Photosynthetic performance associated with phosphorus availability in mats of *Didymosphenia geminata* (Bacillariophyceae) from Patagonia (Argentina and Chile)

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ABSTRACT: Recently reported blooms of *Didymosphenia geminata* from rivers in Argentinean and Chilean Patagonia, together with non-typical blooms reported from lake systems, have the potential to alter community and ecosystem dynamics of aquatic environments in the region. In an effort to quantify key aspects of ecosystem function of *D. geminata* mats in Patagonia, we describe for the first time photosynthesis–irradiance (*P-E*) curves of this species, associated *P-E* parameters (R, P_{max} and α) and their relationship to other functional (alkaline phosphatase activity [APA] enzyme activity and chlorophyll *a*) and environmental variables (soluble reactive and organic phosphorous, electrical conductivity). Mats of *D. geminata* from lakes and rivers representing east and west sides of the Andes from 40°S to 46°S (i.e. much of current known distribution) varied mostly in the α and P_{max} parameters of *P-E* curves. Southern systems showed a higher α and lower P_{max} parameters than more northern sites on the east slope of the cordillera. None of the *P-E* curves showed photoinhibition. *P-E* parameters for the two lakes fell within the range of values for riverine mats; although, soluble reactive P was notably higher in the lakes. We used two different statistical approaches to evaluate associations across environmental, functional or *P-E* parameters, finding a strong relation between APA and P_{max} and a weak one between soluble reactive phosphorus, pH, dissolved organic phosphorus and functional parameters. Our results do not contradict previous observations on the importance of P availability in communities of *D. geminata*; although the variability in response across *P-E* parameters suggests a more complex mechanism of regulation than implied in the existing literature.

KEY WORDS: Diatoms, Invasive species, Phosphorus, Photosynthesis–irradiance curves

INTRODUCTION

The benthic diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt has attracted considerable attention as a general nuisance and probable invasive species of inland waters in temperate and boreal zones. It has become a major concern for its tendency to form conspicuous blooms (predominantly extracellular stalk material) in oligotrophic aquatic systems, with potential for detrimental impacts to recreational fishing (Spaulding & Elwell 2007) and ecosystem function (Reid & Torres 2014). While its native status in South America has been debated (Taylor & Bothwell 2014), blooms in Patagonia appear to be a recent phenomenon and also have shown a clear expanding range. The first report of blooms of *D. geminata* was in May 2010, in River Futaleufú Chile (CIEP 2010). It was subsequently detected upstream, in August 2010 in Argentina (Sastre *et al.* 2013) and in November 2011 in the River Manso (Río Negro Province, Argentina) (M.E. Alemanni, pers. comm. 2011) and in Chimehuin and Collon Cura Rivers (Neuquén Province, Argentina) (Abelli Bonardi *et al.* 2012). Its current range spans over 2000 km, from Tierra del Fuego (52°S) to the Bio Bio watershed (32°S). In January 2013, massive blooms were found in Lake Nahuel Huapi (Río Negro Province,

Argentina), the first published record of massive growth in a South American lake (Beamud *et al.* 2013). In general, lentic populations have drawn much less attention from researchers, with scarce documentation of invasive populations and only a few studies from within its native range (e.g. Lake Baikal, Flower 2004 in Whitton *et al.* 2009; Turkey, Sahin 2000; Russia, Medvedeva 2001; and in the Lake District in England, Godward 1937 in Whitton *et al.* 2009).

Cells of *D. geminata* attach to the substratum with an extracellular stalk composed primarily of sulphated polysaccharides (Gretz 2008) extruded from an apical pore at the base of the cell. When cells divide, the stalk bifurcates and forms a branched structure with stalks intercalating and coalescing to form an aggregate dense mat that contains algae along with macroinvertebrates, detritus and sediments. A wide range of niche parameters have been reported (summarized in Cullis *et al.* 2012). The most commonly cited factors for the development of blooms include oligotrophic streams influenced by snowmelt regime, which are circumneutral to basic pH, and stable base flow index (e.g. lake outlets) (Spaulding & Elwell 2007; Kilroy *et al.* 2008). In general, rapid increase in algal biomass in streams is usually associated with eutrophic conditions; hence, the striking appearance of blooms under oligotrophic conditions has drawn considerable attention and some controversy (Bothwell & Kilroy 2011; Kilroy & Bothwell 2011, 2012; Bothwell *et al.* 2012). The Didymo paradox (Sundareshwar *et al.* 2011), considered an unusual phenomenon in
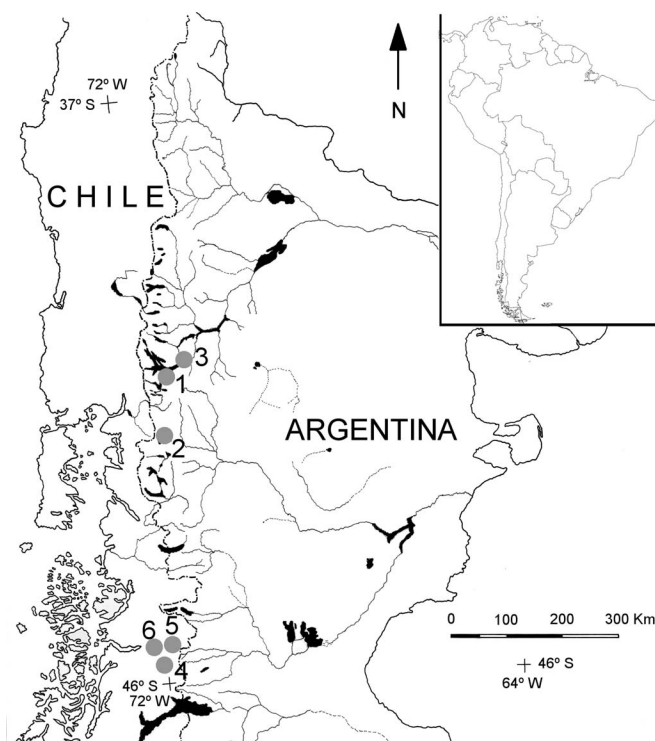
Fig. 1. Map of Northern Patagonia showing sampling sites of *Didymosphenia geminata* in Argentina ([1] Lake Nahuel Huapi; [2] River Azul; [3] River Limay) and Chile ([4] Lake Frío; [5] River Coyhaique; [6] River Claro).

