Cytomorphometric characterization of a population of *Ceratium hirundinella* fa. a*ustriacum* (Dinophyta) during a bloom in a reservoir of the Province of Buenos Aires, Argentina

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With 4 figures and 3 tables in the text

ABSTRACT

Ceratium hirundinella (O. F. MÜLLER) DUJARDIN is a dinoflagellate frequently found in freshwater plankton in Argentina. It may form -together with cyanobacteria- massive blooms, particularly in autumn-winter. From 1997 until now, *C. hirundinella* appeared in Paso de las Piedras reservoir, Province of Buenos Aires, bringing about problems to the water-for-consumption potabilization process. On account of the fact that *C. hirundinella* is an extremely variable species, we have attempted for the first time on this species a statistical analysis of individuals during a massive bloom in May 2000. The specific aims of the present study were i) to determine the average biovolume and ii) to determine whether or not there are correlations among the dimensions of the different horns. The present statistical characterization allowed us to conclude that the studied population belongs to *C. hirundinella* fa. *austriacum.*

All samples analyzed had four horns. The statistical analysis of the data collected in the present study indicates that the length of the fourth horn is the one that exhibits the highest relative variance. In previous studies, no such a marked positive correlation between the lengths of the antapical and postequatorial horns was detected as that observed in our study by means of the principal component analysis.

Keywords: Biovolume, bloom, *Ceratium hirundinella, Ceratium hirundinella* fa. *austriacum,* cytomorphometry.

Running title: *Ceratium hirundinella* fa. *austriacum* cytomorphometry.

INTRODUCTION

Ceratium hirundinella (O. F. MÜLLER) DUJARDIN is a dinoflagellate frequently found in freshwater plankton. Together with cyanobacteria it may form massive blooms (CANTER-LUND & LUND 1995). In temperate areas of the northern hemisphere, these massive blooms occur mainly in summer (HUTCHINSON 1967; SIGEE et al.1998). In the southern hemisphere and particulary in Argentina *C. hirundinella* forms massive blooms in autumn-winter (BUSTAMANTE et al. 2000; PRÓSPERI 2000).

It is one of the most abundant organisms in nitrogen- and phosphate-enriched environments (CANTER-LUND & LUND 1995) as well as in hard water reservoirs and eutrophic lakes (GRAHAM & WILCOX 2000). Populations consisting of motile, flagellate stages become important either in summer or in winter, whereas during the rest of the year they remain as benthic cysts in sediments (REGENFORS & ANDERSON 1998). In Argentina, it was originally found in the lakes located in the southern area of this country (THOMASON 1963, TELL 1985).

C. hirundinella evidences a complex morphological variability. BACHMANN (1911) identified seven morphotypes taking into account the size, shape and number of horns, making it clear that they were not different species. Following the same trend, SCHRÖDER (1920) – in turn – identified nine morphotypes, to which later SCHILLER (1937) referred as "Variationstypen". HUBER-PESTALOZZI (1950) identified ten morphotypes described as forms. Also, more than one species was recognized as a result of the morphological variability of *C. hirundinella* (see CALADO & LARSEN 1997). Consequently, the variety *C.*

hirundinella var. *furcoides* LEVANDER, a valid taxon in the opinion of LANGHANS (1925), was an independent species for SKUJA (1948). Since then, a large part of the literature on *C. hirundinella* corresponds, in fact, to *C. furcoides* (LEVANDER) SKUJA (CALADO & LARSEN 1997). In spite of the complex infra-specific taxonomy that typifies *C. hirundinella*, a statistical analysis aiming a cytomorphometric characterization of the species subtaxa was never done.

From 1997 until now, recurrent massive blooms of C. *hirundinella* have taken place in the reservoir of Paso de las Piedras, Province of Buenos Aires, bringing about serious problems to the water-for-consumption potabilization process (TROBBIANI 2001, TROBBIANI & PARODI 2001). *C. hirundinella* has also been recorded in San Roque reservoir, Province of Córdoba during an autumn-winter bloom (BUSTAMANTE et al. 2000, PRÓSPERI 2000). An early and correct identification of this organism is of a paramount importance for a premature detection of its individuals and thus for taking correct decisions and measures to prevent or palliate massive proliferations. Thus morphological parameters of local populations are essential for a swift and precise determination. In the present article, individuals from a massive population occurring in this reservoir during May of 2000 were statistically studied regarding both its morphological variability and the sources of such variability. The specific purposes of the present study were to determine the average biovolume of the individuals and also to determine whether or not correlations could be found among the dimensions of the different horns, as all these parameters have been previously taken into account to recognize different morphotypes. There are no previous similar statistical studies in a population of the species.

