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Otolith $\delta^{15}N$ Distinguishes Fish from Forested and Agricultural Streams in Southern Illinois

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ABSTRACT

We investigated the ability of otolith stable nitrogen isotope ratio ($\delta^{15}N$) to discriminate among fishes from southern Illinois streams that differed in proportions of watershed agricultural land. Otolith $\delta^{15}N$ was nearly as effective as muscle $\delta^{15}N$ in identifying fishes from individual sites with different percentages of agricultural land cover; both had accuracies of >75%. However, because of the relatively low N content of otoliths, substantial amounts (~8 mg) of otolith material are required for $\delta^{15}N$ analysis compared to fish muscle tissue (~0.3 mg), which precludes the use of otolith $\delta^{15}N$ as an indicator of dietary and environmental history for small fishes.

Measurements of otolith $\delta^{15}N$ have not previously been reported, but nitrogen is present in otoliths because they contain small amounts of protein (Campana 1999). If otolith $\delta^{15}N$ reflects fish diet and environment like muscle $\delta^{15}N$, then otolith $\delta^{15}N$ could be used as an indicator of a fish's dietary and environmental history that would not be subject to subsequent metabolic alteration, as is tissue $\delta^{15}N$. Otolith and muscle $\delta^{15}N$ could also potentially be measured simultaneously to provide insights into both recent (from muscle $\delta^{15}N$) and past (from otolith $\delta^{15}N$) diets and environments.

We collected fishes from one site in each of six streams in southern Illinois during September and October 2006. Sampling locations encompassed a broad range of watershed land cover, from primarily forested to predominantly agricultural catchments. The selected streams were in separate watersheds, which strongly limited the possibility of fish movement among these streams. Species collected included bluegill (*Lepomis macrochirus*), longear sunfish (*L. megalotis*), green sunfish (*L. cyanellus*), and warmouth (*Chaenobryttus gulosus*), and six to eight individuals (63-154 mm total length) were collected from each stream. Fishes were euthanized with MS-222, placed on ice for transport to the laboratory, and stored at -10° C until removal of otoliths and muscle samples.

Sagittal otoliths were removed using forceps, blotted to remove organic residue, rinsed with distilled water, and air dried. One otolith per fish was retained for stable isotope analysis when the mass of an individual otolith was > 8 mg (corresponds to fish > ~95 mm total length); otherwise, both sagittal otoliths from an individual fish were combined to provide sufficient material for analysis. A dorsal muscle plug (~0.5 g) was removed from each fish and dried at 60º C for 48 h. Otolith and muscle samples were ground to a flour-like consistency using a mortar and pestle. Nitrogen isotopic composition ($\delta^{15}N$) of samples was analyzed with a Costech ECS 4010® elemental analyzer interfaced with a ThermoFinnigan Delta Plus XP® isotope ratio mass spectrometer. Mean coefficient of variation among replicate measurements was 3.1% for otolith samples and 1.9% for muscle samples.

We found a highly significant positive correlation between muscle $\delta^{15}N$ and otolith $\delta^{15}N$ for individual fish ($p<0.0001$), with muscle $\delta^{15}N$ averaging 1.1‰ (± 0.2 ‰ SE) higher than otolith $\delta^{15}N$ within individual fish ($p<0.0001$). No significant differences in mean otolith $\delta^{15}N$ or mean muscle $\delta^{15}N$ were detected among species within each of the three sites where multiple individuals of two species were collected (Wilcoxon rank-sum test; $p>0.1$ in all cases).

Percentage of agricultural and percentage of forested land in the watershed for each sampling site were determined from the Illinois Department of Agriculture's Land Cover of Illinois 1999-2000 GIS database (IDA 2007), and these ranged from 18 to 74% for agricultural land and from 16 to 84% for forested land. Both fish muscle and otolith $\delta^{15}N$ were positively correlated with percentage of watershed agricultural land ($r = 0.89$ and 0.81, respectively, $p<0.0001$; Fig. 1) and negatively associated with percentage of forested land in watersheds ($p<0.0001$). One-way analysis of variance followed by Duncan's multiple range test showed that mean otolith $\delta^{15}N$ and mean muscle $\delta^{15}N$ were both significantly different among sites ($p<0.0001$).

