Vol. 52: 145–153, 1989 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published March 2

Inefficient feeding by *Calanus helgolandicus* and *Temora longicornis* on *Coscinodiscus wailesii*: quantitative estimation using chlorophyll-type pigments and effects on dissolved free amino acids

S. Roy[1, 2, *], R. P. Harris[3], S. A. Poulet[1]

[1] Centre d'étude d'océanographie et de biologie marine, C.N.R.S., Station biologique, F-29211 Roscoff, France
[2] INRS-Océanologie, 310 Allée des Ursulines, Rimouski, Québec, Canada, G5L 3A1
[3] Plymouth Marine Laboratory, Citadel Hill, Plymouth PL1 2PB, United Kingdom

ABSTRACT: Inefficient or 'sloppy' feeding behavior by copepods results in algal cell fragmentation and ingestion of only a fraction of the debris produced. In this work, 2 copepod species, *Calanus helgolandicus* and *Temora longicornis*, were fed the large diatom *Coscinodiscus wailesii* to investigate this process in the laboratory. Conditions favoring sloppy feeding (large prey size and high food concentration) were used to set the upper limits of such effects. Chlorophyll-type pigments tracked particulate debris production. Influence of food cell breakage upon dissolved free amino acids (DFAA) was also assessed. Serial filtrations following food and grazer removal after short grazing sessions showed that, for *C. helgolandicus* and *T longicornis* respectively, 26 % and 35 % of the pigments contained in the cells removed during feeding were recovered as > 0.7 µm particulate debris (most debris were < 10 µm). Debris were composed only of cell fragments as feces were absent due to the short grazing session. DFAA total concentration and extracellular composition were not modified significantly by copepod grazing action, suggesting a rapid and selective uptake of amino acids by bacteria and cells. Analysis of pigments in the experimental specimens indicated low levels (5 to 20 %) compared to levels of chlorophyll ingested (estimated on the basis of cell disappearance), suggesting further losses in debris smaller than 0.7 µm or pigment transformations in the copepods.

INTRODUCTION

Laboratory grazing experiments generally rely on algal cell removal to estimate the feeding activity of herbivorous zooplankton, with the underlying assumption that this action does not modify the particles (O'Connors et al. 1976). However, a number of observations have shown that this is not always the case, herbivores being able to dismember long algal chains (O'Connors et al. 1976) and fragment cells by breaking them open and eating only a fraction of this food (Conover 1966, Lampert 1978). The latter process has been termed 'sloppy feeding' and Williams (1981) has pointed out the scarcity of quantitative data on this process and its possible importance in the microbial food chain. As Cushing & Vucetic (1963) also indicated, 'superfluous feeding', as they termed the destruction of algae during feeding, will result in liberation of quantities of particulate matter and dissolved organic matter into the water. This process is thus important both from the point of view of accurately determining ingestion and from that of DOM production during grazing (Lampert 1978, Olsen et al. 1986, Dacey et al. 1986). It is also important in modifying the size spectra of food particles by fragmenting algal cells into smaller-sized debris (or long chains into smaller ones), thus affecting food availability to herbivores of different sizes.

Lengthy laboratory incubations have in many cases been avoided by field measurements of pigment levels in the guts of herbivorous zooplankton (e.g. Mackas & Bohrer 1976). Comparisons between field and laboratory techniques have however been inconclusive, pointing to our lack of knowledge of the reactions altering algal pigments immediately before, during and after ingestion (Conover et al. 1986). 'Sloppy' feeding is a process which might affect results from the 2 tech-

* Addressee for reprint requests (Rimouski, Canada)

0171-8630/89/0052/0145/$ 03.00

niques differently, since if mechanical disruption of the food occurred gut pigments will reflect more what was ingested than will cell removal from the food suspension.

In an effort to assess quantitatively the importance of 'sloppy feeding', we carried out a series of feeding experiments under conditions favoring its occurrence. Dagg (1974), in a study of the loss of prey body contents during feeding by a predator, noted that such inefficient feeding behavior was most likely to occur when feeding at the upper end of the prey size spectrum and at high food concentrations. Following such reasoning, we used an extremely large diatom cell, at high food concentrations, in order to obtain an upper limit value for particulate debris production, traced with chloropigments. The influence of food cell breakage upon dissolved free amino acids, compounds known to be involved in chemosensory feeding (Poulet & Ouellet 1982, Gill & Poulet 1988), was also assessed here.

