δ^{15}N and δ^{13}C in the Mondego estuary food web: Seasonal variation in producers and consumers

Alexandra Baeta [a,*], Rute Pinto [a], Ivan Valiela [b], Pierre Richard [c], Nathalie Niquil [c], João C. Marques [a]

[a] IMAR – Institute of Marine Research, Department of Zoology, University of Coimbra, 3004-517 Coimbra, Portugal
[b] The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543, USA
[c] Centre de Recherche sur les Ecosystèmes Littoraux Anthropisés – UMR6217 CNRS, IFREMER, Université de La Rochelle, Avenue Michel Crépeau 17042 La Rochelle, France

ARTICLE INFO

ABSTRACT

Assessments of temporal variation in stable carbon and nitrogen ratios were used to examine seasonal trends of the water column and benthic food webs in the Mondego estuary (Portugal). There was a marked seasonality in weather and water column conditions, including nutrient supply and chlorophyll concentrations. In spite of the pronounced environmental changes, we found little evidence of seasonal variation in δ^{13}C and δ^{15}N of producers and consumers in the Mondego estuary, with a few notable exceptions. Nitrogen isotope ratios in macrophytes (*Zostera noltii*, *Ulva* sp., *Enteromorpha* sp., and *Gracilaria* sp.), and in two grazers (*Idotea chelipes* and *Lekanesphaera levii*) increased during late summer, with the highest δ^{15}N values being measured in July, during a period of elevated temperatures and drought, which may have favored high rates of denitrification and heavier δ^{15}N values. The results suggest that stable-isotope values from macrophytes and selected grazers are useful as tracers of seasonal changes in nitrogen inputs into estuaries, and that those of consumers reflect other factors beyond seasonal variations in N and C sources.

© 2008 Published by Elsevier Ltd.

1. Introduction

Increased anthropogenic delivery of nutrients to water bodies, both freshwater and estuarine, has caused detrimental changes in habitat, food web structure, and nutrient cycling (Valiela et al., 1997; Cole et al., 2004). The resulting eutrophication has many adverse effects within the estuaries (D'Avanzo et al., 1996; Hauxwell et al., 2003). Increased N loading can lead to the loss of important estuarine habitats such as seagrass meadows (Hauxwell et al., 2003). Eutrophic estuaries can also suffer from hypoxia and anoxia (Zimmerman and Canuel, 2000), and phytoplankton and macroalgal blooms (Hauxwell et al., 2003).

To better understand management of water quality, it is important to know the sources, as well as the amount of inputs of the nutrient limiting production. In the Mondego estuary, as in most estuarine ecosystems, there is evidence that at least for macroalgal growth, nitrogen is the limiting factor (Teichberg et al., submitted for publication). δ^{15}N has proven useful as a tracer of the major source of nitrogen entering coastal waters. Joint use of δ^{15}N and δ^{13}C has further shown promise as a tool that helps to explain how the external N sources, as well as the C sources, move up into estuarine food webs. Application of these isotopic ratios has largely remained an item of research rather than a management tool (Peterson and Fry, 1987; Cole et al., 2004). The practical utility of stable-isotopic ratios to some degree depends on the relative sensitivity of δ^{15}N and δ^{13}C to seasonal variation.

Stable-isotopic N ratios might, in addition, change with increased temperatures as we might find seasonally, but also as might be forced by global atmospheric warming. Microbial processes such as denitrification are strongly affected by temperatures (Valiela, 1995), and higher denitrification could result in notable fractionation of δ^{15}N. This indirect linkage could furnish heavier N that is taken up by producers.

Some studies reported that δ^{15}N and δ^{13}C of producers showed seasonal variation (Riera and Richard, 1996, 1997; Fourqurean et al., 1997; Kang et al., 1999; Adin and Riera, 2003; Machás et al., 2003; Riera and Hubas, 2003; Pruell et al., 2006), while others did not (McClelland and Valiela, 1998; Cole et al., 2004). Similarly, some studies showed variation in consumers (Goering et al., 1990; Riera and Richard, 1996, 1997; Buskey et al., 1999; Kang et al., 1999; Carman and Fry, 2002; Kibirige et al., 2002; Moens et al., 2002; Adin and Riera, 2003; Machás et al., 2003; Riera and Hubas, 2003; Vizzini and Mazzola, 2003, 2005), and others did not (Goering et al., 1990). Knowledge of seasonal variation in stable-isotopic ratios is important as a reflection of biogeochemical and ecological processes, as well as in regard to sampling schedules and expected variability for applied monitoring schedules.

In this paper, we examine the seasonal variation in N and C stable-isotopic ratios of producers and consumers within the food

---

* Corresponding author. Tel.: +351 239 837797; fax: +351 239 823603.
  E-mail address: asbaeta@ci.uc.pt (A. Baeta).

web of the Mondego estuary, and compare the changes in ratios in organisms to the seasonal changes in temperature, precipitation, dissolved nutrients, and in phytoplankton chlorophyll we measured in the Mondego ecosystem. This comparison aims to discern the degree to which seasonally varying driving factors might be manifest in the isotopic ratios of the food web, as well as identify the components of the food web that might be reasonably reliable indicators of changes in nutrient enrichment and warming.

2. Methods

2.1. Study site

The Mondego estuary is a relatively small (1600 ha), warm-temperate, polyhaline, intertidal system located on the Atlantic coast of Portugal, and consists of two arms, north and south (Fig. 1). The southern arm is characterised by large areas of intertidal mudflats (almost 75% of the area) exposed during low tide. The system receives agricultural runoff from 15,000 ha of upstream cultivated land (mainly rice fields) and supports a substantial population, industrial activities, salt-works, and aquaculture farms, and is also the location of the Figueira da Foz city harbour, which constitutes a tourism centre. All these activities have imposed a strong anthropogenic impact. A mixture of inputs from sewage effluent, agricultural runoff, as well as releases from maricultural activity contributes to the nutrient loads entering the Mondego estuary.

