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Feeding ecology of redfish (*Sebastes* sp.) inferred from the integrated use of fatty acid profiles as complementary dietary tracers to stomach content analysis

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Abstract

In the northern Gulf of St. Lawrence (nGSL), redfish (*Sebastes mentella* and *Sebastes fasciatus* combined) are at record levels of abundance following the strong recruitment of three consecutive cohorts in 2011–2013 and have become by far the most abundant demersal fish in the region. Understanding redfish trophic relationships is essential for the effective management and conservation of species in the nGSL ecosystem. To date, description and quantification of redfish diet in the region have been restricted to conventional stomach content analysis (SCA). Using analysis of fatty acid (FA) profiles as complementary dietary tracers, the authors conducted multivariate analyses on 350 livers of redfish which were collected in combination with stomach contents during a bottom-trawl scientific survey in August 2017. The predator FA profiles were compared to those of eight different redfish prey types identified as dietary important with SCA. Results suggested similitude between SCA and FA results, with zooplankton prey being more related to small (<20 cm) and medium (20–30 cm) redfish (16:1n7, 20:1n?, 22:1n9 and 20:5n3) than large (≥30 cm) ones, whereas shrimp prey seemed more related to large redfish size classes (18:2n6 and 22:6n3) relative to the small and medium ones. Although the SCA offers a glimpse in the diet only based on the most recently consumed prey, analysis of FA profiles provides a mid-term view indicating pelagic zooplankton consumption on calanoid copepod and confirming high predation pressure on shrimp. This study constitutes the first attempt of combining FA with SCA to assess the diet of redfish, highlights the benefits of FA as a qualitative tool and suggests improvements for future studies.

KEYWORDS
diet, Gulf of St. Lawrence, liver tissue, prey

1 | INTRODUCTION

Insights into predator–prey dynamics are a key element in the knowledge of ecosystem structure and function to ensure effective management and protection of commercial species (*e.g.*, Arditi & Ginzburg, 2012; Braga *et al.*, 2012; Nielsen *et al.*, 2018). This is of particular interest in the current context of ecosystem conservation with environmental changes, including warming waters and changes in

species composition, which leads to the following question: what are the main prey of key consumers in relation to their respective abundance and availability? To address this issue, several approaches, such as visual- and DNA-based diet analyses, as well as biomarkers based on stable isotopes and fatty acids (FAs), have been developed to measure the ingested or egested prey and estimate the assimilated fraction of prey (*e.g.*, Amundsen & Sánchez-Hernández, 2019; Baker *et al.*, 2014; Braga *et al.*, 2012; Hyslop, 1980; Iverson, 2009; Nielsen *et al.*, 2018).

Stomach content analysis (SCA) is an approach that is particularly relevant to assess fish diet composition because fish generally swallow their prey whole (Amundsen & Sánchez-Hernández, 2019). Although SCA allows the visual identification of ingested prey and their relative importance in the diet, providing inferences on ecological traits, such as feeding mode and prey preferences of a predator, it is subject to several biases (*e.g.*, Baker *et al.*, 2014; Bowen & Iverson, 2013; Braga *et al.*, 2012; Hyslop, 1980; Iverson, 2009; Iverson *et al.*, 2004). First, it only provides a snapshot of the last few most recent meals, so that large sample sizes are required to potentially inform on various prey contributions. Even with sufficient sample sizes, the portrait of diet produced from SCA is restricted to the spatial scale and time period of sampling unless stomachs are collected over different areas and seasons. Further, different prey may be digested at different rates, and the relative contribution of quickly digested prey, such as larvae or soft-bodied organisms, may be underestimated (Amundsen & Sánchez-Hernández, 2019; Baker *et al.*, 2014; Hyslop, 1980). The frequency of partial or complete regurgitation can also be high, which, in addition to stomachs that were truly empty, can reduce the sample size considerably compared to the number of stomachs collected. Furthermore, partial regurgitation results in incomplete prey samples. For instance, physoclist fish species have a closed swim bladder and therefore cannot adapt to rapid changes in pressure. As a result, they are extremely sensitive to barotrauma when brought to the surface rapidly (Jarvis & Lowe, 2008), and partial or total regurgitation is frequent. Although fish showing signs of regurgitation, with food in their mouth or evaginated stomach, are often discarded during sea sampling, it is impossible to determine if the collected stomachs classified as empty are from individuals that had not fed recently or if the contents were regurgitated. Partial regurgitation increases uncertainty in observed contribution of different prey types to the diet.

Under the paradigm "you are what you eat" (*e.g.*, Bradshaw *et al.*, 2003; Bundy *et al.*, 2011), it is recommended to use trophic biomarkers to complement the direct examination of stomach contents to infer trophic relationships (*e.g.*, Bowen & Iverson, 2013; Dalsgaard *et al.*, 2003; Peterson & Fry, 1987; Pethybridge *et al.*, 2018). Different biochemical tracers contribute different types of information about diets. Isotopic compositions are commonly used to delineate trophic structure and examine the ecological dynamics of communities, whereas FA analyses, on the contrary, are used primarily to assess the most important food sources (*e.g.*, Dalsgaard *et al.*, 2003; Peterson & Fry, 1987; Pethybridge *et al.*, 2018). Thus, the analysis of FA profiles has emerged as a tool to provide additional clues about feeding habits and diet assimilation in predators like fish (Dalsgaard *et al.*, 2003; Iverson *et al.*, 2004). FAs are the main molecular building blocks of most of lipids, and some such as the longer, unsaturated chains are transferred in a conservative manner when passing from producer to consumer organisms in the form of neutral lipids (energetic reserve of lipid stores) before integration in polar lipids (structural lipids having physiological functions) (Budge *et al.*, 2006; Dalsgaard *et al.*, 2003; Iverson, 2009; Tocher, 2003). Furthermore, most marine consumers cannot synthetize certain essential fatty acids (EFAs) in sufficient amount to meet their physiological needs (Parrish, 2013), implying that they must be acquired through diet. As these are stored unaltered and accumulate over time, feeding habits of a predator might be inferred by the FA composition, providing information on prey items ingested over a period that can reach several weeks, depending on the tissue sampled and metabolism of the species (*e.g.*, Budge *et al.*, 2006; Dalsgaard *et al.*, 2003; Fraser *et al.*, 1989; Iverson, 2009; Iverson *et al.*, 2004; Kirsch *et al.*, 1998; Parrish *et al.*, 2000; Pethybridge *et al.*, 2018). Thus, the FA integration period, *i.e.*, the turnover rate of FAs, depends on the ability of different tissues to accumulate lipids and varies based on temperature and predator physiological traits, such as the energy requirements or their reproductive status (Budge *et al.*, 2011; Dalsgaard *et al.*, 2003; Kirsch *et al.*, 1998). The appropriate samples useful in diet determination are tissues that serve as a fat energy depot (neutral lipids), such as blubber or liver (Budge *et al.*, 2006; Iverson, 2009; Budge *et al.*, 2011). Tissues, such as skin, that contain more structural FAs (polar lipids) should be avoided for diet studies. Moreover, understanding the metabolic role of FAs stored in predators is an important consideration for the use of FAs in studying food webs (Iverson, 2009). For example, species with a high metabolic rate and limited space to store energy reserves will have faster energy lipid replacement, as demonstrated for example in copepod or krill species (Budge *et al.*, 2006; Norrbin *et al.*, 1990). Large organisms have lower metabolic requirements relative to their mass than small ones (Garvey & Whiles, 2016). Therefore, the analysis of FA profiles has now been applied to several marine predatory species, such as fish and marine mammals (*e.g.*, Bradshaw *et al.*, 2003; Budge *et al.*, 2006; Drazen & Sutton, 2017; Iverson, 2009; Parrish *et al.*, 2015; Parzanini *et al.*, 2018; Pethybridge *et al.*, 2018; Couturier *et al.*, 2020; Jackson *et al.*, 2021).

In the Gulf of St. Lawrence (GSL), an inland sea of the north-west Atlantic Ocean, two sympatric redfish species coexist in the deep waters: the Acadian redfish, *Sebastes fasciatus* (Storer 1854) and the deep-water redfish, *Sebastes mentella* (Travin 1951) (Senay *et al.*, 2022). These two species are morphologically 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 principal species are referred to as redfish (*Sebastes* sp.) hereafter. After a 20-year period of low abundance, redfish have rebounded to record levels with the strong recruitment of three consecutive annual cohorts in 2011–2013 to become the most abundant demersal fish species in the region, accounting for more than 80% of the total biomass sampled in the northern Gulf of St. Lawrence (nGSL) bottom-trawl scientific survey

(Bourdages *et al*., 2022). Genetic analysis performed on the most abundant 2011 cohort indicated that 91% of these fish were *S. mentella* (Senay *et al*., 2021). This sudden resurgence of redfish is expected to have important implications for the nGSL ecosystem, including massive predation on its main prey species, increased food supply for its predators and increased competition with several other groundfish species (*e.g*., Brown-Vuillemin *et al*., 2022; Senay *et al*., 2021).

