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Estimation of nitrogen stable isotope turnover rate of *Oncorhynchus nerka*

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Synopsis

Estimation of the isotopic turnover rate in the tissues of organisms allows us to estimate the temporal relationship between the isotope ratio of an organism and its prey, and to analyze seasonal variations of food webs and migratory patterns of animals. We analyzed the isotopic turnover rate in the tissue of lacustrine sockeye salmon, *Oncorhynchus nerka*, of three age classes in a laboratory food alteration experiment to clarify the isotopic turnover rate. The contribution of growth to isotopic turnover was higher in young and fast-growing lacustrine sockeye salmon than in older and slow-growing fish. Maturation masks the relationship between the contributions of growth and metabolism to isotopic change. Our estimate of the isotopic time lag of lacustrine sockeye salmon can be used to research trophic relationships of anadromous sockeye salmon.

Introduction

Studying the trophic relationships between organisms and their prey is an important approach to understanding aquatic ecosystems (Persson et al. 1992, Vander Zanden et al. 1999a). The trophic relationship between organisms and their prey can be analyzed by gut content analysis and by stable isotope analysis. Gut content analysis has the major disadvantage that it provides only a snapshot of the diet, yet it has the advantage of giving useful taxonomic data about prey items. In contrast, stable isotopes in animal tissue provide integrated information on trophic relationships based on the long term, assimilated diet (Hesslein et al. 1993). Stable isotopes can also be used to determine the nutritional contributions of some prey that are poorly quantified in gut content analysis, such as detritus (Keough et al. 1998). Also, stable isotope analysis is a powerful tool for revealing the trophic relationships of polyphagous feeders, such as lycosid spiders (Oelbermann & Scheu 2002).

Although there are many advantages to stable isotope analysis, one disadvantage occurs when temporal changes in the trophic relationship occur. There is a time lag before the stable isotope value in the tissue reflects the change from one food source to the new source (Hesslein et al. 1993, Frazer et al. 1997, MacAvoy et al. 2001, Maruyama et al. 2001). This time lag is determined by the isotopic turnover rate in the tissue. Many fishes demonstrate a degree of plasticity in prey item choice and change their diet seasonally and/or in response to prey availability. The isotopic turnover rate in many animals is not known and stable isotope values are interpreted without information on turnover rates in food web studies (Vander Zanden et al. 1999a, b, Yoshii et al. 1999), although studies about isotopic turnover rates in fishes have increased recently (Vander Zanden et al. 1998, MacAvoy et al. 2001, Maruyama et al. 2001).

Isotopic turnover rate can be divided into growth and metabolic tissue replacement components (Hesslein et al. 1993, MacAvoy et al. 2001, Maruyama et al. 2001). Animals with a high growth rate have been shown to have high rates of isotopic turnover (Frazer et al. 1997, Herzka & Holt 2000), whereas the isotopic turnover rate in broad whitefish, *Coregonus nasus*, is low owing to the slow growth rate (Hesslein et al. 1993). Maruyama et al. (2001) hypothesized that the relative contribution of metabolic turnover might be higher in slow-growing than in fast-growing fish, and showed that the contribution of metabolic turnover is important.

The lacustrine sockeye salmon, *Oncorhynchus nerka*, is a common species in fisheries in oligotrophic lakes of northern Japan. This species tends to change diet from large zooplankton, *Daphnia* spp., to other less optimal prey with changes of prey availability (Takamura et al. 1999, Sakano et al. 2001). Determination of isotopic turnover rate is important for understanding the temporal feeding relationship between lacustrine sockeye salmon and their prey. In particular, the nitrogen isotope ratio plays a more important role in estimating the trophic position than the carbon isotope ratio (Post 2002). The purpose of this study is to estimate the nitrogen isotopic turnover rate of lacustrine sockeye salmon in three age classes in which growth rates varies.

Materials and methods

The lacustrine sockeye salmon used in this study were reared at the Toya Lake Station for Environmental Biology, Hokkaido University from eggs of spawners caught in Lake Toya. On 13 June 1999, we randomly selected 50 fish each of age 1+ (BW; 9.5–15.3 g), age 2+ (BW; 71.3–169.9 g) and age 3+ (BW; 168.5–308.2 g) lacustrine sockeye salmon were from this stock. We placed each age class of fish in a round, fiber-reinforced, plastic tank (180 cm diameter, 80 cm height, 2000 kl).

Table 1. Changes of food type used in this experiment.

