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Seasonal phytoplankton response to increased temperature and phosphorus inputs in a freshwater coastal lagoon, Southern Brazil: a microcosm bioassay

Resposta sazonal do fitoplâncton ao aumento de temperatura e aporte de fósforo em uma lagoa costeira de água doce no sul do Brasil: um bioensaio em microcosmo

Mariana Coutinho Hennemann and Mauricio Mello Petrucio

Laboratório de Ecologia de Águas Continentais, Departamento de Ecologia e Zoologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Campus Universitário, s/n, Trindade, CEP 88010-970, Florianópolis, SC, Brazil
e-mail: mari.henn@gmail.com, petrucio@ccb.ufsc.br

Abstract: Aim: the present study aimed at assessing the response of phytoplankton biomass (as chlorophyll-*a*) to simulated conditions of increased water temperature and phosphorus (P) inputs in Peri lagoon, a subtropical coastal lagoon dominated by *Cylindrospermopsis raciborskii* most of the year; **Methods:** microcosm experiments were conducted in thermostatic light chambers during 3 and 7 days in austral summer, autumn and winter 2009. Control temperatures were tested against 3 and 5 °C rises in water temperature in each season. In each temperature treatment, three P concentrations were tested: control (non-enriched, ~1.5 $\mu g.L^{-1}$ PO_4^{3+}) and enrichments of four (+6 $\mu g.L^{-1}$ PO_4^{3+}) and eight (+12 $\mu g.L^{-1}$ PO_4^{3+}) times natural concentrations; **Results:** the results showed that P enrichments alone did not increase chlorophyll-*a* concentrations, but temperature increases significantly elevated phytoplankton biomass in autumn and winter microcosms. Water temperature increases were followed by significant elevations in the oxygen saturation levels in all microcosms and seasons. The combined effect of increased temperature and P enrichments resulted in the highest chlorophyll-*a* levels also in autumn and winter. Summer microcosms seem to have been negatively affected by the experimental conditions (too high water temperatures); **Conclusions:** the bioassays showed that global temperature rises can significantly elevate the phytoplankton biomass in Peri coastal lagoon, especially in colder months and if followed by increased P inputs, what can lead to major ecological consequences to the water body and to water supply issues in the region.

Keywords: chlorophyll-*a*, *Cylindrospermopsis*, global changes, oligotrophic lake, cyanobacteria.

Resumo: Objetivos: o presente estudo teve como objetivo avaliar a resposta da biomassa fitoplanctônica (como clorofila-*a*) a condições simuladas de aumento da temperatura da água e do aporte de fósforo (P) na lagoa do Peri, uma lagoa subtropical costeira dominada por *Cylindrospermopsis raciborskii*; **Métodos:** experimentos em microcosmo foram conduzidos em câmaras incubadoras com controle de temperatura e fotoperíodo por 3 e 7 dias no verão, outono e inverno de 2009. Temperaturas controle foram comparadas com aumentos de 3 e 5 °C na temperatura da água. Em cada tratamento, três concentrações de P foram testadas: controle (não-enriquecida, ~1,5 $\mu g.L^{-1}$ PO_4^{3+}) e enriquecimentos de quatro (+6 $\mu g.L^{-1}$ PO_4^{3+}) e oito (+12 $\mu g.L^{-1}$ PO_4^{3+}) vezes a concentração natural na lagoa; **Resultados:** os resultados mostraram que o enriquecimento por P sozinho não provocou aumento nas concentrações de clorofila-a, mas a elevação da temperatura da água aumentou significativamente a biomassa fitoplanctônica nos microcosmos no outono e no inverno. O aumento da temperatura foi acompanhado por aumento significativo na saturação de oxigênio na água em todos os microcosmos e estações do ano. O efeito combinado do aumento da temperatura e do enriquecimento com P resultou em concentrações de clorofila-a ainda mais elevadas também no outono e no inverno. Os microcosmos desenvolvidos no verão parecem ter sido afetados negativamente pelas condições experimentais (temperaturas muito elevadas); **Conclusões:** os bioensaios mostraram que o aumento da temperatura global pode elevar significativamente a biomassa fitoplanctônica na lagoa do Peri, especialmente em meses mais frios e se acompanhada por aumento no aporte de P, o que pode levar a alterações ecológicas profundas no corpo d'água e a problemas no abastecimento de água da região.

Palavras-chave: clorofila-*a*, *Cylindrospermopsis*, mudanças globais, lago oligotrófico, cianobactérias.

1. Introduction

Coastal lagoons are considered ecologically and economically important environments due to their high productivity rates and intense human use for aquaculture, recreation and waste disposal (Spaulding, 1994) and because they are important contributors to local and regional weather stability, preservation of biodiversity and also as water suppliers (Esteves et al., 2008). In spite of their importance and multiple uses, increased nutrient inputs resulting from human activities have been shown to impact coastal lagoons worldwide (Taylor et al., 1995).

The phytoplankton community is the focal point for biological studies of eutrophication for several reasons such as the rapid response to increased nutrient availability and the strong influence that the composition of the phytoplankton community can have on water quality. In this sense, a predictive understanding of the effects of increased nutrients on the phytoplankton community is of great importance (Cottingham et al., 1998).

