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The utilization of periphyton by *Clarias gariepinus* (African catfish) attached on net, plastic, and stone substrates in aqua dams

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ABSTRACT

This study investigated the utilization of periphyton by *Clarias gariepinus* stocked in aqua dams with net (N), plastic (P), and stone (S) substrates. Three feeding regimes were designed. In the first feeding regime, the fish were fed twice a day, every day and designated as N100, P100, and S100. In the second regime, the fish were fed every other day, designated as N50, P50, and S50. In the third feeding regime, the fish were fed every third day, designated as N33, P33, and S33. The periphyton abundance was significantly different across all substrates, with the net substrate registering the highest abundance (R = 0.4108, $P = 0.0001$; ANOSIM). *Microcystis* spp. and *Chlorella* spp. were the most abundant and dominant across all substrates. However, the fish selectively fed on insects and *Chironomidae* larvae. Diatoms, *Difflugia* spp., and *Microcystis* spp. dominated the fecal matter. High growth rates comparable to the control were observed in fish fed every third day in aqua dams with stone (S33). The large lower jaw extensibility (132 ± 25.88%) enabled the catfish to scoop periphyton from the stone substrates. Based on the observed high growth rates and effective periphyton utilization, it is recommended that African catfish be cultured in periphyton-based aquaculture with stone 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 essential 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 [5-7]. Periphyton is a complex community of bacteria, algae, zooplankton, phytoplankton, fungi, and 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 [10-12]. Periphyton not only serves as food but also improves water quality [13-16]. 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 [18-20]. 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 [22], freshwater prawns [23], and mullets [24,25]. 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 [26] reported a net yield of 6700 kg/ha/year in a periphyton-based system compared to a net yield of 2340 kg/ha/year in the control system without periphyton substrates in polyculture systems. A study [27] 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 [28] reported a 42% reduction in commercial feed input in ponds with periphyton substrates covering 75% of the pond surface area. 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 [9,29]. Thus, it has been successfully used as a cost-effective method to reduce commercial feed inputs in aquaculture. However, to date, the use of periphyton to reduce commercial feed input in African catfish farming remains less addressed in aquaculture compared to tilapia.

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Tilapia species are commonly used in periphyton-based aquaculture due to their ability to efficiently ingest and digest periphyton of various sizes [6,17,30]. This ability is attributed to their morphological mouth features, which are effective for scraping, combing, and sucking various natural food items [21]. Cyprinid species, such as *Labeo* and *Garra*, also possess specialized mouth features that enable them to forage with their bodies parallel to the substrate. These species have inferior transverse mouths with a sharp cutting edge, characterized by thick papillose lips, a vomero-palatine organ, prominent rostral features, and labial folds [21]. The feeding apparatus of the African catfish is poorly documented. Studies [31-34] have primarily reported on the morphology of the cranium of the African catfish. This study will compare the feeding apparatus of the African catfish to that of tilapias.

The substrate structure plays a crucial role in determining consumer–resource interactions [35]. More complex substrates can hinder the movement of the fish, thereby reducing their grazing rate on periphyton [36]. As substrate complexity increases, the ability of fish to graze from the substrate decreases. Therefore, the mouth structure of the cultured fish species requires 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 colonization and abundance, demonstrating that higher substrate complexity leads to increased periphyton biomass [37-40]. This study aims to determine whether African catfish can utilize periphyton from net, plastic, and stone substrates in aqua dams.

In the wild, African catfish feed on a wide spectrum of food items, ranging from phytoplankton to other fish species [28,41]. Periphyton is a conglomeration of various food items. The omnivorous feeding habits of African catfish make them a good candidate for periphyton-based aquaculture. However, no studies have been undertaken to evaluate the utilization of periphyton by the African catfish in aqua dams. It is hypothesized that the type of substrate the periphyton is attached to affects the ability of African catfish to utilize it as food. The main objective of this study was to investigate the utilization of periphyton by African catfish from net, plastic, and stone substrates in aqua dams. The specific objectives of the study were:

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

2. MATERIALS AND METHODS

2.1. Study Site

The study was conducted in experimental aqua dams (7000 L) at the Aquaculture Research Unit of the University of Limpopo, South Africa (23.8888° S, 29.7386° E). Experiments were carried out under real field conditions for 6 weeks. The study was approved, and a certificate (no. AREC/05/2023: PG) was issued by the University of Limpopo's Animal Ethics Committee before the commencement of the experiments.

