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Microphytobenthos of the northern Gulf of Mexico hypoxic area and their role in oxygen dynamics

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MICROPHYTOBENTHOS OF THE NORTHERN GULF OF MEXICO HYPOXIC AREA AND THEIR ROLE IN OXYGEN DYNAMICS

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ABSTRACT

The presence or absence of microphytobenthos on the seafloor provides clues about whether benthic oxygen evolution contributes significantly to the oxygen budget of the hypoxic area in the northern Gulf of Mexico. Hypoxia (oxygen < 2 mg l^{-1}) creates inadequate concentrations of dissolved oxygen to support most organisms, such as fish, shrimp and crabs, and occurs over large areas of the Louisiana continental shelf from spring through summer in most years. Oxygen production by benthic autotrophs may offset a decline in oxygen concentrations if there is a functioning community and sufficient light. I sampled three stations (14, 20 and 23 m depths) ~ 100 km west of the Mississippi River over three hypoxic annual cycles (2006 – 2008), and 11 stations along a 14 - 20 m contour on the shelf in late-July in 2006, 2007 and 2008. I used microscopy and high-performance liquid chromatography to estimate the biomass and composition of phytoplankton and microphytobenthos. The potential seasonal oxygen production was estimated in 2007 and 2008 by incubating coupled light/dark sediment cores and bottom water from two stations. The sediment community (cells > 3 μm) differed from those in the water column and were frequently benthic pennate diatoms and filamentous cyanobacteria (58-88% seasonally and 1-99% in mid-summer). The concentration of microphytobenthic biomass was usually < 2.0 μg g dry sed^{-1}, and various biotic parameters were influenced by light at the seafloor. Declines in dissolved oxygen over a seasonal cycle in 2007 and 2008 were affected more by the initial dissolved oxygen concentration than by the presence of microphytobenthos that could generate oxygen. The sediment (1.2 - 27.3 mmol O_2 m^{-2} d^{-1}, n = 97) and bottom-water (1.1 - 17.5 mmol m^{-2} d^{-1}, n = 23) oxygen consumption rates were within the range of the few previously-reported data. This work adds to these data and also provides the only sediment oxygen consumption rates at fixed sites over seasonal time scales. These results provide critical input to three-dimensional, physical-biological models of oxygen dynamics for this hypoxic area.

CHAPTER 1

INTRODUCTION

Low oxygen, commonly referred to as hypoxia, naturally occurs in some aquatic ecosystems worldwide, and it can be caused or exacerbated by human activity (Diaz and Rosenberg 2008). The commonly used definition of hypoxia in the northern Gulf of Mexico is dissolved oxygen ≤ 2 mg O_2 l^{-1} based on lack of demersal catch in bottom trawls below this level (Renaud 1985, Rabalais et al. 1991). Hypoxic bottom water, commonly known as the "Dead Zone," has been detected off the coast of Louisiana, USA, since the early 1970s (Rabalais et al. 2002a) and now extends over 20,000 km^2 in mid-summer (Rabalais et al. 2007a). Smaller and more ephemeral hypoxic water masses are also found less frequently in mid-summer off the Texas and Mississippi coasts (Rabalais et al. 2002a, 2007a), but are expanding in frequencty and extent (N. N. Rabalais, unpubl. data).

Bottom-water hypoxia develops in the northern Gulf of Mexico from the interaction of: (1) nutrient-enhanced primary production, and (2) stratification resulting from freshwater discharge and solar warming. Beginning in late winter through spring, the Mississippi and Atchafalaya rivers discharge high nutrient loads into the coastal region (Rabalais & Turner 2006). Nitrogen and phosphorus loads in the rivers have increased since the 1950s (Turner et al. 1998, Rabalais et al. 2002b), resulting in high primary productivity (> 300 g C m^{-2} y^{-1}) on the adjacent continental shelf, thus making it eutrophic (Sklar & Turner 1981, Lohrenz et al. 1990, Lehrter et al. 2009). The phytoplankton community that develops is composed of a high biomass of diatoms, such as *Skeletonema* and *Chaetoceros* in the spring and late summer, and of high densities of picocyanobacteria during most of the summer (Dortch et al. 2001). About half of the primary production sinks through the water column to the bottom as fecal pellets. The remainder fluxes via diatom chains and aggregates of diatoms and/or picocyanobacteria (Qureshi 1995, Dortch et al. 2001). This high flux of organic matter occurs mostly in the spring (Justić et al. 1993, Redalje et al. 1994, Rabalais et al. 2002b) and increases the respiratory demand in the bottom water and sediments, which leads to oxygen depletion (Turner & Allen 1982). The stratification

of the surface and bottom water acts as a barrier to re-aeration of the bottom water, thus increasing the likelihood of hypoxia.

Benthic photosynthesis from microphytobenthos (benthic microalgae and cyanobacteria) on the sediment surface can increase bottom-water oxygen concentrations (Graneli & Sundbäck 1986, Jahnke et al. 2000) and may prevent bottom-water anoxia (Bierman et al. 1994, Dortch et al. 1994). The amount of oxygen produced from benthic primary productivity in the hypoxic area of the northern Gulf of Mexico was estimated to be 58-141 mg O_2 m^{-2} d^{-1} at four sites in July 1991 (Dortch et al. 1994). These data have been used in preliminary oxygen budgets (Dortch et al. 1994, Rowe 2001). Benthic and bottom-water photosynthesis during summer hypoxia has the potential to equal 23% of the daily sediment oxygen consumption (Dortch et al. 1994). Quiñones et al. (2007), however, suggested that this value would not have significantly affected their estimation of bottom-water respiration rates.

Microphytobenthos are commonly found in sub-tidal and shallow continental shelf sediments (Sundbäck & Jönsson 1988, Cahoon et al. 1990, Nelson et al. 1999, Totti 2003, Cibic et al. 2007, Reiss et al. 2007). The pennate diatoms in sediments were distinctively different from the planktonic algal community of Onslow Bay, North Carolina (Cahoon et al. 1990). Benthic diatoms were abundant and included the genera *Pleurosigma*, *Gyrosigma* and *Navicula* (Totti 2003), possibly because they can photosynthesize at low light levels (Paterson 2001). Benthic cyanobacteria have also been found below diatoms in the sediments, perhaps because their phototactic gliding mobility assists in reaching light (Shilo & Fattom 1984). Benthic cyanobacteria are also known to dominate in environments with varying pH, Eh, and both oxygen and hydrogen sulfide concentrations (Shilo & Fattom 1984). Diatoms (*Pleurosigma* spp., *Gyrosigma* spp., and *Navicula* spp.) dominated the top 1 cm of the sediment in the summer, but filamentous cyanobacteria (Oscillatoriales) were also present in northern Adriatic Sea sediments beneath the eutrophic and hypoxic area influenced by the Po River (Totti 2003). Similar microphytobenthos may be common in the northern Gulf of Mexico inner shelf sediments where eutrophication and oxygen depletion have also worsened in the last 40 years (Rabalais et al. 2007b).

Microphytobenthos in the hypoxic region of the Louisiana continental shelf may influence oxygen dynamics if sufficient light reaches the seafloor. MacIntyre et al. (1996) suggested that, in shallow-water aquatic systems with nutrient-rich and high turbidity waters, light availability may be the main control on photosynthesis by microphytobenthos. Sufficient light penetration is essential for a viable microphytobenthic community. Recent studies of the inner continental shelf of the northern Gulf of Mexico suggested that significant amounts of light reach the seafloor, but this amount varies among sites and years. Quiñones-Rivera et al. (2010) found that Secchi disk depths exceeded station depths about 30% of the time in late-July, 2003, but not in late-July, 2002. The euphotic zone reached the seafloor on 32 to 71% of the Louisiana shelf area studied in 2005-2007 by Lehrter et al. (2009).

This dissertation examines if microphytobenthos were present on the frequently hypoxic continental shelf off the coast of Louisiana. Specifically, I wanted to determine their seasonal and spatial distribution, the environmental conditions that may regulate their presence, and how they may impact the oxygen fluxes in the bottom water and sediment. In Chapter 2, I compared the seasonal composition of phototrophic cells in the water column to the sediment surface at three stations 100 km west of the Mississippi River delta to determine if microphytobenthos were present on the sediment surface. I also examined the environmental variables that affect the microphytobenthos biomass and density. This chapter is in press at in the journal *Marine Ecology Progress Series*. Chapter 3 describes the microphytobenthic biomass, density and composition along a 14-20 m contour on the Louisiana continental shelf where hypoxia is typically present in mid-summer. Most research on microphytobenthos off the Louisiana coast has been limited to areas south of Terrebonne Bay and around Ship Shoal (Grippo et al. 2009, 2010). Chapter 3 discusses data on microphytobenthos biomass and density in a larger geographic area. Chapter 4, discusses results from an investigation of whether microphytobenthic photosynthesis is important to oxygen dynamics in the hypoxic area of the northern Gulf of Mexico. It includes results from incubated light/dark sediment cores representative of typical light levels reaching the seafloor and the associated nutrient fluxes and microbial biomass. Lastly, in

Chapter 5, I provide a summary of my findings and the broader implications should environmental conditions change.

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CHAPTER 2

SEASONAL MICROPHYTOBENTHOS ON THE HYPOXIC NORTHERN GULF OF MEXICO CONTINENTAL SHELF*

INTRODUCTION

Hypoxic bottom water (≤ 2 mg l^{-1}), commonly known as the "Dead Zone," has been detected in the northern Gulf of Mexico off the coast of Louisiana, USA, since the early 1970s (Rabalais et al. 2002) and now extends over 20,000 km^2 in mid-summer (Rabalais et al. 2007a). Smaller and more ephemeral hypoxic water masses are also found less frequently in mid-summer off the Texas and Mississippi coasts (Rabalais et al. 2002, 2007a), but are expanding in areal extent (N. N. Rabalais, unpubl. data). Hypoxia develops from the interaction of (1) nutrient-enhanced primary production and (2) stratification resulting from freshwater discharge and thermal warming. Beginning in late winter through spring, the Mississippi and Atchafalaya rivers discharge high loads of nutrients (Rabalais & Turner 2006) into the coastal region supporting high primary productivity, > 300 g C m^{-2} y^{-1} (Sklar & Turner 1981, Lohrenz et al. 1990, Lehrter et al. 2009). The phytoplankton community that develops is composed of high densities of diatoms, such as *Skeletonema* and *Chaetoceros* in the spring and late summer, and of high densities of picocyanobacteria during most of the summer (Dortch et al. 2001). A high proportion of the primary productivity (~ 50%) from spring through fall sinks to the bottom primarily as fecal pellets (average = 55% of particulate organic flux) (Qureshi 1995), diatom chains, and aggregates of diatoms and/or picocyanobacteria (Dortch et al. 2001). This high flux of organic matter increases the respiratory demand in the bottom water and sediments, and leads to oxygen depletion (Turner & Allen 1982). The stratification between the surface and bottom water, acts as a barrier to re-aeration of the bottom water, thus increasing the likelihood of hypoxic water formation.

The bottom waters have the potential to become anoxic within 4 weeks or less if the organic matter supply is sufficient and[1] bottom-water temperatures are warm enough (Turner et al. 1998). If there is no mixing of the stratified layers, the time to reduce the bottom-water oxygen concentration

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from about 6 to less than 2 mg l^{-1} (based on decline of continuous oxygen concentration data) is 18, 11, or 9 days in April, May and July, respectively (Rabalais et al. 2007a). Murrell & Lehrter (2011), using below-pycnocline total respiration, estimated that 22 days were required for the lower water column to go from saturation to hypoxia.

Benthic photosynthesis is one process that would affect oxygen concentrations in the bottom water and could be why severely depleted bottom-water oxygen values (< 0.05 mg O_2 l^{-1}) and generation of hydrogen sulfide seldom occur, e.g. only five percent of the stations sampled from June through September between 1985-2005 (Rabalais et al. 2007a). Dortch et al. (1994) proposed that pelagic or tychopelagic phytoplankton species may be settling onto the sediment surface and photosynthesizing in the hypoxic region. Cahoon et al. (1990), however, found that the amount of chlorophyll *a* in the South Atlantic Bight sediments could not be explained by settling of phytoplankton alone and proposed that microphytobenthos may be responsible for some of the benthic oxygen production.

Microphytobenthos are common to sub-tidal and shallow continental shelf sediments (Cahoon et al. 1990, Totti 2003, Cibic et al. 2007). Pennate diatoms dominated the sediments of Onslow Bay, North Carolina, and the benthic algal community differed from the planktonic algal community (Cahoon et al. 1990). Benthic diatoms were usually the most abundant, which included the genera *Pleurosigma*, *Gyrosigma* and *Navicula* (Totti 2003), possibly because they can photosynthesize at low light levels (Paterson 2001). Benthic cyanobacteria have also been found below diatoms in the sediments perhaps because their phototactic gliding mobility assists in reaching light (Shilo & Fattom 1984). Benthic cyanobacteria are also known to dominate in environments with varying pH, Eh, and both oxygen and hydrogen sulfide concentrations (Shilo & Fattom 1984). In the summer, diatoms (*Pleurosigma* spp., *Gyrosigma* spp., *Navicula* spp.) dominated the top 1 cm of the sediment, but filamentous cyanobacteria (Oscillatoriales) were also present in northern Adriatic Sea sediments, a eutrophic and hypoxic area influenced by the Po River (Totti 2003). Similar microphytobenthos may be common in the northern

Gulf of Mexico inner shelf sediments where eutrophication and oxygen depletion have also worsened (Rabalais et al. 2007b).

A viable microphytobenthic community existed on shallow, sandy shoals and nearby muddy sites off the central coast of Louisiana (Grippo et al. 2009, 2010). On Ship Shoal, a bathymetric high sand relief, they found relatively high sediment chlorophyll *a* concentrations in the spring and summer because of the presence of benthic diatoms, but few settled phytoplankton compared to the nearby muddy sites (Grippo et al. 2009). There was a higher percentage of benthic diatoms on the shoals even though the chlorophyll *a* concentrations did not differ among their sites (Grippo et al. 2010).

Sufficient light penetration is essential for a viable microphytobenthic community. Recent studies from the inner continental shelf of the northern Gulf of Mexico suggested that significant amounts of light reach the seafloor, but this amount varies among sites and years. Quiñones-Rivera et al. (2010) found that Secchi disk depths exceeded station depths about 30% of the time in late-July, 2003, but not in late-July, 2002. The euphotic zone reached the seafloor on 32 to 71% of the Louisiana shelf area studied in 2005-2007 by Lehrter et al. (2009). The high nitrogen levels in the Mississippi River in springtime stimulate increased phytoplankton production as measured in surface waters south of Terrebonne Bay (Rabalais et al. 2007a). These spring phytoplankton blooms could potentially decrease the amount of light penetrating through the water column and inhibit benthic photosynthesis and oxygen production. Microphytobenthos in the hypoxic region of the Louisiana continental shelf may influence oxygen dynamics if sufficient light reaches the seafloor.

My objective was to identify and quantify microphytobenthos, not just diatoms, in typical innershelf sediments in an area of frequent summer hypoxia over three annual cycles of hypoxia formation and maintenance. I sampled the seasonal variability of microphytobenthos on the seafloor of the inner continental shelf off coastal Louisiana, at stations C4, C6B and C8, 100 km west of the Mississippi River. These stations are within the depth (14-, 20- and 23-m, respectively) where hypoxia occurs, in sediments typical of this depth on the continental shelf and are in an area that frequently

becomes hypoxic (Fig. 2.1) (Rabalais et al. 2002, 2007a). My null hypothesis was that the microalgal community composition at the sediment surface was similar to the water column (surface or bottom) due to sinking phytoplankton. Alternatively, I hypothesized that the sediment surface would have a unique community composition dominated by microphytobenthos. I also hypothesized that the deepest and least frequently hypoxic station was more likely to have a well-developed microphytobenthic community because of less shading by high phytoplankton biomass (farther from nutrient source) and a lower likelihood of sediment resuspension.

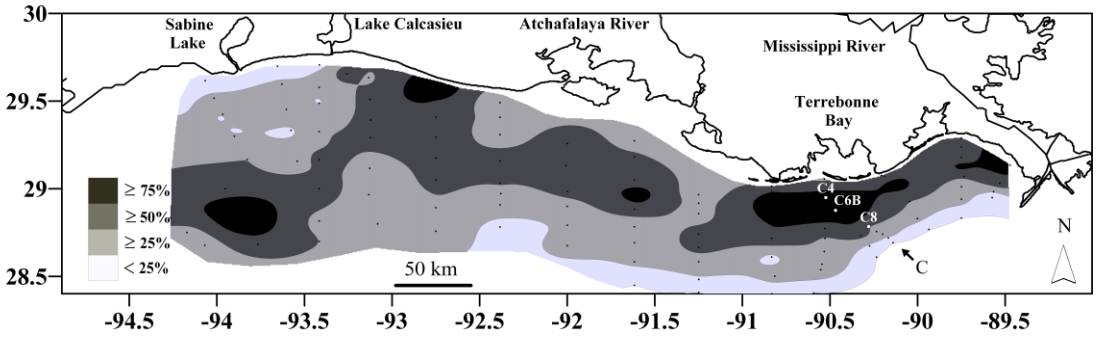

Fig. 2.1. Frequency of mid-summer bottom-water hypoxia ($\leq$ 2 mg l^{-1}) off the coast of Louisiana and Texas for 60 to 80 stations sampled during the summer from1985 to 2008. Stations C4, C6B and C8 are labeled on the C transect. The frequency distribution is updated and modified from Rabalais et al. (2002, 2007a).

MATERIALS AND METHODS

Study Area

Three stations were studied along the C transect located south of Terrebonne Bay, LA, an area that is usually hypoxic during summer (Fig. 2.1). Stations C4 (~14 m depth, 28:57.00′ N, 90:31.46′ W), C6B (~20 m depth, 28:52.18′ N, 90:28.04′ W) and C8 (~23 m depth, 28:47.30′ N, 90:16.60′ W) were sampled bimonthly from June 2006 to July 2008, except in summer 2006 when I sampled more frequently.

Field Collection

Several environmental parameters were measured with a multiparameter sonde (Hydrolab Surveyor 3 or YSI 6820) through the water column and as close to the seafloor as possible. A Biospherical Instruments Inc. profiling natural fluorometer (PNF-300) was used to determine the photosynthetically available

radiation (PAR) at the seafloor and on the research vessel (reference PAR). The percent surface PAR reaching the seafloor was calculated by using the reference PAR (mean ≈ 1800 μmol photons m^{-2} s^{-1}, *n* = 36) measured from the ship at the average local times of: 1500-1600 (C4), 1300-1400 (C6B), and 0600-0900 (C8). The percent PAR calculations were used instead of the absolute PAR values to correct for differences in time of day. Attenuation coefficients using the reference PAR and bottom PAR were also calculated.

I sampled the surface water with a bucket and the bottom water with a 5-l Niskin bottle (about 0.5 m above the seafloor). Water samples were filtered on 47 mm diameter Whatman GF/F filters which were kept in liquid nitrogen until further processing for phytopigment analyses. Surface and bottom water for microscopic analyses were preserved in a nalgene bottle containing 1 ml gluteraldehyde (50%) and filled to 100 ml with filtered sea water. Bottom water samples were analyzed for nitrate-nitrite, ammonium, silicate and phosphate on a Lachat auto-analyzer II system (8000 series) with an autosampler (ASX-400 series) according to EPA methodology (Methods 353.2, 350.1 and 365.2).

I collected sediment at each station from five intact GOMEX box cores (0.5 m high, 0.3 m long, 0.3 m wide, surface area 0.09 m^2). An 'intact' box core retained overlying water and the sediment surface was visibly undisturbed. Station C8 was more challenging to sample than the other stations due to the sandy nature of the sediment and occasionally little overlying-water remained. Subsamples were taken using two acrylic core tubes (7.6 cm diameter) from the middle of the box cores to avoid potentially disturbed edges. The top 0.5 cm was removed from each subcore with a precision core extruder (Fuller & Butman 1988), because light usually penetrates only the top millimeters of the sediment (MacIntyre et al. 1996). The water was carefully removed by pipette from the core sediment surface before the first subcore 0.5-cm slice was extruded. The sediment was homogenized in a Petri dish and used to fill two cyrovials (1.8 ml each) for pigment analysis (stored in liquid nitrogen) and two cryovials (1.25 ml each, stored at 4 ºC) for total organic carbon (TOC) analysis. Because I expected

variability within box cores and among box cores, five replicates were analyzed for sediment pigments and three for sediment TOC. The rest of the sediment (~ 17 ml of slurry) from the first subcore was preserved for microscopy in a 125 ml nalgene bottle with 1 ml of gluteraldehyde (50%) and filtered sea water to make a total of 100 ml. Only one sample per station was taken for microscopic analysis. The second subcore from each box core was sampled for grain size analysis, but only three of the five replicates were analyzed.

Laboratory Analyses

Pigments were extracted in a dark room by sonication in cold HPLC-grade 100% methanol for water samples and sonication with cold HPLC-grade 100% acetone for sediment samples. The filtered (0.2 µm) extract was injected into a Waters® high-performance liquid chromatography (HPLC) system equipped with a 600 controller, 600 pump, 996 photodiode array detector and 474 fluorescence detector based on the methods of Wright et al. (1991). The water content of the sediment samples was minimized by pipetting water from the core sediment surface before extruding and was considered a minimal effect on concentration. I found high levels of pigment degradation products in the sediment and used three columns (Waters® Nova-pak® C_{18} 3.9 × 150 mm, a Rainin Microsorb™ C_{18} and a Vydac® Reverse-Phase C_{18}) to separate and identify pigments. Sediment samples were run for 75 minutes with a gradient elution of 80:20 methanol: ammonium acetate, 90:10 of acetonitrile:water, and 100 percent ethyl acetate. Only one column (Waters® Nova-pak® C_{18} 3.9 × 150 mm) was needed for phytopigment analysis of the water samples, and they were run for 30 minutes on the same gradient elution. I used retention times and visible absorption spectra from DHI Lab standards as well as data and graphic sheets from Jeffrey et al. (1997) to help identify the pigments present. Some phytopigments were left out of the water and sediment analysis because the concentrations were zero or minimal for the majority of the samples. These included: neoxanthin, lutein, myxoxanthophyll, canthaxanthin, echinenone and prasinoxanthin.

The sediment percent total organic carbon by weight was determined with a Perkin Elmer CHN Model 2400 elemental analyzer after drying and grinding the sediment and acidifying to remove calcium carbonates (Hedges & Stern 1984). To determine sediment grain size by weight, I removed organics with 6% hydrogen peroxide, dispersed the sediments in hexametaphosphate and wet sieved (63 μm) to separate the sand from the mud.

Microscopy

Pigment data do not differentiate between water column phytoplankton and microphytobenthos. Thus I used epifluorescence microscopy to determine the community composition of surface and bottom water and sediment samples (adapted from Dortch 1998). The water samples were size fractionated by filtering onto 0.2, 3.0 and 8.0 μm polycarbonate filters. The 3.0 μm and 8.0 μm filters were stained with 0.03% proflavine to highlight the nuclei and chloroplasts. No stain was used on the 0.2 μm fraction to facilitate the identification of the natural pigments phycoerythrin (PE/Low Phycourobilin = PE/Low Pub and PE/High Phycourobilin = PE/High Pub) and phycocyanin (PC). All size fractions were counted on an Olympus BH-2-RFCA epifluorescence microscope with blue and green excitation. The 0.2 and 3.0 μm fractions were counted within a week at 1000 × magnification. The 8.0 μm filter was frozen to count later at 200 × magnification with epifluorescence and also transmitted light to help with identification. Each filter was counted until either one hundred cells or 100 views were reached. Identification of all cells was taken to the lowest level possible. Cell counts were converted to number per liter.

The sediment samples were resuspended, and 0.5 ml of the sediment slurry was removed, rinsed with distilled and deionzed water and centrifuged to remove picocyanobacteria and other small cells that were decanted onto 0.2 and 3 μm filters. A separate sediment slurry (2 - 4 ml) sample was needed to extract the larger cells. Ludox® HS-40 was added to the pellet to separate the larger cells from the sediment by density centrifugation (Totti 2003, Blanchard et al. 1998). Proflavin (0.03%) vital stain was added prior to filtration onto an 8 μm filter. The same counting and identification methodology

employed for the water samples was used for the sediment samples to ensure data compatibility. Cell counts were converted to cells per gram of dry sediment (cells g dry sed $^{-1}$).

Niches were assigned to each microalgal taxon as suggested by Round et al. (1990), Tomas (1997), and Komárek et al. (2003). The pelagic niche was assigned to organisms living in the water column, tychopelagic to cells that adapt to both the water and sediment and benthic to cells associated with the sediment (Cahoon et al. 1994). I characterized the community using niches instead of shape of the cells, such as centric versus pennate, because of the presence of filamentous cyanobacteria and to avoid confusion with the centric diatoms that live in the sediments and the pennate diatoms that live in the water.

Statistical Analyses

A community composition analysis was performed using the software Plymouth Routines in Multivariate Ecological Research (PRIMER) version 6.0 (Clarke & Warwick 2001). The monthly data were standardized to percents to develop the Bray-Curtis similarity index and analyzed by developing a non-parametric multi-dimensional scaling (MDS) plot to determine if the phytoplankton and microphytobenthic composition of the sample types surface water (SW), bottom water (BW) and sediment surface (SS) were similar at each station. MDS plots help visualize composition similarity by utilizing distance relationships. The closer the points are to each other, the more similar. These plots also provide stress values to suggest how well the MDS configuration fits the data. Since the MDS plots do not report quantified group differences, I used analysis of similarity (ANOSIM) with 999 permutations (random re-sampling of data) to help determine if the sample type (e.g. SW, BW, SS) from the monthly compositions for each station were significantly different. These tests help to determine if the sediment surface is different than the water column.

I also used the exploratory analysis SIMPER (similarity percentages) to determine the total average dissimilarity between sample types and the percent contribution of the top taxa contributing to the separation based on a Bray-Curtis similarity index of the percent data. The SIMPER test determines

which taxa groups are responsible for the differences in sample types (e.g. SW, BW, SS) and thus, provides useful ecological information on why they may differ. A seasonal analysis was conducted by grouping the months into four seasons of three consecutive months beginning in January. My null hypothesis for all tests was that there was no difference among sample type, stations, or seasons when $\alpha = 0.05$. To test for significant differences among stations, an analysis of variance (ANOVA) was performed on the natural log transformed pigment and density data to meet assumptions of normality when using the proc mixed statement in SAS 9.1. If significance was detected, a post-hoc Tukey-Kramer test allowed for pairwise comparisons. To determine correlations among environmental variables and samples a correlation biplot based on principal component analysis (PCA) was developed using the correlation matrix on standardized data and an α decomposition of 0.5 for a symmetrical plot with the 'proc princomp' statement and biplot macro in SAS 9.1. Plots were created and analyzed in SigmaPlot version 10.

RESULTS

Phytopigment Composition - Chlorophylls and Derivatives

The concentration of chlorophyll *a* (an indicator for biomass of photosynthetic organisms) in sediments was variable, but generally less than 2 μg g dry sed^{-1} at all stations except at station C8 in summer 2006 when the chlorophyll *a* levels were high (~ 4 μg g dry sed^{-1}; Fig. 2.2). The mean sediment chlorophyll *a* concentrations at stations C4 (n = 70), C6B (n = 69) and C8 (n = 70) were 0.67, 0.36 and 0.99 μg g dry sed^{-1}, respectively. The concentration of chlorophyll *a* at station C6B was significantly lower than at stations C8 ($t_{206,\ 0.05}$ = -3.74, p = 0.0007) and C4 ($t_{206,\ 0.05}$ = 2.76, p = 0.0174). The concentration of chlorophyll *a* at station C4 was, in general, highest in the summer (e.g. August 2006, September 2007, July 2008) and lowest in spring for most years. Similarly, the concentration of chlorophyll *a* at station C6B was higher in the late summer, fall and winter (e.g. August 2006, September 2007 to January 2008). The trends in concentrations varied among the years and the lowest values, often close to zero, occurred from winter to summer in 2007 (e.g. January-July 2007) and spring to summer in 2008

(March-May 2008). The sediment chlorophyll *a* concentration at station C8 was highest (about 4 × higher compared to the rest of the samples) in summer 2006, low (1.5 μg g dry sed^{-1}) in fall 2006 and winter 2007, and lowest (1 μg g dry sed^{-1}) in spring and summer 2008. The spring peaks of chlorophyll *a* were evident in the surface waters of all stations. The concentrations of chlorophyll *a* in bottom-waters were lower than in the surface water, and there were some spring chlorophyll *a* peaks as well. There was no relationship between the high levels of chlorophyll *a* in the water column and the high levels of chlorophyll *a* in the sediment surface for all stations (Fig. 2.2).

The concentration of pheophytin *a* and pyropheophytin *a* (degradation products of chlorophyll *a*) in surface sediments, tended to be as high, or higher, than the concentration of chlorophyll *a* (data not shown). The concentration of degradation products was less than 2.5 μg g dry sed^{-1} at all stations. The concentration of pheophytin *a* at station C4 was greater than the concentration of chlorophyll *a* in March and November 2007 and July 2008, whereas peaks of pheophytin *a* occurred at station C6B in winter 2007 and summer 2008. The sediment surface at station C8 had the lowest values of the chlorophyll *a* derivatives except in July 2006, and the concentrations of chlorophyll *a* were greater than pheophytin *a*. Pyropheophytin *a* concentrations were usually less than pheophytin *a* concentrations in the sediment surface at all stations. The concentration of pheophytin *a* was also lower (~ 3 ×) than chlorophyll *a*, and no phyropheophytin *a* was present in the surface and bottom-water samples.

Phytopigment Composition - Carotenoids

The sediment surface phytopigment composition was dominated by fucoxanthin during most months at all stations (Fig. 2.2). The mean concentration of fucoxanthin was 2.42 μg g dry sed^{-1} at C4 (n = 70), 2.71 μg g dry sed^{-1} at C6B (n = 69) and 1.14 μg g dry sed^{-1} at C8 (n = 70). The concentration of fucoxanthin was significantly less at C8 compared to stations C4 ($t_{206,\ 0.05}$ = 5.03, p = 0.0001) and C6B ($t_{206,\ 0.05}$ = 5.83, p = 0.0001). The concentration of fucoxanthin followed a general seasonal pattern of higher concentrations in the summer/fall and was lower in the spring (Fig. 2.2). Other common pigments present on the sediment surface were zeaxanthin, diadinoxanthin and 19'-

hexanoyloxyfucoxanthin (not shown), which tended to make up the rest of the sediment pigment pool at all stations (Fig. 2.2). Stations C4 and C6B had the highest combined carotenoid levels. The concentration of zeaxanthin followed a seasonal pattern with increasing concentrations in the summer and fall months compared to the rest of the year at all stations.

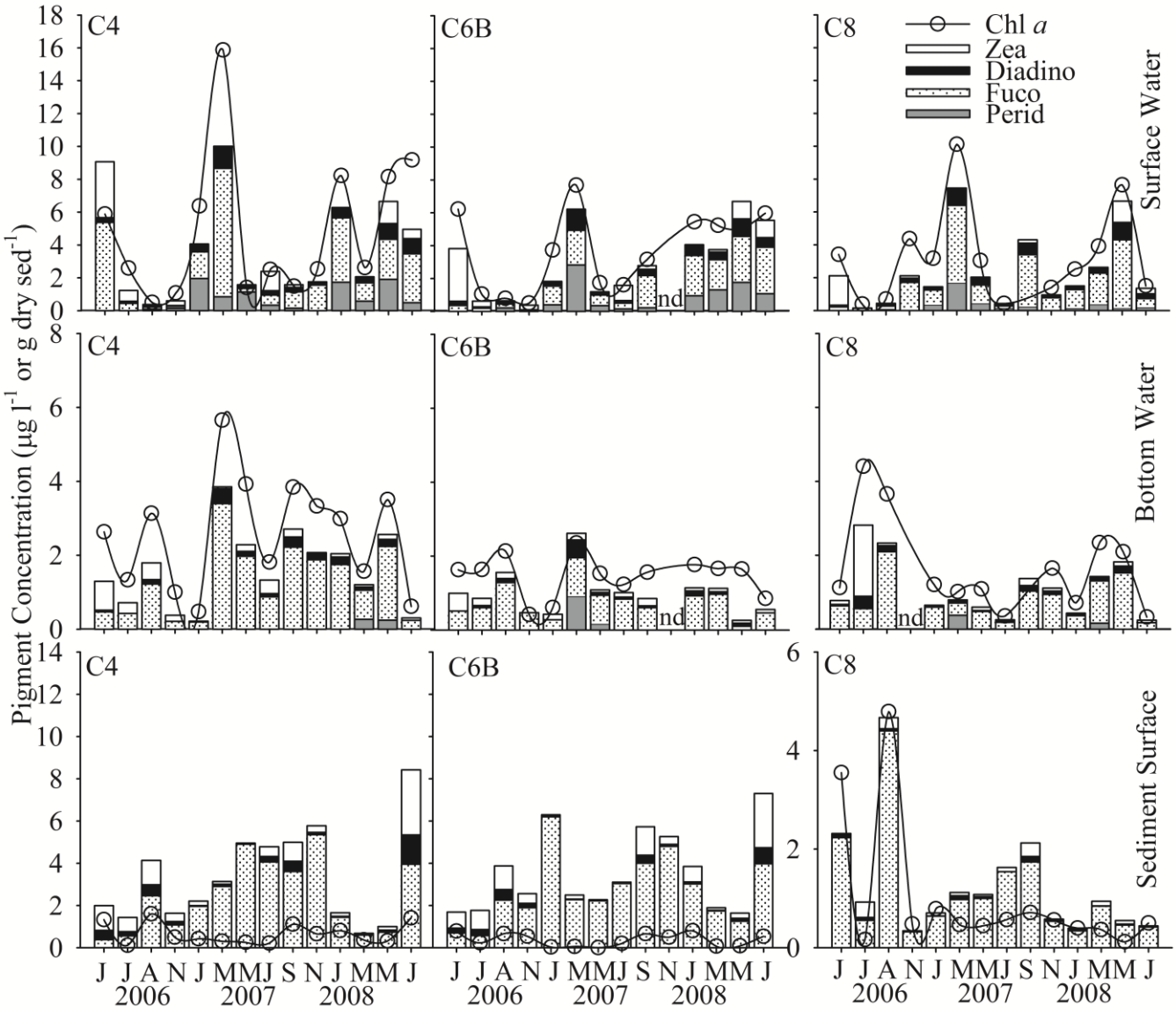

Fig. 2.2. Phytopigment concentrations in the surface water, bottom water and sediment surface from June 2006 to July 2008 at stations C4, C6B and C8. The carotenoids are represented in the stacked bars (Zea = zeaxanthin, Diadino = diadinoxanthin, Fuco = fucoxanthin, Perid = peridinin) and chlorophyll *a* (Chl *a*) is the line. nd = no data. The month abbreviations start with June, July, August and November in 2006 and in 2007 with January; remaining samples were taken bimonthly. Note the different Y axes. Identifiable, but low, concentrations of other pigments were not graphed, including alloxanthin, β-carotene, diatoxanthin, violaxanthin, and 19'-hexanoyloxyfucoxanthin.

The surface water and bottom water tended to have more pigments with higher concentrations present (Fig. 2.2) than the sediment surface and included additional pigments, such as peridinin and β-carotene (not shown), that were not commonly found on the sediment surface. The surface water and bottom water were similar to the sediment surface by having high concentrations of fucoxanthin during most months.

