

Light versus food supply as factors modulating niche partitioning in two pelagic mixotrophic ciliates

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Abstract

We studied the vertical distribution of two mixotrophic ciliates, *Ophrydium naumanni* and *Stentor araucanus* in two contrasting summer seasons (strong vs. mild windy years) to determine whether differences in vertical mixing affect their success by changes in light availability for their endosymbiotic algae. Field experiments were done to evaluate the effect of light climate on the photosynthetic efficiency. To test for a potential overlap in food niche we studied prokaryote abundance and examined food vacuole contents of the ciliates, using the catalyzed reported deposition–fluorescence in situ hybridization (CARD-FISH). *S. araucanus*, a species resistant to ultraviolet radiation, was present in the epilimnion. In contrast, *O. naumanni* preferred the metalimnetic layers and was more abundant in years with deeper thermoclines. Variation in the diffuse extinction coefficient was significantly correlated with *S. araucanus* abundance, suggesting a shading effect of this dark ciliate. The expected extinction coefficient due to *Stentor*, measured by spectrophotometric analyses, did not differ from that observed in nature. *O. naumanni* was found to be photosynthetically efficient at low light intensities and susceptible to photoinhibition at epilimnetic light irradiances. Conversely, *S. araucanus* needed a high light supply to maintain endosymbiotic algal photosynthesis and was favored during years of relatively shallow thermocline and high epilimnetic mean irradiance. CARD-FISH analysis revealed that *O. naumanni* fed on *Archea*, *Eubacteria*, and picocyanobacteria. In contrast, *S. araucanus* did not feed on prokaryotes. Based on these results, we suggest that light climate, created by temporal or spatial variations in thermocline depth, is a key factor modulating niche partitioning for mixotrophic ciliate species.

Nutrients, mixing intensity, mixing depth, and light are environmental factors that have a large effect on the ecological dynamics of plankton. Although plankton communities are generally suspended in a well-mixed medium, a vertical light gradient is present even in the most well-mixed body of water (Passarge and Huisman 2002). Therefore, within aquatic ecosystems, light may have a complex pattern of spatial and temporal variability (Litchman 2003; Stomp et al. 2007), and fluctuation in irradiance may affect photosynthesis and respiration

(Quéguiner and Legendre 1986; Ferris and Christian 1991; Falkowski and Raven 1997) as well as growth rates (Litchman 2000, 2003). In temperate lakes, summer stratification is characterized by a wind-mixed surface layer that is isolated from colder deep waters by a marked thermal discontinuity at the metalimnetic level. Wind action is important in determining mixing depth; therefore, a lake's epilimnion can undergo periods of heating during hot and calm weather and periods of strong mixing by wind. Vertical mixing can lead to a shortage of light if planktonic organisms are frequently mixed down to the bottom, whereas stratification enhances light supply by decreasing mixing depth (Diehl 2002). Thus, temporal heterogeneity in vertical mixing can affect the diversity and composition of phytoplankton communities (Diehl et al. 2002).

Planktonic mixotrophic ciliates have a mixed nutrition, which combines autotrophic and heterotrophic nutrition in a single individual. These autotrophic and heterotrophic abilities enable mixotrophs to completely recycle matter and nutrients inside a single cell (Kooijman et al. 2004; Troost et al. 2005). In mixotrophic ciliates, the photosynthetic capability can be acquired by sequestering chloroplasts from ingested prey or by acquiring photosynthetic cells as endosymbionts (Reisser et al. 1985); thus, mixotrophic ciliate species can be affected by changes in light availability. Because mixotrophic ciliates can also eat either bacteria or other protists (Jürgens and Simek 2000; Kisand

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and Zingel 2000; Simek et al. 2000), niche overlap in mixotrophic ciliates will be mainly based on light and prey availability. At present little is known about the competitive interactions among mixotrophs in nature, although some experiments have studied the interactions between mixotrophs and either obligate heterotrophs or autotrophs (Rothhaupt 1997; Pálsson and Daniel 2004; Katechakis et al. 2005).

Andean large deep lakes in North Patagonia are extremely transparent, have very low diffuse attenuation coefficients of photosynthetically active radiation (K_d PAR), and have large euphotic zones reaching ~50-m depth (Pérez et al. 2002). During summer thermal stratification, these lakes have extended mixed layers that are caused by strong western Patagonian winds that reach speeds of >43 km h⁻¹ on a daily basis for up to 41% of the year (Baigún and Marinone 1995). Interannual variability in wind speed may produce changes in the summer thermocline depth and consequently in the epilimnetic mean irradiance. Different regimes of temporal variability in light supply can lead to changes in the phytoplankton community composition (Litchman 1998; Huisman et al. 1999; Diehl et al. 2002). However, planktonic photosynthetic biomass of Andean deep lakes is dominated by two mixotrophic ciliates species (*Ophrydium naumannii* Pejler and *Stentor araucanus* Foissner and Woelfl) living autotrophically with endosymbiotic algae (Queimaliños et al. 1999; Woelfl and Geller 2002). Although these two species are present throughout the euphotic zone, their relative abundances are differentially distributed with depth in the water column (Modenutti et al. 2004, 2005). Size, shape, and biovolume of the two mixotrophic ciliates differ greatly. *S. araucanus* is a large conical ciliate (180 ± 17 μ m in diameter and 260 ± 23 μ m in length, mean \pm SE, $n = 100$) that appears as dark dots to the naked eye because of the presence of dark blue-green cortical granules (Foissner and Woelfl 1994). *O. naumannii* is an elongate tubular cell (fully extended specimens, 98 ± 17 μ m in length and 22 ± 3 μ m in width, mean \pm SE, $n = 100$) with an overall green tinge due to zoochlorellae in the zooids. Higher *S. araucanus* abundances in the epilimnion may imply that such a large and dark cell would have a shading effect on the lower levels of the water column. Moreover, we observed that ciliates can alternate their relative abundances giving the appearance of “*Stentor* years” and “*Ophrydium* years.” We argue that the temporal heterogeneity in vertical mixing would affect these mixotrophic ciliate species by changing light availability for their endosymbiotic algae.