Significant correlations that we observed between both muscle and otolith $\delta^{15}N$ and percentage of agricultural land were likely due to differences in $\delta^{15}N$ signatures of stream water dissolved inorganic N that were passed through food webs to fish (Anderson and Cabana 2005). The mechanism underlying differences in fish $\delta^{15}N$ signatures among streams draining primarily forested and predominantly agricultural lands in our study area is unknown. However, other researchers have noted elevated $\delta^{15}N$ of stream water nitrate associated with agricultural activities (Harrington et al. 1998, Lake et al. 2001, Chang et al. 2002), suggesting that anthropogenic sources of nitrate in streams draining watersheds with higher percentages of agricultural land use were likely responsible for observed differences in fish $\delta^{15}N$ among streams. Our results indicated no significant differences in $\delta^{15}N$ among species that occupied the same trophic level (insectivores) within sites; thus, combining species for analysis of inter-site differences in $\delta^{15}N$ was justified.

Linear discriminant function analysis indicated classification accuracies of 75-100% for assigning individual fish to streams draining watersheds with low (<20%), moderate (25-40%), and high (>60%) percentages of agricultural land based on muscle $\delta^{15}N$ (Table 1). Corresponding classification successes using otolith $\delta^{15}N$ ranged from 58 to 91% and were lower than those of muscle $\delta^{15}N$ in all cases. Classification accuracies for individual fish to stream types were higher for sites with <20% and >60% watershed agricultural land use compared to streams with intermediate (25-40%) percentages of watershed agricultural land. Misclassifications were most common among sites with low and moderate percentages of watershed agricultural land use. Only one individual collected from a site with a high percentage of agricultural land was not assigned to that site category (based on otolith $\delta^{15}N$), and only one individual from a site with an intermediate percentage of watershed agricultural land was misclassified as having been captured in a stream with >60% agricultural land in its watershed.

Otolith and muscle $\delta^{15}N$ are both potential indicators of fish environmental history when differences in producer $\delta^{15}N$ among habitats are passed through food webs to fish. Otoliths are metabolically inert and will retain $\delta^{15}N$ signatures of previous environments and diets permanently, although because of their relatively low N content (~0.15 % N) substantially more otolith material (~8 mg) is required for $\delta^{15}N$ analysis compared to fish muscle tissue (~0.3 mg). The relatively large amount of otolith material required for $\delta^{15}N$ analysis precludes detailed reconstructions of fish dietary and environmental history that are possible with stable isotope ratios of other elements (Joukhadar et al. 2002, Kennedy et al. 2002, Whitledge et al. 2007). However, reconstruction of fish dietary and environmental history using $\delta^{15}N$ could be accomplished by using multiple tissues and structures (possibly including otoliths) with different N turnover rates (Hobson 1999, Logan et al. 2006,

MacNeil et al. 2006). For fish whose otoliths substantially exceed 8 mg, $\delta^{15}N$ of subsamples from the otolith core region could provide insights into past diet and habitat use that would otherwise be impossible to obtain when signatures from former diets in metabolically active tissues are obscured by $\delta^{15}N$ of more recent diets.