2.2. The Feeding Protocols of 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 using a completely randomized design. Each substrate was replicated 3 times, resulting in a total of 27 aqua dams. African catfish (200.2 ± 22.74 g; mean ± SD) were stocked in the aqua dams. Aqua dams are a static production system, and water lost through evaporation is replenished every 2 weeks. To determine the extent to which African catfish utilized periphyton, three feeding regimes were implemented. The first feeding regime served as the control treatment, where the African catfish were fed commercial pellets twice a day, every day. The fish were fed only commercial pellets daily, without any periphyton substrates in the aqua dams. This represents the standard feeding regime at fish farms, and it is equivalent to a 100% feeding regime. This feeding regime was designated as 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), while periphyton was available for the fish from the deployed substrates. This was equivalent to a 50% feeding regime. This second feeding regime was designated as N50 for the net substrate, P50 for the plastic substrate, and S50 for the stone substrate. In the third feeding regime, the African catfish were fed commercial pellets every 3rd day, twice a day, with periphyton also available in the aqua dams. This was equivalent to a 33% feeding regime. This feeding regime was designated as N33 for the net substrate, P33 for the plastic substrate, and S33 for the stone substrate. The amount of commercial pellets fed to the fish in all aqua dams was recorded consistently. Temperature and dissolved oxygen levels 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 ± SD) and dissolved oxygen was 9.76 ± 3.05 mg/L in all the treatments.

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

To determine the periphyton abundance and taxonomic composition on the net, plastic, and stone substrates in the aqua dams, periphyton samples were collected by carefully scraping the substrate (9 cm^2 surface area). 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 scraping an equal surface area (9 × 9 cm^2) on all substrates. The scraped periphyton was subsequently mixed with 20 mL of distilled water to enable enumeration under a light microscope using an improved double Neubauer chamber (W-Germany, 0.100 mm depth, 0.0025 mm^2). 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 and periphyton abundance was expressed as number per ml, and the graphs were plotted using the logarithm of the number per ml and the frequency of occurrence of genera.

The 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. The 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.

$$Frequence\,of\,occurence\ (\%) = \frac{The\,number\,of\,ml\,in\,which\,spp\,is\,found}{Total\,number\,of\,ml\,examined} \times 100 \quad (1)$$

$$Dominance\,index = \frac{The\,abundace\,of\,periphyton\,taxon}{Total\,abundance\,of\,all\,periphyton} \times frequency\,of\,occerence\,of\,taxon \quad (2)$$

2.3. Growth Performance Indices, Food Selection Indices, and Feeding Intensity of African Catfish in Aqua Dams with Net, Plastic, and Stone Substrates

African catfish were allowed to utilize periphyton for 6 weeks in aqua dams with net, plastic, and stone substrates 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 measured using a bench scale. The standard length of the fish was determined using a tape measure to the nearest 0.5 mm. The weights and standard length of the fish were used to calculate growth performance indices. Three African catfish from each treatment group were then sacrificed and gutted to remove the stomachs. The stomachs were cut open to remove all contents into a petri dish and the periphyton found the stomach contents were identified to genus under a light microscope (Zeiss, Axiolab, Germany). The stomach contents were analyzed under a light microscope to identify the periphyton items ingested by the fish. The stomach fullness was ranked on a scale from 0 to 5, with 0 indicating an empty stomach and 5 indicating a full stomach. Additionally, the distal intestine was gutted from the sacrificed African catfish (mentioned above) and cut open to remove all contents into a petri dish. The periphyton found in the fecal matter was identified to genus level under a light microscope. The fecal matter was collected from the distal intestine of the fish and analyzed under a light microscope to identify which periphyton 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)

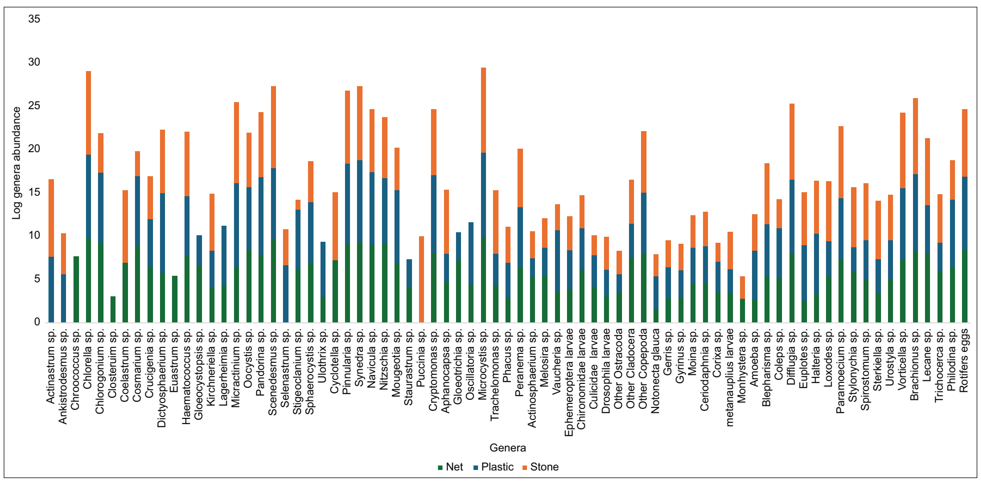

Figure 1: Natural logarithm of the species list of periphyton in aqua dams with net, plastic, and stone substrates.

