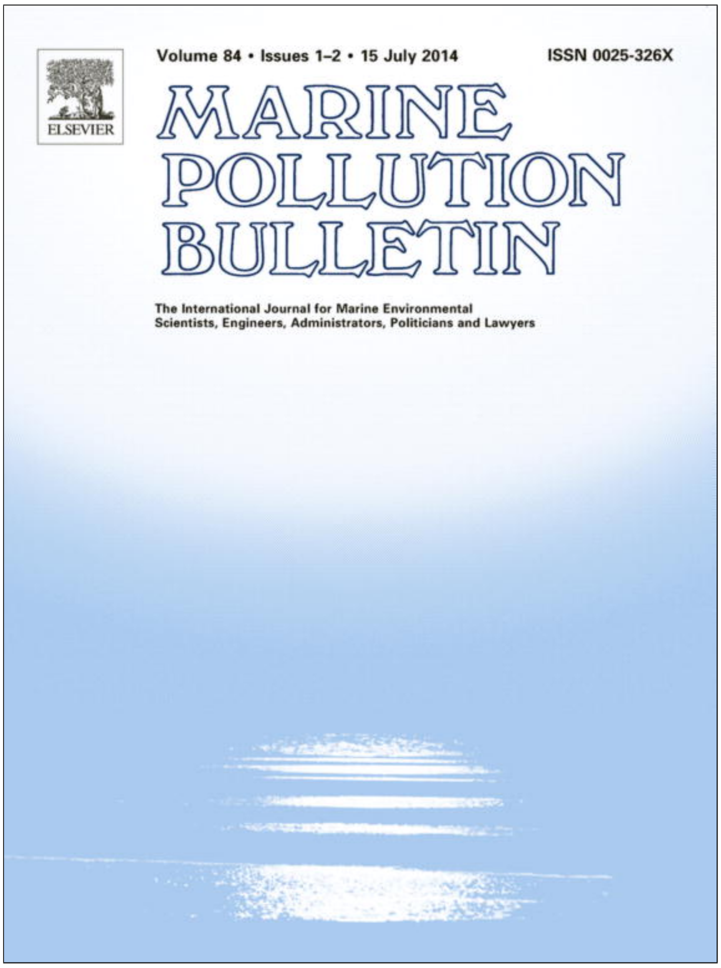
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δ^{15}N variation in *Ulva lactuca* as a proxy for anthropogenic nitrogen inputs in coastal areas of Gulf of Gaeta (Mediterranean Sea)

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ABSTRACT

We tested the capacity of *Ulva lactuca* to mark N sources across large marine areas by measuring variation in its δ^{15}N at several sites in the Gulf of Gaeta. Comparisons were made with the macroalga *Cystoseira amentacea*. Variation of δ^{15}N values was assessed also in the coastal waters off the Circeo Natural Park, where *U. lactuca* and *C. amentacea* were harvested, as these waters are barely influenced by human activities and were used as reference site. A small fragment from each frond was preserved before deployment in order to characterize the initial isotopic values. After 48 h of submersion, *U. lactuca* was more responsive than *C. amentacea* to environmental variation and δ^{15}N enrichment in the Gulf of Gaeta was observed. The spatial distribution of δ^{15}N enrichment indicated that different macro-areas in the Gulf were affected by N inputs from different origins. Comparison of the δ^{15}N values of fragments taken from the same transplanted frond avoided bias arising from natural isotopic variability.

1. Introduction

Marine coastal areas are among the most productive and exploited ecosystems on Earth and are consequently subject to multiple stressors. Over the last century, inputs of inorganic nutrients (particularly nitrogen) and organic matter, which commonly occur in combination with each other (McClanahan et al., 2005; Carreiro-Silva et al., 2009), have become two of the most important stressors affecting aquatic systems, with direct or indirect effects on their chemical, physical, and biological properties (Cottingham, 1999; Gray et al., 2002; McClanahan et al., 2005; Crain et al., 2008; O'Gorman et al., 2012). Inorganic nitrogen inputs mainly derive from agricultural runoff carrying fertilizer, while the organic inputs consist of dissolved and particulate forms of nitrogen associated with decomposing organisms and human and animal waste (McClanahan et al., 2005). Agricultural runoff and untreated sewage increase the rate of primary production in marine coastal areas (Doering et al., 1995; Taylor et al., 1999; Bowen and Valiela, 2001), which can lead to large blooms of phytoplankton and/or opportunistic macroalgae (Nixon and Buckley, 2002), degrading seagrass and macroalgal communities, altering N cycling and reducing water quality.

