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Sourcing and Evaluating the Use of Detritus as a Supplementary Diet for Bivalve Aquaculture Using Stable Isotopes and Fatty Acid Biomarkers

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SOURCING AND EVALUATING THE USE OF DETRITUS AS A SUPPLEMENTARY DIET FOR BIVALVE AQUACULTURE USING STABLE ISOTOPES AND FATTY ACID BIOMARKERS

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An Abstract of the Dissertation Presented
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Detritus is a ubiquitous component of ecosystems and an important fuel for secondary production. Due to the extractive nature of bivalve aquaculture, detritus is often incorporated into carrying capacity and growth models for cultured bivalves. However, despite the complexity and difficulty in obtaining direct measurements, detritus is often treated as a homogeneous food source in models. Further understanding the role detritus plays in the diet of cultured bivalves could lead to more comprehensive and accurate models as well as more informed site selection for growers. The purpose of this study was to assess the abundance, bioavailability, and contribution of detritus to the diet of a commercially important bivalve (*Mytilus edulis*) in a northern temperate bay (Saco Bay, ME USA) using a combination of lipid fatty acid biomarkers and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). Both macroalgal (6.9 ± 0.1%) and vascular plant (4.8 ± 0.1%) detritus contributed to the particulate organic matter of Saco Bay and could supplement essential fatty acids (FA) or their precursors to consumers able to digest them. Mussels in Saco Bay may have been limited by the availability of the essential fatty acid 20:5ω3 (EPA) and incorporated macroalgal detritus as a small part (5 to 11%) of their diet. Macroalgae contained large proportions of the essential FAs 20:4ω6 (7 to 18%) and 20:5ω3 (8 to 25%) which may supplement the dietary needs of mussels. The original source of primary production had more influence on the bioavailability of lipid and FAs than the state of decay. The bioavailability of lipid and FAs from

Ascophyllum nodosum (9 ± 3%) was significantly lower than that of *Spartina alterniflora* (56 ± 19%) and *Isochrysis galbana* (48 ± 2%), likely due to the presence of polyphenols and structural alginates that interfered with lipid solubilization. Although it contains essential FAs and its contribution into the diet of *M. edulis* suggest macroalgae could be a good supplemental diet for bivalve aquaculture, the concentration of secondary metabolites (>3% wt/wt) and alginates needs to be considered due to their anti-nutritional effects.

DEDICATION

I'd like to dedicate this work to Yuki for giving me the motivation and drive needed to persevere, and to Charlie, who was a great help while collecting samples. I hope he has a long and fulfilling retirement.

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LIST OF ABBREVIATIONS

ANF – Antinutritional factor

ANOVA – Analysis of variance

ARA – Arachadonic acid (20:4ω6)

CDOM – Chromophoric dissolved organic matter

CHL – Chlorophyll

CMC – Critical micelle concentration

DAG - Diacylglycerol

DHA – Docosahexaenoic acid

DIN – Dissolved inorganic nitrogen

DOM – Dissolved organic matter

DON – Dissolved organic nitrogen

DW – Dry weight

EHAA – Enzyme hydrolysable amino acid

EPA – Eicosapentaenoic acid

FA – Fatty acid

FAME – Fatty acid methyl ester

FFA – Free fatty acid

GC – Gas chromatography

HI – Hydrolysis index

LI – Lipolysis index

LOBO – Land ocean biogeochemical observing

Logit – $log\frac{x}{1-x}$

MSR – Molar solubilization ratio

MUFA – Monounsaturated fatty acid

NERACOOS – Northeast regional association of coastal ocean observing system

NMID – Non-methylene interrupted diene

OM – organic matter

PAR – Photosynthetically active radiation

PCoA – Principal coordinate analysis

POM – Particulate organic matter

PERMANOVA – Permutational analysis of variance

PERMDISP – Homogeneity of multivariate dispersion

PUFA – Polyunsaturated fatty acid

PL – Phospholipid

QFASA – Quantitative fatty acid signature analysis

RE – Recovery efficiency

REMORG – Remaining organic matter

SAT – Saturated fatty acids

SELORG – Selected organic matter

SIMPER – Similarity percentages

STC – Sodium taurocholate

TAG - Triacylglycerol

CHAPTER 1

INTRODUCTION

Detritus has long been recognized for its ubiquity within ecosystems and importance in the microbial loop, secondary production and stabilization of food webs (Odum, 1969; Mann, 1988; Duggins et al., 1989). To differentiate detritus from the broader term seston (defined as all suspended particulate matter), we operationally define detritus as any decaying or dead organic matter sweated from parent material, along with any associated bacteria. Living bacteria associated with dead and decaying organic matter have been included in our definition of detritus due to their critical role in altering particle size and nutritional value (Mann, 2000). On average over 50% of primary production in both terrestrial and aquatic ecosystems enters detrital pathways, making detritus a crucial component of ecosystem energy flow (Cebrian and Lartigue, 2004). Secondary production can result from the consumption of autochthonous detritus (Baird and Ulanowicz, 1989) or subsidized by allochthonous detritus imported from other ecosystems, as in estuaries (Dias et al., 2016), where detritus is frequently a primary food source (McLusky 1981, Schlacher & Wooldridge 1996). Whether autochthonous or allochthonous in origin, detritus consumption within ecosystems supports a greater diversity and higher biomass of species than would be possible by herbivory alone (Hairston & Hairston 1993, Moore et al. 2004).

Due to the abundance of primary producers, one of the largest sources of detritus is uneaten primary production. In an extensive review of detrital production in terrestrial and marine habitats, the rates of primary production were highly correlated with detrital production (Cebrian & Lartigue 2004). Additionally, nutrient concentrations (N and P) of primary producers are strongly positively correlated with herbivory rates as well as decomposition rates (Cebrian and Lartigue, 2004). Chemical composition, carbon-to-nitrogen ratios, nutrient composition and lignin content have all been used as predictors for

decomposition rates (Swift et al. 1979, Coleman et al. 1983, Melillo et al. 1984, Moore et al. 2004). Another important factor to consider when assessing nutritional quality of detrital material is the concentration of anti-nutritional factors (ANF); defined as "substances generated in natural feed stuffs by the normal metabolism of species by different mechanisms (e.g., inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exert effects contrary to optimum nutrition" (Kumar, 1992). Polyphenols are an example of an ANF, polyphenols are secondary metabolites produced by vascular plants and macroalgae (Ragan and Jensen, 1978; Ragan and Glombitza, 1986; Kumar, 1992) and their presence can inhibit grazing (Duggins and Eckman, 1997) and adversely affect consumers digestive processes (Zimmer, 1997). A primary factor limiting the amount of energy organisms can derive from detrital particulates is their ability to digest them (Arambalza et al., 2010, 2014). In addition, digestibility of detrital particles is not homogeneous across species and their chemical composition affects digestibility by consumers (Arambalza et al., 2010, 2014).

The importance of detritus, whether allochthonous or autochthonous, in energy and nutrient cycling has led to its frequent and necessary incorporation into ecosystem models (e.g., Atlantis (Fulton et al., 2004) and EcoPath (www.ecopath.org)). Models incorporating detritus vary in design, from food-web trophic structure models (Coll et al., 2015; Feng et al., 2018), to carrying capacity models (Byron et al., 2011a), to organismal bioenergetic models (Hawkins et al., 2013a). While frequently considered by models, detritus is often treated as a "black box" whose quantity is estimated based on uneaten primary production. Additionally, modeled detritus is treated as a homogeneous pool of organic matter in terms of particle size, biochemical composition and nutritional value, which does not reflect the reality of its variable nature. Treating detritus as a homogeneous pool of organic matter can result in over or under-estimating its importance, particularly for carrying capacity and growth estimates where varying compositions of detrital material has direct effects on digestibility and absorption efficiencies.

Bivalve aquaculture researchers frequently employ carrying capacity and growth models to estimate the production capacity of particular bays or estuaries. Bivalve aquaculture is inherently extractive; it relies upon resources already present in environments to grow organisms. All bivalves ingest detritus as part of their diet to varying degrees (Langdon and Newell, 1990; Bustamante and Branch, 1996; Tallis, 2009; Ezgeta-Balić et al., 2012). However, large variations exist in detrital contributions to bivalve diets. Detritus can comprise the majority of a bivalve's diet (Bustamante and Branch, 1996) or only a minor fraction (Langdon and Newell, 1990). Although the quality of detrital particulates for bivalve consumers depends on their source and age (Duggins and Eckman, 1997), the availability of higher quality food-sources also plays a role in detrital consumption. For example, detrital contribution to the diets of *Mytilus edulis* and *Crassostrea gigas* varied from 12 to 95 % when measured and was negatively correlated with phytoplankton concentration (Hawkins et al., 2013a). In addition to source and age, detrital consumption varied over time depending on the availability of fresher organic material. As such, detailed and accurate carrying capacity and growth models of bivalve aquaculture require some way of estimating the quantity, quality, and seasonality of the primary diet (phytoplankton) as well as the available detrital resources which may contribute to the diet of the cultivated species.

A better understanding of the reliance of cultured bivalves on detrital resources could allow for better carrying capacity and growth models and better site selection practices. Currently site selection for bivalve aquaculture primarily focuses on the availability of phytoplankton, often based on measurements of chlorophyll-*a* from samples, continuous fluorometry and satellite imaging (Snyder et al., 2017). Although some measurements of detrital availability can be incorporated, e.g. remaining organic matter (REMORG; Hawkins et al., 2013b), they necessarily treat detritus as homogeneous. The reason for this necessary simplification is that there can be numerous sources of detrital material for any given ecosystem and it is usually not feasible or cost effective to measure and model every source.

This simplified approach is understandable from a grower's perspective; however, for ecologists and ecosystem managers seeking to construct carrying capacity models of bivalve aquaculture, more detail in detrital contributions is warranted. As ecosystem engineers, bivalves can have profound effects on their surrounding ecosystem (Karatayev et al., 2002), i.e. seston depletion (Dowd, 2003). As such, by treating detritus as a homogeneous pool of organic matter we risk over or under-estimating food availability, which could lead to ill-advised management decisions. Better understanding how bivalves interact with available food resources will also allow for more informed decisions when siting bivalve aquaculture.

The purpose of this study was to assess the organic matter availability, primary and detrital, in a northern temperate estuary (Saco Bay, ME USA) as well as determine how the available organic matter contributes to the diet of *Mytilus edulis*, a commercially important bivalve species. I use lipid fatty acid biomarkers in combination with stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to determine the composition of particulate organic matter and its contribution to the diet of intertidal *M. edulis* in Saco Bay. Additionally, I assessed the bioavailability of lipids and FAs, estimated by the ability to be solubilized in sodium taurocholate, from two major detrital resources (marsh grass and macroalgae). The goal of this study is to determine which detrital resources may be important for bivalve aquaculture, to narrow down which detrital resources warrant further study and incorporation into ecological carrying capacity and growth models.

CHAPTER 2

CHANGES IN PARTICULATE ORGANIC MATTER DYNAMICS IN A TEMPERATE BAY (SACO BAY, ME USA) DETERMINED USING STABLE ISOTOPE AND FATTY ACID ANALYSES

2.1 Introduction

Particulate organic matter (POM) is a complex resource derived from many sources, which fuels coastal, estuarine, and pelagic food webs. Components of POM range from living phytoplankton and zooplankton to bacterial aggregates (Biddanda, 1988) and dead and decaying detrital material from phytoplankton (Alldredge and Gotschalk, 1989), zooplankton (Davoll and Silver, 1986), macroalgae (Duggins et al., 1989), salt marshes (Mann, 1988; Bergamino and Richoux, 2014) and other terrestrial sources (Kristensen et al., 2008). Each component contributing to POM represents a resource, of varying quality, for organisms capable of exploiting them, e.g., filter feeders. Understanding POM dynamics within an ecosystem can lead to insights into ecosystem function. For example, allochthonous inputs of detrital POM or dissolved organic matter (DOM) can lead to secondary production resulting in net heterotrophic food webs observed in estuaries (Griffith and Raymond, 2011).

The amount and quality of POM determines how much, and what kinds, of secondary consumers can be supported, i.e. herbivores, detritivores, or omnivores. While phytoplankton production serves as the primary food source for herbivorous zooplankton and filter feeders, omnivores and detritivores also consume detrital POM (Diodato and Hoffmeyer, 2008). Filter feeding bivalves ingest both macroalgal and marsh grass detritus (Lucas and Newell, 1984; Newell and Langdon, 1986; Bustamante and Branch, 1996). Similarly, bacterial aggregates (Alber and Valiela, 1996) and vascular plant detritus (Dias et al., 2016) contribute to the diets of primary consumers. Unsurprisingly, due to its ephemeral nature, the quality and quantity of POM vary by season (Danovaro and Fabiano, 1997). The ratio of fresh-to-detrital organic matter also affects detrital consumption by bivalves (Hawkins et al., 2013a).

The composition of POM will affect which fatty acids (FA) are available to consumers. Essential FAs, such as 20:5ω3 (EPA), 22:6ω3 (DHA) and 20:4ω6 (ARA) are important drivers of ecosystem stability and are important for many consumers, such as bivalves and crustaceans (Arts et al. 2001; Arts et al. 2009; Parrish 2009). Essential FAs primarily originate from marine primary producers such as diatoms, dinoflagellates and macroalgae (Parrish 2009; Kelly and Scheibling 2012; Parrish 2013), while vascular terrestrial plants are associated with long chain saturated FAs, such as 24:0, and shorter chain polyunsaturated FAs (PUFA), especially 18:2ω6 and 18:3ω3 (Budge et al. 2001; Kelly and Scheibling 2012). Although some invertebrate consumers are able to elongate PUFA (18:2ω6 and 18:3ω3) into metabolically essential FAs (20:5ω3, 22:6ω3 and 20:4ω6), most marine consumers must obtain them from their diet (Sargent et al. 1999; Hall et al. 2006; Kelly and Scheibling 2012). Changes in the abundance of vascular plant and marine POM will directly affect the abundance and quality of FAs available for consumers.

Characterizing the composition of POM is required to properly assess the resources available to consumers. Understanding the available POM resources allows the construction of carrying capacity models for consumers, e.g. bivalves (Jiang and Gibbs, 2005; Byron et al., 2010, 2011a; Outeiro et al., 2018; Kluger et al., 2019), and can be used to define and quantify a "resource unit" within larger social-ecological system frameworks (Ostrom 2009; Johnson et al. 2019). As such, understanding POM dynamics is important for ecosystem functioning as well as understanding how human activities function and interact within ecosystems.

Quantifying phytoplankton abundance and production using estimates of chlorophyll-*a* from buoy measurements and satellite imagery is a routine practice, e.g. aquaculture site selection (Snyder et al., 2017). In contrast, understanding the dynamics of detrital POM is far more difficult. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) have been successfully used to determine source contributions to POM (Bergamino and Richoux, 2014) as have lipid and fatty acid biomarkers (Budge and Parrish 1998). Used together, stable

isotopes, lipid, and fatty acids biomarkers are powerful tools for determining the sources of POM to consumer diets. Stable isotopes provide insight into the source and pathways of organic carbon and nitrogen in an ecosystem. On the other hand, because lipids are the densest form of energy in marine ecosystems and due to necessity of certain FAs to consumer physiology (Parrish, 2013), lipid and FA biomarkers provide insight into the transfer of energy and essential metabolites in ecosystems.

The purpose of this study was to gather baseline data as well as qualitatively and quantitatively assess the available POM of a northern temperate bay (Saco Bay, Maine USA) with large riverine inputs (Saco River). We collected size-fractionated POM (≥100 and <100 μm), zooplankton, and macrophyte primary producers (macroalgae, marsh grass, and terrestrial plants) from Saco Bay, ME USA, and analyzed their lipid, FA, and stable isotope composition to determine their contributions to the POM pool. Our goal was to describe the qualitative and quantitative organic matter dynamics of the bay as well as the availability of essential FAs. To our knowledge, this is the first characterization of nearshore POM in the Gulf of Maine that employs both FA and stable isotope analysis.

2.2.0 Methods

2.2.1 Study site and sample collection

Saco Bay is a northern temperate bay located in southern Maine, USA, which directly receives freshwater from both the Saco and Scarborough Rivers (Figure 2.1). The Saco River provides the majority of the freshwater to the bay (Jacobson et al., 1987; Barber, 1995; Kelley et al., 2005) and there are numerous salt marshes located around Saco Bay, including Scarborough marsh which is the largest in Maine (Jacobson et al., 1987; Kelley et al., 2005). In addition, six wastewater treatment plant outfalls that discharge into the bay, serving a population of 67,000 (US Census 2010).

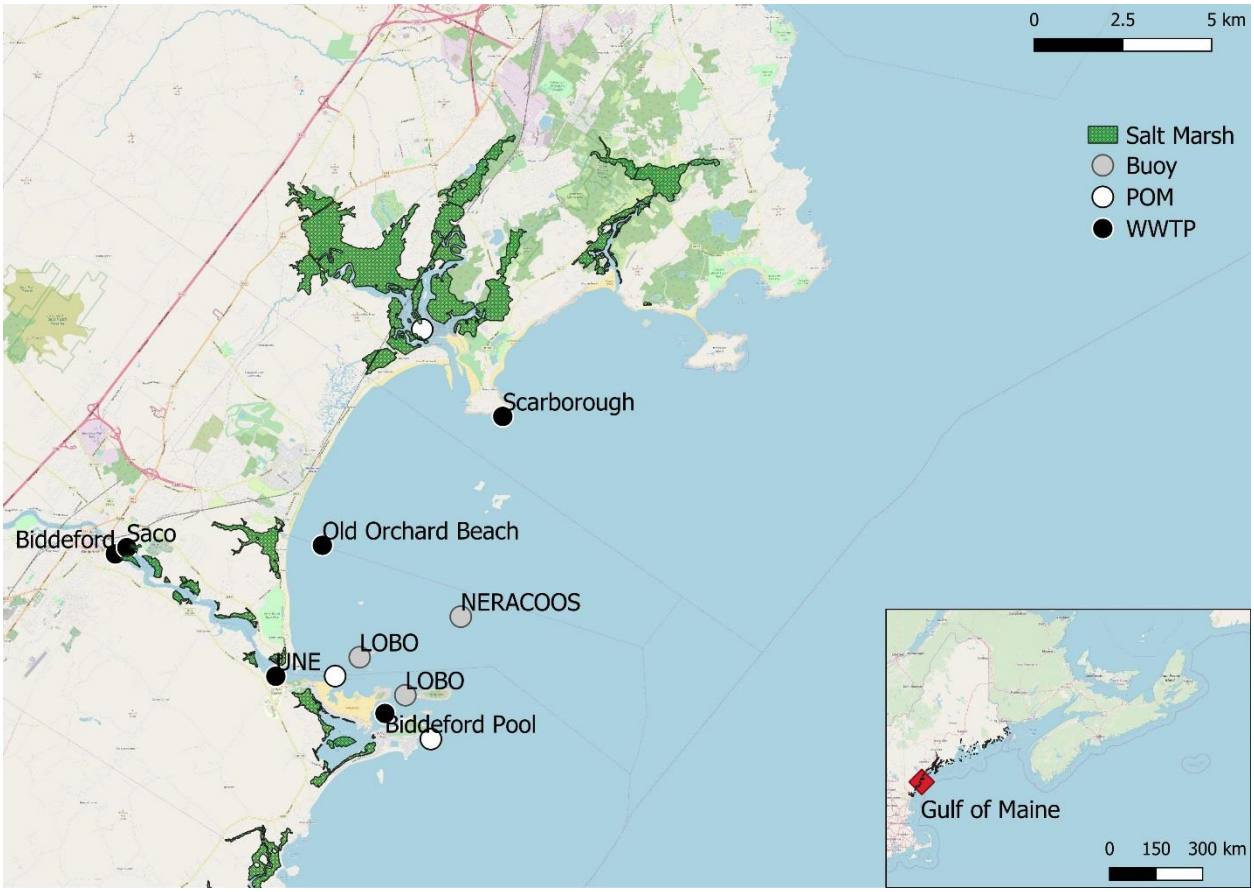

Figure 2.1. Map of Saco Bay. Contains the location of Land Ocean Biological Observatory (LOBO) buoys, Northeast Regional Association of Coastal Ocean Observing System (NERACOOS) buoy, particulate organic matter (POM) sampling sites, wastewater treatment plant (WWTP) outfalls, and salt marshes fringing Saco Bay, ME USA.

We deployed three oceanographic buoys in Saco Bay during 2016 (Figure 2.1). Two Land Ocean Biogeochemical Observing (LOBO; SeaBird Scientific) buoys and one Northeast Regional Association of Coastal Ocean Observing System (NERACOOS; Wallinga et al., 2003) buoy which provided hourly measurements of water velocity, temperature, salinity, photosynthetically active radiation (PAR), chromophoric dissolved organic matter (CDOM), dissolved oxygen, nitrate, pH, chlorophyll-*a*, optical attenuation (turbidity) and backscatter. To ground-truth buoy measurements, biweekly water quality samples were collected from buoy locations for POM, chlorophyll-*a,* dissolved nutrients, primary productivity, zooplankton species composition, as well as phytoplankton species composition, identified using FlowCam (Fluid Imaging Technologies, Inc.) samples analyzed at the Bigelow Laboratory for Ocean

Sciences (Boothbay ME, USA). Biovolume estimates used methods from Sieracki et al. (1989) and algorithms from Burger and Burge (2008) and Chang et al. (2004), while biomass calculations followed carbon content to biovolume functions by Menden-Deuer and Lessard (2000).

In addition to ground-truthing samples, POM was collected from buoy sites along with three additional sites throughout the bay during 2016 and 2017 (Figure 2.1). Samples were size-fractionated (≥100 and <100 µm) *in situ* in the water column, returned to shore, and sub-sampled for chlorophyll-*a*, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), lipid and fatty acid biomarkers. For endmember analysis, we also collected zooplankton, macroalgae (*Saccharina latissima, Ascophyllum nodosum, Chondrus crispus*), and vascular plants (oak leaf-litter and *Spartina alterniflora*) throughout the bay for stable isotope ($\delta^{13}C$ and $\delta^{15}N$), lipid and fatty acid biomarkers.

2.2.2 Chlorophyll-*a*

Chlorophyll-*a* samples were collected on pre-combusted 1.2 µm GF/C Whatman filters and stored at -20°C until analysis. Chlorophyll-*a* content was determined fluorometrically using a Turner Designs TD700 fluorometer. Samples were homogenized in 90% acetone using a glass tissue grinder and refrigerated in the dark overnight. Afterwards, samples were vortexed and centrifuged at 2400 rpm for 10 min before reading on the fluorometer. Samples were acidified by adding two drops of 5% hydrochloric acid to determine phaeopigment concentration (Strickland and Parsons, 1972; Parsons et al., 1984; Welschmeyer, 1994; Arar and Collins, 1997). The fluorometer was calibrated using quantified standards from Turner Designs (part # 10-850). Selected organic matter (SELORG; or organic matter associated with phytoplankton), and remaining organic matter (REMORG; or non-phytoplankton associated organic matter), were calculated as $SELORG = \frac{CHL\ x\ 50}{0.38}$ and $REMORG = POM - SELORG$ as described by Hawkins et al. (2013). Although Hawkins et al. (2013) recommended using a carbon-to-chlorophyll (C:CHL) ratio of 12 when calculating REMORG, to avoid SELORG estimates exceeding total

POM. In this study we used a more conservative and widely used C:CHL ratio of 50 (Taylor et al., 1997) to avoid underestimating phytoplankton contributions to POM.

2.2.3 Stable isotope analysis and interpretation

Stable isotope samples were collected by filtering POM onto pre-combusted and pre-weighed 1.2 μm GF/C Whatman filters. Filters were dried overnight at 60°C or until constant weight. Dried samples were stored in desiccation chambers at room temperature until processed and sent for analysis. Prior to encapsulation in tin, samples were exposed to hydrochloric acid fumes for 24 h to remove carbonate carbon. Endmember tissues were stored at -20°C until processed and sent for analysis. Tissue samples were prepared by drying overnight at 60°C, or until a constant weight, once dry tissues were crushed into a fine powder. Subsamples (1.0 ± 0.2 mg) of powder were encapsulated and sent to the University of California's Davis Stable Isotope facility for analysis using a PDZ Europa ANCA-GSL elemental analyzer along with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

To determine POM contributions of endmembers, we constructed dual-isotope Bayesian mixing models with R Studio (2019; version 1.0.136, 2009-2016) using the stable isotope analysis in R (SIAR, ver. 4.2) package. Bayesian mixing models in SIAR use Markov Chain Monte Carlo methods to generate true probability distributions of possible endmember contributions based on their isotopic values and fractionation factors with incorporated uncertainty (standard deviation) of measurements (Parnell et al., 2010). Diatom and dinoflagellate $\delta^{13}C$ values of -18 ± 2‰ and -24 ± 1‰ were used based on previously reported values from George's Bank (Fry and Wainright, 1991). No fractionation factors were used when determining endmember contributions to POM; this assumes decay did not alter isotopic signatures. Diatom and dinoflagellate $\delta^{15}N$ values were estimated from zooplankton $\delta^{15}N$ by subtracting fractionation due to one trophic level (3.4‰). Due to similarities of $\delta^{13}C$ and $\delta^{15}N$ values between diatoms and macroalgae, they were combined in mixing models. Modeled endmember contributions

were reported as mean, minimum, and maximum of 95% Bayesian credibility intervals, within which represent the range of values that a given estimate from a single model iteration has a 95% probability of falling.

2.2.4 Lipid class and fatty acid analysis

A chloroform:methanol (2:1) modified Folch extraction was used to extract lipids from samples and an Iatroscan Mark V TLC-FID with silica coated Chromarods was used to analyze lipid composition (hydrocarbons, steryl/wax esters, methyl esters, ketones, triacyclglycerols, free-fatty acids, alcohols, sterols, diacylglycerols, acetone-mobile polar lipids, and phospholipids) as described by Parrish (2013). Aliquots of total lipid extracts were transesterified into fatty acid methyl esters (FAME) using concentrated sulfuric acid and by heating samples to 100°C for one hour. FAME composition was determined using an Agilent 7890A Series GC with an FID detector equipped with a 30 m (0.25 µm internal diameter) ZB wax+ column (Phenomenex, US) using helium as the carrier gas (2 ml min^{-1}). Column temperature began at 65°C for 30 sec, then ramped to 195°C at a rate of 40°C min^{-1}, and held for 15 min. Temperature then ramped to 220°C at a rate of 2°C min^{-1} and held for 3.25 min. Injector temperature started at 150°C and ramped at a rate of 200°C min^{-1} until reaching a final temperature of 250°C, while the detector remained a constant 260°C. Fatty acid retention times were determined with a Supelco, 37 component FAME mix (Product number 47885-U).

Permutational multivariate ANOVA (PERMANOVA) was used to determine statistically significant ($p \leq 0.05$) groupings between sample groups and similarity percentages (SIMPER) along with cluster analysis was used to determine similarity within groups and dissimilarity among groups. Homogeneity of multivariate dispersions (PERMDISP) was tested and, when necessary, data were square root transformed prior to analysis to meet the assumption of homogeneity of multivariate dispersion. All multivariate statistical tests including transformations were completed using Primer 7 with the PERMANOVA+ package (ver. 7.0.13, Quest Research Limited). All data are shown as average ± 1

standard error, unless stated otherwise. Proportions (%) of diatom and macroalgal FAs markers identified based on principal coordinates analysis (PCoA) and SIMPER analysis were used to separate combined diatom/macroalgal estimates from stable isotope mixing models, e.g.

$Diatom\ contribution = Combined\ estimate\ x\ (\frac{Diatom\ FAs}{Diatom\ FAs + Macroalgal\ FAs})$.

2.3.0 Results

2.3.1 Endmember biomarker identification and isotopic signatures

Of the top five FAs driving within group similarity, all marine endmembers (zooplankton and macroalgae) contained some essential FAs (20:5ω3, 22:6 ω3, and 20:4ω6), while vascular plant endmembers did not (Table 2.1). Major FAs responsible for similarity among the three macroalgae species were 18:1ω9 and the essential FA arachidonic acid (20:4ω6), while 18:2ω6 and 18:3ω3 drove similarity between vascular plant sources (*S. alterniflora* and oak leaves). The essential FAs, 20:5ω3 and 22:6ω3, both contributed to the within group similarity of <100 µm POM (15%) and zooplankton (34%). For most consumers, due to the lack of essential FAs vascular plant detritus will be of lower nutritional value.

Table 2.1. Endmember biomarkers. Proportion of fatty acids (%) and contribution to similarity (%) between samples of <100 µm particulate organic matter (POM), zooplankton, *Saccharina latissima*, *Ascophyllum nodosum*, *Chondrus crispus*, oak leaves and *Spartina alterniflora* from Saco Bay, Maine USA, throughout 2016 and 2017.

Group	Fatty acid	Average abundance (% Fatty acids)	Contribution to similarity (%)
<100 µm POM	16:0	18	24
(61% average similarity)	16:1ω7	9	10
	22:6ω3	8	9
	20:5ω3	7	8
	14:0	6	6
Zooplankton	22:6ω3	19	19
(80% average similarity)	16:0	16	18
	20:5ω3	15	16
	16:1ω7	9	8
	14:0	7	7
S. latissima	20:4ω6	18	19
(84% average similarity)	16:0	12	14
	20:5ω3	13	14
	18:4ω3	11	10
	18:1ω9	9	8
A. nodosum	18:1ω9	34	35
(90% average similarity)	20:4ω6	11	12
	16:0	11	11
	14:0	10	10
	20:5ω3	8	8
C. crispus	20:5ω3	26	26
(89% average similarity)	16:0	23	25
	16:1ω7	9	9
	20:4ω6	7	7
	18:1ω9	6	5
S. alterniflora	18:3ω3	38	40
(81% average similarity)	18:2ω6	19	21
	16:0	17	20
Oak	22:2	26	28
(75% average similarity)	18:3ω3	16	20
	16:0	11	14
	18:2ω6	5	6
	24:0	4	4

Separating diatom and macroalgal endmembers is difficult because, in addition to similarities between $\delta^{13}C$ and $\delta^{15}N$ values, many of the principal FAs for macroalgae identified by SIMPER are shared with diatoms and dinoflagellates (Parrish et al., 2005; Pepin et al., 2011; Parrish, 2013). Of the top five FAs contributing to similarity between macroalgal species, 20:5ω3 and 16:1ω7 have been used as markers for diatoms while 18:1ω9 and 18:4ω3 have been used as dinoflagellate markers (Parrish et al. 2005; Pepin et al. 2011; Parrish 2013). The only FA that appeared in the top five FAs for each species of macroalgae that has not been used as either a diatom or dinoflagellate marker is the essential FA 20:4ω6, which has been previously described as a marker for red algae and kelp (Kelly et al. 2012; Kelly and Scheibling 2012; Parrish 2013). However, using a single essential FA as a biomarker is not ideal.

Reflecting its high lability, 20:4ω6 made up only a very small proportion of the FAs (0 to 0.9%) in <100 µm POM. Additionally, as an essential FA, it will quickly be absorbed by other organisms leading to difficulties in using it alone as a biomarker. To determine if there were other suitable FA biomarkers for macroalgae, the FA profile of all three species sampled in this study were compared to literature values for dinoflagellates (Nichols et al. 1984; Mansour et al. 1999; Leblond and Chapman 2000; Mansour et al. 2003) using PCoA and SIMPER analysis. The PCoA grouped the macroalgal species together based on 20:4ω6, 20:4ω3, 20:2ω6, 18:1ω9 and 18:3ω6 while dinoflagellates were separated based on 22:6ω3, with 18:2ω6 and 18:3ω3 separating out vascular plant sources (Figure 2.2). Of the identified FAs only 20:4ω6 and 18:1ω9 consistently appeared in SIMPER analyses as showing dissimilarity among the three macroalgae species and literature values for dinoflagellates. Each FA was present in higher proportions in macroalgae (20:4ω6: 13.0 ± 0.2%, 18:1ω9: 20.0 ± 0.5%) than dinoflagellates (20:4ω6: 0.1 ± 0.01%, 18:1ω9: 3.4 ± 0.1%) and together they explained 18% of the dissimilarity among the groups. In particular, *A. nodosum* had high proportions of 18:1ω9 (33.6 ± 0.3%). Although 20:2ω6, 20:4ω3 and 18:3ω6 helped separate macroalgae from dinoflagellates in PCoA, and not in the results from SIMPER, they have potential as biomarkers for macroalgae. All three FAs (20:2ω6, 20:4ω3 and 18:3ω6) are

precursors to essential FAs, 20:2ω6 and 18:3ω6 are precursors to 20:4ω6 and 20:4ω3 is a precursor to 20:5ω3 (Nichols and Appleby 1969; Gurr and James 1980; Khozin et al. 1997; Bergé and Barnathan 2005; Kelly and Scheibling 2012). All three FAs (20:2ω6, 20:4ω3 and 18:3ω6) have also been previously used as algal or macroalgal markers (Kharlamenko et al. 1995; Kelly and Scheibling 2012; Parrish 2013).

