



Greenland Shark (*Somniosus microcephalus*) Stomach Contents and Stable Isotope Values Reveal an Ontogenetic Dietary Shift

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Current knowledge on the feeding ecology of the Greenland shark (*Somniosus microcephalus*), a potential top predator in arctic marine ecosystems, is based on small sample sizes as well as narrow size ranges of sharks. Therefore, potential size-related feeding patterns remain poorly documented. Using stomach content data ($N = 88$) and stable isotope values of white muscle tissue ($N = 40$), this study evaluates the diet of sharks ranging in size from 81 to 474 cm (total length). The importance of prey categories (“Fish,” “Mammal,” “Squid,” “Crustacean,” and “Other”) was evaluated based on the reconstructed prey biomass of the stomach contents. Stable isotope values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranged between -14.4 to -19.9‰ and 11.8 to 17.2‰ , respectively. The importance of each prey category was estimated by the Index of Relative Importance (IRI). Our findings suggest that the smallest Greenland sharks (<200 cm) feed on lower trophic level prey, predominantly squids. Larger sharks (>200 cm) mainly feed on higher trophic level prey such as seals, epibenthic and benthic fishes including gadoids (Gadidae), skates (Rajidae), righteye flounders (Pleuronectidae), lumpfish (Cyclopteridae), wolffish (Anarhichadidae), and redfish (Sebastidae). Redfish were, however, only found to be important in the largest sharks sampled (>400 cm). In addition to demonstrating ontogenetic shifts in their feeding preferences, this study supports that Greenland sharks are capable of active predation on fast swimming seals and large fishes.

Keywords: Greenland shark, diet, feeding ecology, stomach content, isotopes

INTRODUCTION

During the past 10 years, the Greenland shark (*Somniosus microcephalus*) has been subject to increased scientific attention due to its role as a long-lived, highly migratory, and relatively abundant top predator in arctic marine food webs (MacNeil et al., 2012; Campana et al., 2015; Nielsen et al., 2016). The Greenland shark is distributed across the Arctic and in the cold temperate

waters of the Atlantic and reaches more than 5 m in length. (Compagno, 1984; Mecklenburg et al., 2018). Although they are occasionally reported in shallow waters (<30 m, Harvey-Clark et al., 2005; Eriksen, 2011), Greenland sharks predominantly occur in deep waters (>200 m, Fisk et al., 2012; Campana et al., 2015), where they have been recorded as deep as 2,992 m near the mid-Atlantic Ridge (Porteiro et al., 2017). In Greenland waters, Greenland sharks are most often encountered at depths of 400–700 m (Nielsen et al., 2014).

Previous studies have investigated the diet and trophic interactions of Greenland sharks by analyzing stomach contents, stable isotopes, and fatty acids (Fisk et al., 2002; Yano et al., 2007; McMeans et al., 2010; Leclerc et al., 2012; McMeans et al., 2013; Nielsen et al., 2014). These studies are mostly based on relatively few individuals ($N < 50$) from 200 to 400 cm in length, with limited information on larger individuals. Ontogenetic dietary shifts have been documented for other large predatory sharks such as sevengill (*Notorynchus cepedianus*), white (*Carcharodon carcharias*), and tiger sharks (*Galeocerdo cuvier*) (Lowe et al., 1996; Ebert, 2002; Estrada et al., 2006), and preliminary data suggest that small Greenland sharks have a lower trophic position compared to larger sharks (Fisk et al., 2002). Dietary differences across Greenland shark size thus becomes relevant to investigate.

Size distribution of Greenland sharks appears to vary spatially. While sharks <400 cm seem to dominate in the Canadian Arctic, northern Greenland and the Svalbard Archipelago (Norway), larger sharks >400 cm seem to be more common off southern Greenland, Iceland, and Newfoundland (Nielsen et al., 2014; Campana et al., 2015; Lydersen et al., 2016; Devine et al., 2018).

This study documents the feeding ecology of the Greenland shark, investigated across a large size range, by analyzing stable isotopes and stomach contents. Specifically, we identified prey in stomach contents to the lowest possible taxonomical level, estimated their biomass, and calculated the relative importance of prey through ontogeny. Stable isotopes levels were used to determine whether prey composition was reflected in the ^{15}N signal. Elaborating on the feeding ecology of different sized Greenland shark, currently listed as Near Threatened in the IUCN Red List of Threatened Species and as Data Deficient in the Norwegian Red List (Henriksen and Hilmo, 2015; IUCN, 2018), will allow for a more complete understanding of the species' role as an important predator in arctic ecosystems.

MATERIALS AND METHODS

Sampling

Greenland sharks ($N = 78$) from Greenland waters were caught from 2013 to 2017 as part of the international collaboration project “Old and Cold – Biology of the Greenland shark.” Sampling was obtained by the Greenland Institute of Natural Resources (GINR) annual fish surveys (RV Pamiut, RV Sanna), the TUNU IV and V Expeditions (RV Helmer Hanssen) (Christiansen, 2012), the commercial trawler FV Sisimiut, and targeted Greenland shark expeditions with RV Porsild and RV Sanna. All sampling was carried out in accordance with laws, regulations and authorization from the Government of

Greenland (Ministry of Fisheries, Hunting and Agriculture, document number 565466, 935119, 20179208, C-17-129, C-15-17, and C-13-16). Overlap in both sampling area and season (West and East Greenland, inshore and offshore, May–September) allowed us to pool stomach content data with an additional 30 specimens caught in 2012 [reported separately in Nielsen et al. (2014)], increasing the total number of sharks included in this study to 108. All sharks were measured (total length, TL, cm) and the sex determined from the presence (males) or absence (females) of claspers. All shark lengths in this study are reported as TL. To evaluate possible size-related dietary differences, sharks were grouped into six “shark size groups” (TL bins); <200 cm, 201–250 cm, 251–300 cm, 301–350 cm, 351–400 cm, and >400 cm. As in previous studies, data for males and females were combined (see Yano et al., 2007; McMeans et al., 2010; Leclerc et al., 2012; Nielsen et al., 2014). Bin ranges were chosen as “juvenile” sharks of both sexes have previously been defined as those <200 cm (Hussey et al., 2015). In this study (and in general), sharks >400 cm are strictly females and further also potentially mature [cf. Yano et al. (2007) suggesting that males mature at ~300 cm and females mature at >400 cm]. Shark size groups between 200 and 400 cm are thus composed by both immature males and females and potentially mature males.

