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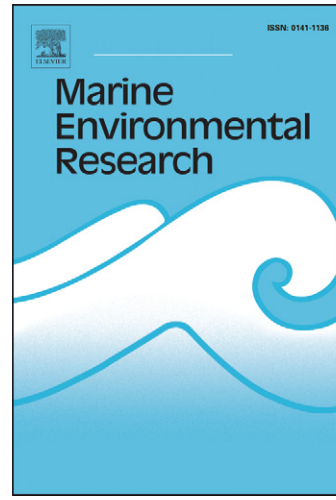


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# $\delta^{15}$N and $\delta^{13}$C in the Mondego estuary food web: seasonal variation in producers and consumers

Alexandra Baeta[1,*], Rute Pinto[1], Ivan Valiela[2], Pierre Richard[3], Nathalie Niquil[3], João C. Marques[1]

[1]IMAR - Institute of Marine Research, Department of Zoology, University of Coimbra, 3004-517 Coimbra, Portugal

[2]The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543, USA

[3]Centre de Recherche sur les Ecosystèmes Littoraux Anthropisés – UMR6217 CNRS, IFREMER, Université de La Rochelle, Avenue Michel Crépeau 17042 La Rochelle, France

*Corresponding author; phone: +351 239 837797; Fax: +351 239 823603

asbaeta@ci.uc.pt

**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 $\delta^{13}$C and $\delta^{15}$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., *Gracilaria* sp.), and in two grazers (*Idotea chelipes*, *Lekanesphaera levii*) increased during late summer, with the highest $\delta^{15}$N values being measured in July, during a period of elevated temperatures and drought, which may have favored high rates of denitrification and heavier $\delta^{15}$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.

*Keywords*: Mondego estuary; $\delta^{13}C$; $\delta^{15}N$; Coastal eutrophication; Seasonality; Estuarine species

## 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 like 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). $\delta^{15}N$ has proven useful as a tracer of the major source of nitrogen entering coastal waters. Joint use of $\delta^{15}N$ and $\delta^{13}C$ have 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 $\delta^{15}N$ and $\delta^{13}C$ to seasonal variation.

Stable isotopic N ratios might, in addition, change with increased temperatures such 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 $\delta^{15}N$. This indirect linkage could furnish heavier N that is taken up by producers.

Some studies reported that $\delta^{15}N$ and $\delta^{13}C$ 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 web of the Mondego estuary, and compare the changes in ratios in organisms to the seasonal changes in temperature, precipitation, dissolved nutrients, and 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 15000 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., 2004 a; 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 (Lillebo 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) 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 were immediately filtered (Whatman GF/F glass-fibre filter) and stored frozen at -18 ºC 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 derive 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 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 (450ºC, 4h) 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 to 300 individuals. Seagrass leaves and roots, and macroalgae were collected by hand and gently cleaned of epiphytic material. Macroinvertebates 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 to 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 removed 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 $\delta^{13}C$ and $N_2$ in air for $\delta^{15}$ N) following the formula: $\delta^{13}C$ or $\delta^{15}N$= [($R_{sample/}$ $R_{standard}$)-1] x $10^3$, where $R$ is $^{13}C/^{12}C$ or $\delta^{15}N/^{14}N$. The analytical precision for the measurement was 0.2‰ for both carbon and nitrogen.

The data were analyzed using ANOVA to test the null hypothesis that there were no significant differences in either the $\delta^{15}N$ or $\delta^{13}C$ composition of each group/species among seasons (autumn, winter, spring, 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 ºC) (Fig. 2, top) were brought about by a northern incursion of an African air mass. The sampling period was also 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 four-fold 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 was 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 composition. These taxa included 5 primary producers, POM, sediment, 21 macroinvertebrates species, 2 meiofauna groups, 8 fish species, and 7 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, 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 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 favoured high denitrification rates.

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 turn over rather quickly. The importance of the results of Figure 6 are that if indeed global atmospheric warming increases water temperatures in estuaries such as the Mondego, we can expect a gradually 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 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 & 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, 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.

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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** | Abreviation | $\delta^{13}C$ 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 edulis* | Me | -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 diversicolor* | 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 |
| *Sagitta friderici* | Sf | -18.2±0.6 | 11.1±0.3 | 6 |
| Zoeae (brachyura) | Zo | -18.7±0.4 | 9.0±0.2 | 6 |

Table 2. ANOVA results testing seasonal differences for C and N isotope ratios of groups/species collected in the Mondego estuary.* = p < 0.05, ** = p < 0.01; the absence of * means no significant differences.

| 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 edulis* | 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 |

## Figure captions

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

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.

