

**EVALUATION OF PERIPHYTON UTILIZATION BY *OREOCHROMIS*
MOSSAMBICUS AND *CLARIAS GARIEPINUS* IN AQUA DAMS IN LIMPOPO
PROVINCE, SOUTH AFRICA.**

BY

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RESEARCH THESIS

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2025

DEDICATION

I dedicate this thesis to my beloved sister, Tsakani Hlongwane. Thank you for your persistent encouragement from the earliest days when I struggled to grasp the fundamentals of writing my name, to the present moment. This thesis is a testament to your unwavering dedication and love.

DECLARATION

I declare that the EVALUATION OF PERIPHYTON UTILIZATION BY OREOCHROMIS MOSSAMBICUS AND CLARIAS GARIEPINUS IN AQUA DAMS is my work and that all the sources that I have used or quoted have been indicated and acknowledged using complete references and that this work has not been submitted before for any other degree at any other institution.



15/11/2024

Khathutshelo Cathrine Hlongwane

Date

PLAGIARISM DECLARATION

The thesis has been submitted to the Turnitin module and I confirm that my supervisors have seen my report and any concerns revealed by such have been resolved with them.



15/11/2024

Khathutshelo Cathrine Hlongwane

Date

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ABSTRACT

The high cost of fish feed in developing countries makes it imperative to develop aquaculture technologies that can produce natural food in production systems without increasing input costs. Periphyton-based aquaculture promotes the growth of periphyton on submerged substrates in water, which serves as a preferred natural food for herbivorous and omnivorous fish such as tilapia and African catfish. Tilapia and African catfish are one of the most widely cultured species in Africa. In this study, the utilisation of periphyton by *Oreochromis mossambicus* (Mozambique tilapia) and *Clarias gariepinus* (African catfish) in aqua dams with net, plastic and stone substrates was investigated. The study aimed to reduce the cost of fish feed in rural aquaculture by enhancing the utilisation of periphyton by Mozambique tilapia and African catfish in aqua dams. Locally available materials were used as substrates for growing periphyton in the aqua dams.

Periphyton dynamics in periphyton-based aquaculture are influenced by several factors thus, it was important to first determine the composition of periphyton in aqua dams before stocking the fish. The growth of periphyton on substrates in water also enhances the growth of bioseston in the water column. Therefore, the effect of substrates on periphyton and bioseston was explored simultaneously to fully understand the periphyton dynamics in aqua dams in summer and winter. Net, plastic and stone were deployed separately in the nine aqua dams in a completely randomised design in winter and summer. The experiment ran for 21 days in each season. The periphyton and bioseston were categorised as periphytic algae, metazoans, ciliates, protists, and aquatic invertebrates to easily distinguish the periphyton and bioseston communities.

Periphytic algae was the most abundant category on all substrates in both winter and summer probably because they have adaptive features that enable them to thrive in various environments. The composition of periphyton and bioseston in the aqua dam with net, plastic and stone during winter and summer showed that substrate, season and grazing played a significant role. The composition and abundance of periphyton and bioseston were significantly higher ($P < 0.05$, ANOSIM) on the net and lowest on

the stone substrate in both winter and summer. The net substrate also had the highest periphyton biomass (in terms of dry matter) while the stone substrate had the lowest in both summer and winter. This was because the texture, surface area and position of the substrate influenced the abundance and composition of periphyton and bioseston in aqua dams. Summer had higher periphyton and bioseston abundance than winter because the temperature was optimal for their growth. Network analysis was later used to determine the pattern of the community structure of periphyton and bioseston in aqua dams in winter and summer and showed a weak community structure. This was caused by low genera diversity due to the similarities in water quality parameters and the high abundance of periphytic algae in both seasons. This indicated that the genera on one substrate were most likely to be found on another substrate despite the season.

Subsequent to understanding factors affecting periphyton and bioseston composition in aqua dams with substrates, fish were then stocked into the aqua dams. To determine the utilisation of periphyton and bioseston by the fish in the aqua dams, three feeding regimes were designed. In the first feeding regime, the fish were fed every day (twice a day) and this was designated: N100, P100, and S100. The first feeding regime (N100, P100 and S100) represented the control because fish are normally fed twice every day at fish farms. In the second feeding regime, the fish were fed commercial pellets every other day (twice a day), and this was designated: N50, P50, and S50. In the third feeding regime, the fish were fed every third day (still twice a day), and this was designated N33, P33, and S33. Mozambique tilapia fingerlings (5.44 ± 0.81 g, mean $\pm$ SE) were stocked into the aqua dams with net, plastic and stone substrates in a completely randomised design for six weeks. Unexpectedly, specific growth rate and condition factor were significantly ($P < 0.05$, MANOVA) higher in aqua dams with plastic substrates. In prior experiments in aqua dams without fish, the net substrate had the highest periphyton biomass. However, the net substrate did not result in the highest fish growth rate (Chapter 4). This was probably because periphyton and bioseston in aqua dams with plastic substrate were accessible and of high quality. This shows that the periphytic algae, metazoans, ciliates, protists, and aquatic invertebrates provided sufficient protein, energy, and lipids to compensate for the nutritional value of the deprived commercial pellets. Furthermore, it was concluded

that commercial pellets can be reduced by 50 to 67 % in aqua dams with periphyton substrates.

Mozambique tilapia of three different size groups (2.62 ± 0.01 g, 5.08 ± 0.02 g and 25.24 ± 0.29 g; mean $\pm$ SE) were further observed for 24 hours to determine their feeding preferences between periphyton and bioseston in fibreglass tanks. The results showed that Mozambique tilapia did not show a preference between periphyton and bioseston, the fish was not actively selecting any food items. This was because Mozambique tilapia can nip and scrap periphyton from substrates and also filter feed on bioseston using gill rakers. Furthermore, the most abundant and dominant periphyton and bioseston in the fibreglass tanks were also abundant in the stomachs of all tilapia size groups. This indicates that Mozambique tilapia was ingesting the most abundant periphyton and bioseston. Diatoms were also abundant and dominant in the stomachs of all tilapia size groups but also abundant in the faecal matter. This indicates that diatoms were not digested, probably because tilapia lacked silica-reinforced teeth to break the diatom's cell wall.

The utilisation of periphyton and bioseston by African catfish was also explored. The same experiments and feeding regimes mentioned above were adopted to determine if African catfish is a good candidate for periphyton-based aquaculture. African catfish (200.2 ± 22.74 g, mean $\pm$ SD) were also stocked in the aqua dams in a complete randomised design for six weeks. The results showed that in aqua dams with net and plastic substrates, the highest specific growth rate was recorded in N100 and P100. However, in aqua dams with stone substrates, the highest specific growth rate was recorded in S33. Both N100 and P100 represented the control where the African catfish were fed commercial pellets twice, every day. The fish in S33 aqua dams were fed commercial pellets every third day. This indicates that catfish in S33 were able to utilise periphyton on stone substrates since the growth rate was comparable with the controls. This was attributed to its large lower jaw extensibility which enabled it to scoop periphyton from the stone substrates. This suggests that stone substrates can be used in aqua dams stocked with African catfish to reduce commercial pellet input by 67 %. In the prior experiments, the stone substrate had the least periphyton

composition and biomass, but this suggests that its nutritional value was sufficient to compensate for the nutritional value of the deprived commercial pellets. The results also showed that each substrate significantly influenced the growth performance of African catfish differently. However, there was no distinct pattern to conclude where the fish grew faster between the net, plastic and stone substrates. This was probably because the net substrate had the highest periphyton abundance and plastic had more genera of animal origin while the periphyton on the stone was more accessible to African catfish to easily scoop.

The results also showed that African catfish selectively fed on insects, Chironomidae larvae, and Cladocera. Furthermore, insects and Chironomidae were important food items in the diet of African catfish across the different substrates. This was because African catfish have predatory behaviour and commonly prefer food items of animal origin. However, diatoms, *Microcystis* sp. and *Diffugia* sp. dominated the faecal matter indicating that African catfish lack gastric juices and silica-reinforced teeth to digest them.

Lastly, an aquaculture recirculating system was designed to determine the impact of periphyton-based biofilters on water quality parameters. To determine the efficacy of a periphyton-based biofilter in maintaining optimal water quality parameters for tilapia culture, Mozambique tilapia (5.00 ± 1.14 g, mean $\pm$ SD) was stocked in the tanks. Net, plastic and stone were used as biofilters in duplicate in a completely randomised design. Nitrifying bacteria were not seeded but the periphyton was allowed to form naturally for three weeks before commencement of the experiment. The water quality parameters were monitored daily for three weeks to determine the overall performance of the periphyton-based biofilters. The water quality parameters were also determined for a period of 24 hrs at four-hour intervals (once every week) to monitor the diurnal dynamics in the periphyton-biofilters.

The results showed the diurnal mean levels mirrored the average water quality parameters across the periphyton-biofilters over the period of three weeks. Nitrate

levels significantly varied ($P < 0.05$) between the periphyton biofilters, net periphyton-biofilter registered the highest nitrate levels and stone registered the lowest levels. This indicates that nitrification effectively occurred in the net periphyton-biofilter contributing to the accumulation of nitrate. However, the accumulated nitrate was within the optimum range for farming tilapia. Nitrite was significantly higher ($P < 0.05$) in the plastic periphyton-biofilter than in net and stone periphyton-biofilter. High nitrite levels probably indicate there was imbalanced nitrite-oxidising microbial activity which led to nitrite accumulation in the plastic periphyton-biofilter. Nitrite can be toxic to tilapia when it's above 1.0 mg/l. However, the recorded level of nitrite was within the optimum range for farming tilapia, below 0.5 mg/l. The stone periphyton-biofilter registered the highest ($P < 0.05$) ammonia levels and plastic registered the lowest levels. The accumulation of ammonia indicates that nitrification was not effective and inadequate in the stone periphyton-biofilter. Even though ammonia was highest in the stone periphyton-biofilter, it was still within the farming range for tilapia. The total phosphate recorded in the periphyton-biofilter was slightly higher than that recorded in the aqua dams. This suggests that the periphyton community on the plastic substrate was not dominated by algae that can take up phosphate in the form of inorganic phosphorus. Dissolved oxygen and pH levels were optimum for tilapia and water purification processes in all the biofilters. The net was an effective periphyton-based biofilter since it was able to maintain the toxic ammonia and nitrite at minimal levels.

This study recommends using periphyton substrate in aquaculture to reduce feed costs. Durable plastic substrates can be used in aqua dams stocked with tilapia to reduce commercial pellet input by 50 to 67 %. Stone substrates can be used in aqua dams stocked with African catfish to reduce commercial pellet input by 67 %. Nonetheless, the use of other substrates needs to be explored. Furthermore, the study recommends using periphyton-based biofilters to maintain the water quality parameters optimum for fish production in a recirculating system. In this study, the net was the most efficient periphyton-based biofilter and we recommend that the net periphyton-biofilter be tested in a large-scale production system. Further experiments should be conducted to determine the feeding preference of African catfish between periphyton and bioseston.

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chroe.; Scenedesmus sp.-Scened.; Selenastrum sp.- Selen.;
 Sphaerocystis sp.- Sphaer.; Amphipleura sp.- Amphi.; Cyclotella sp.- Cyclo.;
 Frustulia sp.- Frustulia; Pinnularia sp.- Pinn.; Rhoicosphenia sp.- Rhoico.;
 Synedra sp.- Synedra; Melosira sp.- Melosira; Navicula sp.- Navicula;
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 Gyrinidae–Gyrinidae ;Corixa sp.- Corixa; Amoeba sp.-Amoeba; Diffugia sp.-
 Diffugia; Halteria sp.-Halteria; Paramoecium sp.- Param.; Vorticella sp.-
 Vorticella; Brachionus sp.- Brachionus; Lecane sp.- Lecane; Philodina sp.-
 Philodina; Rotifers eggs-R.eggs; Aphanocapsa sp.-Aphano.; Oscillatoria sp.-
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 Mallomonas sp.- Mallomonas; Dictyosphaerium sp.- Dictyosph. ; Cyclotella
 sp.- Cyclo.; Pinnularia sp.- Pinn.; Mougeotia sp.- Moug.; Cryptomonas sp.-
 Crypt.; Aphanocapsa sp.- Aphano.; Coelosphaerium sp.- Coeloshp.;
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CHAPTER 1: GENERAL INTRODUCTION

1. INTRODUCTION

1.1 General Introduction

Aquaculture fish farmers are challenged with the high cost of fish feed, mostly in developing countries. Most fish farmers in Southern Africa depend on imported expensive fish feeds. As a result, the procurement of fish feed contributes to over 60 % of the total farm production costs (FAO, 2016; El-Sayed, 2020). Various initiatives, with varying degrees of success, have attempted to reduce the cost of fish feed by using locally sourced plant and animal-derived ingredients (Hoffman et al., 1997; Hlophe & Moyo, 2014; Mwimanze & Musuka, 2014; Rapatsa & Moyo, 2017; Tabassum et al., 2021; Alofa et al., 2023; Nephale et al., 2024). However, none of these alternatives had a commercial uptake. Consumers are reluctant to eat fish that have been fed feed containing ingredients such as housefly maggots or crickets. Therefore, it is imperative to develop aquaculture technologies that can produce natural food in production systems without increasing input costs. Periphyton-based aquaculture is an alternative way to improve fish production by enhancing the growth of natural food in ponds or similar impoundments (Chikorela et al., 2019; Muthoka et al., 2021). Periphyton-based aquaculture is thriving in Brazil, Bangladesh, Nepal, Israel, Indonesia, Pakistan, and India (Signor et al., 2015; Mohapatra et al., 2016; Tortolero et al., 2016; David et al., 2022). In Africa, it has only been tested in West Africa (Welcomme, 1972; Garg, 2005) and it has not been tested in Southern Africa.

Periphyton-based practices can be traced back to traditional fishing methods in West Africa, adopted by fishermen in open waters to attract and capture fish in large natural water bodies as such seas, lakes and reservoirs (Kapetsky, 1981; Azim et al., 2005). Currently, it is gaining traction in closed aquaculture production systems as a preferable natural food source for herbivorous and omnivorous aquatic animals. It is primarily used to reduce the cost of fish feed by decreasing commercial pellet feed inputs for different species in different aquaculture production systems (van Dam et al., 2002; Guttman, 2019; Biswas et al., 2022). Various substrates are deployed in production systems to grow the periphyton that fish and other cultured species directly graze on. Periphyton are ubiquitous and can be found in almost every aquatic ecosystem ranging from small ponds to large production tanks (Azim & Asaeda, 2005). Aquaculture species that are widely used in periphyton-based culture include carp,

tilapia, and prawns because they can effectively digest most plankton material (Garcia et al., 2016; Rai et al., 2018; Tammam et al., 2020; David et al., 2022). Recently, periphyton-based aquaculture was used for the production of mullet fingerlings in brackish water ponds (Biswas et al., 2017; Biswas et al., 2022). The multicomplex community of periphyton can also sustain the culture of more species in polyculture production systems (Azim et al., 2001a & 2002; Sahu et al., 2021). Several studies have reported higher fish yield and growth rates in ponds with periphyton substrates compared to ponds without substrates (Azim et al., 2005; Jha et al., 2018; Tammam et al., 2020). Periphyton-based aquaculture is not only effective in improving fish growth but, also in improving and maintaining the health of cultured fish (van Dam et al., 2002; Abwao et al., 2014; Garcia et al., 2017). This is attributed to some periphyton organisms within the community that can produce antimicrobial and bioactive compounds that function as immunomodulators or immunostimulants (Anusha et al., 2020; Mani et al., 2021; Saleh et al., 2022).

Periphyton-based aquaculture also plays a substantial role in nutrient cycling, where it actively consumes a significant proportion of available carbon, nitrogen, and phosphorus in water and converts them into food (periphyton) for cultured species. In conventional aquaculture ponds, a limited portion of carbon (13%), nitrogen (29%) and phosphorus (16%) inputs are utilised by the cultured species, while the majority of these nutrients accumulate in the water (Avnimelech & Ritvo, 2003). This leads to environmental concerns, and it highlights the need for sustainable practices. Periphyton-based aquaculture through periphyton promotes the uptake of nitrogenous compounds, leading to increased dissolved oxygen and a concomitant decrease in ammonia and nitrite levels, while enhancing the growth of autotrophic microorganisms through photosynthesis (Dodds, 2003; Jesna et al., 2018; Patkar et al., 2021). Moreover, periphyton photosynthesis can increase pH by up to 1 unit and can take up orthophosphate in water (Dodds, 2003). Periphyton has also been reported as the major contributor to primary production in ponds due to its species composition, which is dominated by attached green algae. It is an important ecosystem component due to its competition with other primary producers of carbon, light and nutrients. Periphyton improves the quality of water by nitrification enhancement, oxygen production, decomposition of organic matter, ammonium and nitrate uptake, phosphate uptake

and trapping suspended solids (Azim et al., 2001b; van Dam et al., 2002; Khatoon et al., 2007; Milstein et al., 2008; Chikorela et al., 2019). A study by Crispim et al. (2009) showed that macrophytes efficiently absorb nitrogen, but not phosphate from the water, whereas periphyton is efficient in capturing both. Through periphyton nutrient recycling, periphyton-based aquaculture is currently used to absorb excess nutrients from the water, reducing the risk of water pollution and eutrophication in several water bodies.

Periphyton-based aquaculture system is gaining acceptance due to its dual benefits of nutrient recycling, through the uptake and assimilation of nutrients by periphyton, and improved fish health and growth. Thus, rendering it a superior approach to other methods used to enhance natural food in aquaculture production systems. Another alternative method that has been used to enhance the growth of natural food in aquaculture is using organic or inorganic (chemical) fertilisers in ponds. This is the traditional way of promoting the growth of plankton and insects which provide food for fish in ponds (Boyd, 1981; Green, 2015; Boyd, 2018). This method involves adding organic or inorganic (chemical) fertilisers that contain the necessary nutrients required to stimulate the growth of plankton and aquatic insects (Wurts, 2000; Jana et al., 2001). Organic fertilisers such as chicken, cow, and pig manure also serve as a direct food source for some fish (Knud-Hansen, 1998). However, this method is not widely used because it can also lead to the proliferating growth of pathogenic bacteria such as *Aeromonas* sp. and *Pseudomonas* sp. in water (Sugita et al., 1985; Jinyi et al., 1987). Moreover, manure overdose leads to severe depletion of dissolved oxygen, high ammonia, and generation of hydrogen sulfide and methane leading to stress and sometimes death of the cultured fish (Yip & Wong, 1977; Singh et al., 1991). Chemical fertilisers have the disadvantage of being insoluble in water thus, deteriorating the water quality and affecting the health of culture fish. Therefore, the introduction of other methods such as biofloc culture has been investigated as an alternative to pond fertilization for increasing fish production while avoiding water quality deterioration.

Another method of enhancing the production of natural food for fish in aquaculture production systems is biofloc culture technology. Biofloc culture gained traction in the

2000s as an aquaculture technology used to cultivate natural food using wastewater. Biofloc is a natural food agglomerate composed of bacteria, algae, fungi, protozoa, rotifers, nematodes, hypotrichs, and shell moults (Panigrahi et al., 2018). The biofloc is directly consumed by the fish as a high protein source of food while it effectively removes nitrogenous waste products in water by directly converting it to bacterial biomass. The biofloc system is effective in maintaining optimum water quality, allows higher stocking densities, and reduces the need for water exchange in a system. However, it requires a certain level of skills to daily properly maintain the carbon and nitrogen ratio in water and to prevent nitrite accumulation. The energy cost is also high since it constantly requires aeration and mixing of the water (Ogello et al., 2021; Khanjani et al., 2024). Both biofloc and periphyton-based cultures are effective in improving the water quality by reducing ammonia and other nitrogenous metabolites and removing toxic compounds in the water. Therefore, other studies integrated biofloc and periphyton-based culture into one culture system (Asaduzzaman et al., 2009 & 2010; Cavalcante et al., 2017; Rezende et al., 2018). However, the integrated system was not as effective in improving fish growth and water quality as the separate systems. Periphyton-based aquaculture alone is superior to biofloc culture since it has the ability to effectively improve water quality, fish health and production without additional cost from carbon source and aeration. Moreover, it has an economic advantage over biofloc culture due to the easy availability of substrates because farmers can use any hard material at their disposal as substrates. However, there are some challenges associated with periphyton-based aquaculture.

The major challenge affecting periphyton-based aquaculture is temperature and sunlight availability. Periphyton growth, abundance and composition in the wild vary depending on temperature and sunlight. The shallow littoral zone has higher periphyton abundance and species composition than a deeper littoral zone because it receives more sunlight and has warmer temperatures (Azim et al., 2005; Hofmann et al., 2020). Substrate structure, type and nature (biodegradable or non-biodegradable) also affect the biomass and diversity of periphyton. Our study showed that net substrate had the highest periphyton biomass compared to plastic and stone (Hlongwane et al., 2024). Nurfadillah et al. (2023) reported that artificial substrates support better growth and development of periphyton than organic-based substrates

in aquaculture production systems. The availability and nutrient variations also affect the development of periphyton and usually lead to a shift in taxonomic composition and density (Azim et al., 2005; Hao et al., 2020). This is because periphyton take up nutrients from the water for their growth.

Periphyton-based aquaculture is not suitable for all aquatic species, as a result, most studies focus on *Tilapia* species. *Tilapia* species are commonly used in periphyton-based aquaculture, their mouth morphological features can scrap, comb, and suck various natural food items (Azim et al., 2005). They have pharyngeal jaws, oral jaws with teeth, and jaws with high premaxilla protrusibility and lower jaw extensibility (Sibbing & Witte, 2005; Skelton, 2001; Azim et al., 2005). Other species such as African catfish have different mouth morphological apparatus that may cause them to respond differently to periphyton-based aquaculture. This is because the mouth size, jaw movements, and tooth structure give each species the ability to feed more efficiently on certain prey. Another aspect that makes *Tilapia* species ideal for periphyton-based aquaculture is its physiological adaptability to digest periphyton. Tilapias have a stomach pH of 1.4 which lyses the cell wall of most plankton so it can effectively digest them (Vasconcelos et al., 2018; Temesgen et al., 2022). Moreover, *Tilapia* species have longer intestines compared to catfish species, while catfish have bigger stomachs than tilapia species. The size of the stomach and the length of the intestine play an important role in the digestion of food since they ultimately determine how long the food will be exposed to digestive enzymes. The stomach of African catfish has a pH that ranges from 1 to 3 depending on the water temperature (Moawad et al., 2017; Nurhayati et al., 2020). It is clear from these adaptations that African catfish may exhibit a different response to periphyton utilisation compared to tilapia. However, both tilapia and African catfish have some common feeding adaptations.

Tilapia species and African catfish are both naturally opportunistic omnivores and will feed on the most available food. Zayed and Mohamed (2004) showed that gill rakers of tilapia and African catfish are both covered with a layer of mucous that aids in trapping planktonic (bioseston) organisms during filter feeding. They both have a pharyngeal bone that has rough and sharp teeth (Skelton, 2001). Thus, making both

species suitable for periphyton-based aquaculture. However, the morphology of the gill rakers, particularly the number, shape and spacing can influence African catfish to exhibit distinct response to bioseston utilisation compared to tilapia. This study will use both Mozambique tilapia (*O. mossambicus*) and African catfish (*C. gariepinus*) to determine the utilisation of periphyton in aqua dams deployed with net, plastic and stone substrates.

1.2 Research problem

Most fish farmers in developing countries are still procuring expensive fish feed to sustain their fish farms. Several efforts, most without success, have been made in the past to reduce the cost of feed by using cheap local ingredients. Therefore, it is important to propose new alternatives that will reduce production costs while improving fish production. Hence, this study proposes the use of periphyton-based aquaculture as an alternative way to improve fish production while reducing input costs. One of the factors affecting periphyton-based aquaculture is determining which substrates are most suitable. Very little information is available on the biomass and composition of periphyton biofilm on net, stone and plastic substrates in southern Africa, therefore it needs to be investigated. Another factor affecting periphyton-based aquaculture is species since not all fish species can be cultured in periphyton-based aquaculture. Fish need a high degree of specialisation in the feeding, filtering, and masticating apparatus to efficiently graze on periphyton in periphyton-based aquaculture. Tilapia and African catfish are good candidates for periphyton utilisation because they are capable of efficiently ingesting and digesting a wide spectrum of food items ranging from phytoplankton to other fish species (Welcomme, 1985; Biswas et al., 2022). Periphyton is a conglomeration of different food items they feed on. Moreover, tilapia and African catfish are among the most widely cultured fish species in Africa (Moffitt & Cajas-Cano, 2014; Pouomogne, 2024). Seasonality has also been identified as a major factor influencing periphyton biomass and composition because of their short reproduction cycle. The sub-tropical climate in countries where periphyton-based aquaculture is thriving is similar to that in southern Africa (Signor et al., 2015; Mohapatra et al., 2016; Tortolero et al., 2016; David et al., 2022). Therefore, it is important to explore the potential of periphyton-based aquaculture in southern Africa.

The main objective of this study was to determine the utilisation of periphyton on stones, plastics and nets by *O. mossambicus* and *C. gariepinus* in aqua dams. The overall objectives of the study were to determine:

- ❖ the effect of net, plastic, and stone substrates on the diversity, biomass, and abundance of periphyton and bioseston in aqua dams.
- ❖ the effect of periphyton and bioseston on growth performance of *O. mossambicus* and *C. gariepinus* in aqua dams with net, plastic, and stone substrates.
- ❖ the feeding preference of *O. mossambicus* and *C. gariepinus* utilising periphyton and bioseston in aqua dams with net, plastic, and stone substrates in aqua dams.
- ❖ the biofiltration performance of net, plastic, and stone substrates as periphyton-biofilters.

1.3 Thesis Layout

The thesis is assessing the use of periphyton-based aquaculture to improve fish growth and water quality. This thesis is divided into seven chapters, where the utilisation of periphyton on three different substrates by *O. mossambicus* and *C. gariepinus* in aqua dams is addressed and the performance of periphyton as a biofilter is explored.

Chapter 2

This chapter focuses on the literature review of the study, where relevant literature on periphyton-based aquaculture is explored. The literature on the effect of periphyton on fish yields, the effect of different substrates on periphyton, the improvement of water quality by periphyton, and other methods used to grow natural food were reviewed.

Chapter 3

In this chapter, the composition and biomass of periphyton and bioseston in aqua dams with net, plastic and stone substrate was explored. This chapter determined and compared the diversity, biomass, abundance, dominance, and network of periphyton and bioseston in aqua dams with net, plastic and stone substrates in winter and summer.

Chapter 4

This chapter explored the utilisation of periphyton and bioseston by *O. mossambicus* in aqua dams with periphyton substrates. The chapter evaluated the growth performance of *O. mossambicus*, the feeding preference and also evaluated food selection by Mozambique tilapia utilising periphyton and bioseston in aqua dams with net, plastic and stone substrates.

Chapter 5

In this chapter, the ability of *C. gariepinus* to utilise periphyton and bioseston in aqua dams with net, plastic and stone substrates was explored. The chapter investigated whether African catfish could utilise periphyton and bioseston in aqua dams with net, plastic and stone substrates. It also evaluated the growth performance of African catfish, the feeding preference, and food selection by *C. gariepinus*. The difference in the feeding structure of African catfish and tilapia species was also highlighted.

Chapter 6

This chapter explored the performance of a periphyton-based biofilter system in a recirculation aquaculture system. This chapter compared the ability of net, plastic and stone to function as an effective biofiltration system to maintain optimum water quality.

Chapter 7

This is the general discussion, summing the findings from the previous chapters and comparing these to other published works. Recommendations on future studies are given and an overall conclusion and limitations of the study is presented.

Chapter 8

A complete reference list of all the sources cited in the thesis is given in this chapter.

CHAPTER 2: LITERATURE REVIEW

CHAPTER 2: LITERATURE REVIEW

The quality and quantity of periphyton in periphyton-based aquaculture are influenced directly and indirectly by a broad array of factors. Some of the most commonly reported factors are nutrient concentrations, nature and structure of the substrate, colonisation time, grazing pressure, species type, sunlight, temperature and season. The interaction among these factors is pivotal and complex in the periphyton community, thus some factors cannot be addressed individually. For instance, the nature (biodegradable or non-biodegradable) and structure of the substrate provide attachment sites for periphyton and can influence the establishment and development of periphyton. Sunlight and ambient temperature are the greatest influencers of water temperature while nutrients and temperature drive the biological processes and growth of periphyton. Seasonal change is expected to affect the production and composition of both periphyton and bioseston through its direct effect on the water quality parameters.

Hao et al. (2020) showed that seasonal temperature had a significant effect on the abundance and composition of periphyton in the experimental tanks. Studies by Rodríguez and Pizarro (2015), Kazanjian et al. (2018) and Zhu et al. (2022) agree that seasonality is a major limiting factor that causes variation in periphyton abundance due to changes in the temperature. Change in temperature affects the biomass and diversity of periphyton because of their short reproduction cycle (Winder & Cloern, 2010; Omidí et al., 2019; Hao et al., 2020; Puts et al., 2023). Several studies reported that biomass and diversity of periphyton increased with temperature (Piggott et al., 2015; Hao et al., 2020; Singh et al., 2024). The growth rate of fish stocked in periphyton-based aquaculture production systems will also be affected by seasonal changes in the composition of periphyton because the proximate composition of periphyton will vary with season, and this will affect the growth rate of the fish (van Dam et al., 2002). The current research will conduct experiments under real culture conditions in aqua dams to determine the influence of seasonal temperature on periphyton abundance. Studies done under real culture conditions also showed that periphyton abundance differed among seasons (Signor et al., 2015; Piñosa, 2018; Jha et al., 2018).

In aquaculture, the substrates that can be used include tree branches, aquatic plants, bamboo poles, PVC pipes, glass tubes, common reeds, jute sticks, sugarcane bagasse, and wood (Azim et al., 2005; Khatoon et al. 2007; Gangadhara & Keshavanath, 2008; Tammam et al., 2020). However, the texture, nature, and position of the substrate in the water column play a major role in the abundance of periphyton. In the current study, stones, plastics and nets will be the target substrates for periphyton-based aquaculture in aqua dams to determine periphyton abundance associated with different substrates. Several studies have shown that the use of biodegradable and nonbiodegradable substrates has a great influence on the growth and biomass of periphyton on the substrate (Gangadhara & Keshavanath, 2008; Zhang et al., 2013; Martins et al., 2014; Rosa et al., 2022). For instance, Piñosa (2018) showed that plastic stool substrate could grow sufficient periphyton for 1000-1500 fingerlings to graze on for three to four months during dry seasons. Azim et al. (2003) used glass as substrates for periphyton growth in 1000 L plastic tanks and showed that periphyton biomass on glass substrates was sufficient to double the specific growth rate and tilapia yield over a period of eight weeks. Miao et al. (2021) and Nurfadillah et al. (2023) reported that artificial substrates support better development and biomass of periphyton. On the contrary, other studies have shown that biodegradable substrates enhanced higher periphyton biomass in different production systems (Keshavanath et al., 2001; Keshavanath et al., 2004; Gangadhara & Keshavanath, 2008). However, most biodegradable substrates decompose before the experiments are concluded.

Bamboo is the most widely used biodegradable substrate due to its ability to enhance high periphyton biomass, its availability in the tropics, ease to use and have long durability (Azim et al., 2002; Azim et al., 2005; Khatoon et al., 2007; Keshavanath et al., 2012). For instance, Khatoon et al. (2007) reported the highest periphyton biomass and densities in ponds with the bamboo ($1137.2 \pm 0.6 \mu\text{g m}^{-2}$) substrates as compared to PVC pipe ($929.6 \pm 0.6 \mu\text{g m}^{-2}$), plastic sheet ($684.2 \pm 1.2 \mu\text{g m}^{-2}$), ceramic tile ($657.0 \pm 0.6 \mu\text{g m}^{-2}$) and fibrous scrubber ($79.1 \pm 0.6 \mu\text{g m}^{-2}$). Amisah et al. (2008) also reported better biomass on bamboo substrate ($6.82 \pm 2.79 \mu\text{g/cm}^2$) as compared to

green shade net ($5.24 \pm 2.27 \mu\text{g}/\text{cm}^2$), high-density polyethylene sheets ($4.64 \pm 2.06 \mu\text{g}/\text{cm}^2$), low-density polyethylene sheets ($4.51 \pm 2.08 \mu\text{g}/\text{cm}^2$) and polyvinyl chloride pipes ($3.10 \pm 1.57 \mu\text{g}/\text{cm}^2$). Keshavanath et al. (2001) reported the best periphyton biomass ($0.56 \pm 1.20 \text{ mg cm}^{-2}$, DM) and fish yield ($491 \text{ kg ha}^{-1} 90 \text{ d}^{-1}$) in tanks with bamboo poles, as compared to PVC pipes ($0.09 \pm 0.36 \text{ mg cm}^{-2}$ & $450 \text{ kg ha}^{-1} 90 \text{ d}^{-1}$) and sugarcane bagasse ($0.20 \pm 0.59 \text{ mg cm}^{-2}$ & 100 % mortality). These studies showed that bamboo substrates can enhance better periphyton biomass than most nonbiodegradable substrates. Hao et al. (2022) showed that bamboo poles can be used to control nuisance cyanobacteria blooms by enhancing the growth of competitors that consume cyanobacteria in aquatic systems. Due to these attributes, bamboo poles as substrate for periphyton have been suggested as a superior alternative substrate to improve fish production in periphyton-based aquaculture (Amisah et al., 2008). However, in Southern Africa, bamboo is not locally available thus net, plastic and stone will be used due to their availability.

The type of substrate used in periphyton-based aquaculture also affects the nutritional quality of periphyton. Studies have shown that periphyton contains 27.19 % crude protein, 18 % lipid and 52 % carbohydrates (Nielsen et al., 1997; van Dam et al., 2002; Muthoka et al., 2021). However, depending on the type of periphyton dominating the substrate, the quality of periphyton will significantly change. For instance, van Dam et al. (2002) reported that periphyton grazed from coral reefs had the lowest protein and energy content because it was mixed with sediment material whereas periphyton with a high abundance of bacteria, zooplankton and insects had the highest nutritional quality. Ledger and Hildrew (1998) reported that periphyton on stone substrates have as low as 2–3 % protein, 0.04–0.29 % lipid, and 29–33 % carbohydrate. On the other hand, the protein content of the periphyton growing on bamboo was reported to be 50 % higher than that on the jute stick and hizol tree branches (Azim et al., 2002). According to Kirk (2011), the light intensity can also affect the quality of periphyton growing on substrates. Light intensity changes depending on the season and geographical location. This means similar substrates can have different periphyton quality depending on the geographical location. This study will compare the periphyton growing on net, plastic and stones in Southern Africa.

Zhang et al. (2013) reported that substrate type, colonization time, or substrate morphology, biodegradable and non-biodegradable substrates have less effect on periphyton biomass if the nutrient levels are sufficient. However, Pacheco et al. (2022) suggested that sufficient nutrient alone without sunlight is not sufficient to stimulate periphyton growth. Results of other studies agree that nutrient availability and sunlight act synergistically to stimulate the periphyton biomass on substrates (Rosemond et al., 2000; Azim et al., 2005; Rodríguez & Pizarro, 2015; Pacheco et al., 2022).

Periphyton has been reported to improve water quality in different aquaculture production systems. A study by Kumar et al. (2017) in earthen ponds showed that periphyton was able to improve water quality by reducing ammonia nitrogen, nitrite, nitrate and biological oxygen demand. Another study by Patkar et al. (2021) showed periphyton lowered the low concentrations of ammonia and nitrite while keeping dissolved oxygen and pH optimum in periphyton-based ponds. Even in other studies, Milstein et al. (2009), Richard et al. (2010), and Jha et al. (2018), where water quality was monitored but was not the main objective, periphyton kept the water quality parameters within the desired range of the cultured species. These studies were done in ponds to determine the utilisation of periphyton by fish and periphyton was able to maintain optimum water quality while improving the growth of the cultured fish.

Periphyton can reduce the concentration of nitrogenous and phosphorus compounds, dissolve organic carbon content, and reduce the turbidity in different production systems (Azim et al., 2002; Khatoon, 2007; Singh et al., 2017; Ma et al., 2018; Oberholster et al., 2022). Due to the proven effectiveness of periphyton in keeping the water quality optimum, initiatives have been made to use periphyton in biofilters. For instance, Shahar and Guttman (2021) integrated *Ulva fasciata* biofilter with a periphyton biofilter and reported that periphyton was able to remove nitrate and increase the concentration of dissolved oxygen in water. Guttman et al. (2019) also reported that *Ulva*-periphyton biofilter were able to remove up to 97 % of ammonia from the system and also efficiently removed nitrate from the water. Another study by Guttman et al. (2018) also showed that periphyton integrated with *Ulva* sp. can

effectively reduce the amount of total nitrogen and total ammonia in a production system. However, these studies integrated *Ulva fasciata* and periphyton as one biofilter in mariculture. There is a notable absence of scientific literature exploring the use of periphyton-based stand-alone biofilters in recirculating aquaculture systems. Thus, the current study will evaluate the effectiveness of a periphyton-based biofiltration system deployed in fibreglass tanks, with the objective of determining the overall performance and feasibility of this biofiltration system.

Periphyton-based aqua has the potential of improving fish yield and survival rates (Keshavanath et al., 2001; Milstein et al., 2008; Abwao et al., 2014; Garcia et al., 2016; David et al., 2021; Zhao et al., 2023; Lowanshi et al., 2023). However, the success of periphyton-based aquaculture depends on the species cultured because not all fish are able to graze on periphyton. Fish used in periphyton-based aquaculture must have morphological and physiological adaptations to graze on periphyton. Fish species that are phytoplanktivorous, herbivores, omnivores, detritus and benthos feeders are said to be effective in utilising periphyton (van Dam et al., 2002; Azim et al., 2005). These fish species are also able to filter feed on bioseston. Bioseston is an important part of the periphyton community in periphyton-based aquaculture because the growth of periphyton enhances the growth of bioseston in the water column. 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). Tilapia species are commonly used in periphyton-based aquaculture systems, whereas catfish, despite their benthic feeding behaviour and natural predilection for periphyton and bioseston in their natural diet, are noticeably underrepresented in such systems. Thus, the current study will comparatively assess the suitability and performance of African catfish (*Clarias gariepinus*) and Mozambique tilapia (*Oreochromis mossambicus*) in periphyton-based aquaculture in aqua dams deployed with net, plastic, and stone substrate.

Extensive research has been done on periphyton-based aquaculture using tilapia species because it has morphological and physiological adaptations to utilise periphyton (Huchette et al., 2000; Azim et al., 2005; Garcia et al., 2016; Hlongwane et

al., 2024). For instance, Huchette et al. (2000) reported a significant reduction in periphyton biomass after 30 g tilapia was introduced in the cages in a periphyton-based aquaculture. Similar results were reported by Azim et al. (2003), Keshavanath et al. (2004) and Sakr et al. (2015), where the introduction of tilapia fingerlings significantly reduced the abundance of periphyton in tanks. The current study will determine the effect of grazing on the biomass and diversity of periphyton and bioseston by Mozambique tilapia in aqua dams with net, plastic and stone substrates. The diet of African catfish and other catfish species in the wild also shows that these species can readily utilise natural food items that are part of the periphyton and bioseston community. For instance, Dadebo et al. (2014) also showed that African catfish in the wild feed on other fish, zooplankton, phytoplankton, detritus, macrophytes, insects and gastropods.

The ontogenetic development of fish can lead to shifts in their periphyton and bioseston preferences in periphyton-based aquaculture. Studies have shown that most fish fry are largely carnivorous, feeding on zooplankton, aquatic larvae and even terrestrial insects that fall into the water (Njiru et al., 2004; Tareke et al., 2021; Temesgen et al., 2022). Tilapia fingerlings gradually begin taking more plant foods, including bioseston (phytoplankton) and macrophytes as they grow. Dadebo et al. (2014) showed that African catfish from a lake fed on insects and bioseston (zooplankton) when still fingerlings, larger African catfish largely feed on other fish, detritus, and plants. A study by Haque et al. (2022) reported that in the wild, catfish showed feeding plasticity and opportunistic feeding behaviour with a shift from carnivorous to omnivorous feeding nature depending on the size of the mouth. Mozambique tilapia and African catfish are opportunistic, changing their diet to whatever food items are abundant or available. An evaluation of the feeding habits and dietary preferences of Mozambique tilapia and African catfish in confined aqua dams with net, plastic and stone would provide valuable insight into their trophic interactions in periphyton-based aquaculture. This study will use Mozambique tilapia fingerlings and subadults to determine if there is a change in diet of different size groups.

The paucity of information on a promising aquaculture production system prompted this study. Mozambique tilapia and African catfish are the most widely cultured species in Southern Africa and their potential to utilise periphyton colonising stone, plastic and nets has not been evaluated before. It is hypothesised that if enough periphyton grows on substrates both Mozambique tilapia and African catfish can utilise the periphyton and this will lead to reduced feed cost.

Chapter 3:

**Composition of periphyton and bioseston in
aqua dams with net, plastic and stone
substrates during winter and summer.**

3. Composition of periphyton and bioseston in aqua dams with net, plastic and stone substrates during winter and summer.

3.1 INTRODUCTION

Periphyton-based aquaculture can potentially improve fish production in developing countries without increasing production costs. Periphyton-based aquaculture is a sustainable aquaculture practice that exploits periphyton as natural food for cultured species. It not only serves as food but also improves water quality (Milstein et al., 2008; Martins et al., 2014; Chikorela et al., 2019). The term periphyton has been defined several decades before but some definitions don't include most of the attached animal organisms (Wetzel, 1983). However, the definition was later improved to include all organisms found in periphyton. Van Dam et al. (2002) and Azim et al. (2005) defined it as the complex community of attached aquatic biota on submerged substrates, including associated non-attached organisms and detritus. Therefore, periphyton is a complex community of bacteria, phytoplankton, zooplankton, benthic organisms, fungi, organic detritus, and other invertebrates and their larvae (Azim et al., 2005).

Periphyton community can be categorised into five different groups which include periphytic algae, metazoan species, Ciliates, protists, and invertebrates (and their larvae) (Figure 3.1). It grows naturally on biodegradable and non-biodegradable substrates submerged in water and most aquatic species directly graze on it. 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). The growth of periphyton in water also enhances the growth of bioseston in the water column. Bioseston is also an important source of food for fish species in periphyton-based aquaculture. Bioseston are all the living autotrophic and heterotrophic organisms suspended in water such as phytoplankton, zooplankton, bacteria, and protozoa (Hildreth, 1980; Zieritz et al., 2019). Thus, the periphyton community can include the attached organisms (periphytic algae) and suspended organisms (bioseston) (Figure 3.1). The composition of periphyton and bioseston in production systems is influenced by various factors such as the type of substrate used, with biodegradable and non-degradable exerting distinct

effects on the community structure. In the wild, periphyton colonises on biodegradable and non-degradable substrates such as submerged leaves, macrophytes, stones, sand, gravel, and rocks (Zhang et al., 2013).

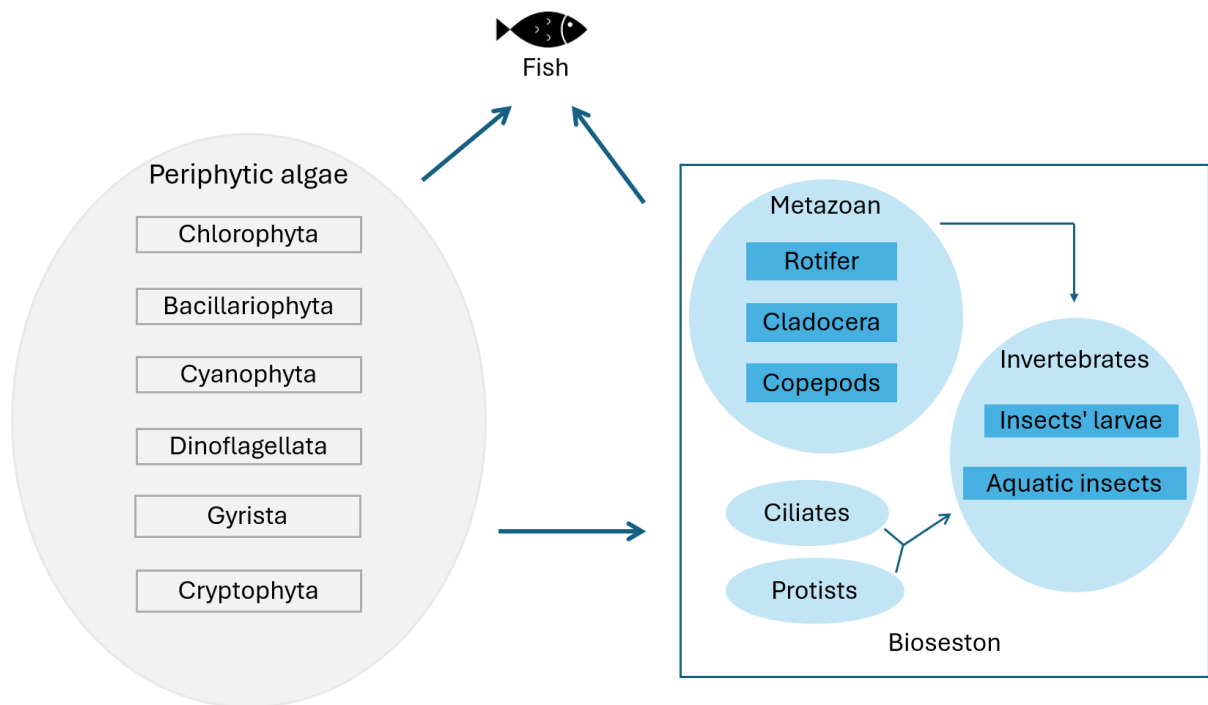


Figure 3. 1: The periphyton and bioeston community on substrates and the water column. The direction of the arrows indicates the flow of energy in the aqua dams.

The colonisation of periphyton on any substrate, whether biodegradable or non-degradable, generally starts with the deposition of organic substances and rapid bacterial attachment, followed by colonisation of algae and invertebrates then finally by filamentous algae periphyton development (Pfeiffer et al., 2013; Ferreiro et al., 2014; Vlaičević et al., 2021). However, biodegradable substrates commonly have high species composition because natural substratum leach nutrients that are absorbed by periphyton colonising the substrate. This means periphyton have access to both organic nutrients leaching from the substrate and inorganic nutrients from the water column. Thus, increasing the availability of phosphate, nitrate, sulphate and other nutrients for periphyton to utilise for growth. Several biodegradable substrates have been tested for periphyton production in periphyton-based aquaculture and a majority of them have resulted in increased fish production (Azim et al., 2005; Gangadhara &

Keshavanath, 2008; Asaduzzaman et al., 2009; Keshavanath et al., 2012; Tortolero et al., 2016). However, not all biodegradable substrates can be used in periphyton-based aquaculture because most can decompose before the fish production cycle ends. Moreover, macrophytes can also release distinct allelochemicals to inhibit periphyton growth (Hilt, 2006) and compete with periphyton for nutrients in the water (Zhang et al., 2018). Bamboo substrates are commonly used in periphyton-based aquaculture due to their proven ability to produce high-quality periphyton, their availability in the tropics, ease of use, and long durability (Azim et al., 2005; Khatoon et al., 2007; Keshavanath et al., 2012). Due to these attributes, bamboo commonly has the highest ($0.6 - 1.5 \text{ g/cm}^2$) periphyton biomass compared to other biodegradable substrates (Keshavanath et al., 2001; Azim et al., 2005). However, in Southern Africa, bamboo is not locally available. Therefore, the potential of other substrates needs to be tested.

Most non-biodegradable substrates have long durability compared to biodegradable substrates, but they cannot serve as organic nutrient supplier to periphyton. Several studies have reported improved fish production due to periphyton in systems with non-biodegradable substrates compared to systems without (Keshavanath et al., 2001; Azim et al., 2005). The most investigated non-biodegradable substrates include PVC pipes, glass, plastic material, ceramic tiles, glass fibre filters, plastic imitation of macrophytes and custom-designed materials such as aqua mats (Bratvold & Browdy, 2001; van Dam et al., 2002; Azim et al., 2005). Some studies have reported glass tubes and slides to be a suitable substrate for colonisation by periphytic algae in periphyton-based aquaculture (Danilov & Ekelund, 2001; Azim et al., 2005). On the other hand, other studies reported mosquito screens and plastic materials (PVC) as suitable substrates for periphyton colonisation (Richard et al., 2009; Azim et al., 2005). Nonetheless, the most important features when choosing a substrate for periphyton-based aquaculture are substrate availability, durability, complexity and texture. For instance, several studies have shown complex substrates lead to high periphyton biomass (van Dam et al., 2002; Casartelli & Ferragut, 2018; Hao et al., 2020). Moreover, substrates with longer durability can enhance periphyton growth for the whole production cycle of the cultured species. In southern Africa, very little information is available on the biomass and composition of periphyton on available

material that can be used as substrates in periphyton-based aquaculture. Greenhouse shade nylon net, high-density polyethylene (HDPE) plastic bottles, and Riverstone are a few of the substrates that can play an important role in aquaculture in the region due to their availability and durability. The current study will use (net, plastic and stone) substrates due to their availability, different textures, complexity and durability. Due to the difference in texture and complexity of these substrates, each substrate is expected to have different periphyton composition and abundance.

The net substrate has been used in several studies to determine its effect on the abundance and composition of periphyton in periphyton-based aquaculture systems. Biswas et al. (2022) used a white nylon net to determine the optimum surface area required for the growth of periphyton biomass serving as natural food in polyculture and reported that a net substrate equivalent to 75 % of pond surface area was most appropriate to maximise fish production. Milstein et al. (2009) used net substrates equivalent to 50 % of the pond surface to improve natural food production for tilapia and reported an improved fish growth rate in ponds with net substrates. However, the net substrate does not always result in high periphyton abundance and biomass. Dutta et al. (2013) showed that nylon net substrates had lower ($0.20 \text{ g}/0.02 \text{ m}^2$) periphyton biomass compared to palm leaf ($0.43 \text{ g}/0.02 \text{ m}^2$). Another study by Amirtharaj et al. (2022) compared the periphyton biomass on the net substrate with other substrates (bamboo poles, polyvinyl chloride pipes, low- and high-density polyethylene plastic sheets) and reported higher periphyton biomass on the bamboo poles ($6.82 \pm 2.79 \mu\text{g}/\text{cm}^2$) then followed by net substrates ($5.24 \pm 2.27 \mu\text{g}/\text{cm}^2$). It is evident from the literature that different types of net have been used in periphyton-based aquaculture and each type has yielded different composition and abundance. The net substrate has been used but the quantity of periphyton was different in different studies therefore, it is important to determine to what extent can it be used to boost periphyton production when deployed in aqua dams. In this study, nylon black nets will be used to determine periphyton composition and abundance in aqua dams.

