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Biophysical Factors Affecting Habitat Suitability for Crassostrea virginica

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BIOPHYSICAL FACTORS AFFECTING HABITAT SUITABILITY FOR *CRASSOSTREA VIRGINICA*

by

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ABSTRACT

Oyster reefs provide a variety of important ecosystem services. However, the mortality rate of eastern oyster, *Crassostrea virginica*, the dominant species that produces oyster reefs in the northern Gulf of Mexico, is increasing at an alarming rate due to a variety of abiotic and biological factors. I examined how biophysical factors, including the less-studied fatty acid profiles of the suspended particulate matter on which oysters feed, influenced morphometric condition of *C. virginica*.

I sampled suspended particulate matter (SPM) and oysters in-situ in the western Mississippi Sound, which historically supported the majority of oyster production in Mississippi waters. Sampling was conducted from April to November 2018 and covered the main growing and spawning season of the eastern oyster. I studied the seasonal pattern of biophysical variables and morphometric condition of eastern oysters. I then developed a probabilistic model to predict morphometric condition of oysters based on in situ biophysical variables. Particularly, I evaluated if the inclusion of a food quality variable improves model performance in predicting the morphometric condition of oysters. The results showed that SPM was best predicted by wind stress, bottom temperature, and bottom salinity. However, the biophysical factors could not predict fatty acids of the SPM, except the ratio of phytoplankton polyunsaturated fatty acids (PUFA) to terrestrial PUFA. The ratio was best predicted using SPM, photosynthetic active radiation, and bottom temperature. The biophysical factors that were included in the best model to predict oyster condition included length, salinity, total PUFA, SPM, and monthly river discharge. Results of this research support the hypothesis that oyster condition is positively affected by both food quality and food quantity.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER I – INTRODUCTION

1.1 Background

Oyster reefs are extremely important to coastal ecosystems. They act as ecosystem engineers in their capacity to control the availability of resources by physically altering habitat, not just structurally, but also through filtration (zu Ermgassen et al. 2012), nutrient cycling (Higgins et al. 2013), and influencing hydrodynamic circulation (Lenihan 1999, Reidenbach et al. 2013). As a keystone species, oysters produce reefs supporting high species diversity by providing a substrate for benthic organisms (Shervette and Gelwick 2008), refuge from predators (Scyphers and Powers 2013), and spawning/nursery habitat for aquatic species (Tolley and Volety 2005). In addition, oysters support economically valuable fisheries around the world.

Unfortunately, oysters have experienced massive global declines over the past century (La Peyre et al. 2014, Pine et al. 2015). For instance, the western Mississippi Sound no longer supports an oyster fishery (Powell, pers. comm.). There are numerous factors speculated to contribute to oyster declines in the Gulf of Mexico (GOM) (La Peyre et al. 2014, Camp et al. 2015, Pine et al. 2015) including increased water temperatures (Powell 2017), disease (Powell 2017), salinity fluctuations (DeHaan et al. 2012, Petes et al. 2012), increased predation (Petes et al. 2012), fishing activities (Lenihan and Peterson 1998), toxic contaminants (Lytle and Lytle 1982, Elston et al. 2005, McCrea-Strub et al. 2011), tropical cyclones (Berrigan 1988, Livingston et al. 1999), and hypoxia (Lenihan and Peterson 1998); however, these factors are not mutually exclusive. These different factors can interact to complicate the evaluation of why oysters have declined dramatically. For example, high salinities have been associated with

increased predation mortality (Petes et al. 2012); dredging activities have been associated with increased hypoxia mortality (Lenihan and Peterson 1998); and the combination of increased temperature and poor food quality have been associated with increased disease mortality (Hégaret et al. 2004, Pernet et al. 2014).

The collapse of the western Mississippi Sound population was caused by a combination of recruitment failures and mass mortality events. These oyster reefs have experienced multiple stressors in recent years including Hurricane Katrina in 2005, the Deepwater Horizon Oil Spill in 2010, and increases in Dermo (a disease caused by *Perkinsus marinus*) attributed to rising sea-surface temperature (Powell 2017). The most recent 2019 record breaking Bonnet Carré Spillway openings have coincided with a near total mortality event in the western Mississippi Sound (USM 2019).

To improve understanding of oyster mortality in Mississippi and more broadly, it is important to understand how environmental factors affect oyster physiology. Understanding the underlying stressors will be critical for any future restoration and management efforts. Oyster habitat suitability models (HSMs) are receiving increased attention in the northern Gulf of Mexico (Soniat et al. 2013, de Mutsert et al. 2016, Linhoss et al. 2016, Hijuelos et al. 2017); however, these models have so far only examined physical and chemical factors affecting habitat suitability. While these approaches are insightful, inclusion of data related to oyster food availability into these physico-chemical HSMs could improve model predictions and subsequent management decisions.

1.2 Oyster Feeding

Understanding nutritional requirements for oysters has been a fundamental goal in oyster biology for decades. The classical approach to studying oyster diet involved washing the entire alimentary canal and examining the contents using microscopy (Galtsoff 1964). This method indicated that oyster diet consisted mostly of detritus (Savage 1925); however, this method had major drawbacks because flagellates and bacteria were rapidly digested and impossible to enumerate. In fact, bacteria were later found to be suitable prey in feeding experiments (Kincaid 1938). To further complicate matters, some prey was found to pass undigested through the digestive tract (Galtsoff 1964). Further challenges in understanding oyster nutritional requirements arise from the fact that oysters compensate for poor food quality by increasing feeding (Urban and Kirchman 1992, Barillé et al. 1997). Additionally, oysters may increase clearance rates to compensate for low concentrations of suspended particulate matter (SPM) (Dupuy et al. 2000). However, at high SPM concentrations, clearance rates decline at roughly 25 mg L^{-1} and feeding ceases at 1 g L^{-1} (Fulford et al. 2017).

Oyster feeding is complex and selective. Water is pumped through the mantle cavity using cilia to either the gills or labial palp (Bayne 2017), where particles are selected based on size and chemical interactions between lectins in the mucus covering the feeding organs and carbohydrates on the surface of plankton cells (Pales Espinosa et al. 2009). Along ciliated tracts, selected particles are transported to the mouth, and rejected particles are exported as pseudofeces (Pales Espinosa et al. 2009). After ingestion, further sorting of particles occurs in the stomach. Small, dense particles are transported to the intestinal groove and excreted as intestinal feces; large, less-dense

particles are broken down, transported to digestive tubules, and excreted as glandular feces (Bayne 2017).

However, feeding selectivity in oysters is still poorly understood. In *Crassostrea gigas,* Cognie et al. (2003) determined that oyster gills retain particles ranging from 4 µm to greater than 250 µm. However, retention of particles as small as 0.5 µm is also possible as the result of particle aggregation (Kach and Ward 2008). Indeed, Sonier et al. (2017) found that *C. virginica* captured and assimilated picophytoplankton (<2 µm) in radiolabeling experiments. Regardless, preferential selection of particles occurs on the lower end of the size-range available to oysters (Tamburri and Zimmer-Faust 1996). Tamburri and Zimmer-Faust (1996) found a negative linear relationship between particle size and ingestion rates of *C. virginica* fed particles ranging 10–410 µm. It should not be overlooked, however, that prey selectivity in oysters is influenced by a variety of factors besides particle size, such as wettability (Rosa et al. 2013) and chemical interactions with lectins (Pales Espinosa et al. 2009).

Considering the complexity of oyster feeding, researchers looked at alternative methods for determination of the diet. One such method was stable isotope analysis (Haines and Montague 1979, Riera and Richard 1996). Sullivan and Moncreiff (1990) did an extensive examination of stable isotopes in a Mississippi estuary, and isotopic values indicated that *C. virginica* belonged to the deposit/suspension feeding group and exhibited similar values to phytoplankton. Chanton and Lewis (2002) conducted similar research in Apalachicola Bay, Florida and concluded that oysters were water column feeders exhibiting selective assimilation of particulates, and this was significantly affected by water flow. Wilson et al. (2010) later compared two sites in the upper reaches

of Apalachicola Bay, FL and found spatial heterogeneity in isotopic profiles. The freshwater-dominated site showed a significantly higher fractional contribution of detrital carbon compared to the marine-influenced site, suggesting food switching depending on salinity and SPM conditions. Wrast (2008) examined trophodynamics in Lavaca Bay, TX and found that oyster isotopic ratios largely resembled that of particle organic matter (POM). Isotopic analysis proved useful for estimating the relative contributions of detritus, phytoplankton, and bacteria to oyster nutrition but gave little insight into the relative contributions of different phytoplankton functional groups (PFGs).

Plankton community composition largely depends on hydrodynamic regimes. Mixed regimes, with high turbulence and often light-limited, are dominated by phytoplankton with large cell sizes, such as diatoms. Stratified regimes, with low turbulence and often nutrient limited, are dominated by smaller phytoplankton taxa, such as flagellates and cyanobacteria (Kiørboe, 1993). Within these regimes, phytoplankton communities are largely regulated by light, nutrients, and temperature (Dalsgaard et al. 2003).

1.3 Lipids

Lipids have a critical role in the storage of photosynthetic energy and membrane fluidity for planktonic organisms. Lee et al. (1971) were the first to demonstrate the conservative transfer of plankton fatty acid trophic markers (FATMs) to consumers. The fatty acid profile of a consumer gives insights into the consumer's diet, because certain fatty acids are passed conservatively to higher trophic levels with little modification. Not only has this expanded understanding of oyster feeding ecology, but it has also helped advance our understanding of oyster nutrition and how fatty acids are linked to oyster

physiological status, disease resistance, and reproductive potential. Research is limited regarding FATMs in *C. virginica*. To date, the only published study that simultaneously examined the lipid compositions of wild *C. virginica* and particulate organic matter was conducted by Chu et al. (1990) in the York River, Virginia. They examined seasonal variations in lipids among different oyster tissues, as well as particulate organic matter (POM), and concluded that oyster lipid compositions were related to reproductive cycles and food lipid supply. Sonier et al. (2017) examined the importance of picophytoplankton in oysters cultured in cages. Research involving FATMs for other oyster species is less sparse (Langdon and Waldock 1981, Soudant et al. 1999, Pernet et al. 2012, 2014).

Lipids in *C. virginica* have been of interest for decades. Lipids regulate oyster homeostasis, therefore, oyster lipid reserves are critical to oyster tolerance to environmental stressors. Most of these reserves are obtained through the food that oysters ingest. Early work focused on the lipid composition of adults. Ackman et al. (1971) examined the lipid composition in oysters, as well as the chain-length distribution of saturated fatty acids. Sidwell et al. (1979) examined seasonal variations of oyster cholesterol and total lipids at both Atlantic and Gulf of Mexico sites. Swift et al. (1980) examined the temporal distribution of neutral lipid classes in eastern oyster tissues. More recent research has focused on all life stages, including juveniles (Wikfors et al. 1984, 1996, Pernet et al. 2008), larvae (Chu and Webb 1984, Gallager and Mann 1986, Gallager et al. 1986, Génard et al. 2011) and eggs (Glandon et al. 2016). Three fatty acids are generally considered essential and must be obtained from dietary sources in oysters: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) (Langdon and Waldock 1981, Ronquillo et al. 2012, da Costa et al. 2016); though, strictly

speaking, oysters do have limited capacity to biosynthesize EPA and AA (da Costa et al. 2016).

Stress tolerance in larval *C. virginica* relies on energetic reserves of neutral lipids (Zhang et al. 2016). Gallager et al. (1986) compared the lipid compositions of healthy and starved *C. virginica* larvae and found triacylglycerol (TAG) was preferentially catabolized during starvation. They suggested that lipid content could serve as an indicator of physiological condition and a predictor for metamorphosis success. Gernard et al. (2011) examined the energy metabolism and lipid composition of larval *C. virginica* in an aquaculture experiment with an antibiotic treatment. During a massive mortality event in the control group, untreated larvae had higher levels of non-methylene interrupted (NMI) dienoic fatty acids (22:2 NMI), lower levels of polar lipids, and a lower peroxidation index of polar lipids. The authors proposed that the increase in 22:2 NMI may be a compensating mechanism for decreased DHA content and oxidative stress. Additionally, increased energy metabolism activity (citrate synthase and cytochrome oxidase) showed a positive relationship with TAG accumulation.

The relationship between lipid reserves and physiological stress response is less understood for juvenile and adult *C. virginica*. Ivanina et al. (2013) compared the total lipid content of adult *C. virginica* exposed to normal and elevated levels of CO_2 (hypercapnia) and found significantly lower lipid levels in the hypercapnia group after two weeks of exposure at 27 ºC. Research has also examined how oyster fatty acid composition is related to seasonal reproductive cycles (Chu et al. 1990) and immune response (Hégaret et al. 2004). Chu et al. (1990) examined lipid class and fatty acid composition of *C. virginica* maintained in a flow-through system in a Virginia estuary.

They found proportions of EPA and DHA in the visceral mass remained elevated during the spawning season even after levels of EPA and DHA had decreased in the POM. Their results, along with similar observations in other studies (Trider and Castell 1980), led the authors to conclude that oyster fatty acid composition is largely related to seasonal reproductive cycles.

Beyond this, it is useful to look at other crassostreid species for insights. Pernet et al. (2010) suggested that increased saturation of adult *C. gigas* fatty acids and increased arachidonic acid content were associated with mortality events in 2008 off the coast of France. Tamayo et al. (2014) compared the fatty acid composition between healthy juvenile *C. gigas* and juveniles exposed to an ostreid herpesvirus at two temperatures (13.0 ºC and 20.6 ºC). Exposed oysters had reduced TAG at higher temperatures and reduced sterols at both temperatures, despite energetic enzyme activity not being significantly affected by disease exposure. In a field experiment, Pernet et al. (2014) found that juvenile *C. gigas* TAG reserves were inversely correlated with disease mortality and positively correlated with the relative contribution of diatoms to the diet. Evidence also shows that aerobic metabolism on TAG substrates represents the dominant energy source in the digestive gland of quiescent oysters, rather than fermentation on glycogen substrates (Mayrand et al. 2017).

Polar lipids (PL) in oyster gill mitochondria have been of special interest, due to their importance in maintaining osmotic balance in response to temperature (Chu and Greaves 1991, Gillis and Ballantyne 1999, Pernet et al. 2007a, 2007b) and salinity (Ballantyne and Moyes 1987, Glémet and Ballantyne 1995). Furthermore, the degree of

saturation in the polar lipids is negatively correlated with basal metabolic rate and positively correlated with growth (Pernet et al. 2008).

Polar membrane fluidity is sensitive to fluctuations in temperature, so to maintain osmotic balance, ectotherms must regulate membrane lipid composition through a process known as homeoviscous adaptation (Sinensky 1974, Pernet et al. 2007a). The theory of homeoviscous adaptation predicts that polar membrane lipids will increase in saturation as temperatures increase (Sinensky 1974). Much of this regulation in oysters must occur by altering the distribution of polyunsaturated fatty acids (PUFA) among lipid classes (e.g., TAG, polar lipids, and sterols), because radiolabeling experiments indicate oysters only have limited ability to elongate and desaturate PUFA over short timescales (Waldock and Holland 1984, Chu and Greaves 1991). In experimental trials, the degree of saturation in the polar lipids (PL) of oyster gills was affected by temperature at both daily and seasonal timescales (Pernet et al. 2007a, 2007b). Another experimental trial indicated that decreased temperatures result in a net increase in total PUFA in the polar lipids of oyster gill mitochondria, including increases of n-3 PUFA in phosphatidylethanolamine, n-6 PUFA in cardiolipin, and total PUFA in phosphatidylinositol (PI) (Gillis and Ballantyne 1999).

In addition to thermoregulation, lipid metabolism in oysters is also affected by salinity. In experiments comparing the lipid composition of oysters maintained at different salinities, oysters maintained at high salinities had significantly higher proportions of the negatively charged polar lipids, cardiolipin, and PI, and lower levels of n-6 fatty acids in all polar lipid classes except phosphatidylcholine (PC) (Glémet and Ballantyne 1995). The authors concluded that the more negatively charged polar

membranes encountered at high salinities could be an adaptive control for the higher intracellular concentrations of inorganic cations (particularly K^+) or a mechanism to adjust the action of membrane-bound enzymes. No lipid-transporting proteins have been identified in bivalves; therefore, the decreased solubility of free fatty acids at low salinities can limit the transport of triacylglyceride stored in the hepatopancreas to other tissues (Ballantyne and Moyes 1987).

