Aquat Ecol (2011) 45:483–492
DOI 10.1007/s10452-011-9369-5

Preservation effects on stable isotope ratios and consequences for the reconstruction of energetic pathways

Jun Xu · Qiang Yang · Meng Zhang · Min Zhang · Ping Xie · Lars-Anders Hansson

Received: 21 February 2011 / Accepted: 8 August 2011 / Published online: 18 August 2011

Abstract Stable isotope analysis provides a powerful tool for describing the energetic pathways in a variety of ecosystems. However, isotope ratios of animal tissues can be altered by preservation methods, potentially leading to biased estimates of energy pathways when they are not taken into account. Here, we investigated the direct preservation effects of formalin, ethanol, NaCl, and drying on the δ^{13}C and δ^{15}N of fish muscle tissues, as well as the ultimate effects on the reconstruction of the energy pathways. All preservation methods, except drying, had significant impacts on δ^{13}C and δ^{15}N values. The effects of preservation appear to be highly taxa-specific and no significant time-dependent variations in nearly 2-year duration of preservation. δ^{13}C and δ^{15}N values were generally changed dramatically within the early stage of the preservation process and became stable over a relatively long-term preservation. Using an isotopic balance mixing model, the isotope-based food web reconstruction reveals that, without preservation correction, the importance of the pelagic energetic pathways for the fishes could be misestimated, except for the drying preservation. These results highlight that preservation can bias the interpretation of food web reconstruction results.

Keywords Carbon and nitrogen · Preservation · Food web reconstruction · Trophic relationships · Energy flows

Handling Editor: Thomas Mehner.

J. Xu (✉) · Q. Yang · P. Xie (✉)
Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, No. 7 Donghu South Road, Wuchang District, Wuhan 430072, Hubei Province, People's Republic of China
e-mail: xujun@ihb.ac.cn

P. Xie
e-mail: xieping@ihb.ac.cn

J. Xu · L.-A. Hansson
Institute of Ecology, Lund University, Ecology Building, 223 62 Lund, Sweden

M. Zhang
Jiangxi Academy of Environmental Sciences, Nanchang 330029, China

M. Zhang
College of Fisheries, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

Introduction

Stable isotope analysis (SIA) in food web studies is now common, and the consistent measurement of isotopic signatures at the individual, population, and species levels can be used to determine the trophic position and energy source of organisms within food webs (e.g., Sierszen et al. 2003; Yeager and Layman 2011; Hornung and Foote 2008). Routinely, the stable nitrogen isotope ratio (δ^{15}N) of consumers is elevated

by about 3–4‰ relative to their food sources during trophic transfer (Post 2002). The stable carbon isotope ratio (δ^{13}C) exhibits little or no trophic level enrichment (0–1‰) and is used to identify the sources of primary production for consumers (Vander Zanden and Vadeboncoeur 2002; Xu et al. 2008). For example, distinct δ^{13}C values of pelagic, benthic, and terrestrial sources are transferred up the food chain, and the consumer δ^{13}C values can then be used to estimate source contributions (Hecky and Hesslein 1995).

Widespread interest has been focused on the alteration of ecosystem structure and function induced by natural and human activities. Using SIA, the change in trophic level and the pathway of energy flow were reported, suggesting significant ecological disruptions in food webs (e.g., Perga and Gerdeaux 2003; Schmidt et al. 2009; Solomon et al. 2008; Vander Zanden et al. 1999). However, many of studies suggest that small isotopic shifts can be associated with large ecosystem disturbances over long periods of time. For example, Perga and Gerdeaux (2003) found the changes in the δ^{13}C of whitefish determined from a scale collection were only within 3‰ although the data extended over the recovering period from eutrophic to oligotrophic state of Lake Geneva. Meanwhile, although interpreting isotope values from the same study with the same treatment method is reasonable, the effects of different sample preservation methods prior to analysis may preclude the comparison of results from investigations differing in time and space (Barrow et al. 2008; Syvaranta et al. 2008; Ventura and Jeppesen 2009). Therefore, the effects of different preservation methods on stable isotope values of the archived samples should be a concern because this may affect the reconstruction and interpretation of the changes in past food webs (Barrow et al. 2008; Kelly et al. 2006; Syvaranta et al. 2008; Ventura and Jeppesen 2009).

Many studies reported the effects of sample preservation on a variety of aquatic organisms, including fishes, benthic invertebrates, and zooplankton (e.g., Barrow et al. 2008; Bosley and Wainright 1999; Kelly et al. 2006; Ventura and Jeppesen 2009). The reported isotopic shift is typically small (within 2‰) and comparable with the isotopic shifts observed in ecosystem reconstruction using the isotopic signatures of archived sample material (Pruell et al. 2003; Solomon et al. 2008; Wainright et al. 1993). Previous studies have shown that the effects of preservation on stable isotopes depend on organisms and preservatives and are often inconsistent and variable (Barrow et al. 2008; Kelly et al. 2006; Schmidt et al. 2009; Syvaranta et al. 2008, 2010). These methodological inconsistencies inhibit our ability to appropriately pool data across studies and may adversely affect data interpretation within studies.