Determining the origin, fate and distribution of anthropogenic discharges in the sea is crucial to assessment of the self-purification capacity of coastal zones and to water quality management. Standard analyses of coastal waters have been used systematically in monitoring programs to track nutrients in the water column and to monitor eutrophication. However, these methods can be ineffective when nutrient loads are rapidly diluted by hydrodynamic forces and/or removed by microbial and plant uptake. Furthermore, they are labour-intensive and expensive (Jones et al., 2001; Burford et al., 2003; Sarà et al., 2004) and cannot distinguish between different N sources. A variety of indicators/indices, such as vegetation abundance responses to nutrient load (Ballesteros et al., 2007; Krause-Jensen et al., 2008), have also been developed to quantify the extent of pollution or eutrophication. However, these indices are unable to detect pollution in its early stages or pulsing sources of N after rapid dilution. In contrast, the stable nitrogen isotope ratio (δ^{15}N) is increasingly employed as a sensitive indicator of N sources in many ecosystems, and the biological characteristics of macroalgae, such as their fast growth and rapid turnover of nutrients in their tissues, make these organisms appropriate probes for detecting the origin of N pollutants by means of stable isotope analysis (SIA). Benthic macroalgae have been shown to be reliable indicators of anthropogenic nutrient loads in aquatic ecosystems as they assimilate nutrients in the water column and

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accumulate them in their tissues, integrating continuous and pulsed nutrient loadings (Jones et al., 2001; Cohen and Fong, 2005; Cole et al., 2005; García-Sanz et al., 2010).

Macroalgal $\delta^{15}N$ value accurately reflects N inputs from terrestrial sources (McClelland et al., 1997), as these inputs are not fractionated during uptake (Naldi and Wheeler, 2002; Cohen and Fong, 2005). Natural and anthropogenic N sources (the latter including fertilizer, sewage and manure) differ in terms of their $\delta^{15}N$ value (Kreitler, 1975, 1979; Kreitler and Jones, 1975; Heaton, 1986; Mariotti et al., 1982; Korom, 1992) and consequently algal $\delta^{15}N$ values can reflect the relative contribution of these different sources in limiting conditions (Grice et al., 1996; Elliott and Brush, 2006). This information helps to improve our understanding of how nitrogen enters a water body and how it is subsequently used by primary producers, which is of great importance in assessing the impacts of anthropogenic vs. 'natural' sources of nutrient inputs in marine systems (Rogers, 2003; Kamer et al., 2004; Savage and Elmgren, 2004).

The aim of this study, which was performed in two geographically close Mediterranean coastal areas, was to assess variation in the $\delta^{15}N$ value of the opportunistic attached macroalga *Ulva lactuca* (Ulvales, Ulvaceae) in response to various anthropogenic pressures. If such a link can be demonstrated, then the $\delta^{15}N$ value of this macroalga, which is found all over the world and is commonly used as an ecological indicator, could be used as a good proxy for the origin of nitrogen-based nutrients in marine waters. Comparisons were made with the attached macroalga *Cystoseira amentacea* (Fucales, Cystoseiraceae), which is not usually found in polluted waters and is thus a key biological element for assessing the ecological status of coastal waters in accordance with the European Water Framework Directive (WFD, 2000/60/EC).

2. Materials and methods

2.1. Study habitat

The two study areas (Gulf of Gaeta, *location A*, and Circeo, *location B*, used as a reference site; Fig. 1) are located along the west coast of Central Italy in the Mediterranean Sea and are characterized by different levels of anthropogenic disturbance. Specifically, the Gulf of Gaeta, with an area of 61 km^2, is delimitated to the north-west by the town of Sperlonga (41°15′49.89″N, 13°25′37.83″E) and to the south-east by the Garigliano river estuary (41°13′23.36″N, 13°45′40.66″E). It is affected by strong urbanization, the river Itri, with a drainage basin of 160.69 km^2, and intensive fish and mussel farming on the north-western side and by the heavily polluted waters of the Garigliano (which has a drainage basin of 4984 km^2) on the south-eastern side. Circeo, with an area of 9 km^2, is located off the Circeo promontory (included in the Circeo National Park; 41°13′30.40″N, 13°3′13.56″E), 30 miles north-west of the Gulf of Gaeta. This area has similar wind and sun exposure to the Gulf but is subject to lower anthropogenic pressure due to the legal protection regime and the absence of estuaries or effluents. Fecal bacterial loading was negligible in this area, whereas 90 MPN *Escherichia coli*/100 ml and 30 *Enterococchus* spp. u.f.c./100 ml were detected in the waters off the Garigliano river estuary in the Gulf (unpublished data courteously supplied by ARPA). Inorganic nitrogen levels were mostly low in both study areas, often below the sensitivity of field monitoring instruments (ammonium <19 μg/L and nitrite <0.6 μg/L), with the exception of nitrates, which at the time of the experiment were <0.6 μg/L in Circeo and 150.5 ± 3.5 μg/L on average in the Gulf of Gaeta. Average total nitrogen concentrations were 166.0 ± 2.1 μg/L in Circeo and 291 ± 7 μg/L in the Gulf of Gaeta (all chemical data from ARPA) and the average temperature was ca. 15 °C in the two areas. The sea bed in the two areas was characterized by variable proportions of mud, sand and rock.