1.4 Objectives

Considering the role that dietary lipids may have on oyster fitness, this dissertation examined the relationship between environmental parameters in the western Mississippi Sound, including seston and lipid availabilities, and oyster condition. The overarching hypothesis was that oyster condition can be better predicted when accounting for both food quality and food quantity than accounting for food quantity alone. I studied the seasonal pattern of suspended particulate matter (SPM, a proxy for seston quantity) (Chapter II) and SPM PUFA concentrations (a proxy of seston quality) (Chapter III) at six oyster reef locations in the western Mississippi Sound. Then, I investigated the temporal dynamics of morphometric condition of oysters at these locations and developed a predictive model of oyster weight, with environmental factors, including SPM and PUFA concentrations, as well as oyster lengths as covariates (Chapter 4). A conceptual model shows the relationships among different key variables studied in this dissertation (Fig. 1.1).

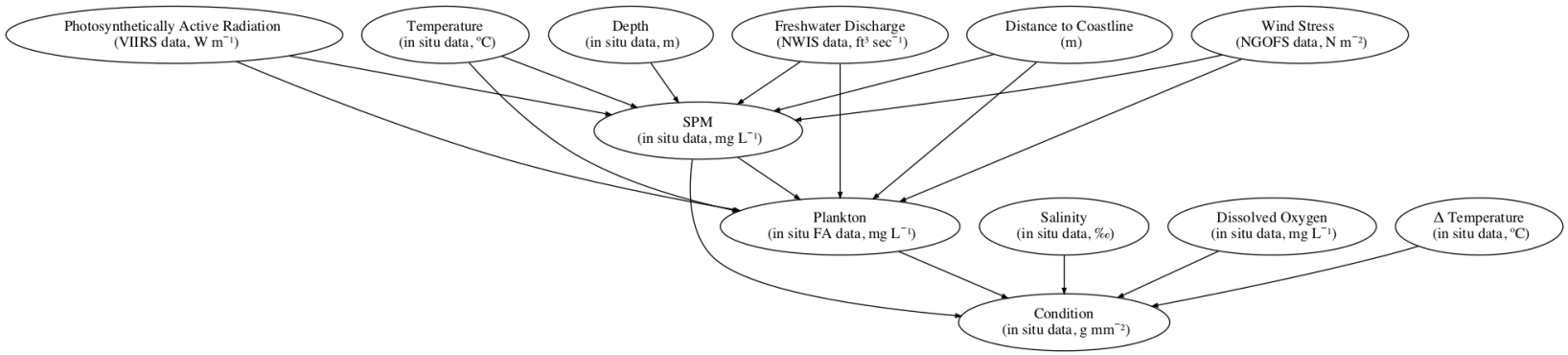

Figure 1.1 *A conceptual diagram of relationships among covariates that were used in predictive models for suspended particulate matter (SPM), phytoplankton fatty acids (FAs), and oyster condition.*

Data sources and measurement units are denoted in parentheses.

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CHAPTER II – SUSPENDED PARTICULATE MATTER AT MISSISSIPPI SOUND OYSTER REEFS

2.1 Introduction

Understanding the food resources of oysters requires knowledge of what is available in the seston. In coastal waters, the seston is composed mainly of plankton, detritus, and suspended sediments, the latter of which are the result of riverine inputs and resuspension of bottom sediments (Miller and McKee 2004). Seston provides many ecological benefits, such as providing allochthonous sources of mineralized nutrients to phytoplankton and vegetated habitats (Green and Coco 2014). The transport of seston within estuaries is a complex process involving tidal and wind driven water flows and primarily mediated by resuspension, settlement, flocculation, and river discharge (Kranck and Milligan 1992). Flocculation is the aggregation of fine particles into "flocs" and is caused by the collision of fine particles. It is an important process mediating both particle size and the concentration of seston in estuaries and has important effects on oyster feeding environments. Flocculation is a function of seston concentration and turbulent shear stresses, which in microtidal estuaries are dominated by the effects of wind and surface waves (Van Leussen et al. 1988, Whipple et al. 2006, Manning et al. 2017). Flocculation also increases at higher salinities because of attractive forces resulting from the interaction of negatively-charged clay particles with positively-charged sodium ions. Additionally, cohesive forces are strengthened by organic extracellular polymeric substances excreted from phytoplankton and bacteria (Manning et al. 2017). Large organic flocs may be an important food resource for oysters (Bayne 2017b).

Living phytoplankton, particularly diatoms, are the most important food source for oysters (Bayne 2017b). In clear water estuaries, benthic microplankton (microphytobenthos) may support up to half of total autotrophic production (Blackford 2002); however, in less-turbulent conditions, the resuspension of the microphytobenthos is limited (Hsieh et al. 2000). Phytoplankton concentrations are regulated by light, nutrients, and temperature. The light available to the phytoplankton is a function of photosynthetically active radiation (PAR), depth, and turbidity and is often the limiting factor for phytoplankton production in well-mixed estuaries (Dalsgaard et al. 2003).

Organic detrital particles may be degraded dead plankton or plant matter that originated from aquatic or terrestrial sources (Bayne 2017a) or biodeposits (feces and pseudofeces) (Lowe et al. 2014). Local detrital production in estuaries can be formulated as the combination of biodeposit production and phyto- and zooplankton mortality minus the losses from sinking and grazing; however, the transport of detrital material to and from other locations (including terrestrial locations) is often a significant contributor to the seston (Grant et al. 2007). Detrital particles alone, particularly old refractory-carbon rich detrital particles, have low nutritive value for oysters; however, the colonization of these particles by bacteria over time can substantially enhance the nutritive value through remineralization (Bayne 2017a). Depending on the type of detritus and the level of bacterial colonization, detritus may contribute from 2–60% of oyster carbon demand (Crosby et al. 1990). Organic detritus may be particularly important to bivalves during periods of low phytoplankton availability (Grant et al. 2007).

Besides being the only food source for oysters, the seston can affect oyster biology by regulating oyster clearance and consumption rates (Hofmann et al. 1992,

Fulford et al. 2007, Suedel et al. 2014, Ehrich and Harris 2015, Gernez et al. 2017) and by acting as a carrier of toxic contaminants (Lytle and Lytle 1982, Elston et al. 2005, Loh et al. 2018). The clearance or filtration rate is the volume of water cleared of a particular particle size range within a unit of time. The food consumption or ingestion rate differs from the clearance rate, in that the ingestion rate does not include the rejected particles with low organic content (Bayne 2014).

Extremely high seston concentrations are not beneficial for oysters (Le Peyre et al. 2019). Both natural (e.g. storms, flooding) and anthropogenic processes (e.g., dredging, freshwater diversions) can result in increases of suspended sediments that are detrimental to oyster reefs. Suspended particulate matter (SPM) concentrations as low as 100 mg L^{-1}, especially of small size classes of SPM, may have sublethal effects on oysters by decreasing clearance and food consumption rates (Shumway et al. 2003, Gernez et al. 2017, Le Peyre et al. 2019) as well as oxygen consumption rates (Shumway and Koehn 1982, Le Peyre et al. 2019). Additionally, increased seston loads are often associated with lower organic content because high seston loads are often the result of resuspended particulate inorganic matter (Bayne 2017). Also, high levels of sediment deposition can result in burial and subsequent death of adult oysters (Comeau 2014) and inhibit recruitment of oyster larvae (Thomsen and McGladdery 2006). Note, however, research suggests that oysters can adapt to highly turbid areas by developing smaller gills and larger labial palps (Barillé et al. 2000). The effect of seston concentrations on oyster ecology is, therefore, dependent on the seston's size classes and magnitude of its concentrations.

Bayne (2017) defines oyster *diet* as the "component of the seston that is available to the oyster for ingestion" and *ration* as the fraction of the diet that is "actually ingested and digested". In this context for diet, seston quantity can be estimated as SPM, the fraction of the seston that is actually measured; however, this measurement is dependent on sampling method and type of filters used (Bayne 2017). Additionally, SPM is composed of both particulate organic matter (POM) and particulate inorganic matter (PIM), and the preponderance of denser inorganic particles means that SPM measurements give little insight into the quantity of digestible food available for oysters (Adams et al. 2019). Therefore, SPM indicates potential food availability and can overestimate food resources.

Considering the importance of seston to ecosystem processes, many studies have described SPM distributions and dynamics in estuaries, including within the Mississippi Sound (Cai et al. 2012, Lin et al. 2012, Bera 2014, O'Brien 2019); however, previous research has not attempted to develop predictive models of SPM concentrations at oyster reefs within the western Mississippi Sound and Bay St. Louis. The exceptions are remote sensing models that are useful for estimating in situ SPM concentrations in real-time (Miller and McKee 2004, Nechad et al. 2010, Merritt 2016) and are useful for validating numerical models, The objective of this chapter was to investigate factors affecting SPM concentrations from spring to fall at six western Mississippi Sound oyster reefs. SPM will be used as a covariate in a morphometric condition model for oysters, as a proxy for potential food availability (Chapter IV).

2.2 Hypothesis

SPM concentrations can be best predicted by some or all of the following variables: freshwater discharge, distance to coastline, wind stress, photosynthetically active radiation, depth, temperature, salinity, and dissolved oxygen, with consideration of spatial and temporal effects. In addition, freshwater discharge is often related to sediment supplies from upland sources, therefore distance to coastline can serve as a proxy for salinity in estuarine areas.

2.3 Materials and Methods

2.3.1 Summary

Water samples were taken at live and dead oyster reefs from May to November at six sites in 2018. Only three sites were sampled in April, right after the Bonnet Carré Spillway closed, so these were not included in the analysis. Water samples were filtered to determine suspended particulate matter (SPM) concentrations at each site. Based on this data, a model was developed that predicts SPM concentrations based on physical conditions at oyster reefs.

2.3.2 Study Area

Oyster reefs cover over 20 km^2 of the area west of the Gulfport Ship Channel in the Mississippi Sound. Notable reefs include St. Joe's, Telegraph, Pass Marianne, Square Handkerchief, and St. Stanislaus Reefs. Water samples were taken monthly from Apr to Nov and covered the main growth and reproductive season during 2018 at six oyster reef locations within the western Mississippi Sound (Fig. 2.1). These stations covered a variety of salinity regimes occurring within the western Mississippi Sound and represented the majority of historical oyster reefs. The western Mississippi Sound is a

shallow tide-dominated system that, until recently, supported a valuable oyster fishery, but the fishery has been in decline since facing multiple stressors including Hurricane Katrina, the Deepwater Horizon Oil Spill, and increases in Dermo attributed to rising sea-surface temperatures (Powell 2017). The western Mississippi Sound was historically influenced by natural freshwater inputs from nearby sources, including the Pearl River, Jourdan River, Wolf River, and Pascagoula River (Sanial et al. 2019). However, in recent years, managed openings of the Bonnet Carré Spillway in response to sporadic flooding events has led to unprecedented freshwater inputs from the Mississippi River (Sanial et al. 2019). The most recent Bonnet Carré Spillway openings have coincided with near total oyster mortality in the western Mississippi Sound (USM 2019). Note that the opening of the Bonnet Carré Spillway from 8 Mar 2018 to 30 Mar 2018 had a substantial effect on salinities and other water quality parameters for the early part of the sampling period.

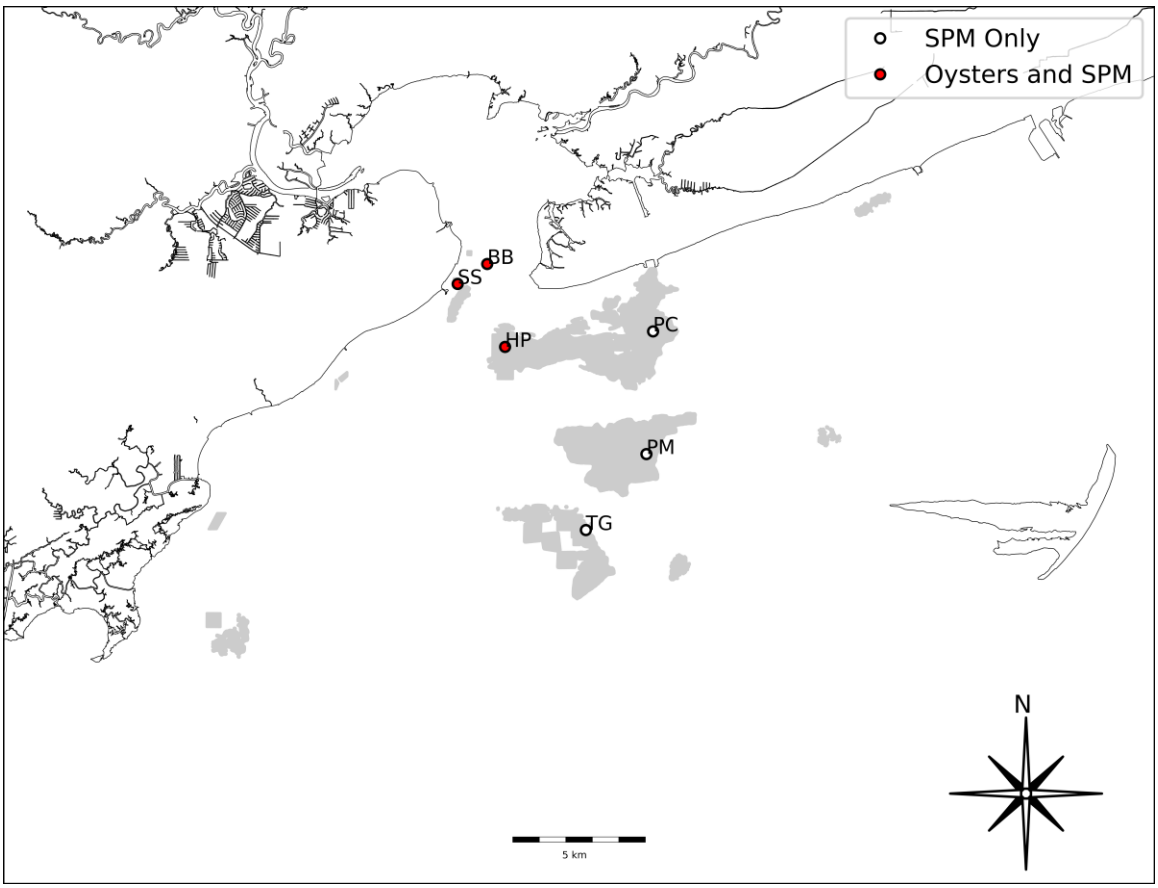

Figure 2.1 *Map of sampling locations in the Mississippi Sound.*

Light gray depicts oyster habitat. White circles depict locations where only SPM was sampled; red circles depict locations where SPM and oysters were sampled for analysis. SS: St. Stanislaus Reef, BB: between the bridges; HP: Henderson Point Reef; PC: Pass Christian Reef; PM: Pass Marianne Reef; TG: Telegraph Reef.

2.3.3 Sample Collection and Preprocessing

Surface water samples (3 L each) were collected at each location using a bucket and rope and maintained at 4–8 ºC in three 1-L amber Nalgene jars. A YSI was used to collect the in-situ bottom temperature, bottom salinity, and bottom dissolved oxygen data.

Water was filtered for SPM following guidelines in APHA (1999). Prior to filtration, 47-mm Whatman GF/F glass fiber filters were pre-ashed for 2 hours at 475 ºC in a muffle furnace and weighed. For each water sample, each filter was rinsed with

deionized water prior to filtration to minimize leaching. Replicate water samples from each sampling location each month were combined, and two subsamples were vacuum-filtered through the filters until the filters were adequately covered in SPM and filtration flow rate was greatly reduced. Target filtration time was 3–10 minutes, which resulted in replicate water volumes of 300–550 mL. Filters were frozen at −80 ºC until analysis for SPM.

2.3.4 Suspended Particulate Matter

To determine SPM concentrations, duplicate filters were dried for 1 hr at 105 ºC and weighed. Suspended particulate matter was determined as the initial mass of the filter subtracted from the mass of the filter with sample after filtration. The concentration of SPM was then determined by dividing this value by the corresponding volume of filtered water sample.

2.3.5 Model Development

A hierarchical Bayesian model was used to predict suspended particulate matter from freshwater discharge, distance to coastline, wind stress, photosynthetically active radiation, depth, bottom temperature, PAR and dissolved oxygen (Fig. 2.2). Sampling location was used as a grouping variable, with distance from coastline and depth as a covariates at this level. Prior to the model development, potential multicollinearity was examined using pair-wise Pearson's correlation coefficients (r) among the potential covariates. Because dissolved oxygen was highly correlated with bottom temperature (r = -0.83), it was removed from the candidate covariates.