Community Composition

The sediment surface community composition (at all levels of size fractions) differed from the surface and bottom water at all three stations based on the different taxonomic composition that produced separation among the sample types in the MDS plots (plots not shown, but statistics reported in Table 2.1). Stress values ~ 0.10 from the MDS plots indicate the goodness-of-fit was reasonable. Some of the *R* values from the ANOSIM analysis were low (~ 0.25), but statistically significant, suggesting differences among sample types although overlap was identified in the MDS plot. The only null hypothesis not rejected, with regard to similarity, was between surface and bottom water at station C8 ($R = 0.04$, $p = 0.143$). The sediment surface community (all size fractions) composition varied seasonally only at station C4 with spring versus winter and summer versus winter being significantly different (C4 global $R = 0.33$, $p = 0.015$, spring vs. winter $R = 0.57$, $p = 0.029$, summer vs. winter $R = 0.46$, $p = 0.016$; C6B global $R = 0.19$, $p = 0.124$; C8 global $R = 0.27$, $p = 0.065$).

Picocyanobacteria (cell size 0.2-3 μm) dominated the community composition in all sample types and at all stations, and were higher (percentages) in sediments than in surface and bottom waters (data not shown). The picocyanobacteria contributed a mean of 99% and varied between 97.5 to 99.9% of the total sediment community cell density at all stations. The seasonal picocyanobacteria density peaks were evident in spring/summer when they were about seven times greater than the mean densities (not including peaks). The mean sediment picocyanobacteria densities differed among the stations (C4 = 1.4×10^7, C6B = 1.7×10^7, C8 = 4.3×10^6, $F_{2,38} = 3.78$, $p = 0.0319$, $n = 41$) with densities at station C8 significantly lower than station C6B ($t_{38,0.05} = 2.73$, $p = 0.0254$). The combined surface and bottom

water mean percentage of total picocyanobacteria cells contributing to the community was 85% (range = 15-99%). The highest densities of picocyanobacteria in surface and bottom waters at all stations were usually during the summer months of July-September.

Table 2.1. Comparison of microalgal community composition by size fractions between sample types, sediment surface versus surface water (SW) and sediment surface versus bottom water (BW) at stations C4, C6B and C8. The multidimensional (3D) scaling (MDS) plot stress value indicates the goodness-of-fit for the SW, BW, and SS data. The ANOSIM *R* statistic and *p* value test for differences between the sample types (e.g., SS vs. SW). SIMPER allows for an analysis of the total average dissimilarity (Avg. Diss.) between the sample types and the percent contribution (Taxa Con. %) of the top taxa contributing to the separation (Avg. Diss.). Bold values indicate the SS average taxa abundance was greater than the SW or BW average taxa abundance. Taxa abbreviations: Pico. = picocyanobacteria (PE/Low Pub + PE/High Pub, > 3 μm), F.Cy. = filamentous cyanobacteria, P.-n. = *Pseudo-nitzschia* spp., and C.Di. = centric diatoms (< 10 μm). Missing samples include: C4 SW 6/06, C6B SW 6/06, C8 SW 6/06, C4 BW 6/06, C6B BW 6/06, C8 BW 6/06 and C8 SS 7/06. SW = surface water, BW = bottom water and SS = sediment surface.

				ANOSIM		SIMPER		
Stat.	Size (μm)	MDS 3D stress	Sa. Ty.	*R*	*p*	Avg. Diss.	Taxa	Taxa Con. %
C4	0.2, 3.0, 8.0	0.09	SW	0.53	0.001	70.8	Pico.	**31.1**
C6B		0.07		0.42	0.001	64.5	Pico.	**32.5**
C8		0.10		0.28	0.001	53.4	Pico.	**31.9**
C4			BW	0.71	0.001	58.4	Pico.	**33.8**
C6B				0.25	0.002	53.0	Pico.	**33.2**
C8				0.30	0.001	48.2	Pico.	**31.6**
C4	3, 8.0	0.09	SW	0.55	0.001	76.7	Pico.	**35.1**
C6B		0.08		0.51	0.001	72.6	Pico.	**34.7**
C8		0.09		0.60	0.001	80.9	Pico.	**40.8**
C4			BW	0.18	0.002	51.1	Pico.	**34.3**
C6B				0.12	0.024	34.3	Pico.	34.9
C8				0.18	0.003	41.0	Pico.	**35.2**
C4	8.0	0.13	SW	0.76	0.001	93.4	F.Cy.	**7.5**
C6B		0.15		0.83	0.001	94.8	F.Cy.	**8.7**
C8		0.17		0.75	0.001	94.2	P.-n.	5.8
C4			BW	0.62	0.001	90.2	F.Cy.	**8.1**
C6B				0.56	0.001	88.3	F.Cy.	**9.2**
C8				0.45	0.001	91.8	C.Di.	4.3

By analyzing the combined size fractions (0.2, 3 and 8 μm), I found that the density of picocyanobacteria contributed the most and dominated the dissimilarity of the community composition between the sample types (SS vs. SW and SS vs. BW) for all stations (Table 2.1). The Bray-Curtis average dissimilarity for all size fractions (0.2, 3 and 8 μm) among the sample types ranged between 53-

70 for all stations. An average dissimilarity value of 100 indicates complete difference, while a zero represents no difference. Thus, these values suggested the communities differed. The high densities of sediment picocyanobacteria (with PE/Low Pub + PE/High Pub) present on the 3 and 8 μm filters, as seen by the top taxa in Table 2.1, caused the separation between the sediment and water column communities at all stations. Eliminating the picocyanobacteria from the 8 μm fraction analysis caused the average dissimilarity in most cases, to increase from 50-70 to 90 for all stations. This pattern was evident with the *R* values from the ANOSIM as well, indicating that the community composition differed more among the larger than the smaller cell sizes (Table 2.1). In addition, the top taxon in the 8 μm fraction contributed more evenly (e.g. ~ 10 %) to the community composition difference, while in the smaller fractions (0.2 and 3.0 μm) I found the top taxon made up a larger proportion of the contribution (e.g. ~ 30 %).

I omitted the data on picocyanobacteria density from further analysis so that the majority of the larger cells could be examined in greater detail. Diatoms represented the majority of the larger cells in the sediment surface community at all stations for most of the time (Fig. 2.3). Compared to the water column, the sediment surface was less diverse in taxa and contained few cryptomonads, phytoflagellates and dinoflagellates. The highest density (9. 8 × 10^4 cells g dry sed^{-1}) of diatoms (*Skeletonema tropicum*, *Skeletonema costatum* and pennate diatoms > 3.0 μm) present on the sediment surface was from station C4 in November 2007. Station C6B also had peaks of diatom density (6.2 and 7.0 × 10^4 cells g dry sed^{-1}) in November of 2006 and 2007, with the common organisms being *Pleurosigma* spp. (~30,000 cells g dry sed^{-1}), *Nitzschia* spp., *Navicula* spp. and centric diatoms (20-30 μm diameter) in 2006 and *Skeletonema tropicum*, *Skeletonema costatum* and centric diatoms (10-20 μm diameter) in 2007. The mean diatom densities among the stations were not significantly different ($F_{2,38} = 0.37$, $p = 0.6961$). Station C8 did not have the peaks in diatom density, as opposed to the other two stations, but had higher median monthly densities (C8 median = 9698, $n = 13$; C6B median = 6412, $n = 14$; C4 median = 6629, $n = 14$). The most common diatoms (due to high density per month) present on the sediment surface at

station C8 were benthic pennates of the genera *Amphora*, *Lyrella/Fallacia*, *Navicula* and *Pleurosigma*. The common pelagic taxa found on the sediment surface at C8 were *Asterionellopsis*, *Chaetoceros*, *Skeletonema tropicum* and centric diatoms (10-30 µm diameter).

The community in the sediment surface also differed from the surface and bottom water column (with picocyanobacteria omitted) because of the presence of filamentous cyanobacteria (Fig. 2.3), whose densities were sometimes greater than diatoms (e.g. C4 July 2008, C6B November 2006). The presence of filamentous cyanobacteria was common at stations C4 and C6B, but not at station C8. March 2007 and June 2008 were the only two sample dates that included filamentous cyanobacteria were present at station C8. Other cyanobacteria, such as *Merismopedia* spp. and *Anabaena* spp., made up the cyanobacterial community at station C8 during summer, 2006.

The respective niche (pelagic, tychopelagic, or benthic) of the autotrophic cells (no picocyanobacteria) in the sediment surface community was dominated by benthic types (Fig. 2.4). At all stations, a general seasonal sediment pattern was evident that included a higher percentage of benthos in the summer and a larger proportion of pelagic or tychopelagic cells in the fall and winter. The surface water and bottom water communities were dominated by pelagic phytoplankton. Abundant benthic types (7×10^4 to 1.8×10^5 cells g dry sed^{-1}) were found in the bottom water at station C6B in July 2007 and at station C8 in August 2006; few to no benthic cells were found in the surface water at any station.

Environmental Parameters

The mean sediment composition varied among the stations (n = 42 for each station). Stations C4 and C6B were the muddiest (% mud: station C4 = 90.5% ± 1.3 Std. Err., station C6B = 92.4% ± 0.11 Std. Err.) and station C8 had the lowest mean mud content (23.6% ± 4.3 Std. Err.). Station C4 was less muddy in summer 2006 and winter/spring 2008, station C6B varied little, and station C8 was the most variable with one month (July 2006) being muddy. The muddier stations had higher mean sediment TOC (station C4 = 1.29%, station C6B = 2.00%) compared to station C8 (0.59%) which varied the most in organic content consistent with the differing percentage of mud. The % surface PAR reaching the

seafloor was less than 1% for all stations except at station C4 in August 2006 (Fig. 2.5). The highest % surface PAR values were during the spring and summer at all stations. The PAR (μmol photons m^{-2} s^{-1}) range for each station was: station C4 = 0-83 (*n* = 12), station C6B = 0-10 (*n* = 13) and station C8 = 0.1-22 (*n* = 10). The attenuation coefficients for each station ranged from: station C4 = 0.25-0.68, station C6B = 0.25-0.63, and station C8 = 0.21-0.38 with a total mean for all stations of 0.40 (*n* = 35). The bottom-water environmental parameters followed a typical seasonal pattern (Fig. 2.6) with the concentration of dissolved oxygen (mg l^{-1}) at all three stations being lower in the summer and higher in the winter; bottom-water temperatures also followed a typical seasonal cycle. Summer hypoxia was more frequent at stations C4 and C6B than C8. The salinity at station C4 varied the most and variation lessened with increasing depths (stations C6B and C8). The pH of the bottom water was lower in the spring and summer months (~ 7.7 for all stations) and increased in the fall and winter to ~ 8 (data not illustrated).

I constructed a PCA biplot (Fig. 2.7) with vectors representing abiotic and biotic variables to determine which variables were correlated. Opposite lying vectors are negatively correlated, small angles between vectors are highly correlated, and perpendicular vectors are uncorrelated. The longer lengths of vectors indicate higher variability. The station samples (C4, C6B and C8) were spread among the variables to which they were related, and the station samples that contained higher values were found closer to that variable label. According to the PCA biplot (Fig. 2.7), the PAR and bottom-water temperature had the highest correlation (e.g. small angles between vectors) with the sediment biotic variables. Of all the nutrients analyzed, silicate had the highest correlation (smallest angle between vectors) with the sediment biotic variables. The sediment characteristics (% sand and % TOC) were not correlated with the sediment biotic variables (e.g. perpendicular vectors) and sediment TOC was inversely related to % sand and depth (e.g. opposite lying vectors). In general, stations C6B and C4 were more closely related with each other than either was with C8 among the variables measured.

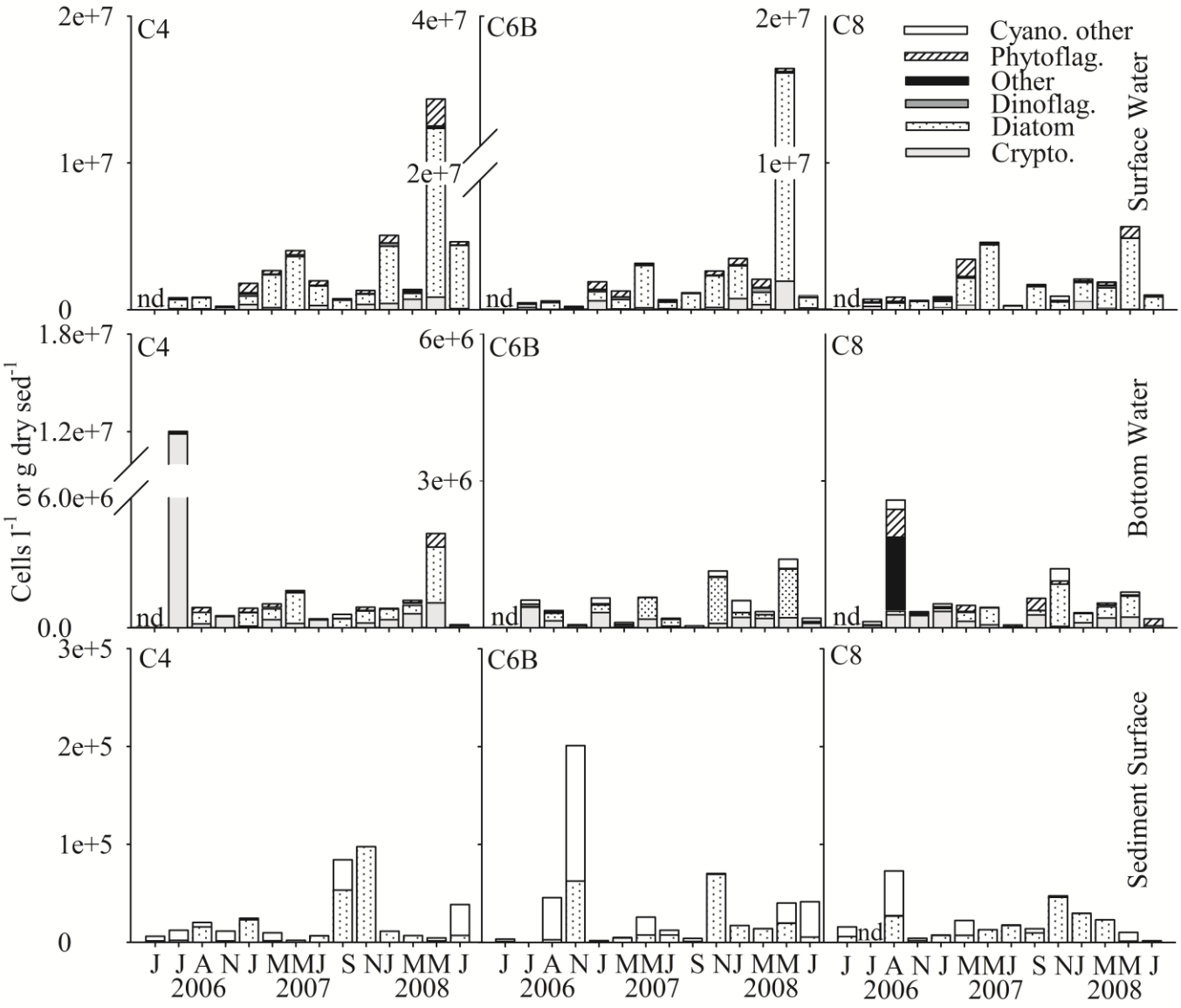

Fig. 2.3. Density of microalgae by taxonomic group: cyanobacteria-other (no picocyanobacteria), phytoflagellates, other (chlorophytes, ciliates, euglenoids, silicoflagellates, ebriids and raphidiophytes), dinoflagellates, diatoms, and cryptomonads for stations C4, C6B and C8 sampled from surface water, bottom water and sediment surface from June 2006 to July 2008. nd = no data. The month abbreviations start with June, July, August and November in 2006, in 2007 with January, and the rest represent bimonthly sampling. Note the different Y axis ranges.

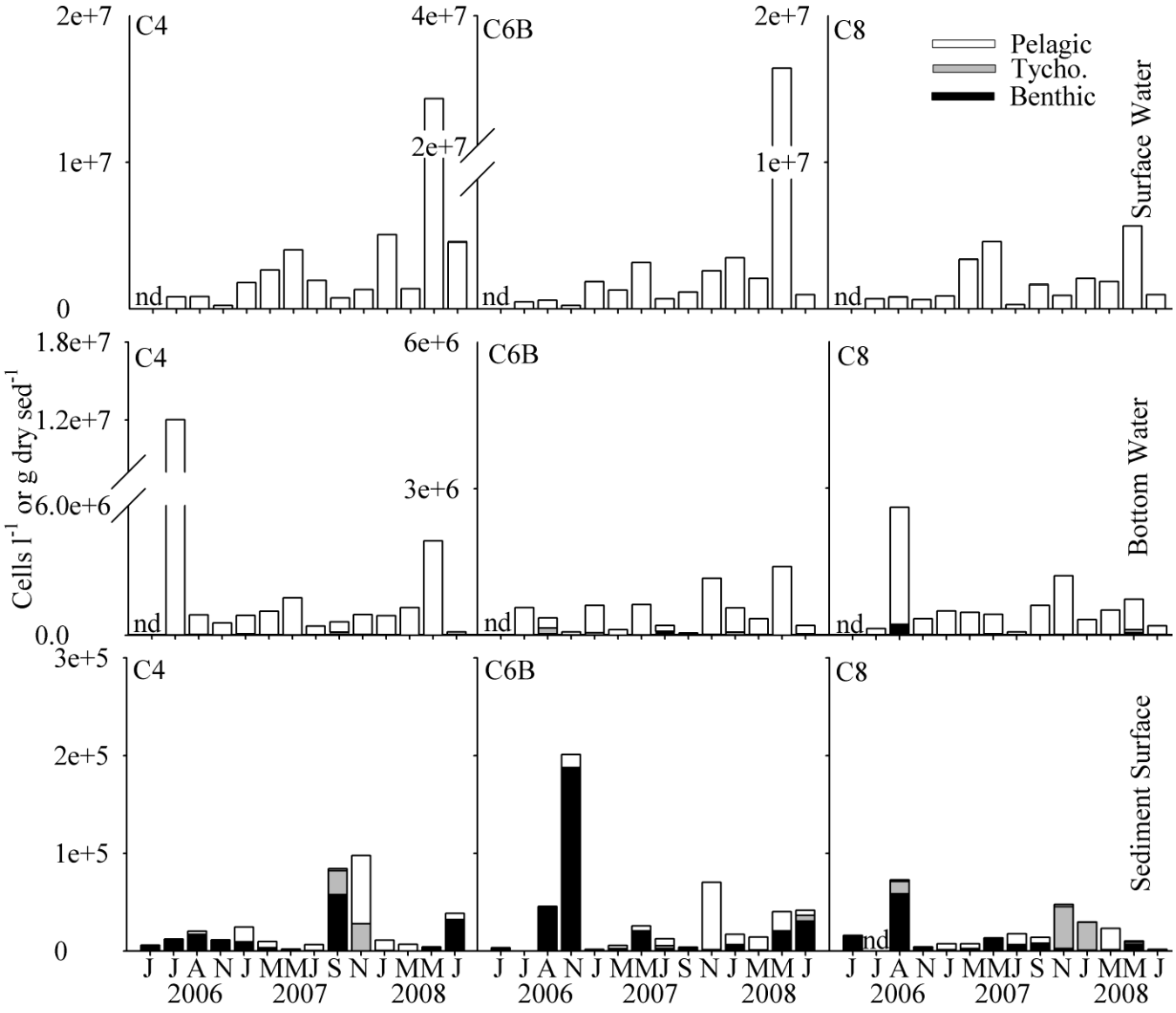

Fig. 2.4. Common niches (pelagic, tychopelagic or benthic) of phytoplankton and microphytobenthos (no picocyanobacteria) observed in the surface water, bottom water and sediment surface at stations C4, C6B and C8 from June 2006 to July 2008. nd = no data. The month abbreviations start with June, July, August and November in 2006, in 2007 with January, and the rest represent bimonthly sampling. Note the different Y axis ranges.

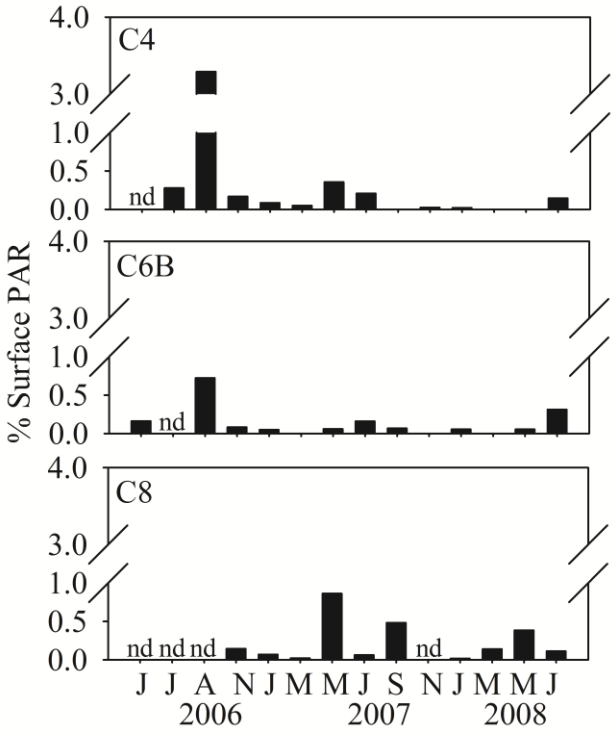

Fig. 2.5. Percent of surface photosynthetically available radiation (PAR) reaching the seafloor from June 2006 to July 2008 at stations C4, C6B and C8. nd = no data due to darkness at sampling. The month abbreviations start with June, July, August and November in 2006, in 2007 with January, and the rest represent bimonthly sampling.

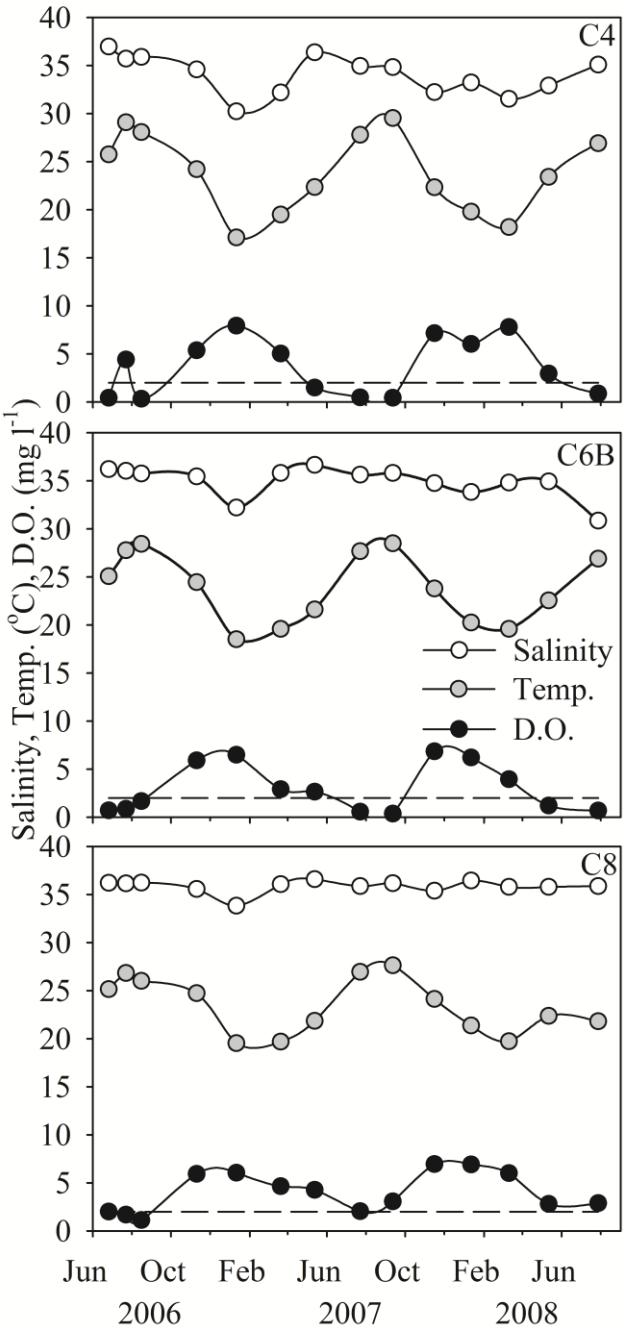

Fig. 2.6. Seasonal variation of bottom-water salinity, temperature (°C) and dissolved oxygen (D.O., mg l^{-1}) at stations C4, C6B and C8 from June 2006 to July 2008. The dashed line at 2 mg l^{-1} is a reference for hypoxic conditions.

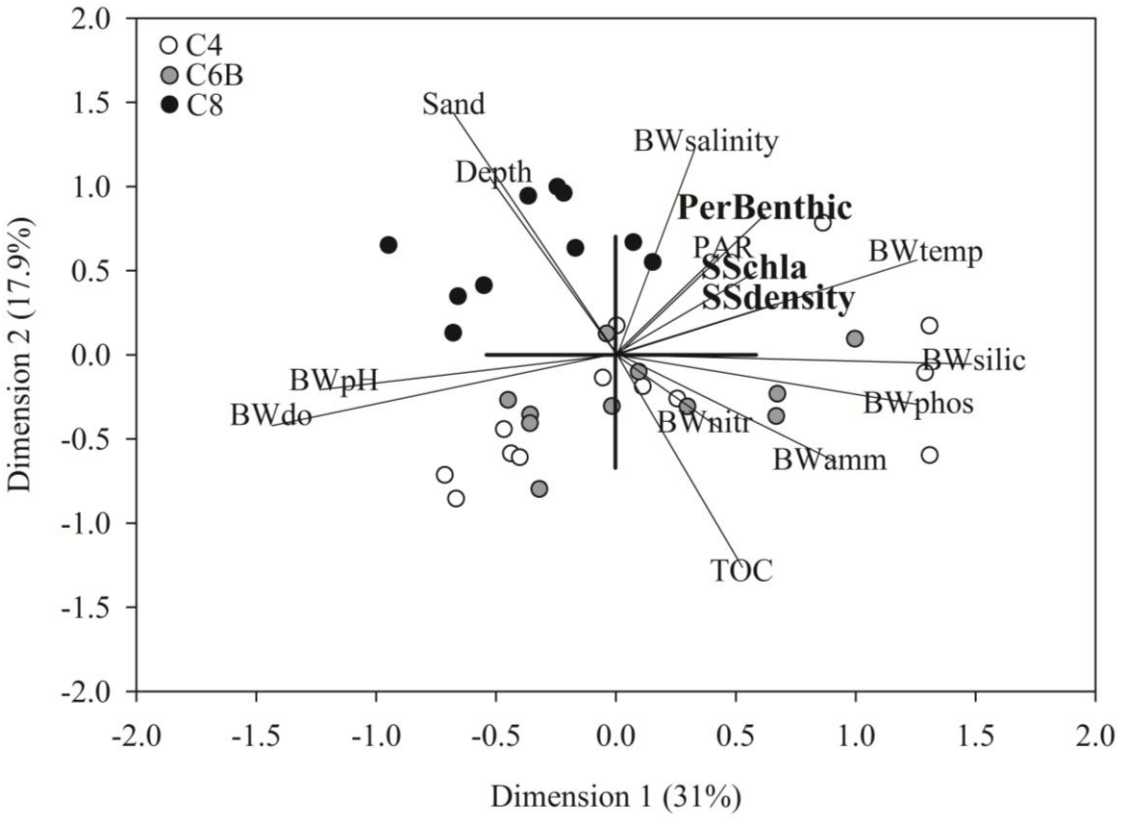

Fig. 2.7. Principal component analysis (PCA) biplot of sediment and bottom-water abiotic variables and sediment biotic (bold) variables as vectors (n = 15) and station samples as points (n = 34). The abiotic variables included: sediment percent sand, depth, bottom-water salinity, seafloor photosynthetically available radiation (PAR), bottom-water temperature, bottom-water dissolved inorganic nutrients (silicate, phosphorus, ammonium and nitrate-nitrite), sediment percent total organic carbon, bottom-water pH and dissolved oxygen. The sediment biotic variables included: percent benthic cells, sediment-surface chlorophyll *a* and sediment-surface benthic cell density. Perpendicular vectors are uncorrelated, small angles between vectors are highly correlated and opposite lying vectors are negatively correlated. Longer lengths of vectors indicate higher variability. Sediment samples were removed from analysis if the data set was incomplete mostly due to no PAR data, which included: C4 6/06, C6B 6/06, C6B 7/06, C8 8/06, C8 6/06, C8 7/06, C8 8/06 and C8 11/07.

DISCUSSION

This study covered a broader spatial area, more representative sedimentary characteristics, and seasonal and inter-annual variability among microphytobenthos than any study to date within the hypoxic area in the northern Gulf of Mexico (Grippo et al. 2009, 2010). Grippo et al.'s studies were confined mostly to sandy nearshore shoal areas with some surrounding muddy areas in regions that were intermittently hypoxic due to their higher bathymetric relief. Their samples were also temporally limited. My stations were in areas that were frequently hypoxic (Fig. 2.1), and at similar depths of other areas on the shelf

that are also hypoxic. The % mud in sediments along an 18-m isobath at 11 stations from the Mississippi River to the Texas/Louisiana border range from 25 to 99%, but are more commonly > 75%, and have a % TOC content ranging from 0.5-2.0% , and average about 1.5% (Baustian et al. unpubl. data). The stations I sampled ranged from 24-92% mud and 0.6-2.0% TOC, which is characteristic of the larger region where hypoxia forms.

I hypothesized that the sediment surface microalgal community would be similar to the surface and bottom water as a result of high primary productivity in the surface waters and the subsequent settling of phytoplankton that fuels hypoxia, but I reject this hypothesis. Instead, microphytobenthos were common on the sediment surface, especially in the summer along the C transect. Although I did not measure benthic oxygen production in this study, I suspect that the Dortch et al. (1994) suggestion that benthic photosynthesis could potentially contribute to oxygen dynamics was possible, based on the presence of microphytobenthos and the potentially-significant irradiance values. I estimated that the net oxygen produced (based on Gattuso et al. 2006) at the highest irradiance (80 μmol photons m^{-2} s^{-1}) for 8 hours of daylight and 1 m deep water column would be about 1.5 mg O_2 l^{-1} d^{-1}. This could influence oxygen dynamics when the sediment oxygen demand is low. For example, if oxygen consumption is at the low end of bottom-water respiration rates (0.02 – 6.96 mg O_2 l^{-1} d^{-1}) (Rabalais et al. 1994, Turner et al. 1998) and sediment respiration rates (0.2 – 4.6 mg O_2 l^{-1} d^{-1}) (Chapter 4), then the amount of oxygen produced could exceed bottom respiration, thus affecting hypoxia formation.

The dominant microphytobenthos (> 3 μm) on the sediment surface were benthic diatoms and filamentous cyanobacteria that were different from the water-column phytoplankton. Even though the time scales may affect differences in community composition between water and sediment due to the varying settling rates of phytoplankton cells, fecal pellets and aggregates, the community composition difference was dominated by the presence of benthic cells. Much of the settled phytoplankton is packaged in fecal pellets (which can sink through a 20-m water column in one day) and others are senescent diatoms (Dortch et al. 2001). The sinking rate of the senescent diatom cells is dependent on

their size and their silica content (Dortch et al. 2001). Other pelagic phytoplankton, such as dinoflagellates, are either not grazed or are remineralized in the upper water column, because the concentration of peridinin in surface waters is not reflected in bottom waters or sediments. A similar situation is likely for cryptomonads, commonly part of the phytoplankton community off the central coast of Louisiana (Dortch et al. 2001). I do not presume to infer that the cells collected in the monthly samples from the surface waters are exactly representative of the cells in the bottom waters or in the sediment surface collected at the same time, but I do think that a correlation among these communities is more likely than a 2-month lag between surface communities and those of the lower water column and sediment surface (Qureshi 1995, Dortch et al. 2001).

The microphytobenthos are a common component of sub-tidal benthic systems, including areas of hypoxia, e.g. the northern Adriatic Sea (Totti 2003, Cibic et al. 2007), the Kattegat (Graneli & Sundbäck 1986), and the northern Gulf of Mexico (Grippo et al. 2009, 2010, this study). Grippo et al. (2009, 2010) reported a higher percentage of benthic diatoms (based on pennate versus centric ratios) on the sediment surface. They also found a higher percentage of microphytobenthos on shallow (< 11 m), sandy shoals compared to off-shoal muddier areas, at their non-hypoxic sites west of my study area (50-150 km). They did not document the concentration of filamentous cyanobacteria, which I found to be abundant possibly because of their adaptation to fluctuating environmental variables such as oxygen and pH (Shilo & Fattom 1984). Larson & Sunbäck (2008) have shown that benthic diatoms can survive hypoxic conditions in laboratory settings and assist in restoring sediment oxygen levels.

Microphytobenthos are known to persist and photosynthesize in light levels as low as 0.1% of the surface light and their photosynthetic response to increasing light levels can be rapid (Graneli & Sundbäck 1986, Paterson 2001, Gerbersdorf et al. 2004, McGee et al. 2008). The seafloor light levels were low at all of my sites; the combined station average PAR was 5 μmol photons m^{-2} s^{-1}, or about 0.2 % of the surface (reference) PAR (n = 36). The % surface PAR levels reaching the seafloor observed in the Lehrter et al. (2009) study were higher, ranging between 2-6 % (for areas with > 1 % light at the

seafloor). I suggest that the limited light measurements I have probably did not fully capture the variability in light at each station. To my knowledge, no continuous light data have been reported for the inner continental shelf of the northern Gulf of Mexico, and this type of data would help improve my understanding of the potential for photosynthesis in the bottom water and on the seafloor. Nevertheless, the light data I do have suggest that light reaches the seafloor, especially in the summer. The results of the PCA biplot analysis (see Fig. 2.7) revealed a positive correlation between PAR at the seafloor and the percent benthic cells, chlorophyll *a* concentration, and benthic cell density, thus suggesting that light levels influence the presence and density of the microphytobenthic community. In addition, the spectral light quality can also affect the taxonomy of the community (Ploug et al. 1993, Cahoon 1999), and the rates of photosynthesis and oxygen evolution may be affected by these low light levels (MacIntyre et al. 1996).

Picocyanobacteria, containing either phycocyanin or phycoerythrin, were present in surface sediments at all stations. Cyanophytes with phycobillins are efficient at gathering low light (Brock 1973), which may be why they are present in the turbid, low-light environment of the northern Gulf of Mexico. Due to their small cell size they make up a small fraction of the phytoplankton biomass (chlorophyll *a*, Dortch et al. 2001). Some of the community composition similarity among the sample types (SW, BW, SS) was due to the density of picocyanobacteria cells, which was documented as dissimilarity values increased with the removal of picocyanobacteria. Their presence in the sediment surface is probably due to direct sinking via diatom/picocyanobacteria aggregates (Dortch et al. 2001) because summer peaks were found in all sample types (SW, BW, SS). Also, the high abundance of PE picocyanobacteria at the 3 and 8 μm filter size of the sediment samples indicates that these cells were part of aggregates. They were not abundant on the smaller filter size (0.2 μm) which would indicate a flux of individual cells. Dortch (1998) also found that a high percentage (up to 60 %) of the picocyanobacteria (mostly PE) in water samples were captured on the large filter sizes (3 and 8 μm) and

proposed that they were part of cell aggregates. Few benthic studies evaluate the smaller autotrophs (< 3.0 μm) and may be missing a portion of the community, at least in abundance values.

