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Incorporation of carbon and nitrogen isotopes in age-0 walleye (*Sander vitreus*) tissues following a laboratory diet switch experiment

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Abstract: Trophic dynamics are often described by following the exchange of naturally occurring isotopes through aquatic communities. However, without experimentally derived isotopic turnover rates and discrimination factors for each species, tissue, and life stage, these trophic models can be misleading. We conducted a laboratory diet shift experiment to describe isotopic turnover and discrimination in age-0 walleye (*Sander vitreus*) dorsal muscle and gutted carcass samples. Although turnover of dietary $\delta^{13}C$ (half-life: 10–12 days) and $\delta^{15}N$ (half-life: ~13 days) signatures was relatively rapid, the diet change was undetected in both tissues during a short transitional period (up to 1.2 times shorter in muscle). Our discrimination estimates generally conform to those of other fishes (Δ_{Carbon} = 0.91, $\Delta_{Nitrogen}$ = 1.6), but were 30%–50% higher in muscle tissues than in gutted carcass samples. The assumption that young walleye tissues are in equilibrium with their diet is untrue for weeks following a diet shift, and when incorporated, discrimination factors differ between tissues. We provide tissue-specific parameters that remove uncertainty associated with the analysis of field collected isotopic age-0 walleye data.

Résumé : La dynamique trophique est souvent décrite en suivant les échanges d'isotopes présents dans la nature au sein de communautés aquatiques. Sans taux de renouvellement isotopique ou facteurs de discrimination établis expérimentalement pour chaque espèce, tissu et étape du cycle biologique, ces modèles trophiques peuvent toutefois ne pas refléter la réalité. Nous avons mené une expérience de changement de régime alimentaire en laboratoire pour décrire le renouvellement et la discrimination isotopiques dans des échantillons de muscle dorsal et de carcasse éviscérée de doré jaune (*Sander vitreus*) de moins d'un an. Si le renouvellement des signatures alimentaires de $\delta^{13}C$ (demi-vie : 10–12 jours) et de $\delta^{15}N$ (demi-vie : ~13 jours) est relativement rapide, le changement de régime alimentaire est indétectable dans les deux tissus durant une courte période de transition (jusqu'à 1,2 fois plus courte dans le muscle). Les valeurs de discrimination estimées correspondent généralement aux valeurs obtenues pour d'autres poissons ($\Delta_{carbone}$ = 0,91, Δ_{azote} = 1,6), mais sont 30–50 % plus élevées dans les échantillons de tissu musculaire que dans ceux de carcasse éviscérée. L'hypothèse voulant que les tissus de jeunes dorés jaunes soient en équilibre avec leur alimentation n'est pas avérée pour plusieurs semaines suivant un changement de régime alimentaire et, quand ils sont intégrés, les facteurs de discrimination diffèrent pour différents tissus. Nous présentons des paramètres propres au tissu qui réduisent l'incertitude associée à l'analyse de données isotopiques de terrain obtenues pour des dorés jaunes de moins d'un an. [Traduit par la Rédaction]

Introduction

Stable isotope analyses track the exchange of naturally occurring isotopes in animal tissues and, using consumer–resource ratios and assumed discrimination factors, infer energy sources and trophic relationships (DeNiro and Epstein 1978, 1981; Post 2002; McIntyre and Flecker 2006). Isotope signatures act as dietary tracers of assimilated organic matter and provide quantitative measures of trophic interactions among food-web components through space and time (DeNiro and Epstein 1978, 1981; Vander Zanden et al. 2015; Franssen et al. 2017). Although other elements have been applied to isotope studies, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are the most commonly used in aquatic systems (DeNiro and Epstein 1978, 1981; Fry and Arnold 1982; Post 2002; Vander Zanden et al. 2015).

Following a diet change, the isotopic compositions of new resources are assimilated into animal tissues over some uncertain period of time via metabolic pathways and somatic growth (Boecklen et al. 2011; Vander Zanden et al. 2015). Inferences from isotopic data assume that, when sampled, consumer tissues have reached isotopic equilibrium with their diet (Pinnegar and Polunin 1999; Post 2002; Vander Zanden et al. 2015). Fishes opportunistically feed on abundant prey, alter food sources during movements, and undergo multiple ontogenetic diet shifts (Ross 2013). If individuals retain isotopic signatures of previous prey items for long periods before new diets are assimilated, the equilibrium assumption can be violated (Herzka and Holt 2000; MacAvoy et al. 2001; Gaye-Siessegger et al. 2004; Vander Zanden et al. 2015; Franssen et al. 2017).

A large number of laboratory isotopic diet shift experiments have estimated trophic half-lives for diverse taxa and tissues (see Boecklen et al. 2011; Vander Zanden et al. 2015). Although these rates are still unknown for most fishes and tissues, research shows

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Table 1. Mean isotopic values and standard deviations (SD) measured throughout the diet switch experiment for age-0 walleye (*Sander vitreus*) tissues with comparison to brine shrimp diet and artificial diet signatures.

			$\delta^{13}C$		$\delta^{15}N$	
Source	Tissue	*n*	Mean	SD	Mean	SD
Experimental diet	Brine shrimp — sample 1	5	–20.80	0.03	12.16	0.09
	Brine shrimp — sample 2	5	–21.01	0.04	12.34	0.08
	Artificial diet	10	–21.59	0.10	8.62	0.14
Before diet switch (day –1)	Dorsal muscle	4	–18.66	0.15	16.12	0.16
	Gutted carcass	4	–18.86	0.25	15.52	0.10
Initial (day 1)	Dorsal muscle	6	–18.55	0.07	16.14	0.34
	Gutted carcass	6	–18.50	0.09	15.66	0.23
Final (day 36)	Dorsal muscle	10	–20.33	0.07	11.49	0.29
	Gutted carcass	10	–20.69	0.09	10.37	0.29

Note: Brine samples were measured twice during the course of the study, before the 7-week acclimation period and prior to the experimental diet switch, to measure consistency of diet signatures.

that isotopic half-lives have varied widely among elements, species, tissues, and life stages (Gaye-Siessegger et al. 2004; Jardine et al. 2005; McIntyre and Flecker 2006; Boecklen et al. 2011). The variability in isotopic half-lives has hindered the development of robust predictive models for all fish species and tissue type combinations (Post 2002; Boecklen et al. 2011; Vander Zanden et al. 2015). However, a recent review suggests that fish size strongly influences the rate of isotopic turnover, which occurs more quickly in small individuals (Hesslein et al. 1993; Buchheister and Latour 2010; Vander Zanden et al. 2015). In the absence of experimentally derived isotopic turnover rates and discrimination factors, widely applied trophic models are potentially misleading (Post 2002; Martinez del Rio et al. 2009; Boecklen et al. 2011; Bond and Diamond 2011).