To date, the description and quantification of prey in redfish diet of the nGSL, as well as most studies in the North Atlantic, have been performed with SCA (*e.g*., Brown-Vuillemin *et al*., 2022; González *et al*., 2000; Ouellette-Plante *et al*., 2020; Steele, 1957). Stomach samples of nearly 7000 individuals made it possible to perform a detailed comparison of diet composition between a period of low redfish abundance (1993–1999) and the recent period of redfish resurgence (2015–2019), despite the large proportion of empty stomachs typical from a deep-water physoclist species (Brown-Vuillemin *et al*., 2022). Identification of stomach contents for the period of record abundance (2015–2019) showed that zooplankton, principally copepods of the genus *Calanus*, represented the main prey category for small (<20 cm) redfish. With increasing size, redfish shifted to larger prey items. Notably, shrimp consumption increased when redfish reached 25 cm and became dominant for fish ≥30 cm. Large redfish (≥30 cm) preyed on two shrimp species in particular: pink glass shrimp (*Pasiphaea multidentata*) and northern shrimp (*Pandalus borealis*) (Brown-Vuillemin *et al*., 2022; Ouellette-Plante *et al*., 2020; Senay *et al*., 2021). These studies suggest that the large 2011–2013 recruitment will have an increasing impact on shrimp populations and also compete with other resident groundfish stocks of the nGSL, such as Greenland halibut (*Reinhardtius hippoglossoides*) and Atlantic cod (*Gadus morhua*).

The objective of this study was to describe the feeding ecology of redfish in the nGSL ecosystem with the use of short-term information from SCA and mid-term diet composition estimated from the analysis of FA profiles in redfish liver tissue collected in August. By examining redfish stomach and FA composition for different size classes and subareas of the nGSL, this study is the first to document redfish diet with the combination of both methods.

2 | MATERIALS AND METHODS

2.1 | Study area and sample collection

Redfish were sampled on the 2017 August randomly depth-stratified trawl survey conducted by Fisheries and Oceans Canada (DFO) and covering the estuary and nGSL. Three distinct subareas were distinguished in this study: (a) the deepest part of the Laurentian Channel (LC) which extends from Cabot Strait to the centre of the nGSL, (b) the north-east Gulf (NEG) including the Esquiman and Anticosti Channels and (c) the north-west Gulf (NWG) comprising the estuary and the western part of the LC (Figure 1). The survey vessel, CCGS *Teleost*, was equipped with a *Campelen 1800* trawl with a 13 mm net liner. Details of bottom-trawl surveys, sampling and protocol can be found in Bourdages *et al*. (2018). For each haul, individuals were selected from a sample of the redfish catch and were stratified by length classes, and only those with no signs of regurgitation or feeding within the trawl were retained. Fish manipulations were carried out based on the recommendation of the Canadian Council of Animal Protection (Batt *et al*., 2005). Each redfish was measured (fork length, FL in millimetre, converted into centimetre for this paper) and weighed (g) upon capture.

For this study, a total of 350 redfish were targeted for stomach content and FA profile analyses (Table 1). Because FAs are transported to the liver (Brindley, 1991), it is the primary organ of lipid deposition and storage. Thus, to investigate redfish FA profiles, at least 500 mg (estimated visually) of liver tissue was collected and immediately preserved in a dichloromethane:methanol solution (2:1, v:v) (Folch *et al*., 1957; Meyer *et al*., 2017). Both samples (stomach and liver) were immediately frozen (−20 and −80°C for stomachs and livers, respectively) with a unique identification label until analysis in the laboratory. In parallel, eight different main prey species, already identified as important prey of redfish (Brown-Vuillemin *et al*., 2022), were randomly collected in catches during the same trawl survey and frozen whole (−80°C) for determination of their FA profiles. Targeted prey species were two fish species, capelin (*Mallotus villosus*) and redfish (*Sebastes* sp.), two shrimp species, northern shrimp (*P. borealis*) and pink glass shrimp (*P. multidentata*), three amphipod species of genus *Themisto* (*Themisto compressa*, *Themisto libellula* and *Themisto abyssorum*) and one copepod genus (*Calanus* sp.).

2.2 | Redfish stomach content analyses

In the laboratory, each stomach was thawed, dissected and examined. Empty stomachs ($n = 137$, Table 1) were excluded from further analyses. All prey in non-empty stomachs ($n = 213$, Table 1) were sorted, weighed and identified to the most precise taxonomic level possible using a binocular microscope and keys and identification guides (*e.g*., Campana, 2004; ICES, 2014; Squires, 1990; Vassilenko & Petryashov, 2009) by personnel at the Maurice Lamontagne Institute, led by Claude Nozères (DFO).

2.3 | Analysis of fatty acid profiles from redfish liver and prey samples

Lipids were extracted from redfish liver samples based on the modified Folch procedure (Folch *et al*., 1957), as described in Parrish (1999) and designed as a classic and robust method for the recovery of lipids from marine tissues (Couturier *et al*., 2020). Lipids were extracted by grinding with a dichloromethane/methanol (CH_2Cl_2:MeOH) solution (2:1, v:v). Lipid phases (neutral and polar) were separated by centrifugation of dichloromethane/methanol solutions for 2 min at 2000 rpm. For the 350 redfish livers (Table 1), only neutral lipids were retained, which in marine fishes constitute predominantly an energetic lipid reserve and are preferred for resolving dietary contributions of different prey items because they reflect trophic

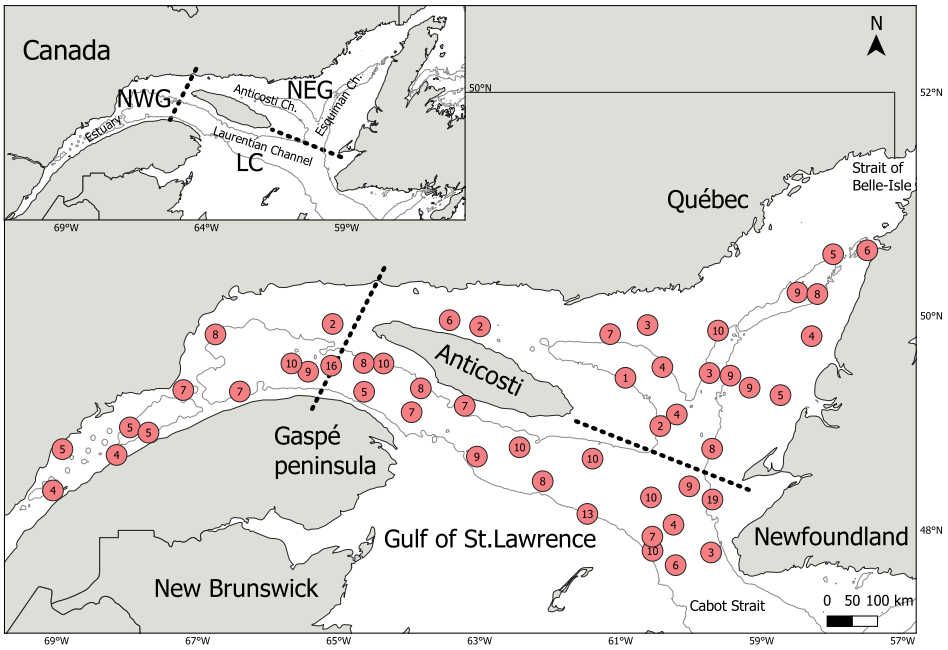

FIGURE 1 Map of the study area showing sampling hauls ($n = 50$) with the number of redfish (*Sebastes* sp.) stomachs and livers sampled ($n = 350$) during the 2017 August trawl survey in the Gulf of St. Lawrence. The grey line indicates the 250 m depth isobath. The three subareas considered for the analysis are delimited by the dotted lines with north-west Gulf (NWG), Laurentian Channel (LC) and north-east Gulf (NEG)

TABLE 1 Number of redfish liver tissues and stomachs with the percentage of empty stomachs and the number of stomachs containing prey collected during the 2017 August trawl survey in the northern Gulf of St. Lawrence based on three redfish size classes and subareas

Size class	Liver tissue and stomach collected and analysed	Liver for FA analysis			% empty stomach	Stomach containing prey			
		NWG	LC	NEG		Total	NWG	LC	NEG
<20	159	47	67	45	36	102	24	48	30
20–30	96	28	36	32	33	64	21	26	17
≥30	95	7	61	27	51	47	4	27	16
Total	350	82	164	104	39	213	49	101	63

Abbreviations: FA, fatty acid; LC, Laurentian Channel; NEG, north-east Gulf; NWG, north-west Gulf.

influences (Dalsgaard *et al.*, 2003; Fraser *et al.*, 1989; Parrish *et al.*, 1995). The neutral fraction was retrieved on silica gel columns hydrated with 6% deionized water. Columns were preconditioned using 10 ml of methanol and 10 ml of dichloromethane before the elution of neutral lipids using 10 ml of a dichloromethane/methanol solution (98:2, v:v) (Marty *et al.*, 1992). For prey, FAs were extracted from the whole animal, as eaten by redfish, after homogenization in a blender. As the objective was to characterize the FA composition of prey organisms, analyses of total (*i.e.*, including both neutral and polar fractions) lipids were performed (Iverson *et al.*, 1997; Kirsch *et al.*, 1998). Each prey species was treated in four or five replicates. For larger species (capelin, redfish and northern and pink glass shrimp) one individual was used per replicate and *c.* 1 g of homogenate was taken to determine FA profiles. For prey with an individual mass of less than 1 g (*Themisto* sp. and *Calanus* sp.), several individuals were taken together per replicate to obtain sufficient sample mass. Fatty acid methyl esters (FAME) were prepared based on the method described by Lepage and Roy (Lepage & Roy, 1984) using sulphuric acid and methanol (2:98, v/v) at 100°C for 10 min. Finally, lipid extracts were purified on silica gel columns using 3 ml of hexane and diethyl ether (1:1, v:v) to remove free sterols (Mejri *et al.*, 2014).

