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Patterns of stable carbon isotope turnover in gag, *Mycteroperca microlepis*, an economically important marine piscivore determined with a non-lethal surgical biopsy procedure

James Nelson · Jeffrey Chanton · Felicia Coleman · Christopher Koenig

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Abstract To determine the feasibility of using stable isotopes to track diet shifts in wild gag, *Mycteroperca microlepis,* populations over seasonal timescales, we conducted a repeated measures diet-shift experiment on four adult gag held in the laboratory. Fish were initially fed a diet of Atlantic mackerel, *Scomber scombrus,* (mean $\delta^{13}C = -21.3‰ \pm 0.2$, $n=20$) for a period of 56 days and then shifted to a diet of pinfish, *Lagodon rhomboids*, (mean $\delta^{13}C = -16.6‰ \pm 0.6$, $n=20$) for the 256 day experiment. We developed a non-lethal surgical procedure to obtain biopsies of the muscle, liver, and gonad tissue monthly from the same four fish. We then determined the $\delta^{13}C$ value of each tissue by isotope ratio mass spectrometry. For the gonad tissue we used the relationship between C/N and lipid content to correct for the influence of lipids on $\delta^{13}C$ value. We observed a significant shift in the $\delta^{13}C$ values of all of the tissues sampled in the study. Carbon turnover rates varied among the three tissues, but the shift in diet from mackerel to pinfish was clearly traceable through analysis of $\delta^{13}C$ values. The turnover rates for muscle tissue were 0.005‰ day^{-1}, and for gonad tissue was 0.009‰ day^{-1}. Although it is generally thought that tissue turnover rates in ectotherms are driven primarily by growth, we found that metabolic rate can be a major factor driving tissue turnover in adult gag.

Keywords Biopsies · Surgery · Lipid correction · Trophic shift

J. Nelson (✉) · J. Chanton
Department of Oceanography, Florida State University, Tallahassee, FL 32306, USA
e-mail: nelson@ocean.fsu.edu

F. Coleman · C. Koenig
FSU Coastal and Marine Laboratory, 3618 Coastal Highway 98, St. Teresa, FL 32358-2702, USA

Introduction

For most animals, the menu is always changing. Permanent changes in diet typically occur with ontogenetic habitat or diet shifts (Mullaney and Gale 1996; Herzka et al. 2001; Bosley et al. 2002; Post 2003; Post et al. 2007; Logan and Lutcavage 2008). Other changes in diet are temporary, occurring either during annual migrations or with seasonal changes in prey availability (Hobson 1999; Herzka 2005). Determining the timing and magnitude of diet changes can provide information on an animal's life history, food web interactions, and energy flow within and among ecosystems.

Stable isotope analysis has become an important tool for ecologists in addressing a range of topics from food web dynamics to migration patterns (DeNiro and Epstein 1978; Fry and Parker 1979; Peterson and Fry 1987; Chanton and Lewis 2002; Fry 2006). Stable isotopes themselves (e.g., those of carbon, sulfur, nitrogen) occur naturally in biological

material. Primary producers fractionate environmental stable isotope ratios to their own characteristic values according to various environmental characteristics such as, CO_2 availability and temperature. In aquatic species the primary producers' carbon ratio ($^{13}C/^{12}C$) is incorporated into consumer tissues with little fractionation (+0.5–1‰) (DeNiro and Epstein 1978; Fry and Parker 1979; Peterson and Fry 1987; Chanton and Lewis 2002; Post 2002; Fry 2006).

Following a dietary change, the $\delta^{13}C$ values of an animal's tissues will shift towards that of the new diet and given enough time will come to isotopic equilibrium. The time to equilibrium is dependent upon the turnover rate for that tissue. Two processes dictate tissue-specific turnover rates: (1) growth, or the dilution of the previous $\delta^{13}C$ values by addition of new tissue created from the isotopically distinct diet; and (2) metabolic turnover, due to replacement of old tissue by new tissue created from the new diet (Fry and Arnold 1982; Hesslein et al. 1993; Phillips and Eldridge 2006). The interaction of growth and metabolic maintenance gives each tissue type a unique turnover rate (Fry and Arnold 1982; Hesslein et al. 1993; Phillips and Eldridge 2006; Guelinckx et al. 2007). To accurately determine the timing and magnitude of a diet shift, it is essential to know the tissue-specific turnover rates for the organism in question as well as how variability in growth and metabolic activity can alter that rate.

