

## Consequences of large temporal variability of zooplankton $\delta^{15}\text{N}$ for modeling fish trophic position and variation

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### Abstract

We use a temporal integration model (TIM) to determine how estimates of trophic variation, using  $\delta^{15}\text{N}$ , depend on consumer growth dynamics and temporal isotopic variation ( $\delta^{15}\text{N}$ ) of food sources. Consumers are rarely in isotopic equilibrium with their food sources, so instantaneous comparisons between the  $\delta^{15}\text{N}$  of a consumer and its diet provide little information about trophic variation, even if the trophic positions of the diet are known. In this paper, we focus on the trophic link between zooplankton and planktivorous fish. We first review the extent of temporal variability of zooplankton  $\delta^{15}\text{N}$ , then examine the consequences of this variability for understanding the isotopic composition of planktivorous fish communities. We use time series of  $\delta^{15}\text{N}$  for *Daphnia*, calanoid copepods, and particulate organic matter ( $>200\ \mu\text{m}$ ) to generate theoretical diets for a model juvenile sockeye over a typical growing season. We use a TIM to predict the isotopic trajectory of individual juveniles feeding on these diets and explore how variance in growth rate and isotopic enrichment ( $\Delta_{\delta\text{N}}$ ) can affect estimates of trophic position and intrapopulation isotopic variability. In general, we found that using a seasonal average of *Daphnia*  $\delta^{15}\text{N}$  to estimate the trophic position of planktivorous fish is nearly equivalent to using a TIM. However, temporal variation in the  $\delta^{15}\text{N}$  of food sources, coupled with individual differences in the growth rate of consumers, can contribute to intrapopulation isotopic variation of consumers and lead to correlations between consumer size and  $\delta^{15}\text{N}$ .

Ecologists are increasingly using average  $\delta^{15}\text{N}$  values to estimate the trophic position of consumers and are using the variance in  $\delta^{15}\text{N}$  (intraspecific or interspecific) to estimate trophic variation. The most reliable method for estimating trophic position is to measure the elevation of  $\delta^{15}\text{N}$  from a baseline ( $\delta^{15}\text{N}_{\text{base}}$ ), divide by an average enrichment per trophic level ( $\Delta_{\delta\text{N}}$ ), and add the trophic position of the baseline ( $\lambda_{\text{base}}$ ) (Post 2002). Using a baseline helps account for large intersystem variation in  $\delta^{15}\text{N}$  that is unrelated to trophic variation (Vander Zanden and Rasmussen 1999; Post 2002; Matthews and Mazumder 2003). However, residual variation of  $\delta^{15}\text{N}$  (i.e., intrapopulation variation) may not be synonymous with trophic variation because of large temporal, compositional, and spatial isotopic variability of food sources (Maruyama et al. 2001; Melville and Connolly 2003; Needoba et al. 2003) and large variance in  $\Delta_{\delta\text{N}}$  (Post 2002). In a recent review, Vanderklift and Ponsard (2003) suggest that taxa specific estimates of the variance in  $\Delta_{\delta\text{N}}$  may improve our ability to account for intrapopulation variation of  $\delta^{15}\text{N}$ . In the present study, we investigate how antecedent temporal variability in the  $\delta^{15}\text{N}$  of a consumer's food sources affects

estimates of trophic position and the interpretation of intrapopulation isotopic variance.

Temporal, spatial, and compositional variability of the consumer's food sources affect how we choose  $\delta^{15}\text{N}_{\text{base}}$  (Cabana and Rasmussen 1996; Post 2002; Matthews and Mazumder 2003). For example, measuring the trophic position of fish in lakes may require a baseline for both the pelagic (e.g., mussels, bulk zooplankton, *Daphnia*) and benthic (snails, *Chironomids*) food chains (Post et al. 2000; Matthews and Mazumder 2003; Vadeboncoeur et al. 2003) because fish use both pelagic and benthic resources. This approach relies, in part, on the assumption that consumers are in temporal isotopic equilibrium with each baseline.

Current baseline approaches for estimating the trophic position of freshwater fish address temporal variability of  $\delta^{15}\text{N}$  in various ways. Intuitively, researchers trade off their sampling effort with how well they expect their baseline to match the temporal variability in the  $\delta^{15}\text{N}$  of the consumer's food sources. For example, Cabana and Rasmussen (1994) used a size fraction of zooplankton ( $<250\ \mu\text{m}$ ) as a baseline to estimate the trophic position of fish in order to help account for the high temporal variance in  $\delta^{15}\text{N}$  of primary producers. Post (2002) extended this approach to account for spatial variability at the base of lake food webs by using mussels and snails as baseline species for pelagic and littoral lake habitats, respectively. Recently, we suggested using *Daphnia* as a baseline for the pelagic habitat (Matthews and Mazumder 2003), but we did not provide a general model to measure the trophic position of planktivorous fish. Here we compare two approaches to estimate trophic variation of planktivorous fish using either a seasonal average or a time series of *Daphnia*  $\delta^{15}\text{N}$ . These approaches are general and are applicable to other systems where it is possible to make repeated measurements of a consumer's food source or base-

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line and measure or model the growth dynamics of the consumer.

Individual zooplankton species exhibit large seasonal variation in  $\delta^{15}\text{N}$  (Graham 1997; Leggett et al. 2000). How do we account for this large variation in our estimates of fish trophic position and trophic variation using  $\delta^{15}\text{N}$ ? Diet switch experiments confirm that the isotopic turnover time of consumers frequently lags the isotopic turnover time of their diets (Hesslein et al. 1993; Herzka and Holt 2000; Harvey et al. 2002). As a result, we expect isotopic disequilibrium between planktivores and zooplankton if there is considerable temporal variation in the  $\delta^{15}\text{N}$  of zooplankton. Using a seasonal average of zooplankton  $\delta^{15}\text{N}$  as  $\delta^{15}\text{N}_{\text{base}}$  will help mitigate this disequilibrium. However, estimates of fish trophic position might be sensitive to the pattern of temporal variation in zooplankton  $\delta^{15}\text{N}$ , and so in some cases a seasonal average may not be appropriate. In other words, the isotopic disequilibrium between zooplankton and fish will depend on the antecedent temporal variation of zooplankton  $\delta^{15}\text{N}$  and the isotopic turnover of fish tissue.

In this paper, we first survey the literature to quantify the extent of temporal variation in the  $\delta^{15}\text{N}$  of zooplankton. Second, we present data on the temporal variability of  $\delta^{15}\text{N}$  in zooplankton communities from six sites in four lakes from a previous study (Matthews and Mazumder 2003). Third, we use a temporal integration model (TIM) from Harvey et al. (2002) to calculate  $\delta^{15}\text{N}_{\text{base}}$  and to explore how intrapopulation isotopic variation depends on individual variation in consumer growth dynamics. For this application, we use juvenile sockeye in Lake Washington as a model system. In Lake Washington and other coastal lakes, juvenile sockeye are obligate planktivores and selectively feed on different zooplankton taxa (Mazumder and Edmundson 2002; Ballantyne et al. 2003). In addition, we can realistically model fry growth over a time period (May to November) that matches many studies that have repeatedly sampled the  $\delta^{15}\text{N}$  of multiple zooplankton species. Finally, we compare two models, a seasonal average model (SAM) and a TIM, that both use time series of zooplankton  $\delta^{15}\text{N}$  to estimate the trophic position and trophic variation of fish.

## Methods

*Literature survey: assessing the extent of zooplankton  $\delta^{15}\text{N}$  variability*—We summarized data from 15 lakes to quantify the extent of temporal variation in the  $\delta^{15}\text{N}$  of size fractions of plankton and individual taxa of zooplankton. To our knowledge, this is an exhaustive survey of the available literature. We used Grafula 3 (v2.10) to digitize data from figures of published papers and from a thesis (see Web Appendix 1: <http://www.aslo.org/lo/toc/vol50/issue.5/1404a1.pdf>). We classified all size fractions of plankton as particulate organic matter (POM) and aggregated all zooplankton species into broad taxonomic groups.

