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DNA Metabarcoding Improves the Taxonomic Resolution of Visually Determined Diet Composition of Beaked Redfish (*Sebastes* sp.)

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ABSTRACT

Beaked redfishes (*Sebastes fasciatus* and *Sebastes mentella*) of the northwest Atlantic have recently reached record abundance levels in the estuary and northern Gulf of St. Lawrence, dominated by *Sebastes mentella*. Knowledge of their diet composition is essential to understand the trophic role that these groundfish play in the ecosystem. The objective of the present study was to compare the performance of visual examination and DNA metabarcoding of stomach contents of the same individual redfish caught in the estuary and northern Gulf of St. Lawrence. Using a universal metazoan mitochondrial cytochrome c oxidase subunit I (COI) marker, we identified a total of 24 taxonomic groups, composed of 22 species and two genera in the content of 185 stomachs with DNA metabarcoding. We compared these results to the 25 prey types, eight identified at the genus and nine at the species level, obtained with visual stomach content analysis (SCA). While both techniques revealed a similar diet composition, our results showed that the SCA and DNA metabarcoding perform differently for particular prey categories, both in terms of detectability and taxonomic resolution, as well as in the estimated relative importance of weight and occurrence in the diet. The use of DNA metabarcoding along with SCA validates and improves the taxonomic resolution of visually determined prey, which supports the concept that both techniques provide useful complementary information on the diet of redfish and likely other fish species.

1 | Introduction

Understanding how the feeding habits of specific species can affect whole communities is fundamental to characterize the dynamics of food webs and the functioning of ecosystems (e.g., Cohen et al. 1993; Ings et al. 2009; Arditi and Ginzburg 2012). Documenting the dietary preferences of abundant species within a given ecosystem is important to support an in-depth

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†Deceased.

understanding of its functioning, including valuable ecological information on trophic structure, predation and competition relationships, energy flow, habitat use, as well as temporal variability of these features. Knowledge of trophodynamics is thus essential in developing robust ecosystem approaches to species management (e.g., Duffy et al. 2007; Arditi and Ginzburg 2012; Long, Charles, and Stephenson 2015) such as, for example, anticipating bottom-up or top-down effects on the population dynamics of marine predators or identifying predators that could be sensitive to the loss of key prey availability (e.g., Spitz, Ridoux, and Brind'Amour 2014; Brown-Vuillemin et al. 2022).

The classic approach used to study predator diet composition consists of visually inspecting digestive tract (usually stomach) contents and identifying prey taxa at the highest taxonomic resolution possible, depending on the condition of ingested prey and the experience of an examiner. Stomach content analysis (SCA) provides an estimation of the contribution of the different prey taxa to the diet and an assessment of how diet composition varies in space and time or according to intrinsic factors such as predator size. A challenge with SCA, even for well-trained taxonomists, is that examined contents may be in various states of digestion, with the most digested prey being difficult to identify, especially if soft-bodied and lacking distinctive hard parts. Biased identification caused by advanced digestion state can distort diet composition, leading to over- or under-representation of certain prey taxa, by assigning prey to certain species when others are possible, or by generalizing to broad groups when specific kinds are present, especially for digested crustaceans and fishes (e.g., Hyslop 1980; Baker, Buckland, and Sheaves 2014; Amundsen and Sánchez-Hernández 2019).

Another approach for obtaining information on diet composition is DNA metabarcoding, which uses high-throughput sequencing (HTS) to produce DNA-based species identification. This method enables the identification of prey by using universal PCR primers that simultaneously amplify the DNA of diverse groups of taxa from a mixed sample (stomach content or feces), even in predators characterized by a diverse diet (e.g., Symondson 2002; Pompanon et al. 2012). This technique is increasingly being used to complement classic SCA and is helpful in refining taxonomic resolution in morphologically similar taxa (e.g., Dunn et al. 2010; Carreon-Martinez et al. 2011; Côté et al. 2013; Oyafuso, Toonen, and Franklin 2016; Jakubavičiūtė et al. 2017; Aguilar et al. 2017; Dahl et al. 2017; Riccioni et al. 2018; Coker et al. 2023; Roy and Boulding 2024; Vasiliadis et al. 2024). However, using DNA metabarcoding on stomach contents may be limited in some cases by the DNA degradation caused by digestion (Symondson 2002; Jakubavičiūtė et al. 2017), the detection probabilities, which can vary by species (Olds et al. 2016), the false detection of prey species from environmental DNA, and the possible absence of validated reference sequences for some prey taxa in public databases (Jakubavičiūtė et al. 2017; Sakaguchi et al. 2017), resulting in imprecise identification of prey taxa. Although the choice and design of primer pairs is critical for the success of DNA metabarcoding projects, any given pair of primers might not perfectly match the DNA sequence of all prey species or have enough resolution to discriminate some groups of closely related species (e.g., Ekrem, Willassen, and Stur 2007; Piñol, Senar, and Symondson 2018; Corse et al. 2019). Another limitation of DNA metabarcoding is the qualitative nature of this technique, relative to the quantitative SCA. Several factors may obscure the quantitative estimates, such as differential DNA extraction success and survival rates of prey tissue during digestion, variation in cell and DNA quantity in different tissues, or the use of different pipelines and data processing procedures (e.g., Pompanon et al. 2012; Lamb et al. 2018; Riccioni et al. 2018; Cuff et al. 2021; Stapleton et al. 2022). The combination of DNA metabarcoding and SCA can offer significant advantages over the use of either method alone. These approaches enable the detection of a wider range of prey taxa and can complement each other, enhancing the overall resolution and reliability of diet studies to improve our understanding of food webs and trophic dynamics in ecosystems.

Following strong recruitment events during the period 2011–2013, the previously collapsed populations of beaked redfishes, composed of two sympatric species, the Acadian redfish *Sebastes fasciatus* (Storer 1854) and the deepwater redfish *Sebastes mentella* (Travin 1951), reached record high levels (principally for *S. mentella*) in the estuary and northern Gulf of St. Lawrence (ENGSL), accounting for more than 80% of the total biomass sampled by Fisheries and Oceans Canada (DFO) on the multispecies trawl survey in recent years (Senay et al. 2021, 2023; DFO 2022). While this exceptionally high biomass level is of potential interest for commercial fishing activities in the coming years, it is also anticipated to have significant implications for functioning of the ENGSL ecosystem. This includes increased predation pressure on forage species, such as the pink glass shrimp (*Pasiphaea multidentata*) and northern shrimp (*Pandalus borealis*), enhanced interspecific competition with other groundfish species, and a rise in cannibalism observed in large redfish (> 30 cm) diet reflecting density-dependent control at high redfish densities (Senay et al. 2021; Brown-Vuillemin et al. 2022, 2023).

In the context of developing an ecosystem-based approach to the management of Canadian fisheries (Pepin et al. 2022), the trophic impact of redfish on other components of the ENGSL ecosystem would benefit from detailed and accurate diet modeling analyses. The objective of this study was to validate the DNA metabarcoding approach in complement to the current description of redfish diet composition based on SCA. In particular, we addressed the following questions: (1) how do SCA and DNA metabarcoding compare in terms of prey taxonomic resolution in the same individuals? (2) is the detection of size-related shifts in diet composition method dependent? and (3) does DNA metabarcoding improve the detection of certain prey taxa, and thus provide a more complete picture of redfish diet?

2 | Materials and Methods

2.1 | Study Area and Collection of Redfish Stomach Content

In the ENGSL, two beaked redfish species coexist in the deep waters: Acadian Redfish, *Sebastes fasciatus* and Deepwater Redfish, *Sebastes mentella* (DFO 2022). A third species, the non-beaked *Sebastes norvegicus* (historically, *Sebastes marinus*) is considered to be infrequent in the region. *S. mentella* and *S. fasciatus* are morphologically very similar, and individuals cannot be assigned to species based on morphological traits. Often not distinguished in both scientific surveys and commercial fisheries (Senay et al. 2021), the two species are referred to as redfish (*Sebastes* sp.) hereafter.

