

Spatiotemporal Variations in Abundance and Biomass of Planktonic Ciliates Related to Environmental Variables in a Temporal Pond, Argentina

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Gabriela C. Küppers and María C. Claps (2012) Spatiotemporal variations in abundance and biomass of planktonic ciliates related to environmental variables in a temporal pond, Argentina. *Zoological Studies* 51(3): 298-313. This report describes the structure and seasonal dynamics of ciliated protozoa associated with variations in physicochemical characteristics of the environment in a temporary pond in Buenos Aires Province, Argentina. Plankton samples were obtained, and physicochemical variables were measured monthly for 2 yr. In total, 50 planktonic ciliates were recorded. The highest species richness occurred during the pond's filling and stable-hydric phases. Upon the pond's desiccation, the number of ciliate species decreased, with the lowest values being recorded in spring; while the highest abundance and biomass values were observed before drought periods. Ciliate diversity tended to be higher after a drought but decreased with pond desiccation. Most ciliate species were rare and found during filling periods. *Vorticella convallaria*, *Pelagostrobilidium wilberti*, and *Coleps hirtus* were dominant; *Cyclidium glaucoma*, *Strobilidium caudatum*, *Pseudochilodonopsis piscatoris*, *Limnostrombidium viride*, *L. pelagicum*, and *Chilodonella* sp. were common; and *Pelagostrombidium mirabile* and *Rhabdostyla* sp., an epibiont on cladocerans, were occasional. The 1st axis and the sum of all axes in the canonical correspondence analysis explained a significant portion of the variance in the ciliate data. Autumn and winter samples were grouped together corresponding to the highest conductivities, high precipitation, and low temperatures, properties which characterized the filling and stable-hydric periods. Species were mainly distributed according to conductivity and temperature gradients along the 1st canonical axis. The structure and temporal dynamics of planktonic ciliates from this temporary pond varied with changes in the physicochemical characteristics of the environment which were determined by flooding and desiccation.
<http://zoolstud.sinica.edu.tw/Journals/51.3/298.pdf>

Key words: Ciliophora, Temporary water body, Buenos Aires Province, Ecology.

Temporary bodies of water are natural environments that experience recurrent drought phases, but with biota that are well adapted to deal with water loss through drought-survival mechanisms such as diapause and the formation of resting structures (Williams 2006). Bodies of water of this type are spatially and temporally heterogeneous, exhibiting diel variations in their physicochemical characteristics, such as temperature increases from insolation and pH fluctuations as a result of algal and macrophyte

photosynthesis (Podrabsky et al. 1998, Echaniz and Vignatti 2010). Ciliated protozoa are able to colonize and survive after the complete loss of water in such environments because many species can produce resting cysts under unfavorable conditions (Foissner 1987). Although ciliates are ubiquitous components of the microbial food web, and temporary waters can be widespread within Buenos Aires Province, Argentina during wet climatic conditions, ecological studies focusing on these microorganisms have not been carried out.

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In fact, ciliates from temporary bodies of freshwater have only scarcely been investigated worldwide (Andrushchyn et al. 2003, Williams 2006). In Argentina, ecological research on freshwater ciliates has been restricted to ultraoligotrophic Andean lakes of Patagonia (Modenutti 1997, Modenutti et al. 2000 2004 2005 2008, Modenutti and Pérez 2001, Modenutti and Balseiro 2002), with the rest of the territory remaining almost completely unexplored. Most of Buenos Aires Province is included within the Pampean Phytogeographic Province (Cabrera 1994) and is characterized by a wet temperate climate with a mean annual precipitation of 800-1100 mm (Auge et al. 2002). On the basis of the behavior of its subterranean waters, Buenos Aires Province mostly belongs to the wet Chacopampean Plateau Hydrogeologic Province, which contains one of the most exploited hydrogeologic units in the country, the Puelche Aquifer (Auge 2004). Under wet climatic conditions, temporary ponds and pools are widespread in this province with their water supply coming mainly from rainfall, although subterranean waters can also contribute to the water input.

The aim of the present study was to describe the structure and spatiotemporal dynamics of planktonic ciliated protozoa in a temporary pond, in relation to variations occurring in certain physicochemical characteristics of the environment.

MATERIALS AND METHODS

Study site and sampling

The study site is located about 40 km south of La Plata City, Buenos Aires Province, Argentina (35°05'S, 57°48'W), along one of the margins of Provincial Route 36 (Küppers et al. 2007). The pond is a temporary body of water that goes through prolonged drought phases in summer along with some minor pulses of short dry periods during an annual cycle. In stable-hydric periods, the pond is about 40 m long by 4 m wide, with a depth in the center of about 1 m. One area of the pond is colonized by aquatic vegetation, such as *Typha latifolia* L.; while the remaining areas either consist of open water or are covered by other macrophytes, such as *Ludwigia peploides* (Kunth) Raven and *Altenanthera philoxeroides* (Martius) Griseb., during certain periods. Sampling was conducted from Jan. 2004 to Dec. 2005 at 3 representative sites according to the depth and

retention of water by the substrate in order to test a possible desiccation gradient along the main axis of the pond. The shallowest zone, termed P1, tended to become dry faster than the others, while the center of the body of water and the zone dominated by *T. latifolia*, respectively called P2 and P3, were both deeper and retained more water.

Duplicate plankton samples were taken monthly by means of a wide-mouth 5-L bottle, from which 250 ml was fixed in situ with 2% (v/v) acetic Lugol's solution in order to perform a quantitative analysis (Finlay and Guhl 1992). The rest of the sample was qualitatively analyzed in the laboratory by means of live observations, establishment of cultures, and silver impregnation with protargol (see below).

Physicochemical variables

Temperature, pH, total dissolved solids (TDS), and electrical conductivity were measured with a multiparameter probe (Horiba U21, Kyoto, Japan) at each sampling site. The dissolved oxygen (DO) concentration was estimated by the Winkler method (Clesceri et al. 1998). The depth was also measured at each sampling site, and diel-rainfall data were obtained from the Servicio Meteorológico Nacional (available at <http://www.smn.gov.ar>).

Cell counting and identification of planktonic ciliates

Fixed samples were allowed to settle by gravity in 10-ml chambers for 24 h, in order to scan the contents of the entire chamber under an inverted microscope at magnifications of 150× and 600× (Utermöhl 1958). To identify ciliates, live samples were observed under stereo- and bright-field microscopes. Cultures were established in Petri dishes with bottled spring water along with crushed wheat kernels to promote bacterial growth as a food source for the ciliates. Silver impregnation with protargol was performed according to Wilbert (1975). The taxonomic scheme followed Lynn (2008) with identification mainly based on Kahl (1930 1931 1932 1935), Foissner et al. (1991, 1992, 1994, 1995, 1999), Foissner and Berger (1996), and specific taxonomic papers.

Biomass

Ciliate biomass was calculated from the numerical abundance, mean cell biovolumes, and

a carbon-conversion factor. Biovolumes were determined by measuring 30-150 Lugol-fixed cells of each species and approximating the cell shapes to geometrical figures (Hillebrand et al. 1999). This value was multiplied by a factor of 1.4 to correct for the shrinkage caused by the fixative used (Müller and Geller 1993). The carbon-conversion factor for ciliates is 0.19 pg C/ μm^3 for Lugol-fixed cells according to Putt and Steocker (1989).

