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The role of phytoplanktonic size fractions in the microbial food webs in two north Patagonian lakes (Argentina)

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Introduction

A current concept concerning organic matter dynamics within pelagic food webs consists of two processes: the traditional food web (nutrients – phytoplankton – zooplankton – fish), which considers that the phytoplankton is the dominant 'producer', and the microbial loop (AZAM et al. 1983), which implies that a portion of matter and energy flows through unicellular organisms, such as bacteria, to mixotrophic and heterotrophic protists, bearing in mind that several phytoplanktonic organisms can also be consumers. Based on these two concepts, the small-sized fractions of phytoplanktonic communities play fundamental roles in the pelagic trophic interactions. In particular, in oligotrophic waters the autotrophic picoplankton can dominate the phytoplanktonic biomass and production (STOCKNER & ANTIA 1986, STOCKNER 1988), and at the same time, together with bacteria, constitute the prey for the protistan assemblage. The nanoplanktonic size fraction, to which most of the mixotrophic protists belong, also represents the food for herbivorous zooplanktonic organisms.

Although the larger phytoplanktonic fraction (microphytoplankton, >20 µm) is not usually included in the microbial food webs, some species of the colonial *Dinobryon* must be also considered as mixotrophic, since they can be bacterivorous (BIRD & KALFF 1986, CARON et al. 1993, JONES 1994). In contrast, several dinoflagellate species are also considered as mixotrophic, but as they consume larger prey, such as small algae and ciliates, they are only indirectly linked with the microbial webs (BOCKSTAHLER & COATS 1993, JACOBSON & ANDERSON 1996, HAVSKUM & HANSEN 1997, SANDERS et al. 2000).

In Andean Patagonic lakes, which have differing limnological features, such as DOC concentrations, light climate and thermal regime, the phytoplankton community consisted of many genera, such as *Chrysochromulina*, *Ochromonas*, *Dinobryon*, *Gymnodinium*, *Peridinium*, *Cryptomonas* and *Rhodomonas* (QUEIMALIÑOS 1993, QUEIMALIÑOS et al. 1999, MODENUTTI et al. 2000) that have been described as mixotrophic in the literature (SANDERS & PORTER 1988, TRANVIK et al. 1989, BORAAS et al. 1992, JONES 1994). Therefore, the aim of this study was to determine the relative importance of these taxa in the phytoplankton of two North Patagonian lakes of different trophic conditions, and, by performing grazing experiments, to determine if either of them are mixotrophic.

Methods

Study site and field sampling

Two lakes with contrasting limnological properties were selected for this study. Lake Moreno Oeste (41° 5' S, 71° 33' W; 758 m a.s.l.) and Lake Morenito (41° 3' S, 71° 30' W; 758 m a.s.l.) are adjacent environments that belong to the Nahuel Huapi system (Patagonia, Argentina).

Lake Moreno Oeste (area, 6 km^2, z_{max} = 90 m) is a warm monomictic deep and ultraoligotrophic lake, with a marked thermocline at around 30-m depth, and temperatures between 15 and 17 °C in the epilimnion, and 7 °C in the hypolimnion. The epilimnetic chlorophyll *a* concentration was lower than 1 µg L^{-1}, and total phosphorus remained between 2 and 4 µg L^{-1}, while dissolved organic carbon concentration was ≤0.6 mg L^{-1} (QUEIMALIÑOS et al. 1999, MODENUTTI et al. 2000). In contrast, Lake Morenito (area, 0.83 km^2, z_{max} = 12 m) is shallow and continuously cold polymictic (since it occasionally freezes), and during the study period the temperature was always homogeneously distributed, varying from 15 to 21 °C. In addition, this lake had higher concentrations of chlorophyll *a*, TP and DOC (~5 µg L^{-1}, 12 µg L^{-1} and 2 mg L^{-1}, respectively) (MODENUTTI et al. 2000), indicating a different trophic condition to that of Lake Moreno Oeste.

