Experimental determination of tissue turnover rates and trophic discrimination factors for stable carbon and nitrogen isotopes of Arctic Sculpin (*Myoxocephalus scorpioides*): A common Arctic nearshore fish

Mark B. Barton¹,⁎, Steven Y. Litvin⁵, Johanna J. Vollenweider⁶, Ron A. Heintz⁶, Brenda L. Norcross⁷, Kevin M. Boswell³

¹Department of Biological Sciences, Marine Sciences Program, Florida International University, 3000 NE 151st St, North Miami, FL 33181, USA
²Monterey Bay Aquarium Research Institute, 7700 Sandholdt Rd, Moss Landing, CA 95039, USA
³Auke Bay Laboratories, Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, 17109 Pt. Lena Loop Rd, Juneau, AK 99801, USA
⁴Institute of Marine Science, College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 131 O'Neill, Fairbanks, AK 99775, USA

ARTICLE INFO

Keywords:
- Stable isotope analysis
- Trophic enrichment factor
- Food web
- Incorporation rate
- Isotopic routing
- Lipid correction

ABSTRACT

Rapid environmental changes occurring in the Arctic nearshore are expected to have significant effects on food web structures. Land-fast ice cover limits the effectiveness of monitoring methods during winter months, precluding understanding of how seasonal Arctic nearshore food webs operate. Tissue-dependent stable isotope analysis (SIA) offers an efficient and cost-effective approach to monitoring changes in food webs during ice-covered months but requires controlled laboratory experiments to determine isotopic turnover rates and trophic discrimination factors (TDFs) of different tissues. We propose that Arctic Sculpin (*Myoxocephalus scorpioides*) be used to investigate Arctic nearshore trophodynamics given its opportunistic feeding habits and consistent residence within nearshore Arctic habitats. We present the first tissue-dependent SIA experiment on an Arctic marine fish species and show that δ¹⁵N values of fin (or liver) and muscle of Arctic Sculpin may be used to identify shifts in low-trophic-level resource availability between 56 and 122 days before sacrifice. Furthermore, TDFs were determined for carbon isotopes (1.87‰), but results for nitrogen (1.23‰ to 3.23‰) suggest that TDFs in Arctic fish may be highly dependent on lipid content of their diet. We observed similar turnover rates between liver and fin tissues (56 and 58 days, respectively), and suggest it may not be necessary to sample both, making it possible to use Arctic Sculpin for tissue dependent analyses with potentially non-lethal sampling of fin and muscle tissues. The use of Arctic Sculpin as an indicator species can increase the understanding of food web structure and aid in monitoring changes to lower-trophic level prey availability as ecosystem dynamics are affected by climate change.

1. Introduction

Climate change and associated repercussions such as decreasing sea ice cover, storm-driven marine mixing, and changes in freshwater input to marine environments are expected to affect Arctic marine food webs (AMAP, 2008; Berkman and Young, 2009; Grebmeier, 2012; Divoky et al., 2015). In response to these imminent threats, much work has been done to better understand the ecology of Arctic marine ecosystems, but until recently, the very nearshore (< 15 m depth) has been largely excluded from these efforts despite the known vulnerability of these habitats (Dunton et al., 2014, 2012, 2006; Gundlach and Hayes, 1978; McTigue and Dunton, 2014). These ecosystems are inhabited by multiple endangered and protected marine mammals and sea birds that are important resources for subsistence hunters and fishermen in the Arctic (Brewster and George, 2010). It is expected that these environmental changes will initiate changes in Arctic food web structures as lower-latitude species ranges extend northward (Grebmeier et al., 2006) and timing and productivity of primary production cycles change (Gradinger and Bluhm, 2010). It is unclear how these changes will impact nearshore wildlife, but previous research has shown that even the smallest perturbations in food web structure can have drastic cascading effects on ecosystems (Pace et al., 1999; Fortin et al., 2005; Mumby et al., 2012), highlighting the need to better characterize food web structure for making predictions of future conditions and protection strategies for these relatively pristine Arctic ecosystems.

A prerequisite for quantifying shifts in ecosystem structure, is to
recognize the annual variability in trophic dynamics in nearshore Arctic habitats under current climatic conditions. Land-fast ice cover limits sample collection from October to June in the Arctic nearshore and consequently, little is known about the variability in trophodynamics outside the open-water summer period. Stable isotope analysis (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can be used as an ecological tracer during the ice-covered period of Arctic ecosystems because it records an integrated signal from weeks (e.g. blood or liver) to years (e.g. bone collagen) of an organism’s trophic status (Hobson and Clark, 1992; Fry, 2006; Madigan et al., 2012). Therefore, SIA provides insight into trophic ecology in nearshore food webs at different timescales or during periods when sampling is hindered.

