CHAPTER 5

"Carbon flow dynamics in the pelagic community of the Sau Reservoir"

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ABSTRACT

Changes in the pelagic community structure and activity along the longitudinal axis of the eutrophic Sau Reservoir (Catalonia, NE Spain) were studied between 1996 and 1999. Samples were taken from several transects from river to dam, measuring dissolved organic carbon (DOC), bacterial abundance and production, chlorophyll *a* concentration, heterotrophic nanoflagelate (HNF) and ciliate abundances and their grazing rates, and zooplankton density. The role of microbial and classical food chains (i. e. based directly on phytoplankon) were compared in the Sau Reservoir by analysing river-to-dam gradients in biomass and carbon and their temporal changes. The detritic metabolic pathway was more important near to the inflow, due to high allochthonous organic matter loads allowing the rapid development of the microbial food web. Protozoans (HNF and ciliates) consumed most of the bacterial production (i.e. >50 %) in the reservoir. As opposed to the systems of lower trophic status ciliate carbon biomass and bacterivory contributions were larger than those of the HNF. We estimated species-specific ciliate growing rates on bacteria and distinguished several periods with high importance of distinct ciliate communities.

Key words: reservoir, longitudinal gradients, plankton, carbon flow, microbial food web

INTRODUCTION

During the past two decades, microbial studies have convincingly demonstrated the existence of a diverse microbial community in aquatic planktonic ecosystems where microbial production is integrated in the pelagic food web at all levels (GAEDKE *et al*., 1995). Also, the microbial loop mediates carbon flow from bacterioplankton to zooplankton. Bacteria are relevant members of the limnetic planktonic food web, both in terms of biomass and production share (SOMMARUGA and ROBARTS, 1997). Bacterial consumers, mostly protozoans but also metazoans, explores the biomass produced by the microbial food webs, which can transport large quantities of carbon in freshwater lakes (WEISSE and MÜLLER, 1990) and constitute the route whereby allochthonous DOC enters the classical food web (JANSSON *et al*., 2000).

The relative contribution of microbial production to total organic carbon in the planktonic food web (i.e., the link-sink issue) is still under discussion, in the light of recent findings about the microbial food web (PORTER *et al*., 1979; SHERR and SHERR, 1988; BERMAN, 1990; PORTER, 1996; GAEDKE *et al*., 1995; HART *et al*., 2000). In marine (GASOL *et al*., 1997) and freshwater (DEL GIORGIO and GASOL, 1995) systems, a parallel reduction in the ratio of total heterotrophic (bacteria + protozoa + mesozooplankton) biomass to total autotrophic biomass with increasing autotrophic planktonic biomass has been observed. Also, RIEMANN and CHRISTOFFERSEN (1993), suggest the increasing importance of the microbial food chain compared to the classical food chain along a gradient of increasing productivity. To explain these observations it is need of further studies to examine the microbial trophodynamics along an eutrophication gradient.

KIMMEL (1983) suggested that the sequence of allochthonous dissolved organic matter (DOM)-to-bacterioplankton-to-macrozooplankton appeared unlikely to be a major organic carbon pathway in reservoirs with significant phytoplankton crops. Nonetheless,

our results from the Sau Reservoir (COMERMA *et al*., 2001) suggest a longitudinal web DOM-to-bacteria-to-heterotrophic nanoflagellates (HNF)-to-ciliates-to-zooplankton could be an important pathway through which allochthonous organic carbon is entering the reservoir food web.

The present case-study, from the eutrophic canyon shape Sau Reservoir contributes to this issue by yielding carbon biomass and flux data in a pelagic ecosystem dominated by high organic inputs. Singularly, a new focusing on carbon flux dynamics has raised new interest in detailed studies of longitudinal changes, which are high important in reservoirs (ŠIMEK *et al*., 1998; ARMENGOL *et al*., 1999; COMERMA *et al*., 2001).

SAMPLING AND METHODOLOGICAL REMARKS

The carbon (C) flow within the pelagic food web in the Sau Reservoir during the 1996-1999 period was investigated using empirically based data. Samplings covered all seasons (July 1996, April 1997, July 1997, October 1997, December 1997, February 1998, May 1998 and April 1999).

HEJZLAR (personal communication) measured biodegradable dissolved organic carbon (BDOC) in the Sau Reservoir by method of SERVAIS *et al*. (1987), estimating it in 20 % of the total DOC.

Original measurements of microbial plankton body sizes were converted to units of C using several conversion factors cleaned from the literature for each group (Table 5.1). Constant C:Volume conversion factors for different protistan plankton groups over large size ranges will cause systematic errors in biomass estimates (MENDEN-DEUER and LESSARD, 2000). Carbon content in algae was estimated from in situ chlorophyll a concentrations. Carbon in zooplankton was estimated from dry weight using conversion factors from the literature (Table 5.1).

Bacterial production and protozoan bacterivory (millions of cells per day) were converted to biomass produced and consumed (mg C l^{-1} day^{-1})

by multiplying both variables, production and bacetrivory, by the *in situ* bacterial carbon content (fg C $cell^{-1}$) measured each time.

Group	Reference	Value
Bacteria	NORLAND, 1993	$C = 0.12 \cdot V^{0.72}$
HNF	BØRSHEIM and BRATBAK, 1987	$C = 0.22 \cdot V$
Ciliates	PUTT and STOECKER, 1989	$C = 0.14 \cdot V$
Phytoplankton	PETERSON, 1978	$C = 37.5 \cdot$ [*Chl. a*]
Zooplankton	LATJA and SALONEN, 1978	C = 50 % DW

Table 5.1
Conversion factors used to calculate biomass (mg C l^{-1}) in the plankton of the Sau Reservoir. Carbon biomass (C) for bacteria, HNF and ciliates are in pg C $cell^{-1}$, volume (V) is in μm^3. Carbon units (C) for phytoplankton and zooplankton are derived from chlorophyll *a* concentrations ([*Chl.* a]), in mg m^{-3}, and from dry weights (DW), in g l^{-1}, respectively.

RESULTS

The most important fraction of organic carbon in the lacustrine zone of the Sau Reservoir was in the dissolved form. The DOC concentration ranged between 1 and 6 mg l^{-1} (Fig. 5.1). Higher values than these of both DOC and POC were recorded at the surface in summer under stratification conditions. Chlorophyll *a* maxima generally coincided with the organic carbon peaks (e.g. in 1998). On the other hand, in dry years such as 1999 (max. depth 25-30 m), the combined effects of phytoplankton excretion, strong river influence and release from the sediment lead to high DOC and POC concentrations throughout the water column.

All values available from the Sau Reservoir were used to estimate epilimnetic biomasses and activities through space and time (Fig. 5.2). The theoretical size of an organism on the x-axis and trophic level on the y-axis determine a position of a plankton group in the figures.

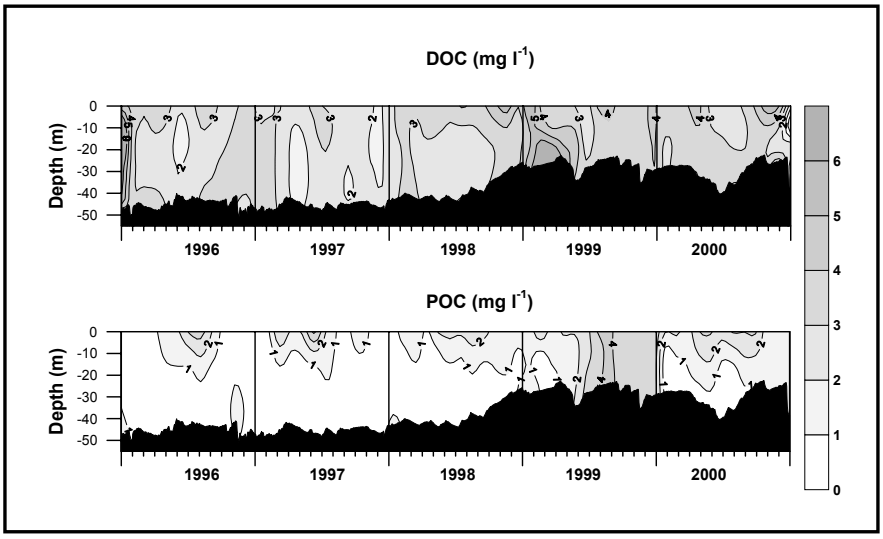

Figure 5.1
Dissolved organic carbon (DOC) and particulated organic carbon (POC) concentrations in the water column in the lacustrine zone of the Sau Reservoir during the 1996-2000 period.

Trophic level concept was established and calculated for Lake Constance by GAEDKE *et al.* (1995) and we have used this concept in order to show a scheme of the pelagic food web.

