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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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; DOLENEČ 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...

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 δ¹⁵N 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
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; SIGLE & 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 δ15N values relative to POM of unaffected marine environments, due to the alteration of δ15N of dissolved N pool by anthropogenically-derived N wastes with typically high δ15N 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); δ15N 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 assess the impact of effluents on the δ15N 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 δ15N 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 predominantly 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 samples at other sampling locations were 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
Bay (PB-1, 2, 4, 5, 6, 7 and 8) (Fig. 1). To get the strongest possible $\delta^{15}\text{N}$ 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 °C 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 ($\delta$) notation as permil (‰) difference between the sample and reference ratios as follows:

$$\delta^{15}\text{N} = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \times 1000$$

where $R = ^{15}\text{N}/^{14}\text{N}$. Laboratory working standards (urea and ammonium sulphate solution) were calibrated against IAEA N-1 and IAEA N-2 reference materials with $\delta^{15}\text{N} +0.4$ 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),
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 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).

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

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

<table>
<thead>
<tr>
<th>Sampling group</th>
<th>Sampling site</th>
<th>$\delta^{15}N$ ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROFF 1</td>
<td>Reef Flat Lumbarda*</td>
<td>+ 4.3</td>
</tr>
<tr>
<td>ROFF 4</td>
<td>Murter Sea I</td>
<td>+ 4.7</td>
</tr>
<tr>
<td>ROFF 4/1</td>
<td>Murter Sea I</td>
<td>+ 4.6</td>
</tr>
<tr>
<td>KB 5/1</td>
<td>Kosirina Bay</td>
<td>+ 4.8</td>
</tr>
<tr>
<td>KB 5/2</td>
<td>Kosirina Bay</td>
<td>+ 4.6</td>
</tr>
<tr>
<td>KB 5/3</td>
<td>Kosirina Bay</td>
<td>+ 4.9</td>
</tr>
<tr>
<td>PB 8</td>
<td>Pirovac Bay II</td>
<td>+ 7.1</td>
</tr>
<tr>
<td>PB 8/1</td>
<td>Pirovac Bay II</td>
<td>+ 7.8</td>
</tr>
<tr>
<td>PB 8/2</td>
<td>Pirovac Bay II</td>
<td>+ 7.1</td>
</tr>
<tr>
<td>PB 8/3</td>
<td>Pirovac Bay II</td>
<td>+ 7.1</td>
</tr>
<tr>
<td>PB 8/4</td>
<td>Pirovac Bay II</td>
<td>+ 7.3</td>
</tr>
<tr>
<td>PB 9</td>
<td>Reef Flat Spličak</td>
<td>+ 7.4</td>
</tr>
<tr>
<td>PB 9/1</td>
<td>Reef Flat Spličak</td>
<td>+ 7.1</td>
</tr>
<tr>
<td>PB 9/2</td>
<td>Reef Flat Spličak</td>
<td>+ 7.4</td>
</tr>
<tr>
<td>PB 9/3</td>
<td>Reef Flat Spličak</td>
<td>+ 7.3</td>
</tr>
<tr>
<td>PB 9/4</td>
<td>Reef Flat Spličak</td>
<td>+ 7.3</td>
</tr>
<tr>
<td>PB 10</td>
<td>Sustipanac Island</td>
<td>+ 8.0</td>
</tr>
<tr>
<td>PB 10/1</td>
<td>Sustipanac Island</td>
<td>+ 7.3</td>
</tr>
</tbody>
</table>
δ¹⁵N of POM collected in Kosirina Bay (KB) are, however, only slightly enriched in ¹⁵N compared to the unaffected reference and other offshore sampling sites (ROFF). The POM sampled in Pirovac Bay (PB) was consistently enriched in ¹⁵N 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 δ¹⁵N of POM show a general trend toward the more positive values of δ¹⁵N from June to August only in Pirovac Bay (PB - group), while the δ¹⁵N 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 δ¹⁵N of POM in Kosirina Bay is presented in Fig. 2. Enrichment in ¹⁵N was observed from June to July while in August the δ¹⁵N values were less positive than in July (Fig. 2).

