Transactions of the American Fisheries Society 131:337–342, 2002

Preservation Effects on Stable Isotope Analysis of Fish Muscle

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Abstract.—We evaluated the effect of salt and formalin–ethanol sample preservation on carbon and nitrogen isotopic signatures of fish muscle tissue. We found statistically significant effects of the tissue preservation technique on both $\delta^{13}C$ and $\delta^{15}N$; however, the magnitude of change was small and directionally uniform. Isotopic shifts were similar to those observed in previous studies in which formalin was used to preserve samples of quail blood and muscle and sheep blood. Because salt preservation caused minimal isotopic shifts (+0.13‰ $\delta^{13}C$, +0.72‰ $\delta^{15}N$), we propose salt as an easy, inexpensive preservation technique for biological samples collected in remote field settings. Specimens preserved with formalin and ethanol were minimally affected by preservation (−1.12‰ $\delta^{13}C$, +0.62‰ $\delta^{15}N$) and therefore may be suitable for ecological applications of stable isotope analysis when carbon and nitrogen sources are differentiated by more than 2‰. Further research is required to evaluate potential long-term storage effects of formalin fixation and alcohol preservation on isotopic signatures of fish tissues.

Stable isotope ratios of carbon and nitrogen are natural biomarkers useful to evaluate sources of energy and organic carbon (Peterson and Fry 1987; Forsberg et al. 1993; Bootsma et al. 1996; Gu et al. 1997; Stapp et al. 1999), trophic position (Minagawa and Wada 1984; Hobson and Welch 1992; Vander Zanden and Rasmussen 1999), physiological condition (Hobson et al. 1993), migration patterns (Kline et al. 1990; Hansson et al. 1997), and anthropogenic impacts to ecosystems (Cabana and Rasmussen 1996; Fry 1999). Stable isotope analysis can provide valuable insights into ecosystem processes, species interactions, and community dynamics. The currently accepted protocol for storing isotope samples (freezing) inhibits the analysis of samples from remote field locations and preserved material archived in museums.

To date, four published studies have examined preservation effects on stable isotope ratios (Junger and Planas 1993; Hobson et al. 1997; Bosley and Wainright 1999; Ponsard and Amlou 1999) with somewhat inconsistent results. Junger and Planas (1993) found no significant difference between $\delta^{13}C$ values of fresh and formalin-preserved invertebrates. Their sample size was small ($n = 5$; mean effect not reported), and their study design appears not to have controlled for individual variation. Hobson et al. (1997) evaluated the effect of ethanol and formalin preservation on quail muscle, quail and sheep blood, and other tissues, using a repeated-measures design to control for variation among individuals ($n = 5$). Ethanol-preserved muscle showed nonsignificant depletion in ^{13}C (mean difference = −0.44 parts per thousand [‰]) and nonsignificant enrichment in ^{15}N (mean difference = 0.40‰). Hobson et al. reported a nonsignificant effect of formalin preservation on $\delta^{15}N$ (mean difference = 0.04‰); however, they observed a significant enrichment of $\delta^{13}C$ by between −0.94‰ and −1.78‰ for formalin-preserved quail muscle and quail and sheep blood. Bosley and Wainright (1999) compared $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue from individual fish randomly assigned to various treatments, including a treatment similar to standard museum preservation (i.e., fixation in 10% formalin followed by transfer to 90% ethanol). They reported statistically significant differences between frozen and formalin–ethanol-preserved samples for both $\delta^{13}C$ (mean difference = −2.17‰) and $\delta^{15}N$ (mean difference = 1.41‰). Given their small sample size ($n = 3$) and the potential for within-population variation (Gu et al. 1997), these findings must be interpreted with caution. Ponsard and Amlou (1999) evaluated the effects of ethanol, formalin, and saltwater, among others, on the isotopic composition of *Drosophila* reared in controlled laboratory environments. Given the small body size of *Drosophila*, they were unable to control for potential interindividual variation. Nonetheless, they found the isotopic composition of *Drosophila* preserved in saltwater was not significantly different from that of frozen *Drosophila* samples. Preservation of *Drosophila* in ethanol and formalin for 6 weeks had nonsignificant effects on $\delta^{15}N$ values; *Drosophila* preserved in formalin and ethanol, however, had $\delta^{13}C$ values significantly more negative (mean difference =

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Received May 1, 2001; accepted October 8, 2001

−2.69‰ and −1.17‰, respectively) than those of frozen individuals.