In each study area, several sampling sites were chosen at two bathymetries (5 m and 12 m) on inshore-offshore transects: 6 sampling sites on 3 transects in Circeo and 16 sampling sites on 8 transects in the Gulf of Gaeta. Transect positioning in the Gulf was based on remote-sensing hydrological surveys to identify areas with a high probability of being affected by inputs from urban, agricultural and livestock-rearing outflows due to superficial runoff and river drainage (see Fig. 1 and Supplemental Material for Method details). This allowed us to identify the main outflow routes and, subsequently, four subareas in the Gulf, hereafter called (from north-west to south-east) Vendicio, Formia, Scauri and Garigliano (Fig. 1).

2.2. Field work

Fronds of *U. lactuca*, widely present in less wave-exposed intertidal coastal areas of both locations, and *C. amentacea* var. *stricta*, occurring in the supralittoral fringe of the reference location, were both collected from the reference location on 18 March 2012 and randomly deployed in replicates at all sampling sites on 19 March.

A small fragment from each frond of each species was cut and conserved (at −80 °C) before deployment and was subsequently used to determine the natural intraspecific variability of the initial $\delta^{15}N$ value (T_0) and to allow the final value of each sample to be compared to its corresponding initial value. The remaining fragments were singly housed in rigid plastic cages (1 cm mesh), which were tagged and suspended in the water column at ~70% light (Secchi disk depth = 2–6 m) about 50–90 cm below the water surface, using a combination of buoys, ropes and weights. In each sampling site three replicate plastic cages with *U. lactuca* and three with *C. amentacea* were submerged. Since the *U. lactuca* and *C. amentacea* cages deployed at the two sampling sites closest to the fish farm were removed twice by persons unknown, comparison in the northern Vendicio area relied only on samples from the other two sampling sites, which were located more than 1.2 km away from the farm. After 48 h of submersion (T_1), time enough for complete turnover of N in *U. lactuca* according to literature (Runcie et al., 2003) and for $\delta^{15}N$ equilibrium according to our preliminary tests (see Supplemental Material), samples were collected and transported to the laboratory in an ice-box. Fragments were frozen at −80 °C and then lyophilized and homogenized to a fine powder with a ball mill (Fritsch Pulverisette 23 with zirconium oxide ball) for the analysis of N and C stable isotope ratios.

Stable isotopes were determined in each sample by continuous-flow isotope mass spectrometer (Isoprime100, Isoprime Limited, Cheadle Hulme, UK) coupled with an elemental analyzer (Elementar vario MICRO CUBE, Elementar, Hanau, D). Each fragment (2.0–2.5 mg dry-weight) was analyzed individually. 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) in accordance with the formula $\delta R\,(‰) = [(R_{sample} - R_{standard})/R_{standard}] * 10^3$ (Ponsard and Arditi, 2000), where R is the heavy-to-light isotope ratio of the element ($R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$). Results were monitored with reference to an internal standard calibrated to International Atomic Energy Agency reference materials (Caffeine: IAEA-CH6).

