Vol. 384: 147–157, 2009
doi: 10.3354/meps08019

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published May 29

Oligochaetes as a possible entry route for terrigenous organic carbon into estuarine benthic food webs

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ABSTRACT: Estuaries receive large quantities of terrestrially derived organic material, yet the current view is that such terrestrial carbon is unimportant for temperate estuarine benthic trophic dynamics beyond microbial processing. This consensus, however, may derive from a lack of consideration in estuarine food web studies of taxa with evolutionary affinities to freshwater systems where these taxa process terrestrial detritus. Here, we used a multiple stable isotope approach (carbon, nitrogen and sulphur) in 3 similar estuarine systems to test whether taxa with high (oligochaetes), medium (amphipods) and low (marine polychaetes and other crustacean taxa) evolutionary associations with freshwater systems differed in their assimilation of carbon derived from contrasting detrital sources. Oligochaetes had isotopic signatures significantly different to those of other organisms, yet not significantly different from tree and ground plant signatures, demonstrating that they assimilate terrestrial carbon. In contrast, amphipods and marine taxa had isotope signatures that indicated a reliance on marine algal carbon, independent of where in the estuary they were sampled, suggesting that, unlike oligochaetes, these taxa do not have an inherent physiological ability to successfully assimilate terrestrial material. These findings indicate that terrestrial carbon can play a significant role in estuarine systems, with oligochaetes providing the metazoan entry route for this carbon source into food webs, and that evolutionary detritivore–detritus associations may influence present-day trophic dynamics within estuaries.

KEY WORDS: Stable isotopes · Estuary · Carbon · Nitrogen · Sulphur · Food web · Oligochaeta · Detritus

INTRODUCTION

Temperate estuaries have amongst the highest levels of secondary production of any ecosystem, in particular the high biomass of invertebrates inhabiting extensive intertidal mudflats. In turn, this production supports large populations of migrating birds (Prater 1981) and juvenile fish (Attrill & Power 2002), which give estuarine systems a high conservation status. Although planktonic and benthic primary production (particularly microalgae) may contribute to the productivity of some estuaries and coastal systems (Chanton & Lewis 2002, Kang et al. 2003), benthic production in temperate estuaries of the northern hemisphere is primarily fuelled by allochthonous detritus (Deegan & Garritt 1997), which can originate from open marine, terrestrial and freshwater sources, as well as bankside vegetation, such as saltmarsh, and seagrass. In temperate estuaries, seasonal inputs of terrestrial carbon can be vast, particularly in the form of leaf litter, and can contribute up to 90% of the total particulate organic carbon in these systems (Eddins 2001, Goni et al. 2003, Gibbs 2008).

Stable isotope techniques have enabled the relative importance of different carbon sources within the estuarine food web to be investigated (e.g. Haines & Montague 1979, Deegan & Garritt 1997, Paterson & Whitfield 1997, Chanton & Lewis 2002). These studies have

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all concluded that terrestrially derived material does not contribute greatly to estuarine benthic secondary production, despite the large amount of terrigenous carbon entering the system. In particular, a major study on a range of consumers (macroinvertebrates and fish) in an estuary in Massachusetts, USA (Deegan & Garritt 1997) reported that terrestrial organic matter was of minimal importance; a conclusion supported by further studies (Mulkins et al. 2002, Martineau et al. 2004). Similarly, organic matter of terrestrial origin does not appear to contribute significantly to the diet of meiofauna taxa such as nematodes (Moens et al. 2002). Estuarine bacteria, however, do utilise terrigenous material (Hullar et al. 1996, Coffin & Cifuentes 1999), but although these heterotrophes may be consumed by detritivores, it has been suggested that this carbon is lost in respiration and not transferred into the food webs (Ducklow et al. 1986, Rolff & Elmgren 2000).

Terrestrial matter has been shown to contribute to some other marine food webs, such as Baltic planktonic systems (Rolff & Elmgren 2000), benthic assemblages off the Rhone delta (Darnaude et al. 2004) and some mangrove-dominated tropical estuaries (Newell et al. 1995, Chong et al. 2001), although for these systems the evidence is equivocal (Bouillon et al. 2002, Kieckbusch et al. 2004). Considering the vast amount of terrigenous carbon entering temperature estuarine systems, we speculated that an important trophic link may have been overlooked in temperate estuarine studies. In particular, we hypothesise that those animals within the estuarine benthic food web most likely to be able to utilise terrestrial carbon would be those with historic affinities to freshwater systems (Attrill & Rundle 2002), where they feed on terrestrial detritus. Here we investigate this hypothesis by comparing stable isotope signals in 3 types of estuarine taxa, i.e. those with (1) high evolutionary affinities with freshwater systems — the oligochaetes, which have colonised upper-mid estuaries from a terrestrial/freshwater route and primarily feed on detrital material in freshwater (Schmid-Araya et al. 2002) — (2) medium freshwater affinity — amphipod crustaceans, which have evolved from marine systems into freshwater where species (e.g. *Gammarus pulex*) are considered to be consumers of terrestrial detritus (Cummins & Klug 1979) — and (3) low freshwater affinity — other marine taxa (see 'Materials and methods').

To test whether either group (1 or 2 above) could be a repository for terrestrial carbon in estuaries, we sampled 3 similar estuaries to determine the fate of detrital material in relation to the taxa of interest. The aim was to test our evolutionary hypothesis and not to undertake a full food-web study. Hence, we sampled primary producers, detrital sources and main benthic invertebrates and utilised multiple stable isotope analyses of carbon, nitrogen and sulphur, the latter providing additional analytical resolution when investigating food sources and the fate of terrigenous material in estuarine food webs (Connolly et al. 2004).