METHODS

Two zooplankton species were tested: the copepods *Calanus helgolandicus* (size ca 3 mm) and *Temora longicornis* (size ca 1 mm). These were net-collected in February 1987 from local waters off Plymouth, United Kingdom, and starved in filtered seawater for 24 h before the experiments. The algal food suspension (ca 5 to 6 cells ml^{-1}) was prepared from a culture of the large diatom *Coscinodiscus wailesii* kept at the Marine Biological Association laboratory, Plymouth, and diluted with filtered seawater. These cells have an average diameter of 295 µm, with a thickness of 130 µm. Hence an indication of the relative sizes of grazers and algal diet is given by the fact that the cell diameter represents ca 10 % of the total body length of *C. helgolandicus* and 29 % of *T. longicornis*.

Three separate grazing experiments were performed under replicated conditions. Each comprised a control bottle (1 l) containing only the food suspension, and a *Calanus helgolandicus* and a *Temora longicornis* feeding bottle (1 l) containing both the food suspension and 200 hand-picked specimens (adult females). Incubations lasted 45 min and took place in the dark at 15 °C with slow rotation of the bottles. This short time period minimized contributions from fecal pellets to the particulate debris pool being monitored since feces production did not start in this time. This approach was also used by Lampert (1978).

Cells were counted microscopically at the beginning and end of the incubations in samples from every bottle, giving the number of cells consumed in 45 min. The presence of debris was recognized by measuring chloropigments in the particles collected after removing both zooplankton and phytoplankton cells at the end of incubations (on 45 µm mesh Nitex) and comparing between control and feeding bottles. Serial filtrations of the full content of each bottle covered 3 size fractions: > 10 µm (10 µm Nuclepore filters), 1 to 10 µm (1 µm Nuclepore filter) and 0.7 to 1 µm (Whatman GF/F filter), giving information on the relative sizes of the debris produced. Triplicate samples of each size fraction were taken for pigment analysis but were later pooled for a better chromatographic signal. Copepods removed at the end of the feeding period were separated from phytoplankton cells and also analyzed for pigment. The *Coscinodiscus wailesii* cultures were sampled just before each experiment to measure cellular pigment content, using a variant of Mantoura & Llewellyn's (1983) technique. A Perkin Elmer Pecosphere C18 column (3.3 cm, 3 µm) was gradient-eluted with the ion-pairing solution P and a 60 % methanol: 40 % acetone solution (Mantoura & Llewellyn 1984) at a flow rate of 1.5 ml min^{-1} for 10 min. Detection was done fluorometrically (Dupont 836 fluorescence detector; excitation: 430 nm; emission: > 600 nm). Recovery of pigments on 1 µm and GF/F filters was measured separately on a suspension of manually broken (glass pestle) cells. It averaged 71 % (± 18 %) thus showing that if cells were broken, the filters used would collect most of the broken cell parts, assuming that breakage of cells in this manner produces similar debris to that due to copepods.

Dissolved free amino acids were measured in triplicate samples, from the filtered seawater (FSW) utilized for the preparation of the food cell suspension, and from the filtrates of both the *Coscinodiscus wailesii* suspension and the suspensions containing the copepods, at the start and at the end of the experiments. Samples were taken at the same time for DFAAs and for pigments, and samples (1 ml) originating from 10 µm Nuclepore filtrates were collected in ultraclean vials and stored at −20 °C prior to analysis of 8 amino acids (ASP: aspartic acid, GLU: glutamic acid, ASN: asparagine, SER: serine, GLN: glutamine, HIS: histidine, GLY: glycine and ARG: arginine) following an HPLC technique (Lindroth & Mopper 1979).