Since these ciliates are heterotrophic, we also explored the potential overlap in their food niche. Previous work showed that *O. naumannii* preys on particles <1 μ m (Modenutti and Balseiro 2002), and this fraction in Andean lakes was composed almost exclusively of picoprokaryotes (Callieri et al. 2007). In the larger species *Ophrydium versatile* it has been clearly determined that the beating cilia of the buccal apparatus draw bacteria and only occasionally small eukaryotic cells into the buccal cavity (Goff and Stein 1981). *S. araucanus* was observed to feed on large (>2 μ m) eukaryotic cells (Modenutti pers. obs.), and

Foissner and Woelfl (1994) reported that it also preys on long bacterial rods. Considering these statements, the potential overlap of *O. naumannii* and *S. araucanus* food niches would be mainly based on picoprokaryotes.

In order to clarify these possible reasons for niche partitioning, we examined the populations of both mixotrophic ciliates: the peritrich *O. naumannii* and the heterotrich *S. araucanus* during two contrasting summer seasons (strong windy vs. mild windy years). The variation in ciliate abundances was analyzed in relation to thermocline depth and light intensity. We studied the vertical distribution and, with field experiments, the photosynthetic efficiency of the two mixotrophic ciliates to evaluate the possibility of a niche partitioning along the water column. We also considered picoprokaryotes as a possible prey group for food niche overlap. Therefore, we studied the vertical distribution of prokaryotes and we characterized the populations present in Lake Moreno by catalyzed reporter deposition–fluorescence in situ hybridization (CARD-FISH). By the same method we compared the food vacuoles content of the two mixotrophic ciliates in order to assess differences in the type of ingested prey (Medina-Sánchez et al. 2005).

Methods

Study area—Lake Moreno Oeste ($41^{\circ}5'S$ and $71^{\circ}33'W$, 758 m above sea level) is located within Nahuel Huapi National Park (Patagonia, Argentina). Lake surface area is 6 km², and maximum depth is 90 m. The thermal regime is warm monomictic, remaining stratified from late November until April (spring–summer months) (Queimaliños et al. 1999; Modenutti et al. 2000). Lake Moreno is ultraoligotrophic, with low dissolved organic carbon concentration (0.46 mg L⁻¹) and high photosynthetically active radiation (PAR) and ultraviolet radiation (UVR) transparency (K_d PAR, 0.14 m⁻¹ and K_d 305, 0.82 m⁻¹) (Morris et al. 1995). The euphotic zone extends up to 35-m depth, and blue-green light prevails in deep waters (Pérez et al. 2002). Epilimnetic chlorophyll *a* (Chl *a*) concentrations are low, less than 1 μ g L⁻¹; however, Chl *a* increases to ~ 2 μ g L⁻¹ at the metalimnion creating a deep chlorophyll maximum at the metalimnion (Queimaliños et al. 1999). Total phosphorus varies between 2 and 4 μ g L⁻¹, and neither nutrient concentrations nor oxyclines were observed (Modenutti et al. 2000).

Field work—Plankton was sampled during the summer 1998–1999 (November–March) and 2003–2004 (December–February) approximately every 2 weeks. Temperature and light vertical profiles (0 to 50 m) of UV bands (305, 320, 340, and 380 nm) and PAR (400–700 nm) were measured with a PUV 500B submersible radiometer (Biospherical Instruments). Water samples were obtained from a central sampling point located at the deepest part of Lake Moreno Oeste. All sampling was carried out at midday, 1 h before astronomical noon. Samples were obtained with a 2-liter Ruttner bottle collected from 0 to 52 m at 4-m intervals (1998–1999) and at 0-, 5-, 10-, 15-, 20-, 30-, and 40-m depth during the summer 2003–2004. A volume of 250 mL was

sampled for ciliate enumeration and 60 mL for total bacteria quantification. Ciliate samples were preserved in acid Lugol's iodine solution (2% v/v). Bacteria samples were preserved adding filtered (0.2 μm) formaldehyde solution (2% v/v).

In the summer of 2004 we also sampled Lake Moreno on three occasions (January, February, and March) at 5 m (epilimnion) and 25- or 35-m depth (metalimnion) to identify the prokaryotic assemblage and visualize labeled prey inside the food vacuoles of the mixotrophic ciliates, using the CARD-FISH method. Samples for ciliates were immediately fixed with 0.5% of Lugol solution, formaldehyde (final concentration 2% v/v), and drops of 3% sodium thiosulfate (Sherr and Sherr 1993); this preservation protocol was only used for food vacuoles examination. Samples for bacteria were processed as mentioned.

Laboratory determinations—Light absorption by *Stentor* was measured directly with a double beam spectrophotometer Shimadzu UV2450. Individuals of *Stentor* at known concentrations were placed in filtered lake water (0.2 μm) in 1-cm quartz cuvettes, and the mean absorption from 400 to 700 nm was measured against filtered (0.2 μm) lake water as blank. Six different scans were run for assessing enough replicates for the estimation.

Enumeration of ciliates was performed following the Utermöhl technique with an inverted microscope (Olympus IX70) using 50-mL Utermöhl chambers and was carried out by scanning the entire surface of the chamber at $\times 200$ magnification. Ciliate identification was performed following Foissner and Woelfl (1994) and Foissner et al. (1999).

