Nutrient uptake efficiency by macrophyte and biofilm: practical strategies for small-scale fish farming

Eficiência de captura de nutrientes por macrófitas e biofilme: estratégias úteis para a cultura de peixes em pequena escala

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Abstract: Aim: The aim of this study was to evaluate the efficiency of macrophytes (*Eichhornia crassipes*) and peryphyton (biofilm) on the removal of nitrogen and phosphorus. **Methods:** We used independent 500 L containers with 300 g of *E. crassipes* (macrophyte treatment) and plastic bands (surface area: 9 m²) (biofilm treatment). A treatment containing only water was also employed (control treatment). Each treatment was replicated three times. We compared nutrient concentrations and four other environmental variables, on the first day of the experiment and on the 45th day. **Results:** Biofilm was highly effective in removing nutrients, and, albeit to a less extent, macrophytes also played an important role. **Conclusions:** We suggest that, given a periodic, relatively effortlessly monitoring, is employed, these strategies can be used for small-scale fish farming with a positive cost-benefit relation.

Keywords: nitrogen, phosphorus, *Eichhornia crassipes*, periphyton, bioremediation.

Resumo: Objetivo: O objetivo desse trabalho foi avaliar a eficiência de macrófitas (*Eichhornia crassipes*) e perifíton (biofilme) na remoção de nitrogênio e fósforo. **Métodos:** Nós utilizamos recipientes de 500 L independentes com 300 g de *E. crassipes* (tratamento das macrófitas) e faixas de plástico (área superficial: 9 m²) (tratamento do biofilme). Um tratamento contendo apenas água também foi empregado (tratamento controle). Cada tratamento foi replicado três vezes. Nós comparamos as concentrações de nutrientes, e de quatro outras variáveis ambientais, no primeiro dia de experimento e no 45º dia. **Resultados:** O biofilme foi bastante eficaz na remoção de nutrientes, e, apesar de em menor grau, as macrófitas também desempenharam um papel importante. **Conclusões:** Nós sugerimos que, com um monitoramento periódico de esforço relativamente pequeno, essas estratégias possam ser utilizadas em culturas de peixes em pequena escala com uma relação custo-benefício positiva.

Palavras-chave: nitrogênio, fósforo, *Eichhornia crassipes*, perifíton, bioremediação.

1. Introduction

Whether in large or in small-scales, fish cultures are amongst the most prominent activities in tropical reservoirs. Nonetheless, like other economic activities, the consequences of fish farming may be potentially negative to the environment (Buschmann, 2001). The impacts on the aquatic ecosystems are the results of three general processes, namely, resource depletion, environmental alteration and residual production (Berveridge, 1996). A rapid increase in nutrient concentrations caused by aquaculture activities is a central concern, and has motivated a substantial amount of studies (e.g. Beveridge, 1984, 1996; Nava, 1990; Arana, 1997; Boyd, 1999; Tovar et al., 2000; Baccarin and Camargo, 2005). In fish ponds, nutrients tend to increase via fish food introduction and this may temporarily, or even permanently, raise the trophic levels of surrounding aquatic environments (Schimittou, 1977).

Many studies which evaluated different modes of eutrophication prevention or restoration in aquatic ecosystems have been accomplished so far (e.g. Brix, 1993; Sebetich and Ferriero, 1997; O'Grady and Duff, 1998; Chacon-Torres, 2000). However, controversial conclusions have been drawn so far and, therefore, further studies are frequently encouraged (e.g. Petrucio and Esteves, 2000).

Plants, particularly macrophytes and periphyton (biofilm), are amongst the most efficient organisms in removing dissolved nutrients in aquatic ecosystems. The former is mostly represented by vascular plants and few large algae (Ikusima and Gentil, 1997), while the latter is a complex microbiota community (algae, bacteria, fungi, animals, inorganic and organic detritus) attached to inorganic or organic, living or dead substrata (Wetzel, 1983a).

