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Uptake of sedimentary organic matter by the deposit-feeding Baltic amphipods *Monoporeia affinis* and *Pontoporeia femorata*

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ABSTRACT: Some benthic deposit-feeders mainly eat freshly deposited phytodetritus, while others feed more on older material that has been mixed with the sediment and modified by diagenetic processes before being ingested. We studied the uptake of sedimentary foods of different ages by the Baltic amphipods *Monoporeia affinis* (Lindström) and *Pontoporeia femorata* Kröyer in laboratory experiments using 3 isotopic tracers. The amphipods were offered fresh ^{14}C-labelled diatoms spread on top of a thin unlabelled sediment layer, underlain by 1 yr old sediment to which ^{13}C- and ^{15}N-labelled diatoms had been added. Thus, ^{14}C uptake represented surface feeding on fresh organic material, and ^{13}C:^{15}N uptake subsurface feeding on aged phytodetritus. Experiments using a single species only or mixed species were conducted in spring with 1 yr old adults and in summer with 3 mo old juveniles. Adult *M. affinis* (initial dry mass 1.6 mg) took up ~5 times more ^{14}C than *P. femorata* (initial dry mass 1.7 mg), indicating that *M. affinis* depended more on fresh phytodetritus, while *P. femorata* had significantly higher ^{13}C:^{15}N uptake, showing a greater reliance of this species on aged organic matter from the deep sediment. In experiments, adult *P. femorata* consistently fed at depth in the sediment, whereas adult *M. affinis* modified feeding depth depending on food quality. Juveniles (0.1 mg initial dry mass) of both species had similar tracer uptake and fed both on surface and subsurface sediment, suggesting greater potential for interspecific food competition in juveniles than in adults. Juveniles of both species had higher mass-specific ^{14}C uptake than adults. Single species treatments had higher ^{14}C uptake than mixed treatments in both adults and juveniles, indicating food competition at the higher density of the mixed treatments.

KEY WORDS: ^{14}C · ^{13}C · ^{15}N · Deposit-feeding · Amphipods · Food quality · Baltic Sea · *Monoporeia affinis* · *Pontoporeia femorata*

INTRODUCTION

In temperate waters, deposit-feeding animals derive their nourishment primarily from phytodetritus supplied by seasonal phytoplankton blooms (Elmgren 1978, Levinton & Bianchi 1981, Goedkoop & Johnson 1996). After a bloom settles to the bottom sediment, diagenetic processes soon start to change its chemical composition (Graf 1987, Goedkoop et al. 1997). Due to bioturbation by deposit-feeders, some organic matter is often mixed down into deeper layers of the sediment (Hansen & Blackburn 1992, Gullberg et al. 1997), where anoxia slows the rate of decomposition (Andersen 1996, Bianchi et al. 2000, van de Bund et al. 2001). There are indications that benthic deposit-feeders differ in their use of freshly deposited and older organic matter in the sediment (Rudnick 1989, Widbom & Frithsen 1995, Ólafsson & Elmgren 1997, Byrén et al. 2002). In addition, deposit-feeders may obtain some nutrition from organisms in the sediment, e.g. bacteria and meiofauna (Elmgren 1978, Uitto & Sarvala 1990, Ejdung et al. 2000). In areas like the Baltic Sea, where most of the annual input of phytodetritus follows the spring phytoplankton bloom, deposit-feeders tend to be food limited for much of the year (Cederwall 1977, Elmgren 1978, Uitto & Sarvala 1991, Lehtonen & Andersin 1998).

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Uptake of sedimentary organic matter of different origin or quality by deposit-feeders can be studied using tracers (Lopez & Crenshew 1982, Fry & Sherr 1984). The radioactive carbon isotope ^{14}C is one of the most frequently used tracers in biological research and is commonly used in feeding or food web studies (Rudnick 1989, Widbom & Frithsen 1995, van de Bund et al. 2001). Stable isotope ratios, e.g. $\delta^{13}C$ and $\delta^{15}N$, are other common tracers in food web research, and can use either natural variations in isotopic ratios (Owens 1987, Guiguer & Barton 2002) or manipulated ratios (Levin et al. 1999). Enrichment with the heavy isotope is often used in nutrition studies (e.g. Preston et al. 1996).

In the northern Baltic Sea proper, soft bottom macrobenthic abundance is often dominated by the deposit-feeding amphipods *Monoporeia affinis* (Lindström) and *Pontoporeia femorata* Kröyer (Ankar & Elmgren 1976, Laine et al. 1997). After the recruitment of juveniles in March and April, the abundance of these amphipods can reach 10 000 m^{-2} (Uitto & Sarvala 1991), but is commonly around 2000 m^{-2} (Ankar & Elmgren 1976, Cederwall 1999). *M. affinis* is a glacial relict of freshwater origin, common on bottoms deeper than 20 m, while the marine *P. femorata* is mostly found below 30 m (Segerstråle 1950, Järvekülg 1973). Both amphipods are active bioturbators (Elmgren et al. 1986) and live mainly in the upper 5 cm of the sediment, with *P. femorata* on average found in deeper sediment (Hill & Elmgren 1987). *M. affinis* swims more actively than *P. femorata*, and has a higher respiration rate and fecundity (Cederwall 1979). Adults of both species were earlier thought to feed on surface sediment (Lopez & Elmgren 1989), but *P. femorata* was recently shown to be mainly a subsurface feeder (Byrén et al. 2002). This suggests that the 2 species are likely to use partly different food resources and that *P. femorata* may rely more on old organic material found deeper in the sediment. Whether juveniles differ in depth of feeding in the sediment has not been studied previously.