Different periphyton substrates have been reported as appropriate to maximise periphyton growth due to their surface area texture, mesh size and colour. Plastic

substrates are smooth in texture and will lead to different periphyton compositions and biomass as compared to the net substrate. Richard et al. (2009) reported higher periphyton biomass on net substrates than on smooth substrates. Amirtharaj et al. (2022) also reported the lowest periphyton biomass on low ($4.51 \pm 2.08 \mu\text{g}/\text{cm}^2$) and high-density polyethylene ($4.64 \pm 2.06 \mu\text{g}/\text{cm}^2$) plastic as compared to bamboo ($6.82 \pm 2.79 \mu\text{g}/\text{cm}^2$) and net ($5.24 \pm 2.27 \mu\text{g}/\text{cm}^2$) substrates. Signor et al. (2015) determined periphyton biomass on wood, black plastic, acrylic, fibreglass, ceramics and glass and reported low biomass in summer and winter on the plastic substrate. Mohapatra et al. (2016) expounded on different textures (polyethylene, polypropylene, fibre-reinforced and acrylic plastic) of plastic to determine the periphyton abundance and reported the lowest abundance on fibre-reinforced plastic ($7.10 \pm 0.26 \text{ ml}/0.1 \text{ m}^2$) followed by polyethylene plastic ($4.43 \pm 0.35 \text{ ml}/0.1 \text{ m}^2$). The current study will use HDPE plastic bottles to determine periphyton composition and abundance in aqua dams. Aqua dams are increasingly becoming important in the culturing of fish, and it is a unique production system in southern Africa. An introduction of plastic substrates in aqua dams may enhance the production of tilapia in the region.

Stones are naturally used as periphyton substrates in natural water bodies and rarely used in periphyton-based aquaculture. A study by Lowe and Gale (1980) reported high ($2.2 \times 10^4 \text{ cell}/\text{mm}^2$) periphyton abundance on Riverstone substrate in the Susquehanna River. Periphyton abundance also differs on stone substrates depending on the position of the stone in the water body. Simangunsong et al. (2015) reported different periphyton abundance on stone substrates in the upstream ($630 \text{ cell}/\text{cm}^2$), midstream ($1.499 \text{ cell}/\text{cm}^2$) and downstream ($4.545 \text{ cell}/\text{cm}^2$) of Salo River. Another study in natural water by Trbojević et al. (2021) deployed artificial (glass, ceramic, willow and yew tree tiles) substrates in Sava Lake and concluded that they are potentially a good alternative for natural (pebbles and stones) substrate since there was no significant difference in species abundance among the substrates. Biggs (1989) showed that the position of the substrate in the river and the flow of the river affects the periphyton biomass. It is evident that stone substrates lead to different periphyton abundance depending on the position and depth of the water body. Therefore, it is important to determine periphyton composition and abundance on stones also in periphyton-based aquaculture systems. The colonisation of periphyton

on stones has only been investigated in rivers and lakes but not investigated in periphyton-based aquaculture systems yet is a readily available substrate that can be deployed in aquaculture production systems.

The deployment of periphyton substrates increases the average density of biosestons such as phytoplankton, zooplankton, and planktonic bacteria (Umesh et al., 1999; Mridula et al., 2003; Azim et al., 2005). This indicates that bioseston abundance responds to the presence of substrates in water. For instance, Konan-Brou and Guiral (1994) reported that the phytoplankton (bioseston) biomass in lagoons with periphyton substrate was eight times higher than in surrounding lagoon waters without substrates. This is because both phytoplankton (bioseston) and periphyton are primary producers and respond similarly to nutrient concentration and temporal variation. The response of phytoplankton and periphyton to various nutrient concentrations has been well-documented in various water bodies (Havens et al., 1996; Zębek, 2013; Rodríguez & Pizarro, 2015; Lanza et al., 2024). Furthermore, there is adequate literature showing that the nature of the substrate has a significant effect on the quantity and quality of periphyton (Azim et al., 2005; Zhang et al., 2013; Amirtharaj et al., 2022). However, previous studies did not look at how the nature of periphyton substrates also affects the abundance and composition of bioseston in aquaculture systems. Therefore, this chapter will investigate whether the nature of the substrate affects bioseston abundance and composition in aqua dams.

Grazing by metazoan, ciliates, protists, and invertebrates has also been identified as a key factor affecting the periphyton and bioseston abundance (van Dam et al., 2002; Azim et al., 2005; Ackermann et al., 2011). In this study, the composition and abundance of periphyton and bioseston will be investigated in the aqua dams with periphyton substrates without fish, thus grazing by metazoans and invertebrates will have a great effect. This is because metazoans primarily feed on periphytic algae (Ackermann et al., 2011). Therefore, intense grazing can lead to less diverse periphytic communities. Hillebrand and Kahlert (2001) reported that grazing had stronger effects on periphyton biomass and composition than nutrient enrichment. On the contrary, Gresens (1995) reported that grazers (chironomid midge larvae, snails,

Cladocera) did not significantly decrease the biomass of periphytic algae. However, not all components of the periphyton assemblage are equally susceptible to grazing. A study by Ackermann et al. (2011) showed that grazing by metazoans can reduce diatom abundance while the abundance of other periphyton with prostrate basal cells is unaffected by grazing. Grazing reduces periphyton biomass, however, algal productivity is enhanced by grazing because self-shading and competition for nutrients are reduced (van Dam et al., 2002; Azim et al., 2005). This is because grazing removes dead or senescent algae and keeps the algal assemblage in a productive, early successional stage (van Dam et al., 2002).

The season is also one of the factors that affect the biomass and diversity of periphyton and bioseston because of their short reproduction cycle (Winder & Cloern, 2010; Omid et al., 2019; Hao et al., 2020; Puts et al., 2023). Several studies reported that the biomass of periphyton and bioseston increased with temperature (Piggott et al., 2015; Hao et al., 2020; Singh et al., 2024). However, some studies contradicted this assertion (Borduqui et al., 2008; Ferragut et al., 2010; Hao et al., 2018). For instance, Pretorius et al. (2016) reported that the mean zooplankton biomass was significantly higher in winter (17.1 mg dry weight m^{-3} , DW) than in summer (9.5 mg DW m^{-3}). On the other hand, Shchapov and Ozersky (2023) reported that zooplankton abundance was the lowest in winter (0.9 ± 0.6 Ind. L^{-1}) and highest in summer (14.2 ± 15.1 Ind. L^{-1}). Periphytic algae also increase in biomass and abundance when the temperature increases (Azim et al., 2005). The effect of temperature on periphyton-based aquaculture has been documented in most parts of the world. However, there is very little information on the effect of seasonality on the composition and abundance of periphyton and bioseston in periphyton-based aquaculture systems in southern Africa. Periphyton-based aquaculture has been thriving 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. For instance, Brazil and India have hot summers (ranging from 20 to 40 °C) and cool winters (5 to 20 °C) (World Data Info, 2024). Similarly, South Africa has summer temperatures that range from 15 to 40 °C and winter ranges from 2 to 26 °C (World Data Info, 2024). Therefore, it is important to

explore the potential of periphyton-based aquaculture in southern Africa. This chapter will therefore focus on:

- Determining the composition and abundance of periphyton and bioseston in aqua dams with net, plastic and stone substrates.
- Determining the effect of seasons on periphyton and bioseston composition and biomass in aqua dams with net, plastic and stone substrates.
- Determining the pattern of the community structure of periphyton and bioseston in aqua dams in winter and summer using Network analysis.

3.2 MATERIALS AND METHODS

3.2.1 Study site

The study was conducted in aqua dams (7000 L) (Figure 3.2) at the Aquaculture Research Unit of the University of Limpopo, South Africa (23.8888° S, 29.7386° E). Aqua dams are plastic production systems that lack sediments and allochthonous material at the bottom. The university is located in the northernmost part of South Africa, Limpopo Province. The weather in Limpopo province is generally hot, having an average minimum temperature of 11 °C in winter and a maximum temperature of 40 °C in summer (World Data Info, 2024).



Figure 3. 2: Aqua dams used to conduct the experiments at Aquaculture Research Unit of University of Limpopo

3.2.2 Experimental setup

Nine aqua dams with net, plastic and stone were used to determine the composition and abundance of periphyton and bioseston in winter and summer. Aqua dams were made of a strong, flexible high-density polyethylene geomembrane material that is ultraviolet light resistant (Figure 3.2). Each aqua dam measured one meter in height and had a radius of 1.5 meters. The aqua dams were cleaned and filled with municipal water up to 80 cm and fertilised with chicken manure at a rate of 0.26 kg m⁻² (Jha et al., 2008). There was no aeration in the aqua dams, and water lost through evaporation was replaced weekly with matured water (water with no chlorine) from a reservoir tank. Net, plastic and stone were deployed separately in the aqua dams in a completely randomised design (Figure 3.3). The net substrate was a rough black greenhouse shading net made from nylon material, with a shading percentage between 70 and 75

%. The surface area of the net was 24.07 m². The plastic substrates were smooth 1 Liter bottles made of high-density polyethene (HDPE) and one bottle had a surface area of 0.0467 m². The stone substrates were smooth river stones, each with a surface area of 0.038 m². Weights (sinkers) were used to ensure that the net substrate remained submerged in the water. The 1 Liter bottles were bundled together in groups of ten, and weights were also used to keep them submerged in the water column. The 1 Liter bottles were cut open at the bottom to facilitate sinking. Each aqua dam was deployed with one net (with a surface area of 24.07 m²), 98 stones, and five bundles of plastic substrates. The deployment of each substrate was replicated three times in winter and summer. The experiments were conducted under natural field conditions during the summer and winter months. The summer months start from December to February while winter is in June until August. The experiment was conducted when it was colder in winter for 21 days and hottest in summer for 21 days.

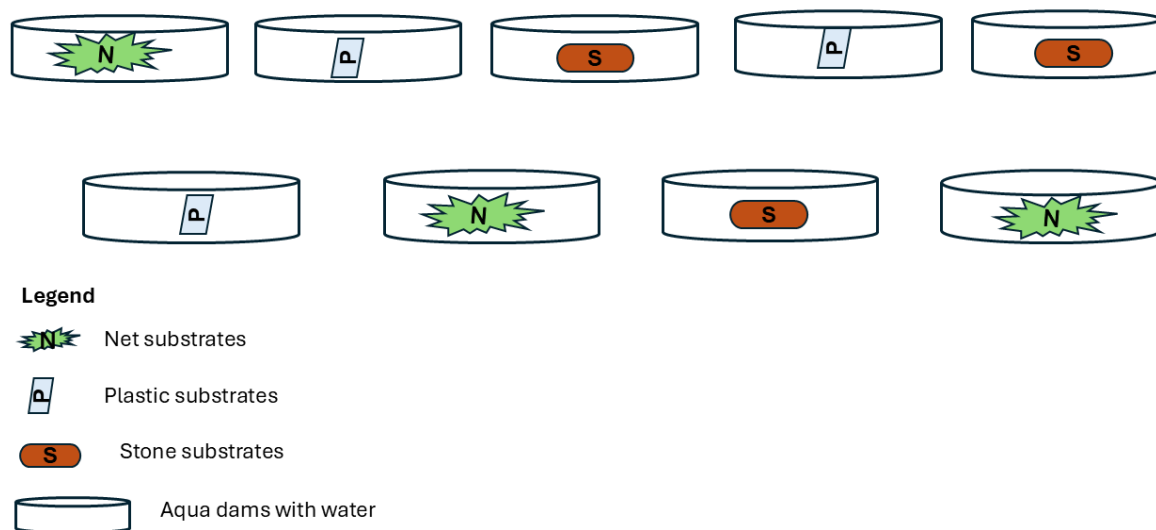


Figure 3. 3: The schematic diagram of the complete randomised design experimental set up of the aqua dams with net, plastic, and stone substrates.

3.2.3 Determination of periphyton abundance on net, plastic, and stone substrates in aqua dams.

Periphyton was allowed to colonise on nets, stones, and plastics for 21 days in aqua dams. On day 21, periphyton samples were collected by carefully brushing the substrate surface area (9 x 9 cm²) with a toothbrush to determine the periphyton

taxonomic composition. Samples from net and plastic substrates were collected from three depths: 20 cm, 50 cm, and 75 cm below the water surface. For the stone substrate, three random stones were sampled from different locations at the bottom of the aqua dam, which is 80 cm from the water surface. Three random stones were sampled since all stones were at the bottom, and none were on the surface of the water. The samples collected from the three depths (net and plastic) and locations (stone) were aggregated to form a single sample. The sample collection was standardized by brushing an equal surface area ($9 \times 9 \text{ cm}^2$) on all substrates. The periphyton brushed from the substrate was diluted with 25 ml distilled water for taxonomic composition under a light compound microscope (Leica E24) using an improved double Neubauer chamber W- Germany 0.100 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 four main squares then the value was 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 larger for the counting chamber. For this, a sample of 1 ml was loaded into the petri dish and examined with a light microscope (Zeiss, Axiolab, Germany). The periphyton was identified to genus level and was expressed as a number per cm^2 . Species counting was done according to standard procedure: All the species that occur in colonies like *Microcystis* sp. were counted as colonies. Filamentous species like *Mougeotia* sp. were counted as filaments. And those that occurred like *Cosmarium* sp. as cells were counted as cells.

Additionally, another set of periphyton samples was collected by brushing the surface of the substrate ($3 \times 3 \text{ cm}^2$) at the bottom (20 cm), middle (50 cm), and surface (75 cm) of the water column to determine the dry matter. The stone substrate samples were taken from three random stones at various locations on the aqua dam's bottom, ensuring standardisation in sample collection. The samples were dried in an oven according to method 10300 of APHA (1998). This method involved drying periphyton at 105 °C to constant weight and then igniting for 1 hour at 500 °C, the next step involved re-wetting the ash with reagent-grade water and drying to constant weight at 105 °C. The container used for drying the sample had a surface area of 0.00514 m^2 .

The frequency of occurrence and dominance index of the enumerated periphyton was calculated using the formula below. The frequency of occurrence was calculated separately per substrate, each substrate's frequency of occurrence is out of 100%. In the present chapter, only periphyton species with a dominance index of > 5 were considered.

$$\text{Frequency of occurrence (\%)} = \frac{\text{The number of ml in which spp is found}}{\text{Total number of ml examined}} \times 100$$

$$\text{Dominance index} = \frac{\text{The abundance of periphyton taxon}}{\text{Total abundance of all periphyton} \times \text{frequency of occurrence of taxon}}$$

3.2.4 Determination of bioseston abundance in aqua dams with net, plastic and stone substrates.

Samples were collected 20 cm below the water surface in the aqua dams with net, plastic and stone substrates using a truncated cone-shaped, silk bolting cloth net. The net used to collect bioseston had a mesh size of 71 µm. To collect the samples, water was filtered through the net. The cap of the plankton net was rinsed into a 5 L bucket using water from the aqua dam, and the contents were then decanted into 250 ml sample bottles. These 250 ml bottles contained the bioseston samples. The sample bottles were stored in a cool room at 4 °C until analysis under a light microscope using the counting chamber and petri dish as mentioned above (subsection 3.2.3). The bioseston was identified to genus level and was expressed as number per ml. The frequency of occurrence and dominance index of the enumerated bioseston was calculated as above (subsection 3.2.3).

3.2.5 Determination of water quality parameters in aqua dams

Water quality was monitored on a weekly basis. The water samples were collected using clean, pre-rinsed bottles to collect water from 40 cm below the surface of the water. Water quality parameters monitored are dissolved oxygen (mg/l), temperature (°C), pH, salinity (ppt) and electrical conductivity (ms/sc) using a YSI multi-probe meter. Transparency (cm) was measured using a Secchi disc. Nitrogen ammonia

(mg/l) was analysed according to method number 8038 of DOC316.53.01078 (Hach, 2012), and nitrate (mg/l) was analysed according to method number 8171 of DOC316.53.01069 (Hach, 2012). Total phosphate (mg/l) was analysed according to method number 8048 of DOC316.53.01119 (Hach, 2012).

3.2.6 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 igraph and vagan package, respectively. Network analysis was used to study the patterns of relationships and the network structure of the periphyton and bioseston 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).

A two-way Analysis of similarities (ANOSIM) was also performed in R using the vegan, MASS, effective, tidyverse, magrittr, and dplyr packages. Two-way ANOSIM was used to determine the significant difference between periphyton and bioseston composition on net, plastic and stone in the aqua dams. It was also used to determine significant differences in dry matter. Two-way ANOSIM was used because the data were not normally distributed, and the data had two factors (season and substrate). The normality and homogeneity of the water quality parameters were tested using Shapiro-Wilk and Levene tests, respectively using Statistical Package and Service Solutions (SPSS version 27). The water quality parameters data met the assumptions of normality and homogeneity. A two-way analysis of variance (ANOVA) was used to test for significant differences in the water quality parameters data using SPSS.

3.3 RESULTS

The species on the substrates were categorised to determine the most abundant category in the community of periphyton and bioseston in the aqua dams. Periphytic algae was the most abundant category on all substrates in both winter and summer (Table 3.1). The net substrate had the highest periphyton and bioseston abundance in both winter and summer, and the stone substrate had the lowest abundance. Protists and ciliates were more abundant in summer than in winter in the aqua dams. Protists and ciliates were most abundant in the aqua dams with stone in summer. Invertebrates were most abundant in aqua dams with the net substrate (in summer). Metazoan was also most abundant in summer in aqua dams with the net substrate (Table 3.1).

Table 3. 1: The categorised periphyton (no./cm², mean $\pm$ SD) and bioseston (no./ml, mean $\pm$ SD) in aqua dams with net, plastic and stone substrate during winter and summer

	Winter			Summer		
	Net	Stone	Plastic	Net	Stone	Plastic
Periphytic algae	35.10 $\pm$ 1.06	18.72 $\pm$ 0.63	30.02 $\pm$ 1.02	169.08 $\pm$ 7.52	125.83 $\pm$ 4.74	166.92 $\pm$ 6.16
Invertebrates	3.32 $\pm$ 0.36	1.08 $\pm$ 0.15	1.12 $\pm$ 0.17	27.90 $\pm$ 5.68	0.30 $\pm$ 0.09	2.830.49
Protists	0.54 $\pm$ 0.17	0.87 $\pm$ 0.26	1.46 $\pm$ 0.83	17.48 $\pm$ 1.08	18.33 $\pm$ 6.86	9.95 $\pm$ 0.29
Ciliates	0.15 $\pm$ 0.07	0.14 $\pm$ 0.06	-	31.00 $\pm$ 7.05	33.17 $\pm$ 4.38	10.923.05
Metazoans	6.36 $\pm$ 1.23	2.97 $\pm$ 0.47	1.76 $\pm$ 0.35	54.92 $\pm$ 8.97	-	0.57 $\pm$ 0.10
Total	45.47$\pm$0.98	23.77$\pm$0.53	34.35$\pm$0.85	300.38$\pm$7.26	177.62$\pm$4.40	191.18$\pm$5.07

Invertebrates were all the aquatic invertebrates and larvae (Ephemeroptera larvae, Chironomidae larvae, Diptera larvae, Drosophila larvae, *Notonecta glauca*, *Rhantus* sp., *Gerris* sp., Gyrinidae, *Corixa* sp., *Lumbricus* sp.).

Periphyton categories were subsequently identified at the genus level. The net substrate had the highest periphyton genera abundance while the stone substrate had the least abundance in winter and summer (Table 3.2). Shannon diversity index showed that genera diversity was comparable across the substrates in both winter and summer (Table 3.2). However, periphyton abundance on the net, plastic and stone substrates in winter and summer were significantly different from each other ($R = 0.6362$, $P = 0.0001$; ANOSIM).

Table 3. 2: The mean periphyton abundance (no./cm²) on net, plastic, and stone in winter and summer

	Winter			Summer		
	Net	Stone	Plastic	Net	Stone	Plastic
<i>Ankistrodesmus</i> sp.	-	-	0.36	0.90	0.00	0.00
<i>Chlorella</i> sp.	3.29	0.90	1.78	15.27	16.28	21.05
<i>Chlorogonium</i> sp.	0.42	-	1.34	-	-	6.38
<i>Coelastrum</i> sp.	1.39	0.03	0.87	11.47	-	-
<i>Cosmarium</i> sp.	0.21	-	0.04	1.38	5.02	-
<i>Dictyosphaerium</i> sp.	-	-	0.63	-	0.63	-
<i>Haematococcus</i> sp.	-	-	0.68	-	-	-
<i>Kirchneriella</i> sp.	-	-	0.51	-	-	-
<i>Micractinium</i> sp.	-	-	0.35	-	-	11.73
<i>Oocystis</i> sp.	1.43	0.42	0.14	-	7.03	-
<i>Pandorina</i> sp.	1.00	-	-	-	-	-
<i>Schroederia</i> sp.	-	0.23	-	-	-	-
<i>Scenedesmus</i> sp.	1.71	0.57	0.84	25.70	15.87	21.72
<i>Selenastrum</i> sp.	1.56	1.10	2.11	-	-	-
<i>Sphaerocystis</i> sp.	1.76	0.06	0.48	-	7.10	-
<i>Amphipleura</i> sp.	2.54	-	2.37	-	-	-
<i>Cyclotella</i> sp.	0.84	0.55	0.27	5.50	4.37	7.25
<i>Frustulia</i> sp.	-	0.62	-	-	-	-
<i>Pinnularia</i> sp.	1.80	2.21	3.03	3.17	-	4.48
<i>Rhoicosphenia</i> sp.	0.13	-	-	-	-	-
<i>Synedra</i> sp.	1.67	1.00	1.27	1.58	-	4.55
<i>Melosira</i> sp.	0.06	-	-	-	-	-
<i>Navicula</i> sp.	2.81	1.72	3.13	-	-	5.77
<i>Nitzschia</i> sp.	1.56	2.06	3.08	10.92	8.43	7.48
<i>Mougeotia</i> sp.	0.10	-	0.02	24.92	-	2.73
<i>Peridinium</i> sp.	-	-	0.07	-	-	-
<i>Mallomonas</i> sp.	-	-	-	-	-	2.22
<i>Vaucheria</i> sp.	-	0.32	-	-	-	-
<i>Chamaesiphon</i> sp.	-	1.56	-	-	-	-
<i>Aphanocapsa</i> sp.	-	-	-	-	-	12.45
<i>Coelosphaerium</i> sp.	-	-	-	-	7.40	-
<i>Oscillatoria</i> sp.	-	-	-	-	5.47	0.42
<i>Gloeotrichia</i> sp.	-	0.07	0.40	-	-	-
<i>Microcystis</i> sp.	3.56	1.33	3.77	25.47	5.68	13.87
<i>Spirochaete</i> sp.	2.28	1.02	0.84	-	-	-
<i>Bacillus</i> sp.	2.10	1.04	0.49	18.07	8.51	11.45
<i>Legionella</i> sp.	2.14	0.14	0.44	0.38	0.12	0.32
<i>Nostoc</i> sp.	-	0.07	-	-	-	-
Total	34.36	17.05	29.31	144.72	91.91	133.87
Diversity index	2.9099	2.8102	2.8849	2.4250	2.5992	2.6692

Bioseston was also identified to the genus level. The aqua dams with net substrate had the highest bioseston abundance in winter and summer (Table 3.3). Shannon

diversity index showed that the aqua dams with net substrate had higher genera diversity in both winter and summer. The bioseston abundance in aqua dams with the net, plastic and stone substrates in winter and summer were significantly different ($R = 0.7457$, $P = 0.0001$; ANOSIM).

Table 3. 3: The mean bioseston abundance (no./ml) in aqua dams with net, plastic, and stone in winter and summer

	<i>Winter</i>			<i>Summer</i>		
	Net	Stone	Plastic	Net	Stone	Plastic
<i>Ephemeroptera larvae</i>	0.62	0.08	0.18	8.77	-	1.48
<i>Chironomidae larvae</i>	0.53	0.17	0.18	17.75	0.30	0.70
<i>Diptera larvae</i>	0.95	0.31	0.17	0.52	-	-
<i>Drosophila larvae</i>	0.84	0.43	0.56	-	-	-
<i>Notonecta glauca</i>	0.08	-	0.03	-	-	-
<i>Rhantus sp.</i>	0.22	-	-	-	-	-
<i>Gerris sp.</i>	0.07	-	-	-	-	-
<i>Gyrinidae</i>	0.01	-	-	-	-	-
<i>Corixa sp.</i>	-	0.07	-	-	-	-
<i>Lumbricus sp.</i>	-	-	-	0.87	-	0.65
<i>Amoeba sp.</i>	-	-	0.01	7.07	-	3.02
<i>Diffugia sp.</i>	0.20	0.49	1.44	5.28	4.80	3.33
<i>Vorticella sp.</i>	0.34	0.38	-	5.13	13.53	3.60
<i>Halteria sp.</i>	0.15	-	-	-	-	-
<i>Euplotes sp.</i>	-	-	-	16.88	10.35	4.58
<i>Loxodes sp.</i>	-	-	-	-	4.43	-
<i>Stylonychia sp.</i>	-	-	-	5.30	8.45	-
<i>Paramoecium sp.</i>	-	0.14	-	8.82	9.93	6.33
<i>Brachionus sp.</i>	0.03	0.88	0.03	19.85	-	-
<i>Lecane sp.</i>	0.28	-	-	23.93	-	0.33
<i>Philodina sp.</i>	0.01	-	-	-	-	-
<i>Rotifers eggs</i>	0.23	0.57	0.71	10.57	-	-
<i>Ceriodaphnia sp.</i>	0.51	-	-	-	-	-
<i>Daphnia sp.</i>	0.67	-	-	-	-	-
<i>Diaphanosoma sp.</i>	0.31	-	-	-	-	-
<i>Leptestheria sp.</i>	0.06	-	-	-	-	-
<i>Bunops sp.</i>	0.03	-	-	-	-	-
<i>Actinosphaerium sp.</i>	0	0	0	3.06	9.51	0
<i>Peranema sp.</i>	0.25	1.16	0.36	8.31	12.55	14.06
<i>Trachelomonas sp.</i>	0	0	0	0.63	0.44	1.29
<i>Cryptomonas sp.</i>	0.48	0.51	0.33	11.5	11.28	15.65
<i>Cladocera</i>	-	0.15	-	0.57	-	0.10
<i>Copepods</i>	4.22	1.36	1.02	-	-	0.13
Total	11.11	6.73	5.04	155.67	85.72	57.32
Diversity index	2.2044	2.1659	1.7489	2.3163	1.7455	1.9605

Cladocera-unidentified & body fragments of Cladoceran. Copepods-Unidentified & body fragments of copepods

The dominance index was later determined to identify the most dominant periphyton and bioseston genera in the aqua dams. *Microcystis* sp., and *Chlorella* sp. (periphytic algae) were the most dominant periphyton on all substrates in winter (Table 3.4). *Scenedesmus* sp. (periphytic algae) was the most dominant periphyton on all substrates in summer. Copepods (metazoan) were the most dominant bioseston on all substrates in winter. *Euplotes* sp. (ciliates) was most predominant in all aqua dams in summer. The plastic substrates in winter were dominated by *Diffflugia* sp. while *Vorticella* sp. was most dominant in summer in aqua dams with the stone substrate (Table 3.4).

Table 3. 4: Dominance index of periphyton and bioseston composition in aqua dams with net, plastic and stone in winter and summer

	Winter			Summer		
	Net	Stone	Plastic	Net	Stone	Plastic
Periphyton						
<i>Chlorella</i> sp.	9.57	5.27	6.09	7.03	17.72	15.72
<i>Coelastrum</i> sp.	2.03	0.03	0.98	5.28	-	-
<i>Oocystis</i> sp.	2.08	1.25	0.24	-	7.65	-
<i>Scenedesmus</i> sp.	4.14	2.24	1.43	17.76	17.26	16.22
<i>Sphaerocystis</i> sp.	3.42	0.11	0.81	0.00	5.15	-
<i>Pinnularia</i> sp.	4.37	10.80	8.60	0.73	-	2.23
<i>Synedra</i> sp.	2.43	1.96	2.17	-	-	2.87
<i>Navicula</i> sp.	5.46	5.06	8.91	7.54	6.12	3.73
<i>Nitzschia</i> sp.	1.52	10.09	8.75	17.22	-	1.36
<i>Mougeotia</i> sp.	0.05	-	0.01	-	-	9.30
<i>Aphanocapsa</i> sp.	-	-	-	-	5.37	-
<i>Coelosphaerium</i> sp.	-	-	-	17.60	2.06	6.91
<i>Microcystis</i> sp.	10.36	7.78	10.71	-	-	-
<i>Spirochaete</i> sp.	5.52	3.01	0.95	4.16	6.18	2.85
<i>Bacillus</i> sp.	5.10	2.04	0.83	7.03	17.72	15.72
Bioseston						
Ephemeroptera larvae	5.56	0.40	1.80	3.75	-	0.58
Chironomidae larvae	4.76	1.25	1.83	7.60	0.23	0.82
Diptera larvae	8.52	3.10	1.12	0.11	-	-
Drosophila larvae	7.59	4.27	5.55	-	-	-
<i>Diffugia</i> sp.	0.60	2.43	23.84	1.13	1.87	2.59
<i>Vorticella</i> sp.	0.51	1.86	-	2.20	15.79	2.80
<i>Euplotes</i> sp.	-	-	-	7.23	12.07	5.35
<i>Stylonychia</i> sp.	-	-	-	2.27	6.57	-
<i>Paramoecium</i> sp.	-	0.35	-	3.78	11.59	2.46
<i>Brachionus</i> sp.	0.09	6.56	0.44	12.75	-	-
<i>Lecane</i> sp.	1.26	-	-	15.37	-	0.13
Rotifer eggs	1.71	5.66	7.05	6.79	-	-
<i>Cryptomonas</i> sp.	2.20	2.56	2.23	4.93	13.16	12.17
<i>Peranema</i> sp.	0.75	8.63	1.21	5.34	14.64	16.41
<i>Actinosphaerium</i> sp.	-	-	-	1.31	7.40	-
Copepods	37.95	16.77	10.07	-	-	0.05
Copepods-Unidentified & body fragments of copepods						

The frequency of occurrence was then used to identify the most frequently occurring periphyton genera in aqua dams. Periphytic algae were the most frequently occurring in winter (*Chlorella* sp. and *Microcystis* sp.) and summer (*Scenedesmus* sp. and *Chlorella* sp.) in all aqua dams (Figure 3.4).

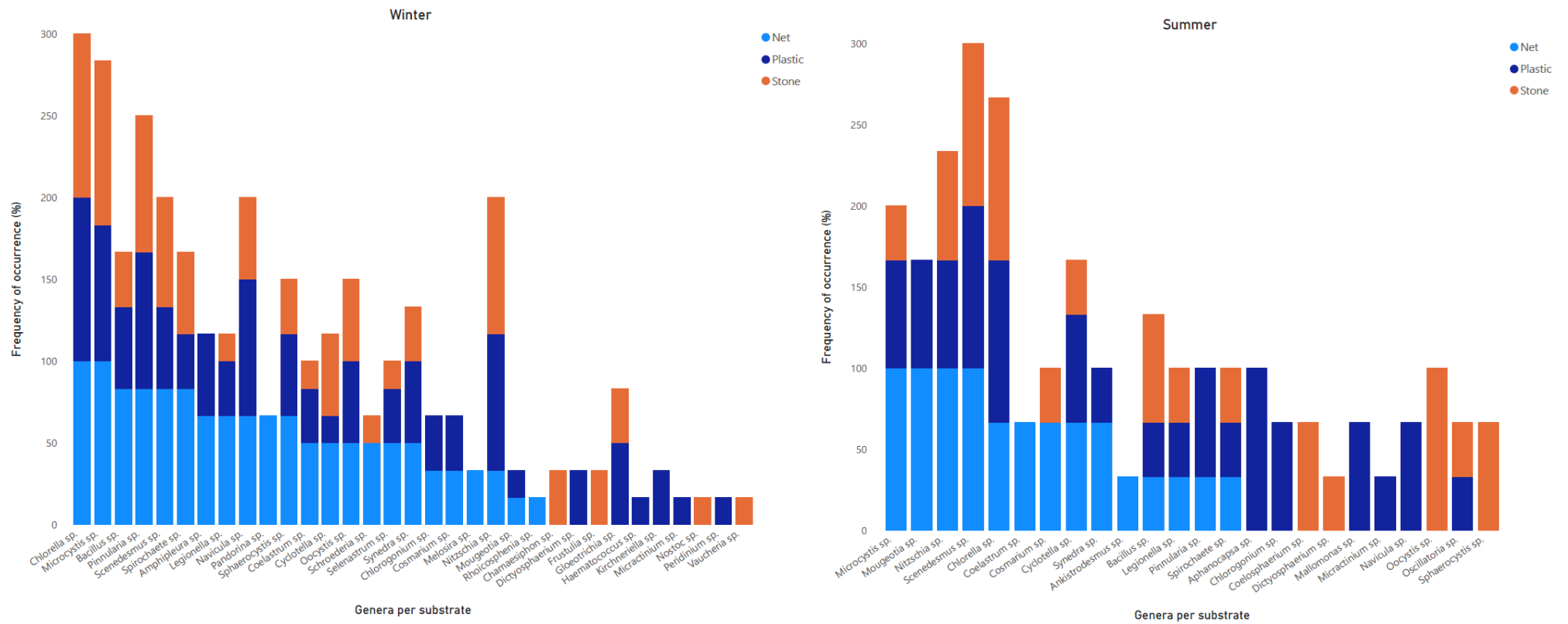


Figure 3. 4: The frequency of occurrence (%) of the periphyton on net, plastic and stone substrates in winter and summer.

The frequency of occurrence of bioseston was also determined to identify the most frequently occurring bioseston genera in the aqua dams. The most frequently occurring bioseston in winter were the copepods (metazoan) species and *Drosophila* larvae (invertebrates), Figure 3.5. The most frequently occurring genera in summer were *Euplotes* sp. and *Peranema* sp. (Figure 3.5).

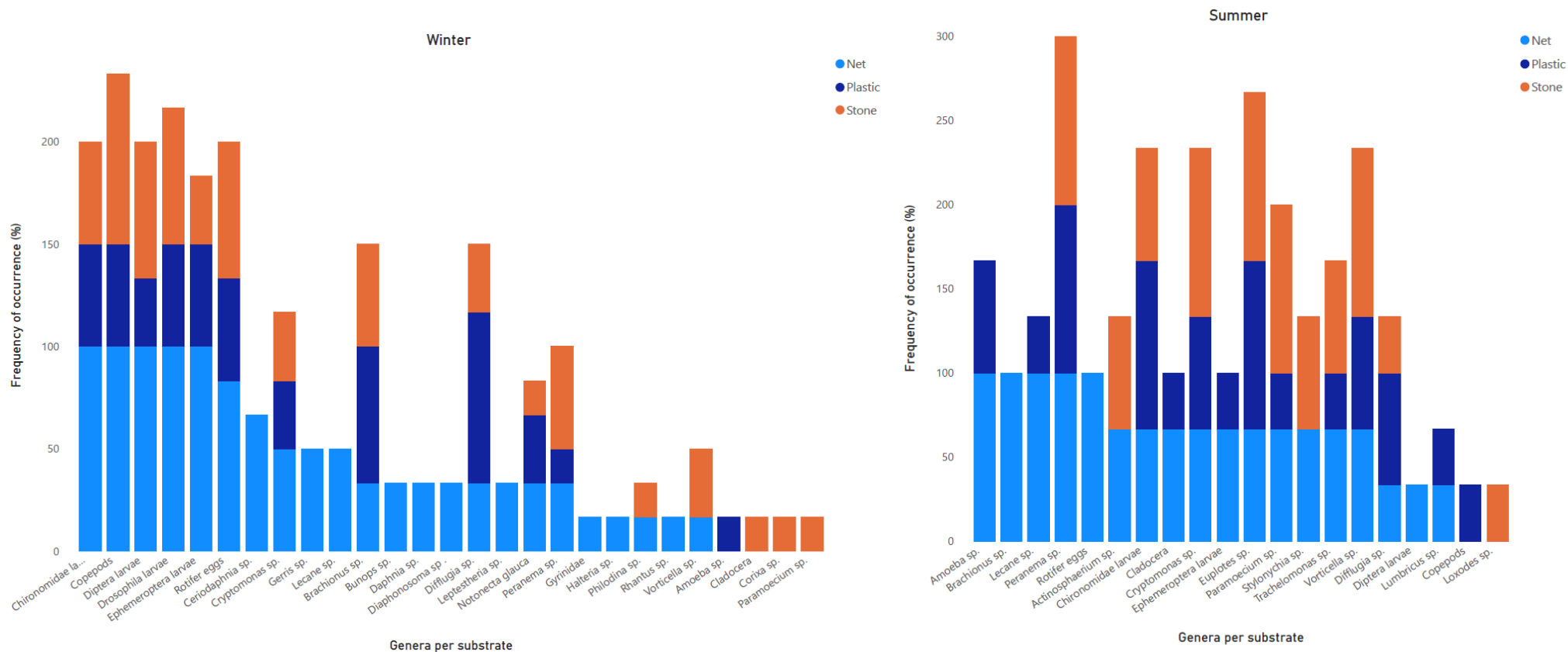


Figure 3. 5: Frequency of occurrence (%) of bioseston in aqua dams with net, plastic and stone in winter and summer. (Cladocera and copepods-body fragments and unidentified species)

The effect of substrate on periphyton biomass was determined. Periphyton biomass in terms of dry matter was determined to identify which substrate had the highest biomass in the aqua dams. The net substrate had the highest periphyton biomass, whereas the stone substrate had the lowest in both winter and summer (Figure 3.6). The dry matter on all substrates was significantly ($R = 0.4519$, $P = 0.003$; ANOSIM) less in winter compared to summer.

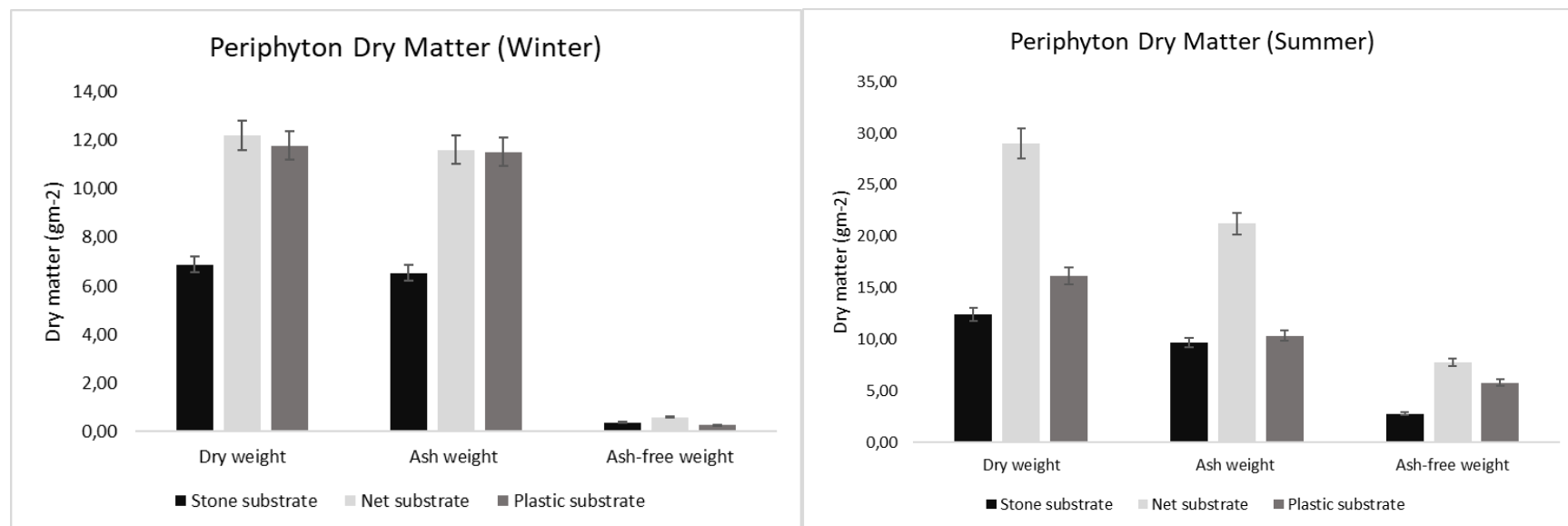


Figure 3. 6: Mean ($\pm$ SD) periphyton dry matter from aqua dams stocked with stone, net and plastic substrates for 21 days without fish grazing in winter and summer

Network analysis was used to determine the pattern of the community structure of periphyton and bioseston in aqua dams in winter and summer. The associations among genera in the communities were not complex. The network had a loose network structure with a fair closeness (0.6470) and betweenness centrality of 0.5209, reasonable degree centrality (0.7229), smaller edge density (0.0591), and small modularity of -0.0316 (Figure 3.7). The periphyton and bioseston community on the net substrate was more abundant in both seasons compared to plastic and stone substrates. The network structure connection shows that similar genera were most dominant in both winter and summer on all substrates (Figure 3.7).

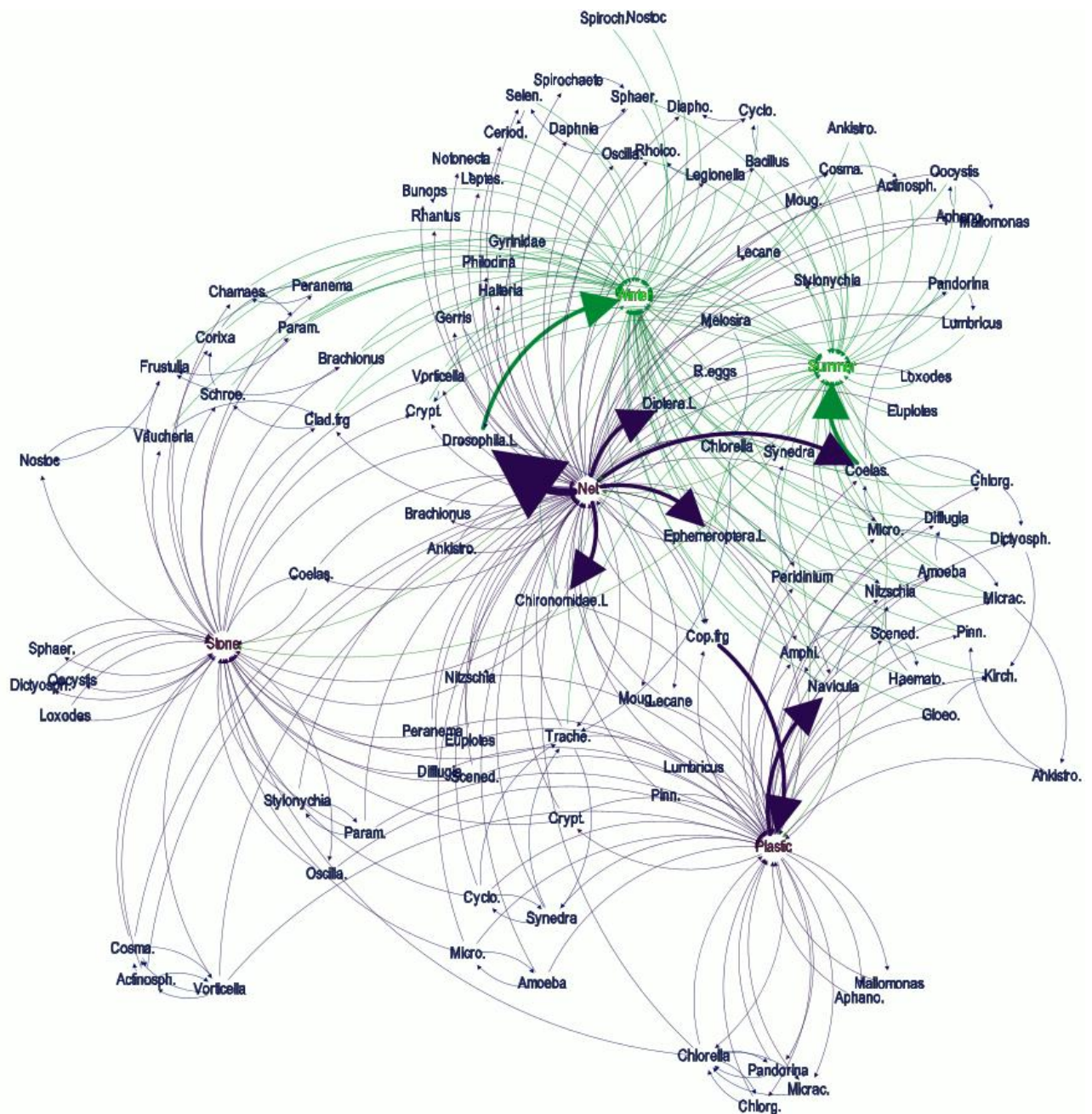


Figure 3. 7: The network structure of the periphyton and bioeston community in aqua dams with net, plastic and stone substrates in winter and summer. Abbreviation used: Ankistrodesmus sp.-Ankistro.;Chlorella sp.- Chlorella; Chlorogonium sp.- Chlorg.;Coelastrum sp.- Coelas.; Cosmarium sp.- Cosma.; Dictyosphaerium-Dictyosph.; Haematococcus sp.-Haemato.; Kirchneriella sp.- Kirch.; Micractiniumsp.- Micrac.; Oocystis sp.- Oocystis; Pandorina sp.-Pandorina; Schroederia sp. Schroe.; Scenedesmus sp.-Scened.; Selenastrum sp.- Selen.; Sphaerocystis sp.- Sphaer.; Amphipleura sp.- Amphi.; Cyclotella sp.- Cyclo.; Frustulia sp.- Frustulia; Pinnularia sp.- Pinn.; Rhoicosphenia sp.- Rhoico.; Synedra sp.- Synedra; Melosira sp.- Melosira; Navicula sp.- Navicula; Nitzschia sp.- Nitzschia; Mougeotia sp.-Moug.; Cryptomonas sp- Crypt.; Chamaesiphon sp.- Chamaes.; Gloeotrichia sp.- Gloeo.; Microcystis sp.-Micro.; Spirochaete sp.-Spiroch.; Bacillus sp.- Bacillus; Legionella sp.- Legionella; Nostoc sp. - Nostoc; Peridinium sp.- Peridinium; Peranema sp.- Peranema;

Vaucheria sp.-Vaucheria; Ceriodaphnia sp.-Ceriod.; Daphnia sp.-Daphnia; Diaphanosoma sp.-Diapho.; Ephemeroptera larvae- Ephemeroptera.L; Chironomidae larvae- Chironomidae.L; Diptera larva- Diptera.L; Drosophila larvae- Drosophila.L; Copepods fragments- Cop.frg; Cladocera fragments- Clad.frg; Notonecta glauca- Notonecta; Rhantus sp.- Rhantus; Leptestheria sp.- Leptes.; Bunops sp.- Bunops; Gerris sp.- Gerris; Gyrinidae–Gyrinidae ;Corixa sp.- Corixa; Amoeba sp.-Amoeba; Diffugia sp.- Diffugia; Halteria sp.-Halteria; Paramoecium sp.- Param.; Vorticella sp.- Vorticella; Brachionus sp.- Brachionus; Lecane sp.- Lecane; Philodina sp.-Philodina; Rotifers eggs-R.eggs; Aphanocapsa sp.- Aphano.; Oscillatoria sp.- Oscilla.; Trachelomonas sp.- Trache.; Actinosphaerium sp.- Actinosph.; Mallomonas sp.- Mallomonas; Dictyosphaerium sp.- Dictyosph. ; Cyclotella sp.- Cyclo.; Pinnularia sp.- Pinn.; Mougeotia sp.- Moug.; Cryptomonas sp.- Crypt.; Aphanocapsa sp.- Aphano.; Coelosphaerium sp.- Coeloshp.; Oscillatoria sp.- Oscilla.; Microcystis sp.-Micro.; Lumbricus sp.- Lumbricus; Stylonychia sp.-Stylonychia.

The effect of the different substrates on water quality parameters in winter and summer was also determined. The water quality parameters in the aqua dams were not significantly affected ($P > 0.05$) by the interaction of the substrates with seasons (winter and summer) (Table 3.5). Dissolved oxygen was significantly lower ($P < 0.05$) in aqua dams with the net substrate in winter. The aqua dams had significantly lower ($P < 0.05$) levels of electrical conductivity, salinity, pH, and total phosphate in winter than in summer (Table 3.5).

Table 3. 5: Mean $\pm$ SD of water quality parameters in aqua dams with net, plastic and stone substrates in winter and summer.

	Net		Plastic		Stone		P values		
	<i>Winter</i>	<i>Summer</i>	<i>Winter</i>	<i>Summer</i>	<i>Winter</i>	<i>Summer</i>	<i>substrate</i>	<i>season</i>	<i>interact</i>
Transparency (cm)	78.00 $\pm$ 1.73	70.00 $\pm$ 17.32	61.33 $\pm$ 19.13	60.00 $\pm$ 0.0	61.33 $\pm$ 14.0	70.00 $\pm$ 0.0	0.194	0.969	0.501
Temperature ($^{\circ}$ C)	14.32 $\pm$ 0.61	25.50 $\pm$ 0.43	13.05 $\pm$ 1.32	26.70 $\pm$ 0.91	13.93 $\pm$ 0.95	26.96 $\pm$ 0.86	0.484	<0.001	0.082
DO (mg/l)	4.40 $\pm$.27	3.11 $\pm$ 1.01	6.39 $\pm$ 1.19	8.10 $\pm$ 0.49	5.18 $\pm$ 1.08	6.82 $\pm$ 3.20	0.002	0.634	0.072
EC (ms/sc)	154.5 $\pm$ 9.2	210.3 $\pm$ 33.1	151.3 $\pm$ 22.7	178.7 $\pm$ 15.3	164.4 $\pm$ 11.8	185.3 $\pm$ 5.2	0.307	0.002	0.268
Salinity (ppt)	0.07 $\pm$ 0.00	0.10 $\pm$ 0.01	0.07 $\pm$ 0.01	0.08 $\pm$ 0.00	0.08 $\pm$ 00	0.08 $\pm$ 01	0.279	0.018	0.363
pH	7.03 $\pm$ 0.01	7.17 $\pm$ 0.01	7.04 $\pm$ 0.01	7.17 $\pm$ 0.01	7.04 $\pm$.01	7.15 $\pm$.01	0.244	<0.001	0.066
Nitrogen (mg/l)	0.02 $\pm$ 0.0	0.03 $\pm$ 0.02	0.06 $\pm$ 0.03	0.04 $\pm$ 0.02	0.03 $\pm$ 0.01	0.07 $\pm$ 0.02	0.066	0.372	0.077
Nitrate (mg/l)	3.47 $\pm$ 0.10	4.06 $\pm$ 0.25	3.49 $\pm$ 0.27	3.40 $\pm$ 010	3.71 $\pm$ 0.66	3.73 $\pm$ 0.20	0.219	0.280	0.190
Total phosphate (mg/l)	2.57 $\pm$ 0.20	6.11 $\pm$ 0.72	2.52 $\pm$ 0.28	6.46 $\pm$ 0.86	2.67 $\pm$ 0.16	6.96 $\pm$ 0.84	0.400	<0.001	0.563

Abbreviation used : DO- dissolved oxygen; EC- electrical conductive; interact-interaction between season and substrate. Each mean represents three samples from three replicate dams. Each mean represents samples from 3 replicates.

