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Use of stable nitrogen isotope signatures of anthropogenic organic matter in the coastal environment: A case study of the Kosirina Bay (Murter Island, Croatia)

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ABSTRACT

Stable nitrogen isotope ratios of particulate matter POM, zooplankton and selected biota such as *Mytilus galloprovincialis* were used to assess the impact of anthropogenically derived organic matter from untreated domestic sewage, municipal and industrial effluents on the coastal ecosystem of Kosirina Bay (Murter Island). The differences in $\delta^{15}N$ values observed in POM and organisms collected in Kosirina Bay were compared to POM and biota sampled at unaffected sites from the southern part of Kornati Island and highly impacted Pirovac Bay. This revealed only very minor effects of anthropogenic inputs of nutrients and organic matter, most probably derived from a sewage outfall south of Tužbina Island.

Keywords: particulate organic matter (POM), zooplankton, *Mytilus galloprovincialis*, sewage, Kosirina Bay, Murter Island, Central Adriatic

1. INTRODUCTION

Expanding urbanisation and other anthropogenic activities, such as industry, traffic, agriculture and tourism in coastal areas have resulted in increased nutrient inputs to coastal ecosystems (HOWARTH et al., 2002; DOLENEC et al., 2006a, PRUELL et al., 2006; CARMICHAEL et al., 2004, VALIELA & BOWN, 2002, COSTANZO et al., 2001; VIZZINI & MAZZOLA, 2006). The major source of anthropogenic nutrient input to many near shore ecosystems could be attributed to inputs from sewage treatment facilities as well as to untreated domestic sewage and municipal and industrial wastes (NIXON et al., 1986; BACHTIAR et al., 1996; ALONSO-RODRIGEZ et al., 2000, CARMICHAEL et al., 2004; HADWEN & ARTHINGTON, 2007). This increase in nutrient load has resulted in N enrichment of aquatic ecosystems, which could lead to the eutrophication of many coastal ecosystems and consequent deterioration of aquatic communities

(HOWARTH, 1998; RABALAIS, 2002; SPIES et al., 1989; VIZZINI & MAZZOLA, 2006; DAVIS & KOOP, 2006).

In recent literature, substantial attention has been paid to evaluating the environmental impact of anthropogenic inputs and organic matter originating from different sewage effluents (HANSSON et al., 1997; TUCKER et al., 1999; RISK & ERDMANN, 2000; HEIKOOP et al., 2000; SIGLEO & MACKO, 2002; ROGERS, 2003; COSTANZO et al., 2003; VIZZINI & MAZZOLA, 2006; DOLENEC et al., 2006a; 2007). This leads to increased concentrations of dissolved nutrients and particulate organic matter (POM), and consequently to increased primary production in the water column (EVGENIDOU & VALIELA, 2002 and references therein). The extent and dispersal of anthropogenic inputs is obviously dependent on several factors, such as the quantity and quality of waste, hydrography, hydrodynamic regime and other environmental features of the area affected by the discharge, (e.g. the physical and chemical characteristics of the water column and sediments, depth) (VIZZINI & MAZZOLA, 2006; SUTHERLAND et al., 2001; ALONGI et al., 2003; KRESS et al., 2004). The POM derived from sewage effluents has typically higher δ^{15}N values relative to POM of unaffected marine environments, due to the alteration of δ^{15}N of dissolved N pool by anthropogenically-derived N wastes with typically high δ^{15}N values (FOGG et al., 1998), and processes that may alter the isotope composition of the dissolved N-pool, such as nitrification, denitrification, N-fixation, etc. (KENDALL, 1998); δ^{15}N of POM can therefore be used to trace the impact areas of anthropogenically derived nitrogen inputs (COSTANZO et al., 2001; GASTON et al., 2004; DOLENEC et al., 2006a; 2007). Previous studies have shown that sewage effluents significantly affect the nitrogen isotope composition of all N pools; dissolved inorganic and organic nitrogen, microbial populations (COSTANZO et al., 2000; DOLENEC et al., 2006a) and macrofauna (DOLENEC et al., 2007, RISK & ERDMAN, 2000; DOLENEC et al., 2005; 2006a,b; 2007).

The present study investigates the impact of anthropogenically derived sewage effluents and their possible transport patterns in the semi-enclosed Pirovac Bay and the coastal part of the Murter Sea (Central Adriatic, Croatia). Expansion of the human population during the tourist season not serviced by adequate municipal infrastructure also represents an additional load of sewage waste on marine coastal ecosystems of Murter Island. Tourist regions without adequate municipal infrastructure discharge sewage wastes directly into the sea. The aim of the present study was:

1) to identify the possible effects of sewage effluents on POM and on resident biota in Kosirina Bay in the western part of Murter Island;

2) to asses the impact of effluents on the δ^{15}N of POM and biota in Kosirina Bay, which may receive sewage effluents by prevalently current-derived mass transport from the south east where the main pollution sources are located;

3) to create maps of the δ^{15}N values of POM, which would enable determination of the geographic extent of a possible anthropogenic impact in Kosirina Bay.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in Kosirina Bay (KB-1 to KB-10) on the southwestern coast of Murter Island (Fig. 1). As reference areas, the semi-enclosed Pirovac Bay (PB-1 to PB-8) and offshore locations south of the Kornati Islands (ROFF-1 to ROFF-4) were also sampled.