Total bacteria enumeration was performed by staining with fluorochrome 4', 6-diamidino-2-phenylindole (DAPI; final concentration 2% v/v) according to Porter and Feig (1980). Counting was performed on polycarbonate black membrane filters (0.2- μm pore size) at $\times 1,250$ magnification in an Olympus BX50 epifluorescence microscope using ultraviolet (UV) light (U-MWU filter). A minimum of 1,000 bacteria per sample were counted and processed with an image analysis system (Image ProPlus; Media Cybernetics).

Photosynthetic efficiency—Photosynthetic efficiency was calculated for each ciliate species based on the determination of Chl *a*, net primary production, and PAR irradiances at different incubation depths. For individual Chl *a* content at each sampling date, six groups of 100 *O. naumannii* and 100 *S. araucanus* were carefully picked up with a micropipette under a stereomicroscope, rinsed in 0.2- μm filtered lake water, and placed on a GF/F filter. Samples were extracted with hot ethanol (Nusch 1980) and measured with a 10-AU fluorometer (Turner Designs). To measure individual primary production, we conducted field incubation experiments. Net primary production (NPP) was measured using the ^{14}C technique (1.22 kBq $\text{NaH}^{14}\text{CO}_3$ mL^{-1}) (Steeman Nielsen 1951). Dark bottle measurements were substituted by the "time 0" organic ^{14}C measurement by adding the isotope to the dark bottle and immediately filtering and analyzing (Fahnenstiel et al. 1994). Incubations were carried out in 20-mL quartz tubes held in

a frame, within the euphotic zone at the depth where each ciliate species develops. *O. naumannii* incubations were performed at 5- and 10-m (epilimnion) and 30-m depth (metalimnion). *S. araucanus* incubations were carried out at 0.30-, 10-, and 20-m depth. Radiation was measured at each incubation depth with the PUV 500B submersible radiometer. The protist species in each vial (40 *O. naumannii* and 20 *S. araucanus* in 0.2- μm filtered lake water) were placed in four replicates each.

CARD-FISH for the characterization of prokaryotic assemblage of Lake Moreno—CARD-FISH was performed using horseradish peroxidase-labeled probes and tyramide signal amplification as described by Pernthaler et al. (2002). Water samples of 10 mL were filtered through 0.2- μm Nuclepore polycarbonate filters (25-mm diameter). Filters were then rinsed twice with deionized water, embedded in low-gelling-point agarose, allowed to air dry, and stored at -20°C until further processing. Then, filters were carefully cut and the portions were subjected to cell permeabilization with lysozyme and achromopeptidase (LA permeabilization) according to the protocol by Sekar et al. (2003).

For prokaryotic assemblage characterization, six different group-specific oligonucleotide probes (Thermo-Hybrid) were targeted to *Archea* (ARCH915), to the domain *Bacteria* (EUB338), to β -*Proteobacteria* (BET42a), to *Cytophaga*-like bacteria (CF319a), and to the class *Actinobacteria* (HGC69a). In addition the probe, NON338 was used as a control. The proportion of formamide in the hybridization buffer was 55% in all cases except for ARCH915 (40%) and HGC69a (35%). After the hybridization, the filter sections were washed in a pre-warmed washing buffer at 37°C for 15 min with the appropriate amount of sodium chloride depending on the proportion of formamide in the hybridization buffer (Sekar et al. 2003). The filters were counterstained with DAPI (1 mg:100 mL) and inspected with an Olympus BX 50 epifluorescence microscope at $\times 1,250$ magnification. At least 10 randomly selected microscopic fields were counted for the enumeration of the FISH-stained bacteria and for the total DAPI stained bacteria, and the percentage value of each bacterial taxa was calculated.

CARD-FISH for visualization of prey inside food vacuoles—Ciliates were separated under a stereomicroscope with a high magnification objective ($\times 100$). One hundred individuals of each species were transferred to polycarbonate membrane filters (1- μm pore size). The same procedure was carried out in four replicates for each species for each date (January, February, and March 2005). We also used CARD-FISH protocol following Medina-Sánchez et al. (2005). This method allows the visualization of specific labeled prey inside the food vacuoles of the ciliates. We used the same set of probes for characterizing the prokaryotic assemblage in lake water.

Calculations—The mean irradiance within the mixolimnion (I_m) was computed for each UV band (305, 320, 340, and 380 nm) and PAR (400–700 nm) wavebands, following

Table 1. Light conditions in Lake Moreno Oeste during the two summer seasons (1998–1999 and 2003–2004). Z_{therm} , thermocline depth; K_d , extinction attenuation coefficient (m^{-1}); I_0 , irradiance at 0-m depth (units, 305 nm, $\mu\text{W cm}^{-2} \text{nm}^{-1}$; PAR, $\mu\text{mol photon m}^{-2} \text{s}^{-1}$); I_m , mean irradiance of the mixolimnion (units as I_0). p is the probability value of a t -test. Values are given as mean \pm SE of all samples in the season.

	1998–1999 $n = 9$	2003–2004 $n = 7$	t	df	p
Z_{therm}	27.7 \pm 0.92	15.8 \pm 0.71	9.33	14	<0.001
K_d PAR	0.14 \pm 0.003	0.16 \pm 0.002	4.17	14	=0.001
K_d 305	0.66 \pm 0.017	0.77 \pm 0.005	4.88	14	<0.001
K_d 320	0.59 \pm 0.011	0.65 \pm 0.009	3.66	14	<0.003
K_d 340	0.45 \pm 0.010	0.50 \pm 0.007	3.30	14	=0.006
K_d 380	0.29 \pm 0.008	0.32 \pm 0.003	2.79	14	=0.015
I_m PAR	199.3 \pm 20.68	542 \pm 48.3	7.38	14	<0.001
I_m 305	0.05 \pm 0.01	0.16 \pm 0.018	6.15	14	<0.001
I_m 320	1.11 \pm 0.13	2.48 \pm 0.239	5.41	14	<0.001
I_m 340	2.82 \pm 0.32	6.10 \pm 0.567	5.44	14	<0.001
I_m 380	6.78 \pm 0.73	15.86 \pm 1.432	6.22	14	<0.001

Helbling et al. (1994).

$$I_m = I_0 \frac{1 - e^{(-K_d Z)}}{K_d Z}$$

where I_0 is the irradiance at the surface, K_d is the diffuse attenuation coefficient (for the corresponding wavelength band), and Z is the depth of the mixed layer. The diffuse attenuation coefficients (K_d) were estimated as regression coefficients from light profiles obtained with the radiometer in the field.