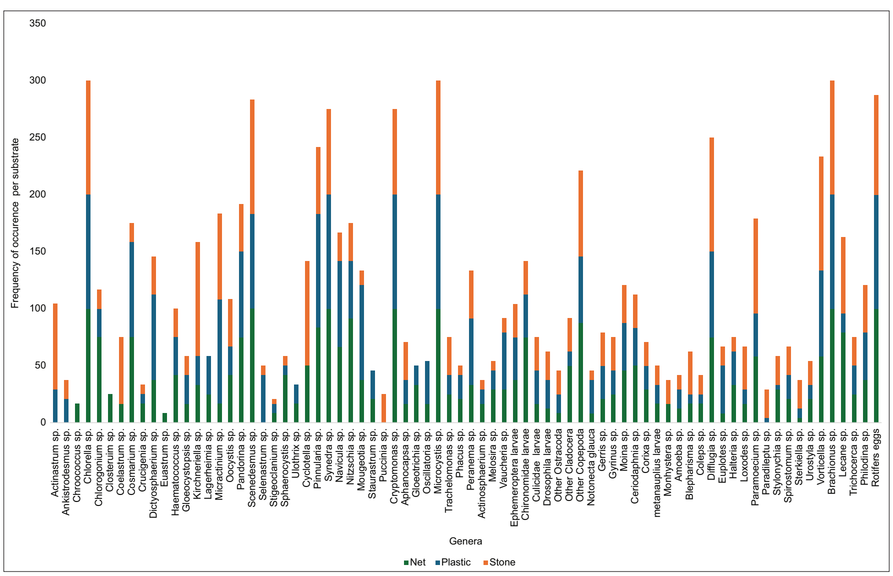

Figure 2: Frequency of occurrence of the species list of periphyton in aqua dams deployed with net, plastic, and stone substrates.

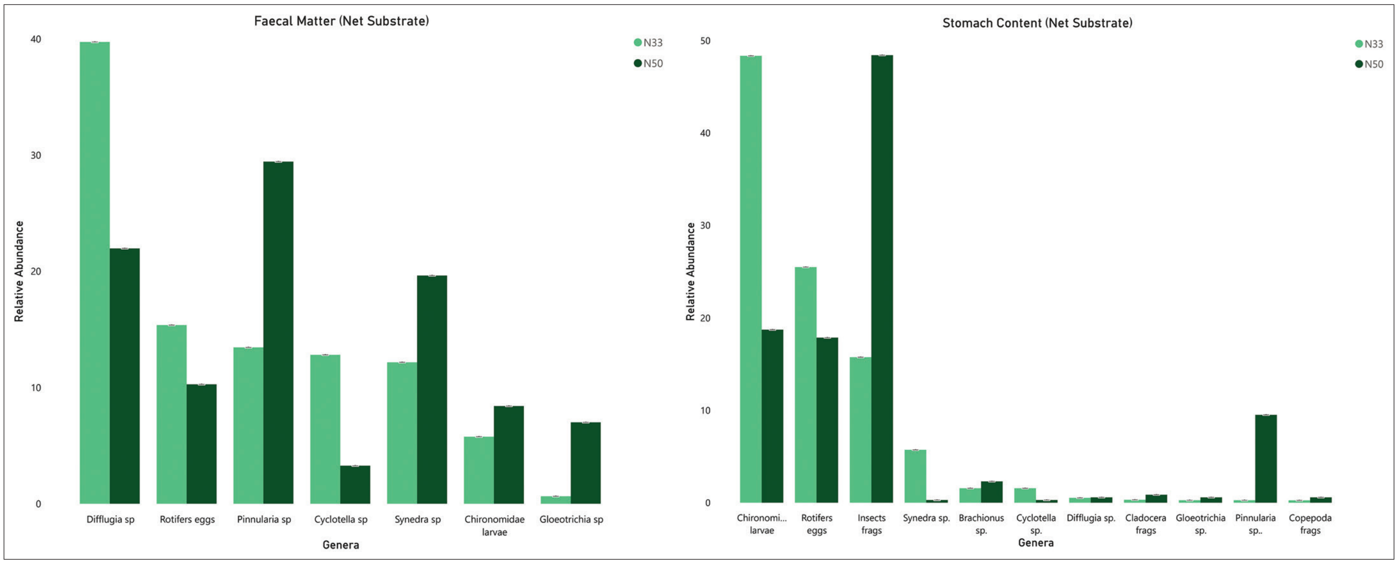

Figure 3: Periphyton genera found in the stomach contents and fecal matter of African catfish in aqua dams with net substrates.

