Amino acids
Copepods
Faeces

Acides aminés
Copépodes
Fécès

Free amino acids in copepod faecal pellets

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ABSTRACT

By measuring water soluble extracts of free amino acids (DFAA) in the diets and faecal pellets of copepods using high performance liquid chromatography (HPLC), it was found that the molar composition of these molecules in the faeces produced depends on the type of food selected and reflects the hydrolysis of food proteins during digestion. On average, four amino acids (ASP, SER, GLY and LEU) together accounted for 63% of total DFAA in the faecal pellets of a variety of copepod species. The diversity of DFAA in copepod faecal pellets was higher than in sea water or in particles and phytoplankton cells ingested by copepods. These first results on DFAA in copepod faecal material indicate the importance of zooplankton faeces in the flux of dissolved nitrogen in pelagic ecosystems,

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RÉSUMÉ

Acides aminés libres associés aux fécès de copépodes

L'analyse par chromatographie liquide haute pression (HPLC) des extraits hydrosolubles d'échantillons de pelotes fécales de copépodes, de particules et de cultures phytoplanctoniques montre que la composition en acides aminés dissous libres (DFAA) des fécès dépend de la nature du régime alimentaire sélectionné par les copépodes. Cette composition est aussi le reflet de la digestion des protéines alimentaires par les copépodes. En moyenne, quatre acides aminés (ASP, SER, GLY et LEU) constituent 63% du total des 16 acides aminés dissous mesurés dans les échantillons pour diverses espèces de copépodes. La diversité des acides aminés libres dans les fécès est supérieure à celle observée dans l'eau de mer, dans les particules ou les cellules phytoplanctoniques formant les régimes alimentaires. Ces premiers résultats témoignent de l'importance du matériel fécal zooplanctonique dans le flux de l'azote dissous en milieu marin,

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INTRODUCTION

Zooplankton faecal pellets have been analyzed for various organic compounds including total nitrogen, total organic carbon and carbohydrates (e. g.: Johannes, Satomi, 1966; Honjo, Roman, 1978; Paffenhöfer, Knowles, 1979; Tanoue *et al.*, 1982; Small *et al.*, 1983; Checkley, Entzeroth, 1985), fatty acids (Tanoue *et al.*, 1982; Corner *et al.*, 1986), pigments (Currie, 1962) and amino acids (Cowey, Corner, 1966; Tanoue *et al.*, 1982; Liebezeit, 1985). Depending on the group of compounds studied, minor or major differences have been reported between the composition of organic particulate matter ingested by zooplankton and the composition of faecal pellet produced on such diets. However, previous studies demonstrated no significant difference between potential food and faeces in terms of amino acid composition (Cowey, Corner, 1966; Tanoue *et al.*, 1982). Similar conclusions were reached when the amino acid composition of sinking particles was related to primary productivity (Lee, Cronin, 1984). These authors hydrolyzed their samples with hydrochloric acid (6N) for 24 hours prior to amino acid analysis. Hence, it should be emphasized that this extraction procedure reflects the amino acid composition of proteins and peptides in addition to dissolved free amino acids in particles or in faeces.

Because of the importance of zooplankton faecal material in the transfer of organic matter in the marine food web, particularly in the vertical flux from surface to deep waters (Bishop *et al.*, 1978; Urrere, Kuauer, 1981), and of the recycling of faecal material in surface waters (Krause, 1981; Welschmeyer, Lorenzen, 1985), the differentiation between the dissolved and combined fraction of organic compounds in particles and in zooplankton faecal pellet demands additional investigation.

Amino acids can account for 10 to 60% of the particulate organic carbon in marine waters (Lee, Cronin, 1984; Wakeham *et al.*, 1984; Liebezeit, 1985). By sinking in particles to the sea floor, amino acids can provide nitrogen to benthic organisms. The fraction of the dissolved organic nitrogen identified as amino acids may range from 13 to 30% of the dissolved total nitrogen (Braven *et al.*, 1984). However, since dissolved free amino acids are labile molecules they constitute a smaller percentage of dissolved organic matter with increasing depth, mainly due to microheterotrophic utilization in the euphotic zone (Carlucci *et al.*, 1985). Therefore, the turnover in the water column of these compounds should depend on their chemical availability. However, there is evidence in the recent literature (Small *et al.*, 1979; Bruland, Silver, 1981), that faeces may sink very quickly ($\sim$, $>100\,m.day^{-1}$), meaning that even labile fractions of the organic matter may reach the ocean floor.

