Differential processing of anthropogenic carbon and nitrogen in benthic food webs of A Coruña (NW Spain) traced by stable isotopes

Antonio Bode*[1], Consolación Fernández[2], Carmen Mompeán[1], Santiago Parra[1], Fernando Rozada[1,3], Joaquín Valencia-Vila[1] and Inés G. Viana[1]

* e-mail: antonio.bode@co.ieo.es

[1] Instituto Español de Oceanografía. Centro Oceanográfico de A Coruña. Apdo. 130. 15080 A Coruña, Spain

[2] Unidad de Ecología. Departamento de Biología de Organismos y Sistemas. Universidad de Oviedo. 33071 Oviedo, Spain

[3] Present address: Instituto de Ganadería de Montaña, CSIC-Universidad de León, Finca Marzanas, Ctra. Grulleros, 24346 León, Spain

Abstract

In this study the effect of inputs of organic matter and anthropogenic nitrogen at small spatial scales were investigated in the benthos of the Ria of A Coruña (NW Spain) using stable carbon and nitrogen isotopes. This ria is characteristically enriched in nutrients provided either by marine processes (as coastal upwelling) or by urban and agricultural waste. Stable isotope composition in trophic guilds of infaunal benthos revealed spatial differences related to their nutrient inputs. The main difference was the presence of an additional chemoautotrophic food web at the site with a large accumulation of organic matter. The enrichment in heavy nitrogen isotopes observed in most compartments suggests the influence of sewage-derived nitrogen, despite large inputs of marine nitrogen. Macroalgae (*Fucus vesiculosus*) resulted significantly enriched at the site influenced by estuarine waters. In contrast, no differences were found in mussels (*Mytilus galloprovincialis*), thus suggesting a major dependence on marine nutrient sources for this species. However, the estimations of anthropogenic influence were largely dependent on assumptions required to model the different contributions of sources. The

measurement of stable isotope signatures in various compartments revealed that, despite anthropogenic nutrients are readily incorporated into local food webs, a major influence of natural marine nutrient sources cannot be discarded.

Keywords: $\delta^{15}N$, $\delta^{13}C$, subtidal, intertidal, wastewater, upwelling, chemoautotrophy

1. Introduction

Coastal food webs are increasingly altered by pressures from eutrophication and pollution as most of the human population concentrates near the coast. Anthropogenic nutrients, as those derived from urban and agricultural wastewaters have been identified as one of the main causes of changes in the structure and composition of food webs because of their impact in the nutrient cycles (McClelland and Valiela, 1998; Castro et al., 2007). The excess nutrients may lead to large increases in primary production and clear signs of eutrophication, including hypoxia when the remineralisation of the produced organic matter exhausts the oxygen in the water, but often the changes remain unnoticed because some coastal ecosystems are already highly productive. Estuaries, for instance, display enhanced production and specific food webs related to their diverse inputs of nutrients from marine and terrestrial sources. Similarly, coastal upwelling causes elevated levels of primary production by the input of significant amounts of nutrients from deep waters.

The Galician coast (NW Spain) is one paradigmatic region to study the effects of the anthropogenic and natural sources of nutrients. It is located at the northern limit of the eastern boundary upwelling system of the N Atlantic and characterised by high primary production due to the input of nutrient-rich deep ocean waters near the coast (Alvarez-Salgado et al., 2002). The fertilising effect of the upwelling is amplified by the rias and bays that retain and exchange water with the shelf and favour rapid mineralisation of the produced organic matter (Alvarez-Salgado et al., 1997). Estuarine zones in the rias are generally small because of the low flow of most rivers in this region (Rio Barja and Rodriguez Lestegás, 1996) and upwelling dynamics

dominate nutrient fluxes (Nogueira et al., 1997). However, most of the urban and industrial population of Galicia concentrates near the rias. Near 57% of a total population of 1.5 million inhabitants lives in the two major urban areas of Vigo and A Coruña (Precedo Ledo et al., 2008) located inside two of the main rias.

The Ria of A Coruña is formed by a bay of 15.7 km^2 and a small estuarine zone (Ria do Burgo) in the mouth of the river Mero (Cosme de Avilés and Prego, 1995). The river basin drains 385 km^2 but its mean annual flow is only 6.6 m s^{-1}. The marine influence is high in the bay (Cabanas et al., 1987) reflecting the nutrient dynamics driven by the winter mixing and summer upwelling in the nearby shelf (Casas et al., 1997; Bode et al., 2004a, b). The influence of estuarine waters is restricted to the inner bay where there was also an effect of the harbour infrastructures causing enhanced nutrient and phytoplankton concentrations (Varela et al., 1994; Varela and Prego, 2003). Urban population near the ria amounts ca. 250,000 inhabitants according to the Spanish Official Population Census (http://www.ine.es/inebase) thus having a large potential impact on nutrient inputs to coastal waters. Urban and industrial developments in the area collect and treat wastewater in a water treatment plant recently improved with secondary and tertiary treatment (http://augasdegalicia.xunta.es/es/edars/ACOR.html). This plant is designed for a population of up to 600,000 inhabitants and the treated waters reach the open ocean through a submarine outfall located outside and 7.5 km west of the mouth of the bay. Nevertheless the possible influence of diffuse inputs of nutrients to the ria by local point sources, occasional leaks in the sanitation system or overflows during storms is not known.

Discrimination between nutrient sources for species or food webs can be made by measuring the natural abundance of stable isotopes. As light isotopes are mobilised faster in chemical reactions than heavy ones, each molecule has a characteristic isotopic signature reflecting the pathways followed in its formation. The reactants are progressively enriched in heavy isotopes while the products are relatively depleted. The isotope enrichment of the reactant (ε) is characteristic of each reaction (Mariotti et al., 1981; Ruby et al., 1987; Wasser et al., 1998; Needoba et al., 2004) and allows for a differentiation of nutrients with different origins (Heaton, 1986). Nitrogen from

urban and agricultural wastewaters is generally enriched in heavy isotopes because of the large fractionation associated with nitrification (Mariotti et al., 1981). This feature was employed in numerous studies to determine the influence of wastewater in coastal systems (McClelland and Valiela, 1998; Savage and Elmgren, 2004; Castro et al., 2007; Bode et al., 2011; Viana et al., 2011; Viana and Bode, 2013). Similarly carbon isotopic signatures reflect the origin of the organic matter in marine food webs (Spiro et al., 1986; Cifuentes et al., 1988; Dando and Spiro, 1993; Machas et al., 2003; Martineau et al., 2004; Bode et al., 2006; Malet et al., 2008; Sakamaki and Richardson, 2008; Bode et al., 2011). However, previous studies focus on a few species or benthic compartments, thus limiting the generalisation of their conclusions to the whole ecosystem.

The objective of the present study is to determine the effect of inputs of organic matter and anthropogenic nitrogen at small spatial scales in the Bay of A Coruña (NW Spain). For this purpose the natural abundance of stable carbon and nitrogen isotopes was examined in different compartments, including water, seston, subtidal sediments and infauna and intertidal organisms. Each compartment was selected as representative of nutrient effects at instantaneous (surface water) or longer time-scales (sediments and benthic organisms). Intertidal benthos was chosen as indicator of the contribution of different nutrient sources in surface water, while subtidal infauna was intended to reflect nutrient sources near the sediment. The seasonal variability was taken into account by sampling across annual seasons and the estimations of anthropogenic contributions included variations in isotopic fractionation.

