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Baseline

Stable isotope variations in benthic primary producers along the Bosphorus (Turkey): A preliminary study

Edoardo Calizza [a,*], Yelda Aktan [b], Maria Letizia Costantini [a], Loreto Rossi [a]

[a] Laboratory of Trophic Ecology, Department of Environmental Biology, Sapienza University of Rome, via dei Sardi 70, 00185 Rome, Italy
[b] Istanbul University, Fisheries Faculty, Ordu Cad. No. 200, 34470 Laleli, Istanbul, Turkey

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ABSTRACT

The Bosphorus Strait is a dynamic and complex system. Recent evidences showed nitrogen and heavy metal concentrations to follow opposite patterns across the Strait, suggesting a complex spatial organisation of the anthropogenic disturbance in this system. Here, we provide isotopic information on the origin and transportation of dissolved nitrogen along the Bosphorus. C and N isotopic and elemental analyses were performed on specimens of *Ulva lactuca* and associated epiphytes sampled in five locations across the Strait. Variations in C and N isotopic signatures were observed in *U. lactuca*, pointing to a decrease in the availability of anthropogenic organic dissolved nitrogen along a north-south direction. Conversely, epiphytes did not show isotopic or elemental patterns across the Strait. These results suggest that preliminary stable isotope surveys in extended costal systems basing on *U. lactuca* can represent a valuable tool to focus meaningful targets and hypotheses for pollution studies in the Mediterranean region.

Coastal marine systems represent complex environments subjected to multiple anthropogenic and natural pressures affecting water quality, habitat integrity, biodiversity organisation and related ecological processes (Halpern et al., 2008). Among others, the Bosphorus (Turkey) represents a highly dynamic system where surface waters flow from the Black Sea in the North to the Sea of Marmara in the South (Tuğrul and Polat, 1995). Freshwater inputs coupled with high concentration of dissolved nutrients characterise the northern part of the Strait, whereas the industrial and sewage discharge from the city of Istanbul, coupled with an intense ship traffic, harm the water quality in the southern part (Tuğrul and Polat, 1995; Balkıs et al., 2012; Aktan et al. 2014). Recent baseline evidences showed nitrogen loadings and heavy metals concentration to follow opposite geographic patterns within the Strait, pointing to a complex spatial organisation of the anthropogenic disturbance in this system (Balkıs et al., 2012; Aktan et al., 2014). Further details on the hydro-ecology and environmental features characterising the Bosphorus can be found in Tuğrul and Polat (1995), Balkıs et al. (2012), and Aktan et al. (2014). In a such complex environmental condition, the identification of meaningful hypotheses and targets for explorative and routine pollution monitoring studies over an extended coastal area is no easy task. The aim of this preliminary baseline study is to provide stable isotope-related information regarding the origin, transportation and uptake by benthic primary producers (i.e. macroalgae and epiphytes) of the dissolved nitrogen along the Bosphorus. Stable isotopes of nitrogen (^{15}N:^{14}N, expressed as $\delta^{15}N$) in macroalgae have been shown to be a powerful tool to track the origin and extent of nutrient loadings in coastal areas (Dailer et al., 2010; Orlandi et al., 2014; Jona-Lasinio et al., 2015). Indeed, anthropogenic organic nitrogen is relatively enriched in ^{15}N ($\delta^{15}N$ varies between +6‰ and +38‰) with respect to synthetic/fertiliser-derived nitrogen ($\delta^{15}N$ varies between −4‰ and +4‰), and both of them can be distinguished from atmospheric-derived (i.e. natural) nitrogen on the basis of their $\delta^{15}N$ signatures (Dailer et al., 2010). In particular, the green macroalga *U. lactuca* has been recently demonstrated to be an effective bioindicator of nutrient pollution in Mediterranean coastal areas subjected to multiple pollution sources (Orlandi et al., 2014; Jona-Lasinio et al., 2015).

