

# $\delta^{15}\text{N}$ of particulate organic matter and *Anemonia sulcata* as a tracer of sewage effluent transport in the marine coastal ecosystem of Pirovac Bay and the Murter Sea (Central Adriatic)

## $\delta^{15}\text{N}$ v partikulatni organski snovi in *Anemoni sulcati* kot sledilo transporta odpadnih voda v priobalnem delu Pirovaškega zaliva in Murterskega morja (srednji Jadran)

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**Abstract:** The present study shows that the stable isotopic composition of nitrogen in particulate organic matter (POM) and in selected marine organisms, such as the sea anemone *Anemonia sulcata*, may be excellent parameters for detecting and monitoring human sewage inputs into the marine coastal ecosystems of Pirovac Bay and the Murter Sea. The  $\delta^{15}\text{N}$  values of POM and *A. sulcata* tissue were significantly higher at sites in the semi-enclosed Pirovac Bay and in the coastal part of the Murter Sea (Central Adriatic) affected by sewage, compared to the off-shore reef flats of the Murter Sea, the coastal parts of the Kornati Islands and at a pristine reference location at the Lumbarda Reef Flat.  $^{15}\text{N}$  enrichment was as high as 7.0 ‰ in POM and 7.7 ‰ in *A. sulcata* tissue and is significantly larger than the natural  $\delta^{15}\text{N}$  variability of the same species at unaffected locations. Geochemical maps of  $\delta^{15}\text{N}$  values were created, which could be useful in monitoring the influence of human sewage impacts in marine coastal ecosystems. Maps of sewage nitrogen distribution could also be used for planning municipal and industrial waste management in the region and to assist in monitoring the efficiency of environmental protection measures after the construction of a new wastewater treatment plant in Murter.

**Izvilleček:** Rezultati pričujoče raziskave kažejo, da je izotopska sestava dušika v partikulatni organski snovi (POM) in v nekaterih morskih organizmih, kot je naprimer anemona *Anemonia sulcata* odličen parameter za sledenje in monitoring komunalnih in drugih odpadkov v morskih priobalnih ekosistemih Pirovaškega zaliva in Murterskega morja (srednji Jadran). Vrednosti  $\delta^{15}\text{N}$  v partikulatni organski snovi in v tkivu *A. sulcata* na območju Pirovaškega zaliva in v priobalju Murterskega morja, ki sta onesnažena predvsem z odpadki iz septičnih sistemov so bistveno večje v primerjavi z referenčno neonesnaženo lokacijo - plitvino Lumbarda na odprtem delu Kornatskega arhipelaga. Partikulatna organska snov iz onesnaženih območij vsebuje za 7,0 ‰, tkiva *A. sulcata* pa za 7,7 ‰ večji  $\delta^{15}\text{N}$  v primerjavi s POM in tkivom anemon iz referenčne, neonesnažene lokacije. Omenjena obogatitev je znatno večja v primerjavi z variabilnostjo  $\delta^{15}\text{N}$  v primerkih iste vrste na neonesnaženih lokacijah. Na podlagi dobljenih podatkov so bile pripravljene tudi geokemične karte porazdelitve parametra  $\delta^{15}\text{N}$ , ki so uporabne za monitoring vpliva odpadnih voda iz septičnih sistemov na priobalne ekosisteme na tem območju. Omenjene geokemične karte bodo koristile tudi pri načrtovanju izpusta nove čistilne naprave na otoku Murter in v kasnejši fazi tudi za ugotavljanje njene efikasnosti, glede na zmanjšanje onesnaženja obalnih ekosistemov na tem območju z odpadnimi vodami.

**Key words:** sewage, pollution monitoring, nitrogen, stable isotope, particulate organic matter, *Anemonia sulcata*, Pirovac Bay, Murter Sea- Central Adriatic

**Ključne besede:** odpadne vode, monitoring onesnaženosti, dušik, stabilni izotop, partikulatna organska snov, *Anemonia sulcata*, Pirovaški zaliv, Murtersko morje, srednji Jadran

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## INTRODUCTION

Increased nitrogen loading in particulate organic matter (POM) and sediments is commonly related to the eutrophication of coastal seas worldwide. A significant component of marine eutrophication in many near-shore environments can be attributed to inputs of anthropogenic nitrogen from untreated domestic sewage and municipal and industrial effluents (LEE AND OLSEN, 1985; NIXON ET AL., 1986; BACHTIAR ET AL., 1996; COSTANZO ET AL., 2001). Managing the effects of sewage entering marine ecosystems has become one of the major environmental challenges of today. Septic systems for household waste disposal and the tourist infrastructure (hotels, camps, marinas) are the predominant point sources of

contaminants in the semi-enclosed Pirovac Bay and in the coastal part of the Murter Sea, Dalmatia. The expansion of the human population during the tourist season not serviced by an adequate municipal infrastructure represents an additional impact of human sewage on the marine coastal ecosystems of Murter Island, creating an increasing demand for on-site sewage treatment system on Murter Island.

