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Contributions of the periphyton to the growth of Nile tilapia (*Oreochromis niloticus*) fingerlings on different fixation substrates: an ecological approach

Contribuições do perifíton no crescimento de alevinos de tilápia-do-nilo (*Oreochromis niloticus*) em diferentes substratos para fixação: uma abordagem ecológica

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ABSTRACT

Knowing about the ecological aspects involved in the commercial breeding of aquatic organisms becomes an important tool to make aquaculture more productive and less impactful. Thus, periphyton taxon composition and biomass on different substrates and the influence of these on water quality and growth parameters of Nile tilapia fingerlings were examined. An experiment with three treatments (substrates for growth of periphyton: geomembrane, polyethylene terephthalate (PET), and bamboo) and a control (without substrate), each with five replicates, was conducted in a greenhouse with controlled aeration and temperature. Each mesocosm was populated with ten tilapia fingerlings with an average weight of 2.3 g for 30 days. Water quality parameters were not significantly different among treatments but remained within that established by the environmental legislation. In all treatments, 36 periphyton taxa were observed. The bamboo substrate was the most diverse, which could be attributed to the fact it was a natural substrate. Regarding fish growth, there was a significant difference among the treatments, with the PET treatment having a higher condition factor (kn). The bamboo substrate was good for colonization concerning alga diversity; however, fish in the PET treatment and control exhibited higher performance and algae consumption values, respectively.

Keywords: periphytic algae; ecologic aquaculture; commercial fish feeding.

RESUMO

Conhecer os aspectos ecológicos que estão envolvidos na criação comercial de organismos aquáticos é ferramenta importante para tornar a aquicultura mais produtiva e menos impactante. Assim, este trabalho avaliou a composição de táxons, a biomassa do perifíton em diferentes substratos de fixação, a influência destes na qualidade de água e o desempenho zootécnico de alevinos de tilápia-do-nilo. Um experimento com três tratamentos (diferentes substratos para crescimento do perifíton: geomembrana, polietileno tereftalato [PET] e bambu) e um controle (sem substrato), com cinco repetições cada, foi montado em uma casa de vegetação com temperatura e aeração controladas. Cada mesocosmo foi povoado com dez alevinos com peso médio de 2,3 g, por 30 dias. Os peixes foram alimentados com ração comercial, cuja taxa de arraçoamento foi 30% menor do que a recomendada pelo fabricante, para estimular o consumo de perifíton. Os parâmetros de qualidade da água não foram significativamente diferentes entre os tratamentos, mas mantiveram-se em conformidade com o estabelecido pela legislação brasileira. Em todos os tratamentos, foram encontrados 36 táxons de perifíton. O substrato bambu foi o mais diverso, considerando-se o número de táxons encontrados, o que pode ser atribuído ao fato de ser ele de origem natural. O substrato geomembrana apresentou crescimento perifítico superior aos demais, com maior quantidade de biomassa final. O único resultado significativamente diferente entre os tratamentos foi o tratamento PET com maior valor de fator de condição (kn), que indicou melhores condições de bem-estar dos peixes. O substrato bambu mostrou-se bom para colonização em relação à riqueza de algas perifíticas, todavia os peixes dos tratamentos PET e controle mostraram melhores valores de desempenho e consumo de algas, respectivamente, em relação aos outros tratamentos avaliados.

Palavras-chave: algas perifíticas; aquicultura ecológica; alimentação de peixes comerciais.

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Introduction

Sustainable aquaculture requires innovation and good environmental management, whereas aquaculture productivity depends on the project and an appropriate method to reduce its impact on the aquatic environment (Putro et al., 2020). Thus, knowing the ecological aspects that are involved in the commercial breeding of aquatic organisms becomes an important tool to make aquaculture more productive and less impactful.

In fish farming, as well as in other aquatic ecosystems, different aquatic organisms grow together along with the fish. These elements, constantly invisible to the fish farmer, compete for space, food, oxygen, and nutrients, cause disease, and also contribute to the ecological balance of the production environment (Russo et al., 2021).

