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Contribution of zooplankton lipids to the flux of organic matter in the northern Adriatic Sea

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ABSTRACT: Analyses of particulate material collected by sediment traps moored at a location in the northern Adriatic Sea in 1991 revealed the presence of zooplankton fatty acids, even though zooplankton and other 'swimmers' killed by the trap's preservative were carefully removed. Laboratory experiments were conducted to (1) prove the existence of zooplankton lipids within fecal pellets, (2) exclude the possibility of incomplete separation of swimmers and other material as eventual contamination with polyunsaturated fatty acids in fecal pellets, (3) evaluate the importance of zooplankton lipids to mass flux and (4) reveal the mechanisms which lead to excretion of undigested organic matter, in this case polyunsaturated fatty acids. Our results show that the main source of fatty acids found in mass flux were zooplankton lipid droplets inside fecal pellets. The predominant fatty acids of zooplankton fecal pellets were saturated acid 16:0, monounsaturated acid 18:1 and polyunsaturated acid 22:6. Lipid composition of fecal pellets was compared with those of zooplankton and phytoplankton. Aliquots of collected fecal pellets were stained with Nile Red in order to visualize lipid droplets within fecal pellets.

KEY WORDS: Zooplankton · Fecal pellets · Fatty acids · Sediment traps · Lipid droplets

INTRODUCTION

Passively sinking large organic aggregates (mass flux) are one of the major mechanisms which control cycling of organic matter in the sea. One important factor regulating the mass flux in the sea is zooplankton, influencing sedimentation via production of large particles, e.g. fecal pellets (Fowler & Knauer 1986, Noji 1991). These are discrete dense packages of diverse material, sometimes enclosed in a peritrophic membrane (Gauld 1957).

The mass flux is estimated by direct collection of sinking particles over a chosen time interval, e.g. days to months. For this purpose sediment traps are commonly used by oceanographers worldwide (Knauer et al. 1979, Honjo 1980). Chemical analyses of collected particles with the 'swimmers' separated are necessary because these animals actively enter the traps within the upper 500 m and are killed by the trap's preservative, causing errors in the assessment of mass flux (Honjo 1980, Knauer et al. 1984, Harbison & Gilmer 1986, Lee et al. 1988, Hargrave et al. 1989, Michaels et al. 1990). To avoid such errors, zooplankton and all other swimmers should be carefully removed from samples.

Our analyses of 'swimmer-free' particles collected by sediment traps positioned in the northern Adriatic Sea during 1991 revealed very similar composition to that of zooplankton. The principal goal of this study was to identify the source of these fatty acids found in sediment trap material. Our assumption was that the major source of this material was zooplankton lipid droplets which remain 'trapped' in fecal pellets. Earlier studies concerned with qualitative changes in organic composition of the plant food as it passes through the guts of the animals have been particularly important for identifying organic inputs, especially lipids, to marine sediments (Tanoue et al. 1982, Prahl et al. 1984, Volkman et al. 1989). To test this hypothesis we conducted laboratory experiments and collected fecal pellets from fresh natural zooplankton community as described by Bochdansky & Herndl (1992) and immediately analyzed for fatty acids by gas chromatography. In addition we compared the fatty acid composition in the

fecal pellets from copepods fed on phytoplankton monocultures of green algae *Dunaliella* sp., eustigmatophyte *Nannochloropsis* sp. (marine *Chlorella* sp.) and prymnesiophyte *Isochrysis* sp. under controlled laboratory conditions, to elucidate the influence of the zooplankton diet on the fatty acid composition of the fecal pellets and to eliminate any doubts that trap samples separated from swimmers were eventually contaminated with zooplankton fragments, moults, eggs or other artifacts which might serve as a source of polyunsaturated fatty acids (PUFA).

Aliquots of collected fecal pellets were stained with Nile Red (NR) for epifluorescence microscopy. NR is a hydrophobic fluorophore that binds specifically to neutral lipids (Fowler & Greenspan 1985, Cooksey et al. 1987). When viewed under epifluorescent illumination, lipid droplets stained by NR show a bright yellow/orange fluorescence. The high specificity of NR in staining lipids makes it an ideal alternative to chemical extraction techniques (Carman et al. 1991) for determining neutral lipids.

MATERIAL AND METHODS

Sediment trap samples: *in situ* experiment. Sediment trap samples were collected at a station in the northern Adriatic Sea (45° 2.8' N, 13° 19.0' E). Trap openings were positioned at 23 m depth in a 37 m water column. Design of the trap system used is described by Puškarić et al. (1992). Traps were prefilled with brine solution containing 5% formalin to preserve the deposited material. Sediment traps were recovered monthly from 24 April to 5 August 1991.