MATERIALS AND METHODS

Our approach was to assess large-scale variation between taxa that, if our hypothesis is supported, should be apparent above and beyond any smaller scale variation within or between estuaries. Our sampling and analytical protocols were, therefore, specifically designed to detect variation at an aggregate level and differences at the taxonomic rather than the individual level. Replicate samples were collected from 3 similar estuaries along the Devon coast in southwest England: the tidal reaches of the rivers Yealm, Erme and Avon (Fig. 1). All 3 river systems arise in the moorlands of Dartmoor National Park and flow southwards for comparatively short distances (Yealm = 16 km; Erme = 20 km; Avon = 24 km) through moorland and light grazing pasture/wooded areas, before discharging into their estuaries which open to the English Channel. Catchments are small (Yealm = 55 km^2; Erme = 44 km^2; Avon = 102 km^2), have low, and similar, levels of human occupation and impact and are fed primarily by groundwater or overland drainage. All 3 estuaries are of similar length (Yealm = 5 km;

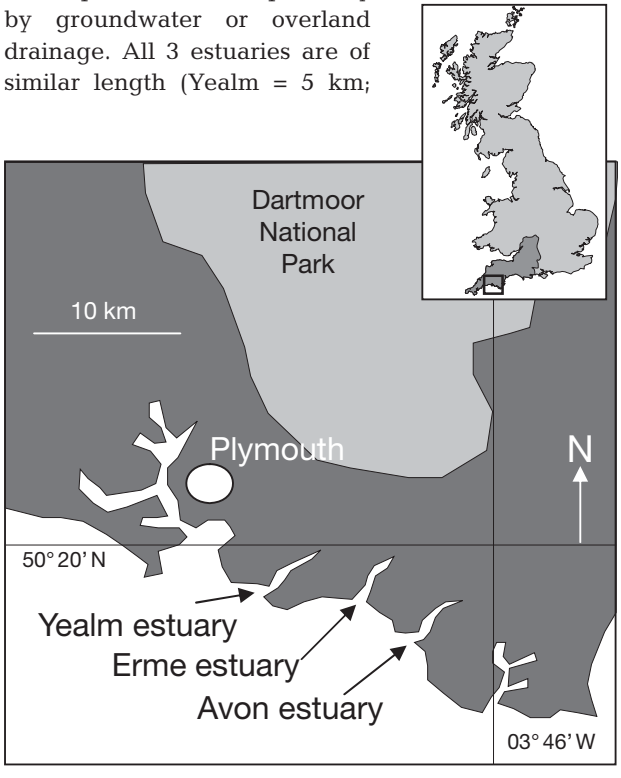

Fig. 1. South Devon, UK, indicating position of the 3 similar estuaries studied

Erme = 3 km; Avon = 4 km) and character (e.g. tidal range, salinity regime and sediment pattern). Within each estuary, 4 separate locations were sampled, representing freshwater (upper tidal limit, FW), upper estuary (UE), mid-estuary (ME) and lower estuary (LE; estuary mouth, fully saline conditions). Sites were evenly spaced along each estuary, and salinity (high and low tide), temperature and pH were recorded at each location.

Field sampling and analytical methods followed those described elsewhere (e.g. Haines & Montague 1979, Peterson 1999, Bouillon et al. 2000, Rolff & Elmgren 2000, Zah et al. 2001, Post 2002), but are described briefly below. The aim of the present study was not to investigate or construct food webs within each estuary, but to address our hypotheses concerning the fate of terrestrial material. Hence, we focused on sampling 4 major taxonomic groups: oligochaetes (evolved into estuaries from a terrestrial/freshwater route); amphipod crustaceans (mainly *Gammarus* spp., evolved into freshwater from a marine/estuarine route); the ragworm *Nereis diversicolor* and either mysids (mainly *Neomysis integer*) or the brown shrimp *Crangon crangon* (all from a marine route and not penetrating freshwater in these systems), depending on which was common at each site (if at all; neither were present at some Avon sites). These invertebrates were collected with repetitive kick samples, use of hand dip-nets or by hand picking from shallow or exposed tidal flats at low tide. All samples were categorised to major taxonomic group in the field, retained in water to allow gut clearance and transported back to the laboratory for identification to species. Due to the destructive nature of the identification process for oligochaetes (i.e. the need to mount on slides and clear with chemicals), they were not identified below family (Lumbriculidae, Tubificidae and Enchytraeidae).

Seston was assessed by collecting a minimum of 10 l of site sample water and filtering with the aid of a peristaltic pump through precombusted Whatman 0.5 m quartz fibre filters. The concentrated seston and filters were then dried at 60°C, acidified to pH 2.5–2.8 with HCl and sealed in scintillation vials for stable isotope analysis. Macrophytes and algae removed or scraped from rocks were rinsed in dilute HCl (1 M) to remove inorganic carbon. Invertebrates and epiphytic material were removed prior to drying by gentle washing with deionized water. Sediment and the accumulated detrital layer were collected by scraping the top 0.5 to 1 cm of the substrate at randomly chosen locations. Coarse particulate organic matter (CPOM) was sampled by allowing drifting material in the water column to be collected in a 500 µm sieve. Collected materials were pulverized and repeatedly acid washed in dilute HCl (1M) to remove the inorganic fraction. Samples of the few existing saltmarsh plants (e.g. *Spartina*, *Salicornia* and *Aster*) and the more extensive surrounding terrestrial vegetation (grasses, bushes and trees) were collected randomly from areas adjacent to each sample site, identified, rinsed in deionized water and subsequently dried at 60°C. Algal and/or macrophyte samples (where present) were taken from the lower freshwater reaches of each river. Seagrass *Zostera marina* was only present in small amounts in the mouth of the Yealm. This was sampled, but not included in full analyses due to its comparative scarcity.

Stable isotope ratios are expressed as delta values (δ) and are measures of the parts per thousand difference (‰) between the isotope ratio of a sample and that of the appropriate international standard according to the formula:

$$\delta^{13}C,\ \delta^{15}N \text{ or } \delta^{34}S = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$$

where R = $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$. Samples that are lower (or more negative) are depleted in the heavier isotope (^{13}C, ^{15}N or ^{34}S). Higher (or less negative) samples are enriched and contain more of the heavier isotopes. The international standards used for carbon and nitrogen isotope signatures included carbonate rock from the Peedee Belemnite formation (Craig 1957) and nitrogen gas in the atmosphere (Mariotti 1983). NBS-123 (Sphalerite Bamata ZnS) was used for sulphur, with values corrected for secondary drift using National Institute of Standards and Technology (NIST) organic reference materials 1577b (bovine liver) and 2976 (mussel) and correction protocols outlined in Fry et al. (2002). By agreement, all international standards are set at a value of 0‰.