Biodegradable dissolved organic carbon (BDOC) ranged between 0.4-0.6 mg C l^{-1}. No clear seasonal trend was detected nor spatial pattern across the epilimnion of the reservoir. BDOC did not seem to be factor limiting growth of microbes. The highest planktonic biomass and activities were measured during the warm seasons (spring-summer) in all years (Fig. 5.2) suggesting both variables are directly influenced by water temperature. Bacteria reached its highest biomass in the riverine zone (0.4 and 0.2 mg C l^{-1} in spring-summer and autumn-winter, respectively) and generally decreased downstream. Bacterial production followed the same seasonal pattern and longitudinal gradient as found for bacterial biomass. Carbon production by bacteria varied between 0.017 and 0.734 mg C l^{-1} day^{-1}, comprising 95-275 % and 31-53 % of the total bacterial carbon standing stock in warm and cold periods, respectively. The biomass of heterotrophic nanoflagellates (HNF) and their bacterivory activity were substantial in the river during the warm periods and negligible in winter. In the riverine zone, the community of HNF occurring

during the spring-summer periods grazed 38 % of the total bacterial carbon production and similarly in autumn-winter they grazed 31 %.

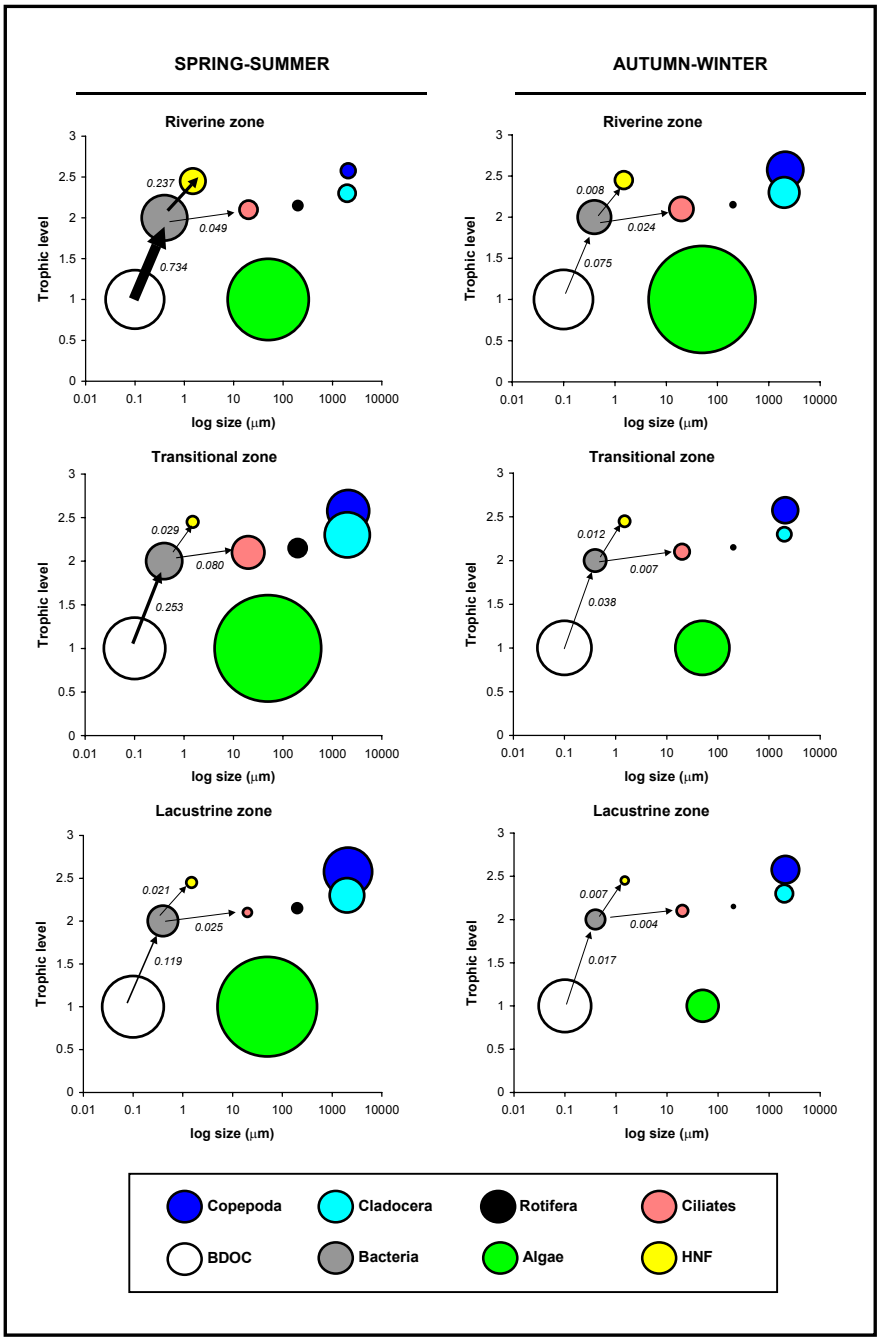

Figure 5.2
Mean biomass (bubbles, mg C l^{-1}) and microbial activities (arrows, mg C l^{-1} day^{-1}) in the pelagic community across the Sau Reservoir from samplings during the 1996-2000 period (Jul-96, Apr-97, Jul-97, Oct-97, Dec-97, May-98 and Apr-99), grouped into spring-summer and autumn-winter periods. Feb-98 has been excluded. The area of bubbles and the thickness of rows are directly proportional to the values they represent. Bubbles of the legend correspond to a biomass of ~ 0.170 mg C l^{-1}. Theoretical values of trophic level and body size are from GAEDKE *et al*. (1995).

Ciliate biomass was higher in the riverine and, mostly, in the transitional zones than downstream. They consumed an average of 61 % of the total bacterial carbon production. This percentage varied depending on the dominant ciliate group.

The major contributors to ciliate biomass were peritrichs (Fig. 5.3), mostly genus *Epistylis* and *Vorticella*. The relative proportions of the main groups in the Sau Reservoir to total ciliate biomass (Fig. 5.3) varied between samplings. Results in Fig. 5.3 show the total abundance of main taxa, pooling together results from all sampling stations. In Jul-97 and Oct-98, Prostomatids had higher biomasses than the rest of ciliate groups. Mainly Peritrichs were dominant in Feb-98 and May-98.

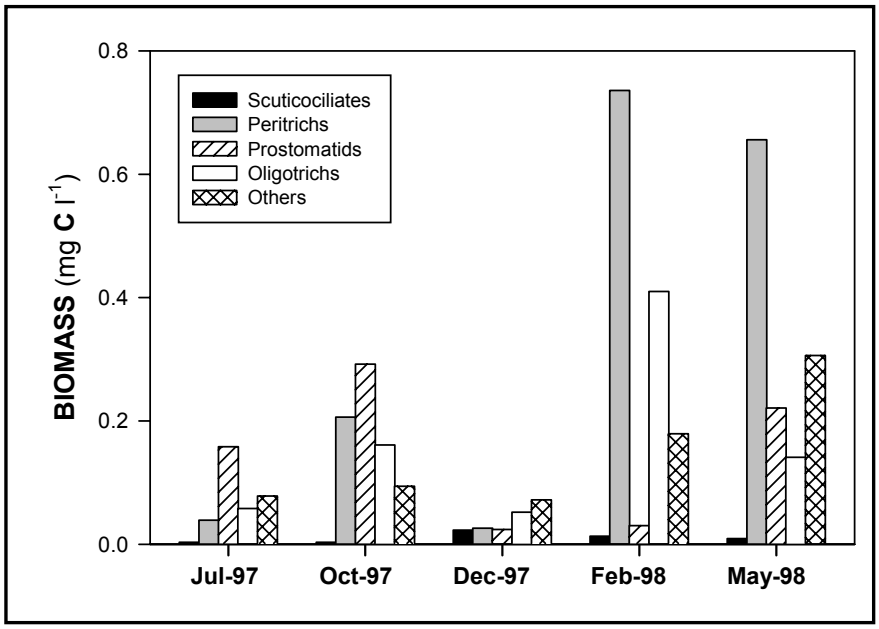

Figure 5.3
Ciliate biomass (mg C l^{-1}) in the epilimnion of the Sau Reservoir in Jul-97, Oct-97, Dec-97, Feb-98 and May-98. Hatching of bars show biomass for the different main ciliate groups found in samplings (Scuticociliates, Peritrichs, Prostomatids, Oligotrichs and others).