2. Methods

2.1 Subtidal sediments and infauna

Samples of subtidal sediments and infauna were collected bimonthly (January 2010 to November 2011) at stations B2 (9 m depth) and DB (17 m depth) using R/V Lura (Fig. 1). These stations we representative of the spatial variability of infaunal benthos in the area (López-Jamar and Mejuto, 1985). At each sampling date five box-core samples (Bouma-type box-corer,

sampling area = 0.0175 m^2) were pooled to obtain representative estimates of infaunal species composition and biomass (López-Jamar et al., 1986). The upper 2 cm of the core sample sediments were analysed for total organic matter content (ash-free dry weight) and granulometric characteristics (Buchanan, 1984). In addition particulate organic carbon and nitrogen concentrations (POC and PON) and stable isotope abundance were determined in subsamples of these sediments. The infaunal samples were sieved through a 0.5 mm mesh, anaesthetized with magnesium chloride (7% w/v), and then preserved in 5% buffered formaldehyde previously containing Rose Bengal as a staining agent to facilitate the sorting of organisms. Specimens intended for stable isotope determinations were obtained from additional box-core samples, sorted immediately after sampling in the laboratory and kept for 24 h in small aquaria containing filtered seawater to facilitate evacuation of gut contents. For trophic analysis species were classified in trophic guilds (filter feeders, deposit feeders, omnivores and carnivores) from the information provided in the literature.

2.2. Water

Temperature, salinity and chlorophyll-fluorescence profiles of water above each subtidal sediment stations were obtained at each sampling date using a CTD SBE-25. Fluorescence was converted to chlorophyll-a concentrations after calibration with acetonic extracts of discrete water samples collected with Niskin bottles. In addition, surface water nutrients were determined in samples collected monthly during 2011 at stations W1 and W2, located near the sediment stations and, according to previous studies (Cabanas et al., 1987; Varela et al., 1994; Varela and Prego, 2003), representative of marine and estuarine end-members, respectively (Fig. 1). Additional water samples were collected near the coast concurrently with samples of intertidal organisms. Dissolved nitrate, nitrite and ammonium concentrations were determined by segmented-flow analysis (Casas et al., 1997).

2.3. Intertidal benthos

Samples of the brown alga *Fucus vesiculosus* and the mussel *Mytilus galloprovincialis* were collected monthly during 2010 and 2011 at two intertidal sites (Fig. 1). Mera was located at the outer bay in the less urbanized part of the study area. In contrast, Ria do Burgo was located in the area of direct estuarine influence and heavy urban population. Algal samples for stable isotope determinations were collected from the apical 2 cm of the thallus of 25-35 cm long specimens, while mussel samples were collected from the adductor muscle of 40-50 cm (shell length) individuals at both sites.

2.4. Stable isotope analysis

Sediment and biological samples for stable isotope determination were dried (50 ºC, 24 h), ground to a fine powder and weighted (± 0.002 mg). The isotopic composition of total nitrate (NO_3^-+NO_2^-) was determined by previous conversion into ammonium and later recovery of ammonium on a solid phase (Ahad et al., 2006). The procedure is an adaptation of the diffusion method (Sigman et al., 1997) involving the incubation of samples in two steps. First, aliquots of the samples were incubated (50 °C, 1 week) in the same collecting flask without cap to reduce the volume and concentrate nitrate. Ashed MgO was added to raise pH above 9.7 to remove ammonia by volatilization. In the second step (50 °C, 2 weeks), ashed Devarda´s alloy was added to the reduced volume sample to convert nitrate and nitrite into ammonium. The high pH (>11) of the mixture ensured also the conversion of ammonium into ammonia gas that was collected on a sterilized glass-fibre disk (Whatman, GF/F), acidified with 0.5 ml of 0.25N H_2SO_4 and hooked on a needle fixed to the inner side of the flask (Slawyk and Raimbault, 1995). After the incubation, the disk filters containing ammonium sulphate were dried and prepared for isotopic analysis. The stable isotope composition of ammonium was determined in another aliquot of the water samples by an adaptation of the diffusion method (Holmes et al., 1998). This method involves gas-phase diffusion as described for the second step of the total

nitrate extraction. In all cases corrections for isotopic fractionation during the whole incubation and diffusion steps were made (Holmes et al., 1998).

Samples were placed in tin capsules and introduced into an isotope-ratio mass spectrometer (Thermo Finnigan Mat Delta Plus) via an element analyser (Carlo Erba CHNSO 1108). Isotopic results are expressed in delta notation ($\delta^{15}N$ or $\delta^{13}C$) relative to atmospheric N or VPDB (Bode et al., 2011). Precision (SE of 5 replicates) was better than 0.05 ‰ for either $\delta^{15}N$ or $\delta^{13}C$. The coefficient of variation of triplicate sample aliquots was always <2%. Precision for $\delta^{15}N$ determinations in both nitrate and ammonium was better than 0.4 ‰.

The contributions of anthropogenic (f_a) and marine ($1-f_a$) sources to nitrogen composition of primary producers ($\delta^{15}N_{P)}$ were estimated using a mixing model of two end members:

$$\delta^{15}N_P = f_a\, \delta^{15}N_a + (1-f_a)\, \delta^{15}N_m - \varepsilon$$

where $\delta^{15}N_P$ is the isotopic composition of phytoplankton, microphytobenthos or macroalgae, estimated from the analysis of seston, surface sediments or *F. vesiculosus*, respectively. The isotopic composition of nitrogen sources was represented by the mean value of total nitrate in surface marine waters ($\delta^{15}N_m$) or anthropogenic nitrogen ($\delta^{15}N_a$). As it was difficult to obtain representative samples from the diffuse sources of anthropogenic nitrogen in the study area, we used a mean $\delta^{15}N_a$ value obtained from measurements in samples of urban wastewater released from several water treatment plants in the study region (17.8±0.6‰ se, n=7). The model included an isotopic enrichment factor (ε) between the source (nitrate) and the product (primary producer nitrogen). Several values of ε were employed to constrain the estimated contributions.

2.5. Statistical analysis

Differences between stations in the values of the environmental variables were determined using non parametric ANOVA (Mann-Whitney U) while differences in isotopic composition among trophic guilds were analysed using parametric ANOVA and a posteriori tests (Dunnett C). As the main objective of this study is to compare different locations in the study area, all values

from the same sampling site were averaged. The species composition of the infaunal communities in subtidal sediments was analysed using a cluster analysis (Bray-Curtis distance, group average method) on log-transformed species biomass for each sampling date. Only species contributing >0.1% to total biomass were selected for this analysis. The contribution of species to similarity within each station was determined using the procedure SIMPER of PRIMER statistical package (Clarke and Warwick, 2001).

3. Results

3.1. Water and sediments

Surface water from the station W2 was significantly less saline than water from W1 but otherwise both stations have similar mean values of SST, nitrogenous nutrients or chlorophyll (Table 1). The influence of the estuarine waters was more evident in the seston composition, as POC and PON values at W2 waters were ca. 2.5 times higher than those of W1, having also an excess of carbon as indicated by the C:N ratio. However, there were no significant differences in the average isotopic composition of seston or water at both stations.

The water collected at both intertidal sampling sites did not show either differences in the water variables analysed, except in the $\delta^{15}N$ values of nitrate that were significantly lower at the estuarine site (Table 1).

Similar results were found when comparing water characteristics above both infauna sampling stations, as both have equivalent temperature, salinity, and integrated chlorophyll values (Table 2). The only significant difference was in the average amount of light reaching the sediment surface (21% at St. B2 and 7% at St. DB).

Despite the similarities in water-column properties, there were large differences in sediments from the infaunal stations (Table 3). Sediments from St. DB showed a mixture of sands but were characterised mainly by a larger fraction of mud and organic matter than those from St. B2. Total organic matter (%AFDW), carbon ($\%C_{total}$) and nitrogen content at St. DB were >5,

>2 and >6 times those of St. B2. Mean $\delta^{13}C_{org}$ values and $\delta^{15}N$ were equivalent for both stations (Table 3).

3.2. Subtidal infauna

Infauna at St. DB contained less species (86 species, Supplement Table S1) than at St. B2 (115 species). Some of the species (generally filter and deposit feeder molluscs) appeared only at one of the stations. For instance, *Myrtea spinifera* and *Thyasira flexuosa*, both containing symbiotic bacteria were almost exclusive of St. DB, while *Pharus legumen* and *Chamelea gallina* were only found at St. B2 with large biomass values (Table 4). However, the largest biomass values were generally reached at St. DB. The large differences in species composition and biomass allowed a clear separation between stations at low similarity level (Fig. 2). Comparatively, samples from the same station collected at different times showed larger similarities than samples from different stations. Most of the separation was due to molluscs (*M. spinifera*, *Nucula* sp.) and polychaetes (*Notomastus latericeus, Euclymene oerstedi, Chaetozone setosa)*, with larger contributions to similarity within stations (Table 4).