Microscopy is clearly useful to estimate the cell density of different types of microphytobenthos and their niches. Additionally, identification of phytopigments helps to indicate and estimate the potential biomass of taxonomic groups of microphytobenthos. For example, the major carotenoid present in the sediment at all stations was fucoxanthin, which is a primary indicator pigment for diatoms, but prymnesiophytes, raphidophytes and some dinoflagellates with endosymbionts also contain fucoxanthin (Jeffrey et al. 1997). My microscopic analysis verified high diatom densities, but these densities were not high in the summer, unlike the high summer fucoxanthin concentrations. This inconsistency could be due to the size of the diatoms and the pigment content per cell or the slower degradation of fucoxanthin in anoxic conditions (Hodgson et al. 1997). Lastly, it is important to note that the sediment pigment pools are influenced by sources of live, senescent and dead cells (phytoplankton and microphytobenthos), fecal pellets, aggregates, and environmental conditions (e.g. oxygen, temperature, PAR) (Sun et al. 1993, Hodgson et al. 1997, Hansen & Josefson 2001). This makes it difficult to distinguish the source, but microscopy helps estimate the potential living contribution.

The range in mean sediment chlorophyll *a* values (0.36 to 0.99 μg g dry sed^{-1}) was similar to other studies that had microphytobenthos present; caution, however, is advised when comparing results from different methodology (spectrophotometry vs. HPLC). In Onslow Bay, North Carolina, for example, the mean sediment chlorophyll *a* value, based on spectrophometric methods, at 20-29 m depth was 0.67 μg g dry sed^{-1} (Cahoon et al. 1990), and in coastal Massachusetts it ranged between 0 to 2.5 μg g dry sed^{-1} (Cahoon et al. 1999). I found the concentration of chlorophyll *a* in sediments was usually less than the total cartenoid pigment concentration (especially fucoxanthin). McGee et al. (2008) suggested that benthic diatoms may adapt to low light levels by producing fucoxanthin concentrations resulting in high fucoxanthin:chlorophyll *a* ratios. They found that this ratio in Onslow Bay, North

Carolina increased from ~ 2:1 to 5:1 in shallow sites (< 35 m, 81 μmol photons m^{-2} s^{-1}) compared to deeper, lower light sites (63 m and 2.34 μmol photons m^{-2} s^{-1}). The fucoxanthin:chlorophyll *a* ratio (*n* = 5) for samples with > 75 % benthic cells were 2.4:1 (station C4), 3.5:1 (station C6B) and 1.4:1 (station C8), which is comparable to the 2:1 ratio value of the shallow sites in the McGee et al. (2008) study. Microphytobenthos on the Louisiana continental shelf may be producing higher concentrations of fucoxanthin than chlorophyll *a* to adapt to the low light levels on the seafloor.

Station C8 had the highest sediment chlorophyll *a* levels (0.99 μg g dry sed^{-1}), which is supportive of my prediction for a well-developed microphytobenthos community there. The lowest frequency of hypoxia occurred there along with overall less frequent mid-summer hypoxia (~ 25-50%) in the past 23 years than at stations C4 and C6B which experienced hypoxia > 75% of the time. I predicted that the microphytobenthos at station C8 were less likely to be light limited due to less phytoplankton shading, but all stations had similar chlorophyll *a* levels in the surface water during my study. Stations C4 and C6B had finer sediments that might be more susceptible to physical resuspension by wave activity, bottom currents and shrimp trawling, thus affecting the light levels to the bottom. However, the sediment characteristics (% sand or % TOC) at the three stations were not correlated with the presence of microphytobenthos (see Fig. 2.7). The variables that correlated with the presence of microphytobenthos were the seafloor PAR, bottom-water temperature and salinity. This suggests that microphytobenthos are more likely to be present when higher levels of seafloor PAR, warmer temperatures and higher salinity exists, i.e., during summer when hypoxia occurs.

The presence of the benthic cells, primarily pennate diatoms, during summer hypoxia may also be enhanced by hypoxia-related conditions. High nutrient fluxes from remineralized organic matter (Rabalais & Turner 2006, Rabalais et al. 2007a, Baustian et al. unpubl. data) in the sediments could support diatom communities where light is sufficient. The silicate concentrations in bottom-water doubled, reaching 40-80 μM when oxygen levels fell from 2 to 1 mg l^{-1} (Rabalais & Turner 2006). Thus, the silicate necessary for diatom frustule formation was more available during extremely low

oxygen conditions, 0-1 mg $^{-1}$, than in non-hypoxic conditions, and could support a community of benthic diatoms (e.g. Sigmon & Cahoon 1997). In addition, hydrogen sulfide toxicity and the mortality of benthic infauna occur in hypoxic conditions (Baustian & Rabalais 2009 and references therein). A reduction in grazing pressure by macroinfauna (Miller et al. 1996, Middelburg et al. 2000) could result in higher densities of microphytobenthos compared to areas with a functioning macroinfaunal community in normoxic bottom water. The digestive tracts of some surface deposit feeding polychaetes from one of these stations contained pennate diatoms (Baustian unpubl. data) and from near-by sites (Grippo et al. 2011) and the density of these polychaetes decreased with hypoxia (Baustian & Rabalais 2009). Alternatively, the benthic grazing pressure may be less on microphytobenthos with excess primary production from the water column (Grippo et al. 2011). Suitable conditions for growth of benthic diatoms coupled with decreased grazing pressure would support a healthy microphytobenthic community, and especially larger pennate forms.

Another reason why microphytobenthos might be present on the hypoxic continental shelf is due to the release of organic molecules from the sediment during mineralization of phytodetritus. This organic substrate could provide benthic diatoms with an alternative nutritional mode via heterotrophy or mixotrophy when environmental conditions, such as light, are not favorable (Round et al. 1990, Cahoon et al. 1994).

My observations indicate that microphytobenthos are present on the sediment surface throughout the year, but more so in the summer, and that the benthic community differs from the water column phytoplankton community in the northern Gulf of Mexico. Because of their presence, I propose that microphytobenthos present during the summer in my study area and elsewhere in similar depths where hypoxia occurs may produce enough oxygen to significantly influence the bottom-water oxygen budget. Measurements of the oxygen fluxes under light and dark conditions would be useful for quantifying the net yield of oxygen from the benthic community.

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CHAPTER 3

MICROPHYTOBENTHOS ALONG THE LOUISIANA CONTINENTAL SHELF DURING MID-SUMMER HYPOXIA

INTRODUCTION

Bottom-water hypoxia, commonly defined as ≤ 2.0 mg l^{-1} and also known as the "Dead Zone," occurs from March to October off Terrebonne Bay located ~ 100 km west of the Mississippi River delta. It forms a continuous band of low oxygen water along the Louisiana continental shelf and onto the adjacent Texas shelf in mid-summer (Rabalais et al. 2002, 2007). The high spring discharge from the Mississippi and Atchafalaya rivers, which mostly flow westward with the Louisiana Coastal Current in winter and spring, provides the nutrient enrichment and stratification that promotes high primary productivity (> 300 g C m^{-2} y^{-1}) along the inner and mid continental shelf (Sklar &Turner 1981, Lohrenz et al. 1990, Lehrter et al. 2009). The senescing phytoplankton, organic detrital aggregates and mostly fecal pellets (Qureshi 1995), beginning in the spring fuel the microbially-mediated aerobic decomposition and mineralization of organic carbon producing the persistent oxygen depletion below the pycnocline (Turner & Allen 1982, Turner et al. 2008, Murrell & Lehrter 2011). Hypoxia develops because the microbial respiration exceeds the supply of dissolved oxygen due to little to no oxygen input (atmospheric or primary production) from the upper layer across the strong pycnocline to the bottom layer or from horizontally advected transport of oxygenated waters. Hypoxia is commonly found between the depths of 5 - 30 m on the Louisiana continental shelf (Rabalais & Turner 2001), and the mean areal extent of hypoxia measured in mid to late-July 1985-2010 was 13,800 km^2 (modified from Rabalais et al. 2007; http://www.gulfhypoxia.net). The largest hypoxic zone recorded was in 2002, covering about 22,000 km^2 (Rabalais et al. 2007). The hypoxic area has averaged 18,800 km^2 since 1993 unless the area is perturbed by tropical storms or unusual shelf circulation patterns.

Microphytobenthos, the benthic microalgae and cyanobacteria, are common in subtidal areas and continental shelves around the globe (Cahoon 1999, Underwood & Kromkamp 1999). Some of these areas include the western Atlantic (Cahoon et al. 1990, Nelson et al. 1999), Dogger Bank, North Sea

(Reiss et al. 2007), Kattegat (Sundbäck & Jönsson 1988), and the northern Adriatic Sea (Totti 2003, Cibic et al. 2007), which also experiences hypoxia. Photosynthetic available radiation (PAR) tends to be the principle factor limiting their presence and production (Chapter 2, MacIntyre et al. 1996, Blackford 2002).

Microphytobenthos are important food resources for micro- and macroheterotrophs, including deposit-feeding infauna (Marsh et al. 1989, Reis et al. 2007) and can contribute benthic carbon basal-resources for macroinfauna (Grippo et al. 2011) and red snapper in the northern Gulf of Mexico (Wells et al. 2008). Microphytobenthos can decrease the benthic inorganic nutrient fluxes out of the sediment (Sundbäck et al. 1991). Also, benthic diatoms can regulate silicate fluxes and potentially reduce Si fluxes by > 80% during daylight hours (Sigmon & Cahoon 1997 and references within).

Microphytobenthos contribute to the total primary production of marine systems and sometimes can exceed intregrated water column primary production (Cahoon & Cooke 1992, Nelson et al. 1999, Totti 2003, Grippo et al. 2009). They also can affect dissolved oxygen budgets in the bottom water (Jahnke et al. 2000). This last point is especially relevant to the hypoxic northern Gulf of Mexico, because oxygen evolution at the seafloor could affect dissolved oxygen fluxes that are used to predict hypoxia formation and subsequent maintenance (for review see Justić et al. 2007).

Microphytobenthos were common and had high densities and biomass in the late summer/early fall off the central coast of Louisiana among sandy shoals and deeper muddier sites, although different categorization schemes were used (Grippo et. al. 2009, Grippo et al. 2010, Chapter 2). The seasonal presence of microphytobenthos along a frequently-hypoxic transect ~ 100 km to the west of the Mississippi River delta were correlated with higher PAR levels, warmer temperatures and higher salinity of the bottom water, coincident with lower river discharge and reduced wave activity when hypoxia typically occurs (Chapter 2). The broader distribution of microphytobenthic communities and related environmental conditions along the Louisiana continental shelf were not known until this study.

Phytopigments, such as chlorophylls, carotenoids and phycobiliproteins used by autotrophs for photosynthesis (either in light harvesting or photoprotection) can be useful biomarkers to determine the presence of major taxa of microphytobenthos (Brotas & Plante-Cuny 2003, Bidigare et al. 2005, Reiss et al. 2007). The degradation products of chlorophylls, such as pheophytins, can also be useful to determine grazing, cell senescence and sediment diagenic processes. Sediment phytopigment pools are not just influenced by living microphytobenthos, but also by settling surface phytoplankton, senescing phytoplankton and microphytobenthos, fecal pellets and other allochthonous detrital material. Studies identifying the presence of microalgae are most definitive when a dual approach of pigment analysis and microscopic identification is used (Irigoien et al. 2004, Havskum et al. 2004).

I describe the estimated biomass, density and composition of microphytobenthos along the Louisiana continental shelf where hypoxia is typically present in mid-summer. I also wanted to verify that the primary producers on the sediment surface were benthic and not the settled phytoplankton, which are common in this highly productive area (Dortch et. al. 1994, 2001, Chapter 2). My null hypothesis was that the presence of microphytobenthos and their density and biomass (as chlorophyll *a*) would be similar along the continental shelf. The alternate hypothesis was that microphytobenthic densities and biomass (estimated by chlorophyll *a*) probably vary along the continental shelf due to seafloor PAR, sediment characteristics and mixing events that could resuspend sediment and the associated microphytobenthos.

MATERIALS AND METHODS

Field Collection

I collected sediment for microphytobenthos on the annual, mid-summer shelfwide cruises to map hypoxia and related environmental variables (Rabalais et al. 2007). Sampling occurred from the Mississippi River to Lake Calcasieu, LA along a 14 to 20 m depth contour in late-July of 2006, 2007 and 2008.

Water column environmental parameters were measured with a SeaBird CTD deployed within 1 m of the seafloor. A Hydrolab Surveyor 3 or YSI 6820 was used to obtain data as close to the seafloor as possible (0.1 to 0.5 m). The bottom waters for the 2006 stations I4, J4 and K4 were sampled only with the CTD. I used data from a Biospherical Instruments Inc. profiling natural fluorometer (PNF-300) to determine the photosynthetically available radiation (PAR) at the seafloor and on the research vessel (reference PAR) when ever possible during 24-h operations. The percent surface PAR reaching the seafloor was calculated by using the reference PAR (mean ≈ 1600 μmol photons m^{-2} s^{-1}, n = 12) measured from the ship. The percent PAR calculations were used instead of the absolute PAR values to correct for variations in time of day. Attenuation coefficients using the reference and bottom PAR were also calculated.

I sampled the surface water with a bucket and the bottom water with a 5-l Niskin bottle at 0.5 m above the seafloor. Water samples for phytopigments were filtered on 47 mm diameter Whatman GF/F filters and stored in liquid nitrogen. Surface and bottom water for microscopic analyses were preserved in a nalgene bottle containing 1 ml gluteraldehyde (50%) and filled to 100 ml. Bottom water samples were analyzed for nitrate + nitrite (BWNO3+NO2), ammonium (BWNH4), silicate (BWSi) and phosphate (BWPO4) on a Lachat auto-analyzer II system (8000 series) with an autosampler (ASX-400 series) according to EPA methodology (Methods 353.2, 350.1 and 365.2).

I collected sediment at each station from five 'intact' GOMEX box cores (0.5 m high, 0.3 m long, 0.3 m wide, surface area 0.09 m^2, Boland and Rowe 1991). An 'intact' box core retained the overlying water and the sediment surface was visibly undisturbed. Subsamples were taken using two acrylic core tubes (7.6 cm diameter) from the middle of the box cores to avoid potentially disturbed edges. The top 0.5 cm was removed from each subcore with a precision core extruder (Fuller & Butman 1988) because light usually penetrates only the top millimeters of the sediment (MacIntyre et al. 1996). The first subcore sediment slice was homogenized in a Petri dish to fill two cyrovials (1.8 ml each, stored in liquid nitrogen) for pigment analysis and two cryovials (1.25 ml each, stored at 4 ºC) for total

organic carbon (TOC) analysis. Five replicates were analyzed for sediment pigments and three for sediment TOC. The rest of the sediment (~ 17 ml of slurry) from the first subcore was preserved for microscopy in a 125 ml nalgene bottle with 1 ml of gluteraldehyde (50%), and filtered sea water was added to make a total of 100 ml. Only one sample per station was taken for microscopic analysis. The second subcore from each box core was sampled for grain size analysis, but only three of the five replicates were analyzed.

Bottom-water salinity, temperature, and dissolved oxygen measurements were recorded at station C6C (28.8686 Latitude, -90.4903 Longitude, located 2.2 km west of station C6B) from June–August in 2006, 2007 and 2008 at 15 min intervals by a multiparameter water quality sonde (YSI 6600 EDS) located 1 m above the seafloor. I calculated the daily means of bottom-water parameters to help determine mixing events, prior, during and after the shelfwide cruises.

Laboratory Analyses

Pigments were extracted in a dark room by sonication in cold HPLC-grade 100% methanol for water samples and sonicated with cold HPLC-grade 100% acetone for sediment samples. The filtered (0.2 µm) extract was injected into a Waters® high-performance liquid chromatography (HPLC) system equipped with a 600 controller, 600 pump, 996 photodiode array detector and 474 fluorescence detector based on the methods of Wright et al. (1991). The water content of the sediment samples was minimized by carefully pipetting water from the core sediment surface before extruding. The water content was considered to have a minimal effect on calculating the concentration. I found high levels of pigment degradation products in the sediment and used three columns (Waters Nova-pak C_{18} 3.9 × 150 mm, a Rainin Microsorb C_{18} and a Vydac Reverse-Phase C_{18}) to separate and identify pigments. Sediment samples were run for 75 minutes with an elution gradient of 80:20 methanol:ammonium acetate, 90:10 of acetonitrile:water, and 100 percent ethyl acetate. Only one column (Waters Nova-pak C_{18} 3.9 × 150 mm) was needed for phytopigment analysis of the water samples, and they were run for 30 minutes on the same elution gradient. I used retention times and visible absorption spectra from DHI

Lab standards as well as data and graphic sheets from Jeffrey et al. (1997) to help identify the pigments present. Some phytopigments were left out of the water and sediment analysis because the concentrations were zero or minimal for the majority of the samples. These pigments included: neoxanthin, lutein, myxoxanthophyll, canthaxanthin, gyroxanthin, echinenone and prasinoxanthin.

The percent total organic carbon by weight in sediment was determined using a Perkin Elmer CHN Model 2400 elemental analyzer after drying and grinding the sediment and acidifying to remove calcium carbonates (Hedges & Stern 1984). I removed organics with 6% hydrogen peroxide, dispersed the sediments in hexametaphosphate and wet sieved (63 μm) to separate the sand from the mud to determine sediment grain size by weight (Folk 1974).

Microscopy

I used epifluorescence microscopy to determine the phytoplankton community composition of surface and bottom water and sediment samples (adapted from Dortch et al. 1997, Dortch 1998). The water samples were size fractionated by filtering onto 0.2, 3.0 and 8.0 μm polycarbonate filters. The 3.0 μm and 8.0 μm filters were stained with 0.03% proflavine to highlight the nuclei and chloroplasts. No stain was used on the 0.2 μm fraction to facilitate the identification of the natural pigments phycoerythrin (PE/Low Phycourobilin = PE/Low Pub and PE/High Phycourobilin = PE/High Pub) and phycocyanin (PC). All size fractions were counted on an Olympus BH-2-RFCA epifluorescence microscope with blue and green excitation. The 0.2 and 3.0 μm fractions were counted within a week at 1000× magnification. The 8.0 μm filter was frozen to count later at 200× magnification with epifluorescence and also transmitted light to help with identification. Each filter was counted until either one hundred cells or 100 views were reached. Identification of all cells was taken to the lowest level possible. Cell counts were converted to number per liter.

The sediment samples were resuspended, and 0.5 ml of the sediment slurry was removed, rinsed with distilled and deionzed water, and centrifuged to remove picocyanobacteria and other small cells that were decanted onto 0.2 and 3 μm filters. A separate sediment slurry (2 - 4 ml) sample was needed

to extract the larger cells. Ludox® HS-40 was added to the pellet to separate the larger cells from the sediment by density centrifugation (Totti 2003, Blanchard et al.1998). A proflavin (0.03%) vital stain was added prior to filtration onto an 8 μm filter. The same counting and identification methodology employed for the water samples was used for the sediment samples. Cell counts were converted to cells per gram of dry sediment (cells g dry sed $^{-1}$).

Niches were assigned to each microalgal taxon as suggested by Round et al. (1990), Tomas (1997) and Komárek et al. (2003). The pelagic niche was assigned to organisms living in the water column, tychopelagic to cells that adapt to both the water and sediment and benthic to cells associated with the sediment (Cahoon et al. 1994). I characterized the community using niches instead of shape of the cells, such as centric versus pennate, because of the presence of filamentous cyanobacteria and to avoid confusion with the centric diatoms that live in the sediments and the pennate diatoms that live in the water.

Statistical Analyses

My null hypothesis for all tests was that there was no difference among stations, or years (α = 0.05). To test for significant differences among stations, an analysis of variance (ANOVA) was performed on the natural log-transformed pigment, % mud and density data to meet assumptions of normality, as well as % TOC when using the 'proc mixed' statement in SAS 9.1 (SAS Institute, Inc. 2003). If significance was detected, a post-hoc Tukey-Kramer test allowed for pairwise comparisons. A simple linear regression was also performed on the natural log-transformed phytopigment data with environmental conditions and cell densities. A community composition analysis was performed using the software Plymouth Routines in Multivariate Ecological Research (PRIMER) version 6.0 (Clarke & Warwick 2001). The benthic cell data were standardized to percents and log-transformed to develop the Bray-Curtis similarity index and analyzed with a non-parametric multi-dimensional scaling (MDS) plot to determine if sediment surface microphytobenthic composition was similar among stations and years. MDS plots help visualize composition similarity by utilizing distance relationships. The closer the

points are to each other, the more similar. These plots also provide stress values to suggest how well the MDS configuration fits the data. Because the MDS plots do not report quantified group differences, I used analysis of similarity (ANOSIM) with 999 permutations (random re-sampling of data) to help determine if the compositions among stations over the years were significantly similar or different. To determine correlations among environmental variables and samples, a correlation biplot based on principal component analysis (PCA) was developed using the correlation matrix on standardized data and an α decomposition of 0.5 for a symmetrical plot with the 'proc princomp' statement and biplot macro in SAS 9.1. All figures were created in SigmaPlot version 11.

RESULTS

Shelfwide Cruises and Environmental Conditions

Eleven stations were sampled during cruises in late-July 2006, 2007 and 2008 (Table 3.1, Fig. 3.1). The stations were ~ 40 km apart and spanned across the Louisiana continental shelf along a 14 - 20 m isobath. Ten stations were sampled in 2006 and 5 stations in each of 2007 and 2008. Four stations were sampled in all years: C6B, D3, E2A and G3. Of the ten stations sampled in 2006, three of them were during daylight and seven of them during nighttime. Fewer stations were sampled in the following years to coincide with daylight so that seafloor PAR data could be maximized. In 2007, all five stations were sampled during daylight hours, and in 2008, only three of five stations had seafloor PAR measurements.

A low pressure system in the southwestern Gulf of Mexico raised winds in the study area to ~ 15 m s^{-1} and seas to 3 m during the 2006 cruise (7/21-7/28) and while sediment sampling (7/22 – 7/27). After sampling stations E2A, F3 and H3, sustained high winds and seas mixed the water column at stations that were sampled consecutively: J4, K4, I4 and G3 and the pycnocline was deeper at these sites (Fig. 3.2). Re-oxygenation of the bottom waters also occurred farther south offshore and to the west of the sampling grid (N. Rabalais pers. comm.) and resulted in a thin layer of low oxygen water near the

bottom towards the end of the cruise on the western edge. The estimated total size of hypoxia was 17,280 km^2 (Fig. 3.1, http://www.gulfhypoxia.net/Research/Shelfwide%20Cruises/2006/).

Table 3.1. Station coordinates (decimal latitude and longitude), depth (m) and bottom-water dissolved oxygen (mg l^{-1}), salinity and temperature (°C) in late-July 2006, 2007 and 2008. nd = no data. Bold values indicate CTD data were used.

			Depth (m)			DO (mg l^{-1})			Salinity			Temp (°C)		
Sta	Lat	Lon	06	07	08	06	07	08	06	07	08	06	07	08
A4	29.13	-89.75	19.4	nd	nd	0.9	nd	nd	34.7	nd	nd	28.1	nd	nd
B6	28.99	-90.08	nd	21.6	nd	nd	1.1	nd	nd	35.4	nd	nd	27.6	nd
C6B	28.87	-90.47	18.4	20.2	19.6	0.9	0.4	0.7	34.9	35.7	30.8	27.7	27.7	27.0
D3	28.72	-90.83	17.0	18.0	17.8	1.5	4.2	0.6	34.8	34.9	35.4	27.5	28.5	25.9
E2A	28.74	-91.25	15.2	14.8	14.6	4.6	1.0	0.2	34.8	34.9	34.9	28.4	28.3	26.9
F3	28.88	-91.62	18.5	nd	nd	0.4	nd	nd	34.9	nd	nd	26.7	nd	nd
G3	28.98	-92.00	20.2	20.1	19.5	2.2	0.5	0.7	36.0	35.8	35.0	26.6	28.2	25.8
H3	29.16	-92.38	13.0	nd	nd	0.1	nd	nd	34.5	nd	nd	26.7	nd	nd
I4	29.18	-92.75	**18.9**	nd	nd	**0.6**	nd	nd	**33.3**	nd	nd	**27.9**	nd	nd
J4	29.29	-93.08	**15.0**	nd	17.4	**1.9**	nd	0.2	**33.7**	nd	32.5	**28.7**	nd	27.1
K4	29.33	-93.42	**14.5**	nd	nd	**5.6**	nd	nd	**30.3**	nd	nd	**29.4**	nd	nd

The offshore conditions were stormier than average from mid-June to mid-July prior to the 2007 mapping cruise (7/21 – 7/28), which disrupted the maintenance of hypoxia as evident from the bottom DO concentrations frequently above 2 mg l^{-1} at the continuous monitoring station, C6C (Fig. 3.3). The mixing of the bottom-water pre-cruise could have resulted in a smaller hypoxic area (20,500 km^2) (Fig. 3.1) than predicted (http://www.gulfhypoxia.net/Research/Shelfwide%20Cruises/2007/). The seas were calmer than earlier in the week while sediment sampling (7/22 – 7/25), but on 7/26 wave conditions prevented sediment sampling at station I4.

The sediment sampling (7/25 – 7/29) in 2008, occurred during calm conditions after the passage of Hurricane Dolly across the Gulf of Mexico from Yucatan to Brownsville, Texas on July 21-23. The storm probably reaerated the western area of the station grid (N.N. Rabalais, pers. comm.). The total area of hypoxia was 20,720 km^2 (Fig. 3.1, http://www.gulfhypoxia.net/Research/Shelfwide%20Cruises/2008/).

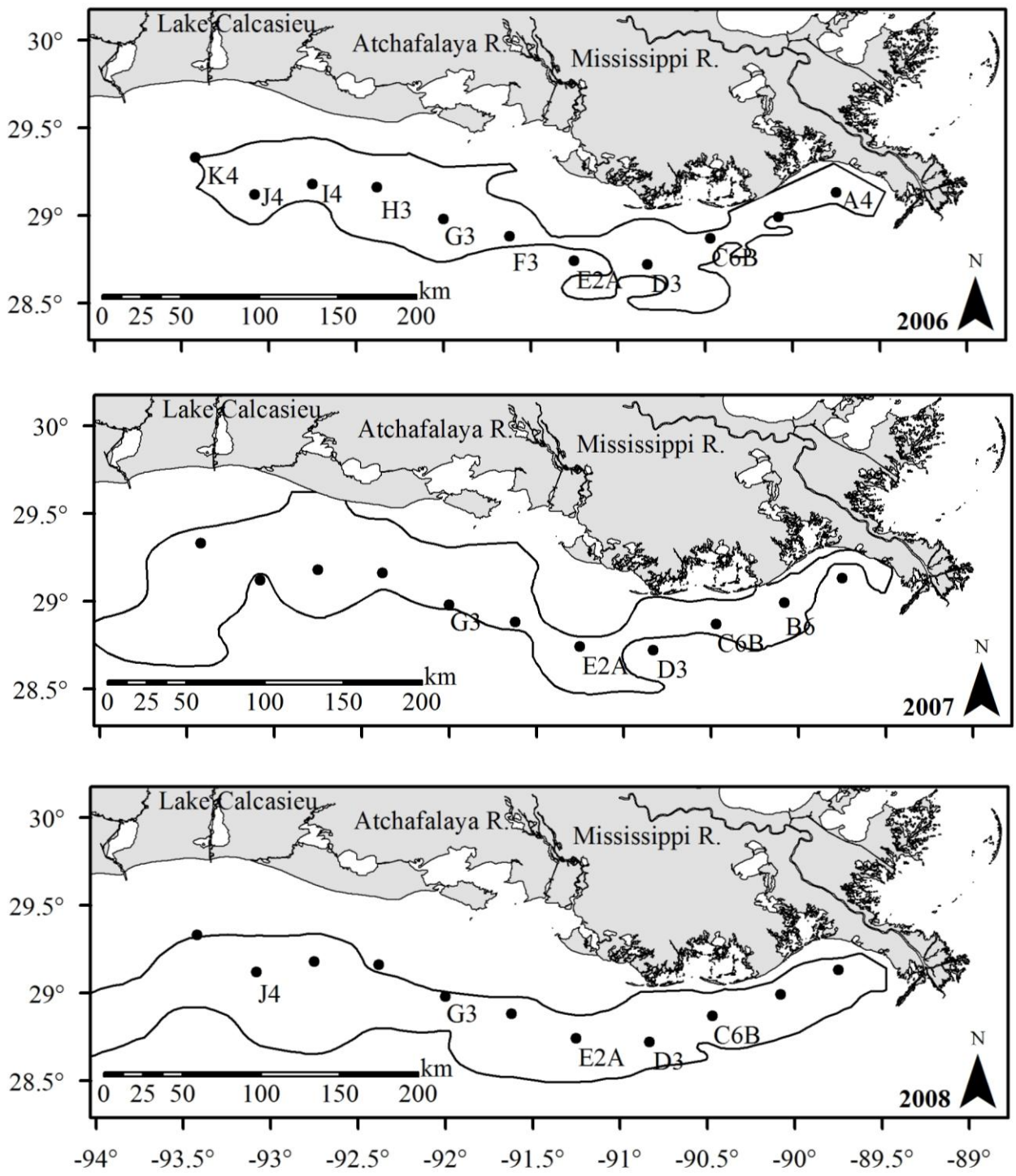

Fig. 3.1. Map of stations and outlines of the hypoxic area ($\leq$ 2 mg O_2 l^{-1}) along a 14 - 20 m contour along the Louisiana continental shelf in late-July 2006, 2007 and 2008. Only stations labeled were sampled in the respective year.

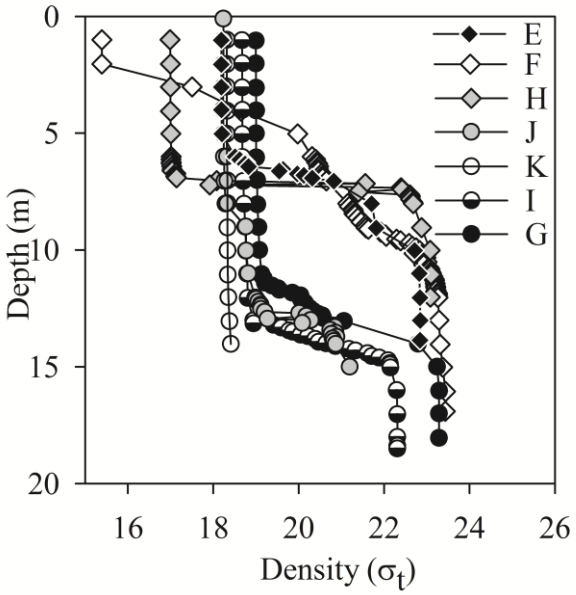

Fig. 3.2. Water-column density (σ_t) profiles from stations (E, F, H) prior to a mixing event, represented as diamonds and after (J, K, I, G, represented as circles) during late-July 2006. Stations listed in order of sampling date and are indicated by the transect (see Table 3.1).

The mean depths of the stations were: 2006 = 16.8 m, *n* = 10; 2007 = 18.8 m, *n* = 5; 2008 = 17.8 m, *n* = 5 (Table 3.1), and for all years it was 17.5 ± 0.5 std. err. The DO ranged from: 0.06 – 5.6 mg O_2 l^{-1} for the three summer collections. Only stations C6B and J4 were hypoxic for all collections of the stations sampled more than once. Seven of 10, 4 of 5 and 5 of 5 stations sampled in 2006, 2007 and 2008, respectively, had hypoxic bottom-water. The mean salinity of the bottom water at all stations was 34.6 ± 0.3 std. err., *n* = 20. The lower salinity values (e.g., ~ 33) were observed at the more western stations (e.g., I4, J4, K4), and verifies the deepening of the pycnocline and oxycline. The bottom-water temperatures were also similar among the stations with an overall mean of 27.5 °C, ± 0.2 std. err., *n* = 20.

The % surface PAR measurements taken at the seafloor were below 1% of the limited PAR data available (Fig. 3.4). The highest percent PAR (0.9%) was at station D3 in 2007, and the lowest (0%) was at stations I4 and K4 in 2006. The range in values was 0 – 22 μmol photons m^{-2} s^{-1}, and the mean was 4 μmol photons m^{-2} s^{-1}, *n* = 12. The highest seafloor PAR levels were at the mid-shelf stations D3 (2007) and E2A (2006). The PAR values were variable with no consistent pattern among stations and

years of four stations sampled consecutively. The mean bottom-water attenuation coefficient was: 0.35 m^{-1} (± 0.02 std. err., n = 10). The lowest bottom-water attenuation coefficient was 0.26 m^{-1} at station D3 in 2007.

Sediments from most stations (9 of 11) were primarily mud, > 75% (Fig. 3.5). Two stations, D3 and E2A, were the sandiest and lower in total organic carbon (TOC) (< 1.5%). The percent TOC at station G3 was also low (< 1.5%) despite a higher mud content (> 75%). Stations E2A and D3 of the four stations sampled consecutively were more variable in % mud and the % mud was lower for both stations in 2006 compared to 2007 and 2008. Overall, the mean station % TOC of the sediment was positively related to the % mud (r^2 = 0.73, p = 0.0001, n = 20). The mean % mud per station differed for all years ($F_{10, 49}$ = 11.70, p = 0.001), and stations D3 and E2A were similar to each other but significantly lower than the rest of the stations. There was also a significant difference among the mean % TOC values by station ($F_{10, 49}$ = 26.60, p = 0.001). Stations D3 and E2A were once again similar to each other, but were significantly different from the majority of the stations, except for station G3.

Water Column - Phytopigments

The surface chlorophyll *a* concentrations ranged between 0.31 – 12.32 μg l^{-1} with a mean of 4.41 μg l^{-1} (n = 20), and tended to be similar among the stations in all years, except for stations E2A and G3 in 2008 (Fig. 3.6). The range in bottom-water chlorophyll *a* concentrations was between 0.63 - 17.94 μg l^{-1} with a mean of 4.86 μg l^{-1} (n = 20). The bottom-water chlorophyll *a* concentrations were greater than coincident surface water chlorophyll *a* concentrations due to the 2006 more western-shelf stations (H3, I4, and K4) that were almost three times higher than the mean surface-water chlorophyll *a* concentrations. The pheophytin *a* concentrations, a biomarker for chlorophyll degradation, were higher in the bottom water than the surface water and were correlated with chlorophyll *a* concentrations in both the surface and bottom water (r^2 = 0.71, p = 0.0001, n = 20; r^2 = 0.67 p = 0.0001, n = 20, respectively) (data not shown).

The accessory pigments were diverse in the surface water, and the total concentration was mostly < 10 μg l^{-1}. Common pigments were fucoxanthin, zeaxanthin, diadinoxanthin and perididin (Fig. 3.7). Other pigments, such as alloxanthin and 19'-hexanoyloxyfucoxanthin, were also present, but at lower concentrations. The same pigments that made up the total pigment pool in the surface waters were common in all years. There were higher total pigment peaks in 2008 than 2006 and 2007 at stations C6B, E2A and G3. The peaks of carotenoids, > 10 μg l^{-1} in the bottom-water samples, were more frequent than in the surface water samples. Fucoxanthin dominated the bottom-water samples, but peridinin, zeaxanthin and diadinoxanthin were also common. There were peaks of pigments in the bottom water in 2006 at the more western stations H3, I4, and K4, consistent with the higher chlorophyll *a* concentrations. The bottom-water pigment concentrations were low at most stations in 2007 and 2008 other than one peak of total pigments in 2007 at station E2A.