RESULTS AND DISCUSSION

Results of pigment analyses are shown in Figs. 1 to 3. In the case of phytoplankton cells and particulate debris, chlorophyll (chl) *a* was quantitatively dominant (> 85 % of all pigments detected). There were no clear qualitative differences between chromatograms from healthy *Coscinodiscus wailesii* cells and from debris collected after 45 min feeding by *Calanus helgolandicus* and *Temora longicornis*. Quantitatively however,

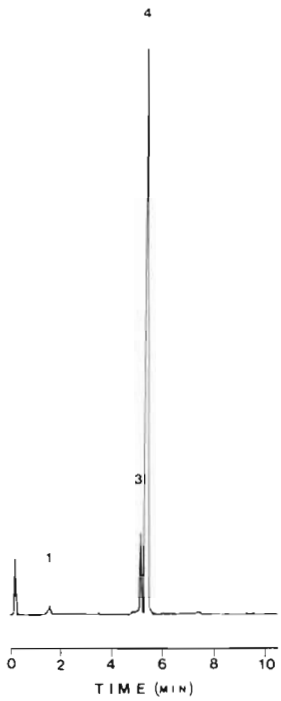

Fig. 1. HPLC fluorescence chromatogram obtained from 50 ml of a 5.5 cells ml^{-1} suspension of *Coscinodiscus wailesii* (algal diet) filtered on a 10 µm Nuclepore filter and extracted in 2.5 ml of acetone. Injection volume = 100 µl. Other technical details in 'Methods'. Peak identification for this and subsequent figures: (1) chl *c*, (2) pheophorbide *a*, (3) chl *a* allomer, (4) chl *a*, (5) pheophytin *a*

the presence of zooplankton caused an increase in the debris collected on the 1 µm and GF/F filters (Fig. 2). Pigments found in the copepods varied considerably from the above results: Fig. 3 shows chromatograms from 200 starved *C. helgolandicus* (identical for *T. longicornis*) and 200 of each copepod collected after the grazing session. In this case, chl *a* is still present (peak no. 4) but no longer dominant. Pheophorbide *a* and pheophytin *a* (peaks no. 2 and 5) also show up and the amounts of pigment vary according to the zooplankton species, *T. longicornis* containing considerably more pigment than *C. helgolandicus* under the same conditions (same number of specimens analyzed). Table 1 summarizes this information.

We estimated the importance of particulate debris production due to inefficient feeding by comparing pigment found in the debris (in terms of chl *a* – the other pigments can be neglected because of their minimal contribution to the pigment pool) with pigments contained in the cells removed from suspension after 45 min feeding. The debris pigments represent the sum of the 3 size fractions used (Table 1, 'Sum' column), while pigments in cells removed were calculated from the number of cells that disappeared and the cellular content of chl *a* for *Coscinodiscus wailesii*, measured before each experiment.

Averaging the 3 experiments, a significant difference in debris production was observed between the grazing and the control bottles for both species (*t*-test, $p < 0.05$). Total debris production due to copepod grazing (corrected for debris present in the control bottles) represented 26 % of pigments in cells removed from the flasks for *Calanus helgolandicus* and 35 % for *Temora longicornis*. These losses are somewhat larger than those calculated by Conover (1966) (15 % for *Calanus hyperboreus* feeding on *Thalassiosira fluviatilis*) or Lampert (1978) (17 % for *Daphnia pulex* feeding on *Asterionella formosa*) but they also represent different quantities: our values refer to a direct measurement of particulate debris while those of Conover and of Lampert are in terms of DOC increases. Paffenhöfer & Knowles (1978) found that, for *Eucalanus pileatus* nauplii (Stages N IV and N V) feeding on *Rhizosolenia alata f. indica*, 29 % of food cells appeared as frustules, presumably due to breakage during feeding. In contrast, Corner et al. (1972), studying *C. helgolandicus* feeding on the diatom *Biddulphia sinensis*, found no evidence of damage to these cells outside the copepod, and no loss of soluble nitrogen and phosphorus was observed. However, *B. sinensis* has significantly smaller cells (100 × 4 µm) than those used in our experiments. It is also possible that *B. sinensis* cells are more difficult to break than those of *Coscinodiscus wailesii* due to shape or silica valve thickness (Paffenhöfer & Knowles 1978). Our experimental conditions were designed to favor inefficient feeding, thus the loss values obtained probably represent upper limits. We do not expect, but cannot verify, that subsequent feeding on the fragments produced could affect our values greatly, since only 24 % of the cells were removed from suspension within 45 min for *C. helgolandicus* (14 % for *T. longicornis*), so that food availability was high during the experiment, making it unlikely that the copepods would encounter fragments more frequently than whole cells.