Researchers have examined isotopic turnover rates following diet shifts in a variety of taxa including mammals (Tieszen et al. 1983; Ayliffe et al. 2004), birds (Hobson and Clark 1992), marine invertebrates (Fry and Arnold 1982), and fish (Hesslein et al. 1993; Herzka and Holt 2000; Bosley et al. 2002; Guelinckx et al. 2007). These studies found turnover rates that varied widely with half lives as short as 27 days for gerbil, *Meriones unguienlatus,* muscle to > 1 year for broad whitefish, *Coregonus nasus*, muscle (Tieszen et al. 1983; Hesslein et al. 1993). The major conclusion from each of these studies is that tissue turnover rates are both life-stage and species specific. For example, juveniles undergoing rapid growth generally have higher turnover rates than adults of the same species (Herzka and Holt 2000; Bosley et al. 2002).

The available information on turnover rates is limited for ectotherms and essentially non-existent for large piscivorous fish (Bosley et al. 2002; Logan et al. 2006; Guelinckx et al. 2007; Suring and Wing 2009). The majority of the existing studies suggest that isotopic turnover in fish tissue is driven primarily by growth (Hesslein et al. 1993; Herzka and Holt 2000; MacAvoy et al. 2001; Bosley et al. 2002). Thus, low isotopic turnover rates in fish such as broad whitefish suggest that a dietary shift would take years to be expressed in their tissues (Hesslein et al. 1993). However, more recent work on other species suggests otherwise. In two temperate marsh species, sand gobies, *Pomatoschistus minutus*, (Logan et al. 2006) and killifish, *Fundulus heteroclitus,* (Guelinckx et al. 2007), metabolism significantly influenced tissue turnover, whereas in red rock lobster, *Jasus edwardsii*, and blue cod, *Parapercis colias*, both slow growing ectotherms, metabolism contributed as much as 37% to the isotopic turnover observed (Suring and Wing 2009). Thus the factors that influence the balance between the effects of metabolism and growth on tissue turnover rates in fish are unclear.

Although growth and basal metabolism drive tissue turnover, other factors can influence the stable isotope composition of tissues, thereby confounding calculation of tissue turnover rates. For example, variation in lipid content can significantly alter observed $\delta^{13}C$ values (Sweeting et al. 2006). Lipids can be depleted by approximately 7‰ in ^{13}C relative to the carbon source (DeNiro and Epstein 1977; Sweeting et al. 2006; Post et al. 2007). Fish store these lipids in repository organs such as the liver and mesentery (Sheridan 1988). Lipid content can vary greatly (~30%) depending on physiological condition, life stage, tissue type, or activity of the animal (e.g., spawning, migration).

The most direct way to determine lipid content is by the Folch method for lipid extraction (Folch et al. 1957). However, a much less labor-intensive method involves using a mathematical correction for the lipid effect on $\delta^{13}C$ values (McConnaughey and McRoy 1979; Sweeting et al. 2006; Post et al. 2007; Logan and Lutcavage 2008). Once the lipid-free C/N ratio is established, a correction factor for $\delta^{13}C$ values is derived for lipid content, and the values can be properly interpreted and turnover rates calculated. In this study we develop an arithmetic lipid correction for $\delta^{13}C$ values of the gonad tissue to correct for the increased lipid content during spawning season.

The primary objective of this study was to evaluate the isotopic turnover rates of muscle, liver, and gonad tissue in gag, *Mycteroperca microlepis*, and determine

if the rates are rapid enough to be useful in tracking seasonal changes in gag diet. With the exception of the work on blue cod and lobster by Suring and Wing (2009) no published information exists on the stable isotope tissue turnover rates of large slow growing ectotherms. The fish in our study have slow growth rates and thus will provide insight into the relative rate of metabolic turnover in the tissues examined. The secondary objective was to develop a non-lethal surgical procedure that could be used to repeatedly sample an individual fish's tissues for stable isotope analysis. Following this experiment the fish were donated to a children's aquarium in Tennessee. This technique has obvious advantages in situations where consumptive sampling is neither possible nor desired (e.g. for rare species or within marine protected areas). Although in the wild it is unlikely that the gag will make a 100% switch to seagrass derived prey items, we hypothesize that gag tissue turnover is sufficiently rapid to allow us to determine diet shifts, and that stable isotopic analysis will be a useful tool for elucidating trophic connections between inshore and offshore habitats.

