Journal of
Plankton Research

plankt.oxfordjournals.org

J. Plankton Res. (2016) 00(00): 1–12. doi:10.1093/plankt/fbw029

Influence of lake trophic conditions on the dominant mixotrophic algal assemblages

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Received October 22, 2015; accepted March 28, 2016

Corresponding editor: John Dolan

Mixotrophic protists combine photo-autotrophy and phago-heterotrophy. Even though only in oligotrophic systems mixotrophs overcome strictly autotrophs, they are represented all along the trophic spectrum. We hypothesize that lakes with different chlorophyll *a* (Chl*a*) and dissolved organic carbon (DOC) concentrations harbor different assemblages of mixotrophs. We examined the composition of mixotrophs in 24 lakes in two Patagonian regions (Argentina), with a range of conditions of Chl*a* and DOC. The categorization of a predominant type of nutrition in each mixotroph was based on published evidence. Additionally in four lakes we analyzed the bacterivory activity of mixotrophs by performing ingestion experiments. We found potentially mixotrophic taxa in all lakes, however the assemblages varied depending on their trophic-DOC features. Primarily heterotrophic mixotrophs (e.g. chrysophytes) attained a higher proportion in oligotrophic lakes, declining towards higher trophic status. In contrast, primarily autotrophic mixotrophs (e.g. cryptophytes) dominated in eutrophic systems. Mixotrophs actively ingested bacteria in all experiments, however cell-specific grazing rates were higher in oligotrophic lakes. Mixotrophs accounted for more than half of total flagellate grazing in oligo-mesotrophic environments. Our results suggest that there is a replacement of mixotrophic taxa along a gradient of increasing trophic state, from primarily heterotrophic to primarily autotrophic mixotrophs, whereas their relative contribution to total flagellate grazing decreases.

KEYWORDS: mixotrophs; phytoplankton; lakes; trophic status; DOC

INTRODUCTION

Mixotrophic protists are organisms capable of obtaining energy and/or nutrients by both phototrophic autotrophy and heterotrophy (e.g. Jones, 2000). Technically, the heterotrophic activity may involve osmotrophy and/or phagotrophy. However, most phytoplankton taxa are in part osmotrophic, particularly due to the uptake of amino acids and other primary metabolites, thus using

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this as a discriminating feature of mixotrophy in planktonic protists appears baseless (Flynn *et al.*, 2012). In consequence, in this paper, we refer, as in most of the ecological literature, to phototrophic and phagotrophic nutrition. Mixotrophic protists play a key role in the planktonic food web due to their substantial contribution to the flow of carbon and other nutrients in aquatic ecosystems (Mitra *et al.*, 2014).

The success of mixotrophs over autotrophs may vary according to environmental conditions. It has been experimentally demonstrated that mixotrophs may overcome strictly autotrophic and heterotrophic protists under poor nutrient conditions (Katechakis and Stibor, 2006; Tittel *et al.*, 2003). Similarly, in a previous study we found that, along a nutrient-DOC gradient of lakes in Patagonia (both at local and regional scales), mixotrophs thrived in relation to autotrophs in oligotrophic systems, while this tendency is reversed in eutrophic systems (Saad *et al.*, 2013). Oligotrophic environments are favourable for mixotrophs due to their nutrient limitation. In addition, in oligotrophic humic lakes, light can also limit photosynthesis; thus prey ingestion subsidizes carbon and/or micronutrients in these environments. At higher trophic status phytoplankton is not subjected to these stressors, so these environments enable algae to develop at the expense of photosynthesis, whereas the expensive mixotrophic strategy (Raven, 1997) would be disadvantageous in less restrictive environments. Another interesting explanation was proposed by Wilken *et al.* (Wilken *et al.* 2014) showing that in oligotrophic systems photoheterotrophic growth could be favoured in some groups of algae, namely where light is primarily utilized as the source of energy and organic matter as source of carbon. In terms of economy, this new vision gives less weight to previous suggestions of the high cost of maintaining both physiological nutrition systems, as the ingestion of prey and assimilation of organic C allow reduction of C fixation in the Calvin–Benson cycle and therefore also a reduction in the content of biochemical structures involved in the light reaction (Wan *et al.*, 2011).

Mixotrophic algae form neither a homogeneous taxonomic nor ecological group. As with all current eukaryotic groups of algae they originated by means of primary, secondary or tertiary endosymbiotic processes (Keeling, 2004). Specifically, mixotrophs express the ancestral phagotrophic mode of nutrition. This secondary evolution to mixotrophy has occurred independently over time, varying the extent of phototrophy and phagotrophy among different groups (Raven *et al.*, 2009).

In a first approach, Jones (Jones, 1994) put forward a classification of mixotrophs, setting them along the spectrum of nutritional strategies between absolute autotrophy and absolute heterotrophy. Later on, different conceptual models were proposed based on their nutritional behavior (e.g. Jones, 1997; Jones, 2000; Stoecker, 1998). Despite some differences among these approaches, all of them agree that different mixotrophs may differ in their degree of heterotrophy due to specific environmental limitations: light, nutrients, prey availability or a combination of these (Bergström *et al.*, 2003). Some mathematical models attempt to determine under which conditions such specialization toward pure phototrophs and pure phagotrophs would take place (de Castro *et al.*, 2009; Troost *et al.*, 2005), however, only a few studies have considered assemblages of mixotrophs along different environmental conditions in the field.

Chrysophytes are frequently positioned in the heterotrophic portion of the nutritional spectrum. For example, *Ochromonas*-like species carry out photosynthesis in addition to grazing bacteria in conditions that bacteria are not abundant or nutritious enough to support growth (Boëchat *et al.*, 2007; Pålsson and Daniel, 2004). In other taxa, such as *Dinobryon* spp., light availability presumably promotes photosynthesis and phagotrophy is a complementary nutrient input in oligotrophic conditions (Caron *et al.*, 1993, Jones and Rees, 1994, Rottberger *et al.*, 2013). By contrast, cryptophytes are commonly located towards the autotrophic end of the gradient; although several species of this group ingest bacteria, phagotrophy seems to be a way to acquire essential nutrients (Unrein *et al.*, 2007; Urabe *et al.*, 2000), whereas its use in energy gain is limited (Tranvik *et al.*, 1989). Other groups of mixotrophs, like the haptophytes, were not included in these first classifications, but based on newer literature they can be located at an intermediate position on the gradient. Unrein *et al.* (Unrein *et al.*, 2014) recently demonstrated the key role of this group as bacterivores in marine ecosystems. On the other hand, Dictyochophyceae of the Order Pedinellales were also found to be a relevant phagotrophic group in some oligotrophic Patagonian and Antarctic lakes with moderate to high bacterivory rates (Gerea *et al.*, 2016). Prasinophyceae is a group that for a long time was considered to be strictly autotrophic; however, more recently some taxa were found to have the ability to ingest prey to supplement photosynthesis for growth (Bell and Laybourn-Parry, 2003; McKie-Krisberg *et al.*, 2015), and even can potentially contribute greatly to predation in Arctic marine environments (McKie-Krisberg and Sanders, 2014).

Although it has been observed that the success of mixotrophs over autotrophs decreases along an increasing gradient of lake trophic state and DOC (Saad *et al.*, 2013), they are represented all along the trophic spectrum. However, considering the variable contribution of phagotrophy and phototrophy to cell nutrition among mixotrophs allowing a differential fitness to develop in each environment, it is expected that different assemblages of phagotrophically active mixotrophs occurring will be found along an environmental gradient. Thus, primarily autotrophic mixotrophs would dominate at higher trophic state environments, while primarily heterotrophic mixotrophs would prevail in oligotrophic systems. Moreover, ingestion rates would decrease at higher lake trophic states, as environmental conditions for photosynthesis improve.