Fish	Regular food	Experimental food
Age 1+	No.1 for fry*	Krill
Age 2+	No.4 for adult*	No.2 for fry*
Age 3+	No.5 for adult*	No.2 for fry*

Asterisks indicate commercial trout food (Oriental Yeast Industry Co., Ltd., Chiba, Japan).

The isotope turnover rate experiment started 20 June 1999 and ended 22 September 1999. Before the isotope turnover rate experiment, we had fed the lacustrine sockeye salmon of age 1+, age 2+ and age 3+ a commercial trout food of No. 1 for young fish, No. 4 for adult and No. 5 for adult (Oriental Yeast Industry Co., Ltd., Chiba, Japan), respectively (Table 1). We fed krill, *Euphausia superba*, to age 1+ fish and commercial trout food of No. 2 for young fish to age 2+ and 3+ fish as an experimental food from the initiation of the experiment. We fed the experimental food ad libitum during this experimental period. There was no control group in this experiment, but our main focus was on the contribution of growth and metabolism on isotopic changes by comparing among isotopic changes of the three age classes that differed growth rate. Previous research has revealed that the stable isotope ratio in the control group did not change with time (Harvey et al. 2002). Therefore, we only compared the growth and isotope change among the three age classes. We used spring water in the experiment. Water temperature in each tank was 9.9–13.4°C (mean water temperature 11.0 ± 0.8°C).

We randomly sampled three fish from each tank on day 0, 4, 8, 14, 31, 57, 72 and 94. We measured the length and mass immediately after capture, and dissected the dorsal white muscle from each fish for stable isotope analysis. We dried samples of dorsal muscle at 60°C for 48 h, and homogenized them with a mortar and pestle into a fine powder in preparation for isotopic analysis. We extracted lipids from the powder sample using 2:1 chloroform–methanol solution (Bligh & Dyer 1959) before the analysis.

We carried out stable isotope analyses using an element analyzer (EA1110, Fisons Instruments, Milan, Italy) and an isotope ratio mass spectrometer (Delta-252, Finnigan MAT GmbH, Bremen, Germany) at the National Institute for

Environmental Studies, Tsukuba, Japan. Stable isotope ratios were expressed in delta (δ) notation; $\delta^{15}N = [(R_{sample}/R_{standard})-1] \times 10^3$, where $R = {}^{15}N/{}^{14}N$. The standard material was atmospheric nitrogen for $\delta^{15}N$.

To estimate the turnover rate of lacustrine sockeye salmon, we used a turnover model composed of growth and metabolism (Hesslein et al. 1993, MacAvoy et al. 2001). The turnover model including the growth and metabolic components is described by the following equation:

$$C = C_n + (C_0 - C_n) \times e^{-(k+m)t}$$

where C is the $\delta^{15}N$ value of fish when sampled, C_n is the expected value for a fish in equilibrium with its new diet, C_0 is the initial $\delta^{15}N$ value of fish, m and k are the metabolic and growth contribution to isotope turnover, and t is time. We calculated growth rate (k) of each age class using the following simple exponential growth model:

$$W = W_0 \times e^{kt}$$

where W_0 is the mass of lacustrine sockeye salmon on day 0, W is the mass on day 4, 8, 14, 31, 57, 72 or 94. We estimated the metabolic turnover (m) by fitting the predicted growth rate (k) to the turnover model. Time lags of the $\delta^{15}N$ shift in tissue (for convenience, number of days required to show a shift equal to an 80% change from the isotope ratio of the initial diet to the expected isotope ratio of the new diet) were calculated by solving the follow equation:

$$t^* = \ln 5/(k+m).$$

We estimated growth rate and turnover rate by non-linear least-square method using simplex search method. We used analysis of covariance to compare between the growth patterns and turn over of age classes. We calculated Akaike's information criterion (AIC) to compare the goodness of turnover models with and without metabolic contribution.

Results

The growth pattern of lacustrine sockeye salmon in each of the three age classes was different (Figure 1). Lacustrine sockeye salmon of age 1+ increased their body mass by 10-fold in the 94 day period. However, lacustrine sockeye salmon in the other age classes did not grow as rapidly. The slopes of simple exponential growth models (i.e., growth rate) for each of the three age classes differed (ANCOVA: $F = 2.79$, d.f. = 2,55, $p = 0.07$). Growth rate of age 1+ lacustrine sockeye salmon was higher than that of the other aged lacustrine sockeye salmon (Age 1+: $k_1 = 0.026$, Age 2+: $k_2 = 0.008$, Age 3+: $k_3 = 0.003$, Scheffe's test: Age 1+ vs. Age 2+; $p < 0.05$, Age 2+ vs. Age 3+; $p > 0.05$, Age 3+ vs. Age 1+; $p < 0.05$). Growth rate decreased in older lacustrine sockeye salmon, although the difference between age 2+ and 3+ was not significant.

