^{15}N signal of *Aplysina aerophoba* as a tracer of anthropogenic nitrogen in the Murter Sea and Pirovac Bay (Central Adriatic)

^{15}N v *Aplysini aerophobi* kot sledilo antropogenega dušika v Murterskem morju in Pirovaškem zalivu (srednji Jadran)

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Abstract: Stable nitrogen isotopes were used to study the environmental impact of sewage-discharge and fish-farming-derived nitrogen in the Murter Sea and in the semi-enclosed Pirovac Bay (Central Adriatic). The results suggested that the effluents from the septic systems of Murter Island and the coastal part of Pirovac Bay discharged into the adjacent coastal ecosystems, which together with the fish-farm-derived fecal material and the feed wastage affected the stable nitrogen isotopic composition of the marine sponge Aplysina aerophoba. Furthermore, the δ^{15}N signature of Aplysina aerophoba can be useful in tracing the untreated municipal and other sewage-derived nitrogen flow in marine coastal ecosystems and the dispersion of ^{15}N loadings generated by the inflows from fish farms.

Izvleček: Članek obravnava izotopsko sestavo dušika v morski spužvi Aplysina aerophoba iz Murterskega morja in Pirovaškega zaliva (srednji Jadran). Rezultati raziskave kažejo, večje vrednosti parametra δ^{15}N v Aplysini aerophobi iz priobalnih delov Murterskega morja in Pirovškega zaliva, ki so posledica vnosa izotopsko težjega dušika ^{15}N z netretiranimi komunalnimi in drugimi odplakami ter izpustov iz ribjih farm. Dobljeni podatki nakazujejo tudi možnost uporabe omenjene spužve za monitoring vpliva odpadnih voda na obalne ekosisteme.

Key words: Aplysina aerophoba, municipal sewage, fish farming activities, nitrogen isotope composition, Murter Sea, Pirovac Bay, Central Adriatic
Ključne besede: Aplysina aerophoba, komunalne odplake, ribje farme, izotopska sestava dušika, Murtersko morje, Pirovški zaliv, srednji Jadran

INTRODUCTION

Increased nitrogen loadings have been implicated in eutrophication occurrences worldwide. A significant component of marine eutophication in many near shore environments can be attributed to the inputs of anthropogenic nitrogen from untreated domestic sewage, as well as municipal and industrial effluents (BACHTIAR, et al., 1996; COSTANZO, et al., 2001; LEE and OLSEN, 1985; NIXON, et al., 1986). In the past decade, the increasing number of fish-culture industries in the Mediterranean and Adriatic area has also begun to create environmental problems due to the impact caused by fish-farming waste. Many papers have shown how fish-farm biodeposition products can negatively affect the sediment chemistry and the community dynamics of seagrasse benthic macrofauna, meiofauna, and benthic bacteria (DOLENEC, et al., 2007; 2006; HOLMER, 1991; LA ROSA, et al., 2001; MIRTO, et al., 2002; PERGENT, et al., 1999; RUIZ, et al., 2001; SARA, et al., 2004).

The near-shore environments of the Murter Sea and the semi-closed Pirovac Bay are among the most impacted areas in northern Dalmatia. The area is exposed to and affected by municipal and industrial sewage from Murter Island and the coastal parts of Pirovac Bay. The aquaculture located near Vrgada Island (Murter Sea) is an additional source of the increased amounts of dissolved and particulate nutrient loads, especially of organic phosphorus (P) and nitrogen (N).

Stable isotopes of biogenic elements (C, N, S) have been used successfully for assessing and monitoring aquatic eco-system quality. Stable isotope ratios can provide information about trophic structure, resource partitioning, habitat usage and species migration (FRY et al., 1977; KNEIB and STIVEN, 1980; ZIEMAN et al., 1984; MUSCATINE et al., 1989; THOMAS and CAHOON, 1993; NEWELL et al., 1995; DEEGAN AND GARRITT, 1997; MONCREIFF and SULLIVAN, 2001; VIZZINI et al., 2002; VIZZINI and MAZZOLA, 2002) and determine major nutrient sources in natural food webs (SHEARER and KOHL, 1993; COFFIN et al., 1994).

