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Autochthonous and allochthonous contributions to mesozooplankton diet in a tidal river and estuary: Integrating carbon isotope and fatty acid constraints

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Abstract

We examined the carbon sources used by bacteria and mesozooplankton in the Scheldt River and estuary (Belgium, The Netherlands) using a combined stable isotope and fatty acid composition approach. Water samples were collected monthly at six stations during 1 yr and analyzed for ^{13}C of dissolved inorganic carbon, dissolved organic carbon (DOC), and particulate organic carbon (POC). Mesozooplankton was determined up to family, genus, or species level and analyzed for ^{13}C and fatty acid content. Suspended particles were analyzed for phospholipid fatty acids and their ^{13}C contents to estimate isotope ratios of phytoplankton groups and heterotrophic bacteria. The carbon isotope signatures of DOC, POC, and bacterial biomass were similar and significantly enriched relative to those of diatoms and green algae, pointing to allochthonous subsidies as an important carbon source for bacteria. The contribution of algae to zooplankton diets as estimated from isotope ratios and fatty acid profiles averaged 41% and 75% respectively, and did not differ significantly among stations, taxa, or age categories. Mesozooplankton relies primarily on grazing on phytoplankton and direct consumption of particulate organic matter. Mesozooplankton appears to receive little of its carbon from DOC via bacteria.

Tidal rivers and estuaries are heterotrophic ecosystems where local primary production is less than community respiration. This heterotrophy is sustained by upstream and lateral supply of detrital (often terrestrial) resources in the form of dissolved organic carbon (DOC) and particulate organic carbon (POC). These systems are therefore characterized by oxygen depletion and high carbon dioxide pressures and emissions (Frankignoulle et al. 1998), and by high rates of bacterial secondary production and respiration (Heip et al. 1995; Del Giorgio and Pace 2008). The availability of two distinct sources of carbon to consumers (local primary production and external subsidies) allows uncoupling of primary and secondary production and has major consequences for food web dynamics (Pace et al. 2004). Comparison of seasonal dynamics and of rates of primary and bacterial secondary production has revealed that bacteria in tidal rivers and estuaries primarily depend on external subsidies (Findlay et al. 1991; Goosen et al. 1997). Moreover, carbon isotope characterization of bacteria, phytoplankton, and detritus has provided independent confirmation that external detrital carbon inputs support bacterial growth in tidal rivers and estuaries (Boschker et al. 2005). Together with mass balance studies (Soetaert and Herman 1995; Del Giorgio and Pace 2008), these collective findings have resulted in the view that aquatic community metabolism and bacterial secondary production in these systems are primarily governed by external carbon supply. However, the importance of detrital resources to pelagic metazoan consumers is still a subject of discussion.

A number of studies have documented that even in tidal rivers and estuaries with high detritus to phytoplankton ratios, phytoplankton carbon governs zooplankton dynamics because of nutritional quality aspects and selective feeding (Müller-Solger et al. 2002; Sobczak et al. 2005). Consistently, stable isotopes studies have revealed strong selectivity of metazoan consumers and decoupling of metazoan and bulk detritus dynamics (Martineau et al. 2004; Delong and Thorp 2006). However, mass-balance considerations and ecosystem modeling have shown that mesozooplankton in estuaries has to consume detrital carbon in order to obtain sufficient energy (Escaravage and Soetaert 1995).

Limnologists have extensively investigated the relative importance of allochthonous vs. autochthonous carbon resources for lacustrine food webs using stable isotopes at natural abundance (Grey et al. 2001) as well as deliberate tracers (Pace et al. 2004). These studies revealed major but variable terrestrial support of lake food webs. The variable contributions of external resources to zooplankton could be attributed to differences in the trophic status of the lakes (Cole et al. 2006).

Stable isotope studies of estuarine organic matter have revealed multiple organic matter sources that vary spatially as well as seasonally (Middelburg and Herman 2007).

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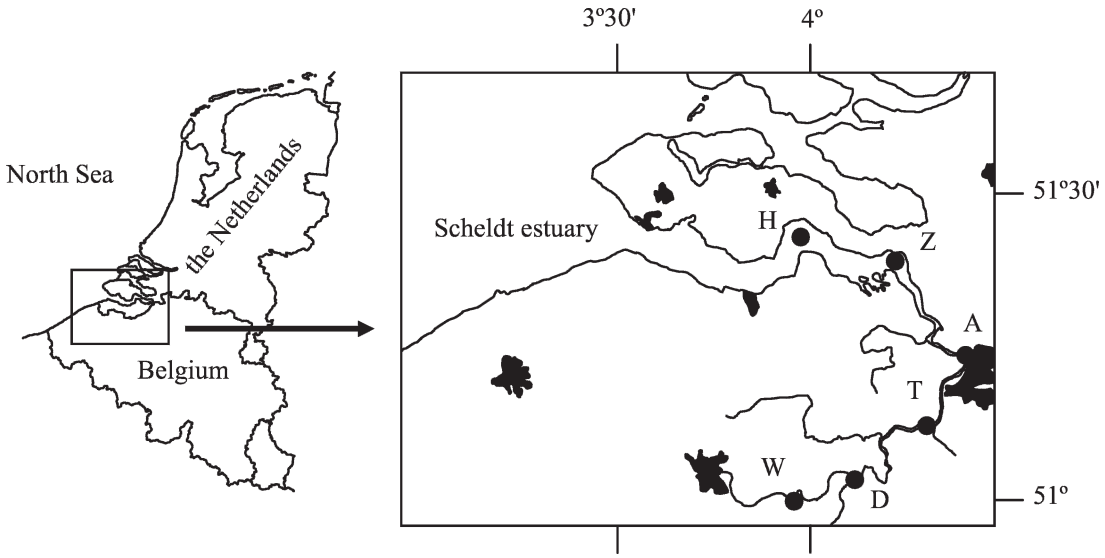

Fig. 1. The Scheldt estuary and tidal river with six sampling stations (H = Hansweert, Z = Zandvliet, A = Antwerpen, T = Temse, D = Dendermonde, and W = Wetteren).

Heterotrophic consumers are considered to reflect the carbon isotope signature of their diet, and it is therefore pivotal to resolve the isotopic signature of the primary food sources. For zooplankton this implies determining at least the isotopic signature of phytoplankton, heterotrophic bacteria, and detritus.

This poses a major challenge in turbid rivers and estuaries, and most studies therefore compare the isotope signatures of zooplankton with those of bulk particulate organic matter or size classes (Kerner et al. 2004; Martineau et al. 2004). Overlapping size classes and aggregated particles complicate physical separation of algae from bacteria and detritus. Estimation of phytoplankton end-member values from the isotope composition of dissolved inorganic carbon (DIC) and isotope fractionation factors is also associated with uncertainty, because fractionation is variable and not well known for natural communities (Pace et al. 2004; Cole et al. 2006). Compound-specific isotope analysis of biomarkers, molecules specific for certain groups of organisms, now makes it possible to resolve the isotopic composition of bacteria and phytoplankton (Boschker and Middelburg 2002; Van den Meersche et al. 2004; Boschker et al. 2005). If the difference in carbon isotope signatures of phytoplankton, bacteria, and detritus is large enough, it is possible to trace how much of zooplankton carbon comes from these various sources.

