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Tissue-specific isotope turnover and discrimination factors are affected by diet quality and lipid content in an omnivorous consumer

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ABSTRACT

Tissue stable isotopes can be used for dietary reconstruction provided that the factors influencing turnover rates and trophic discrimination factors (TDFs) between consumer tissues and diet are known. This experiment quantified $\delta^{13}C$ and $\delta^{15}N$ dynamics in muscle and liver tissues in lab-reared juvenile marine fish after a switch from a high quality control diet to medium and low quality diets with decreasing protein and lipid contents. Turnover of $\delta^{15}N$ in the liver was strongly influenced by metabolism, equilibrating 3× faster compared to muscle for both diets. Nitrogen TDFs were dependent on diet quality, with values ranging from 3.0–6.5‰ in the muscle and 1.5–3.0‰ in the liver. The effects of mathematical lipid correction on $\delta^{13}C$ turnover and discrimination were examined by developing novel empirical equations involving C:N ratios and lipid $\delta^{13}C$ values. Lipid correction affected estimates of isotope turnover in the low quality diet treatment, with lipid-corrected muscle carbon isotopes equilibrating to diet 2× faster than non-corrected muscle, due to lipid retention increasing turnover estimates for non-corrected values. Conversely, lipid-corrected liver half-lives were 4.4× slower than non-corrected liver because lipid catabolism increased turnover rates for non-corrected values. A shift in control fish liver carbon TDFs of 2.1 and 1.7‰ for non-corrected and lipid-corrected values, respectively, between the beginning and end of the experiment was attributed to a 32% increase in lipid content. These results demonstrate that metabolic routing of lipid macromolecules strongly influences tissue-specific turnover, and is important to consider when reconstructing trophic dynamics.

1. Introduction

Stable isotopes are powerful tools for tracing diets and studying food webs across a wide diversity of organisms, from butterflies and elephants on land (Hobson et al., 1999; van der Merwe et al., 1990) to shrimp and tuna in the sea (Fry, 1983; Graham et al., 2010). Unique isotope signatures at the base of the food web are passed to higher trophic levels (with some modifications), and thus provide a means of identifying trophic pathways. Many migratory species transit across dietary 'isoscapes,' that vary as a function of underlying natural biochemical (e.g. photosynthetic pathway) and geochemical (e.g. geologic weathering) processes, as well as variations in anthropogenic inputs (e.g. fertilizer, sewage wastewater) (Hobson et al., 2010). Examples of isoscapes include gradients in $\delta^{13}C$ values of dissolved inorganic carbon and primary producers, which differ regionally between the ocean and the coast (Fry et al., 1984), and locally between fresh water, estuarine and marine habitats (Fry, 2002). Nitrogen isotope gradients are often due to the dominance of denitrification, nitrification or fixation processes transforming the pool of available N (Holl et al., 2007; Macko et al., 1984; McClelland et al., 1997; Sigman et al., 2009) or due to anthropogenic inputs of ^{15}N enriched nitrogen sources (McClelland et al., 1997; Schlacher et al., 2007). Provided turnover rates are known, isotopic clocks from tissues record migration timing, as movement between habitats with isotopically distinct food resources (which comprise isoscapes) is reflected in tissues of mobile consumers (Herzka, 2005; Hobson et al., 2010).

Accurate interpretations of diet histories from tissue-specific stable isotope ratios require a detailed understanding of the factors that affect 1) the time frame represented by the tissue analyzed (i.e. turnover rate) and 2) the isotopic offset between the tissue and the diet, or trophic discrimination factor (Hobson et al., 2010). Tissue turnover rates are

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influenced by growth via the accumulation of new tissue through mass gain and by metabolism via the breakdown and replacement of existing tissue (Fry and Arnold, 1982; Herzka, 2005; Hesslein et al., 1993). Models have been developed that estimate the relative contribution of growth (Fry and Arnold, 1982) and metabolism (Hesslein et al., 1993) to isotope turnover (reviewed by Boecklen et al., 2011). In general, tissues with fast growth and high metabolism have quicker turnover rates. For instance, the high metabolic activity of liver tissue leads to more rapid turnover than muscle in mammals (Sponheimer et al., 2006), birds (Hobson and Clark, 1992), bony fishes (Buchheister and Latour, 2010; Logan et al., 2006; Suzuki et al., 2005), and elasmobranchs (MacNeil et al., 2006; Malpica-Cruz et al., 2012). Trophic discrimination factors (TDF = $\delta_{tissue} - \delta_{diet}$) are influenced by many variables including the element of interest (e.g. ^{13}C or ^{15}N), taxonomic group (e.g. mammals, birds, fish), specific tissue (e.g. blood, liver, muscle), and diet (e.g. protein and lipid content) as reviewed by Martínez del Rio et al. (2009). Environmental temperature and the amount of food consumed can also affect TDF (Barnes et al., 2007). Even within an individual animal and specific tissue type, TDF can vary as a function of growth rate (Trueman et al., 2005). Given the range of variables that influence both turnover rate and TDF, and the importance of both factors for accurately determining trophic relationships, there have been recent calls for more controlled laboratory experiments to explore the dynamics of turnover rates and TDFs (e.g. Martínez del Rio et al., 2009; Boecklen et al., 2011).