Although stable isotope analyses offer unique insight into fish trophic ecology and can benefit fisheries management, including adult walleye (*Sander vitreus*), the technique is fraught with concerns when applied to novel species, tissues, and life stages (Overman and Parrish 2001; Boecklen et al. 2011; Fincel et al. 2014, 2015). Herein, we provide the first estimates of nitrogen and carbon isotopic turnover rates and discrimination factors in two age-0 walleye tissues. Walleye typically undergo a series of ontogenetic diet shifts in the first growing season, initially consuming zooplankton, then macroinvertebrates, and finally fish (Mathias and Li 1982; Johnson and Mathias 1994; Chipps and Graeb 2011). Walleye feeding habits (Fox 1989; Overman and Parrish 2001; Chipps and Graeb 2011; Bethke et al. 2012; Fincel et al. 2015) and population dynamics (Sogard 1997; Uphoff et al. 2013) are largely influenced by prey size and availability during their first growing season. Precise understanding of the dynamics of isotopic turnover and discrimination may allow managers to ascertain the conditions in which walleye growth and survival are maximized during the critical, first growing season.

We used a laboratory diet switch that mimics the ontogenetic dietary transitions of young walleye during their first growing season to estimate isotopic discrimination and turnover (Graeb et al. 2005). Although most studies use dorsal white muscle for isotopic analyses in fishes (Vander Zanden and Rasmussen 2001; Boecklen et al. 2011), obtaining enough tissue can be problematic for small individuals (Minagawa and Wada 1984; Pinnegar and Polunin 1999). Isotopic relationships between muscle and gutted carcass samples were compared to assess tissue-specific variability. The specific objectives of this study were to (*i*) empirically estimate the turnover rates and discrimination factors of nitrogen and carbon in dorsal muscle and gutted carcass samples using the best available model; (*ii*) compare the dependence of isotope signatures on growth and metabolism in both tissues; and (*iii*) describe the relationship between stable isotope ratios of carbon and nitrogen sampled from dorsal muscle and gutted carcass samples. This isotopic analysis will contribute to better descriptions of walleye ontogeny and may improve management outcomes for ecologically and economically valuable fisheries (Post and Evans 1989; Rice et al. 1993; Sogard 1997).

