

Study about Top-Down and Bottom-Up Controls in Regulating the Phytoplankton Biomass in a Eutrophic Reservoir in Northeastern Brazil

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ABSTRACT

This study aims to analyze the effects of nutrients and predation by zooplankton on phytoplankton biomass (chlorophyll *a*) in a eutrophic reservoir in Brazil (Apipucos Reservoir, State of Pernambuco), through experiments in microcosms. For this, samples of water were placed in 1 L Erlenmeyer flasks and kept for seven days. Treatments included the addition of nutrients (nitrogen combined with phosphorus and isolated additions of nitrogen and phosphorus), with presence and absence of zooplankton and a control which contained the reservoir water without any manipulation. The addition of nutrients did not stimulate phytoplankton growth. However, zooplankton significantly decreased phytoplankton biomass in the treatments it was added to (p < 0.05). The results of this study showed that for the reservoir studied, predation by zooplankton is the most significant factor in the regulation of phytoplankton, contradicting several studies which show that phytoplankton biomass is more strongly controlled by nutrients (bottom-up control) than by predation (top-down control).

Keywords: Chlorophyll a; Nutrients; Zooplankton; Tropical Regions

1. Introduction

Top-down (predation) and bottom-up (nutrients) controls have received considerable attention in recent years, mainly due to disputes about which one is more effective in regulating the phytoplankton community. The availability of nutrients has been considered the main regulatory force of phytoplankton structure and biomass [1,2], since the top-down effects, according to [3], are stronger at the top of the food web and weaken gradually toward the base. Thus, it is believed that the phytoplankton biomass is more strongly controlled by nutrients (bottom-up) than predation (top-down). However, studies have also shown that phytoplankton can be strongly regulated by zooplankton [4-8].

Many studies have demonstrated that the addition of nutrients, especially nitrogen (N) and phosphorus (P), can significantly increase the growth of phytoplankton, and influence the taxonomic composition and size class distribution of these organisms [2,9-12]. Zooplankton can have both direct and indirect effects on the phytoplankton community, through predation and by the re-

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cycling of nutrients, respectively [5]. Predation generally causes a decrease in phytoplankton biomass thus influencing primary productivity. However, it can also cause positive effects on phytoplankton, since it can stimulate the growth of inedible algae that have a greater competitive advantage for nutrients when the biomass of edible algae decreases [13].

Studies dealing with top-down and bottom-up controls on phytoplankton in reservoirs and lakes have been conducted in temperate regions, but are rare in tropical environments. Due to the need for broadening and deepening understanding of the structure and dynamics of phytoplankton in these regions, this study aims to analyze the effects of nutrients and predation exerted by zooplankton on the phytoplankton biomass (chlorophyll *a*) in a eutrophic reservoir in northeastern Brazil (Apipucos Reservoir, State of Pernambuco), through experiments in microcosms.

2. Materials and Methods

2.1. Study Areas and Sampling

The Apipucos Reservoir (8°01'14"S e 34°56'00"W) is a

eutrophic ecosystem belonging to the Capibaribe river basin, the main river in the state of Pernambuco, Brazil (Figure 1). It has a total area of 2.9 km², a volume of 556,375 m³ and an average depth of 2.5 m [14]. It has been built for flood containment and recreation, and consists of two interconnected subsystems. The occurrence of free-floating macrophytes is expressive, represented mainly by Eichhornia crassipes (Mart.) for its historical, cultural and environmental values; this water body is considered an "Environmental Protection Area". However, the organic (untreated sewage release) pollution input is high, resulting in elevated eutrophication levels in this system [15]. The annual cycle of the region is characterized by two seasons regulated by precipitation: a rainy season (March to August) and a dry season (September to February).

