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Influence of bacterial density and water temperature on the grazing activity of two freshwater ciliates

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SUMMARY

1. The influences of bacterial density and water temperature on the grazing activity of the ciliates *Uronema* sp. and *Colpoda inflata* were studied. The conditions assayed were two prey densities (10^6 and 4×10^7 bacteria ml^{-1}) and three water temperatures (10, 15 and 22 °C).
2. The response of the ciliates was measured from changes in protistan biovolumes and specific clearance rates. At high prey density, both ciliates showed lower biovolumes as water temperature increased, while at low prey density this tendency was minimized.
3. At the intermediate temperature of 15 °C both ciliates filtered ten times more body volume when bacteria were scarce; however, the ingested bacteria were fewer than at high prey density. At low prey density, a decrease from 15 to 10 °C evidenced different strategies of the two ciliates, which led to a similar ingestion of bacteria: *C. inflata* reduced its specific clearance rates and increased its biovolume, while *Uronema* sp. did not show changes. At high prey density, an increase from 15 to 22 °C caused lower biovolumes and a noticeable increase in specific clearance rates in both ciliates, indicating opportunist behaviour.

Introduction

In aquatic systems, there is a constant flux of matter and energy among the components of the microbial loop (Pomeroy, 1974; Azam *et al.*, 1983; Sanders *et al.*, 1989). Some authors (Larsson & Hagström, 1982) indicate that from 60 to 100% of the phytoplanktonic exudates are channelled towards the bacterial trophic level and transformed into new bacterial production, which in turn nourishes organisms of other trophic levels, such as flagellate and ciliate protists (Porter *et al.*, 1985; Bloem *et al.*, 1989). These planktonic protists may ingest from a very low value up to more than 100% of bacterial production (Porter *et al.*, 1988; Tranvik, 1989), and therefore bacterivory by phagotrophic protists may be a decisive factor in the control of bacterial density in aquatic systems (Güde, 1986; Verity, 1986; Nagata, 1988).

The grazing capacity of ciliates in aquatic systems is well documented (Albright *et al.*, 1987; Sherr & Sherr, 1987; Sanders *et al.*, 1989; Bernard & Rassoulzadegan, 1990; Carlough & Meyer, 1991), and several authors have recognized the influence of prey density (Taylor, 1978; Fenchel, 1980a; Jackson & Berger, 1985) and water temperature (Sherr, Sherr & Rassoulzadegan, 1988) on this activity. However, most of these authors obtained their results in natural aquatic systems rather than in an experimentally controlled system. Therefore, other factors that affect protistan bacterivorous activity, in addition to prey density and/or water temperature, may make it difficult to determine the relative influence of these factors on the overall changes observed.

In this study, we analyse the influence of bacterial prey density and water temperature on the grazing activities of ciliates. We test under laboratory conditions the grazing activity of two ciliates isolated from a freshwater system subject to different combinations of prey density and water temperature.

Materials and methods

From the shallow waters of the Butrón River, two ciliates were isolated and identified as *Uronema* sp. and

Colpoda inflata (Stokes, 1884) Kahi, 1931. *Uronema* sp. is a common and abundant ciliate in the Butrón River: it has been observed throughout the year in water samples, making up to 18% of the total ciliate density (unpublished data). *Colpoda inflata* is less abundant, accounting for up to 5% of the community of ciliates.

In order to estimate the effect of bacterial density and water temperature on the grazing activity of these phagotrophic ciliates, we designed six treatments to simulate different environmental conditions. Each treatment was assayed three times. We assayed three water temperatures, 10, 15 and 22 °C, and two bacterial densities, 10^6 bacteria ml^{-1} and 4×10^7 bacteria ml^{-1}. The temperatures were chosen on the basis of previous work in the Butrón River (Iriberri *et al.*, 1993), where a cold and a warm situation have been characterized yearly, with respective mean water temperatures of 10.3 and 19.0 °C. The water temperature of 15 °C corresponds to the mean annual value in the river. Regarding prey density, 10^6 bacteria ml^{-1} represented a condition of scarcity. This prey density has been reported as enough to allow the grazing activity of ciliates (Sherr & Sherr, 1987). For the high prey density experiments, we took into account work by Fenchel (1980b) which indicated optimum growth rates for ciliates when the bacterial prey ranged from 1.1×10^6 to 5.0×10^6 bacteria ml^{-1}. In addition, similar low and high bacterial densities have been estimated in the Butrón River during the cold and warm situations, respectively, (Barcina *et al.*, 1991; Iriberri *et al.*, 1993).