FAME solutions were analysed using gas chromatography–mass spectrometry (Thermo Fisher Scientific Inc., GC model Trace GC Ultra and MS model ITQ900, Mississauga, ON, Canada) equipped with a Supelco Omegawax 250 capillary column (30 m × 250 μm × 0.25 μm film thickness, Bellefonte, PA, USA) at Institut des Sciences de la Mer (ISMER) of the Université du Québec à Rimouski (UQAR). Initial oven temperature was 100°C for 2 min. Temperature was then increased to 140°C for 1 min, after which it increased at a rate of 10°C min^{-1} until it reached 270°C for 15 min. Injector temperature was 90°C, and a constant helium flow of 1.0 ml min^{-1} was used. A volume of 1 μl was injected. FAs were then identified by comparing retention times and mass spectrum with known standards calibration curve with concentration ranging from 0.5 to 20 μg ml^{-1} (Supelco 37 Component FAME Mix Supelco Inc., Bellefonte, PA, USA). FA peaks were then quantified using the Xcalibur v.2.1 software (Thermo Scientific, Mississauga, ON, Canada) and described using the standard shorthand nomenclature of C:DnX, where C is the number of carbon atoms, D is the number of double bonds and nX indicates the position of the double bond closest to the terminal methyl group (n? was used when the position was unknown). A total of 19 FAs were reported in prey and redfish samples and were expressed as the mean percentage (%FA) of total

FA. Reporting results per gram of liver tissue was not possible because liver sample could not be weighed with sufficient precision while at sea.

2.4 | Data analysis

2.4.1 | Redfish size and spatial analysis

Redfish stomach data and FA composition were analysed as a function of redfish size using three major classes: small (<20 cm), medium (20–30 cm) and large redfish (≥30 cm), and based on three subareas: LC, NEG and NWG (Figure 1 and Table 1).

2.4.2 | Diet composition

SCA is described in detail in Brown-Vuillemin *et al.* (2022). Briefly, to assess the contribution of a prey to the diet of redfish, the mean partial stomach fullness index of prey i (PFI_i) (Lilly & Fleming, 1981; Orr & Bowering, 1997) transformed into a percentage ($\%FI_i$, percentage

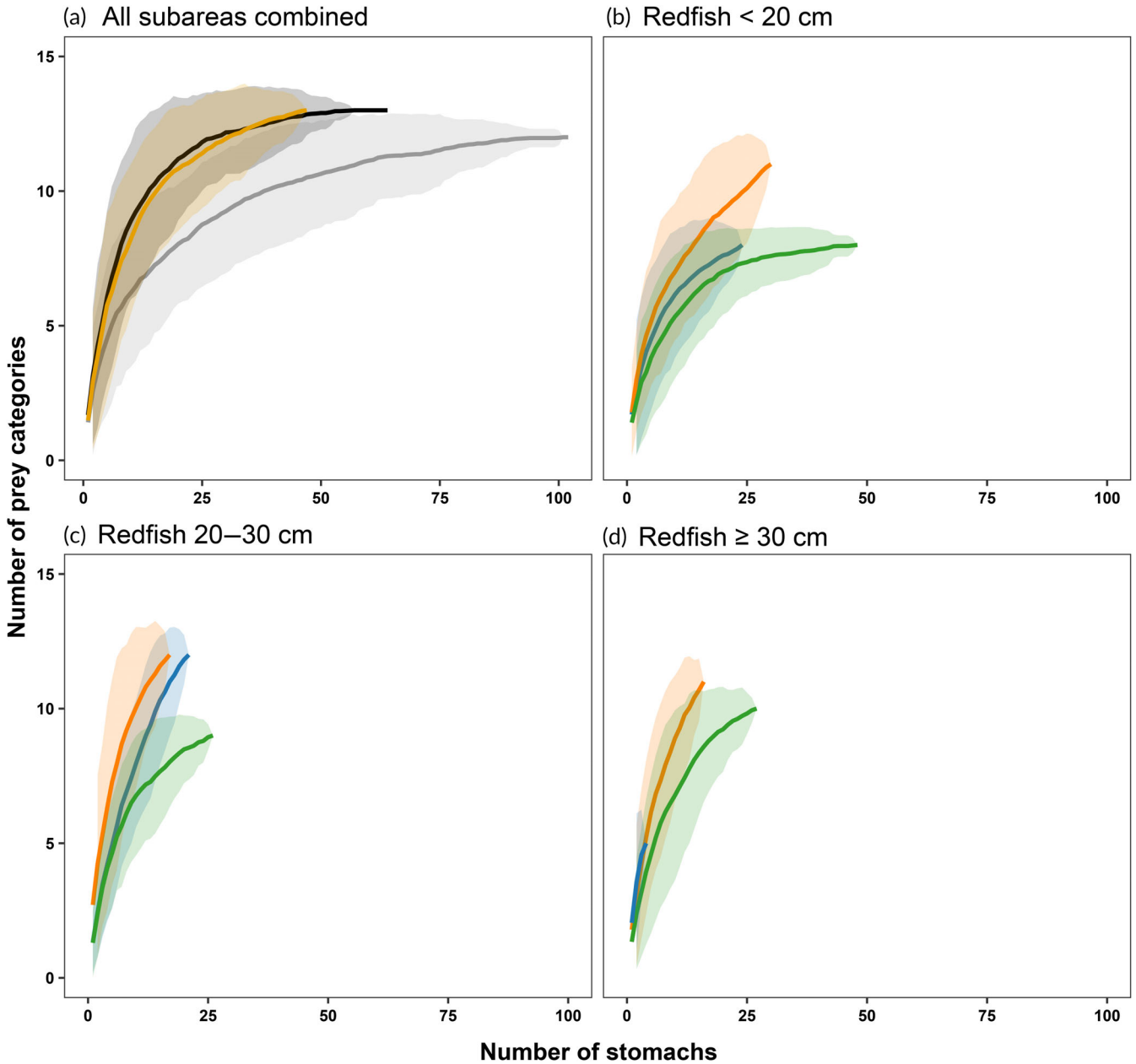

FIGURE 2 Cumulative prey category–stomach curves and C.I. of 95% upper and lower with *b* values through the last five sub-samples for (a) three size classes and (b–d) based on each size class and subarea for stomach content data. (a) ——, <20 cm ($b = 0.005$); ——, <20–30 cm ($b = 0.000$); ——, ≥30 cm ($b = 0.041$); (b) ——, NWG ($b = 0.114$); ——, LC ($b = 0.026$); ——, NEG ($b = 0.170$); (c)——, NWG ($b = 0.242$); ——, LC ($b = 0.098$); ——, NEG ($b = 0.238$); (d) ——, NWG ($b = 0.987$); ——, LC ($b = 0.108$); ——, NEG ($b = 0.373$). LC, Laurentian Channel; NEG, north-east Gulf; NWG, north-west Gulf

fullness index, Bernier & Chabot, 2012) was calculated for each prey taxa in the redfish stomach. Equations used are available in Supporting Information Table S1.

To visualize and interpret the relationship between the relative composition (% of total) of individual FA in redfish among size classes and subareas, non-parametric multidimensional scaling (nMDS) ordinations were performed (Clarke & Warwick, 2001). The Bray–Curtis dissimilarity measure (Legendre & Legendre, 2012) was used to assess groupings within the data set. The same procedure was conducted to analyse %FA data of potential prey item sources. To test for differences among factors, non-parametric distanced-based permutation multivariate analysis of variance (PERMANOVA) was conducted (Anderson, 2014). After significant PERMANOVA results ($P < 0.05$), multiple pair-wise comparisons were used to identify differences ($P < 0.05$). Similarity percentage analysis (SIMPER, Clarke, 1993) was used to identify which FA contributed most to dissimilarities among factors. The authors designated a cut-off of FA that characterized up to 80% of dissimilarities. All analyses were conducted using the R software version 4.0.1 (R Core Team, 2020) and packages "vegan" (Oksanen *et al*., 2019), "ggplot2" (Wickham, 2016) and "ggpubr" (Kassambara, 2020).