Stomach Contents

Each stomach was removed and contents examined following the procedure described in Nielsen et al. (2014), with each prey item counted, measured, weighed, and identified to the lowest possible taxonomical level. All prey items were grouped into five prey categories: “Fish,” “Mammal,” “Squid,” “Crustacean,” and “Other.” The “Other” category included birds and all invertebrates except squids and crustaceans. The remaining stomach contents were designated “Non-prey items” and encompassed small rocks/stones, fishing gear, macro algae, metal pieces, fragments of Porifera, small bivalves (<1 cm), and scavenging lysianassid amphipods. For all “Non-prey items” only frequency of occurrence was calculated.

Reconstructed Biomass

In an attempt to reflect the initial (undigested) quantity of prey consumed, a “reconstructed biomass” was calculated for all prey items (except mammals, see explanation below). For each fish prey item, the reconstructed biomass was calculated from species or genus-specific length-weight relationship acquired from FishBase (Froese and Pauly, 2018). Squid size (pen length) and reconstructed biomass were calculated from beak size according to Zumholz and Frandsen (2006). No attempt was made to reconstruct the initial biomass of any mammal prey items (Nielsen et al., 2014) as it would be unrealistic to assume that an entire large marine mammal, potentially weighing hundreds or thousands of kilograms, was consumed by the shark. Therefore, for calculation purposes, the reconstructed biomass for each mammal prey item was set to be the actual wet mass as this would conservatively reflect the initial quantity consumed. Whenever possible, body morphometrics and wet mass of intact cnidarians (sea anemones), molluscs (gastropods), crustaceans (amphipods and decapods), echinoderms (sea urchins, sea stars,

brittle stars, and sea cucumbers), and birds were used to estimate initial wet mass of digested specimens. The proportion of reconstructed biomass for each prey category (i.e., “Fish,” “Mammal,” “Squid,” “Crustacean,” and “Other”) was plotted as mean values (\pm SE) for each of the six shark size groups.

Index of Relative Importance

The index of relative importance (IRI) for prey items was calculated from the number of prey items (N), the reconstructed biomass of prey items (B) and the frequency of occurrence of prey items (F) expressed as proportions (%), as described in Nielsen et al. (2014). For shark size groups with significantly different ^{15}N levels (cf. size group <200 cm, see section “Results”) the %IRI was calculated for each prey type. %IRI was also calculated separately for each of the six shark size groups and presented as a summarized %IRI-score for prey categories (“Fish,” “Mammal,” “Squid,” “Crustacean,” and “Other”) as well as at family level within these categories.

Stable Isotopes

Stable isotope analysis of Greenland shark muscle tissue poses two challenges. Firstly, the muscle tissue is lipid rich which demands either chemical lipid extraction or mathematical correction to avoid bias from lipid carbon (Shipley et al., 2017). Secondly, shark muscle contains urea and trimethylamine N-oxide (TMAO), which tend to be depleted in ^{15}N relative to protein N (Carlisle et al., 2017; Shipley et al., 2017). In order to compare data with the three main studies that present Greenland shark isotope values (Fisk et al., 2002; Hansen et al., 2012; and McMeans et al., 2013), we did not extract the TMAO, but performed lipid extraction. Hence, the $\delta^{13}\text{C}$ values presented are from lipid extracted samples, whereas the $\delta^{15}\text{N}$ values are from non-lipid extracted samples, thereby circumventing both the effect of $\delta^{13}\text{C}$ depleted lipid on $\delta^{13}\text{C}$ values and the potential effects of lipid extraction on $\delta^{15}\text{N}$ values.

White muscle tissue (5–10 g) sampled dorsal to the vertebrae and anterior to the first dorsal fin, was dried at 60°C for at least 48 h and split in two aliquots. One sample was lipid extracted using 5 ml of a 2:1 chloroform/methanol mixture which was put into a sealed vial. The sample was allowed to extract for 24 h after which the supernatant was removed. This procedure was repeated three times. The vial and sample was then rinsed with another 5 ml of the chloroform/methanol mixture, and the rinsed sample was allowed to dry for 24 h in a fume hood. Both the lipid extracted and non-lipid extracted samples were pulverized in an agate mortar and 0.4–1 mg replicate samples of both aliquots were packed in tin capsules. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were standardized using a Gelatine A (Gel-A) standard with known isotopic values of $\delta^{15}\text{N} = 5.4\text{‰}$ and $\delta^{13}\text{C} = -21.8\text{‰}$. In order to correct for daily offsets and drift, two or three internal 0.2–0.7 mg Gel-A standards were assayed every nine or ten tissue samples.