The aim of this study was to assess the anthropogenic effects on the marine sponge *Aplysina aerophoba* in the Murter Sea and Pirovac Bay by using nitrogen isotope analysis. *Aplysina aerophoba* is one of the most abundant sponges; it is very widespread on the shoal rocky bottom in the coastal part of the Adriatic Sea and therefore very representative of the investigated area. As a bethic sessile invertebrate the sponge is relatively non-mobile and thus tends to be representative of the area being sampled (REYNOLDS et al., 1995).

Materials and methods

Study area

The study area of about 380 km^2 is located in the northern Dalmatia Adriatic, Croatia. It represents the inshore and offshore parts of the Murter Sea, the semi-enclosed Pirovac Bay, the archipelago of the Kornati Islands and the offshore islands of the main islands Žirje and Žut (Figure 1).

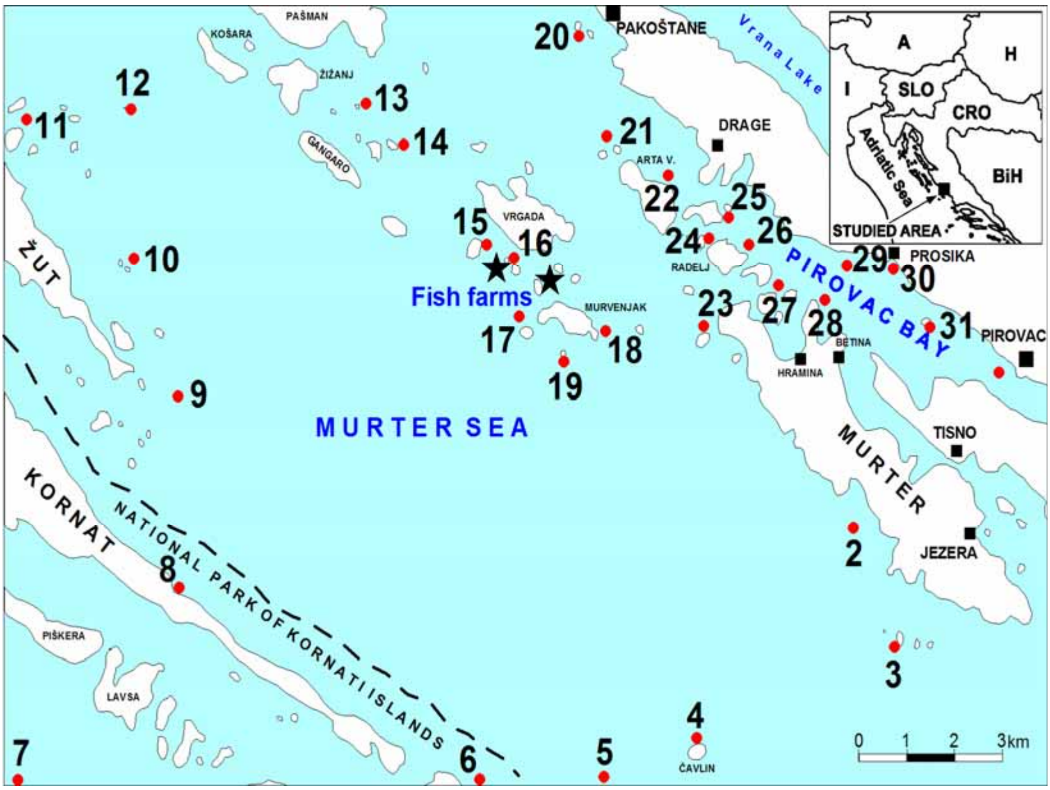

Figure 1. Map of the study area in the Murter Sea and Pirovac Bay (Central Adriatic) showing sites of *Aplisina aerophoba* sampling (★Fish farms)
Slika 1. Geografska karta vzorčnih točk *Aplysine aerophobe* v Murterskem morju in Pirovaškem zalivu - srednji Jadran (★Ribje farme)

The open sea around the Kornati Islands and the islands Žirje and Žut is relatively free from anthropogenic impact, while the coastal part of Murter Sea and Pirovac Bay are exposed to untreated municipal, industrial and to a lesser extent to agricultural pollution from the nearest small cities, such as Pirovac, Tisno, Jezera, Betina and Murter, which increased the amount of nitrogen loading in the particulate organic matter (POM) and the sediments of this region. Marinas in Jezera, Betina and Hramina provide additional inputs of anthropogenic nitrogen from untreated faecal sewage and industrial effluents.

