

# Stable carbon isotope fractionation in the marine copepod *Temora longicornis*: unexpectedly low $\delta^{13}\text{C}$ value of faecal pellets

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**ABSTRACT:**  $^{13}\text{C}$  fractionation effects in an experimental food chain were determined by performing a series of mesocosm experiments with the copepod *Temora longicornis* and using flagellates as food. The bulk copepods were enriched in  $^{13}\text{C}$  by 1.2 to 2.3‰ and the  $\text{CO}_2$  respired was enriched by 0.8‰, compared to the isotopic composition of the food. Faecal pellets, however, were strongly depleted in  $^{13}\text{C}$  by 4.3 to 11.3‰, in isotopic mass balance with the  $^{13}\text{C}$  enrichment of the copepod body and of the respired carbon dioxide. Compound-specific carbon isotope analyses indicated that solvent extractable sterols and alkenones were isotopically light in carbon in both the copepod body and in the faecal matter. These lipids reflected the isotopic nature of the consumed food, indicating that they are not fractionated in the copepod. The residual fraction of the faecal pellets (after extraction) showed the largest isotopic change, being depleted in  $^{13}\text{C}$  by about 16‰ compared to the same fraction in the diet. Curie point pyrolysis analyses indicated that proteins were the major constituents of this residual material from both the copepods and their faecal pellets. When food sources of different isotopic composition were alternately supplied to the copepods, the faecal pellet residue showed a relatively slow turnover rate compared to the alkenones, which were completely egested by the copepods. A similarly slow turnover rate was observed in the residual material from copepods, suggesting that it must be proteins that are being fractionated by the copepod *T. longicornis*. The low  $\delta^{13}\text{C}$  value of faecal pellets adds another variable to the stable carbon isotopic signature of particulate organic carbon in the pelagic environment.

**KEY WORDS:** *Temora* · Carbon · Isotope · Fractionation · Faeces · Respiration · Lipid · Protein

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## INTRODUCTION

Since the early 1980s,  $^{13}\text{C}/^{12}\text{C}$  isotope ratios of bulk tissue of organisms have been routinely used as tracers to determine the pathways of carbon within pelagic food webs (see Fry & Sherr 1984 for a review). Previous work by DeNiro & Epstein (1978) showed that animals under controlled laboratory conditions are slightly

enriched in  $^{13}\text{C}$  relative to their diet, amounting to 0.5 to 1.0‰ per trophic level. Accordingly, isotopic studies on marine food webs have shown that benthic animals and zooplankton are similarly  $^{13}\text{C}$ -enriched relative to the particulate organic carbon (POC) (e.g. Fry & Sherr 1984, Fry 1988). In these studies, the  $\delta^{13}\text{C}$  value of POC is often used as an 'isotopic reference point', with the assumption that most POC is derived from phytoplankton. However, egesta and body remains of mesozooplankton after death contribute a significant proportion of the POC (e.g. Wiebe et al. 1976, Honjo & Roman 1978, Suess 1980, Roy et al. 2000), although actual amounts vary with location, depth and season (see review by Corner et al. 1986).

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In the case of copepods, their faecal pellets may partly remain in the pelagic zone, being recycled there by microheterotrophs and also by mesozooplankton (coprophagy) (Urban-Rich et al. 1999). Depending on diet, pellet size, turbulence regime and water depth, a significant proportion may rapidly sediment and thereby supply the deep sea with comparatively fresh organic matter (Mauchline 1998, Urban-Rich 2001). Therefore, when carbon isotopic signatures are determined in food web studies, it is important to know the contribution of faecal pellets to the  $\delta^{13}\text{C}$  value of POC, which may largely depend on the degree of fractionation during digestion and assimilation in the grazers. In the present research we address the following specific questions: (1) How do the carbon isotopic values of algae, copepods and faecal pellets compare? (2) What are the major biochemical components of faecal pellets? (3) Is there a different assimilation, fractionation and turnover rate of different food components? (4) Is it possible to establish an isotopic mass balance? To the best of our knowledge, this is the first attempt to establish an isotopic mass balance for an aquatic organism.

We conducted a series of controlled laboratory experiments with cultured marine algae and copepods. The algal food and the copepods, eggs, exuviae, faecal pellets and respired  $\text{CO}_2$  were analysed for their  $^{13}\text{C}$  composition. The  $^{13}\text{C}$  composition of the residual matter (after extraction) of some of these materials was determined and compared to that of solvent-extracted lipids measured by Grice et al. (1998) in related work. The main biochemical classes in the faecal pellets and exuviae were determined in comparison to those of the algae and the copepods.

## MATERIALS AND METHODS

**Culture conditions.** Broodstock of the copepod *Temora longicornis* (Müller) was continuously cultured in the laboratory at  $15^\circ\text{C}$ . The cryptophyte *Rhodomonas* sp. (4.8 to 8.5  $\mu\text{m}$  equivalent spherical diameter, ESD) and the haptophyte *Isochrysis galbana* (3.6 to 7.0  $\mu\text{m}$  ESD), both grown non-axenically in chemostats, were supplied as food in surplus of the copepods' needs ( $>300 \mu\text{g C l}^{-1}$ ). The heterotrophic dinoflagellate *Oxyrrhis marina* (10.4 to 19.0  $\mu\text{m}$  ESD) was grown in a separate 2-stage continuous culture using *Rhodomonas* sp. as a single food source. *O. marina* also occurred in the copepod stock cultures, competing with the copepods for the same food algae, but at the same time *O. marina* was an important food source for the copepods. In the experiments with single species of algae as food, *O. marina* was carefully removed. For further details on culture conditions, see Klein Breteler & Gonzalez (1986, 1988).