We suggest that the conclusions of earlier authors (*i.e.* Cowey, Corner, 1966; Tanoue *et al.*, 1982) related to the amino acid composition of zooplankton faeces should be extended on the light of new analytical techniques such as those used in the present work.

MATERIAL AND METHODS

The seawater, copepods and particles used in the experiments were collected on several occasions in July and October 1984 in the coastal surface waters of the English Channel near Plymouth (UK) and Roscoff (France). Cultures of phytoplankton provided to copepods during the feeding experiments (*Cryptomonas maculata, Thalassiosira weissflogii*) were similar to those analyzed by Moal *et al.* (1985) and Martin-Jézéquel *et al.* (1985). At Roscoff, copepods isolated from wild populations were dominated by species and developmental stages of *Acartia* sp., *Temora longicornis, Centropages* sp. and to a lesser extent by *Calanus helgolandicus*, depending on the time of sampling. At Plymouth, experiments were conducted with adult female *Calanus helgolandicus.*

The procedures for feacal pellet collection was the following. Copepods were kept in filtered seawater (Millipore, 0.45 µm pore size) for 12 to 48 hours prior to experimental feeding. After the feeding period (12-14 hours) they were then removed from the incubators and transferred to another incubator containing filtered sea water. Twelve hours later, faecal pellets were gently concentrated using Nitex sieves (25, 50, 63, 200 µm mesh size) and eggs and debris were removed from samples with a micropipette under binocular control. Samples of faeces were finally collected on Millipore filters (13 mm, 0.45 µm) and stored at −20°C prior to analysis of dissolved free amino acids (DFAA). Using this procedure we obtained a first category of faecal material presumably reflecting the *in situ* diet of naturally occurring particles. A second set of faecal pellets (originating from a unialgal diet) was obtained with the same population of starved copepods kept in filtered sea water to which saturating levels of food (10^4-10^5 cells.ml^{-1}) comprising cultures of *Cryptomonas maculata* or of *Thalassiosira weissflogii* were added. At the end of the 24 hours feeding period, faecal material was once again removed from the incubator, collected and stored following the procedure described above.

Samples of filtered seawater and particles (<200 µm) obtained at the same time as the copepods were collected in 5 ml acid-washed vials and concentrated on Millipore filters (13 mm, 0.45 µm) and stored at −20°C prior to the DFAA analysis. All particle and faeces samples were ground for DFAA extraction in a tissue grinder with 1 ml ultra clean distilled water at room temperature and centrifuged for 3 minutes at 3000 RPM. A constant volume (50 µl) of the supernatant was analyzed. The measurements of DFAA in water, particles and faeces extracts were achieved using the high-performance liquid chromatography (HPLC) method described by Lindroth and Mopper (1979) and by Dawson *et al.* (1985). Traces of amino acids in blank filters were subtracted from all individual results. Single measurement of DFAA was carried out on each sample. Variability $|2.\sigma t/\bar{x}.100|$ of the measurements, evaluated on the basis of 5 replicates of standard samples, amounted to 7% for total DFAA concentration and to less than 10% for aspartic acid (ASP), glutamic acid (GLU), asparagine (ASN), serine (SER), histidine (HIS), arginine (ARG), glycine (GLY) and tyrosine (TYR). The variability for glutamine (GLN), alanine

Table 1

Dissolved free amino acid composition, expressed as percentage molar composition of sea water. Samples collected simultaneously with the natural particulate material (Tab. 2). Sampling location: Roscoff. Dates-1: 17.07.84; 2: 20.07.84; 3: 31.07.84; 4: 18.10.84; 5: 26.10.84 (for abbreviations of the 16 DFAAs see method section). — : undetected.