In each of these studies, $\delta^{13}C$ values declined and $\delta^{15}N$ values increased after sample preservation. Freezing is not an option for field studies conducted in remote locations over long periods of time. In those situations, tissue preservation with formalin, alcohol, or salt provides an alternative. In addition, biological specimens housed in natural history collections could be used for stable isotope analysis. Such materials and associated records in natural history collections document biodiversity and ecological relationships through time.

Given the potential usefulness of preserved samples for stable isotope studies, we further evaluated the effect of sample preservation on carbon and nitrogen isotopic signatures. We compared $\delta^{13}C$ and $\delta^{15}N$ signatures of frozen fish muscle samples with those of salt-preserved tissues and of tissues fixed in 10% formalin then transferred to 70% ethanol, the latter being the standard preservation technique for fish specimens (Lundberg and McDade 1990). We hypothesized that salt (NaCl) preservation would not have a significant effect on $\delta^{13}C$ and $\delta^{15}N$ (Ponsard and Amlou 1999). We expected formalin (CH_2O)–ethanol (C_2H_6O) preservation to shift carbon isotope signatures through the retention of preservative carbon (Hobson et al. 1997; Ponsard and Amlou 1999).

Methods

We collected 16 individual fishes representing four species—hardhead catfish *Arius felis* ($n = 1$), spotted seatrout *Cynoscion nebulosus* ($n = 1$), gizzard shad *Dorosoma cepedianum* ($n = 2$), and striped mullet *Mugil cephalus* ($n = 12$)—from an estuarine lagoon, Matagorda Bay, Matagorda County, Texas. Immediately after capture, fishes were euthanized and two pieces of muscle tissue (~2 g) were removed from the dorsum. Both pieces of muscle were rinsed in distilled water for 10 s and then placed in 2.5-in × 3-in Minigrip Reclosable Plastic Bags (Consolidated Plastics Co.). One muscle sample was randomly selected and covered with approximately 20 g of noniodized Morton Salt (>99.1% NaCl), ensuring that salt was in contact with the entire surface area of the muscle sample. The second muscle sample was held on ice for less than 4 h and then frozen. The remainder of the fish was preserved according to standard protocol for natural history collections (2 weeks in 10% formalin followed by transfer to 70% ethanol; Lundberg and McDade 1990). After 6 weeks, a third muscle tissue sample was removed from the dorsum of each formalin-fixed, ethanol-preserved fish specimen. Thus, triplicate samples (frozen, salted, and formalin–ethanol preserved) were obtained from each individual.

Samples were prepared for stable isotope analysis after 6 weeks of preservation. The samples were soaked and then rinsed with distilled water to remove excess preservative (Mullin et al. 1984). Frozen samples were rinsed only. Salt-preserved samples were rinsed in distilled water and then soaked in distilled water for 4 h. Formalin–ethanol-preserved samples were rinsed with distilled water and then soaked in distilled water for 48 h. All samples were then dried at 60°C for approximately 48 h. Once dry, samples were ground to a fine powder with a mortar and pestle. Approximately 0.40 mg of powdered fish muscle was loaded into UltraPure tin capsules (Costech, Valencia, California) and analyzed for percent carbon, percent nitrogen, and isotope ratios (^{13}C:^{12}C and ^{15}N:^{14}N) in a Delta Plus/Carlo Erba EA 1108 continuous-flow isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) in the Texas A&M University Stable Isotope Laboratory (Department of Rangeland, Ecology and Management, College Station, Texas). Results of isotope analyses are reported as parts per thousand relative to standards (Pee Dee belemnite for carbon, atmospheric N_2 for nitrogen) by calibration with SRM 141 d (National Institute of Standards and Technology, Standard Reference Materials Program, Gaithersburg, MD) every eighth sample. Estimated standard error of the mean for SRM 141 d replicates was less than 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$.