continental waters, may not after all be an uncommon phenomenon in aquatic systems (Alcoverro *et al.* 2000). According to Bothwell *et al.* (2012), blooms occur only under P limitation, with soluble available P explaining the timing and spatial distribution of the blooms (Bothwell *et al.* 2014). Nevertheless, the timing and magnitude of nuisance blooms (e.g. excessive polysaccharide stalk production) can affect the ecology, hydrology, biogeochemistry and geomorphology, together with non–steady state aspects, of river dynamics. Consequently, these blooms represent a challenge and an opportunity for research on aquatic ecosystems.

Modelling the productive potential of an algal assemblage requires an accurate quantification of the efficiency with which resources such as light and nutrients are used. In turn,

these functional aspects are important in assessing the structure and function of aquatic ecosystems (Jones *et al.* 2014). Ecophysiological responses of organisms to irradiance changes and the estimation of photosynthesis rates *in situ* (Hill 1996) are usually studied employing non-linear relationships between photosynthetic rate and irradiance (photosynthesis–light curves, or *P-E* curves). These curves can be used to characterize photosynthetic aspects of the community, such as irradiance levels needed to saturate photosynthetic rates or photosystem efficiency. The *P-E* curve reflects the ability of the community to respond to short-term light fluctuations (time-scale of seconds to minutes), and the characteristics of this curve are dependent on the photo-acclimation or light exposure history (Geider 1993). Parameters describing *P-E* curves and corresponding environmental conditions are useful in understanding the processes and the physiology of natural algal assemblages, specifically how photosynthetic rates respond to changing environmental conditions (Cote & Platt 1983). In the interest of better understanding blooms of *D. geminata*, we describe for the first time *P-E* curves of mats from Patagonian aquatic environments (Argentina and Chile), together with the relationship between *P-E* parameters (R, P_{max} and α) and other functional (alkaline phosphatase activity [APA] enzyme activity and chlorophyll *a*) and environmental variables (soluble reactive and organic phosphorous, pH, electric conductivity). Recently documented mats from lake systems present an additional opportunity for comparison with local river populations. Finally, *P-E* performance and functional and environmental variables were evaluated across a representative geographic range in South America (much of the currently known extent, east and west sides of the Andes from 40°S to 46°S).

MATERIAL AND METHODS

Aquatic systems from Patagonia with blooms of *D. geminata* were sampled from lake and river systems from east and west slopes of the Andes in late spring (Fig. 1; Table 1). In each water body, pH and electrical conductivity (EC; Orion 3Star) were determined *in situ*. Stones with attached mats of *D. geminata* (1–4 cm thick) were collected from the littoral zone of lakes and from river beds (0.50 m depth) and stored in plastic containers. Surficial water samples were collected at

Table 1. Characterization of the rivers and lakes sampled: description, location, sampling dates and phenology of mats of *D. geminata*. (A) Argentina; (C) Chile.

Site	Description	Coordinates	Sample date	*D. geminata* phenology
Lake Frío (C)	Mesotrophic lake shallow (Z_{max} 12 m)	45.6685°S 71.9311°W	Dec. 2014	Senescent mats in wave exposed shores, 20 wk since max lake level
River Coyhaique (C)	Mesotrophic river cold steppe	45.5669°S 72.0286°W	Dec. 2014	Mature to senescent mats, 9–10 wk since snowmelt flood pulse
River Claro (C)	Oligotrophic river mountain/ snowmelt	45.5748°S 72.0811°W	Dec. 2014	Small colonies, strongly pigmented, < 4 wk post snowmelt
Lake Nahuel Huapi (A)	Oligotrophic lake deep (Z_{max} 450 m) snowmelt	41.1271°S 71.3509°W	Jan. 2014	Mature mats in wave exposed shores, 16 wk since max lake level
River Azul (A)	Oligotrophic river mountain/ snowmelt	42.0963°S 71.6285°W	Dec. 2013	Mature mats 9–10 wk since snowmelt flood pulse
River Limay (A)	Oligotrophic river mountain/ snowmelt	41.0569°S 71.1447°W	Dec. 2013	Mature mats strongly pigmented 9–10 wk since snowmelt flood pulse

Table 2. Values of pH, electrical conductivity (EC, μS cm^{-1}), soluble reactive P (SRP, μg P l^{-1}), dissolved organic P (DOP, μg P l^{-1}), phosphatase enzyme activity (APA, μmol *p*NPP g^{-1} h^{-1}) and chlorophyll *a* (mg Chl *a* g^{-1} AFDM) in rivers and lakes sampled. (A) Argentina; (C) Chile.

	pH	EC	SRP	DOP	APA	Chlorophyll
Lake Nahuel Huapi (A)	6.8	45.7	24.6	2.8	11.1	0.42
River Azul (A)	7.5[1]	53[1]	0.8	7.5	333.5	0.45
River Limay (A)	6.9	43.2	1.4	0.8	63.7	0.42
Lake Frío (C)	7.4	78.2	4.1	4.3	1.7	0.15
River Coyhaique (C)	7.7	80.9	1.3	4.8	0.6	0.46
River Claro (C)	7.6	44.1	3.6	n.d.	3.1	0.70

[1] Data from Miserendino & Pizzolon (2003).

each site and stored in high-density plastic bottles previously washed with deionized distilled water. All samples were analysed in the research labs of respective field sites (Centro CIEP, Coyhaique, Chile; CRUB-INIBIOMA, Bariloche, Argentina). Mats of *D. geminata* were cored using a 50 ml centrifuge tube (area = 7.06 cm^2) immediately on arrival at the laboratories, and subsamples were mounted on slides for morphological identification with a microscope at ×1000 magnification. Soluble reactive phosphorus (SRP) was analysed on filtered samples (membrane cellulose-acetate filters; 0.45 μm) by the ascorbic acid method (APHA 2005). Total dissolved phosphorus (TDP) was determined by the potassium persulfate acid oxidation method on filtered samples. Dissolved organic phosphorus (DOP) was estimated as a difference between TDP and SRP. Chlorophyll *a* concentration was determined spectrophotometrically from buffered acetone (90%) extracts directly from the *D. geminata* mats or on glass fibre filters (APHA 2005).