The results presented are a part of a comprehensive study employing different tools such as geochemistry, hydrology, microbiology, etc., to investigate the impact of sewage effluents and their possible transport pattern in the investigated area of Pirovac Bay and the Murter Sea. The distribution of sewage effluents in marine

ecosystems can be mapped using various parameters, such as salinity, nutrient concentrations, bacteria, organic matter composition, radioisotopic tracers, dye fluorescence, water current measurements and nitrogen isotope composition in water and sediments (SWEENEY ET AL., 1980; LINDAU ET AL., 1989; SMITH-EVANS AND DAWES, 1996). The nitrogen stable isotopic composition of marine plants (COSTANZO ET AL., 2001; DOLENEC ET AL., 2005) and higher organisms (MOORE ET AL., 1996; HANSSON ET AL., 1997; TUCKER ET AL., 1999; COSTANZO ET AL., 2001) or POM (HEIKOOP ET AL., 2000) has also been used as a tracer of anthropogenic pollution. Here we present an approach that shows the extent and fate of bio-available sewage nitrogen in coastal marine ecosystems, also enabling identification of the location of the pollution source. It is based on the assumption that (1) the nitrogen isotope composition ( $\delta^{15}\text{N}$ ) of POM (representing a mixture of phytoplankton, bacteria, micro-zooplankton and detritus) is affected by sewage-derived nitrogen enriched in  $^{15}\text{N}$ , and (2) tissues of organisms of the same species or groups of organisms with a similar position in the food web, in our case *A. sulcata* individuals, reflect the  $\delta^{15}\text{N}$  of their N source (WADA ET AL., 1991).

Stable nitrogen isotopes have been widely used to trace dissolved and particulate nutrients derived from animal wastes, septic systems and waste water treatment plants, as they physically and biologically move through ecosystems (RISK AND ERDMANN, 2000; HEIKOOP ET AL., 2000). Benthic and benthic feeding animals, as well as other organisms from sewage-impacted areas have shown  $\delta^{15}\text{N}$  values distinct from those collected at unaffected reference sites (VAN DOVER ET AL., 1992; SPIES ET AL., 1989; MOORE

ET AL., 1996; HANSSON ET AL., 1997; TUCKER ET AL., 1999; COSTANZO ET AL., 2001). Stable isotopes of nitrogen can thus be used to distinguish between natural and anthropogenic nitrogen sources in the environment or ecosystems (MARIOTTI ET AL., 1984; TUCKER ET AL., 1999; SIGLEO AND MACKO, 2002). For illustration,  $\delta^{15}\text{N}$  values of nitrate of commercial fertiliser typically range from  $-2.5$  to  $+2.0$  ‰, organic soil nitrate ranges from  $-2$  to  $+9$  ‰, and human and animal wastes range from  $+10$  to  $+22$  ‰ (KREITLER AND JONES, 1975; KREITLER AND BROWNING, 1983; HEATON, 1986; BARRETT ET AL., 1999). Generally,  $\delta^{15}\text{N}$  values of  $\text{NO}_3 > +10$  ‰ are regarded as being indicative of the presence of faecal N (BARRETT ET AL., 1999). Treated sewage shows  $\delta^{15}\text{N}$  values around  $+10$  ‰ (HEATON, 1986). Marine POM in pristine oligotrophic environments exhibits  $\delta^{15}\text{N}$  values distinctly lower than that collected in areas impacted by fin-fish aquaculture (SARA ET AL., 2004). Increased  $\delta^{15}\text{N}$  values of about  $+8$  ‰ were measured in POM near the inflows from septic systems in the port of Murter (DOLENEC ET AL., 2005); similar values were reported for POM dominated by untreated faecal matter of Jepara Bay ( $+7.9$  ‰, HEIKOOP ET AL., 2000).

