Nonlethal Sampling of Sunfish and Slimy Sculpin for Stable Isotope Analysis: How Scale and Fin Tissue Compare with Muscle Tissue

MAUREEN H. KELLY* AND WILLIAM G. HAGAR
Department of Biology, University of Massachusetts, Boston, Massachusetts 02125, USA

TIMOTHY D. JARDINE AND RICHARD A. CUNJAK
Canadian Rivers Institute, Department of Biology, University of New Brunswick, Fredericton, New Brunswick E3B 5A3, Canada

Abstract.—We found that the sampling of tissues that do not result in the death of the fish, such as scale and fin tissue, may be substituted for muscle tissue in stable isotope analysis (SIA) of fishes. Comparisons were made between the values of $\delta^{15}$C and $\delta^{15}$N found in muscle tissue with the corresponding scale tissue of three sunfish species (bluegill Lepomis macrochirus, pumpkinseed L. gibbosus, and redbreast sunfish L. auritus) and with caudal fin tissue of slimy sculpin Cottus cognatus. The fish showed strong linear correlation in $\delta^{13}$C values between their nonlethally sampled scale or fin tissue and their muscle tissue (combined sunfish: $r = 0.97$; slimy sculpin: $r = 0.84$). Sunfish $\delta^{15}$C values were higher in scale tissue than in muscle tissue and required a correction factor for converting the scale values to the muscle values (regression equation: $y = 1.1673x + 1.0531$). Slimy sculpin $\delta^{15}$C fin and muscle values were similar and did not require a correction factor. The correlation of $\delta^{15}$N values between the tissues was also strong in both sunfish ($r = 0.94$) and slimy sculpin ($r = 0.90$). A correction factor was needed to convert $\delta^{15}$N values from scale to muscle in the three sunfish species ($y = 0.8504x + 2.6698$) and from fin to muscle in slimy sculpin ($y = 1.2658x + 3.3234$). Results of this study and other literature support the use of nonlethally sampled tissues for SIA of fish. These methods may be used for investigations of rare and endangered species and also allow for analysis of archived fish scales.

Stable isotope analysis (SIA) has become a reliable method for quantifying trophic relationships and dietary habits of animals, including fishes, in the wild. The ratios of heavy-to-light stable isotopes found in organisms are used to follow the flow of energy and nutrients through an ecosystem. The amount of $^{13}$C in an organism increases a negligible amount (0–1%) from its diet and is used to identify the base of the carbon source for the organism (Peterson and Fry 1987; Post 2002). The larger increase of $^{15}$N from diet to consumer (3–5%) is useful when quantifying an organism’s trophic position (Minagawa and Wada 1984; Vander Zanden et al. 1997; Post 2002). Certain biochemical reactions in an organism discriminate against the heavier isotopes, causing a fractionation in the amounts of isotopes in tissues. For example, $^{13}$C is discriminated against in lipogenesis, causing lipid-containing tissues to be lower in $^{13}$C values than other tissues (DeNiro and Epstein 1977). Urea and ammonia have been shown to contain less $^{15}$N than the tissues of the organism that excreted them (Macko et al. 1982).

Muscle has been the tissue of choice for SIA studies of fish (Pinnegar and Polunin 1999), but other tissues, such as liver, heart, brain, and gonadal tissues, have also been analyzed. The sampling of these tissues almost always requires killing the fish. Nonlethally sampled tissues, such as scale or fin tissue, are potential substitutes for muscle and other lethally sampled tissues. The stable isotope values of scale and fin tissue can be compared with known values in lethally sampled tissues (Hobson and Clark 1993; Shannon et al. 2001), and nonlethally sampled tissues could become the standard for sampling in the future. Sampling these tissues allows researchers to study populations of fish without affecting their numbers, without removing their resources from the ecosystem, and without reducing their gene pools. As early as 1985, Estep and Vigg (1985) stated that the consistent difference in $\delta^{13}$C between scale and muscle tissue could be an important nondestructive tool for distinguishing wild fish from fish reared in different hatcheries. For conservation value, the use of nonlethally sampled tissues would allow the study of endangered and locally rare species.