To determine the activity of phosphatase enzymes that catalyse the hydrolysis of organic phosphorus (APA), we followed the methodology proposed by Ellwood & Whitton (2007) with modifications described below. Mats were refrigerated and analysed within 24 h after sampling, after carefully removing detritus from the mats. Four-Nitrophenyl phosphate bis (cyclohexylammonium) salt (bis-*p*Npp) was used as substrate for the phosphatase enzyme. Approximately 30 mg of *D. geminata* mats were placed in flasks with 2.9 ml of buffered (20 mM Tris solution, pH 8.0) culture medium (modified Chu No. 10 – Chu 1942, with ethylenediaminetetraacetic acid as a chelator and without P or N). The assay was initiated with the addition of 0.2 ml of substrate (100 μM final concentration) and incubated for 20 min at low light in a shaking bath at 20°C. For each site, three replicates were processed for the following combinations: *D. geminata* + Medium + Substrate, *D. geminata* + Medium and Medium + Substrate. To finish the incubation and develop the colour of the product, an aliquot was extracted from each flask, and 2.5 ml of NaOH (1 M) was added. The concentration of the product was measured spectrophotometrically (410 nm). After the analysis, *D. geminata* material was processed for ash free dry mass (AFDM), at 60°C followed by 400°C, weighed on a Mettler Toledo (AB204) analytical balance.

Photosynthetic rates (*PR*) were determined as oxygen exchange using a microsensor (Microx TX3, Presens, Germany). Three mat replicates with water from each

sampling site were incubated in a 2 ml chamber at 20°C. To avoid oxygen micro-gradients inside the chamber, gentle stirring was provided by a magnetic stirrer. To estimate *PR*, the samples were incubated at increasing levels of light irradiance, which ranged from 0 to 1000 μmol m^{-2} s^{-1} of photosynthetic active radiation (PAR; calibrated against a Li-Cor radiometer equipped with a submersible quantum sensor LI-192SB). Each level of irradiance was applied until steady state oxygen production was observed (*c*. 5 min). The hyperbolic tangent function of Jassby & Platt (1976):

$$PR = P_{max}\tanh(\alpha E/P_{max}) + R$$

was used to describe the relationship between photosynthesis and irradiance. In this equation, *PR* is the rate of photosynthesis per unit of chlorophyll *a*, α is the initial slope of the *P-E* curve, *E* the photon flux density, P_{max} the maximum rate of gross photosynthesis and *R* the rate of dark respiration. I_k was calculated as the ratio P_{max}:α (Talling 1957) corresponding with the point at which the linear slope of the light saturation curve intersects the plateau.

The data were fitted by least square mean adjust using QtiPlot program. Data ordinations were performed with CANOCO version 5 for Windows (ter Braak & Smilauer 2012). Principal component analysis (PCA) was applied to examine the associations between the environmental variables (SRP, DOP), functional variables (APA, chlorophyll *a* of *D. geminata* mats) and the values of the parameters obtained from *P-E* curves (*R*, I_k, P_{max} and α). Pearson's (*r*) correlation coefficients were used to determine the degree of association between all the variables and parameters determined.

RESULTS

Table 2 shows pH, conductivity, SRP, DOP, APA rates and chlorophyll *a* (Chl *a*) in mats of *D. geminata* from lakes and rivers sampled. The pH ranged from 6.8 (Lake Nahuel Huapi) to 7.7 (River Coyhaique). Conductivity ranged from 43.1 μS cm^{-1} at River Limay to 80.9 μS cm^{-1} at River Coyhaique. The highest concentration of SRP was found in Lake Nahuel Huapi (24.6 μg P l^{-1}), while the other aquatic systems showed values below or equal to 4 μg P l^{-1}. DOP values were lower than 5 μg P l^{-1}, with the exception of River Azul (7.5 μg P l^{-1}). River Azul had the highest rate of APA (333.5 μmol *p*NPP g^{-1} h^{-1}). Chl *a* concentration in mats of *D. geminata* were similar in all the Argentinean systems (0.42–0.45 mg Chl *a* g^{-1} AFDM), while in Chilean systems the values were more variable (0.15–0.70 mg Chl *a* g^{-1} AFDM).