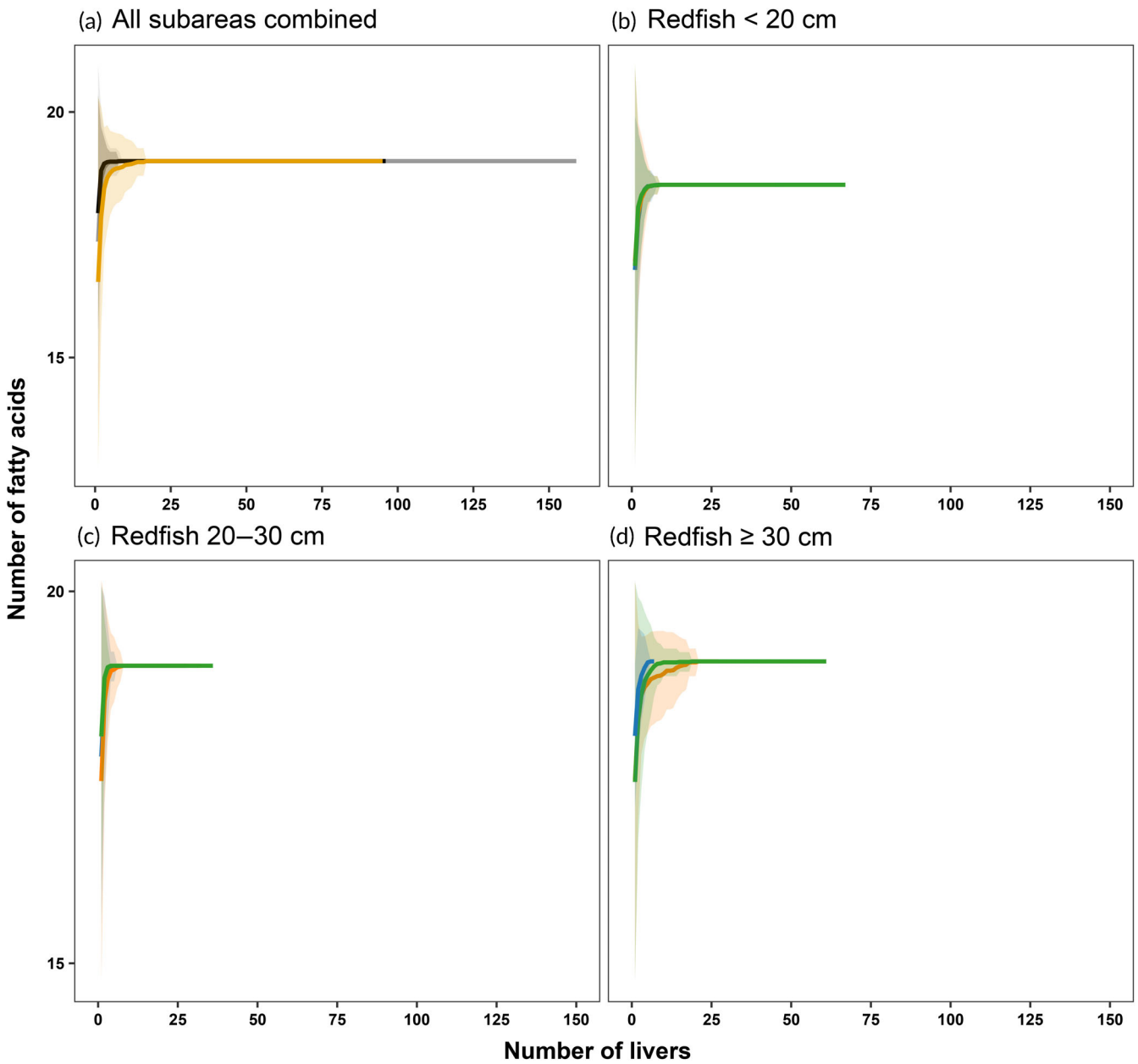

FIGURE 3 Cumulative curves of liver fatty acid and C.I. of 95% upper and lower with *b* values through the last five sub-samples for (a) three size classes and (b–d) based on each size class and subarea for fatty acid data. (a) ——, 20 cm ($b = 0.000$); ——, 20–30 cm ($b = 0.000$); ——, ≥30 cm ($b = 0.000$). (b-c) ——, NWG ($b = 0.000$); ——, LC ($b = 0.000$); ——, NEG ($b = 0.000$). (d) ——, NWG ($b = 0.114$); ——, LC ($b = 0.000$); ——, NEG ($b = 0.000$). LC, Laurentian Channel; NEG, north-east Gulf; NWG, north-west Gulf

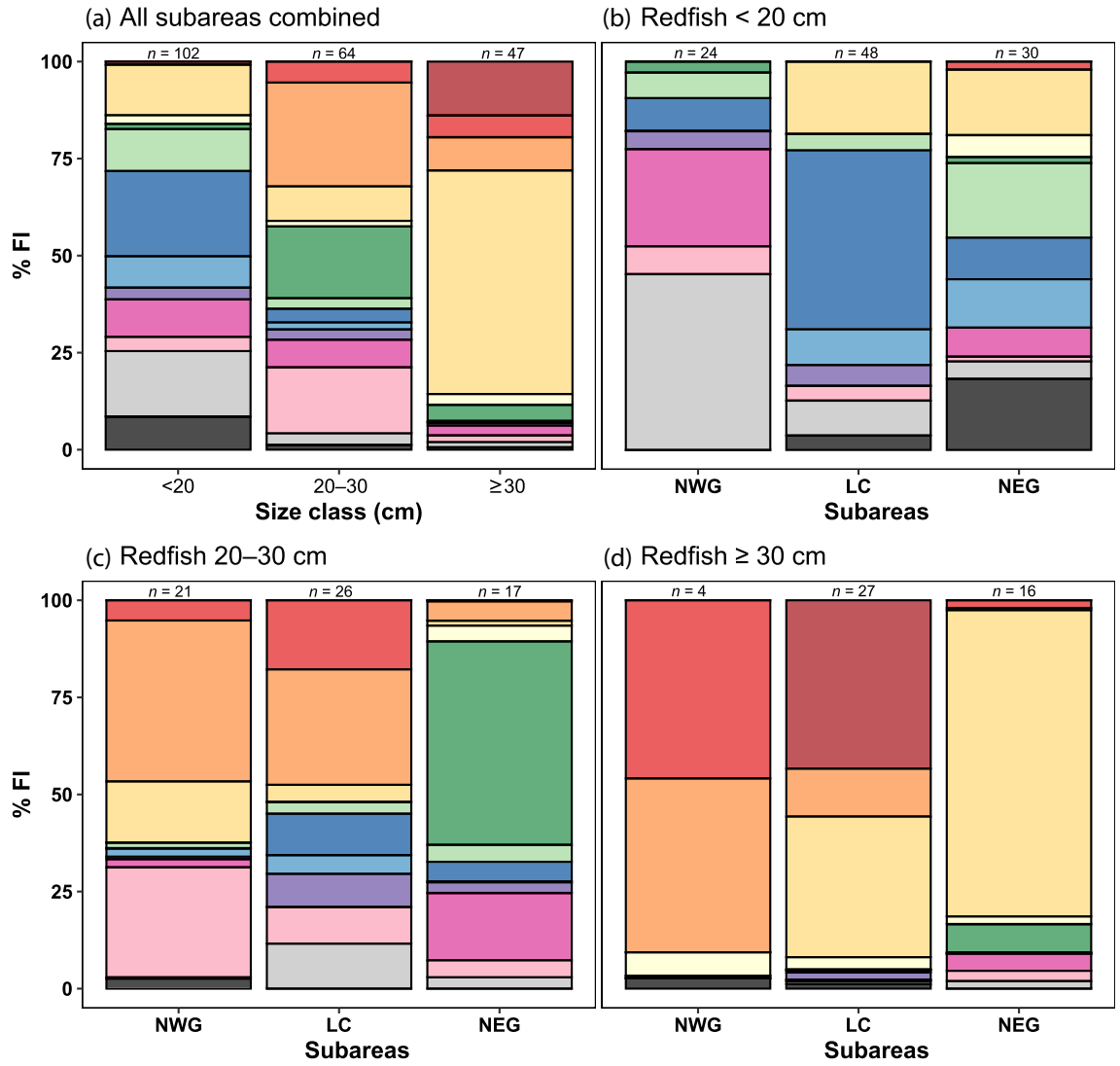

FIGURE 4 Contribution of the 14 prey categories to redfish diet in visual examination of stomach contents, expressed as percentage of fullness index (%FI) for (a) three redfish size classes in all subareas combined and (b–d) based on each size class and subarea. Stomach sample size is indicated on each panel. Prey categories: ■, *Sebastes* sp.; ■, Other Fish; □, *P. multidentata*; □, Other Shrimp; ■, *Themisto libellula*; □, Other Amphipods; ■, *Calanus* sp.; ■, Mysids; ■, *Thysanoessa* sp.; ■, *Pandalus borealis*; ■, Other Copepods; ■, Other Euphausiids; □, Other Invertebrates; ■, Unidentified Material

2.4.3 | Sample size sufficiency

Cumulative curves (Ferry & Cailliet, 1996) were calculated to assess whether the number of redfish samples was sufficient to describe the diet identified with SCA and analysis of FA profiles. Cumulative prey curves plot the total number of prey categories or FA found *vs.* the total number of stomachs or livers analysed. Sample size was considered sufficient once the curve reached an asymptote, and the slope of the linear regression (*b*) through the last five sub-samples was ≤0.05, which signified acceptable levelling off of the prey curve for diet analyses (Brown *et al.*, 2012; Ferry & Cailliet, 1996). Curves were computed after 100 randomizations of the original data (%FI or %FA) to generated means and associated 95% C.I.

3 | RESULTS

3.1 | Sample size sufficiency

The sufficient sample size was much greater for stomachs (SCA) than for livers (FA). Prey category–stomach curves reached a stable asymptote for small (<20 cm, $b = 0.005$) and medium (20–30 cm, $b = 0.000$) redfish but was not yet completely stable ($b = 0.041$) for the large redfish that had the fewest redfish number of samples ($n = 47$; Figure 2a). When examined by size class and subarea, only small redfish (<20 cm) in LC, which were twice as many samples as any other group, produced a curve nearing the asymptote ($b = 0.026$), thus indicating they were sufficient for a robust description of regional diet for

a given size class (Figure 2b–d). In contrast to the stomach content data, curves of liver FA performed adequately and reached more rapidly a stable asymptote ($b = 0.000$) for most of the size class – subarea combinations (Figure 3), with the exception of large redfish (≥30 cm) in NWG ($b = 0.114$), because of the scarcity of this size class in this subarea ($n = 7$; Figure 3d). Overall, the prey category–stomach curves suggested that at least 50 redfish stomachs by group were needed to approach asymptote, whereas the curves of liver FA suggested that only about 10 samples are sufficient for a representative sample.