However, stable carbon isotope analysis allows assessment only of the origin of incorporated carbon, and it usually does not resolve the pathway via which carbon ends up in mesozooplankton: e.g., direct uptake of POC cannot be distinguished from indirect uptake via bacteria or microzooplankton. Such a distinction is necessary because detritus enters aquatic ecosystems in the form of DOC and POC. Allochthonous support of metazoans by DOC involves uptake by bacteria and subsequent transfer up in the food web (Cole et al. 2006). To distinguish between direct and indirect dependence of zooplankton on detrital resources, one either has to combine deliberate tracer experiments with models (Cole et al. 2006) or use additional techniques that provide complementary information. Fatty acid compositions have been used extensively as trophic markers to infer the diet of metazoans (Dalsgaard et al. 2003).

In this paper, we report on the carbon transfers among algae, bacteria, detritus, and mesozooplankton in the turbid, tidal Scheldt River and estuary. The carbon isotope composition of DIC, DOC, POC, diatoms, green algae, heterotrophic bacteria, and zooplankton will be used to estimate the terrestrial subsidy of riverine and estuarine plankton communities. Through the combination of stable isotope analysis and fatty acid profiling of zooplankton, we aim to characterize the importance of and interactions among the herbivorous, bacterial, and detrital pathways. Moreover, we report a full seasonal cycle at six stations, because phytoplankton production and zooplankton communities and diets may vary over the season and within the system.

Methods

Study site and sampling—The river Scheldt has a length of 350 km and a catchment area of ~22,000 km^2, which consists mainly of alluvial plains and has more than 10 million inhabitants in Northern France, Belgium, and the southwest Netherlands. The Scheldt can be divided into the nontidal upper Scheldt and a tidal portion (170 km) that extends from Vlissingen near the mouth to Ghent where sluices stop the tidal influence. This study covers the tidal river and upper part of the Scheldt estuary (Fig. 1). The tidal Scheldt is shallow (7–14 m), generally well-mixed, but turbid because of high suspended matter concentrations maintained by tidal mixing. As a consequence the photic zone is shallow and primary production is light-limited in this nutrient-rich system. Nevertheless, algal biomass can build up to high levels (Muylaert et al. 2000).

The Scheldt River and estuary receive nutrients and organic matter from upper Scheldt and Rupel drainages as well as from lateral sources (Soetaert et al. 2006). The

Scheldt is a highly heterotrophic system characterized by oxygen undersaturation and carbon dioxide supersaturation (Frankignoulle et al. 1998) and high rates of respiration and bacterial secondary production (Boschker et al. 2005).

From February to October 2005, six stations were sampled monthly down the tidal river: from river stations Wetteren (salinity [S] < 1) and Dendermonde ($S < 1$) via upper estuary stations Temse ($S = 0.5$–3) and Antwerpen ($S = 1.5$–7.5) to euhaline stations Zandvliet ($S = 5$–15) and Hansweert ($S = 15$–20). Surface water (upper 1 m) in the downstream stations was sampled with a 20-liter Niskin bottle from the ship RV *Luctor*. Water from that same depth was pumped up and mesozooplankton was collected from it using mesh sizes of 200 μm (adults) and 50 μm (juveniles). Mesozooplankton was defined as animals with a size between 50 μm and 2 mm, and consisted almost exclusively of small crustaceans from the groups of Copepoda and Cladocera. In the riverine stations Wetteren and Dendermonde, samples were taken from a dock and a pontoon, respectively. Nets were deployed to collect mesozooplankton directly from the water, using the same mesh sizes used on board the ship.

Sample analysis—Suspended particulate matter (SPM) was obtained by filtering ~1 liter over preweighed and precombusted GF/F filters (nominal hole size approximately 0.7 μm), and dried at 60°C. These samples were decarbonated within silver cups and then analyzed for $\delta^{13}C$ using a Carlo Erba elemental analyzer (EA) coupled online to a Finnigan Delta S isotope ratio mass spectrometer (IRMS). Samples for DIC and $\delta^{13}C$-DIC were collected in 50-mL-headspace vials using an overflow technique and preserved with mercury chloride. In the laboratory, a He headspace was created and the concentration and isotopic composition of carbon dioxide in the headspace was measured using an EA coupled to a Finnigan Delta XL IRMS (Moodley et al. 2000). Samples for DOC (20 mL) were filtered through precombusted GF/F filters and stored frozen. DIC was removed by adding sulfuric acid in excess and subsequently stripping with helium. DOC samples were analyzed with a Skalar Formacs LT total organic carbon analyzer coupled through a ConFlo II interface to a Finnigan Delta S IRMS. The calibration standards were prepared with potassium phthalate dissolved in purified water (MilliQ). Chlorophyll *a* (Chl *a*) and additional pigments were analyzed by high-performance liquid chromatography (Van den Meersche et al. 2004). Chl *a* and SPM concentration data for tidal river and upper estuarine stations were kindly provided by Tom Maris of the University of Antwerpen.

About 2 liters of water was filtered through GF/F filters for subsequent measurement of phospholipid fatty acid (PLFA) concentration and isotopic composition. Briefly, lipids were extracted in chloroform-methanol-water using a modified Bligh and Dyer method, and part of the extract was fractionated on silicic acid into different polarity classes. The total fatty acid and PLFA fractions were derivatized to fatty acid methyl esters, and concentrations were determined by gas chromatograph-flame ionization detection. The $\delta^{13}C$ of individual PLFAs was measured using gas chromatography-combustion IRMS (Middelburg et al. 2000; Van den Meersche et al. 2004).

Mesozooplankton samples were transferred to the freezer immediately upon harvesting. They were subsequently rinsed twice in pure, deionized water, sorted per species and per development stage, transferred to tin cups, and dried at 60°C. Around five adult copepods were combined per tin cup; more individuals were used for smaller organisms. These mesozooplankton samples were analyzed for $\delta^{13}C$. For mesozooplankton-rich samples, fractions were kept separately; a number of individuals (20 for *Eurytemora* and *Eudiaptomus*, 30–100 for *Acartia* and *Daphnia*) were cleaned following the same procedure as for isotope analysis, and subsequently analyzed for total fatty acid content (as described above).

Conversions and estimates—Isotope ratios of algae and bacteria were estimated by averaging group-specific PLFA using the relative concentrations as weights. The use of PLFA for microbial taxonomy has been well established in the Scheldt estuary (Middelburg et al. 2000; Boschker et al. 2005; Dijkman and Kromkamp 2006). The branched PLFA i14:0, i15:0, ai15:0, and i16:0 are specific for bacteria, and their weighted average $\delta^{13}C$, using PLFA concentrations as weights, is used as a proxy for bacterial $\delta^{13}C$ ($\delta^{13}C_{bact}$; Boschker and Middelburg 2002). A fractionation of 3‰ (Hayes 2001) was assumed between PLFA and whole-cell signatures. The polyunsaturated PLFA 16:2ω4, 16:3ω4, 20:5ω3, and 22:6ω3 occur predominantly in diatoms, dinophytes, and haptophytes, and are further referred to as "diatoms," whereas the 16:3ω3, 16:4ω3, and 18:3ω3 occur primarily in chlorophytes and cryptophytes, and are further referred to as "green algae" (Dijkman and Kromkamp 2006). The isotopic composition of the total algal pool ($\delta^{13}C_{algae}$) was estimated by weighting the $\delta^{13}C$ values of algal-specific fatty acids 16:2ω7, 16:2ω4, 16:3ω4, 16:3ω3, 16:4ω3, 18:3ω3, 20:0, 18:4ω3, 18:5ω3, 20:4ω6, 20:5ω3, 24:0, 22:5ω3, and 22:6ω3 with their respective concentrations.