Coastline data was obtained from the Global Self-consistent, Hierarchical, High-resolution Geography database (GSHHS), and distance to coastline was calculated based

on projected distances (transverse Mercator in the WGS 84 coordinate reference system) from shore using the pyproj library in Python 3. Upstream river discharge data was obtained from the USGS National Water Information Web Interface for two nearby stations: Pearl River at National Space Technology Laboratories (USGS 02492620) and Wolf River near Landon, MS (USGS 02481510). Mean daily discharge rates ($ft^3\ sec^{-1}$). was calculated for each month and at each station and then the freshwater discharge rates at the two stations were averaged to represent freshwater inputs in each month. Mean daily discharge rates for the Bonnet Carré Spillway were obtained from the USACE New Orleans District Website and was applied in April of 2018. Photosynthetically active radiation (PAR) data from the Suomi National Polar-orbiting Partnership's (S-NPP) Visible and Infrared Imager/Radiometer Suite (VIIRS) sensor was obtained from the NASA Goddard Space Flight Center OceanColor website's Level 3 Browser. Wind stress was used to model turbulence effects on sediment resuspension. Modeled wind velocity data at 1/100º (about 1 km) resolution were obtained from the Northern Gulf of Mexico Operational Forecast System (NGOFS). Wind stress (τ) in N m^{-2} was calculated as

$$\tau = C_D \rho_{air} U^2,$$

where U is wind speed (m s^{-1}) at 10 m above sea surface, ρ_{air} is a constant representing the density of air (1.22 kg m^{-3}), and C_D is a dimensionless drag coefficient set to 0.0013 (Kraus 1972).

Full models with all the covariates, with and without the grouping variables, were compared using the posterior predictive loss (PPL) (Spiegelhalter et al. 2002, Hobbs and Hooten 2016). Once the best grouping variables were determined, we compared the full models to the reduced models based on deviance information criterion (DIC). The lower

the PPL or DIC, the better the model predicts. Power of the final model was analyzed using the pwr package function pwr.f2.test() in R.

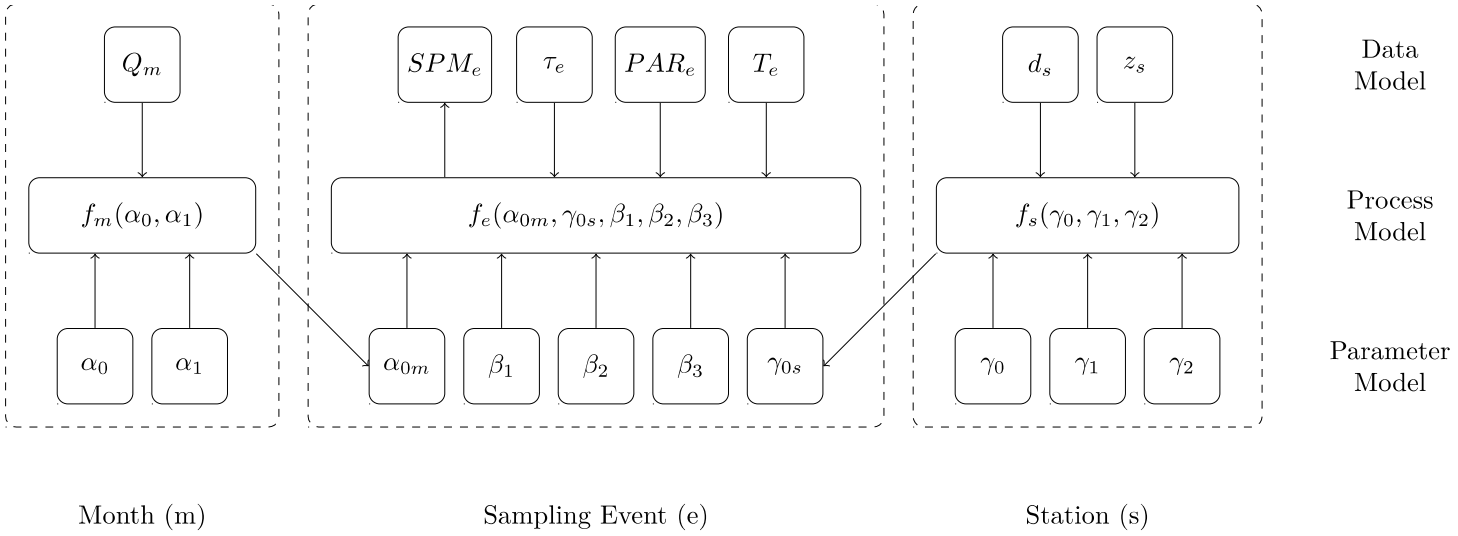

Figure 2.2 *A conceptual directed acyclic graph (DAG) of the multilevel structure for a predictive model of suspended particulate matter (SPM).*

The month (*m*) compartment has the parameters α_0 and α_1, which include the covariate for river discharge (Q_m). The station (*s*) compartment has the parameters γ_0, γ_1, and γ_2, which include the covariates for distance to shore (d_s) and depth (z_s). The sampling event (*e*) compartment has the parameters α_{0m}, γ_{0s}, β_1, β_2, and β_3, which are the parameters for the monthly intercept, the station intercept, wind stress (τ_{ms}), photosynthetically active radiation (PAR_{ms}), and bottom temperature (T_{ms}), respectively.

2.4 Results

2.4.1 Summary

The best model selected for predicting SPM was a single-level model that included wind stress, bottom salinity, and bottom temperature as the covariates (Table 2.3).

2.4.2 Environmental Data

Suspended particulate matter (SPM) concentrations during the 2018 project period ranged 16.5–59.7 mg L^{-1} for the study area (Fig. 2.3). SPM concentrations near Bay St. Louis peaked in August (Henderson Point: 46.9 mg L^{-1}; St. Stanislaus: 41.9 mg L^{-1}, Between Bridges: 44.6 mg L^{-1}); SPM concentrations further from the bay peaked in September (Pass Christian: 47.9 mg L^{-1}; Pass Marianne: 52.6 mg L^{-1}) and October (Telegraph: 59.7 mg L^{-1}) (Fig. 3). SPM concentrations were lowest for all sampling sites in November (Henderson Point: 22.6 mg L^{-1}; St. Stanislaus: 18.2 mg L^{-1}; Between Bridges: 16.5 mg L^{-1}; Pass Christian: 26.2 mg L^{-1}; Pass Marianne: 26.2 mg L^{-1}), with the exception of Telegraph Reef, which experienced the lowest SPM concentration in June (26.9 mg L^{-1}). Salinities at the sampling locations ranged from 10.29 to 28.42 ppt during the study period, with lowest salinities observed during May and highest salinities observed during August (Fig. 2.4). A summary of environmental variables that were measured for the study period can be found in Table 2.1.

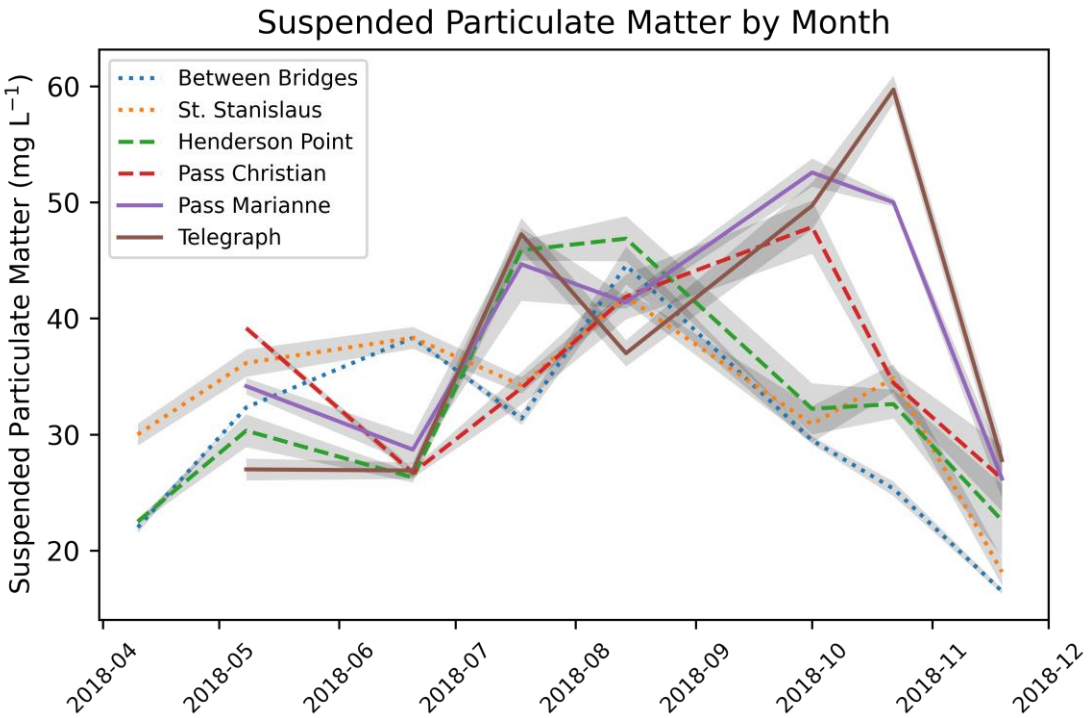

Figure 2.3 *Monthly suspended particulate matter (SPM) concentrations (mg L^{-1}) by sampling site.*

Solid lines represent the two most offshore sites; dashed lines represent intermediate sites; and dotted lines represent the most inshore sites. The shaded areas represent the area ±1 standard deviation.

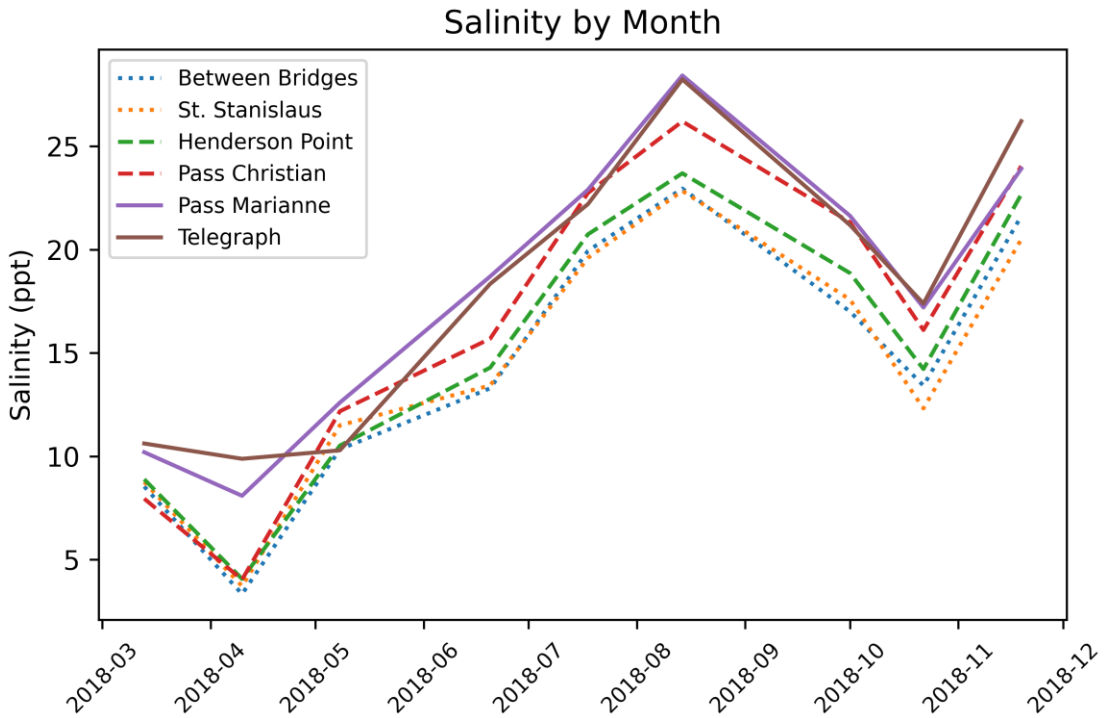

Figure 2.4 *Monthly salinity (ppt) by sampling site.*

Solid lines represent the two most offshore sites; dashed lines represent intermediate sites; and dotted lines represent the most inshore sites.

Table 2.1 *Summary of environmental variables measured for the study period.*

month	station	SPM (mg L^{-1})	bottom temperature (°C)	bottom salinity (ppt)	dissolved oxygen (mg L^{-1})	wind stress (N m^{-2})	PAR (einstein m^{-2} d^{-2})	depth (m)	distance from shore (m)	monthly discharge (ft^3 sec^{-1})
5	BB	32.3	25.1	10.34	6.88	0.008	45.1	2.8	1374	7772
5	SS	36.2	25.4	11.5	6.27	0.008	45.1	2.1	588	7772
5	HP	30.3	25.1	10.52	6.95	0.0118	44.4	3.5	2528	7772
5	PC	39.2	25	12.18	6.55	0.0093	48.6	2.8	2692	7772
5	PM	34.2	25.1	12.6	7.06	0.0165	49.2	1.7	6962	7772
5	TG	27.0	25.1	10.29	7.34	0.0217	47.2	1.5	8819	7772
6	BB	38.3	29.1	13.29	7.25	0.0004	52.7	2.8	1374	6586
6	SS	38.3	28.8	13.43	6.18	0.0003	52.7	2.1	588	6586
6	HP	26.3	29	14.29	6.56	0.0027	53.9	3.5	2528	6586
6	PC	26.7	28.9	15.69	5.57	0.0025	54.5	2.8	2692	6586
6	PM	28.7	28.9	18.7	6.2	0.0064	56.2	1.7	6962	6586
6	TG	26.9	28.8	18.34	6.67	0.0076	55.9	1.5	8819	6586
7	BB	31.4	29.2	19.94	5.21	0.0018	49.7	2.8	1374	7776
7	SS	34.3	29.2	19.6	5.22	0.0022	49.7	2.1	588	7776
7	HP	45.9	29.3	20.74	4.49	0.0083	49.9	3.5	2528	7776
7	PC	34.0	29.5	22.74	4.7	0.0094	49.4	2.8	2692	7776
7	PM	44.7	29	22.9	5.7	0.0206	51.2	1.7	6962	7776
7	TG	47.3	28.8	22.2	5.75	0.0227	48.7	1.5	8819	7776
8	BB	44.6	29.9	22.95	5.44	0.0012	32.5	2.8	1374	6281
8	SS	41.9	29.7	22.84	5.16	0.0013	32.5	2.1	588	6281
8	HP	46.9	29.7	23.69	6.1	0.0017	34.7	3.5	2528	6281
8	PC	41.9	29.9	26.2	4.97	0.002	36.6	2.8	2692	6281
8	PM	41.4	29.8	28.42	4.72	0.0047	36.7	1.7	6962	6281
8	TG	37.0	29.6	28.23	5.65	0.0062	36.3	1.5	8819	6281
9	BB	29.5	27.6	17	6.62	0.0106	38.7	2.8	1374	9059
9	SS	30.9	27.6	17.58	5.55	0.0114	38.7	2.1	588	9059
9	HP	32.2	27.9	18.86	6.71	0.016	39.3	3.5	2528	9059

Table 2.1 Continued

month	station	SPM (mg L^{-1})	bottom temperature (°C)	bottom salinity (ppt)	dissolved oxygen (mg L^{-1})	wind stress (N m^{-2})	PAR (einstein m^{-2} d^{-2})	depth (m)	distance from shore (m)	monthly discharge (ft^3 sec^{-1})
9	PC	47.9	27.8	21.32	5.86	0.0183	38.8	2.8	2692	9059
9	PM	52.6	27.8	21.63	6.41	0.0306	38.3	1.7	6962	9059
9	TG	49.7	28.3	21.18	6.77	0.0347	39.4	1.5	8819	9059
10	BB	25.3	21.7	13.42	7.59	0.0373	28.4	2.8	1374	4634
10	SS	34.9	21	12.32	7.62	0.0404	28.4	2.1	588	4634
10	HP	32.6	21.9	14.22	7.79	0.0428	27.9	3.5	2528	4634
10	PC	34.4	22	16.11	7.41	0.0437	29.2	2.8	2692	4634
10	PM	50.0	21.7	17.2	7.58	0.0692	31.2	1.7	6962	4634
10	TG	59.7	21.9	17.39	7.55	0.0784	31	1.5	8819	4634
11	BB	16.5	14.6	21.62	7.85	0.0055	26.8	2.8	1374	13942
11	SS	18.2	14.5	20.5	6.84	0.0058	26.8	2.1	588	13942
11	HP	22.6	14.2	22.67	8.17	0.0054	26.9	3.5	2528	13942
11	PC	26.2	14.3	24.04	8.55	0.0036	25.3	2.8	2692	13942
11	PM	26.2	14.1	23.91	9.46	0.0057	25	1.7	6962	13942
11	TG	27.8	15.7	26.21	8.44	0.0079	25.4	1.5	8819	13942

2.4.3 Models

A total of 14 model structures were compared (Table 2.2). The best model structure was selected as the model structure with the lowest posterior predictive loss. For multi-level models, covariates were grouped into some combination of month, station, and sampling event compartments, Month and station are either nested or additive. Additionally, models were compared using the representation of freshwater influence as either bottom salinity (represented either by a linear or a quadratic function) or the combination of distance from shore and monthly discharge. The model structure that was selected was the single-level model, using salinity to represent freshwater influence.