MATERIALS AND METHODS

Samples were collected from Paso de las Piedras reservoir, at 38º-39° S and 61º-62° W, located at 58 km from the city of Bahía Blanca, Province of Buenos Aires, Argentina, during a massive bloom in autumn of 2000.

Sampling date	Apr. 7	May 4	May 11	May 18	Jun. 1	Jun. 9
Water temperature (ºC)	10.5	10	12	7	9.5	7.6

They were collected using a 30-µm-mesh plankton net and fixed *in situ*. Some of the samples were fixed in Lugol´s solution and some in 1%-glutaraldehyde in natural medium.

Identification was made following HUBER-PESTALOZZI (1950). The Lugol´s-fixed samples were observed by contrast-phase microscopy and by stereoscopic microscopy. The glutaraldehyde-fixed samples were dehydrated in an acetone series and dried in a critical point dryer (BOLTOVSKOY 1995, TROBBIANI & PARODI 2001). They were subsequently observed by scanning electron microscopy.

Statistical analysis: Both the length of the apical, antapical, postequatorial, and fourth horn and the diameter of the cingulum (N= 93) were measured in order to calculate the biovolume of the individuals. The latter was calculated according to the following formula (WETZEL & LIKENS 1995):

1) $$\pi / 12 \, (L1.A^2+D^3+2B.L4^2+L2.C^2)$$

where L1 is the length of the apical horn; A is the width of the basis of the apical horn; D is the diameter of the cingulum; L4 is the length of the fourth horn; B is the width of the basis

of the fourth horn; L2 is the length of the antapical horn; and C is the width of the basis of the antapical horn (Fig. 1).

The following parameters were calculated: centralization and dispersion measurements, variance, deviations and relative variance. In addition, correlation and principal component analyses (PLA 1986) were conducted using 5 measurements taken from 93 samples whose parts could be totally observed. The following variables were determined (Fig. 1) L1: length of the apical horn; L2: length of the antapical horn; L3: length of the postequatorial horn; L4: length of the fourth horn; and D: diameter of the cingulum.

Nutrient concentrations were determined at the Laboratorio de Química de la Autoridad del Agua (ADA) in the city of Bahía Blanca, Province of Buenos Aires, Argentina.

RESULTS AND DISCUSSION

The cellular length fluctuated between 150-200 μm (155 ± 5 μm) and the average cellular biovolume was 42791.39 μm^3. In the literature, individuals' length ranged between 150-300 μm (GIRBAL et al. 2000, HUBER- PESTALOZZI 1950). In the samples analyzed in the present study, individuals exhibited a smaller size with respect to that of the samples studied by GIRBAL et al. (2000) in Los Molinos and San Roque reservoirs in the Province of Córdoba. Nevertheless they showed a robust cingulum having an average measurement (media) of the diameter of 52.00 ± 6.46 μm (Table 1), although with lower highest-lowest

dimensions (64.4-36.8 μm) than those mentioned for the *forma* by HUBER-PESTALOZZI (1950) (75-60 μm).

The apical horn was 81.70 ± 9.26 μm long and it was truncated. The hypotheca exhibited always three horns of different length, namely, the antapical horn, the postequatorial horn, and the fourth horn, which is sometimes reported to be absent (HUBER-PESTALOZZI 1950, HUTCHINSON 1967). The antapical horn was 48.00 ± 6.03 μm long, it was straight and it had an acute apex. The postequatorial horn was 41.10 ± 6.75 μm long, it was inwardly curved and it had an acute apex, and the fourth horn, which was 13.00 ± 6.10 μm long, was outwardly curved and it also had an acute apex (Table 1). Our stastistical analysis of the collected data demonstrates that the length of the fourth horn evidenced the highest relative variance (46.99% - Table 1). This characteristic of the fourth horn may explain the reason why it is regularly not found in other populations of this species in the northern hemisphere (HUTCHINSON 1967).

