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Tissue-diet discrimination factors of isotopic ratios ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) in two brittle star species: Effect of reproductive state, diet and tissue composition

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ABSTRACT

Tissue-diet discrimination factors (TDDFs) for carbon ($\Delta\delta^{13}C$) and nitrogen ($\Delta\delta^{15}N$) were estimated for two brittle star species (*Ophiocomina nigra* and *Ophiothrix fragilis*) in tissues of two body compartments (disk and arms) according to different diets (fish muscle, mussel and macroalgae). Variations in TDDFs are studied in the light of physiological changes, as two phases were revealed over the course of the experiment, i.e. a gonadal rest and a gonadal maturation. Overall, the average $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values ranged from -4.1 ± 0.2 to 1.5 ± 0.2‰ and from -1.8 ± 0.2 to 4.0 ± 0.3‰, respectively. Variability in TDDFs revealed little differences between species but appeared to be mainly driven by the diet and the physiological state of the organisms to a lesser extent. $\Delta\delta^{13}C$ in gonadal resting organisms were systematically *ca.* 0.8‰ lower than in gonadal maturing species. TDDF values for organisms fell within the range of expected values, except for $\Delta\delta^{13}C$ with macroalgae diet and $\Delta\delta^{15}N$ with fish muscle diet. As revealed by %N and CN ratios, high dietary-protein seemed to play a key role in explaining $\Delta\delta^{15}N$ values, while $\Delta\delta^{13}C$ seemed to be affected by differential assimilation efficiency in dietary components. We also suggested a possible effect of isotopic signature of the diet on TDDFs showing that the more enriched the diet in $\delta^{15}N$ and $\delta^{13}C$, the lower the TDDF.

1. Introduction

Throughout ecological studies, carbon and nitrogen stable isotope analyses have proven to be a powerful tool to understand trophic relationship between a consumer and its food sources in ecosystems. This approach is based on the principle that carbon and nitrogen isotope ratios in animal's tissues closely reflect those in their diets with a slight enrichment of heavier isotopes (^{13}C, ^{15}N) because of preferential respiration of lighter ^{12}C and excretion of lighter ^{14}N (DeNiro and Epstein, 1978, 1981). Tissue-diet discrimination factor (hereafter denoted as Δ, also TDDF) is calculated as the difference in isotope composition between animal's tissue and its diet (e.g., Cerling and Harris, 1999). These factors were considered as constant among organisms with stepwise $\delta^{13}C$ and $\delta^{15}N$ enrichment occurring with each successive trophic level in food webs (DeNiro and Epstein, 1978; Minagawa and Wada, 1984) leading to tissue-diet discrimination factors of *ca.* 1‰, for ^{13}C and ca. 3.4‰, for ^{15}N. However, the rate at which animals incorporate isotopes from their diet can differ depending on species, tissue, body size (Carleton and Martínez del Rio, 2005), nutrient composition of a diet (DeNiro and Epstein, 1978; McCutchan et al., 2003; Vanderklift and Ponsard, 2003), protein turnover in the tissue (Martinez del Rio et al., 2009; Tieszen et al., 1983), and nutritional stress conditions (Hobson et al., 1993). For these reasons, the use of isotope analyses for dietary studies may be influenced by these factors and lead to consistent errors about trophic relationships between a consumer and its diet. After a call for more laboratory experiments by Gannes et al. (1997), substantial variations in $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values have been demonstrated in several feeding investigations and have been mainly focused on terrestrial animals for a wide range of taxa and consumer classes such as mammals (Caut et al., 2008; DeMots et al., 2010; Tieszen et al., 1983), birds (Hobson and Bairlein, 2003; Polito et al., 2011; Therrien et al., 2011) or insects (Wehi and Hicks, 2010). The TDDFs for both carbon and nitrogen were also investigated among aquatic species (including marine organisms), but mainly for species of economic importance. Examples include fishes for aquaculture (Pinnegar and Polunin, 1999), cultivated mollusks (Dubois et al., 2007; Yokoyama et al., 2008), and large crustaceans (Suring and Wing, 2009). Overall, this tendency may lead to an imbalance in the knowledge of trophic ecology between terrestrial and aquatic ecosystems. Consequently, estimates of $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ should now focus more widely on aquatic species within different taxonomic groups and consumer class, even though laboratory experiments for some marine organisms are more difficult to conduct over several months under controlled conditions.

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While food availability is known to affect reproductive status of marine invertebrates (Lefebvre et al., 1999; Martinez-Pita et al., 2011; Pavanelli et al., 2010) or animal maintenance (Bourgoin and Guillou, 1990), relationships between isotopic composition variability and physiological status of animals are poorly known. A few field studies however revealed seasonal changes in isotopic composition of organisms or in specific tissues in relation to its physiology (Lorrain et al., 2002; Malet et al., 2007). Taking into account the physiological state of organisms appears to be of importance in order to provide accurate information in TDDFs.

To investigate factors affecting TDDFs in marine invertebrates, two very common and widely distributed co-occurring brittle star species (ophiuroid, echinoderm) were used as biological models. The brittle star *Ophiocomina nigra* (Abildgaard, 1789) is considered to be an opportunistic consumer (Fontaine, 1965), whereas *Ophiothrix fragilis* (Abildgaard, 1789) is predominantly a suspension-feeder (Davoult and Migné, 2001; Warner and Woodley, 1975). These species are trophically plastic and hence their feeding ecology depends on a large diversity of food sources. Consequently, investigating the trophic relationship of brittle star species and their diet requires an assessment of tissue-diet discrimination factors. To our knowledge, this study is the first experiment to determine carbon and nitrogen tissue-diet discrimination factors in brittle star species. A laboratory experiment was conducted where *O. nigra* and *O. fragilis* were fed with different types of foods under controlled conditions. This investigation aimed (1) to provide inter-specific tissue-diet discrimination factors ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) in two marine invertebrates, (2) to test whether diet affects changes in isotopic turnover and $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ patterns in tissues and (3) to test how changes in physiological state affects $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$.