Here we combine the radioactive isotope ^{14}C and the stable isotopes ^{13}C and ^{15}N as tracers to study deposit-feeding in these 2 amphipods, and in particular, to distinguish between uptake of fresh (^{14}C-labelled) and aged (^{13}C-:^{15}N-labelled) organic material in the sediment. The aim was to test whether, by feeding on deeper sediments, *Pontoporeia femorata* primarily uses older organic material, while the surface-feeding *Monoporeia affinis* mainly uses fresh organic material, recently deposited on the sediment surface. We also tested whether the 2 species influence each other in terms of feeding behaviour, and whether there are differences in feeding between juvenile and adult amphipods.

MATERIALS AND METHODS

Field collections. Animals and sediment were collected with a benthic sled (Blomqvist & Lundgren 1996) at depths of 35 to 45 m near the Askö Laboratory, northwestern Baltic Sea proper (58° 49′ N, 17° 38′ E). Sub-adult amphipods, born in spring the previous year and expected to become reproductive in the coming autumn, for brevity called adults, were collected in late March and early April, and juveniles (young-of-the-year) in late May 2002. Although of similar appearance and size, the 2 species studied are easily identified, since *Pontoporeia femorata* has a dorsal spine which *Monoporeia affinis* lacks. Before use, the amphipods were stored in natural sediment (no food added) in aerated Baltic seawater (salinity 6.5) at 5°C, under the same daily light cycle as in the experiments.

Algal culture and preparation of fresh and aged sediment. The diatom *Skeletonema costatum* (Greville) was cultured in nutrient medium (Guillard 1975) for 11 d in artificial seawater (salinity 15) at 17°C, under a 16:8 h light:dark (L:D) cycle, and labelled by replacing 25% of $NaHCO_3$ in the medium with 25% (0.33 mCi) $NaH^{14}CO_3$ (Amersham; specific activity 54.0 mCi $mmol^{-1}$) (Kester et al. 1967). After harvesting the algae by settling in a separatory funnel in the dark for 5 h at 4°C, remaining dissolved ^{14}C was removed from the water phase by centrifuging the algae at 2500 rpm (ca. 350 *g*) for 10 min, rinsing with filtered brackish seawater, and repeating 3 times. The resulting radioactivity in the algae was 8.2×10^6 dpm (disintegrations per minute) ml^{-1} for the adult and 1.6×10^6 dpm ml^{-1} for the juvenile amphipod experiments. Labelling with ^{13}C and ^{15}N was done the same way, except that all carbon and nitrogen in the nutrient medium were replaced by enriched $NaH^{13}CO_3$ (98%) and $Na^{15}NO_3$ (99.6%), respectively.

The fresh sediment was collected in March 2002, sieved through a 0.5 mm metal mesh to remove macrofauna, and stored aerated at 5°C for 1 mo before the first experiment. The sediment was 66% water, with 2.4% C and 0.4% N in its dry mass. The aged sediment was collected in March 2001, sieved through a 0.5 mm mesh, and labelled with stable isotopes by mixing ^{13}C- and ^{15}N-labelled *Skeletonema costatum* thoroughly with the sediment. The sediment was then stored in the dark at 5°C under 10 cm of aerated Baltic seawater (salinity 6.5) for 1 yr. The aged sediment was 78% water, with 4.1% C and 0.6% N in its dry mass. Evaporation losses during aging were replaced with Baltic seawater, raising the salinity of the sediment to 20. Extra experiments in spring 2003 (28 d, 10 replicates/treatment) started with elevated sediment salinity (20). The results showed that normal sediment salinity (~7) was restored within 14 d under the conditions of the

experiment, and that elevated initial sediment salinity had no significant effect on carbon uptake from the sediment compared to controls at normal salinity (7) for either *Monoporeia affinis* (ANOVA, $F_{1,18}$ = 0.18, p = 0.68) or *Pontoporeia femorata* (ANOVA, $F_{1,18}$ = 1.46, p = 0.24).

Adult amphipod experiment. The adult amphipod experiment began on April 23, 2003 and lasted 28 d. Each plastic experimental jar (380 ml, sediment area 46 cm^2) received 110 g of the wet old ^{13}C- and ^{15}N-labelled sediment (ca. 3 cm deep). Then 50 g of wet fresh unlabelled sediment (ca. 1 cm deep) was carefully spread on the top, followed by gentle addition of water and then 1 ml of ^{14}C-labelled algae (total activity 8.2 × 10^6 dpm) spread evenly on the sediment surface with a Pasteur pipette. Sediment depth was 4.1 ± 0.2 cm (±SD). Amphipods were picked in batches of 3, with damaged ones replaced after checking under a stereo-microscope. After adding 2 randomly selected batches (6 individuals) per species, the jars were connected to a seawater supply (5°C, salinity 6.2, 11.0 ± 0.1 ml min^{-1}), and kept under a daily light cycle (14:10 h L:D), which is similar to field conditions.

Three treatments were used, with *Monoporeia affinis* and *Pontoporeia femorata* either individually or mixed, with 11 replicates in single species treatments and 22 in the mixed treatment. The amphipod density used, 1300 m^{-2}, is often found in the study area (Ankar & Elmgren 1976, Cederwall 1999; Table 1). After the experiment, amphipods were left overnight without sediment to empty their gut, and then counted, dried individually at 60°C, weighed and analysed per replicate. In the mixed treatment, independence of data between species was assured by using half the replicates for analyses of *M. affinis* and the rest for *P. femorata*. Half the survivors of a species from each jar were analysed for ^{14}C and the other half for ^{13}C and ^{15}N. If the survivor number was uneven, an extra specimen was used for ^{14}C-analysis.