The maximum water depth in Kosirina Bay is between 12 and 14 m, and in Pirovac Bay between 23–26 m. Water salinity varies from 28.2 to 36.3 permil in Pirovac Bay while in Kosirina Bay and offshore locations salinity is between 36.2 and 38.3 permil. The average water temperature during the sampling period in Kosirina Bay varied between 20 and 24°C in Pirovac Bay between 22 and 26°C and at offshore locations between 21 and 25°C.

Kosirina Bay is a typical shallow coastal area potentially affected by predominately current derived anthropogenic nutrients and outflow of municipal sewage from the tourist centre of Jezera. One of the closest pollution sources is a direct outfall of municipal sewage southeast of Tužbina Island (SOJ in Fig.1). Dominant currents flow parallel to the shore.

2.2. Sampling

Particulate organic matter (POM), considered as a potential food source for net zooplankton, anemones and mussels, was sampled by vacuum filtering 10 to 20 L of seawater collected at 10 locations in Kosirina Bay (KB-1 to KB-10), as well as at pristine offshore locations south of the Kornati Islands (ROFF-1, 2 and 3), and in the anthropogenically most affected semi-enclosed Pirovac Bay (PB-3, 4, 6, 7 and 8), which receives major inputs of sewage from inadequate domestic septic tank systems, as well as from hotel facilities, autocamps, marinas and small shipyards (Fig. 1). To determine the influence of sewage outfall on the isotopic composition of POM, three water samples were taken in the vicinity of the discharge. The first outfall is located South of Kosirina Bay (SOJ) at a depth of 40 m, the second is near the Hramina marina (SOM) at a depth of 15 m and the third outfall lies south of the village of Betina (SOB) also at a depth of 15 m. Samples at these three outfall locations were taken at a depth of 5 m directly above the sewage release. Water sample at other sampling locations was taken from 1 m below the surface. Samples were filtered through Whatman GF/F glass microfibre filter. POM sampling in Kosirina Bay, Pirovac Bay and Kornati Islands was carried out in June, July and August 2006.

Zooplankton was collected in June 2006 in three different areas: Kosirina Bay (KB-5), at the offshore locations in the Murter Sea (ROFF-1 and between ROFF-3 and 4) and in Pirovac Bay (PB-3, 4 and 6) (Fig. 1). Zooplankton was sampled by towing a net (mesh size: 125 μm) for approximately 30 min. Samples of zooplankton were collected in triplicate at two sites within each locality.

Individual *Mytilus galloprovincialis* were sampled in Kosirina Bay (KB-4) and at different sites at the offshore locations of the Murter Sea (ROFF-1, 2 and 3) and Pirovac