Table 1: The dominance index of periphyton in the aqua dam with net, plastic and stone stocked with African catfish.

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

Copepods are unidentified species and fragments.

Table 2: 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.

Identified food items	Net		Plastic		Stone	
	N50	N33	P50	P33	S50	S33
Chlorella spp.	-	-	2.41	0.00	0.04	0.03
Oocystis spp.	-	-	3.49	0	-	-
Dictyosphaerium spp.	-	-	1.89	7.57	-	-
Sphaerocystis spp.	-	-	4.65	0	-	-
Pandorina spp.	-	-	-	-	13.70	3.77
Scenedesmus spp.	-	-	0.17	0	0.99	0.63
Cyclotella spp.	0.03	0.18	-	-	-	-
Pinnularia spp.	9.80	0.28	-	-	2.58	0.31
Synedra spp.	0.03	2.71	-	-	3.38	0.87
Navicula spp.	-	-	-	-	0.22	1.67
Gloeotrichia spp.	0.07	0.03	-	-	-	-
Microcystis spp.	-	-	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
Brachionus spp.	2.11	1.11	-	-	-	-
Difflugia spp.	0.07	0.06	6.58	20.72	0.07	0.26
Rotifers eggs	18.42	27.15	0.26	0.80	-	-

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 by the percentage of full stomachs per treatment. Ranging from 0% to 100%. The growth performance indices calculated include:

Average Daily Weight Gain (ADWG) = Final Weight Gain (g)/Time (3)

Percentage Weight Gain = [(Final Weight – Initial Weight)/|Initial Weight|] × 100 (4)

Specific Growth Rate (SGR) = (ln Final Weight – ln Initial Weight)/Time (5)

Condition Factor = (Weight/Length^3) × 100 (6)

Survival Rate (%) = (Final Number of Fish/Initial Number of Fish) × 100 (7)

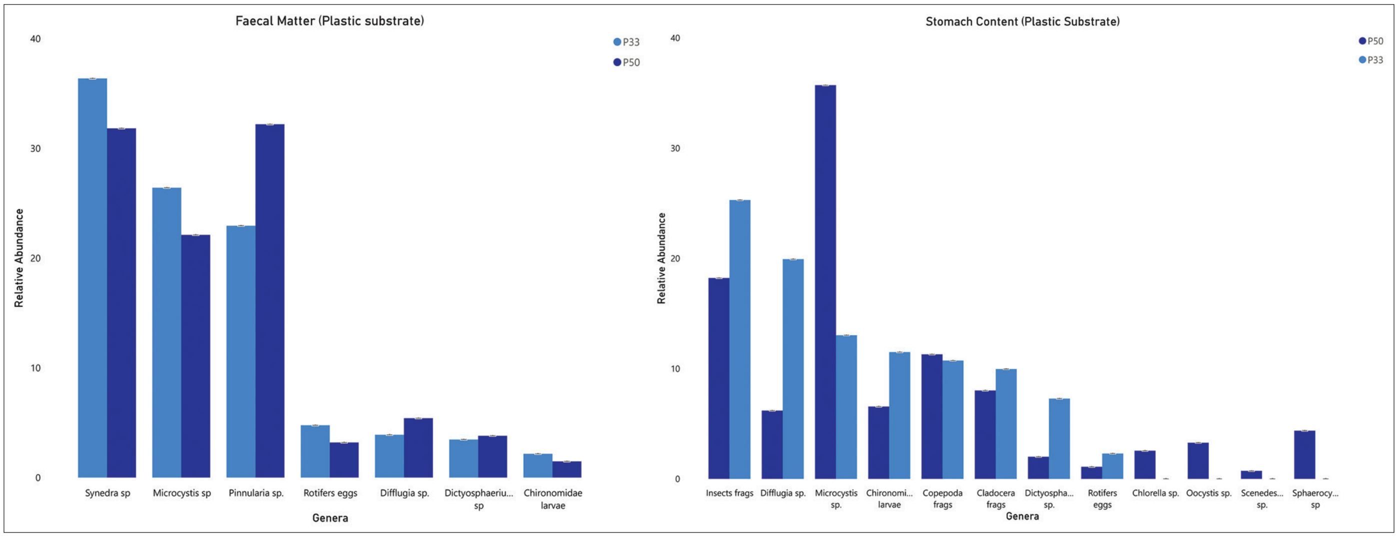

Figure 4: Periphyton genera found in the stomach contents and fecal matter of African catfish in aqua dams with plastic substrates.

