

# Eutrophication in Portuguese estuaries evidenced by $\delta^{15}\text{N}$ of macrophytes

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**ABSTRACT:** Terrestrial inputs of nitrogen in estuaries are increasing and altering salt marsh primary production, plant communities and associated biota. We used N isotope ratios and %N in *Spartina maritima*, *Halimione portulacoides*, *Zostera noltii* and below-canopy sediments to study eutrophication caused by anthropogenic land-derived nitrogen in the Mondego and Mira estuaries, Portugal. In spite of different land use practice intensities between estuaries, there were no marked differences in nitrate concentrations in the water column, but ammonium, total dissolved nitrogen and dissolved organic nitrogen concentrations were greater in the Mondego estuary. Sediment and shoot N contents suggest a general pattern of high nitrogen regimes in Mondego and Mira, and point to a somewhat higher delivery of nitrogen into the Mondego. However, these parameters were not sensitive indicators of level of eutrophication. In contrast,  $\delta^{15}\text{N}$  ratios in plant shoots differed significantly and were higher in Mondego for all 3 plant species. Plant  $\delta^{15}\text{N}$  signatures were therefore more sensitive sentinels of land-derived nitrogen load. The parallel increase of  $\delta^{15}\text{N}$  in shoots and sediments indicated that both shoots and sediments followed and reflected the level of eutrophication and some larger whole estuary difference in N loads. Thus, nitrogen isotopic signatures in macrophytes and sediments were useful indicators of land-derived nitrogen in estuaries.

**KEY WORDS:** Land-derived nitrogen ·  $^{15}\text{N}$  · Rooted macrophytes · Mondego estuary · Mira estuary

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## INTRODUCTION

Estuarine ecosystems receive nutrients from the land. The terrestrial inputs include nitrogen, the element that largely controls growth of producers in coastal estuarine systems. Increases in nitrogen loads to shallow waters directly increase primary production by phytoplankton and macroalgae and indirectly decrease production by seagrasses (Brun et al. 2003). Such changes in producers then lead to other changes that propagate up through estuarine food webs. Increased nitrogen delivery also affects salt marsh communities and dynamics (Deegan et al. 2002). Thus, evaluation of eutrophication of estuaries and effective management of nutrient loadings is a relevant and important issue.

The use of stable isotopes is a powerful technique that is cost-effective and informative, and has been

widely applied in ecology (Cloern et al. 2002, Kang et al. 2003, Cole et al. 2005). Natural abundance and fractionation of  $\delta^{15}\text{N}$  can be useful in linking land-derived nitrogen sources to rivers (Chang et al. 2002) and estuaries (Cole et al. 2004); the method is also valuable in assessing trophic chains (McClelland & Valiela 1998b) and food sources (Carmichael et al. 2004).

$\delta^{15}\text{N}$  signatures have been particularly useful in assessment of trophic position of consumers. The nitrogen isotopic ratios of consumers within a trophic level become successively enriched by about 3‰ relative to their food substrate (Carmichael et al. 2004), allowing determination of consumer trophic position and identification of nitrogen sources entering ecosystems (wastewater, atmospheric deposition and fertilizers have different  $\delta^{15}\text{N}$  signatures). Nitrogen isotope signatures become heavier as the N loads and contribution by wastewater increase (Carmichael & Valiela

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2005, Cole et al. 2005). Cole et al. (2004) found that  $\delta^{15}\text{N}$  of *Spartina alterniflora* and other macrophytes increased uniformly with wastewater inputs and dissolved inorganic nitrogen (DIN) concentrations in the water column across a geographic range. Nitrogen stable isotope measurements on the red alga *Catenella nipae* were used as a means of discerning sewage nitrogen in the environment in Australia (Costanzo et al. 2005). Nutrient discharges from sewage treatment plants have been related to  $\delta^{15}\text{N}$  in the macroalga *Fucus vesiculosus* (Savage & Elmgren 2004).

We used the stable isotope approach to study eutrophication caused by anthropogenic land-derived nitrogen in 2 Portuguese estuaries. Human land use practices on the watersheds of the Mondego River are more

intensive than on the watershed of the Mira estuary (Table 1). The upstream areas of the Mondego estuary (Fig. 1a) support a variety of human activities, including dense urban development, harbor construction, industry and agriculture. Nutrient loading and algal blooms have occurred frequently in the Mondego estuary, with reduction of seagrass cover and changes in consumer populations and trophic food web dynamics (Verdelhos et al. 2005). Long-term changes in the estuary reveal a clear reduction in the salt marsh area over the past 6 decades due to urban and industrial expansion (Castro & Freitas 2006).