Materials and methods

Capture of wild gag

Four adult gag (one male, three females) (Table 1) were captured using chevron fish traps (2.0 mH, 1.5 mW, 0.6 m D) at depths of 80–100 m fished from an 18 m commercial fishing vessel in during late June 2006 approximately 60 miles south of Panama City, FL, in the Madison Swanson Marine Reserve in the northeastern Gulf of Mexico. Fish trapped on the bottom were raised to a depth of ~30 m where a diver vented each fish's swim bladder with a pole spear outfitted with a special 1-cm diameter point designed to penetrate the fish only to about 3 cm through the body cavity wall. This venting method maximizes survival by circumventing swimbladder embolism (Parker et al. 2006). Once vented, the fish were slowly brought to the surface, placed in a 500 L baffled live well with continuous aeration and circulation onboard the vessel, then transported to the Florida State University Coastal and Marine Laboratory (FSUCML) in St. Teresa, Florida. At the FSUCML, they were held in a closed 20 000 L raceway at 22°C and 35‰ salinity under a natural photoperiod. The initial lengths and weights for the four gag used in this experiment were: Gag 1 Female, 81.5 cm, 7080 g; Gag 2 Male, 99 cm, 12 770 g; Gag 3 Female, 65.5 cm, 3650 g; Gag 4 Female, 78.5 cm 6320 g.

After an initial acclimation period of a few days, the gag became quite gregarious, displayed no signs of stress, and readily fed on dead fresh fish. We therefore believe that the stress of captivity was minimal and did not significantly alter our results.

Stable isotope analysis

Stable isotope analysis was performed using a continuous flow ThermoFinnigan DeltaPlus mass spectrometer coupled to a CHN analyzer at the National High Magnetic Field Laboratory in Tallahassee, Fl. Prior to stable isotope analysis, all samples were dried at 50°C for approximately 48 h and then homogenized using an electric grinder (using the same method described for prey species). Approximately 50 μg of tissue was wrapped in tin capsules and analyzed for $\delta^{13}C$ values as well as%C and%N. All isotope values are reported in the following notation:

$$\delta^{13}C = \left[\left(R_{sample}/R_{standard}\right) - 1\right]*1000 \quad (1)$$

where R is $^{13}C/^{12}C$ and the $\delta^{13}C$ are relative the PeeDee Belemnite standard. The standard error for repeated measurements was ±0.1 ‰.

Table 1 Sex, length and growth data for gag, *Mycteroperca microlepis*, kept in captivity at the Florida State University Coastal and Marine Laboratory during the 256 day experiment

Fish	Sex	Initial total length (cm)	Final length (cm)	Weight initial (g)	Final weight (g)	Weight gain (g)	% weight gain
Gag 1	F	81.5	83	7080	7480	400	5.6
Gag 2	M	99	101	12770	13560	790	6.2
Gag 3	F	65.5	71	3650	4660	1010	27.7
Gag 4	F	78.5	79.5	6320	6560	240	3.8

Diet treatments

To determine if dietary changes in gag could be tracked on seasonal time scales using stable isotopes, the captured fish were fed alternately on a diet of pelagic Atlantic mackerel, *Scomber scombrus,* followed by a diet of benthic pinfish, *Lagodon rhomboides,* an estuarine species known to feed in seagrass beds (Chanton and Lewis 2002). Mackerel were obtained from a bait supplier and pinfish were captured by trawl and fish trap from seagrass beds adjacent to the FSUCML during 1 month in summer and then frozen for use in the winter. Twenty randomly chosen specimens of each prey type were evaluated for species-specific $\delta^{13}C$ values. Preparation for analysis involved drying whole fish at 50°C. The dried specimens were then ground with an electric grinder, and $\delta^{13}C$ values determined using isotope ratio mass spectrometry. The mean and standard deviation of the twenty individuals of each food type is given below.

The mean $\delta\ ^{13}C$ values for mackerel (−21.3‰± 0.2‰, n=20) and pinfish (−16.6‰±0.6‰, n=20) used in the feeding trials were significantly different (t-test, $p<0.00001$). The captured gag were fed the mackerel diet for 8 weeks to ensure that gag were in good condition and acclimated to captivity prior to surgery. This pelagic-derived diet mimicked the diet that wild fish consume offshore at the time of capture (Nelson unpublished data). Following this acclimation period, the captured fish were then fed for 10 months on a diet of pinfish. We began feeding the gag pinfish in September, consistent with the time that wild fish would begin to encounter seagrass-derived prey in the offshore environment during the pre-spawning time for gag.

Tissue biopsies were taken once at the end of the pelagic diet phase and then approximately monthly during the benthic diet phase to determine the rate of tissue turnover from a pelagic to a seagrass-derived $\delta^{13}C$ value. We did not assume that the gag were in equilibrium with the mackerel diet. Rather, we hypothesized that we would be able observe a diet shift reflected by changes in their tissue isotopic values. It is unlikely that these fish were consuming significant quantities of pinfish at the time of their capture because the pinfish egress from seagrass beds to deeper water occurs in the fall, not in the summer, when these fish were captured. No gonad samples were taken from the male gag because the sperm duct was too small to allow a biopsy of the testes to be taken.

