Arch. Hydrobiol. Beih. Ergebn. Limnol.	37	187-194	Stuttgart, September 1992

Chemostats used to model the microbial food web: evidence for the feedback effect of herbivorous metazoans*

By HARTMUT ARNDT, HANS GÜDE, MIROSLAV MACEK and KARL OTTO ROTHHAUPT

With 3 figures in the text

Abstract

Five successive chemostats of algae (*Monoraphidium*)/bacteria, a heterotrophic flagellate (*Spumella*), and a rotifer (*Brachionus*) were combined according to a trophic cascade in order to test the hypothesis that the main flux of DOC from phytoplankton to bacteria is via egestion and excretion by herbivores rather than via exudated DOC from intact algae. Laboratory experiments revealed that bacteria and protozoans remained at low concentrations and metabolic activities until the algae were damaged by rotifer grazing. In the last chemostat where rotifers had been removed microbes grew up even more indicating both fuelling and grazing of microbes by rotifers. Evidence is presented that grazing activity of herbivores should be considered as an important source of microbial nutrition.

Introduction

The establishment of the concept on the "microbial loop" (cf. AZAM et al. 1983) was followed by an extensive debate in the literature regarding the importance of the microbial web as a link or a sink for the carbon flux in pelagic environments (e.g. SHERR et al. 1987). Up to now the grazing activities of metazooplankters and the interactions within the microbial web have been viewed mainly as a transfer of energy and organic matter to higher trophic levels. If attention is concentrated on the major part of grazers' food that is degraded rather than on the small part of organic matter that can be kept and transferred by metazoans on high energetic levels, quite another view of the function of metazooplankters results. This changed view has already been proposed by the re-evaluation of the digestion theory by JUMARS et al. (1989). For a limnetic plankton community it was calculated that approximately the same amount of carbon exudated by phytoplankton can be supplied to the microbial web by the sloppy feeding of metazoans and about twice this amount can be supplied by excreted carbon from metazoans (cf. ARNDT 1991).

*This work was supported by a research fellowship of the Humboldt Foundation to H. A.

0071-1128/92/0037-0187 $ 2.—

Until recently this assumed feedback effect of metazooplankton feeding has not been tested under experimental conditions (GÜDE 1988). To test this effect under defined laboratory conditions we used a system of combined chemostats. A small green algae served as a model organism for phytoplankton, a heterotrophic flagellate as a model organism for a bacterivorous protozoan, and a rotifer as a representative of microfiltrating metazooplankters. Our hypothesis was the following: Exudated DOC by undamaged phytoplankton should allow only a limited growth of bacteria and bacterivores, but the algal carbon should be available to microbes if algae are processed by herbivores.

Material and methods

A simple scheme of the chemostat system is illustrated on the top of Fig. 1. The volume of the chemostats ranged from 4 l for the algal chemostat, 450 ml for the second and third stage and 200 ml for the fourth and fifth stage. The algae were continuously illuminated by fluorescent tubes and supplied by a mineral CHU 12 medium. The second chemostat on the system was kept in the dark to prevent further algal growth. All chemostats were run under sterile conditions, so that only those bacteria strains, which were inoculated together with the monoxenic eucaryote cultures, could enter the system. The system was installed in a temperature controlled room at 20°C. In Fig. 1, dilution rates indicated below the symbols of the chemostats were obtained by peristaltic pumps from the algal medium to the fourth chemostat system and by a periodic overflow system from the fourth chemostat to the final outflow. From the fourth (HF rot) to the fifth chemostat (HF end) a sterile sieving system was installed to prevent rotifers from being transferred to the fifth chemostat. The fourth and the fifth chemostat were run simultaneously as a control (B cont; B end) without a rotifer inoculum. Five ml subsamples were taken under sterile conditions every day. On day 14 after establishing relatively stable standing stocks of organisms uptake rates of bacteria regarding four different radioactive substrates were analyzed. ^{3}H-thymidine (20 nM), ^{3}H-leucin (20 nM), ^{14}C-glucose (70 nM) and ^{14}C-amino acids (70 nM; algal protein hydrolysate; Amersham) were supplied for 15 minutes in paralleles of 2 ml subsamples of each chemostat and analysed by LSC counting. Algae and rotifers were counted and measured under an inverted microscope, whereas bacteria and flagellates were analysed after DAPI-staining on black nuclepore filters under an epifluorescence microscope (Zeiss).