All the samples were analyzed at Department of Bioscience, Center for Geomicrobiology, University of Aarhus, Aarhus, Denmark using comparable techniques to previous stable isotope analysis on Greenland sharks (e.g., Fisk et al., 2002). The samples were measured by means of Isotope Ratio Mass Spectrometry in combination with an Element Analyzer and an operational

interface (Thermo Electron Corporation Flash EA 1112 series and Thermo Scientific Delta V Plus Isotope Ratio MS). Results are expressed in a δ notation as the deviation from international standards in parts per thousand (‰) according to the formula:

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

where X is either ^{13}C or ^{15}N , R_{sample} is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ in the sample, and R_{standard} is the ratio for the given standards. Standards for $\delta^{15}\text{N}$ were calibrated against atmospheric air. Statistical analysis was carried out in the statistical computing program R (R Development Core Team, 2017). Analysis of variance and *post hoc* Tukey HSD test were used to evaluate the variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between shark size groups. The level of significance was $P < 0.05$.

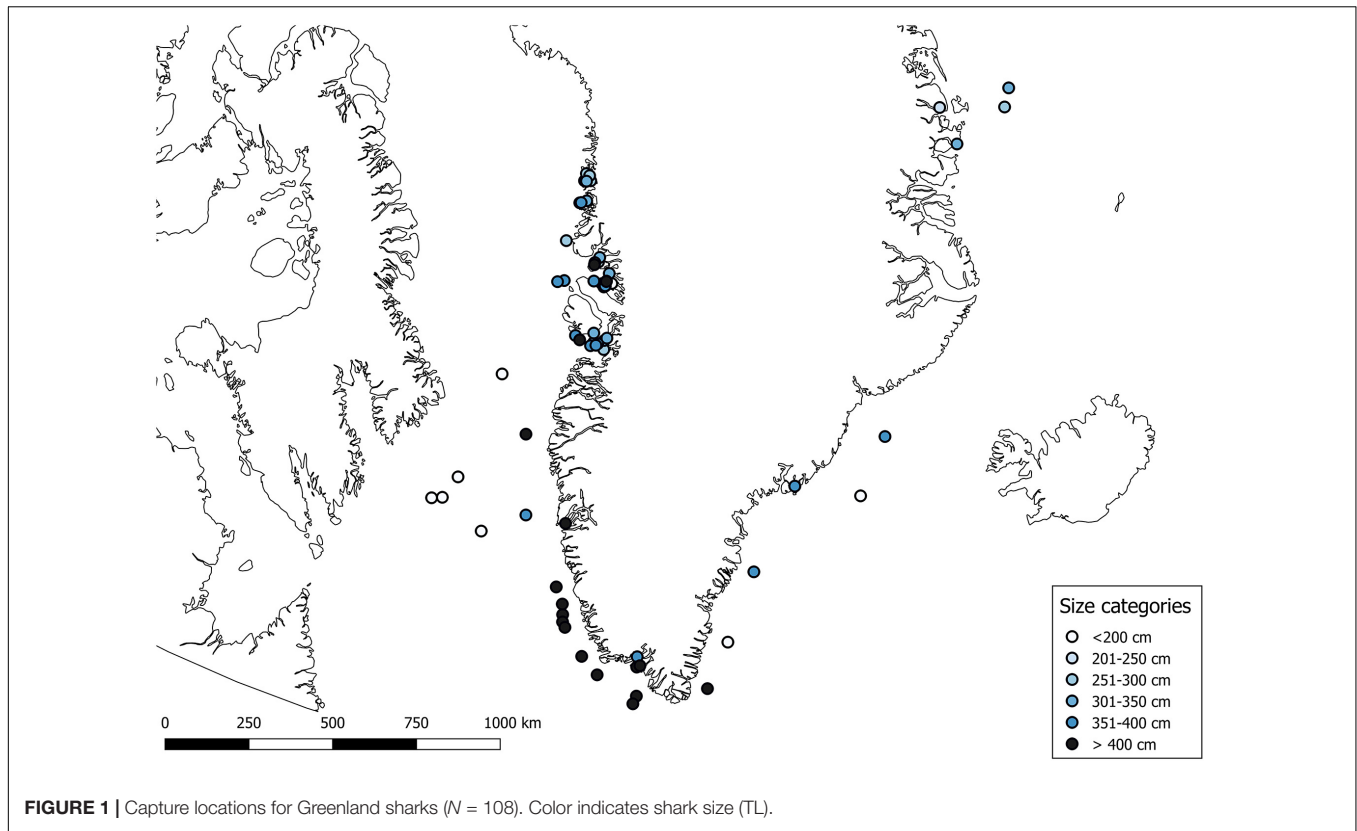
RESULTS

Sampling and Stomach Contents

A total of 108 Greenland sharks were sampled from May to September 2012–2017 in inshore and offshore waters (Figure 1). Males measured from 104 to 372 cm ($N = 27$) and females from 81 to 474 cm ($N = 79$) (Supplementary Table S1). Due to wounds inflicted by conspecifics during capture, the sex of two sharks could not be determined. Stomachs were available from 102 specimens. Since fourteen stomachs were empty, 88 stomachs from sharks ranging in size from 81 to 474 cm were included in the stomach content analysis (Table 1 and Supplementary Table S1). The total wet biomass of stomach contents was 462.3 kg and ranged between 0.029 and 52.0 kg for individual stomachs (mean \pm SD: 5.3 ± 9.1 kg). In total, 3.5 kg was categorized as “Non-prey items” and of the remaining 458.8 kg of stomach contents, 96.9 % was assigned to one of the following prey categories: “Fish,” “Mammal,” “Squid,” “Crustacean,” or “Other.” The remaining 3.1% was made up by non-identifiable digested biological material. Of the 88 stomachs analyzed, a total of 697 prey items were identified representing 57 different prey types (Table 2).

