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Are you what you eat? Effects of trophic discrimination factors on estimates of food assimilation and trophic position with a new estimation method

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ABSTRACT

A key factor for estimates of assimilation of resources and trophic position based on stable isotope data is the trophic discrimination factor (TDF). TDFs are assumed based on literature reviews, but may vary depending on a variety of factors, including the type of diet. We analyzed effects of alternative TDFs on estimates of assimilated resources and trophic positions for an omnivorous fish, *Jenynsia multidentata*, that reveals dietary variation among locations across a salinity gradient of a coastal lagoon in southern Brazil. We also compared estimates of foods ingested vs. foods assimilated. Food assimilation was estimated using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of food sources and consumer muscle tissue and an isotopic mixing model (SIAR); consumer trophic position (TP) was estimated from consumer and production source $\delta^{15}N$ values. Diet was estimated using an index of relative importance based on frequency of occurrence and volumetric and numeric proportions of food items from stomach contents. The effect of variation in TDF on food assimilation and TP was tested using three alternative TDFs reported in review papers. We then created a new method that used food source-specific TDFs (reported separately for herbivores and carnivores) weighted in proportion to estimated assimilation of resources according to mixing model estimates to estimate TP (hereafter TP_{WAR}). We found that plant material was not assimilated in a proportion similar to its importance in the diet of fish at a freshwater site, and the new method yielded best assimilation estimates. Animal material made greatest contributions to fish biomass irrespective of TDFs used in the mixing model. The new method produced TP estimates consistent with differences in estimated food assimilation along the salinity gradient. Our findings support the idea that food source-specific TDFs should be used in trophic studies of omnivores, since the method improved our ability to estimate trophic position and resource assimilation, two important ecological indicators.

1. Introduction

Analysis of elemental stable isotopes is widely used for estimation of flows of organic material in food webs (DeNiro and Epstein, 1981, 1978), with carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) particularly useful owing to (1) their abundance in organic material and (2) relatively predictable shifts in isotopic ratios between tissues of foods and consumers (Fry, 2006). This shift during the process of food digestion, incorporation and assimilation has been termed *trophic fractionation*, and an estimate of its magnitude is called the trophic discrimination factor (TDF) (Post, 2002). TDF is a critical parameter used for estimation of both food assimilation and consumer trophic position. Most researchers now estimate food assimilation using isotopic mixing models that adopt Bayesian statistical approaches that can incorporate uncertainty associated with TDF and other inputs (Bessa et al., 2014; Bond and Diamond, 2011; Parnell et al., 2013). Computation of trophic position based on isotopic data relies on values assigned for TDFs as well as appropriate isotopic references (i.e., trophic position baselines) (Post, 2002; Qu et al., 2016).

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Almost 20 years after a call for more laboratory experiments (Gannes et al., 1997; Wolf et al., 2009), studies have revealed multiple factors affecting TDFs, including variation in food quality (e.g., protein content, amino acid profile), metabolic state (e.g., anabolic vs. catabolic), food intake rate, developmental stage, body mass, sex and even controversial ones, such as isotopic composition (Caut et al., 2009; Florin et al., 2011; Gaye-Siessegger et al., 2007; Kelly and Martínez del Rio, 2010; McMahon et al., 2010; Newsome et al., 2011; Poupin et al., 2011; Robbins et al., 2005; Wessels and Hahn, 2010). The trophic discrimination factor for nitrogen (TDF_N) can vary considerably between trophic levels, with values often greater for primary consumers and smaller for higher trophic levels (Hussey et al., 2014). Several reviews of TDF_N showed inconsistent results when comparing consumers fed plant material with those fed foods derived from animal tissue (Caut et al., 2009; Post, 2002; Vander Zanden and Rasmussen, 2001; Vanderklift and Ponsard, 2003). Studies involving fish have revealed different TDF_N for herbivores and carnivores, with the latter generally having lower values (Madigan et al., 2012; Varela et al., 2011) and herbivores having higher TDF_N compared to values reported in the literature (Lujan et al., 2011; Mill et al., 2007). However, laboratory experiments can test only a few parameters simultaneously, and trophic ecology is influenced by multiple factors. Moreover, captive studies often provide food ad libitum, which increases excretion rate, a factor that strongly influences TDF_N (Mill et al., 2007; Olive et al., 2003; Ponsard and Averbuch, 1999)

To the best of our knowledge, no prior study has addressed the relationship between TDF and food quality (i.e. animal origin vs. plant origin) for animals that change diet along temporal or spatial environmental gradients. To investigate the influence of TDF on estimation of trophic position and food assimilation, we analyzed isotopic and dietary variation of an omnivorous fish in relation a salinity gradient in a coastal ecosystem. The one-sided livebearer, *Jenynsia multidentata* (Jenyns, 1842), a dominant species in fresh and brackish waters along the coast of southern South America (Bastos et al., 2014; Garcia et al., 2004), feeds on both plants (e.g., algae and seagrass) and animals (e.g., microcrustaceans, insects, polychaetes) (Aranha and Caramaschi, 1999; Mai et al., 2006). Plant and animal material differ in nutritional quality, with animal tissue generally containing more protein, and plant tissues containing large fractions of cellulose and other compounds that are difficult or expensive for most animals to digest. Based on our findings, we propose a new approach for estimating trophic position that uses outputs from stable isotope mixing models and takes into account food-specific TDFs.