We were able to grow algae with a relatively constant isotopic composition by using stable continuous cultures with a constant air supply (cf. standard deviations in Table 1). For the heterotrophic *Oxyrrhis marina* we assumed a  $\delta^{13}\text{C}$  value of  $-13.3 \pm 0.4\text{‰}$  (mean  $\pm$  SD,  $n = 5$ ) observed in a continuous culture by van Rooy (1989). The isotopic composition of the mixed diet used to feed the copepod stock culture was calculated as the average value of *O. marina* and the 2 food species present in this mixture (see Table 1).

**Feeding experiments.** To determine isotopic fractionation of the consumed food, nearly mature *Temora longicornis* (copepodite stages CIV, V, and adults) were adapted for a period of 4 d to a single food source of constant isotopic composition, during which they largely matured. The copepods were transferred to 22 l of filtered (2  $\mu\text{m}$ ) seawater and fed with *Isochrysis galbana* or *Rhodomonas* sp. at a concentration of  $\sim 300 \mu\text{g C l}^{-1}$ . A nylon mesh with 200  $\mu\text{m}$  apertures was suspended in the 22 l tank about 2 cm from the bottom, to separate the faecal pellets from the copepods. Every other day, the copepods, a quarter of the seawater (and algal food), and the 200  $\mu\text{m}$  mesh were transferred into a new tank of 2  $\mu\text{m}$  filtered seawater. Several hundreds of faecal pellets were collected, typically every 2 d under dim light, by sequential filtration (200, 50 and 20  $\mu\text{m}$  mesh), washed with double-distilled water and centrifuged to remove any floating debris. The remaining debris was removed with a Pasteur pipette under a dissecting microscope. All samples of faecal pellets were frozen to minimise bacterial degradation (Honjo & Roman 1978), and combined to obtain sufficient material for analysis. The experiments were carried out in the dark at  $15^\circ\text{C}$  for a period of up to 1 wk. At the end of the experiment, most of the copepod bodies and an aliquot of the algae from the overflow of the continuous cultures were also collected, frozen and retained for analyses.

In one of the experiments with *Isochrysis galbana* as food, the  $\delta^{13}\text{C}$  value of the food was altered, using 2 *I. galbana* cultures with different isotopic compositions, to trace the flow of carbon through the experimental food chain. One algal culture was supplied only with air, while the other was supplied with a mixture of air and isotopically light  $\text{CO}_2$ . The isotopic composition of the second culture was allowed to stabilise for a period of 2 wk prior to the experiment. *Temora longicornis* were fed with the first (standard) culture of *I. galbana* during 4 d of adaptation and during the following first 4 d of the experiment. Thereafter, the carbon isotopically light *I. galbana* culture was fed to the copepods for 6 d, after which the food was switched back to the first *I. galbana* culture for a further 4 d. Collection and treatment of samples of algae, copepods and faecal pellets were the same as in the other feeding experiments.

Exuviae and eggs of copepods were collected from the copepod broodstock, using a dissecting microscope, and analysed similarly to the other materials.

Lipids were extracted from part of the material and the  $\delta^{13}\text{C}$  values of sterols and alkenones (cf. Grice et al. 1998) and of the residual material (this study) were determined by isotope ratio monitoring-gas chromatography-mass spectrometry (irm-GCMS). The macromolecular composition of the material was determined by pyrolysis-gas chromatography-mass spectrometry (py-GCMS), as described below.

**Faecal pellet degradation experiments.** To study possible bacterial degradation of faecal pellets, an aliquot of faecal pellets collected from the feeding experiments with *Isochrysis galbana* as food was retained in seawater at room temperature for 1 wk (referred to as 'aged pellets'). For comparison, 160 newly voided faecal pellets were collected from adult copepods from the broodstock, which were rinsed and incubated for 1 h in <1 l of water.

**Respiration experiments.** Because of the very high background of dissolved inorganic carbon (DIC) in seawater, specific precautions were required to measure the minor changes of  $\delta^{13}\text{C}$  due to respiration of the copepods. For this reason, we used a very high concentration of copepods. Prior to the experiment, 2 batches of 2250 and 2360 mostly adult *Temora longicornis* were collected from the stock culture and concentrated in 100 ml of filtered (2  $\mu\text{m}$ ) seawater. Two controls consisted of filtered seawater without copepods. As a second precaution, the experiment was carried out under  $\text{N}_2$  atmosphere in an incubator. DIC was removed from the seawater used for the incubation by lowering the pH to 1.4 with 12M HCl and autoclaving at 120°C for 1 h. The incubation water was cooled and its oxygen content restored by bubbling with  $\text{O}_2$  for 2 h. The pH was restored to 8.1 in the incubator, using a freshly prepared 10M NaOH solution.

All subsequent handling and incubation took place under  $\text{N}_2$  atmosphere in the incubator. Copepods and controls were filtered on 100  $\mu\text{m}$  gauze and quickly rinsed with a few ml of the carbonate-free incubation water. They were resuspended in 100 ml of this water and flushed into a narrow-neck bottle, leaving a head-space of about 20 ml. The bottle was closed with a rubber cap. Two 5 ml samples of the remaining carbonate-free water were taken for initial DIC analysis (blank value). The bottles with copepods and the controls were incubated for 4 h at room temperature. After incubation, a 4 ml sample was taken from each bottle for final DIC analysis. The water was acidified to pH 0.25 with 2.5 ml 18M  $\text{H}_2\text{SO}_4$ , and the bottles were securely closed. Attached to the cap, a small plastic cup was inserted in the head-space of the bottles,

which contained a 12 mm combusted Whatman GF/C filter soaked with 0.05 ml 10M NaOH (Schuster et al. 1998). The  $\text{CO}_2$  was collected on the filter over a 24 h period. Then, the filter was wrapped in aluminium foil, and stored in a small vial under  $\text{N}_2$ . Thereafter, the incubator was opened and the bulk carbon isotopic composition of the material collected on the filter was analysed within a few h. At the end of the experiment, the number of *Temora longicornis* was counted and the final pH was measured.