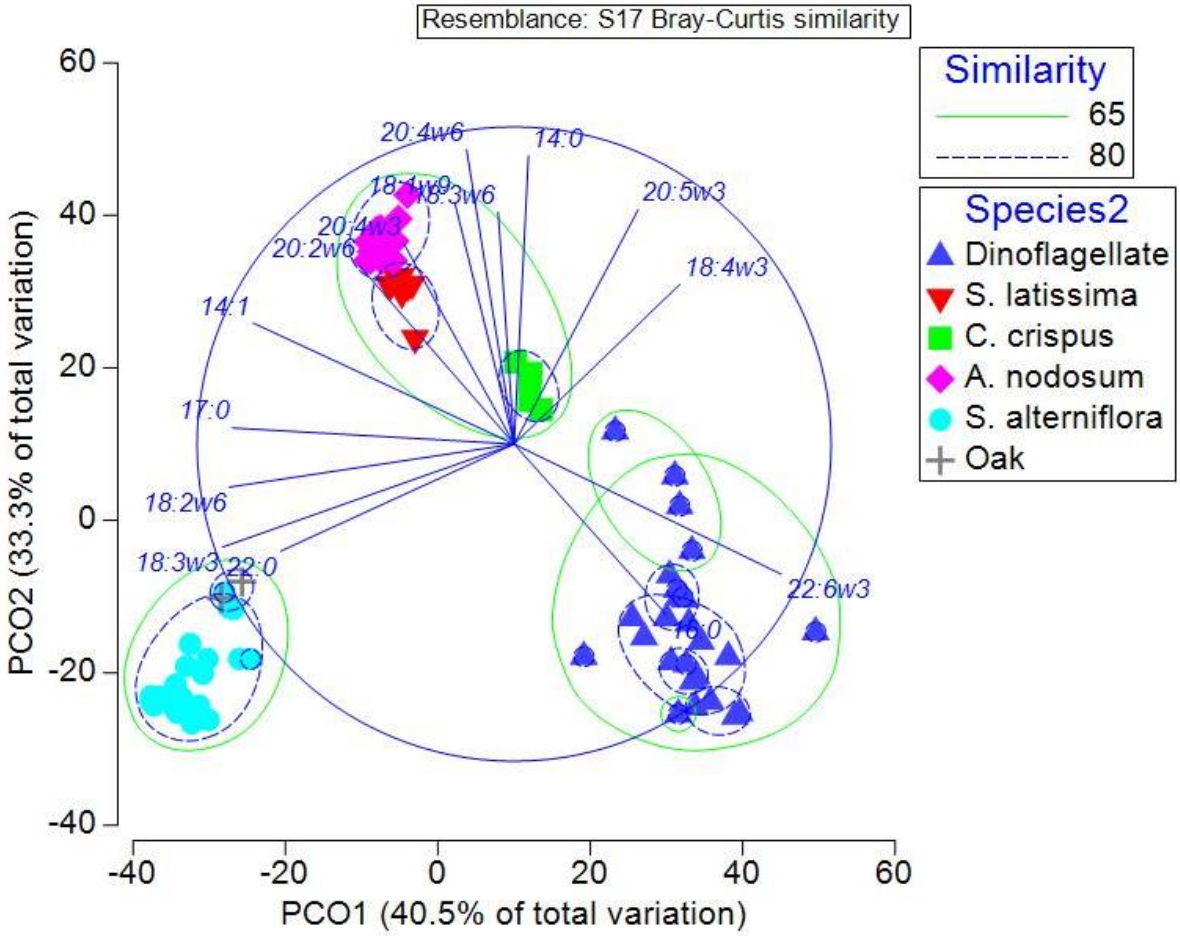

Figure 2.2. Principal coordinates analysis of fatty acid profiles. Contains three macroalgae species (*Ascophyllum nodosum, Saccharina latissima* and *Chondrus crispus*), terrestrial endmembers (*Spartina alterniflora* and oak leaves) and dinoflagellates (Nichols et al., 1984; Mansour et al., 1999, 2003; Leblond and Chapman, 2000).

There were distinct differences in the isotopic composition of most endmembers (Table 2.2). The isotopic composition of vascular plants (*S. alterniflora* and oak) was distinctly different from one another and marine endmembers. *Spartina alterniflora* was heaviest in terms of $\delta^{13}C$ (-13.3 ± 0.1 ‰), while oak was isotopically the lightest (-30.5 ± 0.2 ‰). In contrast, although macroalgae were distinct

from vascular plants, all three macroalgal species had similar $\delta^{13}C$ values (*A. nodosum*: -19.0 ± 0.1 ‰, *S. latissima*: -18.1 ± 0.1 ‰, *C. crispus*: -19.6 ± 0.1 ‰). Zooplankton were isotopically lighter than macroalgae in terms of $\delta^{13}C$ (-21.3 ± 0.1 ‰) but were more enriched in $\delta^{15}N$ than any other endmember (8.6 ± 0.1 ‰). Although the $\delta^{13}C$ of <100 µm POM resembled marine sources (-19.4 ± 0.1 ‰), owing to its varied nature POM $\delta^{13}C$ values had a wide range of values (-12 to -25 ‰).

Table 2.2. Endmember isotopic composition ($\delta^{13}C$ and $\delta^{15}N$). Contains particulate organic matter (<100 and >100 µm) and all endmembers (zooplankton, macroalgae, and vascular plants) collected from Saco Bay, Maine USA, throughout 2016 and 2017.

	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Endmember	**Average**	**St. dev.**	**St. error**	**Average**	**St. dev.**	**St. error**
Particulate organic matter						
<100 µm POM	-19.4	3.7	0.1	7.0	2.8	0.1
>100 µm POM	-21.9	1.5	0.1	8.1	0.9	0.1
Marine						
Zooplankton	-21.3	1.5	0.1	8.6	1.3	0.1
A. nodosum	-19.0	1.5	0.1	5.6	1.1	0.1
S. latissima	-18.1	2.5	0.1	5.6	0.9	0.1
C. crispus	-19.6	2.1	0.1	4.6	0.4	0.1
Vascular plants						
S. alterniflora	-13.3	1.1	0.1	2.8	3.4	0.1
Oak	-30.5	1.2	0.2	-2.6	0.6	0.1

2.3.2 Seasonal trends

2.3.2.1 Late spring to early summer (May and June)

The late spring and early summer season of Saco Bay were characterized by high nutrient (Figure 2.3) and low chlorophyll-*a* concentrations (Figure 2.4). The highest concentrations of dissolved organic nitrogen (DON; 46.7 ± 0.3 µM N) and dissolved inorganic nitrogen (DIN; 6.3 ± 0.2 µM N) occurred during May and decreased throughout June. Chlorophyll-*a* concentrations were at their lowest (<0.1 µg l^{-1}) in late spring and early summer. Although chlorophyll-*a* concentrations were low, there was an elevated concentration of phaeopigments (1.6 µg l^{-1}) during May 2017, likely the tail-end of the spring bloom. Cillates were the most abundant phytoplankton (28.8 ± 0.9 µg C l^{-1}) during the May to June period, followed by dinoflagellates (10.6 ± 0.3 µg C l^{-1}) and then diatoms (1.1 ± 0.1 µg C l^{-1}) based on FlowCam biomass estimates (Figure 2.5).

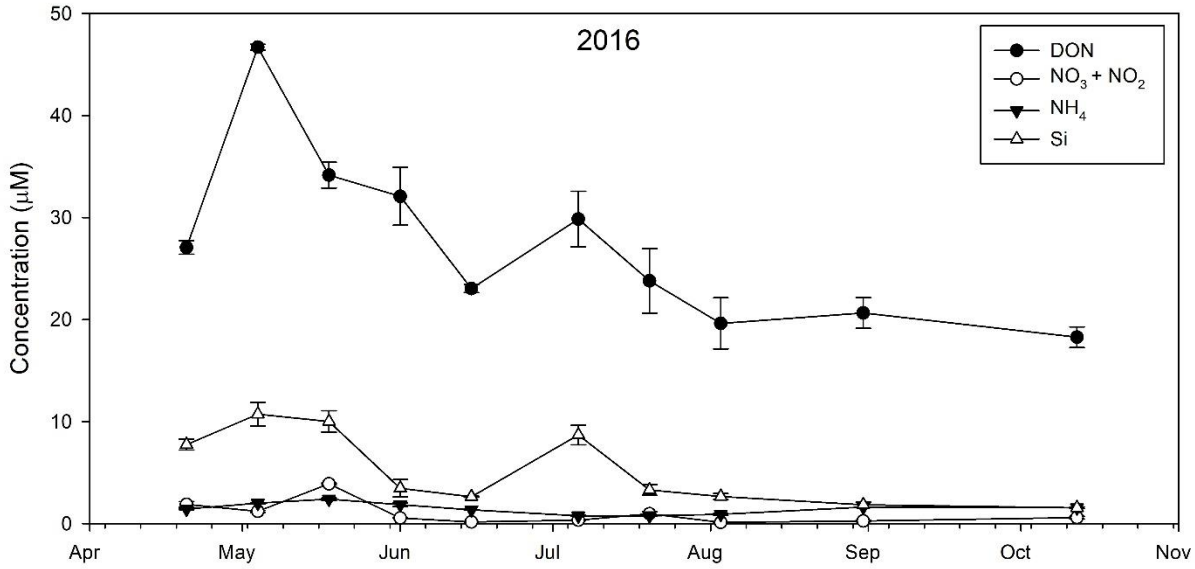

Figure 2.3. Concentration of dissolved nutrients. Concentration (µM) of dissolved organic nitrogen (DON), inorganic nitrogen (NO_3^- and NO_2^-), ammonium (NH_4^+) and silica (Si) within Saco Bay, Maine USA, throughout 2016. Error bars represent ± 1 standard error.

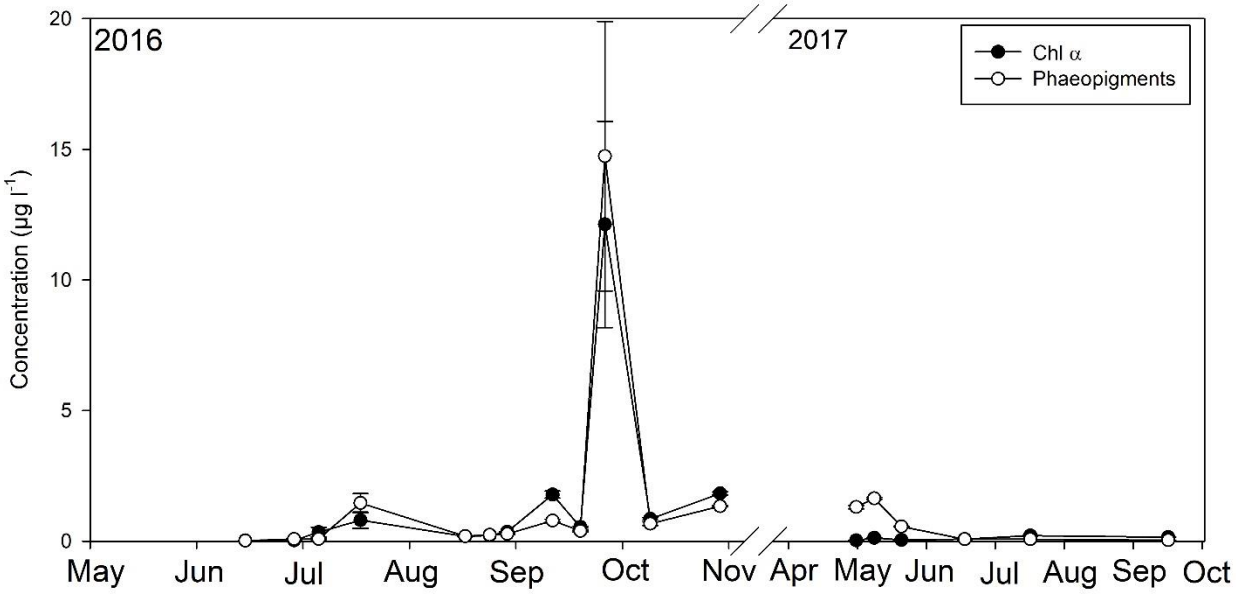

Figure 2.4. Chlorophyll-*a* and phaeopigment concentrations (µg l^{-1}). Measured in Saco Bay, ME USA, throughout 2016 and 2017. Error bars represent ± 1 standard error.

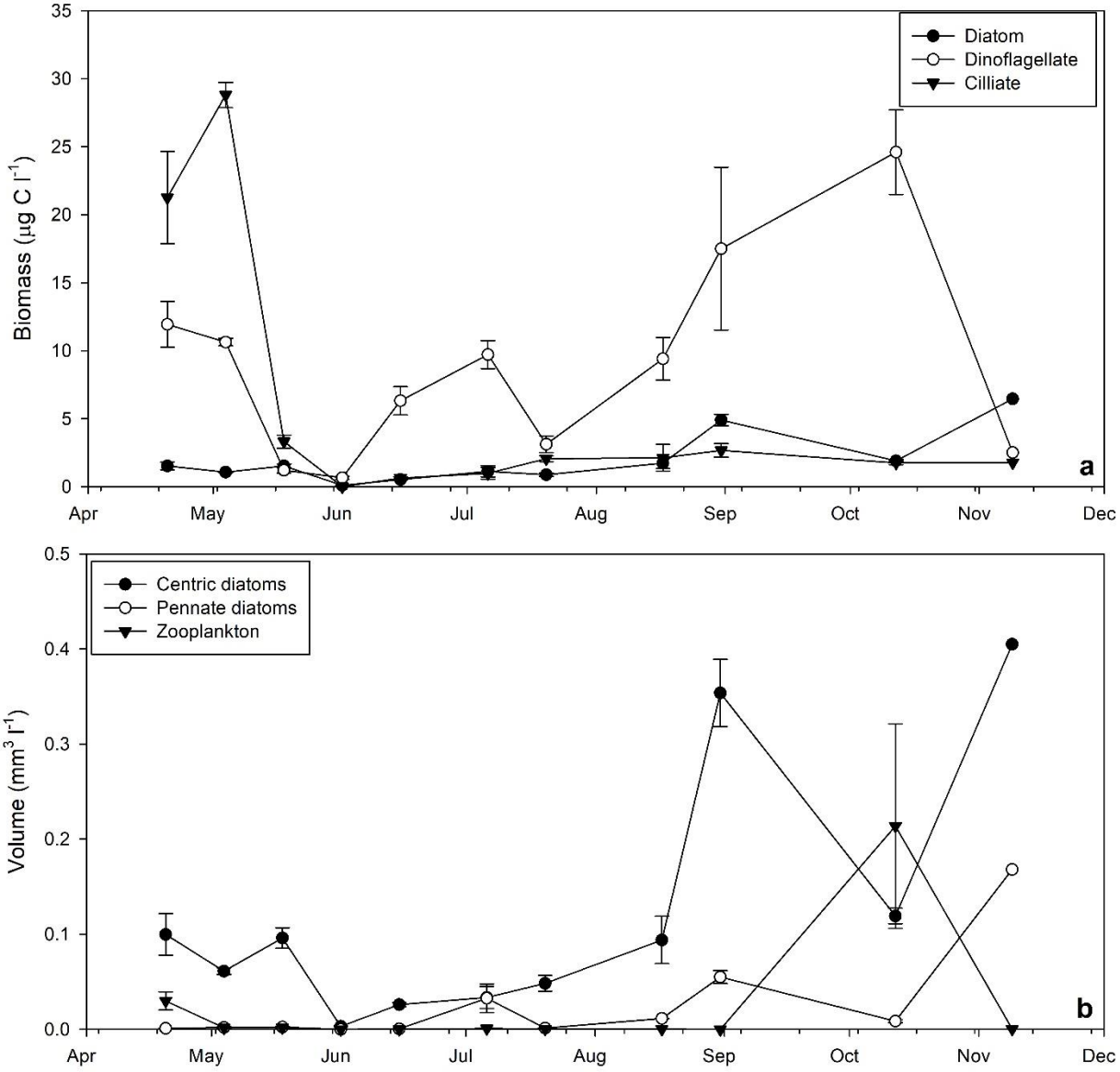

Figure 2.5. Selected components of the plankton community composition in Saco Bay, ME USA, throughout 2016. (a) Biomass (µg C l^{-1}) of diatoms, dinoflagellates, and ciliates. (b) Volume (mm^3 l^{-1}) of centric diatoms, pennate diatoms, and zooplankton. Error bars represent ± 1 standard error.

Low phytoplankton abundances led to proportionally larger contributions of macroalgal (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3 and 20:4ω6) and vascular plant FAs (18:2ω6 and 18:3ω3) to <100 µm POM. The concentration of diatom FAs (16:1ω7, 16:4ω1 and 20:5ω3) during June was 0.3 µg l^{-1} and comprised 7.9 to 11.9% of <100 µm POM FAs (Figure 2.6 and 2.7), while the concentration of dinoflagellate FAs (22:6ω3 and 18:4ω3) ranged from 0.2 to 1.2 µg l^{-1} and comprised 9.9 to 10.9 % of POM FAs (Figure 2.6 + 2.7). Although the concentrations of macroalgal (0.2 to 1.2 µg l^{-1}) and vascular plant (0.1 to 0.9 µg l^{-1}) FAs were not significantly elevated compared to other seasons, their proportional contribution to <100 µm POM FAs was higher than any other time-period (Figure 2.6 + 2.7). Stable isotope modeled estimates of endmember contributions to <100 µm POM corroborated the largest proportional contributions of macroalgal and oak detritus during June (Figure 2.8). Additionally, SELORG comprised only a small proportion (<13%) of POM during early summer corroborating low phytoplankton contributions to POM (Figure 2.9).

Larger proportional contributions of macroalgal detritus during late spring and early summer resulted in higher ratios of 20:4ω6-to-20:5ω3 and 22:6ω3. The highest ratio of 20:4ω6-to-20:5ω3 (0.21 ± 0.06) occurred during June 2017, while the highest ratio of 20:4ω6-to-22:6ω3 (0.25 ± 0.04) occurred in April 2017. Although the ratio of 20:4ω6-to-20:5ω3 and 22:6ω3 were lower in 2016, they were still higher in May and June than any other season (Figure 2.10). All three species of macroalgae had large proportions of 20:4ω6 (7 to 18 %) which led to the incorporation of 20:4ω6 as a biomarker for macroalgae (Table 2.1). Although pennate diatoms can also be sources of 20:4ω6, the ratio of 20:4ω6 to another diatom biomarker (16:1ω7) was elevated in late spring and early summer (Figure 2.10). Additionally, the ratio of another macroalgal marker (18:1ω9) to 16:1ω7 was also elevated (Figure 2.10) suggesting 20:4ω6 was from macroalgal detritus and not diatoms.

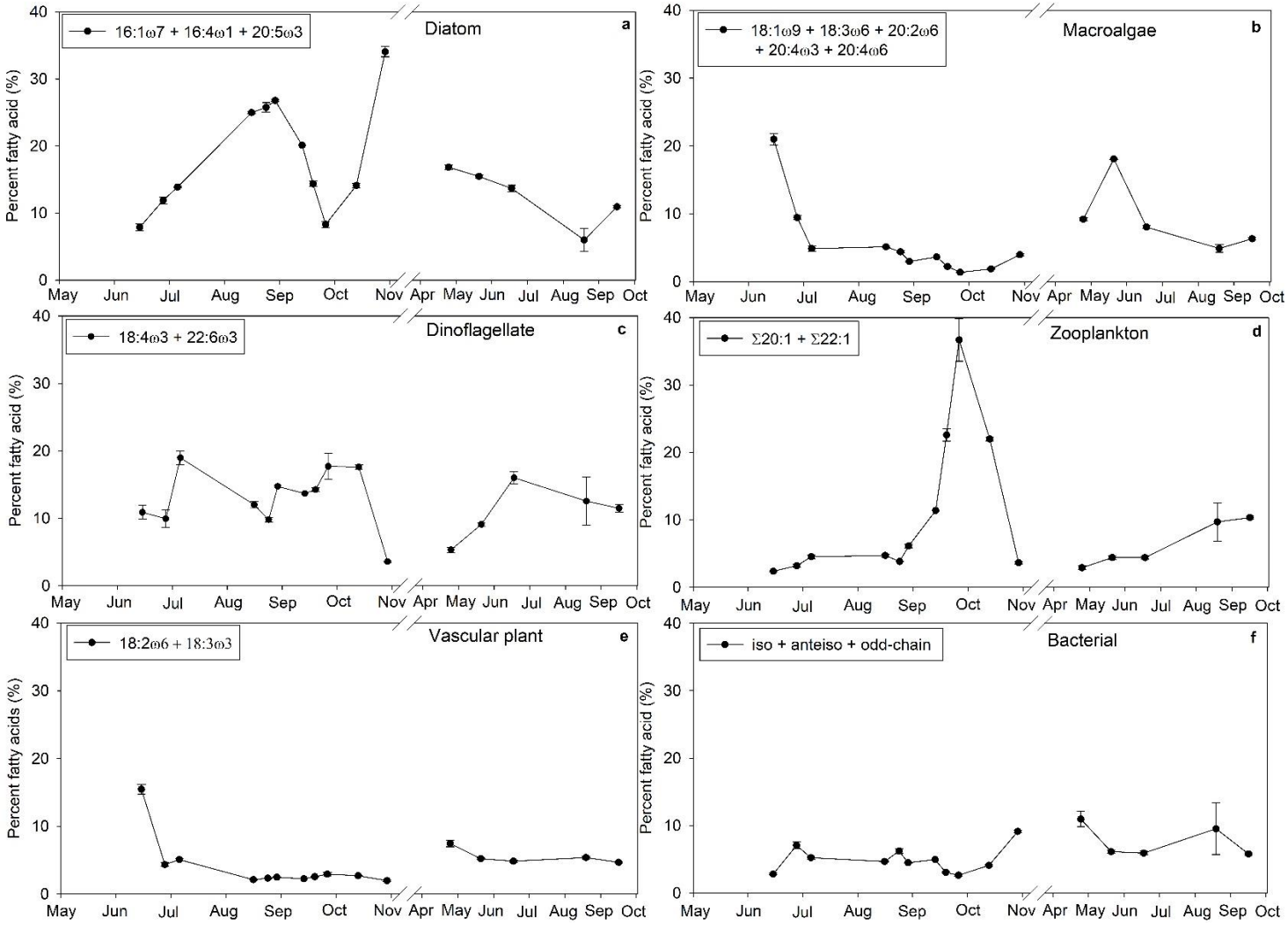

Figure 2.6. Percentage of biomarker fatty acids in particulate organic matter. Proportions (%) of fatty acids in <100 µm particulate organic matter from Saco Bay, Maine USA, throughout 2016 and 2017. (a) Diatom markers (16:1ω7, 16:4ω1 and 20:5ω3). (b) Macroalgal markers (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3 and 20:4ω6). (c) Dinoflagellate markers (22:6ω3 and 18:4ω3). (d) Zooplankton markers (Σ20:1 and Σ22:1). (e) Vascular plant markers (18:2ω6 and 18:3ω3). (f) Bacterial markers (*iso, anteiso* and odd-chain FAs). Error bars represent ± 1 standard error.

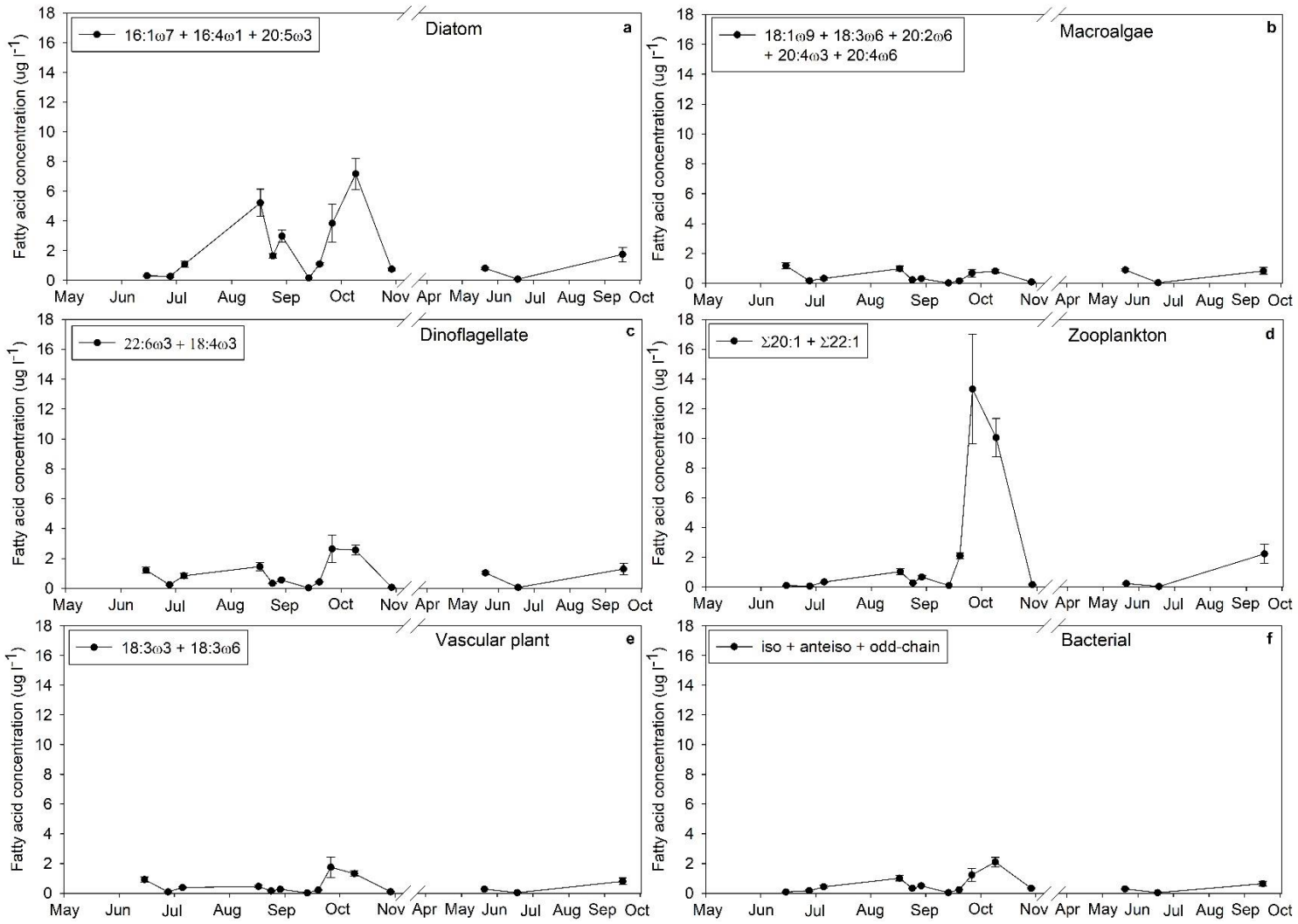

Figure 2.7. Concentration of biomarker fatty acids in particulate organic matter. Concentrations (µg l^{-1}) of fatty acids in <100 µm particulate organic matter from Saco Bay, Maine USA, throughout 2016 and 2017. (a) Diatom markers (16:1ω7, 16:4ω1 and 20:5ω3). (b) Macroalgal markers (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3 and 20:4ω6). (c) Dinoflagellate markers (22:6ω3 and 18:4ω3). (d) Zooplankton markers (Σ20:1 and Σ22:1). (e) Vascular plant markers (18:2ω6 and 18:3ω3). (f) Bacterial markers (*iso, anteiso* and odd-chain FAs). Error bars represent ± 1 standard error.

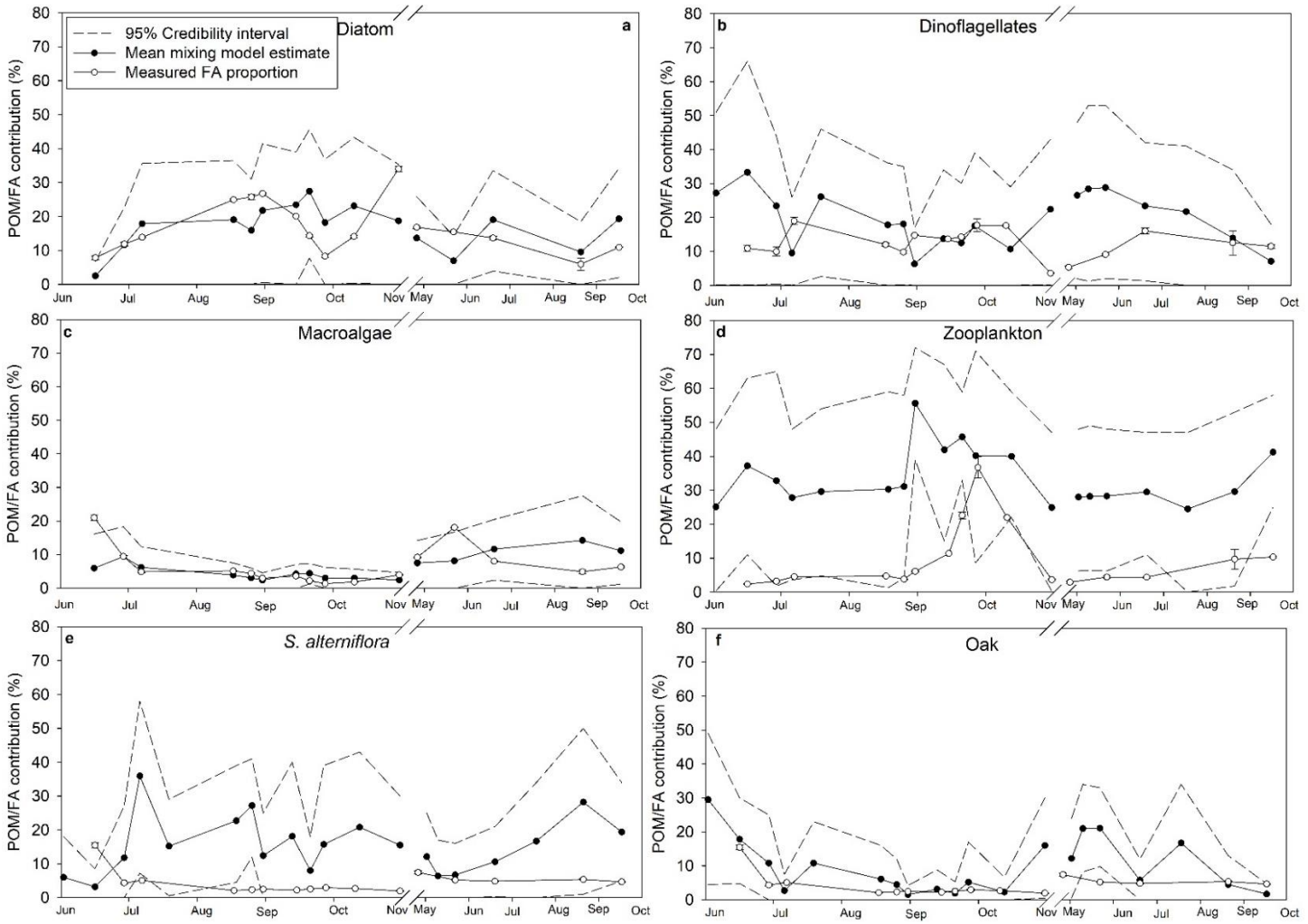

Figure 2.8. Comparison of model estimates and fatty acid biomarkers. Dual-isotope ($\delta^{13}C$ and $\delta^{15}N$) Bayesian mixing model endmember contributions (%) to <100 µm particulate organic matter within Saco Bay, Maine USA, throughout 2016 and 2017 in comparison to measured fatty acid biomarkers. Modeled estimates are shown as mean, minimum, and maximum contributions of 95% Bayesian credibility intervals, while fatty acid data are shown as average ± 1 standard error. (a) Diatom. (b) Dinoflagellates. (c) Macroalgae. (d) Zooplankton. (e) *S. alterniflora*. (f) Oak.