*The temporal variation of  $\delta^{15}\text{N}$  for individual zooplankton taxa*—We collected zooplankton from six sites in four coastal lakes on Vancouver Island, British Columbia: Sooke Lake (SOL), Shawnigan Lake (SHL), Council Lake (COL), and Elk Lake (ELL). In SOL and SHL, we sampled every 2 or

3 weeks from June to November 2001 at two stations, a deep basin (SOL-D [70 m] and SHL-D [53 m]) and a shallow basin (SOL-S [22 m] and SHL-S [27 m]). These two lakes are morphologically similar (SOL, 6.1 km<sup>2</sup>; SHL, 5.5 km<sup>2</sup>) and are in adjacent catchments (within 4 km). They are temperate, warm monomictic, and typically have mean summer epilimnetic chlorophyll *a* concentrations <2  $\mu\text{g L}^{-1}$  (Davies et al. 2004a). We sampled zooplankton from Council Lake and Elk Lake eight times between May and September 2001. Council Lake is a 0.16 km<sup>2</sup> (maximum depth 17 m) oligotrophic lake, and Elk Lake is a 2.46 km<sup>2</sup> (maximum depth 20 m) mesoeutrophic lake. Detailed morphometrics (Spafard et al. 2002), nutrient dynamics (Nowlin 2003; Davies 2004), and productivity of these systems (Davies et al. 2004b) are available elsewhere.

We collected zooplankton for isotopic analysis with a large Wisconsin net (50-cm diameter, 64- $\mu\text{m}$  mesh) from the entire water column or from a maximum depth of 30 m. We used a smaller net (30-cm diameter, 64- $\mu\text{m}$  mesh) to collect zooplankton for identification and enumeration. Zooplankton handling, sorting, and stable isotope analysis is described in detail in Matthews and Mazumder (2003). Briefly, we picked different zooplankton taxa from a bulk zooplankton size fraction, dried them at 60°C, and packaged them in tin capsules. The number of individuals per sample varied depending on the species and size, to approximate 1 mg of dry zooplankton tissue for isotopic analysis. Zooplankton biomass was calculated using an optical counting program (Z-count), and published length weight regressions (Culver et al. 1985; Yan and Mackie 1987).

We collected POM >200  $\mu\text{m}$  from the epilimnion and metalimnion using a 6-m section of Tygon tubing and a vertically oriented Niskin sampler, respectively. We filtered at least 20 liters of lake water through a 200- $\mu\text{m}$  Nitex mesh and then backwashed the POM onto precombusted (550°C for 1 h) 25-mm Whatman GF/C filters. Filters were dried overnight at 60°C and packaged in tin cups.  $\delta^{15}\text{N}$  analyses were conducted on an isochrom continuous flow isotope ratio mass spectrometer coupled to a Carlo Erba elemental analyzer at the University of Waterloo Environmental Isotope Lab with a precision of <0.1‰.

*TIM for predicting the trophic position of fish*—We modified a model developed for predicting the temporal change in isotopic composition following a diet switch between two food sources with different isotopic compositions (Hesslein et al. 1993; Harvey et al. 2002). We used Eq. 1 to calculate the isotopic signature of a fish at day  $t$  ( $\delta_t$ ),

$$\delta_t = \frac{\delta_{t-1} \times B_{t-1} + (B_t - B_{t-1}) \times [(\delta_{fs-1} + \delta_{fs})/2 + f]}{B_t} - \left\{ m \times \left[ \delta_{t-1} - \left( \frac{\delta_{fs-1} + \delta_{fs}}{2} + f \right) \right] \right\} \quad (1)$$

where  $B$  is the biomass,  $\delta_{fs-1}$  is the isotopic signature of the food source on day  $t - 1$ ,  $m$  is a metabolic turnover constant, and  $f$  is the fractionation factor between the food source and the consumer. To simplify the data requirements of bioenergetics analysis (Harvey et al. 2002), we coupled the model

with simulated growth trajectories of sockeye juveniles (*Oncorhynchus nerka*) in Lake Washington (Ballantyne et al. 2003). In Lake Washington, sockeye grow from 1 to 12.5 g from the beginning of May to November (Ballantyne et al. 2003). We modeled sockeye juvenile growth using a simple recursive logistic growth model, with a maximum juvenile weight of 12.5 g and a growth rate ( $\mu$ ; g d<sup>-1</sup>) that we chose at random from a normal distribution (typically,  $\mu = 0.03$ ,  $\sigma_\mu^2 = 0.01$ ). We used the variance in specific growth rate of juvenile sockeye from Ballantyne et al. (2003) to parameterize our simulations of growth dynamics. For the purposes of our simulations, we assumed that juveniles at the start of the simulation (1.0 g) were in isotopic equilibrium with their zooplankton diet. We used  $m = 0.0005$  in all of our simulations (following Harvey et al. 2002) and performed a sensitivity analysis of this parameter ( $m = 0.0005$  to 1.0). All simulations were done using S-plus 2000.

To include natural variability in trophic fractionation among individual sockeye juveniles, we randomly chose an enrichment factor for each juvenile from a normal distribution ( $\Delta_{\delta N} = 3.4\text{‰}$ ,  $\sigma_\Delta^2 = 0.17$ ), where  $\Delta_{\delta N}$  is the average enrichment per trophic level (Post 2002) and  $\sigma_\Delta^2$  is a field estimate of the variance in trophic enrichment of  $\delta^{15}\text{N}$  for lake trout (*Salvelinus namaycush*) (Vander Zanden and Rasmussen 2001). This field estimate is similar to a more recent estimate for muscle tissue of fish raised on a fixed diet ( $\sigma_\Delta^2 = 0.12$ ,  $n = 6$ ) from Vanderklift and Ponsard (2003). We also conducted simulations using an interspecific estimate, not specific to freshwater fish ( $\sigma_\Delta^2 = 0.98$ ; Post 2002), in order to address the model's sensitivity with respect to variation in trophic enrichment.

*Simulated fish diets for the TIM*—For each simulation, we projected the isotopic trajectory of 1,000 fish fed on one of four theoretical diets: (1) POM >200  $\mu\text{m}$ , (2) only *Daphnia*, (3) only calanoids, and (4) the predicted  $\delta^{15}\text{N}$  of the macrozooplankton community. In Council Lake, we included a simulated diet of only *Holopedium*. We linearly interpolated the  $\delta^{15}\text{N}$  for each simulated diet to get a daily resolution of  $\delta^{15}\text{N}$  between sampling dates. To calculate the predicted  $\delta^{15}\text{N}$  of a macrozooplankton diet, we used the percentage biomass composition (Table 1), the percentage nitrogen, and the  $\delta^{15}\text{N}$  of different zooplankton taxa to predict the  $\delta^{15}\text{N}$  signature of the macrozooplankton community. For each sampling date, we calculated the percentage contribution of nitrogen from each taxon as the proportion of the biomass times its nitrogen content. We normalized the proportions to sum to one and multiplied them by the observed  $\delta^{15}\text{N}$  for the corresponding zooplankton taxa. We then calculated the predicted  $\delta^{15}\text{N}$  of the macrozooplankton community as the sum of the contributions of  $\delta^{15}\text{N}$  by each taxon. Because of the low densities of cycloids and *Holopedium* at certain times of year and the small size of cycloids we were unable to make isotopic measurements of these taxa for all of the sampling dates. Previous research demonstrates that the difference in  $\delta^{15}\text{N}$  is typically <0.5‰ between *Daphnia* and *Holopedium* (Matthews and Mazumder 2003) and <1.0‰ between calanoids and cycloids (Web Appendix 1). Therefore, when the abundances of *Holopedium* and cycloids were low (typically <15% of biomass), we substituted the

Table 1. Taxonomic composition of zooplankton biomass for Sooke Lake Reservoir (shallow [SOL-S] and deep [SOL-D]), Shawnigan Lake ((shallow [SHL-S] and deep [SHL-D]), Council Lake (COL), and Elk Lake (ELL). Percentages are a seasonal average of the percentage of the total zooplankton biomass on each sampling date.