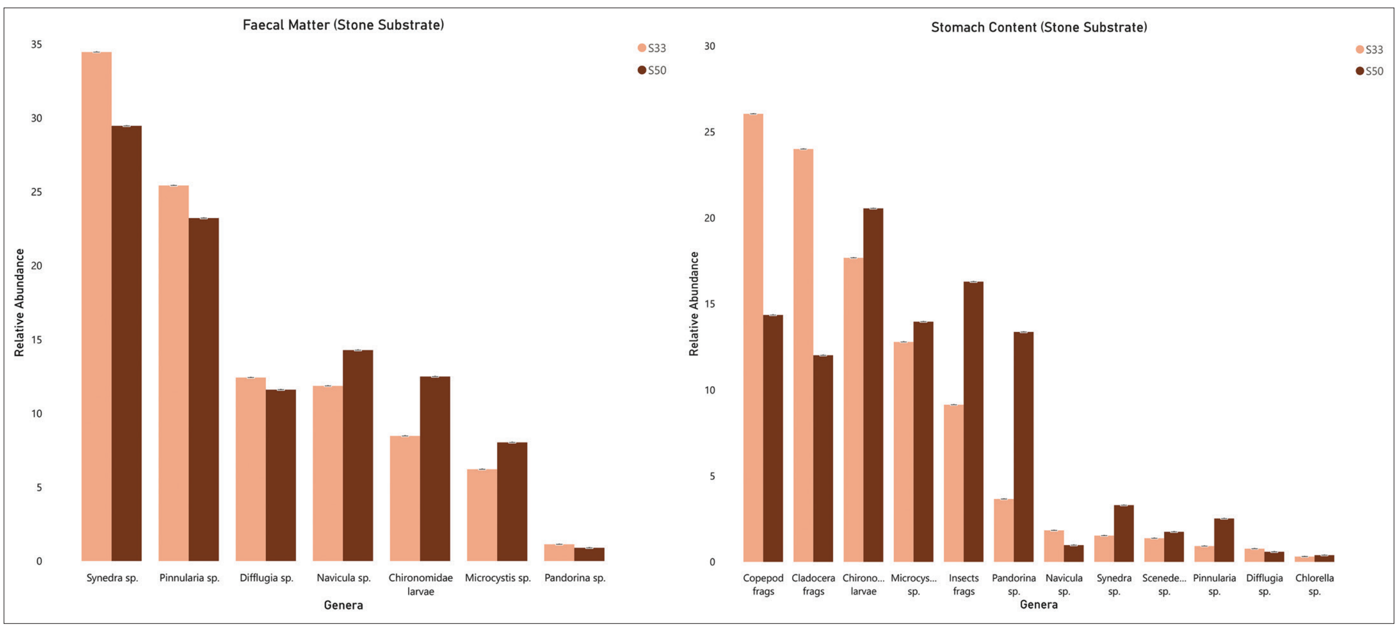

Figure 5: Periphyton genera found in the stomach contents and fecal matter of African catfish in aqua dams with stone substrates.

A modified percentage Index of Relative Importance (%IRI), adopted from [42], was used to determine the importance of food items in the stomachs of African catfish across all treatments:

$$\%IRI = \%NO * \%FO \quad (8)$$

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), defined as:

$$CSI = \frac{g_{i}/b_{i}}{\sum_{j=1}^{n} g_{j}/b_{j}}, i = 1, \ldots, n \quad (9)$$

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 contents and the 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 calculations for all periphyton species in the stomach contents. The CSI 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.

2.3.1. The feeding structures of African catfish and tilapia species

A morphological analysis of the feeding structures of African catfish was conducted and compared to those of *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 the methods described by [43,44]. The width of the mouth aperture was expressed as a percentage of

Table 3: Chesson selectivity index of periphyton items in the stomachs of African catfish from aqua dams with net, plastic, and stone substrates.