Surgical procedure

To obtain tissue samples, we anesthetized the fish in a 100 $mg{\cdot}L^{-1}$ solution of MS-222 for 3 min or until the fish was unresponsive to touch (Murray 2002). The fish were then placed in a 100×20 cm padded cradle, ventral side up, and their gills were perfused with a 50 $mg{\cdot}L^{-1}$ solution of MS-222. A small incision was made at the insertion point of the left pectoral fin directly above the liver, and a biopsy probe inserted to collect a 20 mg sample of liver tissue. Muscle tissue was collected similarly by biopsy through an incision on the caudal peduncle. Gonad tissue was sampled by inserting a small tube in the vent and using suction to remove 20 mg.

It was not possible to conduct a typical stable isotope tissue turnover experiment in which several individuals are sacrificed at regular intervals due to facility constraints. By using a repeated surgical technique on the four individuals we planned to minimize the variability between individuals and maintain statistical power despite our small sample size.

Determining lipid content

To assess the effect of lipid content on the $\delta^{13}C$ values of gag tissues, we sampled gonad, liver and muscle tissue from field-caught adult gag (n=20), similar in size to those used in the in vitro experiment. Field-caught gag tissue samples were lipid extracted with a modified Folch method (after Folch et al. 1957), using 1.5 mg of tissue for each extraction. Each sample was placed in a 1.5 ml micro-centrifuge tube and covered with 90 μL of a 2:1 chloroform: methanol solution. This mixture was then sonicated for 5 min and centrifuged following the methods of (Sweeting et al. 2006). The resultant biphasic sample consisted of the supernatant containing the lipid and a pellet of lipid—free tissue. The supernatant was removed by suction and the process repeated to ensure full extraction of lipid. The pellet was then dried at 50°C for 48 h and then weighed. The lipid content was determined by weight difference. Samples of untreated and lipid extracted tissues from the same individuals were then analyzed to determine the

change in $\delta^{13}C$ value and C/N ratio caused by lipid content (Sweeting et al. 2006).

Modeling tissue-specific $\delta^{13}C$ turnover rates

We modeled carbon turnover rates for each tissue as a function of time using the following equation (Fry and Arnold 1982):

$$\delta_t = \delta_f + (\delta_i - \delta_f)e^{(-vt)} \quad (2)$$

where:

δ_t=the stable isotope value of the tissue at time t; δ_i=the initial tissue value; δ_f=the tissue value when in equilibrium with the new diet; and v=the turnover rate as a function of time.

There are two primary influences on tissue turnover rates: growth and metabolic rate (Fry and Arnold 1982). To determine the relative contribution of growth and metabolic turnover to the changes observed in the $\delta^{13}C$ values we used the following equation that separates the two components (Fry and Arnold 1982).

$$\delta_t = \delta_f + (\delta_i - \delta_f)(W_t/W_i)^C \quad (3)$$

where:

δ_t is the stable isotope value of the tissue at time t; δ_i is the initial tissue value; δ_f is the tissue value when in equilibrium with the new diet (e.g. −16‰); W_t is the fish weight at time t; W_i is the initial weight of the fish; and C is the level of metabolic turnover, the unknown variable in the equation.

In our study we observed a slight enrichment in some of the final tissue $\delta^{13}C$ values relative to the mean $\delta^{13}C$ value of the diet. When in equilibrium with their diet aquatic animal tissues, $\delta^{13}C$ values can be enriched between 0 to 1‰ relative to their diet (Post 2002). For this study an equilibrium value of −16.0‰ was used for δ_f to calculate each turnover rate using a least squares non-linear regression model. This value factors in 0.6‰ enrichment relative to the mean $\delta^{13}C$ value of the pinfish diet. This enrichment factor was chosen because within the range of observed $\delta^{13}C$ enrichment factors for aquatic animals observed in previous studies (Post 2002). The values of V, C, and the enrichment factor where determined by using a least squares nonlinear regression to fit the model to the observed values. The regression analysis was done using the R statistical package (http://www.r-project.org).

Results

$\delta^{13}C$ tissue turnover rates

As hypothesized, we observed a shift in the stable isotope values for each tissue examined over the 256 day experiment (Fig. 1). Tissue specific turnover rates are calculated below using Eq. 2.

Liver

No turnover rates were calculated for liver tissue due to the large amount of variation observed for isotope values. No lipid corrected values were calculated for liver tissue because there is not a significant relationship between liver tissue lipid content and C/N ratio (Fig. 2b).

