δ^{15}N in deployed macroalgae as a tool to monitor nutrient input driven by tourism activities in Mediterranean islands

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Abstract

Mediterranean Sea is among the world's leading tourist destinations; however, the sharp increase in tourists during the high season may affect coastal seawater. The main aim of this study was to evaluate the occurrence and temporal variation of anthropogenic nutrients in coastal seawater in relation to tourist flows in three Mediterranean islands (Cyprus, Sicily and Rhodes), through short-term macroalgae deployments, coupled with $\delta^{15}N$ analysis and GIS mapping. In all islands, an overall increase in macroalgae $\delta^{15}N$ occurred over the deployment carried out in August in the tourist sites, suggesting the presence of anthropogenic nutrients. Decreasing $\delta^{15}N$ values occurred at increasing distance from the coastline in two out of the three islands (Cyprus and Sicily). This study revealed the usefulness of the approach used in the assessment of tourism impact in terms of trophic enrichment and its potential to support competent authorities for the development of sustainable coastal management plans.

Keywords

biomonitoring, indicators, stable isotopes, eutrophication, GIS, *Cystoseira*

1 Introduction

Mass tourism is a rather recent phenomenon that is leading to considerably high density of visitors within restricted spaces and periods. Tourism income has greatly increased in recent years bringing economic benefits to many countries, but there are substantial environmental threats and costs associated with it (Davenport and Davenport, 2006). Nevertheless, the overall understanding of the interaction between tourism and environment, especially in marine and coastal areas is quite poor and fragmented. An example is represented by beaches, as they provide ecosystem services being important recreation tourism icons but, at the same time, are among the most damaged natural ecosystems worldwide (Hall, 2001). The over-crowding at the beaches increases the anthropogenic pressure on the adjacent marine coastal zone, leading to deterioration of water quality and aquatic life (Garcia and Servera, 2003; Hall, 2001). The potential consequent impairment of coastal ecosystem services necessarily triggers a vicious circle that, inevitably, will damage the tourism industry itself (Drius et al., 2019).

One of the main threats potentially driven by coastal tourism is the increase in organic and nutrient load linked to the abrupt rise in population during the high tourism season. Especially when sewage treatment systems are absent, inadequate or malfunctioning, excess input of organic matter and nutrients may enter the marine coastal areas, thereby leading to eutrophication, which may be followed by several secondary detrimental effects (Castro et al., 2007; Nixon, 1995; Signa et al., 2015).

Suitable approaches to track the excess of nutrients of anthropic origin in marine coastal areas include the use of macroalgae, which are proper biological indicators, due to their high uptake and assimilation rate of bioavailable nutrients (Hurd et al., 2014), together with their wide distribution, abundance and sessile life (Cole et al., 2005). In this context, an appropriate technique to assess the presence and the extent of anthropic nutrients in macroalgae exposed to nutrient-enriched seawater is the analysis of nitrogen stable isotope ratio ($\delta^{15}N$). Stable isotopes of nitrogen and carbon represent powerful and highly informative tools that have been widely used in environmental studies and food web ecology to trace the origin of nutrients and organic matter in natural systems (Castro et al., 2007; Signa et al., 2012; Vizzini and Mazzola, 2006). In particular, $\delta^{15}N$ is useful in the identification of nitrogen sources entering marine ecosystems (e.g. atmospheric deposition, wastewater, fertilizers), as marine, terrestrial and anthropogenic nutrients and organic matter have different $\delta^{15}N$ signatures (Castro et al., 2007; Cole et al., 2005; Olsen et al., 2010). Anthropogenic nitrogen is typically more ^{15}N-enriched than terrestrial and marine sources due to the isotopic fractionation that occurs during nitrogen transformations (e.g. ammonia volatilisation and denitrification) and leaves the residual nitrogen pool enriched in ^{15}N (Heaton, 1986). In this context, marine macroalgae are able to integrate spatial and temporal variability of $\delta^{15}N$ of dissolved nitrogen and their isotopic composition provides information on the origin of nitrogen exploited (natural *vs*. anthropogenic) (Cole et al., 1995, 2005; Costanzo et al., 2001).

If $\delta^{15}N$ of naturally growing macroalgae is broadly used to monitor anthropogenic nitrogen input into aquatic ecosystems (e.g. Derse et al., 2007; Lin et al., 2007; Morris et al., 2009; Savage et al., 2005), a step beyond is represented by the deployment approach consisting in short-term macroalgae incubations in selected areas to assess the change of their $\delta^{15}N$ values over the deployment period, according to local environmental conditions (e.g. Costanzo et al., 2001; García-Seoane et al., 2018). The main advantages of this approach, compared with the analysis of naturally occurring macroalgae, derive from the wide coverage of the area that can be monitored, coupled with a low spatial variability of the initial macroalgae $\delta^{15}N$, as the algae used for the deployment are collected from the same site of origin (Alquezar et al., 2013) and the known period of exposure (García-Seoane et al., 2018). This approach was successfully set up in many areas worldwide to detect and map anthropogenic nutrient sources and plumes, especially in case of potential sewage (Costanzo et al., 2005, 2001), agricultural (Deutsch and Voss, 2006) industrial (Alquezar et al., 2013) and aquaculture (García-Sanz et al., 2011, 2010) impacts. Most of these studies used the opportunistic bloom-forming Chlorophyta *Ulva* spp. because of its acknowledged high nitrogen removal efficiency and incorporation rate (Pedersen and Borum, 1997) that ensure a rapid and efficient response to nutrient enrichment. Nevertheless, experimental manipulation of opportunistic macroalgae suffers from two main constraints: i) availability and biomass may strongly vary across the year (García-Seoane et al., 2018); ii) they need a preliminary acclimation step in oligotrophic seawater to allow a complete turnover/depletion of the internal nitrogen pool (Dailer et al., 2010; Orlandi et al., 2014), as they occur prevalently in nutrient enriched areas. On the other hand, perennial macroalgae, such as Fucales, show slower nutrient uptake and growth rates than opportunistic ones (Martínez et al., 2012), but dominate in most temperate areas across the year (Mannino et al., 2014) and are more sensitive to anthropogenic disturbance (Arévalo et al., 2007; Ballesteros et al., 2007). Therefore, their use in biomonitoring studies is less frequent, although it may represent a valid potential (García-Seoane et al., 2018), especially for bypassing the acclimation step, which is not always feasible in routine monitoring.

Here, the genus *Cystoseira* C. Agardh was used to detect the occurrence and temporal variation of anthropogenic nutrients in coastal seawater in relation to tourist flows in three Mediterranean islands, using the deployment approach and nitrogen stable isotope analysis. We hypothesised that seasonality of tourism in Mediterranean islands may lead to a variation in anthropogenic nutrients in coastal seawater that can be effectively recorded in deployed macroalgae through an increase in $\delta^{15}N$.

2 Methods

2.1 Study areas and experimental approach

The study was carried out in three Mediterranean islands: Cyprus and Sicily (Italy) in 2017 and Rhodes (Greece) in 2018 (Fig. 1).

The experimental campaigns, based on short-term macroalgae deployments, were conducted in three periods: putatively before the tourist period (i.e. June), during the tourist peak (i.e. August) and at the end of the tourist period (i.e. October), in three experimental sites per island. The sites were selected in order to compare a potentially impacted site (e.g. featured by popular beaches and large tourist infrastructures), hereafter Tourist site, with two reference sites where tourist activities and infrastructures are negligible (hereafter Control 1 and Control 2 sites).

In Cyprus (Fig. 1a), Sunrise beach, which is located in the small town of Protaras, was selected as Tourist site, while the two Control sites were identified in the southernmost rocky peninsula of Cavo Greco, which is National Forest Park since 1993 and is scantily frequented by tourists. In Sicily (Fig. 1b), Giardini Naxos beach was chosen as Tourist site, and the two Control sites where chosen along the long remote beach of Fondaco Parrino, as there are no tourist infrastructures and tourist frequency is negligible. In Rhodes (Fig. 1c), the beach of the resort village of Faliraki was selected as Tourist site and the southernmost beaches of Traganou and Afandou, which are poorly frequented by tourists, were selected as Control sites.

The experimental fields were constituted by georeferenced grids of 30 points (Tourist sites) and 21 points (both Control sites), which were distributed, at the distance of 50 m each other, along three parallel transects (T1, T2, T3). The transects were, in turn, distant 100, 200 and 300 m from the coastline in Cyprus and Rhodes and 200, 250 and 300 m in Sicily, due to differences in local regulations and restrictions posed by the competent Authorities. Each point corresponded to the exact position where the macroalgae were deployed within removable devices made of a single nylon net bag, which was anchored to the bottom with a ballast and kept straight in the water column at a depth of 1.5 m with a buoy, to ensure good light conditions. The exposure time was 3 days, which is a good tradeoff that allows macroalgae to detect the spatial distribution of $\delta^{15}N$, before other factors (e.g. light, siltation and biofouling) might affect their response (Costanzo et al., 2005; Huntington and Boyer, 2008).

Three intertidal macroalgae species of the genus *Cystoseira* were used for the experiment: based on their local availability, *C. humilis*, *C. amentacea* and *C. compressa* were chosen respectively in Cyprus, Sicily and Rhodes. The genus *Cystoseira*, one of the most representative canopy-forming Fucales (Phaeophyceae) in the Mediterranean Sea, is used as bioindicator of seawater quality due to its sensitivity to anthropogenic pressure (Ballesteros et al., 2007). Moreover *Cystoseira* species have a similar response to nutrient enrichment (Sales and Ballesteros, 2009) showing also a comparable pattern in nutrient uptake and accumulation (Sales et al., 2011).

In all islands, entire macroalgae thalli of the selected species were sampled before the onset of the experiment from a rocky shore characterized by overall pristine conditions, hereafter called Collection site, where the macroalga was present with high abundance throughout all the experimental periods. All collection sites, placed respectively in the Cavo Greco peninsula in Cyprus, in the locality of Addaura in Sicily and of Ladiko in Rhodes, were characterized by exposed rocky shores facing north-east, low depth (2 m maximum) and very scant presence of bathers throughout the year. Moreover, the presence of allochthonous nutrient input can be ruled out at all collection sites.