**Chemical analyses.** Bulk stable carbon isotopic compositions of the samples were determined by automated on-line combustion (Carlo Erba CN analyser 1502 series) followed by conventional isotope ratio-mass spectrometry (Fisons Optima).

Compound-specific stable carbon isotopic composition of the solvent-extractable (ultrasonification with methanol/dichloromethane) lipids of algae, faecal pellets and copepods were determined. Specific fractions of the extracts were analysed by gas chromatography (GC), GCMS and irm-GCMS. Details of extraction, further purification, derivatisation of the fractions and their analysis are described by Schouten et al. (1998). Bulk and compound-specific stable isotopic compositions were analysed in duplicate. Standard deviations of  $\delta^{13}\text{C}$  values due to analytical errors generally were ca. 0.1‰ for bulk values and <0.6‰ for individual compounds.

The macromolecular composition of samples was determined with a FOM-4LX Curie point pyrolysis unit directly coupled to a Hewlett-Packard 5890 gas chromatograph (py-GC), equipped with a cryogenic unit. A CP Sil 5 column (0.45  $\mu\text{m}$  film thickness, 25 m  $\times$  0.32 mm) was utilised. Samples were pressed onto ferromagnetic wires with a Curie temperature of 610°C. Components were eluted using a temperature program of 0 to 320°C at a rate of 3°C min<sup>-1</sup> and held isothermally for 10 min at 320°C. Py-GCMS was performed using the same conditions as described for py-GC and GCMS.

Water samples for DIC analyses were preserved with  $\text{HgCl}_2$  and analysed using a flow-injection technique with a rapid-flow analyser (TRAACS 800), equipped with a dialyser of 10 cm length and a spectrophotometer (Hall & Aller 1992).

## RESULTS

### Distribution of $\delta^{13}\text{C}$ of algae, copepods and faecal pellets

In feeding experiments with *Temora longicornis*, the distribution of  $\delta^{13}\text{C}$  values in the copepod and its faeces was measured in relation to the  $\delta^{13}\text{C}$  value of the

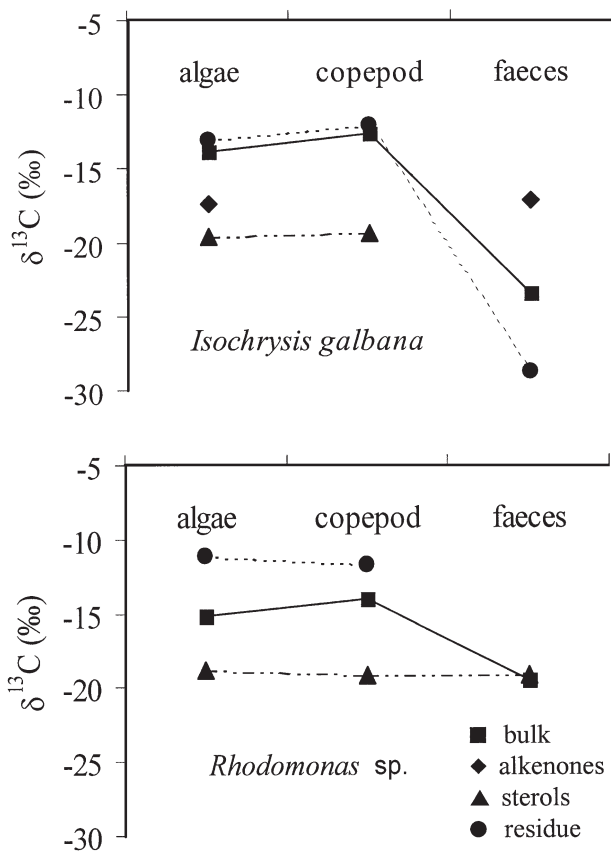


Fig. 1. *Temora longicornis* fed with *Isochrysis galbana* and *Rhodomonas sp.* Stable carbon isotopic compositions of bulk material, extracted lipids (alkenones and sterols) and residue (after extraction) in 'feeding experiments' (cf. 'Materials and methods'). Data of lipids were taken from Grice et al. (1998). Data represent average values of different experiments or sampling dates. For details cf. Table 1

*Isochrysis galbana* or *Rhodomonas sp.* used as the food. The bulk matter of *I. galbana* and *Rhodomonas sp.* had a  $\delta^{13}\text{C}$  value of  $-13.9$  and  $-15.2$ ‰, respectively (Fig. 1, Table 1). After 1 wk of feeding, the copepods were 1.2 to 1.3‰ enriched in  $^{13}\text{C}$  relative to the diet. The  $\delta^{13}\text{C}$  of the faecal pellets was  $-23.5$  to  $-19.5$ ‰ and, therefore, significantly depleted in  $^{13}\text{C}$  relative to the algal food and the copepods (Fig. 1). The depletion was particularly strong with *I. galbana* as food.