Table 2.2 *Comparison of model structures for predicting suspended particulate matter.*

sal and sal2 are the parameters for salinity and squared salinity.

event covariates	**month covariates**	**station covariates**	**random effects structure**	**posterior predictive loss**
wind, PAR, temp, discharge, dist, depth			none	0.00626
wind, PAR, temp, sal, depth			none	0.00603
wind, PAR, temp	discharge	dist, depth	month/station	0.01097
wind, PAR, temp			month/station	0.01060
wind, PAR, temp	discharge	dist, depth	station/month	0.01059
wind, PAR, temp			station/month	0.00986
wind, PAR, temp	discharge	dist, depth	non-nested	0.00666
wind, PAR, temp			non-nested	0.00657
wind, PAR, temp, sal		depth	station	0.00694
wind, PAR, temp, sal, sal^2		depth	station	0.00720
wind, PAR, temp, dist, depth	discharge		month	0.00604
wind, PAR, temp, dist, depth			month	0.00603
wind, PAR, temp, discharge		dist, depth	station	0.00687
wind, PAR, temp, discharge			station	0.00683

Using a the single-level model structure, covariates were excluded step-wise based on deviance information criteria (DIC) until no further model improvement could

be made. (DIC: -284.9, Table 2.3). The backward-selection procedure selected for wind stress, bottom temperature, and bottom salinity to be included in the final model predicting SPM (Figs. 2.5–2.6). All three covariates had a positive effect on SPM, with probabilities of the parameter being greater than zero close to 100.0%, 94.6%, and 96.8% for bottom temperature, bottom salinity, and wind stress, respectively. Sample sizes were deemed adequate based on power analysis of the final model (β=0.99).

Table 2.3 *Step-wise comparison of model covariates for predicting suspended particulate matter.*

Bolded is the best model.

event covariates	**random effects structure**	**mean deviance**	**penalty**	**penalized deviance**
Step 1				
PAR, temp, sal, depth	none	-279.6	6.341	-273.3
wind, temp, sal, depth	none	-293.0	6.331	-286.7
wind, PAR, sal, depth	none	-281.0	6.317	-274.7
wind, PAR, temp, depth	none	-290.3	6.319	-284.0
wind, PAR, temp, sal	none	-293.1	6.307	-286.8
Step 2				
PAR, temp, sal	none	-277.7	5.272	-272.4
wind, temp, sal	**none**	**-294.7**	**5.265**	**-289.5**
wind, PAR, sal	none	-282.3	5.283	-277.0
wind, PAR, temp	none	-290.9	5.264	-285.7
Step 3 (no improvement)				
temp, sal	none	-274.4	4.222	-270.1
wind, sal	none	-275.0	4.205	-270.8
wind, temp	none	-289.2	4.234	-284.9

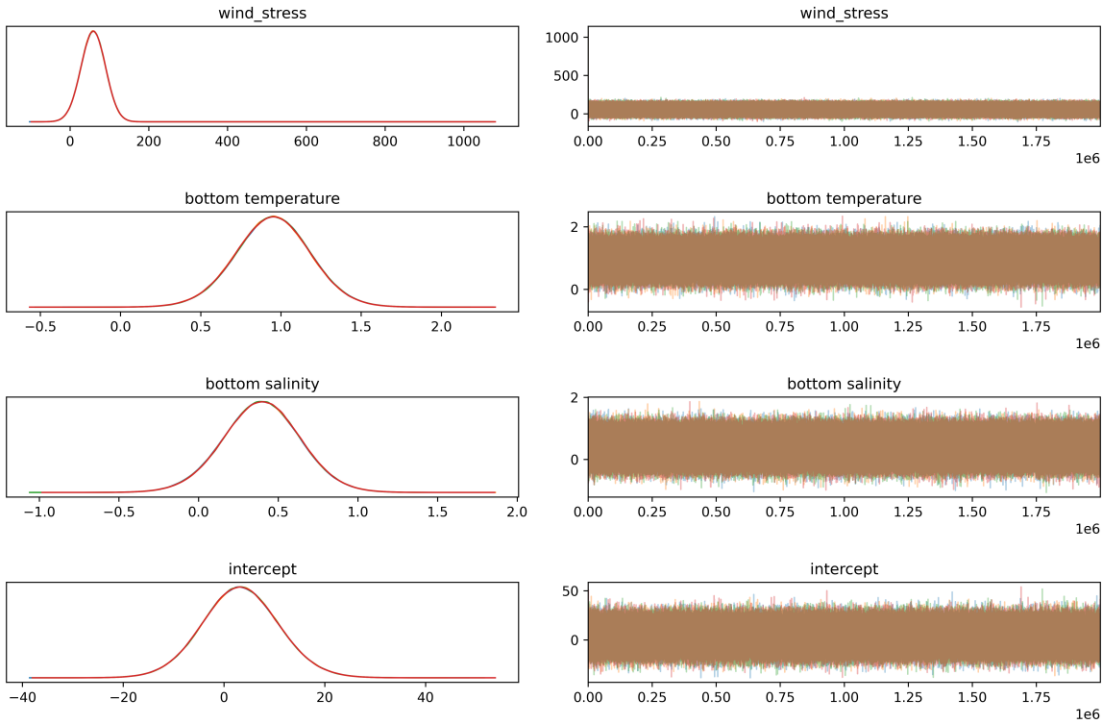

Figure 2.5 *Trace and density plots for the final model parameters.*

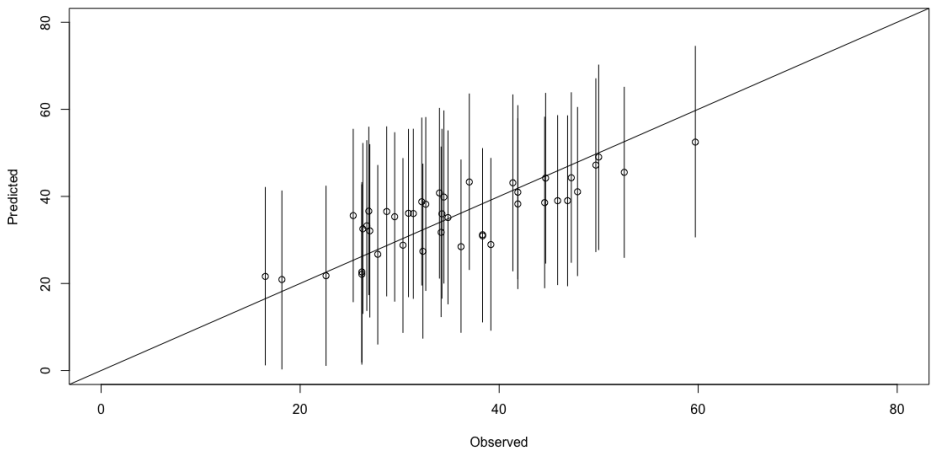

Figure 2.6 *Plot of predicted vs. observed suspended particulate matter concentrations (mg L^{-1}).*

The solid diagonal line represents an ideal 1:1 relationship. The open circles represent the medians on the y-axis and the vertical lines represent 95% credible interval of predictions.

2.5 Discussion

The present research used a Bayesian approach to model SPM concentrations in the western Mississippi Sound from environmental variables that were readily obtained with basic hydrological instrumentation or from existing public data sources. The Bayesian model found that wind stress, temperature, and salinity were useful predictors of SPM concentrations during the 2018 study period within the western Mississippi Sound.

Wind speed and river inflow are two key variables affecting fluid velocities and are often cited as important drivers of SPM in estuaries (Markensten and Pierson 2003), which is consistent with the results presented here. The observation that wind was positively correlated with SPM concentrations in a shallow water body with high organic content is not unexpected and agrees with many other observational studies of SPM in shallow estuaries (Fegley et al. 1992, Wilson-Ormond et al. 1997, Thoresen 2004). As a major factor in fluid velocities, wind affects bed shear stress and sediment resuspension (Markensten and Pierson 2003), which affects both the quantity and composition of the seston. Bed shear stress is also affected by surface roughness, which oyster reefs notably provide (Green and Coco 2014). Although unfortunately not examined here, sediment properties such as grain size and cohesivity are unquestionably important to inorganic SPM concentrations, particularly in shallow and sedimentologically-diverse estuaries.

In the final model selected, river inflow was approximated by bottom salinity. Salinities varied more temporally than spatially and were elevated during the summer months of the study period. This is likely due to the increasingly shoreward wind direction during the summer coupled with lower freshwater inputs. A single salinity

parameter was selected over the quadratic form, but this could relate to the limited salinity range encountered within the study area (10.29–28.42 ppt). The positive relationship between salinity and SPM was not expected, because lower salinities have been associated with increased particulate matter in the study area (Cai et al. 2012). It is possible that an unmodeled interaction between temperature and salinity was responsible for this, because higher salinities were observed during the warmest months of the study period. Indeed, the positive relationship between SPM and temperature may suggest SPM became increasingly dominant during the warmer months because of temperature-induced increases in phytoplankton production.

Seston quantity is a key parameter in mechanistic models of aquatic ecosystems, oyster bioenergetics, and oyster population dynamics (Powell et al. 1995, Filgueira et al 2014). Though model results presented here are based on limited sampling both spatially and temporally in atypical environmental conditions, they provide a valuable snapshot of factors affecting seston quantity, an important biological determinant of oyster fitness, during the period leading up to the ultimate collapse of the western Mississippi Sound oyster population that occurred the following year.

Because this model was regionally calibrated over a matter of months, model parameters should not be applied to different locations and times. Further research would be necessary to determine the variability and suitability of the parameters in the western Mississippi Sound over multiple years. Future work should examine the effect of localized grazing on SPM at oyster reefs by sampling non-reef control sites in the same area. Additionally, model predictions may be improved if in situ wind measurements are used rather than the modeled wind velocities used here. Finally, the inclusion of tidal

currents would likely help model a more complete picture of fluid velocities that affect resuspension of particulate matter. The exclusion of certain covariates from the final model does not mean that these variables are necessarily inconsequential. It only means that their predictive power on variability in SPM was too low given the limited temporal and spatial scope of the data. It should be noted that SPM measurements were not corrected for salinity, which likely created a significant source of error for the SPM estimates (Stavn et al 2009).

The Bayesian approach used here could easily be applied to other regions and improve predictions of SPM concentrations in response to different environmental scenarios for ecosystem forecasting. Furthermore, the predictive models of SPM may be linked to spatial hydrodynamic models, because transport is a key mechanism for SPM dynamics. However, implementing numerical hydrodynamic models of SPM is a huge undertaking, and because of this, the development of a mechanistic model of SPM was outside of the scope of the current research. In the final chapter of this dissertation, in situ SPM concentrations will be related to oyster condition in the western Mississippi Sound.

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CHAPTER III – POLYUNSATURATED FATTY ACID AVAILABILITIES AT MISSISSIPPI SOUND OYSTER REEFS

3.1 Introduction

Seston quality is an often-ignored variable in oyster bioenergetic models. This may be partly due to lack of spatial data on food quality. The quality of the seston depends on its capacity to meet consumer nutritional requirements and can be represented indirectly using various ratios (e.g., chlorophyll content, carbon:nitrogen, and carbon:chl *a*) (Bayne 2014) or more directly using biochemical analysis (e.g., fatty acid composition) (Navarro and Thompson 1995). Most studies of the seston in respect to the eastern oyster have used indirect methods for estimating seston quality. Berg and Newell (1986) examined seasonal seston quality using ratios of POC, PON, and energy content (determined using a dichromate-sulfuric acid digestion) of SPM in the Choptank River, Maryland with respect to oysters. They found that decreases in food quality were caused by increases in inorganic matter but concluded that selective feeding by oysters made food quantity more biologically relevant. The indirect measurement of seston quality showed large temporal variability. Fegley et al. (1992) examined sub-daily fluctuations in seston quality over an 11-hr period in the Great Sound, New Jersey. They found that seston quality indicated by the ratio of C or N to SPM was highest around low tide and mid-flood tide. Wilson-Ormond et al. (1997) also examined sub-daily fluctuations in seston quality in Galveston Bay, Texas nine times over the course of a year. Food quality indicated by the ratio of organic carbon to Chl *a* and organic nitrogen and food quantity was highest in spring and late summer/early fall. In contrast to the limited work of Fegley et al. (1992), Wilson-Ormond et al. (1997) found that food quantity and quality were

affected by diel rather than tidal cycles. Food quality was typically highest during mid-morning and early evening, although there were some inconsistencies between the two food quality measures (ratio of organic carbon to Chl *a* and organic nitrogen). The authors concluded that oyster populations are limited by food quantity and water flow rates, rather than food quality. Thoreson (2004) examined seston quantity and quality (carbon to nitrogen ratio, percent phaeopigment, and organic carbon to Chl *a* ratio) at five sites in the Duplin River, Georgia over multiple temporal scales (tidal, lunar, and seasonal). Although Thoreson (2004) found significant patterns in seston quantity and quality at all timescales, relationships were inconsistent and site-specific. The author also observed spatial gradients in seston quantity and quality. Similar gradients in food availability (measured as the sum of labile carbohydrate, particulate protein, and lipid) were observed at 15 sites in Delaware Bay by Powell et al. (2012), who suggested that food supply was depressed at sites with high oyster biomass because of the top-down influence of oyster feeding. Powell et al. (2012) observed a peak in food availability in the spring with a smaller peak in the fall, noting that food supply could not be predicted on the basis of physical variables alone.

While such investigations give insights into oyster nutritional environments, empirical support for the use of indirect measures of seston quality is weak. For example, C:N ratios are not strongly associated with growth in bivalves (Flaak and Epifanio 1978, Carmichael et al. 2004), which appears to be more limited by energy than protein (Hawkins and Bayne 1991). Additionally, Chl *a* is a poor measure of food quality because oysters are dependent on food sources other than phytoplankton (Soniet et al. 1998). Though previous research emphasizes the importance of food quantity, it also

demonstrates the variabilities related to the indicators used in food quality. Determining seston quantity is relatively straightforward, but proper determination of seston quality in respect to oyster nutrition requires more direct methods.

A fatty acid trophic marker approach provides a versatile way to characterize the nutritional quality of the seston. This approach not only discriminates among phytoplankton, heterotrophic, and terrestrial sources of carbon but can further partition phytoplankton into phytoplankton functional groups (PFGs). Generally, in light-limited conditions, phytoplankton increase saturated fatty acids (SFAs) in the storage lipids. During the exponential growth phase of a phytoplankton bloom, when nutrients are not limiting, phytoplankton accumulate polyunsaturated fatty acids (PUFA) in the membrane lipids. Phytoplankton respond to increased temperature by increasing the saturation of membrane lipids (Dalsgaard et al. 2003). The saturated fatty acids 14:0 and 16:0 are abundant across most phytoplankton taxa; however unsaturated fatty acids are often characteristic of specific PFGs (Reuss and Poulsen 2002). For example, diatoms have elevated 16:1n-7 and 20:5n-3 (EPA) and dinoflagellates have elevated 18:4n-3 and 22:6n-3 (DHA) (Peters et al. 2006). Additionally, bacteria have high levels of branched-chain FA, and terrestrial sources have high levels of 18:3n-3 (ALA) and 18:2n-6 (LA) (Copeman et al. 2009).

In addition to providing detailed information on the seston, phytoplankton fatty acid trophic markers (FATM) are also relevant to oyster nutrition, reproduction, and stress response (Pernet et al., 2010; Filimonova et al., 2016). Limited fatty acid data exist for marine and estuarine waters in other regions, such as Texas (Rooker et al. 2006, Turner and Rooker 2006), Virginia (Chu et al. 1990), Washington (Lowe et al. 2014),

Nova Scotia (Budge et al. 2014), Newfoundland (Copeman et al. 2009), and Greenland (Reuss and Poulsen 2002), but no studies have estimated fatty acid compositions of the seston in the northcentral GOM.

