Rates and components of carbon turnover in fish muscle: insights from bioenergetics models and a whole-lake ^{13}C addition

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Abstract: Stable isotopes are widely employed to describe energy flow in aquatic communities, though interpretation of results can be confounded by the fact that organisms integrate over vastly different time scales. We used results from a 56-day whole-lake ^{13}C addition and a bioenergetic modeling approach to estimate dorsal muscle carbon turnover rates in a natural setting for three sizes of bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and yellow perch (*Perca flavescens*). Generally, dynamic $\delta^{13}C$ models with a metabolic tissue replacement term were better supported than models predicting isotopic change from growth alone, except when relative growth rates were highest (age 0 bluegill). Across species and size classes, the percentage of carbon change due to tissue replacement was variable (2%–80%) and independent of fish size. The half-life of $\delta^{13}C$ in age 0 fishes was similar and ranged from 8 to 18 days. In contrast, adult tissue half-lives were much longer (116–173 days). Based on these and previously published estimates, fish mass (g) was a strong predictor of fish carbon turnover rates, λ: $\log(\lambda) = -3.65 - 0.20\log(\text{mass})$, $r^2 = 0.71$.

Résumé : Les isotopes stables servent couramment à décrire le flux d'énergie dans les communautés aquatiques, même si le fait que les divers organismes les intègrent à des échelles temporelles extrêmement différentes peut introduire de la confusion dans l'interprétation des résultats. Nous utilisons une addition expérimentale de ^{13}C de 56 jours dans un lac entier et une méthodologie de modélisation bioénergétique pour estimer dans un environnement naturel les taux de remplacement du carbone dans le muscle dorsal chez trois tailles de crapets arlequins (*Lepomis macrochirus*), d'achigans à grande bouche (*Micropterus salmoides*) et de perchaudes (*Perca flavescens*). En général, les modèles dynamiques de $\delta^{13}C$ avec un terme de remplacement métabolique des tissus fonctionnent mieux que les modèles qui prédisent les changements isotopiques à partir de la seule croissance, sauf lorsque les taux de croissance relative sont à leur maximum (les crapets arlequins d'âge 0). Le pourcentage de changement de carbone dû au remplacement des tissus est variable (2–80 %) et indépendant de la taille des poissons chez les différentes espèces et les classes de taille. La demi-vie de $\delta^{13}C$ est semblable chez tous les poissons d'âge 0 et varie de 8–18 jours. En revanche, les demi-vies dans les tissus adultes sont beaucoup plus longues (116–173 jours). D'après les présentes estimations et d'autres publiées antérieurement, la masse du poisson (g) est une bonne variable prédictive des taux de remplacement du carbone (λ) chez les poissons: $\log(\lambda) = -3{,}65 - 0{,}20\log(\text{masse})$, $r^2 = 0{,}71$.

[Traduit par la Rédaction]

Introduction

The relationship between carbon stable isotopes in fish tissues and their diets has proven a valuable tool for tracing energy flow in food webs (DeNiro and Epstein 1978; Peterson and Fry 1987). The $\delta^{13}C$ of a fish's tissue conservatively reflects the $\delta^{13}C$ of its diet over longer time periods than direct measurements such as stomach content analysis (Vander Zanden et al. 1997). These properties facilitate tracing energy across multiple trophic levels, such as identifying primary production sources supporting fish growth (Forsberg et al. 1993). However, uncertainty about the length of time that tissues reflect diet $\delta^{13}C$ can cause doubt whether observed $\delta^{13}C$ differences owe to changes in trophic pathways or simply shifts in dietary $\delta^{13}C$ that are not completely reflected in tissue $\delta^{13}C$ (O'Reilly et al. 2002). Understanding how this dynamic value varies across common gradients, such as fish size, would facilitate ecological inference and increase the utility of stable isotopes for quantifying energy flow in aquatic food webs (Martínez del Rio et al. 2009).

The rate of carbon isotope turnover (λ) in an organism or tissue results from the addition of new tissue and metabolic replacement of existing tissue. To date, many fish $\delta^{13}C$ diet-switch studies concluded growth was primarily responsible for $\delta^{13}C$ change, and metabolic replacement had a negligible effect on turnover, but noted that results may not be consistent across all fish life stages (Hesslein et al. 1993; Herzka and Holt 2000; Perga and Gerdeaux 2005). Experiments on older or larger fishes and those with lower specific growth

Received 2 December 2009. Accepted 30 November 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 8 February 2011.
J21544

Paper handled by Associate Editor Marc Trudel.

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rates found replacement to be a major proportion of total turnover, in some cases accounting for 80% of isotopic change in dorsal muscle tissue (Suzuki et al. 2005; Tarboush et al. 2006; Logan et al. 2006). Knowledge of the principal factors that influence the relative importance of tissue replacement and growth in isotopic change would provide a mechanistic basis for predicting dynamics of $\delta^{13}C$ change in fishes (Martínez del Rio et al. 2009).

The relationship between rates of carbon isotope change and simple allometric and physiologic metrics, such as body size or temperature, remains poorly understood. In similar-sized fishes, higher water temperature reduced the half-life of carbon in muscle tissue (Bosley et al. 2002; Witting et al. 2004), supporting speculations by Tieszen et al. (1983) that metabolic activity is positively correlated with λ. The evidence for the negative relationship between body mass and turnover is consistent across studies. The isotopic half-life of larval fishes typically ranges from days to weeks and increases to months in larger fishes (Hesslein et al. 1993; MacAvoy et al. 2001), although many estimates on larger fishes are poorly constrained. Part of our knowledge gap on carbon turnover in fishes may simply owe to logistical laboratory constraints of conducting diet-switch experiments on larger individuals.

A lack of diversity in analytical approaches may also limit our understanding of how common factors, such as growth and size, influence isotopic change in fishes. Standard isotopic diet-switch experiments conducted in laboratory settings have been critical in providing isotopic rates of change, yet such studies most often use early life stages individuals, held under ideal temperatures for growth with ad libitum food sources. Such experiments offer the benefit of controlled conditions but have a harder time mimicking natural systems where fish incur metabolic costs associated with searching for food and predator avoidance. A few studies have estimated rates of isotopic change in natural settings by taking advantage of predictable changes in the isotopic value of fish diets associated with larval development or migration. (Vander Zanden et al. 1998; Maruyama et al. 2001). These studies more accurately reflect natural conditions experienced by fishes, but require a known, unidirectional change in diet isotopic value and usually assume exponential fish growth. An alternative approach to estimating isotopic rates of change uses daily fish growth and consumption estimates from bioenergetic models paired with isotopic time series of fish tissue and diet (Harvey et al. 2002). In contrast with the exponential models used by most isotopic turnover studies, this flexible method does not require a static change in the isotopic value of the diet and can account for temporal variation in fish growth rates owing to temperature or food quality. While not widely applied, this approach has the potential for estimating rates of isotopic change under a range of natural conditions.

We took advantage of an experimental, whole-lake ^{13}C addition to estimate carbon isotope ($\delta^{13}C$) turnover rates (λ) over a broad range of sizes (0.06–434 g) of three common north-temperate fishes. The experiment's original intent was to determine the relative importance of lacustrine and terrestrial energy sources supporting lake food webs. The added ^{13}C, in the form of sodium bicarbonate, drastically increased the $\delta^{13}C$ of primary production and created a contrast between the $\delta^{13}C$ of invertebrates consumed by fish and fish muscle tissue $\delta^{13}C$. We developed a simple mass balance model using bioenergetic-derived growth dynamics to estimate carbon isotope change rates in a natural setting, across a range of fish sizes and species. Using fish tissue and diet $\delta^{13}C$ time series, we evaluated the importance of growth and tissue replacement (m) to isotopic change by comparing models where m was equal to zero (isotopic change due to growth only) and where m was a constant proportion over the season. We combined our results with previously published carbon isotope change studies to evaluate and establish relationships among λ, fish size, and water temperature.

