Received: 29 September 2022 | Accepted: 29 March 2023

DOI: 10.1111/jfb.15389

REGULAR PAPER

JOURNAL OF FISH BIOLOGY fsbi

Isotopic turnover in polar cod (*Boreogadus saida*) muscle determined through a controlled feeding experiment

Amanda Fern Ziegler[1,2] | Bodil A. Bluhm[1] | Paul E. Renaud[3,4] | Lis L. Jørgensen[2]

[1]Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway, Tromsø, Norway

[2]Institute of Marine Research, Fram Centre, Tromsø, Norway

[3]Akvaplan-niva, Fram Centre for Climate and the Environment, Tromsø, Norway

[4]University Centre in Svalbard, Longyearbyen, Norway

Correspondence
Amanda Fern Ziegler, Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway PO Box 6050, Langnes N-9037 Tromsø, Norway.
Email: amanda.f.ziegler@uit.no

Funding information
Norges Forskningsråd, Grant/Award Number: 276730

Abstract

Polar cod (*Boreogadus saida*) is an important trophic link within Arctic marine food webs and is likely to experience diet shifts in response to climate change. One important tool for assessing organism diet is bulk stable isotope analysis. However, key parameters necessary for interpreting the temporal context of stable isotope values are lacking, especially for Arctic species. This study provides the first experimental determination of isotopic turnover (as half-life) and trophic discrimination factors (TDFs) of both $\delta^{13}C$ and $\delta^{15}N$ in adult polar cod muscle. Using a diet enriched in both ^{13}C and ^{15}N, we measured isotopic turnover times of 61 and 49 days for $\delta^{13}C$ and $\delta^{15}N$, respectively, with metabolism accounting for >94% of the total turnover. These half-life estimates are valid for adult polar cod (>3 years) experiencing little somatic growth. We measured TDFs in our control of 2.6‰ and 3.9‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, and we conclude that applying the commonly used TDF of ~1‰ for $\delta^{13}C$ for adult polar cod may lead to misrepresentation of dietary carbon source, while the use of 3.8‰ for $\delta^{15}N$ is appropriate. Based on these results, we recommend that studies investigating seasonal shifts in the diet of adult polar cod sample at temporal intervals of at least 60 days to account for isotopic turnover in polar cod muscle. Although isotopic equilibrium was reached by the fish in this study, it was at substantially lower isotope values than the diet. Additionally, the use of highly enriched algae in the experimental feed caused very high variability in diet isotope values which precluded accurate calculation of TDFs from the enriched fish. As a result of the challenges faced in this study, we discourage the use of highly enriched diets for similar experiments and provide recommendations to guide the design of future isotopic turnover experiments.

KEYWORDS
enrichment, half-life, fractionation, stable isotopes, isotopic routing

1 | INTRODUCTION

Stable isotope analysis has become increasingly popular in recent decades for determining dietary carbon sources and trophic relationships within food webs. Analytical methods continue to advance (Phillips *et al.*, 2014; Stock *et al.*, 2018), (Cucherousset & Villéger, 2015; Layman *et al.*, 2007) (Parnell *et al.*, 2013; Quezada-Romegialli *et al.*, 2018), yet the application of these methods and interpretation of their outputs still requires knowledge of (a) the trophic discrimination factor (TDF) and (b) the isotopic turnover time

(Fry *et al*., 1982). The TDF describes the observed fractionation of isotopes between the diet and tissue of a consumer, and is crucial for the calculation of trophic position (DeNiro & Epstein, 1978, 1981; Peterson & Fry, 1987; Post, 2002). TDFs of $\delta^{13}C$ and $\delta^{15}N$ are generally accepted to be ≤1‰ and 3‰–4‰, respectively (DeNiro & Epstein, 1978; Hobson & Welch, 1992; Minagawa & Wada, 1984; Post, 2002; Vanderklift & Ponsard, 2003), yet these values can vary substantially by species, tissue type, diet composition, temperature and physiological condition of the organism (Boecklen *et al*., 2011; Canseco *et al*., 2021; Caut *et al*., 2008; Godiksen *et al*., 2019; Maitland *et al*., 2021; Suring & Wing, 2009; Vanderklift & Ponsard, 2003). Isotopic turnover is the incorporation of isotopes from a consumer's diet into the consumer's tissue until equilibrium with the diet is reached. It is typically reported as half-life, or the time required for a 50% change between the initial isotopic composition of the consumer and that at equilibrium with a new diet (Reviewed by Carter et al., 2019). Isotopic turnover is controlled primarily by growth and metabolism (Hesslein *et al*., 1993; Trueman *et al*., 2005), both of which are affected by temperature (Brown *et al*., 2004; Gillooly *et al*., 2001). Marine ectotherms living in cold environments (*e.g*., polar regions or the deep sea) should have longer isotopic turnover times relative to endotherms because of depressed metabolic rates (Maitland *et al*., 2021). Because taxa living in these environments are difficult to sample, they are not well represented by the temperature-dependent turnover models that currently exist. For example, a recent meta-analysis of isotopic turnover studies that developed temperature- and body mass-dependent turnover models covered a temperature range of 1 to 42°C but only two studies provided data covering <10°C (Thomas & Crowther, 2015). Although the body of available data of isotopic turnover is growing, species-specific isotopic turnover times are still lacking for most key Arctic taxa, including polar cod (Boecklen *et al*., 2011; Madigan *et al*., 2021; Martínez Del Rio *et al*., 2009; Thomas & Crowther, 2015; Vander Zanden *et al*., 2015).

Polar cod (*Boreogadus saida*) is the most abundant Arctic marine fish and is particularly abundant in the northern and eastern Barents Sea (Gjøsæter *et al*., 2009; Hop & Gjøsæter, 2013). Adult polar cod are much smaller than other gadids, and often only reach a length of 20 cm at maturity (after 2–3 years) and a typical maximum length of 30 cm (Bergstad *et al*., 1987 and references therein; Hop & Gjøsæter, 2013). While polar cod diet consists primarily of zooplankton (Ajiad & Gjøsæter, 1990; Cusa *et al*., 2019), this species also undergoes an ontogenetic migration; larvae and juveniles are epi- to meso-pelagic and consume sympagic (sea-ice associated) and pelagic zooplankton (Geoffroy *et al*., 2016; Kohlbach *et al*., 2017; Rand *et al*., 2013), while adults (>2 years) are bentho-pelagic and consume benthic invertebrates and fish in addition to zooplankton (Renaud *et al*., 2012). Polar cod is of particular interest in the Arctic marine food web because they efficiently link lower trophic levels they consume as prey to top predators (*e.g*., mammals and seabirds) (Falk-Petersen *et al*., 1990; Hop & Gjøsæter, 2013; Pedersen, 2022; Pedersen *et al*., 2021). Polar cod are likely to experience diet and habitat shifts in future due to continued borealization of Arctic fish communities, increased competition for resources (Eriksen *et al*., 2015; Fossheim *et al*., 2015; Renaud *et al*., 2012) and increased water temperature (Marsh & Mueter, 2020). For example, a recent decrease in Barents Sea polar cod stock has been correlated with warming water temperatures and decreased sea ice concentrations, which reduce polar cod recruitment success (Gjøsæter *et al*., 2020). This may be because juvenile polar cod rely on sea ice structures to escape predation and conserve energy (Gradinger & Bluhm, 2004). Concurrent reductions in Arctic zooplankton that are key prey for juvenile polar cod (*e.g*., *Themisto libellula*) may further reduce the biomass of polar cod in the Barents Sea in the future (Dalpadado *et al*., 2012) and other zooplankton species (*e.g*., euphausiids) may become more important in the diet of polar cod (Cusa *et al*., 2019). The northward expansion of boreal fish species that are better acclimated to warmer water (*e.g*., Atlantic cod, *Gadus morhua* and Capelin, *Mallotus villosus*) may also outcompete polar cod in terms of growth (Marsh & Mueter, 2020) and feeding (McNicholl *et al*., 2016; Orlova *et al*., 2009). Given that polar cod represent an integral trophic link within the Arctic marine food web (Hop & Gjøsæter, 2013; Pedersen *et al*., 2021), determination of species-specific isotopic turnover times and TDFs could improve the accuracy of polar cod trophic level calculations and better constrain the temporal context of their diet as inferred from stable isotope analysis.

This study presents the first experimentally derived estimates of isotopic turnover and TDFs of $\delta^{13}C$ and $\delta^{15}N$ in polar cod (*Boreogadus saida*). We compare the experimentally derived parameters to published values for other fish and Arctic species, as well as to values calculated using published models. In addition, we discuss the challenges associated with generating a consistent, isotopically enriched experimental diet and provide recommendations for the design and execution of future turnover experiments.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

All procedures for the capture, maintenance and sacrifice of fish used in this experiment complied with the Norwegian Regulations on Animal Experimentation. The research facility, 160—Forskning og innovasjonsstasjonen Kraknes (FISK), is approved by the Norwegian Food Safety Authority and holds an aquaculture permit for polar cod issued by the Norwegian Directorate of Fisheries (TT 0044).

