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Kieler Meeresforsch., Sonderh. 5, 370 – 375. Kiel 1981

Feeding experiments with bacteria, ciliates and harpacticoid copepods

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Abstract

Many benthic ciliates are known to feed upon bacteria in the marine environment. These may in turn be utilized as food by harpacticoid copepods which graze upon them. Simple non-tracer feeding experiments were carried out in the laboratory with various representatives of these organisms. The growth rates of the ciliate *Uronema* sp. with different species of bacteria as food, including oil-degrading bacteria, were compared. Results of feeding experiments with the harpacticoid copepod *Tisbe holothuriae* fed on *Uronema* sp. show an uptake rate of 12 – 192 ciliates copepod^{-1} h^{-1}. The ecological implications are discussed.

Introduction

The importance of bacteria in the marine environment is well known, due to such classic works on marine microbiology as that of ZoBELL (1946). Bacteria break down organic compounds, bring about their remineralization and convert organic material into bacterial substance, thus making it available as nutrients for organisms at higher trophic levels. Therefore bacteria may be considered as both consumers and also as producers in the marine environment.

Ciliated protozoa are among the most widespread representatives of the microfauna. They are known to feed upon algae, detritus and bacteria (see FENCHEL 1968 and the references therein). Indeed, ''All free-living ciliates do possess a mouth and do feed on particulate food in nature'' (FENCHEL 1968, p. 75). In addition to Fenchel's extensive studies on marine benthic ciliates and their nutrition, there has also been much research done in the laboratory using bacteria as a food source for these organisms. In particular the cosmopolitan ciliate *Uronema* has been the subject of many studies, and will readily feed upon a wide variety of bacteria species (FENCHEL 1968, HAMILTON and PRESLAN 1969, ASHBY 1976, BERK et al. 1976, PARKER 1976, and BERK et al. 1977).

In 1963 GUNKEL (personal communication) noticed the development of a large number of ciliates in an enrichment culture of oil-degrading bacteria from the North Sea. In this single bottle, in which the ciliates had developed, the numbers of oil-degrading bacteria counted were lower by a factor of ten than in a similar bottle in which no ciliate growth was noted. The ciliates were identified as a species of *Euplotes*; a sessile form, *Vorticella*, was also found in large numbers.

In view of the fact that *Uronema* is able to utilize many different bacteria as food, as mentioned above, the question was raised as to how this ciliate would react when oil-degrading bacteria were offered as a nutrient source.

A further question was raised as to which animals, e.g., representatives of the benthic meiofauna such as harpacticoid copepods, could in turn be acting as predators on bacterivorous ciliates. It has already been shown that some planktonic copepods are able to feed on ciliates in the laboratory (BERK et al. 1977, HEINLE et al. 1977), and an interesting study has been made on the food chain relationships between *Daphnia* (Phyllopoda) and a bacterivorous *Paramecium* (TEZUKA 1974). Little is known, however, about the feeding of benthic copepods on ciliates and the role the latter may play in the nutrition of these animals in nature.

It is hoped that the results of feeding experiments presented here involving the lowest members of the food web will add to the available information on the relationships between these organisms in the benthic ecosystem.

Material and Methods

Bacteria

The bacteria used in the experiments include the following: List-7, Gram-negative, non-pigmented rod-shaped bacteria isolated from intertidal sediments at List/Sylt (North Sea);

Serratia sp., Gram-negative, red pigmented bacteria isolated from sublittoral sediments near Helgoland; *Escherichia coli* B, as an example of the enterobacteria (members of this family have been frequently mentioned in the literature as a laboratory food for protozoa); two mixtures of oil-degrading bacteria, including one from chocolate mousse (oil-water emulsion) formed after the "Amoco Cadiz" oil spill off the coast of Brittany in 1978, and another mixture collected from surface waters of the southern North Sea. Both mixtures of oil-degrading bacteria were kindly supplied by W. Gunkel.

All bacteria were maintained on yeast extract-peptone agar (ZoBell 2216E). In addition, for use in the experiments shown in Figure 2 only, the chocolate mousse oil-degrading bacteria were cultivated on two different solid media prepared with Ekofisk oil, obtained from W. Gunkel: Oxoid "Ionagar" No. 2 and silica gel medium containing N and P salts (GUNKEL, unpublished).