Lake site	Percentage of zooplankton biomass: average (SE)				
	<i>Daphnia</i> spp.	Calanoids	<i>Holopedium</i>	Cycloids	Other
SOL-S	54.8 (3.8)	21.5 (2.7)	0	18.2 (1.9)	4.4 (1.3)
SOL-D	26.1 (2.8)	55.9 (2.7)	0	13.0 (2.8)	3.8 (0.5)
SHL-S	50.0 (2.7)	15.0 (1.9)	11.1 (3.6)	16.8 (1.2)	7.1 (1.0)
SHL-D	50.7 (3.7)	23.2 (1.9)	1.7 (1.2)	14.0 (1.6)	10.3 (1.3)
COL	36.5 (5.5)	33.8 (3.3)	24.1 (6.3)	0	5.6 (1.9)
ELL	68.4 (8.1)	19.6 (5.0)	0	5.0 (2.6)	7.1 (2.6)

$\delta^{15}\text{N}$  of *Daphnia* and calanoids for *Holopedium* and cycloids, respectively. We acknowledge that the generality of the small differences between these taxonomic groupings has not been thoroughly tested.

To get a broader range of isotopic patterns for our diet simulations, we linearly interpolated time series of *Daphnia*  $\delta^{15}\text{N}$  for each lake in our literature survey (Web Appendix 1). We interpolated the  $\delta^{15}\text{N}$  of *Daphnia* for each day from May to September (day of year [DOY]: 121 to 243), and separately for June to October (DOY: 152 to 273). We generated these two sets of times series because the sampling patterns over the season differed among lakes. We used these two sets in our TIM simulations to evaluate the effect of intrapopulation variation in juvenile sockeye growth rate ( $\sigma_\mu^2$ ) on the magnitude and pattern of intrapopulation variation of juvenile sockeye  $\delta^{15}\text{N}$ .

*Comparison of baseline approaches for estimating the trophic position of fish*—We compared two baseline approaches to predict the  $\delta^{15}\text{N}$  of sockeye juveniles, both based on time series of zooplankton  $\delta^{15}\text{N}$ . First, we used a seasonal average of the  $\delta^{15}\text{N}$  for each diet and added an average fractionation factor of 3.4‰ (Post 2002) to predict the mean  $\delta^{15}\text{N}$  of juveniles (SAM). Second, we used the TIM, with  $f = 3.4\text{‰}$ , to predict the  $\delta^{15}\text{N}$  of juveniles based on the observed temporal isotopic variability of different diets. We interpreted differences between these predictions ( $\Delta_{\text{SAM-TIM}}$ ) as the potential amount of error in our estimates of fish trophic position depending on our model selection. We calculated  $\Delta_{\text{SAM-TIM}}$  (in ‰ units) for all the simulations we performed in this paper, including those done as part of the sensitivity analysis.

## Results

*Quantification of temporal variation from previous studies*—Results from the literature survey support conclusions reached by Matthews and Mazumder (2003) regarding isotopic differences among zooplankton taxa within a lake. Despite large isotopic variation for individual taxa among lakes, within a lake the  $\delta^{15}\text{N}$  of zooplankton follow established

feeding behaviors (Web Appendix 1). Typically, the more herbivorous zooplankton (like *Daphnia*) have the lowest  $\delta^{15}\text{N}$  signatures, followed by variably omnivorous copepods (calanoids and cyclopoids) and invertebrate predators (*Bythotrephes*, *Chaoborus*, and *Leptodora*). However, there is considerable unexplained variation in the relative differences between taxa among and within lakes (Web Appendix 1). The seasonal range of  $\delta^{15}\text{N}$  for *Daphnia* varied from 1.3‰ at SOL-D to 10.9‰ in Crooked Lake (Fig. 1).

**Results and interpretation of the TIM**—We used the temporal patterns in the  $\delta^{15}\text{N}$  of zooplankton taxa from our six study sites (Fig. 2) as the theoretical diets for juvenile sockeye and used the TIM to predict the  $\delta^{15}\text{N}$  of juveniles (Fig. 3). Each simulation generated a distribution of  $\delta^{15}\text{N}$  values for juvenile sockeye feeding exclusively on a defined diet. The degree of separation between the regions of predicted  $\delta^{15}\text{N}$  varied among lakes (Fig. 3) and was greatest in Elk Lake. The diets made up of *Daphnia* and POM typically overlapped, except in Council Lake (2001) where POM and *Holopedium* diets overlapped. In general, the mean difference between peaks depended on differences in the  $\delta^{15}\text{N}$  between diets and the pattern of temporal variation in the diet (Fig. 2). The variance of each peak depended primarily on the variance in trophic enrichment ( $\sigma_{\Delta}^2$ ) (Fig. 4); however, the variance in Fig. 4A results from individual sockeye juveniles growing at different rates on a fixed diet whose  $\delta^{15}\text{N}$  is increasing over the season (from SOL-D).

Variation in growth rates among individuals ( $\sigma_{\mu}^2$ ) led to an increase in intrapopulation variance by the end of the simulation. This was particularly noticeable when the intrapopulation variation in isotopic enrichment was negligible (as in Fig. 5;  $\sigma_{\Delta}^2 = 0.008$ ). This result was insignificant when we used higher estimates of  $\sigma_{\Delta}^2$  (e.g., 0.17, as in Fig. 3). Given the pattern of temporal variation in *Daphnia*  $\delta^{15}\text{N}$  for Council Lake (2002), fast growing fish had a higher  $\delta^{15}\text{N}$  than slow growing fish at the end of the simulation (Fig. 5). Therefore, variation in growth rate, coupled with an increasing  $\delta^{15}\text{N}$  of *Daphnia* early in the time series, generated a positive relationship between juvenile size and  $\delta^{15}\text{N}$  (see Fig. 6A). This was a general result for *Daphnia* time series that had abrupt increases in  $\delta^{15}\text{N}$  early in the season (Fig. 1A). Abrupt decreases in *Daphnia*  $\delta^{15}\text{N}$  early in the season (Fig. 1B) led to negative relationships between juvenile size and  $\delta^{15}\text{N}$  (Fig. 6A). The total amount of intrapopulation variation of juvenile sockeye  $\delta^{15}\text{N}$  at the end of the simulations was positively related to the intrapopulation variation in growth rate ( $\sigma_{\mu}^2$ ), but the relationship depended on the specific pattern of *Daphnia*  $\delta^{15}\text{N}$  (Fig. 6B). Overall, the timing and interaction between juvenile sockeye growth and change in *Daphnia*  $\delta^{15}\text{N}$ , along with individual differences in isotopic enrichment, can contribute to isotopic variation among individual juvenile sockeye.