Losses in the dissolved fraction (i.e. pigments in particles passing through GF/F filters) were not directly measured but an estimate of these losses was made by mechanically disrupting *Coscinodiscus wailesii* cells and filtering serially on 1 µm and GF/F filters. Comparison of chl *a* values for intact cells and debris collected on both filters shows a 71 ± 18 % recovery of pigment. This value decreased to 60 ± 7 % if the broken cell suspension was allowed to age in the dark at 15 °C for 24 h. Thus a maximum loss of 30 % could be accounted for by pigments passing through GF/F filters in our 45 min experiments, assuming similarity of debris production by the copepods and by mechanical disruption.

Pigment levels found in the copepods were surpris-

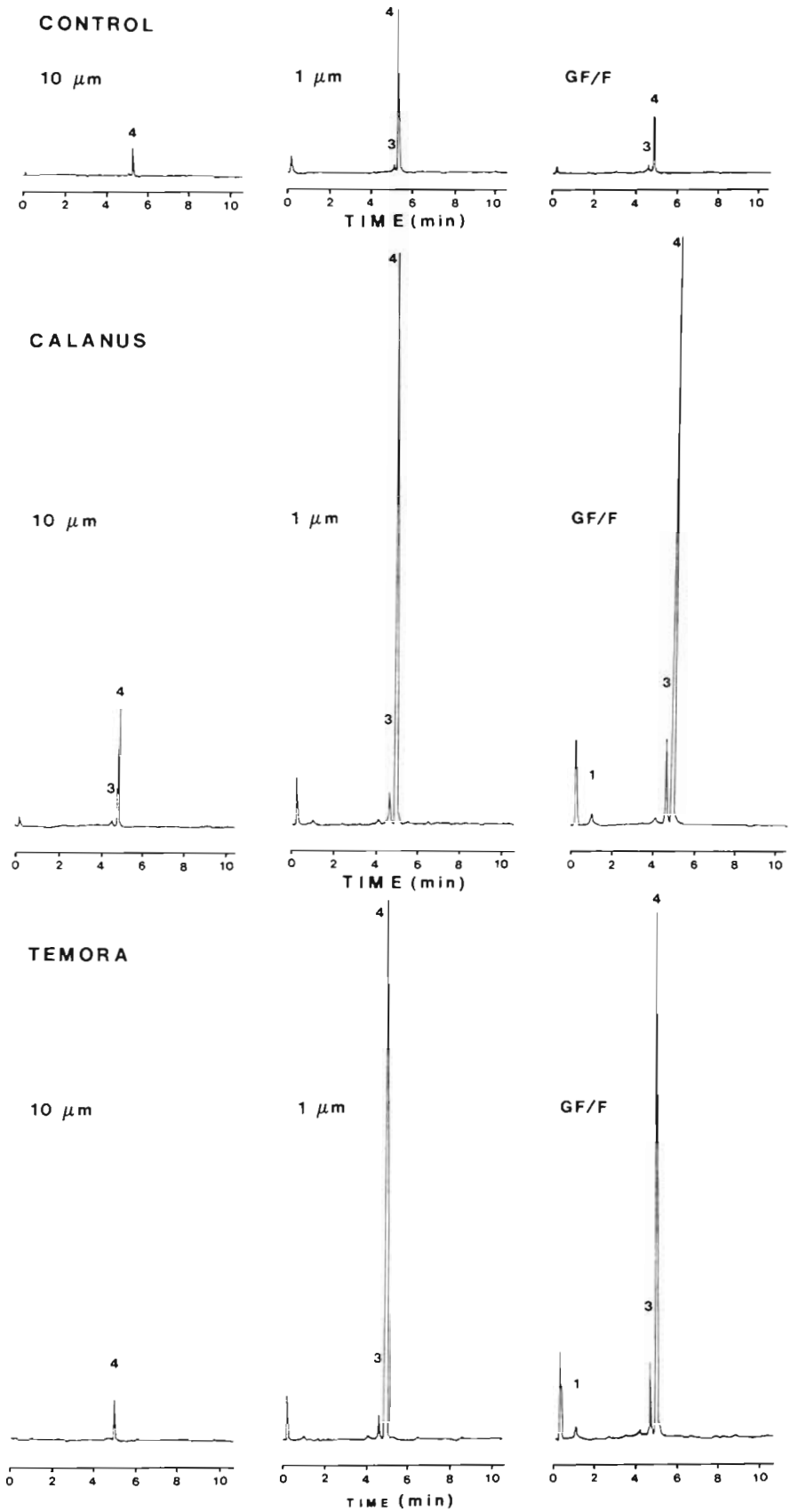

Fig. 2. HPLC fluorescence chromatograms of debris obtained from the control bottle (no copepods added), from the *Calanus helgolandicus* bottle and from the *Temora longicornis* bottle at the end of 45 min incubation. The 1 l bottle contents were filtered successively on 10 μm, 1 μm and GF/F filters after removal of copepods and residual phytoplankton cells (or only phytoplankton cells for the control bottle) on a 45 μm mesh Nitex. Technical details and peak identification as in Fig. 1

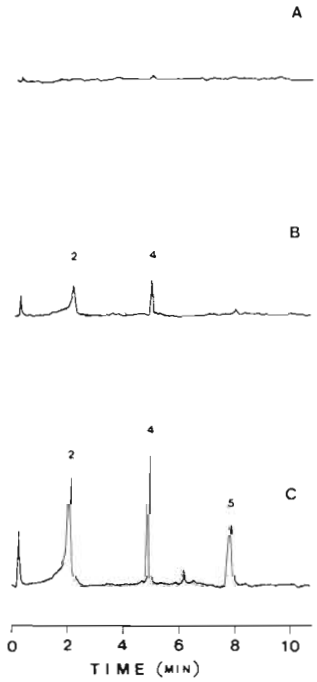

Fig. 3. HPLC fluorescence chromatograms from the pigments contained in the copepods used in the grazing experiments (collected at the end of the feeding session). (A) Extracts from 200 starved (24 h) *Calanus helgolandicus* collected on a 10 µm Nuclepore filter. (B) Extracts from 200 *C. helgolandicus* fed for 45 min on *Coscinodiscus wailesii*. (C) Extracts from 200 *Temora longicornis* fed for 45 min on *C. wailesii*. Technical details and peak identifications as in Fig. 1

