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Competition for inorganic nutrients among five species of Amazonian phytoplankton in batch culture: Both nitrate and phosphorus accounts

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ABSTRACT

Competitive abilities for inorganic nutrients of five Amazonian phytoplankton species (*Closterium libellula*, *Trachelomonas hispida*, *Navicula* sp., *Pinularia subcapitata* and *Lyngbya* sp.) were tested under batch culture conditions. Data were collected from monocultures in order to fit models of growth rate (Monod-like for nitrogen, and Droop-like for internal phosphorus) and nutrient uptake (Monod-like in every case). A multinutrient interspecific competition model was built, and its accuracy was tested with data from a competition experiment using all the species together in batch culture. Competitive ability for nitrate differed quite a lot among these species. However, competitive abilities for phosphate storage did not show great variation according to Q_{min} values. Winners of competition resulted to be those species with lower K_s values for nitrate (*P. subcapitata* and *Lyngbya* sp.) and among these, the model showed that the best competitor for phosphorus would dominate (*Lyngbya* sp.). This shows that phosphorus storage was the most successful strategy among a group of the two best competitors for nitrate. However, in the competition experiment, both species were apparently coexisting at the end. Since model predicted dominance of *Lyngbya* sp. alone, it is most likely that the duration of the experiment was not enough to show exclusion of *P. subcapitata*. Alternative explanations would be that some remineralization of nutrients could have occurred, or that some unknown chemical interactions (allelopathy) were influencing our results.

KEYWORDS: phytoplankton, interspecific competition, resource competition theory, internal storage

INTRODUCTION

Diversity of phytoplankton has intrigued ecologists since Hutchinson's famous "paradox of the plankton" [1]: How to explain the high diversity observed in communities of these organisms if just a few resources (light, inorganic nutrients and a few other elements) are being shared? As a consequence of the attempt to solve this paradox, ecological theory showed great development in the field of interspecific resource competition, using phytoplankton as an experimental or theoretical model [2, 3, 4, 5, 6, 7, 8, 9]. Different authors pointed out several features of planktonic systems that should be considered in order to explain the paradox of the plankton: a though for more heterogeneous environments than previously

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realized, the impact of grazers, the role of nutrient storage, and the demonstration of the possibility to obtain complex dynamics (chaos) in the presence of three resources [7, 8, 9, 10, 11, 12, 13].

The earlier competition models were using Monod based models [14] for the growth rate of phytoplankton. An important assumption of this model is that inorganic nutrients are directly taken from the extracellular environment in order to be used inside the cells. This approach has been shown to be accurate for many experimentally manipulated systems, like chemostats in steady state, and also in other cases for inorganic nutrients that are not accumulated inside the cells, like nitrate.

However, phytoplankton growth could also be supported by intracellular storage of nutrients [15]. This is particularly true for phosphate limited growth. Phosphate could be accumulated by "luxury" uptake and stored in the cells as polyphosphates. Considering this, many experimental studies of competition employed Droop based models accounting for internal storage capacity [4, 5, 6, 10, 16, 17]. These studies showed intracellular storage to be an important factor to be taken into account, because predictions for the outcome of competition may vary when employing either Monod or Droop – based models of competition [10, 16]. In those models accounting for internal storage capacity, the best competitors for a particular nutrient would be those able to grow with the smaller intracellular quota of this nutrient.

In Amazonian lakes, especially those that are flooded by the Amazon River, it is common to find high diversity of phytoplankton, which may reach hundreds of species [18, 19, 20, 21]. This contrasts with the low mineralization that is recorded in lakes of northern South America [22, 23].

In the present work, we are attempting to explain the outcome of interspecific competition for inorganic nutrients observed in laboratory experiments with five relevant freshwater phytoplankton species from the Colombian Amazonia (*Closterium libellula*, *Trachelomonas hispida*, *Navicula* sp., *Pinularia subcapitata* and *Lyngbya* sp.). Monocultures were used to calculate the parameters of a Monod-based model of interspecific competition for nitrogen. The same was done for the Droop model in order to find out which species was the best competitor considering internal storage of phosphorus. Because nitrogen is not usually stored by phytoplankton (with the exception of some cyanobacteria), it was not considered for the Droop model. A multinutrient competition model was built and its outcome compared with the result of a competition experiment performed with a mix of all five species in batch culture.