The isotopic composition of the different species matched their trophic guild, with lower $\delta^{15}N$ and $\delta^{13}C$ values associated to filter and deposit feeders and larger values to predators (Table 4). Mean values were significantly different between trophic guilds and stations (Fig. 3). The filter feeders containing symbiotic bacteria (FFs, found only at St. DB) were significantly depleted in ^{15}N and ^{13}C when compared with other filter feeders and also with other guilds. The ANOVA of $\delta^{15}N$ by stations and trophic guilds (only those shared by both stations) revealed significant differences between trophic guilds ($P<0.001$) and these differences varied between stations (interaction between guild and station, $P<0.001$), while no differences resulted between stations when values for all trophic guilds were pooled ($P>0.05$). Filter feeders had lower mean $\delta^{15}N$ values than all other guilds at St. B2, while at St. DB they had similar values to deposit feeders and both guilds were significantly less enriched in ^{15}N than both omnivores and predators (Fig.

3). Values of $\delta^{13}C$ varied significantly between stations ($P<0.001$) but not between guilds ($P>0.05$), as deposit feeders and omnivores were depleted in ^{13}C at St. DB.

There were large similarities in food webs operating at both stations when compared by plotting mean isotopic values for the likely sources of organic matter (seston and surface sediments) and guilds for each station (Fig. 4). The main difference was the presence of filter feeders containing symbiotic bacteria at St. DB, which were mostly unconnected with the other guilds. Besides FFs, the isotopic space delimited by sources and guilds of both stations largely overlapped, with a sensible reduction at St. DB due to a lower ^{13}C content in deposit feeders.

The average isotopic enrichment between organic matter sources and guilds and between adjacent guilds in the food web was larger for deposit feeders (>4.5‰ in ^{15}N and >8‰ in ^{13}C) than for other guilds at both stations (Fig. 5). Large enrichment was also found between filter feeders and carnivores in ^{15}N while ^{13}C values did not vary between most guilds.

3.3. Intertidal benthos

The similar composition of waters at both intertidal sampling sites (Table 1) was also reflected in $\delta^{15}N$ values of macroalgae and mussels (Fig. 6). Only *F. vesiculosus* from the estuarine waters of the Ria do Burgo had significantly lower values than those from the outer bay site of Mera, while the values for mussels were equivalent at both sites.

3.4. Anthropogenic N inputs

Using the mean $\delta^{15}N$ value for nitrate from surface waters at stations W1 and W2 as the marine end member (Table 1) and the reference value for urban wastewater as the anthropogenic end member, the estimated contribution of anthropogenic nitrogen to seston, sediments and mussels was generally <30% by assuming a fractionation factor of 3‰ (Fig. 7). Only *F. vesiculosus* from the Ria do Burgo had contributions exceeding 30%. However, larger contributions of anthropogenic nitrogen (>70%) will be expected if isotopic fractionation increased to 6‰.

4. Discussion

4.1. Organic matter sources

Water, seston and sediment properties indicate a large influence of marine waters in the study area, despite a small decrease in salinity at some stations reflecting some influence of estuarine waters. This result is consistent with those from previous studies that stress the marine character of the bay of A Coruña, not a typical Galician ria (Cabanas et al., 1987; Varela et al., 1994). The low flow of freshwater to the bay by the main tributary river restricts the estuarine influence to the small Ria do Burgo, located in the southern end of the bay (Cosme de Aviles and Prego, 1995) while the presence of a large dock restricts water exchanges in the harbour area (Gómez-Gesteira et al., 1999). The composition of seston reflects its predominantly marine origin, with mean molar C:N values between 7 and 8 typical of coastal waters outside the bay (Casas et al., 1997; Bode et al., 2004b). The small excess of carbon relative to nitrogen near the estuary suggest a measurable influence of organic matter derived from macrophytes and terrestrial detritus, as found in other estuaries (Machas et al., 2003; Martineau et al., 2004; Sakamaki and Richardson, 2008). However, the influence of phytoplankton and microphytobenthos is likely to be high, as evidenced by the high integrated chlorophyll measured above infaunal sampling stations and relatively low C:N ratios. These high levels of chlorophyll were already described for the inner part of the bay (Varela and Prego, 2003) and may be explained by the transparency of the waters and relatively high nutrient concentrations that allow for the growth of microalgae in the whole water column. Vertical CTD profiles in this study (not shown) revealed an increase of fluorescence near the bottom of infaunal sampling stations suggesting the resuspension of benthic microflora. Furthermore, $\delta^{13}C$ values of seston were similar at both stations and typical of marine phytoplankton (Malet et al., 2008; Bode et al., 2011).

Grain size and location inside the bay were the main factors determining the accumulation and type of organic matter in sediments. Organic matter accumulated mainly at the station with fine

sediments, which was located in the harbour (St. DB) and under the influence of marine seston with lower total concentration and C:N values than estuarine seston. In contrast, the station with coarse sediments and under the influence of estuarine seston (St. B2) accumulated less organic matter. Elevated C:N and $\delta^{13}C_{tot}$ values in bulk sediments of St. B2 cannot be interpreted as indicative of organic matter of different quality or origin as organic matter at St. DB. Instead, both stations shared organic matter of a similar origin, as they have equivalent $\delta^{13}C_{org}$ signatures with values close to those of seston. Larger differences in $\delta^{13}C$ between sediments and seston will be expected if there were significant amounts of sediment organic matter derived from terrestrial or estuarine plants (Machas et al., 2003; Martineau et al., 2004). Sediments receiving mostly in situ deposition of local particles have $\delta^{13}C$ values similar to seston (Cifuentes et al., 1988; Bode et al., 2011).

With the exception of the presence of species containing symbiotic bacteria at St. DB, the similar structure of infaunal food webs at the two sampling sites is consistent with the dominance of marine sources of organic matter. The almost exact match between the areas delimited by sources and guilds in the $\delta^{15}N$: $\delta^{13}C$ plots is indicative of a major correspondence in structure and trophic niche breadth between the two sites. Otherwise, differences in trophic diversity and structure at community level would produce different shapes and less overlaps between the areas delimited in these plots (Layman et al., 2007; Bode et al., 2011). Small or non significant variations in $\delta^{13}C$ across sources and guilds also points to the use of a single source of organic matter in the food webs. This similarity, however, do not exclude differences between both communities (high species richness at St. B2, high biomass at St. DB) that can be attributed to the large accumulation of organic matter at St. DB, as noted by previous studies (Lopez-Jamar et al., 1986; 1995).

The isotopic composition of *M. spinifera* and *T. flexuosa* revealed a different source of organic matter. These molluscs were generally described as filter feeders but their filtration systems are transformed to host symbiotic bacteria capable of chemoautotrophy (Dando et al., 1985; Dando and Southward, 1986; Dando and Spiro, 1993). The symbionts require reduced compounds

(such as methane or sulphide present in organic-rich anoxic sediments) for their autotrophic production (Dufour and Felbeck, 2006), but the host molluscs require oxygen for respiration and particulate organic matter from the overlying waters. To meet both requirements these species are located in sediment layers with low sulphide content but also low oxygen, having long siphon tubes allowing the flux of surface and deep interstitial water (Dando and Southward, 1986). The symbiotic bacteria use sulphide produced by the anoxic degradation of organic matter by free bacteria to drive carbon fixation using CO_2 dissolved in interstitial waters. *T. flexuosa* has been described in A Coruña as an early colonist (López-Jamar et al., 1995) reaching up to 22,000 ind. m^{-2} after major inputs of organic matter, such as oil spills (López-Jamar and Parra, 1997). The highly depleted $\delta^{13}C$ signature found for these species in the present study (ca. -28‰) indicate that they obtain a large fraction of their biomass carbon from other sources than those used by other filter feeders found at the same station. The carbon produced by sulphur-oxidising chemoautotrophic bacteria has a lower $\delta^{13}C$ than ambient CO_2 because of the discrimination against ^{13}C by the enzyme ribulose-bisphosphate carboxylase (Ruby et al., 1987). Therefore $\delta^{13}C$ values reported for tissues of filter feeders obtaining most of their organic matter from sulphur-oxidising symbionts ranged from -23 to -31‰ while those of most benthic invertebrates ranged from -16 to -20‰ (Spiro et al., 1986). These ranges are consistent with our findings in A Coruña (Table 4) and suggest that the symbiont-containing molluscs pertain to a food web separated from the rest of infauna at St. DB. However the significant depletion in ^{13}C found for deposit feeders and omnivores at this station suggest that at least some of the organic matter derived from chemosynthesis enters the main food web, likely via detrital particles released by the bacteria-hosting molluscs.