Table 1. *Monoporeia affinis* and *Pontoporeia femorata*. Experimental survival and animal mass. Initial adult dry mass was 1.6 ± 0.5 mg for *M. affinis* and 1.7 ± 0.4 mg for *P. femorata*. Values are mean ± SD

Variable	Adult experiment		Juvenile experiment	
	Single species	Mixed	Single species	Mixed
Survival (%)				
M. affinis	80 ± 21	85 ± 15	81 ± 13	82 ± 17
P. femorata	86 ± 15	76 ± 21	83 ± 12	77 ± 17
Final dry mass (mg)				
M. affinis	2.2 ± 0.6	2.2 ± 0.7	0.11 ± 0.02	0.10 ± 0.01
P. femorata	2.2 ± 0.6	2.4 ± 0.6	0.10 ± 0.01	0.09 ± 0.02

Juvenile amphipod experiment. This experiment began on June 5, 2002 and lasted 22 d. The experimental jars (105 ml, sediment area 13 cm^2) first received 30 g (ca. 2 cm deep) of wet aged sediment (as in the adult experiment) after which 8 g (ca. 0.5 cm deep) of wet fresh unlabelled sediment was carefully spread on top. This was followed by gentle addition of seawater, and then 1 ml of ^{14}C-labelled algae (total activity 1.6 × 10^6 dpm) spread evenly on the sediment surface with a Pasteur pipette. Sediment depth was 2.5 ± 0.1 cm (±SD). Then 2 batches of 5 amphipods per species, checked for damage under a stereo-microscope, were added and the jars connected to a seawater supply (5.2°C, salinity 6.2, 12.0 ± 0.1 ml min^{-1}). A daily light cycle of 16:8 h L:D approximated field conditions.

The 3 treatments were *Monoporeia affinis* only, *Pontoporeia femorata* only and both species mixed, with 10 individuals (7700 m^{-2}) of each species per replicate. At the end of the experiment, surviving juveniles were allowed to empty their gut overnight, and were then counted, pooled, dried at 60°C, weighed, and analysed. The experiment was designed to give independence of data for species and isotope analyses. The mixed treatment had 28 replicates, and half were used for each species. Due to their small size, juveniles were analysed for ^{13}C and ^{15}N by replicate, giving a value based on all intact survivors, with half the 14 replicates for each species from each treatment used for ^{14}C, the rest for ^{13}C and ^{15}N.

Radioactive isotope analysis. Amphipods for ^{14}C analysis were transferred individually (adult experiment) or by replicate (juvenile experiment) to 20 ml scintillation vials with 1 ml tissue solubiliser (Lumasolve, Lumac). After solubilisation in 50°C overnight, a 10 ml scintillation cocktail (Hionic-Flour, Packard) was added and samples were counted in a liquid scintillation counter (1214 RackBeta, LKB Wallac).

Elemental and stable isotope analysis. Amphipod and sediment samples were dried at 60°C, mortled, and a weighed subsample analysed for ^{13}C and ^{15}N using an Elemental Analyser (EuroEA3024, Eurovector), coupled on line to an Isoprime isotope ratio mass spectrometer (Micromass UK) at the Department of Natural Science, Örebro University, Sweden.

The nitrogen and carbon isotope ratios are expressed in the ‰ notation, using the equation:

$$\delta R(‰) = [(R_{sample}/R_{standard})-1] \times 10^3 \quad (1)$$

where R is the ratio between the heavy and light isotopes (^{13}C:^{12}C or ^{15}N:^{14}N). The stable isotope ratio, denoted by δ, is defined as the deviation in ‰ from an international reference standard (VPDB, Vienna PeeDee Belemnite for C, and atmospheric nitrogen gas for N). Higher δ values indicate a higher proportion of

the heavy isotope, and hence a smaller proportion of the light isotope, while a lower δ value indicates a lower proportion of the heavy isotope. C:N ratios were calculated from C and N mass contents obtained from the Elemental Analyser during the stable isotope analysis.

Remaining labelled phytodetritus. In the aged sediment (1 yr) remaining labelled phytodetritus could be calculated from its ^{13}C and ^{15}N content. Eqs. (1) and (2) gave ‰^{13}C and ‰^{15}N in the aged sediment,

$$F = (\delta + 1000) \times \frac{R_{standard}}{(\delta + 1000) \times R_{standard} + 1000} \quad (2)$$

The culture medium contained 98% ^{13}C and 99.6% ^{15}N, from each of which 1.5% was subtracted as an estimate of remaining light isotope from the seed phytoplankton population to estimate $\delta^{13}C$ and $\delta^{15}N$ in the algae before addition to the sediment. The total carbon content in the old sediment was 4.1%, of which only 0.03% was derived from the ^{13}C-labelled fraction, and 0.59% N, of which only 0.1% was from the ^{15}N-labelled fraction. The C:N ratio of the remaining labelled aged phytodetritus was 2.2, compared to 7.0 for the old sediment as a whole.

Uptake was calculated as the difference in average individual content of an isotope before and after the experiment, and thus includes new somatic growth, possible microbial growth on the exoskeleton and label associated with particles left in the gut.

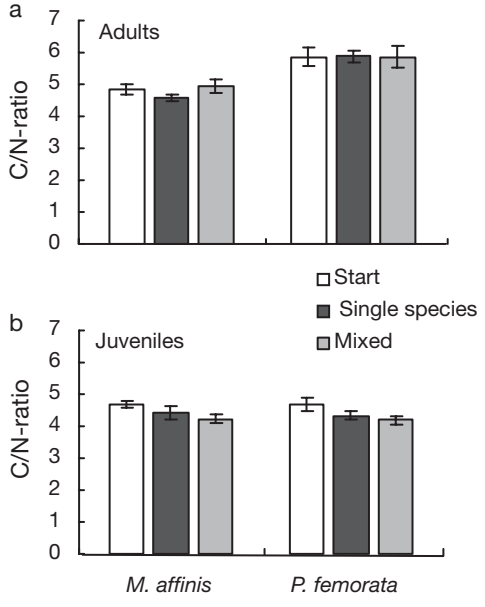

Fig. 1. *Monoporeia affinis* and *Pontoporeia femorata.* Mean (±SE) of C:N ratios in the (a) adult and (b) juvenile experiments

Statistical analyses. Data were analysed using 2-factor ANOVAs with species and culture type (single species vs. mixed species) as factors. Increase in mass was tested with a 1-factor ANOVA for adult amphipods. All data were tested for homogeneity of variance with Cochran's *C*-test, and log-transformed if needed, to obtain variance homogeneity. No significant interaction effects were found. The multiple comparison Tukey HSD test for unequal n was then used for ^{14}C analyses in both adult and juvenile experiments.