The interpretation of stable isotope values is dependent on a thorough understanding of metabolic and growth processes that assimilate isotopes from prey into consumer tissues. Controlled lab experiments can measure the time it takes for a tissue isotopic composition to turnover (tissue turnover rates: TTRs), and the isotopic difference between prey and consumer (trophic discrimination factors: TDFs), to provide temporal context of isotopic values among tissue types and investigate trophic structure (Tieszen et al., 1983; Hobson and Clark, 1992; Madigan et al., 2012). Previous controlled feeding studies on fish have reported TTRs ranging from a few days (Bosley et al., 2002) to over a year (Gaston and Suthers, 2004) depending on tissue type and life-stage. A TDF of 3.4‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$ are used across a variety of marine species and ecosystems (Post, 2002); however, environmental conditions can impact metabolic rates, TTRs and TDFs (Vander Zanden and Rasmussen, 2001). For species that live in extreme environments the canonical TDF's might not be applicable, and differential assimilation of macromolecules from dietary materials into different tissues can cause variability in trophic discrimination among species, environmental conditions, or diet types (Gannes et al., 1997; Vander Zanden and Rasmussen, 2001). Many SIA studies have been conducted on the topic of TTRs and TDFs, but few have focused on Arctic marine organisms, and none of them have focused on Arctic marine fish. We present the first experimental data to examine the incorporation of stable isotopes in Arctic marine fish tissues, and establish Arctic Sculpin (*Myoxocephalus scorpioides*) as an indicator species for assessing trophodynamics of Arctic nearshore habitats relating to environmental change as they (1) are commonly observed in a variety of Arctic nearshore habitats and can be sampled easily and cost effectively from shore with beach seines or fish traps (Mecklenburg et al., 2010). (2) Sculpins are opportunistic feeders (Link and Almeida, 2002; Gray et al., 2017) and are expected to assimilate materials from all available prey resources into their own tissues. (3) Their high site fidelity suggests that their tissues would be representative of the habitat in which they are sampled (Gray et al., 2004).

2. Methods

2.1. Husbandry

Juvenile Arctic Sculpin (< 50 mm; n = 86), were collected by beach seines from nearshore Arctic lagoons and barrier island shores near Point Barrow, Alaska during July and August 2014, and transported alive to climate-controlled holding tanks at Florida International University, Miami, FL. Throughout the duration of the experiment, fish were kept in 511 tanks with a bottom surface area of 0.25 m². Water temperatures were maintained at 8°C to mimic summer conditions in the Arctic nearshore (< 15 m depth) when fish growth is expected to be maximized (Fonds et al., 1992; Vollenweider et al., 2016). No > 5 individual fish were kept in each of the 22 tanks at any given time during the experiment. Fish were fed a diet of homogenized Antarctic Krill (*Euphausia superba*; $\delta^{13}C = -23.72 \pm 0.09‰$, $\delta^{15}N = 4.92 \pm 0.21‰$, ©San Francisco Bay Brand fish foods) during a 36-day quarantine and acclimation period. Feeding was minimized to twice per week during this period to prevent rapid tissue turnover from fast growth rates during the juvenile stage of Arctic Sculpin.

To track individual growth rates throughout the experiment, each specimen was assigned to a holding tank and tagged using a subcutaneous Visible Implant Elastomer Tag (Northwest Marine Technology, Inc.) in one of four colors on the left or right side of the anal fin. Every 7 days following the start of the experiment each fish was weighed to the nearest mg, and length was measured to the nearest 0.01 mm total length (TL). Fish that developed ailments that might affect growth or metabolic rates were culled from the experiment ($n = 10$) and their data were not used in subsequent analyses.

Our approach to quantifying isotopic turnover in Arctic Sculpin tissues was adapted from Tieszen et al. (1983). After the acclimation period (day 0), half ($n = 43$) of the fishes' diets were switched from Antarctic Krill to a homogenized fish diet ($\delta^{13}C = -21.72 \pm 0.27‰$, $\delta^{15}N = 15.36 \pm 0.16‰$) consisting of Pacific Capelin (*Mallotus catervarius*) and Arctic Sand Lance (*Ammododytes hexapterus*) collected by beach seine in Point Barrow, AK during July and August 2014. All diets were homogenized in one single batch and frozen for use through the entire experiment. Both diets had similar protein content (Krill: 70%, Fish: 72% dry weight) but lipid content were different (Krill: 6%, Fish: 24% dry weight) and will be referred to as LLK (Low Lipid Krill) and HLF (High Lipid Fish). The HLF diet was derived of fish collected in the natural habitat of Arctic Sculpin and represents what is naturally available to them. The LLK diet is derived solely of Antarctic Krill.

There are numerous krill species available as prey in the Arctic nearshore, which have similar protein and lipid content as the LLK diet used in this study (Vollenweider et al., 2016), but Antarctic Krill was used because it was more readily available.

Arctic Sculpin were fed ad libitum for the duration of the experiment. Ten randomly selected individuals were sacrificed on day 0 before the start of the experiment, and five randomly selected individuals were sacrificed from each diet treatment using MS-222 (as directed by Florida International University Institutional Animal Care and Use Committee) at 2, 5, 10, 21, 42, 81, and 147 days after the start of the experiment, and were frozen whole at −80°C prior to further processing (Tieszen et al., 1983; Logan et al., 2006; Madigan et al., 2012). All tissues and rays from the caudal and pectoral fins, the entire left side filament with the skin and bones removed, and whole livers were collected for fin, muscle, and liver tissue samples, respectively.

2.2. Stable isotope analysis

Tissues were dried at 50°C for at least 7 days and weighed every 2 days until a consistent weight was obtained, indicating that all moisture was removed. Samples were homogenized to a uniform consistency using a mortar and pestle, weighed into tin capsules (0.4–0.7 mg of dry homogenate), and sent to the Florida International University Southeast Environmental Research Center Stable Isotope Laboratory. The samples were analyzed using Elemental Analysis – Isotope Ratio Mass Spectrometry (EA-IRMS), with a Delta C Finnigan (Thermo Fisher Scientific) and Conflco II dilution interface (Thermo Fisher Scientific) coupled to a NA-1500 NC Carlo ERBA elemental analyzer (Fisons Instruments). Precision and accuracy of the instruments were measured with NIST standard IAEA-CH-6 for $\delta^{13}C$ ($−10.25 \pm 0.05%$), and IAEA-N1 for $\delta^{15}N$ (0.4 ± 0.2‰). Three in-house standards were also used: Bovine Liver, Citrus Leaves, and Glycine. Error based on standard deviations of internal glycine standards ranged 0.09–0.21‰ for $\delta^{15}N$ and 0.07–0.10‰ for $\delta^{13}C$. Stable isotope values are expressed in delta-notation ($\delta$) as per mil (%r) relative to the standard Vienna PeeDee Belemnite (VPDB; $^{13}C$) and atmospheric nitrogen ($^{15}N$) as shown below:

$$\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000, \quad (1)$$

where $q$ is the mass number of the isotope, $X$ is the element of the
isotope, and R represents the ratio of heavy to light stable isotopes for carbon ($^{13}\text{C}$/$^{12}\text{C}$) and nitrogen ($^{15}\text{N}$/$^{14}\text{N}$) within the sample and standards.

