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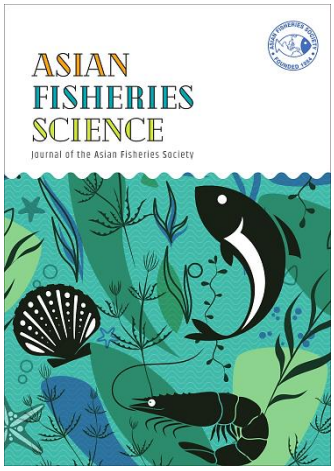

Periphyton Technology Enhances Growth Performance and Delays Prolific Breeding of Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758), Juveniles

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Abstract

This study evaluated the effect of periphyton technology (PPT) on the growth performance and breeding schedule of *Oreochromis niloticus* (Linnaeus, 1758) juveniles. Six ponds, each measuring 81 m^2 were used for the study. The ponds were applied with agricultural lime at a rate of 4 $g.m^{-2}$, and fertilised using chicken manure to facilitate primary productivity. The PPT ponds were fitted with two-metre-long eucalyptus poles of 5 cm diameter placed at 50 cm intervals with the regular addition of molasses as a carbon source. Tilapia juveniles were stocked at a density of 3 $fish.m^{-2}$ in all ponds and fed on a commercial diet of 20 % crude protein (CP) twice daily at 3 % body weight. Fish were sampled weekly for growth and survival data and bi-weekly for fecundity estimates. The PPT-ponds registered significantly higher survival rate (97.50 ± 0.35 %), mean weight (150.69 ± 0.99 g), specific growth rate (SGR) (2.75 ± 0.01), and feed conversion ratio (FCR) (1.29 ± 0.01), than the control ponds, which registered survival (91.15 ± 0.88 %), mean weight (99.23 ± 0.96 g), SGR (2.29 ± 0.00), and FCR (1.58 ± 0.01). There was significantly higher fecundity in the PPT-ponds (2.28 ± 0.09 $g.fish^{-1}$) than control (1.74 ± 0.06 $g.fish^{-1}$), with prolific spawning starting 4 weeks earlier in the control ponds than in the PPT-ponds. This study demonstrated the potential of PPT for enhancing tilapia growth while delaying prolific breeding behaviour. Further studies should explore PPT in replacing synthetic hormones for sex-reversal of tilapia fry in hatcheries.

Keywords: biofilm, bioflocs, survival activity index (SAI), regenerative aquaculture, single cell proteins

Introduction

Aquatic food consumption has become a common debate in the global food system analysis and blue revolution debate due to many health benefits attributed to fish proteins (Naylor et al., 2021). To maintain the current human aquatic food demand, there is a need to expand and intensify the aquaculture sector in the next five decades (Henriksson et al., 2018). The intensification of aquaculture production requires implementing of sustainability-driven policies framed within the blue bio-economy powered by nature-based solutions and blue-biotechnology (Becker and Calado, 2021). The information on regenerative fish production technologies that ensure faster and higher production volumes with minimal input cost should be made locally available to the scientific community, policymakers and fish farmers.

In developing countries, the aquaculture sector faces several challenges, including limited land, water, seed and feeds, which have led to production stagnation (Munguti et al., 2014; Ogello and Munguti, 2016). Fish feed is an important factor in the aquaculture industry that now accounts for 50–70 % of total production cost (Munguti et al., 2021). Most of the fish feeds currently used by small scale fishermen are inefficient as only about 30 % of nutrient inputs are converted into harvestable products (Gross et al., 2000; Krishnani et al., 2019). There is a need for regenerative or bio-circular aquaculture technologies that can generate natural food materials within the system to sustain the aquaculture units, with limited

supplementary feeding, which can be done with low-quality and cheap diets.

Culture systems such as periphyton technology (PPT) are considered sustainable and regenerative systems due to the generation of highly nutritious natural food materials within the system. Periphyton technology is a concept derived from traditional fishing methods in West Africa and was recently improved in Bangladesh and India by introducing substrates in the polyculture of Indian carps (Beveridge et al., 1998; Yadav et al., 2017). The principle of PPT involves using underwater substrates e.g., bamboo sticks on which a community of bacteria, fungi, protozoa, snails, chironomids, mayflies, oligochaetes and crustaceans colonise (Azim et al., 2002; Abwao et al., 2014). The substrates provide sufficient surface area for the growth of periphyton communities, which are direct food sources for the cultured fish (Miao et al., 2021), and facilitate good water quality (Beveridge et al., 1998; Li et al., 2019a). The growth of the microbial community is enhanced by maintaining a higher carbon-nitrogen ratio (C/N) of about 10–15 (Ogello et al., 2018; Guo et al., 2020), which is best achieved through the addition of carbohydrate (molasses) or low-protein supplemental diet (Avnimelech, 1999; Tinh et al., 2021). The periphyton community also convert total ammonia nitrogen (TAN) generated in the system to bacterial biomass by heterotrophic bacteria (Aisyah et al., 2021) or converts it into nitrite in the presence of oxygen and later into nitrate by nitrifying bacteria allowing other microorganisms to form less harmful molecules (Abakari et al., 2020).

Oreochromis niloticus is the main species cultured in ponds because of its rapid growth and ability to graze at lower food chains. However, tilapia has a problem of early sexual maturation and prolific breeding that leads to stunted growth (Fashina-Bombata and Megbowon, 2012). Hormonal (i.e., 17α-methyl testosterone) sex-reversal technique is a popular method currently used in many hatcheries to prevent prolific breeding in ponds (El-Greisy and El-Gamal, 2012; Megbowon and Mojekwu, 2014; Jensi et al., 2016). However, the potential effect of hormonal sex-reversal in the ambient environment has yielded mixed results, hence the need for other techniques. Periphyton is nutritionally attractive as it contains 27 % crude protein, 18 % lipid and 52 % carbohydrates which are better than most commercial feeds used for the growth of *O. niloticus*, thus all nutrient components make their representation on the periphytic microhabitat (Ogello et al., 2014). Since tilapia depends on natural foods in the form of plankton, periphyton (Abdel-Fattah, 2020) and microbial floc (Mugwanya et al., 2021), this study hypothesises that PPT will enhance the growth performance of cultured fish due to the high quality of nutrition. Quality nutrition is a factor of high fecundity and quality of tilapia eggs. However, the adoption of PPT in local aquaculture initiatives has not been adequately explored, and many technical and biological functions are not yet clear for fish farmers. This study aims at evaluating the effects of PPT on the growth performance and breeding behaviour of *O. niloticus* cultured in earthen ponds.