ingly low: transforming pheophorbide and pheophytin *a* into terms of chl *a*-equivalents and summing all these to compare with chl *a* ingested (estimated from the number of cells removed multiplied by the cellular concentration of chl *a*) gives values of 5 % for *Calanus helgolandicus* and 21 % for *Temora longicornis*. If cell number alone had been monitored, we might have concluded that, on average, the 200 *C. helgolandicus* in the experimental bottle had eaten 3.39 µg chl *a* and, for *T. longicornis*, 2.00 µg, while pigment analysis of the grazers shows that these same *C. helgolandicus* contained only 0.17 µg chl *a*-equivalent and *T. longicornis*, 0.42 µg, after feeding 45 min. Considering that the copepods ate inefficiently and produced pigment-containing debris could account for a maximum loss of 56 % (26 % as particulate debris + 30 % as 'dissolved') for *C. helgolandicus* and 65 % for *T. longicornis*, which would leave at least 30 to 40 % pigments to be found in the copepods' guts. The reasons why the zooplankton contained only 5 to 20 % pigments are not clear: perhaps debris losses are larger than evaluated here, as would be the case if small pigmented debris (< 0.7 µm) were underestimated by the manual destruction of *Coscinodiscus wailesii* cells. On the other hand, these low pigment levels can be compared to results obtained by Conover et al. (1986) where pigments recovered in feces were less than 10 % of those ingested (calculated from pigment removal during grazing). Similarly, recent studies by Helling & Baars (1985), Wang & Conover (1986), Dagg & Walser (1987) and Kiørboe & Tiselius (1987) have all concluded that the gut fluorescence method may underestimate algal ingestion rates of herbivorous copepods due to pigment destruction in the animal. What happens to pigments when they are inside the animals is not clear, but substantial absorption/adsorption/transformation may be implied as was observed recently for mussels (Hawkins et al. 1986).