Redfish were sampled in August 2017 during the annual random depth-stratified trawl survey conducted by DFO onboard the CCGS *Teleost*, which covers the lower estuary and northern GSL (Figure 1). The survey vessel was equipped with a *Campelen 1800* trawl with a 13-mm net liner. Details on sampling design and protocol can be found in Bourdages et al. (2018). For each haul, a length-stratified subsample of individual redfish was taken, and only those individuals with no obvious signs of regurgitation were retained for further analyses. Upon capture, each redfish was measured (fork length, FL) to the nearest millimeter and weighed to the nearest gram. Stomachs were excised at sea from the esophagus to the pyloric sphincter, individually labeled and bagged, and stored frozen (−20°C) until analysis in the laboratory. A total of 185 non-empty stomach contents of redfish were selected for combined SCA and DNA metabarcoding analysis.

2.2 | Stomach Content Analysis

Methods for SCA were detailed in Brown-Vuillemin et al. (2022). Briefly, stomachs were thawed for visual analysis at the Maurice-Lamontagne Institute (Mont-Joli, Canada) and each prey taxa found in the stomach contents was weighed and identified to the lowest taxonomic level possible under a dissecting microscope and using keys and identification guides (e.g., Squires 1990; Campana 2004; Vassilenko and Petryashov 2009; ICES 2014). The dissection tools and platform were carefully cleaned with alcohol (90%) between each stomach content analyzed and all disposable tools like gloves and paper towels were discarded after handling each SCA sample. Stomach contents were immediately and individually refrozen (−20°C) until DNA analysis in the laboratory.

2.3 | DNA Metabarcoding of Stomach Contents

2.3.1 | Selection of Primers and Sample Processing

Each stomach content was completely and homogeneously ground to collect at least 20 mg of material, taking care to avoid contamination by using disposable tools and cleaning the platform with 90% alcohol between each sample. DNA was extracted from the 185 redfish stomach contents using a DNeasy Blood & Tissue Kit (Qiagen 69,504), according to manufacturer's instructions. A pair of universal metazoan mitochondrial cytochrome c oxidase subunit I (COI) primers was chosen as the barcoding region for prey species identification that has been developed and tested on a broad array of marine species (Hebert et al. 2003; Ward et al. 2005; Aguilar et al. 2017; Dahl et al. 2017; Roy and Boulding 2024): the forward mICOIintF (5′ GGWACWGGWTGAACWGTWTAYCCYCC 3′) (Leray et al. 2013) and reverse jgHCO2198 (5′ TAIACYTCIGGRTGICCRAARAAYCA 3′) (Geller et al. 2013). DNA amplifications (313 bp) were performed in a single-step PCR using tagged primers consisting of Illumina adapters, indexed barcodes for each sample, and the COI primers. PCRs were done in three replicates per sample. The final reaction volume for each replicate was 25 µL, including 12.5 µL of 2X Qiagen Multiplex Mastermix, 5.5 µL diH$_2$0, added to 2 µL of each primer (at 10 µM), and 3.0 µL of DNA. Extraction negative controls as well as PCR negative controls were also amplified. PCRs were performed

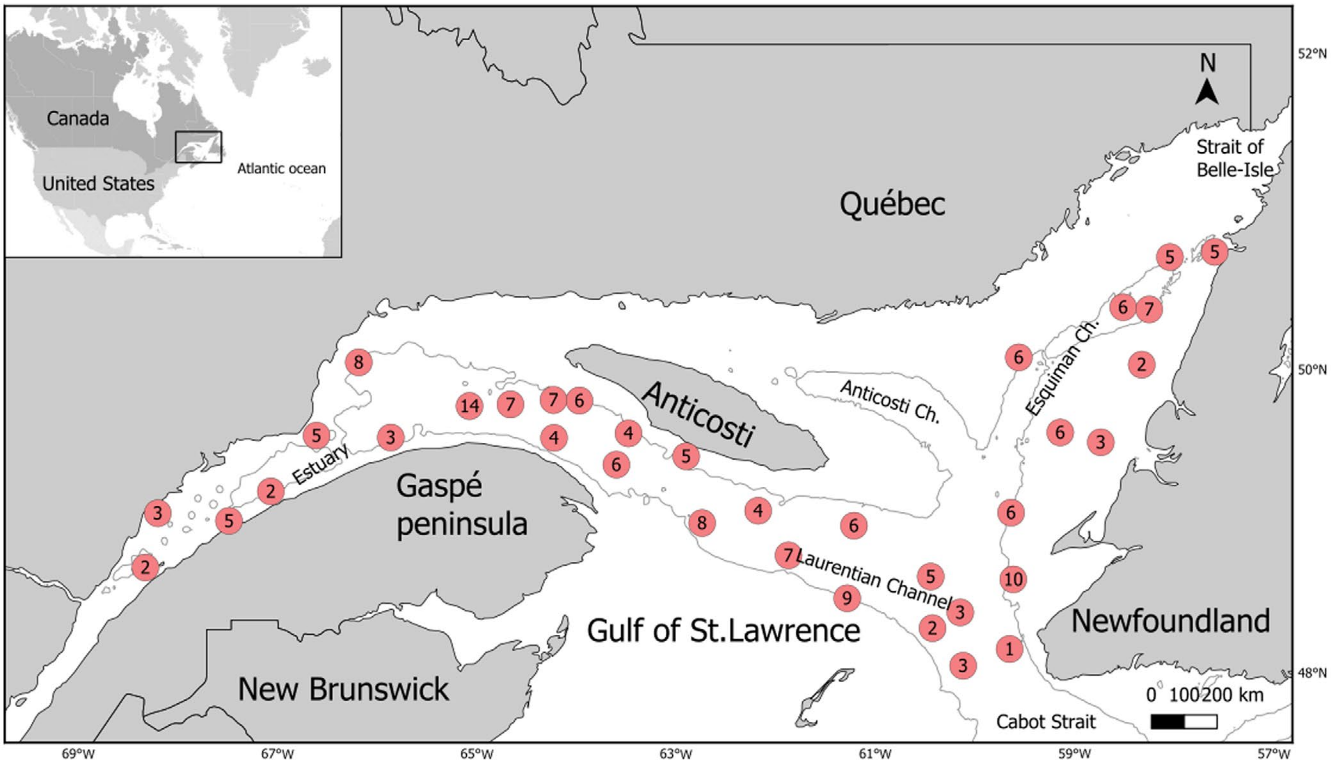

FIGURE 1 | Map of the study area showing sampling hauls where redfish stomachs were sampled for the present study (n = 35) with the number of redfish stomachs preserved (n = 185) during the August 2017 depth-stratified randomly trawl survey in the estuary and northern Gulf of St. Lawrence. The gray line indicates the 250 m isobaths.

following these cycling conditions: 95°C for 15 min, followed by 35 cycles (94°C for 30 s, 52°C for 90 s, 72°C for 60 s), and a final elongation at 72°C for 10 min. All amplifications were visualized on a 1.5% agarose gel for quality and contamination control. Two negative controls were treated and extracted as regular samples and sequenced to check for DNA contamination. The AxyPrep Mag PCR Clean-up kit (AxyGEN Biosciences) was used to remove primer dimers and all samples were then quantified using AccuClear Ultra High Sensitivity DNA Quantitation Kit (Biotium) using a Tecan Spark 10 M Reader and pooled in equal quantities (15 ng/μL). Illumina MiSeq sequencing was performed at the Genomic Analysis Platform of the IBIS (Institut de Biologie Intégrative et des Systèmes www.ibis.ulaval.ca) and located at Université Laval using a paired-end "MiSeq Reagent Kit V3" (Illumina, San Diego, USA; sequence length = 300 bp) and following the manufacturer's instructions. For sequencing, the amplicon pool was diluted to 4 nM with molecular grade water, denatured, and then sequenced at 10 pM following manufacturer's instructions inclusive of spiking the samples with 15% of PhiX.