The highest mean values (8.1 ‰) of δ¹⁵N 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 δ¹⁵N of POM values were observed in Kosirina Bay in July. The average values of δ¹⁵N 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 δ¹⁵N 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 δ¹⁵N 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 δ¹⁵N 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 δ¹⁵N values were obtained to make a reliable group-to-group comparison. However, the data undoubtedly indicated that δ¹⁵N values of zooplankton collected in Pirovac Bay were considerably enriched in ¹⁵N and statistically significantly different from the δ¹⁵N 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 δ¹⁵N (Tab. 3). The presented data clearly show that the Mytilus galloprovincialis from the highly impacted Pirovac Bay exhibited δ¹⁵N 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 δ¹⁵N signal was detected in mussels collected at the reference and other offshore locations. Mytilus galloprovincialis from these sampling sites (ROFF - group) had δ¹⁵N in the range +3.3 to +3.8 ‰, with a mean value of +3.57 ‰. The analysis of δ¹⁵N 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.

Table 3: δ¹⁵N values of Mytilus galloprovincialis collected in the Murter Sea, Kosirina Bay and Pirovac Bay (Central Adriatic) in August 2006.

<table>
<thead>
<tr>
<th>Sampling group</th>
<th>Sampling site</th>
<th>30–50 mm δ¹⁵N ‰</th>
<th>50–80 mm δ¹⁵N ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROFF</td>
<td>Reef Flat Lumbarda*</td>
<td>+ 3.3</td>
<td>+ 3.6</td>
</tr>
<tr>
<td>ROFF</td>
<td>Sedlo Island</td>
<td>+ 3.6</td>
<td>+ 3.8</td>
</tr>
<tr>
<td>ROFF</td>
<td>Reef Flat Čavlin</td>
<td>+ 3.8</td>
<td>+ 3.8</td>
</tr>
<tr>
<td>KB</td>
<td>Kosirina Bay</td>
<td>+ 4.2</td>
<td>+ 4.1</td>
</tr>
<tr>
<td>KB</td>
<td>Kosirina Bay</td>
<td>+ 4.1</td>
<td>+ 4.1</td>
</tr>
<tr>
<td>KB</td>
<td>Kosirina Bay</td>
<td>+ 4.3</td>
<td>+ 4.2</td>
</tr>
<tr>
<td>PB</td>
<td>Reef Flat Kušija</td>
<td>+ 6.0</td>
<td>+ 6.6</td>
</tr>
<tr>
<td>PB</td>
<td>Reef Flat Arta</td>
<td>+ 6.6</td>
<td>+ 8.1</td>
</tr>
<tr>
<td>PB</td>
<td>Port of Murtar 1</td>
<td>+ 7.7</td>
<td>+ 7.1</td>
</tr>
<tr>
<td>PB</td>
<td>Port of Murtar 2</td>
<td>+ 7.2</td>
<td>+ 8.2</td>
</tr>
<tr>
<td>PB</td>
<td>Reef Flat Spiličak</td>
<td>+ 7.3</td>
<td>+ 7.3</td>
</tr>
<tr>
<td>PB</td>
<td>Prosika coast</td>
<td></td>
<td>+ 6.8</td>
</tr>
<tr>
<td>PB</td>
<td>Sustipanac Island</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>Betina (Marina)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In order to compare the distribution of δ¹⁵N 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 δ¹⁵N 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 δ¹⁵N 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 δ¹⁵N values were obtained to make a reliable group-to-group comparison. However, the data undoubtedly indicated that δ¹⁵N values of zooplankton collected in Pirovac Bay were considerably enriched in ¹⁵N and statistically significantly different from the δ¹⁵N 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 δ¹⁵N (Tab. 3). The presented data clearly show that the Mytilus galloprovincialis from the highly impacted Pirovac Bay exhibited δ¹⁵N 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 δ¹⁵N signal was detected in mussels collected at the reference and other offshore locations. Mytilus galloprovincialis from these sampling sites (ROFF - group) had δ¹⁵N in the range +3.3 to +3.8 ‰, with a mean value of +3.57 ‰. The analysis of δ¹⁵N 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 δ¹⁵N values of POM in June (a), July (b) and August 2006 (c) throughout Kosirina Bay (Murter Island).