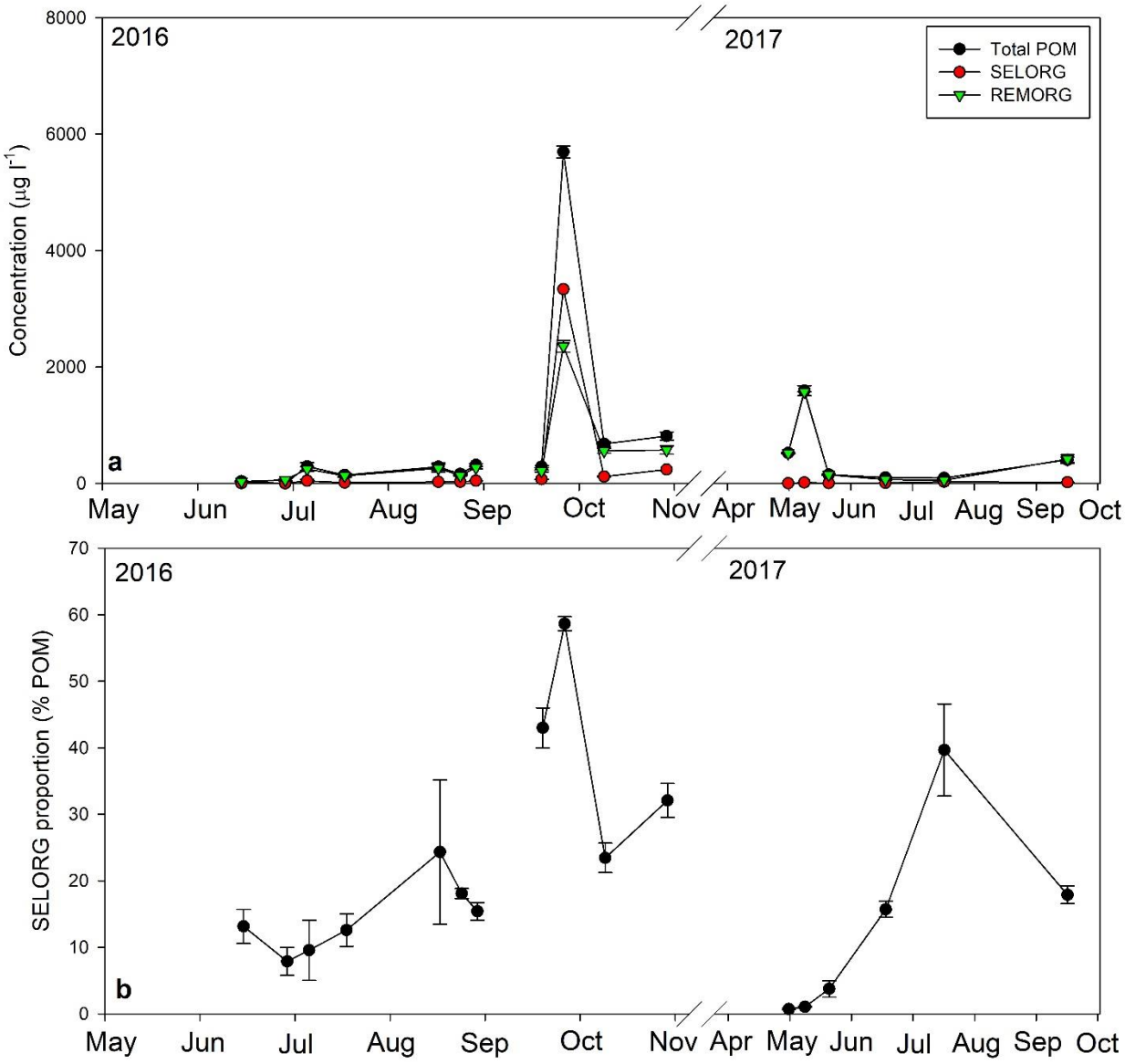

Figure 2.9. SELORG and REMORG concentrations. Particulate organic matter (POM) concentrations within Saco Bay, Maine USA, throughout 2016 and 2017. (a) Total POM, selected organic matter (SELORG), and remaining organic matter (REMORG) concentrations (mg l^{-1}). (b) Proportion of POM composed of SELORG (%), assuming a C:Chl of 50. Data shown are average ± standard error.

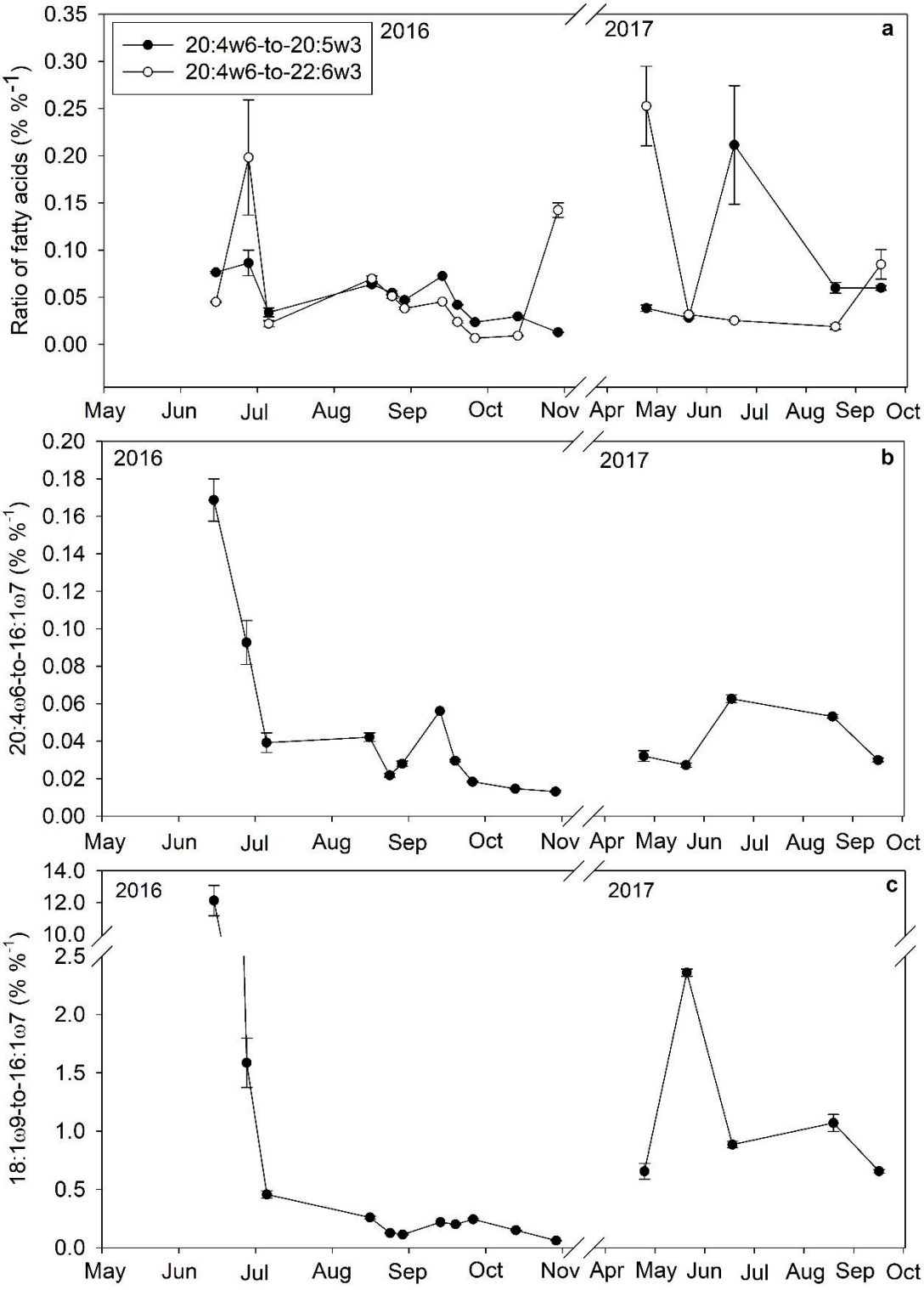

Figure 2.10. Ratios of biomarker fatty acids in particulate organic matter. The ratio (% $\%^{-1}$) of 20:4ω6-to-20:5ω3 and 22:6ω3 (a), the ratio of 20:4ω6-to-16:1ω7 (b), and the ratio of 18:1ω9-to-16:1ω7 in <100 µm particulate organic matter within Saco Bay, Maine USA, throughout 2016 and 2017.

2.3.2.2 Summer (July and August)

Phytoplankton productivity in Saco bay increased throughout the summer months. While chlorophyll-*a* concentrations were still low (<0.8 µg l^{-1}) during summer months, they were elevated compared to May and June (<0.1 µg l^{-1}; Figure 2.4). Although chlorophyll-α concentrations increased during the summer, SELORG still comprised only a small proportion (<18%) of POM (Figure 2.9). Increases in chlorophyll-*a* concentrations were accompanied by increases in the concentration of diatom and dinoflagellate FAs as well as increases in the proportional contribution of diatom and dinoflagellate FAs to <100 µm POM FAs (Figure 2.6 and 2.7). Increases in the proportional contribution of diatoms throughout the summer to <100 µm POM was corroborated by increases in diatom and decreases in macroalgal and oak detritus contributions based on isotope model estimates (Figure 2.8). Additionally, the maximum biomass productivity (P_{max}) increased throughout the summer, increasing from 10.0 ± 1.6 µg C^{-1} d^{-1} in June until reaching a maximum of 116.1 ± 8.4 µg C^{-1} d^{-1} at the end of August.

Although dinoflagellates were the most abundant phytoplankton, diatoms became increasingly abundant throughout the summer. Dinoflagellates (3.1 to 17.5 µg C l^{-1}) were more abundant during summer months than diatoms (0.9 to 4.9 µg C l^{-1}) and ciliates (0.6 to 6.0 µg C l^{-1}; Figure 2.5). There was a slow increase in the biomass of diatoms between July (1.1 ± 0.4 µg C l^{-1}) and August (4.9 ± 0.4 µg C l^{-1}; Figure 2.5). Increases in diatom biomass were corroborated by increases in diatom FA indices (16:1-to-16:0 and ΣC16-to-ΣC18) and a decrease in the ratio of 22:6ω3-to-20:5ω3 (Figure 2.11), which has been used to indicate dinoflagellate dominance (Pepin et al., 2011). Although generally centric diatoms were more prevalent than pennate diatoms, there was an increase in pennate diatoms during July based on FlowCam volumes (Figure 2.5).

Increased productivity of phytoplankton during summer months was likely due to nutrient availability. Although there was a general trend of decreasing nutrient concentrations throughout the summer, there was a relative increase in nutrient concentrations in early July (DON: 29.8 ± 2.7 µM N, Si:

8.7 ± 1.0 µM) compared to June (DON: 23.0 ± 0.4 µM N, Si: 2.6 ± 0.5 µM; Figure 2.3). Accompanying the increases in nutrient availability there was an increase in the C_{16} PUFA ratio (Figure 2.11). The C_{16} PUFA ratio ([16:2ω4 +16:3ω4 + 16:4ω3 + 16:4ω1] to [16:0 + 16:1ω7 + 16:1ω5 + 16:2ω4 + 16:3ω4 +16:4ω3 +16:4ω1]) is a measure of diatom physiological status. The C_{16} PUFA ratio was highest (19.3 ± 2.3) towards the beginning of July, suggesting fresh phytoplankton. In addition, the ω3 and PUFA content of <100 µm POM also increased and were at their highest (33.7 ± 0.9 % and 42.5 ± 0.6 % respectively) during July, again suggesting fresh phytoplankton (Figure 2.11). The proportion of free-fatty acids (FFA), a lipid breakdown indicator, also increased from 7.5 ± 1.5 % to a maximum of 16.6 ± 0.9 % throughout the summer. In conjunction with the proportion of FFAs, the lipolysis index (LI: [(FFA + alcohols) (acyl lipids + alcohol)$^{-1}$]), which shows the breakdown of total acyl lipids (Parrish, 1998), increased from 8.8 ± 0.8 % in mid-June to 22.8 ± 1.4 in late August, suggesting that degradation of organic material may be behind the increased nutrient concentrations. The proportion of FFA and diatom FAs were both positively correlated ($p = 0.03$, $R^2 = 0.11$ and $p < 0.001$, $R^2 = 0.24$ respectively) with the $\delta^{15}N$ of <100 µm POM (Figure 2.12). The highest $\delta^{15}N$ occurred in August, suggesting the possibility of isotopically heavy anthropogenic nitrogen (Figure 2.12). In addition, the proportion of diatom FAs was positively correlated ($p < 0.001$, $R^2 = 0.35$) with the proportion of FFA (Figure 2.12c).

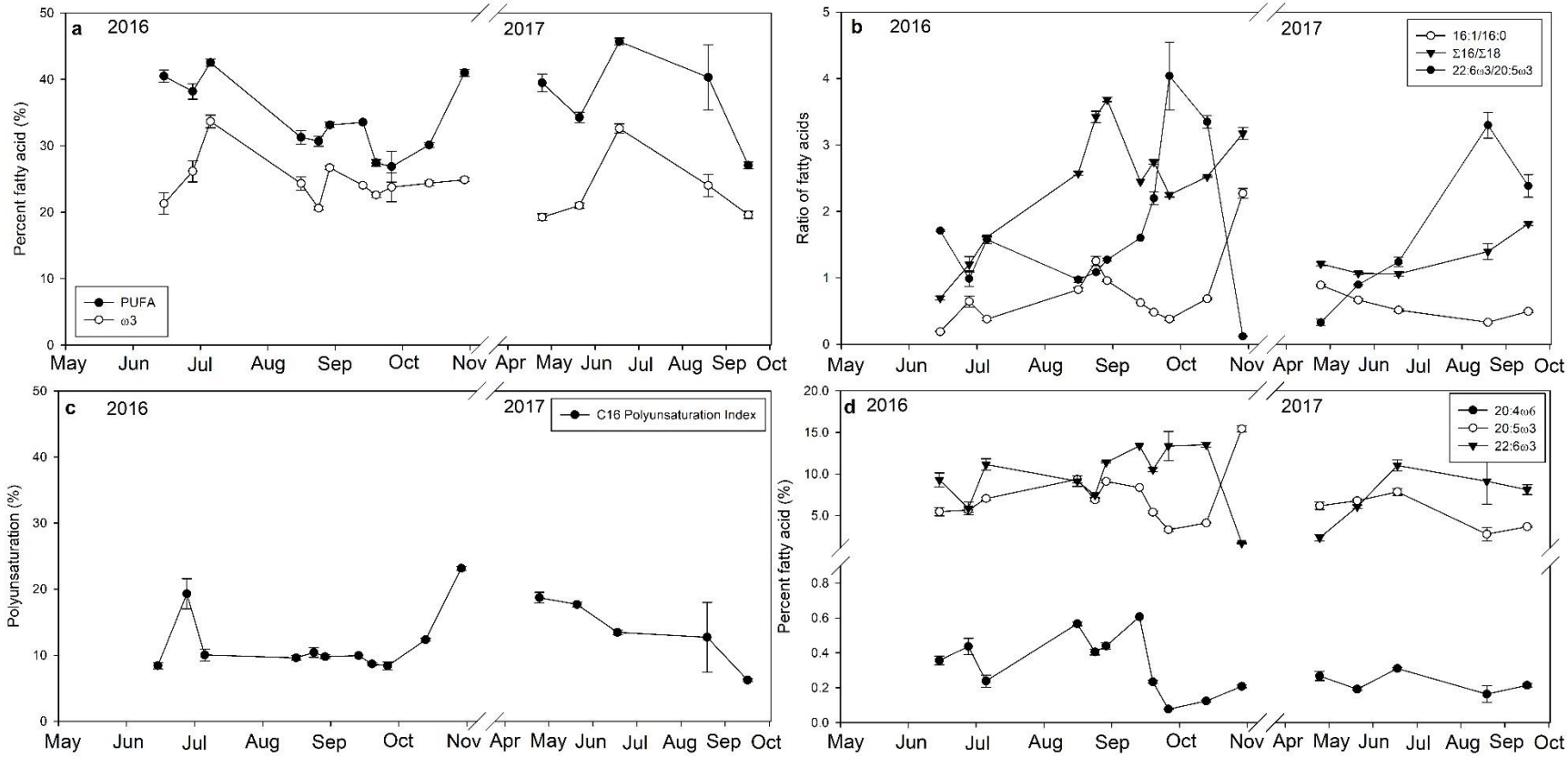

Figure 2.11. Summary of particulate organic matter fatty acids. Proportions (%) of fatty acids in <100 µm particulate organic matter from Saco Bay, Maine USA, throughout 2016 and 2017. (a) Proportion of ω3 and polyunsaturated fatty acids (PUFA). (b) Diatom (16:1/16:0, Σ16/Σ18) and dinoflagellate (22:6ω3/20:5ω3) indices. (c) C_{16} PUFA ratio (16:2ω4 +16:3ω4 + 16:4ω3 16:4ω1 to 16:0 + 16:1ω7 + 16:1ω5 + 16:2ω4 + 16:3ω4 +16:4ω3 +16:4ω1). (d) Proportion of essential fatty acids (20:4ω6, 20:5ω3, and 22:6ω3). Error bars represent ± 1 standard error.

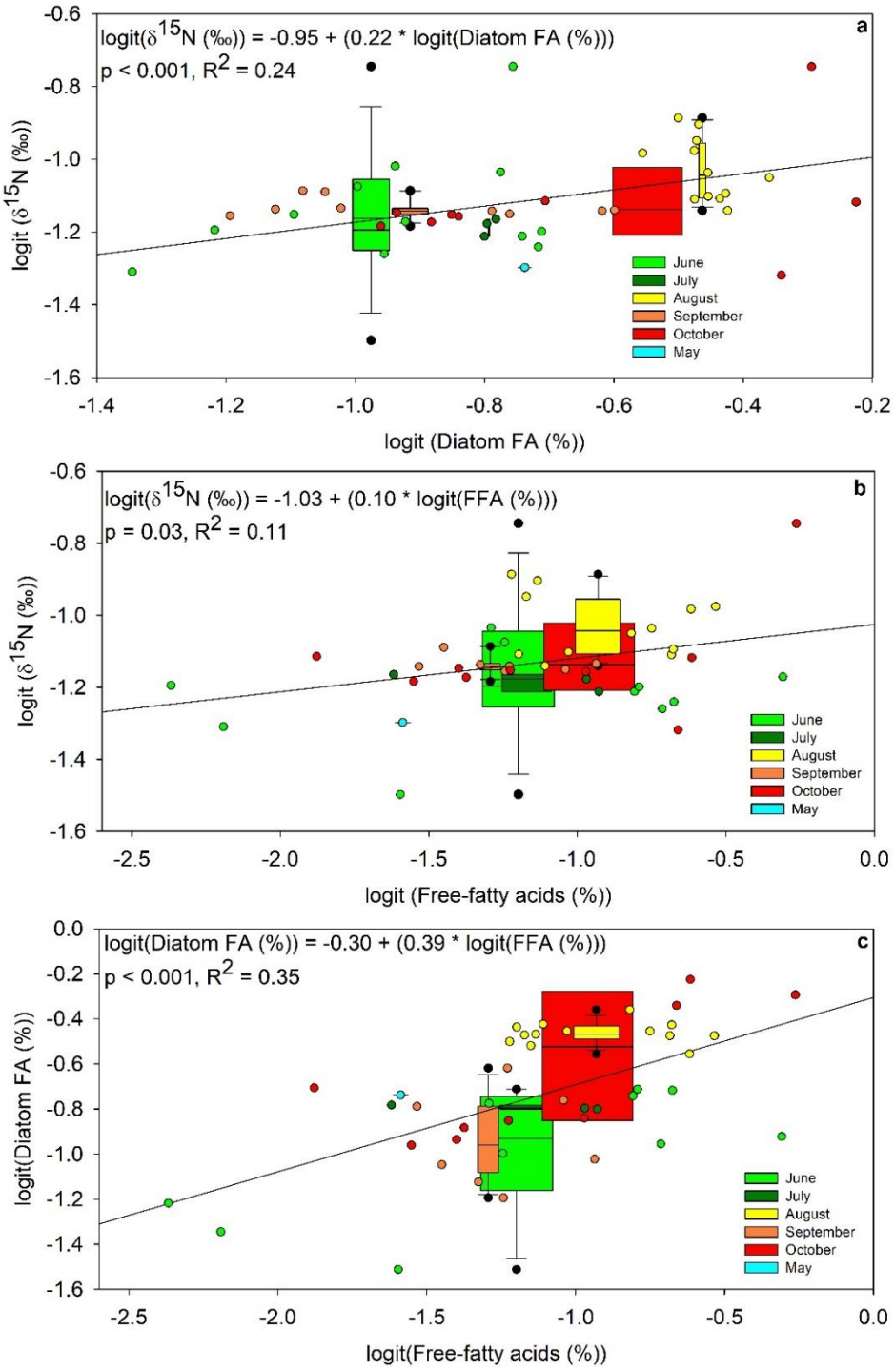

Figure 2.12. Particulate organic matter $\delta^{15}N$ regressions. Significant ($p < 0.05$) regressions between $\delta^{15}N$ (logit(‰)) and the proportion of (a) diatom FAs (16:1ω7, 16:4ω1 and 20:5ω3; logit(%)), (b) free fatty acids (FFA; logit(%)), and between (c) diatom FAs and FFA in <100 µm particulate organic matter from Saco Bay, Maine USA, throughout 2016 and 2017. Center lines of boxplot represent median, box height is interquartile range, error bars represent first and third quartile, box width represents coefficient of variation, and black circles represent outliers.

2.3.2.3 Fall (September to November)

Saco Bay experienced a large phytoplankton bloom throughout the fall due to the breakdown of stratification in the bay. The highest chlorophyll-*a* and phaeopigment concentrations (12.1 ± 3.9 μg l^{-1} and 14.7 ± 5.1 μg l^{-1} respectively) recorded for the bay occurred in late September (Figure 2.4). High chlorophyll-α concentrations resulted SELORG contributions (58.6 ± 1.0 %) to POM exceeding REMORG for the first time (41.4 ± 1.0 %; Figure 2.9). Dinoflagellate and diatom biomasses also peaked during the fall (24.6 ± 3.1 μg C l^{-1} and 4.9 ± 0.4 μg C l^{-1} respectively; Figure 2.5). Coinciding with high biomasses, the concentrations of dinoflagellate (2.6 ± 0.3 μg l^{-1}) and diatom (7.2 ± 1.1 μg l^{-1}) FAs were also higher during fall than at any other period (Figure 2.7). The density difference between the surface and bottom water of the Bay decreased from 1.6 ± 0.5 kg m^{-3} to 0.6 ± 0.2 kg m^{-3} at the end of August which likely triggered the phytoplankton bloom formation. The concentration of chlorophyll-*a* was significantly negatively correlated ($p < 0.01$, $R^2 = 0.35$) with the density difference between the surface and bottom water of the bay (Figure 2.13).

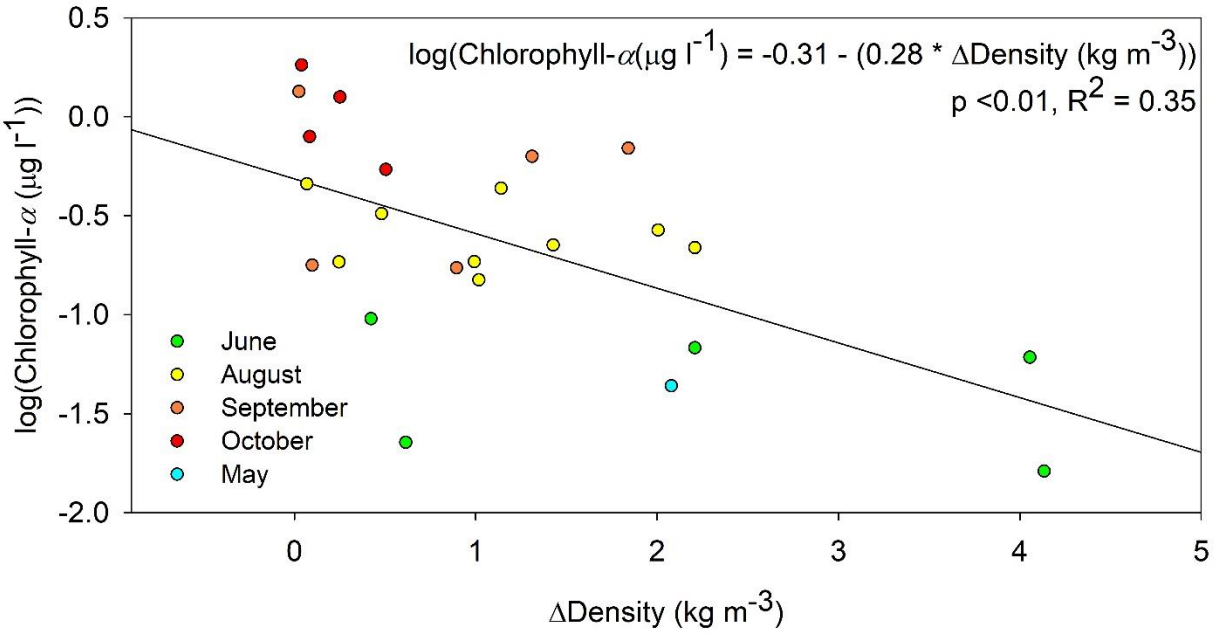

Figure 2.13. Chlorophyll-*a* and density regression. Significant ($p < 0.01$) regression between the chlorophyll-α concentration ($\log(\mu g\ l^{-1})$) and the density difference ($kg\ m^{-3}$) between the surface and bottom water in Saco Bay, Maine USA, throughout 2016 and 2017.

Even during the fall bloom, dinoflagellates were the most abundant phytoplankton. Although the concentration of diatom FAs continued to increase until the chlorophyll-*a* maxima in October, the proportion of diatom FAs was highest (26.7 ± 0.1 %) at the beginning of September and did not coincide with the chlorophyll-*a* maxima (Figure 2.4 + 2.6). Corroborating that diatom's proportional contribution to <100 µm POM was greatest at the beginning of September, both diatom indices (16:1-to-16:0 and ΣC16-to-ΣC18) reached their maxima (1.25 ± 0.1 and 3.7 ± 0.1 respectively) in August and at the beginning of September (Figure 2.11). Additionally, the 22:6ω3-to-20:5ω3 ratio was the lowest (1.3 ± 0.1) at the beginning of September and increased rapidly as the bloom progressed towards the chlorophyll-*a* maxima (Figure 2.11). Dinoflagellates were also more abundant (24.6 ± 3.1 µg C l^{-1}) than diatoms (1.9 ± 0.1 µg C l^{-1}) during the fall based on FlowCam biomass estimates, which was true even at the beginning of September (17.5 ± 6.0 µg C l^{-1} and 4.9 ± 0.4 µg C l^{-1} respectively) when diatom's proportional contribution to POM was highest (Figure 2.5).

Large abundances of zooplankton were indicative of the fall bloom and zooplankton were important concentrators of lipid in the water column. The volume of zooplankton within Saco Bay was generally low (<0.1 $mm^3\ l^{-1}$) but increased (0.2 ± 0.1 $mm^3\ l^{-1}$) during the bloom (Figure 2.5). Like volume estimates, zooplankton FAs (Σ20:1 and Σ22:1) were also generally low (<1.0 $\mu g\ l^{-1}$) before increasing to a maxima (13.3 ± 3.7 $\mu g\ l^{-1}$) during the bloom (Figure 2.7). The proportion of zooplankton FAs was significantly positively correlated ($p = 0.003$, $R^2 = 0.53$) with the chlorophyll-*a* concentration of the bay (Figure 2.14a). The proportion of zooplankton FAs was also significantly positively correlated with the lipid concentration ($p = 0.002$, $R^2 = 0.55$) of <100 µm POM (Figure 2.14b).

Although phytoplankton and zooplankton FAs dominated the water column during the fall, the concentrations of detrital FAs in the water column were the same, or higher, than summer values. During the peak of the bloom when the concentrations of diatom, dinoflagellate, and zooplankton FAs were at their highest, the concentrations of vascular plant FAs and bacterial FAs were also high (Figure 2.7). The concentration of vascular plant and bacterial FAs were both low at the start of September (0.3 ± 0.1 $\mu g\ l^{-1}$ and 0.5 ± 0.1 $\mu g\ l^{-1}$ respectively) and increased towards the end of the September and early October to their highest recorded concentrations (1.7 ± 0.7 $\mu g\ l^{-1}$ and 2.1 ± 0.3 $\mu g\ l^{-1}$, respectively). In addition, although there was no substantial increase during the bloom period, the concentration of macroalgal FAs in the water column during October (0.8 ± 0.1 $\mu g\ l^{-1}$) was comparable to June (1.1 ± 0.2 $\mu g\ l^{-1}$) and August (0.9 ± 0.1 $\mu g\ l^{-1}$; Figure 2.7).

Although the proportion of SELORG increased during the bloom period, the concentration of REMORG also increased during the bloom. Like the proportion of phytoplankton FAs dominating POM FAs masking increases in the concentrations of detrital FAs, the concentration of REMORG in the water column increased from the beginning of September (0.3 ± 0.1 mg l^{-1}) to a maximum in late September (2.3 ± 0.1 mg l^{-1}) even as its proportional contribution decreased (84.6 ± 1.3 % to 41.4 ± 1.0 %; Figure 2.9). It should also be noted, that although in this study we generally associated diatom and

dinoflagellate FAs with fresh phytoplankton, diatom and dinoflagellate biomarker FAs would also be present in phytodetritus. The REMORG concentration of <100 µm POM was positively correlated with macroalgal ($p < 0.01$, $R^2 = 0.19$) and vascular plant FAs ($p < 0.007$, $R^2 = 0.21$) as well as diatom ($p < 0.001$, $R^2 = 0.45$), dinoflagellate ($p = 0.003$, $R^2 = 0.24$), and zooplankton FAs ($p < 0.001$, $R^2 = 0.34$), suggesting detrital material from all biomarker sources contributed to <100 µm POM (Figure 2.15a). In contrast, the proportion of macroalgal ($p = 0.01$, $R^2 = 0.21$), vascular plant ($p = 0.02$, $R^2 = 0.16$), and detrital FAs (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3 and 20:4ω6, 18:2ω6 and 18:3ω3, *iso, anteiso*, odd-chained; $p = 0.01$, $R^2 = 0.20$) were negatively correlated with the concentration of REMORG, suggesting that when REMORG is low (e.g. outside of bloom periods) there is proportionally more macroalgal and vascular plant detrital material (Figure 2.15b).

The $\delta^{13}C$ of <100 µm POM became isotopically heavier during the fall bloom due to a combination of diatoms and vascular plant (i.e. *S. alterniflora*) detritus. The $\delta^{13}C$ of <100 µm POM became isotopically heavier during the fall phytoplankton bloom, increasing from -19.1 ± 0.1 ‰ at the beginning of September to a maximum of -13.8 ± 0.2 ‰ in October, which coincided with concentration increases in diatom and vascular plant FAs. The $\delta^{13}C$ of <100 µm POM was significantly positively correlated with the concentrations of both diatom ($p < 0.01$, $R^2 = 0.21$) and vascular plant FAs ($p < 0.05$, $R^2 = 0.12$; Figure 2.16). Additionally, the $\delta^{13}C$ of <100 µm POM was significantly positively correlated with the concentration of zooplankton FAs ($p < 0.001$, $R^2 = 0.56$), presumably due to zooplankton grazing on diatoms and vascular plant detritus (Figure 2.15).

