



Detrital Subsidies in the Diet of *Mytilus edulis*; Macroalgal Detritus Likely Supplements Essential Fatty Acids

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Detritus is a frequent, poorly defined, component of bivalve growth and carrying capacity models. The purpose of this study was to determine the proportional contributions of detrital material derived from primary producers (phytoplankton, macroalgae, *Spartina alterniflora*, and terrestrial leaf litter) to particulate organic matter (POM) and blue mussel's (*Mytilus edulis*) diet within a temperate bay (Saco Bay, ME, United States). We assessed which detrital sources, if any, warranted incorporation into modeling efforts. Stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and fatty acid biomarkers (FA) of mussels, size fractionated ($<100\ \mu\text{m}$) POM, and primary producer endmembers (phytoplankton, *Saccharina latissima*, *Ascophyllum nodosum*, *Chondrus crispus*, *Spartina alterniflora* and leaf litter) collected between 2016 and 2017 were used to estimate endmember contributions to POM and mussel diets. Based on FAs dinoflagellates were the most abundant phytoplankton in Saco Bay, even during the fall diatom bloom. Diatoms within the bay were primarily centric, but pennate diatoms were at times present in the water column (e.g., in September). Following abundances of dinoflagellates, and centric and pennate diatoms, 22:6 ω 3 (DHA) was the most abundant essential FA ($8.6 \pm 0.1\%$ total FAs), followed by 20:5 ω 3 (EPA: $7.0 \pm 0.1\%$) and 20:4 ω 6 (ARA: $0.3 \pm 0.1\%$). On average, phytoplankton derived organic matter contributed $22.1 \pm 0.3\%$ of the total POM in the bay. The concentration of non-fresh phytoplankton organic matter, or remaining organic matter (REMORG), was positively correlated with all endmember biomarkers. However, the proportion (%) of vascular plant, macroalgal, and detrital FAs was negatively correlated with the concentration of REMORG. This finding suggests in periods of low productivity, vascular plant and macroalgal detritus are proportionally more important contributors to POM. Mussels were broad spectrum omnivores, consuming phytoplankton, zooplankton, and detrital material. Detrital contributions to mussel diets were important (minimum of 16% of diet). Although small, macroalgae's dietary contribution (8%) to *M. edulis* may be important. Macroalgal detritus contained

essential FAs (20:5 ω 3 and 20:4 ω 6) that could supplement mussel diets, as *M. edulis* in Saco Bay were likely limited by 20:5 ω 3. Consideration of how macroalgal detritus affects the availability of essential FAs in POM may be useful to incorporate into aquaculture site selection.

Keywords: *Mytilus edulis*, stable isotopes, fatty acids, detritus, macroalgae, aquaculture, particulate organic matter

INTRODUCTION

Bivalves, such as the blue mussel (*Mytilus edulis*), are an ecologically and economically important group of filter-feeding animals whose growth (Grant, 1996; Hawkins et al., 2002, 2013) and ecological carrying capacity (Byron et al., 2011a,b; Kluger et al., 2016; Outeiro et al., 2018) have been modeled based on food availability and other environmental variables. Detritus is often incorporated into food web and bivalve growth models because a large proportion of ecosystem energy moves through detrital food webs (Cebrian and Lartigue, 2004). We operationally define detritus as any dead or decaying matter shed from a parent organism along with associated bacteria. Detritus is difficult to measure, so models frequently must indirectly estimate the size of the detrital pool. For example, organic matter (OM) that is not live phytoplankton defined as remaining organic matter (REMORG) in the bivalve growth model Shellsim, which is treated as a homogeneous entity (Hawkins et al., 2013).

Of course, detritus is not a homogeneous pool of organic matter. The physical and biochemical composition of different detrital particulates determines their rate of degradation and residence time in ecosystems (Cebrian and Lartigue, 2004) as well as their bioavailability for bivalve consumers (Grant and Cranford, 1991; Duggins and Eckman, 1997; Arambalza et al., 2010; Dethier et al., 2014). Not incorporating this complexity into models may cause under or overestimations of detrital importance. The magnitude of the discrepancy caused by the assumptions of a homogeneous detrital pool of organic matter may be small enough relative to other sources of variability to not cause a major problem in bivalve growth models. However, when used in ecosystem models to determine carrying capacities intended for regulators and decision makers such over-simplifications can have real repercussions. For example, intensive bivalve aquaculture exceeding carrying capacity can result in bay-wide seston depletion (Grant et al., 2005; Comeau et al., 2008). By assuming all detrital material is equally valuable for a bivalve consumer we risk overestimating or underestimating the available food resources.

The major primary producers that contribute to detrital pools in temperate estuaries are phytoplankton, macroalgae and salt marsh grasses. A common proxy for nutritional quality of detrital material is the ratio of carbon-to-nitrogen, which correlates with herbivory rate on the parent material (Cebrian, 1999) as well as predicts decomposition rate (Swift et al., 1979; Coleman et al., 1983; Melillo et al., 1984; Cebrian, 1999; Moore et al., 2004). Phytoplankton, the primary diet for most bivalves, represents the highest quality material with relatively low C:N ratios (9 ± 5 weight weight⁻¹) (Cranford and Grant, 1990;

Enríquez et al., 1993; Kitazato et al., 2000; Beaulieu, 2002) while macroalgae and marsh grass detritus represent progressively lower and more variable quality material with corresponding C:N ratios of 30 ± 20 and 61 ± 34 respectively (Enríquez et al., 1993; Krumhansl and Scheibling, 2012). Although lower in quality than phytoplankton based on C:N, both marsh grass detritus (Lucas and Newell, 1984; Peterson et al., 1985; Newell and Langdon, 1986; Mann, 1988; Langdon and Newell, 1990; Decottignies et al., 2007) and macroalgal detritus (Bustamante and Branch, 1996; Fredriksen, 2003; Allan et al., 2010) have been shown to contribute to bivalve diets. Macroalgal detritus in particular can play an important role in the food webs of nearshore ecosystems (Duggins et al., 1989; Hill et al., 2006; Kaehler et al., 2006; Tallis, 2009; Von Biela et al., 2016) and may represent a valuable resource for bivalves during periods of low phytoplankton production.

Laboratory feeding trials using macroalgal detritus suggest current models of bivalve feeding may be underestimating this potential food source. For example, bivalves display moderate to high absorption efficiencies (41–87%) which increases with age of detrital particulates (Stuart et al., 1982; Cranford and Grant, 1990). Detrital particles consisting of a single cell formed from macroalgae, single celled detritus (SCD; Uchida, 1996), were capable of supporting equivalent or greater growth compared to phytoplankton diets when comprising 50–90% of larval diets in a shellfish hatchery, suggesting a synergistic effect between macroalgal detritus and phytoplankton in the diet (Camacho et al., 2004; Carboni et al., 2016). The synergy of phytoplankton and macroalgal detritus when used together, suggests that macroalgal detritus may supplement something which is lacking in phytoplankton which could be related to their fatty acid (FA) profiles (Carboni et al., 2016). While laboratory examinations of macroalgal detritus use by bivalves looks promising, demonstrating the importance of macroalgal detritus to bivalves *in situ* is far more difficult.

The interplay between aquaculture growth and carrying capacity models, which are reductionist by necessity, and the ecological understanding that bivalves can consume a wide variety of material is interesting and potentially informative. On one hand, if models can adequately predict the growth and carrying capacity of bivalves using solely phytoplankton, why bother with the unnecessary complexity of detrital food sources? On the other hand, if phytoplankton is the only resource required for bivalve growth, why do they ingest detrital food sources? Are detrital food sources an unnecessary component of bivalve diets, and merely ingested accidentally? Or do detrital foods supplement bivalve diets with something lacking in phytoplankton? For example, could inclusion of macroalgal

detritus into a bivalve's diet lead to a more optimized diet than solely phytoplankton as previously suggested? Though answers to these questions are likely to be species, and even location, specific, they are ecologically relevant questions and their answers could help further our understanding of bivalve nutritional requirements while also improving our capacity to model the growth and interactions of bivalve aquaculture with the environment.

Because directly tracking detrital material use by bivalves *in situ* is exceedingly difficult, many studies rely on stable isotope analysis (SIA) (Duggins et al., 1989; Hill et al., 2006; Kaehler et al., 2006; Tallis, 2009; Von Biela et al., 2016). SIA is well suited to distinguishing between C_3 and C_4 photosynthetic plants, as well as between marine and terrestrial primary producers (O'leary, 1988; Ehleringer and Cerling, 2001; Mortazavi et al., 2005; Fry, 2006). In addition, because fractionation causes $\delta^{15}N$ values of consumers to become more enriched than their prey, $\delta^{15}N$ values are useful for estimating trophic level (Post, 2002; Fry, 2006). However, one potential complexity when interpreting $\delta^{13}C$ is that some key primary producers in marine ecosystems can display a wide range of $\delta^{13}C$ values. For example, diatoms display a wide range of $\delta^{13}C$ values depending on their phase of growth (Fry and Wainright, 1991) which can overlap with macroalgal values and lead to ambiguities about the importance of macroalgal detritus (Miller and Page, 2012).

Another powerful tool to track organic matter in ecosystems is lipid biomarkers. Lipid classes (composition, quantity, and ratios) have a long history of use as biomarkers in ecological and biogeochemical studies (see review by Parrish, 2013) and individual FA have been used in oceanographic studies as biomarkers for a large variety of organisms (Kelly and Scheibling, 2012; Parrish, 2013 and references within). For example, lipid biomarkers have been used to determine the shifting contributions of diatoms and dinoflagellates within the water column, as well as detrital inputs into marine sediments (Budge et al., 2001). Lipid FA biomarkers have also been used to determine the contribution of mangrove detritus to suspended particulate matter (Bachok et al., 2003), and compound specific SIA of FA biomarkers has been used to determine the source and age of particulate organic matter (POM; McIntosh et al., 2015; Taipale et al., 2015). Additionally, lipid biomarkers have frequently been used to determine the composition of bivalve diets (Bachok et al., 2003, 2009; Guest et al., 2008; Allan et al., 2010; Ezgeta-Balić et al., 2012; Irisarri et al., 2014; Wang et al., 2014). When used in conjunction with stable isotopes FA biomarkers become a versatile tool that can help determine primary producer contributions to food webs (Carreón-Palau et al., 2013).

We contend that by using SIA and FA biomarkers together, complex organic matter pathways in coastal ecosystems can be parsed more accurately. The purpose of this study was to determine the contributions of detrital particulates from phytoplankton, macroalgae, and vascular plants (marsh grass and terrestrial leaf litter) within a northern temperate bay to the diet of intertidal *M. edulis* using a combination of stable isotopes and lipid biomarkers. Our aim was to more accurately represent

organic matter pathways for consideration in bivalve feeding and carrying capacity models.

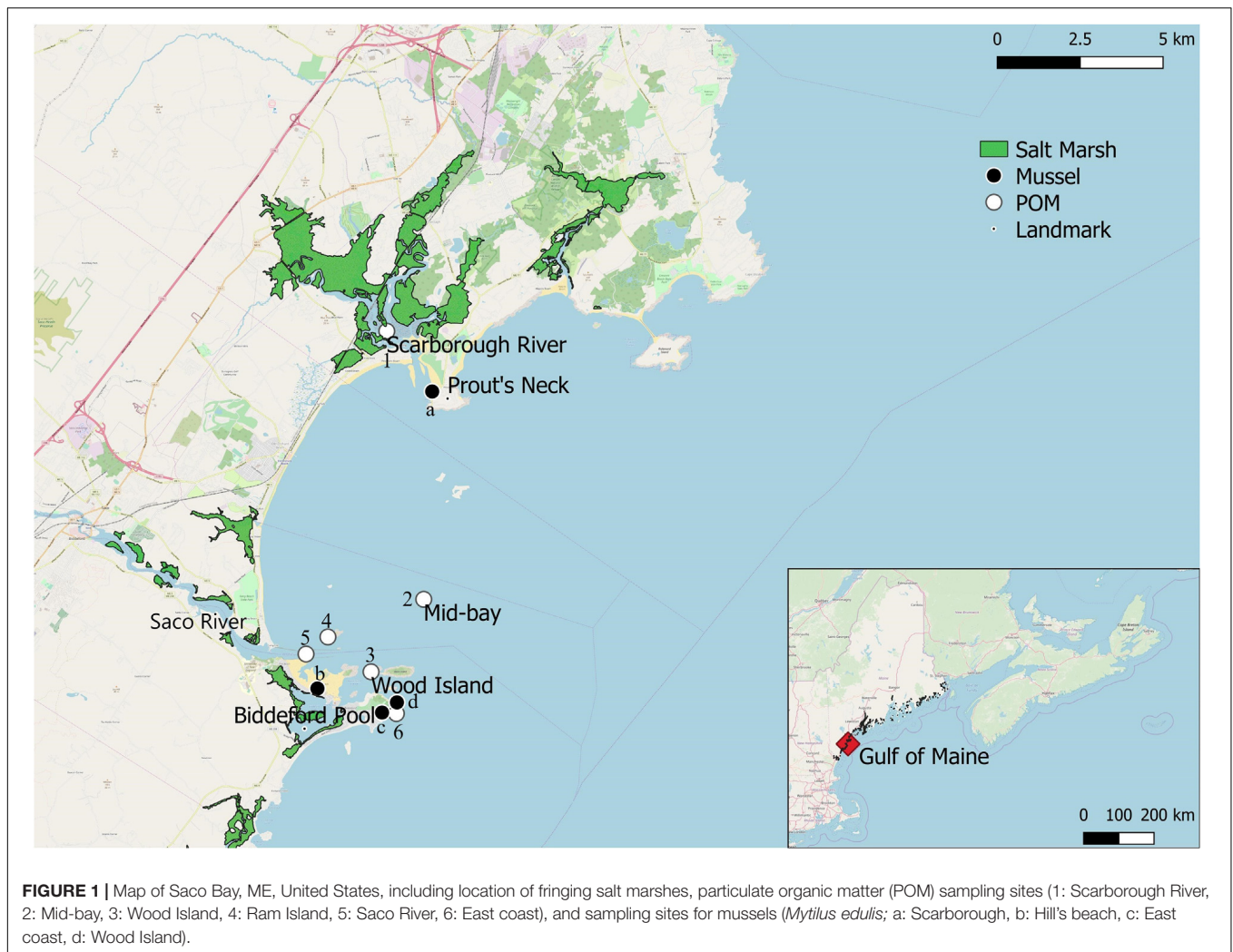
To determine the contributions of macroalgae and vascular plants to mussel diets, we collected fresh samples of each from Saco Bay, ME, United States, along with live *M. edulis*. Lipids were extracted from *M. edulis*, vascular plant, and macroalgal endmembers and quantified via Iatroscan analysis before being derivatized into fatty acid methyl esters (FAME) and identified with gas chromatography. Multivariate statistics were used to determine the relative contributions of each endmember to the diet of *M. edulis*, based on the proportions of FA biomarkers from each producer (identified via SIMPER analysis and previous literature). In addition, the stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of *M. edulis* and each endmember was used to construct a dual-isotope Bayesian mixing model, which estimated primary producer contributions to the diet of *M. edulis*.