Data analyses

The relative occurrence of species was determined by means of the Olmstead-Tukey test (Sokal and Rohlf 1979), which considers the abundance and frequency of occurrence of a species. Accordingly, species were classified as dominant when the relative abundance and frequency of occurrence were higher than the arithmetic mean for both parameters; common when the relative frequency of occurrence was higher than the corresponding arithmetic mean; occasional, when the relative abundance was higher than the corresponding arithmetic mean; and rare, when the relative abundance and frequency of occurrence were lower than their respective arithmetic means.

The Kolmogorov-Smirnov normality test was first performed (Zar 1996). Then, a factorial analysis of variance (ANOVA) was used to assess significant differences in physicochemical variables and ciliate abundance, biomass, and species richness throughout the study period and between sampling stations.

The diversity index of Shannon and Wiener (H') and evenness (E') (Magurran 1988) were calculated by means of the program, Mvsp 3.1 (Kovach 2002), in order to describe the relationship between the species richness and abundance distribution among species throughout the study period.

To elucidate the relationship between ciliate abundances and physicochemical variables, a multivariate analysis was conducted by means of the program CANOCO 4.1 (ter Braak and Šmilauer 1998). Ciliate-species data were analyzed by a detrended correspondence analysis to determine the length of the gradient of the axes. Since the gradient was 4.04 standard deviation units for the 1st axis, a canonical correspondence analysis (CCA) was performed (ter Braak and Prentice 1988). Colinearity among the environmental data was checked on the basis of the variance-inflation factor (VIF < 20). Finally, a stepwise forward

selection and Monte-Carlo permutation test (with 499 unrestricted permutations) were used to select environmental variables that were significantly correlated ($p < 0.05$) with the axes (ter Braak and Šmilauer 1998). The analysis was carried out with square-root-transformed numerical abundances and log-transformed physicochemical variables, except for pH. Species with an occurrence frequency of 1 and an abundance of < 500 individuals (ind.)/L were excluded from the analysis in order to minimize the influence of rare taxa. Before the CCA, Pearson's correlation coefficients were calculated to determine redundant parameters among the environmental variables which could then be discarded.

Optima and ranges of tolerance to the physicochemical variables were calculated for species with an occurrence frequency of > 20% by means of the respective equations for the weighted average and weighted standard deviation (Potapova and Charles 2003).

RESULTS

Hydric changes and succession of aquatic communities

During the study period, the body of water experienced 3 prolonged droughts, in the summers of 2004 (from Jan. to Apr.) and 2005 (from Jan. to Mar.) and from mid-spring 2005 through summer 2006, with minor drought pulses occurring in Oct. 2004 and May 2005. During these desiccation periods, amphibious macrophytes developed, such as *Alternanthera philoxeroides* and *Ludwigia peploides*. These plants persisted in the pond throughout the 1st dry summer, during the filling phase in autumn, and until July 2004. Site P3 was colonized by *Typha latifolia*, which tended to expand so as to occupy a progressively larger area by the end of the sampling period. During the stable-hydric phase in winter, the macrophytes decomposed to produce a major input of organic matter to the pond. Subsequently, in their absence, open waters predominated at sites P1 and P2, while *T. latifolia* continued to occupy a substantial area at P3.

Physicochemical variables

The water was well oxygenated (47%-90% oxygen saturation) with DO concentrations of 3.31-11.24 mg/L (Fig. 1). The temperature varied

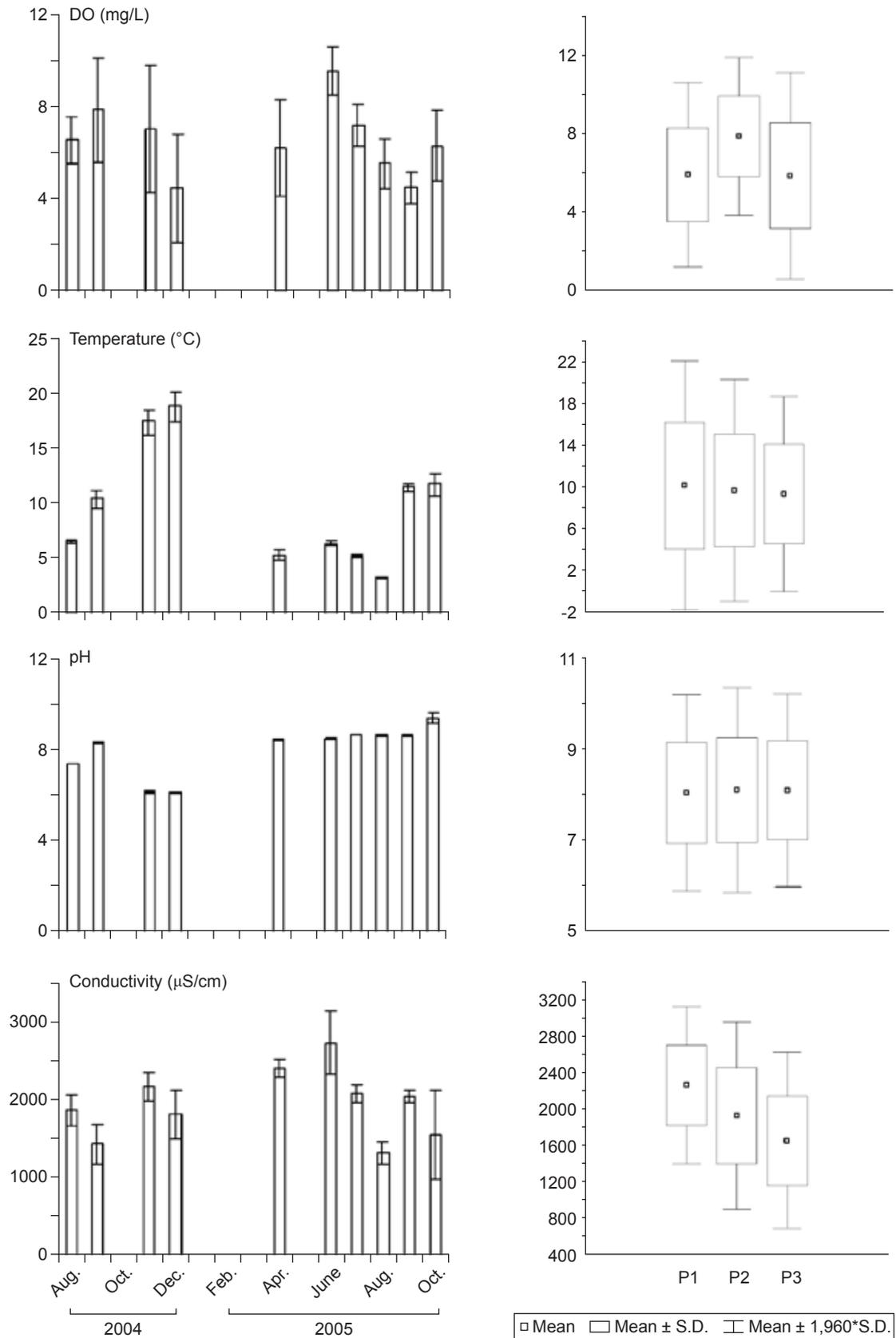


Fig. 1. Physicochemical variables in the temporary pond under study in 2004 and 2005.