RESULTS

Adult amphipod experiment

Survival was 76 to 86%, with no significant differences between species or treatments (2-factor ANOVA, $p > 0.05$; Table 1). Individual mass increased significantly during the experiment by about 40% for *Monoporeia affinis* (ANOVA, $F_{2,30} = 11.5$, $p < 0.01$) and by 33% for *Pontoporeia femorata* (ANOVA, $F_{2,27} = 10.8$, $p < 0.01$), but no significant biomass differences between treatments were found for either species (Tukey HSD for unequal n, $p > 0.05$; Table 1). No significant difference in mass was found between the species, either initially or at the end of the experiment ($p > 0.05$). *P. femorata* had significantly higher C:N ratios than *M. affinis* in all treatments, but there were no significant within species differences between treatments or over time (2-factor ANOVA, $F_{1,44} = 28.9$, $p < 0.05$; Fig. 1).

Monoporeia affinis clearly fed more on surface sediment than *Pontoporeia femorata*, and took up about 5 times more ^{14}C ($p < 0.001$; Table 2), whether single species or mixed species (Tukey HSD for unequal n,

Table 2. *Monoporeia affinis* and *Pontoporeia femorata.* Two-factor ANOVA results for the adult experiment. ns: not significant

Variable	Factor	df_{effect}	df_{error}	*F*	p-value
^{14}C	Species	1	38	86.58	<0.001
	Treatment (single species/mixed)	1	38	9.24	<0.05
	Species × Treatment	1	38	2.49	ns
^{13}C	Species	1	36	7.01	<0.05
	Treatment (single species/mixed)	1	36	0.12	ns
	Species × Treatment	1	36	0.02	ns
^{15}N	Species	1	36	20.69	<0.001
	Treatment (single species/mixed)	1	36	0.04	ns
	Species × Treatment	1	36	0.14	ns

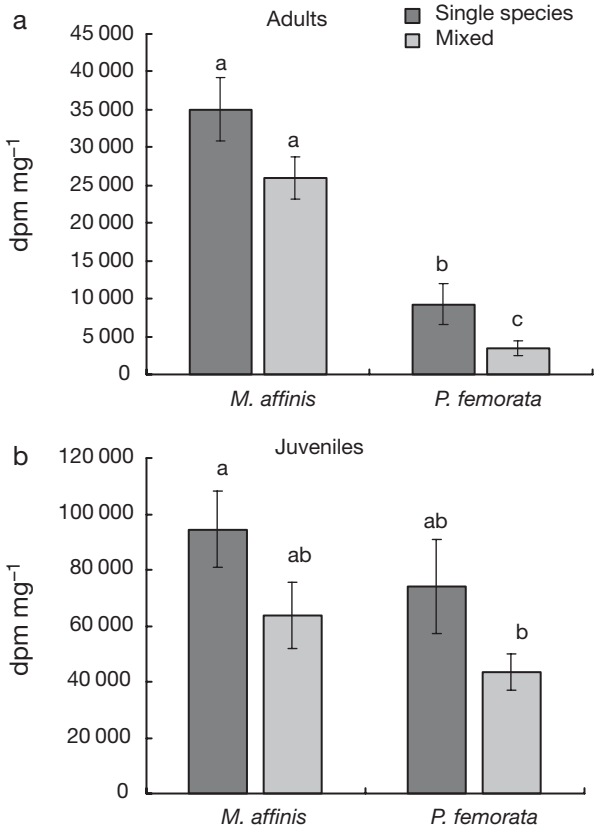

Fig. 2. *Monoporeia affinis* and *Pontoporeia femorata*. Mean (±SE) of ^{14}C uptake as dpm mg^{-1} (dry mass) in (a) adult and (b) juvenile experiments. Note different scales on *y*-axis. Different letter codes denote significant differences (Tukey test for unequal n, $p < 0.05$)

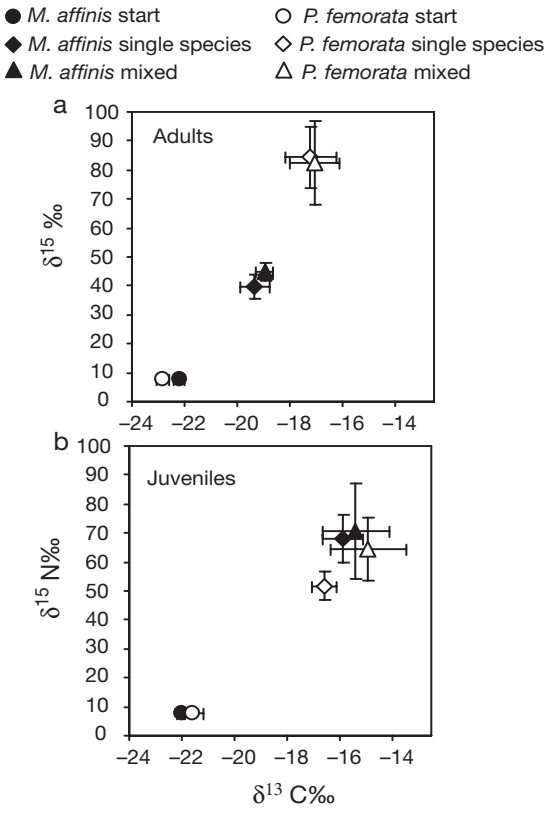

Fig. 3. *Monoporeia affinis* and *Pontoporeia femorata*. Mean $\delta^{13}C$ and $\delta^{15}N$ in *M. affinis* (filled symbols) and *P. femorata* (open symbols) in (a) adult and (b) juvenile experiments. The start value for juvenile *P. femorata* has been moved half a unit to the right to separate the points. Vertical and horizontal bars show ±SEM

