Scientific Paper

^{15}N and ^{13}C Enrichment in *Balanus perforatus*: Tracers of Municipal Particulate Waste in the Murter Sea (Central Adriatic, Croatia)

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Received 15-03-2006

Abstract

Stable isotopic compositions of nitrogen and carbon in barnacles *Balanus perforatus* from the Pirovac Bay (Central Adriatic), highly impacted by untreated municipal waste, and from unaffected sites from the southern part of the Kornati Islands were compared. The differences in δ^{13}C and δ^{15}N values observed in organisms from both areas suggest that this benthic invertebrate could be a potential biomonitor for sewage-derived nutrients in coastal seas. Both nitrogen and carbon were enriched in heavy isotopes in organisms collected in Pirovac Bay, as well as in those from the area around the fish farm at Vrgada Island. These point toward an increased input of ^{15}N and ^{13}C enriched anthropogenic and/or aquaculture derived nitrogen and carbon into the local marine ecosystem. It is suggested that benthic filter feeders, such as *B. perforatus,* could be useful sentinels for detecting anthropogenically-derived inputs of nutrients into the coastal marine ecosystems. However, to get a better insight into the transfer rates of anthropogenic C and N into the food web, more extended research on a larger population is needed, as well as a detailed investigation of seasonal variation of abundance and isotopic composition of particulate organic matter as their presumed food source.

Key words: *balanus perforatus*, particulate organic matter, nitrogen, carbon, stable isotopes. sewage, aquaculture.

1. Introduction

Coastal parts of Murter Sea and Pirovac Bay (Central Adriatic) are exposed to the inputs of anthropogenic nitrogen from untreated domestic and municipal wastes, camping sites and marinas. Additional nutrient loadings occur in the summer season due to intensive tourism, not serviced by adequate municipal infrastructure (sewage systems, wastewater treatment).

By measuring the stable isotopic composition of light elements, such as C, N, S, O and H in marine biota from polluted and unpolluted sites, one can discriminate between marine and terrestrial based organic matter from sewage effluents.[1-8] Variation in the isotopic composition of biogenic elements in living organisms depends on the isotopic composition of their diet, metabolic pathways, and kinetic models of reaction dynamics.[9]

In the trophic network among animals, the δ^{15}N values (representing the stable isotopic composition of N) of their tissues systematically increase by 1.3 to 5.3‰ per trophic level.[9-11] This means, that an organism's δ^{15}N value is generally by some permil more positive than that of its source dietary N. On the other hand, the δ^{13}C values of animals vary resembling those of their diet.[12-14] There seems to be little or no change in the relative abundance of ^{13}C between trophic levels following the primary producer to primary consumer link, so that this isotope is useful as an indicator of sources of C for primary productivity.[15] These facts further suggest that δ^{13}C values are an indicator of the origin of the consumed food substrate(s), since δ^{13}C remains relatively unchanged between successive trophic level, while δ^{15}N signal is a better tracer of the trophic level.[16]

Stable isotope ratios of nitrogen and carbon can further be used to detect the human sewage derived organic matter in the marine environment.[1, 2, 14] Ecosystems with different loadings of sewage derived carbon and nitrogen should exhibit differences in δ^{13}C and δ^{15}N values at each tropic level. Identical trophic structures utilising carbon and nitrogen sources with different isotopic composition should also clearly reflect these differences. Ecosystems with no or minimal sewage input should exhibit relatively low δ^{13}C and δ^{15}N values, while those strongly impacted by human faecal matter from septic systems show increased δ^{15}N and δ^{13}C values, as a result of the utilisation of ^{15}N and ^{13}C enriched N and C at the base of the trophic structure.[17, 18]

In this study, we used δ^{15}N and δ^{13}C values of barnacles (*Balanus perforatus*) and their potential food sources (particulate organic matter - POM), to determine whether these sessile benthic invertebrates are suitable as biomonitors for the anthropogenic nitrogen pollution of the coastal ecosystems of the Adriatic Sea.