Results

The search for suitable organisms which could be cultured under combined chemostat conditions and which were typical representatives of lake plankton revealed a combination of the chlorophyte *Monoraphidium minutum* (Culture Collection Göttingen), the heterotrophic nanoflagellate *Spumella* sp. (isolated by K. JÜRGENS, Plön), the rotifer *Brachionus rubens* (isolated by K.O. ROTHHAUPT, Plön) and the bacterial assemblage which grew in cultures of these organisms.

The chemostat system (see Fig. 1, upper panel) revealed relatively stable population densities up to one month. Chemostats were arranged in a way that trophic groups are combined according to a trophic cascade: The first three steps were arranged as a model of the matter flux from algae to protozoans; the fourth step should serve as a model for the connection between the microbial and the macrobial web. Before the fifth chemostat rotifers were sieved out to obtain informations on the feedback effects of metazoans on the microbial web. Control chemostats (B cont and B end) showed that trans-

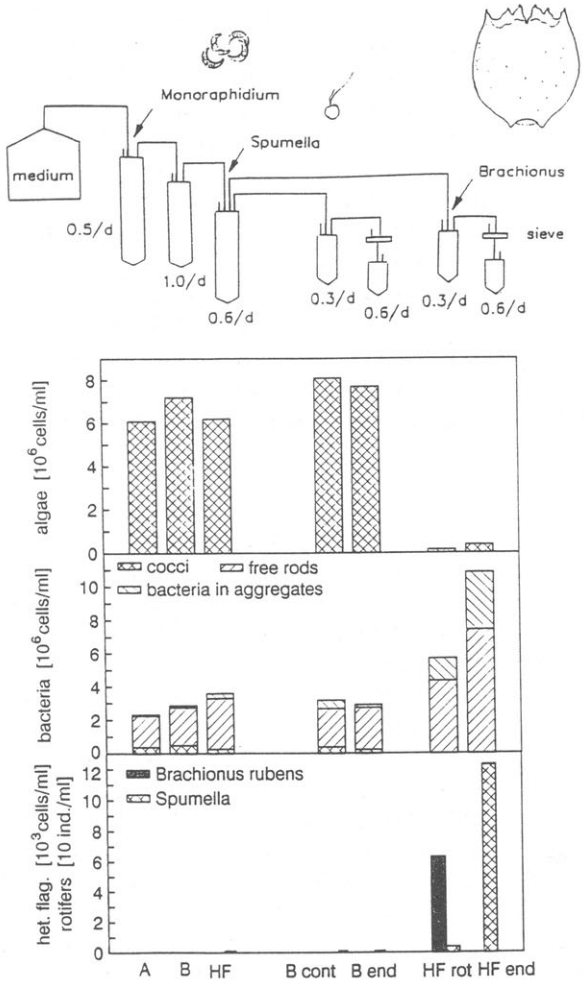

Fig. 1. Simplified scheme of a chemostat system (upper panel) and concentrations of organisms in the different chemostats at the 14th day after establishing relatively stable population densities.

port via the system did not have a significant effect on populations. A periodic siphon outflow from the rotifer chemostat lead to a homogeneous removal of rotifers. An accumulation of detritus could not be fully prevented in this chemostat.

In Fig. 1 the abundance of organisms is illustrated for day 14, when relatively stable population densities were established in all chemostats. The algae alone supported only a small amount of bacteria. When the heterotrophic flagellate was added, its growth rate at low bacterial concentrations was low and only low concentrations could be registered. However, even at these low concentrations of flagellates changes in the bacterial community occurred: bacterial aggregates increased and small cocci decreased. Both of these changes were significant ($p<0.05$). This is a typical phenomenon accompanied with pro-

tozoan predation (for review cf. GÜDE 1989). Considerable changes in the system occurred only when herbivores (*Brachionus*) were added. Though *Brachionus* feed effectively on heterotrophic flagellates and bacteria (ARNDT, JÜRGENS & ZIMMERMANN, unpubl.), both of these components increased significantly ($p<0.01$) in abundance. The bacterial assemblage changed even more towards aggregated forms. Cocci were negligible and rods in aggregates were three times more abundant ($p<0.001$). Except for thymidine, specific rates of substrate uptake of bacteria (Fig. 2) were more than four times higher than in the preceding chemostats. In the fifth chemostat, where rotifer grazing on microbial components was lacking, bacteria and protozoans increased significantly ($p<0.01$) again even when the dilution rate was twice the previous dilution rate. The important bacterial grazer in this chemostat were heterotrophic flagellates, which may have caused the further increase in bacterial aggregates. Though the specific uptake rates of bacteria for leucine, glucose and the mixture of amino acids remained the same as in the preceding chemostat, the thymidine uptake (as a measure of cell division) increased significantly.