Around Vrgada Island are the fish cages with European sea brass (*Dicentrarchus labrax*), gilt-head sea bream (*Sparus aurata*) and Atlantic blue fin tuna (*Thunnus thynnus*). The annual production is approximately 450 tons of *Dicentrarchus labrax* and *Sparus aurata,* as well as 1000 tons of *Thunnus thynnus*. The amount of food consummed each year is about 900 tons of peletted food for the sea brass and sea bream and about 7200 tons of fresh and frozen fish for the blue fin tuna. Fish-farming activities are the source of N-rich inputs such as fish excreta and uneaten food.

As the reference site we chose Lumbarda reef flat, which is situated in the open sea, 3 km west of the island Sedlo (Figure 1). Results from previous research confirmed that the ecosystems around Lumbarda are not affected by environmental pollution (DOLENEC et al., 2006).

Sample collection

The individuals of *Aplysina aerophoba* were collected by scuba diving from 26 locations at depths of approximately 2-5 m. To obtain the strongest possible $\delta^{15}N$ signal, the sampling period was limited to the peak of the summer tourist season (the last two weeks in August 2005). A strong light intensity, high temperatures and efficacious nutrients ensure that the increased primary production is high in August. As a result of intensive tourist activities, the input of untreated human and industrial sewage is also at its highest during that period. The sampling sites were located in the Murter Sea and the semi-enclosed Pirovac Bay (sampling sites PB) as well as around the Vrgada Island, close to the fish farms (sampling sites FF) and in the coastal parts of the small isolated Islands of Kornati, Žut and Žirje (sampling sites ROFF). The reference site was selected in the Lumbarda reef flat. Between five and eight individuals were sampled at each location.

Sample preparation and stable nitrogen isotope analysis

Fresh *Aplysina aerophoba* samples were placed in plastic bags and refrigerated immediately after collection to await further processing. All the *Aplysina aerophoba* individuals were size and weight matched (basal diameter 1.5 cm; height 10 cm) in order to avoid possible isotope effects caused by ontogenetic dietary shifts (DENIRO and EPSTEIN, 1981; MUSCATINE and KAPLAN, 1994, LOJEN et al., 2005) or differences in age (OWENS, 1987), which could also affect the nitrogen isotopic composition. For the isotope analyses only the topmost 1-cm part of five different individuals of *Aplysina aerophoba* from each sampling site were chosen. The selected parts of all the samples were carefully examined with a binocular microscope for the presence of other sponge-dwelling organisms (small worms and crabs), which were separated from their host. Located in a higher trophic level, worms and crabs have different nitrogen isotopic composition than sponges, and their presence in *Aplysina aerophoba* distorts the original nitrogen isotopic signal. *Aplysina aerophoba* samples thus represent the combined tissues of the topmost 1-cm part of five individuals and the associated micro-organisms.

In the laboratory the sponge samples were first rinsed with deionized water to remove salts and sediment particles and then freeze-dried for a minimum duration of 72 h. Lyophilized samples were crushed and homogenised in an agate mortar to avoid heavy-metal contamination. Powder samples packed in tin capsules were preserved in desiccators at room temperature prior to the isotope analysis. The samples from each location were analysed individually.

The nitrogen isotopic composition of the *Aplysina aerophoba* samples was measured using a Europa 20-20 continuous-flow isotope-ratio mass spectrometer with an ANCA SL preparation module (PZD Europa Ltd., U.K.). The results are expressed in the standard δ^{15}N notations as the relative per mille (‰) difference between the sample and the nitrogen standard (atmospheric nitrogen, $\delta^{15}N = 0$ ‰). The analytical precision (1 standard deviation) of triplicate analyses of IAEA N-1 and N-2 standards was better than ± 0.16 ‰. The precision (1 standard deviation) of the duplicate isotopic analyses of samples was within ± 0.2 ‰.

Data analysis

The differences between the sampling sites from Pirovac Bay and the coastal part of the Murter Sea (PB group) as well as the reference and other offshore-location sampling sites (ROFF group) and fish farms (FF group) were initially tested using an analyses of variance (one-way ANOVA) followed by Tukey´s Honesty Significant Difference (HSD) test to compare the data between the nitrogen isotope composition of the *Aplysina aerophoba* from among the sampling-site groups. The results were accepted as significant when $p < 0.05$.

