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Using stable nitrogen isotopes in *Patella* sp. to trace sewage-derived material in coastal ecosystems

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ABSTRACT

Coastal environments are often exposed to different anthropogenic contaminants that can cause evident differences in coastal ecosystems. For this reason the use of various organisms as an indicator offers an important ecological study. In this survey the limpet *Patella caerulea* was examined as a potential anthropogenic bioindicator in coastal marine ecosystems using nitrogen isotope composition measurements. The results indicated generally significant variations between sampling sites. Lower δ^{15}N values were measured in less polluted areas, while at potentially polluted sites δ^{15}N enrichment was observed. The lowest δ^{15}N values were observed near a larger town where a well-regulated purification plant system was in operation and removing undesirable substances (i.e. nitrates) from the sewage. The results suggest that the limpets are useful indicators for tracing anthropogenically derived organic matter from coastal areas in marine ecosystems. Additionally, relatively small differences in δ^{15}N values were observed in different sizes of limpets, which suggest a rather uniform diet over the organism's size spectrum. δ^{15}N variations between tissues in an individual organism were practically negligible.

1. Introduction

Stable isotopes have been used effectively in ecological studies to trace the impact of different components, and consequently potential pollutants, on ecosystems, as well as to trace food webs. They provide a powerful tool for source determination and following anthropogenically derived material (from animal waste, septic systems, sewage treatment plants, etc.) from their source to different segments in the environment (Rau et al., 1981; Heaton, 1986; Tucker et al., 1999; Heikoop et al., 2000; Risk and Erdmann, 2000; Costanzo et al., 2001; Sigleo and Macko, 2002; Sarà et al., 2004; Vizzini and Mazzola, 2004; Vizzini et al., 2005; Fertig et al., 2009; Lassauque et al., 2010; Matsuo et al., 2010; Sherwood et al., 2010; Xu and Zhang, 2012). In these studies, various organisms (from primary producers to upper consumers) were used to understand the influence on the environment and determine the most applicable organism to function as an anthropogenic tracer.

Dillon et al. (2005) found a δ^{15}N value of approximately -5.4 ± 2.6‰ in ammonium from rainwater sources, while wastewater ammonium had values ranging from +16 to +25‰ (Cifuentes et al., 1988; Desimone and Howes, 1996). The mean $\delta^{15}NO_3$ values from rainwater were -0.4 ± 2.1‰, while from municipal wastewater the outfall was from $+4.83 \pm 4.52$‰ (Dillon et al., 2005; Dillon and Chanton, 2008). Previous studies on nitrogen isotope composition (δ^{15}N) indicated significant differences between organisms exposed to anthropogenic sewage particles and naturally occurring materials (Tucker et al., 1999; Sarà et al., 2004, and references therein). It was established that nitrogen isotopic signatures of organisms (POM, macroalgae, seagrass, mussels, corals, fishes, etc.) from anthropogenically impacted areas were usually enriched compared to unpolluted sites (Tucker et al., 1999; Heikoop et al., 2000; Costanzo et al., 2001; Vizzini and Mazzola, 2004, 2006; Vizzini et al., 2005). Also δ^{15}N values indicate trophic relationships among organisms in diverse ecosystems (Hobson et al., 2002; Iken et al., 2005; Corbisier et al., 2006; Žvab et al., 2010; Lemos Bisi et al., 2012). Furthermore, variations in δ^{15}N could also be related to size and/or age differences (Miniwaga and Wada, 1984; Wada et al., 1993; Jennings et al., 2002; Vizzini and Mazzola, 2002), differences in depth (Muscatine and Kaplan, 1994) and seasonal effects (Costanzo et al., 2001).

Previous studies on the nitrogen isotope composition (δ^{15}N) of several marine organisms (particular organic matter, zooplankton, anemone, barnacles, sea grass, sponge, mussels) were used to trace anthropogenic pollution in the Adriatic Sea (Dolenec and Vokal, 2002; Dolenec et al., 2005, 2006a, 2006b, 2006c, 2007, 2011; Rogan et al., 2007; Žvab et al., 2010). Our survey was focussed on the limpet *Patella caerulea*, which is a rather common organism, especially along the rocky eastern coast of the Adriatic Sea. Coastal