In general, benthic sessile invertebrates are relatively non-mobile and thus tend to be representative for the sampled area.[19-21] Barnacles inhabit a rigid calcareous shell (carapace or mantle) that grows without malting. For the purpose of this research, only the acorn barnacle *Balanus perforatus* was chosen. The preliminary data on its nitrogen isotopic composition indicated a significant difference between anthropogenically and fish farming impacted locations in the Murter Sea, compared to the pristine localities (Dolenec et al., unpublished). The focus of the newly developed monitoring programme was a further evaluation of the coastal seawater quality and the assessment of human sewage impacts on the benthic invertebrate population in this part of the Central Adriatic using stable isotopes.

2. Experimental

2.1. Study area and sample collection

The individuals of acorn barnacles of *B. perforatus* were collected by scuba diving from the sea at a depth of up to 1 m at five localities in the semi-enclosed Pirovac Bay (sampling sites PB 7-11, Fig. 1), highly impacted by the human sewage effluents from the septic systems, as well as at the pristine off-shore reference site (Lumbarda Reef Flat, sampling site ROFF - 1) and at three other isolated offshore reef flats (Puh, Balun and Dužac Island, sampling sites ROFF 2-4), which were not affected by human activities. The *B. perforatus* samples were collected from the same depth also around the small isolated Islands of Špinata and Rakita (sampling sites FF 5 and 6) close to the fish farm at Vrgada Island, with an annual production of about 450 t of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) and 1000 t of tuna (*Thunnus thynnus*).

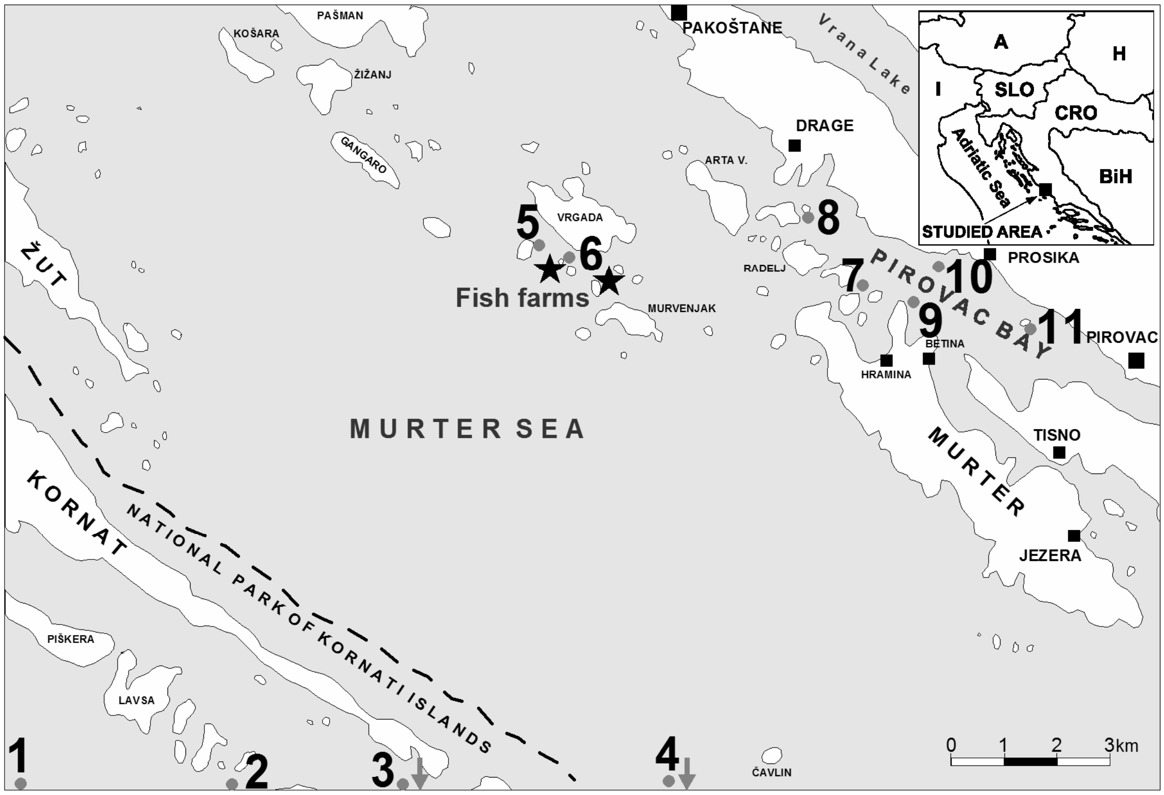

Figure 1. Map of the study area in Murter Sea and Pirovac Bay (Central Adriatic) showing sampling sites.

