Efectos de la estructura trófica sobre el fitoplancton y perifiton en lagos someros subtropicales y templados

Juan Pablo Pacheco Esnal

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Juan Pablo Pacheco Esnal

jp@cure.edu.uy

Orientadores:

Dr. Néstor Mazzeo. Departamento de Ecología. Facultad de Ciencias. Universidad de la República. Uruguay.mazzeobeyhaut@yahoo.com

Dra. Irina Izaguirre. Laboratorio de Limnología. Departamento de Ecología, Genética y Evolución. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Instituto IEGEBA (CONICET-UBA), Argentina. iri@ege.fcen.uba.ar

Tribunal:

Dr. Matías Arim (Presidente) Prof. Agregado. Laboratorio de Biodiversidad. Facultad de Ciencias – CURE, UdelaR

Dra. Carla Kruk. Prof Adjunta. Facultad de Ciencias – CURE, UdelaR

Dra. Vera Huszar. Prof. Titular. Museo Nacional – Universidad Federal de Rio de Janeiro.

RESUMEN

El fitoplancton y el perifiton fotosintético presentan un rol fundamental en el funcionamiento de lagos someros. El conocimiento de los factores que estructuran estas comunidades resulta imprescindible para entender los efectos potenciales de procesos como la eutrofización o el calentamiento global sobre estos ecosistemas. Los controles descendentes por consumo directo o aquellos efectos en cascada promovidos sobre estos productores primarios pueden presentar diferencias sustanciales entre climas, asociados a estructuras tróficas diferenciales.

Mediante experimentos en mesocosmos en lagos en combinación con estudios de campo, se analizaron comparativamente los efectos directos y en cascada promovidos por peces omnívoros y macroinvertebrados sobre la biomasa y composición del fitoplancton y perifiton entre lagos someros subtropicales y templados.

Se evidenciaron para ambos climas importantes efectos en cascada promovidos por peces pequeños sobre el fitoplancton, disminuyendo las tallas grandes de zooplancton y limitando así la capacidad de herbivoría. Esto promovió la dominancia de grupos fitoplanctónicos de gran tamaño como cianobacterias y dinoflagelados, y de muy pequeño tamaño como cianobacterias unicelulares y criptofitas pequeñas (capaces de evitar o compensar el consumo). Por otra parte, en ausencia de peces el zooplancton de ambos climas presentó composiciones similares con una alta proporción de macrofiltradores, lo que se reflejó en biomasas muy bajas de fitoplancton.

No se observaron efectos directos o en cascada de la presencia de macroinvertebrados sobre el perifiton o fitoplancton. Tampoco fueron observados efectos directos o en cascada de los peces o macroinvertebrados sobre el perifiton, por lo que las diferencias entre climas en esta comunidad podrían asociarse a otros factores (ej: requerimientos metabólicos).

Las respuestas diferenciales del fitoplancton de los ecosistemas de diferentes regímenes climáticos fue dependiente de la estructura trófica, encontrándose respuestas similares a los mismos tratamientos más allá de diferencias climáticas. Los resultados sobre el perifiton no son concluyentes, siendo necesario aumentar el conocimiento de los factores estructuradores de esta comunidad y las interacciones con el fitoplancton, especialmente en lagos subtropicales donde la información al respecto es prácticamente inexistente.

SUMMARY

Phytoplankton and photosynthetic periphyton have a fundamental role in the functioning of shallow lakes. Knowledge of the factors structuring these communities is essential to understand the potential effects of processes such as eutrophication and global warming on these ecosystems. Top-down controls due to direct consumption or cascading effects on these primary producers, may present substantial differences between climates derived from differential trophic structures.

Using a combination of mesocosm experiments in lakes and field data, we comparatively analyzed the direct and cascade effects promoted by omnivorous fish and macroinvertebrates on the biomass and composition of phytoplankton and periphyton between subtropical and temperate shallow lakes.

We observed a markedly cascading effects promoted by small fish on phytoplankton in both climatic conditions, reducing the large-sized zooplankton and therefore limiting grazing on phytoplankton. This, also promoted the dominance of large-sized groups in phytoplankton, as cyanobacteria and dinoflagellates, and very small as unicellular cyanobacteria and small cryptophytes. In the absence of small fish, zooplankton of both climates presents similar compositions with a high proportion of large sized zooplankton groups, which resulted in very low phytoplankton biomass.

No effect of macroinvertebrates on phytoplankton biomass or composition was registered and no direct or cascade effects promoted by fish or macroinvertebrates in periphyton were evident, and the differences between climatic conditions in this community could be associated with other factors (eg metabolic requirements).

Phytoplankton responses were not different among climates, with similar patterns for the same treatments, as a clear response of the trophic structure regardless of climatic conditions. The results on the periphyton are not conclusive, being necessary to increase the knowledge of the structural factors of this community and the interactions with the phytoplankton, especially in subtropical shallow lakes where the information about it is practically nonexistent.

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ÍNDICE

1. INTRODUCCIÓN

1.1 Eutrofización y estados alternativos en lagos someros

El aumento en la carga de nutrientes en los lagos (eutrofización) lleva a cambios en su estructura y funcionamiento (Hutchinson 1973; Wetzel 2001). A medida que este proceso avanza, se generan múltiples características adversas como disminución de la transparencia, déficit de oxígeno disuelto y aumento en la frecuencia y duración de floraciones potencialmente tóxicas de fitoplancton, asociado al incremento de la biomasa fitoplanctónica y dominancia de cianobacterias (Wetzel 2001; Khan & Ansari 2005; Smolders *et al.,* 2006). En lagos poco profundos (someros), la eutrofización se evidencia inicialmente por el aumento de la abundancia y biomasa de productores primarios, transitándose en múltiples ocasiones se desde un estado de aguas claras con dominancia de plantas sumergidas hacia otro de aguas turbias con dominancia de fitoplancton (Scheffer *et al.,* 1993; Jeppesen *et al.,* 1998). Ambas configuraciones, pueden alternarse en un amplio rango de nutrientes conformando estados alternativos (Scheffer *et al.,* 2001) que perduran en el tiempo por diferentes mecanismos fisicoquímicos y biológicos (Carpenter *et al.* 1987; Scheffer 1998; Hargeby *et al.,* 2004). Generalmente, el pasaje de un estado a otro ocurre de forma repentina por cambios bruscos en el sistema, asociado a perturbaciones externas o modificaciones en la trama trófica u otros mecanismos de la dinámica interna (Brodersen *et al.,* 2008; Brönmark *et al.,* 2010).

1.2 Rol del fitoplancton y perifiton en la estabilidad de estados alternativos

El estudio de los mecanismos vinculados a la estabilidad de los estados alternativos, se ha basado clásicamente en la interacción entre las plantas sumergidas y el fitoplancton, enfocándose mayormente en la competencia por luz y nutrientes (Balls *et al.,* 1989; Jeppesen *et al.,* 1998; Vadeboncoeur & Steinman 2002) así como en los efectos alelopáticos (ej: Vanderstukken *et al.,* 2011). En presencia de altas densidades de plantas, el desarrollo del fitoplancton se ve limitado por múltiples mecanismos estabilizadores (Richard *et al.,* 1984; Balls *et al.,* 1989; Scheffer *et al.,* 1993), por lo que el pasaje hacia estados de agua turbia está vinculado necesariamente a una disminución drástica en la abundancia de plantas sumergidas (Brönmark *et al.,* 2010).

En este sentido, algunos autores sugieren que el perifiton puede tener un rol fundamental en el proceso de eutrofización regulando el pasaje de estados de aguas claras a turbias, básicamente modificando el umbral donde ocurre dicha transición (Phillips *et al.* 1978; Hough *et al.,* 1989; Daldorph & Thomas 1995; Jones & Sayer 2003). El crecimiento del perifiton sobre las macrófitas limitaría el crecimiento de estas últimas, fundamentalmente por sombreado. Esto favorecería el desarrollo de fitoplancton, el cual a su vez por sombreado y competencia por nutrientes disueltos pasa a limitar el desarrollo de las macrófitas y del perifiton (Jones & Sayer 2003; Sayer 2010). En este sentido, se ha observado en experimentos de laboratorio que la adición de pulsos de nutrientes favorece preferencialmente al perifiton sobre las macrófitas (Jones *et al*. 1999; Jones *et al*. 2000; Jones *et al*., 2002). Evidencia paleolimnológica indica estados con altas biomasas de perifiton previos a estados de aguas turbias por fitoplancton (Osborne & Moss 1977). Estos antecedentes sugieren mecanismos de facilitación de los estados de aguas turbias por parte del perifiton.

Sin embargo, otros estudios (ej: Hansson 1988, Vadeboncoeur *et al.,* 2001) demuestran que el perifiton juega un papel fundamental limitando el pasaje a estados de aguas turbias por competencia con el fitoplancton. El perifiton es capaz de secuestrar grandes cantidades de nutrientes desde la columna de agua (Hansson, 1990) estimándose que es responsable de aproximadamente el 60% del fósforo capturado en zonas vegetadas (Richardson & Marshall 1986; Dodds 2003). Además, su actividad aumenta el pH del agua generando la precipitación de fósforo hacia los sedimentos en forma de fosfato de calcio (Jones *et al.,* 2000; Dodds 2003).

Por otra parte, la elevada actividad fotosintética en el sedimento oxigena la interfase agua-sedimento evitando la liberación de fósforo. En este sentido, Van Luijn *et al.* (1995) observaron que los sedimentos con perifiton liberan cantidades de nutrientes mucho menores que aquellos sin perifiton, limitando la disponibilidad de nutrientes en la columna de agua utilizable por el fitoplancton. Así, la competencia por nutrientes (Hansson 1990) y luz (Vadeboncoeur & Steinman 2002) serían controles que limitarían los estados con altas abundancias de ambos productores primarios. En este sentido, Liboriussen & Jeppesen (2003) encontraron que en lagos claros la productividad primaria de microalgas y cianobacterias estaría mayormente asociada al perifiton, mientras que en lagos turbios la producción es casi exclusivamente pelágica.

Los mecanismos involucrados en los controles sobre el perifiton y el fitoplancton en relación a las concentraciones de nutrientes y los niveles de herbivoría, siguen siendo poco claros, impidiendo elaborar modelos y predicciones acerca del funcionamiento de los lagos someros ante la eutrofización (Vadeboncoeur & Steinman 2002). El escaso conocimiento acerca de estos mecanismos, puede llevar a una errónea subestimación del papel del perifiton y en general de los procesos bentónicos-litorales en el funcionamiento ecosistémico (Lowe 1996; Vadeboncoeur & Steinman 2002). A pesar de la relevancia de estos procesos en el funcionamiento de lagos someros (Carpenter 1983; Vander Zanden *et al.,* 2011), aún son escasos los estudios que analicen conjuntamente al fitoplancton y perifiton en el proceso de eutrofización, particularmente en relación a la estructura trófica, lo cual limita la elaboración de modelos y predicciones de funcionamiento de los lagos someros (Vadeboncoeur & Steinman 2002; Sanchez *et al.* 2010).

1.3 Estructura trófica y funcionamiento de lagos someros

Clásicamente se ha establecido que las tramas tróficas de los ecosistemas acuáticos son estructuradas por dos tipos de controles principales: la disponibilidad de recursos (controles ascendentes o “bottom-up”) y aquellos derivados del consumo por niveles tróficos superiores (controles descendentes o “top-down”; *sensu* McQueen *et al.,* 1986; Carpenter *et al.,* 1987). Estos mecanismos pueden tener importantes efectos sobre la estabilidad de los estados alternativos en lagos someros, tanto directamente como mediante la alteración de las características claves del sistema (Scheffer *et al.,* 1993; Carpenter & Kitchell 1996; Jeppesen *et al.,* 1998). A modo de ejemplo, las plantas sumergidas, pueden afectar indirectamente la producción microalgal y mantener estados de aguas claras mediante la provisión de refugio para el zooplancton y macroinvertebrados herbívoros que consumen sobre el fitoplancton y perifiton respectivamente, afectando su producción (Jeppesen *et al.,* 1998). Los peces, por otra parte, pueden ejercer diferentes roles, afectando diferencialmente la producción de fitoplancton o perifiton mediante mecanismos vinculados a: resuspensión de sedimentos y nutrientes, liberación de nutrientes por excreción, consumo directo o por interacciones tróficas indirectas (Watson *et al.,* 2003; Stein *et al.* 1995). Altas biomasas de peces zooplanctívoros por ejemplo, llevan a la disminución de las biomasas de zooplancton y dominancia de tallas pequeñas que escapan al consumo por peces. Esta predominancia de tallas de zooplancton de pequeño tamaño, lleva a menores presiones de herbivoría y al aumento de la biomasa del fitoplancton, por lo cual los peces zooplanctívoros generan un claro efecto en cascada positivo sobre el fitoplancton (Timms & Moss 1984, Iglesias *et al.,* 2008, Mazzeo *et al.,* 2010, Sinistro 2010).

El rol regulador de los controles en cascada sobre la producción fitoplanctónica ha sido ampliamente estudiado en lagos (Carpenter *et al*., 1987; Northcote 1988; Mc Queen *et al*., 1989) refiriéndose mayormente a vías descendentes en la cadena trófica: peces piscívoros – peces zooplanctívoros – zooplancton – fitoplancton (Carpenter & Kitchell, 1988; Jeppesen *et al*., 1997). En experiencias de biomanipulación de la trama trófica, se ha observado una importante limitación de la producción fitoplanctónica por efectos en cascada promovida por peces piscívoros, consumiendo sobre los zooplanctívoros y aumentando así la presión de herbivoría sobre el fitoplancton (With & Wright, 1984; Hansson *et al*., 1998; Søndergaard *et al*., 2008; Mazzeo *et al.,* 2010).

Por otra parte, algunos autores han señalado que la presencia de los peces omnívoros puede tener efectos directos o en cascada aumentando las biomasas de perifiton. Estos efectos pueden ser explicados por dos mecanismos principales: consumo de macroinvertebrados herbívoros por parte de los peces, liberando de presión de herbivoría al perifiton (Philips *et al*. 1978; Timms & Moss 1984; Beklioglu & Moss 1996; Liboriussen *et al.,* 2005) y enriquecimiento en nutrientes por excretas de peces (Blanco 2001; Blanco *et al.,* 2004).

Otros estudios sin embargo, señalan que no se observan efectos directos o en cascada promovido por la presencia de peces sobre el perifiton. Hansson (1992) estudió en un gradiente latitudinal de lagos someros templados y árticos, los factores estructuradores del perifiton en relación al estado trófico y presencia de peces, encontrando que la biomasa de perifiton se relaciona con los niveles de nutrientes y biomasa fitoplanctónica, pero no con la presencia de peces (ausencia de efectos directos o en cascada). Asimismo, abordajes experimentales han evidenciado que los factores abióticos (niveles de luz y nutrientes) y no las interacciones tróficas son los principales determinantes de la biomasa de perifiton (Hill & Harvey 1990; Bécares et al., 2008). Sin embargo, estos efectos presentan importantes diferencias en relación a las condiciones climáticas, derivadas de la conjunción de factores biogeográficos (Mittelbach *et al.,* 2007), patrones de especiación (Rohde 1992), diversidad (Hillebrand 2004), requerimientos energéticos (Allen *et al.,* 2002; Brown *et al.,* 2004) y diferencias en la estructura de la trama trófica entre climas (Arim 2007; Meerhoff *et al.,* 2007; Teixeira de Mello *et al.,* 2009; Jeppesen *et al.,* 2010).

1.4 Diferencias en la estructura trófica de lagos someros de diferentes regímenes climáticos

Las importantes diferencias en el funcionamiento de ecosistemas, asociada a los patrones de biomasa y riqueza entre regímenes climáticos, han sido ampliamente discutidas en ecología (ej: Rohde 1992; Hillebrand *et al.,* 2004), siendo asociada a la acción de factores aislados (ej: hipótesis del domino medio de Colwell & Lees 2000) o a la combinación de varios factores estructuradores actuando en conjunto (Gaston & Blackburn 2000). Algunos de estos patrones diferenciales entre climas, pueden tener importantes consecuencias sobre el ecosistema. Kosten *et al.* (2012) observó para un total de 143 lagos en una transecta latitudinal, que los lagos someros en latitudes menores presentan mayores probabilidades de una alta representación de cianobacterias en el fitoplancton, aunque las biomasas totales de fitoplancton no presentan una relación con la latitud, patrón que también fue encontrado experimentalmente por Weyhenmeyer *et al.* (2001).

Parte de esta variabilidad, podría ser debida a diferencias en la estructura trófica entre sistemas de diferentes climas. Algunos autores indican por ejemplo que las tramas tróficas en sistemas cálidos son más cortas o truncadas que en climas fríos (Arim *et al.,* 2007; Iglesias *et al.,* 2011). En lagos someros, se han observado importantes diferencias en la estructura trófica en función de los regímenes climáticos, lo cual tiene efectos importantes sobre la estabilidad de los estados alternativos y la vulnerabilidad al proceso de eutrofización (Meerhoff *et al.,* 2007; Havens *et al*., 2009; Jeppesen *et al.,* 2010).

A diferencia de lo que ocurre en lagos templados, en lagos subtropicales las plantas sumergidas presentan baja capacidad de refugio para herbívoros, ya que en ellas se refugian y alimentan peces omnívoros en grandes densidades (Meerhoff *et al.,* 2003; Meerhoff *et al.,* 2006). Asimismo, la omnivoría parece tener un rol fundamental como estructurador de las tramas tróficas en lagos cálidos (Attayde & Menezes 2008; Teixeira de Mello *et al.* 2009; González-Bergonzoni *et al.,* 2012). En una amplia revisión de 269 ecosistemas de ecosistemas acuáticos de agua dulce y salobre en un gradiente latitudinal amplio (41°S, 81,5°N), González-Bergonzoni *et al.* (2012) encuentran un fuerte incremento en la omnivoría de peces hacia latitudes menores, asociándolo a una multicausalidad de mecanismos vinculados a la historia biogeográfica, mayores requerimientos metabólicos, densidad de peces y mayores interacciones tróficas en climas cálidos.

Estas altas densidades de omnívoros pequeños en lagos cálidos (Branco *et al.,* 1997), con múltiples eventos reproductivos a lo largo del año (Paugy & Lévêque 1999), no estarían sometidos a importantes presiones de consumo debido a la baja representación de peces piscívoros en lagos de climas cálidos (Lazzaro 1997; Meerhoff *et al.,* 2007; Jeppesen *et al.,* 2010). Las abundancias diferenciales de estos peces omnívoros, pueden generar efectos diferenciales en los niveles tróficos más bajos entre lagos de diferentes regímenes climáticos.

Por un lado, estos peces ejercen un importante consumo sobre el zooplancton en sistemas cálidos, reflejándose en bajas abundancias de macrofiltradores (ej: *Daphnia*) y dominancia de organismos de pequeña talla (Meerhoff *et al.,* 2007; Lacerot *et al.,* 2010). Como consecuencia, el consumo de fitoplancton, por parte del zooplancton, estaría limitado de manera importante (Iglesias *et al.,* 2008; Sinistro 2010; Van Leeuwen *et al.,* 2013; Lacerot *et al.,* 2013).

Iglesias *et al.* (2011) encontraron que en ausencia de peces, originadas por mortandad de peces o exclusiones, los lagos subtropicales pueden presentar macrofiltradores como *Daphnia* con una alta capacidad de herbivoría sobre el fitoplancton. La evidencia de campo y experimental indica que la presencia de estos macrofiltradores está restringida a periodos cortos de tiempo, pudiéndose comprobar esta hipótesis a través de las estructuras de resistencia en sedimentos o directamente con una alta frecuencia de muestreo (semanal o quincenal) (Iglesias *et al.,* 2011; Gerhard *et al.,* 2016).

Experimentos de biomanipulación en sistemas subtropicales, han mostrado importantes efectos en promovidos por la presencia de peces piscívoros (*Hoplias malabaricus*) que vía descendente en cascada trófica, limitan de manera importante al fitoplancton (Mazzeo *et al.,* 2010) aunque estos efectos promovidos por piscívoros serían menos marcados en sistemas subtropicales que en templados (Jeppesen *et al*., 2005).

Por otra parte, estos peces pueden tener efectos diferenciales sobre el perifiton según el clima. En lagos templados, se ha demostrado que los peces pequeños pueden ejercer un control sobre los macroinvertebrados herbívoros asociados a las plantas, liberando de herbivoría y favoreciendo indirectamente el desarrollo de perifiton (Brönmark & Weisner 1992; Jones & Waldron 2003;Jones & Sayer 2003; Liboriussen *et al.,* 2005). Mientras que en lagos cálidos la mayor relevancia de la omnivoría-herbivoría, puede asociarse a un importante consumo directo de los peces sobre el perifiton, generando el efecto contrario sobre las biomasas de perifiton en relación a lagos templados (Jeppesen *et al.,* 2010; González-Bergonzoni *et al.,* 2012).

1.5 Efectos de la estructura trófica sobre atributos morfológicos-funcionales del fitoplancton y perifiton

Las diferencias en la estructura trófica entre lagos someros de diferentes climas se reflejan en las comunidades de fitoplancton y perifiton presentes. En lagos someros subtropicales, se ha observado que el zooplancton de pequeña talla, estructura al fitoplancton hacia la dominancia de organismos de gran tamaño menos consumidos, como largos filamentos o colonias (Pacheco *et al.,* 2010; Pacheco *et al.* en prep. Cap. IV). Además, se ha observado que la herbivoría por macroinvertebrados es capaz de modificar la fisonomía de la comunidad perifítica, favoreciendo la dominancia de organismos mucilaginosos o de hábitos postrados, los cuales son menos susceptibles al consumo (ej: Steinman 1991; Steinman *et al*., 1992; Walton *et al.,* 1995; Steinman 1996). Así los efectos generados por una determinada estructura trófica llevan a cambios en las características morfológicas-funcionales de los organismos dominantes en estas comunidades de productores primarios, como el largo total, relación superficie/volumen, presencia de mucílago, etc. Por ello, los abordajes basados en las formas de vida y caracteres morfológicos de los organismos, pueden reflejar de mejor manera que los abordajes taxonómicos clásicos los efectos de los factores ambientales sobre el crecimiento, reproducción y asimilación de nutrientes del perifiton (Steinman *et al.,* 1992; Steinman 1996) y fitoplancton (Weithoff 2003; Vogt *et al.* 2010). En este contexto, resulta pertinente señalar que estos rasgos funcionales son propiedades de los organismos que pueden ser medidas y que influencian una o más funciones esenciales, tales como reproducción, crecimiento, entre otros (Weithoff, 2003).

Gran parte de los patrones anteriormente descritos, así como la generalidad de los estudios clásicos en limnología, han sido referidos a procesos pelágicos en lagos profundos de sistemas templados. Por otra parte, el estudio de las interacciones entre los componentes tróficos en procesos bentónicos – litorales, han sido clásicamente mucho menos abordados, a pesar de la gran relevancia que pueden tener en lagos someros (Carpenter *et al*., 2001; Schindler & Scheuerell 2002; Vadeboncoeur *et al*., 2002).

El conocimiento de estos mecanismos en lagos someros subtropicales, con características diferentes en su estructura trófica, es sustancialmente menor. Si bien se ha avanzado mucho en la comprensión de las diferencias en la estructura y funcionamiento trófico entre lagos someros subtropicales y templados (Meerhoff *et al.*2007; Lacerot *et al.* 2010), son escasos los estudios analizando los efectos sobre el perifiton y el fitoplancton (Vadeboncoeur & Steinman 2002; Sanchez *et al.,* 2010).

Considerando el rol fundamental de las interacciones entre estas comunidades en los procesos bentónicos-litorales, y en general en el funcionamiento de los lagos someros (Carpenter 1983; Lowe 1996; Vadeboncoeur & Steinman 2002) resulta sumamente necesario ahondar en la comprensión de los factores que determinan la biomasa y composición de las comunidades de perifiton y fitoplancton en relación a la estructura trófica de estos ecosistemas. En particular, es importante aumentar el conocimiento de los mecanismos causales de estas diferencias entre climas, mediante abordajes conjuntos a partir de datos de campo y experimentales. Estas características, podrían determinar vulnerabilidades diferenciales a la eutrofización en lagos someros de diferentes condiciones climáticas y en consideración de escenarios de calentamiento global.

2. OBJETIVOS & HIPÓTESIS

2.1 Objetivo General

Analizar comparativamente los efectos directos y en cascada promovidos por peces omnívoros y macroinvertebrados sobre la composición y biomasa del fitoplancton y perifiton en lagos someros subtropicales y templados.

2.2 Objetivos específicos

Objetivo 1: Comparar los efectos directos y en cascada promovidos por peces omnívoros y macroinvertebrados sobre la composición y biomasa fitoplanctónica entre lagos someros subtropicales y templados.

Hipótesis 1: Las diferencias en la composición y biomasa de fitoplancton entre lagos de diferentes regiones climáticas se deberán a los efectos en cascada promovidos por los peces omnívoros. La ausencia de peces llevará a mayores biomasas de zooplancton de tallas grandes, que ejercerá un mayor pastoreo sobre el fitoplancton, reflejándose en menores biomasas fitoplanctónicas y dominancia de grupos tolerantes a la herbivoría en ambos climas.

$H_0$1: En ausencia de peces existirán diferencias significativas en la composición morfológica-funcional y biomasa de fitoplancton entre climas.

Objetivo 2: Comparar los efectos directos y en cascada promovidos por peces omnívoros y macroinvertebrados sobre la composición y biomasa perifítica, entre lagos someros subtropicales y templados.

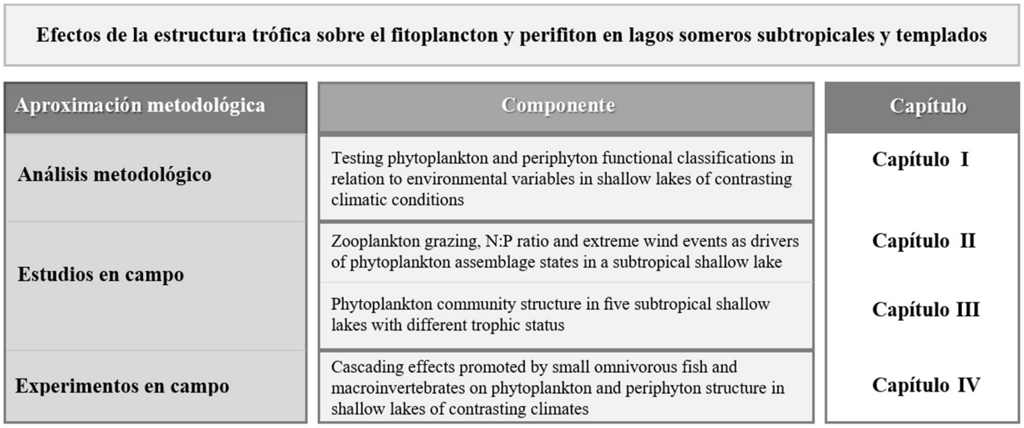
Hipótesis 2: Los efectos de la herbivoría por parte de los peces y macroinvertebrados sobre el perifiton serán mayores en sistemas subtropicales que en templados, limitando su crecimiento y llevando a la dominancia de grupos resistentes a la herbivoría, mientras que en ausencia de peces y macroinvertebrados los lagos subtropicales y templados presentarán similar composición y abundancia de perifiton.

$H_0$2: En ausencia de peces y macroinvertebrados, existirán diferencias significativas en la composición y biomasa de perifiton entre climas.

3. ESTRUCTURA DE LA TESIS

Esta tesis se conforma en base a capítulos referidos a las principales actividades de investigación realizadas en el marco del programa de Maestría. Cada componente, en formato de artículo científico, está asociado a un tipo de aproximación metodológica abordada (Fig. 1).

El contenido de los capítulos se sintetiza en la sección 4 *Componentes de estudio* y posteriormente en la sección 5. *Conclusiones y perspectivas*, se resaltan los principales aportes y perspectivas de esta tesis en la temática de estudio.



Efectos de la estructura trófica sobre el fitoplancton y perifiton en lagos someros subtropicales y templados

Aproximación metodológica	Componente	Capítulo
Análisis metodológico	Testing phytoplankton and periphyton functional classifications in relation to environmental variables in shallow lakes of contrasting climatic conditions	Capítulo I
Estudios en campo	Zooplankton grazing, N:P ratio and extreme wind events as drivers of phytoplankton assemblage states in a subtropical shallow lake	Capítulo II
	Phytoplankton community structure in five subtropical shallow lakes with different trophic status	Capítulo III
Experimentos en campo	Cascading effects promoted by small omnivorous fish and macroinvertebrates on phytoplankton and periphyton structure in shallow lakes of contrasting climates	Capítulo IV

Figura 1. Estructura general de la tesis: aproximaciones metodológicas y componentes considerados.

El primer capítulo consiste en el análisis de componente metodológico sobre el que se basan los componentes de estudios en campo y experimentales de esta tesis. En particular, se analiza la capacidad predictiva de los esquemas de clasificación funcional para ambas comunidades (Capítulo I). Los resultados de este capítulo son considerados para el desarrollo de los capítulos siguientes.

Los Capítulos II y III, se centran en el estudio en campo de los factores estructuradores del fitoplancton en lagos someros subtropicales, incluyendo los efectos del consumo del zooplancton sobre la abundancia y composición del fitoplancton.

En el Capítulo IV se analizan experimentalmente mediante mesocosmos, los efectos directos y en cascada promovidos por peces omnívoros y macroinvertebrados sobre la abundancia y composición del fitoplancton y perifiton en lagos someros subtropicales y templados.