3.2 | Stomach content composition of redfish

A total of 32 different prey types were identified in redfish stomachs (Supporting Information Table S2). Only six main prey specific taxa identified at the genus or species level had important dietary contributions (taxon identified by an asterisk, Supporting Information Table S2) and were retained in the following analyses: *Sebastes* sp., *P. borealis*, *P. multidentata*, *T. libellula*, *Calanus* sp. (*Calanus hyperboreus* was grouped into the genus) and *Thysanoessa* sp. Prey taxa less important in the diet were assigned to one of eight broad categories: other fish, other shrimp, other amphipods, other copepods, mysids, other euphausiids, other invertebrates and digested/unidentified material.

3.2.1 | Effect of redfish size and spatial variability on stomach composition

The relative importance of the six main specific taxa and eight broad prey categories, used for the following redfish diet description inferred from SCA, changed with redfish size (Figure 4). The main size-related shift observed was from zooplankton species in small (<20 cm) redfish to shrimp and fish in large (≥30 cm) redfish. There were also spatial differences in the relative contribution of the dominant prey categories across size classes, specifically for *Calanus* copepods, *Themisto* amphipods, *Thysanoessa* krill, *Sebastes* fish, *P. borealis* and *P. multidentata* shrimp (Figure 4).

The diet of small (<20 cm) redfish was dominated by copepods (%FI = 30), principally with *Calanus* sp. (%FI = 22). Other major prey categories were *P. multidentata* shrimp, amphipods and *Thysanoessa* krill (%FI = 13; 12 and 10, respectively). Taking subareas into account, there were high contributions of *Thysanoessa* krill (%FI = 25) and other invertebrates (%FI = 45) in NWG, *Calanus* sp. in LC (%FI = 46) and a mix of amphipods (%FI = 21), shrimp (%FI = 23) and copepods (%FI = 23) in NEG.

A transition to shrimp was observed in the diet of medium (20–30 cm) redfish, with *P. borealis* as the main prey (%FI = 27). This was followed by the amphipod *Themisto libellula* (%FI = 19) and other euphausiids (digested euphausiidae and *Meganyctiphanes norvegica*, %FI = 17). *P. borealis* was mainly found in stomachs collected in NWG and LC (%FI = 41 and 30, respectively), whereas *T. libellula* was exclusively found in NEG and in very high amounts (%FI = 52).

The diet of large (≥ 30 cm) redfish was strongly dominated by shrimp and fish categories (%FI = 88). The shrimp *P. multidentata* was the major prey type consumed overall (%FI = 58) and was the predominant prey in NEG (%FI = 79). Cannibalism was observed in large redfish (%FI = 14), but this was only observed in LC (%FI = 43). Stomachs from NWG were dominated by other fish (mainly *A. risso*) (%FI = 46) and the shrimp *P. borealis* (%FI = 45), but these results are based on only four redfish.

3.3 | Fatty acid profiles of prey

Multivariate analyses were performed on the 19 FAs recorded in prey samples (Supporting Information Table S3). Significant variations in FA composition were found among the different prey ($F_{7,30} = 17.13$; $P = 0.001$). Overall, the following 13 FAs were responsible for 80% of the dissimilarity among prey (SIMPER analyses): 14:0, 16:0, 18:0, 20:0, 16:1n7, 17:1 n?, 18:1n9, 20:1 n?, 22:1n9, 24:1n9, 18:2n6, 20:5n3 and 22:6n3 (Figure 5). All prey species differed significantly from each other in their FA composition, except that *Sebastes* sp. could not be distinguished from *M. villosus*, *T. compressa* and *T. abyssorum* ($P > 0.05$; Supporting Information Table S4). Pair-wise analyses and nMDS showed that *Calanus* sp. was largely differentiated from other prey taxa and was associated mainly with the monosaturated FA (MUFA) 16:1n7 and the polyunsaturated FA (PUFA) 20:5n3 (26% and 12%, respectively; Supporting Information Table S3). Similarly, the two shrimp species *P. borealis* and *P. multidentata* were differentiated from other prey species mainly due to the influence of the MUFA 24:1n9 (2.8% and 3.7%, respectively) and the PUFA 18:2n6 (2.5% and 3.6%, respectively) and 22:6n3 (6.1% and 6.8%, respectively; Supporting Information Table S3). The amphipod *T. libellula* was also distinct from other prey taxa (pair-wise $P < 0.05$) and seemed to be associated with SFA 14:0 (7.8%) and MUFA 20:1n? (12.3%; Supporting Information Table S3).

3.4 | Fatty acid profiles of redfish livers and relation with prey

The FA profiles of all livers (350 redfish, Table 1) were dominated by MUFA, which comprised 75% of FA contents. The 18:1n9 (28.4% of the total FA) was the main FA, followed by 22:1n9 (17.8%), 20:1n? (16.4%), 16:1n7 (11.2%) and the saturated FA (SFA) 16:0 (8.9%) (Supporting Information Table S5).

3.4.1 | Effect of redfish size on fatty acid signatures

Multivariate analysis showed variations in FA composition among the three redfish size classes ($F_{2,347} = 68.11$; $P = 0.001$) (Figure 6a). Pair-wise comparisons indicated differences ($P = 0.001$) among all size classes (Supporting Information Table S5). SIMPER analyses identified six FAs that explained at least 80% of the dissimilarities among size

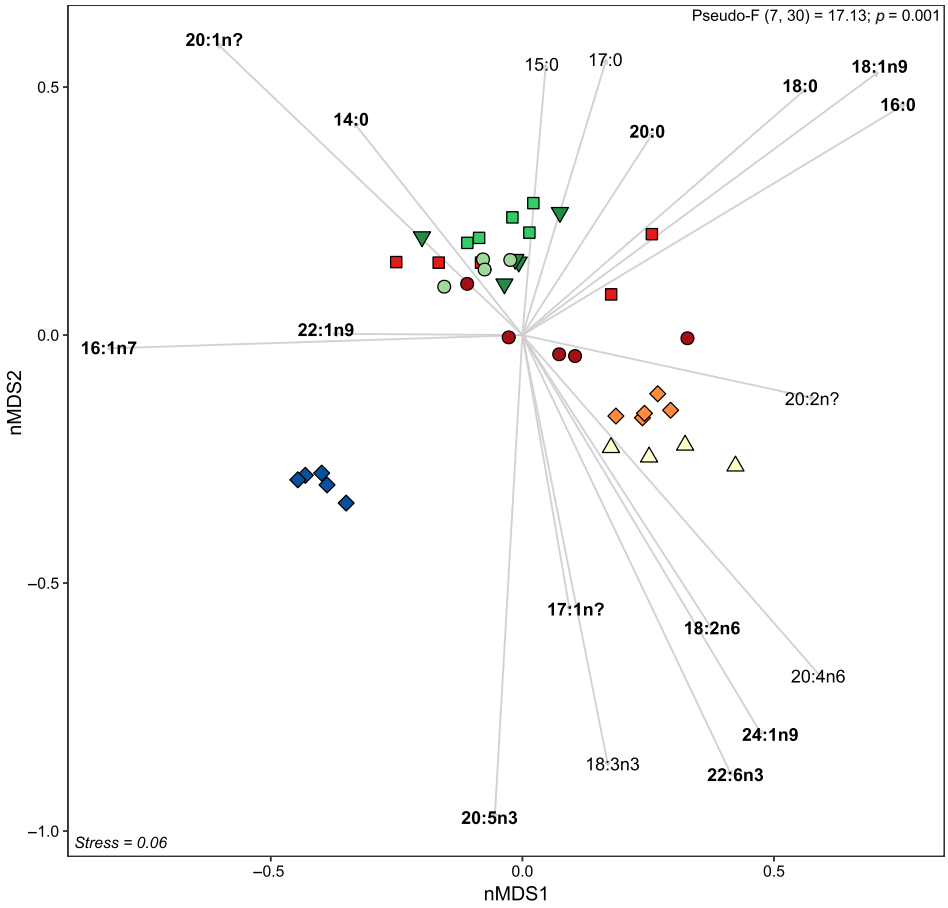

FIGURE 5 Non-parametric multidimensional scaling (nMDS) ordinations and -parametric distanced-based permutation multivariate analysis of variance (PERMANOVA) results (pseudo-F with degrees of freedom and residuals) of the fatty acid composition in the prey species. FA in bold represent up to 80% of dissimilarities (SIMPER analyses). Prey: ●, *Mallotus villosus*; ■, *Sebastes* sp.; ◆, *Pandalus borealis*; △, *Pasiphaea multidentata*; ▼, *T. compressa*; ○, *Themisto libellula*; ■, *T. abyssorum*; ◆, *Calanus* sp.

classes including 16:0, 16:1n7, 18:1n9, 20:1n?, 22:1n9 and 20:5n3. According to nMDS, the FA signatures of small (<20 cm) redfish were associated with the MUFA 22:1n9 and 20:1n? (21.7% and 18.4%, respectively, Supporting Information Table S5) where 20:1n? could be linked with the profile of *T. libellula* (Figures 5 and 6a). The FA signatures of medium (20–30 cm) redfish were influenced by the MUFA 16:1n7 (12.1%) and the PUFA 20:5n3 (5.9%), which were related to FA signatures of *Calanus* sp. (Figures 5 and 6a). The FA signatures of large (≥ 30 cm) redfish were influenced by the MUFA 18:1n9 (36.7%), followed by the SFA 16:0 (11.2%) which could be linked with FA profiles of fish prey more than those of amphipods (Figure 5 and 6a), based on the knowledge acquired through the SCA (Figure 4 and Brown-Vuillemin *et al.*, 2022). To a lesser extent, the FA signatures of large redfish also seemed influenced by the FAs that were instrumental in discriminating the shrimp species *P. borealis* and *P. multidentata* from other prey species (18:2n6 and 22:6n3, Figures 5 and 6a).