Phytoplankton growth is dependent mainly on adequate light intensity and nutrients availability (Huovinen et al., 1999). The most common nutrient limiting photoautotrophic production in freshwater ecosystems is phosphorus, which reaches the systems from sources such as inflow, agricultural run-off and non-point sources (Esteves, 1998; Flöder et al., 2006).

There are several known and unknown direct and indirect effects of climate change on water quality. Climate change will significantly alter functioning of shallow water bodies and seasonal patterns in water quality (Carvalho and Kirika, 2003), because higher average temperatures will lead to elevated biological rates (Choi, 1998), and not only the life cycle of individual species, but also the dynamics of entire food webs may be profoundly affected by climate change (Scheffer et al., 2001). Further understanding of the effect of climate change and the eutrophication process and specially their interactions are required to predict impacts and to start developing management and restoration tools (Carvalho and Kirika, 2003).

This study aimed at understanding the response of the phytoplankton biomass (as chlorophyll-*a* concentration) to simulated conditions of increased water temperature and phosphorus concentration in Peri coastal lagoon. Three hypotheses were formulated: 1) Increased temperature will significantly increase phytoplankton biomass in the microcosms, since higher temperatures lead to elevated biological rates; 2) Phosphorus enrichments will lead to higher phytoplankton biomass in the P-limited Peri lagoon; and 3) The combined effect of increased temperature and phosphorus concentration on water will produce higher chlorophyll-*a* levels than the individual effect of the two treatments.

2. Material and Methods

2.1. Study site

Peri lagoon is located in South Brazil, Santa Catarina State, in the southeastern portion of Santa Catarina island (27° 44' S and 48° 31' W), Florianópolis. It has a surface area of 5.7 km² surrounded by mountains covered by Atlantic Rain Forest in the south, west and north portions and by a sandy Restinga in the east portion. The lagoon presents a maximum depth of approximately 11.0 m, average depth of 7.0 m, and no direct sea water influence (freshwater all over the year). It is a non-stratifying water body and presents a relative spatial homogeneity concerning water quality features (Hennemann and Petrucio, in press).

The lagoon and surroundings (including almost the entire drainage basin) are inside an environmentally protected area ("Parque Municipal da Lagoa do Peri") with a restricted human use and occupation since 1981. Since 2000, the lagoon supplies potable water to a significant percentage of the inhabitants of Santa Catarina island. The climate in the area is characteristically subtropical.

A concerning issue in the lagoon is the increasing monthly phytoplankton densities from 3,079-41,246 individuals.mL^{-1} observed in 1996 (Laudares-Silva, 1999) to 40,305-116,961 individuals.mL^{-1} in 2004 (Grellmann, 2006). The establishment and increasing dominance of the potentially toxic cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju, which augmented from a maximum density of less than 18,000 individuals.mL^{-1} in 1996 to 85,613 individuals.mL^{-1} in 2004 and increased its dominance from a maximum of 56% to 85% in the same period (Laudares-Silva, 1999; Grellmann, 2006) is also a matter that deserves careful monitoring and attention. Chlorophyll-*a* increased from an annual mean of 13.9 µg.L^{-1} (Laudares-Silva, 1999) to 19.2 µg.L^{-1} (Grellmann, 2006) and the number of taxa found monthly decreased (from 10-29 in 1996 to 11-21 in 2004), which indicates

that *C. raciborskii* is increasing and probably also outcompeting other species and changing the phytoplankton composition. In spite of the alterations on phytoplankton densities, no strong variations on trophic relevant water quality parameters were observed in the last 15 years in the lagoon (Hennemann and Petrucio, in press).

The lagoon is strongly P-limited according to previous studies (Laudares-Silva, 1999) and samplings carried out during the present study. Further information on water quality trends in Peri lagoon is available in Hennemann and Petrucio (in press).

2.2. Experimental design

Three microcosm experiments were conducted in laboratory during 7 days in March 2009 (summer), May 2009 (autumn) and July 2009 (winter) to assess the influence of increased temperatures and phosphorus (*P*) concentrations on the phytoplankton community of Peri lagoon in different seasons. Samples from the lagoon were collected 12 hours previous to the start of the experiments and were kept at room temperature in the dark.

To test the effects of increasing temperatures, three thermostatic light chambers were used. All had the same characteristics and same light intensities (5% of solar incidence on the water surface). Light measurements were made by a luxmeter (Extech 401025). Microcosms were incubated in 1 L glass flasks for 3 and 7 days with 14/10 hours (summer), 13/11 hours (autumn) and 12/12 hours (winter) light/dark photoperiods. The flasks were swirled once a day to resuspend plankton. The first chamber was kept at environmental (control) temperature (28 °C in summer, 23 °C in autumn, and 18 °C in winter experiments), the second was kept +3 °C warmer (31, 26 and 21 °C in summer, autumn and winter, respectively), and the third one, +5 °C warmer (33, 28 and 23 °C). In each incubating chamber, three P concentrations were tested: control (environmental concentration, around 1.5 $\mu g.L^{-1}$), four times P enrichment (+6 $\mu g.L^{-1}$ of KH_2PO_4), and eight times P enrichment (+12 $\mu g.L^{-1}$ of KH_2PO_4). So a factorial design of 3 x 3 was performed (three temperature treatments X three P treatments), with triplicates of each treatment.