4. COMPONENTES DE ESTUDIO

4.1 Análisis metodológico: capacidad predictiva de las clasificaciones taxonómicas y funcionales del fitoplancton y perifiton (Capítulo I)

El estudio de los patrones de diversidad en ecología, ha mostrado en los últimos años un importante aumento en el uso de aproximaciones ecológicas o basadas en atributos funcionales de los grupos (McGill *et al.,* 2006). Estas clasificaciones funcionales, consideran características morfológicas, fisiológicas y ecológicas que se asocian a las respuestas de los grupos a los principales gradientes ambientales (McGill *et al.,* 2006). Algunas características morfológicas frecuentemente utilizadas en estos esquemas ecológicos de clasificación, como el tamaño (ej: Arim *et al.,* 2007), tienen un claro correlato con la fisiología e interacción de los grupos en relación al ambiente, vinculándose directamente con sus demandas energéticas (Elton 1927; Brown *et al.,* 2004), capacidad de consumo o de ser consumido (Brooks & Dodson 1965; Jennings & Warr 2003), restricciones en el número de niveles tróficos (Pimm 1982; Hairston & Hairston 1993), reciclaje de nutrientes (Hall *et al.,* 2007), entre otros.

En ecosistemas acuáticos, el uso de los esquemas de clasificación funcional de modo alternativo a las aproximaciones taxonómicas clásicas, es cada vez más frecuente (Litchman & Klausmeier 2008; Heino 2010). La base de su amplio uso parecería radicar en la capacidad de superar la extrema fragmentación de los grupos taxonómicos, sin un correlato directo frecuente con el funcionamiento ecosistémico (Huszar & Caraco 1998; Mc Gill *et al*., 2006; Litchman & Klausmeier 2008), reduciendo la información y reflejando de mejor manera procesos ecológicos subyacentes (Weithoff 2003; Litchman & Klausmeier 2008; Heino 2010).Un ejemplo de esto, lo constituye la evidencia de comportamiento caótico en el plancton cuando es analizado a nivel de especie (Scheffer *et al*., 2003; Beninca *et al.,* 2008).

Atributos morfológicos-funcionales como el tamaño han mostrado ser factores de excepcional relevancia en el funcionamiento de lagos someros (Hilldrew *et al.,* 2007), por ejemplo como estructuradores de las tramas tróficas (Jones & Jeppesen 2007). En este sentido, diferentes componentes de esta tesis son abordados a partir de esquemas de clasificación morfológicos – funcionales, tanto para el fitoplancton (Capítulos II y III) como para el perifiton (I y IV), como indicadores adecuados de los procesos ecológicos estudiados. Sin embargo, el análisis de la respuesta composicional del fitoplancton y perifiton ante factores estructuradores es escasamente estudiados o desconocidos (especialmente en lagos someros subtropicales), por lo que se requiere consideraciones importantes respecto a las aproximaciones a emplear. De este modo, se disminuye el error metodológico evidenciándose de mejor manera los efectos de los mecanismos ecológicos estudiados.

En este capítulo, se analizó la capacidad predictiva de los diferentes esquemas de clasificación (funcional y taxonómica) empleados en la tesis, en relación a los principales gradientes ambientales en los sistemas de estudio. Primeramente, se realizó una breve revisión del uso de los esquemas de clasificación funcional de fitoplancton: grupos funcionales (FG) de Reynolds *et al.* (2002), grupos morfo-funcionales (MFG) de Salmaso & Padisák (2007) y los grupos morfológicos-funcionales (MBFG) de Kruk *et al.* (2010). Para esto se realizó una revisión de artículos hasta el 2013, en revistas indexadas en las bases: JSTOR, Science Direct, Scopus, SpringerLink, Directory of Open Access Journals y Google Scholar, basada en combinaciones de las palabras clave: "functional / traits / groups / classification / approach / morphology / approach" en conjunto con una revisión de artículos que citan los esquemas anteriormente descritos.

Posteriormente, se realizó el análisis de la capacidad predictiva de estos diferentes esquemas de clasificación funcional de fitoplancton, así como, un nuevo sistema de clasificación propuesto para el perifiton fotosintético (Tabla I), en relación a los principales gradientes ambientales, a partir de datos de campo de 16 lagos someros en un gradiente latitudinal (36°48 S - 56°10' N) en Uruguay, Argentina y Dinamarca.

Para los diferentes esquemas de clasificación empleados, los datos fueron analizados en términos de presencia – ausencia del grupo, biomasa (estimada por biovolumen) y transformado por Logaritmo de la biomasa + a (a= menor dato del conjunto de datos considerado).

Tabla I: Grupos morfológicos-funcionales propuesto para el perifiton fotosintético MPG (total 28 grupos; ver explicación en texto Capítulo I).

Afiliación taxonómica	**Morfotipo**	**Código**
1- Cyanobacteria	1a – Filamentos simples	1a FilUnb
	1b – Filamentos con células especializadas	1b FilNos
	1c – Filamentos ramificados sin mucílago	1c FilBran
	1d – Filamentos ramificados mucilaginosos	1d FilMuc
	1e – Colonias mucilaginosas	1e CyaMuc
	1f – Unicelular	1f CyaUni
2- Diatomea (Bacillariophyta)	2a – Unicelular chica <30 µm	2a SmDia
	2b – Unicelular grande >30 µm	2b LDia
	2c – Colonias en empalizada	2c PalDia
	2d – Filamentos	2d FilDia
	2e – Colonias en ramilletes	2e BouqDia
3- Chlorophyta	3a – Unicelular chica < 30 µm	3a SmChlo
	3b – Unicelular grande > 30 µm	3b LChlo
	3c – Flagelados unicelulares	3c FlaUni
	3d – Flagelados coloniales	3d FlaCol
	3e – Colonias desnudas pequeñas<30 µm	3e SmNaCol
	3f – Colonias desnudas grandes >30 µm	3f LNaCol
	3g – Colonias pequeñas mucilaginosas <30 µm	3g SmMucCol
	3h – Colonias grandes mucilaginosas >30 µm	3h LMucCol
	3i – Filamentos simples	3i UnbChlo
	3j – Filamentos ramificados	3j BranChlo
4- Conjugatophyceae	4a – Unicelulares	4a DesUni
	4b – Filamentosas	4b DesFil
5- Xantophyta	5a – Unicelular	5a XanUni
	5b – Filmentosas	5b XanFil
6- Chrysophyta	6a – Unicelulares	6a ChrUni
	6b – Colonias	6b ChrCol
7- Dinophyta		7 Dino

Se observó un importante incremento en el uso de los esquemas de clasificación funcional para el fitoplancton en los últimos años, mayormente vinculado al uso del esquema de grupos funcionales de Reynolds *et al.* (2002; Fig. 2) posiblemente debido a ser el primero en aparecer.

Respecto a la capacidad explicativa de las diferentes clasificaciones funcionales, todos los esquemas considerados presentaron una alta capacidad predictiva de la variabilidad ambiental, similar entre ellos y a la vez similar a la clasificación taxonómica (Tabla II, Fig. 3). La aplicación del logaritmo a los datos de biomasa, mostró un aumento en la capacidad predictiva de los esquemas de clasificación funcional (Fig. 3, ver texto en Cap. I).

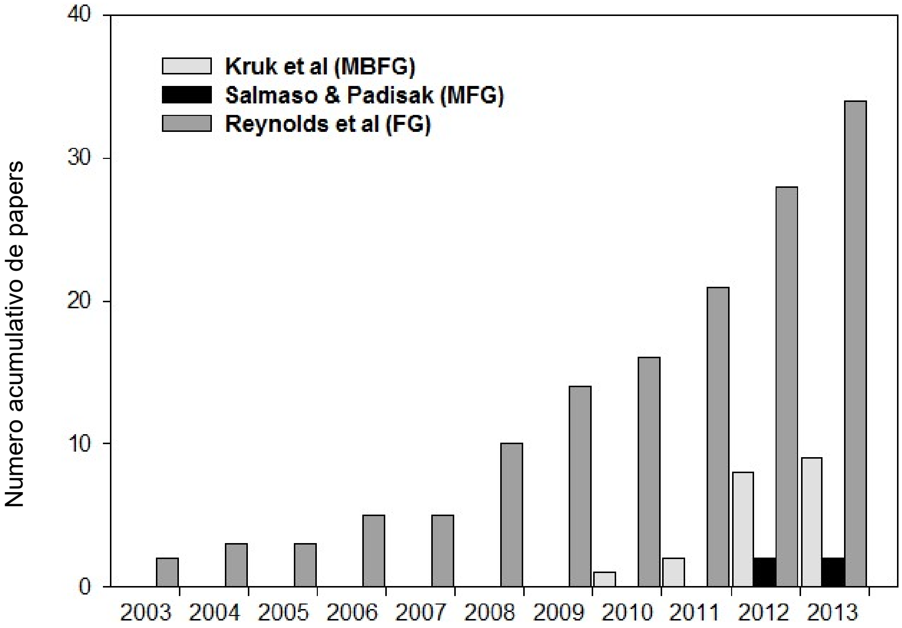


Figura 2. Uso de los diferentes esquemas de clasificación functional de fitoplancton en publicaciones en revistas indexadas internacionales hasta el 2013, considerando las clasificaciones de Reynolds *et al*., (2002); Salmaso & Padisák (2007); Kruk *et al*. (2010). El eje Y representa las abundancias acumulativas.

Tabla II. Capacidad predictiva promedio de los diferentes esquemas de clasificación funcional y de resolución taxonómica de fitoplancton y perifiton en relación a las principales variables fisicoquímicas de los sistemas estudiados. Valores basados en estadístico R ANOSIM, calculados a partir de la biomasa (biovolumen), Logaritmo de la biomasa (Log Bm+a) y presencia –ausencia. Todos los R fueron significativos (P <0.05) (ver texto en Cap. I).

Variables Físico-químicas	0,97		
Fitoplancton	Biomasa	Log (Bm + a)	Presencia - Ausencia
Género	0.76	0.78	0.65
Especie	0.73	0.71	0.64
MBFG Kruk *et al.* 2010	0.31	0.75	0.59
FG Reynolds *et al.* 2002	0.27	0.68	0.62
MFG Salmaso & Padisák 2007	0.30	0.59	0.57
Periphyton			
Género	0.53	0.55	0.32
MPG Perifiton	0.44	0.57	0.26

Dentro de los diferentes esquemas de clasificación funcional analizados del fitoplancton, la clasificación en grupos morfológicos-funcionales de Kruk *et al.,* (2010) presentó la más alta capacidad predictiva de la variabilidad ambiental, incluso más alta que la capacidad predictiva a nivel de especies si se considerándose los datos log transformados.

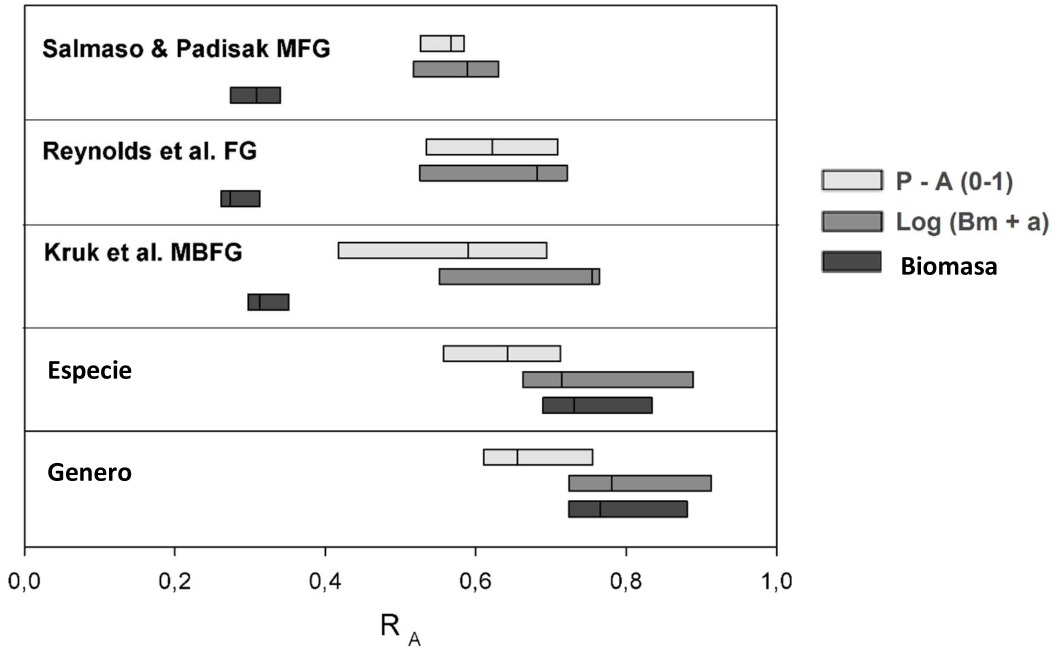


Figura 3. Capacidad predictiva de los esquemas de clasificación funcional del fitoplancton basados en el estadístico R_A de ANOSIM considerando biomasa, Logaritmo de la biomasa + a (a= menor valor del conjunto de datos), y presencia – ausencia (P-A). Promedio (línea) y rango de datos (rango del plot). Todos los Ra presentaron significancia estadística $P< 0.005$.

Estos resultados contrastan con lo encontrado en los estudios de Gallego *et al*. (2012) e Izaguirre *et al.* (2012), en cuanto al análisis de la capacidad predictiva para un grupo de lagos en España y Argentina respectivamente. Sin embargo, coincide con los resultados obtenidos por Kruk *et al.* (2011), donde también se consideraron diferentes situaciones climáticas, en un amplio conjunto de 211 lagos en un gradiente latitudinal, que abarcó desde regiones subpolares hasta tropicales.

El esquema de FG (Reynolds *et al.*, 2002) ampliamente aceptado, de gran uso recientemente, frecuentemente destacado por su alta capacidad predictiva (O´Farrell *et al.,* 2003; Caputo *et al.,* 2008; Becker *et al.,* 2010; entre otros) y que ha sido revisado y validado para una gran variedad de ecosistemas (Padisák *et al.,* 2009), no presentó sin embargo mayor capacidad predictiva que el esquema de Kruk *et al.* (2011). Considerando los altos requerimientos en cuanto a experiencia y tiempo para la clasificación en FG de Reynolds (42 grupos), la cual no es menor a la identificación taxonómica (Gallego *et al.,* 2012; Izaguirre *et al.,* 2012), los MBFG de Kruk con solo 7 grupos fácilmente identificables en base a atributos morfológicos, resulta en una alternativa sumamente conveniente en estudios de diversidad y monitoreo en relación a los gradientes ambientales en lagos someros.

El esquema de clasificación morfológico – funcional propuesto en esta tesis para el perifiton (MPG: grupos morfo-funcionales de perifiton) presentó una buena capacidad predictiva de la variabilidad ambiental y climática, similar a la de la clasificación taxonómica a nivel de género (Tabla II). Con solo 28 grupos fácilmente identificables, con un mínimo nivel de reconocimiento taxonómico (Tabla I), esta clasificación funcional del perifiton reduce la incertidumbre taxonómica asociada a errores de clasificación o debida a la carencia de claves de identificación regionales, a la vez que presenta una alta capacidad predictiva de los principales gradientes ambientales.

Basado en estos resultados, fueron aceptados como buenos descriptores de los patrones de diversidad, a los esquemas de clasificación MBFG de Kruk para el análisis de los patrones de composición de fitoplancton (Capítulos III y IV) y el esquema de clasificación propuesto en esta tesis para el caso del perifiton (Capítulo IV).

4.3 Estudios de campo: Rol del zooplancton como factor estructurador del fitoplancton en lagos someros subtropicales

En este componente (resumen de Capítulos II y III) se analizan los factores estructuradores del fitoplancton en lagos someros subtropicales, incluyendo los efectos del zooplancton sobre el fitoplancton. Este control, ampliamente debatido en cuanto a su relevancia en lagos subtropicales, es analizado mediante estudios de campo, en función del estado trófico de los lagos, frecuencias de muestreo y variabilidad interanual.

4.3.1 Rol del zooplancton, relación de nutrientes y factores de disturbio, sobre la composición del fitoplancton en un lago somero subtropical (Capítulo II)

En este componente se analizaron los principales factores determinantes de diferentes ensambles de fitoplancton en un lago somero subtropical (Laguna Blanca), como modelo de estudio, durante un largo periodo (Julio 2010 – Noviembre 2013) con una alta frecuencia de muestreo (quincenal en algunos periodos). Dicha contribución procura establecer los principales factores que gobiernan la aparición y permanencia de diferentes ensambles de especies de fitoplancton (Sommer *et al.*1993; Naselli-Flores *et al.,* 2003) que definen en gran medida las condiciones del lago y por lo general no estan necesariamente vinculados a limitación por recursos en el caso de sistemas meso y eutróficos (controles Bottom-up; Albay & Akcaalan 2003; Rojo& Alvarez-Cobelas 2003).

Laguna Blanca, mostró una clara alternancia de diferentes conformaciones del fitoplancton, algunos de ellos con alta dominancia de pocas poblaciones y elevadas biomasas (Figura 4) conformando ensambles fitoplanctónicos (Sommer *et al.* 1993; Naselli-Flores *et al.* 2003):

Estado I. Altas biomasas y dominancia de cianobacterias: observado durante el primer año de estudio, donde ocurre un bloom persistente de *Cylindrospermopsis raciborskii* (Cyanobacteria, Grupo MBFG III).

Estado II: Bajas biomasas de varios grupos, Bacillariophyta, Euglenoides, Cyanobacteria y Desmidiáceas en co-ocurrencia: *Closterium acicularis*, *Staurastrum leptocladum* (Chlorophyta), algunas colonias pequeñas de *Microcystis aeruginosa* y filamentos de *Planktolyngbya spp.* (Cyanobacteria). en conjunto con diatomeas y euglenoides. Este ensamble ocurrió desde Julio 2011 - Abril 2012.

Estado III: Biomasas medias a altas, con dominancia de Desmidiáceas (*Closterium acicularis*) y cianobacterias filamentosas (*Limnothrix*; *Cylindrospermopsis raciborskii*). Desde Mayo 2012 - Julio 2012.

Estado IV: Biomasas medias a bajas, dominancia de Chlorophyta (*Coelastrum*, *Scenedesmus,* entre otros). Desde Julio 2012 - Enero 2013, cuando se da un recambio abrupto en la composición fitoplanctónica (< 2 semanas) asociado a un evento extremo de viento (>100 km.h^{-1}).

Estado V: Altas biomasas fitoplanctónica, con amplia dominancia de *Ceratium furcoides* (Dinophyceae; > 95% del biovolumen relativo). Hasta fines del 2013.

Los factores determinantes de los cambios en los ensambles de fitoplancton en Laguna Blanca, pueden asociarse a 3 tipos de controles o forzantes ecológicos:

1) Factores de disturbio: eventos extremos de vientos desencadenando recambios composicionales del fitoplancton y alternancia entre estados.
2) Capacidad de incorporación y acumulación diferencial de nutrientes por parte de algunos grupos de fitoplancton.
3) Consumo por zooplancton, generando importantes cambios en la estructura y biomasa del fitoplancton.

Al igual que lo encontrado en otros estudios (ej:Albay & Akcaalan 2003; Rojo& Alvarez-Cobelas 2003) para el caso de Laguna Blanca, los principales mecanismos determinantes de estos estados o conformaciones, no estuvieron vinculados con competencia entre grupos, sino que se vincularon a capacidades de las especies dominantes para evitar el consumo o capacidad de aprovechamiento diferencial o de acumulación de nutrientes, definiendo estados "estables" del fitoplancton en el sentido descrito por (Sommer *et al.,* 1993).

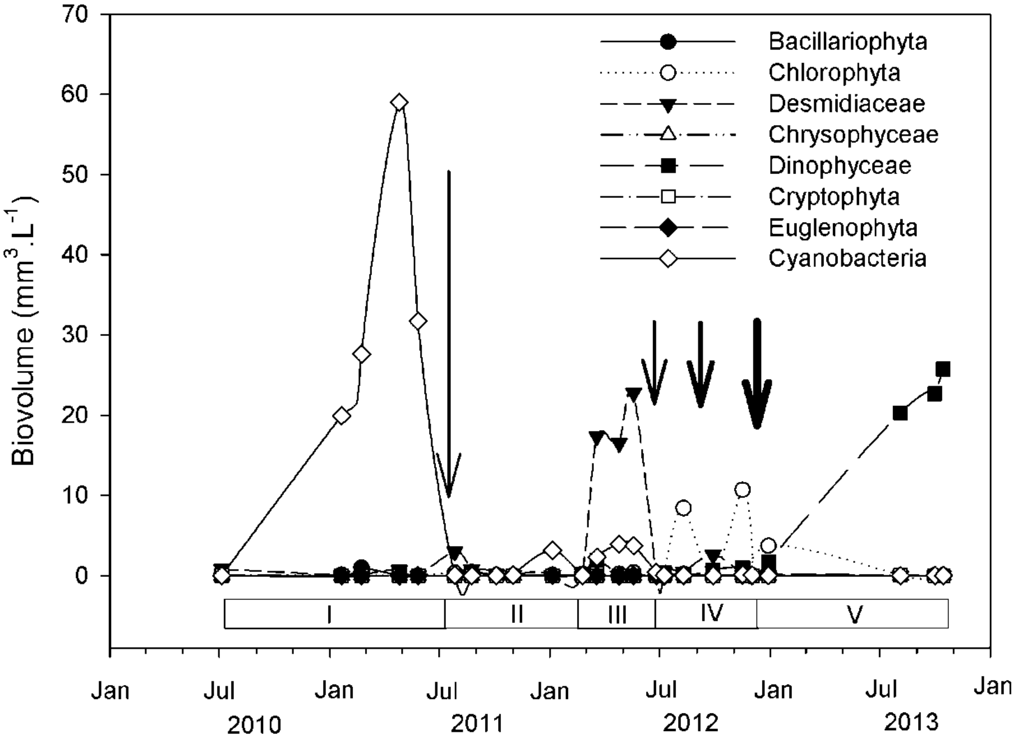


Figura 6. Dinámica temporal de la composición del fitoplancton desde Jul-2010 hasta Nov-2013 en Laguna Blanca. Las diferentes fases del fitoplancton se demarcan por los números I-V. Las flechas indican eventos de viento mayores a 65 Km.h^{-1}.

Los eventos de viento, fueron un factor sumamente importante asociados al recambio rápido entre ensambles de fitoplancton, que no pueden atribuirse a cambios típicos de sucesiones ecológica (Reynolds & Walby 1975) sino que tendrían un importante componente de resuspensión y crecimiento a partir de estructuras de resistencia en sedimentos (Hansson *et al.* 1994).

Por otra parte, en un ambiente con amplia disponibilidad de nutrientes (eutrófico), las variaciones en la relación de nutrientes (relación N:P) y el uso diferencial de estos por parte de algunos grupos como los dinoflagelados, con importante capacidad de acumulación de fósforo ante situaciones de bajas relaciones N:P (Carvalho *et al.*, 2007), fueron factores determinantes en la configuración fitoplanctónica (Fig. 7 y texto y figuras en Capítulo II).

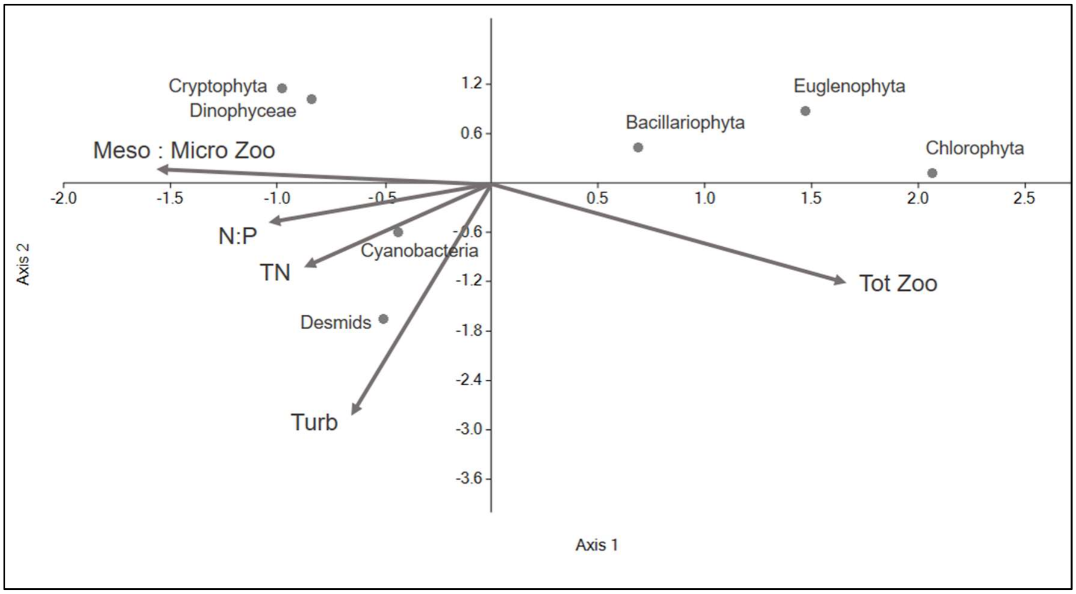


Figura 7. Biplot del análisis discriminante de redundancia (RDA) de la composición del fitoplancton en Laguna Blanca en relación a las variables ambientales explicativas: **Turb** Turbiedad, **TN:** Nitrógeno Total, **TN : TP**: relación Nitrógeno Total / Fósforo Total, **Meso:MicroZoo:** relación mesozooplancton / microzooplancton, **Tot Zoo:** Abundancia total de zooplancton.Los 2 primeros ejes explicaron el 82.3% del total de la varianza (λ1= 58.44; λ2= 23.82).

El tercer control considerado fue la presión de herbivoría por parte del zooplancton. Tanto las abundancias totales de zooplancton, como la dominancia de grupos de mayor tamaño, fueron factores fundamentales determinando la alternancia entre ensambles de fitoplancton (Fig. 7, datos mostrados en el Capítulo II).

A pesar de que en lagos someros de regiones cálidas ha sido ampliamente registrada la escasa capacidad por parte del zooplancton de ejercer una presión de consumo suficiente sobre el fitoplancton, debido a la predominancia de grupos de pequeño tamaño (e.g. Iglesias *et al.* 2008, Sinistro 2010, Lacerot *et al.,* 2010; Lacerot *et al.,* 2013), en este estudio el zooplancton presentó un rol muy importante estructurando la comunidad. En particular, la aparición repentina y por periodos cortos de tiempo de zooplancton de tallas grandes (ej: *Daphnia sp.*), ejerció un importante control por consumo favoreciendo a grupos de Cianobacterias y Dinophyta de gran tamaño, así como Cryptophyta de muy pequeño tamaño. Este patrón de aparición de macrofiltradores como *Daphnia*, fue observado mediante muestreos de alta frecuencia de muestreo (quincenal) en laguna Blanca por Gerhard *et al.* (2016) y ante la exclusión o mortandad de peces en lagos subtropicales por Iglesias *et al* (2011). La aparición de estos grupos de mayor tamaño de zooplancton se vincularía a la emergencia desde estructuras de resistencia en sedimentos (Iglesias *et al.,* 2011; Gerhard *et al.,* 2016), mientras que su desaparición se vincula al importante consumo de estos por parte de la gran carga de peces omnívoros en el sistema (Gelos *et al.,* 2010).

La composición de zooplancton dominada por grupos de mayor tamaño (mayor relación mesozooplancton:microzooplancton) favoreció recambios composicionales hacia la dominancia de organismos de las siguientes clases de tamaños:

1) Grupos de gran tamaño (GALD> 150µm) difícilmente consumibles, que escapan a la herbivoría y presentan altas capacidades de acumular nutrientes, alcanzando altas biomasas en sus fases de dominancia: Cianobacterias filamentosas (principalmente *Cylindrospermopsis raciborskii*), Desmidiáceas (*Closterium acicularis*) y Dinoflagelados (*Ceratium furcoides*). Grupos asociados a estrategias tipo *K*.

2) Grupos de muy pequeño tamaño, que compensan la pérdida por consumo con altas tasas metabólicas – reproductivas: Cryptophyta (*Plagioselmis nannoplanctica*). Asociado a estrategias *r*.

En la Figura 8 se sintetizan los factores de disturbio y estructuradores (controles) asociados a las principales características de los ensambles fitoplanctónicos resultantes.

Los eventos extremos de viento, principalmente del cuadrante S y SW, son factores de disturbio importantes, desencadenando modificaciones importantes en las condiciones del ambiente que disparan los recambios composicionales. Las relaciones de nutrientes (relación N:P) y condiciones de turbiedad, actuando en conjunto con el consumo por diferentes tallas de zooplancton, son los factores estructuradores de la comunidad fitoplanctónica hacia los diferentes ensambles (Fig. 8).

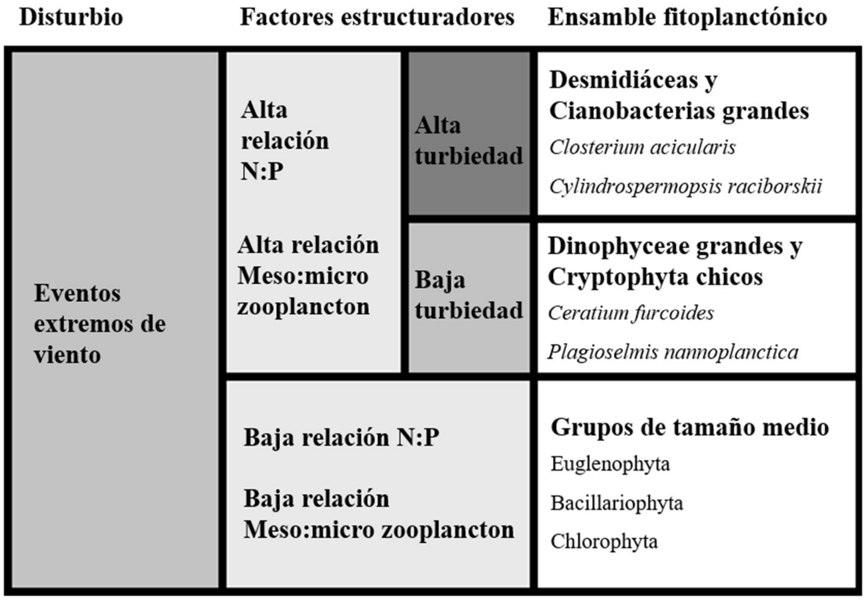


Figura 8. Principales factores que determinarían los ensambles fitoplanctónicos en Laguna Blanca, Uruguay. Eventos de viento S-SW >65km.h^{-1}, relación N:P : relación Nitrógeno Total / Fósforo Total, Relación Meso:micro: relación mesozooplancton / microzooplancton.