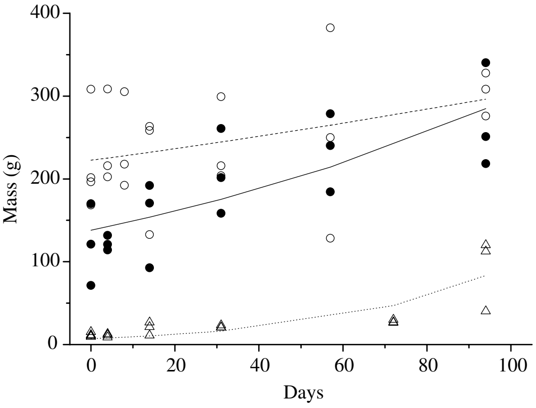

Figure 1. Wet mass of age 1+ (open triangles), age 2+ (solid circles) and age 3+ (open circles) lacustrine sockeye salmon as a function of days from the initiation of the experiment. Fitted exponential growth model gives growth rate of age 1+ ($k_1 = 0.026$), age 2+ ($k_2 = 0.008$) and age 3+ ($k_3 = 0.003$).

$\delta^{15}N$ values in age 1+ lacustrine sockeye salmon decreased following the change in food source from the commercial trout food to krill (Figure 2). In age 2+ and 3+ lacustrine sockeye salmon, $\delta^{15}N$ values were similar and enriched over the initial values.

Regression curves of $\delta^{15}N$ value on days from the initiation of the experiment were determined by assigning the predicted growth rate without metabolism (m) (Age 1+: $r^2 = 0.81$, Age 2+: $r^2 = 0.75$, Age 3+: $r^2 = 0.71$). The percentages of total variation in $\delta^{15}N$ explained by the fitted regression were improved by incorporating the metabolic contribution (Age 1+: $r^2 = 0.85$, Age 2+: $r^2 = 0.87$, Age 3+: $r^2 = 0.73$), and these improvements decreased AIC in all age classes

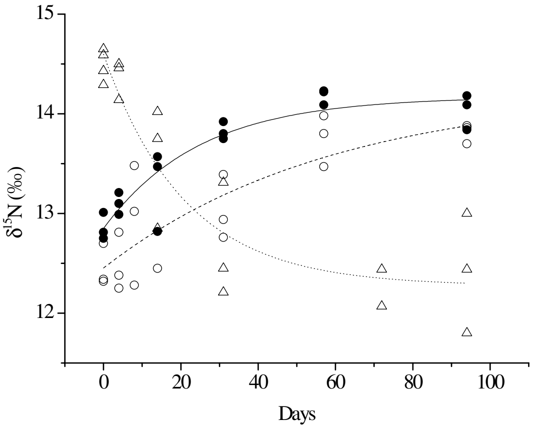

Figure 2. δ^{15}N value of white dorsal muscle of age 1+ (open triangles), age 2+ (solid circles) and age 3+ (open circles) lacustrine sockeye salmon as a function of days from the initiation of the experiment with regression curves of the growth and metabolism model.

(Age 1+: 26.0–24.5, Age 2+: 192.7–172.4, Age 3+: 251.5–201.7). The gain of the coefficient of determination was low except for age 2+. These regression curves differed among the three age classes (ANCOVA: $F = 49.87$, d.f. = 2,51, $p < 0.01$). Metabolic turnover did not decrease with age (Age 1+: $m_1 = 0.022$, Age 2+: $m_2 = 0.032$, Age 3+: $m_3 = 0.014$). The time lag for the δ^{15}N in tissue calculated by growth and metabolic turnover became longer with increasing age (Age 1+: 33.8 days, Age 2+: 40.0 days, Age 3+: 95.2 days).

Discussion

The change in nitrogen isotope in our study became faster with increasing growth rate (Figures 1 and 2), paralleling other published work without comparing turnover rates among groups with varying growth rate. The isotopic turnover of krill is rapid because they have a high growth rate (Frazer et al. 1997). Herzka & Holt (2000) found a rapid turnover in red drum, *Sciaenops ocellatus*, larvae due to their very high growth rate. Hesslein et al. (1993) showed that cultured whitefish juveniles had very slow turnover rates, and they attributed over 90% of the observed nitrogen isotopic change to growth. It is clear that growth is the dominant factor of isotopic change in fish muscle tissue.

