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Critical evaluation of trophic discrimination factors using compound-specific stable isotopes in a Northwest Atlantic shelf marine food web

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ABSTRACT: Quantifying food web structure and interactions is important for understanding food web ecology, conservation biology and management. Trophic position (TP) is a useful metric for these purposes, describing an organism's hierarchical role within the food web. TP can be estimated using the ratio of nitrogen isotopes ($\delta^{15}N$) of specific amino acids within animal tissue. An important component of the TP calculation is the trophic discrimination factor (TDF), which represents the enrichment of ^{15}N with each trophic step. Early work suggested a fixed TDF throughout the food chain, but more recent studies indicate that this may not be appropriate. This study focuses on a shelf marine food web in the Northwest Atlantic, where we used bulk and amino acid-specific $\delta^{15}N$ to determine TDFs for 4 feeding guilds spanning 3 trophic levels. Additionally, we compared TDFs between different tissues: muscle, liver and homogenised whole organism. TDFs calculated from amino acid $\delta^{15}N$ decreased with increasing TP: 6.9 ± 2.3‰ (mean ± SD) for zooplankton, 5.1 ± 1.5‰ for pelagic fish, 4.5 ± 1.0‰ for Atlantic cod and 3.1 ± 1.0‰ for harp seals. This resulted from a combined effect of reduced ^{15}N enrichment of trophic amino acids and ^{15}N enrichment in the source amino acid phenylalanine among higher TP consumers. Finally, there was no evidence for differences in TDF between tissue types. By providing trophic guild-specific TDF values, our study enhances the accuracy of TP estimates and supports the development of amino acid isotope analysis as a tool for assessing food web structure across diverse taxa in the Northwest Atlantic.

KEY WORDS: Food web · Trophic position · Trophic discrimination factor · Stable nitrogen isotopes · Amino acids · Tissue comparison

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1. INTRODUCTION

Trophic connections between consumers and prey underpin ecosystem structure, function and stability (Polis & Strong 1996, Post 2002a, Worm & Duffy 2003). Quantifying these connections is vital for our understanding of climate- and/or anthropogenically mediated impacts on marine communities and helping to inform ecosystem-based fisheries management (Walters & Maguire 1996, Preisser 2008, Lynam et al. 2017). Trophic structure describes the partitioning of biomass between trophic levels and can be quantified using trophic position (TP), a continuous measure of the hierarchical role of a species within a food web that accounts for trophic omnivory (Vander Zanden & Rasmussen 1996, Hussey et al. 2014). Traditional methods to calculate TP in marine taxa include stomach content analysis and bulk stable nitrogen isotope analysis on an organism's tissues; the former provides a recent snapshot of diet and the latter provides time-integrated dietary information. TP estimates generated from $\delta^{15}N_{bulk}$ values require samples from both

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the base of the food web and the consumer, and increase by around 1.7 to 4‰ with each trophic step (Pinnegar & Polunin 1999, Post 2002b, Lorrain et al. 2015, Canseco et al. 2022, Sabadel & MacLeod 2022):

$$TP_{bulk} = TP_{base} + \frac{(\delta^{15}N_{consumer} - \delta^{15}N_{base})}{TDF_{bulk}} \quad (1)$$

where TP_{base} is the estimated TP of an organism used to estimate a baseline value in the food web, e.g. TP_{base} = 1 for primary producers and 2 for primary consumers. $\delta^{15}N_{consumer}$ and $\delta^{15}N_{base}$ are the measured bulk isotopic values of the target consumer and baseline organisms, respectively. An important component of the TP_{bulk} calculation is the trophic discrimination factor (TDF_{bulk}), which represents the enrichment of ^{15}N with each trophic step. It is often difficult to accurately determine $\delta^{15}N$ values at the base of marine food webs (hereafter referred to as the baseline) due to the dynamic nature of ecosystem biogeochemistry and multiple nutrient sources available to phytoplankton (e.g. NH_4^+, NO_3^-, N_2), each potentially distinct and spatiotemporally variable in their isotopic composition (Post 2002b, Lorrain et al. 2015).

Compound-specific stable nitrogen isotope analysis of amino acids (CSIA-N-AA) is a relatively new approach that has the potential to circumvent the baseline-related complications associated with bulk stable isotope analysis (SIA). This is because CSIA-N-AA measures the $\delta^{15}N$ values of individual amino acids (AAs) within the organism's tissue, which can be divided into 'source' and 'trophic' AAs. Source AAs experience minimal levels of enrichment in ^{15}N and so reflect the base of the food web or baseline, i.e. the $\delta^{15}N$ of nitrate incorporated by phytoplankton in marine food webs (McClelland & Montoya 2002, de la Vega et al. 2021a). Conversely, trophic AAs experience significant enrichment in ^{15}N with each trophic step (up to 8‰, Chikaraishi et al. 2009) and thus reflect the feeding activity and metabolism of the consumer (McClelland & Montoya 2002). The combination of baseline and metabolic information contained within a single tissue sample allows for an integrated TP estimate, i.e. the difference in $\delta^{15}N$ between trophic and source AAs, while also accounting for the trophic discrimination factors (TDFs) between consumer and resource, and between primary producers and nitrogenous sources (McClelland & Montoya 2002, Chikaraishi et al. 2007, 2009). TP using $\delta^{15}N_{AA}$ values can be calculated with:

$$TP_{AA} = \frac{(\delta^{15}N_{trophic} - \delta^{15}N_{source}) - \beta}{TDF} + 1 \quad (2)$$

where $\delta^{15}N_{trophic}$ represents the isotopic value of one or more (averaged) trophic AAs, $\delta^{15}N_{source}$ represents the isotopic value of one or more (averaged) source AAs, β represents the intrinsic difference of $\delta^{15}N_{trophic} - \delta^{15}N_{source}$ in primary producers (TP = 1), and TDF is the trophic discrimination factor that describes the enrichment of ^{15}N in a trophic AA relative to a source AA with each trophic step.

Although CSIA-N-AA is a promising approach for estimating time-integrated trophic structure, the universality of applying a single TDF to all occupants of a food web remains unclear. Realistic TP_{AA} values have been estimated in zooplankton, gastropods, leatherback turtles *Dermochelys coriacea*, yellowfin tuna *Thunnus albacares* and lanternfishes (McClelland & Montoya 2002, Schmidt et al. 2004, Chikaraishi et al. 2007, McCarthy et al. 2007, Olson et al. 2010, Choy et al. 2012, Seminoff et al. 2012). However, conflicting studies have shown an underestimation of TP_{AA}, especially in higher marine consumers such as elasmobranchs, penguins, teleosts, pinnipeds and cetaceans (Lorrain et al. 2009, Dale et al. 2011, Germain et al. 2013, Bradley et al. 2015, Hetherington et al. 2017, Matthews et al. 2020). The underestimation of TP in marine food webs can be attributed to interspecific differences in TDFs, which appear to decrease with increasing TP (Germain et al. 2013, Bradley et al. 2015, Hetherington et al. 2017, Matthews et al. 2020). A single ecosystem TDF (canonically identified as 7.6‰ by Chikaraishi et al. 2007, 2009) may therefore not be applicable throughout entire marine food webs (McMahon & McCarthy 2016).

Though often related to TP, the mechanisms underpinning inter-specific TDF discrepancies appear to be associated with the mode of nitrogen excretion (ammonia vs. uric acid or urea) and diet composition/quality, e.g. protein content, or similarity between diet and consumer tissue (Mill et al. 2007, Germain et al. 2013, Hoen et al. 2014, Nielsen et al. 2015, McMahon & McCarthy 2016). Furthermore, TDF variability may also be affected by $\delta^{15}N$ values of source AAs, such as phenylalanine (Phe). The general consensus is that $\delta^{15}N$ fractionation of Phe with each trophic step is minimal, previously reported as ~0.4 ± 0.5‰ (mean ± SD; Chikaraishi et al. 2009). However, recent evidence indicates non-negligible fractionation of source AAs in higher marine consumers (2.3 ± 1.2‰), potentially contributing to lower overall TDF values (Nuche-Pascual et al. 2018, Matthews et al. 2020). Finally, evidence from bulk stable isotope studies indicate that TDFs may also be affected by tissue type, likely due to differences in tissue synthesis and physiological capabilities (Ankjærø et al. 2012, Mohan

et al. 2016, Stephens et al. 2023). However, studies exploring tissue-specific variability in AA TDFs remain lacking.

Most of the growing literature on CSIA-N-AA focuses on isolated components of disparate ecosystems (McClelland & Montoya 2002, Schmidt et al. 2004, Chikaraishi et al. 2007, Popp et al. 2007, Seminoff et al. 2012, Germain et al. 2013, McMahon et al. 2015a, de la Vega et al. 2021a), with few examples encompassing more than 2 trophic levels (Nielsen et al. 2015, Hetherington et al. 2017). To provide a more holistic approach within a single food web, we determine TDFs for 10 taxa in the Northwest Atlantic (NWA) shelf sea food web, organised into 4 trophic guilds based on feeding behaviour: zooplankton, pelagic fish, demersal fish and a marine mammal predator. These taxa span approximately 3 TPs (Sherwood & Rose 2005). The aims of this study were threefold: (1) to generate trophic guild-specific TDFs that can be used to accurately determine TP for a range of consumers in the NWA shelf food web; (2) to assess whether these guild-specific TDFs provide a more representative TP estimate compared to the ubiquitous application of the canonical 7.6‰ TDF (Chikaraishi et al. 2007, 2009); (3) analyse muscle, liver and homogenised whole organisms to investigate whether TDFs differ between tissue types. In addition, we investigated the extent to which $\delta^{15}N_{Phe}$ fractionates between trophic guilds and determined whether this contributes to TDF variability. Quantifying TDFs in this way contributes to the development of CSIA-N-AA as a tool for assessing ecosystem-wide food web structure.

