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SHORT NOTE

Preliminary assessment of Greenland halibut diet in Cumberland Sound using stable isotopes

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Abstract We provide preliminary carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope assessment of the Greenland halibut (*Reinhardtius hippoglossoides*) diet in Cumberland Sound, with focus on two possible prey sources: pelagic represented by capelin (*Mallotus villosus)* and epibenthic represented by shrimp (*Lebbeus polaris*). The δ^{13}C for the Greenland halibut stock indicated a pelagic carbon source in Cumberland Sound while stable isotope mixing models, IsoSource and MixSIR, indicated a 99% dietary composition of capelin relative to the shrimp. The δ^{15}N did not vary across Greenland halibut size ranges and placed them at a fourth trophic position relative to a primary herbivore. This study provides the starting point for more elaborate Cumberland Sound research on the local Greenland halibut feeding ecology by confirming pelagic feeding and establishing relative trophic position as well as identifying stable isotopes as a useful tool for the study of diet in cold water fish species.

Keywords Stable isotope · Feeding ecology · Greenland halibut · Cumberland Sound

Introduction

The use of naturally occurring stable isotopes can provide information on feeding location (e.g. benthic or pelagic) and trophic relationships of aquatic organisms because stable isotope ratios in consumer tissues can be related to the ratios in their diet (DeNiro and Epstein 1978, 1981). Little change between trophic positions occurs for δ^{13}C, but can be used to distinguish benthic/inshore food webs from pelagic/offshore food webs—δ^{13}C value tend to be more enriched in benthic/inshore systems (Hobson and Welch 1992; Hobson et al. 1995; France 1995). Because enrichment of the heavy isotope of nitrogen occurs with trophic position at a relatively constant rate, relative trophic positions can be derived from δ^{15}N (Michener and Schell 1994). Stomach content analysis, the conventional method for assessing diet, only provides a snapshot of organisms in the diet and often suffers from empty stomachs, whereas stable isotopes provide an integrated measure of energy flow through trophic pathways and can overcome empty stomachs (Post 2002).

The Greenland halibut of Cumberland Sound, Nunavut, are targeted by an artisanal, Inuit longline fishery that is currently expanding its commercial nature and extending its fishing seasons. Although many studies have focused on Greenland halibut diet, they have been generally restricted to stomach content analysis with little use of stable isotopes. Attempts to assess Greenland halibut diet in Cumberland Sound via stomach contents have been historically unsuccessful due to the prevalence of empty stomachs. In addition, Greenland halibut prey selection depends on many factors, and diet changes with area, depth and/or predator size (Bowering and Lilly 1992; Sólmundsson 1993; Rodríguez-Marin et al. 1997). Pedersen and Riget (1993) suggest that Greenland halibut feed on the most abundant prey within its habitat, preventing assumptions on feeding ecology of unstudied Greenland halibut stocks.

Here, we provide a preliminary assessment of the Greenland halibut diet in Cumberland Sound. Fisk et al. (2002) used a very limited sample size to place the

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Cumberland Sound stock at a fourth trophic level with a pelagic carbon source. In this study, we were able to increase the sample size and focus efforts on two known potential prey items, capelin (*Mallotus villosus*) and shrimp (*Lebbeus polaris*). Capelin represent a pelagic, surface-feeding organism (Templeman 1948) while shrimp are known to be epibenthic feeders (Birkely and Gullicksen 2003)—thereby allowing these species to act as proxies of their respective habitats. Copepods (*Calanus hyperboreus*) and scallops (*Chlamys islandica*) were also included in this study, representing the baseline for pelagic and benthic carbon sources, respectively, and a trophic position of 2 (i.e. primary herbivores; Hobson et al. 2002).

Methods

Greenland halibut samples were collected in April 2008 in the Cumberland Sound fishery via bottom longline. All halibut were measured for total length and assigned to size classes. Because of the current discrepancy in aging Greenland halibut (e.g. 50 cm Greenland halibut could grow ~1–5 cm per year depending on aging method; Treble 2008), six size classes were created with 5 cm intervals: <55, 55–60, 60–65, 65–70, 70–75, >75. Stomach contents, if any, were identified as close to species level as possible. Shrimp and copepod samples were collected from Cumberland Sound in April 2008 and capelin and scallop samples were collected in August 2008. Shells of shrimp and scallops were removed before analysis.

