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Release of aminoacids and inorganic nutrients by heterotrophic marine microflagellates

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ABSTRACT: Heterotrophic microflagellates isolated from the Baltic Sea and grown under laboratory conditions were shown to release dissolved free amino acids (DFAA) when grazing bacteria. Flagellates released ^{3}H-amino acids when fed ^{3}H-leucine-labelled bacteria, and concentrations of amino acids increased in the experimental medium. Serine showed a strong positive correlation with flagellate feeding. Aspartic acid, glutamic acid and ornithine also increased more than other amino acids. During consumption of bacteria, the flagellates released 13 % of the ingested nitrogen as ammonia, and 30 % of the ingested phosphorus as phosphate. In a field experiment off Scripps Pier, we measured bacterial production, flagellate abundance, and concentration of DFAA over a 28 h period. The concentration of DFAA showed a covariation with the flagellate numbers. Results from our field and laboratory experiments suggest that flagellates may be a source of DFAA in the sea.

INTRODUCTION

Microbial activity greatly influences the distribution of organic compounds in the sea. Marine bacteria are capable of rapid growth using the ambient pool of dissolved organic carbon (DOC) (Ammerman et al. 1984, Hagström et al. 1984). Organic monomers and macromolecules serve as the first link in a micro-heterotrophic food chain, an important pathway for the recycling of matter in the pelagic ecosystem (Azam et al. 1983).

One class of organic monomers commonly found in sea water is the dissolved free amino acids (DFAA) (Lee & Bada 1977, Lindroth & Mopper 1979, Mopper & Lindroth 1982) and a rapid turnover of this pool has been demonstrated (Hollibaugh et al. 1980). Free amino acids are released into sea water by zooplankton and phytoplankton (Webb & Johannes 1967, Hellebust 1974, Mague et al. 1980, Bidigare 1983, Hammer & Brockman 1983). Marine bacteria are capable of using the nanomolar concentrations of amino acids found in sea water (Williams et al. 1976, Dawson & Gocke 1978, Fuhrman & Azam 1982). However, the quantitative relations between organisms and the amino acid pool are less clear.

The ecological importance of microflagellates in the pelagic ecosystem has been shown by Fenchel (1982a, b, c, d). He demonstrated that microflagellates are capable of grazing bacteria at a consumption rate high enough to effectively control the concentration of pelagic bacteria (Fenchel 1982d). It has been suggested that by consuming bacteria, flagellates are responsible for remineralizing organic matter to inorganic nutrients (Johannes 1965) and may play an important role in the pelagic food chain (Williams 1981, Taylor 1982, Azam et al. 1983, Sherr et al. 1983).

Many protozoans, including heterotrophic microflagellates, are known to release organic as well as inorganic nitrogen compounds as metabolic end products (Lui & Roels 1970, Aaronson et al. 1971, Hellebust 1974, Taylor et al. 1985). The object of our study was to investigate the possible role of microflagellates as a source of amino acids in the sea.

MATERIALS AND METHODS

Field data from Scripps Pier. Seawater samples were collected from Scripps Pier (May 1982), over a 28 h period. Amino acid samples were filtered immediately

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through a 0.22 µm Millipore filter and frozen until analysis. ^{3}H-thymidine incubations were run immediately on unfiltered water. Samples for direct counts were preserved for later analysis.

Feeding experiments. Microflagellates were isolated from unfiltered Baltic Sea water by incubating the sea water with added food bacteria in the dark at room temperature. Flagellates were abundant in the cultures after 3 to 5 d. Repetitive isolation of flagellates yielded a monoculture (*Ochromonas* sp.). The individual cells had a diameter of 2.5 µm and a cell volume of about 10 µm^3. Mixed marine bacteria from 'sea water cultures' (Ammerman et al. 1984) and *Escherichia coli* strain NC3 (grown in glycerol minimal-salts medium, filtered with 0.2 µm Nuclepore filters under low vacuum, washed and resuspended in sea water) were used as food sources for the growth of flagellates.