4.3.2 Composición funcional del fitoplancton en lagos subtropicales de diferente estado trófico y turbiedad (Capítulo III)

Estos controles de las biomasas y composición del fitoplancton, analizados en su variación interanual mediante alta frecuencia de muestreo para un lago somero, son analizados en este componente en cinco lagos someros subtropicales en Uruguay, con diferentes estados tróficos y turbidez (Capítulo III).

En este caso, se consideró un conjunto mayor de lagos y una frecuencia estacional, con el fin de analizar los patrones de variación de la biomasa y composición de fitoplancton y sus principales factores reguladores en lagos someros subtropicales. En el análisis se incluyeron lagos con diferentes niveles de nutrientes, turbiedad y biomasa de fitoplancton. En estos sistemas, se tomaron mediciones estacionales de los principales parámetros fisicoquímicos, se analizaron concentraciones de nutrientes, y se registraron las abundancias y composición de fitoplancton y zooplancton.

En el conjunto de lagos seleccionados, la abundancia y composición de tallas de zooplancton presentaron importantes diferencias tanto entre lagos como entre estaciones del año (Fig. 9).

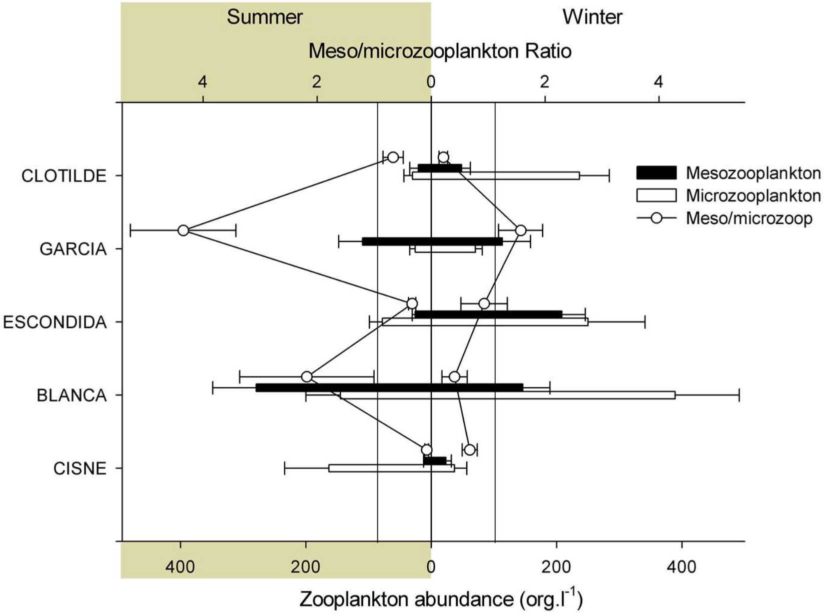


Figura 9. Abundancia media de zooplancton y relación meso:microzooplancton en invierno y verano, en los 5 lagos estudiados (Clotilde, García, Escondida, Blanca, Cisne). Mesozooplancton: copépodos calanoides + cladóceros, Microzooplancton: nauplios + rotíferos. Las barras de error representan 1 desvío estándar.

Al igual que lo observado en el capítulo II, el zooplancton fue un factor de gran relevancia determinando la composición del fitoplancton, es este caso evaluado en términos de grupos morfo-funcionales (MBFG) (*sensu* Kruk *et al.,* 2010) (Fig. 10).

El análisis de la composición funcional, permitió evidenciar de manera adecuada factores directamente vinculados con la presión de *grazing* o pastoreo, por ejemplo el tamaño del organismo (Brooks & Dodson 1965). En este sentido, las mayores abundancias de zooplancton total, presentaron una correlación positiva con grupos de fitoplancton de gran tamaño como III o VII (representados por *Cylindrospermopsis raciborskii* y *Microcystis aeruginosa* respectivamente) y del grupo I de pequeño tamaño, (pequeñas cianobacterias Chroococcales; Fig. 10). Asimismo, la menor palatabilidad de las cianobacterias, puede haber promovido su dominancia por presentar menor presión de consumo. En resumen, estos grupos, son claramente aquellos que pueden evitar el consumo debido a su gran tamaño o compensar la pérdida por con altas tasas de crecimiento dado su pequeño tamaño.

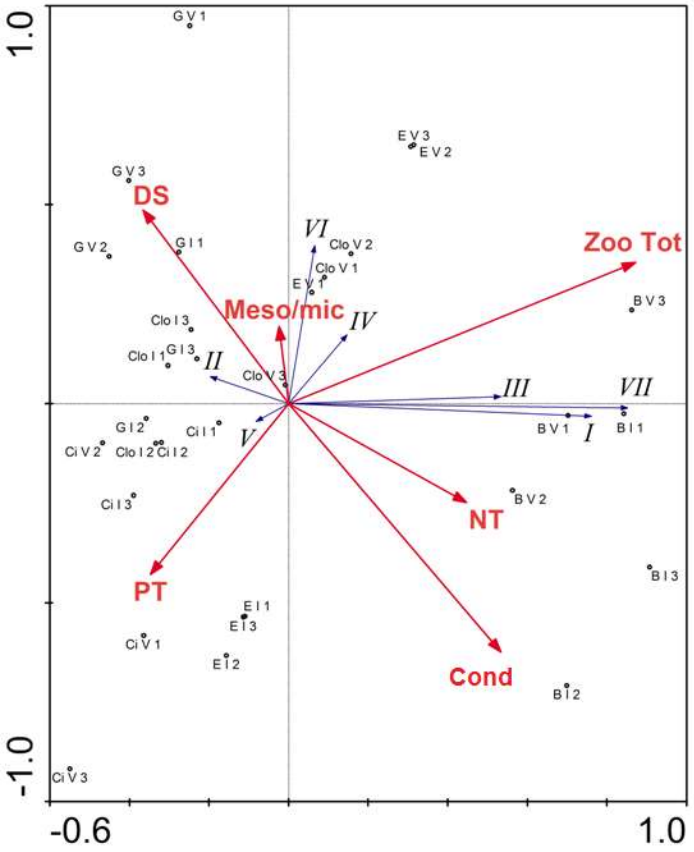


Figura 10. Diagrama triplot del RDA de composición funcional del fitoplancton en relación a las principales variables ambientales explicativas para los diferentes lagos estudiados y estaciones del año.Abreviaciones: DS: profundidad de disco de Secchi, Cond: Conductividad, PT: Fósforo total, NT: Nitrógeno total, Zoo Tot: biomasa total de zooplancton, Meso/mic: relación mesozooplancton / microzooplancon (Mesozooplancton: copépodos calanoides + cladóceros, Microzooplancton: nauplios + rotíferos). Los puntos refieren a los diferentes lagos durante V: verano y I: invierno. Los 2 primeros ejes explicaron el 53,9% de la varianza (λ1= 0.508; λ2= 0.032).

Estos resultados coinciden con lo encontrado por Jeppesen *et al,* (2005) en experiencias de biomanipulación en diferentes climas, encontrando para lagos subtropicales que si bien el zooplancton dominante suele ser de pequeño tamaño, puede tener importantes efectos estructuradores del fitoplancton, favoreciendo la dominancia de cianobacterias de gran tamaño.

El patrón observado, es coincidente con el descrito en el Capítulo II, donde el alto consumo por zooplancton de mayor tamaño estructura al fitoplancton hacia tallas muy grandes o muy pequeñas. Sin embargo, en este caso la relación Meso/microzooplancton no se asoció con la dominancia de los grupos de gran tamaño debido a la ausencia de macrofiltradores como *Daphnia sp.* Si bien varios estudios indican que la limitada capacidad de control de la biomasa fitoplanctónica en lagos subtropicales es debida a la dominancia de zooplancton de pequeño tamaño, derivada del consumo de las tallas mayores por las altas densidades de peces omnívoros (VanLeeuwen*et al.,* 2007; Meerhoff *et al.,* 2007; Jeppesen *et al.,* 2010; Lacerot *et al.,* 2013), los resultados de estos estudios en campo indican un importante rol del zooplancton estructurando la composición fitoplanctónica en lagos someros subtropicales.

Los resultados de los capítulos II y III permiten hipotetizar que la frecuencia de muestreo estacional no es adecuada para observar los efectos de la presencia de filtradores de gran tamaño como *Daphnia sp*. en sistemas subtropicales, dado su presencia esporádica y baja persistencia temporal. En segundo término, se concluye que en lagos subtropicales el zooplancton tanto de pequeño tamaño, como la presencia esporádica de macrofiltradores (como *Daphnia*), puede presentar importantes efectos estructurando al fitoplancton hacia la dominancia de grupos de gran tamaño como dinoflagelados y cianobacterias.

4.4 Experimentos en campo

Para el análisis de los mecanismos involucrados en los patrones observados en campo, así como para la prueba de las hipótesis consideradas, resulta imprescindible el abordaje experimental en los propios ecosistemas, con estructuras de gran tamaño donde se pueda experimentar cambios en las condiciones naturales del ecosistema (mesocosmos; Lampert & Sommer 2007).

Para esto, se analizaron los efectos directos y en cascada promovidos por peces pequeños omnívoros y macroinvertebrados (camarones y anfípodos) sobre el fitoplancton y perifiton, mediante experimentos en mesocosmos en lagos someros de climas contrastantes (Templado: Dinamarca, Subtropical: Uruguay; Capítulo IV).

4.4.1 Efectos promovidos por peces y macroinvertebrados sobre el fitoplancton y perifiton de lagos someros subtropicales y templados (Capítulo IV)

El estudio experimental en mesocosmos en lagos someros de climas contrastantes, conforma un elemento central de esta tesis, debido a que representa el testeo *in situ* de los mecanismos promovidos por peces omnívoros y macroinvertebrados sobre el fitoplancton y el perifiton. En este estudio, se seleccionaron 3 lagos someros subtropicales (Uruguay 35°S) y 3 lagos someros templados (Dinamarca 56°N) de condiciones limnológicas aproximadamente pareadas (cobertura de macrófitas, transparencia, y biomasa de fitoplancton). En cada lago, se manipularon las abundancias de 2 especies de peces omnívoros y 1 especie de macroinvertebrados (crustáceos; Tabla III), de acuerdo a un diseño factorial de 4 tratamientos con 4 réplicas (4x4, total 96 mesocosmos).

Tabla III. Abundancias de peces omnívoros y macroinvertebrados incluidos en los tratamientos, densidades basadas en: [+] Liboriussen *et al.* (2005), * Teixeira de Mello *et al.* (2009), [¤] Wilhelm & Schindler (1999).

	Subtropical	**Templado**
Pez sp. 1	*Cnesterodon deccemaculatus* 50 *	*Gasterosteus aculeatus* 12 [+]
Pez sp. 2	*Jenynsia multidentata* 40 *	*Perca fluviatilis* 6*
Macroinvert.	*Palaemonetes argentinus* 120*	*Gammarus lacustris* 240 [¤]

Los experimentos, fueron llevados a cabo en mesocosmos transparentes de PVC que encerraron un volumen de lago de aproximadamente 1000 litros con plantas sumergidas artificiales (similares a *Myriophyllum sp.*), en densidades de 75% de PVI (Fig. 11 y 12), colocados sobre una pasarela de madera a modo de muelle de fijación, en la zona litoral de los lagos estudiados (Fig. 12).

En estos, se removieron todos los peces y macroinvertebrados posibles mediante pesca eléctrica y red de mano, y luego de un periodo de colonización de las plantas artificiales por perifiton de dos semanas, se incluyeron las densidades de macroinvertebrados y peces referidas en tratamientos:

- Peces omnívoros (+**F**)
- Macroinvertebrados (+**I**)
- Peces omnívoros + Macroinvertebrados (**+F+I**)
- Control, sin peces ni macroinvertebrados (**C**)
- Open, control con plantas artificiales pero sin mesocosmo (abierto)

Los muestreos se realizaron dentro e inmediatamente por fuera de los mesocosmos, en condiciones previas a la introducción de peces y macroinvertebrados (T0), y a las 7 semanas de la introducción (Tf), considerándose parámetros fisicoquímicos, nutrientes y muestras de zooplancton, fitoplancton y perifiton.

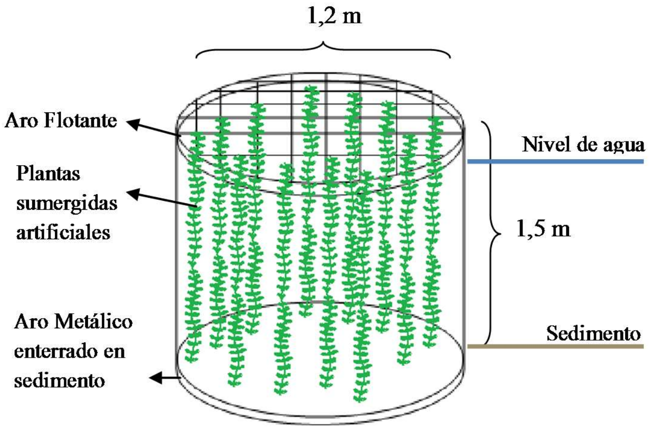


Figura 11: Esquema de mesocosmos utilizados. Las líneas de la izquierda indican el nivel del agua y del sedimento. Las plantas sumergidas se sujetan a una red en la superficie del agua, colgando hasta el fondo. El aro metálico en la parte inferior está enterrado en el sedimento. Los mesocosmos se sujetaron a muelles de madera en la zona litoral de los lagos.

Fitoplancton:

Se encontraron importantes efectos promovidos por la presencia de pequeños peces omnívoros en lagos de ambas condiciones climáticas vía cascada trófica: peces omnívoros – zooplancton – fitoplancton. La presencia de estos peces, provocó una disminución significativa de las abundancias de zooplancton en todos los tratamientos con peces (F y F+I) en relación a aquellos sin peces (F= 4,22; P= 0.002).

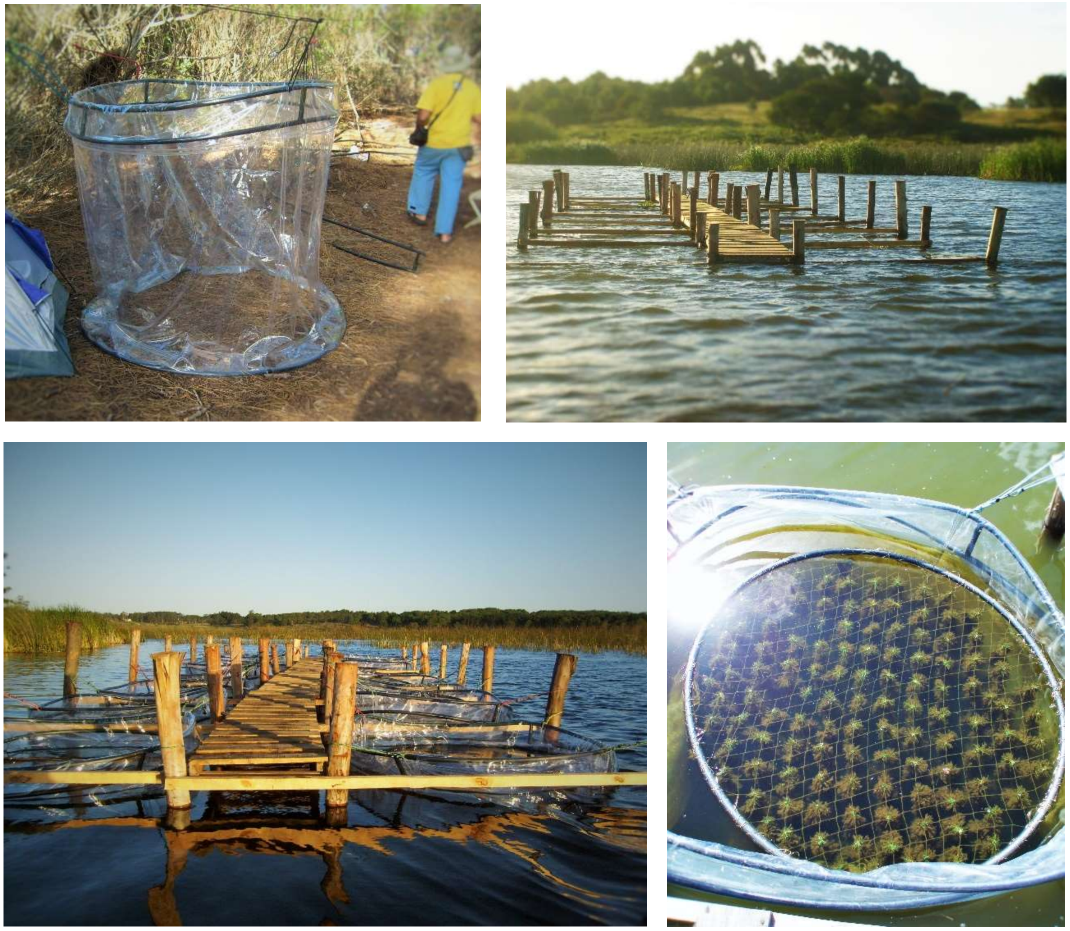


Figura 12. Imágenes de las estructuras y mesocosmos utilizados para los experimentos en lagos (Diario, Blanca y Nutrias en Uruguay; Stigsholm, Kogleaks y Bolling en Dinamarca). Arriba a la iquierda: mesocosmo transparente en PVC con aros plásticos. Arriba a la derecha: muelle de madera sobre la zona litoral de Laguna Blanca. Abajo a la Izquierda: muelle de madera con mesocosmos colocados sobresaliendo de la superficie del agua. Abajo a la derecha: vista superior del interior del mesocosmo con plantas artificiales ya colonizadas por perifiton (previo al comienzo de los experimentos).

Particularmente, los peces consumieron preferencialmente sobre el zooplancton de mayor tamaño (Cladóceros) en lagos de ambos climas (Figura 2 del Capítulo IV). Esto, se reflejó en mayores biomasas de fitoplancton, en comparación con los tratamientos sin peces (solo invertebrados o control; Fig. 13). Los efectos en cascada de la presencia de peces sobre el fitoplancton fueron observables tanto a nivel de biomasa (Fig. 13) como de composición en ambos climas (Tabla IV, Fig. 14).

Este patrón estructurador de los peces pequeños sobre el zooplancton, ha sido ampliamente registrado en sistemas subtropicales (VanLeeuwen*et al.,* 2007; Meerhoff *et* *al.,* 2007; Jeppesen *et al.,* 2010; Lacerot *et al.,* 2013) y es consistente con lo observado en los estudios en campo en esta tesis (Capítulos II y III), donde el zooplancton es estructurado por el consumo de peces promoviendo la dominancia de tallas más pequeñas disminuyendo, así como, la presión de consumo sobre el fitoplancton.

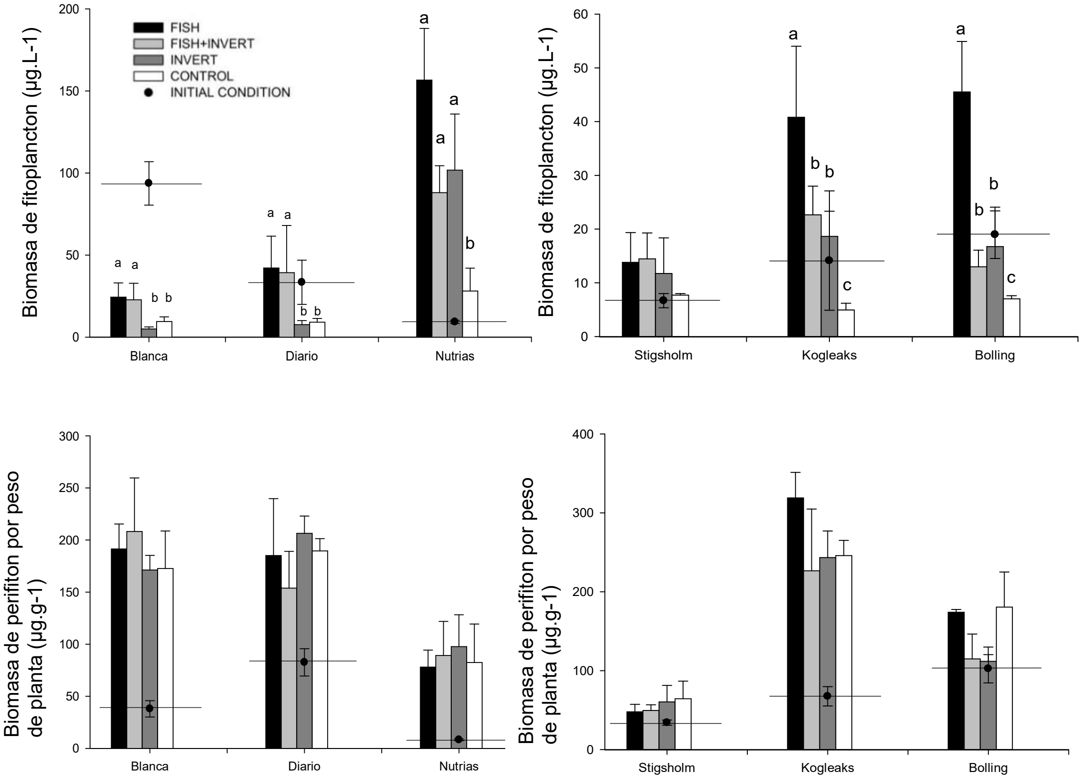


Figura 13. Biomasas de fitoplancton (gráficos superiores) y perifiton (gráficos inferiores) en lagos someros subtropicales (izquierda) y templados (derecha) para tratamientos experimentales con peces y macroinvertebrados. Las líneas horizontales en cada lago indican las condiciones iniciales (previo a la introducción de macroinvertebrados y peces. Las barras indican las condiciones finales. Las barras de error representan ± 1 error estándar. Las letras sobre las barras indican los grupos generados en los análisis pos hoc pareados. Tomado de Iglesias *et al. En prep.*

Los claros efectos en cascada vía: peces omnívoros- zooplancton- fitoplancton, encontrados en este estudio, son coincidentes con los estudios clásicos de McQueen *et al.* (1986). En coincidencia con Jeppesen *et al.* (2005), estos efectos fueron más marcados en lagos templados, donde la presencia de peces en los tratamientos promovió biomasas mayores a las encontradas en el exterior de los mesocosmos (condiciones del lago), mientras que en lagos subtropicales las biomasas en presencia de peces no presentaron diferencias significativas con aquellas en el exterior de los mesocosmos (Fig. 14).

Este patrón podría asociarse a mayores biomasas de piscívoros en lagos templados en relación a los subtropicales (Meerhoff *et al*., 2007; Jeppesen *et al.,* 2010), donde el efecto del consumo de piscívoros sobre los planctívoros libera de consumo al zooplancton, llevando a menores biomasas de fitoplancton. Por otra parte, en lagos subtropicales, las menores abundancias de piscívoros y mayores de omnívoros (Lazzaro 1997; Meerhoff *et al.,* 2007; Teixeira de Mello *et al.,* 2009; Gonzalez-Bergonzoni *et al.,* 2012) se estaría reflejando en biomasas similares de fitoplancton entre los tratamientos con peces (omnívoros) y las condiciones externas del lago. Esta limitada capacidad de consumo de fitoplancton por parte del zooplancton promovida por efectos en cascada de los peces omnívoros es la misma descrita frecuentemente en estudios de campo en lagos subtropicales (Iglesias *et al.* 2008; Sinistro 2010; Lacerot *et al.* 2013; entre otros).

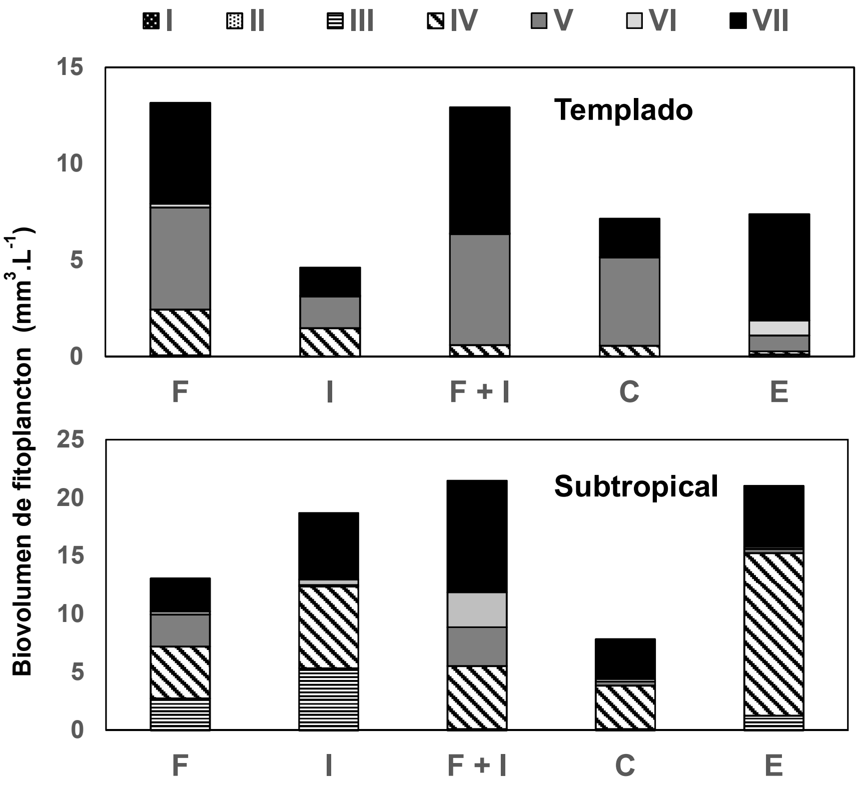


Figura 14. Composición funcional de fitoplancton (de acuerdo a Kruk *et al.* 2010, MBFG I a VII) en los diferentes tratamientos considerados (F: Peces omnívoros, I: Macroinvertebrados, F+I: Peces omnívoros + Macroinvertebrados, C: Control, E: Condiciones del lago externas a los mesocosmos) en lagos Templados (gráfica superior) y Subtropicales (gráfica inferior) donde fueron llevados a cabo los experimentos.

En términos de composición, las diferencias entre tratamientos fueron mayormente evidenciadas considerando la composición morfológico-funcional del fitoplancton (MBFG, Kruk *et al.*, 2010), en comparación con la composición taxonómica (Tabla IV). Esto es coincidente con los resultados del Capítulo I, que señalaban que este tipo de clasificación funcional puede presentar una mayor capacidad predictiva de factores ambientales que los esquemas de clasificación taxonómico.

En lagos de ambos climas, se observaron diferencias significativas en la representación de colonias mucilaginosas de cianobacterias (pertenecientes al MBFG VII) en presencia de peces, lo cual coincide con estudios previos donde el zooplancton en presencia de omnívoros, estructura al fitoplancton hacia la dominancia de grupos de gran tamaño (ej: Jeppesen *et al.,* 2005). Esto, coincide con los patrones encontrados en campo en esta tesis (Capítulos II y III) donde el zooplancton de sistemas subtropicales (alta densidad de omnívoros), estructura al fitoplancton hacia grupos de gran tamaño.

Tabla IV. Significancia de las diferencias en la composición taxonómica y funcional de fitoplancton y perifiton en relación al lago, condiciones climáticas y tratamiento, basado en análisis de similitud (ANOSIM – índice Bray Curtis). R: estadístico R del ANOSIM, P: p-valor definido para un intervalo de confianza del 95%, NS: no significancia estadística en las diferencias (R< 0,25 y/o P> 0,05). Esquemas de clasificación: * Grupos morfológicos-funcionales de Kruk *et al.,* 2010, ** Grupos morfológicos-funcionales de perifiton basados en Pacheco *et al.* (En prep. Capítulo IV esta tesis).

		Lago		**Clima**		**Tratamiento**	
		R	P value	R	P value	R	P value
Fitoplancton	Taxonómico	0.86	0.0001	0.31	0.0001	0.25	0.0001
	Funcional *	0.72	0.0001	NS	-	0.46	0.0292
Perifiton	Taxonómico	0.82	0.0001	0.63	0.0001	NS	-
	Funcional **	0.74	0.0001	0.53	0.0001	NS	-

Perifiton

Para el caso del perifiton, contrariamente a lo esperado no encontramos efectos directos ni en cascada promovidos por la presencia de peces omnívoros o macroinvertebrados sobre la biomasa o composición de estos productores primarios (Fig. 13, Tabla IV). Si bien estudios previos en climas templados han descrito la ausencia de efectos de los peces omnívoros sobre el perifiton, (Hansson, 1992; Brönmark & Vermaat 1998; Bertolo *et al.,* 2000; Bécares *et al.,* 2008) existe múltiple evidencia que los efectos de consumo directo (peces omnívoros – perifiton) son esperables para lagos subtropicales (Iglesias *et* *al.,*2007; Meerhoff *et al.,* 2007; Jeppesen *et al*., 2010), debido a la omnivoría y preferencia de hábitat asociados con macrófitas en dichos sistemas (Teixeira-de Mello *et al.,* 2009).

Mediante experimentos en mesocosmos en lagos europeos, en un importante gradiente latitudinal y de estado trófico, Bécares *et al.,* (2008), resaltó la ausencia de controles descendentes promovidos por peces sobre el perifiton, explicando que los principales factores determinantes del perifiton se vinculan a la turbiedad y niveles de nutrientes en los lagos. Esto coincide por lo encontrado por Hannson (1992), en un gradiente de lagos europeos y subarticos (lagos con y sin peces), y por Hill & Harvey (1990) mediante abordajes experimentales, que establecen que el perifiton no presenta respuesta directa o vía cascada trófica a la presencia de peces, sino que sus biomasas son explicadas por las temperaturas y los nutrientes disponibles.

En el sentido contrario a lo hipotetizado, Blanco (2001) y Blanco *et al.,* (2004) resaltan que los peces pueden tener efectos positivos sobre el perifiton aumentando su biomasa por liberación de nutrientes de sus excretas.

Gran parte de la variabilidad en la biomasa (ver Tabla III Capítulo IV), así como en la composición (Tabla IV) del perifiton fueron explicados exclusivamente por el factor clima.