MATERIALS AND METHODS

Study Site and Sample Collection

We collected samples from Saco Bay (Figure 1), which is located in southern Maine, United States, with its northern-most border framed by Scarborough marsh and Biddeford Pool as the southern boundary (Reynolds and Casterlin, 1985). Saco Bay has a mean tidal range of 2.7 m (Jensen, 1983; Kelley et al., 2005) and the primary source of freshwater is the Saco River. The Saco River is the sixth largest river discharging into the Gulf of Maine (Tilburg et al., 2011) with discharge rates varying between 40 and 620 $m^3 s^{-1}$ and a mean of 100 $m^3 s^{-1}$ (Barber, 1995; Kelley et al., 2005). The only other significant river discharging into the bay is the Scarborough River, which has an average discharge of only 3.1 $m^3 s^{-1}$ (Figure 1; Jacobson et al., 1987; Kelley et al., 2005).

Water samples for POM were obtained from six sites throughout Saco Bay in 2016 and 2017 (Figure 1). POM was size fractionated ($<100 \mu m$) *in situ* in the water column with a custom made sampling device. Although *M. edulis* is capable of ingesting particulates $> 100 \mu m$, we targeted POM $< 100 \mu m$ in this study because we were interested in small detrital particulates, which are able to remain in the water column for extended periods and be exported to other ecosystems. Additionally, the 100 μm cut-off excluded large zooplankton which may have overwhelmed any detrital signal. The sampling device filtered water through a 100 μm pre-screen and a 1 μm collection mesh while being slowly towed vertically through the water column, suction was provided by a small pump aboard the vessel. Water depth for all sampling sites was $<10 m$ and the entirety of the water column was sampled, with exception to the mid-bay site which had a depth of 31 m and only the top 15 m of the water column was sampled due to limitations of the sampling device. On average, $374 \pm 146 l$ were filtered per location when collecting $< 100 \mu m$ POM. Once captured on the 1 μm mesh, POM was gently rinsed into a Nalgene bottle using filtered (0.45 μm) seawater. Separate 1 μm meshes were used per sample location, after sampling meshes were dried at 60°C for 24 h and weighed to account for any particulates that may have adhered to them. Concentrated slurries of POM were



stored in a cooler of ambient water until subsampled on land for each analysis. Replicate subsamples were filtered onto 1.2 μm GF/C Whatman filters for each analysis. Samples were analyzed for dry weight, ash-free dry weight, chlorophyll- α , stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), and FA biomarkers. In 2016 POM was collected biweekly from all six sites, while in 2017 POM samples were only collected monthly from three sites: Wood Island, the mid-bay site, and the mouth of the Scarborough River. For number of samples analyzed per site for each analysis and sampling date see **Supplementary Table S1**.

Intertidal blue mussels and macroalgae (*Saccharina latissima*, *Ascophyllum nodosum* and *Chondrus crispus*) as well as a C_4 photosynthetic marsh grass *Spartina alterniflora*, were collected biweekly from four sites in 2016 and monthly in 2017: the outer northern perimeter of Biddeford pool, the outer eastern perimeter of Biddeford pool, near Wood island, and Prout's Neck near the Scarborough River (**Figure 1**). All mussels, *A. nodosum*, and *C. crispus* samples were collected by hand from the intertidal zone during low-tide, no depth was recorded but the mean tidal range for Saco Bay is 2.7 m (Jensen, 1983; Kelley et al., 2005). Kelp samples were obtained opportunistically when washed into the inter-tidal zone via wave action proceeding from storm events

and collected from an experimental aquaculture lease near Wood Isle. *S. alterniflora* samples were collected from the Scarborough marsh near Prout's neck. Terrestrial leaf litter (composed primarily of *Quercus* spp.) was sampled once in the fall of 2016 to represent C_3 photosynthetic plants. For a breakdown of the number of samples analyzed for mussels and each endmember per site and sampling location see **Supplementary Tables S2, S3**. Macroalgae sampled within this study represent system dominants, fucoid algae, such as *A. nodosum*, and *C. crispus* dominate the intertidal area in the North western Atlantic and kelps, such as *S. latissima*, dominate the subtidal zone (Stephenson and Stephenson, 1972; Chapman and Johnson, 1990). Macroalgal and *Spartina* samples were scraped with a razor to remove epiphytes and rinsed in deionized water prior to processing for stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and FA analysis. Zooplankton were collected with vertical tows of a 200 μm zooplankton net. Afterward, zooplankton were phototaxically separated from settling detritus in a graduated cylinder, by shining a beam of light horizontally through the water surface and decanting off aggregating photophilic zooplankton. For a breakdown of the number of zooplankton samples analyzed per site, per sample date see **Supplementary Table S4**.

Chlorophyll- α

Chlorophyll- α samples were collected on pre-combusted 1.2 μm GF/C Whatman filters and stored at -20°C until analysis. Chlorophyll- α content was determined fluorometrically using a Turner Designs TD700 fluorometer calibrated with quantified standards (part # 10-850). Chlorophyll- α was extracted using 90% acetone and acidified with 5% hydrochloric acid (Strickland and Parsons, 1972; Parsons et al., 1984; Welschmeyer, 1994; Arar and Collins, 1997). Selected organic matter (SELOGR; or organic matter associated with phytoplankton), and remaining organic matter (REMORG; or non-phytoplankton associated organic matter), were calculated as $SELOGR = \frac{CHL \times 50}{0.38}$ and $REMORG = POM - SELOGR$ as described by Hawkins et al. (2013). Hawkins et al. (2013) recommended using a carbon-to-chlorophyll (C:CHL) ratio of 12 when calculating REMORG to avoid SELOGR estimates exceeding total POM; however, we used a more conservative and widely used C:CHL ratio of 50 (Taylor et al., 1997) to avoid underestimating phytoplankton contributions to POM.

Lipid Class and Fatty Acid Analysis

Modified Folch extractions were used to extract lipids from tissues or filtered POM using a chloroform-to-methanol ratio of 2:1 as described by Parrish (2013). Lipid quantification was determined using an Iatroscan Mark V TLC-FID. Heat (100°C for 1 h) and concentrated sulfuric acid were used to transesterify subsamples of total lipid extracts into FAME. An Agilent 7890A Series GC with an FID detector equipped with a 30 m (0.25 μm internal diameter) ZB wax + column (Phenomenex, US) was used to determine FAME composition; retention times were determined with a Supelco 37 component FAME mix (Product number 47885-U). Helium was used as the carrier gas at 2 ml min^{-1} while column temperature began at 65°C for 0.5 min then ramped to 195°C at a rate of $40^{\circ}\text{C min}^{-1}$ and held for 15 min. Column temperature was then ramped to 220°C at a rate of $2^{\circ}\text{C min}^{-1}$ and held for 3.25 min. Injector temperature started at 150°C and ramped at a rate of $200^{\circ}\text{C min}^{-1}$ until reaching a final temperature of 250°C , while the detector remained a constant 260°C .

We used Primer 7 with the PERMANOVA + package (ver. 7.0.13, Quest Research Limited) to perform principal coordinates analysis (PCoA), similarity percentages (SIMPER), cluster analysis, permutational multivariate analysis of variance (PERMANOVA) and homogeneity of dispersion tests (PERMDISP). Data were plotted with PCoA while SIMPER and cluster analysis were used to determine similarity and dissimilarity within and among endmember groups. Statistically significant groupings ($\alpha = 0.05$) were determined using PERMANOVA and prior to multivariate tests PERMDISP was used to test for homogeneity of multivariate dispersion. When necessary, FA data were square root transformed to improve homogeneity. Biomarker FAs for each endmember group as identified from previous studies, are given in Table 1. Data shown are mean ± 1 standard error, unless otherwise indicated. Percentage (%) of diatom and macroalgal FA markers, identified based on PCoA and SIMPER analysis, were used to separate

TABLE 1 | Fatty acid biomarkers used for each endmember in this study along with literature source of previous use.

Endmember	Fatty acid biomarker	Source
Macroalgae	18:1 ω 9	Kelly and Scheibling, 2012; Parrish, 2013
	18:3 ω 6	Kelly and Scheibling, 2012; Parrish, 2013
	20:2 ω 6	Kharlamenko et al., 1995
	20:4 ω 3	Kelly and Scheibling, 2012; Parrish, 2013
	20:4 ω 6	Kelly and Scheibling, 2012; Parrish, 2013
Vascular plants	18:2 ω 6	Kelly and Scheibling, 2012; Parrish, 2013
	18:3 ω 3	Kelly and Scheibling, 2012; Parrish, 2013
Dinoflagellates	22:6 ω 3	Kelly and Scheibling, 2012; Parrish, 2013
	18:4 ω 3	Kelly and Scheibling, 2012; Parrish, 2013
	22:6 ω 3-to-20:5 ω 3 > 1	Pepin et al., 2011
Diatoms	16:1 ω 7	Kelly and Scheibling, 2012; Parrish, 2013
	16:4 ω 1	Dalsgaard et al., 2003; Parrish, 2013
	20:5 ω 3	Kelly and Scheibling, 2012; Parrish, 2013
	22:6 ω 3-to-20:5 ω 3 < 1	Pepin et al., 2011
Zooplankton	20:1	Kelly and Scheibling, 2012; Parrish, 2013
	21:1	Kelly and Scheibling, 2012; Parrish, 2013
Bacteria	Odd-chained FAs	Kelly and Scheibling, 2012; Parrish, 2013
	<i>Iso</i> and <i>anteiso</i> FAs	Parrish, 2013; George and Parrish, 2015

the combined diatom/macroalgal estimates from stable isotope mixing models as follows:

Diatom contribution

$$= \text{Combined estimate} \times \left(\frac{\text{Diatom FAs}}{\text{Diatom FAs} + \text{Macroalgal FAs}} \right).$$

Stable Isotope Analysis

Initial processing of stable isotope samples for POM was identical to dry weights processing. POM was filtered onto pre-combusted and pre-weighed 1.2 μm GF/C Whatman filters, dried for 24 h (60°C), or until a constant weight, and stored in desiccation chambers until processing and sending for analysis at University of California's Davis Stable Isotope facility. Hydrochloric acid fumes were introduced to dried POM samples for 24 h to remove carbonate carbon prior to encapsulation in tin. Due to difficulties in obtaining enough POM for all analyses, we did not send duplicate unacidified samples of POM for analysis. Mussel and endmember tissues were prepared by drying for 24 h at 60°C , or until a constant weight. Once dry, tissues were crushed into a fine powder and subsamples (1.0 ± 0.2 mg) of powder were encapsulated and sent for analysis. Samples were compared to laboratory reference materials, which were calibrated against international reference materials (IAEA-600, USGS-40, USGS-41, USGS-42, USGS-43, USGS-61, USGS-64, and USGS-65). Prior to processing endmember tissues were stored at -20°C .

Analysis of variance tests were conducted using R-Studio (Version 3.6.1). Additionally, we constructed Bayesian stable isotope mixing models, which use Markov-Chain Monte Carlo methods to generate probability distributions for possible dietary

contributions (Parnell et al., 2010), using the SIA package in R (SIAR, ver. 4.2). Fractionation factors of $0.4 \pm 1.0\%$ and $3.4 \pm 1.0\%$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively established by Post (2002), were used when determining mussel diets and no fractionation factors were used when determining POM composition. Phytoplankton $\delta^{15}\text{N}$ values were estimated by subtracting one trophic level (3.4%) from zooplankton $\delta^{15}\text{N}$ values. Previously reported $\delta^{13}\text{C}$ values from George's Banks in the Gulf of Maine were used, $-18 \pm 2\%$ and $-24 \pm 1\%$ for diatoms and dinoflagellates respectively (Fry and Wainright, 1991). Diatoms and macroalgae were combined in mixing models due to similarities in their isotopic values. Modeled endmember contributions are reported as mean, minimum, and maximum contributions of 95% Bayesian credibility intervals, which represent the range of values within which an estimate from a single iteration of the model has a 95% probability of falling. All stable isotope data, measured and modeled, are reported as mean ± 1 standard deviation, while all other data are reported as mean ± 1 standard error. Regressions were constructed using SigmaPlot (2008; version 11.2.0.5 Systat Software, Inc.).