2. Methods

2.1. Field collections and sample processing

Samples were obtained monthly from April 2008 to May 2009 at Lagoa do Peixe National Park (LPNP) located on the coastal plain of Rio Grande do Sul state, Brazil (Fig. 1). Three sites were surveyed in Lagoa do Peixe: (1) lagoon mouth (LM) – the narrow channel that intermittently connects the main lagoon with the sea, (2) estuarine zone (EZ) – a mixohaline area located between the mouth and upper freshwater reaches, and (3) freshwater wetland (FW) fringing the upstream boundary of the lagoon (Fig. 1).

Jenynsia multidentata specimens were captured using a beach seine (9-m long, 2.4-m high, mesh size = 13 mm in wings and 5 mm in center) and beam trawl (0.9 × 0.9 m opening, with size mesh = 5 mm). Captured specimens were immediately euthanized in an ice bath, transported to the lab on ice, and then stored in a freezer. After thawing, each specimen was measured (total length, TL, mm), weighed (g) and dissected to remove the digestive tract for stomach contents analysis. Approximately 5 g of muscle tissue was extracted from the dorso-lateral region of each specimen for isotopic analysis. For specimens <30 mm TL, a composite sample of muscle tissue from 2 to 5 individuals was obtained in order to have sufficient material for analysis of isotopic composition.

In order to estimate trophic positions based on stable isotope ratios of nitrogen ($\delta\ ^{15}N$), tissue samples were obtained for basal production sources at each survey location (leaves from floating, emergent, and submerged macrophytes; filamentous algae; periphyton; suspended particulate organic matter (POM)). Additionally, major dietary items of *J. multidentata*, such as polychaete worms, amphipods and insects, were collected manually from sediments and macrophytes, and tissue was obtained for isotopic analysis.

2.2. Estimates of ingested resources

A total of 121 stomachs were analyzed to quantify the relative importance of food items ingested by *J. multidentata*. A stereoscopic binocular microscope was used to identify food items to the lowest feasible taxonomic level. Inorganic material and partially digested, unidentifiable organic matter were recorded as present or absent and excluded from subsequent analyses. The relative importance of each food category was calculated by the Index of Relative Importance (IRI) (Pinkas et al., 1970). We recorded the frequency of occurrence (F) of food categories in stomachs as percentages of total stomachs examined (Hyslop, 1980). For each stomach sample, we recorded the number of items or major fragments of each food category (N) and the area (mm^2) (A) of each item or category when material was spread evenly over a Petri dish at a depth of approximately 1 mm. If an item was thicker than 1 mm, the item was broken into smaller pieces to achieve a thickness of 1 mm (Hellawell and Abel, 1971). When thickness was <1 mm, thickness was estimated visually (e.g., 0.25, 0.5, or 0.75 mm). The volume (V) of each food category then was calculated as thickness x A. IRI was calculated using the formula: IRI = %F • (%N + %V), where %N was the ratio between the total number of a given food category and the total number among all categories items from all stomachs in the sample, and%A was the ratio between the total area occupied by a given food item or category and the total area occupied by all food items from all stomachs in the sample. Finally, IRI was expressed as a percentage (%IRI) obtained from the ratio between the calculated IRI for a given food category and the total sum of the IRI calculated for all food categories.

2.3. Effects of TDFS on estimates of food assimilation and trophic position

Muscle tissue samples were obtained from the flanks of *J. multidentata* specimens captured from the three survey sites (Table S1, supplementary material). Muscle samples and whole bodies of invertebrates (n = 48), samples of filamentous algae (n = 7), periphyton (n = 32) and macrophytes (n = 59) were rinsed with distilled water to remove foreign material. POM samples (n = 42) were obtained by filtering water through a pre-combusted (450 °C, 4 h) Whatman glass fiber filter (GF/F) with the aid of a manual vacuum pump (Table S1, supplementary material). Samples were placed in sterile Petri dishes, and dried in an oven at 60 °C for a minimum of 48 h. Dried samples were ground to a fine powder with a mortar and pestle and stored in clean Eppendorf tubes. Sub-samples were pressed into Ultra-Pure tin capsules (Costech, Valencia, CA) and sent to the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, for measurement of stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). Stable isotope ratios ($R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$) were compared to internal laboratory standards and then reported as parts per thousand (‰) relative to the correspond-