In Feb-98 oligotrichs also contributed significantly to total ciliate biomass, in particular little individuals (around 25 μm of diameter) of the genus *Rimostrombidium*, which achieved densities >400 cells ml^{-1} in the transitional zone (Fig. 5.4). To high light this, we excluded Feb-98 values from Fig. 5.2 and plotted them separately (see Fig. 5.4). Flagellate, phytoplankton and zooplankton biomasses in Feb-98 were similar to those in autumn-winter (compare Figs. 5.2 and 5.4). Ciliate biomass and

ciliate grazing rates were very high during Feb-98 (0.027-0.241 mg C l^{-1} and 0.042-0.175 mg C l^{-1} day^{-1}) compared to those during the rest of samplings, thus e. g. ciliates (mainly genus *Vorticella* and *Rimostrombidium*) consumed between 103 and 388 % of the total bacterial carbon production.

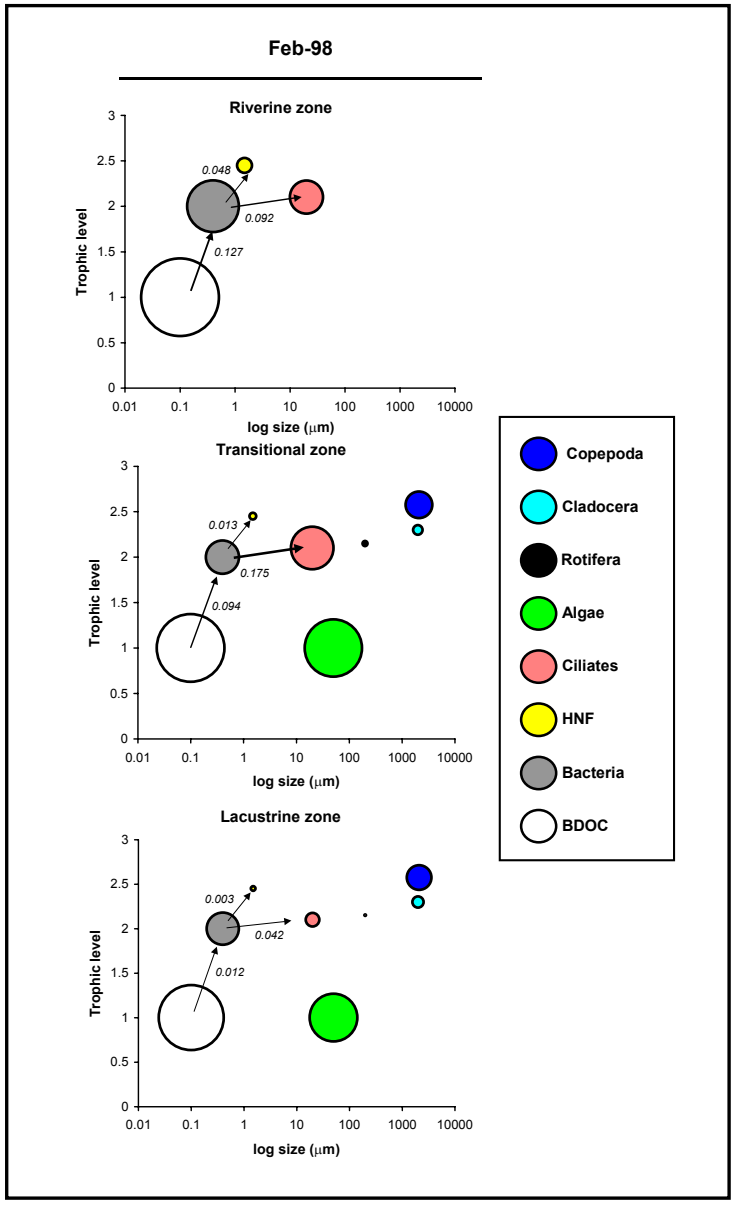

Figure 5.4
Mean biomass (bubbles, mg C l^{-1}) and microbial activities (arrows, mg C l^{-1} day^{-1}) in the pelagic community across the Sau Reservoir in Feb-98. The area of bubbles and the thickness of rows are directly proportional to the values they represent. Bubbles of the legend correspond to a biomass of ~ 0.170 mg C l^{-1}. Theoretical values of trophic level and body size are from GAEDKE *et al.* (1995).

Phytoplankton biomass in the reservoir had two maxima, one in the riverine zone, due to the allochthonous algal impute brought by the river especially during autumn-winter periods, and the other in the transitional zone, due to autochthonous algal production (see Fig. 5.2).

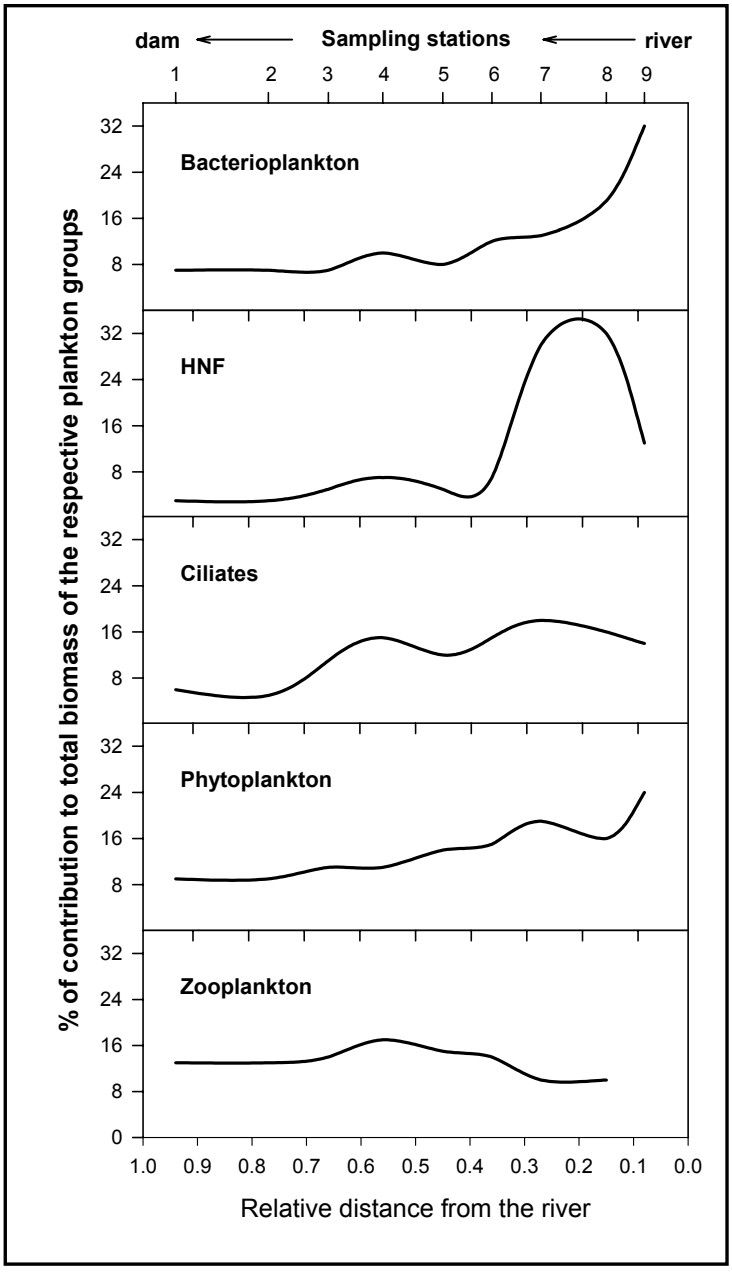

Figure 5.5
Relative proportions (in %) of bacteria, heterotrophic nanoflagellates (HNF), ciliates, phytoplankton and zooplankton biomass (mg C l^{-1}) of the total biomass in each group down a river-to-dam axis in the Sau Reservoir. The sum of the nine values through the reservoir for each line is =100%. Means are of eight longitudinal transects (Jul-96, Apr-97, Jul-97, Oct-97, Dec-97, Feb-98, May-98 and Apr-99).

The peak in zooplankton biomass (Fig. 5.2) mostly overlapped the phytoplankton biomass maximum in the transitional and lacustrine zones. Rotifers achieved the maximum biomass (0.051 mg C l^{-1}) in the transitional zone of the reservoir in spring-summer, but were the group with the smallest contribution to total zooplankton biomass. The biomass of copepods was usually larger than that of cladocerans.

The overall changes in biomass of the main members of the pelagic food web were due to the specific development of different groups down the longitudinal axis of the reservoir (Fig. 5.5). During all seasons, bacterial biomass mostly peaked in the river (station 9), and then decreased downstream by a factor of up to ~ 6.

Most HNF and ciliate biomass developed between the two consecutive downstream stations from the river (i. e. stations 8 and 7). The steep increases in biomass, by factors 6 and 3, respectively, were likely a response to the very high input of growing bacteria with the river (cf. Figs. 5.5 and 5.6; ŠIMEK *et al*., 1998 and Chapter 4). Phytoplankton biomass was high in the river (station 9) and station 7, decreasing downstream (Fig. 5.5). Largest increases in zooplankton biomass occurred between stations 7 and 4.