Labelled mixed-bacteria were obtained from seawater cultures, incubated eight hours with ^{3}H-leucine (0.5 µCi ml^{-1}; 40 Ci mmole^{-1}, Ammersham). Bacteria were washed free of excess label by filtration through a 0.2 µm Nuclepore filter under low vacuum. Care was taken to keep the filter wet and the bacteria submerged in medium at all times. Flagellates were fed the ^{3}H-leucine-labelled bacteria in an attempt to measure excretion of low-molecular-weight organic compounds. Bacteria and flagellates were mixed and samples taken at intervals. Flagellates were retained on a 3 µm filter, and bacteria on a 0.2 µm filter. Both filters and filtrate were transferred to scintillation vials and counted. Instagel (Packard Instruments) was used as liquid scintillant for both filters and water samples. Subsamples of the filtrates were freeze-dried and the residue run through a cation exchanger (Dowex 50W-X8) in the H^+ state. Primary amines were eluted with 1.5N NH_4OH.

As a control, an axenic culture of *Ochromonas* sp. was used to examine the ability of this flagellate to take up amino acid from its medium. Flagellates grown in liquid media (modified after Ford 1958) were transferred to sterile-filtered sea water by repeated gravity filtration and rinsing on a 1 µm filter. ^{3}H-Leucine was then added to the sea water to a final concentration of 1, 10 and 100 nM. Samples were taken at intervals over a 2 h period, precipitated in cold TCA (5 % trichloroacetic acid), and filtered. Radioactivity on the filter and in the filtrate was measured as in the feeding experiment. In addition, the capacity of *Escherichia coli* to assimilate amino acids was tested in the same way with 1 nM ^{3}H-leucine.

Cell counts. Bacteria and flagellates were counted using epifluorescence microscopy and ethidium bromide staining (Hagström et al. 1984). Organisms 2 to 5 µm in length with 2 visible flagella were counted as heterotrophic flagellates. Between 30 and 45 microscopic fields were examined under a total magnification of 625×.

Cell-volume measurements. In laboratory experiments, stained cells were photographed (Kodak technical pan film 2415, Zeiss M 63 camera) in an epifluorescence microscope, magnification on negative: 315×. Negatives were projected on a graphics tablet (Tektronix 4956), and cell-volume data analysed with a Tektronix 4051 computer. In the field study, we assumed a mean bacterial cell size of 0.15 µm^3 to calculate bacterial production (Ammerman et al. 1984).

Bacterial production. Bacterial production was estimated using the frequency of dividing cells (FDC) technique (Hagström 1984) and the ^{3}H-thymidine procedure of Fuhrman and Azam (1982). ^{3}H-thymidine (1.85 µCi, 52 Ci mmole^{-1}, New England Nuclear) was added to 5 ml samples and incubated at ambient temperature for 60 min. Samples were radioassayed using Instagel scintillation fluid.

Chemical analyses. Phosphate, nitrate, ammonia and total nitrogen and phosphorus were measured using routine methods (Carlberg 1972). Dissolved free amino acids (DFAA) and total hydrolyzable amino acids (THAA) were determined by high performance liquid chromatography. Primary amino acids were measured using precolumn derivatization with *o*-phthaldialdehyde (Lindroth & Mopper 1979, Jones et al. 1981). Proteins and peptides were measured after hydrolyzing dried water samples in 6 N HCl at 110 °C for 24 h in an N_2-flushed, sealed ampule.

RESULTS

Field data from Scripps Pier

In the field study, 8 samples of surface sea water were collected from Scripps Pier over a 28 h period. We measured bacterial growth, flagellate numbers and amino acid pool size in these samples (Fig. 1). Bacterial numbers showed no dramatic changes during this time. The growth rate of the bacteria showed 2 pronounced maxima when using either the FDC or the ^{3}H-thymidine technique. The mean generation time was about 24 h, corresponding to a production of about 5.9 × 10^4 bacteria ml^{-1} h^{-1} (Hagström 1984). The minimum growth rate in the afternoon of the first day coincided with a decrease (28 %) in bacterial numbers. Flagellate numbers, although more irregular, showed an overall increase of 100 % in 21 h. The concentration of dissolved free amino acids (DFAA) tended to vary with the flagellate numbers (Fig. 1 B, C). Although the concentration of total and of individual free amino acids covaried with flagellate abundance, the relative amino acid composition did not. Serine, glycine, and