Table 1. δ^{15}N values of *Balanus perforatus* and blue-green algae colonizing the barnacle shells, collected at sampling sites in Pirovac Bay and Murter Sea (*B. perforatus* - pooled sample of soft tissues of five individuals; *selected tissue - thoracic appendages - cirri).

Sample No.	Sample type	Sampling site	Sample Group	δ^{15}N (‰)	STD ±	δ^{13}C (‰)	STD ±
1 (ref.)	*B. perforatus*	Reef Flat Lumbarda	ROFF	+ 4.5	0.2	- 21.6	0.2
	B. perforatus	Reef Flat Lumbarda	ROFF	+ 4.9	0.2	- 21.1	0.1
	B. perforatus	Reef Flat Lumbarda	ROFF	+ 4.4	0.2	- 21.6	0.9
	*B. perforatus**	Reef Flat Lumbarda	ROFF	+ 4.7	0.3	- 19.7	0.2
	Blue green algae	Reef Flat Lumbarda	ROFF	+ 3.1	0.3	- 18.8	1.6
2	*B. perforatus*	Reef Flat Puh	ROFF	+ 5.1	1.0	- 22.0	0.4
	B. perforatus	Reef Flat Puh	ROFF	+ 5.4	0.2	- 20.0	0.1
	Blue green algae	Reef Flat Puh	ROFF	+ 2.4	0.3	- 25.0	3.1
3	*B. perforatus*	Reef Flat Balun	ROFF	+ 5.6	0.1	- 21.3	0.3
	B. perforatus	Reef Flat Balun	ROFF	+ 5.5	0.3	- 22.3	0.2
	B. perforatus	Reef Flat Balun	ROFF	+ 5.4	0.2	- 21.8	0.2
	*B. perforatus**	Reef Flat Balun	ROFF	+ 5.8	0.1	- 19.3	0.5
	Blue green algae	Reef Flat Balun	ROFF	+ 3.1	0.3	- 19.8	1.6
4	*B. perforatus*	Dužac Island	ROFF	+ 5.3	0.2	- 22.5	0.3
	B. perforatus	Dužac Island	ROFF	+ 5.1	0.4	- 25.4	0.9
	Blue green algae	Dužac Island	ROFF	+ 2.8	0.1	- 21.4	0.2
5	*B. perforatus*	Špinata Island	FF	+ 6.4	0.1	- 20.4	0.4
	B. perforatus	Špinata Island	FF	+ 6.5	0.2	- 20.5	0.2
6	*B. perforatus*	Rakita Island	FF	+ 6.6	0.2	- 20.8	0.3
	B. perforatus	Rakita Island	FF	+ 6.8	0.1	- 18.3	0.3
7	*B. perforatus*	Vinih Island	PB	+ 8.2	0.3	- 18.5	0.1
	B. perforatus	Vinih Island	PB	+ 8.0	0.2	- 18.5	0.2
	Blue green algae	Vinih Island	PB	+ 5.9	0.2	- 19.6	0.4
8	*B. perforatus*	Reef Flat Arta	PB	+ 7.7	0.2	- 20.6	0.1
	Blue green algae	Reef Flat Arta	PB	+ 5.5	0.5	- 18.9	0.3
9	*B. perforatus*	Cap of Gradina	PB	+ 8.7	0.3	- 20.4	0.3
	B. perforatus	Cap of Gradina	PB	+ 8.2	0.2	- 19.4	0.4
10	*B. perforatus*	Reef Flat Spličak	PB	+ 9.4	0.3	- 20.2	0.1
	B. perforatus	Reef Flat Spličak	PB	+ 10.0	0.1	- 18.9	0.1
	*B. perforatus**	Reef Flat Spličak	PB	+ 9.6	0.1	- 18.5	0.3
11	*B. perforatus*	Sustipanac Island	PB	+ 9.4	0.2	- 20.4	0.5
	B. perforatus	Sustipanac Island	PB	+ 9.6	0.1	- 19.6	0.1