Materials and methods

The ^{13}C addition was conducted in Crampton Lake, which is located in a forested watershed of the University of Notre Dame Environmental Research Center (46°13′N, 89°32′W) in Michigan's Upper Peninsula. This oligotrophic 26 ha lake has a maximum depth of 18.5 m and a mean depth of 3.5 m. Bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and yellow perch (*Perca flavescens*) dominate the fish community (>95% biomass), with less abundant populations of pumpkinseed (*Lepomis gibbosus*), johnny darter (*Etheostoma nigrum*), golden shiner (*Notemigonus crysoleucas*), and central mudminnow (*Umbra limi*). The lake shore is not developed, and the lake receives limited angling pressure and no harvest. We added ^{13}C in the form of sodium bicarbonate ($NaH^{13}CO_3$, >99% ^{13}C content) to the lake's upper mixed layer each morning (0700 h) for 56 days from 13 June to 7 August 2005. The $NaH^{13}CO_3$ was dissolved in gas-tight carboys filled with lake water, then pumped directly into the lake from a moving boat at a depth of 0.7 m while the boat traveled in a path that covered the entire lake area. This practice promoted dispersions throughout the mixed layer (Pace et al. 2007). Lake water temperature was measured every hour at 1.5 m using three HOBO Pendant data loggers deployed in different areas of the littoral zone. The daily average of these three measurements was used in bioenergetics models.

Sampling and measurement of $\delta^{13}C$, diet, and growth

Fish collections for muscle tissue $\delta^{13}C$ and diet were made every 5–14 days using angling and boat electrofishing. Bluegill (ages 0 and 2), largemouth bass (ages 0, 1, and 6), and yellow perch (ages 0 and 1) were collected from late May through the beginning of November 2005. Age 5 bluegill and age 2 yellow perch were sampled through 8 October 2005. Additional diet samples were collected from age 2 bluegill, age 1 yellow perch, and age 0 largemouth bass from early May through June 2006 to supplement the early season diet descriptions for these age classes. The particular species and age classes were chosen based on our ability to consistently sample within an age class and to represent a broad ontogenetic range in each species. We also used a purse seine in the deepest portion of the lake during June–August 2005 to capture age 0 yellow perch and bluegill that were not present in littoral areas during midsummer. Purse seines did not capture any larger fishes. Dorsal muscle tissue was dissected from individual fish (n = 1–5) in each species and age class, cleaned of scales and skin, rinsed with deion-

ized water, dried at 60 °C for 72 h, and homogenized. Isotope analyses of fish tissue were performed on isotope ratio mass spectrometers at the University of California – Davis stable isotope laboratory. The $\delta^{13}C$ data are reported in per mil (‰) notation relative to Pee Dee Belemnite.

We used gastric lavage, within 30 min of capture, to collect diets from fish greater than 80 mm total length. Contents were flushed into mesh-lined funnels (153 μm) and thoroughly rinsed with deionized water to ensure ^{13}C-labelled lake water did not contaminate diet samples. Smaller fish were euthanized with an overdose of MS-222, stored on ice, and stomachs were dissected in the laboratory. Diet collections were made during three time periods (0600–0800, 1300–1500, 1900–2100) to account for potential diel diet variability. The number of samples that characterized the weekly diet of each species and age class ranged from 4 to 104 per week (average 13.5). We quantified individual fish diets by dry mass proportion for each of 25 diet taxa. Benthic diet classifications included Amphipoda, Decapoda, Diptera, Ephemeroptera, Gastropoda, Hirundinea, Lepidoptera, Megaloptera, Odonata, Trichoptera, and Veneroida. Pelagic taxa consisted of *Chaoborus* sp., Cladocera (large and small), and Copepoda. Terrestrial prey consisted of invertebrates and vertebrates (Rodentia). Prey fish, bluegill, yellow perch, largemouth bass, and johnny darter (*E. nigrum*) were identified to species and categorized as young of year (YOY) or age 1+. Intact diet items were separated, counted, dried at 60 °C for 72 h, and weighed to the nearest milligram. For partially digested or masticated items, unique body parts were counted and dry mass was extrapolated using the average dry mass of a diet taxon calculated from dried, whole items.

The $\delta^{13}C$ of fish diet items was determined using freshly consumed, whole invertebrates from fish diet samples, benthic invertebrates collected directly from the lake substrate, and oblique zooplankton tows. Invertebrate samples collected from fish stomachs consisted of whole diet items from a given date. Diet-collected samples were thoroughly rinsed with deionized water to reduce contamination from other diet contents, combined, dried at 60 °C for 72 h, and homogenized. We measured the $\delta^{13}C$ of Odonata and Diptera from benthic grab samples collected during the ^{13}C addition (Solomon et al. 2008). Samples for zooplankton and *Chaoborus* sp. $\delta^{13}C$ were collected weekly using oblique net hauls in the upper mixed layer. Individual invertebrate taxa were separated under a dissecting microscope, dried, and homogenized (Pace et al. 2007). We did not explicitly measure the $\delta^{13}C$ of *Bosmina* sp. and *Chydorus* sp. but assumed the $\delta^{13}C$ time series of primary producers from Pace et al. (2007) approximated the $\delta^{13}C$ for these small zooplankton. Johnny darters were rare in fish diets, and we did not explicitly measure their $\delta^{13}C$. We assumed the age 1 yellow perch samples approximated the $\delta^{13}C$ of these small, benthic-oriented fishes. Isotope analyses of invertebrates were performed at the University of California – Davis stable isotope laboratory (Davis, California).

Annual growth during 2005 for each species and age class was estimated from transverse-sectioned sagittae otoliths collected from fishes during May and June 2006. Otoliths were mounted in epoxy, and a transverse section (~200 μm) was cut using a low-speed saw. Interpreted annuli were measured along a radius centered at the origin and oriented perpendicular to annual growth marks. The change in length of an individual fish was estimated from the last annual growth increment (2005) using the direct-proportion back-calculation method (Schramm et al. 1992). The change in biomass for each fish was calculated from species-specific length–weight relationships developed over the course of the study. We used the average change in biomass for each species and age class to represent growth in the bioenergetics models. Age 0 fishes recruited to our sampling gear in the middle of the summer. We used the average weight of 20 or more individuals, from the date of first capture, to represent the starting weight in age 0 bioenergetic models.

Model methods

In this study, fish diet $\delta^{13}C$ and fish growth rate were dynamic, rather than static as in most stable isotope diet-switch experiments. Because these values were changing over the course of the study, we could not use an exponential model to estimate λ. Instead, we used a bioenergetic-based growth model and measurements of fish diet $\delta^{13}C$ to predict fish muscle $\delta^{13}C$. We compared our estimated $\delta^{13}C$ time series with observed time series of dorsal muscle tissue $\delta^{13}C$ and compared models with and without metabolic replacement parameter (m) that increased the rate at which the fish reflect the $\delta^{13}C$ of their diet.