Dissolved inorganic nitrogen (DIN) values ranging from 20 to 100  $\mu\text{M}$  were frequently observed before 1998 (Flindt et al. 1997, Verdelhos et al. 2005); this is

Table 1. General characteristics of Mondego and Mira estuarine watersheds

	Mondego estuary watershed	Mira estuary watershed	Source
People $\text{km}^{-2}$ (2001)	165	15	INE (2001)
Resident population (2001)	62601	26106	INE (2001)
Number of residences (2001)	37596	18336	INE (2001)
Land derived N-loading	540 $\text{kg N ha}^{-1} \text{yr}^{-1}$ (South Arm)	280 $\text{kg N ha}^{-1} \text{yr}^{-1}$	Flindt et al. (1997), Costa et al. (2001), Castro et al. (2007)
Human activities	Urban and industrial development, salt-works, aquaculture, intensive agriculture, tourism, urban fisheries, harbour development	Tourism and urban development on river mouth, aquaculture, cattle breeding, fisheries	

Table 2. Literature data on  $\delta^{15}\text{N}$  (‰) from *Spartina* and *Zostera* species (shoots), and sediments, and dissolved inorganic nitrogen concentrations in the water column (WC DIN)

Species	Shoots	Sediment	$\delta^{15}\text{N}$ fractionation	WC DIN ( $\mu\text{M}$ )	Site	Country	Source
<i>S. alterniflora</i>	3.3	2.2	1.1		Sage Lot Pond	USA	Martinetto et al. (2006)
	4.4			1.9		USA	McClelland & Valiela (1998a)
	6.0				Sapelo Island	USA	Peterson & Howarth (1987)
	6.0			1.8	Quashnet River	USA	McClelland & Valiela (1998a)
	6.8			12.6	Mashpee River	USA	Cole et al. (2005)
	7.6			3.5	Childs River	USA	McClelland & Valiela (1998a)
	7.7				Great Pond	USA	Cole et al. (2005)
	7.8	4.3	3.5		Quashnet River	USA	Martinetto et al. (2006)
	8.1			4.6	Green Pond	USA	Cole et al. (2005)
	8.2	4.9	3.2		Childs River	USA	Martinetto et al. (2006)
<i>S. foliosa</i>	10.3				Tijuana estuary	Mexico	Fry et al. (2001)
	10.5	4.5			San Francisco estuary	USA	Cloern et al. (2002)
<i>S. maritima</i>	8.8				Ria Formosa	Portugal	Machás & Santos (1999)
<i>Z. capricorni</i>	8.8				Moreton Bay	Australia	Grice et al. (1996)
<i>Z. marina</i>	-1.6			1.9	Sage Lot Pond	USA	McClelland & Valiela (1998a)
	0.5			1.8	Quashnet River	USA	McClelland & Valiela (1998a)
	2.0	2.2	-0.2		Sage Lot Pond	USA	Martinetto et al. (2006)
	5.0			3.5	Childs River	USA	McClelland & Valiela (1998a)
	9.7			7.0	Tomale Bay	USA	Fourqurean et al. (1997)
	14.1	10.4			Kwangyang Bay	Korea	Kang et al. (2003)
<i>Z. noltii</i>	4.5				Ria Formosa	Portugal	Machás & Santos (1999)

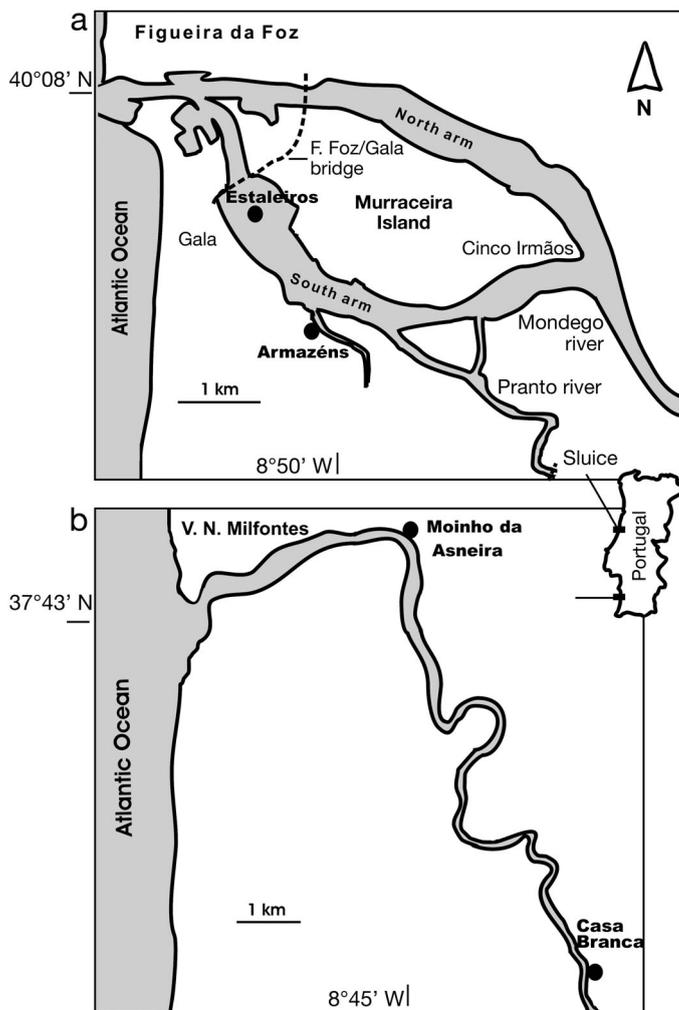


Fig. 1. The (a) Mondego and (b) Mira estuaries. (●) Sampling sites

relatively high in comparison with other sites (Table 2). In 1998, some protection and rehabilitation measures were implemented and environmental quality improved subsequently, but DIN concentrations again increased in the period 2000 to 2001 (Verdelhos et al. 2005). Nitrogen sources may include enriched discharge from farm fields in upstream areas through sluice management.