3.4 DISCUSSION

The net substrate had the highest periphyton genera abundance probably because of its position in the aqua dams. The net and plastic were close to the water's surface, in the euphotic zone layer that has the highest sunlight for photosynthesis. Periphytic algae are photosynthesizing species and are the first to proliferate in various aquatic systems even when exposed to sunlight. Dutta et al. (2013) also reported high periphyton growth on substrates when they were closest to the pond's surface. The texture and surface area of the net also provided sufficient surface for the attachment of periphytic algae. Levy et al. (2017) reported high periphyton abundance on nets in Israel and suggested that the net substrate allowed the periphyton to settle and develop on both sides of the net.

The abundance of periphytic algae on the net substrates also leads to high periphyton dry matter (biomass). The periphyton could easily adhere to the rough texture of the net substrate, which led to a high periphyton biomass. This agrees with Milstein et al. (2008) who reported that periphyton growth was greater on nets with a fine mesh (5.3-9.6 mg/cm²) than with a coarse mesh (3.7 - 4.0 mg/cm²) or on smooth plastic surfaces (1.4 - 2.6 mg/cm²). The periphyton biomass (28.96 ± 2.37 g/m², dry weight) reported in this study is higher than what was reported by Dutta et al. (2013) on nylon nets (0.20 g/0.02 m²) but lower than what was reported by Milstein et al. (2008) on nets (53 - 96 g/m²). It is worth noting that the plastic substrate was also in the euphotic zone but had lower abundance compared to the net due to its smooth surface area. However, periphyton biomass on the net substrate was higher compared to other biodegradable substrates used in other studies. Dutta et al. (2013) reported 0.43 g/0.02 m² (21 g/m²) periphyton biomass on palm leaf and Amirtharaj et al. (2022) reported 6.82 ± 2.79 µg/cm² (0.0686 g/m²) on bamboo poles. Gangadhara and Keshavanath (2008) also reported a lower periphyton dry matter of 0.897 mg/cm² (8.97 g/m²) on bamboo compared to the current results. This may suggest that net is a better periphyton substrate than these biodegradable substrates. In this chapter, the periphyton biomass (dry matter) on the net might also be explained by the high mineral content and dietary fibre from periphytic algae. Diatoms are also part of periphytic algae and have high silica content which contributes to ash-free weight. Green algae and diatoms have

high mineral content because they absorb minerals from water and retain them for growth (Liu, 2017).

The stone substrate had the lowest abundance of periphyton in winter and summer probably because they were completely submerged in the disphotic zone of the aqua dam. This means that the growth of periphytic algae is limited on the stone at the bottom of the aqua dams because of insufficient light. The high levels of DO, EC, nitrate, nitrogen, and total phosphate in aqua dams with stone substrates might indicate that the periphytic algae were not effective in utilising the nutrients. Moreover, unlike most natural substrates, stones are non-biodegradable and do not leach nutrients to support the growth of periphytic algae. The texture of the stone might also have influenced the abundance of periphytic algae. Schneck et al. (2011), Bergey et al. (2010) and Palmer et al. (2010) reported that benthic algae were influenced differently by the smoothness and roughness of substrate at the bottom of streams. Schneck et al. (2011) showed that total species richness was higher on rough than on smooth substrates and rough substrates had higher species richness and density of filamentous, motile, and metaphyton on substrates at the bottom of a stream in Rio Grande do Sul, southern Brazil.

Periphytic algae were the most frequently occurring in winter (*Chlorella* sp. and *Microcystis* sp.) and summer (*Scenedesmus* sp. and *Chlorella* sp.) in all aqua dams. *Chlorella* sp., *Microcystis* sp. and *Scenedesmus* sp. had the highest dominance and frequency of occurrence compared to other genera, in all the aqua dams. The abundance and dominance of these genera suggest that they have adaptive features that enable them to thrive in various environments. For example, *Scenedesmus* sp. has hooks that enable it to easily attach to the smooth texture of the substrate. Moreover, the periphytic algae were able to utilise nutrients in the aqua dams despite the temperature. This is because the optimal temperature for *Scenedesmus* sp., *Chlorella* sp., *Microcystis* sp. is between 22 °C and 35 °C (Ruiz-Martínez et al., 2015; Lindberg et al., 2022) but they are resistant to temperatures as low as 5 °C (Yang et al., 2020; Lindberg et al., 2022). The temperature recorded in this study in both winter and summer was within the optimum and tolerant range of periphytic algae. Canovas

et al. (1996) also reported the dominance of *Chlorella* and *Scenedesmus* in spring, autumn and winter in ponds in Meze, southern France. The temperature in Meze, southern France can be as low as 5 °C in winter, and high as 28 °C in summer (Weather Spark, 2024) and is similar to that of Limpopo province. In Limpopo province, winter temperatures range between 4.4. and 22.2 °C. The aqua dams had significantly lower levels of dissolved oxygen, electrical conductivity, salinity, pH, and total phosphate in winter than in summer. This probably indicates that the summer had more photosynthesizing species that increased the pH and DO levels. The accumulation of total phosphate, electrical conductivity and salinity also indicates that photosynthesizing species had sufficient nutrients for growth in summer.

The most dominant periphytic algae in winter and summer across the substrates was probably because they were resistant to grazing by aquatic invertebrates, metazoan, protists and ciliates. Grazing pressure depends on the size of the grazer, abundance, and feeding mechanism (Montemezzani et al., 2015). Therefore, the size, abundance and structure of the prey also play a major role in grazing, with smaller prey being consumed faster than bigger ones. Genera such as *Chlorella* sp., *Microcystis* sp., *Oocystis* sp. and *Aphanocapsa* sp. might be hard to consume at a fast rate since they occur in colonies. *Scenedesmus* sp. since they have hooks that can be used as defence and protection against grazing. *Mougeotia* sp. is filamentous and may be laborious to consume. Thus, they were more dominant across the substrates. Moreover, due to the high dominance of these periphytic algae, the dry matter was higher on the net substrate.

Invertebrates (Ephemeroptera larvae, Chironomidae larvae, Diptera larvae, *Drosophila* larvae, *Notonecta glauca*, *Rhantus* sp., *Gerris* sp., Gyrinidae, *Corixa* sp., *Lumbricus* sp.) were identified on all substrates but were most abundant on the net substrate in summer. Metazoans (*Brachionus* sp., *Lecane* sp., *Philodina* sp., rotifer eggs, *Ceriodaphnia* sp. *Daphnia* sp. *Diaphanosoma* sp., *Leptestheria* sp., *Bunops* sp., copepods and Cladocera) were also most abundant in summer in aqua dams with the net substrate. This was probably because metazoans and aquatic invertebrates are considered predators of periphytic organisms (Montemezzani et al., 2015), thus they

were more abundant on the net in summer when the food source (periphytic algae) was most abundant. This agrees with Ackermann et al. (2011) and Fröh et al. (2011) who reported that metazoans are effective grazers on periphytic algae on substrates at the bottom of the river Rhine in Germany. Hillebrand et al. (2000) also reported that invertebrates' larvae and metazoans dramatically reduced periphytic algae biomass to only a few percent of the total biomass in the Western Baltic Sea. Lawrence et al. (2002) also reported that aquatic invertebrates can graze off the entire superstructure of periphyton and reduce it to a thin layer of bacterial cells in rivers.

Protists and ciliates are also strong grazers of periphytic algae and bacteria (Weisse, 2017; Sommer et al., 2022). Protists and ciliates were more abundant in summer than in winter in all aqua dams probably because of high abundance of their food source (periphytic algae and bacteria) in summer. Moreover, *Euplotes* sp. (ciliates) and *Peranema* sp. were most dominant on all substrates in summer. *Peranema* sp. was most frequently occurring in summer probably because its optimum temperature for growth is above 22 °C (Saito et al., 2003). Yuan et al. (2024) also reported a high abundance of protozoa species in warmer water temperatures at Lake Liangzi in China. Vlaičević et al. (2021) also reported high ciliates abundance on glass sides in summer due to increased nutrients and temperature in Lake Sakada in Croatia. The dominance of *Diffugia* sp. and *Vorticella* sp. might be also explained by the high abundance of periphytic algae. This agrees with Chuen-chi (1994) who reported that the occurrence of the bioseston depends on water temperature and food abundance in a pond. In summer, the aqua dams with stone substrate had higher protists (*Vorticella* sp., *Diffugia* sp., *Amoeba* sp.) and ciliates (*Halteria* sp., *Euplotes* sp., *Loxodes* sp., *Stylonychia* sp., *Paramoecium* sp.) abundance probably because these groups are detritivores and bacterivores. The abundance of protists and ciliates on bottom substrates did not differ from those reported by Wilbert (1969), Foissner (2004), and Ackermann et al. (2011). This was probably because protists and ciliates are found at the bottom of ponds due to their ability to maintain fertility in the sediments by releasing nutrients when they digest bacteria (Foissner, 2004).

Copepods were the most dominant bioseston in all aqua dams with substrates in winter probably because low temperatures were optimum for their growth. Other studies have reported high copepod abundance in association with low water temperatures around 15 °C (Haberman & Haldna, 2017; Hansen et al., 2010). In this chapter, the winter temperature was around the same range. Furthermore, their abundance in winter might suggest that they were sufficient food and few predators. Copepods primarily feed on algae and also prey on ciliates, small rotifers and bacteria (Montemezzani et al., 2015). However, copepods can consume plant detritus, bacteria and decomposing materials in water when the food chain shifts due to low temperatures (Montemezzani et al., 2015; Harfmann et al., 2019). The death and decomposition of most organisms due to low temperatures probably lead to increased food availability for copepods.

The network structure of the periphyton and the bioseston community identified on three substrates in winter and summer had small modularity. The small modularity might be explained by the fact that the net substrate was connected to most genera in both seasons. Its texture and surface area lead to the highest periphyton and bioseston abundance. The difference in the texture and position of the substrates in the aqua dams also resulted in differences in periphyton ($R = 0.9863$) and bioseston ($R = 0.5931$) composition. Moreover, the small modularity indicates that the genera on one substrate were most likely to be found on another substrate despite the season. This was because only periphytic algae were the most abundant and dominant on all substrates in both winter and summer. Shannon diversity index had a similar diversity range for periphyton (2.4250 to 2.9099) in both summer and winter and for bioseston (1.7455 to 2.2044) in both seasons. Low diversity might be explained by the similarities in water quality parameters. Periphyton and bioseston respond similarly to water quality parameters due to an overlap in the genera that make up the periphyton and bioseston community. Garg (2005) and Lamprecht et al., (2022) also reported that the periphyton community includes phytoplankton, zooplankton and other bottom organisms in combination with microbial biofilms. This definition confirms a strong overlap between periphyton and bioseston species. The main distinction is that periphyton includes all organisms that live attached to substrates while bioseston organisms live in the water column. However, most periphyton dislodge from a

substrate and become part of the bioseston community. On the other hand, most bioseston can be found crawling within the substrate surface area even though they are not attached. This causes an overlap in the periphyton and bioseston genera. In this study, some of the genera classified as periphytic algae were not attached to substrates using attachment organs but were found within the periphyton samples in abundance. However, the network analysis of the periphyton and bioseston communities did not show an overlap between the two communities.

In conclusion, the texture, surface area and position of the substrate influenced the abundance and composition of periphyton and bioseston in aqua dams. The net substrate had the highest periphyton and bioseston biomass and the stone substrate had the lowest. Seasons also influenced the abundance and composition of periphyton and bioseston. Summer had higher periphyton and bioseston abundance than winter. Periphytic algae was most abundant and dominant in both winter and summer on all substrates indicating their resistance to grazing pressure by the metazoans, ciliates, protists, and aquatic invertebrates. Furthermore, protists and ciliates were more abundant in summer than in winter. The network structure of periphyton and bioseston community was weak indicating that the genera on one substrate were most likely to be found on another substrate despite the season. This might have been caused by low genera diversity due to the similarities in water quality parameters and the high abundance of periphytic algae in both seasons.

CHAPTER 4:

**The utilisation of periphyton and bioseston by
Mozambique tilapia (*Oreochromis
mossambicus*) in aqua dams with net, plastic
and stone substrates.**

4. The utilisation of periphyton and bioseston by Mozambique tilapia (*Oreochromis mossambicus*) in aqua dams with net, plastic and stone substrates

4.1 INTRODUCTION

Periphyton-based aquaculture is becoming a sustainable aquaculture practice that exploits periphyton as natural food for herbivorous and omnivorous species. Periphyton-based aquaculture involves using submerged substrates on which a community of natural food such as bacteria, fungi, protozoa, chironomids and algae colonise (Azim et al., 2005; Abwao et al., 2014). It is primarily used to reduce the high cost of fish feed by decreasing or replacing the input of pellets. The growth of periphyton on substrates in water also enhances the growth of bioseston in the water column. *Tilapia* species are commonly used in periphyton-based aquaculture because of their ability to scrap periphyton from substrates and filter feed on bioseston. Moreover, *Tilapia* species are predominantly used in periphyton-based aquaculture due to their ability to effectively digest periphytic algae and bioseston materials. *Tilapia* species have a stomach pH below 1.4, which lyses the plankton cell walls (Rivera Vasconcelos et al., 2018; Temesgen et al., 2022). *Tilapia* species also have morphological adaptations such as small-notched teeth, toothbrush-like pharyngeal teeth, premaxillary protrusibility, low jaw extensibility, and extended intestines that enable them to graze, masticate, and digest periphyton (Azim et al., 2005).

Tilapia species have two movable upper jaws (premaxilla and maxilla) and a lower mandibula jaw. These jaws have high protrusibility and extensibility and are used for a wide array of adaptive functions when feeding from substrates (Azim et al., 2005). It also has two pharyngeal teeth used for triturating the ingested food items and gill rakers essential for filter feeding (Skelton, 2001). The gill rakers of tilapia can be short and widely spread or long and numerous with long gill filaments and lamellae depending on the species (Beyruth et al., 2004; Zayed & Mohamed, 2004; Ibrahim et al., 2015). The fish use gill rakers to secrete mucous to trap bioseston through filter feeding. Another aspect necessary for digestive function is the physiological response to the ingested food items. Tilapia is physiologically equipped to digest periphyton and

bioeston due to the structure of the digestive tract. The stomach of tilapia can be segmented into the esophagus-gastric lumen passage, descending glandular portion, fundic portion, ascending glandular portion, and gastric-pyloric transition portion (Palladino et al., 2023). Each segment of the stomach plays a vital role in excreting necessary gastric acid and digestive enzymes to create a highly acidic ($\text{pH} < 1.4$) stomach environment that digests algae and bacteria. Moreover, tilapia species have long intestines. The size of the stomach and the length of the intestine play an important role in the digestion of food items since they ultimately determine how long the food will be exposed to gastric acid.

Some studies have reported significant growth of tilapia in ponds with periphyton substrates as compared to ponds without substrates. For instance, 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. Tammam et al. (2020) recorded the best growth rates, feed efficiency, body composition, immune response, and overall health status of tilapia in ponds with periphyton substrates. A study by Milstein et al. (2013) reduced commercial feed input by 30-40 % without significantly affecting tilapia growth rate in ponds with periphyton substrates as compared to ponds without periphyton substrates. Azim et al. (2002) reported a net yield of $6700 \text{ kg ha}^{-1} \text{ year}^{-1}$ in a periphyton-based system compared to a net yield of $2340 \text{ kg ha}^{-1} \text{ year}^{-1}$ in the control system without substrate in polyculture systems. Maximum production of $8000 \text{ kg ha}^{-1} \text{ year}^{-1}$ of tilapia was achieved in acadja-enclosed in the Ebrie Lagoon, Ivory Coast (Hem & Avit, 1994). This is because periphyton's nutritional quality is adequate to support the dietary needs of some fish species (van Dam et al., 2002; Azim & Asaeda, 2005; Muthoka et al., 2021).

The dietary needs of most fish species change with stages of development, and this induces ontogenetic dietary shifts and selectivity (Njiru et al., 2004; Tareke et al., 2021). Tilapias are among the species that naturally undergo the ontogenetic dietary

shift in the wild (Lowe-McConnell, 2000; Haque et al., 2022). At an early stage of development (the standard length of 2–3 cm) tilapia feed more on zooplanktons then later change to feed on phytoplankton, macrophyte and detritus at the standard length of 6–7 cm (Rao et al., 2015). Changes in the dietary preferences of a fish species largely depend on the size of the fish, the size of the prey and the availability of preferred prey items in the associated environment (Temesgen et al., 2022; Haque et al., 2022). In the wild, some fish species exhibit habitat shifts in search of preferred food items while some species can switch to food items that are more readily available when their preferred food source is scarce (Araújo et al., 2016; Birnie-Gauvin et al., 2017). Tilapias are opportunistic feeders and will utilise the most readily available food items. Therefore, the dominance of certain food items in the environment might correspond with their predominance in the stomach of tilapia. The evaluation of the feeding preferences of Mozambique tilapia in periphyton-based aquaculture production systems is vital for discovering the trophic interactions in confined production systems.

Periphyton and bioseston are rich in vitamin precursors, growth promoters and essential fatty acids, which are readily absorbed and utilised by aquatic organisms (van Dam et al., 2002; Awasthi et al., 2006). Thus, periphyton-based aquaculture is commonly used to partially or replace commercial pellets thereby reducing production costs, especially for small-scale fish farmers. However, the growth performances of the cultured fish depend on the abundance and accessibility of periphyton on the substrates in the production system. The complexity of the substrate must not hinder grazing by cultured fish. In the wild, periphyton can grow on rocks, sediments, and macrophytes which aquatic organisms can easily graze on (Azim et al., 2005). In aquaculture, the selection of substrate plays a crucial role in maximizing periphyton and bioseston biomass production. Although a range of hard materials are used as substrates in periphyton-based aquaculture, only substrates that optimise periphyton and bioseston growth and biomass are preferred. Furthermore, substrates that are used in periphyton-based aquaculture must be able to produce high-quality periphyton, must be locally available and easy to use, and have long durability to complete the fish production cycle. The most investigated substrates in periphyton-based aquaculture include bamboo, PVC pipes, glass tubes, common reeds, jute

sticks, sugarcane bagasse, and wood (Azim et al. 2005, Khatoon et al. 2007). The current study used net, plastic, and stone, due to their local availability, to determine the growth performance of Mozambique tilapia utilising periphyton in aqua dams.

The deployment of periphyton substrates increases the average density of biosestons such as phytoplankton, zooplankton, and planktonic bacteria (Umesh et al. 1999, Mridula et al. 2003, Azim et al. 2005). Van Dam et al. (2002) also reported that, based on the stomach content, tilapia does not utilise periphyton only but also bioseston (nanoplankton). Dempster et al. (1993) reported that the mean ingestion rate of natural food by tilapia was higher ($2 \text{ mg g}^{-1} \text{ h}^{-1}$) when it was offered as periphyton than when it was offered as phytoplankton (bioseston), and the mean ingestion rate of natural food was 25 times higher ($5 \text{ mg g}^{-1} \text{ h}^{-1}$) when periphyton and bioseston were offered together. This is because tilapia species have two mechanisms to capture and eat their food items. It can capture food using visual predation and filter-feeding through the gill rakers (Beveridge & Baird, 2000). Filter feeding enables them to also feed on small bioseston organisms while visual predation is effective in feeding from the substrates and on larger bioseston species. Tilapias are also opportunistic omnivorous feeders and will feed on food items that are most available and abundant in water (Beveridge & Baird, 2000). Most studies done in periphyton-based aquaculture did not determine whether tilapia was utilising periphyton or bioseston. This chapter will determine whether Mozambique tilapia prefers feeding on periphyton or bioseston. The objectives in this chapter are:

- To determine the effect of substrate on growth performance of Mozambique tilapia utilising periphyton and bioseston in aqua dams with net, plastic, and stone in aqua dams.
- To determine whether Mozambique tilapia of different size groups preferred periphyton or bioseston in the aqua dams with substrates.

4.2 MATERIALS AND METHODS

4.2.1 Study site

The study site is described in Chapter 3, section 3.2.1. The experiments for this chapter were conducted from January 2022 to March 2023 at Aquaculture Research Unit.

4.2.2 The utilisation of periphyton and bioseston by Mozambique tilapia in aqua dams with net, plastic and stone

The effect of substrate on the growth performance of Mozambique tilapia was determined by deploying net, plastic and stone substrates separately in the 7000 L aqua dams. Twenty-seven aqua dams were used in triplicates in a complete randomised design (Figure 4.1). Details about the texture and positioning of the substrates in the aqua dams can be found in Chapter 3, sections 3.2.2 and 3.2.3. The aqua dams were fertilised with chicken manure at the rate of 0.26 kg/m² (Jha et al., 2008) before the fish was stocked. The fertilisation occurred once at the beginning of the experiment, and the fish were introduced seven days later. There was no aeration in the aqua dams, and water lost through evaporation was replaced weekly with matured water (water with no chlorine) from a reservoir tank. Tilapia fingerlings (5.44 ± 0.81 g, mean ± SE) were obtained from the Aquaculture Research Unit's recirculating system and stocked at the density of 2.12 fish m⁻² in all the aqua dams with different substrates.

To determine the utilisation of periphyton and bioseston by Mozambique tilapia, three feeding regimes were designed. Three distinct experiments were carried out, each with three feeding regimes, which resulted in a total of nine aqua dams. These experiments were not conducted simultaneously but took place in the same season, from January 2022 to March 2023, using all twenty-seven aqua dams. The first feeding regime served as the control treatment, where the fish were fed only commercial

pellets twice daily, without any periphyton substrates in the aqua dams. This is the normal feeding regime at fish farms, and it is equivalent to a 100% feeding regime. This feeding regime was designated N100 for the net substrate, P100 for the plastic substrate, and S100 for the stone substrate. In the second feeding regime, the fish were fed commercial pellets every other day (twice a day) with periphyton substrates in the aqua dam, and this was equivalent to a 50 % feeding regime. The feeding regime was designated N50 for the net substrate, P50 for the plastic substrate and S50 for the stone substrate. The third feeding regime involved feeding the fish commercial pellets every third day (still twice a day), equivalent to a 33% feeding rate. The third feeding regime was designated N33 for the net substrate, P33 for the plastic substrate and S33 for the stone substrate. Three distinct experiments were carried out, each with three feeding regimes (Figure 4.1), which resulted in a total of nine aqua dams. These experiments were not conducted simultaneously but took place in the same season, from January 2022 to March 2023, using all twenty-seven aqua dams. The amount of commercial pellets fed to the fish in all treatments was recorded consistently. Each experiment in the aqua dams ran for six weeks. The temperature was 26.81 ± 2.29 °C (mean $\pm$ SD), and dissolved oxygen was 5.65 ± 1.83 mg/l in all the aqua dams.

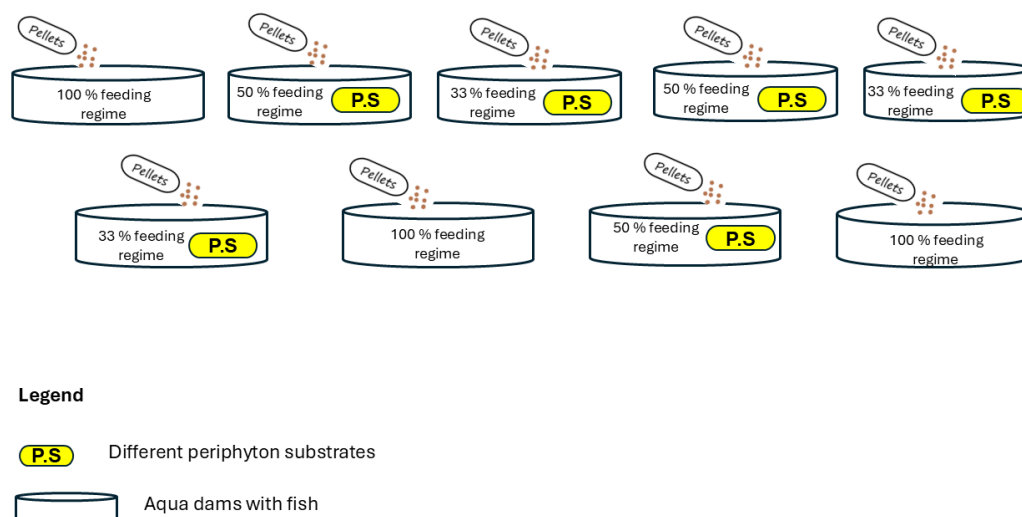


Figure 4. 1: A schematic representation of the complete randomised design of the experimental setup used to determine the utilisation of periphyton and abioseston by fish

4.2.3 Determination of periphyton and bioseston in aqua dams with Mozambique tilapia

The collection of periphyton and bioseston samples was carried out as described in Chapter 3, Section 3.2.3. and 3.2.4. Samples were collected every three weeks. The frequency of occurrence and dominance index of the identified periphyton and bioseston was calculated using the formula Chapter 3, Section 3.2.3. In this chapter, only those genera of periphyton and bioseston with a dominance index of > 5 were considered in the analysis.

4.2.4 Determination of growth performance of Mozambique tilapia utilising periphyton and bioseston in aqua dams

To evaluate the growth performance of the Mozambique tilapia in the aqua dam with substrates (section 4.2.2), tilapia were allowed to utilise periphyton and bioseston for a period of six weeks before measuring the growth performance indices. The total weight (to the nearest 0.01 g) of all the fish in the aqua dams was determined using a bench scale. The standard length of the fish was also determined using a tape measure to the nearest 0.5 mm. This was done before the fish were stocked into the aqua dams and at termination of the experiments. The recorded weights and standard-length measurements were then used to calculate the following growth performance indices:

Average daily weight gain = final weight gain (g)/time

Percentage weight gain = [(final weight – initial weight)/initial weight] × 100

Specific growth rate (SGR) = (ln final weight – ln initial weight)/time

Condition factor = (weight/length^b) × 100, b was the value obtained from length-weight relationship

Survival rate (%) = (final number of fish/initial number of fish) × 100

4.2.5 The utilisation of periphyton and bioseston by different size groups of Mozambique tilapia in fibreglass tanks with net substrate

To determine whether Mozambique tilapia of different size groups utilised the same periphyton and bioseston, the experiment was conducted in twenty-four 90 L fibreglass

tanks in a completely randomised design. The net substrates from the aqua dams described in section 4.2.2 were used in the fibreglass experiment. The fibreglass tanks were installed with heaters and oxygen air stone diffusers. The temperature was kept at 28 °C in the fibreglass tanks. Three different tilapia sizes (2.62 ± 0.01 g, 5.08 ± 0.02 g, and 25.24 ± 0.29 g; mean $\pm$ SE) were used for this experiment. The fish were starved for 24 hours before being stocked in fibreglass tanks. The fish were starved to obtain a complete gut evacuation and a clean digestive tract before commencing experiments in the fibreglass tanks. The net substrate was used for this experiment because it gave the highest periphyton abundance and composition in Chapter 3.

The periphyton and bioseston were harvested from the two-feeding regime (N50 and N33) as described in section 4.2.2 and were designated N50.B (bioseston from the N50 aqua dams) and N33.B (bioseston from the N33 aqua dams), the periphyton was designated N50.P and N33.P (Figure 4.2). The net substrates, with a surface area of 900 cm², from aqua dams described in section 4.2.2 were placed in the fibreglass tanks. One piece of net substrate was placed in the fibreglass tanks for fish to graze. The other net (900 cm²) was enumerated by scraping the periphyton from the substrate, it was never placed inside fibreglass tanks. The periphyton was dried and weighed, according to method no. 10300 of APHA (1998) to determine the amount of periphyton on the substrate. A similar procedure was done for bioseston, where bioseston was inoculated into fibreglass tanks for fish to filter feed on after it was counted under the microscope using a counting chamber. After the fish filter fed on the bioseston, the bioseston was harvested from the fibreglass tanks using a 71 μ m plankton net and counted under a light microscope.

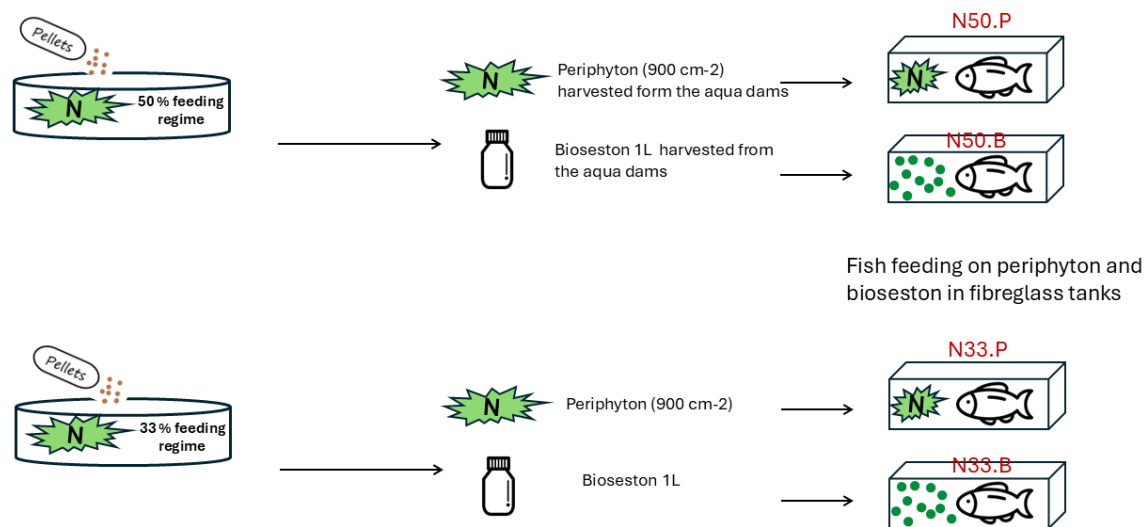


Figure 4. 2: The schematic representation of the utilisation of periphyton and bioseston in fibreglass tanks with different fish sizes.

Four tilapias of each size were stocked per fibreglass tank and allowed to graze on periphyton and filter feed on bioseston for 12 hours. After 12 hours of grazing, the net (900 cm²) substrates in all the fibreglass tanks were collected and enumerated. The periphyton was enumerated by scraping the remaining periphyton on the substrate (from the fibreglass tank), then dried and weighed to determine how much periphyton was eaten by the fish. This was done by calculating the weight difference before and after grazing. The remaining bioseston was then harvested from the fibreglass tank after the fish had filter-fed and counted again under the microscope (Chapter 3, section 3.2.4). Faeces were collected directly from the fibreglass tanks by siphoning, and thereafter the fish were harvested from the fibreglass tanks. Fish were then sacrificed and gutted to remove the stomach. The stomach content was analysed under a light microscope to identify the periphyton items ingested by the fish. The faecal matter was also analysed under a light microscope to determine which periphyton items were not consumed by the fish. The analysis of stomach contents and faecal matter is reported for African catfish from aqua dams (N50, P50, S50, N33, P33, and S33) with periphyton substrates, while no data is provided from the control group (N100, P100, and S100). This omission occurred because the African catfish in the control group

were only fed commercial pellets without access to periphyton on the substrates. The food items identified in the stomach were used to calculate the food selectivity as follows:

Chesson selectivity index (CSI):

$$CSI = \frac{g_i/b_i}{\sum_{j=1}^n g_j/b_j}, i = 1, \dots, n,$$

Where n is the total number of periphyton species in the stomach contents; g and b are the proportional representation of periphyton/bioseston species i or j in the stomach content and water (environment), respectively. The CSI value for a specific periphyton/bioseston species i reflects its presence in the stomach contents relative to the environment, divided by the sum of the equivalent calculation for all periphyton/bioseston species in the stomach content. The Chesson selectivity index value ranges from -1 to +1, with -1 indicating complete avoidance of the food item, 0 indicating a random selection of the food and 1 indicating exclusive feeding on that species.

The prominent value (Norton & Schmitt, 1978) was used to determine whether tilapia preferred feeding on periphyton or bioseston in fibreglass tanks. The following calculations were used to determine the prominent value per treatment:

$$\text{Prominent value of periphyton} = \frac{\text{Periphyton density}}{\sqrt{\text{frequency of occurrence}}}$$

$$\text{Prominent value of bioseston} = \frac{\text{Bioseston density}}{\sqrt{\text{frequency of occurrence}}}$$

4.2.6 Data and Statistical Analysis

Microsoft Excel and Power BI Desktop were used to plot graphs. Shannon-Wiener diversity index was used to determine species diversity using R (version 2024.4.2-764), using the 'igraph'. Normality and homogeneity of growth performance were tested using Shapiro-Wilk and Levene tests, respectively (Statistical Package and Service Solutions [SPSS] version 28). 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 indices in aqua dams. Tukey's post hoc analysis was used to determine which means were significantly different from each other (SPSS version 28). The data were tested at the level of significance of 0.05. Multivariate analysis of variance (MANOVA) and analysis of similarities (ANOSIM) were also performed in R using the vegan, MASS, effective, tidyverse, magrittr, and dplyr packages. MANOVA was used to determine the significant difference in the growth performance indices between the substrates (net, plastic, and stone). ANOSIM was used to determine if there were similarities in periphyton abundance on net, plastic, and stone substrates in aqua dams. ANOSIM was also used to determine the significant difference between periphyton and bioseston in the aqua dams and the food items identified in the stomachs.

4.3 RESULTS

4.3.1 The utilisation of periphyton and bioseston by Mozambique tilapia in aqua dams

The periphyton and bioseston in the aqua dams with substrates were classified to phyla level to determine the most abundant groups. Chlorophyta, Bacillariophyta, and Cyanophyta were the most abundant phyla in all aqua dams with substrates, stocked with Mozambique tilapia (Table 4.1). The aqua dams with plastic substrate had the highest periphyton and bioseston abundance. Aquatic arthropods, copepods, and rotifers were most abundant in aqua dams with plastic substrate. Protozoa were most abundant on the plastic substrate, followed by the stone substrate (Table 4.1).

Table 4. 1: Periphyton and bioseston abundance in aqua dams with net, plastic and stone substrates, stocked with Mozambique tilapia.

	NET		Plastic		Stone	
	Abundance	Relative abundance (%)	Abundance	Relative abundance (%)	Abundance	Relative abundance (%)
Periphyton						
Chlorophyta	102.05	78.21	94.50	56.07	42.16	32.03
Bacillariophyta	18.00	13.80	25.03	14.85	61.78	46.94
Charophyta	2.20	1.69	3.73	2.21	3.39	2.58
Coscinodiscophyta	0.64	0.49	0.00	0.00	0.00	0.00
Cyanophyta	6.45	4.94	44.38	26.33	22.81	17.33
Heterokontophyta	0.00	0.00	0.89	0.53	1.18	0.90
Gyrista	1.14	0.87	0.00	0.00	0.28	0.21
Total	130.48	100.00	168.53	100.00	131.61	100.00
Bioseston						
Euglenozoa	0.76	2.05	0.00	0.09	0.32	1.23
Cryptophyta	2.69	7.23	0.00	0.00	0.00	0.00
Annelida	0.00	0.00	0.06	0.09	0.32	1.23
Arthropoda	7.84	21.05	17.06	25.27	0.18	0.71
Copepod	0.34	0.91	6.86	10.17	0.00	0.00
Cladocera	2.00	5.38	2.10	3.11	0.00	0.00
Protozoa	3.54	9.52	17.76	26.30	16.91	66.09
Rotifer	20.05	53.85	23.60	34.96	7.86	30.74
Total	37.23	100.00	67.44	100.00	25.58	100.00

Periphyton and bioseston were subsequently identified to genera level to determine the most dominant genera in the aqua dams with substrates stocked with Mozambique tilapia. *Scenedesmus* sp. and *Cosmarium* sp. were the most dominant genera on the

net substrate (Table 4.2). *Lyngbya* sp. and *Chlorella* sp. were the most dominant periphyton genera on the plastic substrate. The stone substrate was dominated by *Lyngbya* sp. and *Chlorogonium* sp. The most dominant genera were also most frequently occurring and abundant in the aqua dams with net, plastic, and stone substrates (Table 4.2).

Table 4. 2: Periphyton abundance (no./cm²), frequency of occurrence (FO %) and dominance index (Di) in aqua dams with substrates stocked with Mozambique tilapia

PERIPHYTON	NET			PLASTIC			STONE		
	no./cm ²	FO %	Di	no./cm ²	FO %	Di	no./cm ²	FO %	Di
<i>Chlorella</i> sp.	8.88	100.00	6.86	25.66	100.00	15.23	5.94	100.00	4.52
<i>Chlorogonium</i> sp.	0.46	16.67	0.06	5.58	100.00	3.31	18.71	100.00	14.25
<i>Cosmarium</i> sp.	15.54	100.00	12.02	12.21	100.00	7.25	0.48	16.67	0.06
<i>Micractinium</i> sp.	12.92	83.33	8.33	6.31	66.67	2.49	0.00	0.00	0.00
<i>Pandorina</i> sp.	14.97	100.00	11.58	10.13	100.00	6.01	3.17	50.00	1.21
<i>Scenedesmus</i> sp.	27.40	100.00	21.19	0.26	16.67	0.03	0.22	16.67	0.03
<i>Selenastrum</i> sp.	0.06	16.67	0.01	17.98	100.00	10.67	6.71	100.00	5.11
<i>Diatoma</i> sp.	0.00	0.00	0.00	9.73	100.00	5.77	16.44	100.00	12.51
<i>Rhoicosphenia</i> sp.	0.10	22.22	0.02	4.84	83.33	2.39	13.97	100.00	10.64
<i>Surirella</i> sp.	0.00	0.00	0.00	6.16	100.00	3.65	16.15	100.00	12.30
<i>Navicula</i> sp.	8.10	100.00	6.26	0.11	16.67	0.01	0.09	16.67	0.01
<i>Nitzschia</i> sp.	3.09	100.00	2.39	1.19	33.33	0.23	12.15	100.00	9.25
<i>Lyngbya</i> sp.	0.13	22.22	0.02	34.41	100.00	20.42	21.35	100.00	16.26

The most dominant bioseston genera were *Lecane* sp. and *Philodina* sp. on the net substrate (Table 4.3). *Euplotes* sp. and Cladocera were the most dominant genera on the plastic substrate. The stone substrate was dominated by *Euplotes* sp. and *Trichocerca* sp. The frequency of occurrence and abundance mirrored the dominance index. (Table 4.3)

Table 4. 3: Bioseston abundance (no./ml), frequency of occurrence (FO %) and dominance index (Di) in aqua dams with substrates stocked with Mozambique tilapia

BIOSESTON	NET			PLASTIC			STONE		
	no./ml	FO%	Di	no./ml	FO%	Di	no./ml	FO%	Di
Ephemeroptera larvae	2.50	100.00	6.73	0.00	0.00	0.00	0.00	0.00	0.00
Chironomidae larvae	2.71	100.00	7.30	0.13	16.67	0.03	0.00	16.67	0.00
Cladocera	1.65	100.00	4.45	10.78	83.33	13.07	0.15	33.33	0.19
Copepod	0.00	0.00	0.00	6.38	83.33	7.73	0.00	0.00	0.00
<i>Diffugia</i> sp.	2.53	100.00	6.82	0.00	0.00	0.00	0.00	16.67	0.00
<i>Euplotes</i> sp.	0.00	0.00	0.00	10.98	100.00	15.98	16.07	100.00	61.92
<i>Brachionus</i> sp.	0.47	66.67	0.84	5.68	100.00	8.27	1.11	83.33	3.56
<i>Colurella</i> sp.	0.14	100.00	0.38	7.53	100.00	10.95	1.84	66.67	4.73
<i>Lecane</i> sp.	8.62	100.00	23.23	0.00	0.00	0.00	0.00	0.00	0.00
<i>Trichocerca</i> sp.	1.96	83.33	4.39	6.92	100.00	10.07	3.65	83.33	11.71
<i>Philodina</i> sp.	7.19	100.00	19.38	0.00	0.00	0.00	0.00	0.00	0.00

Cladocera-unidentified & body fragments of Cladocera, copepods-unidentified & body fragments of copepods

Growth performance indices were determined to monitor the effect of periphyton growing on the net, plastic and stone in the growth of Mozambique tilapia in aqua dams. In the aqua dams with net substrate, specific growth rate (SGR) and condition factor of Mozambique tilapia were higher in N50 than in N33 and N100 treatments (Table 4.4). However, there was no significant difference ($P > 0.005$) in all the growth performance indices.

Table 4. 4: The growth performance indices (mean $\pm$ SE) of Mozambique tilapia in aqua dams deployed with net substrate.

	Treatments			
	N100	N50	N33	P value
Initial weight (g)	5.47 $\pm$ 0.95	4.73 $\pm$ 0.23	5.07 $\pm$ 0.42	0.397
Final weight (g)	14.88 $\pm$ 5.44	17.28 $\pm$ 2.91	17.26 $\pm$ 4.41	0.751
ADWG (g)	0.57 $\pm$ 0.14	0.72 $\pm$ 0.01	0.69 $\pm$ 0.03	0. 468
Percentage weight gain (%)	186.5 6 $\pm$ 80.04	263.72 $\pm$ 24.71	238.42 $\pm$ 37.26	0.602
SGR	2.27 $\pm$ 0.77	3.06 $\pm$ 0.15	2.87 $\pm$ 0.25	0.520
Condition factor	1.73 $\pm$ 0.28	1.92 $\pm$ 0.27	1.81 $\pm$ 0.31	0.730
Survival rate (%)	97.33 $\pm$ 4.62	98.67 $\pm$ 2.31	92.67 $\pm$ 4.16	0.212

Abbreviation used: ADWG= Average daily weight gain, SGR = Specific growth rate. Each mean represents samples from 3 replicate aqua dams. Superscript letters indicate significant values which differed from each other

In aqua dams with plastic substrates, the specific growth rate (SGR) of Mozambique tilapia was higher in the P50 followed by the fish in the control (P100) treatment (Table 4.5). The condition factor of Mozambique tilapia was higher in the P100 followed by fish in the P50 treatment. Mozambique tilapia in P33 had the lowest SGR and condition factor compared to P100 and P50. All the growth performance indices were not statistically significant ($P > 0.005$).

Table 4. 5: The growth performance indices (mean $\pm$ SE) of Mozambique tilapia in aqua dams deployed with plastic substrate.

	Treatment			
	P100	P50	P33	P value
Initial weight (g)	6.28 $\pm$ 0.46	5.98 $\pm$ 0.83	6.08 $\pm$ 0.56	0.944
Final weight (g)	24.80 $\pm$ 3.80	24.82 $\pm$ 0.48	17.99 $\pm$ 0. 2.47	0.189
ADWG (g)	0.72 $\pm$ 0.07	0.75 $\pm$ 0.03	0.64 $\pm$ 0.06	0.467
Percentage weight gain (%)	307.01 $\pm$ 84.86	331.50 $\pm$ 60.43	203.93 $\pm$ 56.14	0.428
SGR	3.21 $\pm$ 0.57	3.42 $\pm$ 0.35	2.56 $\pm$ 0.46	0.449
Condition factor	2.42 $\pm$ 0.46	2.17 $\pm$ 0.35	1.82 $\pm$ 0.34	0.586
Survival rate (%)	96.22 $\pm$ 2.32	96.10 $\pm$ 3.57	100 $\pm$ 0.00	0.488

Abbreviation used: ADWG= Average daily weight gain. SGR = Specific growth rate. Each mean represents samples from 3 replicate aqua dams. Superscript letters indicate significant values which differed from each other

On the other hand, Mozambique tilapia in aqua dams with stone substrates had higher SGR in the S33 treatment than fish in S50 and S100 (Table 4.6). The condition factor was higher in fish in the control (S100) treatment. All the growth performance indices were not significantly different ($P > 0.005$).

Table 4. 6: The growth performance indices (mean $\pm$ SE) of Mozambique tilapia in aqua dams deployed with stone substrate.

	Treatment			
	S100	S50	S33	P value
Initial weight (g)	5.00 $\pm$ 0.14	5.16 $\pm$ 0.16	5.25 $\pm$ 0.14434	0.533
Final weight (g)	11.07 $\pm$ 0.17	11.31 $\pm$ 1.05	12.22 $\pm$ 0.38	0.472
ADWG	0.54 $\pm$ 0.01	0.53 $\pm$ 0.06	0.56 $\pm$ 0.01	0.791
Percentage weight gain (%)	121.76 $\pm$ 7.12	120.62 $\pm$ 26.61	133.06 $\pm$ 8.93	0.848
SGR	1.89 $\pm$ 0.07	1.84 $\pm$ 0.30	2.01 $\pm$ 0.09	0.825
Condition factor	1.96 $\pm$ 0.04	1.65 $\pm$ 0.12	1.71 $\pm$ 0.02	0.071
Survival rate (%)	95.00 $\pm$ 0.00	96.66 $\pm$ 0.00	95.83 $\pm$ 2.88	0.702

Abbreviation used: ADWG= Average daily weight gain. SGR = Specific growth rate. Each mean represents samples from 3 replicate aqua dams. Superscript letters indicate significant values which differed from each other

The MANOVA was used to determine the significant differences in the growth performance indices of Mozambique tilapia utilising periphyton and bioseston in aqua dams with net, plastic and stone. The growth performance indices of Mozambique tilapia in the aqua dams with substrates were significantly different ($P = 8.763 \times 10^{-10}$, MANOVA) from each other. The partial Eta Squared value showed that the effect size was 0.81 at a 95 % confidence interval [0.69, 1.00]. The Mozambique tilapia in aqua dams with plastic substrate showed higher growth performance indices (Figure 4.3). The Linear Discriminant Analysis (LDA) was used as a post hoc test and it showed the proportion of trace of 0.7199 and 0.2801 for LD1 and LD2, respectively (Figure 4.3).

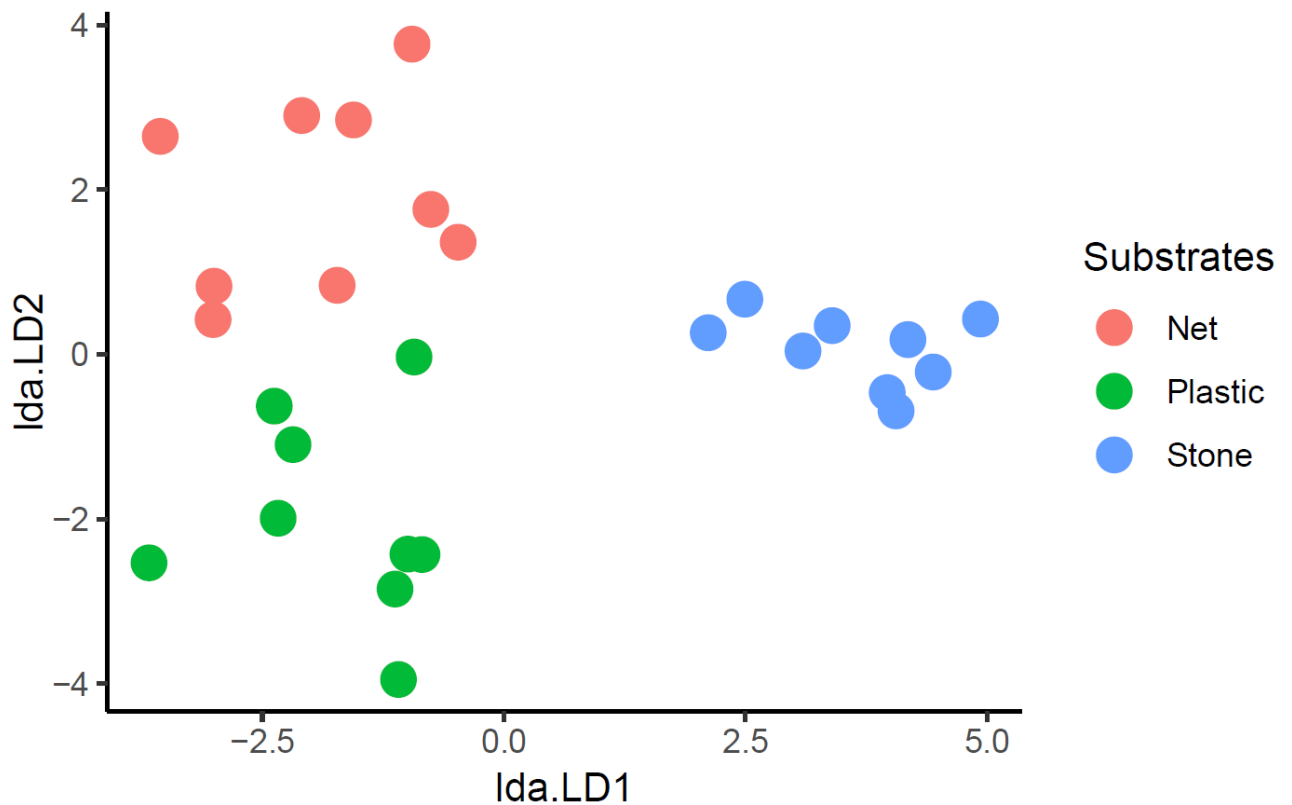


Figure 4. 3: The scatter plot of Linear Discriminant Analysis (LDA) of growth performance indices of Mozambique tilapia in aqua dams with net, plastic and stone substrate

4.3.2 The utilising periphyton and bioseston by Mozambique tilapia in fibreglass tanks with net substrates

The stomach content of different size groups (2 g, 5 g, 25 g) of Mozambique tilapia was analysed to determine the abundance of periphyton and bioseston items that the fish ingested. The faecal analyses were carried out to determine the food items the fish did not digest. *Scenedesmus* sp., *Chlorogonium* sp., *Gloeotrichia* sp., *Chlorella* sp. and *Navicula* sp. were the abundant periphyton and bioseston genera in the stomach of 2 g (Figure 4.4). The faecal matter was dominated by *Navicula* sp., *Nitzschia* sp., *Synedra* sp. and *Pinnularia* sp. (Figure 4.4).