All *B. perforatus* individuals were size matched (basal diameter 1.2 cm, height 2 cm) to avoid possible isotope effects caused by differences in age, which generally affect the nitrogen and carbon isotopic composition of organisms.[22] *B. perforatus* individuals were sampled in last two weeks in August 2005 at the peak of the tourist season, when the primary production is increased due to the increased input of nutrients and favourable light conditions. Fresh *B. perforatus* samples were placed into plastic bags and stored at -20°C until further processing. In the laboratory, soft tissues of barnacles were separated from their shells and freeze-dried. Five individuals were pooled to form one sample. Dry samples were preserved in desiccators at room temperature until the analyses were carried out. To obtain a further insight into the environmental impact of anthropogenic nitrogen in the coastal part of Pirovac Bay on the primary producers, the nitrogen and carbon isotopic compositions of blue-green algae colonizing the barnacle shells were also analysed. Algae were scrapped from the shells, oven-dried at 50 °C overnight and kept dry until further processing.

Particulate organic matter (POM), considered as a potential food source for barnacles, was sampled at a depth of 1 m, at different sampling sites around the reef flats in the open sea (12 samples, ROFF group), fish cages (10 samples, FF group) and in Pirovac Bay (20 samples, PB group). Aliquots of 5 l of water were filtered through Whatman GF/C glass fibre microfilter. Samples were collected monthly from April to August 2005. Filtered POM were freeze-dried and preserved in a desiccator at room temperature until the analyses were carried out.

2.2 Nitrogen and carbon stable isotope analyses

For stable isotopic analyses of organic carbon, dry samples were pulverised, homogenised and soaked in 3N HCl at 50 °C for 3 h to remove carbonates, rinsed with deionised water and dried. Pulverised and homogenised samples for the N isotope analyses remained untreated. Nitrogen and carbon isotopic compositions were measured using a Europa 20-20 continuous-flow isotope ratio mass spectrometer with an ANCA SL preparation module (PDZ Europa Ltd., U.K.). They are expressed as relative δ values in‰, i.e. the difference in parts per mil of the isotopic ratios ^{15}N/^{14}N and ^{13}C/^{12}C of samples from those of the reference materials (atmospheric nitrogen for N and VPDB for C). The analytical precision of isotopic analyses was within ± 0.2‰ for nitrogen and ± 0.1‰ for carbon.

3. Results

The measured δ^{15}N and δ^{13}C values of *B. perforatus* are shown in Table 1. The whisker plots of δ^{15}N and δ^{13}C values of *B. perforatus* specimen are shown in Fig. 2, while the whisker plot of δ^{15}N values for POM collected from April to August 2005 is presented in Fig. 3.

The *B. perforatus* samples can be divided into three statistically different groups: reference and off-shore sites (ROFF sites, 1 - 4), fish farm sites (FF sites, 5 and 6) and Pirovac Bay sites (PB sites, 7-11). The division was made based on the mean δ^{15}N values of the group members (5.14 ± 0.44‰, 6.58 ± 0.17‰ and 8.88 ± 0.66‰ for ROFF, FF and PB sites, respectively). The Tukey's HSD test showed that all groups differ among each other at $p < 0.01$. The mean δ^{13}C values of the ROFF, FF and PB sites were 21.55 ± 1.59‰, 20.00 ± 1.15‰ and 19.50 ± 0.86‰, respectively. A statistically significant difference was found between ROFF and PB sites ($p = 0.0033$), while no significant difference could be observed nor between ROFF and FF sites, neither between FF and PB sites.

The results shown in Table 1 and Fig. 2 indicate that the δ^{15}N values of soft tissues of a single *B. perforatus* individual and/or of pooled samples of five individuals were significantly higher at the anthropogenically affected PB sites (7-11) relative to those from the reference site at the Lumbarda Reef Flat (site ROFF 1), as well as at other isolated offshore reefs, such as Puh and Balun Reef Flats, and the Island of Dužac (sampling sites ROFF 2-4). The ^{15}N enrichment was as high as 5.6‰. Slightly lower enrichment in ^{15}N (up to 2.4‰) was detected in *B. perforatus* samples collected around the fish cages (sampling sites FF 5 and 6).

