

Long-term variation in isotopic baselines and implications for estimating consumer trophic niches

Christopher T. Solomon, Stephen R. Carpenter, James A. Rusak, and M. Jake Vander Zanden

Abstract: Carbon and nitrogen stable isotope ratios are increasingly used to study long-term food web change. Temporal variation at the base of the food web may impact the accuracy of trophic niche estimates, but data describing interannual baseline variation are limited. We quantified baseline variation over a 23-year period in a north-temperate lake and used a simulation model to examine how this variation might affect consumer trophic niche estimates. Interannual variation in C and N stable isotope ratios was significant for both benthic and pelagic primary consumer baselines. Long-term linear trends and shorter-term autoregressive patterns were apparent in the data. There were no correlations among benthic and pelagic C and N baselines. Simulations demonstrated that error in estimated fish trophic niches, but not bias, increased substantially when sampling of baselines was incomplete. Accurate trophic niche estimates depended more on accurate estimation of baseline time series than on accurate estimation of growth and turnover rates. These results highlight the importance of previous and continued efforts to constrain bias and error in long-term stable isotope food web studies.

Résumé : Les rapports d'isotopes stables de carbone et d'azote servent de plus en plus à évaluer les changements à long terme dans les réseaux alimentaires. Une variation temporelle à la base du réseau alimentaire peut avoir un impact sur la précision des estimations de niche trophique, mais il existe peu de données pour décrire la variation d'une année à l'autre de la ligne de base. Nous avons calculé la variation de la ligne de base sur une période de 23 ans dans un lac de la zone tempérée nord et nous utilisons un modèle de simulation pour examiner comment cette variation peut affecter les estimations des niches trophiques des consommateurs. La variation interannuelle des rapports d'isotopes stables de carbone et d'azote est significative pour les lignes de base des consommateurs primaires, tant benthiques que pélagiques. Il existe des tendances linéaires à long terme et des patrons d'autorégression à court terme dans les données. Il n'y a pas de corrélation entre les lignes de base de carbone et d'azote benthiques et pélagiques. Les simulations montrent que l'erreur, mais non la distorsion, dans les niches trophiques estimées des poissons augmente de façon substantielle lorsque l'échantillonnage des lignes de base est incomplet. Les estimations précises des niches trophiques dépendent plus d'une estimation précise de la série chronologique des lignes de base que de l'estimation précise des taux de croissance et de renouvellement de la biomasse. Ces résultats illustrent l'importance des efforts passés et actuels pour réduire l'erreur et la distorsion dans les études à long terme des réseaux alimentaires basées sur les isotopes stables.

[Traduit par la Rédaction]

Introduction

Understanding long-term change in the structure of food webs, and in the positions of consumers within food webs, is an important problem for ecological theory and application (Polis and Winemiller 1996; de Ruiter et al. 2005). Many important drivers of food web change act slowly, and thus, long-term studies are important tools for understanding their effects (Franklin 1989). Stable isotope analyses provide a powerful and increasingly used tool for describing food web change over long periods of time. Such studies have offered unique insights into the patterns, drivers, and consequences of variation in trophic structure, at temporal extents

up to a century or more, in both marine (Wainright et al. 1993; Pruell et al. 2003; Becker and Beissinger 2006) and freshwater ecosystems (Vander Zanden et al. 2003; Gerdeaux and Perga 2006; Maguire and Grey 2006).

Stable isotope techniques are founded on three principles: consumers reflect the stable isotope ratios of their resources, basal food web resources are often isotopically distinct, and there is a relatively fixed change in isotope ratio with each trophic transfer between resource and consumer (Peterson and Fry 1987). By measuring the stable isotope ratios of a consumer and of basal resources (which are also referred to as food web "end members" or "baselines"), researchers can therefore quantify the consumer's trophic position and

Received 21 January 2008. Accepted 25 April 2008. Published on the NRC Research Press Web site at cjfas.nrc.ca on 23 September 2008.
J20378

C.T. Solomon,¹ S.R. Carpenter, J.A. Rusak, and M.J. Vander Zanden. Center for Limnology, University of Wisconsin, 680 N. Park Street, Madison, WI 53706, USA.

¹Corresponding author (e-mail: ctsolomon@wisc.edu).

relative reliance on the different resources. In lakes, for instance, benthic and pelagic primary producers represent importantly different basal resources that also have distinct stable isotope ratios (France 1995; Hecky and Hesslein 1995). Measurements of the C and N stable isotope ratios of fishes reveal that many receive support from both benthic and pelagic food chains (Vander Zanden and Vadeboncoeur 2002).

Baseline stable isotope ratios vary over time. In lakes, planktonic and benthic primary consumers (zooplankton and zoobenthos) are commonly used as baselines (Vander Zanden and Rasmussen 1999; Post 2002). Many studies have demonstrated that physical, biogeochemical, and biological processes induce considerable within-year variability in zooplankton stable isotope ratios of C (Zohary et al. 1994; Gu et al. 1999; Leggett et al. 1999), N (Gu et al. 1994; Leggett et al. 2000), or both C and N (Yoshioka et al. 1994; Lehmann et al. 2004; Perga and Gerdeaux 2006). There is also evidence for within-year variation in zoobenthos stable isotope ratios (Grey et al. 2004; Hershey et al. 2006). Yet because most of these studies have focused on intraannual processes and have therefore spanned 2- to 3-year periods at most, data describing interannual variation in baseline stable isotope ratios are very limited. Available data indicate that C and N stable isotope ratios of zooplankton may vary interannually by several per mille (‰) or more (Zohary et al. 1994; Maguire and Grey 2006).

Researchers have recognized the need to consider how temporal baseline variation can affect interpretation of consumer stable isotope ratios. Accurate estimates of baselines are critical for distinguishing whether changes in consumer stable isotope ratios are due to ecological change or simply to variation in baseline isotope ratios (Cabana and Rasmussen 1996). For instance, a decrease in consumer $\delta^{15}\text{N}$ over time could indicate a shift by that consumer to feeding at a lower trophic position; or it could indicate a change in the inorganic N source utilized by primary producers, without any shift in the trophic position of the consumer. Unfortunately, assembling complete time series of baseline stable isotope data is often extremely challenging or impossible for long-term studies because of limited representation of aquatic primary consumers in many museum collections. Consequently, researchers have used a number of approaches to estimate baseline time series and to consider possible biases and errors. These include collecting multiple years of contemporary baseline isotope data (Pruell et al. 2003), using available historical museum samples of primary consumers either archived directly or dissected from the guts of preserved fish (Vander Zanden et al. 2003), estimating baselines from long-lived primary consumers that integrate temporal changes (Cabana and Rasmussen 1996), using supplementary data on environmental variables that suggest the presence or absence of change in baseline isotope ratios (Wainright et al. 1993; Gerdeaux and Perga 2006), and examining stable isotope ratios of organic matter in sediment cores (Chandra et al. 2005).