The samples were obtained on eight dates between December 1998 and April 1999 at the central point of both lakes, from 0 to 52 m at 4-m intervals in

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Lake Moreno Oeste, and from 0 to 8 m at 2-m intervals in Lake Morenito.

Samples for bacterial enumeration were fixed and stained with 4',6-diamidino-2-phenylindole (DAPI, final concentration 0.2% w/v) according to PORTER & FEIG (1980). Samples for autotrophic picoplankton were fixed with formaldehyde–cacodylate. Both bacteria and picoplankton were quantified on 0.2-µm black membrane filters (Poretics) at 1000× magnification on an Olympus BX50 epifluorescence microscope, using UV light (U-MWU filter) and blue light (U-MWB filter), respectively.

Phytoplankton samples were fixed with Lugol's solution and were counted with an inverted microscope using 50-mL Utermöhl chambers. The nanoplanktonic cells (GALD <20 µm) were quantified by scanning transects at 400×, while microphytoplankton was enumerated by scanning the entire chamber surface at 100×.

Grazing experiments

Four series of in situ grazing experiments were performed on November 28, December 18 and January 12. Three of them were carried out in Lake Moreno Oeste, while the other one took place in Lake Morenito. In these experiments, fluorescently labeled bacteria (FLB) were used, and were prepared following the methods of SIMEK & STRASKRABOVA (1992). The bacteria were concentrated by filtering water from each lake through 2-µm pore size filters, then through 0.22-µm filters, and were loosened from the filters by sonication. Afterwards, the bacteria were killed at 60 °C and stained with 5-[(4,6-dichlorotriazin-2-yl) amino] fluorescein according to the methods of SHERR et al. (1987), and frozen in aliquots.

The experimental design consisted of an in situ incubation of one set with two replicates. Samples of 250 mL were dispensed in 300-mL glass-stoppered flasks. After an acclimatization of 20 min, FLB were added to the two flasks at a final concentration of 1.02×10^5 mL^{-1} (equivalent to 6% of total bacterial abundance), followed by gentle mixing. After 5, 10, 15, 20 and 40 min of incubation, 40-mL subsamples were obtained from each flask, and fixed with 0.5% of Lugol's solution, 2% of formaldehyde and several drops of 3% sodium thiosulphate, according to SHERR & SHERR (1993). Following this, 40-mL samples were stained with DAPI and filtered through 1-µm black polycarbonate filters (Poretics), in order to determine the number of ingested prey. This enumeration was performed within 3 days after fixation, under an epifluorescence microscope (Olympus BX50). The uptake or clearance rates were obtained by calculating a regression of the linear portion of the uptake curve, which was attained by plotting the ratio of prey algal cell^{-1} versus time. The clearance rates per cell per hour were estimated by dividing these clearance rates by the concentration of FLB µL^{-1}. When these values were multiplied by the in situ abundance of the mixotrophic species mL^{-1}, the result was the assemblage clearance rate.

Results and discussion

During the studied summer period, the nanoplankton was always more abundant than the microphytoplankton in both lakes (Figs. 1A and B). In the ultraoligotrophic lake, densities fluctuated between 60 cells mL^{-1} in February and March 1999, and 1200 cells mL^{-1} in December 1998 (Fig. 1A), and it was always dominated by *Chrysochromulina parva* Lackey, which generally contributed more than 70% to total phytoplankton abundance. This species, which was noted for the first time in South America by DÍAZ & LORENZO (1990), is the most significant member of the nanoplanktonic fraction in North Patagonian lakes (QUEIMALIÑOS 1993, 1997, MODENUTTI et al. 1998, QUEIMALIÑOS et al. 1999). Other species that constituted the nanoplanktonic assemblage in the deep lake were *Rhodomonas lacustris* (Pascher & Ruttner) Javornicky and *Gymnodinium* aff. *varians* Maskell. It was observed that the cryptophycean clearly preferred deep strata, since its maximum densities were found at 30- to 40-m depth, in agreement with the cryptophyceans' low-light requirements (KLAVENESS 1990). On the other hand, the microphytoplankton remained at very low densities, and mainly consisted of dinoflagellates, such as *Gymnodinium paradoxum* Schilling, *G. uberrimum* (Allman) Kofoid & Sweizy and *Peridinium* sp.