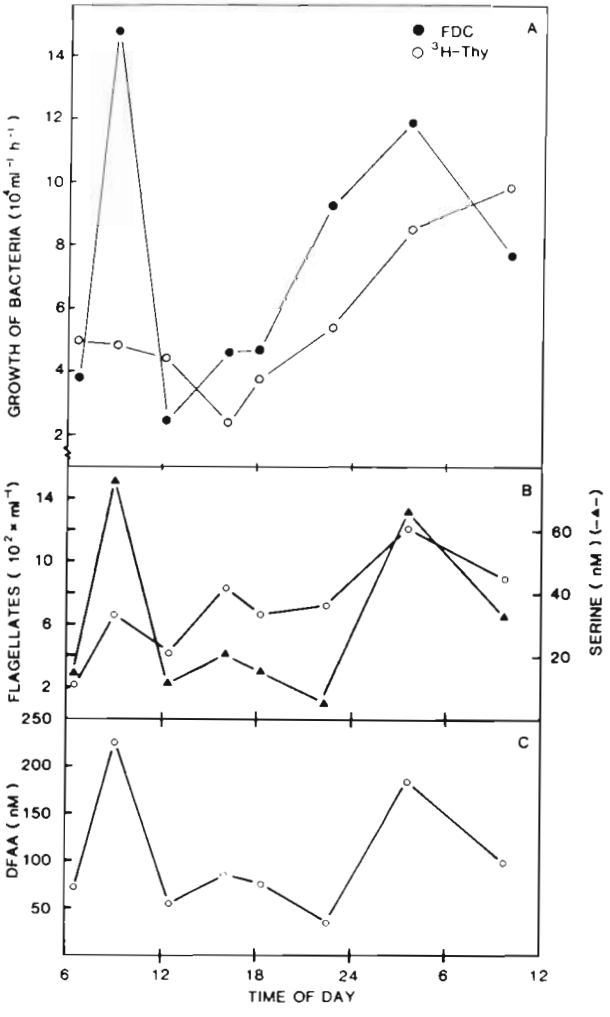

Fig. 1. Covariation between dissolved free amino acids (DFAA) and heterotrophic microflagellates during a diel study off Scripps Pier in May 1982. (A) Bacterial production; (B) flagellate numbers (○), concentration of serine (▲); (C) concentration of DFAA

alanine were the dominant amino acids (mol%) (Table 1). However, only the relative concentration of serine showed a pronounced correlation with flagellate abundance (Fig. 1 B). Protein measured as total hydrolyzable amino acids (THAA) did not show a similar pattern of variation (data not shown).

Feeding experiment

Scripps Pier results suggest that microflagellates might be releasing amino acids after feeding on bacteria. To investigate this idea, we measured free amino acid concentrations in the culture medium while flagellates (*Ochromonas* sp.) were fed cultured seawater bacteria. The concentration of DFAA increased while flagellates were grazing the bacteria. In a control culture which contained no flagellates, the amino acid concentration decreased because bacteria were growing and thus removing the available amino acids (Fig. 2). Ignoring bacterial growth, the biomass of bacteria consumed in our feeding experiment roughly corresponded to the biomass of flagellates produced. Initial flagellate and bacterial numbers were 8.5×10^3 ml^{-1} and 4.6×10^6 ml^{-1}, respectively. About 50 bacteria were consumed by each new flagellate, as calculated from the endpoints of the growth curves. However, since bacteria were growing during the experiment (the control bacterial doubling time was 10 h), this is an underestimate of the total ingestion.

To try to overcome the problem of bacterial growth during the course of the flagellate feeding experiment, we used *Escherichia coli* as a food source for the flagellates. When growing cultures of *E. coli* were transferred to filtered, autoclaved sea water from a minimal-salts medium supplemented with glycerol, no further growth occurred. However, these *E. coli* cultures showed significant potential for uptake of amino

Table 1. Mole % amino acids and total DFAA concentrations off Scripps Pier. (After May 1982)