We used a repeated-measures analysis of variance (ANOVA) model to control for individual variation and to test for within-subject effects ($n = 16$) among the three preservation techniques (frozen, salted, formalin–ethanol-preserved) for $\delta^{13}C$ and $\delta^{15}N$. Bonferonni post hoc tests were used to evaluate all pairwise comparisons. Analyses were computed by using SPSS statistical software, with significance set at $\alpha = 0.05$.

Results

We found a significant effect of tissue preservation technique on both $\delta^{13}C$ ($P < 0.001$) and $\delta^{15}N$ ($P < 0.001$). A posteriori pairwise comparisons for $\delta^{13}C$ indicate that salt-preserved $\delta^{13}C$ values did not differ significantly from frozen sample values (Table 1; Figure 1A; mean difference $\delta^{13}C = 0.13 \pm 0.06$; $n = 16$, $P > 0.15$). Formalin–ethanol-preserved samples had significantly more

TABLE 1.—A posteriori pairwise comparisons of isotopic values between fish muscle tissue samples preserved with different techniques (n = 16).

Method 1	Method 2	Mean difference	Standard error	P
Carbon isotopes ($\delta^{13}C$)				
Salted	Frozen	0.13	0.06	0.16
Formalin–ethanol	Frozen	−1.12	0.07	0.001
Formalin–ethanol	Salted	−1.25	0.05	0.001
Nitrogen isotopes ($\delta^{15}N$)				
Salted	Frozen	0.72	0.04	0.001
Formalin–ethanol	Frozen	0.62	0.04	0.001
Formalin–ethanol	Salted	−0.10	0.02	0.002

negative $\delta^{13}C$ values than did the frozen samples (mean difference $\delta^{13}C = -1.12 \pm 0.07$; $n = 16$, $P < 0.001$). Comparisons of $\delta^{15}N$ indicate salted samples had significantly higher $\delta^{15}N$ values than did the frozen samples (Table 1; Figure 1B; mean difference $\delta^{15}N = 0.72 \pm 0.07$; $n = 16$, $P < 0.001$), and formalin–ethanol-preserved samples had significantly higher $\delta^{15}N$ values than the frozen samples (mean difference $\delta^{15}N = 0.62 \pm 0.04$; $n = 16$, $P < 0.001$). Finally, compared with the salted samples, formalin–ethanol-preserved samples had significantly more negative $\delta^{13}C$ values (mean difference $\delta^{13}C = -1.25 \pm 0.05$; $n = 16$, $P < 0.001$) and slightly but consistently and significantly lower $\delta^{15}N$ signatures (mean difference $\delta^{15}N = -0.10 \pm 0.02$; $n = 16$, $P < 0.002$).

Triplicate samples from two individuals were analyzed from each treatment to evaluate the effect of preservation on the variability of isotopic values. The standard error of the mean for replicates did not vary in any consistent manner among treatment groups and was consistently less than 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Discussion

As expected, preservation method affected isotopic signatures, but the magnitude of change was highly uniform in direction and magnitude. Salt preservation did not appreciably alter the carbon isotopic composition of fish muscle tissue (mean effect = +0.13‰), but significantly increased nitrogen signatures (+0.72‰). Formalin–ethanol preservation, the standard protocol for specimens in natural history collections, altered both carbon (−1.12‰) and nitrogen signatures (+0.62‰). Even so, observed shifts in isotopic composition were consistent and small relative to observed ecological variation. The high degree of uniformity in isotope ratio shifts associated with preservation implies that comparisons and ecological interpretations can be made, provided the materials compared are handled in the same manner. Preserved materials may be most appropriate for ecological applications of stable isotope analysis in which contrasting elemental sources are differentiated by more than 2‰. Our samples were preserved for only 6 weeks; longer preservation might increase variation. Although more research is needed to evaluate the potential influence of long-term storage in preservatives, Mullin et al. (1984) found no consistent temporal affect of formalin preservation on $\delta^{15}N$ signatures for zooplankton samples preserved for as long as 2 years.