2.3.2 | Bioinformatics and Data Processing

We used Barque (https://github.com/enormandeau/barque) to examine the biodiversity of stomach contents at the species level. Shortly, raw reads were trimmed with trimmomatic v0.36 (MINLEN:100, CROP:200). Forward and reverse reads were merged using flash v1.2.11 (−z, −m 30, −M 280). Amplicons were extracted using the primer information and expected amplicon size range for the COI1_mlCOIintF_jgHCO2198 primer (300, 325). Reads of each sample were denoised with vsearch v2.15.1 and the unoise3 algorithm. Chimeras were then removed using vsearch v2.15.2 using the default values. All the animal bins (barcode index number) were downloaded from the BOLD database on 2023-09-12 and all the COI fragments corresponding to the used primers were incorporated into the database used by Barque v1.8.5 (https://boldsystems.org/index.php/Public_BarcodeIndexNumber_Home). We annotated each unique read of each sample to the most similar species of the database if the sequences had at least 98% similarity. In cases of ties, referred to as multiple hits, when more than one species from the database had the same distance from the queried sequence, all the tied annotations were reported. Barque then produced a table of prey taxa counts (including the multiple hits) for each sample. Reads that had equal similarity scores for more than one species were assigned to a higher taxonomic level since it was impossible to discriminate species with certainty (e.g., *Ammodytes* sp. for the multiple hits referencing *Ammodytes americanus* and *Ammodytes dubius*). Read counts from negative controls have been subtracted from the results of prey taxa for each species.

2.3.3 | Taxonomic Sequence Matches From Redfish Stomachs

The complete list of the 104 taxonomic sequence matches detected in stomach contents is provided in Table S1. A total of 29 taxonomic sequence matches (% relative read abundance, %RRA ≥ 0.1), representing 99.6% of the total number of the 104 taxonomic sequence reads, were kept for further analyses. However, five matches were not retained: genera-level Arthropoda (insect or crustacean) and unidentified taxa were not useful for prey identification and were thus discarded. *Anisakis simplex* (a parasite nematode of stomach) and *Eukrohnia hamata* (cosmopolitan chaetognath) were discarded since they were not considered as target prey for redfish. While redfish themselves are a known prey item from visual SCA, it was not possible to determine in the DNA metabarcoding data whether sequences identified as *Sebastes* sp. were the result of cannibalism (Brown-Vuillemin et al. 2022) or cross-contamination from the sampled individuals themselves, and thus *Sebastes* sp. matches were also discarded. While cannibalism can be ecologically significant, the distinction between cannibalism and predator detection is impossible with metabarcoding markers (Cuff et al. 2022). Overall, 24 taxonomic sequence matches (91.1% of the total number of sequenced reads, Table S1), for 22 species and two genera identified, were considered as likely redfish prey and compared to the taxa identified through the visual SCA (Table 1).

2.4 | Data Analysis

To provide taxonomic comparisons for both analyses, each prey taxa was assigned to one of the following eight broad categories: fish, shrimp, amphipod, copepod, mysid, euphausiid, other invertebrates, and unidentified material (Table 1). The unidentified material category was only recorded during SCA identifications to represent prey items too digested to be identified in another broad category (Table 1).

Following Brown-Vuillemin et al. (2022), redfish diet composition data were analyzed as a function of redfish size using three classes: small (< 20 cm, *n* = 95), medium (20–30 cm, *n* = 53), and large redfish (≥ 30 cm, *n* = 37), for both visual and molecular methods. In order to compare the relative importance of prey from SCA and DNA metabarcoding data, the diet of redfish was described using two metrics: (1) measure of relative contribution and (2) measure of occurrence (presence/absence) of prey taxa in the diet.

2.4.1 | Relative Contribution

To assess the contribution of prey taxa to the diet of redfish for the SCA data, the mean partial stomach fullness index of prey i (PFI_i) (Lilly and Fleming 1981; Orr and Bowering 1997) transformed into a percentage ($\%\mathrm{FI}_i$, percentage fullness index, (Bernier and Chabot 2012) was calculated for each prey taxa in the redfish stomach using the following five equations:

$$\mathrm{PFI}_{ij} = \mathrm{M}_{ij} \times \mathrm{L}_j^{-b} \times 10^4 \quad (1)$$

where M_{ij} is the mass of prey i in redfish j, L_j is the FL (cm) of redfish j, and b is the specific allometric exponent calculated for redfish (b = 3.19), corresponding to the slope of the linear relationship of log(mass) and log(FL) of redfish (Brown-Vuillemin et al. 2022). The mean PFI of prey i was then obtained as follows:

TABLE 1 | Diet composition of redfish expressed in percentage fullness index (%FI), relative read abundance in percent (%RRA), percentage occurrence (%FOO), and percentage of relative occurrence (POO) according to SCA and DNA metabarcoding of the 185 redfish.

		SCA			DNA metabarcoding		
Prey categories	**Prey taxa**	**%FI**	**%FOO**	**POO**	**%RRA**	**%FOO**	**POO**
Fish		**2.13**	**4.32**	**3.02**	**6.45**	**27.03**	**11.52**
	Digested fish	1.50	2.16	1.39	—	—	—
Perciformes	*Melanostigma atlanticum*	0.63	2.16	1.39	6.00	21.62	5.05
	Ammodytes sp.	—	—	—	0.10	1.08	0.25
Gadiformes	*Enchelyopus cimbrius*	—	—	—	0.35	4.32	1.01
Shrimp		**44.32**	**20.54**	**14.34**	**22.28**	**44.32**	**18.89**
	Digested shrimp	1.97	3.78	2.44	—	—	—
Pandalidae	*Pandalus borealis*	17.31	6.49	4.18	3.91	18.38	4.29
	Pandalus montagui	—	—	—	0.86	2.70	0.63
	Pandalus sp.	0.33	1.08	0.70	—	—	—
Pasiphaeidae	*Pasiphaea multidentata*	24.71	10.81	6.97	17.50	31.89	7.45
Amphipod		**7.82**	**17.84**	**12.45**	**2.93**	**25.41**	**10.83**
	Digested amphipod	0.14	1.08	0.70	—	—	—
Hyperiidae	*Themisto abyssorum*	0.76	1.62	1.05	—	—	—
	Themisto compressa	0.38	1.62	1.05	1.97	20.00	4.67
	Themisto libellula	1.18	1.08	0.70	0.96	9.73	2.27
	Themisto sp.	5.35	14.59	9.41	—	—	—
Copepod		**18.65**	**51.89**	**36.23**	**42.73**	**90.27**	**38.48**
	Digested copepod	0.25	1.62	1.05	—	—	—
	Digested calanoid	4.00	14.59	9.41	—	—	—
Calanidae	*Calanus hyperboreus*	0.90	3.24	2.09	37.13	85.95	20.08
	Calanus finmarchicus	—	—	—	0.38	18.92	4.42
	Calanus glacialis	—	—	—	0.32	22.70	5.30
	Calanus sp.	12.55	32.97	21.25	3.40	37.30	8.71
Aetideidae	*Gaetanus tenuispinus*	—	—	—	0.08	5.41	1.26
Euchaetidae	*Paraeuchaeta norvegica*	0.12	0.54	0.35	0.82	29.73	6.94
Metrinidae	*Metridia longa*	—	—	—	0.60	16.22	3.79
	Metridia sp.	0.84	5.95	3.83	—	—	—
Mysid		**3.00**	**12.97**	**9.06**	**17.91**	**35.14**	**14.98**
	Digested mysid	T	1.08	0.70	—	—	—
Mysidae	*Boreomysis arctica*	—	—	—	17.91	35.14	8.21
	Boreomysis sp.	2.96	11.35	7.32	—	—	—
	Pseudomma sp.	0.03	0.54	0.35	—	—	—
Euphausiid		**8.05**	**5.95**	**4.15**	**4.60**	**29.73**	**12.67**
	Digested euphausiid	1.91	3.78	2.44	—	—	—
Euphausiidae	*Meganyctiphanes norvegica*	1.25	2.16	1.39	1.53	21.08	4.92