2.2 | Fish collection and acclimation

Polar cod were collected using a Campelen trawl fitted with a fish lift from the Barents Sea (north of the Polar Front) and Hornsund, Spitsbergen in January 2018. Once on board, fish were treated daily with Halamid® disinfectant and kept in flow-through tanks of seawater until transferred to the aquaculture facility at Kraknes, Tromsø (Akvaplan-niva, Axcentive SARL, Bouc-Bel-Air, France) where they acclimated to captivity. The general health of the fish was monitored throughout the duration of captivity and fish were treated with dilute

formalin solutions (0.01%) for *Tricodina* parasites once each in 2018 and 2020. Fish were fed a dry broodstock pellet feed (Vitalis CLEAN, Skretting AS, Norway) which was given by hand three times per week. After 3 years (in January 2021), the remaining adult polar cod were transferred randomly to four 300 l experimental tanks and two replicate tanks were assigned to each group: a control (T1) and an isotopically enriched treatment (T2). For the next 4 months, the fish acclimated to the new conditions in preparation for the experiment. They were fed the same dry broodstock pellet diet as previously, and experienced the ambient temperature, salinity and oxygen of the surrounding fjord waters and the light regime of Svalbard offset by 1 week. By April 2021, each tank contained a similar number and total biomass of fish. We refrained from tagging fish to reduce stress and minimize mortality, hence individual fish weights were not tracked through the experiment.

2.3 | Experimental diet

Ten days prior to the start of the experiment, the fish were acclimated to a moist broodstock diet (Tromsø Fiskeindustri AS, Tromsø, Norway) which would allow for better integration of the isotopic label. This moist feed had slightly higher lipid and lower protein content compared to the dry broodstock pellet (Table 1). Experimental fish feed was made by mixing the moist broodstock feed with powdered microalgae (average 0.14 wt.%) in small batches (0.5–1 kg) using a food processor. In the feed for T2, *Spirulina* powders labelled with ^{13}C (>97 atom %, Cambridge Isotope Laboratories NLM-8401-PK) and ^{15}N (>98 atom %, Cambridge Isotope Laboratories CLM-8400-PK, Andover, Massachusetts, USA) were added to create the dual-labelled feed. To account for the natural $\delta^{13}C$-depletion and nutrient content of the *Spirulina*, unlabelled *Arthrospira platensis* (Spirulina powder, Midsona Norge, Oslo, Norway; Supernature) was added to the feed for T1. After the microalgae were fully blended into the food, approximately 100 ml of MilliQ water was added to form a dough which was then split into feeding portions equivalent to approximately 1%–2% of the fish biomass per treatment, pressed into a solid cake and frozen at −20°C until use. The isotopic and elemental composition of the experimental food for the treatments is given in Table 2 (see Supporting Information Table S1 for all data).

2.4 | Sampling procedure and analysis

The experiment began on 26 April 2021 and was conducted for 151 days until 24 September 2021. During the experiment, fish were fed three times per week by thawing the control and treatment food, then adding small pieces to the tanks by hand. Any unconsumed food was removed from the tanks within 48 h during cleaning. Samples of the feed were collected every 21 days throughout the experiment for isotopic analysis to assess the effect of storage on isotopic composition (Supporting Information Figure S1 and Table S1). Three fish from each treatment were removed on specified days (1, 7, 13, 19, 25, 31, 43, 55, 67, 79, 91, 103, 115, 127, 139 and 151 days) after the introduction of the new diet. Isotopic enrichment of fish muscle was expected to occur relatively quickly at the beginning of the experiment, so sampling intervals were shorter (every 6 days) and increased after the first month (to every 12 days). The removal of fish alternated between the tanks

TABLE 1 Diet compositions of the different broodstock diets used during the pre-experiment and experimental periods

Diet description	Use in experiment	Product/producer	Protein (%)	Lipid (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N
Dry broodstock pellet	Pre-experiment period	VitalisCLEAN, Skretting AS	59	11	-22.9	7.67	4.7
Moist broodstock (+ *Spirulina*)	10 days prior to experiment, +*Spirulina* for duration	Tromsø Fiskeindustri AS	46	16	-23.4*	9.17*	5.0*

*Indicates values that correspond to the moist broodstock feed + Spirulina.

TABLE 2 Summary of isotopic ($\delta^{13}C$, $\delta^{15}N$) and elemental (carbon and nitrogen) compositions of polar cod muscle and fish food for the two experimental treatments

		$\delta^{13}C$ (‰ ± S.D.)	$\delta^{15}N$ (‰ ± S.D.)	Carbon (% ± S.D.)	Nitrogen (% ± S.D.)	*n*	C:N
T1 Control							
Food	Mean	−23.4 ± 0.09	9.17 ± 0.14	46.6 ± 0.7	9.34 ± 0.12	21	5.0
Fish	Day 1	−20.8 ± 0.2	12.6 ± 0.2	47.0 ± 0.1	14.0 ± 0.2	3	3.4
	Day 151	−20.8 ± 0.1	13.1 ± 0.3	49.4 ± 2.3	15.0 ± 0.4	4	3.2
T2 Enriched							
Food	Mean	9.51 ± 12.0	205.9 ± 174	48.5 ± 4.27	9.84 ± 1.38	23	4.9
Fish	Day 1	−20.7 ± 0.2	12.9 ± 0.1	45.5 ± 0.5	13.7 ± 0.1	3	3.3
	Day 151	−17.5	25.9	45.3	13.7	1	3.3

Note: A mean of all food samples analysed throughout the experiment is given and the data for fish are given as means at the start (day 1) and the end of the experiment (day 151). All isotope values are reported in ‰ (± standard deviation), elemental compositions are reported in % (± standard deviation) and sample sizes of all measurements are given (*n*). C:N, %carbon/%nitrogen.

within each treatment to ensure that the number of fish per aquarium and the total number of fish per treatment remained approximately equal throughout the experiment. After random selection and removal, fish were humanely sacrificed with a sharp blow to the head and were immediately weighed, measured (total length) and frozen whole (−20°C). Fish that died naturally but did not appear unhealthy ($n = 7$) were also collected, measured, frozen and processed identically to other sampled fish. Frozen fish were later dissected to isolate approximately 2 g (wet weight) of dorsal muscle tissue. All fish muscle and food samples were freeze dried for 48 h (Labconco, model series 70020, 2.5 l capacity, −50°C), ground and homogenized using a mortar and pestle, and approximately 0.5 mg packaged into analytical tins for isotopic analysis. All samples were analysed for stable isotopes of carbon and nitrogen using a continuous-flow isotope ratio mass spectrometer (CF-IRMS) with a Thermo Scientific Flash 2000 elemental analyser and Thermo Scientific Conflow IV interfaced with a Thermo Scientific DeltaV^{Plus} Mass Spectrometer (Alaska Stable Isotope Facility, University of Alaska Fairbanks Water & Environmental Research Center, USA). Isotopic values were reported in δ notation (Equation 1) where R is the ratio of heavy:light isotopes of the element of interest X (*e.g.*, $^{13}C/^{12}C$) relative to the international standards Vienna PeeDee Belemnite (carbon) and Air (nitrogen). The instrument precision was <0.2‰ for all measurements.

$$\delta^n X\,(‰) = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 1000 \quad (1)$$

The isotopic values of $\delta^{13}C$ for fish muscle were not lipid-corrected as the C:N ratio of fish tissue was low (<3.5 as suggested by Post, 2002), and lipid content of polar cod muscle is only approximately 3% since lipid is primarily stored in the liver rather than muscle in this species (Hop & Gjøsæter, 2013). Similarly, isotopic values of $\delta^{13}C$ from diet samples were not lipid-corrected because a minimal (ca. 1‰) effect on $\delta^{13}C$ values has been documented for plants and animals with a lipid content of 16% (Post *et al.*, 2007).

2.5 | Data analysis

There were no systematic differences in environmental conditions (Supporting Information Figure S2) or isotopic compositions of fish muscle (Supporting Information Figure S3) between tanks within each treatment, thus data were pooled to the treatment level for all analyses. Two isotopic measurements of food from the enriched treatment on days 64 and 148 were removed from the dataset as they were an order of magnitude higher than any other measurements (>300‰ $\delta^{13}C$ and >9000‰ $\delta^{15}N$). TDFs for carbon and nitrogen ($\Delta^{13}C$ and $\Delta^{15}N$) were calculated using Equations 2 and 3 where $\delta^{13}C_{fish}$ and $\delta^{15}N_{fish}$ are the mean final isotopic composition of the fish muscle and $\delta^{13}C_{diet}$ and $\delta^{15}N_{diet}$ are the overall mean isotopic compositions of the food (Table 2).

$$\Delta^{13}C = \delta^{13}C_{fish} - \delta^{13}C_{diet} \quad (2)$$

$$\Delta^{15}N = \delta^{15}N_{fish} - \delta^{15}N_{diet} \quad (3)$$

To determine the isotopic turnover time, the raw and mean $\delta^{13}C$ and $\delta^{15}N$ data for T2 were fitted with nonlinear least squares regressions using the "nls" function following Equation 4 (nls model starting values for $\delta^{13}C$ model $a = 11$, $b = 0.1$, $c = -23$ and the $\delta^{15}N$ model $a = 15$, $b = 0.2$, $c = 45$). This equation describes the time-dependent change in isotopic composition where δ_t is the isotopic value at time t, the coefficient a is equivalent to the difference between the initial and asymptotic isotope value, λ is the decay constant (‰ d^{-1}) and c is the asymptotic isotope value.

$$\delta_t = ae^{-\lambda t} + c \quad (4)$$

$$t_{½} = \frac{\ln(2)}{\lambda} \quad (5)$$

$$\delta_z = ae^{-(g+m)t} + c \quad (6)$$

The resulting λ value from each model was used to calculate the isotopic turnover time ($t_{½}$ reported in days) using Equation 5 (Table 3). Despite the expectation of no fish growth because of the adult age of the fish in this experiment and minimal feeding, we also calculated the proportion of total isotopic turnover attributed to growth and metabolism using Equation 6 (Kaufman *et al.*, 2008). This equation provides a quantitative assessment of growth contributing to turnover which can be directly compared with other studies. In this equation, g is the growth rate calculated as $g = \ln(W_f/W_0)/t$. Here, W_0 and W_f are the initial and final body weight of polar cod, respectively, calculated from a linear regression of measured body weights from the enriched treatment, where t is the time in days for a start time of 0 days and end of 151 days (Supporting Information Figure S4, equation in B). The total turnover (λ) is equal to the sum of the turnover attributed to growth