In the early 1990s, the southern arm was almost silted up in the upstream areas, causing the river discharge to flow essentially through the northern arm. Consequently, the water circulation in the southern arm became mostly dependent on the tides and on the small freshwater input from a tributary, the Pranto River, artificially controlled by a sluice (Marques et al., 2003). In 1990–1992, the communication between the two arms of the estuary became totally interrupted in the upstream area due to the completion of stonewalls in the northern arm banks. Following this interruption, the ecological conditions in the southern arm suffered a rapid deterioration. The combined effect of an increased water residence time and of nutrient concentrations became major driving forces behind the occurrence of seasonal blooms of Ulva sp. and a concomitant severe reduction of the area occupied by Zostera noltii beds, previously the richest habitat in terms of productivity and biodiversity (Marques et al., 1997, 2003). The shift in benthic primary producers affected the structure and functioning of the biological communities, and through time such modifications started inducing the emergence of a new selected trophic structure, which has been analysed in abundant literature (e.g. Dolbeth et al., 2003; Cardoso et al., 2004a,b; Patrício et al., 2004).

From 1998 to 2006 several interventions were carried out to ameliorate the condition of the system, namely, by improving water circulation, which was followed by a partial recovery of the area occupied by Z. noltii and the cessation of green Ulva sp. blooms (Lillebø et al., 2005, 2007).

2.2. Sample collection and preparation

To assess water quality of the Mondego waters, we collected water samples on a monthly basis at two sites (Zostera site and bare sediment site; Fig. 1), from November 2005 to July 2006. In a companion paper (Baeta et al., submitted for publication) we established that there were no differences in nutrients or chlorophyll concentrations in samples taken from the two sites, and so here we treat the samples as replicates. In each sample, we measured concentrations of nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}), and the concentration of chlorophyll a. Samples
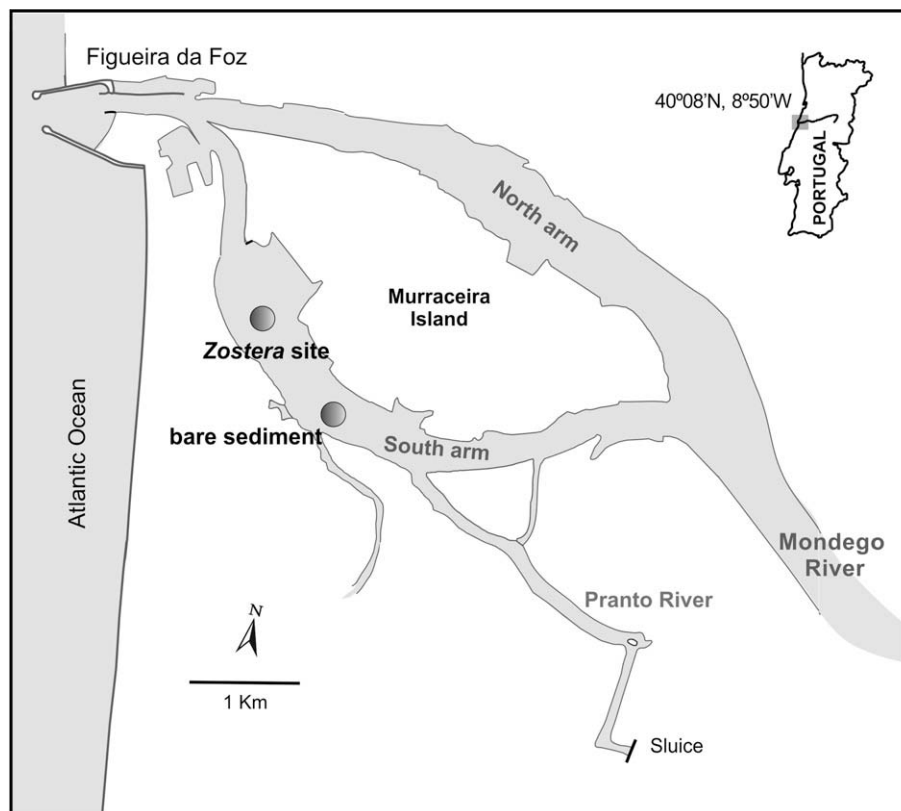

Fig. 1. Mondego estuary map showing sampling sites: Zostera and bare sediment sites (grey circles).

were immediately filtered (Whatman GF/F glass–fibre filter) and stored frozen at −18 °C until the analysis following standard methods described in Limnologisk Metodik (1992) for (NH$_4^+$) and PO$_4^{3-}$, and in Strickland and Parsons (1972) for NO$_3^-$, and nitrite NO$_2^-$. The phytoplankton chlorophyll *a* determinations were performed by filtering 0.5–1.0 l of water through Whatman GF/F glass–fibre filters (Parsons et al., 1985). In the field and during transportation to the laboratory, samples were stored on ice and protected from light. Data on monthly precipitation and air temperature were derived from the nearby city of Coimbra (Instituto de Meteorologia, Coimbra forecast station).

We measured stable-isotopic values in components of the Mondego food web, including particulate organic matter (POM), sedimentary organic matter (SOM), meiofauna, seagrass, macroalgae, macrobenthos, zooplankton, and the fish in each of the two sites. To evaluate the seasonal variation in the isotopic values, we repeated the sampling in November (2005), and in February, May, and July (2006) at the two sites in the south arm of the estuary. Water samples for POM were collected monthly.

POM was obtained by filtering 0.5–1 l of seawater, from a depth of 0.5 m below the surface, onto precombusted (450 °C, 4 h) Whatman GF/F filters (0.45 μm pore size) with a low pressure vacuum pump. Sediment samples from the upper 1 cm were collected with an acrylic corer (31 mm of diameter), and analysed for the isotopic composition. For the meiofauna, sediment samples were collected, and the top 3 cm of each sediment core was then passed through 500 μm and 38 μm sieves. Meiofauna were examined from the 38 μm fraction, and samples for isotopic analysis were composites of 50–300 individuals. Seagrass leaves and roots, and macroalgae were collected by hand and gently cleaned of epiphytic material. Macroinvertebrates were also taken manually from each site, and held in filtered sea water for 24 h to allow their guts to clear.

Zooplankton was collected by towing a Bongo net (0.5 m diameter, 200 μm mesh size) against the current for 20 min. The zooplankton samples for isotope analysis were composites of 20–200 individuals. Resident (*Atherina boyeri*, *Pomatoschistus microps*, *Pomatoschistus minutus*, *Syngnathus abaster*, *Syngnathus acus*) and transient (*Dicentrarchus labrax*, *Solea solea*) fish species were collected using a 2 m beam trawl, with 5 mm stretched mesh size on the cod end. The trawls were carried out during the night, at low water during spring tides, but only at the bare sediment site. These mobile taxa (fish) could easily manage the short distance between the two sites (Fig. 1), so it was not considered worthwhile to collect samples at the two sites.

