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Food web of the intertidal rocky shore of the west Portuguese coast – Determined by stable isotope analysis

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ABSTRACT

The characterization of food web structure, energy pathways and trophic linkages is essential for the understanding of ecosystem functioning. Isotopic analysis was performed on food web components of the rocky intertidal ecosystem in four sites along the Portuguese west coast. The aim was to 1) determine the general food web structure, 2) estimate the trophic level of the dominant organisms and 3) track the incorporation of organic carbon of different origins in the diet of the top consumers. In this food web, fish are top consumers, followed by shrimp. Anemones and gastropods are intermediate consumers, while bivalves and zooplankton are primary consumers. Macroalgae *Bifurcaria bifurcata*, *Ulva lactuca*, *Fucus vesiculosus*, *Codium* sp. and phytoplankton are the dominant producers. Two energy pathways were identified, pelagic and benthic. Reliance on the benthic energy pathway was high for many of the consumers but not as high as previously observed in subtidal coastal food webs. The maximum TL was 3.3, which is indicative of a relatively short food web. It is argued that the diet of top consumers relies directly on low levels of the food web to a considerable extent, instead of on intermediate levels, which shortens the trophic length of the food web.

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1. Introduction

Rocky reefs are widely recognized has providers of goods and services for humans, like defence against storms, recreation and fisheries (Duarte, 2000). It is also acknowledged that these habitats are threatened in many parts of the world, by population pressure, urbanization, pollution, climate warming and rising sea levels (Thompson et al., 2002; Helmuth et al., 2006).

The Portuguese west coast presents numerous rocky reefs, some of them with a large intertidal area, harbouring rich biological communities (Boaventura et al., 2002). This is a highly hydrodynamic coastline, with a semilunar tidal regime. General knowledge on the distribution patterns of dominant species exists (Boaventura et al., 2002) and some communities and biological groups have been studied in depth (e.g. Saldanha, 1974; Marques et al., 1993; Cruz, 1999; Range and Paula, 2001; Flores et al., 2002; Silva et al., 2003; Dias et al., 2014; Mendonça et al., 2015; Vinagre et al., 2015).

This coastal area is under important pressure from urbanization, human effluents and illegal fisheries. However, fast coastal receding from erosion is probably the most visible challenge that policy makers have to deal with, and rising sea levels the most immediate threat to the ecosystems therein. Monitoring of falling cliffs is underway, along with the construction of peers and artificial sand addition in some areas.

Intertidal living resources have an important socio-economical role along the Portuguese coast, being highly exploited by professional and recreational fishermen. Stakeholders generally acknowledge the need for management of these habitats' exploitation, however studies on the structure and dynamics of the intertidal food web, which should be the basis for management, are still missing.

Rocky reefs support diverse communities that encompass all major taxonomic groups. Early marine biologists focused much of their attention on this habitat because of its high biodiversity, complex dynamics and easy accessibility. Food web theory has been built mostly through the investigation of the community dynamics of rocky shores (e.g. Paine, 1966, 1974; Menge, 1976).

Stable isotope analysis, $\delta^{13}C$ and $\delta^{15}N$ in particular, has been increasingly used for the description of food webs in a wide variety of ecosystems because they allow the estimation of the food sources used by organisms over time, allowing a characterization of their feeding habits and trophic levels (e.g. Kwak and Zedler, 1997;

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Riera et al., 1999).

The nitrogen isotope ratio is a marker of trophic position, since $\delta^{15}N$ values increases by 2.5–4.5‰ from prey to predator (Owens, 1987; Peterson and Fry, 1987; Post, 2002), being thus useful for the depiction of the food web basic topology. The $\delta^{13}C$ isotope is useful to elucidate the origin and pathways of organic matter in food webs, an issue of crucial importance in the understanding of ecosystem's functioning, since it informs on what energy pathway top consumers rely. This is possible when the primary sources are isotopically distinct, allowing the identification of the pelagic and benthic contributions to the food web (Hobson et al., 2002) and the tracing of particulate organic through the food web (Le Loc'h and Hily, 2005). Because primary consumers reflect the isotopic composition of their food sources (Post, 2002), they can be used as an isotopic baseline to estimate the reliance of the upper consumers on prey with benthic affinity (Sherwood and Rose, 2005; Le Loc'h et al., 2008).

Coastal ecosystems' trophic structure and pelagic–benthic coupling are well studied using stable isotopes, however studies on the rocky intertidal food web are scarce (e.g. Hansson et al., 1997; Post, 2002; Darnaude et al., 2004; Vizzini and Mazzola, 2006; Le Loc'h et al., 2008; Riera et al., 2009; Schaal et al., 2011; Vinagre et al., 2008, 2011; 2012a; Richoux and Ndhlovu, 2014).

The aim of the present study is to 1) determine the general food web structure, 2) estimate the trophic level of the dominant organisms and 3) track the incorporation of organic carbon of different origins through the estimation of the reliance on benthic affinity prey of the top consumers, in the rocky intertidal ecosystem of the Portuguese west coast.

