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Consumption of periphyton and bioseston by Mozambique tilapia in aqua dams with three different substrates

Khathutshelo C. Hlongwane*, Ngonidzashe A. G. Moyo, Mmaditshaba M. Rapatsa-Malatji

Aquaculture Research Unit, School of Agricultural and Environmental Sciences, Faculty of Science and Agriculture, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga 0727, South Africa

ABSTRACT: Periphyton-based aquaculture can reduce feed input costs. Here we characterised the periphyton and bioseston formation in aqua dams stocked with net, plastic, and stone substrates. The consumption of periphyton and bioseston from the net substrate by Mozambique tilapia *Oreochromis mossambicus* fingerlings was evaluated. Three experiments were conducted. In the first experiment, net, stone, and plastic were deployed in triplicates in aqua dams. The net substrate registered the highest (7.74 ± 1.45 g m^{-2}, ±SE) periphyton biomass. Network analysis showed connectedness between the substrates. The degree centrality showed that the net substrate had the highest score, indicating that there were more groups of species with similar functions growing on the net substrate. The net substrate was subsequently used in the second experiment to determine the consumption of periphyton and bioseston by tilapia fingerlings in aqua dams. Three treatments were assigned: N100 (fish fed 100% commercial diet); N50 (fish fed 50% commercial diet); and N33 (fish fed 33% commercial diet). Growth performance did not differ significantly among the treatments, although N50 showed a trend for a higher growth. This suggests that periphyton may be capable of nutritionally compensating for the partial withdrawal of commercial feed. A third experiment was conducted in fibreglass tanks to determine the preference of tilapia between periphyton and bioseston. The prominent values showed that tilapia preferred to feed on bioseston. The best growth performance was achieved in a periphyton-based aquaculture system, and deployment of the net substrate is recommended in tilapia ponds.

KEY WORDS: Periphyton consumption · Tilapia growth · Natural food · Diversity · Aquaculture · Bioseston · *Oreochromis mossambicus*

1. INTRODUCTION

The high cost of fish feed makes most aquaculture enterprises unprofitable in developing countries (Moyo & Rapatsa 2021). Some developing countries have aquaculture feed-manufacturing companies that are fully operational, but these companies lack readily available low-cost raw materials and depend on imported expensive ingredients such as vitamins, premixes, and fish meal (Hasimuna et al. 2019). Therefore, there is a need to develop aquaculture technologies that can produce natural food in production systems without increasing input costs. Periphyton-based culture is an alternative way to improve fish production by enhancing natural food in a pond (Chikorela et al. 2019, Muthoka et al.

*Corresponding author: kchlongwane@gmail.com

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2021). Periphyton is a complex community of bacteria, algae, fungi, and organic and inorganic detritus attached to submerged substrates (van Dam et al. 2002). It serves as a significant food source for most aquatic animals in their natural habitat, and the nutritional quality is adequate to support the dietary needs of some fish species (Azim et al. 2001, Azim & Asaeda 2005, Piñosa 2018). Periphyton not only provides natural food, but it improves the health of the fish and sequesters excess nutrients in the water (van Dam et al. 2002, Azim et al. 2005, Khatoon et al. 2007). Bioseston is also an important source of food for fish species in periphyton-based aquaculture. Bioseston comprises all the living autotrophic and heterotrophic organisms suspended in the water column, such as phytoplankton, zooplankton, bacteria, and protozoa (Hildreth 1980, Zieritz et al. 2019). The growth of bioseston and periphyton under farming conditions can ultimately reduce supplemental feed inputs (Sakr et al. 2015, Tammam et al. 2020, Sahu et al. 2021). However, the factors affecting periphyton development in fish ponds are poorly understood in southern Africa.

One of the factors affecting periphyton biomass and composition is the nature of the substrate. Very little information is available on the biomass and composition of periphyton biofilm on net, stone, and plastic substrates in southern Africa. These 3 substrates are readily available and can play an important role in aquaculture in the region. The most investigated substrates include bamboo, PVC pipes, glass tubes, common reeds, jute sticks, sugarcane bagasse, and wood (Azim et al. 2005, Khatoon et al. 2007). Bamboo substrate has been shown to have higher densities and more diverse periphyton communities (Azim et al. 2005, Khatoon et al. 2007, Uddin et al. 2007, Amisah et al. 2008, Shafi et al. 2021, Hao et al. 2022). The deployment of periphyton substrates increases the average density of bioseston such as phytoplankton, zooplankton, and planktonic bacteria (Umesh et al. 1999, Mridula et al. 2003, Azim et al. 2005). Dempster et al. (1993) reported that the mean ingestion rate of natural food by tilapia was higher (2 mg g^{-1} h^{-1}) when it was offered as periphyton than when it was offered as phytoplankton, and the mean ingestion rate of natural food was 25 times higher (5 mg g^{-1} h^{-1}) when periphyton and phytoplankton were offered together. However, most previous studies did not look at the composition, patterns of relationships, and network structure of the periphyton and bioseston on the substrates.

Periphyton-based culture has been thriving in some countries, particularly in Brazil, Bangladesh, Nepal, Israel, Indonesia, and India (Signor et al. 2015, Mohapatra et al. 2016, Tortolero et al. 2016, David et al. 2022). The sub-tropical climate in these countries is similar to that in southern Africa. Therefore, it is important to explore the potential of periphyton-based culture in southern Africa. The growth of periphyton and phytoplankton depends mainly on environmental conditions such as water temperature, light, and nutrients (Rodríguez & Pizarro 2015). Thus, in a balanced ecosystem, bioseston and periphyton supply base energy for the food web through primary production, providing sufficient natural food for fish such as tilapia.