The present statistical characterization allowed us to conclude that the studied population belongs to *C. hirundinella* fa. *austriacum.* This *forma* had never been found before in the territory of Buenos Aires Province. C. *hirundinella* fa. *austriacum* has been recorded in the provinces of Chubut, Neuquén and Río Negro in Argentina (TELL 1985).

The morphological variability of *C. hirundinella* has been attributed to changes in environmental factors, such as temperature (HUBER-PESTALOZZI 1950), water turbulence, and concentration of nutrients (PEARSALL 1929, HUTCHINSON 1967, WETZEL 1983). Nevertheless, HAMALOUI et al. (1998) claim that the environmental alterations seem not to be the only agent responsible for the marked cyclomorphism observed in this species.

HUBER-PESTALOZZI (1950) demonstrated experimentally that temperature is the key factor determining the cell size and the number of horns of the hypotheca. The results of the present study agree with the results of HUBER-PESTALOZZI (1950) in individuals cultured with temperatures ranged between 10-12ºC, similar to the temperatures of the present study. He found that 52% of the individuals exhibited lenghts of 152-200 μm and normally were constructed with 3 horns but frequently showed the development of the fourth horn. Thus they were very similar to the individuals studied here. Nevertheless, according to HUTCHINSON (1967) and HAMALOUI et al. (1998), hight nutrient concentrations may exert an influence on the number of horns of the hypotheca. In this respect, it is noteworthy that the water quality in the Paso de las Piedras reservoir has undergone increasing alterations mainly as a result of the nutrients and agrochemical residues used to work on the land in this area and which reach the reservoir from the Sauce Grande River basin and mainly from "El Divisorio" creek (PARODI et al. 2007). The nutrient analyses conducted in the present research revealed high concentrations of NO_3 (1.4-2.0 mg.l^{-1}) and SRP (0.002-0.097 mg.l^{-1}).

Correlation matrix among variables: The positive correlations between the lengths of the antapical (**L2**) and postequatorial horn (**L3**) as well as between the lengths of the apical (**L1**) and the fourth horn (**L4**) could be deduced whereas the remaining correlations can be considered null. This can be seen in the representation of the variables in the plane of the first two principal components (Table 2 and Fig. 2).

Analysis of the principal components of the correlation matrix: The first component (**CP1**) represents the length of the antapical (**L2**) and the postequatorial horn (**L3**). Previous research studies (HUTCHINSON 1967) did not find such a marked positive

correlation between the lengths of the antapical horn and the postequatorial horn as that oberved in our study through the principal component analysis. Experimental evidence reveals that in some temperate areas with regular summer seasons, the variability in the length of all the horns is produced under certain ranges of temperature (HUTCHINSON 1967). The second component (**CP2**) corresponds to the length of the apical horn (**L1**) and that of the fourth horn (**L4**). The third component (**CP3**) is almost exclusively the diameter of the cingulum (**D**). It has not been drawn in the plane of the first two components because it is poorly represented (5.10 %) (Fig. 3, Table 3). The accumulated percentage of the first 3 components ranged 78.24 (eigenvalues = 1.5394; 1.3629; 1.0098; 0.6293; 0.4586).

Individuals with a positive **CP1** may have higher **L2** and **L3** values with respect to average values. Those with a positive **CP2** may have **L1** and **L4** values higher than the average ones. Those individuals with a positive **CP3** (in white), may have a **D** higher than the average value (Fig. 4)**.**

CONCLUSIONS

The statistical analysis of the data collected in the present study indicates that 1) the individuals belong to *C. hirundinella* fa. *austriacum*, 2) all individuals showed the development of the fourth horn, 3) the length of the fourth horn is the one that exhibited the highest relative variance and 4) no such marked positive correlation between the lengths of the antapical and postequatorial horns was detected in previous studies in the species as that observed in our study of the principal component analysis.

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REFERENCES

BACHMANN, H. (1911): Das Phytoplankton des Süßwassers mit besonderer Berücksichtigung des Vierwaldstättersees. Gustav Fischer, Jena.