Stable carbon isotope values were corrected for the effects of lipid content in sample tissues (Sweeting et al., 2006).

$$\delta^{13}\text{C}_{\text{protein}} = \delta^{13}\text{C}_{\text{sample}} + \left(6\% \times \frac{\% \text{ lipid}}{100}\right)$$

(2)

where $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{sample}}$ are the stable carbon isotope value of lipid-free and bulk sample tissue, respectively (Sweeting et al., 2006). This method makes two assumptions: (1) $\delta^{13}\text{C}$ of lipids are 6‰ lower than proteins, and (2) the lipid-extracted tissue and pure protein are isotopically identical (McConnaghy and McRoy, 1979; Sweeting et al., 2006; Logan et al., 2008). Lipid corrections were not applied to diet samples because the fish consumed the dietary materials whole and we cannot assume that carbon from dietary lipids is not incorporated into the consumers proteins (Martínez del Río et al., 2009; Mohan et al., 2016). Carbonate corrections also were not applied to food sources despite them consisting of ground whole organisms because previous works found no significant effect of acidification on samples of fish or crustaceans (Bosley and Wainright, 1999; Pomerleau et al., 2014).

Because % lipid was not determined for all samples (see 2.3 Lipid Content) due to limited sample size, a linear model was fit between % lipid content and C:N ratios ($n = 56$, $R^2 = 0.70$, $p < .001$) for the samples in which both measurements were available (Fin: $n = 3$, Liver: $n = 28$, Muscle: $n = 26$). The resulting model equation was used to predict % lipid content from C:N ratios for all the samples for which lipid content was not available: 

$$\text{Predicted% lipid} = \frac{C}{N} \times 2.04 - 3.01$$

(3)

2.3. Lipid content

Lipid analysis was conducted using the microcolorimetric sulfophosphovanillin method on fish diet samples and Arctic Sculpin tissues (van Handel, 1985; Lu et al., 2008). For each sample, 10–20 mg of homogenized dry tissue was placed into a glass vial to which 2 ml of 2:1 (v/v) chloroform-methanol solution was added. Vials were capped and sonicated in a Branson 3510-DTH Ultrasonic Cleaner for 30 min. A 1:10 dilution of each sample was made and three 100 μl aliquots of the diluted sample were transferred to a glass 96-well plate. Solvent was evaporated on a hot plate at 100 °C for 10 min. 20 μl of concentrated sulfuric acid was added to each well and the samples were incubated at 100 °C for 10 min, after which time the plate was cooled to room temperature. 280 μl of vanillin-phosphoric acid reagent (6.8 mM vanillin, 2.6 M phosphoric acid) was added to each sample and incubated at room temperature (23 °C) with gentle shaking for 30 min. The absorbance at 490 nm was recorded for each well using a Perkin Elmer Victor3 1420 Multilabel Counter spectrophotometer. With each batch of samples, a solvent blank was run through the entire procedure to assess interference ($< 0.30$ mg) and 2 samples of dried walleye pollock (Gadus chalcogrammus) reference material with known lipid content were analyzed to ensure accuracy (average 5.7% error). Total lipid was calculated by comparison of the absorbance values to a lipid:absorbance 10-point calibration curve generated using Menhaden oil (Sigma-Aldrich). Percent lipid values were reported as the averages of the triplicate samples.

2.4. Protein content of diet

Protein content of the Arctic Sculpin diets was measured following the Dumas method using a LECO nitrogen determinator TruSpec CHN [1] (Association of Official Analytical Chemists, 2002). Replicate aliquots of > 0.07 g dried homogenate were dropped into a heated furnace (950 °C) and flushed with oxygen for rapid and complete combustion. Expelled nitrogen was quantified, and protein content was estimated by multiplying total nitrogen by a conversion factor of 6.06 (LECO Instruction Manual 2001). Quality assurance samples included one EDTA and two atmospheric blanks for every 10 sample analysis. Additionally, a replicate sample and Meat1546 (National Institute of Standards) was included with each batch of 17 samples. If quality assurance samples exceeded prescribed limits (15% variation for reference samples, 0.1% protein for blanks, and 1.5 standard deviation for replicates), samples were re-analyzed. Protein values of replicates were averaged.

2.5. Tissue specific isotopic turnover rates

TTRs were derived from exponential models fit to stable isotope values for each tissue, isotope, and diet type (Tieszen et al., 1983; Hobson and Clark, 1992; Madigan et al., 2012; Rosenblatt and Heithaus, 2013):

$$\delta_i = ae^{-\lambda t} + c,$$

(4)

where $\delta_i$ is the stable isotope value at time t, $\lambda$ is a rate constant derived from the fitted model, a is the difference in % between the initial stable isotope value ($\delta_0$) and the steady-state final stable isotope value (c). These models were fit to estimate $a$, $\lambda$, and c using nonlinear least square with the CRAN-R ‘stats’ package, function ‘nls’ (R Team, 2013). Time needed to achieve a percentage (α) of isotopic turnover was estimated for each tissue:

$$t_{\alpha/100} = \frac{\ln \left(1 - \frac{\alpha}{100}\right)}{\lambda},$$

(5)

where $\alpha$, the percentage of isotopic turnover was set to 50 (half-life) and 90, and $\lambda$ is a rate constant derived from Eq. (4).