Estas diferencias encontradas en las biomasas y composición del perifiton entre regímenes climáticos, que no pueden ser explicadas por los efectos de la estructura trófica, pueden deberse a los factores explicados en el Capítulo II. Estos factores, como ser limitaciones metabólicas en la biomasa hacia climas más cálidos (Brown *et al.,* 2004) y un aumento en la riqueza con la temperatura ambiente (Allan *et al.,* 2002), pueden explicar de manera complementaria los patrones diferenciales de riqueza y biomasa del perifiton en función de la latitud en lagos someros.

Es importante resaltar, que la presencia de macroinvertebrados (anfípodos en lagos templados y camarones en subtropicales) no presentó ningún efecto notorio sobre la abundancia o composición de fitoplancton o perifiton. Contrariamente a lo esperado, basado en trabajos de Collins & Paggi (1998) para camarones en lagos subtropicales, o por Wilhelm & Schindler (1999) para anfípodos en lagos templados, la ausencia de efectos sobre el perifiton podría deberse a una sobre-simplificación de las interacciones tróficas en los mesocosmos, o una mala asignación de los roles tróficos de los invertebrados utilizados. Varios autores señalan que los camarones en lagos someros subtropicales, puede ejercer un importante consumo sobre el zooplancton (Boschi 1981; Collins & Paggi 1998; Collins 1999). Sin embargo, los efectos sobre el fitoplancton en los mesocosmos no son claros y parecerían restringirse exclusivamente a mayores biovolúmenes en el fitoplancton de los tratamientos con invertebrados en relación a los controles (Fig. 14), aunque este patrón no es observado a nivel de biomasa (Fig. 13).

El análisis de las interacciones tróficas en mesocosmos en lagos someros de climas contrastantes nos permitió evidenciar:

1) Un importante control ejercido por peces omnívoros sobre las tallas grandes de zooplancton, limitando la capacidad de consumo del fitoplancton, reflejándose en la biomasa y composición del fitoplancton. Si bien los efectos de la presencia de peces omnívoros es similar entre lagos de ambos climas, parecieran ser especialmente marcados en lagos templados.

2) Ausencia de efectos directos o en cascada promovidos por la estructura trófica sobre el perifiton. Mientras que las importantes diferencias en biomasa y composición entre climas puede asociarse a variaciones latitudinales no relacionadas con la estructura trófica.

3) Ausencia de efectos directos o en cascada de los macroinvertebrados (crustáceos) sobre el fitoplancton y perifiton en ambos regímenes climáticos.

Si bien los macroinvertebrados considerados en este componente no presentaron efectos descendentes sobre el fitoplancton o perifiton, los macroinvertebrados filtradores bentónicos puede ejercer un importante control sobre el fitoplancton (Strayer *et al.,* 1999; Elliott *et al.,* 2008), especialmente en lagos someros dada la estrecha relación del componente bentónico en el funcionamiento de estos ecosistemas (Vadeboncoeur *et al*., 2002).

5. CONCLUSIONES Y PERSPECTIVAS

La combinación de los diferentes abordajes considerados en los capítulos de esta tesis, nos permitieron evidenciar importantes patrones derivados de los efectos de la estructura trófica sobre el fitoplancton y perifiton en lagos someros de diferentes regímenes climáticos.

1) Los peces omnívoros promueven importantes efectos en cascada sobre el fitoplancton, debido a un alto consumo de zooplancton especialmente de tallas grandes, lo cual libera de consumo al fitoplancton y se traduce en mayores biomasas (Capítulo IV). Si bien los efectos de la presencia de peces omnívoros es similar entre lagos de ambos climas, parecieran ser especialmente marcados en lagos templados (Capítulo IV). De acuerdo a lo planteado en la Hipótesis 1, las diferencias en biomasa y composición del fitoplancton entre lagos de diferentes regiones climáticas se debieron fundamentalmente a los efectos en cascada promovidos por los peces omnívoros, disminuyendo las abundancias y modificando la estructura de tallas del zooplancton.
2) El zooplancton, es un factor estructurador fundamental de la composición fitoplanctónica en lagos someros subtropicales (Capítulos II y III). Tanto el zooplancton de pequeño tamaño, como la presencia esporádica de macrofiltradores (ej: *Daphnia*), estructura al fitoplancton hacia la dominancia de rangos de tallas extremos: grupos de gran tamaño como dinoflagelados y cianobacterias, y grupos de pequeño tamaño con altas tasas de crecimiento
3) Al contrario de lo esperado, no se encontraron efectos de los peces omnívoros o macroinvertebrados sobre la biomasa o composición de perifiton. Se rechaza entonces la Hipótesis 2 respecto a los efectos esperados sobre la biomasa y composición de perifiton promovidos por peces y macroinvertebrados (Capítulo IV).
4) Las diferencias entre climas en la biomasa y composición del perifiton, que no fueron explicadas por la presencia de peces omnívoros y macroinvertebrados, estarían asociadas a factores climáticos no considerados en este estudio tales como requerimientos metabólicos o patrones de distribución biogeográficos.

Si bien los efectos de la estructura trófica sobre la biomasa del fitoplancton parecen ser claros, resulta necesario ahondar en los mecanismos que determinan su composición, especialmente en términos comparativos entre regiones climáticas contrastantes. Asimismo, debe aumentarse de manera importante la comprensión de los factores determinantes de la composición y biomasa del perifiton diferencial entre regiones climáticas subtropicales y templadas. En particular, en relación a los efectos promovidos por diferentes estructuras tróficas y condiciones ambientales (ej: gradiente trófico), en sistemas cálidos, donde estos estudios son prácticamente inexistentes.

El estudio de estos factores, abordados en términos composicionales y considerando las interacciones entre perifiton y fitoplancton en lagos someros, puede resultar en importantes insumos acerca de las respuestas de estas comunidades ante procesos como el calentamiento global y la eutrofización.

6. Capítulo I

Testing phytoplankton and periphyton functional classifications in relation to environmental variables in shallow lakes of contrasting climatic conditions

Juan Pablo Pacheco, Irina Izaguirre, Laura Sánchez & Néstor Mazzeo

Abstract

We explore the performance of morphological-functional classification of phytoplankton (Kruk, *et al*., 2010; Salmaso & Padisák 2007, Reynolds *et al.*, 2002) periphyton, and taxonomic groups on their predictive ability in relation to environmental conditions in 16 shallow lakes of contrasting climatic conditions.

With this objective, we proposed a new morpho-functional classification for photosynthetic periphyton that consider morphological traits and main taxonomic groups (total of 28 groups).

In addition, a brief review was conducted to see how used are different classifications of phytoplankton, and on which type of freshwater ecosystems.

The classification scheme of functional group proposed by Reynolds *et al.* (2002) has been the most widely used. Shallow lakes and reservoirs are the type of freshwater ecosystem where all the ecological approaches have been used more frequently.

In this study, we found that all ecological classifications have a similar predictive ability between them, and were similar to those obtained taxonomical approaches.

Also in the case of periphyton, morphological-functional groups of periphyton proposed in this paper (MPG), showed a similar predictive ability of the environmental variability to that obtained with taxomic-based approach.

In the case of phytoplankton, both classifications of MFG (Salmaso & Padisák, 2007) and MBFG (Kruk *et al.,* 2010) showed a predictive ability similar or even higher than taxonomic classification.

Considering the ease of use, ecological approaches based on morphology can significantly reduce the error in the data derived from taxonomic uncertainty or about functional aspects of groups, associated with classifications that are more complex as taxonomic-based or functional approach.

Keywords: morphology based functional classification, functional traits, ecological approaches, phytoplankton and periphyton functional classification

Introduction

The current relevance and increasing need for assessing biodiversity in freshwater ecosystems, have been limited by the requirements of time, expertise and money that requires a high degree of taxonomic determination (Dudgeon *et al*., 2006).

The recent use of alternative approaches to taxonomic classification, to analyze patterns of diversity is becoming more frequent in different types of freshwater ecosystems (review in: Litchman & Klausmeier 2008, Heino 2010).

For phytoplankton, there are different types of these approaches mainly based on: a lower level of taxonomic resolution (e.g. Heino & Soininen, 2007), use of indicators groups (Soininen *et al.,* 2009) and ecological classifications or based on attributes directly related with ecological characteristics (Salmaso & Padisák, 2007; Padisák *et al*., 2009; Reynolds *et al*., 2002; Kruk *et al*., 2010).

The basis of the use of these alternative approaches would seem to lie on the simplicity of use, reducing the large number of taxonomic groups into smaller groups, while maintaining or even improving the predictive capability regarding environmental gradients (Huszar & Caraco 1998; Mc Gill *et al*., 2006; Litchman & Klausmeier 2008; Heino 2010). An example of this constitutes the evidence of chaotic behavior of plankton when is analyzed at the species level (Scheffer *et al*., 2003; Beninca *et al.,* 2008) so the use of higher levels of taxonomic determination or the use of functional approaches may result more appropriate evidencing environmental gradients.

Approaches based on lower taxonomic resolution are frequently used in multiple communities (see Gallego *et al*., 2012 and references therein) mainly linked to the impossibility of achieve a higher level of identification, than to the explicit knowledge of their ability to summarize information or to better reflect the ecological processes.

Functional approaches, which consider main characteristics and ecological requirements of groups, reflects the functional diversity of the ecosystem instead the species diversity. Thereby, they reduce and integrate information and may reflect better underlying ecological processes than classical taxonomic approaches (Weithoff 2003; Litchman & Klausmeier 2008, Heino 2010).

Based on these ideas, different functional and morphological-functional classification systems have been developed for phytoplankton. One of the widely known and used is the Functional Groups (FG) classification by Reynolds *et al.* (2002) which firstly proposed 31 groups based on morphology and ecological characteristics in relation to tolerance and sensitivity to different environmental gradients. This classification has been revised and updated by Padisák *et al.* (2009), and now 42 groups can be recognized. Besides, Salmaso & Padisák (2007) developed a system 31 Morphological phytoplankton functional groups (MFG) for two deep lakes in Italy, based on the combination of main taxonomic groups with morphological and functional traits. Here, the criteria adopted to discriminate the groups include the traits proposed by Weithoff (2003): motility, potentiality of mixotrophy, specific nutrient requirements, size and shape, and the presence of envelopes.

More recently, Kruk *et al.* (2010) has developed a classification based on morphological characters associated to functional and physiological characteristics, summarizing in only 7 groups the diversity of phytoplankton in relation to environmental characteristics. This functional approach has shown good predictive ability in relation to environmental characteristics (Kruk *et al.,* 2011). However, this has been discussed regarding their predictive ability in vegetated or with high number of mixotrophs lakes (Izaguirre *et al.,* 2012).

In the case of periphyton (referring only to photosynthetic component) the development of functional groups has been relegated to lotic ecosystems and to similar classifications than used in ecology of terrestrial plants, based on life forms or few functional attributes (e.g. Steinmann *et al.*, 1992). Despite its limited development and testing, these morphological - functional periphyton classifications have shown a very good predictive ability of the environmental characteristics, such as the capability to prevent or reduce grazing (e.g. Steinman 1991; Steinman *et al.,* 1992; Walton *et al.,* 1995).

Considering the key role of the periphyton in the benthic-littoral processes in shallow lakes (e.g. Jones 2002, Jones & Sayer 2003, Liboriussen *et al.*, 2005) the development of morphological-functional groups for periphyton in shallow lakes, may allow to better analyze ecological processes that occur in these ecosystems and the role of periphyton within them.

This paper, first briefly reviews the use of ecological approaches for phytoplankton proposed by Reynolds *et al.* (2002), Salmaso & Padisák (2007) and Kruk *et al.* (2010). Subsequently, we analyze the predictive ability of these classification approaches for phytoplankton, a new morphological-functional scheme proposed for periphyton and taxonomic level, in relation to environmental characteristics in shallow lakes of different climatic conditions (Uruguay, Argentina and Denmark).

Materials and methods

Use of ecological classifications of phytoplankton in freshwater ecosystems

To find out the current level of use of the ecological classification approaches of phytoplankton in freshwater systems, we conducted an extensive literature review of scientific articles on indexed journals up to 2013 considering the databases: JSTOR Science Direct, Scopus, SpringerLink, Directory of Open Access Journals and Google Scholar. In these browsers, we considered as search criteria the following combinations of words:

1. *Phytoplankton + Functional + Group / Classification / Trait / Approach*
2. *Phytoplankton + Morphology / Group / Trait / Approach*

Of all the papers found for this primary review, we only considered those relating to classification schemes proposed by: Reynolds *et al.* (2002), Salmaso & Padisák (2007) and Kruk *et al.* (2010). These papers were sorted according to the classification approach, publication year, country, and type of ecosystem included in the study categorized into Reservoirs, Rivers, Shallow Lakes, Deep Lakes and Ponds.

This review could not be performed for periphyton ecological classification due to the low number of papers that addressed this topic.

Predictive ability of the taxonomic and ecological classifications in relation to environmental characteristics

A total of 16 shallow lakes were considered in different climatic conditions, 8 in Uruguay (34°40'- 34°53' S), 5 in Argentina (35°32'- 36°48 S) and 3 in Denmark (55°58' - 56°10'

N) including clear-vegetated, turbid due to phytoplankton and inorganic turbid (due to resuspension) lakes for each climatic condition. In these lakes the following variables were measured *in situ*: conductivity, pH, dissolved oxygen, and turbidity. In the laboratory we determined: Alkalinity, Phytoplankton and Periphyton biomass (as Chlorophyll a), and nutrients (Total Phosphorus, Reactive Soluble Phosphorus, Total Nitrogen) according to Nusch (1980), Valderrama (1981) and APHA (2005).

For those analysis concerning physicochemical variables and phytoplankton we considered all the lakes, while for those related to periphyton we excluded 5 lakes in Uruguay due to lack of compositional data.

Phytoplankton samples were mostly collected from vertical integration of the water column, except for the lakes in Argentina where were collected subsuperficially. Periphyton were collected from comparable artificial substrates in all cases (colonization time 1 month aprox.) less than 1 m deep and were standardized per unit area (Biovolume / surface). Phytoplankton and periphyton samples were preserved in acid Lugol 1% until they were analyzed in laboratory.

Counting were performed by random fields at 100X to 400X in Olympus CKX 41 inverted microscope in sedimentation chambers (Utermöhl 1958). The algal volumes were calculated according to Hillebrand (*et al.* 1999) and biovolume was estimated as the individual volume of each species by its abundance. Picoplanktonic (less than 2 µm) and tychoplankton organisms were not considered. Biomass was estimated from biovolume, assuming unit specific gravity

We performed a comparative analysis of the predictive ability of phytoplankton ecological classifications (Reynolds *et al.,* 2002; Salmaso & Padisák 2007; Kruk *et al.,* 2010), morphological-functional groups of periphyton proposed in this work (MPG) was, and taxonomic groups in relation to environmental characteristics.

In order to define the periphyton morpho-functional groups, we first consider the main taxonomic groups as integrators of the principal functional and morphological characteristics (e.g. cell wall, composition of pigments). Within these major taxonomic groups, we followed a method for the definition of the functional groups based on that proposed by Salmaso & Padisák (2007) for phytoplankton, and adding particular morphological aspects of the periphyton. The criteria used to discriminate periphyton morpho-functional groups included life form and presence of mucilage (based on

Steinmann *et al.,* 1992) size and ability to obtain nutrients from different sources (Weithoff, 2003).

The combination of these criteria in the differentiation of 28 morphological-functional groups is presented in Table I.

Table I: Morpho-functional groups proposed for photosynthetic periphyton (see explanation in text).

Taxonomic group	**Morphology**	**assigned code**
1- Cyanobacteria	1a – Unbranched filaments	1a FilUnb
	1b – Filaments with specialized cells (Nostocales)	1b FilNos
	1c – Non- mucilaginous branched filaments	1c FilBran
	1d – Mucilaginous branched filaments	1d FilMuc
	1e – Mucilaginous colonies	1e CyaMuc
	1f – Unicellular	1f CyaUni
2- Diatoms (Bacillariophyta)	2a – Small unicellulares <30 µm	2a SmDia
	2b – Unicelulares >30 µm	2b LDia
	2c – Palisaded colonies	2c PalDia
	2d – Filaments	2d FilDia
	2e – Bouquet-like colonies	2e BouqDia
3- Chlorophyta	3a – Small unicellular < 30 µm	3a SmChlo
	3b – Large unicellular > 30 µm	3b LChlo
	3c – Unicellular flagellates	3c FlaUni
	3d – Colonial flagellates	3d FlaCol
	3e – Small naked colonies <30 µm	3e SmNaCol
	3f – Large naked colonies >30 µm	3f LNaCol
	3g – Small mucilaginous colonies <30 µm	3g SmMucCol
	3h – Large mucilaginous colonies >30 µm	3h LMucCol
	3i – Unbranched filaments	3i UnbChlo
	3j – Branched filaments	3j BranChlo
4- Conjugatophyceae	4a – Unicellular desmids	4a DesUni
	4b – Filamentous desmids	4b DesFil
5- Xantophyta	5a – Unicellular	5a XanUni
	5b – Filamentous	5b XanFil
6- Chrysophyta	6a – Unicellular	6a ChrUni
	6b – Colonies	6b ChrCol
7- Dinophyta		7 Dino

Data analyses

The physical and chemical data were standardized by subtracting the mean and then dividing by the standard deviation of the data set, to remove trends resulting from excessive differences between measurement units.

All the classifications considered were analyzed comparatively in terms of: biomass (estimated as biovolume), logarithm of biomass: Log (x + a) where "x" is biomass

calculated in terms of biovolume and "a" is the smallest value of biomass present in the data, and finally by presence / absence (P-A) of the different groups.
For the analysis of environmental gradients in selected lakes, a principal component analysis (PCA) was performed by selecting from the set of environmental variables, those directly linked to the trophic status and light environment ecosystem: nutrients, phytoplankton biomass, periphyton biomass, and turbidity.

Similarity analysis (one-way ANOSIM) a non-parametric procedure to tests for significant differences between groups, were performed to compare classification schemes and physico-chemical variables. These analyzes were performed using Euclidian distance for physico-chemical variables, Bray-Curtis index for community composition, and Jaccard for Presence-Absence data (Clarke 1993) considering a confidence interval of 95% ($P < 0.05$). Variables directly related with community biomass as Chl_a or Turbidity, were excluded from the ANOSIM. High predictability was considered when R values > 0.50; medium predictability when $0.5 > R > 0.25$ and barely predictable when $R < 0.25$ based on Clarke & Gorley (2001). Multivariate analyses were carried out using Past 3.X version software (Hammer *et al.,* 2001).

Results

Use of ecological classifications of phytoplankton in freshwater ecosystems

A total of 38 papers from international and regional indexed journals were found, including the use of the referred ecological approaches of functional phytoplankton between 2003 and 2013. The cumulative number of papers using different functional classification approaches for phytoplankton, showed an exponential pattern ($R^2 = 0.984$; $P <0.001$).

These studies have mostly been conducted in recent years, since 2008 in Brazil and since 2011 in the case of China (Fig. 1). Most of these papers were from shallow lakes (n= 18) and reservoirs (n= 12) mainly in Brazil and China (> 47 % of the papers) followed by Argentina (Fig. 1).

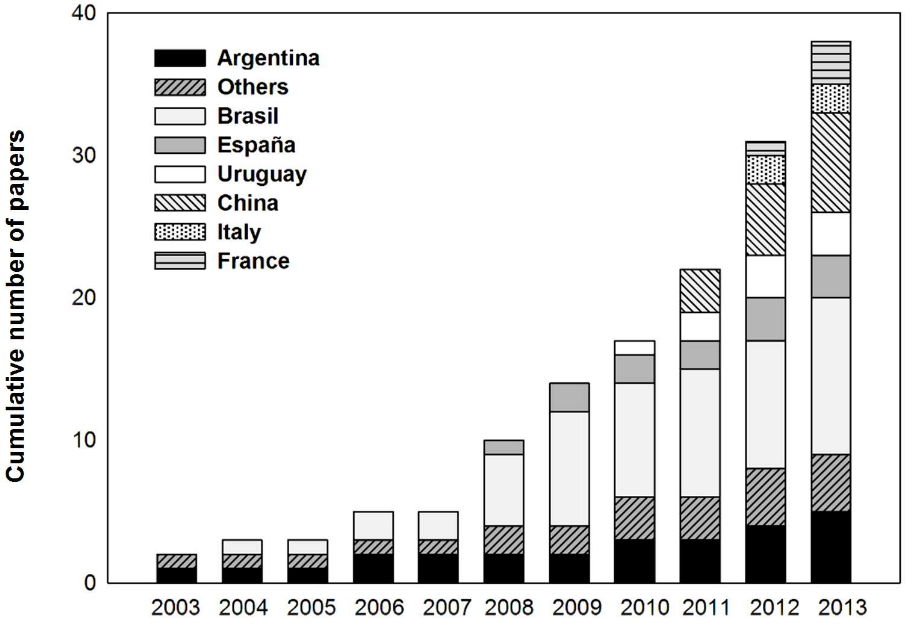


Figure 1. Number of published papers by country considering (morpho-) functional classification schemes of phytoplankton to 2013 (Reynolds *et al*., 2002; Salmaso & Padisák 2007, Kruk *et al*., 2010). Note that the number of papers is cumulative over the years.

Since its publication in 2002, the FG (Reynolds *et al.,* 2002) is being used frequently, increasing its use since 2008 (Fig. 3) especially in Brazil, Spain and France (data not shown).

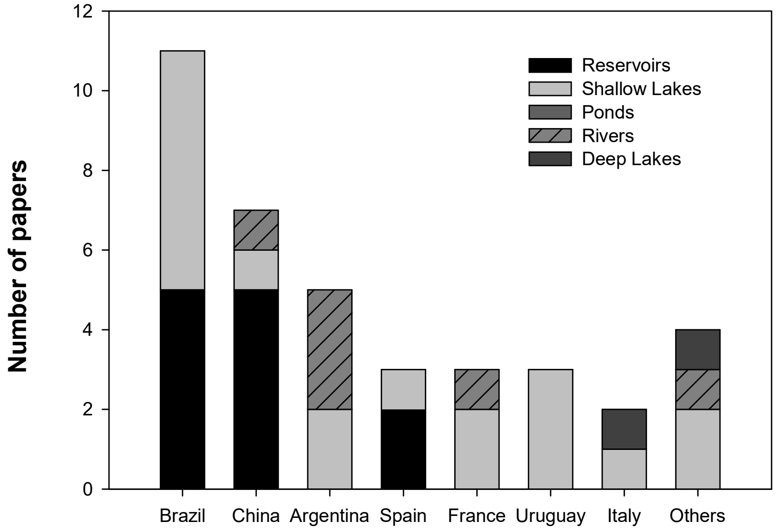


Figure 2. Number of published papers considering (morpho-) functional classification schemes of phytoplankton (based on: Reynolds *et al*., 2002; Salmaso & Padisák 2007, Kruk *et al.,* 2010) by country and type of freshwater ecosystem to 2013.

The MBFG (Kruk *et al.,* 2010) and MFG (Salmaso & Padisák 2007) have been much less used, although the classification in MBFG (Kruk *et al.,* 2010) groups had a significant increase especially since 2012.

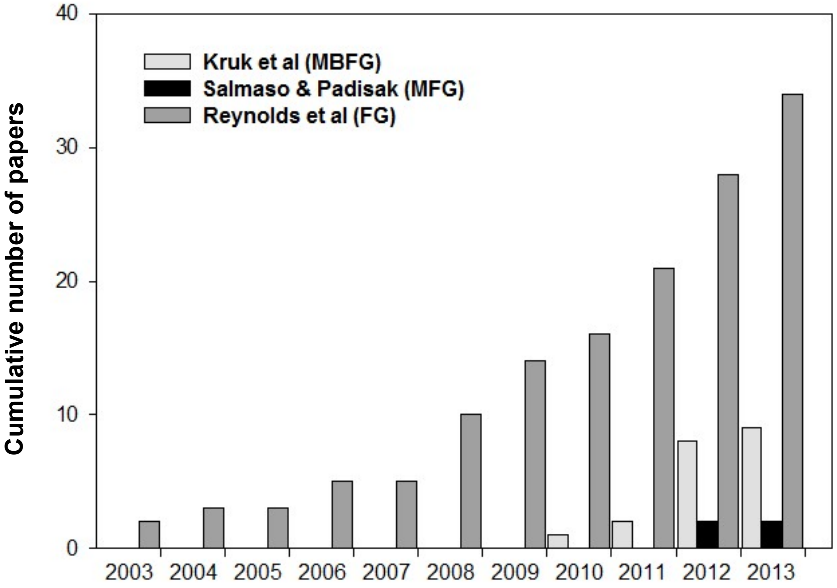


Figure 3. Number of published papers considering the different (morpho-) functional classification schemes of phytoplankton to 2013 (Reynolds *et al*., 2002; Salmaso & Padisák 2007, Kruk *et al*., 2010). Note that the number of papers is cumulative over the years.

Predictive ability of the taxonomic and ecological classifications in relation to environmental characteristics

The principal component analysis showed a clear gradient of trophic status and light environment, considering the environmental variables: turbidity, total Nitrogen, total Phosphorus, Soluble Reactive Phosphorus, Phytoplankton and Periphyton biomass (as Chlorophyll a) (Fig. 4). In the PCA, axis 1 was positively associated with lakes with high turbidity, both inorganic and due to phytoplankton (Phyto Chl_a) with high nutrient levels.

Instead, axis 1 was negatively correlated with clear waters lakes, with low levels of nutrients and high biomass of periphyton (Chl_a Periphyton). Periphyton was negatively correlated with turbidity and phytoplankton, showing that in turbid lakes, both for inorganic or phytoplankton, periphyton can not reach high biomasses (Fig. 4).

Ecological approaches exhibited high predictive capabilities, similar to those obtained by classifications based on taxonomic groups (R > 0,5; Table II). Lower predictive ability values were obtained when considering the data based on biomass (biovolume) for all ecological classifications considered (Table II; Fig. 5). However, this pattern was not observed for taxonomic based classifications (species and genus for phytoplankton and only genus for periphyton).

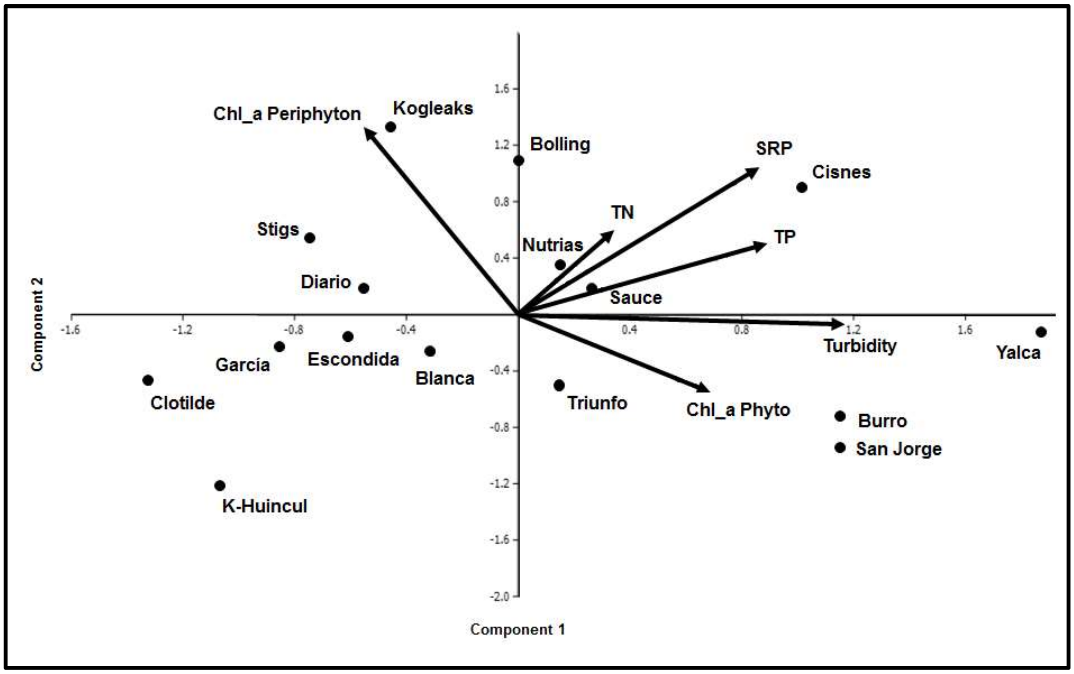


Figure 4. Principal Component Analysis of the main environmental variables related to trophic state of the 16 lakes considered. Environmental variables: Turbidity, Total phosphorous (TP), Soluble Reactive Phosphorus (SRP), Total Nitrogen (TN), Biomass of photosintetic periphyton (Chl_a Periphyton) and Phytoplankton (Chl_a Phyto).

Furthermore, the application of log data to biomass increased on average by 40% the predictive ability of all functional classifications for phytoplankton and 23% for periphyton. Also, considering only the presence or absence of the groups in the ecological approaches, a predictive ability of c.a. 60% was obtained for phytoplankton while for periphyton was only 26%.

Table II. Mean predictive ability of different (morpho-) functional classifications and taxonomic resolution considered for phytoplankton and periphyton, in relation to environmental variables (upper row). R anosim values calculated from Biomass (biovolume), Log (biomass +a), and Presence – Absence. All the R values were significative (P <0.05) (see text).

Physico-chemical			0,97
Phytoplankton	Biomass	Log (Bm + a)	Presence - Absence
Genus	0.76	0.78	0.65
Species	0.73	0.71	0.64
MBFG Kruk *et al*. 2010	0.31	0.75	0.59
FG Reynolds *et al*. 2002	0.27	0.68	0.62
MFG Salmaso & Padisák 2007	0.30	0.59	0.57
Periphyton			
Genus	0.53	0.55	0.32
MPG Periphyton (this paper)	0.44	0.57	0.26

Considering the taxonomic composition, the predictive ability was slightly higher for genus than species level for phytoplankton.

The best performance referred to abilities was associated to taxonomic classification at genus level (R = 0.78) followed by MBFG (Kruk *et al.,* 2010; R = 0.75), both from log transformed data.