BOLTOVSKOY, A. (1995): Técnicas de Microscopía Electrónica de Barrido: Aplicación a las Microalgas. -In: ALVEAL, K., FERRARIO, M. E., OLIVEIRA, E.C. & SAR, E. (eds.): Manual de Métodos Ficológicos, p. 119-138. Universidad de Concepción, Chile.

BUSTAMANTE, M. A., RUIBAL, A. L., LÓPEZ, F., GRANERO, Y., HEMBRECHT, J., BUSSO, F., BONFANTI, E., GIRBAL, A. & SIMONIM, M. E. (2000): Estudio de la evolución trófica del Embalse San Roque. Centro de la Región Semiárida (Córdoba). Instituto Nacional del Agua y del Ambiente. Empresa de Aguas Cordobesas. -In: Seminario Internacional: Identificación y control de algas en la producción de agua potable, p. 1-8. Córdoba, Argentina.

CALADO, A. J. & LARSEN, J. (1997): On the identity of the type of the genus *Ceratium* SCHRANK (Dinophyceae), with notes on *C. hirundinella*. Phycologia **36**: 500-505.

CANTER-LUND, H. & LUND, J.W.G. (1995): Freshwater Algae. -360 pp., Biopress Limited, England.

GIRBAL, A., LAMMEL, E. & BUSSO, F. (2000). Utilización de permanganato de potasio para mitigar un bloom de *Ceratium hirundinella*. Gerencia Calidad de Aguas Cordobesas. -In: Seminario Internacional: Identificación y control de algas en la producción de agua potable, p.15-20. Córdoba, Argentina.

GRAHAM, L. E. & WILCOX, L. W. (2000): Algae. -640 pp. Prentice Hall, Upper Saddle River.

HAMALOUI, S., COUTÉ A., LACROIX, G. & LESCHER-MOUTOUE, F. (1998): Nutrient and fish effects on the morphology of the dinoflagellate. - Comptes Rendus de l´Académie des Sciences Série III, Sciences de la Vie **321**: 39-47.

HUBER-PESTALOZZI, G. (1950): Cryptophyceen, Chloromonadinen, Peridineen. -In: THIENEMANN, A. (ed.), Die Binnengewässer - 310 pp. E. Schweizerbart´sche Verlagsbuchhandlung, Stuttgart.

HUTCHINSON, E. G. (1967): A Treatise on Limnology. – 695 pp. John Wiley and Sons, New York. London. Sydney.

LANGHANS, V. H. (1925): Gemischte Populationen von *Ceratium hirundinella* (O.F.M.) SCHRANK und ihre Deutung. - Arch. Protistenk. **52**: 585-602.

MARGALEF, R. (1983): Limnología. - 1010 pp. Ediciones Omega, Barcelona.

PEARSALL, W. H. (1929): Form variation in *Ceratium hirundinella* O. F. M. - Proc. Leeds Phil. Soc. **1**: 432-439.

PARODI, E. R., SINISCALCHI, A. G., ALIOTO, R., BELLEGGIA, F., SCHEFER, J. C. & LÓPEZ, N. (2007): Análisis de una alternativa de reducción biológica de nutrientes de un afluente del Embalse Paso de las Piedras. CONAGUA :1-6, Argentina (in CD).

PLA, L. E. (1986): Análisis Multivariado: Método de Componentes Principales. Secretaría General de la OEA, Washington, D.C., Serie Matemática – Monografía **27**: 1-89.

PRÓSPERI, C. H. (2000): Competencia entre cianoficeas y pirroficeas en el embalse San Roque. -In: Seminario Internacional: Identificación y control de algas en la producción de agua potable, p.1-5. Córdoba, Argentina.

REGENFORS, G. D. & ANDERSON, R. D. (1998): Environmental and endogenous regulation of cyst germination in two freshwater dinoflagellates. J. Phycol. **34**:568-557.

SCHILLER, J. (1937): Dinoflagellatae (Peridineae) in monographischer Behandlung. In: Kolkwitz, R. (ed.), Rabenhorst´s Kryptogamen-flora von Deutschland, Österreich und der Schweiz, 2nd ed., 10 (3), Part 2, p. 1-589. Akademische Verlagsgesellschaft, Leipzig.