Such approaches have been instrumental in developing the unique insights that long-term food web studies can offer. Yet the magnitude and potential importance of the biases and errors that they seek to constrain remain unclear. To understand fully the implications of incomplete baseline

data for long-term studies of consumer trophic niches, new data and analyses are needed. In this study, we present a 23-year time series of benthic and pelagic primary consumer baselines for a north-temperate lake. We fit statistical process models to these time series to derive general descriptions of the patterns of interannual variation. The empirical data provide the basis for a simulation model designed to assess how incomplete baseline data and inaccurate growth and tissue turnover data affect bias and precision of consumer trophic niche estimates in long-term food web studies.

Materials and methods

Collection and statistical modeling of empirical data

We measured C and N stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; henceforth, collectively δX) in archived samples of benthic and pelagic primary consumers collected from Sparkling Lake, Wisconsin, between 1981 and 2003 by the North Temperate Lakes LTER project (see Magnuson et al. 2006). Primary consumers provide good isotopic baselines because they integrate over some of the variability in primary producer stable isotope ratios (Vander Zanden and Rasmussen 2001; Post 2002).

Benthic macroinvertebrates collected with Dendy colonization samplers during August of each year were preserved in 70% ethanol (lter.limnology.wisc.edu/dendy05.shtml). Individual gammarid amphipods and heptageniid mayflies of the genus *Stenonema* were removed from these samples, rinsed with distilled water, dried at 60 °C for 1 day, and analyzed whole on an isotope ratio mass spectrometer at the University of California Davis Stable Isotope Facility (Davis, California). We selected these two taxa for analysis so that our benthic baseline would reflect changes in the isotope ratios of both detrital and algal resources in benthic habitats. Individuals were analyzed singly when possible or in small groups when necessary to achieve sufficient mass. We ran one to three samples of each of these taxa of zoobenthos in each year (except 1989, when no samples were available).

Depth-integrated zooplankton samples were collected with a Wisconsin net throughout the year, predominantly during the ice-free season (80 μm mesh, n usually between 4 and 8 depending on the year), and preserved in 3%–5% formalin (prior to 2001) or 80% ethanol (2001–2003) (lter.limnology.wisc.edu/bio_field_protocol05.shtml). We representatively subsampled these samples for isotopic analysis after filtration through a 250 μm mesh to ensure that only larger zooplankton (primarily *Daphnia* and large copepod species) were analyzed isotopically.

To account for the effects of sample preservation, we adjusted observed zooplankton $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by +1.1‰ and +0.8‰ when samples were preserved in formalin and by +0‰ and +0.8‰ when samples were preserved in ethanol (Feuchtmayr and Grey 2003). We did not adjust observed $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of zoobenthos (Sarakinis et al. 2002).

We described patterns of variation in time series of baseline δX by comparing fits of the data to four plausible process models consistent with current thinking about isotope behavior in food webs (Table 1). The simplest model describes each year's δX as a random draw from a normal distribution; this represents the pattern of baseline variation

Table 1. Comparison of fits for each of four model types fit to each of four time series of baseline stable isotope data.

	Zoobenthos $\delta^{13}\text{C}$	Zoobenthos $\delta^{15}\text{N}$	Zooplankton $\delta^{13}\text{C}$	Zooplankton $\delta^{15}\text{N}$
Model 1: $Y_t = B_0 + \varepsilon_t$	86.4	31.6	94.3	73.5
Model 2: $Y_t = B_0 + B_1 \times t + \varepsilon_t$	79.6	31.2	96.1	59.3
Model 3: $Y_t = \phi \times Y_{t-1} + \varepsilon_t$	90.9	34.6	104.7	63.4
Model 4: $Y_t = B_0 + B_1 \times t + \phi \times Y_{t-1} + \varepsilon_t$	81.5	31.0	97.3	58.8

Note: Table values give the Akaike information criterion for each fit; lower values indicate better fits. Y_t is the mean stable isotope ratio of C or N in zoobenthos or zooplankton in year t , ε_t is the error at time t , with $\varepsilon \sim N(0, \sigma^2)$, B_0 and B_1 are parameters for the intercept and slope of a linear regression, and ϕ is an autoregressive memory parameter describing the strength of the connection between the stable isotope ratio in year t and year $t - 1$.

that is implicitly assumed by many retrospective stable isotope studies. Two models of intermediate complexity describe δX as changing either linearly or autoregressively over time. Finally, the fourth model includes both a linear trend and an autoregressive memory term. We fit each model to each baseline time series by maximum likelihood, compared models using the Akaike information criterion, and generated confidence intervals for parameter estimates using nonparametric bootstrapping. For most of our analyses, we considered only zooplankton samples collected during the ice-free season each year (usually $n = 4-6$), as this probably represents the most relevant pelagic baseline estimate for fish growth (Perga and Gerdeaux 2005). However, we also considered whether model fits were qualitatively different if we included all zooplankton samples or only summertime (May through August) samples.

Simulation model

When isotopic baseline variation occurs at a time scale similar to that of the ecological process of interest, researchers must consider both the time series of baseline δX and the consumer's rates of tissue growth and turnover to interpret consumer stable isotope ratios accurately (Harvey et al. 2002; Carpenter et al. 2005; Matthews and Mazumder 2005). This is because the δX of a given fish in a given year depends both on the baseline δX during each year of its life and on the proportion of its current biomass that comes from each year's growth. These "mass proportions", in turn, are determined by tissue growth and turnover rates. In long-term studies, researchers typically estimate (explicitly or implicitly) both of these inputs: they interpolate a baseline time series from available data and approximate consumer growth and turnover rates with reasonable guesses. In addition, they estimate the isotopic fractionation associated with trophic transfers from prey to predator. We developed a simulation model to explore how the accuracy of these inputs affects the accuracy and precision of a researcher's inferences about the consumer's trophic niche. The model is described briefly here; additional details and a flow chart are given in Appendix A.

The first phase of the model tracks a population of fish through time, calculating the δX of the average individual of each age in each year. The second phase mimics the implementation of a research project interested in long-term change in consumer trophic niches (specifically τ , the trophic position, and p , the percent reliance on benthic resources). A "researcher" measures the δX of fish from each year and calculates their p and τ using estimates of their mass proportions, the trophic fractionation of isotope ratios, and the baseline δX time series. As in a real study, the researcher's estimates of these three factors may be imperfect.