Researchers need to know how scale and fin tissues compare in isotope values with muscle and other tissues to be able to substitute them in previous and current food web studies (Pinnegar and Polunin 1999; Perga and Gerdeaux 2003). To better understand the degree of isotope fractionation between different tissues in individual fish and to guide the choice of tissues for future studies, we compared the $\delta^{13}$C and $\delta^{15}$N values of scale and muscle tissue in benthopelagic...
centrarchid fishes from two ponds in Massachusetts (bluegill Lepomis macrochirus and pumpkinseed L. gibbosus) and two ponds in New Brunswick (pumpkinseed and redbreast sunfish L. auritus). We also compared caudal fin tissue and muscle tissue of a benthic cottid, slimy sculpin Cottus cognatus, collected from two streams in New Brunswick. Finally, we compared our results for scale and fin tissue with those of previous studies to determine the generality of the outcome across several species of fish.

Fish were sacrificed in this study to compare the amounts of $^{13}$C and $^{15}$N in lethally as well as nonlethally sampled tissues. However, in previous studies that sampled only scale or fin tissue, the fish were returned to the water without apparent negative effects (Gjerde and Refstie 1988; Conover and Sheehan 1999; Tyus et al. 1999; Pratt and Fox 2002). If scale and fin tissues provide adequate data for analysis, the unnecessary sacrifice of sampled organisms may be avoided.

Methods

Pumpkinseeds and bluegills were collected from Maquan Pond (42°03’38”N, 70°51’08”W) and Furnace Pond (42°03’20”N, 70°49’40”W) in southeastern Massachusetts. Five pumpkinseeds were collected in Mills Pond (45°53’9”N, 66°00’49”W) adjacent to Grand Lake and five redbreast sunfish were caught in Yoho Lake (45°46’35”N, 66°52’05”W); both of these ponds are located in New Brunswick. All sunfish were collected by angling. Scale and muscle tissue were extracted from individual fish, except for one pooled sample of five age-2 pumpkinseeds from Maquan Pond. Collection of slimy sculpin was by electrofishing in two stream reaches of the Miramichi River, New Brunswick. Site 1 was located in Catamaran Brook (CBK; 46°52’47”N, 66°06’66”W), a third-order brook that drains to the Little Southwest Miramichi River. Site 2 was located in Stewart Brook (SBK; 46°57’47”N, 65°39’66”W), a second-order brook that drains directly to the main Northwest Miramichi River.

Sacrificed fish were put on ice within 1 h of capture and were kept frozen at −20°C in the laboratory. When processed, the fish were slightly thawed and rinsed with water. Scales were removed from various locations above and below the lateral line and dried at room temperature for 48 h. After drying, whole scales were filed using a slim taper file. To obtain the muscle tissue, the flesh was cut on the left side of the fish under the dorsal fin and the skin was removed. A 2.5- × 1.0-cm rectangle of muscle tissue was excised, dried at 60°C for 48 h, and ground with a mortar and pestle. Fin tissue from the slimy sculpin was obtained by taking a small sample of caudal tissue with scissors; the tissue was then dried and cut to appropriate weights for SIA. The Ecosystem Center Laboratory at the Marine Biological Laboratory, Woods Hole, Massachusetts, performed the isotope analysis for the U.S. sunfish and the Stable Isotopes in Nature Laboratory (SINLAB), University of New Brunswick, performed the isotope analysis on sunfish and slimy sculpin from Canada.

For SIA, we chose not to acid wash our scale or fin tissue. Although some of the carbon in scales is inorganic carbonate, Bunn et al. (1995) reported that acid washing may alter the $\delta^{15}$N values of natural materials and increase the variability of the isotope values. The fact that both $\delta^{13}$C and $\delta^{15}$N values of scale tissue have been shown to increase with acid treatment (Perga and Gerdeaux 2003) suggests that it is not just the inorganic carbon that is removed with acid treatment.