Sediment Surface - Phytopigments

The mean sediment chlorophyll *a* concentrations were highly variable among stations/years and the concentrations were mostly < 1.0 μg g dry sed^{-1} (Fig. 3.6, bottom panel). In 2006, the sediment chlorophyll *a* ranged from 0.22 – 2.42 μg g dry sed^{-1} and averaged 0.88 ± 0.17 std. err. μg g dry sed^{-1} (*n* = 49). Samples for 2007 averaged 0.47 ± 0.09 std. err. μg g dry sed^{-1}(*n* = 25) and for 2008 averaged 0.59 ± 0.09 std. err. (*n* = 24). The highest mean sediment chlorophyll *a* concentration was at station D3 in 2006 (2.4 μg g dry sed^{-1}), which was significantly higher than station B6 in 2007 (0.08 μg g dry sed^{-1}) ($F_{19, 78} = 2.87$, $p = 0.0006$). Due to the highly variable concentrations among the sites and among the years, the mean sediment chlorophyll *a* per year did not significantly differ ($F_{2,95} = 1.99$, $p = 0.1417$). At the four stations (e.g., C6B, D3, E2A and G3) sampled each year, the mean chlorophyll *a* concentrations were not consistently different among stations or years.

Common sediment surface carotenoids, typical of major taxa, were mainly fucoxanthin and zeaxanthin, but diadinoxanthin, 19' hexanoyloxyfucoxanthin, and alloxanthin were present (Fig. 3.7). No peridinin was present on the sediment surface at any station. Fucoxanthin is a major light-harvesting

pigment common in diatoms (Halldal 1970), but it is also used by prymnesiophytes, raphidophytes and some dinoflagellates (Jeffrey et al.1997). Zeaxanthin is commonly utilized by cyanobacteria, prochlorophytes, green algae, chrysophytes and raphidophytes (Jeffrey et al. 2007). Diadinoxanthin is a major pigment found in diatoms and used for photoprotection (Halldal 1970), but prymnesiophytes, some chrysophytes and dinoflagellates may also contain it. The pigment, 19' hexanoyloxyfucoxanthin is a light-harvesting pigment common to prymnesiophytes and some dinoflagellates, and alloxanthin is a major light-harvesting pigment of cryptomonads.

The carotenoid concentrations on the sediment surface were commonly < 5 μg g dry sed^{-1} (Fig. 3.7). There were no patterns of sediment phytopigments by year or station but there were significant differences among stations and years ($F_{19,78}$ = 6.91 p = 0.0001). In 2006, higher concentrations of fucoxanthin were present at station K4 than the stations H3, E2A, C6B and A4. In 2007, stations C6B, D3 and E2A had similar high fucoxanthin concentrations (Fig. 3.7). In 2008, most of the sediments had a low total pigment pool (< 2 μg g dry sed^{-1}), except for station C6B which had significantly higher concentrations > 5 μg g dry sed^{-1}. Concentrations of zeaxanthin were higher in 2006 and 2008 than in 2007 ($F_{19,78}$ = 14.36, p = 0.0001). Sediment samples from the 2006 tended to have a higher number of pigments than the other two years (Fig. 3.7). The four stations that were sampled each year tended to have fucoxanthin as the highest concentration, but the pigment concentrations varied among years with no consistent trends.

The sediment total pheopigments (degradation products of chlorophyll *a*) were pheophytin *a* and the more degraded form, pyropheophytin *a* (common in sediment, Otsuki et al. 1993), were commonly ≤ 2 μg g dry sed^{-1} (Fig. 3.8). Sediment pheophytin *a* contributed the most (> 52%) to the total pheopigment pool (data not shown). The highest total sediment pheopigment concentration was at station C6B in 2008, which was almost three times higher than the remainder of the samples ($F_{19,78}$ = 4.51, p = 0.0001). Station E2A in 2007 was significantly higher ($F_{18, 74}$= 2.98, p = 0.0005) than 2006

stations A4, G3, I4, K4 and station C6B 2007 when station C6B 2008 was excluded from statistical analysis. Overall, most of the stations had similar total pheopigment concentrations.

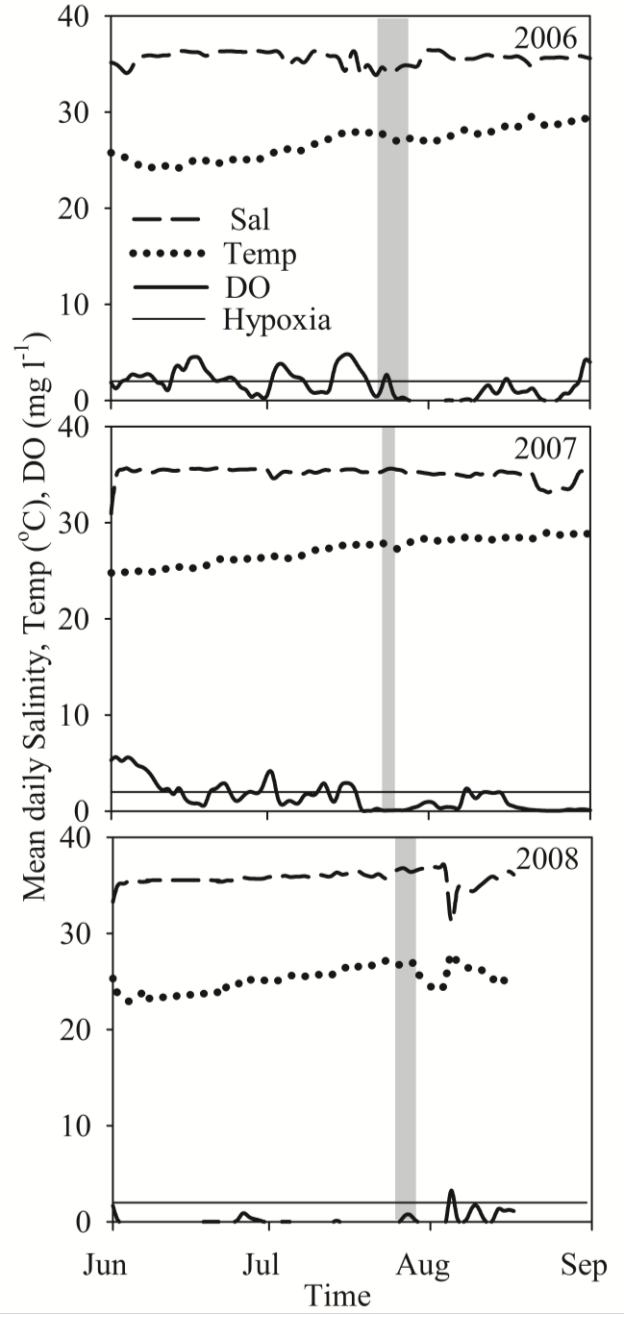

Fig. 3.3. Daily mean bottom-water salinity, temperature (°C) and dissolved oxygen (DO, mg l^{-1}) at station C6C (located ~ 2.2 km west of station C6B) from June through August 2006, 2007 and 2008. Thin horizontal line at 2 mg l^{-1} is a reference line for hypoxic conditions. In 2008, no data for the rest of August due to Hurricane Gustav. Shaded areas represent dates when sediment sampling occurred.

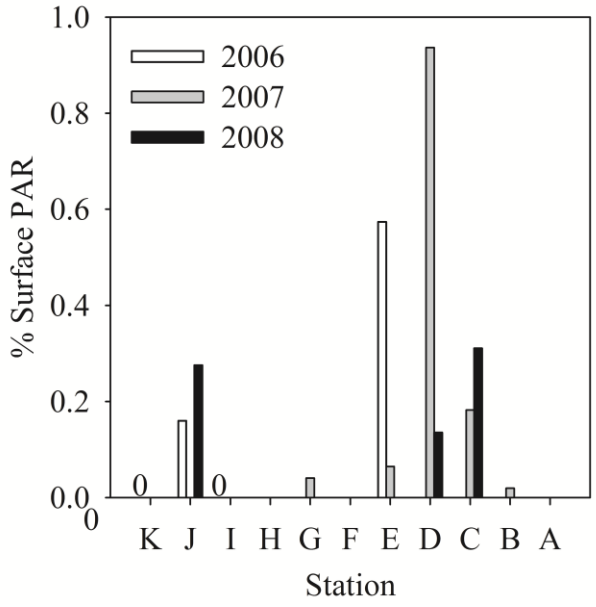

Fig. 3.4. Percent of surface photosynthetically available radiation (PAR) reaching the seafloor at stations (12 of 20 stations sampled) along the Louisiana continental shelf during late-July in 2006, 2007 and 2008. The % surface PAR at stations K4 and I4 in 2006 were zero. Stations are indicated by the transect letter (K to A, west to east, along a 14 - 20 m isobath, Table 3.1). No data for 2006 stations A, C, D, E, G and H and for 2008 stations, E and G.

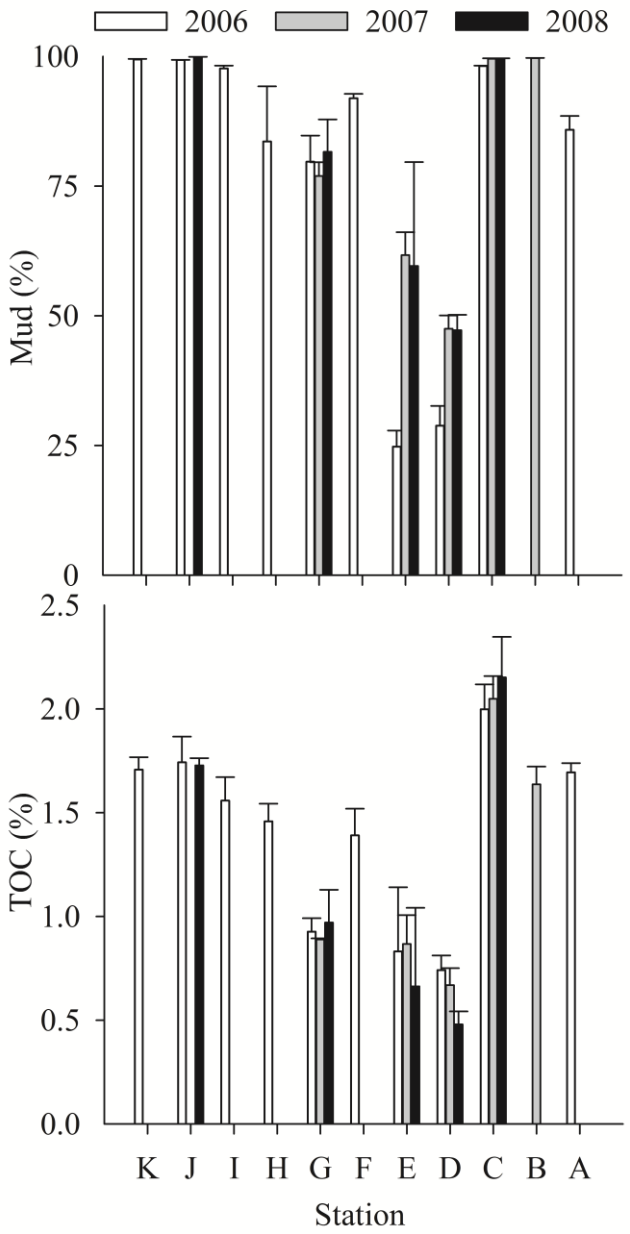

Fig. 3.5. Mean percent of mud (+ std. err.) and total organic carbon (TOC) (+ std. err.) (*n* = 3) of sediment collected at stations along the Louisiana continental shelf during late-July 2006, 2007 and 2008. Stations are indicated by the transect letter (K to A, west to east, along a 14 - 20 m isobath (Table 3.1).

Water Column – Microscopic Analysis

Picocyanobacteria were the most abundant water column cells (0.2, 3 and 8 µm combined) at 96% of the cell totals and ranged between 53-99% of total cells among surface and bottom-water samples (data not shown). Most of the > 3 µm cells in the surface and bottom water were pelagic phytoplankton cells (Fig. 3.9). Diatoms were dense in the phytoplankton of the surface and bottom water in 2007 and 2008,

but phytoflagellates, cryptomonads and dinoflagellates were also common. Dinoflagellates were more abundant and frequent in the surface waters than in the bottom waters (Fig. 3.10). In 2006 at station G3, the surface water had a small proportion of benthic cells which were mostly *Navicula* (length less than 50 μm) with fewer *Pleurosigma*. The bottom water had a higher frequency of benthic cells present than the surface water. The highest densities of benthic cells in the bottom water in 2006 were at stations H3 and K4.

Sediment Surface – Microscopic Analysis

Picocyanobacteria were common and highly dense in the sediment samples (0.2, 3 and 8 μm combined). Both PE and PC picocyanobacteria were found in all size fractions. The mean total density of picocyanobacteria was three orders of magnitude higher than the mean > 3.0 μm total density. I deleted the 0.2 μm size fraction data from further analyses to focus on the larger cells (> 3.0 μm), which are usually reported in microphytobenthic studies.

Most of the cells > 3 μm on the sediment surface at all stations and in all years were benthic (> 50%, 15 of 20 samples), with proportions ranging from 1 – 99 % and a mean of 67% (n = 20) (Fig. 3.9). The range in densities of benthic cells for all samples was: 7-104,000 cells g dry sed^{-1}. The mean densities (cells g dry sed^{-1}) for each year were: 19,000 in 2006, 6000 in 2007, and 43,000 in 2008. The two density peaks were at station D3 in 2006 and E2A in 2008. Stations C6B, D3, E2A and G3 (sampled each year) had no consistent pattern in density among years. In 2006 and 2007, station D3 had high densities but they were low in 2008. Stations in 2007 tended to have a higher proportion of non-benthic cells on the sediment surface. The most dense pelagic diatoms (> 10 % of the total density) on the sediment surface were centric diatoms (diameter commonly 20 - 30 μm). The centric diatom *Coscinodiscus* and the chain-forming diatom *Skeletonema* occurred most frequently, 80% of the sediment samples.

Microphytobenthos were made up of two major taxa, diatoms and cyanobacteria-other (Fig. 3.10). The highest density of benthic diatoms (> 10 % of the total density) belonged to the genera:

Nitzschia, *Gyrosigma*, *Pleurosigma* and *Bacillaria* (Fig. 3.9), and *Pleurosigma* was present most often, 18 of 20 samples. The two common cyanobacteria-other group consisted of filamentous cyanobacteria and the colonial, *Merismopedia*. I found no benthic dinoflagellates, phytoflagellates or cryptomonads on the sediment surface. In 2006 and 2007, diatoms were more dense than cyanobacteria-other but not in 2008. Among all the years, stations D3 and E2A had the highest benthic cell densities.

The benthic cell (> 3.0 μm) community composition was not significantly different among the years (global $R = 0.157$, $p = 0.07$) (Fig. 3.10). The samples from 2006 and 2007 were more variable than 2008 as indicated by the greater distances among the stations (Fig. 3.11). The common genera of the microphytobenthos did vary among the years at stations C6B, D3, E2A and G3 that were repeatedly sampled. Station C6B in all three years had low benthic diatom densities (*Pleurosigma* and *Navicula*) but in 2008 more cyanobacteria-other were present. At station D3, a higher density of benthic diatoms were present in 2006 (*Nitzschia, Gyrosigma* and *Bacillaria*) than 2007 (*Navicula* and *Pleurosigma*) and 2008 (*Pleurosigma* and *Amphora*). The cyanobacterial-other densities at station D3 were increasingly higher in 2007 and 2008. Station E2A had low benthic cell densities in 2006 (*Amphora* and *Pleurosigma*) and in 2007 (*Navicula*, *Fallacia*), but in 2008 cyanobacteria-other dominated. Station G3, similar to station C6B, had cyanobacteria-other densities increasingly abundant in 2006 - 2008 with the opposite pattern for diatoms; for all years *Pleurosigma* was common. See Fig. 3.12 for images of microphytobenthos.

I constructed a PCA biplot to help determine if any environmental conditions (abiotic variables) were affecting the estimated biomass and density of microphytobenthos (biotic variables) (Fig. 3.13). For this analysis a complete data set is needed, therefore only 12 of 20 station samples were used that had seafloor PAR. When the vectors (variables) were opposite lying they were negatively correlated, small angles between vectors were highly correlated, and perpendicular vectors were uncorrelated. The longer lengths of vectors indicate higher variability. The station samples were spread among the variables to which they were related, and the station samples that contained higher values were found

closer to that vector label. According to the PCA biplot (Fig. 3.12), the biotic variable sediment chlorophyll *a* had the highest correlation (e.g., small angles between vectors) with the seafloor PAR and bottom-water DO. The other biotic variables, percent benthic cells and benthic cell density were more correlated with bottom-water nutrients, specifically BWNH4 and BWPO4. The sediment characteristic (% TOC) was not highly correlated with any of the biotic variables. Some of the station samples, D3, E2A, J4 and K4, seemed to be located closer to the labels of the biotic vectors, which indicated they had higher values of those biotic variables. When a PCA biplot was created with all the samples (n = 20) and no PAR data were included (plot not shown), I found the densities of benthic diatoms to be more correlated with seafloor chlorophyll *a* and bottom-water dissolved oxygen concentrations. The cyanobacteria-other were more correlated with the bottom-water nutrient concentrations and more negatively correlated with bottom-water dissolved oxygen concentrations.

DISCUSSION

Microphytobenthos, mainly pennate diatoms and filamentous cyanobacteria, were present at all stations along the Louisiana continental shelf in depths of 14 to 20 m during late-July 2006, 2007 and 2008. The benthic community was different than the phytoplankton-dominated surface and bottom water composition. High densities of sediment picocyanobacteria were found on the larger size fraction filters, which I and others (Chapter 2, Dortch 1998) attribute to cell aggregates. The benthic community tended to shift from pennate diatoms and filamentous cyanobacteria in 2006 and 2007 to mostly filamentous cyanobacteria in 2008. During the last summer, bottom-water oxygen concentrations were consistently low and cyanobacteria densities drove the correlation with bottom-water nutrient concentrations, which increase under hypoxic conditions (Rabalais & Turner 2006, Chapter 4). Low PAR and near aneraerobic conditions (probably due to the low mixing events or calm conditions) in 2008 probably favored cyanobacteria presence (Brock 1973, Stal 1995, Ladakis et al. 2006). During these 2008 conditions, filaments of *Beggiatoa*, the sulfide-oxidizing bacteria were also commonly found among the cyanobacteria filaments and further indicated reduced conditions at the sediment surface.

The sediment chlorophyll *a* concentrations were highly variable along the continental shelf and among years probably due to the natural patchiness of benthic sedimentary structure and distribution of forms of organic matter and changing environmental conditions in the water column and sediment. For example, parameters such as seafloor PAR, dissolved oxygen concentrations, nutrient concentrations, resuspension events and grazing by benthic fauna could impact sediment chlorophyll *a* concentrations (Sun et al. 1991, 1993, Underwood & Kromkamp 1999, Wolfstein et al. 2000, Totti 2003).

The sediment-surface chlorophyll *a* concentrations were positively correlated with seafloor PAR according to the PCA biplot (Fig. 3.13). This suggests that the sediment chlorophyll *a* pool was probably more influenced by viable microphytobenthos or bottom-water phytoplankton than the sinking of fecal pellets or senescing phytoplankton. In addition, the other biotic variables (benthic cell density and % benthic cells) separated from sediment chlorophyll *a* and seafloor PAR and were more correlated with BWNH4 and BWPO4 concentrations (Fig. 3.13). The density of microphytobenthos may be more influenced by the nutrients available in order to build biomass and for cell division (Flöder at al. 2006), and these nutrient concentrations commonly increase under hypoxic conditions (Rabalais & Turner 2006, Chapter 4). The reason why BWNO3+NO2 concentrations were not highly correlated with the population metrics was that BWNO3+NO2 concentrations were low under hypoxic conditions, probably due to denitrification (Chapter 4), and were not available to microphytobenthos.

During the seasonal study at stations along the C transect, the biotic variables (chlorophyll *a*, density and % benthic cells) were all highly correlated with the seafloor PAR and bottom-water temperature (Chapter 2). Some of the values for environmental conditions increased during the summer which is typical of seasonal data. Many of the environmental parameters were not variable (e.g., temperature, salinity) because sampling was targeted for the expected period of maximal hypoxia extent, and therefore they were not highly correlated with the biotic variables. The exception was variability of environmental parameters during mixing events. During the seasonal study (Chapter 2), the microphytobenthos were inversely correlated with DO, meaning greater densities during the summer

when low oxygen occurs. Most of the stations were hypoxic (9 of 12) during the summer in this shelfwide study, and the stations with higher bottom-water DO were positively correlated with sediment chlorophyll *a*. These results may infer that photosynthetic oxygen production occured at stations E2A in 2006 and D3 in 2007 because bottom-water DO was highly correlated with seafloor PAR and sediment surface chlorophyll *a*. The higher bottom-water DO concentrations at station K4 2006 likely resulted from reaeration (mixing events, Fig. 3.2) and not *in situ* bottom-water/sediment photosynthesis.

The shelfwide PAR measurements were within the range of other northern Gulf of Mexico studies (Grippo et al. 2009, 2010, Chapter 2) which measured seafloor PAR between 0-83 μmol photons m^{-2} s^{-1}, and included some sites with less than 1% of surface irradiance (Chapter 2). Grippo et al. (2010) recorded mean % surface light values that were higher than my values in August 2007, with a range between 0.3 – 3.4% and the sandier, shallow shoals, ranging between 0.7 – 7.4% among the shallower and deeper muddier stations surrounding sandy shoals near the transects D and E. Lehrter et al. (2009) estimated up to ~ 70% of their study area (common depths of 10 to 50 m, which were within my transects of A - K) had > 1% of light on the bottom during 2005-2007 in the months of March – September. The % surface PAR values tended to be lower than these cited studies, which could be due to environmental conditions but also due to calculation differences. I used the surface/reference PAR measured on the ship and Lehrter et al. (2009) used the value just below the surface water, which tends to be less than the surface/reference PAR, and thus would increase the bottom % light value. Grippo et al. (2010) found a large range in the mean attenuation coefficients, 0.24 – 4.37 m^{-1} in August 2007 among all sites, as did Lehrter et al. (2009), 0.19 – 1.01 m^{-1}, in their study. The same station (C6B) was investigated in Chapter 2, with estimated attenuation coefficients between 0.25-0.63 m^{-1}. The attenuation coefficients for this study (0.26 – 0.47 m^{-1}) were similar to what was discovered in Chapter 2 but did not reach the higher values of the Grippo et al. (2009, 2010) and Lehrter et al. (2009) studies probably due to differences in environmental conditions (e.g., depth, turbidity, surface water chlorophyll *a*) of the stations and methodology/calculations, as seen with the % surface PAR.

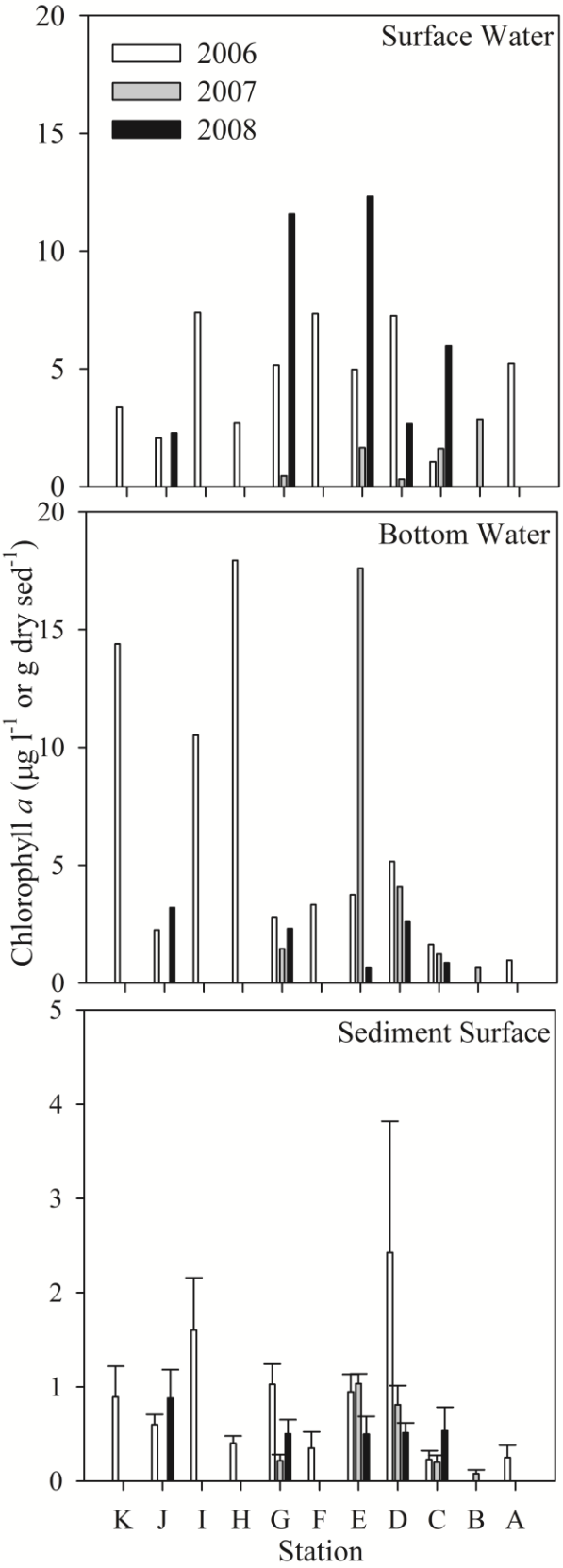

Fig. 3.6. Surface water (µg l^{-1}, top panel, *n* = 1), bottom water (µg l^{-1}, middle panel, *n* = 1) and mean (+ std. err.) sediment surface (µg g dry sed^{-1}, bottom panel) (*n* = 5) chlorophyll *a* at stations along the

Louisiana continental shelf during late-July 2006, 2007 and 2008. Note different ranges in y-axes. Stations are indicated by the transect letter (K to A, west to east, along a 14 - 20 m isobath, Table 3.1).

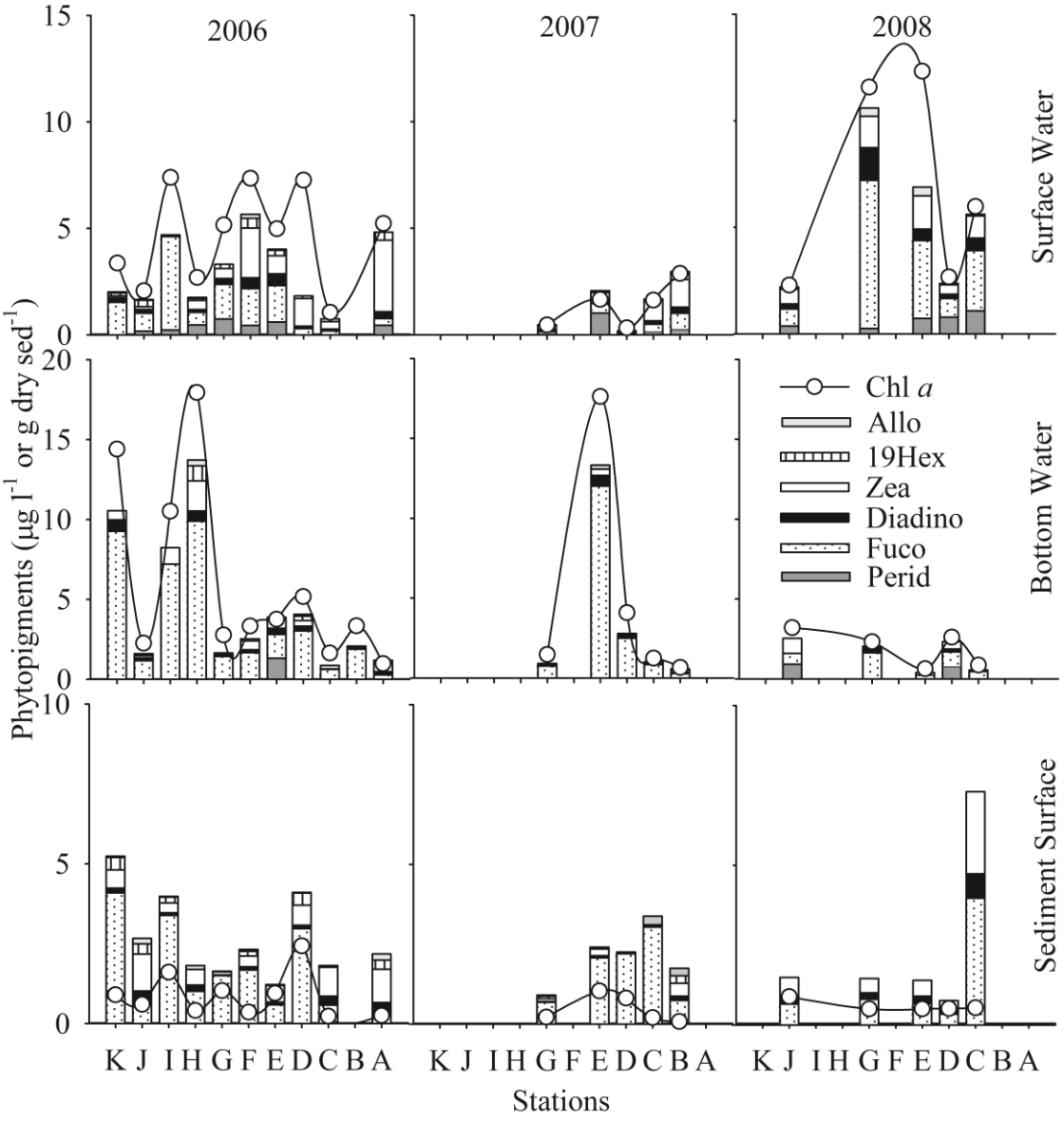

Fig. 3.7. Phytopigment concentrations in the surface water, bottom water and sediment surface from stations sampled in 2006, 2007 and 2008 from the mouth of Mississippi River along the Texas-Louisiana continental shelf to the Texas-Louisiana state border. The carotenoids are represented in the stacked bars (Allo = alloxanthin, 19 Hex = 19'-hexanoyloxyfucoxanthin, Zea = zeaxanthin, Diadino = diadinoxanthin, Fuco = fucoxanthin, Perid = peridinin), and chlorophyll *a* (Chl *a*) is the line. Note the different y- axes. Identifiable but low concentrations of other pigments (β-carotene, diatoxanthin, and violaxanthin) were not plotted. Stations are indicated by the transect letter (K to A, west to east, along a 14 - 20 m isobath, Table 3.1).

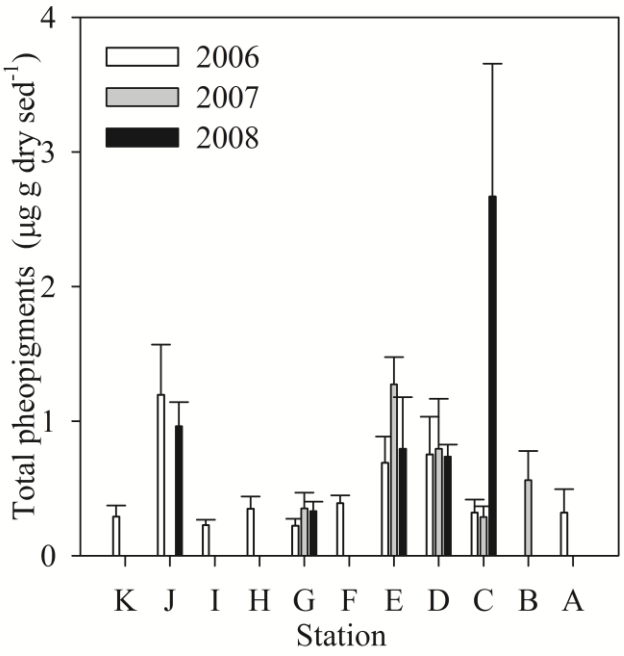

Fig. 3.8. Sediment mean (+ std. err.) total pheopigments (pheophytin *a* + pyropheophytin *a*, µg g dry sed^{-1}) at stations sampled along a 14-20 m isobath from the Mississippi River to the Louisiana/Texas border in late-July 2006, 2007 and 2008. Stations are indicated by the transect letter (K to A, west to east, Table 3.1).

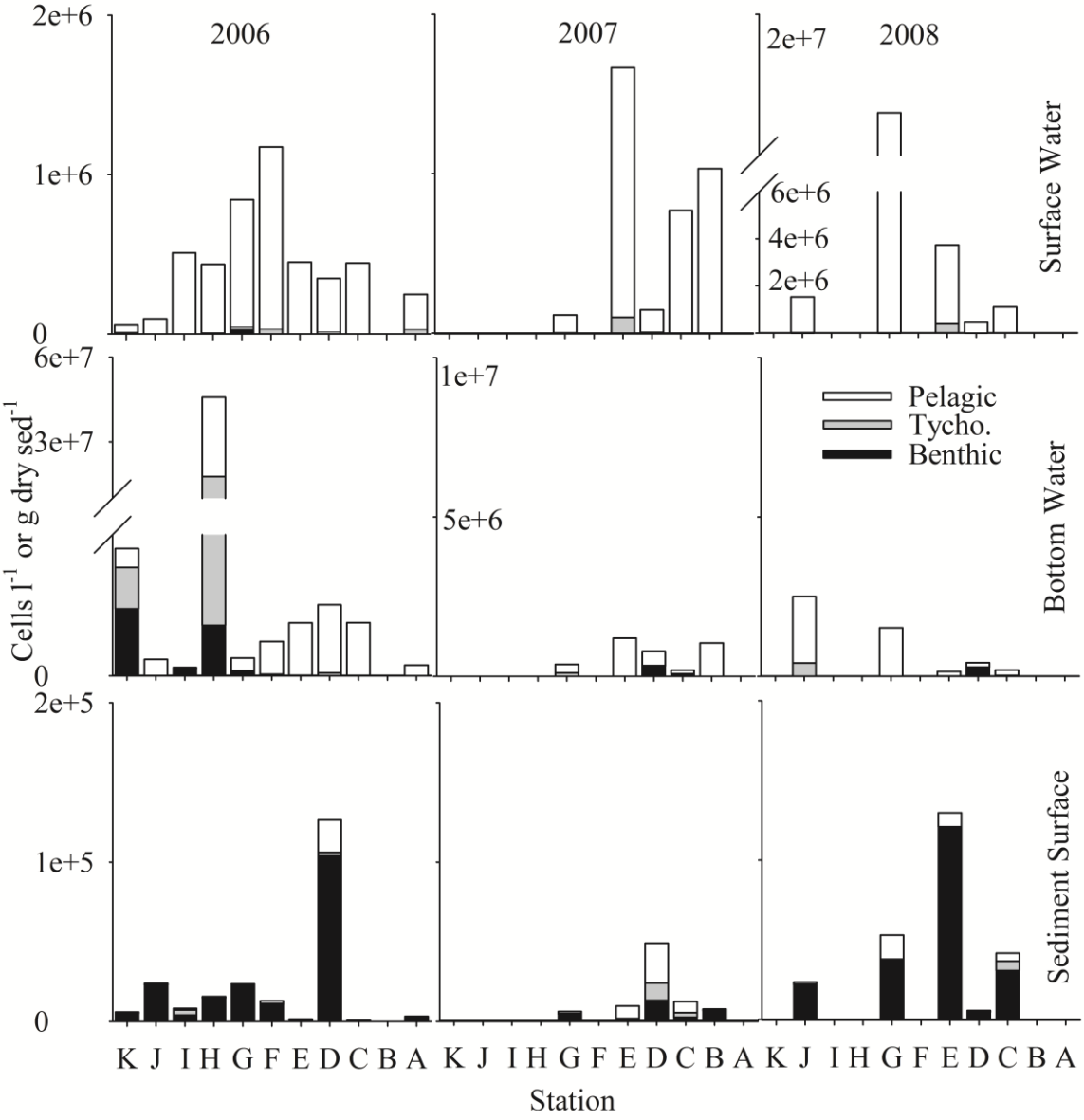

Fig. 3.9. Pelagic, tychopelagic and benthic cells (> 3 μm) in the surface water, bottom water and sediment surface at stations sampled along the Louisiana continental shelf during late-July 2006, 2007 and 2008. Station C6B in 2006 had a low density of ~ 800 cells g dry sed^{-1} with the benthic cells contributing only 7 cells. Note different ranges in y-axes. Stations are indicated by the transect letter (K to A, west to east, along a 14 to 20 m isobath, Table 3.1).