Reconstructed Prey Biomass and Index of Relative Importance

The total reconstructed biomass was 635.3 kg, adding an additional 176.5 kg to the observed biomass. The reconstructed biomasses for prey categories showed that “Squid” dominated the diet for Greenland sharks <200 cm, whereas “Fish” and “Mammal” were of major importance for all larger size groups (Figure 2). For these (i.e., sharks >200 cm), “Fish” constituted approximately 70% of the reconstructed biomass, and “Mammal” became gradually more prevalent with body length, increasing from 10 to 20% (Figure 2). For sharks <200 cm, armhook squid (*Gonatus* spp.) was present in all stomachs as the most dominant prey item (%IRI = 93.7, Table 2). For sharks >200 cm, Atlantic cod (*Gadus morhua*) (%IRI = 26.6) and unknown teleost (%IRI = 17.6) were the main prey followed by Greenland halibut (*Reinhardtius*



hippoglossoides), skates (Rajidae), lumpfish (*Cyclopterus lumpus*), harp seal (*Pagophilus groenlandicus*), armhook squid, and spotted wolffish (*Anarhichas minor*) with %IRIs ranging from 3.2 to 7.9 (Table 2).

The summarized %IRI for each prey category is presented in Table 3 for the six shark size groups. Common to the four largest shark size groups (from 251 to 300 cm and larger), was that gadoids (dominated by Atlantic cod), Unknown teleost, and seal (dominated by harp seal) had a %IRI > 5 (Table 3). In contrast, righteye flounders (dominated by Greenland halibut) only had a %IRI > 5 for the 251–300 cm and 301–351 cm shark size groups (%IRI of 29.8 and 10.7, respectively), whereas righteye flounders had a %IRI < 2.5 for the two largest sizes classes (Table 3). Redfish (Sebastidae) were only important in the largest sharks >400 cm (%IRI > 5). Skates were of least importance in the largest sharks >400 cm (%IRI = 2.0), compared to sharks between 201 and 400 cm (%IRI range between 10.3 to 19.8, Table 3).

Stable Isotopes

Samples for stable isotope analysis were available from 40 sharks measuring between 81 and 474 cm (Supplementary Table S1, Table 1). White muscle $\delta^{13}\text{C}$ values ranged from -14.4 to -19.9‰ and $\delta^{15}\text{N}$ between 11.8 and 17.2‰ (Supplementary Table S1, Figure 3). While there was no significant difference in $\delta^{13}\text{C}$ values among size groups (ANOVA, $F_{4,34} = 0.86$, $P = 0.5$), $\delta^{15}\text{N}$ values differed significantly (ANOVA, $F_{4,34} = 5.8$, $P < 0.05$, Figure 4). Sharks <200 cm had significantly lower $\delta^{15}\text{N}$ values compared

to the three largest size groups (*post hoc* Tukey HSD test $P < 0.05$) whereas the four largest size groups (251–300 cm, 301–350 cm, 351–400 cm, >400 cm) were not statistically different (Figure 4). As the $\delta^{15}\text{N}$ value for the 201–250 cm size group was based on only one individual, it was not included in the ANOVA.

DISCUSSION

This study investigated the ontogenetic shift of the diet of Greenland sharks and found that the smallest sharks (<200 cm) generally fed at a lower trophic level compared to larger sharks, both in terms of prey items and isotopic $\delta^{15}\text{N}$ levels. This finding

TABLE 1 | Overview of number of sharks for each analysis (stomach contents and stable isotopes) in each of the six size bins.

Size bin	Sample size, N	
	Stomach contents	Stable isotopes
<200	8	8
201–250	2	1
251–300	17	5
301–350	25	12
351–400	18	6
>400	18	8
Total	88	40

TABLE 2 | %F = frequency of occurrence, %N = number of prey items, %B = reconstructed biomass, and %IRI = Index of relative importance, for sharks <200 cm (*N* = 8) and >200 cm (*N* = 80).