Stable isotope values are expressed in delta notation (δ) and are determined by the equation

$$\delta_{\text{sample}} = \left( \frac{R_{\text{sample}}}{R_{\text{reference}}} - 1 \right) \times 1,000,$$

where $R$ is the ratio of the heavier isotope : lighter isotope found in the sample and reference material ($R = ^{13}C/^{12}C$ or $^{15}N/^{14}N$). The reference material for carbon is the Peedee Belemnite Limestone Formation, and the reference material for nitrogen is atmospheric nitrogen. Positive delta values show a greater proportion of the heavier isotope than the reference, while negative delta values show a lesser proportion of the heavier isotope than the reference. The delta value is expressed in parts per thousand (‰). Samples were calibrated with International Atomic Energy standards CH6, CH7, N1, and N2. Standard deviations of replicate samples were never greater than 0.1‰ for carbon and 0.2‰ for nitrogen.

The three species and the four sites of sunfish were combined to examine Pearson’s correlations between muscle and scale tissue for $\delta^{13}$C and $\delta^{15}$N. For slimy sculpin, data from the two sites were combined to examine $\delta^{13}$C and $\delta^{15}$N correlations between muscle and fin tissue. Simple linear regression models were used to determine the relationship between muscle tissue $\delta^{13}$C and $\delta^{15}$N values and the corresponding values in scale or fin tissues. Statistical analyses were done with the Statistical Package for the Social Sciences (SPSS 2003). Throughout the rest of this paper, “sunfish” denotes all three species of sunfish from all four sites and “slimy sculpin” denotes the fish from both sites.

Results

Both sunfish and slimy sculpin showed strong correlations in $\delta^{13}$C and $\delta^{15}$N values between their
muscle and scale or fin tissue (Figures 1, 2). Linear regression models for all relationships tested (sunfish $\delta^{13}$C: $F = 530, p < 0.001$; slimy sculpin $\delta^{13}$C: $F = 69, p < 0.001$; sunfish $\delta^{15}$N: $F = 258, p < 0.001$; slimy sculpin $\delta^{15}$N: $F = 122, p < 0.001$) were highly significant. Regression slopes were tested for equality with a 1:1 ratio (slope = 1.0) between muscle tissue values and the scale or fin tissue values. All slopes were significantly different from 1.0 ($t$-test: $p < 0.05$).

To test the hypothesis that correction factors were necessary to convert the values of scale or fin tissues to the values of muscle tissues, paired $t$-tests were performed on $\delta^{13}$C and $\delta^{15}$N values of slimy sculpin and sunfish. Both groups of fish showed significant differences in the values of scale or fin and muscle tissues: sunfish $\delta^{13}$C ($p < 0.001$), sunfish $\delta^{15}$N ($p < 0.001$), slimy sculpin $\delta^{13}$C ($p < 0.005$), and slimy sculpin $\delta^{15}$N ($p < 0.001$). Since the slopes of the regression model lines did not equal 1.0 for either group of fish or for either isotope measured, a single correction factor could not be used for each group of fish. Researchers will need to sacrifice a few fish to determine the actual conversion factor for their individual species and locations.

The high correlation of the sunfish $\delta^{13}$C values is notable because this data set represents the values of three species of sunfish from four ponds, two in the USA and two in Canada (Figure 1). Literature values for anadromous, saltwater, and freshwater fishes from wide-ranging locations show similar mean increases in scale $\delta^{13}$C values over muscle values (Table 1). The difference in $\delta^{13}$C between these two tissues is probably a result of the discrimination of $^{13}$C in lipogenesis because of the substantial lipid content in muscle tissue and the lack of lipid in scale tissue (DeNiro and Epstein 1977; Jobling et al. 1998).

Unlike scales, fin tissue is a mixture of bone, muscle, and cartilage and would be expected to contain some lipid, but not as much as muscle tissue. The $\delta^{13}$C values of slimy sculpin fin tissue, however, were very close to those of the muscle tissue. Although there was a statistical difference between the $\delta^{13}$C values of fin and muscle tissue in slimy sculpin, the difference was small and the use of a correction factor is probably not warranted. This pattern of comparable values between fin and muscle tissue has held up over a variety of species (Rounick and Hicks 1985; McCarthy and Waldron 2000; Jardine et al. 2005; Table 1).