4.2. Nitrogen inputs

The measured values of inorganic nitrogen concentrations were high when compared to values typical of surface waters near the shelf and outside the bay (up to 6 μM-NO_3^- and 2 μM-NH_4^+, Casas et al., 1997) but maximum values were similar to those found in other rias (Nogueira et al., 1997). However, there were no significant differences in mean concentrations because of the

large variability, particularly at the estuarine influence area. Other field studies pointed out high nutrient concentrations in the harbour area, in the inner part of the bay, which may lead to potential eutrophication (Varela and Prego, 2003). Modelling studies suggested that these accumulations were likely caused by a longer residence time of waters in the harbour area compared to that in the estuarine area (Gómez-Gesteira et al., 1999). Nitrogen inputs from the river, with an average flow of 6.6 m^3 s^{-1} (Cosme de Avilés and Prego, 1995), are likely to be low because of the large volume of the bay (0.24 km^3) and tidal mixing (Gómez-Gesteira et al., 1999).

The stable isotope composition of surface waters indicated a homogeneous distribution suggesting the existence of a single source, as mean $\delta^{15}N$ was 4.0‰ for nitrate and between -0.2 and 1.0‰ for ammonium. Only nitrate from shallow waters in the intertidal estuarine zone were slightly depleted suggesting an additional nitrate source. The values were, however, typical of marine waters in the Atlantic, as nitrate $\delta^{15}N$ in the 200-500 m layer was between 4 and 6‰ (Liu and Kaplan, 1989). This result is consistent with the large input of nitrate from Eastern North Atlantic Central Waters by the upwelling in Galicia (Alvarez-Salgado et al., 2002). Other nitrate sources would have more depleted or enriched isotopic signatures. For instance, $\delta^{15}N$ of nitrogen derived from atmospheric nitrogen fixation is generally -2‰ (Landrum et al., 2011) while the $\delta^{15}N$ of chemical fertilisers varies between 1 and 2.5 (Heaton, 1986). Nitrogen fixation is not expected in the study area because of the sufficient supply of nitrogen. Similarly, even the lowest $\delta^{15}N$ values measured in our water samples are far from those of chemical fertilisers, which is consistent with the preferential use of natural fertilisers (e.g. manure) in agriculture fields from Galicia (Lopez Periago et al., 2002). The low $\delta^{15}N$ of molluscs containing symbiotic bacteria can be attributed to the preferential uptake of light nitrogen remineralised from the excess organic matter at St. DB, as has been previously reported for similar species in other ecosystems (Southward et al., 2001). This finding, along with the depleted carbon signatures, further supports the hypothesis of a different food web operating in the subsurface layers of the sediment at this station.

Nitrate derived from wastewaters and manure is characteristically enriched in ^{15}N because of the large fractionation associated with nitrification and dinitrification processes (Mariotti et al., 1981) with both nitrate and ammonium $\delta^{15}N$ generally above 10‰ (Tucker et al., 1999; Savage and Elmgren, 2004). The value assumed for $\delta^{15}N_a$ is comparable with that found in wastewaters from other areas even it exceeded our measurements in the water, sediments or biota. This would indicate that there were no large inputs from urban or agricultural wastewaters to the bay, despite of the large urban population. This is supported by the small enrichment in ^{15}N (<2‰) found between nitrate, seston and surface sediments (Tables 1 and 3). However, there were relatively high enrichments in some guilds (e.g. DF) or species (*F. vesiculosus*), suggesting the potential impact of nitrogen from wastewater, at least in the inner zone of the bay. The enrichment could be explained by the accumulation of heavy nitrogen in structural tissues with low turnover rates while light nitrogen is rapidly exchanged with the water, as found in other studies (Savage and Elmgren, 2004). In addition, diffuse sources of heavy nitrogen may affect benthic organisms at small spatial scales. Recent measurements of $\delta^{15}N$ in nitrate from interstitial waters in upper 10 cm of sediments from the inner estuary (unpub. results) produced values (mean±se 17.2±0.9‰, n=3) which were close to those found in wastewaters. This suggests that estuarine sediments play a major role of in the remineralisation of diffuse inputs of wastewater nitrogen. Our estimations indicate an average low input of wastewater (anthropogenic) nitrogen for seston or sediments, and even for *F. vesiculosus* in the estuarine zone, but these estimations were largely dependent on the isotopic fractionation employed. Assimilation of nitrate is generally associated with isotopic fractionation factors between 0-20‰, both in phytoplankton (Waser et al., 1998; Needoba et al., 2004) and macroalgae (Naldi and Wheeler, 2002) but most estimates are near 5‰. In contrast assimilation of ammonium is assumed to have almost no fractionation at the low ambient concentrations normally found in marine waters (Waser et al., 1998). Therefore the use of a mean fractionation factor of 3‰ in our estimates of anthropogenic nitrogen inputs represents a conservative compromise between the assimilation of both nitrate and ammonium by phytoplankton and macroalgae. Notwithstanding the upwelling pulses of nitrate in the study area, studies with isotopic tracers

indicated that at least phytoplankton uses equivalent amounts of nitrate and ammonium at seasonal time scales (Bode et al., 2004a). The resulting low influence of anthropogenic nitrogen is consistent with previous studies in the Galician coast (Bode et al., 2006; 2011; Viana et al., 2011; Viana and Bode, 2013). However, the large influence of the value of the isotopic fractionation factor employed in the estimated contribution of different nitrogen sources is not generally acknowledged in pollution studies (Savage and Elmgren, 2004; Lamb et al., 2012) and our results add to the growing evidence that $\delta^{15}N$ values alone do not provide an unequivocal proof of a major influence of anthropogenic nitrogen on coastal ecosystems.

Acknowledgements

We are grateful to the crew of R/V Lura for their collaboration during water and sediment sampling. Nutrient concentrations were determined by R. Carballo and sediment characteristics by J. F. Feijoo. We also acknowledge the participation of A.F. Lamas in sample collection and preparation for stable isotope analysis and of M.C. Vázquez in the analysis of infaunal species. Stable isotope determinations were made by the Servicio de Apoyo a la Investigación of the Universidad de A Coruña (Spain). This research was funded by projects ANILE (CTM2009-08396 and CTM2010-08804-E) of the Plan Nacional de I+D+i (Spain), and RADIALES of the Instituto Español de Oceanografía (IEO, Spain). C.M. and I.G.V. were supported by FPI fellowships from IEO and from Ministerio de Economía y Competividad (Spain), respectively.

References

Ahad, J., Ganeshram, R., Spencer, R., Uher, G., Gulliver, P., Bryant, C., 2006. Evidence for anthropogenic ^{14}C-enrichment in estuarine waters adjacent to the North Sea. Geophysical Research Letters 33(8), doi: 10.1029/2006GL025991.

Alvarez-Salgado, X.A., Castro, C.G., Pérez, F.F., Fraga, F., 1997. Nutrient mineralization patterns in shelf waters of the Western Iberian upwelling. Continental Shelf Research 17, 1247-1270.

Alvarez-Salgado, X.A., Beloso, S., Joint, I., Nogueira, E., Chou, L., Pérez, F.F., Groom, S., Cabanas, J.M., Rees, A.P., Elskens, M., 2002. New production of the NW Iberian shelf during the upwelling season over the period 1982-1999. Deep Sea Research 49(10), 1725-1739.