To reach the initial density of ciliates required for the experiments, we incubated a volume of the routine culture (ciliate with accompanying bacteria) in sterile dehydrated cereal leaf infusion medium. This culture was incubated in a rotary shaker in the dark at 75 r.p.m. and at the temperature at which the experiment was to be carried out. After 6 days of incubation, the ciliates were in stationary phase of growth, with densities of about sixty ciliates ml^{-1} in the case of *Uronema* sp. and 130 ciliates ml^{-1} in the case of *C. inflata*. This culture of protists was concentrated by centrifugation, using a gentle treatment designed to cause no injury to the protists and to provide a maximum efficiency of concentration. Protist injury after centrifugation was detected by direct observation testing for cell integrity and the capacity for movement. For *Uronema* sp., we centrifuged 40-ml centrifuge tubes at 840 *g* for 5 min, obtaining 83% of the protists in the 5 ml of the bottom of each tube. In the case of *C. inflata*, the conditions were 475 g and 5 min, and the efficiency was 77%. After this first centrifugation, we washed the protists with Neff's solution (Finlay, Rogerson & Cowling, 1988) and centrifuged again. The final volume of concentrate was 25 ml. From this concentrate of ciliates, we adjusted the initial density in 750 ml of Neff's solution to be between two and seven ciliates ml^{-1}, values found in the Butrón River in previous studies (Barcina *et al.*, 1991; Iriberri *et al.*, 1993). Neff's solution was used due to the absence of organic nutrients in its composition, which prevents the quick growth of bacteria and therefore allows the control of bacterial prey densities without variations in the physiology of the ciliates.

The flasks were incubated under each of the combined conditions of water temperature (± 1 °C) and prey density for 72 h, in an orbital rotary shaker at 50 r.p.m. in the dark, to acclimatize the protists to the experimental conditions. During this period, we did not detect growth of the two ciliates when prey availability was low, so protistan abundance remained at two ciliates ml^{-1}. When prey was abundant, ciliate density increased up to a maximum of 15.2 ciliates ml^{-1}. Bacterial density in the flasks was monitored every 12 h using acridine orange epifluorescence direct counting (Hobbie, Daley & Jasper, 1977), at a magnification of × 1250. Bacteria present in at least twenty randomly selected fields were counted. If the count was lower than the desired value, we added a volume of suspension of heat-killed bacteria; if the count was higher, we diluted with sterile Neff's solution. The concentrate of heat-killed bacteria was obtained from free-living bacterioplankton of the Butrón River, as described in Iriberri *et al.* (1993). The mean value and variability of these counts were $1.11 \times 10^6 \pm 0.25 \times 10^6$ bacteria ml^{-1} in the low prey-density experiments and $4.24 \times 10^7 \pm 0.31 \times 10^6$ bacteria ml^{-1} in the high prey-density experiments.

After 72 h of incubation at the desired prey density and water temperature, we estimated (i) the cell volumes of *Uronema* sp. and *C. inflata*, and (ii) the protistan grazing rates on bacteria by using the technique of uptake of monodispersed fluorescently labelled bacteria (FLB) (Sherr, Sherr & Fallon, 1987).

Protistan cell volumes

We measured the maximum length and widths of thirty to fifty ciliates from samples fixed in Lugol (0.5% final