$p < 0.05$). Single species treatments generally had higher ^{14}C uptake than mixed treatments ($p < 0.05$; Table 2), and *P. femorata* had significantly higher ^{14}C uptake in single species than in mixed treatments (Tukey HSD for unequal n, $p < 0.05$; Fig. 2). On the other hand, *P. femorata* took up significantly more of both ^{13}C and ^{15}N than *M. affinis* (^{13}C: $p < 0.05$; ^{15}N: $p < 0.001$; Table 2), demonstrating more feeding on old sediment, and greater uptake of both carbon and nitrogen from the aged phytodetritus. Both treatments of *P. femorata* differed in $\delta^{15}N$ from both treatments of *M. affinis* (Tukey HSD for unequal n, $p < 0.05$; Fig. 3).

Table 3. $\delta^{13}C$, $\delta^{15}N$ and C:N ratio for ^{14}C-labelled algae and sediment. Values are mean ± SD

Variable	Algae	New sediment	Old sediment
$\delta^{13}C$	−29.5 ± 0.1	−24.3 ± 0.3	0.5 ± 0.2
$\delta^{15}N$	2.9 ± 0.5	5.4 ± 0.4	266 ± 3
C:N	5.21 ± 0.05	6.92 ± 0.43	6.98 ± 0.03

The analysis of the stable isotopes ^{13}C and ^{15}N in cultured, stable-isotope-labelled algae, in new sediment and in old sediment to which the labelled algae had been added before aging for 1 yr (Table 3), demonstrated that strong labelling had been achieved. This allowed the proportion of new amphipod somatic growth (assumed equal to biomass increase), which was based on uptake from new and old sediment, to be estimated, given some assumptions. The mass of added fresh diatoms was not measured, but could be roughly estimated to be 0.14 g C m^{-2} from an earlier identical culture (Byrén et al. 2002). Based on the ^{14}C uptake, carbon from fresh algae would then account for only 0.8% of increase in mass in *Monoporeia affinis* and 0.2% in *Pontoporeia femorata*, and could be ignored in further calculations. Separate 2 source mixing models for ^{13}C and ^{15}N were used to estimate uptake from the 2 remaining sources, new and old sediment. The $\delta^{13}C$ of new somatic growth was calculated as:

$$\delta^{13}C_{prod} = \frac{(Tot\ C_{end} \times \delta^{13}C_{end}) - (Tot\ C_{start} \times \delta^{13}C_{start})}{(Tot\ C_{end} \times \delta^{13}C_{start})} \quad (3)$$

and analogously for $\delta^{15}N$. The isotopic proportions from new (f_A) and old (f_B) sediment were calculated from:

$$f_A = \frac{\bar{\delta}_M - \bar{\delta}_B}{\bar{\delta}_A - \bar{\delta}_B} \quad and \quad f_A = 1 - f_A \qquad (4)$$

where $\bar{\delta}_M$ is the isotopic signature of the new somatic mass produced (mean of single species and mixed treatments), and $\bar{\delta}_A$ and $\bar{\delta}_B$ the signatures for new (A) and old (B) sediment. Results are given in Table 4.

Juvenile amphipod experiment

Survival was 77 to 83%, with no significant difference between species or treatments (2-factor ANOVA, $p > 0.05$; Table 1). There were no significant differences between species in C:N ratio, but there was a significant effect among treatments (2-factor ANOVA, $F_{2,31} = 4.42$, $p < 0.05$), with a decrease in mixed treatments during the experiment (Tukey HSD for unequal n, $p < 0.05$; Fig.1).

A treatment effect was found for ^{14}C uptake, which was higher in single species treatments than in mixed ones ($p < 0.05$; Table 5), with *Monoporeia affinis* in single species treatments having significantly higher ^{14}C uptake than *Pontoporeia femorata* in mixed treatments (Tukey HSD for unequal n, $p < 0.05$; Fig. 2). There were no significant differences in uptake of ^{13}C or ^{15}N between species or treatments (2-factor ANOVA, $p > 0.05$; Fig. 3).

Table 4. *Monoporeia affinis* and *Pontoporeia femorata.* Proportion new body mass, calculated from ^{13}C and ^{15}N values from the 2 sources, new (f_A) and old (f_B) sediment

	f_A	f_B
$\delta^{13}C$		
Monoporeia affinis	0.54	0.46
Pontoporeia femorata	0.14	0.86
$\delta^{15}N$		
Monoporeia affinis	0.59	0.41
Pontoporeia femorata	0.00	1.00

Table 5. *Monoporeia affinis* and *Pontoporeia femorata.* Two-factor ANOVA results for the juvenile experiment. ns: not significant

Variable Factor	df_{effect}	df_{error}	*F*	p-value
^{14}C Species	1	24	2.53	ns
Treatment (single species/mixed)	1	24	5.73	<0.05
Species × Treatment	1	24	<0.001	ns