TABLE 3 Isotopic turnover estimates in polar cod

	a (‰)	c (‰)	λ (day^{-1})	$t_{½}$ (days)	g (day^{-1})	m (day^{-1})	Growth (%)	Metabolism (%)
^{13}C	−10.2 ± 7.58	−10.5 ± 8.65	0.0114 ± 0.019	61.0 (22.5, 86.3)	6.53 x 10^{-4}	0.0107 (7.38 x 10^{-3}, 0.0301)	5.75 (2.12, 8.13)	94.3 (91.9, 97.9)
^{15}N	−40.0 ± 23.0	53.3 ± 26.8	0.0142 ± 0.022	48.8 (19.1, 88.7)	6.53 x 10^{-4}	0.0136 (7.16 x 10^{-3}, 0.0356)	4.60 (1.80, 8.37)	95.4 (91.6, 98.2)

Note: Nonlinear least squares regression model coefficients a, c and λ from Equation (4) are given (±95% confidence intervals) and the isotopic turnover in days ($t_{½}$) calculated based on Equation (5) with upper and lower limits calculated based on 95% confidence intervals of λ. The calculated parameters g and m from Kaufmann et al. (2008) are provided and based on these estimates the percentage contribution by growth and metabolism to total turnover was calculated with 95% confidence intervals given in parentheses for these parameters.

(g) and turnover attributed to metabolism (m), thus $m = \lambda - [\ln (W_f/W_0)/t]$. The proportion of growth and metabolism contributing to total turnover was then calculated for each isotope using the absolute value of the λ estimates and the 95% confidence intervals from each model regression (Table 3). Finally, to test whether fish body size could have a significant effect on turnover time estimates, we conducted correlation analyses between nls model residuals for both $\delta^{13}C$ and $\delta^{15}N$, and fish body weight using the Spearman correlation. All statistical analyses were conducted in R (R Core Team, 2021) and figures were made using ggplot2 package (Wickham & Chang, 2014).

We compared estimates of TDF and turnover time from this experiment calculated using Equations (2)–(4) to values calculated using published equations from recent meta-analyses of fishes (Canseco *et al.*, 2021; Caut *et al.*, 2009; Hussey *et al.*, 2014), as well as models accounting for the allometric relationship between body weight and temperature (Thomas & Crowther, 2015). Model equations and values are given in Table 4.

3 | RESULTS

3.1 | $\delta^{13}C$ and $\delta^{15}N$ and TDFs

The isotopic composition of polar cod muscle in the control remained constant throughout the experiment, varying by 0.7‰ and 1.7‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. On average, the isotopic composition of polar cod muscle was −20.8 ± 0.2‰ and 12.6 ± 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, at the beginning of the experiment and −20.8 ± 0.1‰ and 13.1 ± 0.3‰ $\delta^{13}C$ and $\delta^{15}N$, respectively, at the end (Table 2). This resulted in a total change of both mean $\delta^{13}C$ and $\delta^{15}N$ of ≤0.5‰ over 151 days. Food for the control also had a consistent isotopic composition throughout the experiment with a narrow range of isotopic values that varied by <0.6‰ for both $\delta^{13}C$ and $\delta^{15}N$. TDFs of carbon ($\Delta^{13}C$) and nitrogen ($\Delta^{15}N$) for the control were 2.6‰ and 3.9‰, respectively, and were only 0.3‰–0.8‰ higher than values calculated using other published models (Table 4).

The isotopic compositions of polar cod muscle in the enriched treatment changed by approximately 3‰ for $\delta^{13}C$ and >13‰ for $\delta^{15}N$ when comparing mean isotope values at the beginning and end of the experiment (Table 2). However, variability between replicates was much greater compared to the control. The greatest change for individual fish was 17.3‰ for $\delta^{13}C$ and 80‰ for $\delta^{15}N$ after 127 days of the experiment. Food for the enriched treatment was also more variable than that of the control, resulting in inaccurate estimates of trophic discrimination with values of −27.1‰ for $\Delta^{13}C$ and −180‰ for $\Delta^{15}N$. Possible reasons for such high variability and the impact on other calculations are presented in the discussion.

3.2 | Isotopic turnover of carbon and nitrogen

Isotopic turnover time (half-life) estimates from nonlinear regressions were 60 and 49 days for $\delta^{13}C$ and $\delta^{15}N$, respectively (Table 3). Both regressions had wide confidence intervals for the predicted isotopic compositions reflecting the variability of enrichment in individual fish (grey shading in Figure 1 and values in Table 3). The turnover time estimates calculated using Equations (4) and (5) were lower than those calculated using the models of Thomas and Crowther (2015), which were estimated at over 90 days for both $\delta^{13}C$ and $\delta^{15}N$, but of similar magnitude to those estimated from the equation in Vander Zanden *et al.* (2015) (Table 4). The model presented by Vander Zanden *et al.* (2015) assumes that the turnover of carbon and nitrogen occur at the same rate, but the turnover time for $\delta^{13}C$ calculated in this study was 12 days longer than for $\delta^{15}N$.

TABLE 4 Trophic discrimination factors ($\Delta^{13}C$, $\Delta^{15}N$ in ‰) and isotopic turnover (half-life $t_{½}C$, $t_{½}N$ in days) values for polar cod

Model equation	$\Delta^{13}C$	$\Delta^{15}N$	$t_{½}C$	$t_{½}N$	Model reference
$\Delta^n X = \delta^n X_{fish} - \delta^n X_{diet}$ (control)	2.6	3.9			This study
$\Delta^n X = \delta^n X_{fish} - \delta^n X_{diet}$ (enriched)	-27.1	-180			This study
$\Delta^{15}N = -0.281 * \delta^{15}N_{diet} + 5.879$		3.29			Caut *et al.*, 2009
$\Delta^{15}N = -0.27 * \delta^{15}N_{diet} + 5.92$		3.39			Hussey *et al.*, 2014
$\Delta^{15}N = 7.72 - 0.07 * T - 0.29 * \delta^{15}N_{PDB} + PCF$		3.38			Canseco *et al.*, 2021
$\Delta^{13}C = -0.248 * \delta^{13}C_{diet} - 3.4770$	2.31				Caut *et al.*, 2009
$\Delta^{13}C = -0.75 - 0.11 * \delta^{13}C_{PDB}$	1.82				Canseco *et al.*, 2021
$t_{½} = \ln(2)/\lambda$ (enriched)			61.0	48.8	This study
$\log_{10}(^{13}Ct_{½}) = 1.6668 + 0.1935 * \log_{10}(BM) + -0.0153 * BT$			92.4		Thomas & Crowther, 2015
$\log_{10}(^{15}Nt_{½}) = 1.6884 + 0.1933 * \log_{10}(BM) + -0.0149 * BT$				97.5	Thomas & Crowther, 2015
$\ln(t_{1/2}) = 0.21 * \ln(BM) + 3.23$			67.1	67.1	Vander Zanden *et al.*, 2015
$\log_{10}(^{13}Ct½) = -3.65 - 0.22 * \log_{10}(BM)$			39.9		Weidel *et al.*, 2011

Note: $\Delta^{13}C$ and $\Delta^{15}N$ are given for control (T1) while half-lives ($t_{½}$) of C and N are given for enriched treatment (T2). Discrimination factors were calculated for both treatments using Equations (2) and (3) and compared with values calculated using models published in the literature. Isotopic turnover times were calculated using Equations (4) and (5) for the enriched treatment and compared with values calculated using models published in the literature.
Abbreviation: $\delta^{13}C_{PDB}$, T1 diet initial $\delta^{13}C$; $\delta^{15}N_{PDB}$, T1 diet initial $\delta^{15}N$; BM, mean body mass of fish in T2; BT, mean water temperature during the experiment; PCF, prey-type conversion factor for fish pellets (−1.20‰); T, mean water temperature during the experiment.

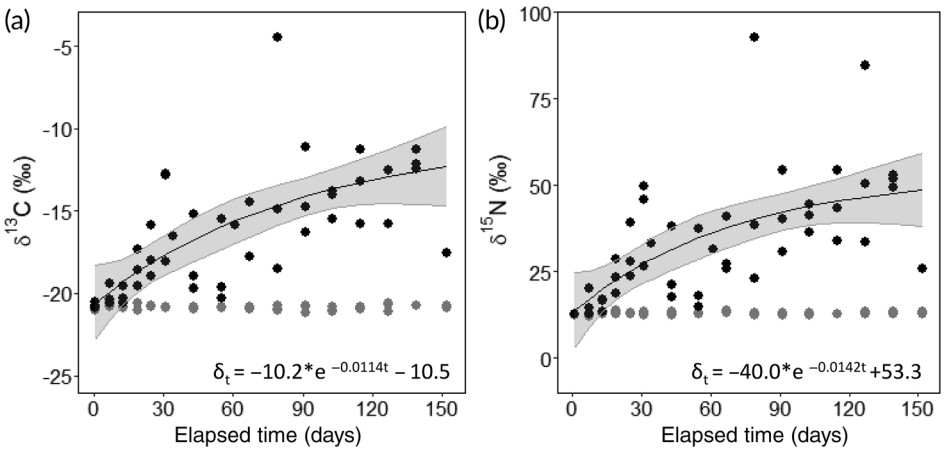

FIGURE 1 $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values of polar cod muscle during the isotope turnover experiment in the two experimental treatments, T1 in grey and T2 in black. Nonlinear regressions fitted to the data of the enriched treatment (T2) are shown by the black lines and the grey shaded regions represent the 95% confidence intervals. Regression equations are provided in the bottom-right of each plot. ●, T1 (control); ●, T2 (enriched).