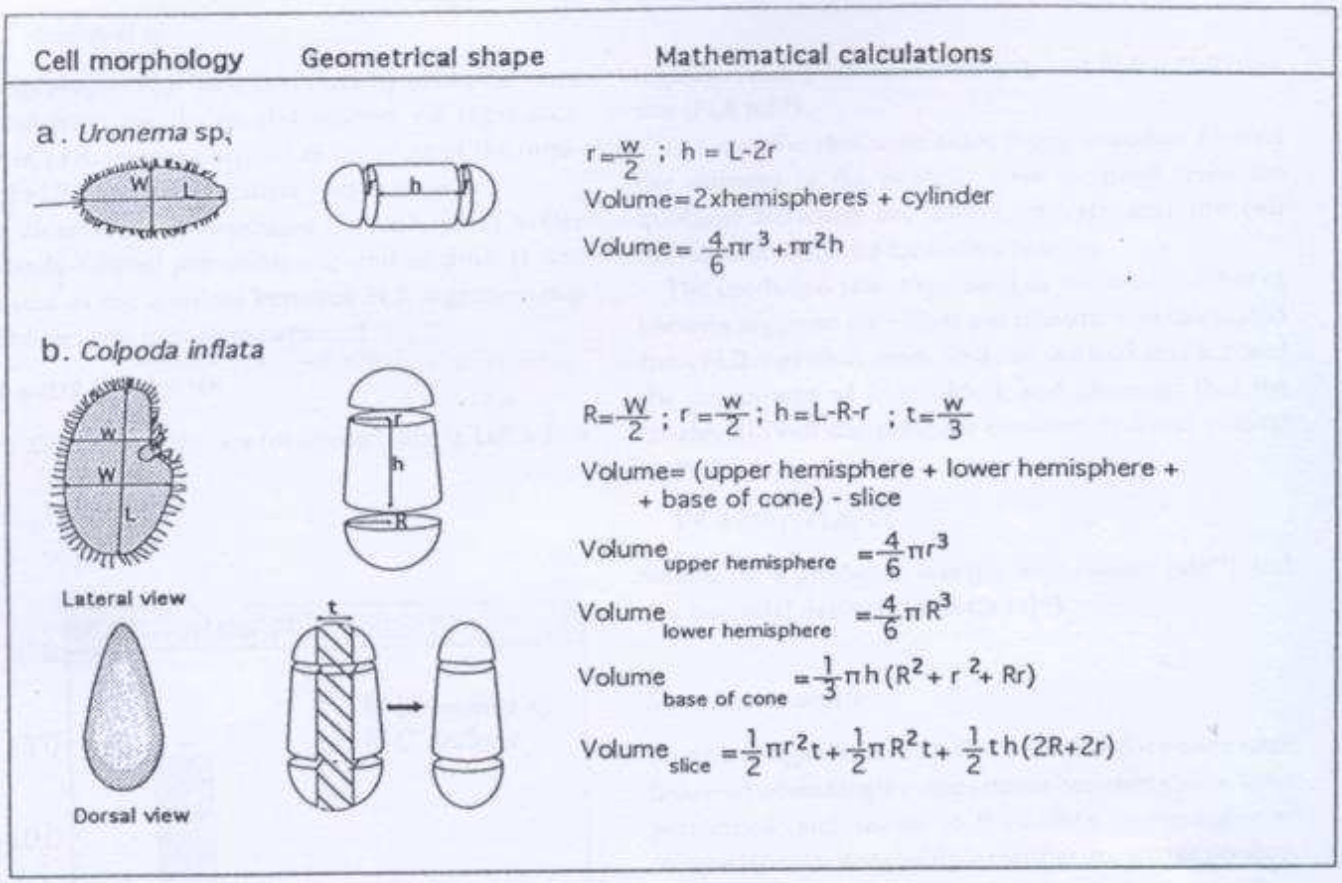

Fig. 1 Geometrical assumptions used to estimate the cell volumes of *Uronema* sp. and *C. inflata*.

concentration) formalin (3% final concentration) (Sherr *et al.*, 1988). As fixatives are known to affect cell volume estimates (Putt & Stoecker, 1989), we assumed that the effect of Lugol–formalin was the same for the two ciliates and constant irrespective of the incubation conditions. The volumes were calculated by assuming geometrical shapes. *Uronema* sp. is an oval-shaped, spheroidal ciliate. Its volume was assumed to be similar to a cylinder bearing two equal hemispheres at its extremes (Fig. 1a). *Colpoda inflata* is a kidney-shaped, laterally flattened ciliate and the length and widths measured corresponded to its lateral view. We likened its biovolume to a geometrical figure composed of a base of a cone with one big and one small hemisphere at its extremes. However, to obtain a better fit to the flattened shape of the ciliate, we subtracted a central slice from the 'spherical' volume (Fig. 1b), taking into account the descriptions given by Foissner *et al.* (1991) and our observations.

Grazing estimations

A volume of suspension of FLB from bacterioplankton of the Butrón River was added to the microcosms to achieve a low percentage (between 2.4 and 9.2%) of the bacterial density in each experiment. The flasks were incubated for 1 h at the accurate temperature. During the first 20 min, 10-ml subsamples were taken at 2-min intervals, and at 5–10-min intervals during the next 40 min. Subsamples were preserved with alkaline Lugol–formalin, and stored for at most 2 weeks at 2 °C in the dark until microscopical processing.