The Mira estuary (southwest of Portugal) (Fig. 1b) is relatively undisturbed by nutrient and chemical pollution, receiving a total of approximately 280 kg of N  $\text{ha}^{-1} \text{yr}^{-1}$ , about half the N load received by the Mondego estuary (Table 1). In the mid 1980s, aquaculture farms were constructed in the lower part of the Mira estuary. The village of Vila Nova de Milfontes has been growing continually in recent decades, mostly due to tourism. Some added nutrients may enter the system from wastewater coming from housing in

upstream areas, aquaculture, and summer activities near the mouth of the river. Despite these pressures, salt marshes have remained almost unaltered since 1958 (Castro & Freitas 2006) and marsh variation seems to follow natural dynamics rather than changes in anthropogenic land use.

To assess the level of eutrophication of these 2 estuaries, we measured  $\delta^{15}\text{N}$  in *Spartina maritima*, *Halimione portulacoides*, *Zostera noltii* and in sediments below their canopies, as well as %N of tissues and nutrient concentrations in estuarine water. We also compared the performance of these different indicators of nutrient regime to establish their relative utilities in assessing eutrophication of estuaries.

## MATERIALS AND METHODS

**Study sites.** The Mondego estuary is located in the centre of Portugal (40° 08' N and 8° 50' W). Near the mouth (Fig. 1a), the river is divided in 2 arms with distinct sedimentology and hydrodynamic characteristics. Murraceira Island separates these 2 arms and for many decades has supported a number of agriculture practices. Figueira da Foz harbor is located on the north arm; it has a maximum depth of 8 m and is a frequently used navigation channel. The south arm is shallower with maximum depth of 4 m and is largely silted-up in the upstream areas. Consequently, the circulation in this arm depends mainly on tides and small freshwater inputs via the Pranto River, a tributary of the Mondego River. Salt marsh habitats are found in both arms, with larger stands located in the south arm near the Cinco Irmãos bifurcation. The area of salt marsh between the river mouth and the bifurcation was 129 ha in 1998 (Castro & Freitas 2006).

The River Mira estuary is a relatively small tidal estuary located on the southwest of Portugal (37° 43' N, 8° 45' W) (Fig. 1b). It runs for over 32 km and near the mouth it can reach about 400 m width; it has a mean depth of about 6 m. The depth varies from 5 to 10 m in the lower and middle sections and reaches less than 3 m at the upper limit of the tidal influence. The fringing marsh vegetation that borders the middle and downstream areas of the estuary is dominated by *Spartina maritima* populations. There are also extensive subtidal beds of *Zostera noltii*, a habitat with important ecological roles in estuarine dynamics.

**Sampling and chemical analysis.** To assess ambient eutrophication we measured  $\delta^{15}\text{N}$  and %N of 3 dominant plant species, and nitrogen content of sediments and water in each estuary. Shoots of the seagrasses *Spartina maritima* (Curtis) Fernald, *Zostera noltii* Hornemann, and *Halimione portulacoides* (L.) Aellen (cordgrass) and sediment below the canopy of each

species (about 20 cm depth) were collected in 2001, 2002, and 2003 in the Mondego and Mira estuaries (Fig. 1). Two sites were chosen for each study area, one in the low marsh, which included *Z. noltii* meadows, and one in upper marsh, where *Z. noltii* was not found (Estaleiros and Armazéns in Mondego, and Moinho Asneira and Casa Branca in Mira). A total of 100 samples (50 plant tissue and 50 sediment) were collected for N and isotope analysis from the 4 sites chosen. In the laboratory, shoot samples were dried at moderate temperature (60°C) to constant weight and ground to a fine powder with a MF 10.1 cutting grinding head in the case of plant tissues, and with a mortar and pestle in the case of sediments.

Nitrogen isotopes and %N content in both plants and sediments were measured using an Isoprime gas isotope rationing mass spectrometer (Micromass) coupled to an automatic N analyser (EuroEA Eurovector). Isotopic values are expressed in delta notation ( $\delta$ ) defined as the part per thousand (‰) deviation of the isotopic ratio of the element in the sample ( $^{15}\text{N}/^{14}\text{N}$ ) from atmospheric  $\text{N}_2$ :

$$\delta X\text{‰} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where X =  $^{15}\text{N}$  and R is  $^{15}\text{N}/^{14}\text{N}$ . In each estuary, we measured concentrations of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , total dissolved nitrogen (TDN), particulate nitrogen (PN) and

dissolved organic nitrogen (DON) in 72 samples. Samples were collected every 3 mo in the river water column at each site during ebb tide between summer 2000 and spring 2003, as described in Castro (2005).