2. MATERIALS AND METHODS

2.1. Sample collection and processing

Fish (capelin *Mallotus villosus*, herring *Clupea harengus*, redfish *Sebastes* spp., turbot *Reinhardtius hippoglossoides* and Atlantic cod *Gadus morhua*) and invertebrate (copepod, hyperiid, gammarid and euphausiid) samples for the trophic guild TDF comparison were collected from the east/northeast Newfoundland shelf in 2014 as part of the autumn (October to December) multispecies trawl sampling effort by the Department of Fisheries and Oceans Canada (DFO) Newfoundland Labrador Region (St. John's, NL) (Fig. 1, Table 1). Sampling stations were selected based on a stratified random sampling design. A Campelen 1800 shrimp trawl was deployed at each station and towed at 2.8–3.2 knots for 15 min. Some inshore samples (such as some herring and capelin) were selected opportunistically from other DFO programmes during the same few autumn months to help expand the data set. Wherever possible, uniform fish total lengths were selected to help remove variability attributed to ontogenetic shifts (Table 1), although this was not possible in all cases (e.g. with turbot) because sample number was too low. For fish, dorsal muscle samples were taken between the operculum and first dorsal fin. Invertebrate zooplankton samples were considerably smaller and so were taken as whole organisms; amphipods and euphausiids constituted ~5 individuals per sample and ~20 individuals were used for copepods. Freeze-dried samples were stored at −20°C, before being analysed for bulk SIA at The Stable Isotope Laboratory of Memorial University in 2014. An aliquot of each sample was shipped to the Liverpool Isotopes for Environmental Research laboratory (LIFER lab), Liverpool, UK, in 2019 for CSIA-N-AA.

Harp seals *Pagophilus groenlandicus* were taken by licensed sealers under the Marine Mammal Regulations of the Canada Fisheries Act in collaboration with DFO (Fig. 1). Muscle tissue was selected for the inter-guild TDF comparison, as well as liver for the comparison between tissues. Harp seal muscle samples from the month of February (2015) were chosen

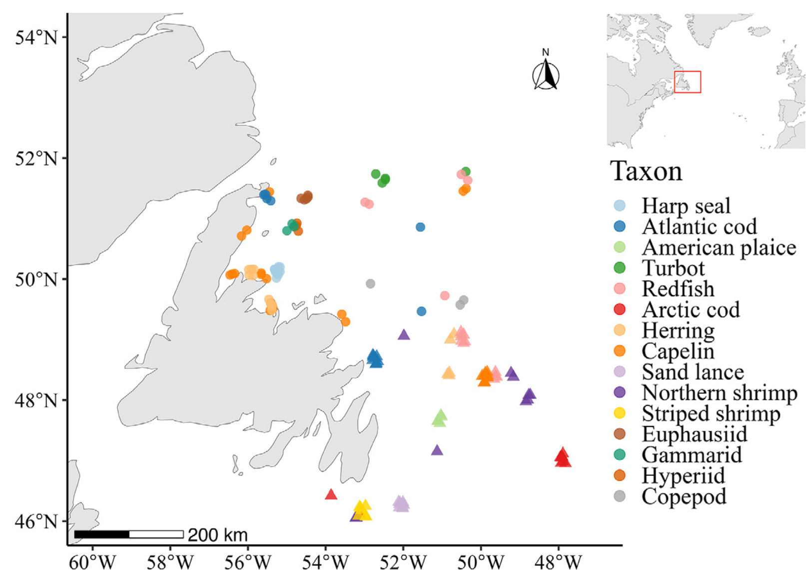

Fig. 1. Sampling area off the east coast of Newfoundland, Canada, where fish and invertebrate samples were collected in October–December 2014 (circles) and May–June 2019 (triangles). Harp seal samples were collected in January–February 2015 (circles). Points are jittered for visual clarity

Table 1. Mean (±1 SD) total length, $\delta^{15}N_{bulk}$ and trophic position (TP_{bulk}, calculated from Eq. 1). The trophic discrimination factor (TDF_{bulk}) was sourced for each taxon based on literature-derived values and the R package 'SIDER'. Taxa were organised into trophic guilds based on their feeding ecology, length and the categories used by Sherwood & Rose (2005). Lengths were not determined (ND) for zooplankton. This table features samples that were exclusively collected for the trophic guild comparison component of the study; fish and invertebrate samples were collected in October–December 2014 and harp seals were collected in February 2015

Trophic guild	Taxon	N samples	Length (cm)	$\delta^{15}N_{bulk}$	TP_{bulk}	TDF_{bulk}	TDF_{bulk} reference
Zooplankton	Copepod	3	ND	9.0 (0.2)	2.9 (0.1)	3.4	Post (2002b)
	Hyperiid	4	ND	8.7 (1.0)	2.8 (0.3)		
	Gammarid	3	ND	5.1 (1.5)	1.7 (0.4)		
	Euphausiid	5	ND	9.1 (0.6)	2.9 (0.2)		
Pelagic fish	Capelin	18	17 (1)	12.4 (0.5)	3.8 (0.1)	3.7	Canseco et al. (2021)
	Herring	10	33 (2)	12.1 (0.3)	3.7 (0.1)		
	Redfish	5	23 (12)	11.6 (0.6)	3.6 (0.2)		
	Turbot	5	34 (14)	12.3 (0.5)	3.7 (0.1)		
Atlantic cod	Atlantic cod	6	78 (15)	14.4 (0.7)	4.9 (0.2)	2.8	Sherwood & Rose (2005)
Harp seal	Harp seal	12	163 (11)	14.7 (0.6)	4.9 (0.2)	2.9	SIDER (Healy et al. 2018)

because muscle likely has a turnover rate of approximately 4 mo (Vander Zanden et al. 2015, de la Vega et al. 2021a) and therefore selecting samples harvested in December or January might have incorporated isotopic values from feeding activity further north during the summer months (Fig. 2). This would have impeded our ability to compare taxa within a connected food web on the Newfoundland shelf. Furthermore, selecting samples after February may have encompassed individuals during a period of weight loss, induced by reduced feeding during the annual pupping, breeding and moulting periods (Fig. 2; Lydersen & Kovacs 1996, Chabot & Stenson 2002, Sjare & Stenson 2010, Stenson et al. 2016, 2020). During periods of fasting, catabolism can occur, which increases tissue $\delta^{15}N$ values and would compromise our TDF interpretations (Newsome et al. 2010). Samples were freeze-dried and stored at −20°C in Liverpool in preparation for bulk and compound-specific SIA.

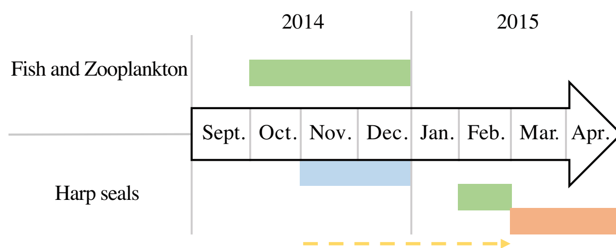

Fig. 2. Sampling design for fish, zooplankton and harp seals, specifically for the inter-guild comparison component of the study. Green represents sampling months, blue indicates harp seal arrival on the Newfoundland shelf following their southerly migration from the Canadian Arctic and Greenland, and orange represents the annual pupping and moulting months. The dashed yellow arrow indicates the 4 mo integration time of dietary N isotopes into harp seal muscle tissue (Vander Zanden et al. 2015, de la Vega et al. 2021a)

For tissue comparison, Atlantic cod (n = 10), American plaice *Hippoglossoides platessoides* (n = 5), redfish (n = 10), Arctic cod *Boreogadus saida* (n = 10), capelin (n = 10), herring (n = 5), sand lance *Ammodytes americanus* (n = 10), northern shrimp *Pandalus borealis* (n = 10) and striped shrimp *P. montagui* (n = 8) were sampled in spring (May and June) 2019 from the southeast Newfoundland shelf (Fig. 1). Harp seal samples were taken from northern Newfoundland in early 2015 (both January and February for the tissue comparison) and an aliquot of muscle and liver was taken from each individual. Fish had an aliquot of muscle and liver removed, before the remaining whole organism was blended in a Sorvall 17 150 Omni Mixer Homogenizer. For invertebrate samples, only muscle and homogenised whole organism were used for analysis. Freeze-dried samples were stored at −20°C in Liverpool in preparation for bulk and compound-specific SIA.

2.2. Instrumental analysis for bulk $\delta^{15}N$ data

Bulk SIA of $\delta^{15}N$ for fish and invertebrates from 2014 was carried out at The Stable Isotope Laboratory of Memorial University. Freeze-dried and homogenised samples were weighed into tin cups and analysed using a Carlo Erba elemental analyser, which was linked to a Delta V Plus isotope ratio mass spectrometer (IRMS; Thermo Scientific) through a ConFlo III interface. The combustion reactor (chro-

mium oxide and silvered cobaltous oxide) was maintained at a temperature of 1050°C and the reduction reactor (Cu) was set at 600°C. For isotopic analysis, scale calibration for $\delta^{15}N$ was performed with EDTA #2 ($\delta^{15}N$ = −0.83 ± 0.05‰; mean ± SD) and caffeine (USGS 62; $\delta^{15}N$ = 20.17 ± 0.08‰), both from Indiana University, Bloomington. Casein (B2155: 13.32 ± 0.40% N; $\delta^{15}N$ = 5.98 ± 0.12‰), acquired from Elemental Microanalysis (Devon, UK), was analysed several times during a run as a quality control sample.

Additionally, bulk SIA of $\delta^{15}N$ was performed on harp seals from 2015 and on fish and invertebrate samples (for the tissue comparison element) from 2019 at the University of Liverpool. This was achieved using an elemental analyser (Costech) coupled to a Delta V IRMS. Isotope values are reported in standard δ-notation (‰) relative to air (nitrogen). $\delta^{15}N$ scale calibration was performed with USGS40 (= −4.52 ± 0.03‰) and USGS41a ($\delta^{15}N$ = 47.55 ± 0.06‰). Furthermore, an internal standard of ground prawn *Penaeus vannamei* with well characterised $\delta^{15}N$ values (6.8‰) was analysed every 10 samples to monitor precision ($\delta^{15}N$ = 6.45 ± 0.09‰).