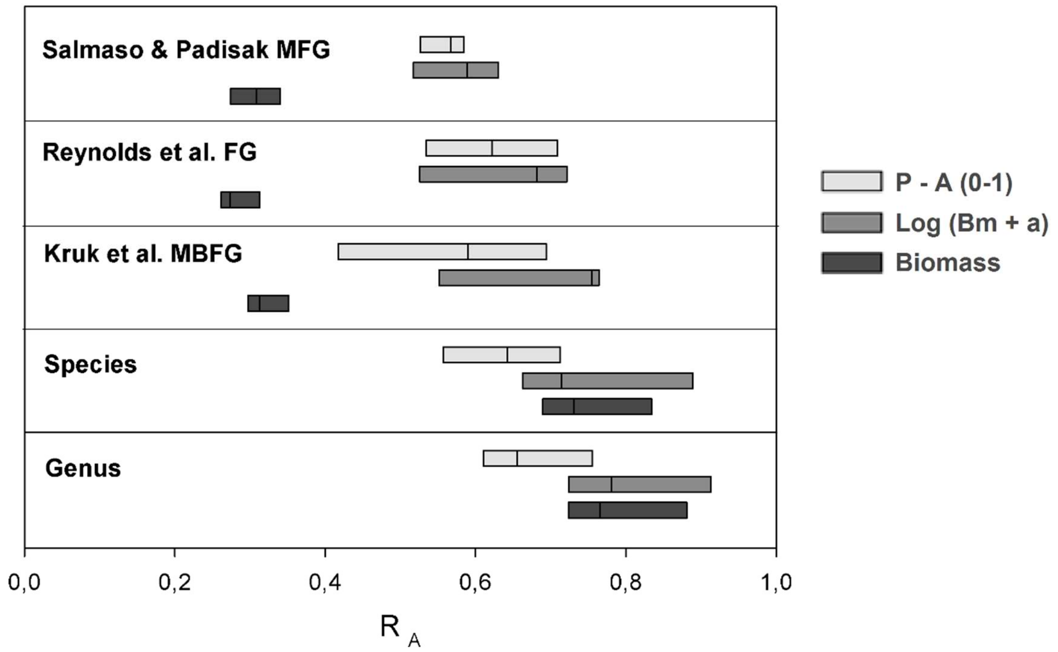


Figure 5. Predictive ability of different (morpho-) functional classification schemes and taxonomic levels considered for phytoplankton. R_A refers to ANOSIM "R" values calculated from Biomass, Log (biomass +a), and Presence – Absence (see text). Lines in each boxplot indicates the mean of data range, and standard error is indicated by the limits of each box. All the R values were significative (P <0.05).

In this sense, the classification in MBFG (Kruk *et al.,* 2010) had the highest predictive ability of different ecological classifications considered, and was even higher than those obtained for species level (Table II, Fig. 5). Morphological-functional classification of periphyton proposed in this paper (MPG), exhibited a high predictive capacity, similar to that obtained with taxonomic classification at genus level (Table II).

Discussion

The use of ecological classifications has been increasing in recent years, with an exponential increase since 2003, showing that have been widely accepted as good descriptors of phytoplankton diversity in relation to environmental variables.

While FG were the most used classification for phytoplankton, this would be due to this classification was the first published and after a time of testing has shown good performed, leading to their widespread use in recent years. The use of MBFG shows a significant increase since its publication, and in consideration of ease of use (only 7 groups based on morphological characteristics) and high predictive capacity (Kruk *et al.,* 2011) probably this number will increase significantly in the next years.

The lakes considered in this study were distributed in different climatic conditions and levels of nutrients, turbidity and biomass of phytoplankton and periphyton. This would be associated with lakes: clear with low levels of nutrients, turbid inorganic and turbid by phytoplankton, the latter two with high levels of nutrients. These contrasting environmental situations constitute an adequate model for the analysis of the predictive ability of the different classifications considered. The inverse pattern exhibited between phytoplankton and periphyton biomasses, coincides with other studies, where in clear lakes microalgal primary productivity is mostly associated with periphyton, while in turbid lakes production is almost exclusively pelagic. This would be associated with competition for light and nutrients between these communities (e.g. Richardson & Marshall 1986; Vadeboncoeur & Steinman 2002; Liboriussen & Jeppesen 2003; Dodds 2003). In this study, all considered ecological approaches exhibited a similar predictive ability to those taxonomic classifications. Although, in this study as in other studies in phytoplankton and other communities, higher taxonomic levels (genus) were the best predicting environmental variability (Gallego *et al.,* 2012 and references therein) the ecological approaches exhibited similar performance to taxonomic based approaches for both phytoplankton and periphyton classifications.

Ecological approaches were however highly sensitive to biomass data without log transformation. The application of log transformation to data biomass decreasing the over-dispersion of data, would have a more marked effect on the ecological than in taxonomic classifications possibly associated with smaller number of functional groups compared to the taxonomic group (genus or species). This may generate an extremely high integration of biomass in a few groups which dispersion would be greatly increased. While the log transform is not recommended in discrete counts data (O'Hara & Kotze 2010) in this paper has shown a fundamental role by increasing the predictive ability of the ecological approaches.

In communities such as phytoplankton and periphyton, aggregated distribution patterns generate high means and over-dispersion in data. In these cases, the logarithm transformation can be a simple method, which as noted in this paper, can significantly increase the ability to explain patterns of composition in relation to environmental gradients. In this study, the application of log data composition had much greater effects than the differences arising from using one type or another functional classification.
Also, the application of generalized linear models with Poisson (Gallego *et al.*, 2012) quasi-Poisson or negative binomial distributions (O'Hara & Kotze 2010) have shown good performance managing composition data with over-dispersion.

Periphyton morphological-functional classification proposed in this study (MPG), exhibited good performance for shallow lakes with different environmental and climatic conditions. The predictive abilities of this classification were similar to those of taxonomic genus level, but with the advantage of having only 28 groups of easier classification than taxonomic-based classifications. In addition, it is simple to use with minimal taxonomic knowledge, which reduces the error from taxonomic uncertainty or arising from the absence of local or regional identification keys. However, this classification must be tested to assess their applicability considering a larger data set, integrating other types of ecosystems in addition to shallow lakes.

The MBFG (Kruk *et al.,* 2010) exhibited the highest predictive capability, being even higher than at the species from log transformed biomass data. However, the good predictive ability of this classification scheme, supporting findings of Kruk *et al.* (2011) may be influenced by the limnological characteristics of the selected lakes. In this sense, it is important to consider that the predictive ability of MBFG scheme has been referred as low by Gallego *et al.* (2012) for 87 artificial ponds in Andalusia and by Izaguirre *et al.,* (2012) in vegetated or with high number of mixotrophs lakes in the Pampa Plain region of Argentina. Therefore, their applicability must be tested in a larger set environmental variables and climatic conditions, in order to clarify what are the environmental characteristics that limit their predictive capacity.

Functional groups proposed by Reynolds *et al.* (2010), widely accepted because their good predictive ability in relation to environmental conditions in various aquatic ecosystem (O´Farrell *et al.,* 2003; Caputo *et al.,* 2008; Becker *et al.,* 2010 among others), has been revised and validated (Padisák *et al.,* 2009). Even earlier versions of this system have presented greater predictive ability than taxonomic approaches (Huszar & Caraco 1998). In this study, FG exhibit high predictive ability, but comparatively similar to the other ecological and taxonomical-based approaches. This is relevant given the high requirements of taxonomic and functional knowledge required for classification in FG (Izaguirre *et al.,* 2012) and considering that is not less time consuming than higher taxonomic classification level. In comparison, the MBFG has a predictive capacity even higher than FG, and is much easier to use as they consider only 7 groups based on easily observable morphological traits, also without extensive taxonomy or functional knowledge. In this classification, the error resulting from taxonomic uncertainty is lower, while adequately reflected general compositional patterns in relation to environmental variability.

As a general pattern, classifications based mostly in morphology traits such as MFG, MBFG and MPG (periphyton classification presented in this work) have shown to be good indicators of the composition of phytoplankton and periphyton in relation to the environmental variability, similar or even higher to taxonomic approaches.

Conclusions

Use of ecological classifications has increased continuously since their appearance. Most studies on this topic considered the FG classification of Reynolds *et al.* (2002). In this study, we found that all ecological classifications have a similar predictive ability between them, and were similar to those obtained with classifications based on taxonomy. Also in the case of periphyton, morphological-functional groups of periphyton proposed in this paper showed a similar predictive ability of the environmental variability to that obtained with taxomic-based approach.

In the case of phytoplankton, both classifications of FG (Salmaso & Padisák, 2007) and MBFG (Kruk *et al.,* 2010) showed a predictive ability similar or even higher than taxonomic classification.

Considering the ease of use, ecological approaches based on morphology can significantly reduce the error in the data derived from taxonomic uncertainty or about functional aspects of groups, associated with classifications that are more complex as taxonomic-based or functional approach.

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7. Capítulo II

Zooplankton grazing, N:P ratio and extreme wind events as drivers of phytoplankton assemblage states in a subtropical shallow lake

Juan Pablo Pacheco, Carlos Iglesias, Miriam Gerhard, Néstor Mazzeo, Mariana Meerhoff, Guillermo Goyenola, Claudia Fosalba & Irina Izaguirre

Abstract

Factors associated to the stability of different stable states in shallow lakes, show important differences in subtropical climates in relation to temperates, which make them more susceptible to turbid water states along the trophic gradient. Many of these factors are associated to the occurrence and persistence of phytoplankton blooms, mostly probable in eutrophic and hypereutrophic conditions. In this study, we analyze the conjunction of different factors, such as disturbance events, and those related to top-down and bottom-up controls, that drives the occurrence and persistence of different phytoplankton assemblage states in a subtropical shallow lake using as a tap water source.

From Jul 2010 to Oct 2013, many sampling campaigns were carried out in Lake Blanca (Uruguay), including high frequency periods (every two weeks from Jul 2011 to Jan 2013). Water samples from three points along a pelagic transect on the maximum lenght were taken by vertically integration of water column.

Physical and chemical variables, zooplankton and phytoplankton were analyzed in conjunction in relation to the occurrence and persistence of different phytoplankton assemblage states.

During the study period, the studied lake (L. Blanca) experienced high biomass states with dominance of Cyanobacteria (*Cylindospermopsis raciborskii* and *Microcystis aeruginosa*), Desmids (*Closterium acicularis*) and, finally high biomass and dinoflagellate dominance (*Ceratium furcoides* >96% biovol), alternating with clear water phases, with lower biomass of chlorophytes, diatoms and euglenoids.

Nutrients N:P ratios and zooplankton grazing were the main factors stabilizing the five phytoplankton assemblage states observed, whereas extreme wind events were related to drastic changes in biomass and compositional replacement.

Although small sized organisms dominated the zooplankton in Lake Blanca, larger zooplankton displayed an important structuring role on phytoplankton composition, contrary to what has been frequently discussed for subtropical shallow lakes. That, led to the dominance of large phytoplankton groups (non-edible, GALD >150µm, K strategist, e.g. *Ceratium furcoides*) that escape from grazing, or small sized groups which compensate the loss by grazing with high growth rates (r strategist, e.g. *Plagioselmis nannoplanctica*). These types of controls are directly linked to those mentioned in other studies as stabilizers of phytoplankton assemblage states, which are not directly associated with competition between phytoplankton groups. These mechanisms are still scarcely recognized as important drivers of phytoplankton composition in subtropical shallow lakes.

Several mechanisms, related to the effect of wind resuspension on the replacement of phytoplankton species, associated with resistance structures, and about the appearance of large zooplankton in short periods are further discussed in this paper in the context of mechanism driving and stabilizing phytoplankton assemblage states.

Keywords

Phytoplankton composition, phytoplankton assemblage states, top down and bottom up controls.

Introduction

Patterns in phytoplankton assemblages and dominance of particular groups derives from the conjunction of various mechanisms related with bottom-up and top down controls, including nutrient and light availability, zooplankton grazing among others (e.g. Reynolds, 1993).

Several studies have pointed out the existence of different types of steady states in phytoplankton composition, defined by Sommer *et al.* (1993) as stable dominance of few species (maximum of three) for a period of several weeks (Naselli-Flores *et al.,* 2003). These steady states are not necessarily explained by competition between groups and can be explained mostly by other processes e.g. grazing (Albay & Akcaalan, 2003; Rojo & Alvarez-Cobelas, 2003).

Although in phytoplankton ecology the "motionless" meaning linked to steady state phases are rarely attained, little variability in species dominance across time has been described regularly in shallow eutrophic lakes, mainly associated to Cyanobacterial blooms (e.g Mischke & Nixdorf, 2003; Nixdorf *et al.,* 2003).

The concept of steady state phytoplankton assemblages referred to eutrophication is closely related to patterns of dominance in turbid state, proposed by Scheffer *et al.* (1993) in the context of the alternative equilibria theory for shallow lakes. This theory proposes that shallow lakes can alternate between two states in a wide range of nutrient availability, one characterized by clear waters and dominance of submerged macrophytes, and other of turbid waters with dominance of phytoplankton (Scheffer *et al.* 1993, Jeppesen *et al.,* 1998, Scheffer *et al.* 2001). Both states are stabilized by different physico-chemical and biological mechanisms, basically related to "top down" and "bottom up" controls (ej. Carpenter *et al.* 1987, Scheffer 1998, Hargeby *et al.* 2004).

Alternating from one state to another is associated with catastrophic changes in the ecosystem, such as extreme weather events or changes in trophic structure (Brodersen *et al.*, 2008, Brönmark *et al.* 2010). The conjunction of these factors determine the stability of the alternative states, which is closely related to vulnerability to eutrophication of these ecosystems.

The relevance of controls related to the availability of resources (bottom-up) as well as those related to the trophic structure (top-down) on the composition of phytoplankton has been widely described (e.g. McQueen *et al*., 1986; Mazumder & Havens, 1998; Benndorf *et al.,* 2002; Hunt & Matveev, 2005; Romo *et al.,* 2005). In particular, the impacts on the community structure of phytoplankton derived from grazing by zooplankton has been widely described (e.g. Carpenter & Kitchell, 1988; Carpenter, Kitchell, & Hodgson, 1985; Gliwicz 2002; Pace, Cole & Carpenter, 1998; Sommer *et al.,* 2001).

Zooplankton grazing usually generates a diminishing in phytoplankton biomass over edible sizes, which can lead to an increase in those nonedible groups. Non-edible phytoplankton, as small-sized with fast growth rates (r groups) and large-sized, with slow growth rates but high nutrient storage capacity (K groups) can take advantage of the availability of nutrients and light, since the competition pressure with other groups diminishes (Sinistro 2007). Nonetheless, different sizes of zooplankton can exert different top-down effects on phytoplankton composition (Sommer *et al.,* 2003).

However, these mechanisms present important differences in subtropical shallow lakes. Shallow lakes in warmer climates, have high densities of small omnivorous fish (Branco *et al.* 1997, Paugy & Lévêque, 1999) that can exert a substantial consumption on herbivorous zooplankton (Meerhoff *et al.* 2003, 2006, 2007).

Consequently, this leads to the lack of macro-filters in zooplankton, and the dominance of small sized species ("microzooplankton" Lacerot *et al.* 2010) with a significantly limited grazing pressure capacity on phytoplankton (Iglesias *et al.* 2008, Sinistro 2010). These characteristics could adversely affect the stability of clear water states in subtropical shallow lakes, since the increase in nutrient supply cannot be compensated by a significant grazing rate. Furthermore, many studies suggests that stabilizing mechanisms of these alternative states could be responsive to the climate change effects (Scheffer *et al.,* 2001; Mooij *et al.,* 2005; Jeppesen *et al.,* 2007).

Therefore, the study of disturbance factors and controls driving steady states in phytoplankton composition is essential to understand stability mechanisms linked to eutrophication in subtropical shallow lakes.

This is particularly relevant in those lakes whose priority ecosystem services could be directly affected by drastic changes in phytoplankton composition and/or biomass.

In this paper, we analyze the main factors driving the emergence and persistence of different phytoplankton assemblage states in a subtropical shallow lake, mainly used as a tap water source.

Methods

Study area

Lake Blanca (34°54' S; 54°50' W, Area: 67,7 ha) is a subtropical shallow lake (maximum depth: 3,2m) located in Uruguay, in the the southeast coastal zone, that is actually used as a tap water source (Kruk *et al.* 2006; Kruk *et al.,* 2009). It is a polimyctic eutrophic ecosystem, which has experienced several episodes of phytoplankton blooms, mostly corresponding to cyanobacteria, limiting its use as a tap water source. During 2006, the lake showed a persistent bloom of the cyanobacteria *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii* in codominance, in high biomass throughout the year; both species were potentially toxic, which led to the suspension of water supply (Pacheco *et al.*, 2010).

Physico-chemical and biological variables

Sampling campaign were carried out on many instances from Jul 2010 to Oct 2013, with periods of high-frequency with sampling every two weeks from Jul 2011 to Jan 2013.

Three sampling pointswere taken along a pelagic transect on the maximum length. On each sampling point, the entire water column was vertically integrated using a sampling tube (10cm diameter), taking samples for physico-chemical analysis, zooplankton and phytoplankton. Also, *in-situ* variables: temperature (Temp), dissolved oxygen (DO), pH, conductivity (Cond), Turbidity (Turb), were measured using a multiparameter probe YSI 650 MDS. Water samples for chlorophyll_a estimation (*Chl_a*) were filtered *in situ* by GF / C until extraction and laboratory analysis according to Nusch (1980), Chemical samples, were frozen until they were analyzed in laboratory. Alkalinity (Alk), Chlorophyll- a (*Chl_a*) and nutrients: Total Nitrogen (TN), Nitrate (NO_3), Ammonia (NH_4), Total Phosphorus (TP) and Soluble Reactive Phosphorus (SRP) were measured according to Valderrama (1981) and APHA (2005).

Samples for zooplankton quantification were taken filtering 10 L of column-integrated water sample through a 50µm mesh net. Counting was performed according to Paggi & de Paggi (1974) and, when possible, individuals were classified to species level.

Both, phytoplankton and zooplankton qualitative samples were taken by using a 20µm 68µm mesh plankton nets respectively. Zooplankton and quantitative phytoplankton samples were preserved with acid Lugol and, qualitative samples were fixed in 1% Formalin. Mesozooplankton:microzooplankton ratio from count data (ind.L^{-1}) was calculated as an indirect measure of zooplankton grazing on phytoplankton.

Phytoplankton samples were counted in random fields at 100X to 400X in Olympus CKX 41 inverted microscope in sedimentation chambers (Utermöhl 1958). Counting was performed reaching at least 100 individuals of the most frequent species (Lund *et al*., 1958) considering the organism as the unit (cell, colony or filament). The volume was calculated according to Hillebrand *et al*. (1999) and biovolume was estimated as the individual volume of each specie by the abundance. Picoplanktonic (less than 2 µm) and tychoplankton organisms were not counted. Dinoflagellates cells were treated with NaClO 20% to separate plates for species idenfitication.

Meteorological historical series were obtained from the nearest (15 km, "Laguna del Sauce Airport" weather station, INUMET) weather station, considering: air temperature, precipitation, direction, average and maximum wind speed in the analysis.

Data analysis

All environmental variables were standardized by substracting the mean and then dividing it by the variance. Species with a lower contribution than 5% of the total community biomass for each sampling time were excluded from the analysis. Phytoplankton biovolume data were Log10 (x + a) transformed, where "a" corresponds to the minimum non-zero value of the variable in each case.

A Detrended Correspondence Analyses (DCA) was performed to evaluate the variables gradient lengths in order to choose the appropiate analysis. Cosidering that the longest gradient was 3.2, RDA (Multivariate Redundancy Discriminant Analyses, Leps & Smilauer 2003) was performed, considering phytoplankton composition as response variables and the environmental variables: Temperature, pH, Conductivity, Turbidity, Secchi Disk Depth, Alkalinity, NT, NH4, NO3, TP, SRP, O2, %O2, TN:TP ratio, Total Zooplankton and Mesozooplankton:microzooplankton ratio, as explanatory variables. The significance of each performed classification were tested using Monte Carlo simulations with 499 unrestricted permutations. PERMANOVA considering physico-chemical and zooplankton variables were performed to analyze the significance between different periods defined from the composition of phytoplankton. Similarity analysis (one-way ANOSIM) were performed to compare differences between phytoplankton composition states considering: physico-chemical variables, phytoplankton and zooplankton composition. Subsequently, to test the differences between consecutive periods, paired test were performed. Complementary to ANOSIM, a Correspondence Analysis (CA) was performed to support the statistical differences between phytoplankton assemblage states, considering the composition of phytoplankton in terms of biovolume. All these analyzes were performed using Euclidian distance for physico-chemical variables and Bray-Curtis index for community composition, and variables directly related with phytoplankton biomass as Chl_a or Turbidity were excluded (Clarke 1993). For ANOSIM, they were considered as significant differences R values higher than 0,5, and a confidence interval of 95% ($P<0,05$). Statistical analyses were carried out using the software CANOCO 4.5 for Windows (Leps & Smilauer 2003) and Past 3.X version (Hammer *et al.,* 2001).

Results

The lake exhibited no thermal stratification during the period considered, and seasonality in water temperature was observed. The range of turbidity, Secchi disk depth and chlorophyll a, showed the occurrence of high and low turbidity states, some of them associated with important phytoplankton biomass. Table I summarizes the mean, maximum and minimum of the main physico-chemical characteristics and phytoplankton biomass of Lake Blanca during the sampling period considered (2010-2013).

Table I. Main physico-chemical characteristics of Lake Blanca during the studied period. Temp: Water Temperature (ºC), Cond: Conductivity (µS.cm-1), SD: Secchi Disk Depth (m), Turb: Turbidity (NTU), pH, Alk: Alkalinity (mgCaCO3.L-1), DO: Dissolved Oxygen ($\mu g.L^{-1}$), % DO: Dissolved Oxygen (percentage), Chl_a: Chlorophyll a ($mg.L^{-1}$), TN: Total Nitrogen ($\mu g.L^{-1}$), NO3: Nitrate ($\mu g.L^{-1}$), NH4: Ammonia ($\mu g.L^{-1}$), TP: Total Phosphorus ($\mu g.L^{-1}$), SRP: Soluble Reactive Phosphorus ($\mu g.L^{-1}$). Max: máximum, Min: Minimum.

	Temp	Cond	SD	Turb	pH	Alk	DO	% DO	*Chl_ a*	TN	NO_3	NH_4	TP	SRP
Mean	18,0	338,2	1,0	5,6	8,0	91,0	9,1	94,3	1,84	1077,6	111,8	24,0	52,0	15,1
Max	28,4	380,0	1,7	10,6	9,0	140	11,5	141,5	6,17	3015,1	348,1	133,0	76,6	39,5
Min	8,7	286,0	0,3	2,4	7,4	64	5,6	67,0	0,17	643,8	25,8	4,5	18,6	1,0

During the considered period, phytoplankton biomass was highly variable, with periods of high biomass alternated with low biomass (Fig. 1). In addition, community composition was also highly variable over time, and five different states defined by the dominance of different groups and biomass levels were identified:

State I. High biomass and dominance of cyanobacteria: during this period, a persistent bloom of *Cylindrospermopsis raciborskii* was present and it lasted the first studied year.

State II: Low biomass of many groups. Bacillariophyta, Euglenoids, Cyanobacteria and Desmids co-ocurred: *Closterium acicularis*, *Staurastrum leptocladum*, some small colonies of *Microcystis aeruginosa* and *Planktolyngbya* spp. accompanied by many diatoms and euglenoids species characterized the community during this period that lasted from month Jul 2011 to Apr 2012.

State III: Medium to high biomass, dominance of Desmids and filamentous cyanobacteria: *Closterium acicularis* accompanied mainly by filamentous cyanobacteria of the genus *Limnothrix*, together with *Cylindrospermopsis raciborskii*. From May 2012 to Jul 2012.

State IV: Medium to low biomass, dominance by Chlorophyta: multiple groups of chlorophytes (*Coelastrum*, *Scenedesmus,* among others) at low biomasses characterized this phase, from Jul 2012 to Jan 2013, when an abrupt change occured in the phytoplankton composition in an extremely short period (< 2 weeks). This transition occurred in coincidence with an extreme wind episode (>100 km.h^{-1}) and was pronounced by a period of very low phytoplankton biomass, mainly diatoms and euglenoid and resuspended sediment and cysts in the samples.

State V: High biomass, overall dominance of *Ceratium furcoides* (Dinophyceae): this phase started with a co-ocureence of several dinoflagellate species (*Ceratium hirundinella*, *Ceratium furcoides* and *Peridinium* sp.) in low biomasses together with diatoms and euglenoid. However, *Ceratium furcoides* presented a very rapid growth reaching high biomass in a short period (<2 weeks) while the other dinoflagellates diminished and then disappeared. *Ceratium furcoides* represented *ca.* 96% of the community biovolume and its dominance lasted until Oct 2013.

These states, according to phytoplankton composition, exhibited a matched pattern with extreme wind events, identified from visual check of the largest deviations of meteorological data (>65 km/h; component S and SW).

Some of the observed transitions among phytoplankton states were asociated to the occurrence of these wind events, especially the end of state I, III and IV. In addition, a decrease in biomass was observed during the state IV associated with an extreme wind event, but instead of a state transition in this case led to the recovery of Chlorophyta (Fig. 1).

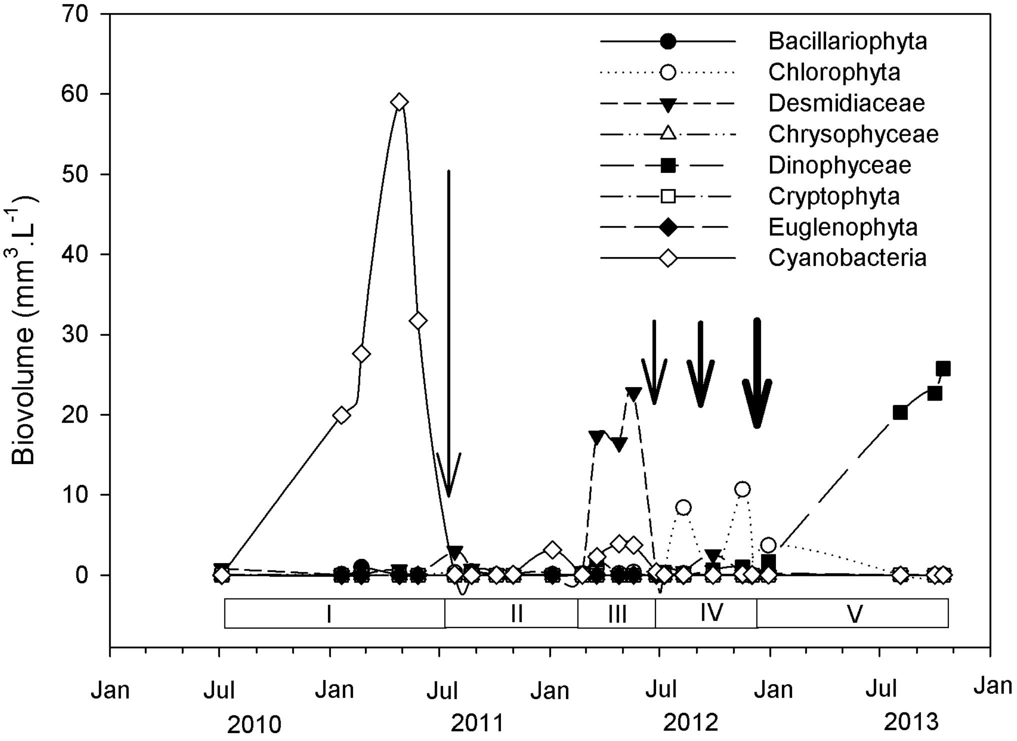

Figure 1. Temporal dynamics in phytoplankton composition during the studied period. Different phytoplankton states are denoted by latin numbers I to V. Arrows indicates extreme wind events from S-SW, and the width of them represent wind speed from 65 to 102 km.h^{-1}.

The statistical significance of the different phytoplankton assemblage states tested by one-way ANOSIM, showed significant differences between consecutive states (P I-II= 0,013; P II-III= 0,014; P III-IV= 0,036; P IV-V= 0,015) although with a low overlapping degree (R= 0,566, P= 0,0002; Fig. 2 b).

The fist two axis in the Correspondence Analysis explained 67% of the total variance (λ1= 40,0; λ2= 27,2) differentiating statistically between different phytoplankton states (Table II).

Table II. Correspondence scores of the phytoplankton composition of the first two axes (λ1 and λ2) and the variance explained, based on Correspondence Analysis of phytoplankton assemblages in Lake Blanca

	λ 1 (40,0 %)	λ 2 (27,2%)
Bacillariophyta	0,741	0,199
Chlorophyta	-0,570	1,960
Desmids	0,266	0,614
Chrysophyceae	-0,946	2,036
Dinophyceae	-1,564	-0,968
Cryptophyta	-0,412	0,220
Euglenophyta	-1,093	1,820
Cyanobacteria	1,013	-0,629

Moreover, the physico-chemical characteristics differed significantly between the different phytoplankton states considered (F= 5,36; P= 0,007; P I-II= 0,036; P II-III= 0,006; P III-IV= 0,007) except between periods IV and V (P = 0,566; Fig. 2 a).

The physico-chemical characterization of the five different states based on phytoplankton composition and biomass are shown in Figure 3. An increase in the transparency (as Secchi disk depth) accompanied by changes in levels of chlorophyll a and turbidity were observed (Fig. 3 a).

