BENTHIC PRIMARY PRODUCTION IN COASTAL SALT MARSH SYSTEMS

by

LILIANA EUGENIA VELASQUEZ

(Under the Direction of Samantha B. Joye)

ABSTRACT

Benthic microalgae are a central component of shallow coastal habitats and they may account for a significant fraction of the total primary production in these ecosystems. While several factors act in concert to generate the high photosynthetic rates observed in benthic microalgae, available data suggest light is of primary importance. Nutrient availability may also be important because increasing human population in the coastal zone has led to an oversupply of nutrients to aquatic habitats, in particular estuaries. The present study focused on the effects of light and nutrients on benthic primary production at two coastal Georgia sites: the Duplin River and the Satilla River. Benthic primary production and biomass along the Duplin varied over space and time. Nutrient addition experiments led to increased primary production by benthic microalgae (indicating nutrient limitation) with the addition of nitrogen and phosphorus at Sapelo Island but not at the Satilla River site.

INDEX WORDS: Benthic Microalgae, Primary Production, Biomass, Duplin River, Satilla River, Nutrient Addition

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DEDICATION

I dedicate this thesis to my loving, supportive husband Jamie, who not only had to put up with me while I was under the stress of finishing my writing and passing my defense but also had to tolerate my rampant hormones due to my pregnancy. To our new baby Parker Javier who has taught me a new meaning of love. To my parents Jesus and Elssy and Brother Juan who have supported me in everything I have set out to accomplish in my life.

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INTRODUCTION

Through the collective activity of benthic microalgae, marsh plants, submerged macrophytes and phytoplankton, salt marsh ecosystems are some of the most productive in the world (Valiela 1984, Söderbaum 1996). These various primary producers fix carbon over a wide area throughout the year. Benthic microalgal production is often comparable to that of marsh plants (Pinckney and Zingmark 1993a) or water column plankton (Cadée and Hegeman 1974), despite the fact that they inhabit only the upper few millimeters of sediments. The term "benthic microalgae" refers to a diverse assemblage of microscopic photosynthetic autotrophs that inhabit surficial sediments in aquatic habitats. Benthic microalgae inhabit fresh and marine sediments at depths where waters are clear enough or shallow enough to allow sufficient light to reach the sediment surface. The light required to support benthic primary production is approximately 1% of the surface incident radiation (Cahoon and Cooke 1992). This minimum light intensity may be reached at depths of even 70-75m in clear shelf waters, as was shown on the continental shelf of the South Atlantic Bight (Nelson et al. 1999). Habitats supporting benthic production include streams, shallow lakes, wetlands and shallow coastal waters including estuaries and the continental shelf (Cahoon and Cooke 1992, Dodds et al. 1999). The benthic microalgal community is often dominated by diatoms (Pomeroy 1959, Williams 1962, Pinckney et al. 1994). In estuaries benthic microalgae may inhabit sandy beaches, intertidal mudflats, shallow subtidal substrates, and the understory of marsh grass (Pinckney and Zingmark 1993b). Benthic microalgae may account for more than half of the system-wide primary production in estuarine

and coastal systems (Laursen et al. 2002), can affect sediment stability (by the production of complex mucus structures) (Miller et al. 1996) and are important components of nutrient cycles (Rizzo et al. 1992). Their importance to coastal systems is indisputable and accordingly, benthic microalgal photosynthetic production should be quantified and controls on the process evaluated.

Many factors influence benthic microalgal productivity including light, temperature, grazing, vertical migration of microalgae and nutrient availability (Table 1). Of these factors, light is possibly the best understood and is often considered most important (Pomeroy 1959, Van Raalte et al. 1976, Moorhead et al. 1997, Barranguet et al. 1998). The amount of irradiance (intensity of light) reaching the benthic microalgae may vary with season, time of day, cloud cover and *Spartina* cover (Pomeroy 1959, Valiela 1984). *Spartina alterniflora* (smooth cordgrass) is the dominant macrophyte of intertidal marshes of the southeast United States. The intertidal marsh may be divided into three zones delineated by light environment: the bank (or mudflat), levee marsh and high marsh (Fig. 1). The intertidal bank is micro-vegetated, occupied by microscopic algae, and receives full illumination at low tide. The levee marsh is occupied by dense, tall *Spartina* (>2m), whereas the high marsh is occupied by sparse, short *Spartina* (~25cm). Due to the *Spartina* cover, the benthic microalgae inhabiting the levee and high marsh sediments receive considerably less light than the benthic microalgae inhabiting the bank sediment. A study of Georgia intertidal marshes reported summer light ranges at the sediment surface of 1600-2000, 300-500 and 300-400 μE for the bank, levee marsh and high marsh respectively, demonstrating the difference in illumination between the micro-vegetated and *Spartina* zones (Whitney and Darley 1983).

Benthic microalgae are able to adapt to diverse light intensities physiologically, principally by varying the amount of primary and accessory pigments (Pinckney et al. 1995).

Photosynthesis is carried out by photosynthetic units comprised of a light collecting antenna of accessory pigments and a reaction center made up of chlorophyll *a* (Valiela 1984). The adaptation to light intensities by the photosynthetic units, termed photoacclimation, may be responsible for observed variations in photosynthetic activity (Barranguet et al. 1998). For example, at low light levels, accessory pigments (which reflect the size of antenna) increase, improving an organism's ability to harvest light (known as photosynthetic efficiency) (Valiela 1984). Full sunlight, on the other hand, may cause photoinhibition or a decrease in photosynthetic rates. Benthic microalgae are capable of maintaining maximum photosynthetic rates over a wide range of light intensities (Rasmussen et al. 1983) suggesting that benthic microalgae are not photoinhibited (Cadée and Hegeman 1974, Van Raalte et al. 1976, Colijn and de Jonge 1984, Blanchard et al. 1997). However, some studies have shown photoinhibition in benthic microalgae (Davis and McIntire 1983, Whitney and Darley 1983).

Table 1. Some factors affecting primary production by benthic microalgae.

Factor	**Site of Study**	**Reference**
Vertical migration of microalgae	Tagus Estuary, Portugal	Brotas et al. 2003
Light, temperature	Westerschelde, Netherlands	Barranguet et al. 1998
Temperature, light, functional chlorophyll *a*	Wadden Sea, Netherlands	Cadée and Hegeman 1974
Nutrients	Swedish west coast	Nilsson et al. 1991
Tidal stage, sun angle	North Inlet Estuary, SC, US	Pinckney and Zingmark 1991
Grazing	Great Sippewissett Marsh, MA, US	Connor et al. 1982

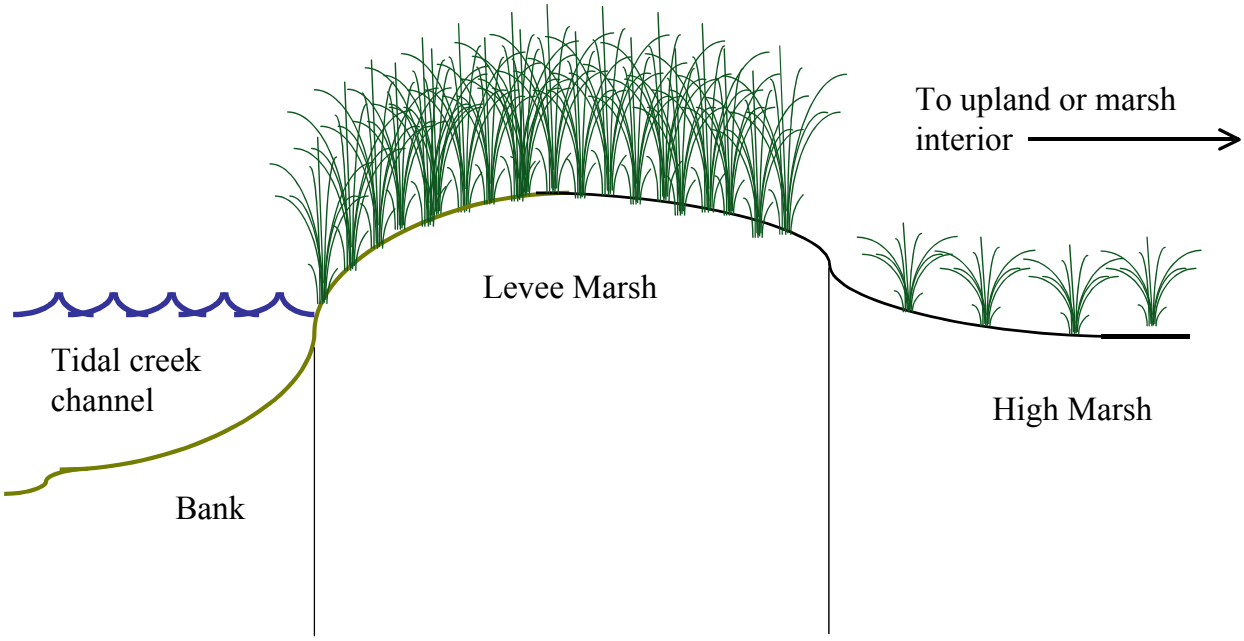

Figure 1. Schematic cross section of the three intertidal zones showing the levee marsh with tall, dense *Spartina* and the high marsh with short, sparse *Spartina*.

The contrast in vegetation cover of the three marsh zones not only results in varying degrees of irradiance, but also in varying temperatures. Temperature of the sediments fluctuates according to season, time of day and plant cover. Although interannual variation in temperature occurs, the highest production values occur generally during summer, associated with increasing temperatures, while lowest values occur in the winter (Davis and McIntire 1983, Colijn and de Jonge 1984, Barranguet et al. 1998). Optimal temperatures for benthic production rates fluctuate depending on the time of year from 15˚C in March to no less than 30˚C in September (Rasmussen et al. 1983). The fluctuating optimal temperatures may be the result of varying enzyme efficiency. For example, increasing temperatures may increase enzyme efficiency thus resulting in higher photosynthetic rates in the warmer months. In the Westerschelde's intertidal

sediments light saturation was correlated with temperature, suggesting temperature regulated benthic primary production (Barranguet et al. 1998). Conversely, in the Great Sippewissett Marsh, no correlation was found between primary production and sediment surface temperature (Van Raalte et al. 1976). The shifting optimal temperatures for benthic production rates along with the opposing results in the Westerschelde and Great Sippewissett Marsh studies may result from the fact that seasonal changes in temperature are closely associated with changes in sunlight (Barranguet et al. 1998). Deciphering whether light or temperature regulates primary production can be difficult and the co-variation of the two factors suggests that temperature may be more important as a covariate, than as an independent factor (Valiela 1984). For instance, high temperatures may favor a particular diatom species (Williams 1962), may facilitate the uptake of nutrients or may increase the performance of enzymes (Valiela 1984). Temperatures favoring the growth of particular diatom species would allow those species to dominate, causing a change in the assemblage's taxonomic composition. These changes in the community species composition may in turn alter the photosynthetic parameters due to physiological differences between species.

Another factor that could contribute to the variability of benthic primary production rates is grazing. Benthic microalgae provide more assimilable food than the higher plants such as *Spartina* (Van Raalte et al. 1976, Miller et al. 1996) and as a result, benthic microalgae are consumed directly by herbivores. Consequently, the primary food source for many meiofauna, juvenile macrofauna, fiddler crabs and snails in intertidal sediments is benthic microalgae. An example of the substantial effects increased grazing pressure can have on a microalgal community is the seasonal reduction in benthic production that occurs after spring blooms (Williams 1962, Pinckney and Zingmark1993a). Grazers may also have a fertilizing effect on

benthic microalgae. For example, grazing by snails may result in increased nitrogen availability to benthic microalgae, thereby stimulating diatom growth (Connor et al. 1982). Snails may also selectively feed on particular diatom species therefore affecting the species composition of the benthic microalgae (Connor et al. 1982). Not only can grazing influence production rates but the activities of the grazers can disturb the sediment on which the benthic microalgae reside and consequently affect productivity (the result of infauna activity is referred to as bioturbation) (Valiela 1984). Bioturbating activities, e.g., burrowing, building feeding pits, or surficial tracking as well as grazing, may contribute to the spatial and temporal variability of benthic microalgal biomass and accordingly, contribute to the variability of photosynthetic rates (Miller et al. 1996, Nelson et al. 1999).