All samples were rinsed with Milli-Q water (filters with POM were rinsed with ammonium formate to remove the salts), and then freeze-dried. When dry, samples were ground (filters with POM were kept whole) into a homogenous powder using mortar and pestle, and combined to make single composite samples of each species/group per site per sampling date. Samples were then weighed, and loaded into tin capsules. Whole organisms were used in all cases except for bivalves and decapods, the shells of which were removed, and for fish, only muscle of the dorsal region was analysed. No acidification was applied to the samples to avoid alterations in the isotopic signal (Mateo et al., 2008).

Samples were analysed using an EA-IRMS (Isoprime, Micromass, UK). Isotopic values were expressed in the δ unit notation as deviations from standards (Vienna Pee Dee Belemnite for δ^{13}C and N$_2$ in air for δ^{15}N) following the formula: δ^{13}C or δ^{15}N = $[(R_{sample}/R_{standard})-1] \times 10^3$, where R is ^{13}C/^{12}C or ^{15}N/^{14}N. The analytical precision for the measurement was 0.2‰ for both carbon and nitrogen.

The data were analysed using ANOVA to test the null hypothesis that there were no significant differences in either the δ^{15}N or δ^{13}C composition of each group/species among seasons (autumn, winter, spring, and summer).

3. Results and discussion

3.1. Seasonal ambient conditions

Weather varied substantially during the sampling period (Fig. 2). Temperatures varied according to the season, with, on average, winter maxima around 15 °C rising to 25 °C in summer. In addition, during the period over which we sampled the estuary there were marked departures from average conditions. In particular, during our last sampling interval in July, very warm temperatures (near 40 °C) (Fig. 2, top) were brought about by a northern incursion of an African air mass. The sampling period was also the one during which Portugal suffered a lengthy drought, relative to average long-term precipitation (Fig. 2, bottom).

There was a marked seasonality to conditions in the water column (Fig. 3). Nitrate concentrations were high during winter, and diminished about fourfold during the warmer months. Concentrations of NH$_4^+$ and PO$_4^{3-}$ were usually much lower than those of NO$_3^-$ (Fig. 3, top). N/P generally above the 16:1 Redfield ratio during colder months and below it in warmer months. This suggests that throughout the winter months, P supply might have been the limiting nutrient, while during the summer, N might limit producer growth (Fig. 3, middle). Chlorophyll *a* concentrations peaked in spring, perhaps drawing down nitrate concentration during warmer months (Fig. 3, bottom).

3.2. Seasonal changes in isotopic values

We collected 45 different taxa and analysed their stable carbon and nitrogen isotopic compositions. These taxa included five primary producers, POM, sediment, 21 macroinvertebrate species,
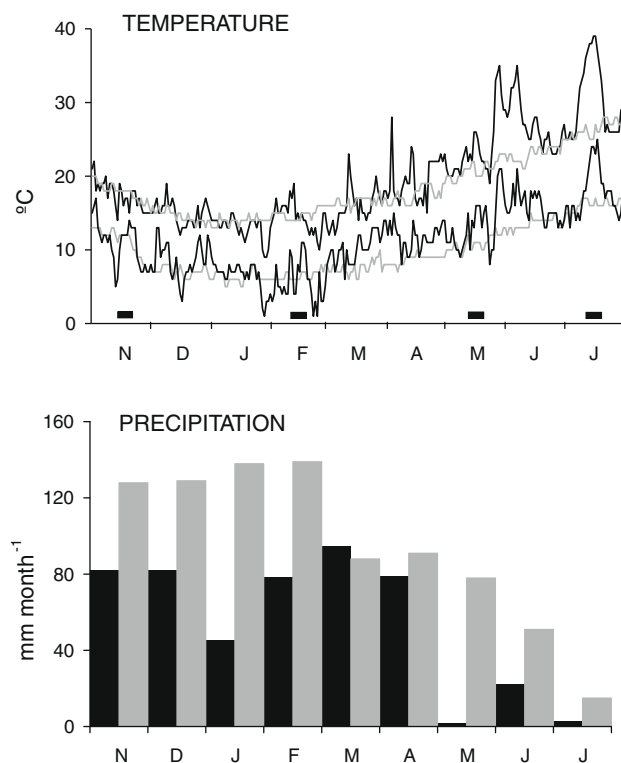

Fig. 2. Top: daily maximum and minimum air temperature, from November 2005 to July 2006 (black lines), and daily maximum and minimum air temperature means for 1961–1990 (grey lines). Black rectangles show when were the sampling periods. Bottom: precipitation values, from November 2005 to July 2006 (black bars), and precipitation means for 1961–1990 (grey bars).
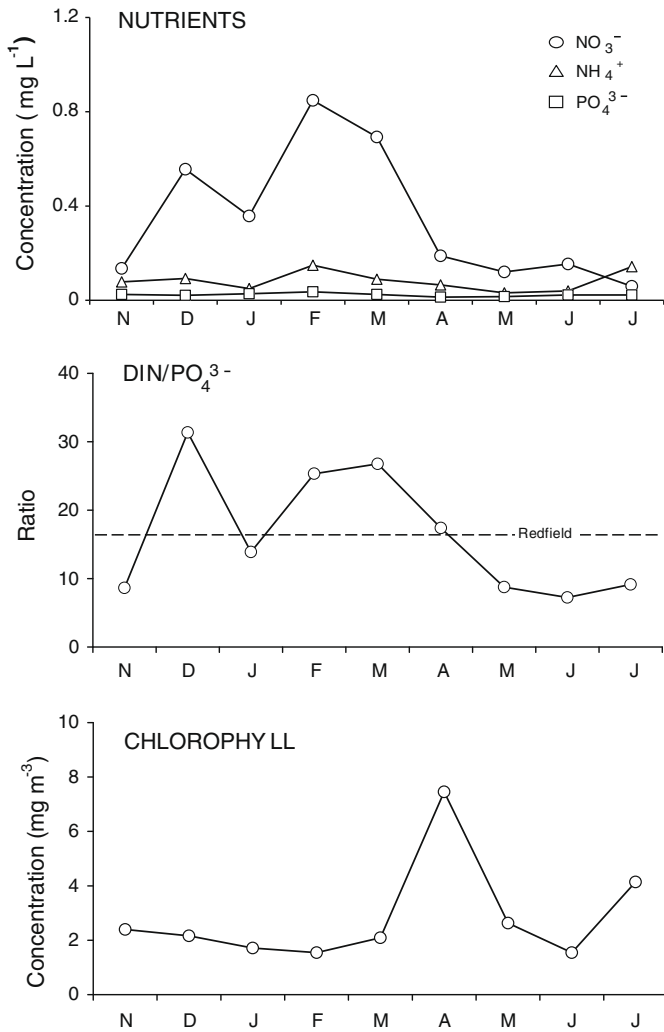
Fig. 3. Mean of nitrate, ammonium, and phosphate (top), DIN/PO$_4^{3-}$ (middle), and phytoplankton chlorophyll a (bottom) concentrations, from the two sampling sites, *Zostera* and bare sediment sites, in the Mondego estuary, from November 2005 to July 2006.