Sedimented particles consisted of amorphous detrital material, fecal pellets and skeletal remains of various phytoplankton species. Traps contained numerous copepods and amphipods. All animals had intact antennae and tissue showing no signs of decomposition (observation before their removal), suggesting that they actively entered the trap and had been killed by the preservative. All swimmers were removed from the wet sample, prior to analysis, by small forceps with sharpened ends under a dissecting microscope at magnification 30 to 50×. The samples were screened through 300 µm mesh, from which larger swimmers were removed, and remaining particles were returned to the screened sample after the swimmer fraction < 300 µm was also removed.

Collection of fecal matter: NR procedure and feeding experiment. The zooplankton community used for the experiment was collected by hauling a net (1 m mouth diameter, 250 µm Nitex mesh) horizontally through the top 5 m for 2 min at the station located 1.5 nautical miles west of Rovinj, Croatia, in June 1992 and March to June 1993. Material in the collection cup was rinsed by 2 l Whatman GF/F filtered seawater (FSW) and placed in a tank with 5 l FSW. Zooplankton were starved for at least 2 h in order to empty the guts. Upon arrival in the laboratory, the zooplankton size fraction between 200 µm and 1 mm was separated by screening collected zooplankton through 1 mm mesh and then rinsing onto 200 µm mesh by passing the sample and 10 l GF/F FSW over the separated zooplankton. Microscopic examination revealed that most of the guts were empty. A ¼ aliquot of the total separated and rinsed zooplankton sample was filtered onto preweighed GF/F filter, rinsed 3 times with 200 ml distilled water and dried at 50 °C for 24 h for dry weight estimates. The remaining material was added to the 8 l phytoplankton culture. The tank was rolled on a rolling table (Shanks & Edmondson 1989), at slow speed (4 rpm) in the dark for 12 h at 22 °C. Zooplankton were then isolated from the tanks by screening (200 µm), separated from the culture material with 10 l GF/F FSW and placed in the collection funnels in GF/F FSW. Aliquots of the fecal pellets were analyzed by gas chromatography or stained with NR, respectively, viewed under epifluorescence microscopy and subsequently filtered on preweighed GF/F filter for dry weight estimates. Zooplankton from collection funnels were filtered on precombusted GF/F filters and extracted as for gas chromatography analysis. The procedure was the same in all feeding experiments, including feeding on algal monocultures. Fecal pellets were collected using plexiglass funnels screened with a 120 µm nylon mesh (Bochdansky & Herndl 1992). The zooplankton community mainly consisted of small copepods *Acartia clausi* (more than 95%). Collected fecal pellets were examined under a dissecting microscope at 30 to 50× magnification prior to filtration on precombusted (380 °C for 2 h) GF/F glass fibre filters. Microscopic examination revealed that the samples were free from contamination of alternative sources of PUFA, e.g. zooplankton fragments, moults, eggs or other artifacts. The types of fecal pellets found most commonly in our experiments are shown in Fig. 1. Subsequently, the filters with fecal pellets were extracted.

For NR staining of fecal pellets the collected zooplankton community was starved in GF/F FSW in collection funnels for 30 min. Fecal pellets were suspended in 5 ml GF/F FSW and 100 µl NR stock solution (2.5 mg of NR added to 100 ml p.a. grade acetone) was added to fecal pellet suspension. Staining of neutral lipids was complete after 30 min. Fecal pellets were examined at 400× using a Nikon Microphot SA microscope with an epifluorescence attachment. A standard blue excitation method was used (main wavelength 495 nm), consisting of Nikon excitation

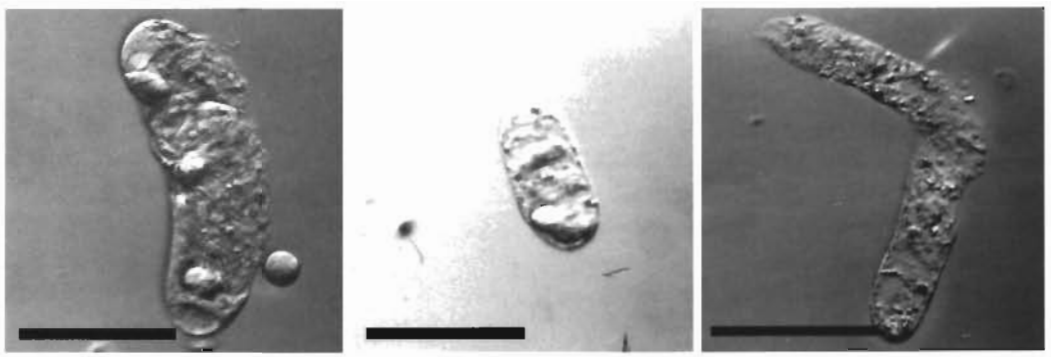

Fig. 1. Most common types of fecal pellets collected in this experiment. Scale bars = 50 µm