Stable nitrogen isotopes are also useful in tracing organic matter through food webs. Animals raised on diets with a known nitrogen composition preferentially incorporated  $^{15}\text{N}$  rather than  $^{14}\text{N}$ , producing proteins enriched in  $^{15}\text{N}$  relative to the food (MINAGAWA AND WADA, 1984; DENIRO AND EPSTEIN, 1981). In the trophic network among animals,  $\delta^{15}\text{N}$  values of their tissues systematically increase by  $1.3$  to  $5.3$  ‰ per trophic level (MINAGAWA AND WADA 1984; WADA ET AL. 1991, 1993; LAJTHA AND MICHENER 1994).

This study was designed with the following aims:

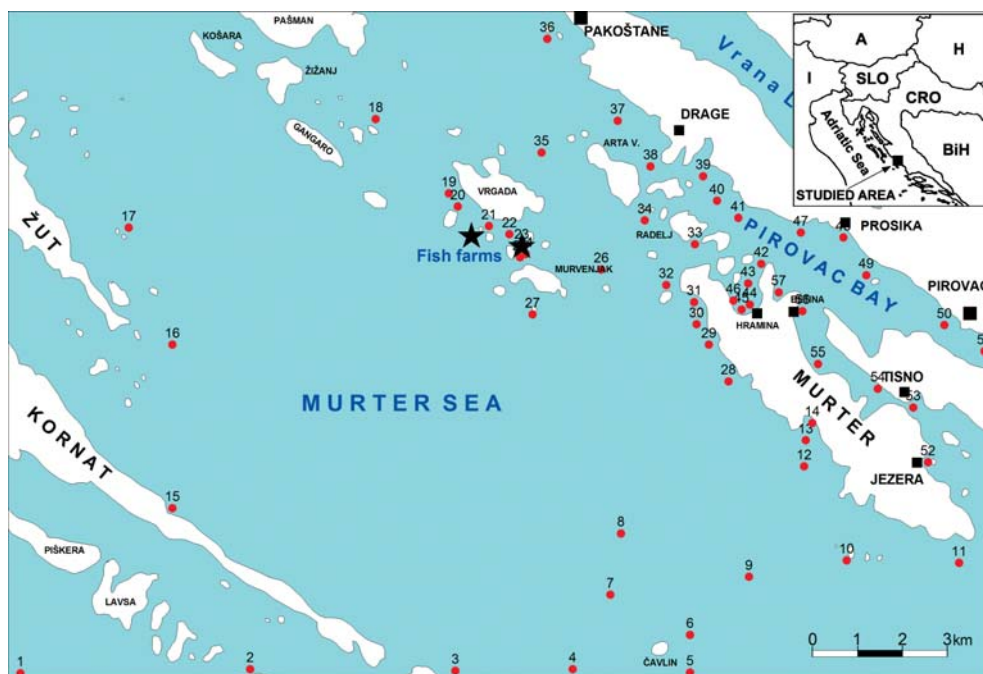
1) to test the hypothesis that the  $^{15}\text{N}$  content of POM and *A. sulcata* tissue collected near-shore and off-shore along the coastal part of the semi-enclosed Pirovac Bay and Murter Sea (Central Adriatic) is a reliable indicator of anthropogenic nitrogen impact arising from mostly untreated domestic and industrial wastes discharged into the coastal marine environment;

2) to create maps of  $\delta^{15}\text{N}$  values, which would enable determination of the geographical extent of anthropogenic impact on the adjacent area of the Murter Sea arising from transport by currents.

## MATERIALS AND METHODS

POM considered as a potential food source for *A. sulcata* was sampled at 1m depth at 57 localities of the Murter Sea and semi-enclosed Pirovac Bay in August 2005 (Fig. 1). 5 l samples were filtered through glass fibre filters (GF/F, Whatman).

Individuals of *A. sulcata* were collected by scuba diving from the sea at depths of approximately 2 - 5 m at 31 localities in the same area (Fig. 2). All sampled individuals were size-matched (basal diameter 3 - 4 cm; tentacles extending 10 - 15 cm) and weighed (fresh weight: 40 - 50 g, dry weight after freeze-drying 8 - 10 g) to avoid possible isotope effects caused by ontogenetic dietary