RESULTS

The results of the δ^{15}N determination in *Aplysina aerophoba* are shown in Table 1 and represent the average nitrogen composition of the topmost 1-cm part of the *Aplysina aerophoba* individuals collected in August 2005. The mean, minimum and maximum δ^{15}N values together with the variance for Pirovac Bay and the coastal part of the Murter Sea (PB group) and the fish farms (FF group), as well as the reference and offshore (ROFF group) samples are shown in Table 2 and Figure 2. The data from Tukey´s HSD test are summarized in Table 3.

The *Aplysina aerophoba* samples can be divided into two statistically different groups: the reference and offshore sites (ROF sampling sites 3-12 and 18, 19) and the Murter Sea and Pirovac Bay sites (PB sampling sites 2 and 20-28) together with the fish-farm sites (FF sampling sites 13-17). The division was made on the basis of the mean δ^{15}N values of the group members (1.38, 3.95 and 4.78 ‰ for the ROFF, PB and FF groups, respectively). Tukey´s HSD test showed a statistically significant difference between the ROFF and PB and/or the FF sites, while no significant difference could be observed between the PB and FF sites (Table 3).

The results shown in Table 1 indicate that the $\delta^{15}N$ values of the *Aplysina aerophoba* tissues varied between – 0.7 and + 5.7 ‰, with a spread of 6.4 ‰ and a mean value of + 2.96 ‰. The first group of *Aplysina aerophoba* samples (ROFF sites) included individuals from the unaffected Lumbarda reference site (**sampling site 7), from the open-sea locations in the Murter Sea (sampling sites 4-6), as well as from the reef flats near the offshore Kornati Islands (sampling site 8) and the Žut Island (sampling sites 8-12). The $\delta^{15}N$ values of this group vary between - 0.7 and + 3.0 ‰, with a mean value of + 1.38 ‰. The second group (PB sampling sites) represents the *Aplysina aerophoba* individuals with slightly elevated $\delta^{15}N$ values in the range of + 3.0 to + 5.1 ‰ and a mean of + 3.95 ‰. These samples were mostly collected from the coastal part of Murter Island and from Pirovac Bay. However, in the inner part, the most impacted part of Pirovac Bay, the marine sponge *Aplysina aerophoba* was not present. We can find *Aplysina aerophoba* only near the inner side of the islands, which separate Pirovac Bay from the Murter Sea. The third group (FF sampling sites) consists of *Aplysina aerophoba* individuals with the highest $\delta^{15}N$ values, which vary from + 4.1 to + 5.7 ‰, while the mean is + 4.78 ‰ (Table 1, Figure 1).

Table 1. δ^{15}N values of *Aplysina aerophoba* collected in the Murter Sea and Pirovac Bay - Central Adriatic in August 2005 (average sample of the topmost 1-cm part of five different individuals)
Tabela 1. Vrednosti δ^{15}N v *Aplysini aerophobi* v avgustu 2005, na območju Murterskega morja in Pirovaškega zaliva - srednji Jadran (vzorec predstavljajo najvišji, 1 cm veliki deli petih različnih prstastih tvorb)

Sample No.	Sampling site	Sample group	δ^{15}N (‰)	σ ±
1	Pirovac (coast)		-	-
2	Murter Island SE	3 PB	+ 3.5	0.1
3	Kukuljari Islands	1 ROFF	+ 2.0	0.1
4	Reef Flat Čavlin	1 ROFF	+ 1.5	0.3
5	Nozdra Island	1 ROFF	+ 0.4	0.2
6	Reef Flat Puh	1 ROFF	+ 0.4	0.1
7*	Reef Flat Lumbarda**	1 ROFF	- 0.7	0.1
8	Bikarijca (coast)	1 ROFF	+ 0.6	0.1
9	Reef Flat Kamenjar	1 ROFF	+ 1.3	0.2
10	Dinariči Islands	1 ROFF	+ 1.9	0.1
11	Gustac Island	1 ROFF	+ 1.5	0.1
12	Reef Flat Galijolica	1 ROFF	+ 3.0	0.2
13	Ošljak Island	2 FF	+ 4.1	0.2
14	R. Kotula Island	2 FF	+ 4.5	0.3
15	Špinata Island***	2 FF	+ 5.5	0.1
16	Rakita Island***	2 FF	+ 5.7	0.1
17	Gira Island	2 FF	+ 4.1	0.2
18	Murvenjak Island	1 ROFF	+ 2.2	0.1
19	Vrtlič Island	1 ROFF	+ 2.4	0.1
20	Žavinac Island	3 PB	+ 3.6	0.2
21	Sestrice Islands	3 PB	+ 3.0	0.1
22	Arta V. Island	3 PB	+ 3.4	0.2
23	Prišnjak V. Island	3 PB	+ 3.5	0.1
24	Prišnjak M. Island	3 PB	+ 3.9	0.2
25	Arta M. Island	3 PB	+ 4.0	0.3
26	Radelj Island	3 PB	+ 4.5	0.2
27	Vinik Island	3 PB	+ 5.0	0.1
28	Cap of Gradina	3 PB	+ 5.1	0.1