(Continues)

TABLE 1 | (Continued)

Prey categories	Prey taxa	SCA %FI	SCA %FOO	SCA POO	DNA metabarcoding %RRA	DNA metabarcoding %FOO	DNA metabarcoding POO
	Thysanoessa inermis	—	—	—	2.71	9.19	2.15
	Thysanoessa raschii	—	—	—	0.36	6.49	1.52
	Thysanoessa sp.	4.90	1.08	0.70	—	—	—
Other invertebrates		**10.46**	**22.16**	**15.47**	**3.09**	**27.03**	**11.52**
	Digested crustacea	10.24	21.62	13.94	—	—	—
Cephalopoda	*Rossia* sp.	0.22	0.54	0.35	—	—	—
Cumacea	*Diastylis lucifera*	—	—	—	0.72	3.24	0.76
	Eudorella emarginata	—	—	—	0.68	3.24	0.76
Ostracoda	*Boroecia borealis*	—	—	—	0.25	13.51	3.16
	Discoconchoecia elegans	—	—	—	0.94	5.41	1.26
Gastropoda	*Limacina helicina*	—	—	—	0.49	4.86	1.14
Digested/unidentified		**5.56**	**7.57**	**5.28**	—	—	—
	Unidentified taxa	5.56	7.57	4.88	—	—	—

Note: The contribution of the eight broad prey categories is shown in bold.

$$\mathrm{PFI_i} = \frac{1}{\mathrm{N}} \times \sum_{j=1}^{\mathrm{N}} \mathrm{PFI_{ij}} \qquad (2)$$

where N is the number of redfish.

Percentage fullness index was further calculated as:

$$\%\,\mathrm{FI_i} = \frac{\mathrm{PFI_i}}{\mathrm{TFI}} \times 100 \qquad (3)$$

where total stomach fullness index (TFIj) was the sum of all $\mathrm{PFI_i}$ for a redfish j.

TFI was calculated as:

$$\mathrm{TFI_j} = \sum_{i=1}^{\mathrm{I}} \mathrm{PFI_{ij}} \qquad (4)$$

$$\mathrm{TFI} = \frac{1}{\mathrm{N}} \times \sum_{j=1}^{\mathrm{N}} \mathrm{TFI_j} \qquad (5)$$

where I represent the number of different prey taxa found in the sample.

To assess the contribution of prey taxa to the diet of redfish for the DNA metabarcoding data, the relative sequence read abundance transformed into a percentage ($\%\mathrm{RRA_i}$) using the sequence counts (Deagle et al. 2018) was used and calculated as:

$$\%\,\mathrm{RRA_i} = \frac{\text{Number of sequence reads for taxa i}}{\text{Total number of sequence reads}} \times 100$$

2.4.2 | Occurrence

For both methods, the percent frequency of occurrence (%FOO) of prey taxa was calculated as:

$$\%\,\mathrm{FOO_i} = \frac{\text{Number of stomach containing taxa i}}{\text{Total number of stomachs}} \times 100$$

Then, the relative percentage of occurrence (POO) of each taxon was calculated to provide a convenient view of each taxon's contribution as a percentage of total diet. %FOO, which does not sum to 100%, was simply rescaled so that the sum across all taxa was 100% (Deagle et al. 2018).

$$\mathrm{POO_i} = \frac{\mathrm{FOO_i}}{\text{Total FOO}} \times 100$$

3 | Results

3.1 | Taxonomic Comparisons Using the Two Stomach Content Analysis Methods

DNA metabarcoding analysis overall identified more taxa at the species level ($n=22$) compared to the SCA ($n=9$) (Table 1, Figure 2). A total of eight prey species was identified with both methods included: one fish, *Melanostigma atlanticum*; two shrimp, *Pandalus borealis* and *Pasiphaea multidentata*; two amphipods, *Themisto compressa and T. libellula*; two calanoid copepods, *Calanus hyperboreus* and *Paraeuchaeta norvegica,* and one euphausiid, *Meganyctiphanes norvegica* (Table 1, Figure 2).

DNA metabarcoding detected additional taxa not identified with SCA: two fish species, the demersal rockling *Enchelyopus cimbrius* and the pelagic sand lance *Ammodytes* sp., one shrimp species *Pandalus montagui*, five small zooplankton species (two

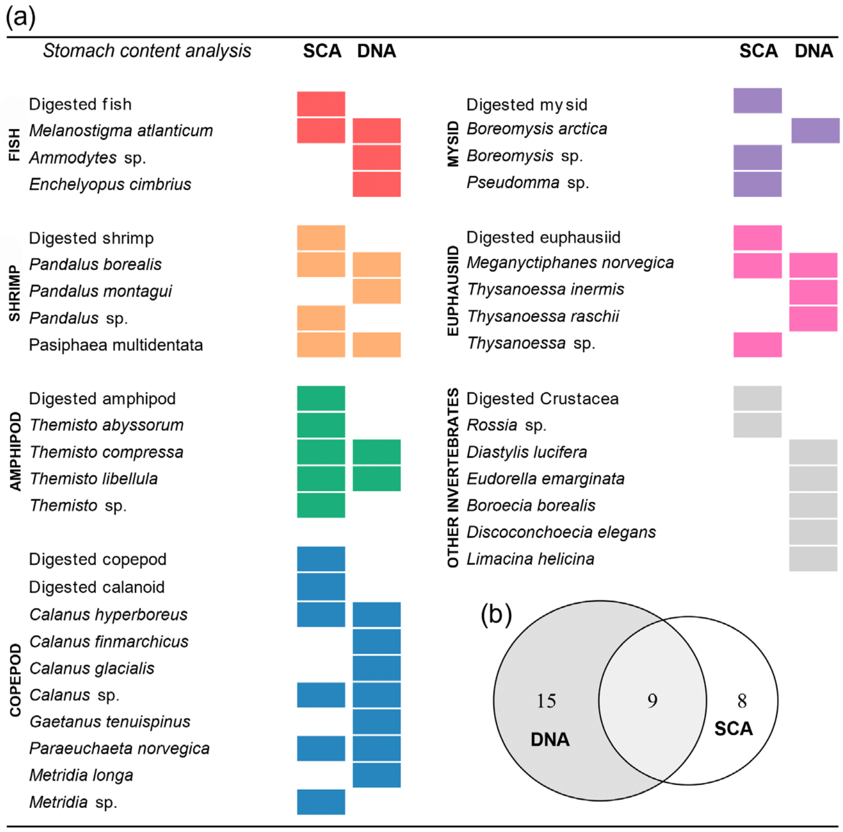

FIGURE 2 | (a) Heat map (presence/absence) of the different prey taxa identified in redfish diet according to SCA and DNA metabarcoding. (b) Venn diagram showing the overlap of the number of prey taxa detected by both SCA and DNA metabarcoding methods.

Cumacea, *D. lucifera*, *E. emarginata*; two Ostracoda, *B. borealis*, *D. elegans*; and one Gastropoda species *L. helicina*). Some taxa identified by SCA were resolved to the species level with metabarcoding, including mysid *Boreomysis* sp. as *B. arctica* and euphausiid *Thysanoessa* sp. as *T. inermis* and *T. raschii*. DNA metabarcoding identified four additional copepod species, *Calanus finmarchicus*, *Calanus glacialis*, *Gaetanus tenuispinus*, and *Metridia longa*, that were recorded at genus (*Calanus* sp., *Metridia* sp.) or more general level (digested copepod and calanoid) in SCA. Conversely, the mysid *Pseudomma* sp. and the amphipod *T. abyssorum* (though identified in the complete list of the 104 taxonomic sequences matches) (Table S1, %RRA < 0.1) was only observed in SCA (Table 1, Figure 2).