We analyzed the mixotrophic assemblages in lakes of two Patagonian regions in Argentina, exhibiting different trophic state and DOC conditions. Additionally, the bacterivory impact of the main mixotrophs was studied in four contrasting lakes (deep clear, humic, clear-vegetated and phytoplankton-turbid), performing short-duration ingestion experiments with fluorescently labelled prey in order to evaluate whether all mixotrophic taxa actively graze in lakes with different features.

METHOD

Study area

Patagonia has a wide variety of lakes with different morphometry, optical properties, dissolved organic matter and nutrient content (e.g. Baigún and Marinone, 1995; Díaz *et al.*, 2000; Modenutti *et al.*, 2010; Pérez *et al.*, 2010; Quirós and Drago, 1985). Our study was conducted in two areas of Austral Patagonia that clearly reflect such diversity: Strobel Plateau (Santa Cruz) and Tierra del Fuego.

We studied 24 freshwater bodies in Santa Cruz and Tierra del Fuego provinces (Fig. 1), whose geographic position is presented in the Supplementary Data online (Table SI). The island of Tierra del Fuego (54°33'S; 67° 30'W) is located in the southernmost region of South America. The region has a great diversity of environments such as steppes, peatlands and mountains; the climate is in general temperate cold and wet, with local variations due the orography of the zone. The lakes surveyed in this study are located in the Argentinean portion of the island, particularly in the South West mountainous area, where lakes were originated by glacial or periglacial activity, and valleys are covered with peat bogs and ponds (Iturraspe and Urciuolo, 2000). This region also shows a strong influence of the North American beaver *Castor canadensis* that generates large landscape-level changes (Anderson *et al.*, 2009); floodings associated with beaver dams rise to ponds that exhibit the highest trophic status among the freshwater environments of the SW region of Tierra del Fuego.

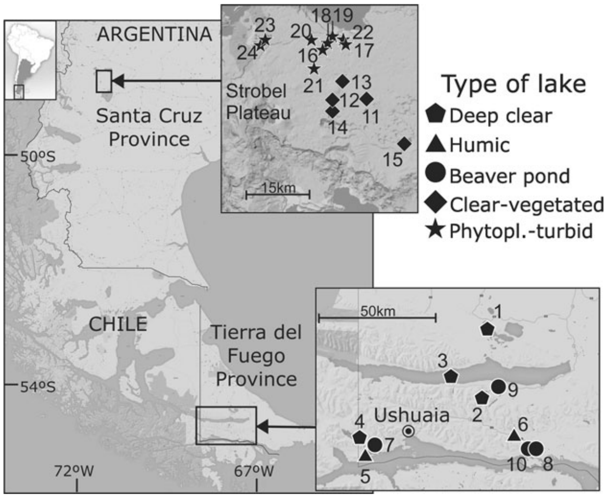

Fig. 1. Location of the studied water bodies classified according to the type of system. Deep clear lakes: 1-Yehuin, 2-Escondido, 3-Fagnano and 4-Acigami. Humic lakes: 5-Negra and 6-Victoria. Beaver ponds: 7-Pond a, 8-Pond b, 9-Pond c and 10-Pond d. Clear-vegetated lakes: 11-S64, 12-S94, 13-Nieve, 14-Justita and 15-Coloradas. Turbid shallow lakes with high phytoplankton biomass (phytoplankton-turbid): 16-Herradura, 17-Temp, 18-9, 19-8, 20-Verde, 21-Campamento, 22-Potrerillo, 23-Rod 19 and 24-Pifie.

Strobel Lake Plateau (48°50'S; 71°20'W), located at the North West of the province of Santa Cruz, comprises a basaltic bed caused by tectonic activity during Tertiary and Quaternary (Panza and Franchi, 2002). This region is exposed to a high wind stress and very low temperatures during colder months. Precipitation is less than 300 mm per year, thus most of the over 1500 shallow water bodies in this area exhibit temporary characteristics. However, large and permanent lakes are also present in the region, some of which have been commercially stocked with rainbow trout (*Oncorhynchus mykiss*) (Lancelotti *et al.*, 2009).

Sampling and physico-chemical analyses

A total of 24 water bodies representatives of five lake types were selected: 4 deep clear lakes (Yehuin, Escondido, Fagnano and Acigami); 2 humic lakes (Negra and Victoria); 4 beaver ponds (Pond a, Pond b, Pond c and Pond d); 5 clear-vegetated shallow lakes (S64, S94, Nieve, Justita and Coloradas); and 9 turbid shallow lakes with high phytoplankton biomass, called phytoplankton-turbid hereinafter (Herradura, Temp, 9, 8, Verde, Campamento, Potrerillo, Rod 19 and Pifie). In most lakes samples were collected during three sampling campaigns in the case of Tierra del Fuego (October 2008, January 2010 and April 2012), and during two sampling campaigns in the Strobel Plateau (November 2011 and February 2013).

Temperature, pH and conductivity *in situ* measurements were carried out with a Horiba D-54 meter (Horiba, Kyoto, Japan), and dissolved oxygen with HI 9146 Hanna portable meter (Hanna Instruments, 150 PD, Italy). Samples for the determination of dissolved nutrient concentration were filtered through Whatman GF/F filter (Whatman, Maidstone, UK) and determined using a Hach^{TM}DR/2800 spectrophotometer (Hach Company, CO, USA) with their reagent kits. For nitrate plus nitrite (NO_3-N + NO_2-N) we used the cadmium reduction method, for ammonium (NH_4-N) the salicylate method and for soluble reactive phosphorus (SRP) the ascorbic acid method. Dissolved inorganic nitrogen (DIN) was calculated as the sum of ammonium, nitrate and nitrite. In all the lakes studied, sampling and measurements were obtained from about 30 cm below the surface.

Aliquots from the same filtered water were stored at 4°C (previously acidified) and then used for dissolved organic carbon (DOC) determination by the high temperature Pt catalyst oxidation method (Shimadzu analyzer TOC-5000A, SM 5310B technique, detection limit: 0.1 mg L^{-1}) according to Sharp *et al.* (1993).

Using GF/F filtered samples, concentration of chlorophyll *a* (Chl*a*) was determined by spectrophotometry before and after acidification (HCl 0.1 N) (to perform the correction for phaeopigments); extractions of pigments were carried out using hot ethanol (60–70°C) and overnight storage at 4°C. Methodological recommendations and equation for Chl*a* determination were based on Marker *et al.* (1980). To calculate the vertical photosynthetically active radiation (PAR) attenuation coefficient (K_d), PAR measurements were performed using a LI-COR radiometer with a submersible spherical quantum sensor (Li-193 SA, Li-Cor PAR, NE, USA). K_d was determined according to Kirk (1994).

Microbial community analyses

Phytoplankton samples for quantitative analysis were fixed with 1% acidified Lugol's iodine solution. Counts were performed according to Utermöhl (Utermöhl, 1958) using an inverted microscope Olympus CKX41 at x400 magnification. The complete procedure for phytoplankton counts is detailed in Saad *et al.* (Saad *et al.*, 2013). The counting error was estimated according to Venrick (Venrick, 1978) and algal biovolumes were calculated using appropriate geometric formulae according to phytoplankton shapes (Hillebrand *et al.*, 1999). Trophic identity of different mixotrophic taxa was obtained from bibliographic references of observational and experimental studies where tolerances towards physical and chemical properties of the environment and ingestion behavior were evaluated (e.g. Caron *et al.*, 1993; Jones and Rees, 1994; Jones, 1994; Rottberger *et al.*, 2013; Tranvik *et al.*, 1989; Unrein *et al.*, 2007; Urabe *et al.*, 2000).

Samples for epifluorescence microscopy were preserved with filtered cold glutaraldehyde 10% (1% final concentration). Fixed samples were filtered through 0.2 µm and 0.8 µm polycarbonate black filters for heterotrophic bacteria and heterotrophic flagellates (HFs) respectively. Cells were stained with DAPI (4,6-diamidino-2-phenyl-indole; 10 mg mL^{-1} final concentration) (Porter and Feig, 1980). Counts were performed with UV excitation using an Olympus epifluorescence microscope (Olympus BX40, Tokyo, Japan) at x1000 magnification. A blue filter was used to confirm the lack of Chl*a* of cells in HF counts.

