ARTÍCULO ORIGINAL

ISSN 0325-7541
Revista Argentina de Microbiología (2012) 44: 43-48

Selective grazing by protists upon enteric bacteria in an aquatic system

MARÍA S. DOMÍNGUEZ* [1], ALICIA H. ESCALANTE[2], ALICIA M. FOLABELLA[1], ÁNGELA S. ZAMORA[1]

[1] Laboratorio de Microbiología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP);
[2] Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP). Funes 3250 (7600) Mar del Plata – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Argentina.
**Correspondencia. E-mail. soledaddominguez7@gmail.com*

ABSTRACT

It is well known that protozoan grazing can be an important agent of mortality for suspended bacteria, both in marine and freshwater environments. Considering that the presence of fecal contamination is a frequent phenomenon in these environments, and that *Escherichia coli* and the genus *Enterococcus* are indicators of microbiological water quality, the effect of protozoan grazing on *E. coli* and *Enterococcus faecalis* in Los Padres Lagoon waters (Buenos Aires, Argentina, 37º 56´30" S, 57º 44´30" W) was herein analyzed. Microcosm assays were carried out, simulating lacustrine conditions, confronting suspensions of autochthonous bacterivorous protozoans with suspensions of autochthonous and collection strains of *E. coli* and *E. faecalis*, combined and individually. Daily counts were made for evaluating bacterial survival and the number of ciliates. The results obtained indicate that there is a preferential sequence for bacterial removal in the water, where *E. faecalis* is more grazing-resistant than *E. coli*. Moreover, it was noted that the origin of bacterial strains influenced their sensitivity for grazing, at least in the short term (*e.g.* the collection strains were less affected). We conclude that protozoan grazing can modify the relative abundance of fecal indicator microorganisms, thus altering the results of water quality studies.

Key words: grazing, *Escherichia coli*, *Enterococcus faecalis*, protozoans, selectivity

RESUMEN

Predación selectiva de bacterias entéricas por protistas en un ambiente acuático.

Está bien establecido que la predación por protozoos puede ser un factor importante de mortalidad para las bacterias en suspensión, tanto en ambientes marinos como de agua dulce. Considerando que la contaminación fecal es un fenómeno frecuentemente observado en estos ambientes, y que *Escherichia coli* y miembros del género *Enterococcus* son indicadores de calidad microbiológica del agua, se analizó el efecto de la predación por protozoos sobre *E. coli* y *Enterococcus faecalis* en aguas de la Laguna de los Padres (Buenos Aires, Argentina, 37º 56´30" S, 57º 44´30" W). Se realizaron ensayos a microcosmos, simulando el ambiente lagunar, enfrentando suspensiones de protozoos bacterívoros autóctonos con suspensiones de cepas autóctonas y de colección de *E. coli* y *E. faecalis*, en forma individual y combinada. Diariamente se efectuaron los recuentos correspondientes para elaborar las curvas de supervivencia. Los resultados obtenidos indican que existe una secuencia en la eliminación de cepas bacterianas por bacterivoría, siendo *E. faecalis* más resistente a la predación que *E. coli*. Además, se observó que el origen de las cepas condiciona su sensibilidad a la predación, ya que las cepas provenientes de los cultivos de colección resultaron menos afectadas. Se concluye que la bacterivoría por protozoos puede modificar la abundancia relativa de los microorganismos indicadores de contaminación y, por ende, los resultados de los estudios de calidad del agua.

Palabras clave: Predación, *Escherichia coli*, *Enterococcus faecalis*, protozoos, selectividad

INTRODUCTION

Protozoans, particularly flagellates and ciliates, are the main bacteria predators in aquatic environments (2, 8, 11). They have also an important role in ecosystem functioning, as they are ubiquitous and abundant in any habitat, and constitute a food resource for metazooplankton. Predation by protists is mentioned as a major mortality factor for planktonic bacteria in freshwater as well as in marine environments (9, 10, 11). The potential growth of these predator populations may be as great as those of their preys (16).

