal growth. Chicken manure also contains different hormones such as 17$\beta$-estradiol and testosterone that are important for culturing zooplankton communities (Finlay-Moore et al., 2000; Hakk et al., 2005; Shemesh & Shore, 1994). These hormones influence the population growth, mixis induction, and body size of zooplankton species (Preston et al., 2000; Yang & Snell, 2010). Therefore, a hypothesis about whether chicken manure extract (CME) and biofloc technology (BFT) can influence the growth, reproductive traits, and fatty acid composition of *Artemia* was formulated and tested in this study.

## 2 | MATERIALS AND METHODS

### 2.1 | Source of artemia cysts

*Artemia* was first inoculated on the Kenyan coast, around Malindi area about 4 decades ago courtesy of the Kenya-Belgium Project (KBP), implemented by the Kenya Marine and Fisheries Research Institute (KMFRI) and University of Ghent, Belgium (Rasowo & Radull, 1986). The *Artemia* species have since then adapted to local conditions and developed permanent populations in Malindi area (Ogello et al., 2013, 2014). *Artemia* is currently being used by salt companies and the local community to purify salt. For this study, *Artemia* cysts were sourced from Khadzuhoni salt pans in Malindi.

### 2.2 | Experimental design

To establish sufficient *Artemia* nauplii for the experiment, *Artemia* hatching was achieved by the use of natural seawater in a 5-L glass bottle, to which 10 g of cysts was added, and placed on a rotor at four cycles $min^{-1}$ for 24 h under constant illumination using fluorescent lamps (2000 lux). Freshly hatched instar I nauplii were harvested for the experiment. About 1000 nauplii were batch cultured in each 1-L glass jar containing 500 ml of natural seawater (i.e., stocking density of 2 nauplii/ml). *Artemia* was cultured under laboratory conditions using three treatments, that is, green water technology (GWT), biofloc technology (BFT), and normal algal conditions (control-C). The composition of each treatment is described in Table 1.

**TABLE 1** Composition of each treatment used for the experiment

| Treatment | Composition | Quantity applied | References |
|---|---|---|---|
| Control (C) | *Tetraselmis* spp. | $7.0 \times 10^6$ cells $ml^{-1}$ daily | Hagiwara et al. (1994) |
| GWT | CME + C | 0.5 g $l^{-1}$ of CME | Ogello et al. (2015) |
| BFT | GWT + Molasses | depending on TAN | |

Abbreviations: BFT, biofloc technology; CME, chicken manure extract; GWT, green water technology; TAN, total ammonium nitrogen.

## 2.3 | Management of the treatments

Molasses were regularly added to BFT based on total ammonium nitrogen (TAN) concentration to maintain a C/N ratio of 10 from the 5th day of culture. During the first 2 days after stocking, *Artemia* was fed with fresh *Tetraselmis tetrathele* algae, for conditioning, after which each treatment was applied. *Artemia* cultures were maintained at 28.0 ± 1°C using an electrically heated water bath with constant aeration and light (2000 lux) for 30 days. An inoculum of *T. tetrathele* was obtained from the laboratory of zoology at Maseno University, Kenya, and the algae were cultured following a standard protocol (Coutteau, 1996), where a continuous culture method was used. Fertilized seawater was continuously pumped into the *T. tetrathele* growth chamber at a steady, predetermined rate and the excess culture was simultaneously washed out, allowing maintenance of the culture close to the maximum growth rate.

## 2.4 | Survival studies

Upon hatching, 50 instar I nauplii were transferred into different cylindrical shaped vials (45 ml) filled with natural seawater and were fed with three replicated treatments described in Table 1 throughout the tests. No artificial aeration was used, but to ensure sufficient oxygen levels, one-third of water renewal was done upon survival determination every 2 days. Final survival was determined at the end of the experiment by counting the number of live *Artemia* in each culture flask.

Survival activity index (SAI) experiments were performed to determine *Artemia* nauplii survivability during starvation. Here, cysts harvested from each treatment were hatched in the laboratory using the protocol and conditions described above. About 20 *Artemia* nauplii from each treatment were placed in a 200 ml beaker containing 100 ml of natural seawater at 28 ± 1°C in total darkness, without aeration and feeding. Dead *Artemia* nauplii were counted and removed every 24 h until total larval mortality was reached. Triplicate observations were used to calculate the SAI using the equation of Shimma and Tsujigado (1981):

$$\mathrm{SAI} = \frac{1}{N}\sum_{i=1}^{K}(N - hi) \times i,$$

where $N$ is the total number of examined larvae, $hi$ is the cumulated mortality by $i$-th day, and $K$ is the number of days elapsed until all larvae died due to starvation.

## 2.5 | Growth studies

*Artemia* growth data was measured in terms of length, which was started from the 5th day after stocking and then repeated every 2 days. A sample of 10 *Artemia* was taken randomly from each jar. *Artemia* was first treated with Lugol's solution before taking total body length measurements from the tip of the head to the end of the telson through a dissecting microscope. As the animals grew, a dissecting microscope was used for measurement with the aid of a digitizer (KD 4300, Graphtec corp., Japan).

## 2.6 | Population density

*Artemia* population density was monitored daily from the batch cultures described above. Each day, three-1 ml of culture water was sampled from each glass jar, fixed with Lugol's, and then *Artemia* numbers were counted under a stereo microscope at ×25 magnification to estimate population density. In the first exponential growth phase, partial harvesting was done by replacing 50% of the culture medium with new seawater and diet. *Artemia* was left to regenerate without additional inoculation.

## 2.7 | Specific growth rate

The specific growth rate (SGR) was calculated as follows: $r = [\ln N_t - \ln N_o]/t$, where, $N_o$ is the initial population density, $N_t$ is the population density after the time ($t$), and $t$ is the time in days (i.e., day 16). To determine the stability of the cultures, the coefficient of variation (CV) of the mean SGR was computed as standard deviation (SD)/mean SGR. The CV was calculated on day 16 for each diet.

## 2.8 | Microbiology

The *Artemia* culture medium was separately screened for bacteria under sterile conditions. The medium was serially diluted up to $10^{-4}$ after filtering out *Artemia* and residues using the 10 $\mu$m net. For platting, the Zobell marine agar (Difco™ 2216; Becton, Dickinson & Co. France) was used. Then, 0.1 ml of the respective diluted samples was inoculated over the surface of the solidified agar in triplicates. The plates were incubated upside down (to avoid vapour condensation

on the agar) at 32°C for 48 h. The bacterial colony-forming units (CFU) were calculated as CFU $ml^{-1}$ = (No. of colonies × dilution factor)/inoculated volume (ml).

