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Colonization of a Neotropical Reservoir (Córdoba, Argentina) by *Ceratium hirundinella* (O. F. Müller) Bergh

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Blooms of *Ceratium hirundinella* (O. F. Müller) Bergh. have been detected in different water bodies in the Neotropical Region since 1990. The colonization began in southern lakes, and during the last decade the dinoflagellate arrived and bloomed in subtropical reservoirs. In this context the colonization of *C. hirundinella* and its population development have been analyzed from its first record in the Río Tercero Reservoir (February 1999 to February 2001). Phytoplankton and physicochemical samples were obtained from three sampling stations at the Reservoir, one in the outlet of the water cooling channel of the nuclear power plant, and one in the nearest tributary (Quillinzo River). Two blooms of *C. hirundinella* were detected during the warm seasons with temperatures higher than 18°C, and pH ranging between 8.5 and 8.9. Environmental conditions such as certain light intensity range and percentage of dissolved oxygen mentioned as favorable for *Ceratium* development were always recorded in Río Tercero Reservoir. Cysts were observed in spring and summer months. Another dinoflagellate (*Peridinium gatunense* Nygaard) bloomed in previous summer in this water body but its population density decreased during the invasive phase of colonization of *C. hirundinella. Asplanchna girodi*, became the dominant zooplankter after the first bloom of *C. hirundinella.* We believe that the presence of this dinoflagellate in the Neotropical Region could be a regional phenomenon associated with some dispersal mechanisms and favorable local conditions for its proliferation like those recorded in the Rio Tercero Reservoir.

Keywords : *Ceratium hirundinella*, first record, phytoplankton dynamic, Río Tercero Reservoir, rotifer predator.

Introduction

Ceratium hirundinella is a widespread freshwater dinoflagellate in temperate and subtropical lakes and in reservoirs of the northern hemisphere, as mentioned by Margalef (1983). The records of this alga remain limited in the southern hemisphere. Since 1990 this alga has been recorded in South-Chilean lakes (Soto & Lembeye 1999) and, since 1996, dense blooms have been detected in Argentinean reservoirs: in western Patagonia and the south-eastern Pampean Plains (Guerrero & Echenique 1997), and in Córdoba province (Ruibal et al. 1999, Girbal et al. 2000, Prosperi 2000, Bustamante et al. 2002). Since thirty years of limnological studies, the presence of *C. hirundinella* was first detected in Río Tercero Reservoir in 1999 (Casco et al. 2002). *Ceratium hirundinella* populations typically have their peak during summer (Heaney & Talling 1980, Reynolds 1984, Ramón & Moyá 1987, Kawabata & Kagawa 1988, Lindstrom 1992). However, other peaks, including blooms, have been recorded during winter in Spanish reservoirs (Pérez-Martinez & Sánchez-Castillo 2001, 2002) and in the winter-spring period in Lake Kinneret (Pollingher et al. 1993). The maximum development of *C. hirundinella* was frequently related to stratification periods (Harris et al. 1979, Heaney & Talling 1980, Lindstrom 1992) but in the Spanish reservoirs it was also, a common species during stratified as well as mixed conditions (Margalef et al. 1976, Pérez-Martinez & Sánchez-Castillo 2001, 2002).

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Planktonic populations of *C. hirundinella* can be inoculated from fugitive cells in the water column, germinating cysts or cells that have reached the reservoir from the tributaries. The formation of cysts is a characteristic of this group of algae which allows their survival in sediments and promotes the presence of the dinoflagellate in subsequent years. Thus the cysts are over-wintering forms in temperate zones and over-summering forms in subtropical areas (Pollingher et al. 1993). The ability of cysts to survive and disperse makes it important to determine the first appearance of this species in a particular water body and also to know the environmental conditions prevailing at the time (Pollingher 1988).

The morphological and eco-physiological characteristics of these algae, which define the successful strategy of dinoflagellates in comparison to nanoplanktonic algae were described by Pollingher (1988). Because of their independent mobility they suffer low sedimentation losses, accumulate in the water column where light conditions are best, and they can obtain nutrients throughout the water column. Dinoflagellates can also have two or three generations in circumstances of low phosphorus concentration due to their capacity for luxury phosphorus consumption when sufficient nutrients are available. Moreover, these algae have long generation times and their losses due to grazing are low by comparison with those of other planktonic algae (Pollingher 1988).