Previous work has demonstrated that another source of variability in photosynthetic rates is vertical migration (Pinckney and Zingmark 1991, Pinckney et al. 1994, Brotas et al. 2003). Many species of benthic microalgae are able to vertically migrate in the sediments. As the benthic microalgae migrate up and down they move in and out of the photic zone (Pinckney et al. 1994, Brotas et al. 2003). The photic zone is the portion of the sediment in which the benthic microalgae are able to photosynthesize and is at most 2-3 mm thick (MacIntyre et al. 1996). Changes in the amount of biomass present in the photic zone lead to variations in community photosynthetic rates (Pinckney and Zingmark 1991). Thus, photosynthetic rates, as well as photosynthetic efficiency, may vary as a function of migratory rhythms (Pinckney et al. 1994). Vertical migration may also ensure protection from herbivory (Pinckney et al. 1994), reduce photoinhibition and desiccation, can facilitate access to nutrients deeper in the sediments (Barranguet 1998) and may prevent resuspension due to tides or wind-induced waves (Miller et al. 1996). Various hypotheses are posed to explain the dynamics of diatom migration patterns,

including tidal and light cycles (Pinckney and Zingmark 1991), salinity, temperature and light changes (Brotas et al. 2003), or temperature, light and tide regimes (Saburova and Polikarpov 2003).

Nutrients, mainly nitrogen and phosphorus but also possibly silica, are considered important factors regulating primary production and thus have been the focus of many benthic primary production studies (Van Raalte et al. 1976, Pinckney et al. 1995, Meyer-Reil and Köster 2000, Laursen et al. 2002, Sundareshwar et al. 2003). When a nutrient becomes limiting, its rate of supply will determine the rate of primary production (Camacho and de Wit 2003). Although some have suggested that nutrients do not limit most species of benthic microalgae due to high rates of remineralization within the sediment (Williams 1962, Nilsson et al 1991), others have shown stimulation of benthic primary production and biomass with the addition of nutrients (Van Raalte et al. 1976, Darley et al. 1981, Pinckney et al. 1995). Nutrients are added to coastal systems by activities such as river run-off, atmospheric deposition and groundwater flow (D'Elia 1987, Cloern 2001, Sundareshwar 2003). Estuaries in particular receive more nutrient inputs per unit surface area than any other type of ecosystem (NRC 1993); although in the vegetated habitats, macroalgae and vascular plants may outcompete the benthic microalgae for nutrients (Van Raalte et al. 1976, MacIntyre et al. 1996). When nutrients are limiting, the addition of nutrients in moderation may be beneficial, as nutrients stimulating benthic primary production may fuel a biomass increase of the higher trophic levels including economically valued species. However, excess nutrient enrichment can become a problem. For instance, excess nitrogen and phosphorus may lead to silicate limitation. Silicate limitation in turn may favor other algae over silica-requiring diatoms (Meyer-Reil and Köster 2000) causing a shift in the composition of the phototrophic community. Most diatoms are considered a good quality food source as compared

to cyanobacteria which include many toxic or inedible species that thrive under nutrient enriched conditions (Paerl et al. 2003). Thus, changes in the phototrophic community may have negative cascading effects on the microfaunal and meiofaunal communities (Pinckney et al. 1995). However, it has been indicated in other studies that diatoms may receive enough silica from the sediment (Pomeroy and Imberger 1981, Nilsson et al. 1991). Other consequences of increased nutrients on benthic communities are increased oxygen consumption (possibly leading to hypoxia or anoxia), decreased penetration depth of oxygen into the sediment and increased concentrations of inorganic and organic substances (Meyer-Reil and Köster 2000).

In summary, benthic primary production is an important component of coastal ecosystem food webs that is poorly constrained in many coastal environments. Although previous research has shown light to be a prevailing environmental control on benthic primary production, other factors are also important. The interactions between these factors are complex and the extent to which they control benthic productivity may differ among sites, studies and on temporal scales. In addition to biological, physical and chemical environmental factors, the benthic microalgae must cope with the consequences of human impacts. Understanding the response of benthic microalgal production to nutrient enrichment has important implications for understanding carbon flows in coastal ecosystems. Benthic microalgae are a vital component of shallow aquatic systems that warrant future and detailed study.

Objectives:

1. To determine benthic microalgal primary production rates and biomass at three intertidal zones in a relatively pristine salt marsh (Moses Hammock, Sapelo Island, Georgia, USA).
2. To determine the effects of added nutrients on benthic microalgal production at two sites, Moses Hammock (Sapelo Island) and Dover Bluff (Satilla River), in coastal Georgia.

Site descriptions (Fig. 2)

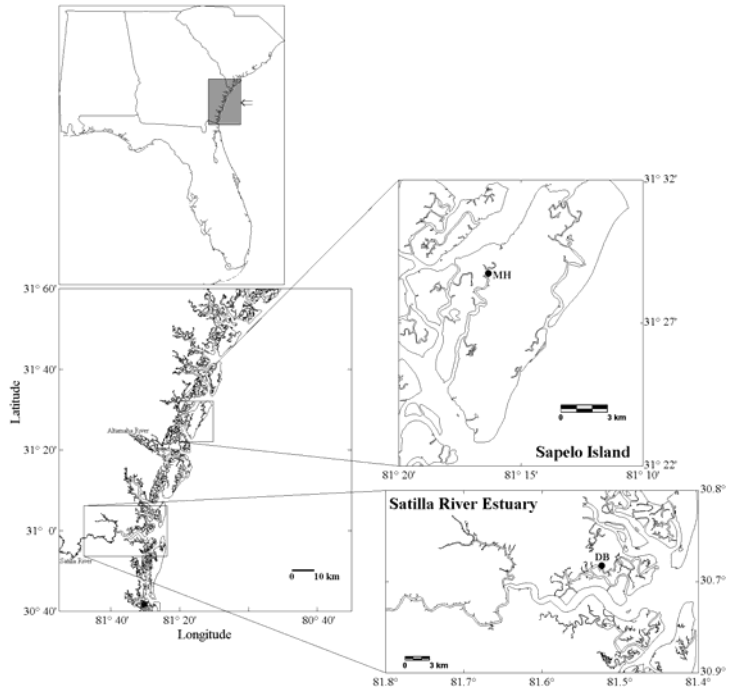

Figure 2. A map showing the locations of the two study sites. The Moses Hammock site lies approximately 40 miles from the Dover Bluff site.

Moses Hammock (MH), Sapelo Island, Georgia

Sapelo Island is a fairly undisturbed undeveloped barrier island off the Georgia coast. The Moses Hammock marsh area is on the west side of Sapelo and is a focus site of the Georgia Coastal Ecosystem Long Term Ecological Research (GCE-LTER) program. The study site borders the Duplin River and is subject to 2-3m diurnal tides. The fine silty sediment is colonized by pennate diatoms that are apparent as a brown biofilm on the sediment surface. Macrovegetation in the marsh is mostly *Spartina alterniflora*.

An advantage of the Sapelo Island study area is the long record of scientific study and ecosystem preservation established by the University of Georgia Marine Institute (UGAMI) in 1954 and continuing to the present. The current study contributes to the knowledge base of the Sapelo Island research and to the LTER data in which primary production is a focus.

Dover Bluff (DB), Georgia

Dover Bluff lies along Umbrella Creek, a tributary of the Satilla River. A residential development lies adjacent to the marsh and each home employs a septic tank to handle household wastes. The septic effluent flows through an upland leach field (<50 feet) and then enters the marsh. Umbrella Creek is subject to 2-3m tides. The fine, silty sediment is colonized by pennate diatoms. Macrovegetation in the adjacent marsh is mostly *Spartina alterniflora*.

The difference in the level of development of the two study sites provides contrasting nutrient loading rates to the marshes and to the benthic microalgae. It is possible that the sewage effluent at DB has an enrichment effect, as was the purpose of the nutrient addition experiment. The nutrient data in Table 2 (Weston et al. in press) demonstrate that DB sediment porewater NOx (NO_2+NO_3) concentrations were lower than the experimental additions; however, the NH_4

and PO_4 frequently reached levels higher than those added in the experiment. Thus, the developed site (DB) is potentially replete with nutrients and the microalgae therefore not experiencing nutrient limitation. In contrast, the MH site is potentially receiving a lower nutrient load and the microalgae there may experience nutrient limitation

Table 2. NH_4, NOx and PO_4 averages for MH and DB overlying water and sediment porewater. Data from Weston et al. in press.

	NH_4 (µM)	**NOx (µM)**	**PO_4 (µM)**
DB overlying water	10.72 ± 8.78	4.46 ± 5.50	5.89 ± 5.12
MH overlying water	3.00 ± 2.43	0.76 ± 0.23	1.69 ± 0.29
DB porewater	309.46 ± 429.11	7.80 ± 16.44	137.26 ± 148.67
MH porewater	45.30 ± 40.30	9.52 ± 11.31	6.65 ± 7.39

Hypotheses

1. Primary production estimates will vary seasonally and spatially as a result of the light regime provided by the presence or absence of *Spartina*.
2. Nutrient additions (nitrogen plus phosphorus) will increase both gross primary production rates and steady state oxygen concentrations in intertidal sediments.

METHODS

Estimation of primary production

Sampling for primary production and associated variables was completed quarterly in an attempt to capture the variation in rates due to seasonal effects including variations in temperature, light, nutrients and grazing (Table 3).

Sediment push cores (inner diameter 4 cm, 5 cm tall) were collected during low tide at Moses Hammock from three transects that traversed the three zones of the marsh (bank, levee marsh, high marsh) and covered a 100 m^2 area (Fig. 3). The cores were collected along the transects between August 2003 and May 2004. Photosynthetically active radiation (PAR) measurements were made with a Li-Cor (LI-1400) light meter at each area where the push cores were collected. Temperature (with a digital thermometer) and pH (by lightly touching a pH strip to the sediment) of the surface sediment were also measured at sampling area. The sediment cores were transported to a laboratory at the University of Georgia (UGA) where they remained outside under partial shade to prevent photoinhibition and limit evaporation while maintaining the natural light-dark cycle and temperatures. Water from the site was added periodically to the surface of the cores to prevent desiccation. Gross primary production measurements were conducted indoors at field temperatures.

Table 3. Schedule of sampling and data collected in this study. Note: In addition to the data collected in this table climate data was available from a weather station at the LTER sites.

Date	Site	Data collected
August 2003	MH	-Primary production estimates, chlorophyll *a*, pheopigments, porosity, organic matter concentrations and PAR for the bank and levee marsh
November 2003	MH	-Primary production estimates, chlorophyll *a*, pheopigments, porosity, organic matter concentrations and PAR for the bank, levee marsh and high marsh
February 2004	MH	-Primary production estimates, chlorophyll *a*, pheopigments, porosity, organic matter concentrations and PAR for the bank, levee marsh and high marsh
May 2004	MH	-Primary production estimates, chlorophyll *a*, pheopigments, porosity, organic matter concentrations, PAR, pH, temperature for the bank, levee marsh and high marsh
	DB	-Primary production estimates for the bank before nutrient additions and at 24h, 48h and 11 days after nutrient additions -Steady state oxygen concentrations for the bank in light and dark before nutrient additions and at 24h, 48h and 11 days after nutrient additions -Chlorophyll *a*, pheopigments, porosity, organic matter concentrations before and after nutrient additions
June 2004	MH	-Primary production estimates for the bank before nutrient additions and at 24h, 48h and 10 days after nutrient additions -Steady state oxygen concentrations for the bank in light and dark before nutrient additions and at 24h, 48h and 10 days after nutrient additions -Chlorophyll *a*, pheopigments, porosity, organic matter concentrations before and after nutrient additions
July 2004	MH	-Primary production estimates, chlorophyll *a*, pheopigments, porosity, organic matter, PAR, pH and temperature for the bank, levee marsh and high marsh Development of photosynthesis versus irradiance curves