Our stable isotope mixing model did not account for the effects of lipid depletion on mussel $\delta^{13}\text{C}$ values and assumed 1 trophic level (3.4% $\delta^{15}\text{N}$) difference between the $\delta^{15}\text{N}$ values of phytoplankton and zooplankton, this assumes no heterotrophic feeding by zooplankton. To test the validity of these assumptions, we constructed an alternative model incorporating a 1.75 trophic level (5.95% $\delta^{15}\text{N}$) difference between phytoplankton and zooplankton, to account for heterotrophic feeding by zooplankton, as well as mussel $\delta^{13}\text{C}$ values corrected for lipid content (based on a lipid-to-protein depletion of 6.5% : Logan et al., 2008). Additionally, as lower fractionation rates (2.3%) have been reported for poikilotherms (McCutchan et al., 2003), we constructed another mixing model with a lower (2.3%) fractionation between zooplankton and phytoplankton, as well as for mussel consumers. The estimates of all models were compared to assess the effects of our assumptions on our conclusions.

RESULTS

Phytoplankton Community and Detrital Contributions to POM

The phytoplankton community of Saco Bay was dominated by dinoflagellates, followed by centric and then pennate diatoms, which directly affected the availability of essential FAs. The average concentration of dinoflagellates ($9.3 \pm 0.3 \mu\text{g C l}^{-1}$) was significantly greater (one-way ANOVA; $p < 0.01$, $n = 30$) than that of diatoms ($1.7 \pm 0.1 \mu\text{g C l}^{-1}$) and the concentration of dinoflagellates was almost always greater than diatoms (Figure 2A). The ratio of 22:6 ω 3-to-20:5 ω 3 confirmed dinoflagellates as the dominant phytoplankton (Figure 2B). A 22:6 ω 3-to-20:5 ω 3 ratio > 1 indicates a greater abundance of dinoflagellates than diatoms and the 22:6 ω 3-to-20:5 ω 3 ratio of Saco Bay was almost always > 1 . Based on volume estimates, centric diatoms ($0.1 \pm 0.01 \text{mm}^3 \text{l}^{-1}$) were statistically more prevalent (t -test; $p = 0.02$) in the water column than pennate

diatoms ($0.02 \pm 0.04 \text{mm}^3 \text{l}^{-1}$; Figure 2C). Both centric and pennate diatoms bloomed in the fall which was followed by an increase in the volume of zooplankton (Figure 2C). However, even during the fall diatom bloom when chlorophyll levels reached their maximum ($12.1 \pm 3.9 \mu\text{g l}^{-1}$), the biomass of dinoflagellates ($17.5 \pm 6.0 \mu\text{g C l}^{-1}$) was greater than that of diatoms ($4.9 \pm 0.4 \mu\text{g C l}^{-1}$). Consistent with the hierarchy of phytoplankton abundance, 22:6 ω 3 (DHA: $8.6 \pm 0.1\%$) was significantly (one-way ANOVA, *post hoc* Tukey; $p < 0.001$, $n = 101$) the most abundant essential FA, followed by 20:5 ω 3 (EPA: $7.0 \pm 0.1\%$) and 20:4 ω 6 (ARA: $0.3 \pm 0.1\%$).

REMORG comprised material from all primary producers and was most abundant during and preceding the fall phytoplankton bloom. The concentrations of all endmember biomarker FAs were significantly ($p < 0.05$) positively correlated with the concentration of REMORG (Figure 3A). Although REMORG comprised a significantly (t -test, $p < 0.001$) larger proportion of POM in summer ($84 \pm 0.4\%$, $n = 40$) than during the fall ($69 \pm 0.7\%$, $n = 34$), the average concentration of REMORG during the fall ($0.5 \pm 0.02 \text{mg l}^{-1}$) was significantly higher (t -test, $p < 0.001$) than summer concentrations ($0.1 \pm 0.01 \text{mg l}^{-1}$). Higher REMORG concentrations during the fall suggest that phytoplankton were more important contributors to REMORG than vascular plants and macroalgae. The proportion of vascular plant (18:3 ω 3 and 18:2 ω 6; $p = 0.02$, $R^2 = 0.16$), macroalgal (18:1 ω 9, 18:3 ω 6, 20:2 ω 6, 20:4 ω 3, 20:4 ω 6; $p = 0.01$, $R^2 = 0.21$), and detrital FAs (*iso*, *anteiso*, odd-chained, 18:1 ω 9, 18:2 ω 6, 18:3 ω 3, 18:3 ω 6, 20:2 ω 6, 20:4 ω 3, 20:4 ω 6; $p = 0.01$, $R^2 = 0.20$) were significantly negatively correlated with REMORG concentration (Figure 3B).

The concentration of macroalgal FAs was significantly (t -test, $p = 0.02$) higher during the spring ($0.6 \pm 0.1 \mu\text{g l}^{-1}$, $n = 25$) than fall ($0.4 \pm 0.1 \mu\text{g l}^{-1}$, $n = 40$) and macroalgal detritus became a more important source of essential FAs during late spring and early summer due to significantly (t -test, $p < 0.001$) smaller contributions from phytoplankton FAs (diatom: $0.3 \pm 0.1 \mu\text{g l}^{-1}$, dinoflagellate: $0.7 \pm 0.1 \mu\text{g l}^{-1}$) compared to the fall (diatom: $2.4 \pm 0.1 \mu\text{g l}^{-1}$, dinoflagellate: $1.1 \pm 0.1 \mu\text{g l}^{-1}$). All three species of macroalgae contained large proportions of the essential FA 20:4 ω 6 (*A. nodosum*: $11.5 \pm 0.1\%$, *S. latissima*: 17.7 ± 0.3 , *C. crispus*: $7.1 \pm 0.3\%$) as well as smaller proportions of 20:5 ω 3 (*A. nodosum*: $7.7 \pm 0.1\%$, *S. latissima*: $12.9 \pm 0.2\%$, *C. crispus*: $25.9 \pm 0.9\%$). Macroalgal detritus contributions to POM in June and July increased the proportion of 20:4 ω 6 relative to other essential FAs (20:5 ω 3). During June and July when macroalgal FAs comprised a larger proportion of POM FAs, the 20:4 ω 6-to-20:5 ω 3 ratio was significantly (t -test, $p < 0.001$) higher in early summer (0.1 ± 0.01 , $n = 27$) than fall (0.03 ± 0.01 , $n = 40$; Figure 4A). Although pennate diatoms can also be sources of 20:4 ω 6, the ratio of 20:4 ω 6 to a diatom biomarker (16:1 ω 7) was also significantly higher (t -test, $p < 0.001$) in late spring and early summer (5.5 ± 0.1) than fall (0.2 ± 0.1 ; Figure 4B) in addition another macroalgal marker (18:1 ω 9) to 16:1 ω 7 (Figure 4C) was also significantly higher (t -test, $p < 0.001$) in late spring and early summer (0.11 ± 0.01) than fall (0.02 ± 0.01) suggesting 20:4 ω 6 within the water column was from macroalgal detritus and not diatoms.

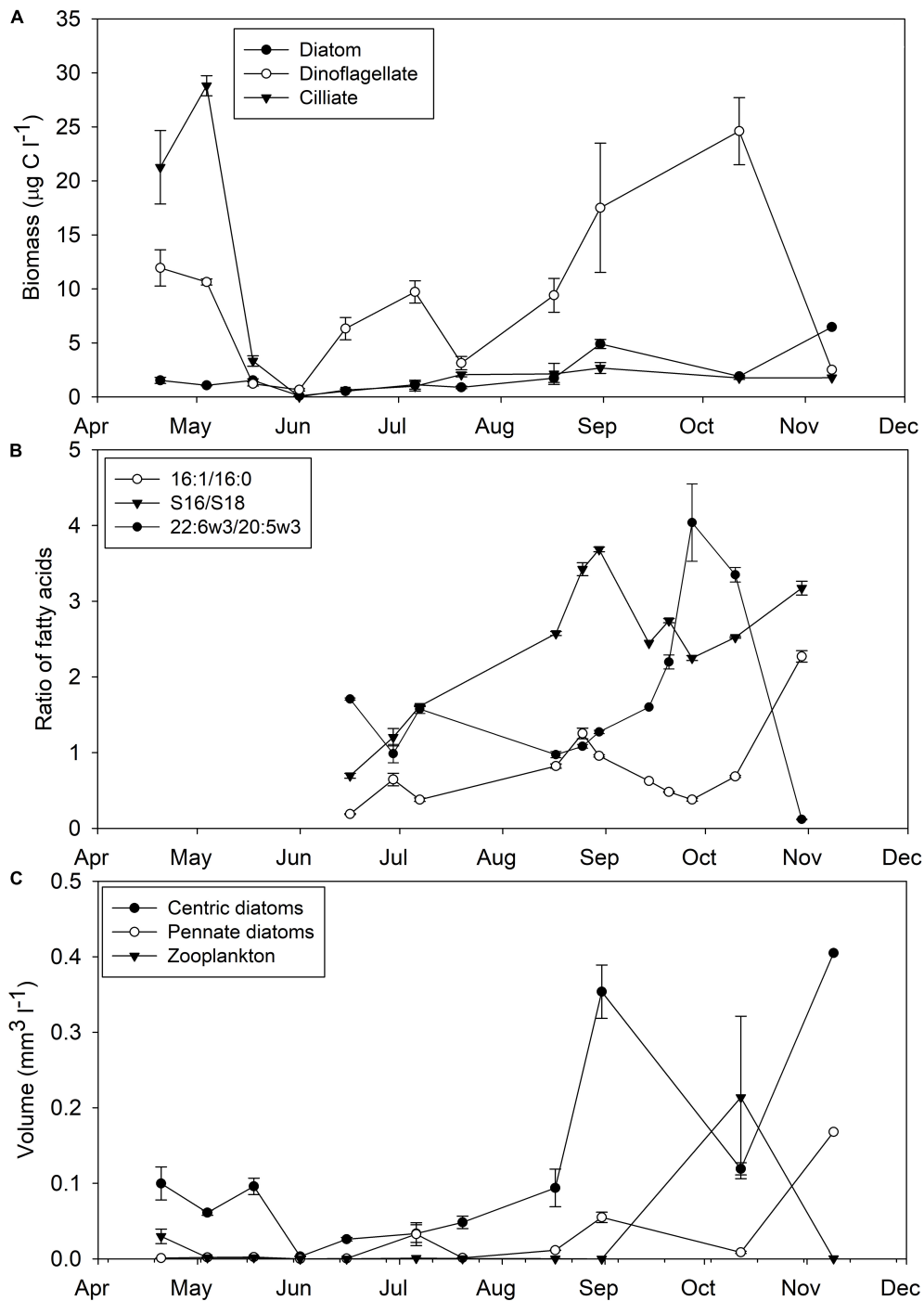
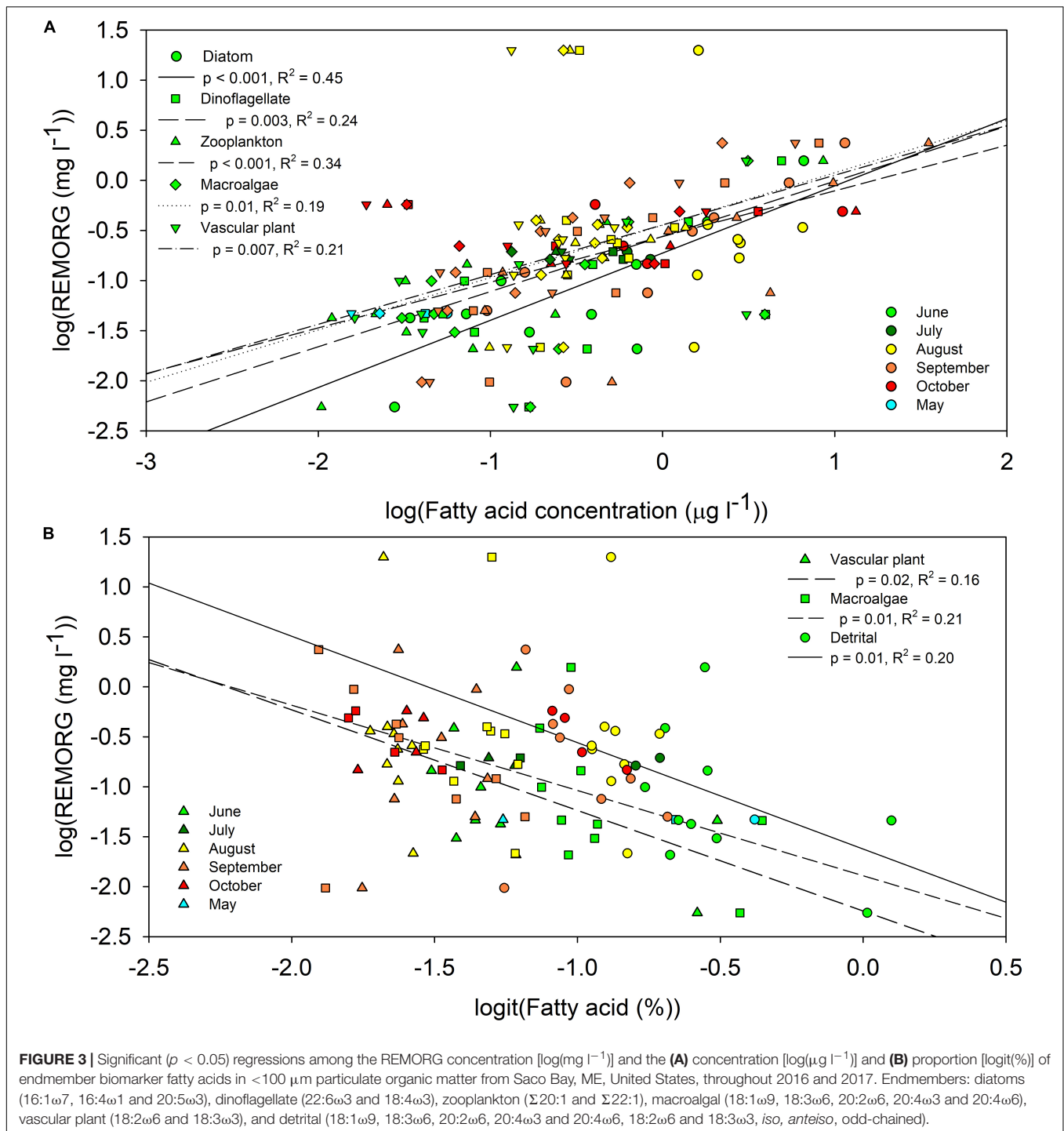


FIGURE 2 | Selected components of the plankton community composition in Saco Bay, ME, United States, throughout 2016. **(A)** Biomass ($\mu\text{g C l}^{-1}$) of diatoms, dinoflagellates, and ciliates. **(B)** Diatom fatty acid indices (16:1-to-16:0 and Σ 16-to- Σ 18) and a dinoflagellate index (22:6 ω 3-to-20:5 ω 3). **(C)** Volume ($\text{mm}^3 \text{l}^{-1}$) of centric diatoms, pennate diatoms, and zooplankton. Error bars represent ± 1 standard error.