**Figure 1.** Map of the study area in the Murter Sea and Pirovac Bay (Central Adriatic) showing sites of POM sampling

**Slika 1.** Geografska karta vzorčnih točk partikulatne organske snovi (POM) v Murterskem morju in Pirovaškem zalivu (srednji Jadran)

shifts (DENIRO AND EPSTEIN, 1981; MUSCATINE AND KAPLAN, 1994) or age (OWENS, 1987). Only pale green- coloured individuals with purple tentacle tips were chosen. To avoid the small seasonal differences in  $\delta^{15}\text{N}$  values of anemone tissue observed during a preliminary study (DOLENEC AND VOKAL, UNPUBLISHED), we limited our analyses in this study to *A. sulcata* individuals collected during August 2002 (DOLENEC ET AL., 2005). Fresh *A. sulcata* samples were placed in plastic bags and stored at  $-20^\circ\text{C}$  till further processing. Each sample was weighed prior to use in subsequent experiments. Samples of POM and *A. sulcata* were freeze-dried for  $>72$  h and stored in a dessicator at room

temperature. *A. sulcata* samples were crushed and homogenised by grinding in an agate mortar prior to analyses.

Nitrogen isotope composition of all samples was measured using a Europa 20-20 mass spectrometer with an ANCA SL preparation module (PDZ EUROPA LTD., U.K.). The results were expressed in the standard  $\delta^{15}\text{N}$  notation in permil (‰) relative to atmospheric nitrogen. The analytical precision (1 standard deviation) of triplicate analyses of IAEA N-1 and N-2 standards was better than  $\pm 0.16$  ‰. Precision (1 standard deviation) of duplicate isotope analyses of samples was within  $\pm 0.2$  ‰.



**Figure 2.** Map of the study area in the Murter Sea and Pirovac Bay (Central Adriatic) showing sites of *Anemonia sulcata* sampling

**Slika 2.** Geografska karta vzorčnih točk *Anemonie sulcate* v Murterskem morju in Pirovaškem zalivu (srednji Jadran)

## RESULTS

The results of  $\delta^{15}\text{N}$  determination in POM are listed in Table 1, while Table 2 shows the nitrogen isotope composition of *A. sulcata* (whole single animal).  $\delta^{15}\text{N}$  of POM ranged from + 2.7 to + 9.7 ‰. Similar  $\delta^{15}\text{N}$

values in the range from + 4.0 to + 11.9 ‰ were measured in *A. sulcata*.

The regional distribution pattern of POM  $\delta^{15}\text{N}$  values in August 2005 is shown on Fig. 3, while the regional distribution pattern of  $\delta^{15}\text{N}$  values of *Anemonia sulcata* individuals collected in the year 2002 is presented in Fig. 4.

**Table 1.**  $\delta^{15}\text{N}$  values of particulate organic matter (POM) collected in the Murter Sea and Pirovac Bay - Central Adriatic in August 2005 (\* reference site)

**Tabela 1.** Vrednosti  $\delta^{15}\text{N}$  v partikulatni organski snovi (POM) v avgustu 2005 na območju Murterskega morja in Pirovaškega zaliva - srednji Jadran (\* referenčna lokacija)

Sample No.	Sampling site	$\delta^{15}\text{N}$ (‰)	Sample No.	Sampling site	$\delta^{15}\text{N}$ (‰)
1	Reef Flat Lumbarda*	2.7	30	Podvrške - Bakarela	6.8
2	Sedlo Island	2.8	31	Podvrške - Port	4.9
3	Reef Flat Bačvica	3.0	32	Prišnjak V. Island	4.0
4	Samograd Island	3.6	33	Radelj Island	5.1
5	Reef Flat Kablinac	3.6	34	Prišnjak M. Island	4.3
6	Čavlin Island	3.8	35	Vrgada / Arta	4.6
7	Reef Flat Čavlin	3.5	36	Žavinac Island	4.6
8	Murter Sea I	3.8	37	Drage	4.6
9	Murter Sea II	4.2	38	Reef Flat Kušija	6.4
10	Kukuljari Island	4.0	39	Reef Flat Arta	5.1
11	Cap of Murter S	3.9	40	Pirovac Bay I	7.2
12	Tužbina Island	4.4	41	Pirovac Bay II	8
13	Kosirina Bay I	4.5	42	Cap of Gradina	7.2
14	Kosirina Bay II	5.7	43	Port of Murter	5.9
15	Bikarijca (coast)	2.8	44	Hramina (Marina)	7.5
16	Reef Flat Kamenjar	3.0	45	Luke	8.5
17	Dinariči Islands	3.5	46	Luke / Vinici	7.3
18	Runjava Kotula	-	47	Reef Flat Splitski	6.8
19	Špinata Island	4.5	48	Prosika (coast)	8.8
20	Obrovanj Island	4.6	49	Sustipanac Island	9.7
21	Reef Flat Kamičić	4.5	50	Port of Pirovac	6.2
22	Fish farms I	5.1	51	Makirina Bay	5.1
23	Fish farms II	4.5	52	Port of Jezera	6.3
24	Fish farms III	3.4	53	Tisno I	4.8
25	Fish farms IV	5.5	54	Tisno II	5.4
26	Visovac Island	4.5	55	Plitka Vala	5.4
27	Vrtlič Island	3.6	56	Port of Betina	5.0
28	Pod Raduč	4.8	57	Betina (Marina)	6.1
29	Slanica Bay	4.9			