Samples for water temperature, dissolved oxygen (oxygen saturation), pH, conductivity, alkalinity, soluble reactive phosphorus (*SRP*), total phosphorus (*TP*) and chlorophyll-*a* (chl-*a*) concentrations were taken at the start of incubation (T = 0) and after three and seven days of experiment.

2.3. Laboratory analysis

Water temperature, conductivity, pH and dissolved oxygen were measured with specific probes (WTW Multi350i and Digimed DM-3P, DM-4P and DM-22). Nitrite (Golterman et al., 1978), nitrate (Mackereth et al., 1978), ammonium (Koroleff, 1976), SRP (Strickland and Parsons, 1960) and total phosphorus and nitrogen (Valderrama, 1981) concentrations were determined in laboratory from filtered and unfiltered frozen water samples kept in polyethylene bottles at -20 °C. Chl-*a* concentrations were obtained by filtering 500 mL water samples through glass fiber filters Millipore AP40 using the method and equation described by Lorenzen (1967).

2.4. Statistical analysis

The individual effect of increased temperature and phosphorus enrichments on the measured parameters was tested by one-way analysis of variance (ANOVA), followed by Tukey HSD post-hoc. The combined effect of temperature and phosphorus was tested by two-way ANOVA, followed by Tukey HSD post-hoc. Pearson's correlation was performed to test if temperature and phosphorus increases had positive or negative effect on the other variables.

3. Results

3.1. Water quality

Table 1 shows the mean values for some water quality parameters in Peri lagoon in the three months microcosm experiments were carried out. Nitrite and nitrate were in very low concentrations (less than 1.0 $\mu g.L^{-1}$) and are therefore not shown. Molar TN:TP ratios were always higher than 130, demonstrating strong P-limitation according to Guildford and Hecky (2000).

3.2. Increased temperature responses

Temperature in the Summer experiment was positively correlated to oxygen saturation (R = 0.89). Oxygen saturation (and dissolved oxygen concentration, data not shown) significantly increased in all treatments in all days following the sequence: control 28 < 31 < 33 °C (Figure 1a). Significant pH and chl-*a* decreases ($p < 0.01$) in relation to initial values (Table 2) were detected in most temperature treatments. Chl-*a* significantly dropped from initial concentration (~18 $\mu g.L^{-1}$) in all treatments at day 7 and in treatment 31 °C

Table 1. Water quality parameters measured in Peri lagoon in the three months of microcosm experiments.

	March 2009	May 2009	July 2009
Water temperature (°C)	27.4	22.0	18.0
Oxygen saturation (%)	92.3	95.7	87.9
Secchi depth (m)	1.1	0.9	1.0
pH	7.1	7.0	6.7
Conductivity (µS.cm^{-1})	71.4	69.4	71.2
Alkalinity (mEq.CO_2.L^{-1})	0.03	0.05	0.04
SRP (µg.L^{-1})	1.6	1.4	1.7
Ammonium (µg.L^{-1})	8.0	19.6	15.5
TP (µg.L^{-1})	14.2	13.3	15.7
TN (µg.L^{-1})	917.2	785.7	989.6
TN:TP molar ratio	143	134	140
Chlorophyll-*a* (µg.L^{-1})	19.3	4.7	16.5