## RESULTS

Nitrogen concentrations in water, and nitrogen content of sediments and macrophytes showed that putative differences in land-derived inputs to the 2 estuaries led to some differences in nitrogen regimes (Table 3). These variables also pointed to rather high nitrogen loading of the Mondego and Mira estuaries (Table 3).

There were no clear seasonal or inter-annual trends in  $\delta^{15}\text{N}$  values or N loading and we pooled all data. N concentrations in the water column varied seasonally (data not shown; see Castro 2005 for details).

The concentrations of  $\text{NH}_4^+$ , DON, and TDN in the Mondego water column were significantly higher than those in Mira, but no significant differences were observed for nitrate or particulate nitrogen (Table 3).

Differences in nutrient inputs to the 2 estuaries are also evident in the contrasting %N content of sediments (Table 3). Sediments collected in Mondego below *Spartina maritima* and *Halimione portulacoides*

Table 3. Means ( $\pm$ SE) of water column concentrations of inorganic and organic nitrogen species ( $\text{NO}_3^-$ : nitrate,  $\text{NH}_4^+$ : ammonium, TDN: total dissolved nitrogen, PN: particulate nitrogen, DON: dissolved organic nitrogen), %N and  $\delta^{15}\text{N}$  signatures in shoots of 3 salt marsh plant species and in sediments below the canopies. ANOVAs tested for significant differences between estuaries with 2 stations per estuary as duplicates. ns: not significant

Water column concentrations	Mondego		Mira		Differences between estuaries
	Estaleiros	Armazéns	Moinho Asneira	Casa Branca	
$\text{NO}_3^-$ ( $\mu\text{M}$ )	28.2 $\pm$ 6.1	33.8 $\pm$ 7.0	21.3 $\pm$ 2.7	35.3 $\pm$ 5.1	$F_{1,46} = 0.18$ , ns
$\text{NH}_4^+$ ( $\mu\text{M}$ )	29.8 $\pm$ 2.6	34.7 $\pm$ 4.3	19.3 $\pm$ 1.6	16.4 $\pm$ 1.8	$F_{1,46} = 28.2$ , $p < 0.001$
TDN (ppm)	4.0 $\pm$ 0.6	3.9 $\pm$ 0.7	1.9 $\pm$ 0.6	2.3 $\pm$ 0.3	$F_{1,22} = 12.2$ , $p < 0.001$
PN (ppm)	4.6 $\pm$ 0.5	3.5 $\pm$ 0.7	3.3 $\pm$ 0.8	3.7 $\pm$ 0.5	$F_{1,22} = 0.70$ , ns
DON (ppm)	3.1 $\pm$ 0.7	2.8 $\pm$ 0.6	1.2 $\pm$ 0.5	1.3 $\pm$ 0.4	$F_{1,22} = 9.2$ , $p < 0.01$
%N in sediment below canopy of					
<i>Spartina maritima</i>	0.29 $\pm$ 0.01	0.41 $\pm$ 0.01	0.26 $\pm$ 0.01	0.26 $\pm$ 0.01	$F_{1,18} = 52.1$ , $p < 0.001$
<i>Halimione portulacoides</i>	0.49 $\pm$ 0.01	0.28 $\pm$ 0.01	0.38 $\pm$ 0.01	0.27 $\pm$ 0.01	$F_{1,18} = 8.95$ , $p < 0.01$
<i>Zostera noltii</i>	0.20 $\pm$ 0.04	–	0.24 $\pm$ 0.00	–	–
%N in shoots of					
<i>Spartina maritima</i>	2.61 $\pm$ 0.16	2.29 $\pm$ 0.10	2.47 $\pm$ 0.12	2.33 $\pm$ 0.11	$F_{1,18} = 0.00$ , ns
<i>Halimione portulacoides</i>	1.69 $\pm$ 0.16	1.86 $\pm$ 0.14	1.48 $\pm$ 0.08	1.41 $\pm$ 0.08	$F_{1,18} = 20.8$ , $p < 0.001$
<i>Zostera noltii</i>	3.36 $\pm$ 0.09	–	3.31 $\pm$ 0.11	–	–
$\delta^{15}\text{N}$ (‰) in sediment below canopy of					
<i>Spartina maritima</i>	4.4 $\pm$ 0.4	6.2 $\pm$ 0.6	4.2 $\pm$ 0.4	4.4 $\pm$ 0.6	$F_{1,18} = 3.17$ , ns
<i>Halimione portulacoides</i>	6.4 $\pm$ 0.3	5.2 $\pm$ 0.6	5.0 $\pm$ 0.2	3.7 $\pm$ 0.1	$F_{1,18} = 12.0$ , $p < 0.01$
<i>Zostera noltii</i>	3.9 $\pm$ 0.3	–	3.6 $\pm$ 0.4	–	–
$\delta^{15}\text{N}$ (‰) in shoots of					
<i>Spartina maritima</i>	9.0 $\pm$ 0.5	10.2 $\pm$ 0.5	5.8 $\pm$ 0.3	5.9 $\pm$ 0.4	$F_{1,18} = 71.2$ , $p < 0.001$
<i>Halimione portulacoides</i>	8.8 $\pm$ 0.4	8.2 $\pm$ 0.4	7.4 $\pm$ 0.3	7.1 $\pm$ 0.4	$F_{1,18} = 11.7$ , $p < 0.01$
<i>Zostera noltii</i>	8.2 $\pm$ 0.3	–	4.9 $\pm$ 0.5	–	–