To search for isotopically light components in faecal pellets, lipids were extracted and the isotopic composition of both extract and residue were compared to extracts from algae and copepods (Fig. 1). Since lipids are known to be isotopically light components of organic matter, their contribution was considered first (Grice et al. 1998). Alkenones, which are present in *Isochrysis galbana*, are not digested by copepods and hence only occurred in the faecal pellets. Sterols occurred in measurable quantities in the algae and the copepods, but not in faecal pellets produced by copepods fed *I. galbana*. The  $\delta^{13}\text{C}$  values of sterols and alkenones in copepods or faecal pellets were relatively low ( $-18$  to  $-20$ ‰), but similar to those in the algae (Fig. 1), indicating that these lipids are not fractionated isotopically by copepod metabolic processes (Grice et al. 1998). The residues of algae and copepods that remained after extraction were slightly enriched in  $^{13}\text{C}$  compared to the bulk organic matter. In contrast, the lipids in faecal pellets appeared to be isotopically heavy components of the bulk organic matter, whilst the residual matter remaining after extraction was even more strongly depleted in  $^{13}\text{C}$ . Obviously, this residual matter was isotopically modified and responsible for a significant part of the low bulk  $\delta^{13}\text{C}$  value of the faecal pellets.

Table 1. *Temora longicornis*. Stable carbon isotopic compositions ( $\delta^{13}\text{C}$ ,‰) of algal diets, and of the copepod body, faecal pellets, eggs, exuviae and respired  $\text{CO}_2$ . In experiments with monospecific algae as food, the data represent values of the bulk material and its residue after lipid extraction. In experiments with a mixed diet of the same algae and the heterotrophic dinoflagellate *Oxyrrhis marina*, the bulk food value was calculated as the average of the other algae (as shown) and *O. marina* ( $-13.3 \pm 0.4$ ‰, observed by van Rooy 1989). The other data on the mixed diet regime were obtained from materials collected directly from the broodstock culture, including a short-term incubation for production of fresh faecal pellets and  $\text{CO}_2$ . Means  $\pm$  SD of different experiments or sampling dates, no. of observations in parentheses, blank values not determined

Diet	Food bulk	Copepod bulk	Faecal pellets bulk	Eggs bulk	Exuviae bulk	Respired $\text{CO}_2$
<i>Isochrysis galbana</i>	$-13.9 \pm 0.0$ (2)	$-12.6 \pm 0.7$ (2)	$-23.5 \pm 1.6$ (2)			
<i>Rhodomonas sp.</i>	$-15.2$ (1)	$-14.0$ (1)	$-19.5$ (1)			
Mixture	$-14.1$	$-11.8 \pm 0.5$ (8)	$-25.4 \pm 2.6$ (4)	$-20.6$ (1)	$-13.4$ (1)	$-13.3 \pm 0.1$ (2)
	Residue	Residue	Residue			
<i>I. galbana</i>	$-13.1 \pm 0.8$ (3)	$-12.1 \pm 1.6$ (2)	$-28.8 \pm 0.1$ (2)			
<i>Rhodomonas sp.</i>	$-11.2$ (1)	$-11.7$ (1)				

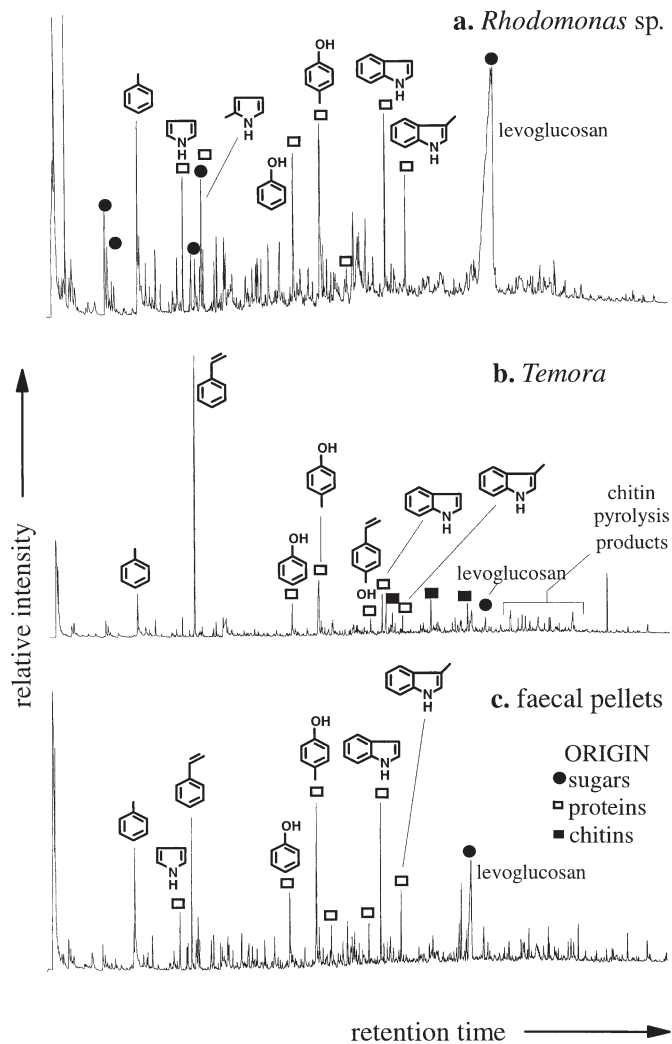


Fig. 2. Total ion current of flash pyrolysates of the residual matter (after extraction) of (a) *Rhodomonas* sp., (b) *Temora longicornis* fed on *Rhodomonas* sp., and (c) faecal pellets egested from *T. longicornis* fed on *Rhodomonas* sp.