Salt preservation is a suitable preservation technique with little influence on isotopic composition of fish muscle tissue. Although formalin fixation followed by ethanol preservation resulted in a greater magnitude of isotopic shifts, these shifts would be insignificant for ecological comparisons between source end members with divergent carbon and nitrogen signatures, for example, C_3 versus C_4 plants (Fry and Sherr 1984), marine versus terrestrial inputs (Stapp et al. 1999; MacAvoy et al. 2000), pelagic versus littoral inputs (Vander Zanden and Rasmussen 1999), and effects of anthropogenic nutrient loading (Fry 1999). Preservation-associated isotopic ratio shifts were small (0.13–1.25‰ for $\delta^{13}C$ and 0.10–0.72‰ for $\delta^{15}N$; Table 1) relative to the observed intraspecific variation observed in this study (0.02–7.15‰ for $\delta^{13}C$ and 0.01–5.82‰ for $\delta^{15}N$), the previously observed within-population variation (i.e., 1.9–3.6‰ for $\delta^{13}C$ and 1.9–3.1‰ for $\delta^{15}N$; Gu et al. 1997), and the extent of variation typically exploited in ecological interpretations (Vander Zanden et al. 1998; Doucett et al. 1999; Stapp et al. 1999; Vander Zanden and Rasmussen 1999; Weinstein et al. 2000). For example, Vander Zanden and Rasmussen (1999) observed considerable variation in carbon (2.1–6.7‰) and nitrogen (1.5–3.6‰) isotopic signatures among conspecific autotrophs from different localities.

One possible mechanism for explaining the effect of formalin–ethanol preservation on carbon isotopic signature of tissue is the loss of lipids or proteins or both. Loss of lipids should result in more positive $\delta^{13}C$ values (DeNiro and Epstein 1977), because lipids contain less ^{13}C than muscle tissue does (Degens et al. 1968; McConnaughey and McRoy 1979). Our results, however, are concordant with those of others (Hobson et al. 1997; Bosley and Wainright 1999), who found samples preserved in formalin and ethanol to be more neg-