In the present study, resident *U. lactuca* specimens having a surface area of circa 77–80 cm^2 were sampled in five distant locations ranging from the Black Sea side to the Sea of Marmara side of the Bosphorus (Fig. 1). Such leaf surface represents the most frequently encountered size characterising *U. lactuca* across the study area, and specimens visibly exceeding the lower or the upper limit of the above mentioned range were intentionally avoided.

* Corresponding author.
E-mail address: edoardo.calizza@uniroma1.it (E. Calizza).

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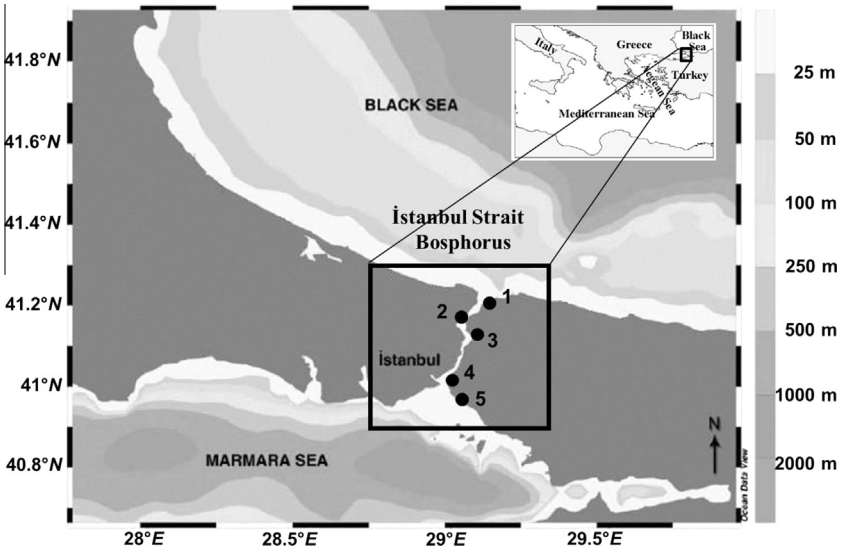

Fig. 1. Geographic map and sampling stations in the Bosphorus (Turkey). Sampling stations (black dots) are numbered from 1 to 5 along a north to south direction: i.e. from the Black Sea side to the Sea of Marmara side of the Strait. Previous baseline studies (Balkıs et al., 2012; Aktan et al., 2014) showed increasing water salinity and heavy metal concentration, and decreasing dissolved nutrient concentration from the Black Sea side to the Sea of Marmara side of the Bosphorus.

Samplings occurred on November 2014 and all sampling sites were visited the same day. In conjunction with the macroalga, also epiphytic algae (hereafter "epiphytes") found on collected specimens of *U. lactuca* were isolated and considered for the analyses. Epiphytes were obtained from *U. lactuca* leaves by gently scraping with a blade, and rinsing in distilled water and frozen. Three replicate samples of *U. lactuca* were analysed for each sampling site, whereas the low biomass of epiphytes growing on the specimens of *Ulva* forced us to pool together all epiphytes at each sampling site in order to obtain a sufficient amount of dry weight for the elemental and isotopic analysis. All samples were analysed twice, in which 2 ± 0.2 mg of dry weight for each analysis were used. C and N elemental and isotopic analyses were performed by using an Elementar vario-MICRO CUBE analyser coupled with an Isoprime 100 mass spectrometer, operating as a continuous flow system. Isotopic ratios were expressed in 'δ' units as the relative difference (in parts per thousand) between the sample and conventional standards (atmospheric N_2 [Air] for ^{15}N; PD-belemnite [PDB] carbonate for ^{13}C). Measurement errors were found to be typically smaller than ±0.05‰ for both $\delta^{13}C$ and $\delta^{15}N$. Further details on samples processing and laboratory analyses can be found in Calizza et al. (2013), Orlandi et al. (2014) and Careddu et al. (2015).