Amino acid	Time of day 6:00	9:00	12:00	16:00	18:00	22:00	3:00	10:00
Asp	11.4	7.0	1.1	10.3	13.1	2.6	3.8	11.3
Glu	3.3	3.9	4.0	5.9	6.9	5.4	1.7	6.0
Ser	21.0	34.2	21.1	25.6	24.9	15.9	37.7	26.7
Gly/Thr	25.6	19.0	13.8	24.4	15.9	20.4	20.5	26.4
Ala	12.9	15.2	16.7	11.6	3.2	37.2	5.9	11.4
Val	4.3	3.0	18.3	11.8	6.2	9.1	6.9	3.4
Ile	3.2	3.7	3.1	1.0	2.2	3.3	2.1	1.9
Leu	2.4	2.0	4.6	2.1	3.2	6.1	4.5	2.6
Orn	1.8	8.0	15.5	6.0	10.9	–	10.0	–
Total concentration (nM)	70.9	223	52.9	82.3	60.7	31.2	177	122

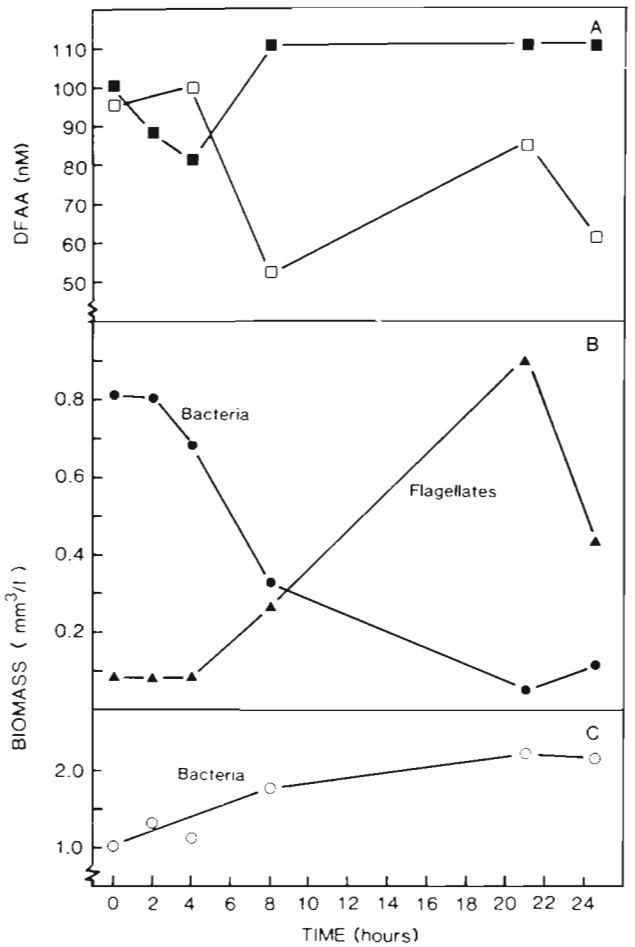

Fig. 2. Growth of flagellates fed a mixed sea-water culture of bacteria: (A) DFAA concentration; (B) bacterial and flagellate biomass; (C) control culture of bacteria with no flagellates. Open symbols: control culture; solid symbols: flagellate-bacteria culture