Summing the pigment levels found in the copepods (5 % of ingested pigments for *Calanus helgolandicus*, 21 % for *Temora longicornis*) and those detected as particulate debris (26 % of ingested pigments for *C. helgolandicus*, 35 % for *T. longicornis*) suggests that up to between 44 and 69 % (i.e. 100 % minus the previous sum) of pigments originally present in ingested cells could have ended up as debris smaller than 0.7 µm. These are maximum values since they assume no effects of gut passage on pigments. Thus, the overall maximum production of particulate and 'dissolved' debris in this experiment could represent 79 to 95 % of pigments present in cells ingested. These very high values, admittedly maximized by our experimental procedure, indicate the potential importance of 'sloppy feeding' in natural waters. Food breakage both changes the particle size spectrum and is a source of dissolved organic material which may be used quickly by bacteria (Fuhrman 1987) and phytoplankton cells.

The concentration of DFAAs per cell in *Coscinodiscus wailesii* is one of the highest among diatoms (Martin-Jézéquel et al. 1988). Thus, if cell breakage occurs upon feeding over a short time interval, we expect to be able to detect it. The addition of *C. wailesii* to culture medium did not modify significantly the initial background level of DFAAs in seawater (Fig. 4). Comparison between the concentrations of 8 amino acids measured in the food suspension controls and in the grazing bottles, at the end of the 45 min experiment, shows however no significant difference after grazing, even if pigment data bring evidence of 'sloppy feeding'. In fact, DFAAs seem to decrease in concentration (in 2 of the 3 experiments) when copepods are added to the food suspensions. Knowing the intracellular concentration of free amino acids in the cells of *C. wailesii* (Martin-Jézéquel et al. 1988) and the number of cells lost during the feeding session (Table 1), and assuming, as a maximum value, that all cells 'lost' were broken by the copepods, the addition of DFAA would be in the range of 42 to 122 n*M*, corresponding to 56 to 163 n*M* h^{-1} (Table 2). Our results do not show such an increase. The relative composition of the 8 amino acids measured

Table 1. *Calanus helgolandicus* and *Temora longicornis* grazing experiments. Results given for 3 separate feeding experiments using ca 5 cells ml^{-1} suspension of *Coscinodiscus wailesii*. The % values given in parentheses represent the proportion of pigments in copepods or in debris relative to the pigments lost during the 45 min feeding period (estimated on the basis of cell counts)

Expt	Bottle	Cells lost[a]	Pigments in animals[b] (µg)	Pigments in debris[c] (µg)				
				10 µm	1 µm	GF/F	Sum	Grazing –control[d]
I	Control	–	–	0.017	0.099	0.059	0.175	–
	C. helgolandicus	1093 (2.77 µg)	0.171 (6.2 %)	0.043	0.246	0.401	0.690	0.515 (18.6 %)
	T. longicornis	587 (1.49 µg)	0.475 (32.0 %)	0.038	0.156	0.316	0.510	0.335 (22.6 %)
II	Control	–	–	0.010	0.080	0.037	0.127	–
	C. helgolandicus	1357 (3.90 µg)	0.190 (4.9 %)	0.073	0.408	0.885	1.366	1.239 (31.8 %)
	T. longicornis	1116 (3.21 µg)	0.314 (9.8 %)	0.038	0.562	0.353	0.953	0.826 (25.7 %)
III	Control	–	–	0.025	0.113	0.033	0.171	–
	C. helgolandicus	1679 (3.49 µg)	0.153 (4.4 %)	0.073	0.452	0.571	1.096	0.925 (26.5 %)
	T. longicornis	621 (1.29 µg)	0.461 (35.7 %)	0.027	0.467	0.396	0.890	0.719 (55.7 %)

[a] Value determined from cell counts before and after grazing. In brackets: corresponding value in terms of chl *a*
[b] Zooplankton collected on a mesh after grazing and analyzed for pigments. Value given in µg chl *a* (other pigments transformed into chl *a*-equivalents)
[c] Debris collected by filtration after removal of zooplankton and cells with 45 µm mesh. Values (µg chl *a*) represent pigments collected on the various filters. 'Sum' represents the sum of pigmented debris on all 3 filters
[d] Values (µg chl *a*) for debris due to 'slopy feeding' corrected for debris present in the control flask (i.e. Sum–Control)