Despite the relatively high number of studies describing the effects of preservation substances, the question of their ultimate effects on the interpretation of the energy pathways of organisms has not been addressed directly. Thus far, very few studies have considered the potential influence of preservation effects on ecosystem reconstruction. Therefore, the main objective of the present study is to explore the potential effects of preservation on the estimates of energy sources in a food web. To this end, we first investigated the effects of drying, formalin, ethanol, and salt (NaCl) on δ^{13}C and δ^{15}N of fishes with different trophic positions. These four preservation methods were chosen based on the consideration that these methods are easy to access in the remote areas where electricity and high-power apparatuses are usually not existed. Second, we determined whether any species-specific or time-dependent changes can be observed in the δ^{13}C and δ^{15}N of fishes with different preservation treatments. Finally, we used an isotope mixing model to reconstruct the energy pathways for each of species to illustrate to what extent the common archived materials used for stable isotope analyses will affect the reconstruction of the changes in ecosystem structure and function.

Materials and methods

Fish sampling

Adult fish samples of Chinese perch (*Siniperca chuatsi* Basilewsky 1855), bighead carp (*Hypophthalmichthys nobilis* Richardson 1845), and grass carp (*Ctenopharyngodon idellus* Valenciennes, 1844) were collected by fishermen from the Mushan area of Lake Chaohu in Anhui Province, Central China, in December 2003 (Xu et al. 2007); the samples were then immediately taken to the laboratory for cleaning and processing (Xu et al. 2005, 2007, 2008). Chinese perch is a piscivorous fish in a benthic–pelagic

habitat; bighead carp is a planktivorous fish in a pelagic habitat; and grass carp is an herbivorous fish in a benthic habitat. We chose these three fish species because of their distinct energetic pathways in the lake food web. Three individuals for each fish species were obtained to test the preservation effects. A filet of dorsal muscle (white muscle) was removed from each individual, cut into pieces, and placed into 10-ml-acidized glass bottles. White muscle tissues have been shown to be representative of the overall stable isotope values in fish (Hesslein et al. 1993) and are widely used in isotope ecology (Post 2002; Vander Zanden and Vadeboncoeur 2002).

Preservation experiment

Samples were divided into those that were dissected, dried (60°C to a constant weight), and analyzed immediately upon collection, which were used as the control samples to be compared with all preserved samples (hereafter "the control"), and those preserved using four treatments by drying (oven-dried at 60°C to a constant weight and sealed in desiccator with silica gel), or immersed in ethanol (70%), formalin (4%), or in supersaturated NaCl solution at room temperature for 13, 31, 63, 99, 127, 165, and 625 days. After each preservation treatments had completed, samples, except for the drying treatments, were carefully rinsed in distilled deionized water for 1 min before oven-drying at 60°C to a constant weight for SIA. The samples were ground to a fine homogenous powder using a mortar and pestle. The mortar and pestle were acid washed and dried to prevent cross-contamination between samples.

Stable isotope analysis

The isotopic composition of the carbon and nitrogen in the samples was analyzed using a Carlo Erba NC-2500 elemental analyzer coupled with a Delta Plus (Finnigan) isotope ratio mass spectrometer via continuous flow II interface. Global reference materials were carbonatite (IAEA-NBS18) and graphite (IAEA-USGS24) for δ^{13}C and ammonium sulfate (IAEA-USGS25 and IAEA-USGS26) for δ^{15}N. On a daily basis, an internal working standard, urea ($\delta^{13}C = -49.44‰$, $\delta^{15}N = -1.53‰$), was used for both δ^{13}C and δ^{15}N analyses. Three to six measurements of each sample were taken using the mass spectrometer to calculate the final carbon and nitrogen isotope composition of 20% samples as replicates. To ensure consistent calibration of the instrument, standard materials were analyzed at an interval of about 10 samples. Blank samples were run through the instrument after every 5 to 10 samples to clear the system of residual gases (i.e., carbon dioxide). The analytical error for replicate samples was approximately ±0.1‰ for δ^{13}C and ±0.2‰ for δ^{15}N.

Mass balance modeling

Large-body primary consumers are considered less sensitive to temporal fluctuations of the isotopic compositions of primary producers and can reflect the base of benthic and pelagic food webs, providing an isotopic baseline for estimating energetic pathways of higher trophic level consumers in lake ecosystems (Cabana and Rasmussen 1996; Post 2002; Vander Zanden et al. 1999; Xu et al. 2005, 2010). Therefore, surface-grazing freshwater snail *Bellamya aeruginosa* and filter-feeding freshwater clam *Corbicula fluminea*, which were sampled simultaneously with the three fish species used here and experienced the same way of pre-treatment as the controls samples in this study, were used to quantify the isotopic baseline for benthic and pelagic energetic pathways. The stable isotope values of these end-members (or baselines) are reported in a previous study on this lake (Xu et al. 2007). The δ^{13}C values of the three fish species studied here were converted into proportions of pelagic and benthic energetic pathways with a Bayesian isotopic mixing models using the software SIAR (Parnell et al. 2010). When calculating mixing models, SIAR takes into account the natural variation and uncertainty in both the sources and the fishes and generates robust probability estimates of source proportions. Trophic enrichment factors for carbon and nitrogen along the trophic transfer are set as 0.4 and 3.4‰ as suggested by Post (2002).