Composition molaire (%) en acides aminés dissous libres de l'eau de mer filtrée. Les échantillons ont été récoltés en même temps que le matériel particulaire (tab. 2). Lieu d'échantillonnage: Roscoff. Époques-1: 17.07.84; 2: 20.07.84; 3: 31.07.84; 4: 18.10.84; 5: 26.10.84 (*cf.* matériel et méthode pour l'abréviation des 16 acides aminés).

	Sample number				
DFAA	1	2	3	4	5
1 ASP	5.52	2.95	1.60	2.42	1.65
2 GLU	3.33	3.85	1.92	0.97	1.59
3 ASN	1.09	1.30	–	–	–
4 SER	15.45	8.24	4.17	6.32	3.70
5 GLN	–	–	–	–	–
6 HIS	–	–	–	–	–
7 GLY	19.97	6.66	4.81	9.12	2.99
8 ARG	6.60	6.84	6.88	8.84	7.90
9 ALA	2.09	1.01	0.91	1.39	0.91
10 TYR	2.88	2.88	2.08	2.64	1.73
11 ABA	–	2.47	1.79	–	1.11
12 VAL	10.90	9.23	17.00	16.70	15.53
13 PHE	8.53	18.95	20.98	18.00	17.57
14 LEU	12.66	16.89	16.77	13.88	16.10
15 ORN	–	–	–	–	–
16 LYS	10.96	18.73	21.10	19.71	29.24

(ALA), valine (VAL), phenylalanine (PHE), leucine (LEU) and ornithine (ORN) and lysine (LYS) ranged between 10 and 23%. Variability for alpha amino butiric acid (ABA) was not estimated. The Kolmogorov-Smirnov test (Siegel, 1956) was used to evaluate significant differences (D_{max}) between the composition of DFAA in particles, in phytoplankton and in the faecal material. The degree of similarity between two chromatograms is given by D_{max}, knowing that $D_{nm}(x) = |F_n(x) - G_m(x)|$ and $D_{max} = \max D_{nm}(x)$, where $F_n(x)$ and $G_m(x)$ are the cumulative frequencies and where (x) is the rank (1 to 16) of the DFAAs in each chromatogram (n and m) containing N = 16 amino acids. Results for DFAA are given in molar % because the weight of particulate and faecal materials was not measured.

RESULTS AND DISCUSSION

Tables 1, 2 and 3 summarize the relative molar composition (as % of the total) of 16 DFAA measured in seawater, in the intracellular pool of phytoplankton cells, natural particulate material and copepod faecal

Table 2

Dissolved free amino acid composition, expressed as percentage molar composition of natural particulate material (P) and the faecal pellets (F) produced by copepods feeding on it. Sampling dates as in Table 1. *Dominant copepod species in the samples,* 1: Acartia *sp.,* Temora longicornis; 2: Acartia *sp.,* Temora longicornis; 3: Acartia *sp.;* 4: Centropages *sp.,* Calanus helgolandicus; 5: Calanus helgolandicus. – : *undetected.*

Composition molaire (%) en acides aminés dissous libres des pelotes fécales (F) produites par les copépodes dont le régime alimentaire est constitué de particules naturelles en suspension (P). Les époques d'échantillonnage sont identiques à celles du tableau 1. Espèces de copépodes dominants dans les échantillons; 1: *Acartia* sp., *Temora longicornis*; 2: *Acartia* sp., *Temora longicornis*; 3: *Acartia* sp.; 3: *Centropages* sp., *Calanus helgolandicus*; 5: *Calanus helgolandicus.*