The rate of bacterial elimination from waters can be strongly influenced by the ability of microorganisms to digest bacteria. Gram-negative bacteria are more easily eliminated than gram-positive ones, probably due to the difficulty to digest the complex cellular wall of the latter (6). Several authors (1, 8, 15) have concluded that the rate of bacterial elimination in waters is related to cell size and morphology, indicating that bacterivorous organisms could decrease the number of bacteria if they have the appropriate size. Group formation or mucus production can constitute a disadvantage for this phenomenon.

Predation by protozoans would influence the morphological structure, taxonomic composition and physiological conditions of bacterial communities in aquatic ecosystems, producing changes in the distribution of cellular sizes, in such a way that bacteria become greater or smaller than normal. The most resistant morphotypes seem to be the filaments and the microcolonies, as they are not consumed or consumed at substantially lower rates in the presence of alternative preys (10, 15). Due to protozoan predation upon bacteria, a temporal increase of filamentous forms would occur, representing more than 40 % of the total bacterial biomass (13). Some authors assumed that predation-resistant forms are those which stabilize the bacterial biomass in natural aquatic ecosystems (1).

Fecal contamination indicators (*Escherichia coli* and enterococci) are used worldwide to determine microbiological water quality for human drinking and recreational uses. Given that fecal contamination is a frequently observed phenomenon in freshwater and marine environments (17), the purpose of the present study was to determine the existence of selective bacterivory in protozoans from Los Padres Lagoon waters (Buenos Aires Province, Argentina) upon two fecal contamination indicators, *E. coli* and *Enterococcus faecalis*, evaluating the influence of origin, size, cellular wall and morphology of bacteria on protozoan predation.

MATERIALS AND METHODS

Los Padres Lagoon is a shallow eutrophic lake located at the eastern border of Sierra de Los Padres, in Buenos Aires province, Argentina (37° 56' 30" S%57° 44' 30" W). Its surface area is 2.16 km^2, with a mean depth of 1.24 m (14). Its basin area has an intensive agricultural land use and the lake can be considered eutrophic (4).

The microcosm assays were carried out with a pool of planktonic bacterivorous protozoans, and bacterial strains, *E. coli* and *E. faecalis*, from two origins:

a) "Collection": belonging to the Laboratory of Microbiology (*E. coli* ATCC 25922 and *E. faecalis* ATCC 29212).

b) "Autochthonous": isolated from Los Padres Lagoon waters.

Bacterial species were selected according to their morphology, arrangement and type of cellular wall. Their importance in public health was also considered, as they are used as indicators of fecal contamination.

The assays were carried out in Erlenmeyer flasks (simulating lacustrine microhabitats) maintained at environmental temperature, discontinuous shaking, and no direct natural light. The components of each assay and the control were as shown: assay 1 (E1), protozoans + autochthonous *E. coli* strain; assay 2 (E2), protozoans + autochthonous *E. faecalis* strain; assay 3 (E3), protozoans + autochthonous *E. coli* + *E. faecalis* strains; assay 4 (E4), protozoans + collection *E. coli* strain; assay 5 (E5), protozoans + collection *E. faecalis* strain; assay 6 (E6), protozoans + collection *E. coli* + *E. faecalis* strains; initial condition control (ICC), protozoans + autochthonous bacteria. This ICC assay, including protozoans plus all the bacteria inhabiting in the lagoon water original sample, was carried out in order to assess the effect of predation in the presence of alternative preys.

Suspensions of autochthonous protozoans were confronted with bacterial strain suspensions, either individually and combined, in aged, filtered and sterilized lagoon waters (SLW). To obtain SLW, the treatment was as follows: during three months the lagoon water was maintained in dark condition, and it was later filtered to remove the deposited matter and sterilized at 121 °C during 15 minutes.

Preparation of bacterial inoculum

In order to reach a minimum bacterial concentration of 10^6 CFU/ml in the assays, similar to that generally found in natural waters (12), dense strain suspensions were placed as bacterial inoculum. The bacterial concentration of each suspension was estimated by spectrophotometry at μ = 520 nm (Bausch & Lomb, Spectronic 20). The extrapolation of the absorbance values on calibration curves was previously done. These suspensions were added to the assays at the beginning and at the time of reinoculation.