2.3. Sample preparation for analyses of $\delta^{15}N$ in amino acids (AAs)

Freeze-dried samples were homogenised using a pestle and mortar, then prepared for CSIA-N-AA following the methods of Corr et al. (2007) and de la Vega et al. (2021a). Briefly, ~10 mg of homogenised tissue were spiked with an internal standard, norleucine (65 µl of 5 mg ml^{-1}) and hydrolysed (6 M HCl) at 100°C for 20−24 h. Once cooled, the hydrosylate was filtered and lipids were extracted with dichloromethane (DCM):hexane (3:2, v/v); the acidic phase was retained via phase separation, and this was repeated 3 times. The cleaned hydrosylate was propylated using a solution of isopropanol:acetyl chloride (4:1, v/v) and heated at 100°C for 1 h. The reaction was quenched by placing the propylated hydrosylates in −20°C for 10 min. Reagents were removed under a gentle stream of $N_{2(g)}$. The propylated hydrosylates were acylated using acetone:triethylamine:acetic anhydride (5:2:1, v/v) and heated at 60°C for 10 min. Acylating reagents were removed under a gentle stream of $N_{2(g)}$ and the sample was dissolved in 2 ml of ethyl acetate. Phase separation was completed by adding 1 ml of saturated NaCl to the solution and vortexing for 30 s. The organic phase was collected and passed through a Pasteur pipette containing $MgSO_4$. This step was repeated twice. Solvents were removed under a gentle stream of $N_{2(g)}$ and the resulting derivatised AA was stored under DCM at −20°C prior to analyses.

2.4. Instrumental analysis for $\delta^{15}N$ of AAs

$\delta^{15}N_{AA}$ values were measured using a Trace Ultra gas chromatograph (GC) with a ConFlo IV interface and Delta V Advantage IRMS (Thermo Fisher). The Cu/Ni combustion reactor was maintained at 1000°C, and a liquid nitrogen trap removed CO_2 from the sample stream. A split/splitless injector (set in splitless mode at 260°C) introduced each sample to a HP Innowax column (Agilent, 30 m × 0.25 mm ID × 0.5 µm film thickness), which separated the AAs. The programmed sequence for the GC was 50°C for 2 min, increase to 180°C at 10°C min^{-1}, then increase to 260°C at 6°C min^{-1} and hold at this temperature for 16.7 minutes. Ultra-high purity helium was used as the carrier gas at a flow rate of 1.4 ml min^{-1}. Ion intensities of 28, 29 and 30 were monitored while the $\delta^{15}N$ values for each AA peak were automatically calculated using Isodat version 3.0 (Thermo Fisher). These calculations were based on comparisons with a standard reference N_2 gas, measured 4 times at both the start and end of each sample analysis.

Each sample was analysed in duplicate and re-run if the mean $\delta^{15}N_{AA}$ values fell outside the expected measurement error (>1.5‰). Precision and accuracy were determined using a mixed standard prepared from 9 AAs (alanine [Ala], valine [Val], leucine [Leu], glycine [Gly], norleucine [Nle], aspartic acid [Asp], proline [Pro], glutamic acid [Glu] and phenylalanine [Phe]) with known $\delta^{15}N$ values (University of Indiana, USA, and SI Science, Japan). However, due to complications with seed oxidation on the GC-IRMS, coelution in sample chromatography and poor reproducibility, it was necessary to omit Ala, Val and Pro from further analysis. The mixed standard was analysed after every 2 sample injections and precision and accuracy are reported in Table 2.

Results are reported in per mil (‰) in delta (δ) notation relative to N_2. Raw $\delta^{15}N_{AA}$ sample values were corrected using Eq. (3) (McCarthy et al. 2013, de la Vega et al. 2021a). This technique accounts for how individual AAs respond to the column's stationary phase and is calculated from the difference between the measured $\delta^{15}N_{AA}$ values of the closed mixed standard and their established $\delta^{15}N_{AA}$ values:

$$\delta^{15}N_{\text{sample reported}} = \text{Avg. } \delta^{15}N_{\text{sample measured}} - (\delta^{15}N_{\text{std. measured}} - \delta^{15}N_{\text{known}}) \quad (3)$$

Table 2. Mean (±1 SD) $\delta^{15}N$ of amino acids for each taxon. Also shown is the mean standard deviation of each amino acid in the mixed standard (precision) and the mean $\Delta\delta^{15}N_{observed-known}$ in the mixed standard (accuracy). n: number of samples; Glu: glutamic acid; Asp: aspartic acid; Leu: leucine; Phe: phenylalanine. This table features samples that were exclusively collected for the trophic guild comparison component of the study; fish and invertebrate samples were collected in October–December 2014 and harp seals were collected in February 2015

Trophic guild	Taxon	n	Glu	Asp	Leu	Phe
Zooplankton	Copepod	3	20.0 (0.8)	16.2 (0.5)	14.2 (1.5)	4.7 (0.8)
	Hyperiid	4	22.6 (1.5)	19.5 (0.8)	19.8 (2.4)	3.3 (1.0)
	Gammarid	3	18.2 (2.2)	13.7 (1.0)	12.8 (0.9)	5.3 (0.9)
	Euphausiid	5	20.6 (1.4)	17.1 (1.3)	18.8 (5.6)	4.8 (0.4)
Pelagic fish	Capelin	18	19.3 (2.1)	19.8 (2.2)	22 (1.4)	4.6 (0.9)
	Herring	10	18.9 (0.4)	20.5 (0.9)	22.8 (1.3)	4.6 (1.1)
	Redfish	5	19.5 (3.2)	18.3 (1.9)	22.9 (3.0)	5.5 (0.6)
	Turbot	5	22.2 (1.4)	19.2 (1.6)	26.7 (1.6)	5.6 (1.4)
Atlantic cod	Atlantic cod	6	25.3 (0.8)	26.9 (0.7)	26.3 (0.9)	6.7 (0.7)
Harp seal	Harp seal	12	20.5 (1.6)	21.8 (0.9)	23.1 (1.7)	7.8 (1.1)
Standards	Mean precision (1 SD)	25	±0.9	±0.6	±1.1	±0.7
Standards	Mean accuracy (observed − known)	25	3.2	−0.9	1.1	2.5

where Avg.$\delta^{15}N_{sample\ measured}$ is the average $\delta^{15}N$ for an AA in the sample (n = 2), $\delta^{15}N_{std.\ measured}$ is the $\delta^{15}N$ for the AA in the nearest mixed standard, and $\delta^{15}N_{known}$ represents the known elemental value for the same standard AA, reported by the University of Indiana and SI Science and checked offline in the LIFER lab using a Costech elemental analyser coupled to a Delta V IRMS.

2.5. Determination of TDFs

TDF values were empirically estimated by rearranging Eq. (2), following Bradley et al. (2015):

$$\Delta^{15}N_{trophic-Phe} = TDF_{AA}(TP - 1) + \beta \quad (4)$$

where $\Delta^{15}N_{trophic-Phe}$ is the difference in $\delta^{15}N$ between a trophic AA and Phe, TDF_{AA} is the enrichment of the trophic AA (in this study Glu, Asp and Leu) relative to Phe with each trophic step, TP is an independent measure of mean TP of each taxon (as estimated from bulk SIA with Eq. 1), and β (the intercept) is the difference in $\delta^{15}N$ between the trophic AA and Phe in primary producers (Nielsen et al. 2015). Phe was exclusively used as the source AA because Gly has been found to be unreliable for this purpose, especially in non-plankton consumers (McMahon & McCarthy 2016, Matthews et al. 2020). Plotted as a linear function, the slope (TDF_{AA}) was derived for each taxonomic group and used as the TDF. For average TDF_{AA} values (Glu, Asp and Leu), we used a combined standard deviation, generated by square-rooting the sum of squared TDF_{AA} standard deviations.

The parameter TP in Eq. (4) has previously been sourced using various methods, including stomach content analysis and ecosystem models (Bradley et al. 2015, Hetherington et al. 2017). For our independent TP estimates, we used bulk $\delta^{15}N$ values generated at Memorial University. We calculated TDF_{bulk} using Eq. (1), wherein TP_{base} was estimated using copepods with a value of 2.5 to account for the occurrence of possible omnivory (Olson et al. 2010, Espinoza et al. 2017). TDF_{bulk} was generated from literature-derived bulk TDFs that best matched our target taxa (Table 1). When generating amino acid TDF estimates for different tissues (Eq. 4), the TP_{bulk} parameter was averaged for all tissues per individual because TDF_{bulk} from Eq. (1) was unknown for each specific tissue.

To test whether TDF coefficients differed between trophic guilds, linear regression models (with a fixed β intercept) were compared using a one-way ANOVA (Type I). One model included an interaction term between TP and trophic guild and the other did not; α was set at 0.05. Subsequent planned comparisons were performed using the 'emmeans' package (v. 1.8.7) in R to determine if the different slopes (representing the guild-specific TDFs) were significantly different. Pairwise p-values were adjusted using the Tukey method. This procedure was repeated for each trophic AA.

To investigate whether TDF variability was driven by inter-guild differences in the fractionation of trophic AAs, baseline corrected $\delta^{15}N$ values ($\delta^{15}N_{trophic-Phe}$) were compared between trophic guilds. This was achieved by running a linear mixed-effects model with 'id' employed as a random effect to account for

repeated measures (Glu, Asp and Leu) per individual. Assumptions of normality and homoscedasticity were assessed visually from quantile-quantile (QQ) and residual vs. fitted plots. Furthermore, we compared these models using a likelihood ratio test. We again utilised the 'emmeans' package to assess planned comparisons of $\delta^{15}N_{trophic-Phe}$ between trophic guilds, whereby Tukey's method was used to adjust p-values and confidence intervals for multiple comparisons.

2.6. Comparing Phe between trophic guilds

To investigate whether TDF variability was also influenced by fractionation of source AAs, differences in $\delta^{15}N_{Phe}$ between trophic guilds were explored using a 1-way ANOVA (Type II). Visual and statistical (Shapiro—Wilk and Levene's tests) inspection of $\delta^{15}N_{Phe}$ data showed a normal and homogeneous distribution. This was followed by Tukey's HSD post hoc test.