Statistical analysis

Mixed model approach was selected to investigate factor effects and interactions among them. In this

model, preservation treatment, species, and time were set as fixed factors, and the individuals of each species were set as a random effect. A Bonferroni pairwise comparison tests were used to prevent the prevalence of false positives after testing for data normality and variance homogeneity. To test the effects of the preservation method and preservation time on the stable isotope values of different fishes, a one-way ANOVA was selected with Bonferroni pairwise comparison tests after testing for data normality and variance homogeneity. The Bonferroni multiple comparison test was also used to check whether the four preservation types of the three fish species caused significant changes in the δ^{15}N and δ^{13}C of preserved fish samples. These analyses were based on the difference between the individual preserved samples and the individual control samples. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS Inc. 2009).

Results

Trophic level and energetic pathways

The δ^{13}C and δ^{15}N values of all species as control samples were ranged from −27.2 to −23.2‰ and from 12.6 to 16.5‰ (Fig. 1). According to the δ^{15}N values, herbivorous grass carp (13.1 ± 0.4‰) and planktivorous bighead carp (13.3 ± 0.1‰) occupied a relatively low trophic level compared with the piscivorous Chinese perch (16.4 ± 0.1‰) (Student's *t* test, $P < 0.010$ for both). Chinese perch and grass carp had similar δ^{13}C values (−23.9 ± 0.9 and −24.1 ± 0.4‰, Student's *t* test, $P = 0.336$), whereas bighead carp exhibited significantly lower δ^{13}C values (−26.4 ± 0.7‰) than both Chinese perch and grass carp (Student's *t* test, $P < 0.010$ for both). Both δ^{15}N and δ^{13}C values indicated that differences in energetic pathways could be expected in these

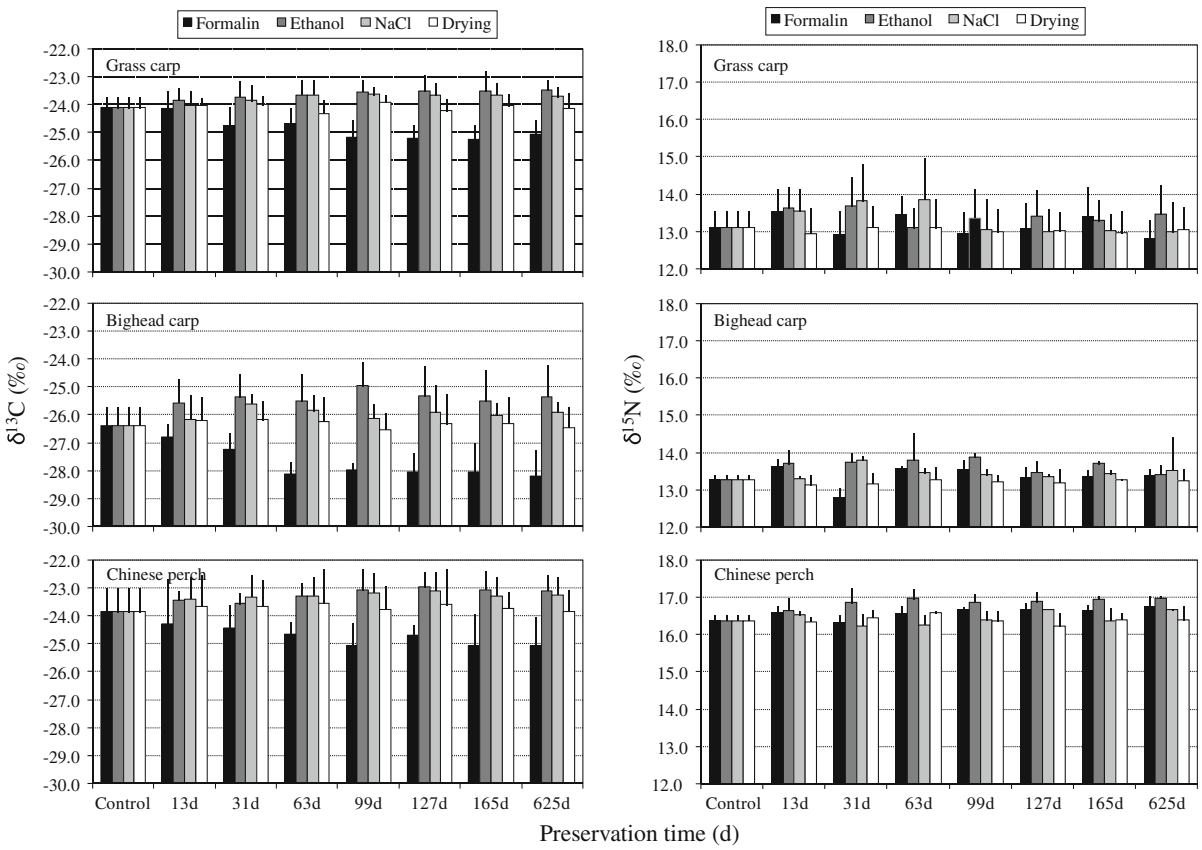

Fig. 1 Changes in δ^{13}C and δ^{15}N values after a series of preservation times by drying, or in formalin, ethanol, or NaCl. The *bars* showed the standard deviations

interested fish species, compared with the pelagic and benthic baselines (10.5 ± 0.7‰ and −22.4 ± 0.4‰ for snail *Bellamya aeruginosa*; 8.5 ± 1.5‰ and −29.82 ± 0.5‰ for freshwater clam *Corbicula fluminea*).