The macroalgae collected at the Collection site were: i) analysed for $\delta^{15}N$ to record the isotopic signature at the onset of the experiment (Day 0, n = 5), representing the isotopic baseline to which compare the $\delta^{15}N$ of the deployed macroalgae at the end of the experiment; ii) deployed in the Collection site using the same type of device and for the same duration as in the Tourist and Control sites with the purpose to check any effect of the experimental procedure (procedural control) on the macroalgae performance, by comparing their $\delta^{15}N$ signature at the end of the deployment on Day 3 (Day 3 - *in situ* Control, n = 5) with that of further samples naturally present in the Collection site and contextually collected (Day 3, n = 5); iii) deployed in the Tourist and Control sites following the experimental design illustrated above (n = 72).

At the end of the third day, the deployment devices were collected from the Tourist and Control sites, macroalgae thalli were carefully removed from the net bags, rinsed with distilled water and stored in the cold until the arrival to the laboratory. Furthermore, during both phases of deployment and retrieval of the net bags at the experimental sites, main physicochemical variables of seawater (temperature and salinity) were recorded using a multiparameter probe. Surface seawater samples were also collected in triplicate (10 L each) from each transect to obtain the total nitrogen content and the background isotopic signature of the suspended particulate organic matter (POM).

2.2 Sample processing and laboratory analysis

Once in the laboratory, only the apical portion of the frond of each thallus, corresponding to the newly grown tips, was selected for the isotopic analysis, as apical tips of perennial macroalgae integrate nutrient concentration and isotopic values of seawater nutrients during their growing period (Viana and Bode, 2013). Then, apical tips were quickly rinsed with distilled water to remove any external material and gently scraped with a scalpel to remove epiphytes, when necessary. Seawater samples were prefiltered at 200 μm and then filtered on precombusted (450°C, 4h) filters (GF/F Whatman, pore size 0.45 μm) and rinsed with distilled water. Macroalgae subsamples and POM filters were then oven dried at 60°C for 48 hours and subsequently ground to a fine powder using a micro-mill. An aliquot of each ground sample was packed in tin capsules and analysed for total nitrogen (TN%) using an Elemental Analyser (Thermo Flash EA1112) and $\delta^{15}N$ using an Isotope Ratio Mass Spectrometer (Thermo Delta IRMS Plus XP) coupled to an Elemental Analyser. Nitrogen stable isotope ratio was expressed in δ unit notation, as parts per mil deviation from the international standard (atmospheric N_2) as follows:

$$\delta^{15}N = [(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard}) - 1] \times 10^3.$$

Analytical precision based on the standard deviation of replicates of internal standards (International Atomic Energy Agency IAEA-CH-6) was 0.1‰.

2.3 Data analysis and statistics

Permutational univariate analysis of variance (PERMANOVA - PRIMER 6 v6.1.10 & PERMANOVA+ β20; Anderson et al., 2008) was used to test for total N and $\delta^{15}N$ differences among periods and sites in each island for the suspended particulate organic matter (POM) based on Euclidean distance matrices obtained from untransformed data. A two-factor design (factor Period fixed with three levels: June, August, October; factor Site with three levels: Tourist, Control 1, Control 2; fixed and orthogonal) was set for each island.

$\delta^{15}N$ values of the macroalgae collected at the Collection site in each island were compared between Periods and Days through PERMANOVA based on Euclidean distance matrices obtained from untransformed data. In particular, we tested for the isotopic variation: i) due to the experimental procedure on the macroalgae performance (by comparing Day 3-*in situ* Control vs. Day 3) and ii) naturally occurring across the same 3-days of the experiment (by comparing Day 0 vs. Day 3). To do this, a two-factor design (factor Period fixed with three levels: June, August, October; factor Day with three levels: Day 0, Day 3 and Day 3 – *in situ* Control; fixed and orthogonal) was set.

$\delta^{15}N$ values of the deployed macroalgae were compared in each island, period, site and transect, with the correspondent baseline through t-test between independent groups (STATISTICA v.10). The baseline adopted was the mean $\delta^{15}N$ value of the macroalgae from the Collection site at Day 0 in each period, except in the case of Cyprus in October where the mean $\delta^{15}N$ value of the macroalgae collected at Day 3 was used as baseline, due to the significant influence of the meteorological conditions (i.e. intense raining) during the 3-day deployment (see results for further details). Homogeneity of variance was previously checked through Levene test. Afterwards, the isotopic variation occurred at the end of the deployment period (i.e. the difference between the $\delta^{15}N$ of the deployed macroalgae and the $\delta^{15}N$ of the baseline) was calculated for each sample and expressed as $\Delta\delta^{15}N$.

The relationship between the isotopic variation of the deployed macroalgae and the total nitrogen content of POM, proxy for the nitrogen load and availability in seawater (Signa et al., 2012), was explored separately by island through simple linear regression using POM TN as the independent variable and $\Delta\delta^{15}N$ as the dependent variable. Both variables were averaged by transect. Residual analysis was run to test the model assumptions and robustness and to identify potential outliers. Additionally, $\Delta\delta^{15}N$ in the Tourist site was compared with two indicators of tourist flow, hotel accommodations (only for Cyprus and Sicily) and international arrivals of passengers at the closest airport (Larnaka, Catania and Rhodes, for Cyprus, Sicily and Rhodes respectively). Tourism data have been obtained from the Ministry of Agriculture, Rural Development and Environment of Cyprus, Taormina Etna Consortium, and the Municipality of Rhodes.

The analysis of spatial patterns of $\Delta\delta^{15}N$ was conducted through Quantum GIS (version 2.18.7) using the Inverse distance weighted (IDW) interpolation technique, which allows to determine cell values using a linearly weighed combination of a set of sample points where the weight is a function of inverse distance (Philip and Watson, 1982; Watson and Philip, 1985). With the purpose to realize a tool for transferring complex ecological information to competent authorities, $\Delta\delta^{15}N$ values were ranked into 5 classes (corresponding to different colours) across all the study areas, sites and periods. In more detail, the five classes were set according to current literature concerning isotopic variation of deployed *Cystoseira* spp. (García-Sanz et al., 2011, 2010; Orlandi et al., 2014); the contours of these 5 categories indicate the occurrence and the extent of plumes of ^{15}N-enriched [$\Delta\delta^{15}N$ positive values: warm colour scale with 4 classes indicating low (0-0.5‰), moderate (0.5-1‰), high (1-1.5‰) or very high (> 1.5‰) isotopic enrichment] *vs.* ^{15}N-depleted [$\Delta\delta^{15}N$ negative values: cold colour with 1 class indicating variations < 0‰)] nutrients in the coastal sites over the experimental periods.

3 Results

3.1 Environmental characterization

Temperature and salinity followed a common temporal trend in Cyprus, Sicily and Rhodes. In particular, both seawater temperature and salinity varied similarly across the three sites of each island, increasing from June to August, and decreasing in October (Table 1).

Mean total N of suspended particulate organic matter (POM) showed the highest values and higher temporal variability in Sicily peaking in August in all sites (Fig. 2, Appendix S1). POM TN content in Rhodes was higher in August and at the Tourist site than in the others periods and sites. In contrast, no temporal or spatial differences were highlighted in Cyprus (Fig. 2, Appendix S1). In both Cyprus and Sicily, mean POM $\delta^{15}N$ peaked in August in the Tourist site and then significantly dropped in October in both Tourist and Control sites (Fig. 2, Appendix S2). Also in Rhodes, the highest values were recorded in the Tourist site, although only before and at the end of the tourist peak (June and October), but a common pattern across sites was not identifiable (Fig. 2, Appendix S2).

3.2 Macroalgae incubation experiment

The three macroalgae species *Cystoseira humilis, C. amentacea* and *C. compressa*, collected from the Collection site during the different collection days (Day 0 and Day 3) respectively in Cyprus, Sicily and Rhodes, showed different $\delta^{15}N$ values across periods, ranging respectively from -0.4 to 1.2 ‰ (mean value 0.5 ± s.d. 0.4‰), from 6.7 to 7.7 ‰ (7.3 ± 0.3‰) and from 1.6 to 3.5 ‰ (2.2 ± 0.4‰).

Permutational univariate analysis of variance (PERMANOVA) revealed significant differences in the macroalgae $\delta^{15}N$ values for the interaction of the factors Period and Day at the Collection site in all islands (Cyprus: $F_{(4, 36)} = 10.7$, $p = 0.001$; Sicily: $F_{(4, 36)} = 5.1$, $p = 0.019$; Rhodes: $F_{(4, 36)} = 4.3$, $p = 0.004$, Appendix S3). Nevertheless, in all islands and periods, the comparison between $\delta^{15}N$ values of the macroalgae collected at Day 3 and the procedural control (Day 3 – *in situ* Control) showed no significant differences ($p > 0.05$, Appendix S3), indicating that the experimental procedure did not influence the $\delta^{15}N$ signature of the macroalgae. In contrast, the comparison between Day 0 and Day 3 revealed significant differences only in Cyprus in October, with significantly higher $\delta^{15}N$ at Day 0 than at Day 3 probably due to intense raining occurred during the experiment implementation, which, passing through agricultural fields, may have contributed to runoff of ^{15}N-depleted nitrogen. Consequently, to remove the effect of this natural variability, the mean $\delta^{15}N$ value of the macroalgae collected at Day 3 (instead of those collected at Day 0) was set as baseline to which compare the $\delta^{15}N$ of the deployed macroalgae at the end of the experiment. Similarly, the higher $\delta^{15}N$ observed at Day 0 in October in Sicily and Rhodes, compared with the other periods, may be due to adverse weather and sea conditions during the days immediately before the experiment, as rainfall and high water mixing may have led to ^{15}N-enriched nitrate replenishment in the upper water layer (Michener and Shell, 1994).