Ingestion experiments

In situ short-term ingestion experiments were conducted in four lakes, two from Tierra del Fuego and two from the Strobel Plateau with contrasting nutrient-DOC contents: Victoria (humic lake) and Escondido (deep clear) in April 2012; S94 (clear-vegetated) and Herradura (phytoplankton-turbid) in February 2013.

Ingestion rates of the main phagotrophic protists in each lake were measured using plastic bottles of 1 L of capacity and fluorescently labelled bacteria (FLB) as prey. FLB was prepared from *Pseudomonas extremaustralis* (Lopez *et al.*, 2009) obtained from a three-day culture of the strain in E2 minimal medium (Lageveen *et al.*, 1988) supplemented with sodium octanoate 0.25% w/v and stained with DTAF (1.2 mg mL^{-1} final concentration) following the Sherr *et al.* (1987) procedure. Each microcosm was prepared with 1 L of prefiltered (55 µm net) natural water that had been taken from the euphotic zone of each lake, plus the tracer cells (at a final concentration of 18% of natural bacteria population density). Experiments in each lake were incubated in triplicate around noon; subsamples were taken from the microcosms immediately after the addition of the FLB (T0), at 15 (T1), 45 (T2) and 90 (T3) minutes after starting the experiment. Each subsample was fixed with cold, 4% glutaraldehyde (2% final concentration); then 5 mL and 2 mL of each sample was filtered through a polycarbonate black filter of 0.8 µm and 0.2 µm pore size respectively, and stained with DAPI. All counts were done using an epifluorescence microscope (Olympus BX40) at x1000. Phagotrophs were counted on the 0.8 µm pore filter and the number of FLB ingested was counted at the same time. Free FLB and total bacteria were counted on the 0.2 µm pore filter.

For each group of phagotrophs, about 50 cells were counted when possible (in less represented taxa between 20 and 30 cells). Filters of 0.8 µm pore size from T0 were used to eliminate the bias of the FLB that could be counted as ingested and were on the surface of the phagotroph. The FLB ingestion rate of each phagotroph was determined according to Šimek *et al.* (Šimek *et al.*, 1995) from the slope of the linear part of the regression between the average number of tracer particles per protist and time (this linear portion in most of the cases was from T0 to T3 = 45 min). Clearance rate (CR) was obtained by dividing the FLB ingestion rate by the FLB concentration used. Cell-specific grazing rate (CSGR) for each protist was estimated multiplying CR by prey concentration (natural + added bacteria) with the assumption that both prey were ingested at the same rate. Grazing impact (GI) of each phagotroph population was determined multiplying CSGR by each phagotroph's abundance. Mean biovolumes of phagotrophs and prey (natural and added) were estimated from size measurements photographically (Infinity 1, Lumenera®) and by image-processing software (Infinity software 6.0, Lumenera®), applying volume formulae according to Hillebrand *et al.* (Hillebrand *et al.*, 1999). A minimum of 20 individuals was measured for each group in each lake.

Statistical analyses

Multivariate analysis of variance (MANOVA) for trophic and DOC variables was applied to test differences among lake types, and comparisons were performed using Hotelling test. Natural log transformation was applied to meet multivariate normality and equality of covariance assumptions.

RESULTS

Lake groups

Lakes surveyed were grouped in five categories according to their salient limnological features: deep clear lakes, humic lakes, beaver ponds, clear-vegetated lakes and phytoplankton-turbid lakes (Table I). The positions of the lakes are plotted in Fig. 2 according to their DOC and Chl*a* concentrations.

Deep clear lakes and humic lakes had the lowest Chl*a* values (mean values: 0.9 µg L^{-1} and 0.8 µg L^{-1} respectively). The first group comprises large water bodies (mean area: 177 km^2), characterized by low conductivity values (mean: 100 µS cm^{-1}) and K_d (mean: 0.79 m^{-1}). The latter also showed low conductivity values (mean: 59 µS cm^{-1}) and the lowest pH (mean 6.6) of the dataset. These two groups differ primarily due to the relatively high concentration of colored DOC in humic systems derived from the high biomass of *Sphagnum* sp. growing in these environments (mean DOC: 9.32 mg L^{-1}).

Beaver ponds are a heterogeneous group of water bodies with different degrees of impact of beavers (*C. canadensis*) and are the smallest water bodies of the dataset (mean 0.05 km^2) and highly influenced by surrounding areas. Consequently, they showed high variation within and among them in their water chemistry and physical properties (Chl*a* = 0.29 µg L^{-1} to 33.53 µg L^{-1}; DOC = 6.9 mg L^{-1} to 24.8 mg L^{-1}; K_d = 0.73 m^{-1} to 3.3 m^{-1}).

Clear-vegetated lakes and phytoplankton-turbid lakes, both on the Strobel Plateau (Santa Cruz), showed mesotrophic to eutrophic conditions. The first group comprises fishless systems with different coverage of macrophytes (mainly *Myriophyllum quitense*), representing the most pristine lakes from this zone. This group of

Table I: Location, altitude, area and limnological variables measured in each type of the studied water bodies

Type	Deep clear	Humic	Beaver pond	Clear-vegetated	Phytoplankton-turbid
N	4	2	4	5	9
Province	TF	TF	TF	SC	SC
Altitude (masl)	63 (20–120)	66 (29–103)	23 (11–43)	879 (749–930)	890 (881–901)
Area (km^2)	177.4 (56.4–645)	0.1 (0.09–0.2)	0.05 (0.001–0.1)3)	0.4 (0.01–2)	0.2 (0.09–0.7)
Temp. (°C)	7.4 (4.5–10.7)	9.2 (4.9–15.9)	9.9 (6.9–14.4)	16.8 (11.3–21)	14.1 (10.3–18)
pH	7.2 (6–8)	6.6 (6–7.4)	7.4 (6.3–8.7)	8.8 (8.3–9.9)	8.4 (8.1–8.9)
Cond. (µs cm^{-1})	100 (69–197)	60 (35–77)	366 (110–1284)	423 (79–1937)	538 (192–1227)
DO (mg L^{-1})	11.1 (9.8–11.7)	10.5 (8.9–12.1)	11.3 (7.7–13.6)	11.8 (9.3–19.3)	10.2 (7.8–17.8)
Chl*a* (µg L^{-1})	0.9 (0.1–4)	0.8 (0.1–2.2)	9.2 (0.3–33.5)	4.4 (0.2–11.6)	11.5 (2.4–55.2)
DIN (mM)	3.55 (0.71–9.99)	2.84 (ND–9.28)	2.84 (0.71–5.68)	7.85 (1.43–50.68)	7.13 (2.84–17.13)
SRP (mM)	1.29 (0.32–3.23)	1.29(ND-2.91)	1.29 (0.32–2.58)	29.26 (0.97–41.33)	17.84 (0.65–29.06)
DOC (mg L^{-1})	4.9 (2.1–9.3)	9.3 (6.4–12)	13.8 (6.9–24.8)	30.2 (3.9–84.8)	41.8 (18.9–98.6)
K_d (m^{-1})	0.8 (0.4–1.5)	1.6 (0.8–2.8)	2.3 (0.7–3.3)	1.7 (0.6–3.8)	2.4 (0.6–6.8)

Most of lakes were sampled in summer of 2011 and 2013. For each group, mean values with ranges (between brackets) are reported. TF, Tierra del Fuego; SC, Santa Cruz; DO, dissolved oxygen; DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphorus; DOC, dissolved organic carbon; and K_d, vertical PAR extinction coefficient.