Isotopic fractionation during primary production ($\varepsilon_{CO2\text{-}algae} = \delta^{13}C_{CO2} - \delta^{13}C_{algae}$) was calculated with $\delta_{CO2} = \delta_{DIC} + 0.1141 \times T - 10.78$ (Zhang et al. 1995), implicitly assuming that algae take up dissolved CO_2 and not bicarbonate, the bulk compound of DIC. Fractionation during mesozooplankton food assimilation was assumed to be 0‰ ± 1‰ (Vander Zanden and Rasmussen 2001), meaning that the isotopic signature of the diet equals the isotopic signature of the mesozooplankton plus or minus an error of 1‰: $\delta^{13}C_{diet} = \delta^{13}C_{mesozooplankton} \pm 1‰$.

The fraction of autochthonous carbon (algae) in mesozooplankton diet was estimated assuming that the zooplankton isotopic signature is a weighted average of its food sources, plus a fractionation factor. We distinguished two sources: autochthonous carbon (algae, with signature $\delta^{13}C_{algae}$) and allochthonous carbon (with signature $\delta^{13}C_{allochthonous}$ estimated in various ways: either equal to the signature of bacteria or detritus or at a fixed value; *see* Results). This results in the following formula for the autochthonous fraction in the mesozooplankton diet:

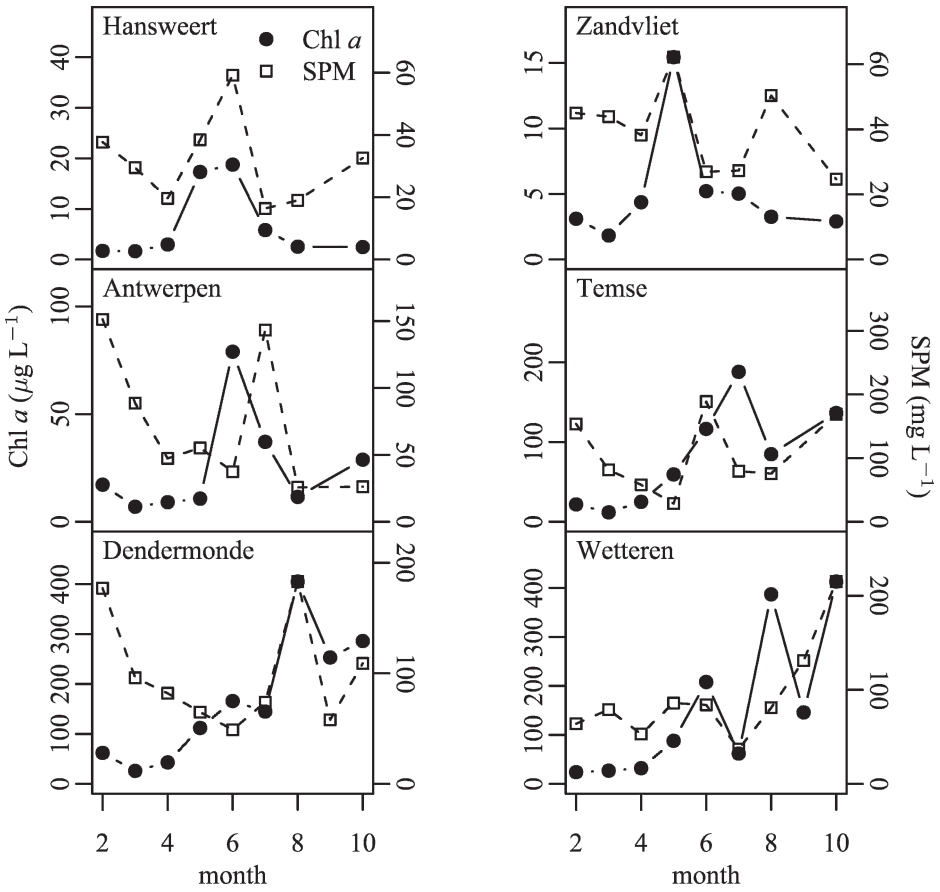

Fig. 2. Chl *a* (μg L^{-1}) and suspended particulate matter (SPM) (mg L^{-1}) concentrations in the estuary from February until October 2005. Data for Dendermonde and Wetteren were obtained from the OMES database (Tom Maris, Universiteit Antwerpen).

$$f_{autochthonous} = \frac{\delta^{13}C_{allochthonous} - \delta^{13}C_{mesozooplankton} - \phi}{\delta^{13}C_{allochthonous} - \delta^{13}C_{autochthonous}} \quad (1)$$

where ϕ accounts for fractionation during zooplankton carbon uptake.

Standard deviations of the fractions were estimated by taking random samples from normal distributions with the appropriate means and standard deviations for each $\delta^{13}C$ value, and subsequently taking means and standard deviations from the set of resulting fractions.

From mesozooplankton fatty acid profiles, we estimated the relative contribution of algae and bacteria to mesozooplankton diets using the Bayesian Compositional Estimator (Van den Meersche et al. 2008). The fatty acid compositions of six phytoplankton groups and bacteria were used as input (Dijkman and Kromkamp 2006).

Results

General estuarine features—Particulate organic matter (not shown) and SPM (Fig. 2) were highest in the upper estuary and tidal river, with maximum concentrations at Temse. Chl *a* concentrations showed clear seasonality and longitudinal gradients. The lowest concentrations were found at Zandvliet, with higher concentrations downstream and in particular upstream (Fig. 2). Maximum Chl *a* concentrations occurred in May to June in the euhaline stations (Hansweert and Zandvliet) and June to July in the upper estuary (Antwerpen and Temse). The tidal river stations (Dendermonde and Wetteren) were characterized by two phytoplankton blooms, one in early summer and one in late summer.

DIC and algal isotope signatures—The isotopic composition of DIC varied from −12.4‰ at station Temse to −3.3‰ at station Hansweert (Fig. 3) and clearly reflected mixing between a heavy marine and a depleted freshwater DIC pool. This gradual shift in $\delta^{13}C$-DIC was reflected in the $\delta^{13}C$ values of diatoms and green algae that also became about 7‰ heavier downstream (Fig. 3). The apparent isotopic fractionation ($\varepsilon_{CO2\text{-}algae}$) was consequently rather uniform over the different stations and over time. The $\delta^{13}C$ values of diatom-specific and green PLFA and thus their fractionation factors differed significantly (F = 572.23, $p < 2.2e{-}16$): $\varepsilon_{CO2\text{-}algae}$ = 15.6‰ ± 3.3‰ for diatoms and $\varepsilon_{CO2\text{-}algae}$ = 23.0‰ ± 3.7‰ for green algae (Table 1). Pigment analyses and PLFA composition analysis revealed phytoplankton communities dominated by diatoms, with green algae contributing minor fractions to the total phytoplankton carbon biomass (on average <23%, and <10% in 85% of the samples). Consequently,