Effects of different factors and their interactions

Mixed model was selected to investigate the effects of different factors (fish species, preservation type, and time) and their effects on δ^{13}C and δ^{15}N values (Table 1). The main effects caused by the preservation type were statistically significant for δ^{13}C values ($df = 3$, $F = 62.6$, $P < 0.001$). The main effects caused by the fish species were statistically significant for both δ^{13}C and δ^{15}N values ($df = 2$, $F = 369.8$, $P < 0.001$ for δ^{13}C and $df = 2$, $F = 1{,}858$, $P < 0.001$ for δ^{15}N). Nonetheless, the preservation effect caused by time is non-significant for both δ^{13}C and δ^{15}N values (see Fig. 1). In the second-order interactions, the preservation type and time of interaction are significant for δ^{13}C values ($df = 21$, $F = 2.156$, $P < 0.005$), reflecting the highly divergent behavior of different preservation types along different preservation time. The preservation type and fish species of interaction are nearly significant for δ^{13}C values ($df = 6$, $F = 2.077$, $P = 0.058$). This is reflected in the highly divergent patterns of different fish species preserved with different preservation methods. No significant interactions were found in the interaction between preservation time and fish species ($df = 14$, $F = 0.731$, $P = 0.742$ for δ^{15}N and $df = 14$, $F = 0.171$, $P = 1.000$ for δ^{13}C), and in the interaction among preservation type, time, and fish species ($df = 42$, $F = 0.520$, $P = 0.993$ for δ^{15}N and $df = 42$, $F = 0.141$, $P = 1.000$ for δ^{13}C).

Species-specific and time-dependent isotopic shift in different preservation types

Compared with the control samples, one-way ANOVA detected the significant impacts of preservation types on δ^{15}N and δ^{13}C of Chinese perch, grass carp, and bighead carp. Significant impacts of preservation on δ^{13}C of grass carp are $df = 3$, $F = 22.28$, $P < 0.001$; δ^{15}N and δ^{13}C of bighead carp are $df = 3$, $F = 7.970$, $P < 0.001$ and $df = 3$, $F = 27.119$, $P < 0.001$, respectively; and δ^{15}N and δ^{13}C of Chinese perch are $df = 3$, $F = 13.318$, $P < 0.001$ and $df = 3$, $F = 11.041$, $P < 0.001$, respectively. Multiple comparisons with Benferroni correction indicated formalin were significantly different from all other preservation methods for carbon isotopic shift in three fish species (Table 2). One-way ANOVA indicates that preservation time (from 13 to 625 days) did not significantly affect the difference in stable isotope values between control and preserved samples. Therefore, we gave a general view of the evident impacts of preservation types on shifts in δ^{15}N and δ^{13}C of Chinese perch, grass carp, and bighead carp with averaged preservation shifts (difference between preserved and control) of all individual of each fish species in each preservation methods at each time (from 13 to 625 days) (Fig. 2),

Table 1 Mixed model of preserved fish samples' δ^{15}N and δ^{13}C among fish species, preservation time, and preservation methods (significance at $P < 0.05$)

Source	δ^{15}N				δ^{13}C			
	df (I)	*df* (II)	*F*	*P*	*df* (I)	*df* (II)	*F*	*P*
Preservation type	3	12	2.287	0.131	3	192	62.60	<0.001
Preservation time	7	188	1.030	0.411	7	192	0.305	0.951
Fish species	2	188	1,858	<0.001	2	192	369.8	<0.001
Preservation type × time	21	188	0.914	0.573	21	192	2.156	<0.005
Preservation type × fish species	6	188	0.555	0.766	6	192	2.077	0.058
Preservation time × fish species	14	188	0.731	0.742	14	192	0.171	1.000
Preservation type × time × fish species	42	188	0.520	0.993	42	192	0.141	1.000
Error	192				192			

df (I) numerator *df*, *df* (II) denominator *df*

Table 2 ANOVA multiple comparisons of the preservation shifts of the four different preservation methods on δ^{15}N and δ^{13}C of each fish species compared with values from control samples ($\Delta\delta^{15}$N and $\Delta\delta^{13}$C)