Periphyton is a universally recognized term that designates all organisms attached to a submerged substrate, usually dominated by single-cell, colonial, or filamentous photosynthetic organisms, both prokaryote and eukaryote (Inyang et al., 2018). According to Wetzel (1990), algae constitute approximately 90% of the periphyton, dependent on variation according to environmental conditions (other microorganisms comprise the remaining 10%). This characteristic has attracted the attention of researchers for its potential use in aquaculture.

Most early-stage fish species, particularly herbivorous and omnivorous fish, use these organisms as a protein, vitamin, and mineral-rich food source (Pérez, 1992; Van Dam et al., 2002). These attributes confer these organisms economic and environmental importance.

In this respect, the periphytic community also plays an important role in water quality and in the trophic state bioindicator thanks to its ability to accumulate substances (such as nutrients and pollutants) in its biomass (Neal et al., 1967; Tedeschi and Chow-Fraser, 2021). This feature allows the periphyton to operate as a biofilter, directly and positively influencing nutrient cycling (Azim et al., 2001, 2004).

Feed and fertilizers are used during the production cycle in the normal daily operation of fish farms, but they may be expensive, and their dosage is often poorly calculated. An excess of these products in the water leads to an incomplete utilization by the fish, and as waste, they contribute to eutrophication and compromise water quality and fish cleanliness (Carballo et al., 2008). Therefore, the use of substrates that increase the area of periphyton growth in fish farming tanks can be an effective tool to reduce the nutrient load stemming from production residues (Biswas et al., 2018), is a naturally available food source, and, consequently, reduces the use of artificial diet, thereby alleviating production costs (particularly for small-scale producers) (Garcia et al., 2017).

In this regard, experiments that evaluate the contribution of the periphyton to the initial growth phases of commercial fish, the possible lower impacts on water quality, and optimization of integrated systems are fundamental to improving the management tools that minimize environmental and economic impacts caused by the exclusive use of artificial diets, particularly in extensive production.

The goal of this study was to evaluate the taxon and biomass composition of the periphytic community on different fixation substrates, as well as their influence on water quality and on the growth parameters of Nile tilapia (*Oreochromis niloticus*) with the hypothesis that the periphyton substrates in the mesocosms would contribute to the zootechnical performance of the fish.

Material and Methods

The experiment was carried out in a greenhouse facility located at EMBRAPA Agropecuária Oeste, in Dourados, state of Mato Grosso do Sul, for 30 days between April and May 2017. Light (50% shade), temperature (air conditioning), and constant aeration of the water were controlled to provide an environment for the experiment that was almost the same as the natural one.

A total of twenty 100 L polyethylene boxes were used, submitted to constant aeration, and distributed in a completely randomized experimental design with four treatments and five replicates: control (without periphyton-fixating substrate), bamboo substrate, geomembrane substrate High-Density Polyethylene (HDP — Thickness: 5 mm), and polyethylene terephthalate (PET) bottle substrate. The substrates were chosen because of the ease of access and handling, low cost, and the presence of mechanical action-resistant surfaces for scraping. In each treatment, a 30 × 40 (cm) rectangular structure with a base of galvanized wire covered with antirust paint was employed for the fixation of the different periphyton growth substrates. This set occupied approximately 10% of each box's total area. The substrates contained within the wireframe base measured 30 × 5 cm and were kept fully immersed. The set was attached to one of the edges of each tank.

The fish were purchased from a local fish farm and acclimatized for 7 days. Each box was stocked with ten fish, with an average weight of 2.3 ± 0.01 g, fed with commercial feed (a 40% crude protein feed at the beginning of the experiment, followed by a 4 mm pelleted feed with 36% crude protein, according to the growth of the fish) at a dosage corresponding to 70% of the manufacturer's recommendations to stimulate periphyton consumption. The total daily feed amount was 10% of the fish biomass. The feeding frequency was four times per day at 7 a.m., 11 a.m., 1 p.m., and 4 p.m.