2.7. Calculating TP from AAs

TP was calculated using Eq. (2) for each individual sample and averaged across trophic guild (Tables 1 & 2). This was repeated with each trophic AA individually (Glu, Asp and Leu) and with averaged trophic AA $\delta^{15}N$ values. TDFs from this study, along with β estimates from Nielsen et al. (2015), were matched to each AA in each trophic guild.

2.8. Tissue comparison

To investigate whether tissue type influenced TDFs, bulk SIA was conducted between tissue types of each taxon (Section 2.1). One- and 2-way ANOVAs were performed to determine if there were differences in $\delta^{15}N_{bulk}$ values between species and tissue type. Akaike's information criterion (AIC) was used to assess models with and without an interaction term between taxa and tissue. A post hoc Tukey test was then used to examine tissue differences for each taxon. Harp seals, pelagic fish (Arctic cod, capelin, herring and sand lance), demersal fish (American plaice and Atlantic cod) and shrimp were analysed separately so that models met assumptions of normality and homogeneity of variance, as assessed with QQ and residual vs. fitted plots. A Kruskal-Wallis test was used to determine if there were tissue-specific differences in demersal fish, followed by a post hoc Dunn's test for multiple comparisons. All p-values were adjusted for multiple comparisons.

Based on the results from bulk SIA, harp seal, Atlantic cod, Arctic cod and capelin tissues were selected for tissue comparison of $\delta^{15}N_{AA}$ values. Following the methods described in Section 2.5. TDFs were compared between muscle, liver and homogenised whole organism. Only muscle and liver were compared for harp seals. Additionally, a 2-way ANOVA (Type II) was run to compare $\delta^{15}N$ between tissue types, controlling for AA and taxon. All data analysis was conducted in R version 4.2.3.

3. RESULTS

3.1. Bulk $\delta^{15}N$ data

Bulk stable nitrogen isotope data were important for calculating the AA TDFs and are presented in Table 1. Mean bulk $\delta^{15}N$ ranged between 5.1 and 9.1‰ for zooplankton, 11.6 and 12.4‰ for pelagic fish, 14.4‰ for Atlantic cod and 14.7‰ for harp seals. Combining these data with literature-derived TDFs for target taxa allowed us to calculate bulk TP (TP_{bulk}) (Table 1) using Eq. (1). Mean TP_{bulk} was between 1.7 and 2.9 for zooplankton, 3.6 and 3.8 for pelagic fish, and 4.9 for Atlantic cod and harp seal.

3.2. AA $\delta^{15}N$ data

Average $\delta^{15}N$ of each trophic AA (Glu, Asp and Leu) ranged from 16.9 to 20.5‰ for zooplankton, 19.4 to 23.0‰ for pelagic fish, 25.3 to 26.9‰ for Atlantic cod and 20.5 to 23.1‰ for harp seals (Table 2, Fig. 3). Combined baseline-corrected trophic AA values ($\delta^{15}N_{trophic-Phe}$) differed between trophic guilds (likelihood ratio test, chi-squared = 39.43, $p < 0.001$) and were enriched in all higher TP consumers compared to lower TP consumers, except for harp seals, which were depleted compared to Atlantic cod and pelagic fish, and not significantly different from zooplankton (Fig. 4; Table S1 in the Supplement at www.int-res.com/articles/suppl/m747p019_supp.pdf).

Average $\delta^{15}N$ of the source AA (Phe) was 4.5‰ for zooplankton, 4.9‰ for pelagic fish, 6.7‰ for Atlantic cod and 7.8‰ for harp seals (Table 2, Fig. 3). $\delta^{15}N_{Phe}$ was lower in zooplankton and pelagic fish compared to Atlantic cod and harp seals (ANOVA, $F_{3,67}$ = 31.852, $p < 0.001$; Fig. 3). $\delta^{15}N_{Phe}$ in harp seals was higher by 3.3 and 2.9‰ compared to zooplankton and pelagic fish, respectively (Tukey HSD, $p < 0.001$).

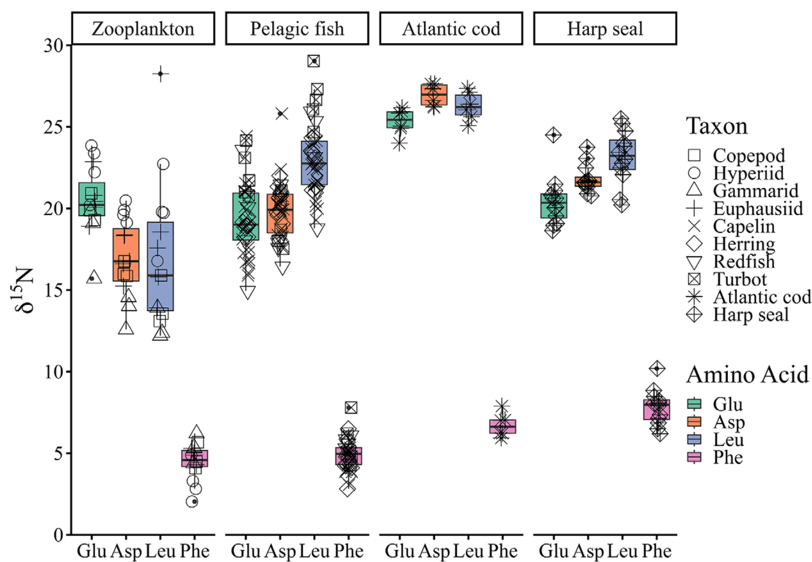

Fig. 3. δ^{15}N of glutamic acid (Glu), aspartic acid (Asp), leucine (Leu) and phenylalanine (Phe) for each trophic guild. Taxa within each trophic guild are defined by shape. For each box, the horizontal line represents the median, the box spans 50% of the data (interquartile range), whiskers define the upper and lower data range (excluding outliers), and black dots represent outliers

$\delta^{15}N_{Phe}$ in Atlantic cod was enriched in ^{15}N by 2.2 and 1.9‰ compared to zooplankton and pelagic fish, respectively (Tukey HSD, $p < 0.001$). $\delta^{15}N_{Phe}$ did not differ between zooplankton and pelagic fish (Tukey HSD, $p = 0.685$; Fig. 3), or between Atlantic cod and harp seals (Tukey HSD, $p = 0.161$; Fig. 3).

3.3. Comparison of TDFs between trophic guilds

Empirical TDF estimates (based on Eq. 4) indicated differences in TDFs between the trophic guilds (Figs. 5 & 6). Zooplankton had the highest TDFs (6.3–7.7‰), pelagic fish and Atlantic cod had intermediate TDFs (4.3–6.2 and 4.0–4.7‰, respectively), and harp seals had the lowest TDFs (2.5–3.6‰; Figs. 5 & 6). Average TDF values (± combined SD) for zooplankton, pelagic fish, Atlantic cod and harp seals were 6.9 ± 2.3, 5.1 ± 1.5, 4.5 ± 1.0 and 3.1 ± 1.0‰, respectively. For each AA, TDFs differed between most trophic guilds, with the exception of pelagic fish and Atlantic cod for Glu and Asp, and between zooplankton and pelagic fish for Leu (Table 3).

3.4. TP calculations using a single TDF vs. using a guild-specific TDF

Applying a single TDF of 7.6‰ (Chikaraishi et al. 2007, 2009) led to consistently low (<3.1) TP estimates across all trophic guilds (Fig. 7). However, applying a guild-specific TDF increased TP estimates for all guilds except zooplankton, which had an almost identical (7.7‰) $TDF_{Glu-Phe}$ to Chikaraishi et al. (2009) (Figs. 6 & 7). The differences between TP estimates generated from a single 7.6‰ TDF vs. a guild-specific TDF was 1.2 for pelagic fish, 1.8 for Atlantic cod and 2.7 for harp seals (Fig. 7). Compared to exclusively using Glu for the TP estimate, averaging all 3 AAs marginally reduced the SD by between 0.03 and 0.2‰, except for zooplankton, where the SD increased by 0.1‰ (Fig. 7).

3.5. Comparison of TDFs between tissue types

There was evidence for differences in bulk δ^{15}N between tissues for harp seals (ANOVA, $F_{1,12} = 6.283$, $p = 0.028$), pelagic fish (ANOVA, $F_{2,115} = 45.896$, $p < 0.001$) and demersal fish (Kruskal-Wallis, chi-squared = 23.4, df = 2, $p < 0.001$), but not for shrimp (ANOVA, $F_{1,33} = 3.148$, $p = 0.085$; Fig. 8). Liver was enriched by a mean of 1.0‰ compared to muscle in harp seals, but depleted by a mean

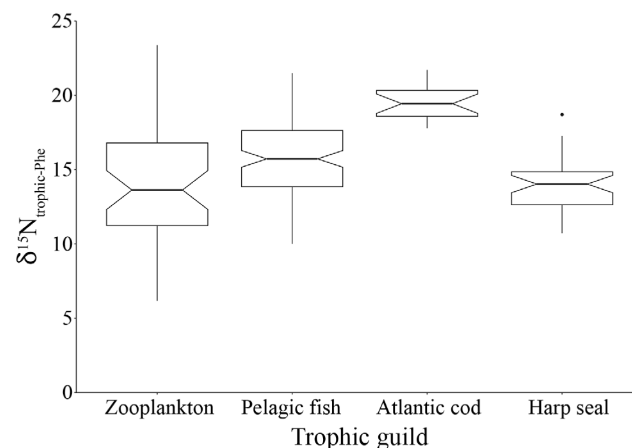

Fig. 4. Baseline-corrected trophic amino acid stable isotope values ($\delta^{15}N_{trophic-Phe}$) for different trophic guilds. Trophic amino acids (glutamic acid, aspartic acid and leucine) were combined. In each box, the horizontal line represents the median, the box spans 50% of the data (interquartile range), whiskers define the upper and lower data range (excluding outliers), and the dot represents an outlier. The notches in the boxes represent the comparison interval around the median values. Samples were collected in 2014 by the Department of Fisheries and Oceans in the Northwest Atlantic