Bioenergetics models partitioned the measured annual growth into daily growth estimates and estimated taxon-specific consumption for each fish species and age class (Table 1). Bioenergetics model inputs included the average mass change over the season as estimated from otolith back-calculations, measured weekly diet proportions, literature values for predator and prey energy densities, and the daily mean water temperature at 1.5 m. We used previously published bioenergetics model parameters for bluegill (Kitchell et al. 1974), largemouth bass (Rice et al. 1983), and yellow perch (age 1, age 2 — Kitchell et al. 1977; age 0 — Post 1990). We assumed predator and prey energy densities were constant over time and obtained values from the literature (Cummins and Wuycheck 1971) and those included in Hanson et al. (1997). We used the carbon to nitrogen ratio (C:N), from stable isotope analysis, to assess our assumed energy density temporal consistency within a prey category and to corroborate the magnitude of differences in literature-based energy densities among prey. We included spawning losses of 15% of wet mass in age 5 bluegill and age 6 largemouth bass bioenergetics models (Kitchell et al. 1974; Rice et al. 1983). Bioenergetics models were run at a daily time step from 19 May 2005 through 25 October 2005 for all age classes except YOY, which started on the first day of capture, 17 July 2005, 9 July 2005, and 27 June 2005 for bluegill, largemouth bass, and yellow perch, respectively (Table 1).

Fish diet $\delta^{13}C$ was calculated as a weighted average using observed diet taxa $\delta^{13}C$ time series and bioenergetics estimates of daily taxon-specific consumption. Fish's diet $\delta^{13}C$ was calculated each day according to

$$\text{DIET } \delta^{13}C_{(t)} = \frac{\sum_j \left(\delta^{13}C_{j(t)} \cdot Q_{j(t)}\right)}{Q_{(t)}} \quad (1)$$

Table 1. Species- and age-specific sample sizes for diet and growth characterization, bioenergetics models start masses, end masses, model duration, estimated proportion of maximum consumption, and citation for bioenergetics model parameters.

Species	Age	Diet (*N*)	Growth (*N*)	Mass start (g)	Mass end (g)	Model duration	Prop. max. consump.	Bioenergetics model
Bluegill	0	102	18	0.06	1.20	17 July – 4 Nov.	0.86	Kitchell et al. 1974
	2	278	21	5.38	15.97	19 May – 4 Nov.	0.52	Kitchell et al. 1974
	5	407	11	72.70	108.60	19 May – 8 Oct.	0.53	Kitchell et al. 1974
Largemouth bass	0	55	25	0.52	4.60	9 July – 4 Nov.	0.59	Rice et al. 1983
	1	177	27	5.60	42.60	19 May – 4 Nov.	0.45	Rice et al. 1983
	6	400	14	413.00	474.00	19 May – 4 Nov.	0.26	Rice et al. 1983
Yellow perch	0	110	10	0.11	2.60	27 June – 4 Nov.	0.49	Post 1990
	1	161	23	2.24	9.13	19 May – 4 Nov.	0.39	Kitchell et al. 1977
	2	56	8	11.16	17.32	19 May – 8 Oct.	0.32	Kitchell et al. 1977

where $\delta^{13}C_{j(t)}$ is the $\delta^{13}C$ of diet item j, consumed on day t, $Q_{j(t)}$ represents the daily mass of diet item j, consumed on day t, and $Q_{(t)}$ is the total mass consumed on day t. $\delta^{13}C_{j(t)}$ was derived from observed or linearly interpolated diet item $\delta^{13}C$ throughout the experiment. For days that we did not measure diet item $\delta^{13}C$, we linearly approximated values between single $\delta^{13}C$ measurements or the average $\delta^{13}C$ if more than one sample was analyzed from a given day.

The $\delta^{13}C$ of the fish on day $t + 1$ is predicted from a mass-weighted mixing equation for $\delta^{13}C$ ($\delta^{13}C$ mass at time $t + 1$ is equal to $\delta^{13}C$ mass carried over from time t + $\delta^{13}C$ mass in growth during the time interval plus the ^{13}C mass in tissue replacement during the time interval)

$$(2a)\qquad \delta^{13}C_{\text{fish}(t+1)} \cdot M_{(t+1)} = \delta^{13}C_{\text{fish}(t)} \cdot [(1-m)\cdot M_{(t)} \cdot \Delta_t] + \left(\delta^{13}C_{\text{diet}(t)} + D\right) \cdot G_{(t)} + \left(\delta^{13}C_{\text{diet}(t)} + D\right) \cdot m \cdot M_{(t)} \cdot \Delta_t$$

which gives the following expression for $\delta^{13}C_{\text{fish}(t+1)}$:

$$(2b)\qquad \delta^{13}C_{\text{fish}(t+1)} = \frac{\delta^{13}C_{\text{fish}(t)} \cdot [(1-m)\cdot M_{(t)} \cdot \Delta_t] + \left(\delta^{13}C_{\text{diet}(t)} + \text{D}\right)\left(G_{(t)} + m \cdot M_{(t)} \cdot \Delta_t\right)}{M_{(t+1)}}$$

In eqs. 2*a* and 2*b*, $\delta^{13}C_{\text{fish}(t)}$ is the calculated fish $\delta^{13}C$, $M_{(t)}$ is the fish mass on day t, and $M_{(t+1)}$ is the fish mass on day $t + 1$. The values for $G_{(t)}$, $M_{(t)}$, and $M_{(t+1)}$ are based on the species- and age-specific bioenergetics models described above. More specifically, $G_{(t)}$ represents fish growth (in grams) at time t, and $M_{(t)}$ and $M_{(t+1)}$ represent fish mass (g) at time t and $t + 1$, respectively. $\delta^{13}C_{\text{diet}(t)} + D$ (discrimination factor between diet and muscle tissue) represents the $\delta^{13}C$ of newly added tissue and replaced tissue, and the initial fish $\delta^{13}C_{\text{fish}}$ is the mean of $\delta^{13}C$ measurements on the first day tissue samples were analyzed. We use the term "discrimination factor", as proposed by Martínez del Rio et al. (2009), to describe the difference in the isotopic composition between an animal's tissue and its diet. We assumed a $\delta^{13}C$ discrimination factor between diet and fish dorsal muscle tissue of 0.80 (Vander Zanden and Rasmussen 2001). The parameter m is proportion of fish mass that is turned over via metabolic replacement each day, and Δ_t is the time step in days (Δ_t = 1 day) over which the model is evaluated. To determine if metabolic tissue replacement was important in the rate of isotopic change, we compared models where metabolic replacement (m) was either zero (growth only) or constant through time using the Akaike information criterion (AIC) for least squares (Burnham and Anderson 1998). We calculated the uncertainty in our estimates of m by bootstrapping model residuals ($\delta^{13}C_{\text{observed}} - \delta^{13}C_{\text{predicted}}$). For each bootstrap iteration, we resampled the vector of residuals with replacement, we added the resampled error to each fish tissue $\delta^{13}C$ observation in the time series, and we refit the model (eq. 2) to create a distribution of m estimates (Efron and Tibshirani 1993). Uncertainty in m is represented as the standard deviation of these 1000 estimates. All analyses were performed with the R statistical package (R Development Core Team 2008).