The preserved subsamples were stained with diamidino-phenylindol (DAPI) (Porter & Feig, 1980). The filters were first observed under UV light at a magnification of × 200, and when a ciliate was located, the incident light was changed to blue light, which allowed the counting of FLB inside it. Between thirty and sixty ciliates were inspected for FLB ingestion in

each subsample. FLB ingestion rates by protozoa were obtained from the slopes, determined via regression analysis, of the linear portions of the plots of the numbers of FLB ingested per ciliate versus time.

The clearance rate expresses the volume of water completely filtered per ciliate per unit of time. It was calculated as the quotient between FLB ingestion rate and FLB density in each experiment.

$$CR = (UR/FLB) \times 10^6$$

where: CR = clearance rate (nl ciliate^{-1} min^{-1}), UR = FLB ingestion rate (FLB ciliate^{-1} min^{-1}), and FLB = FLB density (FLB ml^{-1}).

The specific clearance rates (body volumes filtered per minute) of the protists were obtained from the quotient between the clearance rate and the cell biovolume observed for each situation.

The predation rate, expressed as the total number of bacteria ingested per ciliate per minute, was calculated from FLB ingestion rates. To do so, we took into account the percentage of FLB added, and assumed that the ciliates did not discriminate between FLB and natural bacteria.

$$PR = UR/(FLB/B)$$

where: PR = predation rate (bacteria ciliate^{-1} min^{-1}), and B = bacterial density (bacteria ml^{-1}).

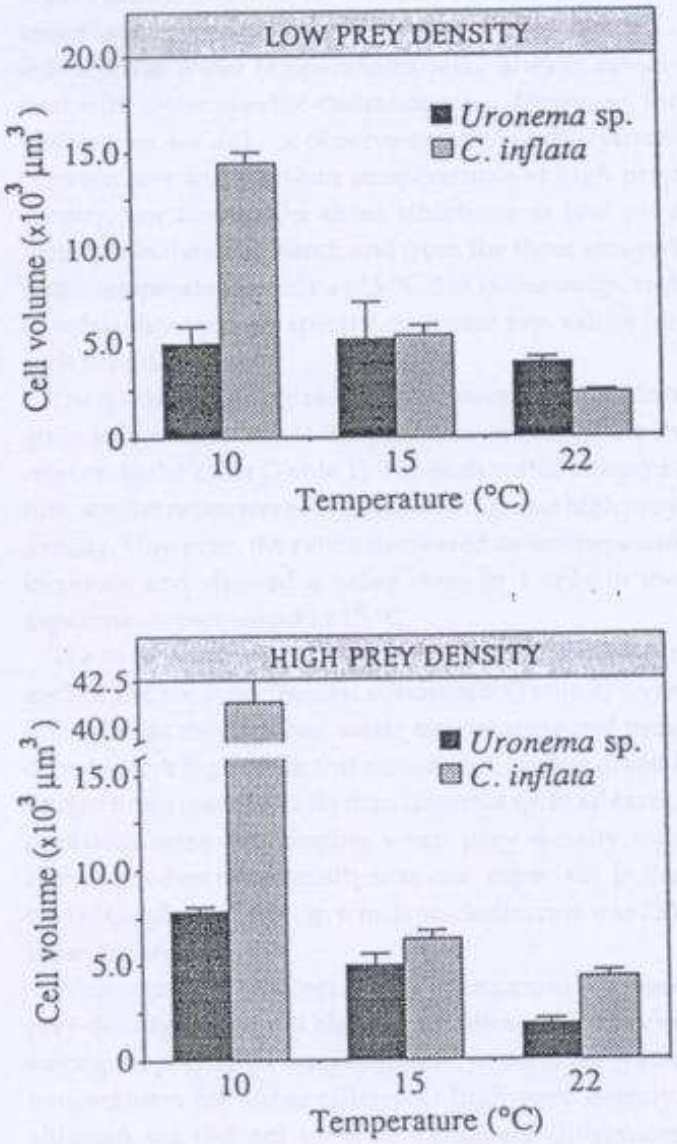

Fig. 2 Cell volumes of *Uronema* sp. and *C. inflata* for each water temperature at low and high prey density. Error bars are SE.