## 2.9 | Reproductive tests

The mass batch culture was carried out until the pre-adult stage was reached in a similar set-up as indicated above. When riding couples were seen, it was noted as the time in days when sexual maturity was attained at the earliest in those animals. Immediately when couples were observed, 10 couples from each treatment were removed from the mass culture bottle and every single couple was transferred into a 50 ml Falcon tube for closer examinations. The *Artemia* couples were exposed to the same conditions as in the culture bottle. The number of cysts and/or nauplii released per female was counted and recorded daily. The data collected were used to determine the pre-reproductive and reproductive period, total offspring per female, offspring per female per day, the total number of broods per female, oviparous and ovoviviparous brood size, and brood interval in days.

## 2.10 | Fatty acid analysis

To obtain substantial biomass for fatty acid analysis, mass cultures were up-scaled into 20-L glass aquariums using similar treatments for 60 days. *Artemia* biomass was regularly harvested from each treatment and weight was measured. A total of 300 g of freshly harvested *Artemia* biomass from each treatment was preserved under frozen conditions and transported to Polucon Company Ltd., Mombasa, Kenya, for fatty acid analysis. Fatty acid methyl esters (FAME) were prepared using the AM/C/107 method in which the sample methanolysis was prepared at 100°C for 2 h after the addition of 2 M hydrogen chloride methanol. FAME was extracted by n-hexane. Gas chromatography analysis was performed using a GC-2010 (Shimadzu Scientific Instruments, Inc.) equipped with an HR-SS-10 column (Shinwa Chemical Industries, Ltd.). The column temperature was regulated at 150–220°C. Individual fatty acids were quantified utilizing the response factor of 15:0 fatty acids as the internal standard (Folch et al., 1957). The analysis essentially followed protocols by Laakso on fatty acid analysis. The fatty Acid values were expressed as g/100 g artemia dry weight.

## 2.11 | Data analysis

The data were analyzed using R statistical software (version 3.2.1 of the R Foundation for Statistical Computing Platform © 2015). The Bartlett test was used to test for the homogeneity of variances. One-way analysis of variance (ANOVA) was used to test the effects of each diet on water quality, *Artemia* population densities, growth (length), and reproductive traits. Where significant differences were detected, Tukey's HSD Post hoc test was performed to locate the differences at $p < 0.05$. Only one sample was analyzed per treatment.

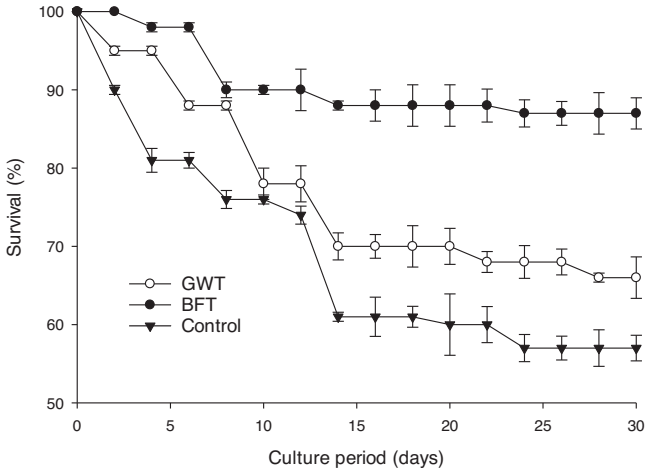


**FIGURE 1** The proportion of surviving *Artemia* cultured in green water technology (GWT), biofloc technology (BFT), and Control using normal seawater for 30 days. Values are presented as means ± SE ($n = 3$).

# 3 | RESULTS

## 3.1 | Survival

As shown in Figure 1, *Artemia* survived in all treatments for 30 days. There was a significant effect of treatments on *Artemia* survival ($p < 0.05$), with *Artemia* cultured in BFT having a higher survival (87.1 ± 3.2%) than those cultured in GWT (66.2 ± 2.9%) and Control (57.9 ± 1.3%). There was a significantly higher survival activity index (SAI) of *Artemia* nauplii hatched from cysts harvested in BFT (8.23 ± 0.33 days) than in GWT (6.34 ± 0.11 days) and Control cultures (5.24 ± 0.42).

## 3.2 | Growth

There was a significant effect of the treatments on *Artemia* size ($p < 0.05$), with *Artemia* cultured in the BFT treatment having a higher length than those grown in GWT and control from day 10 to 30 (Figure 2). The final mean lengths were 12.1 ± 0.2, 10.1 ± 0.4, and 7.5 ± 0.1 mm for *Artemia* cultured in the BFT, GWT, and Control cultures, respectively.

## 3.3 | Population density

There was a significant effect of treatments on *Artemia* population density ($p < 0.05$). The population density of *Artemia* cultured using BFT was higher than those cultured in GWT and Control on days 14, 16

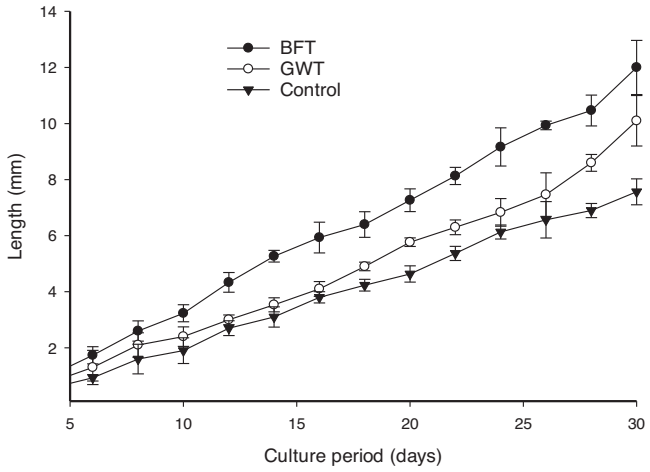


**FIGURE 2** Growth curves of *Artemia* cultured in green water technology (GWT), biofloc technology (BFT), and Control using normal seawater for 30 days. Values are presented as means ± SE ($n = 3$).