METHODS

Phytoplankton species and culture conditions

Species were collected in lake Yahuarcaca (4 º 12'11 .76 south latitude and 69 º 57 '31.89" west longitude at an altitude of 71 meters), which is a lake flooded by the Amazon River. Single cells of five different species were isolated and then, monospecific cultures were established in order to be used in the experiments. These species belong to the following groups: Cyanobacteria (*Lyngbya* sp.), Euglenophyta (*Trachelomonas hispida*), Zygophyceae (*Closterium Libellula*) and Bacillariophyta (*Pinularia subcapitata* and *Navicula* sp.). These species are usually found in flooded meadows in the shores of this lake, although *T. hispida* is also present in the open waters.

These species were cultured in 250 mL flasks placed in growth chambers under a 12:12 light:dark cycle and 28°C. There were two treatments: phosphorus limitation (900 µM nitrate, 1 µM phosphate) and nitrogen limitation (4 µM nitrate, 5 µM phosphate) with two replicates each. The competition experiment with a mix of all five species was performed with 900 µM nitrate and 5 µM phosphate. In order to control carbon dioxide limitation, pH was daily monitored in all cultures.

Cell counts were performed on a daily basis. Five replicates of 2 mL each were taken from the flask and counted under an inverted microscope.

Nutrient analysis

External nitrogen and phosphorus were measured every day. One replicate of 25 mL samples was filtered in GF/F Whatman filters and stored at

-20ºC until they were analyzed. Analysis was performed in a BRAN-LUEBBE autoanalyser. Cell carbon and nitrogen content were measured daily for each species; one replicate of 10 mL of culture was collected every day on pre-combusted 13 mm diameter GF/F Whatman filters, stored at -20ºC and analyzed in a Fisons EA 1108 CHN analyzer. Cell phosphorus content was measured daily for each species; one replicate of 10 mL of culture was collected on GF/F Whatman filters. These filters were digested on 2% HCl and then analyzed in an ICP-OES Perkin Elmer Optima 4300 DV.

Models

Growth rate (μ) was fitted to a Monod model for the case of external nitrogen, with the following equation:

$$\mu = \mu max * S_N / (K_s + S_N)$$

Where μmax is the maximum growth rate, S the concentration of substrate, K_s a half-saturation constant and the highest external concentration of nutrient that makes growth = 0

Growth rate (μ) was fitted to a Droop-like model for the case of intracellular phosphorus, with the following equation:

$$\mu = \mu max(1 - Q_{min}/Q)$$

Where μ is the growth rate, μmax the maximal growth rate, Q is the cell quota for the nutrient, and Q_{min} the minimum cell quota.

For nutrient uptake (V), a Monod–based model was fitted for external nitrate:

$$V_N = vmax_N * S_N / (H_{sN} + S_N)$$

Where $vmax$ is maximum uptake rate, S is the external nitrate concentration and H_s a half-saturation constant.

The equation for phosphorus uptake is a bit different, since uptake increases with external concentration but diminishes with internal concentration:

$$V_P = [\, vmax_P * S_P / (H_{sP} + S_P)][(Q_{max} - Q)/(Q_{max} - Q_{min})]$$

Where $vmax_P$ is maximum uptake of phosphorus, S is phosphorus concentration.

Maximal growth rate was estimated as the slope of the linear regression of cell daily cell density (Ln transformed) per day during the days of highest cell division rate. In an analogous way were estimated maximum uptake rate for phosphate and nitrate, with data from nutrient uptake per cell. The rest of the parameters were fitted using least square regression. Mortality was not expected to be an important parameter creating differences in species behaviour. It was not estimated and instead, a value of 0.1 was set for all the species.