Bluegill, pumpkinseed, and redbreast sunfish scale $\delta^{15}$N values can be used for SIA with a correction factor, but other species may fractionate $^{15}$N between muscle and scale differently. Our values between scale and muscle tissues in $\delta^{15}$N were consistent with results for whitefish Coregonus lavaretus (Perga and Gerdeaux 2003), but significantly differed from published values for sockeye salmon Oncorhynchus nerka, chum

**Discussion**

The values of $\delta^{13}$C and $\delta^{15}$N in the scale and muscle tissues of sunfish and in the fin and muscle tissues of slimy sculpin were strongly correlated, leading us to conclude that the values of these stable isotopes in scale and fin tissues can be substituted for the values in muscle tissues in these and other species of fish. Correction factors were needed in three of the four categories we tested; only the $\delta^{13}$C values in the slimy sculpin could be used interchangeably without a correction factor. Researchers will need to sacrifice a few fish to determine the actual conversion factor for their individual species and locations.
Table 1.—Literature results on the differences between δ^{13}C and δ^{15}N values in scale or fin and muscle tissues of fish. Values are means ± SDs except where noted with SE. Values without parentheses are our calculations; values in parentheses denote other researchers’ reported data, when scales were acid washed or muscle tissue was lipid normalized.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>n</th>
<th>δ^{13}C (%)</th>
<th>δ^{15}N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scale–muscle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bluegill, pumpkinseed and redbreast sunfish</td>
<td>Massachusetts and New Brunswick</td>
<td>38</td>
<td>2.7 ± 0.7</td>
<td>-1.3 ± 0.6</td>
</tr>
<tr>
<td>Whitefish</td>
<td>European lakes</td>
<td>144</td>
<td>2.7 (4.0)</td>
<td>-1.5 (0.2)</td>
</tr>
<tr>
<td>Sockeye salmon</td>
<td>Alaska</td>
<td>89</td>
<td>3.0 (3.7 ± 0.5)</td>
<td>-0.1 ± 0.6</td>
</tr>
<tr>
<td>Chum salmon</td>
<td>Southeast Alaska</td>
<td>25</td>
<td>3.2 (3.9 ± 0.7)</td>
<td>-0.2 ± 0.6</td>
</tr>
<tr>
<td>Pink salmon</td>
<td>Southeast Alaska</td>
<td>23</td>
<td>2.6 (3.2 ± 0.6)</td>
<td>-0.2 ± 0.5</td>
</tr>
<tr>
<td>Chinook salmon</td>
<td>Southeast Alaska</td>
<td>15</td>
<td>3.0 (3.7 ± 0.4)</td>
<td>-0.1 ± 0.6</td>
</tr>
<tr>
<td>Coho salmon</td>
<td>Southeast Alaska</td>
<td>12</td>
<td>2.9 (3.6 ± 0.3)</td>
<td>-0.4 ± 0.4</td>
</tr>
<tr>
<td>Cui-ui</td>
<td>Nevada</td>
<td>6</td>
<td>2.6 ± 0.4</td>
<td>-0.2 ± 1.4</td>
</tr>
<tr>
<td>Haddock</td>
<td>Georges Bank</td>
<td>2</td>
<td>3.2 ± 0.6 (SE)</td>
<td>0.0 ± 0.2 (SE)</td>
</tr>
<tr>
<td>Redfinned bully</td>
<td>Hutt River, New Zealand</td>
<td>2</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Upland bully</td>
<td>Hutt River, New Zealand</td>
<td>2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td><strong>Fin–muscle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slimy sculpin</td>
<td>Canadian rivers</td>
<td>30</td>
<td>-0.3 ± 0.5</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>Brown trout</td>
<td>European rivers</td>
<td>38</td>
<td>0.1</td>
<td>-0.4</td>
</tr>
<tr>
<td>Brook trout</td>
<td>Pointe Wolfe River, Canada</td>
<td>28</td>
<td>0.7 ± 0.9</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>Atlantic salmon</td>
<td>Miramichi River, Canada</td>
<td>24</td>
<td>0.2 ± 0.4</td>
<td>-0.8 ± 0.4</td>
</tr>
<tr>
<td>Redfinned bully</td>
<td>Hutt River, New Zealand</td>
<td>2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Upland bully</td>
<td>Hutt River, New Zealand</td>
<td>2</td>
<td>-0.3</td>
<td></td>
</tr>
<tr>
<td>Longfinned eel</td>
<td>Hutt River, New Zealand</td>
<td>2</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Shortfinned eel</td>
<td>Hutt River, New Zealand</td>
<td>2</td>
<td>-0.1</td>
<td></td>
</tr>
</tbody>
</table>