**Comparison of baseline approaches for estimating the trophic position of fish**—To compare different approaches of predicting the  $\delta^{15}\text{N}$  of a planktivore, we computed the difference in predictions between the TIM and a SAM ( $\Delta_{\text{SAM-TIM}}$ ). In general, the average differences were small (Fig. 7). For a juvenile sockeye feeding exclusively on

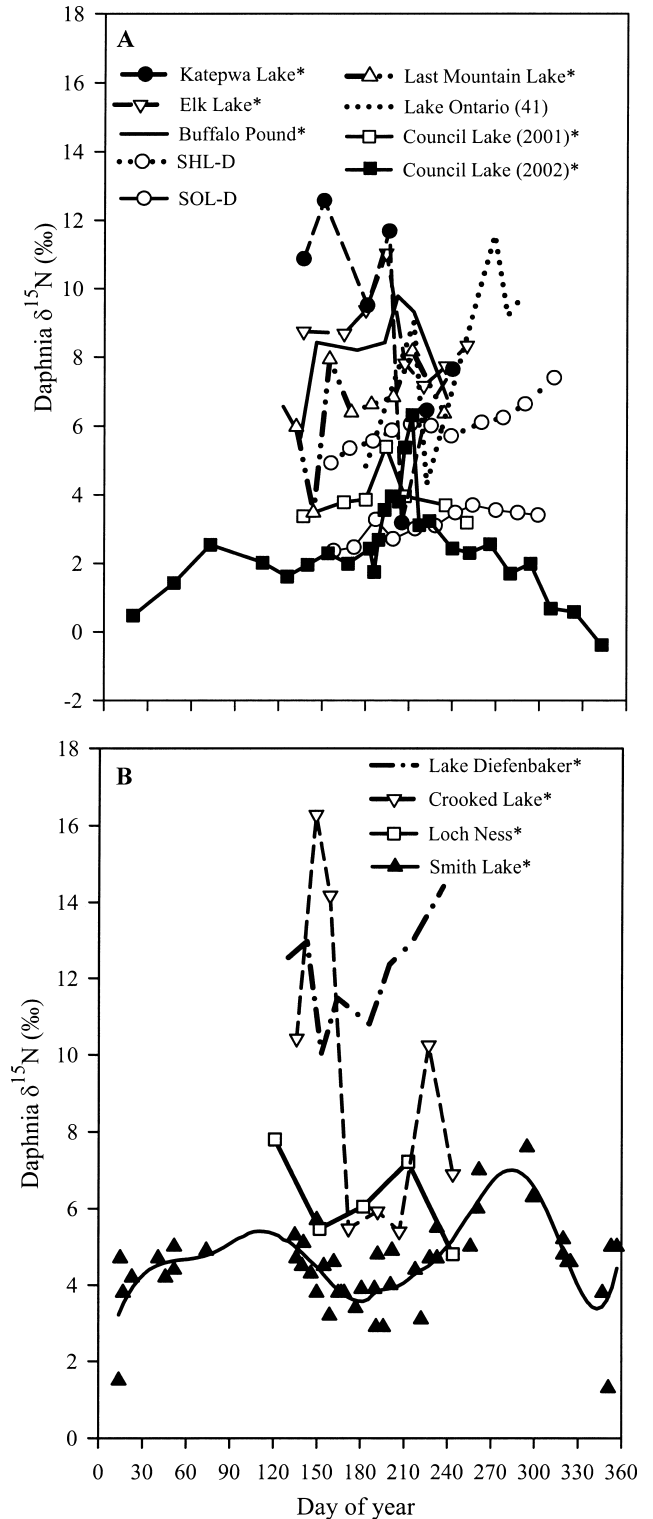


Fig. 1. Time series of *Daphnia*  $\delta^{15}\text{N}$  from a literature survey of the seasonal variability of zooplankton  $\delta^{15}\text{N}$ . The figure is split based on the simulation analyses in Fig. 6. (A) Time series of *Daphnia*  $\delta^{15}\text{N}$  that resulted in either a significant positive relationship ( $p < 0.05$ ) between juvenile sockeye size and  $\delta^{15}\text{N}$  (denoted by an asterisk) or no relationship. (B) Time series with a significant negative relationship between juvenile sockeye size and  $\delta^{15}\text{N}$  (Fig. 6A). The line through Smith Lake is a LOWESS plot (Tension = 0.5) for multiple years of data.

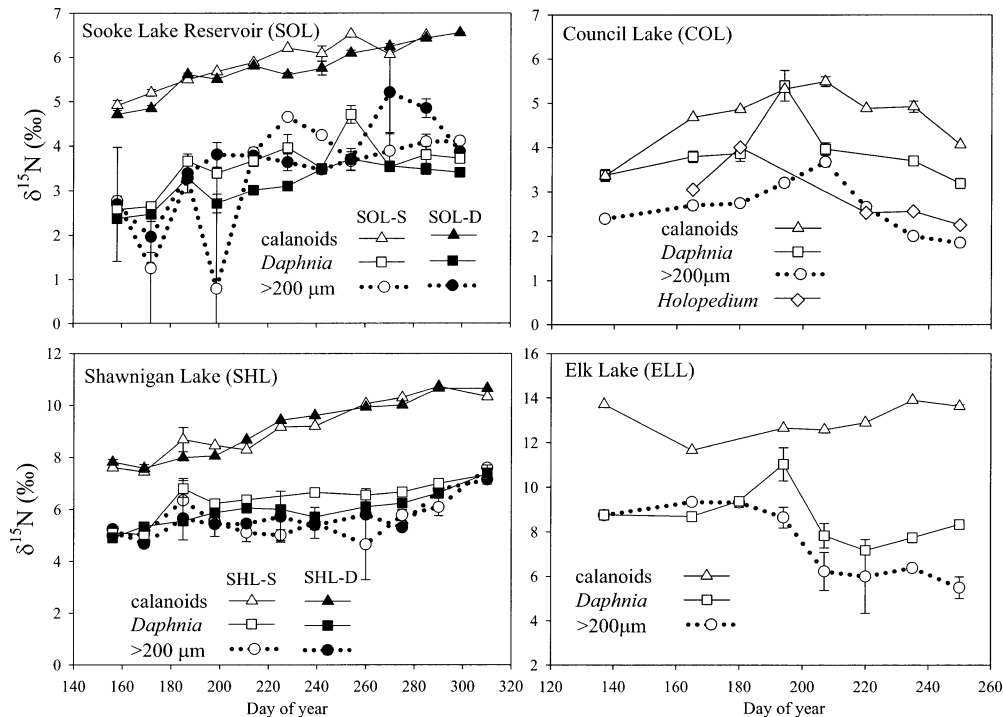


Fig. 2. Temporal changes in the  $\delta^{15}\text{N}$  of *Daphnia*, calanoids, *Holopedium*, and POM  $>200\ \mu\text{m}$ . Error bars are on  $\pm 1$  SE of the mean. When error bars are not present, only a single sample was analyzed for that sampling date. Filled symbols for SHL and SOL are the deep sites (from Matthews and Mazumder 2003), and open symbols are the shallow sites (see text).

*Daphnia*, the average  $\Delta_{\text{SAM-TIM}}$  among lakes was insignificant (mean =  $0.06\text{‰}$ , range =  $-0.66$  to  $0.68$ ,  $N = 30$ ). Among all simulated diets the average difference was similarly small but had a wider range (mean =  $0.10\text{‰}$ , range =  $-0.9$  to  $1.6$ , Fig. 7).

## Discussion

*Specific taxa or bulk size fractions for  $\delta^{15}\text{N}_{\text{base}}$ ?*—Previous approaches for estimating the trophic position of fish relied to some degree on size fractions of zooplankton (Cabana and Rasmussen 1994; Vander Zanden et al. 1999; Post 2002). Different zooplankton taxa have unique isotopic signatures (Meili et al. 1996; Matthews and Mazumder 2003) and can exhibit distinct seasonal patterns of  $\delta^{15}\text{N}$  (Graham 1997; Leggett et al. 2000; Grey et al. 2001; Fig. 2). But does the added precision of separating different zooplankton taxa warrant the extra effort for estimating fish trophic position? In general this depends on the specific research question and must be tailored to a particular study system (Post 2002).