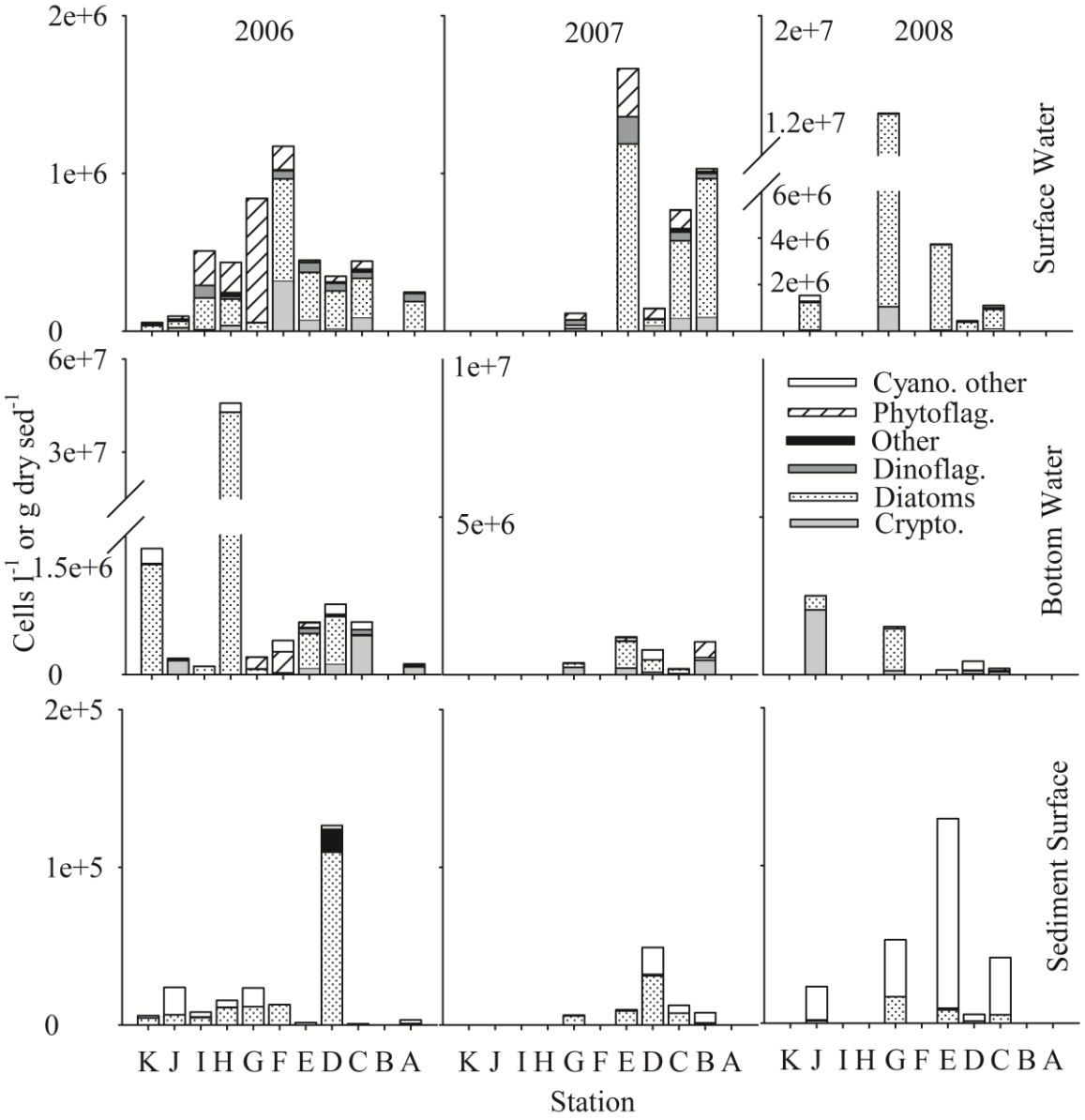

Fig. 3.10. Density of phytoplankton and microphytobenthos by taxonomic group: cyanobacteria-other (no picocyanobacteria), phytoflagellates, other (chlorophytes, ciliates, euglenoids, silicoflagellates, ebriids and raphidiophytes), dinoflagellates, diatoms, and cryptomonads for stations sampled from surface water, bottom water and sediment surface at stations along the Louisiana continental shelf in late-July 2006, 2007 and 2008. Note the different y-axes. Stations are indicated by the transect letter (K to A, west to east, along a 14 - 20 m isobath, Table 3.1).

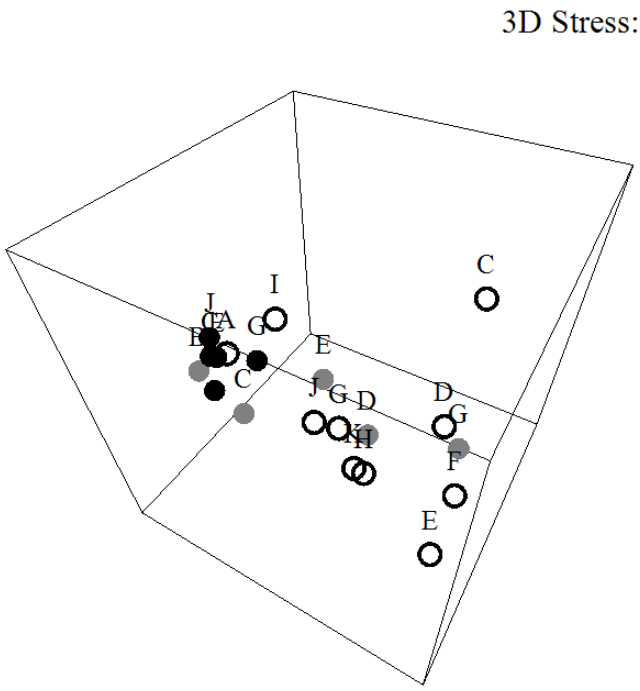

Fig. 3.11. Multi-dimensional (3D) scaling ordination of benthic cells (microphytobenthos, > 3 μm) from stations sampled along a 14 – 20 m isobath from the Mississippi River to the Louisiana/Texas border in late-July 2006, 2007 and 2008. Samples are represented as open circles in 2006 (n = 10), gray circles in 2007 (n = 5) and black circles in 2008 (n = 5). Stations are indicated by the transect letter (see Table 3.1).

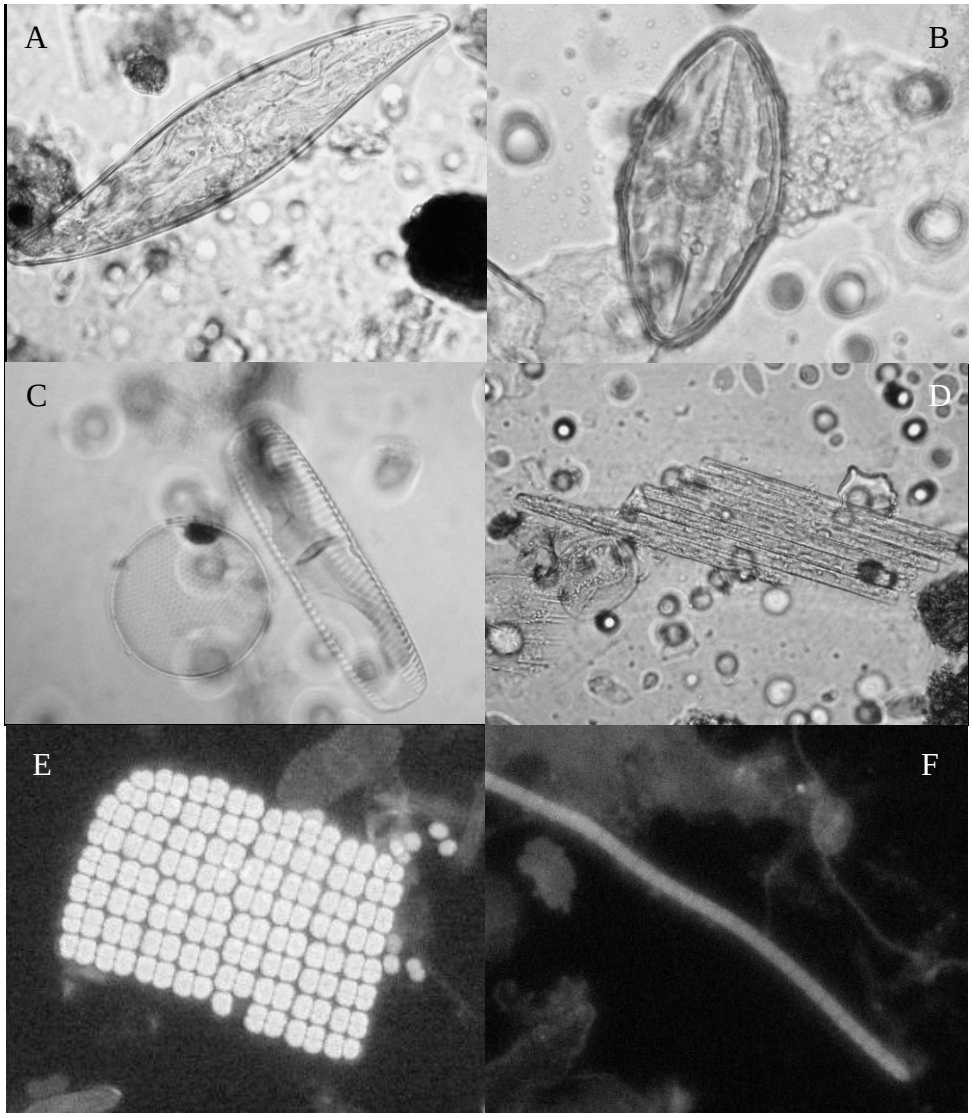

Fig. 3.12. Microphytobenthos along the Louisiana continental shelf in depths of 14 to 20 m in late-July 2006, 2007 and 2008. A = *Pleurosigma*, B = *Mastogolia*, C = *Coscinodiscus* (centric diatom, no chloroplasts) next to *Navicula* (with chloroplasts), D = *Bacillaria*, E = *Merismopedia* and F = filamentous cyanobacteria-other.

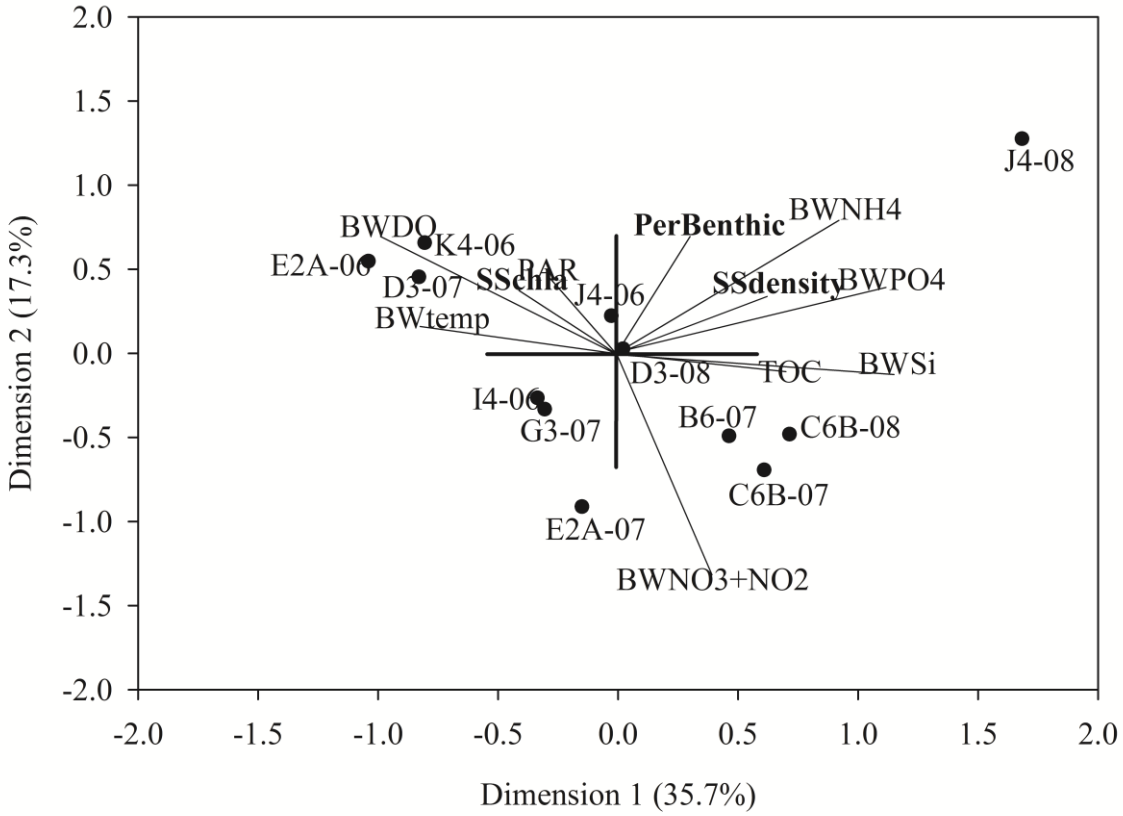

Fig. 3.13. Results of the principal component analysis (PCA) biplot of sediment and bottom-water abiotic variables and sediment biotic (bold) variables as vectors (n = 11) and station samples as points (n = 12). The abiotic variables included depth, seafloor photosynthetically available radiation (PAR), bottom-water temperature, bottom-water dissolved inorganic nutrients, sediment percent total organic carbon, and bottom-water dissolved oxygen. The sediment biotic variables included: percent benthic cells, sediment-surface chlorophyll *a* and sediment-surface benthic cell density. Perpendicular vectors are uncorrelated, small angles between vectors are highly correlated and opposite lying vectors are negatively correlated. Longer lengths of vectors indicate higher variability. Sediment samples were removed (n = 8) from analysis if the data set was incomplete due to no PAR data, see Fig. 3.3.

Samples from stations D3 and E2A tended to be closer to the biotic vector labels and correlated with seafloor PAR and bottom-water nutrients (PCA plot), had the highest PAR levels, benthic cell densities and sediment chlorophyll *a* concentrations. With this evidence, I reject my null hypothesis that microphytobenthos are similar along the shelf. Perhaps stations D3 and E2A are outside of the Mississippi and Atchafalaya riverine influenced surface chlorophyll *a* maxima, which tends to be in higher salinity surface waters (Lohrenz et al. 1999, Rabalais et al. 2002, Dagg & Breed 2003) which would allow more PAR to the seafloor.

Sandier sediments may be correlated with the higher biomass and density of microphytobenthos at stations D3 and E2A. Yet, I found the sediment chlorophyll *a* were not correlated with sediment characteristics, and past studies suggest there is little agreement about sediment grain size affecting microphytobenthos and their biomass (as estimated by sediment chlorophyll *a)* (for review see Cahoon et al. 1999, Underwood and Kromkamp 1999). The seasonal study of microphytobenthos within an area of frequent hypoxia (Chapter 2) also suggested that sediment grain size was not a major factor influencing the microphytobenthos (estimated biomass and density) when comparing a sandy-sediment station to muddier stations. Lastly, the stations D3 and E2A may be in an area that has higher seafloor PAR levels perhaps due to less sediment resuspension (few mud-size particles), which could influence the higher biomass and densities of microphytobenthos.

Bierman et al. (1994) suggested from modeling studies that the western shelf has a different light attenuation-depth-primary productivity relationship. They suggest this is due to the broader shelf and shallower depths west of the of the Atchafalaya River plume compared to the narrower and deeper area west of the Mississippi River delta (their model box numbers 15-21 with depths of 5-21 m would include my stations). I did not find higher sediment chlorophyll *a* or microphytobenthos in this area probably because the seafloor PAR levels were lower at stations G-K during my sampling. The concentrations of bottom-water chlorophyll *a* were frequently high from station H3 westward, especially in 2006, probably due to the mixing conditions, which also increased the bottom-water DO concentrations above 2 mg l^{-1}. For example, the wave activity during the 2006 shelfwide cruise that mixed the water column and deepened the pycnocline (Fig. 3.2) also probably resuspended the sediment-containing benthic diatoms (as suggested by the < 15 % transmission on the bottom measured from CTD), which contributed to high bottom-water chlorophyll *a*, fucoxanthin, and benthic cells at the 2006 western stations of H3, I4 and K4. Microphytobenthos do not only contribute to the primary production of the sediment surface, but they can be resuspended and contribute to the bottom-water phytoplankton biomass and possibly to primary production. It was difficult to determine if the

resuspension event negatively affected the sediment microphytobenthos biomass and density at those stations in 2006 because the chlorophyll *a* concentrations did not differ from other stations in 2007 and 2008.

Hypoxia and the associated impacts on the benthic fauna and nutrient fluxes may produce a refuge for microphytobenthos. Grazing pressure on microphytobenthos (Miller et al. 1996, Moodley et al. 2000) may be reduced when benthic macrofauna and meiofauna populations decline due to low oxygen (Murrell & Fleeger 1989, Baustian & Rabalais 2009). Higher densities of filamentous cyanobacteria are known to occur under absent or reduced grazing pressure (Stal 1995, Ladakis et al. 2006). Reduced shrimp bottom-trawling also occurs during hypoxia, which would otherwise resuspend the sediment which contains the benthic cells and attenuate PAR. Some of the benthic nutrient fluxes, like BWNH4 and BWPO4 can increase in hypoxic bottom water (Rabalais & Turner 2006, Chapter 4) and be available to microphytobenthos, like the filamentous cyanobacteria in 2008. The hypoxic bottom-water nutrient concentrations could also impact the microphytobenthic community structure. When the ratio of BWSi to BWNO4+NO3 falls below one (Turner et al. 1988), the BWSi concentrations may be insufficient to form diatom frustules, which could select for a more non-diatom benthic community structure, as seen with cyanobacteria in coastal tidal flats (Pinckney et al. 1995, Barranguet et al. 1997).

I conclude that microphytobenthos, commonly pennate diatoms and filamentous cyanobacteria are present on the eutrophic, low light Louisiana continental shelf along the 14-20 m contour where hypoxia commonly forms in summer. The sediment chlorophyll *a* concentrations and benthic densities were most closely related to seafloor PAR and ammonium and phosphate. Microphytobenthos present along the continental shelf could, in turn, influence benthic processes, such as secondary production, nutrient fluxes and importantly oxygen dynamics.

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CHAPTER 4

OXYGEN CONSUMPTION RATES FROM LIGHT AND DARK SEDIMENT CORES IN THE HYPOXIC AREA OF THE NORTHERN GULF OF MEXICO

INTRODUCTION

The low-oxygen region, known as hypoxia (≤ 63 mmol O_2 m^{-3} or ≤ 2 mg O_2 l^{-1}), on the northern Gulf of Mexico continental shelf is currently the world's second largest human-caused coastal hypoxic area, with a bottom-water extent up to 22,000 km^2 in mid-summer (Rabalais et al. 2007a, Diaz & Rosenberg 2008). The hypoxic area develops due to the influence of the fresh water and nutrients discharged from the Mississippi and Atchafalaya rivers. These rivers have been delivering increased nitrogen and phosphorus loads since the 1950s (Turner and Rabalais 1991, Rabalais et al. 2002a, Turner et al. 2007), resulting in high primary productivity, > 300 g C m^{-2} y^{-1} along the adjacent continental shelf (Sklar & Turner 1981, Lohrenz et al. 1990, Lehrter et al. 2009) and increased carbon accumulation and worsening oxygen conditions (Sen Gupta et al. 1996, Turner et al. 2004, Rabalais et al. 2007b).

The flux of fixed carbon to the seafloor is high (~ 50%) and primarily in the spring (Justić et al. 1993, Redalje et al. 1994, Rabalais et al. 2002b). The high quality carbon substrate (e.g., senescent diatoms, fecal pellets and marine aggregates) is decomposed by aerobic bacteria, increases the respiration rate in the bottom water (Turner et al. 1998) and drives the hypoxia formation and maintenance. Respiration in the bottom water and sediment causes a high oxygen demand below the pycnocline (Murrell & Lehrter 2011) that is not replenished by oxygen diffusion from the well-oxygenated surface waters across a strong pycnocline (Justić et al. 1993).

The range in sediment oxygen consumption rates (overlying water + sediment) from past studies conducted in the northern Gulf of Mexico hypoxic region include: 4 - 32 mmol O_2 m^{-2} d^{-1} (or about 125 - 1080 mg O_2 m^{-2} d^{-1}) (Dortch et al. 1994, Miller-Way et al. 1994, Morse & Rowe 1999, Rowe 2001, Murrell & Lehrter 2011). These rates were dependent on bottom-water dissolved oxygen (DO) concentrations and temperature (Rowe 2001, Rowe et al. 2002, Hetland & DiMarco 2008, Murrell & Lehrter 2011). If there is no mixing of the stratified layers or advection of oxygenated waters into the

area, the time to reduce the bottom-water oxygen concentration from about 6 to less than 2 mg l^{-1} is 18, 11, or 9 days in April, May and July, respectively (Rabalais et al. 2007a). Numerous models predict hypoxia formation and maintenance (Bierman et al. 1994, Justić et al. 2002, Scavia et al. 2003, Hetland & DiMarco 2007, Wang and Justić 2009), but better estimates of oxygen consumption rates at a more frequent temporal scale are needed to refine these models. In addition, few models include benthic primary production to help forecast conditions of hypoxia (see review, Justić et al. 2007).

Benthic primary production from resident sediment microalgae can be a significant component of primary production on continental shelves (Cahoon & Cooke 1992, Cahoon & Laws 1993, MacIntyre et al. 1996, Cahoon et al. 1999, Nelson et al. 1999, Jahnke et al. 2000, Totti 2003). The presence of microphytobenthos (benthic microalgae and cyanobacteria) has been reported on the northern Gulf of Mexico inner continental shelf (Grippo et al. 2009, 2010, Chapter 2). Microphytobenthos density and biomass (estimated from chlorophyll *a*) were higher during summer along a transect ~ 100 km west of the Mississippi River delta where hypoxia frequently occurs, and phytoplankton densities (pelagic and tychopelagic) were more common on the sediment surface in fall and winter (Chapter 2).

Benthic photosynthesis can increase bottom-water oxygen concentrations (Graneli & Sundbäck 1986; Jahnke et al. 2000) and may prevent bottom-water anoxia (Bierman et al. 1994, Dortch et al. 1994). The amount of oxygen produced from benthic primary productivity in the hypoxic area of the northern Gulf of Mexico has been estimated as 1.8 – 4.4 mmol O_2 m^{-2} d^{-1} (58-141 mg O_2 m^{-2} d^{-1}) from four sites in July 1991 (Dortch et al. 1994) and has been used in preliminary oxygen budgets (Dortch et al. 1994, Rowe 2001). During summer hypoxia, benthic and bottom-water photosynthesis have the potential to offset 23% of the daily sediment oxygen consumption (Dortch et al. 1994). However, Quiñones et al. (2007) suggested this value was too low to significantly affect their estimation of bottom-water respiration rates. I surmise that the sediment oxygen demand is greater than any possible benthic oxygen production, because the net effect is hypoxia development. However, I contend that

oxygen production must occur given the presence of microphytobenthos (Chapter 2) and sufficient light levels (Lehrter et. al. 2009).

My goal was to determine whether oxygen production plays a role in oxygen dynamics in the hypoxic area of the northern Gulf of Mexico. I also sought to determine the oxygen consumption rates that lead to bottom-water hypoxia by incubating sediment cores representative of seasonal conditions on the hypoxic Louisiana inner shelf, including typical light levels reaching the seafloor. In addition, I wanted to investigate the associated nutrient fluxes and microbial biomass. Based on the abundance and distribution of microphytobenthos (Chapters 2, 3), I predicted that when sufficient levels of photosynthetically available radiation (PAR) reached the seafloor, benthic photosynthesis and oxygen production would occur. My null hypothesis was that PAR would have no effect on the sediment community microphytobenthos (estimated chlorophyll *a* and density) oxygen dynamics of the incubated bottom water and sediment cores, nutrient fluxes and microbial biomass. I also hypothesized that the variables (e.g., oxygen consumption rates, nutrient fluxes, and microbial biomass) would not be significantly different among stations and months. My results will support the ongoing hypoxia modeling efforts in the northern Gulf of Mexico (see Justić et al. 2007 for a review) and, more specifically, the development of three-dimensional coupled, physical and hydrodynamic water quality models (Hetland & DiMarco 2008; Justić & Wang 2009; Wang & Justić 2009), in that they will provide process rates of oxygen dynamics across a range of environmental parameters and seasonal variability.

MATERIALS AND METHODS

Field Collection

I sampled two frequently hypoxic stations, C4 (~ 14 m depth, 28:57.00′ N, 90:31.46′ W) and C6B (~ 20 m depth, 28:52.18′N, 90:28.04′ W) during the spring and summer of 2007 and 2008 (Fig. 4.1). I measured the water column properties with a YSI 6820 multiparameter sonde, which allowed me to sample close to the seafloor (e.g. 0.5 m above). In 15 Aug 2008 I sampled station C4 with a SeaBird CTD/rosette system for which the oxygen probe measures approximately 1-1.5 m above the seafloor. A

Biospherical Instruments Inc. profiling natural fluorometer (PNF-300) was also deployed to the bottom to determine the PAR. The reference PAR (mean, n = 15 for all sampling events ~ 2100 μmol m^{-2} s^{-1}) measured from the ship was used as the surface PAR instead of the top half-meter of the water column, which is highly variable. To correct for different sampling times the seafloor PAR was reported as percent of surface PAR. Additional monthly environmental data (N.N. Rabalais et al., unpubl. data, Chapter 2) were used to supplement the environmental background information when sediment cores were not collected.

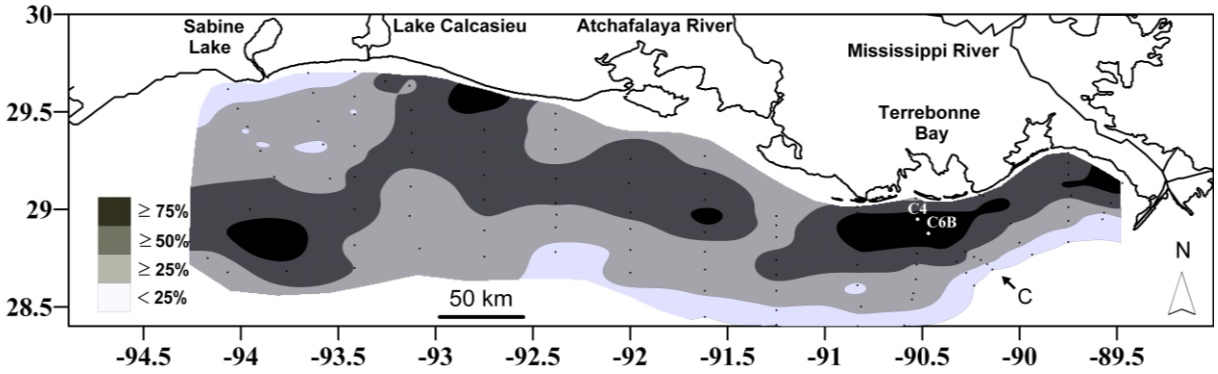

Fig. 4.1. Frequency of mid-summer bottom-water hypoxia (≤ 2 mg l^{-1}) off the coast of Louisiana and Texas for 60 to 80 stations sampled during mid-July from 1985 to 2008. Stations C4 and C6B are labeled on the C transect. The figure is updated and modified from Rabalais et al. (2002 a,b) and Chapter 2.

I used a Niskin bottle to collect bottom water samples (~ 2 L) about 0.5 m from the seafloor. The *in situ* bottom water was also sampled for dissolved nutrient concentrations, ammonium (NH4), nitrate and nitrite (NO3 + NO2), silicate (Si), and phosphate (PO4), microscopic counts of phytoplankton and microphytobenthos (Chapter 2) and phytopigment analyses.

I took three intact, water-overlying GOMEX box cores (0.5 m high, 0.3 m long, 0.3 m wide, surface area 0.09 m^2; Boland & Rowe 1991) at each station. Sediment cores for incubation were removed from the box cores by hand-pushing two (one for light and one for dark) acrylic core tubes (12.7 cm ID, 30 cm tall) to obtain a sediment height of 12 cm and about 2 L of overlying water. Possibly disturbed edges of the box core were avoided. Each light-dark pair was removed from the same box core (e.g., box core A = core 1 and 2, box core B = 3 and 4, and box core C = 5 and 6). The cores were

capped on top and on the bottom by neoprene-bonded acrylic lids; the top lid was equipped with an attached magnetic stir bar (30 rpm). Long plastic screws and wing nuts helped to seal the lids to the cores. The bottom-water samples (~ 2 L, acrylic cores permanently sealed on top and bottom and similar magnetic stir bar). The sediment cores were topped off with bottom water if needed. All cores were kept in an *in situ* bottom-water temperature, shaded bath, and the top lid was left unplugged to prevent oxygen consumption, until incubation began. In June and July 2008 the oxygen concentrations were very low (~ 6.3 mmol m^{-3} or 0.2 mg l^{-1}), and oxygen was artificially added to the sediment cores by using an aquarium air pump (> 63 mmol m^{-3} or 2 mg l^{-1}) so oxygen consumption rates could be detected.

The upper 0.5 cm of *in situ* sediments was precision extruded from a smaller core (7.6 cm diameter) that was removed from each box core, homogenized in a Petri dish, and used to fill two cyrovials (1.8 ml each) for phytopigment analysis. The remaining sediment from the smaller core was preserved in gluteraldehyde for microalgal cell identification and quantity (one per station).

Incubation Experiments

Incubators were placed outside on a dock at the Louisiana Universities Marine Consortium (LUMCON) in Cocodrie, Louisiana, USA. Each incubator represented a station and held six sediment cores and two water samples. Half of the samples were covered with thick black plastic (dark cores), and the other half were left uncovered for natural light penetration. The dark and light sediment and water samples were incubated for 20^{+} hours to allow for natural nighttime (~ 6 hour and ~ 4 hour segment) and daytime periods (~ 12 continuous hours); the times are reported as local time (central daylight savings). I placed nylon screens (5 to 11) to reduce the daytime PAR to simulate seafloor conditions following the exponential decay function of: $PAR_E = PAR_{DT}e^{-0.69x}$, where x is the number of screens, PAR_{DT} is the available daytime radiation and PAR_E is the resulting amount of light for the experiment. Each nylon screen decreases the PAR by ~ 50%. The light treatment for the 2007 experiments included the *in situ* light conditions (0.1 - 1 % surface PAR) measured prior to sampling, and the light treatment was kept constant (~ 3 % surface PAR) in 2008 for all experiments. Quantum radiation measured from the

LUMCON weather station (http://weather.lumcon.edu) determined the available daytime PAR reaching the incubators on the dock, and a handheld PAR sensor (Li-COR LI-193SA) inserted into the incubators, underneath the screens, verified the PAR levels reaching the cores.

A chiller/heater connected to the incubators maintained temperatures typical of seafloor conditions. Dissolved oxygen measurements were taken every two hours with a Hach luminescent dissolved oxygen probe that was placed in the top-lid port, otherwise plugged by a rubber stopper between measurements. At the end of the incubation the sediment height was measured to verify the amount of overlying water (L). Dissolved nutrient concentrations were measured from the overlying water in 2008. At the end of the experiment, a smaller core (7.6 cm diameter) was placed in the middle of each core and the top 0.5 cm was removed for analysis of phytopigments (one from each core) and microscopy (one from each station, core 1). The top 1 cm of the remaining ring of sediment was removed from each core for microbial biomass (C and N) in 2008. $NaH^{13}CO_3$ (Isotec® 98 atomic %) was added to the overlying water ($\delta^{13}C$ of dissolved inorganic carbon (DIC): June and July 2008 = ~ 500‰, August 2008 = ~ 1000‰) of the sediment cores at the beginning of the experiment to determine if carbon fixation via photosynthesis was taking place in the bottom water and sediment. Values of $\delta^{13}C$ higher than about -22‰ background (Hedges & Parker 1976, Goñi et al. 1998) were expected if primary production via photosynthesis was occurring (Fry 2006).

Laboratory Analyses

The sediment and water samples were analyzed for phytopigments by high-performance liquid chromatography (HPLC) based on the methods of Wright et al. (1991); for details see Chapter 2.

I used epifluorescence microscopy (adapted from Dortch et al. 1997, Dortch 1998) to determine the microalgal community composition of surface and bottom waters and sediments, and filtered them on 0.2, 3, and 8 μm fractions, but only the 3 and 8 μm fractions were reported. Aggregates of picocyanobacteria in water and sediment samples are commonly found on the 3 and 8 μm filters (Chapter 2) as with this study but those data were not included in the analyses because they do not

contribute much to the biomass (Dortch et al. 2001, Chapter 2). Sediment samples were collected after the experiment to verify if microphytobenthos were still present. I assigned a niche (pelagic, tychopelagic and benthic) (Cahoon et al. 1994) to each taxon to determine if the cell was associated with the sediment or not. For details on the microscopic methods see Chapter 2.

The *in situ* bottom water from the sample sites and the sub-samples of the overlying water removed after incubation from the 2008 cores were frozen and later analyzed for NH4, NO3+NO2, Si and PO4 on a Lachat auto-analyzer II system (8000 series) with an autosampler (ASX-400 series) according to EPA methodology (353.2, 350.1, and 365.2).

Microbial biomass carbon (cellular mass) was determined according to the chloroform cell-lysing fumigation-extraction method (Vance et al. 1987). From each core, two subsamples (fumigated and non-fumigated) of wet sediment (~ 2 g) were extracted with 25 ml of 0.5 M K_2SO_4 for 30 min on a shaker and vacuum filtered onto a 47 mm diameter membrane filter (0.45 µm pore size) to obtain the supernatant, which was analyzed on a Shimadzu online TOC-V_{CSH} with a TNM-1 TN unit and an autosampler (ASI-V). Microbial biomass, including potential autotrophs and heterotrophs, was determined by subtracting the non-fumigated from the fumigated samples with units of g C kg dry sed^{-1}.

To determine carbon fixation by phytoplankton and microphytobenthos in June, July, and August 2008, the overlying water (e.g., 50 - 300 ml) was filtered onto a GF/F 47 mm-diameter, pre-weighed and pre-ashed (400 ºC for 24 hours) filters and the sediment surface was scraped onto similar filters. Both were acidified with HCl overnight to remove carbonates and dried at 60°C. A portion of the overlying-water filters (e.g., ~ 14 mg) and sediment (e.g., 2 - 5 mg) were analyzed for $\delta^{13}C$ on a Finningan MAT DeltaPlus continuous-flow stable isotope mass spectrometer (Fry 2007). A two source mixing model based on the ^{13}C atom percents allowed for estimates of the percent of the available carbon that was fixed in the water and sediment (Fry 2006), which was further converted from mmoles of carbon to oxygen by using photosynthetic quotients of 1 and 1.3 for a range in values (Falkowski & Raven 2007).