Figure 3: Whisker plots of δ¹⁵N values of POM samples collected in June (a), July (b) and August (c) 2006.
septic tanks are pumped out regularly (personal communica-

tion by director of Autocamp Kosirina Boris Paškalin). 

Ongoing sewage effluent discharges (SOM and SOB) 
into Pirovac Bay is therefore likely to lead to a comparatively 
greater δ15N 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 cur-
cents in the Kosirina Bay have the effect of diluting and dis-
tributing the sewage effluents.

Several studies suggested that ecosystems loaded by ef-
fluents derived from human sewage should exhibit differ-
ences in the δ15N signal at each trophic level (RISK & ERD-
MANN, 2000; SPIES et al., 1989; DOLENEC et al., 2006b). 
As the human and animal waste nitrate have a distinguishable 
nitrogen isotopic composition with δ15N values mostly in the 
range between +10 and +22 ‰ (HEATON, 1986; KREITL
ER & BROWNING, 1983), the elevated δ15N values of NO3 > 
+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 δ15N of the N substrate 
pool. elevated δ15N values have been identified in POM and 
marine biota exposed to groundwater contaminated by sep-
tic wastes (McCLELLAND et al., 1997) and sewage efl
uents (COSTANZO et al., 2001, RISK & ERDMAN, 2000; 
DOLENEC et al., 2005; RISK & HEIKOOP, 1997; WAL-
DRON et al., 2001; HOBBON et al., 2002; DOLENEC 
et al., 2006a). Increased δ15N values of about +7.9 ‰ were mea-
sured 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 sys-
tems in the Port of Murter and in Pirovac Bay (DOLENEC 
et al., 2006a). The POM enriched in heavy nitrogen isotopes 
with δ15N values ranging from +6.1 to +8.6 ‰ was also mea-
sured during this study in Pirovac Bay.

The POM generally represents a mixture of detrital ma-
terial of marine and terrestrial origin, phyto- and zooplankton 
and particulate effluents from different sources. The back-
ground POM unaffected by anthropogenic activities may show 
seasonal variations in δ15N by several permil (BODE & AL-
VAREZ- OSSORIO, 2004). During this study, temporal dif-
fferences in the δ15N of POM from reference and offshore 
locations of about 1.2 ‰ were also observed (Tab. 1). How-
ever, these effects did not overprint the δ15N variabil-
ity due to human sewage inputs in the studied area. Temporal vari-
ations during the summer sampling season in the δ15N 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 δ15N 
values. Previous study carried out on δ15N variability in the 
Murer Sea in 2005 revealed that the δ15N 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 δ15N 
values, which tend to be depleted in 15N. This could be to

4. DISCUSSION

The research revealed that POM and aquatic biota collected 
from Pirovac Bay (with a history of direct inputs of anthro-
pogenic sewage effluents), tended to have more positive δ15N 
values relative to POM and biota from Kosirina Bay, (receiv-
ing 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 sys-
tems, which represent no additional impact of human sew-
age on the marine coastal ecosystem of the bay. There is no 
discharge of sewage into the sea because the plastic water
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 δ¹⁵N values of POM detected in July in the NE part of the Kosirina Bay indicated the effluents from the point source septic system, which accidently 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 δ¹⁵N enrichment not only in POM but also in zooplankton and mussels from Pirovac Bay. For example, NO₃ sewage waste shows significantly higher δ¹⁵N values than other NO₃ sources (HEATON, 1986; MACKO & OSTROM, 1994).

Zooplankton from Pirovac Bay (Tab. 2) exhibiting the highest δ¹⁵N 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 δ¹⁵N-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 δ¹⁵N 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 δ¹⁵N, 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 δ¹⁵N 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 Tuzbina Island (SOJ).

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 δ¹⁵N 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 δ¹⁵N 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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