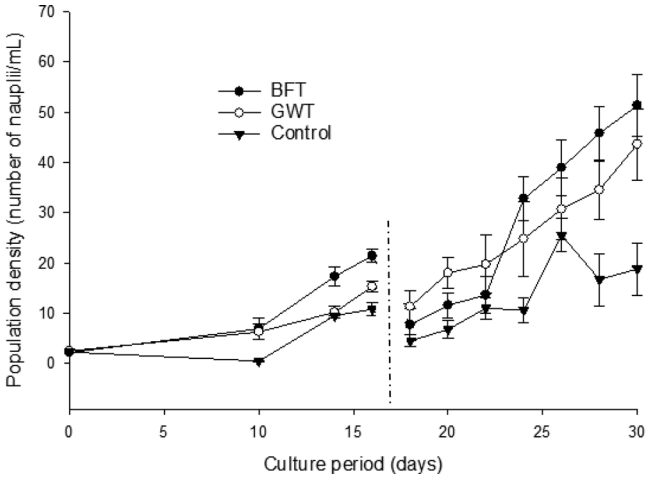


**FIGURE 3** Population density (individuals/ml) of *Artemia* cultured in green water technology (GWT), biofloc technology (BFT), and Control using normal seawater for 30 days. Partial harvesting was done on day 17 by replacing half of the culture medium with new media as indicated by the dotted lines. The values represent mean ± SD. Different letters each day denote significant differences at $p < 0.05$, $n = 3$.

and 20 (Figure 3). At the end of the experiment, the population densities were 45.4 ± 2.3, 26.3 ± 3.1 and 15.1 ± 4.2 nauplii/ml in BFT, GWT and Control cultures, respectively. The *Artemia*-specific growth rates (SGR) were 0.16, 0.12, and 0.11 individuals /day in BFT, GWT, and control, respectively. Meanwhile, the CV was 5.04%, 10.81%, and 10.86% in BFT, GWT, and Control cultures, respectively.

## 3.4 | Biomass

There was a significant effect of treatments on *Artemia* biomass ($p < 0.05$), with *Artemia* cultured in the BFT having higher cumulative biomass than those cultured in GWT and Control. At the end of day 60, the biomass (wet weight) was 722.45 ± 39.33, 577.98 ± 67.34, and 474.23 ± 62.43 g in BFT, GWT, and Control cultures, respectively. *Artemia* productivity was estimated at 36.2, 28.5, and 23.7 g/L after 60 days in BFT, GWT, and control cultures, respectively.

## 3.5 | Microbiology

The colonies are shown in Figure 4. The treatments significantly affected the CFUs ($ml^{-1}$) (one-way ANOVA, $F = 24.11$, $p = 0.01$), where the *Artemia* cultured in the BFT had higher CFU ($1.38 \times 10^7$ CFU/ml) than those cultured in GWT ($1.11 \times 10^7$ CFU/ml) and control-rotifers ($7.3 \times 10^6$ CFU/ml) (Figure 5).

## 3.6 | Reproductive traits

There was a significant effect of treatments on the *Artemia* reproductive traits as shown in Table 2. *Artemia* cultured in the BFT unit had a shorter pre-reproductive period (15.6 ± 0.6 days) and a longer reproductive period (20.9 ± 2.6 days) compared to those cultured in GWT and control (Table 2). The BFT-cultured *Artemia* had higher total broods per female per day (4.3 ± 0.3) than GWT (3.5 ± 0.2) and Control (2.5 ± 0.3). There was a higher ovoviviparous reproduction in BFT (4.4 ± 0.5 broods/female) than in GWT (2.5 ± 0.3) and control (2.4 ± 0.4). However, the control treatment showed higher oviparous reproduction (1.4 ± 0.2 broods/female) than BFT (0.5 ± 0.1) and GWT (0.7 ± 0.2). The total number of offspring per female was significantly higher in the *Artemia* cultured in the BFT unit (73.5 ± 6.1) than those cultured in GWT (66.2 ± 7.9) and Control (42.6 ± 12.1). There was a significantly shorter brood interval in BFT (1.6 ± 0.3 days) than in GWT (1.7 ± 0.3) and control (2.5 ± 0.4).

## 3.7 | Fatty acid composition

The fatty acid composition of *Artemia* was significantly higher in the BFT system than in the GWT and control culture systems. The compositions of myristic C14:0n-8, palmitic C16:0n-3, heptadecanoic C17:1n-7, oleic, C18:1n-9, and linoleic C18:2 n-6 and linolenic C18:3 n-3 acids were significantly higher in the BFT culture system than in the GWT and control systems. The compositions of heptadecanoic acid C17:0n-7, palmitoleic acid C16:1n-7, stearic acid C18:0 n-4, and arachidic acid C20:0 n-6, on the other hand, did not differ significantly between the experimental culture systems as shown in Table 3

The absence of the eicosapentaenoic acid 20:5 n-3 (EPA) which can constitute up to 4%–5% of fatty acids in artemia (based on) can be explained by the lack of capacity at our lab to conduct the analysis. Further explanation is given in Section 4. Only one sample was analyzed per fatty acid due to a lack of capacity at our laboratory to process the samples, resulting in a high cost of sample analysis by the private company.

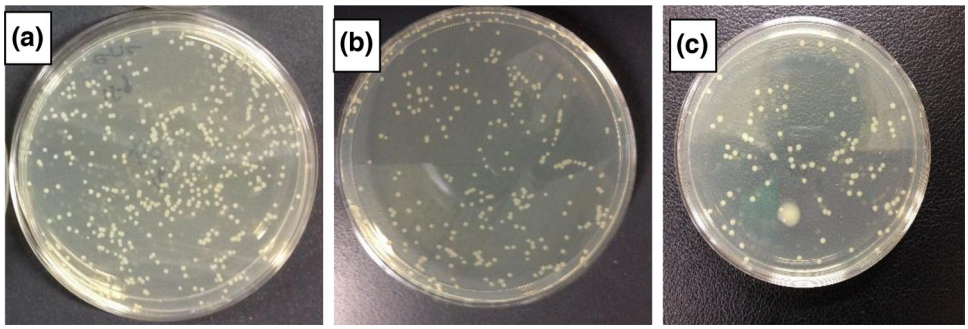


**FIGURE 4** Growth cultures of bacteria isolated from (a) biofloc technology (BFT), (b) green water technology (GWT), and (c) control culture mediums