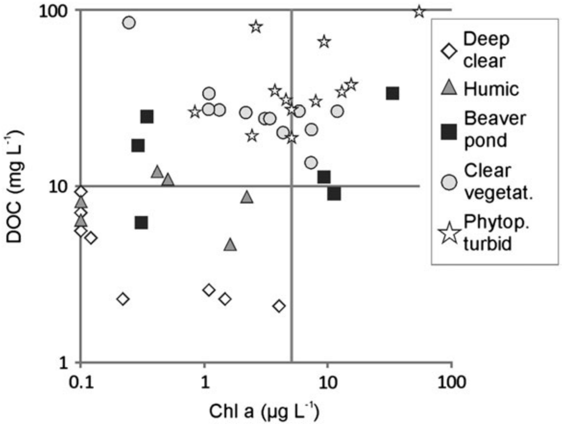

Fig. 2. Biplot between Chl*a* and DOC for each lake.

Table II: Comparisons among type of lakes within MANOVA using Hotelling test

Type	ln(Chl*a*)	ln(SRP)	ln(DOC)	n	Significance
Deep clear	−1.09	−3.37	1.36	8	A
Beaver pond	0.60	−3.38	2.69	6	B
Humic	−0.52	−3.28	2.14	5	C
Clear-vegetated	1.05	−1.49	3.31	12	D
Phytoplankton-turbid	1.82	−1.96	3.59	12	E

Numbers in files conform mean vectors for each type of lake. Means with different scripts are significantly different ($P \leq 0.05$). Chl*a*, chlorophyll *a*; SRP, soluble reactive phosphorus; and DOC, dissolved organic carbón.

lakes had high nutrient content (mean values: DIN = 7.85 mM and SRP = 29.26 mM) that did not result in high Chl*a* values (mean 4.42 $\mu g\,L^{-1}$), probably due to the coverage of macrophytes and the lack of fish. The phytoplankton-turbid lakes also are meso to eutrophic systems (mean values: Chl*a* = 11.54 $\mu g\,L^{-1}$, SRP = 17.84 mM, DIN = 7.13 mM, DOC = 41.81 $mg\,L^{-1}$). They are characterized by scarce macrophyte coverage and most of them are stocked with rainbow trout.

The rationale of the classification of the lakes according to trophic status and DOC variables is supported by the MANOVA analysis, where all lake types significantly differed among each other (Pillai trace = 1.06; $F_{12,114} = 5.18$; $P < 0.0001$) (Table II).

An insight into the assemblages of mixotrophs

A total of seven Cryptophyceae taxa were found, and almost all of them showed the capacity to develop in the five lake types (Table III). *Cryptomonas* spp. and *Plagioselmis* spp. were present in almost all the lakes, while *Chroomonas* sp. and *Hemiselmis* sp. occurred only in few lakes and always had low abundances. Most Chrysophyceae species generally were restricted to oligo and mesotrophic environments; this is the case of *Dinobryon* spp., C*hrysamoeba* sp. and *Chromulina*-like algae, which were found in different environments with low trophic status such as deep clear, humic and some beaver ponds, although they never reached high densities. On the other hand, *Ochromonas*-like algae were well represented in all the lake types. Dictyochophyceae and Haptophyceae were represented by a few species, and both classes were absent in eutrophic environments.

Taking into account the relative abundances, chrysophytes were found in higher proportion in environments with lower Chl*a* and DOC, with a clear decline towards higher concentrations of both variables (Fig. 3). In particular, *Ochromonas*-like species were well represented in almost all lake types, but prevailed in oligotrophic lakes, even constituting the whole mixotroph community, as occurred in the deep clear lake Acigami. More enriched lakes had lower proportions of *Ochromonas*-like than oligotrophic ones, in some lakes accounting for less than 1% of the mixotroph community. Other chrysophyte genera, such as *Dinobryon* spp., were only abundant in some oligo and mesotrophic lakes with intermediate DOC content, while in meso-eutrophic systems this genus was poorly represented or completely absent. *Pseudopedinella* sp. (Dictyochophyceae) was a frequent but less represented taxon, and highest proportions of this group were found in humic lakes; their contribution to the total mixotroph community never exceeded 15%. With a contrasting tendency, cryptophytes were less abundant in oligotrophic lakes, but achieved higher proportions in those systems with high values of DOC and Chl*a*. Inside this group, *Cryptomonas* spp. developed higher relative abundances in eutrophic lakes. In some phytoplankton-turbid lakes they represented close to 70% of the mixotroph community. *Plagioselmis* spp. were present in almost all the lakes, but showed higher proportions in meso and eutrophic environments. *Chroomonas* spp. were only found in considerable quantities in meso and eutrophic lakes, however their maximum contribution was about 20% in a clear-vegetated lake.

Measuring phagotrophic behavior

Different assemblages of mixotrophic flagellates belonging to three algal Classes (Chrysophyceae, Dictyochophyceae and Cryptophyceae) were found in the lakes when the experiment was carried out. Only *Ochromonas*-like and *Plagioselmis nannoplanctica* were present in the four water bodies (Table IV). *Cryptomonas marssonii* and *Pseudopedinella* sp. were found in humic and deep oligotrophic lakes. The Chrysophyceae *Chrysamoeba* sp. and *Dinobryon cylindricum* were only found in lake Victoria (humic), while the

Table III: Mixotrophic flagellate taxon present in the five types of water bodies

	Deep clear	Humic	Beaver pond	Clear-vegetated	Phytoplankton-turbid
Chrysophyceae					
Chromulina sp.		X		X	X
Chrysamoeba sp.		X			
Dinobryon cylindricum		X			
Dinobryon divergens	X	X	X		
Dinobryon sp.	X		X		
Ochromonas-like	X	X	X	X	X
Cryptophyceae					
Chroomonas sp.	X	X		X	X
Cryptomonas marssonii	X	X		X	X
Cryptomonas ovata	X		X	X	X
Cryptomonas sp.	X	X	X		X
Hemiselmis sp.				X	
Plagioselmis lacustris	X	X	X		
Plagioselmis nannoplanctica		X	X	X	X
Dictyochophyceae					
Pseudopedinella sp.	X	X	X	X	
Haptophyceae					
Haptophyceae 1	X	X	X	X	
Chrysochromulina cf. *parva*	X	X			

Crosses (X) represent that the taxon was found at least in one lake corresponding to a specific type of system.

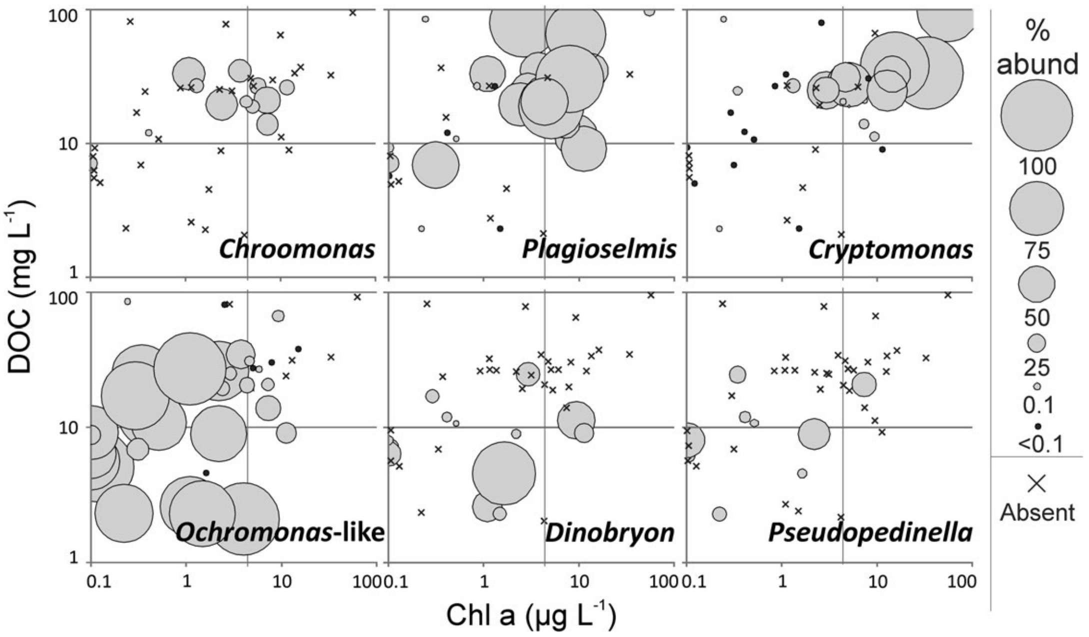

Fig. 3. Percentage of the main mixotrophic flagellate genera abundance to the total mixotrophic flagellates in each lake, according to Chl*a* and DOC concentration.