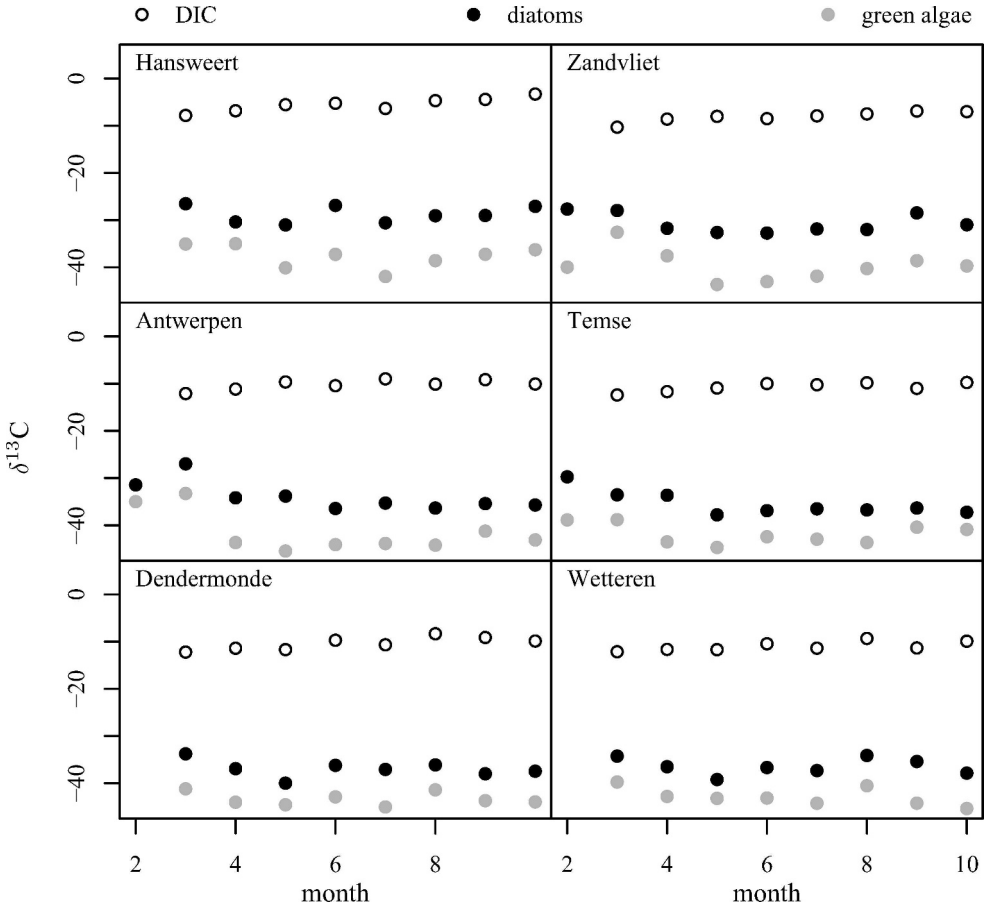

Fig. 3. Carbon stable isotope signatures of dissolved inorganic carbon (white dots), diatoms (black dots), and green algae (grey dots); $\delta^{13}C$ data are expressed in ‰.

Table 1. Carbon isotope fractionation factor for green algae and diatoms relative to carbon dioxide: $\varepsilon_{CO2\text{-}algae}=\delta^{13}C_{CO2}-\delta^{13}C_{algae}$, with $\delta_{CO2}=\delta_{DIC}+0.1141\times T-10.78$ (Zhang et al. 1995).

	Hansweert	Zandvliet	Antwerpen	Temse	Dendermonde	Wetteren
ε green algae						
Mar 05	16.8	12	10.8	16	18.6	17.2
Apr 05	18.5	19.5	23.2	22.6	23.4	21.9
May 05	25.3	26.5	26.7	24.6	23.7	22.4
Jun 05	23	25.8	24.9	23.8	24.5	24
Jul 05	27.3	25.8	26.7	24.5	26.1	24.6
Aug 05	25.3	24.3	25.6	25.4	24.6	22.7
Sep 05	24.3	23.4	23.8	21	26.2	24.5
Oct 05	24			22.2	25.2	26.6
ε diatoms						
Mar 05	8.3	7.4	4.5	10.8	11.2	11.7
Apr 05	13.9	13.7	13.7	12.7	16.3	15.6
May 05	16.2	15.5	15.1	17.7	19.2	18.4
Jun 05	12.7	15.5	17.3	18.2	17.8	17.5
Jul 05	15.9	15.8	18.1	18.1	18.2	17.7
Aug 05	15.8	16	17.8	18.5	19.3	16.3
Sep 05	16.1	13.3	17.9	17	20.5	15.7
Oct 05	14.8			18.6	18.7	19

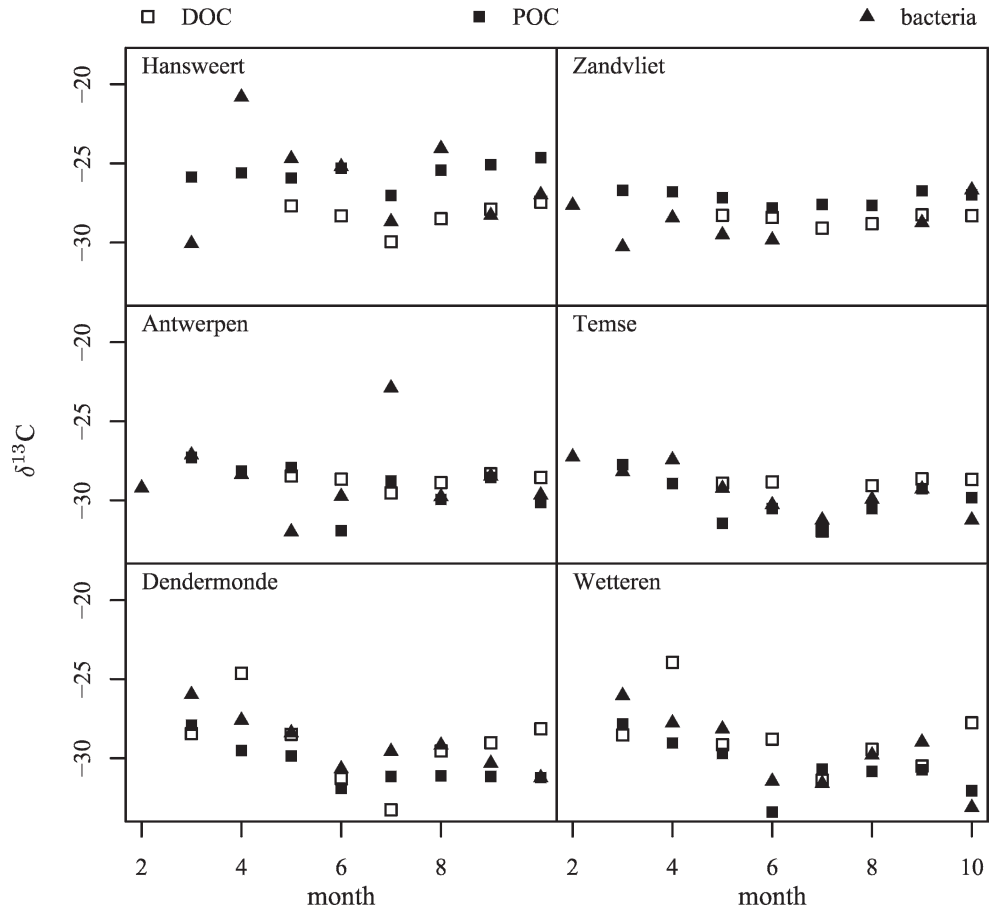

Fig. 4. Carbon stable isotope signatures of dissolved organic carbon (open square), particulate organic carbon (solid square), and heterotrophic bacteria (solid triangle); δ^{13}C data are expressed in ‰.

the total algal isotopic signature was close to that of diatoms and we will therefore use the total algal isotopic signature for further analysis.