**TABLE 2** Reproductive traits of *Artemia* cultured using the biofloc technology (BFT), green water technology (GWT), and the control treatments in normal seawater for 30 days

| Reproductive traits | GWT | BFT | Control |
|---|---|---|---|
| Female pre-reproductive period (days) | $21.8 \pm 0.7^{b}$ | $15.6 \pm 0.6^{a}$ | $22.0 \pm 0.5^{b}$ |
| Female reproductive period (days) | $16.4 \pm 2.3^{b}$ | $20.9 \pm 2.6^{a}$ | $15.8 \pm 1.9^{b}$ |
| Total broods per female per day | $3.5 \pm 0.2^{b}$ | $4.3 \pm 0.3^{a}$ | $2.5 \pm 0.3^{c}$ |
| Ovoviviparous broods per female | $2.5 \pm 0.3^{b}$ | $4.4 \pm 0.5^{a}$ | $2.4 \pm 0.4^{b}$ |
| Oviparous broods per female | $0.7 \pm 0.2^{c}$ | $0.5 \pm 0.1^{a}$ | $1.4 \pm 0.2^{b}$ |
| Total offspring per female | $66.2 \pm 7.9^{a}$ | $73.5 \pm 6.1^{a}$ | $42.6 \pm 12.1^{b}$ |
| Brood interval (days) | $1.7 \pm 0.3^{b}$ | $1.6 \pm 0.3^{b}$ | $2.5 \pm 0.4^{a}$ |

*Note*: Values are presented as means ± SE ($n = 3$).

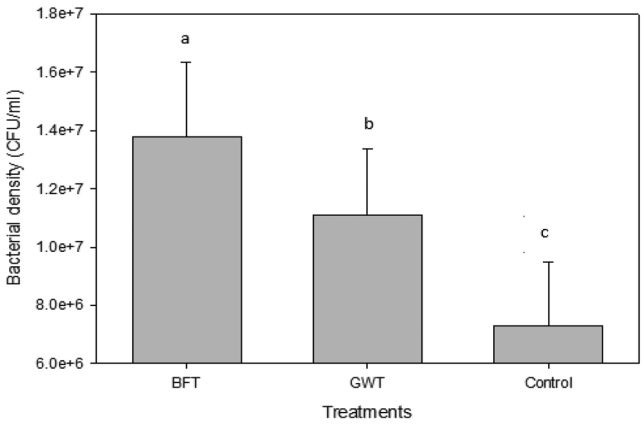


**FIGURE 5** The bacterial colony-forming units (CFU/ml) in the culture medium from biofloc technology (BFT), green water technology (GWT), and control at $\times 10^4$ dilution rate. Different letters indicate significant differences between treatments, $n = 3$.

**TABLE 3** Fatty acid composition (%) in total lipids of dried *Artemia* biomass cultured using the biofloc technology (BFT), green water technology (GWT), and the control treatments in normal seawater for 30 days

| Fatty Acid | Formulation | GWT | BFT | Control |
|---|---|---|---|---|
| | | Units (g/100 g) | | |
| Myristic acid | C14:0 n-8 | 22.64 | 38.82 | 9.51 |
| Pentadecanoic acid | C15:0 n-7 | 0.22 | 0.54 | 0.12 |
| Palmitic acid | C16:0 n-3 | 9.43 | 14.31 | 6.47 |
| Palmitoleic acid | C16:1n-7 | <0.01 | <0.01 | <0.01 |
| Heptadecanoic acid | C17:0 n-7 | 0.02 | 0.03 | 0.01 |
| Stearic acid | C18:0 n-4 | <0.01 | <0.01 | <0.01 |
| Oleic acid | C18:1n-9 | 21.98 | 30.66 | 6.31 |
| Linoleic acid | C18:2 n-6 | 7.11 | 9.38 | 4.33 |
| Linolenic acid | C18:3 n-3 | 4.23 | 5.24 | 2.22 |
| Arachidic acid | C20:0 n-6 | 2.01 | 2.13 | 1.32 |

## 4 | DISCUSSION

With the current growth of the aquaculture sector expected to continue in the next few decades, *Artemia* availability will play a critical role in the improvement of aquaculture, especially the culture of fish and shrimps with high economic returns, such as sea bass (*Lates calcarifer*), sea bream (*Sparus aurata*), *Penaeus monodon*, *Penaeus indicus*, and *Penaeus japonicus* (Dhont & Van Stappen, 2003; Lim et al., 2003). This study has demonstrated that integrated BFT and GWT condition influences the growth and fatty acid composition of *Artemia* under limited algal conditions. In normal situations, *Artemia* is fed on algal pastes,

which have become expensive and fragile to culture in many local fish and shrimp hatcheries. The results of this study indicate that *Artemia* can be cultured using waste-generated bacteria as food for *Artemia*.

There was a higher survival and SAI in the BFT than GWT and control (Figure 1). Higher SAI is an indication that *Artemia* cultured under BFT can withstand longer periods of starvation, which is important for ensuring maximum larval fish feeding in hatcheries. This could be attributed to the presence of huge bacterial biomass in the BFT compared to other treatments. The higher bacterial biomass in the BFT was facilitated by molasses. Being a filter feeder, *Artemia* can feed on a variety of non-algal particles including micro-algae, microbial organisms, protozoa as well as small detritus particles (Fernández, 2001). In addition, the cultures contained minimal algal particles, which presented supplemental food resources for *Artemia* in the BFT treatment. Food shortage causes interactions in *Artemia* populations through competition for the available nutrients, which results in stress, slow growth rate, and eventually mortalities are experienced (Hoa et al., 2021).

The higher *Artemia* growth and population density in BFT could have been a synergistic effect of CME and bacterial biomass within the BFT cultures. It is plausible that CME and molasses in the biofloc system stimulated the growth of algal communities and microorganisms (protozoa and bacteria), respectively, which are direct supplemental feed for *Artemia*. CME is an excellent substrate for bacterial and algal growth associated with organic matter, which promotes the development of a dense population of protozoa (Schroeder, 1980). Further, the addition of a carbon source promotes the generation of sufficient microbial proteins and energy which is very essential for active swimming thus increasing hunting chances for food and obtaining the appropriate oxygen for their metabolism.