Eqs. (4) and (5) were applied separately to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the three tissues (fin, liver, and muscle) of Artic Sculpin from both diet treatments (HLF and LLK). Model parameters $a$, $\lambda$, and c are reported, as well as the number of days needed to achieve turnover half-life and 90% turnover (Table 1).

The turnover rate of an isotope can be separated into the proportion attributable to the addition of new biomass through growth and the replacement of tissues due to metabolic processes. The proportion of turnover driven by growth versus metabolism was estimated using measured weights with a modification of Eq. (4) (Madigan et al., 2012):

$$\delta_i = ae^{-(\delta_i - c)\frac{m}{m'}} + c,$$

(6)

where $\delta_i$ is the difference in % between the initial stable isotope value and the steady-state final stable isotope value (c), and $k'$ is the growth rate constant for each fish for the duration of the experiment. These models were fit for each tissue-isotope combination using nonlinear least squares with the CRAN-R ‘stats’ package, function ‘nls’ (R Team, 2013), to determine $m$, the metabolic rate constant. ($\delta_i - \delta_j$) is analogous to $a$ in Eq. (4), and $\delta_j$ is analogous to $c$; therefore, these values were derived from model fits described for Eq. (4). $k'$ was estimated for each fish using the following equation:

$$k' = \frac{\ln \left(\frac{W_f}{W_i}\right)}{t},$$

(7)

where $W_f$ is the final weight of the fish and $W_i$ is the initial weight of the fish. The following model was fit to estimate the overall growth rate constant for all fish, k:

$$W_f = e^{k't}.$$

(8)

Isotopic turnover is driven by a combination of catabolic and metabolic processes as well as growth (Carleton and Martinez Del Rio, 2010), from which the contribution of each varies across life stages (Madigan et al., 2012). To increase the scope of age classes to which the
Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Food</th>
<th>Isotope</th>
<th>a (%)</th>
<th>λ</th>
<th>c (%)</th>
<th>R²</th>
<th>t₀.₅ (k + m)</th>
<th>t₀.₁ (k + m)</th>
<th>δ (mean ± SD, δ₁₃C or δ₁₅N)</th>
<th>TDF (%)</th>
<th>k</th>
<th>m</th>
<th>Pₓ</th>
<th>Pₓₓ</th>
<th>t₀.₁ k</th>
<th>t₀.₁ m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fin</td>
<td>HLF</td>
<td>C</td>
<td>0.21</td>
<td>20.37</td>
<td>0.02</td>
<td>0.02</td>
<td>0.72</td>
<td>0.72</td>
<td>−23.72 ± 1.95</td>
<td>0.95</td>
<td>0.91</td>
<td>0.91</td>
<td>0.011</td>
<td>0.007</td>
<td>0.61</td>
<td>0.39</td>
</tr>
<tr>
<td>Liver</td>
<td>HLF</td>
<td>C</td>
<td>0.21</td>
<td>20.37</td>
<td>0.02</td>
<td>0.02</td>
<td>0.72</td>
<td>0.72</td>
<td>−23.72 ± 1.95</td>
<td>0.95</td>
<td>0.91</td>
<td>0.91</td>
<td>0.011</td>
<td>0.007</td>
<td>0.61</td>
<td>0.39</td>
</tr>
<tr>
<td>Muscle</td>
<td>HLF</td>
<td>C</td>
<td>0.26</td>
<td>20.02</td>
<td>0.01</td>
<td>0.01</td>
<td>1.00</td>
<td>1.00</td>
<td>−23.72 ± 1.70</td>
<td>0.95</td>
<td>0.91</td>
<td>0.91</td>
<td>0.011</td>
<td>0.007</td>
<td>0.61</td>
<td>0.39</td>
</tr>
<tr>
<td>Fin</td>
<td>LLK</td>
<td>C</td>
<td>0.44</td>
<td>22.05</td>
<td>0.03</td>
<td>0.03</td>
<td>1.67</td>
<td>1.67</td>
<td>−23.72 ± 1.67</td>
<td>0.95</td>
<td>0.91</td>
<td>0.91</td>
<td>0.011</td>
<td>0.007</td>
<td>0.61</td>
<td>0.39</td>
</tr>
<tr>
<td>Liver</td>
<td>LLK</td>
<td>C</td>
<td>1.60</td>
<td>7.62</td>
<td>0.39</td>
<td>0.39</td>
<td>3.52</td>
<td>3.52</td>
<td>−23.72 ± 1.67</td>
<td>0.95</td>
<td>0.91</td>
<td>0.91</td>
<td>0.011</td>
<td>0.007</td>
<td>0.61</td>
<td>0.39</td>
</tr>
<tr>
<td>Muscle</td>
<td>LLK</td>
<td>C</td>
<td>1.82</td>
<td>21.77</td>
<td>0.72</td>
<td>0.72</td>
<td>1.95</td>
<td>1.95</td>
<td>−23.72 ± 1.95</td>
<td>0.95</td>
<td>0.91</td>
<td>0.91</td>
<td>0.011</td>
<td>0.007</td>
<td>0.61</td>
<td>0.39</td>
</tr>
</tbody>
</table>

For δ₁³C in fin tissues on the HLF diet, the model did not properly fit the values (indicated by the negative λ value) and further parameters were not calculated. a is the difference in % between t₀.₁ and the steady-state final stable isotope values, and c is the steady-state final stable isotope of the subject tissue (Eq. (4)). t₀.₁(k + m), and t₀.₁(k + m) are the number of days taken to reach 50% and 90% isotopic turnover, respectively (Eq. (5)). TDF is the trophic discrimination factor derived using Eq. (11). k is the overall growth rate constant derived using Eq. (8), and m is the metabolic rate constant or the difference between λ and k. Pₓ and Pₓₓ are the proportion of isotopic turnover due to growth and metabolism, respectively (Eqs. (9) and (10)). Finally, t₀.₁k and t₀.₁m are the estimated number of days it would take to reach 90% isotopic turnover if turnover was solely driven by metabolism.