Isotopic and FA Composition of *Mytilus edulis*

Mussels within Saco Bay lost lipid and selectively retained certain FAs during summer months. Throughout the entire study, lipid content of *M. edulis* ranged from 0.8 to 5.4% wet

weight (WW) with an average of $3.3 \pm 0.1\%$ (Figure 5A), consistent with previously reported lipid content of *M. edulis* (Table 2). Lipid content in mussels was significantly lower (one-way ANOVA, *post hoc* Tukey; $p < 0.01$) in summer ($20.0 \pm 0.8 \text{ mg g}^{-1}$, $n = 22$) than during spring ($41.5 \pm 2.7 \text{ mg$



g^{-1} , $n = 31$) or fall ($34.9 \pm 0.6 \text{ mg g}^{-1}$, $n = 46$; **Figure 5A**). Additionally, concentrations (mg g^{-1}) of total FAs and all essential FAs (20:4 ω 6, 20:5 ω 3, and 22:6 ω 3) were significantly lower (one-way ANOVA, *post hoc* Tukey; $p < 0.001$) during summer (**Figure 5A**). Although *M. edulis* experienced across the board decreases in FAs, the proportion of two essential FAs (20:4 ω 6 and 22:6 ω 3) and non-methylene interrupted dienes (NMID) were significantly higher (one-way ANOVA, *post hoc*

Tukey; $p < 0.001$) throughout summer (**Figure 5B**), suggesting the importance of these FAs to mussels. Additionally, there were significant decreases (one-way ANOVA, *post hoc* Tukey; $p < 0.001$) in the proportion of 20:5 ω 3 throughout the summer (**Figure 5B**), and 20:5 ω 3 was significantly ($p < 0.01$, $R^2 = 0.2$) negatively correlated with the proportion of NMIDs (**Figure 6**), suggesting mussels may use NMIDs to compensate for 20:5 ω 3 deficiency.

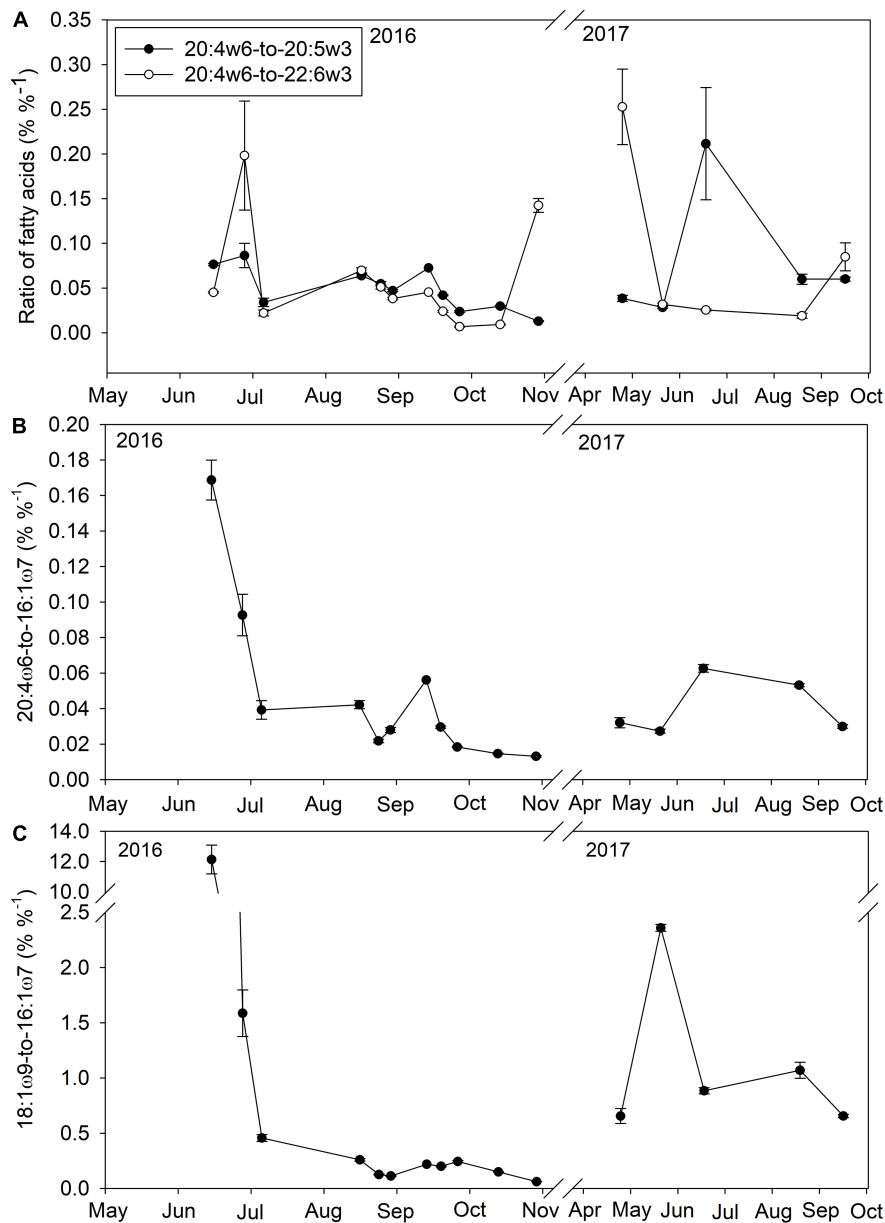


FIGURE 4 | The ratio (% %⁻¹) of the essential fatty acid 20:4ω6 to other essential fatty acids (20:5ω3 and 22:6ω3; **A**), the ratio of 20:4ω6 to a diatom fatty acid (16:1ω7; **B**), and the ratio of a macroalgal fatty acid (18:1ω9) to a diatom fatty acid (16:1ω7; **C**) in <100 μm particulate organic matter within Saco Bay, ME, United States, throughout 2016 and 2017.

The FA composition of *M. edulis* was distinctly different from any individual primary producer endmember (see **Supplementary Table S5** for more detail). The three major FAs (> 10% of total FA) identified in *M. edulis* were 16:0 along with two essential FAs 20:5ω3 and 22:6ω3. This FA profile was significantly different [PERMANOVA, $p(\text{perm}) < 0.05$] than all endmembers. There were several defining FAs for each endmember group (**Table 3**). The most defining difference between macroalgal sources and other endmembers was the amount of 20:4ω6, which comprised a large proportion of macroalgal FAs (*A. nodosum*; $11.5 \pm 0.1\%$, *S. latissima*;

$17.7 \pm 0.3\%$, *C. crispus*; $7.1 \pm 0.3\%$) and only small proportions in vascular plants (*S. alterniflora*; $0.3 \pm 0.1\%$, leaf litter; <0.1%) and consumers (*M. edulis*; $4.7 \pm 0.1\%$, zooplankton; $0.5 \pm 0.1\%$). Additionally, macroalgae (especially *A. nodosum*) had large proportions of 18:1ω9 (*A. nodosum*; $33.6 \pm 0.3\%$, *S. latissima*; $9.0 \pm 0.3\%$, *C. crispus*; $5.7 \pm 0.3\%$) compared to other endmembers (<5%). Both vascular plant endmembers had large proportions of 18:3ω3 (*S. alterniflora*; $37.7 \pm 0.4\%$, Leaf litter; $18.6 \pm 2.5\%$) and 18:2ω6 (*S. alterniflora*; $19.0 \pm 0.2\%$, Leaf litter; $6.0 \pm 0.1\%$). Unsurprisingly, while the *M. edulis* FA profile was distinctly different than any primary producer, the

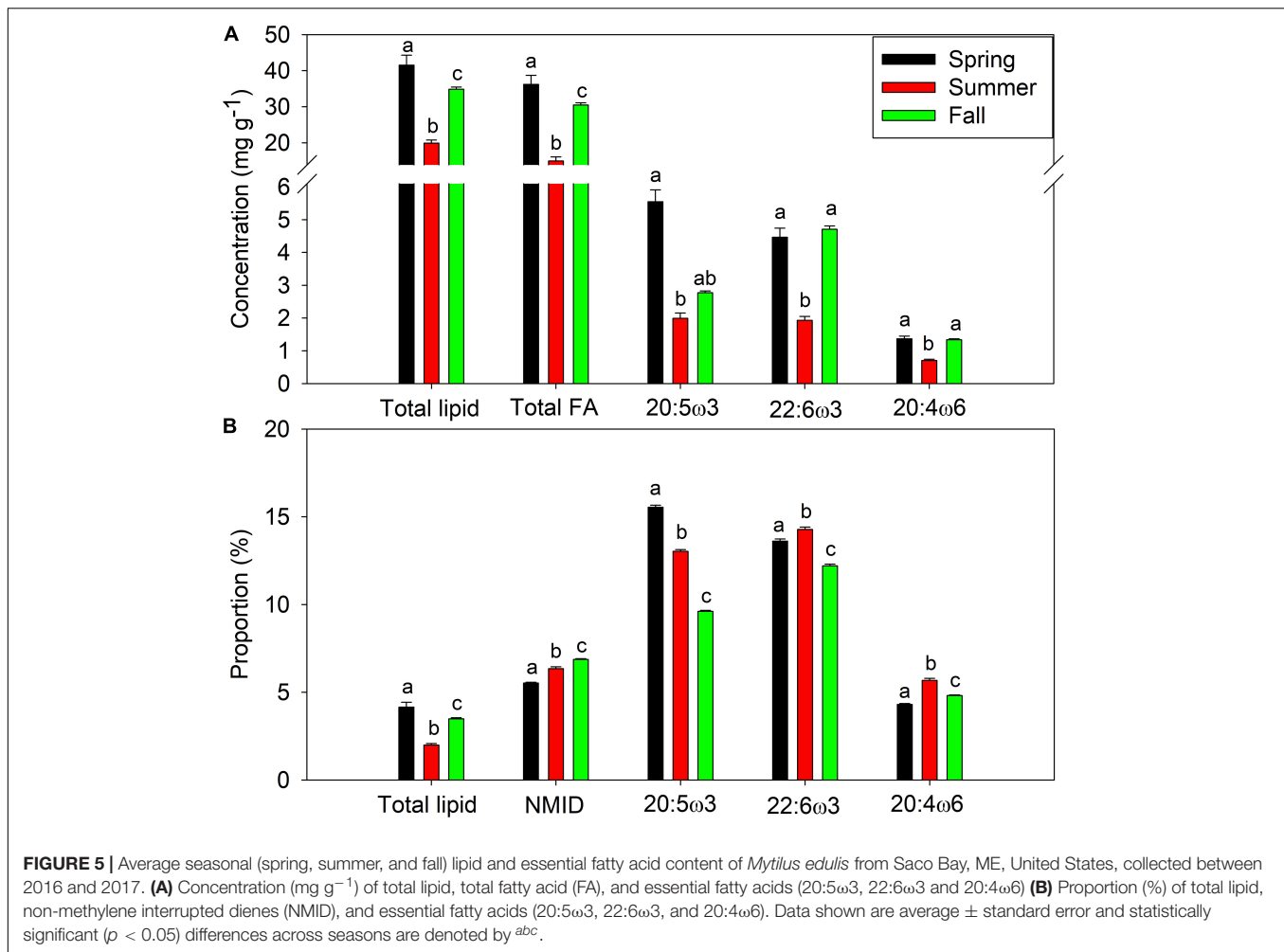


FIGURE 5 | Average seasonal (spring, summer, and fall) lipid and essential fatty acid content of *Mytilus edulis* from Saco Bay, ME, United States, collected between 2016 and 2017. **(A)** Concentration (mg g^{-1}) of total lipid, total fatty acid (FA), and essential fatty acids (20:5 ω 3, 22:6 ω 3 and 20:4 ω 6) **(B)** Proportion (%) of total lipid, non-methylene interrupted dienes (NMID), and essential fatty acids (20:5 ω 3, 22:6 ω 3, and 20:4 ω 6). Data shown are average \pm standard error and statistically significant ($p < 0.05$) differences across seasons are denoted by ^{abc}.

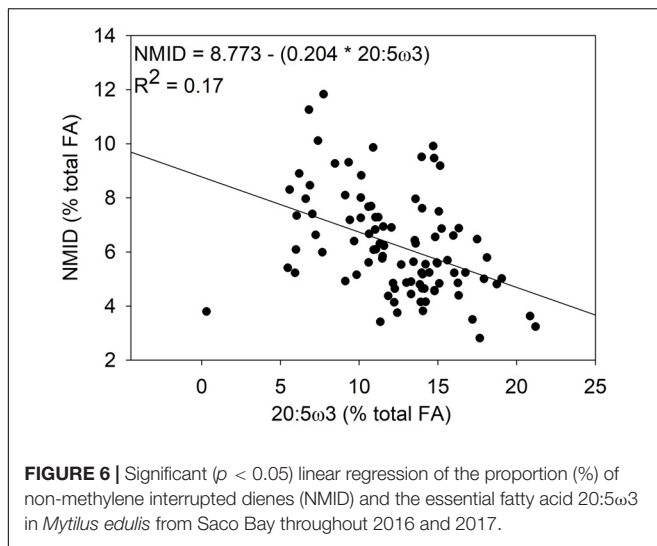
TABLE 2 | Proportions (%) of fatty acids in *Mytilus edulis* measured in this study compared to literature values.