Resembling the biomass changes, most bacterial production in the reservoir was located between stations 9 and 8, near the river inflow (Fig. 5.6), while most protozoan bacterivory occurred between stations 8 and 7. These measured activities are summarized in Figure 5.7 in terms of total carbon produced by bacteria and grazed by protozoan through the reservoir. They both decrease by a factor of about 3 from the riverine to the lacustrine zones.

DISCUSSION

The Sau Reservoir is a deep and narrow river valley reservoir characterized by high organic nutrient inputs. Both its shape and the influence of inputs explain its remarkable river-to-dam gradients in biological variables (i. e. bacterial, HNF, ciliate, phytoplankton and

zooplankton abundances, bacterial production and protozoan grazing rates) measured in the epilimnion (ŠIMEK *et al*., 1998 and 2001; ARMENGOL *et al*., 1999; COMERMA *et al*., 2001). From the eight longitudinal samplings conducted in the 1996-1999 period, clear downstream longitudinal patterns of abundance (Chapter 4) and biomass (Fig.5.5) of the main planktonic organisms (bacteria, heterotrophic nanoflagellates-HNF, ciliates, phytoplankton and zooplankton) were observed.

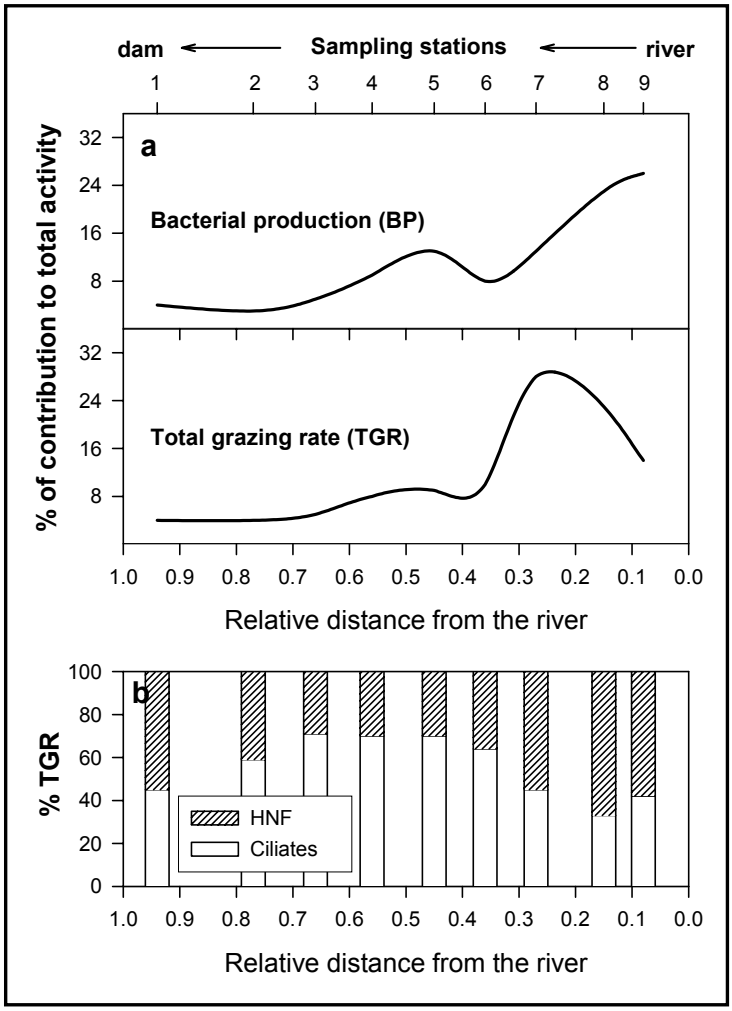

Figure 5.6.
a) Relative proportions (in %) of bacterial production (BP), and protozoan bacterivory (TGR) of the total each activity down a river-to-dam axis in the Sau Reservoir. The sum of the nine values through the reservoir for each line is = 100 %. Means are of eight longitudinal transects (Jul-96, Apr-97, Jul-97, Oct-97, Dec-97, Feb-98, May-98 and Apr-99). b) Relative heterotrophic nanoflagellate (HNF) and ciliate bacterivory of the total grazing rates.

Although the abundance of microorganisms increases with higher nutrient load and primary production (c.f. Fig.5.2), it is believed that the relative contribution of the microbial food web to the carbon flux decreases along eutrophication gradients (WEISSE, 1991; WEISSE and STOCKNER, 1993). However, the spatial heterogeneity in the Sau Reservoir clearly documented that microbes dominate in the riverine zone of this eutrophic system (ŠIMEK *et al*., 1998; ARMENGOL *et al*., 1999; COMERMA *et al*., 2001; GASOL *et al*., 2002). Here, high biomasses and activity rates (bacterial production and protozoan bacterivory, c.f. Figs. 5.2 and 5.7) were found, with their maxima measured close to the river inflow (Figs. 5.6 and 5.7).

Hydrological conditions were determinant factors explaining these biological gradients (Chapter 4). When the river inflow was high, we observed enhanced levels of microbial pollution caused by allochthonous bacterial biomass brought in by the river. In three samplings (Apr-97, Feb-98 and May-98), ~ 50 % of the river water mixed with the epilimnion. Thus, the river during these dates effectively overflowed through the reservoir (Chapter 4). During these samplings, we found the highest values of bacterial biomass and production and, consequently, a marked development of protozoan populations (e. g. ciliates in Feb-98, see Fig. 5.4).

The reservoir has two major sources of dissolved organic carbon (DOC) supporting bacterial growth, i. e. (I) the allochthonous source - originating in the polluted river and yielding the extremely high bacterial production measured in the inflow part (c. f. Figs. 5.2 and 5.6), and (II) the autochthonous organic carbon (i. e. the phytoplankton primary production) likely fuelling most of bacterial production from the transitional zone downstream (ŠIMEK *et al*., 1999).

Protozoans, especially HNF and ciliates, are recognised as major consumers of bacteria (PORTER *et al*., 1985). When all protozoan grazing data were averaged, we estimated that 95 % of bacterial carbon production consumed protozoa in the Sau Reservoir.