Bode, A., Alvarez-Ossorio, M.T., Varela, M., 2006. Phytoplankton and macrophyte contributions to littoral food webs in the Galician upwelling (NW Spain) estimated from stable isotopes. Marine Ecology Progress Series 318, 89-102.

Bode, A., Varela, M., Prego, R., 2011. Continental and marine sources of organic matter and nitrogen for rías of northern Galicia (Spain). Marine Ecology Progress Series 437, 13-26.

Bode, A., Barquero, S., González , N., Alvarez-Ossorio, M.T., Varela, M., 2004a. Contribution of heterotrophic plankton to nitrogen regeneration in the upwelling ecosystem of A Coruña (NW Spain). Journal of Plankton Research 26(1), 1-18.

Bode, A., Varela, M.M., Teira, E., Fernández, E., González, N., Varela, M., 2004b. Planktonic carbon and nitrogen cycling off NW Spain: variations in production of particulate and dissolved organic pools. Aquatic Microbial Ecology 37(1), 95-107.

Buchanan, J.B., 1984. Sediment analysis. In: Holme, N.A.,McIntyre, A.D. (Editors), Methods for the study of marine benthos. Blackwell Scientific Publications, Oxford, pp. 41-65.

Cabanas, J.M., Nunes, T., Iglesias, M.L., Gonzalez, N., Carballo, R., 1987. Oceanografía de la bahía de La Coruña. Boletín del Instituto Español de Oceanografía 4, 21-28.

Casas, B., Varela, M., Canle, M., González, N., Bode, A., 1997. Seasonal variations of nutrients, seston and phytoplankton, and upwelling intensity off La Coruña (NW Spain). Estuarines, Coastal and Shelf Science 44, 767-778.

Castro, P., Valiela, I., Freitas, H., 2007. Eutrophication in Portuguese estuaries evidenced by $\delta^{15}N$ of macrophytes. Marine Ecology Progress Series 351, 43-51.

Cifuentes, L.A., Sharp, J.H., Fogel, M.L., 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. Limnology and Oceanography 33, 1102-115.

Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: An approach to statistical analysis and interpretation. PRIMER-E Ltd., Plymouth (UK), 171 pp.

Cosme de Avilés, A., Prego, R., 1995. Características generales de la Ria de La Coruña y su cuenca fluvial. Monografías de Química Oceanográfica 2, 25-45.

Dando, R., Southward, A.J., Southward, E.C., Terwilliger, N.B., Terwilliger, R.C., 1985. Sulphur-oxidising bacteria and haemoglobin in gills of the bivalve mollusc *Myrtea spinifera*. Marine Ecology Progress Series 23, 85-98.

Dando, P.R., Southward, A.J., 1986. Chemoautotrophy in bivalve molluscs of the genus *Thyasira*. Journal of the Marine Biological Association of the United Kingdom 66(04), 915-929.

Dando, R., Spiro, B., 1993. Varying nutritional dependence of the Thyasirid bivalves *Thyasira sarsi* and *T. equalis* on chemoautotrophic symbiotic bacteria, demonstrated by isotope ratios of tissue carbon and shell carbonate. Marine Ecology Progress Series 92, 151-158.

Dufour, S.C., Felbeck, H., 2006. Symbiont abundance in Thyasirids (Bivalvia) is related to particulate food and sulphide availability. Marine Ecology Progress Series 320, 185-194.

Gómez-Gesteira, M., Montero, P., Prego, R., Taboada, J.J., Leitao, P., Ruíz-Villareal, M., Neves, R., Pérez-Villar, V., 1999. A 2-dimensional particle tracking model for pollution dispersion in A Coruña and Vigo Rías (NW Spain). Oceanologica Acta 22, 167-177.

Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chemical Geology 59, 87-102.

Holmes, R.M., McClelland, J.W., Sigman, D.M., Fry, B., Peterson, B.J., 1998. Measuring ^{15}N-NH_4^+ in marine, estuarine and fresh waters: an adaptation of the ammonia diffusion method for samples with low ammonium concentrations. Marine Chemistry 60, 235-243.

Lamb, K., Swart, P.K., Altabet, M.A., 2012. Nitrogen and carbon isotopic systematics of the Florida Reef Tract. Bulletin of Marine Science 88(1), doi:10.5343/bms.2010.1105.

Landrum, J.P., Altabet, M.A., Montoya, J.P., 2011. Basin-scale distributions of stable nitrogen isotopes in the subtropical North Atlantic Ocean: Contribution of diazotroph nitrogen to particulate organic matter and mesozooplankton. Deep Sea Research 58(5), 615-625.

Layman, C.A., Arrington, D.A., Montaña, C.G., Post, D.M., 2007. Can stable isotope ratios provide for community-wide measurements of trophic structure? Ecology 88, 42-48.

Liu, K.-K., Kaplan, I.R., 1989. The eastern tropical Pacific as a source of 15N-enriched nitrate in seawater off southern California. Limnology and Oceanography 34, 820-830.

López-Jamar, E., Mejuto, J., 1985. Bentos infaunal en la zona submareal de la ría de La Coruña. I. Estructura y distribución espacial de las comunidades. Boletín del Instituto Español de Oceanografía. 2(3), 99-109.

López-Jamar, E., Parra, S., 1997. Distribución y ecología de *Thyasira flexuosa* (Bivalvia, Lucinacea) en las rías de Galicia. Publicaciones Especiales del Instituto Español de Oceanografía. 23, 187-197.

López-Jamar, E., González, G., Mejuto, J., 1986. Temporal changes of community structure and biomass in two subtidal macroinfaunal assemblages in La Coruña bay, NW Spain. Hydrobiologia 142, 137-150.

Lopez-Jamar, E., Francesch, O., Dorrio, A.V., Parra, S., 1995. Long-term variation of the infaunal benthos of La Coruna Bay (NW Spain): Results from a 12-year study (1982-1993). Scientia Marina 59(supplement 1), 49-61.

López Periago, E., Núñez Delgado, A., Díaz-Fierros, F., 2002. Attenuation of groundwater contamination caused by cattle slurry: a plot-scale experimental study. Bioresource Technology 84(2), 105-111.

Machas, 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(4A), 846-856.

Malet, N., Sauriau, P.G., Ryckaert, M., Malestroit, P., Guillou, G., 2008. Dynamics and sources of suspended particulate organic matter in the Marennes-Oleron oyster farming bay: Insights from stable isotopes and microalgae ecology. Estuarine, Coastal and Shelf Science 78(3), 576-586.

Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; Illustration for the denitrification and nitrification processes. Plant and Soil 62, 413-430.

Martineau, C., Warwick, F.V., Frenette, J.-J., Dodson, J.J., 2004. Primary consumers and particulate organic matter: Isotopic evidence of strong selectivity in the estuarine transition zone. Limnology and Oceanography 49(5), 1679-1686.

McClelland, J.W., 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.

Naldi, M., Wheeler, P.A., 2002. ^{15}N measurements of ammonium and nitrate uptake by *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta): comparison of net nutrient disappearance, release of ammonium and nitrate, and ^{15}N accumulation in algal tissue. Journal of Phycology 38, 135-144.

Needoba, J.A., Sigman, D.M., Harrison, P.J., 2004. The mechanism of isotope fractionation during algal nitrate assimilation as illuminated by the $^{15}N/^{14}N$ of intracellular nitrate. Journal of Phycology 40, 517-522.

Nogueira, E., Perez, F.F., Rios, A.F., 1997. Seasonal patterns and long-term trends in an estuarine upwelling ecosystem (Ria de Vigo, NW Spain). Estuarine Coastal and Shelf Science 44(3), 285-300.

Precedo Ledo, A., Míguez Iglesias, A., Fernández Justo, I., 2008. Galicia: el tránsito hacia una sociedad urbana en el contexto de la Unión Europea. Revista Galega de Economía 17(número extraordinario), 1-20.

Rio Barja, F.J., Rodriguez Lestegas, F., 1996. Os rios galegos: morfoloxía e rexime. In: Consello da Cultura Galega (Editor), As augas de Galicia. Consello da Cultura Galega, Santiago de Compostela, pp. 149-211.