Nitrogen stable isotope signature $\delta^{15}N$, total nitrogen TN and C/N ratio of the macroalgae selected as baseline are showed in Table 2. Although the inter-island variability in $\delta^{15}N$, with the lowest values in Cyprus, followed by Rhodes and then Sicily with the highest values, TN was overall in a narrow range, varying from 0.7 ± 0.1% in Cyprus to 1.3 ± 0.2 % in Sicily. Accordingly, the higher C/N ratio (> 40) were recorded in Cyprus, while in Sicily and Rhodes it ranged overall from 20 to 40 (Table 2) .

The comparison between the $\delta^{15}N$ values of the macroalgae deployed at increasing distance from the coastline in each island, site and period, and the baseline $\delta^{15}N$, revealed a significant isotopic enrichment at the end of the 3-day deployment in the Tourist site of Cyprus in all the periods (Fig. 3a). In contrast, macroalgae deployed in Sicily and Rhodes, showed a significant isotopic enrichment at the end of the deployment only in the Tourist site during the tourist peak (August) in transects 1 and 2 in Sicily and only transect 1 in Rhodes. A significant ^{15}N depletion was recorded in the macroalgae deployed in June at the Control 1 in Sicily (transect 1 and 3) and Rhodes (transect 3) and in October at all sites of Rhodes (transect 1 at the Tourist site, transect 2 at the Control 1 and transect 3 at the Control 2) (Fig. 3b, c).

Looking at the variation of δ^{15}N in the macroalgae tissues at the end of the deployment, hereafter $\Delta\delta^{15}$N, georeferenced maps show the temporal and spatial $\Delta\delta^{15}$N trends recorded in the three islands (Figs. 4, 5 and 6), with a slight wider $\Delta\delta^{15}$N range in Sicily (2.37‰: from -1.16 to 1.21‰) than in Rhodes (2.05‰: from -1.36 to 0.69‰) and Cyprus (1.79‰: from -0.43 to 1.36‰). In Cyprus, an isotopic enrichment was evident in the landward transect (100 m from the coastline) of the Tourist site in June and October, and then spread up to transect 3 (300 m from the coastline) in August, when also some $\Delta\delta^{15}$N peaks were recorded in the landward transect. In contrast, at both Control sites, there was only a very small isotopic variation in macroalgae, with low positive or negative $\Delta\delta^{15}$N values (Fig. 4). At the Tourist site in Sicily, positive $\Delta\delta^{15}$N values were evident in the macroalgae deployed in August, especially in the southern part of the bay, where a few enrichment peaks were recorded. In contrast, an overall decrease in the isotopic values of the deployed macroalgae was detected in June, and in both Control sites in August. In October, a minor isotopic variations occurred in the deployed macroalgae, except for the southern part of Giardini Naxos bay, where a slight isotopic enrichment persisted (Fig. 5). Lastly, the maps of Rhodes clearly show that only a low enrichment characterized the high season (August) at the Tourist site, and somewhat spread within 100 m at the Control 1 site, while during the other two periods an overall isotopic depletion in the macroalgae tissues occurred at all sites (Fig. 6).

Linear regression analysis revealed that $\Delta\delta^{15}N$ of the deployed macroalgae significantly increased with increasing POM TN content in Cyprus and Sicily (Cyprus: y = 0.045 + 0.016x, R^2= 0.43, p-value = 0.008; Sicily: y = 0.139 + 0.143x, R^2 = 0.35, p-value = 0.001). In contrast, no significant linear relation emerged in Rhodes (y = 0.053 + 0.015x, R^2 = 0.09, p-value = 0.07). Moreover, mean $\Delta\delta^{15}N$ of the macroalgae deployed in the Tourist sites showed a different trend in the three islands, being overall comparable across periods in Cyprus, peaking in August in Sicily, and dropping in October in Rhodes (Fig. 7). Although data about hotel accommodations are missing in Rhodes, these $\Delta\delta^{15}N$ trends are overall consistent with the tourist flow data, which revealed a high number of visitors in Cyprus and Rhodes since June with a decrease, more marked in Rhodes, in October and a peak in August in Sicily (Fig. 7).

4 Discussion

$\delta^{15}N$ of macroalgae is known to be a useful proxy for anthropogenic nutrients presence in coastal seawater (e.g. Cole et al. 2005, Costanzo et al. 2005, Hurd et al. 2014, García-Seoane et al. 2018) and well before that main ecological changes become observable. Although opportunistic green macroalgae are acknowledged as good indicators of nitrogen supply and sources in coastal systems (Fernandes et al., 2012; Orlandi et al., 2014), this study confirms that also brown macroalgae are reliable indicators of anthropogenic nutrient input in coastal areas through deployment techniques (Alquezar et al., 2013).

The isotopic variation, $\Delta\delta^{15}N$, occurred in *Cystoseira* species at the end of the 3-day deployment period showed relevant spatial and temporal patterns, as well as island-specific differences, which provided indication of the presence of anthropogenic nitrogen nutrients in seawater. This is because macroalgae typically uptake heavier isotopes (^{15}N) when they are more available, quickly integrating them into their tissues (Cole et al., 2005; Costanzo et al., 2001; Fernandes et al., 2012) and show very little or no fractionation during N-uptake and assimilation, unless exposed to high nitrate concentrations (Gröcke et al., 2017; Swart et al., 2014). Although we did not analyse the concentration of nitrogen compounds in seawater, the very low nitrogen content (< 0.4 %) of the particulate organic matter (POM) in all the islands indicates that the coastal areas studied are oligotrophic across all periods.

In Cyprus, the $\Delta\delta^{15}N$ pattern was very evident, and revealed a higher isotopic enrichment in the macroalgae deployed in August than in June and October. Moreover, this pattern was especially evident in the Tourist site, where $\Delta\delta^{15}N$ also decreased at increasing distance from the coast. These findings give a clear clue of the origin and the timing of anthropogenic nutrient spread, which, as expected, seems strictly associated to coastal inputs, thereby to tourism activities. Indeed, tourist arrival in Cyprus starts early in spring and gradually increases up to the peak in August, and then starts to drop from September. Moreover, in the Tourist site investigated in Cyprus, many leisure activities are offered to the beach users, such as lidos, watercrafts and water sports, and the whole town is surrounded by tourist facilities such as restaurants, hotels and resorts, whose guests, along with international arrivals by flight, follow the same temporal pattern as $\Delta\delta^{15}N$. Seasonality of tourism seems to exert a detectable effect on $\delta^{15}N$ of macroalgae, with an evident enrichment, although overall moderate (< 1.4 ‰), compared to the baseline recorded at the beginning of the tourist flow, in June, peaking in August, during the tourist peak, and then decreasing at the end of the tourist season. In contrast, the Cavo Greco peninsula, where Control sites were placed, hosts a protected National Forest Park and agricultural fields, hence no infrastructures are present; tourist attendance is very low even during the high season, and limited to local people and small tourist cruise ships that stop in the coastal area during daily excursions. Accordingly, a low isotopic enrichment was detected in the macroalgae deployed at both Control sites across the periods.

These findings are in accordance with both nitrogen content and isotopic signature of the suspended particulate organic matter (POM) recorded in the coastal waters of Cyprus. The temporal pattern of POM $\delta^{15}N$ (i.e. higher at the Tourist site in August than in October and in the Control sites in both periods), as well as the positive correlation between the $\Delta\delta^{15}N$ of the deployed macroalgae and the POM TN content, suggests the relationship between the presence of anthropogenic nutrients and the macroalgae response at the Tourist site.

The suspended POM in coastal seawater is generally composed by a mixture of detrital material and living phytoplankton, which are known to quickly uptake dissolved nutrients, and then responds to the nutrient load and typology with variations in N content and $\delta^{15}N$ respectively (Signa et al., 2012; Vizzini and Mazzola, 2006). In particular, POM isotopic signature is widely recognized to give indication of the trophic condition of the environment, including the presence of anthropogenic input or events of eutrophication (Cole et al., 2005; Signa et al., 2012).

Although the increase in $\delta^{15}N$ signature of dissolved and particulate nutrients is linked to a number of chemical and biological processes, among which nitrification from excess sewage/nutrient runoff, denitrification or organic decomposition by bacteria and ammonia enrichment from industrial discharges (Costanzo et al., 2005, 2001), we can suppose that direct nutrient enrichment from bathing activities, and potentially undersized wastewater management from the horeca (hotels, restaurants, cafes) infrastructures during the tourist peak may have led to the observed patterns.

Similarly to Cyprus, the isotopic patterns of the macroalgae deployed in Sicily suggest that the coastal activities of the tourist beach of Giardini Naxos represent a source of anthropogenic nutrients into the bay, although mostly restricted to August. The summer increase in tourists and bathers at the beach, coupled with several recreational water activities (boating, water sports) and tourist facilities (hotels, restaurants and private houses) may be responsible for this small-spatial scale pattern, despite the extent of variation is quite moderate. In particular, the presence of a tourist port and breakwaters at the southeastern side may have led to a localised higher availability of ^{15}N-enriched nutrients, which is slightly evident also in October, most probably due to a reduction of the water exchange in the area. Moreover, the macroalgae seasonal trend was exactly consistent with that of POM nitrogen content and isotopic signature, which indeed showed a peak in August, confirming a localised derived increase in ^{15}N-enriched nutrients in the area.