Preservation methods	Formalin $\Delta\delta^{15}N_{grass\ carp}$	Ethanol	NaCl	Formalin $\Delta\delta^{13}C_{grass\ carp}$	Ethanol	NaCl
Ethanol	1.000			<0.001		
NaCl	1.000	1.000		<0.001	1.000	
Drying	1.000	0.414	1.000	<0.001	<0.05	0.288
Preservation methods	**Formalin $\Delta\delta^{15}N_{bighead}$**	**Ethanol**	**NaCl**	**Formalin $\Delta\delta^{13}C_{bighead}$**	**Ethanol**	**NaCl**
Ethanol	<0.05			<0.001		
NaCl	1.000	0.172		<0.001	0.284	
Drying	0.071	<0.001	0.521	<0.001	<0.01	1.000
Preservation methods	**Formalin $\Delta\delta^{15}N_{Chinese\ perch}$**	**Ethanol**	**NaCl**	**Formalin $\Delta\delta^{13}C_{Chinese\ perch}$**	**Ethanol**	**NaCl**
Ethanol	<0.01			<0.001		
NaCl	0.504	<0.001		<0.001	1.000	
Drying	0.078	<0.001	1.000	<0.01	0.691	0.980

Significance at $P < 0.05$

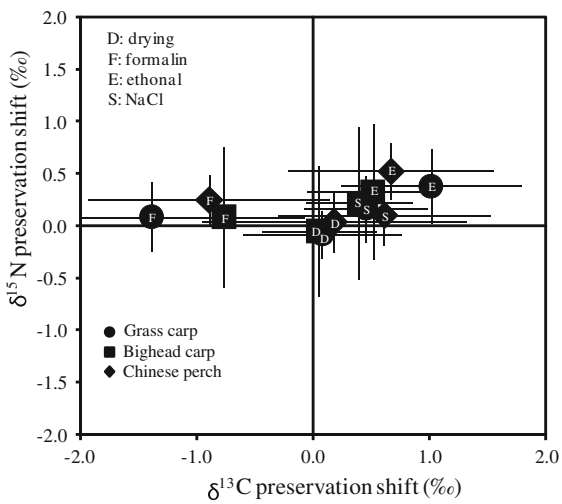

Fig. 2 Preservation shifts in stable isotope values of grass carp, bighead, and Chinese perch as a result of preservation in formalin, ethanol, NaCl, and drying at end of the experiment compared with a control dried on before the beginning of the experiment. *Symbols* and *error bars* are the average ± SD of the preservation shifts (difference between preserved and control values). The *horizontal* and *vertical solid lines* represent the non-effect situation of preservation on isotopic signatures of the samples

showing the either increased or decreased isotopic signatures in different preservation types and fish species.

Energetic pathway quantification

The mixing model reflected differences (Fig. 3) in the contribution of the pelagic energy pathway for grass carp (22–41%), Chinese perch (6–54%), and bighead carp (37–75%) before they were preserved (control samples). One-way ANOVA indicates that there are significant differences among the preservation methods for the percentage of pelagic contribution of Chinese perch ($df = 4$, $F = 66{,}461.90$, $P < 0.0001$), grass carp ($df = 4$, $F = 161{,}152.41$, $P < 0.0001$), and bighead carp ($df = 4$, $F = 169{,}742.39$, $P < 0.0001$). Compared with the situation of unpreserved fishes, multiple comparisons indicated that formalin preservation generally significantly increased the importance of the contribution of the pelagic energy pathway in grass carp (36–47%), Chinese perch (32–44%), and bighead carp (74–85%) ($P < 0.001$ for each fish species). In contrast, ethanol and NaCl preservation significantly decreased (about 5–20%) the importance of the contribution of the pelagic energy pathway ($P < 0.001$ for each fish species of each treatment). Estimation based on the stable isotope values of drying samples showed that the pelagic contribution of grass carp, Chinese perch, and bighead carp were 27–35%, 18–31%, and 50–65%, respectively, and had no significant effect on the

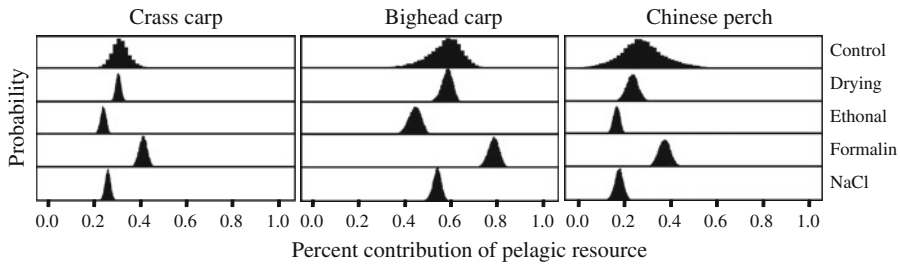

Fig. 3 The histograms showing the posterior model estimates of pelagic contributions for fishes in each preservation methods calculated with a Bayesian isotopic mixing model

contribution of the pelagic energy pathway of all fish species.

Discussion

Although the preservative effects on the stable isotope values of organisms have been widely tested (e.g., Barrow et al. 2008; Kelly et al. 2006; Sweeting et al. 2004; Syvaranta et al. 2008), our study emphasizes the less studied species-, time-, and preservative-dependent shifts of δ^{13}C and δ^{15}N in archived ecological specimens, and their consequences for comprehensive descriptions of historical food webs using stable isotope analysis. Our preservation experiment, which lasted nearly 2 years, indicates that dramatic changes in stable isotope have occurred in three freshwater fishes preserved in liquid chemicals, although the low number of replicates might, to some extent, influence an evaluation of the preservation impacts. The estimation of energetic pathways shows that preservation shifts in isotopic signatures can cause misunderstanding, approximately 20% in maximum, on the importance of energy from the pelagic pathway of fishes with different trophic position.