Particulate and dissolved organic matter and bacterial isotope signatures—The isotope signatures of POC gradually increased downstream from values of −32‰ in Wetteren to −26‰ in Hansweert (Fig. 4) and were distinct from those of algae (Fig. 3). The POC comprises terrestrial organic matter and detritus from algae as well as living algae and bacteria. The more than 5‰ difference in δ^{13}C values between bulk POC and algae in the tidal river and upper estuarine stations suggests that algal material contributed little to the overall POC pool. The δ^{13}C of POC was somewhat more depleted in summer, indicating a larger contribution of phytoplankton to the POC pool during the growing season. POC and Chl *a* were positively correlated along the S gradient; average POC values were 1 mg L^{-1} in Hansweert, 1.5 mg L^{-1} in Zandvliet, 3.6 mg L^{-1} in Antwerpen, and 7 mg L^{-1} in the upstream stations, reaching values as high as 12 mg C L^{-1}. Based on a Chl *a* to C ratio of 30 μg g^{-1} (Muylaert et al. 2001), the contribution of algae to the total POC pool in the upstream stations was generally between 5% and 40%, although it reached up to 70% during phytoplankton blooms. In the downstream stations of Hansweert and Zandvliet, phytoplankton contributions remained under 25% and 10%, respectively, using the same conversion factors. These numbers should be considered rough estimates because the conversion of Chl *a* concentrations to phytoplankton carbon biomass depends on the community structure and environmental conditions (e.g., light; Cloern et al. 1995). Despite this uncertainty and accumulated errors, these estimated small contributions of living algae to total POC are consistent with the differences in δ^{13}C between algae and POC and confirm the dominance of terrestrial subsidies to POC in the upper estuary and tidal river.

The δ^{13}C of DOC closely followed that of the POC pool (Fig. 4). This means that the DOC pool was also dominated by allochthonous input. As bacterial signatures were similar to those of POC and differed from the algal signatures, this indicates that bacteria were mainly growing on allochthonous organic matter. This similarity of POC, DOC, and bacteria and their distinct enrichment relative to algal δ^{13}C suggests that the isotopic signatures of bacteria and algae can be used as reliable proxies for allochthonous and autochthonous organic matter sources, respectively. It also implies that it will not be possible to distinguish whether bacteria or other heterotrophic consumers depend on DOC or POC as their main external subsidy agent.

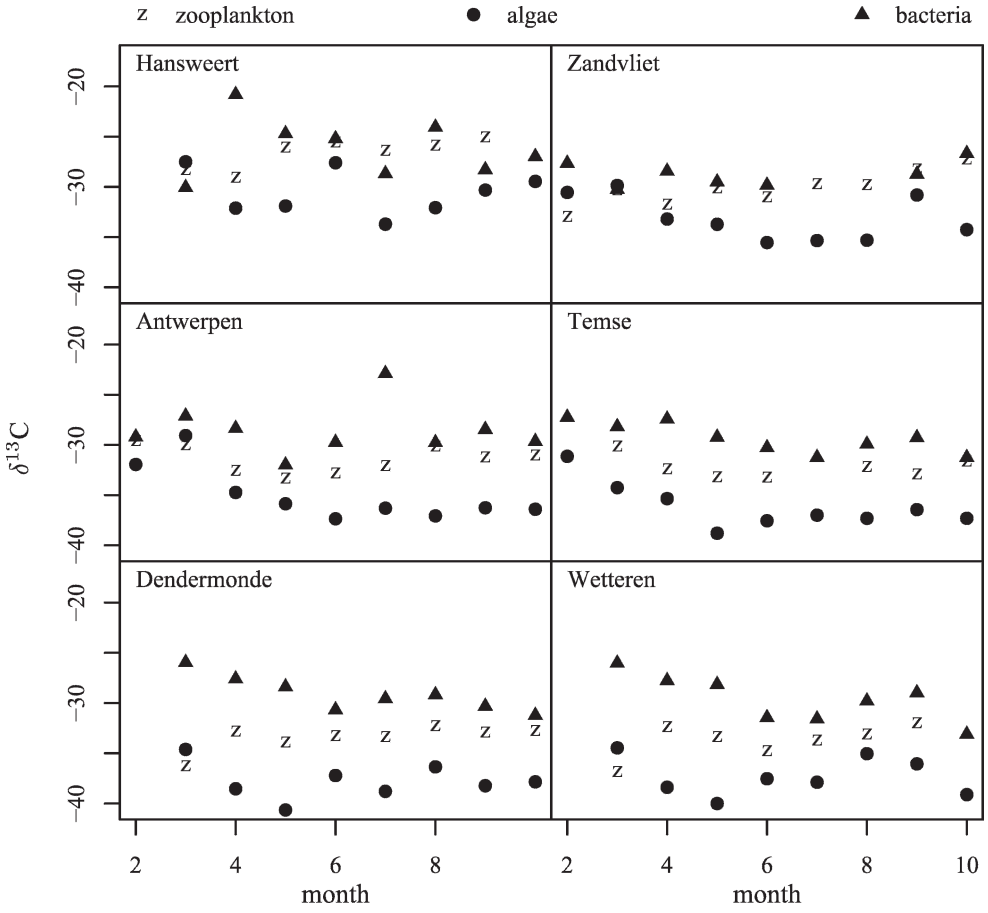

Fig. 5. Carbon stable isotope signatures of total phytoplankton (solid circles), heterotrophic bacteria (solid triangles), and mesozooplankton (z). These mesozooplankton signatures are average values combining different taxonomical groups; *see* Table 2 for mixing model results per group.

Mesozooplankton—The mesozooplankton in the euhaline and upper estuary was dominated by the calanoid copepod *Eurytemora affinis*. At the most downstream station, Hansweert, *Acartia* species were also abundant. The tidal river stations were dominated by Cladocera (genera *Daphnia* and *Bosmina* sp.), but also contained significant numbers of the calanoid copepod *Eudiaptomus* and harpacticoid copepod species.

Carbon isotope signatures of all mesozooplankton groups were in between those of algae and of bacteria, suggesting that mesozooplankton obtained carbon not only via grazing on phytoplankton, but also from allochthonous food sources (Fig. 5). Although the isotope ratio of phytoplankton can be estimated directly from algae-specific PLFA, the isotope ratio of terrestrial organic carbon is not measurable directly, because the POC and DOC pools represent mixtures of allochthonous and autochthonous contributions. We have therefore explored two approaches. First, the δ^{13}C of bacteria can be used as proxy for the δ^{13}C of terrestrial organic matter. This is based on the assumption that bacterial growth on algal exudates or algal detritus can be neglected. Bacterial δ^{13}C was estimated from bacteria-specific PLFA. Table 2 shows the results of an isotopic mixing model for different mesozooplankton groups with bacterial and algal PLFA as proxies for allochthonous and autochthonous carbon. All mesozooplankton groups showed mixed feeding behavior, with an overall mean of 41% dependence on autochthonous resources. No significant differences were found between Copepoda and Cladocera, between adults and juveniles, or among stations (ANOVA, $p > 0.05$). There was also no clear relation with Chl *a* concentrations or with fractions of algae in SPM.

An averaged 41% may seem low, and the results were therefore compared using different assumptions. The bacterial and algal carbon isotope signatures were derived from PLFA isotope values assuming a 3‰ fractionation between fatty acids and total cell. Without such a correction for intracellular isotope fractionation, we would arrive at an average algal contribution of 23% to mesozooplankton diets. Alternatively, we used δ^{13}C values for wastewater and littoral vegetation in the Scheldt that range from −25‰ to −27‰ (Middelburg and Nieuwenhuize 1998) as proxies for terrestrial organic matter and estimated average algal contributions between 56% and 51%, respectively. Clearly, all approaches indicate that autochthonous and allochthonous organic carbon contributed similarly to mesozooplankton diet in the Scheldt River and estuary and that uncertainties in allochthonous end-

Table 2. Means and standard deviations of the fractions of autochthonous carbon in the assimilated carbon of different mesozooplankton taxonomical groups, calculated using mixing models and based on stable isotope signatures of algae-specific PLFA and bacteria-specific PLFA.