SCHRÖDER, B. (1920): Die neuen wesentlichen Formentypen von *Ceratium hirundinella* (O. F. MÜLLER). -Archiv für Naturgeschichte **84** (Abt. A, 8): 222-230.

SIGEE, D. C., KRIVTSOV, V. & BELLINGER, E. G. (1998): Elemental concentrations, correlations and ratios in micropopulations of *Ceratium hirundinella* (Pyrrophyta): an X-ray microanalytical study. J. Phycol. **33**:155-164.

SKUJA, H. (1948): Taxonomie des Phytoplanktons einiger Seen in Uppland, Schweden. Symbolae Botanicae Upsalienses **9**:1-399.

TELL, G. (1985): Catálogo de las algas de agua dulce de la República Argentina. -p. 283. J. Cramer, Vaduz.

THOMASON, K. (1963): Araucarian lakes. Plankton studies in North Patagonia with notes on terrestrial vegetation. Acta Phytogeogr. Suecica. **47**: 1-39.

TROBBIANI, N. M. (2001): Primer estudio citomorfométrico y ultraestructural de *Ceratium hirundinella* (Dinophyta) proveniente del emblase Paso de las Piedras. Thesis to obtain the Bachelor Degree in Biology. -p.59, Universidad Nacional del Sur, Argentina

TROBBIANI, N. M. & PARODI, E. R. (2001): Estructura fina de *Ceratium hirundinella* (Dinophyta) proveniente del embalse Paso de las Piedras. - Bol. Soc. Argent. Bot. **36** (Suppl.): 155.

WETZEL, R. G. (1983): Limnology. -p. 755. Saunders College Publishing. Philadelphia.

WETZEL, R. G. & LIKENS, G. E. (1995). Limnological Analyses. -p.415. Springer, New York.

Figure Legends

Fig. 1. *Ceratium hirundinella*. Scale Bar =10 µm. Photomicrograph with MEB (left) and scheme (right) of the dorsal side of the cell. In the scheme, the parameters for the statistical analysis and biovolume estimate are indicated with lines: L1, L2, L3, and L4, length of horns. A, B, and C, width of the basis of the horns. D, width of the cingulum.

Fig. 2. Representation of individuals among the pairs of variables with highest correlation.

Fig. 3. Representation of variables in the plane of the first two principal components. The first component (CP1) represents the lengths of the antapical (L2) and the postequatorial horn (L3). The second component (CP2) corresponds to the lengths of the apical horn (L1) and that of the fourth horn (L4).

Fig. 4. Representation of individuals in the plane of the first two principal components. Individuals in white have a positive CP3. Individuals in grey have a CP3 near 0. Individuals in black have a negative CP3.

Table 1. Length of the horns and diameter of the cingulum (in μm), variance, deviations and relative variance.

	Apical horn (L1)	Antapical horn (L2)	Postequatorial horn (L3)	Fourth horn (L4)	Cingulum (D)
Highest dimensions	103.50	69.00	59.80	32.20	64.4
Lowest dimensions	59.80	32.20	23.00	2.30	36.8
Average dimensions	81.70	48.00	41.10	13.00	52.00
Variance	79.12	38.03	44.59	37.04	39.69
Deviation	9.26	6.03	6.75	6.10	6.46
Relative variance	11.34	12.56	16.42	46.99	12.43

Table 2. Correlation matrix among variables. ns: non-significant correlation ($p > 0.05$). **: highly significant correlation ($p < 0.01$)

L1	1	ns	ns	**	ns
L2	-0.0216	1	**	ns	ns
L3	-0.0542	0.5205	1	ns	ns
L4	0.3539	0.0856	0.0555	1	ns
D	-0.0185	-0.0602	0.0873	-0.0717	1
	L1	L2	L3	L4	D

Table 3. Correlations between the variables and the first 3 components

Variable	CP 1	CP 2	CP 3	% of reconstruction of each variable With the first 2 CP	With the first 3 CP
L1	0.0460	0.8092	0.2148	65.70	70.31
L2	0.8620	-0.0899	-0.1440	75.11	77.18
L3	0.8543	-0.1842	0.1070	76.37	77.52
L4	0.2539	0.7843	0.0640	67.96	68.37
D	0.0068	-0.2256	0.9630	5.10	97.83