The objective of this chapter is to characterize monthly fluctuations in seston quality, as measured by fatty acid markers for phytoplankton and terrestrial sources, at oyster reefs in the western Mississippi Sound.

3.2 Hypothesis

As food quality is related to food quantity, in situ polyunsaturated fatty acid signals, including nutritionally important eicosapentaenoic acid (20:5n-3 EPA) and docosahexaenoic acid (22:6n-3 DHA) are best predicted by SPM concentration, freshwater discharge, distance from coastline, wind stress, photosynthetically active radiation, and temperature, in the context of time and space.

3.3 Materials and Methods

3.3.1 Summary

Water samples were taken at live and dead oyster reefs. Samples were filtered and analyzed to determine the availability of polyunsaturated fatty acids (DHA, EPA, ALA, and LA) at each site. Then a model was developed that predicts polyunsaturated fatty acid concentrations based on biological, hydrological, climatic, and physical conditions at oyster reefs.

3.3.2 Sample Collection and Preprocessing

Water samples were taken monthly for lipid analysis during 2018 from Apr to Nov that covered the main growing and reproduction season at six oyster reef locations within the western Mississippi Sound (Fig. 2.1). In situ data was collected and two

subsamples were filtered (300–550 mL) for each station as described in the methods of Chapter II. Filters were frozen at −80 ºC until fatty acid methyl ester (FAME) analysis.

3.3.3 Lipid Analysis

3.3.3.1 Fatty Acid Sample Extraction

Filters were cut into eight strips and extracted in 3 mL of 2:1 chloroform:methanol containing 0.005% BHT following Folch et al. (1957). SPM samples were vortexed, allowed to stand for 10 minutes, and centrifuged (900 × G, 2 min), and the supernatant was transferred to a clean 8-mL centrifuge tube using a pipette. Pellets were re-suspended in 1 mL of 2:1 chloroform:methanol and centrifuged, and the supernatant was combined with the supernatant from the first extraction. A 0.88% NaCl solution (1 mL) was added to the combined supernatant and vortexed for 10 s. Tubes were centrifuged, and the lower organic phase was transferred with a pasteur pipette into a clean 8-mL centrifuge tube. The remaining mixture was combined with 1 mL of chloroform and centrifuged, and the lower organic phase was combined with the organic phase from the previous phase separation. These combined organic phases were evaporated under nitrogen in a 25–30 ºC water bath. Each lipid sample was resuspended in 100 µL of chloroform, flushed with nitrogen, capped, and stored at −20 ºC until transmethylation.

3.3.3.2 Transesterification

Fatty acid samples were transmethylated in 1 mL of methanol containing 2.5% v/v sulfuric acid and tricosanoic acid TAG standard (20 µg) for 1.5 hr at 85 ºC. Hexane (1 mL) and 0.88% NaCl (1.5 mL) were combined with the fatty acid methyl ester (FAME) sample. Each sample was vortexed and centrifuged to achieve phase separation.

The upper hexane layer was collected, and the samples were backextracted once more in 1 mL of hexane. Samples were dried and suspended in hexane to achieve approximately 1 mg FAME/mL of hexane. Approximately 100 µL of this suspension was transferred into GC vials with 200-µL inserts for FAME analysis.

3.3.3.3 FAME Analysis

An automated liquid sampler injected 1 µL of sample concentrated at approximately 1 mg/mL into a split injector heated at 250 ºC (David et al. 2005). Separations were achieved using a highly polar capillary column coated with 50% cyanopropyl polysiloxane (0.25-µm film thickness, DB-23, Agilent Technologies, U.S.A.) and detected using flame ionization. Oven temperature began at 50 ºC for 1 min and was increased by 25 ºC/min until reaching 175 ºC. The temperature was then increased by 4 ºC/min until reaching 230 ºC. The final temperature was held for 5 minutes, which produces a total analysis time of approximately 25 min per sample run. Two standards were used to determine retention times for the examined fatty acids (Restek 35066, NuChek GCL490); and a 23:0 standard was used for quantitation of peak area.

3.3.3.4 Chromatogram Processing and Analysis

Chromatograms were processed using OpenChrom Community Edition 1.4 (Wenig and Odermatt 2010). Baselines were corrected using a sensitive nonlinear iterative peak (SNIP) filter. Peak locations were determined using the first derivative peak detector, and each peak was identified by comparing retention times to known external standards. Peak areas were estimated using the trapezoid peak integrator and quantitated based on the internal 23:0 standard concentration. Peaks with an intensity less

than 2700 collectively represented less than 1% of total FA and were excluded from analysis. Fatty acids were quantified as proportions based on the individual chromatogram peak areas divided by the total area under all peaks. Fatty acid masses were calculated as the product of the proportional values and total sample lipids. Fatty acid concentrations were expressed as mg FA/L seawater.

3.3.4 Model Development

Hierarchical Bayesian models were used to predict the concentrations of two indicative phytoplankton fatty acid signals (EPA and DHA) from SPM concentration, freshwater discharge, distance from coastline, wind stress, photosynthetically active radiation, and temperature (Fig. 3.1). Sampling location was used as a grouping variable, with distance from coastline as a covariate at this level. The estimation of freshwater discharge, distance from coastline, wind stress (an important determinant of stratification), and photosynthetically active radiation are described in the previous chapter. Likewise, in situ measurements of SPM concentration and temperature from the previous chapter were used.

Similarly, four other biologically important fatty acid indicators, total phytoplankton FA (EPA+DHA), total terrestrial FA (ALA+LA), total PUFA (EPA+DHA+ ALA+LA), and plankton:terrestrial FA were predicted using hierarchical Bayesian models from freshwater discharge, distance from coastline, and temperature, with distance from coastline as a covariate at the sampling location level. For each FA response variable, eight model structures were compared with the full set of covariates (Table 3.2). Covariates were grouped into some combination of month and station, either nested or non-nested (additive). Additionally, structures were compared using the

representation of freshwater influence as either bottom salinity or the combination of distance from shore and monthly discharge.

Full models with all the covariates, with and without the grouping variables, were compared using the posterior predictive loss (PPL) (Spiegelhalter et al. 2002, Hobbs and Hooten 2016). Once the best grouping variables were determined, we compared the full models to the reduced models based on deviance information criterion (DIC). The lower the PPL or DIC, the better the model prediction. Potential multicollinearity in each model was examined using pair-wise Pearson's correlation coefficients (r) among the potential covariates. Highly-correlated covariates ($r > 0.80$) were removed from the candidate covariates.

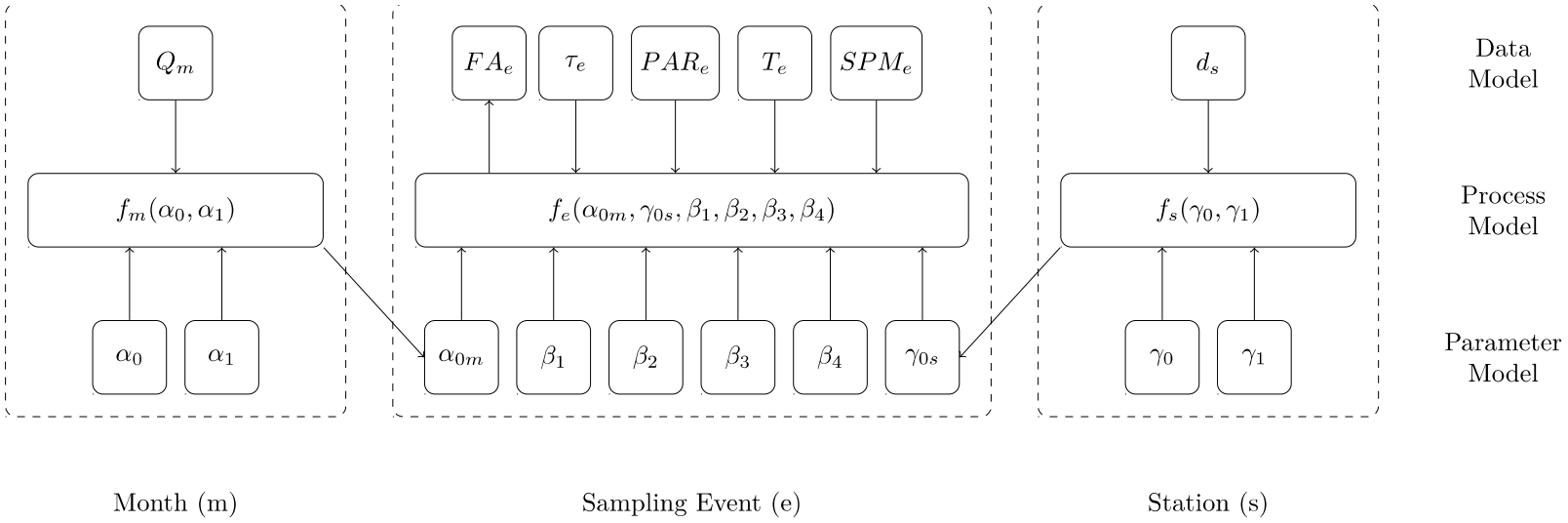

Figure 3.1 *A conceptual directed acyclic graph (DAG) of the multilevel structure for the predictive model of fatty acid concentrations (FA).*

The month (*m*) compartment has the parameters α_0 and α_1, which include the covariate for river discharge (Q_m). The station (*s*) compartment has the parameters γ_0 and γ_1, which include the covariate for distance to shore (d_s). The sampling event (*e*) compartment has the parameters α_{0m}, γ_{0s}, β_1, β_2, β_3, and β_4, which are the parameters for monthly intercept, station intercept, wind stress (τ_e), photosynthetically active radiation (PAR_e), bottom temperature (T_e), and suspended particulate matter (SPM_e), respectively.

3.4 Results

3.4.1 Environmental Data

Samples were analyzed for fatty acids (FA) from six stations during the Apr–Nov 2018 sampling period (Table 3.1). The standards used allowed for the identification of 20 fatty acid species, including the FAs relevant to this study (LA, ALA, EPA, and DHA), though other significant peaks were detected.

EPA concentrations peaked in August for stations within and around Bay St. Louis (BB, HP, SS) and in November for stations (PC, PM, TG) within the Mississippi Sound (Fig 3.2). DHA concentrations peaked earlier at the more riverine-influenced stations in May (SS) and June (BB), but during November at the more marine-influenced sites (HP, PC, PM, TG) (Fig. 3.3). Total phytoplankton PUFA followed a similar pattern to EPA and DHA with early peaks at the more riverine stations in May (SS) and June (BB), but a later November peak at the more marine sites (HP, PC, PM, TG). Terrestrial PUFA concentrations peaked at the more riverine sites in October (BB, SS) and at the more saline sites in November (HP, PC, PM), with the exception of TG, which had a September peak. Total PUFA concentrations peaked at the more riverine sites in May (SS) and October (BB) and at the more marine sites in November (HP, PC, PM, TG). The ratio of phytoplankton to terrestrial PUFA peaked at the more riverine sites in May (BB, SS) and in July (PC), August (HP), October (PM), and November (TG) for the more marine sites.

Table 3.1 *Mean concentrations of some biologically-relevant fatty acids (mg L^{-1}) at oyster reefs during May–Nov 2018.*

phyto: total phytoplankton PUFA (EPA+DHA), terra: total terrestrial PUFA (ALA+LA), PUFA: (EPA+DHA+ALA+LA), and phyto:terra: planktonic:terrestrial PUFA

Sampling Month	station	LA	ALA	EPA	DHA	terra	phyto	PUFA	phyto:terra
May	BB	0.58	0.77	1.48	1.57	1.36	3.05	4.41	2.24
May	HP	0.58	0.71	1.26	1.95	1.30	3.21	4.51	2.47
May	PC	0.30	0.41	0.84	0.92	0.71	1.75	2.46	2.47
May	PM	0.44	0.52	0.98	1.20	0.96	2.18	3.14	2.27
May	SS	0.90	1.00	1.83	3.35	1.90	5.18	7.08	2.73
May	TG	0.38	0.62	1.23	1.20	1.00	2.43	3.43	2.43
June	BB	0.97	1.49	1.51	2.43	2.46	3.94	6.41	1.60
June	HP	0.76	1.34	1.44	2.12	2.10	3.56	5.66	1.70
June	PC	0.96	1.44	1.98	2.78	2.40	4.76	7.16	1.98
June	PM	0.48	0.67	1.56	1.52	1.15	3.09	4.24	2.68
June	SS	0.79	1.58	1.13	1.08	2.37	2.21	4.58	0.93
June	TG	0.68	0.63	1.79	2.23	1.31	4.02	5.34	3.06
July	BB	0.78	1.59	0.89	0.58	2.37	1.47	3.84	0.62
July	HP	0.50	0.52	1.33	0.79	1.02	2.12	3.14	2.07
July	PC	0.56	0.41	2.21	2.12	0.97	4.32	5.30	4.44
July	PM	0.34	0.16	1.17	1.17	0.51	2.34	2.84	4.63
July	SS	0.50	1.06	0.70	0.38	1.56	1.08	2.64	0.69
July	TG	0.35	ND	0.76	0.82	0.35	1.58	1.93	4.56
August	BB	0.59	0.92	2.18	0.91	1.50	3.09	4.60	2.06
August	HP	0.42	0.45	3.28	0.77	0.87	4.05	4.92	4.63
August	PC	0.34	0.47	1.47	0.71	0.82	2.19	3.00	2.68
August	PM	0.39	0.50	0.96	0.79	0.89	1.75	2.63	1.97
August	SS	0.72	1.14	2.12	0.99	1.86	3.11	4.97	1.67
August	TG	0.52	0.72	1.07	0.78	1.24	1.85	3.10	1.49
September	BB	1.18	2.28	1.85	1.06	3.46	2.90	6.36	0.84
September	HP	0.64	1.26	1.78	0.75	1.90	2.53	4.43	1.33
September	PC	0.36	0.41	1.41	0.59	0.78	2.00	2.77	2.57
September	PM	0.58	0.97	1.36	0.69	1.55	2.05	3.60	1.32
September	SS	0.69	1.39	1.20	0.72	2.08	1.92	4.00	0.92
September	TG	0.88	1.39	1.62	0.88	2.27	2.50	4.77	1.10
October	BB	6.39	1.18	1.15	0.69	7.57	1.84	9.41	0.24
October	HP	0.72	0.35	0.90	0.82	1.07	1.72	2.80	1.60
October	PC	0.39	0.22	1.27	1.11	0.61	2.38	2.99	3.91
October	PM	0.21	0.19	1.13	0.82	0.40	1.96	2.36	4.84
October	SS	2.18	0.62	0.71	0.42	2.80	1.13	3.93	0.40
October	TG	0.25	0.16	1.14	0.75	0.41	1.90	2.31	4.62
November	BB	0.37	1.23	0.79	0.71	1.59	1.50	3.09	0.94
November	HP	0.93	2.37	3.06	3.20	3.31	6.26	9.56	1.89
November	PC	1.17	2.27	3.52	3.74	3.43	7.26	10.70	2.11
November	PM	0.84	1.66	3.10	3.59	2.51	6.69	9.20	2.67
November	SS	0.28	0.80	0.54	0.33	1.08	0.87	1.95	0.80
November	TG	0.85	1.35	3.06	3.18	2.19	6.24	8.43	2.85

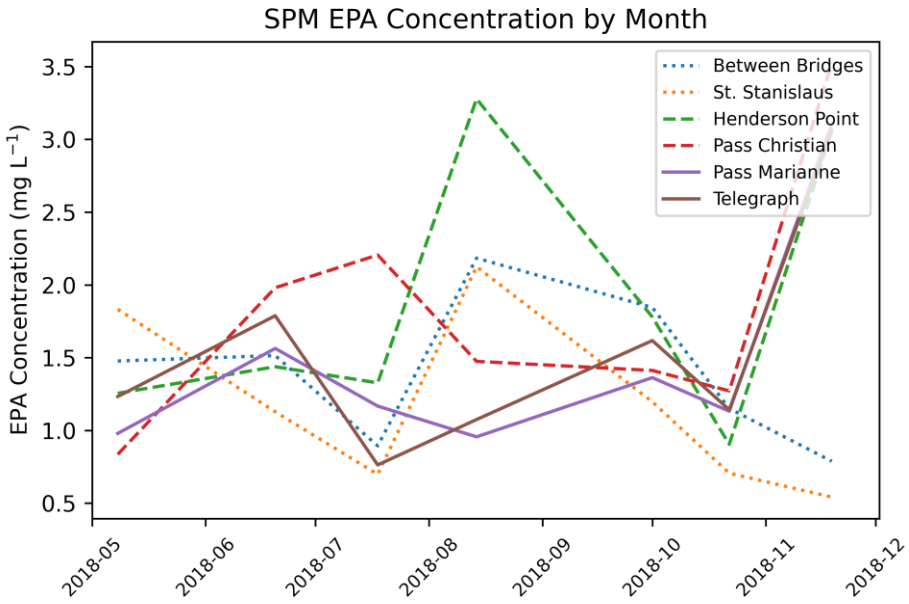

Figure 3.2 *Concentration of EPA by sampling site.*

Solid lines represent the two most offshore sites; dashed lines represent intermediate sites; and dotted lines represent the most inshore sites.