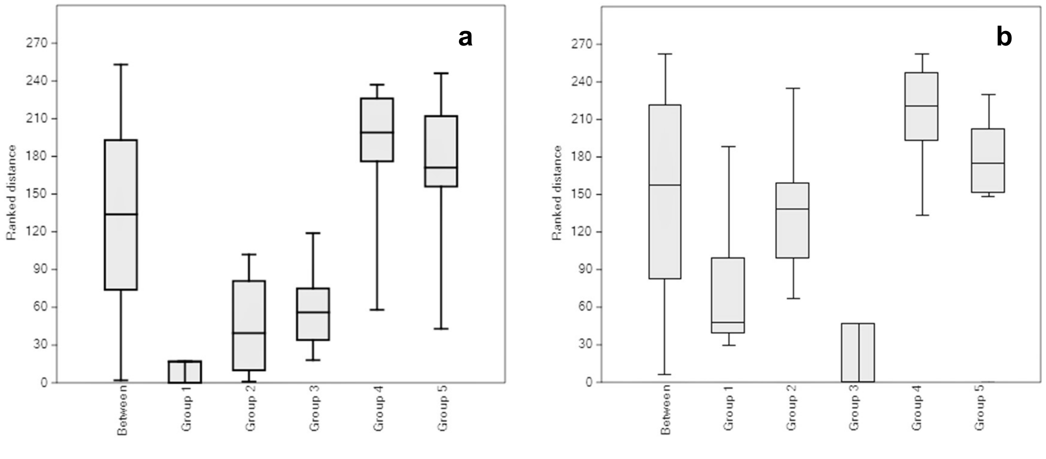

Figure 2. Pairwise biplot of the statistical differences between phytoplankton-defined states based on **a)** physicho-chemical variables, and **b)** phytoplankton taxonomic group composition. Note that groups 1 to 5 refer to states I to V.

A significant decrease in nutrients levels was observed separating states I and II without significative changes in N:P ratio, and an increase in total phosphorus accompanied by a decrease in nitrogen determines low N:P values observed in states III – IV – V (Fig. 3 b).

Besides zooplankton composition did not showed any significant difference between phytoplankton assemblage states I to IV (ANOSIM: R= 0,016; P= 0,387), significant differences were found in the total abundance of zooplankton between states IV-V (PERMANOVA: F=6,35; P= 0,019). Also, the zooplankton composition has sufficient explanatory capacity to have been selected in the RDA in terms of total zooplankton abundance and macrozooplankton:microzooplankton ratio.

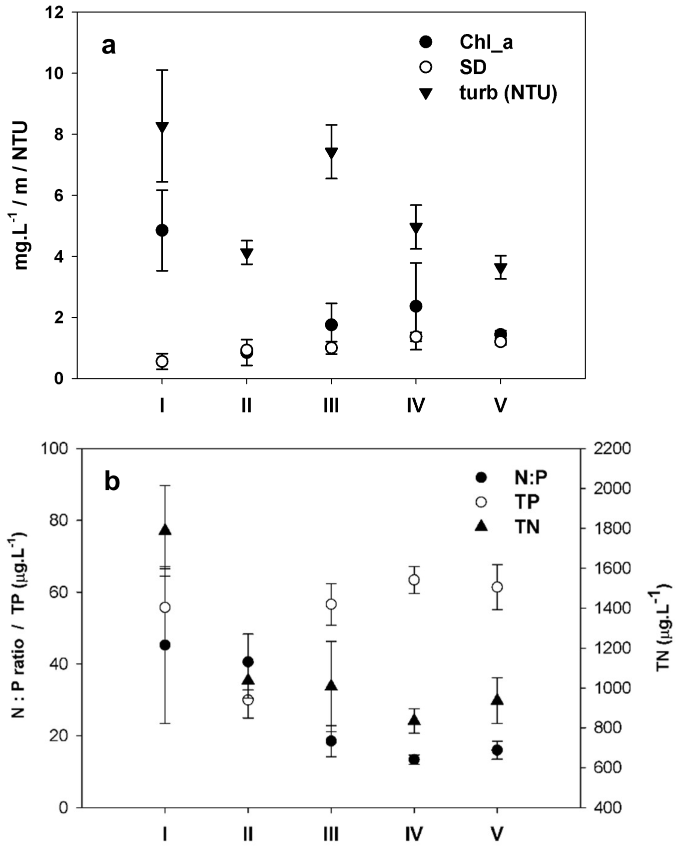

Figure 3: Main physico-chemical variables during the 5 phytoplankton-defined states (I to V, x axis) variables associated with **a)** transparency and phytoplankton biomass: **Chl_a** Chlorophyll a (mg.L^{-1}), **SD** Secchi Disk Depth (m), **Turb** Turbidity (NTU); and **b)** Nutrients: **TN:TP**: Total Nitrogen to Total Phosphorus ratio, **TP** Total Phosphorus and **TN** Total Nitrogen (µg.L^{-1}).

Over the 13 explanatory variables considered in the RDA, only five of them explained enough variance, with an inflation factor lower than 10, to be selected in the analysis (Fig 4). The first 2 axes of the RDA explained 82.3% variance of phytoplankton composition in relation to environmental variables (λ1= 58.44; λ2= 23.82). Nutrients (N and N: P ratio), zooplankton (Total and meso: micro ratio) and turbidity, were the main environmental factors determining the composition of phytoplankton groups.

Cyanobacteria and Desmids, dominant during states I and III respectively, showed a positive association with turbidity, high levels of total nitrogen and high N: P ratios. These also showed a positive correlation with a composition of zooplankton dominated by macrofilters (macrozooplankton:microzooplankton ratio).

Dinophyceae, represented exclusively by *Ceratium furcoides* and Cryptophyta, dominated by *Plagioselmis nannoplanctica*, both mostly represented in state V, showed markedly positive correlation with mesozooplankton:microzooplankton ratio, and negative with total zooplankton (Fig. 4). Meso:micro zooplankton ratio and total zooplankton showed a negative correlation between them, indicating small-sized groups dominance in this community.

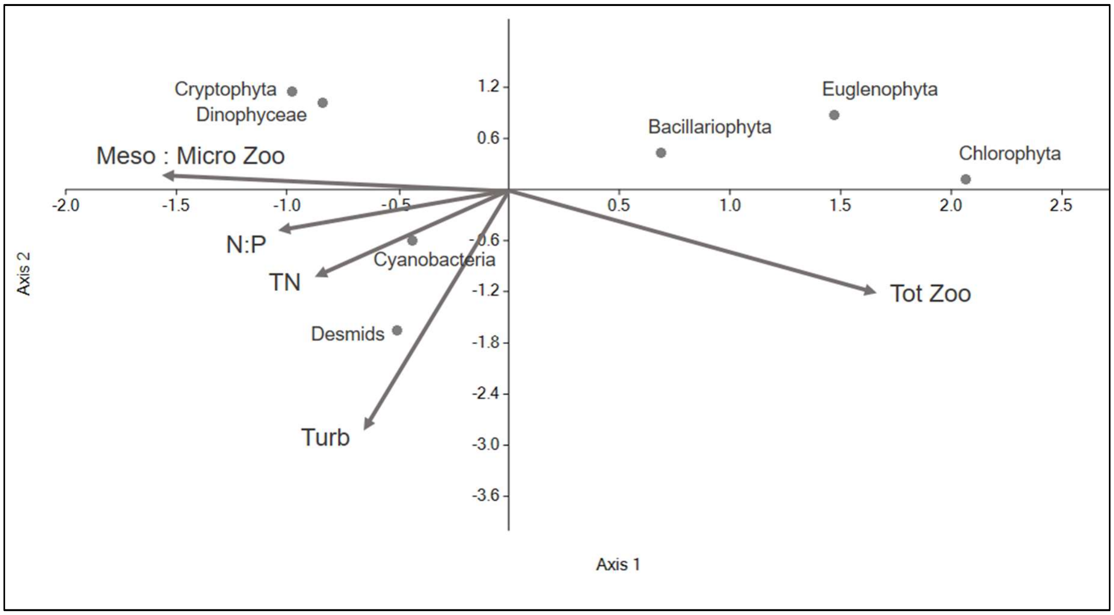

Figure 4. RDA biplot of phytoplankton composition (response variable) and (explaining variables). Selected variables: **Turb** Turbidity, **TN:** Total Nitrogen, **TN : TP** Total Nitrogen / Total Phosphorus ratio, **Meso:Micro Zoo:** mesozooplankton / microzooplankton ratio, **Tot Zoo:** Total zooplankton abundance. Firt two axes explained 82.3% of the total variance (λ1= 58.44; λ2= 23.82).

It is important to emphazise that all groups which showed a positive correlation with a composition of zooplankton dominated by larger sizes (high meso:microzooplankton ratio) were phytoplankton groups represented by large organisms (GALD> 120 µm) e.g.:

Dinophyceae: *Ceratium furcoides*, Cyanobacteria: *Cylindrospermopsis raciborskii*, Desmids: *Closterium acicularis*, or conversely very small organisms as Cryptophyta dominated by *Plagioselmis nannoplanctica*.

On the other hand, diatoms, euglenoids and chlorophytes, corresponding to clear water states (II and IV) showed a positive correlation with the total zooplankton, and negative with turbidity, total nitrogen and larger zooplankton.

Discussion

Phytoplankton composition in Lake Blanca shows high temporal variability, characterized by alternating periods of high and low biomasses, and stable dominance of few species, with a low degree of overlapping between them. This pattern may be associated with steady states phytoplankton assemblages (*sensu* Sommer *et al.* 1993), Regarding relatively constant biomass proposed by Sommer *et al.* (1993) to be considered as "steady" states, which generally is rarely attainable in phytoplankton ecology (e.g. Naselli-Flores *et al.* 2003), in our case, we refer to these states as phytoplankton assemblage states. As it has been observed in other aquatic ecosystems (e.g. Albay & Akcaalan, 2003; Rojo & Alvarez-Cobelas, 2003), Lake Blanca exhibits many key factors stabilizing these phytoplankton assemblage states, which are not directly linked to competition between groups, being mostly referred to grazing or specific capabilities of the species. These factors could be associated with three different types of ecological "filters": 1) disturbance factors, 2) storage and nutrient consumption capacity (related to bottom-up controls) and 3) grazing by zooplankton (related to top- down controls). The first of these ecological filters refers to wind action as a disturbance factor. Alternating patterns in phytoplankton assemblage states with scarce or no overlapping between them can be associated to disturbance factors that generates radical changes in the ecosystem conditions, and consequently trigger a compositional replacement.

In this particular case, the pattern associated between a period of phytoplankton state and extreme wind events would not be associated with slow variations in the system such as changes in nutrient levels, but could be closely related to changes in light conditions and resuspension of sediments. This rapid development would not be linked to a typical ecological succession, but rather it could be associated with a rapid recolonization from the sediment or surface water migration (Reynolds & Walby 1975; Hansson *et al.* 1994). Thus, this behavior of colonization from the sediments can have a significant impact on the dynamics of phytoplankton communities and lead to partially explain the rapid changes in phytoplankton composition (Hansson *et al.* 1994).

The second filter is related with the availability and balance between nutrients. Nutrients are important factors differentiating phytoplankton assemblage states, and in our study particularly the first states were largely differentiated by this factor, with the exception of last replacement (states IV to V). Considering the high availability of nutrients in the system, the balance between these would be the most important factor structuring phytoplankton composition in this level, particularly the N: P ratio, which was distinguished as one of the main explanatory factors in the RDA. This pattern of decreasing values of nitrogen, with the consequent reduction of N: P ratio, has been previously described as a factor that may favor the development of some species such as *Ceratium furcoides* with high nutrient storage capacity as occurred in state V (Matsamura-Tundisi *et al.* 2010).

The third proposed filter refers to zooplankton grazing as an important structuring factor. Both this study, as a previous research (Pacheco *et al.* 2010), showed that zooplankton in Blanca Lake is generally represented by small groups, such as rotifers, small cladocerans and copepods (representative genera *Keratella*, *Bosmina* and *Filinia*).
While in subtropical systems many studies have highlighted the weak capacity of grazing by zooplankton dominated by small sizes (e.g. Iglesias *et al.* 2008, Sinistro 2010), in this work the role of zooplankton grazing is emphasized as an important factor structuring phytoplankton composition. Total abundances of zooplankton and especially the dominance of large sizes were an important factor structuring the phytoplankton community in Lake Blanca. Although the zooplankton of medium to large sizes was scarce, this exerted an important structuring role of phytoplankton, favoring two kinds of strategies tending to overcome this important grazing pressure:

1) Small sized phytoplankton, which compensates loss by grazing with rapid population growth (r strategist) represented in the group of Cryptophyta by small species like *Plagioselmis nannoplanctica.*

2) Sizes large phytoplankton (GALD> 150µm) hardly edible, which escape grazing pressure and with high nutrient storage capacity, thus reaching high population densities (K strategist) e.g.: Dinophyceae: *Ceratium furcoides*, Cyanobacteria: *Cylindrospermopsis raciborskii*, Desmids: *Closterium acicularis*.

While the macrofilter zooplankton is uncommon in this lake, it seems to presents an important structuring role over short periods, which is essential in the functioning of the ecosystem. The sudden appearance of macrofilters in short periods in Lake Blanca, has been registered by Gerhard *et al.,* (2016) associated to high cladoceran richness with rapid temporal replacement. After a period of high consumption, due to some factors such as predation or changes in environmental conditions, the larger zooplankton declines in the lake, so that would not be recorded with a low sampling frequency (Gerhard *et al.,* 2016). This rapid decline of macrofilters may be linked to rapid consumption by the high numbers of small omnivorous fish in this lake (Gelos *et al.* 2010).

Carvalho (*et al*. 2007) described a pattern in phytoplankton composition from a state dominated by large cyanobacteria: *Microcystis aeruginosa, Planktothrix* sp.*, Cylindrospermopsis raciborskii*, to the dominance by *Ceratium furcoides*. The same pattern was observed in this study, and as in this case is also explained by high N:P balances, due to the high phosphorous storage capacity of *C. furcoides*, and capacity to avoid grazing by macrozooplankton, as discussed above.

Although no toxicity has been registered for *C. furcoides*, blooms can lead to significant accumulations causing adverse consequences on ecosystems functioning, such as fish kills attributed to decrease in dissolved oxygen on ecosystems and particularly by saturation of filter systems in water treatment plants (Matsumura-Tundisi *et al.* 2010, Taylor *et al.* 1995). This is particularly relevant in Lake Blanca because it is a system currently used to supply drinking water. Despite some species of this genus have been associated with oligotrophic systems (Starmach 1974; Padisak 1985; Bucka & Zurek 1994) in subtropical systems they are typically associated with eutrophy-hipereutrophy as we found in Lake Blanca. *Ceratium fucoides* exibits the strategy of benthic resistance cysts under unfavorable environmental conditions or as a density dependant processes when the blooms reach high densities and resource limitation occurs. These cysts remain in the sediment surface and can germinate during periods of mixing with favorable environmental conditions, thus constituting an important inoculum able to maintain the population at high densities throughout the year (Bustamante *et al.* 2012). This could be one of the mechanism driving the dominance of *C. furcoides* in the last registered phytoplankton assemblage steady state (V).

It is important to emphasize that the present work represents the first record of *Ceratium furcoides* for Uruguay, so the analysis of factors related to its appearance and persistence are highly remarkable.

In this study, we identified that the conjunction of extreme wind events, nutrient ratios, and grazing by zooplankton are the main factors that drive the emergence and establishment of different phytoplankton assemblage states in a subtropical eutrophic shallow lake. These types of mechanisms are directly associated with the stabilizing mechanisms of these phytoplankton states related to the specific capabilities of the species and derived from grazing pressure.

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Phytoplankton community structure in five subtropical shallow lakes with different trophic status (Uruguay): a morphology based approach

Juan Pablo Pacheco[1,2], Carlos Iglesias[1,2,3,4] , Mariana Meerhoff[1,2,3], Claudia Fosalba[1,2], Guillermo Goyenola[1, 2], Franco Teixeira-de Mello[1,2], Soledad García [1,2], Mercedes Gelós[1,2] & Felipe García-Rodríguez[1,5]
(1)Asociación Civil Investigación y Desarrollo I+D. Iguá 4225, CP 11400, Montevideo, Uruguay.
(2) Depto. de Ecología. Facultad de Ciencias. Universidad de la República. Iguá 4225 C.P. 11400 Montevideo-Uruguay.
(3) National Environmental Research Institute, University of Aarhus. Vejlsøvej 25. DK 8600 Silkeborg, Denmark
(4) Department of Plant Biology, University of Aarhus, Ole Worms Alle, DK 8000, Denmark.
(5) Sección Oceanología. Dpto. de Ecología. Facultad de Ciencias. Universidad de la República. Iguá 4225 C.P. 11400 Montevideo-Uruguay.
Corresponding author: Carlos Iglesias. E-mail: cig@dmu.dk
Running title: Phytoplankton in subtropical shallow lakes with different trophic status

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Abstract

Phytoplankton abundance and biomass can be explained as a result of spatial and temporal changes in physical and biological variables, and also by the externally imposed or self-generated spatial segregation. In the present study, we analyzed contrasting-season changes in the phytoplankton communities of five subtropical shallow lakes, covering a nutrient gradient from oligo-mesotrophy to hypereutrophy, using a morphologically based functional approach to cluster the species.

Six environmental variables accounted for 46% of the total phytoplankton morphological groups variance, i.e., turbidity (Secchi disk), conductivity, total phosphorus, total nitrogen, total zooplankton abundance and herbivorous meso:microzooplankton density ratio. The differences in resource availability and zooplankton abundance among the systems were related with important changes in phytoplankton composition and structure. Within phytoplankton assemblages, adaptations to improve both light and phosphorus/nitrogen uptake were important in nutrient-poor systems; while grazing-avoidance mechanisms, like colonial forms or bigger individuals, seemed relatively important only in eutrophic Lake Blanca, where light was not a limiting factor. However, this was not observed in the nutrient-rich Lake Cisne, where low light availability (due to clay resuspension and dark water color) was identified as the main structuring factor.

Our results suggest that the composition of phytoplankton morphologically-based functional groups appear to reliably describe the trophic sate of the lakes. However, other factors, such as non-biological turbid condition, or zooplankton composition, may interact rendering interpretations difficult, and therefore, deserve further studies and evaluation.

Introduction

Phytoplankton abundance is a result of spatial and temporal changes in physical (e.g. temperature, light, nutrient levels) and biological variables (e.g. grazing pressure, competition), but also of the externally imposed or self-generated spatial segregation (e.g. life cycles) (Roy & Chattopadhyay, 2007). Spatio-temporal variability in nutrient availability can play an important role in determining the phytoplankton distribution and abundance (Reynolds, 1984; Naselli-Flores, 2000; Reynolds, 2006). Over a relatively wide range of nutrient concentration, and depending on the trophic web structure, phytoplankton can dominate the stock of primary producers in shallow lakes (Scheffer et al., 1993). The relative importance of nutrient availability and trophic web structure on phytoplankton composition has been largely recognized (e.g. McQueen et al., 1986;

Mazumder & Havens, 1998; Benndorf et al., 2002; Hunt & Matveev, 2005; Romo et al., 2005). In Uruguay, in particular, former studies on phytoplankton composition and abundance in relation to environmental variables have also confirmed the expected general patterns of variation and distribution observed in other geographical and climatic regions (e.g. Mazzeo et al., 2003; Bonilla et al., 2005; Kruk et al., 2006, 2009b; Vidal & Kruk, 2008, and local references therein).

However, the response of phytoplankton structure and abundance to the above mentioned environmental variables can be analyzed at different taxonomic levels or following different functional group approaches. In particular, Litchman & Klausmeier (2008) pointed out the necessity of using trait-based approaches for the study of phytoplankton, while McGill et al. (2006) highlighted the importance of this approach for the study of environmental gradients. Functional classification of phytoplankton is currently widely used and accepted, as it can explain commonly observed adaptive features to environmental characteristics more accurately than the phylogenetic approach (Reynolds et al., 2002, Kruk et al., 2002; Padisák et al., 2006; Salmaso & Padisák, 2007).

In this sense, Kruk et al. (2009 a) have proposed a phytoplankton classification system using morphologically based functional groups (hereafter MBFG), that summarizes the functional characteristics and captures the variability of the species, while being easier than other classification approaches (e.g. Reynolds et al., 2002). This functional classification is exclusively based on phytoplankton morphology, assuming it reflects different abilities for nutrient and light uptake, growth, and grazing, sedimentation and flushing avoidance mechanisms. The system includes seven groups: **Group I**: small organisms with high surface/volume (S/V) ratio (e.g. *Chlorella, Synechocystis, Chroococcus*); **Group II**: small flagellated organisms with siliceous exoskeletal structures (i.e. Chrysophyceae); **Group III**: large filaments with aerotopes (e.g. *Planktothrix, Anabaena, Cylindrospermopsis)*; **Group IV**: organisms of medium size, lacking specialized traits (e.g. *Closterium, Monoraphidium, Pediastrum)*; group V: unicellular, medium to large-sized flagellates (e.g. Cryptophyceae, Euglenophyceae, Dinophyceae); **Group VI**: non-flagellated organisms with siliceous skeletons (i.e. Bacillariophyceae); **Group VII**: large mucilaginous colonies (e.g. *Botryococcus, Aphanocapsa, Microcystis*).

In the present work, we describe and compare summer and winter phytoplankton communities in five subtropical shallow lakes, covering a wide gradient of nutrient concentration, using the functional MBFG classification. We particularly aimed to test the validity of such a novel morphological classification tool, and then, relate it to both nutrient and light availability as well as to the potential grazing pressure. We hypothesised that phytoplankton abundance and biomass would be trophic- and temperature-dependent, with lower values observed in nutrient-poor systems and in winter, respectively. On the other hand, we expected water transparency to be a key factor for the algal biomass development regardless of nutrient concentration. We also hypothesized that grazing pressure would be an important factor only in the absence of resource limitation.

Materials and methods

We studied five shallow lakes in the southern coast of Uruguay (from 34.75°S-55.83°W to 34.29°S-53.80°W). Except for Cisne Reservoir, all other lakes (Blanca, Escondida, Clotilde and García) were originated after the Holocene marine transgression (García-Rodríguez et al., 2004) and nowadays are completely isolated from the sea. The lakes encompass a trophic gradient from oligo-mesotrophy to hypereutrophy (Kruk et al., 2006; Kruk et al., 2009b), according to Salas & Martino (1991) criteria (Table 1). The five lakes are used, or are under consideration to be used, as drinking water sources for nearby towns.

Sampling campaigns were carried out in summer and winter 2006 (southern hemisphere). In each lake, five liters of water were collected from five equidistant points along each of three randomly selected transects. Samples were taken using a 10-cm diameter tube, by vertically integrating the water column. Temperature, dissolved oxygen concentration (DO), conductivity (Cond), pH and water transparency were registered *in situ* using field sensors and Secchi disc (SD), respectively. Chlorophyll *a* (Chl-*a*) was determined according to Nusch (1980). Alkalinity (Alk), total nitrogen (TN), total phosphorus (TP), reactive soluble phosphorus ($P\text{-}PO_4$), ammonium ($N\text{-}NH_4$), nitrate ($N\text{-}NO_3$) and silicate (Si_2O_4) concentrations were measured according to Valderrama (1981) and APHA (1985). For zooplankton quantification, 20-L pooled samples were filtered through a 50-µm mesh net and fixed with acid Lugol. Counting was made according to Paggi & de Paggi (1974) and, when possible, individuals were classified to species level. The herbivorous mesozooplankton (i.e. calanoid copepods+cladocerans) to microzooplankton (i.e. copepod nauplii+rotifers) density ratios (thereafter meso:microzoolankton) were also calculated for each season. Phytoplankton samples were fixed with acid Lugol, and counting was made in random fields in sedimentation chambers (10 and 20 ml) using an inverted microscope, following the criteria of Ütermohl (1958). In all cases, rarefaction curves were used to establish the necessary number of fields. Biovolume was calculated from the measurements of 30 organisms of each species, in each lake, according to Hillebrand et al. (1999). We also considered the mucilage during cell counting and measuring. Phytoplankton organisms of each sample were sorted into the seven MBFG described above (Kruk et al., 2009 a), considering their maximum linear dimension (MLD), volume (V), surface/volume ratio (S/V), presence of flagella, siliceous structures, mucilage or aerotopes, after classifiying them taxonomically. Biovolume was calculated for each phytoplankton taxon by multiplying population density by their mean specific volume. Community and MBFG biovolume were calculated by integrating the corresponding population biovolume.

Data analyses

Redundancy Analysis (RDA) was performed to analyze the trophic gradient given by the selected lakes. The classical trophic-state descriptors (as in OECD 1982; Salas & Martino, 1991): TP, TN, Chl-*a* and transparency (SD), were used as explanatory variables, while lakes were codified as dummy variables and considered as response variables. The longest gradient in the performed Detrended Correspondence Analysis (DCA) was lower than three standard errors (SE), therefore linear methods were performed (Lepš & Šmilauer, 2003)

Zooplankton community structure was analyzed using two-way ANOVA tests considering two main factors: i) lake (Cisne, Blanca, Econdida, García and Clotilde) and ii) season (summer, winter). In case of relevance, also one-way ANOVA was run for specific lakes (comparing summer vs. winter). Tukey *post hoc* tests were performed in case of significant differences. Cochran' C tests were used to check variance homoscedasticity, and normality was checked with Kolmogorov-Smirnov tests and by visual inspection of the distribution of residuals. When violations to assumptions were detected, data were fourth- root transformed.

To investigate relationships between MBFG and environmental variables, we first performed an exploratory analysis (RDA) using the MBFG as the response variable and all available explanatory variables (i.e. environmental physico-chemistry and zooplankton data). A subset of these variables was chosen using the stepwise manual selection procedure. Selection was refined by examination of the variance inflation factors (VIFs). Those variables that exhibited a VIF>10 (i.e. multiple co-linear variables), were removed one at a time (Wilson et al., 1994). After each removal, RDA was run again and the VIFs were re-examined until no extreme values were observed. Prior to perform direct gradient analyses, all the variables were standardized and log-transformed. The significance of the analyses was tested using the Monte Carlo permutation test with 499 unrestricted permutations, in all cases. Multivariate analyses were run using CANOCO for Windows 4.5 (ter Braak & Šmilauer, 2002).

Results

Explanatory variables

The five lakes were well-mixed and no temperature or oxygen stratification was detected during winter or summer. *In situ* measured values are presented in Table 1. The nutrient gradient ranged from oligo-mesotrophic conditions, in Lake Clotilde, to hypereutrophic, in Lake Blanca (Table 1, Fig. 1). The first two canonical axes of the RDA, which was performed to detect the trophic gradient, accounted for 46% of the total variance (eigenvalues for first and second axis, 0.246 and 0.214, respectively) and were highly significant (Monte Carlo test, F= 8.2, p=0.002). In Lake Cisne, TP, TN and SD values corresponded to eutrophic conditions, but the low Chl-*a* concentrations corresponded to meso-oligotrophy, according to the classification of Salas & Martino (1991).

Table 1. Geographical, morphological, biological and main physico-chemical characteristics of the five studied systems, ordered by increasing TP concentrations from left to right. Values represent in most cases the average of summer and winter values, showing standard error within brackets, except as indicated * (summer and winter values shown). Trophic status codes according to Salas & Martino (1991): O: oligotrophic, M: mesotrophic, E: eutrophic, H: hypereutrophic. Abbreviations, Z max: maximum depth; DO: Dissolved Oxygen; Cond: Conductivity; Alk: Alkalinity TP: total phosphorus; TN: total nitrogen; N:P Ratio: Total Nitrogen to Total Phosphorus ratio; Si_2O_4: Total Reactive Silicates; Chl-*a*: Chlorophyll a Meso:microzoo ratio: Mesozoolankton (calanoid copepods+cladocerans) to microzooplankton (nauplii+rotifers) density ratio.

	Clotilde	***García***	***Escondida***	***Blanca***	***Cisne***
Location	34º15S; 53º47W	34º17S; 53º48W	34º49S; 54º37W	34º54S; 54º50W	34º40S; 55º37W
Origin	Natural	Natural	Natural	Natural	Natural-Damed
Area (ha)	17.7	5.2	10.8	28.7	157.2
Catchment area (ha)	286.7	89.0	479.9	540.6	4885.8
Depth max. (m)*	3.2-4.25	1.8-2.0	3.6	2.1-3.2	1.9-3.5
Temperature (ºC)*	25.8-10.8	24.6-10.7	26.4-12.3	27.1-11.2	21.9-10.7
pH	6.9 (0.3)	7.1 (0.4)	7.1 (0.5)	7.9 (0.8)	6.8 (0.1)
Dissolved O_2 (μg L^{-1})*	7.4-11.1	8.6-11.6	9.7-6.7	8.7-8.7	10.7-9.8
Conductivity (μS cm^{-1})	159.8 (54.7)	128.1 (48.5)	174.3 (49.3)	285.8 (90.4)	184.9 (4.6)
Alkalinity (mg $CaCO_3$ L^{-1})	35.2	29.3	60.0	85.5	49.0
TP (μg L^{-1})	30.7 (16.4)	45.7 (20.9)	57.0 (13.6)	107.4 (20.7)	610.7 (44.8)
TN (μg L^{-1})	606.2 (59.3)	574.5 (102.2)	539.5 (187.0)	1346.2 (304.4)	1061.8 (449.2)
TN:TP ratio	24.0 (10.7)	16.1 (11.3)	9.8 (3.5)	13.3 (5.1)	1.7 (0.7)
Si_2O_4 (μg L^{-1})	2388.2 (1123.1)	1683.2 (746.6)	3933.2 (2620.1)	2596.5 (1315.8)	3693.2 (2575.4)
Secchi Disk depth (m)	1.8 (0.4)	1.7 (0.3)	1.4 (0.1)	0.3 (0.0)	0.2 (0.1)
Chl-*a* (μg L^{-1})	2.2 (0.9)	4.8 (3.2)	7.6 (2.1)	36.4 (7.6)	4.7 (0.9)
Total phytoplankton biovolume (mm^3 L^{-1})*	5.56-2.65	14.65-0.31	12.64-1.45	19.92-26.39	0.002-0.59
Total zooplankton (ind L^{-1})*	388 (53)-53 (31)	210 (66)-135(46)	475 (82)-104 (23)	572 (90)- 432 (60)	72 (34)-189 (66)
Meso:microzoo ratio*	0.2-0.7	1.6-4.4	0.9-0.3	0.4-2.2	0.7-0.1
Trophic status	O-M	M	M-E	E-H	E

Zooplankton total abundance, as well as community structure, exhibited different patterns in the studied systems. Higher mean total abundances occurred in summer (“season” effect, $F_{1,20}$= 55.8, $p<0.0001$), although this pattern occurred only in two of the five lakes (i.e. significant interaction term between “season” and “lake”). The highest average summer-winter values were recorded in Lake Blanca (*post hoc* test: Blanca> Escondida ≥ [Clotilde = García = Cisne], "lake" effect, $F_{4,20} = 36.9$, $p<0.0001$, Table 1). Both Lake Blanca and García showed no significant differences in total zooplankton abundance between summer and winter, but we did register differences in community structure. In both cases, the meso/microzooplankton ratio was >1 in winter, but <1 in summer. Thus, the dominance changed from mesozooplankton during winter to microzooplankton during summer ("season" effect, $F_{1,4}=23.0$, $p<0.01$; $F_{1,4}=1\ 3.1$ $p<0.05$, in Lake Blanca and García respectively, Fig. 2). Both Lake Clotilde and Escondida showed significantly higher total zooplankton abundances during summer, while Lake Cisne showed the opposite pattern ("season" effect, $F_{1,4} = 91.4$, $p<0.001$; $F_{1,4}=9.4$ $p<0.05$; and $F_{1,4}=8.3$ $p<0.05$, respectively, Fig. 2). The meso:microzooplankton ratios also exhibited significant? differences between winter and summer, but never reached values >1. This means that, despite statistical differences, the zooplankton assemblages were always dominated by small-bodied organisms. Both Lake Escondida and Cisne showed significantly higher values of this ratio during summer, while Lake Clotilde displayed the opposite pattern ("season" effect, $F_{1,4}=9.4$, $p<0.05$; $F_{1,4}=57.4$, $p<0.01$; and $F_{1,4}=16.7$, $p<0.05$, respectively).