DISCUSSION

This study found clear differences in preferred food source between adults of *Monoporeia affinis* and *Pontoporeia femorata*. The 5-fold higher ^{14}C uptake by adult *M. affinis* compared with adult *P. femorata* demonstrates a preference for surface feeding on fresh material by *M. affinis*, in agreement with previous experimental studies (van de Bund et al. 2001, Byrén et al. 2002). The uptake of both $\delta^{13}C$ and $\delta^{15}N$, which demonstrates subsurface feeding on aged phytodetritus, was significantly greater in *P. femorata* than in *M. affinis*, indicating that the former relies more on old organic material found at depth in the sediment. This older material is probably more refractory than the fresh organic material settling on the sediment surface. These results confirm that adult *P. femorata* prefer to feed at depth in the sediment, as found by Byrén et al. (2002), but goes further in demonstrating uptake of both carbon and nitrogen from aged organic material from the deeper sediment layer. The old sediment contained live meiofauna (e.g. nematodes and ostracods) even after a year of aging, which demontrates the presence of utilisable organic matter. We still assume that the older sediment had lower food quality than the fresh algae added on top of the sediment, as indicated by its much lower relative organic matter content, and its higher C:N ratio (7.0 vs 5.2). The estimated C:N ratio in the labelled fraction of the old sediment was extremely low, 2.2. This low value may be influenced by remaining inorganic nitrogen (ammonium adsorbed to clay particles), whereas inorganic carbon is lost as carbon dioxide upon drying the sediment for combustion analyses. The labelled fraction added to the old sediment was very small, and thus hardly influenced its C:N ratio.

In both adult and juvenile experiments a treatment effect was found for ^{14}C uptake, with higher uptake in single species than in mixed treatments. This may indicate direct competition for food at higher density, as reported in previous experimental studies (Elmgren et al. 2001, van de Bund et al. 2001), rather than a crowding effect causing interference with feeding. No indication of competition was found at similar amphipod densities in a study where fresh ^{14}C-labelled algae had been mixed down into the sediment (Byrén et al. 2002), and both amphipod species had about the same uptake from subsurface feeding. The present study added no fresh material to the subsurface sediment, from which *Pontoporeia femorata* took up significantly more ^{13}C and ^{15}N than *Monoporeia affinis*. The 2 studies thus differ in terms of age and probable food quality of the subsurface sediment, which could partly explain the interspecific difference in subsurface feeding. That *M. affinis* took up significantly less label than *P. femorata*

from old subsurface sediment in this study, but had similar uptake when both species were offered subsurface sediment amended with fresh algae (Byrén et al. 2002), indicates feeding plasticity in *M. affinis*.

The calculation of carbon and nitrogen uptake from new and old sediment assumes that only the added biomass is newly assimilated material. In reality there is also some turnover of old body material, which results in a slight overestimation of the importance of old sediment. Still, the carbon uptake estimated from ^{13}C seems reasonable, with similar proportions from new (0.54) and old sediment (0.46) in *Monoporeia affinis*, and a preponderance of old sediment (0.86) over new (0.14) in *Pontoporeia femorata*. The nitrogen source apportioning calculated using ^{15}N is similar to that for carbon for *M. affinis* (0.59 new, 0.41 old), but suggests that all newly assimilated nitrogen in *P. femorata* comes from old sediment, which is unlikely, since carbon isotopes indicate some feeding on new sediment.

For both species, the adult C:N ratios resembled those found in nature and did not change during the experiment. A range from 3.7 at the end of winter to a peak of 11 in late summer has been found for *Monoporeia affinis* in the field (Lehtonen 1996, Cederwall & Jermakovs 1999). Adult *Pontoporeia femorata* had a higher C:N ratio than adult *M. affinis*. This is in agreement with the higher lipid content reported by Hill et al. (1992) for this time of the year. In these amphipods, Lehtonen (1996) found a strong positive correlation between C:N ratios and the content of lipids, the major energy storage compounds in both species (Hill et al. 1992). Feeding on old sediment with a high C:N ratio will give adult *P. femorata* proportionally more carbon to store as lipids. This is in agreement with Hill et al. (1992), who found a higher and less variable C:N ratio in *P. femorata* than in *M. affinis*. During winter starvation, lipid stores are more quickly depleted in *M. affinis* than in *P. femorata* (Hill et al. 1992), as expected from its higher rate of metabolism (Cederwall 1979).

There was no clear difference in isotope uptake and hence probably little or no difference in feeding depth between juveniles of the 2 species. The adult and juvenile ^{14}C uptake experiments allow a rough comparison of daily carbon uptake from fresh algae by adult and juvenile amphipods, in spite of differences in the amount of ^{14}C added, individual densities, and experimental jar size (Fig. 4). Since there were no significant differences between treatments within species, all values are pooled. Juveniles had 5-fold higher ^{14}C uptake than adults of *Monoporeia affinis*, and 17 times higher in *Pontoporeia femorata*. A smaller animal has a higher metabolism per unit body mass (Peters 1983), but these differences in ^{14}C uptake are too large to be fully explained by size/metabolism allometry, and also suggest different feeding strategies. Thus, it is clear that *P. femorata* juveniles feed more on surface sediment than adults (Fig. 4), and it is also likely that juvenile *M. affinis* feed more on subsurface sediment than conspecific adults (Fig. 3). In the field, surface feeding by juvenile *P. femorata* in the period during and after the sedimentation of the phytoplankton spring bloom should give them a higher rate of intake of fresh organic matter, and presumably a faster growth rate, than the deep feeding strategy of the adults would allow. Since proteins are essential for growth, feeding on surface sediments with fresh sedimented algae with low C:N ratios could yield proportionally more nitrogen which can be used for growth. This could partly explain the feeding patterns of the juveniles, for which a good strategy would be to grow large as quickly as possible. As in adults, juvenile isotope uptake was lower at the higher density of the mixed treatments, suggesting competition for food. The C:N ratio decreased significantly (4.7 to 4.2) only for juveniles in the mixed treatments, another indication of competition for food.