There are advantages and disadvantages of using size fractions of plankton to estimate the trophic position of fish, as opposed to using a single zooplankton taxon. From our literature survey we found that size fractions of plankton typically had the highest coefficient of variation for a given lake site (14 out of 19 time series in Web Appendix 1). This higher variation likely results from baseline variation in the primary consumers, in addition to changes in the zooplankton composition of the size fraction. In our six study sites, the predicted  $\delta^{15}\text{N}$  of the macrozooplankton community ac-

counted for 82% of the temporal variation in the  $\delta^{15}\text{N}$  of POM ( $>200\ \mu\text{m}$ ) ( $F_{1,52} = 230.9$ ,  $p < 0.001$ ,  $R^2 = 0.82$ ) but was consistently higher than POM by  $\sim 1.5\text{‰}$ . This low  $\delta^{15}\text{N}$  of POM is likely a result of large algae, which we routinely observed in the  $>200\text{-}\mu\text{m}$  size fraction. Similarly in Lake Ontario, Kiriluk et al. (1995) found that the  $\delta^{15}\text{N}$  of net plankton ( $>153\ \mu\text{m}$ ) was  $1.2\text{‰}$  when *Bosmina* and diatoms were abundant but was  $12.1\text{‰}$  when cyclopoid copepods dominated the same size fraction. When there is isotopic heterogeneity within a plankton size fraction, the amount of error in estimates of fish trophic position will partly depend on how well the composition of the size fraction actually represents the diet of the fish. Fish are often size-selective foragers and readily switch between zooplankton taxa depending on availability (Mazumder et al. 1990; Ballantyne et al. 2003). Size fractions of plankton can approximate natural changes in fish diet, if they account for the natural changes in the relative abundance of different zooplankton taxa. Currently we know that different zooplankton taxa have distinct  $\delta^{15}\text{N}$  signatures (Matthews and Mazumder 2003) and that this is a robust pattern among lakes (Web Appendix 1). However, if isotopic differences between zooplankton taxa result from different baseline sources of nitrogen (as opposed to trophic variation), then a size fraction might effectively average out the isotopic variability within the zooplankton community that is unrelated to trophic variation. If this is the case, then a time series of a size fraction may successfully capture baseline variation. This is why it is so critical to distinguish between baseline and trophic variation (Post 2002) and to determine to what extent isotopic

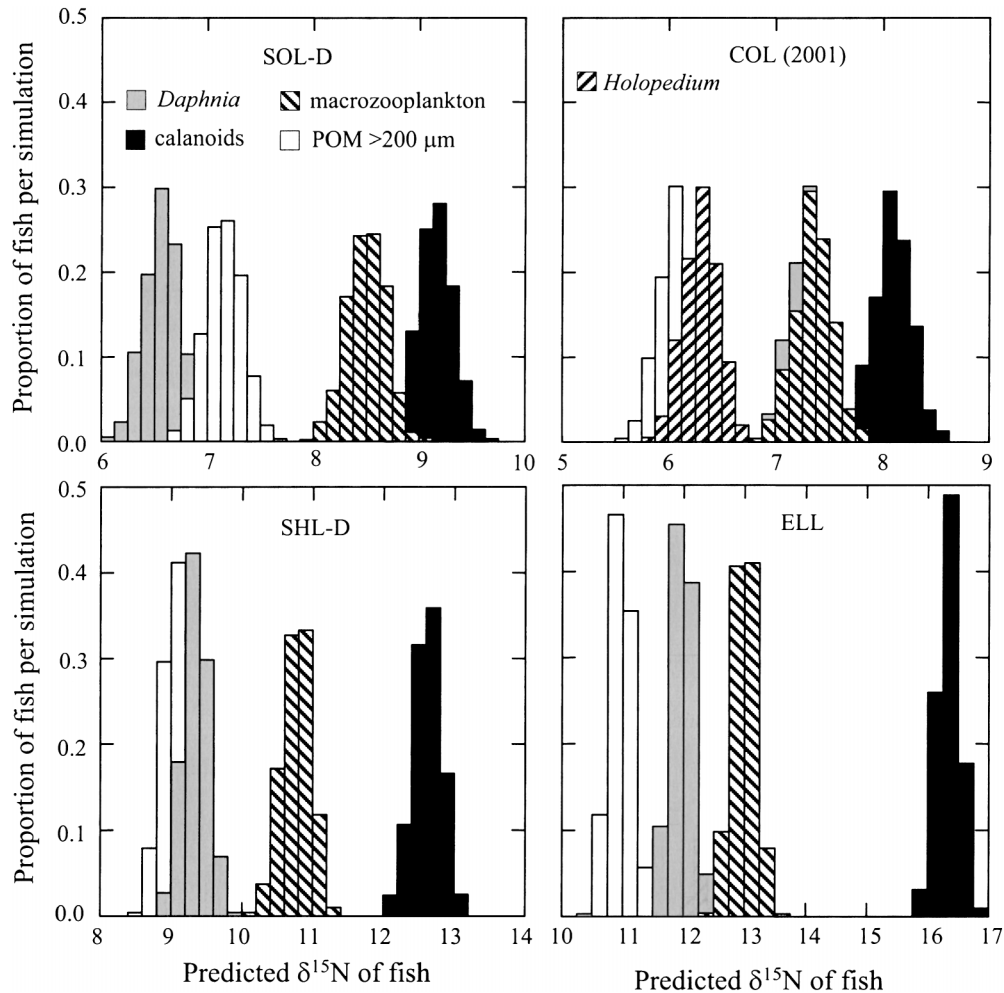


Fig. 3. Each distribution represents a predicted region for a juvenile sockeye feeding exclusively on a defined diet for our four study lakes (POM >200  $\mu\text{m}$ ; *Daphnia*, *Holopedium*, macrozooplankton, or calanoids). The  $^{15}\text{N}$  of macrozooplankton is calculated as described in the text. The TIM simulations were done for each diet category from day 152 to 273 (June to October), with parameters  $\mu = 0.03$ ,  $\sigma_{\mu}^2 = 0.01$ ,  $\sigma_{\Delta}^2 = 0.17$ .

heterogeneity within the zooplankton community actually reflects trophic, rather than baseline, variation.

*Estimating the trophic position of planktivorous fish using time series of plankton  $\delta^{15}\text{N}$* —Temporal variation of zooplankton  $\delta^{15}\text{N}$  is substantial and might be largely unrelated to trophic variation (Fig. 1). Robinson (2001) argues that a consumer's  $\delta^{15}\text{N}$  signature represents the integration of the nitrogen cycle up to the consumer's position in the food web, and Needoba et al. (2003) highlight the uncertainty in the causes of  $\delta^{15}\text{N}$  variation among phytoplankton taxa. Read together, these studies suggest that temporal variation in  $\delta^{15}\text{N}$  of a zooplankton taxon does not necessarily indicate trophic variation. Indeed,  $\delta^{15}\text{N}$  signatures of zooplankton are frequently influenced by changes in nitrogen cycling (Leggett et al. 2000; O'Reilly et al. 2002). A striking example is from Lake Tanganyika, where O'Reilly et al. (2002) demonstrated how a change in the nitrogen source can rapidly change the stable isotope representation of a pelagic food web. Following an upwelling event of isotopically heavy  $^{15}\text{NH}_4$ , zoo-

plankton had a  $\delta^{15}\text{N}$  signature similar to their planktivorous consumers (O'Reilly et al. 2002). It is still unknown whether this type of isotopic distortion is a common feature of lakes. Nevertheless, large temporal variation of zooplankton  $\delta^{15}\text{N}$  (Fig. 1) means that using a seasonal average or a time series of zooplankton  $\delta^{15}\text{N}$  could give different estimates for the trophic position of planktivorous fish.