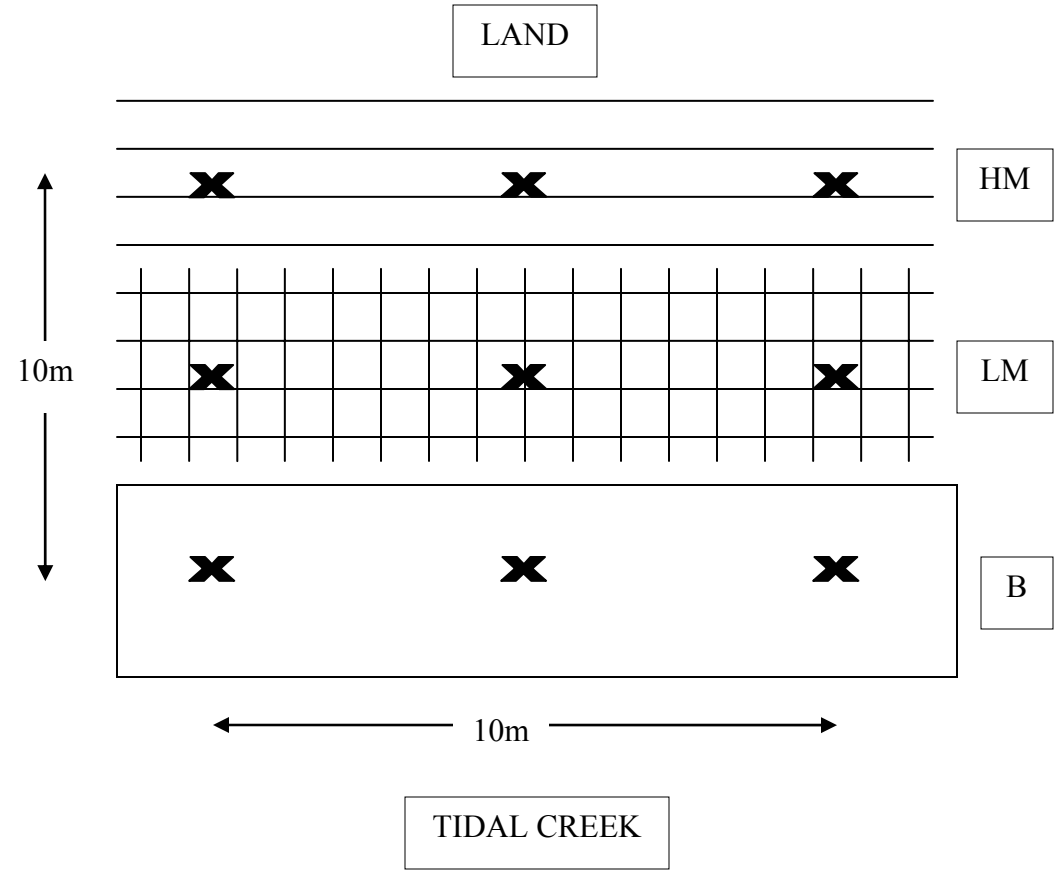

Figure 3. Schematic drawing of the Moses Hammock sampling site showing with "Xs" where sampling occurred (HM: high marsh, LM: levee marsh, B: bank).

Gross primary production rates were measured using Clark-type oxygen microelectrodes maneuvered by a computer-controlled micromanipulator motorized by an Oriel Encoder Mike Controller (Model 18011). Microelectrodes were calibrated in water from the field site that was bubbled with an aquarium pump to achieve 100% air saturation of oxygen (O_2) and with helium to achieve 0% air saturation of O_2. The 100% and 0% readings were used to calibrate the sensor (i.e., to convert the measured voltage to O_2 concentrations). Primary production rates were determined using the light-dark shift technique (Figs. 4-6) (Revsbech et al. 1981). The technique consists of illuminating the sediment until a steady state O_2 concentration was reached. At

steady state, the photosynthetic O_2 production balanced the combined losses due to respiration and diffusion (Revsbech and Jørgensen 1983). Once a steady state O_2 concentration was reached, the light source was blocked, darkening the sediment surface; diffusion and respiration were initially unchanged so the O_2 concentration decreased at a rate equal to the photosynthetic O_2 production rate (Revsbech and Jørgensen 1983). Hence, the decrease in O_2 concentration observed during the first 2-4 seconds of darkness was equal to the rate of gross oxygenic photosynthesis. Rates measured at 100 μm intervals from the sediment-water interface to the depth at which zero O_2 concentration was reached were integrated to provide an estimate of gross primary production. Rates were corrected using sediment density and porosity to yield units of mmol O_2 m^{-2} h^{-1}. Replicate (n=3) O_2 profiles were measured in cores exposed to an irradiance level of 1000 μE. The 1000 μE light level provides nearly saturating light intensities for benthic microalgae (Pinckney and Zingmark 1991, 1993b) with the purpose of obtaining estimates of maximum photosynthetic rates. The level of illumination also coincides with the average light received at the sediment surface on a cloudless day (light levels reach nearly 2000 μE at midday during the summer).

Maximum O_2 concentration, maximum integrated gross photosynthesis and depths of maximum O_2 concentration and zero O_2 concentration were recorded. The depth to which O_2 penetrates the sediment is influenced by several factors including light availability, reduction-oxidation (redox) conditions and organic matter content. Annual estimates of benthic primary production in the bank, levee and high marsh were calculated.

From the measured primary production hourly rates, annual rates were extrapolated by multiplying the hourly rate by the number of daylight hours, which ranged from 10 hours in November to 14 hours in May. The daily rate was then multiplied by 30 to get a monthly rate

then multiplied by three to get a seasonal rate (three months per season). The seasonal rate was then corrected for the relative amount of each marsh zone in the study area (8.8 km^2 of the Duplin watershed is intertidal marsh with 10% bank, 50% levee marsh and 40% high marsh (Darley et al. 1981, Pomeroy and Imberger 1981)). The summation of the seasonal rates then gave the annual production of the Duplin River marshes. Net primary production was estimated to be 90% of gross primary production (Pomeroy 1959, Pinckney and Zingmark 1993a).

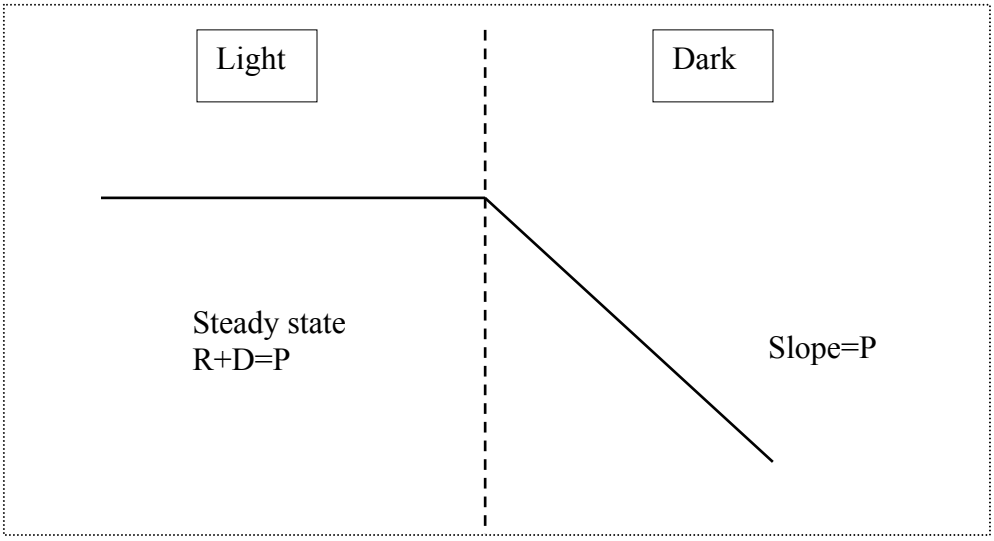

Figure 4. Representation of the light-dark shift technique. The line represents the measured O_2 concentration (R: respiration, D: diffusion, P: photosynthesis).

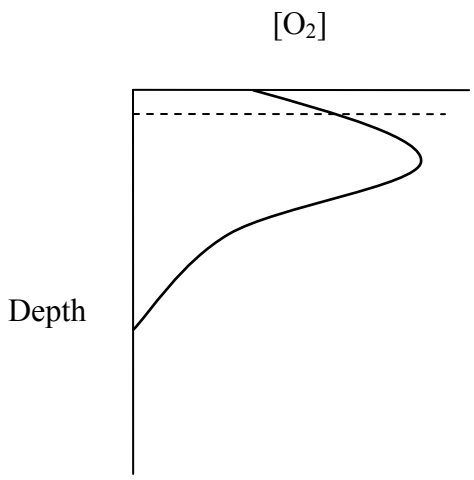

Figure 5. A typical vertical profile of O_2 concentration showing the subsurface peak (dashed line: sediment-water interface).

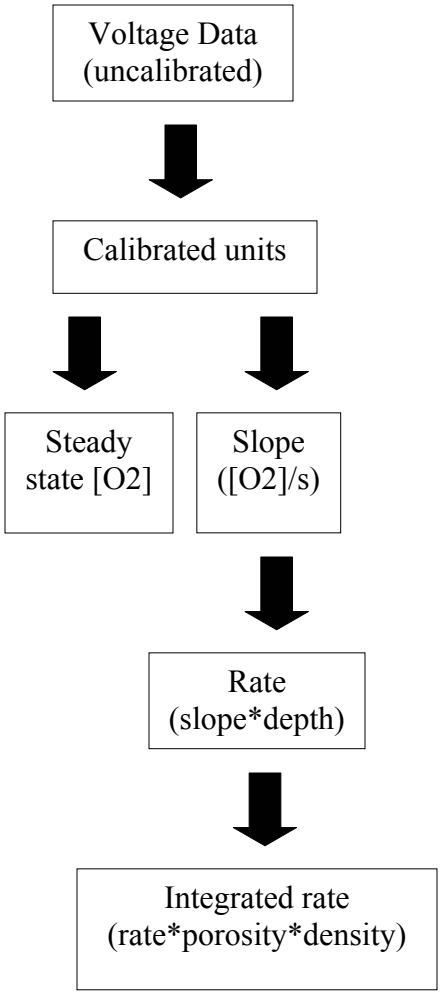

Figure 6. Conversion of voltage data to an integrated rate.

In July 2004, sediment cores from the three Moses Hammock marsh zones were collected and production measurements were made at irradiances from 50 to 2000 μE in order to construct photosynthesis versus irradiance curves (P-I curve, Fig. 7). Three sediment cores were collected from each marsh zone and triplicate profiles measured in each core at each light level then averaged (n=9). Increased irradiance led to increased photosynthetic rates until a maximum was reached, the photosynthetic maximum (P_{max}). Alpha (α), an indicator of photosynthetic efficiency, is defined as the rate of photosynthesis per unit irradiance at low light intensities and

determined as the initial slope of the P-I curve. The saturation onset parameter (I_k) is the estimated irradiance at which light limitation changes to light saturation. A single P-I curve and associated parameters (P_{max}, α, I_k) was obtained for each marsh zone. Gross primary production estimates and the P-I curves provide information on the ability of benthic microalgae (and their pigments) to use available light to support photosynthesis.

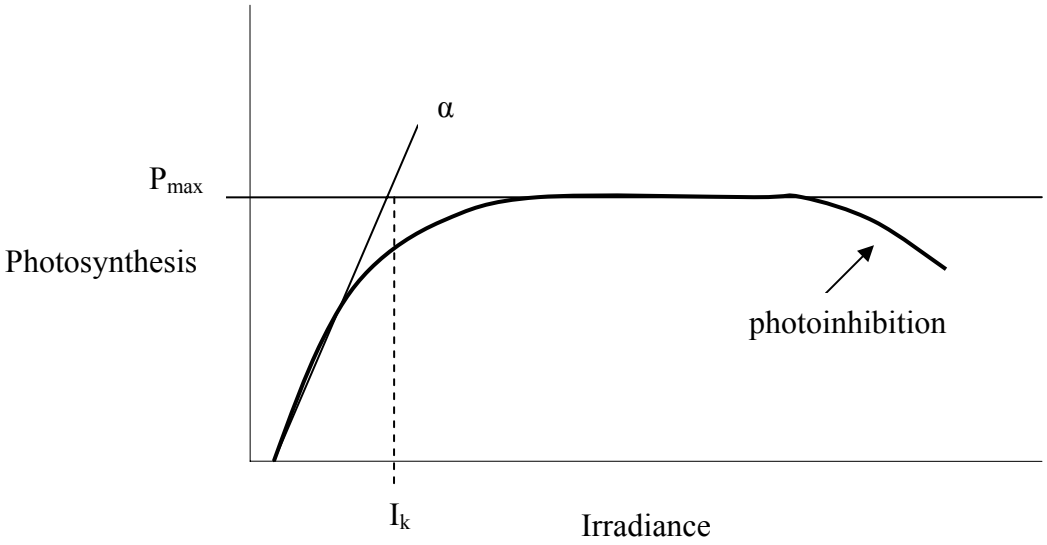

Figure 7. An example of a P-I curve and associated parameters.