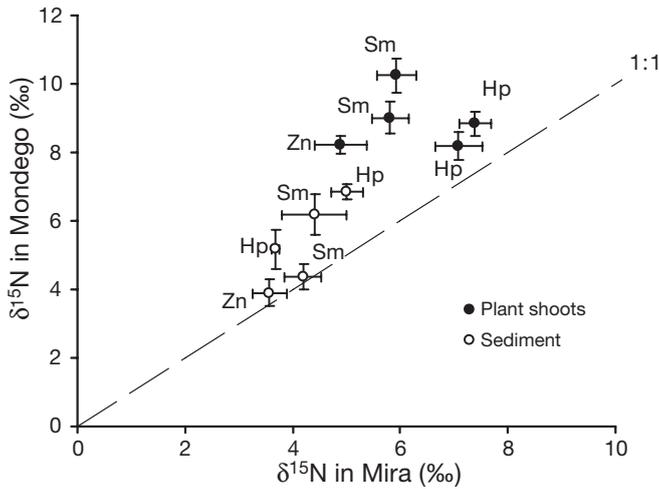


Fig. 2.  $\delta^{15}\text{N}$  (mean  $\pm$  SE) of Mondego plant species and sediments below canopy plotted against those of Mira. Sm: *Spartina maritima*, Hp: *Halimione portulacoides* (each 2 sites per estuary); Zn: *Zostera noltii* (1 site per estuary)

canopies were significantly higher in %N than similar samples obtained from Mira. There were too few samples from *Zostera noltii* sites for adequate tests.

The content of N in macrophyte tissues also hints at richer N sources in Mondego, at least for *Spartina maritima* and *Halimione portulacoides*. In general, however, the %N data point to large N supply in both estuaries (Table 3). In contrast, the  $\delta^{15}\text{N}$  ratios in sediments, and more so in plant shoots, differed consistently and significantly between the 2 estuaries (Table 3). The  $\delta^{15}\text{N}$  values for all 3 plant species were heavier in Mondego than in Mira.

To make the contrast between the 2 estuaries more evident, we plotted the  $\delta^{15}\text{N}$  values of plants and sediments from Mondego against those from Mira in Fig. 2. In all cases the  $\delta^{15}\text{N}$  values from Mondego were heavier than those from Mira. The largest between-estuary differences were for *Spartina maritima*. In addition,  $\delta^{15}\text{N}$  values in plant shoots were heavier than those in the sediments below (ANOVA,  $F_{1,98} = 80.7$ ,  $p < 0.001$ ). The  $\delta^{15}\text{N}$  of shoots was therefore a far more sensitive reflector of degree of human influence than the other variables in Table 3.

To examine potential links between isotopic ratios and ambient conditions, we plotted the  $\delta^{15}\text{N}$  in plants against the concentrations of nitrate and ammonium (Fig. 3), and against the N content of sediments below the canopies (Fig. 4). There were no significant associations that indicated a preponderance of foliar uptake of nitrate (Fig. 3a), but there was a significant association of  $\delta^{15}\text{N}$  and ammonium concentrations in *Spartina maritima* and in *Zostera noltii* (Fig. 3b).  $\delta^{15}\text{N}$  of shoots of *S. maritima* and *Halimione portulacoides* were sig-