Table 1
$\delta^{13}C$ and $\delta^{15}N$ of primary producers and consumers collected from Mondego estuary. Data are sample size (*N*), and mean δ values (±SE), from November 2005 to July 2006.

Group/species	Abbreviation	$\delta^{13}C$ Mean ± SE	$\delta^{15}N$ Mean ± SE	N
Macrophytes				
Enteromorpha sp.	E	−12.9 ± 0.4	12.7 ± 1.2	8
Fucus sp.	F	−16.3 ± 0.5	9.7 ± 0.4	8
Gracilaria sp.	G	−17.4 ± 0.7	9.7 ± 1.0	8
Ulva sp.	U	−11.4 ± 0.3	12.3 ± 0.9	8
Zostera noltii (leaves)	Zl	−12.5 ± 0.1	9.5 ± 1.5	8
Zostera noltii (roots)	Zr	−12.2 ± 0.2	11.3 ± 3.3	8
Particulate organic matter	POM	−22.8 ± 0.2	5.9 ± 0.2	18
Sediment	SOM	−21.31 ± 0.4	4.8 ± 0.4	8
Amphipoda				
Amphithoe valida	Av	−14.8 ± 0.3	10.9 ± 0.2	8
Echinogammarus marinus	Em	−14.7 ± 0.5	10.6 ± 0.3	8
Melita palmata	Mp	−15.5 ± 0.7	9.9 ± 0.2	8
Bivalvia				
Cerastoderma edule	Ce	−19.1 ± 0.2	7.8 ± 0.3	8
Mytilus galloprovincialis	Mg	−19.2 ± 0.4	7.2 ± 0.3	8
Scrobicularia plana	Sp	−17.4 ± 0.5	9.0 ± 0.2	8
Decapoda				
Carcinus maenas	Cm	−16.4 ± 0.3	12.1 ± 0.2	8
Crangon crangon	Ccr	−15.3 ± 0.3	11.6 ± 0.1	8
Gastropoda				
Gibbula umbilicalis	Gu	−12.0 ± 1.5	10.7 ± 1.3	3
Hydrobia ulvae	Hu	−11.5 ± 0.3	9.6 ± 0.1	8
Littorina litorea	Ll	−11.2 ± 0.4	12.2 ± 0.1	4
Isopoda				
Cyathura carinata	Cc	−14.3 ± 0.4	11.7 ± 0.2	8
Idotea chelipes	Ic	−14.5 ± 0.5	9.9 ± 0.5	8
Lekanesphaera levii	Llev	−12.0 ± 0.4	8.7 ± 0.4	8
Meiofauna				
Nematoda	Ne	−16.3 ± 0.5	11.3 ± 0.3	8
Copepoda	Co	−16.3 ± 0.4	10.9 ± 0.3	8
Polychaeta				
Alkmaria romijni	Ar	−16.7 ± 0.7	10.7 ± 0.2	6
Capitella capitata	Cca	−16.4 ± 0.3	10.8 ± 0.3	7
Glycera tridactyla	Gt	−14.3 ± 0.2	13.5 ± 0.3	5
Hediste divers icolor	Hd	−14.2 ± 0.2	11.8 ± 0.3	7
Heteromastus filiformis	Hf	−16.2 ± 0.2	11.5 ± 0.2	7
Nephtys cirrosa	Nc	−15.6 ± 0.7	12.6 ± 0.3	6
Streblospio shrubsolii	Ssh	−16.9 ± 0.7	10.8 ± 0.4	5
Fishes				
Atherina boyeri	Ab	−18.0 ± 0.6	11.7 ± 0.7	2
Dicentrarchus labrax (juv)	Dl	−18.2 ± 0.6	14.2 ± 0.6	2
Diplodus vulgaris (juv)	Dv	−17.9 ± 0.5	15.0 ± 0.3	4
Pomatoschistus microps (juv)	Pm	−22.4 ± 0.3	14.0 ± 0.7	2
P. minutus	Pmi	−17.4 ± 0.4	13.1 ± 0.3	4
Syngnathus abaster	Sa	−15.0 ± 0.9	12.3 ± 0.6	2
S. acus	Sac	−16.2 ± 0.0	11.4 ± 0.9	2
Solea solea (juv)	Ss	−23.5 ± 0.4	14.8 ± 0.6	4
Zooplankton				
Acartia tonsa	At	−23.2 ± 0.9	9.7 ± 0.4	8
Acartia sp.	Asp	−18.5 ± 0.5	8.4 ± 0.2	8
Cladocera	Cl	−21.9 ± 1.0	9.4 ± 0.8	3
Mysidacea (juv)	My	−18.9 ± 0.7	8.1 ± 0.4	4
Pomatochistos sp. (larvae)	Pl	−20.0 ± 0.5	9.8 ± 0.3	3
Sagittafriderici	Sf	−18.2 ± 0.6	11.1 ± 0.3	6
Zoeae (brachyura)	Zo	−18.7 ± 0.4	9.0 ± 0.2	6

two meiofauna groups, eight fish species, and seven zooplankton taxa (Table 1).