	Sample number									
	1		2		3		4		5	
DFAA	P	F	P	F	P	F	P	F	P	F
1 ASP	6.66	13.59	4.21	13.13	0.31	8.23	5.50	5.23	4.30	10.47
2 GLU	4.34	7.98	11.85	8.20	3.33	3.19	–	4.15	13.82	14.88
3 ASN	0.73	0.80	–	1.65	–	1.11	–	0.17	–	2.13
4 SER	21.72	10.98	3.14	16.09	–	12.48	4.10	5.08	6.42	12.02
5 GLN	–	–	–	0.24	–	0.35	–	7.47	–	–
6 HIS	–	–	–	–	–	–	–	–	–	–
7 GLY	14.30	16.17	4.26	25.58	1.86	19.87	27.78	17.10	13.04	21.98
8 ARG	0.88	1.64	–	3.36	–	12.22	–	10.65	2.28	4.28
9 ALA	1.33	3.07	–	3.42	0.21	3.06	–	3.47	2.95	5.71
10 TYR	2.17	5.50	–	5.38	0.40	7.65	–	11.99	–	6.66
11 ABA	–	–	–	–	2.06	0.47	–	–	–	–
12 VAL	–	4.21	–	4.33	–	2.10	–	3.36	–	3.53
13 PHE	–	2.06	–	1.31	–	0.95	–	3.70	–	–
14 LEU	37.92	31.12	68.02	15.84	76.84	26.21	62.63	11.25	57.18	18.35
15 ORN	1.53	–	–	–	–	–	–	6.27	–	–
16 LYS	8.41	2.87	8.52	1.45	14.89	2.11	–	10.19	–	–

Table 3

Dissolved free amino acid composition, expressed as percentage molar composition of laboratory phytoplankton cultures (CT: Thalassiosira weissflogii, *CC:* Cryptomonas maculata) *and the faecal pellets produced by copepods feeding on them (FT and FC respectively). Dates of experiments, and copepod species composition as in Tables* 1 *and* 2. *Experiment* 6 *was performed on* 27.09.84 *in Plymouth using adult female* Calanus helgolandicus. *FCa and FCb represent two independent samples of faecal pellets from animals feeding on the same culture of* Cryptomonas *(CC).* – : *undetected.*

Composition molaire (%) en acides aminés dissous libres des cellules phytoplanctoniques cultivées en Laboratoire (CT: *Thalassiosira weissflogii*, CC: *Cryptomonas maculata*) et des pelotes fécales des copépodes (FT et FC) dont les régimes sont formés de ces algues. Les époques et les espèces de copépodes sont identiques à celles des tableaux 1 et 2. L'expérience 6 a été réalisée à Plymouth le 27.09.84 avec des femelles adultes de *Calanus helgolandicus.* FCa et FCb représentent deux échantillons indépendants de pelotes fécales produites par des copépodes recevant le même régime de *Cryptomonas* (CC).

	Sample number												
	3		4		4		5		5		6		
DFAA	CT	FT	CT	FT	CC	FC	CT	FT	CC	FC	CC	FCa	FCb
1 ASP	7.98	9.35	14.75	20.57	12.40	16.30	5.43	16.48	9.06	19.83	5.90	10.47	11.72
2 GLU	15.47	11.59	24.78	5.89	20.78	2.22	26.43	15.38	15.34	8.62	8.27	8.26	14.27
3 ASN	1.53	3.78	2.34	0.91	0.70	0.54	1.16	1.03	0.84	0.86	4.64	4.68	4.84
4 SER	13.98	13.38	10.41	13.56	13.66	17.89	6.87	0.94	22.93	17.47	10.80	11.73	11.86
5 GLN	6.76	0.66	14.89	0.99	4.38	–	16.44	–	3.23	–	6.68	0.43	0.27
6 HIS	–	–	–	–	–	–	19.33	–	2.04	–	5.34	4.80	3.79
7 GLY	12.97	18.91	8.32	16.75	15.89	16.47	4.82	18.37	15.00	18.73	12.01	20.37	17.58
8 ARG	2.21	1.24	0.44	4.67	1.69	1.96	0.17	1.45	3.50	–	5.09	2.22	1.50
9 ALA	2.74	3.92	1.95	2.92	2.78	2.88	1.99	3.17	2.80	4.69	4.01	4.99	3.85
10 TYR	6.64	7.46	5.65	6.44	9.09	8.97	2.36	4.00	3.36	6.37	5.46	6.36	5.22
11 ABA	–	–	–	–	–	0.69	–	–	–	–	–	0.24	–
12 VAL	4,35	–	3.59	7.07	3.37	5.97	3.61	8.45	2.99	3.06	6.62	8.47	7.97
13 PHE	3.95	–	2.78	5.43	4.65	6.13	0.47	2.11	1.53	–	4.42	4.87	4.48
14 LEU	15.15	28.13	9.54	14.20	10.20	18.10	7.50	15.95	8.41	20.38	9.28	10.82	11.80
15 ORN	4.00	–	–	–	–	–	2.43	1.80	5.44	–	6.06	0.93	0.60
16 LYS	2.28	1.58	0.56	0.59	0.40	1.86	1.00	2.76	3.51	–	5.41	0.36	0.24