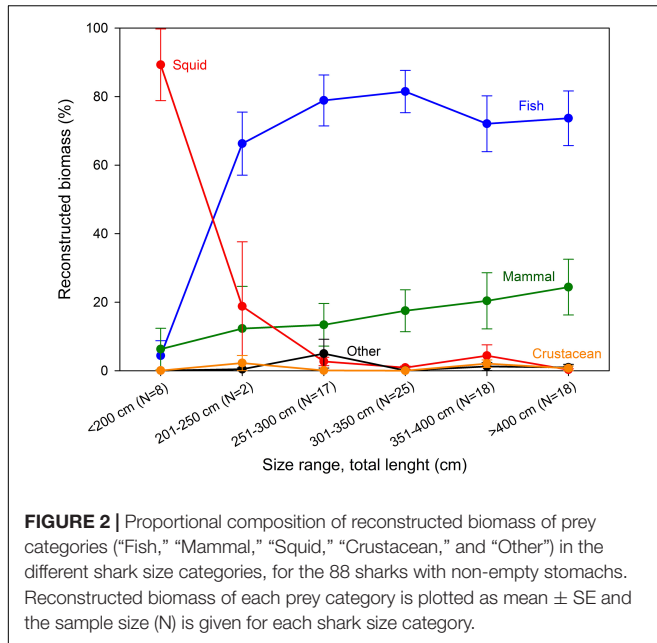
	Family	Common name	% F		% N		% B		% IRI	
			<200 cm	>200 cm	<200 cm	>200 cm	<200 cm	>200 cm	<200 cm	>200 cm
<u>Fish</u>										
<i>Gadus morhua</i>	Gadidae	Gadooids		26.3		20.0		19.2		26.6
<i>Gadus</i> spp.	Gadidae	Gadooids		18.8		4.3		4.3		4.2
<i>Micromesistius poutassou</i>	Gadidae	Gadooids		2.5		3.8		0.7		0.3
<i>Boreogadus saida</i>	Gadidae	Gadooids		2.5		2.1		0.0		0.1
<i>Gadus ogac</i>	Gadidae	Gadooids		1.3		0.3		0.4		<0.1
Unknown teleost				50.0		9.9		3.7		17.6
Rajidae	Rajidae	Skates		36.3		5.8		3.3		8.5
<i>Amblyraja radiata</i>	Rajidae	Skates		8.8		1.7		1.5		0.7
<i>Amblyraja hyperborea</i>	Rajidae	Skates		7.5		0.9		1.5		0.5
Rajidae, egg case	Rajidae	Skates		8.8		1.1		0.0		0.3
<i>Reinhardtius hippoglossoides</i>	Pleuronectidae	Righteye flounders		21.3		6.0		7.0		7.1
<i>Hippoglossoides platessoides</i>	Pleuronectidae	Righteye flounders		2.5		0.8		0.1		0.1
Unknown flounder	Pleuronectidae	Righteye flounders	12.5	1.3	2.2	0.9	0.7	0.2	0.3	<0.1
<i>Cyclopterus lumpus</i>	Cyclopteridae	Lumpfish		22.5		4.4		7.4		6.9
<i>Anarhichas minor</i>	Anarhichadidae	Wolffish		13.8		2.7		6.2		3.2
<i>Anarhichas lupus</i>	Anarhichadidae	Wolffish	12.5	3.8	2.2	0.9	1.9	1.0	0.4	0.2
<i>Anarhichas denticulatus</i>	Anarhichadidae	Wolffish		2.5		0.3		1.8		0.1
<i>Anarhichas</i> spp.	Anarhichadidae	Wolffish		6.3		0.9		0.6		0.3
<i>Sebastes mentella</i>	Sebastidae	Redfish		7.5		2.7		1.6		0.8
<i>Sebastes marinus</i>	Sebastidae	Redfish		1.3		0.9		0.2		<0.1
<i>Sebastes</i> spp.	Sebastidae	Redfish		5.0		0.9		0.2		0.2
<i>Lycodes</i> spp.	Zoarcidae	Eelpouts		8.8		2.1		0.4		0.5
<i>Somniosus microcephalus</i>	Somniosidae	Sleeper shark		1.3		0.2		2.4		0.1
<i>Cottunculus sadko</i>	Psychrolutidae	Fatheads		2.5		0.3		0.1		<0.1
<i>Cottunculus</i> spp.	Psychrolutidae	Fatheads		1.3		0.2		0.1		<0.1
<i>Myoxocephalus scorpius</i>	Cottidae	Sculpins		2.5		0.3		0.1		<0.1
Unknown salmonid	Salmonidae	Salmonids		1.3		0.2		0.3		<0.1
<i>Argentina silus</i>	Argentidae	Argentine		1.3		0.2		0.0		<0.1
<i>Scomber scombrus</i>	Scombridae	Scombrids		1.3		0.2		0.0		<0.1
<i>Macrourus berglax</i>	Macrouridae	Grenadier		1.3		0.2		0.2		<0.1
<i>Myxine glutinosa</i>	Myxinidae	Hagfish		1.3		0.3		0.0		<0.1
<u>Mammal</u>										
<i>Pagophilus groenlandicus</i>	Pinnipeds	Seal		16.3		2.2		14.4		7.0
Unknown seal	Pinnipeds	Seal	25.0	25.0	4.4	3.3	0.1	9.1	0.8	5.9
<i>Erignathus barbatus</i>	Pinnipeds	Seal	12.5	3.8	2.2	0.5	3.7	2.0	0.5	0.2
<i>Pusa hispida</i>	Pinnipeds	Seal		3.8		0.5		1.5		0.2
<i>Cystophora cristata</i>	Pinnipeds	Seal		1.3		0.2		0		<0.1
Unknown whale (blubber)	Cetacea	Whale		2.5		0.3		6.3		0.4
<i>Ursus maritimus</i>	Ursidae	Bear		1.3		0.2		0.1		<0.1
<u>Squid</u>										
<i>Gonatus</i> spp.	Gonatidae	Armhook squid	100	22.5	80.0	10.4	53.3	1.2	93.7	6.8
<i>Histioteuthis</i> spp.	Histioteuthidae		12.5		4.4		40.3		3.9	

(Continued)

TABLE 2 | Continued

	Family	Common name	% F		% N		% B		% IRI	
			<200 cm	>200 cm	<200 cm	>200 cm	<200 cm	>200 cm	<200 cm	>200 cm
<u>Crustacean</u>										
Infraorder										
<i>Eurythenes gryllus</i>	Lysianassida	Amphipod		2.5		2.8		0.0		0.2
<i>Hyas</i> spp.	Brachyura	Crab		10.0		2.4		0.1		0.6
<i>Chionoecetes opilio</i>	Brachyura	Crab		2.5		0.3		0.1		<0.01
<i>Lithodes maja</i>	Brachyura	Crab		1.3		0.2		0.0		<0.01
<i>Pandalus borealis</i>	Caridea	Shrimp		1.3		0.2		0.0		<0.01
<i>Pandalus</i> spp.	Caridea	Shrimp	12.5	1.3	2.2	0.2	<0.1	0.0	0.2	<0.01
<i>Sclerocrangon boreas</i>	Caridea	Shrimp		1.3		0.2		0.0		<0.01
<i>Pasiphaea</i> spp.	Caridea	Shrimp		1.3		0.5		0.0		<0.01
<i>Colossendeis proboscida</i>	Colossendeidae	Marine sea spider	12.5		2.2		<0.1		0.2	
<u>Other</u>										
Unknown auk		Auk		2.5		0.3		0.2		<0.01
Ophiuroidea		Brittle star		8.8		0.1		0.1		<0.01
Holothuroidea		Sea cucumber		2.5		0.3		6.3		<0.01
Arctinaria		Sea anemone		1.3		0.1		2.0		<0.01
Echinoidea		Sea urchin		1.3		0.2		1.5		<0.01
Solasteridae		Sun star		1.3		0.2		0.2		<0.01
Asteroidea		Starfish		3.8		0.2		0.2		<0.01
Gastropoda		Snail		17.5		0.1		0.0		<0.01