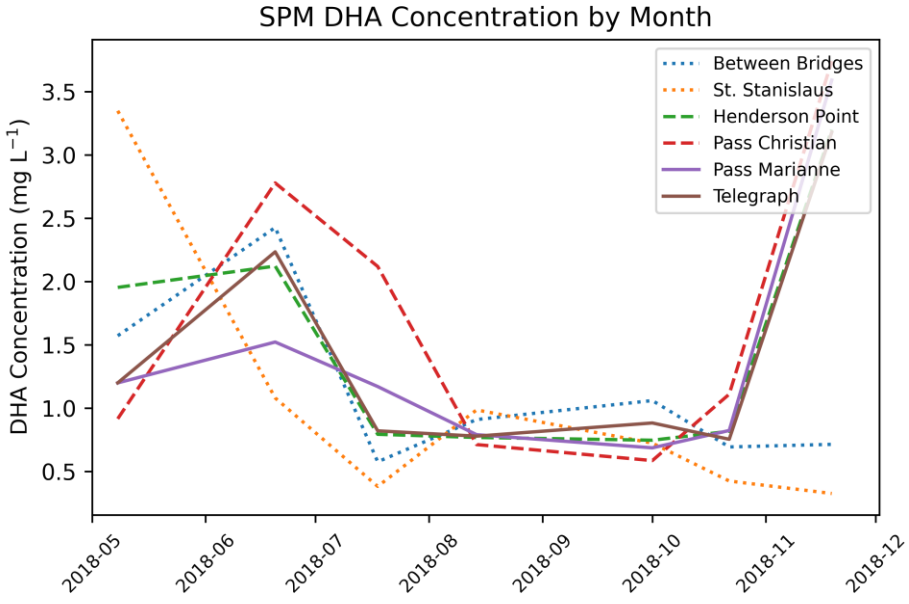

Figure 3.3 *Concentration of DHA by sampling site.*

Solid lines represent the two most offshore sites; dashed lines represent intermediate sites; and dotted lines represent the most inshore sites.

3.4.2 Predictive models of fatty acid concentrations

For EPA, DHA, total phytoplankton PUFA, total terrestrial PUFA, and total PUFA, the model structure that was selected was the single-level model, using salinity to represent freshwater influence. For phytoplankton:terrestrial PUFA, the model structure that was selected was the multilevel model with month and station compartments and freshwater influence represented by distance from shore and total discharge covariates.

Using the selected model structure, covariates were excluded step-wise based on deviance information criteria (DIC) until no further model improvement could be made. For EPA (Table 3.3), DHA (Table 3.4), total phytoplankton PUFA (Table 3.5), total terrestrial PUFA (Table 3.6), and total PUFA (Table 3.7), the backward-selection procedure selected the null models, indicating none of the covariates or combination of the covariates considered could predict these FA measures better than the null model. However, for phytoplankton:terrestrial PUFA , the backward-selection procedure selected the model with SPM, PAR, and bottom temperature as covariates (Table 3.8, *Figs*. 3.4–3.5). SPM and PAR had positive effects on the ratio of phytoplankton PUFA to terrestrial PUFA, with 96.8% and 79.2% probabilities of the parameters being greater than zero, respectively. Bottom temperature had a negative effect, with an 85.3% probability of the parameter being less than zero.

Table 3.2 *Comparison of posterior predictive loss among model structures for predicting EPA, DHA, total phytoplankton PUFA (phyto), total terrestrial PUFA (terra), total PUFA, and the ratio of phytoplankton to terrestrial PUFA.*

event covariates	**month covariates**	**station covariates**	**random effects structure**	**posterior predictive loss**					
				EPA	**DHA**	**phyto**	**terra**	**total PUFA**	**phyto: terra**
SPM, wind, PAR, temp, discharge, dist			none	0.00301	0.00303	0.00314	0.00308	0.00333	101.193
SPM, wind, PAR, temp, sal			none	0.00286	0.00288	0.00299	0.00293	0.00318	99.520
SPM, wind, PAR, temp	discharge	distance	non-nested	0.00445	0.00445	0.00456	0.00451	0.00474	88.479
SPM, wind, PAR, temp, discharge		distance	station	0.00367	0.00369	0.00380	0.00376	0.00402	94.625
SPM, wind, PAR, temp, dist	discharge		month	0.00363	0.00363	0.00374	0.00369	0.00388	96.507
SPM, wind, PAR, temp, sal, sal^2		distance	station	0.00387	0.00390	0.00400	0.00394	0.00422	98.325

Table 3.3 *Step-wise comparison of model covariates for predicting EPA.*

Bolded is the best model.

event covariates	**random effects structure**	**mean deviance**	**penalty**	**penalized deviance**
	Step 1			
wind, PAR, temp, sal	none	-328.4	6.319	-322.1
SPM, PAR, temp, sal	none	-328.4	6.295	-322.1
SPM, wind, temp, sal	none	-328.3	6.332	-322.0
SPM, wind, PAR, sal	none	-328.1	6.354	-321.8
SPM, wind, PAR, temp	none	-328.5	6.332	-322.1
	Step 2			
wind, PAR, temp	none	-330.5	5.308	-325.2
SPM, PAR, temp	none	-330.5	5.264	-325.2
SPM, wind, temp	none	-330.5	5.292	-325.2
SPM, wind, PAR	none	-330.2	5.269	-324.9
	Step 3			
PAR, temp	none	-332.6	4.206	-328.4
wind, temp	none	332.9	4.222	-328.7
wind, PAR	none	332.3	4.209	-328.0
	Step 4			
wind	none	-334.9	3.146	-331.7
temp	none	-334.9	3.162	-331.7
	Step 5			
null	**none**	**-336.8**	**2.099**	**-334.7**

Table 3.4 *Step-wise comparison of model covariates for predicting DHA.*

Bolded is the best model.

event covariates	**random effects structure**	**mean deviance**	**penalty**	**penalized deviance**
	Step 1			
wind, PAR, temp, sal	none	-328.4	6.332	-322.1
SPM, PAR, temp, sal	none	-328.4	6.335	-322.0
SPM, wind, temp, sal	none	-328.3	6.340	-322.0
SPM, wind_stress, PAR, sal	none	-328.2	6.346	-321.8
SPM, wind, PAR, temp	none	-328.4	6.359	-322.1
	Step 2			
wind, PAR, temp	none	-330.5	5.287	-325.3
SPM, PAR, temp	none	-330.5	5.283	-325.2
SPM, wind, temp	none	-330.5	5.283	-325.2
SPM, wind, PAR	none	-330.3	5.259	-325.1
	Step 3			
PAR, temp	none	-332.5	4.226	-328.3
wind, temp	none	-332.6	4.217	-328.3
wind, PAR	none	-332.3	4.238	-328.0
	Step 4			
wind	none	-334.3	3.166	-331.2
temp	none	-334.4	3.164	-331.2
	Step 5			
null	**none**	**-336.3**	**2.098**	**-334.2**

Table 3.5 *Step-wise comparison of model covariates for predicting total phytoplankton PUFA.*

Bolded is the best model.

event covariates	**random effects structure**	**mean deviance**	**penalty**	**penalized deviance**
	Step 1			
wind, par, temp, sal	none	-326.6	6.351	-320.3
SPM, par, temp, sal	none	-326.3	6.370	-319.9
SPM, wind, temp, sal	none	-326.6	6.335	-320.3
SPM, wind_stress, par, sal	none	-326.1	6.328	-319.8
SPM, wind, par, temp	none	-326.7	6.326	-320.4
	Step 2			
wind, PAR, temp	none	-328.7	5.282	-323.4
SPM, PAR, temp	none	-328.1	5.289	-322.9
SPM, wind, temp	none	-328.8	5.270	-323.5
SPM, wind, PAR	none	-328.3	5.274	-323.0
	Step 3			
wind, temp	none	-330.8	4.212	-326.6
SPM, temp	none	-330.3	4.210	-326.0
SPM, wind	none	-330.2	4.231	-326.0
	Step 4			
temp	none	-332.2	3.158	-329.0
wind	none	-332.2	3.170	-329.0
	Step 5			
null	**none**	**-333.7**	**2.118**	**-331.6**

Table 3.6 *Step-wise comparison of model covariates for predicting total terrestrial PUFA.*

Bolded is the best model.

event covariates	**random effects structure**	**mean deviance**	**penalty**	**penalized deviance**
	Step 1			
wind, par, temp, sal	none	-327.4	6.337	-321.1
SPM, par, temp, sal	none	-327.6	6.346	-321.2
SPM, wind, temp, sal	none	-327.4	6.356	-321.0
SPM, wind, par, sal	none	-327.5	6.337	-321.2
SPM, wind, par, temp	none	-327.5	6.331	-321.1
	Step 2			
PAR, temp, sal	none	-329.3	5.270	-324.0
SPM, temp, sal	none	-329.5	5.286	-324.2
SPM, PAR, sal	none	-329.6	5.281	-324.3
SPM, PAR, temp	none	-329.6	5.302	-324.3
	Step 3			
PAR, sal	none	-331.3	4.231	-327.1
SPM, sal	none	-331.5	4.212	-327.3
SPM, PAR	none	-331.6	4.225	-327.4
	Step 4			
PAR	none	-333.3	3.156	-330.1
SPM	none	-333.6	3.185	-330.4
	Step 5			
null	**none**	**-335.2**	**2.116**	**-333.0**

Table 3.7 *Step-wise comparison of model covariates for predicting total PUFA.*

Bolded is the best model.

event covariates	**random effects structure**	**mean deviance**	**penalty**	**penalized deviance**
	Step 1			
wind, par, temp, sal	none	-323.9	6.337	-317.6
SPM, par, temp, sal	none	-323.5	6.345	-317.2
SPM, wind, temp, sal	none	-323.9	6.343	-317.6
SPM, wind_stress, par, sal	none	-323.7	6.337	-317.4
SPM, wind, par, temp	none	-323.9	6.363	-317.5
	Step 2			
wind, temp, sal	none	-325.9	5.268	-320.6
SPM, temp, sal	none	-325.5	5.294	-320.2
SPM, wind, sal	none	-325.4	5.275	-320.1
SPM, wind, temp	none	-326.0	5.268	-320.7
	Step 3			
wind, temp	none	-327.9	4.230	-323.7
SPM, temp	none	-327.6	4.210	-323.4
SPM, wind	none	-327.3	4.227	-323.1
	Step 4			
temp	none	-328.9	3.159	-325.8
wind	none	-328.5	3.166	-325.3
	Step 5			
null	**none**	**-329.8**	**2.096**	**-327.7**

Table 3.8 *Step-wise comparison of model covariates for predicting phytoplankton:terrestrial PUFA.*

Bolded is the best model.

event covariates	random effects structure	mean deviance	penalty	penalized deviance
Step 1				
wind, PAR, temp	month/station	55.07	2028	2083
SPM, PAR, temp	**month/station**	**49.03**	**1846**	**1895**
SPM, wind, temp	month/station	48.31	1874	1922
SPM, wind, PAR	month/station	50.68	2043	2093
Step 2 (no improvement)				
PAR, temp	month/station	54.10	2067	2121
SPM, temp	month/station	47.34	1932	1980
SPM, PAR	month/station	49.82	1961	2011

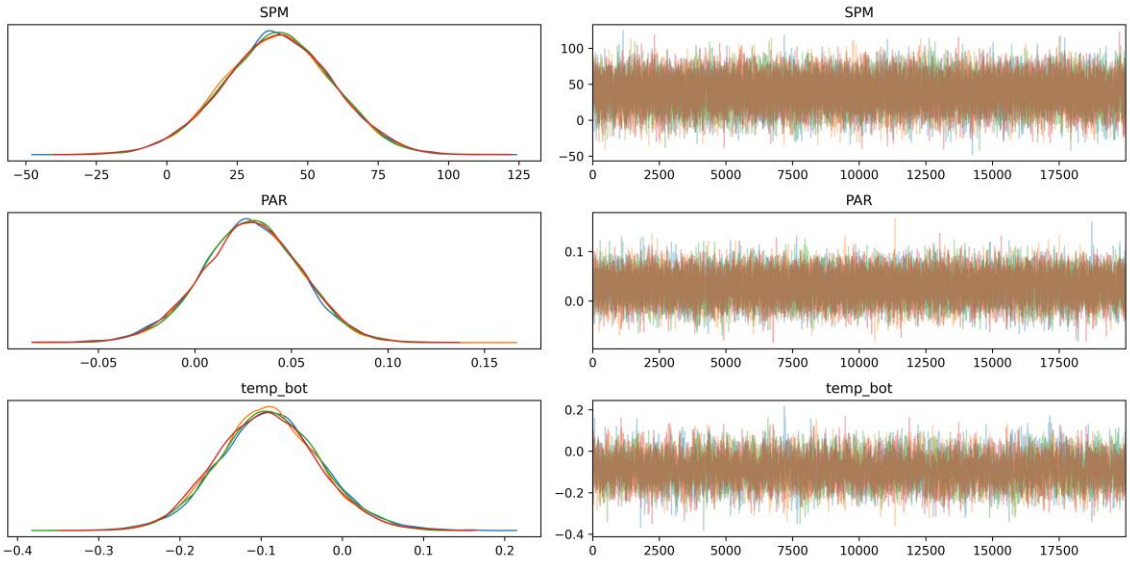

Figure 3.4 *Trace and density plots of the parameters in the final model for phytoplankton:terrestrial PUFA.*

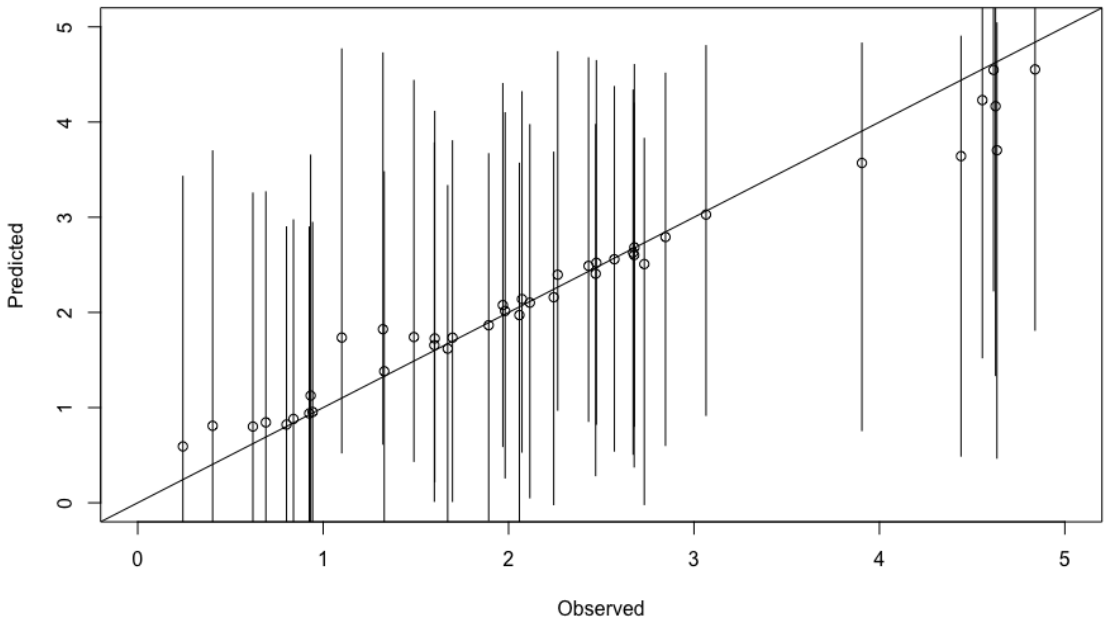

Figure 3.5 *Plot of predicted vs. observed phytoplankton:terrestrial PUFA (dimensionless).*

The solid diagonal line represents an ideal 1:1 relationship. The open circles represent the medians on the y-axis and the vertical lines represent 95% credible interval of predictions.