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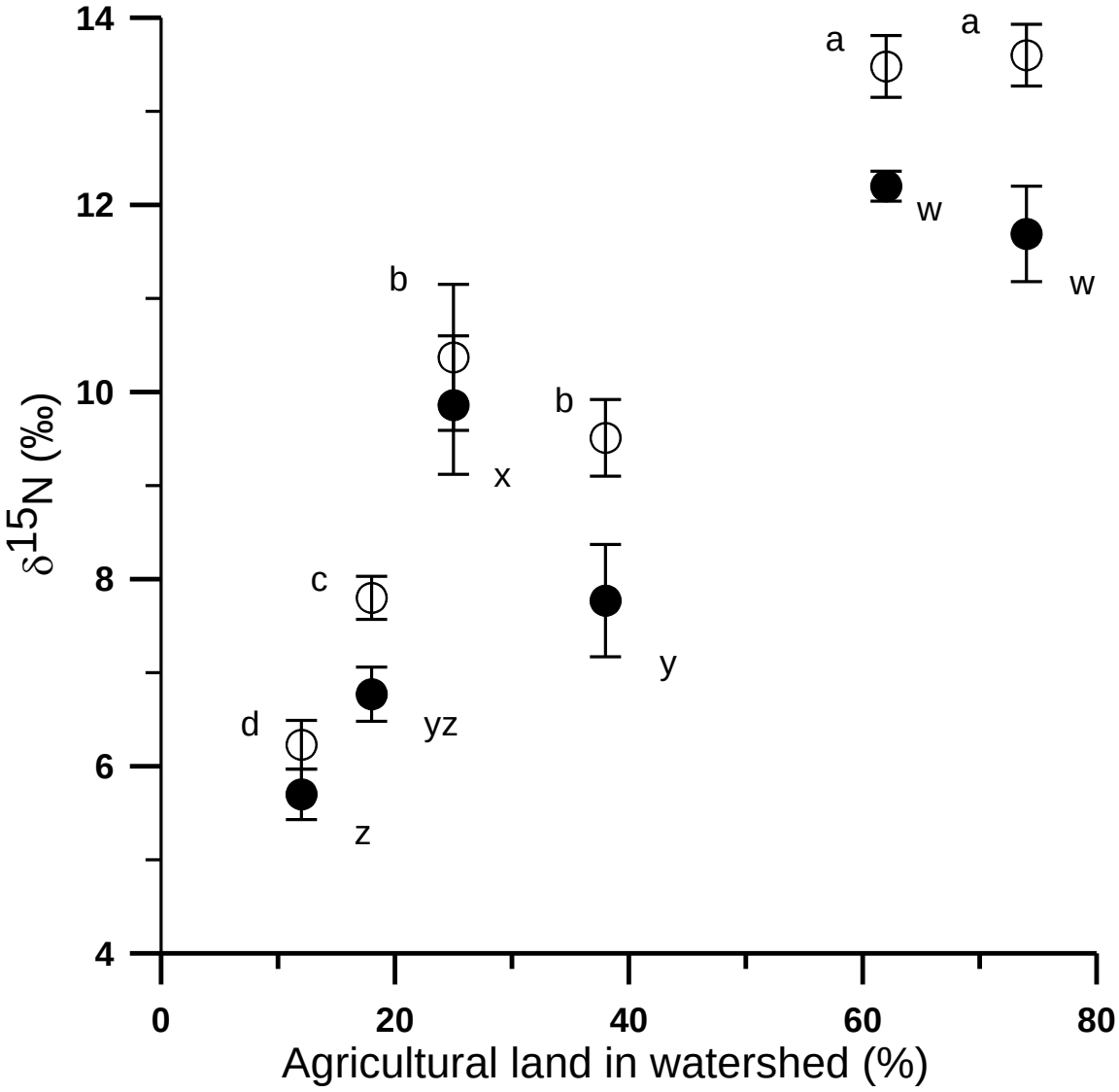
Table 1. Results of linear discriminant function analysis showing classification

accuracy (determined by jackknife procedure) for individual fish to streams draining watersheds with low (<20%), moderate (25-40%) and high (>60%) percentages of agricultural land use (AG) based on otolith $\delta^{15}N$ (‰) and muscle $\delta^{15}N$ (‰). n=number of fish per stream type.

Source stream type	n	Sample type	Assigned stream type			% Correct
			<20% AG	25-40% AG	>60% AG	
< 20 % AG	12	Otolith	10	2	0	83
		Muscle	11	1	0	92
25-40% AG	12	Otolith	4	7	1	58
		Muscle	2	9	1	75
>60% AG	11	Otolith	0	1	10	91
		Muscle	0	0	11	100

Figure Captions

Figure 1. Mean otolith $\delta^{15}N$ (‰, solid symbols ± standard error) and mean muscle $\delta^{15}N$ (‰, open symbols, ± standard error) for sunfishes from six southern Illinois streams in relation to percentage of watershed agricultural land. For a given sample type, means with the same letter are not significantly different ($p>0.05$).


14
12
10
8
6
4
0
20
40
60
80
δ15N (‰)
Agricultural land in watershed (%)
a
a
w
w
b
x
b
y
c
yz
d
z