Sediment chlorophyll *a* was measured spectrophotometrically to estimate active phototroph biomass (Strickland and Parsons 1972). Samples of the top centimeter of the sediment were collected using cut-off 5ml syringes then preserved with $MgCO_3$ and frozen until extraction in 100% acetone for 24 hours. After extraction the samples were centrifuged and absorbance of the supernatant was measured at 750 and 664 nm. Pheopigments, an inactive product of pigment degradation, were determined by adding 100 µl of 1N HCl to the supernatant

waiting two minutes and re-reading the absorbance at 750 and 664 nm. Chlorophyll *a* and pheopigment concentrations were calculated and results expressed in mg m^{-2}. Pigment concentrations were averaged for each area sampled along the transects and for each zone. Accurate estimates of chlorophyll *a* or pheopigments are possible when the concentrations are high, but errors increase as concentrations decline (Lorenzen 1967). In addition, pheopigments in tidal flats are on average one third of the functional chlorophyll but may reach equal concentrations; for this reason it is necessary to distinguish between the two (Cadée and Hegeman 1974). Distinguishing between functional chlorophyll *a* and pheopigments offers a more reliable estimate of the active chlorophyll *a* present that is used in primary production. Furthermore, the chlorophyll *a* estimate was used to calculate the chlorophyll specific primary production. The chlorophyll specific primary production was determined by taking the production value and dividing it by the respective chlorophyll *a* concentration, generating units of mmol O_2 (mg Chl *a*)$^{-1}$ hr^{-1}.

Once the microelectrode measurements were completed, a cut-off 5ml syringe was used to collect the top cm of sediment from the cores to determine sediment density, porosity and organic content. Density is reported as the weight in grams of wet sediment per one cm^3. Porosity was determined as the weight loss of the top cm of sediment after drying at 80 ˚C for 24 hours and reported as g of water (g wet sediment)$^{-1}$. For organic content determination the top cm of sediment was dried at 80 ºC for 24 hours and weighed; the dry sediment was then ashed at 500 ºC for at least 8 hours and reweighed. The weight loss of the ash-free sediment is reported as percent dry weight. Energy-rich organic compounds are produced by autotrophs through photosynthesis. Therefore, primary production represents a potential input of organic matter to

the sediments. Hence, a general correlation between organic matter content and oxygenic photosynthesis was anticipated.

Nutrient addition experiments

Nutrient enrichment experiments were conducted in May for the DB site and June for the MH site. The benthic microalgae at the two study sites were expected to show different responses to nutrient enrichment, because though the two marshes are physiographically and ecologically similar, DB receives higher nutrient loading (Table 2) than does MH. The two study sites were compared to determine whether nutrient enrichment responses from two sites with different nutrient loading rates varied.

Since benthic primary producers at both sites were dominated by diatoms, it was important to examine whether the addition of nitrogen and phosphorus would lead to silicate limitation. Hence, a preliminary experiment was conducted for 10 days with the following three treatments: 1.Control (no nutrients) 2. 500 μM Nitrate + 30 μM Phosphate 3. 500 μM Nitrate + 30 μM Phosphate + 500 μM Silicate. The results demonstrated no difference in biomass or gross primary production between the N+P and the N+P+Si treatment indicating that the N+P addition did not cause Si limitation. Therefore Si was not included in the nutrient addition experiments reported here.

Sediment push cores were collected at the micro-vegetated marsh banks of both Dover Bluff and Moses Hammock. The cores were transported to the UGA laboratory and placed in clear plastic containers and submerged in water with their respective nutrient treatment. Throughout the experiment the containers with the cores were kept in an incubator at a constant temperature of 28˚C (reflecting the *in situ* sediment temperature) and a light–dark cycle of 12h in order to reduce any variability in temperature and light in an attempt to focus on the nutrient

response. Three different treatments were applied to each set of samples: 1. Control: no nutrient additions 2. Low treatment: 100 μM Nitrate + 6 μM Phosphate 3. High treatment: 500 μM Nitrate + 30 μM Phosphate. Nutrient additions were made every other day so as to maintain a constant supply. The nitrogen additions were in the form of KNO_3 and the phosphorus additions in the form of KH_2PO_4. The N:P ratio was 16.7, comparable to the Redfield ratio. Three cores per treatment were used and triplicate profiles of gross oxygenic photosynthesis and oxygen penetration depth measured in each core, giving a total of nine profiles for each treatment. The profiles were evaluated for significant differences between the three treatments using one-way analysis of variance (ANOVA) and t-tests.

Gross primary production and light and dark steady state O_2 concentrations were measured before nutrient additions (0 hours), and at 24 hours, 48 hours and 10 (MH) or 11 (DB) days after nutrient additions. Gross primary production measurements were made at an irradiance of 1000 μE using the light-dark shift technique and integrated over depth, as described previously. Steady state measurements were made after a constant O_2 concentration was achieved at a particular depth. Dark steady state measurements were made without illuminating the sediment cores. The steady state O_2 concentration was recorded at 100 μm intervals from the sediment-water interface to the depth at which O_2 concentration reached zero and integrated over depth. The differences between the light and dark steady state O_2 concentrations were noted as was the depth of zero O_2.

Changes in chlorophyll *a*, pheopigments and chlorophyll *a* to pheopigments ratios were examined by measuring chlorophyll *a* and pheopigments concentrations before and after nutrient addition. A decrease in the chlorophyll *a* to pheopigments ratio indicates increasing senescence

whereas an increase indicates active growth, thus chlorophyll *a* to pheopigments ratios, may be used to interpret the relative health of oxygenic phototrophs (Camacho and de Wit 2003).

RESULTS

Estimation of primary production at Moses Hammock

Chlorophyll *a* concentration and integrated oxygenic photosynthesis rates for the marsh zones at each sampling date are shown in figures 8-11. The distribution of chlorophyll *a* varied significantly by zone (1-way ANOVA, $F=18.10$, $p<0.01$) and sampling date (1-way ANOVA, $F=11.35$, $p<0.01$). Photosynthetic rates also varied significantly by zone (1-way ANOVA, $F=5.37$, $p<0.05$) and sampling date ($F=4.76$, $p<0.01$). Chlorophyll *a* was significantly higher (t-test, $p<0.05$) in the levee marsh sediments throughout the sampling dates compared to the high marsh and bank. The levee marsh also had higher chlorophyll *a* to pheopigments ratios and lower chlorophyll specific primary production (t-test, $p<0.05$) (Table 5). Irradiances at the sediment surface varied between the zones (1-way ANOVA, $F=23.16$, $p<0.01$). (Table 4), with the micro-vegetated bank continually receiving the highest irradiances. It is important to note that variables in Table 4 are one-time measurements observed at low tide during the day of sampling and therefore may or may not be representative of the season in which sampling occurred.

The pH of the surface sediment ranged from 7 to 8.5 during low tide sampling, in accord with a diurnal variation ranging from 7 to 9 and occasionally 10 (Pomeroy 1959). The pH of the Duplin River is about 8 (Pomeroy and Imberger 1981). The pH changes result primarily from consumption of CO_2 and HCO_3 by the microflora in the water and the surface sediments (Pomeroy 1959).

The temperature of surface sediments ranged from 15˚C in November to approximately 40˚C in August (Pomeroy 1959, Table 4). Water temperatures ranged from 15˚C in February to 31˚C in July and air temperatures from 3˚C (February) to 35˚C (July) (weather station data). Water temperature was usually cooler than the sediment temperature during the day.

Rain was nonexistent at MH on sampling days or the days prior to sampling with one exception: during the August sampling less than 5 mm of precipitation fell. Precipitation did not cause any resuspension or disturbance to the marsh sediment prior to collection of sample cores.

The Duplin River salinity ranged from 17 to 30 ‰. Benthic diatoms are tolerant of the wide range in salinities; salinity is not likely to be an important variable in controlling benthic primary production (Williams 1962, Pomeroy et al. 1981).

Pheopigments, chlorophyll *a*, chlorophyll *a* to pheopigments ratios and chlorophyll specific primary production for the MH bank, levee and high marsh for all sampling dates are shown in Table 5. Pheopigments ranged from 78.65 mg m^{-2} (high marsh, May) to 152.88 mg m^{-2} (levee marsh, Aug.). Chlorophyll *a* ranged from 66.74 (bank, Nov.) to 287 mg m^{-2} (levee marsh, Feb.). Chlorophyll *a* to pheopigments ratios were significantly different between zones (1-way ANOVA, $F=17.26$, $p<0.01$) and ranged from 0.84 (bank, Nov.) to 2.40 (levee marsh, Feb.). Chlorophyll specific primary production ranged from 39 (levee marsh, Aug.) to 192 μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1} (bank, Feb.).

Maximum O_2 concentration, maximum integrated gross photosynthesis, depths of maximum O_2 concentrations and depth of zero O_2 for the MH zones for all sampling dates are shown in Table 7. The maximum O_2 concentration was 1268.98 μM (bank, Feb.) and the maximum integrated gross photosynthesis was 29.49 mmol O_2 m^{-2} h^{-1} (high marsh, Nov.).

Depths at which O_2 concentrations reached zero ranged from 900 μm (levee marsh, May) to 4800 μm (bank, Feb.), with the characteristic depth of maximum O_2 concentration being 100 μm.

Porosity ranged from 0.61 (bank, Nov.) to 0.78 g water (g wet sediment)$^{-1}$(high marsh, Feb.) and remained similar within each zone for all sampling dates (Table 6). Organic content remained stable within each zone for all sampling dates although a significant difference in organic content was observed between zones (1-way ANOVA, $F=43.29$, $p<0.01$). The organic content of the bank sediment was significantly lower than that of the levee and high marshes (t-test, $p<0.05$) (Table 6). Sediment characteristics play a significant role in light penetration thus, are indicative of the photic zone which ranged from 200 to 900 μm below the sediment surface.

Average benthic microalgal primary production for the three marsh zones (average for all sampling dates) is shown in Table 9 and compared to results from a similar habitat, North Inlet Estuary, SC (Pinckney and Zingmark 1993a). The estimate for annual chlorophyll *a* and benthic primary production is compared with several other studies in Table 9. For the purpose of comparison the production in oxygen units was converted to carbon units using a photosynthetic quotient (ratio of moles of O_2 produced per moles of carbon fixed) of 1 (Pomeroy 1959, Pinckney and Zingmark 1993a). For benthic microalgal communities net primary production is usually not less than 90% of gross production (Pomeroy 1959). Thus, using the gross annual primary production estimate of 560.09 g C m^{-2} yr^{-1} the net primary production is approximately 504.08 g C m^{-2} yr^{-1}.