The mean PAR absorption $a_{(400-700)}$ *Stentor* mL^{-1} was calculated as

$$a_{(400-700)} = \frac{2.303A_{(400-700)}}{LC}$$

where L is the cell path length in meters and C the concentration of *Stentor* mL^{-1} in the cell.

The estimated extinction coefficient due to *Stentor* (K_{dSt}) was calculated as follows: The K_d without *Stentor* presence, obtained directly from field data in absence of *Stentor*, plus the abundance of *Stentor* (ind. mL^{-1}) times the estimated $a_{(400-700)}$ *Stentor* mL^{-1} .

Photosynthetic efficiency of the two mixotrophic ciliates was calculated as chlorophyll-specific production of each species per irradiance unit at each depth.

Thermocline depth was considered as the depth of the mixing layer (i.e., top of the discontinuity layer). Differences in thermocline depth, mean irradiances, and diffuse attenuation coefficients between the two summer seasons were analyzed using a t -test. Differences in ciliates and total bacteria abundances were tested with a two-way analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used to compare differences in photosynthetic efficiency between both mixotrophic species and slopes in diffuse attenuation coefficient observed versus estimated. All statistical analyses were performed using SigmaStat 3.1 and Statistica 6.0 statistical packages.

Results

Ciliate distribution and abundance—During both summer seasons, Lake Moreno Oeste showed stable temperature

stratification. The thermocline depth varied between the two studied years and was significantly deeper during the 1998–1999 season (Table 1). This was a windy season, while 2003–2004 was a comparatively mild summer. The number of hours with high wind speed ($>30 \text{ km h}^{-1}$) was significantly higher in the 1998–1999 summer, and the ratio (number of hours 1998–1999 : number of hours 2003–2004) increased as wind speed increased (Table 2). In the summer of 1998–1999, the hours with wind speed $>50 \text{ km h}^{-1}$ were 1.5 times that of 2003–2004. Variations in wind action could have resulted in the observed differences in thermocline depth. As a consequence of changes in thermocline depth, the mean irradiance (PAR and UVR) of the mixing layer (I_m) increased significantly during the summer of 2003–2004 (Table 1).

O. naumannii and *S. araucanus* were present during the two studied seasons, but their abundance differed between the two summers. *O. naumannii* was most abundant in the 1998–1999 season, reaching 6 ind. mL^{-1} , and had a ninefold higher abundance than *S. araucanus*. In contrast, the two ciliates codominated during the summer 2003–2004 (~ 2 –3 ind. mL^{-1}) (Fig. 1a,b). The peritrich *O. naumannii* decreased in number three times in 2003–2004. Statistical analyses showed that the abundances of both species were significantly different between years (two-way ANOVA: *S. araucanus* years $F_{1,28} = 44.46$, $p < 0.001$; depths $F_{1,28} = 51.16$, $p < 0.001$; *O. naumannii* years $F_{1,28} = 53.46$, $p <$

Table 2. Wind conditions in Lake Moreno Oeste during the two studied summer seasons 1998–1999 and 2003–2004; expressed as the number of hours with wind speed higher than 20, 30, 40, and 50 km h^{-1} . Ratio of the number of hours in 1998–1999 to the number of hours in 2003–2004. Hourly wind speed data from Servicio Meteorológico Nacional of Argentina.

Wind speed (km h^{-1})	1998–1999 (h)	2003–2004 (h)	Year-ratio
>50	61	40	1.53
>40	387	279	1.39
>30	942	878	1.07
>20	1,503	1,413	1.06