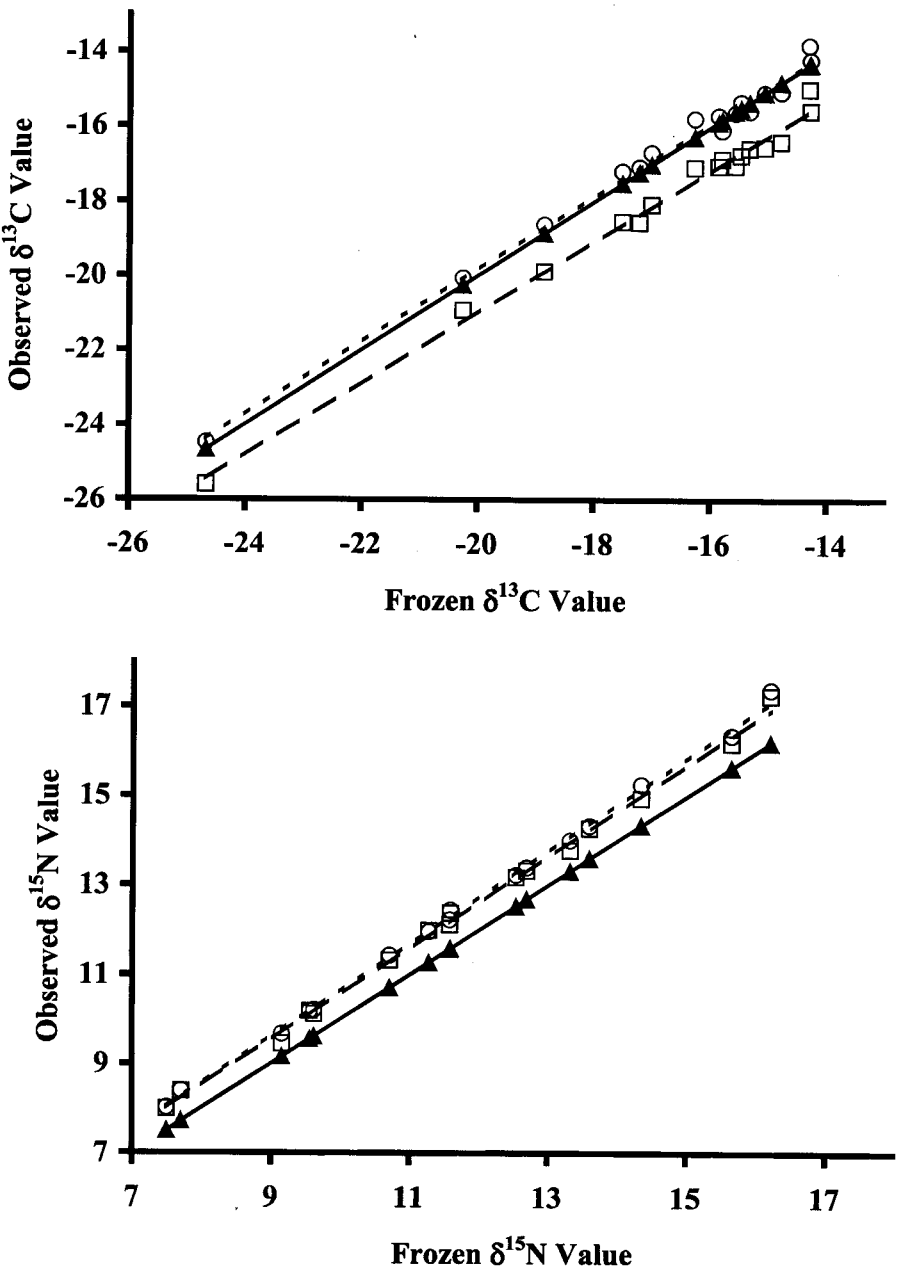

FIGURE 1.—Symbols representing preservation treatments: salted samples (open circles, dotted line), formalin–ethanol samples (open squares, dashed line), and frozen samples (filled triangles, solid line). **(Top)** $\delta^{13}C$ values from preserved samples plotted as dependent variables against frozen-tissue $\delta^{13}C$ values (independent). Fitted linear models: salted $\delta^{13}C = 0.981$ (frozen $\delta^{13}C) - 0.197$, $r^2 = 0.99$; formalin–ethanol $\delta^{13}C = 0.959$ (frozen $\delta^{13}C) - 1.817$, $r^2 = 0.99$. **(Bottom)** $\delta^{15}N$ values from preserved samples plotted as dependent variables against frozen-tissue $\delta^{15}N$ values (independent). Fitted linear models: salted $\delta^{15}N = 1.045$ (frozen $\delta^{15}N) + 0.189$, $r^2 = 0.99$; formalin–ethanol $\delta^{15}N = 1.028$ (frozen $\delta^{15}N) + 0.287$, $r^2 = 0.99$.

ative (depleted in ^{13}C). Our formalin–ethanol-preserved tissues contained lower percentages of carbon relative to their frozen counterparts (n = 16, mean = −3.63‰, SE = 0.31), which is concordant with lipid loss. Depletion in ^{13}C associated with formalin–ethanol preservation was significantly correlated with a decline in percent carbon of fish muscle tissue (n = 16, slope = 0.17, r^2 = 0.41, $P < 0.001$); this is the opposite of the relationship expected if lipid loss were producing observed differences in isotopic signatures of preserved tissues. Alternatively, hydrolysis of proteins by formalin could have affected observed isotopic signatures. Although we did not calculate mass balance, we believe more negative $\delta^{13}C$ values could be the result of uptake of carbon from preservatives (Hobson et al. 1997). If preservative uptake is responsible for altered isotopic signatures, shifts in $\delta^{13}C$ values could depend on the isotopic composition of the chemical stock (manufactured source) used for sample preservation. Duration of sample preservation and preservative concentration also could affect isotope signatures, although the former appears not to have an effect through the first 2 years of preservation (Mullin et al. 1984). Thus, further tests are needed to verify that preservatives obtained from different sources yield the same effect, and to evaluate potential long-term storage effects of formalin fixation and alcohol preservation on isotopic signatures.