The objective of the present study was to describe the colonisation of *C. hirundinella* in Río Tercero Reservoir and to analyse its relationships with some environmental conditions recorded, and with the presence of other phytoplankton components and probable grazers. Another purpose of this paper was to recognize the regional character of the colonisation, given data about this invasion from southern to northern aquatic environments of South America.

Methods

The Río Tercero Reservoir (32° 11' S, 64° 23' W) was built in 1936. Since 1983 it has been regulated by the requirements of a nuclear power plant of 600 MW, which uses the water for cooling. Thus, water level fluctuations in the reservoir have been strongly reduced. The inflow of water from the nuclear plant via a cooling channel (Fig. 1) (up to 7° C warmer than St2, in winter) was revealed by a restricted thermal plume. The reservoir area is 4.3 km^2, with a volume of 5.6 x 10^2 hm^3. The maximum depth is 46.5 m and the mean depth is 12.2 m.

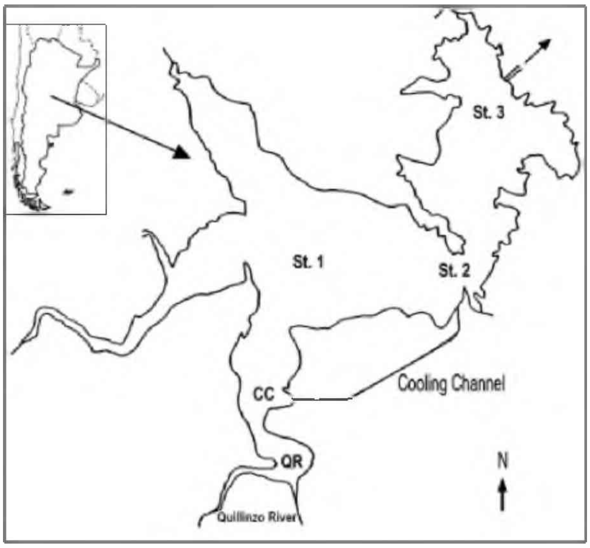

Fig. 1. Location of the sampling stations in the Río Tercero Reservoir : Quillinzo River (QR), Cooling Channel (CC), tributaries inflow (St1), Nuclear Power Plant pumps water for cooling (St2), and near the dam (St3).

Five sampling stations were established : one at Quillinzo River (QR), three in the reservoir (St1 : where the tributaries flow in, St2 : where the Nuclear Power Plant pumps water in for cooling, and St3 : near the dam), and one at the mouth of the cooling channel (CC) (Figure 1).

At St2 a vertical profile was taken based on the extinction of incident light (100, 60, 25, 10, 1%) and including also the aphotic zone for assessing the vertical distribution of parameters and variables. Simultaneously, integrated samples of the photic and aphotic layers were separately obtained at St1 and St3 to evaluate the horizontal distribution of parameters and variables. At the other sites (QR and CC), subsuperficial water samples were collected, considering their shallowness.

The samples were taken every two months between February 1999 and February 2001. Phytoplankton samples were taken with a Van Dorn bottle and counted in 25 ml sedimentation chambers by the Utermöhl method. The biovolumes of algae were obtained using formula proposed by Hillebrand et al. (1999). Chlorophyll "a" was determined by Lorenzen's method (APHA 1995). Zooplankton samples were taken by pumping and sieving 50 L through a net of 35 µm pore size and counting them in Sedgwick-Rafter and Bogorov chambers. Temperature, pH, dissolved oxygen, and conductivity were measured with a Khalsico Surveyor Hydrolab multimeter. Soluble reactive phosphorus and total phosphorus were determined by the ascorbic acid method, the latter after acidic persulfate digestion; nitrates, nitrites, ammonium and total nitrogen by cadmium reduction method and Kiedjal and silica by heteropoly blue method (APHA 1995). The light extinction was measured with a Li-Cor LI-185 B.

Previous data on parameters and plankton were obtained from technical report and papers (Mariñelarena et al. 1998, Casco et al. 2002).

Results

The reservoir can generally be considered as mesotrophic (Table 1) and on some occasions oligotrophic, with a thermal stratification in summer and without any deficit of dissolved oxygen in the bottom layers. The total phosphorus concentration showed interannual fluctuations but without significant differences. The range of nitrate concentrations did not vary during all the sampling period (Figures 2a-b).