*Artemia* cultured in the BFT unit showed higher regeneration and culture stability (coefficient of variation of 5.04%) after partial harvesting (Figure 3). With more stable cultures, several *Artemia* generations can be cultured in ponds while conducting partial harvesting at production peaks (every 2 weeks), without need for fresh inoculations. Higher *Artemia* culture stability is usually a factor of limited pathogenic microorganisms in cultures that would cause culture crush. BFT is known to stabilize cultures by maintaining good water quality and provision of biosecurity advantages (Nootong et al., 2011). However, further studies should be done to determine the ability of BFT-grown *Artemia* to resist pathogenic bacteria. The BFT provided a rich food environment (i.e., bioflocs and algae) that stimulated a faster growth and regeneration of *Artemia* after partial harvesting. This is an important strategy to ensure constant mass production of *Artemia* biomass especially when optimal conditions for oviviparous reproduction are maintained. However, other studies have reported that increased proliferation of bioflocs raises water viscosity, which may reduce *Artemia* swimming activities, food utilization, and higher energy consumption for locomotion, resulting in retarded growth (Toi et al., 2013).

The population density of *Artemia* was higher in the BFT due to ovoviviparous reproduction loop. The high *Artemia* biomass recorded in the BFT cultures shows that the application of BFT is a more efficient technology for *Artemia* biomass production compared to the GWT and control. The high biomass in BFT could be a result of the massive growth of a highly nutritious bacterial community stimulated by the added molasses. Toi et al. (2013) confirmed that an adequate amount of bacteria could be a good source of feed for *Artemia*. This study, therefore, demonstrates that the stimulation of microbial protein through the addition of molasses in BFT could enhance the production of *Artemia* biomass, especially in cases of food shortage. This is because molasses stimulates microbial development in the culture water, thus providing supplemental feed to *Artemia*, leading to high *Artemia* biomass in BFT. In addition, most microbial organisms contain carotenoids mostly found in Haloferax (Chen et al., 2006), glycoprotein, and exopolysaccharides (López-Ortega et al., 2020), which nutritionally might modulate the health of *Artemia*, leading to better performance in terms of biomass and length.

The results of the present study reveal that the BFT treatment improved the reproductive performance of *Artemia*. The provision of mixed diets with microalgae, bacteria, or protozoa microorganisms promotes growth and high rates of mature females obtaining better nauplii due to their nutritional quality. High food abundance in a culture unit is also important because survival and the number of nauplii produced depend on it (Wayne & Maciej, 2001). Earlier research has confirmed that *Artemia* can efficiently feed on bioflocs produced from animal wastes (Sui et al., 2013; Verma et al., 2011). The utilization of CME to stimulate the production of feed for *Artemia* is an attractive alternative due to its low cost. Further, the feeding of Artemia with bioflocs may be a promising method of directly providing probiotics to the digestive tract of the target aqua species (Suzer et al., 2008). In addition, microbial organisms are considered a good source of activating enzymes that enhance the activation of food items (Toi et al., 2014). This, therefore, justifies the significantly higher total number of offspring per female in the *Artemia* cultured in the BFT unit than those cultured in GWT and Control.

According to Browne et al. (1984), *Artemia* has reproductive modes including ovoviviparity and oviparity, and the occurrence of each depends on various environmental conditions, such as variation in temperature, salinity, stress, and the availability of food. Oviparity mostly happens when the *Artemia* have challenges with their living conditions or when the living condition is out of their stand (N. V. Hoa et al., 2007). In the present study, the BFT treatment facilitated the ovoviviparous mode of reproduction, while the control treatment favoured oviparous reproduction. A shorter brood interval in BFT than in control was reported in this study, which could be due to the presence of nutritional microbial feed leading to the fast growth of the *Artemia*, thus reaching sexual maturity within a shorter period compared to *Artemia* reared in GWT and control. The nutrition of the female (fatty acids, ascorbic acids, vitamins, amino acids, and overall food availability), physiological factors (mobilization of energy reserves, and hormones), and ecological factors (environmental conditions such as food availability, water quality, and salinity) are some of the factors that have been confirmed to trigger reproduction (Rocha, 2008). Some of the factors listed above could therefore have contributed to the higher reproductive performance recorded in the BFT treatment in comparison to GWT and control. Bioflocs also not only have a considerable amount of protein but also have highly unsaturated fatty acids (HUFA) and vitamin

C which enhances gonadal maturation and high quantity and quality offspring (Crab et al., 2012; Ekasari et al., 2010). This could be the reason for the higher fecundity recorded in the BFT compared to the GWT and control.

During *Artemia* culture, *Artemia* wastes and organic matter accumulate in the culture medium polluting the environment. It is therefore essential to improve the culture environment to promote higher growth performance of the *Artemia* and enhance the production of biomass as a whole. There are various methods of controlling nitrogenous wastes; however, the stimulation of microbial organisms to grow has been reported to be the best (Avnimelech, 2012). BFT has in earlier studies been reported to improve water quality, biosecurity, and yield of the cultured species through the conversion of nitrogenous compounds to microbial protein, stabilizing the bacterial organisms and lowering the cost of production (De Schryver et al., 2008; Nootong et al., 2011). The addition of molasses as a carbon source which is easily absorbed by heterotrophic bacteria quickly lowers TAN levels through the formation of stable bacterial flocculations.

The presence of HUFA is an important factor that is used in the determination of the nutritional value and marketing of *Artemia* for utilization in the aquaculture industry (Mana et al., 2014). The high levels of mysteric, palmic, linoleic, and oleic fatty acids of the *Artemia* reported in this study confirm earlier studies that have reported the same fatty acids to be the major fatty acids found in bioflocs (Anand et al., 2014; Crab et al., 2010; Emerenciano et al., 2012; Magondu, 2012; Toledo et al., 2014). The absence of the eicosapentaenoic acid 20:5 n-3 (EPA), which can constitute up to 4%–5% of fatty acids in artemia (based on Cabanes, 1992; Tizol-Correa et al., 2006), can be explained by the lack of capacity at our lab to conduct the analysis. The samples were therefore analyzed at a private company (Palucon Company Ltd. in Mombasa, Kenya). The company for some reason failed to test for EPA. The authors acknowledge, however, that this fatty acid could not have been missed in the *Artemia* had the samples been analyzed for it.