Fatty acid	This study	Alkanani et al., 2007		Murphy et al., 2002		
		2000	2001	Site 1	Site 2	Site 3
20:2 NMID	3.4 \pm 0.1	3.7 \pm 0.9	1.0 \pm 1.5	0.5 \pm 0.2	0.3 \pm 0.0	0.3 \pm 0.1
20:4 ω 6	4.7 \pm 0.1	2.8 \pm 0.8	2.8 \pm 1.2	1.5 \pm 0.7	1.9 \pm 0.2	1.8 \pm 0.2
20:5 ω 3	12.3 \pm 0.1	12.0 \pm 2.2	17.0 \pm 3.5	13.3 \pm 3.5	15.1 \pm 0.9	15.4 \pm 0.8
22:2 NMID	2.8 \pm 0.1	3.6 \pm 0.9	2.8 \pm 1.5	1.4 \pm 0.4	1.4 \pm 0.2	1.2 \pm 0.3
22:6 ω 3	14.4 \pm 0.1	21.5 \pm 2.9	20.0 \pm 4.3	18.2 \pm 5.5	21.3 \pm 2.0	24.2 \pm 3.9
Σ Saturated	22.2 \pm 0.1	25.4 \pm 1.8	23.6 \pm 2.3	28.9 \pm 1.2	29.5 \pm 2.7	30.4 \pm 0.6
Σ MUFA	21.1 \pm 0.1	14.5 \pm 3.0	17.7 \pm 4.1	11.9 \pm 4.4	10.4 \pm 1.0	9.6 \pm 0.8
Σ PUFA	54.5 \pm 0.1	61.9 \pm 3.0	60.8 \pm 4.0	44.0 \pm 7.4	49.2 \pm 4.6	50.1 \pm 1.2
Σ ω 3	37.1 \pm 0.1 ^a	47.8 \pm 3.7 ^b	49.5 \pm 3.5 ^b	38.2 \pm 7.2 ^{ab}	41.7 \pm 1.8 ^{ab}	44.6 \pm 1.4 ^{ab}

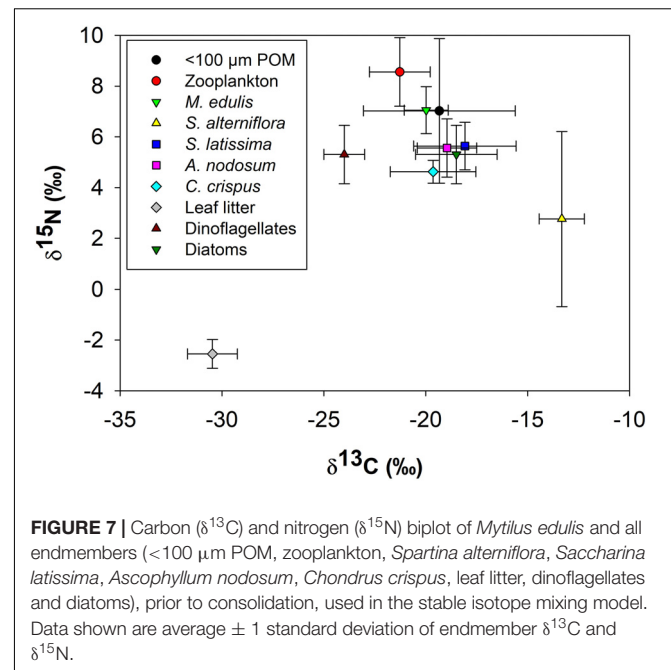
Values shown are average \pm standard error, significant difference ($p < 0.05$) are denoted by ^{abc}, values without superscripts indicate no significant difference across columns.

FA profile of zooplankton was very similar to *M. edulis*. The three major FAs in zooplankton were the same as *M. edulis* (16:0, 20:5 ω 3, and 22:6 ω 3). Although *M. edulis* contained FAs from all endmembers, phytoplankton (diatom and dinoflagellate) were the largest contributors. Diatom (16:1 ω 7, 16:4 ω 1 and 20:5 ω 3) and dinoflagellate (22:6 ω 3 and 18:4 ω 3) FAs comprised

the largest proportions of *M. edulis* FAs, 17.2 \pm 0.1% and 16.9 \pm 0.1% respectively, followed by macroalgae (7.9 \pm 0.1%) and zooplankton (20:1 and 21:1; 6.2 \pm 0.1%) FAs. Vascular plant FAs comprised the smallest proportion (3.3 \pm 0.1%) of *M. edulis* FAs with bacterial FAs (*iso*, *anteiso* and odd-chained) comprising another 4.8 \pm 0.1%.



The isotopic composition of *M. edulis* closely resembled that of $< 100 \mu\text{m}$ POM (Figure 7). The $\delta^{13}\text{C}$ ($-19.4 \pm 3.7\text{‰}$) and $\delta^{15}\text{N}$ ($7.0 \pm 2.8\text{‰}$) of POM $< 100 \mu\text{m}$ varied the most among all endmembers, ranging from -25‰ $\delta^{13}\text{C}$ and 2.9 to 15.2‰ $\delta^{15}\text{N}$. In contrast, the $\delta^{13}\text{C}$ ($-20.0 \pm 1.1\text{‰}$) and $\delta^{15}\text{N}$ ($7.0 \pm 0.9\text{‰}$) of *M. edulis* occupied a very narrow range between -18 to -21‰ and 6.0 to 7.6‰ respectively. All three macroalgal species had similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (combined average $-18.8 \pm 2.0\text{‰}$ and $5.3 \pm 1.0\text{‰}$ respectively). Macroalgal isotopic values were very close to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for diatoms (-18.5‰ and 5.3‰ respectively) based on literature sources and estimated $\delta^{15}\text{N}$ values from zooplankton, which were the most enriched in $\delta^{15}\text{N}$ in this study (Figure 7). Both C_3 and C_4 vascular plants were isotopically distinct from marine endmembers. Leaf litter (C_3) was the most depleted source of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($-30.5 \pm 1.2\text{‰}$ and $-2.5 \pm 0.6\text{‰}$, respectively) while *S. alterniflora* (C_4) was the most enriched in $\delta^{13}\text{C}$ ($-13.3 \pm 1.1\text{‰}$) compared to other endmembers. The $\delta^{13}\text{C}$ of all marine endmembers fell between the values of C_3 and C_4



vascular plants and were more enriched in $\delta^{15}\text{N}$ (Figure 7). The tight clustering of marine endmembers at roughly the mid-point between C_3 and C_4 vascular plants based on $\delta^{13}\text{C}$ made it difficult to determine endmember contributions to POM and mussel diets based on stable isotopes alone.

Endmember Contributions to POM and Mussel Diets

Principal coordinates analysis separated all endmember groups based on their FA profiles (Figure 8). Samples within each endmember group were highly similar ($> 75\%$), except for POM (61% similarity), likely a result of its variable composition. The FA profiles of consumers (zooplankton and *M. edulis*) were similar (65%) to each other, both groups of vascular plants (*S. alterniflora*

TABLE 3 | Proportion (%) of endmember fatty acids in *Mytilus edulis* collected from Saco Bay, ME, United States, throughout 2016 and 2017. Endmembers: Zooplankton (20:1 and 21:1), Vascular plants (18:2 ω 6 and 18:3 ω 3), Macroalgae (18:1 ω 9, 18:3 ω 6, 20:2 ω 6, 20:4 ω 3, and 20:4 ω 6), Diatoms (16:1 ω 7, 16:4 ω 1 and 20:5 ω 3), Dinoflagellates (22:6 ω 3), and Bacteria (iso, anteiso and odd-chained).

	Diatom	Dinoflagellate	Zooplankton	Macroalgae	Vascular plant	Bacterial
Date	%	%	%	%	%	%
5/20/16	19.3 \pm 0.5	15.6 \pm 0.4	6.2 \pm 0.1	9.1 \pm 0.2	3.5 \pm 0.1	4.1 \pm 0.1
6/23/16	15.6 \pm 0.2	15.9 \pm 0.1	6.2 \pm 1.2	8.6 \pm 0.2	2.8 \pm 0.1	4.4 \pm 0.1
7/8/16	13.0	18.1	7.0	10.5	3.3	5.2
9/7/16	17.9 \pm 0.2	15.7 \pm 0.1	6.0 \pm 0.1	7.3 \pm 0.1	3.1 \pm 0.1	5.4 \pm 0.2
10/3/16	15.4 \pm 0.2	17.6 \pm 0.1	6.7 \pm 0.1	8.3 \pm 0.1	3.3 \pm 0.1	5.5 \pm 0.1
12/20/16	19.7 \pm 0.9	14.9 \pm 0.2	5.0 \pm 0.3	5.8 \pm 0.3	2.6 \pm 0.1	4.3 \pm 0.2
5/1/17	21.9 \pm 0.2	16.4 \pm 0.1	5.7 \pm 0.1	6.6 \pm 0.1	2.7 \pm 0.1	4.9 \pm 0.1
6/26/17	19.0 \pm 0.4	16.3 \pm 0.3	6.8 \pm 0.3	7.3 \pm 0.2	2.7 \pm 0.2	4.8 \pm 0.1
9/25/17	10.8 \pm 0.2	20.4 \pm 0.1	6.4 \pm 0.1	8.3 \pm 0.1	5.0 \pm 0.1	3.9 \pm 0.1

Data shown are average ± 1 standard error.

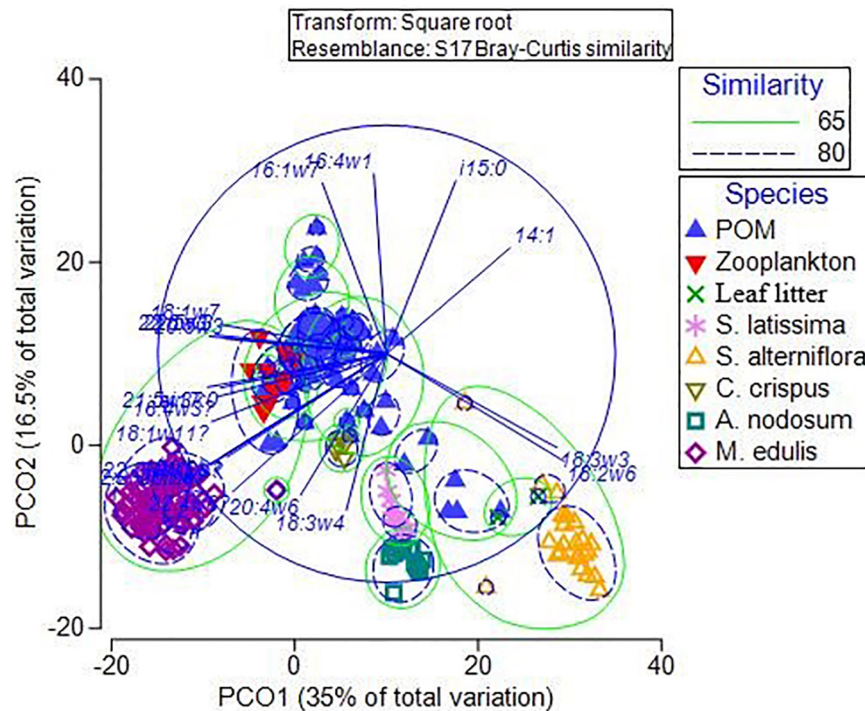


FIGURE 8 | Principal coordinates analysis of fatty acids in <100 μm particulate organic matter (POM), zooplankton, leaf litter, *Spartina alterniflora*, *Saccharina latissima*, *Ascophyllum nodosum*, *Chondrus crispus* and *Mytilus edulis* from Saco Bay, ME, United States, throughout 2016 and 2017. Circled groupings are based on similarity (%) determined from similarity percentage analysis (SIMPER) and cluster analysis.