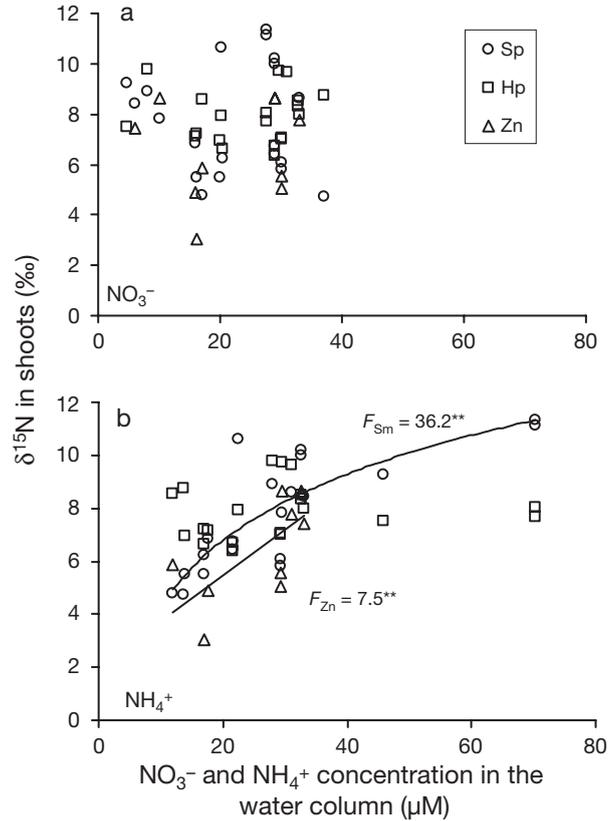


Fig. 3.  $\delta^{15}\text{N}$  from *Spartina maritima* (Sm), *Halimione portulacoides* (Hp) and *Zostera noltii* (Zn) plotted against (a) nitrate ( $\text{NO}_3^-$ ) and (b) ammonium ( $\text{NH}_4^+$ ) water column concentrations (values from both Mondego and Mira estuaries).  $F$  values are from fitted curvilinear regression for Sm and from linear regression for Zn. (Sm:  $y = 3.60\ln(x) - 4.02$ ,  $R^2 = 0.67$ ; Zn:  $y = 0.17x + 1.96$ ,  $R^2 = 0.48$ ). \*\* $p < 0.01$

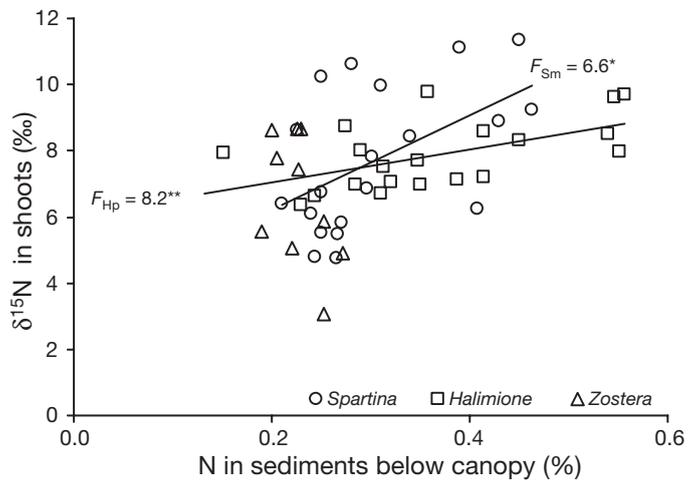


Fig. 4.  $\delta^{15}\text{N}$  from *Spartina maritima* (Sm), *Halimione portulacoides* (Hp), and *Zostera noltii* (Zn) shoots plotted against nitrogen (N) in sediments below canopy in the Mondego and Mira estuaries.  $F$  values are from linear regressions. (Sm:  $y = 14.12x + 3.41$ ,  $R^2 = 0.27$ ; Hp:  $y = 4.99x + 6.05$ ,  $R^2 = 0.31$ ). \* $p < 0.05$ , \*\* $p < 0.01$

nificantly related to nitrogen content of the underlying sediment (Fig. 4).

$\delta^{15}\text{N}$  of all 3 species of macrophytes were heavier than  $\delta^{15}\text{N}$  of bulk sediment (Fig. 5). This comparison shows that in most instances leaf  $\delta^{15}\text{N}$  values were