	Feb 05	Mar 05	Apr 05	May 05	Jun 05	Jul 05	Aug 05	Sep 05	Oct 05
Acartia, adult									
Hansweert				0.47±0.2		0.46±0.22		0.5±0.23	
Zandvliet					0.23±0.08			0.63±0.24	0.24±0.13
Antwerpen					0.26±0.09	0.67±0.18			
Acartia, juvenile									
Hansweert				0.56±0.22		0.42±0.21		0.52±0.24	
Zandvliet					0.21±0.07			0.69±0.24	0.46±0.13
Antwerpen						0.63±0.18			
Eurytemora affinis, adult									
Zandvliet	0.64±0.31	0.4±0.26	0.77±0.16	0.28±0.15	0.12±0.07				
Antwerpen		0.68±0.23	0.7±0.1	0.37±0.17	0.36±0.14	0.65±0.18			0.2±0.11
Temse		0.19±0.12		0.36±0.07					
E. affinis, juvenile									
Zandvliet	0.58±0.34	0.36±0.25	0.61±0.18	0.28±0.14	0.31±0.12				
Antwerpen	0.06±0.05	0.86±0.11	0.69±0.13	0.52±0.19	0.47±0.07	0.68±0.17			0.3±0.11
Temse				0.47±0.08					
Cyclopoida, adult									
Temse							0.35±0.05		0.08±0.05
Dendermonde				0.3±0.04	0.38±0.08	0.4±0.14		0.38±0.11	0.22±0.05
Wetteren			0.41±0.14	0.38±0.05	0.53±0.1	0.28±0.1			
Cyclopoida, juvenile									
Dendermonde			0.35±0.06	0.44±0.05				0.32±0.12	
Wetteren			0.38±0.11	0.39±0.06		0.3±0.1			
Eudiaptomus, adult									
Wetteren				0.52±0.07					
Eudiaptomus, juvenile									
Dendermonde				0.5±0.04					
Bosmina									
Temse							0.31±0.04		
Wetteren				0.47±0.04		0.46±0.09	0.62±0.17		
Daphnia, adult									
Temse							0.26±0.05		
Dendermonde				0.48±0.07					0.26±0.07
Wetteren				0.32±0.2		0.35±0.1	0.56±0.19		
Daphnia, juvenile									
Dendermonde				0.47±0.03				0.29±0.11	
Wetteren						0.27±0.1			

member values do not affect the major outcome that mesozooplankton relied for about half of its carbon biomass on external subsidies.

Fatty acid concentrations and spectra were measured in a selection of mesozooplankton samples, and these were subsequently used in a Bayesian Compositional Estimator (Van den Meersche et al. 2008) to derive the contributions of algal and bacterial fatty acids to mesozooplankton (Table 3). Estimated algal contributions to mesozooplankton fatty acid composition varied from 15% to 95%, with an overall average of 75% and a median of 81%. Figure 6 compares the contribution of algae to mesozooplankton diet as derived from stable isotope and fatty acids analyses. It is clear that these complementary approaches give apparently inconsistent results, with a much higher allochthony fraction based on isotopes than on fatty acid patterns. Hypotheses for the apparent low incorporation of bacterial fatty acids compared to a high intake of allochthonous carbon are discussed below. At the same time, the results from fatty acid analysis confirmed that the bulk of mesozooplankton grazing on algae comprises mainly diatoms and not green algae, as was hypothesized from the total phytoplankton composition (data not shown).

Table 3. Contribution of algae as opposed to bacteria to mesozooplankton diet, estimated with the Bayesian Composition Estimator using fatty acid data.

	Apr	May	Jun	Jul	Aug	Sep
Acartia						
Hansweert		0.96±0.01				
Zandvliet					0.55±0.17	0.91±0.05
Eurytemora						
Zandvliet	0.96±0.02	0.96±0.03				
Antwerpen	0.86±0.05	0.88±0.07	0.8±0.07			
Temse		0.7±0.05				
Dendermonde				0.91±0.02		
Cyclopoida						
Dendermonde	0.81±0.03					
Daphnia						
Dendermonde		0.26±0.04				
Wetteren				0.69±0.04		
Eudiaptomus						
Dendermonde		0.82±0.04				

Discussion

The tidal river and estuary of the Scheldt are among the best studied temperate tidal systems. Tidal estuaries are characterized by intensive mixing, high suspended matter concentrations, and turbid waters limiting primary production. They usually also have high respiration rates exceeding primary production (Heip et al. 1995). Rivers entering these systems deliver riverine algal, bacterial, and mesozooplankton communities as well as riverine, terrestrial, and anthropogenic organic matter. In tidal rivers and estuaries these upstream communities and resources are mixed with autochthonous, endemic communities and newly produced organic matter. The downstream and seasonal trends in phytoplankton, bacteria, and mesozooplankton observed in this study are consistent with literature reports on the Scheldt as well as other turbid, tidal estuaries. The tidal river was characterized by high algal biomass despite high turbidity (Fig. 2), matching observations in the Scheldt (Muylaert et al. 2000) and other systems (Cloern et al. 1985; Cole et al. 1991). The mesozooplankton community of the tidal freshwater section comprised typical freshwater cladocerans *Bosmina* and *Daphnia*, consistent with observations in the Scheldt (Tackx et al. 2004), the Elbe (Kerner et al. 2004), and the San Francisco Bay (Müller-Solger et al. 2002). The endemic estuarine species *E. affinis* dominated mesozooplankton in the upper estuary and co-dominated with *Acartia tonsa*, another endemic estuarine species, at the most saline station, conforming to their well-established estuarine distribution patterns (Soetaert and Van Rijswijk 1993; Heip et al. 1995; Tackx et al. 2004).

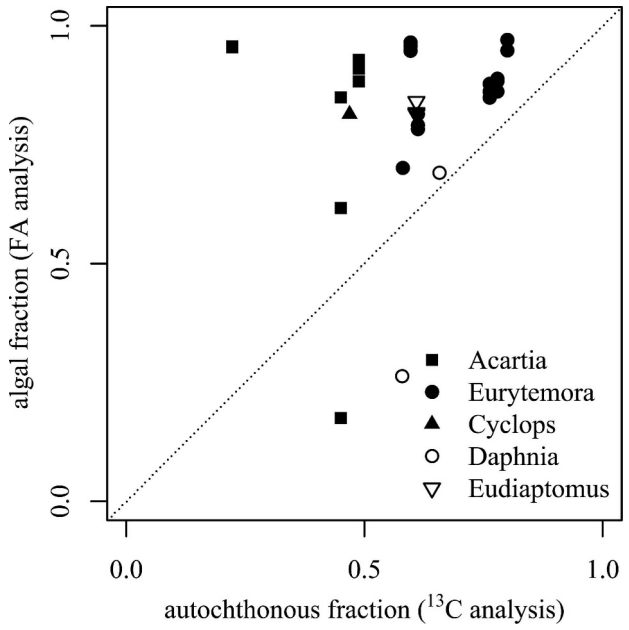

Fig. 6. Comparison of autochthonous contributions to mesozooplankton diets based on stable isotope data and fatty acid profiles. The points shown are the calculated average values in Table 3, plotted in function of the calculated average values in Table 2. Only adult mesozooplankton are shown. The identities of the mesozooplankton groups are indicated in the plot. The dotted line is the 1 : 1 line.