Changes in photosynthetic rates and light irradiance (*P-E*) curves of *D. geminata* mats are shown in Fig. 2. Parameters describing *P-E* curves, estimated with the hyperbolic tangent function, are shown in Table 3. P_{max} of mats of *D. geminata* ranged between 100 and 183 (μmol O$_2$ (mg Chl *a*)$^{-1}$ h^{-1}) in rivers, the lowest value from Lake Frío (96 μmol O$_2$ (mg Chl *a*)$^{-1}$ h^{-1}). The highest value of the initial slope α was found in River Claro (2.7 μmol O$_2$ (mg Chl *a*)$^{-1}$ h^{-1} μmol photon m^{-2} s^{-1}) and the lowest one in Lake Nahuel Huapi (0.5 μmol O$_2$
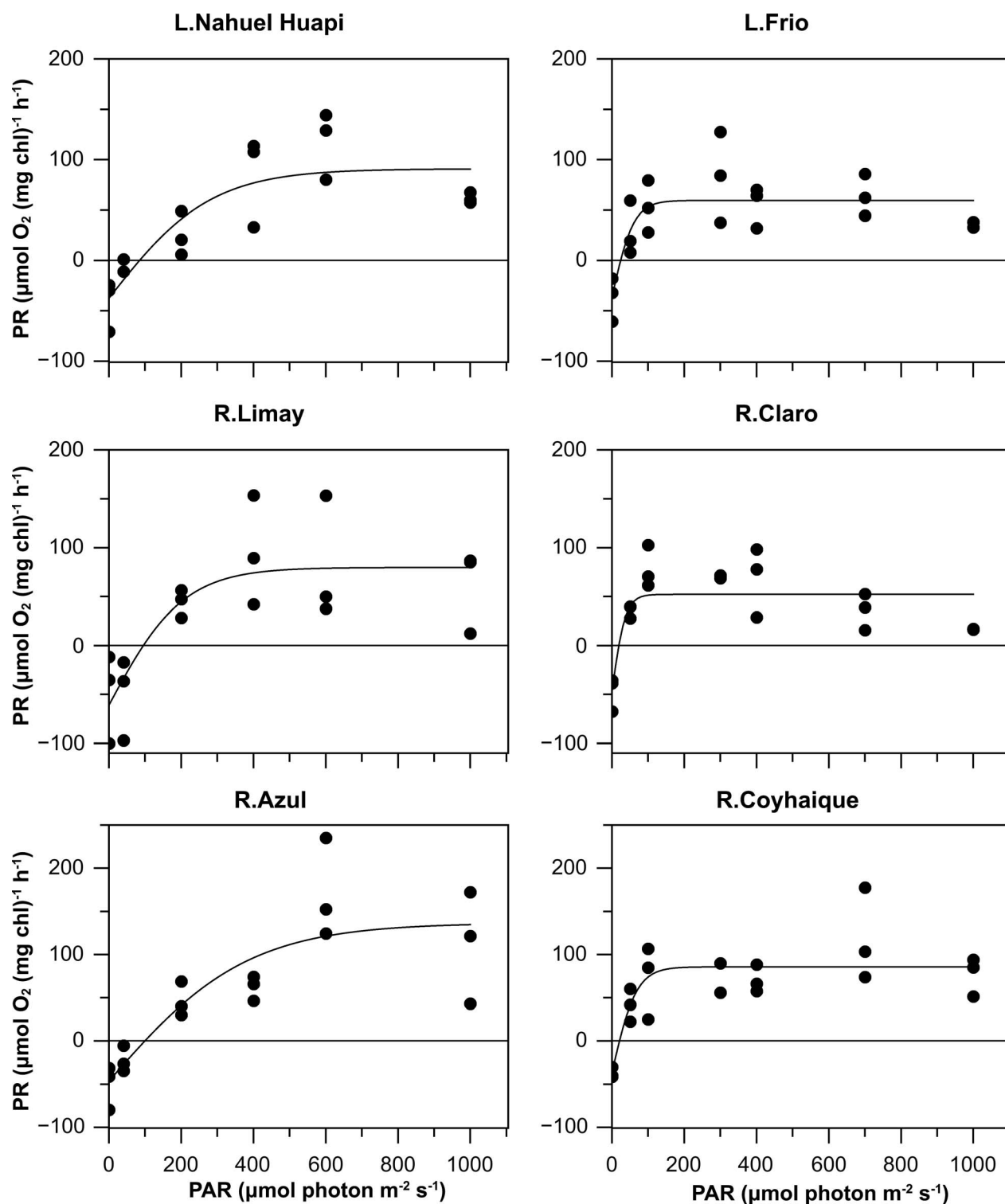
Fig. 2. Photosynthesis–irradiance curves (*P-E*) of mats of *Didymosphenia geminata* from the rivers Limay and Azul (Argentina), Claro and Coyhaique (Chile) and the lakes Nahuel Huapi (Argentina) and Frío (Chile). The lines represent the data fitted to the hyperbolic tangent function (see text).

(mg Chl a)$^{-1}$ h^{-1} μmol photon m^{-2} s^{-1}). Respiration rate R mostly ranged from 37 to 61 μmol O$_2$ (mg Chl a)$^{-1}$ h^{-1} and was slightly higher in River Limay compared with other sites. Light intensity at which photosynthesis is initially saturated (I_k) was lower in Chilean systems (37–67 μmol

photon m^{-2} s^{-1}) compared with Argentinean systems (205–384 μmol photon m^{-2} s^{-1}).

Correlations between environmental and functional variables and *P-E* parameters are shown in Table 4. The only significant ($P < 0.05$) correlations were between APA rates

Table 3. Parameters describing *P-E* curves using the hyperbolic tangent function: $PR = P_{max} \tanh (\alpha*E/P_{max}) + R$. α, initial slope of the *P-E* curve; E, photon flux density; P_{max}, maximum rate of gross photosynthesis; R, rate of dark respiration; I_k, light intensity at which photosynthesis is initially saturated. For all parameter estimates, ± 1 SE is given. R^2 shows the fitting degree of the data to the non-linear *P-E* curves.

	P_{max}	α	R	I_k	R^2
Lake Nahuel Huapi	128.6 ± 20.8	0.46 ± 0.1	−37.7 ± 16.4	281	0.68
River Azul	183.2 ± 30.0	0.48 ± 0.1	−46.4 ± 20.2	384	0.69
River Limay	140.9 ± 26.0	0.69 ± 0.3	−61.1 ± 21.6	205	0.59
Lake Frío	96.4 ± 17.2	1.62 ± 0.7	−36.9 ± 15.6	59	0.57
River Coyhaique	122.7 ± 20.0	1.83 ± 0.7	−37.0 ± 17.9	67	0.63
River Claro	99.6 ± 17.8	2.70 ± 1.5	−47.4 ± 16.1	37	0.58

and P_{max} ($r = 0.90$) and between P_{max} and I_k ($r = 0.90$) and a and I_k ($r = -0.89$). These last two correlations are obvious, yet not truly reliable, since I_k is estimated from P_{max} and α. High, although not significant, correlations were found between DOP and APA rates ($r = 0.66$), P_{max} and DOP ($r = 0.58$), pH and α ($r = 0.68$), EC and R ($r = 0.65$), I_k and APA ($r = 0.79$) and between P_{max} and α ($r = -0.74$).