*Using seasonal averages (SAM)*—The trophic position of an individual fish can be estimated as the elevation of  $\delta^{15}\text{N}$  from the baseline ( $\delta^{15}\text{N}_{\text{base}}$ ) divided by an average enrichment per trophic level, plus the trophic position of the baseline (Eq. 2).

$$\lambda_{\text{base}} + (\delta^{15}\text{N}_{\text{fish}} - \delta^{15}\text{N}_{\text{base}})/\Delta_{\delta\text{N}} \quad (2)$$

where  $\Delta_{\delta\text{N}}$  is the average enrichment per trophic level and  $\lambda_{\text{base}}$  is the trophic level of the consumer used as the baseline (Post 2002). This estimate assumes that the consumer's tissue is in isotopic equilibrium with its baseline (Post 2002). Given the large temporal variability in zooplankton  $\delta^{15}\text{N}$

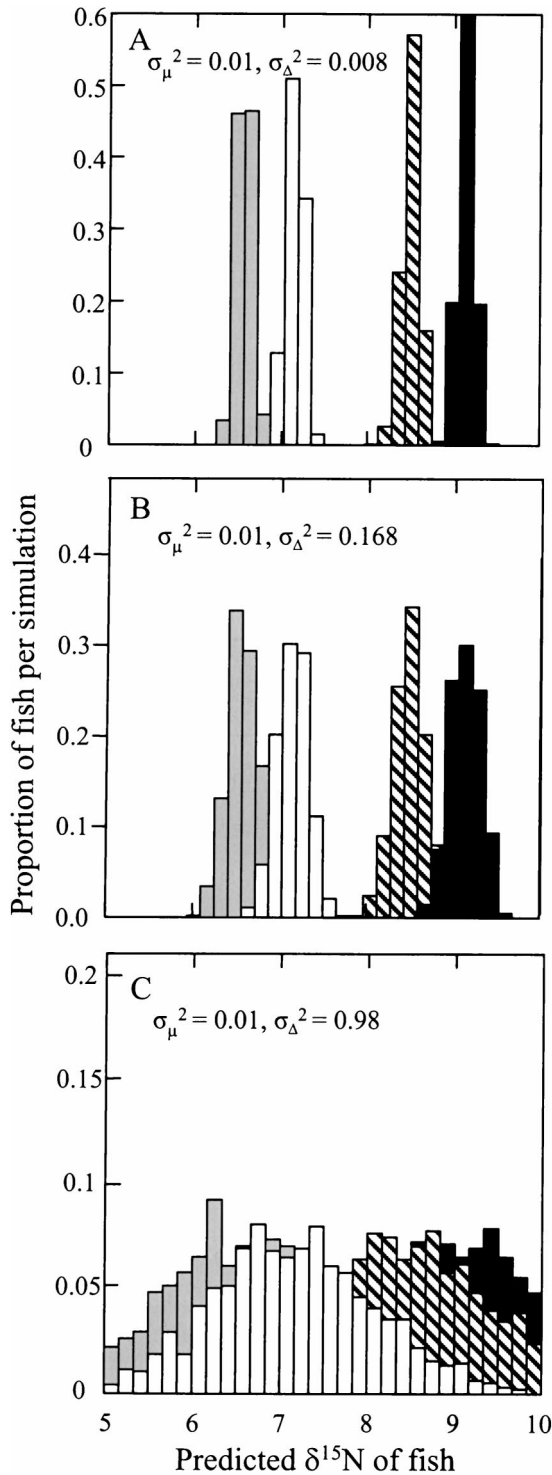


Fig. 4. Sensitivity analysis of the temporal integration model with respect to the variance in trophic fractionation  $\sigma_{\Delta}^2$  for diets at SOL-D. Hatching is the same as in Fig. 3. (A) The amount of variance attributed primarily to differences in growth rate among individual juvenile sockeye. (B) A typical increase in variance if we increase the  $\sigma_{\Delta}^2$  to a value for freshwater fish ( $\sigma_{\Delta}^2 = 0.17$ ) (Vander Zanden and Rasmussen 2001). (C) How the variance changes using an interspecific estimate of variance from Post (2002) ( $\sigma_{\Delta}^2 = 0.98$ ).

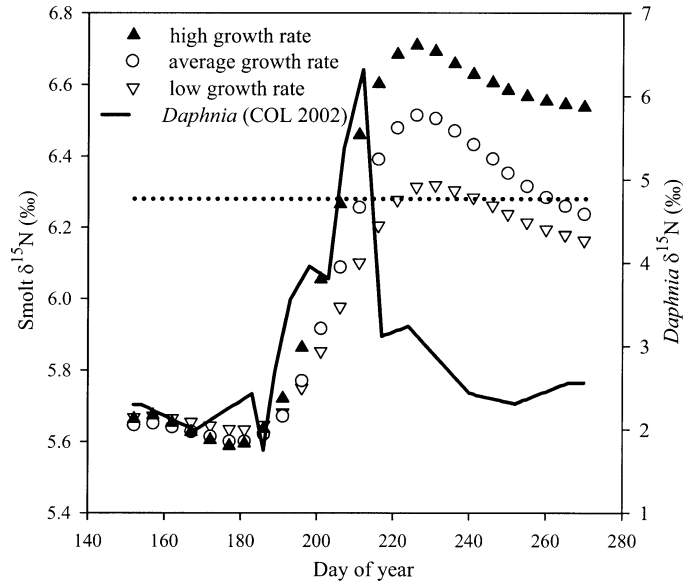


Fig. 5. Isotopic trajectory of juvenile sockeye (symbols—left axis) in a TIM simulation based on *Daphnia*  $\delta^{15}\text{N}$  from Council Lake 2002 (solid line—right axis) (Web Appendix 1). The simulation was from day 152 to 273 (June to Oct), with parameters  $\mu = 0.03$ ,  $\sigma_{\mu}^2 = 0.01$ ,  $\sigma_{\Delta}^2 = 0.008$ . The dotted line (left axis) is the predicted  $\delta^{15}\text{N}_{\text{base}}$  for the planktivore trophic level, calculated as the seasonal average of *Daphnia*  $\delta^{15}\text{N} + 3.4\text{‰}$ . The open circles are a trajectory of a fish with an average growth rate that is similar to the TIM's prediction for  $\delta^{15}\text{N}_{\text{base}}$ . The difference between predictions is  $\Delta_{\text{SAM} - \text{TIM}}$  (Fig. 7).

(Fig. 1; Web Appendix 1), neither point estimates of POM nor point estimates of individual zooplankton species provide robust measures of  $\delta^{15}\text{N}_{\text{base}}$ . As such, a seasonal average is always preferable to point estimates.

In some cases, the seasonal average of single zooplankton taxon can be a useful baseline ( $\delta^{15}\text{N}_{\text{base}}$ ) for measuring the trophic position of fish. *Daphnia* is a convenient baseline zooplankton taxon (Matthews and Mazumder 2003) because we can use a seasonal average of *Daphnia*  $\delta^{15}\text{N}$  for  $\delta^{15}\text{N}_{\text{base}}$  and define  $\lambda_{\text{base}} = \lambda_{\text{Herbivore}} = \lambda_{\text{Daphnia}} = 2$ . This assumes that *Daphnia* is predominantly an herbivore and thus belongs to the second trophic level of the classical pelagic food chain. This model can then be used to estimate the relative trophic position of other zooplankton taxa or other POM size fractions relative to the seasonal average of *Daphnia* using Eq. 2.