To compare our rates of isotope change to other studies, we calculated a single rate of carbon isotope change (λ) that includes the effects of growth and metabolic replacement by simulating tissue $\delta^{13}C$ over a static $\delta^{13}C$ diet switch. We assumed a fish started in equilibrium with its diet at a $\delta^{13}C$ of –25‰ and was switched to a constant diet with a $\delta^{13}C$ of –15‰. For a given species and age, simulations followed the bioenergetic starting masses, growth trajectories, and time periods listed in Table 1. We calculated fish tissue $\delta^{13}C$ with eq. 2, using bioenergetic daily growth estimates, the fitted metabolic replacement parameter (m), and a constant diet of –15‰. We then fit the single-compartment, time-based expo-

nential model of Martínez del Rio and Wolf (2005) to the simulated fish $\delta^{13}C$ estimates. The model was

$$\delta X_{\text{tissue}(t)} = \delta X_{\infty} - \left(\delta X_{\infty} - \delta X_{\text{tissue}(0)}\right) e^{-\lambda t} \quad (3)$$

where $\delta X_{\text{tissue}(t)}$ is the $\delta^{13}C$ at time t, δX_{∞} is the isotopic value of the tissue in equilibrium with its new diet, $\delta X_{\text{tissue}(0)}$ is the isotopic value of the tissue prior to the diet switch, and λ is isotopic turnover, which includes the combined effects of growth and metabolic replacement. We fit λ by minimizing the sum of squares using optimization routines in the R statistical package (R Development Core Team 2008). The half-life of carbon in dorsal muscle was calculated as

$$\text{half} - \text{life} = \frac{\ln(2)}{\lambda} \quad (4)$$

where half-life is represented in days. In these simulations, daily growth followed bioenergetic model estimates, rather than assuming a single exponential growth rate as in many isotope change studies where fish are grown at a single temperature and ration. Accordingly, we could not partition λ into single rates of isotopic change due to growth and metabolic replacement. Instead, we separately summed the daily mass contributions of growth and replacement for each of the 1000 bootstrap iterations (eq. 2). We report the proportional importance of growth and replacement to isotopic change as the mean and standard deviation of these 1000 proportions.

We combined our estimates of λ with those from the literature to determine the relationship among fish size, water temperature, and λ. We restricted our literature search to studies that reported some measure of fish size, water temperature, and λ. For each study or different experimental condition, we used the single reported water temperature, the reported mean temperature, or modal water temperature if a range was given. Fish mass was represented by a single fish mass or a mean mass at the beginning of an experiment. Martínez del Rio et al. (2009), using relationships from Gillooly et al. (2001), proposed that an animal's isotopic incorporation rate should relate to individual mass and temperature-regulated metabolic rate as

$$\lambda \sim m_b^{-\frac{1}{4}} \cdot e^{\frac{-E_a}{kT}} \quad (5)$$

where m_b is fish mass, E_a is the energy of activation of metabolic processes (constant), k is the Boltzmann's constant, and T is temperature (in Kelvin). We evaluated the influences of fish size and temperature using a simplified form of eq. 5, where the natural log of λ is a function of the natural log of mass, where logλ is a function of mass and is temperature represented as an Arrhenius exponential (T^{-1}), or where logλ is a function of mass and temperature is represented with an exponential approximation (T). We compared models using AIC for least squares (Burnham and Anderson 1998).

Results

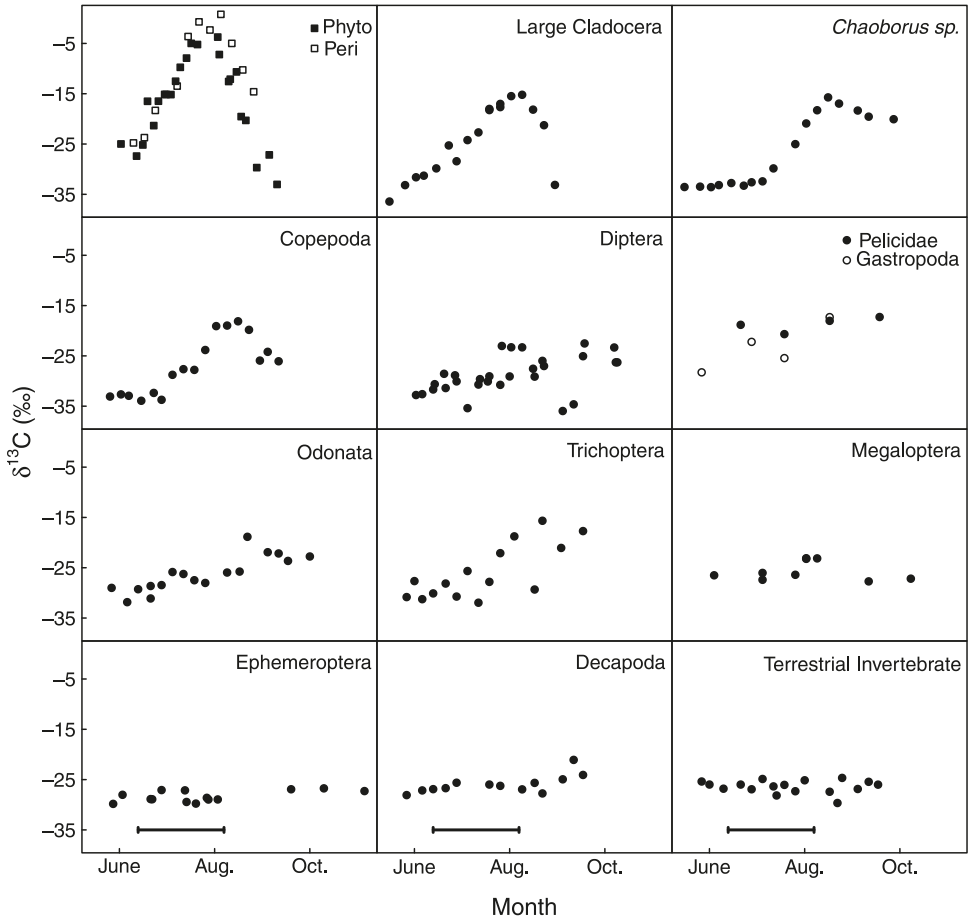
The added sodium bicarbonate (>99% ^{13}C content) elevated the lake water dissolved inorganic carbon $\delta^{13}C$ from an initial value of approximately –12‰ to 5‰–15‰. The $\delta^{13}C$ of both pelagic and benthic primary production rapidly increased 20‰–30‰ during the ^{13}C addition and returned to pre-addition $\delta^{13}C$ levels shortly after the addition ended (Pace et al. 2007). We observed large, consistent increases in zooplankton $\delta^{13}C$ (15‰–20‰), while benthic invertebrate $\delta^{13}C$ increases were less extreme and more variable among groups (0‰–13‰, Fig. 1). The $\delta^{13}C$ of terrestrial diet taxa did not increase over the course of the addition (mean = –26.4‰, 1 SD = 1.2‰; Fig. 1). Increases in invertebrate $\delta^{13}C$ resulted in fish diet $\delta^{13}C$ increases of 10‰–15‰ (Fig. 2).

Of the 25 taxa used to describe the fish diets, six or fewer diet categories represented most of the taxon-specific consumption estimates for any species or age class. Diets of age 2 and age 5 bluegill were chiefly composed of Trichoptera, Odonata, Diptera, and terrestrial invertebrates, while age 0 bluegill consumed small Cladocera (*Bosmina* sp. and *Chydorus* sp.), Trichoptera, and Diptera (Table 2). Ages 1 and 2 yellow perch diets were less diverse, consuming Odonata, Trichoptera, Diptera, and age 0 bluegill (Table 2). Age 0 yellow perch and largemouth bass consumed small Cladocera throughout the season, as well as Odonata and Diptera larvae (Table 2). Age 0 and age 1 yellow perch and bluegill were the primary components in both age 1 and age 6 largemouth bass diets (Table 2). Supplementary diet samples collected in May and June 2006 for juvenile fishes were similar to those collected in 2005 (same taxa) and consisted primarily of small benthic invertebrates (Trichoptera and Diptera) and small Cladocera (*Bosmina* sp. and *Chydorus* sp). We found no linear relationship in the C:N ratio for any of the 16 prey categories for which we had three or more samples of percent carbon and nitrogen ($0.06 < p < 0.75$). Across taxa, our assumed prey energy densities were negatively correlated with the average prey C:N ratio ($p < 0.01$).