filter EX420-490, dichroic mirror DM510 and barrier filter BA520.

For obtaining natural algal cultures, seawater was taken at the sampling site at 5 m depth. Natural phytoplankton culture used in the feeding experiment was mainly composed of *Chaetoceros* sp. To remove larger grazers, the water sample was screened through a 200 µm nylon mesh. Cultures (including phytoplankton monocultures of green algae *Dunaliella* sp., eustigmatophyte *Nannochloropsis* sp. and prymnesiophyte *Isochrysis* sp.) were maintained in 10 l glass bottles (8 l in each tank) in SN medium (Waterbury et al. 1986) at 22 °C and constant irradiation of 320 µE m^{-2} s^{-1} mixed by air bubbling. After the response of the algal community to increased concentrations of nutrients during the exponential phase of growth, at cell densities of 400×10^6 to 700×10^6 cells l^{-1}, a 1 l subsample was filtered onto precombusted GF/F filters for gas chromatography analysis, 200 ml for dry weight estimates (filtered onto preweighed GF/F filter) and 5 ml for phytoplankton cell counts.

Cell counts and dry weight estimates of the phytoplankton culture were done before and after removing the zooplankton from the culture. Cell counts were done at 400×. For dry weight estimates, 200 ml of culture was filtered onto preweighed GF/F filters, rinsed with distilled water and dried at 50 °C for 24 h. The filters were cooled to room temperature in a desiccator for 2 h prior to weighing.

Extraction and analysis. Lipid fractions were obtained from wet samples (up to 1 g dry wt) filtered onto precombusted (380 °C for 2 h) GF/F filters by Soxhlet extractions with dichloromethane (DCM)/methanol 2:1 (v/v) for 24 h (Leenheer 1981). The extracts were partitioned into aqueous and organic phases in separatory funnels by addition of water and DCM, and the water-phase was extracted repeatedly. The extracts were concentrated in a rotary evaporator at a temperature < 40 °C, and transferred into 50 ml screw-capped centrifuge tubes and saponified with 15 ml 1 N KOH in methanol. The tubes were sealed and heated to 100 °C for 30 min. Fatty acids were methylated by adding 10 ml of methanolic BF_3 and the sealed tubes were heated for 5 min at 100 °C. Lipid components were then extracted with hexane, evaporated and transferred into a glass column packed with 2 g of 5% deactivated aluminum oxide over 2 g of 5% deactivated silica (70–140 mesh, Sigma). The column was successively eluted with 10 ml hexane, 10 ml 85:15 hexane:benzene and 8 ml benzene. The fractionation procedure yielded eluants containing aliphatic, aromatic hydrocarbons and fatty acid methyl esters. After evaporation in a stream of nitrogen, each fraction was analyzed using a Hewlett Packard 5730A gas chromatograph and a glass capillary column, coated with methylsilicone (12 m × 0.2 mm), programmed from 70 °C to 270 °C at a rate of 4 °C min^{-1}. Injector and detector temperatures were 250 °C and 300 °C, respectively. Nitrogen was used as the carrier and make-up gas. Peaks were identified by means of reference standard mixtures of fatty acid methyl esters and by comparing relative retention times. The compounds were quantified by internal standard heptadecanoic acid $C_{17:0}$ for fatty acids. The internal standard was added before extraction.

The reproducibility was within 6% for fatty acids for triplicate samples. Recovery efficiencies ranged from 69 to 96% for fatty acids. Blank readings were made daily; all glassware was kept in hot sulfochromic acid and rinsed thoroughly with distilled water and DCM before use.