* LLK-fed sculpin experienced a diet switch 36 days before the start of the experiment (from wild food to the low-lipid krill diet). Therefore, the parameters estimated from those models are only used for discussion of influence of growth vs. metabolism.

For δ₁³C in fin tissues on the HLF diet, the model did not properly fit the values (indicated by the negative λ value) and further parameters were not calculated. a is the difference in % between t₀.₁ and the steady-state final stable isotope values, and c is the steady-state final stable isotope of the subject tissue (Eq. (4)). t₀.₁(k + m), and t₀.₁(k + m) are the number of days taken to reach 50% and 90% isotopic turnover, respectively (Eq. (5)). TDF is the trophic discrimination factor derived using Eq. (11). k is the overall growth rate constant derived using Eq. (8), and m is the metabolic rate constant or the difference between λ and k. Pₓ and Pₓₓ are the proportion of isotopic turnover due to growth and metabolism, respectively (Eqs. (9) and (10)). Finally, t₀.₁k and t₀.₁m are the estimated number of days it would take to reach 90% isotopic turnover if turnover was solely driven by metabolism.

* LLK-fed sculpin experienced a diet switch 36 days before the start of the experiment (from wild food to the low-lipid krill diet). Therefore, the parameters estimated from those models are only used for discussion of influence of growth vs. metabolism.

results can be applied, we estimate the relative contribution of growth and metabolism to isotopic turnover in tissues:

\[
P_x = k / \lambda, \quad (9)
\]

\[
P_m = m / \lambda, \quad (10)
\]

where \(P_x\) and \(P_m\) are the proportion of isotopic turnover caused by growth and metabolism, respectively. This equation assumes that the isotopic turnover driven by metabolism (m) is the proportion of \(\lambda\) that is not driven by growth (k). Therefore, any observed differences in the contributions of growth to isotopic turnover are expected to be driven by metabolism.

2.6. Trophic discrimination factors (TDFs)

TDFs were estimated as the difference between the final steady-state stable isotope value of each tissue type and the mean stable isotope value of the dietary materials that they were fed during the experiment.

\[
\Delta_{Tissue} = c - \delta_{Food}, \quad (11)
\]

where \(\Delta_{Tissue}\) is the TDF for the tissue of interest, \(c\) is the final steady-state stable isotope value determined from Eq. (1), and \(\delta_{Food}\) is the mean stable isotope value of the food type that was fed.

Eqs. (6), (7), (9)–(11) (Madigan et al., 2012) were applied separately to the δ¹³C and δ¹⁵N values of the three tissues (fin, liver, and muscle) of Arctic Sculpin from both diet treatments (HLF and LLK). Eq. (8) was applied to all individual relative weights. Model parameters m and k are reported, as well as the half-life (50%) and 90% isotopic turnover times (t₀.₅, t₀.₁; Table 1). Additionally, the estimated 90% turnover time solely driven by growth or metabolism are reported as t₀.₁k and t₀.₁m, respectively. Furthermore, the relative contribution of growth and metabolism are reported as proportions of the rate constant λ, derived from Eq. (4).

2.7. Ethics statement

All methods and procedures used in these experiments were approved and in compliance with guidelines set by Florida International University Institutional Animal Care and Use Committee (protocol #: IACUC-13-013-CR01).

3. Results

Stable nitrogen isotope values of all tissues (fin, liver, and muscle) reached a steady-state within the 147 days of the diet switch from LLK to HLF, facilitating estimation of time-dependent and growth-dependendent turnover rates, as well as TDFs for δ¹⁵N (Fig. 1A). However, the initial and steady-state stable carbon isotope values of Arctic Sculpin switched to the HLF diet were indistinguishable by Student’s t-test (t₀.₅ = −0.65, p = .55 for fin, t₀.₅ = 0.17, p = .87 for muscle), resulting in poor model fits and difficulty in determining stable carbon isotope turnover rates for all tissues (Fig. 1B). Steady-state stable isotope values of fish feeding on LLK were also observed (c, in Table 1), permitting for accurate calculation of TDFs.

3.1. Time-dependent isotopic turnover

All results referring to tissue turnover rates are based on stable isotope values from Arctic Sculpin that were switched from the LLK to the HLF diet at the start of the experiment, unless the LLK diet is explicitly stated. Lipid corrections were applied to all δ¹³C values of Arctic Sculpin tissues. Average lipid content in fin and muscle were relatively low (3.7% and 5.2%, respectively), but in liver was much higher (21.2%). Bulk and lipid-corrected δ¹³C had strong linear correlation in both fin and muscle (n = 76, R² = 0.92, p < .001; n = 79, R² = 0.91, p < .001) but not liver (n = 81, R² = 0.30, p < .001; Fig. 2). Following the dietary switch at the start of the experiment, all tissues of Arctic Sculpin on the HLF diet reached 90% turnover of nitrogen isotopes within the 147 days of the experiment (Fig. 1A). Fin and liver tissues reached 90% turnover within 56 and 58 days (n = 37, R² = 0.85, p < .05 and n = 39, R² = 0.96, p < .05), respectively (Table 1). Muscle was much slower reaching 90% turnover within 122 days (n = 38, R² = 0.80, p < .05).