reference site **, fish farm***

Table 2. Basic descriptive statistics of nitrogen isotopic composition ($\delta^{15}N$ values) of *Aplysina aerophoba* collected in the Murter Sea and Pirovac Bay - Central Adriatic in August 2005
Tabela 2. Osnovna statistika za izotopsko sestavo dušika ($\delta^{15}N$ vrednosti) v *Aplysini aerophobi* na območju Murterskega morja in Pirovaškega zaliva - srednji Jadran (avgust 2005)

Sample group	$\delta^{15}N$						
	N	**Min**	**Max**	**Median**	**Means**	**S.D.**	**Variance**
1 ROFF	12.00	-0.70	3.00	1.50	1.38	1.04	1.08
2 FF	5.00	4.10	5.70	4.50	4.78	0.77	0.59
3 PB	10.00	3.00	5.10	3.75	3.95	0.70	0.50
All Loc.	27.00	-0.70	5.70	3.40	2.96	1.70	2.89

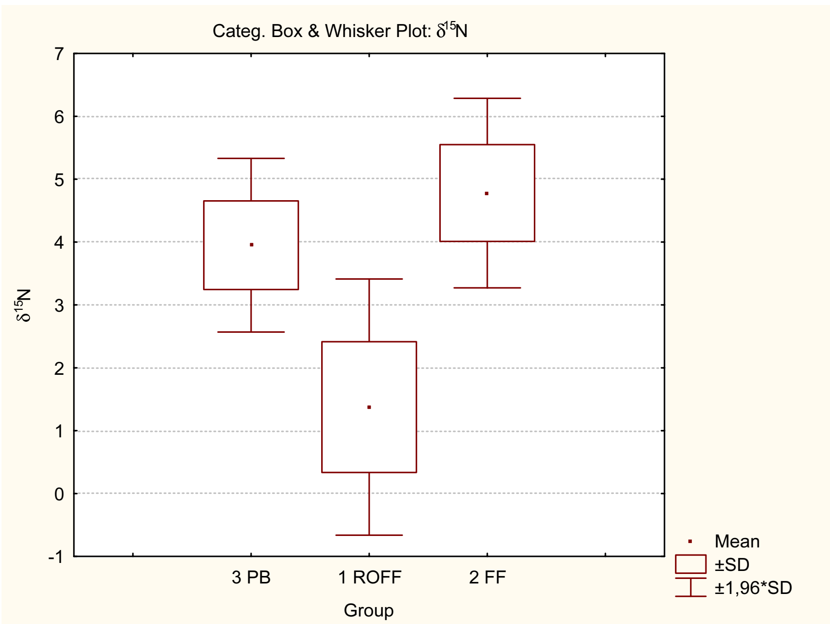

Figure 2. Whisker plots of $\delta^{15}N$ values of *Aplysina aerophoba* (ROFF - reference and offshore locations; FF - fish-farm sampling sites; PB - Murter Sea and Pirovac Bay sampling sites
Slika 2. Whiskerjevi diagrami $\delta^{15}N$ vrednosti v *Aplysini aerophobi* (ROFF – referenčna in oddaljene (od obale) lokacije; FF – vzorčne točke pri ribjih farmah; PB – vzorčne točke v Murterskem morju in Pirovaškem zalivu)

Table 3. Tuckey HSD test; Marked differences (***) of nitrogen isotopic composition (δ^{15}N values) of *Aplysina aerophoba* of ROFF, FF and PB group are significant at $p < 0.05$
Tabela 3. Tuckey-jev HSD test; označene razlike (***) izotopske sestave dušika (δ^{15}N vrednosti) v *Aplysini aerophobi* za skupine ROFF, FF and PB so značilne pri $p < 0.05$