**Table 2.**  $\delta^{15}\text{N}$  values of *Anemonia sulcata* individuals (whole animal; Dolenc et al., 2005) collected in the Murter Sea and Pirovac Bay - Central Adriatic in August 2002 (\* reference site)**Tabela 2.** Vrednosti  $\delta^{15}\text{N}$  v *Anemonii sulcati* v avgustu 2002 (celotni primerek; Dolenc et al., 2005) na območju Murterskega morja in Pirovaškega zaliva - srednji Jadran (\* referenčna lokacija)

Sample No.	Sampling site	$\delta^{15}\text{N}$ (‰)	Sample No.	Sampling site	$\delta^{15}\text{N}$ (‰)
1	Pirovac (coast)	11.9	17	Gira Island	6.1
2	Murter Island SE	6.4	18	Murvenjak Island	5.8
3	Kukuljari Islands	6.1	19	Vrtlič Island	5.4
4	Reef Flat Čavlin	5.8	20	Žavinac Island	7.3
5	Nozdra Island	5.0	21	Sestrice Islands	7.1
6	Reef Flat Puh	4.7	22	Arta V. Island	7.1
7	Reef Flat Lumbarda*	4.2	23	Prišnjak V. Island	6.3
8	Bikarijca (coast)	5.1	24	Prišnjak M. Island	7.8
9	Reef Flat Kamenjar	5.4	25	Arta M. Island	7.6
10	Dinariči Islands	5.6	26	Radelj Island	7.3
11	Gustac Island	5.1	27	Vinik Island	9.8
12	Reef Flat Galijolica	5.8	28	Cap of Gradina	9.5
13	Ošljak Island	5.2	29	Reef Flat Splitski	10.1
14	R. Kotula Island	5.3	30	Prosika (coast)	10.8
15	Špinata Island	6.7	31	Sustipanac Island	11.8
16	Rakita Island	6.3			

From Tables 1 and 2, as well as from Fig. 3 and 4, it is evident that:

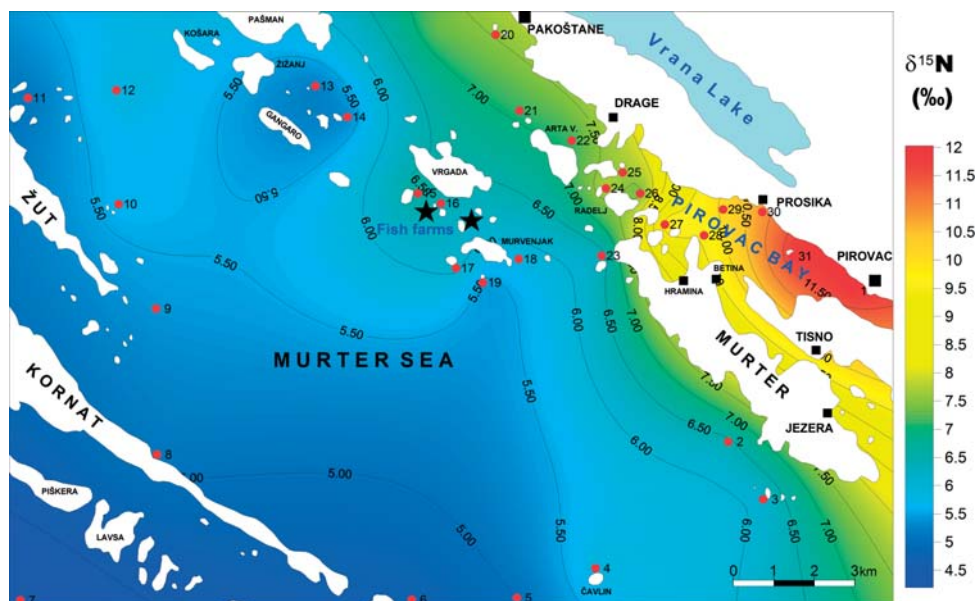
- 1) The variations in  $^{15}\text{N}$  content of both POM and of *A. sulcata* appear to be influenced primarily by the environment in which they were collected.
- 2)  $\delta^{15}\text{N}$  values of POM and *A. sulcata* tissue were significantly higher at the anthropogenically affected sites in the semi-enclosed Pirovac Bay, along the coast of the Murter Sea and the rocky shores of islands close to Murter Island, compared to POM and *A. sulcata* from an unaffected reference site on the shallow Lumbarda reef flat in the open sea (approx. 3 m deep) and those from small uninhabited offshore islands (DOLENC ET AL., 2005).