Viable bacterial cell enumeration in the control and assays was carried out by pour plate (3) by means of the inoculation of a series of decimal dilutions up to 10^{-7} in solid medium selected for each strain. Mac Conkey agar and azide blood agar base (Difco-BD, Buenos Aires, Argentina) were used for *E. coli* and *E. faecalis*, respectively. These media were prepared using SLW as diluting.

Protozoan preparation

Water collected from three randomly selected sites in open waters of Los Padres Lagoon was filtered by means of a 35 mm plankton mesh net. The concentrated plankton net sample was put in sterile tubes for centrifuging (Rolco CM 2036) at 500 r.p.m. during 5 minutes. Pellets were resuspended in SLW up to a final volume of 140 ml. This suspension was divided into seven aliquots of 20 ml each, placed in 1-L Erlenmeyer flasks with 170 ml of SLW. Six of these Erlenmeyers were employed in the assays (E1 to E6) and one for ICC.

Samples from the assays and control were daily taken for counting protozoans and the bacteria under study. Protozoan counts were performed in a 0.3 ml Sedgwick - Rafter chamber under an optic microscope (7). When protozoan density was greater than that allowed for microscopical counts, dilutions were done. The 12th day reinoculations of bacterial strains were placed in the corresponding assays to increase their concentration and to show any evidence of possible bacterivory.

RESULTS

Bacterial count

When comparing the values obtained for *E. coli* of different origins (E1 and E4), although the behavior pattern was very similar until the reinoculation in the

three cases, *E. coli* counts were smaller in the ICC after the 5th day and disappeared at the 9th day (Figure 1a). After reinoculation, the *E. coli* autochthonous strain was quickly eliminated whereas the collection strain persisted, showing a tendency to decrease slowly (Figure 1a).

When analyzing the values obtained in the assays with *E. faecalis* of different origins (E2 and E5), it could be observed that up to the 12th day the tendencies were very similar. *E. faecalis* concentration stabilized at 10^3-10^4 CFU/ml until reinoculation (Figure 1b). After reinoculation, a gradual concentration decrease was observed, though slightly lower for the *E. faecalis* collection strain (Figure 1b).

When comparing the behavior of both authochthonous strains (where they were offered like a sole diet or combined) versus that of the ICC (where they were part of bacteria stock together with other alternative preys) the similitude found in the assays was evident (Figures 1a and 1b).

In the assays with both *E. coli* and *E. faecalis*, their behavior during the first days was similar, independently of the strain origin. On the 9th day, the *E. coli* autochthonous strain disappeared (Figure 1c), while the *E. coli* collection strain disappeared during the 10th day (Figure 1d).

E. faecalis concentration stabilized near a value of 10^3–10^4 CFU/ml until reinoculation (Figures 1c and d).

The *E. coli* autochthonous strain was quickly eliminated (t = 2 days) (Figure 2a), while that from the collection strain persisted until the 15th day at 10^4 CFU/ml concentration (Figure 3a). The behavior of *E. faecalis* strains was very similar (Figures 2b and 3b).

During the counts, it was observed that, at identical incubation time, both *E. faecalis* and *E. coli* autochthonous colonies were smaller than those from collection strains.

Maximum values of protozoan counts corresponded to bacterial reinoculation, always appearing the day after (t = 1 day) (Figures 2a and b).

When considering only the autochthonous strains in relation to the peak of protozoan concentration, *E. coli* disappearance facing *E. faecalis* persistence could indicate greater sensitivity of the former to bacterivory (Figures 2a, b, and c).

Both, *E. coli* and *E. faecalis* collection strains persisted with a tendency to decrease their concentration in the course of time (Figures 3a, b, and c).