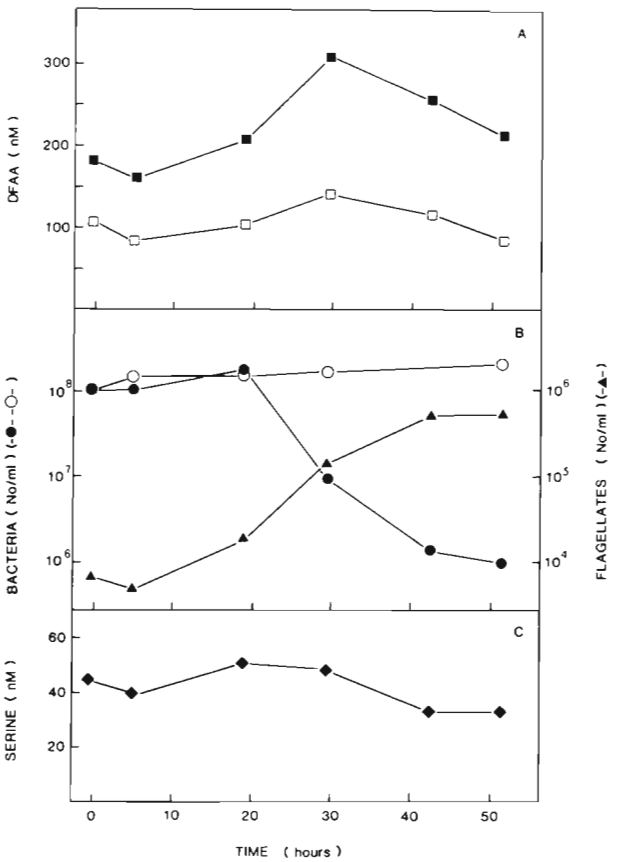

Fig. 3. Growth of flagellates fed *Escherichia coli* resuspended in sea water: (A) DFAA concentrations; (B) bacterial and flagellate numbers; (C) concentration of serine. Open symbols: control culture; solid symbols: flagellate-*Escherichia coli* culture

acids from the medium. When the *E. coli* culture used in the experiment (2.8×10^6 ml^{-1}) was exposed to 1.0 nM of ^{3}H-leucine, the bacteria showed a gradual uptake of the label that reduced the leucine concentration to 0.15 nM in 7 min. After this initial reduction, no further decrease in labelled leucine was seen.

The amino acid results from the *Escherichia coli* feeding experiment were similar to that of the sea-water culture (Fig. 3). Compared to the control, the relative composition of individual amino acids was different in the flagellate culture and varied as total DFAA increased (Table 2). The concentration of serine increased markedly when the flagellates consumed bacteria at a high rate (Fig. 3 B). Aspartic acid, glutamic acid, and ornithine also increased significantly during the feeding experiment (Table 2). Variations in composition were similar, although smaller, in the sea-water bacteria experiment. One surprising feature of our amino acid analyses was the appearance of 4 unknown primary amines in both of the flagellate feeding experiments. These 4 amines were a dominant feature of the chromatograms in the later stages of the experiments indicating that they were being produced by the flagellates. We were unable to identify the amines by retention time, although all common and many uncommon amino acids and amines were tested. Assuming similar response factors to the amino acids, the unidentified amines combined are about the same concentrations as total DFAA.

Remineralization of inorganic nitrogen and phosphorous was also measured during flagellate growth (Table 3). Ammonia increased in concentration during growth, representing 13% of the ingested bacterial nitrogen. No increase in the concentration of NO_3 could be observed. Inorganic phosphorus (PO_4) was released more rapidly, representing 30% of the ingested phosphorus.

We also measured flagellate release of amino acids by feeding flagellates with ^{3}H-leucine-labelled bacteria (Fig. 4). As the labelled bacteria were consumed by the flagellates, the amount of label in the bacterial

Table 2. Mole % amino acids and total DFAA concentrations in *Escherichia coli* feeding experiment

Feeding experiment (flagellates present) Amino acid	Time (hours) 0	5	18.5	29.5	42.5	52
Asp	6.0	9.3	9.6	16.8	11.2	12.4
Glu	8.3	11.4	10.0	14.9	20.4	20.0
Asn	3.7	4.1	4.0	3.5	2.0	1.9
Ser	24.8	24.4	24.4	16.0	12.8	15.1
Gln	1.8	1.6	1.2	3.3	5.6	4.2
Gly/Thr	21.1	22.8	24.0	19.2	19.7	12.1
Arg	1.4	–	1.2	1.1	3.0	2.3
Ala	23.4	14.5	12.0	8.4	8.9	8.7
Tyr/γ–Aba	5.5	6.7	6.8	5.7	5.3	6.0
α–Aba	2.3	3.1	2.4	4.1	4.0	4.9
Orn	1.8	2.1	4.4	7.1	7.2	12.4
Total concentration (nM)	181	160	207	306	252	220
Control experiment (no flagellates)						
Asp	4.1	2.1	5.2	3.3	5.2	5.5
Glu	7.8	11.8	5.0	5.2	5.2	6.9
Asn	3.1	3.3	2.7	2.4	2.1	3.3
Ser	12.9	15.4	18.7	20.7	18.0	16.6
Gln	2.6	–	–	–	–	–
Gly/Thr	27.6	36.6	40.5	40.1	43.4	38.0
Arg	1.3	1.0	0.9	1.1	1.4	1.0
Ala	32.8	18.7	15.0	15.8	14.9	15.2
Tyr/γ–Aba	3.5	5.0	5.5	4.1	3.6	4.1
α–Aba	1.8	2.3	1.5	1.4	1.6	3.5
Orn	2.4	3.5	5.0	4.6	4.7	6.0
Total concentration (nM)	110	88	100	140	120	83