Sediment-water Flux Calculations

Dissolved oxygen fluxes were calculated from the overlying water in the light and dark sediment cores and bottom water samples from the following flux equations (based on Dalsgaard et al. 2000):

Initial and final concentrations (IF), Flux = $((C_n\text{-}C_o) \times V) / (A \times t)$
Time series (all data points) (TS), Flux = $(\alpha \times V) / (A)$

Units defined as:

Flux = mmol m^{-2} d^{-1}
C_0 = concentration at time zero (mmol m^{-3})
C_n = concentration at time n (mmol m^{-3})
t = incubation time (h)
A = area of sediment (m^2)
V = volume of water in core (m^3)
α = slope of the linear regression of concentration (mmol m^{-3}) versus time (h)

The same equations were used to determine the rates during the daytime and nighttime for each light core. This is another way to determine if the light treatment had an effect on the DO fluxes. The nutrient fluxes were calculated using the IF equation and the shipboard collection time was used as the initial time. Sediment oxygen consumption (SOC) rates include the consumption from the overlying water and the sediment. Bottom-water oxygen consumption (BWOC) rates include just the water.

Statistical Analyses

The null hypothesis for all tests was no difference between the light and dark treatment for the stations and months at an alpha level of 0.05 performed with SAS 9.1 (SAS Institute, Inc. 2003). To test for significant differences, analysis of variance (ANOVA) was performed on microbial biomass, carbon fixation rates, natural log transformed pigment, and phytoplankton and microphytobenthos density data to meet assumptions of normality with the 'proc mixed' statement. If significance was detected, a post-hoc Tukey-Kramer test allowed for pairwise comparisons. I used an analysis of covariance (ANCOVA) by 'proc glm' to test for a difference in the slopes of the oxygen consumption rates of the bottom water and sediment treatments (light and dark) versus the initial oxygen concentrations. The interaction (initial DO * treatment) determined if the slopes of the treatments were significantly different. A multiple regression with the r^2 selection method was used to test the effect of temperature and oxygen on SOC rates.

RESULTS

In situ Environmental Parameters

The salinity of the bottom water at stations C4 and C6B was approximately 35 during all sampling events, and the temperature gradually increased through the summer (Fig. 4.2, top panel). The bottom-water DO decreased from about 156 to 15.6 mmol m^{-3} (5 to about 0.5 mg l^{-1}) from spring to early fall as expected. Temperatures were lower during the remainder of the year, and salinity ranged between 30 and 35. No sampling occurred in September 2008 due to Hurricane Gustav.

The photosynthetically available radiation (PAR) on the seafloor at stations C4 and C6B was less than 1% of the surface (reference) PAR at the time of sediment core collection (Fig. 4.2, middle panel). At station C4 the seafloor PAR levels were higher in 2007 compared to 2008, and the levels varied between 0 -18 μmol photons m^{-2} s^{-1}. PAR levels at Station C6B were below 0.5% of surface PAR (range: 0 - 12 μmol photons m^{-2} s^{-1}) for both years, and the highest level of light measured was in August 2007 (11.3 μmol photons m^{-2} s^{-1}) and August 2008 (4.4 μmol photons m^{-2} s^{-1}). The higher PAR levels generally were observed from June - August for both stations and years.

The *in situ* sediment chlorophyll *a* levels were generally less than ~ 5 μg g dry sed^{-1} (Fig. 4.2, bottom panel). At station C4, the 2007 chlorophyll *a* values were frequently higher than in 2008. The highest summer values at C4 were in July 2007 (6.25 μg Chl *a* g dry sed^{-1}) and August 2008 (2.38 μg Chl *a* g dry sed^{-1}). Station C6B had a similar pattern with high values in July 2007 (1.68 μg Chl *a* g dry sed^{-1}) and August 2008 (5.03 μg Chl *a* g dry sed^{-1}), but overall mean chlorophyll *a* at station C6B was significantly lower than C4 (C6B = 0.91 and C4 = 1.87 μg g dry sed^{-1}, $F_{1,48} = 10.06$, $p = 0.0026$). In 2007, the sediment cores were collected after the spring phytoplankton blooms (peak surface water chlorophyll *a* in March) and during a time of lower surface water chlorophyll *a* but in 2008 the surface water chlorophyll *a* levels tended to stay high with multiple peaks. The combined station sediment chlorophyll *a* (log transformed) was significantly correlated with seafloor PAR ($r^2 = 0.33$, $p = 0.02$).

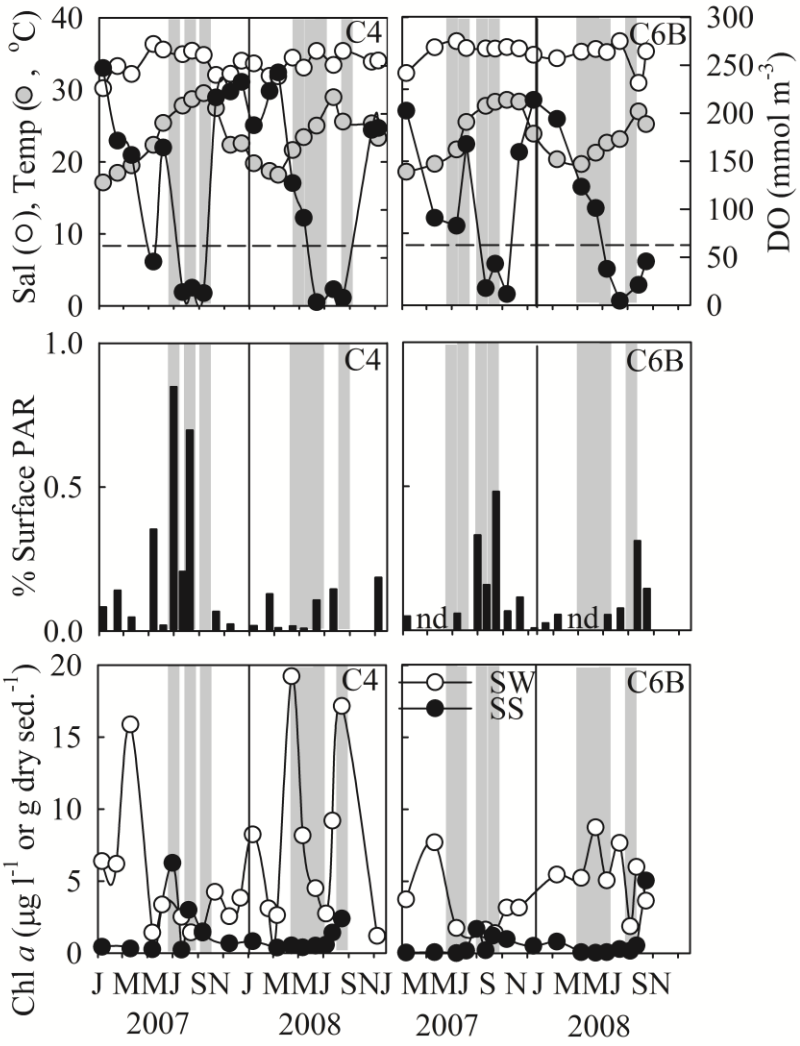

Fig. 4.2. *In situ* bottom-water salinity (open circle), temperature (°C, gray circle), and dissolved oxygen (DO, mmol m^{-3}, filled circle) (top panel), percent surface PAR reaching the seafloor (middle panel) and chlorophyll *a* of surface water (SW, μg l^{-1}) and sediment surface (SS, μg g dry sed^{-1}) (bottom panel) at stations C4 and C6B from January 2007 through August 2008. Dashed line at 63 mmol O_2 m^{-3} is reference for hypoxia (top panel). Gray shaded areas indicate when sediment cores were collected for incubation experiments. nd = no data. The % surface PAR data for 7/8/08 at both stations were not labeled with 'nd' to clarify data presentation. X axis is labeled with bimonthly abbreviations starting with January for each year.

Most of the *in situ* sediment surface cells at stations C4 and C6B were made up of diatoms and filamentous cyanobacteria Fig.4.3). A few (e.g., 40 - 200 cells g dry sed^{-1}) dinoflagellates were present on the sediment surface. Peaks of diatom densities were at station C4 in July and September 2007 and August 2008, and at station C6B in July 2007 and August 2008. Cyanobacteria-other densities in 2007 were higher at station C4 compared to station C6B, and similar cyanobacteria densities existed at both stations in 2008. The bottom water at station C4 and C6B was higher in taxa richness of autotrophic

cells than the sediment surface (data not shown). Station C4 bottom-water composition was composed mostly of phytoflagellates, diatoms, cryptomonads and some cyanobacteria. Diatoms tended to dominate the bottom water at station C6B.

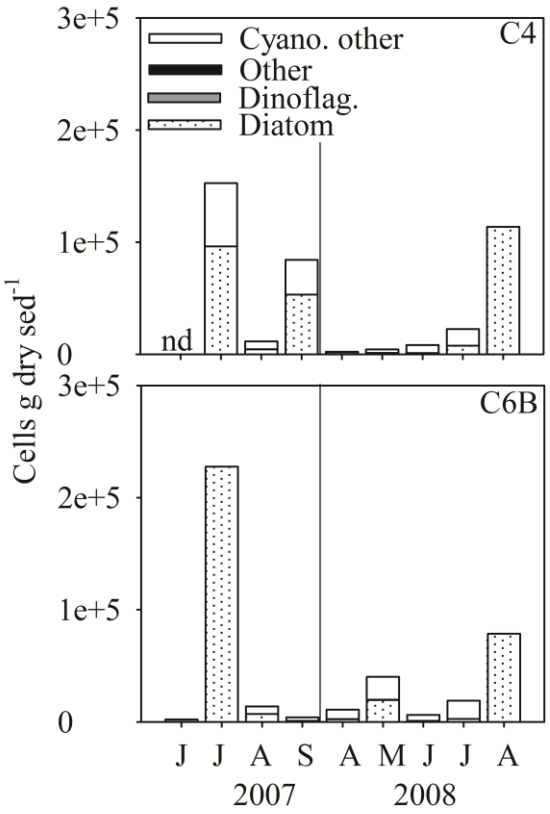

Fig. 4.3. Major taxonomic groups (3 + 8 μm size fractions) observed *in situ* on the sediment surface from June 2007 to August 2008 at stations C4 and C6B. Taxa groups include: Cyanobacteria-other (no picocyanobacteria), other (unidentified autotrophs), dinoflagellates, and diatoms, nd = no data. Month abbreviations represent June – September in 2007 and April - August in 2008.

Benthic cells, indicative of microphytobenthos (3 + 8 μm fractions) were present at both stations in all month/years but in low densities (Fig. 4.4). The benthic percentages ranged between 2- 99% of the total for station C4 and 0.5 - 82% for station C6B, and the mean benthic percentages were, respectively 64% and 50%. At station C4, benthic cell densities were high in July and September 2007 but were lower in 2008. Low densities were observed for both years at station C6B. The majority of the monthly benthic cell densities at both stations were below 2.0×10^4 cells g dry sed^{-1}. The range of *in situ* densities (3 + 8 μm fractions) at station C4 was 2.5×10^2 - 1.5×10^5 cells g dry sed^{-1} and at C6B was

1.8×10^2 - 2.0×10^4 cells g dry sed^{-1}. Pelagic phytoplankton cells were abundant in the sediment surface community in July 2007 and May and August 2008 at station C6B. In August 2008, high pelagic phytoplankton densities were found at station C4. Pelagic cells dominated (C4 mean: 97% and C6B mean: 84%) the bottom-water at both stations (data not shown).

The *in situ* bottom-water nutrient concentrations were similar at both stations from April to August 2008 (Fig. 4.5). The NH4, Si, and, PO4 concentrations increased from April to July and decreased to April levels in August. The NO3+NO2 levels were lowest in June and July when NH4 concentrations were high.

Sediment Core Incubations

Abiotic Conditions

I conducted 17 incubation experiments - eight from station C4 and nine from station C6B (Table 4.1). The range of start time for the incubations was between 18:00 and 01:00, but most started around 23:00 due to the time to return to shore and for experiment preparation. Eight of the experiments ran for 24 hrs, 8 for 22 hours and 1 for 20 hours, with shorter times due to weather conditions. The PAR reaching the incubators during most of the experiments were typical summer light curves with maximum PAR reaching ~ 2000 µmol photons m^{-2} s^{-1} frequently at hour 14 (Fig. 4.6). Six of the 17 experiments (August and September 2007 and August 2008 for both stations) had lower light levels due to clouds and/or thunderstorms, which reduced the natural light reaching the samples. The *in situ* PAR levels at C6B in June 2007 were zero, thus, all samples were treated in the dark. The % surface PAR reaching the sediment surface was measured around noon in each incubator during all the experiments (Table 4.1). The light treatment was reached in 2007 by placing 6^+ screens on the incubators. The % surface PAR was higher than intended for most of the 2008 experiments. I placed fewer screens (5) in 2008 to allow for 3% surface PAR, but higher PAR levels were recorded, which might have been produced from scattering of the allowed higher light levels (Fig. 4.6). In 2008, the observed % surface PAR treatment was acting as if 4 screens were used (instead of 5), which resulted in a > 6% surface PAR level (instead

of 3%) (Table 4.1). The actual % surface PAR treatment (4%) was near the goal of 3% in August 2008, which probably occurred due to the lower source PAR (Fig. 4.6) and potentially lower scattering. The nylon screens also buffered the incubation-water temperatures. The heater/chiller maintained targeted temperatures, especially considering the surrounding air temperature on the dock was commonly ~ 35°C. The only incubator experiment with a large temperature difference compared to the *in situ* temperature was in April when air temperatures dropped during a cold front from an ambient value of 21- 22 ºC to incubated temperatures of ~16 - 19°C.

DO Fluxes

The sediment oxygen consumption (SOC) rates (overlying water + sediment) for all cores (n = 101, C4 7/07 dark core leaked and was omitted) ranged from 1.17 – 27.27 mmol O_2 m^{-2} d^{-1} (37.20 – 872.64 mg O_2 m^{-2} d^{-1}), and the mean was: 10.85 mmol O_2 m^{-2} d^{-1} (347.28 mmol O_2 m^{-2} d^{-1}) for the initial and final (IF) calculations. Similar to the IF rates, the time series (TS) rates ranged from: 0.18 - 25.34 mmol O_2 m^{-2} d^{-1} (0.24 to - 33.79 mg O_2 m^{-2} hr^{-1}). The initial DO range in the overlying water of the sediment cores was 15.00 - 181.89 mmol m^{-3} (0.48 to 5.82 mg l^{-1}).

The pooled (light + dark) SOC rates calculated from the initial and final concentrations were positively and linearly correlated with the initial oxygen levels (r^2 = 0.63, $F_{1, 99}$ = 170.4, p = 0.0001, n = 101) and was a better fit than regressing the average oxygen levels (r^2 = 0.28, p = 0.0001, n = 101) during the incubation or the final oxygen levels (r^2 = 0.05 p = 0.027, n = 101). The time series (TS) SOC rates were not as highly correlated (r^2 = 0.54, p = 0.0001, n = 101) with initial oxygen levels compared to the IF calculations, and I used the IF calculations for subsequent analyses. The pooled SOC rates were more variable at higher initial oxygen levels (> 125 mmol m^{-3} or 4 mg l^{-1}). I thought the artificial oxygen additions in June and July 2008 cores might have introduced additional variability and caused the SOC rates to be variable and affect the regression analysis. Removing the cores with oxygen addition resulted in a 12% decrease of the r^2, therefore I decided to keep the oxygenated cores in the analysis. I also noticed other potential influential outliers in the regression analysis at the higher initial

oxygen levels (Fig. 4.7). Sediment core pairs, box core A: 1, 2 and box core C: 5, 6 collected from station C6B May 2008 had initial oxygen concentrations above 156.26 mmol m^{-3} (5 mg l^{-1}) but did not have DO fluxes as high as predicted from the regression line. The other pair (box core B: 3, 4) had initial oxygen levels below 62.5 mmol m^{-3} (2 mg l^{-1}) and the SOC fluxes fell along the regression line. The bottom-water *in situ* oxygen level at C6B in May 2008 was 12.5 mmol m^{-3} (0.4 mg l^{-1}), and the four cores that had SOC rate outliers had oxygen introduced unintentionally during the cruise compared to the other pair (3-4) that did not. The addition of DO (during collection or transportation to dock) could have unintentionally disrupted the microbial community and their oxygen consumption response to the rapid increase in oxygen levels. I removed these cores (C6B May 2008 cores: 1, 2, 5, 6) from further oxygen flux analyses because they accounted for 15% of the variation.

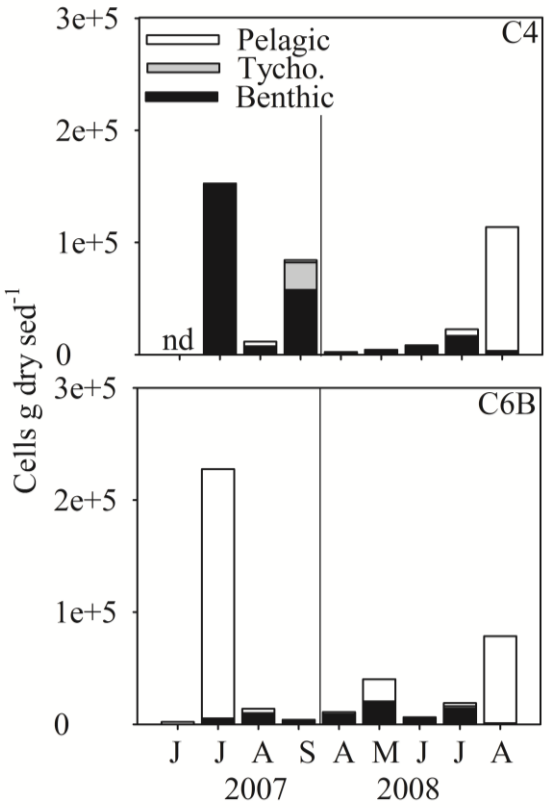

Fig. 4.4. Common niches (pelagic, tychopelagic or benthic) of *in situ* algal cells (3 + 8 μm fractions) observed on the sediment surface from June 2007 to August 2008 at stations C4 and C6B. nd = no data. Month abbreviations represent June - September 2007 and April – August 2008.

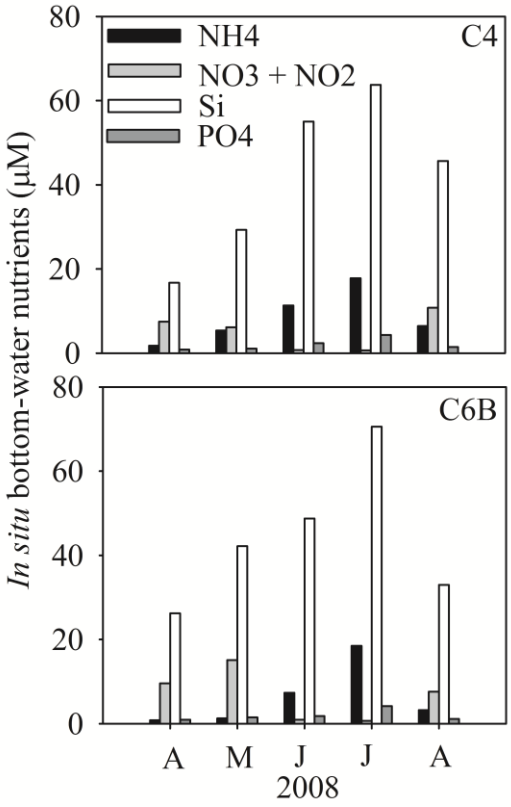

Fig. 4.5. *In situ* bottom-water nutrient concentrations (NH4, NO3 + NO2, Si, and PO4) at station C4 and C6B from April - August 2008.

Table 4.1. Comparison of PAR, dissolved oxygen (DO), and temperature of the *in situ* bottom-water measured prior to the incubation experiments and the mean values (n = 6 for all except C4 7/2/07 n = 5) of the overlying water in sediment cores following incubations. The target % PAR was measured *in situ* for 2007 and kept constant at 3% for 2008. The experiment PAR was the peak levels (% surface) measured at noon. For the *in situ* data, the bold indicates measurements were taken from SeaBird CTD (1-1.5 m above the bottom) instead of the YSI 6820 (within 0.5 m above the bottom). For the incubation data, bold indicates oxygen was artificially added to cores. Local times are in central daylight saving time. nd = no data.

					Target PAR Treat (% surf PAR)	*In situ*		Incubation				Exp PAR Treat (% surf PAR)
						DO (mmol m^{-3})		DO (mmol m^{-3})		Temp (°C)		
Stn	Depth (m)	Yr	Exp Date	Start Time			Temp (°C)	Initial	Final	Initial	Final	
C4	14	07	Jul 2	21:00	1	123.13	27.5	82.69	13.56	28.0	27.7	1
			Aug 10	19:00	1	20.63	28.4	50.21	12.76	28.5	27.1	1.5
			Sep 13	01:00	0.2	13.13	29.5	45.84	15.47	26.7	26.3	nd
		08	Apr 16	23:00	3	127.70	21.6	139.70	75.63	16.7	19.3	9
			May 13	23:00	3	91.57	23.4	107.61	18.70	22.4	23.0	5
			Jun 13	23:00	3	3.75	25.0	**69.07**	20.11	25.9	24.5	7
			Jul 8	18:00	3	4.69	26.4	**68.13**	27.45	28.6	27.2	9
			Aug 16	23:00	3	**8.13**	**25.6**	62.35	17.50	25.9	24.7	4
C6B	20	07	Jun 7	00:25	0	167.51	25.4	133.24	19.84	26.4	26.9	nd
			Jul 2	21:00	0.5	81.57	26.3	73.75	9.12	28.1	27.8	0.5
			Aug 10	19:00	0.5	10.31	27.9	53.39	11.51	29.5	27.2	1
			Sep 13	01:00	0.1	11.25	28.5	63.08	20.05	26.4	26.4	nd
		08	Apr 16	23:00	3	100.94	21.1	141.00	72.30	16.4	19.1	6
			May 13	23:00	3	37.50	22.5	130.58	85.11	22.4	23.7	6
			Jun 13	23:00	3	4.38	23.0	**92.82**	26.04	25.6	24.6	6
			Jul 8	18:00	3	6.56	25.4	**102.82**	25.57	28.0	27.4	11
			Aug 16	23:00	3	45.32	25.1	48.18	11.15	26.3	24.8	4

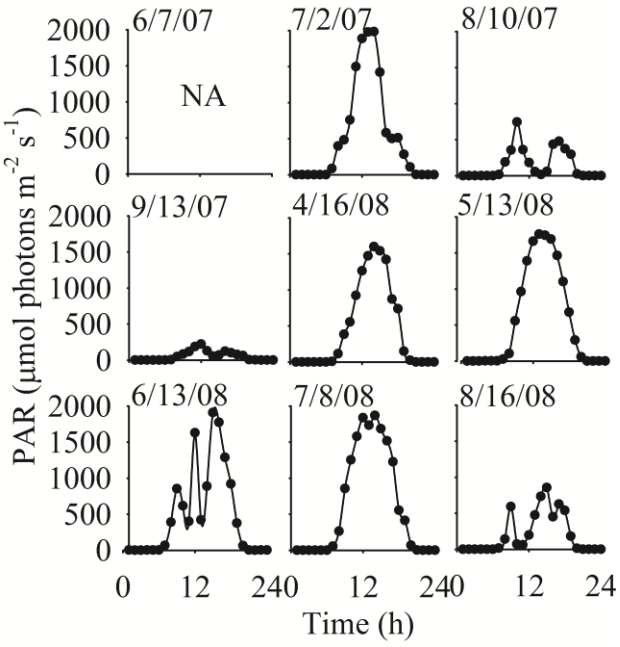

Fig. 4.6. Photosynthetically available radiation (PAR, µmol photons m^{-2} s^{-1}) reaching the outdoor incubators during each experiment in 2007 and 2008. Time is central daylight saving time. All sediment cores were incubated in the dark on 6/7/07 (see text).

I noticed the April 2008 cores were contributing to the high SOC rate variability above 125 mmol m^{-3} (4 mg l^{-1}) (labeled as diamonds in the larger plot of Fig. 4.7). These cores were incubated in temperatures 10°C lower than the others (16.5°C). Therefore, I adjusted the April 2008 SOC rates by calculating a Q_{10} of 1.38. This was done by using cores with similar initial oxygen levels but with temperatures at 26.4°C. By replacing the original April 2008 fluxes with the adjusted fluxes based on the Q_{10} value the r^2 increased by 8%. The mean temperature for all incubations were 26.2°C, and the median was 26.4°C (includes the April Q_{10} adjusted values). The maximum temperature (28.5°C) was in August 2007 and the minimum (23.2°C) was in May 2008. Since most of the cores were incubated ~ 26°C, I did not adjust the remainder of the DO fluxes for temperature differences. Temperature (with the April Q_{10}-adjusted) was not significant (p = 0.0987) and accounted for little of the variation (e.g., 1.8%) when compared to the initial DO concentration (86.6%) in the multiregression analysis.

Oxygen limitation occurred in some of the incubated cores. I observed that the oxygen levels in the overlying water of cores tended to plateau once DO levels reached ~ 15.63 mmol m^{-3} (~ 0.5 mg l^{-1}).

This occurred in ~ 60% of the incubated cores. I removed the plateau and re-calculated the oxygen consumption based on the new "final" concentration and the adjusted time. I found that the DO consumption rates increased two to four times the original (with plateau) SOC rates, and that there was no significant correlation between the SOC rates and the initial oxygen concentrations of the overlying water (r^2 = 0.05). Therefore, I decided not to use the plateau-removed calculations and used the original initial and final (IF) SOC rates (with plateau) for the remainder of the analyses.

I removed the C6B May 2008 SOC rates (n = 4) and temperature-adjusted the April 2008 DO flux rates, which increased the r^2 for the pooled (light + dark) sediment cores to 0.87 and the slope was significant ($F_{1, 95}$ = 615.8, p = 0.0001, n = 97). I combined all sediment cores from stations C4 and C6B in 2007 and 2008 to test if the slopes of the light and dark regressions were significantly different. Testing the slopes was necessary due to the DO covariable. I found no significant difference (all p values > 0.05) between the slopes of the light and dark oxygen flux linear regressions when stations and years were combined, when the stations were examined individually with years pooled, and with years separate for each station. I also binned the SOC rates into % surface PAR treatment categories (0.5-1.5%, 4-5%, 6-7%, and 9-11%), given that the treatment fluctuated, and found no significant difference between the light and dark cores for each category (all p values > 0.05). I combined all the data (no significant difference in the slopes of the treatments for both stations) to test whether station slopes differed, which they did not (p = 0.30). Therefore, all data (dark and light) from both stations were combined to produce one figure that represents the range (e.g., 1.2 – 27.2 mmol O_2 m^{-2} d^{-1}, n = 97) in net SOC rates for my study (Fig. 4.7, small plot).

The mean monthly SOC rates (treatment and station pooled per month) decreased from spring to summer for both years (Fig. 4.8) and the monthly mean rates ranged between 7- 18 mmol m^{-2} d^{-1} (~ 225 - 576 mg m^{-2} d^{-1}, n = 97). When these mean monthly SOC rates were regressed on the mean initial oxygen concentrations of the overlying water, they were positively correlated (r^2 = 0.74, p = 0.003), indicating that the mean monthly SOC rates were dependent on oxygen levels. The months of June, July

and August were sampled in both 2007 and 2008. The only month that did not have similar rates in both years was in June, with 2007 SOC rates greater than 2008.

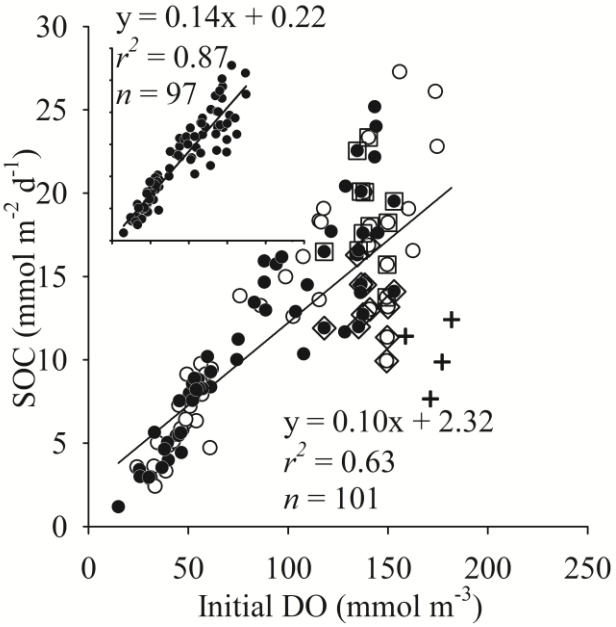

Fig. 4.7. Sediment oxygen consumption (SOC) as a function of the initial DO concentrations sampled in 2007 and 2008 from stations C4 and C6B. The large plot shows all data with open circles representing sediment cores incubated in the light and closed circles in the dark. Crosses indicate outliers (C6B 5/08 core pairs 1-2 and 5-6). Diamonds represent April 2008 SOC raw rates and squares represent April 2008 temperature-adjusted values. The small plot represents all data pooled with the outliers removed (n = 4) and using the April 2008 temperature-adjusted data. The axes on the small plot are the same as the larger plot. The small plot equation in oxygen units of x = mg l^{-1} and y = mg m^{-2} d^{-1} is: y = 136.54x + 7.1

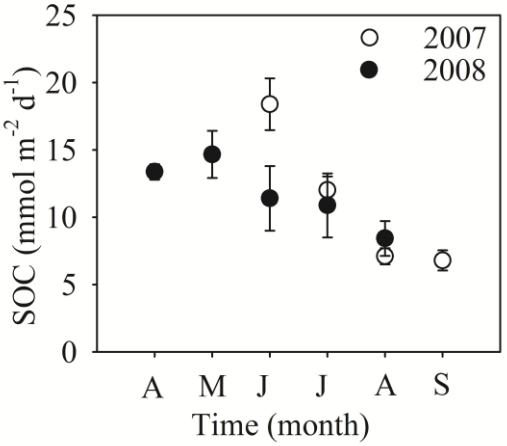

Fig. 4.8. Mean (± std. err.) sediment oxygen consumption (SOC) per month determined from pooled (station and treatment) sediment cores sampled from June to September 2007 (open circle) and April to August 2008 (closed circle). Each month, n = 12, except for: 6/07 n = 6, 7/07 n = 11, and 5/08 n = 8.

Daytime vs. Nighttime SOC Rates

Because I did not find a significant difference in the slopes of the dark vs. light cores, I focused on the light cores to determine if there was a difference in the SOC in the daytime versus nighttime. Sediment cores that were treated in light (n = 48) and that did not plateau prior to the daytime treatment (n = 36) were used to determine if there was an effect on the SOC by the light during the daytime. Once again, the oxygen consumption rates were dependent on initial DO concentrations. The night initial DO concentrations were higher than the day initial DO concentrations because the experiment started at night. Thus, there was little overlap between the day and night rates of the light cores when regressed on initial DO levels. I found the night SOC rates to be more variable than day rates in relation to the initial DO levels. For example, night r^2 = 0.39 and day r^2 = 0.75. Removing samples (8/07, 9/07 and 8/08), because they did not have high PAR levels (see Fig. 4.6), did not increase the r^2. Therefore, to derive some type of estimate on the gross primary productivity of these cores, I separately analyzed the samples that had higher chlorophyll *a* levels (e.g., July 2007 and August 2008; C4 n = 4, C6B n = 6). These had a better regression fit, with the daytime r^2 = 0.72 and nighttime r^2 = 0.89, but the day and night slopes were not significantly different ($F_{1, 16}$ = 0.13, p = 0.73).

Bottom-water DO Fluxes

I treated the water as a core and left the units the same (mmol O_2 m^{-2} d^{-1}) to compare the sediment oxygen consumption rates to the bottom-water oxygen consumption rates because the incubated bottom water equaled the volume of water overlying the sediment core. The incubators had room for only two water samples, and I felt that more replication may be needed. I added BOD bottles in April and May 2008, but there was little change in oxygen concentrations (e.g., IF calculations) for the light and dark treatments, and the results were not included in the analysis. Thus, I have no spring samples for bottom-water oxygen consumption (BWOC) rates. I removed the BOD bottles for further incubations in 2008 and used the two water samples per stations for the remaining monthly experiments. The BWOC rates ranged from 1.13 to 17.48 mmol m^{-2} d^{-1} (1.51 to 23.30 mg m^{-2} hr^{-1}, n = 23), and the mean was 6.64 ±

0.79 std. err. mmol m^{-2} d^{-1} (8.85 ± 1.05 mg m^{-2} hr^{-1}, n = 23) (Fig. 4.9). The common units for BWOC reported in the literature include a volumetric (m^3) unit. I converted the range and mean in rates to units of mmol m^{-3} d^{-1} = 6.25 - 96.57 (0.2 - 3.1 mg l^{-1} d^{-1}) to provide a comparison, and the mean was: 36.39 (~ 1.2 mg l^{-1} d^{-1}). The mean initial DO concentration was 68.1 mmol m^{-3} (~ 2.2 mg l^{-1}). The BWOC rates were correlated with initial oxygen concentrations (r^2 = 0.60, p = 0.001) (Fig. 4.9), and the slopes of the light and dark treatments were not significantly different at each station (C4, $F_{1,6}$ = 4.75, p = 0.07; C6B $F_{1,9}$= 1.08 p = 0.33). When I pooled the light and dark treatments for each station and compared the slopes (C4 vs. C6B) with covarying initial DO concentrations, the two stations differed ($F_{1, 19}$ = 9.59, p = 0.006) (Fig. 4.9).

I added ^{13}C stable isotope as sodium bicarbonate to the overlying water of the sediment cores in June, July and August 2008 to verify if any carbon fixation by photosynthesis was occurring in the bottom water and sediment surface. I decided to add this analysis because the conventional primary production method was not showing any net oxygen production from the previous experiments and I wanted to use a more sensitive method to verify carbon fixation. All of the light-treated fixed particulate organic matter (POM) of the overlying water at station C4 and C6B at the end of the incubations had higher mean isotopic values (> -22 ‰ $\delta^{13}C$) than the dark treatment. The only statistically significant carbon fixation, however, was due to overlying water from station C4 in June 2008 (date* time interaction, $F_{2, 9}$ = 5.74, p = 0.0247), which equaled about 4 - 5 mmol O_2 m^{-3} d^{-1} (~ 0.15 mg l^{-1} d^{-1}). The fixed POM at station C6B was not significantly difference between the treatments ($F_{1,10}$ = 0.11 p = 0.7422). The $\delta^{13}C$ isotopic values for the sediment samples were similar between the light and dark treatment for all experiments (C4: $F_{1,10}$ = 1.40, p = 0.26 and C6B: $F_{1,10}$ = 0.03, p = 0.87).