3.5 Discussion

In this chapter, spatial models of fatty acid concentrations were explored as potential measures of food quality. Spatial models of fatty acid concentrations are limited and have mostly been based on remote sensing data. Prior to this research, no models existed for the Mississippi Sound. The model covariates in this chapter were strictly based on biophysical variables, easily collected in situ. Null models were selected for every individual fatty acid concentration modeled. These results highlight the difficulty in modeling fatty acid concentrations and the general lack of understanding of fatty acid

metabolic processes spatially and temporally. The predictive abilities of these models were not aided by the limited sample sizes in this research.

However, some insights can still be taken from this chapter's results. For example, some relevant seasonal trends in PUFA availabilities were observed and correlated with freshwater influence at the sampling locations. For example, phytoplankton PUFA and total PUFA peaked at more riverine sites in the spring and at more marine sites in the fall. Terrestrial PUFA peaked for all sites in the fall but occurred a month later at the more marine sites. Taken together, the ratio of phytoplankton PUFA to terrestrial PUFA demonstrated a pattern of progression correlated with riverine influence, peaking at the most riverine sites in May (BB, SS), at intermediate sites in July (PC) and August (HP), and at the most marine-influenced sites in October (PM) and November (TG). This seaward "pulse" of the ratio of phytoplankton PUFA to terrestrial PUFA increased as it reached more marine environments later in the fall. While only observational at this point, the existence and implications of such a regime in estuarine ecosystems warrants further investigation.

Additionally, it was possible to model the ratio of phytoplankton FAs (DHA and EPA) to terrestrial FAs (ALA and LA) from biophysical variables. This ratio was positively correlated with SPM and PAR and negatively correlated with temperature. The rate of photosynthesis and phytoplankton growth is dependent on irradiance, nutrients, and temperature (Geider et al. 1998), with light generally being the limiting factor in shallow aquatic environments (Daalsgaard 2003), which explained higher ratio of phytoplankton:terrestrial PUFA. The integration of PAR into the model was therefore critical. PAR is a common variable in marine ecosystem models and an important

component of photosynthesis-irradiance (PI) curves (Jassby and Platt 1976). However, PAR is not the only variable which determines the light environment for phytoplankton. Turbidity (affected mostly by SPM and colored dissolved organic matter) is also a critical variable because of its effect on the attenuation of light in water and, therefore, irradiance at depth. Despite the negative effect of SPM on the light environment, the results suggest a positive relationship between SPM and the ratio of phytoplankton fatty acids to terrestrial PUFA. This suggests that phytoplankton were a greater component of particulate organic matter in the western Mississippi Sound than terrestrial carbon sources.

Bottom temperature was selected as a covariate in the final model. Though temperature upregulates the ratio of Chl *a* to carbon (Geider et al. 1997), the results here indicate that temperature had a negative correlation with the ratio of phytoplankton fatty acids to terrestrial PUFA. This may be counterintuitive, but it is well documented that DHA and EPA are upregulated in polar membranes by lower temperatures during a process known as homeoviscous adaptation (Sinensky 1974, Pernet et al. 2007a).

Improved models of fatty acid distributions in aquatic ecosystems will require better understanding of phytoplankton dynamics as well as factors regulating terrestrial inputs. Phytoplankton dynamic models are strongly influenced by temperature and light, and the Bayesian models presented here also support these as important variables for predicting ratio of FA availabilities from phytoplankton to terrestrial sources. Models of phytoplankton dynamics must also consider nutrient availabilities and grazing. Nutrients would be expected to upregulate phytoplankton production, and grazing would be expected to downregulate phytoplankton production. Unfortunately, measuring nutrient

concentrations and grazing was beyond the scope of this research but has the potential to explain further variation of fatty acid concentrations in the environment.

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CHAPTER IV – BIOPHYSICAL MODEL OF OYSTER CONDITION IN THE MISSISSIPPI SOUND

4.1 Introduction

Condition can be defined as "the ability of an animal to withstand an adverse environmental stress, be this physical, chemical, or biological" (Mann 1978). In attempt to better understand oyster fitness, multiple morphometric, biochemical, and physiological condition indices have been proposed. Morphometric indices do not provide detailed information about metabolic reserves, structural components, or gonadal tissue that other more direct measures of condition can provide (Mann 1978). The most popular morphometric index, the condition index (CI), relates oyster tissue mass to shell cavity volume or mass (Mann 1978). This simple index represents the tissue mass relative to its maximum potential based on the shell size (Mann 1978). However, CI correlates with glycogen levels in *Ostreus edulis* (Gabbott and Stephenson 1974) and *C. gigas* (Li et al 2009). Morphometric condition is also simple, cost-effective, and widely available in public datasets (Filgueira et al. 2013), so only morphometric condition is considered in this chapter.

Morphometric condition has been examined with respect to many factors. Perhaps the best-known influences of oyster condition are seasonal cycles in reproduction. Condition declines significantly during spawning and quickly recovers afterward (Li et al 2009), though it may fall off in cooler months if glycogen stores are depleted (Walne and Mann 1975). Oyster condition also depends on food quantity and quality, as well as physical conditions. Increased oyster condition before spawning is the result of utilization of assimilated nutrients into gonadal tissues. Specifically, eicosapentaenoic acid (EPA)

and docosahexaenoic acid (DHA) have important functions in reproduction (Chu et al. 1990, Soudant et al. 1999). The quantity of nutrients assimilated is a function of the filtration rate, the seston organic content, and the amount of organic matter rejected as pseudofeces (Bayne 2017, Chap 5). Additionally, the filtration rate is a function of seston concentration (SPM), temperature, salinity, and dissolved oxygen (Powell et al. 1992, Fulford et al. 2007).

The effect of feeding environment on oyster condition has been demonstrated across multiple time scales. Schoener and Tufts (1987) observed a relationship between the El Niño Southern Oscillation and condition in *C. gigas*, with a time lag of 5 months. These authors attributed this relationship to decadal-scale changes in plankton productivity. Austin et al. (1993) found that seasonal fluctuations in condition for *C. virginica* were related to both salinity and temperature. Therefore, the use of CI as a direct indicator of fitness is inappropriate and must be viewed in the context of oyster size, reproductive stage, and other prevailing environmental conditions to be physiologically meaningful.

Considering the relationships between environmental conditions and physiological status, understanding temporal and spatial variations in morphometric oyster condition can provide important insights into biophysical factors affecting oyster survival. This chapter examines the condition of oysters across temporal and spatial scales and how it relates to biophysical factors. Seston quantity (SPM) and quality (FA) measures were included as biological predictors, and models were developed to determine the relationship between food quantity and quality and oyster condition. Note

that SPM overestimates the actual food quantity, because not all the fractions of the SPM are viable nutrition sources. The study design facilitated testing the following hypothesis:

H_1: Morphometric condition will be positively correlated with both seston quantity (SPM) and quality (FA measures) when accounting for oyster length, monthly temperature change, bottom salinity, and bottom dissolved oxygen.

4.2 Materials and Methods

4.2.1 Sample Collection and Processing

Live oysters were retained monthly from Apr–Nov at three of the inshore SPM sampling stations within the western Mississippi Sound (Fig. 1). Live oysters were sampled using a bag dredge, and for each reef, eight haphazardly selected live specimens (> 50 mm) were transported to the laboratory on ice. For each oyster, length and width were measured with calipers. The morphometric dimensions are defined as follows. Length is the distance from the umbo to the anterior margin of the shell; width is the maximal distance between the dorsal and ventral margins of the shell parallel to the hinge axis, although width was ultimately not used in the models. Oysters were shucked using a shucking knife and Turtleskin® gloves, taking care to leave the adductor muscle intact. Oyster tissue was blotted dry with paper towels for up to 15 minutes, taking care to also dry the gills and labial palps. Once the tissue was dry to the touch, blotted wet weight was recorded.

4.2.2 Model Development

Hierarchical Bayesian models were used to predict the log of blotted wet weight of oysters from shell length, monthly temperature change, bottom salinity, bottom dissolved oxygen, surface SPM (food quantity), and surface FA (food quality, with separate models for different fatty acid measures) (Fig. 4.1). Sampling location and month were used as grouping variables assuming that the relation of these covariates with oyster conditions is more similar within the same site and within the same month. In situ surface SPM and surface FA concentrations were measured from inshore reefs with data reported in Chapters 2 and 3, respectively. Temperature change was calculated as the current month's bottom temperature minus the previous month's bottom temperature, where a positive value represents increasing temperatures and a negative value represents decreasing temperatures.

Based on the sampling design, a model structure was selected with station nested in month and monthly discharge in the month compartment. Different combinations of covariates were compared among the different models using the deviance information criterion (DIC) (Spiegelhalter et al. 2002, Hobbs and Hooten 2016). The lower the DIC, the better the model prediction. The fatty acid covariates were examined as the current sampling month's value as well as the value lagged by one month. Potential multicollinearity in each model was examined using pair-wise Pearson's correlation coefficients (r) among the potential covariates. Highly-correlated covariates ($r > 0.70$) were removed from the candidate covariates.

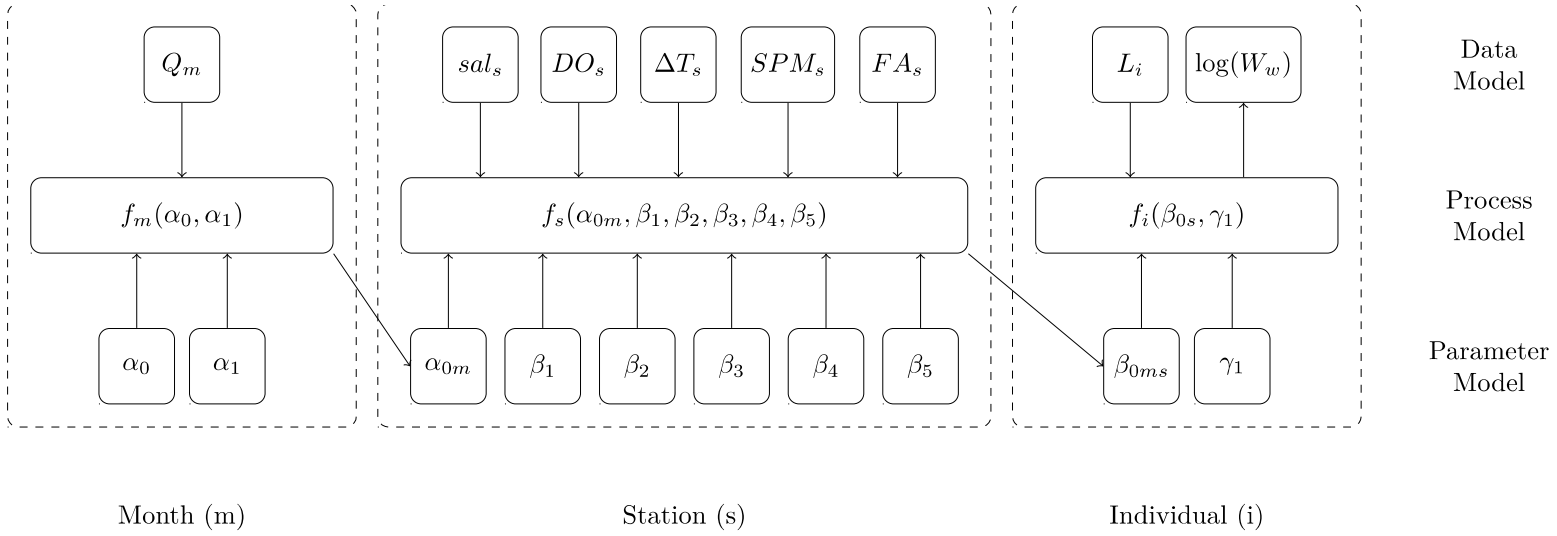

Figure 4.1 *A conceptual directed acyclic graph (DAG) of the predictive model of log(wet weight) (W_W) for oysters.*

The month (m) compartment has the parameters α0 and α1, which include the covariate for river discharge (Qm). The station (s) compartment has the parameters α0m, β1, β2, β3, β4, and β5, which are the parameters for monthly intercept, salinity (sal), dissolved oxygen (DO), monthly change in bottom temperature (ΔT), suspended particulate matter (SPM) concentration, and fatty acid concentration (FA), respectively. The individual (i) compartment has the parameters β0ms and γ1, which are the parameters for the station intercept and shell length (Li), respectively.

4.3 Results

To determine the best fatty acid covariate for predicting oyster condition (analyzed as log weight), the full model was compared using DIC among five fatty acid covariates (EPA, DHA, phytoplankton PUFA, terrestrial PUFA, and total PUFA). Additionally, SPM and bottom salinity were each examined using both a linear function and with an additional quadratic parameter. The model with the lowest DIC had unlagged terrestrial PUFA as the fatty acid covariate with single linear parameters for both SPM and bottom salinity (Table 4.1, DIC=18.1). The model using unlagged total PUFA was selected because the DIC was within 2.0 and total PUFA gives the best representation of nutritional value with respect to the hypothesis (Table 4.1, DIC=18.9). However, multiple models (in bold in Table 4.1), representing all FA covariates tested, predicted morphometric condition as well as the selected model. The one-month lagged fatty acid option performed similarly to the unlagged fatty acid in some models, but using unlagged FA as the covariate avoided data loss associated with having to use the previous month's data.

Table 4.1 *Comparison of fatty acid covariates for predicting oyster weight.*

Lagged FA indicates the previous month's fatty acid concentration was used. Models with a DIC within 2.0 of the lowest DIC are bolded.

event covariates	lagged FA	mean deviance	penalty	penalized deviance
sal, SPM, PUFA, DO, Δtemp, length	**no**	**2.93**	**15.93**	**18.85**
sal, SPM, terra, DO, Δtemp, length	**no**	**2.52**	**15.59**	**18.11**
sal, SPM, phyto, DO, Δtemp, length	**no**	**3.84**	**15.96**	**19.79**
sal, SPM, DHA, DO, Δtemp, length	**no**	**3.93**	**15.86**	**19.79**
sal, SPM, EPA, DO, Δtemp, length	**no**	**3.90**	**15.77**	**19.67**
sal, sal^2, SPM, PUFA, DO, Δtemp, length	no	4.58	16.87	21.45
sal, sal^2, SPM, terra, DO, Δtemp, length	no	4.10	16.66	20.76
sal, sal^2, SPM, phyto, DO, Δtemp, length	no	5.18	17.24	22.42
sal, sal^2, SPM, DHA, DO, Δtemp, length	no	5.19	16.87	22.06
sal, sal^2, SPM, EPA, DO, Δtemp, length	no	5.31	16.54	21.85
sal, SPM, SPM^2, PUFA, DO, Δtemp, length	**no**	**3.03**	**15.97**	**19.00**
sal, SPM, SPM^2, terra, DO, Δtemp, length	**no**	**2.49**	**15.75**	**18.24**
sal, SPM, SPM^2, phyto, DO, Δtemp, length	no	3.90	16.05	19.94
sal, SPM, SPM^2, DHA, DO, Δtemp, length	**no**	**3.94**	**15.85**	**19.79**
sal, SPM, SPM^2, EPA, DO, Δtemp, length	**no**	**3.88**	**15.76**	**19.64**
sal, sal^2, SPM, SPM^2, PUFA, DO, Δtemp,	no	4.54	17.27	21.82
sal, sal^2, SPM, SPM^2, terra, DO, Δtemp, length	no	3.56	16.84	20.41
sal, sal^2, SPM, SPM^2, phyto, DO, Δtemp,	no	5.39	17.03	22.42
sal, sal^2, SPM, SPM^2, DHA, DO, Δtemp, length	no	4.95	17.21	22.16
sal, sal^2, SPM, SPM^2, EPA, DO, Δtemp, length	no	5.43	16.68	22.10
sal, SPM, PUFA, DO, Δtemp, length	yes	3.99	16.21	20.21
sal, SPM, terra, DO, Δtemp, length	**yes**	**3.93**	**16.11**	**20.03**
sal, SPM, phyto, DO, Δtemp, length	**yes**	**3.88**	**15.91**	**19.79**
sal, SPM, DHA, DO, Δtemp, length	**yes**	**3.75**	**15.77**	**19.52**
sal, SPM, EPA, DO, Δtemp, length	**yes**	**3.94**	**15.64**	**19.58**
sal, sal^2, SPM, PUFA, DO, Δtemp, length	yes	5.14	17.08	22.23
sal, sal^2, SPM, terra, DO, Δtemp, length	yes	5.13	17.18	22.31
sal, sal^2, SPM, phyto, DO, Δtemp, length	yes	5.29	17.32	22.61
sal, sal^2, SPM, DHA, DO, Δtemp, length	yes	5.19	17.11	22.30
sal, sal^2, SPM, EPA, DO, Δtemp, length	yes	5.09	16.57	21.66
sal, SPM, SPM^2, PUFA, DO, Δtemp, length	yes	3.83	16.30	20.13
sal, SPM, SPM^2, terra, DO, Δtemp, length	**yes**	**3.89**	**16.20**	**20.09**
sal, SPM, SPM^2, phyto, DO, Δtemp, length	**yes**	**4.05**	**15.96**	**20.01**
sal, SPM, SPM^2, DHA, DO, Δtemp, length	**yes**	**3.87**	**15.80**	**19.67**
sal, SPM, SPM^2, EPA, DO, Δtemp, length	**yes**	**3.92**	**15.59**	**19.52**
sal, sal^2, SPM, SPM^2, PUFA, DO, Δtemp,	yes	5.33	17.17	22.50
sal, sal^2, SPM, SPM^2, terra, DO, Δtemp, length	yes	5.38	17.20	22.58
sal, sal^2, SPM, SPM^2, phyto, DO, Δtemp,	yes	5.25	16.96	22.21
sal, sal^2, SPM, SPM^2, DHA, DO, Δtemp, length	yes	4.99	16.57	21.56
sal, sal^2, SPM, SPM^2, EPA, DO, Δtemp, length	yes	5.21	16.56	21.76

Covariates were excluded step-wise from selected full models based on deviance information criteria (DIC) until no further model improvement could be made (Table 4.2). The backward-selection procedure selected the model with unlagged total PUFA, SPM, bottom salinity (linear), shell length, and monthly discharge as covariates (DIC=17.1, Figs. 4.2–4.3).