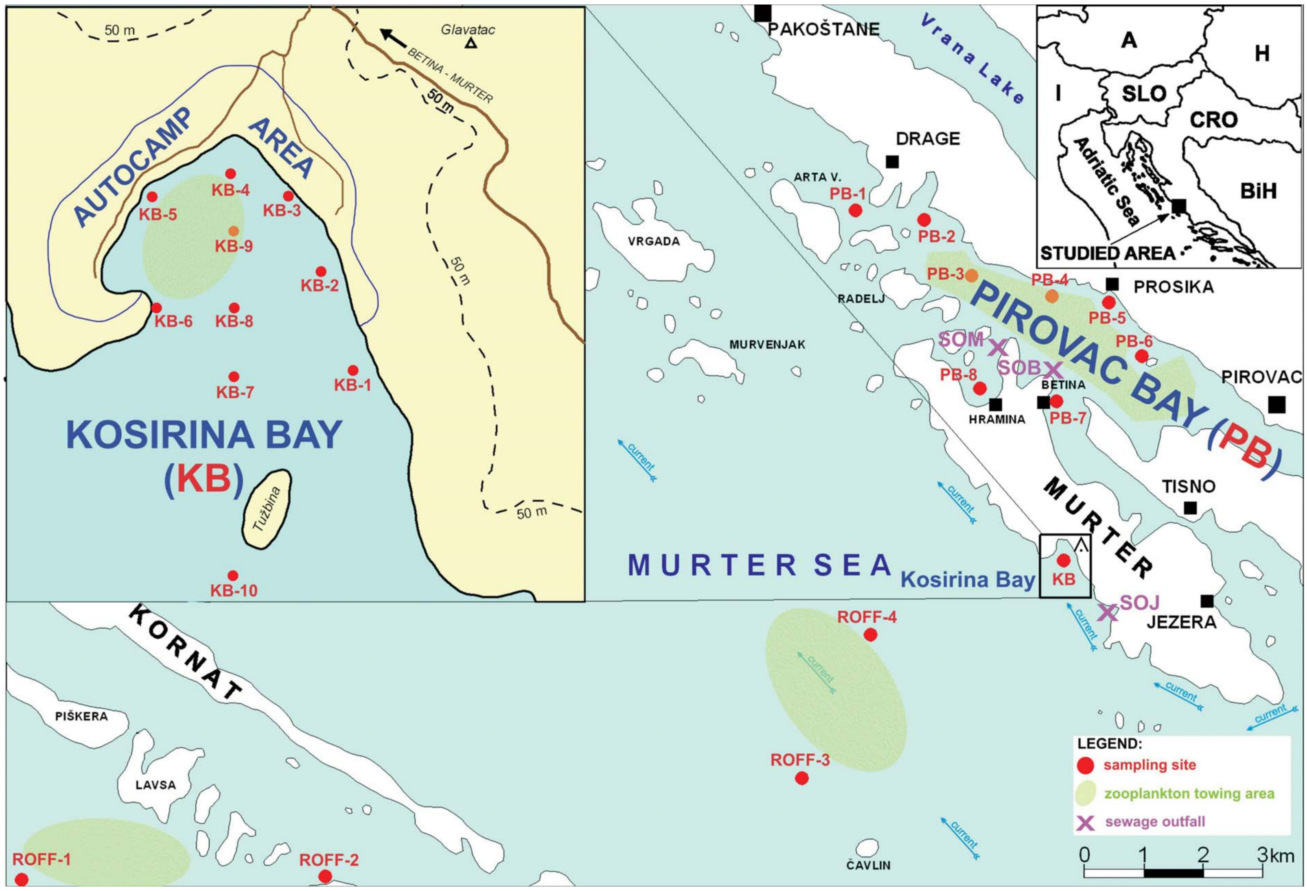

Figure 1: Map of the study area in the Kosirina Bay (Murter Island), Murter Sea, south of the Kornati Island and in the semi enclosed Pirovac Bay (Central Adriatic) showing sites of POM, zooplankton and Mytilus galloprovincialis sampling in June, July and August 2006 (SOJ, SOM, SOB – sewage outfall of Jezera – 40 m depth, Murter – 15 m depth and Betina – 15 m depth).

Bay (PB-1, 2, 4, 5, 6, 7 and 8) (Fig. 1). To get the strongest possible δ^{15}N signal the sampling period for the biota was limited to the peak of the summer tourist season (the last two weeks in August 2006). Primary production is highest in August because of physical parameters including strong light intensity, high temperatures and nutrients. Due to intensive tourist activities, the input of untreated human and other sewage effluents is also maximum during that period. The reference sampling site was selected in the Lumbarda Reef Flat (ROFF-1) in the open sea which is considered to be unaffected by human activities (Fig. 1).

Biota samples were collected by scuba diving at depths of approximately 2–5 m. *Mytilus galloprovincialis* were grouped according to their shell length into two size categories: small (30 to 50 mm long shells) and large (50 to 80 mm shells) to avoid possible isotope effects caused by ontogenetic dietary shifts (DeNIRO & EPSTEIN, 1981; MUSCATINE & KAPLAN, 1994) and differences in age, which could also affect the nitrogen isotopic composition (OWENS, 1987). Each mussel sample consisted of 5 individuals taken at the same sampling site. Upon collection, all the biota samples were immediately frozen and kept at –20 ºC till further processing. They were freeze-dried and pulverised using an agate mortar and pestle. Dry samples were preserved in desiccators at room temperature until the analyses were carried out. Filters of POM were also freeze-dried.

2.3. Isotopic analysis

Isotopic analysis was conducted using a continuous flow-isotope ratio mass spectrometer Europa 20–20 with an ANCA SL preparation module (PDZ Europa Ltd., UK). The results are expressed using standard delta (δ) notation as permil (‰) difference between the sample and reference ratios as follows:

$$\delta^{15}N = [R_{sample} / R_{standard} - 1] \times 1000$$

where R = $^{15}N/^{14}N$. Laboratory working standards (urea and ammonium sulphate solution) were calibrated against IAEA N-1 and IAEA N-2 reference materials with δ^{15}N +0.4 and +20.3 ‰, respectively. The standard deviation of a measurement based on multiple analysis of the working standard was generally < 0.15 ‰.

2.4. Data analysis

Statistica 6.0 data analysis software system (StatSoft Inc) was used for statistical analyses. Analysis of variance (One way ANOVA) was performed to test the differences between the POM and mussels collected at three anthropogenically differently impacted areas:

1) reference and other offshore locations (ROFF - sampling sites),

Table 1: $\delta^{15}N$ of particulate organic matter (POM) collected in Murter Sea, Kosirina Bay, Pirovac Bay and near sewage outfall (SOJ, SOM and SOB) in June, July and August 2006 (* reference sampling site).