The δ¹³C values for fin, liver, and muscle tissues for Arctic Sculpin on the HLF diet did not fit or poorly fit exponential growth models (Eq. (1); Fig. 1B), and TTRs could not be calculated. Fin tissues never reached an isotopic steady state (Fig. 1B). Liver tissue δ¹³C values show an unusual pattern with higher δ¹³C at the beginning of the experiment, reaching an asymptote after 42 days, and then decreasing toward the end of the experiment (Fig. 1B). Though this pattern warrants further discussion, we were unable to estimate turnover rates for liver tissue. The 90% carbon isotope turnover rate for muscle tissues was predicted to be 37 days; however, this estimate is unreliable due to the poor
3.2. Growth-and-metabolic-dependent isotopic turnover

Diet did not affect Arctic Sculpin growth rate (Student’s t<sub>37,40</sub> = 0.74, p = .46). Density dependent effects were not significant based on a linear regression between growth by weight and the number of fish in the tank in the week before sacrifice (n = 76, R<sup>2</sup> = 0.01, p = .75).

Arctic Sculpin sacrificed at the end of the experiment increased 3.9 to 6.1 times their initial weight. Growth rates were highest in Arctic Sculpin that were sacrificed within the first 10 days (k’ = 0.16 ± 0.15) and gradually decreased to a steady growth rate constant (k’ = 0.03) in fish sacrificed near the end of the 147 days of the experiment (Fig. 3).

Diet type influenced the proportion of isotopic turnover driven by metabolism and growth (P<sub>m</sub> and P<sub>g</sub> in Eqs. (9) and (10), respectively). Across all three tissues, metabolism had greater average influence on
turnover in HLF-fed sculpins than in LLK-fed sculpins (Pm mean ± SD: 63 ± 15% and 18 ± 25%, respectively). In HLF-fed sculpin, the majority of turnover in liver and fin was driven by metabolism (73% each), and to a lesser degree in muscle tissue (42%). However, turnover in fin and muscle of LLK-fed sculpins was solely driven by growth (100%), whereas turnover in livers had a greater influence from metabolism (54%). TTRs for δ15N values based on metabolism alone were also calculated from the HLF models, and are approximately 77, 79, and 287 days for fin, liver, and muscle tissue, respectively.

Since δ13C values either did not fit exponential growth models or did not change throughout the experiment, we cannot comment on the role of metabolism and growth in stable carbon isotope turnover in HLF-fed fish. However, for LLK-fed fish, stable carbon isotope turnover was mostly driven by metabolism in fin tissue (64%), and by growth in muscle (61%). Again, because δ13C values from fish on the HLF diet did not fit exponential growth models, these estimates of TTRs based on metabolism alone could not be made for carbon.

3.3. Trophic discrimination factors (TDFs)

TDFs differed between diets for δ15N (range 0.62 to 3.53‰), but consistent for δ13C (range 1.67 to 2.17‰). δ15N TDFs were lower for HLF-fed sculpin (0.62‰ to 2.06‰) than LLK-fed sculpin (2.70‰ to 3.53‰; Student’s t3,3: p = 0.02). As for δ13C TDFs, LLK-fed sculpin ranged between 1.67‰ and 2.17‰. Muscle tissues of HLF-fed sculpin had a similar δ13C TDF value of 1.70‰ (Table 1). When lipid corrections are applied to δ13C of food sources, the range of calculated δ13C TDFs change less for LLK-fed sculpin (TDF range after correction: 1.3‰ to 1.8‰) than for HLF-fed sculpin (~0.3‰ to 0.02‰).

4. Discussion

The estimates of TTRs in Arctic Sculpin measured in three different tissues have the potential to identify trophic shifts that occur on monthly (fin, liver) and seasonal/annual (muscle) timescales in nearshore Arctic systems. Half-life values of δ13N in Arctic Sculpin (17 days for liver, 37 days for muscle; Table 1) resemble those of juvenile midtrophic level consumers from lower latitudes. Previous studies reported δ13N half-lives of 9–18 and 14.4 days for liver, and 27–54 and 19.3–25.7 days for muscle in juvenile Atlantic Croakers (Micropogonias undulates) and Japanese Temperate Bass (Lates japonicus), respectively (Suzuki et al., 2005; Mohan et al., 2016).

Because δ13C values did not fit or poorly fit exponential growth for Arctic Sculpin tissues on the HLF diet, we were unable to determine the TTRs for carbon. This is likely the result of δ13C values being indistinguishable between fish collected for food (Pacific Capelin and Arctic Sand Lance) and Arctic Sculpin, which was not expected. Average LLK-diet δ13C values were approximately 2‰ per mil higher than the average initial stable isotope values of Arctic Sculpin, which resulted in better model fits than on the HLF diet.

The models from Arctic Sculpin on the LLK-diet cannot be used to determine turnover rates; but these models are still eligible to determine TDFs because it only requires the stable isotope values in diet and the isotopic steady-state values of tissues. The influence of metabolism and growth can also be determined because they are proportionally determined from measured growth and the rate constant (λ) estimated from the model.