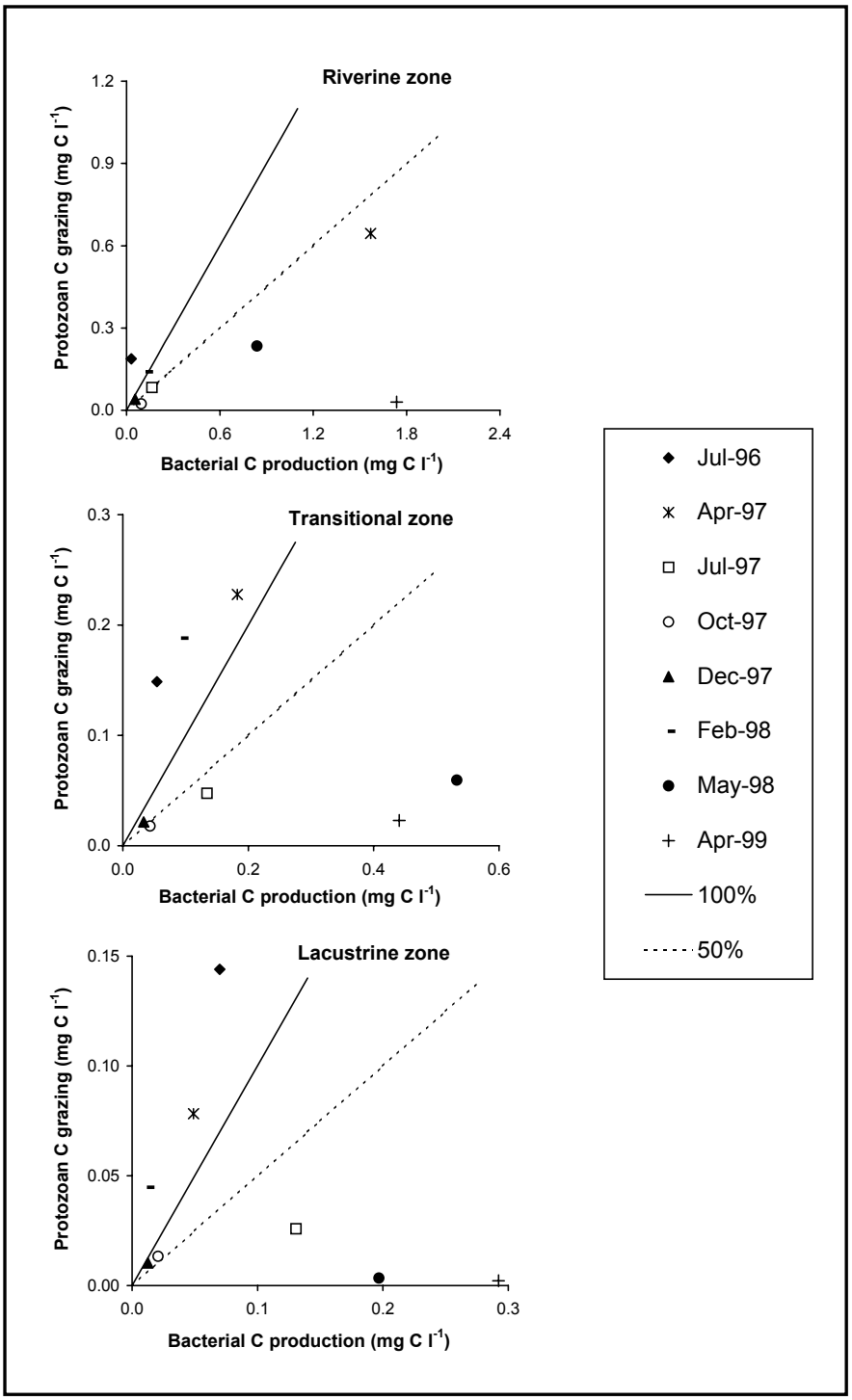

Figure 5.7. Bacterial carbon (C) production and protozoan carbon (C) grazing rates (mg C l^{-1}) measured in the Sau Reservoir during the 1997-1999 period in riverine, transitional and lacustrine areas of the reservoir. Lines show hypothetical responses assuming protozoa consume either 100 % of bacterial production (continuous line) or 50 % (dotted line).

Although HNF have been considered as main consumers of bacteria in the majority of freshwater systems (e. g. BLOEM *et al*., 1989; SANDERS *et al*., 1989), ŠIMEK *et al*. (1999) observed that, with increasing trophy of the systems, ciliates become as important bacterivores as HNF. In the eutrophic Sau Reservoir, HNF and ciliate bacterivory increased with their increases in biomass at the riverine and transitional zones, respectively. Considering the entire reservoir, however, approximately 33 % of the bacterial production was consumed by the HNF, and another 61 % was grazed by ciliates.

Thus, ciliates can play a key role in the Sau Reservoir, mainly during ciliate blooms such that of an oligotrich of the genus *Rimostrombidium* in Feb-98, which, together with the *Vorticella* individuals, consumed >100 % of bacterial production (see Figs. 5.3, 5.4 and 5.6). The peritrich *Epistylis* also showed an annual peak after a clear-water phase, and had marked contributions to the overall protistan bacterivory (Chapter 4). The genus *Halteria* often dominated numerically the pelagic ciliate community in this reservoir (Chapter 4), and it has been described as an important bacterial consumer in meso- to eutrophic freshwaters systems (ŠIMEK *et al*., 2000). Recent studies show that ciliates are significant contributors in the transfer of carbon from picoplankton to higher trophic levels (KISAND and KINGEL, 2000; ŠIMEK *et al*., 2000 and 2001). The most important ciliate bacterivores in freshwaters appear to be, in order of their overall importance: oligotrichs, primarily the omnivorous *Halteria*, peritrichs, and scuticociliates (ŠIMEK *et al*., 2000; Chapter 4). The autochthonous algal carbon produced in the transitional zone of the Sau Reservoir was also an additional food source for some species of ciliates, which achieved a maximum in biomass in this zone of the reservoir (COMERMA *et al*., 2001).

The remarkable microbial activities generated in the riverine and transitional zones of the reservoir could be explained by an enhanced nutrient and organic carbon availability fuelling there the pelagic food webs (c.f. GASOL *et al*., 2002), and it demonstrate an important transfer of organic C to higher trophic levels. In the lacustrine zone of the reservoir, not directly affected by the inflow organic pollution, microbial biomasses were lower than those found upstream, although microbial

activities indicated that there microbial loop might support an efficient transfer of carbon from bacterioplankton to zooplankton. However, further evidence would require direct measurements to confirm the importance of such a pathway. Highest zooplankton biomass was found in the transitional and lacustrine zones of the reservoir (Fig. 5.5). On the other hand, we observed high values of bacterial production at the lacustrine stations in summer, which could not be explained by low protozooplankton grazing rates (Chapter 4). In lacustrine stations during warm seasons (in Jul-97, in May-98 and in Apr-99), protozoans consumed scarcely ~11 % of the total bacterial production (Fig. 5.7). Apart from protozoa, other zooplankters such as rotifers, cladocerans and phytoflagellates, can also be important consumers of bacterioplankton (SANDERS *et al*., 1989). It is suggest that other factors could control microbial communities at the lacustrine stations of this eutrophic reservoir. One of these factors could be the top-down control of bacteria and protozoa by zooplankton. Further work is required on the potentially major forces controlling microbial activities in the lacustrine area during the stratified period (i. e. during warm seasons).

Seasonal shifts in the dominance of autotrophic and heterotrophic production determine the relative strength of the microbial loop in the planktonic food web (PORTER, 1996). Actual data from the Sau Reservoir gives a general trend of carbon flow within the trophic link and some specific features of distinct seasons. A more intensive sampling is needed to examine the opportunistic habits of protozoans (e. g. the blooms of ciliates observed in this reservoir). All planktonic groups had higher C biomass in spring-summer than in autumn-winter. However, the relative proportions of microbial groups and their activities in the pelagic food web increased in autumn-winter. NIXDORF and ARNDT (1993) found protozooplankton were a main regulator of bacteria during the colder season in the eutrophic Lake Müggelsee. From early summer, however, the influence of metazooplankton (i. e. cladocerans) on bacteria was evident. Long-term data collected at station 1 (i. e. near to the dam) in the Sau Reservoir show the development of typical spring peaks in zooplankton abundance. In the short spring clear-water phases, relatively low bacterial and protozoan abundances as well as phytoplankton

biomass were measured. This phenomenon has been well described in the Římov Reservoir (ŠIMEK and STRAŠKRABOVÁ, 1992).

CHAPTER 6

“Bottom-up and top-down factors controlling protozoan growth in a eutrophic reservoir”

ABSTRACT

An experiment with differently top-down and bottom-up manipulated microcosms was conducted during spring 2000 to estimate growth rates of heterotrophic nanoflagellates (HNF) and of main groups of ciliates in the epilimnion of the eutrophic Sau Reservoir (Catalonia, NE Spain). Along with the major factors controlling the growth of protozoan populations we also studied the impact of metazooplankton on the structure of the microbial food web. Depth-integrated samples from the epilimnion were incubated in situ for 72 h and sampled daily in two different types of microcosm. To study resource limitation on growth (bottom-up control), growth rates were calculated in the absence of zooplankton, under three different, dilution-induced food concentrations (25, 50 and 100 %), using dialysis bags. To assess predator effects on growth (top-down control), we compared treatments where different fractions of zooplankton were removed by filtration (>20 μm, >53 μm and without removing any), using polyethylene bottles. Results clearly documented that HNF and ciliate growth rates were controlled mainly by zooplankton predation while only a limited effect of the food resource limitation could be detected in this eutrophic reservoir.

Key words: microcosm, growth rates, heterotrophic nanoflagelates and ciliates

INTRODUCTION

A highly complex combination of bottom-up (nutrient supply) and top-down (grazing) processes control heterotrophic nanoflagellates (HNF) and ciliate populations. Assessing the relative importance of major factors controlling protozoan populations should help to understand carbon flow in planktonic food webs. Moreover, ciliate specific growth rates are still rather rarely reported from systems of high trophic status (MACEK, 1996) though these protozoa frequently play a vital role in nutrient cycling and the control of both primary as well as bacterial productions (SHERR and SHERR, 1987; WEISSE *et al*. 1990; STABELL, 1996).

An interplay of factors such that lake depth, nutrient concentrations, and the thermal regimes of lakes may substantially affect potential food resources and their availability for ciliates and HNF, thus significantly contributing to the development of typical patterns of seasonal succession (PACE, 1982; BARK, 1985; BEAVER and CRISMAN, 1989; BEAVER and CRISMAN, 1990; JAMES *et al*., 1995). PACE (1982) suggested that concentrations of appropriate food resources are important in determining the abundance of heterotrophic ciliates in the plankton. Comerma et al. (in press) described a microbial food web succession from the river inflow to the dam in the Sau Reservoir due to high organic loads, which in turn are regulated by hydrological conditions. PACE (1982) proposed that food resources are probably the major regulator of ciliate populations (diversity, abundance, biomass) in general and the temporal succession of ciliate communities in particular. These studies focused on the bottom-up control (resource limitation) of ciliates while few studies exist directly testing the top-down control (predator control) of ciliate populations (PACE *et al*. 1990; ŠIMEK *et al*. 1990; JÜRGENS *et al*., 1999) although the high predation pressure of zooplankton on the microbial food web is well documented (JÜRGENS and JEPPESEN, 2000; STOECKER and CAPUZZO, 1990). WEISSE and

STOCKNER (1993) showed that the bottom-up control in food webs via substrate/nutrient supply was increasedly replaced by strong top-down interactions along a gradient leading to eutrophy. We hypothesized that top-down forces are the major factors determining the structure of the microbial loop in nutrient unlimited environments such as those in the Sau Reservoir.