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.

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.

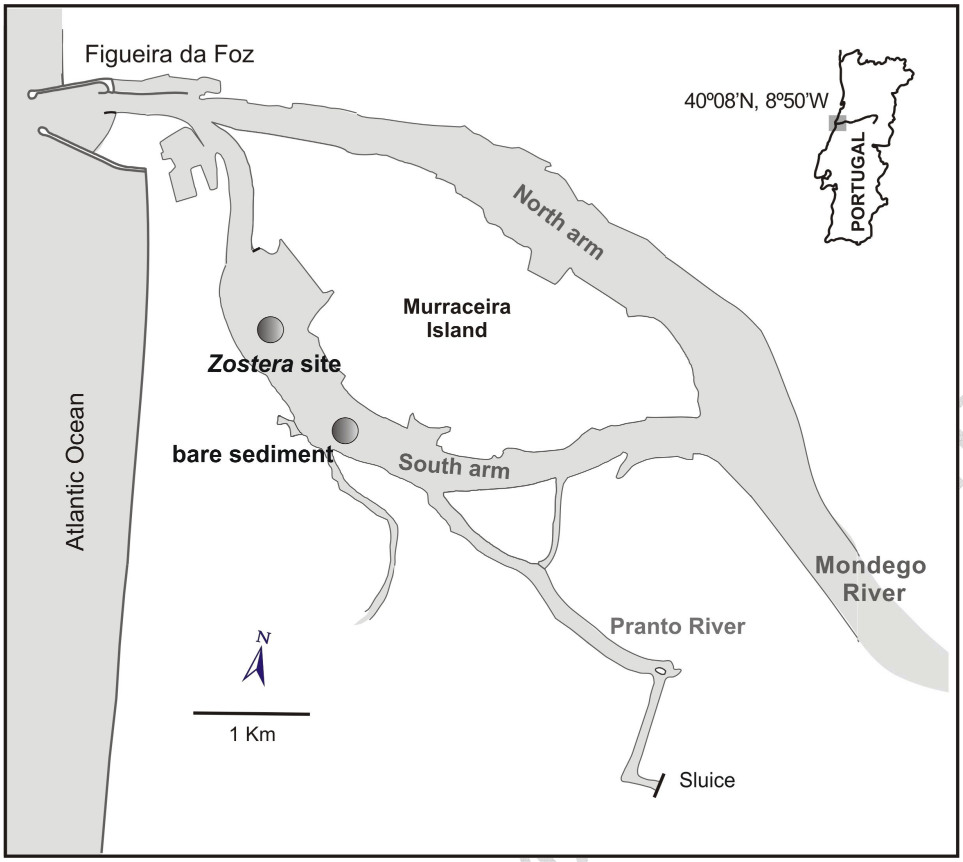
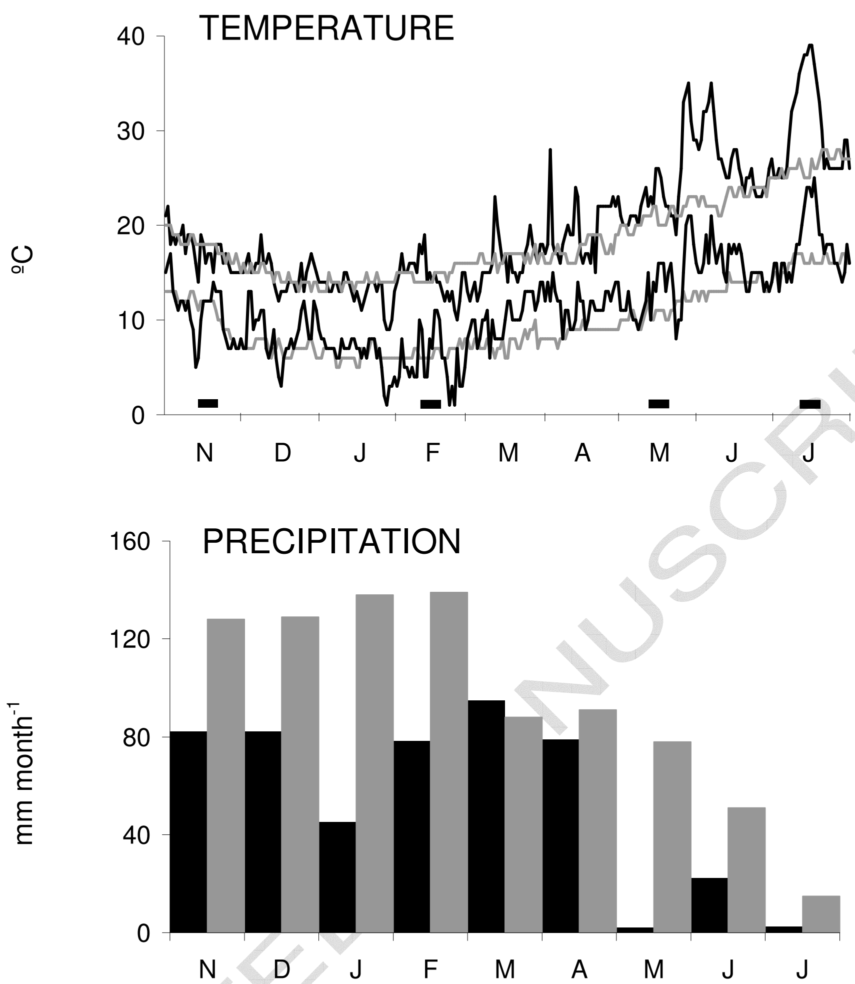