Ruby, E.D., Jannasch, H.W., Deuser, W.G., 1987. Fractionation of stable carbon isotopes during chemoautotrophic growth of sulfur-oxidizing bacteria. Applied Environmental Microbiology 53, 1940-1943.

Sakamaki, T., Richardson, J.S., 2008. Effects of small rivers on chemical properties of sediment and diets for primary consumers in estuarine tidal flats. Marine Ecology Progress Series 360, 13-24.

Savage, C., Elmgren, R., 2004. Macroalgal (*Fucus vesiculosus*) $\delta^{15}N$ values trace decrease in sewage influence. Ecological Applications 14(2), 517-526.

Sigman, D.M., Altabet, M.A., Michener, R., McCorkle, D.C., Fry, B., Holmes, R.M., 1997. Natural abundance level measurement of the nitrogen isotopic composition of oceanic nitrate: an adoption of the ammonia diffusion method. Marine Chemistry 57, 227-242.

Slawyk, G., Raimbault, P., 1995. Simple procedure for simultaneous recovery of dissolved inorganic and organic nitrogen in ^{15}N-tracer experiments and improving the isotopic mass balance. Marine Ecology Progress Series 124(1-3), 289-299.

Southward, E.C., Gebruk, A., Kennedy, H., Southward, A.J., Chevaldonné, P., 2001. Different energy sources for three symbiont-dependent bivalve molluscs at the Logatchev hydrothermal site (Mid-Atlantic Ridge). Journal of the Marine Biological. Association of the United Kingdom 81, 655-661.

Spiro, B., Greenwood, P.B., Southward, A.J., Dando, P.R., 1986. $^{13}C/^{12}C$ ratios in marine invertebrates from reducing sediments: confirmation of nutritional importance of chemoautotrophic endosymbiotic bacteria. Marine Ecology Progress Series 28, 233-240.

Tucker, J., Sheats, N., Giblin, A.E., Hopkinson, C.S., Montoya, J.P., 1999. Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. Marine Environmental Research 48(4-5), 353-375.

Varela, M., Prego, R., 2003. Hydrography and phytoplankton in an isolated and non-pristine ria area: the A Coruña Harbour (NW Spain). Acta Oecologica 24, 113-124.

Varela, M., Prego, R., Canle, M., Lorenzo, J., 1994. The Ria de La Coruña, is hydrologically a ria? Gaia 9, 3-6.

Viana, I.G., Fernández, J.A., Aboal, J.R., Carballeira, A., 2011. Measurement of $\delta^{15}N$ in macroalgae stored in an environmental specimen bank for regional scale monitoring of eutrophication in coastal areas. Ecological Indicators 11, 888-895.

Viana, I.G., Bode, A., 2013. Stable nitrogen isotopes in coastal macroalgae: geographic and anthropogenic variability. Science of the Total Environment 443, 887-895.

Waser, N.A., Yin, K.D., Yu, Z.M., Tada, K., Harrison, P.J., Turpin, D.H., Calvert, S.E., 1998. Nitrogen isotope fractionation during nitrate, ammonium and urea uptake by marine diatoms and coccolithophores under various conditions of N availability. Marine Ecology Progress Series 169, 29-41.

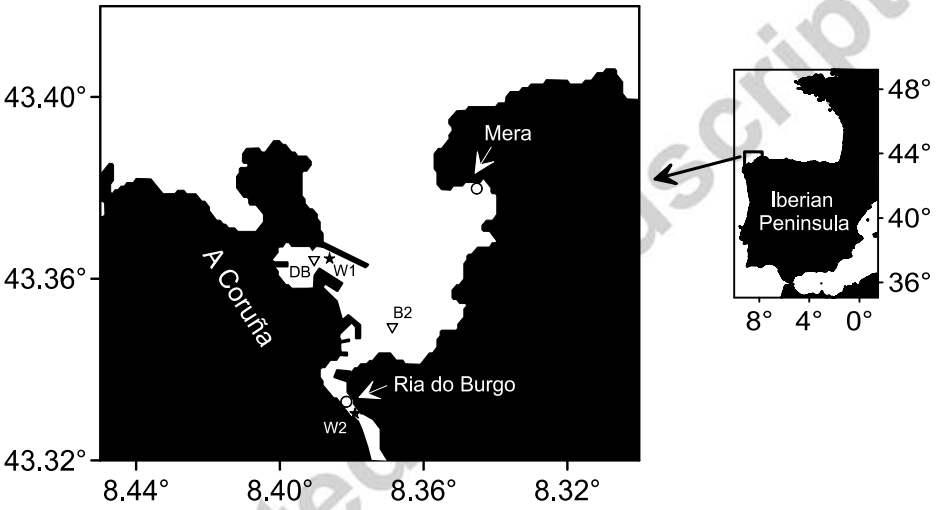

Figure 1. Location of sampling stations for subtidal infauna (DB and B2), intertidal benthos (Mera and Ria do Burgo) and surface water properties (W1 and W2) in the Bay of A Coruña (NW Spain).

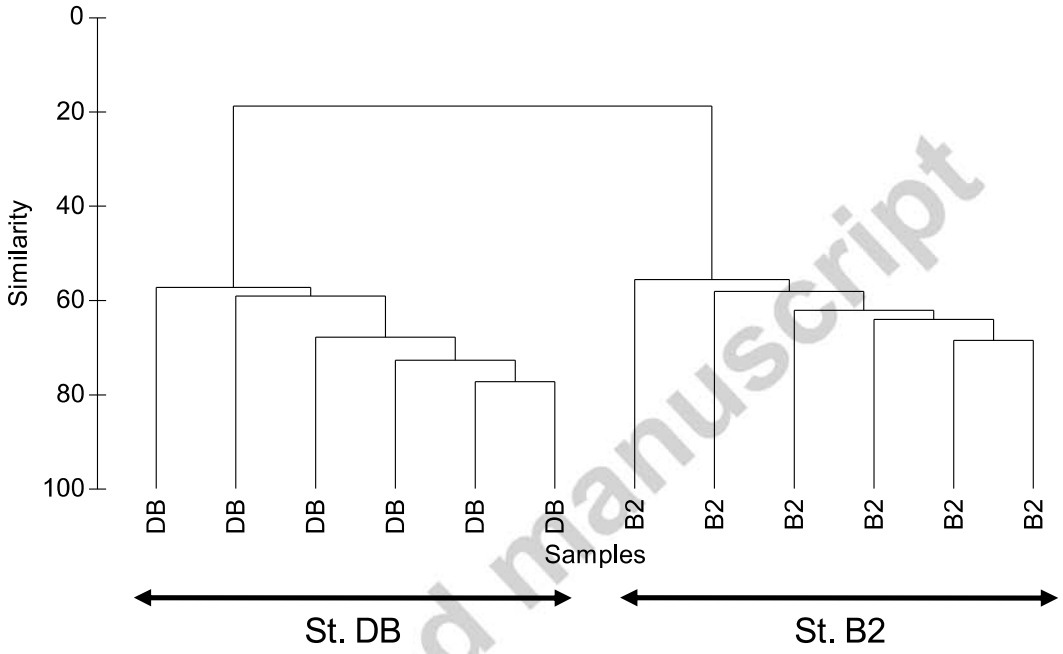

Figure 2. Cluster of infaunal samples (group average method) computed from Bray-Curtis similarity index on log-transformed biomass values of species contributing at least 0.1% to total biomass.