Phytoplankton assemblage structure

We identified a total of 153 species, corresponding principally to green microalgae (Chlorophyceae), diatoms (Bacillariophyceae) and cyanobacteria. Clotilde and Blanca, with 39 and 35 species respectively, were the richest systems, while Lake Cisne was shown to be the poorest (only 13 species). Considering MBFG, Lake Clotilde was again the richest, as all seven groups were registered. Lake Blanca, Escondida and Garcia had six groups and Cisne only four (Fig. 3).

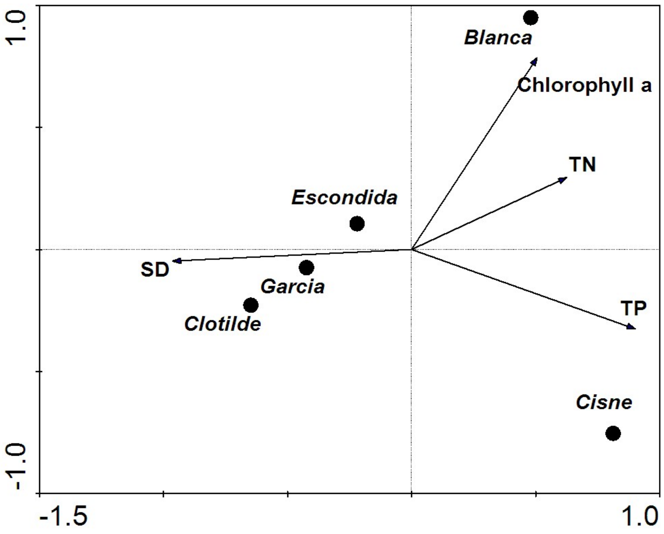

Figure 1. RDA ordination diagram showing the lake trophic gradient (λ axis 1 = 0.256, λ axis 2 = 0.214). Black dots represent the studied lakes. Arrows indicate the direction and intensity of the environmental variables used for trophic state determination according to Salas & Martino (1991), Chorophyll a (Chlo_a), Total Phosphorus (TP) and Total Nitrogen (TN) concentrations and Turbidity (measured as Secchi Disk, SD).

Lake Clotilde was dominated by **Groups IV** (principally Desmids: *Cosmarium* spp., *Euastrum* spp., *Staurastrum* spp. and other green algae, such as *Monoraphidium* spp.) and **VII** (mostly Chlorococcales). Representatives of **Group V** (e.g. *Trachelomonas* spp. and Cryptophyceae) were also registered during winter, while those of **Group VI** (pennated diatoms) were observed in summer (Fig. 3). Lake García showed sharp differences between summer and winter in the composition of MBFG. However, **Groups V** (Cryptophyceae) and **VI** (pennated diatoms) dominated during summer, with a concomitant decrease in total biovolume (Table 1, Fig. 3). **Group VII** (e.g. *Botryococcus* spp. and *Dyctiosphaerium* spp.) was dominant in Lake Escondida throughout the study, but both **Groups V** and **VI** were also abundant during summer and winter respectively (Fig. 3). The lowest biovolume values were registered in Lake Cisne. In summer, only **Groups I** (small Chlorococcales) and **V** (Euglenophyceae and Cryptophycea) were recorded, while in winter we also found representatives of **Groups IV** and VI (Fig. 3).

Lake Blanca was always dominated by individuals of G (Chroococcales: e.g. *Microcystis aeruginosa* and *Aphanocapsa* spp) and **I** (small Oscillatoriales), together with **Group III** (e.g. *Cylindrospermopsis raciborskii* and *Anabaena* spp) in summer (Fig. 3).

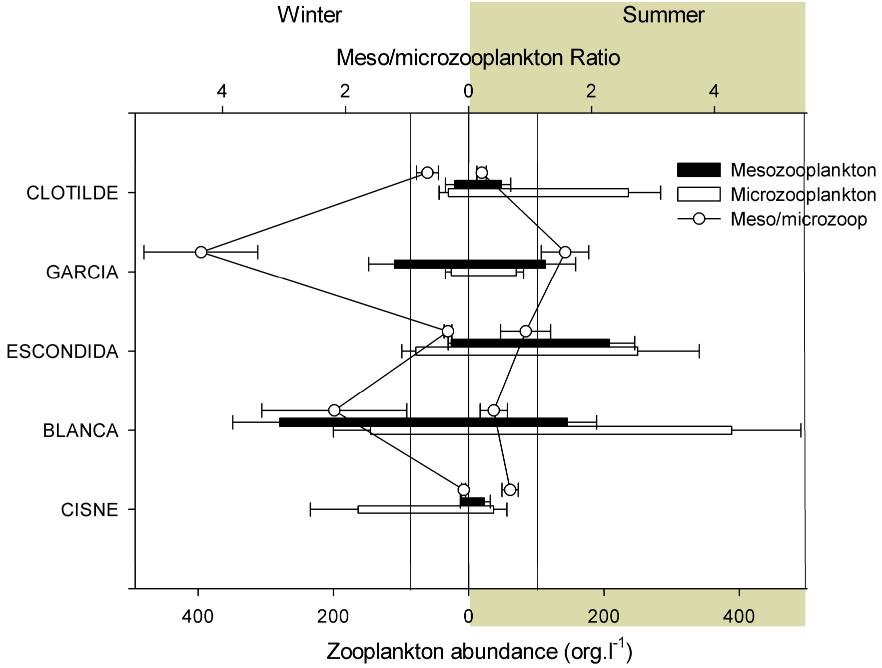

Figure 2. Mean zooplankton abundance according to size, in summer and winter, and the corresponding herbivorous mesozoolankton (calanoid copepods+cladocerans) to microzooplankton (nauplii+rotifers) density ratio. Error bars represent 1 SE. The lakes are ordered by decreasing water transparency from top to bottom.

To stress differences in the community structure and environmental factors between the lakes, six environmental variables were finally chosen after inspection of the VIF in the RDA: total zooplankton density (Tot Zoo), TN, TP, Conductivity, SD, and the meso:microzooplankton ratio (Fig. 4). The eigenvalues for RDA axis 1 (0.508) and axis 2 (0.032) explained 53.9% of the cumulative variance in the species data. The ordination diagram was highly significant (Monte Carlo test, F =18.7, p=0.002). **MBFG II**, **IV** and **VI** were associated to Lake Clotilde, García and Escondida, and exhibited a positive relationship with both water transparency (SD) and zooplankton structure (i.e., meso:microzooplankton ratio). However, these groups appeared to be negatively linked to conductivity and total nutrient concentrations (TP and TN).

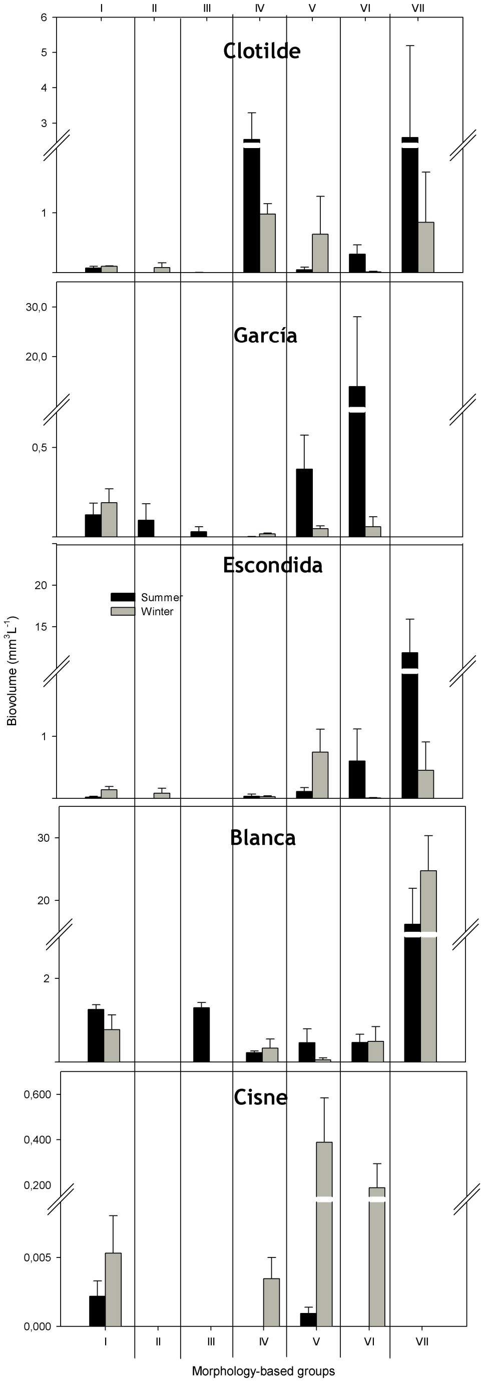

Figure 3. Mean biovolume of the different morphologically based functional groups in each lake and season. The diagram presents the systems ranked by trophic status. Error bars represent 1 SE. Black = summer, grey = winter.

Lake Blanca was characterized by the dominance of **MBFG I**, **III** and **VII**, which were positively associated to total zoo, TN and conductivity, but negatively associated to SD (Fig. 4).

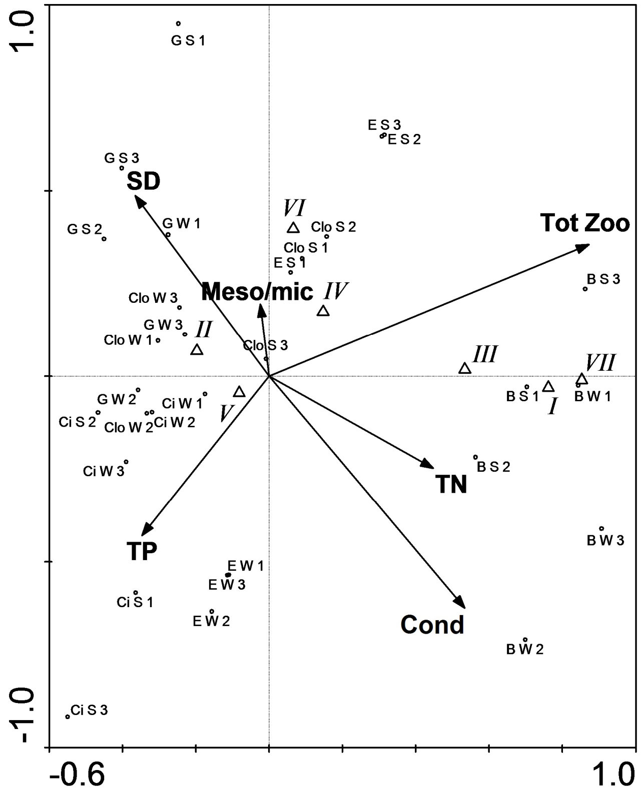

Fig. 4. RDA ordination diagram for morphologically based functional groups, environmental variables and sites. Explain symbols. Abbreviations: I to VII denote each of the seven morphologically based functional groups, SD = Secchi disk depth, Cond = Conductivity, TP = total phosphorus, TN = total nitrogen, Tot Zoo = Total Zooplankton abundance and Meso/mic= Meso/microzooplankton density ratio. Abbreviations of samples: Ci = Lake Cisne, B = Lake Blanca, E = Lake Escondida, G = Lake García and Clo = Lake Clotilde; S and W means summer and winter, respectively. λ axis 1 = 0.508, λ axis 2 = 0.032.

Discussion

In partial agreement with our first hypothesis, the trophic status was significantly related to differences in total phytoplankton biomass, species assemblages and **MBFG** composition. However, we found no relationship between the number of present **MBFG** and lake trophic status.

The variables explaining the pattern of **MBFG** along the trophic gradient were directly related to availability of limiting resources and grazing avoidance strategies. On the low extreme of the trophic gradient, both Lake Clotilde and García showed the lowest Chl-*a* and nutrient concentrations, together with the highest light penetration (as water transparency) level. In these low trophic-level systems, primary production is most likely limited by nutrients.

The nutrient-poor lakes Clotilde and García, and intermediate Escondida were characterized by the occurrence of **Group II**, represented by chrysophyceae, which are associated with low nutrient concentration and high light availability. Such a group may be favored in these systems, because their small size and flagella help them avoid sinking and provide high capacity of nutrient uptake (Reynolds, 1997). Also, the capacity of shifting to facultative mixotrophy and producing resistance propagules (Sandgren, 1991) makes this group highly tolerant to low nutrient levels. However, as also described by Kruk et al (2009 a), **Group II** represented only a small fraction of the phytoplankton assemblage in these oligo-mesotrophic lakes.

Clotilde, the lake of lowest trophic-status, was dominated by **Group IV**, best represented by chlorophytes of the closteriaceae and desmidiaceae family. Together with the low meso:microzooplankton ratio, the dominance of **Group IV** could indicate either a low grazing pressure and/or a selective grazing over smaller phytoplankton sizes. This is because this group is very vulnerable to grazing, as a consequence of their medium size and high nutrient content (Sterner & Elser, 2002). In addition, the oligo-mesotrophic status of Lake Clotilde seems corroborated by the presence of desmid chlorophytes (Gerrath, 2003).

In nutrient-rich systems, cyanobacteria may proliferate under warm temperatures (Robarts & Zohary, 1987; Dokulil & Teubner, 2000; Dowing et al*.,* 2001). In this sense, *M. aeruginosa* (**Group VII**) and *C. raciborskii* (**Group III**) dominated during summer in Lake Blanca, but in winter only high abundances of *M. aeruginosa* were observed. The absence of *C. raciborskii* in winter was evidenced in Lake Blanca since 2004 (Vidal & Kruk, 2008), probably because of high sensitivity to low temperatures (Briand et al., 2002; Chonudomkul et al., 2004; Berger et al., 2007). The zooplankton community structure in Lake Blanca, which was dominated by mesozooplankton in both seasons, could potentially have exerted a stronger grazing pressure here. However, contrary to these expectations, grazing impact was probably weak because of the large body size of phytoplankton **Groups III** and **VII**, which makes them grazing-resistant (Drenner & Hambright, 2002; Domaizon & Devaux, 1999). Also, the dominance of **Group VII** in Lake Blanca can be explained by the capacity of developing high population biomass under conditions of low light penetration and high nutrient availability. The presence of mucilage, and in many cases aerotopes, provides this group with high buoyancy and grazing avoidance capacities. Also, the potential to produce toxins provides high fitness capacity. Nevertheless, we found high biomass of **Group VII** along the trophic gradient, in Lake Clotilde (Oligo-mesotrophic), Escondida (Meso-eutrophic) and Blanca (Eutrophic). **Group VII** is characterized by a large size and very low surface:volume ratio, which make it very sensitive to low resource conditions, as observed in the low trophic level lakes. Unexpectedly, we detected remarkable differences in the taxonomic composition of **Group VII** between systems with different trophic status. This group was dominated by *Dictyosphaerium spp.* and *Botryococcus sp.* in Lake Clotilde and

Escondida respectively. **Group VII** includes species typical of both nutrient-rich and nutrient-poor systems, and therefore, the potential application of the **MBFG** system for trophic classification of lakes would require further evaluation.

Lake Cisne was classified as hypereutrophic, with the low transparency caused by non biological factors (i.e. high water color and sediment resuspension) and representing the main limiting factor for phytoplankton. As found by Bonilla et al. (2005) in a Uruguayan coastal shallow lake with high resuspension levels, an important fraction of the suspended microalgae in Lake Cisne was composed by benthic taxa, mainly represented by pennate diatoms (**Group VI**). The low light penetration might have promoted the dominance of **Group VII (**able to control buoyancy), whereas the low TN/TP ratio (1.7, concentration ratio, Table 1) could have promoted the dominance of nitrogen-fixing cyanobacteria of **Group III** (Smith, 1983; Reynolds, 1999, Kruk et al., 2009 a). However, we observed dominance of cryptophyceae (**Group V**), able to tolerate low light intensities (Kugrens & Clay, 2003), and even diatoms (**Group VI**), that are well adapted to resuspension processes (Witkowski et al., 2000, Kruk et al., 2009 a).

The differences in resource availability and grazing avoidance strategies resulted in important changes in the phytoplankton composition and structure among the systems. The competitive abilities related to maximize light and nutrient uptake seemed to be potentially important at low trophic status, while grazing avoidance mechanisms seemed to be of secondary importance. In eutrophic systems, also resource limitation appeared to be extremely important. According to our second hypothesis, light limitation, particularly when positively correlated with high suspended solid concentration (Kruk et al., 2009b), seemed the strongest force in determining algal biomass in Lake Cisne, independently of season or nutrient availability. In contrast, as proposed by the intermediate control hypothesis (Elser & Goldman, 1991), grazing-avoidance mechanisms could have been important as well in the eutrophic and biologically-turbid Lake Blanca, since **Groups III** (large filaments) and **VII** (large colonies), capable of avoiding grazing, were dominant. However, zooplankton composition was not related to the trophic gradient, not least in winter, as mesozooplankton dominated in both nutrient-poor (Lake García) and nutrient-rich (Lake Blanca) systems. Similarly to previous studies in a larger set of lakes, including the same and other similar lakes (Meerhoff et al., 2007), zooplankton was dominated by small-sized species along the entire trophic gradient, thus resulting in a low grazing pressure over phytoplankton. This community structure, typically found in subtropical lakes, can exert lower impact on phytoplankton structure and biomass than the zooplankton communities typically found in otherwise similar temperate lakes (Huszar et al., 2003; Doyle & Saros, 2005; Jeppesen et al., 2005; Berger et al., 2007).

As pointed out in previous studies (Naselli-Flores & Barone, 2007; Naselli-Flores, Padisák & Albay, 2007, Caputo et al., 2008), the competitive abilities of the different groups were appropriately summarized by their morphological traits. The findings in our study validate the use of the functional classification of phytoplankton based only in easy-to-observe morphological traits, to assess the relationship between phytoplankton communities and environmental variables in lakes of different trophic status.

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9. Capítulo IV

Cascading effects promoted by small omnivorous fish and macroinvertebrates on phytoplankton and periphyton structure in shallow lakes of contrasting climates

Juan Pablo Pacheco, Carlos Iglesias, Néstor Mazzeo, Irina Izaguirre, Mariana Meerhoff & Erik Jeppesen

Abstract

Shallow lakes of different climatic conditions exhibited differences in the trophic structure, which can be reflected in differences in their functioning and vulnerability to eutrophication.

In order to comparatively analyze the role of cascading controls in shallow lakes of contrasting climatic conditions, this study focuses experimentally on the effects of small omnivorous fish and macroinvertebrates (crustacean) on the structure of phytoplankton and periphyton in subtropical and temperate shallow lakes.

Mesocosms experiments, with addition of small omnivorous fish and macroinvertebrates (shrimp and amphipoda) in combined and single treatments, were carried out in three subtropical (Uruguay) and three temperate (Denmark) shallow lakes in a range of limnological features.

Our study suggests the existence of major cascading effects promoted by omnivorous small fish on phytoplankton, both in subtropical and temperate shallow lakes. These cascading effects, mediated by fish predation on larger zooplankton, were observed on the abundance and composition of phytoplankton.

This effect was particularly important in temperate lakes, where the presence of small fish would lead to higher phytoplankton biomasses that those found in the lakes (external conditions) under the predation control by piscivorous fish.

All these effects were exclusively promoted by the presence of fish on treatments, not being observed an effect of invertebrates on biomass or composition of phytoplankton or periphyton.

Furthermore, they were not observed direct or cascade effects promoted by the presence of fish or invertebrates on periphyton biomass or composition. Besides, major variability in periphyton biomass and composition were derived from climatic conditions.

Our results suggest a key role of small fish, by generating higher vulnerability of shallow lakes to phytoplankton-turbid states in the absence of piscivorous, and lack of effects of the presence of fish and invertebrates on the abundance or composition of periphyton.

Key words: trophic food webs, cascading effects, mesocosms, subtropical and temperate lakes

Introduction

Ecosystem functioning is largely determined by trophic interactions between their communities. Classically, food webs are structured by the conjunction of controls based on availability of resources (Bottom-up), and the direct or cascade effects promoted by consumption (Top-down) (e.g. Mc Queen *et al.* 1986). The role of top-down controls on primary producers has been widely evidenced in both marine (e.g. Worm & Myers 2003; Scheffer *et al.*, 2005; Frank *et al.*, 2005; Casini *et al.*, 2009) and freshwater ecosystems (e.g. Carpenter *et al.*, 1987; Northcote, 1988; Mc Queen *et al.*, 1989, among others).

In the case of lakes, it is widely known the important regulatory role of top-down controls on primary producers (Jeppesen *et al.*, 1997; Carpenter & Kitchell, 1988) mainly referred to the cascading effects of fish on phytoplankton. Cascading mechanisms have shown a high capacity to control phytoplankton biomass in biomanipulation of the trophic structure experiences (With & Wright, 1984; Hansson *et al.*, 1998; Søndergaard *et al.*, 2008; Mazzeo *et al.,* 2010). These interactions, as well as the generality of limnological studies, have been mainly focused on the pelagic environment rather than the littoral or benthic, despite the high relevance of the bento-littoral processes in shallow lakes (Carpenter *et al.*, 2001; Schindler & Scheuerell, 2002; Vadeboncoeur *et al.*, 2002).

One example of the relevance of littoral and benthic processes can be given through an iconic case of the functioning shallow lakes, such as the theory of alternative equilibria (Scheffer *et al.* 1993). In clear water states, high densities of macrophytes limit the phytoplankton growth by several mechanisms, thus, the shift to turbid waters must be necessarily linked to a drastic decrease in the abundance of macrophytes (Brönmark *et al.* 2010). Some authors suggest that periphyton can play a key role in this transition, decreasing the resistance mechanisms associated to clear water states (Phillips *et al.,* 1978; Hough *et al*., 1989; Daldorph & Thomas, 1995; Jones & Sayer 2003). Thus, the limitation of macrophytes growth would lead to its collapse, resulting in phytoplankton turbid states (Jones & Sayer 2003; Sayer *et al*., 2010). Experimental studies (Jones *et al.* 1999; 2000; 2002) and paleolimnological evidence (Osborne & Moss, 1977) indicate that turbid states with high phytoplankton biomass were preceded by states with high biomass of periphyton.

However, there are still scarce the studies analyzing interactions between phytoplankton and periphyton on the process of eutrophication in shallow lakes, particularly in relation to the trophic structure (Vadeboncoeur & Steinman 2002; Sanchez *et al.* 2010).

Especially in shallow lakes from warmer climates, these mechanisms are much less known. Shallow lakes in warmer climates have a number of characteristics that could limit the applicability of these theories mostly developed for temperate ecosystems.

Results from biomanipulations suggest that in subtropical lakes cascading effects are not as marked as in temperate lakes (Jeppesen *et al*., 2005). This could be associated to differences in trophic structure between the lakes of these two different climatic conditions (Meerhoff *et al*., 2007; Havens *et al*., 2009), and a higher role of omnivory and herbivory in warmer climates (Lazzaro 1997; Attayde & Menezes, 2008; Teixeira de Mello *et al*., 2009).

In order to comparatively analyze the role of cascading controls in shallow lakes of contrasting climatic conditions, this study focuses experimentally on the effects of small omnivorous fish and macroinvertebrates (crustacean) on the structure of phytoplankton and periphyton in subtropical (Uruguay) and temperate (Denmark) shallow lakes.

We hypothesized that differences in phytoplankton biomass and composition between lakes of different climatic regions will result from cascading effects promoted by the omnivorous fish. In the absence of omnivorous fish, there will be more large-sized zooplankton, with higer grazing on phytoplankton, reflected in lower phytoplankton biomass and dominance of tolerant to grazing groups in both climatic conditions.

We also expected a higher grazing by fish and macroinvertebrates on periphyton in subtropical lakes, limiting their growth and leading to the dominance of resistance to grazing groups, while in the absence of fish and macroinvertebrates subtropical and temperate lakes will exhibit similar periphyton composition and biomass.

Materials and methods

Study area and experimental design

We selected three subtropical (Uruguay 35°S) and three temperate (Denmark 56°N) shallow lakes, approximately paired in their environmental conditions (clear-vegetated, phytoplankton turbid and inorganic-turbid for each climatic condition; Table I).

Table I. Main limnological features of studied systems at initial and final conditions (T0 – TF): surface area, water temperature (Temp), dissolved oxygen (DO), pH, Conductivity, Turbidity, Percentage of Volume Inhabited by submerged plants (PVI), Phytoplankton biomass as Chlorophyll-a (Chl_a), Total Nitrogen (TN) and Total Phosphorus (TP). Data from Iglesias *et al.* In prep.

	Subtropical			**Temperate**		
	Diario	**Blanca**	**Nutrias**	**Kogleaks**	**Stigsholm**	**Bølling**
Temp (ºC)	28.0 - 20.5	22.5 - 21.4	27.0 - 20.2	16.6 - 8.3	15.1 - 10.4	14.7 - 13.9
DO (mg.L^{-1})	9.7 - 7.0	6.7 - 10.4	7.4 - 8.4	5.7 - 9.1	10.5 - 9.3	8.7 - 7.6
pH	8.6 - 7.5	8.4 - 7.61	6.14 - 5.8	6.9 - 7.5	8.4 - 7.5	7.1 - 7.2
Conductivity (mS.cm^{-2})	566 - 617	318 - 316	75 – 82	595 - 542	210 - 185	116 - 116
Turbidity (NTU)	6.3	14.2 - 18.2	26.3 - 39.7	12.4 - 2.1	3.6 - 3.9	14.7 - 17.3
PVI (%)	>75%	<25%	0%	*ca.* 50%	*ca.* 50%	0%
State	Clear - Vegetated	Phytoplankton - turbid	Inorganic-turbid	Clear - Vegetated	Phytoplankton - turbid	Inorganic-turbid
Phytoplankton Chl_a (µg.L^{-1})	15.7 - 9.6	46.5 - 56.1	15.7 - 4.7	8.9 - 4.7	11.4 - 8.2	8.4 - 10.7
TN (µg.L^{-1})	970	1391.5	670	2330	2275	1600
TP (µg.L^{-1})	89.2	65.9	122.5	214.2	55	182.8

In each lake we manipulated the abundances of two mostly planktivorous fish species and one omnivorous macroinvertebrate species (shrimps in subtropical and amphipods in temperate lakes) according to a factorial scheme of four treatments - four replicated. We placed 16 transparent cylindrical PVC enclosures, of 1.2 m in diameter, attached to the bottom of the lake and standing out several centimeters above the water surface. On the top, they were closed with a net to avoid eating fish by birds. The water level within the enclosures ranged from 0.8 to 1.1m during the experiment (average volume= 1000L). Prior to the introduction of fish and macroinvertebrates we removed all submerged plants and fish present.

Inside each mesocom we introduced artificial plants (similar to *Myriophyllum*) at densities corresponding to 75% of PVI two weeks before the first sampling in order to allow colonization by macroinvertebrates and periphyton growth.

Treatments included: Fish (**F**), Invertebrate (Shrimp in subtropical and Amphipoda in temperate lakes; **I**), Fish + Invertebrates (**F+I**), Control (no fish or invertebrates; **C**) (Table II). We selected fish and invertebrates common species in the lakes considered, and in similar abundances to those found in natural conditions according to Teixeira de Mello *et al*. (2009), Liboriussen *et al*. (2005), Wilhelm & Schindler (1999) and Iglesias *et al*. (2008). In all cases, we included individuals of both sexes, and smaller than 7.0cm, to assume predominance of zooplanktivory and benthivory.

Table II. Names and densities of the two species of fish and invertebrates added in the experiments in subtropical and temperate lakes. Densities based on previous data provided by: * Teixeira de Mello *et al.* (2009), [+] Liboriussen *et al.* (2005), [X] Iglesias *et al.* (2008), [¤] Wilhelm & Schindler (1999).

	Subtropical	Temperate
Fish sp. 1	*Cnesterodon deccemaculatus* 50 *	*Gasterosteus aculeatus* 12[+]
Fish sp. 2	*Jenynsia multidentata* 40 *[x]	*Perca fluviatilis* 6 *
Invertebrate	*Palaemonetes argentinus* 120 *	*Gammarus lacustris* 240[¤]

Physico-chemical and biological sampling

In each mesocosm, physic-chemical and biological samples were taken at initial time (T0) and end time (TF) 49 days later. At each sampling time, we measured *in situ* parameters: Temp, pH, Conductivity, turbidity, Dissolved Oxygen, and depth-integrated water samples were taken for physico-chemical analysis, phytoplankton and zooplankton. In laboratory, nutrients (Total Nitrogen, Total Phosphorous, Soluble Reactive Phosphorus) and chlorophyll a from phytoplankton and periphyton were measured according to APHA 2005. Zooplankton samples were taken by filtering of 6 – 8 L through a 50µm mesh net.