The multinutrient interspecific competition model, considering that the nutrient that is limiting growth is defined by Liebeg's "law of the minimum", is as follows:

$$dN/dt = min[\mu(S_N); \mu(Q)]N - mN$$

$$dQ/dt = v_p(S_P, Q) - min[\mu(S_N); \mu(Q)]Q$$

$$dS_N/dt = S_N^{\,0} - \sum v(S_N)N$$

$$dS_P/dt = S_P^{\,0} - \sum v(S_P, Q)N$$

Where N is the density of each of the phytoplankton species, m is mortality, and the rest of the symbols are same as in the equations above. Summation symbol indicates the summation of that value for all the species.

Model simulation was run with Stella software (isee systems, inc.).

RESULTS

Phytoplankton growth and nutrient uptake

In the monocultures, the species reached stationary phase around day 22, six days before the cultures were stopped, so recoding days with the lowest growth rates. The highest value of pH recorded from all monocultures during the whole experiment was 8.95. We consider a pH of 9 as the edge of CO_2 limitation, so we can conclude that our cultures were not CO_2 limited. Total carbon per cell measured in each species is shown in Table 1.

The estimated values of the parameters for the nutrient uptake and growth models are shown in Table 2. Maximum growth rate was estimated as the slope of the linear regression fitted to log-transformed data of growth during the days of maximum growth. Following our results, *Closterium libellula* had the highest maximal growth rate, while other three species (*Trachelomonas hispida*,

Navicula sp., *Pinularia subcapitata*) had very similar values for this parameter. *Lyngbya* sp. had the lowest value, far from all the other species. The values of K_s, and Q_{min} were fitted using least squares regression. According to these parameters, the best competitor for nitrogen would be *P. subcapitata* (given the value of K_s), with *Lyngbya* sp. not far from it (although standard error of the parameter estimate was high), and all the other species were remarkably worse competitors for nitrate. Comparing the ability to grow with the lowest cellular quota of phosphorus, *Lyngbya* sp. would be a better competitor than *P. subcapitata*, but not than *C. libellula* (and *T. hispida* would be very close). *Navicula* sp. and *P. subcapitata* were the worst competitors (Table 2).

Parameters of the equations for nutrient uptake are shown in Table 3. There is no relation with the parameters of the equations of growth.

Table 1. Total carbon per cell measured in the five phytoplankton species under study.

Species	Total carbon (pg C cell^{-1}) mean ± SD
Closterium libellula	371 ± 100
Trachelomonas hispida	258 ± 110
Navicula sp.	473 ± 98
Pinularia subcapitata	370 ± 215
Lyngbya sp.	314 ± 75

Competition experiments

The results for the two replicates of the mixed culture (all species in competition) are shown in Figure 1 (only at stationary phase) and Figure 2*a* (the whole course of the experiment). As these figures show, *Lyngbya* sp. and *Pinularia subcapitata* were the two dominant species in terms of biomass during the stationary phase of the mixed culture. Both species yielded similar values of biomass at the end of the experiment, so there was not a clear winner. The other three species also showed very similar biomass yield

Table 2. Values of the growth parameters estimated for the species under study: maximum daily growth rate (*μmax)*, Half saturation constant for external concentration of substrate in μmols (K_s, Monod model) for nitrogen, minimum quota in mg P cell^{-1}(Q_{min}, Droop model) for phosphorus. SE are standard errors of the estimations.

Species	*μmax*	K_s N	SE	Q_{min} P	SE
Closterium libellula	0.44	7.7	1.9	2.7×10^{-6}	1×10^{-6}
Trachelomonas hispida	0.36	7.4	4	3.7×10^{-6}	1.2×10^{-6}
Navicula sp.	0.34	8.1	4.1	5.3×10^{-6}	2.4×10^{-6}
Pinularia subcapitata	0.31	0.42	0.26	4.5×10^{-6}	2.9×10^{-6}
Lyngbya sp.	0.15	1.1	1.1	3.8×10^{-6}	1×10^{-6}

Table 3. Values of the nutrient uptake parameters estimated for the species under study: maximum rate of uptake (*vmax*) in μmols min^{-1}, Half saturation constant (H_s) in μmols and maximum intracellular quota for phosphorus (Q_{max}) in mg P cell^{-1}.