fraction was reduced to 28 % of the initial value. However, only a small fraction of the label was taken up by the flagellate fraction. When formaldehyde-fixed flagellates were filtered, 86 % of the cells were retained by a 3 µm filter. The remaining cells (14 %) could be collected on a 1 µm filter. Live flagellates may pass the 3 µm filter to a greater degree but this was not demonstrated. Although there was a high background due to clumped bacteria, a large assimilation by the flagellates should have shown a significant increase over background levels. Instead, the label was released into the non-particulate fraction (Fig. 4). In a control, labelled bacteria were incubated iin the absence of microflagellates. No significant excretion of label (< 2 %) could be measured in this culture. The filtrate from the feeding experiment was run through a cation-exchange column to determine whether tritiated amines were present in the released material. Most of the released label was not retained by the ion-exchange column. Since this material was volatile, we assume that it was ^3H_2O produced as a respiration product. About 5 % of the label released was retained by the ion-exchange column.

Table 3. Remineralization of bacterial nitrogen and phosphorus by flagellates

N-content in consumed bacteria (*E. coli*)	4×10^{-14} g cell^{-1}
Ingested bacterial – N	254 ng h^{-1} ml^{-1}
Excreted NH_4	34 ng h^{-1} ml^{-1}
REMINERALIZED N	13 %
P-content in consumed bacteria (*E. coli*)	4.9×10^{-15} g cell^{-1}
Ingested bacterial – P	30 ng h^{-1} ml^{-1}
Excreted PO_4 – P	9 ng h^{-1} ml^{-1}
REMINERALIZED P	30 %

To determine whether the flagellates themselves could affect the amino acid pool through an active uptake of amino acids, we exposed an axenic culture of *Ochromonas* sp. (10^5 cells ml^{-1}) to ^{3}H-leucine in sea water. No significant uptake could be measured when the final leucine concentration was 1 or 10 nM. When the leucine concentration was 100 nM, measurable

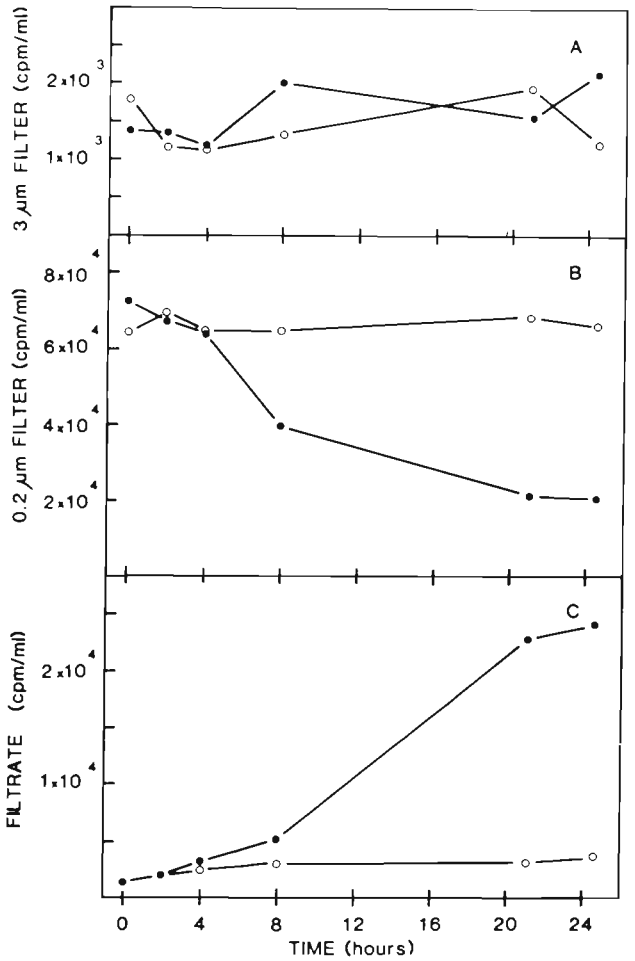

Fig. 4. Size fractionation of label when flagellates were fed ^{3}H-leucine-labelled bacteria. (A) Flagellates retained on a 3 µm filter; (B) bacteria retained on a 0.2 µm filter; (C) filtrate. Open symbols: control culture; solid symbols: flagellate-labelled bacteria culture

uptake occurred. However, turnover time was more than 20 h.