Materials and Methods

Study site and design

The study was conducted at Kenya Marine and Fisheries Research Institute (KMFRI) Sang'oro in Kisumu County, Kenya. Six earthen ponds each measuring 81 m^2, with an average depth of 1 m were used for the study. The ponds were limed using quicklime i.e., calcium carbonate ($CaCO_3$) based on soil pH at 4 $g.m^{-2}$ according to Boyd et al. (2002), and left for 1 week. Two-metre-long eucalyptus poles (a local tree mainly used for fencing and building material) with a mean diameter of 5 cm were used as substrates in the periphyton culture units. The poles were inserted into the pond bottom vertically at an interval spacing of 50 cm, according to Hoque et al. (2018) (Fig. 1). The ponds were filled with water and fertilised using chicken manure to facilitate primary productivity at a rate of 50 $g.m^{-2}$. The manure was enclosed in gunny bags and placed at the inlet area of each pond. The carbon-nitrogen (C:N) ratio of the pond water in the periphyton pond was adjusted to 15:1 using molasses as the main carbon source according to Ogello et al. (2018), and the ponds left for 10 days to allow sufficient production and colonisation of periphyton on the substrates before stocking. This study had two treatments i.e., PPT- and control-ponds which were triplicated. The control ponds had similar treatment and management practices as PPT except for the substrates and addition of molasses.

Fig. 1. Constructed periphyton ponds in KMFRI Sangoro Aquaculture Center, in which *Oreochromis niloticus* were cultured. The inserted eucalyptus poles were used as substrates upon which periphyton community attached for fish to graze.

Experimental fish

Mixed-sex of pure *O. niloticus* post-fingerlings were obtained from KMFRI's hatchery. At the fry stage, the fish were conditioned using live food resources (i.e.,

rotifers, copepods and cladocerans) produced using a live food dispenser operated by biofloc system for 2 weeks (Fig. 2). The fish were introduced to co-feeding program involving live food and a commercial diet (20 % crude protein) until they attained post-fingerling stage of about 12 g. The fish were conditioned, initial measurements taken and then stoked in the ponds. All the ponds were stocked at a density of 3 fish.m^{-2}.

Pond management

There was no water exchange in the ponds to preserve the periphyton community. However, water depth was monitored daily and water was only added to compensate for losses from evaporation. The fish were fed twice daily at 3 % body weight daily ration (at 9.00 am and 3.00 pm) with a commercial diet 20 % CP for 3 months. The 20 % CP diet was obtained from KMFRI's fish feed processing plant. Carbon source (molasses) was periodically added into the PPT ponds to facilitate the proliferation of microorganisms for periphyton development, as shown in Figure 3. Selected physicochemical water parameters i.e., temperature (°C), pH, conductivity (µS.cm^{-1}), and dissolved oxygen (mg.L^{-1}) and total dissolved solids were monitored weekly using a multiparameter water quality meter (WQC-24, DKK-TOA Corporation, Japan). Indophenol blue spectrophotometric method was employed for determination of total ammonia nitrogen (TAN) using spectrophotometer (Varian Cary® 50 UV-Vis Spectrophotometer, Varian, Inc., USA) (Li et al., 2019b).

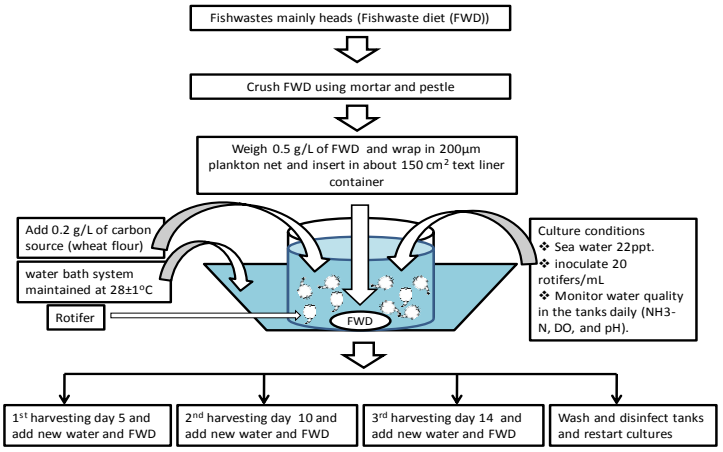

Fig. 2. Live-food dispenser used for production of live food resources using environmental wastes as substrates (Adopted from Ogello et al., 2020).

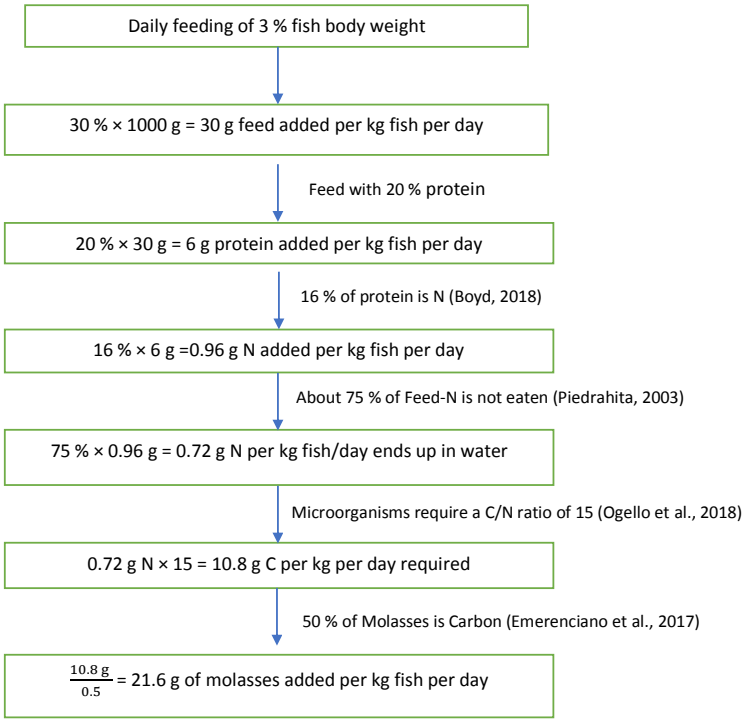

Fig. 3. Schematic calculation of the amount of molasses required to remove total ammonia nitrogen in the periphyton technology ponds.

Data collection

Sampling

Fish was sampled weekly for growth and survival measurements and bi-weekly to determine fecundity. For the growth experiment, 30 fish were randomly sampled from each pond (using seine nets) at each sampling time for length and weight determination before returning them into the pond. For fecundity, 10 fish were sampled and the mouth was checked for eggs. The eggs were weighed and recorded. Prolific breeding behaviour was determined by the time taken to first spawning.

Survival activity index (SAI)

The hatching rate and survival activity index (SAI) experiments were performed to determine the quality of the tilapia eggs and the fish larval survivability during starvation, respectively. Here, 20 eggs were placed in 500 mL beaker containing 300 mL of water at 25 °C in total darkness, without aeration and feeding. After every 24 h, dead larvae were counted and removed until total larval mortality was reached. The treatments were triplicated and observations were used to calculate the hatching rate and SAI. The percentage of eggs that hatched normally was calculated after 24 h using the formula of Pertiwi et al. (2017);

$$\text{Percentage of eggs that hatched normally} = \frac{\text{Normal hatched larvae}}{\text{Total number of eggs}} \times 100$$

SAI was calculated using the equation of Matsuo et al. (2006);

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

where N = total number of examined larvae, hi = cumulated mortality by i-th day and K = number of days elapsed until all larvae died due to starvation. Morphological characteristics of the larval fish samples i.e., total and standard length, eye diameter, body depth and head length (Fig. 4), were measured using a microscopic measurement system that included a stereomicroscope (Discover V8, Zeiss, Germany) equipped with a digital camera (AxioCam HSm, Carl Zeiss, Germany) and image-analysis software (AxioVision 4.8).