Statistical analysis

Statistical significance levels of $P < 0.05$ for t-tests were followed when single comparisons between means were performed, and levels of $P < 0.05/n$ (n = number of comparisons) when multiple comparisons were needed. As the data were not homoscedastic they were log-transformed prior being compared.

Results

Cell volumes of Uronema sp. and C. inflata.

The cell volumes of the two ciliates determined for each situation are shown in Fig. 2. *Colpoda inflata* showed significantly lower biovolumes at increasing temperatures, while *Uronema* sp. only followed this pattern at high prey density. In conditions of low prey density, the cell volume of *Uronema* sp. remained fairly constant in spite of temperature changes.

We also found significant differences between the volume of each ciliate at low and high prey densities in temperatures of 10 and 22 °C. However, at 15 °C not only did each ciliate show similar biovolumes at the two prey densities, but also those values were not significantly different between the two ciliates.

Grazing estimates

Uronema sp. and *C. inflata* filtered significantly more body volumes at low prey density than at high prey

Table 1 Specific clearance rates (SCR) ± SE (n = 3) and ratios between the two ciliates at each water temperature and prey density assayed

	SCR (body volumes min^{-1})				SCR ratio	
	Uronema sp.		*C. inflata*		*Uronema* sp.: *C. inflata*	
Temp. (°C)	Low density	High density	Low density	High density	Low density	High density
10	166 (± 42)	30 (± 5)	60 (± 16)	14 (± 3)	2.8	2.1
15	312 (± 88)	28 (± 3)	327 (± 1)	36 (± 5)	0.9	0.8
22	143 (± 89)	241 (± 3)	647 (± 241)	964 (± 97)	0.2	0.2

density at the lower temperatures assayed, but not at 22 °C (Table 1). When we analysed the specific clearance rates of these two ciliates in each of the conditions of prey availability in order to observe the effect of water temperature, we noticed a different behaviour. For *C. inflata* lower water temperatures were always associated with lower specific clearance rates. However, for *Uronema* sp. we did not observe significant differences between low and medium temperatures at high prey density, nor among the three situations at low prey density. On the other hand, and from the three assayed water temperatures, only at 15 °C did *Uronema* sp. and *C. inflata* show similar specific clearance rate values for each prey density.

The specific clearance rates ratio *Uronema* sp.: *C. inflata* gives information about the response of one ciliate in relation to the other (Table 1). For each water temperature, similar ratios were obtained at low and at high prey density. However, the ratios decreased as temperature increased and showed a value close to 1 only in the experiments performed at 15 °C.

The predation rates of *Uronema* sp. and *C. inflata* for each of the six experimental conditions (Table 2) were quite similar except when water temperature and prey density were highest. In this situation, *C. inflata* grazed sixteen times more bacteria than *Uronema* sp. In all cases, predation rates were higher when prey density was high than when prey density was low, especially in the case of *C. inflata* at 22 °C, in which predation rate was 231 times higher.

With regard to the effect of water temperature, at low prey density we did not observe significant differences among the predation rates estimated at the three water temperatures for either ciliate. At high prey density, although we did not observe significant differences among the predation rates estimated at the low and medium water temperatures for either ciliate, they showed a significantly higher predation rate at 22 than at 15 °C.

Table 2 Predation rates (PR) ± SE (n = 3) of the two ciliates at each water temperature and prey density assayed

	PR (bacteria $ciliate^{-1}$ min^{-1})			
	Uronema sp.		*C. inflata*	
Temp. (°C)	Low density	High density	Low density	High density
10	0.41 (± 10)	8.68 (± 1.49)	0.42 (± 0.11)	20.9 (± 3.9)
15	0.81 (± 0.23)	7.33 (± 0.81)	1.45 (± 0.16)	10.9 (± 1.6)
22	0.34 (± 0.21)	13.3 (± 0.26)	0.90 (± 0.41)	208.3 (± 20.9)

Discussion

The cell volumes of *Uronema* sp. and *C. inflata* varied noticeably depending on the condition of prey density and water temperature assayed. Moreover, these variations were different for each ciliate. *Colpoda inflata* showed a clear tendency to reduce its body size as temperature increased, while for *Uronema* sp. this tendency was less pronounced and even undetectable when prey density was low.