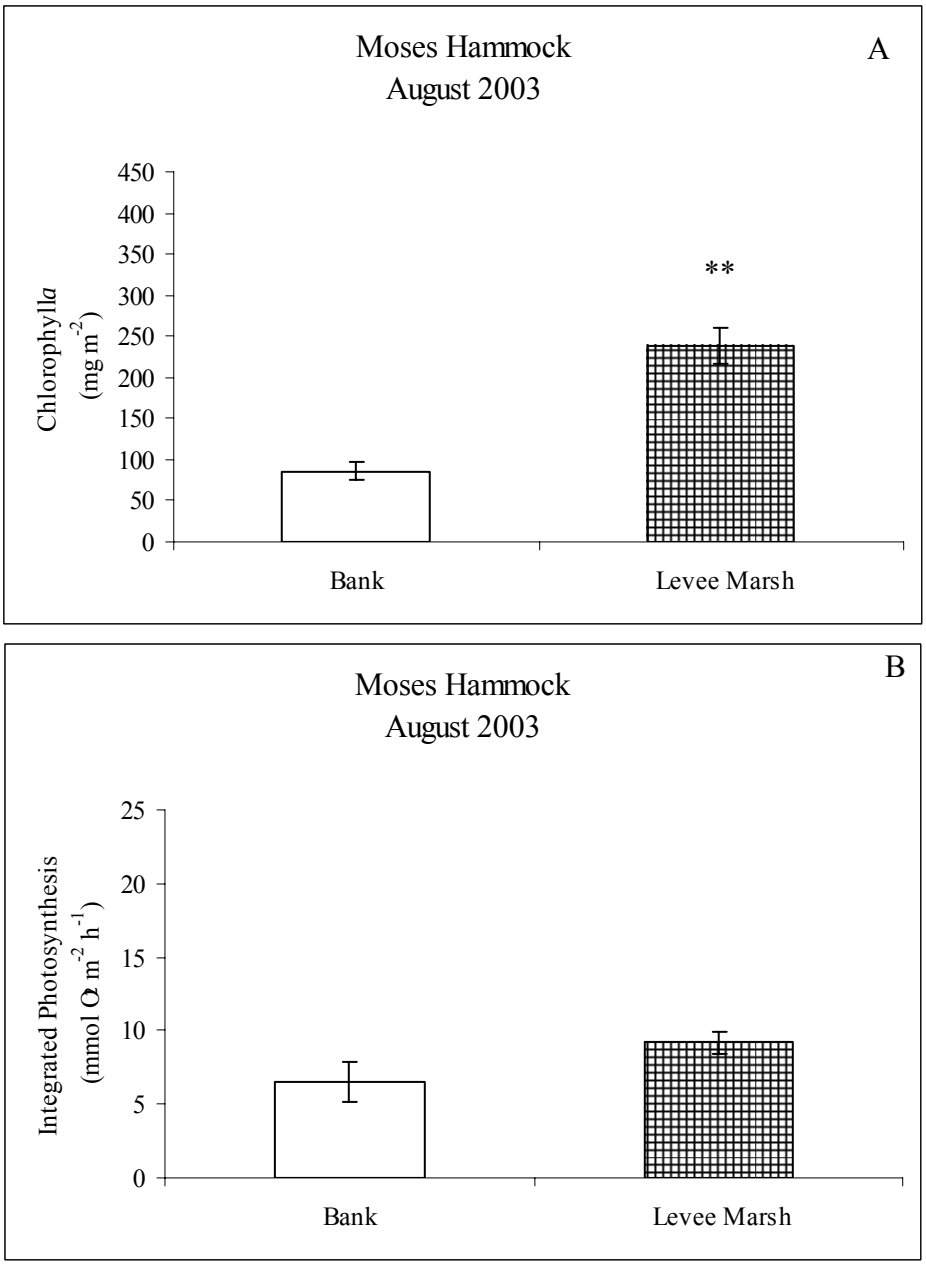

Figure 8. August 2003 chlorophyll *a* (A) and integrated photosynthesis (B) for the MH bank and levee marsh. Statistically significant differences are marked with two asterisks (t-test, $p<0.01$).

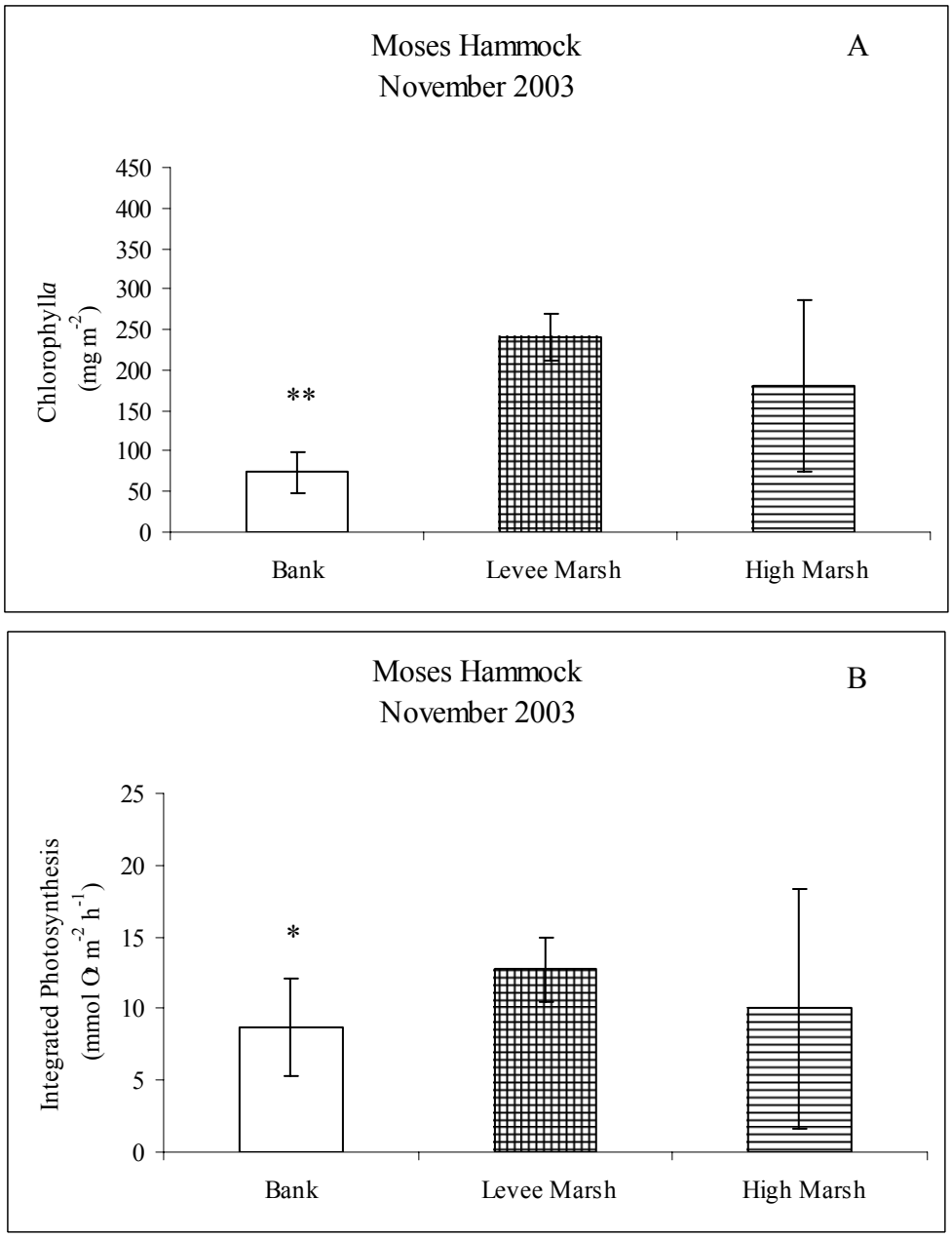

Figure 9. November 2003 chlorophyll *a* (A) and integrated photosynthesis (B) for the MH bank, levee and high marsh. Statistically significant differences are marked with asterisks (t-test, *p<0.05, **p<0.01). Bank values are lower than the levee marsh but similar to the high marsh.

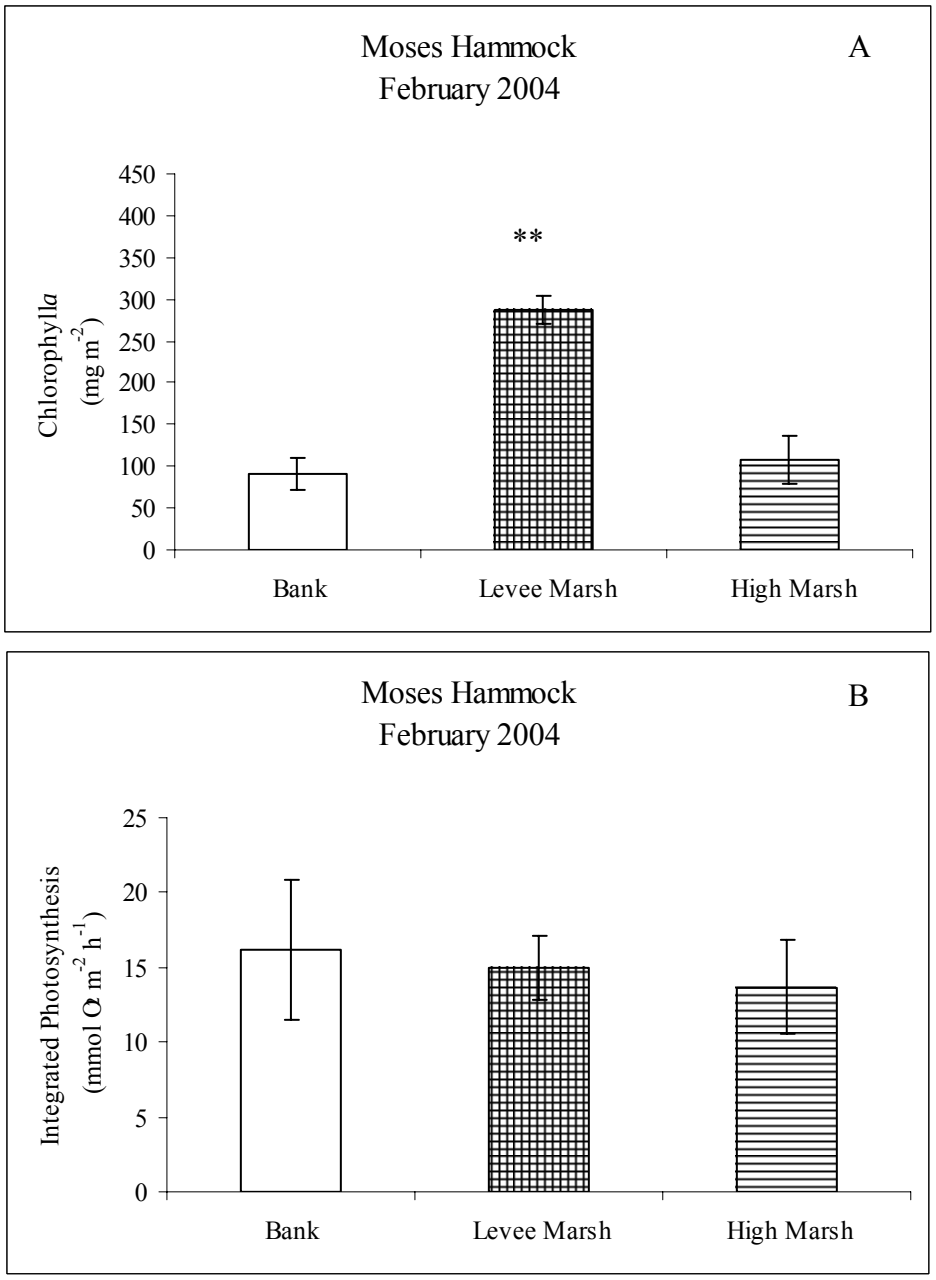

Figure 10. February 2004 chlorophyll *a* (A) and integrated photosynthesis (B) for the MH bank, levee and high marsh. Statistically significant differences are marked with asterisks (t-test, **$p<0.01$).