Chemical preservative-dependent shifts of δ^{13}C and δ^{15}N

The stable isotope values of ecological materials can be shifted by chemical preservatives because of specific mechanisms, including the removal of soluble organic tissue constituents, such as lipids, acids, sugars, and salts, the addition or binding of distinct carbon from preservatives, and the contamination resulting from preservation (Arrington and Winemiller 2002; Barrow et al. 2008; Edwards et al. 2002; Kelly et al. 2006; Sarakinos et al. 2002). However, the scales and direction of these reported effects of preservatives on both δ^{13}C and δ^{15}N have been highly variable and even contradictory (Barrow et al. 2008). Therefore, using various organisms, many studies addressed the significance of possible preservation effects for chemical preservatives, such as formalin, ethanol, formalin–ethanol, salt, lugol, etc. (e.g., Arrington and Winemiller 2002; Barrow et al. 2008; Syvaranta et al. 2008, 2010; Ventura and Jeppesen 2009).

Our results show that the δ^{13}C values of every fish species preserved in formalin were depleted with an average about −1‰ relative to their controls. The negative shift of δ^{13}C has been similarly documented in previous studies and was suggested to be a formalin uptake effect by tissues (Arrington and Winemiller 2002; Edwards et al. 2002; Kelly et al. 2006; Mateo et al. 2008). Formalin increased the δ^{15}N values in our experiments, which can be attributed to the hydrolysis of isotopically light proteins in the high-protein tissue (Sweeting et al. 2004).

The effect of ethanol preservation over time was also evaluated by several studies. Some studies found that ethanol preservation could significantly alter stable isotope values (Barrow et al. 2008; Kaehler and Pakhomov 2001; Ponsard and Amlou 1999; Sarakinos et al. 2002), whereas other studies reported no significant effect on δ^{13}C and δ^{15}N (Barrow et al. 2008; Gloutney and Hobson 1998). We found significantly increased δ^{13}C (0.7‰) and δ^{15}N (0.4‰) values in three fish species preserved in ethanol, suggesting ethanol could cause changes in achieved samples during long-term sample preservation (Barrow et al. 2008; Sweeting et al. 2004). Therefore, before using archived samples with ethanol to reconstruct the historical food web, the effects of ethanol preservation on stable isotopes are suggested to be tested for the interested organisms.

Preservation in a saturated NaCl aqueous solution or in salt is suggested as an alternative when freezing or drying is impossible or inconvenient during field sampling (Arrington and Winemiller 2002; Barrow et al. 2008; Ponsard and Amlou 1999). The short-term storage in saturated NaCl solution is documented to have no effect on either δ^{13}C or δ^{15}N (Barrow et al. 2008; Ponsard and Amlou 1999). However, preservation in saturated NaCl solution was also reported to cause positive shifts on both δ^{13}C and δ^{15}N compared with the controls (Arrington and Winemiller 2002). In the current study, the effects of NaCl as a preservative varied with species and time. δ^{13}C and δ^{15}N of three fish species preserved in salt had significantly positive shifts (0.5 and 0.2‰, respectively), and thus preservation effect on achieved samples preserved in aqueous NaCl is also needed to be considered before using saturated salt preservation (Arrington and Winemiller 2002; Barrow et al. 2008).

Drying-dependent shifts of δ^{13}C and δ^{15}N

Freezing and drying are the most common non-chemical preservation methods in stable isotope ecology. In this study, we evaluated the long-term effects of drying on the stable isotope values, because this method is easier to accessible during the sampling in remote areas than the freezing and freeze-drying. We found that isotope values from samples preserved by drying for each species in the preservation times were not significantly different from the controls. This is comparable with the freezing and freeze-drying methods. For instance, some studies that evaluated the effects of freezing found non-significant shifts in isotope values (Gloutney and Hobson 1998; Kaehler and Pakhomov 2001; Sweeting et al. 2004), while other studies found that both δ^{13}C and δ^{15}N were significantly altered from the control (Barrow et al. 2008; Dannheim et al. 2007; Feuchtmayr and Grey 2003; Syvaranta et al. 2010). Based on these results, drying seems to be the most suitable preservation method over extended periods, compared with the significant bias caused by chemical preservatives, such as salt, formalin, and ethanol preservation, and even freezing methods (Barrow et al. 2008; Kaehler and Pakhomov 2001; Syvaranta et al. 2010).