In Lake Morenito, the nanoplanktonic abundance ranged between 300 and 2850 cells mL^{-1}, with maximum values in January (Fig. 1B), almost duplicating those obtained in Lake Moreno Oeste. In the shallow lake, *C. parva* also dominated the nanoplanktonic assemblage but only in January, because on the other sampling dates it co-dominated with *Rhodomonas lacustris*. Moreover, two other cryptophycean species, *Cryptomonas marssonii* and *C. erosa*, were found in this lake, but their contribution

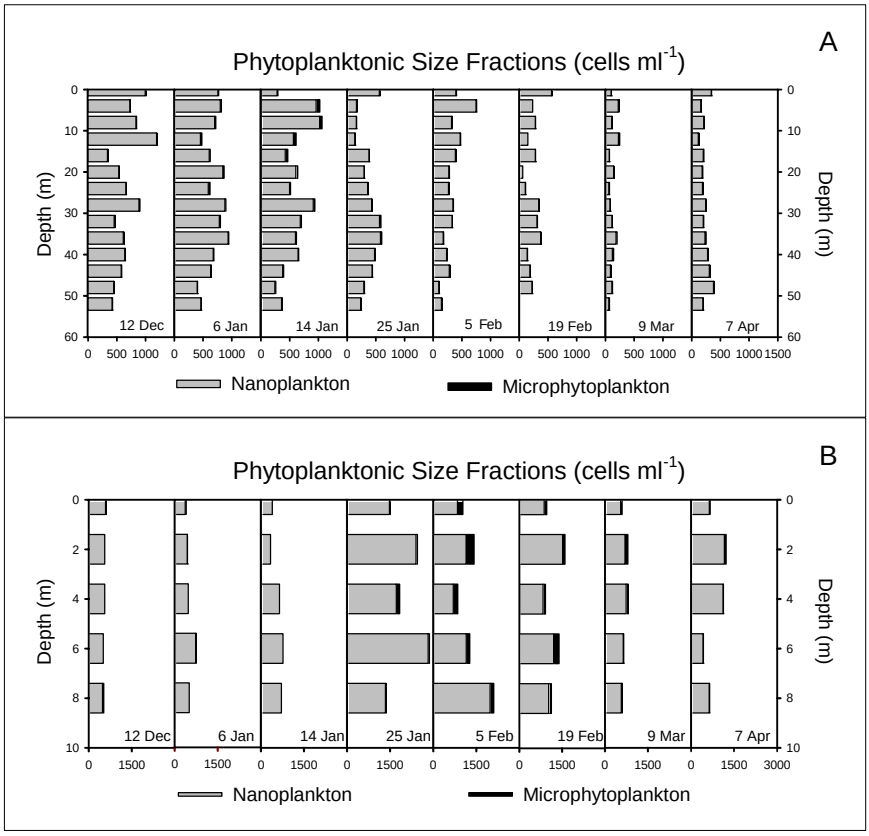

Fig. 1. Nanoplankton and microphytoplankton vertical distributions between December 1998 and April 1999 in (A) Lake Moreno Oeste, and (B) Lake Morenito.

to the total phytoplanktonic abundance was always low (0–16%). With regard to the microphytoplankton, this lake was always dominated by two chrysophycean species, *Dinobryon divergens* Imhof and *D. sertularia* Ehrenb. Although these two species were also present in Lake Moreno Oeste, they never dominated the larger assemblage.