The bottom of each box was siphoned to remove excess waste. Approximately 40% of the water volume was replenished every 2 days. Dissolved oxygen and temperature values were measured daily using a YSI model 55 probe. Once a week, pH, electrical conductivity, and dissolved solids were measured using a Horiba U50 multiparameter probe. Nitrite, ammonia, and orthophosphate concentrations, plus hardness and alkalinity, were measured using Alfakit colorimetric kits. All of these parameters were then compared with CONAMA 357/05, the current legislation available.

The structures containing the substrates were removed weekly to scrape off an area of approximately 10% of the total area with a glass slide. The species composition was qualitatively analyzed by *Transeau* fixation. The taxa of the different substrates were identified under an optical microscope using a 3 mL aliquot per replicate by preparing temporary slides for binocular optical microscopy, Olympus CX41 (Bicudo and Menezes, 2006).

After 30 days, a biometric analysis was performed (weight, and total and standard length). For this procedure, ten fish of each box were euthanized using the benzocaine lethal method (immersion in pH 7-buffered solution), according to the guidelines of Normative Resolution 37/2018 — National Council for Animal Experimentation Control (CONCEA), gutted, and their stomachs were separated for content analysis. Each stomach was immersed in 4% formaldehyde solution in a labeled glass vial for further dietary analysis.

This research was previously certified by the committee on animal research and ethics of Universidade Federal da Grande Dourados under protocol number 51/2016 and approved in a meeting held on November 18, 2016.

In addition to final weight averages, the following data were calculated per treatment. According to Inoue et al. (2014), the weight gain values were obtained by the formula: Final average weight (g) – Initial average weight (g), and the daily weight gain by Daily Weight Gain: Weight gain (g)/ number of days.

The Relative (Kn) or *Le Cren* condition factor of fish in each treatment was calculated using Kn = W observed/W expected, where W observed was the weight of each individual and W expected, the weight determined by the weight-length curve (Le Cren, 1951; Araújo et al., 2011).

To evaluate the existence and proportion of periphyton consumption in each treatment, the stomach contents of the fish were analyzed by the frequency of occurrence method (%FO) using an optical microscope and a *Sedgewick – Rafter* counting chamber; the number of individuals that consumed a given item (feed, different periphyton taxa) was expressed as the percentage of total examined fish with stomach contents (Bowen, 1992). Thus, the number of times each item occurred was treated as a percentage of the total number of occurrences of all items (Hahn and Delariva, 2003).

The effect of the substrates on water quality was tested via a one-way analysis of variance (ANOVA*)*, followed by a Tukey's test ($p < 0.05$) evaluating the physical-chemical parameters. The weight and length data were used to calculate the condition factor followed by a one-way ANOVA to check for significant differences between the factors per treatment. The same biometric data and statistical tests were used to compare all performance results using SigmaStat 4.0 software.

With data on the periphyton taxa composition by substrate, a Cluster analysis was performed to evaluate taxa similarity vs. collection days. For stomach contents data, Permutation Multivariate Analysis of Variance (PERMANOVA) with the Bray-Curtis Coefficient (999 permutations) (Anderson, 2005) was applied to verify the significant differences in the composition of the diet in the different treatments. In addition, the Individual Indicator Value (IndVal) was calculated to show the main fish diet items for each treatment. Principal Coordinate Analysis (PCoA) with Bray-Curtis distance was performed to visualize similarities or differences in the diet composition of fish between treatments. For the aforementioned analyses, software R (R Core Team, 2003) was employed.

Results and Discussion

Water quality complied with the standard established by the CONAMA 357/05 resolution for class 2 water bodies and is shown in Table 1. The parameters remained constant throughout all treatments. The average values were calculated considering the entire experimental period and none of the calculated average values showed a significant difference.

Table 1 – Means and standard deviations of the physical-chemical parameters of water during the 30 days of the experiment in treatments with different periphyton growth substrates.