All dried samples were ground to a fine homogenate using a Retsch MM 2000 ballmill grinder. Between 1 (animal tissue) and 6 (terrestrial plant tissue) mg of dried material was loaded into 5 × 3.5 mm tin cups and combusted in a Carlo Erba elemental analyzer interfaced with a Micromass VG Isochrom continuous-flow isotope ratio mass spectrometer at the Environmental Isotope Laboratory, University of Waterloo. Repeat analyses of commercially available laboratory standards yielded results that were accurate and precise (International Atomic Energy Agency [IAEA] standard CH6; $\delta^{13}C$ (mean ± SD) = −10.2 ± 0.2‰, n = 25; IAEA standard N1; $\delta^{15}N$ = 0.2 ± 0.3‰, n = 25; NBS-123 $\delta^{34}S$ = 17.1 ± 0.3‰, n = 25; NIST 1577b $\delta^{34}S$ = 7.6 ± 0.4‰, n = 25 and NIST 2976 $\delta^{34}S$ = 18.7 ± 0.5‰, n = 25). Replicates of internal laboratory standards were equally accurate and precise. Acceptable accuracy for ecological work of ±0.5‰ suffices for most ecosystems because variability among replicate field samples can often exceed ±0.5‰ (Peterson 1999). Due to lack of sample volume or percent sulphur content, $\delta^{34}S$ values could not be obtained for seston or sediment samples.

Differences of multiple isotopic signatures were investigated in 3-dimensional space utilising multivariate statistical techniques available on the analytical package PRIMER, originally designed for the analysis of community data (Clarke & Warwick 1994). For this novel analysis of isotope data, each element ratio ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) was considered a 'species', with each sample corresponding to an individual organism or carbon source. A similarity matrix was constructed for each pair of samples using the Bray-Curtis similarity index on untransformed data, but with carbon and nitrogen values adjusted to reflect dietary fractionation using the fractionation values reported in Post (2002). The resulting matrix, therefore, contained a % similarity for every pair of organisms or sources, in terms of their isotopic signature. Formal significance testing of differences in isotopic signatures between *a priori* groups (i.e. each taxon or carbon source) was undertaken on this matrix using analysis of similarities (ANOSIM) (Clarke 1993), a multivariate randomisation procedure broadly analogous to ANOVA. The resulting statistic, R, ranges from 0 to 1: 0 if there is no separation in multivariate isotopic signatures between tested groups and 1 if perfect separation occurs. Details of dissimilarities between pairs of taxa and carbon sources were investigated using the similarity percentages (SIMPER) procedure (Clarke 1993).

Investigation of spatial trends in isotopic ratios within individual estuaries showed some variation, but no consistent patterns in carbon sources, live plant material or invertebrates between sites along each estuary (Tables A1 & A2 in Appendix 1). Following multivariate analysis, this within-taxon variability was clearly small compared with between-taxon variability (see 'Results' and Appendix 1). Therefore, samples within estuaries were combined for further analysis. The significance of differences between isotopic signatures of taxonomic groups/carbon sources, and any differences between estuaries, were investigated for each element using 2-factor ANOVA. Isotopic datasets were randomly resampled to provide 4 replicates of each variable in each estuary; this corresponded to the lowest n-value for some parameters (e.g. sediment and CPOM). Occasional missing values were substituted by the mean of the other replicates to allow a balanced design, but this did not affect the variance (Underwood 1997). Animal tissue values were adjusted for fractionation effects to account for trophic enrichment as above prior to statistical analysis. Upper and lower fractionation bounds reflecting the range of fractionation values reported in the literature (laboratory, field and meta-analysis studies; e.g. Post 2002, McCutchan et al. 2003, Vanderklift & Ponsard 2003) were selected for use in analysis. Lower bound fractionation values for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ were 0.4 (Post 2002), 2.3 (McCutchan et al. 2003) and 0.2‰ (Peterson & Fry 1987), respectively. Upper bound fractionation values for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ were 1.0 (DeNiro & Epstein 1978), 3.4 (Minagawa & Wada 1984, Post 2002) and 0.5‰ (McCutchan et al. 2003), respectively. Any significant analyses were further investigated using post-hoc Student-Newman-Keuls (SNK) tests (Underwood 1997) to assess pairwise significance. All analyses were undertaken using GMAV5 for Windows (Centre for Research on Ecological Impacts of Coastal Cities, University of Sydney).

Mass balance computations (Phillips & Gregg 2003) and 2-compartment mixing model analyses (Kline et al. 1998, Phillips & Gregg 2001) were completed to estimate the proportion of terrestrial material in detrital sources and the percent contribution of detrital material to invertebrate signatures (end members were marine macroalgae and the average of terrestrial plants). The percent terrestrial carbon in body tissues was then determined as: (% detrital matter) × (% terrestrial carbon in detrital matter).

RESULTS

The overall pattern of isotopic signatures for primary producers was comparatively consistent among estuaries (Figs. 2 to 4). Marine macroalgae were enriched along all 3 isotopic axes (Table 1), clearly separating this carbon source from terrestrial and freshwater plant material, which had depleted isotope values for all elements. Freshwater values are not plotted on Figs. 2 & 3 as they were extremely depleted (Table 1) and highly separated from all taxa and detritus sources. Isotopic values for saltmarsh plants showed the highest variability, corresponding with the broad range of plant types (and thus photosynthetic pathways) found in this habitat. Whilst this variability could potentially confuse attempts to define main food sources of invertebrates, unlike many estuaries, saltmarsh habitat is extremely rare in the studied steep-sided systems and confined to a few very small patches. It is unlikely, therefore, that saltmarsh production is contributing significantly to estuarine secondary production in these systems, but we sampled these primary producers for the sake of completeness.

All detrital sources (seston, CPOM and sediment) had isotopic signatures placing them between terrestrial plants and marine macroalgae (Figs. 2 to 4a), suggesting that detrital material is a mix of organic matter from terrestrial and marine inputs.