Unlike the aforementioned islands, and although the high tourist flow interesting the island of Rhodes both in June and August, and big resorts located in the beach front of the Tourist site, both the temporal and spatial variability of the isotopic values of the deployed macroalgae were fairly low. $\delta^{15}N$ of the macroalgae showed only a low isotopic enrichment in August at all transects of the Tourist site and in the landward transect of one control site. In all the other sites and periods, no enrichment was recorded by macroalgae, indicating that anthropic nutrients were not recordable. Also the POM isotopic value did not show a clear pattern: although $\delta^{15}N$ was higher in the Tourist site than in Controls, it was lower in August than in June and October, contrarily to expectations. Moreover, although the nitrogen content of POM was significantly higher in August than in June, the lack of correlation with the isotopic variation of deployed macroalgae rules out the anthropic origin of nutrient load recorded. Therefore, despite the presence of numerous tourist facilities along the coast, the demographic increase and the many leisure activities including bathing, boating and water sports, a negligible input of ^{15}N-enriched nutrients occurred in shallow coastal waters in June and August, and no evident spread of anthropogenic nutrients up to offshore areas was highlighted, suggesting that management of tourism and wastewater seems to be efficient also during the tourist peak.

Turning to the extent of the isotopic variation detected in the macroalgae across periods, sites and transects, overall modest variations were recorded, δ^{15}N enrichments ($\Delta\delta^{15}$N) did not exceed 1.4‰ in Cyprus, 1.2‰ in Sicily and 0.7‰ in Rhodes, and were comparable with previous deployment studies that used species of the *Cystoseira* genus. Among these, Orlandi et al. (2014) found an isotopic enrichment up to +1.7‰ of *C. amentacea* incubated for 48 hours in a strongly urbanised coastal area impacted by polluted river discharge. The highest $\Delta\delta^{15}$N found in Cyprus and Sicily was within the range reported by Orlandi et al. (2014), further confirming the uptake of ^{15}N-enriched nutrients by the deployed macroalgae. However, only a few macroalgae samples showed values similar to the highest peaks recorded by Orlandi et al. (2014), not indicating a marked presence of anthropogenic nutrients.

At the same time, the minor isotopic enrichment found in Rhodes is most likely the result of a negligible influence of input of anthropic origin. In contrast, García-Sanz et al. (2010, 2011) found only a low to moderate isotopic enrichment (up to +0.8‰ and +0.7‰ respectively) in *C. mediterranea* deployed at a depth of 5 m for 4-6 days at increasing distance from a fish farm. Although aquaculture waste is a common cause of ^{15}N enrichment in aquatic environments (Vizzini and Mazzola, 2004), the authors attributed the small enrichment to the high baseline values of the macroalgae, coupled with a high nitrogen content, that may have masked the effects of spatial nutrient gradients from fish farms. Although we also recorded a high isotopic baseline in Sicily, we rule out that this may have biased the macroalgae nutrient uptake, as it was not coupled with a high nitrogen content. Rather, in spite of the inter-island baseline variability that may be ascribed to intrinsic background oceanographic factors independent on human activity (Viana and Bode, 2013; Vizzini et al., 2011), low TN content and high C/N ratio were observed in all the macroalgae used as baseline, consistent with previous studies on *Cystoseira* species from oligotrophic coastal areas (Celis-Plá et al., 2014). TN and C/N ratio respectively below and above the critical values of 2% and 10, like those observed in this study, indicate N-limitation (Phillips and Hurd, 2003), condition where high uptake rates are usually observed. In particular, intertidal macroalgae are able to maximize nitrogen acquisition independent on the available nitrogen form, as a strategy to cope with the highly variable and potentially N-limited coastal environment (Phillips and Hurd, 2004).

Although the comparison of the three *Cystoseira* species was out of the scope of the present study, assuming a similar response to nutrient enrichment by the three species used (*C. humilis* in Cyprus, *C. amentacea* in Sicily and *C. compressa* in Rhodes), the match among the temporal pattern of $\Delta\delta^{15}N$ in macroalgae, $\delta^{15}N$ in POM and visitor flows, and the correlation between $\Delta\delta^{15}N$ in macroalgae and the nitrogen content of POM i) suggests the reliability of *Cystoseira* genus in deployment experiments; ii) confirms the greater usefulness of the deployment approach compared to standard monitoring based on nutrients and/or POM characterization. Indeed, although more time consuming, the biomonitoring approach using $\delta^{15}N$ of deployed macroalgae provides space- and time-integrated information about anthropogenic impact, while the analysis of nutrients and/or POM might only provide a snapshot of the highly variable water column conditions.

As previously mentioned, macroalgae widely differ according to their nutritional needs and growth strategy, as well as nutrient metabolism (Pedersen and Borum, 1997). In this context, perennial and slow-growing macroalgae, such as Cystoseiraceae (Phaeophyta) (Mannino et al., 2017), are overall characterized by lower turnover and uptake rates, and have developed nutrient storage pools to cope with low nutrient availability (Martínez et al., 2012). Differently, opportunistic and fast-growing Ulvaceae (Chlorophyta) have a high ability to accumulate and store available nutrients in excess and are characterized by high nutrient uptake and growth rates (Fong et al., 2004; Lubsch and Timmermans, 2018). This opposite metabolism is certainly responsible of the very high isotopic enrichment found in the green macroalga *Ulva* spp. used in other deployment experiments (e.g. from +28 to +45‰ in Dailer et al. 2010 and from +10 to +14‰ in Dailer et al. 2012), as well as the different response of *Cystoseira* vs. *Ulva* ($\Delta\delta^{15}N$ range: +0.5 - +1.7 ‰ and +1.5 - +4.3 ‰ for *Cystoseira* and *Ulva* respectively) incubated in the same anthropized site highlighted by Orlandi et al. (2014).

Nevertheless, field and laboratory experiments reported rapid and significant response of different *Cystoseira* species, once exposed to higher nutrient concentrations. In more detail, Celis-Plá et al. (2014) observed increase in nitrogen content, phenolic compounds and carotenoids in *C. tamariscifolia*. Similarly, Vaz-Pinto et al. (2014), exposing *C. humilis* to high nutrient concentrations in laboratory conditions, found higher growth and nutrient uptake rates than those reported for other perennial species. Lastly, *C. compressa* was found to respond well to high nutrient conditions (Celis-Plá et al., 2015) and to bioaccumulate trace elements (Benfares et al., 2015), confirming its suitability as a biomarker of pollution in coastal areas.

Conclusion

Main findings of this study showed that the manipulative deployment approach with brown macroalgae of the genus *Cystoseira* worked effectively in the detection and monitoring of anthropogenic nutrient enrichment in marine coastal areas driven by tourism activities. However, despite the spatial and temporal patterns emerged in macroalgae $\Delta\delta^{15}N$, its overall extent was rather low or moderate, with only a few high enrichment peaks, depending on the specific site investigated. This suggests a modest influence of anthropogenic activities on nutrient input in coastal seawater in Cyprus and Sicily, which was instead negligible in Rhodes. The final output consisting in georeferenced maps, indeed, summarizes important information that is relatively easy to read and to transfer to competent authorities, helping in facing the major issue of deterioration of water quality due to tourism impact and the consequent impairment of ecosystem services in highly tourist areas. As many aspects of the coastal economy are dependent upon the environmental preservation and recreational quality of the beaches, this approach can represent an early warning system for the development of adequate management plans for a sustainable coastal tourism.

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References

Alquezar, R., Glendenning, L., Costanzo, S., 2013. The use of the brown macroalgae, *Sargassum flavicans*, as a potential bioindicator of industrial nutrient enrichment. Mar. Pollut. Bull. 77, 140–146. doi:10.1016/j.marpolbul.2013.10.013

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods, in: PRIMER-E. Plymouth, UK, pp. 1–214. doi:10.13564/j.cnki.issn.1672-9382.2013.01.010

Arévalo, R., Pinedo, S., Ballesteros, E., 2007. Changes in the composition and structure of Mediterranean rocky-shore communities following a gradient of nutrient enrichment: Descriptive study and test of proposed methods to assess water quality regarding macroalgae. Mar. Pollut. Bull. 55, 104–113. doi:10.1016/j.marpolbul.2006.08.023

Ballesteros, E., Torras, X., Pinedo, S., García, M., Mangialajo, L., de Torres, M., 2007. A new methodology based on littoral community cartography dominated by macroalgae for the implementation of the European Water Framework Directive. Mar. Pollut. Bull. 55, 172–180. doi:10.1016/j.marpolbul.2006.08.038

Benfares, R., Seridi, H., Belkacem, Y., Inal, A., 2015. Heavy Metal Bioaccumulation in Brown Algae *Cystoseira compressa* in Algerian Coasts, Mediterranean Sea. Environ. Process. 2, 429–439. doi:10.1007/s40710-015-0075-5

Castro, P., Valiela, I., Freitas, H., 2007. Eutrophication in Portuguese estuaries evidenced by $\delta^{15}N$ of macrophytes. Mar. Ecol. Prog. Ser. 351, 43–51. doi:10.3354/meps07173

Celis-Plá, P.S.M., Hall-Spencer, J.M., Horta, P.A., Milazzo, M., Korbee, N., Cornwall, C.E., Figueroa, F.L., 2015. Macroalgal responses to ocean acidification depend on nutrient and light levels. Front. Mar. Sci. 2. doi:10.3389/fmars.2015.00026

Celis-Plá, P.S.M., Martinez, B., Quintano, E., García-Sánchez, M., Pedersen, A., Navarro, N.P., Copertino, M.S., Mangaiyarkarasi, N., Mariath, R., Figueroa, F.L., Korbee, N., 2014. Short-term ecophysiological and biochemical responses of *Cystoseira tamariscifolia* and *Ellisolandia elongata* to environmental changes. Aquat. Biol. 22, 227–243. doi:10.3354/ab00573

Cole, M.L., Kroeger, K.D., McClelland, J.W., Valiela, I., 2005. Macrophytes as indicators of land-derived wastewater: Application of a $\delta^{15}N$ method in aquatic systems. Water Resour. Res. 41, 1–9. doi:10.1029/2004WR003269

Cole, M.L., Valiela, I., Kroeger, K.D., Tomasky, G.L., Cebrian, J., Wigand, C., Mckinney, R.A., Grady, S.P., Carvalho de Silva, M.H., Carvalho da Silva, M.H., 1995. Assessment of a $\delta^{15}N$ isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. J. Environ. Qual. 33, 124–132. doi:10.2134/jeq2004.1240

Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., Loneragan, N.R., Thomas, M., 2001. A new approach for detecting and mapping sewage impacts. Mar. Pollut. Bull. 42, 149–156. doi:10.1016/S0025-326X(00)00125-9

Costanzo, S.D., Udy, J., Longstaff, B., Jones, A., 2005. Using nitrogen stable isotope ratios ($\delta^{15}N$) of macroalgae to determine the effectiveness of sewage upgrades: Changes in the extent of sewage plumes over four years in Moreton Bay, Australia. Mar. Pollut. Bull. 51, 212–217. doi:10.1016/j.marpolbul.2004.10.018

Dailer, M.L., Knox, R.S., Smith, J.E., Napier, M., Smith, C.M., 2010. Using $\delta^{15}N$ values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai'i, USA. Mar. Pollut. Bull. 60, 655–671. doi:10.1016/j.marpolbul.2009.12.021

Dailer, M.L., Ramey, H.L., Saephan, S., Smith, C.M., 2012. Algal $\delta^{15}N$ values detect a wastewater effluent plume in nearshore and offshore surface waters and three-dimensionally model the plume across a coral reef on Maui, Hawai'i, USA. Mar. Pollut. Bull. 64, 207–213. doi:10.1016/j.marpolbul.2011.12.004

Davenport, J., Davenport, J.L., 2006. The impact of tourism and personal leisure transport on coastal environments: A review. Estuar. Coast. Shelf Sci. 67, 280–292. doi:10.1016/j.ecss.2005.11.026

Derse, E., Knee, K.L., Wankel, S.D., Kendall, C., Berg, C.J., Paytan, A., 2007. Identifying sources of nitrogen to Hanalei Bay, Kauai, utilizing the nitrogen isotope signature of macroalgae. Environ. Sci. Technol. 41, 5217–5223. doi:10.1021/es0700449

Deutsch, B., Voss, M., 2006. Anthropogenic nitrogen input traced by means of $\delta^{15}N$ values in macroalgae: Results from in-situ incubation experiments. Sci. Total Environ. 366, 799–808. doi:10.1016/j.scitotenv.2005.10.013

Drius, M., Bongiorni, L., Depellegrin, D., Menegon, S., Pugnetti, A., Stifter, S., 2019. Tackling challenges for Mediterranean sustainable coastal tourism: An ecosystem service perspective. Sci. Total Environ. 652, 1302–1317. doi:10.1016/j.scitotenv.2018.10.121

Fernandes, M., Benger, S., Sharma, S.K., Gaylard, S., Kildea, T., Hoare, S., Braley, M., Irving, A.D., 2012. The use of $\delta^{15}N$ signatures of translocated macroalgae to map coastal nutrient plumes: improving species selection and spatial analysis of metropolitan datasets. J. Environ. Monit. 14, 2399–2410. doi:10.1039/c2em10997b

Fong, P., Fong, J.J., Fong, C.R., 2004. Growth, nutrient storage, and release of dissolved organic nitrogen by *Enteromorpha intestinalis* in response to pulses of nitrogen and phosphorus. Aquat. Bot. 78, 83–95. doi:10.1016/j.aquabot.2003.09.006

García-Sanz, T., Ruiz-Fernández, J.M., Ruiz, M., García, R., González, M.N., Pérez, M., 2010. An evaluation of a macroalgal bioassay tool for assessing the spatial extent of nutrient release from offshore fish farms. Mar. Environ. Res. 70, 189–200. doi:10.1016/j.marenvres.2010.05.001

García-Sanz, T., Ruiz, J.M., Pérez, M., Ruiz, M., 2011. Assessment of dissolved nutrients dispersal derived from offshore fish-farm using nitrogen stable isotope ratios ($\delta^{15}N$) in macroalgal bioassays. Estuar. Coast. Shelf Sci. 91, 361–370. doi:10.1016/j.ecss.2010.10.025

García-Seoane, R., Aboal, J.R., Boquete, M.T., Fernández, J.A., 2018. Biomonitoring coastal environments with transplanted macroalgae : A methodological review. Mar. Pollut. Bull. 135, 988–999. doi:10.1016/j.marpolbul.2018.08.027

Garcia, C., Servera, J., 2003. Impacts of tourism development on water demand and beach degradation on the island of Mallorca (Spain). Geogr. Ann. Ser. A Phys. Geogr. 85A, 287–300. doi:10.1111/j.0435-3676.2003.00206.x

Gröcke, D.R., Racionero-Gómez, B., Marschalek, J.W., Greenwell, H.C., 2017. Translocation of isotopically distinct macroalgae: A route to low-cost biomonitoring? Chemosphere 184, 1175–1185. doi:10.1016/j.chemosphere.2017.06.082

Hall, C.M., 2001. Trends in ocean and coastal tourism: The end of the last frontier? Ocean Coast. Manag. 44, 601–618. doi:10.1016/S0964-5691(01)00071-0

Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. Chem. Geol. Isot. Geosci. Sect. 59, 87–102. doi:10.1016/0168-9622(86)90059-X

Huntington, B.E., Boyer, K.E., 2008. Evaluating patterns of nitrogen supply using macroalgal tissue content and stable isotopic signatures in Tomales Bay, CA. Environ. Bioindic. 3, 180–192. doi:10.1080/15555270802537510

Hurd, C.L., Harrison, P.J., Bischof, K., Lobban, C.S., 2014. Seaweed ecology and physiology. Cambridge University Press, Cambridge (UK).

Lin, H.J., Wu, C.Y., Kao, S.J., Kao, W.Y., Meng, P.J., 2007. Mapping anthropogenic nitrogen through point sources in coral reefs using $\delta^{15}N$ in macroalgae. Mar. Ecol. Prog. Ser. 335, 95–109. doi:10.3354/meps335095

Lubsch, A., Timmermans, K., 2018. Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding N:P dynamics in *Ulva lactuca* (Chlorophyta). J. Phycol. 223, 215–223. doi:10.1111/jpy.12612

Mannino, A.M., Vaglica, V., Oddo, E., 2017. Interspecific variation in total phenolic content in temperate brown algae. J. Biol. Res. 90, 26–29. doi:10.4081/jbr.2017.6578

Mannino, A.M., Vaglica, V., Oddo, E., 2014. Seasonal variation in total phenolic content of *Dictyopteris polypodioides* (Dictyotaceae) and *Cystoseira amentacea* (Sargassaceae) from the Sicilian coast. Flora Mediterr. 24, 39–50. doi:10.7320/FlMedit24.039

Martínez, B., Sordo Pato, L., Rico, J.M., Pato, L.S., Rico, J.M., Sordo Pato, L., Rico, J.M., 2012.

Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies. Aquat. Bot. 96, 14–22. doi:10.1016/j.aquabot.2011.09.004

Michener, R.H., Shell, D.M., 1994. Stable isotope ratios as tracers in marine aquatic food webs, in: Lajtha, K., Michener, R.H. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publications, Oxford (UK), pp. 138–157.

Morris, E.P., Peralta, G., Benavente, J., Freitas, R., Rodrigues, A.M., Quintino, V., Alvarez, O., Valcárcel-Pérez, N., Vergara, J.J., Hernandez, I., Pérez-Lloréns, J.L., 2009. *Caulerpa prolifera* stable isotope ratios reveal anthropogenic nutrients within a tidal lagoon. Mar. Ecol. Prog. Ser. 390, 117–128. doi:10.3354/meps08184

Nixon, S.W., 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia 41, 199–219. doi:10.1080/00785236.1995.10422044

Olsen, Y.S., Fox, S.E., Kinney, E.L., Teichberg, M., Valiela, I., 2010. Differences in urbanization and degree of marine influence are reflected in δ^{13}C and δ^{15}N of producers and consumers in seagrass habitats of Puerto Rico. Mar. Environ. Res. 69, 198–206. doi:10.1016/j.marenvres.2009.10.005

Orlandi, L., Bentivoglio, F., Carlino, P., Calizza, E., Rossi, D., Costantini, M.L., Rossi, L., 2014. δ^{15}N variation in Ulva lactuca as a proxy for anthropogenic nitrogen inputs in coastal areas of Gulf of Gaeta (Mediterranean Sea). Mar. Pollut. Bull. 84, 76–82. doi:10.1016/j.marpolbul.2014.05.036

Pedersen, M.F., Borum, J., 1997. Nutrient control of estuarine macroalgae: Growth strategy and the balance between nitrogen requirements and uptake. Mar. Ecol. Prog. Ser. doi:10.3354/meps161155

Philip, G.M., Watson, D.F., 1982. A precise method for determining contoured surfaces. APPEA J. 22, 205–212.