Sampling group	Sampling site		$\delta^{15}N$ ‰		
			June	July	August
ROFF	1	Reef Flat Lumbarda*	+ 3.0	+ 3.2	+ 1.8
ROFF	2	Reef Flat Sedlo	+ 3.6	+ 4.0	+ 2.5
ROFF	3	Reef Flat Čavlin	+ 3.9	+ 4.0	+ 2.8
KB	5	Kosirina Bay KB-1	+ 3.6	+ 4.3	+ 4.8
KB	5	Kosirina Bay KB-2	+ 3.4	+ 4.4	+ 4.0
KB	5	Kosirina Bay KB-3	+ 3.9	+ 6.4	+ 4.5
KB	5	Kosirina Bay KB-4	+ 3.4	+ 3.7	+ 3.3
KB	5	Kosirina Bay KB-5	+ 4.0	+ 5.0	+ 2.9
KB	5	Kosirina Bay KB-6	+ 4.0	+ 5.5	+ 3.1
KB	5	Kosirina Bay KB-7	+ 3.2	+ 3.6	+ 3.9
KB	5	Kosirina Bay KB-8	–	+ 3.9	+ 4.4
KB	5	Kosirina Bay KB-9	+ 3.6	+ 3.6	–
KB	5	Kosirina Bay KB-10	+ 3.6	+ 4.6	+ 4.2
PB	8	Pirovac Bay I	+ 6.1	+ 7.3	+ 8.0
PB	13	Port of Murter	+ 6.1	+ 7.8	+ 7.5
PB	9	Reef Flat Spličak	+ 6.4	+ 8.1	+ 8.5
PB	11	Sustipanac Island	+ 6.7	+ 7.3	+ 8.6
PB	12	Port of Betina	+ 6.4	+ 6.9	+ 7.7
SOJ		Sewage Outfall Jezera		average: + 6.2	
SOM		Sewage Outfall Murter		average: + 7.3	
SOB		Sewage Outfall Betina		average: + 7.2	

2) Kosirina Bay (KB - sampling sites) and
3) Pirovac Bay (PB - sampling sites).

Post-hoc Tukey´s honest (HDS) test was used to examine the differences (determined as significant) by ANOVA. For all statistical tests, significance was accepted at $p < 0.05$.

A contour map was constructed by Kriging, from $\delta^{15}N$ values of particulate organic matter (POM) obtained at each sampling site in the Kosirina Bay, using the SURFER® 8 computer package of Golden Software. This technique had already been successfully used to obtain shoot density maps of *Posidonia oceanica,* as well as for detecting and mapping sewage impacts in different biota (COSTANZO et al., 2001; RUIZ et al., 2001; DOLENEC et al., 2005; 2006a,b).

3. RESULTS

The results of $\delta^{15}N$ determination in POM are listed in Tab. 1, while Tabs. 2 and 3 show the nitrogen isotopic composition of zooplankton and mussels *Mytilus galloprovincialis.* Data presented in Tab. 2 represent the average nitrogen isotopic composition of bulk zooplankton samples, while those in Tab. 3 refer to the $\delta^{15}N$ values of an average mussel (*Mytilus galloprovincialis*) sample consisting of 5 individuals (muscle without digestive gland) taken at the selected sampling sites. The results indicate that the $\delta^{15}N$ values of POM, zooplankton and soft tissue of *Mytilus galloprovincialis* were significantly higher at the anthropogenically most affected

Table 2: $\delta^{15}N$ values of zooplankton collected in the Murter Sea, Kosirina Bay and Pirovac Bay in June 2006.

Sampling group	Sampling site		$\delta^{15}N$ ‰
ROFF	1	Reef Flat Lumbarda*	+ 4.3
ROFF	4	Murter Sea I	+ 4.7
ROFF	4/1	Murter Sea I	+ 4.6
KB	5/1	Kosirina Bay	+ 4.8
KB	5/2	Kosirina Bay	+ 4.6
KB	5/3	Kosirina Bay	+ 4.9
PB	8	Pirovac Bay II	+ 7.1
PB	8/1	Pirovac Bay II	+ 7.8
PB	8/2	Pirovac Bay II	+ 7.1
PB	8/3	Pirovac Bay II	+ 7.1
PB	8/4	Pirovac Bay II	+ 7.3
PB	9	Reef Flat Spličak	+ 7.4
PB	9/1	Reef Flat Spličak	+ 7.1
PB	9/2	Reef Flat Spličak	+ 7.4
PB	9/3	Reef Flat Spličak	+ 7.3
PB	9/4	Reef Flat Spličak	+ 7.3
PB	10	Sustipanac Island	+ 8.0
PB	10/1	Sustipanac Island	+ 7.3

Table 3: $\delta^{15}N$ values of *Mytilus galloprovicialis* collected in the Murter Sea, Kosirina Bay and Pirovac Bay (Central Adriatic) in August 2006.

Sampling group	Sampling site		30–50 mm $\delta^{15}N$ ‰	50–80 mm $\delta^{15}N$ ‰
ROFF	1	Reef Flat Lumbarda*		+ 3.3
ROFF	2	Sedlo Island		+ 3.6
ROFF	3	Reef Flat Čavlin		+ 3.8
KB	4	Kosirina Bay	+ 4.2	+ 4.1
KB	4	Kosirina Bay	+ 4.1	+ 4.1
KB	4	Kosirina Bay	+ 4.3	+ 4.2
PB	6	Reef Flat Kušija		+ 6.0
PB	7	Reef FLat Arta		+ 6.6
PB	13	Port of Murter 1		+ 8.1
PB	13/1	Port of Murter 2	+ 7.7	+ 7.1
PB	9	Reef Flat Spličak		+ 7.2
PB	10	Prosika coast		+ 8.2
PB	11	Sustipanac Island		+ 7.3
PB	12	Betina (Marina)		+ 6.8

sampling sites (PB group) relative to those from the reference site at the Reef Flat Lumbarda (ROFF-1), as well as at other isolated offshore locations, such as Sedlo Island (ROFF-2) and Čavlin Reef Flat (ROFF-3).