We quantified the biases (systematic departures from the true value) and errors (magnitude of scatter around the true value) in \hat{p} and $\hat{\tau}$ across multiple scenarios in which we varied the quality of the researcher's information about these three factors. For the estimated baseline time series, we varied the proportion of years in which the researcher measured baseline δX between one and all of the 23 years of the time series. We also varied whether the sampled years were spaced randomly or evenly and whether the researcher calculated values for unsampled years by linear interpolation or as the mean of sampled years. For the estimated mass proportions, we considered four levels of accuracy: (i) the true mass proportions using the nominal growth and turnover rates, (ii) the worst possible estimate of mass proportions when growth and turnover rate estimates were constrained within $\pm 20\%$ of the nominal rates (determined by maximizing sums of squares), (iii) equal mass proportions (that is, assuming that each year of the consumer's life makes an equal contribution to its mass), and (iv) the worst possible mass proportions when growth and turnover rate estimates were unconstrained (i.e., estimated within 50%–200% and 10%–1000% of the nominal rates, respectively). For the estimated trophic fractionation of C and N isotope ratios, we ran simulations in which the true baseline time series and true trophic fractionation remained fixed, but in each model iteration, the researcher randomly drew estimates of the C and N trophic fractionation from a normal distribution. These distributions were centered on the true trophic fractionations, with standard deviations (1.04‰ for C and 0.41‰ for N) described by field estimates of the variance in trophic fractionation (Vander Zanden and Rasmussen 2001).

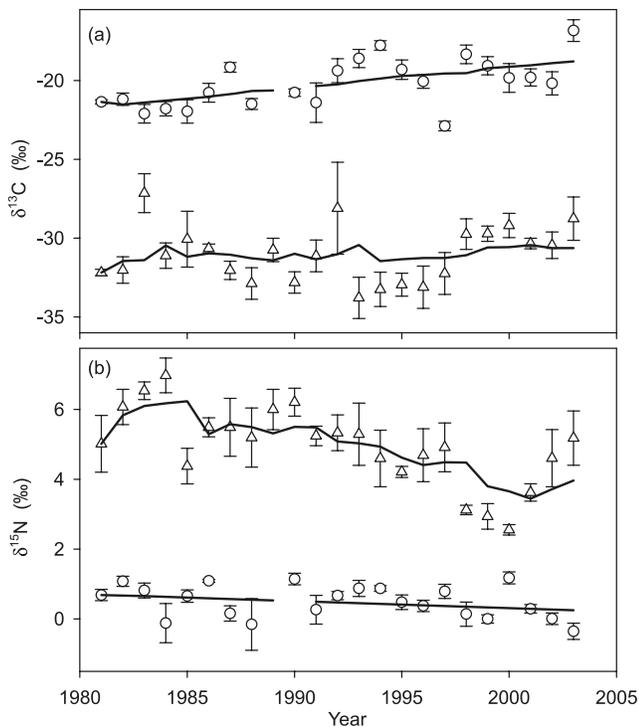
We focused primarily on the effects of baseline and mass proportions estimates because the effects of uncertainty in trophic fractionation have been addressed elsewhere (Vander Zanden and Rasmussen 2001; Post 2002; Matthews and Mazumder 2005). For each scenario, we ran 100 realizations, generating new baseline δX time series each time. In each realization, we calculated the absolute mean bias ("bias") and the standard deviation ("error") of \hat{p} and $\hat{\tau}$ across years for fish of each age class. We then calculated the mean bias and error across the 100 realizations so that we present estimates of the bias and error in \hat{p} and $\hat{\tau}$ across the 23-year time series that do not depend on a particular realization of the baseline-generating process but incorporate the overall effect of that process.

Results

Empirical data

All four of the Sparkling Lake baseline time series

Fig. 1. Mean annual stable isotope ratios of (a) C and (b) N of food web baselines (circles, zoobenthos; triangles, zooplankton) in Sparkling Lake, Wisconsin. Error bars are ± 1 SE. Lines show predictions from the fitted model (model 4), which incorporates a linear trend and temporal autocorrelation.



(Fig. 1) exhibited significant interannual differences in stable isotope ratios (ANOVA: $df = 22, 143$ for zooplankton $\delta^{13}C$ and $\delta^{15}N$, $df = 21, 71$ for zoobenthos $\delta^{13}C$, and $df = 21, 70$ for zoobenthos $\delta^{15}N$, $p < 0.0003$ in all cases). This was true even though seasonal variation resulted in large intraannual variances in zooplankton δX . Pooled intraannual variances were 5.95‰ and 1.15‰ for zooplankton $\delta^{13}C$ and $\delta^{15}N$, respectively, and 1.15‰ and 0.22‰ for zoobenthos $\delta^{13}C$ and $\delta^{15}N$, respectively. Changes in baseline stable isotope ratios were uncorrelated within habitats (benthic versus pelagic) and within elements (C versus N) (Table 2). Both $\delta^{15}N$ time series trended downward over time; in contrast, there was little directional change in zooplankton $\delta^{13}C$ and an upward trend in zoobenthos $\delta^{13}C$ (Fig. 1).

Zooplankton and zoobenthos baselines were clearly different in both C and N stable isotope ratios. On average, mean annual zooplankton $\delta^{13}C$ was 9.94‰ lower than mean annual zoobenthos $\delta^{13}C$ (paired t test: $p < 0.0001$), and mean annual zooplankton $\delta^{15}N$ was 5.20‰ higher than mean annual zoobenthos $\delta^{15}N$ (paired t test: $p < 0.0001$).

Only one time series (zooplankton $\delta^{13}C$) was best described by model 1, the completely random model. Fits to all three of the remaining time series improved substantially with the inclusion of a linear trend in the model, and fits for the two $\delta^{15}N$ time series were improved further by inclusion of an autoregressive memory term (Table 1). There was little qualitative difference in these patterns when we fit models to year-round or summer-only zooplankton data instead of to data from the ice-free season or when we fit

Table 2. Correlations (Pearson's r) among observed time series of baseline stable isotope ratios and p values for test of null hypothesis that correlation is zero.

Comparison	r	p
Within elements		
Zoobenthos $\delta^{13}C$ and zooplankton $\delta^{13}C$	0.03	0.9
Zoobenthos $\delta^{15}N$ and zooplankton $\delta^{15}N$	0.08	0.7
Within habitats		
Zoobenthos $\delta^{13}C$ and $\delta^{15}N$	-0.25	0.3
Zooplankton $\delta^{13}C$ and $\delta^{15}N$	-0.12	0.6

models to data without correcting for effects of preservation in ethanol and formalin (results not shown).

For the purposes of the simulation modeling, we chose to describe all four time series using the most complex model (model 4). All of the simpler models are nested within model 4; thus, by setting some parameters equal to or near zero, model 4 can yield predictions similar to those made by model 2 for zoobenthos $\delta^{13}C$ and by model 1 for zooplankton $\delta^{13}C$. Maximum likelihood parameter estimates, with bootstrapped 95% confidence intervals, are presented for all four time series fit to model 4 (Table 3).

Simulation model

Bias and error in trophic niche estimates declined as the proportion of sampled baseline years increased (Fig. 2). This result held regardless of whether the sampled years were spaced randomly or evenly across the time series and regardless of whether values for unsampled years were inferred by linear interpolation or as the mean of sampled years. For simplicity, we present data only from simulations in which sampled years were evenly spaced and unsampled years were inferred by averaging. Fish age had little effect on bias in \hat{p} and $\hat{\tau}$, but errors were smaller for older fish because the importance of incorrect baseline estimates in any given year was muted by the larger number of years contributing to the biomass of older fish. Bias was relatively low as long as >50% of the baseline years were sampled (<0.02 for \hat{p} and <0.07 for $\hat{\tau}$ for age-5 fish) but increased rapidly as the proportion of sampled years declined below 30%. In contrast, errors in \hat{p} and $\hat{\tau}$ increased rapidly in response to reductions in baseline sampling to maxima of 0.07 and 0.2, respectively (Fig. 2).