Gonad

Using the nonlinear regression model (Eq. 2), the daily turnover rates for the gonad tissue of the female gag (n=3) in the study ranged from 0.002‰ day^{-1} to 0.012‰ day^{-1} (S.D.=0.006) for non-corrected values and from 0.003‰ day^{-1} to 0.019‰ day^{-1} (S.D.= 0.008) for lipid corrected values (Table 2, Figs. 1, 4). The two larger females (No. 1 and 4) had very similar turnover rates for both the non-extracted and lipid extracted values. The smallest female (No. 3) exhibited a much slower turnover rate, more typical of that found for muscle.

Muscle

The daily turnover rates calculated from Eq. 2 for the muscle tissue for each gag in the study ranged from 0.003‰ day^{-1} to 0.011 ‰ day^{-1} (S.D.=0.004) all of which are significant at the 0.05 level (Table 2, Fig. 1). Using Eq. 3 we calculated the relative contributions of growth and metabolic maintenance to the observed tissue turnover. The results of the nonlinear regression indicate that although the entire gag grew during the course of the experiment (Table 1), the muscle tissue turnover rates were dictated primarily by metabolic turnover rather than growth (Fig. 5). Gag 2 had the highest coefficient for metabolic turnover C=−51.1. Gag 1 and 4 had similar values for C of −22.5 and −18.2 respectively. Gag 3 had a metabolic turnover value of C=−4.2, indicating

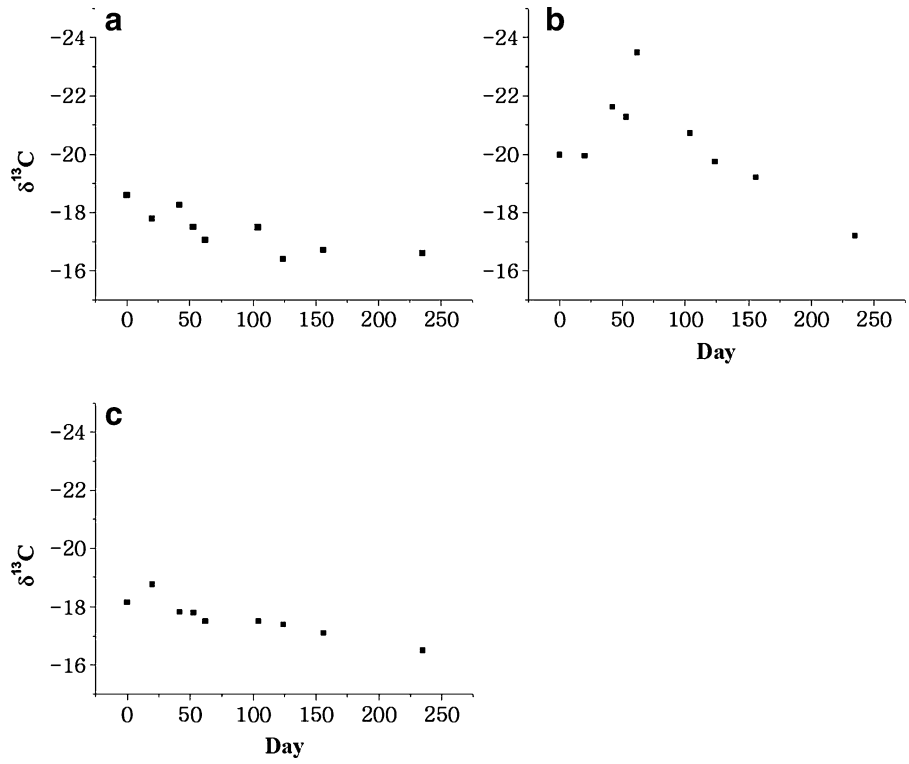

Fig. 1 Plots showing the gags' mean stable isotope values for each sampling period for all non-lipid corrected $\delta^{13}C$ values for each tissue (**a**) gonad, (**b**) liver, and (**c**) muscle tissue over the course of the feeding experiment

that a large portion of the turnover could be explained by growth. All of the values calculated for C were significant at the 0.05 level when fitted using a least squares regression.