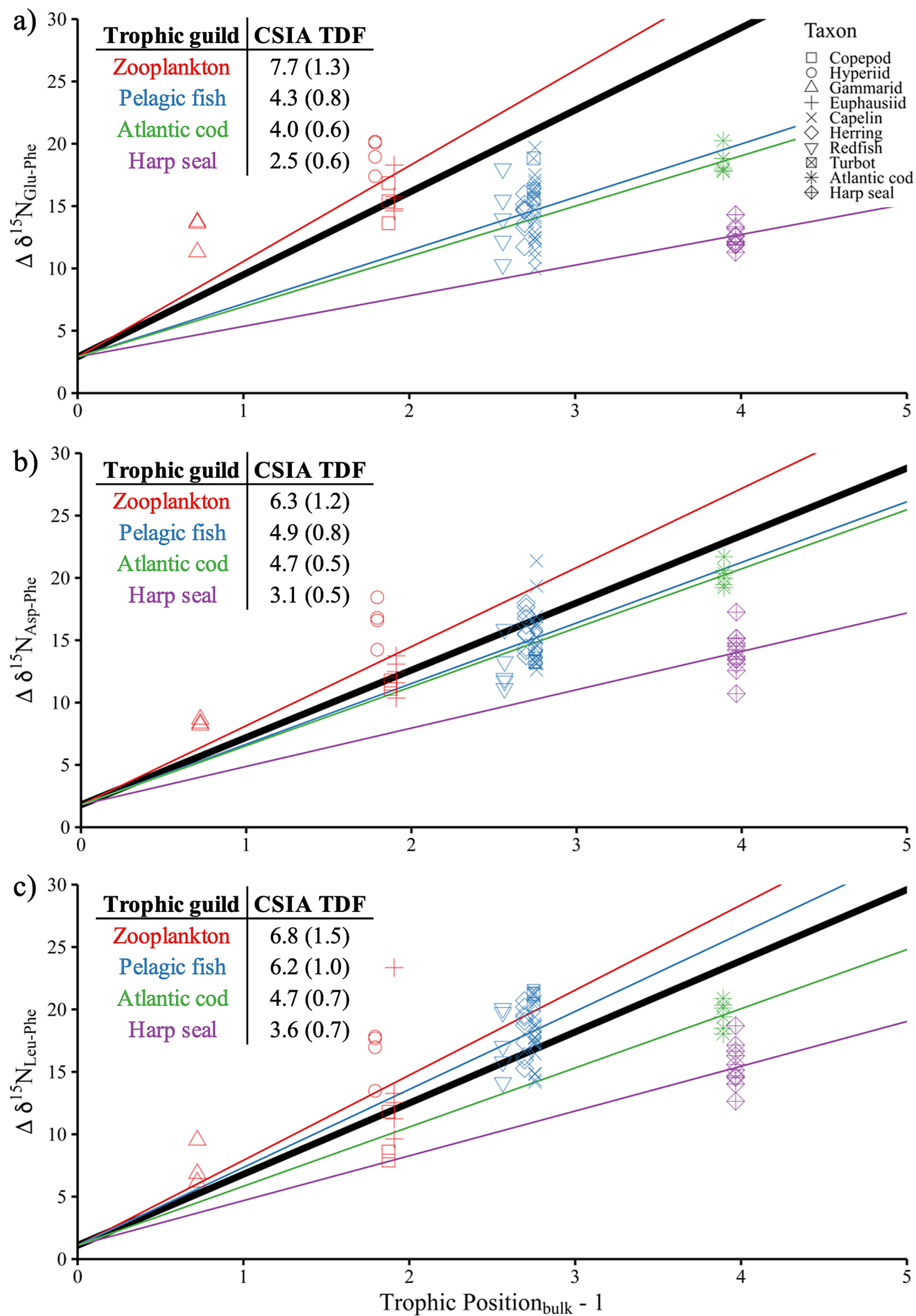

Fig. 5. Linear models (Eq. 4) describing the relationship between trophic position ($TP_{bulk} - 1$) and (a) $\Delta\delta^{15}N_{Glu-Phe}$, (b) $\Delta\delta^{15}N_{Asp-Phe}$ and (c) $\Delta\delta^{15}N_{Leu-Phe}$. TP_{bulk} was calculated for each sample following Eq. (1) and averaged per taxon. Taxa are specified by shape and trophic guilds by colour. The linear slope coefficients are shown in the table insert and represent the trophic discrimination factor (TDF) (±1 SD) of each trophic guild. For comparison, the thick black line shows the average TDF coefficient from Nielsen et al. (2015) for each trophic amino acid: 6.6 for Glu, 5.4 for Asp and 5.7 for Leu. β-values were also specific to each trophic amino acid (Table 3). CSIA: compound-specific stable isotope analysis

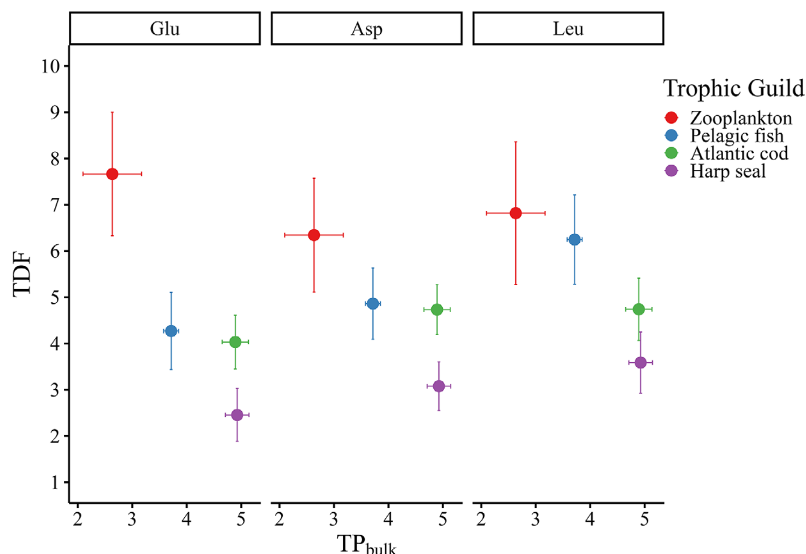

Fig. 6. Mean (±1 SD) trophic discrimination factor (TDF) against the mean (±1 SD) trophic position (TP_{bulk}) of the 4 trophic guilds. TP_{bulk} was calculated using Eq. (1)

of between 1.1 and 1.9‰ compared to muscle and whole organism in Arctic cod (Tukey HSD, $p < 0.001$), capelin (Tukey HSD, $p = 0.002$) and demersal fish (Tukey HSD, $p = 0.003$; Fig. 8). Although sand lance liver was depleted compared to muscle (Tukey HSD, $p = 0.010$), there was no difference between liver and whole organism (Tukey HSD, $p = 0.602$). Finally, there was no difference in bulk $\delta^{15}N$ between tissues in herring (Tukey HSD, $p = 1.00$) or redfish (Tukey HSD, $p = 0.95$; Fig. 8).

AA $\delta^{15}N$ did not differ with tissue type (ANOVA, $F_{2,299} = 0.538$, $p = 0.585$; Fig. S1) and TDF values did not differ between muscle tissue, liver tissue and homogenised whole organism (Table S2). Baseline corrected $\delta^{15}N_{AA}$ results and tissue-specific TDFs for each taxon are provided in Fig. S2 and Table S3.

4. DISCUSSION

In this study, we have shown that TDFs decrease sequentially with increasing TP for multiple taxa in a single shelf marine ecosystem. This offers valuable insight into the variability of TDFs between trophic guilds and provides applicable TDF values for a range of NWA shelf marine taxa, enabling accurate quantification of TP using CSIA-N-AA. Our data support previous findings that show variable TDF values across a range of taxa, which can lead to an underestimation of TP in higher marine consumers (Dale et al. 2011, Bradley et al. 2015, Nielsen et al. 2015, McMahon & McCarthy 2016, Hetherington et al. 2017, Matthews et al. 2020). TDF variability was driven by a combination of effects, namely a reduction in the enrichment of trophic AAs and a notable degree of fractionation of Phe in higher TP consumers, such as Atlantic cod and harp seals. Finally, though differences in bulk $\delta^{15}N$ existed between tissues in some taxa, there was no evidence for tissue-specific differences in the $\delta^{15}N$ of the AAs measured in this study.

4.1. $\delta^{15}N$ enrichment in trophic amino acids

Our data showed a reduction in TDF values with increasing TP, whereby zooplankton had the highest TDF values, pelagic fish had intermediate values, and Atlantic cod and harp seals had the lowest values. This was in part driven by decreasing ^{15}N enrichment of trophic AAs, which undergo significant fractionation during transamination and deamination along metabolic pathways (McMahon & McCarthy 2016). One of the possible mechanisms influencing variable fractionation between the trophic guilds in this study may have been diet quality. Following a review by McMahon & McCarthy (2016), we define diet quality here as the similarity in AA composition between the diet and the consumer, whereby diets that more closely resemble the AA composition of the consumer are considered higher quality (Robbins et al. 2005, 2010). However, diet quality can also be defined as reflecting absolute protein content (i.e. the more protein, the higher the quality of diet) (McMahon et al. 2015b, McMahon & McCarthy 2016). It is widely recognised that diet composition can influence bulk stable isotope values in consumers (Hobson & Clark 1992, Robbins et al. 2005, 2010, Florin et al. 2011), and the 'diet quality' hypothesis posits that TDF values will decrease as diet quality increases (McMahon et al. 2015b, McMahon & McCarthy 2016). There are several potential mechanisms behind this, including assimilative and metabolic fractionation, that relate to the degree of AA transamination and deamination during nitrogen assimilation (McMahon et al. 2015b). Organisms occupying low trophic levels often consume diets that are compositionally different from their own tissues (e.g. zooplankton feeding on phytoplankton) and thus typically need to synthesise much of their own AA pool through transamination

Table 3. Linear model setups (based on Eq. 4) and comparison results per amino acid (AA): glutamic acid (Glu), aspartic acid (Asp) and leucine (Leu). Fixed intercepts, representing the β parameter from Eq. (4), were derived from Nielsen et al. (2015). One-way ANOVAs (Type I) were used to compare models with and without an interaction term, followed by post hoc Tukey HSD tests to compare trophic discrimination factor coefficients between trophic guilds; Zoop: zooplankton; PelFish: pelagic fish