$\delta^{15}N$ of POM collected in Kosirina Bay (KB) are, however, only slightly enriched in ^{15}N compared to the unaffected reference and other offshore sampling sites (ROFF). The POM sampled in Pirovac Bay (PB) was consistently enriched in ^{15}N compared to the POM from the offshore and Kosirina Bay locations. It should also be noted that temporal variation during the summer sampling season in $\delta^{15}N$ of POM show a general trend toward the more positive values of $\delta^{15}N$ from June to August only in Pirovac Bay (PB - group), while the $\delta^{15}N$ signal in POM collected in August is less positive than in July in Kosirina Bay (KB - group) and is less positive than in June and July for offshore locations (ROFF - group) (Tab. 1, Figs. 3 to 5). The distribution pattern of temporal variations during the summer season in $\delta^{15}N$ of POM in Kosirina Bay is presented in Fig. 2. Enrichment in ^{15}N was observed from June to July while in August the $\delta^{15}N$ values were less positive than in July (Fig. 2).

The highest mean values (8.1 ‰) of $\delta^{15}N$ in the POM in Pirovac Bay (PB) and the lowest mean values (2.4 ‰) in the reference and offshore locations (ROFF) were observed in August while the largest variations (SD = 0.9) in $\delta^{15}N$ of POM values were observed in Kosirina Bay in July. The average values of $\delta^{15}N$ in the POM collected at the sewage outfall SOJ, SOM and SOB are 6.2, 7.3 and 7.2 ‰.

In order to compare the distribution of $\delta^{15}N$ values in the POM between periods (June, July and August), the whisker box plot was employed (Fig. 3). As shown in Fig. 3a the POM group samples collected in June exhibit a statistically significant difference in the mean $\delta^{15}N$ values between the ROFF and PB, as well as between the KB and PB sampling sites, while there was no statistically significant variations between the ROFF and KB sites. A similar situation was also observed in July (Fig. 3b), while in August (Fig. 3c), the mean $\delta^{15}N$ values of POM collected in the reference and offshore locations and Kosirina Bay were statistically significantly different from those of the POM from Pirovac Bay. This was also confirmed by Tuckey's HSD test.

Stable nitrogen isotope values for net zooplankton ranged from +4.3 to +8.0 ‰ with a mean value of +6.45 ‰. Too few $\delta^{15}N$ values were obtained to make a reliable group-to-group comparison. However, the data undoubtedly indicated that $\delta^{15}N$ values of zooplankton collected in Pirovac Bay were considerably enriched in ^{15}N and statistically significantly different from the $\delta^{15}N$ of zooplankton from Kosirina Bay (KB - group), and from reference and other offshore locations (ROFF - group) (Fig. 4). This was also suggested by Tuckey's HSD test.

Stable isotope ratios of *Mytilus galloprovincialis* collected at different sampling sites also varied markedly according to each group in $\delta^{15}N$ (Tab. 3). The presented data clearly show that the *Mytilus galloprovincialis* from the highly impacted Pirovac Bay exhibited $\delta^{15}N$ values in the range +6.0 to +8.2 ‰, with a mean value of +7.16 ‰. Considerably lower values which varied from +4.1 to +4.2 ‰, with a mean value of +4.13 ‰ were measured in *Mytilus galloprovincialis* from Kosirina Bay, while the lowest $\delta^{15}N$ signal was detected in mussels collected at the reference and other offshore locations. *Mytilus galloprovincialis* from these sampling sites (ROFF - group) had $\delta^{15}N$ in the range +3.3 to +3.8 ‰, with a mean value of +3.57 ‰. The analysis of $\delta^{15}N$ values measured in mussel tissues during this study for their possible inter group variation showed, according to TUCKEY'S HSD test, a statistically significant difference only between sampling group PB and all the other groups (KB and ROFF groups). However, there were no statistically significant differences among the KB and ROFF groups. As shown in Fig. 5 where the whisker box plot is presented, statistically significant differences existed only between sampling group PB and the KB and/or the ROFF group.

