Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder (*Paralichthys dentatus*)

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Abstract: Migratory and mobile fishes such as summer flounder (*Paralichthys dentatus*) often utilize dietary resources with stable isotope signatures that vary over time and space, potentially confounding diet analyses if tissues with slow turnover are sampled before reaching isotopic equilibrium. A laboratory diet-shift study was conducted using juvenile and young adult summer flounder to (*i*) determine isotopic turnover rates and fractionations of $\delta^{13}C$ and $\delta^{15}N$ in liver, whole blood, and white muscle and (*ii*) estimate the relative importance of growth and metabolic processes on isotopic turnover. Isotopic turnover rates were consistently ranked liver > blood > muscle owing to increased metabolic activities of liver and blood. Carbon and nitrogen half-lives ranged from 10 to 20 days (liver), 22 to 44 days (blood), and 49 to 107 days (muscle), indicating that liver and blood are more useful than muscle as shorter-term dietary indicators for summer flounder and other migratory fishes. Growth-based fractionation estimates of wild flounder tissues ranged from 0.71‰ to 3.27‰ for carbon and from 2.28‰ to 2.80‰ for nitrogen and included the first explicit estimates for isotopic fractionation in fish blood. A generalized model for predicting the time scale of isotopic turnover from growth-based turnover parameters was also developed to help evaluate isotopic equilibrium assumptions of fishes in the field.

Résumé : Les poissons migrateurs et mobiles, tels que le cardeau d'été (*Paralichthys dentatus*), utilisent souvent des ressources alimentaires dont les signatures d'isotopes stables varient dans le temps et l'espace, ce qui peut fausser les analyses de régime alimentaire si on échantillonne des tissus à faible taux de renouvellement avant qu'ils n'aient atteint l'équilibre isotopique. Nous avons procédé à une étude de changement de régime en laboratoire chez des juvéniles et des jeunes adultes du cardeau d'été afin (*i*) de déterminer les taux de renouvellement des isotopes et le fractionnement de $\delta^{13}C$ et de $\delta^{15}N$ dans le foie, le sang entier et le muscle blanc et (*ii*) d'estimer l'importance relative de la croissance et des taux métaboliques dans le renouvellement des isotopes. À cause des activités métaboliques plus importantes du foie et du sang, les taux de renouvellement isotopique suivent généralement l'ordre foie > sang > muscle. Les demi-vies du carbone et de l'azote varient de 10–20 jours (foie), à 22–44 jours (sang) et à 49–107 jours (muscle), ce qui indique que le foie et le sang sont de meilleurs indicateurs alimentaires à court terme que le muscle chez le cardeau d'été et les autres poissons migrateurs. Les estimations du fractionnement relié à la croissance dans les tissus de cardeaux sauvages varient de 0,71–3,27 ‰ pour le carbone et de 2,28–2,80 ‰ pour l'azote et incluent les premières estimations explicites du fractionnement isotopique dans le sang de poisson. Nous mettons au point un modèle généralisé pour prédire le taux de renouvellement isotopique pour les variables du renouvellement reliées à la croissance afin d'aider à évaluer les présuppositions concernant l'équilibre isotopique des poissons en nature.

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Introduction

Stable isotope analysis is becoming an increasingly more common tool for elucidating trophic interactions and energy pathways in food webs (Fry 2006). Stable isotopes act as dietary tracers and can be used to calculate the assimilated contributions of different prey sources to a consumer (Phillips and Gregg 2003; Fry 2006). Dietary calculations and many general conclusions from stable isotope studies are often based on two assumptions: (*i*) sampled tissues are in equilibrium with the sampled diet, and (*ii*) fractionations between the consumed food and the assimilated tissue are known (Post 2002). However, these critical assumptions are often not validated for individual field studies.

The assumption that a consumer is equilibrated isotopically to the sampled diet can be violated easily for many mobile or migratory fish species. Attainment of isotopic equilibrium depends on an animal's isotopic turnover rate and its dietary consistency. Isotopic turnover rate refers to how quickly the isotopic signature in a body tissue changes

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following a dietary change. Proper consideration of turnover rates becomes crucial in assessing diets of the many species that migrate between different coastal, estuarine, or freshwater habitats (e.g., Murdy et al. 1997; Carpenter 2002), because they move between environments with potentially variable isotopic characteristics, rely on seasonally abundant prey types, and generally exhibit ontogenetic shifts in feeding. Aside from increasing the sampling effort needed to adequately characterize prey items (a critical component of isotopic mixing models), dietary changes can prevent a predator from equilibrating isotopically to their prey because isotopic signatures of prey usually vary by species. In addition, the isotopic values of a single prey species can vary over space and time (Perga and Gerdeaux 2005), hindering equilibrium. Dietary inconsistencies (both taxonomic and isotopic) therefore become particularly problematic for migratory species and for species with slow turnover rates, potentially compromising the validity of any conclusions based on stable isotope analyses.

Increasing the temporal resolution of isotopic dietary analyses and minimizing violations of the isotopic equilibrium assumption can be achieved through examination of body tissues with faster turnover rates. Isotopic turnover is mediated by two general processes: the dilution of existing mass by new mass synthesized from recently consumed prey (i.e., growth), and the replacement or conversion of existing tissue using material synthesized from recent diet (i.e., metabolism) (Hesslein et al. 1993). Consequently, tissues that are more metabolically active tend to respond more quickly to dietary changes (Tieszen et al. 1983; Hobson and Clark 1992*a*, 1992*b*), whereas other tissues such as muscle can require over one year to equilibrate to a constant diet (Hesslein et al. 1993; MacAvoy et al. 2001). Although the number of laboratory studies examining turnover rates in fish tissues has increased over the last 10 years (e.g., MacAvoy et al. 2001; MacNeil et al. 2006), many tissues remain understudied relative to muscle. Tissue-specific turnover rates can allow researchers to tailor the sampled tissue to an appropriate time scale for the study's objective. For example, tissues with higher isotopic turnover rates can address prey utilization on shorter temporal scales (Perga and Gerdeaux 2005) or reflect the nutritional contribution of seasonally available migratory prey (MacAvoy et al. 2001). Use of faster turnover tissues may thus help clarify the interpretation of isotopic data for mobile and migratory species experiencing high spatial or temporal heterogeneity in prey isotope signatures.

Calculation of diets from stable isotope ratios of multiple tissues requires appropriate fractionation values for each tissue and isotope. Fractionation refers to the process in which ratios of stable isotopes change between prey and consumer during metabolic processing of consumed material (Fry and Sherr 1984; Fry 2006). Studies often assume fractionations of ~3.4‰ for nitrogen and 0‰–1‰ for carbon stable isotopes (Minagawa and Wada 1984; Post 2002; Sweeting et al. 2007); however, these values are based on muscle or whole-body samples. Fractionation values can vary among tissues (Hobson and Clark 1992*b*) and species (Tieszen et al.1983; Vander Zanden and Rasmussen 2001), but empirical measurements of fractionation values for fish tissues other than muscle are lacking in the literature. To our knowledge, no explicit measurements of fractionation have been published to date for fish blood, and few exist for fish liver (Trueman et al. 2005; Logan et al. 2006; Sweeting et al. 2007). Use of inaccurate fractionations can introduce substantial errors into diet estimates, particularly when the isotopic separation among prey sources is small (Vander Zanden and Rasmussen 2001). Therefore, as isotopic studies become more common and utilize a greater variety of fish tissues, it is crucial to have robust and valid measures of fractionation for the tissue of interest.

In this study, a controlled diet-shift experiment was conducted using summer flounder (*Paralichthys dentatus*), a migratory species that utilizes estuarine and coastal habitats along the eastern seaboard of North America. The objectives were to (*i*) empirically determine the turnover rates and fractionations of carbon (C) and nitrogen (N) stable isotopes in liver, whole blood, and muscle of juvenile and young adult summer flounder, and (*ii*) model the relative contributions of metabolism and growth to the process of isotopic turnover in each tissue. Results from this study facilitate more accurate application of stable isotope techniques to quantify diet and productivity of summer flounder and other migratory fishes by providing critical fractionation values and describing the dietary time scale represented by different body tissues. This study also demonstrates the generic need for more baseline information on tissue-specific turnover rates and fractionations to aid in isotopic studies of fishes, particularly those that are seasonally estuarine-dependent.

Materials and methods

Fish rearing

A group of juvenile, age-0 summer flounder (n = 72) was purchased from a commercial fish hatchery (GreatBay Aquaculture, Portsmouth, New Hampshire). These fish had been spawned from wild broodstock and raised on a similar diet of formulated feed. Hatchery fish were transported from New Hampshire to the Virginia Institute of Marine Science (VIMS) in Gloucester Point, Virginia, and allowed to acclimate to an experimental tank for 3 weeks while maintaining the previous diet. A second group of age-0 juveniles (n = 55) captured from the lower Chesapeake Bay during August and September of 2007 using a bottom trawl was maintained in captivity for 2–3 months on a constant diet of squid. The squid diet mimicked the isotopic signature of food items regularly consumed by juvenile summer flounder in Chesapeake Bay (A. Buchheister, VIMS, unpublished data) and thus maintained the wild fish at roughly the same isotopic values as at initial capture. The hatchery (H) and wild (W) fish were treated separately throughout the experiment because of the differences in their rearing histories and initial isotopic values. The H and W groups of fish were thus maintained separately in 450 gallon recirculating tanks with water filtered to 1 μm obtained from the York River, a tributary of the Chesapeake Bay. Average (± standard deviation, SD) tank temperatures (W group, 19.9 ± 0.7 °C; H group, 20.4 ± 0.7 °C) and salinities (26.1 ± 0.9 ppt) represented intermediate values found within bottom waters of Chesapeake Bay from May to November (Chesapeake Bay Program 2007). Fish were kept on a 13 h light – 11 h dark schedule.