	Control	Geomembrane	Bamboo	PET	NS
Dissolved oxygen ($mg.L^{-1}$)	6.96 ± 0.7	6.97 ± 0.58	6.75 ± 0.94	7.0 ± 0.49	0.84
Temperature (°C)	22.08 ± 0.87	22.1 ± 0.94	22.1 ± 0.84	22.0 ± 0.82	0.99
pH	7.14 ± 0.67	7.05 ± 0.61	7.06 ± 0.6	7.16 ± 0.67	0.99
Alkalinity ($CaCO_3$ $mg.L^{-1}$)	61.28 ± 24.6	55.16 ± 27.5	67.44 ± 11.6	59.0 ± 19.4	0.97
Hardness ($CaCO_3$ $mg.L^{-1}$)	67.36 ± 10.5	70.68 ± 10.6	60.2 ± 20.0	68.0 ± 12.4	0.93
Total ammonia ($mg.L^{-1}$)	0.97 ± 1.11	0.89 ± 1.24	1.07 ± 1.34	0.83 ± 1.13	0.98
Orthophosphate (PO_4 $mg.L^{-1}$)	0.16 ± 0.188	1.24 ± 0.54	1.01 ± 0.61	0.94 ± 0.57	0.64
Nitrite (NO_3 $mg.L^{-1}$)	0.16 ± 0.18	0.16 ± 0.19	0.15 ± 0.18	0.16 ± 0.19	> 1.0
Conductivity ($\mu S.cm^{-2}$)	0.422 ± 0.02	0.421 ± 0.02	0.424 ± 0.03	0.422 ± 0.02	0.99
Total dissolved solids ($mg.L^{-1}$)	0.274 ± 0.01	0.273 ± 0.01	0.275 ± 0.02	0.274 ± 0.01	0.96
Redox Potential (ORP)	191.16 ± 48.6	159.8 ± 93.9	188.5 ± 50.5	193.4 ± 45.9	0.99

NS: significance level.

In this study, substrates occupied approximately 10% of the total mesocosm, which may have been insufficient for the growth of periphyton biomass to have a beneficial effect on water quality parameters.

Previous literature (Keshavanath et al., 2001) reported low values of dissolved oxygen in treatments with natural periphyton growth substrates when compared to an artificial substrate, in that case, the PVC pipe substrate. Azim et al. (2004) showed that the presence of periphyton decreased the nitrogen and total ammonia concentrations, operating as a biofilter in the tanks. In contrast, Andrion (2014) did not observe significant differences between treatments, probably because the periphyton failed to develop because of the presence of suspended particulate material, where the main water parameters remained constant.

Thirty-five taxa were identified in the periphytic community, the majority of which were in the algae group (23) — mainly Chlorophycea, Oedogoneophycea, and Diatomaceae. Other taxa included rotifers (2), fungi (1), copepods (1), protozoa (6), and insects (1). Rotifer eggs were also found, which were classified as a type of food item taxon. The richness of taxa vs. the type of substrate was demonstrated through a similarity analysis (Figure 1).

The dendrogram illustrates which substrates were most similar concerning taxa composition per collection (Figure 1). In the artificial substrates (PET and geomembrane), there was a higher similarity among taxa, probably because of the similar substrate nature (smooth surfaces). Tortolero et al. (2015) described a similar result, wherein natural substrates exhibited a more diverse composition and higher biomass compared to artificial substrates.

The number of taxa found increased in all treatments with time (Figure 2). The increasing colonization was visible, particularly in the bamboo and geomembrane substrates.

This result may be a consequence of the periphytic succession process, as described in studies that evaluated the growth and dynamics of periphyton succession in natural and/or artificial substrates (Siqueira and Rodrigues, 2009; Felisberto and Rodrigues, 2012). Several factors can influence this behavior in natural environments, including the rainfall regime and the roughness of the substrate available for periphytic community growth (Vercellino and Bicudo, 2006). Bergey (2005) demonstrated that the biomass of periphytic algae increased with the number of cracks in the substrate.

