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Elemental turnover rates and isotopic discrimination in a euryhaline fish reared under different salinities: Implications for movement studies

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ABSTRACT

Stable isotopes are useful tools for studying species residency and movement in aquatic environments. Yet, many questions about their use still remain, mostly related to assumptions that should be experimentally validated, such as turnover rates and discrimination factors. Salinity is a key environmental variable that may influence turnover and discrimination factors, but its effects have not been tested. A controlled diet-shift experiment was conducted with whitemouth croakers (*Micropogonias furnieri*), a euryhaline species, to determine the turnover rates and diet-tissue discrimination of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes in muscle tissue. Fish captured in Patos Lagoon, Brazil were distributed into three independent recirculating aquaculture systems with salinities adjusted to represent freshwater, estuarine and marine environments, and fed for 90 days on an isotopically distinct diet. Half-life estimates were longer for both isotopes in the freshwater group ($\delta^{13}C = 25.6$ days, and $\delta^{15}N = 34.6$ days) compared to the estuarine ($\delta^{13}C = 18.7$ days, and $\delta^{15}N = 23.9$ days) and marine treatments ($\delta^{13}C = 17.7$ days, and $\delta^{15}N = 22.3$ days). Overall, carbon isotopic turnover was mainly driven by catabolism, whereas growth was the main factor responsible for nitrogen turnover. Trophic discrimination factors (TDFs) for carbon isotope were similar between fresh and estuarine treatments ($TDF_{final} = 0.82 \pm 0.54$‰ and 0.88 ± 0.58‰, respectively), but higher in marine ($TDF_{final} = 1.44 \pm 0.66$‰). Hence, in addition to providing species-specific isotopic parameters of a widespread sciaenid in the western Atlantic Ocean crucial to elucidate residence time along salinity gradients, these findings provide lab-based evidence of salinity effects on diet-tissue discrimination of a euryhaline species.

1. Introduction

Understanding fish dependence on estuaries and establishing their patterns of migration or movement therein is fundamental for comprehension of the ecology, life history and behavior of these animals, in addition to being prerequisites for their effective conservation and sustainable exploitation (Bennett et al., 2015; Guelinckx et al., 2008; Litvin and Weinstein, 2004). The abundance and stability of fish populations over a year vary as a result of the continued ability of its individuals to move to, from and within bays and estuaries for important foraging and reproductive activities (Kennish and Paerl, 2010; Kerr et al., 2010).

Natural biogeochemical tracers (e.g., stable isotopes) are particularly useful for studying animal movements because they do not require capture-mark-recapture or other tracking methods, and provide spatial and time-integrated information (Rubenstein and Hobson, 2004). Marked differences in stable isotope values can be found along salinity gradients, from freshwater to the sea, and these are incorporated into organisms and their food webs (Deegan, 1993; Fry and Chumchal, 2011; Oliveira et al., 2014). The basic premise underlining the use of stable isotopes in dietary studies is that tissues of consumers will isotopically resemble what is consumed; however, the relationship between isotopic ratios in the diet and that in the tissues is not always straightforward. The dynamics of elemental incorporation depends on a variety of factors, such as nutrient composition of the diet and differences in assimilation (Gaye-Siessegger et al., 2003; Zuanon et al., 2007), tissue turnover rate (Heady and Moore, 2012; Vander Zanden et al., 2015) and isotope trophic discrimination (Buchheister and Latour, 2010; Caut et al., 2013).

Isotopic changes in tissues, in response to dietary or habitat switches, may occur through the dilution of existing mass by new mass (addition) and/or by the synthesis and degradation of existing tissues using material from the new diet (replacement). As a result, the main factors controlling the rate at which an organism's tissues reflect the isotopic signature of its food are the growth of an individual and

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its metabolic activity (Buchheister and Latour, 2010; Hesslein et al., 1993; MacAvoy et al., 2001). Proper consideration of and knowledge about specific turnover rates may determine the length of time that an immigrant to a given habitat will be distinguishable from a longtime resident that has reached isotopic equilibrium, thus being essential to the inference of movement patterns (Guelinckx et al., 2008; Herzka, 2005). Once at equilibrium, trophic discrimination refers to the process by which ratios of stable isotopes change between prey and consumer during metabolic processing of consumed material (Mill et al., 2007; Tieszen et al., 1983). Although it is known that the employment of inaccurate discrimination values may introduce substantial errors in the estimation of contributions by food sources to consumer tissues, many values used in isotopic studies for estuarine species originate from studies conducted with mammals, birds and freshwater animals (Vander Zanden and Rasmussen, 2001; Vanderklift and Ponsard, 2003). This creates a demand for more studies on euryhaline species.

Ideally, both isotopic turnover and trophic discrimination should be validated through controlled experiments, therefore serving as models for field-based results and providing accurate knowledge for the interpretation of the isotopic data gathered in the field (Martínez del Rio et al., 2009). This has not been the case, as the number of observational field studies that apply stable isotopes to ecological problems far surpasses the number of experimental studies designed to clarify the mechanisms behind the patterns found by isotopic ecologists (Wolf et al., 2009). While studies on turnover rates and discrimination factors for fish species have increased over time (Herzka, 2005; Vander Zanden et al., 2015), many questions about their use still remain. For example, abiotic variables (e.g., temperature and salinity) are known to affect metabolic rate through different mechanisms (Claireaux and Lagardère, 1999; Gillooly et al., 2001; Swanson, 1998) and could, therefore, affect turnover rates. The effects of temperature on both isotopic turnover and discrimination have been considered by some studies (Barnes and Jennings, 2007; Bosley et al., 2002; Weidel et al., 2011), but results have been equivocal. Unlike temperature, the influence of salinity on these factors has not yet been tested, even though it is known that differences in salinity values are a key factor determining habitat use and fish distribution in estuaries and coastal lagoons (Jaureguizar et al., 2003; Moura et al., 2012) and may increase the energetic cost of an organism either due to osmoregulation or related physiological processes (Swanson, 1998).