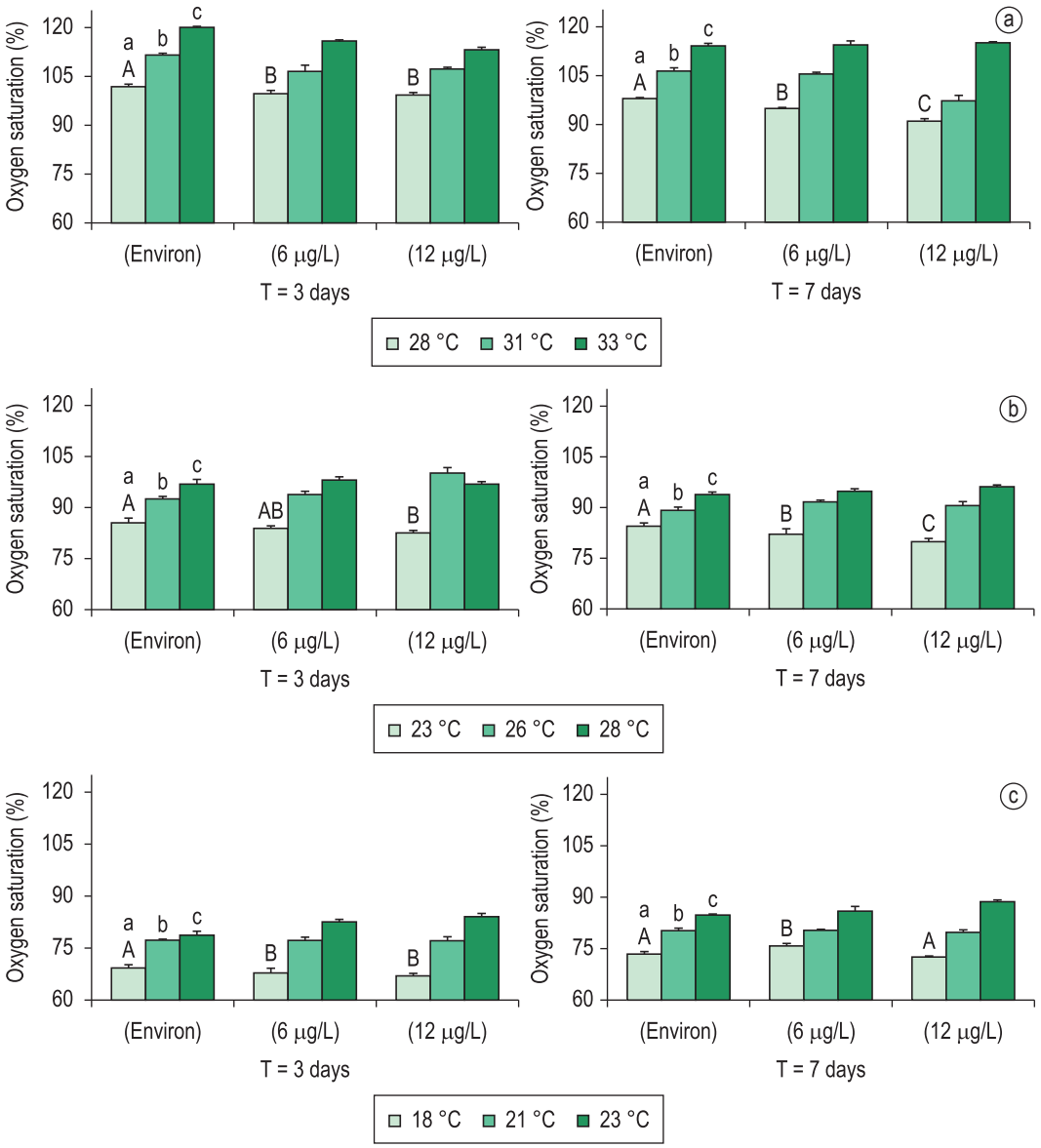

Figure 1. Oxygen saturation at day 3 (left graphics) and day 7 (right graphics) in a) summer; b) autumn; and c) winter microcosm experiments. Different phosphorus concentrations are shown in the x-axis and different temperatures as three scales of green bars (control temperature = light green bars; +3 °C = medium green bars; +5 °C = dark green bars). Error bars denote standard deviations. Different upper cases indicate significant P enrichment effects and different lower cases indicate significant temperature effects.