markedly from one season to the next, ranging 3.27-21.2°C. Slightly acid to alkaline pHs were observed (6.13-9.7). In 2004, the conductivity was high before the summer drought with the highest values recorded at site P1 (2300-2410 $\mu\text{S}/\text{cm}$), whereas in 2005 the highest values were observed during the filling periods at P1 as well (2610-3210 $\mu\text{S}/\text{cm}$). The lowest conductivities were recorded at P3 before the summer drought in 2005 (700 $\mu\text{S}/\text{cm}$). In general, conductivity decreased from P1 to P3 (Fig. 1). TDS showed the same pattern of variation as conductivity, with values ranging 0.05-0.21 g/L. During 2005, the depth at the sampling sites was slightly higher than in 2004 (19-23 vs. 10-17 cm, respectively), but the time of water retention was lower from the time the pond dried out in Nov. rather than Jan. Site P1 tended to dry out before the other two, possibly because of higher water retention by the substrate at P3, since that site had progressively become colonized by *T. latifolia*. In terms of rainfall (Fig. 2), 2004 was the drier year, although the greatest rainfalls did occur in Apr. 2004 (248 mm), which tended to fill the pond. In 2005, 2 rainy periods occurred in spring/summer and autumn/winter, but perhaps high temperatures and evapotranspiration during the summer prevented the pond from completely filling. Throughout the sampling period, the conductivity ($F = 9.35$, $p = 0.000$), temperature ($F = 91.55$, $p = 0.000$), and pH ($F = 358.09$, $p = 0.000$) significantly varied; whereas between sampling sites, only the conductivity ($F = 15.1$, $p = 0.000$) and depth ($F = 6.64$, $p = 0.007$) exhibited significant differences.

Species richness, abundance, and biomass

In total, 50 planktonic ciliates were recorded, belonging to 18 orders (Table 1). The Sessilida,

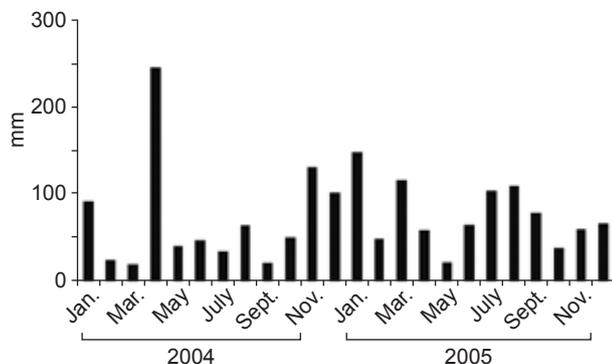


Fig. 2. Monthly accumulated rainfall amounts in the area of the sampling site.

Haptorida, Peniculida, Chlamyodontida, Strombidiida, and Choreotrichida were the most species-rich orders. The highest species richness occurred during the filling and stable-hydric phases in the winter of both years. With increases in temperature and the consequent desiccation of the pond, the number of ciliate species decreased with the lowest values recorded in spring (Fig. 3). In contrast, the highest abundances were observed before the droughts (49,100 ind./L in Sept. 2004 at P3 and 37,350 ind./L in Oct. 2005 at P2), while generally higher values were recorded at P3 (Fig. 3). In 2004, the Halteriida (43.2%), Prorodontida (22.6%), and Strombidiida (17.7%) were the most abundant orders. In 2005; however, the Halteriida (30.9%), Sessilida (19.6%), Choreotrichida (8.9%), and Strombidiida (7.4%) were the most abundant. The remaining orders represented < 5% of the total abundances during both years. The species that made the greatest contributions to the relative abundance of each order were *Halteria grandinella* (Halteriida); *Coleps hirtus* (Prorodontida); *Pelagostrombidium mirabile*, *Limnostrombidium viride*, and *L. pelagicum* (Strombidiida); *Vorticella convallaria* and *Rhabdostyla* sp. (Sessilida); and *Pelagostrobilidium wilberti* and *Strobilidium caudatum* (Choreotrichida).

The highest biomass values were likewise recorded before the droughts (1881 $\mu\text{g C}/\text{L}$ in Sept. 2004 at P3 and 1529 $\mu\text{g C}/\text{L}$ in Oct. 2005 at P2), but a higher biomass was present at P3 throughout the sampling period (Fig. 3). In 2004, the most plentiful orders in terms of biomass were the Strombidiida (30.1%), Prorodontida (12.9%), Sessilida (11.1%), Halteriida (10.7%), and Peniculida (10.5%); while in 2005 the corresponding data were the Sessilida (21.3%), Choreotrichida (16.6%), Strombidiida (16.3%), and Halteriida (13.3%). Species that made the greatest contributions to the total biomass were *Pelagostrombidium mirabile* (Strombidiida); *Pelagostrobilidium wilberti* and *Strobilidium caudatum* (Choreotrichida); *Coleps hirtus* (Prorodontida); *Vorticella convallaria* and *Rhabdostyla* sp. (Sessilida); *Halteria grandinella* (Halteriida); and *Frontonia atra*, *F. leucas*, and *Paramecium caudatum* (Penculida).

Throughout the study period, the number of ciliate species, the abundance, and the biomass significantly varied ($F = 3.65$, $p = 0.009$; $F = 4.19$, $p = 0.005$; $F = 2.98$, $p = 0.026$, respectively), while between sampling sites, differences were not statistically significant.

Diversity

The greatest diversity ($H' = 0.97$) was recorded during a stable-hydric phase in July 2005, characterized by both a high species richness and evenly distributed abundances ($E' = 0.76$). On the contrary, the lowest diversity and evenness were observed in Dec. before the summer drought of 2004 ($H' = 0.18$; $E' = 0.19$). In Nov. 2004, after a short drought interval, ciliate diversity and evenness reached maximum values ($H' = 0.76$; $E' = 0.76$), but the species richness was relatively low, compared to that recorded during stable-hydric periods. In general, the diversity tended to

be higher or at a maximum after droughts, but then decreased along with the desiccation of the pond (Fig. 4).

Occurrence of ciliate taxa

Most (85%) of the planktonic ciliates from the temporary pond under study were rare, i.e., these taxa occurred at low abundances and frequencies (Table 1, Fig. 5). *Halteria grandinella*, hypotrichs, *Vorticella convallaria*, *Pelagostrobilidium wilberti*, and *Coleps hirtus* were dominant; while *Cyclidium glaucoma*, *Strobilidium caudatum*, *Pseudochilodonopsis piscatoris*, *Limnostrombidium*

Table 1. Species list, frequency of occurrence (Occ.), and optima and tolerance range of species with an occurrence frequency of > 20%