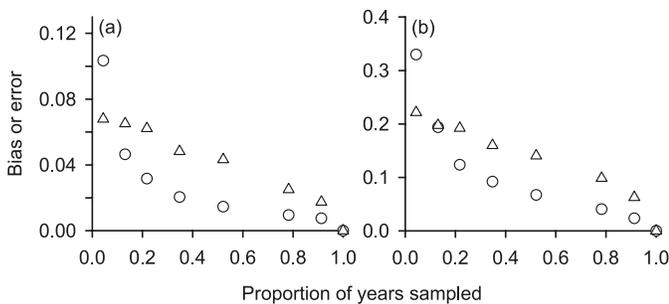
The manifestation of such errors in a particular study could lead to an erroneous inference of variability or long-term change in trophic niche. For example, output is shown from a typical realization of the model scenario in which mass origins were known perfectly and 22% of the years in the baseline time series were sampled (Fig. 3). Although the mean of \hat{p} and $\hat{\tau}$ across the 23-year time series was close to true p and τ , the value of \hat{p} and $\hat{\tau}$ in any given year often differed from the true value. For age-1 fish, estimated trophic position was off by more than half a trophic level in seven separate years and by a full trophic level in two years (Fig. 3b); \hat{p} was often off by >0.10 (Fig. 3a). For age-5 fish, error in \hat{p} and $\hat{\tau}$ in a given year was much less than for age-1 fish as a result of isotopic integration across years. Interestingly, \hat{p} showed a significant increase over time for age-5 fish in this realization even though true p remained

Table 3. Maximum likelihood estimates and bootstrapped 95% confidence intervals (in parentheses) for parameters of the statistical model (model 4) used to describe interannual variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of zooplankton and zoobenthos.

	B_0	B_1	ϕ	σ^2
Zoobenthos $\delta^{13}\text{C}$	-20.89 (-22.98, -13.99)	0.13 (0.04, 0.21)	0.04 (0.00, 0.34)	1.43 (0.47, 2.03)
Zoobenthos $\delta^{15}\text{N}$	0.71 (0.26, 1.12)	-0.02 (-0.05, 0.01)	0.00 (0.00, 0.33)	0.16 (0.07, 0.21)
Zooplankton $\delta^{13}\text{C}$	-25.55 (-32.90, -21.64)	0.02 (-0.10, 0.14)	0.18 (0.00, 0.30)	2.84 (1.05, 4.18)
Zooplankton $\delta^{15}\text{N}$	4.33 (4.45, 7.18)	-0.08 (-0.18, -0.07)	0.33 (0.00, 0.32)	0.53 (0.21, 0.88)

Note: Parameters are intercept (B_0) and slope (B_1) for a linear trend, a memory parameter (ϕ) describing temporal autocorrelation, and the variance (σ^2) of the normally distributed errors.

Fig. 2. Bias (mean absolute bias, circles) and error (SD, triangles) in estimates of trophic niche resulting from incomplete sampling of baseline time series. (a) Bias and error in \hat{p} , the estimated proportional reliance of the consumer on benthic resources; (b) bias and error in $\hat{\tau}$, the estimated trophic position of the consumer. Each point represents the mean across 100 model realizations of the summary statistic (bias or SD) calculated across a 23-year time series for age-5 fish.

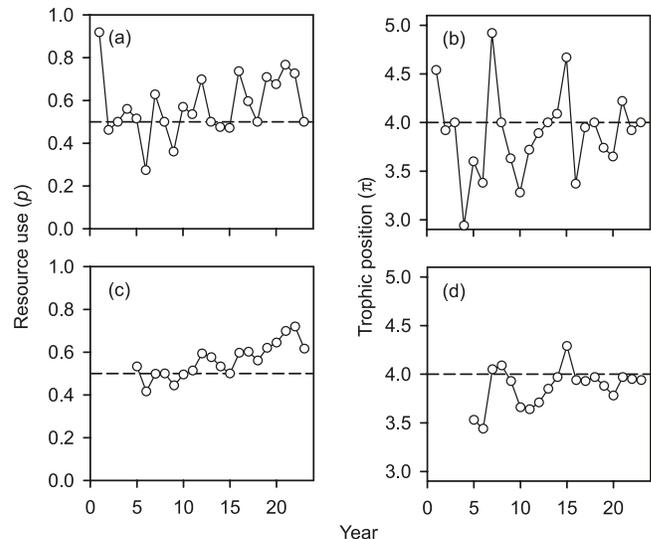


constant, as a result of the vagaries of the estimated baseline time series (Fig. 3c) ($p = 0.002$).

Inaccurate estimates of fish growth and turnover rates generally inflated bias and error in trophic niche estimates; however, this inflation was small as long as growth and turnover rates were reasonably bounded (Fig. 4). Even the worst possible growth and turnover estimates within $\pm 20\%$ of the true rates produced little difference in the estimated mass proportions of the fish (Fig. 4a) and therefore had little effect on bias or error in \hat{p} and $\hat{\tau}$ (Figs. 4b and 4c). On the other hand, when growth and turnover rate estimates were grossly inaccurate (amounting to a misspecification of the form of the growth model, not just of the parameters), inflation in bias, and particularly error, was more substantial (Fig. 4, scenarios *iii* and *iv*). Inflation of error and bias were largest when sampling of the baseline time series was complete and decreased as the quality of the researcher's estimate of the baseline time series decreased (that is, as the proportion of sampled baseline years decreased) (Fig. 4).

Uncertainty about the true trophic fractionation increased the mean bias, but not the error, in trophic niche estimates. Compared with scenarios in which the true trophic fractionation was known, the mean biases in \hat{p} and $\hat{\tau}$ were, respectively, 0.09–0.18 and 0.04–0.27 greater when trophic fractionation was estimated. This bias inflation was not sensitive to the completeness with which the baseline time series was sampled.

Fig. 3. Time series of estimated proportional reliance on benthic resource (\hat{p}) and estimated trophic position ($\hat{\tau}$) for (a and b) age-1 fish and (c and d) age-5 fish in one realization of the simulation model. True p and τ , indicated by the broken line, were held constant at 0.5 and 4.0, respectively. Data are from a model scenario in which estimated growth and turnover rates equal the nominal rates but baseline time series are sampled every fifth year (22% of years).