Isotopic values for invertebrates also demonstrated consistent patterns among estuaries and, within each estuary, variation in isotopic signature for each taxon was small compared with the overall variability. Each taxon is comparatively tightly clustered in Figs. 2 to 4 regardless of location within estuary, justifying pool-

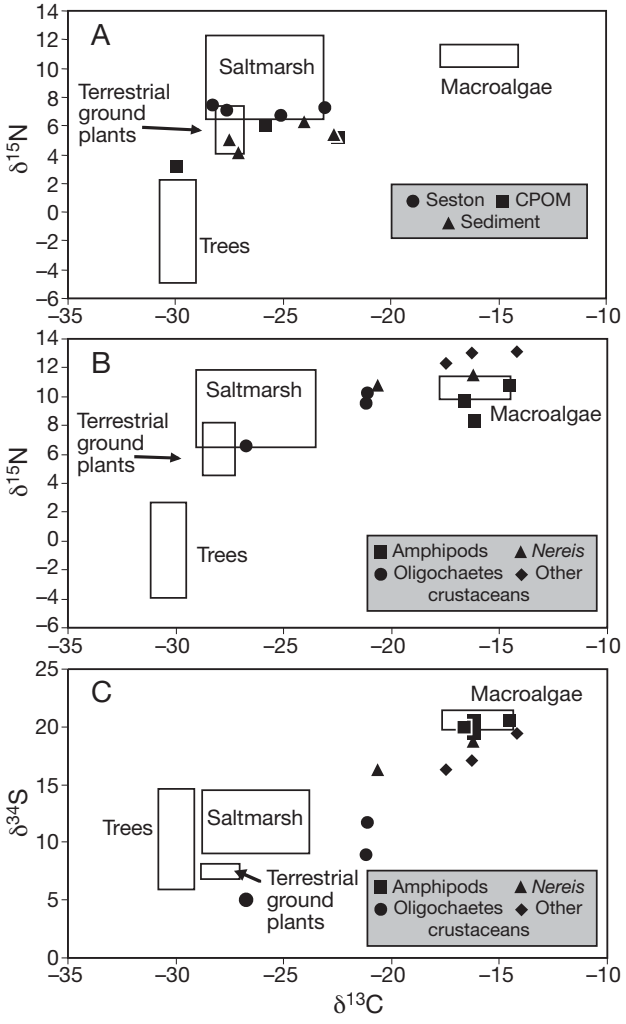

Fig. 2. Scatterplots of stable isotope signatures for samples taken from the Erme estuary, Devon, UK. Boxes indicate range of values for carbon sources; points are mean isotopic values at each sampling site. $\delta^{13}C$ versus $\delta^{15}N$ signatures for (A) detrital material and (B) invertebrate taxa are shown. (C) $\delta^{13}C$ versus $\delta^{34}S$ signatures for invertebrate taxa. CPOM: coarse particulate organic matter

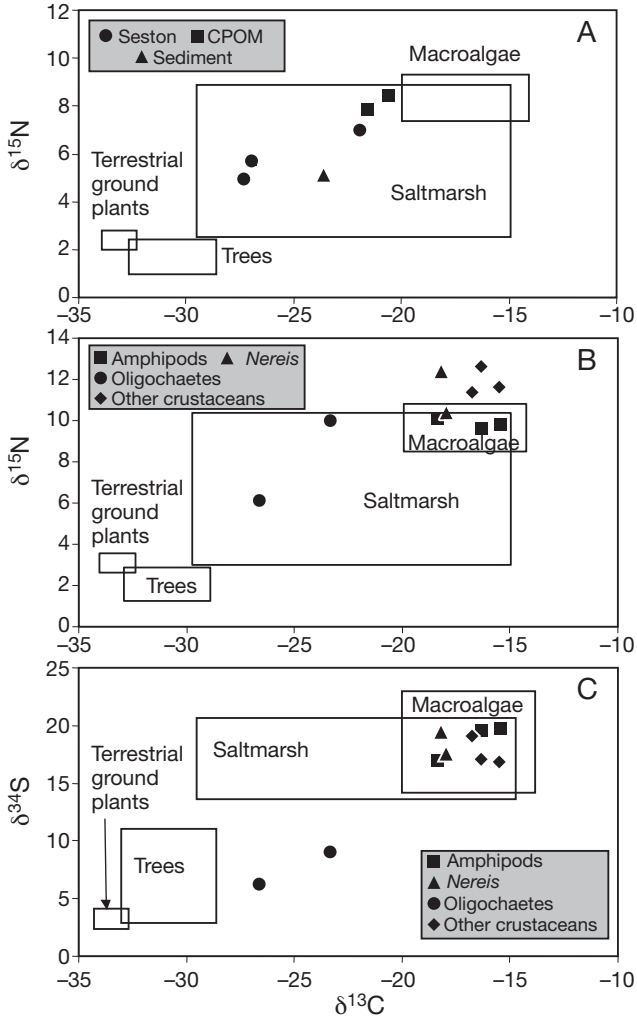

Fig. 3. Scatterplots of stable isotope signatures for samples taken from the Yealm estuary, Devon, UK. Details as for Fig. 2

ing of data for each estuary in ANOVA. For $\delta^{13}C$, amphipods and marine taxa (other crustaceans, *Nereis diversicolor*) had signatures statistically inseparable (SNK, all $p > 0.05$) from marine macroalgae (Figs. 2 to 4, Tables 1 & 2). Similarly, the majority of $\delta^{34}S$ signatures for these taxa were most closely matched to those of marine macroalgae (all $p > 0.05$; Figs. 2 to 4, Tables 1 & 2). Consequently, the carbon and sulphur isotopic signatures for crustaceans and *N. diversicolor* were significantly different (all $p < 0.01$) from any of the 3 main detrital sources in the estuaries (CPOM, seston and sediment; Tables 1 & 2). Oligochaeta signatures, however, were significantly different from all other animals (all $p < 0.05$; Tables 1 & 2), with particularly depleted $\delta^{13}C$ (Figs. 2 to 4B) and $\delta^{34}S$ values (Figs. 2 to 4C) compared with crustaceans and *N. diversicolor*. For these elements, oligochaetes did not have statistically different signatures to any of the detrital sources, trees or terrestrial ground plants, but there was a significant difference between oligochaete signatures and marine macroalgae ($p < 0.01$; Table 1). As expected, nitrogen signatures for most detrital sources were similar with the exception of tree samples, which were significantly lower than other primary producers (all $p < 0.05$; Tables 1 & 2). However, unlike all other invertebrates, the corrected nitrogen signal for oligochaetes was not significantly different from tree values ($p > 0.05$), yet was significantly different from marine algae ($p < 0.01$).