Fig. 3 shows the ordination obtained in the PCA of the environmental variables. The first axis of the PCA explains 42.9% of variance of the data. This showed a gradient for *P-E* parameters P_{max}, α and APA rates that separated southern Chilean systems from northern Argentinean systems. An additional 16.6% was explained by axis 2 and represented a gradient in environmental and functional variables such SRP and Chl *a* and R, mainly separating lakes from rivers (69.5% total variance). All correlations obtained with the Pearson's correlation coefficients (whether significant or not) were reinforced by the PCA results. From this biplot diagram it can be observed that P_{max} was positively related with APA rates and DOP concentration, meanwhile negatively correlated with the initial slope of *P-E* curve α value. High DOP was negatively related to Chl *a*. SRP corresponded with high R and low Chl *a*. Values of α were positively related to Chl *a* and negatively with DOP concentration. Compared across sites, River Azul was characterized by high APA rates, DOP and P_{max}, and low values for α. On the contrary, Lakes Nahuel Huapi and Frío and River Coyhaique were distinguished by high values of SRP and R and low values of Chl *a*, APA rates and P_{max}. Rivers Claro and Limay were characterized by high values of Chl *a* and low values of DOP, SRP and R. River Claro was distinguished by the highest value of α, followed by River Coyhaique.

DISCUSSION

Differences between *P-E* curves for mats of *D. geminata* from the rivers and lakes sampled were primarily related to the initial slope α and P_{max}, while more southern west slope aquatic systems (Chile) showed a higher α and the lowest P_{max}, Argentinean systems showed a reverse trend for these ecophysiological parameters. All the studied rivers and lakes from Argentina and Chile had circumneutral to mildly basic pH values and low conductivity, conforming to previous local studies (Pedrozo *et al.* 1993; CIEP 2010) and global niche parameters for *D. geminata* (Cullis *et al.* 2012). Patagonian aquatic systems in general are considered oligo to mesotrophic (Campos 1984; Pedrozo *et al.* 1993).

Differences found in the parameters describing *P-E* curves could be related to differences in inorganic and organic P availability and chlorophyll *a* content in mats of *D. geminata* of the systems (e.g. lake/river trophic state). Concentrations of SRP in lake populations were notably higher than that associated with blooms worldwide (< 2 µg P l^{-1}, Bothwell *et al.* 2014), particularly in the case of Lake Nahuel Huapi. Here the concentration was higher than the historic average (Diaz *et al.* 1998) and is likely related to the particular bay where *D. geminata* proliferates, which is affected in some degree by anthropic inlets (Baffico 2001). That blooms of *D. geminata* may occur in areas with some nutrient perturbation was also observed elsewhere in Patagonia (CIEP 2010). This could be ascribed to higher probability of introduction in populated areas and promotion of cell division of *D. geminata* and other symbionts by anthropogenic nutrient subsidies at some later point (e.g. these systems are not steady state). That blooms have appeared in somewhat non-ideal habitats but with clear potential for introduction pressure in turn presents some logical support for the possibility that *D. geminata* is invasive. This last point is important, since despite invasive status being recently questioned in the context of potential global drivers of blooms (Taylor & Bothwell 2014), this hypothesis mostly lacks substantive evidence, either for or against the possible native status of *D. geminata* in South America.

The DOP concentration in most of the sites sampled was lower than SRP, which indicates that inorganic P bioavailability is high and of easy access to *D. geminata*. However DOP was more strongly correlated with APA than SRP was for any other functional parameter (although effects of all environmental parameters were not significant). River Limay is the natural outflow of Lake Nahuel Huapi and was recently affected by blooms of *D. geminata* following their appearance in the source lake. This river has historically showed lower P concentrations than the lake (Diaz *et al.* 2007), probably because the lake acts as nutrient trap or because of vertical mixing of lake water supplying the outflow. The other Argentinean aquatic systems reported here have not been as intensively studied as Lake Nahuel Huapi. In the context of cross site comparisons, we speculate that the P values recorded here could be a result of short term, seasonal or local characteristics of sampling sites.

P_{max} is a function of the enzymatic processes in photosynthesis (Cote & Platt 1983), which depend on temperature, nutrient availability, light history, biochemical composition and species composition, among other factors. In our study, the highest P_{max} (River Azul) was reached in

Table 4. Pearson Correlations for the associations between the *P-E* curves parameters and the environmental and functional variables.

	APA	Chl *a*	P_{max}	α	R	I_k
Environmental						
pH	0.12	0.26	−0.13	0.68	0.32	−0.44
EC	−0.22	−0.56	−0.30	0.29	0.65	−0.46
SRP	−0.30	−0.06	−0.12	−0.36	0.39	0.28
DOP	0.66	−0.42	0.58	−0.40	0.45	0.47
Functional						
APA		0.04	0.90[1]	−0.53	−0.26	0.79
Chl *a*			0.07	0.39	−0.32	−0.05
P-E curves						
P_{max}						
α			−0.74			
R			−0.34	0.19		
I_k			0.90[1]	−0.89[1]	−0.22	

[1] Correlations are significant at $P < 0.05$.

systems with low SRP but high DOP and APA rates. Lakes Nahuel Huapi and Frío showed the opposite relation, with higher values of SRP but lower P_{max} compared with the river mentioned. Patagonian systems have among the lowest rates of atmospheric N deposition (Galloway *et al.* 2004) often considered to be N limiting (Diaz *et al.* 2007).

Hill & Boston (1991) noted an inverse relationship between P_{max} and Chl *a* of benthic algae and attributed it to greater self-shading in higher biomass periphyton, a condition that is certainly applicable to typically dense *D. geminata* mats. In our study the absence of a relationship between these two parameters was unexpected.