2.3. Statistical analysis

Differences in $\delta^{15}N$ values between T_0 and T_1 and among sites, and in isotopic enrichment between bathymetries and among sites, were tested by *t*-test or Wilcoxon rank-sum test. The assumption of homogeneity of variances was checked using Cochran's *C*-test,

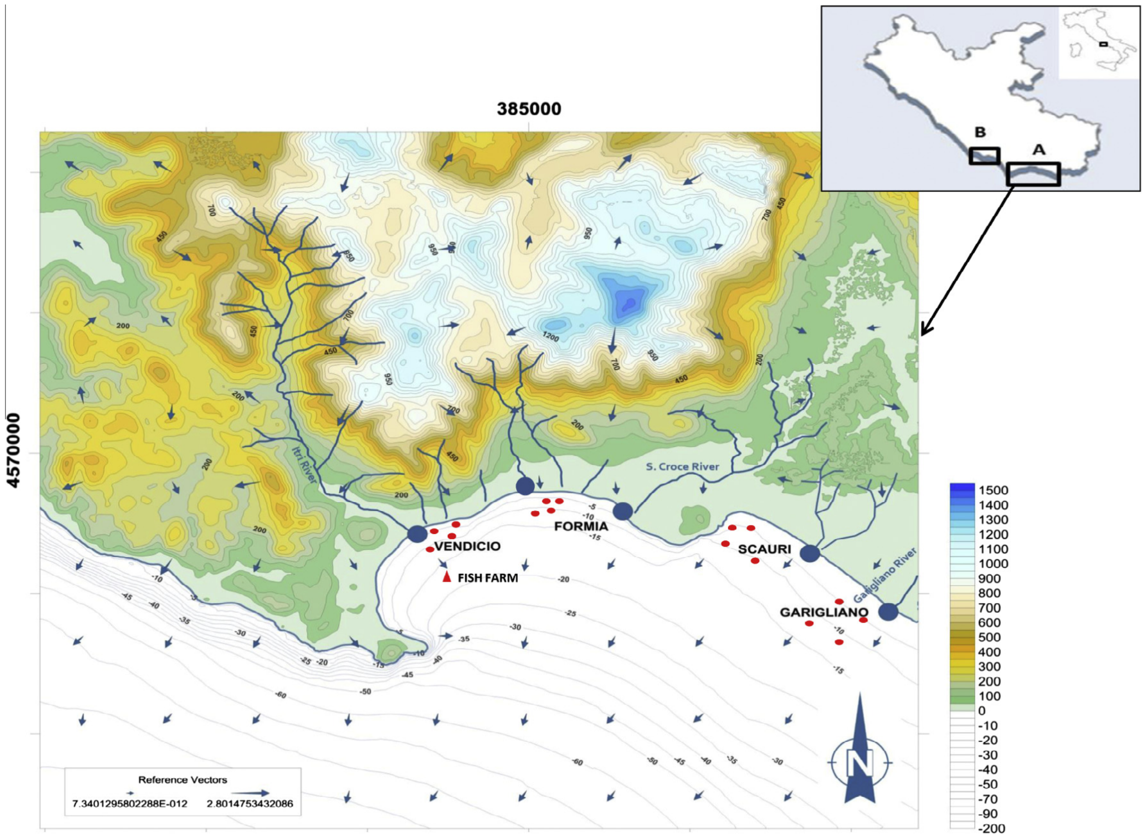

Fig. 1. Study area and location of sampling sites (red points). A Gulf of Gaeta (affected site), B Circeo (reference site). Water deflux routes and directions are shown by arrows (see Supplemental Material for details) and isobaths are reported. The dimension of the arrows are proportional to the steepness of the slopes. Blue points marked the main river mouths. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and data transformations were used where necessary. Spearman Statistical significance was evaluated at $\alpha = 0.05$. The $\delta^{15}N$ values of the macroalgae after 48 h of exposure were analyzed for spatial autocorrelation by Moran's test with uniform spatial weights (Cliff and Ord, 1981) and by distance-based nearest neighbour. Spatial analyses were performed using R software 2.15.2 (geoR and spdep package).

3. Results

3.1. Between-location T_o–T_1 comparisons of $\delta^{15}N$ and $\delta^{13}C$ values

In the reference area (Circeo), the initial N and C isotopic signatures (mean ± S.D.) of macroalgae were $\delta^{15}N = 5.96 \pm 0.54‰$ and $\delta^{13}C = -22.53‰ \pm 2.04‰$ in *U. lactuca*, and $\delta^{15}N = 7.69 \pm 0.39‰$ and $\delta^{13}C = -19.73‰ \pm 2.43‰$ in *C. amentacea*. After 48 h (T_1), $\delta^{15}N$ and $\delta^{13}C$ values were not significantly different in *U. lactuca* (Table 1; *t*-test, n.s.) whereas $\delta^{13}C$ was greater in *C. amentacea* (*t*-test, *p*-value < 0.001).