Figueira da Foz
40º08'N, 8º50'W
PORTUGAL
North arm
Murraceira
Island
*Zostera* site
bare sediment
South arm
Atlantic Ocean
Mondego
River
Pranto River
N
1 Km
Sluice

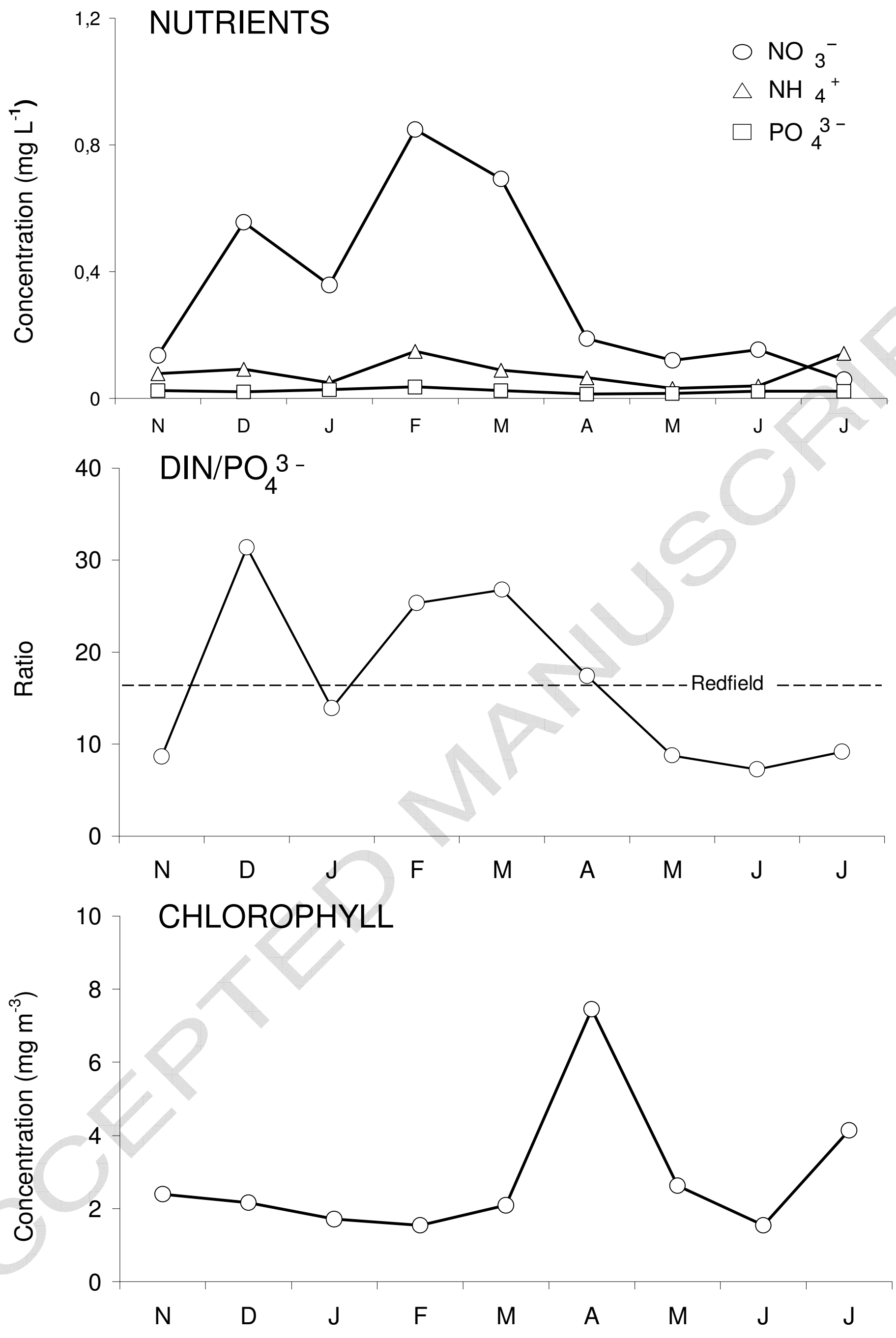
Fig. 1

410


TEMPERATURE
ºC
40
30
20
10
0
N D J F M A M J J
PRECIPITATION
mm month-1
160
120
80
40
0
N D J F M A M J J

411 Fig. 2

412



NUTRIENTS
NO3−
NH4+
PO43−
Concentration (mg L-1)
1,2
0,8
0,4
0
N D J F M A M J J
DIN/PO43-
Ratio
40
30
20
10
0
Redfield
N D J F M A M J J
CHLOROPHYLL
Concentration (mg m-3)
10
8
6
4
2
0
N D J F M A M J J