Metabolism also contributes to isotopic change in muscle tissue, however the contribution of growth accounted for most of the nitrogen isotopic change in our study. The metabolic contribution to isotopic change in mammals and birds is high (Tieszen et al. 1983, Hobson & Clark 1992), as compared to fish and crustaceans (Hesslein et al. 1993, Frazer et al. 1997, Herzka & Holt 2000). Our study shows that the degree of metabolic contribution to the isotopic turnover rate differs with age.

The absence of a strong effect of metabolic turnover on the isotopic composition implies the feasibility of estimation of the isotopic turnover rate in natural populations (Herzka & Holt 2000). In the turnover model of age 1+ fish, the addition of metabolic turnover to the model did not cause substantial increases in the coefficient of determination when compared with that for growth only. The growth rate of age 1+ and age 2+ fish in the 1994 and 1997 cohorts during summer were 0.0056 and 0.013 in the 1994 cohorts, and 0.0077 and 0.011 in 1997 cohorts, respectively (H. Sakano, unpublished data). The growth rate of lacustrine sockeye salmon in Lake Toya is lower than that of laboratory-reared lacustrine sockeye salmon of age 1+, but higher than that of age 2+. For age 1, the time lag to 80% change in natural conditions may become longer (more than 33.8 days) compared with that of reared fish, on account of the lower growth rate in the lake. The coefficient of determination in the turnover model increased by the addition of metabolic turnover to the model in age 2+ fish. Overman & Parrish (2001) also suggested that the metabolic contribution of isotopic turnover is related with age in long-lived walleye, *Stizostedion vitreum*. To estimate the time lag of 80% turnover of age 2+ sockeye salmon, we need to consider the contribution from metabolism. Metabolism in the turnover model is defined as the portion of the existing body weight that is replaced regularly with or without growth (Hesslein et al. 1993). McCarthy et al. (1999) found that the muscle protein synthesis rate increased with temperature by estimating the regression equation between temperature and protein synthesis rate for ectotherms and endotherms. Our reared experimental fish experienced a relatively constant water temperature (11.0 ± 0.8°C). Lacustrine sockeye salmon experienced 8–10°C average temperatures during the summer (Brett 1971). Iida & Mukai (1995) also indicated that lacustrine sockeye salmon tend to live below the thermocline of 7–13°C. Considering these previous studies of the thermal environment of lacustrine sockeye salmon, there is little difference between the temperatures experienced by our cultured fish and their natural conditions. Therefore, a time lag of 80% turnover of age 2+ was calculated as nearly 40 days, as estimated by our diet change experiment.

Older fish tended to be reproductively mature and this means that the allocation of nutritional resources to gonadal maturation, body growth and metabolism affects the isotopic turnover rate. The turnover model of age 3+ fish was not improved by the addition of the metabolic component. Brett (1965) suggested that larger (and older) fish show a lowered metabolic rate. In our study, larger age 3 fish tended to be mature (average gonadosomatic index of age 3+ fishes: male; 3.9, female; 5.7). Unfortunately, there is no information on the allocation of nitrogen to the gonads, body growth and metabolism. Further research should be carried out on this unsolved problem.

In food web studies, it is necessary to explain δ^{13}C and δ^{15}N in consideration of difference of turnover time among life-forms. O'Reilly & Hecky (2002) demonstrated the difference of isotopic turnover time between zooplankton and phytoplankton caused an inversion of the nitrogen isotope signature, which can lead to a misunderstanding in the trophic relationship. This study found that the time lags of isotopic change from an old to a new food item can be estimated by consideration of the relationships between the contribution of growth rate and water temperature affecting metabolism on isotope turnover. The time lags of 80% turnover of age 1+ and age 2+ lacustrine sockeye salmon were estimated at almost 40 days. These results allow us to analyze the seasonal variation of food webs and migratory patterns of lacustrine sockeye salmon, and also to apply it to trophic and migration studies of anadromous sockeye salmon in the North Pacific Ocean.

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References

Bligh, E.G. & W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917.

Brett, J.R. 1965. The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 22: 1491–1501.

Brett, J.R. 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am. Zool. 11: 99–113.

Frazer, T.K., R.M. Ross, L.B. Quetin & J.P. Montoya. 1997. Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: a stable isotope approach. J. Exp. Mar. Biol. Ecol. 212: 259–275.