3.4.2 | Spatial variability on redfish fatty acid signatures

Multivariate analysis performed on redfish livers from different subareas revealed spatial differences in the relationship between FA composition and fish size ($P < 0.05$; Figure 6b–d). For small (<20 cm) redfish, pair-wise comparisons showed that all subareas were different ($P < 0.05$; Supporting Information Table S6). nMDS revealed that the MUFA 20:1n? and 22:1n9 were associated with small redfish from the LC subarea (12.5 and 19.3, respectively) (Figure 6b and Supporting Information Table S5). For medium-sized (20–30 cm) redfish, pair-wise comparisons did not reveal differences between NWG and NEG, but both these subareas were different from LC (Supporting Information Table S6). According to nMDS, medium redfish from LC were associated with the MUFA 16:1n7 and the PUFA 20:5n3 (13.1% and 6.0%, respectively) (Figure 6c). For large (≥30 cm) redfish, pair-wise comparisons indicated that only NWG was significantly different from the two other subareas, due to high a MUFA 18:1n9 contribution (44.5%) (Figure 6d). For each size class, the MUFA 22:1n9 (24%, 20% and 12% for <20, 20–30 and ≥30 cm, respectively) and 20:1n? (19%, 18% and 14% for <20, 20–30 and ≥30 cm, respectively) was always associated to the subarea LC, whereas the MUFA 18:1n9 was always associated with the subarea NWG (27%, 32% and 45% for <20, 20–30 and ≥30 cm, respectively) (Supporting Information Table S5).

4 | DISCUSSION

To the authors' knowledge, this study constitutes the first attempt of combining FA with SCA to assess the diet of redfish. As a boreal

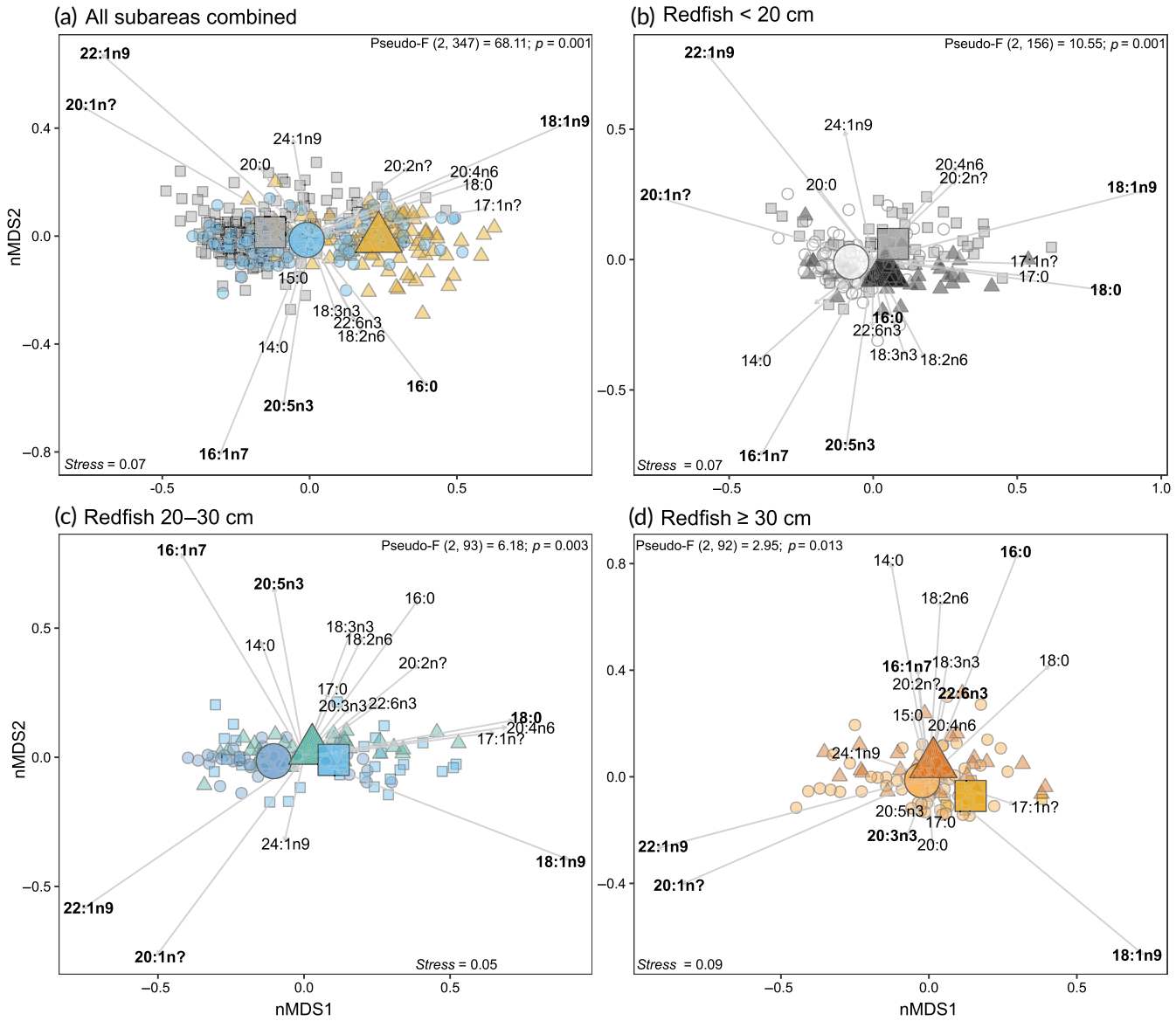

FIGURE 6 Non-parametric multidimensional scaling (nMDS) ordinations and -parametric distanced-based permutation multivariate analysis of variance (PERMANOVA) results (pseudo-F with degrees of freedom and residuals) of the fatty acid composition in redfish livers for (a) three redfish size classes in all subareas combined and (b–d) based on each size class and subarea. FA in bold represent up to 80% of dissimilarities (SIMPER analyses). The centroid for each size class and subarea is represented by the largest symbol. (a) Size class: ■, <20; ●, 20–30; ▲, ≥30. (b) Subarea: ■, NWG; ●, LC; △, NEG (c) subarea: ■, NWG; ●, LC; ▲, NEG (d) subarea: ■, NWG; ●, LC; ▲, NEG. LC, Laurentian Channel; NEG, north-east Gulf; NWG, north-west Gulf

species characterized by relatively low metabolism compared to temperate species exposed to higher temperature, and with a life history involving the accumulation of significant energy reserves, the FA composition of energy reserve lipids in the liver could represent a longer-than-average integration period for redfish (Budge *et al*., 2011; Dalsgaard *et al*., 2003; Kirsch *et al*., 1998). By combining both methods of redfish diet determination and the database of FA composition for the most important redfish prey in nGSL, the authors were able to take advantage of FA as a biochemical tracer and infer diet over a longer time period, probably in the order of several weeks, compared to a few days for SCA.

SCA indicated that redfish captured in 2017 shifted from a zooplankton-based diet to a shrimp- and fish-dominated diet as the size increased, a result consistent with trends described by Brown-Vuillemin *et al*. (2022) for the periods 1993–1999 and 2015–2019. Small zooplankton with copepods (*Calanus* sp.) and macrozooplankton like amphipods (*Themisto* sp.) and euphausiids (especially *Thysanoessa* sp.) dominated the diet of small redfish (<20 cm) and showed decreasing importance with increasing predator size (20–30 cm) as redfish shifted to shrimp (*P. borealis* and *P. multidentata*) and small redfish (cannibalism) in the diet of large (≥30 cm) redfish. FA analyses confirmed size-related changes in diet of redfish in this study.

Multivariate analysis of redfish FA composition showed that a sub-set of FA accounts for a large part of the variation in redfish FA signatures, suggesting dietary changes that can be reported to prey FA signatures. Based on comparisons of prey-redfish FA results, small- and medium-sized redfish (<20 and 20–30 cm) showed FA signatures similar to small and macrozooplankton trophic markers (16:1n7, 20:1n?, 22:1n9 and 20:5n3), whereas large redfish (≥ 30 cm) seemed to be influenced by 18:1n9 and 16:0 (probably fish prey) and showed shrimp-related FA signatures (18:2n6 and 22:6n3).

Similar size-related shifts in redfish diet composition observed in the nGSL (Ouellette-Plante *et al*., 2020; Senay *et al*., 2021; Brown-Vuillemin *et al*., 2022; this study) have also been shown through SCA for other redfish populations (Albikovskaya & Gerasimova, 1993; Dolgov & Drevetnyak, 2011; González *et al*., 2000). Size-related dietary transition from small to large prey items is commonly observed in fishes and is generally attributable to a combination of factors, such as the increase in gape opening and swimming ability (Cook & Bundy, 2010; Sánchez-Hernández *et al*., 2019), changes in energy requirements (Dwyer *et al*., 2010) and changes in habitat use because larger redfish generally occupy deeper waters than smaller ones (*e.g*., Planque *et al*., 2013; Senay *et al*., 2021).