Taxa	Occ. (%)	Optima and tolerance range			
		DO	Temp.	pH	Cond.
Order Heterotrichida					
<i>Blepharisma americanum</i> (Suzuki) Hirshfield, Isquith & Bhandary	1	-	-	-	-
<i>Stentor</i> sp.	1	-	-	-	-
Order Euplotida					
<i>Aspidisca cicada</i> (O.F. Müller) Claparède & Lachmann	15	-	-	-	-
<i>Euplotes</i> sp.	11	-	-	-	-
<i>Euplotoides</i> sp.	6	-	-	-	-
Order Choreotrichida					
<i>Pelagostrobilidium wilberti</i> Küppers, Lopretto & Claps	55	8.1 ± 2.2	7 ± 3	7.8 ± 1.4	2012.5 ± 658.4
<i>Rimostrombidium brachykinetum</i> Krainer	1	-	-	-	-
<i>Rimostrombidium</i> sp.	1	-	-	-	-
<i>Strobilidium caudatum</i> (Fromentel) Foissner	36	6.2 ± 2.1	6.1 ± 1.4	7.9 ± 1.2	2200 ± 457
Order Sporadotrichida					
<i>Stylonychia mytilus</i> (O.F. Müller) Ehrenberg	10	-	-	-	-
Other hypotrichs	85	-	-	-	-
<i>Halteria grandinella</i> (O.F. Müller) Dujardin	95	7 ± 3	7 ± 4	7.4 ± 1.7	1820 ± 542
Order Strombidiida					
<i>Limnostrombidium pelagicum</i> (Kahl) Krainer	30	7.4 ± 1.3	7.7 ± 1.7	6.8 ± 1.3	1686.7 ± 369.6
<i>L. viride</i> (Stein) Krainer	30	7.4 ± 1.3	7.7 ± 1.7	6.8 ± 1.3	1686.7 ± 369.6
<i>Limnostrombidium</i> sp.	1	-	-	-	-
<i>Pelagostrombidium mirabile</i> (Penard) Krainer	23	7.4 ± 2.5	9.2 ± 2.5	8.1 ± 1.6	1530 ± 393
Order Armophorida					
<i>Brachonella spiralis</i> (Smith) Jankowski	1	-	-	-	-
Order Haptorida					
<i>Amphileptus</i> sp.	5	-	-	-	-
<i>Chaenea</i> sp.	3	-	-	-	-
<i>Dileptus</i> sp.	13	-	-	-	-
<i>Spathidium</i> sp.	5	-	-	-	-

Table 1. (continued)

Taxa	Occ. (%)	Optima and tolerance range			
		DO	Temp.	pH	Cond.
<i>Lacrymaria olor</i> (O.F. Müller) Bory de Saint-Vincent	13	-	-	-	-
<i>Lagynophrya</i> sp.	3	-	-	-	-
Order Pleurostomatida					
<i>Litonotus</i> sp.	1	-	-	-	-
Order Cyclotrichiida					
<i>Askenasia volvox</i> (Eichwald) Kahl	13	-	-	-	-
Order Chlamydomontida					
<i>Chilodonella</i> sp.	31	8.5 ± 3.4	13.5 ± 5.4	7.2 ± 1.2	2005.7 ± 516.1
<i>Chilodonella</i> cf. <i>caudata</i>	20	-	-	-	-
<i>Gastronauta membranaceus</i> Bütschli	3	-	-	-	-
<i>Pseudochilodonopsis piscatoris</i> (Blochmann) Foissner	30	8 ± 2	6.9 ± 4.1	7.5 ± 1.5	2177 ± 373
Order Microthoracida					
<i>Drepanomonas</i> sp.	3	-	-	-	-
<i>Microthorax</i> sp.	5	-	-	-	-
Order Colpodida					
<i>Colpoda cucullus</i> (O.F. Müller) Gmelin	13	-	-	-	-
<i>Colpoda inflata</i> (Stokes) Kahl	1	-	-	-	-
Order Cyrtolophosidida					
<i>Platyophrya</i> sp.	3	-	-	-	-
Order Prorodontida					
<i>Coleps hirtus</i> (O.F. Müller) Foissner, Berger & Schaumburg	45	4.3 ± 2.5	16.3 ± 4.7	7.0 ± 1.4	1800 ± 376
<i>Urotricha globosa</i> Schewiakoff	1	-	-	-	-
<i>Urotricha</i> sp.	18	-	-	-	-
Order Peniculida					
<i>Frontonia atra</i> (Ehrenberg) Bütschli	15	-	-	-	-
<i>Frontonia leucas</i> (Ehrenberg) Ehrenberg	8	-	-	-	-
<i>Lembadion lucens</i> (Maskell) Kahl	1	-	-	-	-
<i>Paramecium aurelia</i> O.F. Müller	5	-	-	-	-
<i>P. caudatum</i> Ehrenberg	11	-	-	-	-
Order Philasterida					
<i>Cinetochilum margaritaceum</i> (Ehrenberg) Perty	13	-	-	-	-
Order Pleuronematida					
<i>Cyclidium glaucoma</i> O.F. Müller	35	8.3 ± 2.2	6.9 ± 3.3	8.5 ± 0.5	2371 ± 500
<i>Cyclidium</i> sp.	5	-	-	-	-
Order Sessilida					
<i>Epistylis</i> sp. 1	3	-	-	-	-
<i>Epistylis</i> sp. 2	1	-	-	-	-
<i>Epistylis</i> sp. 3	6	-	-	-	-
<i>Rhabdostyla</i> sp.	5	-	-	-	-
<i>Vorticella convallaria</i> (Linnaeus) Linnaeus	75	5.2 ± 2.6	4.9 ± 3.4	6.4 ± 3.1	2108 ± 405
<i>Vorticella</i> sp.	3	-	-	-	-

Cond., conductivity ($\mu\text{S}/\text{cm}$); DO, dissolved oxygen (mg/L); Temp., temperature ($^{\circ}\text{C}$).

viride, *L. pelagicum*, and *Chilodonella* sp. were common. The euplanktonic species *Pelagostrombidium mirabile* and *Rhabdostyla* sp., an epibiont on cladocerans, were occasionally found. Among the dominant taxa, *Halteria grandinella* was present in the pond throughout the entire study period at abundances ranging 400-16,970 ind./L. The peak value, which occurred

in Sept. 2004, represented 59% of the total abundance recorded during that month. In 2005, *H. grandinella* reached maxima in Aug. (7270 ind./L) and Oct. (7470 ind./L), and constituted 62% and 29% of the respective total abundances recorded during those months (Fig. 3). Hypotrichs were frequent in the pond at abundances ranging 67-1900 ind./L and peaked in Nov. 2004 (883 ind./L,