Species	*vmax*N	*vmax*P	H_s N	SE	H_sP	SE	Q_{max} P	SE
Closterium libellula	2×10^{-5}	4×10^{-5}	0.49	0.49	10.7	5.9	9×10^{-4}	2×10^{-4}
Trachelomonas hispida	2×10^{-6}	6×10^{-6}	0.3	2.2	16	7.6	5×10^{-4}	1×10^{-4}
Navicula sp.	4×10^{-6}	2×10^{-5}	0.28	1.23	24.7	12.1	3×10^{-4}	4×10^{-5}
Pinularia subcapitata	4×10^{-6}	2×10^{-5}	0.5	1.3	11.8	12.5	2×10^{-4}	2×10^{-5}
Lyngbya sp.	4×10^{-6}	4×10^{-6}	1.1	1.6	5.1	3.2	8×10^{-5}	4×10^{-6}

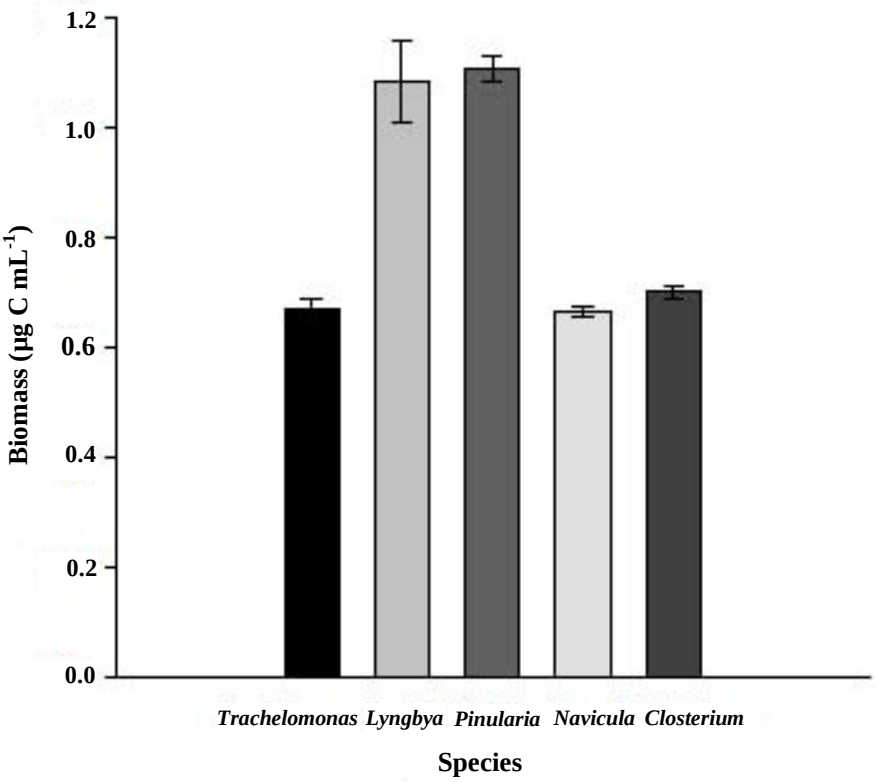

Figure 1. Average biomass ± SD for each species during the three last days (stationary phase) of the competition experiment performed with high initial concentration of nutrients. Means of the two replicates (n = 3) were averaged and standard deviation computed with this two independent values.

between them, and tended to decrease with respect to the two dominant species since the beginning of the stationary phase. These differences were statistically tested with paired Wilcoxon tests (Table 4, Fig. 1). Biomass (µg C mL^{-1}) of species pairs was compared for the last three days of the experiment, when cultures got to stationary phase. This confirms that both *Lyngbya* sp. and *P. subcapitata* were dominating the competition experiments, since they had significantly higher biomass than all others and, at the same time, there were no differences between the two of them (Table 4, Fig. 1). All the other three species did not show significantly different values of biomass among them (Table 4, Fig. 1).