In aquaculture, these fatty acids are very essential in cell synthesis, immune function, reproduction, and endocrine function (Emerenciano et al., 2012; Glencross, 2009). In this context, bioflocs appear to supplement such important fatty acids through natural production which are unavailable in the control and GWT. The fatty acids improve certain effectors of the immune system, including respiratory bursts and coagulation (Aguilar et al., 2012). This influences the resistance of the cultured organisms to infectious organisms including bacteria, viruses, fungi, and parasites (Trichet, 2010), leading to high survival rates. Through bioaccumulation processes, *Artemia* can accumulate fatty acids in their gut, making it a perfect diet for the larviculture of fish and shrimp (Castro et al., 2009; Dhont & Sorgeloos, 2002; Zadehmohseni et al., 2020). However, the BFT developed in this study promises a major leap towards developing a cheap and nutritionally rich diet for *Artemia*. Based on the food value of the bioflocs, the BFT culture fluid can be considered a cheaper enrichment medium for low-quality larval fish foods, for example, bakers' yeast, and may reduce the need for the expensive enrichment emulsions (Ogello et al., 2018). Other homemade emulsion products have been produced using fish oil and egg yolk as cheap ways of enriching *Artemia* cultures, but these products are prone to oxidation and have a short shelf life that limits their application in aquaculture. The bacteria-held polyunsaturated fatty acids (PUFAs) in BFT are more protected against oxidation and provide a variety of other natural nutrients that meet the species-specific nutritional requirements of the *Artemia*, and the larval fish that feed on them.

## 5 | CONCLUSION

Integrated BFT conditions provide an enhanced environment for better *Artemia* survival, growth, reproductive traits, and amino acid composition. This technology promises a major leap towards developing a cheap and nutritionally rich diet for the mass production of *Artemia* biomass compared to algal pastes, which are costlier and more fragile to produce. The BFT condition ensures stable *Artemia* cultures, producing up to 20–40 nauplii/ml biweekly. This presents self-sustaining biotechnology for the production of high density of nutrient-rich *Artemia* without microalgae for aquaculture. BFT reduces the female pre-reproductive period, extends the reproductive period, and promotes ovivivipariy of *Artemia*; hence, it is suitable for mass production of *Artemia* biomass.

### AUTHOR CONTRIBUTIONS

Conceptualization, funding acquisition, resources, and writing—original draft: Erick O. Ogello. Writing—review and editing: Nicholas O. Outa. Data curation: Bramwel O. Mukaburu. Methodology and writing—review and editing: Mavindu Muthoka.

### ACKNOWLEDGEMENTS

This study was funded by the WIOMSA-MASMA project grant No. MASMA/OP/2020/02 awarded to Erick O. Ogello of Maseno University, Kenya. The authors acknowledge the support from laboratory technicians (Mr. James Ojienga, Mr. Peter Adellah) for their assistance in the laboratory work.

### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

### DATA AVAILABILITY STATEMENT

We certify that the data used in this article were collected from this study and can only be availed through the request and permission of the third-party authors.

### ETHICS STATEMENT

We certify that this is our original scientific research work, and it has not been submitted orpublished anywhere. The authors are responsible for all the content in the manuscript.

### ORCID

*Erick O. Ogello* https://orcid.org/0000-0001-9250-7869
*Nicholas O. Outa* https://orcid.org/0000-0002-4085-0398

*Mavindu Muthoka* https://orcid.org/0000-0002-3512-8130

## PEER REVIEW

The peer review history for this article is available at: https://publons.com/publon/10.1002/aff2.89

## REFERENCES

Aguilar, V., Racotta, I.S., Goytortua, E., Wille, M., Sorgeloos, P., Civera, R. & Palacios, E. (2012) The influence of dietarydietary arachidonic acid on the immune response and performance of Pacific whiteleg shrimp, *Litopenaeus vannamei*, at high density. *Aquaculture Nutrition*, 18, 258–271

Anand, P.S.S., Kohli, M.P.S., Kumar, S., Sundaray, J.K., Roy, S.D. & Venkateshwarlu, G. (2014) Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon* effect of dietary supplementation of bio floc on growth performance and digestive enzyme activities in *Penaeus monodon*. *Aquaculture*, 418–419, 108–115. https://doi.org/10.1016/j.aquaculture.2013.09.051

Avnimelech, Y. (2012) *Biofloc technology—a practical guide book* (2nd ed.). Baton Rouge, Louisiana: World Aquaculture Society.

Avnimelech, Y. (2003) Control of microbial activity in aquaculture systems: active suspension ponds. *World Aquaculture*, 34(4), 19–21.

Azam, F., Fenchel, T., Field, J., Gray, J., Meyer-Reid, L. & Thingstad, F. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series*, 10, 257–263.

Azim, M.E. & Little, D.C. (2008) The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 283(1), 29–35. https://doi.org/10.1016/j.aquaculture.2008.06.036

Browne, R.A., Sallee, S.E., Grosch, D.S., Segreti, W.O. & Purser, S.M. (1984) Partitioning genetic and environmental components of reproduction and lifespan in *Artemia*. *Ecology*, 65(3), 949–960. https://doi.org/10.2307/1938067

Castro, T., Sandoval, H., Castro, A., Castro, J., Castro, G., De Lara, R. & Hernandez, L.H. (2009) Monthly assessments of proteins, fatty acids and amino acids in Artemia franciscana cultivated in "Las Salinas de Hidalgo", state of San Luis Potosí, Mexico. *Aquaculture Nutrition*, 15(2), 123–128. https://doi.org/10.1111/J.1365-2095.2008.00574.X

Chen, C.W., Don, T.M. & Yen, H.F. (2006) Enzymatic extruded starch source for the production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by *Haloferax mediterranei*. *Process Biochemistry*, 41(11), 2289–2296. https://doi.org/10.1016/j.procbio.2006.05.026

Coutteau, P. (1996) Microalgae. In: Lavens, P. & P. Sorgeloos (Eds.) *Manual on the production and use of live food for aquaculture*. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 7–48

Crab, R., Chielens, B., Wille, M., Bossier, P. & Verstraete, W. (2010) The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture Research*, 41, 559–567

Crab, R., Defoirdt, T., Bossier, P. & Verstraete, W. (2012) Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture*, 356–357, 351–356. https://doi.org/10.1016/J.AQUACULTURE.2012.04.046

De Schryver, P., Crab, R., Defoirdt, T., Boon, N. & Verstraete, W. (2008) The basics of bio-flocs technology: the added value for aquaculture. *Aquaculture*, 277(3–4), 125–137. https://doi.org/10.1016/j.aquaculture.2008.02.019

Dhont, J. & Van Stappen, G. (2003) Biology, tank production and nutritional value of Artemia. In: Mc Evoy, L. & Støttrup, J. (Edts.), *Live feeds in marine aquaculture*. Blackwell Science, Oxford, pp. 65–121.