Both salt and formalin–ethanol preservation had similar effects on $\delta^{15}N$ values. The specific mechanism that leads to the observed increase in $\delta^{15}N$ after sample preservation is unknown; however, nitrogen enrichment has been observed for several treatments, including ethanol (Hobson et al. 1997), formalin, formalin–ethanol (Bosley and Wainright 1999), and acidification (Bunn et al. 1995). This $\delta^{15}N$ effect of preservation appears to be a general trend. Additional data from fishes collected in South America—bocona *Brycon falcatus* (n = 15), sabalito *B. pesu* (n = 2), boca chica *Semaprochilodus kneri* (n = 14), and caribe cachamera *Serrasalmus manueli* (n = 9)—for which we only have salt- and formalin–ethanol-preserved samples of dorsal muscle, reveal a trend similar in sign and magnitude to that obtained for $\delta^{15}N$ in Table 1 (n = 40, mean difference between salt and formalin–ethanol-preserved fish muscle = −0.21‰, SE = 0.05).

Our results do not indicate an effect of sample preservation on isotopic variation within treatment groups. Previously, Bosley and Wainright (1999) stated that preservatives increased the variability of $\delta^{15}N$ values and decreased the variability of $\delta^{13}C$ values. Standard error of triplicate samples taken from two specimens was consistently less than 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ for each preservation technique. Furthermore, unlike Bosley and Wainright's study, we accounted for interindividual variation. Isotopic variation observed by Bosley and Wainright may have been influenced by small sample size and high between-individual variation, the latter being high in some systems (Gu et al. 1997). Their small sample sizes (treatment group n = 3) carry high risk of type II error.

In conclusion, we propose salt as a suitable preservation technique for fish muscle tissue. Our study suggests material preserved with salt or the same formalin and ethanol stocks is suitable for current ecological applications of isotopic analysis, provided that the samples to be compared are handled in the same way. This contention is reinforced by interspecific uniformity in the sign and similarity in magnitude of shifts observed in our study and in the Hobson et al. (1997) study with quail blood, sheep blood, and quail muscle. Although further evaluation of preservation effects on stable isotope ratios is needed, specimens preserved in natural history collections may be suitable for ecological investigations based on stable isotope analysis. Research also is needed to determine the chemical dynamics responsible for the isotopic differences observed among preservation methods.

Acknowledgments

We thank Thomas Boutton for his assistance with this project. The Nature Conservancy of Texas provided access to the field site. Funding for this study was provided through a Texas A&M University Interdisciplinary Research Initiative grant.

References

Bootsma, H. A., R. E. Hecky, R. H. Hesslein, and G. F. Turner. 1996. Food partitioning among Lake Malawi nearshore fishes as revealed by stable isotope analyses. Ecology 77:1286–1290.

Bosley, K. L., and S. C. Wainright. 1999. Effects of preservatives and acidification on the stable isotope ratios (^{15}N:^{14}N, ^{13}C:^{12}C) of two species of marine animals. Canadian Journal of Fisheries and Aquatic Sciences 56:2181–2185.

Bunn, S. E., N. R. Loneragan, and M. A. Kempster. 1995. Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: implications for food-web studies using multiple stable isotopes. Limnology and Oceanography 40: 622–625.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences of the USA 93:10844–10847.