2. Materials and methods

2.1. Experimental setup and feeding experiment

O. nigra and *O. fragilis* individuals were both collected by scuba diving in the middle of the bay of Brest (48°20′25 N; 04°29′20 W) in October 2009 between 25 and 30 m depth. Bottom water temperature was recorded 15 °C. After field collection, brittle stars were sorted out and placed in independent circular tanks. Each tank contained 20 specimens of homogeneous size of one brittle star species, either *O. nigra* (disk diameter = 1 cm) or *O. fragilis* (disk diameter = 1.5 cm). Each tank consisted of a flow-through cylinder with the bottom composed of a 200 μm mesh. Four tanks of one species were randomly grouped in a larger pool and fed with a specific diet to avoid contamination between food sources. Seawater was pumped continuously and filtered through a sand filter (50 μm) and 2 filtration cartridges (20 μm and 5 μm), so that the whole volume was renewed in less than an hour. Temperature (15 °C), salinity (35) and light (semi-darkness) were kept constant. The experiment was carried out over 3 months. Three diets were tested on *O. nigra* as a proxy for all the trophic levels this species potentially feeds upon: green macroalgae (*Ulva* sp., fresh algae), blue mussel (*Mytilus edulis*, whole body shell) and fish (benthic flatfish *Solea solea*, white muscle). *O. fragilis*, whose diet is rarely composed of decaying fish in the field was fed only macroalgae and mussel. Green macroalgae were collected where ophiuroids were sampled and then rinsed with filtered sea water to remove epibionts, ground very finely with a blender and immediately frozen in small portions at −20 °C. All fish specimens of a homogeneous size were collected at the same time and from the same location (Seine estuary). Fish muscle was removed and frozen at −20 °C. Mussels of a homogeneous size were collected and kept in filtered seawater overnight to clear their stomachs before freezing at −20 °C. Fish muscles and whole body mussels were sliced into small portions and stored frozen before the experiment started. During the experiment all diets were supplied daily *ad libitum* to avoid starvation conditions.

2.2. Sample collection and preparation

All diets (i.e. macroalgae, mussels and fish muscles) were freeze-dried and ground to powder. Four individuals for each species and each feeding treatments were then sampled randomly for day 0 and then after 1, 3, 5, 8, 15, 22, 29, 36, 43, 50, 64, 78, and 92 days. Sampled individuals were kept in filtered seawater overnight to clear their stomachs before being processed. For each date, the specimens were wet weighted, rinsed with distilled water and frozen at −80 °C. Arms and disks for each individual were split and grounded separately to a homogeneous fine powder with a mortar and a pestle after freeze-drying. Arms and disk samples were analyzed separately. Because brittle stars are calcareous organisms, a fraction was decarbonated with 1N HCl for ^{13}C analysis. Because of high lipid content in disk tissues, lipids were removed using cyclohexane (according to protocol in Kojadinovic et al., 2008) for ^{13}C analysis. Lipid-free tissues were dried at 48 °C for 24 h prior to acid treatment. Untreated samples were used for ^{15}N analysis.

Organisms were considered as two body compartments: arms and disk. In ophiuroids, arms are independent from the disk and mainly are composed of muscle tissue, while the disk contains a small proportion of muscle but is mainly composed of digestive and reproductive tissues, the proportions of which vary over time. As a result, arms were considered as muscle tissue and disks were considered either as digestive tissue during gonadal rest or as a mix between digestive and reproductive tissues when gonadal maturation started (see Results).

Isotopic analyses were performed by an isotope ratio mass spectrometer (IRMS) Finnigan MAT Delta Plus coupled with a Carlo EbraNC2500 elemental analyzer at the Isotope Stable Laboratory, Cornell University (New York, USA). The analytical error was 0.2‰, for both N and C (as measured with internal laboratory standards). Stable isotopic data are expressed as the relative per mil differences between the samples and the conventional standard Pee Dee Belemnite (PDB) for carbon and air N_2 for nitrogen, according to the following equation:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000$$

where X ‰ is ^{13}C or ^{15}N abundance and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios.

The difference in isotope composition between a tissue of animal and its diet is represented by a tissue-diet discrimination factor (TDDF, symbolized by Δ) which is calculated at equilibrium as:

$$\Delta_{tissue-diet} = X_{tissue} - X_{diet}, \text{ with } X = \delta^{13}C \text{ or } \delta^{15}N$$

2.3. Tissue-diet discrimination factors of whole animal

Six individuals for each species for the two different physiological states (gonadal rest and gonadal maturation) were sampled, dried at 48 °C for 72 h and weighted. Arms and disk of both species were cut and weighted separately, then burned at 450 °C in a muffle-furnace. The ash-free dry weight of organic matter in arms and disk was then determined by the difference between dry weight and ash weight. Relative mass proportions of the two body compartments (arms and disk) were calculated to estimate TDDFs for carbon and nitrogen ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) of the whole body animal.

Average values of ratios (R) for arms and disk relative to the total body mass were calculated as:

$$R_{tissue} = weight_{tissue}/weight_{whole\ animal};\ tissue = arms\ or\ disk$$