$\delta^{15}N$ in *Ulva lactuca* decreased from the Black Sea side to the Sea of Marmara side of the Bosphorus (i.e. from site 1 to site 5), being negatively correlated with the distance from site 1 ($r = -0.77$, d.f. = 13, $p < 0.001$) (Fig. 2a). Inversely, both the $\delta^{13}C$ and the percentage of Nitrogen (N%) in *U. lactuca* increased along the Strait, being positively correlated with the distance from site 1 ($\delta^{13}C$: $r = 0.90$, d.f. = 13, $p < 0.001$; N%: $r = 0.86$, d.f. = 13, $p < 0.001$). $\delta^{15}N$, $\delta^{13}C$ and N% in epiphytes were not significantly related to the distance from site 1 (p always > 0.05), neither other variation patterns were evident across the five sampling sites considered. On the other hand, localised variations in both isotopic signatures and elemental content in epiphytes were observed between neighbouring sampling sites (Fig. 2b). Accordingly, the relative variation (in percentage) of the above mentioned parameters from site 1 to site 5 was always higher in *U. lactuca* than in epiphytes. $\delta^{15}N$ varied by the 31% and the 8% in macroalgae and epiphytes respectively, whereas the $\delta^{13}C$ varied by the 20% and the 11%, and the N% varied by the 91% and the 71% in macroalgae and epiphytes respectively. The % of carbon (C%) did never vary significantly with the distance from site 1, neither for *U. lactuca* nor for epiphytes (data not shown; r always < 0.10, p always > 0.05). In *U. lactuca*, $\delta^{15}N$ decreased with both the $\delta^{13}C$ ($r = -0.69$, d.f. = 13, $p < 0.01$) and the N% ($r = -0.57$, d.f. = 13, $p < 0.05$), which, in their turn, were positively correlated (i.e. $\delta^{13}C$ vs. N%: $r = 0.84$, d.f. = 13, $p < 0.001$).

The decreasing $\delta^{15}N$ value from north to south along the Bosphorus could be considered indicative of the decreasing influence of nutrient-rich freshwater inputs feeding the Strait along the north to south direction (Dailer et al., 2010; Aktan et al., 2014; Orlandi et al., 2014). Relatively higher $\delta^{15}N$ values have been shown to reflect organic anthropogenic-derived dissolved nitrogen available to *U. lactuca*, which can be associated to estuarine anthropic systems fuelling marine coastal areas (Orlandi et al., 2014; Careddu et al., 2015; Jona-Lasinio et al., 2015), and the northern part of the Bosphorus in particular (Aktan et al., 2014). Previous evidences reporting (i) gradually increasing salinity values along a north to south direction in the Bosphorus (including 4 out of 5 sampling sites among those considered in this study), and (ii) changes in the epipelic algal community across the Strait, including freshwater dwelling species that have been found on the Black Sea side (Aktan et al., 2014), support the hypothesis of the mix of fresh and marine waters and the estuarine origin of the nitrogen assimilated by *U. lactuca* in the northern part. The gradually increase in $\delta^{13}C$ values from the Black Sea to the Sea of Marmara and the significant inverse relationship between $\delta^{13}C$ and $\delta^{15}N$ further support potential freshwater inputs at the base of the observed higher $\delta^{15}N$ values in the northern part of the Strait. Indeed, as a consequence of remineralisation processes of the organic materials acting in terrestrial and freshwater environments, freshwater dissolved organic carbon shows relatively depleted ^{13}C values with respect to those naturally observed in marine waters, such difference potentially affecting $\delta^{13}C$ in coastal marine primary producers (Mook and Tan, 1991; Chanton and Lewis, 1999; Piola et al., 2006; Careddu et al., 2015). The isotopic gradients observed, in conjunction with a higher concentration of dissolved nutrients reported in the northern part of the Strait with respect to the southern one (Aktan et al., 2014), let suppose that the allochthonous (i.e. freshwater-anthropogenic) nitrogen inputs entering the Bosphorus in the Black Sea side are taken up from the water column within the extent of the Strait. Nevertheless, water quality in the Bosphorus is known to be severely affected by a direct urban

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