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Notes

Non-lethal sampling of lake sturgeon for stable isotope analysis: Comparing pectoral fin-clip and dorsal muscle for use in trophic studies

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ABSTRACT

When used to study the feeding ecology of fish, stable isotope analyses generally require sacrificing animals to collect tissue samples. Lethal sampling can be undesirable when studying a species at risk such as lake sturgeon (*Acipenser fulvescens*). We evaluated the feasibility of using pectoral fin clips as an alternative to dorsal muscle tissue in stable isotope studies for adult lake sturgeon. Because lipid content can affect stable isotope ratios, we also determined whether lipid extraction and mathematical normalization affected the relationship between fin-clip and muscle tissue signatures. Dorsal muscle plugs and fin clips were collected from adult lake sturgeon and analyzed for stable isotopes of carbon and nitrogen. Significant, positive relationships were observed between fin and muscle tissues for both $\delta^{15}N$ ($r^2 = 0.43$) and $\delta^{13}C$ ($r^2 = 0.32$). Lipid extraction significantly reduced the among-individual variation, and improved the $\delta^{13}C$ relationship significantly ($r^2 = 0.74$). While general non-linear mathematical lipid normalization did effectively reduce the among-individual variation, a consistent enrichment relative to chemical extraction was observed. An alternative mass balance correction method, however, yielded lake sturgeon specific parameter estimates that facilitated accurate lipid correction. The strong relationships between fin-clip and muscle tissue signatures demonstrate that fin-clips should be considered as good surrogates for muscle tissue, will allow trophic studies to accurately adjust for the effect of differential lipid accumulation and can effectively limit the need for invasive sampling for adult lake sturgeon.

Introduction

Owing to the predictable changes that occur in stable isotope ratios as energy flows through food webs, stable isotope analysis (SIA) has become a commonly used method for obtaining time-integrated information about feeding relationships in food web studies (Peterson and Fry, 1987; Finlay and Kendall, 2007). When used to study the feeding ecology of fish, dorsal white muscle is commonly sampled because it has an intermediate turnover rate, low isotopic variability, and frequently has low lipid content (Pinnegar and Polunin, 1999).

Obtaining dorsal muscle tissue for SIA can pose problems for many species as laws or management plans may constrain, or prohibit, the lethal sampling needed to obtain the tissue (e.g., Haley, 1998; Brosse et al., 2002). On larger species, the utilization of muscle biopsy plugs has been applied (Hanisch et al., 2010; Nelson et al., 2011; Carlisle et al., 2012) as one means of reducing the need for lethal sampling, but biopsies are often viewed as undesirable for juveniles or species of special concern (COSEWIC, 2007).

In place of muscle tissue, a number of studies have considered alternative non-lethal sampling including: mucus (Church et al., 2009), scales (Sinnatamby et al., 2008) and fin clips (e.g., Willis et al., 2013). Fin clip tissue has been considered a suitable surrogate to dorsal muscle tissue in several reviews including: Australian tropical and temperate fishes (Jardine et al., 2011), European freshwater fishes (Tronquart et al., 2012) and North American freshwater species (Willis et al., 2013). Within North American studies salmonids, in particular, dominate in study comparisons (42%) as a result of their threatened status and the associated demands for non-lethal sampling alternatives (Sanderson et al., 2009; Hanisch et al., 2010).

While there is some evidence that regional multi-species models for fin–muscle relationships may be applied in fish food web related studies (Jardine et al., 2011; Tronquart et al., 2012), the fin–muscle stable isotope relationship has frequently been shown to vary by species (Kelly et al., 2006; Hanisch et al., 2010; Willis et al., 2013). Furthermore, fin–muscle relationships often fail (28% of test cases) to exhibit a 1:1 slope indicative of an unbiased conversion of one tissue SIA value to another (Willis et al., 2013). Therefore, while strong relationships between the isotope signatures of fin clip and muscle tissues available in the literature demonstrate that fin clips can be used in place of muscle tissue, evidence of conversion biases and the dominance of salmonid studies indicate the need for further studies on non-salmonid species.

One species for which tissue comparisons have not been made to date is lake sturgeon (*Acipenser fulvescens*). Lake sturgeon population

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sizes have decreased in recent decades due to poor water quality, overfishing and damming (COSEWIC, 2006; Fisheries and Oceans Canada, 2008), with recent studies having identified the need for further non-lethal sampling information on sturgeon diet and foraging in areas where populations are affected by invasive species (McCabe et al., 2006) or other anthropogenic stressors (e.g., Haxton and Findlay, 2008). Furthermore, lake sturgeon represent a unique fin–muscle comparison given the high muscle tissue C:N ratios (typically > 5) as compared to previously examined species (e.g., Tronquart et al., 2012 C:N ≈ 3.5).

A strong relationship has been found between the C:N ratio and lipid content in animals (Post et al., 2007). Lipids are isotopically depleted in carbon relative to carbohydrates and proteins due to differing fractionation during lipid synthesis (DeNiro and Epstein, 1978; Griffiths, 1991). As such, some researchers have employed direct chemical extraction of lipids or mathematical normalization techniques to minimize the bias caused by heterogeneous lipid content among tissue samples (McConnaughey and McRoy, 1979; Sweeting et al., 2006; Hoffman and Sutton, 2010), with the technique being recommended when the C:N ratio is relatively high (above 3.5) or highly variable (Post et al., 2007).