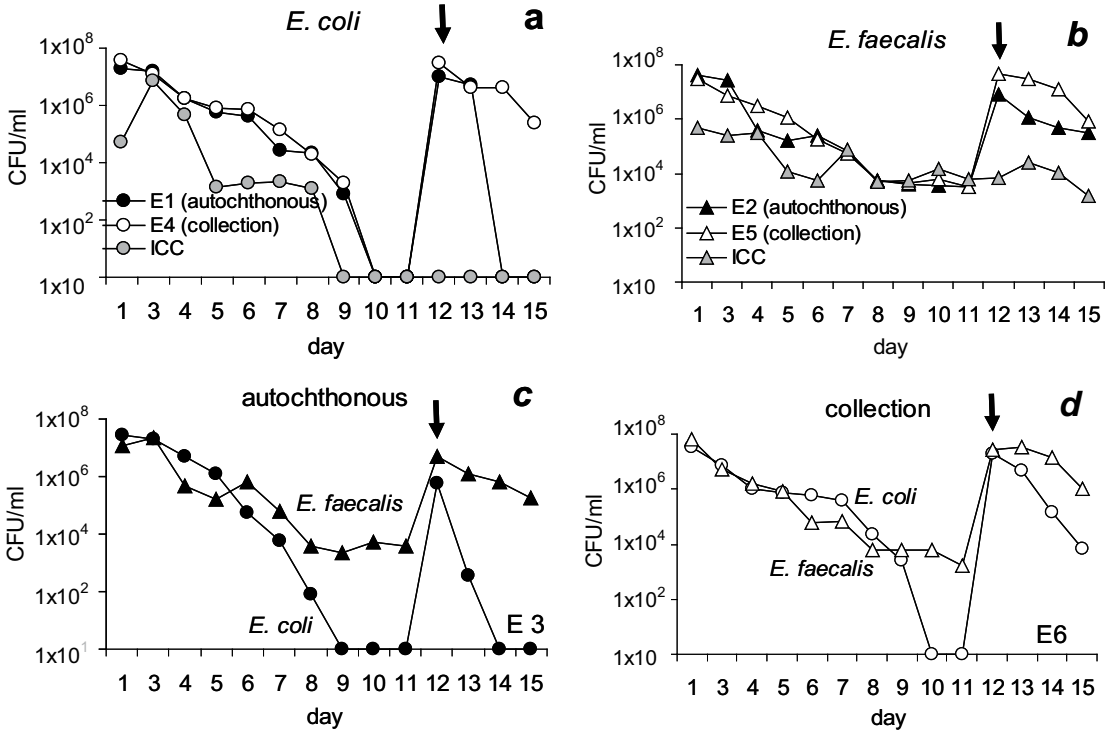

Figure 1. a) *E. coli* counts in E1 and E4 and in Initial Condition Control (ICC). b) *E. faecalis* counts in E2 and E5 assays and in Initial Condition Control (ICC). c) *E. coli* and *E. faecalis* counts in E3 assay; d) *E. coli* and *E. faecalis* counts in E6 assay. The arrow indicates reinoculation