Zooplankton and quantitative phytoplankton samples were preserved with acid Lugol and phytoplankton qualitative samples were fixed in 1% Formalin. Zooplankton counting was performed according to Paggi & de Paggi (1974) and, when possible, individuals were classified to species level. Periphyton samples were taken from the removal of an artificial plant, and extracted by shaking in filtered water. One periphyton sample was used for chlorophyll_a determination, while another was kept in acid Lugol for later laboratory identification and measurement. Phytoplankton and periphyton samples were counted in random fields at 100X to 400X in Olympus CKX 41 inverted microscope in sedimentation chambers (Utermöhl 1958). Counted was performed reaching at least 100 individuals of the most frequent species (Lund *et al.* 1958) considering the organism as the unit (cell, colony or filament). The volumes were calculated according to Hillebrand *et al*. (1999) and biovolume was estimated as the individual volume of each species by the abundance. Periphyton biomasses were standardized as biovolume per plant weight of artificial plant ($\mu g.g^{-1}$). Picoplankton (GALD < 2 μm) organisms were not considered.

In addition to samples from closed mesocosms, we took physicochemical and biological samples from open mesocosms (also with artificial plants, only in temperate lakes) and open waters immediately outside the mesocosms (both climates) to analyze differences between the conditions of the enclosure and the open water.

Phytoplankton was classified both taxonomically and by considering the ecological approach proposed by Kruk *et al* (2010) in Morphologically Based Functional Groups. Periphyton, was classified also considering both taxonomically and ecological approach. The ecological approach for periphyton (MPG Pacheco *et al.* in prep, Chapter VI) proposes 28 groups based on main morphological and functional traits, and this approach was based on a similar classification given by Salmaso & Padisák (2007) for phytoplankton. All added fish and macroinvertebrates were collected by electrofishing after being performed the final sampling.

Data analysis

Environmental variables were centered by subtracting the mean and then standardized dividing by the variance. Species with a lower contribution than 5% of the total biomass for each sampling time were excluded from the analysis. Phytoplankton and periphyton biomass data were log transformed (Log10 (x + a) where "a" corresponds to the minimum non-zero value of the variable in each case). The main limnological gradients of the studied lakes, were analyzed by performing a principal component analysis (PCA) selecting from the set of environmental variables, those directly linked to the trophic status: nutrients, phytoplankton biomass, and turbidity. PERMANOVA (non parametric multiple ANOVA) considering physico-chemical and biological variables were performed to analyze the significance between different treatments and climate for each sampling time (Ways: Treatment / Climate / Time). Similarity analyses (Two-way ANOSIM) were performed to compare differences between phytoplankton and periphyton composition between treatments (Way 1) and climate (Way 2).

Paired test were a posteriori performed, in order to test differences between treatments or isolated effects (e.g. isolated effect of fish: Fish & Fish+Invertebrate vs. Invertebrate & Control). All these analyzes were performed using Euclidian distance for physico-chemical variables and Bray-Curtis index for community composition (Clarke 1993). The effect generated by the enclosure was analyzed separately by comparing the phytoplankton and periphyton biomass (PERMANOVA) and composition (ANOSIM) between open treatments (external and open mesocosms) with controls for each lake. We considered as significant differences R ANOSIM values higher than 0.5 as distinctly separated, 0.25 as separate but overlapping, and not significant to $R < 0.25$. For all these analyzes we selected a confidence interval of 95% ($P< 0.05$). Statistical analyses were carried out using the software CANOCO 4.5 for Windows (Leps & Smilauer 2003) and Past 3.X version (Hammer *et al.,* 2001).

Results

Considered lakes exhibited different states regarding main limnological features, varying among conditions of turbidity, conductivity, nutrients, phytoplankton and periphyton biomass (Table I, Figure 1). Physico-chemical and biological variables selected in PCA explained the 82.17% of the total variance ($\lambda 1= 53.33 + \lambda 2= 28.84$). Temperature ranges were markedly different between the two climatic conditions considered (Subtropical: 20.2 to 28 °C; Temperate: 8.3 to 16.6 °C).

Periphyton biomass exhibited an inverse pattern with phytoplankton and turbidity, indicating conditions mostly associated to states of: clear with high periphyton, phytoplankton turbid with low periphyton and inorganic-turbid with low periphyton or phytoplankton.

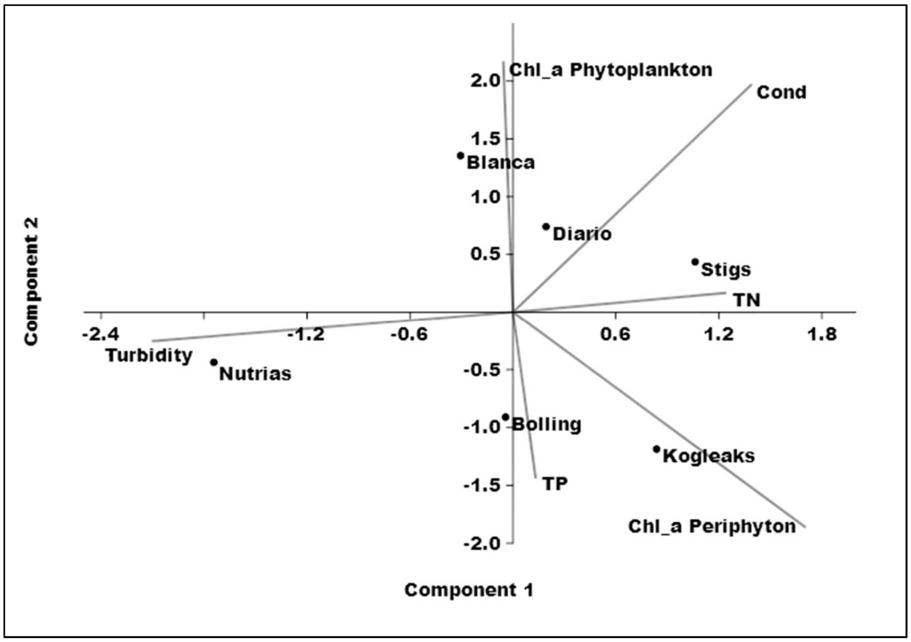

Figure 1. Principal Components Analysis of the main limnological features related with trophic state for the six lakes where the experiments were carried out. Turbidity, Conductivity (Cond), Total Phosphorous (TP), Total Nitrogen (TN), Phytoplankton biomass (Chl_a Phytoplankton), Periphyton biomass (Chl_a Periphyton). Percentage variance explained λ1= 53.33; λ2= 28.84 (Total= 82.17 %).

Treatment with fish (F and F+I) exhibited significant effect on zooplankton, with lower biomasses and lower abundances of cladocerans in both climatic conditions (Fig. 2). Particularly, in subtropical lakes a decline in abundances of calanoid copepod associated with the presence of fish was also observed, although it was not observed in temperate lakes. In general, all treatments with fish addition presented lower total zooplankton abundances than those fishless (F= 4,22; P= 0.002).

Table III. Phytoplankton and periphyton biovolume differences between treatments and climatic conditions based on two-way PERMANOVA using Bray-curtis index. NS: no significant differences.

	Clima		Tratamiento	
	F	P value	F	P value
Fitoplancton	6.05	0.0001	1.06	0.0005
Perifiton	8.32	0.0001	NS	-

As observed in two-way PERMANOVA, phytoplankton and periphyton biomasses showed significant differences derived largely by climatic conditions, while differences between treatments were exclusively restricted to phytoplankton biomass (Table III). Moreover, an increase in the final periphyton biomass in comparison to initial conditions was observed (Fig. 3). As well, no differences in periphyton biomass or composition in relation to treatment were observed considering independently the lakes (data not shown). Moreover, no significant differences were observed in taxonomic or functional periphyton composition in relation to treatments, although these differences were observed in relation to lake and climatic conditions (Table IV).

Table IV. Phytoplankton and periphyton taxonomical and functional composition differences between lakes, climatic conditions, and treatments based on ANOSIM using Bray-curtis index. NS: no significant differences (R< 0.25; P> 0,05). *: Morpho-functional classification based on Kruk *et al.,* 2010, **: Morpho-functional groups of periphyton based on Pacheco *et al.* (in prep.).

		Lake		**Climatic condition**		**Treatment**	
		R	P value	R	P value	R	P value
Phytoplankton	Taxonomical	0.86	0.0001	0.31	0.0001	0.25	0.0001
	Functional *	0.72	0.0001	NS	-	0.46	0.0292
Periphyton	Taxonomical	0.82	0.0001	0.63	0.0001	NS	-
	Functional **	0.74	0.0001	0.53	0.0001	NS	-

In the case of phytoplankton, clear differences were observed both in terms of biomass (Table III, Fig. 3) and taxonomic and functional composition (Table IV, Fig. 4) associated with the presence of fish in the treatments (**F**, **F + I** vs **I**, **C**). Phytoplankton composition was comparatively better to discern between treatments in terms of functional composition (MBFG Kruk *et al.* 2010) than taxonomically (Table IV).

In addition, as in the case of biomass, much of the variability in phytoplankton composition was explained exclusively by climatic conditions (Table IV).

While the effects on phytoplankton biomass were similar in lakes of both climatic conditions, in terms of phytoplankton composition the greatest effects were found in temperate lakes (Fig. 4) where the presence of fish (treatment F and F+I) favored higher biomasses than those found in lake external conditions (E). Moreover, phytoplankton biomass in temperate lakes were similar between control (C) and external conditions (E).

Phytoplankton composition in the presence of fishes exhibited in temperate lakes, higher biomasses of large sized phytoplankton groups, as flagellates (MBFG V) and mucilaginous colonies (MBFG VII).

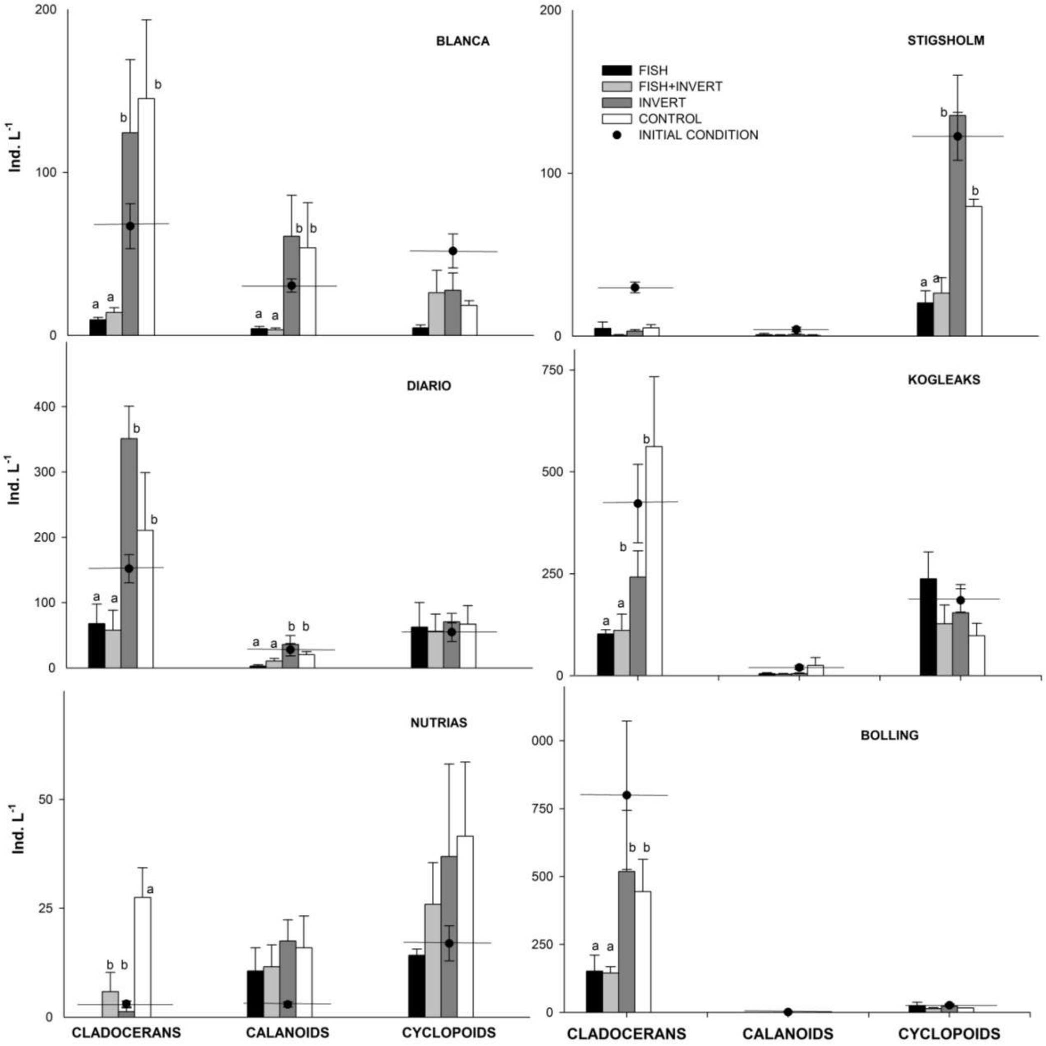

Figure 2. Zooplankton composition in experimental treatments in subtropical (left) and temperate (right) lakes at initial (horizontal lines) and final conditions (49 days after fish and invertebrates addition). Error bars represent ± 1 standard error. Please note the different scales on the y-axes. Letters indicate groupings based on paired post hoc tests when PERMANOVA tests were significant. From: Iglesias et al. (In prep.)

In subtropical lakes, no significant differences were observed in phytoplankton composition between treatments (Fig. 4). Contrarily to what found in temperate lakes, subtropical lakes shown lower phytoplankton biomasses in control treatments (C) than those found in external conditions in the lake (E) indicating a lower zooplankton consumption by zooplankton in external conditions.

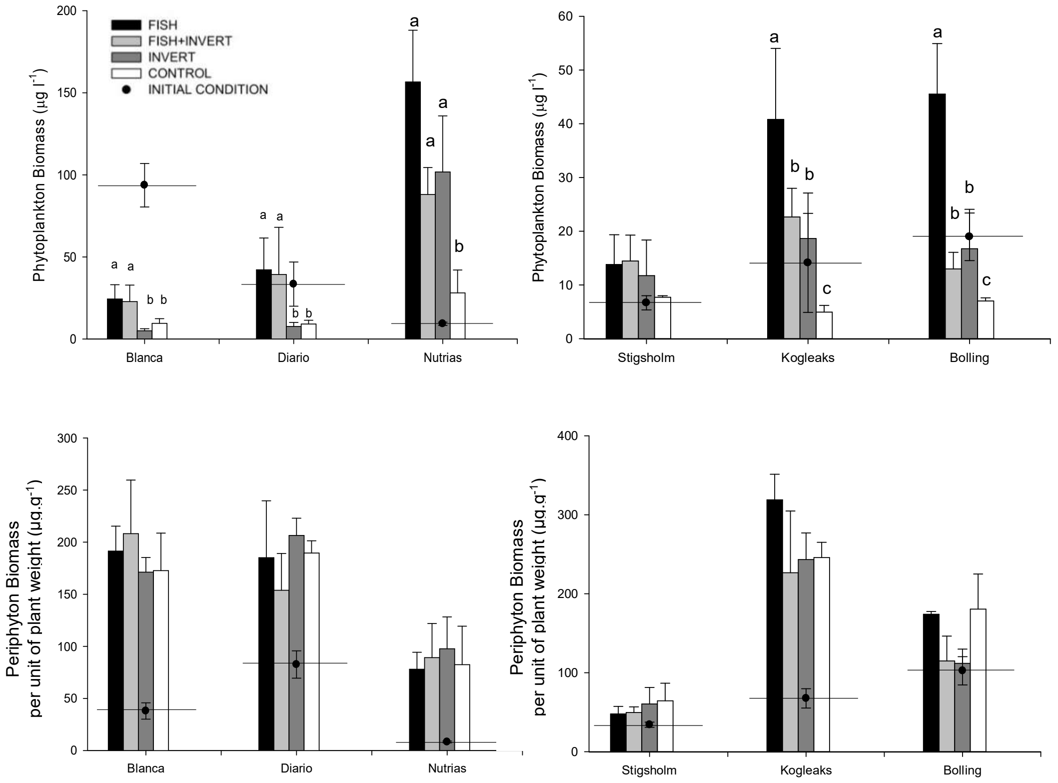

Figure 3. Phytoplankton (upper panels) and Periphyton (lower panels) biomasses in subtropical (left) and temperate (right) lakes at initial (horizontal lines) and final conditions (49 days after fish and invertebrates addition). Error bars represents ± 1 standard error. Pos hoc groups at final time are given by a, b and c. From: Iglesias et al. (In prep.)

In the case of periphyton, no significant differences in terms of biomass or composition were observed between control mesocosms (enclosed) and open mesocosms (external conditions). Also, the presence of invertebrates in treatments (I) showed no effect on the abundance or composition of phytoplankton and periphyton.

Discussion

The range of environmental variability and the different conditions or states found in lakes make them suitable as a study model. However, lakes were not exactly comparable in pairs in their limnological conditions. In this sense, level of nutrients and periphyton were higher in temperate lakes, whereas in subtropical lakes phytoplankton biomass are much higher (e.g. Stigsholm phytoplankton turbid lake in temperate climate, has lower phytoplankton biomass to that observed in Diario, clear - vegetated lake in subtropical climate).

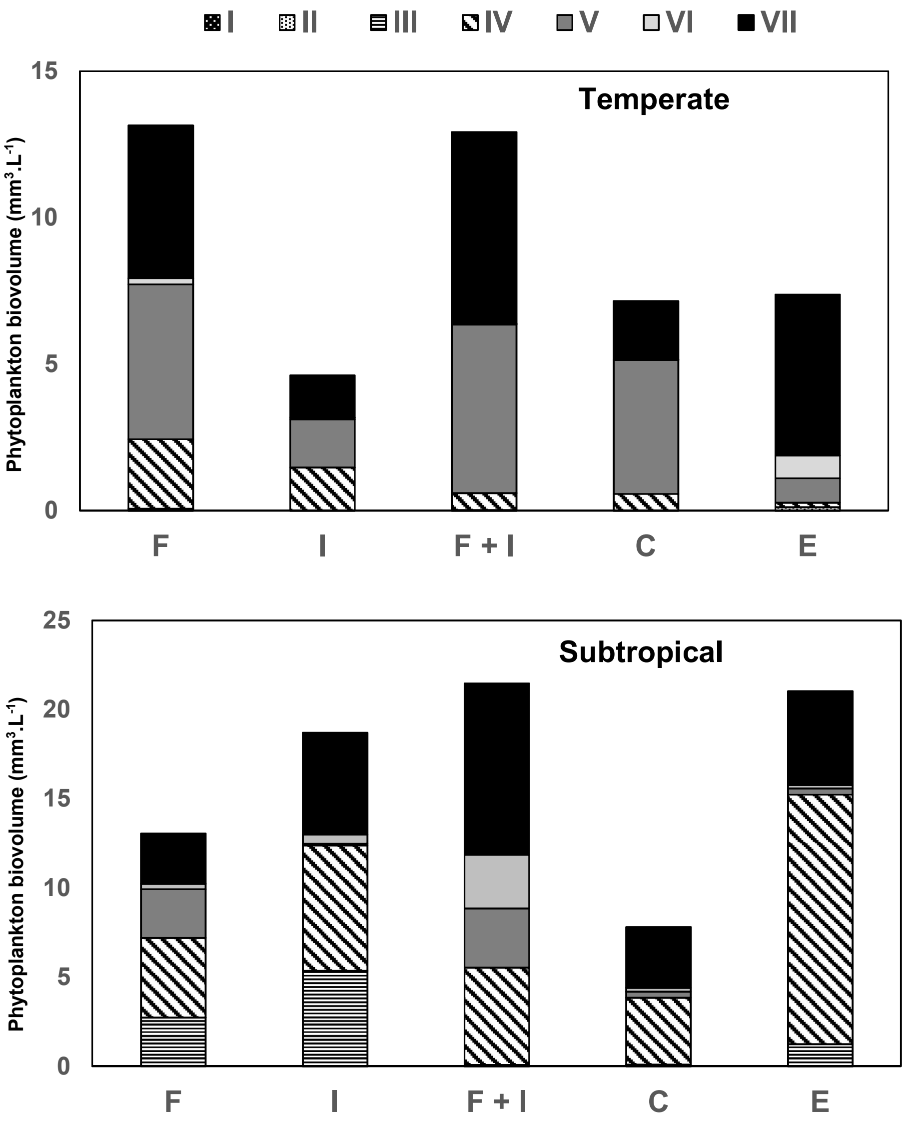

Figure 4. Phytoplankton functional composition (according to Kruk *et al.* 2010, MBFG I to VII) by treatment (F: Fish, I: Invertebrate, F+I: Fish + Invertebrate, C: Control, E: External) for lakes in temperate (upper graph) and subtropical (lower graph) climates, where the experiments were carried out.

Phytoplankton

Significant cascading effects promoted by fishes on phytoplankton biomass and composition were observed in both climatic conditions, via top-down: zooplanktivorous fish – zooplankton – phytoplankton.

In lakes from both climatic conditions, the presence of fishes significantly decreased total abundances of zooplankton and particularly large-sized groups as cladocerans. This led to a lower grazing by zooplankton, generating higher phytoplankton biomasses and compositional changes in the presence of fishes.

This trophic cascade effect promoted by small fish, has been classically described (McQueen *et al.* 1986) and widely registered in subtropical systems (VanLeeuwen *et al.,* 2007; Meerhoff *et al.,* 2007; Jeppesen *et al.,* 2010; Lacerot *et al.,* 2013) where the zooplankton is structured by predation, promoting the dominance of small sized groups with lower grazing impact on phytoplankton.

Similarly to the findings by Jeppesen *et al.* (2005), in this study the effects promoted by fish on zooplankton were more pronounced in temperate lakes. In these ecosystems, fish presence in treatments promoted higher biomass than those found outside of the mesocosms (lake conditions) while in subtropical lakes biomasses in the presence of fish did not present significant differences with those outside the mesocosms.

This pattern could be associated to higher densities of piscivorous in temperate lakes in relation to the subtropical (Meerhoff *et al.,* 2007; Jeppesen *et al.,* 2010), and the higher control by piscivorous on small zooplanktivorous fish in temperate lakes could be evidenced by lower biomasses of phytoplankton.

On the other hand, lower biomasses of piscivorous may not limit the high biomasses of small omnivores existing in subtropical lakes (Lazzaro 1997; Meerhoff *et al.,* 2007; Teixeira de Mello *et al.,* 2009) limiting the grazing exerted on phytoplankton. This pattern found in this experimental approach is the same as has been described for subtropical shallow lakes in field studies (Iglesias *et al.,* 2008; Sinistro 2010; Lacerot *et al.,* 2013; among others).

The lower phytoplankton biomasses in control (C) and invertebrate (I) treatment, would be associated with a high grazing capacity of the zooplankton in subtropical shallow lakes in the absence of small fishes.

According to previous studies (Jeppesen *et al.,* 2005; Jeppesen *et al.,* 2007; Meerhoff *et al.,* 2007) the low grazing capacity exerted by zooplankton on phytoplankton in subtropical lakes would be associated with high zooplaktivory by small omnivorous fish. In these ecosystems, zooplankton grazing effects on phytoplankton is difficult noticeable in field studies (Kruk *et al.,* 2009; Gelós *et al.,* 2010; Pacheco *et al.,* 2010). It has also been observed that in the absence of planktivorous fish (Iglesias *et al.,* 2011) or in experimental conditions with high densities of piscivorous fish (Mazzeo *et al.,* 2010; Sinistro, 2010) large sized zooplankton can exert a significant control over the biomass and structure of phytoplankton in subtropical shallow lakes.

In an interanual study in a subtropical shallow lake Pacheco *et al.,* (*in prep*) observed that the emergence of large zooplankton as *Daphnia* over short periods can play an important role structuring phytoplankton composition to the dominance of large sized groups as filamentous cyanobacteria.

The differences in composition between treatments, which were higher considering functional groups (MBFG Kruk *et al.*, 2010) showed a clear pattern of compositional replacement in the presence of fish in temperate lakes, favoring large groups of phytoplankton, particularly MBFG group VII. Jeppesen *et al.,* (2005) also observed this pattern in phytoplankton composition for subtropical shallow lakes, attributing this to the dominance of small sized zooplankton that dominates in high densities of zooplanktivorous.

Periphyton

Although it was expected an effect derived from climatic condition on periphyton biomass, the main mechanisms proposed for this community were the differences in the trophic structure in lakes of different climatic conditions (Meerhoff *et al*., 2007; Havens *et al*., 2009; Teixeira de Mello *et al*., 2009). However, in the present experimental study we observed no differences in terms of biomass, taxonomic or functional composition of periphyton attributable to the direct or cascading effects promoted by small omnivorous fish or invertebrates.

In subtropical lakes, it was expected that the absence of fish was associated with a greater number of invertebrates, increasing grazing on periphyton, evidenced by lower biomass and dominance of non-edible or resistant to grazing groups. However, this pattern was not observed in terms of periphyton biomass or composition.

As described by several studies in subtropical lakes (Iglesias *et al.,* 2007; Meerhoff *et al.,* 2007; Jeppesen *et al*., 2010) a direct effect of consumption by fish on periphyton was expected due to widespread omnivory and habitat preference of fishes associated with macrophytes (Teixeira-de Mello *et al.,* 2009; Gonzalez- Bergonzoni *et al.,* 2012).

However, previous studies from temperate climates, have described the absence of effects on periphyton due to direct consumption by fish, both in field (Hansson, 1992) as in experimental approaches (Brönmark & Vermaat, 1998; Bertolo *et al.,* 2000; Bécares *et al.,* 2008). Through experiments in mesocosms along a latitudinal gradient in European shallow lakes with different trophic status, Bécares *et al.* (2008) described the absence of top-down controls promoted by fish on periphyton, explaining that the main determinants of periphyton are linked to turbidity and nutrient levels in lakes.

This coincides with the findings by Hanson (1992) in a gradient of European and subarctic lakes, in conditions of presence and absence of fish, and the study of Hill & Harvey (1998) by experimental approaches. Both authors found that fish do not promote any direct or cascade effect on periphyton, and most of the variability in the biomass could be explained by the temperature and nutrient levels. Moreover, in the opposite way to hypothesized, Blanco (2001) and Blanco *et al.,* (2004) showed that fish can have positive effect on periphyton biomass by increased levels of nutrients due to excretion.

Much of the variability in periphyton biomass and composition, were related exclusively to the climatic condition, and can not be explained by effects of the trophic structure derived from omnivorous fish or macroinvertebrates.

These differences could be explained complementarily by the effect of metabolic constraints on the biomass to warmer climates (Brown *et al.,* 2004) and a general increase in taxonomic richness to warmer climates (Allan *et al.,* 2002) in accordance with the findings by Meerhoff *et al*. (2012) for periphyton in a review of latitudinal gradients in shallow lakes.

This lack of control by consumption observed for periphyton may be important in facilitating the development of high abundances over macrophytes, limiting their growth and thereby facilitating the passage to phytoplankton - turbid waters states (Jones & Sayer 2003, Sayer et al. 2010). This mechanism would be particularly important in situations of pulse of dissolved nutrients in the water body, which may favor the development of the macrophyte periphyton as has been observed experimentally by Jones *et al.,* (2002).

Finally, it is important to remark that the presence of invertebrates did not exhibit any noticeable effect on the abundance or composition of phytoplankton or periphyton in any of the lakes considered. This, contrasts with findings by Collins & Paggi (1998) for shrimps in subtropical lakes, and by Wilhelm & Schindler (1999) for amphipods in temperate lakes.

Several authors point out that shrimp may exert a significant consumption of zooplankton in subtropical shallow lakes (Boschi 1981; Collins & Paggi 1998; Collins 1999). However, in this study the effects on phytoplankton are unclear and appear to be restricted exclusively to a higher biovolume in phytoplankton in treatments with invertebrates compared to controls, although this pattern is not observed at the level of biomass (as Chlorophyll a).

This absence of effects on periphyton could be due to an over-simplification of trophic interactions in mesocosms, or misallocation of trophic roles of invertebrates used. Other factors derived from experimental simplifications may have been important in determining the absence of effects of macroinvertebrates. Low total abundances considered in mesocosms, can lead to density estimations are incorrect regarding existing in the lakes.

In this sense, it is highly relevant to increase the understanding of the role of macroinvertebrates and fish on the abundance and composition of periphyton in subtropical lakes, in order to analyze whether the lack of effects could be associated to an oversimplification of natural trophic structure lakes or ambiguous roles of the species concerned.

Conclusions

The results of our study suggests that the presence of omnivorous fish, can promote major cascading effects on the phytoplankton abundance and composition both in subtropical and temperate shallow lakes. This coincides with the hypothesized mechanisms, where the presence of omnivorous fish led to lower zooplankton biomasses and can structure this community towards the dominance of small sizes, which exert less grazing pressure on phytoplankton, and thus facilitating high biomass states.

These mechanisms may be more marked in temperate lakes where treatments with omnivorous fish had higher phytoplankton biomass than those found in external condition lakes (lake conditions) where predation of omnivorous by piscivorous fish would limit this cascading effect.

These effects were promoted exclusively by the presence of fish on treatments, whereas it was not observed any cascading effect promoted by the presence of macroinvertebrates in biomass or composition of phytoplankton or periphyton.

In addition, and contrary to our hypothesis, it was not observed any clear pattern attributable to direct (by consumption) or cascading effects promoted by omnivorous fish or macroinvertebrates on periphyton, and treatments with fish and / or macroinvertebrates respond similarly to control conditions. This contrasts with the expected effect, especially for subtropical shallow lakes, where a higher level of grazing by small fish on periphyton was expected. Besides, major variability in periphyton biomass and composition were derived exclusively from climatic conditions. This may be attributable both to errors in experimental design due to an oversimplification of the food web in lakes, as by differences in metabolic requirements on periphyton between lakes of different climatic regions. This must be analyzed regarding their interaction with the trophic structure between lakes of different climate regimes, in order to elucidate possible effects on periphyton biomass and composition.

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