Overall, metabolism was responsible for >94% of isotopic turnover during this experiment compared with small contributions (< 6%) from somatic growth (Table 3). Although we did not track individual fish growth throughout the study, there was little change in the average length and weight of fish sampled over time and linear regressions revealed slopes (*i.e.*, growth rates) that were not significantly different from zero (Supporting Information Figure S4). Additionally, there was no significant correlation between model residuals and fish body size ($P > 0.05$ for both $\delta^{13}C$ and $\delta^{15}N$), suggesting that there was no systematic effect of body size on turnover. This supports the finding that metabolism and not growth was responsible for the majority of isotopic turnover during this experiment, confirming our expectation since adult fish were used.

3.3 | Fish survival and health

Mortality during the experiment was low ($n = 7$). Water temperatures remained within the thermal tolerance range expected for polar cod (Brennan *et al.*, 2016; Schurmann *et al.*, 1994) and oxygen concentrations were maintained at ≥92% saturation during the experiment, well above the stress threshold of hypoxia (Farrell & Richards, 2009). Fish health was generally good, no fish had visible lesions or abnormalities and individuals that died did not have parasites but were perhaps weakened during the spawning period. Many individuals spawned during the first 2 months of the experiment (personal communication, E. Sztybor, Kraknes Facility, May), but individual reproductive condition was not possible to track since fish were not tagged. There was no evidence for biofouling within the tanks that could have introduced isotopically variable food sources to the fish. We are therefore confident that changes in the isotopic compositions of fish muscle over time are directly related to consumption of the experimental diet and fish physiology.

4 | DISCUSSION

We present the first isotopic turnover experiment of polar cod and the second of any Arctic fish. Our experiment yielded isotopic turnover times of 61 and 49 days for carbon and nitrogen, respectively, which were shorter than expected for adult fish with slow growth. We compared our results with literature values and discussed the potential effects of temperature, body size/growth and metabolic processes on isotopic turnover times. We calculated TDFs of 2.6‰ and 3.9‰ for carbon and nitrogen, respectively, from our control group and we compared these with literature values discussing the impact of diet composition on trophic discrimination. The TDFs calculated from the enriched treatment in our experiment produced erroneous values of −27.1‰ and −180‰ for carbon and nitrogen, respectively, therefore we investigated potential causes. Based on our findings and the challenges we faced in this experiment, we provide an outlook for future ecological studies using bulk stable isotope analysis emphasizing the consideration of isotopic turnover and trophic discrimination, and we provide recommendations for future turnover experiments.

4.1 | Isotopic turnover times

As expected, we found that isotopic turnover times of polar cod muscle were comparable to the only other Arctic fish species for which data exist (*Myoxocephalus scorpioides*), as well as to a closely related gadid, Atlantic cod (*Gadus morhua*), and rainbow trout (*Oncorhynchus mykiss*). The similarity between turnover times reported for *Gadus morhua* juveniles and the adult polar cod in our study was surprising because juveniles typically have faster isotopic turnover compared to adult fish due to more rapid growth and higher rates of muscle tissue production. This suggests that phylogeny may play a role in constraining isotopic turnover, and that turnover times for poorly studied Arctic species may be approximated from closely related species from boreal waters. With only one example, however, this conclusion should be treated as largely speculation. Isotopic turnover in polar cod muscle was also similar to half-lives reported for several fish species that were fed similar diets but were reared at temperatures 7–18°C warmer than in our study (see nitrogen turnover of *Pomatoschistus minutus*, *Lateolabrax japonicus* and carbon turnover of *Paralichthys dentatus*, *Danio rerio* in Table 5), a factor that should decrease turnover times (Tieszen *et al.*, 1983; Weidel *et al.*, 2011). Similarity between turnover times of unrelated fish species from experiments

TABLE 5 Results of published diet-switching studies to measure isotopic turnover ($t_{½}$C and $t_{½}$N in days) and trophic discrimination (Δ^{13}C and Δ^{15}N‰) compared to this study

Species	Diet	(°C)	$t_{½}$C	$t_{½}$N	Δ^{13}C	Δ^{15}N
Onisimus litoralis (A)[a]	Ice algae†	1	18.7	22.4		
	Ice algae†	1	77			
	Ice algae	1		115.5		
	Ice algae†	4	13.9	22.4		
Macoma moesta (M)[b]	Diatoms†	4.6	> 21	> 21		
Nuculana radiata (M)[b]	Diatoms†	4.6	> 21	> 21		
Myoxocephalus scorpioides (C)[c]	Fish	8		37	1.7	2.06
	Krill	8	39	74	1.95	3.53
Boreogadus saida (C)[This study]	Fish-based pellets†	<10	61.0	48.8	2.6	3.9
Clupea pallasi (C)[d]	Fish-based pellets†	10.6				~5
Gadus morhua (C)[‡ e,f]	Mussel	11	~42	77.9	~1.7	5.1
	Fish (planktivore)	11	~49	~53	2.17	~2.5
	Fish (piscivore)	11	~30	~41	0.69	0.76
	Fish-based pellets	4-14			~3	2.6–3.2
Oncorhynchus mykiss (C)[g]	Pellets (Bio Vita)	14		39–52		3.4
Pseudopleuronectes americanus (C)[‡ h]	Brine shrimp	13	4.1	3.9	2.5	2.2
	Brine shrimp	18	2.2	3.1	<1	-0.3
Pomatoschistus minutus (C)[i]	Fish-based pellets	17	24.7	27.8	3.15	
Paralichthys dentatus (C)[‡ j]	Krill	20	68.9	84.9	3.1	2.13
	Krill	20	49.4	106.5	4.79	2.53
Solea senegalensis (C)[‡ k]	Brine shrimp	21.1	2.5–3.5		0.8–2.3	
Lateolabrax japonicus (C)[‡ l]	Pellets (Hirame-S)	23	19.3	25.7	2.4	2.4
Pseudoplatystoma corruscans (C)[‡ m]	Fish	26.7	4.38		0.36	
Danio rerio (C)[n]	Pellets (Purina)	28.5	53.3	147	~2	~3

Note: The first five species are records of Arctic fish and invertebrates, and the remaining rows are of other fish species. The use of isotopically enriched diets is denoted by † (next to diet) and studies where juveniles were used are indicated by ‡ (next to species name). References are given by superscript letters with details in the table footnote. Invertebrate phyla represented: A, Arthropoda; M, Mollusca; C, Chordata.
References: [a] Kaufman *et al*., 2008; [b] Weems *et al*., 2012; [c] Barton et al. 2019a; [d] Miller 2006; [e] Ankjærø *et al*., 2012; [f] Godiksen *et al*., 2019; [g] Heady and Moore, 2012; [h] Bosley et al. 2002; [i] Guelinckx *et al*., 2007; [j] Buchheister and Latour, 2010; [k] Gamboa-Delgado et al. 2008; [l] Suzuki et al. 2005; [m] Furuya et al. 2002; [n] Tarboush et al. 2006.

conducted at higher temperatures but with similar food to our study suggests that isotopic turnover times of fishes may be more affected by diet composition than by water temperature (see further discussion below).

Since no temperature effect was observed, it is possible that the polar cod used in our experiment were well adapted to the seasonally warm water temperatures they were exposed to after several years of captivity in these conditions. The polar cod used in our experiment were acclimated for more than 3 years to temperatures of approximately 3–12°C (4.6–9.7°C during our experiment April–September), which is at the upper end of the expected thermal range for wild polar cod. For example, polar cod are often found in high abundances in association with fronts of the Beaufort Sea where temperatures can reach 9°C and occasionally 14°C or more in the shallow near-shore regions during the peak of summer (Laurel *et al*., 2016). In offshore regions, however, polar cod rarely encounter temperatures >2°C (Laurel *et al*., 2016) and juvenile polar cod living in association with sea ice experience near-freezing temperatures. Despite laboratory evidence that polar cod performance is greatly reduced at a temperature of 6.5°C (Drost *et al*., 2016; Kunz *et al*., 2018), there was no sign of low survivorship, or poor health or swimming ability of the polar cod during our experiment. Furthermore, the temperature increase during the experiment did not cause isotopic enrichment of the polar cod muscle evident from the lack of enrichment in the control which was exposed to the same temperature regime as the enriched treatment. It is also possible that no temperature effect was observed because the temperature increased nearly two-fold by day 60 of the experiment, when the fish were already approaching steady state. Considering these circumstances, we suggest that the turnover time estimates we present here may best represent adult polar cod living in a seasonally warm environment (*e.g*., shallow regions like the Chukchi Sea).

We found surprisingly rapid isotopic turnover in muscle tissue of polar cod otherwise expected to have a slow turnover because of the relatively low temperature, moderate size, adult age and slow growth

of fish used in our study compared to most isotopic turnover studies of fishes. In fact, our experimentally derived turnover times were 31–48.7 days shorter than if calculated from published model equations that consider body mass and temperature. However, most model-derived turnover times were within the confidence intervals of the isotopic turnover times estimated by our experiment, albeit at the upper ends of the intervals (Table 3). One model (Weidel *et al*., 2011) predicted a carbon half-life that was 21 days shorter than observed in our study, but nitrogen turnover was not determined in that study for comparison. The wide confidence intervals (and high uncertainty in turnover time estimates) may be the result of variability in individual fish body size since there is a negative relationship between isotopic turnover time and fish body size (Weidel *et al*., 2011). Despite this general trend, there was no significant correlation between fish mass and the nls model residuals of either $\delta^{13}C$ or $\delta^{15}N$ in this study from which we can robustly assess the cause of isotopic variability. The polar cod used in our study varied in body weight from 72 to 139 g (enriched treatment) and the turnover times calculated from models (Thomas & Crowther, 2015) differed by 12 days due to this difference in size alone. The sensitivity of isotopic turnover models to temperature also increases with increasing body size. For the average adult polar cod in our study (104 g), the calculated turnover times of carbon and nitrogen decreases from 102 to 86 days for the temperature extremes of our experiment (4.6 and 9.7°C, respectively), therefore while the variability in water temperature and body size of the fish in our experiment was not unreasonable for a population of wild polar cod, it may have increased the uncertainty of our turnover time estimates. Nonetheless, we are confident that the isotopic turnover times presented here are valid and provide a reasonable constraint on the temporal scale of bulk stable isotopic measurements from polar cod muscle.