*Using a time series and a TIM*—The purpose of the TIM is to use the temporal variability of consumer food sources explicitly in estimates of trophic position and variation. The TIM scales the temporal variability of the consumer's food source to match the turnover time of the consumer's tissue. For example, to model the zooplankton–planktivore trophic link we could define  $\lambda_{\text{base}} = \lambda_{\text{Daphnia}}$ , but instead of using a seasonal average of *Daphnia*  $\delta^{15}\text{N}$  as  $\delta^{15}\text{N}_{\text{base}}$  we could determine  $\delta^{15}\text{N}_{\text{base}}$  using Eq. 1 and a time series of *Daphnia*  $\delta^{15}\text{N}$ . For example, the TIM can predict isotopic trajectories of planktivores that feed exclusively on *Daphnia*. If we consider the average of these simulated planktivores as the third trophic level, we can use the average  $\delta^{15}\text{N}$  of simulated

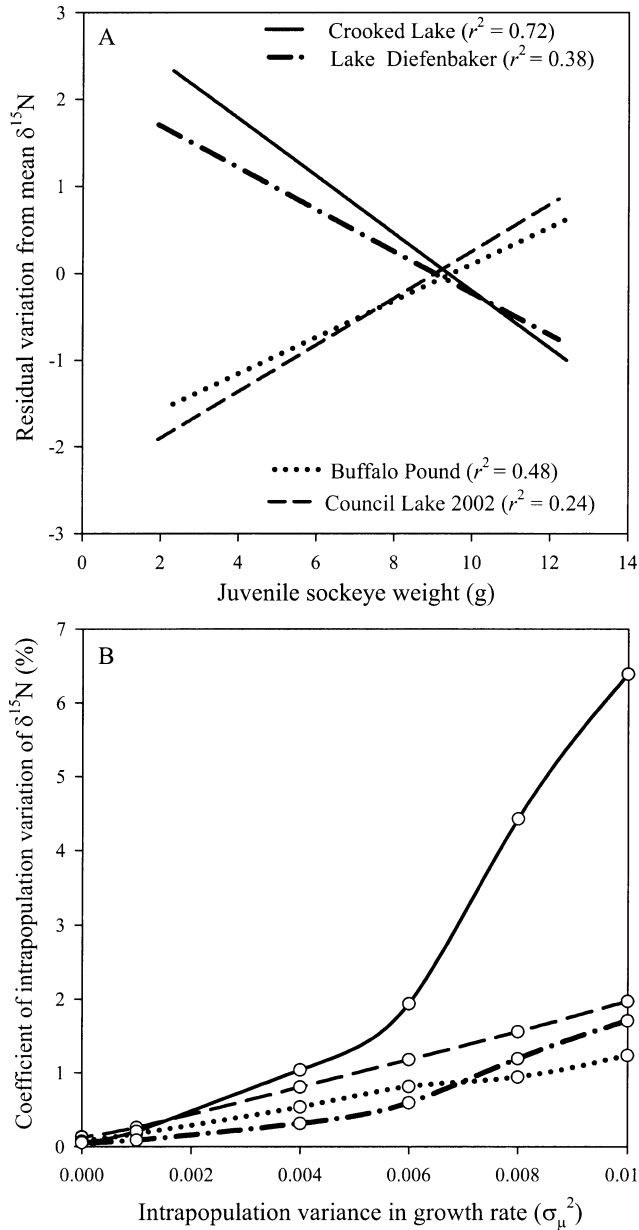


Fig. 6. (A) Lines from linear regressions ( $N = 1000$ ; data points not shown) for four simulations where there was a significant ( $p < 0.05$ ) relationship between juvenile sockeye weight and  $\delta^{15}\text{N}$  at the end of a TIM simulation. Here, all simulations were done from day 121 to 243 (May to September) with parameters  $\mu = 0.03$ ,  $\sigma_\mu^2 = 0.01$ ,  $\sigma_\lambda^2 = 0.17$ . For clarity, intrapopulation variation in  $\delta^{15}\text{N}$  is shown as Z scores for individual juvenile sockeye at the end of the simulation. (B) The effect of increasing variation in growth rate among individuals ( $\sigma_\mu^2$ ) on the total amount of intrapopulation isotopic variation at the end of several TIM simulations. Lines are the same as in (A), as are the parameters  $\mu$  and  $\sigma_\lambda^2$  for the simulations.

planktivores as  $\delta^{15}\text{N}_{\text{base}}$  for the planktivore trophic level (with  $\lambda_{\text{base}} = 3$ ). Used in this way  $\delta^{15}\text{N}_{\text{base}}$  differs slightly from the definition of Post (2002). Here  $\delta^{15}\text{N}_{\text{base}}$  is the average isotopic signature of the modeled consumer that feeds on the trophic level below it. Therefore, fish collected from the field can have isotopic signatures below  $\lambda_{\text{base}} = 3$ . Comparing the var-

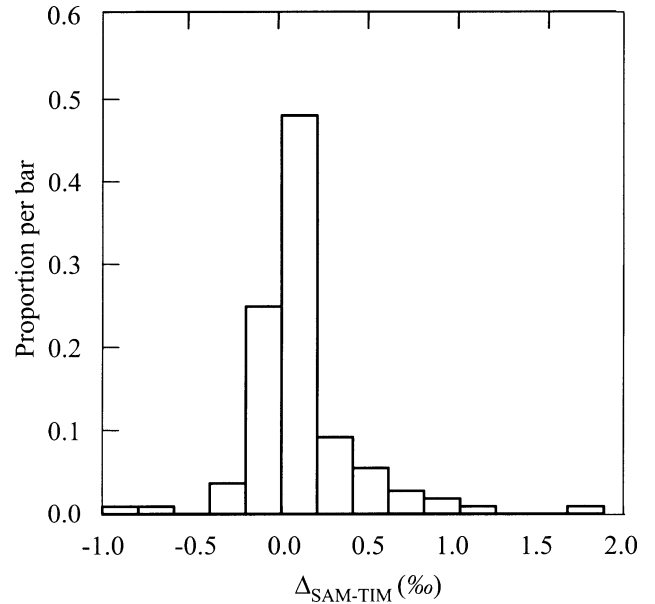


Fig. 7. Histogram of the difference between baseline approaches ( $\Delta_{\text{SAM-TIM}}$  in ‰ units) for calculating the trophic position of juvenile sockeye. Here we plot the results from 108 simulations based on different interpolated time series and model modifications, including all the simulations used to generate Fig. 3 (different diets), Fig. 4 (sensitivity analysis), and Fig. 6 (*Daphnia* only).

iance in  $\delta^{15}\text{N}$  of field estimates around  $\lambda_{\text{base}} = 3$  could then give us some indication of trophic variation at the third trophic level.

**Model sensitivity analysis**—The utility of the TIM depends primarily on the isotopic separation between food sources and the variance in trophic enrichment ( $\sigma_\lambda^2$ ). Variance in growth rate can contribute to intrapopulation variance (variance of peaks in Fig. 4A), but our approach is most sensitive to  $\sigma_\lambda^2$  (compare Fig. 4B,C). Ideally, researchers should use an estimate of  $\sigma_\lambda^2$  that is specific to the consumer of interest. A recent review suggests that this approach may soon be possible given the considerable interest in this area of research (Vanderklift and Ponsard 2003).

The TIM model was not very sensitive to the isotopic turnover constant  $m$  (Table 2) probably because of the large temporal variability of zooplankton  $\delta^{15}\text{N}$  and because we only modeled the growth phase of juvenile sockeye. Several previous diet switch studies suggest that  $m$  has a small impact on isotopic change compared with growth dilution (Hesslein et al. 1993; Vander Zanden et al. 1998). This is particularly the case for poikilotherms such as fish, which have a low basal metabolic rate (Herzka and Holt 2000).

**Using the TIM model**—The TIM model can easily be extended to model the trophic position of consumers in other food chains as long as we scale the temporal isotopic variability of the food source to the turnover time of the consumer. To use this model one would need information about the growth dynamics of the consumer and a temporal series of a baseline taxon or a food source with a known trophic position (such as *Daphnia* for planktivorous fish). Although

Table 2. The effect on the final estimate of fish  $\delta^{15}\text{N}$  resulting from variability in the rate of metabolic tissue turnover. Parameter estimates in the literature for freshwater fish range from 0 to 0.025  $\text{d}^{-1}$  (Hesslein et al. 1993; Harvey et al. 2002). The mean and variance is calculated for a simulation of 1,000 fish that fed only on calanoids at Shawnigan Lake (shallow).