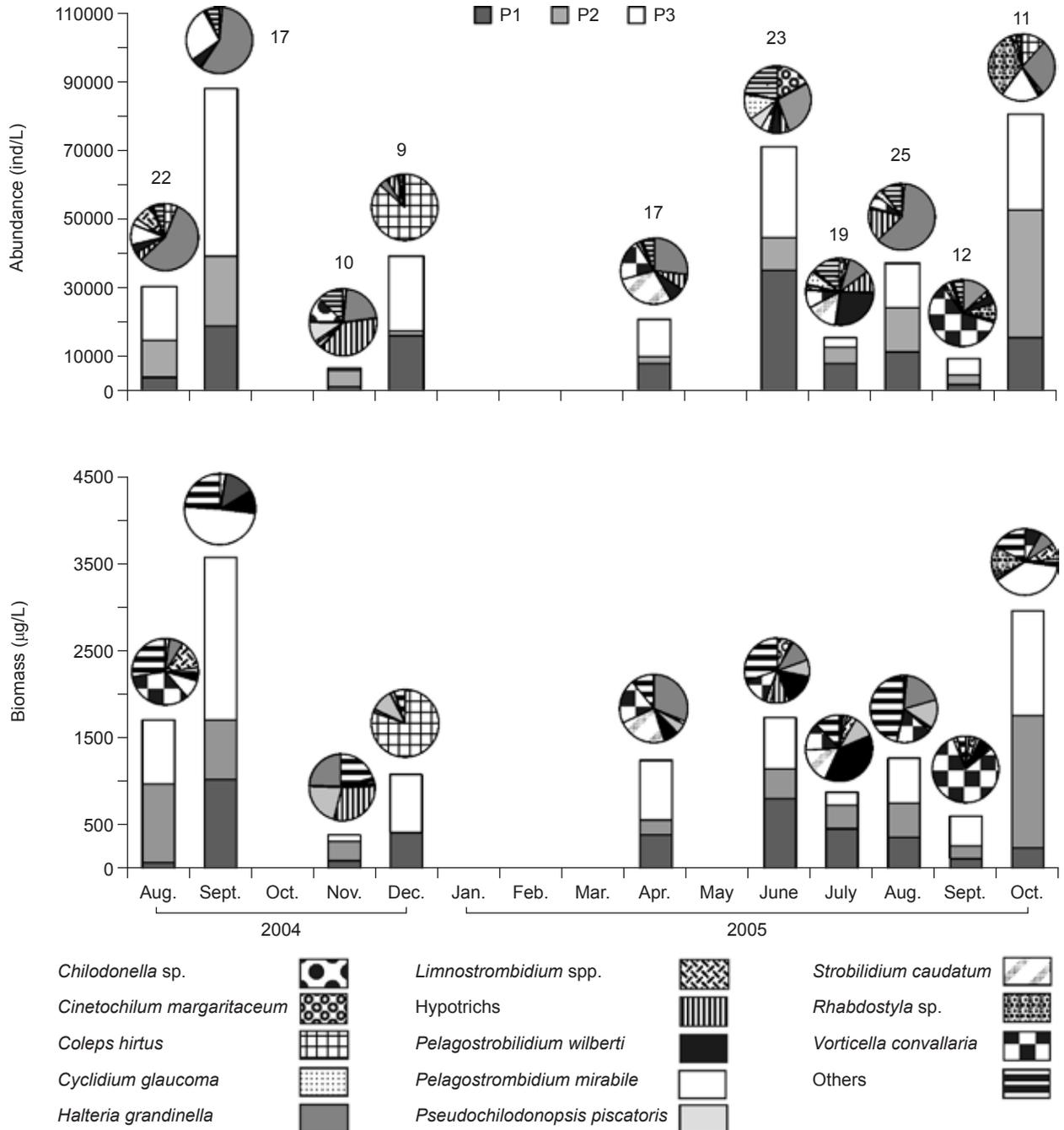


Fig. 3. Absolute and percent (pie plots) abundance and biomass values of ciliate species. Numbers above the abundance bars indicate the species richness.

at 40% of the total abundance) and Aug. 2005 (1900 ind./L, at 17% of the total abundance). *Vorticella convallaria* was more abundant in 2005 than in 2004 (133-1850 ind./L vs. 50-317 ind./L, respectively) and peaked in Apr. (1417 ind./L, at 20% of the total abundance) and Sept. 2005 (1850 ind./L, at 61% of the total abundance). In that last month, its biomass of 287 µg C/L represented 80% of total biomass recorded for the month (Fig. 3). *Pelagostrobilidium wilberti* was described as a new species in this pond (Küppers et al. 2006), and in the present study, occurred at abundances that ranged 33-1467 ind./L.

Maximum abundances were recorded in Sept. 2004 (1467 ind./L, at 5% of the total abundance) and July 2005 (1400 ind./L, at 26% of the total abundance) along with respective maximum biomasses of 116 and 111 µg C/L. In July 2005, the biomass of *P. wilberti* represented 38% of the total biomass recorded that month (Fig. 3). *Coleps hirtus* had 2 abundance maxima, first in Dec. 2004 (11,400 ind./L, at 87% of the total abundance) and then in Oct. 2005 (3150 ind./L, at 12% of the total abundance), before prolonged droughts on both occasions. The highest biomass of this species likewise occurred in Dec. 2004 (292 µg C/L, at 80% of the total biomass) and in Oct. 2005 (81 µg C/L; Fig. 3).

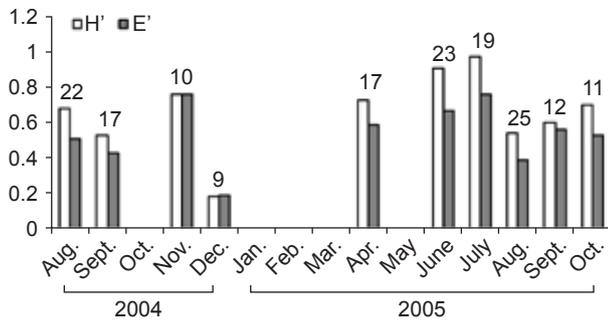


Fig. 4. Ciliate diversity (Shannon and Wiener index, H') and evenness (E') over the study period.

Among the common taxa, *Cyclidium glaucoma* exhibited the highest abundance in June 2005 (2950 ind./L, at 13% of the total abundance) and the highest biomass (5.62 µg C/L). *Strobilidium caudatum* ranged in abundance from 17 to 2070 ind./L, with maximum values recorded in Apr. and July 2005 (2070 and 780 ind./L, respectively), representing 28% of the total abundance in Apr. Whereas the maximum biomass of *S. caudatum* in 2004 was 12 µg C/L during Sept., in 2005, the value reached 135 µg C/L (at 23% of the total abundance) during

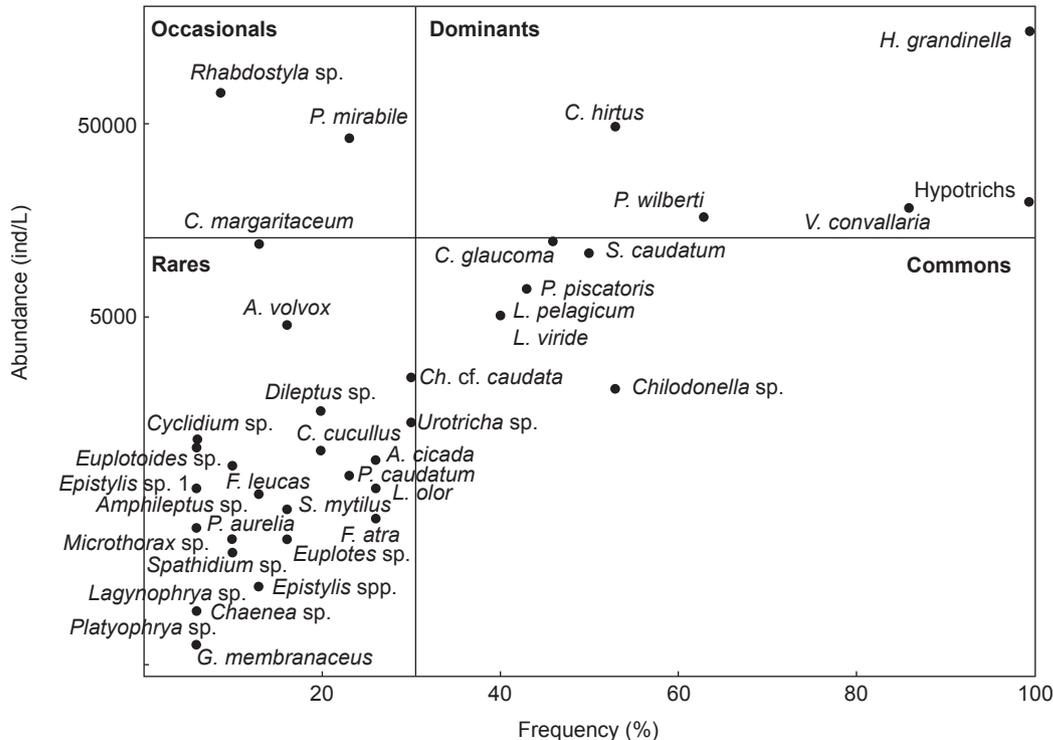


Fig. 5. Frequency of occurrence of ciliate species according to the Olmstead-Tukey test.