cryptophyte *Cryptomonas ovata* was restricted to lake Herradura (phytoplankton-turbid). All these mixotrophic groups ingested prey in the two oligotrophic lakes (Victoria and Escondido) with the highest CSGR in these environments. Particularly, *Pseudopedinella* sp. and *C. marssonii* presented the highest CSGR (*Pseudopedinella* 3.89 bact. flag.$^{-1}$h^{-1} in Escondido and *C. marssonii* 3.92 bact flag.$^{-1}$h^{-1} in Victoria), even higher than HFs. The lowest CSGR were measured in the clear-vegetated and phytoplankton-turbid lakes; in these lakes only *C. ovata* and *P. nannoplanctica* (both of them Cryptophyceae) ingested prey at a measurable rate (Table IV).

Mixotrophs accounted for more than half of the bacteria consumed in oligotrophic and mesotrophic lakes, while their impact decreased in the most eutrophic lake (Fig. 4). Within mixotrophs, chrysophytes exerted a high

Table IV: Main grazing parameters and biovolume (average ± standard deviation) estimated for the different phagotrophic flagellates in the four types of water bodies

	Type	Biovolume ($\mu m^3\ mL^{-1}$)	CSGR ($bact.flag.^{-1}h^{-1}$)	GI ($bact.mL^{-1}h^{-1}$)	CR ($nl\ flag.^{-1}h^{-1}$)
HF < 5 μm					
	Deep clear	633 ± 14	1.2 ± 0.2	639.0 ± 95.9	1.4 ± 0.2
	Humic	3917 ± 260	0.5 ± 0.1	1593.9 ± 321.1	0.5 ± 0.1
	Clear-vegetated	1651 ± 112	0.8 ± 0.1	629.1 ± 82.9	0.7 ± 0.1
	Phytoplankton-turbid	3099 ± 265	0.9 ± 0.1	868.8 ± 16.1	0.4 ± 0.01
HF > 5 μm					
	Deep clear	5525 ± 502	1.9 ± 0.6	183.1 ± 61.2	2.2 ± 0.6
	Humic	5970 ± 959	2.1 ± 0.6	226.1 ± 88.8	2.0 ± 0.6
	Clear-vegetated	15509 ± 1805	1.0 ± 0.3	118.9 ± 53.9	0.8 ± 0.2
	Phytoplankton-turbid	54060 ± 14310	3.3	662.7	1.3
Chrysophyceae					
Chrysamoeba sp.	*Humic*	122332 ± 2329	1.8 ± 0.3	646.6 ± 132.3	1.7 ± 0.3
Dinobryon cylindricum	*Humic*	73945 ± 5682	2.8 ± 1.3	148.4 ± 60.1	2.6 ± 1.2
Ochromonas-like	*Deep clear*	1936 ± 203	1.4 ± 0.2	695.8 ± 180.8	1.6 ± 0.1
	Humic	10446 ± 1690	1.3 ± 0.1	1398.5 ± 241.3	1.2 ± 0.1
	Clear-vegetated	65 ± 6	ND	ND	ND
	Phytoplankton-turbid	959 ± 354	ND	ND	ND
Cryptophyceae					
Cryptomonas marssonii	*Deep clear*	87814 ± 10977	2.3 ± 0.2	84.3 ± 14.6	2.7 ± 0.2
	Humic	34760 ± 16261	3.9 ± 0.4	63.6 ± 36.6	3.7 ± 0.5
	Clear-vegetated	59729 ± 7243	ND	ND	ND
Cryptomonas ovata	*Phytoplankton-turbid*	2354975 ± 380016	1.3 ± 0.4	861.7 ± 152.1	0.5 ± 0.1
Plagioselmis nannoplanctica	*Deep clear*	5112.98 ± 432	1.6 ± 1.2	158.4 ± 105.3	1.8 ± 1.2
	Humic	14715 ± 1517	2.6 ± 0.1	500.4 ± 89.7	2.5 ± 0.2
	Clear-vegetated	44151 ± 9931	1.2 ± 0.3	1136.8 ± 401.1	1.0 ± 0.3
	Phytoplankto-turbid	4666 ± 969	1.0 ± 0.1	105.5 ± 3.1	0.4 ± 0.02
Dictyochophyceae					
Pseudopedinella sp.	*Deep clear*	2288 ± 367	3.9 ± 0.3	250.4 ± 63.6	4.4 ± 0.1
	Humic	2576 ± 1072	3.1 ± 0.5	120.9 ± 58	2.9 ± 0.4

CSGR, Cell specific grazing rate; GI, grazing impact; and CR, clearance rate.

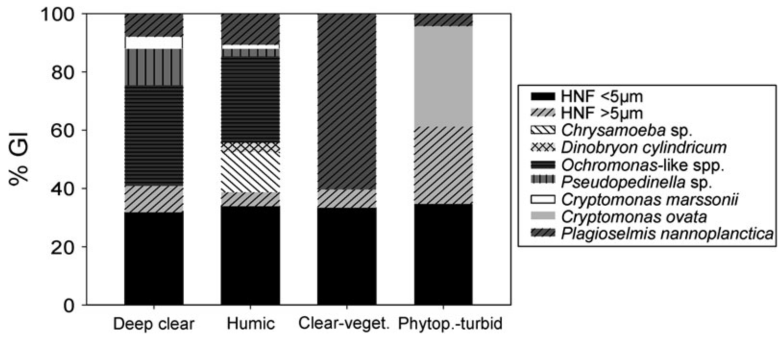

Fig. 4. Contribution of each phagotrophic flagellate taxon to the total GI on the bacterial community in the four water bodies.

impact only in oligotrophic lakes (deep clear and humic). In both systems *Ochromonas*-like cells were the most important grazing algae (on average 53% of the impact among mixotrophs). In the humic lake two other species also contributed: *D. cylindricum* and *Chrysamoeba* sp., respectively accounting for 5% and 22% of the GI within mixotrophs in this lake. The only representative of Dicthyochophyceae, *Pseudopedinella* sp. had a higher impact in the deep clear lake than in the humic one (21 and 4% respectively). In contrast, cryptophytes were the main grazing algae in clear-vegetated and phytoplankton-turbid lakes. *Plagioselmis nannoplanctica* was the only mixotroph-ingesting prey at a measurable rate in the clear-vegetated lake, achieving the highest GI among phagotrophs. *Cryptomonas ovata* had the highest impact in the phytoplankton-turbid lake. It is noteworthy that despite *C. marssonii* exerteing higher CSGR, it did not contribute significantly to the GI due to their low abundances in the oligotrophic systems studied.

DISCUSSION

The two-dimensional ordination of lakes according to autotrophy and allotrophy gradients proposed by Rodhe (Rodhe, 1969), and used by Williamson *et al.* (Williamson *et al.*, 1999), successfully described the heterogeneity of the aquatic environments encompassed in this study. In particular, our results showed a strong correspondence between the trophic conditions of the lakes (in relation to Chl*a* and DOC) and the assemblages of mixotrophic protists occurring.