Growth and survival analysis

The following growth parameters were measured using standard protocols (Khanjani et al., 2017);

$$\text{Mean weight gain (MWG)} = \text{Final mean weight } (W_1) - \text{Initial mean weight } (W_0)$$

$$\text{Specific growth rate (SGR)} = \frac{[\ln(\text{final mean weight}) - \ln(\text{initial mean weight})]}{\text{Time in days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed}}{\text{Body weight gain}}$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish at the end of the experiment}}{\text{Number of fish at the beginning of the experiment}} \times 100$$

Determination of plankton communities

Water samples from the ponds were collected during the last week of the study period. In each pond, the water was collected in three spots at a depth of 25 cm and passed through 50 µ mesh plankton net. The water samples were then put on a measuring cylinder and made up to 100 mL. One millilitre of water sample was then pooled from the measuring cylinder and placed on Sedgewick-Rafter (S-R) chamber. The plankton communities were observed in a light microscope under ×400 magnification. Five fields of the chamber were randomly selected, counted and used to estimate the total number of the plankton communities. The abundance was expressed as the number of individuals 5 mL^{-1}. The zooplankton species were identified using the keys constructed by Jeje (1988), Fernando (1994), while phytoplanktons were identified using the keys compiled by Withford and Schumacher (1973) and Edmonson (1959). Estimation of the total number of planktons was done using the formula;

$$N = (n \times v)/V$$

where, N = Total number of plankton cells per litre of original water; n = Average number of plankton counted in 5 fields; v = Volume of the final concentrate of the sample (mL); V = Volume of total pond water filtered (L) (Patkar et al., 2021).

Proximate analysis of periphyton

Ten poles from each pond were selected randomly on the last week of the experiment. The biofilm attached to the poles (periphyton) was then scraped carefully using a scalpel and emptied on a clean sampling bottle (Fig. 5). The periphyton was then put on aluminium foil and sun-dried before sending to Kenya Agricultural & Livestock Research Organization (KALRO) for proximate analysis.

Statistical analysis

Statistical analysis was performed using R software (version 3.2.1 of the R Foundation for Statistical Computing Platform © 2015 R Foundation). Histograms were plotted to test data normality, while the Bartlett test for homogeneity of variances was used to test for the equality of variance. The effects of PPT on growth performance and breeding of tilapia were analysed with independent t-test. Data was

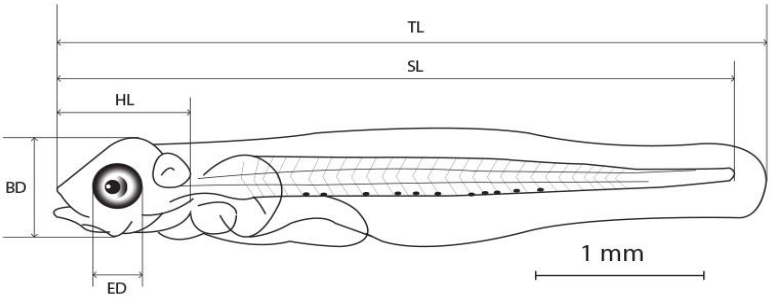

Fig. 4. The five morphological characteristics to estimate larval growth and development of *Oreochromis niloticus*. TL: Total length; SL: Standard length; HL: Head length; ED: Eye diameter; BD: Body length.

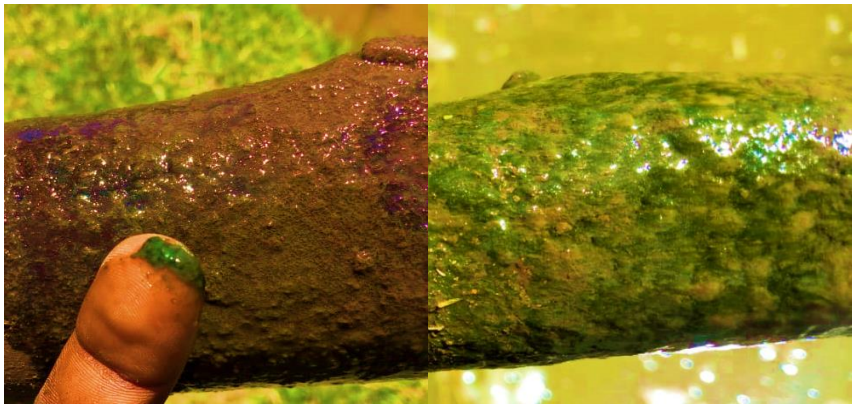

Fig. 5. Periphyton biofilms attached on the eucalyptus substrates from the ponds treated with biofloc materials.

presented using graphs in SPSS version 20. Values were expressed as mean ± standard error of mean (SEM), and the significant differences accepted at $P < 0.05$.

Results

There were significant differences in the final weight, final length, weight gain, survival, SGR and FCR, with fish cultured under PPT condition recording superior performance than those cultured in control. The growth performance indicators of *O. niloticus* cultured under PPT and control treatments are summarised in Table 1. Water quality parameters are presented in Table 2.

Table 1. *Oreochromis niloticus* growth performance indicators comparing the control and periphyton ponds.

Variable	Control	Periphyton
Initial weight (g)	12.35 ± 0.15^a	12.35 ± 0.15^a
Initial length (cm)	8.44 ± 0.09^a	8.44 ± 0.09^a
Final weight (g)	99.23 ± 0.96^a	150.69 ± 0.99^b
Final length (cm)	17.58 ± 0.06^a	21.08 ± 0.21^b
Weight gain (g)	86.88 ± 0.81^a	138.34 ± 0.84^b
Survival (%)	91.15 ± 0.88^a	97.50 ± 0.35^b
Specific growth rate (SGR)	2.29 ± 0.00^a	2.75 ± 0.01^b
Feed conversion ratio (FCR)	1.58 ± 0.01^a	1.29 ± 0.01^b

The values represent mean ± SE. Common superscript in the same row shows that the measurements were not statistically different as determined by unpaired t-test; Different superscripts indicate significant differences at $P < 0.05$; a < b; n = 30.

There was no significant difference in mean body weight within the first 3 weeks of the experiment between PPT- and control fish. However, significant difference was eminent from week 4 to 13, with fish cultured in PPT condition having higher mean body weight than those cultured in control conditions (Fig. 6).

Periphyton technology significantly affected fish fecundity estimates ($t_{71.29} = 5.025$, $P = 0.001$), with the PPT-ponds showing higher mean weight of eggs (2.28 ± 0.09) than the control-ponds (1.74 ± 0.06). There was a significant difference in fish spawning schedule, with fish in control ponds experiencing early spawning (from fourth week) while fish in the PPT started spawning at the eighth week (Fig. 7). There was a statistically significant increase ($P < 0.05$) in the mean weight of eggs across the weeks in periphyton.