Harvey, C.J., P.C. Hanson, T.E. Essington, P.B. Brown & J.F. Kitchell. 2002. Using bioenergetics models to predict stable isotope ratio in fishes. Can. J. Fish. Aquat. Sci. 59: 115–124.

Herzka, S.Z. & G.J. Holt. 2000. Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: potential applications to settlement studies. Can. J. Fish. Aquat. Sci. 57: 137–147.

Hesslein, R.H., K.A. Hallard & P. Ramlal. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by δ^{34}S, δ^{13}C, and δ^{15}N. Can. J. Fish. Aquat. Sci. 50: 2071–2076.

Hobson, K.A. & R.G. Clark. 1992. Assessing avian diets using stable isotopes I: turnover of ^{13}C tissues. Condor 94: 181–188.

Iida, K. & T. Mukai. 1995. Behavior of kokanee *Oncorhynchus nerka* in Lake Kuttara observed by echo sounder. Fish. Sci. 61: 641–646.

Keough, J.R., C.A. Hagley, E. Ruzycki & M. Sierszen. 1998. δ^{13}C composition of primary producers and role of detritus in a freshwater coastal ecosystem. Limnol. Oceanogr. 43: 734–740.

MacAvoy, S.E., S.A. Macko & G.C. Garman. 2001. Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey. Can. J. Fish. Aquat. Sci. 56: 923–932.

Maruyama, A., Y. Yamada, B. Rusuwa & M. Yuma. 2001. Change in stable nitrogen isotope ration in the muscle tissue of a migratory goby, *Rhinogobius* sp., in a natural setting. Can. J. Fish. Aquat. Sci. 58: 2125–2128.

McCarthy, I.D., E. Moksness, D.A. Pavlov & D.F. Houlihan. 1999. Effects of water temperature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*). Can. J. Fish. Aquat. Sci. 56: 231–241.

Oelbermann, K. & S. Scheu. 2002. Stable isotope enrichment (δ^{15}N and δ^{13}C) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effect of prey quality. Oecologia 130: 337–344.

O'Reilly, C.M. & R.E. Hecky. 2002. Interpreting stable isotopes in food webs: recognizing the role of time averaging at different trophic levels. Limnol. Oceanogr. 47: 306–309.

Overman, N.C. & D.L. Parrish. 2001. Stable isotope composition of walleye: δ^{15}N accumulation with age and area-specific differences in δ^{13}C. Can. J. Fish. Aquat. Sci. 58: 1253–1260.

Persson, L., S. Diehl, L. Johansson, G. Andersson & S.F. Hamrin. 1992. Trophic interactions in temperate lake ecosystems: a test of food chain theory. Am. Nat. 140: 59–84.

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

Sakano, H., S. Ban, M. Kaeriyama, H. Ueda & K. Shimazaki. 2001. The interaction between lacustrine sockeye salmon (*Oncorhynchus nerka*) and pond smelt (*Hypomesus nipponensis*) in relation to the decline of zooplankton biomass in Lake Toya, northern Japan. pp. 3881–3885. *In*: W.D. Williams (ed.), Proceedings of the 27th Congress of the International Association of Theoretical and Applied Limnology, Dublin, Ireland.

Takamura, N., H. Mikami, H. Mizutani & K. Nagasaki. 1999. Did a drastic change in fish species from kokanee to pond smelt decrease the secchi disc transparency in the oligotrophic Lake Towada, Japan? Archiv für Hydrobiologie 144: 283–304.

Tieszen, L.L., T.W. Boutton, K.G. Tesdahl & N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ^{13}C analysis of diet. Oecologia 57: 32–37.

Vander Zanden, M.J., M. Hulshof, M.S. Ridgway & J.B. Rasmussen. 1998. Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. Trans. Am. Fish. Soc. 127: 729–739.

Vander Zanden, M.J., J.M. Casselman & J.B. Rasmussen. 1999a. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401: 464–467.

Vander Zanden, M.J., B.J. Shuter, N. Lester & J.B. Rasmussen. 1999b. Patterns of food chain length in lakes: a stable isotope study. Am. Nat. 154: 406–416.

Yoshii, K., N.G. Melnik, O.A. Timoshkin, N.A. Bondarenko, P.N. Anoshko, T.Yoshioka & E. Wada. 1999. Stable isotope analyses of the pelagic food web in Lake Baikal. Limnol. Oceanogr. 44: 502–511.