DISCUSSION

The covariation between amino acid concentration and heterotrophic microflagellate numbers in the samples taken from Scripps Pier (Fig. 1B) led us to suspect that flagellates might be releasing free amino acids. We investigated this possibility in a series of feeding experiments with microflagellates isolated from Baltic sea water using both *Escherichia coli* and mixed sea-water culture bacteria as food.

Our experiments showed that heterotrophic microflagellates remineralize organic matter derived from bacteria which they have consumed (Table 3). Rates of ammonia excretion were similar to those of Sherr et al. (1983), about 2 µmole NH_4^+ (mg dry wt flagellate)$^{-1}$ h^{-1} assuming their dry weight to volume ratio. The *E. coli* N:P ratio was 8.2:1. However, the flagellates excreted ammonia-N and phosphate-P in the ratio 3.8:1, suggesting either that the flagellates were accumulating nitrogen (relative to phosphorus) or that other nitrogen compounds were being excreted also. When we fed ^{3}H-leucine-labelled *E. coli* to flagellates, 45% of the consumed label was released (Fig. 4). Most of the excreted material was undoubtedly water respired during metabolic processes. In this experiment, about 5% of the excreted label could be retained on a cation-exchange column suggesting that amino acids or other primary amines were being excreted.

Our further experiments showed that the concentration of free amino acids (DFAA) increased when flagellates consumed either *Escherichia coli* (Fig. 3) or bacteria grown in sea-water culture (Fig. 2). Control cultures containing bacteria alone with no flagellates showed no increase in amino acid concentration with time. In order to determine whether our filtration procedure caused cell rupture, we took advantage of the fact that the *Ochromonas* sp. used in our experiments contains a chloroplast. *In vivo* fluorescence was measured before and after filtration of a dense flagellate culture (3.5×10^5 ml^{-1}). Insignificant fluorescence (< 3 %) was detected after filtration through either a 1, 0.6, or 0.2 µm filter, indicating that the cells were not damaged. In addition filtration damage due to cell rupture would produce DFAA concentrations which increase with increasing flagellate numbers. Clearly this was not the case in our feeding experiments.

In the Scripps Pier experiment, the hydrolyzed amino acids (THAA) did not show the same correlation with flagellate concentration as did the DFAA, suggesting that THAA concentration is not controlled by the same processes. Since internal cell pools contain protein as well as DFAA, both would be expected to leak out if physical rupture of a cell occurred. Glutamic acid and alanine are the most common amino acids found in bacterial internal pools (Holden 1962, Brown & Stanley 1972, Stanley & Brown 1976). Although glutamic acid increased the most rapidly in our feeding experiments, alanine decreased in importance. Ornithine was also preferentially released by the flagellates. Mopper & Lindroth (1982) found increases in ornithine with depth in Baltic Sea water which they attributed to increased heterotrophic activity. Thus, we feel the release of free amino acids by flagellates is more likely the result of metabolic processes rather than cell rupture from our filtration techniques.

An obvious question in our experiments is why flagellates release free amino acids. Bacterial protein phagocytized by the flagellates is hydrolyzed in food vacuoles, and the amino acids are transported into the cell through the vacuole membrane. Amino acids still present in the food vacuole may be released when the

remains of the bacteria are egested. Rapidly changing serine concentrations were a pronounced feature of both the field data and the feeding experiments. In the field, high serine concentrations coincided with high bacterial growth rate and hence with an increased potential for flagellate predation. In the flagellate cultures, high concentrations of serine occurred when the flagellates consumed bacteria at high rates. Serine is a potent attractant for chemotactic bacteria (Adler 1966) and might represent a chemical signal used by the flagellates.