Feeding status	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
Chlorella spp.	-	-	0.0002	0.00000	0.00001	0.00001
Oocystis spp.	-	-	0.0035	0.00000	-	-
Dictyosphaerium spp.	-	-	0.00038	0.00029	-	-
Sphaerocystis spp.	-	-	0.00365	-	-	-
Pandorina spp.	-	-	-	-	0.00223	0.00553
Scenedesmus spp.	-	-	0.00034	0.00000	0.00007	0.00007
Cyclotella spp.	0.00009	0.00204	-	-	-	-
Pinnularia spp.	0.00037	0.00001	-	-	0.00031	0.00013
Synedra spp.	0.00001	0.00086	-	-	0.00044	0.00016
Navicula spp.	-	-	-	-	0.00039	0.00079
Gloeotrichia spp.	0.00002	0.00044	-	-	-	-
Microcystis spp.	-	-	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
Brachionus spp.	0.000262	0.00064	-	-	-	-
Difflugia spp.	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

Table 4: The growth performance indices (mean±SE) of African catfish in aqua dams with net substrates.

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

ADWG: Average daily weight gain, SGR: Specific growth rate. Superscript letters indicate significant values that differed from each other.

Table 5: The growth performance indices (mean±SE) of African catfish in aqua dams with plastic substrates.

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

ADWG: Average daily weight gain, SGR: Specific growth rate. Superscript letters indicate significant values that differed from each other.

the standard length. The number of gill rakers was also counted. The protrusibility of the premaxilla is presented as the percentage difference in distance from the anterior margin of the lower eye socket to the outer edge of the premaxilla, measured from the tightly closed to the wide-open mouth [43]. The lower jaw extensibility is defined as the distance from the anterior margin of the lower eye socket to the tip of the lower jaw with the mouth closed. This distance is subtracted from the distance with the mouth open and expressed as a percentage of the former [43].

2.4. Statistical Analysis

Microsoft Excel and Power BI were used to plot graphs. The normality and homogeneity of growth performance indices were tested using the Shapiro-Wilk and Levene tests, respectively (Statistical Package for Social Science [SPSS] version 28). Analysis of similarities (ANOSIM), permutational multivariate analysis of variance (PERMANOVA), multivariate analysis of variance (MANOVA), and Shannon diversity index were performed in R (version 2024.4.2-764) using the vegan,

Table 6: The growth performance indices (mean±SE) of African catfish in aqua dams with stone substrates.

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

ADWG: Average daily weight gain, SGR: Specific growth rate. Superscript letters indicate significant values that differed from each other.

Table 7: Comparison of mouth apparatuses of African catfish and tilapia.

Mouth apparatuses	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 rankers	72.4±15.32	20.50±1.29

SL: Standard length. The criteria for grouping mouth apertures are adopted from [43].

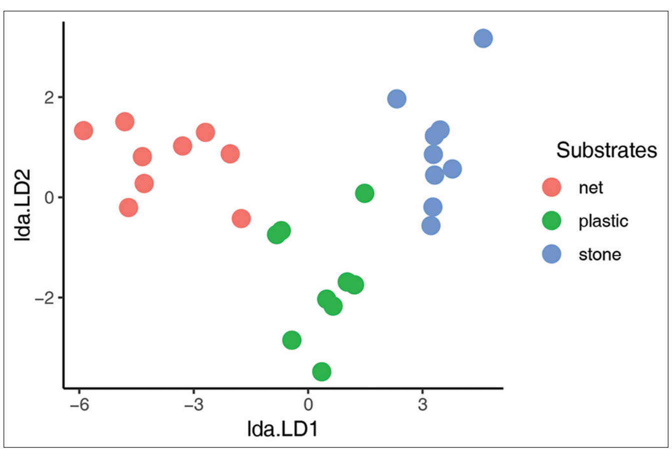

Figure 6: Scatter plot of linear discriminant analysis of growth performance indices of African catfish in aqua dams with net, plastic, and stone substrates.

MASS, effective, tidyverse, magrittr, and dplyr packages. ANOSIM was used to determine whether there were similarities in periphyton abundance on net, plastic, and stone substrates in aqua dams. PERMANOVA was employed to assess the statistical difference between the food items identified in the stomachs of African catfish utilizing periphyton. Additionally, ANOSIM was used to evaluate whether food items identified in the stomach were similar within each treatment or between the treatments. ANOSIM and PERMANOVA were used for data that was not normally distributed. Normality and homogeneity of variance data were tested using the Shapiro-Wilk and Levene tests, respectively. Analysis of Variance was used to test for significant differences in the growth performance of African catfish. 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 a level of significance of 0.05. A MANOVA was conducted to assess the significant differences in the growth performance indices among the substrates (net, plastic, and stone) using a permutation test.