The molecular composition of the residual matter of the algae, copepods, and faecal matter was examined using py-GCMS. The pyrolysate of this residue of *Rhodomonas* sp. contained products derived from proteins and carbohydrates (Fig. 2), which are generally enriched in  $^{13}\text{C}$  relative to lipids (Abelson & Hoering 1961, DeNiro & Epstein 1977, van Dongen et al. 2002). The pyrolysates of the residual matter of *Temora longicornis* and of its faecal pellets both contained products mainly derived from proteins, with few carbohydrate-derived products. The copepods additionally yielded small amounts of chitin (Fig. 2). With *Isochrysis galbana* as food, similar protein, carbohydrate and chitin products were obtained (results not shown).

### Aging of faecal pellets

To ensure that aging of pellets did not affect our  $\delta^{13}\text{C}$  measurements, we followed the isotopic composition of decomposing faecal matter over a 1 wk period. Freshly voided (<1 h) pellets had an average  $\delta^{13}\text{C}$  value of  $-25.4\text{‰}$  (mixed diet, Table 1). After 1 wk in seawater, faecal pellets had a very similar  $\delta^{13}\text{C}$  value of  $-25.9\text{‰}$  (*Isochrysis galbana* diet). Both these fresh and aged pellets were slightly more depleted in  $^{13}\text{C}$  than the untreated pellets from the feeding experiments, which were less than 2 d old (*I. galbana* diet, Table 1). Obviously, bacterial degradation or other effects connected with aging do not explain the low carbon isotopic value of the faecal pellets.

### $\delta^{13}\text{C}$ of eggs and exuviae

To study the potential contribution of other copepod products to POC and sedimenting organic matter, the  $\delta^{13}\text{C}$  value of copepod eggs and exuviae was measured (Table 1). Eggs obtained from the broodstock of copepods, fed a mixed diet, were isotopically light, their  $\delta^{13}\text{C}$  value averaging  $-20.6\text{‰}$ , which reflects the same trend to the average light value of  $-20.7\text{‰}$  measured in the youngest nauplii NI and II of *Temora longicornis* under comparable conditions (van Rooy 1989). The  $\delta^{13}\text{C}$  value in exuviae ( $-13.4\text{‰}$ ) from the broodstock was similar to the  $\delta^{13}\text{C}$  value of the bulk copepods. The pyrolysates of the bulk copepods and of copepod exuviae (data not shown) were both predominantly comprised of products derived from proteins and chitin. Their similar molecular nature may also contribute to the similar isotopic compositions observed.

### Turnover rates of food components

To assess the turnover rates of the different algal components in copepods and their faecal pellets, *Isochrysis galbana* of different isotopic composition were alternately fed to *Temora longicornis*. The  $\delta^{13}\text{C}$  values of the extracted residual cell material from the 2 *I. galbana* cultures differed by 11 to 13‰ (Fig. 3c), which is considerably less than the 30‰ difference found in the lipids extracted from these cultures (Grice et al. 1998) (Fig. 3a,b). This suggests that the residual matter of the algae was not yet in steady state isotopically, even though the  $^{13}\text{C}$ -depleted culture had been supplied with isotopically light  $\text{CO}_2$  for 2 wk. For the initial 4 d, the  $^{13}\text{C}$  contents of the residues of the copepods were identical to those in the *I. galbana* culture on which the copepods had been fed for 4 d prior to the experiment. When the food supply was changed to the isotopically light culture