Two days prior to the start of the diet-shift experiment in early November of 2007, all individuals were marked with unique coded wire tags (Northwest Marine Technology, Shaw Island, Washington) inserted into the blind-side musculature and measured for total length (mm) and weight (mg) (Table 1). The minute tags did not appear to affect fish behavior, and a minimal proportion of fish (2.6%) did not retain their tags (likely due to insertion error), making the coded wire tags ideal for the study. At the start of the experiment, seven fish were sacrificed from each experimental group to obtain baseline isotopic signatures for muscle, liver, and blood. The remaining fish were all switched to a constant diet of commercially purchased krill, *Euphausia superba* (Fishalicious Foods, Custer, Washington), which had substantially different isotopic signatures from both summer flounder groups and their previous diets. Fish were fed up to 5% of their body weight per day in an attempt to replicate realistic growth rates of fish in the wild (Rountree and Able 1992).

To monitor turnover of stable isotopes, four to six individuals from each group were randomly sampled and euthanized at specified time periods. Towards the end of the experiment, up to 10 individuals were sampled to use all animals. All protocols pertaining to sampling and euthanizing of experimental fish were approved by the College of William and Mary's Institutional Animal Care and Use Committee. Fish were sampled at 7, 13, 24, 35, 49, 67, 89, 126, 155, and 180 days from the start of the experiment. Three samples of the krill diet were periodically sampled to assure consistency in the dietary isotopic signature.

Processing of sampled fish

Summer flounder selected for isotopic determination were removed from the water at least 12 h after the previous feeding and measured for total length (mm) and weight (mg). Samples of whole blood (0.5–1 mL) were extracted from the caudal vein using a 25-gauge needle and sterile syringe and then stored in a precombusted glass vial. Fish were then placed in Ziploc bags (S.C. Johnson & Son Inc., Racine, Wisconsin) and euthanized in an ice-water bath. In the laboratory, samples of liver and dorsal muscle (above the pectoral fin on the ocular side) were excised, rinsed with deionized water, and placed in precombusted glass vials. Coded wire tags were recovered from the musculature of each fish to identify individuals. Tissue samples were dried (45 °C for at least 2 days) and ground and then a 0.8–1.2 mg subsample was packaged into a 4 mm × 6 mm tin capsule for stable isotope analysis.

Prepared samples were analyzed at the University of California – Davis Stable Isotope Facility (Davis, California) using a continuous flow isotope ratio mass spectrometer (Europa Hydra 20/20; Europa Scientific, Cambridge, UK). Stable isotope ratios are reported in relation to conventional standards:

$$(1) \qquad \delta X = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \times 1000$$

where X is the stable isotope of C or N, and R is the mass ratio of the heavy to light stable isotope (e.g., $^{15}N/^{14}N$) for either the sample or the standard. The conventional standards used for the analyses were Pee Dee Belmnite for C and air for N, and δX is reported in per mil (‰). Repeated measurements of a calibration standard indicated that instrument precision (SD) was 0.15‰ and 0.08‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Ratios of C to N (C:N) were calculated from measured amounts of C and N in each sample.

Lipid correction for livers

A mathematical correction was used to normalize liver $\delta^{13}C$ values due to the high lipid concentrations in this tissue that bias $\delta^{13}C$ (DeNiro and Epstein 1977; Logan et al. 2008). A subsample of livers was used to characterize the relationship between raw C:N and the bias in $\delta^{13}C$ due to lipids by measuring the isotopic value before ($\delta^{13}C_{NE}$) and after ($\delta^{13}C_{LE}$) lipid extraction (Appendix A). Of a suite of models, the model by McConnaughey and McRoy (1979) best represented the data (Appendix A), and consequently the lipid bias of $\delta^{13}C$ was modeled as

$$(2) \qquad \delta^{13}C_{LE} - \delta^{13}C_{NE} = D\left(\theta + \frac{3.90}{1 + 287/L}\right)$$

$$\text{where} \quad L = \frac{93}{1 + (0.246 \times \text{C:N} - 0.775)^{-1}}$$

In eq. 2, L represents the sample lipid content as a function of C:N, and D represents protein–lipid discrimination. The parameters D and θ were estimated (± standard error, SE) as $D = 6.2883 \pm 1.3519$ and $\theta = 0.0612 \pm 0.1527$. Based on measured C:N, eq. 2 was used to adjust all raw liver $\delta^{13}C$ to values corresponding to a lipid-extracted $\delta^{13}C$. Lipid corrections were not applied to muscle and blood $\delta^{13}C$ values because of relatively low C:N values and limited variability across individuals (mean C:N ± SD: muscle, 3.40 ± 0.13; blood, 4.12 ± 0.39; liver, 14.54 ± 4.68), which suggests that any lipid effects on $\delta^{13}C$ would be negligible in these tissues (Kiljunen et al. 2006).

Growth-based modeling

Changes in stable isotope ratios were modeled as a function of relative growth since the diet switch. The growth-based model predicts the isotopic value of a fish as

$$(3) \qquad \delta_{W_R} = \delta_f + (\delta_i - \delta_f) W_R{}^c$$

(Fry and Arnold 1982), where δ_f is the expected isotopic value when completely equilibrated to the new diet and δ_i is the initial isotopic signature prior to the diet shift. The average isotopic value for the seven fish sacrificed before the diet shift was used as the estimate for δ_i in the model. The relative increase in weight of each fish (W_R) was calculated as the final wet weight divided by the initial wet weight. The variable δ_{W_R} is the measured isotopic value for a fish given its increase in weight, and c is a turnover rate constant. Both δ_f and c were estimated using nonlinear regression (SAS version 9.2; SAS Institute Inc., Cary, North Carolina). In this model, if $c = -1$, then growth is entirely responsible for turnover, whereas if $c < -1$, metabolism is contributing to turnover of the stable isotope in the tissue, with more negative values representing greater contributions by metabolism (Fry and Arnold 1982). A t test was used to test c against a null hypothesis of $c = -1$ (Zar 1999).

Fractionations of $\delta^{15}N$ and $\delta^{13}C$ between the diet and each tissue (Δ_{TISSUE}) were calculated as

Table 1. Initial total length and wet weight of hatchery (H) and wild (W) summer flounder (*Paralichthys dentatus*) sampled for stable isotope analysis.

		Total length (mm)				Wet weight (g)			
Group	*n*	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
H	66	176	19	130	230	58.8	18.3	26.3	124.9
W	48	215	25	131	255	102.0	33.2	29.3	185.3

Note: SD, standard deviation; min., minimum; max. maximum.

$$\Delta_{\text{TISSUE}} = \delta_{\text{f}} - \delta_{\text{d}} \quad (4)$$

(Minagawa and Wada 1984), where δ_{d} is the mean stable isotope signature for samples of krill diet. Standard errors for the fractionation estimate were based on errors associated with the model estimate of δ_{f} ($\text{SE}_{\delta_{\text{f}}}$) and the standard error of the mean for δ_{d} ($\text{SE}_{\delta_{\text{d}}}$):

$$\text{SE}_{\Delta_{\text{TISSUE}}} = \sqrt{\text{SE}_{\delta_{\text{f}}}{}^2 + \text{SE}_{\delta_{\text{d}}}{}^2} \quad (5)$$

The amount of relative growth needed to achieve an α percent turnover of δ^{13}C or δ^{15}N ($G_{\alpha/100}$) was calculated as

$$G_{\alpha/100} = \exp\left(\frac{\ln(1 - \alpha/100)}{c}\right) \quad (6)$$

The growth-based half-life ($G_{0.5}$) is obtained when α = 50% and represents the amount of growth needed for a 50% conversion between the initial and final isotopic values. The fractions of new tissue derived from growth (D_{g}) and from metabolism (D_{m}) were calculated at the midpoint between the old and new isotopic values (Witting et al. 2004):

$$D_{\text{g}} = \frac{2(G_{0.5} - 1)}{G_{0.5}} \quad (7)$$

$$D_{\text{m}} = \frac{(2 - G_{0.5})}{G_{0.5}} \quad (8)$$

However, note that the equations originally published by Witting et al. (2004) were misprinted (D.A. Witting, NOAA Fisheries Restoration Center, 501 West Ocean Boulevard, Long Beach, CA 90802, USA, personal communication, 2008).

Time-based modeling

Changes in stable isotope ratios were also modeled as a function of time since the diet switch. The time-based exponential model (Hesslein et al. 1993) describes the isotope value of a fish at time t (δ_t) as

$$\delta_t = \delta_{\text{f}} + (\delta_{\text{i}} - \delta_{\text{f}})\text{e}^{-(k' + m)t} \quad (9)$$

where δ_{f} and δ_{i} are as previously defined and m is the metabolic turnover constant. The group-specific growth rate constant, k', was estimated by fitting an exponential growth model to the available data:

$$W_{\text{f}} = W_{\text{i}}\,\text{e}^{k't} \quad (10)$$

(Ricker 1979), where W_{i} is a fish's initial wet weight and W_{f} is the final weight when sampled on day t. Because of different initial sizes and different growth rates between groups, the k' parameter was estimated separately for the wild and hatchery groups using nonlinear regression (SAS version 9.2; SAS Institute Inc., Cary, North Carolina). In eq. 9, if turnover is due to growth alone, then m = 0. The values of δ_t, δ_{i}, and t were measured or calculated, and δ_{f} and m were estimated using nonlinear regression.

Diet–tissue fractionations (±SE) were also derived from time-based model estimates of δ_{f} using eqs. 4 and 5. The length of time needed to achieve an α percent turnover of δ^{13}C or δ^{15}N was calculated as

$$T_{\alpha/100} = \frac{\ln(1 - \alpha/100)}{-(k' + m)} \quad (11)$$

(Tieszen et al. 1983). Half-life ($T_{0.5}$) is obtained when α = 50%.