The relationship between water temperature and body size of organisms has been discussed by several authors (Jackson & Berger, 1984; Verity, 1985; Lucas, Probyn & Painting, 1987; Choi & Peters, 1992). At high temperatures, the physiology of organisms suffers several

changes as a consequence of accelerated activity: the metabolic losses (respiration, excretion) increase more quickly than food ingestion rates, and consequently, the growth efficiency becomes lower. Our results concerning lower biovolumes at increasing water temperatures clearly conform to this explanation when prey availability is high. In addition, it is necessary to take into account the growth phase reached by the ciliates under the different treatments. In our work, the two ciliates were inoculated from 6-day, stationary-phased cultures, but in the experiments with high bacterial density, the amount of prey was enough to allow them to grow and reach an exponential phase, after a lag whose length in time depended on water temperature. After 72 h, the higher the temperature was, the further along the exponential phase the cells were. The differences detected in the cell volume of the two ciliates may correspond to a pattern similar to that proposed by Taylor & Berger (1976), who detected noticeable differences in the cell size of the ciliate *Colpidium campylum* inoculated from stationary phase into fresh bacterial medium: this ciliate increased its volume during the lag phase, decreased in size during the exponential phase and reached a minimum and constant biovolume in the stationary phase.

However, in the situation of short supply of bacterial prey, the general effect of water temperature on the biovolume seems to be minimized, as *Uronema* sp. did not show significant differences in its volume at any temperature studied, and the changes in volume detected in *C. inflata* were noticeably lower than in conditions of high prey density. These reduced variations in body size could be explained taking into account that scarcity of prey made the ciliates go on a maintenance phase and, according to Taylor & Berger (1976), the cell volume would tend to be invariable in this state of the cells. In this case the differences in biovolume detected in *Colpoda inflata* could only be due to water temperature, while *Uronema* sp. would be less sensitive to this factor. It is also remarkable that only at the moderate water temperature (15 °C) did *Uronema* sp. and *C. inflata* show similar biovolumes, with no apparent effect of prey availability or growth phase.

The grazing activity of the two ciliates was analysed in terms of specific clearance rates, because their volumes changed with prey density and water temperature. Both ciliates showed higher specific clearance rates at lower water temperatures and prey densities. This might indicate that the high and low bacterial densities assayed represent food levels above and below a critical prey density, defined as the food level associated with the attainment of maximum ingestion rates. Therefore the predation rates observed at high prey density and 10 and 15 °C would be comparable to potential maximum ingestions. This saturating food level could have shifted upwards at 22 °C because the filtering activity showed an increase from the low to the high prey density. This would imply that if conditions were appropriate, both ciliates would be able to graze more bacteria than we observed in this experiment. Such a variation in the critical food concentration with temperature has been detected in zooplankton (Vidal, 1980a, b, c, d) but contradictory results have been observed (Durbin & Durbin, 1992).

To study the influence of bacterial availability on protistan bacterivory, we analysed the ratio between the specific clearance rates of *Uronema* sp. and *C. inflata* at each experimental condition (see Table 1). These ratios were similar at low and high prey density for each water temperature, which indicated that both ciliates reacted in a similar way to this factor. Moreover, the values were close to 1 at the moderate water temperature, 15 °C (0.9 and 0.8 at low and high prey density, respectively), which means that both ciliates exhibited nearly the same specific clearance rate at each prey density. As we have mentioned above, this water temperature was the only one at which *Uronema* sp. and *C. inflata* showed similar biovolumes at low and high prey density. Therefore, the condition of 15 °C provided an ideal situation to analyse the influence of prey density on the grazing activity of *Uronema* sp. and *C. inflata*. A decrease of about 1.4 orders of magnitude in bacterial availability (4×10^7 to 10^6 bacteria ml^{-1}) involved a 10-fold increase in activity (specific clearance rate) of the two ciliates. The energetic cost associated with this increased filtering activity would also become higher, but not as high as to allow the ciliates to obtain the same amount of prey per unit of time as in the plentiful prey situation (0.8 vs. 7.3 bacteria $ciliate^{-1}$ min^{-1} for *Uronema* sp., and 1.5 vs. 10.9 bacteria $ciliate^{-1}$ min^{-1} for *C. inflata*). On the other hand, in the aquatic system of the Butrón River the values of prey density assayed in this work are usually associated with extreme water temperatures (10 and 22 °C), while when water temperature is moderate (15 °C) there are lower changes in bacterial abundance (Barcina *et al.*, 1991). According to our results, the response of the two ciliates to changes in prey density at this moderate temperature

would rely on the regulation of their filtering activity, in such a way that they would clear greater water volumes when prey was scarce and smaller water volumes when prey was plentiful. A similar shift in clearance due to changes in prey densities has been found by several authors (Sherr, Sherr & Berman, 1983; Verity, 1985; Sanders *et al.*, 1989).