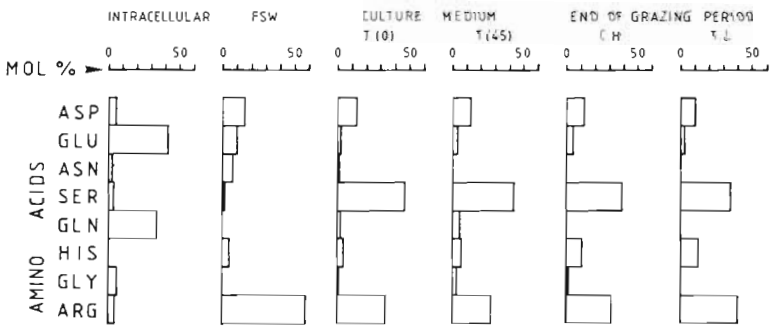

Fig. 4. Amino acid composition, in percent molar units (mol %) relative to total dissolved free amino acids in the samples. Amino acid abbreviations are given in 'Methods'. Intracellular: DFAA composition measured intracellularly in *Coscinodiscus wailesii*; FSW: DFAA composition in filtered seawater used to prepare food suspensions; Culture Medium: DFAA composition at the beginning (T[0]) and end (T[45]) of incubations in control bottles, containing only algae. Righthand 2 histograms (End of Grazing Period): DFAA composition after 45 min feeding in the *Calanus helgolandicus* (C. H.) and *Temora longicornis* (T. L.) bottles

also does not show any strong differences between the control and grazing flasks (Fig. 4), whereas the amino acid composition of the algal cells is quite different from these, with a predominance in the cells of GLU and GLN, 2 amino acids which are almost absent in the control and grazing bottles after 45 min feeding. These data could therefore not effectively demonstrate amino acid enrichment due to 'sloppy feeding' by copepods. A possible explanation of these results, assuming that the pigment results are a valid proof of cell break-up, is a rapid and selective uptake of GLU and GLN during 'sloppy feeding'.

The discrepancy between release of DOC (Lampert 1978, Olsen et al. 1986) and stability of net DFAAs during the feeding of copepods (Table 2) is probably related to several factors, such as the rate of utilization by bacteria and phytoplankton, and the rate of DFAA adsorption onto the debris produced by copepods, which are likely of the same order of magnitude as the rate of enrichment. These processes are suggested by the various studies of Parks (1976), Fuhrman et al. (1980), Amano et al. (1982), Wheeler et al. (1983), Dortch et al. (1984) and Kirchman & Hodson (1986).

Table 2. Concentration (n*M*) of dissolved free amino acids (DFAA) in the 3 feeding experiments with *Calanus helgolandicus* and *Temora longicornis*. Values are means (± SE) of triplicate samples, except for the last 2 columns. Food cells: algal suspension of *Coscinodiscus wailesii*. FSW: filtered seawater. DFAA concentration in FSW was 5.36 (± 1.02) n*M*. Start and End: before and after the grazing session. Potential addition of DFAA due to cell break-up was calculated from the number of cells removed in each grazing session (cf. Table 1) and the average intracellular DFAA concentration of *C. wailesii* in exponential growth computed from Martin-Jézéquel et al. (1988) (0.073 n*M* cell^{-1}). It represents potential maximum input of DFAA in the dissolved phase assuming that 100 % of cells removed were broken

Expt	Suspension of food cells (FSW + algal cells)		Grazing bottles (FSW + cells + copepods)		Potential addition of DFAA due to cell break-up	
	Start	End	*C. helgolandicus*	*T. longicornis*	*C. helgolandicus*	*T. longicornis*
I	63.19 (± 8.81)	43.19 (± 32.38)	34.25 (± 7.73)	21.84 (± 1.64)	79.79	42.85
II	83.28 (± 16.33)	53.34 (± 6.48)	56.29 (± 19.65)	44.40 (± 11.50)	99.06	81.47
III	39.76 (± 16.87)	114.35 (± 83.74)	63.14 (± 8.84)	92.80 (± 6.73)	122.57	45.33