pellets. In the *in situ* seawater samples and in the seawater medium used for the phytoplankton cultures, DFAA composition varied from sample to sample. The pattern of DFAA composition in these samples is similar to that described by Mopper and Lindroth (1982), Poulet *et al.* (1984), Poulet and Martin-Jézéquel (1983), Braven *et al.* (1984) using a similar technique. The reason for measuring DFAA in these samples was to check the background level in seawater in order to compare their occurrence in the particulate phase. It is generally assumed that amino acids dissolved in sea water reflect the net result of the balance between release and uptake processes related to microheterotrophic activity, excretion, "sloppy feeding", adsorption onto particles and lysis of organic matter (*see* review by Poulet *et al.*, 1984). Judging from the evidence currently available, there does not appear to be any similarity between the DFAA composition and abundance measured in seawater and in particles or in phytoplankton collected from that seawater sample (Williams, Poulet, 1986; Martin-Jézéquel *et al.*, 1985). Under experimental conditions, it has been shown that DFAA in the seawater samples could reflect the physiological activity of phytoplankton or bacteria (Hammer *et al.*, 1981; Hammer, Eberlein, 1981; Poulet, Martin-Jézéquel, 1983; Carlucci *et al.*, 1985). Similarly, by breaking particles or faeces during feeding, zooplankton could indirectly release the dissolved organic matter contained in the particulate food into the surrounding seawater as suggested by Lampert (1978) and Williams and Poulet (1986). Such an input of amino acids could modify the composition of DFAA in seawater, judging from the results in Tables 1 to 3. When it occurs in nature, however (Williams, Poulet, 1986), the scale of such a modification should be defined on the basis of the relative phytoplankton and zooplankton standing stocks in time and space, and based on the feeding activity of grazers.

Significant differences were found between the DFAA composition in natural particles and in the faeces produced by copepods which had fed on that stock of particulate matter (Tab. 2 and 4). In contrast, generally no significant differences in molar composition could be found between phytoplankton cells and faecal pellets of copepods fed upon the cultures of *Cryptomonas maculata* and *Thalassiosira weissflogii* (Tab. 3 and 4). Figure 1 illustrates the specific composition of amino acids measured in the diet formed of natural particles and phytoplankton cultures and in the faecal pellets. The molar composition of DFAA in the faecal pellets was similar irrespective of the species of copepods, the type of diet and the date of the experiment (Tab. 5). Therefore, DFAA composition in faeces does not seem to reflect the patterns often reported for the composition of phytoplankton and particles (Martin-Jézéquel *et al.*, 1985; Williams, Poulet, 1986) or even in seawater (Hammer *et al.*, 1981; Mopper, Lindroth, 1982; Poulet *et al.*, 1984; Braven *et al.*, 1984). Previous reports on amino acid composition in food and zooplankton faecal material, showed considerable similarities between the diet and faecal pellets (Cowey, Corner, 1966; Tanoue *et al.*, 1982). Parsons *et al.* (1961) reported similar amino acid composition regardless of size and species of phyto-

Table 4

Values of the Kolmogorov-Smirnov parameter D_{max} and the resulting significance of differences between the DFAA composition of the diet and faecal pellets produced for the natural particulate matter, and laboratory phytoplankton culture experiments (data in Tab. 2 and 3).

Valeurs du paramètre D_{max} du test de Kolmogorov-Smirnov, réalisé sur des couples de chromatogrammes d'acides aminés mesurés dans les régimes et les pelotes fécales des copépodes. Les époques et les symboles sont identiques aux tableaux 2 et 3.