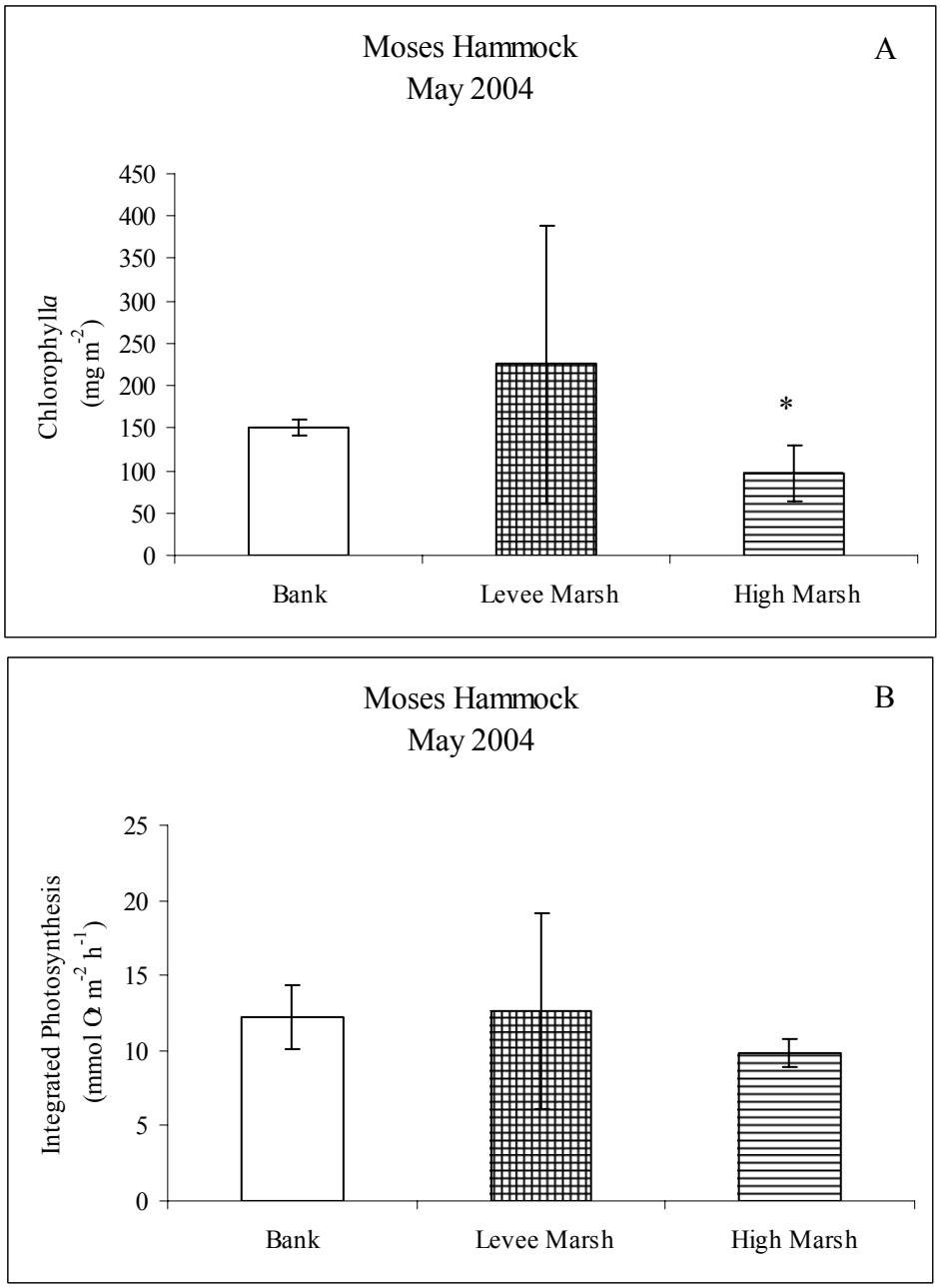

Figure 11. May 2004 chlorophyll *a* (A) and integrated photosynthesis (B) for the MH bank, levee and high marsh. Statistically significant differences are marked with asterisks (t-test, *$p<0.05$). High marsh chlorophyll *a* was lower than the bank but similar to the levee marsh.

Table 4. Sediment pH, PAR and temperature and water temperatures of MH (nd: not determined). Underlined temperatures are from Pomeroy (1959). Note: variables (except those from Pomeroy) are one-time measurements observed at low tide. PAR varied significantly between the different zones (1-way ANOVA, $F=23.16$, $p<0.01$).

Date of sampling	**Zone**	**pH**	**PAR (µE)**	**Sediment temperature (˚C)**	**Water temperature (˚C)**
August 9, 2003	HM LM B	nd	700 550 1900	nd 37 27.5	30
November 21, 2003	HM LM B	nd	450 300 1200	nd 24 26	19
February 27, 2004	HM LM B	nd	350 300 1200	nd 17 22	15
May 17, 2004	HM LM B	8.5 8.5 8	750 200 1000	30.7 29.5 34	25
July 11, 2004	HM LM B	7.5 7 7	460 550 1530	28.9 29.1 34.9	31

Table 5. Moses Hammock average chlorophyll specific primary production, chlorophyll *a* to pheopigment ratio, chlorophyll *a* and pheopigments for the three zones (n=9, ±SD). The distribution of chlorophyll *a* varied significantly by zone (1-way ANOVA, F=18.10, p<0.01) and sampling date (1-way ANOVA, F=11.35, p<0.01). Chlorophyll *a* to pheopigments ratios were significantly different between zones (1-way ANOVA, F=17.26, p<0.01.

Marsh zone and date	**Chlorophyll specific primary production ($\mu molO_2$ (mg Chl *a*)$^{-1}$ h^{-1})**	**Chlorophyll *a*: Pheopigments**	**Chlorophyll *a* (mg m^{-2})**	**Pheopigments (mg m^{-2})**
Bank				
Aug 2003	75 ± 11	0.91 ± 0.18	86.24 ± 35.11	95.15 ± 14.79
Nov 2004	147 ± 48	0.84 ± 0.20	66.74 ± 22.20	86.08 ± 10.80
Feb 2004	192 ± 94	0.92 ± 0.21	90.81 ± 19.81	100.74 ± 23.07
May 2004	81 ± 11	1.20 ± 0.12	150.88 ± 8.94	126.89 ± 17.01
Levee Marsh				
Aug 2003	39 ± 7	1.66 ± 0.47	238.86 ± 27.71	152.88 ± 49.73
Nov 2004	66 ± 26	2.20 ± 0.77	233.80 ± 63.45	120.95 ± 53.27
Feb 2004	52 ± 5	2.40 ± 0.11	287.00 ± 16.19	119.75 ± 12.13
May 2004	73 ± 38	1.98 ± 0.94	225.11 ± 162.77	101.79 ± 51.30
High Marsh				
Nov 2004	61 ± 2	1.56 ± 0.41	170.50 ± 136.01	108.28 ± 47.76
Feb 2004	133 ± 40	1.34 ± 0.38	107.40 ± 28.75	80.90 ± 10.73
May 2004	109 ± 35	1.22 ± 0.11	96.98 ± 32.68	78.65 ± 20.02

Table 6. Moses Hammock average porosity and organic content values for the three zones (n=6, ±SD). Organic content was significantly different between zones (1-way ANOVA, $F=43.29$, $p<0.01$).

Marsh zone and date	Porosity (g water (g wet sed)$^{-1}$)	Organic content (% dry weight)
Bank		
Aug 2003	0.64 ± 0.03	11.37 ± 0.70
Nov 2004	0.61 ± 0.04	8.34 ± 1.28
Feb 2004	0.66 ± 0.07	10.41 ± 2.97
May 2004	0.66 ± 0.01	12.31 ± 0.89
Levee Marsh		
Aug 2003	0.69 ± 0.04	20.49 ± 2.72
Nov 2004	0.69 ± 0.03	19.41 ± 2.31
Feb 2004	0.69 ± 0.01	19.18 ± 2.44
May 2004	0.65 ± 0.04	18.43 ± 4.05
High Marsh		
Nov 2004	0.75 ± 0.06	21.89 ± 3.09
Feb 2004	0.78 ± 0.03	22.26 ± 3.33
May 2004	0.68 ± 0.03	18.19 ± 0.04

Table 7. Moses Hammock maximum O_2 concentration, maximum gross integrated photosynthesis, depth of maximum O_2 concentration and depth of zero O_2 concentration for the three zones (nd=not determined). Differences between each of the four variables were not significant (1-way ANOVAs).

Marsh zone and date	Maximum [O_2] (µM)	Maximum Integrated Gross Photosynthesis (mmol O_2 m^{-2} h^{-1})	Depth of Maximum [O_2] (µm)	Depth of Zero [O_2] (µm)
Bank				
Aug 2003	nd	8.17	nd	nd
Nov 2004	945.61	15.44	500	4300
Feb 2004	1268.98	22.26	200	4800
May 2004	674.57	16.58	100	1600
Levee Marsh				
Aug 2003	nd	14.98	nd	nd
Nov 2004	910.90	16.98	300	3100
Feb 2004	1032.81	22.44	100	2700
May 2004	725.70	21.55	100	900
High Marsh				
Nov 2004	938.96	29.49	100	2800
Feb 2004	511.26	21.93	100	2200
May 2004	630.39	15.12	200	2100

The relationship between irradiance and photosynthesis for the three Moses Hammock zones in July 2004 is shown in Figure 12; the associated parameters are in Table 8.

Table 8. Parameters from the photosynthesis versus irradiance curves in Figure 12.

Zone	P_{max} (mmol O_2 m^{-2} h^{-1})	Irradiance at P_{max} (µE)	I_k (µE)	α	Regression line equation	R^2
B	20.16 ± 3.39	2000	450	0.015	y=8.7577Ln(x)-2.2517	0.9217
LM	25.75 ± 3.35	1600	300	0.029	y=12.584Ln(x)-3.6217	0.8406
HM	16.85 ± 2.92	1200	200	0.022	y=10.155Ln(x)-2.7311	0.8638

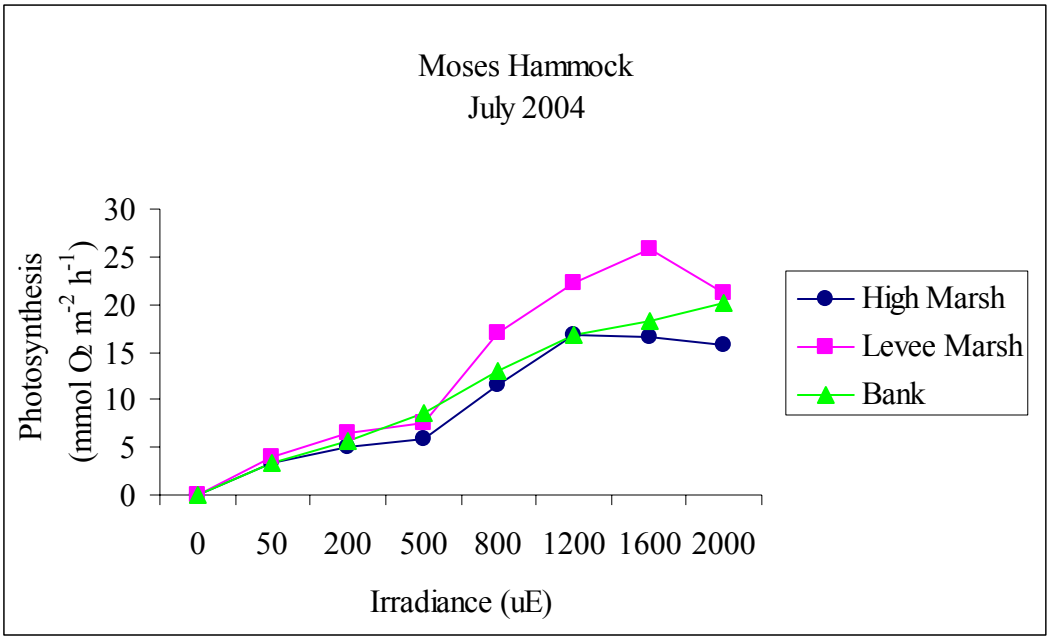

Figure 12. Relationship of irradiance to photosynthesis in the MH bank, levee marsh and high marsh (n=9 for each point).

Table 9. Comparison of benthic primary production in this study (MH) and North Inlet Estuary, SC (Pinckney and Zingmark 1993a).

Zone	Moses Hammock primary production (mmol O_2 m^{-2} h^{-1})	North Inlet Estuary primary production (mmol O_2 m^{-2} h^{-1})
Bank	11.14 ± 4.10	11.11 ± 0.83
Levee Marsh	12.76 ± 2.58	6.99 ± 0.71
High Marsh	11.36 ± 2.06	5.95 ± 0.57

Table 10. Benthic microalgae chlorophyll *a* and photosynthesis values from various studies (nd: not determined).