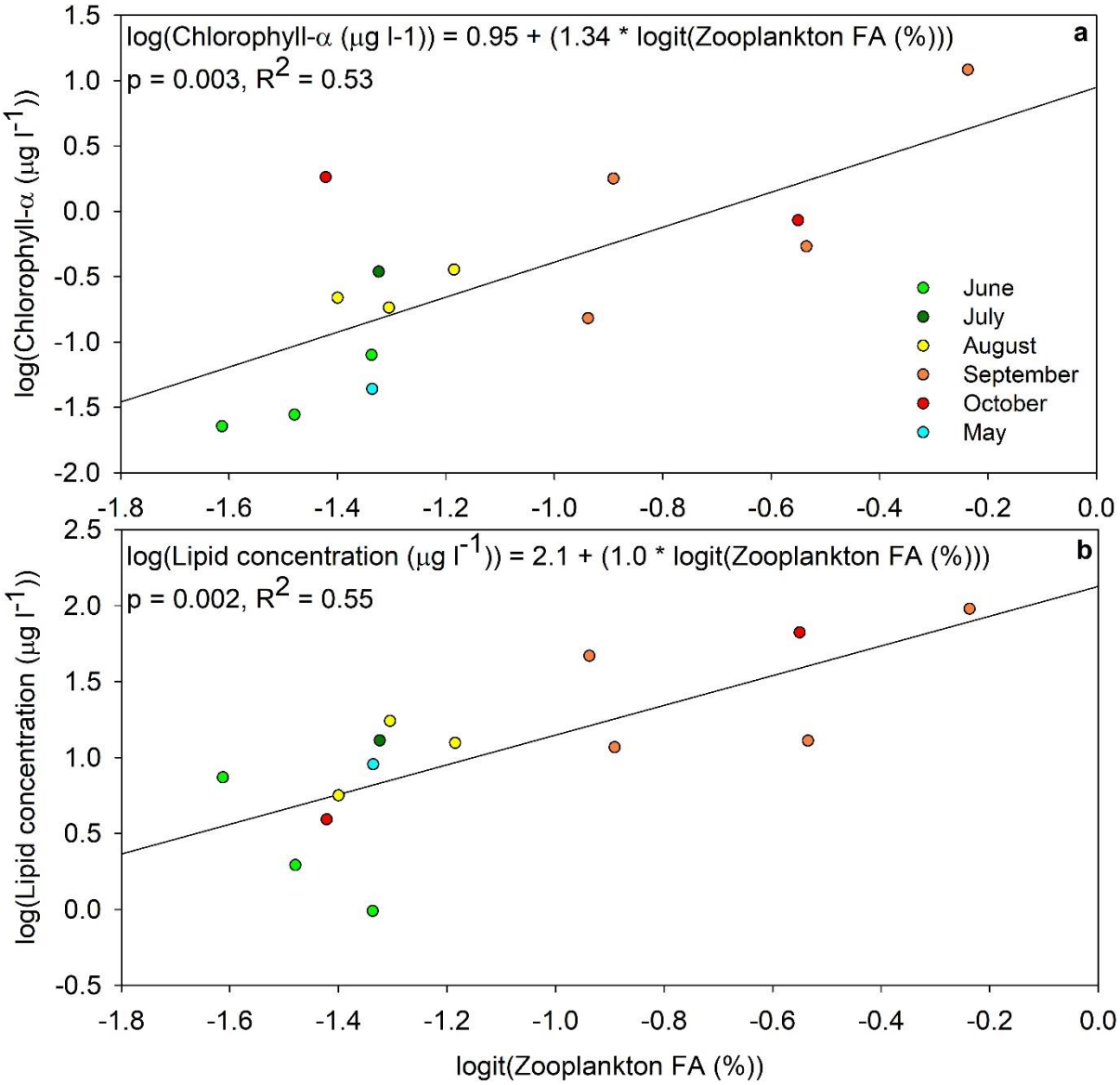

Figure 2.14. Zooplankton biomarker fatty acid regressions. Significant ($p < 0.01$) regressions between zooplankton fatty acids (logit(%)), the chlorophyll-*a* concentration (log(μg l^{-1});a), and the lipid concentration (log(μg l^{-1});b) of <100 μm particulate organic matter from Saco Bay, Maine USA, throughout 2016 and 2017.

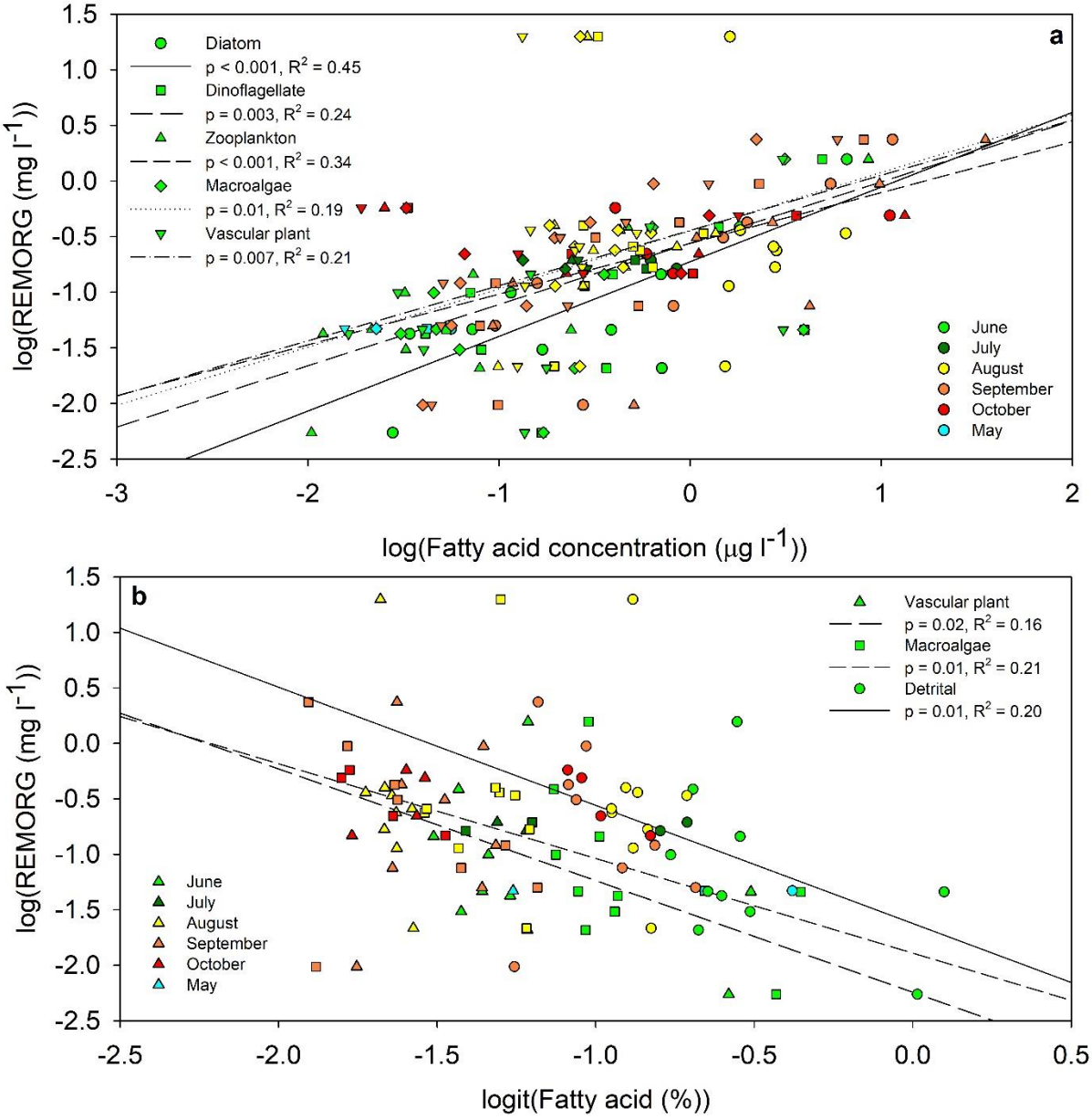

Figure 2.15. REMORG regressions. Significant ($p < 0.05$) regressions among the REMORG concentration (log(mg l^{-1})) and the (a) concentration (log(µg l^{-1})) and (b) proportion (logit(%)) of endmember biomarker fatty acids in <100 µm particulate organic matter from Saco Bay, Maine USA, throughout 2016 and 2017. Diatoms (16:1ω7, 16:4ω1 and 20:5ω3), dinoflagellate (22:6ω3 and 18:4ω3), zooplankton (Σ20:1 and Σ22:1), macroalgal (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3 and 20:4ω6), vascular plant (18:2ω6 and 18:3ω3), and detrital (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3 and 20:4ω6, 18:2ω6 and 18:3ω3, *iso, anteiso*, odd-chained).

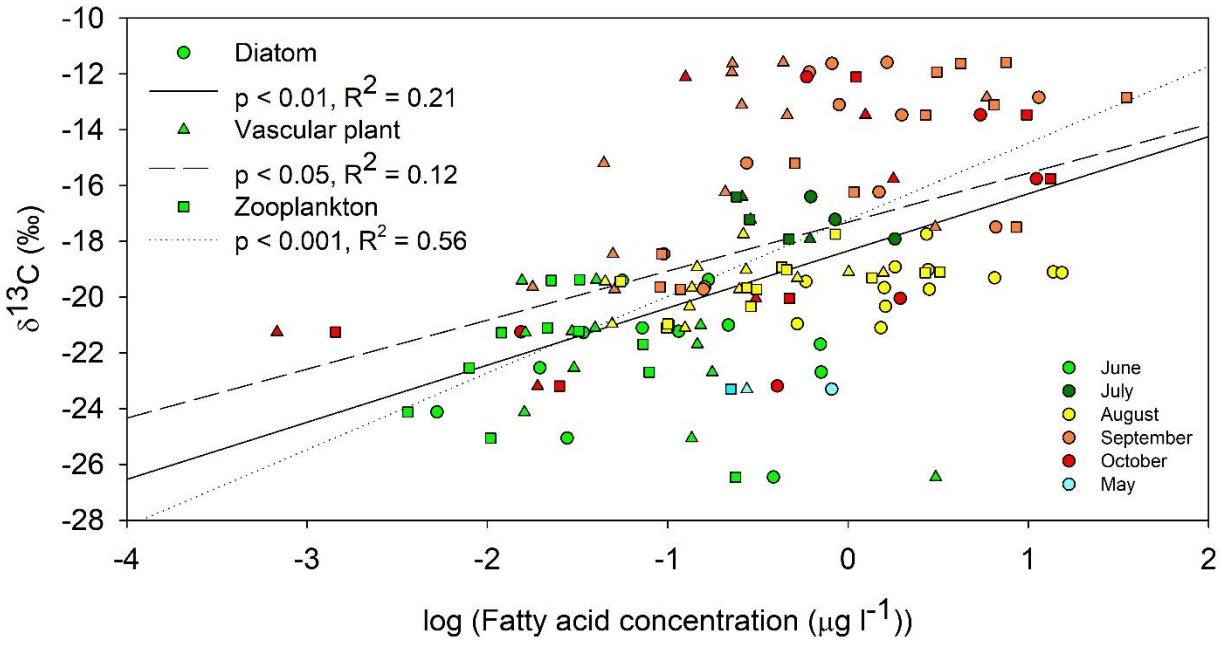

Figure 2.16. Fatty acid biomarker and particulate organic matter $\delta^{13}C$ (‰) regressions. Significant ($p < 0.05$) regressions between $\delta^{13}C$ (‰) and concentration ($\log(\mu g\ l^{-1})$) of endmember fatty acid biomarkers in <100 µm particulate organic matter from Saco Bay, Maine USA, throughout 2016 and 2017. Diatoms (16:1ω7, 16:4ω1 and 20:5ω3), zooplankton (Σ20:1 and Σ22:1), and vascular plants (18:2ω6 and 18:3ω3).

2.4.0 Discussion

2.4.1 POM composition

For a consumer in Saco Bay, most (38.4 ± 0.1%) of the lipids and FAs present in <100 µm POM were associated with phytoplankton and zooplankton. Diatom, dinoflagellate, and zooplankton contributed more to <100 POM than detrital sources based on FAs and isotope modeled estimates (Figure 2.8). Although they comprised a smaller proportion (17.3 ± 0.1%), detrital sources did contribute to the available pool of lipids and FAs in <100 µm POM (Figure 2.6 and 2.7). The positive correlations between REMORG and all endmember biomarker FAs suggests that detrital material from all endmembers contributed to <100 µm POM (Figure 2.15a). However, the negative correlations between REMORG and the proportion of macroalgal, vascular plant, and detrital FAs suggests that these detrital sources become more important contributor's to POM as REMORG concentrations decrease (Figure

2.15b). As the highest concentrations of REMORG coincide with the fall phytoplankton bloom (Figure 2.4 and 2.9a), this suggests that macroalgal and vascular plant detritus would likely be more important to consumers during non-bloom periods as supplemental food-sources.

Based on FA composition, POM in Saco Bay can be broken down into two general categories; marine POM (phytoplankton, zooplankton, and macroalgal detritus) and vascular plant detritus (salt marsh and terrestrial plant). The difference between marine and vascular plant POM for consumers was the presence of essential FAs. While marine detritus is expected to contain some amounts of essential FAs, in varying concentrations depending on the source, vascular plant detritus lacks essential FAs and is instead rich in 18:2ω6 and 18:3ω3 (Table 2.1). The distinction between the FAs of marine and vascular plant detritus is not surprising, it has long been known that vascular plants from marshes and terrestrial sources are poor sources of essential FAs (Phillips, 1984). Due to the differences in their FA compositions, marine and vascular plant detritus offer very different opportunities for consumers.

Although 20:5ω3, 22:6ω3, and 20:4ω6 are considered essential FAs for many consumers, because they lack the ability to synthesize them *de novo* (Sargent et al., 1999; Hall et al., 2006; Kelly and Scheibling, 2012), some invertebrate consumers possess the enzymes required to chain elongate and desaturate 18:2ω6 and 18:3ω3 into 20:5ω3, 22:6ω3, and 20:4ω6 (Waldock and Holland, 1984; Bell et al., 2001; Troch et al., 2012; Da Costa et al., 2015). For consumers unable to synthesize 20:5ω3, 22:6ω3, and 20:4ω6 from precursors, these FAs are considered essential nutrients (they must be obtained in their diet) whereas they are only essential metabolites for consumers able to synthesize them from precursors (Parrish, 2009). Copepods can elongate 18:3ω3 into 20:5ω3 and 22:6ω3 as well as 18:2ω6 into 20:4ω6 (Norsker and Støttrup, 1994; Nanton and Castell, 1998; Stottrup, 2000; O'Keefe, 2002; Troch et al., 2012). Additionally the Pacific oyster, *Crassostrea gigas*, is able to elongate 18:3ω3 into 20:5ω3 (Waldock and Holland, 1984; Da Costa et al., 2015).. Consumers able to synthesize metabolically essential FAs (20:5ω3, 22:6ω3, and 20:4ω6) using their precursors (18:2ω6 and 18:3ω3) will be able to

more readily take advantage of vascular plant detritus than those that cannot. By being able to exploit vascular plant detritus a consumer will have more avenues available to obtain metabolically required FAs. Consumers physiologically capable of exploiting vascular plant detritus will likely benefit from reduced competition, by being less reliant on ephemeral phytoplankton to obtain metabolically essential FAs.

Like other marine POM, macroalgal detritus contains metabolically essential FAs (20:5ω3 and 20:4ω6) which would be available to consumers lacking the elongase and desaturase enzymes. Macroalgae were potentially an important means for consumers to obtain both 20:4ω6 (7 to 18%) and 20:5ω3 (8 to 26%). Macroalgal FAs comprised the largest proportion of <100 µm POM during May and early June, a time when phytoplankton FAs were low (Figure 2.6). Due to the abundance of 20:4ω6 in macroalgae, proportionally larger contributions of macroalgal detritus led to higher ratios of 20:4ω6-to-20:5ω3 and 22:6ω3 (Figure 2.10). Overall macroalgal detritus was less abundant than phytoplankton based on both FAs and stable isotopes (Figure 2.8); however, its consistent presence in <100 µm POM offered a potential avenue for consumers to obtain additional 20:4ω6 and 20:5ω3, especially during spring/early summer.

However, unlike phytoplankton and zooplankton, macroalgae contain large amounts of ANFs in the form of secondary metabolites (Haddad et al., 1992) and difficult to digest structural material (Schiener et al., 2017). Both secondary metabolites and refractory structural material, such as cellulose and lignin's, are potential barriers barring consumers from accessing the essential FAs in macroalgal detritus. These barriers set macroalgal detritus apart from other marine POM, but like vascular plant detritus, macroalgal detritus can become an alternative source of metabolically essential FAs for consumers capable of digesting it. It should be noted that vascular plant detritus also contains ANFs in the form of secondary metabolites and refractory structural carbohydrates (Newell and Langdon, 1986; Haddad et al., 1992). So, for a consumer to obtain metabolically essential FAs from vascular plant

detritus they would need to possess elongase and desaturase enzymes and be capable of dealing with ANFs. As such, vascular plant detritus is likely the most difficult avenue to obtain metabolically essential FAs.

Although macroalgae were an abundant source of 20:4ω6, pennate diatoms could also contribute 20:4ω6 to the water column. Arachidonic acid (20:4ω6) is present in moderate proportions (2 to 6%) in some pennate diatoms (Dunstan et al., 1994). Although centric diatoms were more prevalent in Saco Bay, pennate diatoms were at times present in the water column and could have been a source of 20:4ω6 (Figure 2.5). A well-known FA biomarker for diatoms is 16:1ω7 (Parrish, 2013), and because of their higher proportion of 20:4ω6, pennate diatoms (0.01 to 0.25) have a higher ratio of 20:4ω6-to-16:1ω7 than centric diatoms (0.01 to 0.07; Dunstan et al., 1994). With higher proportional abundances of 20:4ω6 and lower proportional abundances of 16:1ω7 than pennate diatoms, macroalgae have an even higher ratio of 20:4ω6-to-16:1ω7 (*A. nodosum*: 6.8 ± 0.1, *S. latissima*: 4.8 ± 1.3, *C. crispus*: 0.8 ± 0.1). In this study, 18:1ω9 was an abundant FA in macroalgae (especially *A. nodosum*) which led to its use as a biomarker (Table 2.1). By comparing the ratio of the macroalgal marker 18:1ω9 to the diatom marker 16:1ω7 we can estimate whether there were macroalgae or diatom FAs present in the water column. The ratio of 18:1ω9-to-16:1ω7 is low in both centric (0.01 to 0.43) and pennate (0.01 to 0.27) diatoms (Dunstan et al., 1994), while the ratio of 18:1ω9-to-16:1ω7 is much higher in macroalgae (*A. nodosum*: 20.1 ± 0.4, *S. latissima*: 2.4 ± 0.1, *C. crispus*: 0.6 ± 0.1). As such, when both the ratio of 20:4ω6-to-16:1ω7 and 18:1ω9-to-16:1ω7 increase it is indicative of macroalgal material, whereas when only the ratio of 20:4ω6-to-16:1ω7 increases it likely due to pennate diatoms. In the current study, both 20:4ω6-to-16:1ω7 and 18:1ω9-to-16:1ω7 are elevated in late spring and early summer corroborating the presence of macroalgal detritus in the water column, while in the fall only the ratio of 20:4ω6-to-16:1ω7 increased suggesting pennate diatoms were the source of 20:4ω6 (Figure 2.10).

Both FlowCam and FA biomarker analysis demonstrated that dinoflagellates dominated Saco Bay, followed by centric diatoms and then pennate diatoms. The biomass of dinoflagellates was always greater than that of diatoms (Figure 2.5) and the ratio 22:6ω3-to-20:5ω3 was always ≥1 (Figure 2.11b). Based on cell counts, dinoflagellates have previously been reported to be the dominant phytoplankton in the Gulf of Maine (Bigelow, 1926; Marshall, 1984; Fry and Wainright, 1991). Centric diatoms were always more prevalent than pennate diatoms (Figure 2.5). Dinoflagellates predominantly contain 22:6ω3 and diatoms predominantly contain 20:5ω3 (Budge and Parrish, 1998), while 20:4ω6 is lacking in most dinoflagellates (Nichols et al., 1984; Mansour et al., 1999, 2003; Leblond and Chapman, 2000) and only present in some pennate diatoms (Dunstan et al., 1994). Reflecting the dominance of dinoflagellates followed by centric diatoms, 22:6ω3 (8.6 ± 0.1%) was the most abundant metabolically essential FA in POM, followed by 20:5ω3 (7.0 ± 0.1%), while there was relatively little 20:4ω6 (0.3 ± 0.1%; Figure 2.11d). Although each individual consumer will have different essential FA requirements, based on only their abundance we would expect 20:4ω6 to be the most limiting essential FA, followed by 20:5ω3, both of which are present in macroalgae.

Macroalgal detritus consistently contributed to <100 µm POM, likely because macroalgal detritus can arise from a variety of vectors (blade erosion, gamete release, and epithelial shedding), which vary seasonally. Fucoid algae such as *A. nodosum* are known to release gametes during April and May in estuaries and during June for open coastlines like Saco Bay (Keser and Foertch 1982; Milan Keser and Larson 1984). The temperature range of the bay during early June was within the range reported (6 to 15 °C) for *A. nodosum* gamete release (Bacon and Vadas, 1991). *Saccharina latissima* releases spores slightly earlier in the year during May (Lee and Brinkhuis, 1986). Macroalgal gametes could also have contributed to POM during fall since *S. latissima* is known to produce sorus (gamete producing) material in the fall (Lee and Brinkhuis, 1986; Egan and Yarish, 1990). Both FA markers used for macroalgae (18:1ω9 and 20:4ω6) are present in *S. latissima* zoospores, especially 18:1ω9 which occurs in large

proportions (30 to 45%; Steinhoff et al. 2011) corroborating a gamete source of these FAs. Zoospores contain large amount of triacylglycerol's in the form of lipid droplets (Brzezinski et al., 1993), which makes them an energy dense food source. Due to their unicellular nature (Steinhoff, 2012), macroalgal zoospores are presumably easier to digest than their parent material; however, zoospores do possess polyphenols (Steinhoff et al., 2011).

Another likely source of macroalgal detritus is material eroded from blade tips. Erosion rates in macroalgae are positively correlated with temperature (Krumhansl and Scheibling, 2011, 2012). Increased concentrations of macroalgal FAs in late summer coincided with the warmest water temperatures in the bay. In addition to temperature, wave exposure has also been positively correlated with macroalgal erosion rates (Krumhansl and Scheibling, 2011, 2012), which could explain increases in macroalgal FAs during fall when the bay became more turbulent due to seasonal storms (Brown and Beardsley, 1978). Aside from increasing macroalgal detritus production via increased erosion rates, increased wave action may also simply resuspend settled macroalgal detritus and cannot be ruled out as the cause of increased macroalgae FA concentrations in the water column. Additionally, to combat epiphytes macroalgae, such as *A. nodosum*, periodically shed thin sheets of epithelial cells into the water column, representing about 1% of frond biomass per month (Halat et al., 2015), which could help explain the consistent low concentrations of macroalgal FAs.

Like macroalgal detritus, vascular plant FAs were less abundant than phytoplankton FAs but were consistently present at low levels (Figure 2.7e). Unlike macroalgal detritus, vascular plant detritus may have contributed more to POM carbon and nitrogen (Figure 2.8e and f). Although our stable isotope mixing model estimation of *S. alterniflora* contributions are likely an overestimation, diatom/macroalgae and *S. alterniflora* were negatively correlated (R^2 = -0.57) in the isotopic mixing model, so over-estimations of one would lead to reductions in the other. Some of the observed differences in measured FAs and stable isotope model estimates (Figure 2.8) could be explained by

natural processes. While FAs can distinguish between bacteria and other endmembers, mixing models cannot. Heterotrophic bacterial $\delta^{13}C$ is similar to their substrate which can make it difficult to distinguish their contribution using traditional mixing models. Conversion of labile endmember material into bacterial biomass would thus lower their contributions to total FAs while showing little change in $\delta^{13}C$. Although this could explain some of the difference between isotope modeled estimates and measured FAs, the combined contributions of vascular plant FAs and bacterial FAs (10.4%) is still lower than the isotope model estimated contribution of vascular plants (25.4 ± 0.4%). Because many of the zooplankton within Saco Bay are omnivorous, it is also possible that consumption of vascular plant detritus by zooplankton, and consequent reworking into essential FAs, masked the contribution of vascular plants.

The reworking of vascular plant FAs could partially explain why zooplankton FAs were highly positively correlated ($R^2 = 0.56$) with the $\delta^{13}C$ of <100 µm POM (Figure 2.16). However, diatoms were also positively correlated ($R^2 = 0.21$) with the $\delta^{13}C$ of <100 µm POM (Figure 2.16). Diatom $\delta^{13}C$ values are known to become isotopically heavier during periods of rapid growth and nutrient limitation (Fry and Wainright, 1991; Fry, 1996), conditions which are both expected to occur towards the end of a bloom. Nutrient concentrations in the bay and the C_{16} PUFA ratio both corroborate that diatoms likely became nutrient limited as chlorophyll-*a* concentrations reached their maxima at the beginning of October and POM $\delta^{13}C$ became isotopically heavier (Figure 2.4 and 2.11c). The peak in chlorophyll-*a* concentrations and isotopically heavy POM also coincided with increasing proportional contributions of dinoflagellates, an expected successional pattern following nutrient limitation in diatoms (Figure 2.6).

Another possibility which could explain increases in POM $\delta^{13}C$ is benthic diatom contributions. Salt marsh benthic microalgae have isotopically heavy $\delta^{13}C$ (-13.0 ‰) and are important food sources for estuarine consumers (Currin et al., 1995). Microphytobenthos can contribute directly to the water column when resuspended and are commonly associated with pennate diatoms (Cahoon et al., 1992; MacIntyre et al., 1996). It is possible the pennate diatoms present in the water column during

September based on volume estimates and FA ratios were resuspended microphytobenthos (Figure 2.5 and 2.10b). Turbidity within the bay increased throughout September and October (data not shown), suggesting there was likely increased re-suspension. Although re-suspended benthic diatoms could explain the increases in pennate diatoms and changes in the ratio of 20:4ω6-to-16:1ω7, the peak in pennate diatom abundance occurred during September and did not coincide with the isotopically heaviest POM $\delta^{13}C$ in early October. As such, while re-suspended benthic pennate diatoms could explain some of the increases in POM $\delta^{13}C$, there were likely other contributing factors.

Yet another possibility which could explain the isotopically heavier POM $\delta^{13}C$, is the presence of cyanobacteria. Like benthic diatoms, cyanobacteria are known to be isotopically heavy in $\delta^{13}C$ (-14.5 to -8.5 ‰) and are present in salt-marsh estuaries (Calder and Parker, 1973; Fry et al., 1982; Pulich and Scalan, 1987; Currin et al., 1995). In the present study, the abundance of the cyanobacterium *Synechococcus* spp. peaked during July (9.7×10^4 cells ml^{-1}) and decreased until October (0.7×10^4 cells ml^{-1}). If *Synechococcus* spp. was responsible for increases in POM $\delta^{13}C$, we would have expected their abundance to increase during the fall, not decrease, suggesting *Synechococcus* spp. was not responsible for the observed changes in POM $\delta^{13}C$. It should be noted, that the presence of isotopically heavy benthic diatoms or cyanobacteria within the water column at other times of the year, e.g. summer, could also partially explain the over-estimates of *S. alterniflora* contributions in our isotope model compared to measured FA biomarkers.

Given the increased concentration of vascular plant FAs in the water column during October as well as the significant ($R^2 = 0.12$) positive correlation between vascular plant FAs and POM $\delta^{13}C$ (Figure 2.16), albeit weaker than diatoms, it is unlikely that diatoms are solely responsible for the heavier $\delta^{13}C$ of POM. Increases in fall contributions of *S. alterniflora* detritus to the water column are consistent with POM fluxes measured in other salt-marsh estuaries (Dame et al., 1986). Fall increases in vascular plant

FAs would also be consistent with the timing of post-flowering senescent *S. alterniflora* plants as well as the outwelling hypothesis (Odum, 1980; Crosby et al., 2015).

2.4.2 Phytoplankton dynamics

Saco Bay followed the classic northern temperate estuary spring/fall bloom cycle (Thomas et al., 2003). Diatoms began to bloom in September and October when the bay became less stratified, the bloom timing is corroborated by FlowCam diatom biomass estimates (Figure 2.5b), chlorophyll-*a* levels (Figure 2.4), the concentration and proportion of diatom FAs (Figure 2.6 and 2.7), reductions in silicate concentrations (Figure 2.3), and the negative correlation between chlorophyll-*a* and the surface-to-bottom density difference (Figure 2.13). Chlorophyll-*a* in Saco Bay was comparable to previously reported fall chlorophyll-*a* maxima (Feurt et al., 2015). Phytoplankton blooms are known to occur during the spring (March to May) and fall (August to November) within the Gulf of Maine (Thomas et al., 2003) with diatoms comprising the majority of phytoplankton during the spring bloom (Townsend and Thomas 2001).

In contrast to diatoms, dinoflagellates commonly bloom during the summer and succeed diatom blooms in the Gulf of Maine when silicate levels fall and limit diatom growth (Townsend and Thomas 2001; Townsend et al. 2005). Both our FlowCam biomass (Figure 2.5) estimates and dinoflagellates markers (22:6ω3 and 18:4ω3; Figure 2.6) indicate that dinoflagellates dominated the summer and post-fall bloom assemblage. In addition, the 22:6ω3/20:5ω3 ratio (Figure 2.11b), an indicator of dinoflagellate dominance, was also elevated during these times. The C_{16} PUFA ratio first described by Shin et al. (2000) and later modified by Parrish et al. (2005) has been used as an indicator of the physiological status of diatoms. The low C_{16} PUFA ratio in early October 2016 corroborates nutrient limited diatoms as dinoflagellate abundance increased (Figure 2.11c). Nutrient limitation (nitrate, phosphate, and silica) is known to reduce the proportion of ω3 and PUFA in phytoplankton (Harrison et al. 1990; Sukenik and Wahnon 1991; Reitan et al. 1994; Parrish et al. 2005). Coinciding with the

relatively low C_{16} PUFA ratio in October, the proportions of PUFA and ω3 FAs were also low, corroborating that diatoms were nutrient limited (Figure 2.11a). Although the ratio of dinoflagellates-to-diatoms decreased during the diatom bloom in the fall, the 22:6ω3/20:5ω3 ratio remained > 1 (Figure 2.11b). The 22:6ω3/20:5ω3 ratio suggests dinoflagellates were the dominant phytoplankton even during the diatom bloom, a result corroborated by FlowCam estimates of dinoflagellate biomass (µg C l^{-1}) which were greater than diatom estimates for almost all dates sampled (Figure 2.5).

While the C_{16} PUFA ratio and proportions of PUFA and ω3 were low at the end of the 2016 fall bloom, they were elevated in July, a time when diatoms are generally nutrient limited (Townsend et al., 2005). Nutrient concentrations within the bay were low in June; however, they increased in July coinciding with increases in diatom physiological status and marker (16:1ω7, 16:4ω1 and 20:5ω3) abundance increases (Figure 2.3, 2.6, and 2.7). Although diatom biomass within the bay was low during this period (Figure 2.5), the volume occupied by pennate diatoms was relatively high, suggesting their growth was driving the increase observed in FAs. Low precipitation, and thus river discharge, in July suggests that the increased nutrient input into the bay was due to other sources such as oceanic and local wastewater sources. Maine is a popular, summer tourist destination, attracting 35.8 million visitors in 2016 (Maine Office of Tourism, 2016). There are six separate wastewater treatment plant outfalls located within the bay and Saco River which serve an estimated population of 67,000 (US Census 2010). Discharge from Old Orchard Beach's outfall, the largest discharger in the area, increased roughly 40% from May to July 2016 and remained high throughout August until decreasing in September. Free-fatty acids signal lipid breakdown when present in human sewage (Pastore et al., 2015). The proportion of FFA in <100 µm POM increased from 8.6 ± 2.8% in late June to 16.6 ± 0.9% in late August in conjunction with wastewater discharges and was positively correlated (R^2 = 0.35) with diatom FAs (Figure 2.12c).