The relative contributions of growth (k') and metabolism (m) were calculated as the ratio of each parameter to the sum of the two parameters. This calculation yields the proportion of turnover attributable to growth (P_{g}) and to metabolism (P_{m}). A t test was used to test m against a null hypothesis of m = 0 (Zar 1999). To assess growth patterns of the wild and hatchery populations over the duration of the experiment, specific growth rates (k) were calculated for each individual using the exponential growth model, eq. 10, by substituting k for k' (Ricker 1979).

Model fitting and selection

An information-theoretic approach to model selection was used to assess the best parameterizations for the growth- and time-based models. Given the use of two experimental groups (hatchery and wild fish), we were interested in determining whether the groups were best parameterized separately (i.e., group-specific c or m, or group-specific δ_{f}), or whether the data could be pooled and a single parameter estimate deemed valid for both groups. Additionally, models could be parameterized to either include or exclude a metabolic contribution to turnover to examine the relative importance of metabolism on the turnover process in each tissue. Six potential models were generated as follows: model A, group-specific turnover parameter and group-specific δ_{f}; model B, group-specific turnover and a single pooled δ_{f} estimate; model C, single turnover parameter and group-specific δ_{f}; model D, single turnover parameter and a single δ_{f}; model E, no metabolic contribution to turnover (i.e., c = –1 in eq. 3 or m = 0 in eq. 9) and group-specific δ_{f}; and lastly model F, no metabolic contribution to turnover and a single δ_{f} estimate. These six model formulations were fitted to each isotope–tissue combination and assessed separately for the growth- and time-based methods.

Objective evaluations of the best model parameterization were based on Akaike's information criterion corrected for small sample sizes (AIC_{c}) calculated for each model as

$$\text{AIC}_c = n\left(1 + \ln\left(\frac{2\pi \times \text{RSS}}{n}\right)\right) + 2K + \frac{2K(K+1)}{n-K-1} \quad (12)$$

where n is the sample size, RSS is the residual sum of squares calculated using nonlinear regression (SAS version 9.2; SAS Institute Inc., Cary, North Carolina), and K is the number of estimable parameters in the model (Burnham and Anderson 2002; Kimura 2008). AIC_c differences (ΔAIC_c) among the six models were calculated as

$$\Delta\text{AIC}_c = \text{AIC}_i - \text{AIC}_{\min} \quad (13)$$

where AIC_i is the AIC_c of model i, and $\text{AIC}_{\min}$ is the lowest AIC_c of the competing models. The model with the most empirical support generates a $\Delta\text{AIC}_c = 0$, and we only present parameter estimates for the best of the six models. As a general rule of thumb, ΔAIC_c values from 0 to 2 indicate substantial support for the model, ΔAIC_c values from 4 to 7 suggest considerably less support, and ΔAIC_c values >10 indicate essentially no support for that model (Burnham and Anderson 2002).

For each isotope and tissue, regression assumptions of normality were supported by histograms of the residuals and by Kolmogorov–Smirnoff tests conducted at different levels of the independent variable. Levene's tests and residual plots indicated that homogeneity of variance was not supported for the growth- and time-based models of δ^{15}N for muscle and liver or for δ^{13}C turnover in liver. These models were weighted by the inverse of the variance of the response variable to account for the heteroscedasticity in the estimation and fitting procedures (Neter et al. 1990). Because of heteroscedasticity, eq. 10 was also weighted using the inverse of the W_f variance (calculated at each time period) as the weighting factor (Neter et al. 1990).

Results

Growth

Summer flounder grew well, reaching lengths > 300 mm and weights > 375 g during the 180-day experiment (maximum length and weight of 325 mm and 446.0 g, respectively). Several hatchery individuals more than quadrupled in weight (Fig. 1). Some individuals died after jumping out of the tanks, but a total of 66 hatchery fish and 51 wild fish were sampled during the experiment. Most fish sampled after 60 days of the experiment appeared to have reached sexual maturity based on macroscopic staging; however, no fish were reproductively active.

Specific growth rates (k) varied across individuals, across time, and between groups (Fig. 2). Overall, individual growth rates ranged from –0.010 to 0.024 day^{-1}, but only three fish experienced negative growth rates, or a loss of weight. Hatchery fish tended to grow more rapidly than wild fish, exhibiting the highest k values, particularly in the first 50 days of the experiment. Group-specific growth rates (k') reflected this difference between groups with k' values (±SE) of $8.16 \times 10^{-3} \pm 1.65 \times 10^{-4}$ day^{-1} (H group) and $6.51 \times 10^{-3} \pm 2.51 \times 10^{-4}$ day^{-1} (W group). Growth rates increased initially in both groups before leveling off and then decreasing slightly towards the end of the experiment.

Fig. 1. Relative growth (W_R) for individual summer flounder (*Paralichthys dentatus*) from both hatchery (open triangles) and wild (solid circles) groups. Estimated growth trajectories for each group were based on group-specific growth rates, k' (hatchery group, broken line; wild group, continuous line).

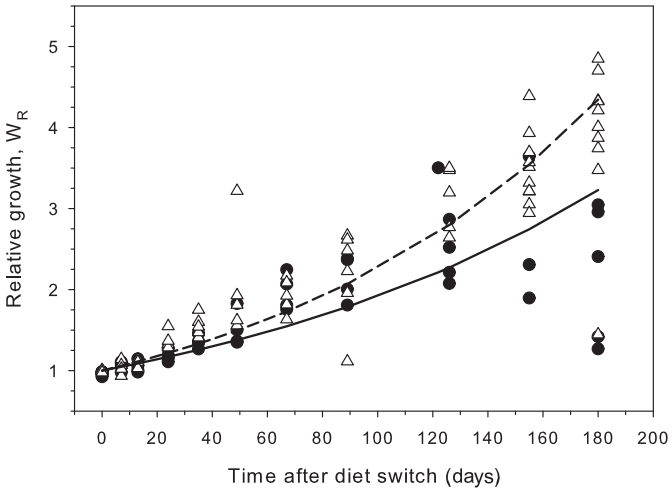
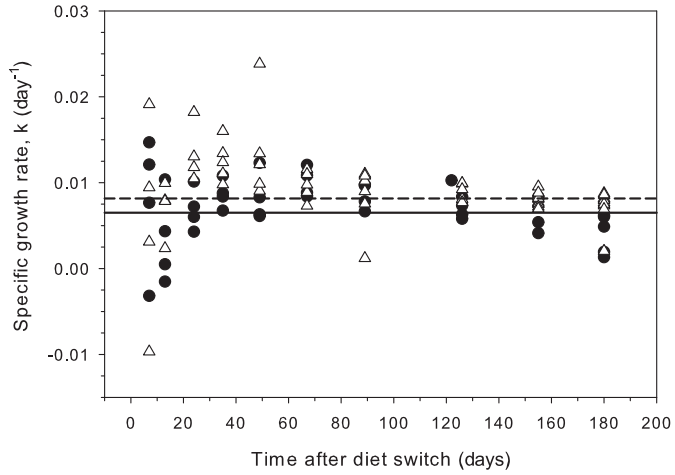


Fig. 2. Specific growth rates (k) calculated for individual summer flounder (*Paralichthys dentatus*) for both hatchery (open triangles) and wild (solid circles) groups. Estimated group-specific growth rates (k') for hatchery (8.16×10^{-3} day^{-1}) and wild (6.51×10^{-3} day^{-1}) groups are represented by broken and continuous lines, respectively.



The decrease in growth rates was particularly evident in the W_R trends of wild fish over time (Fig. 1). Declines in individual growth rates corresponded with reduced consumption rates that were observed during the second half of the experiment, particularly for the wild fish.

Model selection

The best model parameterization (models A–F) varied for each method (growth- vs. time-based), isotope, and tissue combination. For growth-based modeling, liver and blood turnover were best described with separate c and δ_f parameters for hatchery and wild groups (model A; Table 2). Muscle δ^{13}C and δ^{15}N turnover was better parameterized with a single δ_f estimated for both groups; however, for the δ^{13}C model, c was estimated for each group individually (model B), whereas δ^{15}N was best modeled with $c = -1$

Table 2. Comparisons of ΔAIC_c among six turnover model parameterizations that treat hatchery and wild fish groups either separately or combined for each parameter and either allow for a metabolic contribution to turnover or not.

				ΔAIC_c					
Method	Isotope	Tissue	*n*	Model A	Model B	Model C	Model D	Model E	Model F
Growth-based	$\delta^{13}C$	Liver	98	**0.0**	5.4	28.6	31.4	242.6	274.6
		Blood	99	**0.0**	3.1	6.7	4.8	52.9	55.6
		Muscle	99	1.2	**0.0**	0.6	1.9	6.0	4.7
	$\delta^{15}N$	Liver	98	**0.0**	32.4	1.7	30.2	231.5	238.4
		Blood	99	**0.0**	4.3	7.7	6.0	21.6	53.8
		Muscle	99	4.6	2.7	2.9	1.1	0.7	**0.0**
Time-based	$\delta^{13}C$	Liver	101	0.7	**0.0**	9.9	8.1	270.6	273.9
		Blood	103	1.8	2.1	0.3	**0.0**	94.1	93.3
		Muscle	103	**0.0**	5.7	2.4	5.5	11.7	9.9
	$\delta^{15}N$	Liver	101	1.5	27.7	**0.0**	40.0	270.9	317.1
		Blood	103	**0.0**	12.6	1.0	13.6	54.8	60.9
		Muscle	102	2.6	1.4	0.5	1.1	2.1	**0.0**

Note: Models were fitted using the growth- and time-based methods for each isotope, tissue, and group. Brief model descriptions (see text for full details): A, group-specific turnover parameter (*c* or *m*), group-specific δ_f; B, group-specific turnover, single δ_f; C, single *c* or *m*, group-specific δ_f; D, single *c* or *m*, single δ_f; E, no metabolic contribution to turnover ($c = -1$ or $m = 0$), group-specific δ_f; and F, no metabolic contribution to turnover, single δ_f. The best model for each comparison is indicated in bold type.