The stable isotopes in dietary materials assimilated into consumer tissues is dependent on metabolic and growth rates (Hesslein et al., 1993; Carleton and Martinez Del Rio, 2010; Madigan et al., 2012). In juvenile fish, such as the Arctic Sculpin used in this experiment, isotope turnover is expected to be dominated by growth (Bosley et al., 2002; Logan et al., 2006), this was only found to be the case in muscle tissues. Due to rapid catabolism and metabolism, liver has a faster turnover rate than muscle despite having a similar growth rate (Tieszen et al., 1983; Logan et al., 2006; Madigan et al., 2012; Heady and Moore, 2013). This is confirmed as 90% isotopic turnover for nitrogen in HLF-fed sculpin was achieved at 58 days for liver versus 122 days for muscle, and on both diets, metabolism contributed more to isotopic turnover in liver than in muscle. In this experiment, fin tissue δ15N turnover rates were similar as liver, but in previous studies fin tissues have had variable (9 to 22 days; Suzuki et al., 2005; Heady and Moore, 2013) turnover rates in different species, making it important to determine TTRs before using this tissue.

In the past, many studies have used SIA of liver and muscle to identify shifts in diet between short- and long-term temporal scales; however, the fatty composition of liver often increases variability in δ13C values, complicating this identification and making liver a suboptimal choice as a fast turnover tissue (Sweeting et al., 2006; Logan et al., 2008; Mohan et al., 2016). In Arctic Sculpin, livers were also found to be fatty in comparison to fin and muscle (21% versus 4% and 5%, respectively; Table 1). Arithmetic lipid corrections can account for the effects of lipids on δ15N but there is often variability in the isotope values of high lipid tissues that is not explained by lipid content alone (Sweeting et al., 2006; Post et al., 2007; Logan et al., 2008). The higher error in lipid corrections of liver tissues, shown by the poorer correlation between bulk and lipid-corrected δ13C (R² = 0.30, Fig. 3), confirms that tissue is more appropriate than liver to investigate short term shifts in the diet of Arctic Sculpin. Fin tissue clippings and muscle tissue plugs using 14 gauge biopsy needles can be harvested non-lethally (Baker et al., 2004) suggesting it is possible to sample high- and low-turnover rate tissues to capture different time periods of a system without impacting fish populations. However, this method has not been attempted on sculpins, and should be tested before employment in the field.

When using derived TTRs to provide temporal context to isotope values in sculpins, ontogenetic effects may influence metabolic rate. The fish in this experiment were juveniles, the isotopic turnover was largely driven by growth, but in adult fish turnover may be almost exclusively driven by metabolism as growth rates reduce (Fonds et al., 1992; Harvey et al., 2002). For this reason, 90% TTRs based solely on metabolic turnover were reported. These values may give a more appropriate estimate of temporal context to the isotopic composition of adult fish tissue that have reduced growth rates. However, it is unclear how much metabolic rates decrease in sculpin as they reach maturity and these estimates may not be applicable until this relationship is further investigated.

TDFs determined from this experiment may be used to represent isotopic fractionation in food webs. The TDFs estimated for δ13C had minor differences between models and suggests that 1.87% may be an appropriate TDF to represent carbon fractionation in Arctic Sculpin. Fractionation of δ15N was diet dependent, with TDFs in HLF-fed sculpin (1.22 ± 0.61‰) being lower than in LLK-fed sculpin (3.25 ± 0.39‰). The most commonly used δ15N TDF is 3.4‰, but ranges from 0.5 to 5.5‰ have been reported. Based on data presented by Post (2002), the δ15N TDF determined from the HLF-fed sculpin (1.23‰) is in the lower 9th percentile and is uncommonly low. A low δ15N TDF on a natural diet is unlikely because Sculpin are generalists that feed mostly on benthic invertebrates, who’s lipid content is typically lower than that of the HLF diet (Gray et al., 2017). The δ15N TDF for LLK (3.23‰) is closer to the commonly used 3.4‰ value, and may be more appropriate based on lower lipid content of benthic invertebrates in the Point Barrow region (Vollenweider et al., 2016). The δ13C TDF reported for Arctic Sculpin (1.87‰) is in the upper 35th percentile based on data presented by Post (2002) and is relatively uncommon. The use of different δ15N and δ13C TDFs will impact the conclusions drawn from food web models as the predicted trophic positions of consumers and prey would change, and it is important to choose appropriate TDFs when building such a model.

The results of percent contribution of metabolism and growth to isotopic turnover indicated that metabolism is higher when high-lipid diet was fed, suggesting that high quality diets increase metabolic rates.
accounting for the observed trophic discrimination. These results may suggest that the appropriate δ^{15}N TDF to accurately determine the diet of sculpin ranges between 1.23% and 3.23% and is dependent on prey quality (lipid content). Though there are no published data on the feeding ecology of Arctic Sculpin, the congeneric Shorthorn Sculpin (Myoxocephalus scorpius) is likely to have similar prey selection as it has a similar body shape and gape but is more abundant further from shore. Shorthorn Sculpin are generalists that mostly feed on benthic crustaceans and worms, but fish can also be a common prey source depending on the region (Gray et al., 2017). With this in mind, it may be helpful to determine the lipid content of common prey species within a sampling area to determine the correct TDFs for parameterizing food web models.

Mohan et al. (2016) described similar effects of lipid content on TDFs of Atlantic Croakers (Micropogonias undulatus) and suggested that this was likely due to routing of macromolecules from dietary lipid into assimilated proteins. This idea is supported by the fact that TDFs for δ^{13}C calculated for Arctic Sculpin, using stable isotope values of lipid-free dietary materials (protein; HLF = −20.3‰, LLK = −23.4‰), suggests that there is no fractionation between the proteins of the HLF diet and assimilated proteins (average HLFfferent Δ FOOD = 0.1 ± 0.1‰). A TDF this small could indicate that much of the assimilated carbon in proteins may be derived of dietary lipids, and because the routing of lipids in protein synthesis is poorly understood one should not perform lipid corrections on dietary materials or while constructing food web models with multiple trophic levels using whole fish samples (Mohan et al., 2016).