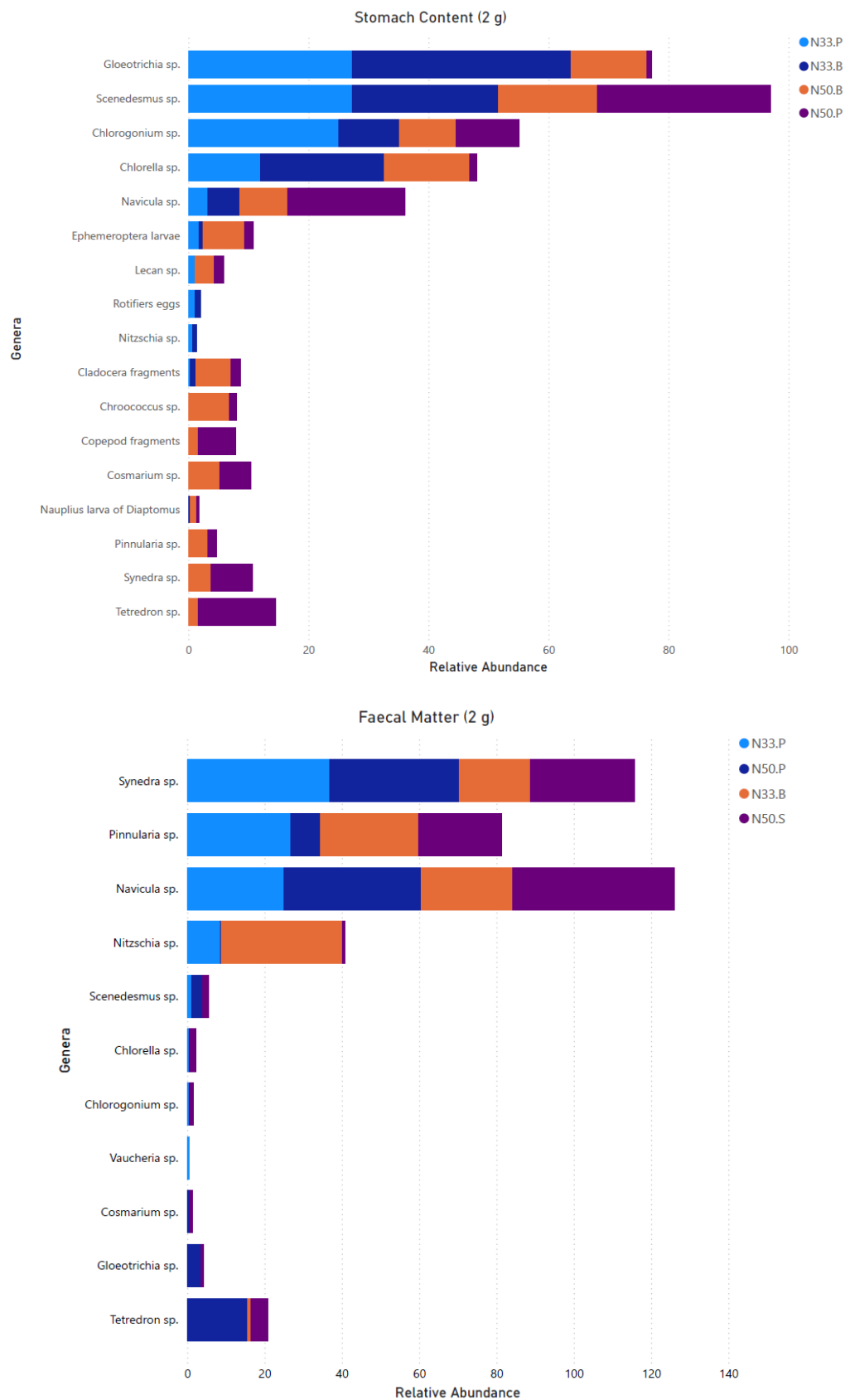


Figure 4. 4: The stomach content and faecal matter of 2 g Mozambique tilapia in fibreglass tanks utilising periphyton (N50.P and N33.P) and bioseston (N50B and N33.B)

Chlorogonium sp., *Chlorella* sp., *Pinnularia* sp., *Nitzschia* sp., and *Navicula* sp. were the most abundant in the stomach of 5 grams tilapia (Figure 4.5). The faecal matter was dominated by *Navicula* sp., *Nitzschia* sp., *Synedra* sp., and *Pinnularia* sp. (Figure 4.5).

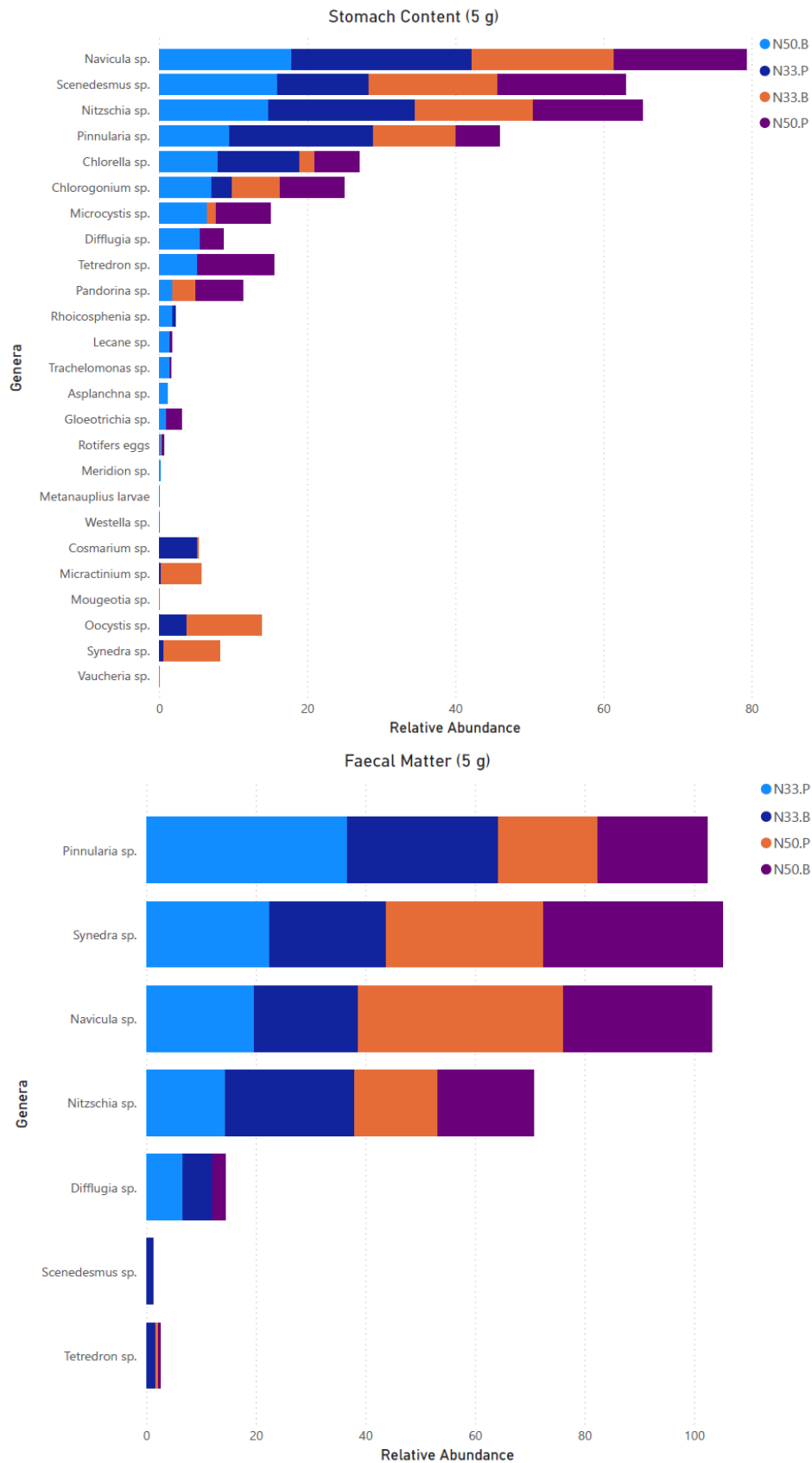


Figure 4. 5: The stomach content and faecal matter of 5 g Mozambique tilapia in fibreglass tanks utilising periphyton (N50.P and N33.P) and bioseston (N50B and N33.B)

The most abundant periphyton and bioseston genera in the stomach of 25 g tilapia were *Scenedesmus* sp., *Lecane* sp., *Pandorina* sp., *Chlorella* sp., and *Navicula* sp. were in the stomach (Figure 4.4). The faecal matter was also dominated by diatoms (Figure 4.4).

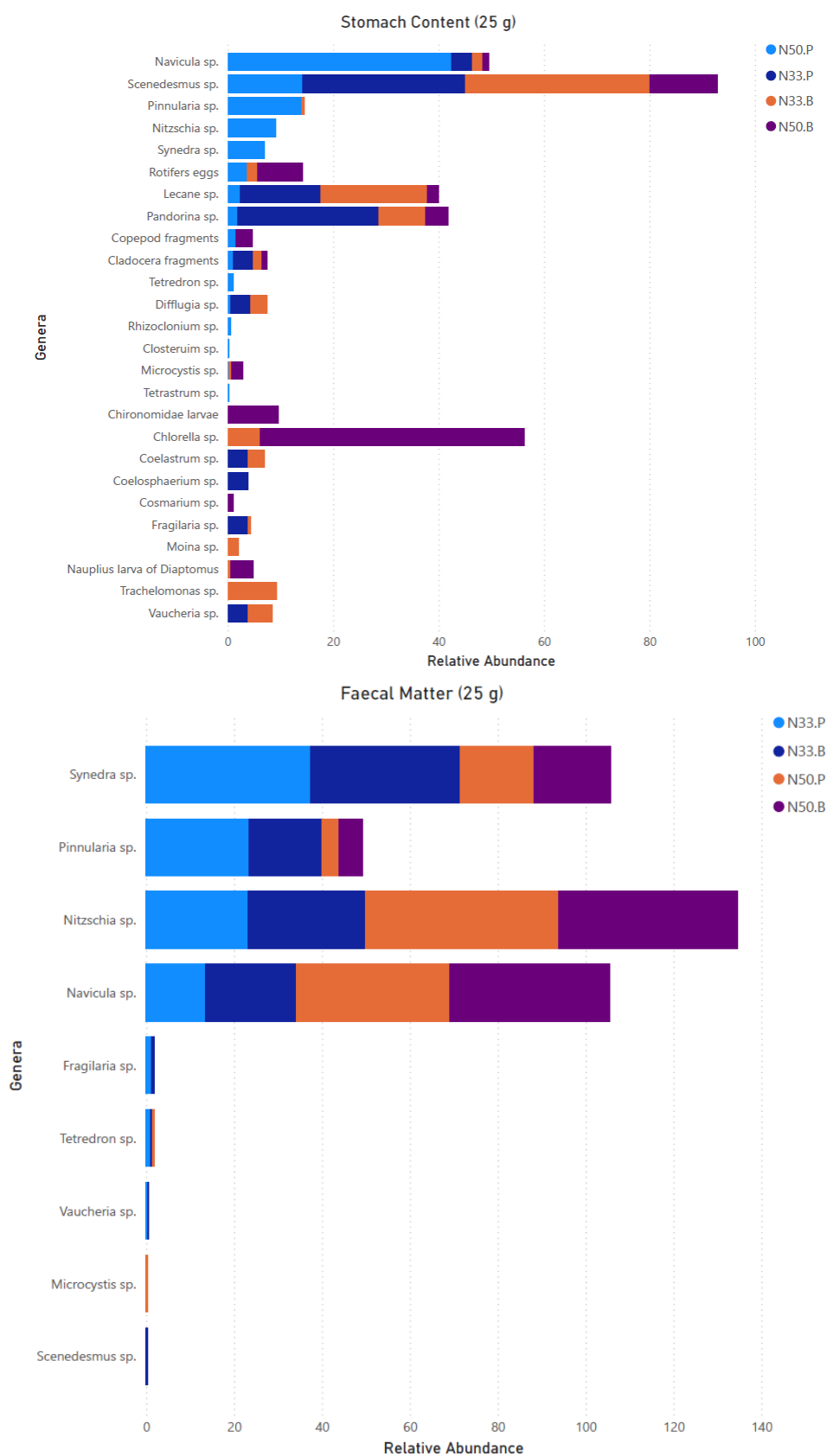


Figure 4. 6: The stomach content and faecal matter of 25 g Mozambique tilapia in fibreglass tanks utilising periphyton (N50.P and N33.P) and bioseston (N50B and N33.B).

Chesson selection index was used to determine the periphyton and bioseston items that were selectively ingested by the fish in fibreglass tanks. There was no active selection of the food items identified in the stomachs in all group sizes of Mozambique tilapia in the fibreglass tanks (Table 4.7 to Table 4.9). The number of food items identified in the stomachs of all size groups was significantly different ($R= 0.6354$, $P= 0.0004$; ANOSIM). The 25 g tilapia, followed by 5 g had a greater number of food items compared to the 2 g tilapia size (Table 4.7 to Table 4.9).

Table 4. 7: The Chesson selection index of food items identified in the stomach of 2 g Mozambique tilapia feeding on periphyton (N50.P and N33.P) and bioseston (N50.B and N33.B) in fibreglass tanks.

	N50.P	N33.P	N50.B	N33.B
<i>Scenedesmus sp.</i>	0.0082	0.0090	0.0034	0.0072
<i>Tetredron sp.</i>	0.2930	-	0.0261	-
<i>Chlorogonium sp.</i>	0.1030	0.2748	0.0673	0.0975
<i>Chlorella sp.</i>	0.0009	0.0137	0.0079	0.0209
<i>Cosmarium sp.</i>	0.0025	-	0.0019	-
<i>Pinnularia sp.</i>	0.0015	-	0.0021	-
<i>Navicula sp.</i>	0.0135	0.0052	0.0040	0.0075
<i>Synedra sp.</i>	0.0472	-	0.0180	-
<i>Nitzschia sp.</i>	-	0.0017	-	0.0012
<i>Gloeotrichia sp.</i>	0.0243	0.6503	0.2858	0.7743
<i>Chroococcus sp.</i>	0.0218	-	0.0925	-
Ephemeroptera larvae	0.0068	0.0054	0.0215	0.0016
Rotifier eggs	-	0.0062	-	0.0045
<i>Lecan sp.</i>	0.0010	0.0017	0.0014	-
Copepod fragments	0.3173	-	0.0584	-
Cladocera fragments	0.1322	0.0320	0.3566	0.0692
Nauplius larva of Diaptomus	0.0270	-	0.0530	0.0162

Table 4. 8: The Chesson selection index of food items identified in the stomach of 5 g Mozambique tilapia feeding on periphyton (N50.P and N33.P) and bioseston (N50.B and N33.B) in fibreglass tanks.

	N50.P	N33.P	N50.B	N33.B
<i>Chlorella sp.</i>	0.0096	0.0296	0.0133	0.0031
<i>Micractinium sp.</i>	-	0.0009	-	0.0087
<i>Oocystis sp.</i>	-	0.3796	-	0.6025
<i>Westella sp.</i>	-	-	0.0001	-
<i>Chlorogonium sp.</i>	0.1739	0.0700	0.1513	0.0969
<i>Pandorina sp.</i>	0.0064	-	0.0020	0.0026
<i>Cosmarium sp.</i>	-	0.0073	-	0.0001
<i>Scenedesmus sp.</i>	0.0100	0.0096	0.0098	0.0079
<i>Tetredon sp.</i>	0.4874	-	0.2537	-
<i>Pinnularia sp.</i>	0.0112	0.1310	0.0192	0.0436
<i>Navicula sp.</i>	0.0253	0.0918	0.0267	0.0421
<i>Rhoicosphenia sp.</i>	-	0.1524	0.1449	-
<i>Synedra sp.</i>	-	0.0200	-	0.1295
<i>Meridion sp.</i>	-	-	0.0222	-
<i>Nitzschia sp.</i>	0.1170	0.1078	0.1230	0.0505
<i>Microcystis sp.</i>	0.0113	-	0.0107	0.0096
<i>Gloeotrichia sp.</i>	0.1274	-	0.0694	-
<i>Trachelomonas sp.</i>	0.0009	-	0.0195	-
<i>Vaucheria sp.</i>	-	-	-	0.0020
Metanauplius larvae	-	-	0.0057	-
Rotifer eggs	0.0046	-	0.0046	0.0009
<i>Diffugia sp.</i>	0.0147	-	0.0273	-
<i>Asplanchna sp.</i>	-	-	0.0944	-
<i>Lecane sp.</i>	0.0002	-	0.0021	-

Table 4. 9: The Chesson selection index of food items identified in the stomach of 25 g Mozambique tilapia feeding on periphyton (N50.P and N33.P) and bioseston (N50.B and N33.B) in fibreglass tanks.

	N50.P	N33.P	N50.B	N33.B
<i>Chlorella sp.</i>	-	-	0.0552	0.0076
<i>Closterium sp.</i>	0.0006	-	-	-
<i>Coelastrum sp.</i>	-	0.0698	-	0.0823
<i>Cosmarium sp.</i>	-	0.0000	0.0008	-
<i>Pandorina sp.</i>	0.0024	0.0125	0.0030	0.0060
<i>Rhizoclonium sp.</i>	0.0488	-		-
<i>Scenedesmus sp.</i>	0.0107	0.0075	0.0052	0.0125
<i>Tetrastrum sp.</i>	0.0223	-	-	-
<i>Tetredron sp.</i>	0.0627	-	-	-
<i>Fragilaria sp.</i>	-	0.1802	-	0.0355
<i>Pinnularia sp.</i>	0.0348	-	-	0.0016
<i>Synedra sp.</i>	0.1249	-	-	-
<i>Navicula sp.</i>	0.0779	0.0045	0.0011	0.0036
<i>Nitzschia sp.</i>	0.0928	-	-	-
<i>Coelosphaerium sp.</i>	-	0.4054	-	-
<i>Microcystis sp.</i>	0.0005	-	0.0023	0.0030
<i>Trachelomonas sp.</i>	-	-	-	0.2908
<i>Vaucheria sp.</i>	-	0.0386	-	0.0684
Chironomidae larvae	-	-	0.0553	-
<i>Moina sp.</i>	-0	-	-	0.2431
Copepod fragments	0.2100	-	0.2328	-
Cladocera fragments	0.2334	0.2495	0.1293	0.1473
Nauplius larva of Diaptomus	-	-	0.4233	0.0345
<i>Diffugia sp.</i>	0.0031	0.0138	-	0.0163
<i>Lecane sp.</i>	0.0039	0.0182	0.0019	0.0349
Rotifer eggs	0.07102	-	0.08996	0.0127

Prominent value was used to determine the feeding preference of Mozambique tilapia between periphyton and bioseston. In N50 tanks, 2 g and 25 g tilapia preferred periphyton and 5 g tilapia preferred bioseston (Table 4.10). In N33 tanks, all size groups (2 g, 5 g and 25 g) preferred bioseston.

Table 4. 10: Prominent values showing feeding preference between periphyton and bioseston by Mozambique tilapia of different size in fibreglass tanks.

	N50.P	N33.P	N50.B	N33.B
<i>2 g</i>	27.3	35.9	20.1	44.3
<i>5 g</i>	187.8	96.0	215.7	127.4
<i>25 g</i>	49.2	4.2	13.8	24.0

4.4 DISCUSSION

Chlorophyta, Bacillariophyta, and Cyanophyta were the most abundant phyla on all the substrates in the aqua dams stocked with Mozambique tilapia. This was probably because green algae (Chlorophyta), diatoms (Bacillariophyta) and cyanobacteria (Cyanophyta) are commonly the main taxa in different water bodies due to their ability to grow in various aquatic environments. This might suggest that these phyla are cosmopolitan. These phyla have been identified on stones, tree roots, sand, macrophyte, glass slides, bamboo, and water column in natural aquatic systems and periphyton-based systems (Figueredo & Giani, 2005; Santos et al., 2018; Santi et al., 2023). This is because species belonging to these phyla have traits that make them competitive and resilient compared to other species. Chlorophyta species do well as long as they are exposed to sunlight (Mette et al., 2011). The aqua dams were outdoors and exposed to sufficient sunlight for the proliferation of Chlorophyta species. Cyanophyta group have the nitrogen-fixing ability that enables them to flourish under varying conditions (Stal, 2017; Paerl, 2017). Chicken manure was added into aqua dams to increase the concentration of nitrogen. Bacillariophyta species have silica cell walls that produce adhesive secretions known as extracellular polymeric substances which probably enabled them to attach to different substrates (Sweat & Johnson, 2013; Watanabe et al., 2022).

The most dominant periphyton genera were *Scenedesmus* sp., and *Cosmarium* sp. on the net substrate. The most dominant genera were also most frequently occurring and abundant in the aqua dams with periphyton substrates. This might suggest that the genera were resistant to grazing by tilapia. This is because these genera proliferate at a fast rate when they are exposed to sunlight, as a result, they withstand grazing pressure. For instance, *Scenedesmus* sp. have spines and some bristles too that act as defence mechanisms to resist predation. Moreover, *Scenedesmus* was probably dominant on the net substrate because these features helped it to attach to the net's mesh. Some *Cosmarium* species' cell walls are ornamented with spines, granules, or spinules that can function as a defence against grazing pressure make them (Gerrath, 2002). *Lyngbya* sp. and *Chlorella* sp. were the most dominant periphyton genera on the plastic substrate. *Chlorella* sp. proliferates in colonies and may be hard to remove at a fast rate through grazing. *Lyngbya* sp. are filamentous

algae protected by a rigid mucilaginous sheath that might help in resist intense grazing pressure. The stone substrate was dominated by *Lyngbya* sp. and *Chlorogonium* sp. *Chlorogonium* sp. were able to resist grazing probably because of the flagella that allow them to swim away from the fish.

Aquatic arthropods, copepods and rotifers were most abundant in aqua dams with plastic substrate. This was probably because the plastic substrate had the highest abundance of green algae (Chlorophyta), diatoms (Bacillariophyta) and cyanobacteria (Cyanophyta) that served as food for the bioseston. Metazoans (copepods, Cladocera, rotifers, protists and ciliates) are key grazers of diatoms and green algae (Montemezzani et al., 2015; Pančić et al., 2019). Protozoa were most abundant on the plastic substrate, followed by the stone substrate. Protozoa are detritivores and bacterivores and are found in planktonic, benthic, and soil biomes (Wiser, 2024). Therefore, their abundance on the plastic substrate was probably because they can thrive in the water column since they have locomotion features (Ackermann et al., 2011; Früh et al., 2011; Vlaičević et al., 2021). They were also abundant on the stone substrate because the stone substrate was located at the bottom of the aqua dams where there is a lot of sediment. The most dominant bioseston genera were *Lecane* sp., *Philodina* sp., *Euplotes* sp., Cladocera, and *Trichocerca* sp. *Euplotes* sp. is a dominant genus in the ciliates and grows well in water bodies with high sediments (Huang et al., 2021). Chicken manure also increased sediment load and nutrients in the aqua dams. Rotifers (*Lecane* sp., *Philodina* sp., and *Trichocerca* sp.) commonly dominate when there is sufficient nutrient concentration, food availability, neutral pH and high temperature (Montemezzani et al., 2015). The dominance of Cladoceran can be attributed to their ability to swim quickly and escape predators. Moreover, the presence of commercial pellets most likely causes tilapia to conserve energy instead of chasing Cladoceran in the aqua dams.

The specific growth rate (SGR) and condition factor of Mozambique tilapia were higher in N50 followed by fish in N33 treatment. This may suggest that the fish were utilising natural food and the nutritional value of the periphyton and bioseston species was able to compensate for the nutritional value of the deprived commercial feed. Similarly,

Milstein et al. (2006) also reported that periphyton compensated for the reduced feed input and no negative effects on tilapia were observed. This suggests that tilapia was able to effectively harvest periphyton from the net substrate. This indicates that the commercial pellet input can be cut down by 67 % in periphyton-based aquaculture. The fish fed on 50 and 67 % less commercial pellets had growth-specific rates comparable to the control. This indicates that up to 67 % of feed can be saved without a significant reduction in tilapia growth rate in aqua dams with net substrates. The current study can save up to 67 %, unlike the study by David et al. (2022) which ultimately saved 50 % of the feed input in periphyton-based systems stocked with tilapia. On the other hand, Milstein et al. (2009 & 2005) reported that up to 40 % of fish feed was saved in their studies using tilapia in ponds with periphyton substrates.

The specific growth rate (SGR) of Mozambique tilapia in aqua dams with plastic was higher in the P50 followed by fish in the control (P100) treatment. This might be explained by the high abundance of aquatic arthropods, protozoa, and rotifer recorded in the aqua dams. These are animal-based species that commonly have higher protein content, carbohydrates, lipids, high unsaturated fatty acids and polyunsaturated fatty acids compared to most algae (Riccardi & Mangoni, 1999; Helland et al., 2003; Jagadeesan et al., 2010). Moreover, green algae were also most dominant in aqua dams with plastic substrates. Green algae such *Chlorella* sp. has been found to have 45.23% protein, 23.43% carbohydrate and 18.12% total lipids (Alagawany et al., 2021). This might indicate that periphyton and bioseston in the aqua dams were nutritionally adequate to sustain the growth of tilapia. This was because tilapia was able to visually feed on periphyton and also filter feed the bioseston in the aqua dams. This probably suggests that commercial pellet input can be cut back by 50 % in periphyton-based aquaculture in aqua dams with plastic substrate. Mozambique tilapia in P33 had the lowest SGR and condition factor compared to P100 and P50. However, it was higher SGR recorded in fish in aqua dams with stone substrates. This suggests that nutritional quality was still sufficient to maintain the growth even if the fish were fed once every third day.

Mozambique tilapia in aqua dams with stone substrate in S33 treatment had higher SGR than fish in S50 and S100. This suggests that even though the periphyton can be limited on the stone substrate, the bioseston that grows in aqua dams also supports the growth of cultured fish. This probably suggests that feeding once every three days had a positive influence on the uptake of periphyton and bioseston by tilapia in aqua dams with stone substrates. This confirms that 67 % of the commercial pellets can be reduced in aqua dams with periphyton substrates. Other periphyton-based aquaculture has shown that commercial pellets can be reduced by 30 to 40 % without affecting the growth rates of the fish (Azim et al., 2005; Milstein et al., 2005; Milstein et al., 2013). Compared to other studies, the current study shows that tilapia can be fed once every three days in a system with periphyton substrates in order to reduce pellet use by 67 %.

The Mozambique tilapia in aqua dams with plastic substrate significantly had higher SGR and condition factor than fish in aqua dams with net and stone substrates. This was probably because of the accessibility of periphyton on plastic substrates. The plastic substrates had smooth surfaces and texture, suggesting that species were easily coming off the substrates when the fish were grazing. Tilapia in aqua dams with stone substrate recorded the lowest SGR and condition compared to plastic and net substrate, while the fish in aqua dams with plastic substrate showed the best growth rate. This suggests that periphyton growing on the plastic substrates was easily assessable and high quality. The plastic substrates had a high abundance of aquatic arthropods, protozoa, and rotifer compared to other substrates. The animal-based species are of high quality because they have high protein, energy, and lipids (Kim et al., 2012). Periphytic algae were also recorded in aqua dams with plastic substrates. The algae are also of good-quality food for fish since they contain high-quality protein, sufficient lipid levels and all essential amino acids necessary for fish's growth (Nagappan et al., 2021; Vijayaram et al., 2024).

The abundance of periphyton and bioseston genera identified in the stomach of all size groups (2, 5, and 25 g) was dominated by green algae (*Scenedesmus* sp., *Chlorogonium* sp., *Chlorella* sp., *Pandorina* sp.). This might suggest that green algae

were resistant to grazing because they were also most abundant in the aqua dams. It is worth mentioning that the green algae were also dominant in aqua dams without fish in Chapter 3. This might suggest that Mozambique tilapia was feeding on the most dominant periphyton and bioseston in the aqua dams. All the green algae identified in the stomachs of all size groups were absent from the faecal matter. This shows that green algae were utilised by all the size groups. However, all the identified diatoms in the stomach were most dominant in the faecal matter. The faecal matter of all tilapia size groups was dominated by *Synedra* sp., *Pinnularia* sp., *Navicula* sp. and *Nitzschia* sp., indicating that tilapia was not digesting the diatoms. This was because diatom silica cell walls (frustules) cannot be chemically broken down by digestive juices and enzymes, animals need to physically break diatom cells in order to assimilate the cell contents (Houki et al., 2018). All tilapias' sizes were not able to digest diatom species because tilapia's pharyngeal bone does not have silica-reinforced teeth used to crack the hydrated silicon dioxide cell wall of diatoms. This is consistent with a study by (Ping & Jiankang, 1994), and Mohamed et al. (2019) that showed that tilapia is unable to digest diatoms. Most diatom-feeding species have various strong organs to crush cell walls such as silica-containing opal teeth, siliceous gastric mills in the stomach, and silica-reinforced teeth on the pharyngeal bone (Xie, 1999 & 2001; Houki et al., 2018). Rohu (*Labeo rohita*) and Common Carp (*Cyprinus carpio*) are some aquaculture species that utilise diatoms because of silica-reinforced teeth on the pharyngeal bone that break down the silica cell wall.

There was no active selection of the food items identified in the stomachs of all group sizes of Mozambique tilapia in the fibreglass tanks. The food items were overlapping and there was no distinct pattern. This confirms that Mozambique tilapia was feeding on the most dominant periphyton in the aqua dams. This suggests that tilapia do not select when feeding on periphyton. This is consistent with a previous study by Huchette et al. (2000) that reported that tilapia had only limited feeding selectivity when feeding on periphyton. Another study by Dempster et al. (1993) showed that tilapia actively feed on periphyton without being selective. The 25 g tilapia followed by 5 g had a greater number of food items compared to the 2 g tilapia size. This was probably because the mouth of 2 g tilapia size was poorly developed compared to 25 g and 5 g fish sizes. It probably had small lower jaw extensibility and premaxilla prostrusibility,

thus its periphyton grazing efficiency was less compared to the other sizes. Otieno et al. (2014) Wakil et al. (2014) and Temesgen et al. (2022) reported that the size of food items and the size and age of fish have a significant influence on the food selection because fish feed on items that can fit into their mouth and what their stomach can digest.

Prominent value showed that in N50 tanks, 2 g and 25 g tilapia preferred periphyton and 5 g tilapia preferred bioseston while in N33 tanks, all size groups preferred bioseston. This suggests that tilapia was nabbing or scraping periphyton from the substrate as much as it was filter feeding on bioseston from the water column. This shows that tilapia can efficiently graze on periphyton and filter feed on the bioseston despite the size of the fish. Tilapia respond positively to feeding on periphyton and bioseston because it has two feeding mechanisms. Beveridge and Baird (2000), Ibrahim et al. (2015), and Attayde and Menezes (2008) have reported that tilapia uses both filter feeding and visual feeding to consume food items because one mode of feeding might not be sufficient to support growth and reproduction of the fish in natural aquatic systems. Furthermore, Dempster et al. (1993) showed that tilapia feed more efficiently when natural food is offered together as periphyton and bioseston (phytoplankton) in aquaculture production systems.

In conclusion, the growth rate and condition factor of tilapia in aqua dam dams with net, plastic, and stone substrates were comparable to the fish in aqua dams without substrate. This indicates that the periphyton and bioseston were nutritionally adequate to support the growth of tilapia in aqua dams. The most abundant and dominant periphyton and bioseston were also identified in the stomachs of all tilapia size groups in fibreglass tanks. This indicates these species were resistant to grazing and that tilapia was feeding on the most abundant food item. However, Mozambique tilapia was not able to digest diatom even though they were ingested in abundance. The fish was not actively selecting any food items when feeding on the periphyton and bioseston in the fibreglass tanks. Moreover, the prominent value showed that tilapia was visually feeding on the periphyton and filter-feeding on the bioseston. The results of this study have shown that Mozambique tilapia in aqua dams with plastic substrate had higher

growth rates and condition factors compared to fish in aqua dams with net and stone substrate. The result of this study suggests that commercial pellets can be reduced by 50 to 67% in aqua dams with periphyton substrates.

CHAPTER 5:

**Is the African catfish (*Clarias gariepinus*) a
good candidate for periphyton-based
aquaculture?**

5. Is the African catfish (*Clarias gariepinus*) a good candidate for periphyton-based aquaculture?

5.1 INTRODUCTION

Periphyton-based aquaculture is primarily used to reduce the high cost of fish feed by decreasing or replacing the input of commercial pellet feed in production systems. Periphyton-based aquaculture enhances the growth of periphyton and bioseston that serve as food for herbivore and omnivore cultured species. *Clarias gariepinus*, Burchell 1822 (African catfish) is one of the most widely cultured species in Africa (Kadye, 2011; Moffitt & Cajas-Cano, 2014; FAO, 2024a), yet it is not commonly used in periphyton-based aquaculture as tilapia and carp species. African catfish is an omnivorous benthic feeder that can feed well from rocks and other substrates from the bottom of the lake in the wild (Welcomme, 1985). African catfish, like tilapia species, are opportunistic feeders that readily feed on different natural food. In the wild, catfish naturally feed on zooplankton, phytoplankton, other fish, higher plants, insects, vegetable debris, crustaceans, plant fibrous roots, and detritus (Dadebo et al., 2014; Santi et al., 2023; Tesfahun & Alebachew, 2023). Most of these food items form part of the periphyton and bioseston community. Thus, African catfish might perform well in periphyton-based aquaculture.

The major challenge with periphyton-based aquaculture is the selection of species to be cultured and substrates to enhance periphyton growth. This is because not all fish species can be cultured in periphyton-based aquaculture since not all fish species can graze periphyton from substrates. In order to graze on periphyton efficiently, fish need a high degree of specialisation in the feeding, filtering, and masticating apparatus (Sibbing & Witte, 2005). *Tilapia* species are among the most cultured species in periphyton-based aquaculture due to their ability to graze on periphyton from the substrates and filter feed on bioseston in the water column. *Tilapia* species have mouth features that allow them to scrap, comb, suck, pecker, or bite-tear various periphyton (Azim et al., 2005). However, tilapia species and African catfish have different mouth morphological apparatus that may cause them to respond differently to utilising periphyton on substrates. Cyprinid species such as *Labeo* and *Garra* also

have specialised mouth features that enable them to forage with the body parallel to the substrate. These species have inferior transversal mouths with a sharp cutting edge, thick papillose lips, vomero-palatine organs, prominent rostral, and labial folds (Azim et al., 2005). The feeding apparatus of African catfish is poorly documented. Studies by van Wassenbergh et al. (2004), Herrel et al. (2005), Zulfahmi et al. (2022), and Murray and Holmes (2022) only reported on the morphology of the cranium of African catfish. This study will compare the feeding apparatus of the African catfish to that of tilapias.

African catfish is one of the representatives of Actinopterygian fishes that show hyostylic jaw suspension (Zulfahmi et al., 2022). This allows them to move the upper and lower jaw without moving the cranium. The upper jaw of African catfish is made up of three regions (maxillary, mandibular region, and mandibular arcus) which protrude to the lateral side and also form a straight line to enlarge cavum brachialis (Zulfahmi et al., 2022). The upper jaws also have curved band vomerine tooth pads with sharply pointed teeth (Skelton, 2001). African catfish also have a lower jaw with high extensibility. However, *Tilapia* species and common carp exhibit a higher degree of morphological specialisation in their feeding apparatus compared to African catfish. This is evidenced by the upper and lower jaw structures which enable them an enhanced premaxilla protrusibility and lower jaw extensibility (Sibbing & Witte, 2005). Tilapia, common carp, and African catfish all have a pharyngeal bone that is used to triturate ingested food items (Skelton, 2001). Moreover, tilapia, common carp, and African catfish all have gill rakers that support filter feeding on bioseston in the water column (Zayed & Mohamed, 2004). Similarities in feeding adaptations might make African catfish a good candidate for periphyton-based aquaculture.

The structure of the substrate plays a crucial role in determining consumer–resource interactions (Taniguchi et al., 2003). More complex substrates will hinder the movement of the fish, thereby reducing the grazing rate on periphyton (Warfe & Barmuta, 2004). When the substrate complexity increases, the ability of the fish to graze from the substrate decreases. Thus, the mouth structure of the cultured fish species needs a certain level of premaxilla protrusibility and lower jaw extensibility to

efficiently graze on periphyton. Previous studies on periphyton-based aquaculture have focused on the impact of substrate structure on periphyton colonisation and abundance, showing that higher substrate complexity leads to increased periphyton biomass (Gosselain et al., 2005; Hao et al., 2017; Casartelli & Ferragut, 2018; Hao et al., 2020). This study will determine African catfish's ability to utilise periphyton from the net, plastic, and stone substrate in aqua dams.

There is a paucity of literature on studies done in periphyton-based aquaculture using African catfish. A single study by Amisah et al. (2008) reported the highest (16.0 kg/100 m²) mean gross fish yield in treatments with bamboo substrates only compared to treatments without substrates (6.0 kg/100 m²). Another different study by Teuling et al. (2017) showed that African catfish can effectively digest algal material effectively as tilapia. This might be attributed to the stomach pH of both species. Tilapia species have a stomach pH that is below 1.4 which lyses the cell wall of most plankton material while the stomach pH of African catfish ranges between 1 and 3 depending on the temperature (Nurhayati et al., 2020). The acid in the stomachs of African catfish and tilapia can lyse periphyton and bioseston cell walls. Both African catfish and tilapia have a pharyngeal bone to grind and triturate the food (Beveridge & Baird, 2000). These adaptations might cause African catfish to respond similarly to periphyton utilisation as tilapia species. The omnivorous feeding habits of African catfish make it a good candidate for periphyton-based aquaculture. No studies have been undertaken to evaluate the utilisation of periphyton by the African catfish in aqua dams. Therefore, this chapter will investigate whether African catfish can effectively utilise bioseston and periphyton in aqua dams with net, plastic, and stone substrates. The main objectives are:

- To determine the effect of substrates on African catfish utilisation of periphyton and bioseston in aqua dams with net, plastic, and stone substrate
- To determine the compatibility of the African catfish's feeding structure to utilise periphyton from net, plastic and stone.

5.2 MATERIALS AND METHODS

5.2.1 Study site

Refer to Chapter 3, section 3.2.1

5.2.2 The utilisation of periphyton by African catfish in aqua dams with net, plastic and stone substrates.

Three substrates (net, plastic and stone) were deployed separately in 7 000 L aqua dams in a completely randomised design. The substrate's texture and position in aqua dams are described in Chapter 3, sections 3.2.2 and 3.2.3. The deployment of each substrate was replicated three times, twenty-seven aqua dams were used. African catfish (200.2 ± 22.74 g, mean $\pm$ SD) were stocked in the aqua dams, 35 fish per aqua dam. Aqua dams are a static production system and water lost through evaporation was replaced weekly with matured water (water with no chlorine) from a reservoir tank. In order to determine the extent to which African catfish utilised periphyton, three feeding regimes were adopted. The same treatments described in Chapter 4 (section 4.2.2) were adopted (N100, P100 and S100 for the control; N50, P50 and S50 for the fish were fed commercial pellets every other day: N33, P33 and S33 the African catfish were fed commercial pellets every third day). Three distinct experiments were carried out, each with three feeding regimes, which resulted in a total of nine aqua dams. These experiments were not conducted simultaneously but took place in the same season, from January 2022 to March 2023, using all twenty-seven aqua dams. Temperature and dissolved oxygen were monitored in all the aqua dams to ensure that they did not become confounding variables. The temperature was 28.41 ± 3.59 °C (mean $\pm$ SD) and dissolved oxygen was 9.76 ± 3.05 mg/l in all the substrates.

5.2.3 Determination of periphyton abundance in the aqua dams with net, plastic and net substrates.

The collection of periphyton samples was carried out as described in Chapter 3, Section 3.2.3. Periphyton abundance was expressed as a number per cm². Frequency of occurrence was calculated to determine the most occurring periphyton genera. The frequency of occurrence was calculated separately per substrate, each substrate's

frequency of occurrence is out of 100%. The frequency of occurrence and dominance index of the enumerated periphyton was calculated using the formula below. Dominance index was calculated to determine the most dominant periphyton genera. In the present study, only periphyton genera with a dominance index of > 5 were considered.

$$\text{Frequency of occurrence (\%)} = \frac{\text{The number of ml in which spp is found}}{\text{Total number of ml examined}} \times 100$$

$$\text{Dominance index} = \frac{\text{The abundance of periphyton taxon}}{\text{Total abundance of all periphyton} \times \text{frequency of occurrence of taxon}}$$

5.2.4 Determination of bioseston in aqua dams with net, plastic and stone substrates.

The collection of bioseston samples was carried out as described in Chapter 3, Section 3.2.4. The frequency of occurrence was determined according to the formula above. Dominance index was calculated to determine the most dominant bioseston genera, only bioseston genera with a dominance index of > 5 were considered. Bioseston abundance was expressed as a number per ml, and the abundance was log-transformed to standardised bioseston abundance. Frequency of occurrence was calculated to determine the most occurring bioseston genera.

$$\text{Dominance index} = \frac{\text{The abundance of bioseston taxon}}{\text{Total abundance of all bioseston} \times \text{frequency of occurrence of taxon}}$$

5.2.5 Growth performance indices and feeding intensity of African catfish in aqua dams with net, plastic and stone substrates.

African catfish were allowed to utilise periphyton and bioseston for six weeks in aqua dams with net, plastic and stone substrates (section 5.2.2) before the growth performance indices were determined. The total weight (to the nearest 0.01 g) of all the African catfish in the aqua dams was determined using a bench scale. The standard length of the fish was also determined using a tape measure to the nearest 0.5 mm. The weight and standard length of the African catfish were recorded before

the fish were stocked into the aqua dams and at termination of the experiments. Three African catfish from each treatment in the aqua dams were then sacrificed and gutted to remove the stomach. The stomach content was analysed under a light microscope to identify the periphyton and bioseston items ingested by the fish. The stomach fullness was ranked from 0 to 5 with a 0 indicating empty stomach and a 5 indicating full stomach. The faecal matter was collected from the distal intestine of the fish and analysed under a light microscope to determine which periphyton and bioseston items were not digested by the fish. The analysis of stomach contents and faecal matter is reported for African catfish from aqua dams (N50, P50, S50, N33, P33, and S33) with periphyton substrates, while no data is provided from the control group (N100, P100, and S100). This omission occurred because the African catfish in the control group were only fed commercial pellets without access to periphyton on the substrates. Feeding intensity was determined because African catfish are nocturnal feeders. Feeding intensity was determined by the percentage of empty stomachs per substrate. The feeding intensity ranged from 0 to 100 percent, with a 0 indicating less intense feeding and a 100 indicating intense feeding. The fish weights and standard-length values were used to calculate the following growth performance indices:

Average daily weight gain = final weight gain (g)/time

Percentage weight gain = [(final weight – initial weight)/initial weight] x 100

Specific growth rate (SGR) = (ln final weight – ln initial weight)/time

Condition factor = (weight/length³) × 100

Survival rate (%) = (final number of fish/initial number of fish) × 100

5.2.6 Modified percentage Index of Relative Importance (%IRI) and food selection indices of African catfish in aqua dams with net, plastic and stone substrates.

A modified percentage index of relative importance was determined because African catfish have a well-defined stomach. A modified percentage Index of Relative Importance (%IRI), adopted from Mahesh et al. (2018), was used to determine the importance of food items in the stomach of African catfish in all treatments calculated by the formula:

$$\%IRI = \%NO * \%FO$$

where %NO is the percentage of the number of the food item, and %FO is the percentage frequency of occurrence of the food items

Food selectivity was calculated using the following Chesson selectivity index (CSI):

$$CSI = \frac{g_i/b_i}{\sum_{j=1}^n g_j/b_j}, i = 1, \dots, n,$$

Where n is the total number of species in the stomach contents; g and b are the proportional representation of periphyton species i or j in the stomach content and water (environment), respectively. The CSI value for a specific species i reflects its presence in the stomach contents relative to the environment, divided by the sum of the equivalent calculation for all species in the stomach content. The Chesson selectivity index value ranges from -1 to +1, with -1 indicating complete avoidance of the food item, 0 indicating a random selection of the food and 1 indicating exclusive feeding on that species.

5.2.7 The feeding structure of African catfish and tilapia species.

Morphological analysis of the feeding structures of African catfish was carried out and compared to *Oreochromis mossambicus* (tilapia species). A total of 18 subadult individuals (from each species) were used. The width of the mouth, premaxilla protrusibility and lower jaw extensibility were measured to the nearest 0.05 mm using methods described by Keast and Webb (1966) and Morris (1981). The width of the mouth aperture was expressed as a percentage of the standard length. The number of gill rakers was also counted. The premaxilla protrusibility is given as the percentage difference in distance from the anterior margin of the lower eye socket to the outer edge of the premaxilla, from the tightly closed to the wide-open mouth (Keast & Webb 1966). The lower jaw extensibility is given as the distance from the anterior margin of the lower eye socket to the tip of the lower jaw with the mouth closed, which was

subtracted from the distance with the mouth open and expressed as a percentage of the former (Keast & Webb 1966).

5.2.8 Data and statistical analysis

Microsoft Excel and Power BI were used to plot graphs. Analysis of similarities (ANOSIM), Permutational multivariate analysis of variance (PERMANOVA) and Multivariate analysis of variance (MANOVA) were performed in R (version 2024.4.2-764) using the vegan, MASS, effective, tidyverse, magrittr and dplyr packages. ANOSIM was used to determine if there were similarities in periphyton abundance on net, plastic and stone substrates in aqua dams. PERMANOVA was used to determine the statistical difference of the food items identified in the stomach of African catfish utilising periphyton on net, plastic and stone substrates in aqua dams. ANOSIM was also used to determine if the food items identified in the stomach were similar within each substrate or between the substrates. Canoco (4.5) was used to run the detrended correspondence analysis (DCA) which was used to identify the relationship between the food items in the stomach and faeces of the fish and the treatments in the aqua dams. Normality and homogeneity of growth performance indices were tested using Shapiro-Wilk and Levene tests, respectively using Statistical Package and Service Solutions (SPSS, version 28). Analysis of Variance (ANOVA) was used to test for significant differences in growth performance. Tukey's post hoc analysis was used to determine which means were significantly different from each other (SPSS version 28). MANOVA was then used to determine the significant difference in the growth performance indices between the substrates (net, plastic and stone).

5.3 RESULTS

Periphyton and bioseston were identified to genera level to determine the most abundant genera in the aqua dams with substrates. *Microcystis* sp., *Chlorella* sp. and *Scenedesmus* sp. were numerically abundant periphyton on all substrates (Table 5.1). The species composition on the net substrates was significantly higher ($R = 0.377$, $P = 0.0001$; ANOSIM) than the composition on plastic and stone substrates.

Table 5. 1: Species list of periphyton in aqua dams deployed with net, plastic and stone substrates

	Net	Plastic	Stone
Periphyton			
<i>Actinastrum</i> sp.	-	6.88	20.93
<i>Ankistrodesmus</i> sp.	-	0.92	0.38
<i>Chroococcus</i> sp.	7.05	-	-
<i>Chlorella</i> sp.	60.39	50.03	51.09
<i>Chlorogonium</i> sp.	35.09	11.10	0.32
<i>Closterium</i> sp.	0.07	-	0.00
<i>Coelastrum</i> sp.	3.43	-	14.43
<i>Cosmarium</i> sp.	23.35	11.35	0.06
<i>Crucigenia</i> sp.	2.21	0.81	0.47
<i>Dictyosphaerium</i> sp.	1.07	33.96	5.07
<i>Euastrum</i> sp.	0.74	-	-
<i>Haematococcus</i> sp.	7.65	3.39	5.53
<i>Gloeocystopsis</i> sp.	2.43	0.11	0.00
<i>Kirchneriella</i> sp.	0.20	0.24	2.31
<i>Lagerheimia</i> sp.	0.25	3.30	-
<i>Micractinium</i> sp.	2.20	51.60	37.87
<i>Oocystis</i> sp.	15.62	4.57	1.80
<i>Pandorina</i> sp.	9.10	25.17	5.88
<i>Scenedesmus</i> sp.	53.81	12.13	43.24
<i>Selenastrum</i> sp.	-	2.60	0.21
<i>Stigeoclanium</i> sp.	1.78	3.00	0.01
<i>Sphaerocystis</i> sp.	3.61	3.43	0.37
<i>Ulothrix</i> sp.	0.07	1.90	-
<i>Cyclotella</i> sp.	4.68	-	8.56
<i>Pinnularia</i> sp.	32.22	33.41	14.73
<i>Synedra</i> sp.	36.79	44.82	17.13
<i>Navicula</i> sp.	27.34	15.15	4.66
<i>Nitzschia</i> sp.	33.75	6.02	3.64
<i>Mougeotia</i> sp.	3.56	14.23	0.44
<i>Staurastrum</i> sp.	0.22	0.08	-
<i>Puccinia</i> sp.	-	-	12.56
<i>Aphanocapsa</i> sp.	0.38	0.09	5.15

<i>Gloeotrichia sp.</i>	4.51	0.08	-
<i>Oscillatoria sp.</i>	0.28	4.42	-
<i>Microcystis sp.</i>	72.26	53.09	61.78
<i>Melosira sp.</i>	0.79	0.08	0.10
<i>Vaucheria sp.</i>	0.13	3.78	0.07
Total	447.0233	401.75	318.76
Diversity index	2.1182	2.0278	2.0086

Brachionus sp. and *Cryptomonas sp.* were the most abundant bioseston on the net and plastic substrates (Table 5.3). *Diffflugia sp.*, *Brachionus sp.*, and *Vorticella sp.* were numerically dominant bioseston on the stone substrate. The species composition was significantly higher ($R = 0.2411$, $P = 0.0001$; ANOSIM) on the stone substrate (Table 5.2).

Table 5. 2: Species list of bioseston in aqua dams deployed with net, plastic and stone substrates

	Net	Plastic	Stone
Bioseston			
<i>Actinosphaerium sp.</i>	0.67	0.03	0.07
<i>Trachelomonas sp.</i>	0.24	0.14	4.88
<i>Phacus sp.</i>	0.06	0.18	0.20
<i>Peranema sp.</i>	1.94	3.55	2.90
<i>Cryptomonas sp.</i>	11.11	26.15	6.60
Ephemeroptera larvae	0.17	0.29	0.16
Chironomidae larvae	1.45	0.44	0.14
Culicidae larvae	0.22	0.13	0.03
Drosophila larvae	0.13	0.11	0.08
Other Ostracoda	0.11	0.03	0.05
Other Cladocera	6.25	0.17	0.52
Other Copepods	10.46	3.61	3.94
Notonecta glauca (backswimmer)	0.02	0.15	0.04
<i>Gerris sp.</i>	0.06	0.12	0.07
<i>Gyrinus sp.</i>	0.06	0.08	0.07
<i>Moina sp.</i>	0.31	0.22	0.14
<i>Ceriodaphnia sp.</i>	0.34	0.24	0.17
<i>Corixa sp.</i>	0.12	0.11	0.03
<i>metanauplius larvae</i>	0.11	0.05	0.25
<i>Monhystera sp.</i>	0.05	-	0.04
<i>Amoeba sp.</i>	0.05	1.00	0.22
<i>Blepharisma sp.</i>	0.74	1.34	3.84
<i>Coleps sp.</i>	0.60	1.05	0.09
<i>Diffflugia sp.</i>	10.55	16.46	21.53

<i>Euplotes sp.</i>	0.04	2.03	1.47
<i>Halteria sp.</i>	0.09	3.54	1.49
<i>Loxodes sp.</i>	0.76	0.18	3.34
<i>Paramoecium sp.</i>	5.08	4.12	13.15
<i>Paradileptu sp.</i>	-	0.11	0.26
<i>Stylonychia sp.</i>	1.33	0.05	3.29
<i>Spirostomum sp.</i>	0.50	0.31	2.39
<i>Sterkiella sp.</i>	0.11	0.16	2.80
<i>Urostyla sp.</i>	0.50	0.32	0.62
<i>Vorticella sp.</i>	5.03	12.84	20.06
<i>Brachionus sp.</i>	13.86	23.18	21.15
<i>Lecane sp.</i>	10.31	0.86	7.31
<i>Trichocerca sp.</i>	1.43	0.08	0.91
<i>Philodina sp.</i>	2.10	8.20	0.31
<i>Rotifers eggs</i>	15.03	16.09	7.89
Total	101.9867	127.6933	132.52
Diversity index	2.1892	2.2036	2.2451

(Other copepods, other Cladocera and other Ostracoda are unidentified species and fragments)

The frequency of occurrence of periphyton and bioseston genera in all aqua dams with substrates mirrored the abundance (Figure 5.1).