Diet Natural particles	Faecal pellets produced	D_{max} (%)
P_1	F_1	13.87
P_2	F_2	59.23**
P_3	F_3	63.42**
P_4	F_4	34.90*
P_5	F_5	38.85*
Diet Phytoplankton cultures	**Faecal pellets produced**	**D_{max} (%)**
Thalassiosira		
CT_3	FT_3	8.29
CT_4	FT_4	25.25
CT_5	FT_5	33.73*
Cryptomonas		
CC_4	FC_4	14.97
CC_5	FC_5	10.77
CC_6	FC_{6a}	10.15
CC_6	FC_{6b}	13.08

Critical values for N=16 are: *$D(n; \alpha)=32.8$ at $\alpha=0.05$; **$D(n; \alpha)=39.2$ at $\alpha=0,01$.

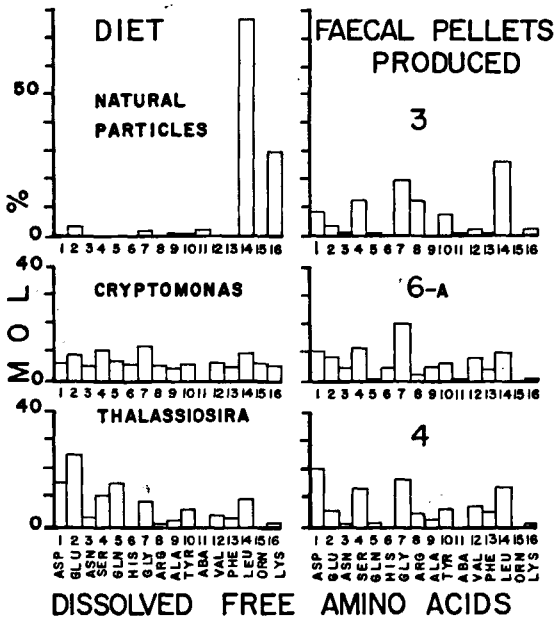


Figure 1

Composition molaire (%) des acides aminés dissous libres (DFAA) des pelotes fécales de copépodes nourris avec du matériel particulaire naturel (3), avec des cultures phytoplanctoniques, monospécifiques, constituées de *Cryptomonas maculata* (6) et de *Thalassiosira weissflogii* (4). Les dates des expériences et les espèces dominantes de copépodes utilisés sont les suivantes, 3: 31.07.84; *Acartia* sp., 6-A: 27.09.84; *Calanus helgolandicus*; 4: 18.10.84; *Centropages* sp., *Calanus helgolandicus.*

Dissolved free amino acid composition,expressed as percentage molar composition of copepod faecal pellets produced by animals feeding on natural particulate material (3), Cryptomonas maculata (6), *and* Thalassiosira weissflogii (4). *Dates of experiments and dominant species used are as follows,* 3: 31.07.84; Acartia *sp.*, 6-*A*: 27.09.84; Calanus helgolandicus; 4: 18.10.84; Centropages *sp.*, Calanus helgolandicus.

Table 5

Values of the Kolmogorov-Smirnov parameter, D_{max}, and the resulting significance of differences between the DFAA composition of faecal pellets produced by copepods feeding on the range of diets illustrated in Tables 2 and 3.

Valeurs du paramètre D_{max} du test de Kolmogorov-Smirnov, réalisé sur des couples de chromatogrammes d'acides aminés mesurés dans les pelotes fécales de copépodes nourris avec des régimes phytoplanctoniques monospécifiques et de particules naturelles en suspension. Les époques et les symboles sont identiques aux tableaux 2 et 3.

Faecal pellet samples		D_{max} (%)
F_1	F_3	10.15
F_2	F_5	4.49
F_2	FT_4	7.54
F_2	FC_{6a}	5.34
F_4	F_5	24.87
F_4	FT_4	19.56
F_4	FC_4	22.32
F_4	FC_{6a}	9.65
F_5	FT_5	9.21
F_5	FC_5	9.36
FT_4	FC_4	8.31
FT_4	FC_{6a}	10.14

Critical value for N = 16 is: $D(n; \alpha) = 32.8$ at $\alpha = 0.05$.

plankton. The discrepancy between our and these authors' results is mainly related to analytical techniques and extraction procedures. The present results reflect the dissolved and water-soluble free amino acids whereas the earlier workers described the product of protein hydrolysis. In our results, the similar pattern of DFAA composition in faeces probably reflects the hydrolysis of proteins by copepods during the digestion process of food (Tab. 5).