It is well established that tilapia are omnivorous filter-feeders that largely graze on plankton species, with phytoplankton as the main dietary component (Figueredo & Giani 2005, Semyalo et al. 2011, El-Otify 2015). Tilapia fry and fingerlings feed on zooplankton, then later change to feed on phytoplankton, macrophytes, and detritus (Trewavas 1982, Egna & Boyd 1997, Beveridge & McAndrew 2000). Thus, tilapia are good candidates for periphyton consumption since they are capable of efficiently ingesting and digesting plankton of various sizes. Tilapia have a stomach pH below 1.4, which lyses the phytoplankton cell walls so they can be effectively digested (Rivera Vasconcelos et al. 2018, Temesgen et al. 2022). Tilapia also have morphological adaptations such as small-notched teeth, fine pharyngeal teeth, and extended intestines that enable them to digest periphyton material (Azim et al. 2005). Consequently, tilapia are commonly used in periphyton-based aquaculture because they can effectively consume natural food (van Dam et al. 2002, Azim et al. 2003, Milstein et al. 2005, Garcia et al. 2016, David et al. 2022).

Despite its growing popularity, the benefits of periphyton-based aquaculture remain inconclusive. Recently, Shafi et al. (2021) showed that the survival rate and fish yield in concrete tanks using bamboo poles and PVC sticks as substrate was significantly higher than that of the control treatment without substrates. Keshavanath et al. (2004) also reported significant growth of tilapia using bamboo poles as substrate and concluded that periphyton-based systems can enhance fish growth and lower production costs. In contrast, other studies have reported that periphyton-based systems are not beneficial to the fish (Huchette et al. 2000, Azim et al. 2003, Uddin et al. 2007, Garcia et al. 2016). For instance, Cavalcante et al. (2017) reported that periphyton has a minimal contribution to the growth of tilapia in periphyton-based systems. Garcia et al. (2016) reported that the benefits of periphyton-based culture are dependent on the size of the fish.

The contradictory results on the benefits of periphyton-based aquaculture make it imperative for studies to be undertaken to investigate the abundance, composition, and network of periphyton community in commonly available substrates. The overall aim of this study was to investigate the consumption of natural food by tilapia. The specific objectives were to characterise the periphyton and bioseston in aqua dams stocked with net, plastic, and stone substrates and subsequently measure the consumption of the periphyton and bioseston by tilapia fingerlings.

2. MATERIALS AND METHODS

2.1. Study site

The study was conducted in experimental aqua dams (plastic dams [containers] used to culture fish in Southern Africa; 7000 l), under real culture conditions at the Aquaculture Research Unit of the University of Limpopo, South Africa (23.8888° S, 29.7386° E). The university is located in Limpopo province in the northernmost part of South Africa, one of the warmest regions in South Africa. It has an average minimum temperature of 11°C in winter and maximum temperature of 40°C in summer. Three experiments were conducted during the summer season from October 2021 to January 2022.

2.2. Expt 1: colonisation of periphyton on different substrates and bioseston growth in aqua dams

The objective of this experiment was to identify periphyton and bioseston associated with different substrates. Network analysis was then used to determine the relationship and the structure of the periphyton community growing on 3 substrates in aqua dams.

Nine aqua dams were cleaned and filled with municipal water. The water in the aqua dams was allowed to mature for 5 d and was then fertilised with chicken manure at 0.26 kg m^{-2} (Jha et al. 2008). The aqua dams had no aeration and water circulation; water lost through evaporation was replaced once a week. Three substrates (stones, net, and plastic) were deployed in the aqua dams, and the experiment was conducted in triplicates in a randomised design. Hereafter, the term 'periphyton' is used to indicate the assemblage of attached organisms on submerged substrates, and 'bioseston' to indicate non-attached phytoplankton and zooplankton in the water column of aqua dams stocked with different substrates.

Periphyton was allowed to colonise on nets, stones, and plastics for 21 d in aqua dams without fish. To collect periphyton samples on Day 21, the periphyton was carefully removed from the substrates using a sharp blade by scraping the surface of the substrate (3 × 3 cm). To determine periphyton biomass based on the dry matter (ash free weight, ash weight, dry weight) the samples were dried in an oven according to method no. 10300 C of APHA (1998). Another set of periphyton samples collected by scraping the surface of the net (9 × 9 cm) at the bottom, middle, and surface was used to determine periphyton taxonomic composition. The periphyton scraped from the substrate was diluted with 20 ml distilled water to determine the taxonomic composition under a light compound microscope (Leica E24) using an improved double Neubauer counting chamber (0.1 mm depth). First, the counting chamber and the coverslip were cleaned with 70% ethanol, and then 0.01 ml (10 µl) of the sample was loaded on the loading groove using a micropipette and counted. The concentration of cells in 1 µl was estimated by dividing the number of counted cells by the volume of the 4 main squares; the value was then multiplied by 1000 to get the number of cells in 1 ml. The periphyton was also counted using a petri dish because some species were too big for the counting chamber; a sample of 1 ml was loaded into the petri dish and counted using a light microscope (Zeiss, Axiolab).