The $\delta^{15}N$ values measured in Saco Bay also indicate that anthropogenic N input was an important driver of POM dynamics. In nearby Casco Bay (the most populous watershed and home to the

largest wastewater treatment outfalls in the state), the $\delta^{15}N$ of nitrate in areas near wastewater outfalls (0.4‰) was elevated in comparison to more pristine areas (-2.7‰; Flynn et al., 2011). If we assume that we can derive $\delta^{15}N$ values by subtracting two trophic levels from zooplankton (Fertig et al., 2014), the resulting $\delta^{15}N$ (1.7‰) is within a similar range to the elevated nitrate values of Casco Bay suggesting the importance of anthropogenic N inputs to this ecosystem. Corroborating that anthropogenic nitrogen may increase $\delta^{15}N$ values, the $\delta^{15}N$ of <100 µm POM was positively correlated with diatom FAs (R^2 = 0.24) and FFAs (R^2 = 0.11; Figure 2.12). It should be noted that oceanic nitrate, with $\delta^{15}N$ values between 2.4 to 5.3 ‰ (Marconi et al., 2015), would also be expected to increase $\delta^{15}N$ over riverine values; however, the correlation between $\delta^{15}N$ and FFA supports the importance of anthropogenically sourced nitrogen in this system.

2.5.0 Conclusions

There were two main categories of POM in Saco Bay, marine POM which contained essential FAs (20:5ω3, 22:6ω3, and 20:4ω6) and vascular plant POM which lacked metabolically essential FAs but was rich in their precursors (18:2ω6 and 18:3ω3). Phytoplankton and zooplankton were the most important sources of lipid and FAs; however, macroalgal and vascular plant detritus became more important contributors to POM outside of phytoplankton bloom periods. Macroalgal detritus could serve as a supplemental source of the essential FAs 20:5ω3 and 20:4ω6, while consumers possessing elongase and desaturase enzymes could make use of 18:2ω6 and 18:3ω3 from vascular plant detritus. Like the rest of the Gulf of Maine there were spring/fall diatom blooms in Saco Bay, followed by a succession of dinoflagellates and zooplankton.

CHAPTER 3

DETRITAL SUBSIDIES TO THE DIET OF *MYTILUS EDULIS*: MACROALGAL DETRITUS LIKELY SUPPLEMENTS ESSENTIAL FATTY ACIDS

3.1.0 Introduction

Bivalves, such as the blue mussel (*Mytilus edulis*), are a major group of filter-feeding animals whose growth (Grant, 1996; Hawkins et al., 2002, 2013a) and ecological carrying capacities (Byron et al., 2011a, 2011b; Kluger et al., 2016; Outeiro et al., 2018) have been modeled based on food availability and other environmental variables. Within ecosystems large proportions of energy in food-webs move through detritus (Cebrian and Lartigue, 2004), which is often incorporated into food web and bivalve growth models. In this study 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, e.g. organic matter (OM) that is not live phytoplankton defined as remaining organic matter (REMORG) in Shellsim, which is treated as a homogeneous entity (Hawkins et al., 2013a).

Detritus is not, however, a homogeneous entity and 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 can cause under or overestimations of detrital importance, which may be acceptable for 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 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) (Cranford and Grant, 1990; Enríquez et al., 1993; Kitazato et al., 2000; Beaulieu, 2002) while macroalgae and marsh grass detritus represent progressively lower 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, 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 to varying degrees. In particular macroalgal detritus 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 the potential food source. For example, bivalves display moderate to high absorption efficiencies (41 to 87%) which increases with age of detrital particulates (Stuart et al., 1982; Cranford and Grant, 1990). Detrital particles consisting of a single cell (SCD) formed from macroalgae were capable of supporting equivalent or greater growth compared to phytoplankton diets when comprising 50 to 90% of larval diets in a shellfish hatchery (Camacho et al., 2004; Carboni et al., 2016).

While laboratory examinations of macroalgal detritus use by bivalves looks promising, the importance of macroalgal detritus to bivalves within natural ecosystems is still uncertain.

Directly tracking detrital material use by bivalves *in situ* is exceedingly difficult so, many studies rely on stable isotope analysis (Duggins et al., 1989; Hill et al., 2006; Kaehler et al., 2006; Tallis, 2009; Von Biela et al., 2016). Stable isotopes are 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). 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 available to ecologists to track organic matter in ecosystems is lipid fatty acid biomarkers. Lipid classes themselves (composition, quantity, and ratios) have a history of use as biomarkers in ecological and biogeochemical studies (see review by Parrish, 2013) and individual fatty acids (FA) have been used in oceanographic studies as biomarkers for a large variety of organisms (Kelly & Scheibling 2012, Parrish 2013 and references within). Lipid FA biomarkers have been used to determine the shifting contributions of diatoms and dinoflagellates within the water column, as well as the 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 stable isotope analysis 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). Lipid FA biomarkers have also 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 reveal primary producer contributions to food webs (Carreón-Palau et al. 2013).

We content that by using SIA, lipid, 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 oaks) within a northern temperate bay to the diet of intertidal *M. edulis* using a combination of stable isotopes and lipid fatty acid biomarkers. Our aim was to more accurately represent organic matter pathways for consideration in bivalve feeding and carrying capacity models.

3.2.0 Methods

To determine the contributions of macroalgae and vascular plants to mussel diets, we collected fresh samples of each from Saco Bay, ME USA, 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*.

3.2.1 Study site and sample collection

We collected samples from Saco Bay (Figure 3.1), which is located in southern Maine, USA, with its northern-most border framed by Scarborough marsh and the southern boundary is Biddeford Pool (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 the 6th 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 3.1; 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 3.1). Particulate organic matter was size fractionated (≥100 and <100 µm) *in situ* in the water column by filtering water through a 100 µm pre-screen and a 1 µm collection mesh. In 2016 POM samples were 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. 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-*a*, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), and lipid and fatty acid biomarkers.

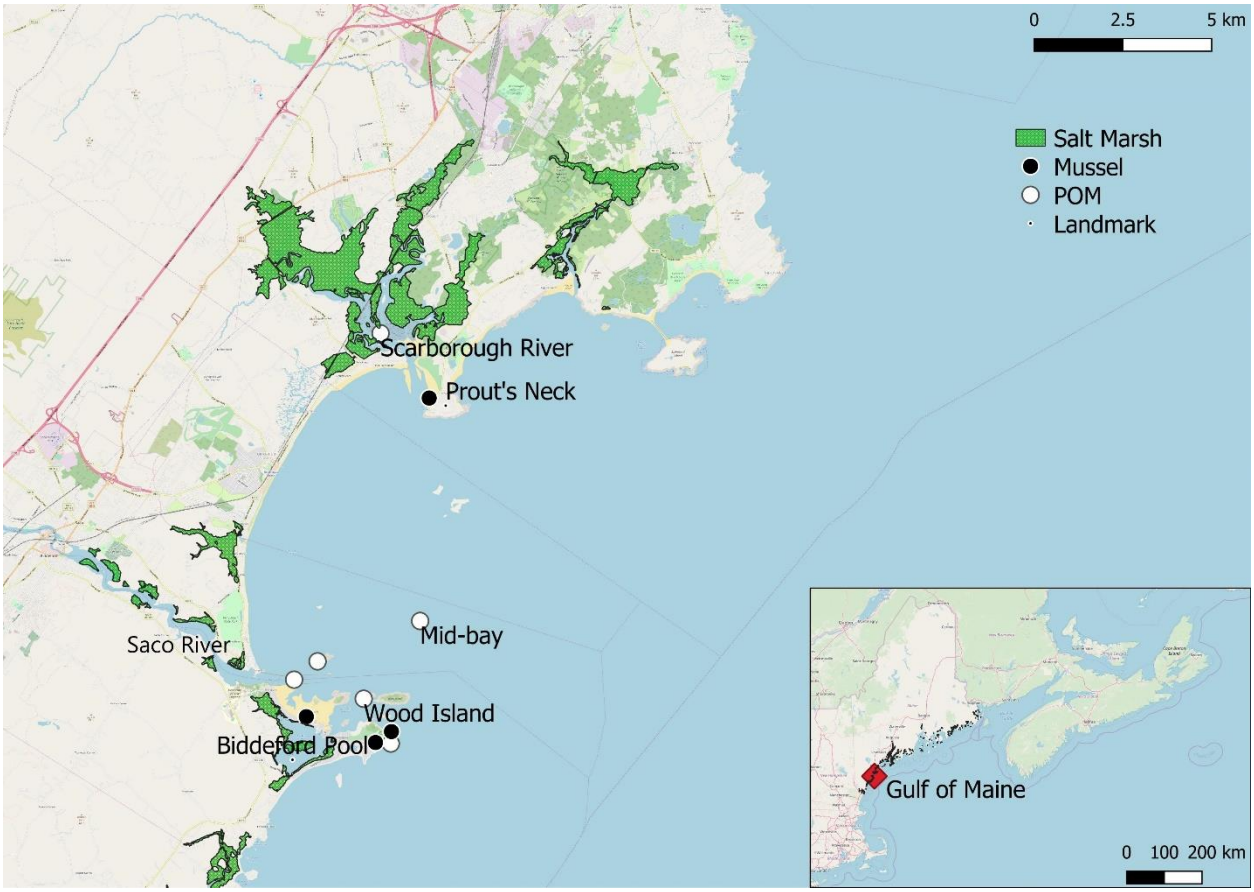

Figure 3.1. Map of Saco Bay, ME USA. Includes locations of fringing salt marshes, particulate organic matter (POM) sampling sites, and sampling sites for mussels (*Mytilus edulis*).

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 other eastern perimeter of Biddeford pool, near Wood island, and Prout's Neck near the Scarborough River (Figure 3.1). Oak tree leaf litter was sampled once in the fall of 2016 to represent C_3 photosynthetic plants. 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). Mussels were shucked and processed for stable isotope ($\delta^{13}C$ and $\delta^{15}N$), lipid and FA

analysis as described below. Macroalgal and *Spartina* samples were scraped with a razor to remove epiphytes and rinsed in deionized water prior to processing for stable isotope ($\delta^{13}C$ and $\delta^{15}N$), lipid, and fatty acid analysis. Zooplankton were collected with vertical tows of a 200 µm zooplankton net. Afterwards, 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.

3.2.2 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 one hour) and concentrated sulfuric acid were used to transesterify subsamples of total lipid extracts into fatty acid methyl esters (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°C min^{-1} and held for 15 min. Column temperature was then ramped to 220°C at a rate of 2°C min^{-1} and held for 3.25 min. Injector temperature started at 150°C and ramped at a rate of 200°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 visualized with PCoA while SIMPER and cluster analysis were used to determine similarity and dissimilarity within and among endmember groups. Statistically significant groupings (α = 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 3.1. Data shown are average ± 1 standard error, unless otherwise indicated. Proportions (%) of diatom and macroalgal FA markers, identified based on PCoA and SIMPER analysis, were used to separate the combined diatom/macroalgal estimates from stable isotope mixing models, e.g. $Diatom\ contribution = \ Combined\ estimate\ x\ (\frac{Diatom\ FAs}{Diatom\ FAs + Macroalgal\ FAs})$.

Table 3.1. Fatty acid biomarkers used for each endmember in this study. Includes literature source of previous use.

Endmember	Fatty acid biomarker	Source
Macroalgae	18:1ω9	(Kelly and Scheibling, 2012; Parrish, 2013)
	18:3ω6	(Kelly and Scheibling, 2012; Parrish, 2013)
	20:2ω6	(Kharlamenko et al., 1995)
	20:4ω3	(Kelly and Scheibling, 2012; Parrish, 2013)
	20:4ω6	(Kelly and Scheibling, 2012; Parrish, 2013)
Vascular plants	18:2ω6	(Kelly and Scheibling, 2012; Parrish, 2013)
	18:3ω3	(Kelly and Scheibling, 2012; Parrish, 2013)
Dinoflagellates	22:6ω3	(Kelly and Scheibling, 2012; Parrish, 2013)
Diatoms	16:1ω7	(Kelly and Scheibling, 2012; Parrish, 2013)
	16:4ω1	(Dalsgaard et al., 2003; Parrish, 2013)
	20:5ω3	(Kelly and Scheibling, 2012; Parrish, 2013)
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)
	Iso and *anteiso* FAs	(Parrish, 2013; George and Parrish, 2015)

3.2.3 Stable isotope analysis

Initial processing of stable isotope samples for POM was identical to how dry weights were processed. Particulate organic matter was filtered onto pre-combusted and pre-weighed 1.2 µm GF/C Whatman filters, dried for 24 hours (60°C), or until a constant weight, and stored in desiccation chambers until processed and sent for analysis. Hydrochloric acid fumes were introduced to dried POM samples for 24 hr to remove carbonate carbon prior to encapsulation in tin (Sn). Mussel and endmember tissues were prepared by drying for 24 hr 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 to the University of California's Davis Stable Isotope facility 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, USGUS-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 of possible dietary contributions (Parnell et al., 2010), using the stable isotope analysis package in R (SIAR, ver. 4.2). Fractionation factors of 0.4 ± 1.0‰ and 3.4 ± 1.0‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively established by Post (2002) were used when determining mussel diets. Phytoplankton $\delta^{15}N$ values were estimated by subtracting one trophic level (3.4‰) from zooplankton $\delta^{15}N$ values. Previously reported $\delta^{13}C$ values from George's Banks in the Gulf of Maine were used, -18 ± 2‰ and -24 ± 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

model estimates, is reported as average ± 1 standard deviation, while all other data is reported as average ± 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 $\delta^{13}C$ values and assumed 1 trophic level (3.4‰ $\delta^{15}N$) difference between the $\delta^{15}N$ values of phytoplankton and zooplankton. To test the validity of these assumptions, a revised model incorporating a 1.75 trophic level (5.95‰ $\delta^{15}N$) difference between phytoplankton and zooplankton as well as mussel $\delta^{13}C$ values corrected for lipid content (based on a lipid-to-protein depletion of 6.5‰: Logan et al., 2008) was constructed. The estimates of both models were compared to assess the effects our assumptions had on the results.

3.3.0 Results

3.3.1 Lipid class and fatty acid composition

The lipids and FA composition of *M. edulis* changed qualitatively and quantitatively among seasons. Lipid content of *M. edulis* ranged from 0.8 to 5.4% wet weight (WW) with an average of 3.3 ± 0.1% (Figure 3.2a), consistent with previously reported lipid content of *M. edulis* (Table 3.2). Lipid content in mussels was 70% lower in mid-summer (July) than during spring (May) or fall (September). Mussels contained high quality lipids, polyunsaturated FAs comprised the bulk (54.5 ± 0.1%; range 49-60%) of identified FAs in *M. edulis,* and *M. edulis* contained a large proportion (37.1 ± 0.1%) of ω3 FAs (Figure 3.2b). The proportion of PUFA (Figure 3.3b), two essential FAs, 20:4ω6 and 22:6ω3, and non-methylene interrupted dienes (NMID) increased throughout summer and fall. Additionally, there was a decrease in the proportion of 20:5ω3 (Figure 3.3a), which was significantly ($p < 0.01$) negatively correlated with the proportion of NMIDs (Figure. 3.4), suggesting mussels may use NMIDs to compensate for 20:5ω3 requirements. In contrast concentrations (μg g^{-1} WW) of all FAs, including essential FAs, decreased throughout the summer before increasing in the fall (Figure 3.3b).

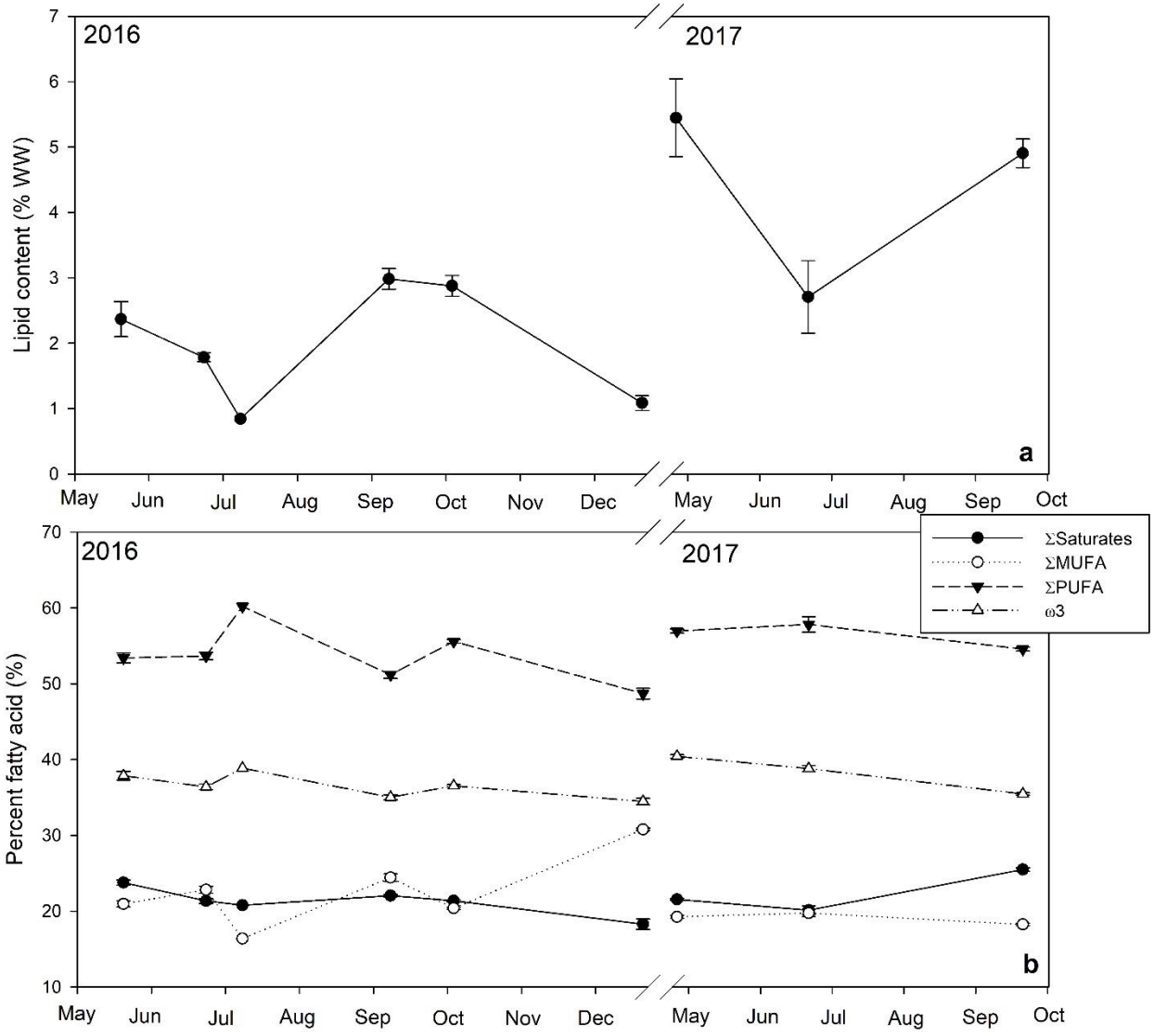

Figure 3.2. Summary of *Mytilus edulis* lipids. Lipid content (% wet weight) and proportion of fatty acids (%) in *Mytilus edulis* from Saco Bay, Maine USA, throughout 2016 and 2017. (a) Total lipid content (% wet weight). (b) Proportion (%) of saturated, monounsaturated (MUFA), polyunsaturated (PUFA), and ω3 fatty acids. Data shown are average ± standard error.

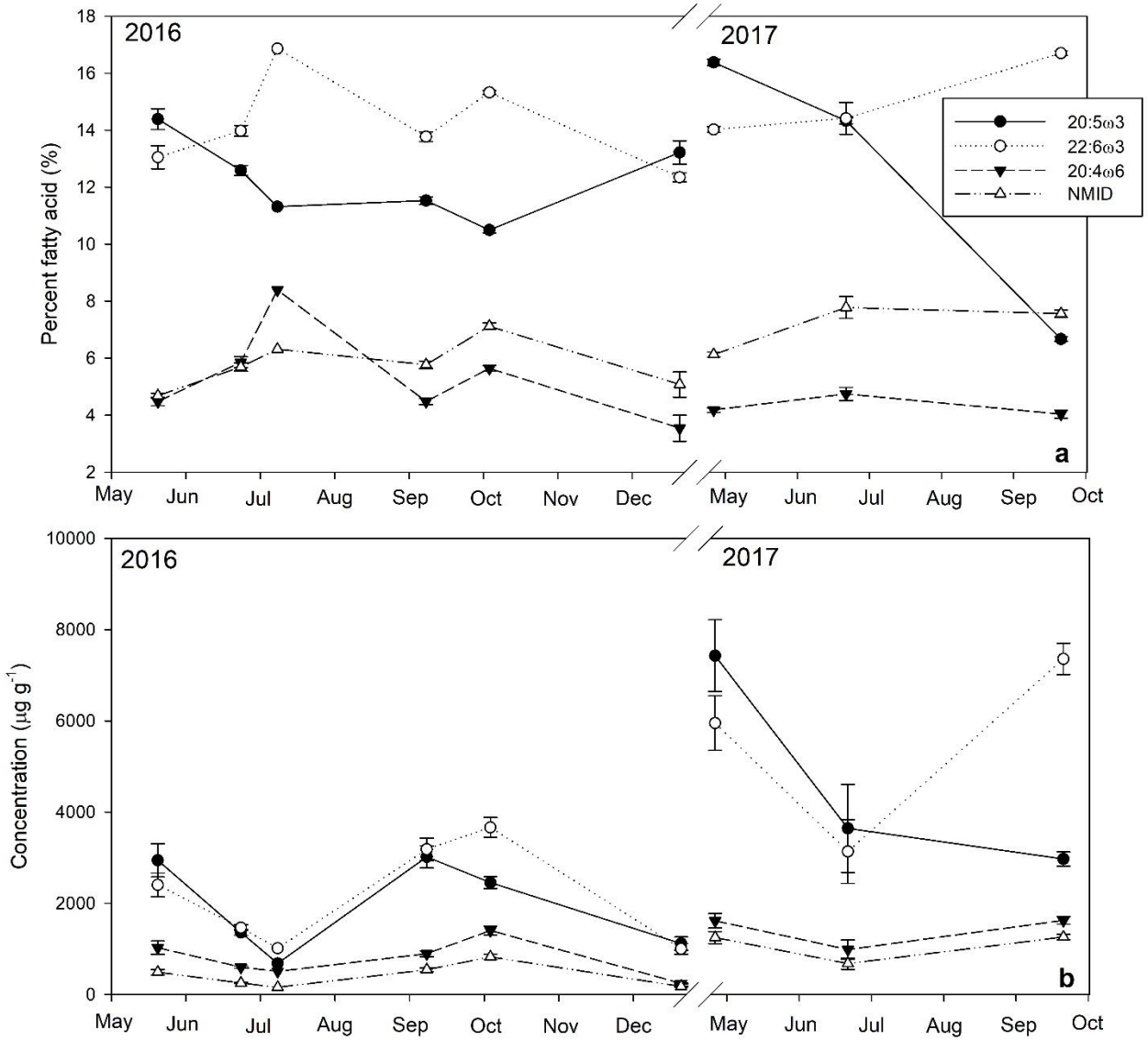

Figure 3.3. Essential fatty acids in *Mytilus edulis*. Proportion (%) and concentration (µg g^{-1} wet weight) of essential fatty acids (20:5ω3, 22:6ω3 and 20:4ω6) and non-methylene interrupted dienes (NMID) in *Mytilus edulis* from Saco Bay, Maine USA, throughout 2016 and 2017. (a) Proportion of total fatty acids (%). (b) Concentration (µg g^{-1} wet weight). Data shown are average ± standard error.

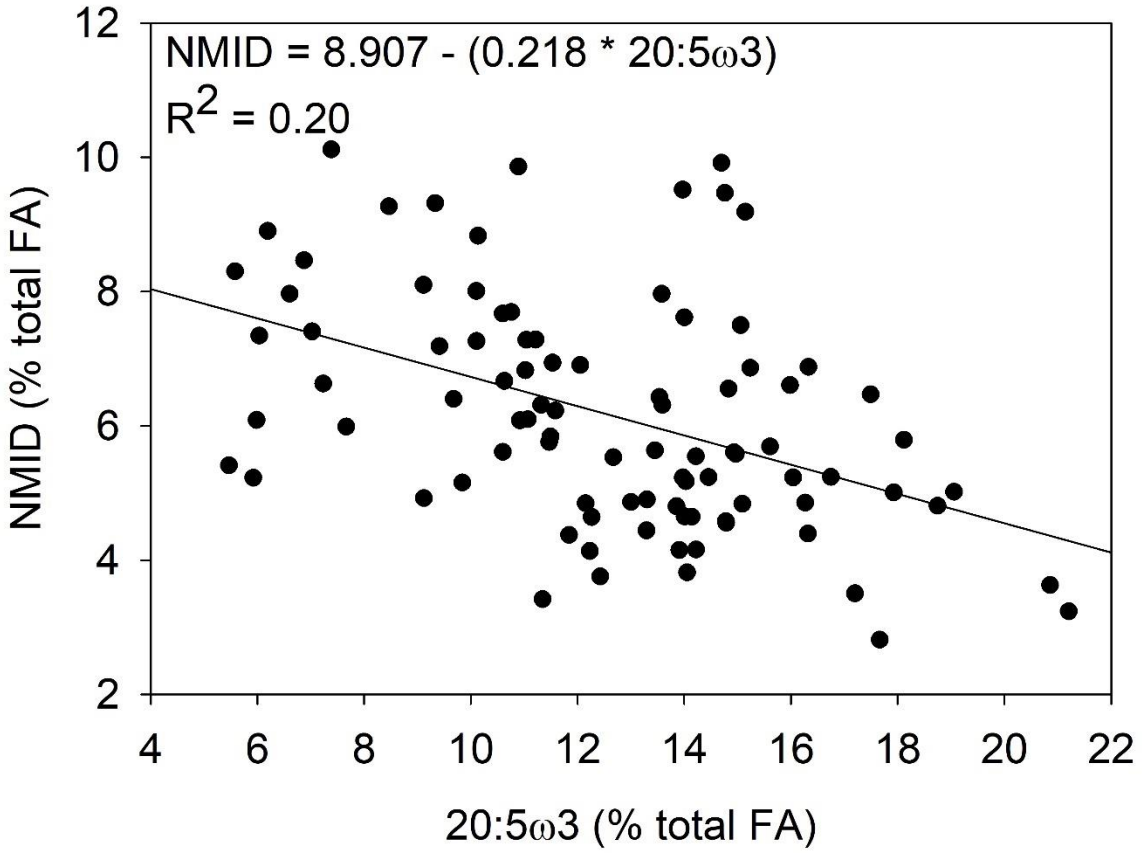

Figure 3.4. Significant ($p < 0.05$) linear regression of the proportion (%) of non-methylene interrupted dienes (NMID) and the essential fatty acid 20:5ω3 in *Mytilus edulis* from Saco Bay throughout 2016 and 2017.

Table 3.2. Proportions (%) of fatty acids in *Mytilus edulis* measured in this study compared to literature values. Values shown are average ± standard error, significant difference ($p < 0.05$) are denoted by ^{abc}.

	This study	Alkanani et al. 2007		Murphy et al. 2002		
Fatty acid		2000	2001	Site 1	Site 2	Site 3
20:2 NMID	3.4 ± 0.1	3.7 ± 0.9	1.0 ± 1.5	0.5 ± 0.2	0.3 ± 0.0	0.3 ± 0.1
20:4ω6	4.7 ± 0.1	2.8 ± 0.8	2.8 ± 1.2	1.5 ± 0.7	1.9 ± 0.2	1.8 ± 0.2
20:5ω3	12.3 ± 0.1	12.0 ± 2.2	17.0 ± 3.5	13.3 ± 3.5	15.1 ± 0.9	15.4 ± 0.8
22:2 NMID	2.8 ± 0.1	3.6 ± 0.9	2.8 ± 1.5	1.4 ± 0.4	1.4 ± 0.2	1.2 ± 0.3
22:6ω3	14.4 ± 0.1	21.5 ± 2.9	20.0 ± 4.3	18.2 ± 5.5	21.3 ± 2.0	24.2 ± 3.9
Σ Saturated	22.2 ± 0.1	25.4 ± 1.8	23.6 ± 2.3	28.9 ± 1.2	29.5 ± 2.7	30.4 ± 0.6
Σ MUFA	21.1 ± 0.1	14.5 ± 3.0	17.7 ± 4.1	11.9 ± 4.4	10.4 ± 1.0	9.6 ± 0.8
Σ PUFA	54.5 ± 0.1	61.9 ± 3.0	60.8 ± 4.0	44.0 ± 7.4	49.2 ± 4.6	50.1 ± 1.2
Σ ω3	37.1 ± 0.1^{a}	47.8 ± 3.7^{b}	49.5 ± 3.5^{b}	38.2 ± 7.2^{ab}	41.7 ± 1.8^{ab}	44.6 ± 1.4^{ab}

The FA composition of *M. edulis* and each primary producer endmember were distinctly different (Table 3.3). The three major FAs (>10% 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(perm) < 0.05) than all endmembers. The most defining FA difference between macroalgal sources and other endmembers was 20:4ω6, which comprised a large proportion of macroalgal FAs (*A. nodosum*; 11.5 ± 0.1%, *S. latissima*; 17.7 ± 0.3%, *C. crispus*; 7.1 ± 0.3%) and only small proportions in vascular plants (*S. alterniflora*; 0.3 ± 0.1%, Oak; <0.1%) and consumers (*M. edulis*; 4.7 ± 0.1%, zooplankton; 0.5 ± 0.1%). Additionally, macroalgae (especially *A. nodosum*) had large proportions of 18:1ω9 (*A. nodosum*; 33.6 ± 0.3%, *S. latissima*; 9.0 ± 0.3%, *C. crispus*; 5.7 ± 0.3%) compared to other endmembers. Both vascular plant endmembers had large proportions of 18:3ω3 (*S. alterniflora*; 37.7 ± 0.4%, Oak; 18.6 ± 2.5%) and 18:2ω6 (*S. alterniflora*; 19.0 ± 0.2%, Oak; 6.0 ± 0.1%). The three major FAs in zooplankton were the same as *M. edulis* (16:0, 20:5ω3, and 22:6ω3). *M. edulis* contained FA biomarkers from all endmembers sampled (Table 3.4). Diatom (16:1ω7, 16:4ω1 and 20:5ω3) and dinoflagellate (22:6ω3) FAs comprised the largest proportions of *M. edulis* FAs, 17.2 ± 0.1% and 16.9 ± 0.1% respectively, followed by macroalgal (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3, and 20:4ω6; 7.9 ± 0.1%) and zooplankton (20:1 and 21:1; 6.2 ± 0.1%) FAs. Vascular plant (18:2ω6 and 18:3ω3) FAs comprised the smallest proportion (3.3 ± 0.1%) of *M. edulis* FAs with bacterial FAs (*iso, anteiso* and odd-chained) comprising another 4.8 ± 0.1%.

Table 3.3. Endmember fatty acids. Proportion (%) and concentration (µg g^{-1} wet weight) of fatty acids for all endmembers: *Saccharina latissima*, *Ascophyllum nodosum*, *Chondrus crispus*, *Spartina alterniflora*, oak leaves, zooplankton and *Mytilus edulis* from Saco Bay throughout 2016 and 2017. Values shown are average ± standard error, sample size denoted by n.