Materials and methods

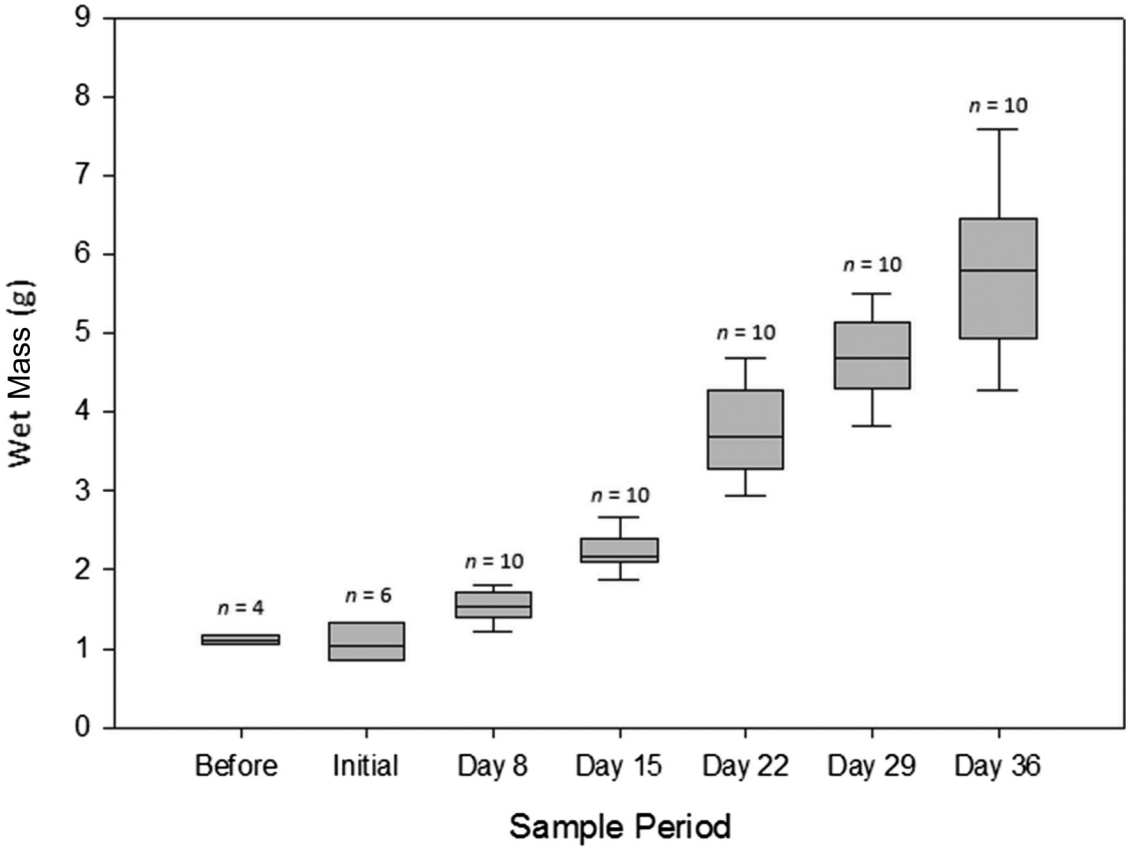
Fish collection and husbandry

The Calamus State Fish Hatchery in Nebraska provided age-0 walleye larvae for this study (mean total length (TL) = 9.0 mm; range: 8.5–9.5 mm). Hatchery staff spawned the walleye from eggs collected from wild brood stock. Walleyes were received by University of Nebraska at Kearney wet laboratory prior to yolk sac absorption and were housed in 38 L aquaria at a density of six individuals per litre. Following the onset of allochthonous feeding, larval walleyes were fed brine shrimp (*Artemia* spp.) ad libitum for 7 weeks to acclimate to laboratory conditions. The acclimation period allowed the walleye to reach lengths representative of those in wild populations when shifting to piscivory (Chipps and Graeb 2011; Uphoff et al. 2013). We assumed that parentally derived isotopic signatures were completely diluted (Vander Zanden et al. 1998) and that isotopic equilibrium was reached with the brine shrimp before the diet switch. The acclimation period approximates the half-lives of nitrogen (mean = 35.9 days, standard error (SE) = 7.1 days) and carbon (mean = 34.3 days, SE = 6.8 days) isotopes in similarly sized fish tissues (Vander Zanden et al. 2015). We maintained a 13 h light : 11 h dark photoperiod and tank temperatures at 20 °C, conditions typical of Nebraska reservoirs during walleye development (Uphoff et al. 2013).

Prior to the start of the diet shift experiment on 13 July 2011, we separated 77 juvenile walleye (mean TL = 54.8 mm, SE = 0.25 mm; range: 54–55 mm) into a 194 L recirculating chamber filled with dechlorinated municipal water. We sacrificed four walleyes from the experimental group prior to the start of the experiment (12 July 2011) to measure the tissue isotopic signatures before the diet switch. The remaining individuals (n = 73) were fed a diet of commercial dry fish feed, hereinafter referred to as "artificial feed" (Otohime C2: 840–1410 μm; composition: >20% fish meal, >35% krill, >15% squid meal; Reed Mariculture, Inc.). Fish were fed approximately 4.4% of their body mass daily, and uneaten food was removed after fish stopped feeding to maintain water quality.

The artificial feed emulated the ontogenetic diet shifts of walleye during their first growing season (Table 1). Stable isotope ratios of nitrogen ($^{15}N/^{14}N$) and carbon ($^{14}C/^{13}C$) were measured from the brine shrimp (n = 5) twice and the artificial feed (n = 10) once to ensure consistency in the dietary isotopic signature. Brine shrimp isotopic signatures were measured at the beginning of the 7-week acclimation period and 4 days prior to the diet switch. The artificial diet isotopic signatures were assumed constant because a single package was used during the experiment. We pooled indi-

Fig. 1. Observed wet mass (g) of age-0 walleye (*Sander vitreus*) before and after experimental diet shift, with number of individuals sacrificed each sample period.



vidual brine shrimp and feed granules and measured a single sample of 3 mg dry mass for each diet.

We randomly euthanized and sampled tissues from 6–10 individuals each week for 5 weeks to monitor the carbon and nitrogen isotope signatures in both tissues (University of Nebraska at Kearney Institutional Animal Care and Use Committee #042811). We sampled fish on day 1 (n = 6; 13 July 2011) and again on experiment days 8, 15, 22, 29, and 36 (Fig. 1). We weighed (g) and measured each sacrificed fish to TL (mm). We withheld food for 12 h prior to sacrifice to allow for gut evacuation. In the laboratory, we excised samples of the dorsal muscle from above the pectoral fin and anterior to the dorsal fin for each sample. We rinsed the dorsal muscle sample and the remaining gutted carcass with deionized water and dried each at 75 °C for 48 h. Because tissues within the body cavity turn over at different rates (Jardine et al. 2005), all visceral organs were removed from the composite gutted carcass sample. Tissue samples were ground with mortar and pestle and packaged into a 4 mm × 6 mm tin capsule before being placed in glass scintillation vials awaiting further analysis.

Sample processing

Prepared tissue samples were analyzed for carbon and nitrogen stable isotopes at the Cornell University Stable Isotope Laboratory (Ithaca, New York) using a Finnigan MAT Delta Plus isotope ratio mass spectrometer. Stable isotope ratios were expressed in delta (δ), in parts per thousand (‰), 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 carbon or nitrogen, and R is the mass ratio of the heavy to light stable isotopes (e.g., $^{15}N/^{14}N$) for both the sample and standard. Standards were Pee Dee Belemnite for carbon and atmospheric nitrogen. Repeated measurements of a calibration standard at 10 sample increments indicated that instrument precision was 0.08‰ and 0.25‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Ratios of carbon to nitrogen (C: N) were calculated from measured amounts in each sample and ranged between 3.2 and 5.2 (mean = 3.7, SE = 0.05). We corrected $\delta^{13}C$ values mathematically to reduce lipid bias (Post et al. 2007; Logan et al. 2008):

$$(2) \qquad \delta^{13}C_{\text{corrected}} = (\delta^{13}C - 3.32 + 0.99) \times C:N$$

Isotopic turnover and discrimination

Several distinct model structures can be used to estimate isotopic turnover and discrimination in consumer tissues following a diet change (Boecklen et al. 2011; Vander Zanden et al. 2015). The different modeling frameworks express isotopic turnover as a function of either mass change or time (Vander Zanden et al. 2015). Because fish growth and metabolic processes can affect isotopic signatures, we used growth- and time-based models to assess isotopic change in age-0 walleye tissues and compared their relative fits.