AA	Linear model setup	Linear models					ANOVA				Tukey test			
		Adj. R^2	SE	*F*	df	Fixed intercept (β)	SS	df	*F*	p	Trophic guild comparison	df	*t*-ratio	p
Glu	$\Delta\delta^{15}N_{Glu-Phe} \sim (TP - 1)$	0.87	4.46	464.1	1, 70	2.9	1046.40	3	68.088	<0.001	Zoop − PelFish	67	9.177	<0.001
											Zoop − Cod	67	8.685	<0.001
											Zoop − Seal	67	13.648	<0.001
	$\Delta\delta^{15}N_{Glu-Phe} \sim (TP - 1)$: trophic guild	0.97	2.26	500.6	4, 67	2.9					PelFish − Cod	67	0.868	0.822
											PelFish − Seal	67	8.515	<0.001
											Cod − Seal	67	5.460	<0.001
Asp	$\Delta\delta^{15}N_{Asp-Phe} \sim (TP - 1)$	0.93	3.50	950.7	1, 70	1.8	563.96	3	43.189	<0.001	Zoop − PelFish	67	4.344	<0.001
											Zoop − Cod	67	4.178	0.001
											Zoop − Seal	67	9.285	<0.001
	$\Delta\delta^{15}N_{Asp-Phe} \sim (TP - 1)$: trophic guild	0.98	2.09	699.8	4, 67	1.8					PelFish − Cod	67	0.513	0.956
											PelFish − Seal	67	9.092	<0.001
											Cod − Seal	67	6.220	<0.001
Leu	$\Delta\delta^{15}N_{Leu-Phe} \sim (TP - 1)$	0.92	4.46	830.0	1, 70	1.1	930.12	3	45.166	<0.001	Zoop − PelFish	67	1.331	0.547
											Zoop − Cod	67	4.290	<0.001
											Zoop − Seal	67	7.308	<0.001
	$\Delta\delta^{15}N_{Leu-Phe} \sim (TP - 1)$: trophic guild	0.97	2.62	634.2	4, 67	1.1					PelFish − Cod	67	4.767	<0.001
											PelFish − Seal	67	10.783	<0.001
											Cod − Seal	67	3.446	0.005

of keto acids (McMahon & McCarthy 2016). This process results in fractionation, rendering the newly formed nitrogenous compounds (used in protein synthesis) enriched in ^{15}N, leading to higher TDF values (McMahon & McCarthy 2016). Since zooplankton in the NWA exhibit a degree of herbivory (representative of a lower-quality diet that incurs more AA synthesis), this could explain why TDFs for these taxa were highest in this study (Marshall & Orr 1972, Pepin et al. 2011, McMahon & McCarthy 2016, Zhou et al. 2021). Conversely, higher marine predators feeding on higher-quality diets with AA compositions that more closely match their own tissue may satisfy their AA requirements via direct isotopic routing, which bypasses the transamination step along with the subsequent fractionation (Schwarcz 1991, Braun et al. 2014, McMahon & McCarthy 2016, de la Vega et al. 2021b). This could explain why harp seals, Atlantic cod and pelagic fish had lower TDF values than zooplankton, owing to their more carnivorous diet. Though there are now numerous controlled feeding studies and reviews supporting this hypothesis (Robbins et al. 2005, 2010, Hoen et al. 2014, McMahon et al. 2015b, Nielsen et al. 2015, McMahon & McCarthy 2016), further research is required to directly quantify the relationship between diet quality and the TDF values observed in this system.

The 'diet quality' hypothesis does not help to explain why the TDFs of harp seals were lower than those of Atlantic cod, since both have a similar diet dominated by fish and invertebrates (Hammill et al. 2005, Link et al. 2009, Stenson 2013, Knickle & Rose 2014). This discrepancy may be explained by mode of excretion (i.e. urea vs. ammonia), which was first proposed by Germain et al. (2013) as a possible driver of TDF variability. TDFs have been shown to be lower in urea-excreting organisms compared to ammonia-excreting organisms (Germain et al. 2013, Nielsen et al. 2015), which may be due to divergent biochemical processes. Urea synthesis includes

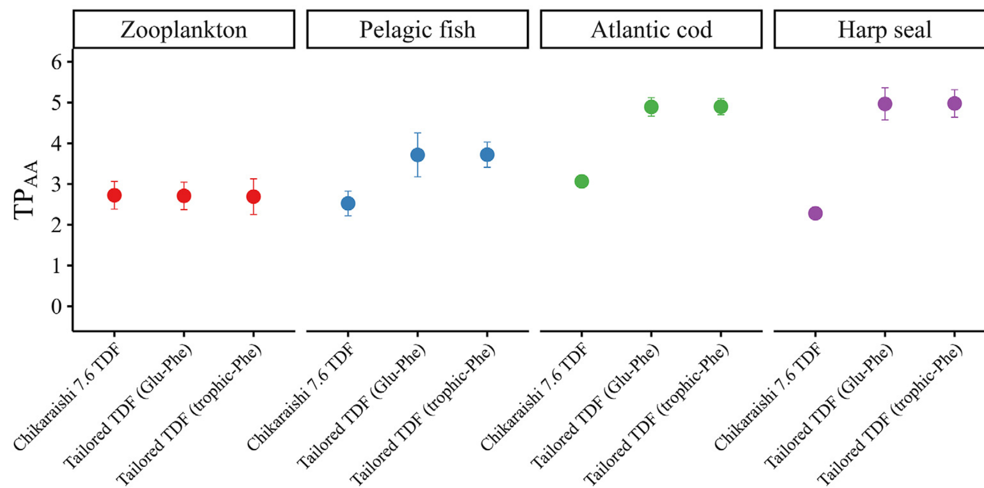

Fig. 7. Comparing trophic position (TP_{AA}) estimates (mean ± 1 SD) using a universal 7.6‰ trophic discrimination factor ($TDF_{Glu-Phe}$) from Chikaraishi et al. (2007, 2009) vs. tailored TDFs for each trophic guild. TP_{AA} was calculated with Eq. (2) using $\delta^{15}N$ of glutamic acid minus phenylalanine (Glu−Phe) and an average $\delta^{15}N$ of trophic AAs minus phenylalanine (trophic−Phe)

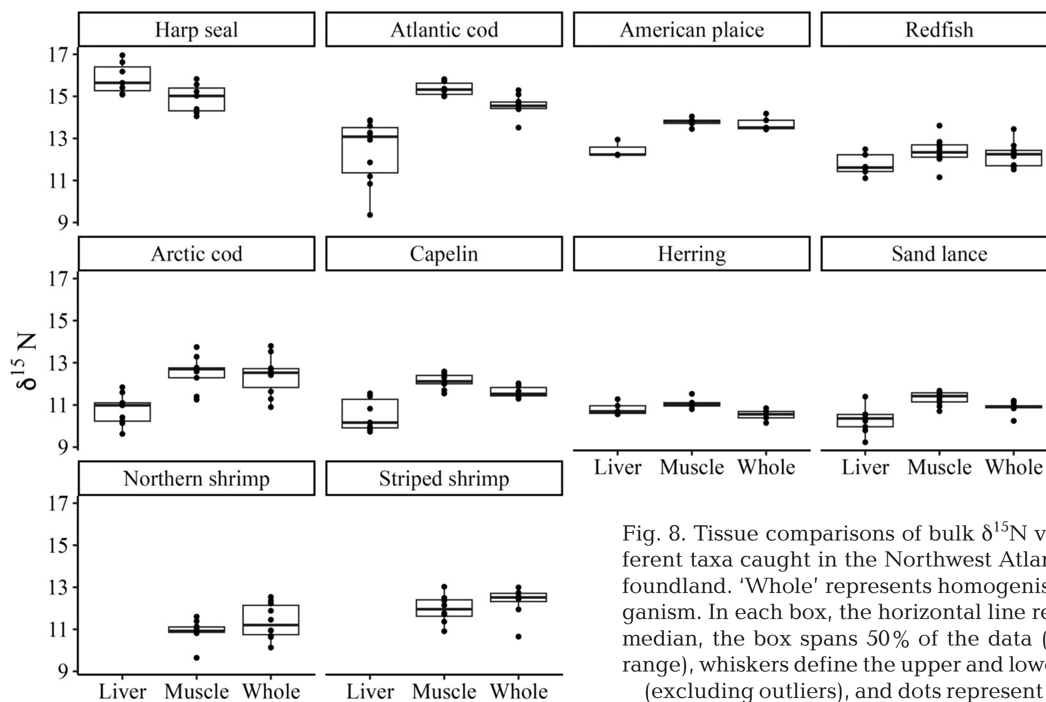

Fig. 8. Tissue comparisons of bulk $\delta^{15}N$ values for different taxa caught in the Northwest Atlantic off Newfoundland. 'Whole' represents homogenised whole organism. In each box, the horizontal line represents the median, the box spans 50% of the data (interquartile range), whiskers define the upper and lower data range (excluding outliers), and dots represent datapoints

more deamination steps or branch points (i.e. the ornithine cycle) compared to ammonia synthesis (Germain et al. 2013, McMahon & McCarthy 2016). Conversion of Glu to ammonia requires one oxidative deamination step via glutamate dehydrogenase, resulting in direct excretion of ^{15}N-depleted ammonia and leaving behind a ^{15}N-enriched residual Glu pool (Germain et al. 2013, McMahon & McCarthy 2016). This corresponds to a large TDF between the consumer tissue and ammonia waste product. Urea/uric acid production involves all of the enzymatic steps of ammonia biosynthesis, plus additional nitrogen-transferring reactions involving Glu to Asp, and Asp to urea (McMahon & McCarthy 2016). This multi-step, multi-reservoir pathway distributes ^{14}N more broadly throughout major biochemical compartments of the urea reaction chain, thereby producing a waste product (urea) that is relatively enriched compared to ammonia. This ultimately corresponds to reduced TDFs in urea-excreting organisms compared to ammonia-excreting organisms (Ger-

main et al. 2013, McMahon & McCarthy 2016). Our observations support this hypothesis because harp seals (urea-excreting) had lower TDFs than Atlantic cod (ammonia-excreting), despite occupying similar TPs. Further research is required to explicitly quantify the relationship between mode of excretion with TDFs, especially given that some studies have not found it to be an important driver (Hoen et al. 2014, Hetherington et al. 2017).