In the Gulf of Gaeta, the isotopic signature of *U. lactuca* was $\delta^{15}N = 5.71 \pm 1.25‰$ and $\delta^{13}C = -22.26 \pm 2.23‰$ at start (T_0) and $\delta^{15}N = 8.15 \pm 1.03‰$ and $\delta^{13}C = -22.42 \pm 2.10‰$ at T_1. There was thus a statistically significant increase in $\delta^{15}N$ (Fig. 2 and Table 1; *t*-test, $p < 0.001$) and no significant change in $\delta^{13}C$ (*t*-test, n.s.). The high ^{15}N enrichment of *U. lactuca* was also evident in comparison with the reference area (Circeo) (Fig. 2; *t*-test, $p < 0.001$). During 48 h of submersion in the Gulf, the coefficient of variation of N^{15} among replicate fronds of *U. lactuca* fell considerably, from 12.37% at T_0 to 1.85% at T_1 in the Vendicio area, from 23.73% to 6.76% in Formia, from 26.40% to 16.00% in Scauri, and from 14.70% to 6.03% in Garigliano.

C. amentacea, which had higher starting values, was much less enriched in $\delta^{15}N$ than *U. lactuca* after 48 h in the Gulf (Table 1; Fig. 2). The variability of ^{15}N among replicate fronds of *C. amentacea* was rather low, varying from a minimum of 3.67% observed at

Table 1
Mean (±S.D.) $\delta^{15}N$ and $\delta^{13}C$ values in *U. lactuca* and *C. amentacea* at T_0 and T_1 in two sampled locations (*n* = number of samples).

Taxon	Site	Time	*n*	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
U. lactuca	Circeo	T_0	16	5.96 ± 0.54	−22.53 ± 2.04
		T_1	16	6.40 ± 0.49	−22.37 ± 2.21
	Gulf of Gaeta	T_0	42	5.71 ± 1.25	−22.26 ± 2.23
		T_1	42	8.15 ± 1.03	−22.42 ± 2.10
C. amentacea	Circeo	T_0	16	7.69 ± 0.39	−19.73 ± 2.43
		T_1	16	7.94 ± 0.65	−16.11 ± 0.82
	Gulf of Gaeta	T_0	42	7.08 ± 0.53	−19.20 ± 2.70
		T_1	42	8.19 ± 0.67	−15.80 ± 1.01

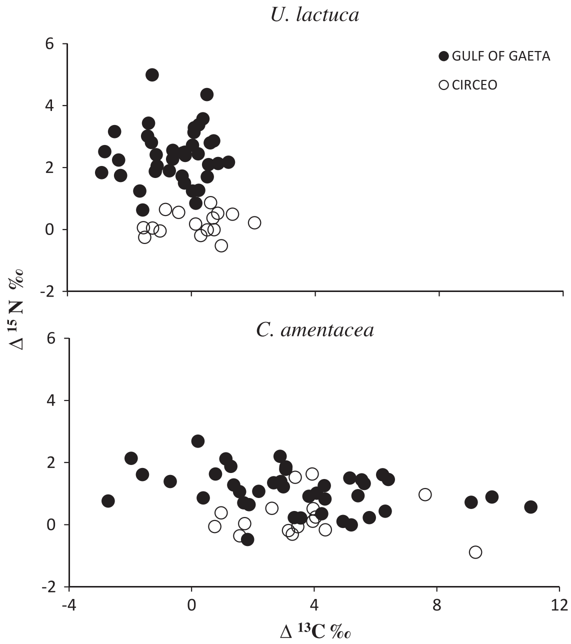

Fig. 2. Biplots of $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ values of *U. lactuca* in two sampling locations (reference and affected sites).

T_0 to a maximum of 10.3% observed at T_1. In contrast, the $\delta^{13}C$ values of *C. amentacea* increased at both locations (Table 1) while $\delta^{13}C$ enrichment in *U. lactuca* was negligible (Fig. 2).

3.2. Comparisons of $\delta^{15}N$ values within the Gulf of Gaeta

U. lactuca $\delta^{15}N$ values were significantly higher after 48 h at all sampling sites in the Gulf (*t*-test, $p < 0.001$), varying between 7.53 ± 0.14‰ and 8.58 ± 1.39‰ in the 4 macroareas with a minimum ^{15}N enrichment of about 2‰ at Formia. The average increase in *C. amentacea* was 1‰ at all sampling areas except the northern Vendicio area, where ^{15}N enrichment was 1.6‰ (Table 2 and Fig. 3).