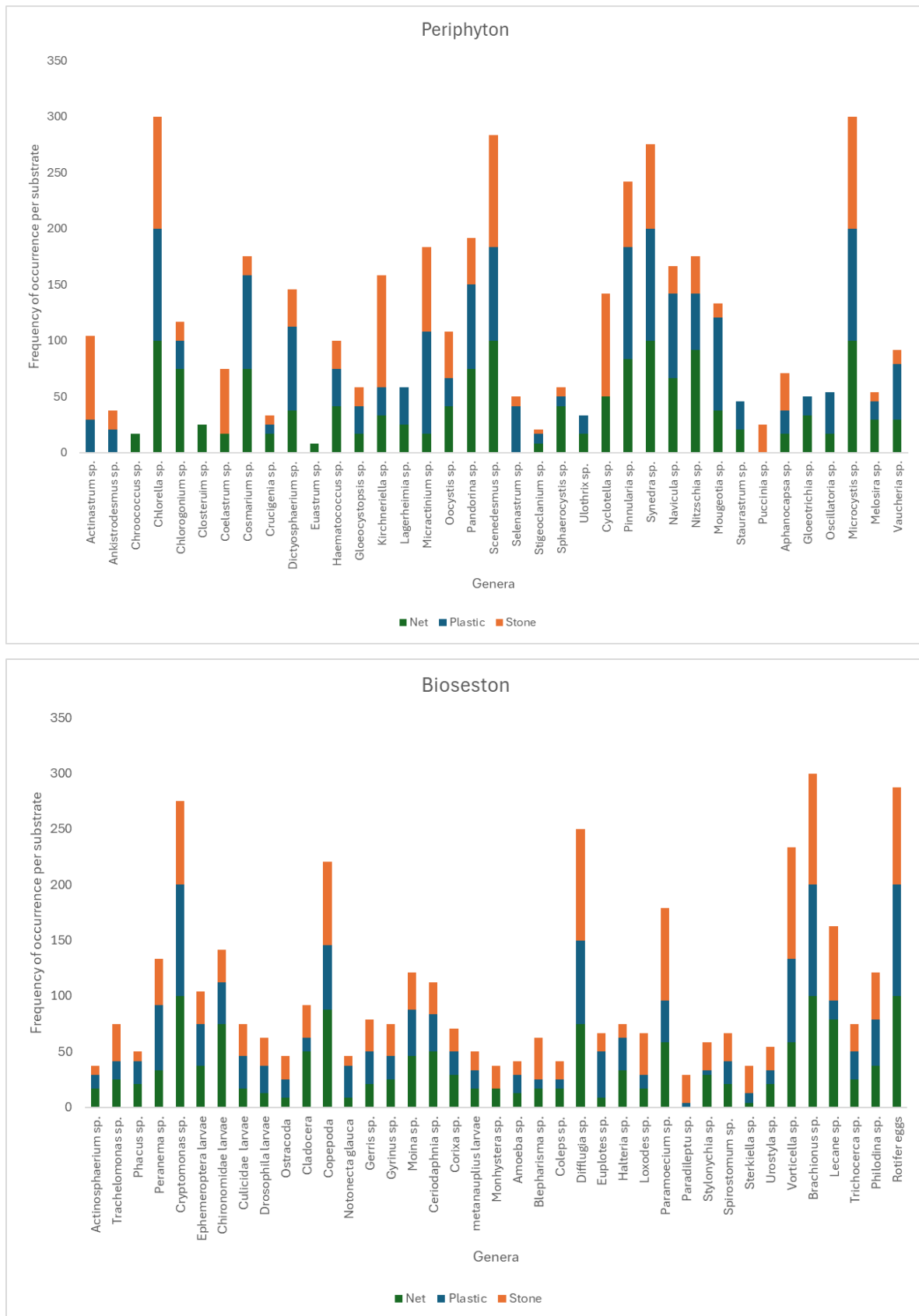


Figure 5. 1: Frequency of occurrence of species list of periphyton and bioseston in aqua dams deployed with net, plastic and stone substrates (Copepods, Cladocera and Ostracoda are unidentified species and fragments)

The dominance index was calculated to identify the most dominant genera across the substrates in all the aqua dams. *Chlorella* sp. and *Microcystis* sp. were the most dominant periphyton on all substrates (Table 5.3). *Brachionus* sp., *Cryptomonas* sp., and *Diffugia* sp. were the most dominant bioseston in all aqua dams with substrates (Table 5.3).

Table 5. 3: The dominance index of periphyton and bioseston in the aqua dam with net, plastic and stone stocked with African catfish.

	Net	Plastic	Stone
Periphyton			
<i>Actinastrum</i> sp.	0.00	0.50	5.07
<i>Chlorella</i> sp.	13.51	12.45	13.31
<i>Chlorogonium</i> sp.	5.89	0.69	0.01
<i>Dictyosphaerium</i> sp.	0.09	6.34	0.44
<i>Micractinium</i> sp.	0.08	11.77	7.40
<i>Scenedesmus</i> sp.	12.04	2.52	11.27
<i>Pinnularia</i> sp.	6.01	8.32	2.24
<i>Synedra</i> sp.	8.23	11.16	3.35
<i>Nitzschia</i> sp.	6.92	0.75	0.32
<i>Microcystis</i> sp.	16.17	13.21	16.10
Bioseston			
<i>Cryptomonas</i> sp.	10.90	20.48	3.74
<i>Copepod</i>	8.98	1.65	2.23
<i>Diffugia</i> sp.	7.76	9.67	16.24
<i>Paramoecium</i> sp.	2.90	1.21	8.27
<i>Vorticella</i> sp.	2.88	7.54	15.14
<i>Brachionus</i> sp.	13.59	18.16	15.96
<i>Lecane</i> sp.	8.00	0.11	3.68
<i>Rotifer</i> eggs	14.74	12.60	5.21

Copepods are unidentified species and fragments

The detrended correspondence analysis (DCA) was used to identify the relationship between the food items in the stomachs of the fish and the treatments in the aqua dams. Detrended correspondence analysis showed that the first two axes explained 56.1 % of the variation in the distribution of food items in the stomach of African catfish, and the total inertia was 0.700 (Figure 5.2). The stomach of African catfish in aqua dams with net was associated with high abundance of Chironomidae larvae, insects and rotifers in both treatments (Figure 5.2). The stomach of African catfish in aqua

dams with the plastic substrate was associated with *Microcystis* sp., copepods and Cladocera (Figure 5. 2). The stomach of African catfish in aqua dams with stone substrate was not associated with any of the food items.

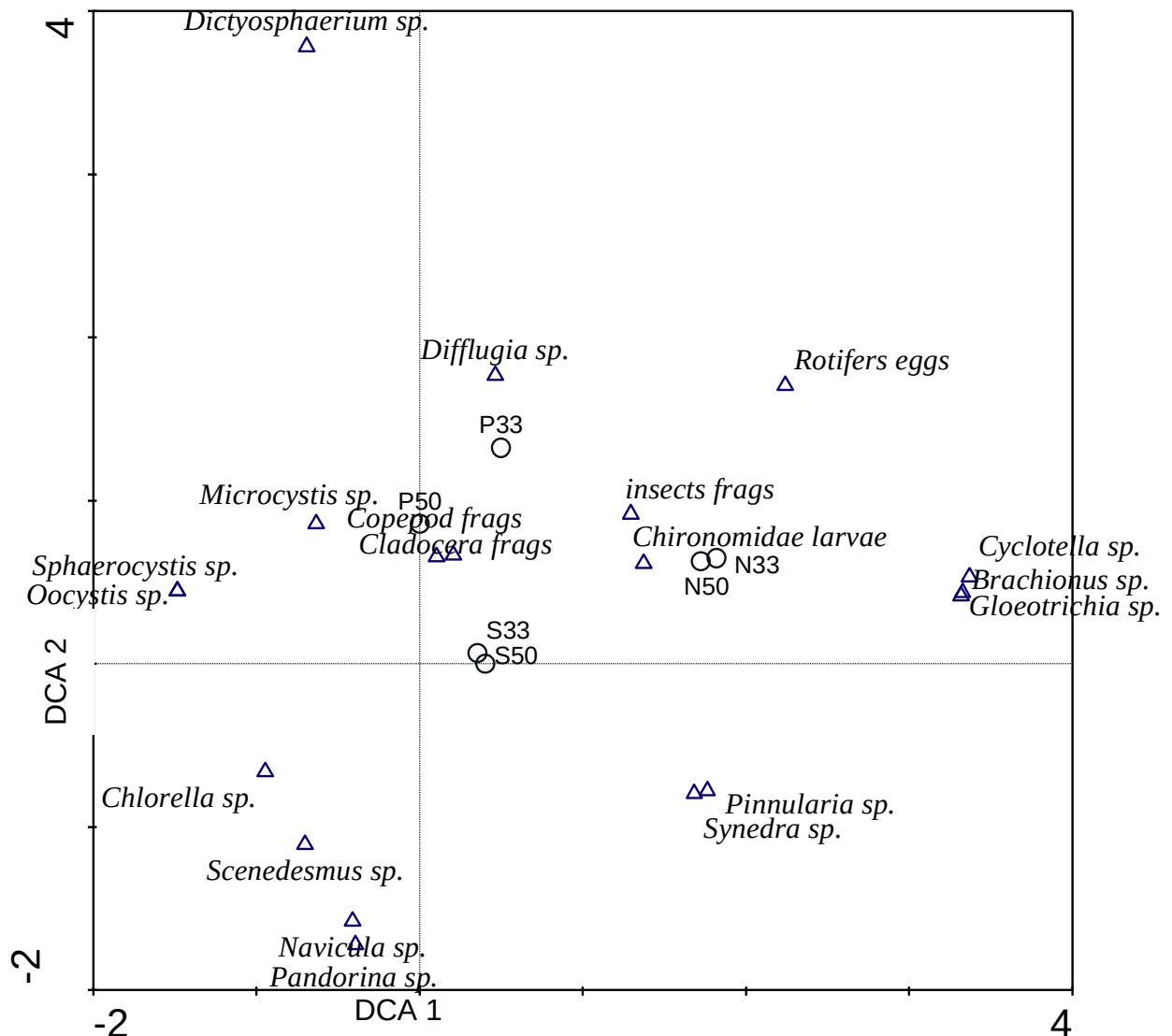


Figure 5. 2: Detrended correspondence analysis (DCA) plot showing the relationship between the food items identified in the stomachs of African catfish and the treatments in aqua dams with net, plastic and stone. Abbreviation used: Copepod frag- copepod body fragment, Cladocera- Cladocera body fragments.

The detrended correspondence analysis (DCA) was used to identify the relationship between the food item in the faecal matter of the fish and the treatments in the aqua dams. The first two axes explained 61.4 % of the variation in the distribution of food

items in the faeces of African catfish, the total inertia was 0.494 (Figure 5.3). *Pinularia* sp., *Navicula* sp., *Synedra* sp., *Diffflugia* sp., Chironomidae larvae, rotifer eggs, *Dictyosphaerium* sp. and *Microcystis* sp. were found in the faecal matter of African catfish in all aqua dams deployed with net, plastic and stone substrate (Figure 5.3).

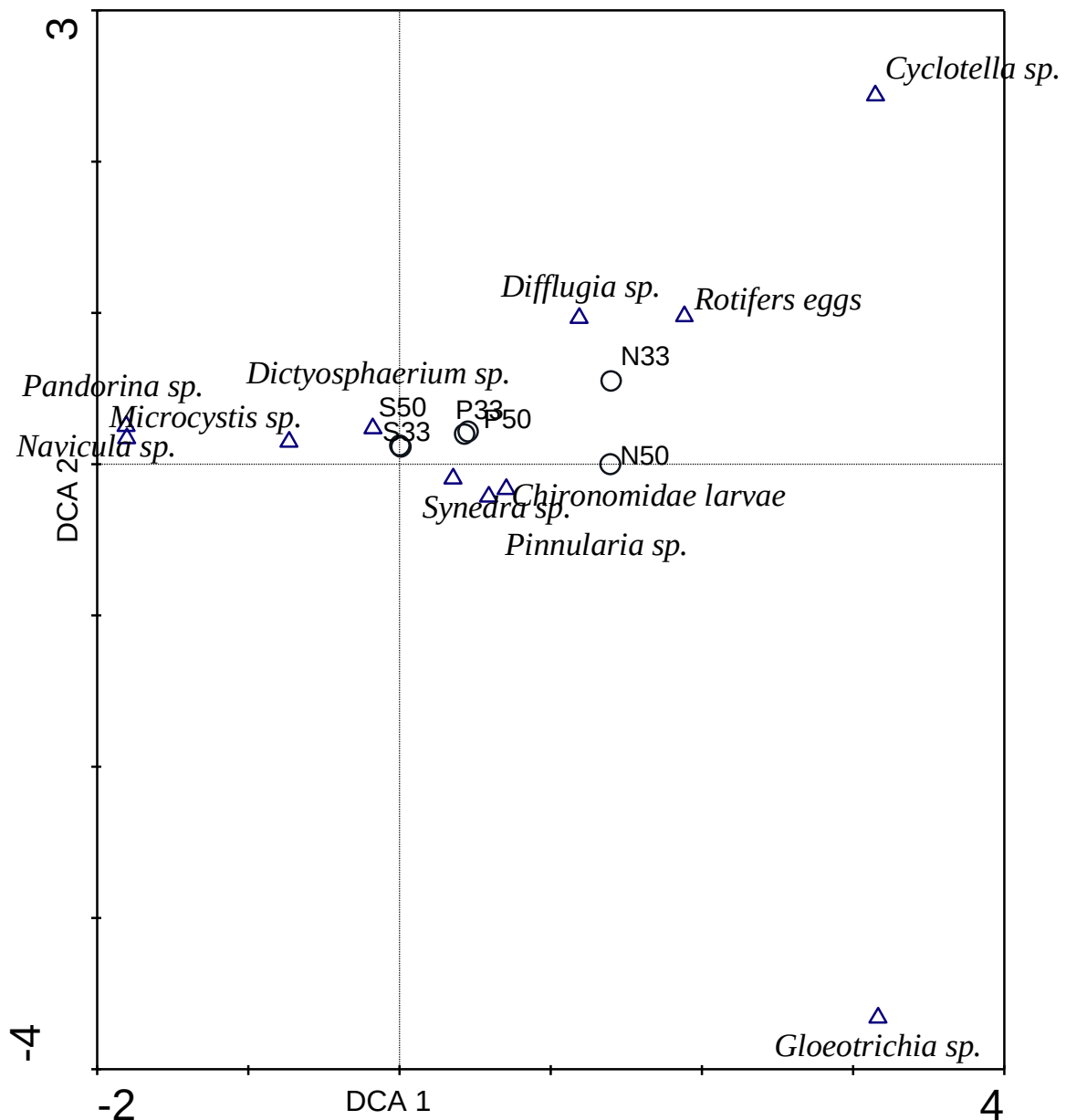


Figure 5. 3: Detrended correspondence analysis (DCA) plot showing the relationship between the food items identified in the faecal matter of African catfish and the treatments in aqua dams with net, plastic and stone.

A modified Index of Relative Importance (%IRI) was used to determine the most important food item for African catfish utilising periphyton and bioseston in the aqua dams. Insects and Chironomidae larvae were the most important food items for African catfish in aqua dams with net substrate (Table 5.4).

Table 5. 4: A modified Index of Relative Importance (%IRI) of food items identified in the stomach of African catfish in aqua dams with net, plastic and stone.

	Net		Plastic		Stone	
	N50	N33	P50	P33	S50	S33
<i>Chlorella sp.</i>	-	-	2.41	0.00	0.04	0.03
<i>Oocystis sp.</i>	-	-	3.49	0	-	-
<i>Dictyosphaerium sp.</i>	-	-	1.89	7.57	-	-
<i>Sphaerocystis sp.</i>	-	-	4.65	0	-	-
<i>Pandorina sp.</i>	-	-	-	-	13.70	3.77
<i>Scenedesmus sp.</i>	-	-	0.17	0	0.99	0.63
<i>Cyclotella sp.</i>	0.03	0.18	-	-	-	-
<i>Pinnularia sp.</i>	9.80	0.28	-	-	2.58	0.31
<i>Synedra sp.</i>	0.03	2.71	-	-	3.38	0.87
<i>Navicula sp.</i>	-	-	-	-	0.22	1.67
<i>Gloeotrichia sp.</i>	0.07	0.03	-	-	-	-
<i>Microcystis sp.</i>	-	-	37.95	13.55	14.30	13.18
Chironomidae larvae	19.31	51.52	3.49	9.56	21.05	18.21
Cladocera fragments	0.20	0.03	8.52	10.36	12.31	24.80
Copepod fragments	0.07	0.03	12.01	11.16	14.69	26.84
Insects fragments	49.90	16.90	18.59	26.29	16.68	9.42
<i>Brachionus sp.</i>	2.11	1.11	-	-	-	-
<i>Diffugia sp.</i>	0.07	0.06	6.58	20.72	0.07	0.26
Rotifers eggs	18.42	27.15	0.26	0.80		

The Chesson selectivity index was calculated to determine whether the food items were ingested at random, actively selected or avoided in the aqua dams. The Chesson selectivity index showed that African catfish in aqua dams with net, plastic and stone substrates selectively fed on insects, Chironomidae larvae and Cladocera while other periphyton genera were fed randomly (Table 5.5). The food items in the stomachs of all African catfish in aqua dams with different substrates were not statistically significant ($F = 0.256$, $Pr = 0.78$; PERMANOVA). The food items were evenly distributed within and between the stomachs of African catfish in all substrates ($R =$

0.0845, $P = 0.1543$; ANOSIM). Feeding intensity was 100 % in African catfish in aqua dams with net substrate (both treatments) (Table 5.5).

Table 5. 5: The Chesson selectivity index of the periphyton items in the stomach of African catfish in aqua dams deployed with net, plastic and stone substrates.

	Net		Plastic		Stone	
	N50	N33	P50	P33	S50	S33
Stomach fullness	0.5 ± 0.0	0.5 ± 0.0	0.3±0.5	0.7±1.1	0.7±1.1	1.4±2.2
<i>Chlorella</i> sp.	-	-	0.0002	0.00000	0.00001	0.00001
<i>Oocystis</i> sp.	-	-	0.0035	0.00000	-	-
<i>Dictyosphaerium</i> sp.	-	-	0.00038	0.00029	-	-
<i>Sphaerocystis</i> sp.	-	-	0.00365	-	-	-
<i>Pandorina</i> sp.	-	-	-	-	0.00223	0.00553
<i>Scenedesmus</i> sp.	-	-	0.00034	0.00000	0.00007	0.00007
<i>Cyclotella</i> sp.	0.00009	0.00204	-	-	-	-
<i>Pinnularia</i> sp.	0.00037	0.00001	-	-	0.00031	0.00013
<i>Synedra</i> sp.	0.00001	0.00086	-	-	0.00044	0.00016
<i>Navicula</i> sp.	-	-	-	-	0.00039	0.00079
<i>Gloeotrichia</i> sp.	0.00002	0.00044	-	-	-	-
<i>Microcystis</i> sp.			0.00368	0.000383	0.00041	0.00043
Chironomidae larvae	0.01802	0.21852	0.03198	0.04788	0.26462	0.25092
Cladocera fragments	0.00011	0.00241	0.14041	0.74348	0.02558	0.23120
Copepod fragments	0.00007	0.00016	0.02292	0.00364	0.00689	0.01317
Insects fragments	0.97853	0.76641	0.79004	0 20221	0.69901	0.49751
<i>Brachionus</i> sp.	0.000262	0.00064	-	-	-	-
<i>Diffugia</i> sp.	0.000086	0.00028	0.00257	0.00156	0.00004	0.00009
Rotifers eggs	0.002245	0.00816	0.00024	0.00055	-	-
Feeding intensity (%)	100	100	33	33	33	67

Growth performance indices were determined to monitor the growth of African catfish in the aqua dams utilising periphyton and bioseston. In aqua dams with net substrates, the fish in the control treatment (N100) exhibited higher SGR and condition factors compared to N50 and N33 (Table 5.6). However, there was no significant difference ($P > 0.05$) in the growth performance indices of African catfish in aqua dams with net substrate, except for percentage weight gain and ADWG (Table 5.6).

Table 5. 6: The growth performance indices (mean $\pm$ SE) of African catfish in aqua dams with net substrate.

	Treatments			
	N100	N50	N33	P value
Initial mean weight	191.33 $\pm$ 4.37	188.66 $\pm$ 10.72	220.66 $\pm$ 15.50	0.160
Final mean weight	490.33 $\pm$ 22.73	415.66 $\pm$ 48.73	407.00 $\pm$ 22.64	0.237
ADWG	0.60 $\pm$ 0.02 ^a	0.53 $\pm$ 0.04 ^{ab}	0.45 $\pm$ 0.00 ^b	0.042
Percentage weight gain (%)	157.05 $\pm$ 17.27 ^a	119.71 $\pm$ 19.79 ^{ab}	84.84 $\pm$ 2.91 ^b	0.042
SGR	2.29 $\pm$ 0.17	1.89 $\pm$ 0.23	1.61 $\pm$ 0.04	0.075
Condition factor	1.65 $\pm$ 0.14	1.32 $\pm$ 0.20	1.03 $\pm$ 0.31	0.236
Survival rate (%)	93.33 $\pm$ 6.66	100 $\pm$ 0.0	100 $\pm$ 0.0	0.422

Abbreviation used: ADWG= Average daily weight gain, SGR = Specific growth rate.

In aqua dams with plastic substrates, the SGR and condition factor of the fish were higher in the control (P100) treatment followed by the P33 treatment (Table 5.7). However, only the mean final weight and condition factor were significantly different across all treatments in aqua dams with plastic substrate ($P < 0.05$).

Table 5. 7: The growth performance indices (mean $\pm$ SE) of African catfish in aqua dams with plastic substrate.

	Treatment			
	P100	P50	P33	P value
Initial mean weight	325.33 $\pm$ 20.07	304.66 $\pm$ 19.74	317.33 $\pm$ 27.96	0.819
Final mean weight	666.66 $\pm$ 42.17 ^a	500.33 $\pm$ 5.84 ^b	627.50 $\pm$ 18.09 ^a	0.011
ADWG	0.50 $\pm$ 0.04	0.39 $\pm$ 0.04	0.49 $\pm$ 0.05	0.255
Percentage weight gain (%)	106.35 $\pm$ 17.14	65.63 $\pm$ 11.03	102.24 $\pm$ 25.53	0.315
SGR	1.88 $\pm$ 0.22	1.31 $\pm$ 0.18	1.81 $\pm$ 0.31	0.281
Condition factor	1.71 $\pm$ 0.06 ^a	1.37 $\pm$ 0.04 ^b	1.61 $\pm$ 0.02 ^a	0.007
Survival rate (%)	100 $\pm$ 0.0	100 $\pm$ 0.0	93.33 $\pm$ 6.66	0.422

Abbreviation used: ADWG= Average daily weight gain, SGR = Specific growth rate.

On the other hand, African catfish in the S33 treatment had higher SGR than S50 and S100 in aqua dams with stone substrates. (Table 5.8). The condition factor was higher in the control treatment (S100). However, there was no significant difference ($P > 0.05$)

in the growth performance indices of African catfish in aqua dams with stone substrates (Table 5.8).

Table 5. 8: The growth performance indices (mean $\pm$ SE) of African catfish in aqua dams with stone substrate.

	Treatment			
	S100	S50	S33	P value
Initial mean weight	285.33 $\pm$ 35.59	301.33 $\pm$ 39.70	239.33 $\pm$ 51.34	0.595
Final mean weight	510.00 $\pm$ 30.74	505.00 $\pm$ 36.82	439.50 $\pm$ 39.11	0.361
ADWG	0.43 $\pm$ 0.06	0.43 $\pm$ 0.05	0.45 $\pm$ 0.10	0.910
Percentage weight gain (%)	84.37 $\pm$ 25.09	71.48 $\pm$ 16.11	96.82 $\pm$ 36.83	0.813
SGR	1.69 $\pm$ 0.37	1.51 $\pm$ 0.26	1.83 $\pm$ 0.54	0.865
Condition factor	1.42 $\pm$ 0.11	1.31 $\pm$ 0.08	1.30 $\pm$ 0.08	0.482
Survival rate (%)	93.33 $\pm$ 6.66	93.33 $\pm$ 6.66	93.33 $\pm$ 6.66	1.000

Abbreviation used: ADWG= Average daily weight gain, SGR = Specific growth rate.

The growth performance indices of African catfish in the aqua dams with net, plastic, and stone substrates were significantly different ($P = 4.827 \times 10^{-8}$, MANOVA). However, there was no distinct pattern to conclude where the fish grew faster among the aqua dams with net, plastic, and stone. The partial Eta Squared value showed that the effect size was 0.76 at a 95% confidence interval [0.60, 1.00]. This shows that each substrate influenced the growth performance of African catfish differently (Figure 5.4). The Linear Discriminant Analysis (LDA) was used as a post hoc test and it showed the proportion of trace of 0.8551 and 0.1449 for LD1 and LD2, respectively (Figure 5.4).

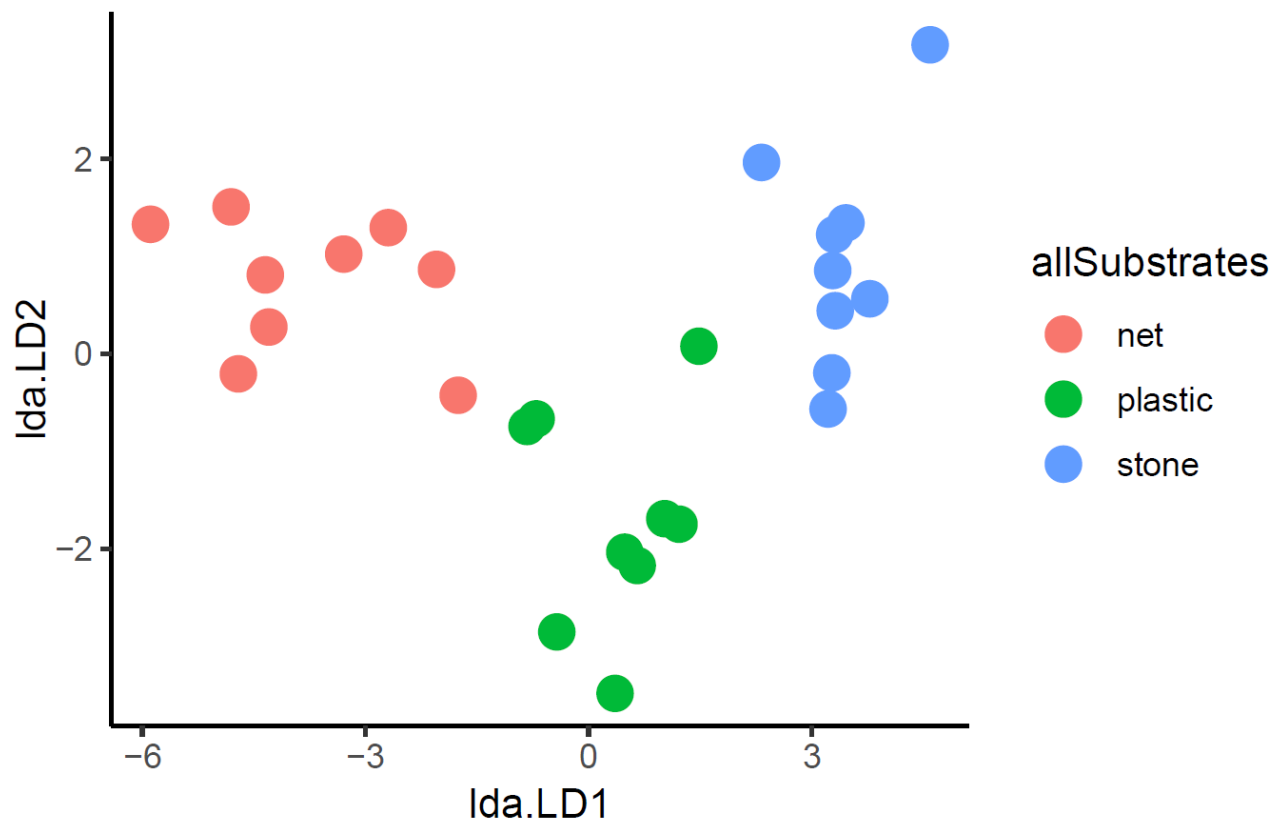


Figure 5. 4: The scatter plot of LDA of growth performance indices of African catfish in aqua dams with net, plastic and stone substrate.

The mouth aperture of African catfish was assessed and compared with the mouth aperture of tilapia to determine its suitability to utilise periphyton and bioseston in aqua dams with net, plastic and stone substrate. The mouth aperture of African catfish was wide while that of tilapia was moderate (Table 5.9). The mouth height in relation to the standard length of the fish was higher in tilapia than in African catfish. The mouth width and depth in relation to the standard length of the fish were relatively similar in both species (Table 5.9). The premaxilla protrusibility was much smaller in African catfish compared to tilapia. However, the lower jaw extensibility was much larger in African catfish compared to tilapia (Table 5.9). The pharyngeal bone was tooth-like with rough and sharp teeth and it was divided into four pads while in tilapia it had three pads, and it was toothbrush-like. The gill rakers of tilapia were short and widely spaced while African catfish have long and narrowly spaced gill rakers.

Table 5. 9: The comparison of mouths apparatus of African catfish and tilapia.

	African catfish	Tilapia
Mouth Aperture	Wide	Moderate
Mouth depth from side	1.04 ± 0.34	1.40 ± 0.22
Mouth height when open	4.5 ± 0.82	2.73 ± 0.36
Mouth width when open	3.36 ± 0.56	1.40 ± 0.24
Mouth depth % of SL	$9,75 \pm 0,40$	$8,29 \pm 1,14$
Mouth height % of SL	$13,09 \pm 1,94$	$16,08 \pm 0,96$
Mouth width % of SL	9.76 ± 0.90	8.25 ± 0.94
Premaxilla protrusibility (%)	5 ± 0.00	52.50 ± 22.17
Lower jaw extensibility (%)	132 ± 25.88	21.25 ± 4.79
Number of gill rankers	72.4 ± 15.32	20.50 ± 1.29

Abbreviation used: SL- standard length. Criteria grouping mouth aperture is adopted from Keast and Webb (1966).

5.4 DISCUSSION

In the wild, African catfish naturally feed on zooplankton, phytoplankton, other fish, higher plants, insects, vegetable debris, crustaceans, plant fibrous roots, and detritus (Dadebo et al., 2014; Santi et al., 2023; Tesfahun & Alebachew, 2023). Periphyton and bioeston are a conglomeration of these food items that African catfish feed on in the wild. *Microcystis* sp., *Chlorella* sp. and *Scenedesmus* sp. were the most abundant and dominant periphyton on all substrates in aqua dams with African catfish. This might suggest they were not ingested by African catfish due to their preference for animal-based food items. African catfish have predatory behaviour and commonly prefer food items of animal origin. However, *Brachionus* sp., *Diffugia* sp. *Cryptomonas* sp., *Vorticella* sp. are of animal origin, yet they were also the most abundant and dominant bioeston in all aqua dams with substrates. This might suggest that were consumed at lower rate due to the presence of commercial pellets. The presence of commercial pellets commonly shifts the preference of fish from feeding on natural food since they use more energy to catch the prey or filter feed compared to feeding on pellets. This agrees with Grover et al. (1989) and Rocha-Loures (2001) who reported that fish consume less zooplankton when commercial pellet is introduced in fish ponds. In this chapter, *Microcystis* sp., *Chlorella* sp. and *Scenedesmus* sp. were classified as periphyton and *Brachionus* sp., *Diffugia* sp., *Cryptomonas* sp., *Vorticella* sp. were classified as bioeston. However, they are commonly classified as phytoplankton and zooplankton, especially in systems without substrates.

Chironomidae larvae, insects, copepod and Cladocera, *Mircocystis* sp. and *Pandorina* sp. dominated the stomach content of African catfish in all aqua dams with net, plastic and stone. The abundance of *Microcystis* sp. and *Pandorina* sp. in the stomach was probably because of the availability of *Microcystis* sp. since it was numerically dominant and most frequently occurring on all substrates. More species of animal origin were identified in the stomach compared to plant-origin species. This was probably because of its predatory behaviour and its preference for animal-based protein. This agrees with Dadebo et al. (2014), Haque et al. (2022), and Tesfahun and Alebachew (2023) who also identified food items of animal origin as the most dominant in the stomach of African catfish in the wild. However, most studies that identified the food items in the stomach of African catfish were not done in periphyton-based

aquaculture. The current study will be the first to establish that African catfish stomach is still dominated by food items of animal origin in periphyton-based aquaculture.

Pinularia sp., *Navicula* sp., *Synedra* sp., *Diffflugia* sp., Chironomidae larvae, rotifer eggs, *Dictyosphaerium* sp. and *Microcystis* sp., were found in the faecal matter of African catfish in all aqua dams deployed with net, plastic and stone substrate. The presence of diatoms (*Pinnularia* sp., *Synedra* sp. and *Navicula* sp.) in the faecal matter was probably because they have silica-reinforced cell walls that are hard to break thus, most fish species cannot assimilate them. Only fish that have silica-reinforced teeth can break the cell walls of diatoms to assimilate them. Both diatoms' cell walls and *Diffflugia*'s tests have biogenic elements (Liang et al., 2024). *Diffflugia* sp was also identified in the faecal matter since they also have shells that are difficult to digest. The high abundance of *Microcystis* sp. in the faecal matter indicates that some of the *Microcystis* sp. were not digested by African catfish. This was probably because most blue-green algae have thick cell walls which is difficult to break down (Hoiczkyk & Hansel, 2000; van Vuuren et al., 2006). This suggests that African catfish lack gastric juices to break down blue-green algae's cell walls. Santi et al. (2023) compared the algal community in the stomachs of African catfish and tilapia and concluded that tilapia consumed more *Microcystis* sp. than African catfish. However, this conclusion was based on the stomach analysis and may not thus reflect the extent to which African catfish can digest *Microcystis* sp. Furthermore, Chironomidae larvae and rotifer eggs were also identified in the faecal matter probably because these food items were ingested in high abundance, but their digestion was sluggish and incomplete. Rotifers eggs have three-layered shells for protection; therefore, it requires a longer time exposure to digestive enzymes for the eggs to be fully digested.

Insects and Chironomidae were the most important food items in the diet of African catfish across the different substrates. Although most studies have indicated that African catfish feed on a variety of food items (Yatuha et al., 2012; Dadebo et al., 2014; Admassu et al., 2015; Haque et al., 2022), in this chapter it selectively fed on insects, Chironomidae, and Cladocera. This indicates that African catfish gravitate toward feeding on animal-based protein. Insects are neustonic organisms and African catfish

were able to easily catch them due to their predatory behaviour. Moreover, African catfish is an efficient benthic feeder thus, it was able to selectively graze on insects and Chironomidae attached to different substrates. A study by Mehring et al. (2017) showed that Chironomidae larvae form U-shaped burrows on substrate or sediments. However, the euryphagous feeding nature of this fish was also evident in this chapter as it was able to randomly feed on periphyton and bioseston ranging from rotifers to periphytic algae such as *Microcystis* sp. Feeding intensity was highest on the net substrates probably because of the availability of periphyton due to the high abundance recorded on the net substrate.

Fish stocked in aqua dams with net (N100) and plastic (P100) substrate recorded the highest growth rate along with fish stocked in aqua dams with stone substrate (S33). Both N100 and P100 represented the control because African catfish are normally fed twice every day. This indicates that catfish was able to efficiently utilise periphyton from the stone substrate since its growth rate was comparable to the control. This suggests substrate affects the utilisation of periphyton by African catfish. African catfish are benthic feeders and can easily utilise periphyton growing on the stone substrates in aqua dams, periphyton was accessible. Even though the stone substrate recorded the least periphyton abundance, African catfish used the lower jaw to scoop periphyton to support the high growth. The quality of the periphyton that colonised the stone substrate was nutritional adequate for the fish's growth. Several studies have reported that the nature of the substrate affects the quality of the periphyton (van Dam et al., 2002; Azim et al., 2005; Amirtharaj et al., 2022). Therefore, the nature of the substrate will ultimately affect the growth of the cultured fish since the growth rate of the fish is mainly determined by the biomass and abundance of the periphyton and bioseston in the system. In this chapter, the permutation test also showed that each substrate significantly influenced the growth performance of African catfish differently. However, there was no distinct pattern to conclude where the fish grew faster between net, plastic and stone substrate. This was probably because the net substrate had the highest periphyton abundance and plastic had more genera of animal origin while the periphyton on the stone was more accessible to African catfish to easily scoop. The substrate used in this study are all non-biodegradable and were able to enhance sufficient periphyton growth to sustain the growth of the cultured fish. This might

suggest that non-biodegradable substrates can be good alternatives for biodegradable substrates in periphyton-based aquaculture.

African catfish had a much smaller premaxilla protrusibility compared to tilapia. This suggests that African catfish had a stronger and fixed upper jaw which limited it to feed on periphyton as compared to tilapia. The premaxilla of African catfish was less flexible thus limiting the fish to some extent to feed on periphyton on the substrate. The higher premaxilla protrusibility of tilapia allows it to easily protrude the upper jaws to nip and scrap periphyton on the substrate. Thus, making tilapia a better periphyton feeder than African catfish. African catfish cannot scrap periphyton from the substrates due to a small premaxilla protrusibility. However, the lower jaw extensibility was much larger in African catfish than in tilapia which allows them to scoop the periphyton from the substrate. The larger lower jaw extensibility also allows African catfish to capture larger, stronger and more active prey such as insects, as identified in this chapter. The larger lower jaw extensibility also allows African catfish to easily engulf a variety of periphyton on stone substrates. The pharyngeal bone also plays an important part in the fish's ability to utilise particular food items. The teeth on the pharyngeal bone of African catfish are tooth-like unlike those on the tilapia which are toothbrush-like. African catfish probably use the teeth on the pharyngeal bone to triturate benthonic periphyton like insects and Chironomidae. The long and narrow-spaced gill rakers enable the fish to filter feed on bioseston. In filter-feeding, mucus is secreted in the gill rakers allowing the fish to feed on bioseston of various sizes (Ibrahim et al., 2015).

In conclusion, African catfish can utilise periphyton in aqua dams. African catfish can efficiently scoop periphyton from stone substrate because of larger jaw extensibility. Insects and Chironomidae were important food items. It selectively fed on insects, Chironomidae larvae, and Cladocera. However, diatoms, *Microcystis* sp. and *Diffugia* sp. dominated the faecal matter indicating that African catfish lack gastric juices and silica-reinforced teeth. Although, the net substrate registered the highest abundance, the highest growth rate was recorded in fish fed every third day with stone substrate (S33). This suggests substrate affects the quality and utilisation of periphyton by the

fish. African catfish is suitable for periphyton-based aquaculture in systems with stone substrates since its growth rate was comparable to the control.

CHAPTER 6:
**The impact of periphyton-based biofilter on
water quality parameters**

6. The impact of periphyton-based biofilter on water quality parameters.

6.1 INTRODUCTION

Periphyton-based aquaculture is primarily used for sustainable fish yield improvement and to reduce dependence on supplementary feed. However, it also plays an important role in nutrient cycling and trophic transfer of nutrients. The periphyton that grows on submerged substrates consists of autotrophic and heterotrophic microorganisms, bacteria, fungi, and algae which fish feed on, but it also acts as *in situ* water purifiers (Thompson et al., 2002; Azim et al., 2005). Periphyton is an essential part of a healthy ecosystem and can retain nutrients, remove toxic substances and metals from open water. Periphyton effectively accumulates nutrients by adsorption into extracellular polymeric substances, cell surface adsorption, and intracellular absorption (Holding et al., 2003; Serra et al., 2009). Due to this attribute, periphyton is commonly used as a bioremediation measure for contaminants in natural aquatic systems (Kanavillil et al., 2012; Tedeschi & Chow-Fraser, 2021). Though periphyton has been successfully applied as bioremediation in contaminated rivers and lakes, information on periphyton as a biofilter for freshwater aquaculture effluents has been limited. In recent years, periphyton has been used as a biofilter to remove effluents from culture tanks in brackish and marine aquaculture (Levy et al., 2017; Guttman et al., 2018). However, most studies combined periphyton with *Ulva* sp. or *Gracilaria* spp. as one biofilter (Guttman et al., 2018 & 2019; Shahara & Guttman, 2021). The current study will use periphyton on net, plastic and stone substrate as a stand-alone biofilter in a fibreglass tank system under field conditions.

Periphyton improves water quality through the photosynthesis of the periphytic organisms that reduces excess carbon dioxide and ammonia, stabilisation of the pH levels, and increases oxygenation and uptake of phosphate (Milstein, 2005; Verdegem et al., 2005; Azim & Little, 2006). The rate at which the water quality is improved depends on the abundance and composition of periphyton growing on the substrate. Periphyton growing on different substrates such as bamboo, jute stick, sugarcane bagasse, paddy straw, glass slides, and PVC pipes have also maintained water quality when they were used to improve fish growth in periphyton-based aquaculture (Wahab

et al., 1999; van Dam et al., 2002; Azim et al., 2004; Rai et al., 2018). For instance, Keshavanath et al. (2001) reported higher dissolved oxygen (DO) levels in systems with PVC substrate (6.4 mg/l) and lower levels of DO in systems with bamboo substrates (4.9 mg/l) but extremely low (1.3 mg/l) DO in systems with bagasse substrates. Umesh et al. (1999) reported lower DO levels in tanks with bagasse substrates (5.5 mg/l) compared to the control tanks without substrate (6.9 mg/l). Dissolved oxygen is critical for the survival and growth of fish in any production system. Therefore, only substrates that can enhance sufficient periphyton growth to maintain DO levels at optimum for the cultured fish should be used in periphyton-biofilter.

Periphyton substrates that are used in periphyton-biofilter must be efficient in the removal of ammonia and nitrite in circulating water. The study by Guttman (2018) reported that a dual *Ulva*-periphyton biofilter can remove up to 76% of the total nitrogen, with a specific removal efficiency of 97% of the TAN and 67% of the nitrate nitrogen. Another study by Levy et al. (2017) reported that periphyton-biofilter was able to remove total ammonia nitrogen (TAN) by up to 80%. Shahara and Guttman (2021) reported that the integration of two sequenced *Ulva fasciata* biofilters (*Ulva-Ulva*) produced more biomass and removed more nitrogen (25 g N wk⁻¹) than the *Ulva*-periphyton (18 g N wk⁻¹) biofilter in mariculture effluent, because *U. fasciata* removed nitrate faster than the periphyton. This is attributed to the ability of periphyton to assimilate nitrite, nitrate, or ammonium, as the sole nitrogen source for growth while improving water quality (Yariv, 2001). The assimilation of different forms of nitrogen by periphyton follows the same pattern whether in freshwater, marine, or brackish water ecosystems.

In aquaculture systems, the source of nitrogen is fish feed, faecal matter and the atmosphere (Kumar et al., 2017; Bai et al., 2023; Chen et al., 2024). However, different forms of nitrogen in aquaculture systems usually accumulate due to high feed input and less water exchange. In aquaculture systems, nitrogen also remains in the forms of ammonium, ammonia, nitrate, nitrite, dissolved organic nitrogen and particulate nitrogen as in natural aquatic ecosystems (Hargreaves, 1998; Schmautz et al., 2021; Chen et al., 2024). The accumulation of ammonia and nitrite is toxic to the cultured

species therefore, they require monitoring to avoid reaching a toxic level in the production systems. The removal of different forms of nitrogen relies on microbial-driven nitrogen removal reactions within the aquaculture production system. Therefore, the innovation of production systems with high nitrogen removal reactions in aquaculture systems is necessary. Research on periphyton-based aquaculture has focused on periphyton composition and the utilisation of periphyton by different aquatic organisms. Only a few studies have investigated periphyton-based biofilters in marine aquaculture and freshwater (Verdegem et al., 2005). This study will investigate periphyton-based biofilters using net, plastic and stone substrates in freshwater tanks. The general aim of this chapter was to investigate the feasibility and efficacy of a periphyton-based biofiltration system for enhancing nitrogen removal from aquaculture systems. The main objective of the chapter is:

- To determine the efficacy of a periphyton-based biofilter in maintaining optimal water quality parameters for Mozambique tilapia culture in a recirculating aquaculture system

6.2 MATERIALS AND METHODS

6.2.1 Study site

Refer to Chapter 3, section 3.2.1.

6.2.2 Experimental system

The experiment was designed to determine the performance of periphyton-based biofilters in a recirculating system. Net, plastic and stone from the Aquaculture Research Unit were used as biofilters. The nature and structure of the net, plastic and stone are described in Chapter 3, section 3.2.2. Six fibreglass tanks (500 L), three water storage tanks (250 L) and three 1.1 kW water pumps (E-que EQ8012; 0.42 brake horsepower) were used to set up the system. The system had three units, each unit consisted of two fibreglass tanks, one water pump and one water storage tank. In each unit, the water storage tank was connected to two fibreglass tanks and a water pump (Figure 6.1). Each fibreglass tank had an outlet pipe connected to the water pump that supplied water to the storage tank. The storage tank inlet pipe was located at the top of the tank (10 cm from the tip/head) and the outlet was at the bottom of the tank (10 cm from the base of the storage tank). The storage tank had an inlet pipe that received water from the fibreglass tank via a pump, and the outlet supplied water to the fibreglass tank by gravity. The water storage tanks had a net, plastic and stone inside which were used as a biofilter. The water passes through the net, plastic or stone inside the water storage tanks before flowing into the fibreglass tanks. From henceforth, water storage tanks with net, plastic or stone will be referred to as biofilters. Therefore, in each unit, water was pumped from the fibreglass tanks into the biofilters and then circulated back to the fibreglass tanks by gravity (Figure 6.1). The systems had no aeration, and water lost through evaporation was replaced once a week. The system was in an open space away from surrounding shades. All the fibreglass tanks are exactly the same, and any differences in water quality are due to the periphyton biofilters.

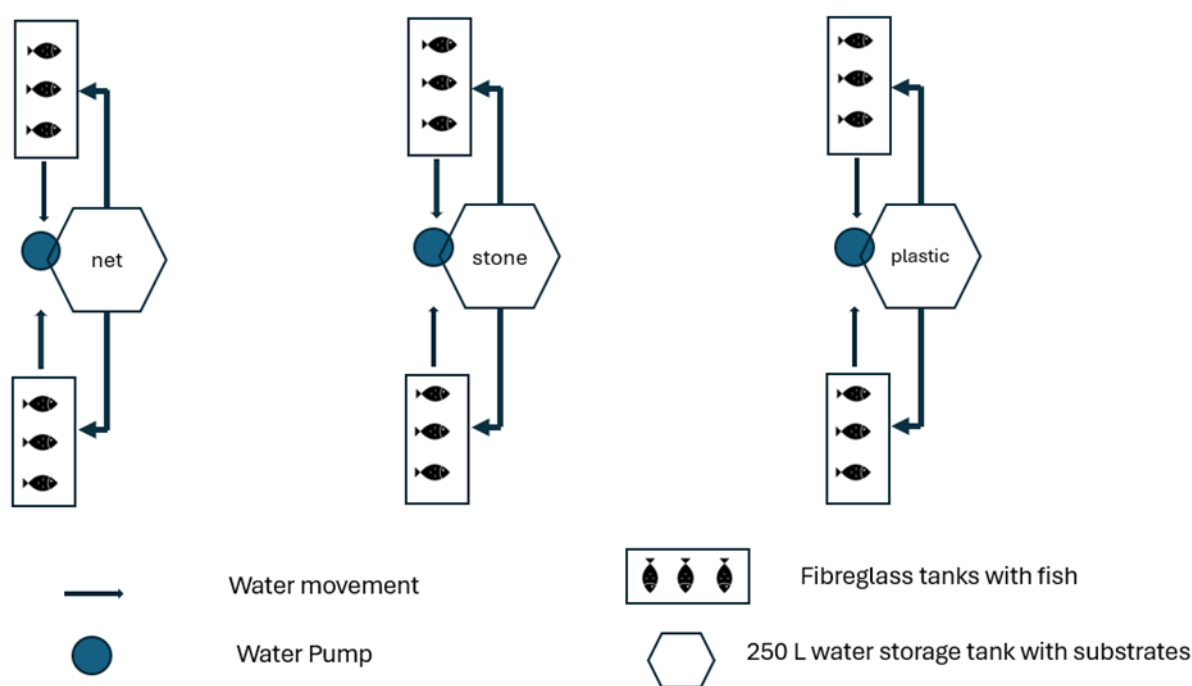


Figure 6. 1: Schematic representation of the periphyton-biofilter experimental system (a top view). Net, plastic and stone inside 250 L tanks indicate type of substrates for periphyton, not to scale

6.2.3 Experimental design

The periphyton-based biofilters were tested in duplicate in a complete randomised design. Nitrifying bacteria were not seeded, the periphyton in the biofilter was allowed to form naturally for three weeks before the commencement of the experiment. To ensure that all biofilters had equal periphyton biomass colonised on each biofilter before the commencement of the experiment, the periphyton biomass was determined on each biofilter. Periphyton samples, in triplicate, were collected weekly by brushing the surface of the substrate ($3 \times 3 \text{ cm}^2$) to determine the periphyton biomass (in terms of dry matter). The samples were dried in an oven to determine biomass according to the standard procedure (APHA, 1998). The experiment commenced when the ash-free weight reached 2.034 ± 0.04 (mean $\pm$ SD) on all biofilters. The fish was introduced into the system after three weeks when the periphyton was well established inside the biofilters. Water quality parameters were not monitored during the time the periphyton was growing, it was monitored after the fish were introduced and acclimatised to the system.