Figure 2*b* shows the results of a simulation of the competition model, projected for 60 days. The values of predicted biomass or cell numbers are quite different than the observed ones. Patterns are qualitatively similar, though some differences will be highlighted in the next section.

DISCUSSION

The values of maximum growth rates (Table 2) were relatively low compared to similar species of phytoplankton, so our species could be considered, in general, not strong competitors with sufficient nutrient supply. The best competitors for each different nutrient are different; there is no relation in being good competitor for one nutrient and being good competitor for the other one (Table 2). So we can talk about the existence of trade-offs in the ability of these species to compete for these nutrients. This means that if the competition experiment is long enough to deplete nutrients to the maximum capacity of the two best competitors for each nutrient, they could coexist.

In the case of parameters for the uptake of nutrients (Table 3) there are not apparent trade-offs either.

Regarding the outcome of the competition experiments, the competitive exclusion of the three “loser” species would be a matter of time,

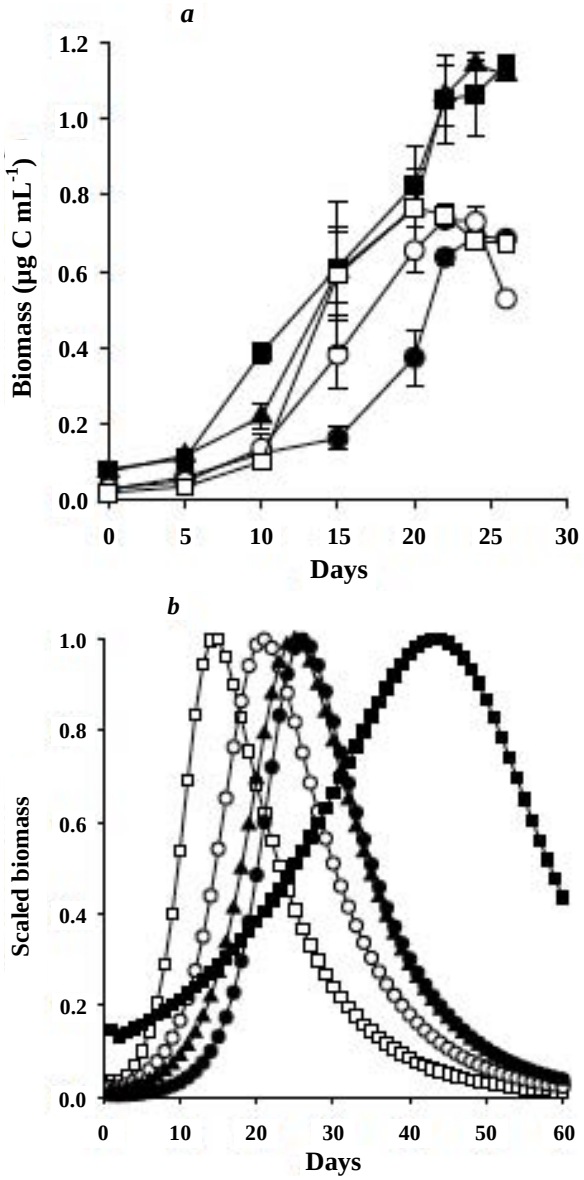

Figure 2. ***a*** Plot of Mean ± SD for the biomass of each species during the course of the competition experiment performed with high initial concentration of nutrients. The two replicates were averaged. ***b*** Same values simulated with the competition model. Values are scaled to the maximum value of each species. Symbols: dark circle (*Trachelomonas hispida*), dark square (*Lyngbya* sp.), dark triangle (*Pinularia subcapitata*), open circle (*Navicula* sp.), open square (*Closterium libellula*).

and the short time scale of the present experiment was not enough to show this exclusion. These results are matching the pattern of dominance predicted by nitrate competitive abilities (see Table 2).