Water samples for bioseston analysis were collected on Day 21 of the experiment. The samples were collected 50 cm below the surface using a truncated cone-shaped, silk bolting cloth net. A 71 µm mesh size net was used to collect bioseston. Samples were collected by filtering the water through the net, which was then rinsed into a 5 l bucket using pond water and then decanted into 250 ml sample bottles. The sample bottles were kept in a cool room at 4°C until analysis under a light microscope using the counting chamber and petri dish as mentioned above. Water quality parameters monitored include dissolved oxygen (mg l^{-1}), temperature (°C), pH, salinity (ppt), and electrical conductivity (mS cm^{-1}) using a YSI multi-probe meter. Transparency (cm) was measured using a Secchi disc. Nitrogen ammonia (mg l^{-1}) was analysed according to method number 8038 of DOC316.53.01078 (Hach 2012), and nitrate (mg l^{-1}) was analysed according to method number 8171 of DOC316.53.01069 (Hach 2012). Phosphate (mg l^{-1}) was analysed according to method number 8048 of DOC316.53.01119 (Hach 2012).

2.3. Expt 2: consumption of periphyton and bioseston by tilapia in aqua dams

The objective of this experiment was to determine the consumption of periphyton and bioseston in aqua dams stocked with Mozambique tilapia *Oreochromis mossambicus* fingerlings, and determine the growth performance of the fish. Based on the network analysis results, the net substrate registered the largest periphyton community. Thus, the net substrate was subsequently used to determine periphyton and bioseston consumption in aqua dams stocked with 5 g tilapia fingerlings.

Three treatments in triplicates in a complete randomised design were used to determine the consumption of periphyton and bioseston by 5 g *O. mossambicus*. The first treatment was the control, in which the fish were fed only commercial feed twice daily, and the aqua dams had no periphyton substrates (no periphyton-based feed). This was equivalent to 100% feeding. In the second treatment, the fish were fed commercial feed every other day twice a day with periphyton-based feed (periphyton substrates in the aqua dam); this was equivalent to 50% feeding. In the third treatment, the fish were fed commercial feed every third day, equivalent to 33% feeding. The feeding regimes were designated N100 for the control, N50 are for aqua dams with a net substrate that received 50% of the recommended feed, and N33 are for aqua dams with a net substrate that received 33% of the recommended feed. Tilapia fingerlings (5.09 ± 0.62 g) were obtained from the Aquaculture Research Unit's recirculating system and stocked at a density of 2.12 fish m^{-2} in all the treatments (N100, N50, and N33). The pilot experiment showed the stocking density of 2.12 fish m^{-2} was optimum when the aqua dams were stocked with net substrate. The experiment ran for 6 wk, and water quality parameters were monitored on a weekly basis. The mean (±SE) temperature was 25.81 ± 2.59°C, dissolved oxygen (DO) was 4.84 ± 1.25 mg l^{-1}, pH was 6.81 ± 0.18, nitrogen ammonia was 1.03 ± 0.84 mg l^{-1}, nitrate was 0.02 ± 0.01 mg l^{-1}, and mean phosphate was 4.00 ± 1.81 mg l^{-1}. At termination, the total fish weights and lengths were also determined to calculate the following growth parameters:

$$\text{Weight gain} = \text{final weight} - \text{initial weight} \quad (1)$$

$$\text{Average daily weight gain} = \text{final weight gain (g)/time} \quad (2)$$

$$\text{Specific growth rate (SGR)} = [(\ln \text{final weight} - \ln \text{initial weight})/\text{time}] \quad (3)$$

$$\text{Feed conversion ratio (FCR)} = \text{feed consumption/weight gain} \quad (4)$$

$$\text{Feed efficiency rate (FER)} = 1/\text{FCR} \quad (5)$$

$$\text{Survival rate (\%)} = [(\text{final number of fish/initial number of fish}) \times 100] \quad (6)$$

$$\text{Condition factor} = [(\text{weight/length}^3) \times 100] \quad (7)$$

2.4. Expt 3: characterisation of periphyton and bioseston in *O. mossambicus* diet

This experiment was undertaken in fibreglass tanks, with the objecive to determine feed preference of 5 g tilapia.

Periphyton and bioseston were harvested from 2 treatments (N50 and N33) as previously described in Expt 2 and were designated N50.B and N33.B for bioseston, and N50.S and N33.S for periphyton (where 'S' indicates being scraped from a substrate) from the N50 and N33 treatments, respectively. The experiment was conducted in triplicate in 12 fibreglass 90 l tanks.

Tilapia fingerlings were starved for 24 h before stocking in the fibreglass tanks inoculated with periphyton and bioseston. The periphyton and bioseston were enumerated as previously described (Section 2.2) before inoculation. Four tilapia fingerlings were stocked per tank and allowed to graze for 12 h. After 12 h of grazing (at the end of the experiment), the bioseston (N50.B and N33.B) and periphyton (N50.S and N33.S) were harvested from all tanks and enumerated again. Faeces were collected directly from the tanks by siphoning, and thereafter the fish were harvested. Fish were sacrificed and gutted to remove the stomach. Humane measures were implemented when the fish were handled and sacrificed in accordance with the University of Limpopo's Animal Ethical Committee. The stomach contents were analysed under a light microscope to identify the periphyton and bioseston items ingested by the fish. The faecal matter was analysed under a light microscope to determine which periphyton and bioseston items were not consumed by the fish. The prominent value (Norton & Schmitt 1978) was used to determine whether tilapia preferred periphyton or bioseston. The following calculations were used to determine the prominent value per treatment:

$$\text{Prominent value of periphyton} = \frac{\text{periphyton density}}{\sqrt{\text{frequency of occurrence}}} \quad (8)$$

$$\text{Prominent value of bioseston} = \frac{\text{bioseston density}}{\sqrt{\text{frequency of occurrence}}} \quad (9)$$

2.5. Data and statistical analysis

Microsoft Excel and Power BI Desktop were used to plot graphs. Network analysis and Shannon-Wiener diversity index were determined in R (version 4.4.2) using the 'igraph' and 'vegan' packages, respectively. Network analysis was used to study the patterns of relationships and the network structure of the periphyton community growing on 3 substrates in aqua dams. Network analysis allows researchers to identify patterns and trends in the relationships between the entities in a network; it operates at multiple levels to describe and make inferences about individual entities of the entire network (Marsden 2005). Normality and homogeneity of growth performance and water quality parameters were tested using Shapiro-Wilk and Levene tests, respectively (Statistical Package and Service Solutions [SPSS] version 27). The data met the assumptions of normality and homogeneity and were therefore not transformed. ANOVA was used to test for significant differences in growth performance and water quality parameters. Tukey's post hoc analysis was used to determine which means were significantly different from each other (SPSS version 27). The data were tested at level of significance of 0.05.