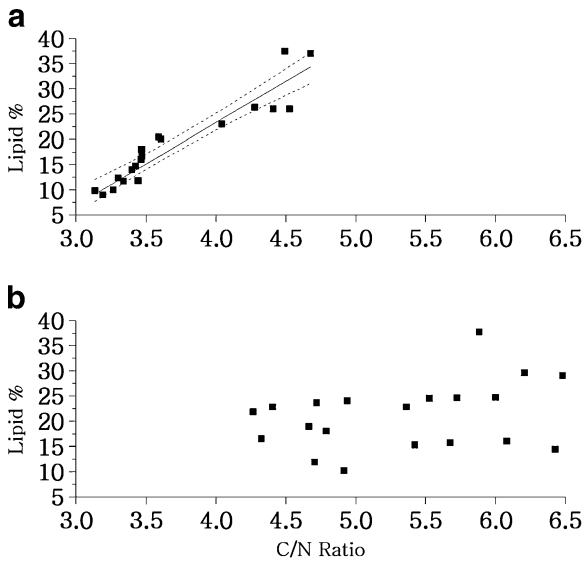

Fig. 2 The relationship between C/N ratio and percent lipid in (**a**) gonad and (**b**) liver tissue of N=20 field-caught gag. Dotted lines represent 95% confidence limits

Correction of $\delta^{13}C$ values for lipid content

To correct the observed $\delta^{13}C$ values for lipid content, the relationship between lipid content, C/N ratio, and the change in $\delta^{13}C$ value (= $\Delta^{13}C$) before and after lipid extraction was examined. The relationship between lipid content and $\Delta^{13}C$ may be explained by the C/N ratio of the unextracted tissue (Fig. 3). The relationship between C/N ratio and $\Delta^{13}C$ for gonad tissue was significant (linear regression, $p<0.0001$, $R^2=0.84$, $n=20$; Fig. 3a) but was not significant for liver tissue (linear regression, $p=0.64$, $R^2=0.012$, $n=20$; Fig. 3b). Muscle contained <10% lipid and therefore did not require lipid correction (Post et al. 2007).

Discussion

Tissue turnover

Based on the turnover rates we calculated we conclude that stable isotope analysis is a viable tool for tracking seasonal diet changes in gag. However, as we will discuss later, due to difficulty in resolving the

Table 2 Mean δ ^{13}C values and turnover rates for gonad, liver, and muscle tissues extracted from gag grouper, *Mycteroperca microlepis*, kept in captivity at the Florida State University Costal and Marine Laboratory. All data were fit to Eq. 2 using non-linear regression for the uncorrected and lipid corrected values. Mean daily turnover rates were calculated using the mean δ ^{13}C values for each sampling period and then fitting the regression to the data. The mean δ ^{13}C value for the mackerel diet used was −21.3 ‰±0.2 ‰, n=20 and the mean δ ^{13}C value for the pinfish diet used was −16.6 ‰±0.6 ‰, n=20

	δ ^{13}C		Non-linear regression (Eq. 2)	Lipid corrected non-linear regression (Eq. 2)
	Initial	Final	Turnover ‰ day^{-1}	Turnover ‰ day^{-1}
Gonad				
Gag 1	−20	−16.7	0.012	0.019
Gag 3	−17.6	−16.9	0.002	0.003
Gag 4	−18.2	−16.3	0.012	0.011
Mean	−18.6	−16.6	0.009 (S.D.=0.006)	0.011 (S.D.=0.008)
Liver				
Gag 1	−18.2	−15.8	NA	NA
Gag 2	−19.3	−17.5	NA	NA
Gag 3	−21.2	−18.3	NA	NA
Gag 4	−21.2	−17.1	NA	NA
Mean	−20	−17.2	NA	NA
Muscle				
Gag 1	−18	−16.6	0.005	NA
Gag 2	−18.1	−16	0.011	NA
Gag 3	−17.9	−17.2	0.003	NA
Gag 4	−18.4	−16.3	0.003	NA
Mean	−18.1	−16.5	0.005 (S.D.=0.004)	NA

influence of lipids on liver δ^{13}C value we feel it cannot be used for our purpose. The mean half-life of the muscle tissue was 138 days with a range of 63–231 days and the mean half-life of the gonad tissue for the three females in the study was 105 days with a range of 36–231 days. The half-lives observed here are more rapid than those observed in Suring and Wing (2009), where they observed half-lives of 280 days for slow growing blue cod. We hypothesize that the wild gag diet switch will occur over a period of 3–4 months. Although the gag may not switch entirely to an inshore derived diet the more rapid turnover rate of the gonad tissue of mature females should be rapid enough to observe the shift using stable isotope analysis. As in the study by Suring and Wing (2009), the observed turnover appears to be primarily driven by basal metabolism and not growth (Fig. 5). There were measurable differences in the tissue specific turnover rates for gag. These differences could be caused by tissue-specific differences in metabolism and/or tissue-specific changes in growth rate (e.g. gonad growth in preparation for spawning).