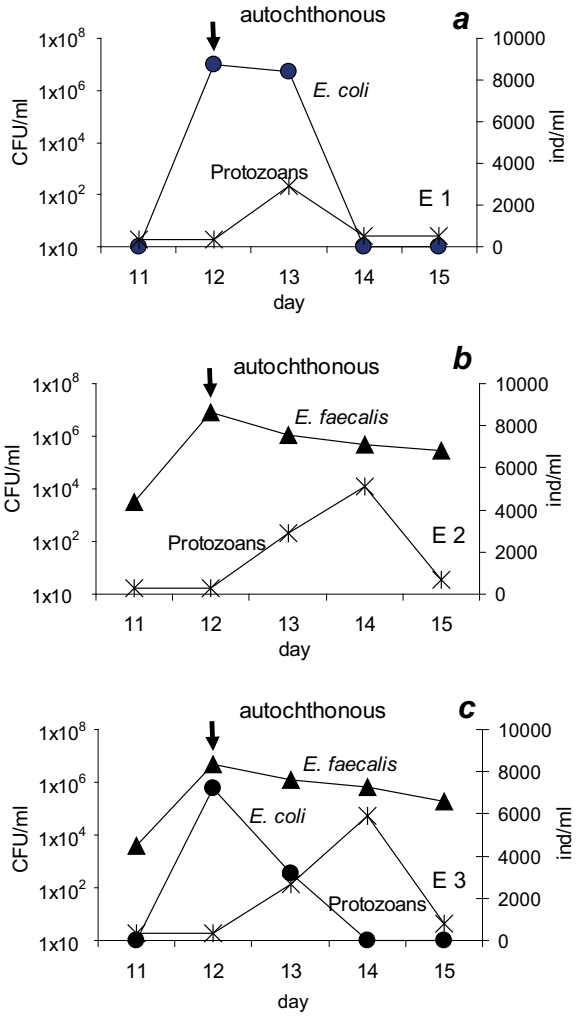

Figure 2. a) Protozoans and *E. coli* counts in E1 assay; b) Protozoans and *E. faecalis* counts in E2 assay; c) Protozoans, *E. coli* and *E. faecalis* counts in E3 assay. All the counts were done after the 11th day

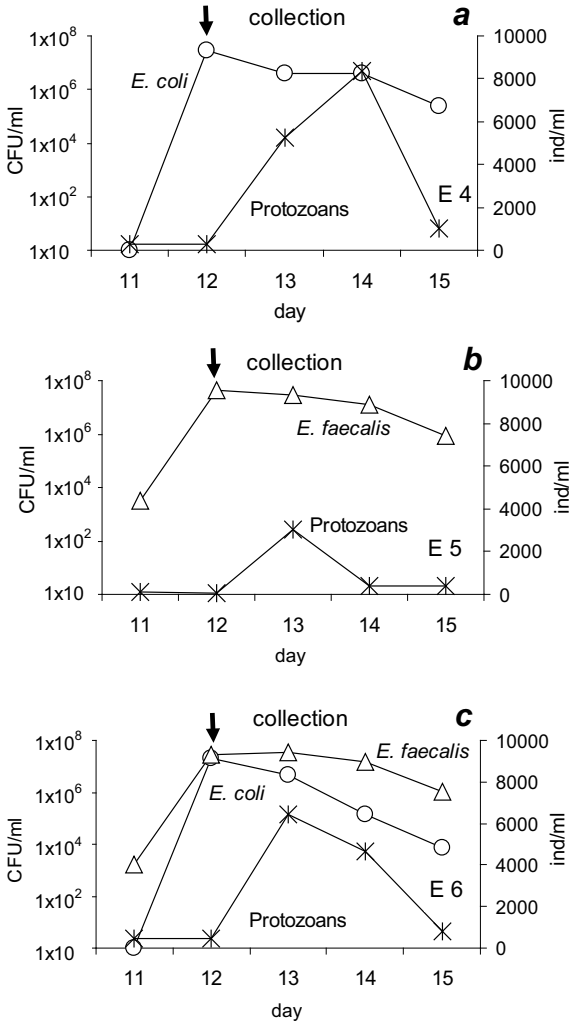

Figure 3. a) Protozoans and *E. coli* counts in E4 assay; b) Protozoans and *E. faecalis* counts in E5 assay; c) Protozoans, *E. coli* and *E. faecalis* counts in E6 assay. All the counts were done after the 11th day

With respect to mixed collection strains, the difference between elimination velocities was noticeable. This difference was also shown when *E. coli* and *E. faecalis* were together; therefore, there would be a greater *E. coli* sensitivity to predation by protozoans (Figure 3c).

Data from ICC counts were shown as from the 1st day, as *E. coli* absence after the 9th day prevented to clearly analyze the tendencies (Figure 4). The noticeable decrease in protozoan concentration would suggest a close association with *E. coli* disappearance.