Species-specific shifts of δ^{13}C and δ^{15}N

Species-specific shifts of δ^{13}C and δ^{15}N in our study were significant. Our results, together with many studies, documented the different effects of preservatives in different species, which resulted in the unpredictable patterns for different species (Barrow et al. 2008; Kaehler and Pakhomov 2001; Kelly et al. 2006; Sarakinos et al. 2002; Vander Zanden et al. 2003). Given the observed differences among species, an appropriate and universal correction factor for differently preserved species based on these data is not recommended. Although the biochemical processes involved in the species-specific variability are not fully understood, different rates of removal and addition of organic constituents in different species during the preservation likely facilitate the changes in stable isotope ratios (Arrington and Winemiller 2002; Barrow et al. 2008; Edwards et al. 2002; Kelly et al. 2006; Sweeting et al. 2004). Nonetheless, the data presented here illustrate the potential pitfalls of long-term species-specific preservation and suggest that preservation effect on stable isotope values of organisms should be evaluated separately when using different species in food web reconstruction studies (Barrow et al. 2008; Kelly et al. 2006).

Time-dependent shifts of δ^{13}C and δ^{15}N

Our preservation experiment, which lasted nearly 2 years, provides additional evidence on the shifts in stable isotope values in fish tissue with different preservation methods. It is reported that the preservation time affected the stable isotope values, which typically exhibited dramatic changes in the early phase of the experiment and remained relatively constant with prolonged preservation time (Edwards et al. 2002; Sarakinos et al. 2002; Sweeting et al. 2004; Syvaranta et al. 2008). In the current study, the preservation time showed no significant effects on the variation of stable isotopes, indicating that the long-term preservation does not increase the variability of the shifts in isotope values of archived samples. Given that the preservation process generally occurs within the early days or weeks of preservation, the estimates of the preservation effect derived from short-term studies might be unsuitable for application to the samples that have been preserved for much longer time periods, such as years (Vander Zanden

et al. 2003). Therefore, when the long-term archived samples are supposed to be used in retrospective study of historical food webs, the correct factors for stable isotope values are suggested to estimate from preservation experiment of a relatively longer time (Sarakinos et al. 2002; Sweeting et al. 2004; Vander Zanden et al. 2003; Ventura and Jeppesen 2009).

Preservation effects on quantifying the energy pathways

Our estimates of consumer energetic pathways with SIAR provide a new perspective on the preservation effect on food web reconstruction using stable isotopes. Our study demonstrates that without preservation correction, the energetic contribution from the pelagic pathways to the fishes were significantly misestimated (about 5–20%) for the specimens that preserved in chemical preservatives, including NaCl, formalin, and ethanol. Accurate estimates of the achieved samples are critical for distinguishing whether the changes of energy flows in the consumer high up the food web are due to the ecological change or simply to preservation shifts in stable isotope values. However, the drying preservation showed no significant changes in the pelagic percentage of energy for all three species, indicating that this method could be suggested as a suitable way of sample storage for long-term stable isotope ecological studies. These results showed that depending on the preservation method, significant changes in diet estimates can already occur, which require caution when translated into ecologically meaningful shifts in trophic interaction and energetic pathways. Alternatively, the presence of the correction factor of isotopic shift would help us verify and explain the ecological implications of small variations in isotope ratios in historical reconstruction (Schmidt et al. 2009; Solomon et al. 2008; Vander Zanden et al. 2003). For achieving the correction factors, one option is suggested to review the related preservation effects on stable isotope values based on previously published experimental studies quantifying the effects of tissue preservation on isotope values (Schmidt et al. 2009; Solomon et al. 2008; Vander Zanden et al. 2003), and the other alternative option is a pilot preservation experiment considering the factors of species, preservation type, and time comparable to museum specimens (Barrow et al. 2008; Kaehler and Pakhomov 2001; Syvaranta et al. 2010).

Conclusion

In conclusion, we propose drying as a suitable preservation technique for isotopic analysis of fish muscle tissue. Our study suggests that although preserved samples may be suitable for ecological investigations based on stable isotope analysis, the evaluation of preservation effects on isotopic signatures is needed to understand the scale and direction, time dependence, and species specification of isotopic shifts in different preservation methods. Such studies can be helpful in determining the suitable correction of the isotopic signatures of preserved specimens, improving the reconstruction and interpretation of the changes in past food webs.

Acknowledgments We are grateful to the associate editor and three anonymous reviewers for detailed, thoughtful, and constructive comments that have improved this paper. This research was partially supported by the National Basic Research Program of China (Grant Nos. 2007CB109205 and 2008CB418001-1), the National Natural Science Foundation of China (Grant No. 30870428), and the Water Pollution Control and Management Project of China (Grant No. 2008ZX07101-001). Funding was also awarded to JX by the Knowledge Innovation Program of the Chinese Academy of Sciences.