(model F). The best parameterizations for time-based modeling were inconsistent across isotope–tissue combinations, with models A, B, C, D, and F selected as the best model at least once (Table 2). All subsequent parameter estimates and model fits presented were based on the best parameterizations identified in Table 2 using ΔAIC_c.

Turnover and fractionation of $\delta^{13}C$

Isotopic turnover of $\delta^{13}C$ in summer flounder tissues was clearly evident over the duration of the experiment and well represented by both growth- and time-based models (Figs. 3, 4). Initial $\delta^{13}C$ values of hatchery and wild fish were similar and approximately 10‰ removed from the mean initial isotopic signature of the krill diet (Table 3). For a given group, turnover was most rapid in liver followed by blood then muscle, as indicated by the more negative values of *c* (growth-based model; Table 4) and higher values of *m* (time-based model; Table 5). However, the magnitude of these parameters for liver and blood were similar, translating into small differences in isotopic trajectories by growth (Figs. 3*a*, 3*b*) or over time (Figs. 4*a*, 4*b*).

Half-lives for $\delta^{13}C$ in liver, blood, and muscle were estimated as 20, 22, and 69 days for hatchery fish and 17, 23, and 49 days for wild fish, respectively (Table 5). A 95% isotopic turnover in liver and blood could occur in as little as 72 or 94 days, whereas 95% turnover in muscle could require up to 298 days. In terms of growth, hatchery fish attained 50% turnover in liver, blood, and muscle after growing 1.3, 1.4, and 2.7 times their initial size, respectively (Table 4). Wild fish needed to grow 1.1, 1.2, and 2.5 times their initial size to attain 50% turnover in liver, blood, and muscle, respectively.

For blood and liver, estimates of the asymptotic isotopic value, δ_f, were relatively consistent between modeling methods and between experimental groups. Differences in δ_f estimates between the time-based and growth-based models were relatively small (<0.8‰) for blood and liver, and H and W groups differed by less than 1‰ (Tables 4, 5). Unlike liver and blood, there was a higher degree of variability in δ_f estimates for muscle, with larger differences up to ~4‰ between estimates. The uncertainty in these estimates was reflected in the higher SE of these parameters.

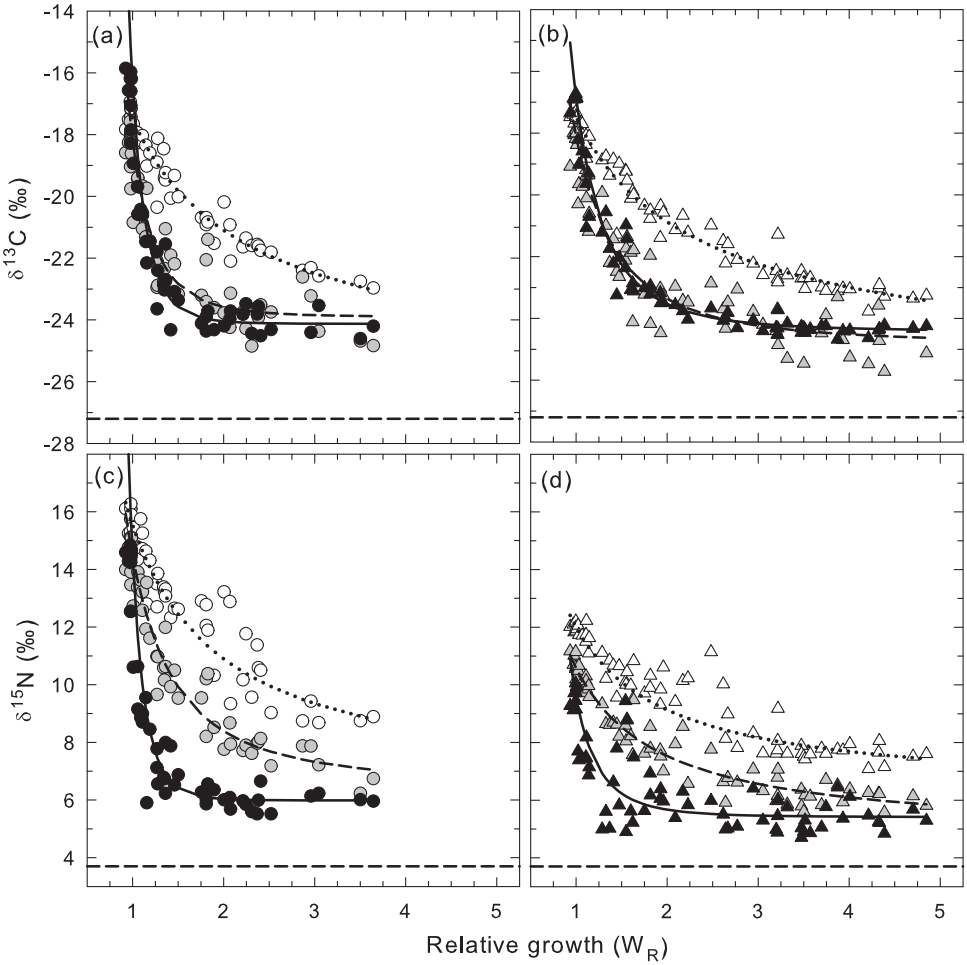
Fractionation estimates for $\delta^{13}C$ varied by tissue, group, and the model used. Liver fractionation values were the most consistent, ranging from 2.76 to 3.05‰ (Tables 4, 5). Fractionation estimates for blood ranged from 2.34‰ to 3.27‰, whereas muscle fractionation varied from 0.71‰ to 4.79‰.

Turnover and fractionation of $\delta^{15}N$

Isotopic turnover of $\delta^{15}N$ in summer flounder tissues was also well represented by growth- and time-based models. Hatchery fish exhibited more depleted initial $\delta^{15}N$ values than wild fish due to the different initial diets of each group; initial values were between 5.9‰ and 11.9‰ more $\delta^{15}N$-enriched than the krill diet (Table 3). Turnover rates of $\delta^{15}N$ reflected the same general patterns seen for $\delta^{13}C$, with liver rates being the fastest (Figs. 3*c*, 3*d*, 4*c*, 4*d*). Values for *m* were more than four times greater for the liver than for the other tissues (Table 5). Half-life was about 10 days for liver compared with approximately 38 and 96 days for blood and muscle, respectively. Growth-based estimates of *c* also indicated a much more rapid turnover in liver, such that a 95% turnover to the new krill diet could occur after an approximate doubling in weight (Table 4). Turnover rates of $\delta^{15}N$ for blood were intermediate between liver and muscle, with muscle consistently responding the slowest to the dietary change. Muscle required over 367 days (Table 5) or over a 20-fold increase in weight (Table 4) to attain 95% of $\delta^{15}N$ turnover, given the observed growth rates.

Estimates of liver δ_f and muscle δ_f were similar across methods, whereas estimates of blood δ_f were less consistent (Tables 4, 5). For example, blood δ_f differences between W and H groups were approximately 1.6‰. Again, the magnitude of the difference between group or method estimates of δ_f seemed to be related to the SE of the parameter estimates.

Fig. 3. Changes in $\delta^{13}C$ and $\delta^{15}N$ of summer flounder (*Paralichthys dentatus*) tissues as a function of relative growth (W_R) after a diet switch. (*a*, *c*) Circles denote data for wild fish; (*b*, *d*) triangles represent hatchery fish. Data and growth-based model fits are shown for muscle (open symbols, dotted line), blood (shaded symbols, broken line), and liver (solid symbols, continuous line). The horizontal broken line indicates the mean value for the krill diet.



Depending on the method used and the experimental group, fractionation estimates varied from 1.45‰ to 2.28‰ (liver), 1.11‰ to 3.86‰ (blood), and 2.13‰ to 2.53‰ (muscle) (Tables 4, 5). Similar to carbon, fractionations of $\delta^{15}N$ also exhibited a group-related trend, with higher estimates for the wild group due to consistently higher δ_f values of wild fish relative to the hatchery group.

Metabolic contributions to turnover

Metabolism played an important role in isotopic turnover in liver and blood. For liver and blood, models that were parameterized to include metabolism (models A–D) were more supported by the data, yielding the lowest ΔAIC_c values (Table 2). The very large ΔAIC_c of the competing models with no metabolism (models E and F) suggest that there is practically no evidence that growth is solely responsible for isotopic turnover in blood and liver. The importance of metabolic processes to turnover in these tissues was further indicated by the significant difference of most m and c estimates from 0 or –1, respectively (Tables 4, 5). According to the growth-based model, metabolism accounted for over 46% of $\delta^{13}C$ turnover in blood, whereas metabolic contributions reached 80% for liver $\delta^{13}C$ turnover (Table 4). Metabolic contributions to $\delta^{15}N$ turnover remained comparable, except for blood of hatchery fish, which appeared to be predominantly growth-driven. Time-based models generated slightly greater estimates of metabolic contributions to isotopic turnover in blood and liver, with values generally over 69% (Table 5).