Statistical analysis of the multiple stable isotope data using ANOSIM revealed that all invertebrate taxa had significantly distinctive 3-dimensional signatures

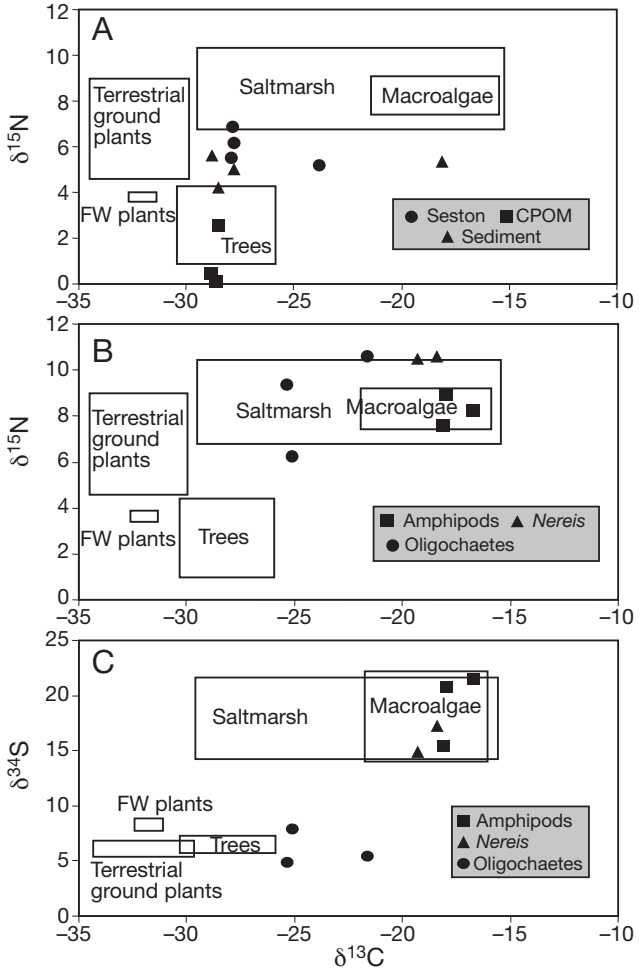

Fig. 4. Scatterplots of stable isotope signatures for samples taken from the Avon estuary, Devon, UK. FW: freshwater. Other details as for Fig. 2

Table 1. Isotopic values (‰, mean ± SD) for primary producers, detrital sources and invertebrates sampled from 3 similar estuaries in south Devon, UK. CPOM: coarse particulate organic matter; other Crustacea: mysids and/or *Crangon crangon*; nm: not measurable

	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Primary producers				
Marine macroalgae	53	−17.29 ± 2.89	9.33 ± 0.95	19.05 ± 3.05
Saltmarsh plants	32	−23.47 ± 5.74	8.11 ± 2.72	15.37 ± 3.30
Freshwater macrophytes	3	−38.11 ± 0.91	7.19 ± 0.81	6.44 ± 1.28
Trees	20	−29.46 ± 1.65	1.16 ± 1.90	7.72 ± 2.85
Terrestrial ground plants	10	−31.03 ± 1.01	5.25 ± 1.26	5.54 ± 0.35
Detrital sources				
CPOM	10	−26.30 ± 2.36	4.22 ± 1.36	2.91 ± 0.48
Seston	53	−25.21 ± 2.37	6.74 ± 1.87	nm
Sediment	12	−25.02 ± 3.17	5.11 ± 0.50	nm
Invertebrates				
Oligochaeta	26	−23.41 ± 2.64	8.71 ± 1.83	6.23 ± 4.14
Amphipoda	48	−17.59 ± 3.08	9.00 ± 1.20	19.29 ± 1.92
Other crustacea	54	−17.22 ± 2.33	11.80 ± 1.16	17.63 ± 1.29
Nereis diversicolor	29	−18.45 ± 1.46	11.02 ± 0.78	17.36 ± 1.64

Table 2. Nested 2-way ANOVA results (source of element [e.g. plant, animal and detritus], estuary) for each element. C, N and S values were adjusted for fractionation effects (see 'Materials and methods'). MS = mean squares, n = 12. For individual source and site data, see Appendix 1

	df	MS	*F*	p
$\delta^{13}C$				
Source	10	329.79	16.21	**<0.001**
Estuary (source)	22	20.34	2.95	**<0.001**
Residual	99	6.90	–	–
$\delta^{15}N$				
Source	10	57.61	9.92	**<0.001**
Estuary (source)	22	5.81	3.10	**<0.001**
Residual	99	1.87	–	–
$\delta^{34}S$				
Source	7	605.54	42.36	**<0.001**
Estuary (source)	16	14.29	4.63	**<0.001**
Residual	72	3.09	–	–

(global r = 0.676, p < 0.001; all pairwise tests p < 0.001), but the average dissimilarity between oligochaetes and all other taxa (26.22 ± 0.98%) was higher than for other possible pairwise combinations (6.41 ± 0.69%). Further analysis of the relative similarities between taxa and carbon source signatures (Table 3) confirmed the earlier conclusion that all crustaceans and *Nereis diversicolor* were most similar to marine macroalgae and had very different signatures from other primary producers and all detrital sources. Conversely, oligochaete multiple signatures showed greatest similarities to seston and terrestrial plant material (ANOSIM pairwise tests, p > 0.05, Table 3), the latter due particularly to equally depleted sulphur values (Table 1).