In the present study we experimentally tested the magnitude of nutrient uptake by macrophyte and periphyton, and proposed these environmental-friendly mechanisms as a means of preventing eutrophication in small-scale fish ponds used for aquaculture.

2. Material and Methods

Experiments were carried out on the margin of the Padre de Azevedo Reservoir, Sapé city, Paraíba State, Brazil (7° 2' 20" S, 35° 11' 15" W). To test the magnitude of nutrient uptake by macrophyte and biofilm we used containers of 500 L with water collected from the reservoir. We prepared three containers with 300 g of manually collected *Eichhornia crassipes* (Mart.) Solms in each (macrophyte treatment) and three containers with eleven arranged plastic bands in each (total surface area: 9.0 m^2) to be colonized with biofilm on their surfaces (biofilm treatment). Another treatment containing only water from the reservoir was used as a control group (control treatment). Experiments were carried out simultaneously and an orthogonal experimental design was employed, with each treatment being totally independent from the others. Following preparation, the experiment was maintained for 45 days and three exact replicates were employed for each treatment. Throughout the study span, the nine containers were kept under similar environmental conditions. To account for productivity saturation by biofilm, which reduces productivity rates, we periodically cleaned the plastic surfaces (i.e. within every 15 days). We employed this interval given the exponential phase is specific for each environmental conditions (Cavalieri et al., 1994).

Water samples were collected on the first and last days of the experiment to determine initial and final nutrient concentrations, respectively. The collected samples were acclimatized in ice, and nutrient concentrations (in $\mu g.L^{-1}$) were determined in the laboratory as follows: ammonia (NH_3) via the phenol-based method; nitrite (NO_2) via the colorimetric method; nitrate (NO_3) via the cadmium reduction method; orthophosphate (OP) via the ascorbic acid method; total phosphorus (TP) via the colorimetric method. Detailed procedures of these methods are described in Clesceri et al. (1999).

At each treatment we measured the magnitude of nitrogen and phosphorus uptake by calculating differences between their initial and final concentrations, following (Equation 1):

$$N_T = C_i - C_f \quad (1)$$

where: N = nutrient uptake (in $\mu g.L^{-1}$); T = total inorganic nitrogen ($NH_3 + NO_2 + NO_3$) or total phosphorous; C_i = initial concentration (day 1); C_f = final concentration (day 45). To account for differences between treatments during the beginning of the study we also calculated uptake rate as a proportion of the initial concentration, namely, percent rate of nutrient uptake. Positive values suggest effective removal of nutrients, whereas negative values suggest that the treatment was not effective on their removal.

The following physical and chemical water variables were also determined during the first and last day of the experiment: electric conductivity using a portable conductivimeter (Technal), water temperature and dissolved oxygen (O_2) using an IPSI oximeter and pH using a portable pHmeter (Handlab).

Data was tested for homogeneity prior to the analyses and, when necessary, was log_{10}-transformed. To determine the influence of each treatment on nutrient concentrations and water characteristics, we used one-way ANOVAs to determine the significance in variation among treatments for the first and last day of study. In case of significance this was followed by Student-Newman-Keuls post-hoc tests. Uptake rate was also compared among treatments using the same procedure. Further, we made pairwise comparisons between the first and last day of study for each variable within each treatment using paired Student's *t*-tests to determine their temporal fluctuations. We used non-metric multidimensional scaling (nMDS) with the Bray-Curtis clustering procedure to test for dissimilarities among treatments. This procedure was followed by an analysis of similarities (ANOSIM), to statistically test the significance of each pairwise dissimilarity, and the SIMPER routine (similarity percentages) to determine the contribution of each variable to the observed dissimilarities (Clarke, 1993).