RESULTS

Mass fluxes of particles collected by sediment traps are given in Table 1, ranging from 0.55 to 0.93 g dry wt m^{-2} d^{-1}, as well as total lipids, fatty acids, PUFA and the total fraction of unsaturated fatty acids. Fatty acid composition observed in sediment trap samples

Table 1 Mass fluxes (dry wt m^{-2} d^{-1}) of particles collected by sediment traps in terms of total lipids, fatty acids, polyunsaturated fatty acids (PUFA) and total fraction of unsaturated fatty acids

Collection period 1991	Total particle flux (g m^{-2} d^{-1})	Total lipids (mg m^{-2} d^{-1})	(% total flux)	Fatty acids (mg m^{-2} d^{-1})	(% total flux)	PUFA (mg m^{-2} d^{-1})	(% total flux)	Unsat. (mg m^{-2} d^{-1})	(% total flux)
25 Apr – 14 May	0.76	9.50	1.25	0.62	0.08	0.09	0.01	0.19	0.02
14 May – 21 Jun	0.73	18.20	2.49	0.43	0.06	0.09	0.01	0.18	0.03
8 Jul – 23 Jul	0.93	255.70	27.49	4.31	0.46	0.76	0.08	1.57	0.17
23 Jul – 5 Aug	0.55	8.25	1.50	1.45	0.26	0.41	0.08	0.46	0.08

Table 2. Fatty acid composition (% of total) of sediment trap samples free of 'swimmers', in zooplankton fecal pellets, phytoplankton used in the feeding experiment and starved zooplankton

Fatty acid	Trap sample				Fecal pellets										Phytoplankton culture	Isolated zooplankton
	1	2	3	4	1	2	3	4	5	6	7	8	9	10		
14:0	3.3	4.2	4.3	1.3	5.9	5.0	4.2	6.9	8.6	5.5	6.6	7.3	6.2	8.1	13.5	7.6
15:0	2.7	2.6	0.8	1.1	2.5	2.4	1.1	2.2	5.0	3.5	3.9	4.1	5.2	1.8	3.7	0.9
16:1	6.6	7.3	4.2	6.2	6.6	6.7	3.1	3.6	5.6	9.5	7.3	7.6	4.5	6.8	34.0	5.6
16:0	33.3	19.5	31.2	20.2	33.8	31.3	30.8	34.0	28.3	28.5	30.7	33.0	30.8	32.1	23.5	19.9
17:0	1.5	0.9	0.8	1.6	0.8	1.1	0.8	1.1	1.8	0.7	0.8	0.6	1.2	0.4	1.7	0.8
18:3	1.3	2.2	0.4	2.5	0.6	0.4	2.2	1.1	1.4	0.2	0.2	0.2	0.5	0.7	0.3	3.0
18:2	1.0	2.3	0.9	2.6	2.7	2.8	3.7	2.3	0.3	0.2	0.3	0.3	0.9	0.7	0.3	3.8
18:1	22.6	34.0	31.9	25.3	12.4	13.0	12.0	6.8	15.9	16.5	13.6	12.7	15.5	13.7	2.0	16.7
18:0	7.2	5.2	3.7	4.4	7.9	6.9	5.1	6.1	11.7	5.3	9.0	9.4	10.5	7.6	6.5	4.6
19:0	1.0	0.4	0.5	0.7	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0
20:5	6.5	9.1	10.2	12.6	2.3	3.3	5.4	3.8	6.3	7.7	7.3	5.6	4.9	7.7	11.9	12.6
20:1	0.1	0.5	0.2	0.1	0.1	0.0	0.0	0.0	0.2	0.1	0.1	0.1	0.2	0.3	0.2	0.1
20:0	1.8	1.6	3.7	3.6	0.5	0.7	0.3	0.6	0.2	0.1	0.0	0.0	0.0	0.5	0.2	0.2
22:6	5.4	8.2	6.3	11.0	21.1	22.5	30.5	30.0	15.1	23.2	20.2	18.4	19.0	20.4	1.7	23.9
22:1	0.1	0.6	0.2	0.5	0.1	0.0	0.0	0.2	0.1	–	–	–	–	–	–	–
22:0	2.6	1.0	0.6	2.5	1.0	1.5	0.5	0.6	–	–	–	–	–	0.2	–	0.2
24:0	2.0	0.5	0.3	3.6	1.8	2.3	0.5	0.6	–	–	–	–	–	–	–	–