3.2 | Importance of Prey Taxa to Redfish Diet Composition

The measures used to estimate the relative contribution and occurrence of the different taxa to redfish diet, %FI in SCA, %RRA in DNA metabarcoding, %FOO and POO for both methods revealed a similar overall importance of the different prey categories (Table 1). For SCA, shrimp was the most abundant prey category (%FI = 44.3), which is led by *P. multidentata* (%FI = 24.7) then followed by Copepod (%FI = 18.7), mainly driven by the genus *Calanus* (%FI = 12.6). These two categories were also the most represented in terms of %RRA in DNA metabarcoding, but in reverse order: Copepods were the most represented category (%RRA = 42.7) dominated by *Calanus hyperboreus* (%RRA = 37.1), followed by shrimp (22.3%) also led by *P. multidentata* (%RRA = 17.5). In terms of occurrence, the copepod category was the most frequently found in stomach contents for both methods (%FOO = 51.9 and POO = 36.2 for SCA and %FOO = 90.3 and POO = 38.5 for DNA metabarcoding) with *Calanus* sp. as the most common genus identified with SCA (%FOO = 33.0 and POO = 21.3) and *C. hyperboreus* with DNA (%FOO = 86.0 and POO = 20.1). The other invertebrates category was the second most frequently reported prey group for SCA (%FOO = 22.2 and POO = 15.5) with a predominance of digested crustacea (%FOO = 21.6 and POO = 13.9). Shrimp was the second most frequent category for DNA metabarcoding (%FOO = 44.3 and POO = 18.9) with *P. multidentata* as predominant species (%FOO = 31.9 and POO = 7.5).

3.2.1 | Redfish Diet Composition as a Function of Fish Size

The same general patterns in size-related dietary shifts were observed both with the SCA and DNA metabarcoding when comparing %FI with %RRA (Figures 3 and 4, Table S2). Relying on these relative contribution measures, we observed the same shift from a dominance of small zooplankton taxa in smaller redfish, driven by copepod, to one of shrimp in the diet of larger redfish. While this size-related pattern was confirmed by occurrence with POO in SCA, it was less apparent in DNA metabarcoding,

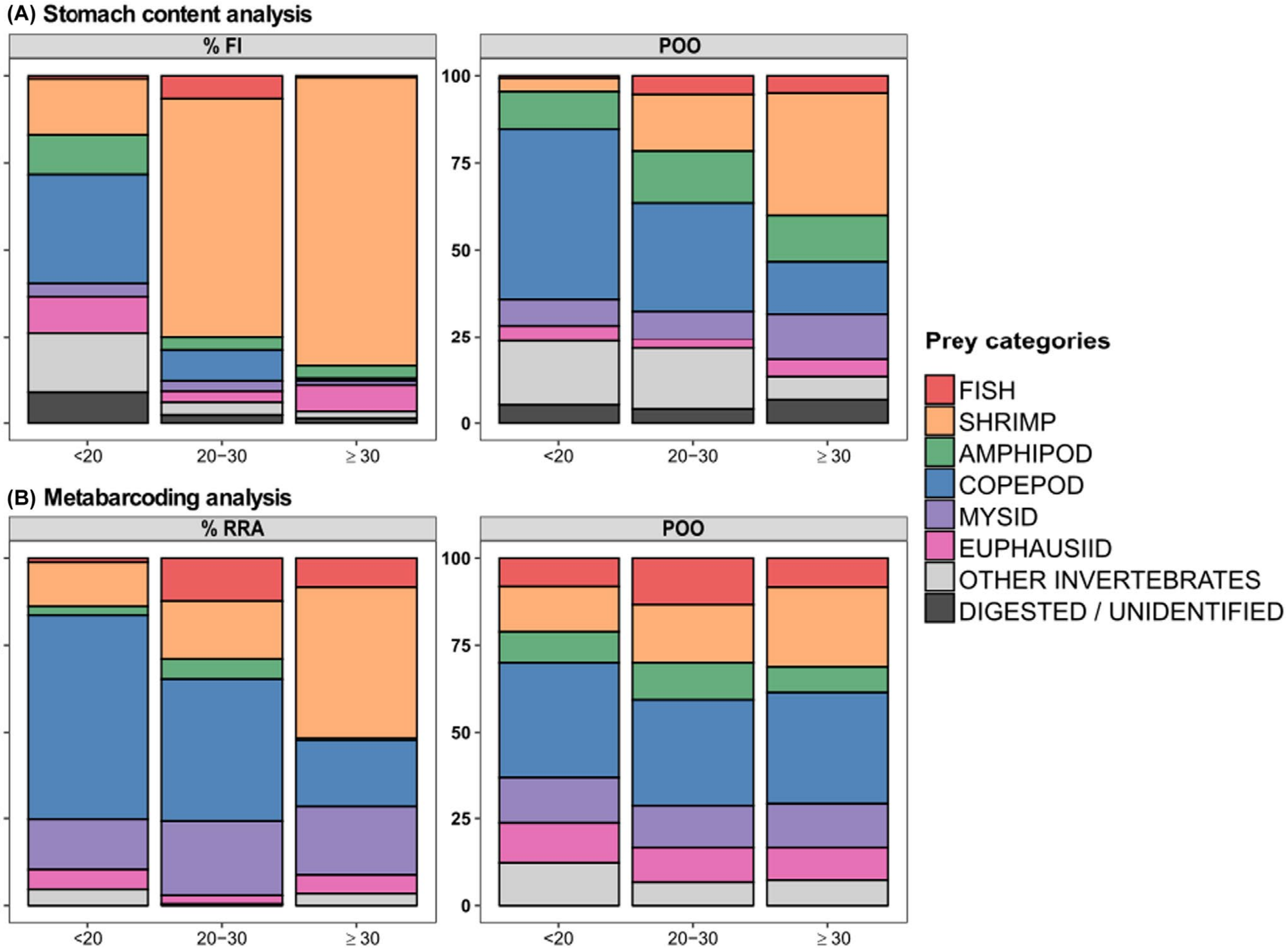

FIGURE 3 | Contribution of the eight prey categories to redfish diet as a function of redfish size classes (small, medium, and large individuals), expressed as percent fullness index (%FI), relative read abundance (%RRA), occurrence (%FOO), and relative occurrence (POO) according to SCA and DNA metabarcoding.

given the occurrence of copepod, which did not show a decreasing trend as the size of redfish increased (POO = 33.3, 30.7, 32.3 for each redfish size < 20, 20–30 and ≥ 30 cm, respectively) (Figures 3 and 5, Table S2).

Both methods showed that the diet of small (< 20 cm) redfish was dominated by copepod in terms of relative contribution (%FI = 31.2 and %RRA = 58.9) and that the importance of this prey category decreased with redfish size although remaining important in DNA metabarcoding relative to SCA for medium-sized redfish (20–30 cm) (%FI = 8.9 and %RRA = 41.1) and large-sized redfish (≥ 30 cm) (%FI = 0.7 and %RRA = 19.4). At the genus level, *Calanus* sp. (%FI = 21.1, POO = 30.3) was identified by SCA. With DNA metabarcoding *C. hyperboreus* (%RRA = 49.6, POO = 18.4) was identified as the most important copepod species in terms of relative contribution and occurrence in the diet of small redfish (Figures 3–5, Table S2).