In light of the above, the aim of the present study was to explore the use of non-lethal sampling for SIA of adult lake sturgeon. A specific objective was to test the hypothesis that there is a significant relationship between the stable isotope measures of pectoral fin-clip and dorsal muscle tissue samples. Furthermore, we sought to address a critical analytical issue (e.g., Arrington et al., 2006; Kiljunen et al., 2006; Logan et al., 2008; Fagan et al., 2011) by evaluating the influence of lipid extraction and mathematical normalization models on the obtained pectoral fin-clip and muscle tissue stable isotope comparisons as a means of determining whether lipid extraction and/or correction is routinely required for lake sturgeon stable isotope related studies.

Methods

The lake sturgeon used in the study were captured on the Rainy River, Ontario (48°36′ N, 93°24′ W) immediately below the International Falls Dam. The Rainy River is located on the Minnesota–Ontario border and flows westward from Rainy Lake to the south end of Lake of the Woods. The fish were collected by the staff of Fisheries and Oceans Canada by boat electro-fishing at night or with 25.4 and 30.5 cm mesh gill nets set overnight in early to late May 2012 and 2013. Nets were set following protocols described in Dubreuil and Cuerrier (1950), i.e., parallel or at an angle to river flow in currents and back eddies and strategically placed to optimize lake sturgeon capture. Collections were completed coincident with lake sturgeon spawning congregations on spawning beds or immediately downstream of the spawning areas.

Fin clips were obtained with a circular paper punch applied to the pectoral fin membrane so as to avoid sampling of the fin ray (Tyus et al., 1999). One or two muscle plugs were taken from each individual using a 3 mm dermal biopsy punch from behind the third dorsal scute to obtain at least 1 mg of muscle tissue (Tyus et al., 1999). The resulting wounds were sealed using 3M Vetbond Tissue Adhesive™. A total of 91 lake sturgeon were biopsied in two sampling seasons (66 in 2012 and 25 in 2013). Muscle and fin clip samples were kept frozen until returned to the laboratory where they were dried at 50 °C for 24–48 h and ground into a fine powder using a mortar and pestle.

Lipids were extracted from half the available muscle tissue from 30 individuals using methanol and chloroform following Folch et al. (1957) as revised by Kauffman et al. (2007). Approximately 0.5 mg of a tissue sample was added to 8 ml of 2:1 (by volume) chloroform:methanol solution; left to soak and centrifuged for (10 min at 1000 ×g) after 12–24 h. The lipid extracted muscle tissue was then rinsed with distilled water and allowed to air dry before SIA.

Fin clip, muscle, and lipid extracted muscle tissue samples were processed for SIA, with all analyses performed at the Environmental Isotope Laboratory, University of Waterloo, on a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a Carlo Erba elemental analyzer (CHNS-O EA1108, Carlo Erba, Milan, Italy). Machine analytical precision was ±0.1‰ and ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, and was determined by repeat analysis of duplicates (one in ten). All resulting measurements are expressed using standard delta notation as parts per thousand (‰) differences with respect to the international reference standards, carbonate rock from the Peedee Belemnite formation for $\delta^{13}C$ (Craig, 1957) and nitrogen gas in the atmosphere for $\delta^{15}N$ (Mariotti, 1983). The carbon-to-nitrogen (C:N) ratio by mass was determined for each sample before and after lipid extraction.

Several lipid correction mathematical models exist in the literature (McConnaughey and McRoy, 1979; Kiljunen et al., 2006; Post et al., 2007; Logan et al., 2008). Models differ both in approach, i.e., lipid normalization, arithmetic mass balance and regression based (Hoffman and Sutton, 2010) and the data sets used for estimation i.e., strictly aquatic, mixed aquatic and terrestrial (Logan et al., 2008) and functional form i.e., linear, non-linear (Fagan et al., 2011). Differences in model approach, however, may matter less than the accuracy with which model parameters are estimated or the specificity of the data used to estimate the model (Logan et al., 2008).

Accordingly here we have chosen two differing approaches. The first entails the use of the commonly applied general non-linear model developed by McConnaughey and McRoy (1979) and modified by Kiljunen et al. (2006) for specific application to freshwater fish. The model was selected because the C:N ratio values used in its estimation were comparable to those observed in this study (3–12). Furthermore, in a comparative analysis of predictive performance, the McConnaughey and McRoy (1979) adjusted model proved statistically superior in terms of having the lowest mean absolute percent predictive error when tested against an extensive lake whitefish (*Coregonus clupeaformis*) data set (Fagan et al., 2011). The use of the model requires the sequential estimation and application of two equations, as defined below.

$$L = \frac{93}{1 + (0.246 \times \mathrm{C:N} - 0.775)^{-1}} \quad (1)$$

$$\delta^{13}C_N = D\left(I + \frac{3.90}{1 + \frac{287}{L}}\right) \quad (2)$$

where L is the proportional lipid content of the sample, $\delta^{13}C_N$ is the lipid-normalized value of the sample, C:N is the ratio of elemental carbon and nitrogen in the sample, $\delta^{13}C$ is the measured value of the sample, D is the isotopic difference between protein and lipid and I is a constant. The parameter values used here were $D = 7.018$ and $I = 0.048$ as re-estimated by Kiljunen et al. (2006).

For the second approach an arithmetic mass balance model using tissue C:N ratios as a proxy for tissue lipid content (Alexander et al., 1996; Fry et al., 2003) was developed and applied. The model can be applied with the estimation of two parameters derived from stable isotope data available for bulk and lipid extracted samples: the $C:N_{protein}$ of

Table 1
Summary statistics giving mean, standard deviation (SD), and minimum and maximum values for weight, total-length and age of sampled lake sturgeon.

	n	Mean	SD	Min	Max
Weight (kg)	68	15.21	6.55	0.2	38.2
Total-length (cm)	68	126.54	20.3	42.5	168.5
Age (y)	55	18.44	6.55	3	33

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