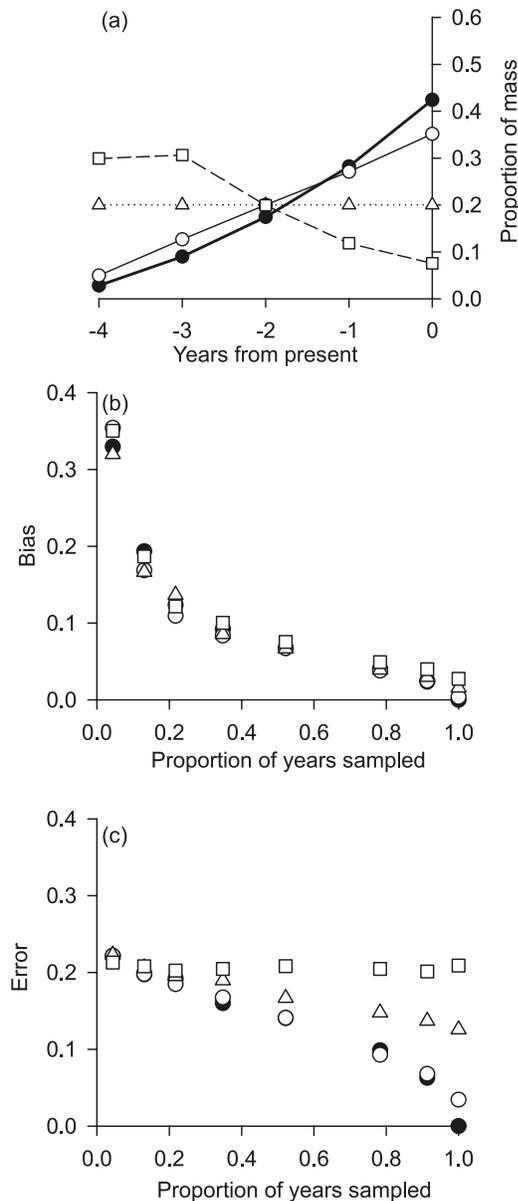
Discussion

Empirical data

Stable isotope ratios of food web baselines vary at multiple time scales. To characterize annual values, we analyzed multiple samples through time for short-lived pelagic primary consumers and replicate samples of longer-lived benthic primary consumers. The point estimates that we report for annual values therefore integrate over much of the isotopic variability at time scales shorter than a year. Despite the uncertainty in these point estimates (intraannual errors), we observed significant interannual variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of both benthic and pelagic baselines in Sparkling Lake.

Patterns of baseline variation not only provide clues to the mechanisms that drive such variation, but also determine the sampling frequency necessary to adequately characterize baseline time series. For three of the four baseline time series in Sparkling Lake, interannual variation was better described by statistical models that included long-term linear trends and (or) temporal autocorrelation than by purely random models. This indicates that the observed variation may have resulted from slow, directional shifts in key driver

Fig. 4. Effect of growth and turnover rate estimates on bias and error in consumer trophic niche estimates. (a) Proportion of the current mass of an age-5 fish that is derived from each year of its life in four scenarios with different growth and turnover rates. Scenarios are (i) nominal growth and turnover rates (solid circles), (ii) the worst possible estimate of mass proportions when growth and turnover rate estimates are constrained within $\pm 20\%$ of the nominal rates (open circles), (iii) growth and turnover are not explicitly estimated, but mass proportions are assumed to be uniform across the consumer's life (triangles), and (iv) the worst possible mass origins when growth and turnover rate estimates were unconstrained (within 50%–200% and 10%–1000% of the nominal rates, respectively) (squares). (b) and (c) Bias and error in $\hat{\tau}$, the estimated trophic position of the consumer, plotted against the proportion of baseline years sampled for each of the growth scenarios shown in Fig. 4a.



processes rather than from stochastic environmental noise around a stationary mean. Although our results provide insight on the statistical form of the processes that drive long-

term baseline change in Sparkling Lake, the identity of these drivers remains unclear. Two potential mechanisms can be excluded. First, the “Seuss effect”, a long-term decline of about $0.016\text{‰}\cdot\text{year}^{-1}$ in the $\delta^{13}\text{C}$ of atmospheric CO_2 owing to fossil fuel combustion, could be dampening, but not driving, the slight increase in $\delta^{13}\text{C}$ that we observed for both zooplankton and zoobenthos (Kroopnick 1985; Quay et al. 2003). Second, although the structure of the Sparkling Lake zooplankton community changed considerably in the early 1980s (to dominance by cyclopoids as a result of an invasive planktivorous fish; Beisner et al. 2003) and although shifts in zooplankton community structure can alter the stable isotope ratios of zooplankton baselines (Matthews and Mazumder 2003), this change does not seem to be detectable in the zooplankton isotopic time series. A variety of other physical, biogeochemical, and biological processes could be driving baseline variation. For zooplankton, a number of these processes have been identified in studies spanning one to a few years (see citations in the Introduction). In Sparkling Lake, the lack of correlation among the time series suggests that either the identity or the effects of these key processes vary among the four habitat-by-element combinations that we considered. Complete analyses of potential drivers, using multiple long-term data sets such as this one, will be necessary to fully understand the causes of interannual variation in baseline stable isotope ratios. Such information could be extremely useful for predicting changes in baselines even when historical samples of baseline organisms are unavailable.

Because long-term baseline data sets are scarce, it is difficult to assess how well the patterns that we observed might represent baseline variation in other systems. As a first cut at comparing interannual baseline variation among systems, we fit the four statistical process models that we considered in this paper to a continuous 19-year time series of zooplankton stable isotope data from Lough Erne (Maguire and Grey 2006, data digitized from their fig. 1). These data come from a single June sample in each year and include a much broader size range of zooplankton than our samples; thus, they may not be directly comparable with our zooplankton data, although the range of variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was similar in the two data sets. As in Sparkling Lake, the random model was the best fit to the Lough Erne zooplankton $\delta^{13}\text{C}$ time series, with both the linear and linear-plus-autoregressive models as plausible alternatives. The linear model was clearly the best fit for the Lough Erne zooplankton $\delta^{15}\text{N}$ time series; in Sparkling Lake, this model explained variation in zooplankton $\delta^{15}\text{N}$ almost as well as the selected linear-plus-autoregressive model. Although this analysis has significant limitations, it does suggest that similar statistical processes may describe interannual baseline variation in different lakes. Identifying key processes that drive variation will enable better comparisons among lakes. For instance, if winter under-ice respiration affects algal $\delta^{13}\text{C}$ by setting the $\delta^{13}\text{C}$ of the dissolved inorganic C pool in the springtime, then lakes with similar dissolved inorganic C pool sizes, morphometry, and ice duration may show some coherence in their interannual baseline $\delta^{13}\text{C}$.

Simulation model

Although inaccurate estimates of consumer growth and

turnover rates did result in inaccurate trophic niche estimates, this effect was strong only when rate estimates were highly imprecise; when rate estimates were reasonably bounded, errors in mass proportions were small and had little effect on trophic niche estimates. Reasonably bounded estimates of growth and turnover rates are usually possible, even in the absence of data specific to the study system, because these processes have been studied extensively. Well-tested models of fish growth and even species-specific parameter values for these models are widely available (Quinn and Deriso 1999; Froese and Pauly 2006). Data describing tissue turnover rates are also substantial and growing rapidly (e.g., Hesslein et al. 1993; MacAvoy et al. 2001; McIntyre and Flecker 2006). Thus, inaccurate estimates of consumer growth and turnover rates will likely be negligible sources of bias and error in most long-term studies.