Mozambique tilapia (*Oreochromis mossambicus*) with a mean weight of 5.00 ± 1.14 g (mean $\pm$ SD) was stocked into the fibreglass tanks with matured water. Matured water is municipal water that was left for 5 to 7 days for chlorine to evaporate prior to use. The stocking density was 3 fish per cubic meter. Tilapia fingerlings were obtained from the Aquaculture Research Unit's aquaculture recirculating system. The fish were allowed to acclimatise for 7 days before the experiment commenced. The water quality parameters in the 7 days acclimatising period were 9.38 ± 1.49 mg/l for dissolved oxygen; 8.22 ± 0.89 for pH; 0.27 ± 0.09 mg/l for nitrogen; 0.08 ± 0.05 mg/l for nitrite; 10.08 ± 4.11 NTU for turbidity; and 6.89 ± 3.66 mg/l for total phosphate. The fish were stocked into the fibreglass tanks to increase the organic input into the system. The fish were fed commercial pellets twice a day to satiation, 10h00 am in the morning and 16h00 pm in the afternoon. From here forth, the biofilter with a net inside will be referred to as net periphyton-biofilter, the biofilter with plastic inside will be referred to as plastic periphyton-biofilter, and with stone will be referred to as stone periphyton-biofilter. The experiment ran for a period of four weeks: one week for fish to acclimatise and three weeks for monitoring water quality. Water quality can change from day to day or even hour to hour depending on the weather and location. Therefore, three weeks was considered sufficient to monitor the change in water quality parameters in this study.

6.2.4 Determination of the water quality parameters in biofilters

The water quality parameters were determined every morning and afternoon to monitor the daily range of the water for 3 weeks. Water quality parameters were monitored daily at 9h00 am and 15h00 pm using a multi-probe YSI meter. The water from the biofilters and fibreglass tanks were both monitored in the first week of the experiment. Water sample analysis was stopped in the fibreglass tanks because the results (readings from the spectrophotometer) were the same for the biofilter and fibreglass tanks. After that, the water samples were only collected from the biofilters. This might have been caused by the size of the systems. Water samples in the biofilters were collected to analyse for nitrogen (as ammonia), nitrite, nitrate and total phosphate. Once every week, the water quality parameters were also determined for 24 hrs every four hours to monitor changes in the water quality parameters in a 24 hrs

cycle. Temperature ranged from 21.18 ± 0.63 °C in the morning to 31.10 ± 0.54 °C in the afternoon.

Water quality parameters monitored are dissolved oxygen (mg/l), and pH, using a YSI multi-probe meter. Turbidity (NTU) was measured using a turbidity meter. Nitrogen as ammonia (mg/l) was analysed according to method number 8038 of DOC316.53.01078 (Hach, 2012) and nitrate (mg/l) was analysed according to method number 8171 of DOC316.53.01069 (Hach, 2012). Total phosphate (mg/l) was analysed according to method number 8048 of DOC316.53.01119 (Hach, 2012).

6.2.5 Data and statistical analysis

Microsoft Excel and Power BI were used to plot graphs. Normality and homogeneity of water quality parameters were tested using Shapiro-Wilk and Levene tests, respectively using Statistical Package and Service Solutions (SPSS, version 27). The water quality parameters data met the assumptions of normality and homogeneity. A two-way analysis of variance (ANOVA) was used to test for significant differences in the water quality parameters data using SPSS.

6.3 RESULTS

6.3.1 The daily average water quality parameters periphyton-based biofilters

The average water quality parameters were analysed to monitor the performance of the periphyton-biofilter. The pH ranged between 8.38 and 9.65. There was no significant difference ($P > 0.05$) in the DO levels between the periphyton-biofilters although plastic recorded marginally higher levels (Table 6.1). Nitrate levels significantly varied ($P < 0.05$) between the periphyton-biofilters, net periphyton-biofilter registered the highest nitrate levels and stone registered the lowest levels. Nitrite was significantly higher ($P < 0.05$) in the plastic periphyton-biofilter than in net and stone periphyton-biofilter. Nitrogen (as ammonia) levels significantly varied ($P < 0.05$) between the periphyton-biofilters, stone periphyton-biofilter registered the highest ammonia levels and plastic registered the lowest levels. Turbidity levels significantly differed ($P < 0.05$) between periphyton-biofilters, it was highest in the stone periphyton-biofilter and lowest in net periphyton-biofilter. Total phosphate significantly varied ($P < 0.05$) between the periphyton-biofilters, plastic periphyton-biofilter registered the highest total phosphate levels and stone registered the lowest levels (Table 6.1). All the measured water quality parameters were within the target range for farming tilapia except for total phosphate, which was above the range (Table 6.1).

Table 6. 1:The daily average water quality parameters (mean $\pm$ SD) in net, plastic and stone periphyton-biofilter.

	Net			Plastic			Stone			P values			TWQR
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	bio	weeks	interact	
DO (mg/l)	9,51 $\pm$,82	9,93 $\pm$ 1,50	10,36 $\pm$,97	10,10 $\pm$ 1,53	10,56 $\pm$ 1,02	10,51 $\pm$ 1,66	9,42 $\pm$,82	9,81 $\pm$ 1,37	10,01 $\pm$,84	0,342	0,075	0,840	3-10
Nitrogen (mg/l)	0,14 $\pm$ 0,06	0,14 $\pm$ 0,01	0,14 $\pm$ 0,00	0,14 $\pm$ 0,02	0,12 $\pm$ 0,04	0,10 $\pm$ 0,05	0,28 $\pm$ 0,08	0,31 $\pm$ 0,02	0,28 $\pm$ 0,11	<0,001	0,009	0,948	0.0-0.60
Nitrite (mg/l)	0,02 $\pm$ 0,01	,02 $\pm$ 0,01	0,01 $\pm$ 0,01	0,12 $\pm$ 0,05	0,13 $\pm$ 0,05	0,15 $\pm$ 0,05	0,01 $\pm$ 0,01	0,01 $\pm$ 0,01	0,01 $\pm$ 0,01	<0,001	0.772	0,665	0.0-0.5
Nitrate (mg/l)	11,21 $\pm$ 1,67	11,36 $\pm$ 1,73	12,03 $\pm$ 1,42	5,37 $\pm$ 1,23	5,95 $\pm$ 1,22	6,83 $\pm$ 1,02	5,48 $\pm$ 3,03	5,29 $\pm$ 3,08	4,09 $\pm$ 2,42	<0,001	0,996	0,440	<200
Turbidity (NTU)	8,94 $\pm$ 0,50	9,70 $\pm$ 2,1	7,40 $\pm$ 1,85	12,32 $\pm$ 2,68	14,22 $\pm$ 3,71	14,34 $\pm$ 2,38	17,91 $\pm$ 3,55	19,01 $\pm$ 3,99	15,42 $\pm$ 1,71	<0,001	0,027	0,101	<3000
TP (mg/l)	8,70 $\pm$ 2,27	8,06 $\pm$ 2,29	8,70 $\pm$ 2,57	9,68 $\pm$ 3,18	9,92 $\pm$ 2,60	8,96 $\pm$ 2,11	4,62 $\pm$ 2,90	5,17 $\pm$ 3,57	4,98 $\pm$ 3,84	<0,001	0,973	0,948	<1.00

Net- net periphyton-biofilter, plastic- plastic periphyton biofilter, stone- stone periphyton-biofilter. Abbreviations: DO- dissolved oxygen, TP- total phosphate, nitrogen- nitrogen as ammonia, bio-biofilters, interact-interaction. TWQR = Target water quality range. Sources for TWQR: Boyd & Tucker, 2015; Leira et al., 2017; Francis-Floyd et al., 2009; Soderberg, 2017; Tucker, 1993; Aquaculture SA, 2003; FAO, 2024b.

6.3.2 The diurnal water quality parameters in periphyton-based biofilters.

The diurnal mean water quality parameters were determined to monitor the daily dynamics of the periphyton-biofilters. In all the periphyton-biofilters, the diurnal mean levels mirrored the average water quality parameters levels recorded above. In the net periphyton-biofilter, pH rose from morning until midday and then started falling after midday until 4h00 am (Figure 6.2). Dissolved oxygen remained relatively constant throughout the 24 hrs although the highest level was reached at midnight. Nitrogen (as ammonia) and nitrite did not change throughout the 24 hrs cycle. Nitrate level initially declined and then rose, its highest level was recorded in the early evening before declining (Figure 6.2).

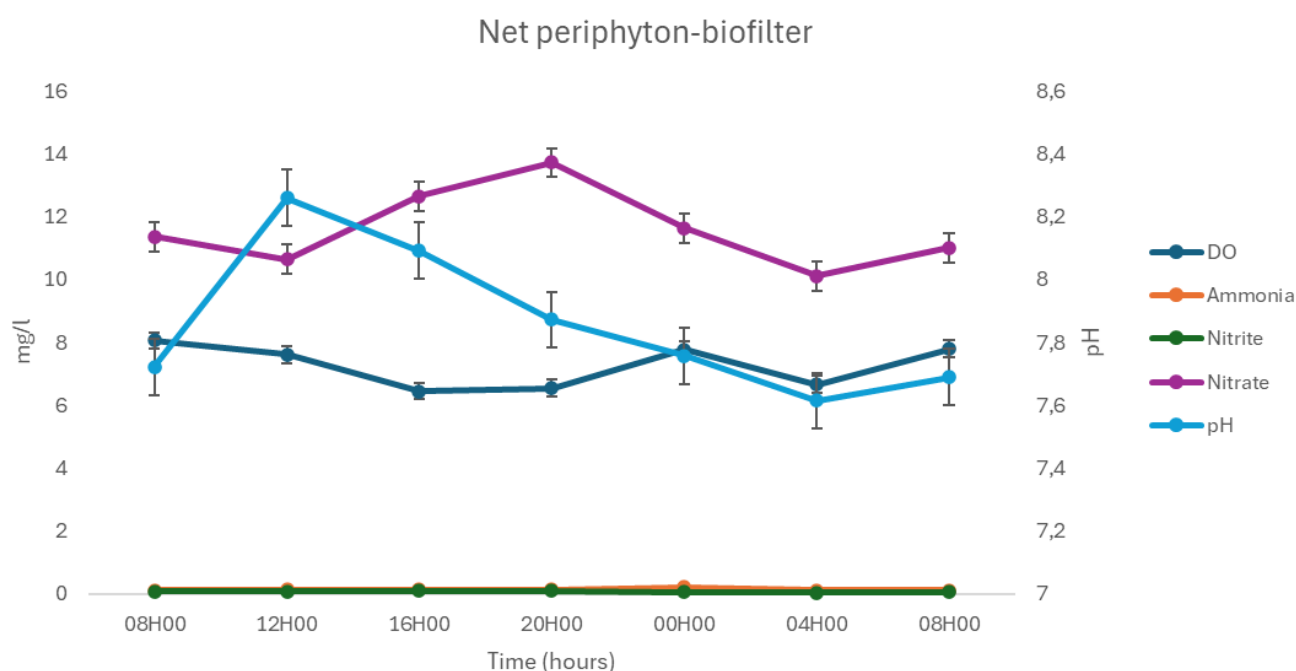


Figure 6. 2: The diurnal cycle of selected water quality parameter in net periphyton-biofilter. Abbreviations used: DO- dissolved oxygen.

In the plastic periphyton-biofilter, pH initially rose in the morning until midday and then remained relatively constant (Figure 6.3). Dissolved oxygen only started declining after midday and then rose at midnight before declining again. Ammonia and nitrite levels remained relatively constant during the day, and then a slight increase was recorded at midnight for ammonia. The nitrate level initially declined until late in the afternoon

and then rose. Its highest level was recorded in the morning before declining (Figure 6.3).

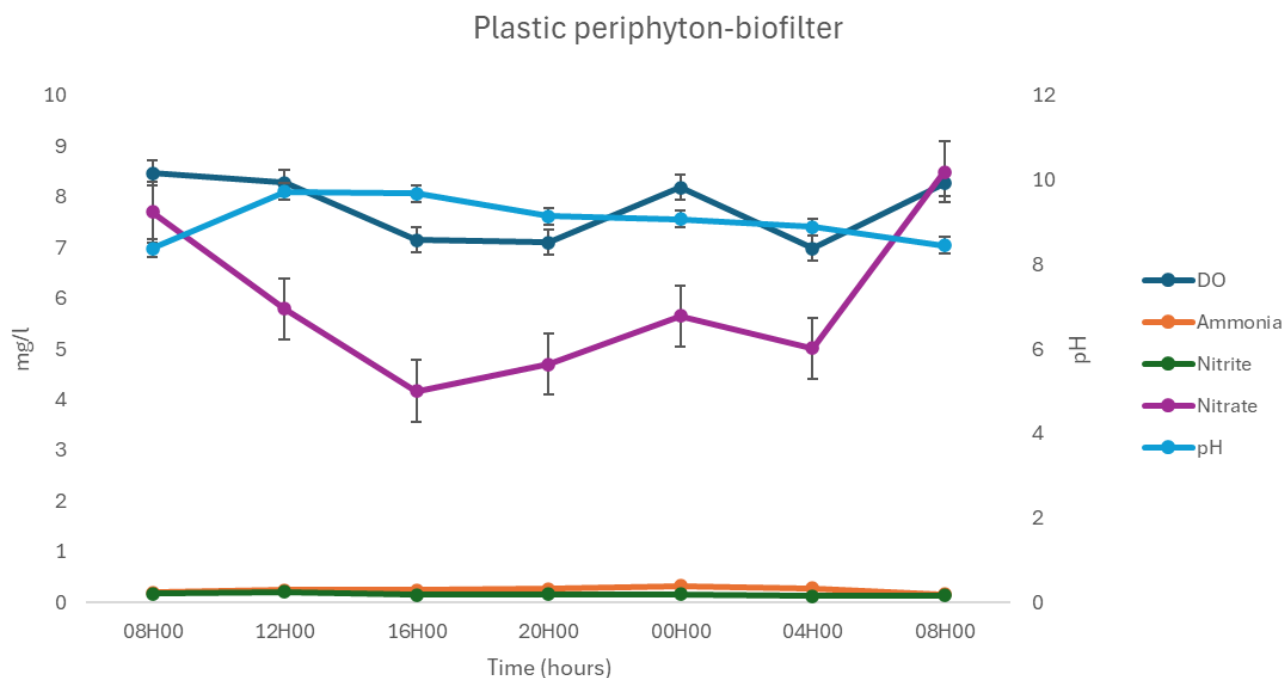


Figure 6. 3: The diurnal cycle of selected water quality parameter in plastic periphyton-biofilter. Abbreviations used: DO- dissolved oxygen.

Similar to the plastic periphyton-biofilter, pH initially rose in the morning until midday and then remained relatively constant in the stone periphyton-biofilter (Figure 6.4). Dissolved oxygen also took a similar pattern as in the plastic periphyton-biofilter. Similar to the net periphyton-biofilter, ammonia and nitrite levels in the stone periphyton-biofilter did not change, but ammonia was relatively higher than nitrite throughout the 24 hrs cycle. Moreover, the level of ammonia was highest (0.287 – 0.475 mg/l) in the stone periphyton-biofilter compared to net (0.120 – 0.222 mg/l) and plastic (0.167 – 0.327 mg/l) periphyton-biofilters. Nitrate level initially rose until early evening and then declined until 4 am.

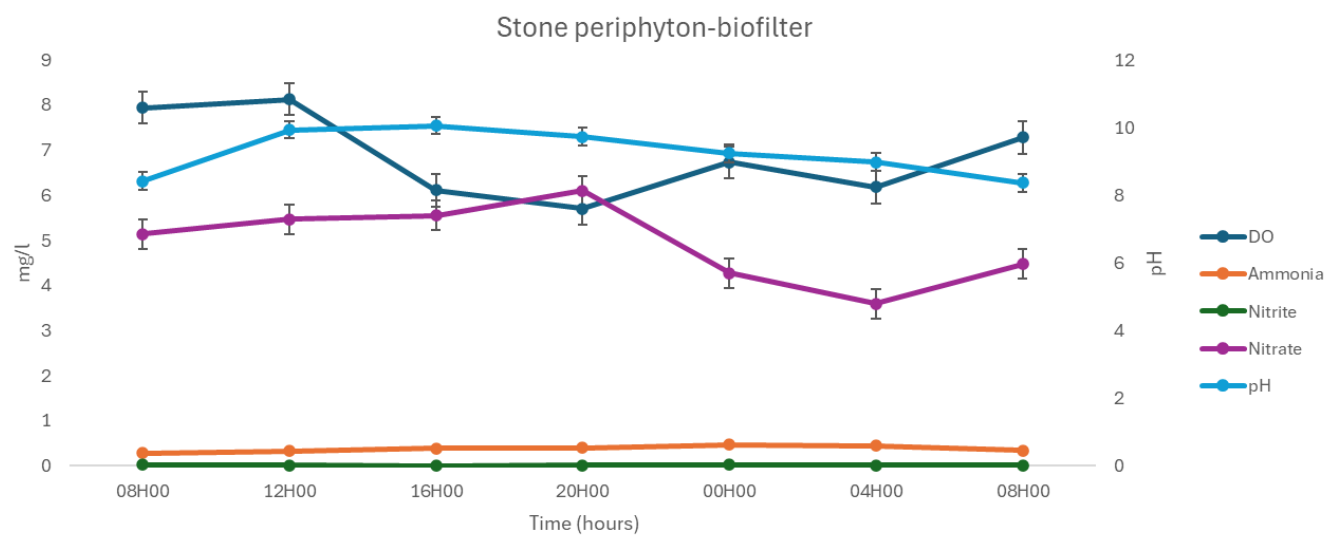


Figure 6. 4: The diurnal cycle of selected water quality parameter in stone periphyton-biofilter. Abbreviations used: DO- dissolved oxygen.

6.4 DISCUSSION

Periphyton and other photosynthesizing organism in aquatic ecosystems mostly improve water quality through nitrogen fixation, nitrification and denitrification. Nitrification is one of the main transversions for the removal of ammonium and nitrite in aquaculture systems. Dissolved oxygen, pH and temperature also play a critical role in the process of ammonium oxidation. In this chapter, the variation of pH ranged between 8.38 and 9.65 in three weeks and the DO range was between 9.67 and 10.39 mg/l. The pH and DO levels were sufficient for nitrification since it was above 7 and 5 mg/l, respectively. There was no aeration, the recirculating system and the DO was optimum for tilapia. This indicates periphyton biofilter is oxygen self-generating. Shahar and Guttman (2021) also reported that periphyton biofilter requires no aeration because it is a self-generation of oxygen. These levels favoured nitrification and periphyton growth in the biofilter. The net periphyton-biofilter had the highest level of nitrate. This indicates that nitrification effectively occurred in the net periphyton-biofilter contributing to the accumulation of nitrate. Nitrate is the end product of nitrification which is a form of nitrogen that is assimilated by periphyton. A study by Ale et al. (2010), Li et al. (2015) and Guttman et al. (2018) showed that many aquatic photosynthetic organisms effectively assimilate nitrogen (as ammonia) over nitrate due to the energetically favoured reactions for uptake, thus leading to the accumulation of nitrate. Ale et al. (2010) suggested that nitrate accumulates because the presence of ammonia inhibits the uptake of nitrate by photosynthesizing organisms. However, Syrett (1981) and Pritchard et al. (2015) indicated that algae can assimilate nitrate in the presence of ammonia. Therefore, its accumulation in this study might suggest that its assimilation by periphyton was slow in the net periphyton-biofilter.

The plastic periphyton-biofilter had the highest nitrite and total phosphate levels in the period of three weeks. Phosphate is essential for periphyton growth and is commonly assimilated by periphyton, but its high level might suggest a slow uptake. This might suggests that the periphyton community on the plastic substrate was not dominated by algae that can take up phosphate in the form of inorganic phosphorus. In aquaculture production systems, phosphorus is mostly taken up by green algae and blue-green algae and then the algae are consumed by the fish (Bai et al., 2023).

Moreover, in traditional fish ponds, phosphate accumulates in the sediments when it's not taken up by algae. Kibria et al. (1997), Pouil et al. (2019) and Yang et al. (2017) reported that there is low phosphate use efficiency by cultured organisms in aquaculture systems, therefore phosphate is released into water or deposited at the bottom making the sediment a potential source of phosphate. However, high phosphate levels are common and not toxic to most fish species unlike ammonia and nitrite (Barak et al., 2003; Neori et al., 2007; Kim et al., 2013). Milstein et al. (2018) also reported the accumulation of total phosphate in periphyton-biofilter systems due to slow phosphate removal by the periphyton in the mariculture system. Asaduzzaman et al (2009) also reported high phosphate concentration due to increased feeding of the cultured species associated with high stocking density. High nitrite levels probably indicate there was imbalanced nitrite-oxidising microbial activity which led to nitrite accumulation in the plastic periphyton-biofilter. The level of ammonia recorded in the plastic-biofilter was lower compared to net and stone periphyton-biofilter. This probably indicates that ammonia-oxidising bacteria were thriving thus converting ammonia to nitrite. Moreover, DO and pH levels were sufficient to support nitrification. Sorokin et al. (2015), Ni et al. (2023) and Daebeler et al. (2023) have reported that ammonia-oxidising bacteria mediate ammonia oxidation at high pH in aquatic environments.

The stone periphyton-biofilter had the highest level of turbidity, pH and ammonia. The accumulation of ammonia indicates that nitrification was not effective in the stone periphyton-biofilter. This probably indicates that the nitrifying bacteria was not as well established and effective as in other periphyton-biofilters for the period of three weeks. Poor nitrification in the stone periphyton-biofilter might be explained by high turbidity. Low turbidity allows water purification processes such as photosynthesis, phosphate cycle, carbon fixation and nitrification to occur effectively due to sufficient sunlight and oxygen generation. On the other hand, high turbidity leads to low nitrogen fixation and nitrification due to reduced photosynthesis rate. This probably explains the high nitrogen levels in stone periphyton-biofilter. Liu et al. (2013), Xia et al. (2016) and Jia et al. (2016) also reported low nitrification processes due to high water turbidity associated with low photosynthesis and low oxygen in water. Xia et al. (2021) reported

that turbidity was an important factor influencing the emission of nitrogen and nitrous oxide after when nitrification and denitrification were complete in rivers.

The diurnal fluctuation of water quality parameters in the net periphyton-biofilter confirmed the average water quality parameters recorded over the period of three weeks. In the net periphyton-biofilter, pH rose from morning until midday and then started falling after midday until 4h00 am. This might be explained by high sunlight intensity at midday which leads to a high photosynthesis rate which lowers the concentration of total dissolved carbon dioxide. Kumar et al. (2017) and Dodds (2003) also reported high pH levels in ponds with substrates due to a high rate of photosynthesis and carbon fixation during the day. The pH decreased as the night progressed suggesting that the dissolved carbon concentration increased since the fish and periphyton were consuming DO. It is also evident by the levels of DO that decreased after midnight. This suggests that the consumption of oxygen by fish and periphyton was higher after midnight. However, the net periphyton-biofilter had a high photosynthesis rate during the day which was sufficient to maintain optimum pH and DO during the night. Shahar and Guttman (2021) reported that periphyton biofilter requires no aeration because it is a self-generation of oxygen. Thus, the pH and DO's target range for farming tilapia was maintained. Sousa et al. (2018), Eгна and Boyd (2017) and Diemer et al. (2010) have shown that it is common for DO and pH to be at lowest at dawn due to high oxygen consumption by the fish and oxygen-producing organisms. Godoy et al. (2021) also reported higher oxygen levels during the day due to high photosynthesis rates. However, in this study, even at its lowest level, DO was still within the optimum range for farming tilapia.

The pH and DO levels were also sufficient to support the nitrification process at all hours of the diurnal cycle in the net periphyton-biofilter. Nitrification is active at pH above 7 (Ni et al., 2023). It is evident by the low level of ammonia that periphyton was purifying in the net periphyton-biofilter. Ammonia did not change throughout the 24 hrs cycle and was within the optimum range for farming tilapia, below 0.60 mg/l (Soderberg, 2017). This indicates that the net periphyton-biofilter was efficient and able to keep ammonia and nitrite at non-toxic level for tilapia since mortality was not

observed. Continuous monitoring of ammonia and nitrite is recommended, as levels above tolerated levels may affect the health and growth of the tilapia. Nitrate level initially declined and then rose, its highest level was recorded in the early evening before declining. The nitrate was significantly higher in the net periphyton-biofilter than other periphyton-biofilters at all hours. However, nitrate accumulation is generally not a concern in aquaculture because tilapia can tolerate as high as 200 mg/l and above (Monsees et al., 2016).

In the plastic periphyton-biofilter nitrite levels remained relatively constant. The level of nitrite was within the optimum range for farming tilapia, below 0.5 mg/l (Leira et al., 2017). Nitrite is toxic to tilapia when it's above 1.0 mg/l (Yanbo et al., 2006; Leira et al., 2017). It reduces the fish's ability to take up oxygen by binding with haemoglobin to produce methemoglobin (Yanbo et al., 2006). Nitrite is an intermediate product of nitrification and denitrification. Its accumulation suggests that there were factors that prohibited the oxidation of ammonia to nitrate (nitrification) or the reduction of nitrate to nitrogenous gases (denitrification) by periphyton. The level of pH, DO and temperature were optimum for the nitrification process. Therefore, this accumulation was probably indicating that the oxidation of nitrite to nitrate was hindered due to imbalanced nitrite-oxidising bacterial (*Nitrobacter*) activity. In the early hours of the morning, nitrite was lowest probably because of the declining level of pH and DO as mention above. This is because nitrite levels are related to the levels of DO and ammonia in the water (Mercante et al., 2018). Ammonia levels remained relatively constant during the day and then slightly increased at midnight. However, it was still within the optimum range of farming tilapia. pH initially rose in the morning until midday and then remained relatively constant while DO fluctuated during the day and night. Nonetheless, the pH and DO were also optimum for tilapia. This shows that the photosynthesis rate was sufficient to keep these water quality parameters optimum. The nitrate level initially declined until late in the afternoon and then rose. Its highest level was recorded in the morning before declining. This suggests that the nitrification process was not constant and inadequate. However, nitrate was still within the optimum range for farming tilapia.

Similar to the net periphyton-biofilter, ammonia and nitrite levels in the stone periphyton-biofilter did not change, but ammonia was relatively higher than nitrite throughout the 24 hrs cycle. The level of ammonia was highest (0.287 – 0.475 mg/l) in the stone periphyton-biofilter compared to net (0.120 – 0.222 mg/l) and plastic (0.167 – 0.327) periphyton-biofilters. According to Soderberg (2017), the level is within the optimum range for farming fish, below 0.60 mg/l. However, according to Francis-Floyd et al. (2009), the optimum range should be below 0.30 mg/l. This shows that the efficiency of the stone periphyton-biofilter is questionable. This indicates that the stone substrate will not be able to keep the ammonia level optimum for farming tilapia in a full production cycle. This probably indicates that the periphyton in the stone was not effectively carrying out the nitrification process, which led to the accumulation of ammonia. This might have been caused by the loss of periphyton and dislodging of nitrifiers (*Nitrosomonas* sp.) from the stones due to the water recirculation. The loss and dislodging of periphyton increased turbidity in the stone periphyton-biofilter. This is confirmed by the highest turbidity recorded over the three weeks. Turbidity can slow down nitrogen nitrification due to limited sunlight. The peak level of ammonia at midnight also shows that sunlight and photosynthesis rate contribute to its accumulation. Xia et al. (2017, 2019) also showed that high turbidity reduces the rate of nitrogen nitrification due to reduced sunlight. Nitrate level initially rose until early evening and then declined until 4 am. However, it was within the optimum range for farming tilapia throughout the diurnal cycle. This might also suggest that the stone periphyton-biofilter was able to maintain levels of nitrite and nitrate lower by anaerobic ammonium oxidation. This might mean that the stone periphyton-biofilter had heterotroph organisms growing.

In conclusion, the diurnal mean levels mirrored the average water quality parameters across the periphyton-biofilters. Nitrification is the main process by which a biofilter purifies water in aquaculture systems. Ammonia levels were within the target range for farming tilapia across the periphyton-biofilter although stone recorded marginally higher levels. This probably indicates that the nitrification process was less effective and inadequate in the stone periphyton-biofilter. The levels of nitrite were also within the target range throughout the three weeks although the plastic periphyton-biofilter recorded slightly higher levels. This was probably because of the imbalance of nitrite-

oxidising bacterial activity (*Nitrobacter*) that hindered the oxidation of nitrite to nitrate. The net periphyton-biofilter recorded a higher level of nitrate throughout the experiment, however, it was still within the target range for farming tilapia. The accumulation of nitrate indicates nitrification was efficiently occurring in the net periphyton substrate. The total phosphate recorded in the periphyton-biofilter was slightly higher than that recorded in the aqua dams due to the presence of sediments. Dissolved oxygen and pH levels were optimum for tilapia and water purification processes in all the biofilters. This was because periphyton biofilter requires no aeration, it is a self-generation of oxygen. The net was an effective periphyton-based biofilter since it was able to maintain the toxic ammonia and nitrite at minimal levels. Therefore, the net periphyton-biofilter was the most efficient biofilter for maintaining optimum water quality for tilapia farming.

Chapter 7: General discussion, recommendations and conclusion

7. GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSION

What are the implications associated with using some substrates in periphyton-based aquaculture to improve fish growth?

Periphyton and bioseston are natural food items of tilapia and other species in the wild. In Chapter 3, the factors affecting the abundance of periphyton and bioseston were identified as substrates, seasons and grazing. The net substrate had the highest periphyton biomass and bioseston abundance. In Chapter 4, the potential of periphyton and bioseston as food items in the diet of tilapia was evaluated. Surprisingly, tilapia growth rate was highest in aqua dams with plastic substrates. This was unexpected because, in the previous experiment, Chapter 3, plastic substrate had a lower periphyton biomass in comparison to net substrate. One of the major challenges of using plastic is that fish may eat part of the plastic, and this can affect their ability to digest. In this study, plastic was disintegrating towards the end of the third experiment. It has been demonstrated in other studies that plastic causes environmental pollution in oceans, seas, lakes, dams and rivers (Sigler, 2014; Schwarz et al., 2019; Horton & Blissett, 2021). Much concern has been raised about plastic pollution in the ocean, but this pollutant also threatens freshwater ecosystems. This is because rivers contribute synthetic polymers to the ocean (Lebreton et al., 2017; Horton & Blissett, 2021).

Numerous studies have shown the presence of plastics in rivers, sediment, and areas associated with inland waters (Sigler, 2014; Fischer et al., 2016; Ebere et al., 2019; Horton & Blissett, 2021). Fish species in rivers and other inland environments around the world have records of plastic ingestion and death from ingestion (Sigler, 2014; Urbanski et al., 2020). The use of this type of plastic in periphyton-based aquaculture can introduce microplastics into aquaculture environments. A study by Zhang et al. (2021) has shown that there was a high concentration (11.49 particles/m) of microplastic in areas with marine aquaculture practice compared to other areas without mariculture (1.57 particles/m). Microplastics have toxic effects on fish's immune system. For instance, microplastics with sizes of 24 nm to 150 µm can cause oxidative damage to the cells of fish and can slow down the development of fish brains

(Mattsson et al., 2015; Espinosa et al., 2019; Wan et al., 2019). However, alternative plastic materials that do not easily break and disintegrate can be used in periphyton-based aquaculture.

The net substrate used in this study did not break easily because it was made of nylon material with long durability. However, it did not result in the best growth for tilapia. This was probably due to lack of accessibility of the periphyton on the net substrate. The accessibility of periphyton to the fish can be restricted when fish get entangled in the net substrate while feeding, limiting the fish to freely graze. In this study, it was observed that within the first 8 hours of stocking, tilapia in the aqua dams with net substrate would get trapped inside an enclosed net edge or hooked to the net by the tip of the fins. However, it did not lead to mortalities. Gilman et al. (2016), Beneli et al. (2020), Do and Armstrong (2023) have shown that even in the wild, fish and other aquatic organisms can get trapped in abandoned nets and this can sometimes lead to mortality.

In this study, the stone substrates were also used in aqua dams stocked with Mozambique tilapia and African catfish. Unlike plastic substrates, stone substrates are not prone to disintegration and can be used unlimitedly in periphyton-based aquaculture. However, it was observed that the stone substrate had the least periphyton biomass and bioseston abundance. Nonetheless, it was sufficient to support the growth of African catfish, not tilapia. This might suggest that the stone substrate was more appropriate for benthic fish like African catfish since they feed best from the bottom.

What is the challenge of using African catfish in periphyton-based aquaculture?

In Chapter 5, the African catfish fed commercial pellets once every third day (S33) in aqua dams with stone substrate recorded a higher specific growth rate comparable to fish that received commercial pellets daily (S100). Furthermore, the specific growth rate was comparable with the fish that received commercial pellets daily in aqua dams with plastic (P100) and net substrate (N100). However, South Africans' consumption

of African catfish is low compared to West African countries like Nigeria due to religion and the quality of meat. African catfish from periphyton-based aquaculture will be most likely rejected because of the quality of the meat. In this study, cyanobacteria were identified as part of the periphyton and bioseston community. Certain genera of Cyanobacteria produce odorous off-flavour compounds such as geosmin and 2-methylisoborneol (Schrader et al., 1998; Schrader & Dennis, 2005).

Prevalence and intensity of off-flavour compounds is high in lentic ecosystem such as aqua dams (Schrader & Dennis, 2005). The off-flavour compounds affect the quality of the meat of fish and render them unmarketable products (Schrader et al., 1998; Schrader, 2022). Producers must hold fish that are determined to be off-flavour for several days to weeks in clean water to remove the odour before selling to the market and this exacerbates the production cost (Schrader & Denni, 2005). The accumulation of geosmin and 2-methylisoborneol in the flesh of fish is more prominent in catfish than in tilapia. This might be because African catfish can be cultured in water with high turbidity as compared to tilapia, it can even survive in muddy water. Therefore, it is most likely to be exposed to water with a high abundance of geosmin and 2-methylisoborneol producing species such as Myxobacteria, actinobacteria, cyanobacteria, proteobacteria, streptomyces, and fungi (Dickschat et al., 2005; Abd El-Hack et al., 2022). This is because the levels of geosmin and 2-methylisoborneol measured in the fish meat often reflect the levels in culture water (Howgate, 2004; Koskela et al., 2020; Abd El-Hack et al., 2022). However, this challenge can be mitigated if periphyton is incorporated into commercial pellets.

Is the nutritional quality of periphyton and bioseston better than commercial pellets?

Poor egg and fingerling quality is one of the major problems that have restricted aquaculture expansion. The fecundity of the fish can vary based on the size of the fish, diet and the production system involved. The egg quality is determined by ecological factors, physiological factors, and nutrition of the female (Rocha, 2008). African catfish do not spawn spontaneously in captivity because of ecological factors such as a rise in water level and inundation of shallow areas does not occur at the fish farms (FAO,

1994). Therefore, African catfish are commonly conditioned with a high-protein diet before artificial breeding in aquaculture. However, in Chapter 5, it was observed that African catfish had a swollen, reddish genital papilla and matured eggs were identified when fish were sacrificed to remove the stomach and the rectum. This was observed only in aqua dams with periphyton substrates. This suggests that periphyton can be used to condition African catfish before artificial breeding. Furthermore, in Chapter 4, numerous tilapia fry were identified in the aqua dams with periphyton substrates at the termination of the experiments. This was because periphyton is of high quality and was able to condition African catfish and tilapia for reproduction. Muthoka et al. (2021) also reported high reproductive performance in periphyton-based ponds due to high-quality periphyton and plankton (bioseston). This suggests that the nutritional value of periphyton is of higher quality than commercial pellets.

Generally, periphyton is nutritionally adequate for fish as it contains 27 % crude protein, 18 % lipid and 52 % carbohydrates which are better than most commercial feeds used in aquaculture (van Dam et al., 2002; Muthoka et al., 2021). Fish in aquaculture are fed different commercial pellets depending on the stage of development because one formulated diet fails to meet all the needs of the fish. For instance, the commercial diet formulated for fry is not the same as for fingerlings and subadults. Furthermore, a different diet is used to condition broodstock before breeding and diseased fish are fed different diets (medicated feeds). However, periphyton can support the growth, reproduction and health of fish at all stages of development (van Dam et al., 2002; Muthoka et al., 2021). This is because some periphyton genera can produce antimicrobial and bioactive compounds that function as immunomodulators or immunostimulants (Anusha et al., 2020; Mani et al., 2021; Saleh et al., 2022). Immunostimulants and bioactive compounds possess various pharmacological functions such as anti-stress, antioxidant, tonic, immune-stimulatory, anti-inflammatory, antimicrobial, antifungal, antiparasitic and antiviral properties (Bulfon et al., 2015; van Hai, 2015; Walker et al., 2020). Other periphyton genera, such as *Ankistrodesmus* sp. and *Coelastrum* sp., can produce astaxanthin (Kaha et al., 2021; Luu et al., 2021). 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,

2024). This group of xanthophyll carotenoids also has essential properties such as anti-inflammatory and antioxidant activities (Kaha et al., 2021).

What is the future implication of using periphyton and bioseston in aquaculture?

Periphyton and bioseston has a proven potential to improve fish growth, reproduction and health due to the dietary stimulants, bioactive compounds and immunostimulants produced by periphyton (Wang et al., 2015; Muthoka et al., 2021; Abwao et al., 2014). Bioseston are also rich in essential nutrients like proteins, lipids, vitamins, minerals, and fatty acids (Taipale et al., 2019; Napiórkowska-Krzebietke, 2017). Therefore, it is essential to incorporate all these growths and immune boosters' benefits into a formulated diet. For periphyton and bioseston to be more accessible for most farmers, especially large commercial farmers, it needs to be formulated in commercial pellets. This will help to distribute cheap high-quality feed and commercialise periphyton in the aquaculture industry. The potential of periphyton biomass to replace dietary fishmeal has not been reported until recently by Savonitto et al. (2021). However, Savonitto et al. (2021) focused on the replacement of fishmeal by periphyton from marine biofilter in the diet of carnivorous marine fish, gilthead seabream. Savonitto et al. (2021) showed that a complete removal of fishmeal from feed resulted in 15 % slower growth but replacing 50 % of fishmeal with waste periphyton improved feed conversion ratio and growth.

The fishmeal replacement by periphyton can be improved to 100 % for opportunistic omnivorous species such as tilapia and African catfish. Several ingredients have been used in fish feed to replace fishmeal (Rapatsa & Moyo, 2017; Rapatsa & Moyo, 2019; Tabassum et al., 2021; Alofa et al., 2023; Nephale et al., 2024), however, none of the alternatives had a commercial uptake. Replacing fishmeal with periphyton and bioseston would benefit both the environment and the aquaculture industry because the amount of wild-caught fish required to supply fishmeal will be reduced.

What are the limitations of this study?

Periphyton-based aquaculture system is a modern concept and eco-friendly approach in closed-water aquaculture that most developing countries can benefit from. However, some substrates require manual and hard labour to install. Periphyton-based aquaculture is economically suitable for countries with water scarcity like South Africa. In this study, the specific growth rate of tilapia in aqua dams with plastic substrates and African catfish in aqua dams with stone substrates showed that commercial pellets can be reduced by 50-67 %. Secondly, the periphyton biofilter was not seeded with nitrifying bacteria but the bacteria naturally established in the biofilters. Moreover, no cost was required to buy the substrates that were used in the aqua dams or biofilters since the substrates are readily available. This shows that periphyton-based aquaculture can cut production costs. However, this study did not develop a cost-benefit analysis, economic efficiency or bioeconomic model. Nevertheless, other studies have shown that a periphyton-based aquaculture practice might be a profitable business (Huda et al., 2002; Azim et al., 2004). This study did not compare the nutritional proximate composition of periphyton growing on the net, plastic and stone substrates in aqua dams. However, other studies have determined the nutritional value of periphyton and proved that the nutritional value of periphyton differs according to the substrate used and abundance (van Dam et al., 2002; Muthoka et al., 2021).

What are the benefits and recommendations of the study?

In Southern Africa, the aquaculture sector faces several challenges, including water scarcity, lack of premium fingerling, and expensive feed, which have led to aquaculture production stagnation in most countries. There is a need to expand and intensify the aquaculture sector in Southern Africa through innovations that improve fish growth without increasing production costs and also promote water reuse through self-purification systems. This study has proved that periphyton-based aquaculture is an alternative way to improve tilapia and catfish production and improve water quality. Therefore, this study recommends using periphyton substrate in aquaculture to reduce feed costs. Specifically, plastic substrates, with long durability made of nylon material, can be used in aqua dams stocked with tilapia to reduce commercial pellet input by 50-67 %. Stone substrates can be used in aqua dams stocked with African catfish to reduce commercial pellet input by 67 %. Nonetheless, the use of other substrates

needs to be explored. Moreover, caution needs to be exercised when selecting substrates to avoid substrates that will leach harmful chemicals into the water or substrates that will disintegrate before the end of the production cycle.

The nutritional composition of fish cultured from periphyton-based aquaculture needs to be determined and compared to evaluate the influence of periphyton on different substrates on meat quality. It is, also, recommended that the use of periphyton-based biofilter to keep the water quality parameters optimum for fish production in a recirculating system and must be tested in a large-scale production system. In this study, the feeding preference of African catfish between periphyton and bioseston was not determined because the fish did not respond to the 24-hour experiment in the fibreglass tanks even after extending the feeding time to 48 hours. This was because the African catfish required more time to acclimatise before feeding on the periphyton and bioseston. Therefore, further experiments should be conducted to determine the feeding preference of African catfish between periphyton and bioseston. It was evident that periphyton's nutritional value is of high quality to support the growth of fish and reproduction. Therefore, it is recommended that further experiments be conducted to determine the breeding behaviour and reproduction performance of Mozambique tilapia and African catfish utilising periphyton in periphyton-based production systems.

In conclusion, this study has shown that periphyton-based aquaculture is an alternative way to improve tilapia and catfish production without increasing production costs in aqua dams. The specific growth rate of tilapia in aqua dams with plastic substrates and African catfish in aqua dams with stone substrates showed that commercial pellets can be reduced by 50-67 %. This study has also shown that periphyton-based biofilters can be used to maintain water quality in freshwater aquaculture production systems. Periphyton growing on net, plastic and stone was sufficient to maintain optimum water quality parameters for farming tilapia in a recirculating aquaculture system. The net periphyton-biofilter was found to be the most efficient periphyton-based biofilter compared to the plastic and stone periphyton-biofilter. This study also recorded significant differences in periphyton biomass and abundance depending on the type of substrate. The net substrate had the highest

periphyton biomass and abundance followed by the plastic substrates in aqua dams. The stone substrate had the least amount of periphyton composition and biomass. The growth of periphyton on substrates also enhanced the growth of bioseston in the water column in the aqua dams. Network analysis showed that the periphyton and bioseston communities produced small modularity in summer and winter, indicating a weak community in the aqua dams. This was caused by low genera diversity due to the similarities in water quality parameters and the high abundance of periphytic algae in both seasons. This indicated that the genera on one substrate were most likely to be found on another substrate despite the season.

Chapter 8: References

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APPENDIX

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

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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 ($7.74 \pm 1.45 \text{ g m}^{-2}$, $\pm \text{SE}$) 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.

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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). Bioeston is also an important source of food for fish species in periphyton-based aquaculture. Bioeston 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 bioeston 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 bioeston 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 ($2 \text{ mg g}^{-1} \text{ h}^{-1}$) 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 ($5 \text{ mg g}^{-1} \text{ h}^{-1}$) 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 bioeston 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, Moha-

patra 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, bioeston 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 (Figueroa & Giani 2005, Semyalo et al. 2011, El-Otifi 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 ($\pm$ SE) temperature was $25.81 \pm 2.59^\circ\text{C}$, dissolved oxygen (DO) was $4.84 \pm 1.25 \text{ mg l}^{-1}$, pH was 6.81 ± 0.18 , nitrogen ammonia was $1.03 \pm 0.84 \text{ mg l}^{-1}$, nitrate was $0.02 \pm 0.01 \text{ mg l}^{-1}$, and mean phosphate was $4.00 \pm 1.81 \text{ mg l}^{-1}$. At termination, the total fish weights and lengths were also determined to calculate the following growth parameters:

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

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

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

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

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

$$\text{Survival rate (\%)} =$$

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

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

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

This experiment was undertaken in fibreglass tanks, with the objective 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.

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 $\pm$ 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

Table 1. Abundance (mean $\pm$ 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
<i>Ankistrodesmus</i>	—	0.90 ± 1.56	—
<i>Chlorella</i>	16.28 ± 0.19	15.27 ± 13.23	21.05 ± 3.40
<i>Chlorogonium</i>	—	—	6.38 ± 10.08
<i>Coelastrum</i>	—	11.47 ± 10.24	—
<i>Cosmarium</i>	5.02 ± 8.69	1.38 ± 1.31	—
<i>Dictyosphaerium</i>	0.63 ± 1.10	—	—
<i>Micractinium</i>	—	—	11.73 ± 20.24
<i>Oocystis</i>	7.03 ± 1.60	—	—
<i>Pandorina</i>	—	—	0.08 ± 0.14
<i>Scenedesmus</i>	15.87 ± 1.87	25.70 ± 7.40	21.72 ± 20.70
<i>Sphaerocystis</i>	7.10 ± 6.16	—	—
<i>Cyclotella</i>	4.37 ± 7.56	5.50 ± 8.59	7.25 ± 7.40
<i>Pinnularia</i>	—	3.17 ± 5.48	4.48 ± 7.55
<i>Synedra</i>	—	1.58 ± 2.16	4.55 ± 7.88
<i>Navicula</i>	—	—	5.77 ± 9.60
<i>Nitzschia</i>	8.43 ± 8.16	10.92 ± 7.08	7.48 ± 8.95
<i>Mougeotia</i>	—	24.92 ± 14.08	2.73 ± 4.69
<i>Aphanocapsa</i>	—	—	12.45 ± 9.49
<i>Coelosphaerium</i>	7.40 ± 6.46	—	—
<i>Oscillatoria</i>	5.47 ± 9.47	—	0.42 ± 0.72
<i>Microcystis</i>	5.68 ± 9.84	25.47 ± 9.41	13.87 ± 12.84
<i>Trachelomonas</i>	0.57 ± 0.49	1.48 ± 2.57	3.33 ± 5.77
<i>Actinosphaerium</i>	9.52 ± 8.24	3.07 ± 5.23	—
<i>Mallomonas</i>	—	—	2.22 ± 3.63
<i>Lumbricus</i>	—	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

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).

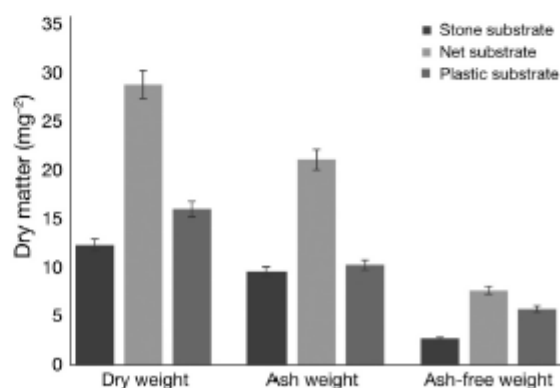


Fig. 1. Mean ($\pm$ SD) periphyton dry matter from aqua dams stocked with stone, net and plastic substrates for 21 d without fish grazing

Network analysis showed that associations among genera in bioseston 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 sub-

strate (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, *Diffugia*, *Euplotes*, *Paramoecium*, and *Vorticella* were abundant on all substrates (Table 2).