Tissue was grounded using a ball mill grinder (SPEX CertiPrep 8000-D ball milling unit, SPEX CertiPrep, Metuchen, NJ, USA). Samples were homogenized in 5 ml of 2:1 chloroform:methanol with 30 s of vortexing, left 24 h, and then decanted for gravimetric determination of lipids (Post et al. 2007). The process was repeated for thorough extraction and solvents were dried-off the tissue via 24 h in an fumehood. Between 400 and 600 µg of tissue was weighed into tin capsules and stable-carbon and nitrogen isotope ratios were provided from a continuous flow isotope ration mass spectrometer (IRMS, Finnigan MAT Delta^{plus}, Thermo Finnigan, San Jose, CA, USA). The standard reference material was Pee Dee Belemnite carbonate for CO_2 and atmospheric nitrogen N_2. The analytical precision (standard deviation) for $\delta^{15}N$ was 0.14 and for $\delta^{13}C$ was 0.05 based on the analysis of NIST standards sucrose ($n = 13$) and ammonium sulfate ($n = 13$).

Because the Greenland halibut were assigned to size classes, the relationship between Greenland halibut size, sex, and $\delta^{15}N$ (muscle tissue) was investigated using a generalized linear model (GLM). Confidence intervals at 95% were calculated to compare Greenland halibut tissue isotope values between size classes and to other food-web species. All analyses were run in R Development Core Team (2008). In addition, trophic position (TP) was determined relative to the copepod, assumed to be a primary herbivore occupying a trophic position of 2 (Hobson et al. 2002). Relative trophic positions of consumers were calculated based on the muscle tissue $\delta^{15}N$ using:

$$\text{Trophic position} = 2 + \left(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{Calanus}}\right)/3.4 \quad (1)$$

and assuming an isotopic discrimination factor among trophic positions of 3.4‰ (Post 2002).

Mixing model software, Isosource (Phillips and Gregg 2003) and MixSIR (Moore and Semmens 2008) was used to assess dietary contributions of both prey species to the diet of the Greenland halibut. These models calculate feasible combinations of primary producer isotope signatures, $\delta^{15}N$ and $\delta^{13}C$, that explain observed consumer isotope signatures. For Isosource, the model source increment was set at 1% and the tolerance at 0.7% with shrimp and capelin as potential prey species. Isosource outputs are reported here as median and 1–99th percentile range of solutions (Bernstead et al. 2006). In MixSIR, we used previously published fractionation values of 1.0 ± 0.4‰ for $\delta^{13}C$ and 3.4 ± 1.1‰ for $\delta^{15}N$ and also included shrimp and capelin as the potential prey items. The maximum importance ratio was below 0.001, indicating model effectiveness in estimating true posterior density (Moore and Semmens 2008). MixSIR results are presented as median, 5th, and 95th percentiles.

Results

We collected a total of 26 Greenland halibut (17 males, 7 females). Total length ranged in size from 51.4 to 85.6 cm with a mean ± 95% confidence interval (CI) of 62.44 ± 2.95 cm. The majority of stomachs was empty (89%) with three stomachs containing shrimp, mysids, or unidentifiable fish. Of species analyzed from the Cumberland Sound food-web for Greenland halibut feeding, Greenland halibut size class 6, >75 cm, had the highest $\delta^{15}N$ (17.13) and scallops the lowest $\delta^{15}N$ (8.58 ± 0.18) (Table 1; Fig. 1). The lowest $\delta^{13}C$ belonged to copepods (−20.52 ± 0.82) and highest $\delta^{13}C$ to scallops (−18.55 ± 0.38) (Table 1). The mean $\delta^{13}C$ value for Greenland halibut was −8.95 ± 0.16‰, with little variation with size (Table 1). Greenland halibut size classes were clumped together in a plot of $\delta^{15}N$ against $\delta^{13}C$, suggesting similarities in diet across sizes.