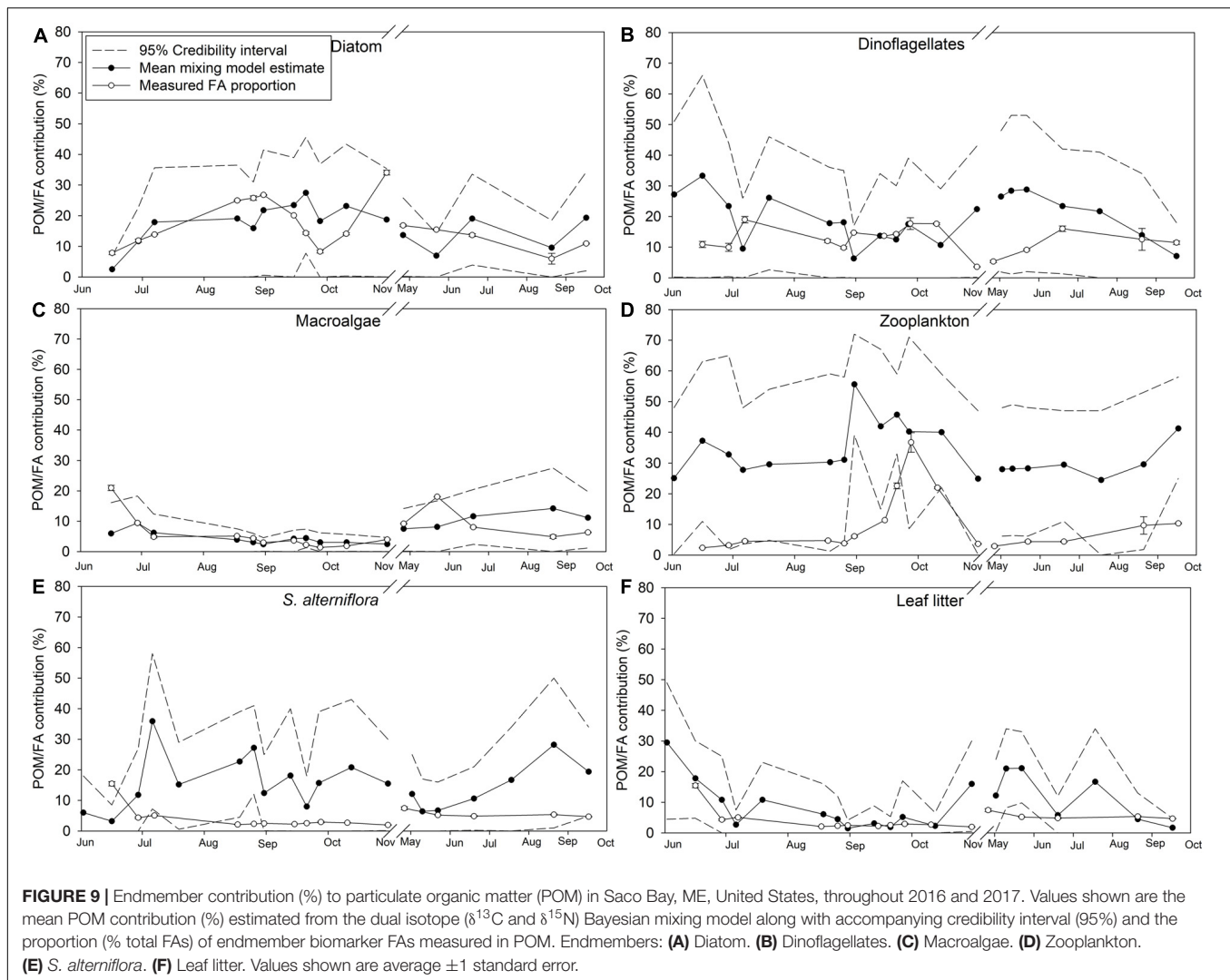
and leaf litter) also resembled each other (65%), while the three macroalgal groups were more loosely grouped together. POM was the most variable group interspersed between all the other groups. However, there was a larger proportion of POM samples grouped closer to zooplankton than either macroalgae or vascular plants, suggesting zooplankton (and presumably phytoplankton) more strongly influenced POM FAs.

Credibility intervals (95%) from the dual isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) Bayesian mixing models estimating endmember contributions to POM and the diet of *M. edulis* were relatively large, suggesting uncertainty in the model (Figures 9, 10). Because diatoms and macroalgae are traditionally difficult to separate based on their $\delta^{13}\text{C}$ ($-18 \pm 2.0\text{‰}$ and $-18.8 \pm 2.0\text{‰}$ respectively), they were initially combined for the model. Taking the combined macroalgal and diatom model output, we used the relative proportions of diatom and macroalgal FAs to estimate their separate contributions to POM and mussel diets. In the updated model, based on mean credibility interval estimates, zooplankton ($33.6 \pm 8.2\%$) were the largest contributor to the POM pool, followed by diatoms ($16.8 \pm 6.5\%$), *S. alterniflora* ($15.6 \pm 8.4\%$), dinoflagellates ($19.4 \pm 7.9\%$), leaf litter ($9.8 \pm 8.1\%$), and finally macroalgal detritus ($6.3 \pm 3.7\%$; Figure 9). The largest proportion ($25.3 \pm 5.4\%$) of *M. edulis* diet was comprised of *S. alterniflora* based on the dual isotope mixing model followed by dinoflagellates ($18.6 \pm 2.3\%$), zooplankton ($17.2 \pm 3.0\%$), diatoms ($16.7 \pm 2.6\%$), leaf litter ($14.8 \pm 2.1\%$), and macroalgae ($8.3 \pm 2.1\%$; Figure 10).

Importantly, the stable isotope mixing model and the FA composition analyses largely agree regarding the contributions of diatoms, dinoflagellates, and macroalgae to both POM and mussel diets (Figures 9A–C, 10A–C). However, there were more discrepancies between the estimated contributions of zooplankton (Figures 9D, 10D) and vascular plant detritus (Figures 9E,F, 10E,F) between the two methods. To test the assumptions of our model, we compared our original estimates with estimates of a revised models incorporating $\delta^{13}\text{C}$ corrections based on mussel lipid content and larger or smaller fractionation values between zooplankton and phytoplankton, as well as between mussels and their diet (Supplementary Table S6). Based on the comparison, we concluded that our model assumptions did not affect our conclusions. To address uncertainty in the model from wide credibility intervals, which frequently approached zero, we used the raw model output (distribution of estimates from all 3×10^4 model iterations) to determine the probability of zero contribution (<1%) to the diet of *M. edulis* for each endmember (Table 4). In general, the probability of zero contribution for most endmembers was low (<1%), with the exception of leaf litter which had significantly higher probabilities ($p < 0.01$) of zero diet contribution than any other endmember.

DISCUSSION

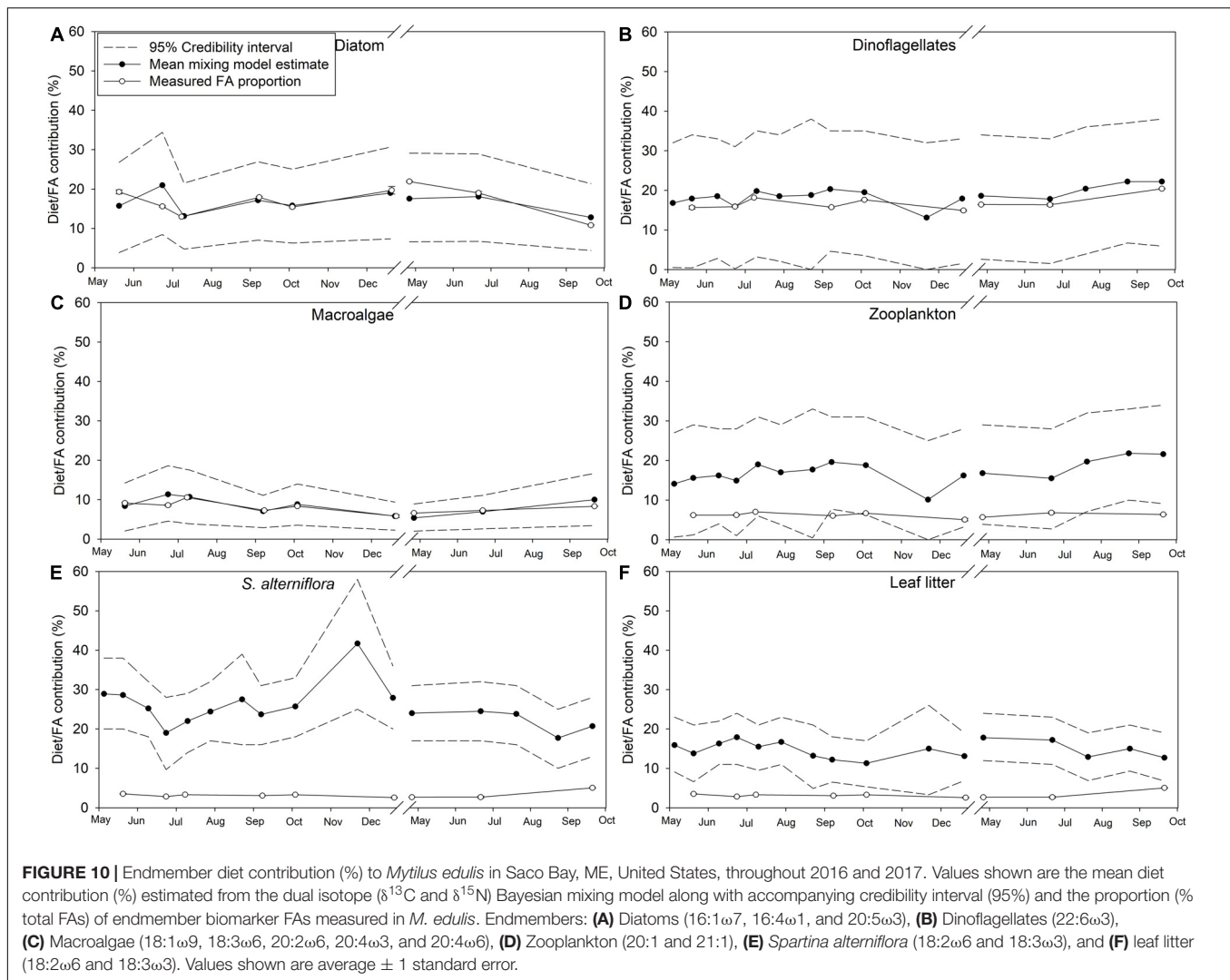
It has long been hypothesized that detritus food chains predominate in salt-marsh ecosystems (Odum, 1980), and more



recently that differential utilization of POM allows filter feeders to partition into trophic niches to reduce competition (Lefebvre et al., 2009; Tallis, 2009; Antonio and Richoux, 2016). Our forensic investigation of intertidal mussel diets clearly shows mussels were broad spectrum opportunistic omnivores and incorporated phytoplankton, zooplankton, macroalgal detritus, and vascular plant detritus into their diets to varying degrees. Using the measured FA biomarkers, along with insights from the Bayesian stable isotope mixing model results, we characterized the potential roles of each food source for *M. edulis* (Figure 11). Unsurprisingly, diatoms, dinoflagellates, and zooplankton were the primary contributors to the diet of mussels. These three food sources explained 40% of *M. edulis* FAs and 53% of their isotopic signature. Mussels primarily obtained the essential FAs 20:5 ω 3, 22:6 ω 3, and to a lesser extent 20:4 ω 6 (depending on resuspension of benthic pennate diatoms) from phytoplankton and zooplankton. In addition to their primary food sources, mussels also incorporated macroalgal detritus into their diet, which accounted for 8% of their FAs and isotopic composition (Figure 10). We suspect that although macroalgal detritus comprised a small proportion of their diet, macroalgal detritus

could be important to *M. edulis* in supplying the essential FAs, 20:4 ω 6 and 20:5 ω 3. Although mussels could obtain 20:5 ω 3 from both diatoms and zooplankton, our results suggest mussels were likely limited by 20:5 ω 3 during summer months and would have incentive to obtain this essential FA from additional sources. In contrast, although bacteria and vascular plants also contributed to the FAs of *M. edulis* (5 and 3%, respectively), they do not supply essential FAs. As such, the role of bacteria and vascular plant detritus is likely only to aid mussels in meeting their energetic or carbon requirements. There were only very low probabilities (<1%) that macroalgal and *S. alterniflora* detritus did not contribute to the diet of *M. edulis* in Saco Bay based on our stable isotope model outputs (Table 4). Although it is likely that *M. edulis* did make some use of leaf litter, the significantly ($p < 0.01$) higher likelihood (<5%) and frequency that leaf litter had zero-contribution to mussel diets compared to all other endmembers suggests leaf litter use may be less important and a more sporadically used food source.

Mussels in Saco Bay were omnivores that obtained some of their dietary requirements through detrital feeding. Although



M. edulis FAs primarily resembled those of dinoflagellates (22:6 ω 3) and diatoms (20:5 ω 3 and 16:1 ω 7) based on their biomarker content in both PCoA and SIMPER analyses, mussels did contain zooplankton (20:1 and 22:1), vascular plant detritus (18:2 ω 6 and 18:3 ω 3), and macroalgal detritus (18:1 ω 9, 18:3 ω 6, 20:2 ω 6, 20:4 ω 3, and 20:4 ω 6) markers (**Table 3**). Zooplankton were the most similar group to *M. edulis*, based on dinoflagellate and diatom markers, similarities which are likely due to a shared diet of phytoplankton. However, *M. edulis* did contain zooplankton FAs ($6.2 \pm 0.1\%$) which could only be obtained by direct consumption of either live zooplankton or zooplankton derived detritus (Dalsgaard et al., 2003). We assume that vascular plant (salt marsh and leaf litter) and macroalgal material present in POM and assimilated by mussels are detrital in origin. In addition to phytoplankton and zooplankton markers, *M. edulis* contained small proportions of macroalgal ($7.9 \pm 0.1\%$), bacterial ($4.8 \pm 0.1\%$), and vascular plant FAs ($3.3 \pm 0.1\%$), suggesting that *M. edulis* on average obtains 22.2% of their FAs from a combination of detritivory and carnivory. Mussels and oysters have previously been reported to make use of non-phytoplankton

food sources such as bacteria, protozoans, zooplankton and detritus based on a variety of analyses, including stable isotopes and FA (Newell and Langdon, 1986; Baldwin and Newell, 1991; Bustamante and Branch, 1996; Davenport et al., 2000; Xu and Yang, 2007; Zhao et al., 2013). Our results further support *M. edulis* not being solely herbivorous. Detrital contributions to the diet of *M. edulis* in this study are comparable to estimated salt marsh detritus contributions to the carbon requirement of oysters (5.5%) and ribbed mussels (31%; Langdon and Newell, 1990), but are lower than previous contributions of kelp detritus (50%) to carbon requirements of *Mytilus galloprovincialis* (Bustamante and Branch, 1996). The latter discrepancy is perhaps the result of Saco Bay's adjacency to Scarborough Marsh, the largest salt marsh in the region, and the abundance of marsh detritus in POM. High contributions of kelp detritus to the diet of *M. galloprovincialis* were in areas where kelp detritus composed large proportions (>65%) of POM, which is not the case in Saco Bay and may explain lower macroalgal contributions to the diet of *M. edulis*.

Although there is little doubt that *M. edulis* consumes phytodetritus, our methods cannot differentiate between

TABLE 4 | The proportion (%) of Bayesian mixing model iterations (30×10^3) that estimated zero contribution (<1%) for each endmember (Diatom, dinoflagellate, zooplankton, macroalgae, *Spartina alterniflora*, and leaf litter) to the diet of *Mytilus edulis* in Saco Bay, ME, United States, throughout 2016 and 2017.