Water samples for the laboratory experiment were collected on the day that each experiment began: January 10, January 30 and February 27, 2012. Eight liters of water were collected from the surface layer at a single point, with the aid of a van Dorn bottle, of which seven liters were filtered through plankton net with mesh opening of 68 μ m to remove most of the zooplanktonic organisms that could interfere with the experimental design. The filtered material was stored in plastic containers and taken to the laboratory where it was used in the experiment. Part of this water was destined for nutrient analysis.

The following abiotic variables were measured *in situ:* water temperature (°C); and dissolved oxygen (mg·L⁻¹), measured through a Schott oximeter, Handylab OX1; water transparency, measured with a Secchi disk; and light intensity (μ mol photons m⁻², s⁻¹), measured with a photometer. The turbidity and pH values were obtained from measurements in the laboratory with a Hanna In-



Figure 1. Geographical location of the Apipucos reservoir, Pernambuco, Brazil.

The concentrations of nitrate (μ g N-NO₃ L⁻¹) and nitrite (μ g N-NO₂ L⁻¹) were estimated according to the method described by [16]; and ammonia nitrogen (N-NH₃ + N-NH₄⁺) [17], and total phosphorus (μ g PT L⁻¹), according to the methodology in [18]. The total nitrogen (TN) was considered as the sum of the concentrations of nitrate, nitrite and ammonia nitrogen.

2.2. Experimental Design

Sub-samples of 700 mL were placed in 1 L Erlenmeyer flasks and kept in the laboratory for seven days under temperature conditions of $25^{\circ}C \pm 1^{\circ}C$, artificial light with a photoperiod of 12:12 (light and dark), and constant oxygenation.

Three experiments were performed, each one with treatments that included the addition of nutrients (nitrogen combined with phosphorus, nitrogen or phosphorus), with the presence and absence of zooplankton. Therefore, for the environment studied, seven treatments were tested (n = 3): two with the addition of nitrogen and phosphorus, with and without addition of zooplankton, treatments NP and NP + Z, respectively; two with the addition of nitrogen, with and without the addition of zooplankton, treatments N and N + Z, respectively; and two with the addition of zooplankton, treatments N and N + Z, respectively; and two with the addition of zooplankton, treatments P and P + Z, respectively, and the final one, water from the reservoir that had not been manipulated (Control).

For treatments with the addition of nutrients, the source of nitrogen was NaNO₃ and of phosphorus KH_2PO_4 , and they were added once on the first day of the experiments (**Table 1**). Zooplanktons added to the treatments were collected in the reservoir using plankton net with mesh size of 68 µm. The initial density added to each treatment was twice the average recorded in the natural environment, based on the results of a recent study conducted in the studied reservoir [19].

2.3. Analysis of Phytoplankton

The phytoplankton biomass was estimated by the amount of chlorophyll *a* (μ g·L⁻¹), according to the methodology in [20] and, for this, 10 mL subsamples were removed from each treatment on the 1st, 3rd, 5th and 7th days of the experiment.

2.4. Statistical Analysis

Statistical differences between treatments were determined using a two-way analysis of variance (ANOVA), with a significance level of p < 0.05, using BioEstat. Version 5.0.

3. Results

3.1. Abiotic Variables and Chlorophyll a

The data of the physico-chemical variables, TN/TP ratio and chlorophyll *a* are shown in **Table 2**. The nutrient analysis in the Apipucos Reservoir showed high concentration of nitrogen in this environment and an average concentration of TN equal to $2229.07 \pm 320.07 \ \mu g \cdot L^{-1}$. Despite this, the TN/TP ratio was low (4.76 ± 0.98).

3.2. Bioassays

The addition of nutrients did not stimulate the growth of phytoplankton. At the end of the experiments, there were no significant differences between the concentration of chlorophyll a in the NP, N and P treatments and their

Table 1. Concentration of nitrogen $(NaNO_3)$ and phosphorus (KH_2PO_4) in the treatments with added nutrients.