Morphological characteristics of larval fish samples

Morphological features of the larval fish samples are shown in Table 3. There were no significant differences in eye diameter, head length and body depth between the fish cultured in PPT and those cultured under control. However, there were significant differences in hatching rate, total and standard length (Table 3). Fish larvae from PPT-ponds survived up to the eighth day of starvation with a survival activity index (SAI) of 8.94 ± 1.7 while all the

Table 2. Water quality parameters in control and periphyton technology ponds.

Variable	Control	Periphyton	Ideal ranges
Temperature (°C)	27.43 ± 0.45[a]	27.76 ± 0.41[a]	20–35 (Ngugi et al., 2007)
pH	8.30 ± 0.19[a]	8.10 ± 0.13[a]	6.5–9.0 (Deswati et al., 2020)
Total dissolved solids (mg.L^{-1})	204.54 ± 0.96[a]	97.15 ± 6.21[b]	<500 (FME, 2001)
Conductivity (µS.cm^{-1})	431.92 ± 16.05[a]	233.54 ± 15.51[b]	100–500 (Russel et al., 2011)
Dissolved oxygen (mg.L^{-1})	6.71 ± 0.18[a]	6.49 ± 0.13[a]	>4.0 (Emerenciano et al., 2017)
Nitrite (mg.L^{-1})	0.07 ± 0.01[a]	0.02 ± 0.01[b]	<1.0 (Emerenciano et al., 2017)
Ammonia (mg.L^{-1})	0.21 ± 0.03[a]	0.06 ± 0.01[b]	<1.0 (Emerenciano et al., 2017)
Ammonium (mg.L^{-1})	0.20 ± 0.04[a]	0.02 ± 0.01[b]	<1.0 (Emerenciano et al., 2017)

Different letters in the same row represent statistically significant differences between the ponds as determined by independent t-test ($P < 0.05$).

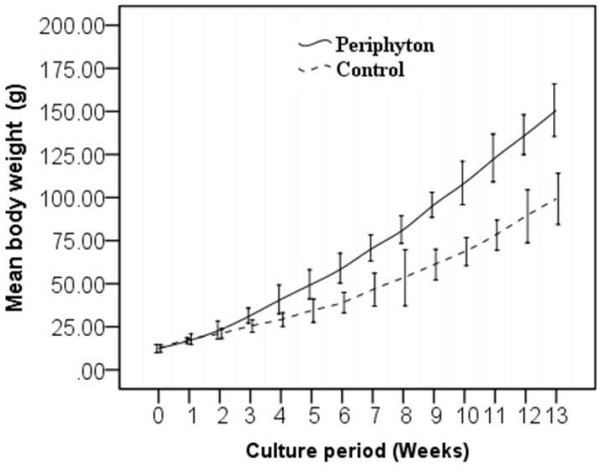

Fig. 6. Growth curves showing mean body weight (g) of *Oreochromis niloticus* cultured in periphyton technology and control-ponds over the experimental period.

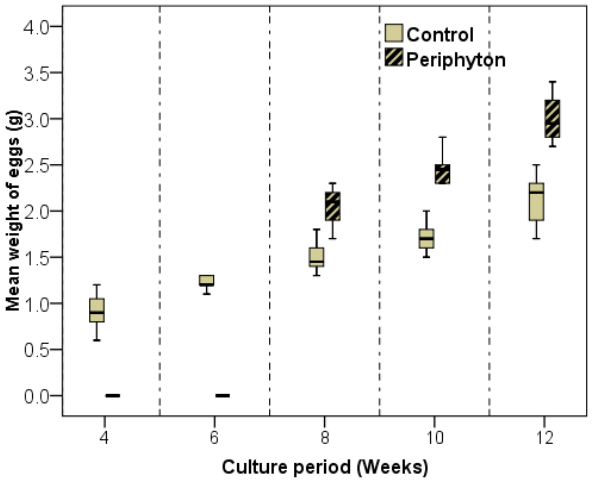

Fig. 7. Mean weight of eggs of *Oreochromis niloticus* sampled weekly from periphyton and control ponds.

Table 3. Morphological features of the *Oreochromis niloticus* larval samples comparing the control- and periphyton ponds.

Variable	Control	Periphyton	P-value
Hatching rate (%)	88.75 ± 0.45[a]	96.40 ± 1.98[b]	0.000
Total length (mm)	5.74 ± 0.06[a]	6.05 ± 0.01[b]	0.000
Standard length (mm)	4.76 ± 0.04[a]	5.33 ± 0.07[b]	0.001
Body depth (mm)	0.71 ± 0.09[a]	0.71 ± 0.10[a]	0.159
Head length (mm)	0.91 ± 0.07[a]	0.92 ± 0.09[a]	0.088
Eye diameter (mm)	0.29 ± 0.03[a]	0.30 ± 0.04[a]	0.232

The values represent mean ± SE. Common superscript in the same row shows that the measurements were not statistically different as determined by unpaired t-test; Different superscripts indicate significant differences at P < 0.05; a > b; n = 25.

control fish died by the fifth day, indicating SAI of 5.29 ± 2.1.

Plankton abundance

Phytoplankton

There was no statistical difference ($P > 0.05$) in phytoplankton mean abundance between control (1061.60 ± 145.46) and the periphyton treatment (689.67 ± 127.71). Cyanobacteria dominated the control ponds while diatoms dominated the PPT ponds (Fig. 8).

Zooplankton

There was significantly higher ($P < 0.05$) mean zooplankton abundance in the PPT-ponds than in control ponds. The PPT- and control-ponds registered zooplankton mean abundance of 2771.83 ± 313.11 and 262.67 ± 16.78, respectively. Rotifera was the most abundant, while Cladocera was the least abundant in control and PPT treatment (Fig. 9).

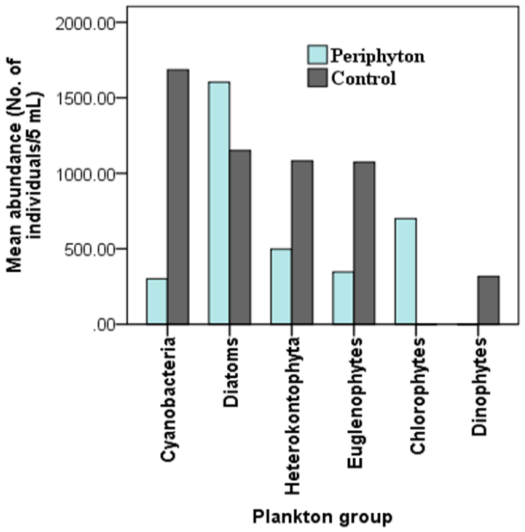

Fig 8. Mean abundance of phytoplankton in both control and periphyton technology-ponds

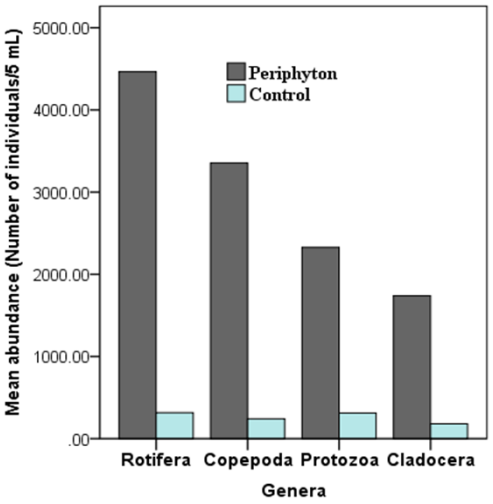

Fig 9. Mean abundance of zooplankton in the control and periphyton technology treatment.