Non-prey items (%F): <200 cm: Amphipods 25; >200 cm: Macro algaea 11.3, Bivalvia 5.0, Porifera 3.8, amphipod 26.3, rock 21.3, fishing equipment 8.8, metal 2.5 For sharks <200 cm, reconstructed biomass is 10.8 kg for 45 prey items. For sharks >200 cm, reconstructed biomass is 624.5 kg for 652 prey items.



mirrors observations by Fisk et al. (2002), who reported lower $\delta^{15}\text{N}$ values in two 135 cm sharks compared to fifteen specimens >250 cm. We also observed that the stomach contents of the <200 cm sharks were mainly armhook squid and the $\delta^{15}\text{N}$ values were similar to that of other squid-feeding predators of

the North Atlantic (Hooker et al., 2001; Mendes et al., 2007). The $\delta^{15}\text{N}$ values of larger sharks >200 cm indicated a diet consisting of higher trophic level prey such as large piscivorous fishes and seals corresponding with stomach content results for these size classes, which primarily consisted of Atlantic cod, Greenland halibut and seals (see stable isotope values for Greenlandic marine food webs in Hansen et al., 2012). Taken together, the stomach contents and $\delta^{15}\text{N}$ analyses suggests an ontogenetic shift from small (<200 cm), primarily squid-eating sharks, to larger (>200 cm), fish and mammal-eating sharks. However, sample size was limited for sharks from 201 to 250 cm, making it challenging to determine at which exact size the ontogenetic shift occurs.

When investigating the feeding ecology of a species, it is important to combine data of stomach contents and stable isotopes because stable isotopes provide an integrated and long-term estimate of trophic position that cannot be inferred from stomach content (Hobson and Welch, 1992; Christiansen et al., 2012). Although, stomach content analysis enables prey species identification and provides a detailed snapshot of recently ingested prey, regurgitation during capture and varying rates of digestion can skew the stomach content results leading to spurious conclusions, especially when sample size is small (Wetherbee et al., 2012). In this study, armhook squid was the single most important prey for the smallest sharks (<200 cm, %IRI = 97.6, Table 2), and was also relatively important in sharks >200 cm (%IRI = 6.8, Table 2), and more specifically, for 351–400 cm sharks (%IRI = 27.1, Table 3). For the

TABLE 3 | %IRI calculated for each prey category and for prey families for each shark size category.

	<200, N = 8		201–250, N = 2		251–300 cm, N = 17		301–350 cm, N = 25		351–400 cm, N = 18		>400 cm, N = 18	
Recons. biomass	10.8 kg	Recons. biomass	2.9 kg	Recons. biomass	83.3 kg	Recons. biomass	153.0 kg	Recons. biomass	96.2 kg	Recons. biomass	289.1 kg	
No. prey items	45	No. prey items	41	No. prey items	109	No. prey items	137	No. prey items	127	No. prey items	238	
	%IRI		%IRI		%IRI		%IRI		%IRI		%IRI	
Fish (%IRI = 0.7)		Fish (%IRI = 59.6)		Fish (%IRI = 79.8)		Fish (%IRI = 88.4)		Fish (%IRI = 56.0)		Fish (%IRI = 81.9)		
Righteye flounders	0.3	Unidentified teleost	20.6	Righteye flounders	29.8	Skates	19.8	Cod	16.7	Cod	56.8	
Wolffish	0.4	Sculpin	2.7	Unidentified teleost	21.5	Unidentified teleost	17.8	Unidentified teleost	9.4	Unidentified teleost	9.6	
		Eelpout	6.5	Cod	14.8	Lumpfish	14.2	Wolffish	8.6	Redfish	5.6	
		Cod	17.1	Skates	10.3	Cod	12.4	Skates	8.4	Lumpfish	3.3	
		Skate	12.7	Wolffish	1.9	Wolffish	12.1	Lumpfish	6.5	Wolffish	3.8	
				Lumpfish	1.1	Righteye flounder	10.7	Sleeper shark	2.5	Skates	2.0	
				Fatheads	0.2	Eelpouts	0.9	Righteye flounders	2.3	Righteye flounder	0.6	
				Eelpouts	0.1	Redfish	0.2	Eelpouts	0.9	Salmon	0.1	
				Redfish	0.1	Fatheads	0.2	Redfish	0.5	Argentine	<0.1	
						Sculpins	0.1	Hagfish	0.2	Grenadier	<0.1	
										Mackerel	<0.1	
Mammal (%IRI = 1.3)		Mammal (%IRI = 7.6)		Mammal (%IRI = 10.4)		Mammal (%IRI = 8.3)		Mammal (%IRI = 14.6)		Mammal (%IRI = 17.1)		
Seal	1.3	Seal	7.6	Seal	10.2	Seal	8.1	Seal	14.6	Seal	16.7	
				Bear	0.2	Whale	0.2	Whale	0.4	Whale	0.4	
Squid (%IRI = 97.6)		Squid (%IRI = 11.8)		Squid (%IRI = 8.6)		Squid (%IRI = 2.8)		Squid (%IRI = 27.1)		Squid (%IRI = 0.1)		
Armhook squid	93.7	Armhook squid	11.8	Armhook squid	8.6	Armhook squid	2.8	Armhook squid	27.1	Armhook squid	<0.1	
Cock-eyed squid	3.9											
Crustacean (%IRI = 0.4)		Crustacean (%IRI = 14.7)		Crustacean (%IRI = 0.9)		Crustacean (%IRI = 0.6)		Crustacean (%IRI = 2.2)		Crustacean (%IRI = 0.9)		
Caridae	0.2	<i>Eurythmes gryllis</i>	14.7	Large amphipod	0.8	Crab	0.3	Crab	1.8	Crab	0.9	
Colossendeidae	0.2			Crab	0.1	Shrimp	0.3	Shrimp	0.4	Shrimp	<0.1	
Other (%IRI = 0)		Other (%IRI = 6.3)		Other (%IRI = 0.2)		Other (%IRI = 0)		Other (%IRI = 0)		Other (%IRI = 0.1)		
		Brittle star	3.8	Bird	0.2					Bird	<0.1	
		Snail	2.5									