Mass balance computations suggested that detrital material (CPOM, seston and sediment) is largely (63%) composed of terrestrial material (tree leaf litter and terrestrial ground plants). Mixing model analysis, completed using a range of fractionation assumptions, determined a range for the percentage contribution of detrital material to invertebrate signatures and revealed high values (78 to 87%) for oligochaetes, with *Nereis diversicolor* having the next highest proportion (18 to 26%). Therefore, from these results, oligochaete tissues are estimated to consist of 50 to 54% terrestrial carbon. In contrast, other marine taxa have comparatively low, but measurable, fractions: amphipods 5 to 10%, other Crustacea 2 to 7% and *N. diversicolor* 11 to 17%.

Table 3. Average percentage dissimilarity (SIMPER output using Bray-Curtis similarity index) between the multiple stable isotope signatures of estuarine taxa and potential food/carbon sources. Lowest dissimilarity for each taxon is highlighted in bold; only C and N were analysed for seston and sediment. Animal C, N and S values were adjusted for fractionation effects prior to analysis (see 'Materials and methods'). CPOM: coarse particulate organic matter

	Oligochaetes	Amphipods	*Nereis diversicolor*	*Crangon crangon*	Mysids
Carbon source					
Trees	13.12	32.52	29.27	34.76	33.57
Terrestrial ground plants	9.30	31.19	27.52	32.97	31.81
Saltmarsh	21.30	15.65	15.65	16.53	16.41
Marine algae	32.76	**8.53**	**9.81**	**8.03**	**6.85**
Detritus source					
CPOM	13.64	38.50	34.72	40.80	39.42
Seston	**8.29**	16.50	13.62	21.40	18.04
Sediment	10.66	16.93	15.69	22.00	19.16

DISCUSSION

We hypothesised that taxa demonstrating evolutionary affinities with freshwater systems, where they are known to utilise terrestrial detrital material, would be the most likely groups to uptake terrigenous carbon into estuarine food webs. Two main groups (oligochaetes and amphipod crustaceans) were targeted and compared with fully marine taxa. Estuarine Oligochaeta sampled in the present study showed clear evidence of acquiring carbon from sources different from other studied estuarine invertebrate taxa, including amphipods which appear to rely on marine macroalgae. A few amphipod species (e.g. *Gammarus pulex* in UK rivers) have evolved into freshwaters systems from a marine origin (*G. pulex* only invading after the last ice age; Green 1968) and have developed the capacity to feed off terrestrial detrital material (Cummins & Klug 1979), although the mechanism by which such species assimilate this carbon has been open to debate. Barlöcher (1983) suggested that *G. fossarum* possessed gut enzymes that were most suitable for digesting leaf litter that had been substantially conditioned, in that it had been part-digested in the stream by an associated microbial assemblage; the enzymes present in the gut were then able to act on the intermediate products from microbial action. Moreover, Barlöcher (1982) also showed that the activity of fungal enzymes persisted for several hours in the presence of *G. fossarum* gut enzymes, suggesting that the microbes may also play a role within the guts of freshwater amphipods. The estuarine amphipods sampled in the present study showed little evidence of assimilating terrestrial carbon, having isotopic signatures consistently similar to marine algae and other marine invertebrate taxa, with mixing model analysis revealing a minimal contribution from terrestrial sources. A recent investigation of stable isotope signatures in amphipods from an estuary in British Columbia also suggested that amphipods in this system were utilising leaf packs for shelter rather than as a food source (Sakamaki & Richardson 2008). It is possible that leaf litter in estuaries is not conditioned by microbial action in a similar way to that in freshwater, perhaps due a change in, or loss of, species of fungi and bacteria associated with leaves when in freshwater. Sakamaki & Richardson (2008) suggested that, in their estuarine system, microbial action is still important in leaf litter processing, but there may be subtle differences in how this breakdown conditions the leaves. Clearly, further research is required to investigate the interaction between microbial conditioning and the assimilation of terrestrial leaf litter by estuarine amphipods.

Oligochaete isotopic ratios, however, were generally statistically inseparable from detrital sources and terrestrial plant material. This suggests that oligochaetes in estuaries are able to assimilate terrestrial carbon (55% of tissue carbon) as well as material from marine sources, resulting in tissue isotopic values very similar to the available detrital sources. We suggest that estuarine oligochaetes have inherited this ability through their evolutionary route, invading into estuaries from terrestrial/freshwater systems (Ruppert & Barnes 1991) where they are key consumers of terrestrial plant material (Ruppert & Barnes 1991, Schmid-Araya et al. 2002). Oligochaeta, therefore, provide a route of entry for terrestrial carbon into temperate estuarine food webs that has not previously been clearly demonstrated, perhaps due to this taxon being marginalised or excluded from previous studies. However, in many estuaries, oligochaetes (e.g. *Heterochaeta costata*, *Limnodrilus hoffmeisteri* and *Tubificoides benedii*) can be present in vast numbers, particularly in upper and middle reaches (Attrill 1998). In organically polluted systems they have been recorded at densities >300 000 m^{-2} (Harrison & Grant 1976) and estuarine oligochaete biomass in Forth estuary mudflats was found to be greater than infaunal mollusc or polychaete production (McLusky & Elliott 2004). We therefore suggest that oligochaetes provide the major, and perhaps the only, metazoan route for the uptake of terrigenous carbon into estuarine systems and, coupled with bacterial utilisation (Hullar et al. 1996, Coffin & Cifuentes 1999), play a key functional role in estuarine carbon cycling that has been underestimated to date.

Two interesting questions arise from these findings. Firstly, why does the clear carbon signature within oligochaetes not appear further up the food web as the carbon is incorporated into predator tissues? Despite their abundance in many temperate estuaries, few estuarine animals feed solely, or mainly, on oligochaetes, as illustrated by food webs constructed for estuarine systems (e.g. Green 1968) and diet analysis of estuarine taxa (e.g. Wheeler 1969). Consequently, isotopic signals will be diluted within predator tissues by other prey taxa that have predominantly marine signatures. An exception is wildfowl, particularly some sea duck species, which have been recorded feeding primarily on tubificid oligochaetes in estuarine mudflats. Harrison & Grant (1976), for example, revealed how species such as pochard *Aythya farina*, mallard *Anas platyrhynchos* and shelduck *Tadorna tadorna* fed extensively on tubificids in the Thames estuary, duck numbers decreasing in line with oligochaete numbers as the system was rehabilitated in the 1960s to the 1970s. If wildfowl (and some waders) are the main predators of oligochaetes within estuaries, then much terrestrial carbon will be removed from the system, shortcutting the main food web, and even be exported back to terrestrial habitats as faeces (Post et al. 1998).