CONCLUSIONS

Several studies have indicated clear differences among benthic deposit-feeders in the use of fresh, newly deposited organic material and older organic matter in the sediment (Rudnick & Oviatt 1986, Rudnick 1989, Widbom & Frithsen 1995, Ólafsson & Elmgren 1997, Byrén et al. 2002). This study is the first to test such differences in controlled laboratory experiments, with both old and new organic material isotopically labelled.

In summary, this study shows that juveniles of both amphipod species studied have similar feeding patterns, and act like generalists in utilising both fresh

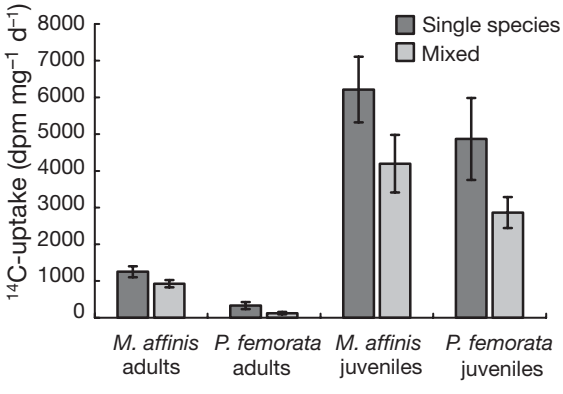

Fig 4. *Monoporeia affinis* and *Pontoporeia femorata.* Mean (±SE) uptake of ^{14}C dry mass in adults and juveniles, both experiments compared. The ^{14}C uptake in the juvenile experiment was recalculated to the same ^{14}C addition cm^{-2} as in the adult experiment

surface organic material, and deeper, older sediment. Both species also recruit at the same time in spring, which suggests that they could potentially compete for food. Such competition is, however, likely less in late spring–early summer, when abundant new organic matter from the recently settled spring bloom is still available in the surface sediment (Bianchi et al. 2002). Later in their life, *Pontoporeia femorata* and *Monoporeia affinis* become more specialised subsurface and surface deposit-feeders, respectively. Adult *P. femorata* rely primarily on older, more deeply burrowed organic material, as suggested by Byrén et al. (2002), whereas adult *M. affinis* feed at a higher rate and use freshly deposited organic matter, normally available primarily at the sediment surface. Feeding at the sediment surface will ensure *M. affinis* immediate access to newly deposited phytodetritus and hence promote rapid growth, but will also increase vulnerability to predators active on or above the sediment surface. By feeding on subsurface food resources of generally lower quality, adult *P. femorata* will probably decrease their risk of predation, at the price of a reduced intake of food, a strategy made possible by their lower respiration rate (Cederwall 1979).

The combination of several isotopic tracers proved useful for experimental testing of differences in depth of feeding and in utilisation of food of different origin. In future studies, this should be useful for differentiating between use of organic matter of different ages or from different sources, also when these are thoroughly mixed.

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LITERATURE CITED

Andersen FØ (1996) Fate of organic carbon added as diatom cells to oxic and anoxic marine sediment microcosms. Mar Ecol Prog Ser 134:225–233

Ankar S, Elmgren R (1976) The benthic macro- and meiofauna of the Askö-Landsort area (northern Baltic proper). A stratified random sampling survey. Contrib Askö Lab Univ Stockholm 11:1–115

Blomqvist S, Lundgren L (1996) A benthic sled for sampling soft bottoms. Helgol Meeresunters 50:453–456

Bianchi T S, Johansson B, Elmgren R (2000) Breakdown of phytoplankton pigments in Baltic sediments: effects of anoxia and loss of deposit-feeding macrofauna. J Exp Mar Biol Ecol 251:161–183

Bianchi TS, Rolff C, Widbom B, Elmgren R. (2002) Phytoplankton pigments in Baltic seston and sediments: seasonal variability, fluxes and transformations. Estuar Coast Shelf Sci 55:369–383

Byrén L, Ejdung G, Elmgren R (2002) Comparing rate and depth of feeding in benthic deposit-feeders: a test on two amphipods, *Monoporeia affinis* (Lindström) and *Pontoporeia femorata* Kröyer. J Exp Mar Biol Ecol 281:109–121

Cederwall H (1977) Annual macrofauna production of a soft bottom in the northern Baltic proper. In: Keegan BF, Céidigh PÒ, Boaden PJS (eds) Biology of benthic organisms. Pergamon Press, Oxford, p 155–164

Cederwall H (1979) Diurnal oxygen consumption and activity of two *Pontoporeia* (Amphipoda, Crustacea) species. In: Naylor E, Hartnoll RG (eds) Cyclic phenomena in marine plants and animals. Pergamon Press, Oxford, p 309–316

Cederwall H (1999) Bottenvatten och mjukbottenfauna (English summary: bottom water and soft bottom fauna). Östersjö '98. Swedish Marine Monitoring in the Baltic Proper Annual Report. Stockholm Mar Res Centre, Stockholm University, p 11–14

Cederwall H, Jermakovs V (1999) Growth and production of three macrozoobenthic species in the Gulf of Riga, including comparisons with other areas. Hydrobiologia 393:201–210

Ejdung G, Byrén L, Elmgren R (2000) Benthic predator-prey interactions: evidence that adult *Monoporeia affinis* (Amphipoda) eat postlarval *Macoma balthica* (Bivalvia). J Exp Mar Biol Ecol 253:243–251

Elmgren R (1978) Structure and dynamics of Baltic benthos communities, with particular reference to the relationship between macro- and meiofauna. Kiel Meeresforsch Sonderh 4:1–22

Elmgren R, Ankar S, Marteleur B, Ejdung G (1986) Adult interference with postlarvae in soft sediments: the *Pontoporeia-Macoma* example. Ecology 67:827–836