Our study found that >94% of the isotopic turnover in adult polar cod muscle was attributed to metabolic processes, supporting previous work on fishes (Guelinckx *et al*., 2007 and references therein). Metabolism is the balance of catabolism (the breakdown of compounds) and anabolism (the synthesis of new, complex compounds from simple molecules). Together, these processes control the rate of protein turnover in muscle tissue (De La Higuera *et al*., 1999; Perga & Gerdeaux, 2005). The source of molecules used in anabolic processes can be from dietary components or products catabolized from other tissues. The isotopic composition of a tissue will therefore reflect the origin of the components used to synthesise it (Gannes *et al*., 1997). In our experiment, we can confirm that fish assimilated isotopically heavy carbon and nitrogen directly from the experimental diet because there was significant enrichment of both elements within the muscle tissue. However, there must also have been catabolic processes occurring in the muscle because the mean weight of fish did not increase, which would occur if anabolic processes dominated turnover. We also observed an increase in the muscle tissue C:N ratio during the first ~50 days of the experiment concurrent with the increases in both $\delta^{13}C$ and $\delta^{15}N$. This could result from an increase in lipid storage within the muscle tissue during the experiment which would primarily retain carbon while excess nitrogen is continuously excreted (Perga & Gerdeaux, 2005). However, we cannot confirm this hypothesis with the data available. We conclude that the metabolic processes involved in protein turnover contributed to the relatively rapid isotopic turnover in adult polar cod muscle tissue that we measured, despite limited growth of the adult fish.

Reproduction can also influence isotopic turnover through changes in feeding rates, catabolism and weight loss, yet we cannot quantify the potential role of those processes. Prior to spawning, polar cod may undergo a brief (1–7 days) cessation of feeding and appear weak (Graham & Hop, 1995). A few polar cod individuals appeared to be preparing for spawning at the start of our experiment, and several spawned during the first 1–2 months of the experiment (E. Sztybor, personal observation). While eggs were periodically observed in the experimental tanks and some individuals were visibly full of eggs during sampling, fish continued to feed throughout the experiment, suggesting that there was only limited, if any, change in feeding behaviour (E. Sztybor, personal observations). Sexually maturing fish can also utilize energy stores of protein and lipid to aid in gonad maturation, and previous experiments with Atlantic cod have shown that energy stores from the liver can be used and replenished quickly in response to starvation or spawning (Karlsen *et al*., 1995). These catabolic processes would be expected to increase isotopic enrichment of individuals, although the greatest enrichment would be expected in gonadal tissue (Jardine *et al*., 2005) and there was no evidence that fish observed bearing eggs had more isotopically enriched muscle tissue than fish without eggs. The shedding of eggs or sperm should result in significant weight reduction as gonad weight can represent up to 22% of total body weight in polar cod (Hop *et al*., 1995; Nahrgang *et al*., 2016). We did not detect significant changes in mean body weight during our study, although individual reproductive information and body weights would be necessary to assess the full impact of reproduction on isotopic turnover in polar cod. Nonetheless, differences in the sex and reproductive condition of individuals in our study may have contributed to the large variability in isotopic values we measured among individuals. We are, however, confident that the isotopic turnover we measured was not detectably affected by reproductive condition because isotopic enrichment in muscle was only observed in the fish from the enriched treatment and not the control where individuals were in the same reproductive condition.

4.2 | Diet composition and isotopic variability

Dietary components can be assimilated at different rates thereby affecting the rate at which these components are incorporated into tissues. In our study, the isotopic label was introduced via enriched *Spirulina* added to the moist broodstock feed. Although *Spirulina* is not part of wild polar cod diet, it has been evaluated as a potential replacement for fish meal in various animal feeds because it contains essential nutrients (including polyunsaturated fatty acids), minerals and a high proportion of protein, which have been shown to support growth through rapid assimilation (Gamboa-Delgado *et al*., 2019;

Holman & Malau-Aduli, 2013; Tokuşoglu & Ünal, 2003). The high amino acid and protein content of the *Spirulina* may have made it more easily assimilated compared to other components of the feed, which would have increased the rate of isotopic enrichment of muscle and decreased the isotopic half-life. Such differential assimilation of dietary components occurs, for example, in Arctic zooplankton such as *Calanus* spp. (Graeve *et al*., 2005) and in fish in aquaculture industries fed pellets similar to the moist broodstock feed used in our study (Blanchet-Aurigny *et al*., 2012; Kusche *et al*., 2018; Tomas *et al*., 2006; Whitledge & Rabeni, 1997). However, the fish in our experiment reached isotopic equilibrium (as indicated by the asymptote in the nonlinear regressions) at values substantially *lower* than expected based on the isotopic composition of the experimental diet. Preferential assimilation of the *Spirulina* would not only have decreased the turnover time but also increased the enrichment of individual fish, thereby decreasing the difference between isotope values of the muscle tissue and diet. Thus, differential assimilation of dietary components cannot fully account for the discrepancy between diet and equilibrium isotope values.

The enrichment of ^{13}C and ^{15}N in the experimental diet may have been overestimated if nutrients from the enrichment process of *Spirulina* were retained on the algal cells. To isotopically enrich microalgae (*e.g*., *Spirulina*), it is typically cultured using enriched nutrients (*e.g*., $Na^{15}NO_3$ and $NaH^{13}CO_3$) which are assimilated into the algal cells during growth. If these nutrients remain on the algal cells, the apparent enrichment of the diet will be greater than that assimilated by the consumer because these nutrients are not bioavailable to fish. Although the enriched *Spirulina* used in this study was produced with culturing methods similar to those described above, a very small amount of enriched nutrients was used in the culturing process and the algal cells were sufficiently rinsed prior to drying (personal communication, Cambridge Isotope Laboratories, May). It is unlikely that enriched nutrients contaminated the *Spirulina* we used and artificially increased the enrichment of the diet. However, we cannot fully discount this possibility and recommend that future studies using enriched microalgae take precautions to ensure that no contamination occurs.

If enriched nutrients were present in the experimental diet we used, it is also possible that they could have leached from the food while it soaked in seawater prior to consumption (Kaufmann et al., 2008). This leaching would reduce the enrichment of food that was consumed relative to the unsoaked diet samples that we analysed and could explain the discrepancy between the isotopic values of the diet and the equilibrium values reached by fish in this study. To test this hypothesis, we measured the isotopic composition of food from our control and enriched treatment after 0, 6, 12, 18, 24 and 48 h of exposure to seawater. We did not observe significant changes in enrichment of either $\delta^{13}C$ or $\delta^{15}N$ over the 48 h, although replicates were again highly variable (see Supporting Information Figure S5 and Table S2 for raw data). Nonetheless, these results do not support the hypothesis that leaching of enriched nutrients from the experimental food can account for the reduced isotopic equilibrium values attained by the fish in our study.

The high variability in diet isotope values we observed may be the result of the heterogeneous distribution of the enriched *Spirulina* within the experimental food. We know of two other studies which used highly enriched algae in experimental food with variability in diet isotope values similar to our study (Kaufman *et al*., 2008; Weems *et al*., 2012). We chose to use a highly enriched (>97 atom %) algal product to create our enriched experimental feed because (a) it was readily available, (b) it was produced under laboratory control and was expected to be more isotopically consistent compared to our own culturing efforts, and (c) we were confident that the *Spirulina* would be assimilated, thereby ensuring a measurable enrichment of the consumer tissue. However, a consequence of using such an enriched product was that we needed to add very little to the fish food, which made it difficult to distribute evenly. This presumably means that the amount of *Spirulina* consumed by individual fish was also highly variable. Different feeding rates among individuals (expected within a population) and differences in the amount of *Spirulina* consumed could have led to a wide range of isotopic equilibrium trajectories of the fish in the experiment. Fish that consumed more *Spirulina* would have experienced greater tissue enrichment and attained a higher equilibrium value compared to fish that consumed less *Spirulina*. There was no evidence of feeding behaviour that actively selected for or against fish food that contained more *Spirulina* (personal communication, E. Sztybor, Kraknes Facility). Because polar cod are small, we were unable to measure the temporal evolution of isotope values of muscle tissue within individual fish by using a nondestructive sampling method. Instead, we sacrificed multiple fish at different times during the experiment, measured the isotopic values and had to assume that the regression analysis of the resulting data would produce an average estimate of turnover for this small population. The confidence intervals of these estimates would then represent the variability among individuals. However, if individual fish were on trajectories for different isotopic equilibria, then our instantaneous isotope measurements could have represented very different stages of isotopic turnover and the regression analysis may not well represent the population average. A recent study of more than 100 California yellowtail showed that individual differences in metabolism and growth caused the estimates of isotopic half-life to vary by hundreds of days (Madigan *et al*., 2021). If we had used a nondestructive sampling method, we may have been able to assess whether individual polar cod had different isotopic equilibrium trajectories which could explain some of the variability in our turnover time estimates.