The second question involves the mechanism behind the uptake of marine algal carbon by the majority of estuarine taxa. All invertebrates in the present study apart from oligochaetes had isotopic signatures similar to marine algae, suggesting they are discriminating in favour of marine carbon which is assimilated into their tissues primarily from this source, regardless of where the individuals were sampled within the 3 estuaries. The taxa studied (*Nereis diversicolor*, shrimps, mysids and amphipods) tend to be detritivores or predators within estuarine systems so will not be feeding directly on attached algal material, which is uncommon upstream of the outer estuary at our sites due to lack of hard substrata. Therefore, marine organisms feeding on detritus from either the sediment or water column must either be actively selecting marine algal particles or ingesting all the detritus/seston, but disproportionately assimilating the fraction that is from marine algae (perhaps because marine carbon is more labile; Osinga et al. 1996). Either strategy could be energetically demanding when estuarine detritus, particularly in upper or mid-estuarine reaches, is primarily composed of terrestrial litter (Eddins 2001, Goni et al. 2003), a situation that may favour oligochaetes. However, in the present study, mass balance computations suggest that terrestrial carbon makes up 63% of detrital material, leaving adequate amounts of carbon for marine taxa to profitably assimilate. Whilst some benthic filter feeders are capable of actively sorting particles, this behaviour tends to be size-based or involves removing food fractions from mineral particles (e.g. Ward et al. 1998). It would seem unlikely that generalist detritivores would be able to actively pre-sort detritus by source; either material is actively sorted in the gut (Newell et al. 1989) or terrestrial matter is passively egested.

CONCLUSIONS

The present study has provided evidence to answer a persistent question in estuarine ecosystem ecology: Where does all the terrestrial carbon go? Through their evolutionary route of colonisation into estuaries, oligochaetes have the ability to assimilate this material and provide an important route for apparently lost terrigenous carbon to enter estuarine food webs.

Acknowledgements. Funding support came from an NSERC discovery grant to M.P.

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Appendix 1. Details of C, N and S stable isotope ratios for carbon sources and taxa at each site within 3 similar estuaries (Yealm, Erme and Avon) in SW England

Table A1. Mean (±SD) stable isotope ratios for each carbon source at each of the sampled estuarine sites. Sites were located from the freshwater–estuary interface (Site 1) to estuary mouth/fully marine conditions (Site 4). CPOM: coarse particulate organic matter. n/a: not applicable

Carbon source	Site	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Trees	Avon 1	–28.09 ± 2.25	2.61 ± 1.70	6.49 ± 0.10
	Erme 1	–29.93 ± 0.51	1.20 ± 3.19	10.20 ± 4.35
	Yealm 1	–30.35 ± 2.20	2.07 ± 0.81	6.48 ± 4.09
Terrestrial plants	Avon 1	–32.02 ± 2.19	6.75 ± 2.19	6.12 ± 0.32
	Erme 1	–28.09 ± 0.84	6.05 ± 1.60	7.33 ± 0.72
	Yealm 1	–32.99 ± 0.00	2.97 ± 0.00	3.18 ± 0.00
Saltmarsh plants	Avon 2	–27.65 ± 0.14	9.24 ± 0.25	13.17 ± 0.39
	Avon 3	–19.93 ± 7.13	8.27 ± 2.10	19.97 ± 1.49
	Erme 2	–25.89 ± 3.25	7.89 ± 2.16	9.39 ± 0.37
	Erme 3	–27.20 ± 1.30	12.00 ± 1.12	14.06 ± 1.88
	Yealm 2	–21.60 ± 8.29	7.95 ± 2.66	17.01 ± 3.48
	Yealm 3	–25.07 ± 0.36	1.28 ± 0.09	n/a
Seagrass	Yealm 4	–8.52 ± 0.05	8.34 ± 0.12	18.04 ± 0.07
Macroalgae	Avon 1	–31.65 ± 0.53	3.60 ± 0.26	8.42 ± 0.18
	Avon 2	–20.75 ± 0.69	7.81 ± 0.09	12.66 ± 0.14
	Avon 3	–20.18 ± 0.83	8.09 ± 0.67	20.69 ± 0.36
	Avon 4	–13.99 ± 2.38	8.64 ± 1.25	20.87 ± 0.13
	Erme 2	–16.68 ± 0.39	9.68 ± 1.24	20.22 ± 0.39
	Erme 3	–16.29 ± 1.42	10.82 ± 0.10	20.24 ± 0.30
	Erme 4	–15.73 ± 2.55	10.02 ± 0.27	21.06 ± 0.38
	Yealm 1	–41.12 ± 1.58	11.80 ± 0.12	7.15 ± 0.20
	Yealm 2	–20.95 ± 1.12	10.74 ± 1.05	19.89 ± 1.48
	Yealm 3	–14.14 ± 2.48	9.05 ± 0.81	20.91 ± 0.44
	Yealm 4	–14.96 ± 3.70	9.00 ± 0.31	14.92 ± 7.40
CPOM	Avon 1	–28.63 ± 0.30	0.12 ± 0.13	2.23 ± 0.03
	Avon 2	–28.89 ± 0.00	0.44 ± 0.00	2.75 ± 0.00
	Avon 3	–28.49 ± 0.00	2.52 ± 0.00	3.18 ± 0.00
	Erme 1	–29.95 ± 0.00	3.14 ± 0.00	2.31 ± 0.00
	Erme 3	–25.80 ± 0.28	6.05 ± 0.25	3.94 ± 0.26
	Erme 4	–22.45 ± 0.00	5.23 ± 0.00	4.56 ± 0.00
	Yealm 2	–26.89 ± 0.00	5.67 ± 0.00	2.45 ± 0.00
	Yealm 4	–21.56 ± 0.00	7.83 ± 0.00	n/a
Water (seston)	Avon 1	–27.89 ± 0.45	5.51 ± 2.65	n/a
	Avon 2	–27.78 ± 0.37	6.18 ± 1.60	n/a
	Avon 3	–27.87 ± 0.30	6.89 ± 2.14	n/a
	Avon 4	–23.80 ± 1.57	5.21 ± 1.50	n/a
	Erme 1	–27.63 ± 0.64	7.07 ± 4.13	n/a
	Erme 2	–28.27 ± 1.13	7.45 ± 0.89	n/a
	Erme 3	–25.12 ± 0.17	6.71 ± 0.17	n/a
	Erme 4	–23.06 ± 1.00	7.26 ± 0.50	n/a
	Yealm 1	–27.35 ± 0.31	4.94 ± 0.23	n/a
	Yealm 2	–26.95 ± 0.64	5.68 ± 0.34	n/a
	Yealm 3	–20.63 ± 0.38	8.46 ± 0.30	n/a
	Yealm 4	–21.95 ± 1.48	6.98 ± 0.15	n/a