Dhont, J. & Sorgeloos, P. (2002) Applications of Artemia. In: Abatzopoulos, T.J., Beardmore, J.A., Clegg, J.S. & Sorgeloos, P. (Eds.) *Artemia: Basic and* applied biology. Springer, Dordrecht, pp. 251–277. https://doi.org/10.1007/978-94-017-0791-6_6

Eryalcin, K. (2018) Effects of different commercial feeds and enrichments on biochemical composition and fatty acid profile of rotifer (*Brachionus Plicatilis*, Muller 1786) and *Artemia Franciscana*. *Turkish Journal of Fisheries and Aquatic Sciences*, 18, 81–90.

Ekasari, J., Crab, R. & Verstraete, W. (2010) Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. *HAYATI Journal of Biosciences*, 17(3), 125–130. https://doi.org/10.4308/HJB.17.3.125

Emerenciano, M., Cuzon, G., Ar?evalo, M., Miquelajauregui, M.M. & Gaxiola, G. (2012) Effect of short-term fresh food supplementation on reproductive performance, biochemical composition, and fatty acid profile of *Litopenaeus vannamei* (Boone) reared under biofloc conditions. *Aquaculture International*, 21, 987–1007.

Faruque, M., Kawser, A. & Quddus, M.M.A. (2010) Characterization and functional role of heat shock protein 26 in *Artemia*. *World Journal of Zoology*, 5(4), 282–294.

Fernández, R.G. (2001) Artemia bioencapsulation I. Effect of particle sizes on the filtering behavior of *Artemia* Franciscana. *Journal of Crustacean Biology*, 21(2), 435–442. https://doi.org/10.1163/20021975-99990144

Finlay-Moore, O., Hartel, P.G. & Cabrera, M.L. (2000) 17B-estradiol and testosterone in soil runoff from grasslands amended with broiler litter. *Journal of Environmental Quality*, 29, 1604–1611.

Folch, J., Lees, M. & Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497–509

Glencross, D.B. (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in Aquaculture*, 1, 71–124.

Hagiwara, A., Hamada, K., Hori, S., & Hirayama, K., (1994). Increased sexual reproduction in Brachionus plicatilis (Rotifera) with the addition of bacteria and rotifer extracts. *J. Exp. Mar. Biol. Ecol*, 181, 1–8.

Hakk, H., Millner, P. & Larsen, G. (2005) Decrease in water-soluble 17$\beta$-estradiol and testosterone in composted poultry manure with time. *Journal of Environmental Quality*, 34, 943–950.

Hoa, N.V., Van, N.T., Anh, N.T., Ngan, P.T., Toi, H. & Le, T.H. (2007) *Study on Artemia culture and its application in aquaculture*. Can Tho University, Agriculture Publishing House.

Hoa, N.V., Giang, H.T., Van, N.T.H., Quynh, H.T. & Toi, H.T. (2021) The combined effect of stocking density and C/N ratio on growth performance and biomass production of Artemia reared in a biofloc system under laboratory culture conditions. *Egyptian Journal of Aquatic Biology and Fisheries*, 25(4), 929–938. https://doi.org/10.21608/EJABF.2021.196216

Lim, C.L., Philippe, D. & Sorgeloos, P. (2003) Recent developments in the application of live feeds in the freshwater ornamental fish culture. 3rd Fish and Shellfish Larviculture Symposium, 227(1–4), 319–331, 10 November.

López-Ortega, M.A., Rodríguez-Hernández, A.I., Camacho-Ruíz, R.M., Córdova, J., López-Cuellar, M.R., Chavarría-Hernández, N. & González-García, Y. (2020) Physicochemical characterization and emulsifying properties of a novel exopolysaccharide produced by haloarchaeon *Haloferax mucosum*. *International Journal of Biological Macromolecules*, 142, 152–162. https://doi.org/10.1016/J.IJBIOMAC.2019.09.087

Magondu, E.W. (2012) Aerobic, anaerobic and anoxic bioflocs from tilapia proximate composition. Nutritional properties and attractiveness as fish feed. MSc Thesis. Wageningen University. Wageningen, Netherlands.

Mana, P.N., Vahabzadeh, H., Seidgar, M., Hafezieh, M. & Pourali, H.R. (2014) Proximate composition and fatty acids profiles of *Artemia* cysts, and nauplii from different geographical regions of Iran. *Iranian Journal of Fisheries Sciences*, 13(3), 761–775.

Manan, H., Moh, J.H.Z., Kasan, N.A., Suratman, S. & Ikhwanuddin, M. (2016) Identification of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp, *Penaeus vannamei*, culture in closed hatchery system. *Applied Water Science*, 7, 2437–2446. https://doi.org/10.1007/s13201-016-0421-4

Nootong, K., Pavasant, P. & Powtongsook, S. (2011) Effects of organic carbon addition in controlling inorganic nitrogen concentrations in a biofloc system. *Journal of the World Aquaculture Society*, 42(3), 339–346. https://doi.org/10.1111/J.1749-7345.2011.00472.X

Ogello, E.O., Nyonje, B. & Stappen, G.V. (2013) Genetic differentiation of Artemia franciscana (Kellogg, 1906) in Kenyan coastal saltworks. *International Journal of Advanced Research*, 2, 1154–1164.

Ogello, E.O., Kembenya, E., Githukia, C.M., M, B.N. & Munguti, J.M. (2014) The occurrence of the brine shrimp, *Artemia franciscana* (Kellog 1906) in Kenya and the potential economic impacts among Kenyan coastal communities. *International Journal of Fisheries and Aquatic Studies*, 1(5), 151–156. https://www.researchgate.net/publication/262723852_

Ogello, E.O., Wullur, S., Sakakura, Y. & Hagiwara, A. (2018) Composting fish-wastes as low-cost and stable diet for culturing *Brachionus rotundiformis* Tschugunoff (Rotifera): influence on water quality and microbiota. *Aquaculture*, 486, 232–239. https://doi.org/10.1016/J.AQUACULTURE.2017.12.026

Ogello, E.O. & Hagiwara, A. (2015) Effectsof chicken manure extract on the population growth, mixis induction and bodysize of the freshwater rotifer Brachionus angularis Gosse 1851. *Asian FisheriesScience*, 28, 174–185.

Persoone, G. & Sorgeloos, P. (1980) General aspects of the ecology and biogeography of *Artemia*. *Ecology Culturing Use in Aquaculture*, 3, 3–24.