In contrast to liver and blood, isotopic turnover of muscle was primarily driven by the growth-related process of dilution. Turnover of muscle $\delta^{15}N$ was best parameterized without a metabolic contribution (model F; Table 2), with growth processes alone able to account for isotopic turnover (D_g = 1 (Table 4); P_g = 1 (Table 5)). Results for muscle $\delta^{13}C$ were less conclusive, although there was some support for growth-based parameterizations that exclude a metabolic component ($\Delta AIC_c < 6.0$ for models E and F; Table 2). Estimates of c for muscle $\delta^{13}C$ turnover were greater than –1 (Table 4), suggesting that turnover occurred more slowly than expected from growth, opposite to the effect that muscle metabolism would have. Time-based estimates of m were not significantly different from 0 for hatchery fish, and metabolism accounted for only 19% of turnover (Table 5), supporting the predominant role of growth in turnover of muscle $\delta^{13}C$.

Table 3. Mean initial isotopic values and standard deviations (SD) for tissues of hatchery (H) and wild (W) summer flounder (*Paralichthys dentatus*) and for the experimental krill diet.

		$\delta^{13}C$		$\delta^{15}N$	
Group	Tissue	Mean	SD	Mean	SD
H	Liver	–16.84	0.07	9.59	0.36
	Blood	–18.08	0.16	10.60	0.42
	Muscle	–17.48	0.12	12.01	0.15
W	Liver	–16.35	0.42	14.51	0.21
	Blood	–18.16	0.38	14.49	0.60
	Muscle	–17.48	0.37	15.55	0.57
Krill	Whole	–27.18	0.87	3.71	0.48

Discussion

Turnover and fractionation in tissues

Differences in turnover rates among the three tissues were driven by variable metabolic contributions to the turnover process. The metabolic parameters in the turnover models technically incorporate any and all turnover processes aside from growth, but there is a good physiological basis for greater metabolic turnover in blood and liver. For example, protein synthesis and degradation rates in fish livers can be one or two orders of magnitude greater than in muscle tissues that are broken down less regularly (Smith 1981; Houlihan et al. 1988; de la Higuera et al. 1999). In ginbuna crucian carp, red blood cells exhibited half-lives of ~51 days, which is slightly longer than our estimates for blood $\delta^{15}N$ turnover; however, half-lives of other blood components (white blood cells and platelets) had half-lives of <5 days (Fischer et al. 1998) and would contribute to reducing turnover in whole blood. In birds, blood plasma proteins, synthesized in the liver, have high turnover rates that translate into short half-lives of isotopes relative to muscle (Hobson and Clark 1992*a*; Hobson and Clark 1993). Some studies demonstrated little or no difference in isotopic turnover rates among fish tissues (e.g., Hesslein et al. 1993; Sweeting et al. 2005; McIntyre and Flecker 2006), but limited sample sizes may have reduced the power to detect differences in some instances. Other studies of fishes support our findings of faster liver and blood turnover relative to muscle (e.g., Suzuki et al. 2005; Logan et al. 2006; MacNeil et al. 2006; Table 6). Metabolic contributions to isotopic turnover in our study reached 79% and 91% for blood and liver, respectively, whereas turnover in muscle was predominantly mediated by growth and simple dilution as shown for most fishes (e.g., Hesslein et al. 1993; Herzka and Holt 2000). Interestingly, metabolic contributions for C turnover in blood were greater than for N turnover, reflecting different turnover rates between the isotopes. Other studies have also documented faster turnover of C relative to N in some tissues (MacAvoy et al. 2001; Suzuki et al. 2005; Guelinckx et al. 2007), likely due to different catabolic processes operating on the various biochemical constituents of tissues. Despite this, AIC and model selection techniques showed very strong support for the role of metabolism in isotopic turnover of both liver and blood, unlike muscle, and this analytical tool provides an objective means to evaluate importance of metabolism to isotopic turnover.

Trophic fractionation between prey and consumer has

Table 4. Parameter estimates (standard error (SE) in parentheses) and calculations from growth-based models of $\delta^{13}C$ and $\delta^{15}N$ turnover in summer flounder (*Paralichthys dentatus*) tissues.

Isotope	Tissue	Model	Group	c (SE)	δ_f (SE)	Δ (SE)	$G_{0.5}$	$G_{0.95}$	D_g	D_m	F_g
$\delta^{13}C$	Liver	A	H	–3.095* (0.158)	–24.42 (0.04)	2.76 (0.09)	1.3	2.6	0.40	0.60	0.99
			W	–6.532* (0.879)	–24.13 (0.09)	3.05 (0.12)	1.1	1.6	0.20	0.80	1.00
	Blood	A	H	–2.204* (0.282)	–24.84 (0.29)	2.34 (0.30)	1.4	3.9	0.54	0.46	0.97
			W	–4.123* (0.624)	–23.91 (0.26)	3.27 (0.28)	1.2	2.1	0.31	0.69	1.00
	Muscle	B	H	–0.686* (0.103)	—	—	2.7	79.0	1.27	–0.27	0.66
			W	–0.744* (0.104)	—	—	2.5	56.0	1.21	–0.21	0.62
			C	—	–26.47 (0.89)	0.71 (0.89)	—	—	—	—	—
$\delta^{15}N$	Liver	A	H	–4.023* (0.824)	5.41 (0.10)	1.70 (0.11)	1.2	2.1	0.32	0.68	1.00
			W	–7.319* (0.998)	5.99 (0.02)	2.28 (0.05)	1.1	1.5	0.18	0.82	1.00
	Blood	A	H	–1.093 (0.206)	4.82 (0.58)	1.11 (0.58)	1.9	15.5	0.94	0.06	0.82
			W	–2.085* (0.227)	6.51 (0.39)	2.80 (0.39)	1.4	4.2	0.57	0.43	0.93
	Muscle	F	H	—	—	—	—	—	—	—	0.79
			W	—	—	—	—	—	—	—	0.73
			C	–1.000	6.24 (0.04)	2.53 (0.06)	2.0	20.0	1.00	0.00	—

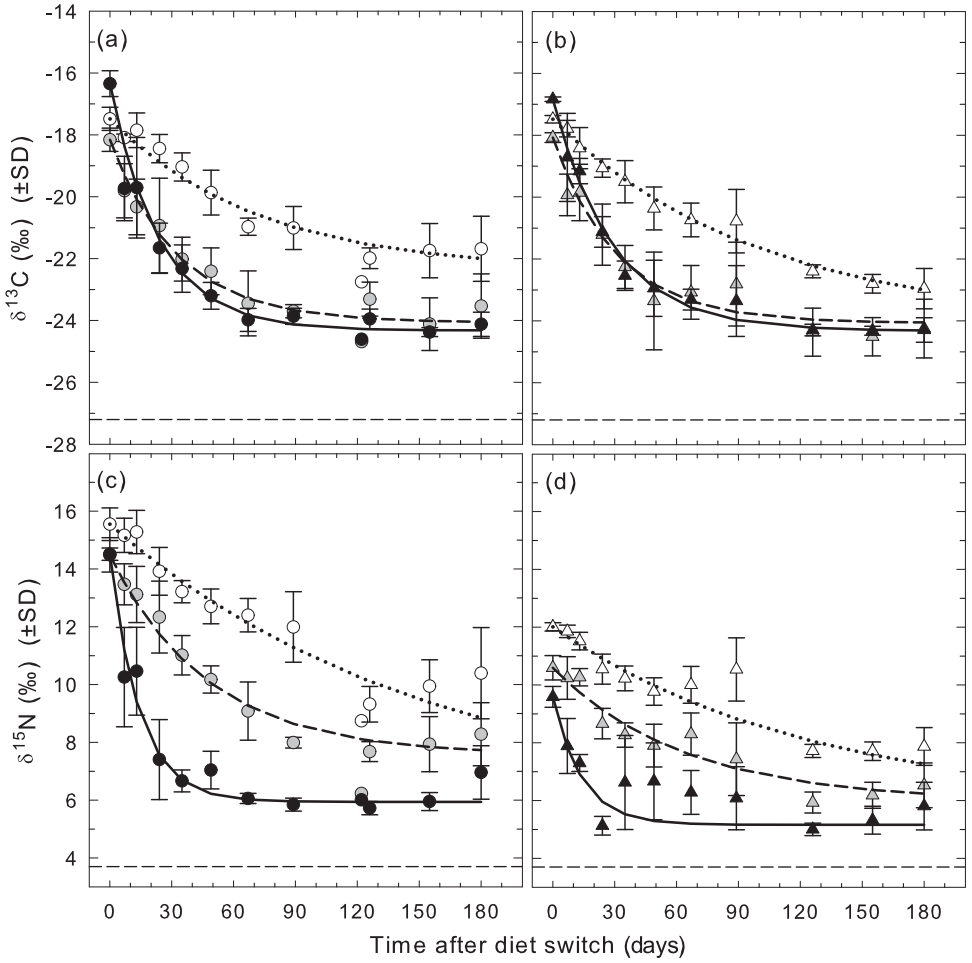
Note: See text for descriptions of models A–F. Abbreviations and parameter estimates: H, hatchery fish; W, wild fish; C, hatchery and wild fish combined; c, turnover constant; δ_f, final asymptotic isotopic value (‰); Δ, fractionation between krill diet and each tissue (‰); $G_{0.5}$, growth-based half-life; $G_{0.95}$, amount of relative growth needed to reach 95% turnover; D_g, proportion of turnover attributed to growth; D_m, proportion of turnover attributed to metabolism; F_g, fraction of complete turnover attained by the end of the experiment based on growth. Turnover constants that are significantly different from –1 ($p < 0.05$) are marked with an asterisk (*).

Table 5. Parameter estimates (standard error (SE) in parentheses) and calculations from the time-based model of $\delta^{13}C$ and $\delta^{15}N$ turnover in summer flounder (*Paralichthys dentatus*) tissues.