There were no significant differences in algal isotopic enrichment between the two bathymetries (Table 2; Wilcoxon *t*-test, n.s.), nor was there any significant relationship between $\delta^{15}N$ and distance from the coastline (Spearman's correlation coefficient, n.s.). At the south-eastern sites, the isotopic difference between T_0 and T_1 in *U. lactuca* was higher than at the north-western sites,

Table 2
Mean (±S.D.) $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ values in *U. lactuca* and *C. amentacea* at two bathymetries in four sampling stations in Gulf of Gaeta (*n* = number of samples).

Taxon	Site	Depth (m)	*n*	$\Delta\delta^{15}N$ (‰)	$\Delta\delta^{13}C$ (‰)
U. lactuca	Vendicio	5	3	2.40 ± 1.26	−0.06 ± 0.22
		12	3	2.47 ± 0.31	0.91 ± 2.21
	Formia	5	6	2.09 ± 0.59	−0.11 ± 0.99
		12	6	1.80 ± 0.57	−0.67 ± 1.28
	Scauri	5	6	2.45 ± 0.36	−0.52 ± 1.27
		12	6	3.27 ± 1.08	−1.54 ± 1.12
	Garigliano	5	6	3.12 ± 1.15	−0.01 ± 0.79
		12	6	2.10 ± 0.74	−0.92 ± 1.17
C. amentacea	Vendicio	5	3	1.11 ± 0.61	2.03 ± 4.00
		12	3	2.13 ± 0.59	2.16 ± 2.64
	Formia	5	6	1.01 ± 0.79	3.57 ± 2.95
		12	6	1.06 ± 0.44	4.55 ± 3.32
	Scauri	5	6	0.75 ± 0.26	4.94 ± 4.98
		12	6	1.33 ± 0.61	4.09 ± 1.79
	Garigliano	5	6	0.97 ± 0.40	2.16 ± 2.06
		12	6	1.03 ± 1.03	2.57 ± 1.35

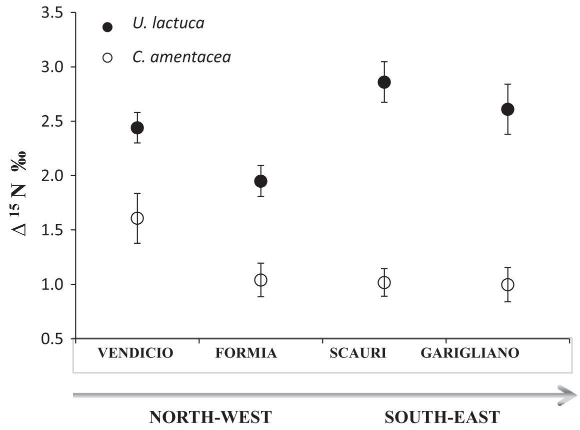

Fig. 3. $\Delta^{15}N$ values of *U. lactuca* and *C. amentacea* in Gulf of Gaeta along geographical gradient (vertical bars = standard error).

generating a north-west–south-east $\delta^{15}N$ gradient, especially at the bathymetry of 5 m (Table 2 and Fig. 3). Two statistically different areas can be distinguished, with Scauri–Garigliano showing higher $\delta^{15}N$ values (8.4‰) than Gaeta–Vendicio (7.6‰) at T_1 (*t*-test; $p < 0.05$). Within each of these two areas, values were found to be spatially autocorrelated up to 1.5 km ($p = 0.08$; Fig. 4).