There was a consistent lack of seasonal pattern in the isotopic values in most compartments of the Mondego ecosystem (Figs. 4 and 5). Thirty of the 37 compartments measured showed no evidence of seasonal changes (Table 2).

For $\delta^{13}C$ only the copepod *Acartia tonsa* showed a seasonal variation, becoming less negative in spring and summer. Buskey et al. (1999) showed that *A. tonsa* living over seagrass beds obtain a larger proportion of their carbon from seagrass than do nearby populations living over muddy bottoms without seagrass. In our study, during the most intense periods of the phytoplankton bloom, $\delta^{13}C$ values in *A. tonsa* became less negative, suggesting that more seagrass carbon might have been entering their diets. On the other hand, the much depleted carbon signatures for the most of the year could be due to a great contribution of terrestrial organic matter, since several studies have shown that terrestrial plants have the most depleted $\delta^{13}C$ signatures, around −26‰ (e.g. Vizzini and Mazzola, 2003).

The $\delta^{15}N$ of producers and consumers consistently lacked significant seasonal variation across most of the growing season (Table 2), and only on one date in July did it become significantly higher ($p < 0.05$) in the $\delta^{15}N$ of producers. The stable-isotopic values of C

and N in these producers and consumers therefore showed no seasonal variation. In contrast, the $\delta^{15}N$ of producers rapidly increased seemingly as a seasonal response to certain conditions.

High nitrogen isotopic signatures found in producers in July may have resulted from seasonal changes in biogeochemical processes, such as denitrification. Denitrification is temperature dependent and takes place under anaerobic conditions. This process may lead to a loss of isotopically light ^{14}N, which enriches
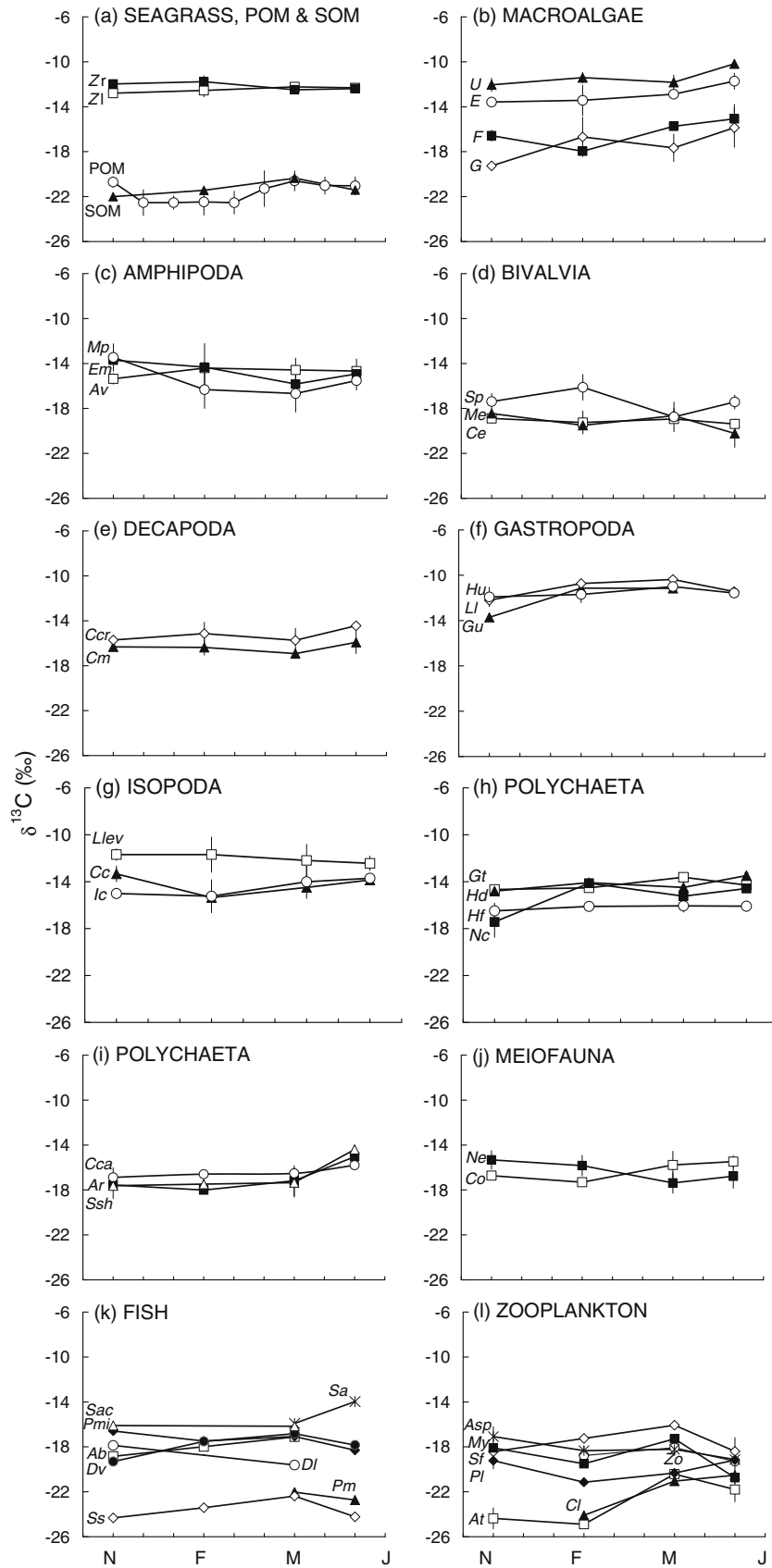
Fig. 4. C stable-isotopic values (mean ± SE) for all the groups/species collected in the Mondego estuary, from November 2005 to July 2006. Abbreviations of species/groups are shown in Table 1.
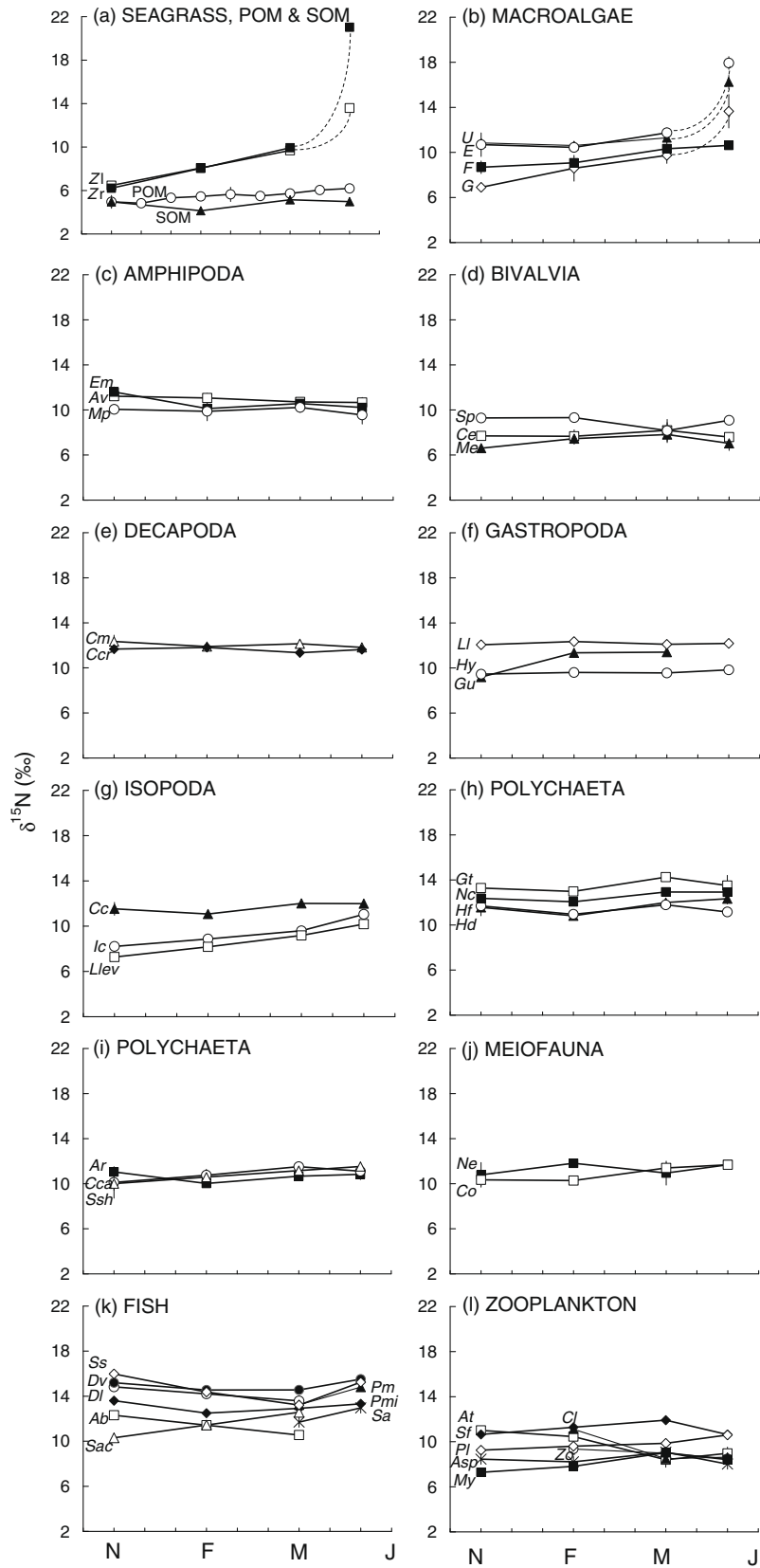
Fig. 5. N stable-isotopic values (mean ± SE) for all the groups/species collected in the Mondego estuary, from November 2005 to July 2006. Abbreviations of species/groups are shown in Table 1.