The δ^{13}C values of *B. perforatus* soft tissue of a single animal and of the pooled samples of five individuals show a small, but insignificant difference between locations affected by sewage or fish farms, and reference and other less impacted sampling sites (Table 1, Fig. 2). However, slightly higher δ^{13}C values were found in barnacles from Pirovac Bay (sampling sites PB 7-11) and to some extent in those collected around the fish cages (sampling sites FF 5 and 6) relative to those living at the reference or at other offshore locations (sampling sites ROFF 1-4). The isotopic data for blue green algae colonizing the barnacle shells are scarce, since it was not always possible to obtain enough material for analyses. However, the preliminary results indicated a significant difference in nitrogen isotopic composition between samples from Pirovac Bay and reference, as well as of offshore locations (Table 1, Fig. 1).

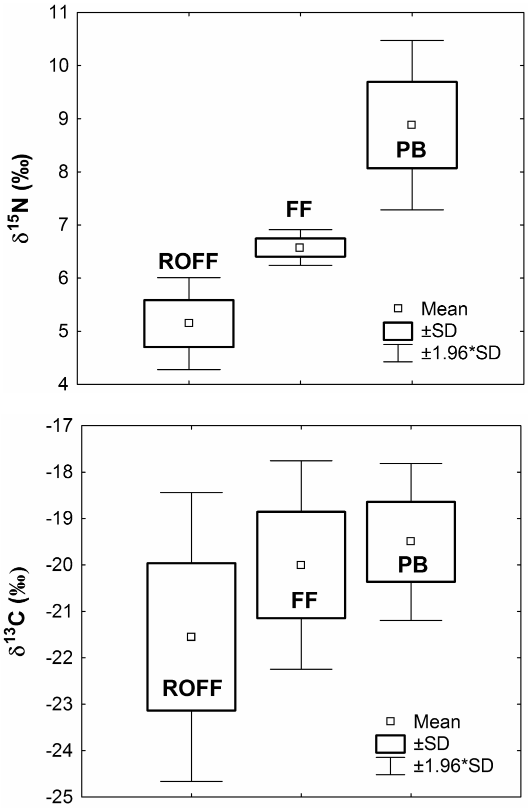

Figure 2. Whisker plots of δ^{15}N and δ^{13}C and values of *Balanus perforatus* (ROFF - reference and offshore locations; FF - fish farm sampling sites; PB - Pirovac Bay).

The specimens from Pirovac Bay yielded a range of δ^{15}N values from + 5.5 to + 5.9‰, while those from the reference and others unpolluted locations exhibited δ^{15}N values in the range between + 2.4 and + 3.1‰. The δ^{13}C values of blue green algae show no significant difference between affected and reference sampling sites. However, those from the pristine, offshore locations are slightly enriched in ^{12}C relative to those from Pirovac Bay.

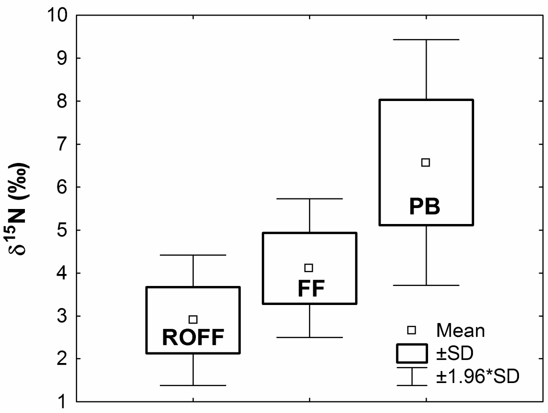

Figure 3. Whisker plot of δ^{15}N values of particulate organic matter - POM (ROFF - reference and offshore locations; FF - fish farm sampling sites; PB - Pirovac Bay).

δ^{15}N values of POM collected at the same sites as organisms (Fig. 3) were significantly higher inside Pirovac Bay and around fish farms than at the unaffected reference and other offshore sampling sites south of the Kornati Islands, including the lighthouse of Blitvenica (sample group ROFF, Fig. 1). The standard deviations of group mean δ^{15}N of POM were much larger than in *B. perforatus* (2.9 ± 0.77‰, 4.11 ± 0.83‰ and 6.57 ± 1.46‰ at ROFF, FF and PB sites, respectively). It should be noted that the seasonal variations in δ^{15}N values of POM show a general trend toward the enrichment in ^{15}N from April to August. The observed enrichment attained up to 5.0‰ in Pirovac Bay, 2.5‰ around the fish cages and 2.9‰ at the reference and other offshore locations. A statistically significant difference in the mean δ^{15}N values of POM was observed between ROFF and PB sites, as well as between FF and PB sites, while the difference between ROFF and FF sites was significant only at $p = 0.066$.