In the Sau Reservoir, microbes dominate the riverine zone of this eutrophic system (ŠIMEK *et al*., 1998; ARMENGOL *et al*., 1999; COMERMA *et al*., 2001; GASOL *et al*., 2002). In the lacustrine zone of the reservoir, not directly affected by organic pollution from the river, microbial biomasses were lower than those found upstream, although microbial activities indicated that there might exist a significant pathway for efficient transfer of carbon from bacterioplankton to zooplankton (Chapter 5). Thus, we tested both the top-down and bottom-up control of HNF and ciliate spring populations in the eutrophic Sau Reservoir, and measured specific ciliate growth rates. A short-term manipulation experiment in microcosms (72 hours) was conducted in the epilimnion of lacustrine areas of the Sau Reservoir during May 2000.

METHODOLOGICAL REMARKS

Microcosms. Two types were used: dialysis bags (alowing relatively free penetration of limiting substrates and nutrients), to test bottom-up control and polyethylene bottles (no penetration of the substrates), to test top-down control on protozoan growth rates.

Dialysis bags (Sigma) had a molecular weight cut-off size of 6,000-8,000 Da and maximal width of 10 cm and were cut in lengths of 30 cm to hold 1200 ml. The bags had been thoroughly washed in hot tap water, rinsed overnight, and then soaked for >3 h in Milli-Q water before use. Then they were filled with 1.2 litres of an integrated epilimnion water sample, which had been filtered through a 20 μm mesh to remove any larger metazooplankton and large ciliates. The dialysis bags were closed

and attached to the inner part of small, wired cages. To reduce food particle concentration for HNF and ciliates (mainly bacteria) we diluted (1:2 and 1:3) selected treatments using epilimnetic depth-integrated water filtered through a 0.2 μm nucleopore filter (max. 15 mmHg pressure, to avoid cell disruption). We diluted food sources since in situ bacterial abundance was sufficiently high in May 2000 (i. e. $5\text{-}6\cdot10^6$ bacteria ml^{-1}).

Polyethylene, transparent, 5-litre bottles were filled with 3 litres of an integrated water sample filtered through a 53 μm mesh to remove metazooplankton. One treatment was additionally passed through a 20 μm mesh. In a third treatment, zooplankton was reintroduced at approximately natural densities (i. e. $3.55\cdot10^6$ ind m^{-2}).

Finally, six treatments were performed as summarised in Table 6.1. Three replicates were conducted per treatment (18 microcosms in total). Note that treatments C and F are the same except for the container used; i.e., dialysis bags penetrable for low molecular compounds versus non-penetrable polyethylene bottles.

CONTROL:	**BOTTOM-UP (Dialysis bags)**		
TREATMENT:	Water filtered through a 20μm mesh and diluted 1:3 with water filtered through a 0.2μm membrane	Water filtered through a 20μm mesh and diluted 1:2 with water filtered through a 0.2μm membrane	Water filtered through a 20μm mesh
NAME OF TREATMENT:	**A**	**B**	**C**
CONTROL:	**TOP-DOWN CONTROL (Polyethylene bottles)**		
TREATMENT:	Water filtered through a 53μm mesh and zooplankton added	Water filtered through a 53μm mesh	Water filtered through a 20μm mesh
NAME OF TREATMENT:	**D**	**E**	**F**

Table 6.1
Experimental design of the manipulation experiment (duration: 72 h) conducted in the Sau Reservoir (Barcelona, Spain) in May 2000.

Microcosms (i. e. dialysis bags or polyethylene bottles) were placed in the reservoir, attached to weights, submerged 1.5 m from the surface at the same site and fixed by buoys. Both types of microcosms were

sampled at times 0, 24, 48 and 72 hours, taking 50 ml subsamples and preserving them with alkaline Lugol's solution (0.5%), and then adding formaldehyde (2%). Samples were decolorized with a few drops of sodium thiosulphate (3%) (SHERR and SHERR, 1993).

Growth rates and doubling times of ciliate and HNF. The population growth rates (μ) can be estimated simply from changes in population density (N_1, N_2) between two observation times (t_1, t_2), assuming exponential growth, using the following expression (LAMPERT and SOMMER, 1997):

$$\mu = \frac{(\ln N_2 - \ln N_1)}{(t_2 - t_1)} \qquad \text{Equation 6.1}$$

The population growth rates (μ) were estimated for HNF and ciliates between 0 and 48 h. of experiment initiation in each treatment. Doubling time (T) was calculated as follows,

$$T = \frac{(\ln 2)}{\mu} \qquad \text{Equation 6.2}$$

Data analysis. Effects of food sources and predation manipulation (treatments A, B, C, D, E and F, see Table 6.1) on the abundance of HNF and ciliates were tested by two-way ANOVA with repeated measures on factor time (0, 24, 48 and 72h).

RESULTS

Epilimnion water temperature was round 19 ºC during the experiment in May 2000. The microcosm experiments were performed during a period of warm, stable conditions and when the reservoir was strongly stratified. Water chemistry of the epilimnion in the reservoir area (column "*in situ*" in Table 6.2) was comparable to that of water in microcosms at the beginning of the experiment (Table 6.2). Dissolved nutrient concentrations did not vary substantially through the experiment.

Also, similar nutrient concentrations were maintained between treatments, except for nitrates, which decreased through the course of the experiment, mainly in the polyethylene bottles (in treatments D, E and F). Chlorophyll *a* concentrations showed no variation neither through time nor between treatments, except in treatments A and B (i. e. original concentrations diluted 33 % and 50 %, respectively) where they tended to increase with time.

		In microcosms											
		A		B		C		D		E		F	
	In situ	0 h.	72 h.	0 h.	72 h.	0 h.	72 h.	0 h.	72 h.	0 h.	72 h.	0 h.	72 h.
Chl. *a* ($mg\ m^{-3}$)	39	11	31±5	25	37±6	47	63±17	48	68±12	46	65±14	47	55±6
DOC ($mg\ l^{-1}$)	2.8	3.7	4.1±0.37	3.3	4.1±0.48	3.1	4.2±0.38	3.1	2.8±0.03	2.8	3.0±0.31	3.1	3.2±0.29
SRP (μM)	0.04	0.10	0.04±0.004	0.05	0.04±0.001	0.05	0.08±0.011	0.09	0.06±0.011	0.06	0.06±0.004	0.06	0.06±0.018
NO_3^- (μM)	33	31	21±3	29	20±1	28	20±2	34	13±5	27	8±5	34	13±7
Cl^- ($mg\ l^{-1}$)	60	49	56±8	49	59±1	60	61±4	60	59±1	48	60±2	59	60±1

Table 6.2
Chemical conditions (DOC: dissolved organic carbon, SRP: soluble reactive phosphorus, nitrate and chloride concentrations) and lake Chlorophyll *a* (Chl. *a*) concentration (*In situ*), at the beginning (0 h.) and end of each treatment incubation (A, B, C, D, E and F). Values are means of three microcosm replicates in each treatment (±sd).

Protozoan growth rates

HNF and ciliate densities were significantly different ($p<0.001$, repeated-measures ANOVA) between treatments (A, B, C, D, E and F), through time (0, 24, 48 and 72 h.). The interaction of treatment and time was also significant (Table 6.3).

	Treatment			Time			Time x treatment		
	df	F	p	df	F	p	df	F	p
Bacteria mcv* (A, B, C, D, E, F)	5	3.7	0.029	3	22.2	<0.001	15	6.2	<0.001
Bacteria mcv* (A, B, C)	2	0.22	n.s.	3	21.9	<0.001	6	5.5	<0.002
Bacteria mcv* (D, E, F)	2	0.08	n.s.	3	5.8	<0.006	6	1.6	n.s.
HNF (A, B, C, D, E, F)	5	214.1	<0.001	3	246.2	<0.001	15	40.1	<0.001
HNF (A, B, C)	2	287.7	<0.001	3	142.0	<0.001	6	28.3	<0.001
HNF (D, E, F)	2	171.3	<0.001	3	150.0	<0.001	6	80.3	<0.001
Ciliates (A, B, C, D, E, F)	5	535.2	<0.001	3	522.8	<0.001	15	62.5	<0.001
Ciliates (A, B, C)	2	1553.9	<0.001	3	614.6	<0.001	6	212.5	<0.001
Ciliates (D, E, F)	2	107.2	<0.001	3	225.9	<0.001	6	14.9	<0.001

* mcv = mean cell volume

Table 6.3
Two-way ANOVA results for effects of food sources (treatments A, B, C, see Table 6.1) and zooplankton predation (treatments D, E, F) over time on bacterial mean cell volumes (mcv), and heterotrophic nanoflagellate-HNF and ciliate abundances.