Properties of amino acids and DOC are perhaps different, and thus should be assessed in relation to the processes listed above. Eppley et al. (1981) showed that the flow of carbon to the dissolved and bacterial size fractions was enhanced by copepod grazing, although it was quantitatively limited. Although DFAAs represent a small fraction of DON produced by phytoplankton (13 to 30 %: Braven et al. 1984), their rate of uptake and their rôle as nutrients for phytoplankton and bacteria are well documented (e.g. Crawford et al. 1974, Wheeler et al. 1974, Fuhrman 1987). In particular, GLU and GLN are known to be rapidly and selectively utilized by bacteria (e.g. Amano et al. 1982). Therefore, we assume that the deficit of DFAAs, as suggested by our results, is related to the selective uptake by bacteria and live cells of *Coscinodiscus wailesii*. In order to avoid a potential artefact or inhibition of the chemosensory feeding of copepods, no antibiotic was added to the cell and copepod suspensions. Thus, bacterial activity could not be inhibited. Roy & Poulet (unpubl.) have run experiments using freshly collected copepod fecal pellets. Addition of DFAAs due to this fecal material, reflecting another pathway linking copepod feeding activity to DFAAs, again could not be effectively measured in the experimental bottles, but was deducted from controls containing the antibiotic chloramphenicol. These type of data show that the pool of DFAAs related to copepod feeding activity (e.g. fecal pellets or 'sloppy feeding') is labile and promptly utilized by bacteria. Therefore, the deficit of DFAAs, equivalent to the potential addition due to cell break-up (Table 2), is an index of the copepod-DFAA-bacteria link. Under non-axenic conditions, increase of DFAAs due to 'sloppy feeding' might be detected only at very small scales of time and space, corresponding to the instantaneous break-up and chemical sphere of the cells.

In summary this study provides a quantitative estimate of the losses taking place when *Calanus helgolandicus* and *Temora longicornis* feed inefficiently on *Coscinodiscus wailesii* under conditions favoring the inefficient use of the food ('sloppy feeding'), so as to define the maximum extent of this phenomenon. Values of 26 % loss for *C. helgolandicus* and 35 % for *T. longicornis* are found when particulate debris bigger than 0.7 µm are collected after short grazing sessions and the pigments in these debris compared with pigments in the cells removed from suspension by the grazers. These values are large enough to merit consideration in grazing investigations, especially since debris smaller than 0.7 µm were not estimated here. The conditions we used deliberately optimized the 'sloppy feeding' process, and cells as large as *C. wailesii* do not normally dominate phytoplankton assemblages. Hence it is difficult to conclude that such inefficient feeding is a general phenomenon. However, production of cell debris is a function of the size of both particulate food and feeder (Paffenhöfer & Knowles 1978). There is evidence (Price et al. 1983) that cells as small as 12 µm may be individually handled by copepods. It is thus possible that similar losses occur when much smaller copepods, or developmental stages, feed on smaller, more numerous diatoms, the relative sizes of grazers and food cells being similar to those in our own experiment. This possibility is suggested by the work of Paffenhöfer & Knowles (1978), where nauplii stages fed by breaking up cells of *Rhizosolenia alata f. indica*, leaving behind empty frustules.

In addition, it is likely that, even in the presence of abundant smaller cells, copepods would gain by eating a few big cells whose contents are equivalent to numerous small cells. This was observed in a study of feeding by *Calanus pacificus* on an equivolume diet of *Coscinodiscus angstii* cells of 45 and 90 µm (Frost 1977). The same carbon values could be obtained by the copepods by eating 78 % of the smaller cells and only 22 % of the big ones. This suggests that 'sloppy feeding' is likely to occur under natural conditions.

Pigments found in copepods collected right after the feeding period in the experiments reported here show very low levels, unexplainable when compared with the number of cells removed from suspension by the grazers, even when 'sloppy feeding' is taken into account. This suggests either the presence of very small pigmented debris or transformations of pigments in the guts of herbivorous zooplankton of a nature still to be determined.

The absence of an increase in DFAA concentration in the grazing bottle points to a rapid utilisation of the amino acids released upon breakage of phytoplankton cells.

Acknowledgements. We gratefully acknowledge the help of Dr R. F. C. Mantoura (IMER, Plymouth) for discussion and the use of his equiment to carry out the pigment analyses. One of us (S. R.) was supported during this work by a post-doctoral fellowship from NSERC (Canada). Dr R. J. Conover is also thanked for comments on the manuscript.

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This article was submitted to the editor

Manuscript received: February 29, 1988
Revised version accepted: December 10, 1988