2. Material and methods

2.1. Study area

Four sites were chosen in the west coast of Portugal, distanced on average 23 km, from south to north: Praia das Maçãs (38°49′37″N, 9°28′12″W), Ericeira (38°58′12″N, 9°25′18″W), Lourinhã (38°14′28″N, 9°20′38″W) and Baleal (39°22′15″, 9°22′19″) (Fig. 1). Sites were located in rocky reefs with an extensive intertidal area, typically dominated by barnacles and mussels (Boaventura et al., 2002). Algal coverage was similar in all the sites, including *Codium* sp., *Fucus vesiculosus*, *Ulva lactuca* and *Bifurcaria bifurcata*.

The study area, which is the westernmost coastal area of Europe, has a north-south orientation, is affected by strong hydrodynamics and is exposed to northwest oceanic swell, which can reach heights of 5 m during winter storms. The tidal regime is semidiurnal with tides that can range up to 4 m.

2.2. Sampling

Surveys were conducted in the intertidal area, in the low mediolitoral, in September 2013, late summer, after 3 months of summer drought to insure prior stable conditions. Samples (3 replicates per site) of water (2 l per replicate) for future POM analyses. Phytoplankton and zooplankton were also collected at each site. Water was collected in mid-water with bottles, nearshore at depths of 1.20 m. Zooplankton was collected from horizontal zooplankton trawls (width = 25 cm), with a 335 μm mesh, with a duration of 5 min. Phytoplankton was collected from horizontal phytoplankton trawls (width = 25 cm), with a 55 μm mesh, with a duration of 5 min. Phytoplankton samples were then sieved through a 250 μm mesh to remove large zooplankton and detritus. Zooplankton and phytoplankton trawls were performed manually in mid-water, nearshore at depths of approximately 1.20 m. Subsamples of phytoplankton and zooplankton were always examined at the microscope after collection, to insure that the sample was mostly composed of phytoplankton or zooplankton, respectively.

Macroalgae and intertidal fauna were collected manually and with hand nets over the rock and in tidal pools. A fishing rod was used to capture nekton in the channels within the rocky shore. Such nekton is part of the rocky shore intertidal food web, since it feeds in the intertidal when the tide goes up. Number of replicates was 3 for macroalgae and 5–8 for animals, per site. Specimens of intertidal fauna were measured (total length for fish, shrimp and bivalves, diameter for anemones and gastropods) (Table 1). Samples were stored at −20 °C and analysed within less than a month.

2.3. Stable isotope analysis

Water samples for POM analysis were filtered until clogged onto pre-combusted filters (Whatman GF/A glass microfiber, nominal pore size 1.6 μm). The whole body of cnidarian species was used after their stomachs were emptied. In the case of molluscs the foot was dissected. Dorsal muscle tissue samples of all fish and shrimp were dissected. Tissues of all species were dried at 60 °C and ground to fine powder with a mortar and pestle.

In samples suspected of having carbonate contamination, such as those of POM, phytoplankton, zooplankton and invertebrate species, a subsample was taken and acidified with several drops of 10% HCl while being observed under a dissecting microscope. If bubbling occurred the sample was acidified, rinsed with distilled water, re-dried at 60 °C and stored in glass vials, following the procedure described by Pinnegar and Polunin (1999). Whenever the test was positive for carbonates, subsamples were acidified and used to obtain $\delta^{13}C$ values, and only those values were used in any calculations using $\delta^{13}C$.

$^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the samples were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) (Preston and Owens, 1983), on a Isoprime (GV, UK) stable isotope ratio mass spectrometer, coupled to an EuroEA (EuroVector, Italy) elemental analyser for online sample preparation by Dumas-combustion.

Isotope ratios were expressed as parts per thousand (‰) according to:

$$\delta X = [(\text{Rsample}/\text{Rstandard}) - 1] \times 10^3,$$

where X is ^{13}C or ^{15}N, R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ and δ is the measure of heavy to light isotopes in the sample.

The standards used were IAEA-N1 and IAEA-600 for nitrogen isotope ratio, and IAEA-CH6 and IAEA-CH7 or IAEA-600 for carbon isotope ratio; $\delta^{15}N$ results were referred to Air and $\delta^{13}C$ to PeeDee Belemnite (PDB). Precision of the isotope ratio analysis, calculated using values from 6 to 9 replicates of laboratory standard material interspersed among samples in every batch analysis, was ≤0.2‰.

2.4. Data analysis

Analyses of variance were conducted in order to test if the POM and phytoplankton values were similar between the four sites, to insure that the data from the four sites could be used in a single food web characterizing this coastal stretch as a whole. A significance level of 0.05 was considered.

The following equation was used to estimate trophic level (TL):

$$TL = \left(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{mean baseline}}\right) \Big/ 3.4 + 1,$$

where 3.4‰ is assumed to be the ^{15}N trophic fractionation factor, according to Minagawa and Wada (1984), Post (2002) and Akin and

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