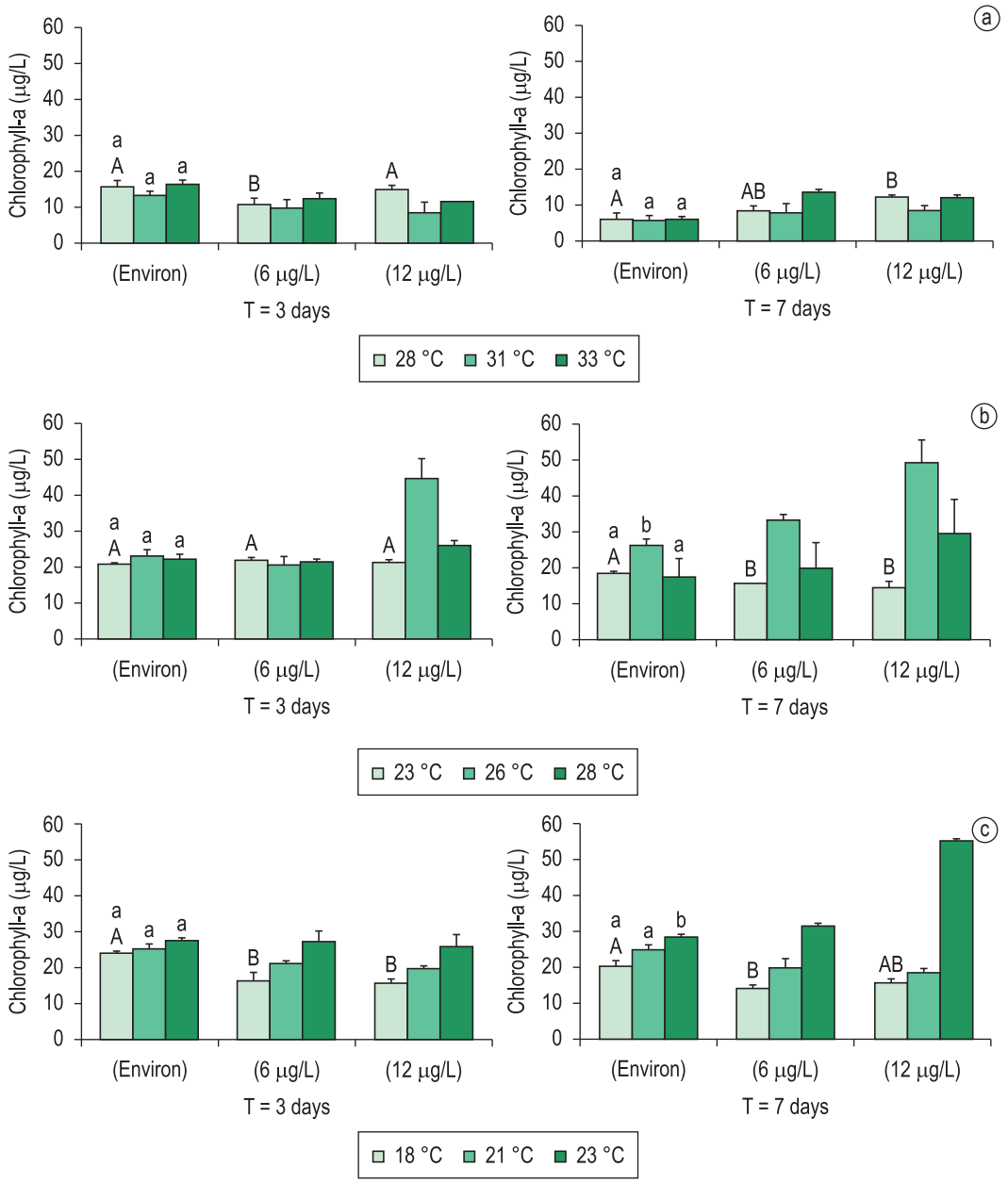

Figure 2. Chlorophyll-*a* concentration at day 3 (left graphics) and day 7 (right graphics) in a) summer; b) autumn; and c) winter microcosm experiments. Different phosphorus concentrations are shown in the x-axis and different temperatures as three scales of green bars (control temperature = light green bars; +3 °C = medium green bars; +5 °C = dark green bars). Error bars denote standard deviations. Different upper cases indicate significant P enrichment effects and different lower cases indicate significant temperature effects.

at day 3 (Figure 2a). Chl-*a* was statistically equal ($p > 0.05$) to controls (28 °C) in all temperature treatments and days.

In the Autumn experiment, temperature was weakly but positively correlated to chl-*a* ($R = 0.27$) and oxygen saturation ($R = 0.88$). Oxygen saturation (Figure 1b) significantly increased with increasing temperature (23 < 26 < 28 °C). Differently from the observed in summer, pH values (Table 2) were significantly higher ($p < 0.05$) in most treatments when compared to initial. Alkalinity (Table 2) significantly increased from the initials at temperature 26 °C in both days. Chl-*a* (Figure 2b) did not increase from initial (~21 µg.L^{-1}) and control concentrations in any temperature treatment and between temperatures, except for day 7, when microcosms at 26 °C were significantly higher ($p < 0.05$) than controls and 28 °C treatments.

Winter experiment showed the strongest response to increasing temperature. Positive

Table 2. Conductivity, pH and alkalinity mean values measured in summer, autumn and winter experiments at days 0, 3 and 7. Phosphorus treatments are shown as columns and temperature treatments as rows. Values in parenthesis indicate standard deviations. (environ) = natural SRP concentrations; (6µg) = 6µg.L^{-1} PO_4^{3+} addition; (12µg) = 12µg.L^{-1} PO_4^{3+} addition.

		pH						Conductivity						Alkalinity					
		3 days			7 days			3 days			7 days			3 days			7 days		
		[environ]	[6 µg]	[12 µg]	[environ]	[6 µg]	[12 µg]	[environ]	[6 µg]	[12 µg]	[environ]	[6 µg]	[12 µg]	[environ]	[6 µg]	[12 µg]	[environ]	[6 µg]	[12 µg]
Summer	T = 0	6.57	6.41	6.29	6.57	6.41	6.29	71.7	72.6	72.6	71.7	72.6	72.6	0.05	0.05	0.04	0.05	0.05	0.04
		(0.06)	(0.01)	(0.03)	(0.06)	(0.01)	(0.03)	(0.4)	(0.8)	(0.3)	(0.4)	(0.8)	(0.3)						
	28 °C	6.30	6.32	6.21	6.15	6.02	5.96	79.4	77.9	69.3	89.3	91.1	79.2	0.04	0.04	0.04	0.04	0.04	0.04
		(0.09)	(0.06)	(0.08)	(0.05)	(0.06)	(0.05)	(1.2)	(0.5)	(1.1)	(1.5)	(1.9)	(1.1)						
	31 °C	6.41	6.45	6.42	6.30	6.29	6.17	75.1	72.4	74.8	85.2	83.7	87.5	0.05	0.05	0.05	0.07	0.06	0.06
		(0.07)	(0.01)	(0.15)	(0.04)	(0.04)	(0.07)	(0.3)	(4.1)	(1.4)	(1.7)	(1.3)	(2.6)						
	33 °C	6.18	6.03	6.11	6.01	5.97	6.05	69.8	68.8	69.3	82.2	83.7	77.4	0.04	0.04	0.04	0.04	0.04	0.04
		(0.08)	(0.06)	(0.11)	(0.01)	(0.04)	(0.00)	(1.2)	(1.6)	(0.3)	(3.0)	(0.8)	(2.4)						
Fall	T = 0	6.97	6.82	6.90	6.97	6.82	6.90	84.2	85.8	86.3	84.2	85.8	86.3	0.04	0.05	0.05	0.04	0.05	0.05
		(0.00)	(0.15)	(0.14)	(0.00)	(0.15)	(0.14)	(0.0)	(0.5)	(0.3)	(0.0)	(0.5)	(0.3)						
	23 °C	7.29	7.12	7.06	7.38	7.16	7.10	85.0	80.7	76.9	90.3	86.1	86.8	0.05	0.05	0.05	0.06	0.05	0.05
		(0.06)	(0.10)	(0.08)	(0.07)	(0.05)	(0.04)	(4.8)	(6.4)	(1.2)	(5.9)	(4.9)	(0.8)						
	26 °C	7.23	7.26	7.88	7.14	7.24	7.20	86.9	81.0	76.3	90.3	92.4	89.6	0.07	0.06	0.05	0.07	0.07	0.07
		(0.04)	(0.05)	(0.16)	(0.09)	(0.12)	(0.09)	(6.7)	(1.8)	(1.4)	(2.1)	(9.3)	(3.3)						
	28 °C	7.20	7.07	7.22	7.14	7.07	6.91	77.6	76.8	78.4	85.3	85.5	85.7	0.05	0.04	0.04	0.05	0.05	0.05
		(0.05)	(0.13)	(0.08)	(0.01)	(0.06)	(0.12)	(1.6)	(1.0)	(0.5)	(2.3)	(2.3)	(2.9)						
Winter	T = 0	6.46	5.45	5.31	6.46	5.45	5.31	87.5	80.9	87.0	87.5	80.9	87.0	0.04	0.04	0.03	0.04	0.04	0.03
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)						
	18 °C	5.68	5.62	5.79	5.66	5.80	5.61	93.0	90.5	89.5	91.5	95.4	96.5	0.03	0.03	0.03	0.06	0.05	0.05
		(0.09)	(0.08)	(0.30)	(0.11)	(0.16)	(0.09)	(3.6)	(6.6)	(2.2)	(1.4)	(1.2)	(1.7)						
	21 °C	5.90	5.79	5.99	5.45	5.70	5.62	87.4	86.1	83.4	96.4	94.9	94.0	0.04	0.03	0.04	0.05	0.04	0.04
		(0.19)	(0.15)	(0.37)	(0.12)	(0.10)	(0.14)	(3.3)	(1.4)	(1.4)	(6.2)	(0.9)	(5.8)						
	23 °C	5.52	5.57	5.55	5.84	6.22	6.00	93.7	88.7	86.4	106.9	99.2	93.7	0.06	0.06	0.05	0.07	0.06	0.05
		(0.25)	(0.16)	(0.09)	(0.08)	(0.09)	(0.03)	(2.5)	(3.5)	(1.5)	(7.5)	(7.1)	(4.6)						