The model that included the metabolic replacement parameter m had a lower AIC than the growth-only model for each species and age class, except age 0 bluegill (Table 3). Support for including m in the yellow perch models was weak, as indicated by small differences between model AIC values (0.4–2.4). We report goodness of fit statistics and estimated model parameters for all the models including metabolic replacement (Table 4). Standard errors of model residuals ranged from 0.27‰ to 0.90‰ and were substantially smaller than the observed increases in fish tissue $\delta^{13}C$ (5‰–20‰, Table 3). Estimates for λ ranged from 0.0060 to 0.0840 $g \cdot g^{-1} \cdot day^{-1}$ and were negatively associated with fish size (Table 4). The exponential model accurately fit the simulated diet-switch data for all species and age classes and yielded half-life estimates that ranged from 8 days for age 0 yellow perch to 173 days for age 6 largemouth bass (Table 4). In our simulations, the proportional importance of growth (0.21–0.96) and metabolic replacement (0.02–0.79) in contributing to overall isotopic change was variable and was not associated with size.

We combined our isotopic change results with those of 13 previously published studies that reported some measure of fish size and water temperature. We excluded one lambda value (0.0006) from Sweeting et al. (2005) as an outlier, given it was sevenfold smaller than the next smallest lambda value that we considered. There was a strong negative relationship between λ and fish mass (Fig. 3; Table 5). Includ-

Fig. 1. $\delta^{13}C$ time series of primary producers (upper left panel; solid squares, phytoplankton; open squares, periphyton) and invertebrate taxa (solid and open circles, all other panels) during the experimental ^{13}C addition, Crampton Lake, 2005. Month labels on the *x* axis represent the first day of each month. The horizontal line above the *x* axis demarcates the timing of the ^{13}C addition. Primary producer $\delta^{13}C$ data, from Pace et al. (2007), is included for reference to secondary consumers and as a proxy for $\delta^{13}C$ of *Bosmina* sp. and *Chydorus* sp. Diptera and Odonata $\delta^{13}C$ data are from both Solomon et al. (2008) and invertebrates collected from fish diets. All other invertebrate samples were collected from fish diets in this study.



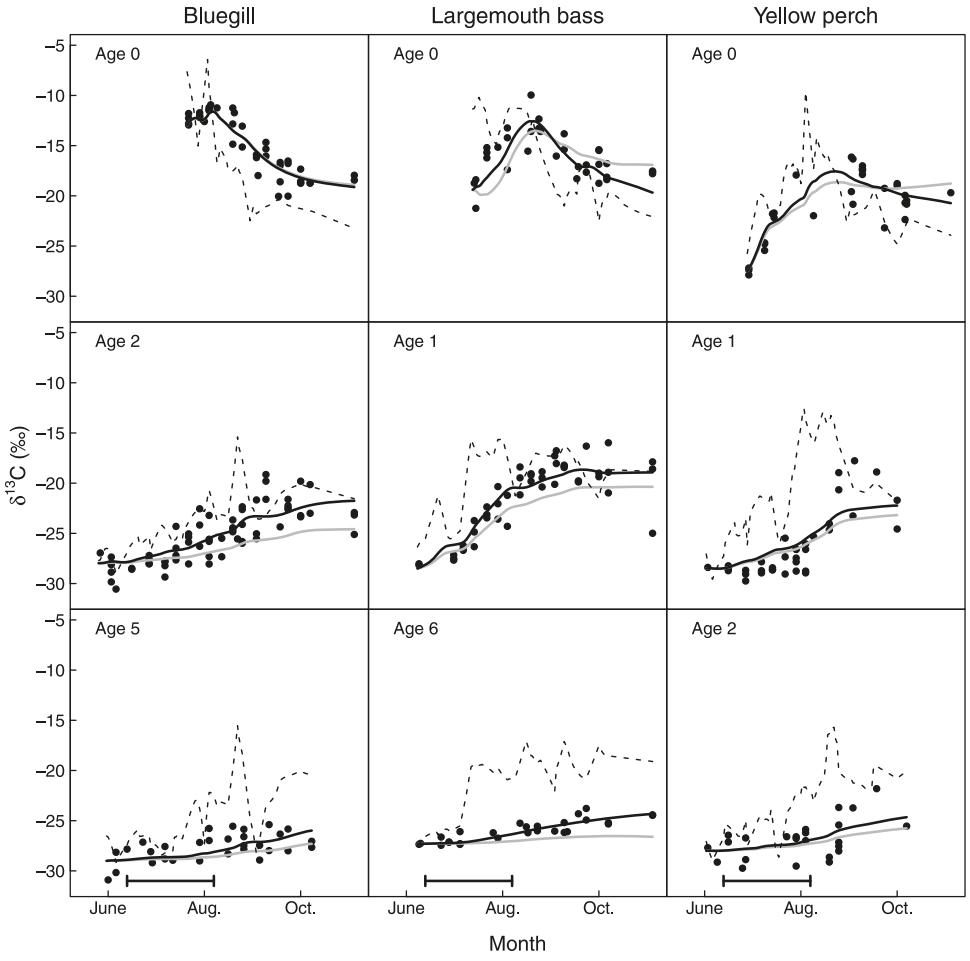
ing water temperature or the inverse of water temperature as an additional explanatory variable did not significantly improve the prediction of turnover rate based on AIC (Table 6).

Discussion

We found that models including a tissue replacement parameter were generally better supported by the $\delta^{13}C$ data than models predicting carbon turnover based solely on growth. There was only weak support for the replacement term in yellow perch, where model AIC values differed by less than 2.5. Our results are consistent with more recent isotopic turnover studies that found metabolic replacement can be a significant component of isotopic turnover (Suzuki et al. 2005; Logan et al. 2006; Tarboush et al. 2006). These conclusions are in contrast with studies that assert growth is the dominant factor influencing $\delta^{13}C$ change in fishes (Sakano et al. 2005; Perga and Gerdeaux 2005). The generalizations about what controls a fish's isotopic turnover rate based on early studies may have been biased by the sizes of organisms used and the conditions under which initial experiments were conducted (Tarboush et al. 2006). Early studies used juvenile fishes, with high growth/body size ratios, held in temperatures and conditions that were optimal for growth (Hesslein et al. 1993; Herzka and Holt 2000). Mass-specific growth rates are maximized in larval and juvenile stages, so studies on these life stages are likely a poor predictor of the relative importance of growth and metabolic replacement to isotopic turnover over the entire life of a fish, particularly older ages. While initial turnover studies concluded that the contribution of metabolic tissue replacement to isotopic turnover was small or negligible, some studies suggested this result may not be consistent in older, slower growing individuals (Fry and Arnold 1982).

The variable influence of tissue replacement in predicting age 0 fish $\delta^{13}C$ dynamics was unanticipated based on previous studies that concluded growth accounted for the majority of isotopic change in juvenile fishes (Vander Zanden et al. 1998; Sakano et al. 2005). Interspecific variability in the bioenergetics model parameter that represents the proportion of maximum consumption (*p* value) may explain differences in the importance of replacement in age 0 fishes. This bioenergetics parameter can be difficult to quantify. It is influenced by a range of physiological factors such as activity and ration quality. A *p* value of 1.0 represents maxi-

Fig. 2. Observed and model-predicted $\delta^{13}C$ values for different age classes of largemouth bass, bluegill, and yellow perch from Crampton Lake, 2005. Points represent dorsal muscle $\delta^{13}C$ observations. The broken line represents the diet $\delta^{13}C$. The solid black line is the model-predicted $\delta^{13}C$ including a term for metabolic tissue replacement. The solid grey line is the model-predicted $\delta^{13}C$ based on growth alone, without metabolic replacement. Month labels on the *x* axis represent the first day of each month. The horizontal line above the *x* axis demarcates the timing of the ^{13}C addition.



mum consumption for a given fish size and temperature. The age 0 bluegill *p* value was 0.88, suggesting bluegill growth over the 2005 season was near the maximum value for the given temperature conditions. Conversely, the *p* values for age 0 yellow perch ($p = 0.40$) and largemouth bass ($p = 0.57$) were much lower than that for bluegill. For both of these species, including the metabolic replacement term increased the model's support, although the support in yellow perch models was weak. Similarly, Witting et al. (2004) found evidence of tissue replacement in juvenile summer flounder with growth rates below their maximum rate (~60%), but lacked statistical power to include replacement as a significant predictor of isotope change. Our results suggest the relative importance of growth and metabolic turnover may be linked to growth efficiencies, where the rate of carbon isotope change is driven by growth when growth efficiencies are high and more related to metabolic rate when growth efficiencies are low. Future turnover studies, whether in natural or laboratory settings, would benefit from treatments that directly address how fish growth rate or growth efficiencies influence the proportional importance of metabolic replacement and growth in isotopic change.