Bias and error resulting from incomplete baseline data may be more important concerns for long-term stable isotope studies than those resulting from inaccurate growth and turnover estimates. In many cases, researchers are interested in understanding changes in consumer trophic niches over long periods during which baseline samples are scarce. For instance, in a previous study in which we sought to understand food web change and restoration potential in Lake Tahoe, we had baseline data available for only two of five time periods spanning >100 years (Vander Zanden et al. 2003). Results from the present study suggest that incomplete baseline data may affect consumer trophic niche estimates, particularly by introducing error. On the positive side, biases in trophic niche estimates resulting from incomplete baseline data will be minimal in many cases. Nonetheless, our results underscore the importance of the measures that researchers have taken to assess and limit biases and errors in consumer trophic niche estimates from long-term stable isotope studies.

Quantitative estimates of the bias and error attributable to incomplete baseline data depend on the extent and pattern of variability in the baseline time series. The simulation results reported here are based on baseline time series similar to those that we observed in Sparkling Lake. To make our results as general as possible, we did not rely strictly on the observed data but instead generated multiple realizations of baseline time series based on the statistical processes that best described the data. Nonetheless, even these generalized statistical process models are parameterized based only on one lake. Our quantitative estimates of bias and error should therefore be interpreted with caution. Qualitatively, however, the simulation results reported here may be quite general. For instance, we observed that biases increased slowly in response to decreases in available baseline data until sampling became quite sparse and that errors increased somewhat more quickly. These patterns occur because small gaps in baseline data do not significantly decrease the overall accuracy of the estimated baseline time series but can introduce localized errors in trophic niche estimates for consumers in the years when baseline data are not available. Similar patterns would be obtained even in systems with patterns of baseline variation different from those observed in Sparkling Lake.

Even when baseline sampling is very sparse, bias from inaccurate baseline estimates is less than what may result

strictly from inaccurate trophic fractionation estimates. For example, in our simulations, realistic levels of uncertainty in trophic fractionation resulted in increases of 0.09–0.18 in mean bias in \hat{p} relative to scenarios in which trophic fractionation was known. In comparison, the mean bias in \hat{p} owing to inaccurate estimates of the baseline time series was <0.11 even when baseline data were available from only 4% of the years of the time series. Other recent studies have observed similar biases in \hat{p} and $\hat{\tau}$ as a result of uncertainty in trophic fractionation (Vander Zanden and Rasmussen 2001; Post 2002).

Researchers who use long-term stable isotope techniques have recognized the need to interpret consumer trophic niche estimates with caution when baseline data are incomplete (Wainright et al. 1993; Vander Zanden et al. 2003; Matthews and Mazumder 2005). However, the limited availability of long, complete baseline time series has precluded quantitative assessment of potential biases and errors. Our empirical data and simulation analysis indicate that long-term stable isotope studies will need to continue to consider issues including baseline data availability, growth and turnover models, and trophic fractionation uncertainty. In addition, there is a clear need for more data describing long-term variation in isotopic baselines and for improved capabilities for understanding and predicting such variation. Such advances will continue to expand the ability of long-term stable isotope studies to reveal insights into food web pattern and process in aquatic systems.

Acknowledgements

W. Fetzer, K. Lord, and J. McCarthy assisted with sample processing. E. Stanley, B. Weidel, and the anonymous reviewers provided valuable comments on the manuscript, and one reviewer suggested the ideas that we presented about dissolved inorganic C pools and interannual variation in isotopes. This research was funded by the Wisconsin Alumni Research Foundation and by graduate fellowships from the University of Wisconsin and the National Science Foundation. We gratefully acknowledge the staff and facilities of the National Science Foundation funded North Temperate Lakes LTER site for the long-term data collection that made this study possible.

References

- Becker, B.H., and Beissinger, S.R. 2006. Centennial decline in the trophic level of an endangered seabird after fisheries decline. *Conserv. Biol.* **20**: 470–479. doi:10.1111/j.1523-1739.2006.00379.x. PMID:16903108.
- Beisner, B.E., Ives, A.R., and Carpenter, S.R. 2003. The effects of an exotic fish invasion on the prey communities of two lakes. *J. Anim. Ecol.* **72**: 331–342. doi:10.1046/j.1365-2656.2003.00699.x.
- Cabana, G., and Rasmussen, J.B. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 10844–10847. doi:10.1073/pnas.93.20.10844. PMID:8855268.
- Carpenter, S.R., Cole, J.J., Pace, M.L., Van de Bogert, M., Bade, D.L., Bastviken, D., Gille, C., Hodgson, J.R., Kitchell, J.F., and Kritzberg, E.S. 2005. Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. *Ecology*, **86**: 2737–2750. doi:10.1890/04-1282.
- Chandra, S., Vander Zanden, M.J., Heyvaert, A.C., Richards, B.C., Allen, B.C., and Goldman, C.R. 2005. The effects of cultural eu-