Temperature has been known to have direct effects on metabolic rate, consumer isotope assimilation rates and TDFs (Fonds et al., 1992; Bosley et al., 2002). Until this is better understood, the results of this experiment may only be applicable to fish collected during summer months when temperatures are similar to those in the experiment. However, stable isotope values in tissues of Arctic Sculpin collected at the beginning of the open-water season represent their diet through ice-covered months (prior to July) despite not knowing the exact turnover time of those tissues. When compared to Arctic Sculpin collected well into the open-water season (August), the difference between the tissues will offer new insight to seasonal variability in trophodynamics of Arctic nearshore fish.

With known TTRs, Arctic Sculpin can be used as a biological indicator to monitor changes in trophodynamics in the Arctic nearshore. Previous studies indicate that earlier spring ice melt is decreasing the time period when ice algae can bloom (Arrigo et al., 2008; Bluhm and Gradinger, 2008; Gradinger and Bluhm, 2010; Iken et al., 2010). This ice algae production is a crucial basal resource during ice-covered months, and much of this production is transported to the benthos to support highly productive benthic communities in the Arctic. As ice continues to melt faster, it is expected that the degree of benthic-pelagic coupling will decrease, causing a major shift from a benthic invertebrate dominated food web to a pelagic fish dominated food web. Using tissue-dependent SIA, the timing and magnitude of such a shift in basal resources can be determined. This method could be applied to monitor any scenario in which a shift in basal resources is expected, such as increasing inputs of terrestrial organic material from melting permafrost or increasing riverine discharge (Schuur et al., 2008; Strauss et al., 2011; Dunton et al., 2012; Barton et al., 2016). There may also be potential to identify shifts in resource utilization by Arctic Sculpin resulting from anthropogenic impacts to lower trophic levels such as those from oil spills or other anthropogenic stressors (Gundlach and Hayes, 1978; Aguilar et al., 2008; Moreno et al., 2013). SIA could be paired with other tissue-dependent analyses to investigate an array of biological responses of Arctic Sculpin to ecological changes associated with climate change including condition (Vollenweider et al., 2016), hydrocarbon contamination (Aas et al., 2000), and stress responses (Pickering and Pottinger, 1989).

5. Conclusions

Our results demonstrate that tissue dependent δ^{15}N values of Arctic Sculpin can be used to identify shifts in prey resource use between approximately 56 and 122 days using fin and muscle tissues, respectively. Liver has a turnover rate similar to fin and given the higher variance in stable isotopic values of liver tissues, fin is a better choice to identify short-term dietary shifts. Furthermore, non-lethal tissue sampling of Arctic Sculpin for SIA could be a viable way to monitor changes in food web structure, basal resource use, or potential impacts of anthropogenic activities.

As previous studies have also shown, there is an apparent influence of diet quality (lipid content) on the isotopic TTRs (Mohan et al., 2016). It is likely that these effects relate to the response of metabolic processes to dietary compositions, but little is known about the specific influence of diet on metabolism of specific isotopes. Furthermore, the effect of temperature on TTRs and TDFs of isotopes in Arctic Sculpin requires more research.

Differences in TDFs were likely driven by metabolic changes caused by diet quality/lipid content, and it is important to consider the quality of available prey in a food web when reconstructing its structure using SIA. Another explanation is that dietary carbon and nitrogen of lipids and proteins are being rerouted to different tissue types in the consumer. This is supported by the fact that the TDFs between dietary protein and assimilated protein is near zero, and it is likely that dietary lipid is being incorporated into consumer proteins; however, the data provided by this experiment cannot adequately address this issue and further investigation is needed.

Given their generalist diet, high site-fidelity and abundance, Arctic Sculpin are a viable biological indicator. This study outlines how tissue-dependent SIA can be used to investigate seasonal changes in trophic structure and resource dependence to help elucidate the effects that climate change and adverse anthropogenic impacts will have on Arctic nearshore food webs.

Acknowledgements

We thank Florida International University Marine Biology for providing the temperature-controlled fish tank facility in which the experiments were carried out. Special thanks to Craig George, Leandra Sousa, Todd Sforno, Robert Suydam, and the North Slope Borough for providing significant logistical support during the collection of Arctic Sculpin in Barrow, AK. Additional thanks to laboratory and field technicians at Florida International University (FIU), NOAA Auke Bay Labs, and University of Alaska Fairbanks who processed the isotope samples and lipid content samples or contributed to field collection efforts, including Robert Darcy, Mark Kleese, Katrina Abel, Alexandra Brownstein, Matthew Callahan, Samuel George, and Ann Robertson. We also thank the FIU Southeast Environmental Research Center Stable Isotope Laboratory for analyzing the Arctic Sculpin tissues. Finally, thanks to our sponsors for providing materials and gear including Columbia®, Engel Coolers®, and Smith Optics®. This is contribution No. 109 from the Marine Education and Research Center in the Institute for Water and the Environment at Florida International University.

Funding: This work was supported by the North Pacific Research Board (grant number 1229, 2012); Bureau of Ocean Energy Management (BOEM), Cooperative Agreement Contract Nos. M12PG00024 (ACES), and M12PG00018 (Arctic EIS) of the U.S. Department of the Interior (Cooperative Agreement Contract M12PG00024, M12PG00018).

References