Date	Diatom/Macro # Obs. < 1% (%)	Diatom # Obs. < 1% (%)	Macroalgae # Obs. < 1% (%)	Dinoflagellates # Obs. < 1% (%)	Zooplankton # Obs. < 1% (%)	<i>S. alterniflora</i> # Obs. < 1% (%)	Leaf litter # Obs. < 1% (%)
5/5/16	0.13	–	–	0.6	0.18	0	0.96
5/20/16	–	0.28	0.62	0.79	0.05	0.01	2.32
6/9/16	0.03	–	–	0.28	0	0	0.38
6/23/16	–	0.04	0.07	0.97	0.05	0.12	0.68
7/10/16	–	0.11	0.16	0.29	0	0	0.5
7/28/16	0.04	–	–	0.36	0	0	0.35
8/22/16	1.11	–	–	1.9	0.05	0.07	3.57
9/6/16	–	0.02	0.11	0.24	0	0	3.06
10/3/16	–	0.07	0.12	0.30	0	0	4.6
11/21/16	1.7	–	–	2.81	4.62	0.01	3.16
12/19/16	–	0.05	0.24	0.39	0.01	0	1.97
5/1/17	–	0.07	0.51	0.27	0.01	0	0.35
6/26/17	–	0.08	0.27	0.39	0	0	0.33
7/25/17	0.05	–	–	0.34	0	0.01	2.3
8/28/17	0.05	–	–	0.23	0	0.04	0.78
9/25/17	–	0.14	0.18	0.28	0	0.01	2.63

Diatoms and macroalgae were originally combined in model estimates, when available fatty acid proportions were used to separate diatom and macroalgae contributions.

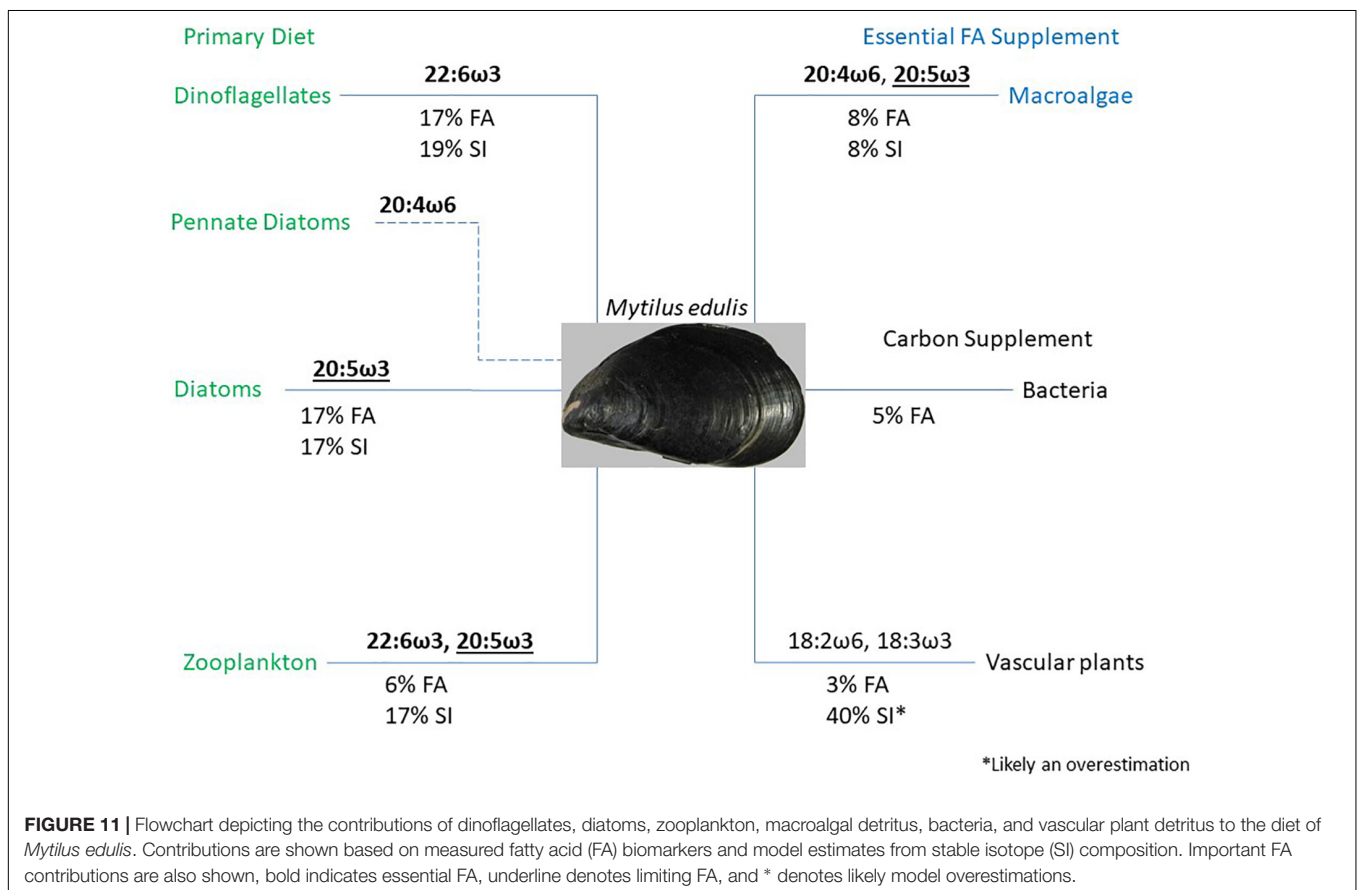


FIGURE 11 | Flowchart depicting the contributions of dinoflagellates, diatoms, zooplankton, macroalgal detritus, bacteria, and vascular plant detritus to the diet of *Mytilus edulis*. Contributions are shown based on measured fatty acid (FA) biomarkers and model estimates from stable isotope (SI) composition. Important FA contributions are also shown, bold indicates essential FA, underline denotes limiting FA, and * denotes likely model overestimations.

contributions of live vs. dead phytoplankton to their diets. By assuming all phytoplankton ingested are fresh our estimates of detrital usage are inherently conservative and underestimate

the importance of detritus. Diatom FAs comprised $17.2 \pm 0.1\%$ of *M. edulis* FAs, which agrees with the range of modeled contributions estimated with stable isotopes (Figure 10A).

Proportions of dinoflagellate FAs in *M. edulis* averaged $16.9 \pm 0.1\%$, which also fell within the range of modeled dietary contributions (**Figure 10B**). Taken together, these results suggest that *M. edulis* obtains at least 34% of their diet from herbivorous grazing. As such, phytoplankton contribute more FAs, and presumably total lipids, to the diet of *M. edulis* than any single other detrital source. This contribution of phytoplankton FAs is slightly lower than previously reported for *M. edulis* (39–46%; Alkanani et al., 2007), again possibly due to the proximity of Saco Bay to Scarborough Marsh. Proportions of saturated FAs, PUFA, MUFA, $\omega 3$, essential FAs and non-methylene interrupted dienes (NMID) in *M. edulis* were also comparable to previously reported values (**Table 2**; Murphy et al., 2002; Alkanani et al., 2007).

While vascular plant FAs contributed only a small proportion to *M. edulis* FAs ($3.3 \pm 0.1\%$) and POM ($4.8 \pm 0.1\%$), vascular plant detritus, particularly *S. alterniflora* detritus, comprised a significant portion of mussel diets ($25.3 \pm 5.4\%$) and POM ($15.6 \pm 8.4\%$) based on stable isotopes (**Figures 9E,F, 10E,F**). There are several possible explanations for this discrepancy: (1) Degraded vascular plant detritus is relatively depleted in lipids compared to their parent material and contribute more to POM and *M. edulis* C than lipids. (2) Vascular plant detritus contributions based on FAs are underestimates due to conversion of vascular plant FAs into bacterial FAs. (3) Diet contributions of diatoms and dinoflagellates are overestimations due to selective retention of FAs (e.g., 22:6 $\omega 3$ and 20:5 $\omega 3$) by *M. edulis*. (4) Inherent differences in what each method measures; FA biomarkers are only a subset of dietary lipids (do not sum to 100%), whereas isotope mixing models consider all C and N sources (an explicit assumption in some Bayesian models). (5) Overlap in endmember stable isotope signatures could mislead the Bayesian mixing model. For example, using an isotopically heavier $\delta^{13}\text{C}$ for diatoms closer to -16 or -14‰ , still within the reported range (Fry and Wainright, 1991), would result in lower estimations of *S. alterniflora* contributions. In our stable isotope mixing model *S. alterniflora* and macroalgae/diatoms were highly negatively correlated ($r = -0.77$), so improper estimation of macroalgae/diatom contributions would lead to overestimations of *S. alterniflora* detritus. Similarly, benthic pennate diatoms are enriched in $\delta^{13}\text{C}$ (-13‰) and their contributions to POM and mussel diets would be misinterpreted as *S. alterniflora* detritus in our isotope model (Currin et al., 1995). Although less prevalent than centric diatoms, pennate diatoms do contribute to the water column of Saco Bay, particularly in September (**Figure 2C**).

It is important to note that due to the difficulty in collecting enough $<100 \mu\text{m}$ POM for analysis, we were unable to analyze duplicate unacidified samples for $\delta^{15}\text{N}$. Although acidification has little effect on $\delta^{15}\text{N}$ values of animal samples (Bosley and Wainright, 1999; Jaschinski et al., 2008), acidification can affect $\delta^{15}\text{N}$ values of POM samples (Barrios-Guzmán et al., 2019). Because our POM samples were acidified, effects of acidification on POM $\delta^{15}\text{N}$ values is a source of error for this study. However, we suspect that it is primarily differences in $\delta^{13}\text{C}$ values driving the results of our mixing model, we base our suspicion on the relatively small changes in estimates when comparing alternative models with both smaller (2.3‰) and larger (5.95‰)

fractionation values (**Supplementary Table S6**). As the shift in $\delta^{15}\text{N}$ values due to acidification is usually minor (0.5‰; Barrios-Guzmán et al., 2019), smaller than the difference in $\delta^{15}\text{N}$ among our alternative models, error due to the effects of acidification on POM $\delta^{15}\text{N}$ values is unlikely to shift model estimates enough to alter the conclusions of this study.

While it is surprising that isotope modeled contributions of *S. alterniflora* detritus to *M. edulis* diets were relatively high, it is not necessarily implausible. Although the eastern oyster (*Crassostrea virginica*) and the ribbed mussel (*Geukensia demissa*) only obtained small amounts of their required carbon from *S. alterniflora* detritus (0.7 and 8.6% respectively), they obtained larger proportions of their carbon requirements (5.5 and 31% respectively) from the associated bacteria (Langdon and Newell, 1990). Minimum isotope modeled contributions of *S. alterniflora* detritus in this study (9.7–25.0%) fall within the upper range of Langdon and Newell's (1990) values suggesting they are feasible. However, the mean (17.7–41.7%) and maximum (25.0–58.0%) estimates seem less likely. Combined bacterial and vascular plant FAs represented 8.1% of mussel FAs, much lower than the 19–29% minimum modeled contributions of vascular plants based on stable isotopes. Attributing bacterial FAs to vascular plant detritus consumption, assumes that all bacterial FAs measured in *M. edulis* were from their tissue and not from bacteria growing on them, as well as that all bacterial FAs ingested by *M. edulis* were from vascular plant sources. Both assumptions are unlikely and, even considering that detritus is likely to be lipid depleted, our model almost certainly overestimated *S. alterniflora* contributions to mussel diets. Higher proportional contributions of vascular plant FAs (~11% total FAs) in the Mediterranean mussel (*Mytilus galloprovincialis*; Zhao et al., 2013), further suggest that lipid depletion of vascular plant detritus is unlikely to fully explain the higher contributions of vascular plant detritus in our model. Due to the close proximity of Scarborough marsh, the largest salt marsh in Maine, and the “outwelling hypothesis” we expected an abundance of *S. alterniflora* detritus for *M. edulis* to consume (Odum and de la Cruz, 1967). Both our measured FAs and model estimates confirm that *S. alterniflora* contributed to POM (**Figure 9E**), although our model estimates for POM are also likely overestimations. Our minimum stable isotope estimates of *S. alterniflora* (9.7–25.0%) are comparable to isotope modeled *S. alterniflora* contributions to mussel diets (10–12%) near other large marshes (Riera, 2007). However, the mean (17.7–41.7%) and maximum (25.0–58.0%) estimates are again much higher, further corroborating they are likely overestimations.

Macroalgae comprised a modest proportion (~10%) of the diet of *M. edulis* (**Figure 10C**). Consumption of macroalgal detritus by *M. edulis* is unsurprising given that macroalgal detritus is known to contribute to nearshore food webs and bivalve diets elsewhere (Duggins et al., 1989; Fredriksen, 2003; Kaehler et al., 2006; Tallis, 2009; Allan et al., 2010; Von Biela et al., 2016). Additionally, macroalgae have been shown to be a long-term dietary component for bivalves in areas with strong seasonal production of phytoplankton. The Antarctic scallop (*Adamussium colbecki*) utilized phytoplankton and zooplankton in the short-term during productive summer seasons, based on gut $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, but their tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ indicated the

use of the red algae *Iridea cordata* as a long-term dietary item (Calizza et al., 2018). Detrital contributions from macroalgae were on the lower end of previously reported values for other bivalves. Macroalgal detritus can contribute small (10–20%) to large (>50%) proportions of bivalve diets (Bustamante and Branch, 1996; Fredriksen, 2003; Allan et al., 2010) and have also been successfully incorporated into hatchery diets at rations of 50–90% (Camacho et al., 2007; Carboni et al., 2016). Lower contributions of macroalgal detritus in this study, could be due to differences in the quality of detrital particulates, or the abundance of salt marsh detritus. Quality of macroalgal detritus varies with species, age, and concentration of secondary metabolites, such as polyphenols (Cranford and Grant, 1990; Duggins and Eckman, 1997). There are large amounts of polyphenols (5–15% DW) in both *A. nodosum* and *S. latissima* (Ragan and Jensen, 1978; Wang et al., 2009). These concentrations are equivalent to or greater than those previously shown to inhibit bivalve grazing of fresh material (Duggins and Eckman, 1997), suggesting polyphenols could have deterred ingestion by *M. edulis*.