Table 1. Abundance (mean ± SD ind. ml^{-1}) of periphyton colonising stone, net, and plastic substrates. Each mean represents samples from 3 replicate aqua dams

Genus	Stone	Net	Plastic
Ankistrodesmus	–	0.90 ± 1.56	–
Chlorella	16.28 ± 0.19	15.27 ± 13.23	21.05 ± 3.40
Chlorogonium	–	–	6.38 ± 10.08
Coelastrum	–	11.47 ± 10.24	–
Cosmarium	5.02 ± 8.69	1.38 ± 1.31	–
Dictyosphaerium	0.63 ± 1.10	–	–
Micractinium	–	–	11.73 ± 20.24
Oocystis	7.03 ± 1.60	–	–
Pandorina	–	–	0.08 ± 0.14
Scenedesmus	15.87 ± 1.87	25.70 ± 7.40	21.72 ± 20.70
Sphaerocystis	7.10 ± 6.16	–	–
Cyclotella	4.37 ± 7.56	5.50 ± 8.59	7.25 ± 7.40
Pinnularia	–	3.17 ± 5.48	4.48 ± 7.55
Synedra	–	1.58 ± 2.16	4.55 ± 7.88
Navicula	–	–	5.77 ± 9.60
Nitzschia	8.43 ± 8.16	10.92 ± 7.08	7.48 ± 8.95
Mougeotia	–	24.92 ± 14.08	2.73 ± 4.69
Aphanocapsa	–	–	12.45 ± 9.49
Coelosphaerium	7.40 ± 6.46	–	–
Oscillatoria	5.47 ± 9.47	–	0.42 ± 0.72
Microcystis	5.68 ± 9.84	25.47 ± 9.41	13.87 ± 12.84
Trachelomonas	0.57 ± 0.49	1.48 ± 2.57	3.33 ± 5.77
Actinosphaerium	9.52 ± 8.24	3.07 ± 5.23	–
Mallomonas	–	–	2.22 ± 3.63
Lumbricus	–	0.87 ± 1.50	0.65 ± 1.13
Total periphyton abundance	93.37 ± 4.97	131.68 ± 8.61	126.17 ± 6.49
Shannon-Wiener diversity index	2.349	2.148	2.456

3. RESULTS

3.1. Expt 1: colonisation of periphyton on different substrates and bioseston growth in aqua dams

The periphyton species diversity index was highest on the plastic substrate (2.456) and lowest on the net substrate (2.148) (Table 1). The net substrate had the highest total periphyton abundance (131.68 ± 8.61 ind. ml^{-1}, mean ± SD), and the stone substrate had the lowest (93.37 ± 4.97 ind. ml^{-1}) among the 3 substrates. *Ankistrodesmus* and *Coelastrum* were exclusively associated with the net substrate. The plastic substrate was exclusively associated with 6 genera (*Chlorogonium*, *Micractinium*, *Pandorina*, *Navicula*, *Aphanocapsa*, and *Mallomonas*), whereas the genera *Dictyosphaerium*, *Oocystis*, and *Coelosphaerium* were exclusively associated with the stone substrate (Table 1). *Scenedesmus*, *Chlorella*, *Cyclotella*, *Nitzschia*, *Microcystis*, and *Trachelomonas* were abundant on all substrates (Table 1). The net substrate had the highest periphyton biomass (7.74 ± 1.45 g m^{-2}), whereas the stone substrate had the lowest (2.75 ± 0.10 g m^{-2}) (Fig. 1).

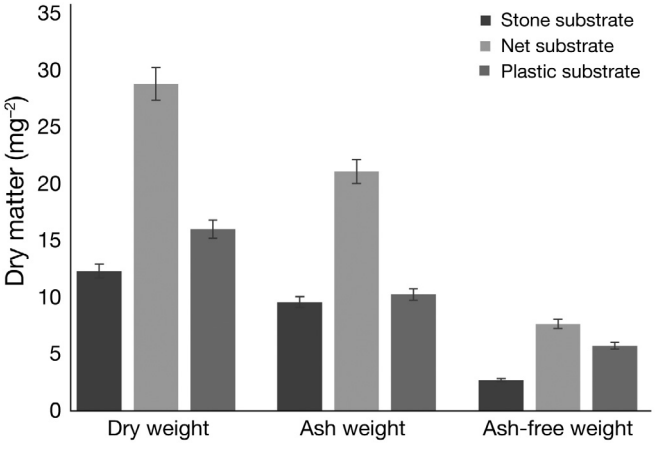

Fig. 1. Mean (±SD) periphyton dry matter from aqua dams stocked with stone, net and plastic substrates for 21 d without fish grazing

Network analysis showed that associations among genera in biosestion and periphytic communities were not complex and had a loose network structure with a fair closeness (0.434) and betweenness centrality of 0.341, reasonable degree centrality (0.635), smaller edge density (0.0587), and small modularity of −0.0531 (Fig. 2). The circle size on the substrate node indicates that the net substrate exhibited a higher proportion of nodes and edges than the stone and plastic substrate.