4.3 | Factors affecting trophic discrimination

The TDFs of carbon and nitrogen from the control in our study were 2.6‰ and 3.9‰, respectively. The TDF of nitrogen was most similar to that of the Arctic sculpin which was fed krill, rainbow trout maintained on a pellet diet, and the universally used values of 3.4‰ and 3.8‰ (Hobson & Welch, 1992; Minagawa & Wada, 1984). The TDF of carbon was most similar to fish species that were fed fish-based pellets (*e.g*., *Lateolabrax japonicus*, *Gadus morhua*, *Pomatoschistus*

minutus) or crustaceans (*e.g.*, *Pseudopleuronectes americanus*, *Paralichthys dentatus*), and substantially higher than the typically referenced ≤1‰ value (Post, 2002). Although temperature is known to affect trophic fractionation in fishes (Canseco *et al.*, 2021), the TDFs calculated from the control group in our experiment seem most comparable to studies based on the experimental diet rather than rearing temperature.

Trophic discrimination of both carbon and nitrogen in consumer tissue increases with decreasing dietary protein content as a result of variable amino acid composition and fractionation (McMahon *et al.*, 2010). The fish-based moist broodstock diet used in our study had moderate protein content (46% crude protein) compared to other diets from experiments that yielded TDFs similar to this study. For example, the krill diet used in the study of Arctic sculpin (Barton *et al.*, 2019b) contained 70% protein and yielded TDFs of carbon and nitrogen of 1.95‰ and 3.53‰, respectively. A feeding experiment with Atlantic cod (Godiksen *et al.*, 2019) used a diet containing 54%–57% protein and measured TDFs of ~3‰ (carbon) and 2.6‰–3.2‰ (nitrogen). However, protein quality (in terms of amino acid composition) can also affect trophic discrimination. Generally, protein quality is considered high when the amount of essential amino acids is sufficient for growth and similar to that of the consumer tissue (Florin *et al.*, 2011; McMahon *et al.*, 2015) and poor when amino acids are limited, necessitating the use of stored proteins (*e.g.*, muscle tissue) as amino acid sources (McMahon *et al.*, 2010). In fact, >80% of the variation in $\Delta^{15}N$ from published studies of mammals and birds can be explained by differences in dietary protein quantity and quality alone (Florin *et al.*, 2011). Although the specific amino acid compositions of the experimental diet or fish used in this study are unknown, ongoing catabolism in the muscle tissue (described previously) suggests that the polar cod were utilizing this stored protein as an amino acid source rather than routing amino acids directly from the diet, resulting in an increased $\Delta^{13}C$ (McMahon *et al.*, 2010, and references therein).

Trophic discrimination of carbon also increases with increasing diet lipid content (Wolf *et al.*, 2015). For example, a dietary lipid increase from 6% to 24% resulted in an increase in $\Delta^{13}C$ of Arctic sculpin muscle from 1.70‰ to 1.95‰ when the two different diets contained similar proportions of protein (Barton *et al.*, 2019a). Similarly, *Gadus morhua* juveniles had $\Delta^{13}C$ values that increased from 0.69‰ to 2.17‰ as a result of increased dietary lipid of fish used as food in the experiment (Ankjærø *et al.*, 2012). The experimental diet used in our study contained a moderate amount of lipid (ca. 16%) which may have contributed to the elevated TDF of carbon that we measured compared to the expected ~1‰. TDFs of wild polar cod are expected to be similar to or higher than TDFs measured in this study, as they feed primarily on zooplankton with slightly higher protein content (52%–59%; Suontama *et al.*, 2007), a similar or lower protein quality (in terms of amino acid similarity to consumer) to that of the experimental diet, but a substantially higher lipid content (Bouchard & Fortier, 2020; Falk-Petersen *et al.*, 2009). These results highlight the need to consider the nutritional composition of the diet used to derive TDF values when comparing to the literature or when choosing TDF values to apply in isotope mixing models.

4.4 | Recommendations for future studies

Polar cod is the most abundant fish in the Arctic, representing an integral link within Arctic marine food webs, and their diet may be altered as prey abundances change in response to continued ocean warming (Fossheim *et al.*, 2015). Seasonal and interannual changes to the Arctic marine ecosystem emphasize the need for continued seasonal sampling and a better understanding of diet composition and plasticity of key Arctic species, including polar cod. Given that complete isotopic equilibrium requires four to five half-lives of exposure to a stable diet, polar cod muscle would require more than 120 days to reach complete isotopic equilibrium with its prey. This ultimately means that the diet composition inferred from bulk stable isotopes of adult polar cod muscle is indicative of the integrated diet over the preceding ca. 4 months and this method cannot be used to resolve dietary changes over a period shorter than the turnover time (half-life) of 1–2 months. Thus, to resolve diet or trophic position differences at a higher temporal resolution, we recommend the use of additional dietary biomarkers (*e.g.*, fatty acids, Budge *et al.*, 2008) or isotope analysis of tissues with more rapid turnover like liver or blood (Hobson & Clark, 1992).

Given that bulk stable isotope analysis has become such a common tool in food-web studies, and interpretations of the data from these studies depend on accurate turnover times and TDFs, species-specific values of these parameters remain valuable. However, given the challenges of experimentation we have documented here, we recommend future isotopic turnover studies aim to (a) assess large sample sizes to better capture population-level variation, (b) utilize nonlethal sampling to trace isotopic turnover in individuals, (c) conduct studies across different life stages to improve our understanding of how growth, metabolism and reproduction affect isotopic turnover, and (d) avoid the use of highly enriched experimental diets and ensure the homogenous distribution of any isotopic labels therein. Additional experiments to better understand the impact of diet composition on trophic discrimination of isotopes are also needed, and diet- or species-specific TDFs should be used in applications such as isotope mixing models whenever possible.

AUTHOR CONTRIBUTIONS

A.F.Z., B.A.B., P.E.R. and L.L.J. conceived and designed the experiments. A.F.Z. analysed the data with guidance from B.A.B., P.E.R. and L.L.J. A.F.Z. wrote the manuscript with significant editing contributions by all authors.

ACKNOWLEDGEMENTS

We would like to thank Thor Arne Hangstad and Emilia Sztybor (Akvaplan-niva, Tromsø) for providing experimental design feedback as well as the laboratory care, feeding and sampling of the fish. We also thank Jens-Petter Jøstensen (Tromsø Fiskeindustri AS) for providing the unlabelled fish feed, and Liova Idahl and Johanna Hovinen (UiT—The Arctic University of Norway) for assisting with sample preparation in the laboratory. We are grateful to Time Howe at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks for providing analytical services and data quality management.

FUNDING INFORMATION

This work was funded by the Research Council of Norway (The Nansen Legacy, #276730) and UiT–The Arctic University of Norway Department of Arctic and Marine Biology.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in DataverseNO at https://doi.org/10.18710/ZADW9B.

ORCID

Amanda Fern Ziegler https://orcid.org/0000-0002-9027-5766

REFERENCES

Ajiad, A., & Gjøsæter, H. (1990). Diet of polar cod, *Boreogadus saida*, in the Barents Sea related to fish size and geographical distribution. *International Council for the Exploration of the Sea: Demersal Fish Committee*, *9*, 1–9.

Ankjærø, T., Christensen, J. T., & Grønkjær, P. (2012). Tissue-specific turnover rates and trophic enrichment of stable N and C isotopes in juvenile Atlantic cod *Gadus morhua* fed three different diets. *Marine Ecology Progress Series*, *461*, 197–209.

Barton, M. B., Litvin, S. Y., Vollenweider, J. J., Heintz, R. A., Norcross, B. L., & Boswell, K. M. (2019a). 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. *Journal of Experimental Marine Biology and Ecology*, *511*, 60–67.

Barton, M. B., Litvin, S. Y., Vollenweider, J. J., Heintz, R. A., Norcross, B. L., & Boswell, K. M. (2019b). Implications of trophic discrimination factor selection for stable isotope food web models of low trophic levels in the Arctic nearshore. *Marine Ecology Progress Series*, *613*, 211–216.

Bergstad, O. A., Jørgensen, T., & Dragesund, O. (1987). Life history and ecology of the gadoid resources of the Barents Sea. *Fisheries Research*, *5*, 119–161.

Blanchet-Aurigny, A., Guillou, M., Pernet, F., Gaffet, J. D., & Dubois, S. F. (2012). Tissue-diet discrimination factors of isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) in two brittle star species: Effect of reproductive state, diet and tissue composition. *Journal of Experimental Marine Biology and Ecology*, *426–427*, 68–77.

Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, *42*, 411–440.

Bosley, K. L., Witting, D. A., Chambers, R. C., & Wainright, S. C. (2002). Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. *Marine Ecology Progress Series*, *236*, 233–240.

Bouchard, C., & Fortier, L. (2020). The importance of *Calanus glacialis* for the feeding success of young polar cod: a circumpolar synthesis. *Polar Biology*, *43*, 1095–1107.

Brennan, C. E., Blanchard, H., & Fennel, K. (2016). Putting temperature and oxygen thresholds of marine animals in context of environmental change: A regional perspective for the scotian shelf and gulf of St Lawrence. *PLoS ONE*, *11*, 1–27.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*, 1771–1789.

Buchheister, A., & Latour, R. J. (2010). Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder (*Paralichthys dentatus*). *Canadian Journal of Fisheries and Aquatic Sciences*, *67*(3), 445–461. https://doi.org/10.1139/F09-196.

Budge, S. M., Wooller, M. J., Springer, A. M., Iverson, S. J., McRoy, C. P., & Divoky, G. J. (2008). Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia*, *157*, 117–129.

Canseco, J. A., Niklitschek, E. J., & Harrod, C. (2021). Variability in $\delta^{13}C$ and $\delta^{15}N$ trophic discrimination factors for teleost fishes: a meta-analysis of temperature and dietary effects. *Reviews in Fish Biology and Fisheries*, *1*, 313–329.