(Continued)

TABLE 3 | Continued

<200, N = 8	201–250, N = 2	251–300 cm, N = 17	301–350 cm, N = 25	351–400 cm, N = 18	>400 cm, N = 18
Main prey items (%IRI > 5) Armhook squid	Main prey items (%IRI > 5) Unidentified teleost, polar cod, <i>Eurythres</i> grylls, armhook squid, Arctic skate, harp seal, Eelpout	Main prey items (%IRI > 5) Unidentified teleost, greenland halibut, Atlantic cod, skates, armhook squid, and harp seal	Main prey items (%IRI > 5) Unidentified teleost, lumpfish, spotted wolffish, skate, greenland halibut, Atlantic cod, harp seal	Main prey items (%IRI > 5) Armhook squid, Atlantic cod, unidentified teleost, spotted wolffish, skate, lumpfish, harp seal	Main prey items (%IRI > 5) Atlantic cod, unidentified teleosts, beaked redfish, harp seal

Reconstructed biomass and number of prey items within each shark size category is presented together with the most important individual prey groups.

reasons described below, we are not convinced that IRI-levels accurately reflect the importance of squids for larger sharks >200 cm. Firstly, one Greenland shark of 351 cm (GS17, **Table 1**) contained 32 squid beaks making this single observation accountable for 29% of all armhook squid observations in sharks >200 cm. Secondly, the contribution of the reconstructed biomass to the squid IRI-score is minimal but driven by a high frequency of occurrence (F) and numerical abundance (N) which is different from sharks <200 cm where all IRI parameters (F, N, and B) were high for squids. Thirdly, squids were much smaller (pen length mean \pm SD: 16.8 \pm 6.88 cm, N = 88) than prey items of >200 cm Greenland sharks typically measuring 21.3–68.8 cm in length (Nielsen et al., 2014). Lastly, the vast majority of squid observations were based on hard-to-digest chitin beaks, which for large sharks, especially, are prone to be derived from secondary stomach contents of large fully swallowed and digested prey like seal, Greenland halibut and Atlantic cod (a “matrijoska effect”). Based on the arguments outlined above, we believe that the importance of squids for sharks >200 cm is overestimated by our IRI calculations. In contrast, however, we believe that squids are very important for <200 cm sharks as indicated by their numerical abundance, frequency of occurrence, reconstructed biomass, and by $\delta^{15}\text{N}$ values. The prominence of Atlantic cod (%IRI = 26.6) in the diet of sharks >200 cm was to some extent, also driven by observations from only few sharks, with 69% of all cod observations (87 of 127 specimens) being present in only two stomachs. However, Atlantic cod were recorded in 26% of all stomachs and are (in contrast to squids) very unlikely to be consumed secondarily. Thus the generally high importance of Atlantic cod seems a reasonable conclusion, which together with skates, seals, Greenland halibut, lumpfish, and wolffish are the most important prey for sharks >200 cm.

Our findings also show that prey fishes differ between shark size groups. For example, righteye flounder (mainly Greenland halibut, **Table 3**) were among the most important prey for sharks between 251 and 350 cm, but of limited importance to larger sharks (i.e., 351–400 and >400 cm). Furthermore, despite redfish being abundant across the Greenland continental shelf (ICES, 2017), they were only an important part of the diet among the largest sharks (>400 cm). Females this size from Iceland shelf waters have also been reported to feed predominantly on redfish (McMeans et al., 2010) suggesting some degree of prey selectivity. For sharks >400 cm, the scarcity or complete lack of deep sea fishes such as Greenland halibut, grenadiers and slickheads (Alepocephalidae) in the stomachs is noteworthy and is supported by depth records from a recent tagging study in Greenland waters (Nielsen, 2018), which found that >400 cm females do not spend much time at depths >800 m. Instead, females this size seem to mainly occupy and forage on the continental shelf and the upper part of the continental slope.

The apparent shift in diet between small and large sharks was corroborated by significantly lower $\delta^{15}\text{N}$ values in sharks of <200 cm compared to sharks >200 cm. As noted in the Section “Materials and Methods” we did not attempt to extract

TMAO and urea from the samples, though these compounds have been shown to lower the $\delta^{15}\text{N}$ values to a variable extent (Carlisle et al., 2017). As we primarily used the isotopic values to compare between shark size groups, we believe any TMAO and urea produced bias in $\delta^{15}\text{N}$ would be expected to have a similar effect across size groups. Hansen et al. (2012) analyzed Greenland shark samples from the same areas around Greenland (lengths 270 to 473 cm) and found average $\delta^{15}\text{N}$ values of 16.7‰ which is similar to our values. Greenland sharks from Svalbard (lengths 245 to 404 cm) were reported

by McMeans et al. (2013) to have average values of 15.9 (‰) ($\delta^{15}\text{N}$) and -18.6 (‰) ($\delta^{13}\text{C}$), which are also very similar to the ones reported here. In contrast, the $\delta^{13}\text{C}$ values found by Hansen et al. (2012) were approximately 2‰ higher (-15.6 ‰ compared to -17.6 ‰ in our study). While the reason for the discrepancy between the two values is not clear, it could be due to varying isotopic baselines in different areas of Greenland