Amphipod category was found to be more important for small (< 20 cm) redfish in terms of relative contribution when assessed by SCA compared to DNA metabarcoding. However, their occurrence was similar between the two methods (%FI = 11.3 and POO = 10.7; %RRA = 2.5 and POO = 8.9) and was mainly assigned to the genus *Themisto* (%FI = 8.2 and POO = 13.5) for SCA and *T. compressa* (%RRA = 2.1 and POO =17.9) for DNA metabarcoding. For the euphausiid category, the observed trend was the same between both methods with a more important contribution in small (< 20 cm) redfish, especially for *Thysanoessa* sp. (%FI = 7.1, POO = 0.7) with SCA, and *T. inermis* according to DNA metabarcoding (%RRA = 4.9, POO = 8.1). Regardless of the method used, the relative importance of other invertebrates category was also higher in small (< 20 cm) redfish (%FI = 17.4 and %RRA = 4.7, POO = 18.3 and 12.2 for SCA and DNA, respectively). While the relative contribution of the mysid category to the redfish diet estimated from the SCA varied between 1.3–3.8 %FI and 7.6–13.3 POO according to different redfish sizes, DNA metabarcoding yielded much higher values for relative contribution, ranging between 14.5 and 21.3 %RRA, with 12.0–13.0 POO represented by one species: *B. arctica* versus *Boreomysis* sp. for SCA. Regardless of the method used, the fish category was most important in medium-sized redfish (%FI = 6.6 and %RRA = 12.3, POO = 5.41 and 13.3 for SCA and DNA, respectively), driven by *M. atlanticum* (%FI = 2.5 and POO = 2.7; %RRA = 12.0 and POO = 7.0) (Figures 3–5, Table S2).

The contribution of shrimp increased with redfish size for both methods and the diet of large (≥ 30 cm) redfish was dominated by this category in terms of relative contribution (%FI = 82.9 and

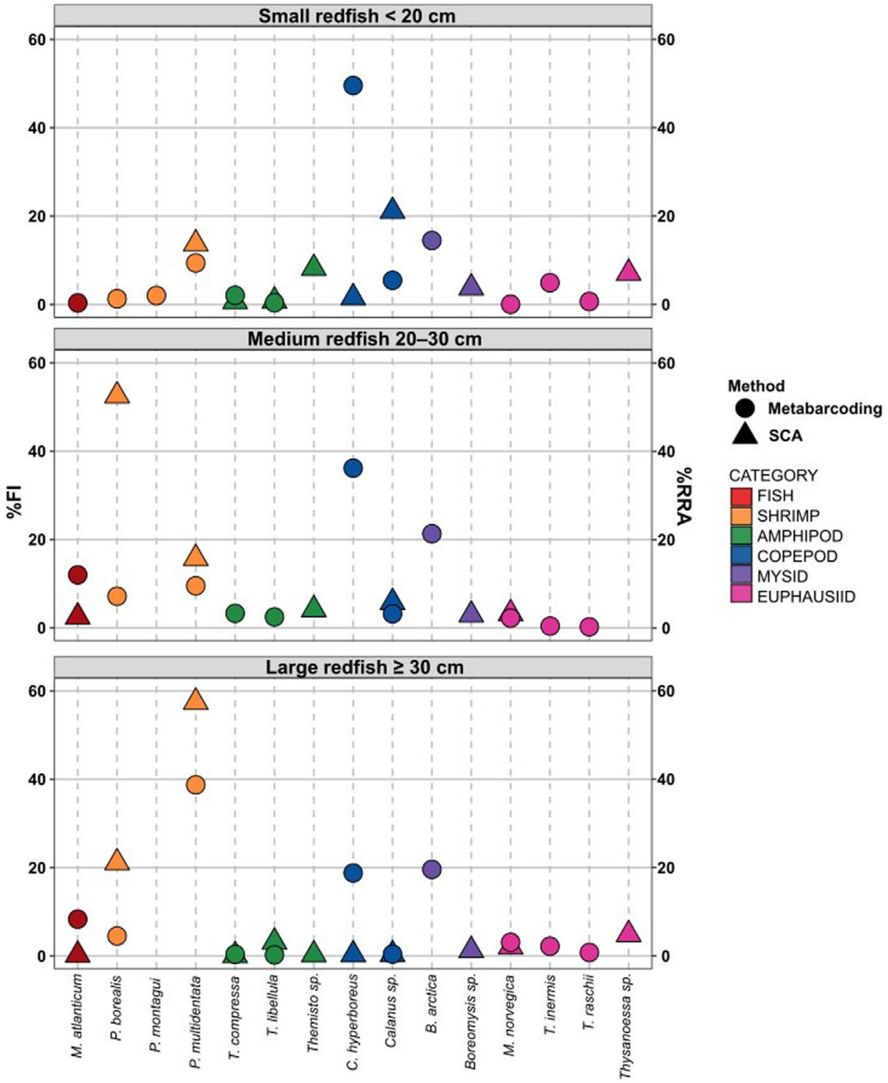

FIGURE 4 | Contribution of the most important prey identified at the species or genus level by categories to redfish diet as a function of size class (small, medium, and large individuals), expressed as percentage fullness index (%FI) and relative read abundance (%RRA) according to SCA and DNA metabarcoding.

%RRA = 43.3). In terms of relative occurrence, shrimp were predominantly represented for SCA (≥ 30 cm, POO = 35) and in second rank, after the copepods, for DNA (≥ 30 cm, POO = 22.9). At the species level, *P. borealis* was the most important shrimp in medium-sized (20–30 cm) redfish for SCA provided higher values than the DNA metabarcoding (%FI = 52.6 and POO = 10.8; %RRA = 7.1 and POO = 5.6). With DNA metabarcoding, *P. multidentata* was the most important shrimp in medium-sized (%RRA = 38.7 and POO = 7.0) compared to SCA (%FI = 15.72 and POO = 4.0) but became the most important shrimp in large (≥ 30 cm) redfish for both methods (%FI = 57.45, POO = 20.9 and %RRA = 38.7, POO = 14.1) (Figures 3–5, Table S2).

4 | Discussion

This study constitutes the first attempt to combine and compare SCA and DNA metabarcoding on stomach content to assess the diet composition of redfish. Our results add to the growing body of evidence that the use of DNA metabarcoding can improve the taxonomic resolution of the prey of marine predators, particularly in concert with the classic SCA (e.g., Dunn et al. 2010; Côté et al. 2013; Oyafuso, Toonen, and Franklin 2016; Jakubavičiūtė et al. 2017; Aguilar et al. 2017; Dahl et al. 2017; Riccioni et al. 2018). Our study confirms redfish as a bathypelagic predator feeding mostly on small zooplankton species with especially copepods and on two shrimp *P. borealis* and *P. multidentata*, which are considered as main prey of redfish following previous SCA-based work (Brown-Vuillemin et al. 2022, 2023).