413 Fig. 3.

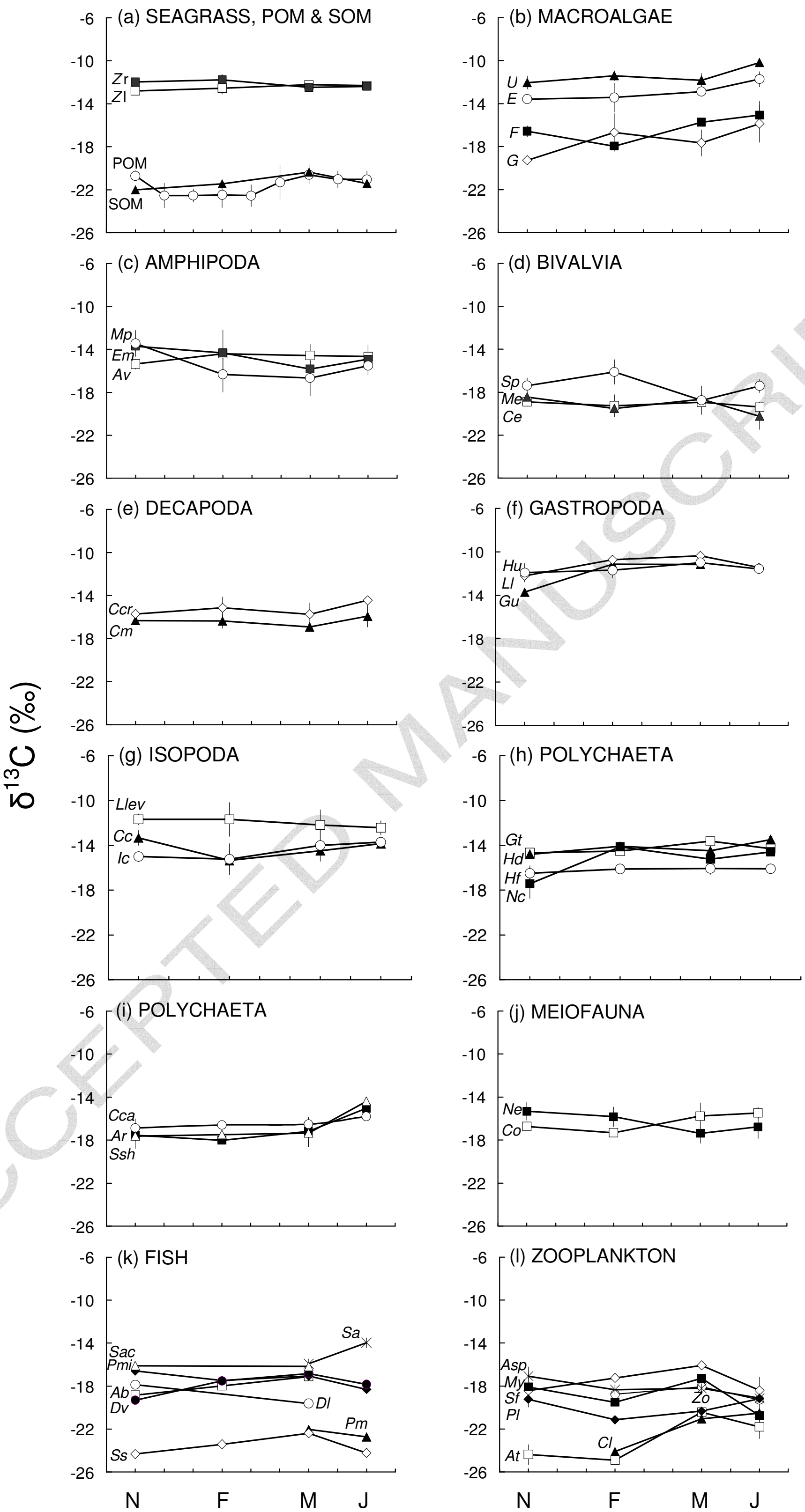