Studies such as one by Osório et al. (2019) showed that the colonization of the periphytic community differed according to the ability of each species to colonize substrates of small or large complexity. Murdock and Dodds (2007) obtained higher values of chlorophyll and higher diversity of algae in more rugged substrates, suggesting that the larger surface area available with rough substrates contributes to the growth and diversity of species in the community, as does the complexity of the substrate.

There were significant differences in diet composition (consumed items), between treatments (PERMANOVA F = 4.5382, p = 0.01), and the IndVal (Table 2) showed that the indicator items of the bamboo treatment were the algae *Monoraphidium* sp, *Pennales*, and *Selenastrum* sp; for geomembrane, there were no indicator items and the fish ingested higher amounts of feed when compared to the other systems evaluated herein. In the PET treatment, the highlighted items were rotifer, and resistance egg groups; in the control group, *Scenedesmus* sp. was the indicator item.

Ordering analysis (PCoA) explained the 42% data variation, and important segregation was observed among treatments containing substrates and the control treatment (Figure 3). In both, the analysis of tilapia stomach contents showed a diversified diet, rich in natural foods. Periphytic algae taxa were consumed, as well as planktonic algae of the *Scenedesmus* genus (Appendix 1), which are one of the most common phytoplanktonic components present in freshwater bodies (Bicudo and Menezes, 2006). These algae were the most consumed natural item by fish in the control treatment, possibly because of the absence of substrate that allows the growth of the periphytic community.

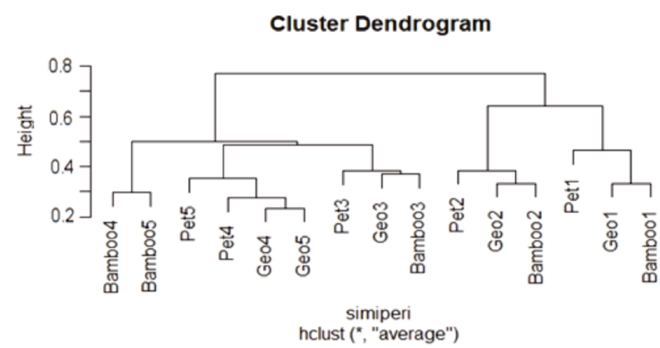

Figure 1 – Cluster dendrogram showcasing the similarity of taxa composition in each periphyton substrate treatment per week (1, 2, 3, 4, 5) throughout the 30-day experiment.
Geo: geomembrane.

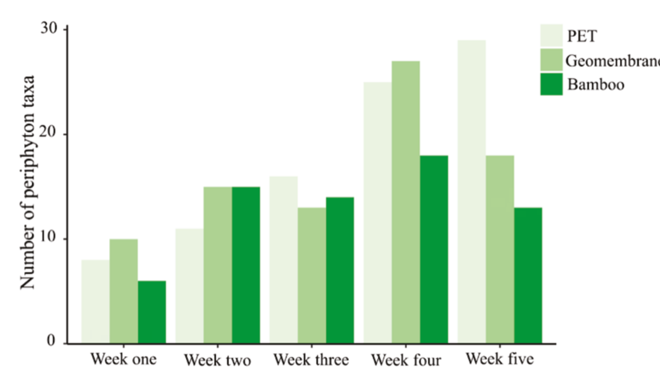

Figure 2 – Relationship between the number of periphyton taxa vs. substrate in the three treatments (bamboo, PET, and geomembrane).

Loures et al. (2001) observed that for tilapia from fish farms fed mainly on feed, at certain times, the consumption of phytoplankton was almost equal to that of the feed provided by the researcher. The phytoplankton and periphytic communities require practically the same nutrients to grow, a higher consumption of phytoplanktonic algae (mainly from the *Scenedesmus* genus) in the control treatment supports the hypothesis that the absence of a substrate in this treatment favored the development of phytoplanktonic algae.