Apr. (Fig. 3). *Pseudochilodonopsis piscatoris* peaked in June 2005 (1650 ind./L) after a short drought pulse during May; likewise, the highest biomass of that species (51 $\mu\text{g C/L}$) was recorded during the same month. *Limnostrombidium* spp. (i.e., *L. viride* and *L. pelagicum*) reached abundances that varied 17-850 ind./L, but maximum abundance values were recorded in Aug. 2004 (850 ind./L) and Oct. 2005 (567 ind./L) along with peak biomass values (113 and 75 $\mu\text{g C/L}$, respectively) in those same 2 mo. *Chilodonella* sp. reached a maximum abundance in Nov. 2004 (280 ind./L); but in 2005, this species exhibited very low, although less variable, abundances (30-50 ind./L) and biomasses (0.31-0.5 $\mu\text{g C/L}$; Fig. 3).

Among the occasional taxa, *P. mirabile* was recorded from July to Sept. 2004, with maximum abundance and biomass in Sept. (7870 ind./L, at 27% of the total abundance and 601 $\mu\text{g C/L}$, at 50% of the total biomass). In 2005, this species was only recorded in Oct. (4970 ind./L, at 18% of the total abundance; 379 $\mu\text{g C/L}$, at 39% of the total biomass). *Rhabdostyla* sp. was registered as an epibiont on cladocerans, but only twice: in Sept. and Oct. 2005, although with a high abundance before a drought period (9217 ind./L, at 35% of the total abundance). In the latter month, the biomass of *Rhabdostyla* sp. represented 15% of the total biomass recorded during that month (Fig. 3).

Ciliate assemblages relative to environmental variables

According to the CCA, ciliate assemblages located along the 1st axes were mainly related to conductivity, rainfall, and temperature. The 1st canonical axis and the sum of all canonical axes explained a significant portion of the variance in ciliate data (F -ratio = 3.21, p = 0.01 and F -ratio = 2.52, p = 0.002, respectively). The environmental variables that were significantly correlated with the canonical axes after performing the Monte-Carlo permutation test were rainfall (F -ratio = 2.82, p = 0.006), conductivity (F -ratio = 2.66, p = 0.006), temperature (F -ratio = 2.43, p = 0.002), DO (F -ratio = 2.32, p = 0.008), and pH (F -ratio = 2.12, p = 0.008). Although inflation factors of all variables included in the analysis were < 20 deviation units, the parameter of TDS was excluded because of its direct relationship with conductivity. The 1st canonical axis explained 34% of the variance of the species-environment relationship, while the 2nd axis accounted for 61% of the cumulative variance.

Conductivity, rainfall, and temperature were correlated with the 1st axis (R = -0.66, -0.59, and 0.55, respectively), while DO was correlated with the 2nd axis (R = 0.66). The pH was correlated with the 3d axis (R = 0.64). Autumn and winter samples were coupled together in relation to the highest conductivity values, high rainfall amounts, and low temperatures, which characterized the filling and stable-hydric periods of the pond (Fig. 6). The spring assemblage (mostly samples from Sept. 2004) was positively related to high pH values and DO concentrations as well as the lowest conductivity and rainfall. This assemblage was related to desiccation of the pond that occurred in Oct. 2004 and was more pronounced during 2005, when the pond completely dried in early Nov. Finally, samples of late spring and early summer of 2004 were grouped in the positive quadrant of the biplot, together with spring 2005 samples (Fig. 6). This assemblage was related to the filling phase, a short drought interval having occurred in Oct. 2004, followed by a refilling of the pond in Nov., but also reflected desiccation conditions, because a complete drought occurred in both Jan. and Nov. 2005. This unstable period was characterized by high temperatures and low DO concentrations and pH values. The species were mainly distributed according to conductivity and temperature gradients along the 1st canonical axis (Fig. 7). *Litonotus* sp., *Drepanotrema* sp., *Cinetochilum margaritaceum*, and *Euplotes* sp. were correlated with the highest conductivities and lowest temperatures, while the contrary occurred with *Rhabdostyla* sp., *Amphileptus* sp., and *P. mirabile*. *Brachionella spiralis* and *C. hirtus* were associated with the lowest DO concentrations and pH values, whereas *Dileptus* sp., *Gastronauta membranaceus*, and *Askenasia volvox* preferred the highest levels of these variables. Most of the dominant and common species were distributed in the filling and stable-hydric periods preferring medium to high conductivity values and low temperatures.

DISCUSSION

According to Williams (2006), the definition of a temporary body of water requires a consideration of the cyclic nature of related droughts, with the duration and intensity of the droughts constituting the best criteria for assigning the term, "temporary". In the pond under study, intense drought periods occurred during the summers, although some

brief dry periods took place in autumn and spring with the persistence of scattered small hollows of water. Hence, the pond underwent annual seasonal droughts during the period of time studied, alternating between wet and dry periods in a predictable pattern.

The water temperature exhibited a wide range of variation as a result of the low depth of the pond and elevated light penetration. Fluctuations in pH values registered may have been related to the accumulation of organic matter before the summer droughts and to the development of aquatic macrophytes, such as *A. philoxeroides* and *L. peploides*, plants which are commonly found in pampean shallow lakes and streams (Lahitte et al. 1997). Values of conductivity and TDS were higher than those characteristic of ponds in which the water supply comes mainly from rainfall (Gibbs 1970, Baca and Threlkeld 2000), thus indicating a possible connection with saline subterranean water. The Puelche and Pampeano aquifers are

characterized by high salinity levels (Auge 1997 2005) and thus possibly contributed to filling the pond. Echaniz and Vignatti (2010) recorded conductivities that ranged 310-3730 $\mu\text{S}/\text{cm}$ in an episodic wetland from La Pampa Province, where the highest values occurred during desiccation periods and were attributable to a concentration process through evaporation. In the pond we studied, the greatest conductivities were recorded during a desiccation period in 2004; this period was characterized by high temperatures, low levels of precipitation, and the consequent concentration of organic matter and ions. In contrast, in 2005, when precipitation increased, the higher conductivity values recorded during the filling and part of the stable-hydric period of the pond were possibly due to infiltration by aquifer water. This latter input would also explain the high conductivities recorded during that period, when dilution from the rainfall would otherwise have been expected.