Bioseston species diversity index was highest on the net substrate (2.546) and lowest on the stone substrate (2.036) (Table 2). The net substrate had the highest total bioseston abundance (150.25 ± 6.67 ind. ml^{-1}), and the plastic substrate had the lowest (53.33 ± 4.73 ind. ml^{-1}). *Anopheles* (mosquito larvae), *Brachionus*, and rotifer eggs were exclusively found on the net substrate (Table 2). The genus *Loxodes* was exclusively associated with the stone substrate. Copepods were exclusively associated with the plastic substrate. *Cryptomonas*, *Peranema*, midge larvae, *Difflugia*, *Euplotes*, *Paramoecium*, and *Vorticella*were were abundant on all substrates (Table 2).

Fig. 2. Network of bioseston and periphytic communities at the genus level from aqua dams with stone, net, and plastic substrates for 21 d. The size of the circle (colour) on the nodes (substrates) indicates the number of edges connected to it. Ash-free weight, ash weight, and dry weight are a biomass measure for the bioseston and periphytic community growing on the substrates. R.eggs: rotifier eggs; ashW: ash weight; Ash-free.w: ash-free weight; dryw: dry weight; Midge.L: midge larvae; Mosquito.L: mosquito larvae; Mayfly.L: mayfly larvae; Cop.B.frag: copepod body fragments; Clad.B.frag: Cladocera body fragments; bact.count: Bacterial count

Table 2. Abundance (mean ± SD ind. ml^{-1}) of bioseston colonising stone, net, and plastic substrates. Each mean represents samples from 3 replicate aqua dams

Genus or group	Stone	Net	Plastic
Cryptomonas	11.28 ± 5.20	11.50 ± 10.06	15.65 ± 4.94
Peranema	12.55 ± 1.29	8.32 ± 6.53	14.07 ± 4.43
Mayfly larvae	–	8.77 ± 7.73	1.48 ± 2.57
Midge larvae	0.30 ± 0.40	17.75 ± 15.39	0.70 ± 0.48
Anopheles sp. (mosquito larvae)	–	0.52 ± 0.89	–
Copepods body fragments	–	–	0.13 ± 0.23
Cladocera body fragments	–	0.57 ± 0.55	0.10 ± 0.17
Amoeba	–	7.07 ± 4.02	3.02 ± 3.03
Difflugia	4.80 ± 8.31	5.28 ± 9.15	3.33 ± 5.30
Euplotes	10.35 ± 3.69	16.88 ± 17.40	4.58 ± 3.81
Loxodes	443 ± 7.68	–	–
Paramoecium	9.93 ± 0.20	8.82 ± 7.73	6.33 ± 10.97
Stylonychia	8.45 ± 7.61	5.30 ± 7.48	–
Vorticella	13.53 ± 3.83	5.13 ± 7.87	3.60 ± 3.23
Brachionus plicatilis	–	14.40 ± 24.94	–
Brachionus sp.	–	5.45 ± 4.27	–
Lecane	–	23.93 ± 9.26	0.33 ± 0.58
Rotifera eggs	–	10.57 ± 5.42	–
Total bioseston abundance	75.63 ± 5.25	150.25 ± 6.67	53.33 ± 4.73
Shannon-Wiener diversity index	2.036	2.546	2.243

Table 3. Mean ± SE of water quality parameters from aqua dams with different substrates. Each mean represents samples from 3 replicate dams. Superscript letters indicate significant values which differed from each other

	Stone	Net	Plastic	p
Transparency (cm)	70.0 ± 0.00	70.0 ± 10.00	60.0 ± 0.00	0.422
Temperature (°C)	26.96 ± 0.49	25.5 ± 0.25	26.7 ± 0.52	0.119
Dissolved oxygen (mg l^{-1})	6.82 ± 1.85^{a}	2.11 ± 0.58^{b}	8.10 ± 0.28^{a}	0.022
Electrical conductivity (mS cm^{-1})	185.36 ± 3.00	210.36 ± 19.11	178.73 ± 8.85	0.238
Salinity (ppt)	0.08 ± 0.00	0.10 ± 0.01	0.08 ± 0.00	0.228
pH	7.15 ± 0.00	7.17 ± 0.01	7.17 ± 0.01	0.069
Nitrogen ammonia (mg l^{-1})	0.07 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.096
Nitrate as N (mg l^{-1})	3.73 ± 0.12^{a}	4.06 ± 0.14^{b}	3.4 ± 0.05^{a}	0.017
Total phosphate (mg l^{-1})	6.96 ± 0.48	6.11 ± 0.41	6.46 ± 0.50	0.480

Except for dissolved oxygen and nitrate ($p < 0.05$), the water quality parameters were not significantly different ($p > 0.05$) among the aqua dams with stone, net, and plastic substrates (Table 3).

3.2. Expt 2: consumption of periphyton and bioseston by tilapia in aqua dams

All growth performance indices (weight gain, average daily weight gain, SGR, condition factor, FER, and FCR) were highest in the N50 treatment, although the differences were not statistically significant ($p > 0.05$) (Table 4).

3.3. Expt 3: characterisation of periphyton and bioseston in *Oreochromis mossambicus* diet

The prominent values showed that tilapia preferred to feed more on bioseston than on periphyton (Fig. 3). The prominent values per treatment were N50.S: 9.0; N33.S: 6.0; N50.B: 10.4; and N33.B: 7.6 (Fig. 3).