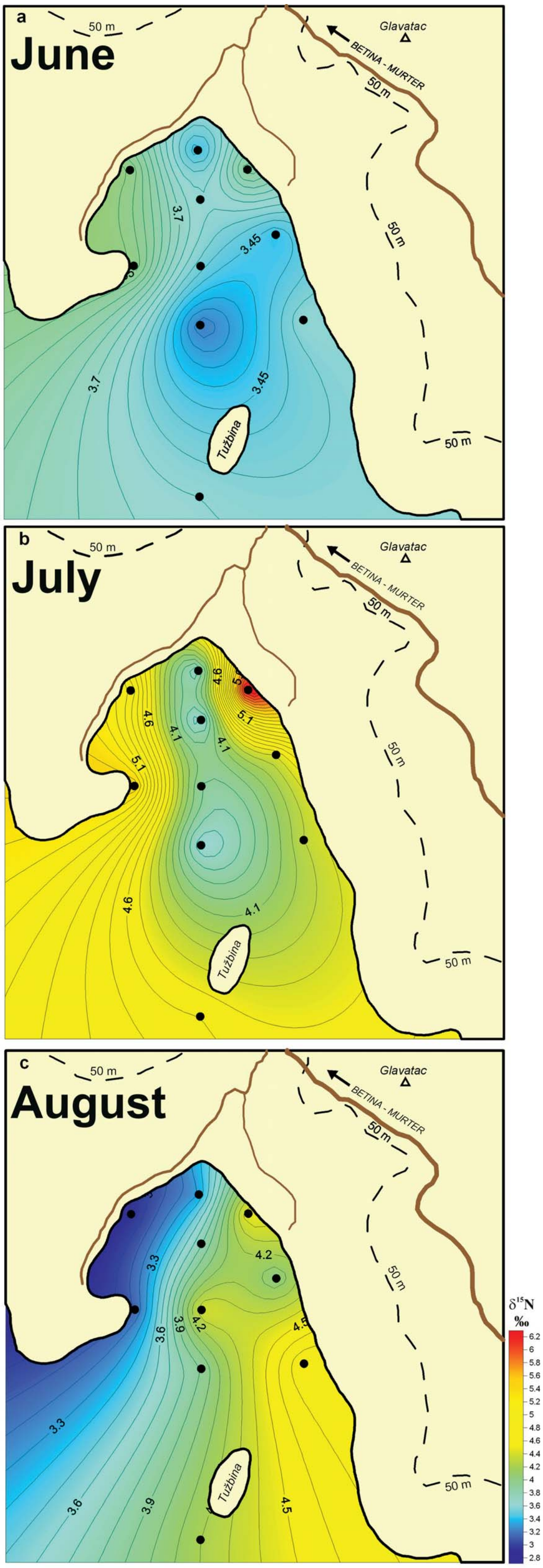

Figure 2: Spatial distribution pattern of $\delta^{15}N$ values of POM in June (a), July (b) and August 2006 (c) throughout Kosirina Bay (Murter Island).

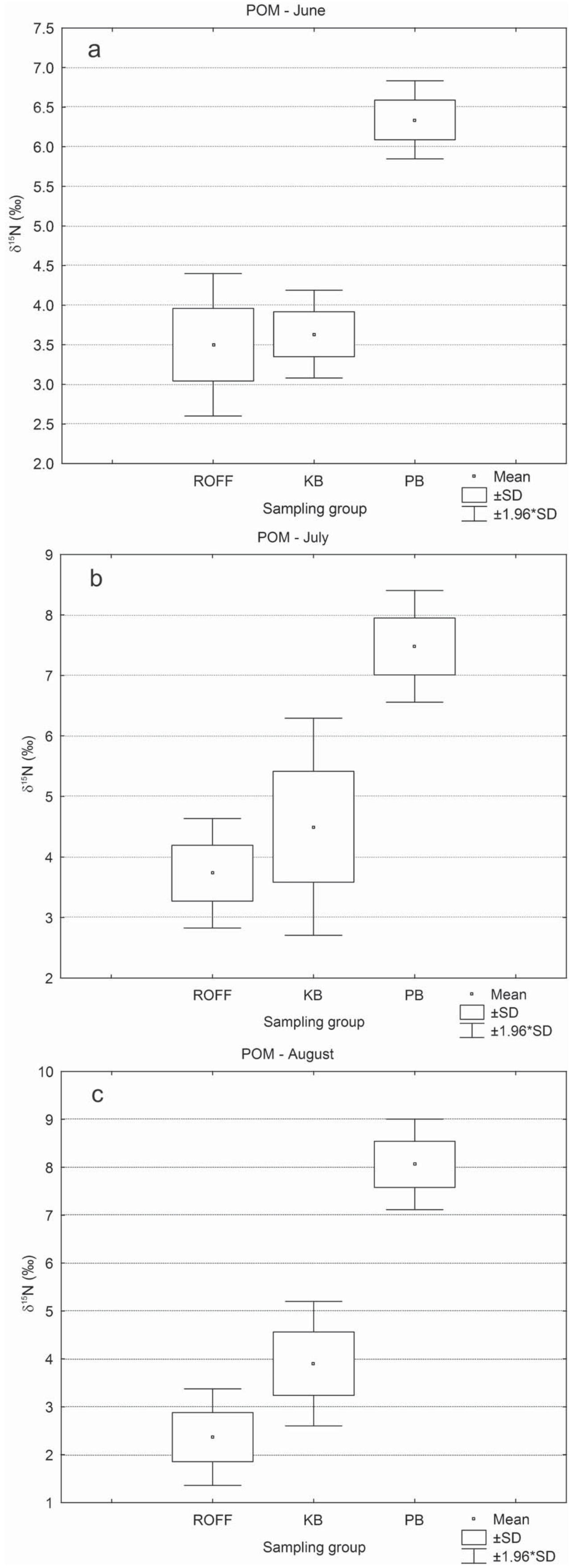

Figure 3: Whisker plots of $\delta^{15}N$ values of POM samples collected in June (a), July (b) and August (c) 2006.