	Saccharina latissima (n = 11)		*Ascophyllum nodosum* (n = 13)		*Chondrus crispus* (n = 5)		*Spartina alterniflora* (n = 23)	
Fatty acid	%	µg g^{-1} WW	%	mg g^{-1} WW	%	µg g^{-1} WW	%	mg g^{-1} WW
14:0	6.6 ± 0.2	72.0 ± 4.4	9.9 ± 0.1	1.4 ± 0.08	4.8 ± 0.1	49.3 ± 3.8	1.0 ± 0.1	0.1 ± 0.1
16:0	12.5 ± 0.2	130.9 ± 8.2	10.7 ± 0.1	1.6 ± 0.1	23.3 ± 0.3	235.0 ± 15.7	17.2 ± 0.1	3.5 ± 0.5
16:1ω7	3.8 ± 0.1	40.5 ± 3.0	1.8 ± 0.1	0.3 ± 0.02	9.0 ± 0.3	91.0 ± 7.2	0.7 ± 0.1	0.1 ± 0.02
16:4ω1	0.2 ± 0.1	1.8 ± 0.1	Trace	Trace	0.8 ± 0.1	8.0 ± 0.8	Trace	Trace
18:0	0.6 ± 0.2	6.9 ± 0.6	0.6 ± 0.1	0.08 ± 0.01	0.9 ± 0.1	9.0 ± 0.5	2.4 ± 0.1	0.3 ± 0.03
18:1ω9	9.0 ± 0.3	109.8 ± 8.2	33.6 ± 0.3	4.7 ± 0.3	5.7 ± 0.3	57.8 ± 5.3	4.8 ± 0.1	0.9 ± 0.1
18:2ω6	7.2 ± 0.2	88.1 ± 6.6	7.5 ± 0.1	1.1 ± 0.07	1.4 ± 0.1	14.5 ± 1.0	19.0 ± 0.2	3.5 ± 0.5
18:3ω3	4.9 ± 0.2	42.3 ± 2.4	3.2 ± 0.1	0.5 ± 0.03	0.9 ± 0.1	8.7 ± 0.9	37.7 ± 0.4	8.0 ± 1.2
18:3ω6	1.7 ± 0.1	22.1 ± 1.9	0.4 ± 0.1	0.06 ± 0.01	0.8 ± 0.1	8.5 ± 0.9	Trace	Trace
18:4ω3	11.0 ± 0.5	98.5 ± 6.1	2.5 ± 0.1	0.4 ± 0.03	1.3 ± 0.1	13.4 ± 1.5	0.1 ± 0.1	Trace
20:1	Trace	0.8 ± 0.05	0.4 ± 0.1	0.05 ± 0.01	0.4 ± 0.1	3.6 ± 0.2	0.4 ± 0.1	0.09 ± 0.01
20:2 NMID	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20:2ω6	0.2 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	0.3 ± 0.02	0.1 ± 0.1	1.4 ± 0.2	0.2 ± 0.1	0.04 ± 0.01
20:4ω3	0.8 ± 0.2	6.8 ± 0.4	0.6 ± 0.1	0.1 ± 0.02	0.7 ± 0.3	7.4 ± 2.7	0.2 ± 0.1	0.01 ± 0.01
20:4ω6	17.7 ± 0.3	195.8 ± 12.0	11.5 ± 0.1	1.7 ± 0.1	7.1 ± 0.3	72.7 ± 6.4	0.3 ± 0.1	0.9 ± 0.2
20:5ω3	12.9 ± 0.2	133.5 ± 8.4	7.7 ± 0.1	1.1 ± 0.08	25.9 ± 0.9	265.6 ± 19.8	0.1 ± 0.1	0.02 ± 0.01
22:1	0.1 ± 0.03	0.3 ± 0.04	0.3 ± 0.1	0.04 ± 0.01	0.3 ± 0.1	3.5 ± 0.3	0.3 ± 0.1	0.12 ± 0.02
22:2 NMID	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22:6ω3	Trace	0.3 ± 0.04	1.0 ± 0.1	0.1 ± 0.01	0.9 ± 0.1	9.3 ± 0.7	0.8 ± 0.1	1.5 ± 0.3
24:0	0.1 ± 0.1	0.6 ± 0.05	0.2 ± 0.1	0.03 ± 0.01	0.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.07 ± 0.01
Bacterial	3.7 ± 0.1	33.3 ± 2.1	1.4 ± 0.1	0.2 ± 0.02	3.6 ± 0.1	36.4 ± 2.6	3.6 ± 0.1	0.6 ± 0.08
Σ Saturated	21.2 ± 0.3	225.6 ± 14.1	22.3 ± 0.1	3.2 ± 0.2	30.7 ± 0.2	310.6 ± 20.9	25.3 ± 0.2	4.4 ± 0.6
Σ MUFA	17.4 ± 0.2	192.0 ± 13.0	38.3 ± 0.2	5.4 ± 0.3	24.0 ± 0.7	240.7 ± 18.3	10.6 ± 0.2	2.0 ± 0.3
Σ PUFA	59.0 ± 0.4	621.0 ± 36.9	38.9 ± 0.2	5.8 ± 0.4	42.7 ± 0.7	435.8 ± 30.3	61.7 ± 0.4	15.9 ± 2.5
Σ ω3	30.0 ± 0.9	286.2 ± 16.4	16.0 ± 0.2	2.4 ± 0.2	30.3 ± 0.9	309.5 ± 21.9	40.0 ± 0.4	11.2 ± 1.8
Total		1060 ± 64		14.5 ± 0.9		1014 ± 69		22.6 ± 3.4

Table 3.3. cont.

	Oak (n = 2)		*Mytilus edulis* (n = 94)		Zooplankton (n = 11)	
Fatty acid	%	mg g^{-1} WW	%	mg g^{-1} WW	%	mg g^{-1} WW
14:0	1.2 ± 0.3	0.3 ± 0.09	3.0 ± 0.1	1.0 ± 0.02	7.2 ± 0.2	1.5 ± 0.2
16:0	12.2 ± 0.6	2.6 ± 0.4	13.7 ± 0.1	4.2 ± 0.09	16.5 ± 0.2	3.2 ± 0.4
16:1ω7	0.1 ± 0.1	0.03 ± 0.01	4.7 ± 0.1	1.8 ± 0.06	8.7 ± 0.3	1.7 ± 0.2
16:4ω1	0.1 ± 0.1	0.03 ± 0.01	0.2 ± 0.1	0.06 ± 0.01	1.0 ± 0.1	0.2 ± 0.03
18:0	1.5 ± 0.1	0.3 ± 0.04	2.7 ± 0.1	0.7 ± 0.01	3.2 ± 0.1	0.7 ± 0.08
18:1ω9	2.2 ± 0.7	0.4 ± 0.1	2.2 ± 0.1	0.7 ± 0.01	2.5 ± 0.1	0.4 ± 0.03
18:2ω6	6.0 ± 0.1	1.3 ± 0.1	2.0 ± 0.1	0.6 ± 0.01	1.4 ± 0.1	0.2 ± 0.02
18:3ω3	18.6 ± 2.5	4.1 ± 0.9	1.3 ± 0.1	0.4 ± 0.01	1.0 ± 0.1	0.2 ± 0.02
18:3ω6	0.1 ± 0.1	0.02 ± 0.01	0.1 ± 0.1	0.04 ± 0.01	0.3 ± 0.1	0.06 ± 0.01
18:4ω3	0.1 ± 0.1	0.03 ± 0.01	2.4 ± 0.1	0.8 ± 0.02	2.7 ± 0.2	0.7 ± 0.1
20:1	0.3 ± 0.1	0.07 ± 0.01	6.0 ± 0.1	1.6 ± 0.02	1.6 ± 0.1	0.3 ± 0.04
20:2 NMID	n.d.	n.d.	3.4 ± 0.1	0.9 ± 0.02	n.d.	n.d.
20:2ω6	3.9 ± 0.5	0.8 ± 0.04	0.6 ± 0.1	0.2 ± 0.01	0.5 ± 0.1	0.1 ± 0.01
20:4ω3	0.9 ± 0.1	0.2 ± 0.05	0.2 ± 0.1	0.07 ± 0.01	0.7 ± 0.1	0.1 ± 0.01
20:4ω6	Trace	0.01 ± 0.01	4.7 ± 0.1	1.2 ± 0.02	0.5 ± 0.1	0.1 ± 0.01
20:5ω3	0.6 ± 0.1	0.1 ± 0.01	12.3 ± 0.1	3.5 ± 0.07	15.3 ± 0.4	4.3 ± 0.7
22:1	0.6 ± 0.1	0.13 ± 0.01	0.1 ± 0.1	0.05 ± 0.01	0.5 ± 0.02	0.1 ± 0.02
22:2 NMID	n.d.	n.d.	2.8 ± 0.1	0.8 ± 0.01	n.d.	n.d.
22:6ω3	0.1 ± 0.1	0.03 ± 0.01	14.4 ± 0.1	4.0 ± 0.06	18.6 ± 0.5	4.9 ± 0.7
24:0	4.1 ± 0.1	0.9 ± 0.08	0.2 ± 0.1	0.02 ± 0.01	0.1 ± 0.1	0.02 ± 0.01
Bacterial	2.7 ± 0.1	0.6 ± 0.04	4.8 ± 0.1	1.3 ± 0.02	4.5 ± 0.1	0.8 ± 0.1
Σ Saturated	26.2 ± 1.1	5.6 ± 0.7	22.2 ± 0.1	6.6 ± 0.1	29.2 ± 0.4	5.8 ± 0.7
Σ MUFA	7.2 ± 0.8	1.5 ± 0.04	21.1 ± 0.1	6.2 ± 0.1	20.1 ± 0.4	3.7 ± 0.5
Σ PUFA	64.5 ± 0.4	13.8 ± 1.1	54.5 ± 0.1	15.1 ± 0.3	48.6 ± 0.8	12.1 ± 1.7
Σ ω3	24.6 ± 2.0	5.3 ± 0.9	37.1 ± 0.1	10.4 ± 0.2	41.0 ± 1.0	10.9 ± 1.5
Total		21.3 ± 1.8		28.6 ± 0.5		22.0 ± 3.0

Table 3.4. Proportion (%) of biomarker fatty acids in *Mytilus edulis*. Proportion (%) of endmember fatty acids in *Mytilus edulis* collected from Saco Bay, ME USA, throughout 2016 and 2017. Endmembers: Zooplankton (20:1 and 21:1), Vascular plants (18:2ω6 and 18:3ω3), Macroalgae (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3, and 20:4ω6), Diatoms (16:1ω7, 16:4ω1 and 20:5ω3), Dinoflagellates (22:6ω3), and Bacteria (iso, anteiso and odd-chained). Data shown are average ± 1 standard error.

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

Principal coordinate analysis was able to separate all endmember groups based on their FA profiles (Figure 3.5). Samples within each endmember group were highly similar (>75%), with exception to POM (61% similarity), likely a result of the varied composition of POM. The FA profiles of zooplankton and M. edulis were similar (65%) to each other, both groups of vascular plants (S. alterniflora and oak) 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.

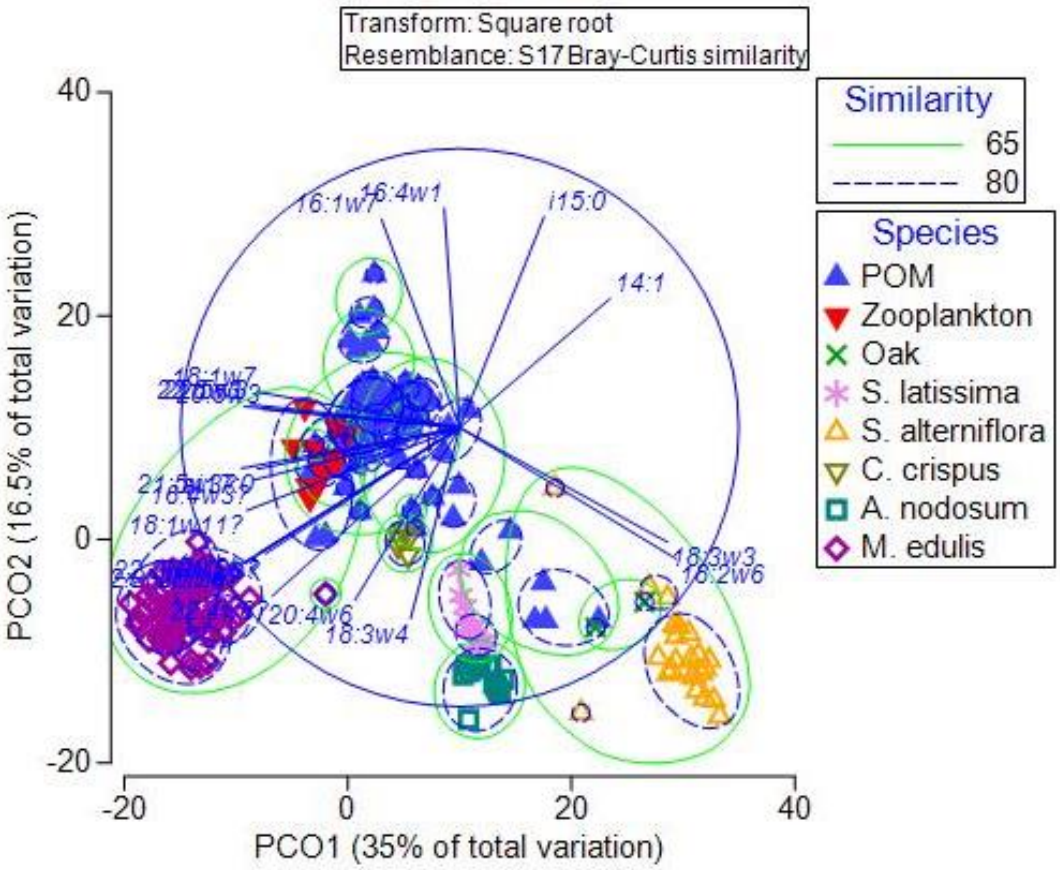

Figure 3.5. Principal coordinates analysis of fatty acids. Includes <100 µm particulate organic matter (POM), zooplankton, oak leaves, *Spartina alterniflora*, *Saccharina latissima*, *Ascophyllum nodosum*, *Chondrus crispus* and *Mytilus edulis* from Saco Bay, Maine USA, throughout 2016 and 2017. Circled groupings are based on similarity (%) determined from similarity percentage analysis (SIMPER) and cluster analysis.

3.3.2 Stable isotopes

The isotopic composition of *M. edulis* closely resembled that of <100 µm POM (Figure 3.6). Unsurprisingly, the $\delta^{13}C$ (-19.4 ± 3.7‰) and $\delta^{15}N$ (7.0 ± 2.8‰) of POM <100 µm varied the most among all endmembers, ranging from -12 to -25‰ $\delta^{13}C$ and 2.9 to 15.2‰ $\delta^{15}N$. In contrast, the $\delta^{13}C$ (-20.0 ± 1.1‰) and $\delta^{15}N$ (7.0 ± 0.9‰) of *M. edulis* occupied a very narrow range between -18 to -21‰ and 6.0 to 7.6‰ respectively. All three macroalgae had similar $\delta^{13}C$ and $\delta^{15}N$ values (combined average -18.8 ± 2.0‰ and 5.3 ± 1.0‰ respectively). Macroalgal isotopic values were very close to the $\delta^{13}C$ and $\delta^{15}N$ values for diatoms (-18.5‰ and 5.3‰ respectively) based on literature sources and estimated $\delta^{15}N$

values from zooplankton, which were the most enriched in $\delta^{15}N$ (Figure 3.6). Both C_3 and C_4 vascular plants were isotopically distinct from marine endmembers. Oak leaf litter (C_3) was the most depleted source of both $\delta^{13}C$ and $\delta^{15}N$ (-30.5 ± 1.2‰ and -2.5 ± 0.6‰ respectively) while *S. alterniflora* (C_4) was the most enriched in $\delta^{13}C$ (-13.3 ± 1.1‰). The $\delta^{13}C$ of all marine endmembers fell between the values of C_3 and C_4 vascular plants and were more enriched in $\delta^{15}N$ (Figure 3.6).

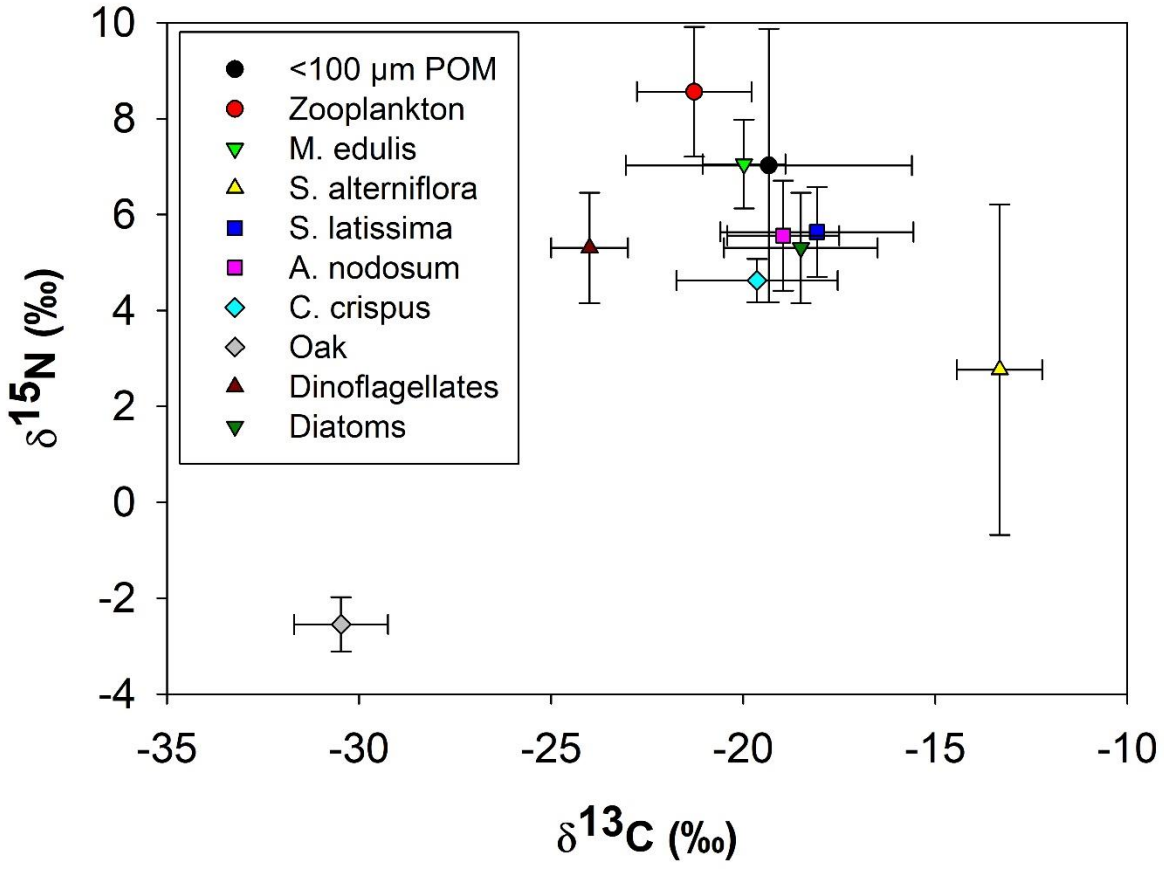

Figure 3.6. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) biplot. Includes *Mytilus edulis* and all endmembers (<100 µm POM, zooplankton, *Spartina alterniflora*, *Saccharina latissima*, *Ascophyllum nodosum*, *Chondrus crispus*, oak leaves, dinoflagellates and diatoms), prior to consolidation, used in the stable isotope mixing model. Data shown are average ± 1 standard deviation of endmember $\delta^{13}C$ and $\delta^{15}N$.

3.3.3 Isotope mixing model

Credibility intervals (95%) from the dual isotope ($\delta^{13}C$ and $\delta^{15}N$) Bayesian mixing models estimating endmember contributions to the diet of *M. edulis* were relatively large, suggesting uncertainty in the model (Figure 3.7). Because diatoms and macroalgae are traditionally difficult to separate based on their $\delta^{13}C$ (-18 ± 2‰ and -18.8 ± 2.0‰ respectively), they were initially combined for the model. Therefore, their combined impact on mussel diets was relatively high (20.1 to 32.3%). In a subsequent model formulation, we used the relative proportions of diatom and macroalgal FAs to estimate their separate impacts on the diet. In the updated model, *S. alterniflora* comprised the largest proportion (18 to 42%) of *M. edulis* diet based on the dual isotope mixing model followed by dinoflagellates (13 to 22%), zooplankton (10 to 22%), diatoms (13 to 21%), oak leaf litter (11 to 18%), and finally macroalgae (5 to 11%).

Importantly, the stable isotope mixing model and the FA composition analysis largely agree regarding the contributions of diatoms, dinoflagellates and macroalgae to mussel diets (Figure 3.7a, b, and c). However, there was more discrepancy between the contributions of zooplankton (Figure 3.7d) and vascular plant detritus (Figure 3.7e and f) between the two methods. Differences between measured FA biomarkers and the stable isotope model are likely the result of two factors: (1) poor assumptions in the stable isotope model or (2) endmembers may contribute differently to the overall C budget of *M. edulis* than to their lipid and FAs. Because credibility intervals of the model were wide, and frequently approaching zero, we used the raw model output to determine the probability of zero contribution (<1%) to the diet of *M. edulis* for each endmember (Table 3.5). In general, the probability of zero contribution for most endmembers was low (<1%), with exception to oak detritus which had significantly higher probabilities ($p < 0.01$) of zero diet contribution.

There were two major model assumptions to evaluate: (1) that there is one trophic level between zooplankton and phytoplankton and (2) that using no lipid correction of *M. edulis* $\delta^{13}C$ values is reasonable. To test these assumptions, we re-ran the Bayesian mixing model using 1.75 trophic levels between zooplankton and phytoplankton and lipid corrections for *M. edulis* $\delta^{13}C$ values. The estimated phytoplankton $\delta^{15}N$ value was 2.7 ± 1.4‰ after assuming a 1.75 trophic level increase of zooplankton consumers. Lipid correction of mussel $\delta^{13}C$ values resulted in an average increase in $\delta^{13}C$ values of 0.17‰ (range 0.05‰ to 0.35‰). Endmember contributions to the diet of *M. edulis* based on the revised model were similar to the original model (Table 3.6). Differences between the original and revised estimated diet contributions were ≤9% for all endmembers and usually ≤5%, suggesting that these two assumptions only minimally impacted our results.

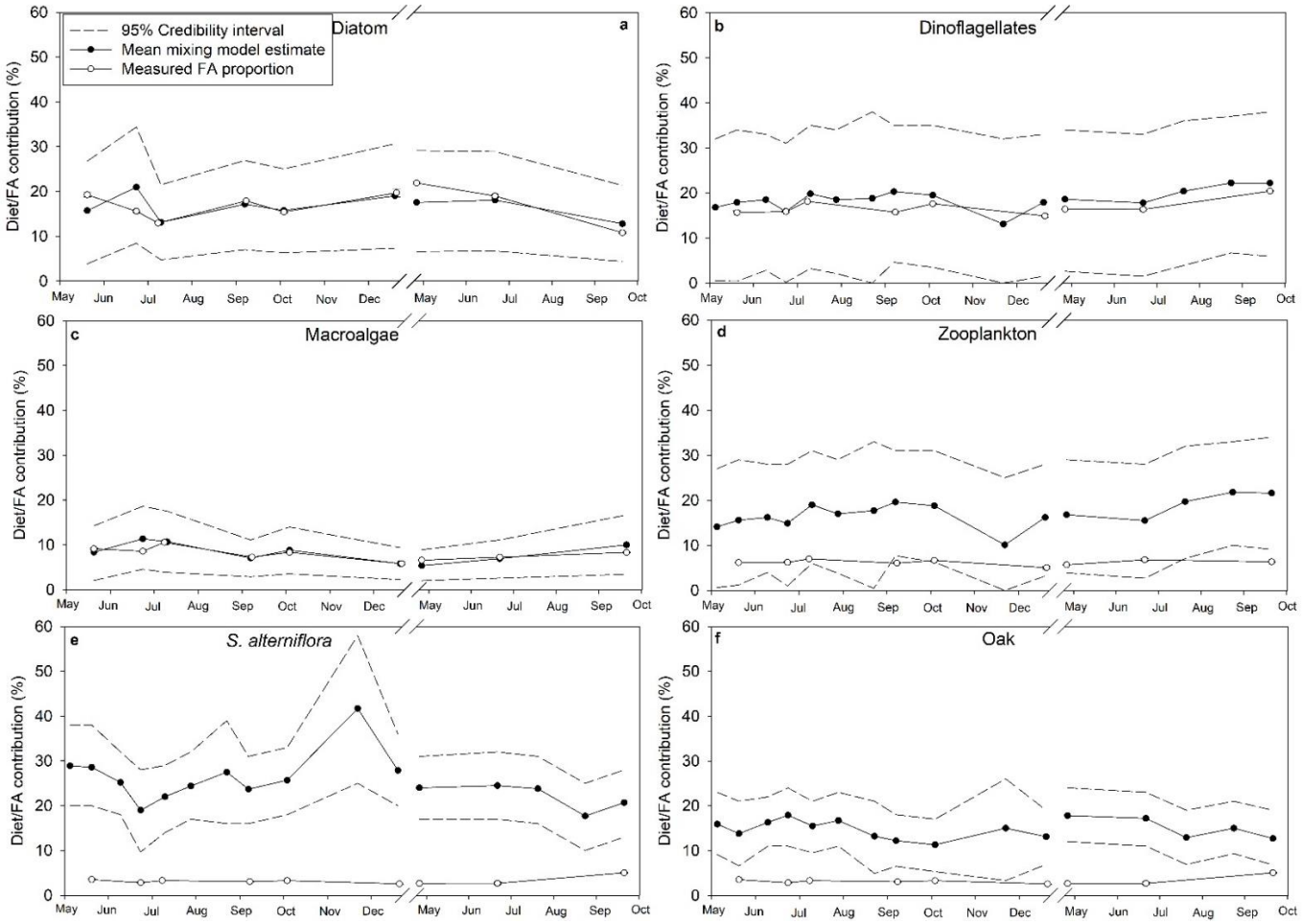

Figure 3.7. Comparison of model estimates and fatty acid biomarkers. Endmember diet contribution (%) to *Mytilus edulis* in Saco Bay, ME USA, throughout 2016 and 2017. Values shown are the mean diet contribution (%) estimated from the dual isotope ($\delta^{13}C$ and $\delta^{15}N$) Bayesian mixing model along with accompanying credibility interval (95%) and the proportion (% total FAs) of endmember biomarker FAs measured in *M. edulis*. Endmembers: (a) Diatoms (16:1ω7, 16:4ω1 and 20:5ω3), (b) Dinoflagellates (22:6ω3), (c) Macroalgae (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3, and 20:4ω6), (d) Zooplankton (20:1 and 21:1), (e) *Spartina alterniflora* (18:2ω6 and 18:3ω3), and (f) oak leaf litter (18:2ω6 and 18:3ω3). Values shown are average ± 1 standard error.

Table 3.5. The probability of zero (<1%) diet contribution. The proportion (%) of Bayesian mixing model iterations (30×10^3) that estimated contributions <1% for each endmember (Diatom, dinoflagellate, zooplankton, macroalgae, Spartina alterniflora, and oak) to the diet of Mytilus edulis in Saco Bay, Maine USA, throughout 2016 and 2017. Diatoms and macroalgae were originally combined in model estimates, when available fatty acid proportions were used to separate diatom and macroalgae contributions.

Date	Diatom/Macro # Obs. <1% (%)	Diatom # Obs. <1% (%)	Macroalgae # Obs. <1% (%)	Dinoflagellates # Obs. <1% (%)	Zooplankton # Obs. <1% (%)	*S. alterniflora* # Obs. <1% (%)	Oak # 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

Table 3.6. Comparison between the original and revised model estimates. Comparison of modeled 95% mean credibility estimate of endmember contribution (%) to the diet of *Mytilus edulis* from our original Bayesian mixing model (assuming 1 trophic level between zooplankton and phytoplankton and no lipid correction for $\delta^{13}C$ values) and an updated model (1.75 trophic level difference and $\delta^{13}C$ values corrected for lipid). Values are averaged across all samplings in 2016 and 2017 and values shown are average ± 1 standard deviation.

	Original model estimate		**Corrected model estimate**	
Contribution to *M. edulis*	**Average (%)**	**Range**	**Average (%)**	**Range**
Dinoflagellate	18.6 ± 0.1	13.1 to 22.	20.8 ± 1.2	17.5 to 22.5
Diatom	16.7 ± 2.6	12.8 to 21.0	17.7 ± 2.9	13.8 to 22.8
Macroalgae	8.3 ± 2.1	5.3 to 11.3	8.8 ± 2.3	5.7 to 12.3
Terrestrial	40.1 ± 5.6	32.7 to 56.7	31.5 ± 5.8	24.1 to 49.0
Zooplankton	17.2 ± 3.0	10.1 to 21.8	22.1 ± 4.2	11.1 to 28.3

3.4.0 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). Mussels incorporated phytoplankton, zooplankton, macroalgal detritus, and vascular plant detritus into their diets in varying degrees. 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 3.5). Although it is likely that *M. edulis* did make some use of oak detritus, the significantly ($p < 0.01$) higher likelihood (<5%) and frequency that oak detritus had zero-contribution to mussel diets suggests oak detritus use may be less important and a more sporadically used food source. 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 3.8). 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 20:5ω3, 22:6ω3, and to a lesser extent 20:4ω6 (depending

on resuspension of benthic 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 3.7c). 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 20:4ω6 and 20:5ω3. 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.

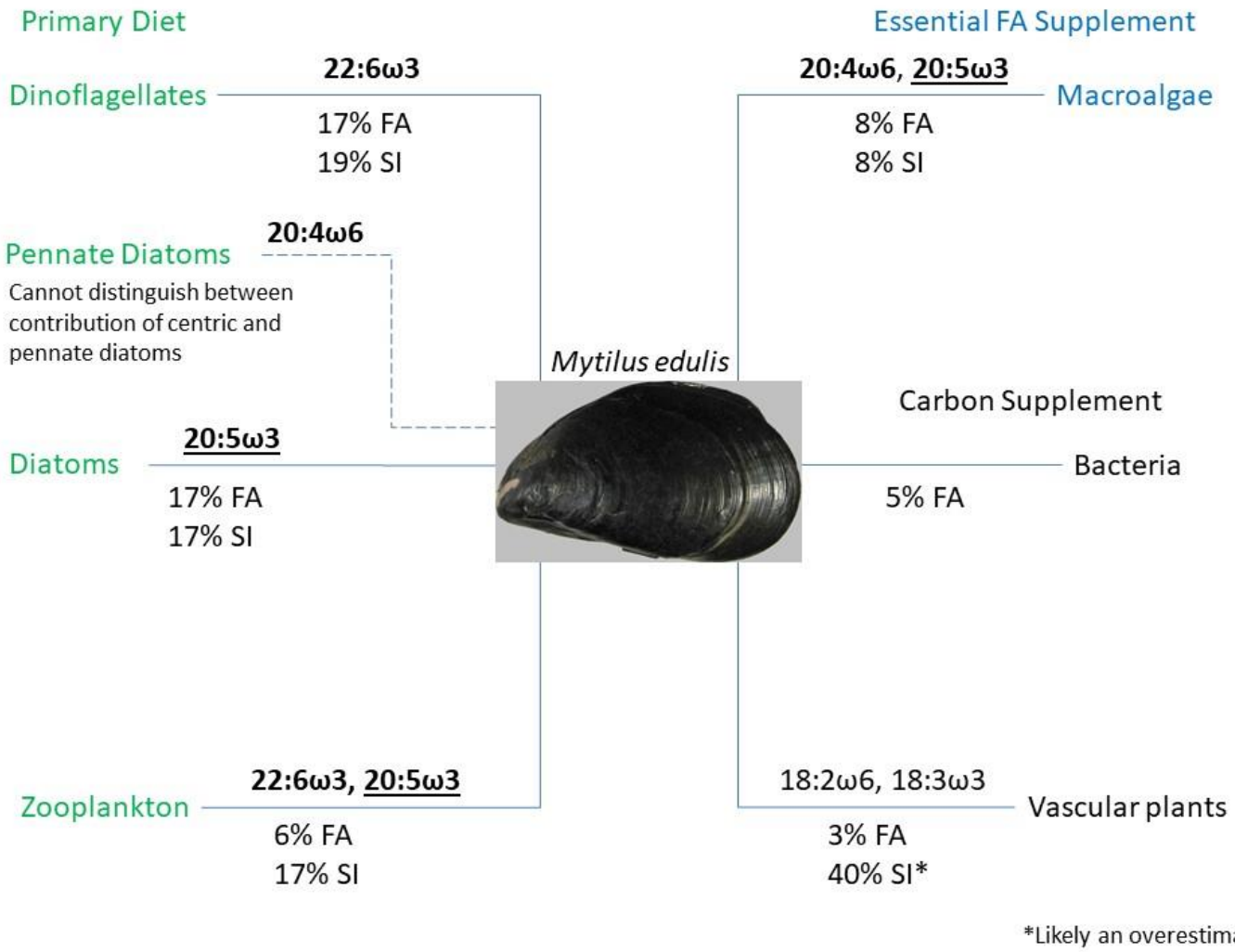

Figure 3.8. Flowchart depicting the contributions of dinoflagellates, diatoms, zooplankton, macroalgal detritus, bacteria, and vascular plant detritus to the diet of *Mytilus edulis*. Contributions are shown based on measured fatty acid (FA) biomarkers and model estimates from stable isotope (SI) composition. Important FA contributions are also shown, bold indicates essential FA, underline denotes limiting FA, and * denotes likely model overestimations.

