Science of the Total Environment 443 (2013) 887–895

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Stable nitrogen isotopes in coastal macroalgae: Geographic and anthropogenic variability

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HIGHLIGHTS

► Anthropogenic versus upwelling nitrogen effect on macroalgal $\delta^{15}N$ was studied.
► The influence of populations and upwelling has not been made before on macroalgal $\delta^{15}N$.
► Natural variability has not been taken into account in most biomonitoring studies.
► Upwelling explains most of the variability in $\delta^{15}N$ in macroalgae.

ARTICLE INFO

Article history:
Received 12 September 2012
Received in revised form 16 November 2012
Accepted 19 November 2012
Available online 14 December 2012

Keywords:
Upwelling
Wastewater
Urban populations
Biomonitors
Fucus
Ascophyllum

ABSTRACT

Growing human population adds to the natural nitrogen loads to coastal waters. Both anthropogenic and natural nitrogen is readily incorporated in new biomass, and these different nitrogen sources may be traced by the measurement of the ratio of stable nitrogen isotopes ($\delta^{15}N$). In this study $\delta^{15}N$ was determined in two species of macroalgae (*Ascophyllum nodosum* and *Fucus vesiculosus*), and in nitrate and ammonium to determine the relative importance of anthropogenic versus natural sources of nitrogen along the coast of NW Spain. Both algal species and nitrogen sources showed similar isotopic enrichment for a given site, but algal $\delta^{15}N$ was not related to either inorganic nitrogen concentrations or $\delta^{15}N$ in the water samples. The latter suggests that inorganic nitrogen inputs are variable and do not always leave an isotopic trace in macroalgae. However, a significant linear decrease in macroalgal $\delta^{15}N$ along the coast is consistent with the differential effect of upwelling. Besides this geographic variability, the influence of anthropogenic nitrogen sources is evidenced by higher $\delta^{15}N$ in macroalgae from rias and estuaries compared to those from open coastal areas and in areas with more than 15×10^3 inhabitants in the watershed. These results indicate that, in contrast with other studies, macroalgal $\delta^{15}N$ is not simply related to either inorganic nitrogen concentrations or human population size but depends on other factors as the upwelling or the efficiency of local waste treatment systems.

1. Introduction

Coastal areas, particularly estuaries, have been subjected to increasing nitrogen loads due to the growing human population and its associated anthropogenic activities (e.g. agriculture, sewage). As a consequence of these activities, coastal ecosystems are under increasing pressures of pollution and eutrophication (Paerl et al., 2006; Vidal et al., 1999). The latter, a problem first limited to enclosed or semi enclosed water bodies, is now being observed in most coastal areas (Cloern, 2001; Druon et al., 2004; Gilbert et al., 2009; Valiela et al., 2000). Determining the origin of the dissolved nitrogen in estuarine environments can be an effective means of evaluating nutrient management policies, and may ultimately lead to more successful environmental regulation of anthropogenic nitrogen (Ahad et al., 2006).

The adverse effects of anthropogenic nitrogen inputs have led to the development of suitable indicators to assess water quality of aquatic ecosystems, both for management or biological issues. Direct quantification of dissolved inorganic nitrogen in water has been frequently used (e.g. Hickel et al., 1993; Paerl et al., 2006; Rabalais et al., 1996). However, nutrient concentrations in the water column alone seem not to be adequate to quantify anthropogenic loads as they are highly variable in time because of rapid consumption by primary producers (Fry et al., 2003). Moreover, changes in nitrogen concentrations may not only be due to anthropogenic inputs but also to natural processes, as coastal upwelling (e.g. Arístegui et al., 2006).

As an alternative to nutrient measurement, the ratio of nitrogen stable isotopes ($\delta^{15}N$) in macroalgae has been increasingly used to quantify the importance of different nitrogen sources for primary producers (Costanzo et al., 2005; Gartner et al., 2002; Lapointe and Bedford, 2007; McClelland and Valiela, 1998; McClelland et al., 1997; Piñón-Gimate et al., 2009; Riera et al., 2000; Savage and Elmgren, 2004; Tucker et al., 1999). Nitrogen has two stable isotopes,

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http://dx.doi.org/10.1016/j.scitotenv.2012.11.065

and its proportion might vary according to the different metabolic routes that a molecule follows, as light isotopes (^{14}N) are mobilized faster by some processes than the heavy ones (isotopic fractionation). For some biological reactions, the reactants are progressively enriched in heavy isotopes while the products are relatively depleted at a rate characteristic of each reaction (Mariotti et al., 1981). Anthropogenic nitrogen sources, like sewage, manure, terrestrial runoff, fish farm waste and groundwater, are often more enriched in ^{15}N than seawater (Heaton, 1986; Jordan et al., 1997; McClelland and Valiela, 1998; Vizzini and Mazzola, 2004; Voβ and Struck, 1997) because of isotopic fractionation during nitrification and volatilization in the case of NH_4^+, or denitrification in the case of NO_3^- (Montoya, 2008). In contrast, nitrogen pools from most agricultural facilities are characterized by depleted isotopic values, as they are synthesized from atmospheric N_2 (Heaton, 1986). Furthermore, $\delta^{15}N$ in macroalgae can also be used to detect the intensity and variability of the anthropogenic nitrogen loading (Cole et al., 2004; Costanzo et al., 2005; Savage and Elmgren, 2004) often related to the degree of urbanization in the watershed (Cole et al., 2004, 2005; McClelland and Valiela, 1998; McClelland et al., 1997).