correlations between temperature and alkalinity (R = 0.53), chl-*a* (R = 0.67) and oxygen saturation (R = 0.89) were found. Oxygen saturation (Figure 1c) significantly increased ($p < 0.05$) in most treatments according to the sequence: control 18 < 21 < 23 °C. Higher pH values (Table 2) than initials were observed at a significant level ($p < 0.05$) in all microcosms, but pH did not respond to temperature rises. Alkalinity (Table 2) was higher ($p < 0.05$) in the higher temperature and at day 7 in relation to day 3. Chl-*a* (Figure 2c) increased from the initial concentrations (~16 $\mu g.L^{-1}$) in all microcosms except for day 7, temperature 18 °C. A tendency of increasing chl-*a* concentrations with increasing temperatures could be observed, but only at day 7, treatment 23 °C was statistically higher ($p < 0.05$) than control temperature (18 °C).

3.3. Phosphorus enrichment responses

In Summer enrichment experiments, pH, conductivity and alkalinity (Table 2) had no clear or strong responses to P additions. Enrichments negatively affected oxygen saturation (Figure 1a) at days 3 and 7 since lower saturations ($p < 0.05$) were observed in [6 $\mu g.L^{-1}$] and [12 $\mu g.L^{-1}$] treatments when compared to non-enriched treatments. Chl-*a* (Figure 2a) dropped from initial concentrations (~18 $\mu g.L^{-1}$) in most P treatments. At day 3, [6 $\mu g.L^{-1}$] P enrichment showed a significant drop in chl-*a* concentrations from non-enriched and [12 $\mu g.L^{-1}$] microcosms. At day 7, [12 $\mu g.L^{-1}$] enrichments increased ($p < 0.05$) chl-*a* concentrations in relation to control.

Autumn enrichment experiments showed no important variations on pH, conductivity and alkalinity (Table 2). A tendency of decreasing oxygen saturation with increasing P concentrations could be observed, but it was only significant ($p < 0.05$) from non-enriched to [12 $\mu g.L^{-1}$] enrichments. Chl-*a* (Figure 2b) remained similar to initial concentrations (~21 $\mu g.L^{-1}$) or significantly dropped (day 7, enriched microcosms). No influence of the P enrichments could be noticed at day 3 experiments, but significant ($p < 0.05$) decreases on chl-*a* concentration occurred in enriched microcosms in relation to control at day 7.

In the Winter microcosms, pH, conductivity and alkalinity (Table 2) maintained similar values in all P treatments as occurred in summer and autumn experiments. However, alkalinity increased from day 3 to 7 ($p < 0.05$) in most treatments. Enrichments seem to have negatively affected oxygen availability at day 3, since saturation decreased ($p < 0.05$) from non-enriched to [12 $\mu g.L^{-1}$] treatments. On the other hand, oxygen saturation increased ($p < 0.05$) from non-enriched to [6 $\mu g.L^{-1}$] microcosms at day 7. Chl-*a* (Figure 2c) only increased ($p < 0.05$) from initial concentrations (~16 $\mu g.L^{-1}$) at day 3, non-enriched microcosms. Enriched microcosms showed lower chl-*a* concentrations ($p < 0.05$) than non-enriched ones at both day 3 and 7 of incubation.