We assume that vascular plant (salt marsh and oak leaf litter) and macroalgal material present in POM are detrital in origin. Although *M. edulis* FAs primarily resembled dinoflagellate (22:6ω3) and diatom (20:5ω3 and 16:1ω7) based on their biomarker content in both PCoA and SIMPER analyses, mussels did contain proportions of zooplankton (20:1 and 22:1), vascular plant detritus (18:2ω6 and 18:3ω3), and macroalgal detritus (18:1ω9, 18:3ω6, 20:2ω6, 20:4ω3, and 20:4ω6) markers (Figure 3.5). 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 ± 0.1%) which could only be obtained by direct consumption of either live zooplankton or zooplankton derived detritus. In addition, *M. edulis* contained small proportions of macroalgal (7.9 ± 0.1%), bacterial (4.8 ± 0.1%), and vascular plant FAs (3.3 ± 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 (Newell and Langdon, 1986; Baldwin and Newell, 1991; Bustamante and Branch, 1996; Davenport et al., 2000). Our results further support that *M. edulis* are not 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).

Although there is little doubt that *M. edulis* consumes phytodetritus, our methods used in this study cannot differentiate between contributions of live *vs* dead phytoplankton to their diets. By assuming all phytoplankton ingested are fresh our estimates of detrital usage are inherently conservative. Diatom FAs comprised 17.2 ± 0.05% of *M. edulis* FAs, which agrees with the range of modeled contributions estimated with stable isotopes (Figure 3.7a). Proportions of dinoflagellate FAs in *M. edulis* averaged 16.9 ± 0.03%, which also fell within the range of modeled dietary contributions

(Figure 3.7b). 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 lipids as well, to the diet of *M. edulis* than detrital sources. This contribution of phytoplankton FAs is slightly lower than that previously reported (39 to 46%; Alkanani et al., 2007)

While vascular plant FAs contributed only a small proportion to *M. edulis* FAs (3.3 ± 0.01%), vascular plant detritus, particularly *S. alterniflora* detritus, comprised a significant portion of mussel diets (minimum 19.3 to 29.1%) based on modeled estimates from stable isotopes (Figure 3.7e and f). There are several possible explanations for this discrepancy: (1) Degraded vascular plant detritus is relatively deplete in lipids compared to their parent material and contribute more to *M. edulis* C requirements 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. 22:6ω3 and 20:5ω3) 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 $\delta^{13}C$ 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 $\delta^{13}C$ (-13‰) and their contributions to 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 (see Chapter 2).

The mixing model used in this study makes several assumptions, which could have influenced the results. We used whole mussel tissues when measuring $\delta^{13}C$ and did not correct for the effect of $\delta^{13}C$ depletion in lipids. Additionally, we estimated the $\delta^{15}N$ of phytoplankton by subtracting one trophic level (3.4‰) from the values measured from mixed assemblages of zooplankton. This assumes all zooplankton graze directly on phytoplankton, which may not be a valid assumption. However, zooplankton within the Gulf of Maine have a large trophic overlap between size classes and only small differences in trophic level were measured (0.5 to 0.75 trophic levels) between zooplankton from 64 to 8000 µm in size (Fry and Quiñones, 1994). Lacking direct measurements of zooplankton trophic levels, it is impossible to tell the trophic distance between phytoplankton and zooplankton. However, the two models tested represent two extremes in model parameters: (1) no lipid $\delta^{13}C$ correction and all zooplankton are grazers (1 trophic level, 3.4‰ $\delta^{15}N$, between zooplankton and phytoplankton), (2) lipid $\delta^{13}C$ correction and all zooplankton incorporate carnivory (1.75 trophic levels, 5.95‰ $\delta^{15}N$, between zooplankton and phytoplankton). By comparing results of the two model extremes we can determine how much our model was affected by our assumptions. With only minimal differences (≤9% to estimated contributions; Table 3.6) between the two models, we conclude that our assumptions did not significantly impact the conclusions of this study.

While it is surprising that isotope modeled contributions of *S. alterniflora* detritus to *M. edulis* diets were so high, it is not impossible. Although *Crassostrea virginica* and *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 to 25.0%) fall within the upper range of Langdon and Newell's (1990) values suggesting they are feasible. However, the mean (17.7 to 41.7%) and maximum (25.0 to 58.0%) estimates seem less likely. Combined bacterial and vascular plant FAs

represented 8.1% of mussel FAs, much lower than the 19 to 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 over-estimated *S. alterniflora* contributions to mussel diets. Due to the vicinity 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). Our minimum contribution model estimates of *S. alterniflora* (9.7 to 25.0%) are comparable to isotope modeled *S. alterniflora* contributions to mussel diets (10 t0 12%) near other large marshes (Riera, 2007). However, the mean (17.7 to 41.7%) and maximum (25.0 to 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 3.7c). 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). Detrital contributions from macroalgae were on the lower end of previously reported values for other bivalves. Macroalgal detritus can contribute small (10 to 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 to 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 to 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. edilus*.

Although macroalgal detritus did not comprise a large proportion of *M. edulis* diets, its role may be significant. Macroalgae have large proportions (7 to 18%) of the essential FA arachidonic acid (20:4ω6; Table 3.3), 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 ω6 FAs, is significantly correlated with mussel growth (Alkanani et al., 2007) and was selectively retained by mussels in the current study (Figure 3.4). Proportions of saturated FAs, PUFA, MUFA, ω3, essential FAs and NMIDs measured in this study for *M. edulis* were comparable to previously reported values (Table 3.2; Alkanani et al. 2007; Murphy et al. 2002).

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 (20:4ω6 and 22:6ω3), suggests that mussels were physiologically stressed and preferentially retaining essential FAs (Figure 3.2 and 3.3). Between May and July mussel lipid content decreased by 70%, a decrease that was generally mirrored across all FAs (SAT: 72%, MUFA: 78%, PUFA: 67%, ω3: 69%). Mussel 20:5ω3 content decreased similarly (77%); however, the decreases in 20:4ω6 (51%) and 22:6ω3 (58%) 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 essential ω3 FAs lacking in their diet (Zhukova and Svetashev, 1986; Zhukova et al., 1992; Alkanani et al., 2007).

Selective retention of FAs could affect interpretation of diet contributions based on biomarker FAs. Both selectively retained FAs (20:4ω6 and 22:6ω3) 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 will 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 of mussels selectively retaining 20:4ω6 and 22:6ω3.

Possibly limited by 20:5ω3, mussels may have used macroalgal detritus to supplement their dietary requirements. Macroalgae contained the essential FA 20:5ω3 in large proportions (8 to 26%; Table 3.3). Eicosapentaenoic acid (EPA; 20:5ω3) is important for maintaining membrane fluidity and there is a strong relationship between 20:5ω3 and temperature in the gills of scallops (Hall et al., 2002). In the current study, only the proportion of the essential FA 20:5ω3 decreased in conjunction with increasing NMIDs (Figure 3.4), suggesting that NMIDs were synthesized to replace 20:5ω3. Based on this negative correlation, 20:5ω3 may have been a limiting FA for mussels. The phytoplankton community of Saco Bay is dominated by dinoflagellates (see Chapter 2), so it is possible that limitations in diatom availability caused the limitation of 20:5ω3 for mussels. As such, it is possible that mussels make use of macroalgal detritus to supplement their diet with 20:4ω6 and 20:5ω3; 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). Macroalgae has 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 to 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 20:4ω6 only occurs in small proportions in most diatoms, some pennate diatoms can have larger proportions (2 to 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 to 4.4%) to the diets of both wild and cultivated bivalves (Riera, 2007). Another bivalve, *Macoma balthica*, has been shown to make use of benthic diatoms in their diet in another Maine estuary connected to the Damariscotta river (Incze et al., 1982). It was hypothesized based on $\delta^{13}C$ values that *M. balthica* was 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 $\delta^{13}C$ values became enriched (see Chapter 2). It therefore seems likely that pennate diatoms contributed 20:4ω6 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 20:4ω6 (see Chapter 2). Considering resuspension of benthic pennate diatoms, macroalgal detritus may not be needed by bivalves as an arachidonic acid supplement; however, through multiple lines of evidence, our study makes it clear that mussels have a diverse diet with multiple pathways to obtain essential FAs.

Results of this study clearly show *M. edulis* in Saco Bay are omnivores and incorporate detrital material into their diet. 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 could help validate macroalgal detritus as an avenue for mussels to obtain essential FAs. Compound specific stable isotope analysis of detrital FA biomarkers could 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.

3.5.0 Conclusions

Mytilus edulis obtained 22.2% of their assimilated lipid from omnivorous feeding, 16% of which 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 to 11%) of *M. edulis* diet, its contribution could be significant. Macroalgal detritus had large amounts of the essential FAs 20:4ω6 and 20:5ω3 and could be an avenue for mussels to supplement their essential FA requirements. Although phytoplankton is a more important food source for bivalves, 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 (e.g. macroalgal detritus) may be a useful resource to consider when siting bivalve aquaculture.

CHAPTER 4

BIOMIMETIC DETERMINATION OF THE BIOAVAILABILITY OF LIPIDS FROM THREE PRIMARY PRODUCERS TO BIVALVE CONSUMERS USING SODIUM TAUROCHOLATE

4.1.0 Introduction

Due to their importance as fishery and aquaculture products, there has been great interest in understanding the growth and dietary requirements of filter-feeding bivalves (Kreeger et al., 1995; Pettersen et al., 2010; Hawkins et al., 2013a). While phytoplankton is viewed as the primary food-source for bivalves, other food-sources are known to contribute to bivalve diets to varying degrees, e.g. macroalgae (Bustamante and Branch, 1996; Duggins and Eckman, 1997) and marsh grass (Lucas and Newell, 1984; Langdon and Newell, 1990). There has been interest in alternative food-sources, other than phytoplankton, by bivalve hatcheries looking to reduce costs associated with culturing live phytoplankton (Uchida and Murata, 2002; Camacho et al., 2004, 2007). Many of the alternative food-sources for bivalves are detrital in nature and their importance, especially in an ecological context, is not yet fully understood.

Bioavailability is an important factor to consider when evaluating the importance of a food source, especially detrital sources. The bioavailability of a given food item is dependent not only on the biochemical composition of the food source but also the ability of the consumer to digest it (Mayer et al., 1995, 1996). Assimilation efficiencies are an effective way to assess the bioavailability of organic matter available to bivalve consumers and multiple studies have measured assimilation efficiencies for bivalves ingesting a variety of material ranging from phytoplankton (Kiørboe et al., 1980) to macroalgal detritus (Duggins and Eckman, 1997) to marsh detritus (Arambalza et al., 2014). Recently, assimilation efficiencies of bivalves have also been measured for specific biochemical components (proteins, carbohydrates, and lipids) of ingested food. Fernández-Reiriz et al. (2017) measured the absorption efficiency of proteins, carbohydrates, and lipids by *Mytilus galloprovincialis* ingesting natural seston.

Measured absorption efficiencies varied with season, which they attributed to changes in the lability of the seston. While these studies are important to understand the energy budgets of bivalve consumers, they may overlook the importance of dietary supplements that are not major dietary components but instead provide essential micronutrients such as essential fatty acids.

Biomimicry and biomimetic assays, which simulate the digestive and enzymatic capabilities of organisms, are another approach used to assess the bioavailability of organic particulates (Choo et al., 1981; Laursen et al., 1996; Bünemann, 2008). Biomimetic assays offer a controlled and more easily manipulated method to estimate the bioavailability of refractory detrital material. Biomimetic assays have been used to measure the amount of enzyme hydrolysable amino acids (EHAA) in sediments (Mayer et al., 1995) as well as within particulate organic matter (Adams et al., 2019). Adams et al. (2019) correlated bioavailable EHAA with estuarine oyster growth and found the amount of bioavailable EHAA could not be explained by phytoplankton alone. In addition, Adams et al. (2019) found oysters to readily take up phytodetrital proteins and suggested that bioavailable detrital proteins may supplement oyster diets. The study by Adams et al. (2019) serves as a good example of the usefulness of using biomimetic assays to understand the contributions of detrital material to the diets of bivalves in lab and field settings.

While proteins are an important dietary component for bivalves and amino-acid derived N may at times be limiting (Kreeger et al., 1995; Knauer and Southgate, 1999), lipids and their fatty acid (FA) constituents, especially polyunsaturated fatty acids (PUFA), are also important for bivalve nutrition and physiology (Leonardos and Lucas, 2000). Several PUFA (22:6ω3, 20:5ω3, and 20:4ω6) are of particular importance due to their roles as essential FAs not only for bivalves but also aquatic food webs (Parrish, 2009). Lipids serve as important substrates for both catabolic as well as anabolic processes. Lipids serve as the densest form of energy in food webs, providing two thirds more energy per gram than protein or carbohydrates (Parrish, 2013). Additionally, lipids and their fatty acid (FA) constituents are essential

components of membranes and are important for mediating membrane fluidity, also known as homeoviscous adaptation (Arts and Kohler, 2009; Parrish, 2013). Essential FAs are correlated with growth in bivalves (Alkanani et al., 2007) and are especially important for bivalve membrane fluidity as well as for adaptation to environmental temperature changes (Hall et al., 2002; Parent et al., 2008). Some essential FAs are most frequently associated with fresh phytoplankton, a fact reflected by the use of 20:5ω3 and 22:6ω3 as biomarkers for both diatoms and dinoflagellates respectively (Kelly and Scheibling, 2012; Parrish, 2013). However, other sources of essential FAs do exist, such as macroalgae, which contain both 20:5ω3 and 20:4ω6 (Kelly and Scheibling, 2012). Macroalgae are known to produce large amounts of detrital material (Krumhansl and Scheibling, 2012), which can be of dietary importance for bivalves and coastal food webs (Bustamante and Branch, 1996; Kaehler et al., 2006). The ingestion of macroalgal detritus by bivalves raises the question, how important are detrital lipids, especially FAs?

Unlike proteins, biomimetic assays have not been used to assess the dietary bioavailability of nutritional lipids, including FAs. To date most bioavailability assays for lipids have focused on organic contaminants (Mayer et al., 1996; Simpson et al., 2006). Due to their hydrophobic nature, the ability to dissolve or solubilize lipids is an important limiting step in their digestion by consumers (Shiau, 1987). Surfactants, which encapsulate hydrophobic materials in micelles or promote emulsification, are an effective way to solubilize lipids (Carey et al., 1983; Lichtenberg, 1985). Emulsification and micelle formation are important steps in the digestion of lipids as they serve as the site of hydrolysis by promoting the binding of co-lipase, phospholipase, and eventually lipases (Carey et al., 1983). As a result, surfactants are frequently found within invertebrate gut fluids (Tugwell and Branch, 1992; Mayer et al., 1997; Smoot et al., 2003). Sodium taurocholate (STC), a commercially available surfactant, was found to adequately mimic the gut fluids of an invertebrate deposit feeder (*Arenicola marina*) for some purposes, e.g. it has been used to assess the amount of bioavailable lipid contaminants within sediments (Voparil and Mayer, 2004). Because of its prior use as a gut fluid proxy, STC is a reasonable candidate to

explore the bioavailability of nutritional lipids. Solubilization by gut-fluids is known to be an excellent predictor of absorption efficiency of lipid contaminants for invertebrates (Ahrens et al., 2001).

Surfactants do not act alone within animal guts; lytic enzymes also lyse large molecules into their smaller constituents. Lipases and phospholipases hydrolyze lipids into smaller constituents, which aids in lipid absorption (Carey et al., 1983). While bivalves are known to possess both lipases (George, 1952) and phospholipases (Hoehne-Reitan et al., 2007), in this study we focus only on the ability of STC to solubilize lipids and FAs. We omitted lipases and phospholipases from our approach due to possible interference caused by lipases when separating lipids by thin-layer chromatography. Due to the omission of lytic enzymes, our study focuses on only a single, but important, mechanistic step in determining the bioavailability of lipids.

The purpose of this study is to determine the relative bioavailability of lipid classes and FAs from several ecologically important primary producers (phytoplankton, marsh grass, and macroalgae). We used STC as a proxy for bivalve gut fluids and tested its ability to solubilize lipids from homogenates of both fresh primary producers and degraded products from them. We compared the lipid class and FAs from fresh primary producers, as well as their degradation products, solubilized by STC with those recovered using conventional non-polar solvent lipid extractions to estimate the bioavailability of lipids and FAs from each source. The relative bioavailability based on results from our biomimetic approach were used to draw inferences into the importance and potential use of detrital material by estuarine bivalves.

4.2.0 Methods

To test the ability of STC to solubilize lipids from three primary producers (*Ascophyllum nodosum, Spartina alterniflora*, and *Isochyrsis galbana*) we produced a slurry of each producer in artificial seawater. Immediately after each slurry was made, sub-samples were taken to represent the fresh primary producers. After the initial sampling, each slurry was allowed to degrade naturally over a

period of two-months and sub-sampled regularly to represent detrital products for each producer. We compared the amount of extractable lipid, extracted using conventional non-polar solvent extractions, to the amount of lipid solubilized by STC. By comparing the amount of extracted lipid to solubilized lipid we calculated a recovery efficiency (RE) of lipids and FA which served as a measure of bioavailability (Figure 4.1).

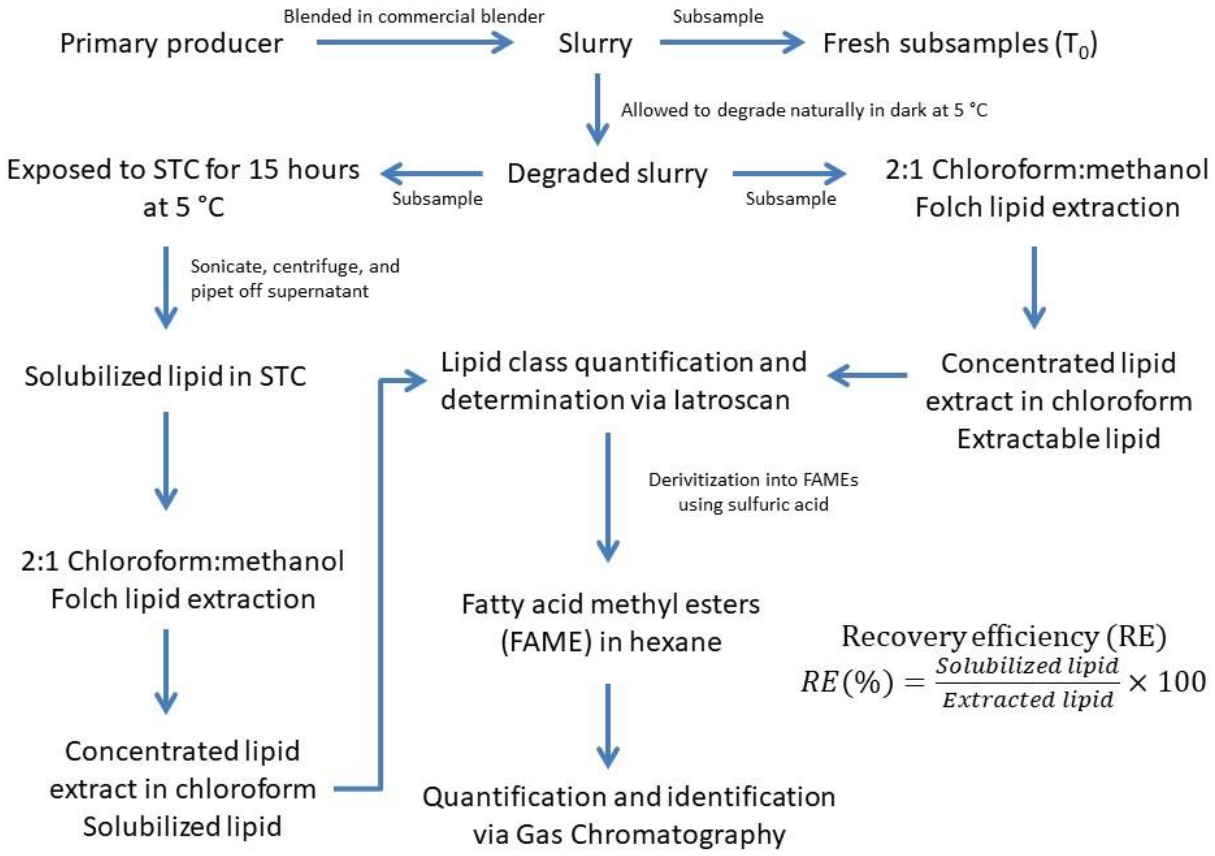

Figure 4.1. Flowchart of the experimental design used to obtain solubilized lipids with sodium taurocholate (STC) and extractable lipids from primary producer slurries. Primary producers used to create slurries were *Ascophyllum nodosum, Spartina alterniflora*, and *Isochrysis galbana*.

4.2.1 Sample preparation and decay

Brown macroalgae (*A. nodosum*) and marsh grass (*S. alterniflora*) were collected by hand from Saco Bay, Me USA. Whole fronds of *A. nodosum* and *S. alterniflora* were cut into smaller fragments, added to 0.45 µm filtered seawater from Saco Bay, and then blended into a slurry using a commercial

blender. Filtered seawater was used as a medium in order to maintain the natural bacterial population of the estuary. The produced slurries, along with a culture of *Isochrysis galbana* (henceforth considered another slurry), were then placed in an open Erlenmeyer flask the dark at 5 °C and allowed to degrade naturally for a period of two months.

Sub-samples from each slurry were collected initially (t_0) and then weekly throughout the first month; afterwards slurries were left for another month before a final sub-sample was taken. Unfortunately, there was only enough material in the *I. galbana* slurry to sample for two weeks. Sub-samples from each slurry were taken for extractable lipid and solubilizable lipid. Sub-samples were taken by passing a known volume of slurry through a 0.45 µm Whatman GF/C filter. Filtered samples for solubilized lipid were placed in chloroform and methanol rinsed test tubes (three rinses each), capped with nitrogen, and stored at -20 °C, while extractable lipid samples were treated similarly except that 1 ml of chloroform was added to each sample prior to storing at -20 °C.

4.2.2 Lipid solubilization using sodium taurocholate

Sodium taurocholate was dissolved in artificial seawater at a concentration of 28.7 mM, well above the critical micelle concentration (CMC) of 2.3 mM (Voparil and Mayer, 2004). Prior to testing the ability of STC to solubilize lipids from primary producers and decay products, we tested the ability of STC to solubilize analytical standards of solid phospholipids (phosphatidylcholine) and triacylglycerols (liquid triolein and solid tripalmitin). Aliquots of STC containing an excess of micelles (molar ratio of STC above CMC:lipids >10) were added to test-tubes containing filtered samples or analytical standards. Filtered samples in STC solutions were then ground using a glass rod until roughly homogeneous, vortexed, and then sonicated for 4.5 min. Once sonicated, samples were capped under nitrogen and placed in the dark at 5 °C for 15 hours. Samples were kept at a constant temperature during the experiment to prevent temperature fluctuations from influencing the extent of solubilization, while a digestion time of 15 h was chosen to represent the maximum gut passage time of *Mytilus edulis* (Hawkins et al., 1990). Afterwards,

samples were re-vortexed, sonicated again for 4.5 min, and then centrifuged at 3000 rpm for 3 min. The resulting supernatant of STC, containing lipids solubilized from the substrate, was pipetted into a lipid-clean test tube, leaving behind any solids and un-solubilized lipids.

Non-polar solvent (chloroform) lipid extractions were performed on the recovered STC to determine the amount of lipid that had been solubilized. The amount of solubilized lipid recovered from STC was compared to extractable lipid of paired samples to determine a lipid RE, calculated as $RE\ (\%) = \frac{Solubilized\ lipid}{Extractable\ lipid} \times 100$. Subsamples of lipid extracts from both extractable lipid and solubilized lipid samples were derivatized into fatty acid methyl esters (FAME) and quantified via gas chromatography (GC). Like lipids, a RE of FAs was determined by comparing the extractable fatty acids to the amount of solubilizable fatty acids, $RE\ (\%) = \frac{Solubilized\ FA}{Extracted\ FA} \times 100$.

4.2.3 Lipid class and fatty acid determination

Lipids were extracted from samples using a modified chloroform:methanol (2:1) Folch extraction as described by Parrish (1999). Filtered samples were homogenized in chloroform:methanol (2:1), vortexed, sonicated for 4.5 mins, and then centrifuged at 3000 rpm for 3 mins. Once centrifuged, the heavier chloroform layer along with dissolved lipids was removed via double pipetting, more chloroform was added to the sample and the entire process was repeated three additional times. The recovered lipid from each subsequent wash was pooled and reduced, via evaporation under nitrogen to prevent lipid oxidization, into a concentrated lipid extract.

Thin-layer chromatography and an Iatroscan Mark V were used for class separation and quantification of lipids as described by Parrish (2013). Additionally, small aliquots of lipid extracts were derivatized into FAMEs by heating (100°C) for one hr with sulfuric acid. Once derivatized, FAMEs were measured using an 7890A Agilent Series GC equipped with a ZB Wax+ column (Phenomenex: 30 m and 0.25 µm internal diameter) along with flame ionization detection (FID) and identified by comparing with a Supelco 37 component FAME analytical standard (Product number 47885-U). Column temperature

began at 65 °C for 0.5 min before ramping to 195 °C at a rate of 40 °C min^{-1}, temperature was then held for 15 min before ramping again to 220 °C at a rate of 2 °C min^{-1} and held for 3.25 min. The detector temperature was a constant 260 °C, while injector temperature started at 150 °C and ramped to a final temperature of 250 °C at a rate of 200 °C min^{-1}. Helium was used as the carrier gas, with a flow rate of 2 ml min^{-1}, and nonadecane was used as an internal standard for FAME quantification.

4.3.0 Results

4.3.1 Lipid solubilization using sodium taurocholate

Using STC we were successfully able to solubilize both phospholipids (PL) and triacylglycerol (TAG) standards. Sodium taurocholate was able to solubilize triolein with a recovery efficiency of 53 ± 19% (n = 6). Although STC was able to solubilize phosphatidylcholine, we were unable to accurately quantify the recovery efficiency of PLs due to contamination interference. We were able to confirm the solubilization of PLs by STC in early standard runs, prior to contamination building up sufficiently on Chromarods to make quantification difficult. The presence of contamination, suspected to be incombustible phytates (Ackman and Woyewoda, 1979), resulted in ghost peaks on chromatograms produced using the Iatroscan Mark V; these ghost peaks overlapped with the PL peak making quantification difficult and lowered our confidence in the reliability of measured PL recovery efficiencies. As a result, although we were able to solubilize PL standards using STC only REs of TAG will be reported. In contrast, our measured REs for acyl FAs will include FAs derived from PL and thus provide some insight into their bioavailability.

Sodium taurocholate was able to solubilize a fraction of the lipids from all three primary producers tested, although the REs varied with producer (Figure 4.2). Averaged throughout the experiment, RE of TAG was significantly (2-way ANOVA $p < 0.01$) higher for *S. alterniflora* (56 ± 19%) and *I. galbana* (48 ± 2%) than they were for *A. nodosum* (9 ± 3%). Although time was a significant factor ($p < 0.01$) with respect to RE, a post-hoc Tukey's test revealed only a single timepoint (day 20) to be

significantly different from the others, likely a result of the high RE for *S. alterniflora*. These results suggest that the initial source of TAG was more important in determining its bioavailability than any effects from the degradation process.

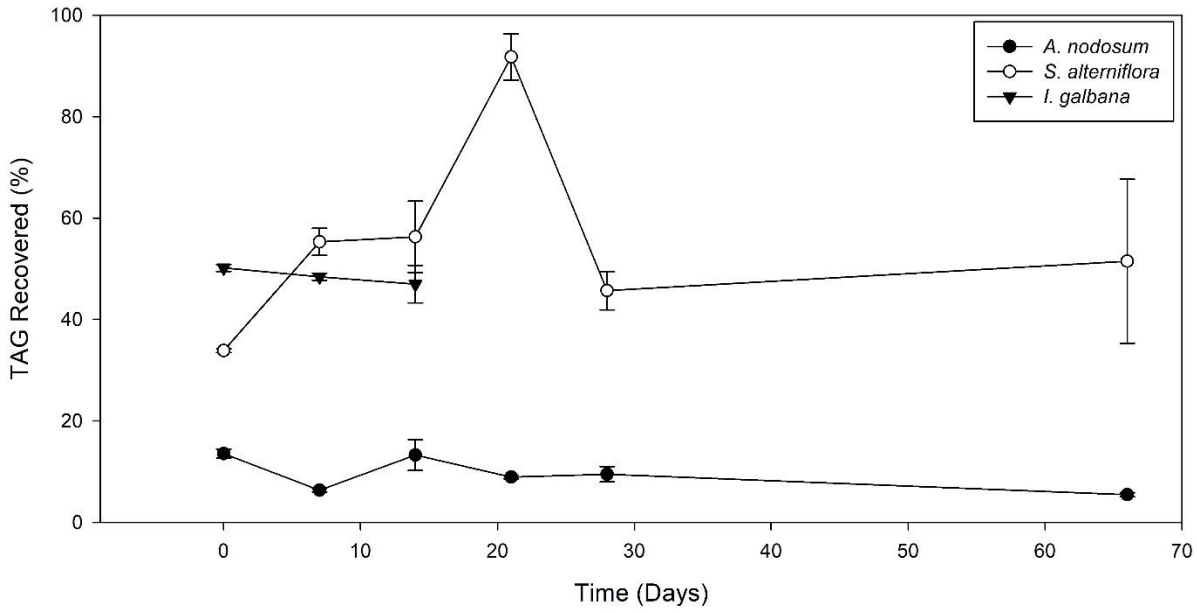

Figure 4.2. Proportion (%) of extractable triacylglycerols (TAG) solubilized by sodium taurocholate. Solubilized TAG, considered bioavailable, from three primary producers (*Ascophyllum nodosum*, *Spartina alterniflora*, and *Isochrysis galbana*). Error bars represent ± 1 standard deviation.

Our inability to completely solubilize TAG from natural substrates in STC may have been due to saturation of STC with lipid. Even though we provided STC micelles far in excess of that of TAG (>50× higher on a molar basis; Table 1), we were unable to completely solubilize the extractable TAG from any of the primary producers. To ensure the validity of our results we tested to see if there were any effects of STC saturation. Subsequent tests with triolein standards yielded solubilized TAG:micelle STC ratios approximately an order of magnitude greater than those from primary producers (Table 4.1). These results suggest that the STC was not saturated with TAG; however, unlike our triolein standards the tested primary producers contain other lipid classes besides TAG. In a further test, we ran replicate samples of fresh *I. galbana* as well as *Nanochloropsis oculate* using 50% higher concentrations of STC.

The resultant REs for samples with increased micelles (*I. galbana* 36 ± 0.3%, *N. oculata* 29 ± 1.3%) were nearly identical to REs of samples with fewer micelles (*I. galbana* 36 ± 0.1%, *N. oculata* 26 ± 0.6%), further suggesting STC saturation was not an issue.

Table 4.1. The molar ratio of triacylglycerols to sodium taurocholate. The initial molar ratio (µmol:µmol) of triacylglycerols (TAG) to sodium taurocholate (STC), above the critical micelle concentration, and the molar solubilization ratio (MSR) of TAG by STC for each primary producer (*Ascophyllum nodosum, Spartina alterniflora*, and *Isochrysis galbana*) and a TAG standard (triolein). Values reported are average ± 1 standard deviation.

	Initial concentration TAG:STC (µmol:µmol)	**Recovered** MSR (µmol:µmol)
A. nodosum	$1.9 \pm 0.8 \times 10^{-2}$	$1.6 \pm 0.4 \times 10^{-3}$
S. alterniflora	$1.7 \pm 1.0 \times 10^{-3}$	$8.6 \pm 2.1 \times 10^{-4}$
I. galbana	$1.5 \pm 0.2 \times 10^{-3}$	$7.3 \pm 1.1 \times 10^{-4}$
Triolein	2.0×10^{-2}	$1.2 \pm 0.2 \times 10^{-2}$

Testing similar STC-to-TAG increases when solubilizing triolein yielded similar results (Figure 4.3). Although there was an initial increase in TAG RE when increasing STC above CMC:TAG from 10 to 20 (REs: 31 ± 13% and 66 ± 7% respectively), there was no subsequent increase in RE (54 ± 11%) when the STC above CMC:TAG was increased to 40. The lack of TAG RE following a subsequent doubling of the STC above CMC:TAG beyond 20, suggests that so long as STC above CMC:TAG is above 20 there will be an excess STC. This result, again suggests that STC saturation was not an issue as the STC above CMC:TAG for our samples was well above 20 (Table 4.1).