The bioenergetics models provide a method to explicitly account for temperature, food and consumer energy densities, and size-specific physiology in isotopic change studies. This flexible approach may be useful in understanding how natural complexities that are difficult to simulate in laboratory conditions influence isotopic rates of change, including temporal variation and dependence on food abundance and quality (Martínez del Rio et al. 2009). The choice of appropriate bioenergetic model parameters is important and can influence analyses and interpretation that depend on these models of fish growth dynamics. For example, the bluegill and yellow perch bioenergetic models used have been shown to underestimate consumption by inaccurately accounting for fish activity costs (Rowan and Rasmussen 1996; Trudel and Rasmussen 2001). These underestimates of consumption would likely have had little influence on our rates of carbon isotope change, as fish diet proportions and annual growth would remain unchanged, and therefore the fish diet $\delta^{13}C$, which drive our model estimates of *m*, would likely not change appreciably. The bioenergetics models used in this study predict the growth pattern for the average fish and therefore cannot account for individual-

Table 2. Species- and age-specific diet composition by dry mass (percentage) for bluegill, yellow perch, and largemouth bass from Crampton Lake, 2005 and spring of 2006.

	Blugill			Largemouth bass			Yellow perch		
Diet taxa	Age 0	Age 2	Age 5	Age 0	Age 1	Age 6	Age 0	Age 1	Age 2
Amphipoda		<1	3			<1		3	3
Bluegill, YOY		<1	4	30	12	11		7	5
Bluegill, age 1+						12			
Chaoborus sp.		<1	5	2					
Cladocera, large	26	8	5	21	3		37	18	<1
Cladocera, small	26	1	<1	7			15		<1
Copepoda	5	3	<1	16			15	<1	<1
Decapoda			2		1	10			4
Diptera	21	22	13	3	2	<1	7	11	9
Ephemeroptera		3	3		<1	1		1	4
Gastropoda			2			<1			
Hirundinea			2						
Johnny darter			1			8			
Largemouth bass, YOY					9	7		2	
Largemouth bass, age 1+						4			
Lepidoptera		<1	3						
Megaloptera	<1		3		<1	<1	2		
Odonata	4	18	16	3	12	2	12	49	53
Terrestrial vertebrate						2			
Terrestrial invertebrate	1	17	16	6	9	3		2	3
Trichoptera	17	25	18	2	3	1	11	5	11
Unidentifiable invertebrates			<1	1		<1			
Yellow perch, YOY			1	10	48	13		1	9
Yellow perch, age 1+					1	25		1	25
Veneroida		3	3		1			1	

Table 3. Sample sizes and Akaike information criterion (AIC) values for $\delta^{13}C$ time series models without a metabolic replacement term ($m = 0$) and where m is constant over time.

Species	Age	$\delta^{13}C$ (N)	$m = 0$, $p = 1$	m = constant, $p = 2$
Bluegill	0	36	9.5	11.2
	2	31	111.1	66.4
	5	36	23.6	16.9
Largemouth bass	0	29	53.2	38.4
	1	42	76.5	48.1
	6	25	16.1	–28.2
Yellow perch	0	25	36.8	34.4
	1	34	52.6	51.6
	2	25	28.4	28.0

Note: The number of penalized parameters (p) used in AIC calculations, including the error term, is noted under each model.

level variability in growth or diet. Additionally, our bioenergetic approach cannot statistically estimate separate rates of isotopic change due to growth and metabolic replacement. In contrast, isotope change studies that employ static diet $\delta^{13}C$ shifts and model growth exponentially or independently measure growth can statistically estimate the importance of these processes in influencing the isotopic change in fish tissue.

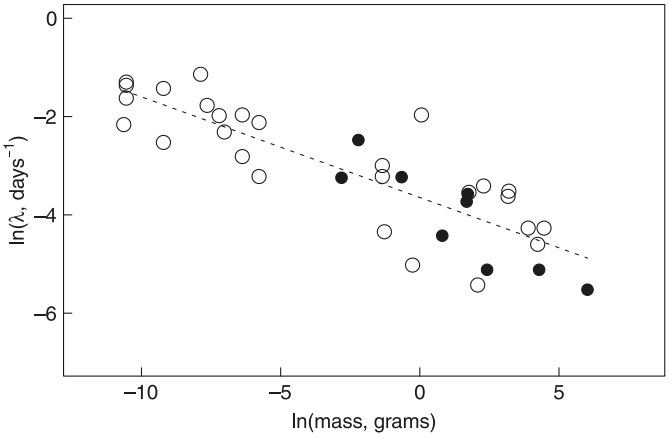
A number of important assumptions may influence our results. First, we did not mathematically correct for or extract lipids from fish tissue samples prior to analysis, although this likely had little effect because lipid content in muscle tissue of our study species is low. Post et al. (2007) concluded lipid correction is not necessary when tissue C:N ratio was <3.5; mean C:N ratios were 3.2, 3.5, and 3.6 for bluegill, yellow perch, and largemouth bass, respectively. Second, most studies of aquatic consumers assume a discrimination factor between diets and tissue of 0‰–1‰ (DeNiro and Epstein 1978). We chose to use a consistent discrimination factor of 0.80, which was the mean estimate from seven fish species (Vander Zanden and Rasmussen 2001). If the true isotopic shift between a fish tissue and diet in our study was closer to zero, our model estimates of half-life decrease on average by 12% (range: –33% to +3%). Conversely, if the isotopic shift is closer to 2.0‰ as suggested by Pinnegar and Polunin (1999) and Barnes et al. (2007), our half-life estimates increase by an average of +30%, although results are variable (range: 20% decrease to 100% increase). Similarly, a higher assumed discrimination factor increases the relative importance of metabolic replacement in isotopic change, while a discrimination factor of zero results in a greater proportion of change being attributed to growth. Well-constrained discrimination factor estimates are critically important for isotopic turnover studies that use dynamic models such as those in this study or for studies with larger individuals, because large fishes will rarely reach equilibrium with dietary $\delta^{13}C$. While bootstrapping provided an estimate for uncertainty around m, this certainly represents a lower bound estimate because we did not explicitly account for variability in growth, diet, diet item $\delta^{13}C$, or discrimination factor. Accounting for these additional sources of variability would not have ultimately

Table 4. Summary of species and age model results for the model that includes a constant tissue replacement (*m*).