Experiments		NaNO ₃ ($\mu g \cdot L^{-1}$)	$KH_2PO_4(\mu g{\cdot}L^{-l})$
Nitrogen + Phosphorus	NP	4019.54	186
Nitrogen	N	15127.14	-
Phosphorus	Р	-	186

Table 2. Physical-chemical variables, TN/TP ratio and chlorophyll *a* at the Apipucos Reservoir.

	Mean (± standard deviation)
Water temperature (°C)	28.53 (±0.21)
Dissolved oxygen (mg \cdot L ⁻¹)	3.67 (±1.69)
Depth (m)	1.20 (±0.15)
Transparency (m)	43.33 (±7.64)
Electrical conductivity $(\mu S \cdot cm^{-1})$	992.33 (±288.12)
Light intensity (µmol photons m^{-2} , s^{-1})	139.25 (±81.97)
Turbidity (UNT)	34.14 (±6.27)
pH	7.10 (±0.16)
Nitrate ($\mu g \cdot L^{-1}$)	304.82 (±15.99)
Nitrite ($\mu g \cdot L^{-1}$)	106.26 (±2.85)
Ammonia nitrogen ($\mu g \cdot L^{-1}$)	1817.99 (±308.43)
Total nitrogen ($\mu g \cdot L^{-1}$)	2229.07 (±320.07)
Total phosphorus $(\mu g \cdot L^{-1})$	472.95 (±34.10)
TN:TP ratio	4.76 (±0.98)
Chlorophyll <i>a</i> (μ g·L ⁻¹)	432.69 ±136.04)

respective control treatments (p > 0.05). Moreover, the zooplankton decreased significantly the phytoplankton biomass in those treatments to which it was added (p < 0.05). In the experiment with combined addition of nitrogen and phosphorus, the final chlorophyll *a* concentration was 886.53 μ g·L⁻¹ in the control, while in treatments NP + Z and Z it was 605.93 μ g·L⁻¹ and 540.87 μ g·L⁻¹, respectively. In the experiment with only the addition of nitrogen, the concentration was 1106.1 μ g·L⁻¹ in the control, 455.47 μ g·L⁻¹ in treatment N + Z and 345.67 μ g·L⁻¹ in treatment **Z**. In the experiment with phosphorus addition, the final concentration of chlorophyll was 947.5 μ g·L⁻¹ in the control, 427 μ g·L⁻¹ in treatment P + Z and 329.4 μ g·L⁻¹ in treatment Z (**Figure 2**).



Figure 2. Average concentration of phytoplankton biomass (±standard deviation) in the experiments with added (a) Nitrogen and phosphorus; (b) Nitrogen; and (c) Phosphorus. Treatment abbreviations are explained in Materials and Methods.

4. Discussion

Studies show that phytoplankton biomass tends to increase with the addition of nutrients, especially nitrogen (N) and phosphorus (P), and decrease due to predation by zooplankton [10,21,22]. In this study, the addition of nutrients had no effect on the phytoplankton biomass, measured by the concentration of chlorophyll a.

Due to the low N:P ratio recorded in the Apipucos Reservoir, the addition of nitrogen, or nitrogen combined with phosphorus, was expected to stimulate the growth of phytoplankton. According to [23], lakes and reservoirs which have an N:P ratio lower than 20 are limited by nitrogen, those greater than 50 are limited by phosphorus, and those between 20 and 50 are co-limited by N and P. A similar pattern was also proposed by [24,25].

Traditionally, phosphorus has been identified as the main limiting factor for phytoplankton growth, at least in temperate regions, while nitrogen is frequently cited as limiting at low latitudes [26]. However, it has been difficult to establish the main limiting nutrient for phytoplankton, because this can vary between different environments and among algal species.