Site	Chlorophyll *a* (mg m^{-2})	Photosynthesis	Reference
Sapelo Island, Ga, USA	183.9 ± 81.8	560.09 g C m^{-2} yr^{-1}	This study
Sapelo Island, Ga, USA	nd	200 g C m^{-2} yr^{-1}	Pomeroy 1959
Dutch Wadden Sea	7.1 µg g^{-1}	100 g C m^{-2} yr^{-1}	Cadée and Hegeman 1974
Falmouth Bay, USA	nd	105.5 g C m^{-2} yr^{-1}	Van Raalte et al. 1976
Ems-Dollard estuary, Netherlands	33-184 (annual mean)	62-276 g C m^{-2} yr^{-1}	Colijn and de Jonge 1984
North Carolina, USA continental shelf	36.4 (average)	52 g C m^{-2} yr^{-1}	Cahoon and Cooke 1992
Westerschelde estuary, Netherlands	5.9 ± 3.2 (sand) 17.3 ± 6.7 (mud) (annual means)	34.5 ± 23.6 mg C m^{-2} h^{-1} (sand) 41.1 ± 11.6 mg C m^{-2} h^{-1} (mud)	Barranguet et al. 1998

Impact of nutrient addition on photosynthesis

The Dover Bluff nutrient experiment illustrated that the nitrate plus phosphate additions did not have a significant effect on integrated photosynthetic rates (1-way ANOVA, $F=1.68$, $p=0.25$) (Fig. 13). However, a decrease in photosynthetic rates was observed at 11 days for the low nutrient treatment (t-test, $p<0.01$). Dark steady state O_2 concentrations displayed a pattern that was difficult to evaluate (Fig. 14). The dark steady state O_2 concentrations decreased after 24 h then increased at 48 h and decreased again at 11 d. The light steady state O_2 concentrations increased significantly in the low nutrient treatment (t-test, $p<0.05$) (Fig. 15). The dark steady state depths of O_2 penetration illustrated the same fluctuating pattern of the dark steady state O_2 concentrations (alternating decreasing and increasing depths). However, O_2 penetrated deeper into the sediment in the low treatment (t-test, $p<0.01$). Nutrient addition significantly affected chlorophyll *a* concentration (1-way ANOVA, $F=16.07$, $p<0.01$). Chlorophyll *a* significantly increased in both the low (t-test, $p<0.05$) and high (t-test, $p< 0.01$) nutrient additions (Fig. 16). Pheopigment levels did not change significantly after nutrient addition. Organic content was similar for all three treatments (15%). The ratio of chlorophyll *a* to pheopigments ranged from 0.90 to 1.50 with significant increases after the low (t-test, $p<0.05$) and high (t-test, $p<0.01$) nutrient treatments. Chlorophyll *a*, pheopigments, chlorophyll *a* to pheopigments ratio, organic content and porosity for the DB and MH nutrient experiments are displayed in Table 11.

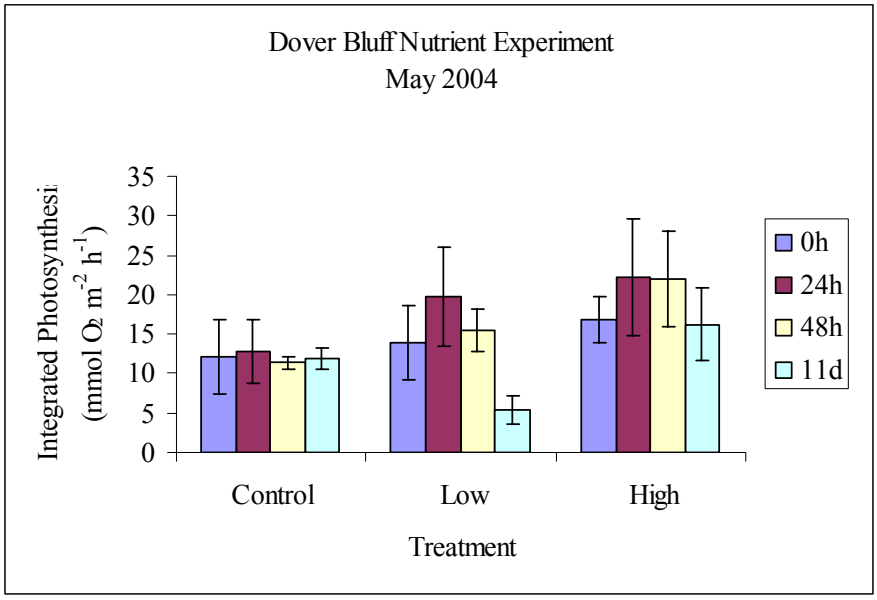

Figure 13. Integrated photosynthesis for the DB nutrient experiment. Nutrient additions had no significant effect on photosynthesis (1-way ANOVA, F=1.68, p=0.25).

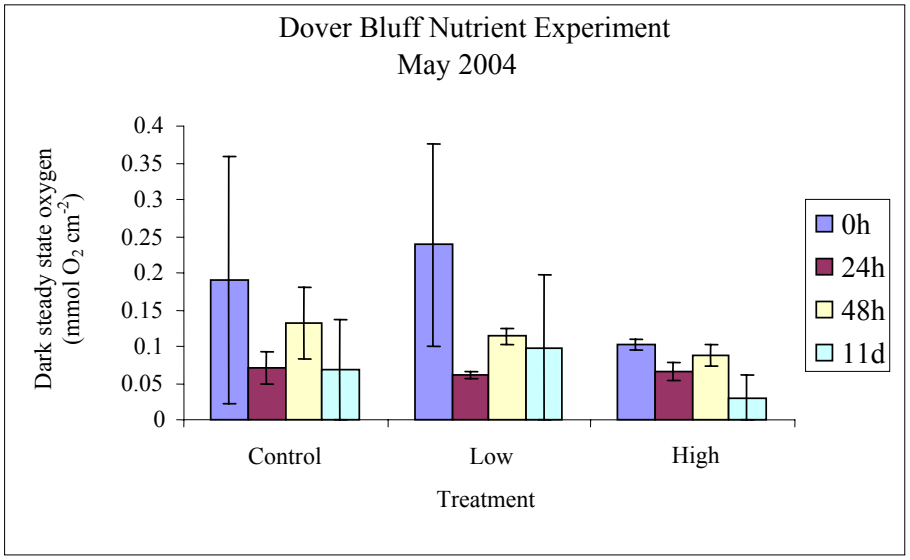

Figure 14. Dark steady state O_2 concentrations for the DB nutrient experiment. Nutrient additions had no significant effect on dark steady state O_2 concentrations (1-way ANOVA, F=1.01, p=0.40).

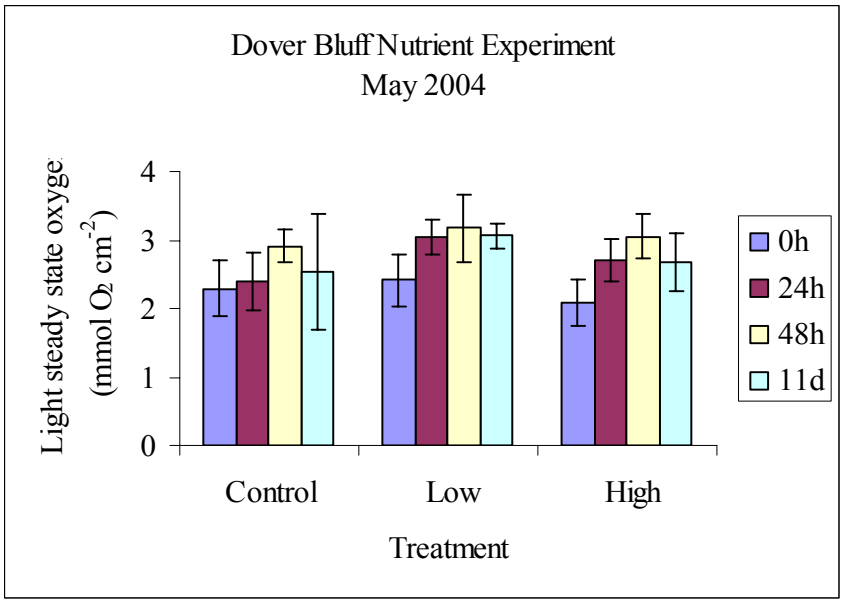

Figure15. Light steady state O_2 concentrations for the DB nutrient experiment. A significant increase was shown in the low nutrient treatment (t-test, $p<0.05$)

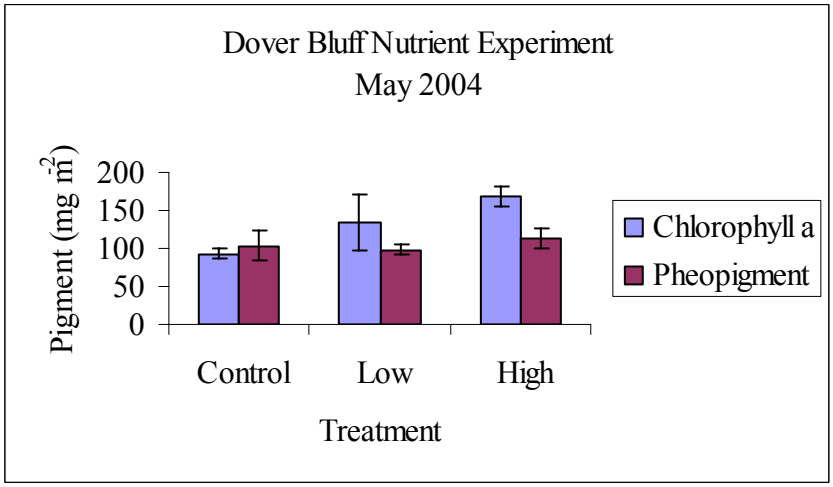

Figure 16. Chlorophyll *a* and pheopigments for the DB nutrient experiment. Chlorophyll *a* significantly increased in both the low (t-test, $p<0.05$) and high (t-test, $p< 0.01$) nutrient additions. Pheopigment levels did not change significantly after nutrient addition.

The Moses Hammock nutrient experiment illustrated nutrient additions had a significant effect on photosynthetic rates (1-way ANOVA, $F=8.95$, $p<0.01$). An increase in photosynthetic

rates with nutrient additions was observed although a decrease occurred at 10 days in the low addition treatment (Fig. 17), as was observed in the Dover Bluff experiment (t-test, $p<0.05$). Dark steady state O_2 concentrations were similar except for a decrease in the high treatment on the last day of the experiment (t-test, $p<0.05$) (Fig. 18). The light steady state O_2 concentrations showed a significant increase after nutrient addition (t-test, $p<0.05$) (Fig. 19). Light steady state O_2 penetration depths increased significantly following low level nutrient addition (t-test, $p<0.05$). Dark steady state depths remained similar except for a decrease in the high treatment on the last day of the experiment (t-test, $p<0.05$). Nutrient addition resulted in significant differences in chlorophyll *a* (1-way ANOVA, $F=8.15$, $p<0.01$) and pheopigment concentrations (1-way ANOVA, $F=3.72$, $p<0.05$). Chlorophyll *a* and pheopigments concentrations increased with nutrient additions (Fig. 20) (t-test, $p<0.05$). In the low nutrient treatment, chlorophyll *a* and pheopigments contents were similar. In the high nutrient treatment the pheopigment content was approximately 60% of the chlorophyll *a* content. Organic content was similar for all three treatments at 5%. The ratio of chlorophyll *a* to pheopigment increased with the increased nutrient additions (t-test, $p<0.05$).

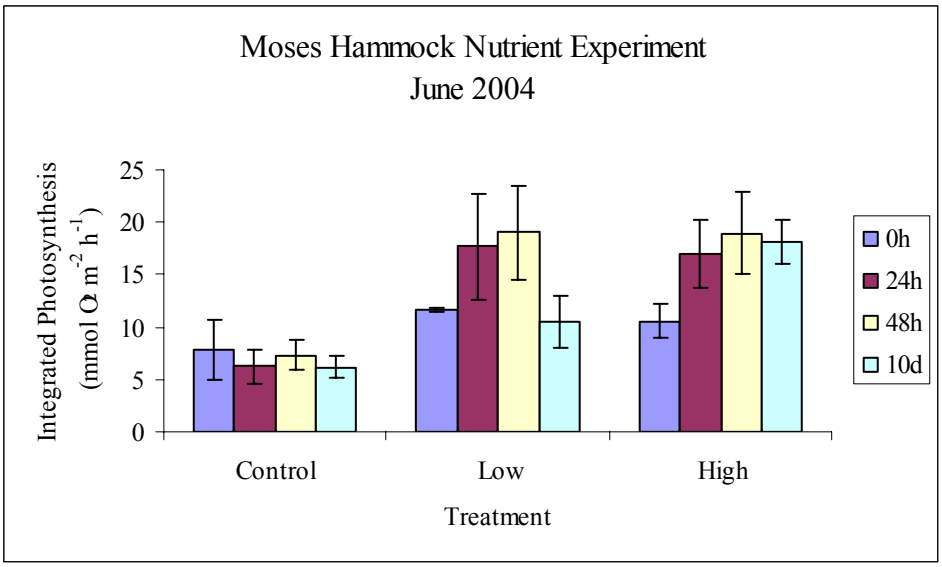

Figure 17. Integrated photosynthesis for the MH nutrient experiment. Nutrient additions had a significant effect on photosynthetic rates (1-way ANOVA, $F=8.95$, $p<0.01$).