4. Discussion

Cerripedia includes the familiar benthic marine animals known as barnacles, as well as some unfamiliar and bizarre parasites.[23] Most cerripedes are regarded as suspension feeders, feeding on phytoplankton and bacteria, although some of them capture small crustaceans and other animals and are therefore classified as predators rather than suspension feeders.[24] The acorn barnacles, such as *B. perforatus* selected for this study, are sessile filter feeders, attached directly to the substrate, that capture their food with thoracic appendages known as cirri.

The stable isotope composition of *B. perforatus* from the investigated area varied over a range of 5.6 - 6.9‰ for both carbon and nitrogen. Shifts in trophic position or feeding patterns are not a likely explanation for the observed variability, since these animals are known to rely on fairly constant diets consisting of a mixture of detritus, phytoplankton and zooplankton.[25-27] The observed variability in *B. perforatus* stable carbon and nitrogen isotopic composition can thus be related, according to Wissel and Fry,[28] to the changes in sources supporting the food web rather than changes in food types or feeding strategies.

Several studies suggested that ecosystems loaded by effluents deriving from human sewage or fish farming should exhibit differences in the δ^{15}N values at each trophic level. As the human and animal waste nitrate have a distinguishable nitrogen isotopic composition with δ^{15}N values mostly in the range between + 10 and + 22‰,[29, 30] the elevated δ^{15}N values of NO$_3$ > + 10‰ are regarded as being indicative for the faecal N origin.[31] This nitrogen is assimilated by primary producers and transferred further into consumers, affecting their N isotopic composition. Elevated δ^{15}N values have been identified in marine biota exposed to ground water contaminated by septic wastes[32] and sewage effluents.[4, 17, 33-39] Increased δ^{15}N values of about + 7.9‰ were also measured in POM dominated by untreated faecal matter of Jepara Bay.[40] Similar values (+ 8.0‰) were found in POM near the inflows from septic systems in the Port of Murter in Pirovac Bay (Dolenec et al., unpublished). On the other hand, organic matter deriving from the sewage effluents with low δ^{15}N values was found near outlets of primary treated sewage,[1, 18, 41, 42] causing the invertebrates to exhibit lower δ^{15}N values in the vicinity of sewage outfalls than at the nearby unpolluted marine locations.

A ^{15}N enrichment has also been found in marine plants and other biota around the fish cages.[8, 33, 43, 44] Marine POM collected in areas impacted by fish farming exhibits δ^{15}N values distinctly higher than that from pristine locations.[7] Therefore it was assumed that the N isotopic composition of POM would influence the range of the δ^{15}N values of the entire food web at affected sampling sites.[45-47] Identical trophic structures - in our case *B. perforatus* individuals - utilising POM with different nitrogen isotopic composition should thus reflect these differences.[11] As the size, depth and the season of collection were taken into account during the sampling procedure, δ^{15}N variability of *B. perforatus* may indicate only the variations in $\delta^{15}N$ values of their diet.

B. perforatus from the reference and other pristine offshore locations show significantly lower $\delta^{15}N$ values ($p < 0.05$) relative to those from Pirovac Bay and from the fish farm sites (Table 1, Fig. 2). This can be explained by the intake of POM of fully marine origin with $\delta^{15}N$ between + 1.5 and + 3.8‰ (mean 2.9‰). On the other hand, *B. perforatus* from Pirovac Bay with increased $\delta^{15}N$ values in the range from + 7.7 to + 10.0‰ (mean + 8.9‰) indicate that their food is enriched in ^{15}N. Therefore we proposed that ^{15}N enrichment in barnacles is due to the contribution of nitrogen from anthropogenic sources, which also accounts for the most of enrichment of POM with ^{15}N in Pirovac Bay. The $\delta^{15}N$ values of POM (+ 4.3 to + 9.7‰, mean + 6.6‰) could be interpreted as a mixture of (1) in-situ plankton, (2) terrestrial organic matter and (3) a prevalent component of organic matter deriving from sewage, enriched in ^{15}N, which masked the original $\delta^{15}N$ values of POM.