was similar to the composition found in fecal pellets (Table 2), i.e. both containing high proportions of PUFA with 20 and 22 C atoms. This similarity is additionally confirmed by the cluster analysis dendrogram shown in Fig. 2. The dendrogram is based on the composition of fatty acids of fecal pellets (FP 1 to 13), zooplankton (ZOO 1 to 4; ZOO1 was fed with natural phytoplankton culture, ZOO2 with *Dunaliella* sp., ZOO3 with *Nannochloropsis* sp. and ZOO4 with *Isochrysis* sp.), natural phytoplankton culture (PHYTO), *Dunaliella* sp. (DUN), *Nannochloropsis* sp. (NAN), *Isochrysis* sp. (IS) and sediment trap samples (TRAP 1 to 4), and the most similar samples are grouped and sorted by the complete linkage method (farthest neighbor). There are 3 distinct groups, of which one represents fecal pellets and zooplankton, one sediment trap samples and one phytoplankton cultures used in the experiments. Fig. 2 shows that fecal pellets are similar to each other and most similar to zooplankton and sediment trap samples. Most importantly, Fig. 2 shows that the phytoplankton cultures

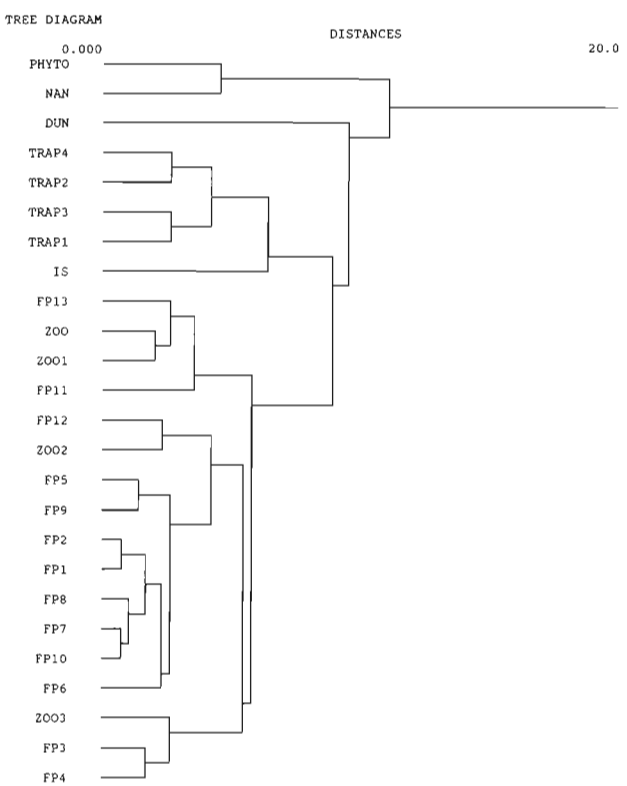

Fig. 2. Cluster analysis dendrogram (complete linkage method — farthest neighbor) of fatty acid composition of sediment trap samples (TRAP), zooplankton fecal pellets (FP), natural phytoplankton culture (PHYTO), *Dunaliella* sp. (DUN), *Nannochloropsis* sp. (NAN), *Isochrysis* sp. (IS) and starved zooplankton (ZOO; ZOO1: then fed with PHYTO; ZOO2: fed with DUN; ZOO3: fed with NAN; ZOO4: fed with IS). Distance metric is Euclidean distance

Table 3. Summary statistics for 3 clusters, output data for dendrogram shown in Fig. 2. TRAP: sediment trap sample; FP: fecal pellet; PHYTO: phytoplankton culture; ZOO: starved zooplankton; DUN: *Dunaliella* sp.; NAN: *Nannochloropsis* sp.; IS: *Isochrysis* sp.

Variable	*F*-ratio	Probability
TRAP1	38.739	0.000
TRAP2	16.322	0.000
TRAP3	24.232	0.000
TRAP4	22.145	0.000
FP1	76.326	0.000
FP2	75.818	0.000
FP3	50.988	0.000
FP4	34.152	0.000
FP5	35.123	0.000
FP6	54.856	0.000
FP7	47.558	0.000
FP8	51.076	0.000
FP9	59.696	0.000
FP10	53.007	0.000
PHYTO	2.559	0.107
ZOO1	30.036	0.000
DUN	7.639	0.004
ZOO2	49.883	0.000
FP11	47.276	0.000
NAN	7.404	0.005
ZOO3	54.054	0.000
FP12	32.861	0.000
IS	10.551	0.001
ZOO3	30.526	0.000
FP13	20.291	0.000

are very distant from all other samples. Statistical support for Fig. 2 is shown in Table 3. From *F*-ratios it can be seen that phytoplankton cultures form a distinct group of samples, different from fecal pellets, sediment trap samples and zooplankton.