A particular area of concern for trophic studies is the effect of lipid content on carbon isotope turnover and fractionation. Lipids are depleted in ^{13}C relative to protein and carbohydrates and many researchers address 'lipid bias' with chemical extraction or mathematical correction techniques. Despite their wide use, lipid correction equations developed are tissue- and species-specific, and no universal lipid correction model exists (Logan et al., 2008; Mintenbeck et al., 2008; Post et al., 2007). Additionally, lipids such as essential fatty acids are important nutritional and source-specific components of food webs (Litzow et al., 2006) and so by extracting lipids before measuring $\delta^{13}C$ values, dietary information may be permanently lost. In contrast, correcting for 'lipid bias' using mathematical equations permits comparison between lipid-corrected and non-corrected carbon isotope values and preserves dietary information linked to the lipid fraction. Moreover, migratory omnivores will potentially feed on a large range of dietary items of variable qualities and lipid content that can introduce considerable complexity into isotope-based dietary reconstructions. Despite the importance of lipids, they are rarely addressed in controlled experiments that quantify isotope turnover rates and TDFs.

In this study, a diet switch experiment was conducted on an omnivorous marine teleost, Atlantic croaker (*Micropogonias undulatus*), that were reared in the laboratory with known diet and growth histories. The experiment was designed to achieve four primary objectives: 1) determine nitrogen and carbon isotope turnover rates of fish muscle and liver tissue using models that partition turnover to growth and metabolism; 2) estimate tissue-specific TDFs and compare to other studies; 3) investigate the influence of diet quality on turnover and TDFs; and 4) examine the influence of lipids on assimilation dynamics using empirically-derived correction equations for $\delta^{13}C$ values based on extracted-lipid $\delta^{13}C$ values and C:N ratios. Results of this study reveal the complex factors that affect tissue-turnover rates and isotopic discrimination, and will facilitate interpretation of tissue isotope data from migratory animals.

2. Material and methods

2.1. Fish husbandry

Atlantic croaker eggs were collected from laboratory brood stock grown under simulated photoperiod and temperature regimes to induce natural spawning at the University of Texas Marine Science Institute (UTMSI), Port Aransas, Texas USA. Eggs were treated with 1-ppm formalin for 30 min to prevent bacterial and fungal infections and hatched in 150 L conical tanks. Rotifers, that were raised on *Isochrysis galbana* algae and enriched over 24 h with RotiGrow Plus (Reed Mariculture Inc.) to increase essential fatty acid content, were fed daily to croaker larvae. Rotifers were added as needed to maintain densities of 5–10 rotifers·mL^{-1}. After 3 weeks, larvae were fed *Artemia* enriched with AlgalMac 3050 (Bio-Marine, Inc.) at starting densities of 0.25 artemia·mL^{-1}, which was increased to 2 artemia·mL^{-1} as fish grew. Larvae were then gradually weaned onto an enriched marine dry feed Otohime EP3 (Reed Mariculture Inc.) that increased in pellet-size from 250 μm–1.7 mm as fish reached juvenile size (60 d of age). On d 60, juveniles were transferred to circular 350 L grow-out tanks and fed Otohime pellets (2.3 mm). After 165 d, fish reached experimental size (110 ± 10 mm total length, 17 ± 5 g) and were transferred into 12 individual 450 L experimental tanks at densities of ~30 fish per tank. Each tank was equipped with an automatic feeder that dispensed pellet food at 09:00, 12:00, 15:00 and 18:00 h daily. After one week of acclimation, all fish were anesthetized with MS-222 (150 mg·L^{-1}), measured for length and mass and tagged individually with Visual Implant Alpha (VIA) IV tags (Northwest Marine Technologies, Inc.). The tags contained unique codes and were inserted into the snout cavity to monitor individual growth rates. Tagged fish were further acclimated for 10 d before initiating the diet switch.

2.2. Experimental protocol

A schematic of the experimental design is presented in Electronic Supplementary Materials (ESM Fig. 1). Experimental diets ("medium" quality and "low" quality) were selected to be a similar pellet size to the control diet but had different protein and fat content and source (terrestrial versus marine), resulting in distinct isotope values (Table 1; ESM Fig. 2). The low quality feed contained 5% lipid and 32% protein, primarily from terrestrial sources and was below the optimal protein level of 45% recommended for Atlantic croaker (Davis and Arnold, 1997). The control feed contained 15% lipid and 48% protein and was primarily from marine sources, while the medium quality diet had 12% lipid and 45% protein from a mixture of terrestrial and marine sources, according to manufacturer information. The isotopic shift in experimental diets was comparable to natural diet shifts a coastal migratory fish could experience following a discrete offshore-to-inshore habitat shift that results in a change in the underlying food web structure. Each diet was randomly assigned to 4 tanks, resulting in 4 replicate tanks per treatment. On experiment d 0, immediately before the diet switch, 12 fish were sacrificed to obtain initial equilibrium isotope values (i.e. baseline values) and estimate TDFs for both muscle and liver tissues (ESM Fig. 2). Food was withheld 24 h before sampling to ensure clearance of stomach contents (Lee et al., 2000). Random samples of 2 fish per tank were collected on d 7, 18, 32, 52, and 104, resulting in 8 fish per time point per diet treatment. Fish were euthanized with a lethal dose of MS-222 and placed on ice until dissection. The Institutional Animal Care and Use Committee (AUP-2013-0083) at the University of Texas at Austin approved all experimental procedures.

2.3. Sample analysis

Each fish was patted dry, weighed (0.01 g) and measured for standard length (SL; mm) and total length (TL; mm). The VIA tag was removed from the snout and fish identification was recorded. The liver and a filet of dorsal white muscle tissue was collected, rinsed with deionized water, weighed, placed in 2 mL vials and frozen at −80 °C. Samples were then freeze dried for 36 h, re-weighed, and ground into a fine powder using a ceramic mortar and pestle. One (±0.2) mg of dried tissue was then packed into tin capsules and sent to the Stable Isotope Facility of the University of California Davis for bulk C and N content and isotope analysis using a PDZ Europa ANCA-GSL elemental analyzer