Along the longitudinal axis of the pond,

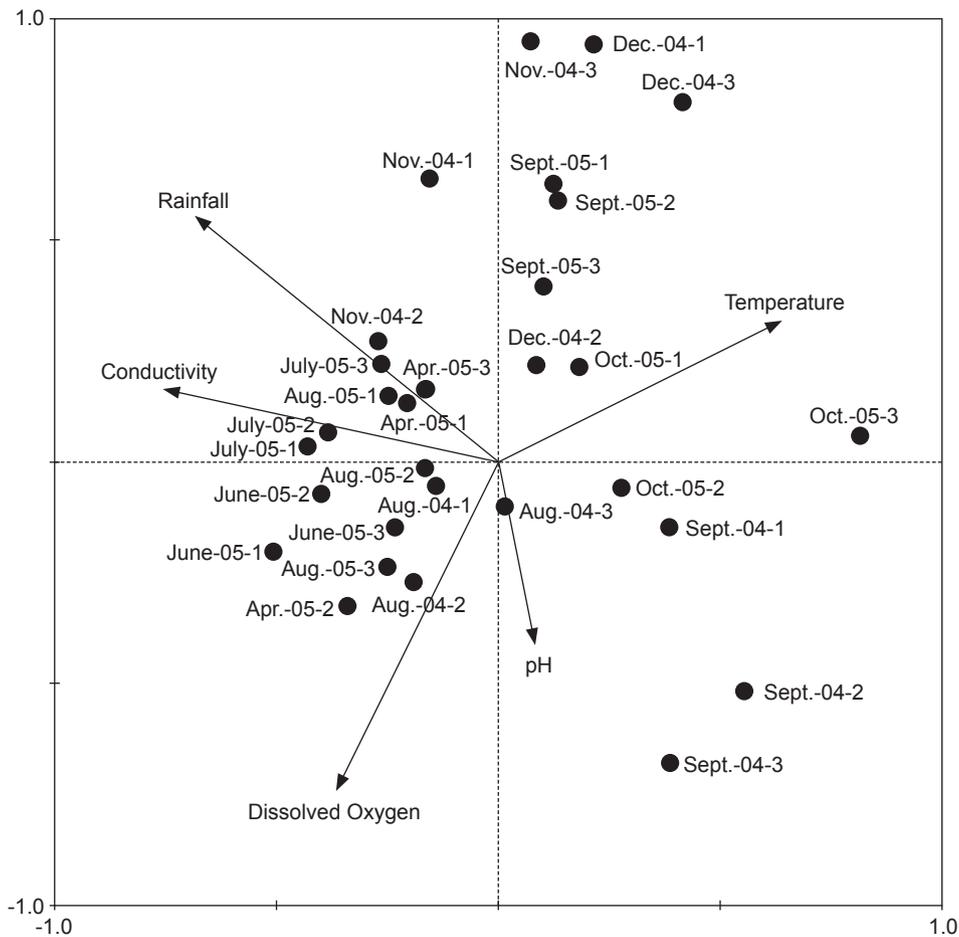


Fig. 6. Canonical correspondence analysis scatterplot of samples and environmental variables. Labels indicate month, year, and sampling point.

from sites P1 to P3, significant differences were observed in conductivity and depth, thus constituting a possible spatial desiccation gradient extending from site P1, which usually dried out earlier than the other 2 sites, through sites P2 to P3, where the persistent presence of *T. latifolia* may have contributed to higher water retention by sediments. Despite these differences, ciliate abundance and biomass values did not

significantly differ between sampling sites. In contrast, significant temporal differences were observed in ciliate abundance and biomass values at each of the sites and in values of temperature, pH, conductivity, and TDS.

Because, as stated earlier, most ecological studies on freshwater ciliates from Argentina were restricted to ultraoligotrophic Andean lakes (Modenutti 1997, Modenutti et al. 2000 2004 2005

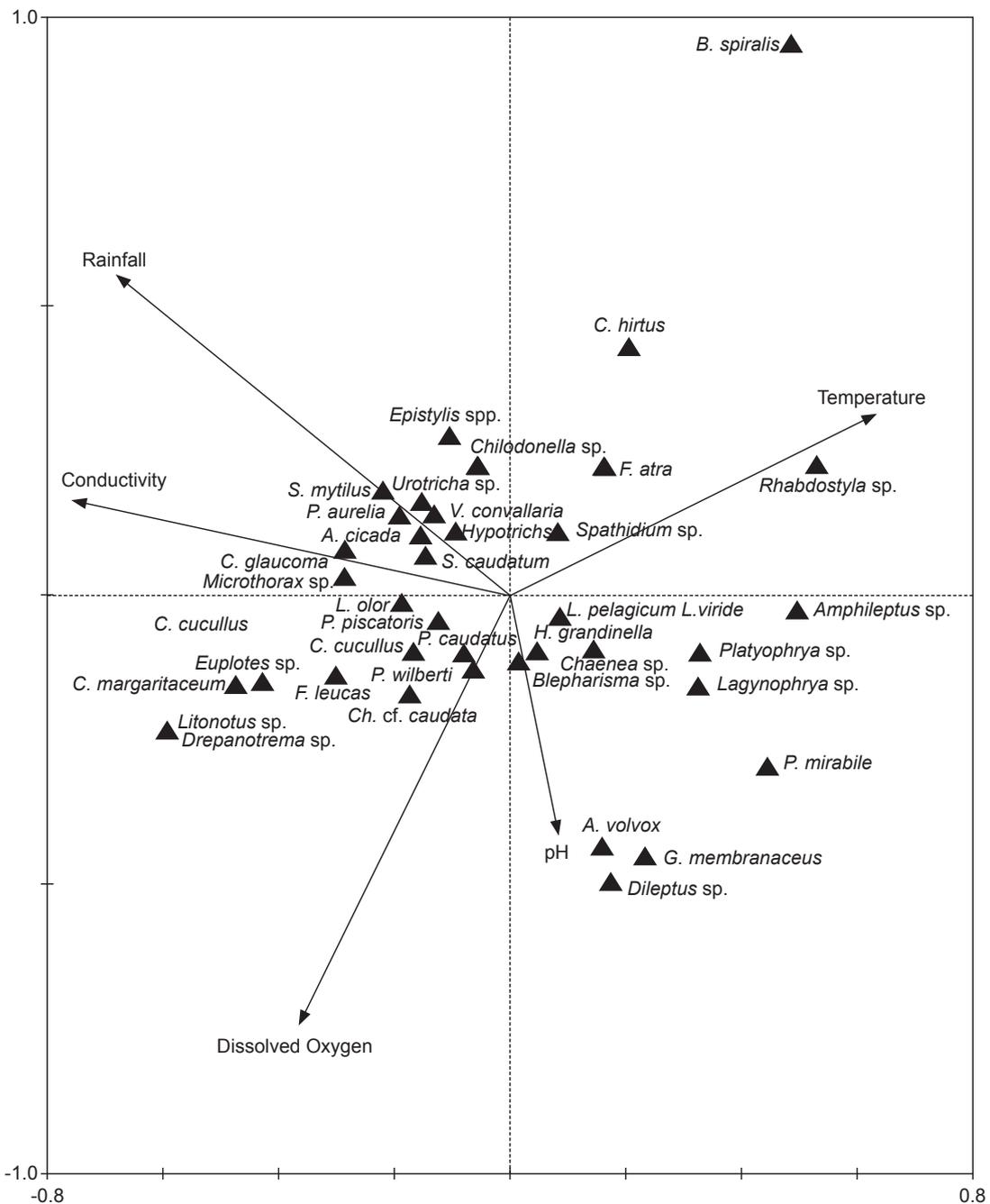


Fig. 7. Canonical correspondence analysis scatterplot of species and environmental variables.