3. Results

During the beginning of the experiment all variables showed similar values among treatments (Table 1). At the end, however, O_2, NH_3, NO_2, OP and TP showed significant differences in their values. In the macrophyte treatment, electric conductivity, temperature, NO_2, OP and TP significantly increased throughout the study, whereas pH, O_2 and NH_3 decreased (Table 1). Further, *Eichhornia crassipes* biomass increased substantially throughout the study (from 300 g to 1144.6 ± 117.9 g: mean ± SE). In the biofilm treatment, electric conductivity and temperature increased, whereas O_2, NH_3, NO_3 and TP decreased. In the control treatment, electric conductivity, temperature, NO_2 and TP increased significantly throughout the study.

The nMDS ordination indicated that, during the beginning of the experiment, all treatments grouped in association, but segregated greatly at the end (Figure 1). During the last day of study, the ANOSIM test revealed significant differences between the control and macrophyte treatments ($R = 0.39$; $p < 0.01$) and the control and biofilm treatments ($R = 0.28$; $p < 0.01$), but not between the macrophyte and biofilm treatments ($R = 0.01$; $p > 0.05$). According to the SIMPER results, 82.4% of the dissimilarity between the control and macrophyte treatments were explained by (ratio average dissimilarity/SD; % contribution) TP (1.38; 23.3), NH_3 (1.47; 19.25), OP (1.82; 18.45), NO_3 (1.57; 11.89) and electric conductivity (1.42; 9.54). Also, 82.1% of the dissimilarity between the control and biofilm treatments were explained by TP (1.18; 25.15), NH_3 (1.48; 15.94), OP (1.58; 15.72), NO_3 (1.36; 15.07) and electric conductivity (1.34; 10.24).

Macrophytes efficiently captured dissolved nitrogen, but not phosphorus from the water, whereas biofilm was efficient in capturing both (Figure 2).

Table 1. Values (mean ± SE) of physical and chemical variables sampled at three experimental treatments (control: C, macrophytes: M and periphyton: P) throughout a 45 days experiment span. ANOVA and Student-Newman-Keuls post-hoc results among treatments (horizontal comparison) are indicated. Pairwise comparison results based on Student's t-tests between days (vertical comparison) for each treatment and each variable are indicated by letters. Where different letters were assigned, significant differences were detected.

Variables	Treatments			ANOVA results			SNK test
	C	M	P	df	F	P	
Electric conductivity							
Day 1	610.0 ± 0.0^{A}	610.0 ± 0.0^{A}	$636.7 \pm 26.7.9^{A}$	2	1.00	NS	C = M = P
Day 45	1433.3 ± 33.3^{B}	1600.0 ± 57.8^{B}	1300.0 ± 100.0^{B}	2	4.60	NS	C = M = P
pH							
Day 1	10.1 ± 0.1^{A}	10.1 ± 0.0^{A}	10.0 ± 0.1^{A}	2	1.50	NS	C = M = P
Day 45	10.2 ± 0.1^{A}	9.8 ± 0.1^{B}	10.0 ± 0.1^{A}	2	4.30	NS	C = M = P
Temperature							
Day 1	27.7 ± 0.4^{A}	27.4 ± 0.3^{A}	27.0 ± 0.0^{A}	2	1.50	NS	C = M = P
Day 45	31.7 ± 0.4^{B}	31.0 ± 0.0^{B}	31.0 ± 0.0^{B}	2	4.00	NS	C = M = P
O_2							
Day 1	10.5 ± 0.4^{A}	11.3 ± 0.4^{A}	11.1 ± 0.2^{A}	2	2.37	NS	C = M = P
Day 45	8.5 ± 0.6^{A}	6.0 ± 0.4^{B}	6.1 ± 0.4^{B}	2	8.50	< 0.05	C ≠ (M = P)
NH_3							
Day 1	233.5 ± 18.6^{A}	212.6 ± 30.4^{A}	258.4 ± 15.4^{A}	2	1.15	NS	C = M = P
Day 45	240.0 ± 65.1^{A}	77.7 ± 30.7^{B}	94.1 ± 4.0^{B}	2	4.60	< 0.05	C ≠ (M = P)
NO_2							
Day 1	0.9 ± 0.2^{A}	1.2 ± 0.4^{A}	0.5 ± 0.2^{A}	2	1.33	NS	C = M = P
Day 45	3.0 ± 0.4^{B}	2.4 ± 0.9^{B}	0.7 ± 0.1^{A}	2	9.87	< 0.05	C ≠ M ≠ P
NO_3							
Day 1	118.4 ± 8.9^{A}	112.3 ± 4.6^{A}	169.7 ± 23.1^{A}	2	4.81	NS	C = M = P
Day 45	94.9 ± 8.5^{A}	140.6 ± 26.2^{A}	87.9 ± 18.8^{B}	2	2.40	NS	C = M = P
Orthophosphate							
Day 1	31.3 ± 2.2^{A}	29.4 ± 2.9^{A}	47.7 ± 17.2^{A}	2	0.98	NS	C = M = P
Day 45	32.5 ± 16.3^{A}	166.4 ± 12.1^{B}	36.5 ± 2.4^{A}	2	41.8	NS	(C = P) ≠ M
Total phosphorus							
Day 1	198.8 ± 43.2^{A}	83.0 ± 32.2^{A}	198.8 ± 28.7^{A}	2	3.83	NS	C = M = P
Day 45	2176.8 ± 487.6^{B}	253.0 ± 46.9^{B}	151.3 ± 17.3^{B}	2	51.34	< 0.001	C ≠ (M = P)