TP concentrations demonstrated that microcosms were successfully enriched by KH_2PO_4 additions (non-enriched: 12-14 $\mu g.L^{-1}$; 6 $\mu g.L^{-1}$ PO_4^{3+} additions: 17-19 $\mu g.L^{-1}$; 12 $\mu g.L^{-1}$ PO_4^{3+} additions: 23-26 $\mu g.L^{-1}$). SRP concentrations did not show significant increases or clear response patterns among controls and enriched experiments (concentrations in microcosms varied between 0.2 and 4.8 $\mu g.L^{-1}$).

3.4. Temperature + phosphorus enrichments effect

Combined effect of temperature and P could be observed in Summer (two-way ANOVA, F = 7.57, $p < 0.000$) at day 3, when chl-*a* concentration significantly decreased in temperature 33 °C from non-enriched to [12 $\mu g.L^{-1}$] treatment. An opposite result was obtained in microcosms at day 7, when higher chl-*a* was detected at higher temperatures (33 °C) and at higher P concentrations (6 $\mu g.L^{-1}$ and 12 $\mu g.L^{-1}$ PO_4^{3+} additions).

Increased temperature of 3 °C (26 °C) and the higher P enrichment (12 $\mu g.L^{-1}$) also significantly increased chl-*a* in Autumn experiments at days 3 and 7 in relation to controls (two-way ANOVA, F = 4.38, $p < 0.000$). pH followed the same pattern, showing higher values at the 26 °C and enrichment [12 $\mu g.L^{-1}$] at day 3. Alkalinity also increased at 26 °C. A tendency of increasing oxygen saturation in higher temperatures and P additions can be observed in autumn microcosms.

A similar response was observed in Winter (two-way ANOVA, F = 6.63, $p < 0.000$) microcosms at day 7, when the highest chl-*a* concentration was observed in the highest temperature (23 °C) and P enrichment (12 $\mu g.L^{-1}$). Chl-*a* significantly increased with temperature in enriched treatments (23 °C > control 18 °C, in microcosms [6 $\mu g.L^{-1}$] and [12 $\mu g.L^{-1}$] at days 3 and 7). Winter experiments also showed a combined but less pronounced effect of temperature and P on oxygen saturation at days 3 and 7 (higher saturation at the highest temperature and P addition of 12 $\mu g.L^{-1}$ when compared to non-enriched microcosm and control temperature 18 °C). PH followed chl-*a* at day 7,

showing increased values in higher temperatures in enriched microcosms.

4. Discussion

4.1. Summer microcosms

Increases observed in oxygen saturation (and dissolved oxygen concentration, data not shown) at both days 3 and 7 of experiment could be an indirect indication of higher primary production rates in higher temperatures.

Significant drops in chl-*a* from initial concentrations and especially after 7 days of incubation indicate that summer microcosms might have negatively interfered in the phytoplankton community. Constant high temperatures (>30 °C) probably negatively affected cyanobacterial growth, as recently demonstrated by Mehnert et al. (2010). In fact, a thin layer was observed attached to the bottom of the flasks at the end of the each incubation period. The thin layer was probably composed by phytoplankton individuals that tried to reach "deeper" waters in order to avoid the high temperatures and by dead cells. This phenomenon could have lead to the lower chl-*a* concentrations found. Oxygen production by photosynthesis probably continued even in the bottom of the flasks, but decreases in pH values were also observed, possibly due to higher CO_2 levels associated to higher respiration rates and bacterial growth to decompose dead phytoplankton.

P enrichments positive effects on chl-*a* at day 7 but not at day 3 could be explained by studies that showed that bacteria are effective competitors for P (Currie and Kalff, 1984) and may sequester P or delay its availability to phytoplankton (Cottingham et al., 1997). Moreover, P enrichments could only be observed through TP concentrations, because no clear effect was noticed on SRP values, probably due to rapid absorption of the dissolved P added to the microcosms by bacteria. *Cylindrospermopsis raciborskii* is also a strong competitor for P, since this species is able to rapidly absorb this nutrient and store it as polyphosphate granules inside the cells for posterior use (luxurious consumption) (Isvánovics et al., 2000). In spite of that, the same authors demonstrated that large P pulses, instead of P continuous supply, can delay *C. raciborskii* cells growth, what could also have happened in our microcosms and caused the delayed response in chl-*a* concentrations.

Higher temperatures and P enrichment at day 3 significantly dropped chl-*a* concentration in relation to controls, but at day 7, chl-*a* increased at 33 °C, in enrichment [12 $\mu g.L^{-1}$]. The lower chl-*a* concentrations in enriched microcosms at day 3 could be a consequence of intense bacteria competition for resources at the beginning of the incubation period, as explained above. On the other hand, at day 7, P may have become available to phytoplankton and increased chl-*a* concentrations.

These results demonstrate that global temperature rises are probably not going to significantly affect phytoplankton biomass in Peri lagoon on summer months, but elevated temperatures combined with increased P inputs to the lagoon may lead to higher primary production rates.