The stomach contents of tilapia were characterised by a high occurrence of *Navicula*, *Nitzschia*, *Scenedesmus*, *Pinnularia*, *Chlorogonium*, *Microcystis*, *Tetredron*, *Difflugia*, and *Chlorella* sp. (Fig. 4).

The faecal matter was analysed to determine if all periphyton and bioseston species identified in the stomachs of tilapia were ultimately digested. The faecal matter was characterised by a high occurrence of *Synedra*, *Pinnularia*, *Navicula*, *Nitzschia*, and *Difflugia* sp. (Table 5).

4. DISCUSSION

Ankistrodesmus and *Coelastrum* were exclusively found on the net substrate. These genera are green algae that can produce astaxanthin (Kaha et al. 2021, Luu et al. 2021), and are widely distributed in water bodies with sufficient sunlight and nutrients. Astaxanthin is a xanthophyll carotenoid that enhances growth, increases feed conversion rates, improves disease resistance, and reduces embryonic mortality in aquatic animals (Lim et al. 2018, Elbahnaswy & Elshopakey in press). This group of xanthophyll carotenoids also has essential properties such as anti-inflammatory and antioxidant activities (Kaha et al. 2021). The periphyton community from the stone substrate was characterised by *Dictyosphaerium*, *Coelosphaerium*, and *Oocystis*. *Coelosphaerium* is a genus of cyanobacteria that can live in conditions with limited sunlight because of their competitive behaviour. Cyanobacteria species produce stable peptides that are resistant to heat, chemical hydrolysis, and oxidation. These species can be cell-bound under dark conditions and persist for months or years (Sivonen & Jones 1999, Weller 2011, Godo et al. 2017). *Dictyosphaerium* and *Oocystis*

Table 4. Growth performance of tilapia fingerlings in aqua dams deployed with net substrates in 3 different treatments. N50: fish were fed commercial feed every other day with periphyton-based food; N33: fish were fed commercial feed once every third day with periphyton-based food. N100: fish were fed only commercial feed every day (without periphyton substrates). Values are mean ± SE

	Treatment			p
	N100	N50	N33	
Initial weight (g)	5.47 ± 0.95	4.73 ± 0.23	5.07 ± 0.42	0.397
Final weight (Week 6)	14.88 ± 5.44	17.28 ± 2.91	17.26 ± 4.41	0.751
Weight gain (g)	9.41 ± 6.13	12.55 ± 2.68	12.20 ± 4.09	0.667
Average daily weight gain (g)	0.22 ± 0.15	0.30 ± 0.06	0.29 ± 0.10	0.667
Specific growth rate	2.21 ± 1.32	3.03 ± 0.30	2.68 ± 0.52	0.524
Feed conversion ratio	2.88 ± 2.85	1.09 ± 0.25	0.89 ± 0.29	0.338
Feed efficiency rate	0.60 ± 0.38	0.96 ± 0.26	1.24 ± 0.50	0.220
Survival rate (%)	97.33 ± 4.62	98.67 ± 2.31	92.67 ± 4.16	0.212
Condition factor	1.73 ± 0.28	1.92 ± 0.27	1.81 ± 0.31	0.733

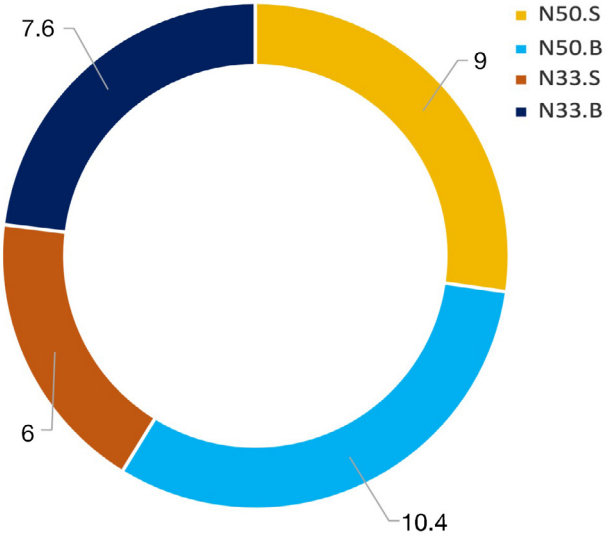

Fig. 3. Prominent values, showing that tilapia preferred to feed more on bioseston than on periphyton. Treatments are described as in Table 4, where 'S' refers to periphyton consumed and 'B' refers to bioseston

were exclusively associated with the stone substrate; these green algae are widely distributed in a variety of water bodies with sufficient nutrient concentrations and benthic sediments (Mette et al. 2011). The plastic substrate exclusively supported *Micractinium*, *Pandorina*, *Navicula*, *Mallomonas*, and *Chlorogonium*. These genera have attachment organs such as flagella and bristles that enable them to easily attach to the plastic substrate, which has a smooth texture. *Navicula* was abundant in the stomach of tilapia, but it was not consumed as food because of the silica cell wall. *Aphanocapsa* was also exclusively found on the plastic substrate because it is a cyanobacteria that can resist various environmental conditions as discussed above.