Parameter $m$	Mean (variance)
0.0005	12.41 (0.054)
0.0070	12.46 (0.052)
0.01	12.49 (0.054)
0.05	12.78 (0.044)
0.10	13.07 (0.036)
0.25	13.56 (0.029)
0.35	13.71 (0.028)
0.50	13.81 (0.028)
0.80	13.81 (0.027)
1.0	13.73 (0.026)

this approach is data intensive, it forces the user to consider the effects of uncertainty in growth rate and trophic enrichment on the accuracy of trophic position estimates using  $\delta^{15}\text{N}$ . Modeling multiple steps in the food chain with this type of approach will likely fail in complex food webs because uncertainties in growth dynamics and trophic fractionation will propagate up the food chain. The approach of Post (2002) is clearly better suited to make inferences about food chain length and the trophic position of top predators. The model we present is intended for interpreting isotopic variation at adjacent trophic levels and for identifying the significance of isotopic differences among individual consumers.

Post (2002) models trophic position of fish using a long-lived primary consumer, such as mussels, as a baseline with  $\lambda_{\text{Mussels}} = 2$ . An advantage of this approach is that a long-lived consumer naturally integrates the changing isotopic signatures of the environment (Post 2002). Long-lived consumers, unlike a coarse time series of zooplankton, continuously sample the environment and might be more likely to pick up short-term baseline variation (O'Reilly et al. 2002). This is particularly useful when you can spatially and temporally match a consumer with a baseline species that has a known feeding behavior (sensu Kling et al. 1992). But there are situations where this might not be possible. For example, to estimate the trophic position of a rapidly growing fish larva (Herzka and Holt 2000), it might be more reasonable to measure the temporal variability of the larvae's food source and use an observed variance in growth rates to interpret intrapopulation trophic variation (Fig. 5). Likewise, TIM could also be useful for feeding experiments to estimate  $\Delta_{\delta\text{N}}$  (Vanderklift and Ponsard 2003), particularly if the  $\delta^{15}\text{N}$  of food sources change over the experiment (as might be the case for live feed).

In using TIM to calculate  $\delta^{15}\text{N}_{\text{base}}$ , it is important to consider whether the added complexity is worthwhile compared with the simpler SAM approach. Among all our simulated diets, the difference between models ( $\Delta_{\text{SAM} - \text{TIM}}$ ) is almost always less than 1‰ (Fig. 7), which amounts to less than half a trophic level (since  $\Delta_{\delta\text{N}} \sim 3.4\text{‰}$ ). In many cases,  $\Delta_{\text{SAM} - \text{TIM}}$  is not much larger than the analytical precision of

replicate field samples. Therefore, in most of the circumstances modeled here, the choice of models will not significantly alter the interpretation of stable isotope data from the field. However, in our model simulations all the fish started at the same biomass and grew over time in a single cohort. Ultimately, the difference between models will depend on the timing of growth among individual consumers, the interaction with the isotopic change in food sources, and the sampling resolution of food sources and consumers. In general, the TIM model is most useful for short time series where large changes in the  $\delta^{15}\text{N}$  of food sources occur early in the growth of the consumer.

It is still an open question how precisely we can expect to, or need to, determine trophic position. For example, how significant is half a trophic level of variation? For studies of contaminant accumulation, half a trophic level can be quite significant, depending on the rate of contaminant accumulation up the food chain. For example, the slope of the relationship between  $\delta^{15}\text{N}$  and mercury (Cabana and Rasmussen 1994) is steeper than several other contaminants (Kidd et al. 1995; Kiriluk et al. 1995), and even a 0.5‰ (<0.2 trophic levels) error in the predicted  $\delta^{15}\text{N}$  of a planktivorous fish can lead to >30% error in the prediction of its mercury concentration. For investigating trophic energetics, a 0.5‰ difference in  $\delta^{15}\text{N}$  is probably inconsequential. However, half a trophic level of variation (~1.7‰) within a planktivorous fish community could be significant, particularly since fish predators at the top of lake food chains only vary by two trophic levels among lakes (Post et al. 2000). Within a population, if an individual fish is half a trophic level higher than the average population, then this could have significant consequences for its overall fitness (Post 2003). Using  $\delta^{15}\text{N}$  to measure trophic position is a continuing challenge for ecologists because there are many sources of intrapopulation variation in  $\delta^{15}\text{N}$  that are unrelated to trophic variation. In general, ecological arguments based on stable isotope variation can be strengthened by using independent sources of dietary data (Matthews and Mazumder 2004) or data on growth dynamics (Post 2003).

*Interpreting intrapopulation and temporal variation of  $\delta^{15}\text{N}$* —Determining whether size-based variation in  $\delta^{15}\text{N}$  is actually related to trophic variation is a common problem in many ecological studies. Body size of fish is often correlated with  $\delta^{15}\text{N}$  (Beaudoin et al. 1999; Fry et al. 1999), and this relationship is commonly interpreted as an ontogenetic shift to higher trophic positions (Guiguer et al. 2002). However, relationships between body size and  $\delta^{15}\text{N}$  can also result from other processes, including shifts in habitat use (Genner et al. 2003), and temporal variability of food sources (Fig. 6A). One of the important features of the TIM is that it can provide a prediction about the expected relationship between body size (or growth rate) and  $\delta^{15}\text{N}$ .

A practical extension of the TIM we used here would be a model that linked consumer growth dynamics with the isotopic composition and abundance of food sources. This would help address covariation between consumer growth rate and  $\delta^{15}\text{N}$  of food sources, which could complicate interpretations of temporal isotopic patterns and intrapopulation variation of consumers. In lakes, for example, if the

food quality of algae declines following a switch from uptake of  $\text{NO}_3^-$  to nitrogen fixation, this may lead to a correlated decline in both the  $\delta^{15}\text{N}$  and abundance of the zooplankton community. In a similar vein, growth rate of juvenile sockeye may be correlated with the  $\delta^{15}\text{N}$  of their food source. If juveniles grow faster by feeding on zooplankton with a higher  $\delta^{15}\text{N}$ , this could lead to a positive relationship between body size and  $\delta^{15}\text{N}$ . To determine whether the resulting intrapopulation variation is related to trophic variation, it is essential to determine to what extent  $\delta^{15}\text{N}$  variation in the plankton community, both among and within species, is attributable to trophic variation.

For calculating the trophic position of planktivorous fish, the SAM is much simpler to use and yields equivalent information as the TIM (Fig. 7). The TIM approach is more widely applicable for determining the integrative feeding history of consumers in general and for interpreting intrapopulation isotopic variation in particular. For example, TIM can provide an estimate of isotopic variation that is unrelated to trophic variation and a prediction about the relationship between consumer body size and  $\delta^{15}\text{N}$ . Different types of temporal variation in food sources, coupled with variation in the growth dynamics of consumers, will lead to different amounts of intrapopulation isotopic variation (Fig. 6B). Large directional change in the  $\delta^{15}\text{N}$  of a consumer's food source, particularly early in a consumer's ontogeny, will lead to more intrapopulation isotopic variation because small differences in growth rate can lead to large isotopic differences in the biomass fixed during this time (Fig. 5). In comparison, smaller random isotopic variation will lead to less variation among individuals, even if individuals are growing at different rates. In either case, the intrapopulation variation resulting from differences in growth rate is unrelated to trophic variation unless the temporal variation in the food source itself is related to trophic variation. Therefore, the TIM can provide an expected variance of a consumer population that feeds on a defined diet over time and so can be used as a null model to test for the presence of trophic variation within a population (*sensu* Matthews and Mazumder 2004). There is an emerging body of literature that is interpreting intrapopulation variability in stable isotopes as evidence of individual diet variation (Gu et al. 1997; Beaudoin et al. 1999). However, these studies do not explicitly state the magnitude of variance required for evidence of individual specialization. Future studies should consider how the TIM model affects the use of isotopic mixing models and our interpretations of intraindividual variation of inert tissues (such as hair, feathers, or scales). In general, the temporal integration modeling approach is critical for linking isotopic variation among individuals to individual differences in diet.

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