Growth-based model

We fit a growth-based model (Fry and Arnold 1982) to walleye isotopic values to quantify the relationship between growth rates and isotopic turnover after consuming an enriched, artificial diet (Buchheister and Latour 2010; Franssen et al. 2017). This model predicts the isotopic signature of consumer tissues given its relative increase in mass as

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

where δ_{W_R} is the predicted isotopic signature of a fish tissue, δ_f is the expected isotopic signature at equilibrium, and δ_i is the initial isotopic value prior to the diet shift. We used the mean isotope signature from the four individuals sacrificed prior to the diet shift for δ_i. The relative increase in wet mass (W_R) was calculated as the walleye wet mass when sampled divided by the mean initial wet mass (mean = 1.10 g, SE = 0.027 g), and c is the curve-fitted turnover rate constant. Values of c equal to –1 indicate that isotopic change is only a result of somatic growth, whereas values of $c < -1$ suggest that metabolic processes influence isotopic turnover rates. A z test was used to test the estimated parameter c against the null hypothesis, $c = -1$ (Zar 2010).

We calculated the amount of change in wet mass needed to achieve α percent turnover of $\delta^{13}C$ and $\delta^{15}N$ ($G_{\alpha/100}$) as

$$G_{\alpha/100} = \exp\left[\frac{\ln\left(1 - \frac{\alpha}{100}\right)}{c}\right] \quad (4)$$

The growth-based half-life ($G_{0.5}$) was obtained when $\alpha = 50\%$ and represents the amount of growth needed to observe 50% conversion in consumer tissues. We calculated the change to sample tissues derived from growth (D_g) and from metabolic activity (D_m) using the half-life (Buchheister and Latour 2010):

$$D_g = \frac{2(G_{0.5} - 1)}{G_{0.5}} \quad (5)$$

$$D_m = \frac{(2 - G_{0.5})}{G_{0.5}} \quad (6)$$

Time-based models

We also estimated changes in stable isotope ratios through time (t) using a time-based exponential model developed by Hesslein et al. (1993):

$$\delta_t = \delta_f + (\delta_i - \delta_f)\exp[-(k' + m)t] \quad (7)$$

where m is a metabolic turnover constant. The growth rate constant k' was derived from an exponential growth model of walleye growth:

$$W_f = W_i \exp(k't) \quad (8)$$

where W_i is the initial wet mass, and W_f is the final mass when sampled on day t. In the time-based model, if turnover is due to growth alone, then m will equal 0. The values of δ_t, δ_i, and t were measured or calculated, whereas δ_f, m, and k' parameters were estimated using nonlinear regression.

We estimated the length of time needed to achieve α percent turnover of $\delta^{13}C$ or $\delta^{15}N$ as follows (Buchheister and Latour 2010):

$$(T_{\alpha/100}) = \left[\frac{\ln\left(1 - \frac{\alpha}{100}\right)}{-(k' + m)}\right] \quad (9)$$

We calculated the isotope half-life ($T_{0.5}$) at $\alpha = 50\%$.

We estimated the relative contributions of growth ($k' = 0.048$) and metabolism (m) as the ratio of each parameter to the sum of the two parameters. This calculation yielded the proportion of turnover attributable to growth (P_g) and metabolism (P_m). A z test was used to test m against the null hypothesis that $m = 0$ (Zar 2010).

Discrimination

Discrimination factors for each isotope and tissue combination were calculated as the difference between the mean isotopic signature of the diet and the predicted isotopic equilibrium. Discrimination of $\delta^{13}C$ and $\delta^{15}N$ between the artificial diet and both dorsal muscle and the gutted carcass sample (Δ_{Tissue}) were calculated following Minagawa and Wada (1984); Buchheister and Latour (2010):

$$\Delta_{Tissue} = \delta_f - \delta_d \quad (10)$$

where δ_d is the mean stable isotope signature for the samples of the artificial diet. Standard error values associated with the fractionation estimate were based on errors related with the model estimate of δ_f (SE_{δ_f}) and the standard error of the mean for δ_d (SE_{δ_d}):

$$SE_{\Delta Tissue} = \sqrt{SE_{\delta_f}^2 + SE_{\delta_d}^2} \quad (11)$$

Model selection

An information-theoretic model selection approach was used to assess relative support for four candidate models: model A: growth-based with a metabolic contribution to turnover; model B: growth-based with no metabolic contribution to turnover (i.e., $c = -1$); model C: time-based with a metabolic contribution to turnover; and model D: time-based with no metabolic contribution to turnover (i.e., $m = 0$). We compared model fit using Akaike's information criterion corrected for small sample sizes (AIC_c; Burnham and Anderson 2002).

Relationship of dorsal muscle signature to gutted carcass signature

Linear regression analysis was used to describe the relationships between tissue types for each isotope. Regression parameters (slope, intercept) were used to develop a predictive model for estimating dorsal muscle isotope values from gutted carcass values. All statistical analyses and parameter estimates were conducted in SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Thirteen fish (18%) died during the study; however, the remaining individuals ($n = 60$) were sufficient to sample stable isotope signatures throughout the study's duration. Walleye mean TL nearly doubled (final TL = 92.9 mm, SE = 1.80 mm) and mean wet mass increased by more than five times during the experimental period (Fig. 1). Estimated growth rates of experimental walleye (specific growth rate (SGR); range: 2.8–6.6%·day^{-1}) mirrored wild age-0 growth rates in Nebraska reservoirs (SGR: 6.1%·day^{-1}; C. Uphoff, unpublished data).

Initial $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures were similar between tissue types, and both tissues were different than the artificial diet ($\delta^{15}N$: $F_{[2,15]} = 5321.42$, $p < 0.01$; $\delta^{13}C$: $F_{[2,15]} = 1712.90$, $p < 0.01$; Table 1). The isotopic signatures of the artificial diet and brine shrimp were significantly different ($\delta^{15}N$: $F_{[1,18]} = 3438.66$, $p < 0.01$; $\delta^{13}C$: $F_{[1,18]} = 2796.99$, $p < 0.01$; Table 1). After the diet shift, isotopic turnover of walleye tissues was evident for both $\delta^{15}N$ and $\delta^{13}C$ (Fig. 2). Time-based models were consistently supported by $\delta^{13}C$ and $\delta^{15}N$ assimilation data measured in muscle and gutted carcass samples (Table 2). The time-based model, parameterized to exclude the influence of metabolism ($m = 0$; model D), performed better than the other candidate models in all cases (Table 2). Model D was, on average, 6.2 times more likely to be the best-fitting model for our data than the next best model in the candidate set (range: 3.05 – 15.00; Table 2). The comparatively large ΔAIC_c values of other competing models suggest that the influence of metabolism on isotopic turnover of these tissues is weak during the rapid growth period evaluated (Table 2).