- 3)  $\delta^{15}\text{N}$  values of POM showed significant differences between  $^{15}\text{N}$  depleted sampling sites (1-8, 15-17 and 27) from the reference location (Reef Flat of Lumbarda) as well as the relatively unaffected offshore part of the Murter Sea and the sampling sites (12-14 and 28-57) from Pirovac Bay and the coastal part of the Murter Island affected due to dissolved faecal organic matter. Samples of POM from the reference site (1) and offshore locations (2-8, 15-17 and 27) had  $\delta^{15}\text{N}$  values in the range between + 2.7 and + 3.3 ‰, while those dominated by untreated faecal matter (12-14 and 28-57) typically had values within the range from + 4.6 to + 9.7 ‰. The  $\delta^{15}\text{N}$  values of POM

collected in the area around the fish farms at the Vrgada Island (sampling sites 19-26) have been determined to vary from + 4.5 to 5.1 ‰. A gradient towards lower  $\delta^{15}\text{N}$  values was observed from the impacted sites around fish cages toward locations further away, as also observed in previous studies (DOLENEC ET AL., 2005).

- 4) *Anemonia sulcata* individuals living on the offshore reef flats or on rocky shores of small isolated islands of the Murter Sea, as well as along the coastal part of the Kornati Islands and small uninhabited islands around the Island of Žut (sample sites 4 to 12) had consistently lower  $\delta^{15}\text{N}$  values (average: + 5.2 ‰; range: + 4.2 to + 5.8 ‰) than individuals living on rocky surfaces of the coastal parts of the islands closer to the coast (sample sites 13 to 19). These lat-

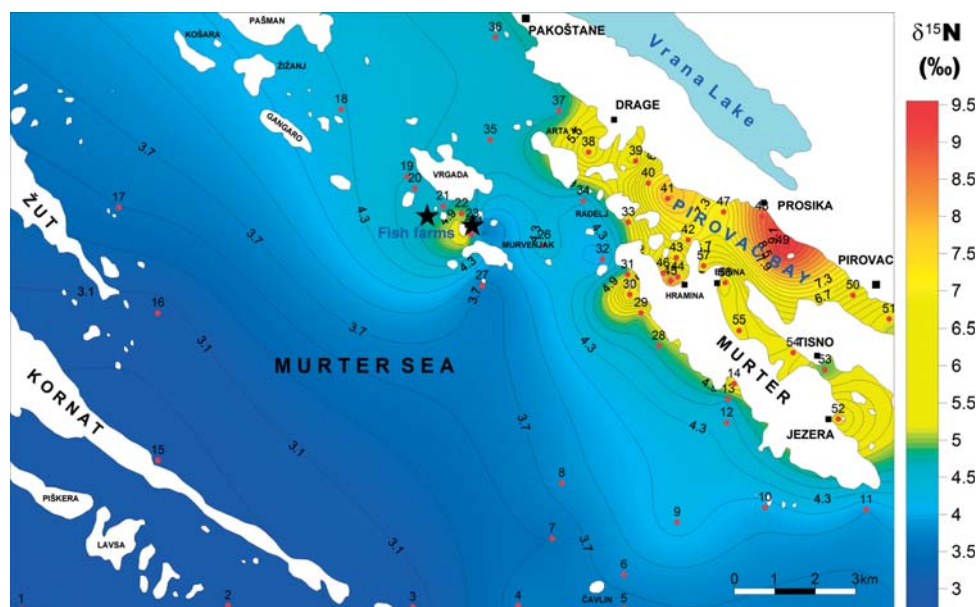
ter had  $\delta^{15}\text{N}$  values with an average of + 5.8 ‰, ranging from + 5.2 to + 6.7 ‰ (Table 2). Slightly enriched  $\delta^{15}\text{N}$  values (up to 2.5 ‰ relative to the reference site) in this region were found in anemones from small islands around fish farms (sample points 15, 16 and 17). Considerably higher  $\delta^{15}\text{N}$  values (average + 6.9 ‰; range + 6.1 to + 7.8 ‰) were measured along the islands separating Pirovac Bay from the Murter Sea (sampling sites 21, 22, 24 and 25), as well as along the coastal part of Murter Island (sampling sites 2 and 23) and the Islands of Kukuljari (sampling site 3). However, the highest  $\delta^{15}\text{N}$  values (+7.3 to +11.9 ‰, averaging +10.2 ‰) were found in *A. sulcata* tissues from the inner part of Pirovac Bay (sampling sites 1 and 26-31), (DOLENEC ET AL., 2005).