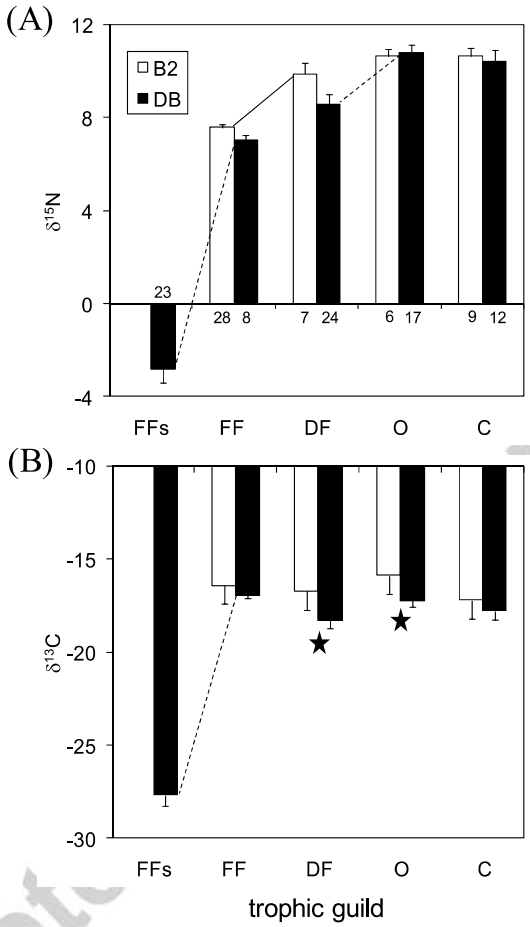

Figure 3. Mean (± se) $\delta^{15}N$ (A, ‰) and $\delta^{13}C$ (B, ‰) in selected infaunal species grouped by trophic guild at stations B2 and DB. FFs: filter feeders with bacterial symbionts, FF: other filter feeders, DF: deposit feeders, O: omnivores, C: carnivores. The numbers indicate the data averaged for each category. Significant differences between stations are indicated by stars (Mann Whitney U test, $P<0.05$) and differences between adjacent guilds for each station (ANOVA and Dunnet C test, $P<0.05$) by continuous (B2) or dashed lines (DB).

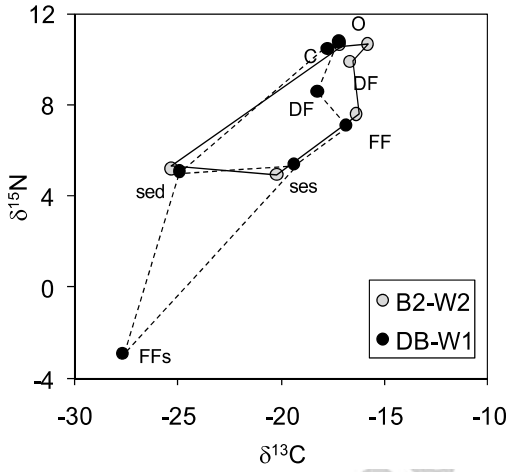

Figure 4. Relationships between mean $\delta^{15}N$ and $\delta^{13}C$ in infaunal trophic guilds, seston (ses) and sediments (sed) of stations in the Bay of A Coruña. Guild codes as in Fig. 3. The polygons include trophic pathways at stations B2 and DB.

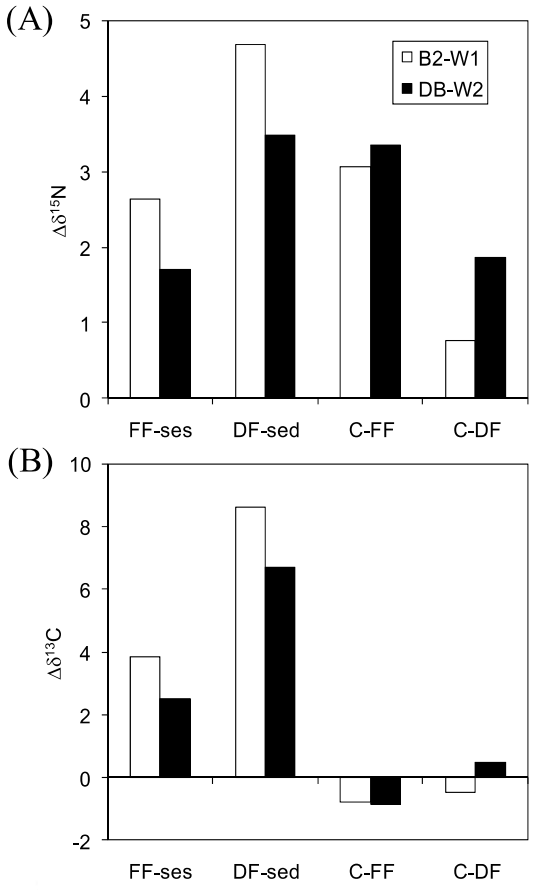

Figure 5. Mean isotopic enrichment for nitrogen (A, $\Delta\delta^{15}N$) and carbon (B, $\Delta\delta^{13}C$) between food sources and infaunal trophic guilds of stations in the Bay of A Coruña. Filter feeders with symbiotic bacteria and omnivores were not included. FF: filter feeders (without symbiotic bacteria), DF: deposit feeders, C: carnivores, ses: seston, sed: sediments.

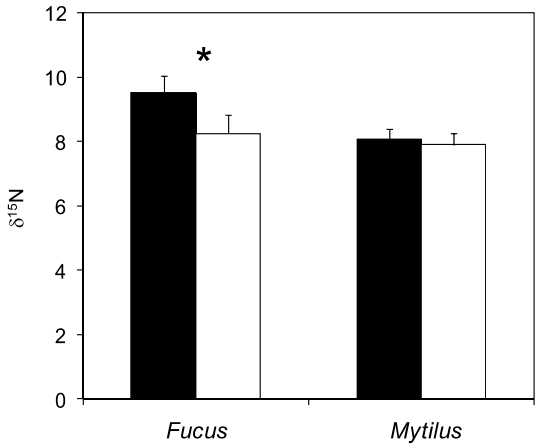

Figure 6. Mean (± se) $\delta^{15}N$ (‰) in *Fucus* and *Mytilus* at intertidal locations in Ria do Burgo and Mera. Significant differences between sites are indicated with an asterisk (Mann Whitney U test, $P<0.05$). n = 15.

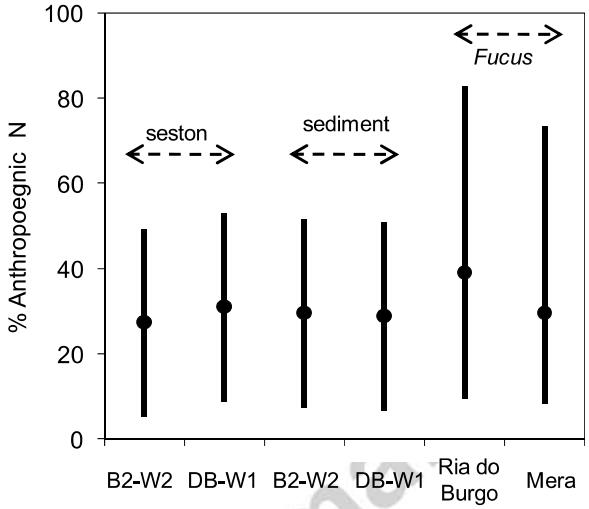

Figure 7. Percent contribution of anthropogenic nitrate to seston, sediment and *Fucus* nitrogen estimated from mean $\delta^{15}N$ values in the studied stations and an isotopic mixing model with marine and anthropogenic end-members. Each bar represent the range of contributions estimated using isotope fractionation values between 0 and 6‰. The dots indicate the estimates with a fractionation value of 3‰.