	1 ROFF	**2 FF**	**3 PB**
1 ROFF	-	***	***
2 FF	***	-	-
3 PB	***	-	-

DISCUSSION

Sponges are primitive metazoans. Instead of organs or tissues they possess amoeboid, omnipotent and phagocytotically active cells that move freely through the sponge matrix, termed the mesohyl. Various micro-organisms have evolved to reside in the sponges, including cyanobacteria, diverse heterotrophic bacteria, unicellular algae and zoochlorellae (FREDERICH et al., 2001 and references therein). Three types of associations of micro-organisms with *Aplysina aerophoba* have been described: 1) cosmopolitan bacteria, which most likely serve as a food source, 2) extracellular bacteria, which are probably specific to the sponge mesohyl, and 3) intracellular bacteria, which permanently reside in the nuclei of specific host cells (VACELET, 1975). The presence of the hosted bacteria can amount as much as to 40 % of the total animal biomass (YOUNG-BEON et al., 2003). *Aplysina aerophoba* sponges also contain high concentrations of brominated metabolites (up to 13 % of the dry weight) with antimicrobial activity (SHARMA AND BURKHOLDER, 1967; FAULKNER, 1978), repellent properties against predators (WEISS et al., 1996), and cytostatic activity in human breast-cancer cell lines (KREUTER et al., 1990). All these compounds, which are probably produced by the sponge itself (EBEL et al., 1997) as well as the hosted bacteria, and the food sources influenced the nitrogen isotope composition of the *Aplysina aerophoba.*

Aplysina aerophoba is a filter feeder, utilizing food particles suspended in the water and captured by the coenocytes. These food particles consist essentially of bacteria, other micro-organisms, and particles of organic debris. The feeding strategies of the suspension filter feeders are assumed to be closely coupled to the seasonal input of POM (IKEN et al., 2001). In marine ecosystems impacted by fish-farm activities or untreated human and animal waste of faecal origin, the effluent related δ^{15}N signal is incorporated into the entire food web (MCCLELLAND and VALIELA, 1998; RISK and ERDMAN, 2000; HEIKOOP et al., 2000; BURFORD et al., 2002). Fish-farm loadings and waste-water nutrients derived from septic systems and animal wastes are generally enriched in the heavy nitrogen isotope ^{15}N. This is due to the nitrogen transformation that typically occurs in such waters before or after discharge (HEIKOOP et al., 2000). Consequently, the POM and biota in marine ecosystems impacted by such anthropogenic nitrogen inputs are also enriched in ^{15}N.

The differences in the $\delta^{15}N$ values of the *Aplysina aerophoba* among all three groups of sites (FF, PB and ROFF) are in the range of 6.4 ‰, which is quite a lot when compared to the literature data for a single species raised on an identical diet. Kidd et al. (1995) reported that the variation in the $\delta^{15}N$ values for arctic lake invertebrates was less than 2.0 ‰. Variations in the $\delta^{15}N$ values in individuals of the same species could be related to size and age effect (MINIWAGA and WADA, 1984; WADA et al., 1991), a depth effect (SAINO and HATORY, 1980; MUSCATINE and KAPLAN, 1994), seasonal effects (MARRIOTTI et al., 1980; COSTANZO et al., 2001), and differences due to varying levels of ^{15}N-enriched fish-farm or human-sewage-derived nitrogen (RISK and ERDMAN, 2000; COSTANZO et al., 2001; MAZZOLA and SARÀ, 2001; VIZZINI and MAZZOLA, 2004; SARÀ et al., 2004). As the size, depth and season of collection were strictly controlled by the sampling design, the observed variations in *Aplysina aerophoba* ^{15}N content appears to be primarily explained by a variation in the extent of the elevated ^{15}N-enriched sewage and/ore effluents input into the investigated area.