Sewage effluents from the septic systems enriched in heavy nitrogen could account for the ^{15}N enrichment not only in barnacles, but also in other biota and POM from Pirovac Bay.[33, 43] For example, NO_3^- from sewage waste shows significantly higher $\delta^{15}N$ values than other NO_3^- sources[29, 41] and thus the overall ecosystem $\delta^{15}N$ increase with the degree of urbanization.[32, 48, 49]

Barnacles from Pirovac Bay (sampling sites PB 9-11) exhibiting the highest $\delta^{15}N$ values indicate a larger input of sewage wastes, which decrease with distance from the inner part of the Bay. This is indicated by lower $\delta^{15}N$ values of *B. perforatus* individuals (sampling sites PB 7 and 8), as well as of other benthic organisms, such as *Anemonia sulcata* and *Aplysina aerophoba*.[33, 43] We may hypothesize that mixing of sewage affected seawater from the inner part of Pirovac Bay with less polluted SE-SW sea current may have diluted the ^{15}N enriched waters of the Bay.

Similarly we can explain the nitrogen isotopic composition of *B. perforatus* from the fish farms (sampling sites FF 5 and 6). Their $\delta^{15}N$ values (range: + 6.4 to + 6.8‰; mean 6.6‰) are significantly higher than those from the reference and offshore locations (sampling sites ROFF 1 - 4) and significantly lower ($p < 0.05$) as compared to barnacles sampled in Pirovac Bay (sampling sites PB 7 – 11, Table 1, Fig. 2). These values reflect the presence of ^{15}N enriched POM derived from fish farming, with $\delta^{15}N$ values from + 3.0 to + 5.5‰ (mean 4.1‰).

$\delta^{13}C$ values are preferably used to indicate the origin of carbon sources rather than as an indicator of the trophic level. The general pattern of inshore benthos-linked food webs being more enriched in ^{13}C compared to offshore, pelagic food webs presents a potentially useful tool.[50] In *B. perforatus,* differences in $\delta^{13}C$ values were found between specimens collected at different locations (Table 1, Fig. 2). The $\delta^{13}C$ values of those collected at the reference site and at other offshore locations (sampling sites ROFF 1 - 4) range from - 19.3 to - 25.4‰ (mean - 21.6‰), while those from Pirovac Bay (sampling sites PB 7 - 11) showed $\delta^{13}C$ values between - 18.5 and - 20.6‰ (mean - 19.5‰). The carbon isotopic composition of the barnacles from the fish farm sites (5 and 6) with the range from - 18.3 to - 20.5‰ (mean - 20.0‰) are similar to those from Pirovac Bay. This indicates that the main carbon source in the open sea is depleted in ^{13}C, while in Pirovac Bay and around fish cages it is somewhat enriched in heavy carbon. Typically, higher $\delta^{13}C$ values in coastal than in offshore food webs were also observed by Hobson.[51] Lojen et al., (unpublished) measured lower $\delta^{13}C$ values of about - 23.0‰ for net plankton from the offshore locations in the Central Adriatic and higher $\delta^{13}C$ values of around - 21.5‰ from the coastal part of Murter Sea.

Two possible processes might account for the higher $\delta^{13}C$ values found in barnacles from Pirovac Bay and around fish farms. First, higher $\delta^{13}C$ values could reflect a carbon source originating from the septic systems and fish farm derived effluents in addition to the marine derived organic carbon. This is in agreement with the observations made by Bachtiar,[52] who found that the carbon isotopic composition of the sewage effluents at the Burlington Skyway Sewage Treatment Plant in Hamilton Harbour exhibit the highest $\delta^{13}C$ values of about - 22.9‰ right at the STP outfall. Higher $\delta^{13}C$ values of - 16.5‰ of composite sewage effluent particulate matter were reported also by Spies.[18] Isotopically heavier C was also found in *Balanus amphitrite* from the fish ponds and closed lagoons relative to those from the open sea.[53] Further investigation showed that the carbon isotopic composition of POM and benthic fauna in fish and/or shrimp ponds is enriched in ^{13}C,[53, 54] most probably due to the presence of remains of pelleted food. Shrimp feed had $\delta^{13}C$ of - 20.7 ± 0.4‰,[54] while pelleted fish food for finfish at Vrgada Island exhibit $\delta^{13}C$ in the range between - 20.1 and - 23.9‰ (Lojen et al., unpublished).