The choice of the item to be eaten by the fish is influenced by the availability of the resource, food preference, and prey size (Sipaúba-Tavares, 1993). Abimorad et al. (2013) found that, while foraging, fish can select the food that better suits their nutritional needs, leaving unsuitable organisms in the substrate. Huchette et al. (2000) observed a similar relationship between the species found in the artificial substrates inserted in the culture environment with the species found in fish stomachs, corroborating what was observed in this study. Rivera Vasconcelos et al. (2018) observed that the magnitude of tilapia predation effect on the phytoplankton and zooplankton communities in tropical lakes is dependent on the biomass and structural size of both communities.

Table 2 – Frequency of occurrence of food items found in the stomach contents of fish per treatment e IndVal significance values ($p < 0.05$).

Items		Bamboo	Geomembrane	PET	Control
Algae	*Monoraphidium* sp.	4.54*	1.8	1.5	0.25
	Scenedesmus sp.	1.95	1.6	0.65	16.5***
	Chlorococcales	0	0.35	0.85	0.25
	Desmodesmus sp.	0.55	0.2	0.04	0
	Pennales	0.95**	0.3	0.1	0.05
	Coelastrum sp.	1.6	1	4.45	2.1
	Johannesbaptista sp.	0.4	0	0	0
	Chroococcales	0.15	0.04	0	0
	Selenastrum sp.	2***	0.1	0.2	1
	Stigeoclonium sp.	0.4	0	0.1	0
	Bulbochaete	0.01	0.03	0	0
	Binuclearia sp.	0.2	0.15	0.02	0
	Gloeotila sp.	0.05	0.05	0	0
	Oedogonium sp.	0.4	0	0	0
	Oocystis sp.	0	0	0	0.1
	Pseudoanabaena	0	0.88	0.15	0.05
	Eudorina	0	0	0.04	0
Protozoa					
	Suctoria	1.05	0.3	1.7**	0.6
	Peritrichida	0.3	0.2	1.45	0
	Synhymeniida	0	0.5	0	0
Rotifers					
	Bdelloidea	3	2	6.2*	1.6
	Eutoratoria	0.3	0.15	1.15	0.15
Other items					
	Arcella	0.05	0.05	0	0
	Scale	0.05	0	0.05	0
	Rotifer eggs	1	0.3	1.4**	0.3
	Chytridiomycetes	0.05	0.5	0.05	0
Feed		81	89.5	79.9	77.05
Total		100	100	100	100

*0.05; **0.01; ***0.001.

RBCIAMB | v.57 | n.2 | June 2022 | 333-342 - ISSN 2176-9478

In the early stages, plankton is the main food source for most fish, in natural environments (Uys and Hecht, 1985) and, in fish farming, it has great importance as a supplementary item without additional costs, which can improve weight gain, condition factor, and immune responses to fluctuating weather conditions and pathogens (Uddin et al., 2009; Cavalcanti et al., 2021).

In this study, there were no significant differences in zootechnical performance ($p > 0.05$) between the different treatments (Table 3). As compared to the Salazar-Torres et al. (2016) experiment, the authors argued that low weight gain stems from a failure to meet the tilapia's energetic demands. Keshavanath et al. (2004), in a 75-day experiment, showed significant differences in the final weight of fish that fed on a system with bamboo substrates for periphyton growth, with fingerlings being able to predate the periphyton. The same may have occurred in our work, that is, the surface area for growth of the periphyton, as well as the duration of the experiment, may have been insufficient to produce a significant effect on the weight and length of the fish.

Still concerning zootechnical performance, PET treatment showed a higher condition factor, with significant differences (ANOVA $F = 275.5$, $p < 0.05$) between treatments (Figure 4). The condition factor is a way to quantify and compare the well-being, fat, or condition of the fish (Vazzoler, 1996). Variations in this indicator can be a result of several factors, including food availability, environmental quality, and the effects of pathogens (Bolger and Connolly, 1989; Ramos et al., 2013; Sarkar et al., 2013; Gouveia et al., 2020).