Periphyton proximate composition

The proximate composition of periphyton is shown in Table 4.

Table 4. Proximate analysis of periphyton biofilm scrapped from the eucalyptus poles in the periphyton ponds.

Composition	Amount (%)
Crude protein	27.26 ± 1.32
Fibre	0.55 ± 0.01
Fat	3.59 ± 0.13
Ash	39.50 ± 0.78
Moisture	12.53 ± 0.28

Discussion

This study has demonstrated the potential of PPT in improving growth performance while delaying prolific breeding behaviour in Nile tilapia, *O. niloticus*. The survival rate of *O. niloticus* in the periphyton treatment (97.50 ± 0.35 %) was significantly higher than the control-ponds (91.15 ± 0.88 %). This could be attributed to quality nutrition from feeding on the high-density plankton community in the PPT ponds. Consumption of zooplanktons and benthos by young tilapia positively influence fish survival rate (Mamaril, 2001). The microbial communities attached to the substrates have been reported to increase fish survival by providing antibiotic substances, probiotics, and vitamins (Azim et al., 2005). Other studies have shown that immunostimulant substances such as lipopolysaccharide, peptidoglycan and beta-glucan in the wall of heterotrophic bacteria increases fish survival (Walker et al., 2020). The substrates used in the PPT ponds may have acted as shelters for the fish hence preventing predation.

Periphyton technology ponds exhibited a positive fish growth parameter index compared to fish cultured under control conditions (Table 1). Fish were regularly browsing on the large periphyton biomass attached to the eucalyptus substrates in the periphyton ponds. This study hypothesised that the attached nutritious periphyton was a highly preferred fish feed that might have contributed to the high tilapia growth rate compared to control ponds. After being grazed, the periphyton mass would quickly regenerate, probably due to the symbiotic relationship between periphyton and phytoplankton. This finding corroborates the findings of other studies, which reported periphyton-based aquaculture as a robust source of high-quality natural feed (Saikia and Das, 2009), and has been used to improve aquaculture production (Keshavanath et al., 2004). Other studies have reported improved growth performance of cultured fish in natural heterotrophic biota (Adineh et al., 2019; Sarkar et al., 2021). Furthermore, other studies have confirmed that microbial organisms are rich sources of dietary stimulants (Wang et al., 2015), bioactive compounds (Ju et al., 2008; Xu and Pan, 2013), growth and immune boosters (Supamattaya et al., 2005; Kuhn et al., 2010) that along with supplemental feed, provide a complete diet for cultured aquatic species (Khanjani and Sharifinia, 2020).

The high growth performance is also attributed to the presence of periphytic algae in the PPT-ponds. According to Dempster et al. (1995), filter-feeding on only planktonic algae may not meet the energy requirements of *O. niloticus*. Being an herbivorous fish, *O. niloticus* species require larger sized food items such as algal-based detritus and benthic algae to augment phytoplankton consumption (Dempster et al., 1993). Becker (2007) reported that microalgae are composed of high nutritional value, superior to conventional plant protein sources. According to Huchette et al. (2000), algae attached to periphyton

substrates and the coexisting zooplankton and bacterial biomass are directly utilised by tilapia species leading to higher growth than in ponds without substrates. The slow growth rates in the control ponds could possibly be due to the dominance of cyanobacteria which have low nutritional value and fish are unlikely to graze on them, possibly due to their toxicity effects (Llario et al. 2018). Malbrouck and Kestemont (2006), from their laboratory and field experiments, reported that aqueous and cell-bound cyanotoxins in fish diets are not good for their behaviour, morphology and physiology.

Studies by Mirzakhani et al. (2019) and Khanjani et al. (2020) reported improved FCR in tilapia cultured in C/N-controlled systems. Sakr et al. (2015) reported a better fish growth performance in treatments with low protein levels in the presence of periphyton substrates than the treatments fed on high protein level in absence of periphyton substrates. Huchette and Beveridge (2003) concluded that the periphyton could only be used to partially replace the pelleted feeds to reduce the production costs. According to Garcia et al. (2016), the introduction of substrates in cages improved the performance of *O. niloticus* using a low-proteinous diet (20 %). Avnimelech (2007) reported that the bacterial biomass stimulated by adding carbon source provides 50 % of the protein requirement of tilapia. Therefore, the high growth rate recorded in the periphyton treatment shows efficient utilisation of the natural microbial feed.

The PPT ponds generally exhibited good water quality, as demonstrated by low ammonia, ammonium, nitrite, and dissolved solids (Table 2). The addition of carbon source in the PPT promoted the proliferation of single cell proteins (SCP), which practically extracts ammonia molecules in the water to make body mass, hence the good water quality in PPT ponds. Good water quality is necessary for faster fish growth rate and survival.

Fecundity is considered as the reproductive capacity of a fish species in each spawning season (Izquierdo et al., 2001; Orlando et al., 2017). Low egg quality is one of the major problems that have restricted aquaculture expansion. Fecundity of *O. niloticus* can vary based on the size of the fish or the farming system involved (Gómez-Márquez et al., 2003; Lupatsch et al., 2010). In the present study, PPT-ponds registered a significantly higher mean weight of eggs than the control-ponds. The PPT treatment also had a significantly higher eggs quality than the control. According to Rocha (2008), egg quality is determined by ecological factors (e.g., water quality), physiological factors (e.g., mobilisation of energy reserves) and nutrition of the female. The lipids and proteins in fish feeds are assimilated into the eggs during yolk formation inform of enzymes, lipoproteins, and enzymes (Orlando et al., 2017). The larvae depend solely on the nutritional reserves as they hatch. The time taken to exhaust the endogenous reserves determines the quality of eggs. Therefore, the higher reproductive performance in PPT-ponds could be attributed to high quality periphyton because the nutrients present in the diet are deposited into the oocytes during yolk formation. The microbial feed also contains vitamin C (Crab et al., 2012) and polyunsaturated fatty acids (Ekasari et al., 2010), which have been reported to contribute to the high quality and quantity of eggs (Dabrowski and Ciereszko, 2001). Ecological factors such as poor water quality also caused fish stress, eventually reducing reproductive performance in the control ponds.

The sustainability of *O. niloticus* culture has been limited by its precocious maturation. In the current study, the use of periphyton substrates seems to be a possible solution to reduce the overpopulation in farmed tilapia. The prolific spawning behaviour started earlier (in the fourth week) in control ponds but was delayed to eighth week in PPT-ponds. This resulted in reduced growth rates in control ponds because tilapia species spend a lot of energy during their generative process, including for the pugnacious behaviour of the males, territorial defence, mating, and mouthbrooding of the eggs. If the energy reserved for the reproductive process is not enough, tissue proteins are mobilised and catalysed to perform these functions (Orlando et al., 2017). Further, the early spawning periods make the ponds overpopulated with fries and fingerlings resulting in competition for feeds and depriving oxygen meant for the cultured tilapia. In the PPT-ponds, the presence of periphyton substrates may have restricted tilapia spawning by reducing lekking activities and preventing early nest formation. This, therefore, reduced the prolific breeding and energy that could be directed to maintaining their reproductive capacity during the early stages of growth was translated to somatic growth. Further studies are needed to explore the use of PPT as an alternative to synthetic hormones that are currently being used to sex-reverse tilapia to obtain all-male populations for culture.