Dzialowski *et al.* (2005) [24], analyzing 19 reservoirs in Kansas, USA, observed that the addition of P stimulated growth of phytoplankton in only 8% of all experiments in bioassays. On the other hand, limitation by N (16%) and co-limitation by N and P (63%) was often observed. Moreover, just as in the present study, the authors observed that in 13% of the reservoirs, the addition of nutrients did not stimulate phytoplankton growth. A similar result was observed by [27], who analyzed five dams along the Manyame River (Harava, Seke, Chivero Lake, Manyame Lake and Bhiri), Mozambique. Nitrogen was the main limiting factor in the Harava, Seke and Manyame Lake dams, and phosphorus was a limiting factor in Bhiri, while no nutrient limited phytoplankton growth in Lake Chivero.

In both studies cited above, light was identified as the main limiting factor in the reservoirs where the addition of nutrients did not stimulate algal growth. The possibility of limitation by light is rarely addressed in bioassays for limiting nutrients in freshwater systems [28]; however there is frequent evidence of limitation by light for phytoplankton in lakes and reservoirs [28-30]. According to [31], when the phytoplankton is limited by light it may show little or no limitation by nutrients.

The availability of these two resources may vary spatially and temporally in most ecosystems [29-32]. According to [32], in periods of intense rainfall there is an excessive discharge of nutrients which can directly lessen the limitation of this feature. Moreover, there is an increase in the discharge and re-suspension of sediments, increasing the intensity of limitation by light, since less penetration of light into the water column can ultimately limit algal growth.

Regarding spatial variation, it is believed that the phytoplankton growing in shallow water sites near heavy water flow are exposed to increased sediment discharge and pulses of nutrients when compared to phytoplankton growing in deeper, thermally stratified areas closer to the dam. Hence, phytoplankton from shallow lotic areas may be more limited by light and less limited by nutrients than phytoplankton from deeper areas [32].

Due to the environmental conditions observed in the Apipucos Reservoir, the results of the experiments suggest a potential limitation by light, since this reservoir is made up of neighborhoods heavily populated by lowincome populations, where sewage is discharged continually, either directly or indirectly [33]. Moreover, its waters are dark and turbid, indicating a high concentration of suspended sediment.

One should also consider the possibility of limitation by another nutrient or a seasonal variation in the limiting resource of the phytoplankton in the Apipucos Reservoir.

While the addition of nutrients had no effect on the phytoplankton biomass, predation exerted by zooplankton caused a significant reduction in the biomass. This result does not corroborate the hypothesis that top-down control exerted by zooplankton in tropical systems is weak when compared to that observed in temperate regions [34]. It is believed that the absence of large herbivorous zooplankton in the tropics, as well as the dominance of small-sized species, are the main reasons responsible for this difference [35,36].

Von Rückertand and Giani (2008) [34], while evaluating the effects of predation of three microcrustaceans *Daphnia laevis*, *Moina micrura* and *Thermocyclops decipiens* on the phytoplankton community in the Pampulha Reservoir (Minas Gerais, Brazil) found that zooplankton was inefficient in controlling phytoplankton. Low *et al.* (2010) [37], when analyzing phytoplankton and zooplankton populations in 12 tropical reservoirs, observed that the zooplankton exerted a top-down control on certain algal communities, such as green algae (Ankistrodesmus, Scenedesmus and Cosmarium) and phytoflagellates (*Peridinium*). Cyanobacteria, with the exception of *Planktothrix*, were not adversely affected by zooplankton.

For a better understanding of the interaction between phytoplankton and zooplankton communities in the Apipucos Reservoir, more information about the various factors that may affect the ability of zooplankton to prey on phytoplankton is necessary, namely, the taxonomic composition and biomass of the zooplankton, body size of the zooplankton and phytoplankton, zooplankton selectivity and efficiency, resilience of the phytoplankton, the relationship between the nutritional requirements of the zooplankton and the nutritional quality of the phytoplanktonic organism [38-44].

5. Conclusion

The results of this study showed that, for the reservoir studied, predation by zooplankton is the most significant factor in the regulation of phytoplankton, contradicting several studies which show that phytoplankton biomass is more strongly controlled by nutrients (bottom-up control) than by predation (top-down control).

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