Mosquito larvae, *Brachionus*, and rotifer eggs were bioseston exclusively associated with the net substrate. The mosquito larvae were found only on the net substrate because they need to breathe air at the water's surface, and the net provided a surface for attachment at the top of the aqua dam. Under normal conditions, mosquito larvae depend on the surface tension of the water for attachment. The presence of *Brachionus* is explained by high periphyton diversity and community structure on the net substrate that served as food for this rotifer genus. The high bioseston abundance of *Loxodes* on the stone substrate is because these ciliated species are detritivores and bacterivores. Because of their diverse feeding and nutritional mechanisms, they do well at the bottom of the pond where light is limited, but they are also found in high abundance in the surrounding water column (Ackermann et al. 2011, Früh et al. 2011, Vlaičević et al. 2021). Copepods in the bioseston were exclusively associated with the plastic substrate because of the high abundance of diatom species recorded on the plastic substrates, which served as food sources for the copepods. Copepods are key grazers of diatoms (Pančić et al. 2019). Thirteen genera were abundant on all substrates because they are commonly found in a variety of water conditions as long as they are exposed to sunlight. These species are commonly abundant in different water bodies, ranging from freshwater oligotrophic to polluted water bodies (Cesarini et al. 2022, Gwos Nhiomock et al. 2022). Thus, the network structure of the periphyton community and the bioseston identified on the 3 substrates was connected due to these 13 genera.

Even though 13 genera were most abundant on all 3 substrates, periphyton biomass in terms of dry matter differed among the substrates. The net substrate had the highest periphyton biomass in comparison with stone and plastic substrates. The high periphyton biomass on the net substrate is explained by the large surface area, rough texture, and strong hydrophobicity of the net. Moreover, the mesh of non-degradable material of the net substrate favoured the trapping of particles and provided an adequate site for periphyton development and attachment points. The net substrate also had a high periphyton diversity because it created an undisturbed environment for a variety of species to

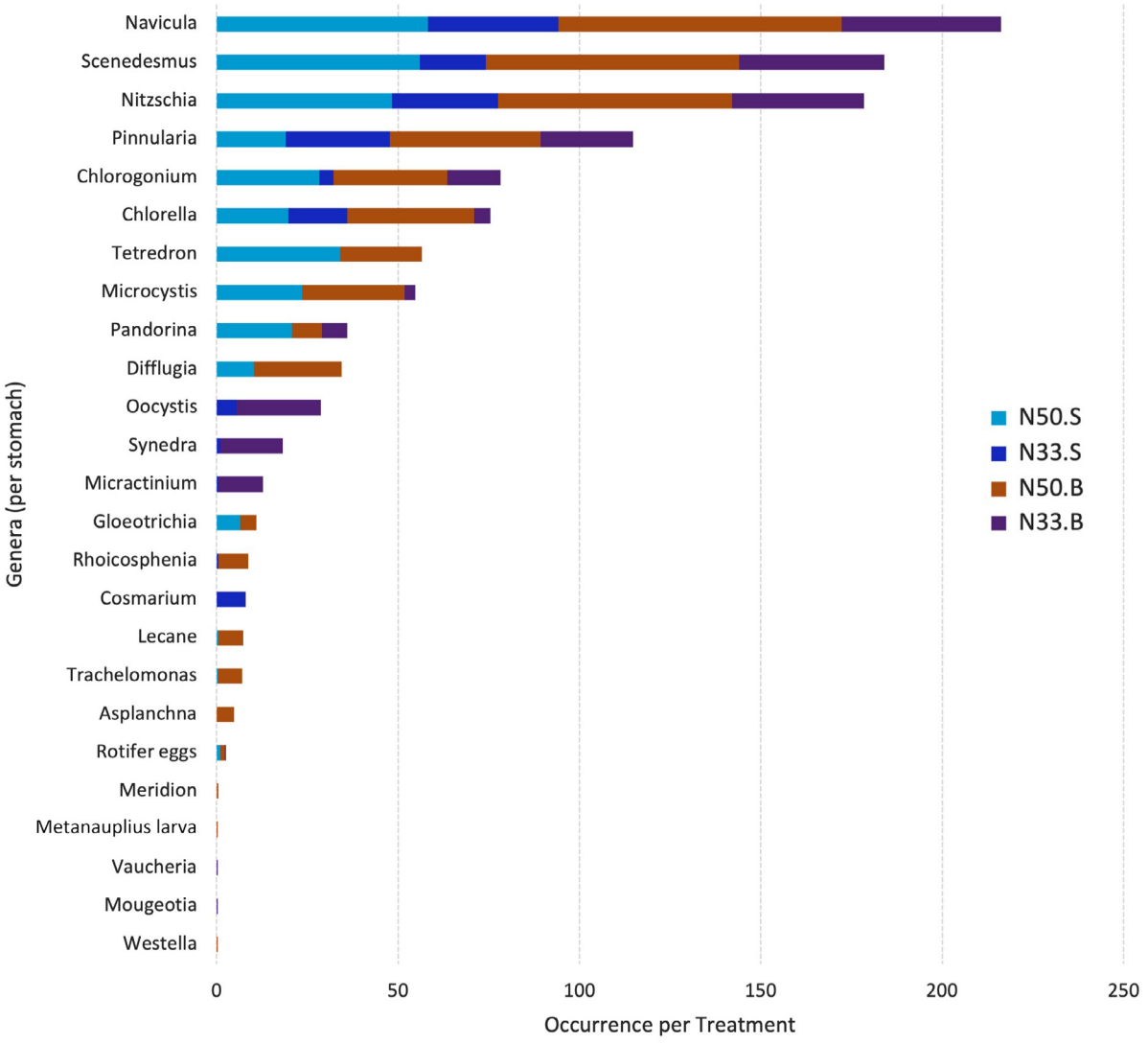

Fig. 4. Frequency of occurrence of periphyton and bioseston genera identified from the stomach of *Oreochromis mossambicus* fingerlings in the net substrate experiment. Treatments are described as in Table 4, where 'S' refers to periphyton consumed and 'B' refers to bioseston