Conclusion

Tilapia grew and performed better in the periphyton technology -ponds than in control ponds. The high growth performance is attributed to better feed utilisation efficiency of the natural feed in the form of plankton and periphyton. The reduced early sexual maturation of tilapia is also a major contributor to the increased growth performance. Therefore, this concept can be used to reduce the costs and negative impacts that may result from the use of 17α-methyl testosterone hormone, which is widely used for sex reversal of Nile tilapia. The present study demonstrated the potential of periphyton technology for sustainable, regenerative aquaculture with the potential of enhancing growth performance indicators while delaying tilapia prolific breeding behaviour, which causes stunted growth. Therefore,

periphyton technology can act as a sustainable approach for ecological fish culture by fish farmers. Further studies should explore PPT in replacing synthetic hormones for sex-reversal of tilapia fry in hatcheries.

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References

Abakari, G., Luo, G., Kombat, E.O. 2020. Dynamics of nitrogenous compounds and their control in biofloc technology (BFT) systems: A review. Aquaculture and Fisheries 6:441–447. https://doi.org/10.1016/j.aaf.2020.05.005

Abdel-Fattah, M.E. 2020. Tilapia culture. Taxonomy and basic biology. 2nd Edition. Academic Press, pp. 21–31. https://doi.org/10.1016/B978-0-12-816509-6.00002-1

Abwao, J., Boera, P.N., Munguti, J., Orina, S., Ogello E. 2014. The potential of periphyton based aquaculture for Nile tilapia (*Oreochromis niloticus L.*) production. A review. International Journal of Fisheries and Aquatic Studies 2:147–152.

Adineh, H., Naderi, M., Hamidi, M.K., Harsij, M. 2019. Biofloc technology improves growth, innate immune responses, oxidative status, and resistance to acute stress in common carp (*Cyprinus carpio*) under high stocking density. Fish and Shellfish Immunology 95:440–448. https://doi.org/10.1016/j.fsi.2019.10.057

Aisyah, Yustiati, A., Andriani, Y. 2021. Evaluation of various sources of bacteria on biofloc productivity. Asian Journal of Fisheries and Aquatic Research 12:30–39. https://doi.org/10.9734/ajfar/2021/v12i230231

Avnimelech, Y. 1999. Carbon and nitrogen ratio as a control element in aquaculture systems. Aquaculture 176:227–235. https://doi.org/10.1016/S0044-8486(99)00085-X

Avnimelech, Y. 2007. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. Aquaculture 264:140–147. https://doi.org/10.1016/j.aquaculture.2006.11.025

Azim, M.E., Verdegem, M.C.J., Khatoon, H., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M. 2002. A comparison of fertilization, feeding and three periphyton substrates for increasing fish production in fresh water pond aquaculture in Bangladesh. Aquaculture 212:227–243. https://doi.org/10.1016/S0044-8486(02)00093-5

Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. 2005. Periphyton: ecology, exploitation and management. CABI Publishing, Cambridge, UK. 319 pp. https://doi.org/10.1079/9780851990965.0000

Becker, E.W. 2007. Micro-algae as a source of protein. Biotechnology Advances 25:207–210. https://doi.org/10.1016/j.biotechadv.2006.11.002

Becker, J., Calado, R. 2021. Aquaculture, fish and fisheries: A new home for the blue revolution. Aquaculture, Fish and Fisheries. https://doi.org/10.1002/aff2.4

Beveridge, M., Verdegem, M., Wahab, M., Keshavanath, P., Baird, D. 1998. Periphyton-based aquaculture and the EC-funded PAISA Project. NAGA, The International Centre for Living Aquatic Resources Management (ICLARM) Quarterly 21:49–50.

Boyd, C., Wood, C. W., Thunjai, T. 2002. Aquaculture pond bottom soil quality management. Pond Dynamics/Aquaculture Collaborative Research Support Program Oregon State University, Corvalis, Oregon 97331-1641, pp. 38–41.

Boyd, C.E. 2018. Protein conversion efficiency in aquaculture. Global Aquaculture Advocate. https://www.aquaculturealliance.org/advocate/protein-conversion-efficiency-in-aquaculture/?headlessPrint=AAAAAPIA9c8r7gs82oWZBA (Accessed 10 January 2021).

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

Dabrowski, K., Ciereszko, A. 2001. Ascorbic acid and reproduction in fish: endocrine regulation and gamete quality. Aquaculture Research 32:623–638. https://doi.org/10.1046/j.1365-2109.2001.00598.x

Dempster, P., Baird, D.J., Beveridge, M.C.M. 1995. Can fish survive by filter-feeding on microparticles? Energy balance in tilapia grazing on algal suspensions. Journal of Fish Biology 47:7–17. https://doi.org/10.1111/j.1095-8649.1995.tb01868.x

Dempster, P.W., Beveridge, M.C.M., Baird, D.J. 1993. Herbivory in the tilapia Oreochromis niloticus: a comparison of feeding rates on phytoplankton and periphyton. Journal of Fish Biology 43:385–392. https://doi.org/10.1111/j.1095-8649.1993.tb00573.x

Deswati, D., Safni, S., Khairiyah, K., Yani, E., Yusuf, Y., Pardi, H. 2020. Biofloc technology: water quality (pH, temperature, DO, COD, BOD) in a flood and drain aquaponic system. International Journal of Environmental Analytical Chemistry. https://doi.org/10.1080/03067319.2020.1817428

Edmonson, W.T. 1959. Freshwater biology. Wiley, New York, USA. 1248 pp.

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 17:125–130. https://doi.org/10.4308/hjb.17.3.125

El-Greisy, Z.A., El-Gamal, A.E. 2012. Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17α-methyltestosterone with respect to the degree of sex stability after one year of treatment. The Egyptian Journal of Aquatic Research 38:59–66. https://doi.org/10.1016/j.ejar.2012.08.005

Emerenciano, M., Martinez-Cordova, L., Martinez-Porchas, M., Miranda-Baeza, A. 2017. Biofloc technology (BFT): a tool for water quality management in aquaculture. In: Water quality, Tutu, H. (Ed.), IntechOpen, pp. 91–109. https://doi.org/10.5772/66416

Fashina-Bombata, H., Megbowon, I. 2012. Proximate composition and breeding description of an unidentified cichlid of Epe Lagoon, Lagos, Southwest, Nigeria commonly called 'Wesafu'. International Journal of Nutrition and Metabolism 4:57–63. https://doi.org/10.5897/IJNAM11.069

Fernando, C.H. 1994. Zooplankton, fish and fisheries in tropical freshwaters. Hydrobiologia 272:105–123. https://doi.org/10.1007/BF00006516

FME (Federal Ministry of Environment). 2001. Guidelines and standards for water quality. Nigerian Publication Federal Ministry of Environment, pp. 114.