Nutrient Fluxes

The light and dark treatments did not significantly affect (all p values > 0.05) the nutrient fluxes at both stations and the treatments were pooled. There was a significant difference with NH4 fluxes ($F_{4,39}$ = 5.41, p = 0.0015) and PO4 fluxes ($F_{4,39}$ = 8.15, p = 0.0001), due to lower fluxes in April and May

compared to the months of June – August (Fig. 4.10). The NO3 + NO2 fluxes ($F_{4,39} = 8.57$, $p = 0.0001$) and Si fluxes ($F_{4,39} = 6.97$, $p = 0.0002$) had significant interactions with month and station. The NO3 + NO2 fluxes were only significantly different between stations C4 and C6B during the months of April and August. The Si fluxes were only different between station C4 and C6B in May and overall stayed the same through time. Most of the nutrient fluxes were positive, indicating an efflux from the sediments into the overlying water. In certain months, NO3 + NO2 and PO4 were being taken up by the sediment (negative fluxes) (Fig. 4.10).

Sediment Biota

At the termination of the incubations, microphytobenthos were present on the sediment surface. However, sometimes their densities were higher or lower than the *in situ* densities (data not shown). Treating the sediment cores in light did not cause sediment pigments (chlorophyll *a*, fucoxanthin, pheophytin *a*, and pyropheophytin *a*) to differ from the dark treated sediment cores during all experiments at station C4 and C6B (all p values > 0.05). Most *in situ* pigment concentrations were similar to the concentrations at the end of the experiments in the light and dark treated sediment cores.

The light and dark treatment did not significantly affect the total microbial biomass (autotrophs + heterotrophs) carbon (p values > 0.42), therefore the data were pooled per month at each station. The microbial biomass carbon at station C6B and C4 ranged between ~ 1.5 – 7.5 g C kg dry sed^{-1} (Fig. 4.11). The microbial biomass carbon at C6B (~ 5 g C kg dry sed^{-1}) over all months was significantly higher than C4 (~ 3 g C kg dry sed^{-1}) ($F_{1, 40} = 63.86$, $p = 0.0001$) (Fig. 4.12). Because there was no significant interaction with station and month, the carbon biomass was pooled by month and indicated significantly lower values in July and August compared to the other months.

DISCUSSION

None of the sediment processes (e.g., oxygen fluxes and nutrient fluxes) or population metrics (e.g., phytopigment concentrations, microphytobenthos density, and microbial biomass) were affected by the light treatment when compared to the dark. I think that the combination of the variability of

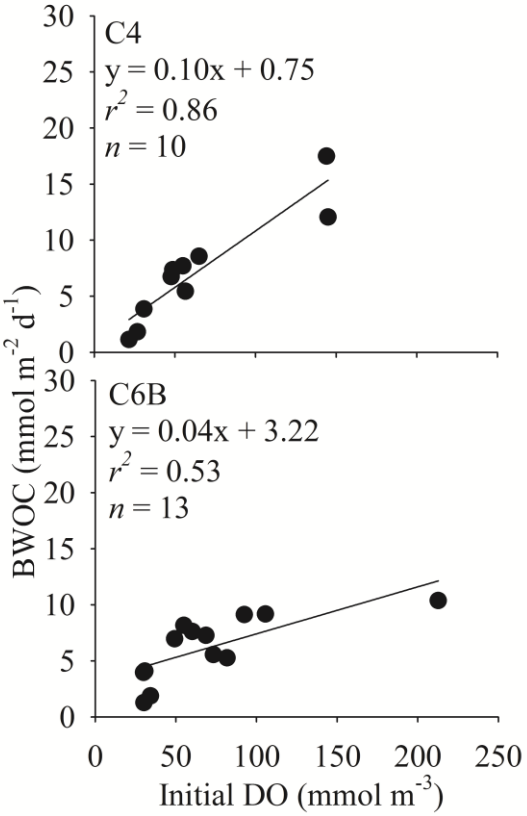

Fig. 4.9. Bottom-water oxygen consumption (BWOC) rates (mmol m^{-2} d^{-1}) as a function of the initial dissolved oxygen (DO) concentrations (mmol m^{-3}) sampled from June - September 2007 and July - August 2008 at station C4 and June - September 2007 and June - August 2008 at station C6B. Light and dark samples were pooled for each station.

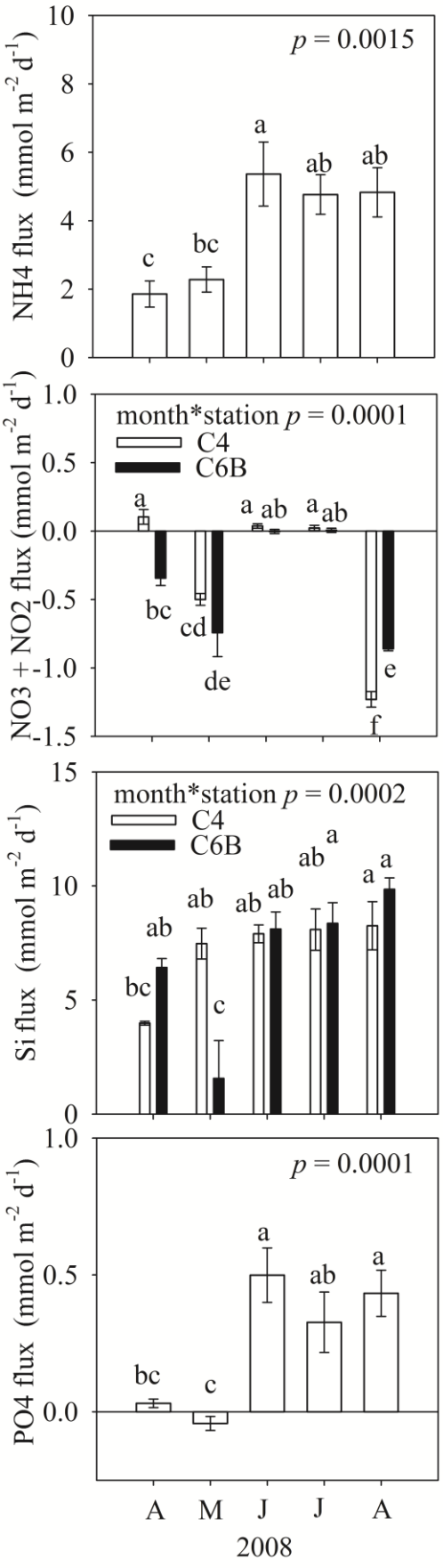
NH4 flux (mmol m-2 d-1)
p = 0.0015
c
bc
a
ab
ab
NO3 + NO2 flux (mmol m-2 d-1)
month*station p = 0.0001
C4
C6B
a
bc
cd
de
a
ab
a
ab
f
e
Si flux (mmol m-2 d-1)
month*station p = 0.0002
C4
C6B
bc
ab
ab
c
ab
ab
ab
a
a
a
PO4 flux (mmol m-2 d-1)
p = 0.0001
bc
c
a
ab
a
A
M
J
J
A
2008

Fig. 4.10. Mean net benthic nutrient fluxes (± std. err.) of NH4, NO3 + NO2, Si, and PO4 from April to August 2008 at stations C4 and C6B. n = 12 for each month when data were pooled, n = 6 per station due to a station*month significant interaction. Similar letters indicate no significant difference.

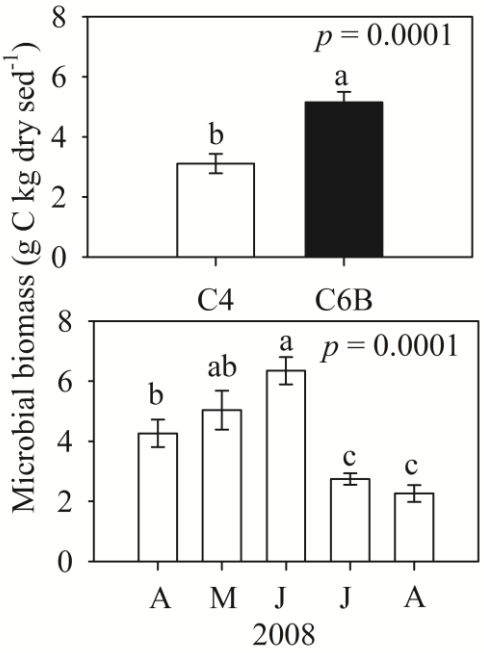

Fig. 4.11. Mean microbial biomass carbon (± std. err.) at stations C4 and C6B (top panel) and stations pooled from April to August 2008 (bottom panel). n = 30 for each station and n = 12 for each month. Similar letters indicate no significant difference.

sediment chlorophyll *a* concentrations (e.g., 0.02 - 6.3 µg dry sed^{-1}), density of microphytobenthos (range: 184 – 151,374 cells g dry sed^{-1}) with the additional variable natural PAR source (e.g., maxima = 60 - 2000 µmol photons m^{-2} s^{-1}) during the experiments made it difficult to measure a significant oxygen evolution response from the light cores.

The microphytobenthos composition (diatoms and filamentous cyanobacteria), densities and estimaged biomass (as chlorophyll *a*) were similar to an earlier year-round study conducted at these sites. The benthic cells were typically under 500,000 cells g dry sed^{-1} (Chapter 2), as seen in this study. Pelagic cells were more common during the summer months (July and August) of this study compared to the same months in Chapter 2. The sediment chlorophyll *a*, indicative of biomass, was commonly under 2 µg g dry sed^{-1} and was significantly twice the mean concentration at station C4 than station C6B, as seen in both studies. But the mean concentrations from this study were higher probably because

most were summer samples versus many more winter samples with low chlorophyll *a* levels that reduced the mean value in the previous study (Chapter 2).

Quantifying net oxygen production from sediments with a high oxygen demand has proven to be challenging. Benthic oxygenic photosynthesis can increase the sediment respiration demand by increasing the dissolved oxygen concentrations or by increasing the availability of excreted low weight organic compounds (Epping & Helder 1997, Middelburg et al. 2005). Oxygen produced can also be consumed within the sediment prior to reaching the overlying water (Epping & Helder 1997). Similar complications are likely present in the northern Gulf of Mexico. Oxygen produced in the sediment cores by microphytobenthos or settled pelagic phytoplankton could be consumed immediately or over the course of dark light conditions by respiration of these and other organisms in the lower water column or in the sediments, resulting in no detectable impact on bottom-water oxygen concentrations. I did not expect net autotrophy, or P/R > 1, of the sediment surface and bottom water due to the low light levels and high oxygen demand that leads to hypoxia, but I did expect to measure some oxygen production by the microphytobenthos present. The sediment oxygen consumption rates were the lowest near anoxic conditions, so positive net oxygen evolution from photosynthesis might be measurable at extremely low oxygen levels. But this idea becomes convoluted during anoxic conditions because the chemical oxygen demand of reduced metabolic end products, for instance, hydrogen sulfide produced from sulfate reduction, can consume oxygen by reacting with free oxygen (Morse & Rowe 1999, Rowe 2001, Murrell & Lehrter 2011).

The stable isotopes provided clues that low levels of estimated carbon fixation/oxygen evolution occurred, but for most cases it was not significantly different between the light and dark treatments. The maximum mean oxygen evolved in the overlying water, equaled about 4 - 5 mmol m^{-3} d^{-1} (~ 0.15 mg l^{-1} d^{-1}) due to overlying-water phytoplankton. But this may not have been detected by the DO probe with a precision of ± 3 mmol m^{-3} (0.1 mg l^{-1}). Therefore, adding ^{13}C to the overlying water of the sediment cores in 2008 verified that the light treatment overall was not significantly affecting the photosynthetic

carbon fixation or oxygen evolution, which matched the conclusion from the conventional method of measuring DO as an indicator of photosynthesis.

My results for oxygen consumption rates added a finer temporal resolution than any prior study in the northern Gulf of Mexico. The SOC rates (overlying water + sediment) of the light and dark cores ranged between 1.17 – 27.27 mmol O_2 m^{-2} d^{-1} (n = 97) and were similar to past studies in this region (1.3 - 32 mmol O_2 m^{-2} d^{-1}), that employed various incubation methods (Dortch et al. 1994, Miller-Way et al. 1994, Morse & Rowe 1999, Rowe et. al. 2002, Murrell & Lehrter 2011). The mean SOC rate (11.50 mmol m^{-2} d^{-1} ± 0.65 std. err.) was equal to the mean value reported in Murrell & Lehrter (2011). The mean initial DO for this study was: 82.63 mmol m^{-3} ± 4.41 std. err., n = 97. Other studies from this area (e.g., Rowe 2001, Rowe et al. 2002, Eldridge & Morse 2008, Murrell & Lehrter 2011) also reported a high dependence of SOC rates on the initial DO concentrations in incubations. The function applied to the oxygen-covarying SOC rates was linear when chambers were placed on seafloor (Rowe 2001) or cores removed for incubation (Murrell & Lehrter 2011), and logarithmic when rates were determined by benthic chambers (Rowe et al. 2002, Eldridge & Morse 2008). The slopes of the simple linear regression varied among the studies, which were: 0.29 (Rowe 2001), 0.094 (Murrell & Lehrter 2011), and 0.14 (this study). Since the slope of the linear regression of this study was higher than Murrell & Lehrter (2011), the number of days to reduce dissolved oxygen from ~ 250 mmol m^{-3} (8 mg l^{-1}) to ~ 63 mmol m^{-3} (2 mg l^{-1}) was 13 days, lower than the estimated 22 days of Murrell & Lehrter (2011). My estimate was within range of 9-18 days of Rabalais et al. (2007a) when oxygen levels decreased from 187.5 mmol O_2 m^{-3} (6 mg l^{-1}) to 63 mmol m^{-3} (2 mg l^{-1}) according to continuously declining DO measurements from deployed meters that followed reaeration events. Rowe et al. (2002) reported a logarithmic fit with a slope of 10.05; r^2 = 0.85. When a logarithmic function was applied to my converted data, I obtained a similar slope = 10.53; r^2 =0.86, n = 97. This dependence on initial oxygen concentration was also likely responsible for the differences in the June 2007 versus June 2008 mean

SOC rates, because the overlying water initial DO levels in 2007 were greater than 2008 even with core aeration in 2008 (2007: 133 mmol m^{-3} versus 2008: 81 mmol m^{-3}).

Oxygen limitation was probably more of a factor affecting the SOC rates than the sediment characteristics (as also seen in Murrell & Lehrter 2011) because the SOC rates were not affected by the significantly higher *in situ* sediment TOC at C6B (2.05% ±0.05 std. err.) than C4 (1.22% ±0.13 std. err., $F_{1, 20} = 41.62$, $p = 0.001$) (Chapter 2). I initially included a station in deeper water with sandier and lower TOC sediments (< 25% mud, ~ 0.6% TOC) but was not able to collect deep-enough sediment cores for incubation. Whether the SOC rate would have been lower (Grant et al. 1991) because of lower TOC or higher because the carbon available in sandy sediments can have higher degradability than finer sediments (Boudreau et al. 2001, Middelburg et al. 2005), remains unknown. The rates I present are within range of the initial SOC rates from the Louisiana shelf measured in the 1990s and within ~100 km west of the Mississippi River (Dortch et al. 1994, Miller-Way et al. 1994, Morse & Rowe 1999, Rowe et al. 2002). I would expect the current rates to be higher due to the increased accumulation of carbon in the sediments west of the Mississippi River (Turner et al. 2004, 2008). However, I did not sample often in the spring when there is higher carbon flux (Redalje et al. 1994) and higher bottom-water DO concentrations (Rabalais et al. 2007a), which would support higher oxygen consumption rates.

I found that the BWOC to be dependent on the initial incubation oxygen concentrations which differed from Murrell & Lehrter (2011) who reported a higher mean initial DO concentration (e.g., 107 mmol m^{-3}) than this study (e.g., 68 mmol m^{-3}). The lower DO concentrations in this study might have produced a limitation for BWOC. Alternatively the incubation methodology (smaller volume BOD bottles in the former study vs. larger volume acrylic containers with a stir bar in this study might have produced different oxygen consumption rates. The range and average BWOC rates (units of mmol m^{-3} d^{-1}) for this study tended to be higher (as much as 10 times) than past studies that used BOD bottles for incubations (for review see Table 4 in Murrell & Lehrter 2011).

This study also differs from others in that I focused on monthly and interannual variability at two stations within a frequently hypoxic area (e.g., > 75% hypoxic in mid-July from 1985-2008 (Fig. 4.1, Chapter 2, updated from Rabalais et al. 2002b) rather than focusing within the hypoxia season or an ad hoc date/geographic location based on cruise logistics. This allowed investigation of the seasonal dynamics through the formation and maintenance of hypoxia. For example, the higher spring (April and May) carbon flux (Redalje et al. 1994) probably influenced the increase in sediment microbial biomass in my samples. April and May were periods of higher DO levels in the bottom water that correlated with higher BWOC and SOC rates. In late spring/early summer (June-July), the carbon flux and DO levels in the bottom water start decreasing and by the end of the summer (August and September) both of these variables probably became a limiting factor for microbes, as suggested by the significantly lower microbial biomass and SOC rates.

I could not simply compare the mean fluxes between the SOC and BWOC rates because they covaried with the initial oxygen levels. Instead I used slopes of the linear regressions for the comparison. The sediment cores (sediment and bottom water) had a higher slope of 0.14 compared to the bottom-water samples, which had a slope of 0.10 at station C4 and 0.04 at station C6B. The higher bottom-water oxygen demand at a shallower site (C4, ~ 14 m) may result from more resuspension of sediments causing a higher respiration demand of bottom water. Also, phytodetritus may also have less time to be mineralized through the shallower water column (compared to station C6B, 20 m) and could potentially increase respiratory demand of the bottom water. One way to estimate the amount of oxygen being consumed by only the sediment is to subtract the two slopes. The sediment-only oxygen consumption slopes were estimated to be about 0.04 and 0.10, or ~ 29% at station C4 and ~ 71% at station C6B, respectively, of the total oxygen consumption (sediment + bottom water). These values (SOC and BWOC) for both stations were below Quiñones-Rivera et al. (2007, 2010) summer (May – October) average estimate of ~ 75% (range of 61-81%) for the portion of oxygen consumed by benthic respiration. They also found that the, the relative contribution of the benthic respiration to be about 42%

during winter (November-April). Using the total integrated water column approach, Murrell & Lehrter (2011) found that sediment consumed about 20% of the total below-pycnocline respiration from April to September. My results agree with theirs when I did the same type of calculation, but I used the simple linear regression function of the SOC and BWOC and a higher DO concentration (200 mmol O_2 m^{-3}). The sediment accounted for 20% of the total with a mean below-pycnocline depth at C6B of 10 m and 18% of the total at station C4, with a mean below-pycnocline depth of 6 m. These are the highest values possible because SOC is negatively correlated with DO, and the sediment percent contribution decreased with lower oxygen concentrations. By using the same calculation methodology, but only using a water column depth of 1 m (similar to Quiñones-Rivera et al. 2007) and at similar high DO concentrations, I found that the sediment accounts for 58% (C4) and 71% (C6B) of the total oxygen consumption. Therefore, my estimates of the percent contributions of the sediment and water oxygen consumption agree with the past studies, but were dependent on the depth of the overlying water used in the calculations. The SOC rates commonly include the bottom water and the sediment. If I subtract the BWOC rates from the SOC rates to estimate sediment-only proportions, then the highest percent sediment-only contribution for C4 (6 m below-pycnocline depth) = 5% and C6B (10 m below-pycnocline depth) = 12%. These sediment-only contributions are lower because I estimated higher BWOC rates than previous studies. Lastly, Eldridge & Morse (2008) found that the sediment initially consumes about 60% of the oxygen which drives the development of hypoxia and then accounts for about 40% of the consumption for the remainder of the summer due to oxygen limitation of aerobic respiration. At first glance, all of these studies suggest there is little consistency among estimates for the contribution of sediment to the total oxygen consumption. Comparing them remains complicated due to the multiple methods for calculating the oxygen consumption rates and for integrating the rates over dissimilar water depths.

Using one oxygen consumption rate for the sediment or bottom-water may erroneously lead to an over- or under-estimation of oxygen consumption that produces and maintains the hypoxic area in the

northern Gulf of Mexico. From my results, both a linear and logarithmic fit accounted for the same variability (linear, r^2 = 0.87 and logarithmic r^2 = 0.86); therefore, it is difficult to determine which one would be better for modeling studies. However, linear regressions tend to be used for simplicity purposes. I recommend that a function that accounts for the covariance of the oxygen concentrations, like the one I found for both the sediment and bottom water, may help fine-tune models for better hypoxia modeling and to predtict hypoxia formation.

Nutrient Concentrations and Fluxes

The *in situ* nutrient concentrations for 2008 in the bottom water followed a similar trend to other areas that experience nutrient regeneration under hypoxic conditions (Childs et al. 2002, Conley et al. 2007). The bottom-water concentrations of Si, NH4, and PO4 increased from April through July 2008 at both stations most likely due to the increased release of nutrients from organic matter mineralization and also due to the reduced mixing of the water column when stratification occurs. Rabalais and Turner (2006) also found the highest concentrations of Si, NH4 and PO4 in overlying bottom water under hypoxic conditions. Nutrient concentrations were lower in the last month (August) of 2008 which was likely due to a mixing event (Tropical Storm Edouard, 5 and 6 Aug 2008) that diluted the bottom-water nutrient concentrations. The physical activity also added oxygen to the bottom water since DO concentrations were above 2 mg l^{-1} during this mixing event (continuous bottom-water oxygen monitoring at nearby station C6C, N.N. Rabalais unpubl. data).

The NO3+NO2 concentrations at both stations decreased during the hypoxic months of June, July and August, probably because nitrate was being used as the next available terminal electron acceptor for denitrification in the sediments. The NO3+NO2 fluxes were the only inorganic nutrient fluxes affected by the intentional bottom-water oxygenation of the sediment cores in June and July 2008. Miller-Way et al. (1994) also documented that nutrient uptake and regeneration in the sediment cores were sensitive to oxygenation. By adding DO to the June and July sediment cores I facilitated a NO3 + NO2 source instead of a sink.

Prior to hypoxia, NO3 + NO2 was the dominant inorganic nitrogen constituent while during hypoxia NH4 was dominant, similar to Conley et al. (2007) and McCarthy et al. (2008). Not only could the increase in NH4 concentrations at both stations during summer hypoxia (June – August) be due to increasing NH4 fluxes via organic matter mineralization, but the less efficient nitrification during hypoxia could be responsible (Childs et al. 2002, 2003, Middelburg & Levin 2009). Dissimilatory nitrate reduction to ammonium (DNRA) is another process that could increase the NH4 concentrations in the bottom water during hypoxia (Childs et al. 2002, Karlson et al. 2005, McCarthy et al. 2008; Middelburg & Levin 2009, Lin et al. 2011).

The PO4 and NH4 fluxes from the sediment had the same increasing trend in the transition from normoxic conditions (April and May 2008) to hypoxic (June – August 2008), which was also observed in Danish coastal waters that experience hypoxia (Conley et al. 2007). Because the PO4 fluxes mirror the NH4 flux trend, I suspect that organic carbon mineralization was probably responsible. The change in redox state of the sediment from normoxia to hypoxia can also cause PO4 to be released from iron oxides, thus increasing the flux of PO4 into the bottom water (Kemp et al. 2005, Conley et al. 2007, Middelburg & Levin 2009).

The majority of the mean sediment Si fluxes did not differ between the stations or over time. The mean Si flux at station C6B in May 2008 was significantly lower compared to the rest of the months at C6B. Four of the six C6B May 2008 sediment cores (1,2,5,6) that were SOC flux outliers (see Fig. 4.7) were also Si flux outliers (see Fig. 4.10). Perhaps whatever affected the bacteria and the associated sediment oxygen consumption also affected the microbially-mediated degradation of diatom-containing phytodetritus. It is interesting that this is the only example of where the Si fluxes and SOC rates were related. The Si fluxes throughout 2008 were mostly similar, even when the SOC rates were decreasing due to oxygen limitation. Higher Si fluxes at low DO concentrations might be expected because Si concentrations tend to be higher at low DO concentrations (Rabalais & Turner 2006) but I did not find higher Si fluxes in hypoxia.

The nutrient flux rates were within the range of previous Gulf studies (Miller-Way et al. 1995, Morse & Rowe 1999, Rowe et al. 2002, Lin et al. 2011) despite differences in incubation methods (e.g., static incubated cores, flow through cores or benthic chambers), DO concentrations, seasons sampled, and station location. Increased nutrient fluxes to the water column can enhance primary production in the bottom waters if there is sufficient light or in surface waters if the nutrients diffuse into the waters above the pycnocline. The Si:N:P flux ratio of 14:10:1 into overlying waters during hypoxia would increase the likelihood of phytoplankton production according to the Redfield ratio (16:16:1) as long as sufficient nutrient concentrations were present, which they were (Redfield et al. 1963).

CONCLUSION

The water column oxygen consumption rates and sediment oxygen consumption rates (water + sediment) in an area of frequent summer hypoxia on the Louisiana continental shelf did not differ when experimental chambers were exposed to experimental conditions of *in situ* light and environmental parameters. Instead, the sediment oxygen consumption rates were most closely related to the initial oxygen concentration of the experiment, both linerarly and exponentially. I attribute this result to the *in situ* variability of the microphytobenthos (density and estimated biomass as chlorophyll *a*) and the fluctuation in light levels of the incubations. If photosynthetic production of oxygen from microphytobenthos was present, it was not a sufficient mechanism to offset high respiration rates or chemical oxygen demand in the eutrophic, hypoxic area of the northern Gulf of Mexico.

These results did provide experimental seasonal rates of oxygen dynamics from the northern Gulf of Mexico hypoxic zone. These rates were consistent with other SOC rates from the hypoxic area and a broader geography, and were similar to other studies with regard to relationships with initial DO concentrations. I provided more information on the relative proportion of BWOC and SOC to total oxygen consumption over a seasonal basis, and provided information on nutrient fluxes over a seasonal cycle of hypoxia. These results provide critical process rates and information for coupled biological-dynamic physical models of hypoxia formation in the northern Gulf of Mexico.

The results from these experiments further documents the presence of viable microphytobenthic communities. My oxygen consumption rate results were consistent with other SOC rates from the hypoxic area over a broader geography, and were similar to other studies with regard to relationships with initial DO concentrations. My work also contributes to the increasing, but quite dissimilar, information on the relative proportion of BWOC and SOC to total oxygen consumption. My work provides the only sediment oxygen consumption rates over seasonal and interannual time scales at geographically restricted locations. Further, I provide critical input to three-dimensional, coupled physical and biological models of oxygen dynaimics for this eutrophic and hypoxic continental shelf.

I recommend refinements for future experiments designed to better quantify benthic oxygen production by microphytobenthos as part of oxygen budgets in the northern Gulf of Mexico hypoxic zone. Sediment cores should be incubated for a shorter interval (< 24 hours), perhaps 10 hours to avoid oxygen limitation. Placing cores in environmental chambers might reduce the variability of temperature and light. Also, using artificial light at one consistent level, or multiple experimental levels expected at the seafloor, will help determine whether a specific light treatment produces a variable and measurable response. Microelectrodes that measure gross oxygen production on the sediment surface (e.g., Revsbech et al. 1988) may provide better detection of oxygen evolution at the sediment-water interface Lastly, oxygen production will depend on the presence/biomass of microphytobenthos regardless of the methods used to document oxygen production.

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CHAPTER 5

SUMMARY

The purpose of this dissertation was to determine if microphytobenthos were present in sufficient quantity to affect oxygen dynamics in the seasonally hypoxic continental shelf of the northern Gulf of Mexico influenced by the Mississippi and Atchafalaya rivers. My first goal was to document the microphytobenthos community seasonally at three stations (C4, C6B and C8) along the C transect ~ 100 km west of the Mississippi River, and also over a broader geography (transects A-K) along the 14-20 m depth contour in summer, and to determine if they differed from the water-column phytoplankton community. Another goal was to examine the environmental conditions that affect microphytobenthos density and biomass. Lastly, I incubated sediment cores in the light and dark to measure rates of oxygen production from benthic photosynthesis as a component of overall oxygen dynamics in the hypoxic area.

In Chapter 1, I provided an introduction to the environmental issue of bottom-water hypoxia and explained the potential importance of microphytobenthos and their impact on bottom-water hypoxia dynamics via photosynthetic production of oxygen.

In Chapter 2, my observations from samples at stations C4, C6B and C8 that were taken between June 2006 to July 2008 indicated that the sediment community (cells > 3 μm) differed from those in the water column and were primarily benthic (58-88%), representing microphytobenthos. Settled pelagic phytoplankton (1-36%) and tychopelagic phytoplankton (5-10%) were also present. The settled phytoplankton were mostly present on the sediment during fall and winter. The abundance of microphytobenthos was positively correlated with light levels on the seafloor and sediment chlorophyll *a* values. Picocyanobacteria, pennate diatoms and filamentous cyanobacteria dominated the sediment community (by density for all cells, sizes between 0.2 – 8.0 μm) during the summer.

In Chapter 3, I investigated the distribution of microphytobenthos along a 14-20 m contour on the continental shelf in late-July 2006, 2007, and 2008. The typical sediment chlorophyll *a* concentrations were less than 1.0 μg g dry sed^{-1}, with the highest mean concentration at a sandier mid-

shelf area, and the lowest mean concentration at a station near the Mississippi River delta. Benthic cells (> 3 μm) were present at all stations (mean of total cells = 67%), but their density varied between 1 – 99%. Pelagic and tychopelagic phytoplankton cells contributed to the remainder of the total cell density (> 3 μm) in the sediments. Benthic diatoms (*Nitzschia*, *Gyrosigma*, *Pleurosigma* and *Bacillaria)* and benthic cyanobacteria (filamentous and colonial) were common (> 3 μm cell size) on the sediment surface. The variability of sediment chlorophyll *a* concentrations and density of microphytobenthos were influenced by seafloor PAR and bottom-water nutrient concentrations.

Microphytobenthos were present on the sediment surface (commonly < 2.0×10^4 cells g dry sed^{-1}) at stations C4 and C6B, indicating the potential for photosynthesis. I did not detect a significant response to light (0.5 -11% of surface PAR) on the bottom water oxygen consumption rates or sediment oxygen consumption rates (water + sediment) of paired light and dark of the inclubated sediment cores (Chapter 4). I attributed this result to the natural variability of the microphytobenthos, and to the variable light treatments of the experiments, which made it difficult to measure a response. These experiments, however, supplemented existing information on oxygen consumption in bottom waters and sediments of the northern Gulf of Mexico. In particular, my experiments provided the first seasonal data for an area that frequently experiences summertime hypoxia.. I found that the sediment (1.17 - 27.27 mmol O_2 m^{-2} d^{-1}, n = 97) and bottom-water (1.13 - 17.48 mmol m^{-2} d^{-1}, n = 23) oxygen consumption rates were dependent on the oxygen concentrations at the beginning of the experiments, as in other studies. Some of the benthic nutrient fluxes (e.g., NH4, NO3+NO2, PO4) increased from prior normoxic conditions after a long exposure to low oxygen. Fig. 5.1 provides a conceptual model that summarizes these results.

From this dissertation research, I documented that 1) microphytobenthos were present on the sediment surface; 2) they differed from the phytoplankton communities in the water column, and 3) they were positively affected by the amount of seafloor PAR. The light climate on the seafloor of the Louisiana continental shelf was variable, spatially and temporally, which affected the density and

estimated biomass (as chlorophyll a) of microphytbenthos. Future research on continuous seafloor PAR values seasonally and geographically would help ecologists better understand the seafloor light climate, and thus, the significance of microphytobenthos to photosynthetic oxygen production.

If eutrophication worsens in the future due to increasing phytoplankton blooms, then the amount of PAR reaching the seafloor may decrease and limit the density and biomass of microphytobenthos. On the other hand, if nutrients from the watershed are mitigated, surface primary production may decrease and the subsequent PAR reaching to seafloor might increase. In this scnario, microphytobenthos may be more important in contributing to the oxygen pool of the northern Gulf of Mexico. The 2008 Action Plan for reducing, and mitigating and controlling hypoxia in the northern Gulf of Mexico and improving water quality in the Mississippi River basin (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force, 2008) recommends a a reduction in nutrients in the Mississippi River watershed in order to decrease the high primary production that fuels hypoxia. The U.S. EPA Science Advisory Board (USEPA 2008) further recommends a 45% reduction in nitrogen and phosphorus from current levels to reach the 2001 and 2008 Action Plan goal of a mid-summer area of hypoxia less than 5000 km^2 over a 5-year running average by the year 2015. The suspended sediment loads to the Gulf decreased by 50% since the completion of dams and other engineering activities on the the Missouri River in the 1950s (Meade & Moody 2010). A continued reduced suspended sediment load combined with reduced phytoplankton biomass may allow more light to the seafloor, resulting in higher microphytobenthos biomass and density. Oxygen evolution from benthic photosynthesis could be a positive feedback to this ecosystem if nutrient loads decrease to the northern Gulf of Mexico.

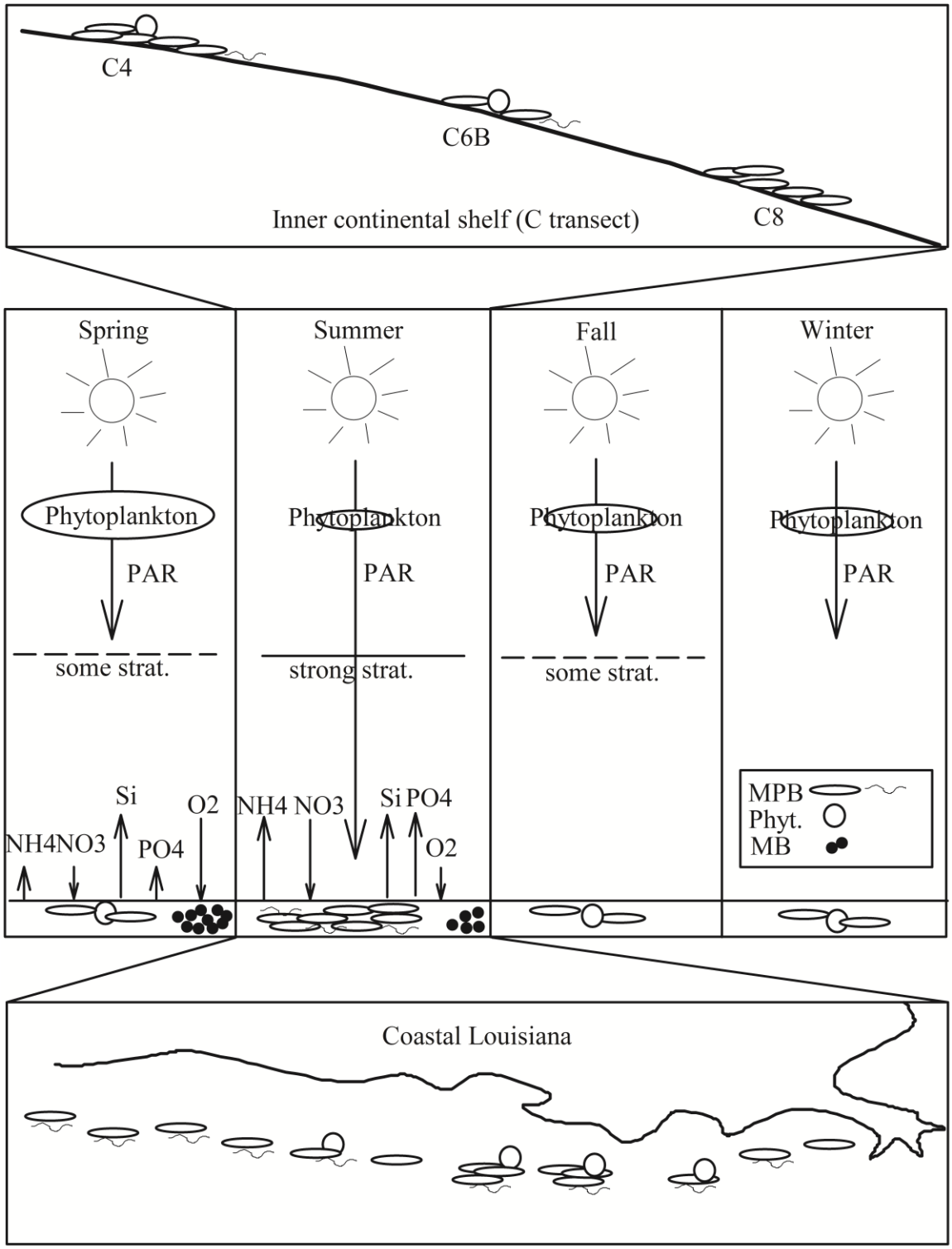

Fig. 5.1. Conceptual model that summarizes the results from my dissertation research on the presence of microphytobenthos and their role in oxygen dynamics on the Louisiana continental shelf. Each block represents a season. PAR = photosynthetically available radiation, strat. = stratification, MPB = microphytobenthos, Phyt. = settled phytoplankton, MB = sediment microbial biomass, NH4 = ammonium flux, NO3 = nitrate and nitrite flux, Si = silicate flux, PO4= phosphate flux, O2 = oxygen flux. The length of arrow represents higher values and arrow head shows direction. Sediment processes and microbial biomass were not measured in fall and winter.