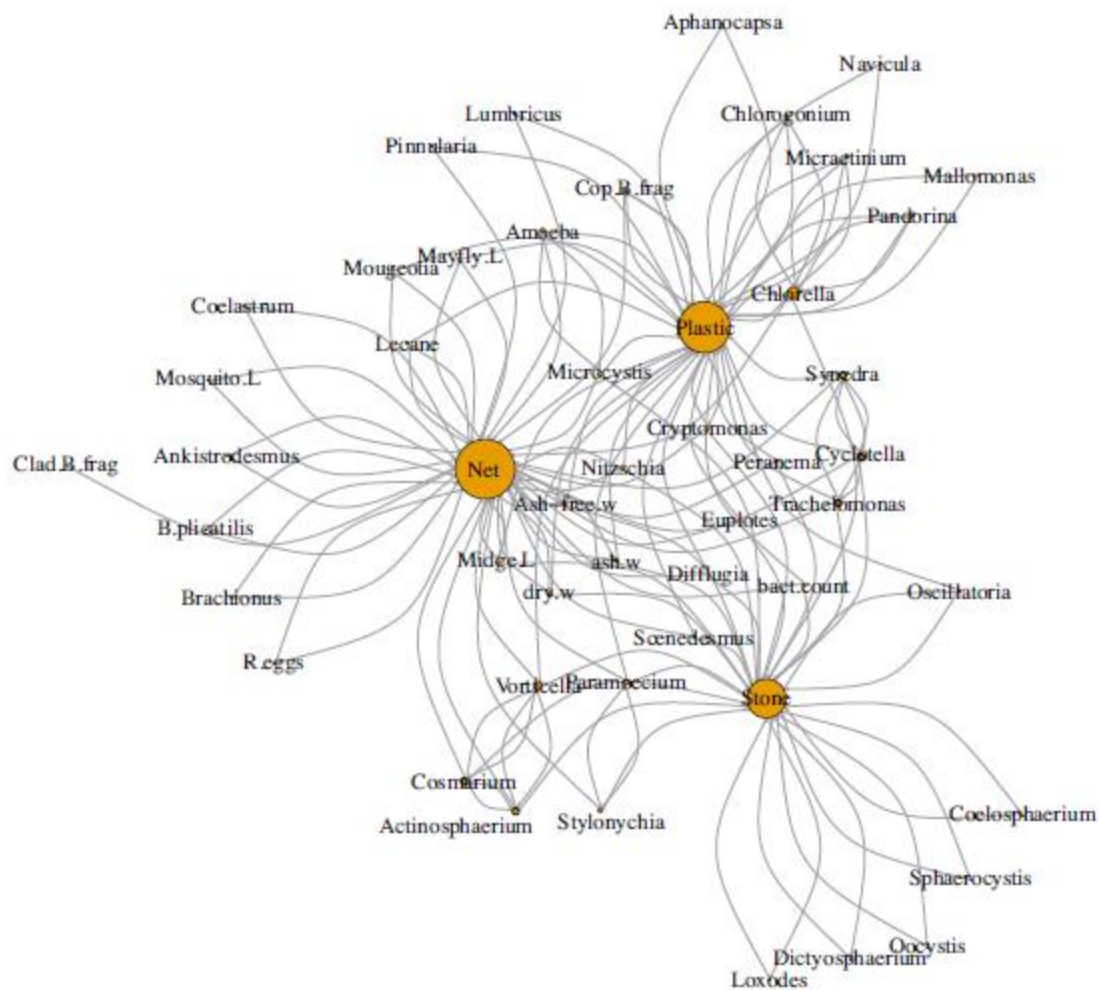


Fig. 2. Network of bioseston and periphytic communities at the genus level from aqua dams with stone, net, and plastic substrates for 21 d. The size of the circle (colour) on the nodes (substrates) indicates the number of edges connected to it. Ash-free weight, ash weight, and dry weight are a biomass measure for the bioseston and periphytic community growing on the substrates. R.eggs: rotifer 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 $\pm$ 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
<i>Cryptomonas</i>	11.28 $\pm$ 5.20	11.50 $\pm$ 10.06	15.65 $\pm$ 4.94
<i>Peranema</i>	12.55 $\pm$ 1.29	8.32 $\pm$ 6.53	14.07 $\pm$ 4.43
Mayfly larvae	—	8.77 $\pm$ 7.73	1.48 $\pm$ 2.57
Midge larvae	0.30 $\pm$ 0.40	17.75 $\pm$ 15.39	0.70 $\pm$ 0.48
<i>Anopheles</i> sp. (mosquito larvae)	—	0.52 $\pm$ 0.89	—
Copepods body fragments	—	—	0.13 $\pm$ 0.23
Cladocera body fragments	—	0.57 $\pm$ 0.55	0.10 $\pm$ 0.17
<i>Amoeba</i>	—	7.07 $\pm$ 4.02	3.02 $\pm$ 3.03
<i>Diffugia</i>	4.80 $\pm$ 8.31	5.28 $\pm$ 9.15	3.33 $\pm$ 5.30
<i>Euplotes</i>	10.35 $\pm$ 3.69	16.88 $\pm$ 17.40	4.58 $\pm$ 3.81
<i>Loxodes</i>	443 $\pm$ 7.68	—	—
<i>Paramoecium</i>	9.93 $\pm$ 0.20	8.82 $\pm$ 7.73	6.33 $\pm$ 10.97
<i>Stylonychia</i>	8.45 $\pm$ 7.61	5.30 $\pm$ 7.48	—
<i>Vorticella</i>	13.53 $\pm$ 3.83	5.13 $\pm$ 7.87	3.60 $\pm$ 3.23
<i>Brachionus plicatilis</i>	—	14.40 $\pm$ 24.94	—
<i>Brachionus</i> sp.	—	5.45 $\pm$ 4.27	—
<i>Lecane</i>	—	23.93 $\pm$ 9.26	0.33 $\pm$ 0.58
Rotifera eggs	—	10.57 $\pm$ 5.42	—
Total bioseston abundance	75.63 $\pm$ 5.25	150.25 $\pm$ 6.67	53.33 $\pm$ 4.73
Shannon-Wiener diversity index	2.036	2.546	2.243

Table 3. Mean $\pm$ 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 $\pm$ 0.00	70.0 $\pm$ 10.00	60.0 $\pm$ 0.00	0.422
Temperature (°C)	26.96 $\pm$ 0.49	25.5 $\pm$ 0.25	26.7 $\pm$ 0.52	0.119
Dissolved oxygen (mg l ⁻¹)	6.82 $\pm$ 1.85 ^a	2.11 $\pm$ 0.58 ^b	8.10 $\pm$ 0.28 ^a	0.022
Electrical conductivity (mS cm ⁻¹)	185.36 $\pm$ 3.00	210.36 $\pm$ 19.11	178.73 $\pm$ 8.85	0.238
Salinity (ppt)	0.08 $\pm$ 0.00	0.10 $\pm$ 0.01	0.08 $\pm$ 0.00	0.228
pH	7.15 $\pm$ 0.00	7.17 $\pm$ 0.01	7.17 $\pm$ 0.01	0.069
Nitrogen ammonia (mg l ⁻¹)	0.07 $\pm$ 0.01	0.03 $\pm$ 0.01	0.04 $\pm$ 0.01	0.096
Nitrate as N (mg l ⁻¹)	3.73 $\pm$ 0.12 ^a	4.06 $\pm$ 0.14 ^b	3.4 $\pm$ 0.05 ^a	0.017
Total phosphate (mg l ⁻¹)	6.96 $\pm$ 0.48	6.11 $\pm$ 0.41	6.46 $\pm$ 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*, *Tetradron*, *Diffugia*, 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 *Diffugia* 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 $\pm$ SE

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

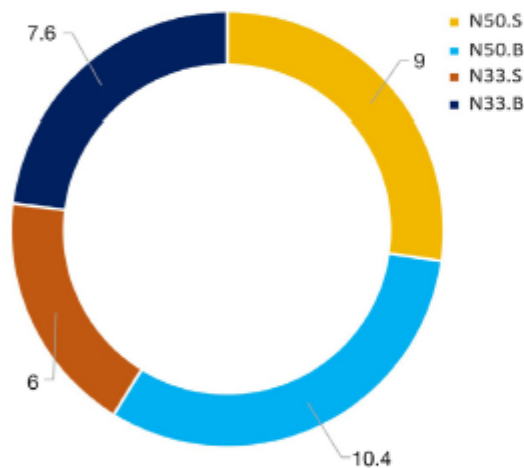


Fig. 3. Prominent values, showing that tilapia preferred to feed more on bioseston than on periphyton. Treatments are described as in Table 4, where 'S' refers to periphyton consumed and 'B' refers to bioseston

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 re-

sist 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, Vlajčević 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 Nhimock 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

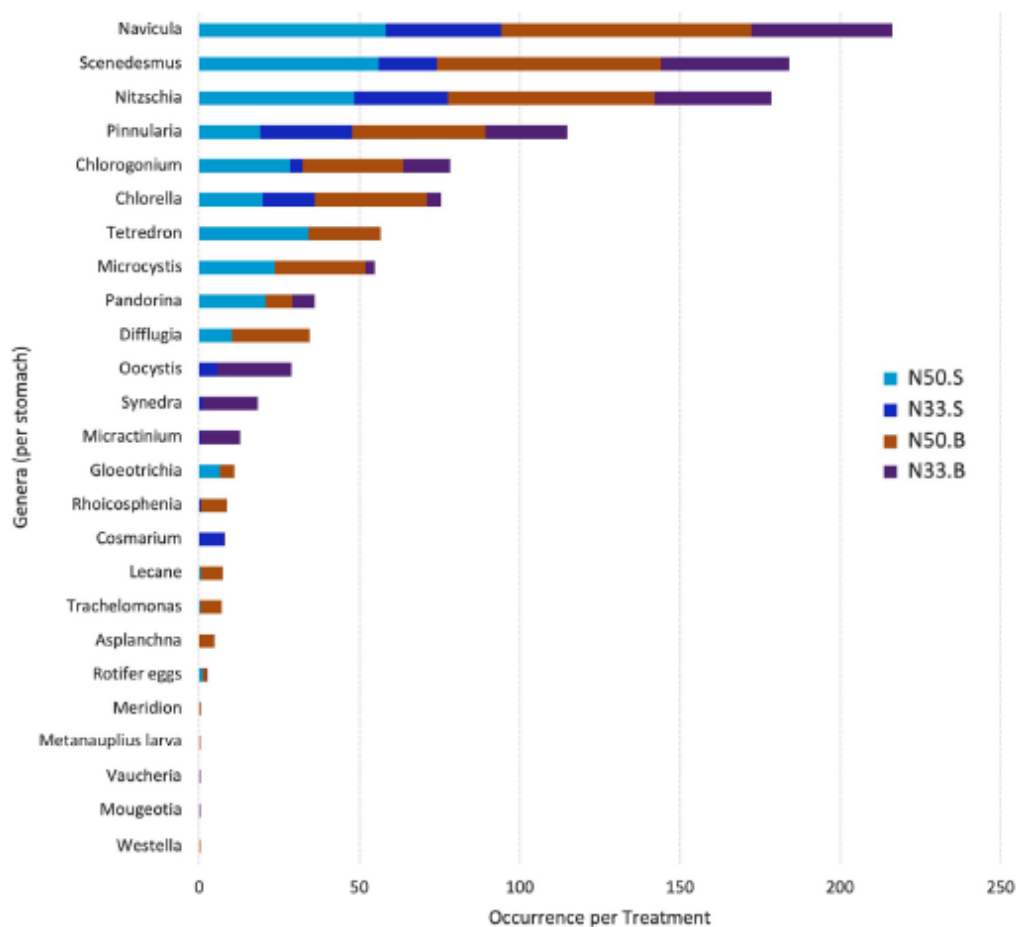


Fig. 4. Frequency of occurrence of periphyton and bioseston genera identified from the stomach of *Oreochromis mossambicus* fingerlings in the net substrate experiment. Treatments are described as in Table 4, where 'S' refers to periphyton consumed and 'B' refers to bioseston

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
<i>Scenedesmus</i>	0	0	0	2.8
<i>Tetredron</i>	0.8	0	0.5	4.3
<i>Pinnularia</i>	37	68	48	75.3
<i>Navicula</i>	65.8	41.3	61.5	54.8
<i>Synedra</i>	52.8	45.8	72.3	60
<i>Nitzschia</i>	32.8	32.8	43.3	65.8
<i>Diffugia</i>	10	20.5	14.3	23
<i>Lecane</i>	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 fin-

gerlings 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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APPENDIX B

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View Submission File Inventory View Attachments Revise Submission Decline to Revise Google Scholar Title Search Similar Articles in Scopus Google Scholar Author Search Scopus Author Search Scopus All Author Search Scopus Title Search Correspondence Send E-mail	AECO-D-24-00102R2	Periphyton dynamics in aqua dams stocked with Oreochromismossambicus in Limpopo Province, South Africa.	23 Apr 2024	12 Apr 2025	02 Apr 2025	Revise	Minor revisions

1 Title: Periphyton dynamics in aqua dams stocked with *Oreochromis mossambicus* in Limpopo
2 Province, South Africa.

3

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19 **ABSTRACT**

20 The study investigated the effect of different substrates, seasonality and grazing by *Oreochromis*
21 *mossambicus* on periphyton composition in aqua dams. Three experiments were conducted. In the first
22 experiment, net, stone, and plastic were deployed in triplicates in aqua dams. The periphyton (mainly
23 attached algae) biomass was high on the plastic (155.40 no./ml) and net (150.63 no./ml) substrate. The
24 net substrate was subsequently used in the second experiment to determine the seasonal variation of
25 periphyton in aqua dams. Periphyton was most abundant in summer and winter. The dominate phylum
26 in winter was Bacillariophyta and in summer it was Chlorophyta (mainly *Scenedesmus* sp.). The
27 network analysis produced a weak connection since a few genera dominated the periphyton biomass
28 across seasons. Periphyton abundance in summer was influenced by DO, EC, temperature, nitrogen,
29 salinity, and transparency, while in winter it was influenced by nitrate. The third experiment was
30 conducted in fibreglass tanks to determine the effect of *O. mossambicus* grazing on periphyton. Stomach
31 and faecal analysis showed that *O. mossambicus* were grazing mostly on *Scenedesmus* across different
32 size groups. However, Chesson selectivity index showed no evidence of food selection. Multivariate
33 analysis showed that season and substrates are the main factors affecting periphyton dynamics in aqua
34 dams. Deployment of net and plastic substrate in aqua dams is recommended in order to reduce feed
35 cost.

36 **Keywords:** Periphyton dynamics, Tilapia, Seasons, Substrates, Grazing, Aqua dams

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35 **Keywords:** Periphyton dynamics, Tilapia, Seasons, Substrates, Grazing, Aqua dams

1 INTRODUCTION

Periphyton-based aquaculture is an emerging low-cost technology. It is a simple and cheap way of producing natural food for herbivorous and omnivorous fishes (Azim et al. 2005; Garcia et al. 2016; El-Sayed 2020). The natural food grows on hard substrates that are deployed or fixed in production systems such as earthen fish ponds. The presence of natural food in a production system enhances fish growth and reduces production costs (Chikorela et al. 2019; Muthoka et al. 2021). Periphyton is a complex community of autotrophic and heterotrophic organisms such as bacteria, fungi, inorganic matter, and organic detritus that is attached to the substratum in water (Wetzel 1983; van Dam et al. 2002). One of the dominant components of periphyton is attached algae. This study will focus on attached algae since the fish species under study (*Oreochromis mossambicus*) mainly feeds on algae. Periphyton has traditionally been investigated in rivers, lakes, and wetlands (Zhao et al. 2023; Giongo et al. 2023). Nutrient concentration, light attenuation, grazing, seasons, and substrate type have been identified as some of the factors affecting periphyton dynamics in these aquatic ecosystems (Hao et al. 2020; Hu et al. 2022; Giongo et al. 2023). However, the key drivers of periphyton dynamics in extensive aquaculture production systems have not been investigated before. The growth performance of fish in periphyton-based aquaculture systems is determined largely by the biomass and diversity of periphyton. An investigation into the factors affecting periphyton dynamics is therefore imperative.

Season affects the biomass and diversity of periphyton because of their short reproduction cycle (Winder and Cloern 2010; Omid et al. 2019; Hao et al. 2020; Puts et al. 2023). Several studies reported that biomass and diversity of periphyton increased with temperature (Piggott et al. 2015; Hao et al. 2020; Singh et al. 2024). However, some studies contradicted this assertion (Borduqui et al. 2008; Ferragut et al. 2010; Hao et al. 2018). There is very little information on the effect of seasonality on the population dynamics of periphyton in periphyton-based aquaculture ecosystem. The growth rate of fish stocked in periphyton-based aquaculture production systems will be affected by seasonal changes in the composition of periphyton because proximate composition of periphyton will vary with season and this will affect the growth rate of the fish (van Dam et al. 2002).

64

65 Grazing has also been identified as a key factor affecting the dynamics of periphytic algae such as green
66 algae, diatoms, and cyanobacteria in lakes and rivers (El-Sayed 2020; Temesgen et al. 2022; Peng et al.
67 2023). Contradictory results on the effects of grazing in the aquatic systems have been recorded.
68 Huchette et al. (2000) showed that tilapia significantly reduced the biomass of periphyton over time in
69 periphyton-based aquaculture. Azim et al. (2003) also reported that periphyton biomass decreased after
70 the introduction of tilapia in periphyton-based tanks. However, Milstein et al. (2008) reported that
71 grazing had no significant effect on the biomass of periphyton in periphyton-based aquaculture. This
72 study will use multivariate analysis to evaluate the key drivers influencing periphyton dynamics in aqua
73 dams. During grazing, selectivity of particular periphyton taxa will influence the population dynamics
74 of those taxa (Vermaat 2005). In periphyton-based aquaculture, tilapia will scrap periphyton from the
75 substrate. It is, therefore, important to determine if *Oreochromis mossambicus* exhibits any selection
76 when it scraps periphyton from the substrate.

77

78 Substrates have been shown to influence the biomass and diversity of periphyton. Hlongwane et al.
79 (2024) investigated periphyton composition on net, plastic, and stone; and indicated that the net
80 substrate had the highest periphyton biomass because the net provided an adequate site for periphyton
81 development and attachment points on the mesh surface. However, the study did not investigate the
82 effect of seasonal variations on periphyton composition. Furthermore, the study did not identify the
83 factors affecting periphyton composition in aqua dams. Nurfadillah et al. (2023) reported that artificial
84 substrates support better growth and development of periphyton than organic-based substrates in
85 aquaculture production systems. In this study stones, plastics, and nets will be the target substrates for
86 periphyton-based aquaculture in aqua dams to analyse periphyton dynamics associated with different
87 substrates. The substrate type coupled with a nutrient source for periphyton growth affects not only the
88 biomass (quantity) of periphyton but also the quality of the periphyton. The quality and quantity are
89 affected by type of substrate deployed in periphyton-based aquaculture (Anix et al. 2017).

90

91 Aqua dams are the most commonly used aquaculture systems in South Africa. This is because the
92 government distributed these production systems in the last decade to promote aquaculture production
93 in rural communities. Aqua dams are made of a strong, flexible high-density polyethylene
94 geomembrane material that is ultraviolet light resistant. This makes them suitable for outdoor
95 aquaculture farming, under real cultural conditions exposed to different weather conditions. However,
96 aquaculture production in aqua dams has remained low and one way to increase production is through
97 periphyton-based aquaculture. The periphyton-based aquaculture not only lowers production costs, but
98 it also improves the growth performance and health of the fish and improves water quality (Azim et al.
99 2005; Shafi et al. 2021; Hao et al. 2022). The main objective of this study was to determine the effect
100 of season, substrate and grazing on periphyton composition in aqua dams stocked with *Oreochromis*
101 *mossambicus*.

102 2 MATERIALS AND METHODS

103 2.1 Study site

104 The study was conducted in experimental aqua dams (7000 L) at the Aquaculture Research Unit.
105 Experiments were conducted for a year under real field conditions, in three different seasons (summer,
106 autumn, and winter). The university is located in the northernmost part of South Africa, known as
107 Limpopo Province. The weather forecast of Limpopo Province is generally hot, having an average
108 minimum temperature of 11 °C in winter and a maximum temperature of 40 °C in summer
109 (WeatherSpark 2023). The study was approved, and a certificate (no. AREC/05/2023: PG) was issued
110 by the University of Limpopo's Animal Ethical Committee before the commencement of experiments.

111

112 2.2 The effect of different substrates on periphyton abundance and dominance.

113 The effect of substrate on periphyton abundance and dominance was determined by deploying net,
114 stone, and plastic (1 litre bottle) in nine aqua dams (7000 L), each treatment was replicated three times
115 in a completely randomised design. The net substrate is made of nylon material and plastic bottles are
116 made out high-density polyethylene (HDPE) plastic. Tap water (municipal water) was allowed to
117 mature in aqua dams for five days. In order to stimulate planktonic development, chicken manure was
118 added at 0.26 kg m⁻² (Jha et al. 2008). The periphyton was allowed to colonise on nets, stones, and
119 plastics for 21 days in aqua dams without fish. Periphyton abundance and water quality parameters were
120 then monitored.

121

122 2.2.1 Periphyton sampling and enumeration

123 Periphyton samples were collected by carefully scraping the substrate's surface area (9 x 9 cm²) to
124 determine periphyton abundance. The periphyton from the substrate was then mixed with 20 ml distilled
125 water before enumeration under a light microscope (Leica E24) using an improved double Neubauer
126 chamber W- Germany 0.100 mm depth, 0.0025 mm². The counting chamber was cleaned with 70 %
127 ethanol, and then 0.01 ml (10 µl) of the sample was loaded on the loading groove using a micropipette

and counted. The loading groove has nine main squares delimited by three lines; the counting is performed in the area delimited by three lines. The concentration of species in 1 μ l was estimated by dividing the number of counted species by the volume of the four main squares. The value was then multiplied by 1000 to determine the total number of species in 1 ml. Petri dish was also used to count periphyton since some species were too big to be counted using a counting chamber. A volume of 1 ml of the sample was enumerated in a petri dish under a light microscope. Only attached algae from the periphyton community is reported in this study. Hereafter, the term 'periphyton' is used to indicate the assemblage of attached algae on submerged substrates. The frequency of occurrence and dominance index of the enumerated periphyton was calculated using the formula below. In the present study, only periphyton species with a dominance index of >5 was considered. Shannon diversity index, species richness, and species evenness were calculated using R (version 4.4.2) using *vegan* and *tidyverse* packages.

140

$$i. \text{ Frequency of occurrence (\%)} = \frac{\text{The number of ml in which spp is found}}{\text{Total number of ml examined}} \times 100$$

142

$$ii. \text{ Dominance index} = \frac{\text{The abundance of periphyton taxon}}{\text{Total abundance of all periphyton} \times \text{frequency of occurrence of taxon}}$$

144

2.2.2 Water quality parameters determination

Water quality parameters were determined by using a *YSI multi-probe meter and spectrophotometer methods* weekly. Dissolved oxygen (DO) (mg/l), temperature ($^{\circ}$ C), pH, salinity (ppt), and electrical conductivity (EC) (mS/cm) were monitored using a *YSI multi-probe meter*. Transparency (cm) was measured using a Secchi disc. Nitrate (mg/l) was analysed according to cadmium reduction method no. 8171 of *DOC316.53.01069*, ammonia as nitrogen (mg/l) according to USEPA *nessler* method 8038 of *DOC316.53.01078*, and phosphate (mg/l) according to *PhosVer 3* method no. 8048 of *DOC316.53.01119* (HACH 2012).

153

154 **2.3 Periphyton seasonal variation in aqua dams with net substrates.**

155 The effect of seasonal variation on periphyton abundance and dominance was investigated during
156 summer, autumn, and winter by deploying nets in aqua dams in a completely randomised design. The
157 net substrates were chosen because it has been shown to be the best periphyton substrate in freshwater
158 tilapia ponds. The aqua dams had no aeration and water circulation, water lost through evaporation was
159 replaced once a week. The summer experiment was conducted in January and February 2022, the
160 autumn experiment was conducted between March and May 2022, and the winter experiment ran from
161 June until July 2022. Periphyton samples were collected to determine the seasonal diversity, abundance,
162 and dominance of species in the aqua dams. The periphyton biomass and water quality parameters were
163 monitored as described in sections 2.2.1 and 2.2.2 respectively.

164

165 **2.4 The effect of tilapia grazing on periphyton in aqua dams**

166 The experiment was conducted using 36 fish in nine 90 L fibreglass tanks in a completely randomised
167 design in triplicate. Three different tilapia sizes (2, 5 and 25 g) were used for this experiment. The fish
168 were starved for 24 hours before being stocked in fibreglass tanks. The fish were starved to obtain a
169 complete gut evacuation and a clean digestive tract before commencing experiments in the fibreglass
170 tanks.

171

172 The net substrates, with a surface area of 900 cm^2 , from aqua dams described in section 2.3 were placed
173 in the fibreglass tanks. One piece of net substrate was placed in the fibreglass tanks for fish to graze on.
174 Periphyton was enumerated from another piece of net (900 cm^2) which was not placed in the fibreglass
175 tanks. Thus, this net was not exposed to grazing by the fish. The periphyton was enumerated by drying
176 and weighing to determine the amount of periphyton on the substrate according to method no. 10300 C
177 of APHA (1998).

178

Four tilapias of each size were stocked per fibreglass tank and allowed to graze on the periphyton on the net substrate for 12 hours. After 12 hours of grazing, the net (900 cm²) substrates in all the fibreglass tanks were taken and enumerated. Periphyton was enumerated by scraping the remaining periphyton on the substrate (from the fibreglass tank), then dried and weighed to determine how much periphyton was eaten by the fish, using weight difference before and after grazing. The fish did not have access to any other food items outside the periphyton on the net. This method is adopted from Dempster et al. (1993), van Dam et al. (2002) and Azim et al. (2003). After grazing, faeces were collected directly from the fibreglass tanks by siphoning, and thereafter the fish were harvested from the fibreglass tanks. All fish (36) were then sacrificed and gutted to remove the stomach. The stomach content was analysed under a light microscope to identify the periphyton items ingested by the fish. The faecal matter was also analysed under a light microscope to determine which periphyton items were not consumed by the fish. The dominance index of periphyton in the stomach was calculated using the formula mentioned in section 2.2.1. The frequency of occurrence of periphyton in the stomach content was calculated using the following formula:

$$\text{i. Frequency of occurrence (\%)} = \frac{\text{The number of stomachs in which a periphyton is found}}{\text{Total number of stomachs containing periphyton}} \times 100$$

Food selectivity was calculated using the following Chesson selectivity index (CSI):

$$\text{ii. } CSI = \frac{g_i/b_i}{\sum_{j=1}^n g_j/b_j}, i = 1, \dots, n,$$

Where n is the total number of periphyton species in the stomach contents; g and b are the proportional representation of periphyton species i or j in the stomach content and water (environment), respectively.

The CSI value for a specific periphyton species i reflects its presence in the stomach contents relative to the environment, divided by the sum of the equivalent calculation for all periphyton species in the

203 stomach content. The Chesson selectivity index value ranges from -1 to +1, with -1 indicating complete
204 avoidance of the food item, 0 indicating a random selection of the food and 1 indicating exclusive
205 feeding on that species.

206

207 **2.5 Multivariate analysis**

208 Network analysis was determined in R (version 4.4.2) using “igraph”, and “test layout” was used for
209 the output plot. Network analysis was used to determine the connection of the periphyton in the three
210 seasons. It was used to study the patterns of relationships and the network structure of the periphyton
211 community growing during winter, summer, and autumn. Canoco (4.5) was used to run the canonical
212 correspondence analysis (CCA). Canonical correspondence analysis was used to determine the
213 relationship between seasonal periphyton species dominance and water quality parameters, effectively
214 identifying dominant periphyton species associated with environmental water quality parameters. The
215 ~~adonis2~~ test was also determined in R using vegan and MASS packages.

216

217 The ~~adonis2~~ test was used to determine the most significant factor (between season, grazing and
218 substrate) affecting periphyton dynamics in aqua dams. Each factor (season, grazing and substrate) was
219 first tested for significance separately and then tested simultaneously. ~~Adonis2~~ was used to test for
220 significance because the data was not normally distributed, it is non-parametric (periphyton
221 composition) data. It was used because it can test categorical and continuous data, and it can perform
222 sequential, marginal and overall tests. The ~~adonis2~~ test also uses additive constants or square-root of
223 dissimilarities to avoid negative eigenvalues but can also handle semi-metric indices that produce
224 negative eigenvalues (Oksanen et al. 2012). The ~~adonis2~~ uses F-tests to perform significance tests using
225 semi-metric and metric distance matrices. The test was run 999 times because the actual sequence of
226 group identities forms one of the possible permutations and therefore is included in the denominator of
227 the probability calculation.

228 3 RESULTS

229 3.1 Periphyton composition in aqua dams with different substrates

230 Seven phyla were identified on the net, stone, and plastic substrates in the aqua dams (Table 1).
231 Chlorophyta was the most abundant phylum in all the substrates. On the stone substrate, the second
232 most abundant phylum was Cyanophyta followed by Euglenophyta. On the net substrate, the second
233 most abundant phylum was Charophyta followed by Cyanophyta. Bacillariophyta and Cyanophyta were
234 the second and third abundant phyla on the plastic substrate (Table 1).

235

236 A total of twelve periphyton genera were most dominant on all the substrates (Table 2). The stone
237 substrate was dominated by *Chlorella* sp. and *Scenedesmus* sp., followed by *Peraenoma* sp. The net
238 substrate was dominated by *Scenedesmus* sp. followed by *Microcystis* sp. and *Mougeotia* sp. The plastic
239 substrate was dominated by *Scenedesmus* sp. and *Chlorella* sp. The Shannon Weiner diversity index
240 and evenness were higher on the net substrate (Table 2).

241

242 3.2 Periphyton seasonal variation in aqua dams with net substrates.

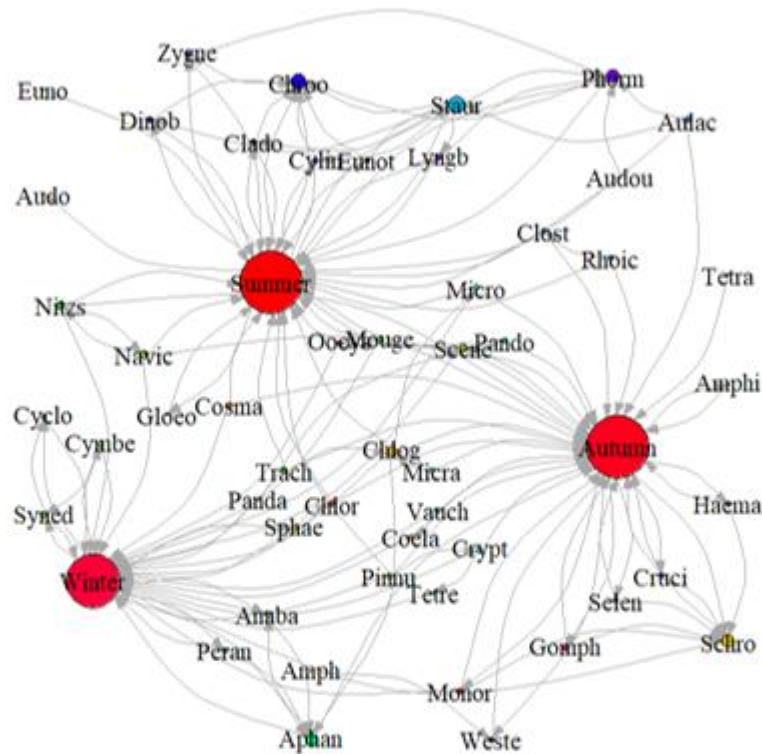
243 Nine phyla were identified on the net substrates across the seasons (Table 3). Bacillariophyta and
244 Chlorophyta were the most abundant phyla in winter and summer, respectively (Table 3). Ten genera
245 were the most abundant across the seasons (Table 4). In winter, *Synedra* sp. followed by *Pinnularia* sp.
246 were most dominant genera. Summer and autumn season were dominated by *Scenedesmus* sp. genera.
247 The Shannon Weiner diversity index and evenness were higher in winter season (Table 4).

248

249 The network analysis showed the connections and pattern of relationship of the periphyton community
250 growing on the net substrate in three seasons. The network analysis produced a weak modularity of
251 0.322, showing that the community of species found in one season were likely to be found in another
252 season (Fig. 1). Based on the degree of centrality, summer and autumn had the higher score, showing

253 that there were more species with similar functions growing on the substrate during these seasons. The
 254 network analysis produced a graph density of 0.063 showing an overall weak connection among the
 255 seasons (Fig. 1).

256



257

258 Fig. 1 Network analysis of periphyton in aqua dams deployed with net substrates in three seasons. Abbreviation
 259 used: ~~Chlor-~~ *Chlorella* sp.; ~~Chlog-~~ *Chlorogonium* sp.; ~~Clost-~~ *Clasterium* sp.; ~~Coela-~~ *Coelastrum* sp.; ~~Cosma-~~
 260 *Cosmarium* sp.; ~~Cruci-~~ *Crucigenia* sp.; ~~Haema-~~ *Haematococcus* sp.; ~~Micra-~~ *Microactinium* sp.; ~~Monor-~~
 261 *Monoraphidium* sp.; ~~Oocys-~~ *Oocystis* sp.; ~~Pando-~~ *Pandorina* sp.; ~~Schro-~~ *Schroederia* sp.; ~~Scene-~~ *Scenedesmus*
 262 sp.; ~~Selen-~~ *Selenastrum* sp.; ~~Tetra-~~ *Tetrasclera* sp.; ~~Weste-~~ *Westella* sp.; ~~Amphi-~~ *Amphipleura* sp.; ~~Gomph-~~
 263 *Gomphonema* sp.; ~~Pinnu-~~ *Pinnularia* sp.; ~~Rhoic-~~ *Rhoicosphenia* sp.; ~~Aulac-~~ *Aulacoseira* sp.; ~~Nitza-~~ *Nitzschia* sp.;
 264 ~~Navic-~~ *Navicula* sp.; ~~Mouge-~~ *Mougeotia* sp.; ~~Crypt-~~ *Cryptomonas* sp.; ~~Vauch-~~ *Vaucheria* sp.; ~~Aphan-~~
 265 *Aphanocapsa* sp.; ~~Staur-~~ *Staurastrum* sp.; ~~Euno-~~ *Eunotia* sp.; ~~Audou-~~ *Audouinia* sp.; ~~Zygne-~~ *Zygnema* sp.;

266 Clado- *Cladophora* sp.; Dinob- *Dinobryon* sp.; Chroo- *Chroococcus* sp.; Micro- *Microcystis* sp.; Gleso-
 267 *Gleasonella* sp.; Cylin- *Cylindrocapsa* sp.; Phorm- *Phormidium* sp.; Lyngb- *Lyngbya* sp.; Eucy- *Eucypris* sp.;
 268 Sphae- *Sphaerocystis* sp.; Cymbe- *Cymbella* sp.; Cyclo- *Cyclotella* sp.; Syned- *Synedra* sp.; Trach-
 269 *Trachelemonas*; Peran- *Peranonea* sp.; Anaba- *Anabaena* sp.

270

271 The environmental water quality was monitored seasonally to determine its influence on periphyton
 272 dynamics in aqua dams. The levels of electrical conductivity, salinity, nitrogen (ammonia), and total
 273 phosphate were highest in summer (Table 5). Nitrate was highest in autumn and winter. The water
 274 quality parameters were statistically significant except for dissolved oxygen (Table 5).

275

276 Canonical correspondence analysis (CCA) showed the relationship between the dominant periphyton
 277 and environmental water quality parameters in three seasons (Fig. 2). The first two Canonical
 278 correspondence analysis axes explained 100 % of the variation of the seasonal periphyton in the aqua
 279 dams. The Canonical correspondence analysis 1 (CCA 1) explained 86.7 % of the variation and CCA 2
 280 explained 13.3 %. The CCA plot showed that autumn was positively associated with *Chlorella* sp. and
 281 *Microactinium* sp. (Fig. 2). Nitrate was associated with *Synedra* sp., *Aphanocapsa* sp. *Microcystis* sp.,
 282 and *Pinnularia* sp. in winter. In summer, environmental water quality parameters (DO, EC, temperature,
 283 nitrogen as ammonia, salinity, and transparency) influenced the high frequency of occurrence and
 284 abundance of periphyton (*Navicula* sp., *Cosmarium* sp., *Pandorina* sp. and *Scenedesmus* sp.) (Fig. 2).

285

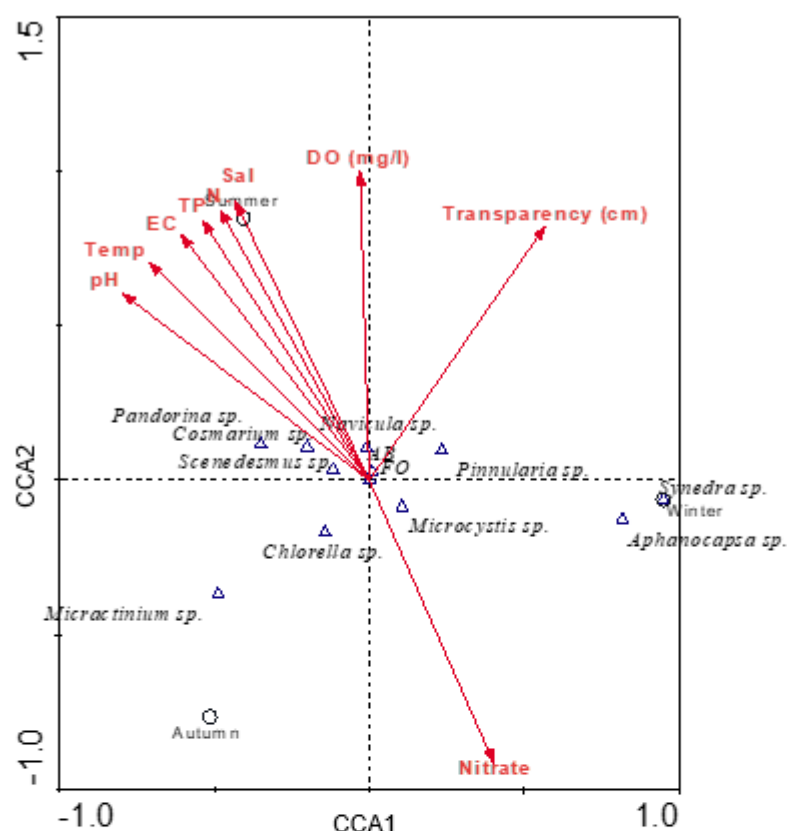
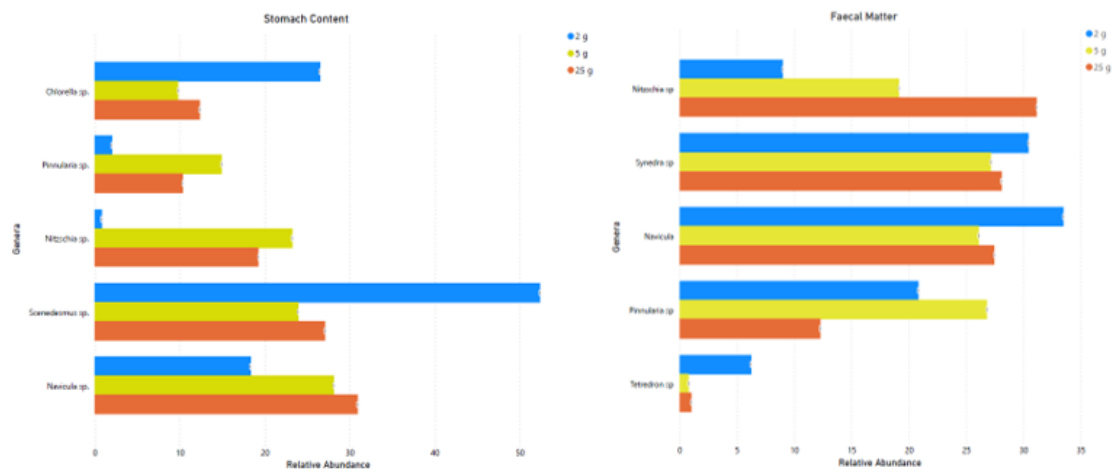


Fig. 2 Canonical correspondence analysis of the seasonal periphyton in aqua dams deployed with net substrates. Abbreviations: DO- dissolved oxygen, temp- temperature, EC- electrical conductivity, TP- total phosphate, sal- salinity, N.- nitrogen as ammonia, AB species abundance, FO- species frequency of occurrence.

3.3 The effect of tilapia grazing on periphyton in aqua dams

The relative abundance of the most dominant periphyton species identified in the stomach content of tilapia showed that *Scenedesmus* sp. followed by *Chlorella* sp. were the dominant genera in the stomach content of 2 g fingerlings (Fig. 3). The stomach content of 5 g fingerlings was dominated by *Navicula* sp. followed by *Scenedesmus* sp. and *Nitzschia* sp. In 25 g fingerlings, *Navicula* sp. was the most dominant and the second most dominant species was *Scenedesmus* sp. (Fig. 3).

:97



:98

:99

Fig. 3 Relative abundance of the most dominant periphyton species in the stomach content and faecal matter of tilapia with different sizes

300 The Chesson selective index showed that all the *O. mossambicus* size groups were non-selective when
301 feeding on the periphyton (Table 6).

302

303 3.4 Multivariate analysis of season, grazing and substrate

304 The ~~adonis2~~ test was used first to test each factor independently to determine the influence of season,
305 grazing and substrate on periphyton dynamics in aqua dams. The periphyton abundance in summer,
306 autumn and winter produced an F value of 8.0176 and a P value of 0.001 (Table 7). The ~~adonis2~~ test
307 also showed a significant (F= 3.4696, P= 0.001) difference in the periphyton abundance in aqua dams
308 due to substrates. Grazing was not significantly different (F= 1.0632, P= 0.369) due to fish sizes (2 g,
309 5 g and 25 g) groups. This indicates that season and substrate were similarly influencing periphyton
310 dominance. When all factors were tested simultaneously, ~~adonis2~~ test showed that periphyton
311 abundance in aqua dams was significantly (F= 17.54, P= 0.001) influenced by seasons, grazing and
312 substrates (Table 7).

313 4 DISCUSSION

314 Chlorophyta was the most abundant phylum on all the substrates and *Scenedesmus* was the most
315 dominant genera across the seasons. This is probably because Chlorophyta is tolerant of diverse
316 environmental conditions (Holzinger and Karsten 2013; Celewicz and Kozak 2022). The aqua dams
317 were outdoors, exposed to the vagaries of the weather. The dominance of *Scenedesmus* on the plastic
318 and net was probably because it has hooks that enable it to easily attach to the smooth texture of the
319 plastic substrate. *Scenedesmus* may have predominated the stone substrate because of its ability to grow
320 in light-limited conditions (Gelin et al. 1997; Bellinger and Sigee 2010; Banerjee et al. 2022). This
321 shows that *Scenedesmus* sp. is a cosmopolitan species, which is why it was dominant on all substrates.
322 Cyanophyta was the second most abundant on the stone and plastic substrates and the third most
323 abundant on the net. Their nitrogen-fixing ability enabled them to flourish under varying conditions
324 (Stal 2017; Paerl 2017). Bacillariophyta was the second most abundant on the plastic substrate. Diatoms
325 have silica cell walls that produce adhesive secretions known as extracellular polymeric substances
326 which probably enabled them to attach to smooth substrates (Sweat and Johnson 2013; Watanabe et al.
327 2022).

328

329 CCA showed the association of season with nutrients (environmental water quality parameters) in aqua
330 dams. Winter was strongly associated with diatoms (*Synedra* sp. and *Pinnularia* sp.), and cyanobacteria
331 (*Aphanocapsa* sp. and *Microcystis* sp.), and nitrate. This was probably because diatoms and
332 cyanobacteria are not sensitive to low temperatures and can bloom in temperatures as low as 11 °C. In
333 Limpopo province, winter temperatures range between 4.4 °C and 22.2 °C. These temperatures are
334 optimal for the proliferation of diatoms (Guttman 2019; Andersen et al. 2020). The dominance of
335 diatoms and cyanobacteria in high nitrate concentrations might be explained by the fact that diatoms
336 and cyanobacteria can efficiently utilise nitrate (Grinten et al. 2004; Liu et al. 2022). In order to
337 stimulate periphyton growth, chicken manure was added to the aqua dams, and this resulted in high
338 nitrate levels. The dominance of diatoms and cyanobacteria in water bodies with high nitrate levels has
339 previously been reported in several studies (Ernst et al. 2005; Andersen et al. 2020; Stief et al. 2022).

340 The dominance of *Chlorella* sp. and *Micractinium* sp. in autumn is explained by the rising temperatures.
 341 Summer was associated high frequency of occurrence and abundance of *Navicula* sp., *Coscinodiscus* sp.,
 342 *Pandorina* sp., and *Scenedesmus* sp. This was probably because the temperature recorded (30.07 °C) in
 343 the aqua dams was optimal for most periphyton identified in summer. For example, the optimal
 344 temperature for *Scenedesmus* sp. and *Navicula* sp. is between 25 °C and 35 °C (Hodaifa et al. 2010;
 345 Ruiz-Martinez et al. 2015; Li et al. 2017).

346

347 The network analysis showed an overall weak connection among the seasons because only three phyla
 348 were most dominant in all the seasons. The network analysis showed that there were more species with
 349 similar functions growing on the substrates during the summer and autumn seasons. This was because
 350 the three seasons were dominated by the same phylum (Chlorophyta), and the species diversity,
 351 evenness and richness were comparable. The dominance of Bacillariophyta in winter could not give the
 352 network community a strong connection because a few genera (*Synedra* sp., *Pinnularia* sp.,
 353 *Scenedesmus* sp., *Chlorella* sp.) were dominant in all seasons. The aqua dams are artificial habitats and
 354 have less diverse periphytic communities as compared to natural habitats. Thus, the network analysis
 355 obtained in this study differs from those reported by Hu et al. (2022), Liu et al. (2022), and Miao et al.
 356 (2021). This was probably because these studies were conducted in natural water habitats that receive
 357 allochthonous material, unlike aqua dams. Moreover, the periphyton community in natural water bodies
 358 grows on submerged macrophytes, tree roots, leaves, and twigs which leach nutrients that enhance the
 359 growth of periphyton (Azim et al. 2005). Several studies have shown that the periphytic community
 360 from natural water bodies is large and more diverse (Azim et al. 2005; Chang et al. 2022; Zhu et al.
 361 2022).

362

363 Chlorophyta and Bacillariophyta dominated the periphyton community in both the aqua dams and fish
 364 stomachs. *Synedra* sp. was only most dominant in the aqua dams while *Navicula* sp. and *Scenedesmus*
 365 sp. were the most dominant genera in both the aqua dams and the stomachs. This probably indicates

that *O. mossambicus* were not selective in their feeding on periphyton, they were feeding on the most dominant periphyton in the aqua dams. *Oreochromis mossambicus* did not select any periphyton genera although *Scenedesmus* dominated the food items in the stomach. *Scenedesmus* sp. was the most readily available genera and probably easily accessible to *O. mossambicus* because it is easily attached to substrates using floatation spines (Konur 2020). This is consistent with a previous study by Huchette et al. (2000) that showed that tilapia showed only limited feeding selectivity when feeding on periphyton. Another study by Dempster et al. (1993) showed that tilapia actively feed on periphyton without being selective. *Chlorella* sp. and *Scenedesmus* sp. were dominant in the stomach of all the size groups, but conspicuously absent from the faecal matter. This shows that these Chlorophyta (*Scenedesmus* sp. and *Chlorella* sp.) were effectively utilised by all the size groups. The dominance of *Navicula* sp. in the stomach content suggests a higher ingestion rate compared to *Synedra* sp., although both genera were likely consumed in significant amounts due to their high dominance in the aqua dams. The dominance threshold (dominance index of >5 was considered) has obscured the true extent of *Synedra* sp. in the stomach. The faecal matter of all size groups was dominated by *Synedra* sp., *Pinnularia* sp., *Navicula* sp., and *Nitzschia* sp., indicating that *O. mossambicus* was not digesting the Bacillariophyta. All tilapias' size groups were not able to digest Bacillariophyta species because of the silica cell wall covering diatom species. This was probably because *O. mossambicus* lack mandibles lined with silica-reinforced teeth used to crack the hydrated silicon dioxide cell wall of diatoms. This is consistent with a study by Ping and Jiankang (1994), and Mohamed et al. (2019) that showed that tilapia is unable to digest diatoms.

Periphyton dynamics in aqua dams were significantly influenced by substrates and seasons. Grazing also influenced the periphyton dynamics to a limited extent. All the substrates used in this study are not degradable and this probably affected the abundance of the periphyton. Periphyton abundance is higher in biodegradable substrates (Gangadhara and Keshavanath 2008; Keshavanath et al. 2012). Both the diversity and abundance of periphyton varied with season. This is consistent with previous studies (Asaduzzaman et al. 2010; Shaikh and Chavan 2013; Tortolero et al. 2016). Periphyton variability

393 across seasons will affect the feeding habits of *O. mossambicus*. *Oreochromis mossambicus* stops
394 feeding at 16 °C (Lowe-McConnell 1987; El-Sayed et al. 1996). Thus, the dominance of some
395 periphyton in winter was not beneficial to *O. mossambicus* since it will not feed in winter.

396

397 **5 CONCLUSION**

398 The periphyton community was dominated by *Scenedesmus* sp. across seasons and on different
399 substrates. The phyla Bacillariophyta also dominated the periphyton community in winter. However,
400 *O. mossambicus* did not digest Bacillariophyta, this is evident from its dominance in the faecal matter.
401 *Oreochromis mossambicus* non-selectively fed on *Scenedesmus* sp. across different sizes (2 g, 5 g, 25
402 g). The periphyton composition was affected by season and substrate. The cultivation of *Scenedesmus*
403 sp. is recommended in order to enhance periphyton-based aquaculture in aqua dams.

404 **6 STATEMENTS AND DECLARATIONS**

405 **Competing interest statement**

406 The authors have no relevant financial or non-financial interests to disclose.

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409 **Data availability statement**

410 Data are available from the corresponding author on request.

411 **Compliance with Ethical Standards**

412 The study was approved and a certificate (no. AREC/05/2023: PG) was issued by the University of
413 Limpopo's Animal Ethical Committee before the commencement of experiments. Humane measures
414 were implemented when the fish were handled and sacrificed in accordance with the University of
415 Limpopo's Animal Ethical Committee.

416 **Authors' contribution statements**

417 All authors contributed to the study conception and design. Material preparation, data collection and
418 analysis were performed by Khathutshelo C. Hlongwane. The first draft of the manuscript was written
419 by Khathutshelo C. Hlongwane. Supervision, review, and editing were performed by Ngonidzashe A.G
420 Moyo and Mxaditshaba M. Rapatsa- Malatji. All authors commented on previous versions of the
421 manuscript. All authors read and approved the final manuscript.

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613 **8 TABLES**

614 **Table 1** The classification of periphyton taxa in aqua dams with different substrates.

	Stone		Net		Plastic	
	No./ml	Relative abundance (%)	No./ml	Relative abundance (%)	No./ml	Relative abundance (%)
Chlorophyta	46.92	40.03	53.33	35.41	61.13	39.34
Charophyta	5.02	4.28	26.30	17.46	2.73	1.76
Bacillariophyta	12.80	10.92	21.17	14.05	29.53	19.00
Euglenophyta	13.12	11.19	9.80	6.51	17.40	11.20
~~Ochromyces~~ Chlorophyta	9.52	8.12	3.07	2.04	2.22	1.43
Cyanophyta	18.55	15.83	25.47	16.91	26.73	17.20
~~Cryptophyta~~ Cryptophyta	11.28	9.63	11.50	7.63	15.65	10.07
Total	117.20	100	150.63	100	155.40	100

615

Table 2 The periphyton composition of the dominate species on different substrates in aqua dams

Substrate	Species	Abundance (no/ml)	Frequency of occurrence	Dominance index	Diversity index	Richness	Evenness
Stone	<i>Chlorella</i> sp.	16.28	100	13.89	2.517	15	1.726
	<i>Oocystis</i> sp.	7.03	100	6.00			
	<i>Scenedesmus</i> sp.	15.87	100	13.54			
	<i>Cryptomonas</i> sp.	11.28	100	9.63			
	<i>Peranema</i> sp.	12.55	100	10.71			
	<i>Actinosphaerium</i> sp.	9.52	66.67	5.41			
Net	<i>Chlorella</i> sp.	15.27	66.67	6.76	2.999	15	2.057
	<i>Coelastrum</i> sp.	11.47	66.67	5.07			
	<i>Scenedesmus</i> sp.	25.70	100	17.06			
	<i>Nitzschia</i> sp.	10.92	100	7.25			
	<i>Mougeotia</i> sp.	24.92	100	16.54			
	<i>Cryptomonas</i> sp.	11.50	66.67	5.09			
	<i>Microcystis</i> sp.	25.47	100	16.91			
	<i>Peranema</i> sp.	8.32	100	5.52			
Plastic	<i>Chlorella</i> sp.	21.05	100	13.55	2.595	18	1.570
	<i>Microactinium</i> sp.	11.73	66.67	5.03			
	<i>Scenedesmus</i> sp.	21.72	100	13.97			
	<i>Cryptomonas</i> sp.	15.65	66.67	6.71			
	<i>Aphanocapsa</i> sp.	12.45	100	8.01			
	<i>Microcystis</i> sp.	13.87	66.67	5.95			
	<i>Peranema</i> sp.	14.07	100	9.05			

Table 4 The seasonal periphyton composition of the dominate species in aqua dams.