Discussion

The hypothesis that herbivores can support the activity of the microbial web could be verified by our chemostat experiments. Additional experiments with the bacterivorous ciliate *Cyclidium* sp. as a protozoan inoculum revealed a similar result (ARNDT et al., unpubl.). Of course, care has to be taken regarding a generalization of these first results with specific laboratory organisms. However, PEDUZZI & HERNDL (in press) regarding marine metazooplankters indicated that in the field microbial activity can be enhanced by the presence of metazoans.

A re-interpretation of published data and theoretical calculations by JUMARS et al. (1989) suggested that bacteria do not obtain the major amount of carbon directly via excreted DOC from algae as currently believed, but through the by-products of animal feeding. In order to test this hypothesis we estimated the carbon flux through the compartments in the rotifer chemostat (Fig. 3). The inflow and outflow concentrations (except for the DOC and detritus pool) were measured. Several assumptions had to be made to estimate the fluxes between the different compartments: It was assumed that equations for steady state conditions in the chemostat can be used to calculate the fluxes of carbon. All biomasses were transferred to carbon values assuming that dry weight is 20% of fresh weight and 50% of dry weight is carbon. It was assumed that heterotrophs respire the same amount of carbon which they transfer into biomass production. The difference of algae inflow and outflow was attributed to the grazing of rotifers (cf. control chemostats). The grazing impact on bacteria and flagellates by rotifers was derived from results of separate grazing experiments, where the relative filtration rate of *Brachionus rubens* regarding bacteria and *Spumella* was compared with that regarding *Scenedesmus* (0.65 times and 1.32 times, resp.). The same relative differences were used with regard to *Monoraphidium* to estimate the grazing rate of *Brachionus* on bacteria (similar size classes) and *Spumella* in the chemostat. The significant grazing pressure on bacteria and flagellates was evident in the fifth chemostat in the absence of rotifers, where bacteria (in the presence of intensive flagellate grazing) and heterotrophic flagel-