Implicit in the classification of mixotrophs along a nutritional gradient proposed by Jones (Jones, 1994) is the likelihood that each group is better able to grow in different environments, mainly under contrasting light conditions, DOC content, inorganic nutrients and prey densities. Our findings based on field data are consistent with this model: for all chrysophyte species the percentage of their abundance in total mixotrophic flagellates decreased towards higher trophic states, whereas cryptophytes showed the opposite tendency. Despite their relatively low abundances, species of the Dictyochophyceae (particularly the Pedinellales) also decreased towards higher trophic states, similarly to the chrysophytes. The mathematical model developed by Crane and Grover (Crane and Grover, 2010) supported these patterns; the model predicted that mixotrophs are favoured by nutrient limitation, persisting in environments with low P supply, but only those with a strategy that is mostly autotrophic remain with high nutrient and light supply.

Phagotrophy has been recognized as a relevant functional trait in the ecology of freshwater phytoplankton (e.g. Weithoff, 2003). Phytoplankton functional classifications, such as the most frequently used of Reynolds *et al.* (Reynolds *et al.*, 2002), and updated by Padisák *et al.* (Padisák *et al.*, 2009), provide a good framework to understand the mixotrophic assemblages found in the different types of lakes. Among cryptophytes the codes X2(*Plagioselmis lacustris*) and Y(*Cryptomonas* spp.) comprise respectively small nanoplankters (mostly unicellular $< 10^3$ μm^3 in volume) frequent in meso-eutrophic environments, such as the clear-vegetated lakes investigated in our study, and larger nanoplanktonic flagellates (10^3–10^4 μm^3 in volume) growing in small and enriched lakes, such as phytoplankton-turbid lakes. In Finnish lakes Lepistö and Holopainen (Lepistö and Holopainen, 2003) found a similar tendency, where variation in cell size and abundance of Cryptophyceae species reflected the different trophic status of the lakes. Small cell size and surface/volume ratio are known to be an adaptation to low nutrient availability (Reynolds, 1984) and confer shorter duration of cell division, both appropriate strategies for the development in areas with frequent environmental perturbations, such as clear-vegetated lakes in this study. Among chrysophytes, *Ochromonas*-like were found in relatively high abundances in oligo to mesotrophic lakes, and according to Reynolds *et al.* (Reynolds *et al.*, 2002) they are in the X3 and X2 groups (small nanoplankters from oligotrophic and meso-eutrophic lakes, respectively). Although they are favored in shallow, mixed and clear environments, in our study they were also present in deep clear and humic systems. Difficulties in the taxonomy of this diverse and small genus with no distinctive morphology under the light microscope probably cause loss of information of environmental specific tolerances. *Dinobryon* was another abundant chrysophyte in humic lakes and ponds from Tierra del Fuego. This group has an obligate requirement for light, and subsidizes phosphorus need from bacterial ingestion (Caron *et al.*, 1993). In agreement with classification group "E", these algae develop in oligo to mesotrophic lakes and have a low tolerance to nutrient enrichment, thus probably explaining the absence of this genus in the meso to eutrophic lakes of the Strobel Plateau. On the other hand, the presence of *Dinobryon* species in slightly acid waters was reported by Kristiansen (Kristiansen, 2005). Environmental characteristics of the humic lakes and ponds from Tierra del Fuego, where mosses develop in the littoral zones, are appropiate for them.

In spite of difference in the mixotrophs developed in environments with different trophic states (e.g. chrysophytes dominate oligotrophic lakes and cryptophytes eutrophic ones), all of them attained higher CSGR in oligotrophic lakes, being highest in the humic system. Moreover, mixotrophs accounted for more than half of the total flagellate grazing in oligotrophic and mesotrophic lakes, while their impact decreased in the most eutrophic lake. These results are in accordance with those obtained in other oligotrophic freshwater and marine systems (e.g. Bell and Laybourn-Parry, 2003; Isaksson *et al.*, 1999; Medina-Sánchez *et al.*, 2004; Unrein *et al.*, 2007; Zubkov and Tarran, 2008). These findings are related to the ecological characteristics of the oligotrophic lakes, where low nutrients limit both primary and bacterial production, and thus the strategy of "eating your competitor" encouraged by mixotrophs on heterotrophic bacteria (regardless the type of mixotroph) may be more successful (Thingstad *et al.*, 1996). Particularly, as expected, in the humic lake we found the highest CSGR, as light-restrictive conditions due to allochthonous dissolved humic acids are coupled with oligotrophy and together would trigger heterotrophic nutrition. This result emphasizes the importance of bacterivory in the flux of matter and energy from allochthonous organic carbon to higher trophic levels in this type of humic systems (Jansson *et al.*, 2000). Although the results of our experiments showed clear tendencies, to give more strength to the conclusions, experiments should be developed encompassing more lakes.

The present results also showed that despite a lower grazing rate per individual, a high abundance of smaller flagellates (either mixotrophic or heterotrophic) resulted in significantly higher GI than larger flagellates. The same result was obtained by Unrein *et al.* (2007) in a coastal marine system. As a general rule in natural environments, smaller organisms usually achieve higher abundance than larger ones, which explains the observed pattern. In addition, heterotrophic bacteria probably do not completely fulfill the nutritional demand of larger organisms that might also graze on larger prey (e.g. photosynthetic picoplankton).

The CSGR of chrysophytes measured in our study are in agreement with the results obtained in previous experiments in lakes of Tierra del Fuego (Saad, 2011) and in other oligotrophic systems from the Northern hemisphere (e.g. Hitchman and Jones, 2000; Isaksson *et al.*, 1999; Medina-Sánchez *et al.*, 2004). In the case of cryptophytes, despite their lower abundance in oligotrophic systems, their high CSGR may be interpreted as a way to subsidize nutrients required for growth, as has been reported in other studies (e.g. Izaguirre *et al.*, 2012; Unrein *et al.*, 2014; Urabe *et al.*, 2000). Cryptophytes can also develop phagotrophy under different environmental conditions, such as a survival response during light limitation in Antarctica (e.g. Thurman *et al.*, 2012). It seems that even though this group generally is able to reach high densities in nutrient-enriched environments at the expense of autotrophy, it also presents the plasticity to grow (but with lower abundances) in oligotrophic environments combining autotrophy with phagotrophy; what remains unknown is whether this behavior is species-specific.

All groups of mixotrophic algae investigated in our study originated from secondary endosymbiosis of red algae (Keeling, 2004). The re-adoption of phagotrophy by mixotrophic algae (de Castro *et al.*, 2009) may be suitable for explaining the distribution of the different mixotrophic algae in the lakes studied. The high abundance of "more heterotrophic" mixotrophs in oligotrophic environments and "more autotrophic" ones in eutrophic systems could result from the obligate reduction of cellular biomass due to specialization of predation on small bacteria in oligotrophic systems.

The experiments performed provide information concerning the relative importance of heterotrophic nutrition in the protists analyzed. Nevertheless, it would be enlightening to complement our data with empirical quantification of the photosynthetic rates in different mixotrophs. However, this is notoriously difficult in natural communities, which limit the possibility of identifying the primary cause triggering grazing, i.e. whether it is the acquisition of nutrients required for photosynthesis or carbon input as a complement for photosynthesis in poor light conditions.

CONCLUSIONS

Mixotrophic algae were found in all lakes, whereas the assemblage of mixotrophs inhabiting each one is linked to the trophic status (i.e. Chl*a* and DOC concentration). Thus, primarily autotrophic mixotrophs (e.g. cryptophytes) dominated eutrophic lakes, whereas primarily heterotrophic mixotrophs (e.g. chrysophytes, *Pseudopedinella*) dominated oligotrophic systems. In addition, regardless of the predominant type of nutrition, all mixotrophs displayed higher cell-specific ingestion rates in oligotrophic lakes, thus mixotrophs were responsible for more than 50% of the total flagellates bacterivory in oligo-mesotrophic systems.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

ACKNOWLEDGEMENTS

We thank the help of our colleagues Rodrigo Sinistro, Inés O´Farrell, Romina Schiaffino, Julio Lancelotti, Magdalena Arias and María Cristina Marinone for their collaboration during field campaigns. We are also grateful to the logistic support of the CADIC-CONICET (Centro Austral de Investigaciones Científicas) and Estancia Laguna Verde (Strobel Plateau).