Although macroalgal detritus did not comprise a large proportion of *M. edulis* diets, its role may still be significant. Macroalgae have large proportions (7–18%) of the essential FA arachidonic acid (20:4 ω 6; see **Supplementary Table S1**), which is found only in small amounts (~1%) in dinoflagellates and most diatoms (Ackman and Tocher, 1968; Nichols et al., 1984; Dunstan et al., 1994; Mansour et al., 1999, 2003; Leblond and Chapman, 2000; Arts et al., 2001). Arachidonic acid, when used in conjunction with other ω 6 FAs, has been significantly correlated with mussel growth (Alkanani et al., 2007) and, importantly, was selectively retained by mussels in the current study (**Figure 5**). Supplementation of 20:4 ω 6 by macroalgal detritus could potentially explain why bivalves fed mixtures of macroalgal detritus and phytoplankton performed better than bivalves fed solely phytoplankton in previous studies (Carboni et al., 2016).

Decreases in lipid and total FA concentrations in *M. edulis* throughout the summer, accompanied by an increase in the proportion of NMIDs and essential FAs (20:4 ω 6 and 22:6 ω 3), suggests that mussels were physiologically stressed and preferentially retaining essential FAs (**Figures 5, 6**). Between spring and summer mussel lipid content decreased by 52%, a decrease that was generally mirrored across all FAs (SAT: 61%, MUFA: 58%, PUFA: 58%, ω 3: 59%) including essential FAs, such as 20:5 ω 3, which decreased 64%. However, the decreases in 20:4 ω 6 (48%) and 22:6 ω 3 (57%) were lower, suggesting selective retention of these FAs. Growth of *M. edulis* has been negatively correlated with the proportion of NMIDs present in their FAs and NMIDs are negatively correlated with essential FAs (Alkanani et al., 2007). Mussels are capable of synthesizing NMIDs *de novo* and are believed to do so to substitute for essential ω 3 FAs lacking in their diet (Zhukova and Svetashev, 1986; Zhukova et al., 1992; Alkanani et al., 2007). The negative correlations between NMID and growth of mussels suggests their synthesis is metabolically costly, considering co-occurring reductions in mussel 20:5 ω 3, mussels were likely stressed and either unable to completely compensate with NMID production, or 20:5 ω 3 was more dispensable

to mussels than 20:4 ω 6 and 22:6 ω 3. In finfish, 20:5 ω 3 is often more dispensable than 22:6 ω 3 and its supplementation into diets does not improve growth (Trushenski et al., 2012), if also true for mussels, supplementation of 20:5 ω 3 may not result in better performance. However, the negative correlations of NMIDs between mussel growth and 20:5 ω 3 suggests supplementing 20:5 ω 3 could improve mussel growth and warrants further study.

Selective retention of FAs could affect interpretation of diet contributions based on biomarker FAs. Both selectively retained FAs (20:4 ω 6 and 22:6 ω 3) were used as biomarkers in this study, for macroalgae and diatoms respectively. By selectively retaining these biomarker FAs, the contribution of both macroalgae and diatoms to mussel diets would appear larger. Effects of consumer metabolism on the interpretation of biomarker FAs is well known and has led to the development of Quantitative Fatty Acid Signature Analysis (QFASA; Iverson et al., 2004). In QFASA, calibration coefficients are used to account for the lipid metabolism of consumers, like trophic retention factors account for changes in consumer stable isotopes. Although useful for dealing with effects of consumer lipid metabolism, controlled feeding experiments are required to compute QFASA calibration coefficients. Due to a lack of controlled feeding experiments, we are unable to calculate calibration coefficients for *M. edulis* consuming diatoms and macroalgae. As such, it is important to recognize that contributions of macroalgal detritus and diatoms to the diet of mussels in this study may be slight over-estimations because mussels selectively retain 20:4 ω 6 and 22:6 ω 3.

Mussels may have been limited by dietary 20:5 ω 3 and consequently used macroalgal detritus to supplement their dietary requirements. Macroalgae contained the essential FA 20:5 ω 3 in large proportions (8–26%; see **Supplementary Material**). Eicosapentaenoic acid (EPA; 20:5 ω 3) is important for maintaining membrane fluidity and there is a strong relationship between 20:5 ω 3 and temperature in the gills of scallops (Hall et al., 2002). In the current study, only the proportion of the essential FA 20:5 ω 3 decreased in conjunction with increasing NMIDs (**Figures 5, 6**), suggesting that NMIDs were synthesized to replace 20:5 ω 3. Based on this negative correlation, 20:5 ω 3 may have been a limiting FA for mussels. The phytoplankton community of Saco Bay is dominated by dinoflagellates (**Figure 2**), so it is possible that limitations in diatom availability caused the limitation of 20:5 ω 3 for mussels. Thus mussels could make use of macroalgal detritus to supplement their diet with 20:4 ω 6 and 20:5 ω 3; this supplemental effect might explain why bivalve growth was found to increase when supplied with a mixed diet of macroalgal detritus and live phytoplankton in previous studies (Camacho et al., 2007). The period in late spring and early summer when mussels were physiologically stressed coincides with when macroalgal detritus contributed proportionally more to POM (**Figure 9**). Stressed mussels, potentially limited in 20:5 ω 3, would be incentivized to make use of macroalgal detritus as an alternative essential FA source. Higher proportional contributions of macroalgal detritus to POM in late spring and early summer also resulted in higher 20:4 ω 6-to-22:6 ω 3 and

20:4 ω 6-to-20:5 ω 3 ratios (**Figure 4**). Macroalgae have a history of use as a supplemental diet in agriculture. Although considered an inferior ingredient, based on proximate composition and low digestibility, macroalgae is still supplemented into animal diets in low amounts (3–10%) due to prebiotic effects which result in increased health and productivity (Evans and Critchley, 2014). Could macroalgal detritus serve a similar role for bivalves and provide small amounts of micronutrients such as essential FAs?

While 20:4 ω 6 only occurs in small proportions in most diatoms, some pennate diatoms can have larger proportions (2–6%; Dunstan et al., 1994). Microphytobenthos production can represent a significant portion of total productivity, which when resuspended can directly contribute to the water column phytoplankton assemblage (MacIntyre et al., 1996). Pacific oysters, *Crassostrea gigas*, are capable of selectively feeding on resuspended pennate diatoms from the microphytobenthos (Cognie et al., 2001). Additionally, microphytobenthos have been shown to contribute (0.4–4.4%) to the diets of both wild and cultivated bivalves (Riera, 2007). Another bivalve, *Macoma balthica*, has also been shown to consume benthic diatoms in another Maine estuary connected to the Damariscotta river (Incze et al., 1982). It was hypothesized based on $\delta^{13}\text{C}$ values that *M. balthica* were feeding on the isotopically heavy (−12.4‰) *Amphipleura rutilans*. Pennate diatoms were present in the water column of Saco Bay in September, a time when POM $\delta^{13}\text{C}$ values became enriched (**Figure 2C**; see Both et al., 2020 for more details) It therefore seems likely that pennate diatoms contributed 20:4 ω 6 to the diet of *M. edulis* during September, potentially explaining some of the discrepancy between stable isotope modeled estimates and measured FAs. However, at other times (e.g., June) when pennate diatoms were less abundant, macroalgal detritus may be a more important source of 20:4 ω 6 (**Figure 2C**). *M. edulis* does not always maximize energy gain in the short-term and can rely upon energy reserves for gametogenesis, or to avoid feeding inefficiently upon largely inorganic particulates (Hawkins et al., 1985). Although macroalgal detritus consumption is unlikely to be as energy efficient as fresh phytoplankton consumption, when physiologically stressed and potentially limited by essential FAs, *M. edulis* may feed on less energetically optimized macroalgal detritus to obtain physiologically important micronutrients such as essential FAs.

Ratios of FA biomarkers in POM confirm that macroalgal detritus was present in the water column during early summer. A well-known FA biomarker for diatoms is 16:1 ω 7 (Parrish, 2013), and because of their higher proportion of 20:4 ω 6, pennate diatoms (0.01–0.25) have a higher ratio of 20:4 ω 6-to-16:1 ω 7 than centric diatoms (0.01–0.07; Dunstan et al., 1994). With higher proportional abundances of 20:4 ω 6 and lower proportional abundances of 16:1 ω 7 than pennate diatoms, macroalgae have an even higher ratio of 20:4 ω 6-to-16:1 ω 7 (*A. nodosum*: 6.8 \pm 0.1, *S. latissima*: 4.8 \pm 1.3, *C. crispus*: 0.8 \pm 0.1). In this study, 18:1 ω 9 was an abundant FA in macroalgae (especially *A. nodosum*) which led to its use as a biomarker (see **Supplementary Material**). By comparing the ratio of the macroalgal marker 18:1 ω 9 to the diatom marker 16:1 ω 7, we can estimate whether there were

macroalgae or diatom FAs present in the water column. The ratio of 18:1 ω 9-to-16:1 ω 7 is low in both centric (0.01–0.43) and pennate (0.01–0.27) diatoms (Dunstan et al., 1994), while the ratio of 18:1 ω 9-to-16:1 ω 7 is much higher in macroalgae (*A. nodosum*: 20.1 \pm 0.4, *S. latissima*: 2.4 \pm 0.1, *C. crispus*: 0.6 \pm 0.1). Consequently, an increase in the ratio of both 20:4 ω 6-to-16:1 ω 7 and 18:1 ω 9-to-16:1 ω 7 indicates the presence of macroalgae, whereas an increase in the ratio of only 20:4 ω 6-to-16:1 ω 7 indicates pennate diatoms. In our study, both 20:4 ω 6-to-16:1 ω 7 and 18:1 ω 9-to-16:1 ω 7 are elevated in late spring and early summer confirming the presence of macroalgal detritus in the water column, while in the fall only the ratio of 20:4 ω 6-to-16:1 ω 7 was elevated suggesting pennate diatoms were the source of 20:4 ω 6 (**Figures 4B,C**). Contributions of macroalgal detritus to POM represent an additional avenue for mussels to obtain 20:4 ω 6 which is not reliant on resuspension of benthic pennate diatoms. Through multiple lines of evidence, our study makes it clear that mussels have a diverse diet with multiple pathways to obtain essential FAs.

It should be noted that, detrital use by *M. edulis* is likely to vary depending on their habitat and the available detrital resources. Different ecosystems will have different types, but also proportions, of detrital material in their POM for *M. edulis* to ingest. For example, the diet of the scallop (*A. colbecki*) varied with both depth and season as available POM shifted (Calizza et al., 2018). This may be particularly important for offshore aquaculture, where nearshore detrital inputs are likely to be reduced. Additionally, although some areas may have similar proportions of detrital inputs from various taxonomic groups (e.g., vascular plant, macroalgae, phytodetritus), the species within those groups may vary (e.g., different species of macroalgae). Detritus produced from different species will have different compositions (e.g., C:N, presence of secondary metabolites, and different FA profiles) which will affect the ability of *M. edulis* to exploit them. For example, the food quality of macroalgal particulates is known to vary with species for *M. edulis* consumers (Duggins and Eckman, 1997), so macroalgal detritus contributions to *M. edulis* will likely vary between ecosystems with different species of macroalgae. As such, it is important to take into consideration both the available sources (taxonomic groups and temporal availability) and composition of detrital resources within an ecosystem when considering their potential value for bivalve consumers.

We show that *M. edulis* in Saco Bay are omnivores and diversify their diet by incorporating multiple detrital sources. However, more work is needed to understand not only when, but why, and in what environments mussels make use of detrital subsidies. Feeding trials to confirm the ingestion and bioavailability of macroalgal FAs would help validate macroalgal detritus as an avenue for mussels to obtain essential FAs. Compound specific SIA of detrital FA biomarkers could also be used to more clearly trace organic matter within ecosystems. Additionally, more work is needed to determine whether detrital subsidies are required to meet mussel metabolic demands. These are important ecological questions and regarding aquaculture siting, if *M. edulis* does not require detrital inputs then siting strictly based on available chlorophyll is valid. However, if

detrital inputs are required, estimates of detrital abundance and quality will be needed to properly predict growth, carrying capacities, and environmental interactions of bivalve aquaculture. Understanding detrital requirements could be particularly important for offshore aquaculture, which may result in growing areas with minimal contributions from nearshore detrital inputs and water depths that inhibit the ability of resuspended material to contribute to bivalve diets.

CONCLUSION

Mytilus edulis obtained at least 22.2% of their assimilated lipid from omnivorous feeding, of which at least 16% was detrital feeding based on FAs. Of all the detrital sources ingested, macroalgal detritus arguably warrants the most attention for further studies. Although macroalgal detritus only comprised a small proportion (5–11%) of *M. edulis* diet, its contribution to *M. edulis* growth could be significant. Macroalgal detritus had large amounts of the essential FAs 20:4 ω 6 and 20:5 ω 3 and could be an avenue for mussels to supplement their essential FA requirements. Despite phytoplankton being a more important food source for bivalves, diversifying their organic matter consumption through detrital food sources offer alternate pathways for mussels to supplement their catabolic energy and essential FA requirements. Although the importance of detrital subsidies is still unclear in an ecological context, detrital material may be a useful resource to consider when culturing bivalves or siting bivalve aquaculture (e.g., macroalgal detritus increasing availability of essential FA in POM).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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AUTHOR CONTRIBUTIONS

AB designed the study, collected and processed the data, and wrote the manuscript. CB, DB, BC-P, and CP advised and guided the development of the study, provided insight and input during data processing, and edited the manuscript. All the authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.561073/full#supplementary-material>

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

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