Fig. 2. Changes in $\delta^{13}C$ (top panels) and $\delta^{15}N$ (bottom panels) of age-0 walleye (*Sander vitreus*) tissues as a function of relative growth (W_R; left panels) and time (days; right panels) since an experimental diet switch. Data (mean ± SD) and growth- and time-based model fits are shown for dorsal muscle (solid circles, continuous line) and gutted carcass (open circles, broken line) samples.

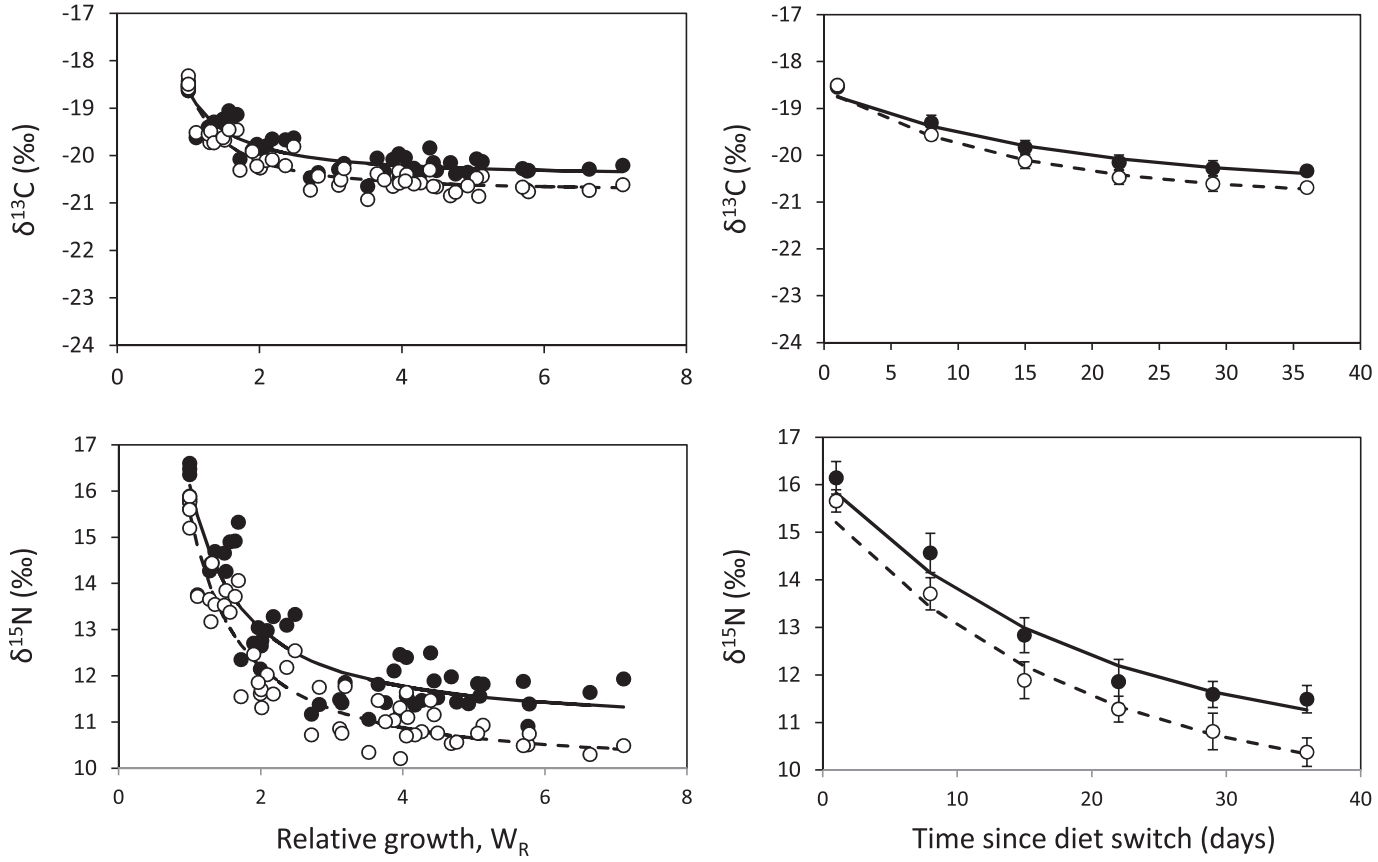


Table 2. Comparisons of ΔAIC_c and W_i values among four isotope turnover models.

	$\delta^{13}C$				$\delta^{15}N$			
	Dorsal muscle		Gutted carcass		Dorsal muscle		Gutted carcass	
	ΔAIC_c	W_i	ΔAIC_c	W_i	ΔAIC_c	W_i	ΔAIC_c	W_i
Model A	9.1	0.01	13.7	0.00	6.9	0.02	7.7	0.02
Model B	3.7	0.19	5.4	0.06	2.5	0.17	9.5	0.01
Model C	2.6	0.11	6.2	0.04	2.3	0.20	2.4	0.23
Model D	0.0	0.70	0.0	0.90	0.0	0.61	0.0	0.75

Note: Model A, growth-based with metabolic contribution to turnover; model B, growth-based with no metabolic contribution; model C, time-based with metabolic contribution to turnover; model D, time-based with no metabolic contribution. For all models, $n = 56$ and $k = 3$.