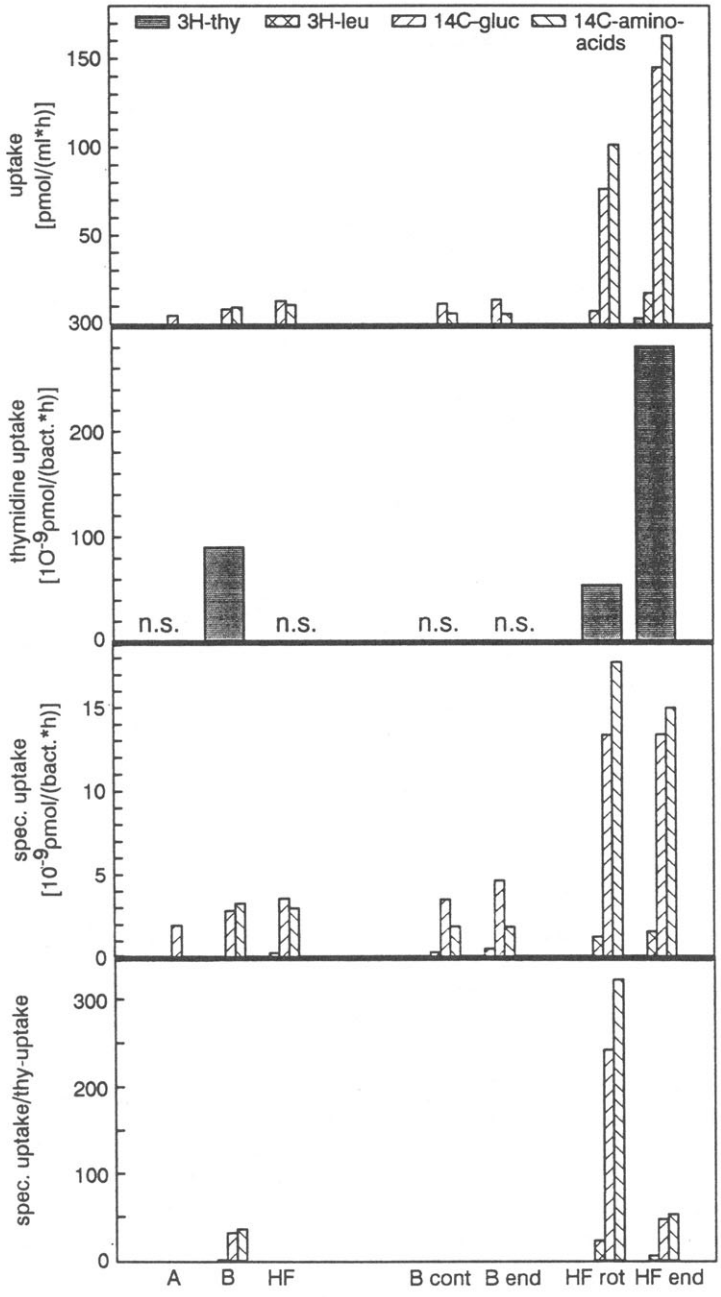

Fig. 2. Uptake rates of bacteria regarding different substrates in the different chemostats at the 14th day after establishing relatively stable population densities (significant thymidine uptake was measured in three chemostats only, n.s. = not significant from zero).

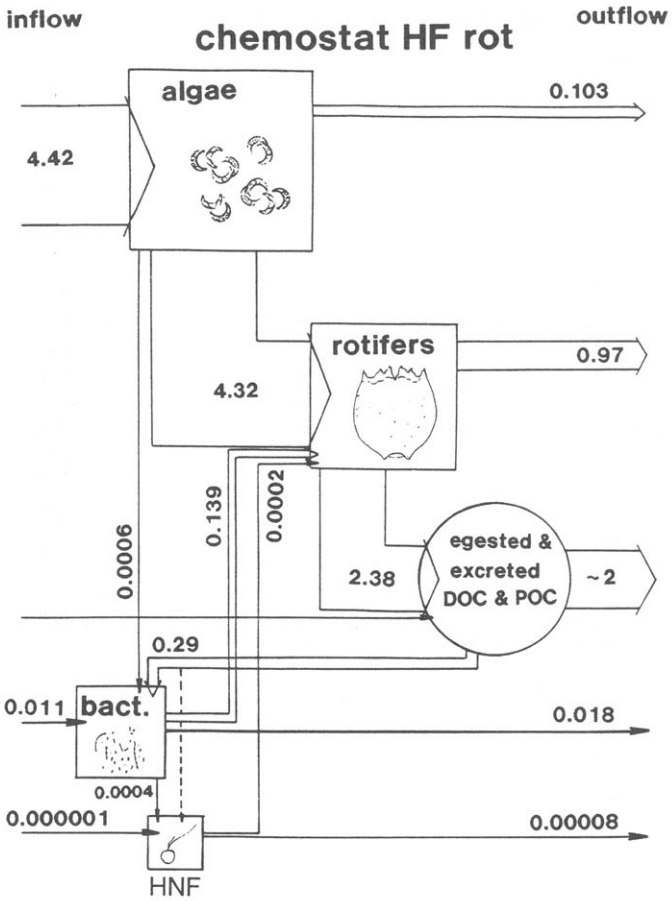

Fig. 3. Scheme of an estimation of the carbon flux (values as $mgC \cdot l^{-1} \cdot d^{-1}$) through the different compartments on the 14th day in the fourth chemostat with rotifers. Note that bacteria are fuelled nearly exclusively by the egestion and excretion products of rotifers.

lates increased at higher dilution rates. The amount of DOC and POC which was egested and excreted by rotifers was calculated as the difference between consumption and the sum of rotifer production (outflow) and respiration. This would mean an assimilation efficiency of rotifers of about 44%, which seems to be an acceptable value. To calculate carbon uptake by the box "bacteria", it was assumed that each algae in the chemostat with rotifers supported the same amount of bacterial biomass as in the first chemostat. This may be an overestimation of the carbon flux via algal exudates, since the exudates have probably already been exhausted in the rotifer chemostat. The uptake from the pool of egested and excreted carbon was calculated as the difference of the sum of loss processes of bacteria (outflow, metazoan grazing, flagellate grazing, respiration = production) and the sum of bacteria inflow and uptake of algal exudates. To reach the estimated production, the doubling time of bacteria have to be about 8 hours. This seems

to be an acceptable value under these chemostat conditions. For the box "heterotrophic flagellates (HNF)" it was assumed that HNF had a filtration rate of 10^5 times their own biovolume per hour to estimate their grazing impact on bacteria. This estimation fits good to the assumption (see above) that carbon losses due to respiration are in the same range as carbon used for production. It is evident in Fig. 3 that the flux of carbon from algae via the rotifers contributed the major portion of the nutrition of bacteria in the fourth chemostat. This conclusion is not affected, even by significant biases of assumptions from real values. The major part of the DOC and POC release of rotifers is probably via excretion. Up to now the "sloppy feeding" was studied for crustaceans only (e.g. LAMPERT 1978), but it probably plays a role in rotifers, too.