2008, Modenutti and Pérez 2001, Modenutti and Balseiro 2002), and the ciliate assemblages from temporary ponds are barely studied worldwide (Andrushchyshyn et al. 2003), comparisons of abundance and biomass values are not possible. Nevertheless, most of the dominant groups of ciliates recorded in this temporary pampean pond were comparable to those found by other authors in permanent eutrophic to mesotrophic ponds and lakes from other geographic locations (Hatano and Watanabe 1981, Laybourn-Parry et al. 1990, Müller et al. 1991, Sime-Ngando and Hartmann 1991, Song 2000, Munawar and Lynn 2002, Carrick 2005, Mieczan 2007). Permanent lakes are typically dominated by members of the Haptorida, Scuticociliatida (Pleuronematida in this study), and Oligotrichida (Halteriida, Choreotrichida, and Strombidiida in this study; Beaver and Crisman 1989). In contrast, in the present investigation, haptorids such as *Amphileptus* sp., *Chaenea* sp., *Dileptus* sp., *Spahtidium* sp., *Lacrymaria olor*, and *Lagynophrya* sp. were recorded only rarely, but the scuticociliate, *Cyclidium glaucoma*, was quite common. In the temporary pond studied here, prorodontids were particularly abundant during the spring, and *Coleps hirtus* was the species that contributed the most to the total abundance. Hatano and Watanabe (1981) found *Coleps* sp. to be dominant in a small pond with an input of leaf litter, although in laboratory experiments, this ciliate was seen to increase in abundance when supplied with dead copepods as food. *Coleps hirtus*, a bacterivore-detritivore, is histophagous on dead vegetal and animal matter (Foissner et al. 1999). In this pond, this species seemed to find optimal conditions for developing its populations in the spring, when the environment was drying out. *Halteria grandinella* was the most abundant species during autumn and winter. This species is cosmopolitan and is found in a wide range of habitats (Foissner et al. 1999). *Limnostrombidium pelagicum* and *L. viride* were common during autumn and winter 2004, but *Pelagostrombidium mirabile* replaced those species with an increase in temperature in spring.

The highest ciliate abundance and biomass values in this temporary pond occurred before drought periods, with the most abundant being *H. grandinella* and species of the Strombidiida and Sessilida (> 20 µm in length). Song (2000) observed a considerably higher abundance and biomass of ciliates in the lake Houhu (China), with maximum values of 87,710 ind./L and 3013.58 µg C/L and the most abundant members being

scuticociliates (*C. glaucoma*) followed by tintinnids and “naked oligotrichs”. In contrast, Sime-Ngando and Hartmann (1991) observed considerably lower ciliate abundance and biomass values in Lake Aydat (France) compared to values from the present study, with maxima of around 7800 ind./L and 190 µg C/L, with the dominant ciliates being scuticociliates (mainly *Cyclidium* sp.) and small oligotrichs (< 50 µm in length).

In the CCA, ciliates were distributed along conductivity and temperature gradients and were grouped in assemblages that corresponded to (i) filling of the pond and the stable-hydric phase after summer droughts, (ii) desiccation of the pond (mainly spring samples), (iii) filling periods after a short drought interval, and (iv) complete prolonged desiccation during the summer that followed. Most dominant and common species were distributed in the filling and stable-hydric periods, and appeared to prefer medium to high conductivities, low temperatures, and high DO. Taxa like *Drepanotrema* sp., *Cinetochilum margaritaceum*, *Euplotes* sp., *Stylonychia mytilus*, and other hypotrichs, as well as *Cyclidium glaucoma*, are mostly bacterivorous; and their presence may have been related to the decomposition of macrophytes that persisted in the pond after the drought as a result of the development of bacteria. *Cyclidium glaucoma* is known to tolerate a wide salinity range (Finlay et al. 2006). This species was found at higher conductivity values in the pond. *Coleps hirtus* and *Brachonella spiralis* were related to the highest temperatures and lowest DO concentrations. *Brachonella spiralis* is known to inhabit anoxic and sulfide-rich environments (Foissner et al. 1992), and those conditions possibly prevailed at the time before the pond's complete desiccation. The histophagous detritivore *C. hirtus*, could have taken advantage of concentrations of dissolved and suspended matter before the summer drought. The occasional *Rhabdostyla* sp. and *P. mirabile* were mainly related to lower conductivities and rainfall that occurred during desiccation of the pond in spring.

Maximum diversity was observed during the filling and stable-hydric phases of the pond together with a high species richness and evenness. The diversity tended to decrease toward the end of the hydroperiod, with the lowest values recorded before a drought in addition to the lowest evenness data, thus indicating the presence of dominant species. Such species were *C. hirtus* in Dec. 2004 and *Rhabdostyla* sp. and *H. grandinella* in Oct. 2005. According to the

intermediate-disturbance hypothesis, diversity maximizes at intermediate levels of perturbation so as to allow the coexistence of competitive and opportunistic species (Connell 1978). If the entire cycle of filling and desiccation of the pond is considered, a disturbance gradient could be defined where only the right-hand side of Connell's bell-shaped curve of disturbance is detected (Adams 2009) upon inspection of figure 4. In this respect, species richness and diversity tended to decline with greater and/or more-frequent disturbances. Many ciliates are able to survive unfavorable environmental conditions by forming resting cysts (Foissner 1987). These dormant structures then persist in the dried bed of the pond until rainfall refills it with water in the next hydroperiod. If the availability of water is to be considered one of the most influential conditions for the development of ciliates in this temporary pond, the filling phase would promote massive excystations of ciliates, with a consequent high species richness ensuing after a drought. During the stable-hydric period, more-complex processes of intra- and interspecific interactions may take place, such as competition and predation, which could have resulted in the dominance of 1 species over others. Finally, when the environment has become unfavorable through desiccation of the pond, along with the consequent changes in physicochemical and biological variables that take place under such conditions, only a few species could persist. Accordingly, repopulation of the pond by most species occurred when the pond was refilling, and they were furthermore considered rare according to the Olmstead-Tukey test. During the rest of the hydroperiod, certain dominant and commonly found species prevailed, such as *H. grandinella*, the hypotrichs, *Vorticella convallaria*, *C. hirtus*, *Pelagostrobilidium wilberti*, *Strobilidium caudatum*, *L. pelagicum*, *L. viride*, and *C. glaucoma*. Ultimately, only a small number persisted, such as *C. hirtus* and *H. grandinella*, with the appearance of an occasional few, such as *Rhabdostyla* sp., after the pond's desiccation. This type of habitat is considered a suitable system for testing ecological concepts (Williams 2006), so the present study could be complemented, in the future, by further laboratory experiments in order to investigate the various assumptions postulated above.

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