4.1 | Taxonomic Resolution in Prey Identification

In diet studies with SCA, taxonomists regularly deal with a large amount of visually unidentifiable taxa consisting of highly digested prey (e.g., digested crustacea, Table 1). The traditional approach in SCA is to be conservative in identification, going to the lowest taxonomical level that a technician feels comfortable recording. In recent years, stomach contents have been documented and photographed, allowing for validation

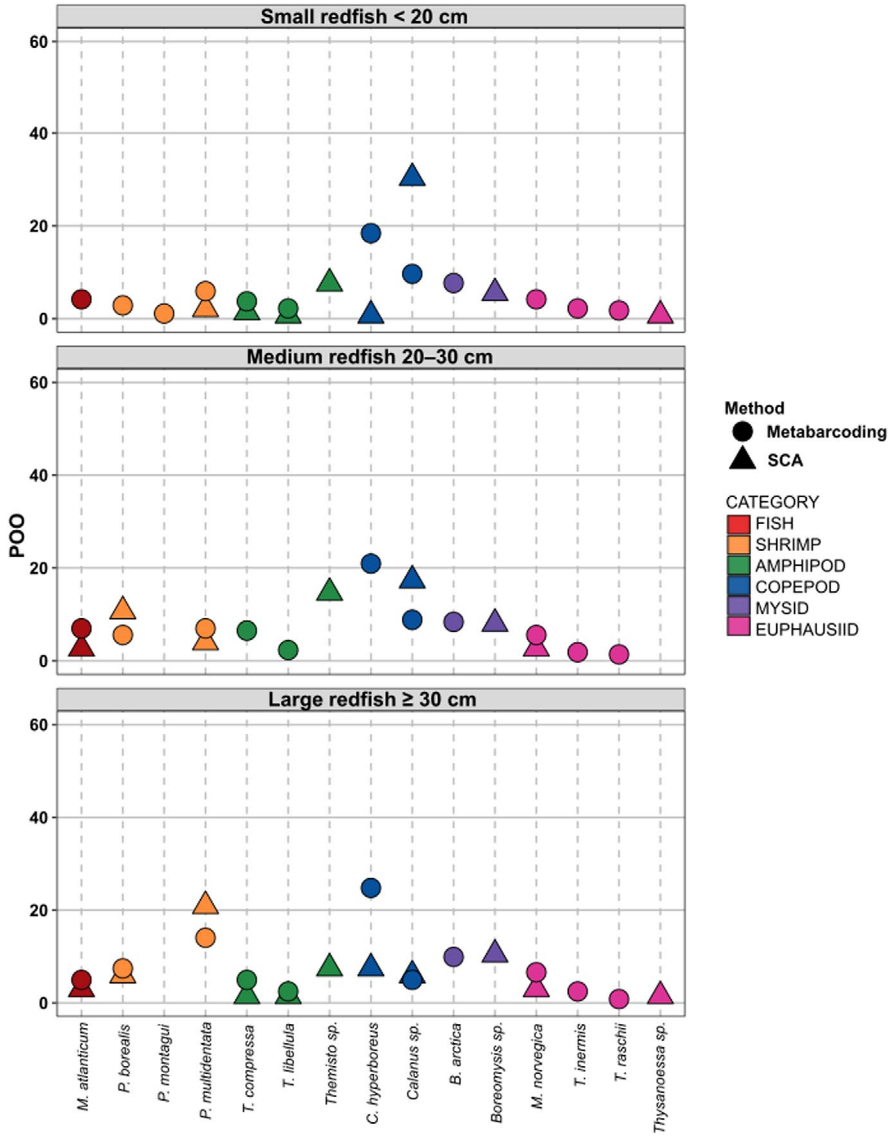

FIGURE 5 | Contribution of the most important prey identified at the species or genus level by categories to redfish diet as a function of size class (small, medium, and large individuals), expressed as a relative percentage of occurrence (POO) according to SCA and DNA metabarcoding.

and updates of taxonomic identifications in later reviews. The incidence of unidentified taxa was noticed in our earlier analysis across time periods, with digested shrimp material more often identified to species level in more recent stomach contents (Brown-Vuillemin et al. 2022). Although taxonomic identification effort and experience can improve accuracy, digested taxa often cannot be reliably assigned to finer taxonomic levels (e.g., genus or species). This limitation may thus introduce biases in the definition of diet composition (e.g., Baker, Buckland, and Sheaves 2014), and therefore, specific contributions of digested items could be more accurately estimated if identified by DNA analysis.

Our results suggest that DNA metabarcoding can complement SCA by enabling the assignment of specific species within broader prey categories, especially for zooplankton that were conservatively identified to the genus level in SCA (e.g., *Calanus*, *Metridia*, *Boreomysis*, and *Thysanoessa*). This improvement with DNA in identification can help in making links with particular species that are monitored in ecosystem studies.

Of concern is the fact that several taxa detected by metabarcoding remained unidentifiable with SCA. DNA metabarcoding enhanced the identification of small, benthic crustacean species of ostracods and cumaceans. Also, some of the detected prey were soft-bodied species (e.g., *Limacina helicina, M. atlanticum*) and could be undetected with SCA. Following validation and confirmation, DNA metabarcoding has the potential for greater taxonomic diversity and resolution for soft-bodied or small prey items, as was also demonstrated in other diet studies (e.g., Clarke et al. 2020; Vasiliadis et al. 2024).

4.2 | Quantitative Prey Assessment

DNA-based studies can be influenced by the detection of secondary or non-target prey, especially in metabarcoding analyses where universal primers maximize the detection of DNA from a broad array of species. This approach may lead to overestimations of certain species if secondary prey or rare items consumed during feeding remain in the analysis (Sheppard et al. 2005;

Oehm et al. 2017; Cuff et al. 2021). Although our methodology included filtering measures (using a relative read abundance filter with removal of %RRA < 0.1) as an attempt to remove artifacts, some non-target prey could have been retained and their importance in redfish diet was exaggerated. Clarke et al. (2020) demonstrated that secondary prey is rarely found in stomach content sequencing data and is typically represented by less than 1% of the total read, yet our study noted occasional detections of low-abundance prey items (e.g., ostracods, cumaceans, gastropods with %RRA < 1; Table 1). This finding raises questions about whether such items represent true dietary targets or if they were ingested by the redfish's primary prey, such as amphipods, euphausiids, or shrimp. For instance, some copepods (e.g., POO of *C. hyperboreus*), identified in large redfish diets, are also essential components of other redfish prey (e.g., *Thysanoessa libellula*, *Meganyctiphanes norvegica*, and *Pandalus borealis*) (Ouellet and Lefaivre 1994; Marion et al. 2008; Schmidt 2010). Future analyses should consider alternative filtering techniques or complementary methods to improve prey specificity, aiming to minimize potential biases in dietary importance attributed to prey.

While DNA metabarcoding has become a well-established tool for dietary studies (Hebert et al. 2003; Carreon-Martinez et al. 2011), there is no consensus regarding to what extent metabarcoding can be quantitative (Lamb et al. 2018) because of the lack of a standardized method for using sequence read counts as a proxy for prey contribution to the diet (Deagle et al. 2018). The concept that DNA metabarcoding provides accurate quantitative information is under debate since variability in technical and biological aspects can generate major sources of bias. For example, cell density, primer mismatch, unequal primer binding with different primer affinities during the PCR, differential quality, and degradation of DNA by digestive enzymes in stomachs, and wrong or missing reference sequences are all factors that can affect the relative read abundance for a given taxon (e.g., Pompanon et al. 2012; Thomas et al. 2016; McInnes et al. 2017; Buglione et al. 2018; Deagle et al. 2018; Stapleton et al. 2022; Damian-Serrano et al. 2022). These complexities underscore the need for standardized methodologies and caution when interpreting DNA read counts as indicators of dietary composition, highlighting the importance of complementary approaches such as SCA to achieve more robust dietary assessments. In light of these challenges, Osowski et al. (2023) used an interesting DNA-based approach to uncover dietary details that are often masked during visual identification of stomach contents. For prey that could not be visually identified at the species level during SCA, a genetic analysis was performed. Assuming that the prey with the highest number of reads represented the most likely dietary component of a particular digested material, the authors were able to relate and quantify the diet observed in the stomachs using DNA barcoding.