Trophic positions were calculated for each species using Eq. 1 and are listed in Table 1. The Greenland halibut was calculated at a trophic position of approximately 4.0 for all

Table 1 Stable-nitrogen and stable-carbon isotope values (mean ± SE) for several food-web components of the Cumberland Sound ecosystem and derived relative trophic position

Species	Size class	*n*	δ^{15}N (‰)	δ^{13} C (‰)	Relative trophic position
Copepods		4 bulk	9.47 ± 0.26	−20.52 ± 0.82	2
Scallop		5	8.58 ± 0.18	−18.55 ± 0.38	Benthic
Shrimp		5	13.24 ± 0.12	−18.74 ± 0.16	3.1
Capelin		7	12.92 ± 0.13	−19.25 ± 0.05	3.0
G. Halibut 1	<55	6	15.85 ± 0.10	−19.05 ± 0.09	3.9
2	55–60	3	16.25 ± 0.10	−18.94 ± 0.01	4.0
3	60–65	8	16.00 ± 0.21	−19.01 ± 0.21	4.0
4	65–70	5	16.35 ± 0.20	−18.93 ± 0.09	4.0
5	70–75	3	16.50 ± 0.29	−18.62 ± 0.39	4.1
6	>75	1	17.13	−18.91	4.3

Trophic position = $2 + (\delta^{15}N_{consumer} - \delta^{15}N_{Calanus})/3.4$

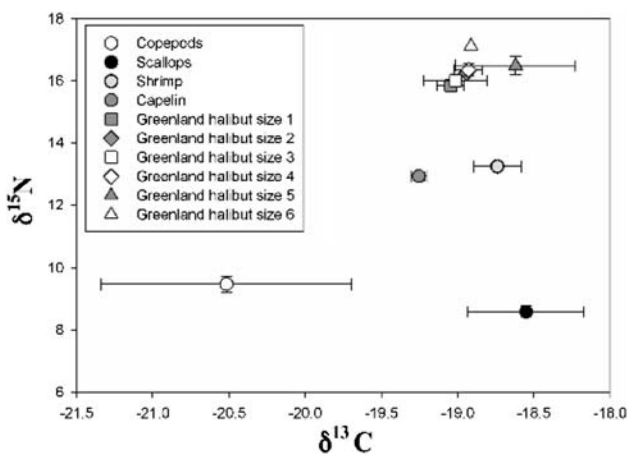

Fig. 1 Stable-nitrogen and stable-carbon isotope values (mean ± SE) of six size classes of Greenland halibut (see Table 1), likely prey species (capelin and shrimp) of the Greenland halibut and two baseline species from the Cumberland Sound ecosystem

size classes (Table 1). Only the largest size class, >75 cm and for which $n = 1$, had a different trophic position (TP = 4.3). Assessment of size in the GLM found length as a significant predictor of Greenland halibut δ^{15}N ($P = 0.0022$), but when a sex effect was also included in the GLM, neither effect was significant (sex $P = 0.9124$, length $P = 0.0893$). The mixing models IsoSource and MixSIR both identified capelin as the main component of the Greenland halibut's diet (Table 2).

Table 2 Model estimates of prey contributions to the diet of Greenland halibut, *Reinhardtius hippoglossoides*, as provided by IsoSource (median, 1st and 99th percentiles) and MixSIR (median, 5th and 95th percentiles) using isotopic discrimination factors 3.4 and 1.0‰ for δ^{15}N and δ^{13}C, respectively

Prey species	IsoSource	MixSIR
Shrimp	0.0 (0.0–0.01)	0.001 (0.0–0.002)
Capelin	0.99 (0.99–1.0)	0.999 (0.998–1.0)

Discussion

Our results suggest that identification of important prey species' habitats for cold water fish species can be estimated from stable isotopes in an unknown system since our results compare to diet assessments in other systems. In addition, trophic position can be determined with a small sample size by directing research according to stomach content analysis from other systems and using prior knowledge of feeding behavior (i.e. pelagic) of the species in question. Greenland halibut are primarily harvested in Cumberland Sound during winter and early spring months, and stomach content analysis has proven ineffective due to the high percentage of empty stomachs during these times of year. Attempts in this study to capture Greenland halibut from Cumberland Sound in summer/fall months were unsuccessful, indicating the usefulness and necessity of stable isotope data to elucidate the prey preferences of Greenland halibut in Cumberland Sound. Because the growth rate of cold water fish species is likely to be low, the turnover rate of stable isotopes in the muscle tissue of these species, and indication of a diet shift, will be slow and on the order of approximately 6 months (MacNeil et al. 2006).