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APPENDIX A

EXTRACTION AND IDENTIFICATION OF SUBTIDAL SOFT-SEDIMENT MICROPHYTOBENTHOS

Method developed by: Melissa M. Baustian and Wendy L. Morrison at the Louisiana Universities Marine Consortium (LUMCON), Cocodrie, LA.

SAMPLING OF MICROPHYTOBENTHOS

1. Collect sediment by using a box core or multi-corer.
2. Insert an acrylic core (e.g., 7.2 cm ID) the box core and remove.
3. Slice off the top 0.5 cm of the sediment core by using a precision core extruder.
4. Place ~ 17 ml of the homogenized sediment slurry into a bottle with 1 ml gluteraldehyde (50%) and add up to 100 ml of filtered (0.2 μm) salt water for preservation.
5. Keep samples refrigerated and in the dark until further processing.

EXTRACTION OF MICROPHYTOBENTHOS FROM SEDIMENT

The following procedures separate microphytobenthos from both fine and coarse sediment. These procedures were developed to extract all possible autotrophs, not just diatoms, including microautotrophs of benthic and pelagic origin. This method is also meant for samples that will be identified and enumerated with an epifluorescence compound microscope. Figure A.1 is a flow chart of the procedures.

Supplies Needed

1. Personal protection gear (laboratory coat, gloves and goggles)
2. Gluteraldehyde preserved sediment samples
3. 15 ml and 50 ml centrifuge tubes (with conical tips)
4. Pipette (1 ml - 5 ml)
5. Distilled and deionized water (DDW) in squirt bottles
6. Vortex (high setting)

7. Ludox® (HS - 40, density = 1.3 g ml^{-1} at 25°C, Sigma - Aldrich) (Warning: Do not allow Ludox® to dry because the fine dust can be toxic if inhaled)
8. Timer (30 seconds and 1 minute intervals)
9. Ring stand with two clamps
10. Sieves (mesh size 10, 35, and 63 μm, made with PVC pipe fittings)
11. Water bath (to rinse Ludox® from sieves and beakers)
12. 50 ml syringes (with a glass tube and short length Tygon tubing as the connector)
13. Proflavin hemi-sulfate stain (0.03%) (one drop per 25ml of solution)
14. Microscope slides (2 per sample)
15. Cover slips
16. Low viscosity microscope immersion oil
17. Filters (0.2, 3, and 8 μm polycarbonate filters, PCF)
18. Vacuum pump
19. Centrifuge (2500 and 4000 RPM)
20. Glass beakers (labeled: "final 63μm", "final 35 μm+10 μm", "63 μm", "10 μm" and "35 μm")
21. Aluminum weighing boats

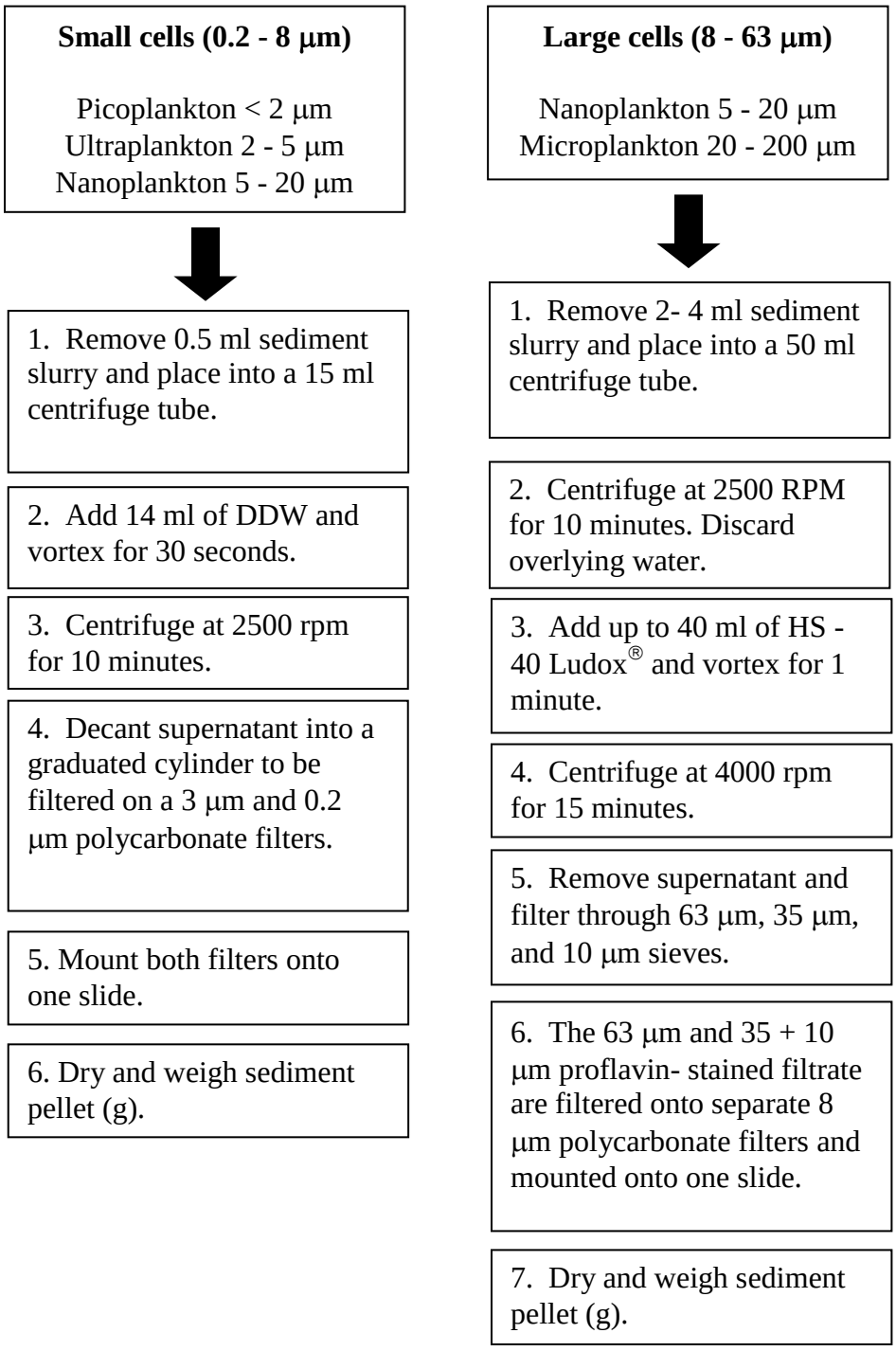

Fig. A.1. A flow chart describing the steps to remove small cells (0.2 - 8 μm) and large cells (8 - 63 μm) from gluteraldehyde-preserved sediment samples that will be examined on an epifluorescence microscope.

Removal of Small (0.2 - 8.0 μm) Autotrophs (e.g. picocyanobacteria and flagellates)

1. Shake bottle vigorously and remove 0.5 ml of sediment slurry from middle of the bottle.
2. Place sample into a 15 ml centrifuge tube
3. Add DDW up to 14 ml and cap
4. Vortex for 30 seconds
5. Centrifuge for 10 minutes at 2500 RPM
6. Remove supernatant immediately to prevent cells from sinking into sediment. Dispense into a labeled beaker. Multiple samples can be done at a time depending on the number of samples the centrifuge holds.
7. On a stacked filtering system (PCF filters) with 3 μm above and 0.2 μm below, add the supernatant. Use vacuum suction to pull solution through both filters.
8. Place filters onto one labeled slide.
9. Dispense the sediment into a pre-weighed aluminum weighing boat with DDW. Vortexing prior to dispensing may ease removal of sediment from centrifuge tube.

   By treating the sediment pellet once with DDW, a mean of 32% ± 5 (n = 12, 95% confidence interval) of small autotrophs are removed (Fig. A.2).

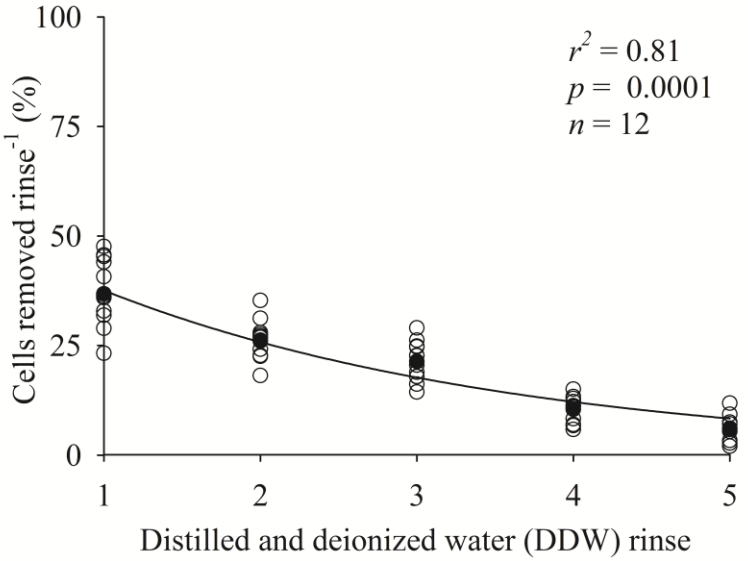

Fig. A.2. The number of small autotrophic cells (size range 0.2 - 8.0 μm) removed after five rinses with DDW. The percentiles were calculated from the average of the 12 samples (C4 = 3, C6B = 6, and C8 = 3). The first rinse removed a mean of 32% ± 5 (95% CI) of small autotrophs. All cells were assumed to be removed by 10 rinses as suggested by preliminary counts that had few cells (e.g. 8 - 30) remaining at the 10th rinse. To save time and costs I only counted 5 rinses for 12 samples involved in the methodology development. The line represents the modeled exponential decay of cells removed.

Removal of Large (8 - 63 μm) Autotrophs (e.g. diatoms)

This method was modified from Blanchard et al. (1988) and Totti (2003).

1. Shake bottle vigorously and remove 2 - 4 ml of sediment slurry from the middle of the bottle. About ~ 2 ml of sediment slurry was removed if the sediment was known to have higher chlorophyll *a* levels and/or fine sediment content.
2. Dispense sample into a 50 ml centrifuge tube and cap.
3. Centrifuge for 10 minutes at 2500 RPM to remove the water from the sample and to prevent dilution of the Ludox®. Discard the overlying water. Microscopic analysis verified no autotrophic cells are present therefore the overlying water can be discarded.
4. Vortex pellet to loosen sediment and add 40 ml of HS - 40 Ludox®.
5. Vortex for 1 minute (use a timer to be consistent) and invert periodically.
6. Centrifuge for 15 minutes at 4000 RPM

7. Remove the supernatant from the tubes with a 50 ml syringe-glass tube apparatus. Use the ring stand to help in this process. The top clamp holds the syringe and bottom clamp holds the centrifuge tube.
8. To remove the large organic matter material (sediment, infauna, etc) place a labeled "63 μm" beaker under a 63 μm sieve and dispense solution from the syringe into the sieve. Microscopic analysis verified large organic matter material was caught on this mesh size which prevented interference of later identification of the smaller autotrophic cells.
9. Rinse the 63 μm particulates caught on the sieve into the labeled beaker "Final 63 μm", add 1 drop of Proflavin (per 25 ml of solution) and filter on 8 μm. Place the filter onto a labeled microscope slide.
10. Place the labeled "35 μm" beaker under the 35 μm sieve and pour the "63 μm" filtrate solution through a 35 μm sieve.
11. Rinse the 35 μm mesh into the beaker labeled "Final 35 μm + 10 μm"
12. Place the labeled "10 μm" beaker under the 10 μm sieve and pour the "35μm" solution onto the sieve. Discard the filtrate from the "10 μm" beaker into a Ludox® waste container.
13. Rinse the 10 μm sieve into a beaker labeled "Final 35 μm + 10 μm"
14. Add 1 drop of proflavin per 25 ml of the "Final 35 μm + 10 μm" solution and pour onto an 8 μm filter. Use a vacuum pump to assist with filtering. Place the filter alongside the "Final 63 μm" filter on the microscope slide.
15. Place the sieves and beakers in a water bath immediately to prevent Ludox® from drying and rinse them to prepare for the next sample.
16. Once the Ludox® has been removed from the sediment pellet dispense into a pre-weighed aluminum boat, dry and weigh to obtain the dry mass (g).

By treating the sediment pellet once with Ludox®, the majority (70% ± 7, n = 11, 95% confidence interval) of the large autotrophs are removed (Figure A.3).

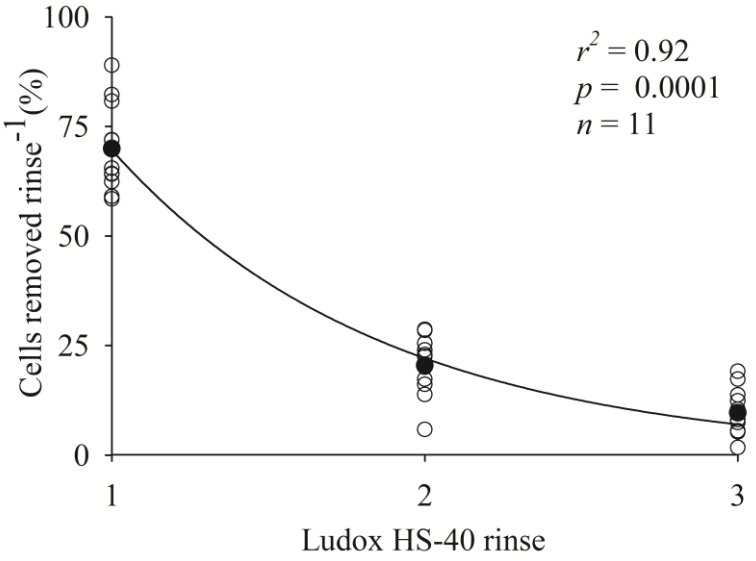

Fig. A.3. The removal of large cells (8 - 63 μm) from sediment samples with Ludox® treatments. The percentiles were calculated from the average of the 11 samples (C4 = 2, C6B = 3, and C8 = 6). One rinse with Ludox® removed the majority of the cells (mean = 70 ± 7, 95% CI). All cells were assumed to be removed by 3 rinses as suggested by preliminary counts, which had few cells (e.g. 2 - 50) remaining at the 3rd rinse. To save time and costs involved in the methodology development only 3 rinses were conducted. The line represents the modeled exponential decay of cells removed.

MICROPHYTOBENTHOS IDENTIFICATION AND ENUMERATION:

This method was adapted from Dortch et al. (1997) and Dortch et al. (1998).

0.2 and 3.0 μm Filters (small size fraction)

1. The 0.2 and 3.0 μm filters need to be identified soon (no more than a week) after extraction because the pigments degrade quickly.
2. At 1000 × magnification identify and count all organisms on the 0.2 and 3.0 μm filters until 100 cells or 100 fields are reached with an epifluorescence microscope (EFM) that includes blue and green excitation light.
3. Slided can be properly disposed once they have been counted.
4. A correction factor is needed because only 33% of the cells were removed during the first rinse (Fig. A.2). The following equation was applied to the initial counts collected from the first rinse in order to calculate an approximate total cell density (cells dry sed $^{-1}$):

   Corrected Cell Density$_{0.2 \text{ and } 3.0 \mu m}$ = (Cell Density$_{\text{initial counts}}$) / (0.33$_{\text{fraction removed}}$)

8.0 μm Filters (large size fraction)

1. Keep 8 μm slides in slide box and frozen until ready for identification and enumeration.
2. Remove slides and allow thawing. At 200 × identify and count all organisms until 100 cells or 100 fields are reached with an epifluorescence microscope (EFM) with blue and green excitation light and transmitted light. Identify and count organisms at 100 × that were not seen at the 200 × count on half the filter. This step allows for identification of large rare species.
3. A similar method (e.g., counting 100 cells) was employed to be able to compare sediment samples to water samples and verified by calculating the coefficients of variation (CV) after 10 cell intervals until 150 cells were reached. The mean CV (calculated over all species) leveled off after 70 cells (± 18, 95% CI), which means the species variablity of the sample was not changing after 70 cells were counted. The mean CV was calculated by evaluating four samples (C4 8/16/06, C8 8/16/06, C4 1/11/07 and C8 1/11/07) that had a combination of low and high abundances and were sampled in different seasons. By counting 100 cells on the 8.0 μm sediment slides the compatibility with water samples was reached and the number of cells counted were greater than the mean CV of 70, thus, this method proved appropriate for these sediment samples.
4. A correction factor is needed because 70% of the cells were removed during the first rinse (Fig. A.3). The following equation was applied to the initial counts collected from the first rinse. To calculate an approximate total cell density (cells g dry sed $^{-1}$):

$$\text{Corrected Cell Density}_{8.0\ \mu m} = (\text{Cell Density}_{\text{initial counts}}) / (0.70_{\text{fraction removed}})$$

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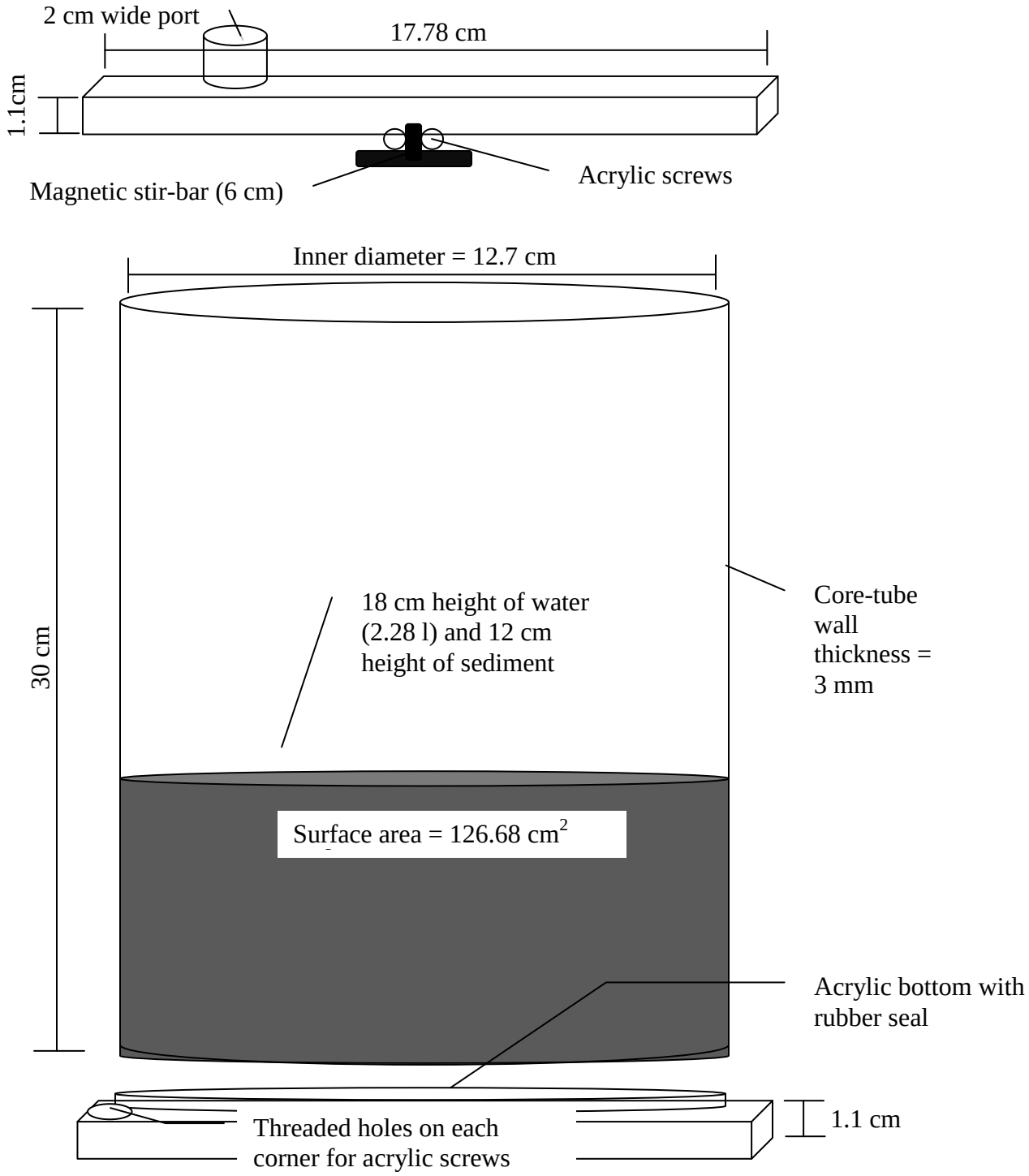
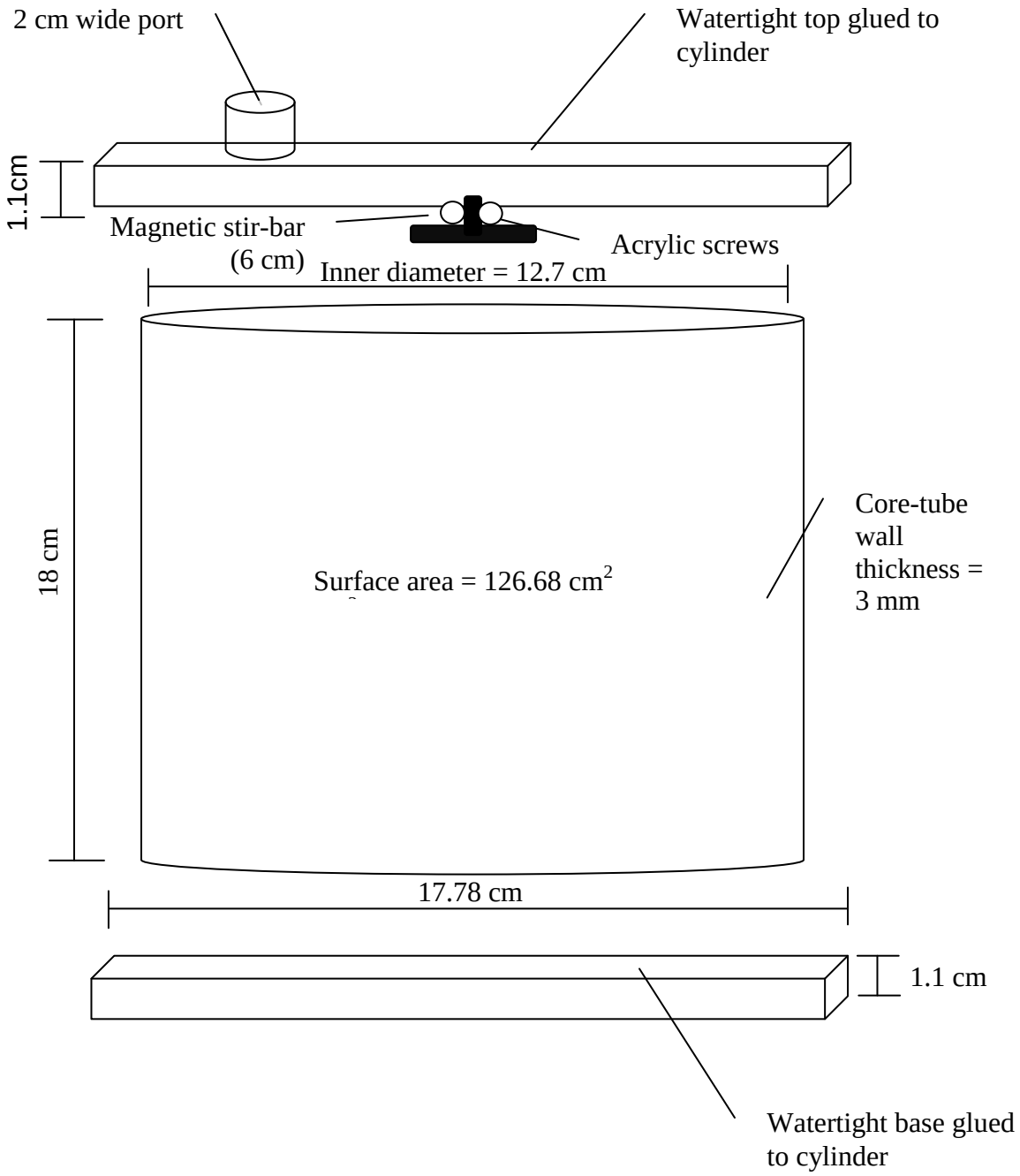
APPENDIX B

DIMENSIONS OF SEDIMENT CORES USED FOR INCUBATION EXPERIMENT



APPENDIX C

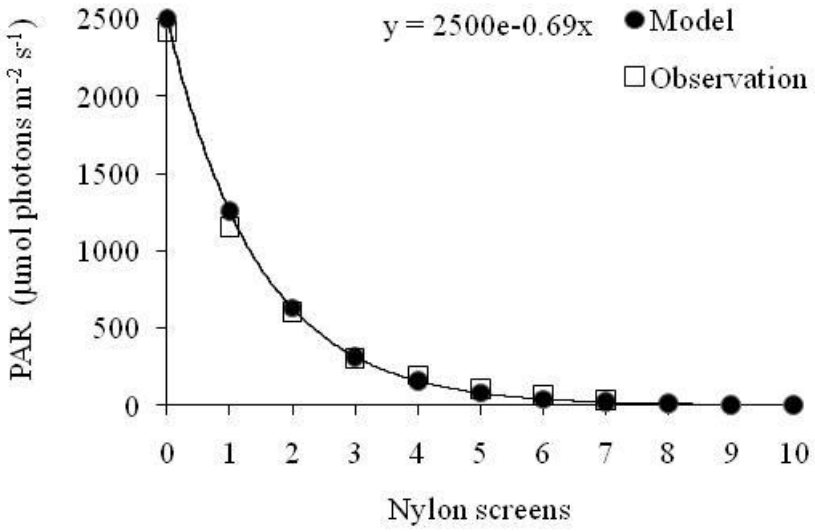
DIMENSIONS OF CONTAINER USED TO INCUBATE BOTTOM WATER



APPENDIX D

LIGHT CURVE USED FOR SEDIMENT CORE INCUBATION EXPERIMENTS

This light curve was developed at LUMCON's dock on 5/9/07 at 4:25 PM using nylon screens and a Li-COR LI-193SA hand held photosynthetically-available radiation (PAR) sensor. An exponential model was used to calculate the number of screens needed to decrease the light in order to simulate *in situ* PAR conditions. Each nylon screen decreases the amount of available light by 49.8%. Data from the quantum radiation measured from the LUMCON weather station (http://weather.lumcon.edu) also helped determine the available PAR reaching the dock during the experiments.



APPENDIX E

SODIUM BICARBONATE STABLE ISOTOPE ($NaH^{13}CO_3$) ADDITIONS TO SEDIMENT CORE INCUBATION EXPERIMENTS

Sodium bicarbonate stable isotope ($NaH^{13}CO_3$) additions were given to sediment cores in June, July and August 2008. $\delta^{13}C$ values higher than about - 22 ‰ background were expected as a result of carbon fixation from photosynthesis. Sediment cores (D, L) from stations C4 and C6B 6/13/08 were incubated for an additional 24 hours with one screen placed on the incubators to allow 50% light, or about a maximum of 1000 μmol photons m^{-2} s^{-1}. D = dark and L = light.

			Overlying water			Sediment		
				Mean $d^{13}C$	Std		Mean $d^{13}C$	Std
Station	Date	Treat.	*n*	(‰)	Err.	*n*	(‰)	Err.
C4	6/13/08	D	2	-16.1	2.6	2	-21.1	0.2
		L	2	-6.3	4.6	2	-21.1	0.4
		50% D	1	-15.5	-	nd	nd	nd
		50% L	1	23.7	-	1	-19.9	-
	7/8/08	D	3	-4.0	2.5	3	-19.4	1.4
		L	3	17.5	1.4	3	-20.7	0.2
	8/16/08	D	3	-1.3	4.8	3	-20.4	0.5
		L	3	14.2	5.0	3	-20.5	0.1
C6B	6/13/08	D	2	-16.5	5.8	2	-20.1	0.9
		L	2	-9.8	2.5	2	-20.7	0.5
		50% D	1	-16.5	-	1	-20.8	-
		50% L	1	-25.8	-	1	-21.7	-
	7/8/08	D	3	-6.1	3.4	3	-20.7	0.3
		L	3	11.0	4.0	3	-20.0	1.1
	8/16/08	D	3	3.1	0.8	3	-21.5	0.3
		L	3	17.8	8.5	3	-21.0	0.2

APPENDIX F

A COPY OF LETTER REQUESTING AND GRANTING PERMISSION FOR PEER-REVIEWED PUBLICATION OF CHAPTER TWO

Gmail by Google

Melissa Baustian <melissa.m.baustian@gmail.com>

Re: Ms. No. 201011107, M 9262 for MEPS

Marianne Hiller <marianne@int-res.com> **Thu, Jun 23, 2011 at 5:16 AM**
To: Melissa Baustian <melissa.m.baustian@gmail.com>

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Dear Nicole Taros,

I am writing to request permission to have this copyrighted accepted-manuscript to be included in my Ph.D. dissertation.

Please let me know what information you need in order to grant permission.

Thank you!

Sincerely,

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APPENDIX G

GENERAL AUDIENCE EXPLANATION OF DISSERTATION RESEARCH

Low oxygen, or hypoxia, develops in the bottom water off the coast of Louisiana during spring and summer due to the Mississippi and Atchafalaya rivers dumping fresh water containing nutrients (nitrogen and phosphorus) into the Gulf of Mexico. These nutrients promote algal (microscopic plants) growth that can turn the surface water green or golden brown. The algae support a productive food web, but for much of the year the algae are not completely consumed. The dying algae and fecal pellets from zooplankton that consume the algae eventually sink through the water column to the bottom while being decomposed by oxygen-consuming bacteria. The separation of the fresh and less dense riverine water floating on top of the saltier and denser marine water prevents oxygen from diffusing into the bottom water. Hypoxia develops in the lower water column because oxygen consumption by bacteria becomes greater than oxygen production by the algae. Low oxygen in the water threatens the survival of animals such as brown shrimp, blue crabs and red snapper because they need oxygen to breathe. Some of these animals can swim away and find other areas with more oxygen, but the small worms, snails, clams and starfish that cannot flee eventually die, which is why this area is known as the "Dead Zone." Some organisms on the seafloor can use light for photosynthesis and produce oxygen. This produced oxygen at the bottom may offset the oxygen consumed by bacterial consumption. These microscopic oxygen-producing organisms are called microphytobenthos (micro=very small, phyto=plant, benthos=bottom dwelling). There are few studies about them and their oxygen-producing ability in the hypoxic area of the northern Gulf of Mexico. The goal of my dissertation project was to determine the community composition of microphytobenthos and the environmental conditions that affect them. I sampled for three years (2006 – 2008), every other month, at three stations located approximately 100 km west of the Mississippi River delta, and once each year in late-July at several stations in 14 to 20 m water depth along most of the Louisiana continental shelf. I took sediment samples from the seafloor and incubated them in natural light conditions that are typical offshore to determine if the microphytobenthos were

generating oxygen through photosynthesis. I found that microphytobenthos were present on the seafloor and became more common in the summer when seafloor light levels were highest. I did not detect any oxygen production from the incubated cores, probably because of the low number of microphytobenthos on the sediment surface and also because of the cloudy days that reduced the outdoor light during my experiments. Overall, my research contributes new information about the ecology and composition of microphytobenthos on the seafloor, and oxygen consumption rates especially in areas with bottom-water hypoxia.

VITA

Melissa Millman Baustian grew up in St. Donatus, an eastern Iowa small town that is nestled between the beautiful rolling hills and the mighty Mississippi River. She attended Dubuque Senior High School and graduated in May 1999. She enrolled at Iowa State University (ISU) in the fall of 1999 to pursue studies in biology and aquatic ecology. While attending ISU she participated in marine science summer courses at Gulf Coast Research Laboratory in 2000, worked as an undergraduate research assistant in the Iowa State University Limnology Laboratory from 2001-2003, and was granted a National Science Foundation Research Experience for Undergraduates at the Marine Biological Laboratory in Woods Hole, Massachusetts, during summer 2002. At Woods Hole, she conducted research on the effects of land-derived nitrogen loads on estuarine shrimp populations. During her undergraduate program, Ms. Baustian was an active participant in the Beta, Beta, Beta Biological Honor Society, Mortar Board Honor Society and the Marine Biology Club, which she helped co-found at ISU. She was honored with the "Outstanding Upperclassman Award" from the ISU Biology/Zoology/Genetics Club in fall 2002 and an "Outstanding Undergraduate Student Award" by the Iowa Water Resources Research Institute in spring 2003, which is given to only one undergraduate in the state of Iowa per year. In May 2003, she graduated with a Bachelor of Science degree in biology. Soon after graduating, she moved to Louisiana in summer 2003 to work with Dr. Nancy Rabalais at Louisiana Universities Marine Consortium (LUMCON) and began collecting samples on her first oceanographic research cruise. In the fall of 2003 she began her Master of Science program at Louisiana State University (LSU) in the Department of Oceanography and Coastal Sciences where she examined the effects of hypoxia on benthic macroinfauna which are potential prey for demersal fish predators. While a master's student she was the vice president of the graduate student organization, Marine Environmental Researchers, and led a team that organized the first LSU-sponsored Graduate Student Symposium at LUMCON. She received her Master of Science degree in August 2005 and soon after started her doctoral studies in the same department with the same advisor. Her doctoral project continued with the topic of benthic ecology in

the hypoxic area, but at a smaller scale, by examining microphytobenthos, the benthic microalgae and cyanobacteria. While a doctoral student, she was active in science education by being a teaching assistant at LUMCON, guest lecturer at LSU, volunteer scientist at teacher workshops and coordinator of the high school student mentoring program, EnvironMentors. During her doctoral studies she received the SEASPACE scholarship for academic excellence in spring 2006, the Dr. Theodore Ford Memorial doctoral scholarship in fall 2008, and the Lipsey Foundation doctoral scholarship in "Recognition of her contribution to the better understanding and preserving of Louisiana's living coastal and marine resources" in spring 2009. She will be receiving her Doctor of Philosophy degree from the Department of Oceanography and Coastal Sciences with a minor in science education on August 5, 2011.