Garcia, F., Romera, D.M., Sousa, N.S., Paiva-Ramos, I., Onaka, E.M. 2016. The potential of periphyton-based cage culture of Nile tilapia in a Brazilian reservoir. Aquaculture 464:229–235. https://doi.org/10.1016/j.aquaculture.2016.06.031

Gómez-Márquez, J.L., Peña-Mendoza, B., Salgado-Ugarte, I.H., Guzmán-Arroyo, M. 2003. Reproductive aspects of *Oreochromis niloticus* (Perciformes: Cichlidae) at Coatetelco Lake, Morelos, Mexico. Revista de Biología Tropical 51:221–228.

Gross, A., Boyd, C.E., Wood, C.W. 2000. Nitrogen transformation and balance in channel catfish ponds. Aquacultural Engineering 24:1–14. https://doi.org/10.1016/S0144-8609(00)00062-5

Guo, H., Huang, L., Hu, S., Chen, C., Huang, X., Liu, W., Wang, S., Zhu, Y., Zhao, Y., Zhang, D. 2020. Effects of carbon/nitrogen ratio on growth, intestinal microbiota and metabolome of shrimp (*Litopenaeus vannamei*). Frontiers in Microbiology 11:652. https://doi.org/10.3389/fmicb.2020.00652

Henriksson, P.J.G., Belton, B., Jahan, K.M., Rico, A. 2018. Measuring the potential for sustainable intensification of aquaculture in Bangladesh using life cycle assessment. Proceedings of the National Academy of Sciences 115:2958-2963.

Hoque, M.I., Rahman, A.K.M.F., Mansur, M.A., Rahman, S. 2018. Effects of periphyton on monoculture of *Puntius gonionotus*. International Journal of Agriculture Research Innovation & Technology 8:13–23. https://doi.org/10.3329/ijarit.v8i2.40551

Huchette, S., Beveridge, M. 2003. Technical and economical evaluation of periphyton-based cage culture of tilapia *(Oreochromis niloticus)* in tropical freshwater cages. Aquaculture 218:219–234. https://doi.org/10.1016/S0044-8486(02)00414-3

Huchette, S.M.H., Beveridge, M.C.M., Baird, D.J., Ireland, M. 2000. The impacts of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. Aquaculture 186:45–60. https://doi.org/10.1016/S0044-8486(99)00365-8

Izquierdo, M.S., Fernandez-Palacios, H., Tacon, A.G.J. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197:25–42. https://doi.org/10.1016/B978-0-444-50913-0.50006-0

Jeje, C.Y. 1988. A revision of the Nigerian species of the genera *Mesocyclops* Sars, 1914 and *Thermocyclops* Kiefer, 1927 (Copepoda: Cyclopoida). Hydrobiologia 164:171–184. https://doi.org/10.1007/BF00008457

Jensi, A., Marx, K.K., Rajkumar, M., Shakila, R.J., Chidambaram, P. 2016. Effect of 17 α-Methyl testosterone on sex reversal and growth of Nile tilapia (*O. niloticus* L., 1758). Ecology, Environment and Conservation 22:1493–1498.

Ju, Z.Y., Forster, I.P., Conquest, L., Dominy, W. 2008. Enhanced growth effects on shrimp (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. Aquaculture Nutrition 14:533–543. https://doi.org/10.1111/j.1365-2095.2007.00559.x

Keshavanath, P., Gangadhar, B., Ramesh, T.J., Van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J. 2004. Effect of bamboo substrate and supplemental feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus* - *Oreochromis niloticus*). Aquaculture 235:303–314. https://doi.org/10.1016/j.aquaculture.2003.12.017

Khanjani, M.H., Alizadeh, M., Mohammadi, M., Sarsangi A.H. 2020. Biofloc system applied to Nile tilapia (*Oreochromis niloticus*) farming using different carbon sources: Growth performance, carcass analysis, digestive and hepatic enzyme activity. Iranian Journal of Fisheries Sciences 20:490–513.

Khanjani, M.H., Sajjadi, M.M., Alizadeh, M., Sourinejad, I. 2017. Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system: the effect of adding different carbon sources. Aquaculture Research 48:1491–1501. https://doi.org/10.1111/are.12985

Khanjani, M.H., Sharifinia, M. 2020. Biofloc technology as a promising tool to improve aquaculture production. Reviews in Aquaculture 12:1836–1850. https://doi.org/10.1111/raq.12412

Krishnani, K.K., Kathiravan, V., Meena, K.K., Sarkar, B., Kumar, S., Brahmane, M.P., Kumar, N., Kailasam, M. 2019. Bioremediation of aquatic toxicants: Application of multi-omic approaches. Advances in Fish Research 7:371–398.

Kuhn, D.D., Lawrence, A.L., Boardman, G.D., Patnaik, S., Marsh, L., Flick, G.J. 2010. Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 303:28–33. https://doi.org/10.1016/j.aquaculture.2010.03.001

Li, D., Li, Z., Wang, C., Wang, T., Xu, X. 2019b. Spectroscopic method for detection and determination of ammonia nitrogen in aquaculture water. IOPScience 4:4–13.

Li, Z., Wang, G., Yu, E., Zhang, K., Yu, D., Gong, W., Xie, J. 2019a. Artificial substrata increase pond farming density of grass carp (*Ctenopharyngodon Idella*) by increasing the bacteria that participate in nitrogen and phosphorus cycles in pond water. PeerJ 7:e7906. https://doi.org/10.7717/peerj.7906

Llario, F., Rodilla, M., Escriva, J., Falco, S., Sebastia-Frasquet, T. 2018. Phytoplankton evolution during the creation of a biofloc system for shrimp culture. International Journal of Environmental Science and Technology 16:211–222. https://doi.org/10.1007/s13762-018-1655-5

Lupatsch, I., Deshev, R., Magen, I. 2010. Energy and protein demand for optimal egg production including maintenance requirements of female tilapia (*Oreochromis niloticus*). Aquaculture Research 41:763–769. https://doi.org/10.1111/j.1365-2109.2009.02229.x

Malbrouck, C., Kestemont, P. 2006. Effects of microcystins on fish. Environmental Toxicology and Chemistry 25:72–86. https://doi.org/10.1897/05-029R.1

Mamaril, A.C. 2001. Zooplankton diversity in Philippine lakes. In: Conservation and ecological management of Philippine Lakes in relation to fisheries and aquaculture, Santiago, C.B., Cuvin-Aralar, M.L., Basiao, Z.U. (Eds.), Southeast Asian Fisheries Development Center, Aquaculture Department, Iloilo, Philippines; Philippine Council for Aquatic and Marine Research and Development, Los Banos, Laguna, Philippines; and Bureau of Fisheries and Aquatic Resources, Quezon City, Philippines, pp. 81–93.