Based on the empirically best supported time-based model, rates of isotopic turnover were equal between tissue types for $\delta^{15}N$ (Table 3; Fig. 2). A larger m value indicated that turnover of $\delta^{13}C$ occurred more quickly in gutted carcass samples than in dorsal muscle tissues (Table 3; Fig. 2). Assimilation rates of the artificial diet were 1.01 times ($\delta^{15}N$; time-based model estimate) and 1.24 times ($\delta^{13}C$; time-based model estimate) faster in the gutted carcass composite than in the dorsal muscle samples (Table 4). Half-lives for $\delta^{15}N$ in the dorsal muscle and gutted carcass tissues were estimated as 13.0 and 13.2 days for age-0 walleye, respectively (Table 4). Further calculation found that a 95% isotopic turnover of $\delta^{15}N$ in age-0 walleye tissue would require approximately 56 days for both tissue types evaluated (Table 4). The amount of time necessary for a 50% conversion of $\delta^{13}C$ in dorsal muscle and gutted carcass composite were estimated as 12.1 and 9.7 days for age-0 walleye, respectively (Table 4). A 95% isotopic turnover of $\delta^{13}C$ in age-0 walleye samples would require nearly 52.4 days for dorsal muscle and 42.1 days for the gutted carcass composite (Table 4). Although not as well-supported by the data, growth-based parameter estimates (Table 3; Fig. 2) and turnover half-lives are provided (Table 5). The half-life for $\delta^{15}N$ and $\delta^{13}C$ for both tissues occurred after individuals reached masses approximately 1.5 times their initial wet mass (Table 5).

Metabolic contributions to $\delta^{13}C$ turnover were apparent for gutted carcass samples when estimated using the time-based model; however, there was no evident contribution of metabolism to the turnover of $\delta^{15}N$ (Table 3). The time-based $\delta^{13}C$ model indicated that metabolic activity was important to isotopic turnover of gutted carcass composite tissues because the turnover constant value (m) was significantly different than zero ($z = 3.85$, $p < 0.001$; Table 3). The time-based model predicted that metabolic activity accounted for 32% of $\delta^{13}C$ turnover and only 9% of $\delta^{15}N$ turnover in the gutted carcass sample (Table 4). In growth-based models, metabolic activity contributed significantly to the turnover of carbon

Table 3. Parameter estimates (standard error (SE) in parentheses) and calculations from the growth- and time-based models of $\delta^{13}C$ and $\delta^{15}N$ turnover in age-0 walleye (*Sander vitreus*) tissues.

Isotope	Tissue	Model	c (SE)	m (SE)	δ_f (SE)	Δ (SE)
$\delta^{15}N$	Dorsal muscle	Growth-based	–1.323 (0.18)	—	10.94 (0.36)	2.32 (0.37)
		Time-based	—	0.005 (0.01)	10.42 (0.19)	1.80 (0.21)
	Gutted carcass	Growth-based	–1.344 (0.22)	—	10.02 (0.29)	1.39 (0.31)
		Time-based	—	0.004 (0.01)	9.42 (0.31)	0.80 (0.33)
$\delta^{13}C$	Dorsal muscle	Growth-based	–1.524 (0.25)*	—	–20.43 (0.11)	1.16 (0.11)
		Time-based	—	0.008 (0.01)	–20.65 (0.11)	0.94 (0.12)
	Gutted carcass	Growth-based	–1.788 (0.19)*	—	–20.74 (0.08)	0.84 (0.08)
		Time-based	—	0.023 (0.08)*	–20.90 (0.08)	0.69 (0.08)

Note: Abbreviations and parameter estimates: c, turnover constant; m, metabolic turnover constant (day^{-1}); δ_f, final asymptotic isotopic value (‰); and Δ, discrimination between the artificial diet and each tissue (‰). Metabolic turnover constants (m and c) that indicate a significant metabolic contribution to tissue turnover at $p < 0.05$ are marked with an asterisk (*).

Table 4. Isotopic ($\delta^{15}N$ and $\delta^{13}C$) turnover rates for age-0 walleye (*Sander vitreus*) tissues following an experimental diet switch.

Isotope	Tissue	$T_{0.50}$	$T_{0.95}$	P_g	P_m
$\delta^{15}N$	Dorsal muscle	13.0	56.3	0.91	0.09
	Gutted carcass	13.2	56.9	0.92	0.08
$\delta^{13}C$	Dorsal muscle	12.1	52.4	0.85	0.15
	Gutted carcass	9.7	42.1	0.68	0.32

Note: Both time-based half-life ($T_{0.5}$) and number of days to reach 95% turnover ($T_{0.95}$), as well as the proportion of turnover contributed to dilution through growth (P_g) and metabolic activity (P_m), are presented.

Table 5. Isotopic ($\delta^{15}N$ and $\delta^{13}C$) turnover rates for age-0 walleye (*Sander vitreus*) tissues following an experimental diet switch.

Isotope	Tissue	$G_{0.50}$	$G_{0.95}$	D_g	D_m
$\delta^{15}N$	Dorsal muscle	1.7	9.6	0.82	0.18
	Gutted carcass	1.7	9.3	0.81	0.19
$\delta^{13}C$	Dorsal muscle	1.6	7.1	0.73	0.27
	Gutted carcass	1.5	5.3	0.64	0.36

Note: The relative increase in growth necessary for 50% ($G_{0.5}$) and 95% turnover ($G_{0.95}$) to the new diet, as well as the proportion of turnover contributed to dilution through growth (D_g) and metabolic activity (D_m), are presented.

in both dorsal muscle and gutted carcass composite tissues (Table 3).

Discrimination

At the start of the study, walleye tissue $\delta^{15}N$ values were approximately 3‰ removed from the brine shrimp diet and nearly 8‰ removed from the mean isotopic signature of the artificial diet (Table 1). Initial values of $\delta^{13}C$ were about 5‰ removed from the artificial diet in both tissues (Table 1). Regardless of the model applied, estimates of $\delta^{15}N$ isotopic equilibrium were dissimilar between tissues (Table 3). Discrimination estimates for $\delta^{15}N$ in the gutted carcass were approximately half of those from dorsal muscle samples (Table 3). The time-based model predicted discrimination of $\delta^{15}N$ as 0.80 ± 0.33 in gutted carcass sample and 1.80 ± 0.21 in dorsal muscle tissue (Table 3). Estimates of $\delta^{13}C$ isotopic equilibrium were more similar, but were still about 30% higher in dorsal muscle tissues than in gutted carcass samples (Table 3). Discrimination of $\delta^{13}C$ was 0.94‰ for dorsal muscle and 0.69‰ for gutted carcass samples (Table 3).