To determine whether excretion by the flagellate-bacteria system may be a significant source of DFAA in the sea, we need to know what percentage of the bacterial nitrogen ingested by the flagellates is released as amino acids. We can estimate the percentage released by flagellates in our *Escherichia coli* feeding experiment in the following manner. The *E. coli* contained 4.0×10^{-14} gN cell^{-1}. During the phase of rapid growth (30 h), the flagellates consumed 1.8×10^8 cells ml^{-1} of bacteria, thus 240 ng N ml^{-1} h^{-1} was ingested. The concentration of DFAA in the flagellate culture over the same time period showed an increase of 100 nM as compared to the control culture without flagellates. Assuming an average of 12.5 % N in amino acids, only 0.02 % of the ingested nitrogen was released as amino acids. In contrast, ammonia release accounted for 13 % of the bacterial nitrogen ingested. Although *E. coli* showed no growth in sea water, this bacterium was capable of taking up amino acids in nanomolar concentrations at high rates. Hence, our experiments measured the net change in amino acid concentration, rather than the total amount of DFAA released. Our calculated release rate is therefore a minimum rate.

To estimate bacterial utpake of DFAA in our *Escherichia coli* culture, we measured ^{3}H-leucine uptake in a flagellate-free culture of this bacterium. The uptake of ^{3}H-leucine was 85 % in 7 min when leucine was present at 1 nM, a turnover time of about 10 min. Given an amino acid concentration of 200 nM in the experiment (Fig. 3), and assuming the leucine turnover time to be valid for the total DFAA pool, the flow of amino acids into the bacteria ($V = f/t \times Sn$) equals 1200 nM h^{-1}. Since the concentration of amino acids remains within a narrow range during the flagellate feeding experiments, we can assume a tight coupling between production of DFAA by flagellates and uptake by *E. coli*. Bacterial uptake of amino acids must therefore be balanced by an equal production by the flagellates. (Uptake of amino acids by the flagellates was negligible in our experiments.) Therefore, flagellates must have produced the 1200 nM h^{-1} of amino acids taken up by the bacteria in addition to the 3.3 nM h^{-1} actually observed. Flagellates that consumed 240 ng ml^{-1} h^{-1} of bacterial nitrogen would thus have lost about 7.3 % of that nitrogen as amino acids.

When sea-water culture bacteria are fed to the flagellates, these bacteria still grow during the experiment and thus complicate calculation of the amount of bacteria consumed. In our flagellate feeding experiment using sea-water culture bacteria, maximal changes in bacterial consumption and DFAA production occurred between 4 and 8 h after the experiment began (Fig. 2). Ignoring bacterial growth and uptake of DFAA, the flagellates released about 7.5 nM h^{-1} between 4 and 8 h (Fig. 2 A, closed symbols). During the same time period, the flagellates ingested 3.5 ng N ml^{-1} h^{-1}, if we assume that the 0.37 mm^3 l^{-1} bacteria consumed were 30 % dry weight with a density of 1 and a nitrogen content of 12.7 %. In this experiment, we can estimate the rate of bacterial uptake of DFAA from the bacterial removal rate observed in the absence of flagellates (Fig. 2 A, open symbols). Between 4 and 8 h, a decrease of about 10 nM h^{-1} occurred. If we assume steady-state uptake and production in the flagellate-bacteria system, then 10 nM h^{-1} uptake by bacteria must be equaled by a similar 10 nM h^{-1} production by flagellates in addition to the 7.5 nM h^{-1} actually observed. This represents 7.5 % of the ingested nitrogen.

We can use the percent of bacterial nitrogen ingested which is excreted by the flagellates to estimate the contribution of flagellates to the change in DFAA concentration observed during our Scripps Pier experiment. We assume that all the bacteria produced during this experiment (3.8×10^{-10} gN ml^{-1} h^{-1}) were consumed by flagellates, and that the percent ingested nitrogen (7.3 to 7.5 %) which was excreted in the feeding experiments are valid for the Scripps Pier bacteria. Thus, heterotrophic microflagellates could have contributed about 45 nM per day to the DFAA pool, a significant percentage of the changes we observed.

While microheterotrophs may play an important role in the energy flow of the pelagic ecosystem, other organisms such as phytoplankton or zooplankton also contribute to the amino acid pool. Hence, simultaneous fluctuations in flagellate and DFAA concentrations may be in response to changing oceanographic conditions which affect other source organisms in the same way. Our study shows that marine heterotrophic microflagellates release free amino acids and play a small but significant role as a source of DFAA in the sea. The reason why these flagellates release amino acids is unknown, but perhaps certain individual amino acids such as serine may function as chemical signals.

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