Total PUFA, SPM, shell length, and monthly discharge had positive effects on oyster weight, while bottom salinity negatively affected oyster weight. Based on the data, there were 89.1%, 90.0%, 100.0%, and 90.4% probabilities that total PUFA, SPM, shell length, and monthly discharge positively affected oyster condition, respectively, and a 99.9% probability that bottom salinity negatively affected oyster condition.

Table 4.2 *Step-wise comparison of model covariates for predicting oyster weight.*

Bolded is the best model.

event covariates	**random effects structure**	**mean deviance**	**penalty**	**penalized deviance**
Step 1				
SPM, PUFA, DO, Δtemp, length	month/station	7.75	17.38	25.13
sal_bot, SPM, PUFA, Δtemp, length	month/station	2.57	15.12	17.68
sal_bot, SPM, PUFA, DO, length	month/station	2.64	15.63	18.27
Step 2				
SPM, PUFA, Δtemp, length	month/station	6.90	16.94	23.84
sal_bot, SPM, PUFA, length	**month/station**	**2.27**	**14.87**	**17.14**
Step 3 (no improvement)				
SPM, PUFA, length	month/station	6.71	16.48	23.19

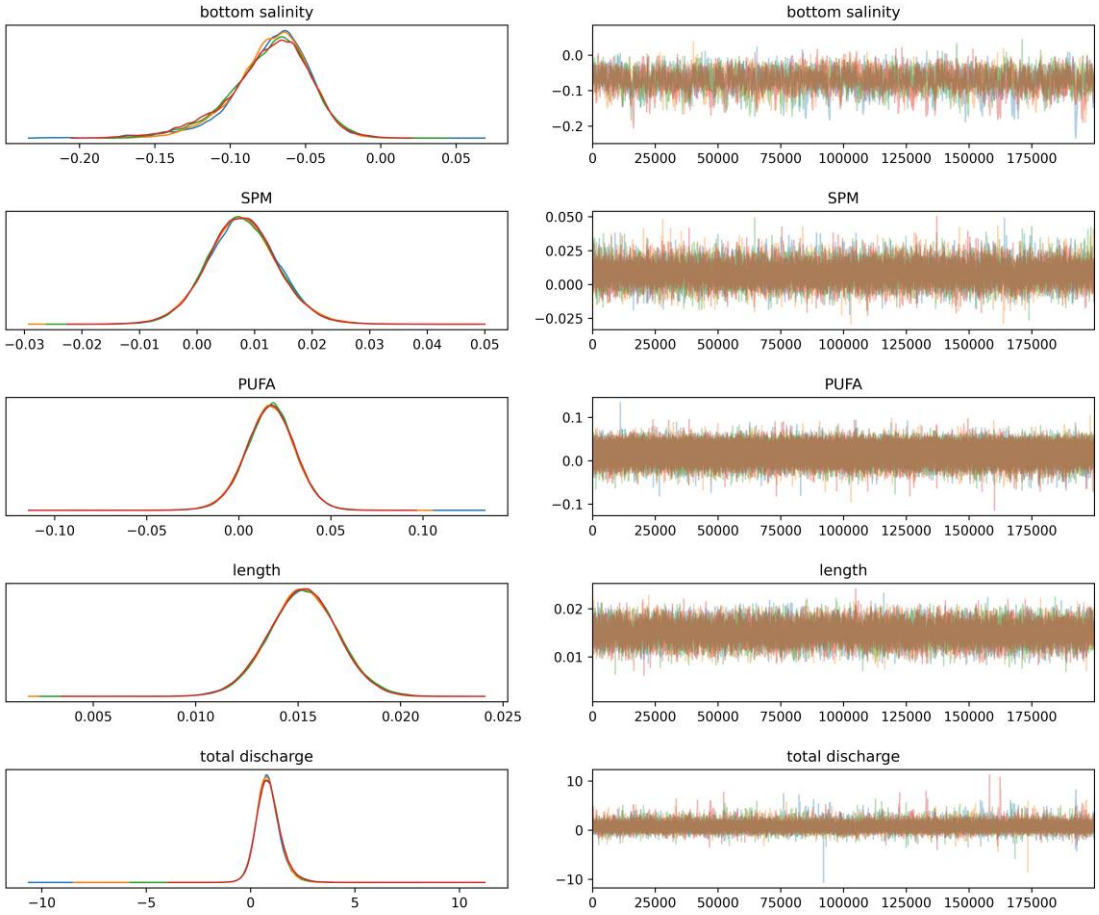

Figure 4.2 *Trace and density plots for the final model parameters.*

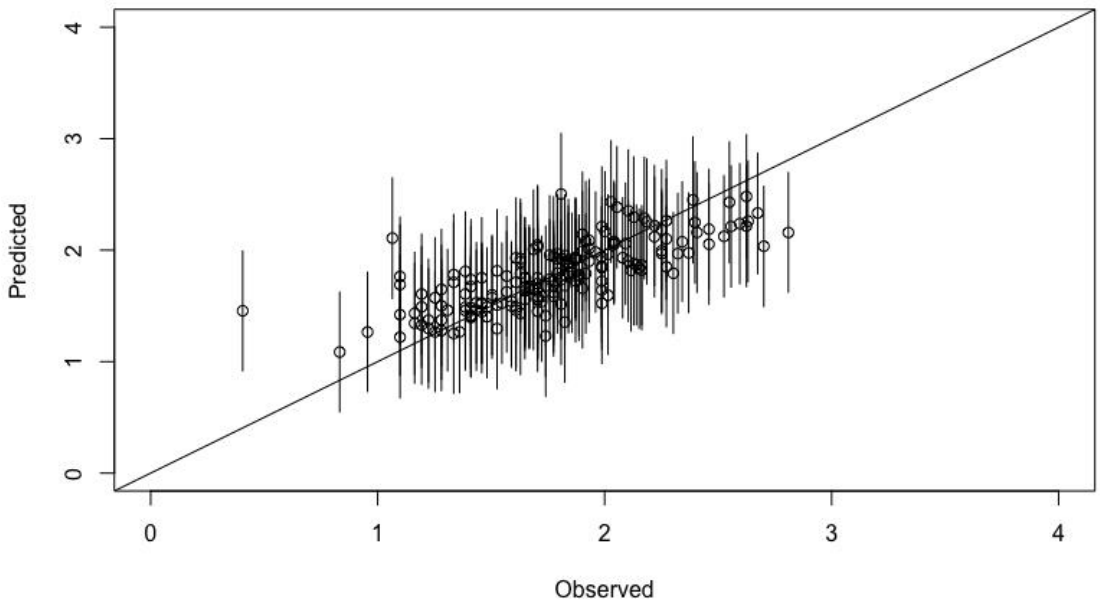

Figure 4.3 *Plot of predicted vs. observed log oyster wet weight (g).*

The solid diagonal line represents an ideal 1:1 relationship. The open circles represent the medians on the y-axis and the vertical lines represent 95% credible interval of predictions.

4.4 Discussion

The condition model presented here, with log(wet weight) as the response variable and shell length as a mandatory covariate, provided a mathematical framework to directly assess oyster condition, without the need for opaque or size-dependent condition indices. Model results support the hypothesis that seston quantity and quality are important factors regulating oyster condition. There were 89.1% and 90.0% probabilities, respectively, that PUFA and SPM had positive effects on oyster condition in the final model. Other researchers have demonstrated the importance of food quality in other regions, particularly with juvenile oysters (Hégaret et al. 2004, Pernet et al. 2014),

and food quantity is a key parameter in many population dynamic models for oysters (Powell et al. 1992). The model selection process chose a linear SPM parameter, and this is likely appropriate given that SPM levels weren't very low (<10 mg L^{-1}) or above levels believed to cause sublethal effects to oyster fitness (>100 mg L^{-1}; see Shumway et al. 2003, Gernez et al. 2017, Le Peyre et al. 2019). It is likely that an additional quadratic parameter for SPM would be appropriate if SPM levels exceeded sublethal levels. It may take from 10 days to 2 months for fatty acids in oyster tissue to turn over in response to dietary changes (Teshima et al. 1987, Delaporte et al. 2005). However, unlagged FA measurements were selected in the final model. It is likely that the 1-month temporal scale of the study was not sufficient for determining the proper time lag for considering the response of oyster condition to feeding conditions. Increasing the sampling frequency to weekly would inform the lagged effect better in future studies.

Salinity was negatively related to condition. This is likely related to homeostatic processes in which oysters reduce tissue water content in higher salinities, and care should be given not to ascribe morphometric condition as a direct measure of oyster fitness or reef-level health. While maintaining homeostasis can be metabolically costly, many healthy oyster reefs exist at high salinities, and low salinity is generally regarded as detrimental to oyster reproduction (Gregory et al. 2023). The selection of a single negative linear salinity parameter in the final model suggests that salinities during the study period (10.3–28.4 ppt) were within a healthy range. The negative impact of salinity was consistent with a positive impact of river discharge. Predictions outside of the salinity range sampled in the study should be done with caution using the model developed here.

Total phytoplankton PUFA peaked at riverine sites in the spring, and progressively at more marine sites through November. The fraction of phytoplankton lipids in SPM was lower during the summer. So, although the decreased condition in summer may be mostly attributable to the dramatic loss of gonadal tissue after spawning, metabolic stress or decreasing food quality cannot be ruled out as contributing factors. Oyster condition begins recovering immediately after spawning in most areas, but this is often not the case in the western Mississippi sound (Powell, pers. comm.). The data observed here show decreased PUFA availabilities and increased SPM concentrations for an extended period after spawning, so poor nutrition may have contributed to the poor recovery of condition that was observed.

The 2018 sampling period was not typical, except in the sense that the past one or two decades were typified by an abundance of extreme natural and anthropogenic disturbances. In March of 2018, just prior to the onset of this research, the Bonnet Carré Spillway was open for a period of 22 days releasing an estimated 1.5 trillion gallons of Mississippi River water toward the primary Mississippi oyster fishing grounds (USACE 2020). Oyster populations were already depleted after a period that included Hurricane Katrina, the Deepwater Horizon Oil Spill, other spillway openings, and outbreaks of Dermo. By the spring of 2019, oyster reefs in the western Mississippi Sound had completely collapsed mainly due to another prolonged opening of Bonnet Carré Spillway. The samples taken here represent some of the last oyster samples taken before that collapse. Unfortunately, there was no baseline knowledge on food quantity or quality prior to this research, so it is impossible to know if these factors may have contributed to the collapse.

The research here presents a simple framework that can be built upon to examine the effects that food supply and food quality have on oyster condition. The data presented here provide evidence that direct measures of food quality, such as FA composition, should be considered in any future oyster modeling efforts.

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CHAPTER V – SUMMARY

The results of this research support the hypothesis that food quantity and food quality are important covariates in predictive models of oyster condition but also highlight the difficulty of modeling both SPM and FA.

A predictive model of SPM was developed with easily obtainable wind stress, bottom temperature, and bottom salinity covariates. Existing remote sensing models of SPM are also easily parameterized and can provide greater spatial and temporal coverage and more reliable estimates of SPM concentrations than the model presented here. However, it is important to understand the factors influencing spatial and temporal variability in SPM concentrations, and the modeling approach presented here provides a simple framework to do so and can be used to make predictions under different environmental conditions, such as altered wind stress, bottom temperature and bottom salinity. During the 2018 sampling period, SPM concentrations at western Mississippi Sound oyster reefs were positively influenced by wind stress and temperature but negatively affected by salinity.

A model was developed for predicting the ratio of phytoplankton PUFA to terrestrial PUFA. During the 2018 sampling period, phytoplankton PUFA was positively affected relative to terrestrial PUFA by SPM concentrations. Predictive models of individual FA availabilities could not be developed, and existing remote sensing models of fatty acid availabilities (e.g., Budge et al. 2014) are not capable of performing in highly turbid coastal environments. Therefore, an important gap in knowledge still exists for being able to predict spatial FA availabilities in critical nearshore habitats, such as oyster reefs. While filling that gap will likely be aided by remote sensing data, it may

require highly compartmentalized, mechanistic, hydrodynamically-coupled plankton dynamic models to be able to predict individual fatty acids in coastal environments with any reliability.

The model of oyster condition presented here is a novel approach that integrates the effects of food quality, useful when mechanistic population dynamic models of oysters that incorporate direct measures of food quality are non-existent. For the 2018 study period, oyster condition at western Mississippi Sound oyster reefs was positively affected by SPM, PUFA, and river discharge but negatively affected by salinity. While the data did not support using a 1-month lag for FA concentrations, it takes 10 days to 2 months for oysters to turn over tissue lipids. Future work should seek to sample at a finer temporal scale to determine the appropriate lag time to best predict oyster condition.

It must be emphasized that the study period occurred directly after a Bonnet Carré Spillway opening, therefore results may significantly differ from a more typical year without such an anthropogenic disturbance. It is unclear if the data in this dissertation provides insights into the collapse of the western Mississippi oyster fishery in 2019. Notably, oyster condition begins recovering immediately after spawning in most areas, but this has not typically been the case in the western Mississippi sound (Powell, pers. comm.). The data observed here show decreased PUFA availabilities and increased SPM concentrations during an extended period immediately following spawning, so poor nutrition should be considered as a potential contributor.

Collectively, this research demonstrates how physical variables, such as wind stress, temperature, and salinity affect oyster feeding environments and how oyster feeding environments, along with physical variables, can affect oyster condition. With the

challenges facing oyster fisheries, future management and restoration efforts should strongly consider the physical conditions and feeding environment at existing and prospective oyster reef locations.

APPENDIX A – Storage and Handling

For tissues subject to lipolysis by enzymes, it is recommended that tissues are frozen quickly and stored under nitrogen in sealed containers at no more than −20 ºC. High levels of unesterified fatty acids, diacylglycerols, phosphatidic acid, or lysophospholipids are an indication that lipolysis has occurred. To minimize oxidation, add BHT to solvents at 50–100 mg/L (Christie and Han 2010). Purified lipid extracts are no longer subject to enzymes but can still oxidize. For long-term storage, lipid extracts should be kept at −20 ºC in glass containers flushed with nitrogen with a relatively non-polar solvent containing BHT. Refrigeration temperatures are acceptable for short-term storage.

APPENDIX B – Hazards

All chemical handling should be conducted under a fume hood. Ether can form very explosive peroxides during long-term storage. Ether should contain a small amount of BHT as a stabilizer and never be kept longer than the expiration date. Chloroform should contain a stabilizer (usually ethanol), or the highly toxic compound, phosgene, may form during long-term storage. Chloroform-methanol is a powerful skin irritant, and most solvents have toxic properties. People handling hazardous chemicals should always have suitable gloves, safety glasses, and a lab coat. Nitrile gloves are permeable to strong solvents such as chloroform, so they should be changed immediately if solvent contact occurs. Flammable solvents should not be handled around ungrounded electrical circuits or open flames, and all chemicals should be stored in an appropriate flame-proof cabinet. Solvents should never be stored with strong acids. When preparing methanolic sulfuric acid, care should be taken to add the acid slowly to the methanol in an ice bath.