FUNDING

This study was financed by projects of the CONICET, Argentina (PIP 418), and University of Buenos Aires (UBACYT Y018) and FONCYT (PICT 2013-0794).

REFERENCES

Anderson, C. B., Pastur, G. M., Lencinas, M. V., Wallem, P. K., Moorman, M. C. and Rosemond, A. D. (2009) Do introduced North American beavers *Castor canadensis* engineer differently in southern South America? An overview with implications for restoration. *Mammal Rev.*, **39**, 33–52.

Baigún, C. and Marinone, M. C. (1995) Cold-temperate lakes of South America: do they fit northern hemisphere models? *Arch. Hydrobiol.*, **135**, 23–51.

Bell, E. M. and Laybourn-Parry, J. (2003) Mixotrophy in the Antarctic phytoflagellate, *Pyramimonas gelidicola* (Chlorophyta: Prasinophyceae). *J. Phycol.*, **39**, 644–649.

Bergström, A.-K., Jansson, M., Drakare, S. and Blomqvist, P. (2003) Occurrence of mixotrophic flagellates in relation to bacterioplankton production, light regime and availability of inorganic nutrients in unproductive lakes with differing humic contents. *Freshwat. Biol.*, **48**, 868–877.

Boëchat, I. G., Wetthoff, G., Krüger, A., Gücker, B. and Adrian, R. (2007) A biochemical explanation for the success of mixotrophy in the flagellate *Ochromonas* sp. *Limnol. Oceanogr.*, **52**, 1624–1632.

Caron, D. A., Sanders, R. W., Lim, E. L., Marras, C., Amaral, L. A., Whitney, S., Aoki, R. B. and Porter, K. G. (1993) Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cylindricum*. *Microb. Ecol.*, **25**, 93–111.

Crane, K. W. and Grover, J. P. (2010) Coexistence of mixotrophs, autotrophs, and heterotrophs in planktonic microbial communities. *J. Theoret. Biol.*, **262**, 517–527.

de Castro, F., Gaedke, U. and Boenigk, J. (2009) Reverse evolution: driving forces behind the loss of acquired photosynthetic traits. *PLoS One*, **4**, e8465.

Díaz, M., Pedrozo, F. and Baccala, N. (2000) Summer classification of southern hemisphere temperate lakes (Patagonia, Argentina). *Lakes Reserv. Res. Manag.*, **5**, 213–229.

Flynn, K. J., Stoecker, D. K., Mitra, A., Raven, J. A., Glibert, P. M., Hansen, P. J., Granéli, E. and Burkholder, J. M. (2012) Misuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. *J. Plankton Res.*, **35**, 3–11.

Gerea, M., Saad, J. F., Izaguirre, I., Queimaliños, C., Gasol, J. M., Unrein, F. (2016) Presence, abundance and bacterivory of the mixotrophic algae *Pseudopedinella* (dictyochophyceae) in freshwater environments. *Aquat. Microb. Ecol.*, **76**, 219–232.

Hillebrand, H., Claus-Dieter, D., Kirschtel, D., Pollingher, U. and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, **35**, 403–424.

Hitchman, R. B. and Jones, H. L. J. (2000) The role of mixotrophic protists in the population dynamics of the microbial food web in a small artificial pond. *Freshwat. Biol.*, **43**, 231–241.

Isaksson, A., Bergström, A.-K., Blomqvist, P. and Jansson, M. (1999) Bacterial grazing by phagotrophic phytoflagellates in a deep humic lake in northern Sweden. *J. Plankton Res.*, **21**, 247–268.

Iturraspe, R. and Urciuolo, A. (2000) Clasificación y caracterización de las cuencas hídricas de Tierra del Fuego. *Actas XVIII Congreso Nacional Del Agua –Río Hondo*, Santiago Del Estero June-2000.

Izaguirre, I., Sinistro, R., Schiaffino, M. R., Sánchez, M. L., Unrein, F. and Massana, R. (2012) Grazing rates of protists in wetlands under contrasting light conditions due to floating plants. *Aquat. Microb. Ecol.*, **65**, 221–232.

Jansson, M., Bergström, A.-K., Blomqvist, P. and Drakare, S. (2000) Allochtonous organic carbon and phytoplankton/bakterioplankton production relationships in lakes. *Ecology*, **8**, 3250–3255.

Jones, H. L. J. (1997) A classification of mixotrophic protists based on their behaviour. *Freshwat. Biol.*, **37**, 35–43.

Jones, R. I. (1994) Mixotrophy in planktonic protist as a spectrum of nutritional strategies. *Mar. Microb. Food Webs*, **8**, 87–96.

Jones, R. I. (2000) Mixotrophy in planktonic protists: an overview. *Freshwat. Biol.*, **45**, 219–226.

Jones, R. I. and Rees, S. (1994) Incluence of temperature and light on particle ingestion by the freshwater phytoflagellate *Dinobryon*. *Arch. Hydrobiol.*, **132**, 203–211.

Katechakis, A. and Stibor, H. (2006) The mixotroph *Ochromonas tuberculata* may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions. *Oecologia*, **148**, 692–701.

Keeling, P. J. (2004) Diversity and evolutionary history of plastids and their hosts. *Am. J. Bot.*, **91**, 1481–1493.

Kirk, J. T. O. (1994) *Light and Photosynthesis in Aquatic Ecosystems*. 2nd edn. Cambridge University Press, Cambridge, p. 509.

Kristiansen, J. (2005) *The Golden Algae: A Biology of Chrysophyceae*. Koletz Scientific Books, Keoneigestin, p. 167.

Lancelotti, J. L., Pozzi, L. M., Yorio, P. M., Diéguez, M. C. and Pascual, M. A. (2009) Fishless shallow lakes of Southern Patagonia as habitat for waterbirds at the onset of trout aquaculture. *Aquat. Cons. Mar. Freshw. Ecosyst.*, **19**, 497–505.

Lageveen, R. G., Huisman, G. W., Preusting, H., Ketelaar, P., Eggink, G. and Witholt, B. (1988) Formation of polyesters by *Pseudomonas oleovorans*; effect of substrates on formation and composition of poly-(R)-3-hydroxyalkanoates and poly-(R)-3-hydroxyalkenoates. *Appl. Environ. Microb.*, **54**, 2924–2932.

Lepistö, L. and Holopainen, A. L. (2003) Occurrence of cryptophyceae and katablepharids in boreal lakes. *Hydrobiologia*, **502**, 307–314.

Lopez, N. I., Pettinari, M. J., Stackebrandt, E., Tribelli, P. M., Potter, M., Steinbuchel, A. and Mendez, B. S. (2009) *Pseudomonas extremaustralis* sp. nov., a poly(3-hydroxybutyrate) producer isolated from an Antarctic environment. *Curr. Microbiol.*, **59**, 514–519.

Marker, A. F. H., Nusch, A., Rai, H. and Reimann, B. (1980) The measurement of photosynthetic pigments in freshwater and standardization of methods: conclusions and recommendations. *Arch. Hydrobiol. Beih. Ergeb. Limnol.*, **14**, 91–106.

Medina-Sánchez, J. M., Villar-argaiz, M. and Carrillo, P. (2004) Neither with nor without you: a complex algal control on bacterioplankton in a high mountain lake. *Limnol. Oceanogr.*, **49**, 1722–1733.

McKie-Krisberg, Z. M. and Sanders, R. W. (2014) Phagotrophy by the picoeukaryotic green alga *Micromonas*: implications for Arctic Oceans. *ISME J.*, **8**: 1953–1961.

McKie-Krisberg, Z. M., Gast, R. J., Sanders, R. W. (2015) Physiological responses of three species of Antarctic mixotrophic phytoflagellates to changes in light and dissolved nutrients. *Microb. Ecol.*, **70**, 21–29.