The vertical distributions of bacterial and picoplanktonic organisms were quantified in order to estimate the in situ prey abundance for the mixotrophic algae. The bacterial densities were homogeneously distributed all along the water column in both lakes, ranging from 0.7×10^6 to 2.4×10^6 cells mL^{-1} in Lake Moreno Oeste, and double this in Lake Morenito. This higher abundance could be attributed to the higher DOC concentration in this shallow lake. In Lake Moreno Oeste, the picoplanktonic abundance ranged from 0.1×10^5 to 1.8×10^5 cells mL^{-1} during the entire studied period, and presented a heterogeneous vertical pattern in January and February, with maximum values at the limit of the euphotic zone, where light incidence was 1% of the surface irradiance. In Lake Morenito, this small-sized fraction was always homogeneously distributed along the water column, probably due to its constant mixing, and was more abundant than in Lake Moreno Oeste, with the greatest difference during early January when it reached 6×10^5 cells mL^{-1}. These higher densities are attributed to the higher trophic condition of Lake Morenito

Table 1. Rates obtained during the grazing experiments carried out in Lake Moreno Oeste in November and December 2000, and in Lake Morenito in January 2001.

Experiment date	Lake	FLP uptake rate (FLP cell^{-1} h^{-1})	Clearance rate (nL cell^{-1} h^{-1})	Assemblage clearance rate (day^{-1})
November 2000	Moreno Oeste	0.890	8.728	0.078
December 2000	Moreno Oeste	0.370	2.055	0.018
January 2001	Morenito	0.400	3.924	0.120

(MODENUTTI et al. 2000).

The results obtained in the grazing experiments revealed that several phytoplanktonic species present in the studied lakes were mixotrophic. Considering that the offered prey was bacterial sized, only the bacterivorous algae were recognized. These species are *Chrysochromulina parva*, *Rhodomonas lacustris*, *Gymnodinium* aff. *varians*, *Cryptomonas marssoni*, and *Dinobryon divergens*. They belong to the nanoplanktonic fraction, with the exception of *Dinobryon divergens*, which is microphytoplanktonic.

Chrysochromulina parva was always found with only one ingested prey, and was responsible for almost 40% of the total ingestion activity in Lake Moreno Oeste. This bacterivorous behavior has been cited in several marine species of this genus (JONES et al. 1994), such as *C. hirta* (KAWACHI et al. 1991). Although most species of dinoflagellates have been described as ingesting larger food items, such as ciliates (BOCKSTAHLER & COATS 1993, JACOBSON & ANDERSON 1996, HAVSKUM & HANSEN 1997), in the present study this small-sized *Gymnodinium* aff. *varians* was found to eat bacteria. Phagotrophy in *Cryptomonas* has been noted by PORTER et al. (1985) and TRANVIK et al. (1989), and in this study *C. marssonii* showed bacterivory.

In Lake Moreno Oeste, *Rhodomonas lacustris* developed its maximum densities in low light conditions. Having observed its phagotrophic behavior, this manifestation can be explained by the presence of picoplanktonic peaks at the same depths.

The phagotrophic ability of the genus *Dinobryon* to ingest bacteria was noted several years ago by BIRD & KALFF (1986), and later JONES & REES (1994) described high clearance rates for *D. divergens*.

The clearance rates obtained, considering all the mixotrophic assemblage, showed maximum values at 15 min of incubation. Although more grazing experiments are necessary to analyze the seasonal fluctuations in the clearance rates of these mixotrophs, these first results (Table 1) are comparable to the considered representative value for marine mixotrophic species, 2.6 nL mixotroph^{-1} h^{-1} (SAFI & HALL 1999). This similarity is probably related to the oligotrophic conditions of both studied lakes, since one potential advantage of mixotrophy is the acquisition of nitrogen and phosphorus from particulate food when concentrations of dissolved nutrients are low (JONES 1997, SANDERS et al. 2000).

Considering the results obtained in the grazing experiments together with the importance of the nanoplanktonic fraction in these two North Patagonian lakes and the dominance of the genus *Dinobryon* in the microphytoplankton of Lake Morenito, it can be concluded that mixotrophy is a prevalent physiological behavioral trait in these ecosystems.

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