The mean, standard deviation and range of values of total phosphorus, nitrogen fractions, silica, conductivity, temperature, chlorophyll "a", dissolved oxygen and pH, measured during the period February 1999-February 2001 are given in Table 1. SRP fraction was undetected in the majority of the sampling occasions, and for this reason was excluded from Table 1.

Despite the fact that the phytoplankton was monitored in the reservoir from 1977, *C. hirundinella* was recorded for the first time in February 1999 at all sampling stations except in the thermal plume. In this sampling occasion, the highest density was recorded at St2 in the layer coinciding with 10% of surface incident light (38 cells ml^{-1} with 60 µE cm^{-2}). *Ceratium hirundinella* was absent in April 1999 and maintained low densities during the winter (Fig. 3a). In August 1999, low densities (115 and 127 cells ml^{-1}) were recorded at St 2 with light intensities of 145 and 348 µE cm^{-2} that represent 25 and 60 % of the surface incident light, respectively. Later that year, in spring, blooms were detected in the euphotic zone of the reservoir, mainly at St2 (283 cells ml^{-1} with a radiance of 225 µE cm^{-2}, 25% of the surface incident light), and at St3 (737 cells ml^{-1} with 279 µE cm^{-2}).

Table 1. Mean, standard deviation and range of some parameters recorded during 1999-2001 from photic and aphotic sectors of sampling stations located in Río Tercero Reservoir (*: undetected).

	mean	SD	range
Total phosphorus (µg L^{-1})	21.3	24.9	*-161.0
P-PO_4 (µg L^{-1})	2.1	3.3	*-24.0
N-NO_3^- (µg L^{-1})	58.6	73.4	*-281.0
N-NO_2^-(µg L^{-1})	2.8	3.9	0,2-20.5
N-NH_4^+(µg L^{-1})	17.3	29.9	2.0-51.0
Silica (µg L^{-1})	5.9	2.1	1.6-11.2
Conductivity (µS cm^{-1})	156.0	27.6	105.0-207.8
Temperature (°C)	18.6	5.0	10.3-26.7
Chlorophyll "a" (µg L^{-1})	7.7	7.2	0.6-36.1
Dissolved oxygen (µg L^{-1})	8.1	1,9	1.2-11.7
pH	8.0	0.4	7.0-8.9

The maximum values of total biovolume and chlorophyll "a" were recorded between November 1999 and January 2000 (5.2×10^{10} µm^3 L^{-1} in January 2000 and 36 µg L^{-1} chlorophyll "a" in November 1999) (Fig 4a and 4c). Peaks of phytoplankton biomass were coincident with the maximum biovolume values of *C. hirundinella* (2.7×10^{10} µm^3 L^{-1} in November 1999 and

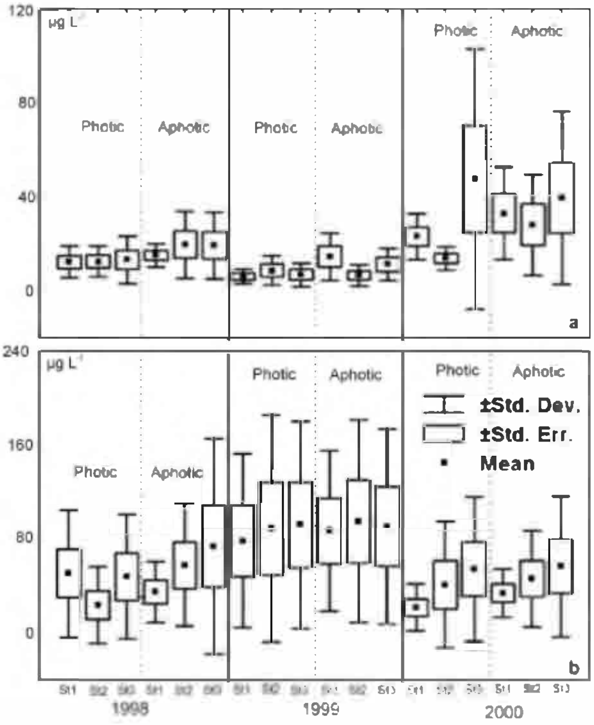

Fig. 2a-b. Variation of nutrients (a : total phosphorus, b: nitrates) in the Río Tercero Reservoir at photic and aphotic zones of the three sampling stations (St1, St2, St3). Period 1998-2000 : bimonthly sampling frequency.