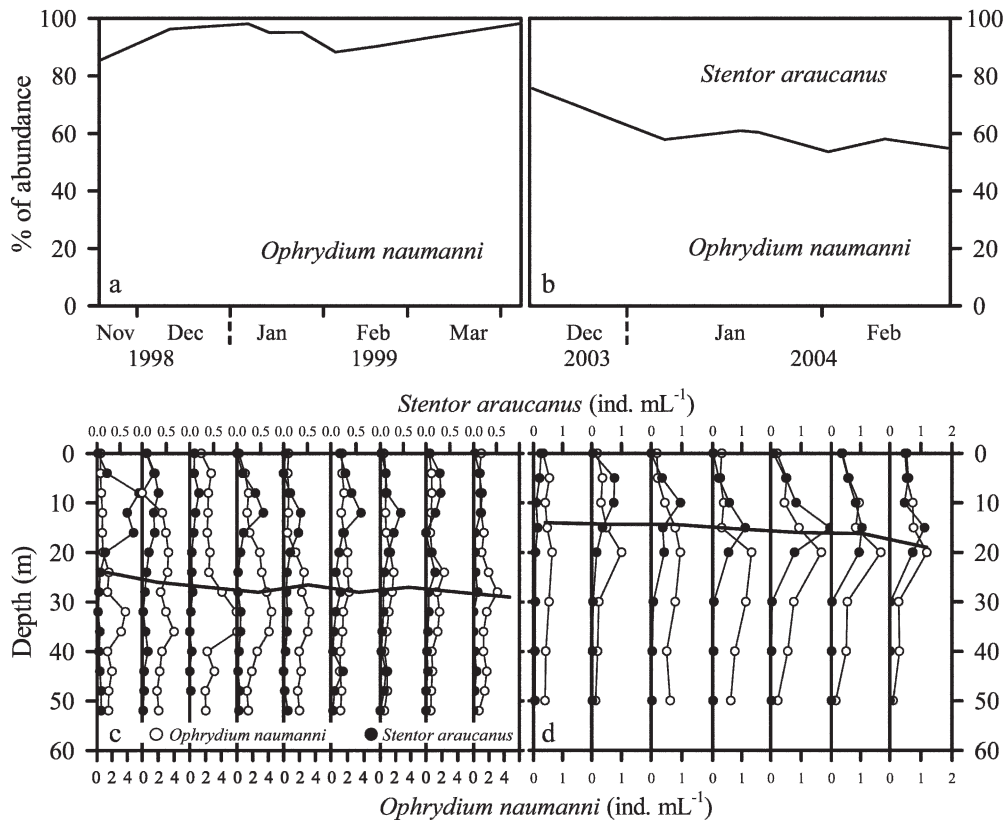


Fig. 1. Percentage of abundance of *O. naumanni* and *S. araucanus* in Lake Moreno Oeste (a) 1998–1999, (b) 2003–2004. Vertical abundance distribution of the two species, and thermocline depth (solid line) (c) 1998–1999, and (d) 2003–2004. Note change in scale of abundance between c and d.

0.001; depths $F_{1,28} = 6.76$, $p = 0.015$), but both species showed recurrent vertical distribution patterns in the two seasons (Fig. 1c,d). The highest abundances of *S. araucanus* were always observed around the 10-m depth in the epilimnion, whereas *O. naumanni* reached its highest abundances in the metalimnetic levels (Fig. 1c,d). Remarkably, in the 2003–2004 season, *O. naumanni* had its maximum abundance at upper depths but remained in the metalimnion (Fig. 1c,d, notice the change in scale in *O. naumanni* abundance between Fig. 1a,c and 1b,d). Additionally, we calculated the total integrated abundances of both ciliates in each sampling date in order to evaluate whether differences of abundances among depths were due to a dilution in a greater volume determined by the deeper mixed layer in 1998–1999. The results indicated that for both ciliates the interannual differences of integrated abundances were significant (*O. naumanni*, $t = 5.51$, $df = 14$, $p < 0.0001$; *S. araucanus*, $t = 3.10$, $df = 14$, $p = 0.008$).

S. araucanus and *O. naumanni* reacted oppositely to a reduction in the depth of the mixing layer (Fig. 2). *S. araucanus* showed higher abundances when the mixing layer was shallower and had higher I_m values (Table 1). In contrast, *O. naumanni* decreased its abundance under such conditions. Noticeably, the diffuse attenuation coefficients of PAR were higher in the 2003–2004 season, with increased *S. araucanus* abundances (Table 1). An analysis of the partial K_d (each 5 m) and the abundance of *Stentor*

at the corresponding layer showed a positive correlation ($r^2 = 0.420$) (Fig. 3). This trend remains unchanged if all data of both seasons are pooled (ANCOVA, $F_{1,113} = 0.941$, $p = 0.334$). In addition, we measured the spectrophotometrical absorbance of *Stentor* cells. Results showed that 1 *Stentor* mL⁻¹ would lead to an increase of $0.036 (\pm 0.0002)$ in the K_d ; thus, we estimated the expected K_d values due to the presence of *Stentor* (K_{dSt}) in the water column. The obtained regression did not differ from that observed in nature (Fig. 3) (ANCOVA, $F_{1,68} = 0.480$, $p = 0.491$).

Photosynthetic efficiency (PE)—The PE, measured as chlorophyll-specific primary production per mol of photons, showed a decrease in both species with the increase of PAR (Fig. 4), although the slopes were significantly different (ANCOVA, $F_{1,23} = 8.01$, $p = 0.009$). At intermediate light intensities (around $270 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), both species showed similar photosynthetic efficiencies (around $1.15 \text{ ng C [ng Chl } a]^{-1} [\text{mol photons m}^{-2}]^{-1}$). However, for intensities lower than $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, the efficiency of *S. araucanus* does not continue to increase. This may imply that *S. araucanus* has reached its maximum possible efficiency of $\sim 1 \text{ ng C (ng Chl } a)^{-1} (\text{mol photons m}^{-2})^{-1}$. On the other hand, the efficiency of *O. naumanni* was highest at lower light intensities but decreased sharply to $0.15 \text{ ng C (ng Chl } a)^{-1} (\text{mol photons m}^{-2})^{-1}$ when light and UVR (Table 1,

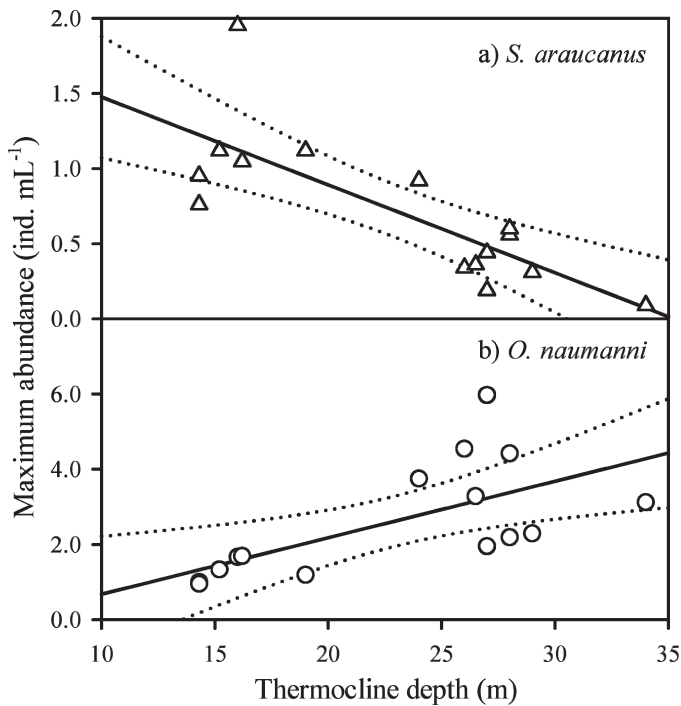


Fig. 2. Relationship between (a) *O. naumanni* and (b) *S. araucanus* maximum abundances and thermocline depth.

see K_d) changed from metalimnetic intensities to epilimnetic ones ($540 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 5-m depth) (Fig. 4). However, at these levels, the PE of *S. araucanus* remained high (Fig. 4).