Table 4. Results of the paired Wilcoxon tests comparing biomass of each species during stationary phase (3 last days of the experiment).

Species pairs	V	p
Closterium-Trachelomonas	11	1
Closterium-Navicula	14	0.56
Closterium-Pinularia	0	0.03
Closterium-Lyngbya	0	0.03
Trachelomonas-Navicula	9	0.84
Trachelomonas-Pinularia	21	0.03
Trachelomonas-Lyngbya	21	0.03
Navicula-Pinularia	0	0.03
Navicula-Lyngbya	21	0.03
Pinularia-Lyngbya	9	0.84

C. libellula and *T. hispida* had the lowest values of Q_{min} for phosphate but they do not appear to dominate. A possible explanation would be their lower competitive ability for nitrogen. *P. subcapitata* is a better competitor than *Lyngbya* sp. For nitrogen and the opposite is true for phosphate. This shows a clear trade off. Resource competition theory predicts that, in this case, there is a point at which coexistence of both species would be allowed if both nitrogen and phosphorus were limiting. This situation is likely to happen in the mixed cultures by the last days of the experiment, during stationary phase. Coexistence should not be discarded to be happening in the experimental data. However, a longer time course of the experiment would be needed in order to confirm this.

The differences in μmax between the five species were not strong enough to show dominance by the fastest-growing species during the period of no nutrient limitation, so there is not a clear winner during this period. In addition, stationary phase arrived quite soon, so the limitation by nutrients was the main factor shaping the observed dynamics.

After analyzing the results of these experiments it would be interesting to compare them with the model output. There are some differences between our experimental and theoretical results that should be remarked. First, the initial increase in biomass of the species is structured according to the maximum growth rate of the species (observed

in monocultures). This pattern is not observed at all in Figure 2*a*. This is not necessarily important, since the resolution of real data doesn't seem to be enough to account for these differences. More importantly, there are discrepancies in the temporal scale: stationary phase of *Lyngbya* sp. arrives much later in the simulation (it wouldn't show in our 26-days experiment). However, the major discrepancy between the simulation and actual data would be perhaps due to the fact that *Pinularia subcapitata* is not dominating together with *Lyngbya* sp. at any point during the simulation. Together with *Trachelomonas hispida*, *P. subcapitata* is the last competitor excluded by *Lyngbya* sp. However, this is not a big discrepancy if we consider that the coexistence between these two species that we observed has to be a transitory phenomenon. That is because our system is not going to reach equilibrium, as it is not a continuous system. Stable coexistence would be predicted only at a certain equilibrium point in the concentrations of nutrients, from which the system should not move. So, it is very likely that, as the simulation shows, *Lyngbya* sp. would be dominating alone at some point if our experiment lasted for more time. This was shown clearly after day 35.

More possible explanations for the observed co-dominance of these two species could be remineralization of nutrients, or allelochemical interactions that would change the dynamics predicted by nutrient competition alone.

The strategy of phosphorus storage has been shown to be particularly important under variable environments as opposite to systems like classic chemostats [4, 5, 6, 10, 24]. This kind of system reaches a steady state in which nutrients in the environment are in equilibrium with nutrients inside the cells. Subsequently, growing populations of microalgae are controlled by external nutrient supply, making the Monod model a good approach for competition. The batch cultures could be considered as variable environments that reach low nutrient concentrations when algal biomass is high, so the Monod model should be considered a worst approach than the Droop model for the study of these systems.

Our results could be relevant in order to explain the abundances of these and similar species in the geographic area of our study. Storage strategists would be favoured in a variable environment in terms of the availability of resources (alternating short periods of high nutrientent availability with periods of depletion). The area of interest match these features, because nutrient concentrations vary through the year and periods of low nutrient availability are common [25].

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