- trophication on the coupling between pelagic primary producers and benthic consumers. *Limnol. Oceanogr.* **50**: 1368–1376.
- de Ruiter, P.C., Wolters, V., and Moore, J.C. (Editors). 2005. Dynamic food webs. Academic Press, Amsterdam, the Netherlands.
- Feuchtmayr, H., and 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. doi:10.1002/rcm.1227. PMID:14648896.
- France, R.L. 1995. ^{13}C enrichment in benthic compared to planktonic algae: food web implications. *Mar. Ecol. Prog. Ser.* **124**: 307–312. doi:10.3354/meps124307.
- Franklin, J.F. 1989. Importance and justification of long-term studies in ecology. In *Long-term studies in ecology*. Edited by G.E. Likens. Springer-Verlag, New York. pp. 3–19.
- Froese, R., and Pauly, D. (Editors). 2006. FishBase. World Wide Web electronic publication. Available from www.fishbase.org [updated June 2006].
- Gerdeaux, D., and Perga, M.E. 2006. Changes in whitefish scales $\delta^{13}\text{C}$ during eutrophication and reoligotrophication of subalpine lakes. *Limnol. Oceanogr.* **51**: 772–780.
- Grey, J., Kelly, A., and Jones, R.I. 2004. High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. *Limnol. Oceanogr.* **49**: 239–244.
- Gu, B.H., Schell, D.M., and Alexander, V. 1994. Stable carbon and nitrogen isotopic analysis of the plankton food web in a subarctic lake. *Can. J. Fish. Aquat. Sci.* **51**: 1338–1344. doi:10.1139/f94-133.
- Gu, B., Alexander, V., and Schell, D.M. 1999. Seasonal and interannual variability of plankton carbon isotope ratios in a subarctic lake. *Freshw. Biol.* **42**: 417–426. doi:10.1046/j.1365-2427.1999.00472.x.
- Harvey, C.J., Hanson, P.C., Essington, T.E., Brown, P.B., and Kitchell, J.F. 2002. Using bioenergetics models to predict stable isotope ratios in fishes. *Can. J. Fish. Aquat. Sci.* **59**: 115–124. doi:10.1139/f01-203.
- Hecky, R.E., and Hesslein, R.H. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *J. North Am. Benthol. Soc.* **14**: 631–653. doi:10.2307/1467546.
- Hershey, A.E., Beaty, S., Fortino, K., Kelly, S., Keyse, M., Luecke, C., O'Brien, W.J., and Whalen, S.C. 2006. Stable isotope signatures of benthic invertebrates in arctic lakes indicate limited coupling to pelagic production. *Limnol. Oceanogr.* **51**: 177–188.
- Hesslein, R.H., Hallard, K.A., and Ramlal, P. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}\text{S}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$. *Can. J. Fish. Aquat. Sci.* **50**: 2071–2076. doi:10.1139/f93-230.
- Kroopnick, P.M. 1985. The distribution of ^{13}C of ΣCO_2 in the world oceans. *Deep-Sea Res. I. Oceanogr. Res. Pap.* **32**: 57–84. doi:10.1016/0198-0149(85)90017-2.
- Leggett, M.F., Servos, M.R., Hesslein, R., Johannsson, O., Millard, E.S., and Dixon, D.G. 1999. Biogeochemical influences on the carbon isotope signatures of Lake Ontario biota. *Can. J. Fish. Aquat. Sci.* **56**: 2211–2218. doi:10.1139/cjfas-56-11-2211.
- Leggett, M.F., Johannsson, O., Hesslein, R., Dixon, D.G., Taylor, W.D., and Servos, M.R. 2000. Influence of inorganic nitrogen cycling on the $\delta^{15}\text{N}$ of Lake Ontario biota. *Can. J. Fish. Aquat. Sci.* **57**: 1489–1496. doi:10.1139/cjfas-57-7-1489.
- Lehmann, M.F., Bernasconi, S.M., McKenzie, J.A., Barbieri, A., Simona, M., and Veronesi, M. 2004. Seasonal variation of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of particulate and dissolved carbon and nitrogen in Lake Lugano: constraints on biogeochemical cycling in a eutrophic lake. *Limnol. Oceanogr.* **49**: 415–429.
- MacAvoy, S.E., Macko, S.A., and Garman, G.C. 2001. Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey. *Can. J. Fish. Aquat. Sci.* **58**: 923–932. doi:10.1139/cjfas-58-5-923.
- Magnuson, J.J., Kratz, T.K., and Benson, B.J. (Editors). 2006. Long-term dynamics of lakes in the landscape: long-term ecological research on north temperate lakes. Oxford University Press, Oxford, UK.
- Maguire, C.M., and Grey, J. 2006. Determination of zooplankton dietary shift following a zebra mussel invasion, as indicated by stable isotope analysis. *Freshw. Biol.* **51**: 1310–1319. doi:10.1111/j.1365-2427.2006.01568.x.
- Matthews, B., and Mazumder, A. 2003. Compositional and inter-lake variability of zooplankton affect baseline stable isotope signatures. *Limnol. Oceanogr.* **48**: 1977–1987.
- Matthews, B., and Mazumder, A. 2005. Consequences of large temporal variability of zooplankton $\delta^{15}\text{N}$ for modeling fish trophic position and variation. *Limnol. Oceanogr.* **50**: 1404–1414.
- McIntyre, P.B., and Flecker, A.S. 2006. Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. *Oecologia (Berl.)*, **148**: 12–21. doi:10.1007/s00442-005-0354-3.
- Perga, M.E., and Gerdeaux, D. 2005. 'Are fish what they eat' all year round? *Oecologia (Berl.)*, **144**: 598–606. doi:10.1007/s00442-005-0069-5.
- Perga, M.E., and Gerdeaux, D. 2006. Seasonal variability in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the zooplankton taxa in two alpine lakes. *Acta Oecol.* **30**: 69–77. doi:10.1016/j.actao.2006.01.007.
- Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* **18**: 293–320. doi:10.1146/annurev.es.18.110187.001453.
- Polis, G.A., and Winemiller, K.O. (Editors). 1996. Food webs: integration of pattern and dynamics. Chapman and Hall, New York.
- Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**: 703–718.
- Pruell, R.J., Taplin, B.K., and Cicchelli, K. 2003. Stable isotope ratios in archived striped bass scales suggest changes in trophic structure. *Fish. Manag. Ecol.* **10**: 329–336. doi:10.1046/j.1365-2400.2003.00369.x.
- Quay, P., Sonnerup, R., Westby, T., Stutsman, J., and McNichol, A. 2003. Changes in the $^{13}\text{C}/^{12}\text{C}$ of dissolved inorganic carbon in the ocean as a tracer of anthropogenic CO_2 uptake. *Global Biogeochem. Cycles*, **17**: 4.1–4.20. doi:10.1029/2001GB001817.
- Quinn, T.J., and Deriso, R.B. 1999. Quantitative fish dynamics. Oxford University Press, New York.
- Sarakinos, H.C., Johnson, M.L., and Vander Zanden, M.J. 2002. A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. *Can. J. Zool.* **80**: 381–387. doi:10.1139/z02-007.
- Vander Zanden, M.J., and Rasmussen, J.B. 1999. Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology*, **80**: 1395–1404. doi:10.1890/0012-9658(1999)080[1395:PCCANA]2.0.CO;2.
- Vander Zanden, M.J., and Rasmussen, J.B. 2001. Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: implications for aquatic food web studies. *Limnol. Oceanogr.* **46**: 2061–2066.
- Vander Zanden, M.J., and Vadeboncoeur, Y. 2002. Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology*, **83**: 2152–2161.
- Vander Zanden, M.J., Chandra, S., Allen, B.C., Reuter, J.E., and Goldman, C.R. 2003. Historical food web structure and restoration of native aquatic communities in the Lake Tahoe (California–Nevada) Basin. *Ecosystems (N.Y., Print)*, **6**: 274–288. doi:10.1007/s10021-002-0204-7.

- Wainright, S.C., Fogarty, M.J., Greenfield, R.C., and Fry, B. 1993. Long-term changes in the Georges Bank food web: trends in stable isotope compositions of fish scales. *Mar. Biol. (Berl.)*, **115**: 481–493. doi:10.1007/BF00349847.
- Yoshioka, T., Wada, E., and Hayashi, H. 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. *Ecology*, **75**: 835–846. doi:10.2307/1941739.
- Zohary, T., Erez, J., Gophen, M., Berman-Frank, I., and Stiller, M. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. *Limnol. Oceanogr.* **39**: 1030–1043.