In addition to size, there were local effects, with differences in diet among subareas observed with the two methods. Usually, diet differences shown by SCA between subareas were for species of a type, *e.g*., copepods *vs*. euphausiids or amphipods or shrimp *vs*. fish. This study showed that the FA composition of redfish changed with increasing size and also based on subarea. Feeding Atlantic cod (*Gadus morhua*) first with squid and then with Atlantic mackerel (*Scomber scombrus*), Kirsch *et al*. (1998) observed that cod FA composition became more similar to that of Atlantic mackerel. Nonetheless, they also noted that cod maintained a specific FA signature different from that of their sole prey. Iverson *et al*. (1997) showed that predatory fish species can be easily differentiated from each other even when predators of similar size and located in the same area are characterized by similar diets. Thus, species-specific differences in the FA signatures of prey and predators support the use of FA signatures to study the diet of redfish, including for the description of size-related and spatial variability. Nevertheless, inferences may be complex to draw because of absolute differences among predator and prey FA signatures attributable to differences in predator metabolism (Iverson, 2009). In future redfish diet studies, stronger conclusion could be made based on long-term feeding trials in controlled conditions.

4.1 | Pelagic zooplankton consumption suggested by *Calanus*-type markers

There is a large body of information on the lipids of calanoid copepods that dominate the zooplankton biomass in several parts of the world's oceans, and which are particularly important in northern temperate and polar latitude pelagic food webs (*e.g*., Dalsgaard *et al*., 2003). Several authors showed that FA composition of calanoid copepods diverge from that of other copepods and other zooplankton species through a lower proportion of 16:0 and 18:0 SFA, which is supported by the results of this study (Supporting Information Table S3). They are also differentiated by their high content of 20:1 and 22:1 MUFA biosynthesized *de novo*, which only strictly herbivorous copepods such as calanoid species of the genus *Calanus* can do in considerable amounts (Brewster *et al*., 2018; Dahl *et al*., 2000; Dalsgaard *et al*., 2003; Falk-Petersen *et al*., 1987; Falk-Petersen *et al*., 1990; Fraser *et al*., 1989; Lee, 1974; Sargent, 1976). Consequently, high levels of $\sum 20:1+22:1$ MUFA (*Calanus*-type and pelagic marine feeding markers) have been used to trace and resolve food web relationships in, *e.g*., hyperiid amphipods, euphausiids and fish, which consume typically large quantities of calanoid copepods (Brewster *et al*., 2018; Dalsgaard *et al*., 2003; Falk-Petersen *et al*., 1987; Falk-Petersen *et al*., 2002; Meyer *et al*., 2019; Sargent, 1976). In this study, the sum of these FAs in the liver tissue of large redfish (24%) is almost half of that found in small redfish (40%), suggesting that the importance of copepods in redfish diet decreases with increasing fish size, possibly reflecting the decreasing payoff of hunting small prey for a larger fish, and/or a reduction in spatial overlap as larger redfish generally occupy deeper waters than smaller redfish. The finding of Voronin *et al*. (2021) offers evidence for the reduction in spatial overlap hypothesis, as MUFA in muscles, including dietary markers of zooplankton (copepods) 20:1 and 22:1, were lower in redfish *S. mentella* sampled at greater depths in the Irminger Sea. In the present study, multivariate analyses indicated that 16:1n7 and 20:5n3 were most useful in discriminating *Calanus* sp. from other potential redfish prey. As, 16:1n7 and 20:5n3 are FA trophic markers of diatoms (Budge & Parrish, 1998; Dalsgaard *et al*., 2003; Viso & Marty, 1993), these results could suggest high diatoms feeding by *Calanus* sp. in nGSL. In this study, 20:1n? was mostly associated to *T. libellula* according to multivariate analysis and could indicate that this species occupying the same food web consumes calanoid copepods in significant quantities in the nGSL, assuming that amphipods cannot biosynthesize 20:1 *de novo*. This probability of calanoid ingestion highlights the fact that no single FA can be assigned uniquely to any one particular species. This needs to be considered when using the FA approach to study specific trophic relationships in an uncontrolled environment and within a complex network (Brett *et al*., 2016).

Feeding on copepods by small (<20 cm) redfish was demonstrated through SCA and was particularly important in the LC subarea (%FI = 55%). Combining SCA and FA signatures, it seems likely that the large contribution of 20:1 and 22:1 MUFA to the FA profile of small redfish is the result of direct consumption of copepods in the LC subarea, rather than secondary ingestion of copepods through amphipods. The FA profiles of medium redfish showed contributions of 16:1n7 and 20:5n3, associated with *Calanus* FA signatures, particularly important in LC subarea. Because FA signatures integrate diet contributions over a longer time period, this could mean small and medium redfish have greater access to this calanoid resource earlier in the summer, whereas copepods were less available in August, when stomachs were sampled, due to the fact that *Calanus* sp. sink in the deep channels in the nGSL, diapausing from August to early spring (Dufour & Ouellet, 2007; Harvey *et al*., 2004).

Accumulation of eicosapentaenoic acid (EPA, 20:5n-3) could be particularly relevant for redfish because this essential FA, which cannot be synthesized *de novo* or in sufficient amounts by marine animals, was found to be retained in the muscles of fast-swimming fish in cold water (Meyer *et al*., 2019; Sargent *et al*., 1987, 2002). Furthermore, EPA is the precursor of several eicosanoids, which are signal molecules playing a role in modulating many biological and biochemical processes (Sargent *et al*., 2002; Tocher *et al*., 1996). Work by Dall *et al*. (1993) suggests that EPA plays an important role in tissue biosynthesis, and Rawn (1989) demonstrated that EPA is required for the synthesis of eicosanoid hormones, which have a wide range of functions, including regulation of steroid biosynthesis, inhibition of gastric secretions and stimulation of smooth muscle contraction. EPA was reported to be important to larval survival and development (Dickey-Collas & Geffen, 1992; Watanabe, 1982). Furthermore, EPA, like DHA, is incorporated in membrane phospholipids to maintain the structural and functional integrity of biological membranes, particularly in cold conditions (Hulbert & Else, 1999). The consumption of calanoids by small redfish may be a feeding trait originating from the larval stage (Burns *et al*., 2020; Burns *et al*., 2021) and a good source of EPA, with mean values observed here of 12% of their total FAs. Work by Burns *et al*. (2020) showed positive selection by redfish larvae on *C. finmarchicus* eggs, supporting the hypothesis of a strong link between larvae and a key calanoid copepod in the GSL ecosystem.

FA analysis thus generated some complementary hypotheses relative to the importance of pelagic zooplankton in the diet composition of small (<20 cm) and medium-sized (20–30 cm) redfish with the Calanus-type markers, but also highlighted the complexity to establish strong and direct trophic links with this method. Like in the example given above with cod diet (Kirsch *et al*., 1998), it would be relevant to understand the influence of FA on redfish by studying in a controlled environment how different diets are reflected in the FA profile of redfish and evaluate which dietary FA may be used for inferring diet.

4.2 | Validation of the predation pressure on shrimp with fatty acid signatures

In SCA, two specific shrimp species stood out in the diet of medium (20–30 cm) and especially of large (≥30 cm) redfish, northern shrimp (*P. borealis*) and pink glass shrimp (*P. multidentata*). These shrimp were the main prey of redfish ≥30 cm, making up to 68% (FI) of the diet. These results were supported by inferences from FA profiles, as contributions of 18:2n6 and 22:6n3 (docosahexaenoic acid, DHA) suggest an integration of shrimp to the diet of large (≥30 cm) redfish, especially in the deep LC and NEG subareas, where redfish catch rates are the highest and where the overlap with the distribution of northern shrimp suggests a strong predation impact (Bourdages *et al*., 2022; Senay *et al*., 2021). As an important food source for several demersal fish species (Parsons, 2005), shrimp form a link between the benthic infauna and higher trophic levels and represent a source of DHA for redfish, as their levels were observed around 7% of their total FAs. The long-chain n-3 PUFA DHA (22:6n3), which is linked with deep- and cold-water habitats (Meyer *et al*., 2019), could serve as a trophic marker for an important trophic link between shrimp and redfish in the nGSL. This FA is critical for neural and visual development in higher trophic-level marine organisms (Bell *et al*., 1999; Navarro *et al*., 1997; Sargent *et al*., 2002), by its involvement in neurotransmission, cell survival and neuro-inflammation prevention (Bazinet & Layé, 2014). DHA tends to increase with trophic level in the marine biome, by its transfer and selective retention when it is consumed (Colombo *et al*., 2017; Kainz *et al*., 2004; Twining *et al*., 2016). Contrary to EPA, which is highly retained in zooplankton species, DHA is highly retained in fish, suggesting that it is the primary synthesis of polyunsaturated FAs in most of marine fish species (Colombo *et al*., 2017). This is particularly important in cold environments, as DHA integration in phospholipid membranes counteracts the rigidity effect linked to low temperature, as already observed in fish (Dey *et al*., 1993; Logue *et al*., 2000; Mejri *et al*., 2021).

Redfish predation on shrimp and implications for the demersal community in the nGSL were raised in Brown-Vuillemin *et al*. (2022), and this new analysis based on FA suggests that shrimp consumption by large redfish is important all summer long and not just in August, when stomachs were collected. Nonetheless, unlike SCA, FA signatures do not allow the quantification of the relative predation mortality of *P. borealis* and *P. multidentata* due to large redfish. More specific studies on the interactions between redfish and shrimp need to be carried out.