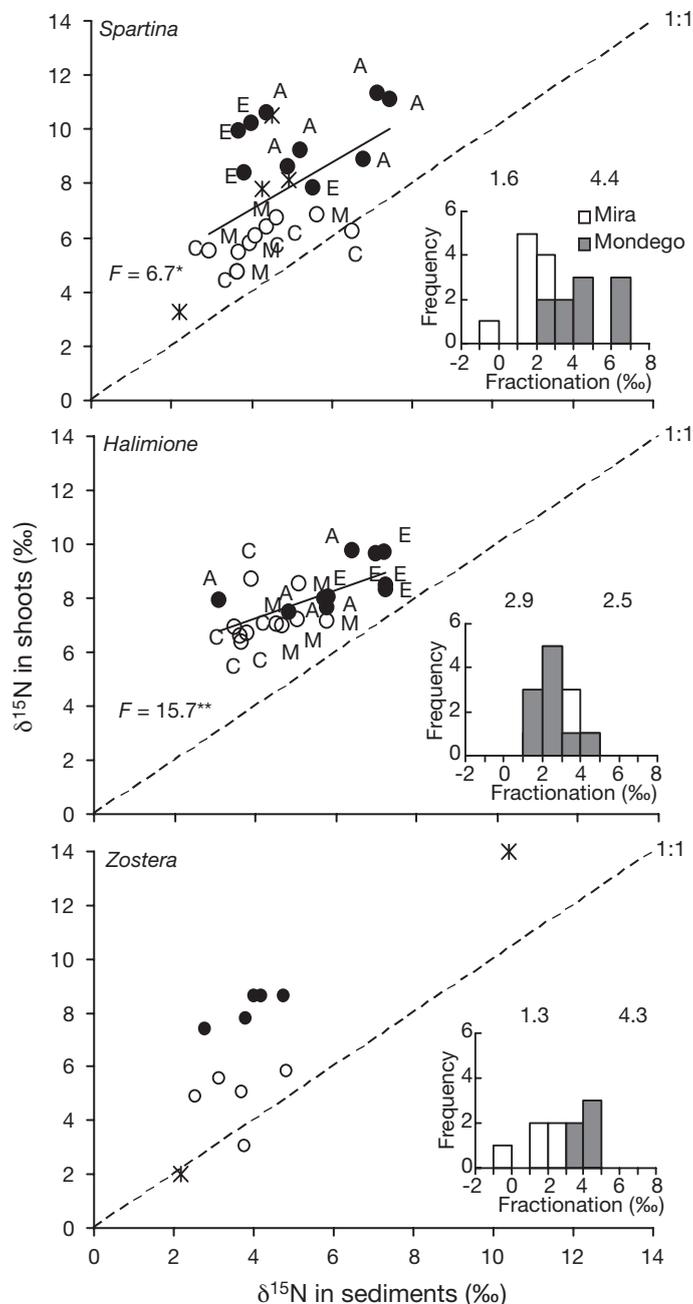


Fig. 5. Plots of  $\delta^{15}\text{N}$  in plant shoots against those of sediments and respective fractionation frequencies (inset histograms). Mondego (●); Mira (○); Data from Cloern et al. (2002), Kang et al. (2003), and Martinetto et al. (2006) (×). A: Armazéns, E: Estaleiros, C: Casa Branca, M: Moinho da Asneira. Numbers above histogram bars are mean fractionation values for the Mondego (right) and Mira (left) estuaries.  $F$ -values are from linear regressions. (*Spartina*:  $y = 0.86x + 3.63$ ,  $R^2 = 0.27$ ; *Halimione*:  $y = 0.52x + 5.17$ ,  $R^2 = 0.47$ ). \* $p < 0.05$ , \*\* $p < 0.01$

larger than sediment  $\delta^{15}\text{N}$  values. The plots of Fig. 5 also revealed a tendency for shoot and sediment  $\delta^{15}\text{N}$  to increase together.

Plant uptake resulted in a clear fractionation (Fig. 5, inset histograms). The fractionation mean of plants was about 3‰, with a wide range of up to 7‰. The means for *Spartina maritima* and *Zostera noltii* from Mondego were about double those for Mira; this was not the case for *Halimione portulacoides*.

## DISCUSSION

Nitrogen concentrations of the water in Mondego and Mira estuaries were quite high in comparison to concentrations reported elsewhere. Mean concentration of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in Mondego and Mira were higher than those found in 90 and 93% (respectively) of estuaries included in a survey of 106 North American water bodies (G. Tomasky, J. York & I. Valiela unpubl. data); similarly, the Mondego and Mira concentrations were higher than those we collated in Table 2. Despite mitigation procedures implemented in the Mondego estuary in 1998, high nitrogen loads are entering the system and the measures now in place are insufficient to maintain environment quality.

The differences in reduced nitrogen compounds in the water column may be related to known but unmeasured inputs from nearby release of human and fish pond effluent, whereas  $\text{NO}_3^-$  is more likely derived from N sources released farther away and subjected to microbial transformations (mineralization) during transport. Whatever the sources and subsequent transformations of  $\text{NO}_3^-$ , they contribute in similar amounts to the 2 estuaries. The differences in reduced nitrogen concentrations, and lack of difference in oxidized forms seem related to the contrasting land uses on the 2 watersheds. Our emphasis on the contrast between Mondego and Mira ought not to overshadow our finding rather high concentrations of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in these estuaries.

Sediment N levels in both estuaries (0.2 to 0.5%) were high compared to developing marshes or habitats under severe N limitation (van Lent & Verschuure 1994, Tyler et al. 2003), but were moderate compared with other studies where values of 1% N or more have been measured in surface sediments (Craft et al. 1999, Struck et al. 2000).

The range of %N in macrophyte shoots (1.4 to 3.4) is within the range found for global surveys (Duarte 1990), but most values in Table 3 lie higher than the 1.8% median values reported by Duarte (1990). In fact, values higher than the median (>1.8% N) have been associated with enriched growth conditions, such as we report for the Mondego and Mira estuaries.

The data on water, sediment and shoot N contents suggest a general pattern of high nitrogen regimes in Mondego and Mira, and point to a somewhat higher delivery of nitrogen to Mondego, following the pattern we might expect owing to the different land use intensities on the respective watersheds. Although there are some uncertainties and variation in the responses to increased land-derived N loadings into these receiving waters, we conclude that these response variables do mirror external inputs and may do so reliably. Results suggest that at least for these highly enriched estuarine systems, nitrogen concentrations in water and nitrogen contents in sediment and plants were indicative of eutrophication level, but were not sufficiently sensitive as indicator signals.

The range of sediment  $\delta^{15}\text{N}$  we report in Table 3 (3.6 to 6.4‰) was quite similar to the range of 3.1 to 7.7‰ found by Deegan & Garritt (1997), and the range of 2.2 to 4.9‰ in the literature data summarized in Table 2.  $\delta^{15}\text{N}$  values between 4 and 6 were reported by Carmichael & Valiela (2005) for estuaries receiving heavier N loads, and between 2 and 4 for less polluted estuaries.