Table A1 (continued)

Carbon source	Site	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Sediment carbon	Avon 1	−28.52 ± 0.00	4.22 ± 0.00	n/a
	Avon 2	−27.77 ± 0.00	5.05 ± 0.00	n/a
	Avon 3	−28.83 ± 0.00	5.62 ± 0.00	n/a
	Avon 4	−18.10 ± 0.00	5.36 ± 0.00	n/a
	Erme 1	−27.07 ± 0.00	4.16 ± 0.00	n/a
	Erme 2	−27.52 ± 0.00	5.02 ± 0.00	n/a
	Erme 3	−24.04 ± 0.00	6.30 ± 0.00	n/a
	Erme 4	−22.64 ± 0.00	5.35 ± 0.00	n/a
	Yealm 2	−26.10 ± 0.00	n/a	n/a
	Yealm 3	−23.59 ± 0.00	5.09 ± 0.00	n/a
	Yealm 4	−22.15 ± 0.00	n/a	n/a

Table A2. Mean (±SD) stable isotope ratios for invertebrate taxa at each of the sampled estuarine sites. Sites were located from the freshwater–estuary interface (Site 1) to estuary mouth/fully marine conditions (Site 4). n/a: not applicable

Taxon	Site	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Oligochaeta	Avon 1	−25.11 ± 0.09	6.23 ± 0.01	7.88 ± 0.46
	Avon 2	−25.34 ± 0.08	9.35 ± 0.03	4.78 ± 0.03
	Avon 3	−21.66 ± 0.00	10.62 ± 0.00	5.42 ± 0.00
	Erme 1	−26.74 ± 0.13	6.61 ± 0.11	5.06 ± 0.12
	Erme 2	−21.19 ± 0.15	9.55 ± 0.29	8.93 ± 0.59
	Erme 3	−21.15 ± 0.00	10.26 ± 0.00	11.71 ± 0.00
	Yealm 1	−26.60 ± 0.03	6.15 ± 0.14	6.29 ± 0.36
	Yealm 2	−23.36 ± 1.09	10.02 ± 1.30	9.02 ± 2.01
	Yealm 3	−19.56 ± 0.25	9.64 ± 0.13	n/a
Polychaeta (*Nereis diversicolor*)	Avon 2	−19.27 ± 0.03	10.49 ± 0.03	14.93 ± 0.07
	Avon 3	−18.41 ± 0.30	10.60 ± 0.08	17.31 ± 0.24
	Erme 2	−20.62 ± 0.21	10.75 ± 0.15	16.25 ± 0.08
	Erme 3	−16.22 ± 0.16	11.53 ± 0.07	18.75 ± 0.21
	Yealm 2	−18.22 ± 0.03	12.38 ± 0.03	19.43 ± 0.12
	Yealm 3	−17.96 ± 0.26	10.37 ± 1.11	17.50 ± 0.39
Amphipoda	Avon 1	−25.65 ± 0.00	7.04 ± 0.00	n/a
	Avon 2	−18.10 ± 0.04	7.60 ± 0.02	15.39 ± 0.20
	Avon 3	−17.94 ± 0.05	8.96 ± 0.04	20.70 ± 0.08
	Avon 4	−16.71 ± 0.03	8.23 ± 0.04	21.45 ± 0.20
	Erme 2	−16.14 ± 0.87	8.24 ± 2.96	19.44 ± 0.14
	Erme 3	−16.65 ± 0.46	9.70 ± 0.57	19.93 ± 0.18
	Erme 4	−14.57 ± 0.74	10.77 ± 0.21	20.52 ± 0.36
	Yealm 2	−18.40 ± 0.43	10.12 ± 0.38	16.96 ± 0.16
	Yealm 3	−15.42 ± 0.41	9.82 ± 0.39	19.72 ± 0.10
	Yealm 4	−16.37 ± 0.28	9.61 ± 0.15	19.50 ± 0.18
Other crustacea (*Crangon crangon*, *Neomysis integer*)	Avon 2	−21.07 ± 0.00	10.06 ± 0.00	n/a
	Avon 3	−20.23 ± 0.00	10.35 ± 0.00	n/a
	Erme 2	−17.50 ± 0.61	12.34 ± 0.91	16.30 ± 0.11
	Erme 3	−16.27 ± 0.46	12.98 ± 0.67	17.08 ± 0.86
	Erme 4	−14.19 ± 0.21	13.13 ± 0.45	19.44 ± 0.19
	Yealm 2	−16.34 ± 0.76	12.62 ± 1.09	17.06 ± 0.27
	Yealm 3	−15.49 ± 1.20	11.59 ± 0.53	16.83 ± 1.82
	Yealm 4	−16.74 ± 0.34	11.36 ± 0.17	19.06 ± 0.05

Editorial responsibility: Matthias Seaman, Oldendorf/Luhe, Germany

Submitted: August 2, 2006; Accepted: March 20, 2009
Proofs received from author(s): May 18, 2009