Phillips, J.C., Hurd, C.L., 2004. Kinetics of nitrate, ammonium, and urea uptake by four intertidal seaweeds from New Zealand. J. Phycol. 40, 534–545. doi:10.1111/j.1529-8817.2004.03157.x

Phillips, J.C., Hurd, C.L., 2003. Nitrogen ecophysiology of intertidal seaweeds from New Zealand: N uptake, storage and utilisation in relation to shore position and season. Mar. Ecol. Prog. Ser. 264, 31–48. doi:10.3354/meps264031

Sales, M., Ballesteros, E., 2009. Shallow *Cystoseira* (Fucales: Ochrophyta) assemblages thriving in sheltered areas from Menorca (NW Mediterranean): Relationships with environmental factors and anthropogenic pressures. Estuar. Coast. Shelf Sci. 84, 476–482. doi:10.1016/j.ecss.2009.07.013

Sales, M., Cebrian, E., Tomas, F., Ballesteros, E., 2011. Pollution impacts and recovery potential in three species of the genus *Cystoseira* (Fucales, Heterokontophyta). Estuar. Coast. Shelf Sci. 92, 347–357. doi:10.1016/j.ecss.2011.01.008

Savage, C., 2005. Tracing the influence of sewage nitrogen in a coastal ecosystem using stable nitrogen isotopes. Ambio 34, 145–50. doi:10.1579/0044-7447-34.2.145

Signa, G., Mazzola, A., Costa, V., Vizzini, S., 2015. Bottom-Up Control of Macrobenthic Communities in a Guanotrophic Coastal System. PLoS One 10, e0117544. doi:10.1371/journal.pone.0117544

Signa, G., Mazzola, A., Vizzini, S., 2012. Effects of a small seagull colony on trophic status and primary production in a Mediterranean coastal system (Marinello ponds, Italy). Estuar. Coast. Shelf Sci. 111, 27–34. doi:10.1016/j.ecss.2012.06.008

Swart, P.K., Evans, S., Capo, T., Altabet, M.A., 2014. The fractionation of nitrogen and oxygen isotopes in macroalgae during the assimilation of nitrate. Biogeosciences 11, 6147–6157. doi:10.5194/bg-11-6147-2014

Vaz-Pinto, F., Martínez, B., Olabarria, C., Arenas, F., 2014. Neighbourhood competition in coexisting species: The native *Cystoseira humilis* vs the invasive *Sargassum muticum*. J. Exp. Mar. Bio. Ecol. 454, 32–41. doi:10.1016/j.jembe.2014.02.001

Viana, I.G., Bode, A., 2013. Stable nitrogen isotopes in coastal macroalgae: Geographic and anthropogenic variability. Sci. Total Environ. 443, 887–895. doi:10.1016/j.scitotenv.2012.11.065

Vizzini, S., Colombo, F., Costa, V., Mazzola, A., 2011. Contribution of planktonic and benthic food sources to the diet of the reef-forming vermetid gastropod *Dendropoma petraeum* in the western Mediterranean. Estuar. Coast. Shelf Sci. 96, 262–267. doi:10.1016/j.ecss.2011.11.021

Vizzini, S., Mazzola, A., 2006. The effects of anthropogenic organic matter inputs on stable carbon and nitrogen isotopes in organisms from different trophic levels in a southern Mediterranean coastal area. Sci. Total Environ. 368, 723–731. doi:10.1016/j.scitotenv.2006.02.001

Vizzini, S., Mazzola, A., 2004. Stable isotope evidence for the environmental impact of a land-based fish farm in the western Mediterranean. Mar. Pollut. Bull. 49, 61–70. doi:10.1016/j.marpolbul.2004.01.008

Watson, D.F., Philip, G.M., 1985. A refinement of inverse ditance weighted interpolation. Geoprocessing 2, 315–327.

Artwork

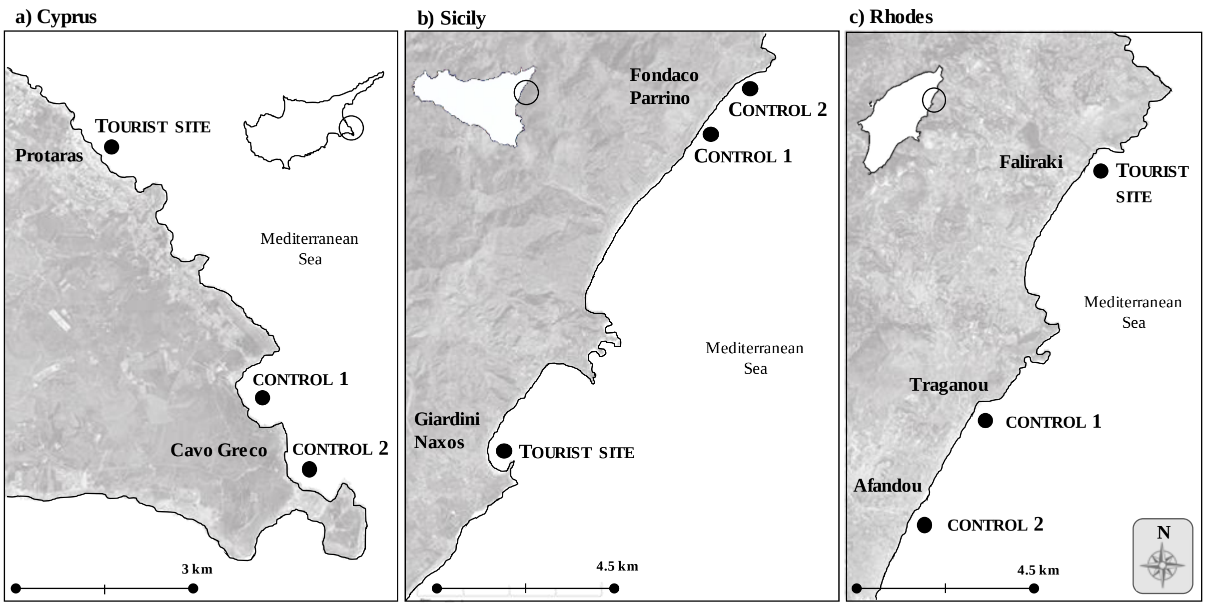


Fig. 1. Experimental sites in a) Cyprus, b) Sicily (Italy) and c) Rhodes (Greece).

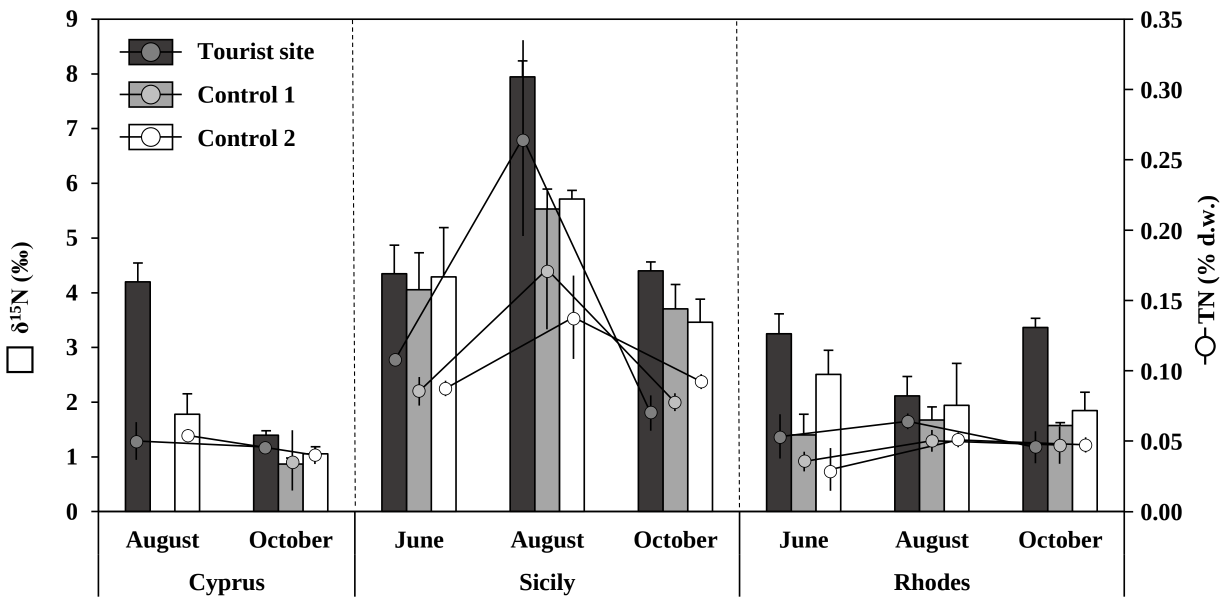
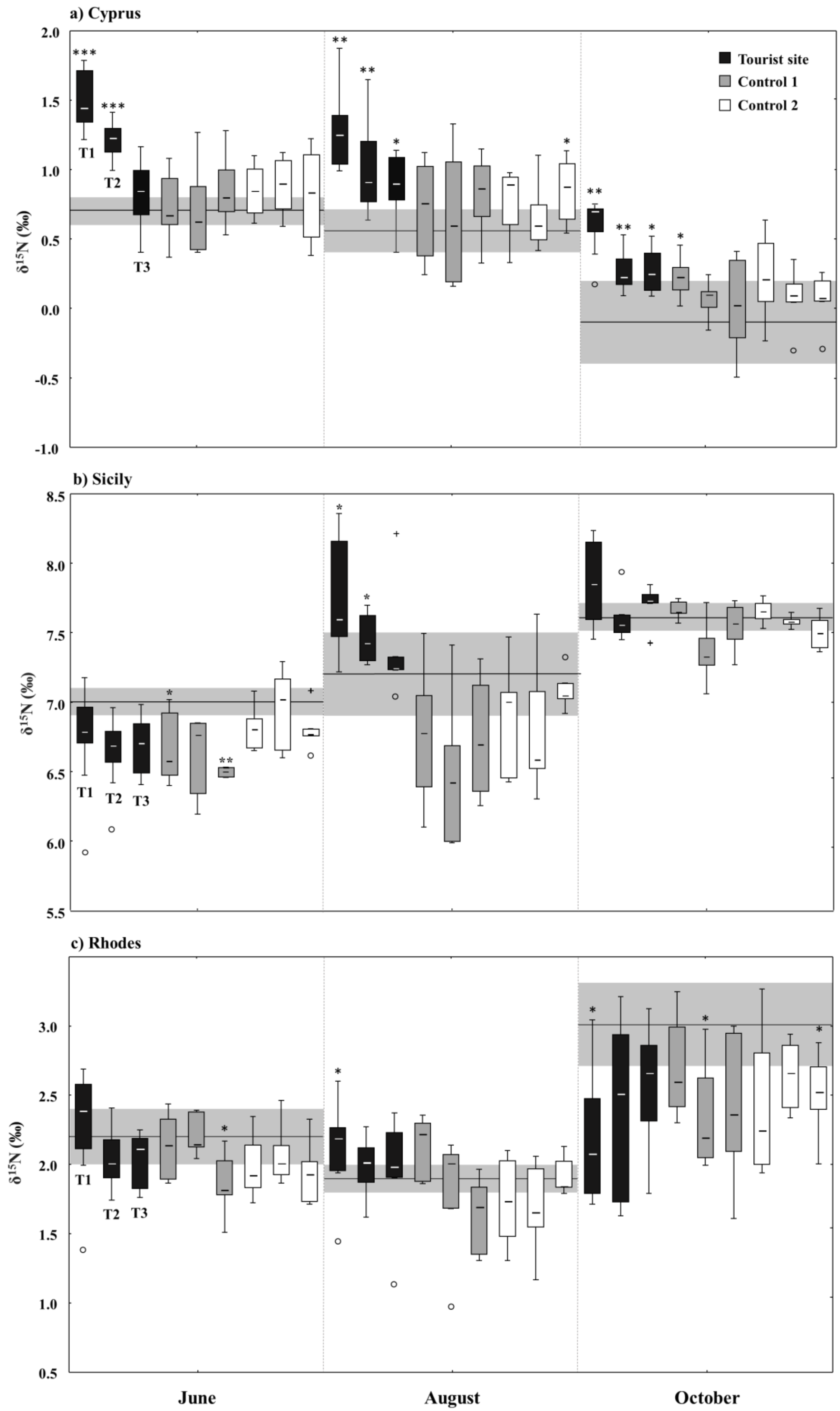