Carter, W. A., Bauchinger, U., & McWilliams, S. R. (2019). The importance of isotopic turnover for understanding key aspects of animal ecology and nutrition. *Diversity*, *11*, 1–20.

Caut, S., Angulo, E., & Courchamp, F. (2008). Discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$) in an omnivorous consumer: Effect of diet isotopic ratio. *Functional Ecology*, *22*, 255–263.

Caut, S., Angulo, E., & Courchamp, F. (2009). Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): The effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, *46*, 443–453.

Cucherousset, J., & Villéger, S. (2015). Quantifying the multiple facets of isotopic diversity: New metrics for stable isotope ecology. *Ecological Indicators*, *56*, 152–160.

Cusa, M., Berge, J., & Varpe, Ø. (2019). Seasonal shifts in feeding patterns: Individual and population realized specialization in a high Arctic fish. *Ecology and Evolution*, *9*, 11112–11121.

Dalpadado, P., Ingvaldsen, R. B., Stige, L. C., Bogstad, B., Knutsen, T., Ottersen, G., & Ellertsen, B. (2012). Climate effects on Barents Sea ecosystem dynamics. *ICES Journal of Marine Science*, *69*, 1303–1316.

De La Higuera, M., Akharbach, H., Hidalgo, M. C., Peragón, J., Lupiáñez, J. A., & García-Gallego, M. (1999). Liver and white muscle protein turnover rates in the European eel (*Anguilla anguilla*): Effects of dietary protein quality. *Aquaculture*, *179*, 203–216.

DeNiro, M. J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, *42*, 495–506.

DeNiro, M. J., & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, *45*, 341–351.

Drost, H. E., Lo, M., Carmack, E. C., & Farrell, A. P. (2016). Acclimation potential of Arctic cod (*Boreogadus saida*) from the rapidly warming Arctic Ocean. *Journal of Experimental Biology*, *219*, 3114–3125.

Eriksen, E., Ingvaldsen, R. B., Nedreaas, K., & Prozorkevich, D. (2015). The effect of recent warming on polar cod and beaked redfish juveniles in the Barents Sea. *Regional Studies in Marine Science*, *2*, 105–112.

Falk-Petersen, S., Hopkins, C. C. E., & Sargent, J. R. (1990). Trophic relationships in the pelagic, Arctic food web. *Proceedings of the 24th European Marine Biology Symposium*, 315–333.

Falk-Petersen, S., Mayzaud, P., Kattner, G., & Sargent, J. R. (2009). Lipids and life strategy of Arctic *Calanus*. *Marine Biology Research*, *5*, 18–39.

Farrell, A. P., & Richards, J. G. (2009). Defining hypoxia: An integrative synthesis of the responses of fish to hypoxia. In *Fish Physiology* (pp. 487–503). San Diego, CA: Elsevier Inc..

Florin, S. T., Felicetti, L. A., & Robbins, C. T. (2011). The biological basis for understanding and predicting dietary-induced variation in nitrogen and sulphur isotope ratio discrimination. *Functional Ecology*, *25*, 519–526.

Fossheim, M., Primicerio, R., Johannesen, E., Ingvaldsen, R. B., Aschan, M. M., & Dolgov, A. V. (2015). Recent warming leads to a rapid borealization of fish communities in the Arctic. *Nature Climate Change*, *5*, 673–677.

Fry, B., Arnold, C., & Pierce, F. (1982). Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia*, *54*, 200–204.

Furuya, V. R. B., Hayashi, C., Furuya, W. M., & Sakaguti, E. S. (2002). Replacement rates of carbon stable isotope (^{13}C) in muscle tissue of pintado, *Pseudoplatystoma corruscans* (Agassiz, 1829). *Zootecnia Tropical*, *20*(4), 461–472.

Gamboa-Delgado, J., Cañavate, J. P., Zerolo, R., & Le Vay, L. (2008). Natural carbon stable isotope ratios as indicators of the relative

contribution of live and inert diets to growth in larval Senegalese sole (*Solea senegalensis*). *Aquaculture*, *280*(1–4), 190–197. https://doi.org/10.1016/j.aquaculture.2008.04.036.

Gamboa-Delgado, J., Morales-Navarro, Y. I., Nieto-López, M. G., Villarreal-Cavazos, D. A., & Cruz-Suárez, L. E. (2019). Assimilation of dietary nitrogen supplied by fish meal and microalgal biomass from *Spirulina* (*Arthrospira platensis*) and *Nannochloropsis oculata* in shrimp *Litopenaeus vannamei* fed compound diets. *Journal of Applied Phycology*, *31*, 2379–2389.

Gannes, L. Z., O'Brien, D. M., & Martinez del Rio, C. (1997). Stable isotopes in animal ecology: assumptions, caveats and a call for more laboratory experiments. *Ecology*, *78*, 1271–1276.

Geoffroy, M., Majewski, A., LeBlanc, M., Gauthier, S., Walkusz, W., Reist, J. D., & Fortier, L. (2016). Vertical segregation of age-0 and age-1+ polar cod (*Boreogadus saida*) over the annual cycle in the Canadian Beaufort Sea. *Polar Biology*, *39*, 1023–1037.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, *293*, 2248–2251.

Gjøsæter, H., Bogstad, B., & Tjelmeland, S. (2009). Ecosystem effects of the three capelin stock collapses in the Barents Sea. *Marine Biology Research*, *5*, 40–53.

Gjøsæter, H., Huserbråten, M., Vikebø, F., & Eriksen, E. (2020). Key processes regulating the early life history of Barents Sea polar cod. *Polar Biology*, *43*, 1015–1027.

Godiksen, J. A., Chung, M. T., Folkvord, A., & Grønkjær, P. (2019). Effects of temperature on tissue—diet isotopic spacing of nitrogen and carbon in otolith organic matter. *Marine and Freshwater Research*, *72*, 1757–1767.

Gradinger, R. R., & Bluhm, B. A. (2004). In-situ observations on the distribution and behavior of amphipods and Arctic cod (*Boreogadus saida*) under the sea ice of the High Arctic Canada Basin. *Polar Biology*, *27*, 595–603.

Graeve, M., Albers, C., & Kattner, G. (2005). Assimilation and biosynthesis of lipids in Arctic *Calanus* species based on feeding experiments with a ^{13}C labelled diatom. *Journal of Experimental Marine Biology and Ecology*, *317*, 109–125.

Graham, M., & Hop, H. (1995). Aspects of reproduction and larval biology of Arctic cod (*Boreogadus saida*). *Arctic*, *48*, 130–135.

Guelinckx, J., Maes, J., Van Den Driessche, P., Geysen, B., Dehairs, F., Ollevier, F., … Ollevier, F. (2007). Changes in $\delta^{13}C$ and $\delta^{15}N$ in different tissues of juvenile sand goby *Pomatoschistus minutus*: A laboratory diet-switch experiment. *Marine Ecology Progress Series*, *341*, 205–215.

Heady, W. N., & Moore, J. W. (2013). Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. *Oecologia*, *172*(1), 21–34. https://doi.org/10.1007/s00442-012-2483-9.

Hesslein, R. H., Hallard, K. A., & Ramlal, P. (1993). Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. *Canadian Journal of Fisheries and Aquatic Sciences*, *50*, 2071–2076.

Hobson, K. A., & Clark, R. G. (1992). Assessing avian diets using stable isotopes I: Turnover of ^{13}C in Tissues. *The Condor*, *94*, 181–188.

Hobson, K. A., & Welch, H. E. (1992). Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. *Marine Ecology Progress Series*, *84*, 9–18.

Holman, B. W. B., & Malau-Aduli, A. E. O. (2013). *Spirulina* as a livestock supplement and animal feed. *Journal of Animal Physiology and Animal Nutrition*, *97*, 615–623.

Hop, H., Graham, M., & Trudeau, V. L. (1995). Spawning energetics of Arctic cod (*Boreogadus saida*) in relation to seasonal development of the ovary and plasma sex steroid levels. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*, 541–550.

Hop, H., & Gjøsæter, H. (2013). Polar cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) as key species in marine food webs of the Arctic and the Barents Sea. *Marine Biology Research*, *9*, 878–894.

Hussey, N. E., MacNeil, M. A., McMeans, B. C., Olin, J. A., Dudley, S. F. J., Cliff, G., … Fisk, A. T. (2014). Rescaling the trophic structure of marine food webs. *Ecology Letters*, *17*, 239–250.

Jardine, T. D., Gray, M. A., McWilliam, S. M., & Cunjak, R. A. (2005). Stable isotope variability in tissues of temperate stream fishes. *Transactions of the American Fisheries Society*, *134*, 1103–1110.

Karlsen, Ø., Holm, J. C., & Kjesbu, O. S. (1995). Effects of periodic starvation on reproductive investment in first-time spawning Atlantic cod (*Gadus morhua L.*). *Aquaculture*, *133*, 159–170.

Kaufman, M. R., Gradinger, R. R., Bluhm, B. A., & O'Brien, D. M. (2008). Using stable isotopes to assess carbon and nitrogen turnover in the Arctic sympagic amphipod *Onisimus litoralis*. *Oecologia*, *158*, 11–22.

Kohlbach, D., Schaafsma, F. L., Graeve, M., Lebreton, B., Allen, B., David, C., … Flores, H. (2017). Strong linkage of polar cod (*Boreogadus saida*) to sea ice algae-produced carbon: Evidence from stomach content, fatty acid and stable isotope analyses. *Progress in Oceanography*, *152*, 62–74.

Kunz, K. L., Claireaux, G., Pörtner, H.-O., Knust, R., & Mark, F. C. (2018). Aerobic capacities and swimming performance of polar cod (*Boreogadus saida Lepechin*) under ocean acidification and warming conditions. *Journal of Experimental Biology*, *221*, 1–11.