4.2. Enrichment of phenylalanine

In our study, we observed increased Phe $\delta^{15}N$ ($\delta^{15}N_{Phe}$) values in Atlantic cod and harp seals compared to zooplankton and pelagic fish, which differed by a mean of up to 3.3‰. Phe is regularly used as a canonical source AA because of its minimal fractionation (0 to 1.5‰) between consumer and prey (McClelland & Montoya 2002, Chikaraishi et al. 2007, 2009, Bradley et al. 2014, McMahon & McCarthy 2016, de la Vega et al. 2021a). However, recent studies on higher marine predators (Pacific yellow tail *Seriola lalandi* and cetaceans) indicate that ^{15}N enrichment in Phe can be larger, potentially in response to prey protein content and digestibility (Nuche-Pascual et al. 2018, Matthews et al. 2020). If so, this has implications for accurately estimating TP in consumers eating a diverse range of diets because TDFs can additionally be affected by diet-driven variations in source AA fractionation. For instance, TDFs in consumers eating a high-quality diet may be reduced through a compression effect characterised by smaller fractionation of trophic AAs and larger fractionation of source AAs (Matthews et al. 2020).

Furthermore, even in cases where ^{15}N enrichment in Phe remains small between each trophic step (1.1 ± 0.5‰, de la Vega et al. 2021a), propagation of this enrichment over 3 or more trophic steps will still impart a significant shift in consumer $\delta^{15}N_{Phe}$, which will need to be accounted for in the assigned TDF value used to calculate TP (Ruiz-Cooley et al. 2014, McMahon & McCarthy 2016). Overall, our observation of increased $\delta^{15}N_{Phe}$ values in predators (Atlantic cod and harp seal) compared with lower trophic levels (zooplankton and forage fish) supports the notion that $^{15}N_{Phe}$ fractionation should not be overlooked as a source of TDF variability.

4.3. Tissue type

A comparison of $\delta^{15}N_{bulk}$ values between tissues showed liver to be enriched in harp seals, but depleted in most of the fish species, including capelin, Arctic cod, sand lance, American plaice and Atlantic cod. To understand these tissue-specific differences further, we used CSIA-N-AA to investigate which AAs were responsible. However, there was no evidence for tissue-specific differences in $\delta^{15}N$ for the AAs measured in this study. This also corresponded to no observed differences in AA TDFs between tissues. Further analysis of a broader range of AAs is required to investigate the underlying reasons for the observed tissue-specific differences in $\delta^{15}N_{bulk}$ values.

The $TDF_{Glu-Phe}$ for harp seal muscle in this study (2.5 ± 0.6‰) was similar to that estimated by Germain et al. (2013; 2.6 ± 1.3‰); however, their $TDF_{Glu-Phe}$ for blood serum was considerably higher, at 4.3 ± 1.2‰. Previous studies have also documented tissue-specific differences in TDFs, primarily using bulk SIA (Hobson & Clark 1992, Hobson et al. 1996, Schmidt et al. 2004, Kurle et al. 2014). Tissue-specific variation in fractionation may occur because different tissues have unique AA compositions and metabolic rates, which draw differentially from AA pools during their formation (Schmidt et al. 2004). It is speculated that tissues with higher metabolic rates (and thus faster isotopic turnover) have larger TDF values because of greater levels of preferential excretion of ^{14}N waste products (Kurle et al. 2014). In addition, these tissues also undergo rapid protein degradation and turnover, resulting in frequent recycling of their proteins (Kurle et al. 2014). This constant recycling exposes their components to enzymatic fractionation, contributing to the accumulation of residual ^{15}N (Kurle et al. 2014). It is therefore mysterious why we observed lower $\delta^{15}N_{bulk}$ values in the livers of certain fish species; further research is required to understand the mechanisms behind this, especially given that no differences in subsequent $\delta^{15}N_{AA}$ values were observed between tissues.

4.4. Caveats

We were unable to include Ala, Val and Pro in our study (Section 2.4). Although the reduction in the number of observable trophic AAs was unfortunate, this does not undermine the findings from the other amino acids, which remain valuable. Future work should investigate whether the pattern observed in the trophic AAs in this study are also reflected in Ala, Val and Pro, especially because this may provide insight into the discrepancy between bulk and compound-specific stable isotope differences in the tissue comparison section of the study. This is especially per-

tinent given the apparent variance in the enrichment of ^{15}N between AAs at certain levels of the food web, such as for micro- and mesozooplankton (Gutiérrez-Rodríguez et al. 2014, Décima et al. 2017). Décima et al. (2017) demonstrated that ^{15}N enrichment of Ala was similar between protistan and metazoan plankton, whereas enrichment of Glu was variable. This suggests that Ala may be more appropriate for quantifying trophic structure in lower trophic level organisms, which has implications for quantifying food web structure more generally. Further research should focus on whether specific AAs should be paired up with taxa depending on their TP in the food web.

4.5. Conclusion

Our study demonstrates a sequential decrease in TDFs across trophic guilds within a single shelf sea ecosystem. These findings help to develop CSIA-N-AA as tool to quantify food web structure, offering valuable TDF values for a diverse range of shelf sea taxa in the NWA. Our results corroborate previous research highlighting variable TDF values among taxa, which can lead to underestimations of TP in higher marine consumers (Lorrain et al. 2009, 2015, Dale et al. 2011, Germain et al. 2013, Matthews & Ferguson 2014, Hoen et al. 2014, Bradley et al. 2015, McMahon & McCarthy 2016, Matthews et al. 2020). TDF variability stemmed from a combination of reduced ^{15}N enrichment of trophic AAs and increased ^{15}N enrichment in Phe in higher TP consumers. Explanations for this remain speculative, but may be related to diet quality and excretion (McMahon & McCarthy 2016). Finally, our analysis revealed no tissue-specific differences in AA TDFs, despite variability in $\delta^{15}N_{bulk}$ values, and the underlying reason for this warrants further investigation. Leveraging CSIA-N-AA alongside an independent measure of TP (e.g. stomach content analysis, ecosystem models or bulk stable isotope analysis) remains a useful solution for estimating TDFs in wild populations (Bradley et al. 2015, Hetherington et al. 2017). Our results will contribute towards building a catalogue of species- and tissue-specific TDFs across a range of taxa and environments that will be useful for future TP_{AA} applications.

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LITERATURE CITED

Ankjærø T, Christensen JT, 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. Mar Ecol Prog Ser 461:197–209

Bradley CJ, Madigan DJ, Block BA, Popp BN (2014) Amino acid isotope incorporation and enrichment factors in pacific bluefin tuna, *Thunnus orientalis.* PLOS ONE 9:e85818

Bradley CJ, Wallsgrove NJ, Choy CA, Drazen JC, Hetherington ED, Hoen DK, Popp BN (2015) Trophic position estimates of marine teleosts using amino acid compound specific isotopic analysis. Limnol Oceanogr Methods 13: 476–493

Braun A, Vikari A, Windisch W, Auerswald K (2014) Transamination governs nitrogen isotope heterogeneity of amino acids in rats. J Agric Food Chem 62:8008–8013

Canseco JA, Niklitschek EJ, Harrod C (2022) Variability in $\delta^{13}C$ and $\delta^{15}N$ trophic discrimination factors for teleost fishes: a meta-analysis of temperature and dietary effects. Rev Fish Biol Fish 32:313–329

Chabot D, Stenson GB (2002) Growth and seasonal fluctuations in size and condition of male Northwest Atlantic harp seals *Phoca groenlandica*: an analysis using sequential growth curves. Mar Ecol Prog Ser 227:25–42

Chikaraishi Y, Kashiyama Y, Ogawa NO, Kitazato H, Ohkouchi N (2007) Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: implications for aquatic food web studies. Mar Ecol Prog Ser 342:85–90

Chikaraishi Y, Ogawa NO, Kashiyama Y, Takano Y and others (2009) Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnol Oceanogr Methods 7:740–750

Choy CA, Davison PC, Drazen JC, Flynn A and others (2012) Global trophic position comparison of two dominant mesopelagic fish families (Myctophidae, Stomiidae) using amino acid nitrogen isotopic analyses. PLOS ONE 7: e50133

Corr LT, Berstan R, Evershed RP (2007) Optimisation of derivatisation procedures for the determination of $\delta^{13}C$ values of amino acids by gas chromatography/combustion/isotope ratio mass spectrometry. Rapid Commun Mass Spectrom 21:3759–3771

Dale JJ, Wallsgrove NJ, Popp BN, Holland KN (2011) Nursery habitat use and foraging ecology of the brown stingray *Dasyatis lata* determined from stomach contents, bulk and amino acid stable isotopes. Mar Ecol Prog Ser 433:221–236

de la Vega C, Mahaffey C, Tuerena RE, Yurkowski DJ and others (2021a) Arctic seals as tracers of environmental and ecological change. Limnol Oceanogr Lett 6:24–32

de la Vega C, Mahaffey C, Yurkowski DJ, Norman L and others (2021b) Biomarkers in ringed seals reveal recent onset of borealization in the high- compared to the mid-latitude Canadian Arctic. Front Mar Sci 8:700687

Décima M, Landry MR, Bradley CJ, Fogel ML (2017) Alanine

$\delta^{15}N$ trophic fractionation in heterotrophic protists. Limnol Oceanogr 62:2308–2322

Espinoza P, Lorrain A, Ménard F, Cherel Y and others (2017) Trophic structure in the northern Humboldt Current system: new perspectives from stable isotope analysis. Mar Biol 164:86

Florin ST, Felicetti LA, Robbins CT (2011) The biological basis for understanding and predicting dietary-induced variation in nitrogen and sulphur isotope ratio discrimination. Funct Ecol 25:519–526

Germain LR, Koch PL, Harvey J, McCarthy MD (2013) Nitrogen isotope fractionation in amino acids from harbor seals: implications for compound-specific trophic position calculations. Mar Ecol Prog Ser 482:265–277