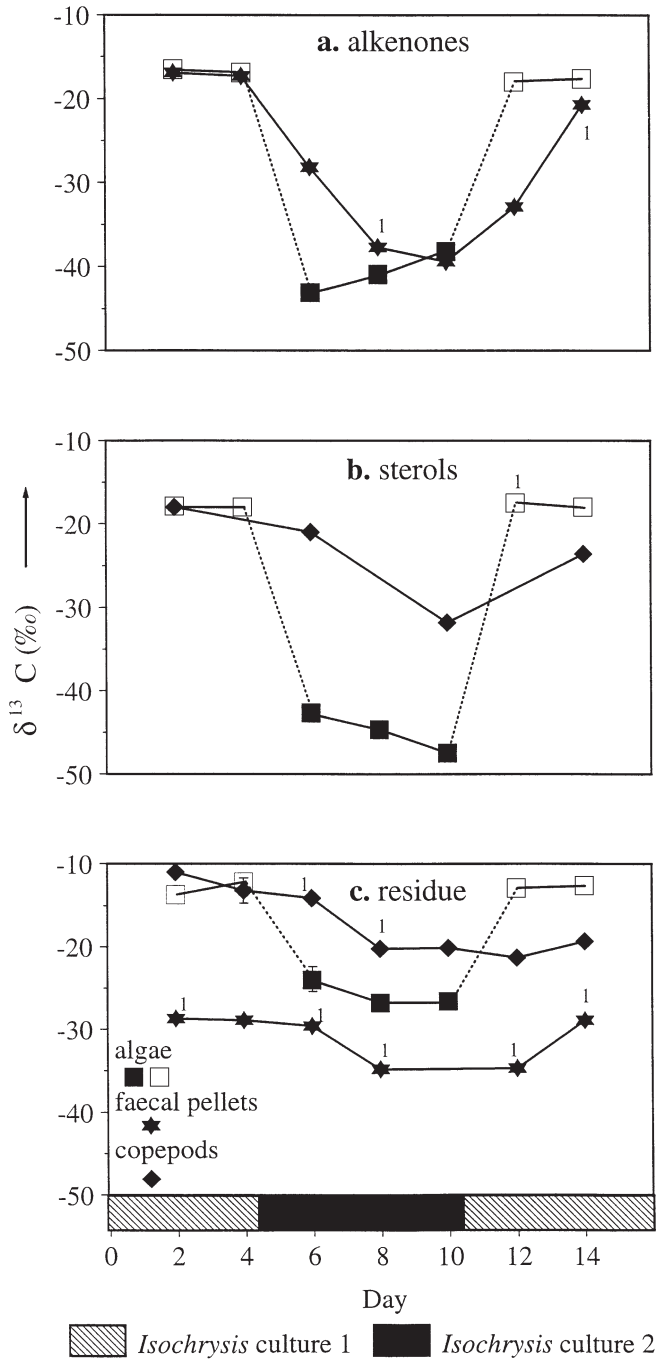


Fig. 3. *Temora longicornis* fed with *Isochrysis galbana* having different isotopic compositions. Stable carbon isotopic compositions of lipids—alkenones (a), sterols (b)—and of the residue after extraction (c) in algae, copepods and faecal pellets during a 2 wk feeding experiment. The copepods were fed an isotopically heavy *I. galbana* Culture 1 (□) during 4 d of adaptation prior to the experiment and in the first and last part of the experiment. From Days 5 to 11, the copepods were fed the isotopically light *I. galbana* Culture 2 (■). Samples of algae, faecal pellets and copepods were taken every 2 d over 2 wk. Samples were analysed in duplicate, except single measurements (1). SD only shown if >0.6‰. Data on lipids from Grice et al. (1998)

for 6 d and back to the original isotopically heavy culture thereafter, the  $\delta^{13}\text{C}$  values of the residual matter in copepods did immediately reflect these changes. Also in the faecal pellets the isotopic composition of the residual material (depleted in  $^{13}\text{C}$ , see Fig. 1) followed a similar trend to that of the residual cell material of *I. galbana*, confirming that the egested faecal carbon is predominantly algal in origin. However, in comparison to the alkenones and perhaps also the sterols (Grice et al. 1998) (Fig 3a,b), there was a lag in the rates of change of the residual matter of the copepod body and faecal pellets. Therefore, this experiment indicates that the isotopic signatures of the different algal components are closely reflected in the copepod body and the egested faecal materials, although the turnover rates of the different materials are not the same.

### $\delta^{13}\text{C}$ value of $\text{CO}_2$ respired by the copepods

We measured the isotopic composition of the carbon respired by the copepods, to detect possible isotopic fractionation of the food due to respiration. At the start of the experiment, the DIC content of the water was very low (blanks in Table 2), indicating that more than 99% of the inorganic carbon present in untreated seawater had been removed. In the controls after 4 h of incubation the DIC concentration had doubled, but was still much lower than in natural seawater. The copepod bottles contained 5 times more DIC than the controls, indicating a clear difference between respired and background  $\text{CO}_2$ .

The quantity of carbon collected in the headspace of the copepod bottles after acidification of the water was 33 to 39% lower than the quantity derived from DIC (Table 2). In the control bottles such a loss of respired carbon was not evident. In one control the quantity recovered was almost twice as much as the DIC present in the water, indicating a contamination of the sample. Therefore, this value and its accompanying  $\delta^{13}\text{C}$  value were excluded. Small droplets of NaOH adhering to the inserted cup were impossible to recover, and this explains part of the loss of respired carbon.

The  $\delta^{13}\text{C}$  value of the  $\text{CO}_2$  produced by the copepods was quite similar in the 2 bottles (Table 2). The average value was  $-13.3\text{‰}$ , which is  $0.8\text{‰}$  enriched in  $^{13}\text{C}$  relative to the mixed diet (Table 1).

## DISCUSSION

### Mass balance

Our experiments indicate that  $^{13}\text{C}$  was strongly depleted in the faecal pellets and eggs, which should be in

Table 2. *Temora longicornis*. Production and isotopic composition of CO<sub>2</sub> respired. The quantity of dissolved inorganic carbon (DIC) in decarbonised seawater was measured before the experiment (Blank), and after 4 h of incubation without (Control) and with (Copepod) copepods. The carbon was recovered by a filter with NaOH, inserted into the headspace of the bottles. The δ<sup>13</sup>C values (‰) of the respired carbon were calculated from the recovered carbon after correction for the control value (excluding 1 value [\*], see text). Data shown represent values from duplicate bottles

Bottle	No. of copepods	DIC		Recovered carbon		Respired carbon
		μM	μg C l <sup>-1</sup>	μg C l <sup>-1</sup>	‰δ <sup>13</sup> C	‰δ <sup>13</sup> C
Blank		13, 15				
Control	0, 0	24, 33	287, 392	299, (685*)	-31.3, (-25.8*)	
Copepod	2250, 2360	171, 179	2048, 2153	1258, 1435	-17.5, -17.1	-13.2, -13.4

isotopic mass balance with the slight <sup>13</sup>C enrichment observed in both the bulk copepods and the respired CO<sub>2</sub>. To establish an isotopic mass balance from the present isotope data of adult *Temora longicornis*, a carbon budget (Table 3) was derived from measurements conducted with copepods fed a mixed diet.