Degens, E. T., M. Behrendt, B. Gotthardt, and E. Reppmann. 1968. Metabolic fractionation of carbon isotopes in marine plankton II. Data on samples collected off the coasts of Peru and Ecuador. Deep-Sea Research 15:11–20.

DeNiro, M. J., and S. Epstein. 1977. Mechanisms of carbon isotope fractionation associated with lipid synthesis. Science 197:261–263.

Doucett, R. R., W. Hooper, and G. Power. 1999. Identification of anadromous and nonanadromous adult brook trout and their progeny in the Tabusintac River, New Brunswick, by means of multiple-stable-isotope analysis. Transactions of the American Fisheries Society 128:278–288.

Forsberg, B. R., C. A. R. M. Arauho-Lima, L. A. Martinelli, R. L. Victoria, and J. A. Bonassi. 1993. Autotrophic carbon sources for fish of the central Amazon. Ecology 74:643–652.

Fry, B. 1999. Using stable isotopes to monitor watershed influences on aquatic trophodynamics. Canadian Journal of Fisheries and Aquatic Sciences 56: 2167–2171.

Fry, B., and E. B. Sherr. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in Marine Science 27: 13–47.

Gu, B. H., C. L. Schelske, and M. V. Hoyer. 1997. Intrapopulation feeding diversity in blue tilapia: evidence from stable-isotope analyses. Ecology 78: 2263–2266.

Hansson, S., J. E. Hobbie, R. Elmgren, U. Larsson, B. Fry, and S. Johansson. 1997. The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. Ecology 78:2249–2257.

Hobson, K. A., R. T. Alisauskas, and R. G. Clark. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. Condor 95:388–394.

Hobson, K. A., H. L. Gibbs, and M. L. Gloutney. 1997. Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. Canadian Journal of Zoology 75:1720–1723.

Hobson, K. A., and H. E. Welch. 1992. Determination of trophic relationships within a High Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series 84:9–18.

Junger, M., and D. Planas. 1993. Alteration of trophic interactions between periphyton and invertebrates in an acidified stream: a stable carbon isotope study. Hydrobiologia 262:97–107.

Kline, T. C., Jr., J. J. Goerning, O. L. Mathisen, P. H. Poe, and P. L. Parker. 1990. Recycling of elements transported upstream by runs of Pacific salmon: 1. $\delta^{15}N$ and $\delta^{13}C$ evidence in Sashin Creek, southeastern Alaska. Canadian Journal of Fisheries and Aquatic Sciences 47:136–144.

Lundberg, J. G., and L. A. McDade. 1990. Systematics. Pages 65–103 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

MacAvoy, S. E., S. A. Macko, S. P. McIninch, and G. C. Garman. 2000. Marine nutrient contributions to freshwater apex predators. Oecologia 122:568–573.

McConnaughey, T., and C. P. McRoy. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Marine Biology 53:257–262.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48:1135–1140.

Mullin, M. M., G. H. Rau, and R. W. Eppley. 1984. Stable nitrogen isotopes in zooplankton: some geographic and temporal variations in the North Pacific. Limnology and Oceanography 29:1267–1273.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Reviews in Ecology and Systematics 18:293–320.

Ponsard, S., and M. Amlou. 1999. Effects of several preservation methods on the isotopic content of *Drosophila* samples. Comptes Rendus de l Academie des Sciences Serie III-Sciences de la Vie-Life Sciences 322:35–41.

Stapp, P., G. A. Polis, and F. Sánchez Piñero. 1999. Stable isotopes reveal strong marine and El Niño effects on island food webs. Nature (London) 401: 467–469.

Vander Zanden, M. J., M. Hulshof, M. S. Ridgway, and J. B. Rasmussen. 1998. Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. Transactions of the American Fisheries Society 127:729–739.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80:1395–1404.

Weinstein, M. P., S. Y. Litvin, K. L. Bosley, C. M. Fuller, and S. C. Wainright. 2000. The role of tidal salt marsh as an energy source for marine transient and resident finfishes: a stable isotope approach. Transactions of the American Fisheries Society 129:797–810.