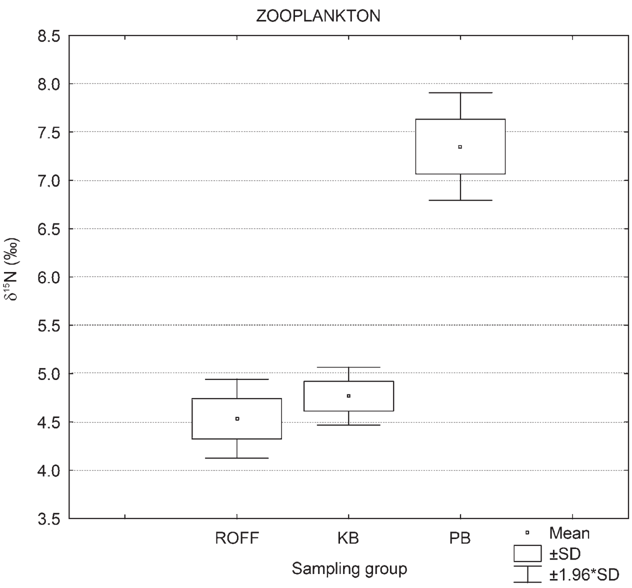

Figure 4: Whisker plots of $\delta^{15}N$ values of zooplankton collected at different sampling sites in June 2006.

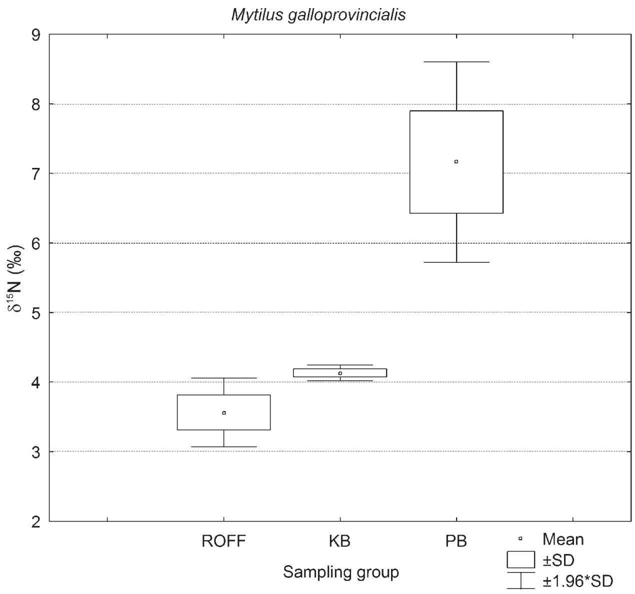

Figure 5: Whisker plots of $\delta^{15}N$ values of *Mytilus galloprovincialis* collected at different sampling sites in August 2006.

4. DISCUSSION

The research revealed that POM and aquatic biota collected from Pirovac Bay (with a history of direct inputs of anthropogenic sewage effluents), tended to have more positive $\delta^{15}N$ values relative to POM and biota from Kosirina Bay, (receiving sewage effluents and organic matter only by the SW-NE current pollutant transport). During the summer in Kosirina Bay is auto camp, which is serviced by adequate septic systems, which represent no additional impact of human sewage on the marine coastal ecosystem of the bay. There is no discharge of sewage into the sea because the plastic water septic tanks are pumped out regularly (personal communication by director of Autocamp Kosirina Boris Paškvalin).

Ongoing sewage effluent discharges (SOM and SOB) into Pirovac Bay is therefore likely to lead to a comparatively greater $\delta^{15}N$ enrichment relative to the south-western side of the Murter Island, including Kosirina Bay, which is open to the sea. Flushing and tidal action as well as predominant currents in the Kosirina Bay have the effect of diluting and distributing the sewage effluents.

Several studies suggested that ecosystems loaded by effluents derived from human sewage should exhibit differences in the $\delta^{15}N$ signal at each trophic level (RISK & ERDMANN, 2000; SPIES et al., 1989; DOLENEC et al., 2006b). As the human and animal waste nitrate have a distinguishable nitrogen isotopic composition with $\delta^{15}N$ values mostly in the range between +10 and +22 ‰ (HEATON, 1986; KREITLER & BROWNING, 1983), the elevated $\delta^{15}N$ values of NO_3 > +10 ‰ are regarded as being indicative of faecal N origin (BARRET et al., 1999). This nitrogen is quickly assimilated by primary producers and transferred further into the entire food web including zooplankton, affecting their nitrogen isotopic composition (WASER et al., 1998a, 1998b), which makes the POM indicative of the $\delta^{15}N$ of the N substrate pool. Elevated $\delta^{15}N$ values have been identified in POM and marine biota exposed to groundwater contaminated by septic wastes (McCLELLAND et al., 1997) and sewage effluents (COSTANZO et al., 2001, RISK & ERDMAN, 2000; DOLENEC et al., 2005; RISK & HEIKOOP, 1997; WALDRON et al., 2001; HOBSON et al., 2002; DOLENEC et al., 2006a). Increased $\delta^{15}N$ values of about +7.9 ‰ were measured in POM dominated by untreated faecal matter of Jepara Bay (HEIKOOP et al., 2000). Similar values of about +8 ‰ were also found in POM near the inflows from septic systems in the Port of Murter and in Pirovac Bay (DOLENEC et al., 2006a). The POM enriched in heavy nitrogen isotopes with $\delta^{15}N$ values ranging from +6.1 to +8.6 ‰ was also measured during this study in Pirovac Bay.