Adult *Temora longicornis* reared under the same conditions as our stock culture and fed with naked flagellates have a weight-specific ingestion rate of carbon of 84 % d<sup>-1</sup> (Klein Breteler et al. 1990). In the adult population (including males) we observed a specific growth rate of 6.0 ± 4.1 % (measured as egg production) and a faecal pellet production of 5.7 ± 0.5 % (mean ± SD, n = 3) of body-C d<sup>-1</sup> (W. C. M. Klein Breteler unpubl.). From these values we derive an approximate carbon budget for adult *T. longicornis* (cf. Table 3) in which 7 % of the ingested carbon is used for growth (as eggs) and 7 % for faeces production, which leaves 86 % for respiration. Applying these proportions to the δ<sup>13</sup>C values in copepods fed a mixed diet (cf. Table 1) and considering that the δ<sup>13</sup>C observed in the eggs is -20.6‰, results in the isotopic mass balance shown in Fig. 4. Mass balance is achieved at a δ<sup>13</sup>C value of the respired CO<sub>2</sub> of -12.7‰, which is consistent with the measured value of -13.3‰ (cf. standard deviations in Table 1). Moreover, the difference between the expected and the observed value will be even smaller if somatic growth occurs (tissue growth of recently moulted adults and spermatophore production by males) in addition to egg production. For

instance, at a somatic growth of 6 % of body-C d<sup>-1</sup> and a δ<sup>13</sup>C value similar to that of bulk adult copepods (-11.8‰), the expected δ<sup>13</sup>C value of the respired CO<sub>2</sub> would decrease to -12.8‰. We thus conclude that our isotope data are consistent with an isotopic mass balance. This supports our laboratory results, showing strongly <sup>13</sup>C-depleted copepod faecal pellets, as well as a probable enrichment in <sup>13</sup>C of respired CO<sub>2</sub>, compared to the food consumed. Both observations are discussed below.

#### Increased <sup>13</sup>C content of respired CO<sub>2</sub>

In the respiration experiments, small-sized bottles with an extremely high density of copepods were used to reduce background CO<sub>2</sub> and allow a short incubation time while avoiding shortage of O<sub>2</sub>. No food was given to the copepods during the experiment. Clearly, these conditions do not allow any quantitative estimate of the respiration rate. The rationale behind the measurements was to ensure that there were no major changes in the substrates respired during the short incubation time, so that the isotopic composition of the

Table 3. *Temora longicornis*. Estimated carbon budget of adults. Data on daily ingestion from Klein Breteler et al. (1990). Growth data represent egg production only. Body carbon represents the summed weight of males and females. Respiration has been calculated as ingestion (100%) minus the percentages of growth and faeces

% of body carbon d <sup>-1</sup>	% of ingested carbon d <sup>-1</sup>		% of ingested carbon d <sup>-1</sup>		
	Ingestion	Growth	Faeces	Growth	Respiration
84	6	6	7	86	7

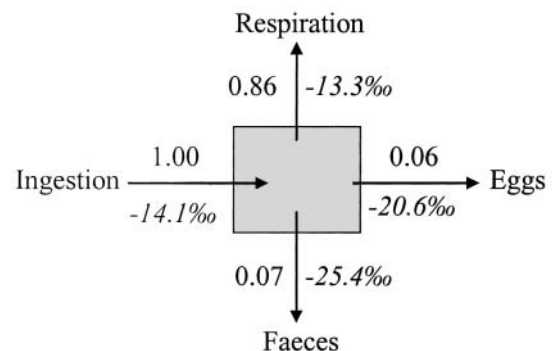


Fig. 4. *Temora longicornis*. Box model of simplified carbon budget (proportion of ingestion) and δ<sup>13</sup>C values measured (in italics, ‰). Isotopic mass balance is obtained at a δ<sup>13</sup>C value of the respired CO<sub>2</sub> of -12.7‰, which was within the accuracy of the carbon isotope method

respired  $\text{CO}_2$  would be due to the food consumed in the stock culture prior to the measurements. The slight 0.8‰ increase that we observed in respired carbon in comparison to the food consumed is in contrast to the slight decrease observed in terrestrial insects (DeNiro & Epstein 1978). However, the latter average decrease included quite a range of values, varying from increases of up to ca. +1.4‰ similar to our data, to decreases by ca. -3.5‰ of respired carbon compared to that of the food. Selective loss of  $^{12}\text{C}$  via respiration has been suggested as one mechanism that could account for the progressive  $^{13}\text{C}$  enrichments observed in food webs (McConnaughey & McRoy 1979, Fry & Sherr 1984). However, there is no *a priori* reason why respiratory carbon should be isotopically light compared to that of the bulk diet. During metabolism  $^{12}\text{C}$  is expected to be lost more readily than  $^{13}\text{C}$  (Hayes et al. 1990), but this concerns a loss from the burnt substrate, which is generally not the bulk food. Substrates such as carbohydrates and lipids in algal food may differ in  $^{13}\text{C}$  content more than originally thought, as appears from observations in *Isochrysis galbana* (van Dongen et al. 2002). Hence, depending on the isotopic routing of substrates (Gannes et al. 1997), respired carbon may have a relatively high  $^{13}\text{C}$  content when it results from utilization of carbohydrates (enriched in  $^{13}\text{C}$ ), while it may be lower when due to utilization of lipids (depleted in  $^{13}\text{C}$ ), in comparison to the bulk diet.

### $\delta^{13}\text{C}$ value of faecal pellets

Our laboratory observations of a strong depletion of  $^{13}\text{C}$  in copepod faecal pellets are unprecedented. Other such laboratory observations have been done on 3 species of insects and 1 snail by DeNiro & Epstein (1978), who showed that the faecal carbon egested from these animals was slightly enriched (on average ~1‰) in  $^{13}\text{C}$  relative to the diet.