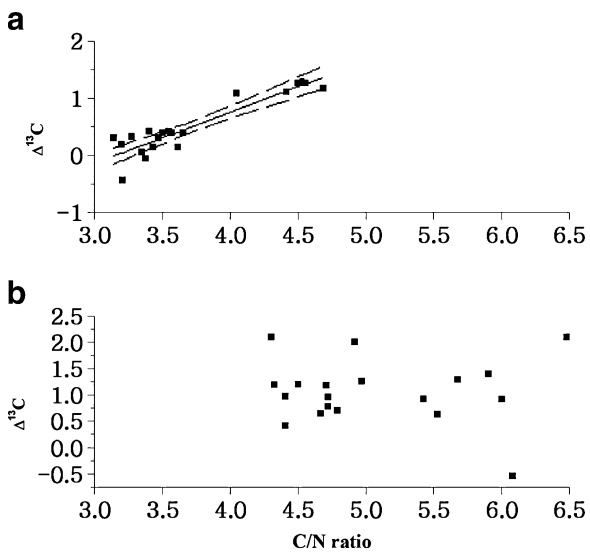

Fig. 3 Relationship between C/N ratio and prior to lipid extraction and the observed change in δ ^{13}C value after lipid extraction of (**a**) gonad tissue and (**b**) liver tissue (Δ ^{13}C). Of the 20 gag grouper in Fig. 2. The dotted lines represent the 95% confidence boundaries

Tissue turnover rates also varied among individuals, suggesting that an individual's size, sex, or life stage is a factor in tissue-specific turnover rates. For example, the daily turnover rate for muscle tissue was higher for the male (Gag 2) than for the females in the study (Table 2). This is most likely due a high basal metabolism in the male gag (Gag 2) (Fig. 5). The

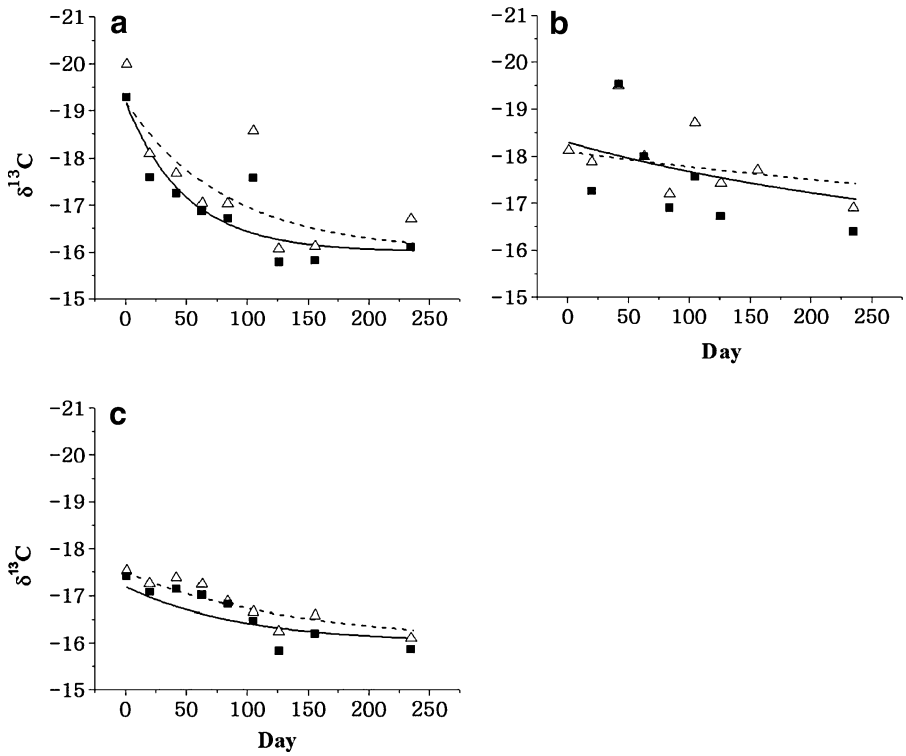

Fig. 4 Change in δ ^{13}C in lipid corrected (solid squares, fitted solid line) and uncorrected (open triangles, fitted dotted line) gonad tissue for (**a**) gag 1, (**b**) gag 3, and (**c**) gag 4 over the course of the 256-day experiment (Eq. 2)

gonad turnover rates for the female gag in the study differed as well. The two large female gag (gag 1 and gag 4) had turnover rates that were more similar and slightly faster than that of the smaller female (gag 3) (Table 2, Fig. 4). Histological analysis of gonad samples indicated that gag 1 and 4 were mature females whereas gag 3 was sexually immature. Immature female fish primarily commit energy to growth rather than to gonad maintenance and egg production (Berkeley et al. 2004). Therefore we would expect gag 3 to have a slower gonad turnover rate.