The initial slope, α, is a function of the photochemical processes in photosynthesis and depends on the quantum yield and on the ability of the cells to trap incident light

Fig. 3. PCA biplot showing the distribution of the sampling sites, the environmental variables and *P-E* parameters. Coordinates of the sampling sites were used as supplementary variables (dashed lines): W (West) and S (South). LNahuHu, Lake Nahuel Huapi; RCoyha, River Coyhaique.

(Cote & Platt 1983). Some of the factors affecting the magnitude of α include cell size, pigment composition, adaptation to light, light quality and nutrient availability (Cote & Platt 1983). In our study, systems with high α showed the highest values of chlorophyll *a*. Falkowski (1981) suggested that nutrient stress may result in decreases in the size and number of photosynthetic units; subsequently an increase in α would be expected with increased nutrient limitation. We observed this association between initial slope and DOP availability for *D. geminata*. Mats from more southern rivers Claro and Coyhaique showed a higher α, indicating a higher photosystem affinity for light compared with the other mats studied.

There was no evidence for photoinhibition, congruent with the lack of photoinhibition in a study of *P-E* curves in Hinds Creek (Hill & Boston 1991). According to these authors, in benthic mats there is compensation between photosynthesis by underlayers and photoinhibition by surface layers. Hill *et al.* (1995) found similar results in a comparison of open zones to *P-E* curves from shaded zones of the same creek. A possible explanation considered by Hill *et al.* (1995) was the presence of photoprotective pigments (e.g. carotenoids) in algal communities of zones exposed to high light irradiances. When these shaded communities were exposed experimentally to high irradiances in the lab, photoinhibition was observed. All sites in our study were from non-shaded conditions. The study of accessory pigments related to photoprotection of high PAR intensities and ultraviolet radiation will be our main focus in future works with *D. geminata*.

Environmental factors influencing the values of I_k include temperature, species composition and previous light history. This parameter varied among populations, generally with lower values for the more southern Chilean sites. Light condition in rivers and streams is subject to high spatial variability, and this particular spatial structure of the river bottom creates high habitat heterogeneity regarding light availability (Uehlinger *et al.* 2000). According to these authors, it is assumed that some fraction of photosynthesis by epilithic, epiphytic and macrophytic algae is light limited, resulting in *P-E* curves lacking light saturation. In contrast, all of the mats we studied exhibited light saturation at higher light intensities. Hill & Boston (1991) proposed that if exposure to high irradiances is long enough, lotic autotrophs
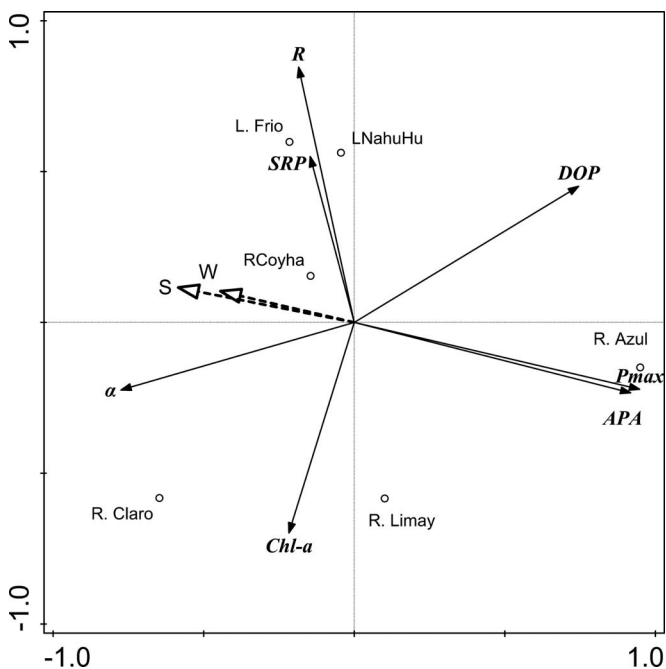
can adapt genotypically or phenotypically to potentially inhibiting light intensities. All our studied sites were unshaded by tree canopy but we cannot account for effect of topography, especially for river sites. Ambient light (PAR) at Lake Nahuel Huapi during our sampling was very high: 2390 μmol photon m^{-2} s^{-1}. Accordingly, we could expect a similar situation to that observed in Hinds Creek, with potential photo-acclimation of mats in the lake.

Several interacting autecological factors, together with dynamic non–steady state habitat processes, may be separately or interactively involved in growth of *D. geminata* vs bloom development. This may have contributed to the great range of observations, speculations and uncertainty regarding the niche of *D. geminata*. Light availability has a positive impact on cellular division rates of *D. geminata* (Bothwell & Kilroy 2011) and length of the stalks (Kilroy & Bothwell 2011). Although cells have been found in water bodies with a wide average temperature range (Spaulding & Elwell 2007), this factor is considered an important one regarding habitat definition of this species (Kumar *et al.* 2009). Low water temperatures could be directly related to functional processes affecting stalk production, activity of phosphatase enzymes located inside the stalks (Whitton *et al.* 2009) and uptake of potentially limiting nutrients, as demonstrated for diatoms in cold ocean regions (Lomas & Gilbert 1999). Conversely, low temperatures from snow melt pulses could be indirectly related to performance via covarying effects on nutrient limitation. Low nutrient concentrations could give a competitive advantage to *D. geminata* compared with other benthic algae (Cullis *et al.* 2012), which would explain oligotrophy as a condition for their massive development. The pH, which is among the more consistent parameters globally (generally >7), may be related to APA and also substrate availability of inorganic carbon for polysaccharides production. In extensive blooms, most of the biomass consists of stalks (Whitton *et al.* 2009). The production of stalks is mainly dependent upon cell production other than nutrient availability, since structural carbohydrates incorporate low amounts of nutrients (Cullis *et al.* 2012). For this reason, excessive stalk production could be observed under P limiting conditions with soluble reactive P concentrations below 2 μg l^{-1} (Bothwell & Kilroy 2011; Kilroy & Bothwell 2011, 2012). As mentioned before, according to Bothwell *et al.* (2012), *D. geminata* blooms occur only under P limitation, and soluble available P would be responsible for the timing and spatial distribution of blooms (Bothwell *et al.* 2014). We concur but add that once the physical and chemical prerequisites are met for stalk production, later conditions may interact or promote various functional or physiological aspects of the life cycle of *D. geminata* and the associated mat community. In this context, it is worth noting that in lakes Nahuel Huapi and Frío and in River Claro, *D. geminata* has been established and has shown chronic blooming for at least the last 2–3 yr, despite P values above the threshold mentioned by Bothwell *et al.* (2012, 2014).