**Figure 3.** Spatial distribution pattern of  $\delta^{15}\text{N}$  values of POM throughout the Murter Sea and Pirovac Bay (Central Adriatic)

**Slika 3.** Prostorska porazdelitev vrednosti  $\delta^{15}\text{N}$  v partikulatni organske snovi (POM) v Murterskem morju in Pirovaškem zalivu (srednji Jadran)





**Figure 4.** Spatial distribution pattern of  $\delta^{15}\text{N}$  values of *Anemonia sulcata* throughout Pirovac Bay and the Murter Sea - Central Adriatic (Dolenec et al., 2005)

**Slika 4.** Prostorska porazdelitev vrednosti  $\delta^{15}\text{N}$  v *Anemonii sulcati* v Murterskem morju in Pirovaškem zalivu - srednji Jadran (Dolenec et al., 2005)

## DISCUSSION

The  $^{15}\text{N}$  enrichment of POM and anemones from the semi-enclosed Pirovac Bay, coastal parts of Murter Island, as well as from the inshore islands that separate Pirovac Bay from the Murter Sea, indicates that their primary food source is affected by heavy nitrogen due to local inputs of untreated sewage effluents into the coastal marine environment. Since sewage-derived wastewater DIN (dissolved inorganic nitrogen) is typically enriched in  $^{15}\text{N}$  and exhibits  $\delta^{15}\text{N}$  values mostly in the range between + 10 and + 22 ‰ (HEATON, 1986), this source term may also be responsible for the  $^{15}\text{N}$  enrichment in phytoplankton. Such enrichment in  $^{15}\text{N}$  due to ground water DIN was found in both primary producers and consumers in estuarine settings of Cape Cod,

Massachusetts (McCLELLAND ET AL., 1997; McCLELLAND AND VALIELA, 1998).  $^{15}\text{N}$  enrichment has also been found in reef molluscs, stomatopods, fishes and corals in 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). Zooplankton and reef particulate organic matter may have higher  $\delta^{15}\text{N}$  values at sewage-polluted sites if  $^{15}\text{N}$ -enriched wastewater is utilised by phytoplankton at the base of the food chain (HANSSON ET AL., 1997). Elevated  $\delta^{15}\text{N}$  values 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; COSTANZO ET AL., 2001).

The spatial distributions of the sewage source indicators such as the  $\delta^{15}\text{N}$  signal in POM and *A. sulcata* tissues allow us to draw conclusions on the impact pattern of sewage effluents and their transport in the coastal part of the Murter Island and Pirovac Bay. In line with the  $\delta^{15}\text{N}$  values of *A. sulcata*, a similar spatial distribution of  $\delta^{15}\text{N}$  was found also in other marine organisms such as *Aplysina aerophoba*, *Balanus perforatus*, *Mytilus galloprovincialis*, *Arca noae*, *Ostrea edulis* and marine plants (*Posidonia oceanica*) from the same sampling sites collected during the years 2004 and 2005 (DOLENEC ET AL., IN PREPARATION). A small difference in the sewage plumes delineated by  $\delta^{15}\text{N}$  values of POM and *A. sulcata* was observed, but the overall trends are the same. POM shows the clearest plume resolution, extending up to several km from the major sources. This undoubtedly suggests that the isotope techniques used in this study are useful not only for showing the actual uptake and assimilation of sewage nutrients by marine organisms and plants, but also in tracing sewage effluent transport in the sampling area under consideration.