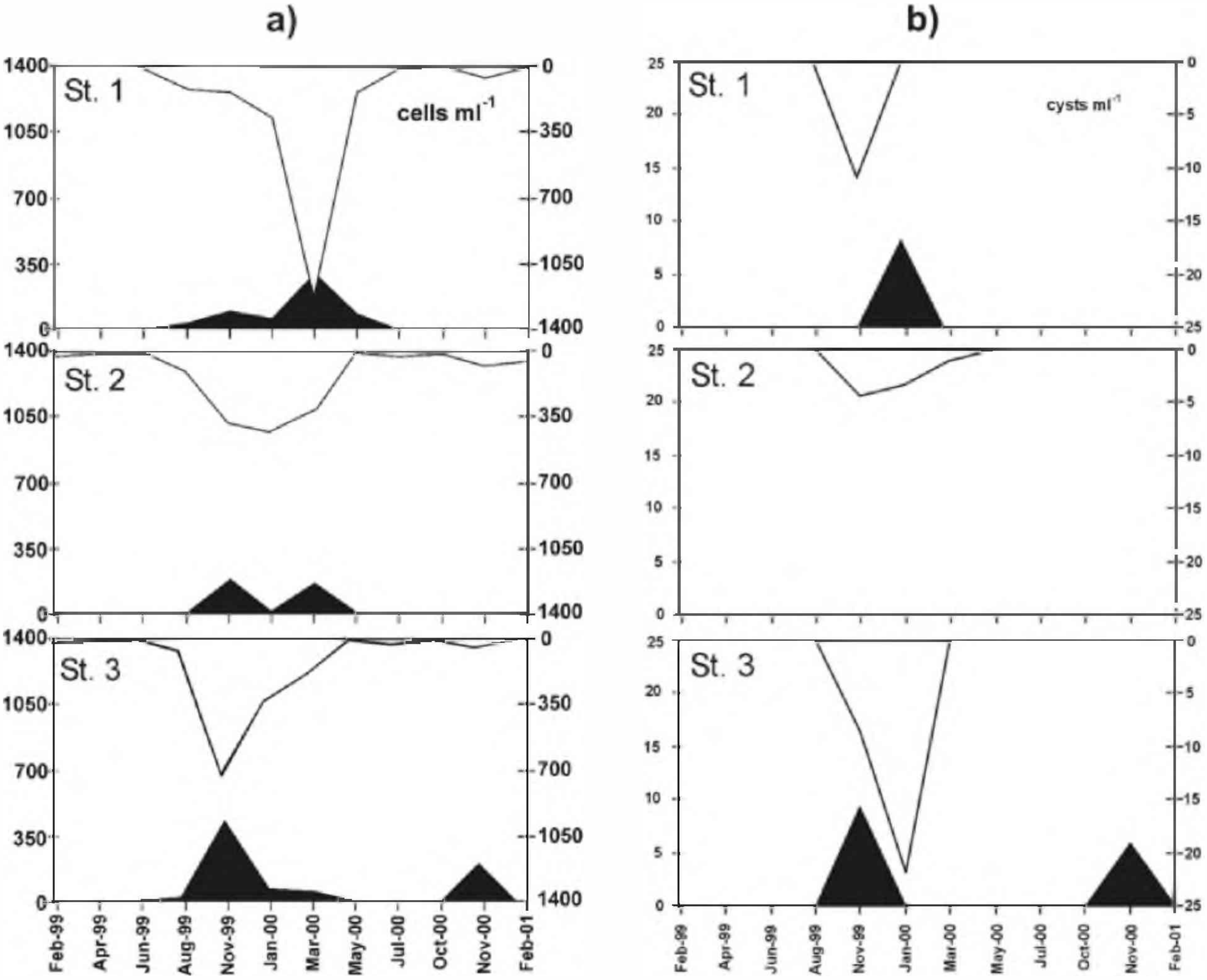

Fig. 3a-b. Distribution of *Ceratium hirundinella* in the Río Tercero Reservoir during the period February 1999-February 2001. white : photic zone, black : aphotic zone (a : cells, b : cysts).