Appendix A. Detailed description of simulation model

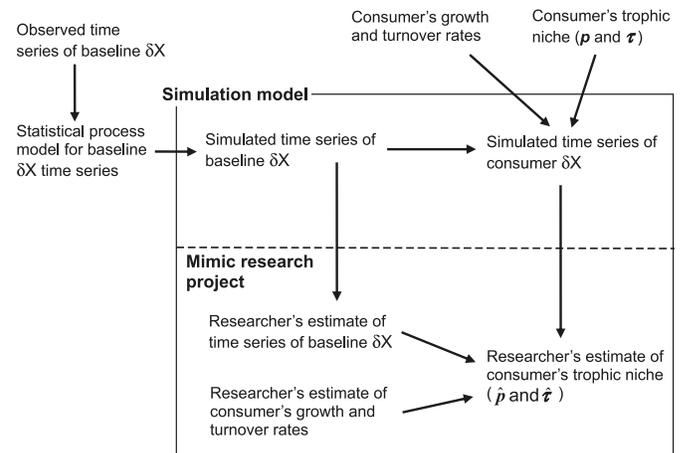
We used a simulation model (Fig. A1) to explore how estimates of consumer trophic niches are affected by the accuracy of (i) estimated time series of stable isotope ratios in food web baselines and (ii) estimated tissue growth and turnover rates of the consumer.

In the first phase of the model, three pieces of information are used to calculate fish δX for each year and age class. First, the fish's trophic niche determines the proportion of its biomass that comes from benthic versus pelagic resources and the number of trophic transfers (and associated isotopic fractionations) that occur between the resources and the fish. The particular values chosen for p and τ are of little consequence because the objective of the model is to determine how accurately the researcher can estimate p and τ (whatever they may be) given imperfect estimates of growth, turnover, and baseline δX . We set $p = 0.5$ and $\tau = 4.0$. Isotopic fractionation occurs with each trophic transfer between end members and fish. Empirical estimates of trophic fractionation vary (Vander Zanden and Rasmussen 2001; McCutchan et al. 2003; Vanderklift and Ponsard 2003), and the effects of this uncertainty on consumer trophic niche estimates have been considered by several authors (Vander Zanden and Rasmussen 2001; Post 2002; Matthews and Mazumder 2005). As a point of comparison, we also investigated these effects in one set of simulations (see description below), but for most simulations, we set trophic fractionation constant at 0.8‰ for C and 3.4‰ for N (Vander Zanden and Rasmussen 2001).

Second, varying baselines mean that the isotope ratios of new tissue depend on the year in which tissue is added. Rates of tissue growth and turnover control the contribution of each year's growth to the total biomass of the fish; we call this the fish's "mass proportions". Again, the particular values for growth and turnover parameters are relatively unimportant because we focus on how the researcher's estimates of these parameters affect the accuracy of \hat{p} and $\hat{\tau}$. We defined fish growth according to a von Bertalanffy age-length relationship and an allometric length-weight relationship using parameter values for yellow perch (*Perca flavescens*), a common north-temperate species. Parameter values were taken from Carlander (1950) and Froese and Pauly (2006) based on original work by Smith (1939) and Hasler (1945). Fish replaced 30.6% of their tissue every year via metabolic turnover based on a daily turnover rate of 0.1% (Hesslein et al. 1993).

Third, given the trophic niche and rates of growth and turnover, fish stable isotope ratios are determined by the

Fig. A1. Flow chart describing steps in the simulation model. The goal of the model is to assess bias and error in estimates of consumer trophic niches (τ , trophic position, and p , the proportional reliance of the consumer on benthic resources) that result from inaccurate estimates of either the true baseline time series or the consumer's growth and turnover rates. The first phase of the model (above the broken line) uses three types of inputs to simulate time series of baseline and consumer stable isotope ratios in C and N (δX). The second phase (below the broken line) mimics the implementation of a research project in which a researcher samples from the baseline and consumer δX time series and calculates estimates of p and τ (\hat{p} and $\hat{\tau}$).



baseline δX time series. We assumed that the observed Sparkling Lake baseline data were just one realization of a stochastic process that generates time series of baseline δX . To make our conclusions about the effects of incomplete baseline data as generalizable as possible, we ran multiple iterations of each model scenario and generated a new realization of the baseline time series in each iteration. Each realization was generated using the statistical model structure that best fit the observed Sparkling Lake data, but each time, the parameter values for the model were selected randomly from the set of bootstrapped parameter estimates (see main text: Methods, Collection and statistical modeling of empirical data). Inter- and intra-annual errors (drawn from empirically estimated distributions) were also added to each baseline realization. Thus, each iteration of the simulation model uses a plausible realization of baseline δX time series that incorporates our uncertainty about both the underlying generating process and the observed data in a given year.

Up to this point, the model is concerned with calculating the "true" δX for fish of each age in each year, given the "true" baseline time series, growth and turnover rates, and trophic niche of the fish. The second phase of the simulation model mimics the procedure that a researcher would use to calculate \hat{p} and $\hat{\tau}$ in a long-term study (Fig. A1). The researcher samples fish δX without error (for simplicity) but may have imperfect estimates of the baseline time series and the mass proportions of the fish. Given the age and stable isotope signature of a fish, the researcher uses the estimated mass proportions and baseline time series to estimate the trophic niche of the fish. This estimate is calculated by using an iterative procedure to find values of \hat{p} and $\hat{\tau}$ that

minimize the squared distance (in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ space) between observed and predicted fish δX (Post 2002).

References

- Carlander, K.D. 1950. Handbook of freshwater fishery biology. Wm. C. Brown Co., Dubuque, Iowa.
- Froese, R., and Pauly, D. (Editors). 2006. FishBase. World Wide Web electronic publication. Available from www.fishbase.org [updated June 2006].
- Hasler, A.D. 1945. Observations on the winter perch population of Lake Mendota. *Ecology*, **26**: 90–94. doi:10.2307/1931918.
- Hesslein, R.H., Hallard, K.A., and Ramlal, P. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}\text{S}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$. *Can. J. Fish. Aquat. Sci.* **50**: 2071–2076. doi:10.1139/f93-230.
- Matthews, B., and Mazumder, A. 2005. Consequences of large temporal variability of zooplankton $\delta^{15}\text{N}$ for modeling fish trophic position and variation. *Limnol. Oceanogr.* **50**: 1404–1414.
- McCutchan, J.H., Lewis, W.M., Kendall, C., and McGrath, C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**: 378–390. doi:10.1034/j.1600-0706.2003.12098.x.
- Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**: 703–718.
- Smith, M.W. 1939. Fish population of Lake Jesse, Nova Scotia. *Proc. N.S. Inst. Sci.* **19**: 389–427.
- Vander Zanden, M.J., and Rasmussen, J.B. 2001. Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: implications for aquatic food web studies. *Limnol. Oceanogr.* **46**: 2061–2066.
- Vanderklift, M.A., and Ponsard, S. 2003. Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichment: a meta-analysis. *Oecologia (Berl.)*, **136**: 169–182. doi:10.1007/s00442-003-1270-z.