The population development of microbes in the fifth chemostat gives an impression of the direct influence (suppression) of metazoan feeding on bacteria and protozoans. Besides the fuelling of microbes via egestion and excretion of metazoans the direct grazing impact should not be overlooked. During the clear-water phase in temperate lakes metazooplankters (daphnids) are able to graze down algae, protozoans, and bacteria (cf. GÜDE 1988, ARNDT & NIXDORF 1991).

The carbon balance in the rotifer chemostat alone cannot reveal a direct estimation of the relative importance of algal exudation versus zooplankton excretion as a bacterial substrate, since exudates may have already been exhausted in the fourth chemostat. Therefore, we related the bacterial production (estimates from the carbon balance at steady state conditions) to the particulate algal production in the first chemostat, where algal exudates were the only substrate for bacteria. We found that bacteria production was only about 0.17% (0.013 mgC/l/d) of the particulate daily algal production. In the rotifer chemostat (which was the chemostat, where additional changes in biomass occurred) about 3.3% (0.16 mgC/l/d) of the daily inflow of algal carbon were transferred to bacterial carbon (about 22% to rotifer carbon). This percentage should have been even higher, if bacteria would not have been as strongly suppressed by rotifers (see bacterial increase in the last chemostat). We conclude that a several times higher amount of carbon could enter the bacteria compartment due to the presence of herbivores. Thus our results can be used as preliminary evidence for the hypothesis of JUMARS et al. (1989).

Exudation rates of algae in the field vary within a broad range of values. Future chemostat experiments are necessary for a better understanding of the relative importance of herbivore excretion under different exudation regimes of algae.

Our chemostat experiments showed that grazing activity by herbivores should be considered as an important source of microbial nutrition. This way of carbon flux seems to be underestimated in the original concept of the microbial loop.

Acknowledgements

We are grateful to critical comments by PETER K. BJØRNSEN and two anonymous reviewers to an earlier version of the manuscript.

References

ARNDT, H. (in press): Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) — a review. - Hydrobiologia, in press.

ARNDT, H. & NIXDORF, B. (1991): Spring clear-water phase in a eutrophic lake: Control by herbivorous zooplankton enhanced by grazing on components of the microbial web. - Verh. Internat. Verein. Limnol. **24:** 879-883.

AZAM, F., FENCHEL, T., FIELD, J.G., GRAY, J.S., MEYER-REIL, L.-A. & THINGSTAD, F. (1983): The ecological role of water-column microbes in the sea. - Mar. Ecol. Progr. Ser. **10:** 257-263.

GÜDE, H. (1988): Direct and indirect influences of crustacean zooplankton on bacterioplankton of Lake Constance. - Hydrobiologia **159:** 63-73.

— (1989): The role of grazing on bacteria in plankton succession. - In: SOMMER, U. (ed.): Plankton Ecology: Succession in Plankton Communities. - pp. 337-364, Springer-Verlag, Berlin, Heidelberg, New York.

JUMARS, P.A., PENRY, D.L., BAROSS, J.A., PERRY, M.J. & FROST, B.W. (1989): Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. - Deep-Sea Res. **36:** 483-495.

LAMPERT, W. (1978): Release of dissolved organic carbon by grazing zooplankton. - Limnol. Oceanogr. **23:** 831-834.

PEDUZZI, P. & HERNDL, G.J. (in press): Zooplankton activity fuelling the microbial loop: differential growth response of bacteria from oligo- and eutrophic waters. - Limnol. Oceanogr.

SHERR, E.B., SHERR, B.F. & ALBRIGHT, L.J. (1987): Bacteria: link or sink? and response by DUCKLOW, H.W., D.A. PURDIE, P.J. LeB. WILLIAMS & J.M. DAVIES. - Science **235:** 88-89.

Addresses of the authors: H. ARNDT, Institut für Limnologie, Österreichische Akademie der Wissenschaften, Gaisberg 116, A-5310 Mondsee, Austria. H. GÜDE, Institut für Seenforschung und Fischereiwesen, Untere Seestraße 81, D(W)-7994 Langenargen, B.R.D. M. MACEK, Institute for Hydrobiology, Czechoslovak Academy of Sciences, Na sádkách 7, CS-37005 C. Budejovice, Czechoslovakia. K.O. ROTHHAUPT, Max-Planck-Institut für Limnologie, Postfach 165, D(W)-2320 Plön, F.R.G.