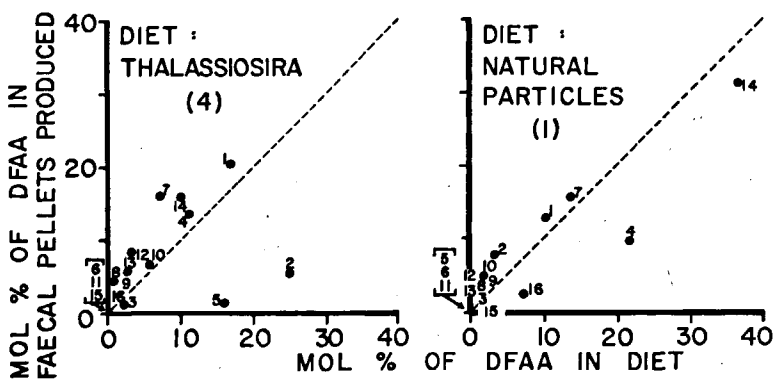
When the food available to copepods is experimentally restricted to a single algal species, it was observed that the DFAA composition in faecal pellets was in general not significantly different from that in the diet (Tab. 4). In contrast, when feeding on natural particles *in situ*, the DFAA composition in faecal pellets was significantly different from that in the stock of particles. Such differences between the two sets of experiments is probably a result of selective feeding by copepods when a choice of food is available in nature (*i. e.* Poulet, 1974; Price *et al.*, 1983). In addition, the difference between the diet and the faecal pellets produced could reflect differences in digestive enzyme activity of the kind reported by Harris *et al.* (1985).

Copepod faecal pellets are generally considered to be of major importance both in the flux of particulate organic matter to the benthic and pelagic food webs (Paffenhöfer, Knowles, 1979; Small *et al.*, 1983; Wakeham *et al.*, 1984; Lee, Cronin, 1984; Welschmeyer, Lorenzen, 1985) and as a source of organic compounds for heterotrophic organisms (Turner, 1979; Hofman *et al.*, 1981; Ittekkot *et al.*, 1984). In terms of dissolved organic matter, very little is known about the importance of faecal pellets in the flux of the organic matter in the sea. Dissolved free amino acids are of major significance as a source of nitrogen for heterotrophic organisms (Bonin, Maestrini, 1981; Admiraal *et al.*, 1984; Carlucci *et al.*, 1985). Williams (1981) drew attention to the problem of assessing the importance of faecal material to planktonic heterotrophs. A number of studies have reported rapid colonization by bacteria and high bacterial numbers in faecal pellets from copepods (Honjo, Roman, 1978; Jacobsen, Azam, 1984) and faecal aggregates from pelagic tunicates (Pomeroy *et al.*, 1984). The presence of DFAAs in faecal pellets will presumably contribute to favourable growth conditions for these bacterial populations. However, it is interesting to note that Jacobsen and Azam (1984) did not observe significantly higher growth rates for bacteria attached to *Calanus pacificus* faecal pellets when compared with free-living bacteria in the surrounding water. It is relevant to note that 4 amino acids (ASP, SER, GLY, LEU) were dominant in the pool of DFAA contained in copepod faecal pellets (Tab. 3 and 4). These four molecules represented, on average, 62.8% and ranged from 38.4 to 76.2% of the total. Two of these amino acids, SER and ASP, were among the four amino acids shown by Williams *et al.* (1976) to have the highest rates of heterotrophic turnover. No information is available for the time-course of individual DFAA concentration within faecal pellets. However, evidence consistent with their rapid utilization by bacteria is provided by Pomeroy *et al.* (1984) who observed a decrease in primary amines coinciding with rapid increase in bacterial populations within pelagic tunicate faecal aggregates. In addition, dissolved free amino acids are labile compounds which can be rapidly turned over in the marine food web. Providing that faecal pellets could be broken down due to sloppy feeding