Gutiérrez-Rodríguez A, Décima M, Popp BN, Landry MR (2014) Isotopic invisibility of protozoan trophic steps in marine food webs. Limnol Oceanogr 59:1590–1598

Hammill MO, Lesage V, Carter P (2005) What do harp seals eat? Comparing diet composition from different compartments of the digestive tract with diets estimated from stable-isotope ratios. Can J Zool 83:1365–1372

Healy K, Guillerme T, Kelly SBA, Inger R, Bearhop S, Jackson AL (2018) SIDER: an R package for predicting trophic discrimination factors of consumers based on their ecology and phylogenetic relatedness. Ecography 41:1393–1400

Hetherington ED, Olson RJ, Drazen JC, Lennert-Cody CE, Ballance LT, Kaufmann RS, Popp BN (2017) Spatial food-web structure in the eastern tropical Pacific Ocean based on compound-specific nitrogen isotope analysis of amino acids. Limnol Oceanogr 62:541–560

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes II: factors influencing diet–tissue fractionation. Condor 94:189–197

Hobson KA, Schell DM, Renouf D, Noseworthy E (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can J Fish Aquat Sci 53:528–533

Hoen DK, Kim SL, Hussey NE, Wallsgrove NJ, Drazen JC, Popp BN (2014) Amino acid ^{15}N trophic enrichment factors of four large carnivorous fishes. J Exp Mar Biol Ecol 453:76–83

Hussey NE, MacNeil MA, McMeans BC, Olin JA and others (2014) Rescaling the trophic structure of marine food webs. Ecol Lett 17:239–250

Knickle DC, Rose GA (2014) Dietary niche partitioning in sympatric gadid species in coastal Newfoundland: evidence from stomachs and C-N isotopes. Environ Biol Fishes 97:343–355

Kurle CM, Koch PL, Tershy BR, Croll DA (2014) The effects of sex, tissue type, and dietary components on stable isotope discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$) in mammalian omnivores. Isotopes Environ Health Stud 50:307–321

Link JS, Bogstad B, Sparholt H, Lilly GR (2009) Trophic role of Atlantic cod in the ecosystem. Fish Fish 10:58–87

Lorrain A, Graham B, Ménard F, Popp B, Bouillon S, van Breugel P, Cherel Y (2009) Nitrogen and carbon isotope values of individual amino acids: a tool to study foraging ecology of penguins in the Southern Ocean. Mar Ecol Prog Ser 391:293–306

Lorrain A, Graham BS, Popp BN, Allain V and others (2015) Nitrogen isotopic baselines and implications for estimating foraging habitat and trophic position of yellowfin tuna in the Indian and Pacific Oceans. Deep Sea Res II 113:188–198

Lydersen C, Kovacs KM (1996) Energetics of lactation in harp seals (*Phoca groenlandica*) from the Gulf of St. Lawrence, Canada. J Comp Physiol B 166:295–304

Lynam CP, Llope M, Möllmann C, Helaouët P, Bayliss-Brown GA, Stenseth NC (2017) Interaction between top-down and bottom-up control in marine food webs. Proc Natl Acad Sci USA 114:1952–1957

Marshall SM, Orr AP (1972) The biology of a marine copepod: *Calanus finmarchicus* (Gunnerus). Springer, Berlin

Matthews CJD, Ferguson SH (2014) Spatial segregation and similar trophic-level diet among eastern Canadian Arctic/north-west Atlantic killer whales inferred from bulk and compound specific isotopic analysis. J Mar Biol Assoc UK 94:1343–1355

Matthews CJD, Ruiz-Cooley RI, Pomerleau C, Ferguson SH (2020) Amino acid $\delta^{15}N$ underestimation of cetacean trophic positions highlights limited understanding of isotopic fractionation in higher marine consumers. Ecol Evol 10:3450–3462

McCarthy MD, Benner R, Lee C, Fogel ML (2007) Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. Geochim Cosmochim Acta 71:4727–4744

McCarthy MD, Lehman J, Kudela R (2013) Compound-specific amino acid $\delta^{15}N$ patterns in marine algae: tracer potential for cyanobacterial vs. eukaryotic organic nitrogen sources in the ocean. Geochim Cosmochim Acta 103:104–120

McClelland JW, Montoya JP (2002) Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. Ecology 83:2173–2180

McMahon KW, McCarthy MD (2016) Embracing variability in amino acid $\delta^{15}N$ fractionation: mechanisms, implications, and applications for trophic ecology. Ecosphere 7:e01511

McMahon KW, Polito MJ, Abel S, McCarthy MD, Thorrold SR (2015a) Carbon and nitrogen isotope fractionation of amino acids in an avian marine predator, the gentoo penguin (*Pygoscelis papua*). Ecol Evol 5:1278–1290

McMahon KW, Thorrold SR, Elsdon TS, McCarthy MD (2015b) Trophic discrimination of nitrogen stable isotopes in amino acids varies with diet quality in a marine fish. Limnol Oceanogr 60:1076–1087

Mill AC, Pinnegar JK, Polunin NVC (2007) Explaining isotope trophic-step fractionation: why herbivorous fish are different. Funct Ecol 21:1137–1145

Mohan JA, Smith SD, Connelly TL, Attwood ET, McClelland JW, Herzka SZ, Walther BD (2016) Tissue-specific isotope turnover and discrimination factors are affected by diet quality and lipid content in an omnivorous consumer. J Exp Mar Biol Ecol 479:35–45

Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mamm Sci 26:509–572

Nielsen JM, Popp BN, Winder M (2015) Meta-analysis of amino acid stable nitrogen isotope ratios for estimating trophic position in marine organisms. Oecologia 178:631–642

Nuche-Pascual MT, Lazo JP, Ruiz-Cooley RI, Herzka SZ (2018) Amino acid-specific $\delta^{15}N$ trophic enrichment factors in fish fed with formulated diets varying in protein quantity and quality. Ecol Evol 8:9192–9217

Olson RJ, Popp BN, Graham BS, López-Ibarra GA and others (2010) Food-web inferences of stable isotope spatial patterns in copepods and yellowfin tuna in the pelagic eastern Pacific Ocean. Prog Oceanogr 86:124–138

Pepin P, Colbourne E, Maillet G (2011) Seasonal patterns in

zooplankton community structure on the Newfoundland and Labrador Shelf. Prog Oceanogr 91:273–285
Pinnegar JK, Polunin NVC (1999) Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. Funct Ecol 13:225–231
Polis GA, Strong DR (1996) Food web complexity and community dynamics. Am Nat 147:813–846
Popp BN, Graham BS, Olson RJ, Hannides CCS and others (2007) Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. Terr Ecol 1:173–190
Post DM (2002a) The long and short of food-chain length. Trends Ecol Evol 17:269–277
Post DM (2002b) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718
Preisser E (2008) Trophic structure. In: Fath B (ed) Encyclopedia of ecology, 2nd edn, Vol 3. Elsevier, p 647–655
Robbins CT, Felicetti LA, Sponheimer M (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144:534–540
Robbins CT, Felicetti LA, Florin ST (2010) The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. Oecologia 162: 571–579
Ruiz-Cooley RI, Koch PL, Fiedler PC, McCarthy MD (2014) Carbon and nitrogen isotopes from top predator amino acids reveal rapidly shifting ocean biochemistry in the outer California current. PLOS ONE 9:e110355
Sabadel AJ, MacLeod CD (2022) Stable isotopes unravel the feeding mode–trophic position relationship in trematode parasites. J Anim Ecol 91:484–495
Schmidt K, McClelland JW, Mente E, Montoya JP, Atkinson A, Voss M (2004) Trophic-level interpretation based on $\delta^{15}N$ values: implications of tissue-specific fractionation and amino acid composition. Mar Ecol Prog Ser 266: 43–58
Schwarcz HP (1991) Some theoretical aspects of isotope paleodiet studies. J Archaeol Sci 18:261–275
Seminoff JA, Benson SR, Arthur KE, Eguchi T, Dutton PH, Tapilatu RF, Popp BN (2012) Stable isotope tracking of endangered sea turtles: validation with satellite telemetry and $\delta^{15}N$ analysis of amino acids. PLOS ONE 7:e37403
Sherwood GD, Rose GA (2005) Stable isotope analysis of some representative fish and invertebrates of the Newfoundland and Labrador continental shelf food web. Estuar Coast Shelf Sci 63:537–549
Sjare B, Stenson GB (2010) Changes in the reproductive parameters of female harp seals (*Pagophilus groenlandicus*) in the Northwest Atlantic. ICES J Mar Sci 67: 304–315
Stenson G (2013) Estimating consumption of prey by harp seals (*Pagophilus groenlandicus*) in NAFO Divisions 2J3KL. DFO Can Sci Advis Sec Res Doc 2012/156. https://publications.gc.ca/collections/collection_2013/mpo-dfo/Fs70-5-2012-156-eng.pdf
Stenson GB, Buren AD, Koen-Alonso M (2016) The impact of changing climate and abundance on reproduction in an ice-dependent species, the Northwest Atlantic harp seal, *Pagophilus groenlandicus.* ICES J Mar Sci 73:250–262
Stenson GB, Haug T, Hammill MO (2020) Harp seals: monitors of change in differing ecosystems. Front Mar Sci 7: 569258
Stephens RB, Shipley ON, Moll RJ (2023) Meta-analysis and critical review of trophic discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$): importance of tissue, trophic level and diet source. Funct Ecol 37:2535–2548
Vander Zanden JM, Rasmussen JB (1996) A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. Ecol Monogr 66:451–477
Vander Zanden MJ, Clayton MK, Moody EK, Solomon CT, Weidel BC (2015) Stable isotope turnover and half-life in animal tissues: a literature synthesis. PLOS ONE 10: e0116182
Walters C, Maguire JJ (1996) Lessons for stock assessment from the northern cod collapse. Rev Fish Biol Fish 6: 125–137
Worm B, Duffy JE (2003) Biodiversity, productivity and stability in real food webs. Trends Ecol Evol 18:628–632
Zhou F, Hirai J, Hamasaki K, Horii S, Tsuda A (2021) Feeding ecology of three euphausiid species in the North Pacific Ocean inferred from 18S V9 metabarcoding and stable isotope analysis. Front Mar Sci 8:756067

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