Table 1. Mean (± se) values of surface water temperature (SST, °C), salinity (SSS), nitrate (NO_3^-, µM), nitrite (NO_2^-, µM), ammonium (NH_4^+, µM), chlorophyll-a (Chla, mg m^{-3}), particulate organic carbon (POC, µM) and nitrogen (PON, µM), seston molar C:N ratio, natural abundance of stable carbon and nitrogen isotopes in seston ($\delta^{13}C_{ses}$ and $\delta^{15}N_{ses}$, ‰) and in ammonium and nitrate ($\delta^{15}N_{NH4}$ and $\delta^{15}N_{NO3}$, ‰) at stations W1 and W2, and intertidal sampling sites at Ria do Burgo and Mera during an annual cycle (number of samples = 11, except for $\delta^{15}N_{NH4}$ and $\delta^{15}N_{NO3}$ where n = 8). P: significance of Mann-Whitney U for differences between stations. n.s.: P>0.05

	W1		W2		
	mean	se	mean	se	P
SST	15.14	0.50	15.06	0.65	n.s.
SSS	35.35	0.25	32.11	1.31	0.025
Chla	1.674	0.334	1.880	0.371	n.s.
NO_3^-	19.78	7.77	24.54	7.01	n.s.
NO_2^-	1.35	0.62	1.37	0.38	n.s.
NH_4^+	4.85	1.68	4.85	0.99	n.s.
POC	15.21	3.25	38.52	7.33	0.009
PON	2.10	0.31	4.87	0.98	0.014
C:N	6.81	0.41	7.96	0.29	0.031
$\delta^{13}C_{ses}$	-19.4	0.4	-20.2	0.5	n.s.
$\delta^{15}N_{ses}$	5.4	0.3	4.9	0.5	n.s.
$\delta^{15}N_{NH4}$	1.1	0.5	-0.2	0.8	n.s.
$\delta^{15}N_{NO3}$	4.0	0.3	4.0	0.6	n.s.
	Ria do Burgo		Mera		
	mean	se	mean	se	P
SST	17.2	1.2	16.8	0.9	n.s.

SSS	30.4	2.4	34.0	0.6	n.s.
NO_3^-	38.62	33.77	14.21	10.65	n.s.
NO_2^-	0.76	0.15	0.53	0.11	n.s.
NH_4^+	4.72	1.13	3.51	1.32	n.s.
$\delta^{15}N_{NH4}$	0.2	0.7	0.2	0.7	n.s.
$\delta^{15}N_{NO3}$	3.1	0.2	4.2	0.3	0.007

Table 2. Mean (se: standard error) values of variables measured in the water column above infauna stations (B2 and DB). SST: sea surface temperature (°C), SSS: sea surface salinity, t_{bottom}: temperature 1 m above the bottom (°C), $Chla_{int}$: water column integrated chlorophyll-a, $\%PAR_{bottom}$: percent of surface irradiance (PAR) at the bottom. number of samples = 12. P: significance of Mann-Whitney U for differences between stations. n.s.: P>0.05

	B2		DB		
	mean	se	mean	se	P
SST	14.95	0.49	14.65	0.52	n.s.
SSS	33.82	0.51	34.43	0.59	n.s.
t_{bottom}	14.82	0.34	14.34	0.28	n.s.
$Chla_{int}$	16.01	5.46	45.17	13.96	n.s.
$\%PAR_{bottom}$	20.81	3.13	6.66	1.37	0.001

Table 3. Mean (se: standard error) values of sediment variables measured at the infauna stations. Q_{50} ϕ: median of the distribution of particle diameter ($\phi = \log_2(mm)$), S_0: selection coefficient, %Coarse sand: percent of >500 µm diameter particles (by weight), %Fine sand: percent of 62-500 µm diameter particles (by weight), %Mud: percent of <62 µm diameter particles (by weight), %AFDW: percent ash-free dry weight, $\%C_{total}$: percent total carbon (including

carbonates), %N: percent nitrogen, C:N: molar C:N ratio, $\delta^{13}C_{total}$: natural abundance of ^{13}C (including carbonates), $\delta^{13}C_{org}$: natural abundance of ^{13}C (organic matter), $\delta^{15}N$: natural abundance of ^{15}N. number of samples = 9. P: significance of Mann-Whitney U for differences between stations. n.s.: P>0.05.

	Station				
	B2		DB		
	mean	se	mean	se	P
Q_{50} ϕ	2.95	0.01	3.62	0.08	0.000
S_0	1.44	0.01	2.06	0.03	0.000
%Coarse sand	1.02	0.14	3.20	0.37	0.000
%Fine sand	91.19	0.46	56.61	1.74	0.000
%Mud	7.79	0.39	40.19	1.90	0.000
%AFDW	2.07	0.18	10.30	0.55	0.000
$\%C_{total}$	1.90	0.16	4.60	0.12	0.000
%N	0.03	0.00	0.20	0.01	0.000
C:N	76.86	7.97	27.41	1.14	0.001
$\delta^{13}C_{total}$	-2.8	0.3	-13.4	0.3	0.000
$\delta^{13}C_{org}$	-25.3	0.1	-24.9	0.1	n.s.
$\delta^{15}N$	5.2	0.1	5.1	0.1	n.s.

Table 4. Mean (se: standard error) values of $\delta^{13}C$, $\delta^{15}N$ and biomass (B, mg fresh weight m^{-2}) for selected infaunal species at stations B2 and DB. The taxonomic group (all species) and trophic guild (only species analysed for stable isotopes) are indicated. %sim: percent contribution of species to Bray-Curtiss similarity for each station (SIMPER analysis, Clarke and Warwick, 2001). n: number of samples (or individuals for stable isotopes). FF: filter feeder, FFs: filter feeder with symbionts, DF: deposit feeder, O: omnivore, P: predator.

			$\delta^{13}C$		$\delta^{15}N$			B2				DB			
Group	species	Guild	mean	se	mean	se	n	%sim	mean	se	n	%sim	mean	se	n
Mollusca	*Pharus legumen*	FF	-15.5	0.1	7.7	0.2	9	16.8	760	529	6	---	---	---	---
Polychaeta	*Paradoneis armata*	---	---	---	---	---	---	11.1	416.8	61.2	6	---	---	---	---
Mollusca	*Chamelea gallina*	FF	-17.4	0.3	7.3	0.2	12	9.0	228	138	5	---	2.6	---	1
Others	Nemertea	---	---	---	---	---	---	8.3	293.4	215.7	7	---	251	227	6
Polychaeta	*Magelona filiformis*	---	---	---	---	---	---	7.3	80.5	23.7	6	---	---	---	---
Mollusca	*Myrtea spinifera*	FFs	-27.5	0.5	-1.9	0.5	18	---	---	---	-	10.4	177	574.5	6
Polychaeta	*Notomastus latericeus*	DF	-17.5	0.5	8.3	0.4	9	---	75.5	74.3	4	8.8	435.1	115.8	7
Polychaeta	*Euclymene oerstedi*	DF	-18.1	0.3	8.7	0.4	7	---	---	---	-	8.3	528.5	233.6	7

Polychaeta	*Chaetozone gibber*	DF	-19.3	0.4	7.6	0.3	8	---	0.3	---	1	8.0	792.6	213.4	7
Mollusca	*Nucula* sp.	---	---	--	---	--	--	---	69.7	68.9	2	7.4	455.8	131.1	6
Polychaeta	*Chaetozone setosa*	---	---	--	---	--	--	---	---	---	0	7.2	100 6.5	808.8	7
Echinodermata	*Leptosynapta bergensis*	DF	-16.6	0.3	11.8	1.5	4	---	155.1	91.8	6	---	75.7	28.8	5
Echinodermata	*Leptosynapta inhaerens*	DF	-16.7	0.4	10.8	0.0	3	---	99.8	---	1	---	362.8	337.0	2
Mollusca	*Abra nitida*	DF	-18.1	--	6.6	--	1	---	0.9	0.6	4	---	131.7	71.2	3
Mollusca	*Corbula gibba*	FF	-16.9	0.2	7.1	0.2	8	---	---	---	--	---	560.6	367.1	3
Mollusca	*Mactra stultorum*	FF	-15.9	0.1	7.9	0.2	7	---	581 7.6	324 1.4	5	---	237 9.2	---	1
Mollusca	*Nassarius reticulatus*	O	-16.5	0.4	10.8	0.3	1 4	---	412 5.4	363.3	2	---	100 0.0	465.5	4

Mollusca	*Thyasira flexuosa*	FFs	28.5	0.3	-6.37	0.75	---	0.3	---	1	---	1	112.1	32.36	--
Polychaeta	*Diopatra neapolitana*	C	17.3	0.3	-10.6	0.45	---	569.2	354.73	---	---	---	---	---	--
Polychaeta	*Glycera* sp.	C	17.5	0.2	-11.3	0.66	---	3.7	3.03	---	369.9	58.52	--		
Polychaeta	*Nephtys hombergii*	C	16.7	0.4	-9.26	0.66	---	384.3	285.66	---	15.01	11.74	--		