Matsuo, Y., Kasahara, Y., Hagiwara, A., Sakakura, Y., Arakawa, T. 2006. Evaluation of larval quality of viviparous scorpion *Sebasticus marmoratus*. Fisheries Science 72:948–954. https://doi.org/10.1111/j.1444-2906.2006.01242.x

Megbowon, I., Mojekwu, T.O. 2014. Tilapia sex reversal using methyl testosterone and its effect on fish, man and environment. Biotechnology 13:213–216. https://doi.org/10.3923/biotech.2014.213.216

Miao, L., Wang, C., Adyel, T.M., Zhao, J., Yan, N., Wu, J., Hou, J. 2021. Periphytic biofilm formation on natural and artificial substrates: comparison of microbial compositions, interactions, and functions. Frontiers in Microbiology 12:684903. https://doi.org/10.3389/fmicb.2021.684903

Mirzakhani, N., Ebrahimi, E., Jalali, S.A.H., Ekasari, J. 2019. Growth performance, intestinal morphology and nonspecific immunity response of Nile tilapia (*Oreochromis niloticus*) fry cultured in biofloc

systems with different carbon sources and input C:N ratios. Aquaculture 512:734235. https://doi.org/10.1016/j.aquaculture.2019.734235

Mugwanya, M., Dawood, M.A.O., Kimera, F., Sewilam, H. 2021. Biofloc systems for sustainable production of economically important aquatic species: A review. Sustainability 13:7255. https://doi.org/10.3390/su13137255

Munguti, J., Obiero, K., Orina, P., Mirera, D., Kyule, D., Mwaluma, J., Opiyo, M., Musa, S., Ochiewo, J., Njiru, J., Ogello, E., Hagiwara, A. 2021. State of aquaculture report 2021: Towards nutrition sensitive fish food production systems. Techplus Media House, Kenya. 190 pp.

Munguti, J.M., Mugiranea, J.K., Ogello, E.O. 2014. An overview of the Kenyan aquaculture sector; current status, challenges and opportunities for future development. Fisheries and Aquatic Sciences 17:1–11. https://doi.org/10.5657/FAS.2014.0001

Naylor, R.L., Hardy, R.W., Buschmann, A.H. 2021. A 20-year retrospective review of global aquaculture. Nature 591:551–563. https://doi.org/10.1038/s41586-021-03308-6

Ngugi, C.C., Bowman, J.R., Omolo, B.O. 2007. A new guide to fish farming in Kenya. Aquaculture Collaborative Research Support Program. Oregon State University, USA. 95 pp.

Ogello, E.O., Munguti, J. 2016. Aquaculture: A promising solution for food insecurity, poverty and malnutrition in Kenya. African Journal of Food Agriculture, Nutrition and Development 16:11331–11350. https://doi.org/10.18697/ajfand.76.15900

Ogello, E.O., Musa, S.M., Aura, C.M., Abwao, J.O., Munguti, J.M. 2014. An appraisal of the feasibility of tilapia production in ponds using biofloc technology: A review. International Journal of Aquatic Science 5:2–3.

Ogello, E.O., Wullur, S., Sakakura, Y., Hagiwara, A. 2018. Composting fishwastes 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., Wullur, S., Sakakura, Y., Hagiwara, A. 2020. Dietary value of waste-fed rotifer *Brachionus rotundiformis* on the larval rearing of Japanese Whiting *Sillago japonica*. E3S Web of Conferences 147:01005. https://doi.org/10.1051/e3sconf/202014701005

Orlando, T., Oliveira, M., Paulino, R., Costa, A., Allaman, B., Rosa, V. 2017. Reproductive performance of female Nile tilapia (*Oreochromis niloticus*) fed diets with different digestible energy levels. Brazilian Journal of Animal Science 46:1–7. https://doi.org/10.1590/s1806-92902017000100001

Patkar, A.R., Manjappa, A.R., Nayak, H. 2021. Dynamics of plankton in substrate-based fish and prawn culture. Journal of Entomology and Zoology Studies 9:175–182. https://doi.org/10.22271/j.ento.2021.v9.i3c.8678

Pertiwi, P., Abinawanto, A., Yimastria, S. 2017. Fertilization rate of Lukas fish (*Puntius bramoides*). AIP conference proceedings 2023:020160. https://doi.org/10.1063/1.5064157

Piedrahita, R.H. 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 226:35–44. https://doi.org/10.1016/S0044-8486(03)00465-4

Rocha, M.J. 2008. Fish reproduction. Science Publisher, California, USA, pp. 9–37. https://doi.org/10.1201/b10747

Russell, M., Shuke, R., Samantha, S. 2011. Effects of conductivity on survivorship and weight of goldfish (*Carassius auratus*). http://departments.juniata.edu/biology/eco/documents/Russell_et al.pdf (Accessed 05 August 2021).

Saikia, S., Das, D. 2009. Potentiality of periphyton-based aquaculture technology in rice-fish environment. Journal of Scientific Research 1:624–634. https://doi.org/10.3329/jsr.v1i3.2114

Sakr, M., Shalaby, M., Wassef, A., El-Sayed, M., Moneim, I. 2015. Evaluation of periphyton as a food source for Nile tilapia (*Oreochromis niloticus*) juveniles fed reduced protein levels in cages. Journal of Applied Aquaculture 27:50–60. https://doi.org/10.1080/10454438.2014.967073

Sarkar, S., Rekha, P.N., Panigrahi, A. 2021. Integrated brackishwater farming of red sea weed *Agarophyton tenuistipitatum* and Pacific white leg shrimp *Litopenaeus vannamei* (Boone) in biofloc system: a production and bioremediation way out. Aquaculture International 29:2145–2159. https://doi.org/10.1007/s10499-021-00739-w

Supamattaya, K., Kiriratnikom, S., Boonyaratpalin, M., Borowitzka, L. 2005. Effect of a *Dunaliella* extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (*Penaeus monodon*). Aquaculture 248:207–216. https://doi.org/10.1016/j.aquaculture.2005.04.014

Tinh, T.H., Momoh, T.A., Kokou, F., Hai, T.N., Schrama, J.W., Verreth, J.A.J., Verdegem, M.C.J. 2021. Effects of carbohydrate addition methods on Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture 543:736890. https://doi.org/10.1016/j.aquaculture.2021.736890

Walker, D.A.U., Suazo, M.C.M., Emerenciano, M.G.C. 2020. Biofloc technology: principles focused on potential species and the case study of Chilean river shrimp *Cryphiops caementarius*. Reviews in Aquaculture 12:1752–1782. https://doi.org/10.1111/raq.12408

Wang, C., Pan, L., Zhang, K., Xu, W., Zhao, D., Mei, L. 2015. Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of *Litopenaeus vannamei* in zero-exchange culture tanks. Aquaculture Research 47:1–12. https://doi.org/10.1111/are.12784

Withford, L.A., Schumacher, G.J. 1973. A manual of freshwater algae. Sparks, Raleigh, North Carolina, USA. 324 pp.

Xu, W.J., Pan, L.Q. 2013. Enhancement of immune response and antioxidant status of (*Litopenaeus vannamei*) juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. Aquaculture 412–413:117–124. https://doi.org/10.1016/j.aquaculture.2013.07.017

Yadav, R., Kumar, P., Saini, V.P., Sharma, B.K. 2017. Importance of periphyton for aquaculture. Aqua Star. Article 2; pp. 38–40.