Natural phytoplankton culture used in the feeding experiment was mainly composed of *Chaetoceros* sp. with cell density 402×10^6 cells l^{-1}; the fatty acid composition used in the feeding experiment contained a very low proportion of the polyunsaturated acid 22:6 (only 1.72%; Table 2), while the concentrations of 16:1, 16:0, 14:0 and 20:5 fatty acids accounted for 83% of the total fatty acid composition. The C_{18} and C_{22} PUFA were minor constituents.

The major fatty acids of *Isochrysis* sp. were 14:0, 16:0, 18:1 and 22:6. A similar fatty acid composition was also reported in other prymnesiophytes (Volkman et al. 1989). The fatty acid composition of *Dunaliella* sp. was typical for most green algae, with C_{16} and C_{18} PUFA being most abundant. The major fatty acids were identified as 16:0, 16:4, 18:2 and 18:3. A distinct feature of *Dunaliella* sp. was that it contained very small amounts of 20:5 and 22:6 PUFA. *Nannochloropsis* sp. contained fatty acids 16:0, 16:1, 18:3 and 20:5 as major components, while 22:6 was found in traces.

Fatty acid composition of zooplankton, isolated after starvation in fecal pellet collection funnels, showed high proportions of 16:0 and 18:1, and 20:5 and 22:6 PUFA (Table 2).

It should be pointed out that fatty acids in fecal pellets collected under natural, as well as under laboratory, conditions were very similar, dominated by 16:0, 18:1 and 22:6 fatty acids which varied between 59.3 and 73.3%. A very high proportion of PUFA varied within a similar range to that found in zooplankton (21.4 to 35.9%).

The distinguishing characteristic of fecal pellets from zooplankton fed on *Dunaliella* sp. and *Nannochloropsis* sp. was that the PUFA characteristic of the algal diet were absent. In contrast, the PUFA composition in fecal pellets showed a much closer resemblance to the zooplankton composition (Table 4).

Fig. 3 shows various types of lipid droplets entrapped within the pellet's membrane stained with NR, and Fig. 1 indicates the most frequent forms of fecal pellets which contained lipid droplets. The analysis of collected fecal pellets by epifluorescence microscopy revealed that 81 to 100% of the pellets contained on average 9 to 10 droplets per pellet (diameters 0.5 to 20 µm).

DISCUSSION

The fatty acid composition observed in sediment trap samples was characterized by a relatively low proportion of saturated fatty acids with 14 C atoms and a much higher proportion of the unsaturated fatty acids with 18 C atoms. The presence of diatoms was indicated with a high proportion of 20:5 fatty acid. A low proportion of the 16:1 fatty acid and 16:1/16:0 ratio argues against the abundance of diatoms. But, as shown by Tanoue (1985), these low proportions characterized small sized-diatoms and fecal pellets. However, it was demonstrated that diatoms, as well as fecal pellets, contain a very low proportion of the polyunsaturated acid 22:6 (Tanoue 1985, Volkman et al. 1989). The 22:6 fatty acid found in our sediment trap samples was assumed to originate directly from zooplankton.

Moreover, the fatty acid composition of fecal pellets collected during our feeding experiments and from starved zooplankton fed in their natural environment in the northern Adriatic Sea was very similar, having an unusually high proportion of PUFA (Fig. 2, Table 2). Almost the same composition was observed in zooplankton fatty acids (Fig. 2, Table 2), being within the range given for various zooplankton species in Morris

Table 4. Fatty acid composition (% of total) in fecal pellets (FP) of zooplankton (ZOO) fed with *Dunaliella* sp. (D), *Nannochloropsis* sp. (NAN) and *Isochrysis* sp. (IS), respectively. TR: traces