Secondly, it is also possible that the high $\delta^{13}C$ values measured in *B. perforatus* from Pirovac Bay and fish cages could be influenced by some other factors, such as temperature or elevated $\delta^{13}C$ values of dissolved inorganic carbon (DIC). For example, the progressive enrichment of the DIC in ^{13}C in the euphotic zone during the spring algal bloom, as well as the increase of the water temperature, could have increased the $\delta^{13}C$ values of phytoplankton, which is an important component in POM.[2] However, further investigation is needed to prove this suggestion. Therefore we propose that ^{13}C enrichment of the *B. perforatus* from Pirovac Bay and around the fish farm is mostly due to the contribution of carbon from sewage and/or fish farm effluents, which possibly affected the surrounding environment by releasing ^{13}C-enriched particulate wastes.

Further indications of anthropogenic pollution are $\delta^{15}N$ and $\delta^{13}C$ values of blue green algae colonizing barnacle's shells. Those from reference and offshore locations are generally enriched in light carbon and nitrogen isotopes and exhibit lower $\delta^{13}C$ (- 18.8 to - 25.0‰; mean - 21.3‰) and $\delta^{15}N$ values (+ 2.4 to + 3.1‰; mean + 2.9‰) relative to those from Pirovac Bay. The isotopically heavier C and N of green algae from Pirovac Bay could be related to the enrichment of DIC with ^{13}C and ^{15}N enriched nitrogen mostly derived from septic systems, since conversion of the sewage urea to the nitrate through hydrolysis may increase the resulting $\delta^{15}N$ values by 5-15‰.[29]

5. Conclusions

The presented study revealed the widespread evidence of the presence of sewage and aquaculture derived particulate and dissolved material in the semi-enclosed Pirovac Bay and in the area around fish farms and its transfer into the local ecosystem. These effluents significantly affected the isotopic composition; especially the $\delta^{15}N$ values of *B. perforatus*, blue green algae and POM, and to a lesser extend the $\delta^{13}C$ values of barnacles and the blue green algae. The presence of effluents deriving from sewage was detected in the entire Pirovac Bay. However, its cessation toward the more open NW part of the Bay was also observed, most probably due to the local hydrodynamic regime, influenced by the prevalent SE-NW sea currents. The *B. perforatus* nitrogen is enriched in ^{15}N relative to their diet (POM). The Δ *Balanus* - POM values ($\delta^{15}N_{Balanus}$ - $\delta^{15}N_{POM}$) range from 2.2 to 2.5‰ regardless of the sampling location and could be explained by the small tropic enrichment at the base of the food web.

6. Acknowledgement

This research was financially supported by the Ministry of Higher Education, Science and Technology, Republic of Slovenia (Research Programmes P1-0195-1555, P1-0143-0106 and a Bilateral Cooperation Project between Slovenia and Croatia for 2005-2006), and Geoexp, d. o. o., Tržič, Slovenia. Thanks also to Dr. Anthony R. Byrne for linguistic corrections.

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Povzetek

Z masnospektrometrično analizo izotopske sestave dušika in ogljika v vitičnjakih vrste *Balanus perforatus* smo raziskovali vpliv neobdelanih komunalnih odplak in izpustov iz ribjih farm na priobalne ekosisteme na območju Murterskega morja (srednji Jadran). Rezultati kažejo, da se vrednosti δ^{15}N in δ^{13}C v tkivu vitičnjakov iz Pirovškega zaliva in priobalja Murterskega morja, ki sta onesnažena predvsem s komunalnimi odplakami, statistično značilno razlikujejo od primerkov z neonesnaženih vzorčnih mest na odprtem delu Murterskega morja in Kornatskega otočja. Dobljeni podatki nakazujejo možnost uporabe omenjenih bentoških organizmov za monitoring vpliva odpadnih voda na obalne ekosisteme.