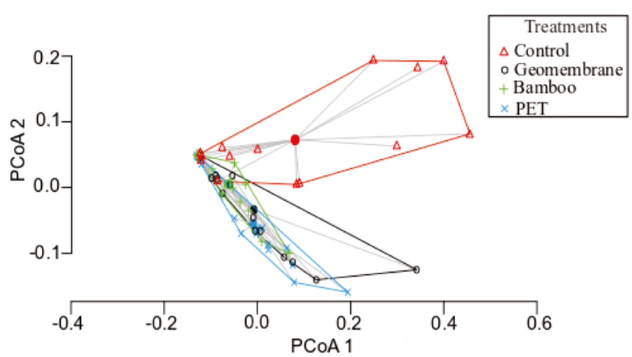

Figure 3 – Principal coordinate analysis (PCoA) using Bray-Curtis dissimilarity of major food items consumed in the different treatments with substrates for periphyton growth and control. The items significantly consumed are in Table 2.

However, it is essential to consider that PET is an artificial material in a natural environment.

In a review, Miloloža et al. (2021) observed that microplastic in freshwater sources may cause various adverse effects such as neurotoxicity, reproductive toxicity, oxidative stress, immunotoxicity, and a decrease in photosynthetic efficiency in living organisms including algae, an important aspect of this study. Exploring the literature, Heindler et al. (2017) analyzed the effects of PET microparticles in copepods and one of the conclusions was that the prolonged exposure to microplastics had severe deleterious impacts on the population viability of these crustaceans.

On the other hand, comparing the several types of plastic, their chemical characterization, and the ecotoxicological effects of chemical additive leaching, Capolupo et al. (2020) demonstrated that the PET plastic contained the lowest number and concentration of measured additives.

Fish subjected to the PET treatment ingested a lower percentage of feed compared to the other two treatments with substrates (79.9%). Moreover, animal items, such as rotifers and protozoa, were also consumed in higher proportions, a fact that may have influenced the better condition of fish (Gomiero et al., 2010; Araújo et al., 2011; Biswas et al., 2017).

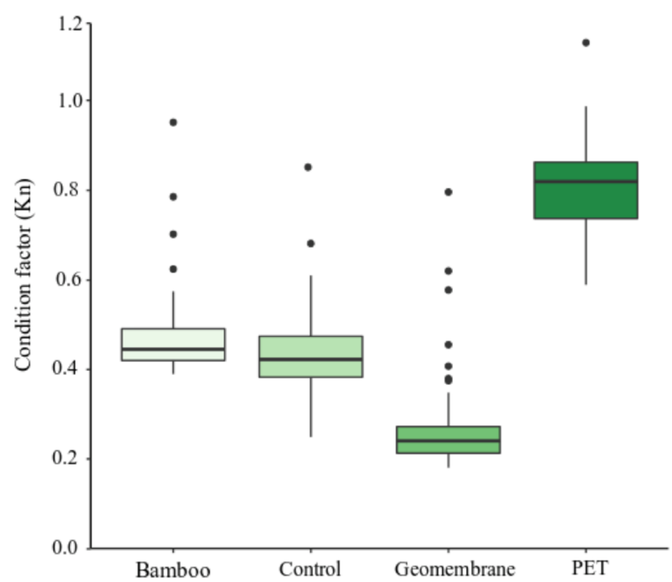

Figure 4 – Fish condition factors per treatment.

Table 3 – Zootechnical performance of tilapia fingerlings in all three substrate and control treatments, and significance represented by the p-value from the one-way ANOVA.