The various heterotrophic consumers, allochthonous and endemic, prokaryotes as well as metazoans, have direct or indirect access to three basic resources: detritus, DOC, and phytoplankton (Fig. 7). Although ecosystem metabolism and bacterial secondary production are fueled mainly by external POC and DOC subsidies in tidal rivers and estuaries (Findlay et al. 1991; Heip et al. 1995; Sobczak et al. 2005), this is not clear for eukaryotic consumers. Metazoans can obtain their carbon requirements via three pathways: (1) by direct grazing on phytoplankton (herbivory), 2) by feeding on particulate detritus (detritivory), or (3) via predation on bacteria that consume POC or DOC (bacterivory), possibly indirectly by feeding on bacterivorous microzooplankton.

Elucidating the relative importance and interactions among these three carbon flow pathways with stable

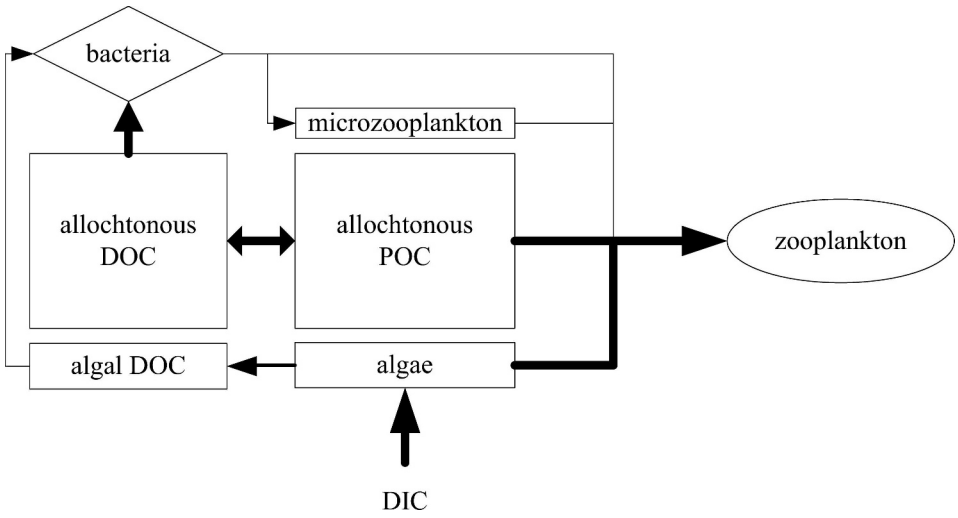

Fig. 7. Schematic picture of the lower plankton food web in the tidal Scheldt River and estuary, including main carbon fluxes to mesozooplankton. Thick arrows represent the most important flow paths.

isotopes requires resolving the isotopic signatures of algae, bacteria, and detritus as well as those of mesozooplankton. This is a daunting task and most approaches are fraught with problems. Many studies assume that algal biomass and algal detritus make up most of the POC pool and use the $\delta^{13}C$ of POC as a proxy for algal $\delta^{13}C$. Although this approach might work in clear water systems (large lakes and open marine systems), it is problematic in turbid systems where allochthonous matter dominates the POC pool (Middelburg and Herman 2007). Our study confirms once again that $\delta^{13}C_{POC}$ cannot be universally used as a proxy for algae, because PLFA clearly show a different picture (Figs. 3, 4). In turbid systems, size fractionation to separate algae, bacteria, and detritus is rather difficult and is limited by overlapping size classes and association of bacteria with detritus (Kerner et al. 2004), but when successfully applied algal signatures were usually depleted relative to detritus (Hamilton and Lewis 1992; DeLong and Thorp 2006). It has been proposed that the carbon isotope signature of herbivores, putative primary consumers, may provide a proxy for algal $\delta^{13}C$ (e.g., suspension feeding bivalves, Vander Zanden and Rasmussen 2001; scrapers, Finlay 2004; and *Daphnia* spp., Marty and Planas 2008). However, this requires highly selective feeding and our results (Fig. 5; Table 2) provide clear evidence that most small metazoan consumers in tidal rivers and estuaries rely on multiple resources. Some studies (Finlay 2004; Marty and Planas 2008) have proposed that the isotope signature of phytoplankton can be estimated from the $\delta^{13}C$ of DIC and isotopic fractionation factors that are a function of algal growth rate and carbon dioxide availability. Our data provide both support and critique to this approach. On the one hand isotope fractionation factors were rather constant among stations and showed limited seasonal variability, implying that a single fractionation factor may be used (Table 1). These stations also differed in DIC and pCO_2 levels, indicating that the pCO_2 dependence of isotope fractionation is rather limited at high CO_2 levels and for natural communities, consistent with observations by Boschker et al. (2005) and Bade et al. (2006). On the other hand, fractionation was taxon-specific, with green algae having much more depleted isotope values than diatoms (Fig. 3). This difference in fractionation factors between taxa (23‰ for green algae and 15.6‰ for diatoms) is consistent with previous observations in the Scheldt estuary (Boschker et al. 2005). This means that the aforementioned fractionation factor depends on the phytoplankton community structure, thus complicating application of this approach to mixed or uncharacterized natural communities. Although the isotope fractionation factor for green algae (23‰) is according to theoretical expectations for algae relying on dissolved CO_2 and using Rubisco to fix CO_2 (25–28‰; Goericke and Fry 1994), the derived fractionation factor for diatoms (15.6‰) was much lower, consistent with other recent field observations (Finlay 2004; Boschker et al. 2005; Bade et al. 2006). This can be explained by bicarbonate (HCO_3^-) uptake by diatoms (Tortell et al. 2008). Because bicarbonate has a higher $\delta^{13}C$ value than CO_2 (Zhang et al. 1995), $\delta^{13}C$ of diatoms will then be enriched relative to green algae relying only on CO_2.

Our study relies on the use of $\delta^{13}C$ of algae-specific PLFA as proxy for $\delta^{13}C$ of algae. This approach has been successfully used in a number of isotope-labeling studies (Middelburg et al. 2000; Pace et al. 2004; Van den Meersche et al. 2004). Application at natural abundance, as in this study, requires not only knowledge of the specificity of certain PLFA for certain algal groups (Boschker et al. 2005; Dijkman and Kromkamp 2006), but also the assumption that the fractionation between PLFA and whole algal cells is known and constant. The $\delta^{13}C$ of diatoms, green algae, and heterotrophic bacteria have been corrected for a 3‰ offset between PLFA and total cells, although it may vary between 2‰ and 5‰ (Hayes 2001). Sensitivity analysis has shown that this uncertainty does not affect the major outcome.