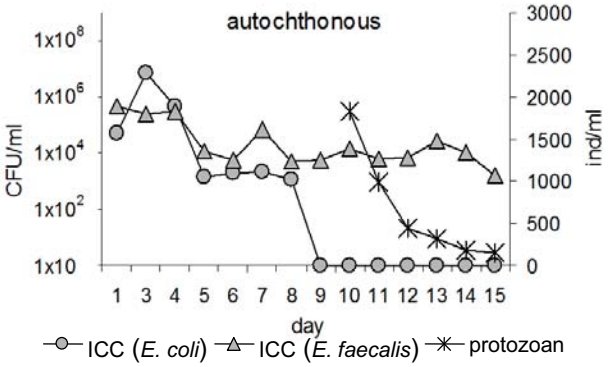

Figure 4. Protozoans, *E. coli* and *E. faecalis* counts in Initial Condition Control (ICC) from the 1st day

DISCUSSION

With regard to the results obtained from the different assays carried out in the present study, predation upon

E. coli would be remarkably greater, while *E. faecalis* would be less sensitive, and capable of maintaining a relatively stable concentration after the first days. This agrees with other authors who considered that complex forms are either not consumed or consumed at substantially lower rates (13). Competition for nutrients and other limiting resources are the major selective forces that promote bacterial adaptations, such as starvation, motility and antibiotic production. Success in the environment, however, is not only defined by growth and reproduction but also by the ability of organisms to avoid, tolerate or defend themselves against natural consumers (9).

The development of smaller size colonies observed in *E. coli* and *E. faecalis* autochthonous strains compared with that of collection colonies could lead to think of cellular size differences. If so, it could be suggested that prey size is the defence mechanism most influencing in this case. The collection strains could present greater cellular sizes than autochthonous ones as they have neither adapted themselves to the lack of resources nor evolved predator evasion mechanism.

Though less evidently, the elimination rates of *E. faecalis* strains differ from each other too, also suggesting here that cellular size is the most important characteristic conditioning prey selection. These phenotypic differences could be understood when considering the observations made by some authors (5, 15, 8). They concluded that species in natural environments used to develop complex forms or modify their cellular size to avoid the range of prey selection of predominant bacterivores.

Bacteria exceeding in size the upper species-specific uptake limit of predators are protected from grazing. On the other hand, despite the decrease of the uptake efficiency of particle size, no lower uptake limits exist. Some phagotrophic flagellates are even able to feed on virus particles (5). This could explain the greater incidence of bacterivory upon autochthonous *E. coli*.

Finally, it has to be remarked that we have worked with bacterivorous protozoan as a functional group, without identifying taxa. Among bacterivorous protists, the bacterivorous nanoflagellates are known to be the major factor influencing both bacterial community structure and bacterial standing stock (15). If the cleaning specific volume of the flagellates is greater than that of the ciliates, the flagellates would probably represent the group determining the bacterial stock in microcosm assays. The similitude found when comparing the behavior of both autochthonous strains in the assays and in the ICC would suggest that, even in the presence of alternative preys, a similar elimination pattern by bacterivory was developed.

As the impact of protist grazing on bacterial communities is based on the complex interplay of several parameters, including grazing selectivity, both differences in sensitivity of bacterial species to grazing and in responses of single bacterial populations to grazing (size and physiology), as well as the direct and indirect influence of grazing on bacterial growth conditions (substrate supply) and bacterial competition (elimination of competitors) (1, 7, 4, 13), the direct extrapolation of the results obtained at laboratory scale is practically inapplicable. Nevertheless, the use of microcosm assays becomes necessary to understand the determining processes of grazing.

CONCLUSIONS

Bacterivory by planktonic protozoans constitutes a regulator mechanism of bacterioplankton abundance.

E. faecalis strains are more grazing-resistant than those of *E. coli*. Strain origin influences bacterial sensitivity to predation. According to the present investigation, the autochthonous strains are more sensitive.

Bacterial prey size might be a major determinant of bacterivory resistance.

Protozoan predation modifies the relative abundance of microorganisms, which are contamination indicators, altering the results of water quality studies.

The sensitivity to bacterivory presents the following sequence: autochthonous *E. coli* > collection *E. coli* autochthonous *E. faecalis* > collection *E. faecalis*.