Species	Age	*m* (SD)	Mean error (‰)	Growth (SD)	Replacement (SD)	λ (day^{-1})	Half-life (days)
Bluegill	0	0.0010 (0.0018)	0.27	0.96 (0.06)	0.02 (0.03)	0.0390	18
	2	0.0143 (0.0026)	0.43	0.32 (0.04)	0.68 (0.03)	0.0240	29
	5	0.0033 (0.0012)	0.27	0.43 (0.08)	0.57 (0.08)	0.0060	116
Largemouth bass	0	0.0228 (0.0058)	0.43	0.42 (0.06)	0.58 (0.06)	0.0395	18
	1	0.0121 (0.0027)	0.85	0.45 (0.09)	0.55 (0.06)	0.0280	25
	6	0.0031 (0.0003)	0.08	0.21 (0.02)	0.79 (0.02)	0.0040	173
Yellow perch	0	0.0131 (0.0076)	0.65	0.46 (0.17)	0.54 (0.17)	0.0840	8
	1	0.0026 (0.0014)	0.90	0.92 (0.10)	0.08 (0.10)	0.0120	58
	2	0.0029 (0.0010)	0.56	0.66 (0.17)	0.34 (0.17)	0.0060	116

Note: We report the fitted value for *m*, with bootstrapped error estimates, the mean error between observed and predicted $\delta^{13}C$ measurements (‰), and the proportion of isotopic change, by mass, due to growth and metabolic replacement processes. Standard deviations (SD) of *m* and the proportions of isotopic change due to growth and replacement were computed with 1000 bootstrap iterations. Additionally, we report the results of the simulated static diet switch, where λ represents the overall isotopic turnover rate, and dorsal muscle half-life is calculated as ln(2)/λ.

Fig. 3. Fish dorsal muscle isotopic turnover is negatively related to fish size. The broken line ($\log(\lambda) = -3.65 - 0.20\log(\text{mass})$, $r^2 = 0.71$) represents the natural log relationship from both literature values (open circles) and values from this study (solid circles) as estimated by ordinary least squares. Note that the slope is similar to –0.25, which is the slope predicted by allometric and metabolic theory (Martínez del Rio et al. 2009).



changed our best estimates of *m*, but would likely increase our confidence bounds for this parameter.

The large $\delta^{13}C$ contrast between fish diets and fish tissue provided by the stable isotope addition was fundamental to estimating isotopic turnover rates in our study system. While isotope additions can be cost prohibitive at the ecosystem scale, researchers have taken advantage of changes in fish behavior and dietary $\delta^{13}C$ to estimate tissue turnover rates in natural systems. Such studies have included migration, rapid development, and seasonal diet changes (Vander Zanden et al. 1998; Maruyama et al. 2001; Perga and Gerdeaux 2005). Bioenergetics models offer an alternative analytical approach for understanding isotopic change in these natural settings.

The combination of frequent diet sampling and the ^{13}C addition identified a less traditional lake food web connection that was critical in predicting juvenile fish $\delta^{13}C$ dynamics. The $\delta^{13}C$ of age 0 bluegill and age 0 largemouth bass peaked at approximately –10‰, while the most enriched invertebrate food source we measured, pelagic zooplankton, only reached –16‰. Our results suggest these fishes did not rely on large Cladocera as their primary food source, but must have obtained energy from a source with higher $\delta^{13}C$. Diet data revealed that small-bodied Cladocera, primarily *Bosmina* sp., were the dominant zooplankton found in age 0 fish diets, but these species were not abundant in pelagic zooplankton samples (Pace et al. 2007). Because we did not specifically measure the $\delta^{13}C$ of these small-bodied zooplankton taxa, we assumed the $\delta^{13}C$ of primary production approximated their carbon isotope value. This assumption is supported by the fast relative growth rates and small body size of *Bosmina* sp. compared with larger Cladocera (e.g., *Holopedium gibberium*, *Diaphanasoma bergei*) (Pennak 1978) and the fact that their midsummer abundance peak in lakes of this region coincided with the ^{13}C addition (Carpenter and Kitchell 1993). If the $\delta^{13}C$ of small-bodied Cladocera were more negative than we assumed, fish diet $\delta^{13}C$ would have been lower, and fish $\delta^{13}C$ time series models would have attributed a higher proportion of isotopic change to metabolic replacement. Subsequent investigations in Crampton Lake have recorded high densities (>1000 individuals per litre) of *Bosmina* sp. at total depths of 1–2 m. Together, these observations suggest that zooplankton heterogeneity between pelagic and nearshore habitats may play an important role in understanding carbon sources supporting fish. An alternative explanation for the high $\delta^{13}C$ values of age 0 fishes is that gape limitation of age 0 fishes restricted them to consume smaller benthic invertebrates, which may have had a higher $\delta^{13}C$ relative to larger conspecifics, but were underrepresented in pooled dietary $\delta^{13}C$ samples. The discrepancy between juvenile fish tissue $\delta^{13}C$ and measured prey $\delta^{13}C$ highlights the importance of dietary $\delta^{13}C$ information to our modeling approach. Future studies employing this approach may find it useful to directly analyze $\delta^{13}C$ from homogenized fish diets rather than separating diet items to be analyzed.

The long tissue half-lives of adult fishes in our study contradict the oft-held assumption of equilibrium between consumers and their diet (Gannes et al. 1997; Tarboush et al. 2006). Our result reinforces the need to consider the diet legacy incorporated into a point estimate for a tissue $\delta^{13}C$ value. This long period of incorporation means the small

Table 5. Previously published carbon turnover rates (λ), half-life, and experimental conditions for studies on fish muscle tissue and whole fish.

Species	λ (%·day^{-1})	SD	Half-life (days)	Temp. (°C)	Mass (g)	Study
Red drum (*Sciaenops ocellatus*)	0.1150		6.0	28.00	2.40×10^{-5}	Herzka and Holt 2000*,†
	0.1380		5.0	28.00	7.40×10^{-4}	Herzka and Holt 2000*,†
	0.0990		7.0	24.00	8.90×10^{-4}	Herzka and Holt 2000*,†
Channel catfish (*Icatalurus punctatus*)	0.0140		49.5	15.00	8.67×10^{1}	MacAvoy et al. 2001
Winter flounder (*Pleuronectes americanus*)	0.1700	0.024	4.1	13.00	4.80×10^{-4}	Bosley et al. 2002*
	0.3200	0.038	2.2	18.00	3.80×10^{-4}	Bosley et al. 2002*
Pintado (*Pseudoplatystoma corruscans*)	0.0066		105.0	26.69	7.70×10^{-1}	Furuya et al. 2002
Japanese flounder (*Paralichthys olivaceus*)	0.1400		5.0	18.50	1.06×10^{0}	Tominaga et al. 2003
	0.0400		17.3	18.15	2.60×10^{-1}	Tominaga et al. 2003
	0.0500		13.9	17.00	2.60×10^{-1}	Tominaga et al. 2003
Summer flounder (*Paralichthys dentatus*)	0.0800	0.02	8.7	13.00	1.00×10^{-4}	Witting et al. 2004*
	0.2400	0.02	2.9	22.00	1.00×10^{-4}	Witting et al. 2004*
	0.0600	0.01	11.6	13.00	1.70×10^{-3}	Witting et al. 2004*
	0.1400	0.02	5.0	22.00	1.70×10^{-3}	Witting et al. 2004*
	0.0400	0.06	17.3	13.00	3.10×10^{-3}	Witting et al. 2004*
	0.1200	0.04	5.8	22.00	3.10×10^{-3}	Witting et al. 2004*
Japanese temperate bass (*Lateolabrax japonicus*)	0.0330		21.0	23.00	9.87×10^{0}	Suzuki et al. 2005
European sea bass (*Dicentrarchus labrax*)	0.0044		157.5	12.00	8.00×10^{0}	Sweeting et al. 2005
Nile tilapia (*Oreochromis niloticus*)	0.0297		23.3	25.00	2.446×10^{1}	Zuanon et al. 2006
	0.0267		26.0	25.00	2.378×10^{1}	Zuanon et al. 2006
Zebra danio (*Danio rerio*)	0.0130		53.3	28.50	2.80×10^{-1}	Tarboush et al. 2006
Sand goby (*Pomatoschistus minutus*)	0.0290	0.011	23.9	17.00	5.85×10^{0}	Guelinckx et al. 2007
Senegalese sole (*Solea senegalensis*)	0.2730		2.5	21.50	2.63×10^{-5}	Gamboa-Delgado et al. 2008*
	0.2560		2.7	21.50	2.63×10^{-5}	Gamboa-Delgado et al. 2008*
	0.1970		3.5	21.50	2.63×10^{-5}	Gamboa-Delgado et al. 2008*
Summer flounder (*Paralichthys dentatus*)	0.0101		69.0	19.90	5.88×10^{1}	Buchheister and Latour 2010
	0.0140		49.0	20.40	1.02×10^{2}	Buchheister and Latour 2010
Bluegill (*Lepomis macrochirus*)	0.0390	0.0018	18.0	21.50	6.00×10^{-2}	This study
	0.0240	0.0026	29.0	21.50	5.38×10^{0}	This study
	0.0060	0.0012	116.0	21.50	7.27×10^{1}	This study
Largemouth bass (*Micropterus salmoides*)	0.0395	0.0058	18.0	21.50	5.20×10^{-1}	This study
	0.0280	0.0027	25.0	21.50	5.60×10^{0}	This study
	0.0040	0.0003	173.0	21.50	4.13×10^{2}	This study
Yellow perch (*Perca flavescens*)	0.0840	0.0076	8.0	21.50	1.10×10^{-1}	This study
	0.0120	0.0014	58.0	21.50	2.24×10^{0}	This study
	0.0060	0.001	116.0	21.50	1.116×10^{1}	This study