4. Discussion

Marine coastal waters are the final recipients of nutrients translocated from land (Howarth, 2008; Swaney et al., 2012), but their precise source and distribution can be difficult, costly and time-consuming to determine. The results of this study show that in Mediterranean coastal waters, anthropogenic sources of nitrogen (N) can be rapidly monitored using the opportunistic macroalga *U. lactuca* as a probe. This macroalga was found to assimilate dissolved N, displaying altered N stable isotope ratios in the polluted area with respect to the unpolluted area after 48-h exposure. Macroalgae directly reflect the availability and isotopic composition of N sources thanks to their capacity to take up and store excess N in their tissues, with little or no fractionation during N uptake across a wide range of nutrient concentrations (Cohen and Fong, 2005; Lin and Fong, 2008). Variability in $\delta^{15}N$ values among replicate fronds of *U. lactuca*, collected from coastal intertidal areas of the reference location, decreased dramatically after 48-h exposure, meaning that the isotopic signature converged to values typical of the deployment sites. This exposure time was much shorter than in similar studies with other algae (Costanzo et al., 2005; García-Sanz et al., 2011). *U. lactuca* has a high surface/volume ratio and a high nitrogen uptake rate (Rosenberg and Ramus, 1984; Taylor et al., 1998, 1999) and thus can assimilate significant amounts of dissolved nitrogen over a comparatively short period of time. Thus, its $\delta^{15}N$ value strongly reflects sources of nutrients assimilated in the recent past (Jones et al., 2001; Cohen and Fong, 2005; Cole et al., 2005). *C. amentacea* showed similar final $\delta^{15}N$ values, but smaller increases ($\Delta\delta^{15}N$) than *U. lactuca* after 48-h exposure in the Gulf due to higher starting values. The latter were higher than those measured in other Mediterranean *Cystoseira* spp. growing in pristine environments (Pantoja et al., 2002) and could be the result of episodic nitrogen input in the past. A similar lack of response was described for another brown alga, *Fucus vesiculosus*, which was unable to reflect nutrient availability gradients (Deutsch and Voss, 2006) and for *Cystoseira mediterranea*, which was unable to uptake fish-farm nitrogen loadings over short time periods (i.e. 2–8 days) (García-Sanz et al., 2010). *Cystoseira* is a perennial alga with a relatively long tissue turnover time and is therefore a good indicator of ambient water nutrient conditions over longer timescales. In

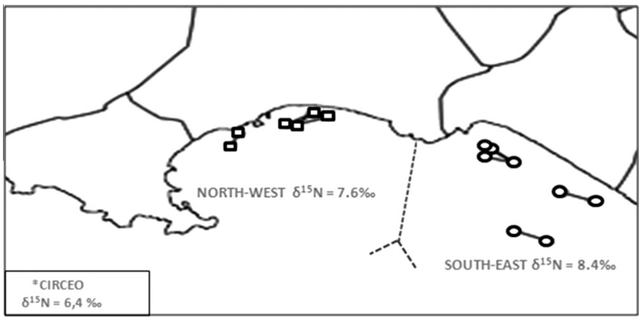

Fig. 4. Spatial analysis of $\delta^{15}N$ values in Gulf of Gaeta performed using R software 2.15.2, * = value for Circeo (reference site).

contrast, the ability to grow quickly when nutrients are available and the rapid turnover of the internal N of *U. lactuca* explain why its $\delta^{15}N$ values reflect more transient and pulsed nitrogen inputs in the water column (Aguiar et al., 2003; Teichberg et al., 2008). Differences in uptake and turnover rates between green and brown algae can be explained by differences in photosynthetic pigments and acclimation abilities. In particular, green algae have high relative content of chlorophyll *b* (Rabinowitch, 1945 and literature cited therein), which makes them more efficient at shallow depths than brown algae. Furthermore, the growth of *Ulva* spp. has been shown to be poorly affected by changes in the light spectrum (Aguilera et al., 1999; Altamirano et al., 2000), which could promote the continuity of tissue turnover also under changing exposure conditions. Morphological differences among macroalgae also can determine differences in their nutrient requirements, uptake kinetics and storage capacity (Runcie et al., 2003; Teichberg et al., 2008). In particular, *U. lactuca* is a bistromatic alga with all cells equally exposed to nutrients, which rapidly assimilates nitrogen and rapidly remobilizes the stored nitrogen when required (Runcie et al., 2003 and literature cited therein).

The rise in the $\delta^{15}N$ value of *U. lactuca* tissue observed in the Gulf of Gaeta was consistent with the enrichment of this isotope with respect to natural sources (e.g. rain), typically observed in the presence of organic sources, either dissolved or particulate, from human and/or animal waste (Costanzo et al., 2001; Cole et al., 2004; Deutsch and Voss, 2006). The Gulf of Gaeta is a typical Mediterranean area affected by several types of nitrogen sources. Specifically, the fish farm in the north-west and the large river mouth in the south-east were the most direct sources of nitrogen influencing the distribution of $\delta^{15}N$ values in macroalgae on opposite sides of the Gulf. However, the values recorded in the northern area were lower than expected from fish farm inputs (Mazzola and Sarà, 2001), probably due to the fact that the cages were farther than 1.2 km away and thus beyond the limit at which the spatial contagiousness of $\delta^{15}N$ value could be detected in macroalgae across the Gulf.