Mitra, A., Flynn, K. J., Burkholder, J. M., Berge, T., Calbet, A., Raven, J. A., Granéli, E., Glibert, P. M., *et al.* (2014) The role of mixotrophic protists in the biological carbon pump. *Biogeosciences*, **11**, 995–1005.

Modenutti, B., Albariño, R., Bastidas Navarro, M., Díaz Villanueva, V., Souza, M. S., Trochine, C., Laspoumaderes, C., Cuassolo, F., *et al.* (2010) Structure and dynamic of food webs in Andean North Patagonian freshwater systems: organic matter, light and nutrient relationships. *Ecología Austral*, **20**, 95–114.

Padisák, J., Crossetti, L. O. and Naselli-Flores, L. (2009) Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. *Hydrobiologia*, **621**, 1–19.

Pålsson, C. and Daniel, C. (2004) Effects of prey abundance and light intensity on nutrition of a mixotrophic flagellate and its competitive relationship with an obligate heterotroph. *Aquat. Microb. Ecol.*, **36**, 247–256.

Panza, J. L. and Franchi, M. R. (2002) Magmatismo Basáltico Cenozoico Extrandino. In Haller M.J. (ed.), *Geología y Recursos Naturales de Santa Cruz. Relatorio del XV congreso Geológico Argentino.* El Calafate, 201:236.

Pérez, G. L., Torremorell, A., Bustingorry, J., Escaray, R., Pérez, P., Diéguez, M. and Zagarese, H. (2010) Optical characteristics of shallow lakes from the Pampa and Patagonia regions of Argentina. *Limnologica*, **40**, 30–39.

Porter, K. G. and Feig, Y. S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943–948.

Quirós, R. and Drago, E. (1985) Relaciones entre variables fisicas, morfometricas y climaticas en lagos patagonicos. *Rev. Asoc. Cienc. Nat. Litor.*, **16**, 181–199.

Raven, J. A. (1997) Phagotrophy in phototrophs. *Limnol.Oceanogr.*, **42**, 198–205.

Raven, J. A., Beardall, J., Flynn, K. J. and Maberly, S. C. (2009) Phagotrophy in the origins of photosynthesis in eukaryotes and as a complementary mode of nutrition in phototrophs: relation to Darwin's insectivorous plants. *J. Exper. Bot.*, **60**, 3975–3987.

Reynolds, C. S. (1984) The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge.

Reynolds, C. S., Huszar, V., Kruk, C., Naselli-Flores, L. and Melo, S. (2002) Towards a functional classification of the freshwater phytoplankton. *J. Plankton Res.*, **24**, 417–428.

Rodhe, W. (1969) Crystallization of eutrophication concepts in Northern Europe. In Eutrophication: Causes, Consequences, Correctives. *Natl. Acad. Sci. Publi.*, **1700**, 50–64.

Rottberger, J., Gruber, A., Boenigk, J., Kroth, P. G. (2013) Influence of nutrients and light on autotrophic, mixotrophic and heterotrophic freshwater chrysophytes. *Aquat. Microb. Ecol.*, **71**, 179–191.

Saad, J. F. (2011) Impacto de depredación de protistas fagotróficos y su relación con la producción primaria y bacteriana en un lago oligotrófico y uno distrófico de Tierra del Fuego (Argentina). Bachellor Thesis. University of Buenos Aires, 45 pp.

Saad, J. F., Schiaffino, M. R., Vinocur, A., O'Farrell, I., Tell, G. and Izaguirre, I. (2013) Microbial planktonic communities of freshwater environments from Tierra del Fuego: dominant trophic strategies in lakes with contrasting features. *J. Plankton Res.*, **35**, 1220–1233.

Sharp, J. H., Peltzer, E. T., Alperin, M. J., Cauwet, G., Farrington, J. W., Fry, B., Karl, D. M., Martin, J. H., *et al.* (1993) Procedures subgroup report. *Mar. Chem.*, **41**, 37–49.

Sherr, B. F., Sherr, E. B. and Fallon, R. D. (1987) Use of monodispersed, fluorescently labeled bacteria to estimate in situ protozoan bacterivory. *Appl. Environ. Microb.*, **53**, 958–965.

Šimek, K., BobkováJ., Macek, M., Nedoma, J. and Psenner, R. (1995) Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. *Limnol. Oceanogr.*, **40**, 1077–1090.

Stoecker, D. K. (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *Europ. J. Protistol.*, **34**, 281–290.

Thingstad, T. F., Havskum, H., Garde, K. and Riemann, B. (1996) On the strategy of "Eating Your Competitor": a mathematical analysis of algal mixotrophy. *Ecology*, **77**, 2108–2118.

Thurman, J., Parry, J., Hill, P. J., Priscu, J. C., Vick, T. J., Chiuchiolo, A. and Laybourn-Parry, J. (2012) Microbial dynamics and flagellate grazing during transition to winter in Lakes Hoare and Bonney, Antarctica. *FEMS. Microbiol. Ecol.*, **82**, 449–458.

Tittel, J., Bissinger, V., Zippel, B., Gaedke, U., Bell, E., Lorke, A. and Kamjunke, N. (2003) Mixotrophs combine resource use to outcompete specialists: implications for aquatic food webs. *PNAS*, **100**, 12776–12781.

Tranvik, L. J., Porter, K. G. and Sieburth, J. M. N. (1989) Occurrence of bacterivory in *Cryptomonas*, a common freshwater phytoplankter. *Oecologia*, **78**, 473–476.

Troost, T. A., Kooi, B. W. and Kooijman, S. A. L. M. (2005) When do mixotrophs specialize? Adaptive dynamics theory applied to a dynamic energy budget model. *Math. Biosci.*, **193**, 159–182.

Unrein, F., Massana, R., Alonso-Sáez, L. and Gasol, J. M. (2007) Significant year-round effect of small mixotrophic flagellates on bacterioplankton in an oligotrophic coastal system. *Limnol. Oceanogr.*, **52**, 456–469.

Unrein, F.,Gasol, J. M., Not, F., Forn, I. and Massana, R. (2014) Mixotrophic haptophytes are key bacterial grazers in oligotrophic coastal waters. *ISME J.*, **8**,164–176.

Urabe, J., Gurung, T. B., Yoshida, T., Sekino, T., Nakanishi, M., Maruo, M. and Nakayama, E. (2000) Diel changes in phagotrophy by *Cryptomonas* in Lake Biwa. *Limnol. Oceanogr.*, **45**, 1558–1563.

Utermöhl, H. (1958) Zur Vervollkomnung der quantitativen Phytoplankton-methodik. *Mitt. Int. Ver. Theor. Amgewan. Limnol.*, **9**, 1–38.

Venrick, E. L. (1978) How many cells to count? In Sournia, A. (ed.), *Phytoplankton Manual.* UNESCO, Paris, France.

Wan, M. X., Liu, P., Xia, J. L., Rosenberg, J. N., Oyler, G. A., Betenbaugh, M. J., Nie, Z. Y. and Qiu, G. Z. (2011) The effect of mixotrophy on microalgal growth, lipid content, and expression levels of three pathway genes in *Chlorella sorokiniana*. *Appl. Microbiol. Biotech.*, **91**, 835–844.

Weithoff, G. (2003) The concepts of 'plant functional types' and 'functional diversity' in lake phytoplankton- a new understanding of phytoplankton ecology?. *Freshwat. Biol.*, **48**, 1669–1675.

Wilken, S., Schuurmans, J. M. and Matthijs, H. C. P. (2014) Do mixotrophs grow as photoheterotrophs? Photophysiological acclimation of the chrysophyte *Ochromonas danica* after feeding. *New. Phytol.*, **204**, 882–889.

Williamson, C. E., Morris, D. P., Pace, M. L. and Olson, O. G.(1999) Dissolved organic carbon and nutrients as regulators of lake ecosystems: resurrection of a more integrated paradigm. *Limnol. Oceanogr.*, **44**, 795–803.

Zubkov, M. V. and Tarran, G. A. (2008) High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. *Nature* **455**, 224–226.