Relationships of stable isotope signatures between tissues

All 60 walleye (TL = 49–101 mm) yielded adequate amounts of both tissues for stable isotope analysis and were used to compare dorsal muscle and gutted carcass composite isotopic values (Fig. 3). Both model structures predicted more rapid turnover of both elements in gutted carcass samples than in dorsal muscle tissues (Fig. 2). There was a significant difference between dorsal muscle and gutted carcass composite isotope signatures for both $\delta^{15}N$ ($F = 145.91$, $p < 0.001$) and $\delta^{13}C$ ($F = 76.9$, $p < 0.001$) when controlling for time. However, a positive linear relationship was observed between tissue signatures for $\delta^{15}N$ (slope = 0.92, intercept = 1.77, $r^2 = 0.94$) and $\delta^{13}C$ (slope = 0.79, intercept = –3.97, $r^2 = 0.85$) isotopes (Fig. 3).

Discussion

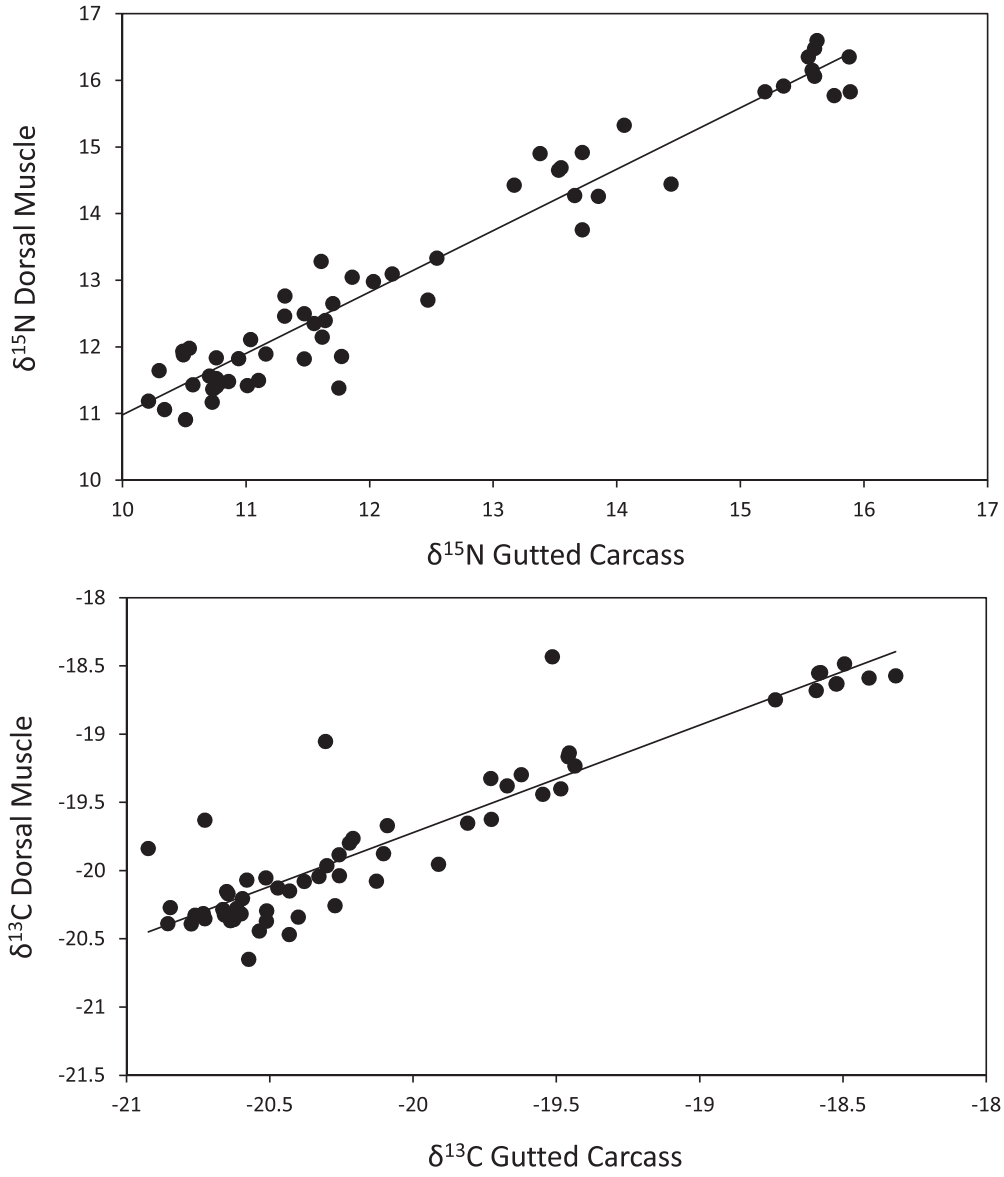
Common to fast-growing age-0 fishes, isotopic turnover occurred relatively quickly in walleye tissues during the diet shift and was only slightly influenced by metabolic processes. Although discrimination factors generally conformed to those of other fishes, they varied by tissue. We provide mathematical corrections to convert isotopic signatures between tissues, when sampling is tissue prohibitive. Even though turnover was fairly rapid in both walleye tissues, the transitional period during which consumer tissues do not fully reflect the diet being consumed should be considered when interpreting field-collected isotopic data (Boecklen et al. 2011; Vander Zanden et al. 2015).