Isotope studies have the advantage over other methods that the data reflect the in situ food assimilation over longer time periods. Several stable isotope studies in heterotrophic rivers have shown that autochthonous carbon dominates pelagic metazoan food webs because allochthonous carbon, albeit abundant, is mostly recalcitrant (Hamilton and Lewis 1992; Delong and Thorp 2006). Estuarine studies also revealed that metazoans preferentially consume locally produced algal material: e.g., in the St. Lawrence estuary (Martineau et al. 2004), in the Elbe estuary (Kerner et al. 2004), and in San Francisco Bay (Cloern et al. 2002). This contemporary view of detritus fueling ecosystem metabolism while metazoan food webs depend on primary production has also been proposed based on more traditional approaches, such as correlations between phytoplankton and mesozooplankton dynamics in the San Joaquin River Delta (Sobczak et al. 2005) or growth efficiency of copepods and Cladocera on algal vs. detritus diets (Müller-Solger et al. 2002; Tackx et al. 2003). Overall, our isotope study revealed that allochthonous and autochthonous contributions to mesozooplankton diet are of similar magnitude. Our results appear to stand apart relative to other studies of the planktonic food webs in rivers and estuaries, but are consistent with observations in lakes (Pace et al. 2004) and highly turbid estuaries. In the turbidity maximum of the Gironde, the maximum mesozooplankton biomass co-occurred with low primary production, and other food sources (detritus, bacteria, protozoa, terrestrial import) were hypothesized (David et al. 2006). In another study involving fractionation experiments in the Scheldt estuary, no significant mesozooplankton grazing pressure was found on algae (Lionard et al. 2005), and Tackx et al. (2003) found that the estuarine copepod *E. affinis* had limited capability of selective feeding in different west European estuaries. These studies in turbid estuaries corroborate our findings that allochthonous carbon can be an essential part of the mesozooplankton diet in turbid estuaries. Detritus is the Scheldt is of relatively high quality (as reflected in high pCO_2, low dissolved oxygen, high nitrogen turnover, and high bacterial secondary production; Frankignoulle et al. 1999; Boschker et al. 2005; Middelburg and Herman 2007) and may thus constitute a good resource for mesozooplankton.

Is there a general trend that mesozooplankton is more selective in low-biomass estuaries and tidal rivers, and relies more on allochthonous carbon sources in turbid estuaries, despite high concentrations of algae? The high concentrations of detritus and DOC may interfere with copepods' prey selection. Chemoreception and mechanoreception of particles (Demott and Watson 1991), known to be mechanisms by which copepods can select food, will work less efficiently in this extremely rich environment because of a low "signal to noise ratio." High background concentrations of organic carbon can interfere with chemoreception of algal cells, whereas mechanoreception detects non-algal particles as well. Selection would involve catching and tasting, a costly activity, so it might be beneficial for the animals not to be too picky and supply their diets with some "junk food."

Although allochthonous and autochthonous carbon contributed similarly to mesozooplankton diet, availability of algal resources was much lower than that of detrital resources, implying that mesozooplankton had to feed selectively on algae. This is consistent with the well-known selective feeding strategies of mesozooplankton (Tackx et al. 2003; Sobczak et al. 2005). Another kind of selectivity, i.e., for specific phytoplankton groups, has been largely ignored throughout this study. The clearly lower isotope signature of green algae can theoretically be used to address this selectivity. However, green algae made up only a small proportion of the total algal community. Inclusion of selective feeding on green algae would imply a larger contribution of allochthonous carbon to mesozooplankton diet. However, accurate partitioning among green algae, diatoms, bacteria, and detrital contributions to mesozooplankton diet is not straightforward using only stable isotope data.

The stable isotope data provide unique and essential information on eventual external carbon subsidies, but little information on whether the mesozooplankton obtained this directly from detritus or via some intermediate steps, e.g., via bacteria. Fatty acid analysis offers complementary results. It also provides an integrative view of the organism's diet, but rather than distinguishing autochthonous and allochthonous carbon, fatty acid profiles allow tracing of the contributions of algae and bacteria to mesozooplankton diet. Estimated algal contribution to mesozooplankton diet based on stable isotope and fatty acids data differed significantly (Tables 2, 3; Fig. 6): fatty acid analysis systematically revealed a higher contribution of algae than isotope analysis, regularly showing minimal incorporation of bacterial fatty acids in mesozooplankton. This difference can be attributed to the low concentration of bacterially derived branched fatty acids found in mesozooplankton and can be explained in a number of ways.

One explanation involves direct grazing on bacteria by mesozooplankton, but these bacterially derived branched fatty acids are metabolized rather than assimilated and incorporated into the cell membranes. Assimilation efficiencies may indeed be fatty acid-specific, and de novo synthesis of (mostly C-20 and C-22) lipids has been reported for calanoid copepods (Dalsgaard et al. 2003). This complicates the use of fatty acids in food web studies, and if branched fatty acids are not incorporated, biased algal-bacteria contributions will then be estimated. The second explanation is based on the premise that free bacterial cells are too small for many mesozooplankters to process; i.e., bacteria must first be consumed by microzooplankton (ciliates and nanoflagellates) before mesozooplankton can profit from them. In marine environments, this pathway can make up to 30% of carbon uptake if phytoplankton is limiting (Calbet and Saiz 2005). In San Francisco Bay, *Acartia* consumed a diverse diet but appeared highly selective for motile prey, especially ciliates and nanoflagellates (Bollens and Penry 2003). Also, developmental stages of *E. affinis* have exerted higher clearance rates on microzooplankton than on phytoplankton (Merrell and Stoecker 1998). However, Ederington et al. (1995) studied the transfer of bacterially derived branched fatty acids to mesozooplankton via ciliates and observed that these compounds can be transferred up the food web without modification. Accordingly, low

concentrations of bacterially derived fatty acids in mesozooplankton likely reflect a low contribution of bacteria to mesozooplankton diet, making both these hypotheses unlikely.

An alternative hypothesis is that detritivorous mesozooplankton directly consumed POC (not via bacteria). Direct consumption of POC would allow a substantial allochthonous contribution to mesozooplankton diets (as inferred from stable isotope signature), while the fatty acid profiles would lack evidence of bacterial contributions. This alternative is fully consistent with all information at hand, but implies that detrital organic matter in the Scheldt estuary has a relatively high nutritional quality so that it can provide energy and nutrients for growth. Given that the tidal Scheldt complex is a highly heterotrophic system in which respiration of allochthonous carbon exceeds local primary production (Frankignoulle et al. 1998), it is likely that at least part of the allochthonous carbon has a high quality and nutritional value. This direct consumption of POC by mesozooplankton in the Scheldt is consistent with observation in temperate freshwater lakes showing that detritivory accounts for 22% to 75% of mesozooplankton carbon acquisition (Cole et al. 2006).

Synthesizing all this information, allochthonous organic matter in turbid estuarine systems is an important food web component, supporting microbial growth and respiration, and thus determining the heterotrophic status of these systems. Although DOC and POC typically contribute similarly to the total allochthonous carbon pool, it appears that little if any of the DOC enters, via bacteria, the metazoan part of the food web. DOC is often considered to comprise two fractions: a small labile fraction and a large non-labile fraction (Del Giorgio and Pace 2008). The small labile fraction is consumed by bacteria and little is transferred up in the food web. A large, more refractory fraction is transported largely unchanged through tidal river and estuary, but may exchange with POC (Middelburg and Herman 2007). POC, mainly detritus and imported resources, supports mesozooplankton for 50%. Mesozooplankton depends for another 50% on local primary production, which represents a small stock, but significant turnover.

In conclusion, it appears that not only prokaryotes but also mesozooplankton profit from the import of organic matter from upstream and lateral ecosystems. However, although bacteria may profit from both DOC and POC, mesozooplankton receive very little of their carbon via the bacterial loop pathway, i.e., from DOC, via bacteria and ciliates. This also implies intermingling of detrital and herbivory pathways. Although these two pathways are often considered and studied separately and only linked via regeneration of nutrients in detritus and subsequent use of these nutrients by primary producers, we show that metazoans directly profit from both resources. This additional complexity of food web subsidization has many consequences for food web dynamics (Cole et al. 2006). Further elucidation of these interactions might require use of deliberate tracer techniques. We also showed that two different approaches to derive herbivory (stable isotope analysis and fatty acid analysis) lead to significantly different results, but that these differences can be reconciled and that their combined application can reveal unique additional information.

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