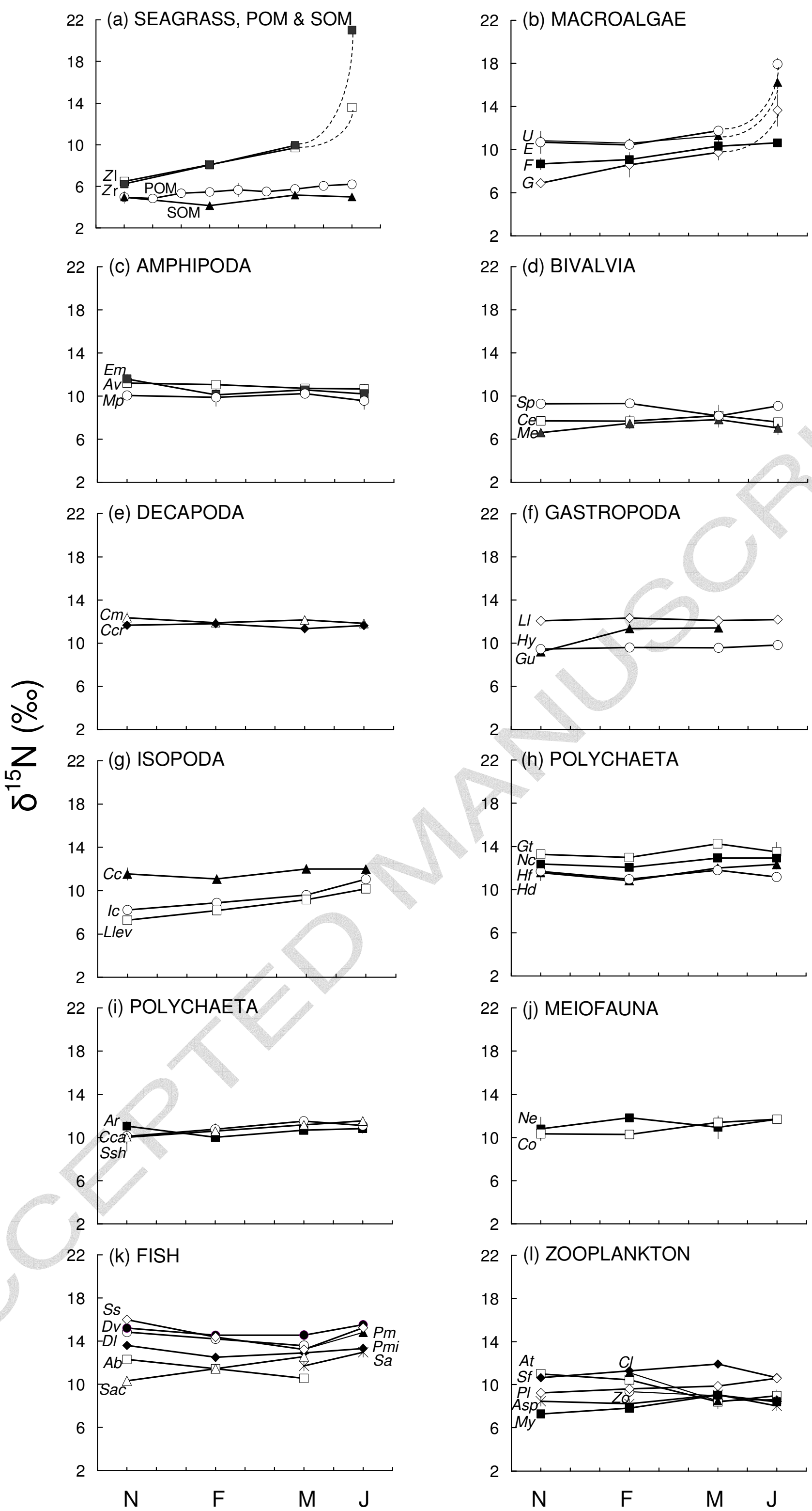

