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Can stable isotopes be used to infer site fidelity of nekton in open coastal areas?

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ABSTRACT

Stable isotope analysis has been applied to the investigation of movement in several species, including marine animals. However, its application to nekton living in open coastal areas is still very scarce. This study aims to test if stable isotope analysis can be used for this purpose over a much wider spatial scale than previously investigated. Stable isotope analysis was used to 1) investigate isotopic variation in shrimp and fish, in 11 sites, along a 160 km coastal stretch, to 2) determine the site fidelity of the individuals within each species, and to 3) test the relation between the body size of the individuals within each species, at each site and the percentage of isotopic deviants. Site fidelity was the highest for the intertidal fish *Gobius paganellus* and *Coryphoblennius galerita*, with 60% and 64% of individuals considered residents, respectively, and lowest for the demersal fish *Diplodus vulgaris* and *Diplodus sargus* with 23% and 33% of resident individuals, respectively. The percentage of isotopic deviants was not correlated with length in any species. Site fidelity was considerably higher than that previously found for other open coastal areas and similar to more structured environments, like coastal ponds. It was hypothesized that the complex tri-dimensional structure of the rocky reefs that occur in this area, often encompassing channels and tide pools, offers conditions favourable to high site fidelity. This study shows that stable isotopes can be used to infer nekton movement in wide open coastal areas.

1. Introduction

Tracking animal movements is a pivotal endeavor for environmental management, conservation and theoretical biology. However, this is particularly difficult in the marine environment, where recapture rates of externally tagged animals are quite low. In addition, many organisms are too small to hold satellite or radio transmitters for direct monitoring (Hobson, 2003).

Stable isotopes are naturally occurring tags that have been used to study movement in several animal groups, from butterflies to birds, shrimp, fish and turtles (e.g. Hobson and Wassenaar, 1997; Fry et al., 1999; Hobson et al., 1999; Herzka, 2005). Large-scale migrations, small-scale movement and site-fidelity have been inferred in various marine species (e.g. Fry et al., 1999; Cherel and Hobson, 2007; Vinagre et al., 2008, 2011a; Carlisle et al., 2012).

The use of stable isotopes relies on the fact that isotope concentrations in animal tissues reflect those in their foodwebs and that they vary spatially in nature. Thus, knowledge on the dynamics of stable isotopes in animal tissues, on the ecology of the target-species and on the temporal and spatial patterns of isotopic signatures across food webs, can be combined to infer animal movements (Hobson, 2003).

A mosaic of isotopic signatures has been identified in estuarine environments, associated with the salinity gradient and the occurrence of distinct habitats and sources of organic matter (Fry, 1983; Fry et al., 2003; Kline et al., 1998; Vinagre et al., 2008, 2011a). This spatial variation in isotopic values is particularly useful for the inference of movement in mobile marine animals, particularly fish and shrimp, since nekton that spends several weeks feeding at a single site should reflect the isotopic signature of that site (Fry et al., 1999, 2003; Herzka et al., 2002). Transient individuals migrating between sites, or that explore simultaneously more than one site, will have a more varied isotopic composition, or intermediate isotopic values between several local isotopic signatures (Fry et al., 1999, 2003; Herzka et al., 2002).

Various studies have explored this environmental mosaic of isotopic signatures and reported different levels of site fidelity or residency among species using different habitats within an estuary, mostly common shrimp and fish (Deegan et al., 1990; Fry et al., 2003; Vinagre et al., 2008; Vinagre et al., 2011a); using estuarine and adjacent coastal habitats (Fry and Parker, 1979; Deegan et al., 1990; Fry et al., 2003; Augley et al., 2007; Leakey et al., 2008) and

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occurring in neighbouring estuaries (Hansson et al., 1997; Kwak and Zedler, 1997; Griffin and Valiela, 2001).

Vinagre et al. (2011b) reported that the site fidelity of coastal fish and cephalopoda could also be studied based on the isotopic mosaic that occurs at coastal areas adjacent to major rivers, since the terrestrial input creates a gradient of spatial isotopic signatures that extends from the estuary to open coastal waters. The number of individuals with a central isotopic range (defined as the range of values indicative of similar feeding location, which is ±1‰ for $\delta^{13}C$ and $\delta^{15}N$ values, according to Fry et al., 1999) was surprisingly high and comparable to more physically structured environments like estuaries. It also revealed that the coastal nekton analysed had a close relation to the 30 km coastal stretch studied.