Fatty acid	D	ZOO	FP	NAN	ZOO	FP	IS	ZOO	FP
Saturated									
14:0	0.9	1.9	4.1	6.5	7.7	14.8	17.9	5.1	8.8
15:0	1.7	1.8	6.5	2.2	9.6	3.8	1.3	0.9	0.7
16:0	29.8	19.3	22.6	28.7	32.4	30.6	22.2	26.5	15.4
17:0	2.3	0.9	5.8	0.1	4.4	2.1	0.6	0.2	0.2
18:0	3.8	6.6	6.3	6.5	5.4	5.9	3.4	5.6	7.5
20:0	0.3	0.6	1.1	0.1	1.2	1.3	0.3	0.7	1.1
22:0	TR	0.4	1.5	0.1	1.6	2.1	0.2	0.3	0.3
24:0	TR	0.2	1.7	0.1	1.4	1.6	0.2	0.3	0.1
Sum %	38.8	31.7	49.6	44.3	63.7	62.2	46.1	39.6	34.1
Monounsaturated									
16:1	1.7	5.6	5.2	22.6	5.3	4.9	4.7	4.1	8.4
18:1	11.4	18.3	14.5	5.1	10.3	10.9	17.8	8.8	14.1
20:1	0.7	0.6	1.2	0.2	1.6	2.9	0.3	0.4	0.9
Sum %	13.8	24.5	20.9	27.9	17.2	18.7	22.8	13.3	23.4
Polyunsaturated									
16:3	TR	TR	TR	TR	TR	TR	1.3	TR	TR
16:4	16.8	TR	TR	TR	TR	TR	TR	TR	TR
18:2	2.9	4.2	4.6	2.2	0.7	1.1	3.9	5.2	6.3
18:3	23.5	6.1	4.7	13.7	0.7	1.9	2.1	4.4	4.6
18:4	TR	TR	TR	TR	0.2	0.6	12.2	3.6	4.8
20:5	3.1	8.6	5.4	11.5	4.8	4.6	0.5	7.9	6.1
22:5	TR	TR	TR	TR	0.3	0.6	1.6	0.8	0.2
22:6	1.1	24.9	14.8	0.4	12.4	10.3	9.5	25.2	20.5
Sum %	47.4	43.8	29.5	27.8	19.1	19.1	31.1	47.1	42.5

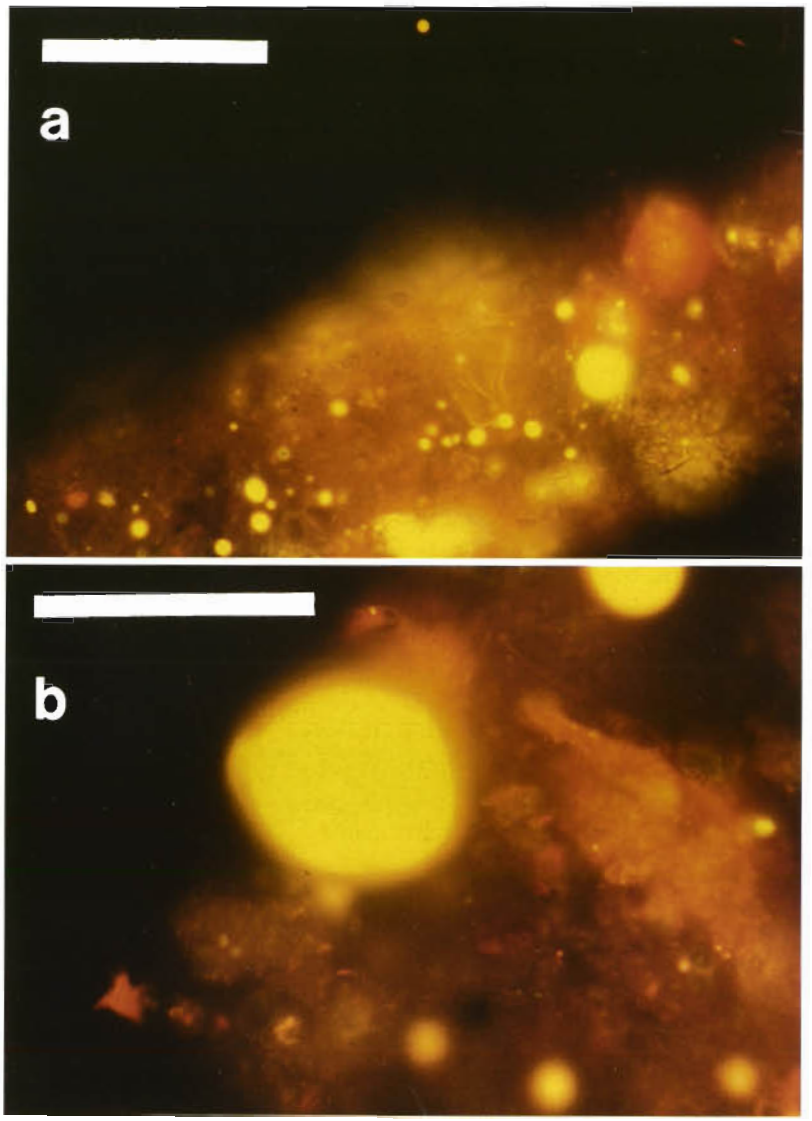

Fig. 3. (a) Numerous small lipid droplets within a fecal pellet, and (b) larger droplets entrapped in the pellet's membrane. Scale bars = 30 µm