The negative relationship found between SRP and P_{max}, and between SRP and Chl *a,* agrees with the previous works mentioned from Bothwell and Kilroy that demonstrate that growth of *D. geminata* is higher when P concentrations are very low. In our case the relationship is explained at a more functional level, since photosynthetic performance α, an indicative of photosystem affinity and efficient use of light, is also related negatively with soluble P. Ellwood & Whitton (2007), however, demonstrated that soluble P deficiencies affecting performance in oligotrophic systems could be offset by organic P available via phosphatase enzyme activity. The associations found in our study between SRP, DOP and APA rates are also clearly coincident with the observations from Ellwood & Whitton (2007). This reinforces the importance of P availability in blooms of *D. geminata* but may be more relevant for cellular processes other than stalk production.

As noted by Reid & Torres (2014), the various proposed mechanisms involved in blooms of *D. geminata* are not necessarily mutually exclusive. While some may be essential (P as the limiting nutrient promoting excess carbohydrate production), other mechanisms may also be important contributing factors in the context of the wide range of niches and dynamic nature of aquatic ecosystems. In conclusion, we highlighted the importance of P availability as a key controlling factor for the growth and blooming capacity of *D. geminata*. In addition, the availability of P may be a clear link to photosynthetic performance and light affinity of the photosystems of this species.

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REFERENCES

Abelli Bonardi M., Baffico G., Beamud G., Castiñeira L., Diaz M., Laffitte L., Laurenzano B., Morzenti P., Muñiz Saavedra J., Pedrozo F., Rivera D. & Temporetti P. 2012. Programa de vigilancia, seguimiento y control de *Didymosphenia geminata* (Lyngbye) Schmidt 1899 en la Provincia del Neuquén. 5th Argentinean Congress of Limnology, Santa Fé, Argentina.

Alcoverro T., Conte E. & Mazzella L. 2000. Production of mucilage by the Adriatic epipelic diatom *Cylindrotheca closterium* (Bacillariophyceae) under nutrient limitation. *Journal of Phycology* 36: 1087–1095.

American Public Health Association (APHA). 2005. *Standard methods for the examination of water and wastewater*. ed. 21. APHA, Washington, DC. 1200 pp.

Baffico G.D. 2001. Variations in the periphytic community structure and dynamics of Lake Nahuel Huapi (Patagonia, Argentina). *Hydrobiologia* 455: 79–85.

Beamud G., Baffico G., Pedrozo F. & Diaz M. 2013. First record of the invasive algae *Didymosphenia geminata* in the Lake Nahuel Huapi: Argentina, Patagonia. *Revista Chilena de Historia Natural* 86: 493–496.

Bothwell M.L. & Kilroy C. 2011. Phosphorus limitation of the freshwater benthic diatom *Didymosphenia geminata* determined from the frequency of dividing cells. *Freshwater Biology* 56: 565–578.

BOTHWELL M.L., KILROY C., TAYLOR B.W., ELLISON E.T., JAMES D.A., GILLIS C.-A., BLADON K.D. & SILINS U. 2012. Iron is not responsible for *Didymosphenia geminata* bloom formation in phosphorus-poor rivers. *Canadian Journal of Fisheries and Aquatic Sciences* 69: 1723–1727.

BOTHWELL M.L., TAYLOR B.W. & KILROY C. 2014. The Didymo story: the role of low dissolved phosphorus in the formation of *Didymosphenia geminata* blooms. *Diatom Research* 29: 229–236.

CAMPOS H. 1984. Limnological study of Araucanian Lakes (Chile). *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie* 22: 1319–1327.

CHU S.P. 1942. The influence of the mineral composition of the media on the growth of planktonic algae. 1. Methods and culture media. *Journal of Ecology* 30: 284–325.

CIEP 2010. *Evaluación preliminar sobre la diatomea invasora exótica Didymosphenia geminata en cuencas de Futaleufú y Provincia de Palena, Región de Los Lagos, Chile*. Preliminary Report, Centro de Investigación en Ecosistemas de la Patagonia, Coyhaique, Chile. 20 pp.

COTE B. & PLATT T. 1983. Day-to-day variations in the spring-summer photosynthetic parameters of coastal marine phytoplankton. *Limnology and Oceanography* 28: 320–344.

CULLIS J.D.S., GILLIS C.-A., BOTHWELL M.L., KILROY C., PACKMAN A. & HASSAN M. 2012. A conceptual model for the blooming behavior and persistence of the benthic mat-forming diatom *Didymosphenia geminata* in oligotrophic streams. *Journal of Geophysical Research* 117: G00N03 DOI:10.1029/2011JG001891.

DIAZ M., PEDROZO F. & TEMPORETTI P. 1998. Phytoplankton of two Araucanian lakes differing in trophic state (Argentina). *Hydrobiologia* 369/370: 45–57.

DIAZ M.M., PEDROZO F.L., REYNOLDS C. & TEMPORETTI P. 2007. Chemical composition and the nitrogen-regulated trophic state of Patagonian lakes. *Limnologica* 37: 17–27.

ELLWOOD N.T.W. & WHITTON B.A. 2007. Importance of organic phosphate hydrolyzed in stalks of the lotic diatom *Didymosphenia geminata* and the possible impact of atmospheric and climate change. *Hydrobiologia* 592: 121–133.