Season	Species	Abundance (no./ml)	Frequency of Occurrence	Dominance index	Diversity index	Richness	Evenness
Winter	<i>Scenedesmus</i> sp.	15.08	100	5.40	2.019	25	1.099
	<i>Pinnularia</i> sp.	25.28	100	9.05			
	<i>Aphanocapsa</i> sp.	16.14	100	5.78			
	<i>Synedra</i> sp.	28.14	100	10.07			
	<i>Microcystis</i> sp.	16.90	100	6.05			
Summer	<i>Pandorina</i> sp.	29.09	100	10.59	1.824	26	0.972
	<i>Cosmarium</i> sp.	29.05	100	10.58			
	<i>Scenedesmus</i> sp.	51.15	100	18.63			
	<i>Navicula</i> sp.	20.00	83.33	6.07			
Autumn	<i>Chlorella</i> sp.	21.69	100	11.46	1.940	29	0.975
	<i>Microactinium</i> sp.	17.28	83.33	7.60			
	<i>Pandorina</i> sp.	14.01	83.33	6.16			
	<i>Cosmarium</i> sp.	12.85	83.33	5.65			
	<i>Scenedesmus</i> sp.	29.21	100	15.43			

622 **Table 5** Mean $\pm$ SE of water quality parameters in aqua dams with net substrates in summer, autumn, and winter

	Summer	Autumn	Winter	P value
Transparency (cm)	70.74 $\pm$ 1.68 ^a	58.51 $\pm$ 2.21 ^b	70.92 $\pm$ 2.05 ^a	<0.001
Temperature (°C)	30.07 $\pm$ 0.18 ^a	20.66 $\pm$ 0.42 ^b	15.64 $\pm$ 0.39 ^c	<0.001
DO (mg/l)	4.83 $\pm$ 0.16	4.39 $\pm$ 0.04	4.57 $\pm$ 0.05	0.102
EC (mS/cm)	287.55 $\pm$ 18.40 ^a	175.48 $\pm$ 10.57 ^b	144.95 $\pm$ 8.31 ^c	<0.001
Salinity (ppt)	0.13 $\pm$ 0.00 ^a	0.09 $\pm$ 0.00 ^b	0.09 $\pm$ 0.01 ^b	0.005
pH	6.80 $\pm$ 0.02 ^a	6.62 $\pm$ 0.02 ^b	6.47 $\pm$ 0.01 ^c	<0.001
Nitrogen as ammonia (mg/l)	1.02 $\pm$ 0.11 ^a	0.11 $\pm$ 0.12 ^b	0.05 $\pm$ 0.00 ^c	<0.001
Nitrate (mg/l)	0.02 $\pm$ 0.00 ^a	3.33 $\pm$ 0.15 ^b	3.21 $\pm$ 0.04 ^b	<0.001
Total phosphate (mg/l)	3.99 $\pm$ 0.24 ^a	1.47 $\pm$ 0.20 ^b	1.09 $\pm$ 0.07 ^b	<0.001

623 Abbreviation used : DO- dissolved oxygen; EC- electrical conductivity. Each mean represents three samples from three
624 replicate dams. Each mean represents samples from 3 replicates. Superscript letters indicate significant values which differed
625 from each other

626 **Table 6** The Chesson selective index of all the species identified in the stomach content of *O. mossambicus*.

	2 g	5 g	25 g
<i>Chlorella sp.</i>	0.128497	0.100731	0.010174
<i>Micractinium sp.</i>	-	0.019344	0
<i>Oocystis sp.</i>	0	0.026046	0
<i>Pandorina sp.</i>	0	0.01563	0.008106
<i>Glosterium sp.</i>	0.164692	0.006893	0.001639
<i>Cosmarium sp.</i>	0	0.001195	0.000253
<i>Chlorogonium sp.</i>	0.131188	0.001211	0
<i>Scenedesmus sp.</i>	0.159778	0.045427	0.016554
<i>Tetradon sp.</i>	0.338996	0.01006	0.005092
<i>Westella sp.</i>	-	-	0.000209
<i>Rhizoclonium sp.</i>	-	-	0.051247
<i>Fragilaria sp.</i>	-	-	0.069549
<i>Pinnularia sp.</i>	0.003626	0.122824	0.044471
<i>Navicula sp.</i>	0.054802	0.136565	0.081701
<i>Rhoicosphenia sp.</i>	-	0.052626	0
<i>Synedra sp.</i>	0.008143	0.171287	0.238995
<i>Trachelomonas sp.</i>	0	0.057348	0.087632
<i>Nitzschia sp.</i>	0.010279	0.138609	0.220536
<i>Mougeotia sp.</i>	0	0.000652	0
<i>Vaucheria sp.</i>	0	-	0.162281
<i>Chroococcus sp.</i>	0	0	-
<i>Microcystis sp.</i>	0	0.093552	0.001562

627

628 **Table 7** The significance differences of periphyton abundance tested using `adonis2` test with seasons, substrates
629 and size as factors.

	Df	Sum of Sq	R ²	F value	Prob (>F)
As factor (seasons)	2	1.4679	0.42209	5.4777	0.001
Residuals	15	2.0097	0.57791		
Total	17	3.4776	1.00000		
As factor (substrates)	2	0.90071	0.3163	3.4696	0.001
Residuals	15	1.94698	0.6837		
Total	17	2.84769	1.0000		
As factor (size)	1	0.37994	0.12415	1.0632	0.369
Residuals	16	2.68028	0.87585		
Total	17	3.06022	1.00000		
As factor (seasons, substrates, size)	2	6.4558	0.40753	17.54	0.001
Residuals	51	9.3855	0.59247		
Total	53	15.8413	1.00000		

630

631 Word count: 7964

APPENDIX C



APPENDIX D

- 1 Subject area: Fisheries
- 2 **Title:** The utilization of periphyton by *Clarias gariepinus* (African catfish) attached on net,
- 3 plastic, and stone substrates in aqua dams



4 ABSTRACT

5 This study investigated the utilization of periphyton by *Clarias gariepinus* (African catfish)
6 stocked in aqua dams with net (N), plastic (P), and stone (S) substrates. Three feeding
7 regimes were designed. In the first feeding regime, the fish were fed twice a day, every day
8 and designated as N100, P100, and S100. In the second regime, the fish were fed every
9 other day, designated as N50, P50, and S50. In the third feeding regime, the fish were fed
10 every third day, designated as N33, P33, and S33. The periphyton abundance was
11 significantly different across all substrates, with the net substrate registering the highest
12 abundance ($R=0.4108$, $P=0.0001$; ANOSIM). *Microcystis* sp. and *Chlorella* sp. were the
13 most abundant across all substrates. However, the fish selectively fed on insects and
14 Chironomidae larvae. Diatoms, *Diffugia* sp., and *Microcystis* sp. dominated the fecal
15 matter. High growth rates comparable to the control were observed in fish fed every third
16 day in aqua dams with stone (S33). The large lower jaw extensibility ($132\pm25.88\%$)
17 enabled the catfish to scoop periphyton from the stone substrates. Based on the
18 observed high growth rates and effective periphyton utilization, it is recommended that
19 African catfish be cultured in periphyton-based aquaculture with stone substrates.

20 Keywords: African catfish, Periphyton, Aqua Dams, Substrates, Utilisation.

21 **ARTICLE HIGHLIGHTS**

- 22 • African catfish are capable of effectively utilizing periphyton from stone
23 substrates.
- 24 • It is well known that African catfish are benthic feeders, thus they were able to
25 utilize periphyton from stones due to the large extensibility of the lower jaw.
- 26 • Several studies have shown how other species (tilapia, carp, freshwater prawns,
27 and mullets) utilized periphyton on different substrates in various production
28 systems. For instance, a study [40] indicated that tilapia can efficiently utilize
29 periphyton from net substrates. These findings suggest that African catfish and
30 tilapia could potentially be cultured together, as they each utilize periphyton from
31 distinct substrates.

1. INTRODUCTION

Clarias gariepinus (African catfish) is one of the most extensively cultured fish species in Africa [1, 2]. However, the increased aquaculture production of this species is impeded by the high cost of fish feed [3, 4]. Therefore, it is important to investigate alternative methods that may help reduce the cost of fish feed. In recent years, periphyton-based aquaculture of tilapia has been promoted as a significant success [5, 6, 7]. Periphyton is a complex community of bacteria, algae, zooplankton, phytoplankton, fungi, and both organic and inorganic detritus attached to submerged substrates [8, 9]. It grows on submerged substrates in water and is nutritionally adequate to support the growth and reproduction of most aquatic organisms due to its nutrients and biologically active compounds [10, 11, 12]. Periphyton not only serves as food but also improves water quality [13, 14, 15, 16]. Moreover, it enhances and maintains the health of cultured fish [9, 15, 17]. This is attributed to certain periphyton organisms within the community that can produce antimicrobial and bioactive compounds, which function as immunomodulators [18, 19, 20]. However, not all fish species can be cultured in periphyton-based aquaculture. To harvest periphyton efficiently, fish need a high degree of specialization in their feeding, filtering, and masticating apparatus [21].

The use of periphyton-based aquaculture as a source of natural food for fish has been widely explored in the production of tilapia [21] carps [57], freshwater prawns [58], and mullets [59, 60]. African catfish, like tilapia and carp species, are opportunistic omnivorous feeders that readily feed on different natural food. Yet it is not commonly used in periphyton-based aquaculture as tilapia and carp species. Most studies [4, 13, 21] have reported optimal growth rates, feed efficiency, body composition, immune response, and overall health status of tilapia in ponds with periphyton substrates. A study [61] reported a reduced commercial feed input by 30-40 % without affecting tilapia growth rate in ponds with periphyton substrates as compared to ponds without periphyton substrates. Another study [54] reported a net yield of 6700 kg ha⁻¹ year⁻¹ in a periphyton-based system compared to a net yield of 2340 kg ha⁻¹ year⁻¹ in the control system without periphyton substrates in polyculture systems. To date, the use of periphyton to reduce commercial feed input in African catfish farming remains less addressed in aquaculture compared to tilapia.

63 *Tilapia* species are commonly used in periphyton-based aquaculture due to their
64 ability to efficiently ingest and digest periphyton of various sizes [22, 17, 6]. This ability is
65 attributed to their morphological mouth features, which are effective for scraping,
66 combing, and sucking various natural food items [21]. Cyprinid species, such as *Labeo*
67 and *Garra*, also possess specialized mouth features that enable them to forage with their
68 bodies parallel to the substrate. These species have inferior transverse mouths with a
69 sharp cutting edge, characterized by thick papillose lips, a vomero-palatine organ,
70 prominent rostral features, and labial folds [21]. The feeding apparatus of the African
71 catfish is poorly documented. Studies [23, 24, 25, 26] have primarily reported on the
72 morphology of the cranium of the African catfish. This study will compare the feeding
73 apparatus of the African catfish to that of tilapias.

74 The substrate structure plays a crucial role in determining consumer–resource
75 interactions [27]. More complex substrates can hinder the movement of the fish, thereby
76 reducing their grazing rate on periphyton [28]. As substrate complexity increases, the
77 ability of fish to graze from the substrate decreases. Therefore, the mouth structure of the
78 cultured fish species requires a certain level of premaxilla protrusibility and lower jaw
79 extensibility to efficiently graze on periphyton. Previous studies on periphyton-based
80 aquaculture have focused on the impact of substrate structure on periphyton
81 colonization and abundance, demonstrating that higher substrate complexity leads to
82 increased periphyton biomass [29, 30, 31, 32]. This study aims to determine whether
83 African catfish can utilize periphyton from net, plastic, and stone substrates in aqua
84 dams.

85 In the wild, African catfish feed on a wide spectrum of food items, ranging from
86 phytoplankton to other fish species [33, 34]. Periphyton is a conglomeration of various
87 food items. The omnivorous feeding habits of African catfish make them a good
88 candidate for periphyton-based aquaculture. However, no studies have been undertaken
89 to evaluate the utilization of periphyton by the African catfish in aqua dams. It is
90 hypothesized that the type of substrate the periphyton is attached to affects the ability of
91 African catfish to utilize it as food. The main objective of this study was to investigate the
92 utilization of periphyton by African catfish from net, plastic, and stone substrates in aqua
93 dams. The specific objectives of the study were:

- 94 • To determine the effect of substrates on African catfish utilization of periphyton
95 in aqua dams with net, plastic, and stone substrates.
- 96 • To determine the compatibility of the African catfish's feeding structure to utilize
97 periphyton from net, plastic and stone substrates.

98 2. MATERIALS AND METHODS

99 2.1 Study site.

100 The study was conducted in experimental aqua dams (7000 L) at the Aquaculture
101 Research Unit of the University of Limpopo, South Africa (23.8888° S, 29.7386° E).
102 Experiments were carried out under real field conditions for six weeks. The study was
103 approved, and a certificate (no. AREC/05/2023: PG) was issued by the University of
104 Limpopo's Animal Ethics Committee before the commencement of the experiments.

105 2.2 The feeding protocols of African catfish in aqua dams with net, plastic, and 106 stone substrates

107 Three substrates (net, plastic, and stone) were deployed separately in 7 000 L aqua dams
108 using a completely randomized design. Each substrate was replicated three times,
109 resulting in a total of 27 aqua dams. African catfish (200.2 ± 22.74 g; mean $\pm$ SD) were
110 stocked in the aqua dams. Aqua dams are a static production system, and water lost
111 through evaporation is replenished every two weeks. To determine the extent to which
112 African catfish utilized periphyton, three feeding regimes were implemented. The first
113 feeding regime served as the control treatment, where the African catfish were fed
114 commercial pellets twice a day, every day. This represents the standard feeding regime
115 at fish farms. This feeding regime was designated as N100 for the net substrate, P100 for
116 the plastic substrate, and S100 for the stone substrate. In the second feeding regime, the
117 fish were fed commercial pellets every other day (twice a day), while periphyton was
118 available for the fish from the deployed substrates. This second feeding regime was
119 designated as N50 for the net substrate, P50 for the plastic substrate, and S50 for the
120 stone substrate. In the third feeding regime, the African catfish were fed commercial
121 pellets every third day, twice a day, with periphyton also available. This feeding regime
122 was designated as N33 for the net substrate, P33 for the plastic substrate, and S33 for
123 the stone substrate. Temperature and dissolved oxygen levels were monitored in all the
124 aqua dams to ensure that they did not become confounding variables. The temperature
125 was 28.41 ± 3.59 °C (mean $\pm$ SD) and dissolved oxygen was 9.76 ± 3.05 mg/l in all the
126 treatments.

127 **2.2.1** *Determination of periphyton abundance in the aqua dams with net,*
128 *plastic, and stone substrates.*

129 To determine the periphyton abundance on the net, plastic, and stone substrates in the
130 aqua dams, periphyton samples were collected by carefully scraping the substrate (9 cm²
131 surface area). The scraped periphyton was subsequently mixed with 20 ml of distilled
132 water to enable enumeration under a light microscope using an improved double
133 Neubauer chamber (W-Germany, 0.100 mm depth, 0.0025 mm²). The counting chamber
134 was sterilized with 70% ethanol before use. Additionally, a petri dish was employed to
135 enumerate larger periphyton species that exceeded the counting chamber's capacity. A
136 1 ml sample volume was examined under a light microscope in the petri dish to ensure
137 comprehensive species counting. Periphyton abundance was expressed as number per
138 ml, and the graphs were plotted using the logarithm of the number per ml and the
139 frequency of occurrence of genera.

140 **2.3 Growth performance indices, food selection indices, and feeding intensity of**
141 **African catfish in aqua dams with net, plastic, and stone substrates.**

142 African catfish were allowed to utilize periphyton for six weeks in aqua dams with net,
143 plastic, and stone substrates before the growth performance indices were determined.
144 The total weight (to the nearest 0.01 g) of all the African catfish in the aqua dams was
145 measured using a bench scale. The standard length of the fish was determined using a
146 tape measure to the nearest 0.5 mm. The weights and standard length of the fish were
147 used to calculate growth performance indices. Three African catfish from each treatment
148 group were then sacrificed and gutted to remove the stomachs. The stomach contents
149 were analysed under a light microscope to identify the periphyton items ingested by the
150 fish. The stomach fullness was ranked on a scale from 0 to 5, with 0 indicating an empty
151 stomach and 5 indicating a full stomach. The fecal matter was collected from the distal
152 intestine of the fish and analysed under a light microscope to identify which periphyton
153 items were not digested by the fish. Feeding intensity was determined by the percentage
154 of empty stomachs treatment. Ranging from 0 to 100 percent. The growth performance
155 indices calculated include:

156	Average Daily Weight Gain (ADWG)=Final Weight Gain (g)/Time	1
157	Percentage Weight Gain = [(Final Weight – Initial Weight)/Initial Weight] x 100	2

158	Specific Growth Rate (SGR) = (ln Final Weight – ln Initial Weight)/Time	3
159	Condition Factor = (Weight/Length ³) × 100	4
160	Survival Rate (%) = (Final Number of Fish/Initial Number of Fish) × 100	5

161 A modified percentage Index of Relative Importance (%IRI), adopted from [35],
 162 was used to determine the importance of food items in the stomachs of African catfish
 163 across all treatments:

$$164 \quad \%IRI = \%NO * \%FO \quad 6$$

165 where %NO is the percentage of the number of the food item, and %FO is the percentage
 166 frequency of occurrence of the food items.

167 Food selectivity was calculated using the following Chesson selectivity index
 168 (CSI), defined as:

$$169 \quad CSI = \frac{g_i/b_i}{\sum_{j=1}^n g_j/b_j}, i = 1, \dots, n, \quad 7$$

170 Where n is the total number of periphyton species in the stomach contents; g and b are
 171 the proportional representation of periphyton species i or j in the stomach contents and
 172 the water (environment), respectively. The CSI value for a specific periphyton species i
 173 reflects its presence in the stomach contents relative to the environment, divided by the
 174 sum of the equivalent calculations for all periphyton species in the stomach contents.
 175 The Chesson selectivity index value ranges from -1 to +1, with -1 indicating complete
 176 avoidance of the food item, 0 indicating a random selection of the food, and +1 indicating
 177 exclusive feeding on that species.

178 2.3.1 The feeding structures of African catfish and tilapia species.

179 A morphological analysis of the feeding structures of African catfish was conducted and
 180 compared to those of *Oreochromis mossambicus* (tilapia species). A total of 18 subadult
 181 individuals from each species were used. The width of the mouth, premaxilla
 182 protrusibility, and lower jaw extensibility were measured to the nearest 0.05 mm using
 183 the methods described by [36, 37]. The width of the mouth aperture was expressed as a
 184 percentage of the standard length. The number of gill rakers was also counted. The

185 protrusibility of the premaxilla is presented as the percentage difference in distance from
186 the anterior margin of the lower eye socket to the outer edge of the premaxilla, measured
187 from the tightly closed to the wide-open mouth [36]. The lower jaw extensibility is defined
188 as the distance from the anterior margin of the lower eye socket to the tip of the lower jaw
189 with the mouth closed. This distance is subtracted from the distance with the mouth
190 open and expressed as a percentage of the former [36].

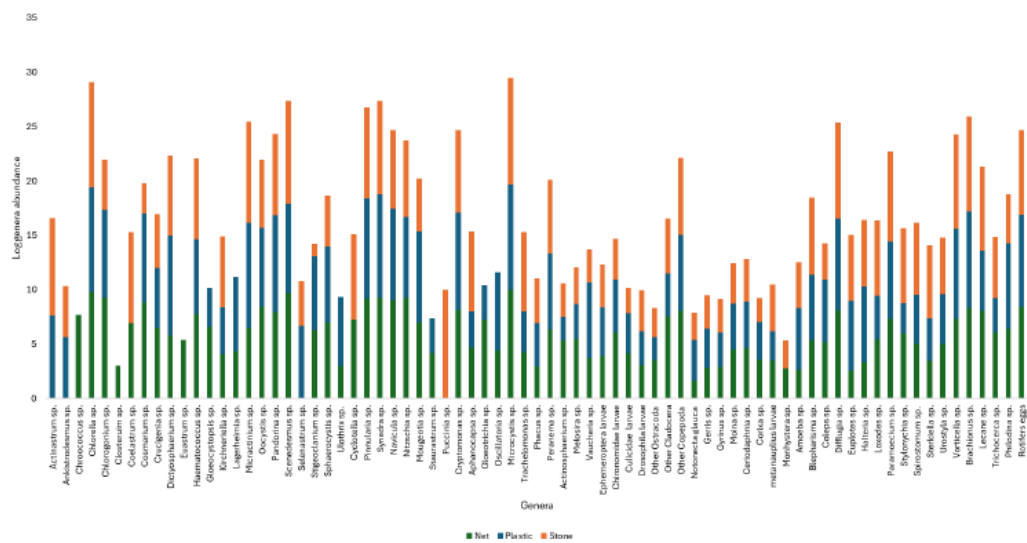
191 **2.4 Statistical analysis**

192 Microsoft Excel and Power BI were used to plot graphs. The normality and homogeneity
193 of growth performance indices were tested using the Shapiro-Wilk and Levene tests,
194 respectively (Statistical Package for Social Science [SPSS] version 28). Analysis of
195 similarities (ANOSIM), permutational multivariate analysis of variance (PERMANOVA),
196 and multivariate analysis of variance (MANOVA) were performed in R (version 2024.4.2-
197 764) using the vegan, MASS, effective, tidyverse, magrittr, and dplyr packages. ANOSIM
198 was used to determine whether there were similarities in periphyton abundance on net,
199 plastic, and stone substrates in aqua dams. PERMANOVA was employed to assess the
200 statistical difference between the food items identified in the stomachs of African catfish
201 utilizing periphyton. Additionally, ANOSIM was used to evaluate whether food items
202 identified in the stomach were similar within each treatment or between the treatments.
203 ANOSIM and PERMANOVA were used for data that was not normally distributed.
204 Normality and homogeneity of variance data were tested using the Shapiro-Wilk and
205 Levene tests, respectively. Analysis of Variance (ANOVA) was used to test for significant
206 differences in the growth performance of African catfish. Tukey's post hoc analysis was
207 used to determine which means were significantly different from each other (SPSS
208 version 28). A MANOVA was conducted to assess the significant differences in the growth
209 performance indices among the substrates (net, plastic, and stone) using a permutation
210 test.

211 3. RESULTS

212 3.1 Species abundance of periphyton in aqua dams with net, plastic, and stone 213 substrates.

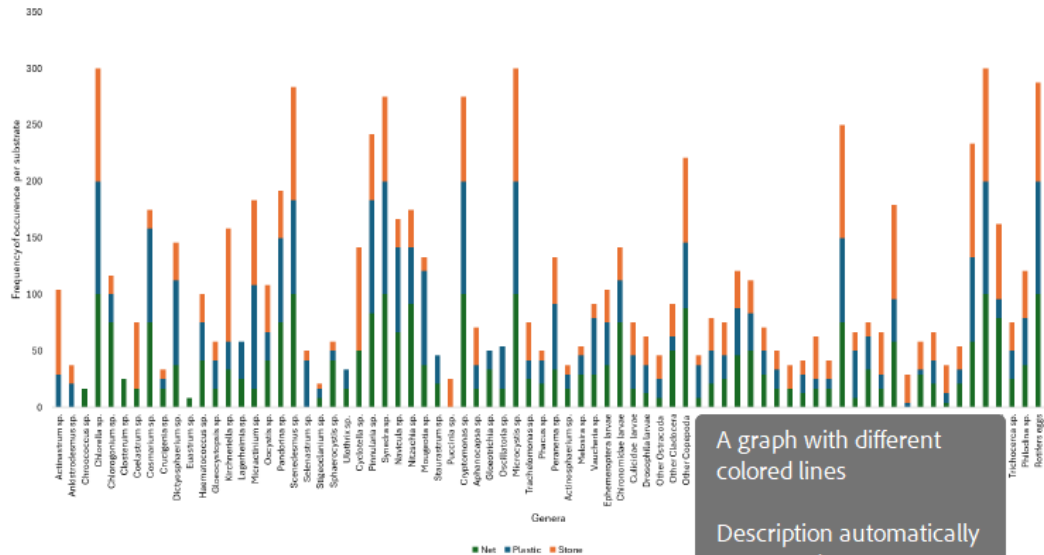
214 A total of 71 periphyton genera were identified on the substrates in aqua dams stocked
215 with African catfish (Figure 1). *Microcystis* sp. and *Chlorella* sp. were numerically
216 dominant across all substrates. The stone substrates exhibited the lowest species
217 abundance (Figure 1). The periphyton species abundance on the net, plastic, and stone
218 substrates was significantly different ($R = 0.4108$, $P = 0.0001$; ANOSIM). The frequency of
219 occurrence of periphyton genera on all substrates mirrored the abundance (Figure 2).



220

221 **Figure 1:** Natural logarithm of the species list of periphyton in aqua dams with net, plastic, and stone substrates.

12



222

223 **Figure 2:** Frequency of occurrence of the species list of periphyton in aqua dams with net, plastic, and stone substrates.

13

224 **3.2 The utilization of periphyton by African catfish in aqua dams with net,**
225 **plastic, and stone substrates**
226 Chironomidae larvae, unidentified insects, and rotifers dominated the stomach contents
227 of African catfish in all aqua dams with net substrates in both N50 and N33 treatments
228 (Figure 3). The fecal analysis of African catfish with net substrates showed that *Diffugia*
229 sp., *Pinnularia* sp., *Synedra* sp., and *Cyclotella* sp. were also the most dominant. A few
230 Chironomidae larvae and rotifer eggs were also identified in the fecal matter of the fish
231 (Figure 3).

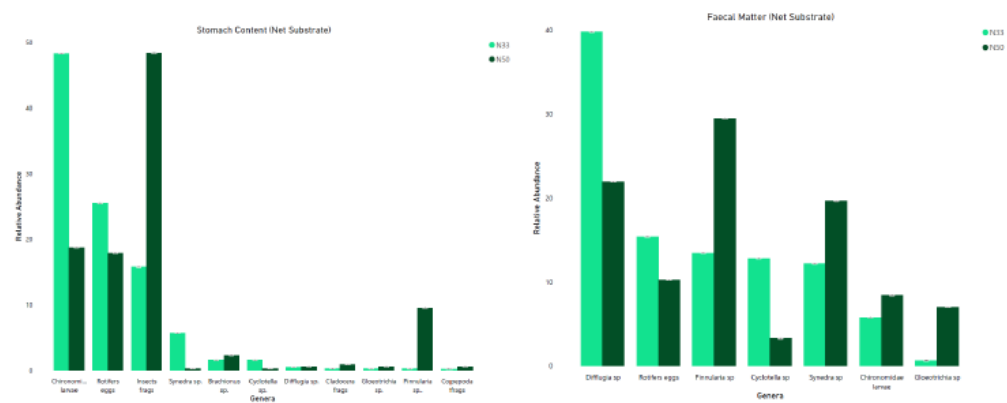
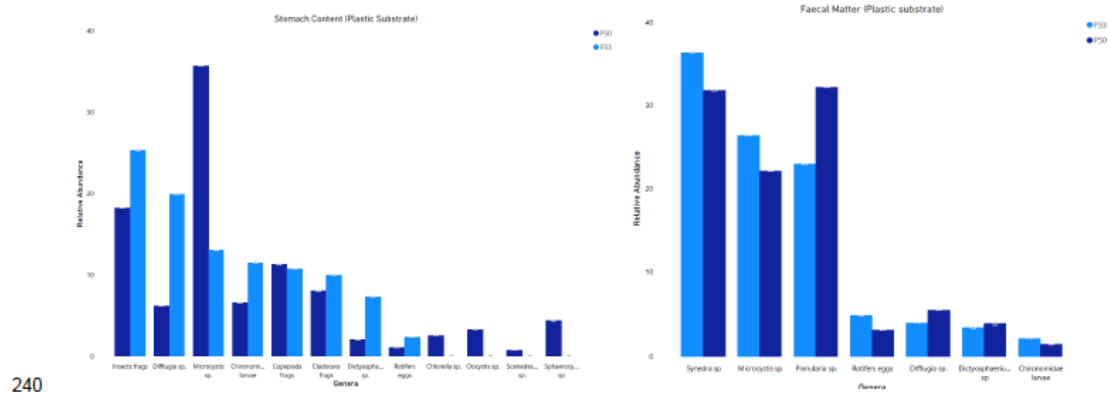


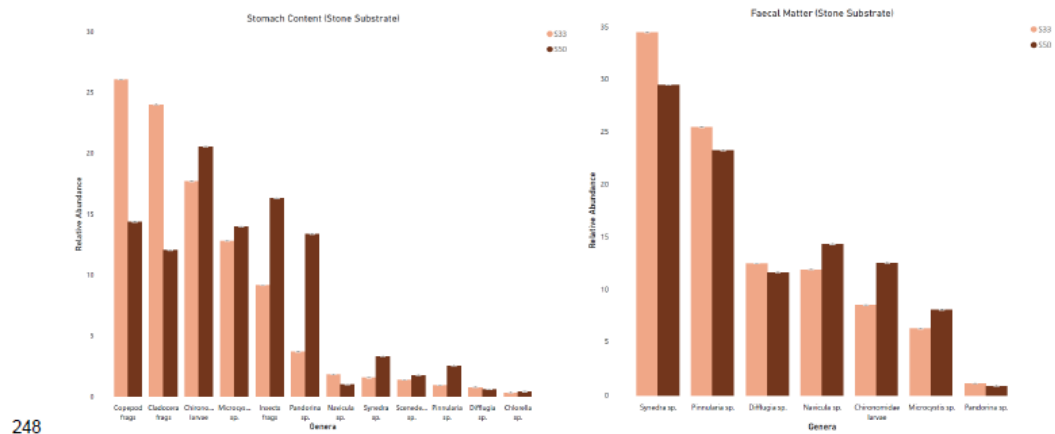
Figure 3: Periphyton genera found in the stomach contents and fecal matter of African catfish in aqua dams with net substrates.

235 The stomach contents of African catfish in aqua dams with plastic were
236 dominated by *Microcystis* sp., unidentified insects, *Diffugia* sp., Chironomidae larvae,
237 unidentified copepod and cladocerans in both the P50 and P33 treatments (Figure 4). The
238 fecal matter of the fish in aqua dams with plastic substrates was dominated by *Synedra*
239 sp., *Pinnularia* sp., and *Microcystis* sp. in both the P50 and P33 treatments (Figure 4).



241 **Figure 4:** Periphyton genera found in the stomach contents and faecal matter of African catfish in aqua dams with plastic substrates.

242 Unidentified copepods and cladocerans, Chironomidae larvae, *Microcystis* sp.,
243 unidentified insects, and *Pandorina* sp. were dominant in the stomach content of the fish
244 in aqua dams with the stone substrate (Figure 5). *Synedra* sp., *Pinnularia* sp., *Diffugia* sp.,
245 and *Navicula* sp. were the most dominant in the fecal matter compared to the stomach
246 content (Figure 5). Overall, the most important food items were unidentified insects and
247 Chironomidae larvae (Table 1).



248 **Figure 5:** Periphyton genera found in the stomach contents and faecal matter of African catfish in aqua dams with stone substrates.

Unidentified insects and Chironomidae larvae were the most important food items for African catfish in aqua dams with net substrates (Table 1).

Table 1: A modified Index of Relative Importance (%IRI) of food items identified in the stomachs of African catfish in aqua dams with net, plastic, and stone substrates.

	Net		Plastic		Stone	
	N50	N33	P50	P33	S50	S33
<i>Chlorella sp.</i>	-	-	2.41	0.00	0.04	0.03
<i>Oocystis sp.</i>	-	-	3.49	0	-	-
<i>Dictyosphaerium sp.</i>	-	-	1.89	7.57	-	-
<i>Sphaerocystis sp.</i>	-	-	4.65	0	-	-
<i>Pandorina sp.</i>	-	-	-	-	13.70	3.77
<i>Scenedesmus sp.</i>	-	-	0.17	0	0.99	0.63
<i>Cyclotella sp.</i>	0.03	0.18	-	-	-	-
<i>Pinnularia sp.</i>	9.80	0.28	-	-	2.58	0.31
<i>Synedra sp.</i>	0.03	2.71	-	-	3.38	0.87
<i>Navicula sp.</i>	-	-	-	-	0.22	1.67
<i>Gloeotrichia sp.</i>	0.07	0.03	-	-	-	-
<i>Microcystis sp.</i>	-	-	37.95	13.55	14.30	13.18
Chironomidae larvae	19.31	51.52	3.49	9.56	21.05	18.21
Cladocera frags	0.20	0.03	8.52	10.36	12.31	24.80
Copepod frags	0.07	0.03	12.01	11.16	14.69	26.84
Unidentified insects frags	49.90	16.90	18.59	26.29	16.68	9.42
<i>Brachionus sp.</i>	2.11	1.11	-	-	-	-
<i>Diffugia sp.</i>	0.07	0.06	6.58	20.72	0.07	0.26
Rotifers eggs	18.42	27.15	0.26	0.80	-	-

The Chesson selectivity index indicated that African catfish in aqua dams with net, plastic, and stone substrates selectively fed on insects unidentified, Chironomidae larvae, and Cladocera, while other periphyton genera were consumed randomly (Table 2). The food items found in the stomachs of all African catfish across different substrate types were not statistically significant ($F = 0.256$, $Pr = 0.78$; PERMANOVA). The food items were evenly distributed within and between the stomachs of African catfish across all substrates ($R = 0.0845$, $P = 0.1543$; ANOSIM). Feeding intensity was 100% in African catfish in aqua dams with net substrates for both treatments (Table 2).

Table 2: Chesson selectivity index of periphyton items in the stomachs of African catfish from aqua dams with net, plastic, and stone substrates.

	Net		Plastic		Stone	
	N50	N33	P50	P33	S50	S33
Stomach fullness	0.5 ± 0.0	0.5 ± 0.0	0.3±0.5	0.7±1.1	0.7±1.1	1.4±2.2
<i>Chlorella</i> sp.	-	-	0.0002	0.00000	0.00001	0.00001
<i>Oocystis</i> sp.	-	-	0.0035	0.00000	-	-
<i>Dictyosphaerium</i> sp.	-	-	0.00038	0.00029	-	-
<i>Sphaerocystis</i> sp.	-	-	0.00365	-	-	-
<i>Pandorina</i> sp.	-	-	-	-	0.00223	0.00553
<i>Scenedesmus</i> sp.	-	-	0.00034	0.00000	0.00007	0.00007
<i>Cyclotella</i> sp.	0.00009	0.00204	-	-	-	-
<i>Pinnularia</i> sp.	0.00037	0.00001	-	-	0.00031	0.00013
<i>Synedra</i> sp.	0.00001	0.00086	-	-	0.00044	0.00016
<i>Navicula</i> sp.	-	-	-	-	0.00039	0.00079
<i>Gloeotrichia</i> sp.	0.00002	0.00044	-	-	-	-
<i>Microcystis</i> sp.			0.00368	0.000383	0.00041	0.00043
Chironomidae larvae	0.01802	0.21852	0.03198	0.04788	0.26462	0.25092
Cladocera frags	0.00011	0.00241	0.14041	0.74348	0.02558	0.23120
Copepod frags	0.00007	0.00016	0.02292	0.00364	0.00689	0.01317
Unidentified insects frags	0.97853	0.76641	0.79004	0.20221	0.69901	0.49751
<i>Brachionus</i> sp.	0.000262	0.00064	-	-	-	-
<i>Diffugia</i> sp.	0.000086	0.00028	0.00257	0.00156	0.00004	0.00009
Rotifers eggs	0.002245	0.00816	0.00024	0.00055	-	-
Feeding intensity (%)	100	100	33	33	33	67

The fish in the control treatment (N100) exhibited higher SGR and condition factors compared to those in treatment N50 and N33 (Table 3). However, there was no significant difference ($P > 0.05$) in the growth performance indices of African catfish in aqua dams with net substrate, except for percentage weight gain and ADWG (Table 3).

Table 3: The growth performance indices (mean $\pm$ SE) of African catfish in aqua dams with net substrates.

	Treatments			P value
	N100	N50	N33	
Initial mean weight	191.33 $\pm$ 4.37	188.66 $\pm$ 10.72	220.66 $\pm$ 15.50	0.160
Final mean weight	490.33 $\pm$ 22.73	415.66 $\pm$ 48.73	407.00 $\pm$ 22.64	0.237
ADWG	0.60 $\pm$ 0.02 ^a	0.53 $\pm$ 0.04 ^{ab}	0.45 $\pm$ 0.00 ^b	0.042
Percentage weight gain (%)	157.05 $\pm$ 17.27 ^a	119.71 $\pm$ 19.79 ^{ab}	84.84 $\pm$ 2.91 ^b	0.042
SGR	2.29 $\pm$ 0.17	1.89 $\pm$ 0.23	1.61 $\pm$ 0.04	0.075
Condition factor	1.65 $\pm$ 0.14	1.32 $\pm$ 0.20	1.03 $\pm$ 0.31	0.236
Survival rate (%)	93.33 $\pm$ 6.66	100 $\pm$ 0.0	100 $\pm$ 0.0	0.422

Abbreviation used: ADWG= Average Daily Weight Gain, SGR = Specific Growth Rate. Superscript letters indicate significant values that differed from each other.

The SGR and condition factor of the fish were higher in the control (P100) treatment, followed by the P33 treatment (Table 4). However, only the mean final weight and condition factor were significantly different across all treatments in aqua dams with plastic substrate ($P < 0.05$).

Table 4: The growth performance indices (mean $\pm$ SE) of African catfish in aqua dams with plastic substrates.

	Treatment			P value
	P100	P50	P33	
Initial mean weight	325.33 $\pm$ 20.07	304.66 $\pm$ 19.74	317.33 $\pm$ 27.96	0.819
Final mean weight	666.66 $\pm$ 42.17 ^a	500.33 $\pm$ 5.84 ^b	627.50 $\pm$ 18.09 ^a	0.011
ADWG	0.50 $\pm$ 0.04	0.39 $\pm$ 0.04	0.49 $\pm$ 0.05	0.255
Percentage weight gain (%)	106.35 $\pm$ 17.14	65.63 $\pm$ 11.03	102.24 $\pm$ 25.53	0.315
SGR	1.88 $\pm$ 0.22	1.31 $\pm$ 0.18	1.81 $\pm$ 0.31	0.281
Condition factor	1.71 $\pm$ 0.06 ^a	1.37 $\pm$ 0.04 ^b	1.61 $\pm$ 0.02 ^a	0.007
Survival rate (%)	100 $\pm$ 0.0	100 $\pm$ 0.0	93.33 $\pm$ 6.66	0.422

Abbreviation used: ADWG= Average Daily Weight Gain, SGR = Specific Growth Rate. Superscript letters indicate significant values that differed from each other.

African catfish in the S33 treatment had a higher SGR (Table 5). The condition factor was higher in the control treatment (S100). However, there was no significant difference ($P > 0.05$) in the growth performance indices of African catfish in aqua dams with stone substrates.

284 **Table 5:** The growth performance indices (mean $\pm$ SE) of African catfish in aqua dams
285 with stone substrates.

	Treatment			P value
	S100	S50	S33	
Initial mean weight	285.33 $\pm$ 35.59	301.33 $\pm$ 39.70	239.33 $\pm$ 51.34	0.595
Final mean weight	510.00 $\pm$ 30.74	505.00 $\pm$ 36.82	439.50 $\pm$ 39.11	0.361
ADWG	0.43 $\pm$ 0.06	0.43 $\pm$ 0.05	0.45 $\pm$ 0.10	0.910
Percentage weight gain (%)	84.37 $\pm$ 25.09	71.48 $\pm$ 16.11	96.82 $\pm$ 36.83	0.813
SGR	1.69 $\pm$ 0.37	1.51 $\pm$ 0.26	1.83 $\pm$ 0.54	0.865
Condition factor	1.42 $\pm$ 0.11	1.31 $\pm$ 0.08	1.30 $\pm$ 0.08	0.482
Survival rate (%)	93.33 $\pm$ 6.66	93.33 $\pm$ 6.66	93.33 $\pm$ 6.66	1.000

286 Abbreviation used: ADWG= Average daily Weight Gain, SGR = Specific Growth Rate. Superscript letters
287 indicate significant values that differed from each other.

288 The growth performance indices of African catfish in the aqua dams with net,
289 plastic, and stone substrates were significantly different ($P = 4.827 \times 10^{-8}$, permutation
290 test). The partial Eta Squared value indicated that the effect size was 0.76 at a 95%
291 confidence interval [0.60, 1.00]. This shows that each substrate influenced the growth
292 performance of African catfish differently (Figure 6). Linear Discriminant Analysis (LDA)
293 was used as a post hoc test, showing a proportion of trace values of 0.8551 for LD1 and
294 0.1449 for LD2, respectively (Figure 6).

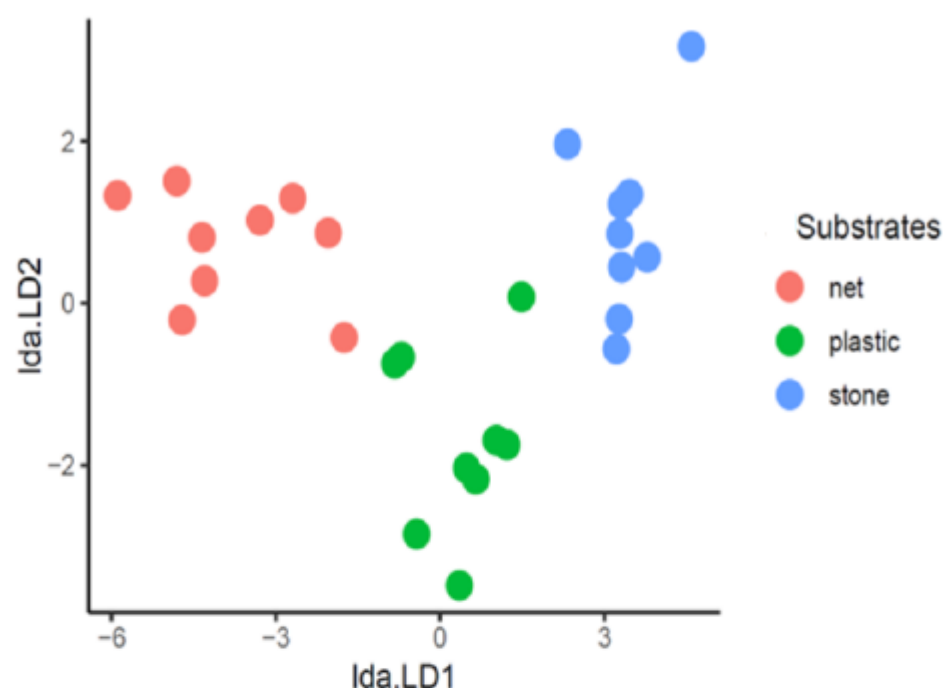


Figure 6: Scatter plot of Linear Discriminant Analysis (LDA) of growth performance indices of African catfish in aqua dams with net, plastic, and stone substrates.

The mouth aperture of African catfish was wide, while that of the tilapia was moderate (Table 6). The mouth height relative to the standard length of the fish was greater in tilapia than in African catfish. The mouth width and depth in relation to the standard length were relatively similar in both species (Table 6). The protrusibility of the premaxilla was much smaller in African catfish compared to tilapia. However, the lower jaw extensibility was much larger in African catfish compared to tilapia (Table 6). The pharyngeal bone was tooth-like featuring rough and sharp teeth, and it was divided into four pads. In contrast, tilapia had three pads a toothbrush-like structure. The gill rakers of tilapia were short and widely spaced, while those of the African catfish were long and narrowly spaced.

308 **Table 6:** Comparison of mouth apparatuses of African catfish and tilapia.

	African catfish	Tilapia
Mouth Aperture	Wide	Moderate
Mouth depth from side	1.04 ± 0.34	1.40 ± 0.22
Mouth height when open	4.5 ± 0.82	2.73 ± 0.36
Mouth width when open	3.36 ± 0.56	1.40 ± 0.24
Mouth depth % of SL	9.75 ± 0.40	8.29 ± 1.14
Mouth height % of SL	13.09 ± 1.94	16.08 ± 0.96
Mouth width % of SL	9.76 ± 0.90	8.25 ± 0.94
Premaxilla protrusibility (%)	5 ± 0.00	52.50 ± 22.17
Lower jaw extensibility (%)	132 ± 25.88	21.25 ± 4.79
Number of gill rakers	72.4 ± 15.32	20.50 ± 1.29

309 Abbreviation used: SL- Standard Length. The criteria for grouping mouth apertures are adopted from [36].

4. DISCUSSION

Microcystis sp. and *Chlorella* sp. were the most dominant on the substrates in the aqua dams. This dominance was likely due to the fact that green algae and cyanobacteria are the primary taxa in various water bodies, owing to their ability to thrive in diverse aquatic environments and their resilience as species [38, 56]. The abundance of periphyton on the net, plastic, and stone substrates was significantly different from one another due to the varying textures of the substrates and their locations at different water levels in the aqua dams. The stones were completely submerged in water, while the net and plastic were positioned closer to the water's surface. The net and plastic substrates were located in the euphotic zone, which had the highest periphyton abundance due to the sufficient light for photosynthesis. In contrast, the stone substrates were positioned in the disphotic zone of the aqua dam, where light was limited due to low transparency. Increased turbidity reduces light penetration, thereby decreasing photosynthesis and primary production. As a result, periphyton on stones rarely grew due to limited light availability, and lower abundance compared to periphyton growing on the net and plastic substrates. Additionally, the texture of the substrates may have contributed to the differences in periphyton abundance. The texture of the net substrates was rough, while the plastic was smooth. As a result, periphyton easily adheres to the rough texture of the net substrate, leading to a higher abundance of periphyton on the net. Similar results have been reported in several studies, which indicated that the net substrates support better periphyton growth in ponds and aqua dams [13, 34, 39,40]. Other studies have also shown that different substrates promote varying levels of periphyton abundance [5, 6, 41, 42].

Unidentified insects and Chironomidae were the most important food items in the diet of African catfish across different substrates. While most studies have indicated that African catfish feed on a variety of food items [43, 44, 45, 46, 47], this study found that they selectively fed on unidentified insects and Chironomidae. The African catfish is an efficient benthic feeder; thus, it is able to selectively graze on unidentified insects and Chironomidae attached to various substrates. However, the euryphagous feeding nature of this fish was also evident in this study, as it was able to feed on planktonic organisms ranging from rotifers to periphytic algae, such as *Microcystis* sp. Despite the high

341 abundance of diatoms on all substrates, they were seldom digested. This might suggest
342 that African catfish lack gastric juices to digest diatoms. Diatoms possess a silica cell
343 wall that is not easily digestible. Only a few species, such as abalone, are capable of
344 ingesting and digesting diatoms [48].

345 The high abundance of *Microcystis* sp. in the fecal matter suggests that some of
346 the *Microcystis* were not digested by African catfish. Most blue-green algae, including
347 *Microcystis* sp., have thick cell walls that are difficult to break down [49, 50]. Recently,
348 [51] compared the algal communities in the stomachs of African catfish and tilapia,
349 concluding that tilapia consumed more *Microcystis* sp. than African catfish. However,
350 this conclusion was based solely on stomach analysis and may not accurately reflect the
351 extent to which African catfish can digest *Microcystis* sp. It is also important to note that
352 in aquaculture, *Microcystis* sp. can be a nuisance, as some species produce toxins
353 [52]. *Diffugia* sp. was also abundant in the stomach and fecal matter of African catfish.
354 These protozoa are related to *Amoeba* sp. and have shells that are difficult to digest.

355 Fish stocked in aqua dams with net (N100) and plastic (P100) substrates recorded
356 the highest growth rate, along with fish stocked in aqua dams with stone substrate (S33).
357 Both N100 and P100 served as the control, as African catfish are typically fed twice a day.
358 This indicates that catfish were able to efficiently utilize periphyton from the stone
359 substrate, as their growth rate was comparable to that of the control treatment. This
360 suggests that the substrate affects the utilization of periphyton by African catfish. African
361 catfish are benthic feeders and can easily utilize periphyton growing on the stone
362 substrates in aqua dams. Even though the stone substrate recorded the least periphyton
363 abundance, African catfish used their lower jaw to scoop up periphyton to support their
364 high growth. This probably suggests that the type of substrate has a more significant
365 impact on the growth of cultured fish than the amount of periphyton present on those
366 substrates. Several studies have reported that the nature of the substrate influences both
367 the quality and quantity of the periphyton [9, 21, 53]. The substrates used in this study are
368 all non-biodegradable. Most studies on periphyton-based aquaculture have used
369 bamboo substrate, which has been reported to support high-quality and quantity
370 periphyton [53, 54, 55]. However, in this study, bamboo substrate was not used due to its

371 limited local availability. Therefore, a comparative analysis of the quality and quantity of
372 periphyton on stone and bamboo substrates is recommended.

373 A fish's ability to digest food is also affected by its mouth structure. African catfish
374 have a wide mouth, which allows them to ingest prey of different sizes. However, their
375 premaxilla protrusibility is low in comparison to that of tilapia. Tilapias can protrude their
376 mouths to scrap periphyton from various substrates. African catfish cannot scrap
377 periphyton from substrates. However, they have a highly extensible lower jaw that allows
378 them to scoop the periphyton from the substrate. The pharyngeal teeth also play an
379 important role in the fish's ability to utilize specific food items. The pharyngeal teeth of
380 African catfish are tooth-like, in contrast to those of tilapia which are more toothbrush-
381 like. The African catfish likely use their pharyngeal teeth to triturate benthic periphyton,
382 such as insects and Chironomidae.

383

384 5. CONCLUSION

385 African catfish can utilize periphyton in aqua dams. They can efficiently scoop periphyton
386 from stone substrates due to their larger jaw extensibility. Unidentified insects and
387 Chironomidae are important food sources even though catfish selectively feed on
388 insects. However, diatoms, *Microcystis* sp., and *Diffugia* sp. dominated the fecal matter,
389 indicating that African catfish lack the gastric juices necessary to break them down.
390 Although the net substrate registered the highest abundance, the highest growth rate
391 was recorded in fish fed every third day with a stone substrate (S33). This suggests that
392 substrate type has a more significant impact on the utilization of periphyton by the fish.
393 This study recommends the use of periphyton-based aquaculture to culture African
394 catfish using stones as substrates in aqua dams or similar impoundments, such as
395 ponds. Further studies are recommended to comparatively assess the efficacy of
396 scraping and scooping feeding approaches in facilitating periphyton utilization in
397 periphyton-based aquaculture.

398 **6. STATEMENTS AND DECLARATIONS**

399 **Competing interest statement**

400 The authors declare that they have no relevant financial or non-financial interests to
401 disclose.

402 **Funding**

403 The research leading to these results received funding from the National Research
404 Foundation under Grant number 141330.

405 **Data availability statement**

406 Data are available from the corresponding author upon request, following the submission
407 of the PhD thesis for review.

408 **Institutional Review Board Statement/ Compliance with Ethical Standards**

409 The study was approved and by the University of Limpopo's Animal Ethical Committee,
410 and a certificate (no. AREC/05/2023: PG) was issued prior to the commencement of
411 experiments. Humane measures were implemented during the handling and sacrifice of
412 the fish in accordance with the guidelines set forth by the University of Limpopo's Animal
413 Ethical Committee.

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