Bacteria and other prokaryotic assemblages as food resources—In the first season (1998–1999) total bacteria abundances were almost uniformly distributed along the water column (mean \pm SE, epilimnion, $1.26 \times 10^6 \pm 9.08 \times 10^4 \text{ cell mL}^{-1}$; metalimnion, $1.23 \times 10^6 \pm 9.07 \times 10^4 \text{ cell mL}^{-1}$), while in the summer 2003–2004 we did observe an increase in bacteria abundance in the metalimnion (epilimnion, $1.17 \times 10^6 \pm 6.47 \times 10^4 \text{ cell mL}^{-1}$; metalimnion, $1.85 \times 10^6 \pm 2.43 \times 10^5 \text{ cell mL}^{-1}$). Comparing both summer seasons and considering all data (nine samplings in 1998–1999 and seven in 2003–2004, with a total of 32 data), we observed that there was a significant interaction between depth and years (two-way ANOVA: depth \times years $F_{1,28} = 6.199$, $p = 0.019$). The a posteriori Tukey test indicated that mean epilimnetic total bacteria abundance did not differ between years ($p = 0.604$). However, in the summer 2003–2004, mean metalimnetic bacteria abundance was higher than in 1998–1999 ($p = 0.006$). Finally, analyzing abundances within the same season we observed that in 1998–1999 epilimnetic and metalimnetic abundances did not differ ($p = 0.790$), but in 2003–2004 the metalimnetic abundances were higher ($p = 0.005$).

During three summer occasions we analyzed the composition of the bacteria and other prokaryotic assemblages using the CARD-FISH method. We observed a dominance of *Bacteria* (EUB338) over *Archaea* (ARCH915), and particularly a decrease of the latter at the metalimnion (Fig. 5). We did not observe differences in

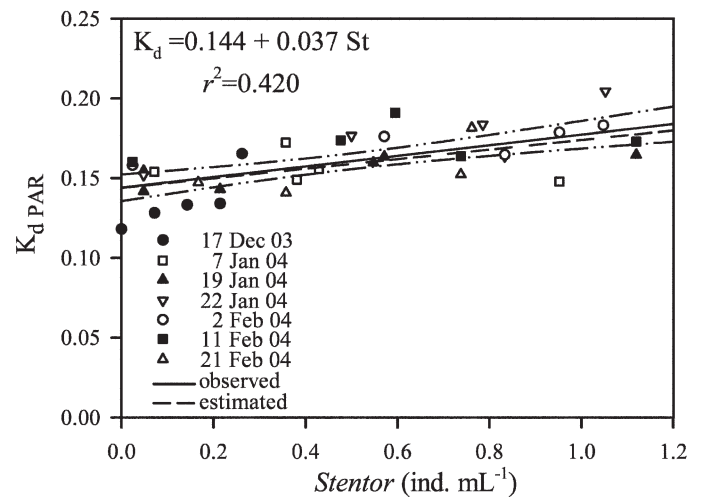


Fig. 3. Relationship between partial K_d values (each 5 m) and the abundance of *S. araucanus* at each depth in the 2003–2004 season in Lake Moreno Oeste. Symbols identify data of same sampling date. Solid line, observed relationship; dash-dot-dot line, 95% confidence limits; dashed line, estimated relationship based on spectrophotometric absorbance of *S. araucanus*.

the proportion between groups (*β -Proteobacteria*, *Cytophaga*-like bacteria, and *Actinobacteria*) in the different sampling dates (Fig. 5).

The combination of procedures was an optimal protocol for detecting prokaryotes ingested by mixotrophic ciliates (Fig. 6). In addition, the microscopic observations confirmed that embedding of filters after the ciliate transfer to the polycarbonate membrane filters prevented cell loss. In the analysis of the ciliate vacuole content, we observed that *O. naumanni* was able to feed on all prokaryote groups. We identified *Archaea* inside food vacuoles as well as the other tested groups (*β -Proteobacteria*, *Cytophaga*-like bacteria, and *Actinobacteria*) and also picocyanobacteria. Unfortunately, the method did not allow us to quantify ingestion of the different prey. The examination of the other ciliate, *S. araucanus*, was surprising since we did not find any marked *Bacteria* or *Archaea* inside its vacuoles (Fig. 6). Similarly, no picocyanobacteria or prokaryote CARD-FISH signal was observed inside the ciliate. We also looked for bacterial rods in the lake and inside ciliates, and we determined that the proportion of larger rods to total cocci was small in the lake (<1%), and we did not observe rods inside the ciliate. Therefore, we were able to determine that prokaryotic cells that can serve as food resources are not shared by the two mixotrophic ciliate species.