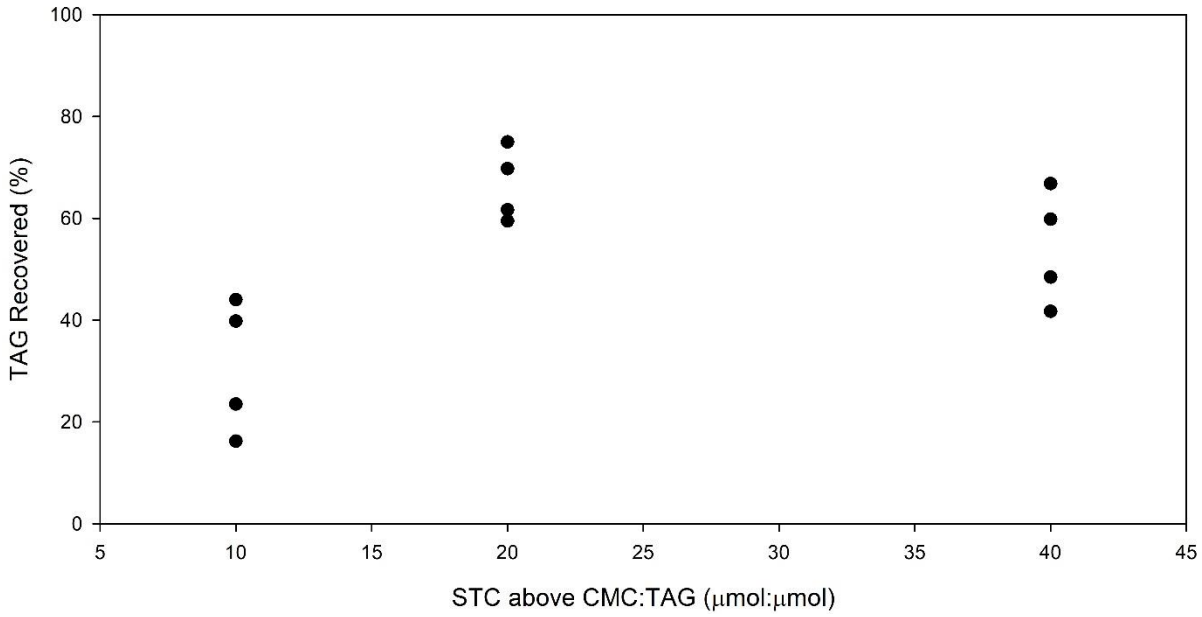

Figure 4.3. Proportion (%) of triolein solubilized by sodium taurocholate (STC) at varying ratios of STC above the critical micelle concentration (CMC) to triolein (μmol:μmol).

At the conclusion of the degradation experiment we found a mass of pulp, consisting of tangled fibrous material, at the bottom of the *S. alterniflora* slurry. This finding suggests that the higher REs of *S. alterniflora*, relative to *A. nodosum*, resulted from sampling only smaller, more easily suspended particulates and not the larger fibrous pulp. To test this possibility, we analyzed samples of the *S. alterniflora* pulp to see if the resultant REs differed. There was no difference in the RE of *S. alterniflora* pulp (48 ± 4%) and that of *S. alterniflora* slurry samples (56 ± 19%), suggesting that particle size did not influence REs and that our sub-sampling method did not influence the results.

Quantification of FA REs using gas chromatography generally corroborated the results of TAG REs obtained from Iatroscan analysis (Table 4.2). In general, both TAG and FA REs were lower for *A. nodosum* than the other producers and the state of degradation did not affect the FA RE (no significant difference between initial and degraded REs), suggesting once again that the original source of lipid was more important than any degradation processes. The FA RE for *A. nodosum* (18 ± 6%) and *S. alterniflora*

(42 ± 13%) were comparable to their TAG REs (9 ± 3% and 56 ± 19% respectively); however, the FARE (20 ± 2%) was lower than the TAG RE (48 ± 2%) for *I. galbana*. Both producer and FA types, bacterial (*iso* , *anteiso*, odd-branched), saturated, monounsaturated, polyunsaturated, and ω3 significantly affected the FA RE ($p < 0.01$); however, a post-hoc Tukey-test found only the FA RE of *S. alterniflora* to be significantly different ($p < 0.01$) than the other producers and there was no consistency in RE differences among FA types (Table 4.2).

Table 4.2. Proportion (%) of fatty acids solubilized in sodium taurocholate from three primary producers (*Ascophyllum nodosum, Spartina alterniflora*, and *Isochrysis galbana*) expressed as fatty acid recovery efficiency (RE). Reported values are average ± 1 standard deviation, * denotes significant differences among producers while ^{abc} denotes significant differences among fatty acid groups.

	A. nodosum	***S. alterniflora***	***I. galbana***
	RE (%)	RE (%)	RE (%)
Total FA	18 ± 6	42 ± 13*	20 ± 2
Bacterial	28 ± 19	36 ± 13	43 ± 9^{a}
Saturated	24 ± 6	47 ± 12^{a}	31 ± 8
MUFA	15 ± 5	37 ± 19	11 ± 9^{b}
PUFA	18 ± 6	37 ± 13	27 ± 8
ω3 PUFA	19 ± 7	20 ± 9^{b}	16 ± 11^{b}

4.3.2 Degradation effects on lipid classes and fatty acids

There were significant ($p < 0.05$) changes in the extractable lipid class composition of all producers throughout the course of the degradation experiment (Figure 4.4). There were no significant differences between the initial total lipid concentration (mg/g WW) and the end of the experiment. For all producers, there was a significant reduction in the proportion of PLs in conjunction with an increase of free fatty acids (FFA) and diacylglycerols (DAG) throughout the course of the degradation experiment (Figure 4.4b - d). Additionally, two measures of lipid degradation, the hydrolysis index (HI) and lipolysis index (LI), significantly increased for all producers throughout the experiment (Figure 4.4e + f). While we did observe the production of DAG for all producers suggesting degradation of TAG, the proportion of DAG remained low (<10% total lipid) and there were no significant decreases in the proportion of TAG

for any producers (Figure 4.4a and c). Although changes in lipid class composition were significant ($p < 0.05$) for all producers, the magnitude of change was greater for *S. alterniflora* and *I. galbana* than for *A. nodosum*, suggesting *A. nodosum* had the slowest degradation rate.

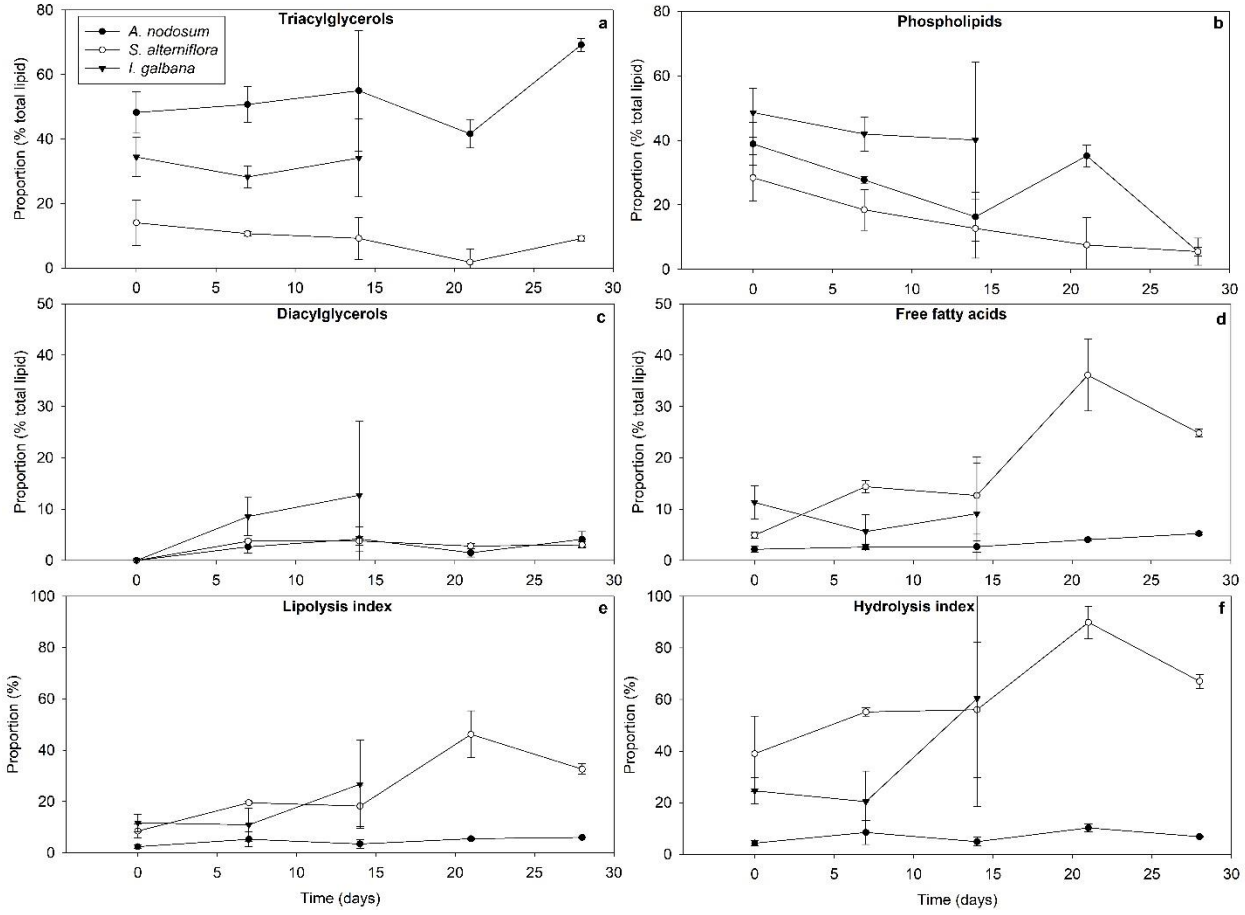

Figure 4.4. Summary of primary producer lipid changes during degradation. Proportion of total lipids (%) of triacylglycerols (TAG), phospholipids (PL), diacylglycerols (DAG), free fatty acids (FFA), as well as the lipolysis (LI) and hydrolysis (HI) index for three primary producers (*Ascophyllum nodosum, Spartina alterniflora*, and *Isochrysis galbana*) as they degrade. Values shown are average ± 1 standard deviation.

Accompanying changes in the lipid class composition, there were significant ($p < 0.05$) changes in the FA composition of *A. nodosum* and *S. alterniflora* (Figure 4.5). There was a significant increase in the proportion of bacterial FAs (*iso*, *anteiso*, and odd-chained) with degradation, although the increase for *A. nodosum* was very small (<1%). As with changes in lipid composition, the magnitude and extent of change was greatest for *S. alterniflora*. In addition to an increased proportion of bacterial FAs, the

proportion of saturated (SAT) and monounsaturated (MUFA) FAs in *S. alterniflora* increased with time, along with a corresponding decrease in the proportion of polyunsaturated (PUFA) FAs (Figure 4.5a – d). These results suggest once again that *S. alterniflora* showed the greatest amount of degradation, corroborating the lipid class results.

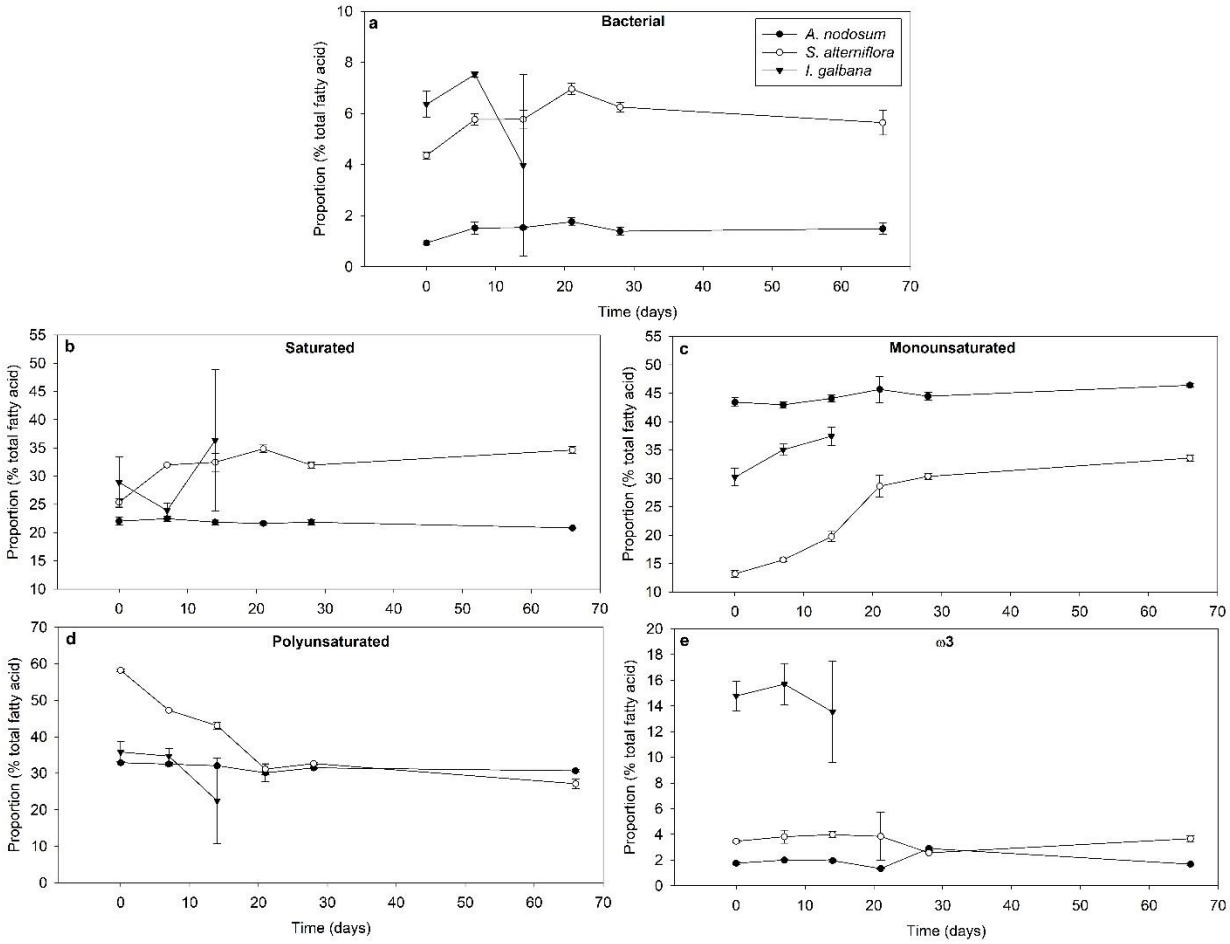

Figure 4.5. Summary of primary producer fatty acid changes during degradation. Proportion (% total fatty acids) of bacterial (*iso, anteiso*, and odd-chained), saturated, monounsaturated, polyunsaturated, and ω3 fatty acids in three primary producers (*Ascophyllum nodosum, Spartina alterniflora*, and *Isochrysis galbana*) as they degrade. Values shown are average ± 1 standard deviation.

4.4.0 Discussion

The amount of lipid solubilizable by STC was always only a fraction of EL (<50%), regardless of primary producer and bioavailability varied with FA types. This is not the first study to show incomplete bioavailability, roughly defined here as RE, of lipids for consumers or differences in bioavailability among lipid and FA types. Copepods are able to digest the majority (>65%) of ingested organic matter when feeding upon diatoms but are less capable (30%) of digesting pigments (Cowie and Hedges, 1996). Zooplankton also digested FAs at high efficiencies (>60%) when feeding on dinoflagellates, though efficiency varied with the type of FA ingested (Harvey et al., 1987). In contrast, although larvae of the oyster *Crassostrea gigas* were able to efficiently assimilate the essential FAs 20:4ω6 and 22:6ω3 (82 and 76% respectively), they had lower assimilation efficiencies (<60%) of other FAs, including the essential FA 20:5ω3 (Da Costa et al., 2015). However, our inability to completely solubilize triolein, which is expected to be fully bioavailable (Carroll and Richards, 1958), even when STC was in excess, suggests REs of 50% or greater likely represents full bioavailability. This is under the assumption that there are no other structural or matrix effects preventing producer lipids from solubilization.

Primary production source was more important than detrital processing in terms of affecting the bioavailability of lipids and their constituent FAs for consumers. Lipids and FAs from *A. nodosum* were consistently less bioavailable than those from *S. alterniflora* and *I. galbana* and bioavailability did not change with time or state of decay, suggesting an inherent difference between *A. nodosum* and the other two producers. It has previously been suggested that the rate limiting step in the solubilization of PLs by STC was membrane permeability and that the presence of cholesterol significantly lowered the permeability of membranes to STC (Ramaldes et al., 1996). Our results would then imply that *A. nodosum* had higher levels of cholesterol and less permeable membranes than *S. alterniflora* or *I. galbana*. However, cholesterol is only a minor component of *A. nodosum* (Knights, 1970; Rayirath et al., 2009), *S. alterniflora* (Lee et al., 1980), and *I. galbana* (Bandarra et al., 2003). As such, it seems unlikely

that the concentration of cholesterol in *A. nodosum* was sufficiently elevated compared to that of *S. alterniflora* or *I. galbana* to result in the observed differences in RE. Other sterols have also been found to decrease membrane permeability to surfactants, dihydrocholesterol and coprostanol both increased membrane resistance to the surfactant sodium dodecyl sulfate (Apel-Paz et al., 2005). The proportion of sterols in *A. nodosum* (7.9 ± 0.2%) was larger than *S. alterniflora* (5.2 ± 0.1%) and *I. galbana* (5.5 ± 0.2%), suggesting that *A. nodosum* membranes were more resistant to surfactant attack. However, the PL:sterol ratio of *A. nodosum* (3.6 ± 3.0) was like that of *S. alterniflora* (3.4 ± 4.1) and both were lower than *I. galbana* (6.2 ± 2.9). Based on this ratio, we would expect *I. galbana* lipids to be the most bioavailable and *A. nodosum* to have comparable bioavailability to *S. alterniflora*. This was not the case, suggesting it is unlikely that membrane permeability is the sole factor affecting the observed bioavailability differences.

Alginates might lower RE of *A. nodosum* relative to *S. alterniflora* or *I. galbana*. Alginic acids can emulsify with bile acids and interfere in the formation of lipid micelles (Dumelod et al., 1999; Rajapakse and Kim, 2011). Incorporation of seaweeds into the diets of humans and rats can decrease the absorption of cholesterol and increase the cholesterol content of feces (Dumelod et al., 1999; Hall et al., 2012; Seal and Mathers, 2019). Structural alginates comprise roughly 15% (wt/wt) of *A. nodosum* (Schiener et al., 2017); if the alginates in *A. nodosum* prevent formation of STC micelles it would explain the lower REs compared to *S. alterniflora* and *I. galbana*. Given that STC is a vertebrate bile salt (Voparil and Mayer, 2004), it seems likely that alginates in *A. nodosum* would be able to interfere with lipid micelle formation as observed previously in humans and rats. Another possible cause of the lower REs for *A. nodosum* compared to the other producers, may be the presence of polyphenols. Polyphenols could interfere with digestion by precipitating gut surfactants of consumers (Zimmer, 1997). Although both *S. alterniflora* (~1.5% wt/wt) and *A. nodosum* (~6% wt/wt) contain secondary metabolites, *A. nodosum* in particular contains much higher concentrations of polyphenols than *S. alterniflora* (Haddad

et al., 1992; Tibbets et al., 2016). It is possible that the higher concentration of polyphenols in *A. nodosum* was enough to precipitate enough STC to cause the reduction observed in REs. Bivalves have been shown to select against particulates with higher polyphenolics (>3% wt/wt) and particulates with higher polyphenolic concentrations resulted in inferior growth rates (Duggins and Eckman, 1997; Levinton et al., 2002). Based on previous studies, we would expect the polyphenol concentration in A. nodosum to be high enough to adversely affect bivalve consumers, a result consistent with our REs.

The observation, that the original source of lipid was more important than the effects of degradation to the bioavailability of nutritional lipids, has several ecological implications. First, unlike conventional wisdom based on C:N ratios (De la Cruz, 1965; Odum and de la Cruz, 1967), the quality of detrital material, based on RE, did not improve with degradation in terms of nutritional lipids, nor were detrital products more bioavailable than their fresh counterparts. If degradation does not increase bioavailability, bivalves may be able to more directly make use of nutritional lipids from macrophytes such as macroalgae and marsh grasses than previously thought. This finding is important because it suggests less processing is required before lipids of shed macrophyte material is bioavailable for bivalves, reducing the likelihood that material is exported out of the ecosystem before it's bioavailable. That is not to say material shed from macrophytes is immediately available to bivalves, other factors including particle size and secondary metabolites, such as polyphenols that inhibit grazing (Duggins and Eckman, 1997), need to be taken into consideration as well. In addition, regarding alternative diets for aquaculture, our results suggest that testing the bioavailability of the parent primary producer is a suitable and relatively easy first step to identifying potential food sources. It should also be noted that, although the bioavailability of nutritional lipids did not change with degradation, the quality of lipids changed with degradation. The loss of PUFA throughout degradation will result in particulates of lower nutritional quality than the those prior to degradation.

Second, although previous studies have found *S. alterniflora* and its detrital products were only assimilated at low efficiencies (<10%) by oysters and mussels based on C and N budgets (Lucas and Newell, 1984; Langdon and Newell, 1990), our results indicate that a fair amount (~50%) of nutritional lipids from *S. alterniflora* are bioavailable. The discrepancy between the assimilation efficiency of C and N and the apparent bioavailability of nutritional lipids raises some interesting questions. Although salt marsh detritus does not contribute significantly to their overall C and N budget, could marsh detritus serve as a lipid and FA supplement for bivalves? Although *S. alterniflora* is deficient in many essential FAs compared to marine primary producers, e.g. 20:5ω3 and 22:6ω3, it is rich in 18:2ω6 and 18:3ω3 which are precursors of essential FAs (Kelly and Scheibling, 2012). While most vertebrates are incapable, or limited, in their ability to chain elongate 18:2ω6 and 18:3ω3 into essential FAs, many invertebrates possess the elongase and desaturase enzymes required to modify dietary FA (Kelly and Scheibling, 2012). *Crassostrea gigas* and their larvae are capable of chain-elongation and desaturation of MUFA into PUFA and have been shown to convert 18:3ω3 into 20:5ω3 (Waldock and Holland, 1984; Da Costa et al., 2015). Through this process, bivalves capable of chain-elongation and desaturation could make use of ingestible particulates of *S. alterniflora* to supplement their essential FA requirements. If bivalves did make use of *S. alterniflora* detritus in this way, marsh detritus may play a more important role in bivalve nutrition than previously thought based on C and N budgets alone. In contrast to *S. alterniflora*, *A. nodosum* and other macroalgae do contain essential FAs, particularly 20:4ω6 and 20:5ω3. While lipids and FA from *A. nodosum* were less bioavailable than the other two primary producers tested, a small proportion (~20%) of PUFA and ω3 FAs were bioavailable. Although we did not detect substantial amounts of 20:5ω3 in *A. nodosum*, 20:4ω6 was recovered with a RE of 17 % (data not shown), suggesting that bivalves could supplement some of their essential FA requirements from macroalgal detritus as well. If bivalves did supplement their essential FA requirements with macroalgal detritus, it

could explain why some studies found higher growth rates of bivalves when fed a combination of macroalgal detritus and conventional phytoplankton diets (Camacho et al., 2007).

We did not observe any changes in the bioavailability of lipid classes and FAs throughout the course of our experiment, but qualitative changes in the lipid and FA composition of producers suggest that some degradation occurred. There were significant increases in DAG, FFA and bacterial FAs, which are all expected products of degradation. The increases of degradation products caused increases in the HI and LI which are used to show the proportion of breakdown of neutral acyl lipids and total acyl lipids respectively (Weeks et al., 1993; Parrish, 1998). The decreases in PL and PUFA that occurred in conjunction with increases in degradation products and indices suggests that PL and PUFA are more susceptible to degradation than TAG, SAT and MUFAs. Taken together, the increases in degradation products and indices show clearly that degradation occurred in our slurries, although to varying degrees. Based on the magnitudes of change observed, *S. alterniflora* underwent the greatest amount of lipid degradation and *A. nodosum* the least. The reduced degradation observed in *A. nodosum* might be attributed to polyphenols. The presence of polyphenols is known to reduce colonization of microbes (Ragan and Glombitza, 1986; Krumhansl and Scheibling, 2012) and their higher concentration in *A. nodosum* could have slowed the degradation process by inhibiting the colonization of microbes more than *S. alterniflora*. Due to the nature of the closed system used in our rot experiment, the bacterial inhibition of polyphenols was likely magnified. Even after they leached from producer tissues, polyphenols would still be present in the seawater medium and be able to inhibit the growth of bacteria. Another potential factor is the sourcing of our filtered seawater; marsh grass and macroalgae are known to degrade at different rates depending on where they are located in an estuary (Quintino et al., 2009). Marsh grasses degraded faster farther inside an estuary and closer to where they grew than macroalgae which degraded faster towards the mouth of the estuary. The intake for the filtered seawater used in this experiment was upstream of the estuary mouth and as a result could have had a bacterial

community primed to more readily decompose *S. alterniflora* than *A. nodosum* as a result. Regardless of the differences in the amount of degradation, degradation of primary producer slurries did occur, and degradation did not significantly affect the bioavailability of lipids and FAs.

Bioavailability measured as RE's in this study may be minimum values because we only measured a single step in the digestion process. We focused on the ability of gut surfactants (STC in this case) to solubilize nutritional lipids. In addition to surfactants, bivalves are known to possess both lipases and phospholipases (George, 1952; Hoehne-Reitan et al., 2007), which will aid the solubilization of lipids by breaking them down into smaller constituent parts. Because we did not include lipases and phospholipases in our digestions, we only measured the initial step in digestion, the ability of gut surfactants to solubilize nutritional lipids. Because co-lipase, phospholipase, lipase, and bile salts all act synergistically (Carey et al., 1983), addition of lipases and phospholipases would be expected to increase the solubilization of lipids and as such, our measured bioavailabilities should be viewed as minima only. Although the addition of lipases and phospholipases would be expected to increase solubilization of nutritional lipids, the increases may not be uniform across lipid sources. Depending on the biochemical composition of each primary producer, lipases and phospholipases may be better or worse at accessing and lysing lipids from each source and further testing will be required to fully understand the bioavailability of nutritional lipids. For example, bivalves possess laminarinases (Brock and Kennedy, 1992), a carbohydrase that breaks down laminarin. Laminarin is an abundant structural carbohydrate in macroalgae (Schiener et al., 2015) and the presence of laminarinases in bivalves suggests they are equipped to digest macroalgal material. As such, bivalves may contain lipases similarly tooled to deal with lipids from macroalgal sources. So, while our study represents a first step in developing a biomimetic approach to assess the bioavailability of nutritional lipids, more work is required to fully represent the digestion process of lipids.

We made the decision to omit lipases, phospholipases, and co-lipase from out digestion because of early difficulties associated with quantifying phospholipids. As previously mentioned, there were ghost peaks that appeared in the same area as PLs in chromatographs obtained from Iatroscan analysis. We initially believed these peaks to be caused by lipases, which prompted us to remove them from our cocktail. It was only later, and after the experiment had begun, that we realized the ghost peaks were not caused by lipases; the now suspected cause is incombustible phytates (Ackman and Woyewoda, 1979). If it is indeed incombustible phytates, and not lipases, that are the cause of ghost peaks it should be possible to re-introduce lipases into the cocktail to better mimic a digestive system. Furthermore, by altering Chromarod development times prior to Iatroscan analysis it may be possible to shift the retention time of PLs away from the ghost peaks to allow for proper quantification. As such, the re-introduction of lipases along with changes in chromarod development times would be easy first steps to begin improving upon the methods used in this study. In addition, more robust bioassays, e.g. feeding trials of live animals using radiolabeled essential FAs or compound specific stable isotopes, to determine digestive performance of bivalves should be conducted to properly ground truth the bioavailabilities measured within this study.

4.5.0 Conclusions

The initial source of nutritional lipids and FAs influenced bioavailability more than the state of decay, of the parent material. The bioavailability of lipids and FAs from *A. nodosum* was lower than those of *S. alterniflora* or *I. galbana*, perhaps due to presence of alginates which interfered with the formation of micelles. Sodium taurocholate was able to successfully solubilize TAG from slurries of primary producers and could serve as a proxy for measuring the bioavailability of nutritional lipids and FAs; however, further refinement, e.g. the addition of lipases and phospholipases, is required to adequately represent the digestive systems of bivalves. Additionally, comparisons of the ability of STC to solubilize lipids with the digestive fluids of bivalves, live animal assays, and bioassays (e.g. ^{14}C-labelling or compound specific stable isotopes) is required to properly assess the viability of our biomimetic approach.

CHAPTER 5

CONCLUSIONS

Saco Bay, ME USA, had a phytoplankton community dominated by dinoflagellates, followed by centric diatoms, which was reflected by the availability of essential fatty acids (FA). As such, 22:6ω3 was the most abundant essential FA followed by 20:5ω3, while there was relatively little 20:4ω6. There were two broad categories of particulate organic matter (POM) in Saco Bay, marine POM (phytoplankton, zooplankton, and macroalgal detritus) which contained essential FA and vascular plant POM which lacked essential FAs but was rich in their precursors 18:2ω6 and 18:3ω3. Macroalgal detritus was differentiated from other marine POM by the presence of secondary metabolites and difficult to digest structural materials; however, it could be a supplemental source of 20:5ω3 and 20:4ω6 for consumers able to digest it. In contrast, vascular plant detritus lacked essential FAs while also possessing secondary metabolites and difficult to digest structural material. While of a relatively poorer quality when compared to macroalgal detritus and other marine POM, consumers possessing FA desaturase and elongase enzymes could potentially make use of vascular detritus to supplement essential FAs.

Mytilus edulis within Saco Bay were omnivores making use of both fresh phytoplankton and zooplankton in their diets as well as detrital sources, which comprised a minimum of 16% of their diet based on FAs. Vascular plant FAs comprised a relatively small proportion of *M. edulis* FAs (3.3 ± 0.01%). In contrast, although macroalgae only comprised a small proportion (5 to 11%) of the diet of *M. edulis*, macroalgal detritus contained large proportions of 20:5ω3 and 20:4ω6. Mussels within the bay may have been limited by 20:5ω3, suggesting their ingestion of macroalgal detritus may have been to supplement their diet with 20:5ω3. More work is needed to determine if detrital supplements, particularly detrital FAs, are required by *M. edulis*.

The initial source of lipid and FAs influenced bioavailability, based on the solubilization ability of sodium taurocholate (STC), more than the state of decay. The bioavailability of lipids and FAs from *Ascophyllum nodosum* was significantly lower than *Spartina alterniflora* or *Isochyrsis galbana*. Differences in bioavailability were perhaps due to the content of alginates and polyphenols which could have interfered with the formation of micelles and precipitate gut-fluids. Although STC was able to solubilize TAG and FAs from slurries of primary producers and could serve as a proxy to measure the bioavailability of nutritional lipids, further development is needed to properly simulate the digestive environment of bivalves.

Based on the results of this study, macrolagal detritus shows the most promise to be incorporated into bivalve aquaculture models. Macroalgal detritus contributed to both POM and the diets of *M. edulis* in Saco Bay and may serve as a supplemental source of essential FAs. However, poor bioavailability of *A. nodosum* lipids and FAs, suggests caution is required when considering macroalgal detritus as a food-source for bivalves. Close attention should be paid to the content of secondary metabolites (>3% wt/wt) and structural material (e.g. alginates) when assessing macroalgal, or any, detritus as a food-source for bivalve aquaculture. Additionally, the digestive capabilities and ability to modify dietary FAs of the cultured bivalve should be considered when considering detrital food-sources.

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