Isotope	Tissue	Model	Group	m (SE)	δ_f (SE)	Δ (SE)	k'	$T_{0.5}$	$T_{0.95}$	P_g	P_m	F_t
$\delta^{13}C$	Liver	B	H	0.0261* (0.002)	—	—	0.00816	20.2	87.5	0.24	0.76	1.00
			W	0.0352* (0.003)	—	—	0.00651	16.6	71.8	0.16	0.84	1.00
			C	—	–24.32 (0.04)	2.86 (0.09)	—	—	—	—	—	—
	Blood	D	H	—	—	—	0.00816	21.6	93.5	0.25	0.75	1.00
			W	—	—	—	0.00651	22.8	98.5	0.21	0.79	1.00
			C	0.0239* (0.003)	–24.07 (0.15)	3.11 (0.17)	—	—	—	—	—	—
	Muscle	A	H	0.0019 (0.002)	–24.08 (0.50)	3.10 (0.51)	0.00816	68.9	297.9	0.81	0.19	0.84
			W	0.0075* (0.002)	–22.39 (0.35)	4.79 (0.36)	0.00651	49.4	213.4	0.46	0.54	0.92
$\delta^{15}N$	Liver	C	H	—	5.16 (0.09)	1.45 (0.10)	0.00816	9.7	42.0	0.11	0.89	1.00
			W	—	5.94 (0.08)	2.23 (0.09)	0.00651	9.9	43.0	0.09	0.91	1.00
			C	0.0632* (0.005)	—	—	—	—	—	—	—	—
	Blood	A	H	0.0076* (0.003)	5.97 (0.32)	2.26 (0.32)	0.00816	43.8	189.5	0.52	0.48	0.94
			W	0.0145* (0.003)	7.57 (0.28)	3.86 (0.29)	0.00651	33.0	142.7	0.31	0.69	0.98
	Muscle	F	H	—	—	—	0.00816	84.9	367.1	1.00	0.00	0.77
			W	—	—	—	0.00651	106.5	460.2	1.00	0.00	0.69
			C	0.0000	5.84 (0.11)	2.13 (0.12)	—	—	—	—	—	—

Note: See text for descriptions of models A–F. Abbreviations and parameter estimates: H, hatchery fish; W, wild fish; C, hatchery and wild fish combined; m, metabolic turnover constant (day^{-1}); δ_f, final asymptotic isotopic value (‰); Δ, fractionation between krill diet and each tissue (‰); k', group-specific growth rate (day^{-1}); $T_{0.5}$, time-based half-life (days); $T_{0.95}$, time needed to reach 95% turnover (days); P_g, proportion of turnover attributed to growth; P_m, proportion of turnover attributed to metabolism; F_t, fraction of complete turnover attained by the end of the experiment based on time. Metabolic turnover constants that are significantly different from 0 ($p < 0.05$) are marked with an asterisk (*).

Fig. 4. Changes in mean $\delta^{13}C$ and $\delta^{15}N$ of summer flounder (*Paralichthys dentatus*) tissues as a function of time (days) since the diet switch. (*a*, *c*) Circles denote data for wild fish; (*b*, *d*) triangles represent hatchery fish. Data (mean ± standard deviation, SD) and time-based model fits are shown for muscle (open symbols, dotted line), blood (shaded symbols, broken line), and liver (solid symbols, continuous line). The horizontal broken line indicates the mean for the krill diet.



been mostly examined for muscle, and the muscle results from this experiment were generally consistent with previous work. Our muscle fractionation estimates of $\Delta^{15}N$ (2.13‰–2.53‰) were less than the intraspecific mean value of 3.4‰ (Vander Zanden and Rasmussen 2001; Post 2002) but located well within a wide literature range ($\Delta^{15}N$, ~–1‰ to 6‰; McCutchan et al. 2003). Our muscle estimates of $\Delta^{13}C$ (0.71‰–4.79‰) also conformed to the broad bounds of published fractionations ($\Delta^{13}C$, –3‰ to 4‰), but time-based estimates were more enriched than the traditionally assumed values of 0‰–1‰ (Post 2002; McCutchan et al. 2003). This discrepancy in $\Delta^{13}C$ may be largely explained by the muscle only reaching 62%–92% of equilibrium by the end of the experiment. With isotopic values not equilibrated to the diet, model estimates of δ_f were associated with relatively large standard errors that translated into reduced precision of muscle fractionation estimates. Additionally, different model parameterizations (models A–F) yielded plausible results, suggesting that the muscle $\Delta^{13}C$ fractionation estimates may not be the most robust. Future field studies planned for summer flounder in Chesapeake Bay may be able to address whether the reduced precision in muscle $\Delta^{13}C$ appears to have any influence on its accuracy.

To our knowledge, our results include the first explicit estimates of $\delta^{13}C$ and $\delta^{15}N$ fractionation for fish blood and some of the first for fish liver. In growth-based models of blood turnover, both C (2.34‰–3.27‰) and N (1.11‰–2.80‰) isotopes of flounder (hatchery and wild) were strongly fractionated from the dietary signal, more so than found in birds ($\Delta^{13}C$, –0.3‰ to 1.2‰; $\Delta^{15}N$, 2.2‰ to 3.1‰; Hobson and Clark 1992*b*) or seals ($\Delta^{13}C$, 1.7‰; $\Delta^{15}N$, 1.7‰; Hobson et al. 1996), particularly for C in wild fish. More research is needed to determine if the large blood $\Delta^{13}C$ documented for summer flounder holds for all fishes. Liver estimates for $\Delta^{15}N$ ranged from 0‰ to 3.5‰ in three studies (Trueman et al. 2005; Logan et al. 2006; Sweeting et al. 2007), corresponding with our estimates of 1.45‰–2.28‰. Due to the $\delta^{13}C$ bias introduced by the high lipid content in fish livers (Pinnegar and Polunin 1999), $\Delta^{13}C$ is best evaluated using data that were lipid-extracted or lipid-corrected, which prevents comparisons to some studies (e.g., Suzuki et al. 2005). Summer flounder in our study exhibited liver $\Delta^{13}C$ of 2.76‰–3.05‰, which was greater than that measured for rainbow trout (1.4‰; Pinnegar and Polunin 1999) or Atlantic salmon (1.6‰; Trueman et al. 2005).

Table 6. Published growth-based turnover constants (c; ± standard error (SE)) for $\delta^{13}C$ and $\delta^{15}N$ in fishes with summary data of the experimental subjects and environmental conditions, including developmental stage (H, hatchery fish; W, wild fish), initial length (L_i, ± standard deviation (SD)), and initial wet weight (W_i, ±SD).

							c (±SE)	
Source	Species	Tissue	Temp. (°C)	Stage	L_i (mm)	W_i (g)	$\delta^{15}N$	$\delta^{13}C$
A	Red drum *(Sciaenops ocellatus)*	Whole	28	Larvae, first feeding	2.6	<<0.001	–0.99±0.29	–0.96±0.35
		Whole	28	Late larvae – early juvenile	4–8	<0.001	–1.13±0.25	–1.96±0.89
		Whole	24	Late larvae – early juvenile	4–8	<0.001	–0.94±0.09	–1.12±0.32
B	Winter flounder *(Pseudopleuronectes americanus)*	Whole	13	Early juvenile	—	—	–1.00±0.22	–0.79±0.25
		Whole	18	Early juvenile	—	—	–1.14±0.34	–1.34±0.48
C	Summer flounder *(Paralichthys dentatus)*	Whole	13 and 22	Larvae, first feeding	—	0	–1.5±0.2	–1.4±0.2
		Whole	13 and 22	Larvae, artemia diet	~8	<0.001	–1.0±0.2	–1.2±0.3
		Whole	13 and 22	Early juvenile, fish diet	39	0.08	—	–3.7±1.5
D	Japanese flounder *(Paralichthys olivaceus)*	Fin	—	Juvenile	—	—	—	–4.8
		Liver	—	Juvenile	—	—	—	–11.4
		Muscle	—	Juvenile	—	—	—	–2.07
E	Mummichog *(Fundulus heteroclitus)*	Liver	18	Juvenile	40–51	0.84	–5.85±0.61	—
		Muscle	18	Juvenile	40–51	0.84	–2.33±0.25	—
F	Sand goby *(Pomatoschistus minutus)*	Heart	16.9	Juvenile	43±1	0.98	–1.55±0.83	–10.59±4.07
		Liver*	16.9	Juvenile	43±1	0.98	–15.97±4.59	–5.15±1.30
		Muscle	16.9	Juvenile	43±1	0.98	–1.19±0.57	–2.25±0.56
G	Summer flounder *(Paralichthys dentatus)*	Blood	20	Juvenile – early adult (H)	176±19	59±18	–1.09±0.21	–2.20±0.28
		Blood	20	Juvenile – early adult (W)	215±25	102±33	–2.09±0.23	–4.12±0.62
		Liver	20	Juvenile – early adult (H)	176±19	59±18	–4.02±0.82	–3.09±0.16
		Liver	20	Juvenile – early adult (W)	215±25	102±33	–7.32±1.00	–6.53±0.88
		Muscle	20	Juvenile – early adult (H)	176±19	59±18	–1.00†	–0.69±0.10
		Muscle	20	Juvenile – early adult (W)	215±25	102±33	–1.00†	–0.74±0.10

Note: Sources are as follows: A, Herzka and Holt 2000; B, Bosley et al. 2002; C, Witting et al. 2004; D, Watanabe et al. 2005; E, Logan et al. 2006; F, Guelinckx et al. 2007; G, this study. Asterisk (*) indicates that liver samples were not corrected for lipid content, which can influence $\delta^{13}C$ measurements. Dagger (†) denotes values that were not estimated but selected as the best parameterization using AIC_c.