5.1x10^{10} µm^{3} L^{-1} in January 2000) (Fig 4a and 4b). High biovolume values were detected at the beginning of the sampling period due to the contribution of *Peridinium gatunense* and *Anabaena spiroides* Kleb. (5.3 x10^{9} and 5.5 x10^{9} µm^{3} L^{-1} respectively)

The maximum phytoplankton density during 1999 occurred at the beginning of the year. The diatoms - *Aulacoseira alpigena* (Grunow) Krammer, *A. granulata* (Ehr.) Simonsen and *Cyclotella meneghiniana* (Kützing) - prevailed numerically throughout the year, followed by Cryptophytes, mainly, *Cryptomonas pusilla* Bachm. Species of *Monoraphidium* (*M. arcuatum* (Korsch.) Hindák, *M. contortum* (Thuret in Bréb) Kom.-Legn., *M. dybowskii* (Wolosz.) Hind. et Kom. Legn., *M. griffithii* (Berk.) Kom.-Legn., *M. minutum* (Näg.) Kom.-Legn. and *M. tortile* (W. et G.S. West) Kom.-Legn.) were present throughout the annual cycle and were relevant due to their abundance and distribution. The minimum of total phytoplankton density was observed in the summer 1999-2000, when *C. hirundinella* bloomed. During the *Ceratium* bloom, nitrogen concentrations diminished to values less than 20 µg L^{-1} in the photic layers and less than 43 µg L^{-1} in the aphotic layers. Following this bloom, the Cryptophytes proliferated, especially *Cryptomonas pusilla.*

In January 2000, there were similar densities of *C. hirundinella* in the photic layers of all sampling sta-