In this study, a controlled diet-shift experiment was conducted with the whitemouth croaker *Micropogonias furnieri* (Desmarest, 1823), an important euryhaline, estuarine-dependent sciaenid widely distributed along the western Atlantic coast from Mexico (20°N) to Argentina (41°S) (Isaac, 1988). This species is an important component of commercial and recreational fisheries in Brazil (where fish landings exceed 40,000 metric tons, Chao et al., 2015), Uruguay and Argentina (Vasconcellos and Haimovici, 2006). Nowadays, stocks are considerably depleted, either due to overexploitation (Cergole et al., 2005; Seeliger and Odebrecht, 2010) and/or climatic events (e.g. ENSO), which may exert a great influence on the distribution of early life stages and recruitment success (Garcia et al., 2001). In estuaries and coastal lagoons, young-of-the-year usually occupy low salinity habitats, from which they move towards more saline areas downstream until reaching maturity, then migrate to sea for reproduction (Costa and Araújo, 2003; Jaureguizar et al., 2003). A previous isotopic study in Patos Lagoon estuary, the largest nursery area for this species in southern Brazil, provided first evidence that whitemouth croaker sub-adults stay in freshwater long enough to have their carbon isotope ratios reflect local autochthonous freshwater food sources (Garcia et al., 2007). A lack of lab-determined values for isotope turnover rates and trophic discrimination, however, hinders a precise inference of residence time periods in estuarine and freshwater conditions experienced by the whitemouth croaker, and other species, as they move along salinity gradients.

Hence, the objectives of this study were to determine the turnover rates and trophic discrimination factors of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotopes in muscle tissue of the whitemouth croaker reared under different salinities. The hypothesis was that isotopic turnover would take longer in the freshwater environment as a result of an enhanced energetic cost due to osmoregulatory activity (Laiz-Carrión et al., 2005). These results will elucidate the temporal utilization of the estuary region and adjacent areas by croakers, and their applicability can be extended to other fish species with the same behavior, helping to better understand life-history patterns, eventually leading to more efficient management and conservation of these species.

2. Material and methods

2.1. Experimental design and fish facilities

Whitemouth croakers were captured from their natural environment in Patos Lagoon estuary, in a site approximately 5 m deep, dominated by silty-clay sediments and where the presence of floating macroalgae depends on estuarine currents and local winds (32°04′0″ S, 52°05′00″ W). The temperature at time of capture was 26 °C and salinity, measured using the Practical Salinity Scale (UNESCO, 1985), was 18. Fish were captured by 5 min hauls using a bottom trawl (15 m head rope, 5 m opening mouth, 13 mm mesh size on the end, and a pair of 10 kg weighted otter doors) towed by a small boat (SISBIO collecting permit no. 33369-1). Immediately after capture, fishes were kept inside a plastic container (170 L) equipped with aeration. Later, they were transferred to a transport unit box (1000 L) where dissolved oxygen concentration was maintained above saturation with air stones and bottled oxygen and, then, transported by truck (25 km) to the Marine Aquaculture Station of the Federal University of Rio Grande (EMA-FURG) (Rio Grande City, Brazil), where the experiment was conducted. Upon arrival, fish were stocked in three 300 L static tanks and, after a 12 h period of acclimation, they were measured to the nearest mm (total length — TL) and weighed (g). Twenty-three individuals were randomly selected, measured and euthanized to determine the initial isotopic composition (δX_i) prior to the diet switch. Fish rearing and euthanasia procedures followed all Brazilian Ethics Committee Guidelines (Proc. 23116.001894/2013-86).

Fish with initial weight between 8 and 9.9 g were selected and randomly distributed into three salinity treatments, at densities of 30 fish/tank (90 fish per treatment). Each treatment consisted of an independent recirculating aquaculture system (RAS), comprised of three tanks (300 L each), a reservoir (sump — 300 L) with bioballs, a skimmer and a sand filter. Initially, to avoid osmotic shock, salinity was held constant in each treatment (~20), approximately the same as the croakers were experiencing in the field. That value was gradually adjusted, either by adding sea water to the system or by diluting the existing water with dechlorinated tap water, until the treatments reached values simulating freshwater, estuarine and marine environment. Salinity was changed at rates of approximately 1.5 (on the practical salinity scale) per day for the freshwater system and 0.5 per day for the estuarine and marine system, reaching values of 3, 16 and 27, respectively, which remained fixed until the end of the experiment. Each tank was equipped with an air diffusor to maintain dissolved oxygen close to saturation. Temperature (thermometer INCOTERM Hg), salinity (refractometer Atago, S/Mill-E), pH (digital pHmeter Mettler Toledo, FiveEasy FE20), alkalinity (titrimetry — APHA, 1998), total ammonia-nitrogen (TAN — Grasshoff et al., 1999) and nitrite (Aminot and Chaussepied, 1983) were monitored daily. The photoperiod was fixed at 14 L:10 D, simulating summer conditions, and flow rate in the RAS was held constant at 60 L h^{-1}.

Because the initial isotope value (δX_i) for $\delta^{13}C$ was $-17.54 \pm 0.95‰$, consistent with a combination of organic matter in suspension, organic matter in the sediment and C_4 plant-derived diet for croakers in Patos Lagoon estuary (Garcia et al., 2007), the experimental diet was formulated using fish meal combined with C_3 plants (rice and soybean) (Table 1). This resulted in a large difference between initial and expected equilibrium values (δX_{eq}), important for adequately