the remaining DIN pool with [15]N. During the unusually warm event in July there was a strong sulfitic smell, which suggested widespread anoxia that could have favored high denitrification rates.

Table 2
ANOVA results testing seasonal differences for C and N isotope ratios of groups/species collected in the Mondego estuary.

Group/species	$\delta^{13}C$		$\delta^{15}N$	
	F	df	F	df
Macrophytes				
Enteromorpha sp.	0.983	3	30.678[**]	3
Fucus sp.	0.822	3	3.990	3
Gracilaria sp.	1.051	3	2.801	3
Ulva sp.	3.615	3	49.339[**]	3
Zostera noltii (leaves)	2.004	3	32.239[**]	3
Zostera noltii (roots)	1.229	3	32.247[**]	3
Particulate organic matter	1.926	3	2.046	3
Sediment	5.412	3	3.294	3
Amphipoda				
Amphithoe valida	0.218	3	0.77	3
Echinogammarus marinus	0.648	3	1.178	3
Melita palmata	1.074	3	4.091	3
Bivalvia				
Cerastoderma edule	0.159	3	0.204	3
Mytilus galloprovincialis	0.797	3	0.899	3
Scrobicularia plana	1.167	3	1.856	3
Decapoda				
Carcinus maenas	0.397	3	0.526	3
Crangon crangon	0.315	3	0.561	3
Gastropoda				
Hydrobia ulvae	0.347	3	1.348	3
Isopoda				
Cyatura carinata	2.063	3	1.797	3
Idotea chelipes	0.532	3	11.204[*]	3
Lekanesphaera levii	0.116	3	10.327[*]	3
Meiofauna				
Nematoda	0.903	3	0.435	3
Copepoda	0.853	3	4.471	3
Polychaeta				
Alkmaria romijni	1.203	2	1.774	2
Capitella capitata	0.659	3	0.669	3
Glycera tridactyla	0.77	3	0.359	3
Hediste diversicolor	4.305	3	0.645	3
Heteromastus filiformis	0.131	3	4.557	3
Nephtys cirrosa	1.943	3	0.552	3
Streblospio shrubsolii	2.966	2	1.746	2
Fishes				
Diplodus vulgaris (juv)	7.428	3	4.261	3
P. minutus	4.668	3	3.875	3
Solea solea (juv)	3.939	3	7.186	3
Zooplankton				
Acartia tonsa	15.897[*]	3	3.628	3
Acartia sp. (marine species)	5.837	3	4.245	3
Sagitta friderici	8.585	2	3.613	2
Mysidacea	4.016	3	1.320	3
Zoea (*C. maenas*)	0.787	2	14.653	2

[*] $p < 0.05$, [**] $p < 0.01$; The absence of * means no significant differences.