For all isotope–tissue combinations, the time-based models were more supported by available data than growth-based methods. Time-based models were also best supported in similar research on turnover processes of other fish species (Bosley et al. 2002; Gaye-Siessegger et al. 2004; Franssen et al. 2017). Variability in individual walleye growth rates had little influence on parameter discrepancies, thereby supporting the use of a single population growth parameter to estimate isotopic turnover dynamics. Although these experimental trials provided considerable support for the time-based model parameter estimates, it may be important to evaluate this relationship when considerable variation in growth rates are induced, such as with alterations to temperature and feeding rates (Bosley et al. 2002; Gaye-Siessegger et al. 2004; Vander Zanden et al. 2015).

Differences in turnover rates between the tissues were relatively small, but turnover always occurred more quickly for $\delta^{13}C$ than for $\delta^{15}N$. Although analogous research has demonstrated the same relationship in other fishes, the tissues evaluated had little metabolic activity (e.g., Hesslein et al. 1993; McIntyre and Flecker 2006; Boecklen et al. 2011). Isotopic turnover likely occurs faster in more metabolically active tissues such as mucus, liver, and blood (Suzuki et al. 2005; Logan et al. 2006; MacNeil et al. 2006; Church et al. 2009; Buchheister and Latour 2010; Chen et al. 2012). Including additional tissue types, presumably with more rapid turnover, may aid in developing more complete dietary histories and further our understanding of fish feeding ecology in early life stages (Overman and Parrish 2001; Jardine et al. 2005). This technique has already proven advantageous for adult walleye (Fincel et al. 2012). The rate in which new diets are integrated into fish tissues is particularly important when studying temporal trophic dynamics, which would remain ambiguous without these tissue and element-specific assimilation parameters (Herzka and Holt 2000; MacAvoy et al. 2001; Franssen et al. 2017).

Similar to studies of other fast-growing ectothermic animals, somatic growth and to a lesser extent metabolic tissue replacement mediated isotopic turnover in young walleye tissues (Fry

Fig. 3. Relationships between dorsal muscle tissue and gutted carcass sample isotopic values for $\delta^{15}N$ (top panel) and $\delta^{13}C$ (bottom panel) from 60 age-0 walleye (*Sander vitreus*).



and Arnold 1982; Hesslein et al. 1993; Buchheister and Latour 2010). Somatic growth has controlled the rate of isotopic dilution in other species (Hesslein et al. 1993; Herzka and Holt 2000; MacAvoy et al. 2001; Bosley et al. 2002; Logan et al. 2006; Buchheister and Latour 2010; Vander Zanden et al. 2015) and largely contributed to isotopic turnover of nitrogen in young walleye. As with other studies of isotopic turnover in fishes, metabolic processes contributed more to carbon assimilation, particularly for gutted carcass samples (Suzuki et al. 2005; Franssen et al. 2017). Several explanations exist to explain different isotopic responses between elements, often citing metabolism and lipid concentrations (MacAvoy et al. 2001; Suzuki et al. 2005; Buchheister and Latour 2010); however, ontogenetic changes to carbon isotopic signatures have been recently recognized (Franssen et al. 2017). Our analysis provides additional evidence that carbon isotope signatures undergo ontogenetic changes over time. Strong support for the time-based models, larger metabolic contributions, and faster turnover of carbon suggest that ontogeny may alter $\delta^{13}C$ isotopic signatures (Franssen et al. 2017). Although somatic growth strongly influences isotopic turnover in walleye tissues, isotopic changes to carbon signatures seem to include additional physiological changes.

Discrimination between prey isotopic compositions and those assimilated into consumer tissues has been primarily studied for dorsal muscle tissues, but several more recent studies have included additional tissues (Jardine et al. 2005; Church et al. 2009; Buchheister and Latour 2010; Chen et al. 2012). Patterns and variability in diet–tissue discrimination factors have been summarized (Vander Zanden and Rasmussen 2001; Boecklen et al. 2011). Discrimination estimates of $\delta^{15}N$ in young walleye tissues were within the range typically found in the literature (1‰–6‰; McCutchan et al. 2003). However, our estimates of nitrogen discrimination were consistently less than the long-standing intraspecific mean (3.4‰; Vander Zanden and Rasmussen 2001; Post 2002). This is potentially related to the relatively high amount of algivorous krill in the artificial diet. In contrast, our estimates of $\delta^{13}C$ discrimination in walleye tissues conformed well to the traditionally assumed values of 0‰–1‰ (Post 2002; McCutchan et al. 2003). Of particular concern to field isotopic studies, the discrimination values for carbon and nitrogen differed between tissues. Variabil-

ity in discrimination factors by tissue is likely a result of diverse biochemical constituents within each tissue (e.g., amino acids, protein, lipids) and allocation of resources during digestion (DeNiro and Epstein 1978, 1981; Post 2002; McCutchan et al. 2003; Buchheister and Latour 2010). Divergent discrimination factors among tissues could have a large influence on trophic inferences if not accounted for.

High variability in isotopic half-lives and discrimination factors have prompted concern about the reliability of trophic models without experimentally derived species and tissue-specific parameters (Post 2002; Martinez del Rio et al. 2009; Boecklen et al. 2011; Vander Zanden et al. 2015). Although generalized models may provide broad patterns, they may inappropriately define diets and trophic positions of novel species, tissues, and life stages (Boecklen et al. 2011; Vander Zanden et al. 2015). Many researchers sample white muscle because of its low lipid concentration and ease of homogenization (Boecklen et al. 2011). But because sufficient dorsal muscle tissue is often difficult to obtain from small-bodied fishes or larval walleye, our correction factor allows the estimation of dorsal muscle signatures from gutted carcass composite samples for both isotopes evaluated.

Although the turnover of nitrogen and carbon isotopes in age-0 walleye tissues was generally rapid, a transitional period exists in which tissues do not fully reflect the diet being consumed. The assumption that young walleye tissues are in equilibrium with their diet may be untrue for weeks following a diet shift. Sizeable error could be incorporated into dietary analyses using stable isotopes if isotopic turnover rates and tissue-specific discrimination factors are not considered. These experimentally derived, species- and tissue-specific turnover rates and discrimination factors will remove uncertainty currently associated with the analysis of field-collected isotopic age-0 walleye data.

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