Figure 2

Comparaison de la composition molaire (%) des acides aminés dissous libres (DFAA) entre les régimes et le matériel fécal produit par les copépodes nourris de cultures de *Thalassiosira weissflogii* (4) et de particules récoltées (1) *in situ*. Les dates des expériences et les espèces dominantes de copépodes utilisés sont les suivantes, 4: 18.10.84; *Centropages* sp., *Calanus helgolandicus*; 1: 17.07.84; *Acartia* sp., *Temora longicornis*. La ligne pointillée indique la limite d'isomolarité entre les régimes et les pelotes fécales. Les nombres 1 à 16 correspondent aux acides aminés des tableaux 1 à 3.

Comparison of the percentage molar composition of the DFAA present in the diet and in the faecal pellets produced by copepods feeding on a laboratory culture of Thalassiosira weissflogii (4) *and natural particulate matter* (1). *Dates of experiments and dominant species-4:* 18.10.84, Centropages *sp.*, Calanus helgolandicus; 1: 17.07.84; Acartia *sp.;* Temora longicornis. *Dotted line indicates* 1:1 *correspondance between percentage occurrence in the diet and in the faecal pellets. Numbers* 1 *to* 16 *correspond to the amino acids listed in Tables* 1 *to* 3.



and coprophagy (Lampert, 1978; Paffenhöfer, Knowles, 1979) or due to bacterial degradation (Turner, 1979; Gowing, Silver, 1983), these dissolved compounds trapped in faeces could be rapidly released and utilized within the water column. Further, the diversity of amino acids in freshly collected faecal pellets (within 12 to 48 hours) was generally higher than in the diet as illustrated in Figure 2 and than in sea water (Tab. 1, 2 and 3). Among the 16 amino acids analyzed, the majority occurred in higher proportions in the faecal pellets compared to the food (Fig. 2). Enrichment of elemental nitrogen in faeces, relative to particulate matter has been reported (Checkley, Entzeroth, 1985). Our observation of higher diversity of amino acids in faecal pellets, together with their dominance by four DFAAs (ASP, SER, GLY, LEU), is also relevant to the recent work of Nott *et al.* (1985). These authors report cyclical changes in the digestive epithelium of *Calanus helgolandicus* resulting in a contribution to the faecal pellets from disintegrating cells of the gut. Similarly, Prahl *et al.* (1984) have reported the presence of lipids in copepod faecal pellets which derive from the animal itself rather than the diet. Hence it is possible that the changes in DFAA composition of faecal pellets observed in the present study may to some extent reflect the contribution of amino acids from the cells of the copepod itself. In this case, one would predict, in view of the observations of Nott *et al.* (1985), a cyclical input of DFAAs into faecal pellets. However, it should be noted that the food species used by Nott *et al.* (1985) to pre-feed their animals, *Scrippsiella trochoidea*, has previously been shown by Huntley *et al.* (1983) to be rejected relative to other cells by *Calanus pacificus*. In fact, ingestion rates on *S. trochoidea* were not much greater on these cells that on polystyrene beads at the same concentration. Similarly the phytoplankton species fed to *Calanus* by Prahl *et al.* (1984), *Dunaliella primolecta*, is unlikely on either geographical or size considerations to form part of this copepod's natural diet. These dietary considerations are relevant as Harris *et al.* (1985) also observed cyclical responses, in this case in terms of digestive enzyme activity, but as a result of transfer to starving conditions. Clearly, further work relating ultrastructural changes in the copepod's gut to digestive enzyme activity, and the DFAA composition of faecal pellets, for animals feeding on naturally occurring diets would be informative. These results constitute the first account of dissolved free amino acids in copepod faecal pellets. They indicate the importance of zooplankton faecal material in the flux of dissolved nitrogen in pelagic systems. The relative importance of coprophagy and microbial breakdown in relation to pellet size and hence sinking rate should be investigated in future studies.

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