Note: Tissue half-life is calculated as ln(2)/λ. Values from this table were used to draft Fig. 3.
*Whole organism sampled as opposed to white muscle tissue.
†Values taken from Herzka 2005.

differences observed in the natural abundance of carbon isotopes between energy sources are of limited use in understanding short-term feeding changes in larger or older life stages. MacAvoy et al. (2001) concluded that long half-lives of carbon in muscle tissue precluded detecting an isotopic signal in adult blue catfish (*Icatlurus furcatus*) feeding on migratory prey fishes with a distinctly different isotopic value. Estimates of tissue half-lives are required to determine the duration of feeding or the difference in $\delta^{13}C$ of the diet necessary to detect a change in the $\delta^{13}C$ of these larger consumers. Conversely, short half-lives of carbon indicate tissue $\delta^{13}C$ measurements may only represent recent dietary $\delta^{13}C$ and do not necessarily integrate dietary information over longer time periods. Fish tissues with short half-lives would be useful for inferring short-term changes in behavior or migration, whereas long half-life tissues would be useful for inferring longer-term phenomena such as average annual food web connections.

Table 6. Model results and parameter values for models relating the natural log of the rate of isotopic change (λ) to the natural log of fish mass (*M*), and water temperature (*T*, in Kelvin).

Model	*N*	r^2	AIC	*a* ± SE	*b* ± SE	*c* ± SE
$\log(\lambda) = a + b\cdot\log(M)$	36	0.71	78.8	–3.65±0.17*	–0.20±0.02*	
$\log(\lambda) = a + b\cdot\log(M) + c_1\cdot(T^{-1})$	36	0.72	80.8	–2.46±7.89	–0.20±0.02*	346.99±2319.04
$\log(\lambda) = a + b\cdot\log(M) + c_2\cdot(T)$	36	0.72	80.7	–15.51±9.65	–0.22±0.03*	0.04±0.03

Note: In the second equation, $c_1 = -E_a/k$. Regression coefficients noted with an asterisk (*) were significant at $p < 0.001$.

Factors affecting carbon turnover rates

In reviewing the state of biological isotope ecology, Martínez del Rio et al. (2009) note ecologists should understand isotopic turnover rates and factors that influence those rates to (*i*) determine the time window through which tissues reflect dietary isotope change and (*ii*) explore resource use variability through tissues with different turnover rates. We would add that understanding relationships between turnover rates and simple gradients, such as body size, increase the utility of stable isotopes to quantify material transport in food webs rather than simply providing qualitative descriptions (Schindler and Lubetkin 2004). Combining rates of carbon isotope change from this and other studies, we established a significant negative relationship between fish size and the rate of isotopic change. McIntyre and Flecker (2006) found similar evidence for the negative scaling of nitrogen isotope change with body size in tropical ectotherms. While the slope of the fish size and carbon turnover rate relationship was similar to –0.25, which has been proposed for some allometric-based metabolic models, it is important to recognize this particular exponent is not universally accepted (Glazier 2010). The intention of our comparison was not to support or reject specific metabolic scaling coefficients, but rather to draw attention to the utility of metabolic theory in understanding isotopic incorporation rates in animals. The proposed allometric relationship relating dorsal muscle λ and fish mass can be used to approximate the value of muscle λ and half-life based solely on fish mass and thereby aid ecologist's interpretation of isotopic patterns.

We expected that including temperature as a predictive variable along with mass would improve our capability to estimate λ (Gillooly et al. 2001). Including the temperature term in the regression model slightly increased the predictive capacity from $r^2 = 0.61$ (mass alone), to $r^2 = 0.62$ (mass and temperature) and, based on AIC, the improvements were not adequate to support the additional parameter in the model. The ranges of temperature and fish size examined likely limited the improvement in the two parameter model. In those studies that reported both a measure of water temperature and fish size, the range of temperatures considered (14–24 °C) would approximately double metabolic rate, whereas the mass range (0.00012–434 g) would cause a sixfold change in metabolic rate (Clark and Johnston 1999). The positive coefficient of temperature in models predicting λ supports the positive influence of temperature on the rate of isotopic change (Bosley et al. 2002; McIntyre and Flecker 2006).

The utility of carbon stable isotopes in defining energy sources and visualizing niche space has been well established (France 1995; Vander Zanden and Vadeboncoeur 2002). To move beyond static linkages and employ carbon stable isotopes to quantify nutrient or energy flux in food webs, we must have an estimate of the dietary legacy that a consumer's $\delta^{13}C$ represents. By taking advantage of a ^{13}C addition in a natural system, we demonstrated this legacy can range from a week to multiple years within a single lake fish community. We found carbon stable isotope change in muscle tissue is not solely regulated by growth, but that metabolic replacement influences the rate at which a consumer's muscle $\delta^{13}C$ comes to reflect the $\delta^{13}C$ of their diet. In short, fish size has a fundamentally important role in the rates of stable isotope changes for fish muscle tissue. By combining bioenergetic models with frequent dietary $\delta^{13}C$ estimates, we established a method to determine isotopic turnover and its components in natural systems where consumers exhibit dramatic changes in dietary $\delta^{13}C$.

Acknowledgements

We thank the University of Notre Dame Environmental Research Center East administration and staff for providing access to Crampton Lake. We thank J. Tetzlaff, M. Provost, K. Amend, J. Fox, S. Jones, N. Preston, J. Coloso, S. Powers, K. McDonnell, T. Van Schyndel, S. Healy, C. Solomon, J. Cole, M. Pace, J. Hodgson, and P. Lisi for field and laboratory support. We thank C. Martínez del Rio, M. Trudel, J. Weidel, and an anonymous reviewer for comments on previous manuscript versions. We thank the staff of the University of California – Davis Stable Isotope laboratory for sample analysis. This work was funded by the National Science Foundation Ecosystems Studies Program (DEB-0414258), the Research Experiences for Undergraduates Program, the Chase Noland Fellowship for Undergraduate Research (University of Wisconsin – Madison), and the Anna Grant Birge Fellowship Program (University of Wisconsin – Madison).

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