The removal of mesozooplankton (all treatments except D) had a clearly positive effect on protozoan populations (Fig. 6.1), which showed a strong and immediate response to zooplankton removal. In the three treatments where organisms larger than 20 μm were removed (A, B and C in Fig. 6.1), increasing HNF and ciliate densities through time were observed. All treatments developed HNF and ciliate populations whose size was proportional to initial concentrations (2-4 times initial densities). Note that the dilutions applied to the treatments A and B affected not only bacterioplankton and phytoplankton, but also the rest of planktonic components, including HNF and ciliates.

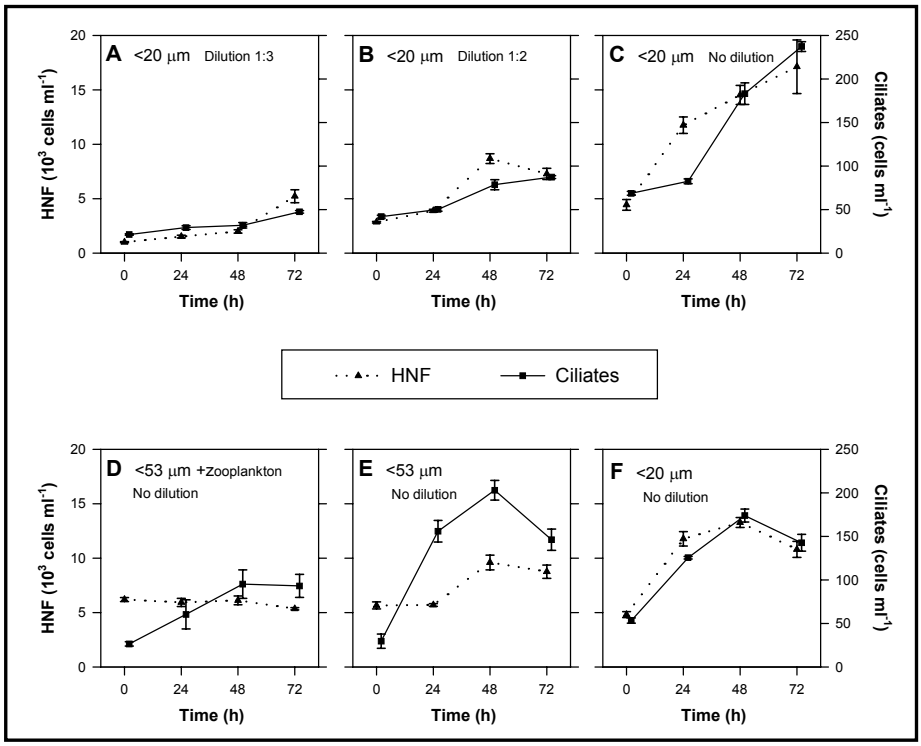

Figure 6.1
Protozoan population dynamics (heterotrophic nanoflagellates-HNF and ciliates) in the microcosms (mean and standard deviations of the 3 replicates). Treatments A, B and C test bottom-up control and treatments D, E and F test top-down control.

In top-down treatments (see Table 6.1) we observed several different growth patterns, depending on the filtration treatment (Fig. 6.1). In treatment D, with zooplankton added, and natural plankton densities, HNF populations did not grow and ciliates increased little during the experiment. When the zooplankton were removed (see treatments E and

F, in Fig. 6.1), HNF and ciliate densities increased significantly by 48 h. of experiment initiation. Between 48 and 72 h., no or negative growth of populations were observed in bottle microcosms. The main difference between treatments C and F (different types of container, dialysis bags versus polyethylene bottles) was the absence of growth in treatment F between 48 and 72 h., while in treatment C, HNF and ciliate populations continued growing. *In situ* incubation of water in dialysis bags (treatments A, B and C) allowed diffusion of dissolved organic matter and exposure to ambient nutrient concentrations. Therefore, no marked substrate limitation could occur in these treatments (HERNDL *et al*., 1993). Dissolved nutrient concentrations did not decrease much, but bacterial populations in bottle microcosms without zooplankton decreased to very low densities compared to those in dialysis bags. Therefore, in order to compare the growth efficiency of protozoans under the different experimental conditions (i. e. dialysis bags and polyethylene bottles), growth rates (Table 6.4) were estimated between 0 to 48 h. experimental time.

Estimated protist growth rates (h^{-1})

	Treatment	μ_{HNF}	$\mu_{Ciliates}$
Bottom-up control	A (<20μm 1:3)	0.014	0.008
	B (<20μm 1:2)	0.023	0.013
	C (<20μm)	0.025	0.020
Top-down control	D (<53μm+zoopl.)	0	0.012
	E (<53 μm)	0.011	0.022
	F (<20μm)	0.021	0.025

Table 6.4
Estimated protist growth rates (heterotrophic nanoflagellates -HNF, and ciliates) in each microcosm.

The highest HNF growth rates were observed in treatments C and F, 0.025 and 0.021 h^{-1}, respectively (cf. Table 6.4), in the absence of predators. No HNF growth was observed in treatments with zooplankton (D in Table 6.4). At the 1:2 dilution treatment (B) there was no significant

effect on HNF growth rate. However, at the 1:3 dilution treatment (C) HNF populations reduced their growth. Ciliates also had highest growth rates in treatments C and F with obviously no top-down control of ciliate populations. Resource dilution as well as zooplankton presence affected negatively the ciliate population growth rates. Total ciliate growth rates were similar in treatments E and F.

Mean bacterial cell volumes seemed to increase with time (see Table 6.3 and Figure 6.2), mainly in treatments B and C, owing to increasing protozoan densities and their grazing pressure on the bacteria. Shifts in bacterial community structure towards grazing-resistant forms (usually larger cells) have been observed under high protozoan grazing pressures (e. g. JÜRGENS, 1997, ŠIMEK *et al*., 2001, ŠIMEK *et al*., 1999).

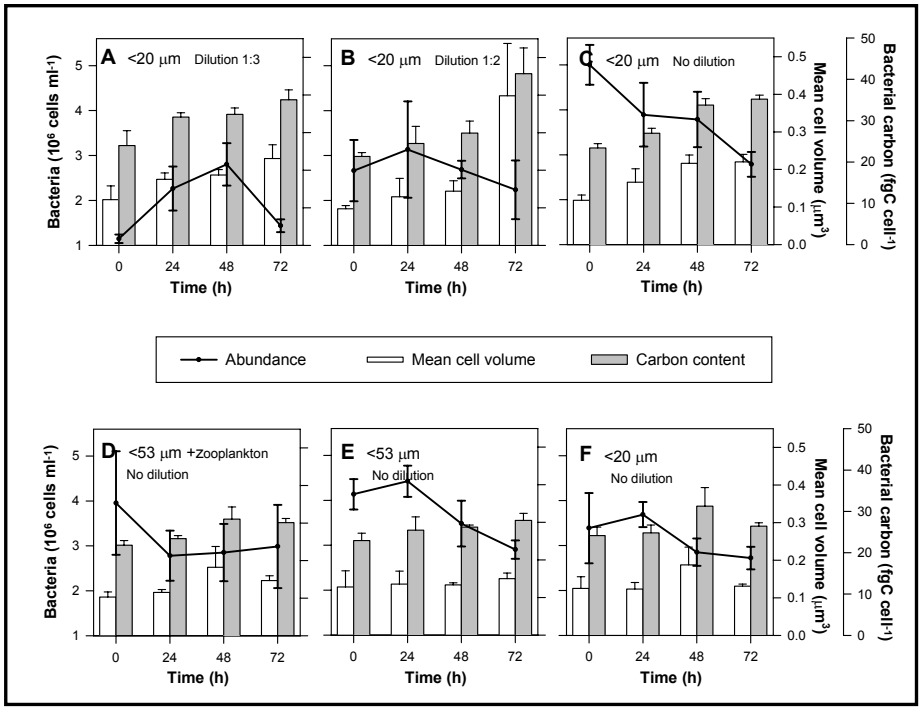

Figure 6.2
Bacterial population dynamics (abundance, mean cell volume and carbon content) in the microcosms (mean and standard deviations of the 3 replicates). For further details see treatments in Table 6.1.