The tissue-specific fractionations that we report are influenced by the different biochemical constituents of the tissues (e.g., amino acids, proteins, lipids), which differ in their own fractionations (DeNiro and Epstein 1977; Macko et al. 1987; McClelland and Montoya 2002). Also, assimilated dietary components are not necessarily allocated equally across all tissues, contributing to tissue-specific fractionations (Gannes et al. 1997). Additional research across a variety of taxa is needed to elucidate the variability in liver and blood fractionation factors of fishes, as has been done for muscle.

Overall, the experimental groups (wild and hatchery) yielded similar relative conclusions regarding tissue turnover rates, confirming that liver and blood respond more quickly to dietary changes than does muscle. In some cases, pooling groups was supported, generating more robust measures of model parameters, but often the groups were more appropriately parameterized separately. For example, both turnover rates and fractionations were typically higher for wild fish. Differences between wild and hatchery groups could be related to a suite of factors, including differences in initial sizes, growth rates, maturity schedules, physiology, environmental histories, and genetic diversity. The differences between groups and the potential influence of these factors are reminders that natural populations of summer flounder likely exhibit variability in both isotopic turnover and fractionation. It may therefore be more prudent for field studies on summer flounder to rely on parameter estimates derived from the wild group as opposed to hatchery fish, but we acknowledge the possibility that isotope dynamics may differ slightly between laboratory and field settings (Vander Zanden and Rasmussen 2001).

Time- vs. growth-based turnover models

Both time- and growth-based turnover models captured the patterns of isotopic changes experienced by the experimental populations, supporting the mechanistic foundations of the models in which somatic growth and metabolic tissue replacement drive isotopic turnover (Fry and Arnold 1982; Hesslein et al. 1993). However, the time-based model relies on a single growth parameter value intended to be representative of the entire population, and it is assumed to be constant through time (Tarboush et al. 2006). Summer flounder in our study experienced large variability in individual growth rates and irregular growth trajectories, characteristics that are common among fish species in natural settings. The variability in individual growth rates likely influenced parameter discrepancies between modeling methods and contributed to a large portion of the residual error in the time-based model. Not surprisingly, time-based model residuals plotted against individual growth rates consistently showed a slight negative relationship, indicating that faster growing fish experienced greater turnover after a given amount of time than slower growing individuals.

Relative to the growth-based model, time-based model turnover rates may not be as applicable to situations that deviate from the experimental conditions. Time-based turnover rate parameters (both k' and m in eq. 9) can differ by temperature and feeding rates because of the different growth rates elicited by these environmental conditions (Bosley et al. 2002; Witting et al. 2004; Watanabe et al. 2005). Turnover rates derived from growth-based models (c in eq. 3), on the other hand, may not drastically suffer from these problems because any variability in growth rates is inherently accounted for by the relative growth variable W_R. For example, Bosley et al. (2002) and Witting et al. (2004) showed that c did not vary greatly between temperature treatments, despite changes in fish growth rates, and Watanabe et al. (2005) made no indication that different feeding and growth rates influenced growth-based turnover rates (their fig. 5). Consequently, application of turnover parameters derived from growth-based models may be more suitable in field conditions where temperatures or growth rates differ from the laboratory setup from which results were generated. Although time-based models can provide valuable information, including relative differences in turnover among tissues, metabolic and growth parameters in these models may be particularly sensitive to any environmental factors that alter fish growth.

Predictive model of turnover times

Knowledge of tissue-specific turnover rates will enable researchers to select tissues suitable for the time scale of the inferences to be made in field studies utilizing stable isotopes. To avoid making an erroneous assumption that a species is in isotopic equilibrium with the sampled diet, it is important to predict the approximate isotopic equilibrium rates for different tissues, especially in migratory fishes. The half-lives calculated from the time-based turnover models may be inadequate for such predictions if field conditions, and consequently growth rates, differ from the laboratory experiment, as described above. Here, we propose a slight reformulation of the growth-based turnover model, eq. 3, to translate turnover rate constants into the more tangible unit of time for field applications.

Growth can be adequately described with an exponential model (eq. 10; Ricker 1979), and this can be substituted for W_R in the growth-based model, eq. 3, yielding

$$\delta_{W_R} = \delta_f + (\delta_i - \delta_f)e^{ktc} \quad (14)$$

For a given percentage of complete turnover (α), δ_{W_R} can be rewritten as

$$\delta_{W_R} = \frac{\alpha}{100}(\delta_f - \delta_i) + \delta_i \quad (15)$$

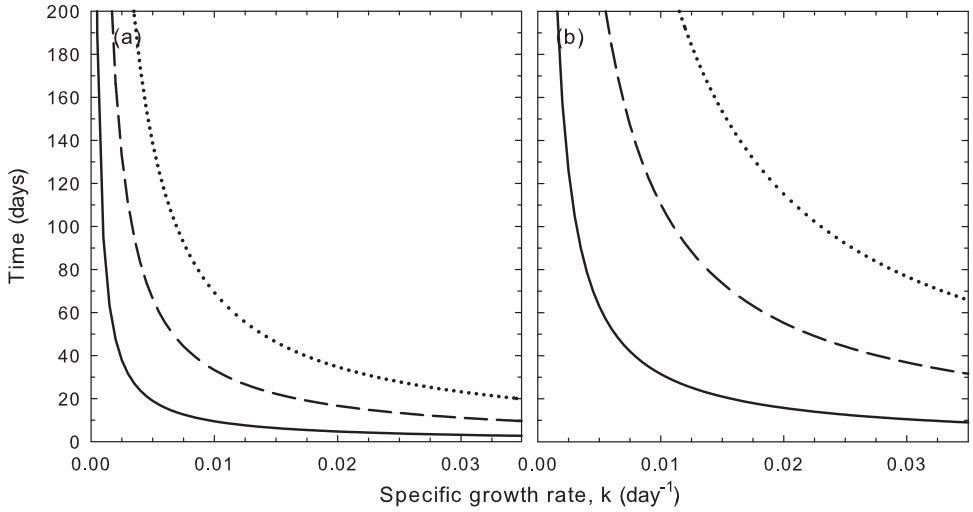
For example, a 50% turnover between the initial and final isotope values would equate to α = 50% and δ_{W_R} would be half way between δ_i and δ_f. After substituting eq. 15 into eq. 14, the following model can be obtained:

$$t_{\alpha/100} = \frac{\ln(1 - \alpha/100)}{kc} \quad (16)$$

where $t_{\alpha/100}$ is the time needed for α% turnover to be completed. The growth-based model fits presented in this paper provide the turnover rate constants, c, for three summer flounder tissues. Assuming that c is relatively constant across different specific growth rates (k), then the time needed for a given amount of turnover to occur (for a specified growth rate) can be modeled using eq. 16.

Plotting this model over a range of realistic growth rates for juvenile summer flounder (Rountree and Able 1992) using turnover estimates of $\delta^{15}N$ emphasizes the differences in temporal scales represented by various tissues (Fig. 5). For

Fig. 5. Projected time to (*a*) 50% and (*b*) 90% turnover of $\delta^{15}N$ for summer flounder (*Paralichthys dentatus*) muscle (dotted line), blood (broken line), and liver (continuous line), based on eq. 16 and estimated *c* parameters for wild fish.



example, given a growth rate of 0.01 day^{-1}, muscle requires ~70 days for 50% turnover, whereas liver reaches 90% turnover in ~30 days at that growth rate. Differences in turnover times among tissues are greatest at lower growth rates, suggesting that tissue selection is particularly important for researchers working on slower growing animals. This model also highlights the important role that growth rates have on mediating the time course in which isotopic equilibrium is achieved.

The assumption that *c* is constant across different growth rates was supported by the lack of a relationship between growth-based $\delta^{15}N$ model residuals and individual growth rates, *k* (ranging from approximately 0 to 0.024 day^{-1}). Also, work on larval and juvenile flatfish did not reveal substantial differences in *c* between temperature treatments, which altered growth rates (Bosley et al. 2002; Witting et al. 2004). We did discover a slight negative relationship between growth-based model $\delta^{13}C$ residuals and individual *k*, suggesting that the consistency of *c* across growth rates may vary by isotope, but a more directed study into this would be needed.

Using eq. 16, expected turnover times can be estimated for a species of interest given information on the growth rate and turnover rate constants (known or assumed). Even though growth-based turnover rate parameters (*c*) remain unexamined for most species and tissues, ranges of possible turnover times can be estimated using published values of turnover rates for species with similar characteristics (e.g., developmental stage, size, life history characteristics) (Table 6).

Application to field studies

Large differences in tissue turnover times in this study reflect the extreme importance of tissue selection for isotopic studies of migratory fishes such as the summer flounder. Summer flounder inhabit estuarine waters such as Chesapeake Bay from spring to fall (~6–9 months) before their spawning migration to the outer continental shelf during late autumn and winter (Murdy et al. 1997). With reasonable growth rates (*k*) of 0.010–0.015 day^{-1}, muscle can require ~154–230 days to reach 90% equilibrium with a constant diet (Fig. 5). Consequently, muscle reflects dietary habits over long time periods that span the majority of typical residency of individuals in the productive estuarine waters. The lack of dietary resolution provided by muscle is compounded by the well-documented seasonal and ontogenetic dietary changes exhibited by summer flounder (Link et al. 2002; Latour et al. 2008). These dietary changes, along with regional movements, tend to exacerbate the heterogeneity of consumed isotopic signals. Such dietary variability is common for estuarine fishes in temperate environments because of the seasonality of productivity and residency in these ecosystems (Murdy et al. 1997), hindering isotopic analyses of tissues with slow turnover. Our results indicate that relative to muscle, liver and blood exhibit faster turnover rates that would benefit field studies of summer flounder and other migratory fishes by responding more rapidly to consumed material and minimizing the confounding factors introduced by movement and dietary variability. Use of blood in isotopic studies of migratory fish may be particularly useful because animals do not need to be sacrificed for sampling, allowing repeated sampling. Combining isotopic analysis of blood with other techniques such as telemetry could present a powerful means for examining habitat utilization, growth, and trophic ecology in mobile species.