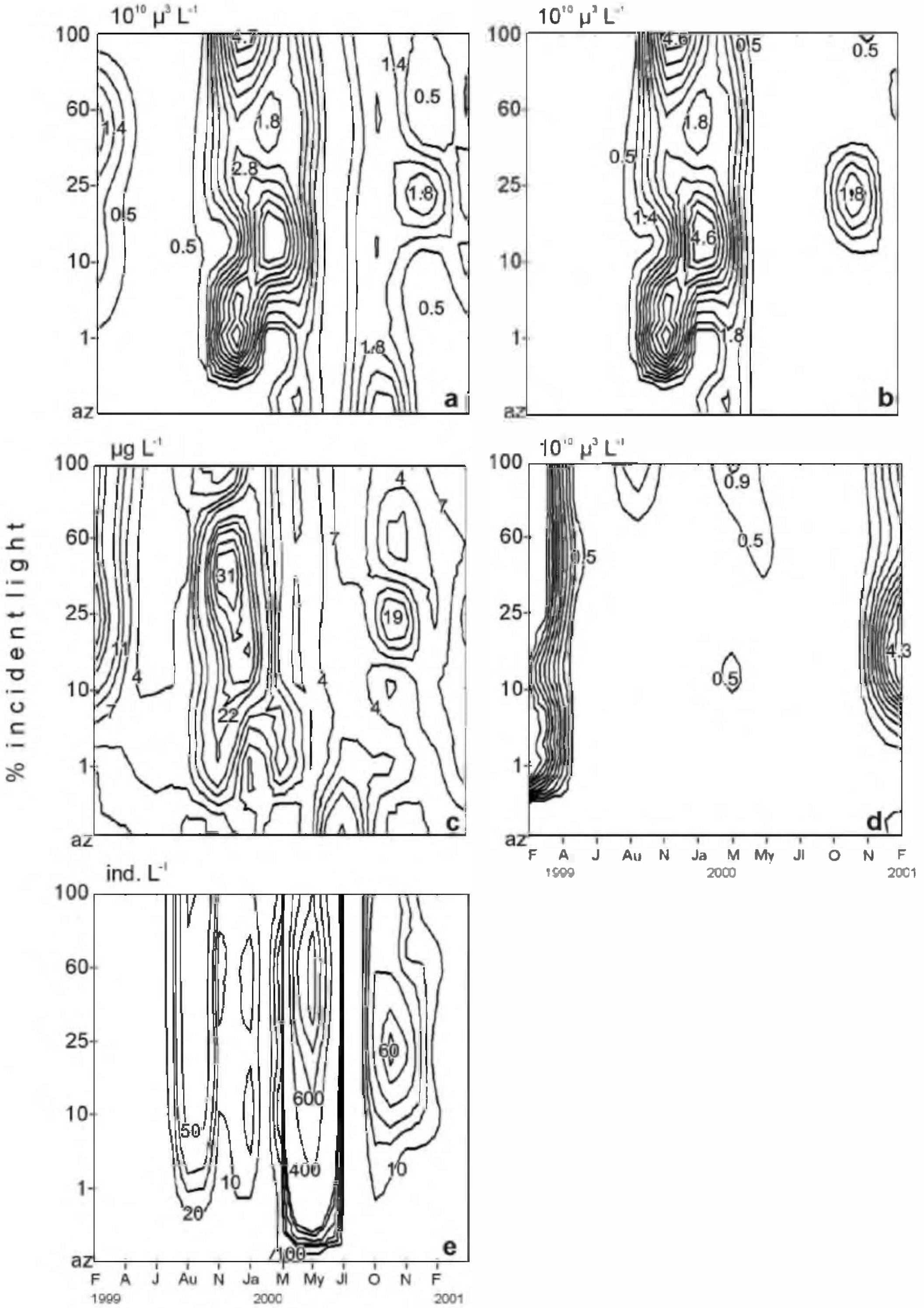

Fig. 4a-e. Isolines of phytoplankton biomass (a), *Ceratium hirundinella* biovolume (b), chlorophyll "a" concentrations (c), *Peridinium gatunense* biovolume (d) and *Asplanchna girodi* (e) densities in the vertical profile of St2 during the sampling period.

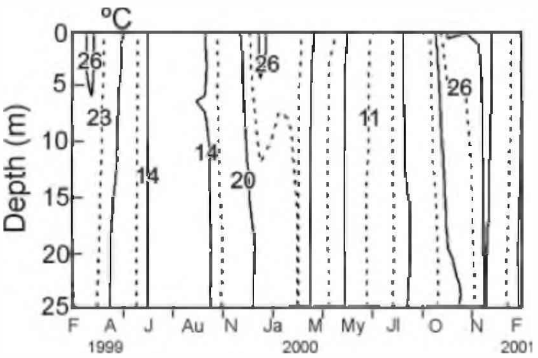

Fig. 5. Isolines of temperature during the sampling period.

tions within the reservoir (300 to 400 cells ml^{-1}) and the lowest densities were recorded in the aphotic layers (7 - 70 cells ml^{-1}). This preference for the photic layers was coincident with the highest temperature range within the water column (6°C) (Fig. 5). In late summer (March 2000), the populations of *C. hirundinella* in the reservoir were significant and a peak was observed at St1 (photic zone), reaching the maximum for the whole sampling period and all stations (1,244 cells ml^{-1}). The population of *C. hirundinella* in the reservoir decreased abruptly in autumn and maintained low densities in winter.

The peak of total phytoplankton occurred in winter 2000 (8.397 cells ml^{-1} in the aphotic layer of St1) and the dominant species was *Actinocyclus normanii* (Greg. Ex Grev.).

In spring 2000 (November), the highest densities of *C. hirundinella* were restricted to the aphotic sector of St3 and to the intermediate layers of the photic zone of St2. The bloom was smaller than that recorded in November 1999. During this second bloom, the nitrogen diminished considerably only in the aphotic layer of St3 (5 µg L^{-1}), where the maximum density of *C. hirundinella* was recorded (Fig. 3a). The SRP fraction was undetectable in both summers in both the photic and aphotic layers.

Table 2. Comparison of minimum and maximum temperature values (°C) recorded in particular environments with presence and blooms of *Ceratium hirundinella*.

	Water temperature	Presence of *C. hirundinella*	Blooms of *C. hirundinella*
North temperate lakes	1-23	6-23	12-23
Bermejales Reservoir	4-25	7-25	7-14
Kinneret lake	13-30	16-25	20-23
Río Tercero Reservoir	10-26	10-26	18-26

Peridinium gatunense, a typical species recorded in the Río Tercero Reservoir since 1977 and responsible for periodic blooms in previous summer, decreased its number during the maximum proliferation of *C. hirundinella* (Fig. 4d) but later in summer 2001 increased its population density to those recorded previously to *C. hirundinella* colonization.

The abundance of *C. hirundinella* was positively correlated with the concentrations of chlorophyll "a" (n: 54, r: 0.42, $p < 0.001$), pH (n: 54, r: 0.41, $p < 0.002$) and temperature (n: 54, r: 0.47, $p < 0.001$), negatively correlated with the concentrations of nitrates (n: 54, r: - 0.43, $p < 0.001$) and nitrites (n: 54, r: - 0.37, $p < 0.006$) and uncorrelated with the light intensity (n:54, r: 0.16).

Ceratium hirundinella was recorded at high densities when pH ranged between 8.5 and 8.9, whereas at pH lower than 8 this alga declined, its populations were lower than 300 ind. ml^{-1}. Blooms were only detected at temperatures above 18°C (Table 2).