From Figures 3 and 4 it is evident that the enrichment decreases with distance from the coast toward open sea ecosystems. Such on-shore to offshore  $\delta^{15}\text{N}$  variations most probably indicate that the sewage-induced  $^{15}\text{N}$  enrichment signal is rapidly attenuated with distance from the sewage sources (within some kilometres for sewage from the inhabited areas of Pirovac Bay and Murter Island). Similar inshore-offshore  $\delta^{15}\text{N}$  variations have also been observed in stomatopods from southwest Sulawesi (Risk and Erdman, 2000) and corals from Indonesia, Zanzibar and the Maldives (HEIKOOP ET AL., 2000; RISK AND ERDMAN, 2000).

The most important characteristic of the Adriatic Sea is the general counter-clockwise water circulation pattern, which is reflected in the spatial distribution of  $\delta^{15}\text{N}$  values of POM and sea anemones (Fig. 2). The strongest  $\delta^{15}\text{N}$  signal is typical of polluted coastal ecosystems, especially those of the semi-enclosed Pirovac Bay. Here  $^{15}\text{N}$  enrichment undoubtedly resulted from wastewater nutrients derived mostly from septic systems in the surrounding villages, tourist centres in Pirovac Bay (Murter, Betina, Tisno, Jezera and Pirovac), marinas (Hramina, Betina and Jezera), and seasonally open auto camps. The  $^{15}\text{N}$  sewage signal of Pirovac Bay was evident up to 6 km NW of the Bay, though it decreases with distance from the shore. It is supposed that mixing of sewage affected seawater from Pirovac Bay with less polluted south-east to north-west sea currents may have diluted the signal from the Bay.

The elevated  $\delta^{15}\text{N}$  signal in the SW coastal part of Murter Island also suggests that pollutants may reach the coastal ecosystems of the Murter Sea from local septic systems in the settlement of Podraduč, the Colentum hotel, as well as by prevalently current-derived mass transport from the south-east where such pollution sources are located (i. e. the cities of Split and Šibenik releasing urban and industrial waste, and the tourist centres of Vodice and Tribunj). It is noteworthy that a similar regional distribution pattern influenced by the counter-clockwise system of the Adriatic Sea currents, showing an onshore to offshore attenuation, was also observed in heavy metal concentrations in the Central Adriatic (DOLENEC ET AL., 1998).

The net transport pattern inferred from the previous isotope tracer indicators such as the  $\delta^{15}\text{N}$  signal in POM (Fig. 3) seem to be compatible with models of two dimensional circulation of a buoyant effluent plume, under the influence of the prevailing tidal and wind-driven current regime. The overall transport direction of sewage-derived effluents with a predominant component of faecal matter is shown to be toward the NW, more or less parallel to the shore. The secondary trend is directed toward the west and southwest and could be explained by wind driven circulation of the surface water and local tidal currents. Preliminary observations during summer 2005 indicated that during SW-NE and NW-SE winds (bura, tramontana), south-westward and north-eastward currents are developed in the coastal area of Pirovac Bay and the Murter Sea, which are capable of redistribution of sewage effluents enriched by  $^{15}\text{N}$  in the above mentioned prevalent directions.

## CONCLUSIONS

Stable nitrogen isotopes enabled us to identify a sewage signal in POM and *A. sulcata* in Pirovac Bay and the Murter Sea and suggested a higher fraction of faecal sewage-derived N in the whole food web in coastal ecosystems. The results of this study further indicate that *A. sulcata* from the most polluted sites are most probably consuming food with a significant sewage component. This is also confirmed by  $^{15}\text{N}$  enrichment in

POM from more polluted sampling sites of Pirovac Bay and the Murter Sea, which represent the base of the food web. The observed variations in POM and *A. sulcata*  $\delta^{15}\text{N}$  values appear to be primarily explained by variation in the extent of domestic and industrial wastes, which have been discharged into the coastal ecosystems of the investigated area. By using  $\delta^{15}\text{N}$  values of POM and *A. sulcata* the anthropogenic nitrogen inputs in many other marine coastal ecosystems could be also easily detected and mapped.

This technique also provides some insight into sewage effluent plume transport, which can be effectively traced by analyses of the spatial distribution of  $\delta^{15}\text{N}$  values of POM. The distribution-transport patterns identified by this study provide a useful preliminary survey of the possible transport/deposition cycles involving domestic and other sewage wastes associated with the proposed construction of a new sewage treatment plant (STP) in Murter.

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