The enrichment of $\delta^{15}N$ in the producers increased significantly with warmer temperatures (Fig. 6), as might be expected if a temperature-dependent process such as denitrification was indeed involved. It is not surprising to find that macrophyte isotopic values show seasonal changes in N supply: isotopic values of macrophyte fronds change in a matter of hours to a few days (Teichberg et al., 2007), since their internal nitrogen pools turnover rather quickly. The importance of the results of Fig. 6 is that if indeed global atmospheric warming increases water temperatures in estuaries such as the Mondego, we can expect a gradual increase in $\delta^{15}N$ in the producers. $\delta^{15}N$ values then could therefore be thought of as indirect indicators of warming.

Nitrogen isotope ratios in two isopods also showed seasonal variation, increasing in July. Both *I. chelipes* and *L. levii* are grazers, feeding on macrophytes (Bamber, 2004), so the increased nitrogen
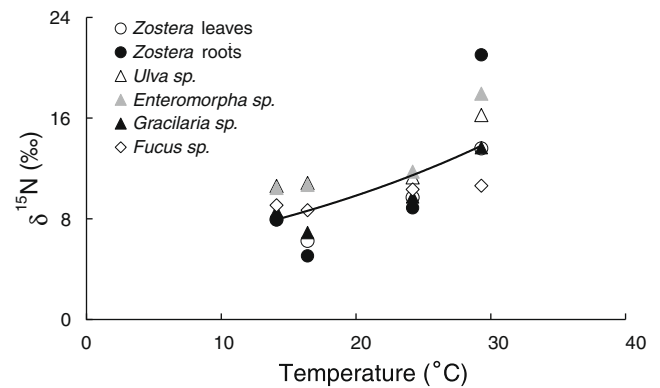

Fig. 6. N stable-isotopic ratio for primary producers (seagrass and macroalgae) collected in the Mondego estuary. N stable-isotopic data are plotted against the air temperature.

isotopic ratio in summer could be due to the enrichment found in the producers during this period, since complete turnover of these populations could occur in a matter of days if all individuals were equally mobile (Shafir and Field, 1980). Accordingly, the lack of seasonal variation of $\delta^{15}N$ enrichment in most consumers of the Mondego food web might be related to a slower turnover of internal N pools in consumers (weeks/months) compared to pools in macrophytes (days), position of species in the food web and omnivores feeding behaviour, and also probably due to the fact that, excepting isopods, the other groups do not feed directly on fresh macrophytes. This suggests that consumer isotopic values constitute a more time-integrated reflection of nitrogen isotopic values, as reported by Vander Zanden et al. (1998).

The results of this study show that there were strong seasonal driving variables in the Mondego estuary. Increased temperature increased $\delta^{15}N$ of important producers; this might be an indirect result of microbial N transformations and suggests that producer $\delta^{15}N$ might become an indicator of larger climatic trends. The influence of this seasonal forcing, as manifest in stable-isotopic ratios of consumers within the Mondego food web, was surprisingly modest, with most species showing no significant seasonal trends. These results suggest that the seasonal variation in the various factors we measured (temperature, precipitation, nutrients, and chlorophyll) within the Mondego was not enough to change isotopic signatures in consumers. This is convenient for monitoring purposes, as it frees sampling protocols from seasonal schedules.

References

Adin, R., Riera, P., 2003. Preferential food source utilization among stranded macroalgae by *Talitrus saltator* (Amphipod, Talitridae): a stable isotopes study in the northern coast of Brittany (France). Estuarine Coastal and Shelf Science 56, 91–98.

Baeta, A., Valiela, I., Pinto, R., Richard, P., Niquil, N., Marques, J.C., submitted for publication. δ^{15}N and δ^{13}C in the Mondego estuary food webs: effects of eutrophication, presence of seagrass, and trophic structure. Marine Biology.

Bamber, R.N., 2004. Temporal Variation and Monitoring of Important Lagoonal Communities and Species in Wales. Bangor, CCW Marine Monitoring Report No. 12, 42p.

Buskey, E.J., Dunton, K.H., Parker, P.L., 1999. Variations in stable carbon isotope ratio of the copepod Acartia tonsa during the onset of the Texas brown tide. Estuaries 22, 995–1003.

Cardoso, P.G., Pardal, M.A., Lillebø, A.I., Ferreira, S.M., Raffaelli, D., Marques, J.C., 2004a. Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. Journal of Experimental Marine Biology and Ecology 302, 233–248.

Cardoso, P.G., Pardal, M.A., Raffaelli, D., Baeta, A., Marques, J.C., 2004b. Macroinvertebrate response to different species of macroalgal mats and the role of disturbance history. Journal of Experimental Marine Biology and Ecology 308, 207–220.

Carman, K.R., Fry, B., 2002. Small-sample methods for δ^{13}C and δ^{15}N analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. Marine Ecology Progress Series 240, 85–92.

Cole, M.L., Valiela, I., Kroeger, K.D., Tomasky, G.L., Cebrian, J.C., Wigand, J., McKinney, R.A., Grady, S.P., Silva, M.H.C., 2004. Assessment of a δ^{15}N isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. Journal of Environmental Quality 33, 124–132.

D'Avanzo, C., Kremer, J.N., Wainright, S.C., 1996. Ecosystem production and respiration in response to eutrophication in shallow temperate estuaries. Marine Ecology Progress Series 141, 263–274.

Dolbeth, M., Pardal, M.A., Lillebø, A.I., Azeiteiro, U., Marques, J.C., 2003. Short- and long-term effects of eutrophication on the secondary production of an intertidal macrobenthic community. Marine Biology 143, 1229–1238.

Fourqurean, J.W., Moore, T.O., Fry, B., Hollibaugh, J.T., 1997. Spatial and temporal variation in C:N:P ratios, δ^{15}N, and δ^{13}C of eelgrass Zostera marina as indicators of ecosystem processes, Tomales Bay, California, USA. Marine Ecology Progress Series 157, 147–157.

Goering, J., Alexander, V., Haubenstock, N., 1990. Seasonal variability of stable and carbon isotope ratios of organisms in a North Pacific bay. Estuarine Coastal and Shelf Science 30, 239–260.