Preston, B.L., Snell, T.W., Robertson, T.L. & Dingmann, B.J. (2000) Use of freshwater rotifer *Brachionus calyciflorus* in screening assay for potential endocrine disruptors. *Environmental Toxicology and Chemistry*, 19, 2923–2928.

Rasowo, J. & Radull, J. (1986) Inoculation of the brine shrimp *Artemia salina* in Kenya: expected impact on aquaculture development. In: Huisman E.A. (ed.) *Aquaculture research in the Africa Region. Proc. Afr. seminar on aquaculture. International Foundation for Science (IFS) Stockholm Sweden*. Pudoc Wageningen, Netherlands, pp. 54–59.

Rocha, M.J. (2008) *Fish reproduction*. Science Publisher. California, USA.

Schroeder, G.L. (1980) Fish farming in manure-loaded ponds, In: Pullin, R.S.V. & Shehadeh, Z.H. (Eds.) *Integrated agriculture-aquaculture*. ICLARM; Conference Proceedings 4, p. 73–86. https://fortuneofafrica.com/ug/fish-farming/

Shemesh, M. & Shore, L.S. (1994) Effects of hormones in the environment on reproduction in cattle. In: Fields, M.J. & Sand, R.S. (Eds.) *Factors affecting net calf crops*. CRC Press Boca Raton, Florida, pp. 287–298.

Shimma, H. & Tsujigado, A. (1981) Some biochemical quality of bred scorpaenoid fish, *Sebastiscus marmoratus*, and activities of their larvae. *Bulletin of National Research Institute of Aquaculture*, 2, 11–20.

Sorgeloos, P. (1980) Life history of the brine shrimp *Artemia*. In: Persoone, G., Sorgeloos, P., Roels, O. & Jaspers, E. (Eds..), *The brine shrimp Artemia, Vol.1. Morphology, genetics, Radiobiology, toxicology*. Universa Press, Wetteren, Belgium, pp 19–22.

Sorgeloos, P., Dhont, P. & Candreva, P. (2001) Use of brine shrimp *Artemia* spp in marine fish larviculture. *Aquaculture*, 200(2001), 147–159.

Sorgeloos, P. & Tackaert, W. (1991) Roles and Potentials of *Artemia* in Coastal Salt works. Proceedings International Symposium on Biotechnology of salt ponds, Tang Gu, PR China, Sept 18–21, 1990, Chang, L. (Ed.), Salt Research Institute, Tanggu. pp 71–76.

Sui, L.Y., Wang, J., Nguyen, V.H., Sorgeloos, P., Bossier, P. & Van Stappen, G. (2013) Increased carbon and nitrogen supplementation in *Artemia* culture ponds results in higher cyst yields. *Aquaculture International*, 21(6), 1343–1354. https://doi.org/10.1007/s10499-013-9637-6

Suzer, C., Çoba, D., Kamaci, H.O., Saka, S., Firat, K., Otgucuoğlu, Ö. & Küçüksaric, H. (2008) *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L) larvae: effects on performance and digestive enzyme activities. *Aquaculture*, 280, 140–145.

Toi, H.T., Boeckx, P., Sorgeloos, P., Bossier, P. & Van Stappen, G. (2013) Bacteria contribute to Artemia nutrition in algae-limited conditions: a laboratory study. *Aquaculture*, 388–391(1), 1–7. https://doi.org/10.1016/J.AQUACULTURE.2013.01.005

Toi, H.T., Boeckx, P., Sorgeloos, P., Bossier, P. & Van Stappen, G. (2014) Co-feeding of microalgae and bacteria may result in increased N assimilation in *Artemia* as compared to mono-diets, as demonstrated by a 15N isotope uptake laboratory study. *Aquaculture*, 422–423, 109–114. https://doi.org/10.1016/J.AQUACULTURE.2013.12.005

Toledo, M., Silva, B.C., Vieira, F.N., Pedreira, J.L., Seiffert, M. & Quadros, W. (2014) Effects of different dietary lipid levels and fatty acids profile in the culture of white shrimp *Litopenaeus vannamei* (Boone) in biofloc technology: water quality, biofloc composition, growth and health. *Aquaculture Research*, 47, 1841–1851. https://doi.org/10.1111/are.12642

Trichet, V.V. (2010) Nutrition and immunity: an update. *Aquaculture Research*, 41, 356–372.

Turcihan, G., Turgay, E., Yardimci, R.E. & Eryalcin, K.M. (2021) The effect of feeding with different microalgae on survival, growth, and fatty acid composition of *Artemia franciscana* matanauplii and on predominant bacterial species of the rearing water. *Aquaculture International*, 29(5), 2223–2241. https://doi.org/10.1007/s10499-021-00745-y

Van Stappen, G., Sui, L., Hoa, V.N., Tamtin, M., Nyonje, B., Rocha, R.D., Sorgeloos, P. & Gajardo, G. (2020) Review on integrated production of the brine shrimp Artemia in solar salt ponds. *Reviews in Aquaculture*, 12, 1054–1071.

Van Stappen, G. (1996) *Introduction, biology and ecology of Artemia and use of cysts*. In: Lavens, P. & Sorgeloos, P. (Eds.) In: Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper 361. Rome, pp. 101–170.

Verma, A.K., Raghukumar, C. & Naik, C.G. (2011) A novel hybrid technology for remediation of molasses-based raw effluents. *Bioresource Technology*, 102(3), 2411–2418. https://doi.org/10.1016/J.BIORTECH.2010.10.112

Wayne, A.W. & Maciej, Z.G. (2001) Limnological control of brine shrimp population dynamics and cyst production in the Great Salt Lake, Utah. *Hydrobiologia*, 466, 119–132.

Yang, J. & Snell, T.W. (2010) Effects of progesterone, testosterone, and estrogen on sexual reproduction of the rotifer *Brachionus calyciflorus*. *International Review of Hydrobiology*, 95, 441–449.

Zadehmohseni, B., Zakeri, M., Yavari, V. & Haghi, M. (2020) Effects of different salinities on amino acid profile in *Artemia franciscana*. *Aquaculture Research*, 5, 1–9.

**How to cite this article:** Ogello, E.O., Outa, N.O., Mukaburu, B.O. & Muthoka, M. (2023) Biofloc and green water condition improves reproductive traits and fatty acid composition of *Artemia franciscana* cultured under limited algal conditions. *Aquaculture, Fish and Fisheries*, 3, 61–70. https://doi.org/10.1002/aff2.89