Fig. 2. Mean $\delta^{15}N$ and TN (± standard deviation, n: 9) of suspended particulate organic matter (POM) of surface water collected from the three study sites of Cyprus, Sicily and Rhodes in the different periods (June, August, October). Data from Cyprus in June (all sites) and August (only Control 1) are lacking because of logistic constraints.



a) Cyprus
b) Sicily
c) Rhodes
Tourist site
Control 1
Control 2
δ15N (‰)
T1
T2
T3
June
August
October

679

Fig. 3. Boxplot of $\delta^{15}N$ values of the macroalgae deployed along three transects at different distance from the coastline [T1: 100, T2: 200, T3: 300 m in Cyprus (n: 5-10) and Rhodes (n: 5-11), T1: 200, T2: 250, T3: 300 m in Sicily (n: 3-12); indicated only below the first three boxes for the sake of simplicity] in the three study sites (Tourist, Control 1 and 2) in June, August and October in Cyprus (a), Sicily (b) and Rhodes (c). Each box contains 50% of the data, the thick horizontal line indicates the median; lower and upper whiskers represent respectively the first and fourth quartiles of the total range and circles and plus symbols represent respectively outliers and extremes of the distribution. Asterisks indicate the significance level of the differences between the $\delta^{15}N$ values of the deployed macroalgae and that of the baseline, according to t-test. p-values: * = p-value< 0.05, ** = p-value< 0.01, *** = p-value< 0.001. Shadow areas overlaying the boxplots at each period indicate the mean and the standard deviation of the site and period specific baseline.

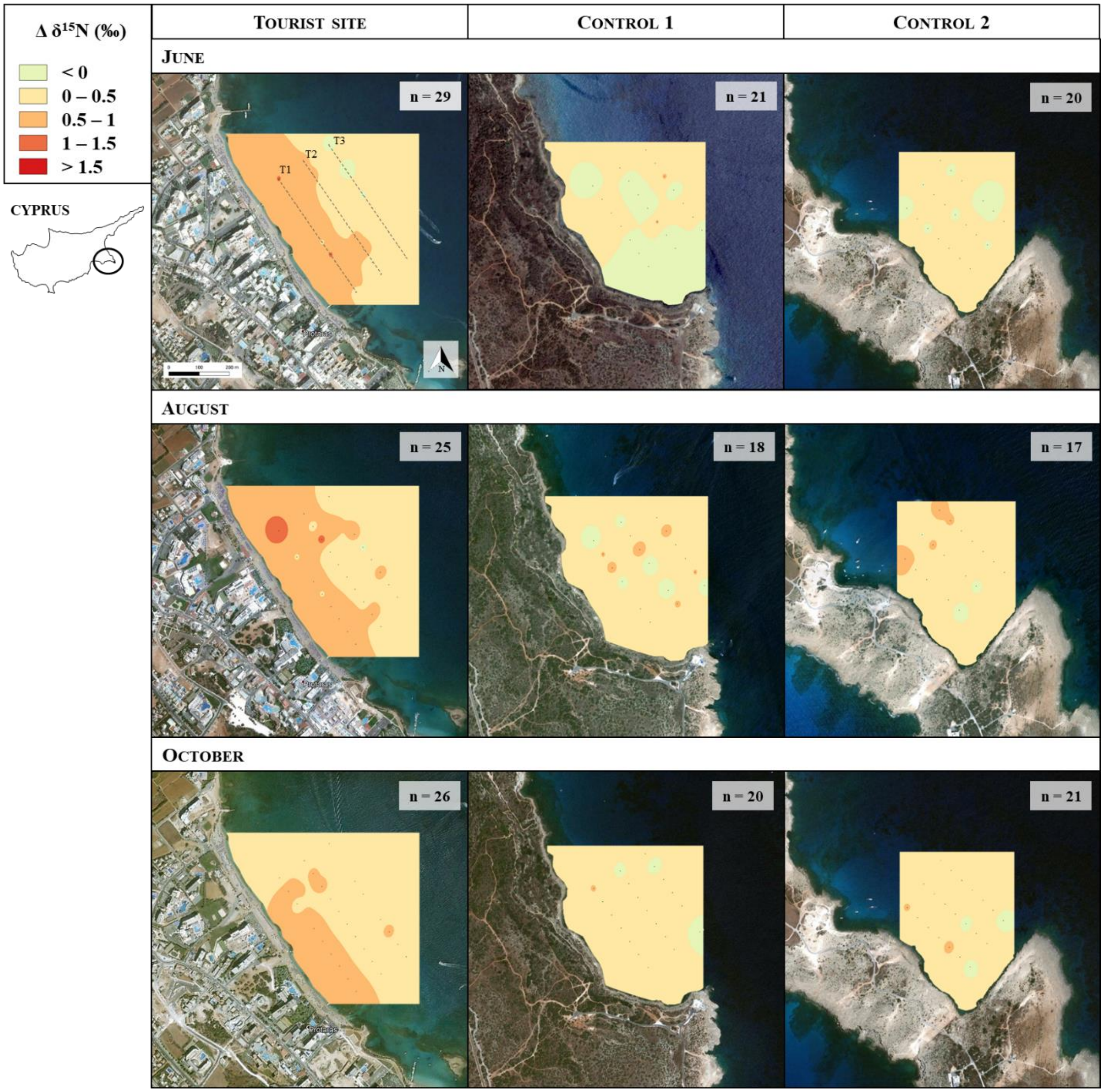


Fig. 4. Georeferenced maps of $\Delta\delta^{15}N$ values in June, August and October 2017 at the Tourist site and Control sites of Cyprus. Dashed lines (superimposed only to the first panel for the sake of simplicity) indicate the transects at different distance from the coastline (T1: 100, T2: 200 and T3: 300 m) where macroalgae were deployed. The number of the macroalgae deployment devices retrieved at each period and site is also indicated.

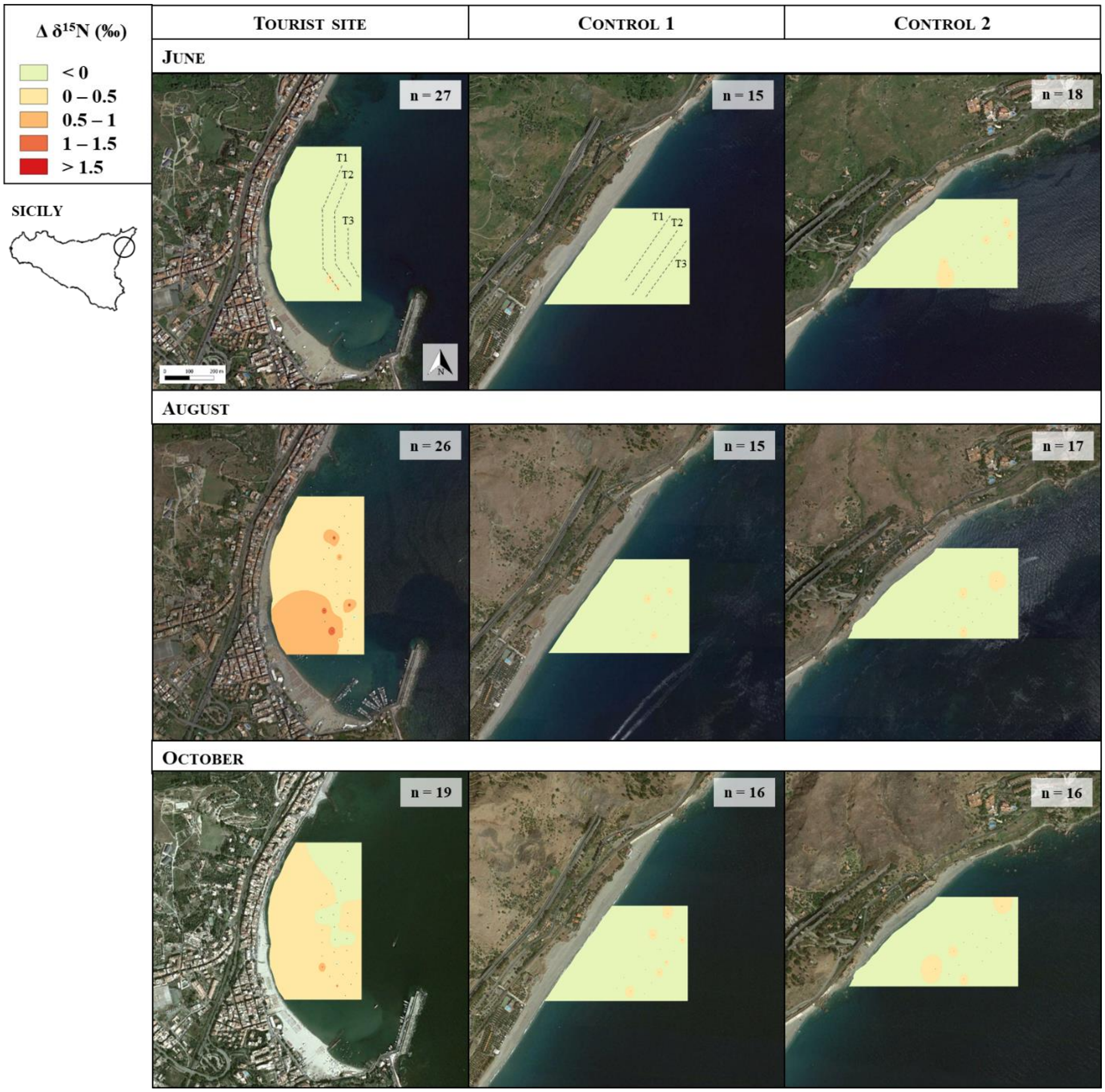


Fig. 5. Georeferenced maps of $\Delta\delta^{15}N$ values in June, August and October 2017 at the Tourist site and Control sites of Sicily. Dashed lines (superimposed only to the first panel for the sake of simplicity) indicate the transects at distance from the coastline (T1: 200, T2: 250 and T3: 300 m) where macroalgae were deployed. The number of the macroalgae deployment devices retrieved at each period and site is also indicated.