The POM generally represents a mixture of detrital material of marine and terrestrial origin, phyto- and zooplankton and particulate effluents from different sources. The background POM unaffected by anthropogenic activities may show seasonal variations in $\delta^{15}N$ by several permil (BODE & ALVAREZ-OSSORIO, 2004). During this study, temporal differences in the $\delta^{15}N$ of POM from reference and offshore locations of about 1.2 ‰ were also observed (Tab. 1). However, these effects did not overprint the $\delta^{15}N$ variability due to human sewage inputs in the studied area. Temporal variations during the summer sampling season in the $\delta^{15}N$ of the POM in Pirovac Bay were affected by human sewage inputs, by about 2.5 ‰ (Tab. 1), and is most probably related to the variable inputs of effluents with more or less constant $\delta^{15}N$ values. Previous study carried out on $\delta^{15}N$ variability in the Murter Sea in 2005 revealed that the $\delta^{15}N$ of POM tended to be lowest in the early part of the summer season, and higher during the late summer (at the end of August) (DOLENEC et al., 2006a). This pattern was not repeated in 2006. During this study, the POM sampled in August 2006 exhibited $\delta^{15}N$ values, which tend to be depleted in ^{15}N. This could be to some extend the result of a very rainy August 2006, with a considerable input of rain water, which can alter the cycling of nutrients in the study area.

Higher $\delta^{15}N$ values of POM detected in July in the NE part of the Kosirina Bay indicated the effluents from the point source septic system, which accidentally at the time of the sampling period affected a limited part of the coastal water (Fig. 2b). However, in August during the peak of the tourist season, the bay was relatively free from anthropogenically-derived nitrogen due to a rainy August 2006 (Fig. 2c).

Sewage effluents from the septic systems enriched in heavy nitrogen could account for the ^{15}N enrichment not only in POM but also in zooplankton and mussels from Pirovac Bay. For example, NO_3^- sewage waste shows significantly higher $\delta^{15}N$ values than other NO_3^- sources (HEATON, 1986; MACKO & OSTROM, 1994).

Zooplankton from Pirovac Bay (Tab. 2) exhibiting the highest $\delta^{15}N$ indicates a larger input of sewage wastes compared with zooplankton sampled in Kosirina Bay and/or on reference and other offshore locations. This indicates a significantly lower input of sewage wastes as well as a considerable dispersion and dilution of the ^{15}N-enriched wastes by sea currents along the SW part of the Murter Island.

Similarly, we can explain the nitrogen isotopic composition of mussels *Mytilus galloprovincialis* (Tab. 3). Their $\delta^{15}N$ are significantly higher at the sampling sites in Pirovac Bay compared to individuals sampled in Kosirina Bay and at reference and/or offshore locations. This also suggest the same direction of change in $\delta^{15}N$, with more positive values in impacted than reference sites (COSTANZO et al., 2001; VIZZINI & MAZZOLA, 2002; 2006).

The spatial distribution of the anthropogenic impact indicators such as the $\delta^{15}N$ signal in POM collected in June, July and August in Kosirina Bay (Fig. 2), revealed a variable but only minor influence of human sewage in the bay. This could be related to anthropogenic organic matter, which mostly reached Kosirina Bay during southerly wind-driven sea currents from the sewage outfall south of Tužbina Island (SOJ).

$\delta^{15}N$ values also offer the possibility of estimating the trophic level of organisms, because $\delta^{15}N$ values generally increase with increasing trophic position (FRY, 1988; HOBSON & WELCH, 1992), but this ^{15}N enrichment is variable; it varies between animal groups and is often diet-related (McCUTCHAN et al., 2003; VANDERKLIFT & PONSARD, 2003). In consumers, the trophic shift for nitrogen is about +1.4 ‰ for consumers feeding on an invertebrate diet and +3.3 ‰ for consumers feeding on a high-protein diet. The nitrogen trophic-shift for carnivorous fish is about +2.6 ‰ (McCUTCHAN et al., 2003; VANDERKLEFT & PONSARD, 2003). The isotopic signature of the zooplankton and mussels follows the signature of POM, although the ^{15}N enrichment in the system POM – zooplankton – *Mytilus galloprovincialis* was not consistent with the predicted trophic pathway. To elucidate discrepancies, a further, more complex study and sampling programme is needed.

5. CONCLUSION

The results of this study provide a contribution to the broad debate on the problems relating to the anthropogenic N loading intensity on the coastal ecosystems of the Murter Sea and Pirovac Bay. This study revealed that ^{15}N abundances in POM and biota were statistically significantly different when comparing sampling sites in the less affected Kosirina Bay and offshore sites, as well as at reference location, with the anthropogenically most impacted Pirovac Bay, which is mostly affected by municipal wastes and untreated effluents from the septic systems. By using the $\delta^{15}N$ signature of POM, a spatial distribution of anthropogenically-derived nitrogen in Kosirina Bay was also mapped. Based on results reported in the present study we can conclude that the shallow water marine ecosystem of Kosirina Bay was practically free from long-term anthropogenic impact of organic load, mostly originating from untreated wastes and effluents from the septic systems. However, to get a better insight into the qualitative or quantitative shifts in the structure of the aquatic food web caused by pollutants, more extended research on the benthic population is needed, as well as a detailed investigation of temporal variations during the summer season of abundance and isotopic composition of POM and zooplankton as their presumed food source.

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