Considering that bacterial number is one of the parameters conditioning water quality for human drinking and recreational uses, the control that protozoan grazing can exert upon bacterial concentrations in waters would be a very interesting point to start the development of bioremediation techniques.

Future *in situ* studies permitting to determine the mortality induced by protozoans would allow to increase the size of the database to be considered when evaluating the risk of the introduction of non-autochthonous species in a water body.

Acknowledgements: We thank A. Berengeno for her field assistance. We also thank A. Licciardo for editing the final version of the manuscript. This project was supported by the Universidad Nacional de Mar del Plata (grant EXA 340/06) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina (grant PIP 02875) to AHE.

REFERENCES

1. Corno G, Jürgens K. Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. Appl Environ Microbiol 2006; 72: 78-86.
2. Fernandes Florêncio de Araújo M. Ecologia do protozooplâncton, bacterioplâncton e virioplâncton em um sistema fluvial-lagunar do Nordeste Brasileiro. Doctoral Thesis 2004. Universidade Federal de São Carlos.
3. Food and Drug Administration. Bacteriological Analytical Manual, AOAC International; 8th edition; 1998, USA.
4. González Sagrario MA, Balseiro E. Indirect enhancement of large zooplankton by consumption of predacious macroinvertebrates by littoral fish. Arch Hydrobiol 2003, 158: 551-74.
5. Hahn MW, Höfle MG. Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol Ecol 2001; 35: 113-21.
6. Iriberri J, Azúa I, Labirua-Iturburu A, Artlozaga I, Barcina I. Differential elimination of enteric bacteria by protists in a freshwater system. J Appl Bacteriol 1994; 77: 476-83.
7. José de Paggi S, Paggi JC. Determinación de la abundancia y biomasa zooplanctónica. In: Lopreto EC, Tell G, editors. Ecosistemas de Aguas Continentales. Metodologías para su estudio. La Plata, Editorial Sur, 1995, p. 315-23.
8. Matz C, Jürgens K. High motility reduces grazing mortality of planktonic bacteria. Appl Environ Microbiol 2005; 71: 921-9.
9. Matz C, Kjelleberg S. Off the hook-how bacteria survive protozoan grazing. Trends Microbiol 2005; 13: 302-7.
10. Muylaert K, Van der Gucht K, Vloemans N, De Meester L, Gillis M, Vyverman W. Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. Appl Environ Microbiol 2002; 68: 4740-50.
11. Nieset J. The effect of protozoan predation on four bacterial communities. Doctoral Thesis 2006. University of Akron.
12. Pedrós-Alió C, Guerrero R. Prokaryotology for the limnologist. In: Margalef R, editor. Limnology Now: a Paradigm of Planetary Problems. Elsevier Science BV, 1994, p. 37-57.
13. Pernthaler J, Zöllner E, Warnecke F, Jürgens K. Bloom of filamentous bacteria in a mesotrophic lake: identity and potential controlling mechanism. Appl Environ Microbiol 2004; 70: 6272-81.
14. Pozzobon MV, Tell G. Estructura y dinámica de la comunidad perifítica sobre *Ricciocarpus natans* (Hepaticae) de la Laguna de Los Padres (Buenos Aires, Argentina). Bol Soc Arg Bot 1995, 30: 199-208.
15. Qinglong LW, Boenigk J, Hahn MW. Successful predation of filamentous bacteria by a nanoflagellate challenges current models of flagellate bacterivory. Appl Environ Microbiol 2004; 70: 332-9.
16. Sherr EB, Sherr BF. Significance of predation by protists in aquatic microbial food webs. Antonie van Leeuwenhoek 2002; 81: 293-308.
17. Vilanova Solà X. Diversitat de les poblacions de coliformes fecals I d´enterococs a les aigües residuals i anàlisi de les modificacions en la composició i l´estructura poblacional a les plantes depuradores. Doctoral Thesis 2005. Universitat de Barcelona.

Recibido 21/06/11 – Aceptado 11/01/12