Ciliate community composition and doubling times

The ciliate community was dominated by the typical planktonic species observed in previous field studies in the Sau Reservoir during spring (Chapter 4). Ciliates were dominated by small oligotrichs (30 % of total ciliate numbers were *Halteria cf. grandinella*, and 25 %, *Rimostrombidium brachykinetum*) and small prostomatids (*Urotricha spp*, *Balanion planctonicum*, 13 % of total ciliate abundance). We estimated ciliate taxon-specific duplication times from the maximum individual growth rates of several species observed in the microcosms (see Table 6.5). Other ciliates present in the plankton were not included in this analysis because of their low densities (e.g. *Askenasia*, *Litonotus* and *Vorticella*) or because of their sensitivity to filtration (e.g. *Tintinnidium* cells can break easily due to their large size and *Epistylis* forms colonies which do not pass easily through filters). Experiments in CARRIAS *et al.* (2001) showed that oligotrichs and tintinnids were very sensitive to manipulation and incubation.

Genus	T (ºC)	Mesh size (μm)	Doubling times (d)	System	Reference
Cyclidium	19	<20	0.55	The eutrophic Sau Reservoir	This study
	18	<100	0.87	The eutrophic Římov Reservoir	Šimek *et al.*, 1996
Halteria	18	<20	0.73	The eutrophic Sau Reservoir	This study
	18	<20	0.75-1.05	The eutrophic Římov Reservoir	Šimek *et al.*, 1999
	18	<100	1.65	The eutrophic Římov Reservoir	Macek *et al.*, 1996
	15	<153	2.10	Lake Michigan	Carrick *et al.*, 1992
Rimostrombidium	19	<20	0.79	The eutrophic Sau Reservoir	This study
(Strobilidium)	15	<30	1.10	Lake Michigan	Carrick *et al.*, 1992
	18	<100	2.04	The eutrophic Římov Reservoir	Macek *et al.*, 1996
	15	<153	2.31	Lake Michigan	Carrick *et al.*, 1992
Tintinnidium	19	<20	0.42	The eutrophic Sau Reservoir	This study
	15	<153	3.65	Lake Michigan	Carrick *et al.*, 1992
Urotricha	6	<10	1.33	Oligo-mesotrophic Lake Pavin	Carrias *et al.*, 2001
	19	<20	0.92	The eutrophic Sau Reservoir	This study
	15	<30	1.65	Lake Michigan	Carrick *et al.*, 1992
	6	<50	1.10	Oligo-mesotrophic Lake Pavin	Carrias *et al.*, 2001
	18	<100	1.07	The eutrophic Římov Reservoir	Macek *et al.*, 1996
	15	<153	1.78	Lake Michigan	Carrick *et al.*, 1992

Table 6.5
Comparison of *in situ* growth rates of planktonic ciliate populations estimated for different size-fractions incubated in lakes or reservoirs reported in literature. Values obtained in this study are compared to those found in the literature.

In assessing in situ protozoan populations growth, size fractionation of prey and predators using nylon mesh screens is routinely used (CARRICK *et al*., 1992; MACEK *et al*. 1996; ŠIMEK *et al*. 1996; JÜRGENS *et al*. 1999; JÜRGENS and JEPPESEN, 2000; CARRIAS *et al*., 2001). Duplication times from these studies are summarised in Table 6.5. The shortest ciliate doubling time, or the most rapid growth rate, was observed in our microcosm experiments (Table 6.5).

Zooplankton

The zooplankton density during the experiment was very high ($3.55 \cdot 10^6$ ind m^{-2}, cf. Figure 6.3). Copepoda was the dominant group (5.8 gr m^{-2}; mainly nauplii and adult *Cyclops abyssorum*). Cladocera was the second group in abundance and biomass (1.2 gr m^{-2}, Fig. 6.3; mainly *Daphnia* sp. and *Bosmina longirostris*). Rotifera (mainly *Keratella cochlearis*, *K. Quadrata* and *Polyartra vulgaris-dolicoptera*) was the least abundant zooplankton group.

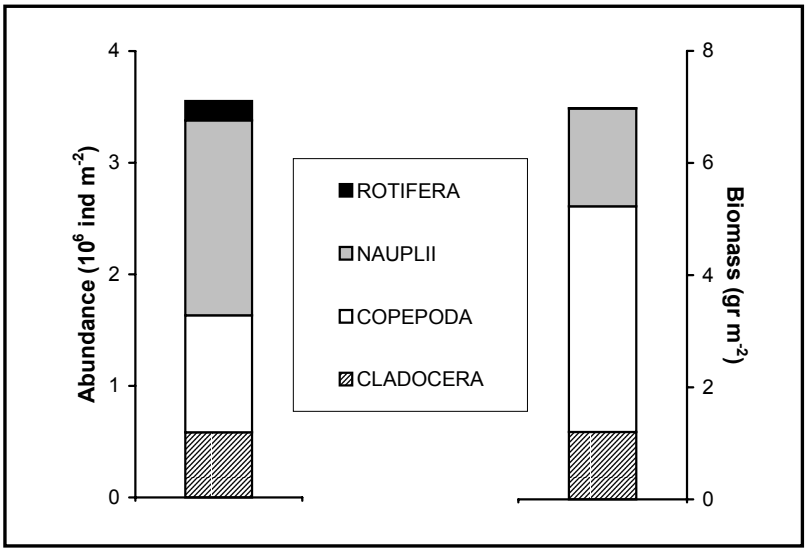

Figure 6.3
Abundance and biomass of metazooplankton in the Sau Reservoir in May 2000.

DISCUSSION

The dominant mesozooplankton during this experiment (Fig. 6.3), mostly copepods (*Cyclops abyssorum*), can cause significant mortality on large ciliates (JÜRGENS and JEPPESEN, 2000; WICKHAM, 1995). The predation impact of *Daphnia* affects a wide prey size-range and most groups of planktonic protozoans (JÜRGENS, 1994). Small-sized forms (15-25μm) and HNF (JÜRGENS *et al*., 1996) are susceptible to microfiltration by grasping rotifer species (ARNDT, 1993). Thus, the zooplankton community had the potential to have a substantial impact on both HNF and ciliate (small and large) populations.

JÜRGENS (1992) examined laboratory and field growth rates of HNF in laboratory and population standing stocks of both HNF and bacteria in the epilimnion of various meso- to eutrophic lakes. The numbers of larger celled bacteria in these lakes (e.g. as in the case of the Sau Reservoir) suggests that in most cases bacterivorous flagellates are not food-limited, although the abundance of HNF seemed to be rather low. The present microcosm experiment in the Sau Reservoir shows that bacterial densities below $2 \cdot 10^6$ cells ml^{-1} reduced the growth capacity of HNF populations (Fig. 6.1). The range of bacterial abundance in lentic areas of the Sau Reservoir during the 1996-1999 period was $2\text{-}7.5 \cdot 10^6$ cells ml^{-1} (the most frequently between 3 and $4 \cdot 10^6$ cells ml^{-1}). Thus, natural bacterial densities in Sau probably do not limit HNF populations in the reservoir, although temporarily we have observed low HNF numbers during 1996-1999 (Chapers 2 and 4). This experiment reveals that metazoans are the main factor controlling HNF populations in the Sau Reservoir, because no growth was observed in the presence of zooplankton when food sources were not limiting. The reduced numbers of HNF in treatment E (<53 μm, see Table 6.1) can also be explained by predation by large ciliates and some rotifer species, which could have easily passed this mesh size.

Ciliate populations also increased a lot in samples without zooplankton (population growth rate μ = 0.025 h^{-1}). From our knowledge of the literature, doubling times measured in Sau are the highest estimates measured in an eutrophic system (cf. Table 6.5). It is important to note, however, the low effectiveness in reducing predator density of the fractionation experiments using size pores larger than 50 μm.

Resources, not only predation, affected ciliate growth rates in our experiment. Resource dilution (treatments A and B) also reduced HNF densities, and small-sized ciliates, dominant in our experiment, which are thought to be important consumers of nanoflagellates (WEISSE, 1990). Moreover, this assumed predation of small ciliates on HNF in treatments A, B, C and D (<20 μm) suggests that estimates of maximum HNF growth rates in this study were underestimated.

Although both together the food supplyes (bottom-up control) and predation intensity (top-down-control) may affect the protozoan community, the relative importance of both in the Sau Reservoir apparently changed over space. High nutrient inputs allowed the development of an important microbial community near to the river inflow (Chapter 4) and high zooplankton densities control microbial densities nearer to the dam. GASOL *et al*. (2002) demonstrated that a complex regulation involving both types of control (i. e. bottom-up and top-down) can occur in a single heterogeneous planktonic system.

Our experiments show a substantial top-down control of protozoan populations in lacustrine parts of the eutrophic Sau Reservoir. Results support the concept of predator control of the microbial food web in eutrophic lakes (HADAS and BERMAN, 1998; JÜRGENS and JEPPESEN, 2000). Protozoan populations of the Sau Reservoir are efficient energy transfers between the bacterial community and the metazooplanton, with ciliates acting specifically as an intermediate step in the energy transfer between HNF and zooplankton.