NS: non-significant.

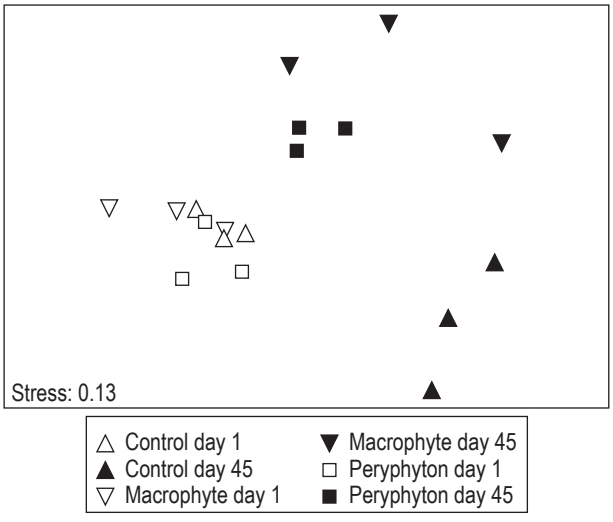

Figure 1. Non-metric multidimensional scaling ordination of three experimental treatments based on values of nine environmental variables.

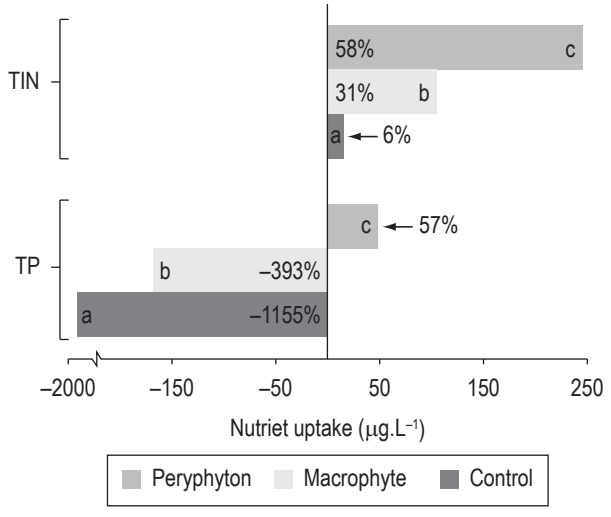

Figure 2. Mean total inorganic nitrogen (TIN) and total phosphorus (TP) uptake rates at three experimental treatments. Comparisons among treatments were significant (ANOVA; $p < 0.05$) and Student-Newman-Keuls post-hoc results are indicated by letters. Where different letters were assigned, significant differences were detected. Percent rate of nutrient uptake (i.e. nutrient uptake relative to initial concentration) are also indicated.