In the absence of species- and situation-specific turnover rates and fractionations, certain considerations can help reduce errors when researchers use literature values in their studies. Although we advocate that field studies using stable isotopes can strongly benefit from complementary laboratory research on turnover rates and fractionations, we acknowledge that it may be impractical in some cases. Given the documented influences of species (Deniro and Epstein 1978, 1981), tissue (Logan et al. 2006), developmental stage (Sakano et al. 2005), and growth rate (Trueman et al. 2005) on turnover rates and fractionations, we recommend that researchers consider these factors, along with taxonomy, lifestyle, and habitat, when using values from previous research. However, the limited available information of this type, especially for growth-based methods (e.g., Table 6), emphasizes the importance of continued research on isotope

dynamics in fishes across habitats, life stages, and tissues, especially for blood and liver.

When using literature values, it should also be common practice to conduct sensitivity analyses to reveal the potential effects that borrowed parameters may have on the conclusions drawn (e.g., Vander Zanden and Rasmussen 2001). In this regard, the parameter estimates that we present, along with eq. 16 and Table 6, may be particularly useful in estimating ranges of possible turnover times and assessing variability in dietary mixing model results. For example, given a diet shift from one prey to another, possible isotopic trajectories for a predator can be predicted using the presented turnover rates and their 95% confidence intervals. Simulations of this type suggest that within-tissue variability in dietary estimates is typically less than ~25% for flounder. Furthermore, diet estimates generated from liver and blood $\delta^{13}C$ at any given point in growth or time are indistinguishable at the 95% confidence level. For $\delta^{15}N$, on the other hand, a wild fish would require a tripling in weight or ~100 days (at a growth rate of 0.01 day^{-1}) before the 95% confidence range of diet estimates from blood would approximate (within 5%) that of the liver because of the faster turnover of nitrogen in the liver. When combined with an understanding of an organism's ecology and behavior, simulations and sensitivity analyses can aid researchers in determining the most suitable tissue and the range of appropriate inference for specific research questions utilizing stable isotopes. Specifically, deviations from the two broad assumptions of isotopic equilibrium and known fractionations can be minimized, thus reducing the sources of error in isotopic analyses, particularly in trophic studies of migratory fishes.

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Appendix A. Correcting liver $\delta^{13}C$ values for lipid content

To characterize the bias introduced into measurements of liver $\delta^{13}C$ due to lipids, 12 liver samples were divided into two aliquots and analyzed both before lipid extraction ($\delta^{13}C_{NE}$) and after lipid extraction ($\delta^{13}C_{LE}$). Lipids were extracted using a solvent mixture of 65% dichloromethane and 35% methanol (Bligh and Dyer 1959) and an accelerated solvent extractor (ASE-200; Dionex Corp., Sunnyvale, California) at 80 °C and 1800 psi. The lipid extract was concentrated to 1 mL (TurboVap 500; Zymark Corp., Hopkinton, Mass.), and its weight was determined gravimetrically to calculate the percent lipid content of each sample. Lipid-free samples were dried and analyzed for stable isotope ratios as described in Materials and methods. Following Logan et al. (2008), the relationship between elemental C:N (for nonextracted samples) and the change in $\delta^{13}C$ due to extraction ($\delta^{13}C_{LE} - \delta^{13}C_{NE}$) was evaluated using four alternative models (the numbering of equations mimics the numbering used by Logan et al. 2008):

$$\text{(A1)} \quad \delta^{13}C_{LE} - \delta^{13}C_{NE} = D\left(\theta + \frac{3.90}{1 + 287/L}\right)$$

$$\text{where} \quad L = \frac{93}{1 + (0.246 \times \text{C:N} - 0.775)^{-1}}$$

$$\text{(A1a)} \quad \delta^{13}C_{LE} - \delta^{13}C_{NE} = \frac{a \times \text{C:N} + b}{\text{C:N} + c}$$

$$\text{(A2)} \quad \delta^{13}C_{LE} - \delta^{13}C_{NE} = P - \frac{P \times F}{\text{C:N}}$$

$$\text{(A3)} \quad \delta^{13}C_{LE} - \delta^{13}C_{NE} = \beta_0 + \beta_1 \ln(\text{C:N})$$

Each of these four models describes the relationship between the raw C:N and the bias introduced by not extracting lipids. Model A1 is based on the McConnaughey and McRoy (1979) model and includes an empirically derived expression for lipid content (L) and a parameter for the protein-lipid $\delta^{13}C$ discrimination (D). Model A1a is a generalized form of eq. A1, with the assumed values accounted for by parameters a, b, and c (Logan et al. 2008). Model A2, based on Fry (2002), has parameters for protein–lipid $\delta^{13}C$ discrimination (P) and $C:N_{lipid\text{-}free}$ (F). And lastly, model A3 characterizes the bias due to lipids as a linear function of the log-transformed raw C:N, with $C:N_{lipid\text{-}free}$ represented by $e^{(-\beta_0/\beta_1)}$ (Logan et al. 2008). All parameters (D, θ, a, b, c, P, F, β_0, and β_1) were estimated using nonlinear regression (SAS version 9.1; SAS Institute Inc., Cary, North Carolina). Akaike's information criterion corrected for small sample size (AIC_c; eq. 12) was used to identify the best model of the set (Burnham and Anderson 2002).

The percent lipid content of liver samples had a positive relationship with the C:N of the samples, supporting the notion that C:N is a suitable proxy for lipid content (Fig. A1). All four models captured the nonlinear trend in $\delta^{13}C_{LE} - \delta^{13}C_{NE}$ (Fig. A2). Although nearly indistinguishable from model A2, model A1 exhibited the lowest AIC_c and was identified as the best model of the set (Table A1). Consequently, all liver sample $\delta^{13}C$ from this study were corrected for lipid content using model A1 with the parameters estimated (±SE) as D = 6.2883 ± 1.3519 and θ = 0.0612 ± 0.1527 (Table A1).

Fig. A1. Percent lipid content of liver samples plotted by raw, non-lipid-extracted C:N.

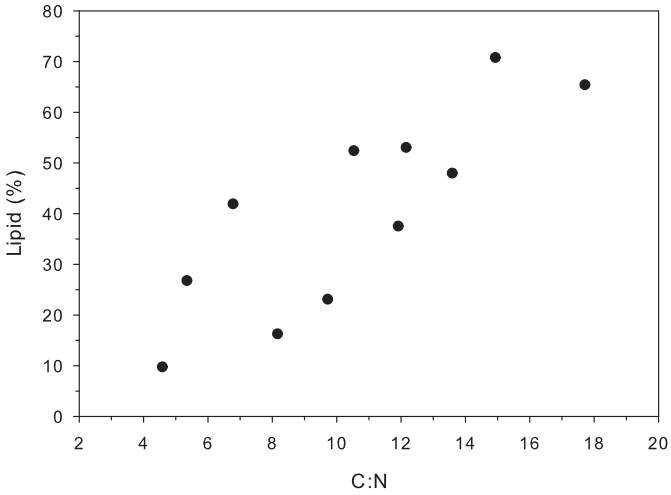

Fig. A2. Relationship between raw C:N and $\delta^{13}C$ bias introduced by not extracting lipids from liver samples. Bias in $\delta^{13}C$ was calculated as the difference between $\delta^{13}C$ for lipid-extracted samples (LE) and non-lipid-extracted samples (NE). Model fits are indicated by different lines (bold continuous line, model A1; broken line, model A1a; continuous line, model A2; dotted line, model A3). Note that models A1 and A2 are overlapping in the figure.

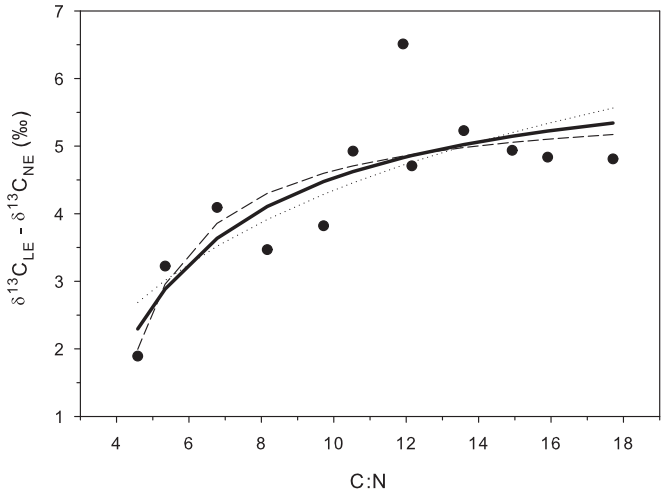

Table A1. Statistics and parameter estimates for the four competing models used to describe the relationship between liver C:N and the bias in $\delta^{13}C$ introduced by not extracting lipids.

Model	RSS	ΔAIC_c	Parameter	Estimate	SE
A1	4.778	**0.000**	D	6.2883	1.3519
			θ	0.0612	0.1527
A1a	4.537	4.093	a	5.6965	0.7851
			b	–21.7933	3.0509
			c	–2.4176	2.0722
A2	4.789	0.028	P	6.4081	0.4821
			F	2.9370	0.4410
A3	5.787	2.299	β_0	–0.5532	1.2458
			β_1	2.1277	0.5299

Note: AIC_c differences (ΔAIC_c) were calculated from residual sum of squares (RSS) for each model, and the best model is indicated in bold type. SE, standard error.

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