FALKOWSKI P.G. 1981. Light shade adaptation and assimilation numbers. *Journal of Plankton Research* 3: 203–216.

GALLOWAY J.N., DENTENER F.J., CAPONE D.G., BOYER E.W., HOWARTH R.W., SEITZINGER S.P., ASNER G.P., CLEVELAND C.C., GREEN P.A., HOLLAND E.A., KARL D.M., MICHAELS A.F., PORTER J.H., TOWNSEND A.R. & VÖRÖSMARTY C.J. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70: 153–226.

GEIDER R.J. 1993. Quantitative phytoplankton physiology: implications for primary production and phytoplankton growth. *ICES Marine Science Symposia* 197: 52–62.

GRETZ M.R. 2008. The stalks of Didymo. In: *Proceedings of the 2007 International Workshop on Didymosphenia geminata* (Ed. by M.L. Bothwell & S.A. Spaulding), pp. 21. Canadian Technical Report of Fisheries and Aquatic Sciences 2795, Nanaimo, Canada.

HILL W. 1996. Effects of light. In: *Algal ecology: freshwater benthic ecosystems* (Ed. by R.J. Stevenson, M.L. Bothwell & R.L. Lowe), pp.121–148. Academic Press, New York.

HILL W.R. & BOSTON H.L. 1991. Community development alters photosynthesis-irradiance relations in stream periphyton. *Limnology and Oceanography* 36: 1375–1389.

HILL W.R., RYON M.G. & SCHILLING E.M. 1995. Light limitation in a stream ecosystem: responses by primary producers and consumers. *Ecology* 76: 1297–1309.

JASSBY A.D. & PLATT T. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography* 21: 540–547.

JONES C.T., CRAIG S.E., BARNETT A.B., MACINTYRE H.L. & CULLEN J.J. 2014. Curvature in models of the photosynthesis-irradiance response. *Journal of Phycology* 50: 341–355.

KILROY C. & BOTHWELL M.L. 2011. Environmental control of stalk length in the bloom-forming freshwater benthic diatom *Didymosphenia geminata* (Bacillariophyceae). *Phycology* 47: 981–989.

KILROY C. & BOTHWELL M.L. 2012. *Didymosphenia geminata* growth rates and bloom formation in relation to ambient dissolved phosphorus concentration. *Freshwater Biology* 57: 641–653.

KILROY C., SNELDER T., FLOERL O., VIEGLAIS C. & DEY, K. 2008. A rapid technique for assessing the suitability of areas for invasive species applied to New Zealand's rivers. *Diversity and Distribution* 14: 262–272.

KUMAR S., SPAULDING S., STOHLGREN T.J., HERMANN K.A., SCHMIDT T.S. & BAHLS L. 2009. Potential habitat distribution for freshwater diatom *Didymosphenia geminata* in the continental US. *Frontiers in Ecology and the Environment* 7: 415–420.

LOMAS M. & GILBERT P. 1999. Temperature regulation of nitrate uptake: a novel hypothesis about nitrate uptake and reduction in cool-water diatoms. *Limnology and Oceanography* 44: 556–572.

MEDVEDEVA L.A. 2001. Biodiversity of aquatic algal communities in the Sikhote-Alin biosphere reserve (Russia). *Cryptogamie, Algologie* 22: 65–100.

MISERENDINO M.L. & PIZZOLON L.A. 2003. Distribution of macroinvertebrate assemblages in the Azul-Quemquemtreu river basin, Patagonia, Argentina. *New Zealand Journal of Marine and Freshwater Research* 37: 525–539.

PEDROZO F., CHILLRUD S., TEMPORETTI P. & DIAZ, M. 1993. Chemical composition and nutrient limitation in rivers and lakes of Northern Patagonian Andes (35.5°–42°S, 71°W) (Rep. Argentina). *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie* 25: 207–214.

REID B. & TORRES R. 2014. *Didymosphenia geminata* invasion in South America: ecosystem impacts and potential biogeochemical state change in Patagonian rivers. *Acta Oecologica* 54: 101–109.

ȘAHIN B. 2000. Algal flora of Lakes Aygir and Balikli (Trabzon, Turkey). *Turkish Journal of Botany* 24: 35–45.

SASTRE A.V., SANTINELLI N.H., BAUER G.A., AYESTARÁN M.G. & UYUA N.M. 2013. First record of the invasive diatom *Didymosphenia geminata* (Lyngbye) Schmidt in a Patagonian Andean river of Argentina. *BioInvasions Records* 2: 11–17.

SPAULDING S. & ELWELL L. 2007. Increase in nuisance blooms and geographic expansion of the freshwater diatom *Didymosphenia geminata*. USGS Open File Report 2007-1425.

SUNDARESHWAR P.V., UPADHYAY S., ABESSA M., HONOMICHL S., BERDANIER B., SPAULDING S.A., SANDVIK G. & TRENNEPOHL A. 2011. *Didymosphenia geminata*: algal blooms in oligotrophic streams and rivers. *Geophysical Research Letters* 38: L10405.

TALLING J. 1957. Photosynthetic characteristics of some freshwater plankton diatoms in relation to underwater radiation. *New Phytologist* 56: 29–50.

TAYLOR B.W. & BOTHWELL M.L. 2014. The origin of invasive microorganisms matters for science, policy, and management: the case of *Didymosphenia geminata*. *BioScience* 64: 531–538.

TER BRAAK C.J.F. & SMILAUER P. 2012. *CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination* (Version 5). Microcomputer Power, Ithaca, New York. 496 pp.

UEHLINGER U., KÖNIG C. & REICHERT P. 2000. Variability of photosynthesis-irradiance curves and ecosystem respiration in a small river. *Freshwater Biology* 44: 493–507.

WHITTON B.A., ELLWOOD N.T.W. & KAWECKA B. 2009. Biology of the freshwater diatom *Didymosphenia*: a review. *Hydrobiologia* 630: 1–37.