4.3 | Complexity of marine food webs for fatty acid analysis

4.3.1 | Importance of a representative sampling

In the marine system, the FA biomarker approach is based on observations that phytoplankton, at the base of the food web, produces essential FA not biosynthesized by consumers, like the DHA and EPA, which are then deposited in species tissue (Budge *et al*., 2006; Dalsgaard *et al*., 2003) *via* trophic accumulation up the food chain (Sargent *et al*., 2002). The current paradigm in ecological FA-related studies is that EPA and DHA are synthesized only by particular phytoplankton taxa (Gladyshev *et al*., 2013; Taipale *et al*., 2013, 2016) and transferred to fish *via* zooplankton, allowing the growth and functions of delicate and complex organs of fishes, *e.g*., muscle, eye, brain and gonads (*e.g*., Arts *et al*., 2001; Tocher, 2003). Phytoplankton composition fluctuates seasonally, and the FA composition varies among classes of phytoplankton (Viso & Marty, 1993; Volkman *et al*., 1989). FA of dietary interest may sometimes reflect the characteristics of the environment, inclusive of water temperature, salinity, incident light and available nutrients, all of which may differ geographically (Budge *et al*., 2002). This can result in spatiotemporal variability in the FA signatures of phytoplankton in a given area and thus the FA composition of food available to redfish may differ among the three subareas of the nGSL. Thus, the use of FA signature to compare the diet of a predator in different areas or subareas requires a prey data set as complete

as possible and ideally with complete spatial coverage (Nozères, 2006) and knowledge of the predator's movements. In this study, the different redfish prey were randomly sampled throughout the nGSL. Nozères (2006) demonstrated that prey FA could vary by size, subarea, season and year in the nGSL. It would be useful to obtain a specific sample of prey earlier in the summer to obtain FA profiles that drive redfish FA profiles in August, for future redfish diet studies regarding summer feeding habits. Moreover, it will be valuable to complement prey FA data by acquiring the FA signatures of other zooplankton species playing a pivotal role in nGSL food webs and which have been identified in the SCA. For example, krill species were important prey for redfish based on SCA in this study. This is supported by another study in the same area based on a much larger sample size (Brown-Vuillemin *et al*., 2022). It would be thus useful to obtain FA profiles for the three main krill species in the study area, *Thysanoessa raschii*, *T. inermis* and *M. norvegica*.

4.3.2 | Ubiquity of fatty acid

Making inferences on links between prey and predators based on FA composition is no simple task. For example, large (≥30 cm) redfish were shown to be associated with 16:0, 18:1n9 and with DHA (22:6n3) which could be attributed to shrimp in the present study. These FAs have important roles in fish physiology, ranging from energy source, hormone mobilization, buoyancy regulation and acting as structural elements (Dalsgaard *et al*., 2003; Ortega & Mourente, 2010).

The lipid signature of large redfish liver was characterized by high content of oleic acid 18:1n9 (37%). According to the results of this study, this FA could constitute the main lipid energy reserve of large redfish in summer. It has been hypothesized that 18:1 FAs have active role in the compensatory response to changes in temperature and depth (Arts & Kohler, 2009; Velansky & Kostetsky, 2008). Marine fish have the ability to synthesize 18:1n9 by desaturation of dietary 18:0 *via* the enzyme Δ9 desaturase (Dalsgaard *et al*., 2003; Sargent, 1976). In the case of redfish, this conversion can be considered negligible given the low amount of 18:0 (0%–6%) and the high availability of 18:1n9 (7%–22%) available among potential prey. The high concentration of 18:1n9 in the liver would then likely result from its incorporation from dietary lipids. Nevertheless, FA 18:1n9 is very abundant in marine environments and a major FA of most marine animals and demonstrated as dominant in carnivorous and omnivorous crustaceans (Falk-Petersen *et al*., 1990). It must be considered that such an FA may influence the lipid imprint of many other species, including the potential prey of redfish. Therefore, FAs such as 18:1n9, 16:0 and 18:0, which are ubiquitous in marine systems and can be biosynthesized by zooplankton and fish or freely absorbed (Dalsgaard *et al*., 2003), are sometimes excluded when assessing diet from FA composition in predator tissues. Nonetheless, the results of this study showed value in considering these FA. For instance, according to the results and the premise that these FAs are incorporated from prey, the contribution of 18:1n9, 16:0 and 18:0 to the FA signatures of large redfish makes it possible to exclude copepods, which are characterized by a low proportion in these FA, as important prey of large redfish because copepods do not seem to be the precursors of this intake.

4.4 | Complementarity of fatty acid profiling and implication for the study of redfish diet

4.4.1 | Sample size considerations

Independent of the method of dietary analysis used, an adequate sample size is required to obtain a representative portrait of the diet composition of predators (*e.g*., Baker *et al*., 2014; Brown *et al*., 2012; Cortés, 1997; Ferry & Cailliet, 1996). Moreover, accurate diet composition analysis requires representative sampling across a predator's geographical range, time and life-history stages, as diet can change spatially, temporally and with specimen size (Hovde *et al*., 2002; Link & Garrison, 2002) as already demonstrated for redfish (Brown-Vuillemin *et al*., 2022). In this study, cumulative prey category–stomach curves showed that results for the large majority of size class and subarea combinations relied on an insufficient number of stomachs containing prey to provide a robust description of the redfish diet composition. This is not surprising because the data used in this study represent a sub-sample and thus a reduced number of stomach contents, of a larger study about redfish diet with SCA (Brown-Vuillemin *et al*., 2022). Characterizing diet and quantifying contribution of different prey items through SCA is a challenging task due to individual variability in stomach contents, the fact that stomachs represent a snapshot of diet based on one or a few last meals before capture and differential digestion of potentially important prey taxa. Successful SCA requires large sample sizes, which is difficult for deep-water predatory fish and in particular physoclist species like redfish and hake, which often regurgitate their stomach contents upon capture, which is more generally common for deep-water predators (Drazen & Sutton, 2017; Pethybridge *et al*., 2011). The FA method offers new perspectives on redfish diet beyond information that can be extracted from SCA alone, by overcoming the effect of barotrauma and the associated regurgitation, which does not prevent a liver tissue to be taken for FA analysis.

The results of the cumulative curves of liver FA showed that only about 10 samples of liver tissue are required to assess redfish diet in a given sector and size class, which highlights the advantage of utilizing FA profiles as diet tracers to overcome the logistical difficulties in the collection of large sample sizes required for SCA. As such, FA analysis represents a cost-effective option to assess diet for redfish or more largely for species that are difficult to obtain due to conservation needs or remote locations as suggested and demonstrated for shark and chimaera species in Pethybridge *et al*. (2011). Nonetheless, SCA will remain an essential step to identify which prey need to be collected for FA analysis and to help interpret the results obtained with this method. To complement SCA and help to refine taxonomical prey resolution, the DNA metabarcoding of stomach contents may assist in avoiding important potential pitfalls of SCA. This method enables the

identification of prey by using "universal" PCR primers that amplify sequence standardized DNA barcode regions from organisms in the stomach contents at high sequence read counts. This method has proven efficient, even for predators characterized by a diverse diet (*e.g.*, Pompanon *et al.*, 2012; Symondson, 2002).

4.5 | Conclusion

Marine environments are complex, and obtaining accurate information on trophic linkages constitutes a difficult task. No single method allows a comprehensive assessment of a predator's diet composition and its inherent variability on various time scales. This study relied on the use of multitrophic markers coupling SCA and FA analysis as a first step for documenting the spatial variability across size classes of the redfish (Supporting Information Figure S1). The authors conclude that FA analysis is promising for assessing seasonal or monthly variation in redfish diet composition integrating all feeding regime. It requires smaller samples of fish, and these could be collected during the fishery, as there is no need for specialized personnel to excise liver. These advantages would come at the cost of a coarser taxonomic resolution than that of SCA, and a possibly reduced spatial coverage relative to redfish distribution, compared with research surveys. These disadvantages could be mitigated with the addition of stomach samples from one or a few additional months, *e.g.*, during the winter scientific surveys initiated by DFO in 2022 for 3 years.

Results of this study support the concept that the combination of several techniques provides the maximum level of information on a predator's feeding ecology. SCA is the only method that can detect cannibalism on small redfish, a behaviour expected to intensify as individuals from the 2011–2013 cohorts become larger and will need to be monitored (Brown-Vuillemin *et al.*, 2022). Nonetheless, FAs provide important information on the nutritional quality of prey, which could be particularly important in the context of climate change within the St. Lawrence system. As noted by Colombo *et al.* (2017), high-latitude marine organisms provide a disproportionately large global share of DHA and EPA to consumers, and an increase in water temperature is predicted to result in decreased proportion of DHA and EPA by the primary producers. Redfish currently reach a modal size about 24 cm (DFO, 2022), corresponding to a shift between a zooplankton-dominated diet and one primarily based on fish and shrimp. Predation on shrimp by large redfish, validated by FA analysis, is thus expected to increase in the short term and accelerate the decline of northern shrimp, which is already impacted by rapidly increasing temperature in the system (Bourdages *et al.*, 2020). Because the biomass and specific composition of prey assemblages are greatly influenced by changes in the structure of water masses, particularly in terms of temperature, it is important to keep acquiring data on redfish diet composition and on the abundance of its main prey to detect future changes in trophic linkages among the main components of the food web. The variability in phenology, abundance and distribution of calanoid copepods and shrimp in relation to the environmental variability will be important to consider for future redfish trophodynamic studies and for the development of marine resource management strategies for the GSL.

AUTHOR CONTRIBUTIONS

All authors conceived the project objectives and methodology. S.B.V. performed the analysis of fatty acid profiles and wrote the first draft of the manuscript. S.B.V., R.T. and D.C. conducted the data analyses. All authors have contributed to the revision and improvements of the manuscript and take responsibility for its content.

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SUPPORTING INFORMATION

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

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