Group	Control	PET	Bamboo	Geomembrane	p-value
Average final weight (g)	13.036 ± 3.54	13.786 ± 4.13	13.31 ± 3.32	13.52 ± 4.28	0.748
Weight Gain (g)	10.86 ± 4.01	11.96 ± 3.97	10.96 ± 4.18	11.37 ± 4.83	0.646
Daily weight gain (g/day/fish)	15.80 ± 5.84	17.11 ± 5.68	15.81 ± 6.03	16.40 ± 6.97	0.748
Specific Growth Rate (%/day)	1.17 ± 0.18	1.21 ± 0.17	1.16 ± 0.22	1.16 ± 0.27	0.684

Enhancement of natural food through the use of substrates in the ponds is a cheap alternative (Milstein et al., 2008; Barlaya et al., 2021). Additionally, in order to meet the growing demand and prevent the depletion of natural resources, aquaculture should become more sustainable (FAO, 2020). Thus, substitutes for fish feed can reduce the impacts of aquaculture feed production (Boyd, 2015). In this sense, considering the consumption of periphyton by fish and the improvement in the observed condition factor of the PET substrate in this work, manipulating natural food can increase the productivity and efficiency of aquaculture production systems, making them more economically and environmentally sustainable.

Conclusion

The study evidenced a higher-taxon richness in the bamboo substrate, where a significant consumption of several groups of algae was also observed. There were no significant differences in water quality between treatments and control, neither there were positive effects of substrate use on these parameters, and this may be related to constant water exchange in the system. According to our results, the PET substrate treatment was a promising activity in this tilapia fingerling system. The type of periphytic organism that grows on the different substrates may be more important than diversity, providing a food supplement that improves the condition of the fish. Moreover, because this type of substrate is cost-free (as it is obtained from recycling), we emphasize the economic and environmental importance of the results obtained, considering the more sustainable production systems. We indicate the use of PET substrate in systems that are homologous to the one exposed in this study and recommend a larger occupation area for the substrate (around 20%) to increase the plankton available and possibly influence the final weight gain, concomitantly with ecotoxicological investigations to provide substantial information about the use of the PET material in these particular activities. This substrate has an exposure time for fish and cultured water that generally lasts around 6 to 8 months. Consequently, we believe that the short period of time this material can be exposed does not influence the health of the fish and water quality.

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Contribution of authors:

ROSA, Y. P. S.: Conceptualization; Data curation; Investigation; Methodology; Formal Analysis; Writing — original draft; Writing – Revision and Editing; RUSSO, M. R.: Conceptualization; Investigation; Writing — original draft; Writing – Revision and Editing; Supervision; Project Administration; Methodology; Formal Analysis; INOUE, L. A. K. A.: Conceptualization; Investigation; Writing — original draft; Writing – Revision and Editing; Supervision; Project Administration; Methodology; Formal Analysis; CAVALCANTI, L. D.: Conceptualization; Data curation; Investigation; Methodology; Formal Analysis; Writing — original draft; Writing – Revision and Editing.

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Appendix 1 – Absence and presence of the taxa found in the three fixation substrates throughout the 30 days of the experiment.

	PET	GEOMEMBRANE	BAMBOO
Arcella			x
Synhymeniida	X		x
Peritrichida	x	x	x
Peniculida	x	x	
Ictio sp			x
Suctoria	x	x	x
Chytridiomycetes	x	x	x
Bdelloidea	x	x	x
Eurotatoria		x	x
Eustigmatales		x	x
Volvocales	x		
Ulotrichales	x	x	x
Eudorina		x	
Chroococales	x	x	x
Oscillatoriales	x		x
Chlorococcales		x	x
Coelastrum	x	x	x
Desmodesmus	x		x
Monoraphidium	x		x
Scenedesmus	x	x	x
Eutetramorus	x	x	x
Pennales	x	x	
Oocystis sp	x	x	
Selenastrum sp	x	x	
Mycrocistis sp	x		x
Anabaena sp		x	x
Bulbochaete sp			x
Rizoclonium sp			x
Closterium sp			x
Oedogonium sp			x
Nauplius			x
Resistance eggs	x	x	x
Diptera larvae			x