a This study.

b Perga and Gerdeaux (2003).

c Values reported in the Perga and Gerdeaux paper for the differences between scale and muscle tissues were 4.0% and -0.2% for δ^{13}C and δ^{15}N, respectively. They acid-washed their scales to remove carbonates, which increased δ^{13}C and δ^{15}N values by 1.3%. We adjusted their values by 1.3% to compare with our scales, which were not acid washed.

d Satterfield and Finney (2002).

e Satterfield and Finney (2002) lipid-normalized their muscle values, which increased δ^{15}N.

f Wainwright et al. (1993).

g Wainwright et al. (1993).

h Composite samples of five fish.

i Rounick and Hicks (1985).


k Jardine et al. (2005).

salmon O. keta, pink salmon O. gorbuscha, Chinook salmon O. tshawytscha, coho salmon O. kisutch, cui-ui Chasmistes cujus, and haddock Melanogrammus aeglefinus (Estep and Vigg 1985; Wainwright et al. 1993; Satterfield and Finney 2002; Table 1). Satterfield and Finney (2002) used only the outer annulus of the scale in their analysis of the salmon species and Estep and Vigg (1985) had a small sample size of six. These factors may have accounted for the less-pronounced differences between scale and muscle δ^{15}N.

Factors that affect the fractionation of δ^{15}N may be location specific. Both sunfish and slimy sculpin had smaller differences between isotope levels in tissues at higher δ^{15}N values (Figure 2). A more complete understanding of the processes that cause the fractionation of δ^{15}N in fish would help us explain these differences in locations. We conclude that slimy sculpin fin values of δ^{15}N can be substituted for muscle values with a correction factor.

Our results, taken with those from previous studies (Estep and Vigg 1985; Rounick and Hicks 1985; Wainwright et al. 1993; McCarthy and Waldron 2000; Satterfield and Finney 2002; Perga and Gerdeaux 2003; Jardine et al. 2005), indicate that slimy sculpin fin tissue and sunfish scale tissue can be used in place of muscle tissue for isotope analysis with the correction factor determined from the linear regression equation stated. Similar scale–muscle and fin–muscle relationships are expected for other fishes but may initially require comparison of tissue before extrapolation. Given that scale and fin tissues have been sampled in a variety of species with relatively little effect on growth and survival (Gjerde and Refstie 1988; Conover and Sheehan 1999; Tyus et al. 1999; Pratt and Fox 2002), we advocate the use of these tissues in dietary investigations using SIA for both δ^{13}C and δ^{15}N. Further study is required to understand the factors that affect
the fractionation of $^{15}$N in organisms and why that fractionation may differ with location.

**Acknowledgments**

We thank Shaheen Kanchwala, Jessica Thomas, Hallie Lee, and Ray Forget for collecting fish in the Massachusetts ponds and helping with the processing of samples. We thank Solange Brait for her consultation on the statistical analyses. Support for this study came from National Science Foundation Research Experience for Undergraduates grant number DBI-0097685 (Jindhart, University of Massachusetts, Boston); North Carolina Sea Grant 2002 (M.H.K.); Fulbright Fellowship 2003 (W.G.H.); Contribution Number 83 of the Catamaran Brook Research Project; and the Canada Research Chairs Program (R.A.C.). Aaron Fraser, Alex Ready, and Dave Parsons assisted in slimy sculpin collections. Anne McGeachy and Mireille Savoie conducted isotope analyses at SINLAB.

**References**