$\delta^{15}\text{N}$  values of plant shoots varied significantly and consistently with different degrees of land use on the respective watersheds. The differences in  $\delta^{15}\text{N}$  ratios in plant shoots follow patterns observed in many other estuaries where plant  $\delta^{15}\text{N}$  values become heavier as the N loads and contribution by wastewater increase (McClelland & Valiela 1998a, Cole et al. 2005). Grice et al. (1996) also reported higher  $\delta^{15}\text{N}$  values in macrophytes from a more eutrophic site (8.6 to 8.8‰) than in a site less anthropogenically influenced (2.6 to 4.5‰). These contrasts imply either a considerably larger input from human or animal wastes, or more intense microbial transformations in the Portuguese estuaries. Processes behind these contrasts remain undefined, and merit further study.

Significant associations between the seagrasses and ammonium concentrations in the water might reflect direct uptake of this reduced nitrogen species from water; such uptake would be energetically advantageous, since it would side-step the need to internally reduce N for conversion to forms suitable for synthesis of amino acids. Cole et al. (2004) found similar relationships between water column DIN concentrations (much lower concentrations than in our sites) and the  $\delta^{15}\text{N}$  values of rooted macrophytes.

The significant relationship between  $\delta^{15}\text{N}$  of shoots and N content of sediments suggests that, as might be expected, root uptake is of considerable importance, though there may also be  $\text{NH}_4^+$  uptake from the water column by leaves. The large scatter of the data in Fig. 4, however, does show that other factors modified the uptake and storage of sediment-derived N by these

macrophytes. It is well known that marsh plant growth, abundance and morphology are strongly linked to available nutrient resources and that most nitrogen is taken up from sediments, with some foliar uptake (Touchette & Burkholder 2003); it is likely that in the estuaries we studied plants access dissolved mineral nutrients through roots and aboveground portions. Nitrogen limits plant growth in most salt marshes (Cloern 2001) and foliar N-uptake can have a significant role in contributing to the overall N requirements of salt marsh plants. In general, there is a preferential uptake of  $\text{NH}_4^+$  over  $\text{NO}_3^-$  from the water column in both seagrasses and marine algae (Bouma et al. 2002).

The  $\delta^{15}\text{N}$  of shoots depended on N content of sediments, but there were important and undefined species-specific differences in sources of N for the plants. Both water and sediment may be sources, mainly for *Spartina maritima* and *Zostera noltii*. *Halimione portulacoides* is a plant that colonizes sediments elevated in relation to the water column; this likely leads to a prevalence of sediment N uptake.

Almost all leaf  $\delta^{15}\text{N}$  values were higher than sediment  $\delta^{15}\text{N}$ . These data may allow a comparison of the fractionation that might be involved in the transfer from sediment to plant tissues assuming that in fact  $\delta^{15}\text{N}$  of bulk sediment does represent the pool from which plants take up nitrogen. It is possible that, instead, plant roots take up nitrogen from the more readily available porewater source, or from the water column (most likely in seagrasses) which may or may not have the same  $\delta^{15}\text{N}$  as bulk sediment.

Rooted macrophytes can use a mixture of N sources (Touchette & Burkholder 2003) and our results suggest that both *Spartina maritima* and *Zostera noltii* must have taken up some of their N from the water column or from porewaters, which we speculate have higher signatures than those from sediments in the Mondego, and probably the same value as the sediments in the Mira. The fact that no differences were observed for N-fractionation in *Halimione portulacoides* between estuaries may support the suggestion that nutrient uptake occurs mainly from sediment sources in this species.

Fractionation seemed more related to isotope signatures in shoots than those from sediments since there were similar  $\delta^{15}\text{N}$  values in sediments in both estuaries. Differences in the way that  $\delta^{15}\text{N}$  of different macrophytes relate to external loads have been reported previously (McClelland et al. 1998a); such different responses have not been explained mechanistically but must be related to physiological contrasts among the different species of macrophytes. In any case, these macrophytes evidenced fractionation values somewhat larger than the 1.4 to 2.1‰ reported for *Spartina alterniflora* and underlying sediment (Deegan & Gar-

ritt 1997), and our compilation of other literature values (−0.2 to 3.5‰, Table 2). Further study of such differences in fractionation and possible links to ambient supply will be of interest.

The parallel increases of  $\delta^{15}\text{N}$  in shoots and sediments may indicate that both shoots and sediments followed and reflected the level of eutrophication and some larger whole estuary difference in N loads even though responsiveness of  $\delta^{15}\text{N}$  of sediments may be constricted by biogeochemical transformations (Ohte et al. 2004).

Coastal eutrophication is a growing problem and development of effective indicators that provide early information and predict how ecosystems will react are needed. Common methods use to study nutrient enrichment rely on water column concentrations, but sometimes poor relations can be found between these data and productivity or abundance of primary producers (Fong et al. 1993, Castro 2005). Our results support the idea that nitrogen isotopic signatures in rooted macrophytes and sediments can be useful indicators of land-derived nitrogen in estuaries but tellingly the range in  $\delta^{15}\text{N}$  values in shoots was wider than that in sediments, so that shoot  $\delta^{15}\text{N}$  may indeed be a more sensitive indicator.

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