The isotopic composition of organisms feeding in either clean or polluted environments also depends upon their position in the food chain. The lowest trophic level TL - 1 consists of phytoplankton with $\delta^{15}N$ values of about + 5 ‰. Sponges, anemones, mussels, small polychaetes and copepods represent the TL - 2 trophic level. Their $\delta^{15}N$ values range between + 5 and + 7 ‰. The $\delta^{15}N$ values of the *Verongia aerophoba* tissue analysed during this study were found to be lower than the reported values for trophic level TL - 2. All *Aplysina aerophoba* individuals analyzed during this study also exhibited $\delta^{15}N$ values that are generally lower relative to the nitrogen isotopic composition of POM (a mixture of phytoplankton, detritus, bacteria and small micro-zooplankton) at the base of the food web (DOLENEC et al., 2006; 2007). This is not consistent with previous studies, which assumed a diet-tissue fractionation factor of between 2.8 and 3.8 ‰, with an average of 3.20 ± 0.14 ‰ (PERSIC et al., 2004) and an enrichment of consumers in terms of ^{15}N with increasing food-web position. Such depletion was already reported for some other species of sponge in oligotrophic environements (*Mycale fistulifera* around the Ardag fish farm in Eilat, Red Sea, LOJEN et al., 2005). The depleted signature in *Aplysina aerophoba*, lower than that of POM, most probably indicated the fractionation of nitrogen isotopes during uptake and assimilation by hosted bacteria (DOLENEC et al., 2007). The symbiotic association between *Aplysina aerophoba* and bacteria, which plays an important role in the nutrition of some shallow-water sponges (REISWIG, 1975; ARILLO et al., 1993), thus leads to lower $\delta^{15}N$ values. Those bacteria may be able to metabolize highly refractory material, which can than be assimilated by the sponge, leading to a depletion in terms of the ^{15}N of its tissue.

Although the ^{15}N depletion due to bacterial metabolism considerably lowered the original δ^{15}N food signal in *Aplysina aerophoba*, the observed variations in δ^{15}N values could be primarily explained in terms of elevated ^{15}N-enriched sewage inputs into the investigated area. This is also supported by the data relating to nitrogen isotope composition in *Anemonia sulcata* and *Balanus perforatus* tissue, which exhibited similar differences between (ROFF), (PB) and (FF) group samples (DOLENEC et al., 2006).

The δ^{15}N values of *Aplysina aerophoba* were significantly higher in the coastal part of the Murter Sea and Pirovac Bay (PB group) and around fish farms (FF group) than they were in the less-affected offshore or pristine sites (ROFF group). Similar ^{15}N enrichment was also found in reef molluscs, stomapods, fishes and corals in SE Asia settings exposed to anthropogenic nutrient pollution (RISK and HEIKOOP, 1997; MENDES et al., 1997; HEIKOOP et al., 2000; RISK and ERDMAN, 2000; WEISS et al., 2002). Elevated δ^{15}N values from other world sites have also been measured in marine plants exposed to ground-water contaminated by septic systems (MCCLELLAND et al., 1997) and sewage effluents (GRICE et al., 1996; UDY and DENNISON, 1997; CONSTANZO et al., 2001). Nutrients derived from septic systems are generally enriched in ^{15}N and exhibit δ^{15}N values of + 10 to + 22 ‰ (HEATON, 1986). Most of the sewage-derived heavy nitrogen ^{15}N in the semi-enclosed Pirovac Bay and in the coastal part of the Murter Sea tend to be related to the inadequate septic systems and wastewater treatment in the surrounding villages, tourist centres (hotel facilities) in Pirovac Bay (Murter, Betina, Tisno, Jezera and Pirovac), marinas (Hramina, Betina and Jezera), and seasonally open auto camps. The increase in the human population during the touristic season represents an additional impact of human sewage on the marine coastal ecosystems of Murter Island.

Furthermore, the fish-farm biodeposition products also affect the water column and sediment chemistry, the seagrass, the meiofauna and the benthic bacteria (FINDLAY and WATLING, 1997; HARGRAVE et al., 1997; PERGENT et al., 1999; PEARSON and BLACK, 2000; LA ROSA et al., 2001; MIRTO et al., 2002; ALONGI et al., 2003, SARÀ et al., 2004, DOLENEC et al., 2004, 2005). Faecal waste material, several organic pollutants, algicides and herbicides, disinfectants, antibiotics, inducing agents, probiotics, etc. are enriched in ^{15}N and thus contribute to the elevated δ^{15}N signal in different organisms close to the fish farms (TACON et al., 1995; DOLENEC et al., 2007). The wastage of feed, which is estimated to be of about 20% (ENELL, 1995), also contributed to the increased δ^{15}N signal in the POM and biota in the impacted area. The effects of fish-farm loading depend on the culture method, the feed type, the farm size, the hydrography, the hydrodynamic regime and other environmental features of the area (PILLAY, 1991).