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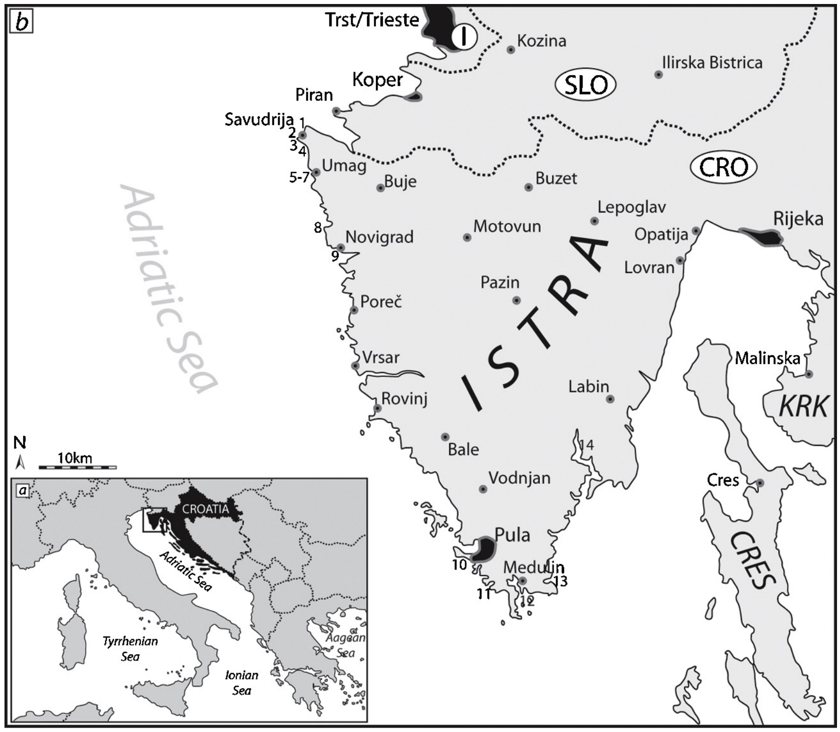

Fig. 1. Position (marked by a box) of the study area in Southern Europe (a) and detailed sampling locations (1–14) along the Istrian coast (b).

areas are exposed to anthropogenic nitrogen inputs from untreated domestic and municipal waste from cities, marinas, tourist areas, and camping sites, as well as aquaculture activities.

This paper presents the results of a survey utilising nitrogen stable isotope analyses to investigate the amount of discharge material (mainly municipal sewage) along the Istrian coast. The main purpose of study was to measure the nitrogen isotope composition of *Patella* sp. (*P. caerulea*) as a potential bioindicator for tracing anthropogenic pollution in the coastal marine environment. Furthermore, the objective was also to detect possible isotopic variations between limpets of different sizes or between different organism tissues.

2. Materials and methods

2.1. Study area

Istria is the largest peninsula in the Adriatic Sea, the northwest-southeast extending arm of the Mediterranean Sea. The Istria Peninsula is located in the northern Adriatic between the Gulf of Trieste in the north and the Bay of Kvarner in the south (Fig. 1a). The area of Istria lies predominately in Croatia, but is also shared with Slovenia and Italy. The coast of the Istria Peninsula is generally rocky with a tidal range of about 1.2 m.

Samples of limpets were collected in the summer of 2008 at 14 locations between Savudrija in the north and the Raša estuary in the south (Fig. 1b). Distances between sampling locations were between a few hundreds of metres (around some bays of cities) up to tens of km (between cities). Because the aim of the study was to observe the impact of different unnatural potential pollutants on limpets, sampling sites were exposed to different sources as well as amounts of anthropogenic inputs, including municipal and industrial sewage from cities, marinas, ports, and tourist facilities that are specially impacted in the summer.

2.2. Limpet Patella sp.

Patella sp. is a limpet with a rounded to oval conical shell, a diameter of up to 60 mm and height around 10 mm. The shell can be greyish-white, smooth, granulated or coarse, with radial and concentric lines. The mantle edge overhangs a shallow groove, extended around the entire body, which houses the pallial gills. The muscle scar is horseshoe-shaped with an anterior opening (Harvey, 2009). It inhabits the medio-littoral zone, attaching to firm substrates including rocks and stones where dense populations form. The shape of the shells and its pedal muscle enable the limpet to live in this ecosystem, and to defy conditions like battering waves and tides. They are fixed on the substrate, and during moist conditions or high tides commonly move around to graze on algae and assorted debris covering the surrounding surface. During low tides they return to their primary spot, and inhabit exactly the same place by following the mucus trail that they deposit. Especially on calcareous rocks, they dig a depression that helps them attach and better shield the organism from dehydration and desiccation during periods of dryness (Fish and Fish, 1996; Hill, 2000; Nakhlé et al., 2006).

Common limpets begin their life as males, becoming sexually mature at around 9 months of age. Most individuals undergo a sex change, typically becoming female at 2 or 3 years of age, although some remain as males. Spawning takes place once a year, usually from October to December. Fertilisation occurs externally; the larvae spend their first few days of life in the water column, after which time they settle on the shore. The lifespan varies, but is between 10 and 20 years (Fish and Fish, 1996; Hill, 2000).

In our research, limpet *P. caerulea* were chosen for isotopic analyses. This species is one of the most abundant limpets in the Atlantic Sea, and inhabits the lower midlittoral (Simunovic, 1970; Della Santina et al., 1993; Santini and Chelazzi, 1995). Due to its limited inhabited area the diet also reflects the specific algal community typical of this zone (Della Santina et al., 1993).