4.2. Autumn microcosms

The same pattern of increased oxygen saturation in higher temperatures was observed in autumn microcosms, probably indicating increased primary production rates as well.

Temperature had no effect at the beginning of the experiment, but at day 7, 3 °C increase in temperature significantly elevated chl-*a* concentration in relation to control and 5 °C rise. This result is in accordance with the results observed by Mehnert et al. (2010), which showed that *C. raciborskii* has an optimum growth at temperatures between 25 and 30 °C. Alkalinity also increased in this treatment, indicating lower CO_2 levels, which is probably related to higher consumption by photosynthesis. These results show that increases in temperature in autumn will probably lead to higher primary productivity, but if increases are too dramatic, effects will be less pronounced probably because several phytoplankton species may be negatively affected when temperatures become too high.

Correlation between temperature and chl-*a* was weak, but is supported by oxygen increases at days 3 and 7 and by chl-*a* increases at day 7.

The apparent negative effect of P on phytoplankton biomass at day 7 was followed by decreases in oxygen saturation levels and on pH values, what can be indicative of lower primary production and higher respiration rates, leading to lower O_2 and higher CO_2 concentrations in the microcosms.

On the other hand, the combined effect of P enrichment [12 $\mu g.L^{-1}$] and rises in temperature of 3 and 5 °C significantly increased chl-*a* concentration and oxygen saturation at day 7. Alkalinity and pH increases at 26 °C followed chl-*a*. High pH values reflect increases in rates of carbon fixation and production of chl-*a* by phytoplankton

(Schelske et al., 1974), while higher alkalinity reflects lower CO_2 levels, as previously explained.

As in summer microcosms, the responses to P enrichments seem to have had a delay apparently by the same reasons (rapid bacterial and cyanobacterial P uptake and delay in phytoplankton growth). Chl-*a* levels did not increase in non-enriched microcosms at the highest temperature but in enriched microcosms, a significant increase could be noticed. P limitation could have prevented further development of the phytoplankton community in non-enriched microcosms.

C. raciborskii have been shown optimal growth rates between 25 and 30 °C (Mehnert et al., 2010). If autumn (and spring) temperatures were elevated by climate changes, it could result in intense cyanobacterial growth with unclear consequences for Peri lagoon and water supplying.

The results from autumn microcosms show that increased global temperatures can significantly alter phytoplankton biomass in Peri lagoon, but combined with increased P inputs the effect can be even greater.

4.3. Winter microcosms

A clear tendency of increasing chl-*a* concentrations with increasing temperature could be noticed in winter microcosms. According to Carvalho and Kirika (2003), responses of annual water quality trends to climate change are difficult to predict, but increasing phytoplankton biomass in colder months are likely to happen. Alkalinity and oxygen saturation had a positive correlation with temperature and significantly responded to increased temperatures in most treatments, indicating CO_2 consumption and O_2 production, respectively.

As observed in the summer and autumn experiments, P additions negatively affected chl-*a* and oxygen saturation. Intense bacterial growth and competition for resources with phytoplankton in enriched microcosms and/or *C. racisborskii* P storage capacity and slow growth response to P inputs are possible explanations for such results.

The combined effect of elevated temperatures and P enrichments lead to significantly higher chl-*a* levels, up to four times higher than the concentrations usually found during colder months. Oxygen saturation (at days 3 and 7) and pH (at day 7) also increased with elevated temperatures and P concentrations, probably reflecting a positive combined effect of these two factors on primary production rates.

These results also suggest that global temperature changes are likely to affect the phytoplankton biomass in Peri lagoon, but if this weather changes are combined with increased sewage inputs or other kinds of P inputs, the outcome can be of a much greater concern.

Several microcosm and mesocosm experimental studies simulating nutrient enrichments and other environmental changes have been developed in the last decades in all kinds of inland waters. Most of these studies also showed no positive influence of P enrichments alone, but significant increases chl-*a* were observed in N and especially N+P enrichments (Taylor et al., 1995; Havens et al., 1996; Camacho et al., 2003; Dzialowski et al., 2005; Sagrario et al., 2005; Hlaili et al., 2006). However, Ramirez-Olvera et al. (2009) found that P and N alternate in limiting a deep saline lake in Mexico, but P enrichments resulted in chl-*a* increases in most microcosm experiments.

Thompson et al. (2008) observed that N additions alone had no effect on chl-*a*, but N additions combined with warmer temperatures resulted in significant chl-*a* increases in microcosms with samples from a Canadian alpine lake, a combined effect similar to the one found by our study.

The results obtained in the present study indicate that global changes are very likely to interfere in the phytoplankton community of Peri lagoon. Temperature rises can lead to increased chl-*a* concentrations in autumn and winter months, but a negative effect on summer phytoplankton community is expected from the results obtained in the microcosms. Small P inputs to the lagoon without other environmental changes will probably have no profound effects on primary producers. On the other hand, the combined effect of global temperature rises and increased P inputs can significantly augment phytoplankton biomass in Peri coastal lagoon in both cold and warm months. These are concerning results since several bloom forming species of Cyanobacteria are present in the lagoon and environmental alterations can lead to toxin production with dramatic consequences to other living organisms within the lagoon and to people that depend on the lagoon as a water supplier.

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