The strongest δ^{15}N signal found in *Aplysina aerophoba* from the coastal part of Murter Island and Pirovac Bay (PB group) suggested the input of untreated domestic and industrial effluents and wastewaters, which were discharged into the coastal ecosystems of the investigated area. The elevated δ^{15}N signal in *Aplysina aerophoba* from the SW coastal part of Murter Island could be related to the current-derived sewage from the cities of

Split and Šibenik as well as from the tourist centres of Vodice and Tribunj. The dispersion of ^{15}N loading generated by aquaculture at Vrgada Island, however, affected the adjacent offshore ecosystems and the $\delta^{15}N$ signal in *Aplysina aerophoba* (FF group) and in other biota (DOLENEC et al., 2006; 2007). A pattern of influence caused by farm waste ^{15}N is a function of the local current speed, the water depth, and the total output from the farms. The last factor is important in determining the real impact on the adjacent environment (IWAMA, 1991).

Aplysina aerophoba from ecosystems located further away from cities, harbours and tourist centres with no appreciable sewage is depleted in ^{15}N. However, the lowest $\delta^{15}N$ values were measured in the more or less pristine offshore reef flats of the Kornati, Žut and Žirje ecosystems with minimal anthropogenic impact (ROFF group). Offshore ecosystems are usually characteristic of highly oligotrophic conditions, where the algal fixation of atmospheric nitrogen is the major source of nitrogen. As a result, the $\delta^{15}N$ signal at the base of the food webs is low and the ^{15}N enrichment of various organisms is also relatively low.

The results of this study further indicated that the enrichment in *Aplysina aerophoba* ^{15}N content decreased with the distance from the coast towards the open-sea ecosystems as well as away from the fish cages. Such onshore-to-offshore $\delta^{15}N$ variations most probably indicate that the sewage-induced ^{15}N-enrichment signal is rapidly attenuated with the distance from the pollution sources. Similar inshore-offshore $\delta^{15}N$ variations were also observed in stomapods from Sulawesi (RISK and ERDMAN, 2000) and corals from Indonesia, Zanzibar and the Maldives (HEIKOOP et al., 2000; RISK and ERDMAN, 2000).

CONCLUSIONS

The results presented here indicate that an inadequate municipal infrastructure and aquaculture activities are the principal sources of ^{15}N loading in the investigated area. The ^{15}N-enriched nitrogen in effluents from the septic systems of Murter Island and the coastal part of Pirovac Bay, which discharge into the adjacent coastal ecosystems, were found to be incorporated into the *Aplysina aerophoba* living in the shallow water of the Murter Sea and Pirovac Bay. Similarly, the fish-farm-derived ^{15}N-enriched waste could also be traced in *Aplysina aerophoba* sampled around the fish farms of the Vrgada and adjacent islands. By using the $\delta^{15}N$ signature of *Aplysina aerophoba* biologically available and therefore ecologically significant, nitrogen can be detected and used to support the monitoring of the human-sewage impact and/or the influence of fish-farm activities in different marine environments. The $\delta^{15}N$ signature of *Aplysina aerophoba* and of other biota as well as in POM could also be useful in planning the wastewater management in the region.

POVZETKI

^{15}N v *Aplysini aerophobi* kot sledilo antropogenega dušika v Murterskem morju in Pirovaškem zalivu (srednji Jadran)

Neprimerna komunalna infrastruktura in aktivnosti v ribjih farmah veljata na podlagi predstavljenih rezultatov za glavna vira obremenitve preiskovanega območja z ^{15}N. Odplake iz septičnih sistemov otoka Murter in priobalnih delov Pirovaškega zaliva se izlivajo v bližnje obalne ekosisteme in vsebujejo povišane vrednosti ^{15}N, kateri se posledično vgrajuje v spužvo *Aplysino aerophobo*, ki živi v plitkih vodah Murterskega morja in Pirovaškega zaliva. Odpadki ribjih farm so obogateni s povišanimi vrednostmi ^{15}N, katere prav tako sledimo v *Aplysini aerophobi*, ki je bila povzorčena okoli ribjih farm v bližini otoka Vrgade in sosednjih otokov. Meritve izotopske sestave dušika v *Aplysini aerophobi* so se izkazale kot dober indikator onesnaženosti različnih morskih ekosistemov s komunalnimi in drugimi organskimi odplakami ter kot odličen pokazatelj obremenitve okolja zaradi marikulturne dejavnosti. Vrednosti parametra. δ^{15}N v *Aplysini aerophobi*, drugi bioti in partikulatni organski snovi (POM) (geokemične karte) lahko uporabimo za pomoč pri novih okoljskih načrtih in konstrukcijah odpadnih sistemov v regiji.

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