From the natural environment no direct evidence exists of isotopic fractionation due to metabolic activity of zooplankton. The few data available only compare the  $^{13}\text{C}$  values of sieved fractions of plankton or POC with those of faecal pellets, ignoring the food that had really been consumed. Thus, the work carried out by Fischer (1991) showed that sinking faecal matter of krill is enriched in  $^{13}\text{C}$  by 2 to 5‰ relative to the plankton, although other pellet material derived from unspecified sources was not enriched in  $^{13}\text{C}$ . Similarly, Checkley & Entzeroth (1985) showed that faecal pellets of copepods are slightly enriched in  $^{13}\text{C}$  relative to the natural suspended POC. However, these faecal pellets were depleted by 2‰ relative to the copepod body. Considering an enrichment of 0.5 to 1.0‰ of the body compared to the food consumed (DeNiro &

Epstein 1978), the data by Checkley & Entzeroth actually indicate an isotopic depletion of faecal pellets compared to the actual diet. A strong depletion of  $^{13}\text{C}$  in copepod faecal pellets would support the explanation by Nakatsuka et al. (1997) that the  $^{13}\text{C}$  content of POC decreases in the sea with increasing water depth. Obviously, in studies with natural plankton it is not easy to establish an accurate isotopic relation between a consumer and its food, particularly in the case of highly selective copepods grazing on a variety of food particles. Hence, controlled laboratory observations such as those presented in this study may contribute to elucidate the possible role of the different foods, which may be assimilated differently and lead to differences in the biochemical composition and  $^{13}\text{C}$  content of faecal pellets (see discussion below).

### Composition of faecal pellets

The present study shows that the residual matter remaining after solvent lipid extraction was isotopically the lightest fraction of the faecal pellets, and was the main carrier of the very low  $\delta^{13}\text{C}$  value of the faecal pellets. Py-GCMS indicated that the nature of this isotopically light fraction of the pellets is predominantly protein. The main lipids present in the faecal pellets were  $\text{C}_{27}$  and  $\text{C}_{28}$  sterols and alkenones (Grice et al. 1998). Although their  $\delta^{13}\text{C}$  value was low, as is the case in fatty acids as well (Schouten et al. 1998), they had the same isotopic composition as the lipids in both the diet and in the copepod bodies. Clearly, no carbon isotopic fractionation of these lipids occurred (Grice et al. 1998). Therefore, fractionation of the assimilated food seems to be restricted mainly to the protein material.

The observed differences in turnover rates of different compounds can serve to trace the metabolic processes involved in isotopic fractionation. Results of the alternating feeding experiment show that the retained and egested material originates from the algal food. The change in the  $\delta^{13}\text{C}$  value of the food led to a corresponding gradual change in the  $\delta^{13}\text{C}$  values of all measured compounds. The time-lag observed may be due to the dilution with the previous food, of which one-quarter of the volume was retained to keep the copepods well-fed. Nevertheless, alkenones reached isotopic stability relatively rapidly (Fig. 3). Since they were not digested (Grice et al. 1998), they were not detectable in the copepod body and passed directly into faecal matter. The slower  $\delta^{13}\text{C}$  response of the sterols results from metabolic turnover of steroid carbon skeletons within the copepods, transforming algal sterols mainly into cholesterol and eventually steroid hormones (Grice et al. 1998). The isotopic composition of the residue (after extraction) of the copepods and



faecal pellets also responded slowly to the change of the algal food. The similarly slow turnover of the residues of copepods and faecal pellets suggests that the residual matter of the pellets mainly consisted of material that had been assimilated by the copepods. Part of this may be due to the cyclic renewal of gut epithelium (Corner et al. 1986, Mayzaud 1986) and the production by the gut wall of the peritrophic membrane for the pellet (Nott et al. 1985). Since the residual matter of the faecal pellets mainly consisted of proteins, it seems that significant isotopic fractionation takes place during protein synthesis in the copepod. Possibly, only few amino acids are involved in the low  $\delta^{13}\text{C}$  value of resynthesised proteins, as appears from the significant depletion by up to ca. 10‰ in 6 amino acids from marine faecal pellets in comparison to the same amino acids in the plankton (Keil & Fogel 2001). It is concluded that, together with isotopically light sterols or alkenones, resynthesised proteins may contribute to strong  $^{13}\text{C}$  depletion in faecal pellets of *Temora longicornis*.

By tracking the isotopic signature of the indigestible alkenones, our study puts emphasis on the need to differentiate between kinetic fractionation due to isotopic discrimination of carbon atoms of a molecule, and processes such as selective digestion of different food components that have different isotopic compositions. The latter may include selective assimilation of aldoses and amino acids (Cowie & Hedges 1996) and selective cleavage of peptide bonds in the gut (Altabet & Small 1990). Also in our study, selective digestion of different food materials may explain the slight differences in  $^{13}\text{C}$  contents of the faecal pellets from different foods (Table 1). Considerable changes in the biochemical composition of faecal pellets are suggested by differences in carbon concentration, C/N ratio and the ratio of dissolved:particulate amino acid carbon in the faecal pellets produced from different foods or during different phases of phytoplankton blooms (see review by Morales 1987, Urban-Rich 2001). In *Oikopleura* feeding on diatoms, the concentrations of polysaccharides and lipids were higher in the faecal pellets than in the food (Bochdansky et al. 1999). Also copepods have difficulty in digesting the cell walls of diatoms (Reinfelder & Fisher 1991), partly depending on the proportion of inorganic matter (Conover 1966). Hence, undigested compounds from diatoms, armoured dinoflagellates and detritus may mask the carbon isotopic depletion of faecal pellets that results from protein metabolism, such as that observed in our laboratory study with easily digestible naked flagellates and microzooplankton as food.

In conclusion, the unexpectedly low  $\delta^{13}\text{C}$  value of faecal pellets we observed is consistent with true isotopic fractionation. The  $\delta^{13}\text{C}$  value of copepod faecal

pellets also depends on the digestibility of the food. Fractionation, and selective feeding and digestion, are significant sources of variability, and complicate the interpretation of stable carbon isotopic signatures in food web studies.

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