Ciliates

Uronema sp. was found as a contaminant in cultures of harpacticoid copepods (*Nitocrella polychaeta*) isolated from the beach at List/Sylt. *Euplotes* sp. was found to occur regularly in laboratory cultures of *Tisbe*. The ciliates were isolated and maintained at 20° C in small covered glass vessels containing 25–50 ml of 95 % autoclaved seawater (S = 30 °/₀₀) filtered through a 0.45 μm membrane filter after autoclaving. Ciliate cultures were diluted and given fresh seawater every 7–14 days. They were fed with loopfuls of live List-7 bacteria removed directly from agar plate cultures. The amount of bacteria added every 4 days was enough to cause a turbidity in the ciliate vessels, i.e. approximately $10^7 - 10^8$ bacteria ml^{-1}.

The ciliates were counted in the following manner: from the experimental vessels an aliquot was removed with a pipette during stirring. The ciliates were then fixed with formalin, filtered onto 0.45 μm membrane filters, stained with erythrosin, and counted under a microscope at 125 x.

Harpacticoid copepods

Four species of harpacticoid copepods were used in the initial feeding experiments: *Tisbe holothuriae*, *Paramphiascella vararensis*, *Nitocrella polychaeta* and *Amphias-*

coides debilis. Tisbe cultures were maintained on a diet of mussel meat; all other harpacticoids received a diet of fish food mixture. The stock cultures were handled according to the methods described in RIEPER (1978).

All feeding experiments were carried out in small covered glass vessels with 25–50 ml autoclaved, aged 95 % seawater, filtered after autoclaving. The temperature was 18–20° C, with a light: dark cycle of 12:12 hrs.

Results and Discussion

Ciliate-bacteria feeding experiments

The first series of experiments was carried out to investigate the relative growth rates of *Uronema* sp. fed on different species of bacteria. The results given in Figure 1 show that the best growth occurred with List-7 bacteria and the mixtures of oil-degrading bacteria, as compared to that with *Serratia* sp. and the control vessels with no additional bacteria. At the start of the experiment, the cultures of List-7 (a) and *Serratia* sp. were 9 days old; List-7 (b) and the oil-degrading bacteria 10 days old. All bacteria were grown on ZoBell 2216E agar medium.

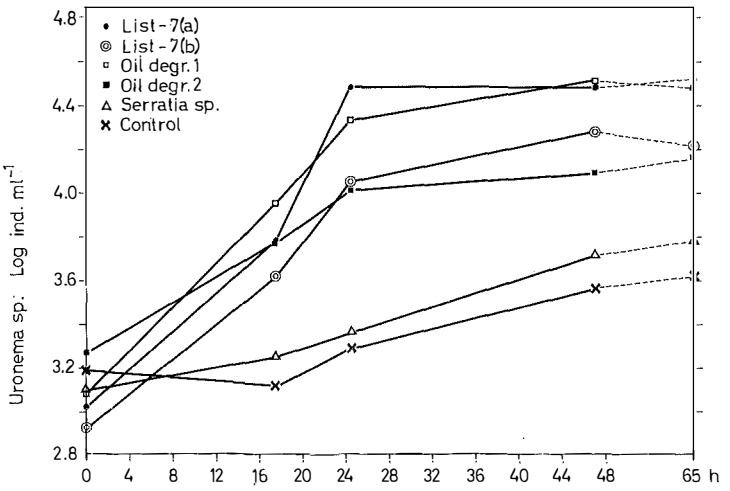

Figure 1

Relative growth rates of the ciliate *Uronema* sp. on different species of bacteria. Oil degr. 1 denotes oil-degrading bacteria from "Amoco Cadiz" chocolate mousse: oil degr. 2, oil-degrading bacteria from North Sea surface waters. All bacteria were cultivated on yeast extract-peptone medium (2216 E)

After it had been shown that *Uronema* sp. is capable of growth on oil-degrading bacteria, an experiment was carried out to see if these ciliates would feed on bacteria which had been cultivated on a medium containing oil. Accordingly the oil-degrading bacteria from the chocolate mousse were grown on two different medie prepared with Ekofisk oil and fed to *Uronema* sp. As a comparison, these bacteria were also cultivated on ZoBell 2216B (ZS), as were List-7 and *E. coli* used in the same feeding experiment.