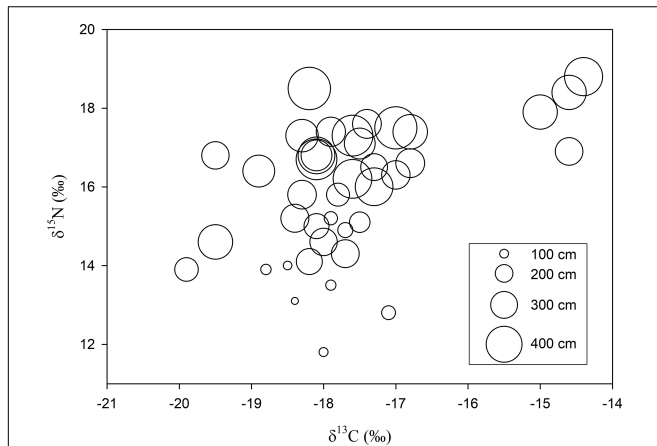


FIGURE 3 | Bi-plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the 40 sharks ranging in size from 81 to 474 cm. The diameter of circles are proportional to the TL (cm) of the individual shark.

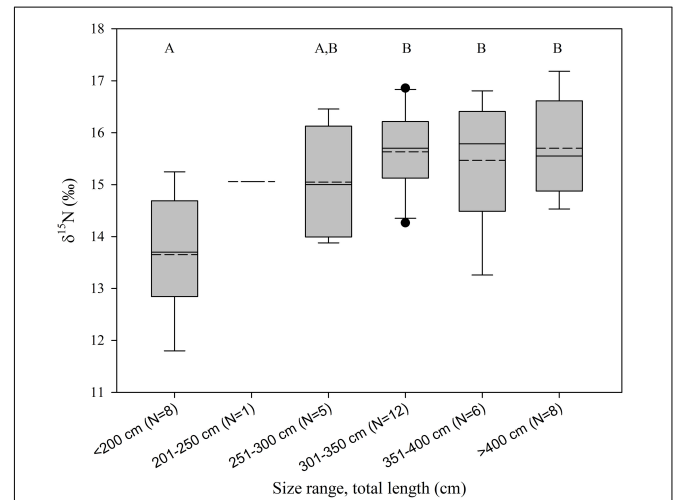


FIGURE 4 | Boxplot [10th percentile, first quartile, median (dashed line), mean (full line) third quartile, and 90th percentile, dots represent outliers] of $\delta^{15}\text{N}$ plotted against shark size categories. Different letters show significant differences between size categories.



FIGURE 5 | (a) The characteristic circular gape shape of a Greenland shark (Photo: J. Nielsen). (b) Harp seals of 14 kg (top) and 19 kg (bottom) found in stomach of a 470 cm Greenland shark (Photo: J. Nielsen). (c) Circular bite marks on a freely swimming beluga whale (from MacNeil et al., 2012). (d) Seal chunks of skin, blubber, meat and ribs from shark stomach (Photo: J. Nielsen).

(Hansen et al., 2012), as well as differences in the average size of the sharks examined in the different studies. Fisk et al. (2002) found $\delta^{15}\text{N}$ values similar to this study, but had more depleted $\delta^{13}\text{C}$ values, which may be attributed to their use of toluene as solvent to remove lipids.

For decades, scientists have noted the discrepancy between the lethargic appearance of the Greenland shark and the potentially faster moving prey species found in their stomachs (Jensen, 1914; Bigelow and Schroeder, 1948; Hansen, 1963; Watanabe et al., 2012) which has raised the question as to whether Greenland sharks are capable of active hunting or mainly feed as scavengers (Leclerc et al., 2012; Nielsen et al., 2014; Edwards et al., in press). Greenland shark scavenging events have been documented (Leclerc et al., 2011) but observations made in our study provide supporting evidence that Greenland sharks also are capable of active predation on fast swimming seals and large epibenthic fishes such as Atlantic cod and Greenland halibut. For example, we have observed circular bite wounds on large prey (Figures 5a,d) matching wounds observed on free swimming marine mammals (Figure 5c, Idrobo and Berkes, 2012 and MacNeil et al., 2012). Furthermore, two intact and freshly ingested seals were found without any scavenging fauna common for carcasses (Figure 5b) suggesting that the seals had been swallowed during a recent hunting event (Leclerc et al., 2012). How Greenland shark catch fast swimming prey such as seals remains to be resolved (Edwards et al., in press), but it has been suggested that predation occurs on seals sleeping in the water column (Leclerc et al., 2012).

In conclusion, Greenland shark display a marked ontogenetic shift in trophic relationships from a squid diet in small sharks (<200 cm) to a diet dominated by fishes and seals in larger sharks (>200 cm). As the diet reflects available prey for given geographic areas and depths, how may we define Greenland shark in terms of feeding behavior? The squid diet for juvenile Greenland sharks is interesting and suggests a specialized feeding behavior where younger animals actively select squid, whereas larger animals appear generalists mainly feeding on demersal fishes and seals in addition to a minor proportion of benthic invertebrates. Clearly better information on the distribution, behavior and environments occupied by Greenland shark of

different size and age is needed to clarify the understanding of the trophic relationships for this migratory and long-lived species.

AUTHOR CONTRIBUTIONS

JN coordinated this work. JN, HK, PB, KP, and JS collected the stomachs. JN and RH analyzed the stomach contents. HK, PG, and RH analyzed the stable isotopes. JN, JC, and RH designed the project and wrote the manuscript. All authors revised, reviewed, and finally approved the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2019.00125/full#supplementary-material>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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