δ13C (‰)
(a) SEAGRASS, POM & SOM
Zr
Zl
POM
SOM
(b) MACROALGAE
U
E
F
G
(c) AMPHIPODA
Mp
Em
Av
(d) BIVALVIA
Sp
Me
Ce
(e) DECAPODA
Ccr
Cm
(f) GASTROPODA
Hu
Ll
Gu
(g) ISOPODA
Llev
Cc
Ic
(h) POLYCHAETA
Gt
Hd
Hf
Nc
(i) POLYCHAETA
Cca
Ar
Ssh
(j) MEIOFAUNA
Ne
Co
(k) FISH
Sa
Sac
Pmi
Ab
Dv
Dl
Pm
Ss
(l) ZOOPLANKTON
Asp
My
Sf
Pl
Zo
Cl
At
-6
-10
-14
-18
-22
-26
N
F
M
J

Fig. 4



(a) SEAGRASS, POM & SOM
Zl
Zr
POM
SOM
(b) MACROALGAE
U
E
F
G
(c) AMPHIPODA
Em
Av
Mp
(d) BIVALVIA
Sp
Ce
Me
(e) DECAPODA
Cm
Ccr
(f) GASTROPODA
Ll
Hy
Gu
(g) ISOPODA
Cc
Ic
Llev
(h) POLYCHAETA
Gt
Nc
Hf
Hd
(i) POLYCHAETA
Ar
Cca
Ssh
(j) MEIOFAUNA
Ne
Co
(k) FISH
Ss
Dv
Dl
Ab
Sac
Pm
Pmi
Sa
(l) ZOOPLANKTON
At
Sf
Pl
Asp
My
Cl
Zo
δ15N (‰)
N F M J


416

Fig. 5

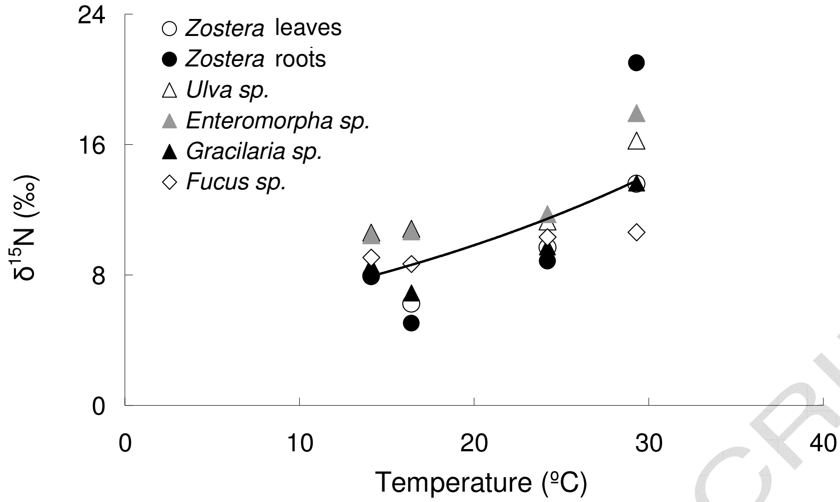

24
16
8
0
δ15N (‰)
0
10
20
30
40
Temperature (ºC)
Zostera leaves
Zostera roots
Ulva sp.
Enteromorpha sp.
Gracilaria sp.
Fucus sp.

Fig. 6