Kusche, H., Hillgruber, N., Rößner, Y., & Focken, U. (2018). The effect of different fish feed compositions on $\delta^{13}C$ and $\delta^{15}N$ signatures of sea bass and its potential value for tracking mariculture-derived nutrients. *Isotopes in Environmental and Health Studies*, *54*, 28–40.

Laurel, B. J., Spencer, M., Iseri, P., & Copeman, L. A. (2016). Temperature-dependent growth and behavior of juvenile Arctic cod (*Boreogadus saida*) and co-occurring North Pacific gadids. *Polar Biology*, *39*, 1127–1135.

Layman, C. A., Arrington, D. A., Montaña, C. G., & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, *88*, 42–48.

Madigan, D. J., Snodgrass, O. E., Hyde, J. R., & Dewar, H. (2021). Stable isotope turnover rates and fractionation in captive California yellowtail (*Seriola dorsalis*): insights for application to field studies. *Scientific Reports*, *11*, 4466.

Maitland, B. M., Del Rio, C. M., & Rahel, F. J. (2021). Effect of temperature on ^{13}C and ^{15}N incorporation rates and discrimination factors in two North American fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, *78*, 1833–1840.

Marsh, J. M., & Mueter, F. J. (2020). Influences of temperature, predators, and competitors on polar cod (*Boreogadus saida*) at the southern margin of their distribution. *Polar Biology*, *43*, 995–1014.

Martínez Del Rio, C., Wolf, N., Carleton, S. A., & Gannes, L. Z. (2009). Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews*, *84*, 91–111.

McMahon, K. W., Fogel, M. L., Elsdon, T. S., & Thorrold, S. R. (2010). Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. *Journal of Animal Ecology*, *79*, 1132–1141.

McMahon, K. W., Thorrold, S. R., Elsdon, T. S., & McCarthy, M. D. (2015). Trophic discrimination of nitrogen stable isotopes in amino acids varies with diet quality in a marine fish. *Limnology and Oceanography*, *60*, 1076–1087.

McNicholl, D. G., Walkusz, W., Davoren, G. K., Majewski, A. R., & Reist, J. D. (2016). Dietary characteristics of co-occurring polar cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) in the Canadian Arctic, Darnley Bay. *Polar Biology*, *39*, 1099–1108.

Miller, T. W. (2006). Tissue-specific response of $\delta^{15}N$ in adult Pacific herring (*Clupea pallasi*) following an isotopic shift in diet. *Environmental Biology of Fishes*, *76*(2–4), 177–189. https://doi.org/10.1007/s10641-006-9020-9.

Minagawa, M., & Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, *48*, 1135–1140.

Nahrgang, J., Storhaug, E., Murzina, S. A., Delmas, O., Nemova, N. N., & Berge, J. (2016). Aspects of reproductive biology of wild-caught polar cod (*Boreogadus saida*) from Svalbard waters. *Polar Biology*, *39*, 1155–1164.

Orlova, E. L., Dolgov, A. V., Rudneva, G. B., Oganin, I. A., & Konstantinova, L. L. (2009). Trophic relations of capelin *Mallotus villosus* and polar cod *Boreogadus saida* in the Barents Sea as a factor of impact on the ecosystem. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *56*, 2054–2067.

Parnell, A. C., Phillips, D. L., Bearhop, S., Semmens, B. X., Ward, E. J., Moore, J. W., … Inger, R. (2013). Bayesian stable isotope mixing models. *Environmetrics*, *24*, 387–399.

Pedersen, T. (2022). Comparison between trophic positions in the Barents Sea estimated from stable isotope data and a mass balance model. *Frontiers in Marine Science*, *9*, 1–18.

Pedersen, T., Mikkelsen, N., Lindstrøm, U., Renaud, P. E., Nascimento, M. C., Blanchet, M., … Blanchet, H. (2021). Overexploitation, recovery, and warming of the Barents Sea ecosystem during 1950–2013. *Frontiers in Marine Science*, *8*, 1–22.

Perga, M. E., & Gerdeaux, D. (2005). 'Are fish what they eat' all year round? *Oecologia*, *144*, 598–606.

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, *18*, 293–320.

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., … Ward, E. J. (2014). Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology*, *92*, 823–835.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*, 703–718.

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montaña, C. G. (2007). Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, *152*, 179–189.

Quezada-Romegialli, C., Jackson, A. L., Hayden, B., Kahilainen, K. K., Lopes, C., & Harrod, C. (2018). tRophicPosition, an r package for the Bayesian estimation of trophic position from consumer stable isotope ratios. *Methods in Ecology and Evolution*, *9*, 1592–1599.

Rand, K. M., Whitehouse, A., Logerwell, E. A., Ahgeak, E., Hibpshman, R., & Parker-Stetter, S. (2013). The diets of polar cod (*Boreogadus saida*) from August 2008 in the US Beaufort Sea. *Polar Biology*, *36*, 907–912.

Renaud, P. E., Berge, J., Varpe, O., Lønne, O. J., Nahrgang, J., Ottesen, C., & Hallanger, I. (2012). Is the poleward expansion by Atlantic cod and haddock threatening native polar cod, *Boreogadus saida*? *Polar Biology*, *35*, 401–412.

Schurmann, H., Steffensen, J. F., & Bushnell, P. G. (1994). Oxygen consumption in four species of teleosts from Greenland: no evidence of metabolic cold adaption. *Polar Biology*, *14*(49), 54.

Stock, B. C., Jackson, A. L., Ward, E. J., Parnell, A. C., Phillips, D. L., & Semmens, B. X. (2018). Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ*, *6*, e5096.

Suontama, J., Karlsen, Ø., Moren, M., Hemre, G. I., Melle, W., Langmyhr, E., … Olsen, R. E. (2007). Growth, feed conversion and chemical composition of Atlantic salmon (*Salmo salar L.*) and Atlantic halibut (*Hippoglossus hippoglossus L.*) fed diets supplemented with krill or amphipods. *Aquaculture Nutrition*, *13*, 241–255.

Suring, E., & Wing, S. R. (2009). Isotopic turnover rate and fractionation in multiple tissues of red rock lobster (*Jasus edwardsii*) and blue cod (*Parapercis colias*): Consequences for ecological studies. *Journal of Experimental Marine Biology and Ecology*, *370*, 56–63.

Suzuki, K. W., Kasai, A., Nakayama, K., & Tanaka, M. (2005). Differential isotopic enrichment and half-life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: Implications for analyzing migration. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(3), 671–678. https://doi.org/10.1139/f04-231.

Tarboush, R. A., MacAvoy, S. E., Macko, S. A., & Connaughton, V. (2006). Contribution of catabolic tissue replacement to the turnover of stable isotopes in *Danio rerio*. *Canadian Journal of Zoology*, *84*(10), 1453–1460. https://doi.org/10.1139/z06-136.

Thomas, S. M., & Crowther, T. W. (2015). Predicting rates of isotopic turnover across the animal kingdom: A synthesis of existing data. *Journal of Animal Ecology*, *84*, 861–870.

Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. *Oecologia*, *57*, 32–37.

Tokuşoglu, Ö., & Ünal, M. K. (2003). Biomass nutrient profiles of three microalgae: *Spirulina platensis*, *Chlorella vulgaris*, and *Isochrisis galbana*. *Journal of Food Science*, *68*, 1144–1148.

Tomas, F., Álvarez-Cascos, D., Turon, X., & Romero, J. (2006). Differential element assimilation by sea urchins *Paracentrotus lividus* in seagrass beds: Implications for trophic interactions. *Marine Ecology Progress Series*, *306*, 125–131.

Trueman, C. N., McGill, R. A. R., & Guyard, P. H. (2005). The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals. An experimental study with Atlantic salmon (*Salmo salar*). *Rapid Communications in Mass Spectrometry*, *19*, 3239–3247.

Vander Zanden, M. J., Clayton, M. K., Moody, E. K., Solomon, C. T., & Weidel, B. C. (2015). Stable isotope turnover and half-life in animal tissues: A literature synthesis. *PLoS ONE*, *10*, 1–16.

Vanderklift, M. A., & Ponsard, S. (2003). Sources of variation in consumer-diet $\delta^{15}N$ enrichment: A meta-analysis. *Oecologia*, *136*, 169–182.

Weems, J., Iken, K., Gradinger, R., & Wooller, M. J. (2012). Carbon and nitrogen assimilation in the Bering Sea clams Nuculana radiata and Macoma moesta. *Journal of Experimental Marine Biology and Ecology*, *430–431*, 32–42.

Weidel, B. C., Carpenter, S. R., Kitchell, J. F., & Vander Zanden, M. J. (2011). Rates and components of carbon turnover in fish muscle: insights from bioenergetics models and a whole-lake ^{13}C addition. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*, 387–399.

Whitledge, G. W., & Rabeni, C. F. (1997). Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*, 2555–2563.

Wickham, H., & Chang, W. (2014). *Package ggplot2: An Implementation of the Grammar of Graphics*. R Package Version 1.0 215. New York, NY: Springer-Verlag.

Wolf, N., Newsome, S. D., Peters, J., & Fogel, M. L. (2015). Variability in the routing of dietary proteins and lipids to consumer tissues influences tissue-specific isotopic discrimination. *Rapid Communications in Mass Spectrometry*, *29*, 1448–1456.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ziegler, A. F., Bluhm, B. A., Renaud, P. E., & Jørgensen, L. L. (2023). Isotopic turnover in polar cod (*Boreogadus saida*) muscle determined through a controlled feeding experiment. *Journal of Fish Biology*, *102*(6), 1442–1454. https://doi.org/10.1111/jfb.15389