References

Arrington DA, Winemiller KO (2002) Preservation effects on stable isotope analysis of fish muscle. Trans Am Fish Soc 131:337–342

Barrow LM, Bjorndal KA, Reich KJ (2008) Effects of preservation method on stable carbon and nitrogen isotope values. Physiol Biochem Zool 81:688–693

Bosley KL, Wainright SC (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. Can J Fish Aquat Sci 56:2181–2185

Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. Proc Natl Acad Sci USA 93:10844–10847

Dannheim J, Struck U, Brey T (2007) Does sample bulk freezing affect stable isotope ratios of infaunal macrozoobenthos? J Exp Mar Bio Ecol 351:37–41

Edwards MS, Turner TF, Sharp ZD (2002) Short-and long-term effects of fixation and preservation on stable isotope values (^{13}C, ^{15}N, ^{34}S) of fluid-preserved museum specimens. Copeia 4:1106–1112

Feuchtmayr H, Grey J (2003) Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. Rapid Commun Mass Spectrom 17:2605–2610

Gloutney ML, Hobson KA (1998) Field preservation techniques for the analysis of stable-carbon and nitrogen isotope ratios in eggs. J Field Ornithol 69:223–227

Hecky RE, Hesslein RH (1995) Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. J North Am Benthol Soc 14:631–653

Hesslein RH, Hallard KA, Ramal P (1993) Replacement of sulphur, carbon and nitrogen of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by δ^{13}C, δ^{15}N and δ^{34}S. Can J Fish Aquat Sci 50: 2071–2076

Hornung JP, Foote AL (2008) Comparing dietary preferences of Bufflehead ducklings in Western Canada through gut content and stable isotope analysis. Aquat Ecol 42:61–70

Kaehler S, Pakhomov EA (2001) Effects of storage and preservation on the ^{13}C and ^{15}N signatures of selected marine organisms. Mar Ecol Prog Ser 219:299–304

Kelly B, Dempson JB, Power M (2006) The effects of preservation on fish tissue stable isotope signatures. J Fish Biol 69:1595–1611

Mateo MA, Serrano O, Serrano L, Michener RH (2008) Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. Oecologia 157:105–115

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS One 5:e9672

Perga ME, Gerdeaux D (2003) Using the δ^{13}C and δ^{15}N of whitefish scales for retrospective ecological studies: changes in isotope signatures during the restoration of Lake Geneva, 1980–2001. J Fish Biol 63:1197–1207

Ponsard S, Amlou M (1999) Effects of several preservation methods on the isotopic content of *Drosophila* samples. Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie 322:35–41

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718

Pruell RJ, Taplin BK, Cicchelli K (2003) Stable isotope ratios in archived striped bass scales suggest changes in trophic structure. Fish Manag Ecol 10:329–336

Sarakinos HC, Johnson ML, Zanden MJV (2002) A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. Can J Zool 80:381–387

Schmidt SN, Vander Zanden MJ, Kitchell JF (2009) Long-term food web change in Lake Superior. Can J Fish Aquat Sci 66:2118–2129

Sierszen ME, McDonald ME, Jensen DA (2003) Benthos as the basis for arctic lake food webs. Aquat Ecol 37:437–445

Solomon CT, Carpenter SR, Rusak JA, Vander Zanden MJ (2008) Long-term variation in isotopic baselines and implications for estimating consumer trophic niches. Can J Fish Aquat Sci 65:2191–2200

Sweeting CJ, Polunin NVC, Jennings S (2004) Tissue and fixative dependent shifts of δ^{13}C and δ^{15}N in preserved ecological material. Rapid Commun Mass Spectrom 18:2587–2592

Syvaranta J, Vesala S, Rask M, Ruuhijarvi J, Jones RI (2008) Evaluating the utility of stable isotope analyses of archived freshwater sample materials. Hydrobiologia 600:121–130

Syvaranta J, Martino A, Kopp D, Céréghino R, Santoul F (2010) Freezing and chemical preservatives alter the stable isotope values of carbon and nitrogen of the Asiatic clam (*Corbicula fluminea*). Hydrobiologia 658:383–388

Vander Zanden MJ, Vadeboncoeur Y (2002) Fishes as integrators of benthic and pelagic food webs in lakes. Ecology 83:2152–2161

Vander Zanden MJ, Casselman JM, Rasmussen JB (1999) Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401:464–467

Vander Zanden MJ, Chandra S, Allen BC, Reuter JE, Goldman CR (2003) Historical food web structure and restoration of native aquatic communities in the Lake Tahoe (California–Nevada) basin. Ecosystems 6:274–288

Ventura M, Jeppesen E (2009) Effects of fixation on freshwater invertebrate carbon and nitrogen isotope composition and its arithmetic correction. Hydrobiologia 632:297–308

Wainright SC, Fogarty MJ, Greenfield RC, Fry B (1993) Long-term changes in the Georges Bank food web: trends in stable isotopic compositions of fish scales. Mar Biol 115:481–493

Xu J, Xie P, Zhang M, Yang H (2005) Variation in stable isotope signatures of seston and a zooplanktivorous fish in a eutrophic Chinese lake. Hydrobiologia 541:215–220

Xu J, Zhang M, Xie P (2007) Size-related shifts in reliance on benthic and pelagic food webs by lake anchovy. Ecoscience 14:170–177

Xu J, Zhang M, Xie P (2008) Stable isotope changes in freshwater shrimps (*Exopalaemon modestus* and *Macrobrachium nipponensis*): trophic pattern implications. Hydrobiologia 605:45–54

Xu J, Zhang M, Xie P (2010) Sympatric variability of isotopic baselines influences modeling of fish trophic patterns. Limnology. doi:10.1007/s10201-010-0327-z

Yeager LA, Layman CA (2011) Energy flow to two abundant consumers in a subtropical oyster reef food web. Aquat Ecol 45:267–277