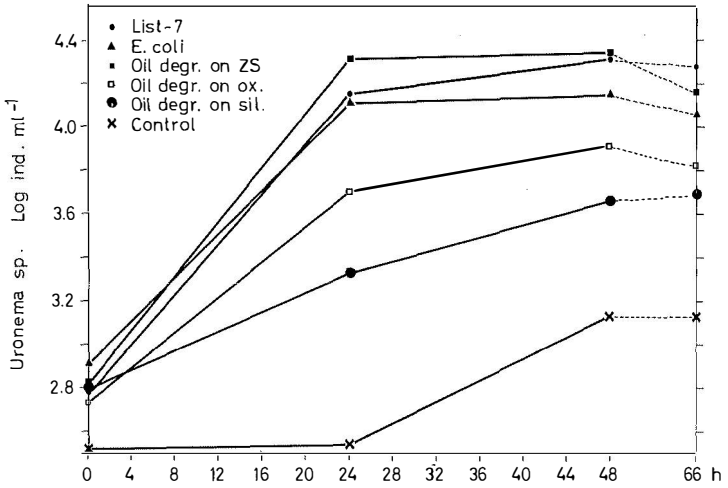

Figure 2

Relative growth rates of the ciliate *Uronema* sp. on different species of bacteria. Ox. = Oxoid "Ionagar" and Sil. = silica gel medium, both prepared with Ekofisk oil. All other bacteria were cultivated on yeast extract-peptone medium (2216 E) (ZS)

All bacteria cultures were 4 days old at the start of the experiment. The results are given in Figure 2. Although the best relative growth took place with the oil-degrading bacteria cultivated without oil, as well as with List-7 and *E. coli*, *Uronema* was also able to grow and reproduce when fed with oil-degrading bacteria which had been cultivated on media prepared with Ekofisk oil. The oil-degrading bacteria did not grow well on the silica gel medium, however, and the ciliates probably received lower numbers of these bacteria than the others.

Since the ciliates did not disregard the bacteria which were grown on oil prior to the experiment, the question could be asked whether or not hydrocarbon residues, if present in any of the bacterial cells, could move up the food chain to higher trophic levels, i.e. small metazoans which prey on the ciliates.

Ciliate-copepod feeding experiments

In order to determine if harpacticoid copepods would graze on ciliates if these were offered as food, a screening experiment was carried out with the copepods *Tisbe holothuriae*, *Paramphiascella vararensis*, *Nitocrella polychaeta* and *Amphiascoides debilis*. Their sizes ranged from approximately 400 μm (*N. polychaeta*) to over 900 μm (*T. holothuriae*). The ciliates tested were *Uronema sp.* (lag phase length 20–30 μm) and *Euplotes* sp. (lag phase length 30–35 μm). The results showed that two of the four harpacticoids apparently did not feed on the ciliates at all: *N. polychaeta* and *P. vararensis*. Another species, *A. debilis*, may possible have fed on *Euplotes* – approximately 19 ciliates copepod^{-1}h^{-1} disappeared from the culture – but this number is relatively small compared to the inaccuracies of the method of counting ciliates, so that it is questionable.

The best results in the feeding experiments were obtained with *T. holothuriae* and the ciliate *Uronema*. *Euplotes* was not taken up by *Tisbe*. In a preliminary experiment (of 19

hrs duration at 18° C) with an initial ciliate concentration of 5 x 10^3 *Uronema* ml^{-1} and 25 *Tisbe* adults and copepodites, there was an uptake of 184 ciliates $copepod^{-1}h^{-1}$.

In the experiments which followed, the initial concentration of ciliates was varied to determine the effect on the rate of uptake. The results of two such experiments are given in Table 1. The concentrations of ciliates and copepods are the average values of 2 parallel vessels per experiment. The stock *Uronema* cultures were maintained on List-7 bacteria.

Table 1

Results of feeding experiments with the harpacticoid copepod *Tisbe holothuriae* on the ciliate *Uronema* sp., which had received List-7 bacteria

Experiment No.	Initial conc. of ciliates ml^{-1}	Ciliates taken up $copepod^{-1}$ h^{-1}	Length of experiment
1 (9/80)	844	12	49.5 hrs
	4 221	109	
	8 442	141	
2 (14/80)	925	0	19 hrs
	2 313	73	
	4 626	175	
	11 565	192	

Table 2

Results of feeding experiments whith the harpacticoid copepod *Tisbe holothuriae* on the ciliate *Uronema* sp., which had received oil-degrading bacteria

Experiment No.	Initial conc. of ciliates ml^{-1}	Ciliates taken up $copepod^{-1}$ h^{-1}	Length of experiment
3 (17/80)	572	22	22 hrs
	5 217	58	

These experiments were then repeated with the difference that *Uronema* was first cultivated for several days with the chocolate mousse oil-degrading bacteria. The results in Table 2 show that *T. holothuriae* will also graze on *Uronema* when these ciliates had previously been fed a mixture of oil-degrading bacteria, although for the higher initial concentration of ciliates the uptake rate of 58 ciliates $copepod^{-1}$ h^{-1} is less than that for ciliates fed the proteolytic List-7 bacteria.