Discussion

During the two seasons (windy vs. mild summer) we observed substantial variation in thermocline depth and differences in the epilimnetic light intensity. Under these conditions, the relative abundance of *S. araucanus* and *O. naumanni* changed drastically. *S. araucanus* showed a vertical distribution with maxima around 10-m depth regardless of thermocline depth, suggesting habitat selection by the ciliate. In the 1998–1999 season there was an extended

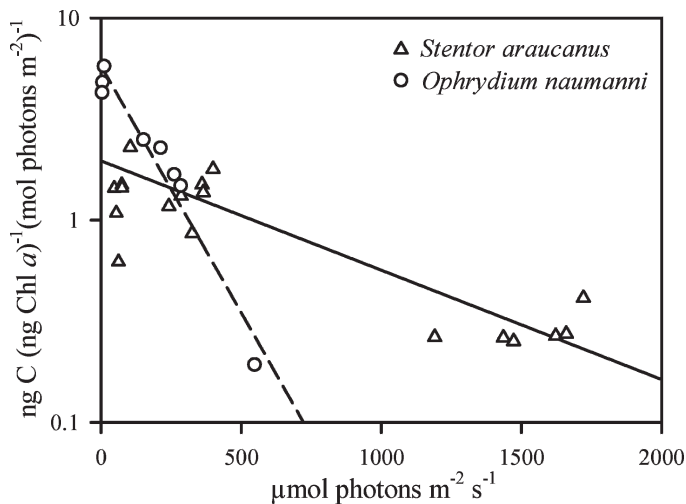


Fig. 4. Field photosynthetic efficiencies of *O. naumannii* (open circles and dashed line) and *S. araucanus* (open triangles and solid line).

epilimnion (30-m depth). In this case, ciliates would be mixed down into the water column and experience a low mean light intensity. Under these conditions, selecting a particular depth would require a substantial energy cost for ciliates, depending on how energetic costs of maintenance are related to resource supply. This situation is in accordance with models for light-limited growth in which biomass of phytoplanktonic taxa will decrease monotonically with increasing mixing depth (Huisman 1999; Diehl 2002). On the contrary, the lower thermocline depth in the second studied season (2003–2004) may reduce the energy cost of maintaining a population in a high light regime and may lead to higher abundances. This change in the thermocline depth, which limits the mixing space where cells of *S. araucanus* can be dragged, may be viewed as a light enrichment as described by Diehl et al. (2002). *S. araucanus* became more abundant when the thermocline depth was lower and the I_m higher. Our data indicated that the high light supply achieved in the epilimnion during the 2003–2004 season was important in the success of *S. araucanus*. In chemostat experiments, Huisman et al. (2002) demonstrated that under light-limited conditions, the steady-state population density was inversely related to mixing depth. In our case, the increase in population density when the mixing depth decreased would indicate that the dark-pigmented *S. araucanus* might be light limited.

S. araucanus has dark blue-green cortical granules with the pigment called stentorin (Foissner and Woelfl 1994). The presence of these dark granules may shade the endosymbiotic algae, since stentorin absorbs PAR mainly between 400 and 600 nm (Moeller 1962). Consequently, this species has a higher critical light level needed to maintain endosymbiotic algal photosynthesis. This was also observed in the analysis of field photosynthetic efficiency, in which *S. araucanus* showed a decrease in efficiency below $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Although the species with lower critical light intensity should be a better competitor (Huisman 1999), the fact that

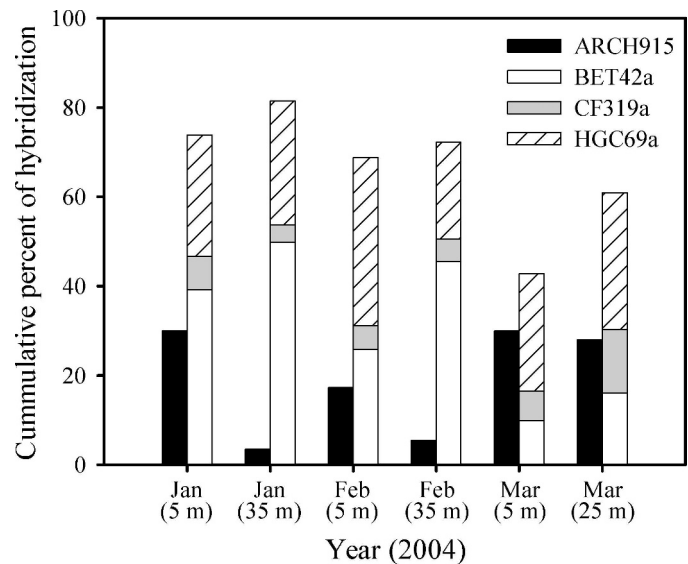


Fig. 5. Prokaryote assemblage composition in Lake Moreno at 5- and 25–35-m depth (metalimnion). ARCH915 = *Archea*, BET42a = β -*Proteobacteria*, CF319a = *Cytophaga*-like bacteria, and HGC69a = *Actinobacteria*.

mixing in the epilimnion will expose organisms to hazardous light levels may affect the outcome of competitive interactions. *O. naumannii* was more abundant when the thermocline was deeper and showed a high photosynthetic efficiency at low light intensity. Thus, the metalimnetic population of *O. naumannii* seems to be a good competitor at this level. However, the upper 10 m of the epilimnetic levels of Lake Moreno Oeste are hazardous because of UVR (Alonso et al. 2004; Modenutti et al. 2004). *S. araucanus* is a particularly resistant species to UV-B radiation because it survived for days in near surface incubations in the field (full sunlight) and in laboratory incubations (UV-B lamp) (Modenutti et al. 1998). The sharp decrease in *O. naumannii* PE observed in our field incubations may result also from the incidence of UV wavelength. Previous research has showed that the net primary production in this species decreased sharply in the upper 5 m of the water column, and 50% of the individuals died after 4 h of full sunlight exposure at 1-m depth (Modenutti et al. 2004; Modenutti pers. obs.). During the mild windy summer of 2003–2004, the epilimnion increased not only in light availability (I_m) but also in the UVR risk for an unprotected species as *O. naumannii*; mean epilimnetic irradiance of 305 nm increased threefold compared with the 1998–1999 season. On the other hand, at epilimnetic irradiances and UVR, the PE of *S. araucanus* remained high because of their high UV resistance (Modenutti et al. 2005).