Besides nutrients from anthropogenic origin, different natural processes also affect inorganic nitrogen concentrations and in consequence macroalgal isotopic values. For instance, algae from mangrove habitats that were exposed to nitrogen derived from N_2 fixation were depleted in ^{15}N while those in habitats with frequent coastal upwelling were relatively enriched (Lamb et al., 2012). In addition, $\delta^{15}N$ in estuarine waters vary as a consequence of freshwater inputs and local biogeochemical processes (Ahad et al., 2006). Because different combinations of sources may produce similar $\delta^{15}N$ values, additional information on factors affecting local nitrogen dynamics is required to obtain unequivocal evidence that significant amounts of anthropogenic nitrogen are affecting the coastal zone.

The regions of Galicia and Asturias (NW Spain, Fig. 1) are characterized by the presence of estuaries and rias sustaining high levels of biological production due to seasonal upwelling fertilization (Arístegui et al., 2006). Each of these rias has also an independent river basin, but the nutrient inputs from these rivers are lower than those from the upwelling (Bode et al., 2011b). The upwelling has a larger impact in the production of western and southern rias (Galicia) because the initial nutrient inputs are amplified by remineralization of organic matter in the shelf and subsequent import with estuarine circulation (Álvarez-Salgado et al., 1997). In contrast, upwelling in the northern coast (Asturias) is generally weaker than in the western coast and limited to the vicinity of major capes (Botas et al., 1990). Upwelling nutrients support a larger fraction of primary production in Galicia than in Asturias (Álvarez-Salgado et al., 2002; Bode et al., 2011a). In consequence, geographic variability in the nitrogen sources, and correspondingly in their isotopic signature, can be expected in NW Spain. Besides, most of the human population concentrates in the coastal zone, which showed large urbanization development in recent years (Viña, 2008). Previous studies of macroalgal $\delta^{15}N$ in this region reported high enrichment near large urban areas and inside the rias, suggesting the influence of nitrogen from wastewater (Bode et al., 2006, 2011b; Carballeira et al., 2012; Viana et al., 2011).

In this study the variability in the isotopic composition of two intertidal macroalgae in relation to concurrent measurements of dissolved inorganic nitrogen concentrations and isotopic composition in the NW coast of Spain was analyzed to determine the relative importance of anthropogenic versus natural nitrogen sources. The effect of the coastal upwelling, as the main natural source of nitrogen, was represented by the geographical distribution of sampling sites along the coast, while the main anthropogenic input was represented by the size of the human population in the watershed as a proxy for wastewater production.

2. Material and methods

2.1. Sampling

Samples were collected in the intertidal along the coast of NW Spain at sites representative of environments with variable influence of the upwelling and in a large range of urban influence (Fig. 1). As upwelling in the northern coast is generally weaker than in the western coast (Botas et al., 1990), an arbitrary reference point located at the sea discharge point of the River Miño (Fig. 1) was used to compute the distance along the coast between each sampling site and this reference point. This distance was intended to indicate the lower input of new nitrogen by the upwelling in the northern coast (Mar Cantábrico, zone I in Fig. 1) compared to those in the western coast (Galicia). In the latter, two zones were considered to investigate potential differences between Rias Baixas (zone III) and other rias (zone II). Sampling sites covered a large range of urban population influence in the watershed (from ~240 to ~246,000 inhabitants) according to Spanish Official Population Census (http://www.ine.es/inebase). Sampling surveys were carried out mostly during spring and summer 2010 and 2011, but some samples from 2006 were

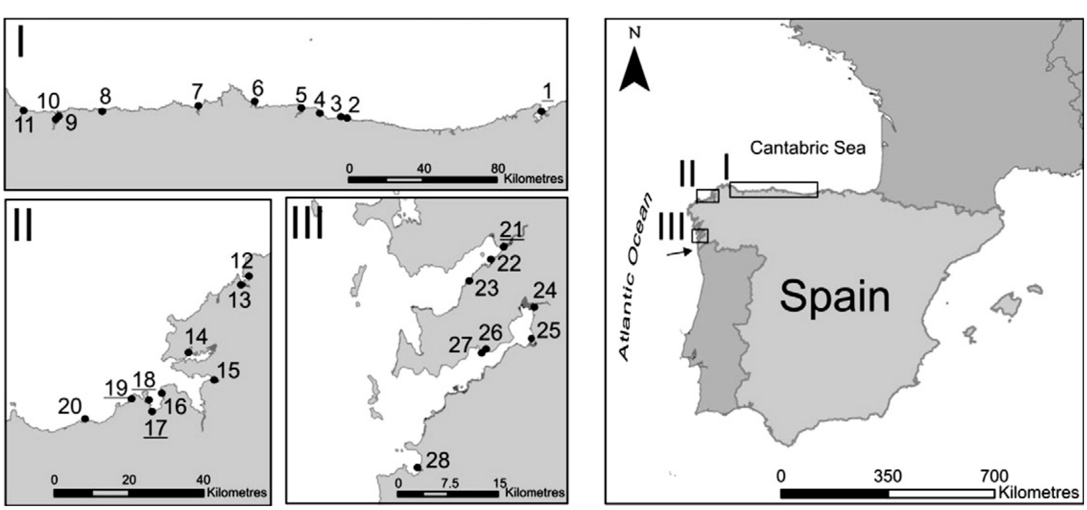

Fig. 1. Location of sampling sites along NW Spain. Three environment types representing coastal sites in large rias (I), sites in or near middle rias (II) and mostly open sea sites at the northern coast (III) were considered. The arrow indicates the River Miño discharge point used as the southernmost reference point to compute intersite distances in this study.