Table 5. Frequency of occurrence of periphyton and bioseston identified from the faeces of *Oreochromis mossambicus* fingerlings. Treatments are described as in Table 4, where 'S' refers to periphyton consumed and 'B' refers to bioseston

Genus	Treatment			
	N50.S	N33.S	N50.B	N33.B
Scenedesmus	0	0	0	2.8
Tetredron	0.8	0	0.5	4.3
Pinnularia	37	68	48	75.3
Navicula	65.8	41.3	61.5	54.8
Synedra	52.8	45.8	72.3	60
Nitzschia	32.8	32.8	43.3	65.8
Difflugia	10	20.5	14.3	23
Lecane	0	0	0.3	0

grow. Several studies have demonstrated that substrate properties, such as surface texture, surface roughness, hydrophobicity, and biocompatibility, are selective factors of periphyton colonisation and biofilm formation on different substrate types (Chen et al. 2013, von Ammon et al. 2018, Miao et al. 2020). The plastic substrate had less periphyton biomass compared to the net because of its high-gloss (smooth) texture. Even though the plastic substrate received sufficient sunlight, the periphyton was not able to easily colonise the plastic substrate because of its smooth texture. The stone substrate had the lowest periphyton ash-free weight because it was completely submerged and at the bottom of the aqua dam where sunlight was limited. Substrates submerged at the bottom of a water

body commonly have low autotrophic periphyton biomass due to limited sunlight (Guariento et al. 2009, Tortolero et al. 2016). However, even under limited sunlight, periphyton on substrates provide natural food to cultured organisms such as tilapia fingerlings.

The growth performance of tilapia fingerlings did not differ significantly among the 3 treatments, although N50 and N33 showed a tendency for higher SGR and better FCR than N100. The SGR was highest on the N50 treatment followed by N33. This may suggest that the nutritional value of the periphyton and bioseston species was able to compensate for the nutritional value of the deprived commercial feed, possibly because the net substrate was exclusively associated with the bioseston (zooplankton) species and green algae species that produce astaxanthin. *Ankistrodesmus* and *Coelastrum* produce astaxanthin, a carotenoid that enhances growth and better feed conversion rates (Luu et al. 2021). Juvenile fish require an animal-based diet at early stages of development, thus tilapia initially feed more on zooplankton then later change to feed on phytoplankton. The higher SGR and better FCR recorded in N50 was perhaps because the periphyton and bioseston found on the net substrate were sufficient to support the growth of tilapia fingerlings. Rotifers can have roughly 52 to 59% protein, 13% fat, and 3.1% n-3 unsaturated fatty acids depending on the food source (Das et al. 2012). These results suggest that tilapia feeding on alternate days (N50) and on every third day (N33) were grazing at a higher rate on periphyton and bioseston between meals of commercial feed. These results agree with other studies reporting that tilapia consume periphyton effectively at an earlier stage of development (Rao et al. 2015, Haque et al. 2022, Temesgen et al. 2022). Similar results were recorded by Garcia et al. (2016), Rodrigues et al. (2019), and David et al. (2022). They found that partial or total feed restriction plus periphyton leads to high survival rates and better FCR for fish in periphyton-based systems than in conventional production systems. This might be because bioseston in the form of phytoplankton and zooplankton were always abundant in periphyton-based systems to support the growth of tilapia. Umesh et al. (1999) and Mridula et al. (2003) also showed that periphyton substrates increase the average density of phytoplankton, zooplankton, and planktonic bacteria (bioseston) in water.

To better understand if tilapia preferred periphyton or bioseston attached to the substrates, the characterisation of periphyton and bioseston in the diet of *Oreochromis mossambicus* was investigated in fibreglass tanks. The prominent values showed that tilapia fingerlings effectively consumed periphyton but preferred bioseston. This suggests that the fish prefer filter feeding, which uses less energy than removing the periphyton from a substrate. This is because tilapia lack feeding apparatus suitable for grazing, such as prominent rostral and labial folds. Such feeding apparatus are prominent in *Labeo congoro* and *L. cylindricus* (Skelton 2001). Azim et al. (2005) also showed that in order to harvest periphyton efficiently, fish need a high degree of specialisation of the feeding, filtering, and masticating apparatus.

The comparison of the periphyton and bioseston items in the stomach and faeces of the fish from the fibreglass tanks confirmed that tilapia was feeding on periphyton and bioseston. The faecal matter indicated that tilapia were unable to digest Bacillariophyta species because of the silica cell wall covering them. This is because tilapia do not have powerful mandibles lined with silica-reinforced teeth used to crack diatom cell walls. Unlike other periphytic species, consumed diatoms are not broken during ingestion and digestion because of the hydrated silicon dioxide cell wall (Pančić et al. 2019).

In conclusion, this study showed that the net substrate was associated with high periphyton biomass and high bioseston diversity in comparison with stone and plastic substrates. Growth of tilapia fingerlings was highest when they were fed every other day (N50), suggesting that periphyton and bioseston species were able to compensate for the nutritional value of the deprived commercial feed. The study also showed that tilapia fingerlings effectively consumed periphyton but preferred bioseston to periphyton. Unlike other periphyton grazers such as *L. cylindricus*, tilapia do not have a thick-lipped, sucking mouth on the underside of the head to effectively scrape periphyton off the substrate. The faecal matter indicated that tilapia were unable to digest Bacillariophyta species. It is recommended that net substrate be deployed in fish ponds and that the fingerlings be fed every other day.

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