The spatial differences in $\delta^{15}N$ enrichment cannot be ascribed to changes in algal metabolism during the experiment since deployment was simultaneous in the sampling areas and the temperature was substantially uniform among sampling sites. The fact that no significant change was observed in the macroalgae deployed in Circeo supports the hypothesis that the isotopic signature of *U. lactuca* was influenced by anthropogenic inputs in the Gulf of Gaeta. Interestingly, while nitrates and total nitrogen were higher than in the reference area, no significant variation in either the chemistry or concentration of nitrogen was observed across the Gulf. Nevertheless, SIA made it possible to distinguish two areas in the Gulf, differing both from each other and from the reference site in terms of N sources. The Circeo area was the closest site of *U. lactuca* beyond the influence of the Gulf and barely affected by land-derived N. The $\delta^{15}N$ value of fronds from this reference site was similar to that of naturally derived marine NO_3^-, as documented by sewage studies (Miyake and Wada, 1967; Cline and Kaplan, 1975; Peterson et al., 1985; Monteiro et al., 1997). The absence of estuaries or effluents excludes possible effects of the increased microbial activity associated with estuarine loadings on the isotopic signature of the nitrogen assimilated by *U. lactuca* (Riera et al., 2000; Raimonet et al., 2013). Other studies found similar isotopic values to be indicative of cesspools, shrimp farm effluents (Lin and Fong, 2008; Dailer et al., 2010), or naturally occurring estuarine levels (Riera et al., 2000) in other algal genera. When focusing on *Ulva* spp., Gartner et al. (2002) reported a $\delta^{15}N$ value of 6.1 to be indicative of natural (i.e. not impacted) conditions, and Dailer et al. (2010) reported a $\delta^{15}N$ value of 9.8 to be indicative of *Ulva* sp. exposed to cesspool-derived nitrogen loadings.

Therefore, it appears that predictable isotopic ranges in this macroalga when taken from uncontaminated sites can serve as a benchmark for studying contamination and planning and verifying the remediation of polluted areas. Specifically, given the high natural intraspecific variability of the $\delta^{15}N$ value in this macroalga, the comparison of isotopic signatures in single individuals before and after exposure as performed in this study yielded accurate results with a reasonably low number of samples.

5. Conclusion

Coastal marine waters are experiencing a rapid decline in quality due to human activities. $\delta^{15}N$ variation in uncontaminated *U. lactuca* can be an effective indicator of exogenous nitrogen loading after 48-h exposure. Comparison of the isotopic signatures of small fragments from the same frond before and after exposure avoids possible bias arising from the natural variability of $\delta^{15}N$ values among fronds, thus permitting the monitoring of larger areas with less sampling effort than would otherwise be necessary. Species of the genus *Cystoseira*, which dominate the Mediterranean upper sublittoral communities, are particularly sensitive to any natural or anthropogenic stress (Bellan-Santini, 1966; Ballesteros et al., 1984; Hoffmann et al., 1988; Soltan et al., 2001) and, therefore, their populations have experienced profound declines over extensive areas (Thibaut et al., 2005). However, our results show that while *C. amentacea* is considered a good indicator of environmental quality and may thus be used in water quality assessment, it is less useful than *U. lactuca* as an indicator of N input variation over short time periods. *Cystoseira* typically has a very low nitrogen uptake rate and large amounts of structural biomass, and so would require longer periods of exposure to assimilate sufficient new nitrogen to alter the average $\delta^{15}N$ value of its fronds. The stable-isotope values in these two macroalgae could be used to delineate the influence of sewage-derived nutrients in coastal areas (Hobbie et al., 1990; Rogers, 1999; Costanzo et al., 2001; Wayland and Hobson, 2001) and to map sewage dispersal over different timescales. However, while the isotopic signature of *Ulva* spp. has already been acknowledged to be highly responsive to pollution (Gartner et al., 2002; Dailer et al., 2010, 2012; Barr et al., 2013), further investigations are necessary to evaluate *C. amentacea* as a useful in situ long-term indicator for N pollution episodes in the pristine habitats where it normally occurs.

In conclusion, our large-scale study shows the usefulness of $\delta^{15}N$ in *U. lactuca* as a proxy for locating anthropogenic sources of nitrogen in disturbed Mediterranean coastal areas. Short-term algal exposure represents an important temporal logistic advantage in such coastal areas characterized by intense tourism and commercial activities, which need to be reduced or interrupted during the assessment. This technique of mapping pulse nitrogen inputs of different origins could be thus used as a baseline for future water quality monitoring and management programmes, but only after defining the best sampling grid to exactly describe the topography of nitrogen inputs and distribution in coastal seas.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2014.05.036.

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