The appearance of *Ceratium* cysts occurred nine months later than the first record of *C. hirundinella* in the reservoir, coinciding with its first detected bloom (Fig. 3a-b). The cysts were found in all parts of the reservoir as well as in the cooling channel, but not in the Quillinzo River. The cysts were observed in the water column from November 1999 to March 2000, with values ranging between 4 and 22 cysts. ml^{-1}. In January 2000, the cyst densities were quite variable among the sampling stations, with maximum values in the photic layers of St3. During autumn and winter the cysts disappeared from the water column. In spring 2000, the

Table 3. Records of the first blooms of *Ceratium hirundinella* in Chilean lakes (L) and Argentinean reservoirs (R) cited in the literature.

year		Locality coordinates	References
1990	Villarrica (L)	39° 20'S 72° W	Soto & Lembeye (1999)
	Puyehue (L)	40° 60' S 70° 30' W	Soto & Lembeye (1999)
	Llanquihué (L)	41° 10' S 73° W	Soto & Lembeye (1999)
	Chiloé (L)	42° 90' S 73° 90' W	Soto & Lembeye (1999)
1996	Ramos Mexía (R)	39° 30' S 69° W	Guerrero & Echenique (1997)
	Arroyito (R)	39° 14' S 68° 40' W	Guerrero & Echenique (1997)
	Paso de las Piedras (R)	38° 20' S 61° 41' W	Guerrero (1998)
1997	La Viña (R)	31° 90' S 65° W	Prosperi (2000)
1997	Tarahuín Cucao	42° 43' S 73° W	Villalobos (2003)
1998-1999	San Roque (R)	31° 22' S 64° 27' W	Ruibal et al. (1999), Girbal et al. (2000), Prosperi (2000), Bustamante et al. (2002)
1999-2000	Los Molinos (R)	31° 50' S 64° 32' W	Rodriguez et al. (2000)
2000	Sunampa (R)	27° 55 S 65° 30 W	Silverio et al (2001)

cysts were restricted to the aphotic sector of St3 and thermal plume, with low numbers. In summer 2000-2001, in coincidence with the lowest densities of cells, the cyst production was minimal (Fig. 3b).

A possible grazer of *C. hirundinella*, *Asplanchna girodi*, was present in the reservoir during its first bloom but at low densities (10-20 ind. L^{-1}). The populations of this predatory rotifer reached their highest densities in May 2000 at St2 (139 - 1,098 ind. L^{-1}). In the other months when *A. girodi* was found, its abundance was lower than 100 individuals L^{-1} (Fig. 4e).

Discussion

Generally, *C. hirundinella* has been considered as a species of warm and stratified water (Harris et al. 1979, Heaney & Talling 1980, Reynolds 1988, Lindstrom 1992, Bustamante et al. 2002) but it was a successful species in Río Tercero reservoir during stratified and mixing periods. Our results were coincident with a regional study covering approximately one hundred Spanish reservoirs, where *C. hirundinella* constituted one of the most abundant species found in stratified as well as mixed periods (Margalef et al. 1976). Pollingher et al. (1993) suggested that the development of *C. hirundinella* occurs at approximately the same temperature range in temperate and subtropical lakes and reported that this dinoflagellate disappears above 25°C in Lake Kinneret. In the Rìo Tercero Reservoir, this species was present during all seasons, between 10°C and 26°C. In the Bermejales reservoir, this taxon can be present during all the year because the temperature ranged between 4 and 25°C (Pérez-Martínez & Sánchez-Castillo 2001) (Table 2).

During the sampling period, the light conditions in the photic zone of the reservoir ranged between values reported as optimal for the development of *C. hirundinella.* In the Balaton lake, highest densities occurred with 126 and 440 µE cm^{-2} (Padisák 1985) whereas they occured in with 150 µE cm^{-2} English lakes (Harris et al. 1979). Our results were coincident with those recorded in Bermejales Reservoir, where light intensity and number of sunny days are higher than in temperate European regions (Pérez Martínez & Sánchez Castillo 2002). In the Río Tercero Reservoir, light availability is not a control factor for this alga during the annual cycle. In Río Tercero Reservoir proliferation of cysts could not be related to unfavourable environmental conditions. *C. hirundinella* showed a proliferation of cysts during the maximum densities of vegetative cells.