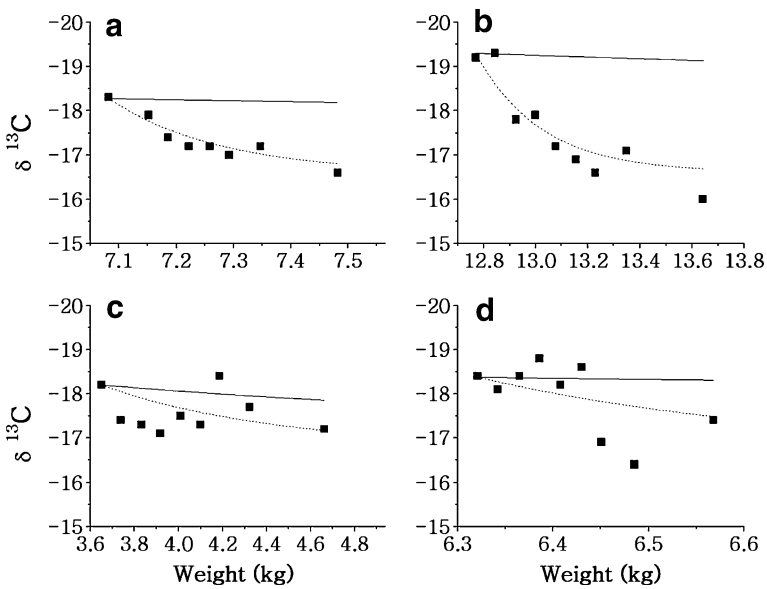

Fig. 5 Change in muscle δ ^{13}C relative to body weight (kg) over the course of the 256-day experiment in (**a**) Gag 1, (**b**) Gag 2, (**c**) Gag 3, (**d**) Gag 4. Solid lines indicate change due to growth (C=−1), and dotted lines indicate change due to metabolic turnover (Eq. 3)

Contribution of metabolism to tissue turnover

Most available information on the influence of growth and metabolism on isotopic turnover rates in ectotherms indicates that growth should dominate tissue turnover rates (Fry and Arnold 1982; Tieszen et al. 1983; Hesslein et al. 1993; Guelinckx et al. 2007). However, our results indicate that metabolic turnover is the primary cause for the observed changes in $\delta^{13}C$ values for muscle tissue (Fig. 5). Unlike other studies, which report half lives on the order of years (Hesslein et al. 1993), we observed half lives of that range from 63–231 days. Although our results may only be applicable to warm temperate or tropical species, they show that maintenance metabolism makes a significant contribution to carbon turnover. These results demonstrate the feasibility of determining changes in diet for adult fish over shorter periods of time. In the future, we intend to examine tissues with a faster metabolic turnover, such as blood plasma (Suring and Wing 2009), in concert with tissues that have a slower metabolic turnover, to provide high resolution information on the timing and magnitude of diet shifts.

Lipid correction

In this study, we observed a significant relationship between the C/N ratio, lipid percent, and change in $\delta\ ^{13}C$ value (Δ) for the gonad tissue but not for the liver tissue (Figs. 2, 3). As others have noted (Post et al. 2007), and we observed in our study the primary cause for our failure to correct for lipids content in liver tissue was the large variation in the C/N ratio of lipid-free liver tissue (Figs. 2, 3). The liver is a complex organ that functions not only in lipid storage, but also in the synthesis and secretion of bile, digestive enzymes, and hormones (Allendorf et al. 1979). How these functions affect lipid content and C/N ratio for the liver is unknown. Given all of the factors that can affect the $\delta\ ^{13}C$ value of liver tissue we concluded that it is not a viable tissue to use in diet shifts studies for gag.

Conclusion

There are two caveats to our results. First, the results are based on a small sample size (N=4); and second, the study lacked a control fish held on a constant pinfish and constant mackerel diets. The reason for these shortcomings is the extreme difficulty we encountered in capturing, transporting, and holding large fish for extended periods of time as well as the holding tank limitations of the laboratory. Despite this we feel the repeated measures technique we developed has significant advantages, such as non-lethality and the ability to elucidate individual isotopic variability, over traditional techniques where individuals are sacrificed at regular intervals. Finally, this work provides preliminary data suggesting that tissue turnover rates can vary among individuals within a species and are influenced by life stage and changes in energy allocation during the life cycle. This information further bolsters the case made by others, (Fry et al. 1999; Herzka and Holt 2000; Bosley et al. 2002; Ayliffe et al. 2004; Guelinckx et al. 2007), that turnover rates are species specific and should be determined before stable isotope values are used to assess changes in diet in wild populations.

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