As has been previously reported (Iriberri *et al.*, 1993), the environmental variation in the Butrón River throughout the year in relation to prey density and water temperature can be reduced to two cases; a cold situation, characterized by low and medium water temperatures (8–14 °C) and low prey density (mean value 3.3×10^6 bacteria ml^{-1}), which may be considered less favourable to the micro-organisms, and a warm situation, with higher water temperatures (20–24 °C) and higher bacterial availability (mean value 8×10^6 bacteria ml^{-1}) which can be considered favourable. This close link between the two factors detected in the Butrón River has already been observed in many other aquatic systems (Goulder, 1980, 1986; Nagata, 1988; Gocke & Rheinheimer, 1988). Thus, the condition of 10 °C and scarcity of prey tested in this study can be considered as a non-favourable situation and the condition of 22 °C and high prey density as a favourable situation.

When we compare the specific clearance rates of *Uronema* sp. and *C. inflata* at 15 versus 10 °C in conditions of scarcity of bacterial prey, we detect different physiological strategies for each ciliate. *Colpoda inflata* significantly reduced its specific clearance rate, although its body size became significantly greater. As a consequence, the amount of ingested bacterial prey was 0.42 bacteria $ciliate^{-1}\ min^{-1}$. In the case of *Uronema* sp., there were no notable changes either in its cell volume or in its specific clearance rate. However, this ciliate also reached an ingestion of 0.41 bacteria $ciliate^{-1}\ min^{-1}$, the same as *C. inflata*. Therefore, in these conditions of low prey density and low water temperature both ciliates may cause the same grazing pressure on the prey community, but *Uronema* sp. will probably be more successful in these conditions than *C. inflata*, because of its ability to obtain the same amount of prey for feeding a smaller body size.

The results obtained at 15 and 22 °C at high prey density allowed us to consider the effect of an increase in water temperature on the activity of the two ciliates. Contrary to what happened in the previous case, these results showed that both ciliates presented a similar physiological response: both of them reduced their cell volumes and increased their specific clearance rates. Therefore, and in spite of their smaller body sizes, *Uronema* sp. and *C. inflata* were able to graze a very large number of bacterial prey: 13.3 bacteria $ciliate^{-1}\ min^{-1}$ for *Uronema* sp. and 208.3 bacteria $ciliate^{-1}\ min^{-1}$ for *C. inflata*. These high prey ingestion rates suggest a typically opportunist behaviour—maximum exploitation of resources when they are abundant enough and environmental conditions are appropriate. However, if we analyse the results obtained for this same increase in water temperature but at low prey density, we see that *C. inflata* did not increase its specific clearance rate significantly and *Uronema* sp. even reduced it. Therefore the two ciliates seem to need both factors, high water temperature and high bacterial prey supply, to be able to show such an opportunist response, because a high prey density would be necessary to compensate for the metabolic losses produced as a consequence of an accelerated activity due to high water temperature. But the two ciliates did not show the same degree of opportunism in these very favourable conditions, as *Colpoda inflata* showed a noticeably higher bacterivorous activity than *Uronema* sp. This enhanced response of *C. inflata* is due to a higher sensitivity than *Uronema* sp. to the water temperature factor rather than to the prey density factor. This may be deduced from the similar ratios of specific clearance rates at the two prey densities obtained at 22 °C (see Table 1), and the decreasing values of those ratios at any prey density when temperature increases.

The opportunist behaviour of the studied ciliates to take advantage of favourable conditions, both physical (water temperature) and nutritional (bacterial prey availability), suggests a potential importance of the ciliate community as a factor of bacterial mortality when environmental conditions change to maximum abundance and/or production of bacteria, which in turn is usually associated with high water temperatures. Moreover, not all the components of the ciliate community will cause the same grazing pressure, but it will depend on the species composition, as can be deduced from this study: *C. inflata* would be able to cause a more intensive grazing pressure on the prey community than *Uronema* sp. On the other hand, when water temperatures and bacterial densities become low, both studied ciliates showed very reduced predation rates. This suggests that when environmental conditions are unfavourable, some other factors, such as flagellate grazing, viral lysis,

etc., would become more important than ciliate grazing in controlling the bacterial community.

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