In addition, the vertical segregation gives *S. araucanus* the advantage of driving light availability for other phototrophs located lower in the water column. Indeed, we observed that the variation in partial K_d depends on *S. araucanus* abundances and that *O. naumannii* abundances decreased in relation with the increase in K_d . *Stentor* advantage is restricted to periods with high epilimnetic

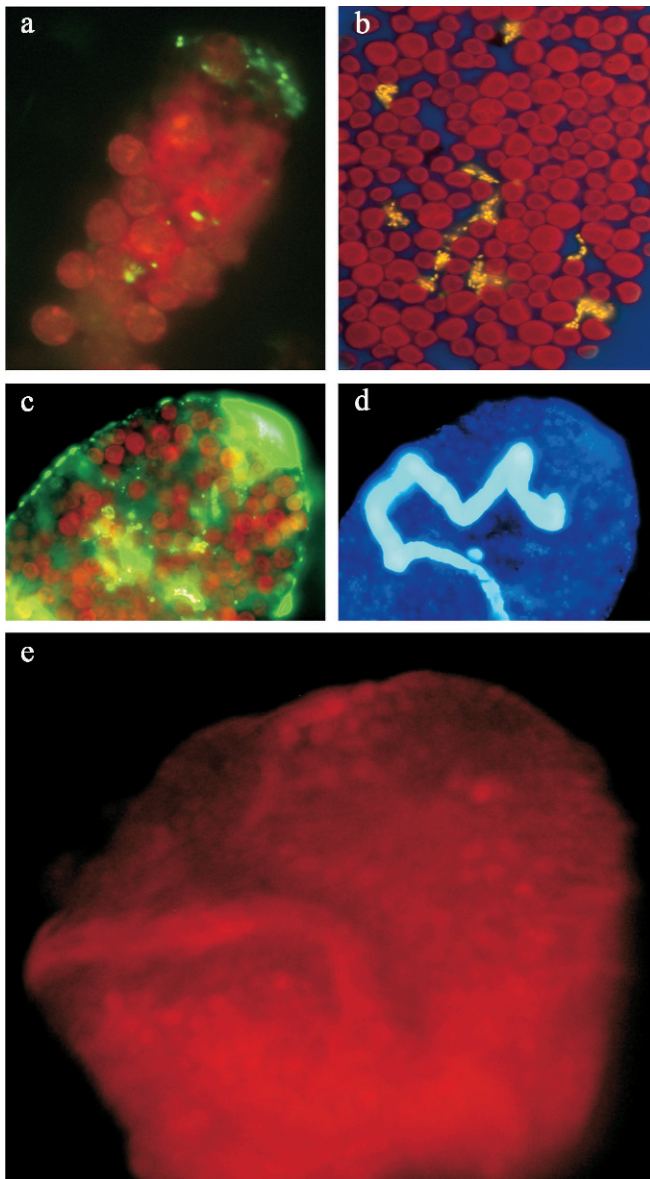


Fig. 6. Epifluorescence microscope photographs. (a) DAPI and CARD-FISH staining with EUB338 probe on *O. naumanni* with blue light (Olympus U-MWB filter). (b) *O. naumanni* with picocyanobacteria prey with blue light. (c) and (d) DAPI and CARD-FISH staining with EUB338 probe on *O. naumanni*, (c) blue light and (d) UV light (Olympus U-MWU filter). (e) DAPI and CARD-FISH staining with EUB338 probe on *S. araucanus* blue light.

mean irradiance because of rather shallow epilimnetic layers. Another possibility is that *S. araucanus* may interfere with particular wavelengths of the light spectrum affecting the quality of metalimnetic light. Blue light prevails in the metalimnion of Andean lakes (Pérez et al. 2002), and the stentorin pigment could change the natural light availability for other phototrophic organisms through the absorbance of short visible wavelengths (Moeller 1962). However, our spectrophotometric scanning on live individuals did not show an increase in the absorption in the green-blue spectra, probably because we analyzed whole

live individuals and not the pigment alone as Moeller (1962) did.

Both ciliates depend more on phototrophy rather than on phagotrophy (Modenutti and Balseiro 2002; Woelfl and Geller 2002), although they ingest food particles (Foissner and Woelfl 1994; Modenutti and Balseiro 2002). In this study, through a direct observation of food vacuoles, we determined that *O. naumanni* grazed on all the prokaryote assemblage including *Archea* and picocyanobacteria. In contrast, we did not identify prokaryotes (neither cocci nor rods) in food vacuoles of *S. araucanus*, indicating that prokaryotes do not constitute a significant proportion of its diet; although it would be able to prey on larger eukaryotic cells. Considering the lack of food overlap (*O. naumanni* feeds on prokaryotes and *S. araucanus* does not), food resource competition between both ciliates would be negligible. In addition, differences in total bacteria abundances could not explain the observed variations in *O. naumanni* abundances. Moreover, the increase in metalimnetic bacteria abundances during the 2003–2004 season implies that the decrease in *O. naumanni* was not due to a decrease in food resources. Finally, the spatial segregation of the two species suggests that the possible competition for food has been reduced.

Our results suggest that light is the main factor in the development of *S. araucanus* and *O. naumanni* populations because light directly affects the photosynthesis of the endosymbiotic algae. In this study, temporal variability in light supply was observed to be important in the outcome of competition between the two mixotrophic species, as previously indicated for phytoplankton community composition (Litchman 1998; Huisman et al. 1999; Diehl et al. 2002). In mixotrophic corals, light gradient may also determine different functional responses of energy loss processes, which are important determinants of species-specific physiological limits to growth and thereby of niche differences (Anthony and Connolly 2004). In the same way, light gradient in Andean lakes may offer an example of niche partitioning in these two mixotrophic ciliates.

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