Elmgren R, Ejdung G, Ankar S (2001) Intraspecific food competition in the deposit-feeding benthic amphipod *Monoporeia affinis* — a laboratory study. Mar Ecol Prog Ser 210: 185–193

Fry B, Sherr EB (1984) Delta ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib Mar Sci 27:13–47

Goedkoop W, Johnson RK (1996) Pelagic-benthic coupling: Profundal benthic community response to spring diatom deposition in mesotrophic Lake Erken. Limnol Oceanogr 41:636–647

Goedkoop W, Gullberg KR, Johnson RK, Ahlgren I (1997) Microbial response of a freshwater benthic community to a simulated event: interactive effects of benthic fauna. Microb Ecol 34:131–143

Graf G (1987) Benthic energy flow during a simulated autumn bloom sedimentation. Mar Ecol Prog Ser 39:23–29

Guiguer KRRA, Barton DR (2002) The trophic role of Diporeia (Amphipoda) in Colpoys Bay (Georgian Bay) benthic food web: a stable isotope approach. J Gt Lakes Res 28: 228–239

Guillard RL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum Press, New York, p 26–60

Gullberg KR, Goedkoop W, Johnson RK (1997) The fate of diatom carbon within a freshwater benthic community — a microcosm study. Limnol Oceanogr 42:452–460

Hansen LS, Blackburn TH (1992) Effect of algal bloom deposition on sediment respiration and fluxes. Mar Biol 112: 147–152

Hill C, Elmgren R (1987) Vertical distribution in the sediment in the co-occurring benthic amphipods *Pontoporeia affinis* and *P. femorata*. Oikos 49:221–229

Hill C, Quigley MA, Cavaletto JF, Gordon W (1992) Seasonal changes in lipid content and composition in the benthic amphipods *Monoporeia affinis* and *Pontoporeia femorata*. Limnol Oceanogr 37:1280–1289

Järvekülg A (1973) Distribution and ecology of local populations of benthic glacial relicts. Oikos(Suppl) 15:91–97

Kester DR, Duedall IW, Connors DN, Pytkovocz RM (1967) Preparation of artificial seawater. Limnol Oceanogr 12: 176–179

Laine AO, Sandler H, Andersin AB, Stigzelius J (1997) Long-term changes of macrozoobenthos in the Eastern Gotland Basin and the Gulf of Finland (Baltic Sea) in relation to the hydrographical regime. J Sea Res 38:135–159

Lehtonen K (1996) Ecophysiology of the benthic amphipod *Monoporeia affinis* in an open-sea area of the northern Baltic Sea: seasonal variations in body composition, with bioenergetic considerations. Mar Ecol Prog Ser 143:87–98

Lehtonen K, Andersin AB (1998) Population dynamics, response to sedimentation and role in benthic metabolism of the amphipod *Monoporeia affinis* in an open-sea area of the northern Baltic Sea. Mar Ecol Prog Ser 168:71–85

Levin LA, Blair NE, Martin CM, DeMaster DJ, Plaia G, Thomas CJ (1999) Macrofaunal processing of phytodetritus at two sites on the Carolina margin: *in situ* experiments using ^{13}C-labeled diatoms. Mar Ecol Prog Ser 182: 37–54

Levinton JS, Bianchi TS (1981) Nutrition and food limitation of deposit-feeders. I. The role of microbes in the growth of mud snails (Hydrobiidae). J Mar Res 39:531–545

Lopez GR, Crenshew MA (1982) Radiolabelling of sedimentary organic matter with ^{14}C-formaldehyde: Preliminary evaluation of a new technique for use in deposit-feeding studies. Mar Ecol Prog Ser 8:283–289

Lopez GR, Elmgren R (1989) Feeding depths and organic absorption for the deposit-feeding benthic amphipods *Pontoporeia affinis* and *Pontoporeia femorata*. Limnol Oceanogr 34:982–991

Ólafsson E, Elmgren R (1997) Seasonal dynamics of sublittoral meiobenthos in relation to phytoplankton sedimentation in the Baltic Sea. Estuar Coast Shelf Sci 45:149–164

Owens, NJP (1987) Natural variations in ^{15}N in the marine environment. Adv Mar Biol 24:389–451

Peters RH (1983) The ecological implications of body size. Cambridge University Press, Cambridge

Preston NP, Smith DM, Kellaway DM, Bunn SE (1996) The use of enriched ^{15}N as an indicator of the assimilation of individual protein sources from compound diets for juvenile *Penaeus monodon*. Aquaculture 147:249–259

Rudnick DT (1989) Time lags between the deposition and meiobenthic assimilation of phytodetritus. Mar Ecol Prog Ser 50:231–240

Rudnick DT, Oviatt CA (1986) Seasonal lags between organic carbon deposition and mineralization in marine sediments. J Mar Res 44:815–837

Segerstråle SG (1950) The amphipods on the coasts of Finland — some facts and problems. Soc Sci Fenn Commentat Biol X 14:2–28

Uitto A, Sarvala J (1990). Perspectives on the ecological factors regulating *Pontoporeia* populations in the northern Baltic Sea. Ann Zool Fenn 27:297–301

Uitto A, Sarvala J (1991) Seasonal growth of the benthic amphipods *Pontoporeia affinis* and *P. femorata* in a Balthic archipelago in relation to environmental factors. Mar Biol 111:237–246

van de Bund WJ, Olafsson E, Modig H, Elmgren R (2001) Effects of the coexisting Baltic amphipods *Monoporeia affinis* and *Pontoporeia femorata* on the fate of a simulated spring diatom bloom. Mar Ecol Prog Ser 212:107–115

Widbom B, Frithsen JB (1995) Structuring factors in a marine soft bottom community during eutrophication æ an experiment with radio-labelled phytodetritus. Oecologia 101: 156–168

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