The minimum temperature for *C. hirundinella* excystment and the appearance of vegetative cells has been reported to be 4°C (Rengefors 1997, Rengefors & Anderson 1998). Since the temperatures of Río Tercero Reservoir were higher than 4°C, and there was no anoxia, the excystement could be possible all the year. In Río Tercero Reservoir, the appearance of this alga was associated with low concentrations of total phosphorus in disagreement with the results of James et al. (1992) in Eau Galle Reservoir (Wisconsin). In Río Tercero Reservoir, during1999, total phosphorus concentration was low and these conditions allowed the development of *C. hirundinella* populations, which could use their ability to extract phosphorus at low concentrations and to accumulate phosphorus over a considerable period. *Ceratium hirundinella* as cyst can immobilise large amounts of nutrients and promotes a decrease in the overall activity of the ecosystem, associated with its long generation time and perennation during several months in the sediments (Pollingher 1988). Moreover, environmental conditions such as nitrate limitation and anoxia, which are mentioned as adverse for *Ceratium* development (Rengefors 1997, Pérez- Martinez & Sánchez-Castillo 2001 ; Pollingher 1988) were not detected in Río Tercero Reservoir.

Despite *Ceratium hirundinella* showed variable patterns within the vertical profile during the sampling period, this alga represented about 50% of the total phytoplankton density in both blooms (November 1999 and November 2000). During the sampling period, the zooplankton showed a decrease in species richness and abundance (Casco et al. 2002) probably associated with the increase of *C. hirundinella* and to the detriment of palatable algae.

The presence of possible grazers (Arndt 1993, Rengefors et al. 1998), which could have exerted pressure on the populations of *C. hirundinella*, was noted in the reservoir. *Asplanchna girodi* showed an increase in its populations, and became the dominant zooplankter in autumn 2000. At the same time, the abundance of the dinoflagellate diminished.

In Spanish reservoirs, the growth of *C. hirundinella* populations could be prevented in summer during an extremely high water outflow (Pérez-Martínez & Sánchez-Castillo 2001, 2002). In contrast to these results, and other reported by James et al. (1992) in a small reservoir of Winsconsin, we detected blooms of *C. hirundinella* in the Río Tercero Reservoir during outflow because it represented only 1% of total volume of reservoir by day.

The arrival of *Ceratium hirundinella* in Río Tercero

Reservoir was not associated with the Quillinzo River because this alga was found only in January and March 2000 in this tributary at low densities. Its contribution to the horizontal distribution of the populations in the reservoir was irrelevant. The cooling channel of the Nuclear Power Plant recycles water to thermal plume from the deeper layers of St2, where high densities of the dinoflagellate were recorded during the whole summer. Viable cells were observed in the channel and a circulation of organisms was promoted between these reservoir zones, enhancing the high densities at thermal plume and perhaps at St1.

It has been commonly stated that cyst formation occurs at the end of a bloom (Dottne-Lindgren & Ekbohm 1975, Heaney & Talling 1980, Pollingher et al. 1993). In the Río Tercero Reservoir, cysts were observed in the water column throughout the summer, coinciding with the great proliferation of vegetative cells. During autumn, the cysts disappeared from the water column at the same time as the density of adult cells diminished abruptly. In the Río Tercero reservoir the size of the inocula did not seem to have an effect on the final population maximum, in agreement with the results of Pollingher et al. (1993).

Since 1990 *C. hirundinella* was found in the southern part of South America both in lakes and reservoirs of different eutrophication degree and belonging to unrelated catchments (Table 3). Taking in account the dispersion pattern from higher to lower latitudes along 1,800 kilometers in 10 years, this event could be linked to the aeroplankton or dispersion mechanisms such as those associated with waterfowl. Finally we can conclude that the appearance of *C. hirundinella* in Neotropical water bodies can be considered as a regional phenomenon. We supposed that the massive cyst formation in summer 1999-2000 constituted the invasive phase of colonisation of this dinoflagellate in the reservoir. We assume that the colonisation or invasive phase of *C. hirundinella* into the reservoir has finished, and blooms with similar values to those detected in November 2000 may be expected in the following years. Similar densities of *C. hirundinella* to those estimated in this second bloom have been termed conspicuous blooms in some eutrophic lakes (Pollingher et al. 1993). We expect that this species will create blooms during subsequent summers, with similar dynamics, associated with some inputs from tributaries and germination of cysts from sediments for which the environmental conditions are always favourable in this reservoir. Meanwhile, the cause that promoted the decline of populations in winter remains unidentified.

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