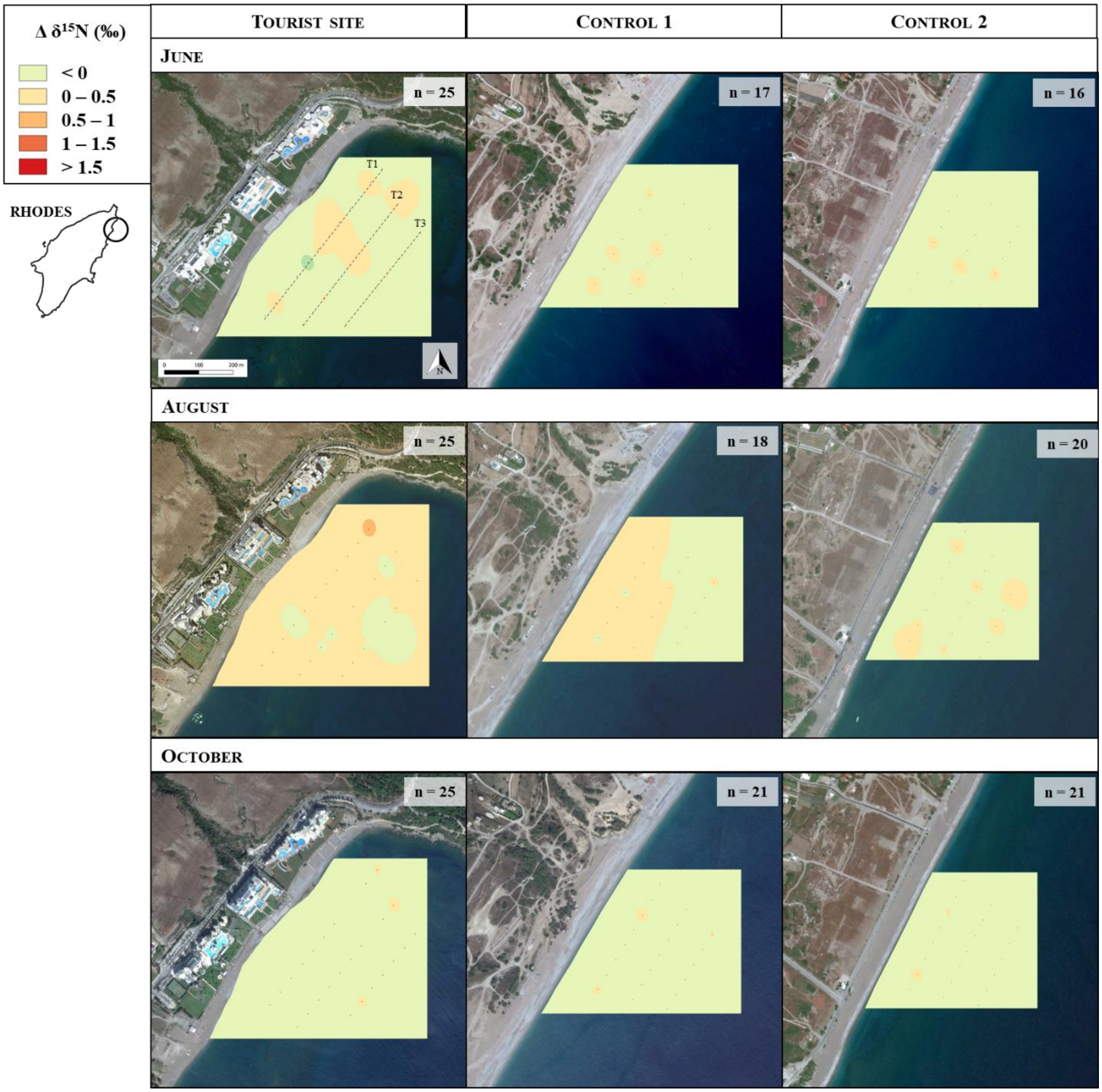


Fig. 6. Georeferenced maps of $\Delta\delta^{15}N$ values in June, August and October 2018 at the Tourist site and Control sites of Rhodes. Dashed lines (superimposed only to the first panel for the sake of simplicity) indicate the transects at distance from the coastline (T1: 100, T2: 200 and T3: 300 m) where macroalgae were deployed. The number of the macroalgae deployment devices retrieved at each period and site is also indicated.

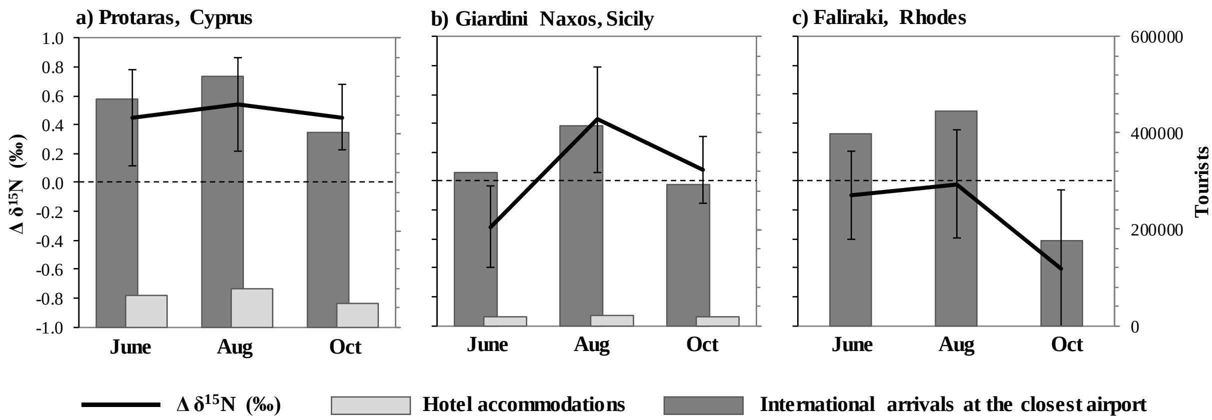


Fig. 7. Mean $\Delta\delta^{15}N$ (± standard deviation, n: 19-29) values recorded in macroalgae deployed at the Tourist sites of a) Cyprus (Protaras), b) Sicily (Giardini Naxos) and c) Rhodes (Faliraki) in the different periods (June, August, October), superimposed to local hotel accommodations (only at Protaras and Giardini Naxos) and International arrivals at the closest airports.

Tables

Table 1. Mean (± standard deviation, s.d., n: 6), minimum (min) and maximum (max) temperature (°C) and salinity (psu) of surface waters in Cyprus, Sicily and Rhodes, in the different periods (June, August, October).

		T (°C)				**S (psu)**			
Island	**Period**	mean	s.d.	min	max	mean	s.d.	min	max
Cyprus	**June**	22.4	0.2	21.9	22.9	38.1	0.2	37.5	38.2
	August	29.0	0.2	28.8	29.4	41.2	0.2	41.0	42.0
	October	25.6	0.3	25.0	26.1	40.7	0.8	38.9	41.0
Sicily	**June**	22.1	0.5	21.3	22.9	38.2	0.6	37.2	38.7
	August	25.9	0.4	25.5	26.7	38.8	0.1	38.7	38.9
	October	22.1	1.1	20.8	24.1	38.4	0.1	38.3	38.6
Rhodes	**June**	26.2	0.3	25.2	26.9	38.4	0.3	38.1	38.6
	August	30.5	0.8	29.3	32.0	41.5	0.8	41.2	41.8
	October	28.3	0.5	26.9	29.0	40.2	0.5	39.8	40.5

Table 2. Mean values (± standard deviation, s.d., n: 5) of $\delta^{15}N$, total N and C/N of the macroalgae used as baseline in all islands (Cyprus, Sicily and Rhodes) and periods (June, August, October).

Island	**Period**	**$\delta^{15}N$ (‰)**		**TN (%)**		**C/N**	
		mean	s.d.	mean	s.d.	mean	s.d.
Cyprus	**June**	0.7	0.1	0.8	0.1	48.7	5.8
	August	0.5	0.2	0.7	0.1	46.4	2.5
	October	-0.1	0.3	0.7	0.1	41.0	4.7
Sicily	**June**	7.0	0.1	1.0	0.0	34.7	1.9
	August	7.2	0.3	1.3	0.2	26.0	5.9
	October	7.6	0.1	0.8	0.0	34.8	2.0
Rhodes	**June**	2.2	0.2	0.8	0.1	40.6	4.2
	August	1.9	0.1	0.9	0.1	24.2	1.7
	October	3.0	0.3	1.2	0.2	21.7	2.8