Many studies conservatively interpret sequence counts as occurrence data. However, occurrence-based diet summaries do not necessarily provide a more accurate representation of overall diet since they can potentially over-emphasize the contribution of rare taxa while downplaying the contribution of major prey (Deagle et al. 2018). Occurrence data can then inflate the importance of taxa that occur at low read counts, including artifacts, producing misleading results even after read count filters (Drake et al. 2022). In the present study, from the comparison between analyses derived from SCA and DNA metabarcoding, we argue that occurrence results derived from metabarcoding cannot be used to interpret dietary size-related trends, notably with the strong occurrence of copepods in large redfish in DNA unlike the shift away from zooplankton observed in SCA, and only %RRA could provide a similar semi-quantitative trend of diet in accordance with the quantitative results obtained from SCA in this study (Figure 3). In fact, the %RRA values for all main prey taxa were relatively close to their respective %FI values, with main prey representing more than 50% of the total diet in terms of both %FI and %RRA. Both methodologies pointed to the same important zooplankton taxa in small redfish (< 20 cm) and DNA metabarcoding allowed us to redefine the most important species: copepods of the genus *Calanus* including *C. hyperboreus* and mesoplankton taxa such as the mysids of the genus *Boreomysis* dominated by the species *B. arctica*, euphausiids of the genus *Thysanoessa* driven by the species *T. inermis*, and amphipods of the genus *Themisto* dominated by the species *T. libellula* and *T. compressa* (Figure 4). In addition to these zooplankton taxa, shrimp consumption was important in medium-sized (20–30 cm) and in large (≥ 30 cm) redfish, with a dominance by *P. borealis* and *P. multidentata*. These results confirmed the dietary shift from zooplankton to shrimp, already observed in previous studies (Ouellette-Plante et al. 2020; Senay et al. 2021; Brown-Vuillemin et al. 2022, 2023). This size-related dietary transition is generally attributed to a combination of factors, such as increased mouth opening and swimming ability (e.g., Cook and Bundy 2010), changes in energy requirements (e.g., Dwyer, Buren, and Koen-Alonso 2010), and changes in habitat use, as larger redfish generally occupy deeper waters than smaller ones (e.g., Planque et al. 2013; Senay et al. 2021). Given that current cohorts of redfish are reaching the size (≥ 24 cm, Senay et al. 2023) corresponding to a shift from a zooplankton-dominated diet to one primarily based on shrimp, consumption of these prey is expected to rise in the short term. This has important implications for the development of management strategies for commercial stocks such as the GSL northern shrimp. Redfish predation on shrimp and its implications for the demersal community in the northern GSL, such as increased interspecific competition, were discussed in Brown-Vuillemin et al. (2022). The variability in the phenology, abundance, and distribution of major redfish prey in relation to environmental variability will be important to consider in future redfish trophodynamic studies and the development of marine resource management strategies for the GSL, as discussed by Brown-Vuillemin et al. (2023). However, in order to use the DNA results of this study in a semi-quantitative way, cannibalism, not observed in this SCA study but previously recorded in large redfish diet (Brown-Vuillemin et al. 2022), should be considered, which is impossible with DNA metabarcoding. To improve our ability to use DNA metabarcoding as a semi-quantitative tool to assess diet composition of redfish, experimental studies could be conducted to evaluate how prey detectability varies among prey species and estimate correction factors to account for different digestion rates and loss of read count data, to assess impact of artifacts on metabarcoding datasets (Deagle et al. 2010; Thomas et al. 2014; Egeter, Bishop, and Robertson 2015; Stapleton et al. 2022; Drake et al. 2022), in addition with corrected and validated reference sequences database.

4.3 | The Importance of Universal Primer Selection

A large number of previous studies confirmed the reliability of COI as a taxonomic tool across a wide diversity of taxa (Hebert et al. 2003; Ward et al. 2005; Dawnay et al. 2007; Dahl et al. 2017; Corse et al. 2019; Roy and Boulding 2024). Our results suggest that the selected universal primers set (mICOIintF, jgHCO2198) allowed for an efficient detection of prey in redfish stomachs, which supports conclusions of other studies on fish (Aguilar et al. 2017; Dahl et al. 2017; Jakubavičiūtė et al. 2017). Even though the COI primers are designed to be taxonomically broad, they may not bind equally to all prey species and could lead to misleading representation of the prey in the diet (Deagle et al. 2007). For diet metabarcoding analysis, Corse et al. (2019) demonstrated that the complementary use of multiple primers targeting the same locus (e.g., COI gene) can describe species diversity with finer taxonomic levels especially when prey composition can be highly variable (e.g., for generalist predators). As a result, the "one-locus-several-primer-sets" strategy (Corse et al. 2019) could be relevant for further analysis of prey-redfish interactions to comprehensively cover the taxonomic diversity of prey (Villsen et al. 2022).

Moreover, the assignment of taxa to higher taxonomic levels in DNA metabarcoding (e.g., *Ammodytes* spp. and *Calanus* spp., Table 1 or Arthropoda and Unidentified taxa, Table S1) may indicate that the reference database could either lack sequences for some species. In such cases, sequences may align with other taxa that are present in the database, resulting in broader taxonomic assignments. On the other hand, inefficiency in primer amplification could also lead to taxa being missed in the analysis. A COI sequence reference database with full taxonomic coverage can minimize unassigned sequences and thus provide a more accurate taxonomic scope essential for placing sequencing results in biological and ecological contexts (Meglécz, 2023). However, achieving this is often challenging in many regions and for various taxonomic groups. In addition, the presence of too many sequences from unrelated regions or environments can also lead to false or ambiguous taxonomic assignments. Mugnai et al. (2023) have shown the importance of having reliable reference databases, that were refined for the local study area and without the presence of unexpected taxa. Creating regional reference libraries could thus enhance assignment reliability and increase the number of species detected in metabarcoding studies (Ortega et al. 2020; Sarkis et al. 2022; Bourret et al. 2023). Even local, specific databases cannot, however, replace the need for barcoding to address gaps in taxonomic coverage; this should be done in collaboration with expert taxonomists by testing primer sets on DNA extracts from key dietary taxa and adding the resulting sequences to reference databases. Global metabarcoding initiatives that aim to obtain COI records may result in more ENGSL species being barcoded in the near future, with several groups being notoriously difficult and in need of validation, either at the taxonomic or geographic (regional library) level (Bourret et al. 2023).

5 | Conclusion

DNA metabarcoding provides a promising complementary approach to SCA for redfish diet composition by confirming the species recorded at genus-level taxa that would otherwise go unconfirmed (Jakubavičiūtė et al. 2017; Riccioni et al. 2018; Cordone et al. 2022). We found that both techniques provided useful and complementary information to estimate the prey ingested directly from the stomach and that they are best used together to inform redfish diet (e.g., Alonso et al. 2014; Matley et al. 2018; Cordone et al. 2022; Lejeune et al. 2022). As sequencing technology and reference databases for different primer sets continue to develop and become increasingly affordable, the use of metabarcoding for diet determination will increase (Stapleton et al. 2022) and taxonomic resolution will keep improving. This will yield an increasingly precise portrait of trophic linkages among species with a comprehensive list of prey. This improved accuracy in fish trophic data will in turn facilitate the implementation of ecosystem-based approaches to resource management (Canals et al. 2024). In contrast, even though SCA can be limiting in terms of taxonomic resolution, it allows for a more quantitative measure of diet composition and can resolve cases of cannibalism, as well as distinguish target and artifact (e.g., environmental signatures) consumption (Canals et al. 2024). Given the complementary nature of the two methods, we advocate for increasing collaborations between taxonomists and molecular ecologists working on reference sequences and applying metabarcoding analyses to assist with modeling diet composition as well as trophic linkages at the ecosystem level.

Author Contributions

All authors conceived the project objectives and methodologies. C.N. conducted the prey taxa identification from the stomach contents. S.B.V. and C.H. performed the analysis of DNA metabarcoding. E.N. performed the bioinformatics data processing. S.B.V. conducted the data analyses and wrote the first draft of the manuscript. All authors have contributed to the writing, revision, and improvements of the manuscript and take responsibility for its content.

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Ethics Statement

Ethical review and approval were not required because the study was conducted in the field using animals killed during surveys.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on Dryad database with a digital object identifier DOI (https://doi.org/10.5061/dryad.37pvmcvpr) and from the corresponding author upon reasonable request (sarah.brown-vuillemin@uqar.ca).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.