4. Discussion

In this work dissolved nutrient uptake was highly effective by biofilm. A similar proportion of total inorganic nitrogen and total phosphorous, 58 and 57%, respectively, was captured during the 45 days of experimentation. The macrophyte *E. crassipes*, on the other hand, was effective in removing nitrogen, particularly NH_3, but not total phosphorous. The nutrients removed by macrophyte and biofilm (NH_3 and TP) are considered the most important in aquatic systems (Wetzel, 1983b).

Although total phosphorous increased during the study in the macrophyte treatment, its final concentration was approximately ten times lower than the observed in the control flasks. This suggests that although macrophytes did not reduce total phosphorus concentration, they precluded a rapid proliferation of this nutrient. Further, decomposed leaves observed at the end of the study released organic material which likely created a nutrient feedback, thus withdrawing their nutrient uptake efficiency. Albeit not significant, the higher electric conductivity values observed at the end of the study in the macrophyte treatment further supports this increase in organic material.

Electric conductivity also increased on the other treatments as a result of regular water evaporation throughout the study. Somewhat high pH values were observed on all treatments, but very little fluctuation was detected. The lack of fluctuation in temperature values among treatments suggests that the presence of macrophytes and the plastic used for biofilm colonization did not increase water temperature. The higher temperature observed at the end of the study likely reflected an arbitrary cause, given that water temperature is highly dynamic and dependent upon factors such as time of day and light intensity. Oxygen concentrations were lower on both macrophyte and biofilm treatments due to higher respiration processes at these treatments.

Our study showed that both biofilm and *E. crassipes* can be successfully employed to enhance dissolved nutrient uptake in tropical aquatic systems, given their low cost and high benefits. Biofilm, has already been cited as a bioeliminator to improve water quality elsewhere (Sládečková and Matulová, 1998). Biofilm communities have important regulatory functions which can drastically alter rates and pathways of ecosystem biogeochemical cycling (Wetzel, 1983b). On the other hand, submerged macrophytes are most effective in reducing chlorophyll-*a* concentrations (phytoplankton), but also nutrients (Petrucio and Esteves, 2000).

The use of macrophytes to purify water sewage in constructed wetlands is increasing, and macrophyte invasions may frequently be a useful tool in improving water quality (Greenway and Wooley, 1999). Nevertheless these activities need to be periodically monitored (Haury et al., 2008). The higher effectiveness of biofilm, as compared to that of *E. crassipes* in our study, may be related to the periodical cleaning of the plastic used as a colonization substratum for biofilm which prevented nutrient-capture saturation. Therefore, a periodical removal of dead *E. crassipes* leaves avoiding decomposition and a consequential nutrient feedback may enhance nutrient uptake. On the other hand, these processes were tested on fish-free environments and, therefore, it is likely that introduction of omnivorous or herbivorous fishes would naturally control biofilm and macrophyte growth. If nutrient removal by fish overcomes their own discharge rates (i.e. nitrogen excretion), than these systems may be used in a somewhat self-sustaining fashion. We suggest that the use of biofilm is more effective in tropical aquatic ecosystems, particularly in northeastern Brazil, given that the number of native fish species which feed on this community is higher than those which potentially feed on macrophytes such as *E. crassipes* (Marinho et al., 2007).

When considering fish farming strategies, future studies are encouraged to specifically determine the amount of biofilm and macrophyte biomass necessary to effectively remove nutrients per kg of introduced fish food. Also, the efficiency of other macrophyte species, particularly submerged ones, need to be investigated.

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