3. RESULTS

3.1. Species Abundance of Periphyton in Aqua Dams with Net, Plastic, and Stone Substrates

A total of 71 periphyton genera were identified on the substrates in aqua dams stocked with African catfish [Figure 1]. *Microcystis* spp. and *Chlorella* spp. were numerically dominant across all substrates. The stone substrates exhibited the lowest species abundance [Figure 1]. The periphyton species abundance on the net, plastic, and stone substrates was significantly different (R = 0.4108, $P = 0.0001$; ANOSIM). The Shannon diversity index of periphyton in aqua dams with net substrates was 2.1823, and in aqua dams with plastic substrates was 2.1322, while in aqua dams with stone substrates was 2.0738. The frequency of occurrence of periphyton genera on all substrates mirrored the abundance [Figure 2].

The dominance index was calculated to identify the most dominant genera across the substrates in all the aqua dams. *Chlorella* spp., *Microcystis* spp. *Brachionus* spp., *Cryptonomas* spp., and *Difflugia* spp. were the most dominant periphyton on all substrates [Table 1].

3.2. The Utilization of Periphyton by African Catfish in Aqua Dams with Net, Plastic, and Stone Substrates

Chironomidae larvae, unidentified insects, and rotifers dominated the stomach contents of African catfish in all aqua dams with net substrates in both N50 and N33 treatments [Figure 3]. The fecal analysis of African catfish with net substrates showed that *Difflugia* spp., *Pinnularia* spp., *Synedra* spp., and *Cyclotella* spp. were also the most dominant. A few *Chironomidae* larvae and rotifer eggs were also identified in the fecal matter of the fish [Figure 3].

The stomach contents of African catfish in aqua dams with plastic were dominated by *Microcystis* spp., unidentified insects, *Difflugia* spp., *Chironomidae* larvae, unidentified copepod and cladocerans in both the P50 and P33 treatments [Figure 4]. The fecal matter of the fish in aqua dams with plastic substrates was dominated by *Synedra* spp., *Pinnularia* spp., and *Microcystis* spp. in both the P50 and P33 treatments [Figure 4].

Unidentified copepods and cladocerans, *Chironomidae* larvae, *Microcystis* spp., unidentified insects, and *Pandorina* spp. were dominant in the stomach content of the fish in aqua dams with the stone substrate [Figure 5]. *Synedra* spp., *Pinnularia* spp., *Difflugia* spp., and *Navicula* spp. were the most dominant in the fecal matter compared to the stomach content [Figure 5]. Overall, the most important food items were unidentified insects and *Chironomidae* larvae [Table 2].

Unidentified insects and *Chironomidae* larvae were the most important food items for African catfish in aqua dams with net substrates [Table 2].

The CSI 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 3]. The CSI of the food items found in the stomachs of all African catfish across different substrate types was 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 3].

The fish in the control treatment (N100) exhibited higher SGR and condition factors compared to those in treatments N50 and N33 [Table 4]. 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 4].

The SGR and condition factor of the fish were higher in the control (P100) treatment, followed by the P33 treatment [Table 5]. However, only the mean final weight and condition factor were significantly different across all treatments in aqua dams with plastic substrate ($P < 0.05$).

African catfish in the S33 treatment had a higher SGR [Table 6]. 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.

The growth performance indices of African catfish in the aqua dams with net, plastic, and stone substrates were significantly different from each other (*P* = 4.827e-08, MANOVA). The partial Eta Squared value indicated a strong effect size of 0.76 at a 95% confidence interval (0.60, 1.00). Indicating that the differences are substantial, meaning the effect of substrate on African catfish's growth was significant and not due to chance. This shows that each substrate influenced the growth performance of African catfish differently [Figure 6]. Linear Discriminant Analysis (LDA) was used to plot the *post hoc* test results, showing a proportion of trace values of 0.8551 for LD1 and 0.1449 for LD2, respectively [Figure 6].

The mouth aperture of African catfish was wide, while that of the tilapia was moderate [Table 7]. 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 7]. The protrusilibilty 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 7]. 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.

4. DISCUSSION

Microcystis spp. and *Chlorella* spp. 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 [45,46]. 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 [13,28,47,48]. Other studies have also shown that different substrates promote varying levels of periphyton abundance [5,6,49,50].

Chlorella spp. and *Microcystis* spp. were the most dominant periphyton on all substrates in aqua dams with African catfish. This might suggest that these species 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* spp., *Difflugia* spp., and *Cryptomonas* spp. are of animal origin, yet they were also the most dominant in all aqua dams with substrates. This might suggest that these species were consumed at a 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. Other studies [51,52] have also reported that fish consume less zooplankton when commercial pellets were introduced in fish ponds.