(1971). However, the analysis of natural phytoplankton culture and monocultures *Dunaliella* sp. and *Nannochloropsis* sp. on which the zooplankton were fed exhibited the lack of 22:6 PUFA. Alternative sources of 22:6 PUFA, such as dinoflagellates or some prymnesiophytes (Volkman et al. 1989), were not present in natural phytoplankton cultures dominated by *Chaetoceros* sp. This can serve as evidence that phytoplankton prey of zooplankton are not the source of the PUFA found in fecal pellets, even if it is present as a contaminant in fecal pellets.

This unusually high proportion of the 22:6 PUFA is inconsistent with the findings of Tanoue et al. (1982), Prahl et al. (1984) and Tanoue & Hara (1986), who showed that unsaturated fatty acids were found to be susceptible to digestion by zooplankton in laboratory experiments. In their experiments, fecal pellets showed very small proportions of 20:5 and 22:6 PUFA. Polyunsaturated fatty acids are a class of compounds that undergo considerable modification. The explanation proposed by Prahl et al. (1984), that animals selectively absorbed PUFA during digestion or that hydrogenation of fatty acids occurred within the gut itself, obviously contributes to the observed changes only for the PUFA taken from the diet. In our feeding experiment the percentage of characteristic dietetic PUFA (16:4, 18:3, 18:4 which are abundant constituents in *Dunaliella* sp. and *Nannochloropsis* sp. respectively) was markedly reduced during the passage of the food material through the gut. In the case when an alternative source of 22:6 PUFA was present in the food, as with *Isochrysis* sp. (9.2% of total), it was difficult to distinguish whether 22:6 PUFA found in fecal pellets originated from the diet or from the zooplankton itself.

Based on the high abundance of lipid droplets in fecal pellets which we determined by means of the NR method, it can be speculated that the actual fatty acid composition of fecal pellets was masked by fatty acid composition of lipid droplets entrapped within the pellets' peritrophic membrane, pointing to the possibility that lipid droplets originated directly from the zooplankton and that contamination with zooplankton fragments, moults and eggs as the source of 22:6 PUFA can be excluded. The analysis of collected fecal pellets exhibited a high degree of volume variation of lipid droplets, e.g. 81 to 100% of the pellets contained 9 to 10 droplets per pellet, most probably directly related to the quantity and proportion of fatty acids in zooplankton.

Our experiments using natural conditions, as well as defined food sources, have shown that the composition of fatty acids in fecal pellets is very similar to zooplankton, i.e. the fatty acids remain unaltered in composition even after digestion. For this reason we assumed that the main source of fatty acids found in fecal pellets were zooplankton undigested lipids. The

NR method indicated that lipid droplets found within fecal pellets (see Figs. 1 & 2) most probably represent the undigested part of the total lipid content of zooplankton. The mechanism of zooplankton excretion of lipid droplets was previously discussed by Nott et al. (1985), explaining this phenomenon with the activity and disintegration of vacuolar B-cells (Arnaud et al. 1978). High abundance of lipid droplets in fecal pellets (and sediment trap samples) found in the northern Adriatic Sea might be related to high productivity of the investigated area, and thus higher grazing and activity of vacuolar B-cells, influencing high production and excretion of lipids in zooplankton guts.

In 1991 fecal pellets contributed up to 30% to the total mass flux in the northern Adriatic Sea (S. Puškarić & S. W. Fowler unpubl. data). The notion that undigested PUFA can remain entrapped within the fecal pellet membrane in the form of droplets and appear as a component of mass flux (Table 1) indicates that the seafloor can be seeded by easily available organic matter. Additionally, entrapping of fecal pellets by marine snow and their subsequent remineralization by heterotrophs can also be an additional source of DOM for upper layers of the water column.

Moreover, including lipid droplets in the mechanism proposed in this paper has ecological significance not only in shallow waters such as the northern Adriatic Sea but might be operative even in greater depths as shown by Saliot et al. (1982) and Wakeham (1982), who found material characterized by biological printings of phytoplankton and zooplankton activities in the equatorial North Atlantic at 5082 m depth and in the Arabian Sea below 1000 m as a component of particulate material.

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