Hauxwell, J., Cebrián, J., Valiela, I., 2003. Eelgrass Zostera marina loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. Marine Ecology Progress Series 247, 59–73.

Kang, C.K., Sauriau, P.G., Richard, P., Blanchard, G.F., 1999. Food sources of the infaunal suspension-feeding bivalve Cerastoderma edule in a muddy sandflat of Marennes-Oléron Bay, as determined by analyses of carbon and nitrogen stable isotopes. Marine Ecology Progress Series 187, 147–158.

Kibirige, I., Perissinotto, R., Nozais, C., 2002. Alternative food sources of zooplankton in a temporarily-open estuary: evidence from δ^{13}C and δ^{15}N. Journal of Plankton Research 24, 1089–1095.

Lillebø, A.I., Neto, J.M., Martins, I., Verdelhos, T., Leston, S., Cardoso, P.G., Ferreira, S.M., Marques, J.C., Pardal, M.A., 2005. Management of a shallow temperate estuary to control eutrophication: the effect of hydrodynamics on the system nutrient loading. Estuarine Coastal and Shelf Science 65, 697–707.

Lillebø, A.I., Teixeira, H., Pardal, M.A., Marques, J.C., 2007. Applying quality status criteria to a temperate estuary before and after the mitigation measures to reduce eutrophication symptoms. Estuarine Coastal and Shelf Science 72, 177–187.

Limnologisk Metodik., 1992. Ferskvandsbiologisk Laboratorium. Københavns Universitet (Ed.), Akademisk Forlag. København, 172p.

Machás, R., Santos, R., Peterson, B., 2003. Tracing the flow of organic matter from primary producers to filter feeders in Ria Formosa lagoon, southern Portugal. Estuaries 26, 846–856.

Marques, J.C., Pardal, M.A., Nielsen, S.N., Jørgensen, S.E., 1997. Analysis of the properties of exergy and biodiversity along an estuarine gradient of eutrophication. Ecological Modeling 102, 155–167.

Marques, J.C., Nielsen, S.N., Pardal, M.A., Jørgensen, S.E., 2003. Impact of eutrophication and river management within a framework of ecosystem theories. Ecological Modeling 166, 147–168.

Mateo, M.A., Serrano, O., Serrano, L., Michener, R.H., 2008. Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. Oecologia 157, 105–115.

McClelland, J., Valiela, I., 1998. Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries. Marine Ecology Progress Series 168, 259–271.

Moens, T., Luyten, C., Middelburg, J.J., Herman, P.M.J., Vincx, M., 2002. Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. Marine Ecology Progress Series 234, 127–137.

Parsons, T.R., Maita, Y., Lally, C.M., 1985. Pigments. In: A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, pp. 101–104.

Patrício, J., Ulanowicz, R., Pardal, M.A., Marques, J.C., 2004. Ascendency as ecological indicator: a case study on estuarine pulse eutrophication. Estuarine Coastal and Shelf Science 60, 23–35.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystems studies. Annual Review of Ecology and Systematics 18, 293–320.

Pruell, R.J., Taplin, B.K., Lake, J.L., Jayaraman, S., 2006. Nitrogen isotope ratios in estuarine biota collected along a nutrient gradient in Narragansett Bay, Rhode Island, USA. Marine Pollution Bulletin 52, 612–620.

Riera, P., Hubas, C., 2003. Trophic ecology of nematodes from various microhabitats of the Roscoff Aber Bay (France): importance of stranded macroalgae evidenced through δ^{13}C and δ^{15}N. Marine Ecology Progress Series 260, 151–159.

Riera, P., Richard, P., 1996. Isotopic determination of food sources of Crassostrea gigas along a trophic gradient in the estuarine bay of Marennes-Oléron. Estuarine Coastal and Shelf Science 42, 347–360.

Riera, P., Richard, P., 1997. Temporal variation of δ^{13}C in particulate organic matter and oyster Crassostrea gigas in Marennes-Oléron Bay (France): effect of freshwater. Marine Ecology Progress Series 147, 105–115.

Shafir, A., Field, J.G., 1980. Importance of a small carnivorous isopod in energy transfer. Marine Ecology Progress Series 3, 203–215.

Strickland, J.D.M., Parsons, T.R., 1972. A Practical Handbook of Seawater Analysis (Bulletin 167, second ed.). In: Fisheries Research Board of Canada, Ottawa, pp. 71–80.

Teichberg, M., Heffner, L.R., Fox, S., Valiela, I., 2007. Nitrate reductase and glutamine synthetase activity, internal N pools, and growth of Ulva lactuca: responses to long and short-term supply. Marine Biology 151, 1249–1259.

Teichberg, M., Fox, S., Olsen, Y., Valiela, I., Martinetto, P., Iribarne, O., Muto, E.Y., Petti, M.A.V., Corbisier, T.N., Soto, M., Páez-Osuna, F., Castro, P., Neto, J., Freitas, H., Zitelli, A., Cardinaletti, M., Tagliapietra, D., submitted for publication. N and P control of macroalgal growth in temperate and tropical estuaries: field experiments with Ulva spp. Limnology and Oceanography.

Valiela, I., 1995. Marine Ecological Processes, second ed. Springer-Verlag, New York. 686p.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K., 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnology and Oceanography 42, 1105–1118.

Vander Zanden, M.J.V., Hulshof, M., Ridgway, M.S., Rasmussen, J.B., 1998. Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. Transactions of the American Fisheries Society 127, 729–739.

Vizzini, S., Mazzola, A., 2003. Seasonal variations in the stable carbon and nitrogen isotope ratios (^{13}C/^{12}C and ^{15}N/^{14}N) of primary producers and consumers in a western Mediterranean coastal lagoon. Marine Biology 142, 1009–1018.

Vizzini, S., Mazzola, A., 2005. Feeding ecology of the sand smelt Atherina boyeri (Risso 1810) (Osteichthyes, Atherinidae) in the western Mediterranean: evidence for spatial variability based on stable carbon and nitrogen isotopes. Environmental Biology of Fish 72, 259–266.

Zimmerman, A.R., Canuel, E.A., 2000. A geochemical record of eutrophication and anoxia in Chesapeake Bay sediments: anthropogenic influence on organic matter composition. Marine Chemistry 69, 117–137.