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 [53-57], this study found that they selectively fed on unidentified insects and *Chironomidae*. The African catfish is an efficient benthic feeder; thus, they are 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* spp. Despite the high abundance of diatoms on all substrates, they were seldom digested. This might suggest that African catfish lack gastric juices to digest diatoms. Diatoms possess a silica cell wall that is not easily digestible. Only a few species, such as abalone, are capable of ingesting and digesting diatoms [58].

The high abundance of *Microcystis* spp. in the fecal matter suggests that some of the *Microcystis* were not digested by African catfish. Most blue-green algae, including *Microcystis* spp., have thick cell walls that are difficult to break down [59,60]. Recently, [61] compared the algal communities in the stomachs of African catfish and tilapia, concluding that tilapia consumed more *Microcystis* spp. than African catfish. However, this conclusion was based solely on stomach analysis and may not accurately reflect the extent to which African catfish can digest *Microcystis* spp. It is also important to note that in aquaculture, *Microcystis* spp. can be a nuisance, as some species produce toxins [62]. *Difflugia* spp. was also abundant in the stomach and fecal matter of African catfish. These protozoa are related to *Amoeba* spp. and have shells that are difficult to digest.

Fish stocked in aqua dams with net (N100) and plastic (P100) substrates recorded the highest growth rate, along with fish stocked in aqua dams with stone substrate (S33). Both N100 and P100 served as the control, as African catfish are typically fed twice a day. This indicates that catfish were able to efficiently utilize periphyton from the stone substrate, as their growth rate was comparable to that of the control treatment. This suggests that the substrate affects the utilization of periphyton by African catfish. African catfish are benthic feeders and can easily utilize periphyton growing on the stone substrates in aqua dams. Even though the stone substrate recorded the least periphyton abundance, African catfish used their lower jaw to scoop up periphyton to support their high growth. This probably suggests that the type of substrate has a more significant impact on the growth of cultured fish than the amount of periphyton present on those substrates. Several studies have reported that the nature of the substrate influences both the quality and quantity of the periphyton [9,21,63]. The substrates used in this study are all non-biodegradable. Most studies on periphyton-based aquaculture have used bamboo substrate, which has been reported to support high-quality and quantity periphyton [26,63,64]. However, in this study, bamboo substrate was not used due to its limited local availability. Therefore, a comparative analysis of the quality and quantity of periphyton on stone and bamboo substrates is recommended.

The high growth observed on African catfish in aqua dams with stone substrates might also suggest optimal nutrient availability between the fish and the periphyton. In aqua dams with low periphyton abundance, there may be a more favourable nutrient balance that supports fish growth. This is because periphyton and fish use similar nutrients (nitrogen, phosphorus and carbon) to support their growth and metabolism. Therefore, with lower periphyton abundance, these nutrients become more readily available in the water to meet the nutritional needs of the fish as they utilize the periphyton. Moreover, this might suggest that low periphyton biomass has an ineffectual effect on fish's growth due to its nutritional quality. A study [9,29] suggested that the nutritional quality of periphyton is generally better than most commercial feeds used in aquaculture. This is because some periphyton genera can produce antimicrobial and bioactive compounds that function as immunomodulators or immunostimulants [18-20]. Immunostimulants and bioactive compounds possess various pharmacological functions such as anti-stress, antioxidant, tonic, immune-stimulatory, anti-inflammatory, antimicrobial, antifungal, antiparasitic and antiviral properties [65-67]. Other periphyton genera, such as *Ankistrodesmus* spp. and *Coelastrum* spp. which were identified in aqua dams, can produce astaxanthin [68,69]. Astaxanthin is a xanthophyll carotenoid that enhances growth, increases feed conversion rates, improves disease resistance, and reduces embryonic mortality in aquatic animals [70,71]. Therefore, the incorporation of all these growths and immune boosters' benefits into a formulated commercial diet would be cost-effective and beneficial to the aquaculture sector, especially in developing countries. This can easily commercialise periphyton and make it more accessible for most farmers, especially large commercial farmers.

The feeding efficiency of fish in aqua dams with net substrates was 100% indicating that all fish stomachs contained food items. This was probably because of the high abundance of periphyton that provided sufficient food for all African catfish in aqua dams. However, the fish from aqua dams with net substrates had fewer food items in their stomachs (0.5 ± 0.0 for N50 and N33) compared to those from aqua dams with stone substrates (0.7 ± 1.1 for S50 and 1.4 ± 2.2 for S33). Interestingly, the fish in aqua dams with stone substrates exhibited lower overall feeding efficiency (33% for S50 and 67% for S33) in comparison to those in aqua dams with net substrates. This discrepancy may be attributed to the African catfish's ability to efficiently graze on periphyton from the stone substrates, due to its specific feeding structures.

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

5. CONCLUSION

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

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

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

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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