Many common coastal species are important for fisheries as target-species or as common prey of target-species. For their effective management it is of pivotal importance that their feeding ranges are well known, so that feeding grounds can also be managed in a sustainable way. However, feeding ranges of such species are generally unknown, especially for their juvenile phases, which occur in shallow waters. Juvenile individuals are usually not easy to study through tagging or telemetry due to their reduced size, this way isotopic studies have the potential to address this gap in knowledge that hinders the appropriate management of coastal species.

The present study aims to test this concept one step further, investigating nekton site fidelity over a much wider spatial scale, 160 km, in the west Portuguese coast. This coastal stretch is influenced by two major rivers and several smaller rivers, thus offering small-scale variability in environmental isotopic composition.

This area harbours local fisheries and is located at a biodiversity hotspot that has been selected has a priority for global conservation efforts (Myers et al., 2000). This way, knowledge on the site fidelity of its most abundant nekton species is valuable for an informed management of natural resources.

The objectives of the present study are to 1) investigate isotopic variation in shrimp and fish, 2) determine the site fidelity of the individuals in relation to 11 sites along a 160 km coastal stretch, using the concept of central isotopic range, as defined by Fry et al. (1999) and 3) test the relation between the body size of the individuals at each site and the percentage of isotopic deviants (individuals outside the central isotopic range).

2. Methods

2.1. Study area

The coastal stretch selected for this study is located in the west Portuguese coast, from the beach of Baleal (39°22′15″, 9°22′19″, site A, Fig. 1) to the beach of Figueirinha (38°29′35″N, 8°56′26″, site K, Fig. 1), encompassing 160 km of open coasts. It is composed mostly of rocky shores and some sandy beaches. The prevailing sediment is coarse sand. The study area, which is the westernmost coastal area of Europe, 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 at eleven sites (Fig. 1), in the intertidal and subtidal area, in September 2013, after 3 months of summer draught to insure prior stable conditions. Organisms were collected within a limited site (one 5 m radius area per site) and many km apart from the next site (Fig. 1). Collection of organisms was carried out in the lower intertidal area during low tide, in tide pools and channels, at a depth of less than 1 m. Shrimp (*Palaemon elegans* and *P. serratus*) and fish (*Gobius paganellus*, *Lipophrys pholis*, *Coryphoblennius galerita*, *Diplodus sargus* and *Diplodus vulgaris*) were collected with hand nets and a fishing rod. Only *D. sargus* and *D. vulgaris* were captured in the subtidal area (depth of less than 1 m). Specimens were measured (total length) (Table 1). Samples were stored at −20 °C and analysed within less than a month.

2.3. Stable isotope analysis

Shrimp and fish dorsal muscle was dissected (individual samples), dried at 60 °C and ground to fine powder with a mortar and a pestle.

$^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the samples were determined by continuous flow isotope 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. 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‰.

Isotope ratios were expressed as parts per thousand (‰) differences from a standard reference material:

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

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.

2.4. Data analysis

The nekton groups under investigation, shrimp and fish, were analysed at an individual level. Differences in length of each species, according to site, were tested using a 1-way ANOVA, followed by Tukey post-hoc tests. Each species was analysed separately. Differences in $\delta^{13}C$ and $\delta^{15}N$ among the sites were analysed separately using a 1-way ANOVA, followed by Tukey post-hoc tests. Each species was analysed separately. The ANOVA assumptions were previously investigated. Normality was investigated through the Shapiro-Wilk's test and homoscedasticity through Levene's test. A significance level of 0.05 was considered in all test procedures. As significant differences in $\delta^{13}C$ and $\delta^{15}N$ values in a specific compartment between sites do not necessarily imply a significant difference of the joint $\delta^{13}C$ and $\delta^{15}N$ isotopic signature, a permutational- MANOVA (PERMANOVA) using a Euclidean distance similarity index was performed to better discriminate site signatures, for each species, as used by Selleslagh et al. (2015).

The central isotopic range was defined for each site and species. The central range is defined as the range of values indicative of similar feeding site, and is ±1‰ for $\delta^{13}C$ and $\delta^{15}N$ values, according to Fry et al. (1999). Laboratory and field studies have shown that animals feeding on the same diet have isotope values within ±1‰ mean site values (Fry and Arnold, 1982; Fry and Sherr, 1984; Fry et al., 1999). In the present study we graphically defined a circular area determined by the mean value ±1‰ for $\delta^{13}C$ and $\delta^{15}N$ values, which allowed the identification of individuals feeding in similar sites, and deviants, i.e. individuals outside the central range, as described by Fry et al. (1999) and Vinagre et al. (2011b). The percentage of individuals of each species within and outside the central range of isotopic values was calculated for all sites.

Linear regressions were calculated for the variation of body size with percentage of deviants of each species, using STATISTICA 12.0. A significance level of 0.05 was considered in all tests.