The results presented in Tables 1 and 2, however, should be regarded with caution for the following reasons: 1) the method of counting the ciliates is unsatisfactory, since parallel samples often show great fluctuations in numbers of ciliates present; 2) in the feeding experiments described above, the number of ciliates in the controls without *Tisbe* did not remain the same. There should not have been growth of the ciliates since the numbers of bacteria present – the ciliate cultures were not axenic – were below the minimum for ciliate feeding (see BERK et al. 1976). There should also not have been a decrease in the controls during the relatively short term experiments (1–2 days). Wherever there was a difference in the initial and final ciliate numbers in the controls, this difference was taken into account in calculating the ciliate uptake by the copepods.

(A more reliable method for determining uptake of ciliates by copepods would be to lable the ciliates with ^{14}C. At the time these experiments were performed, however, it was not possible to use this method in our laboratory.)

Keeping these reservations in mind, the values of 12–192 ciliates copepod^{-1} h^{-1} are reasonable in the order of magnitude. Using data provided by GERLACH (1978), this would represent an uptake of 1.4 – 23.0 μg organic ciliate C copepod^{-1} day^{-1}. This may be compared to results of feeding experiments which showed the carbon requirement of harpacticoid copepods fed with bacteria alone to be 1 to 3.5μg C copepod^{-1} day^{-1} (RIEPER 1978).

In conclusion it may be stated that bacteria are eaten by certain ciliates, which are in turn grazed upon by some benthic copepods – and that possibly residues of substances utilized by the bacteria may move up the food chain in this manner.

Acknowledgements

Sincere gratitude is extended to Dr. E. Hartwig (Hamburg) and Dr. G. Uhlig (Helgoland) for the identification of the ciliates *Uronema* sp. and *Euplotes* sp., and D. Whybrew (Göttingen) for isolating and identifying the harpacticoid copepod *Nitocrella polychaeta*.

References

ASHBY, R.E., 1976. Long term variations in a protozoan chemostat culture. J. exp. mar. Biol. Ecol. **24**, 227–235.

BERK, S.G., R.R. COLWELL, and E.B. SMALL, 1976. A study of feeding response to bacterial prey by estuarine ciliates. Trans. Amer. Micros. Soc. **95**, 514–520.

BERK, S.G., D.C. BROWNLEE, D.R. HEINLE, H.J. KLING, and R.R. COLWELL, 1977. Ciliates as a food source for marine planktonic copepods. Microb. Ecol. **4**, 27–40.

FENCHEL, T., 1968. The ecology of marine microbenthos II. The food of marine benthic ciliates. Ophelia **5**, 73–121.

GERLACH, S.A., 1978. Food-chain relationships in subtidal silty sand marine sediments and the role of meiofauna in stimulating bacterial productivity. Oecologia (Berl.) **33**, 55–69.

HAMILTON, R.D. and J.E. PRESLAN, 1969. Cultural characteristics of a pelagic marine hymenostome ciliate, *Uronema* sp. J. exp. mar. Biol. Ecol. **4**, 90–99.

HEINLE, D.R., R.P. HARRIS, J.F. USTACH, and D.A. FLEMER, 1977. Detritus as food for estuarine copepods. Mar. Biol. **40**, 341–353.

PARKER, J.G., 1976. Cultural characteristics of the marine ciliated protozoan, *Uronema marinum* Dujardin. J. exp. mar. Biol. Ecol. **24**, 213–226.

RIEPER, M., 1978. Bacteria as food for marine harpacticoid copepods. Mar. Biol. **45**, 337–345.

TEZUKA, Y., 1974. An experimental study of the food chain among bacteria, Paramecium and Daphnia. Int. Revue ges. Hydrobiol. **59**, 31–37.

ZoBELL, C.E., 1946. Marine Microbiology. A monograph on hydrobacteriology. Chronica Botanica Co., Waltham, Massachusetts, 240 pp.