Values of δ^{13}C across the sampled species from Cumberland Sound suggested two carbon sources, pelagic and benthic. Consistent with other Artic systems, differences between pelagic and benthic δ^{13}C in Cumberland Sound were tight (Hobson et al. 2002), and benthic organisms in this study were ^{13}C enriched (i.e. more negative δ^{13}C values relative to the pelagic species; Hobson and Welch 1992; Hobson et al. 1995, 2002). Consistent with known habitat and feeding preferences of these organisms, the invertebrates and fish increased on a gradient of $\delta^{13}C$ from pelagic habitat species (copepods, capelin; Templeman 1948; Hirche 1997) to epibenthic (shrimp; Birkely and Gullicksen 2003) to benthic habitat species (scallops; Wiborg 1963). Scallops were the most ^{13}C enriched, suggesting a primarily benthic carbon source. The Greenland halibut $\delta^{13}C$ values across all size classes grouped them with the offshore pelagic species. Despite being a flatfish, pelagic feeding behavior of Greenland halibut in Cumberland Sound is consistent with body morphology (de Groot 1970) and stomach content studies (Bowering and Lilly 1992; González et al. 2006). $\delta^{13}C$ values for Greenland halibut were more enriched than samples from 1999 from Cumberland Sound (Fisk et al. 2002), indicating a more benthic carbon source. Nonetheless, the mixing models, which were based on both $\delta^{13}C$ and $\delta^{15}N$, also indicated pelagic behavior by identifying capelin as the main component (relative to shrimp) in the Cumberland Sound Greenland halibut diet.

Relative values of $\delta^{15}N$ for the species in this study were also consistent with prior knowledge of their feeding ecology. Capelin are a foraging pelagic fish found throughout the Arctic and sub-Arctic zones of the Atlantic and Pacific oceans (Templeman 1948), and a relative trophic position of 3.0 is consistent with their position as secondary consumers (O'Driscoll et al. 2001). Greenland halibut across all sizes were a trophic position of 4.0, consistent with a known dietary preference in other ecosystems for capelin (TP = 3.0) or shrimp (TP = 3.1). In addition, the isotopic discrimination factor of 3.4 and 1‰ appears correct based on the $\delta^{15}N$ and $\delta^{13}C$ observed for the Greenland halibut of Cumberland Sound.

The mixing models identified capelin as the main prey item for the 50–85 cm Greenland halibut sampled in Cumberland Sound, also consistent with previous observations on diet with size: individuals 20–69 cm predominately consume small fish (e.g. herring, capelin). However, it is important to note that not all possible prey species were included in this studies, but rather common species representing pelagic fish and meso-benthic invertebrates. Other species (e.g. Arctic cod (*Boreogadus saida*); Bowering and Lilly 1992) share similar stable isotopes signatures to capelin/shrimp and could likely be important components of the Cumberland Sound Greenland halibut diet. The existence of other prey species at TP = 3 in the Greenland halibut diet is also supported by the fact that capelin represent a relatively new food source to the Greenland halibut of Cumberland Sound, having only been observed there in high numbers in recent years. Although MixSIR (which incorporates multiple sources of uncertainty; Moore and Semmens 2008) still emphasized capelin as important, the systems modeled were incredibly simplified with only two source items included. The addition of other species to the mixing models could reduce the success and/or agreement of the models.

The tendency of Greenland halibut to change diet with size as suggested by previous studies was reflected in the GLM results. Length was a significant predictor of $\delta^{15}N$ in the GLM, implying a change in diet with size. However, most Greenland halibut individuals in this study were 50–75 cm, and, according to other studies, such a range in size would share a similar diet. Thus, the significance of length was driven by the one individual >75 cm. Although only a sample size of 1, the $\delta^{15}N$ for the >75 cm individual is consistent with reported diet switches (e.g. we observed TP <75 cm = 3.9–4.1 and TP >75 cm = 4.3) at a certain size (Bowering and Lilly 1992).

The indication of pelagic feeding from the $\delta^{13}C$ values, the identification of capelin as the main prey item by the mixing models, and the implication by the GLM of transitions in diet with size are all characteristics observed in other studies of Greenland halibut. However, the diet of Greenland halibut can be extremely variable in prey preferences and seasonal diets, such that dietary assumptions cannot be made for Greenland halibut in an unstudied ecosystem. Nonetheless, the variability in diet preferences has been well-documented and can direct small-scale research in unstudied areas.

This study provides the starting point for more elaborate Cumberland Sound research on the local Greenland halibut feeding ecology by confirming pelagic feeding, establishing its relative trophic position, and specifying the importance of capelin as prey.

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