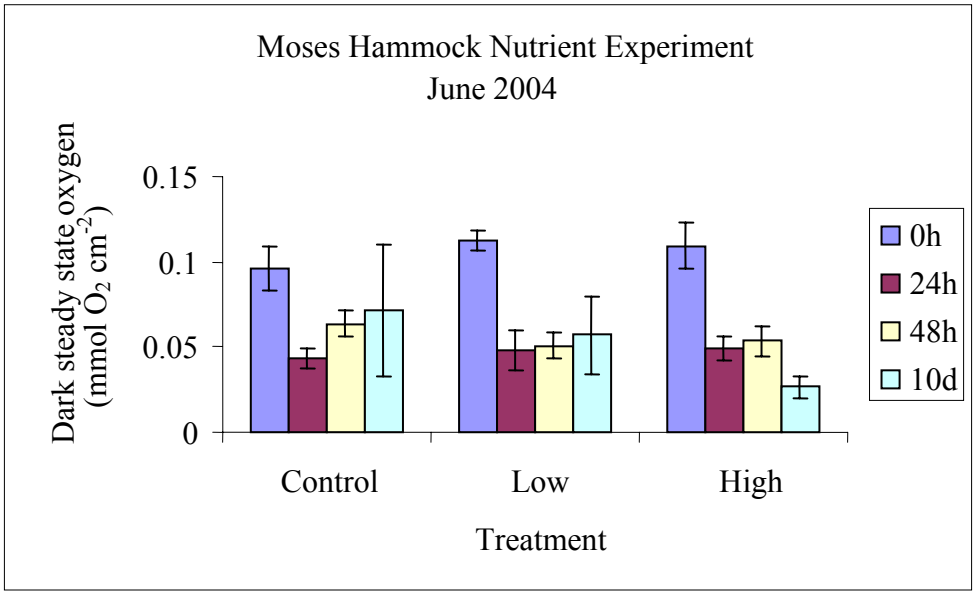

Figure 18. Dark steady state O_2 concentrations for the MH nutrient experiment. Dark steady state O_2 concentrations were similar except for a decrease in the high treatment on the last day of the experiment (t-test, $p<0.05$).

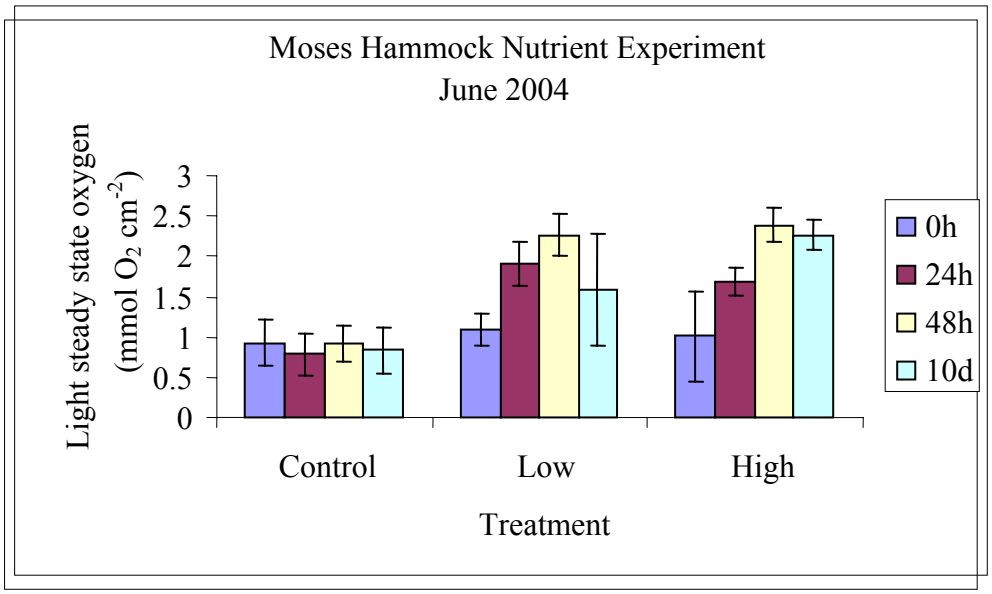

Figure 19. Light steady state O_2 concentrations for the MH nutrient experiment. The light steady state O_2 concentrations showed a significant increase after nutrient addition (t-test, $p<0.05$).

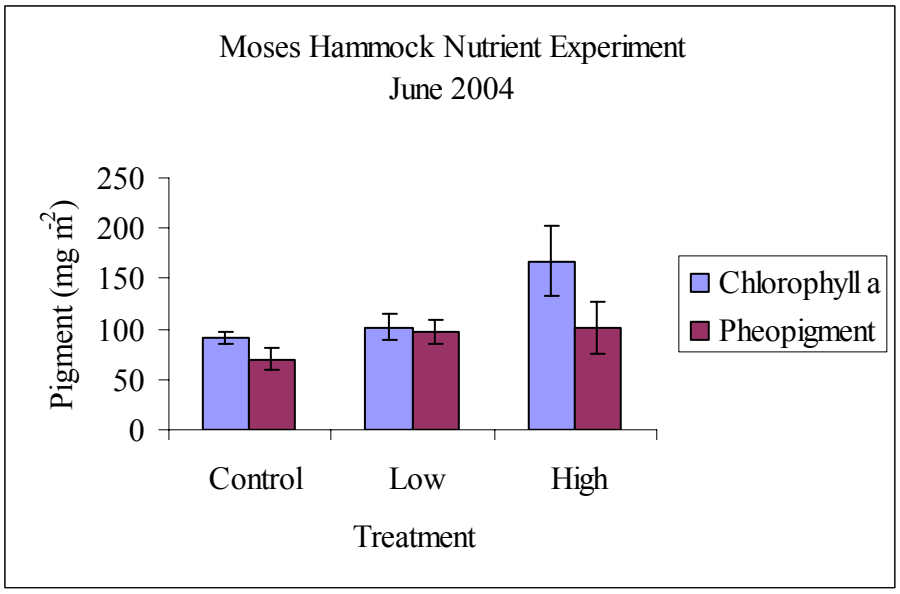

Figure 20. Chlorophyll *a* and pheopigments for the MH nutrient experiment. Chlorophyll *a* and pheopigments concentrations increased with nutrient additions (t-test, $p<0.05$)

Table 11. Chlorophyll *a*, pheopigments, chlorophyll *a* to pheopigments ratio, organic content and porosity for the nutrient experiments (n=6, ±SD). Nutrient additions significantly affected chlorophyll *a* concentration in the DB experiment (1-way ANOVA, $F=16.07$, $p<0.01$) and MH experiment (1-way ANOVA, $F=11.40$, $p<0.01$). Pheopigments concentrations were affected by nutrient additions in the MH experiment (1-way ANOVA, $F=3.72$, $p<0.05$). Chlorophyll *a* to pheopigments ratios were affected in the MH (1-way ANOVA, $F=10.78$, $P<0.01$) and DB nutrient experiment (1-way ANOVA, $F=10.43$, $p<0.01$).

Site and treatment	**Chlorophyll *a* (mg m^{-2})**	**Pheopigments (mg m^{-2})**	**Chlorophyll *a*: Pheopigments**	**Organic content (% dry weight)**	**Porosity (g water (g wet sed)$^{-1}$)**
Dover Bluff					
Control	92.63 ± 6.41	103.59 ± 6.79	0.90 ± 0.12	15.36 ± 0.54	0.78 ± 0.01
Low	133.89 ± 37.45	97.87 ± 12.59	1.38 ± 0.17	15.63 ± 0.40	0.81 ± 0.01
High	168.88 ± 13.72	112.86 ± 8.49	1.50 ± 0.06	15.38 ± 0.78	0.78 ± 0.01
Moses Hammock					
Control	91.62 ± 43.92	71.74 ± 33.23	0.70 ± 0.32	5.40 ± 0.48	0.49 ± 0.00
Low	101.94 ± 12.34	97.26 ± 10.62	1.05 ± 0.06	5.80 ± 1.35	0.52 ± 0.07
High	167.47 ± 32.17	101.53 ± 22.98	1.74 ± 0.53	5.72 ± 1.71	0.49 ± 0.07

DISCUSSION

This study tested the hypothesis that benthic primary production rates would vary seasonally and spatially due to the light regime present at the sediment surface in a salt marsh. Complex relationships were observed between benthic primary production and the factors that regulate production rates. In order to eliminate some environmental factors that may have affected production, the sediment cores were removed from the marsh, thus allowing a greater focus on light as a factor. Larger grazers such as fiddler crabs were removed from the sample cores, although meiofauna remained since it was impossible to remove them without disturbing the sediment. Tidal action, and therefore sediment transport and microalgal resuspension, was eliminated by determining rates in the laboratory. To maintain a moderate environment, a constant, thin layer of water on the sediment surface helped avoid desiccation and large fluctuations of salinity and temperature. Salinity and temperature were monitored periodically and the overlying water replaced as needed to avoid variation.

Primary production rates varied seasonally and spatially as expected. Variation in production rates was observed within single cores because the measurements could not be performed repeatedly at exactly the same spot and the distribution of benthic microalgae is patchy at sub mm scales (Pinckney and Sandulli 1990). Although variation in production rates within a sample core may not have been related to irradiance, production rates between zones and seasons were certainly affected by the *in situ* light regime. As photosynthetic day lengths may range from 6 h in December to 12.2 h in June (Davis and McIntire 1983), benthic microalgae have approximately twice the time to photosynthesize during summer months. The

greater availability of irradiance during the summer months may be why many studies demonstrate a summer peak in primary production (Davis and McIntire 1983, Laursen et al 2002). However, in the present study the primary production maximum (when hourly rates were summed for the three zones) was observed in February. Other studies concur with these results of an early spring primary production peak (Van Raalte et al. 1976, Pinckney and Zingmark 1993a). It is possible that the *Spartina* cover drives reductions in benthic primary production in summer months when plant biomass peaks (Van Raalte et al. 1976). In February, the *Spartina* canopy was at its bare minimum and allowed increased irradiances to reach the sediment surface in the levee and high marshes. Nevertheless, PAR continued to be highest on the bank, as it was throughout this study, but production rates were similar across zones for the February sampling. The similarity in production rates given variable PAR in the three zones suggests that benthic microalgae in *Spartina* zones are more efficient at using the available irradiance than those on the bank, which receives the highest irradiances throughout the year. Data collected from the P-I curves support this idea, revealing a higher α in the levee marsh followed by the high marsh then the bank. The bank microalgae expressed a low α, indicating they were not as photosynthetically efficient at low light intensities, and a high P_{max} which are characteristic of a high-light acclimated population (Valiela 1984, Pinckney and Zingmark 1993b). In contrast, an increased α, lower P_{max} (although similar in the levee) and a lower I_k were observed in the benthic microalgal communities in the *Spartina* habitats; characteristic of a low-light acclimated population. Since the bank microalgae were acclimated to high irradiances, photoinhibition was not observed. However, photoinhibition was observed in the high marsh and levee marsh at irradiances greater than 1200 µE. The highest measured *in situ* PAR in the *Spartina* zones was 750 µE (in the high marsh) and photoinhibition occurred at greater than 1200 µE, so it is unlikely

that benthic microalgae in the *Spartina* zones experience photoinhibition *in situ*. These results suggest that photoinhibition occurs when the shade acclimated communities beneath the *Spartina* are exposed to full sunlight. Furthermore, primary production in the *Spartina* zones appeared to be limited by low *in situ* irradiances, as P_{max} was reached at irradiances higher than those measured *in situ*. Additionally, I_k occurred between 200 and 450 μE, which was consistent with previous studies revealing an I_k between 200 and 400 μE (Davis and McIntire 1983).

The comparison of gross primary production rates in the different marsh zones between this study and that of the North Inlet estuary (Table 9) (Pinckney and Zingmark 1993a) revealed the bank rates are comparable to each other. However, the levee and high marshes of Moses Hammock have higher rates of primary production compared to North Inlet. The North Inlet estuary is a similar habitat to Sapelo Island and the same technique (oxygen microelectrode) was used in both studies to estimate gross primary production rates. When comparing annual primary production estimates from the present study against earlier results from Sapelo Island (Table 10), the current results show more than a twofold higher estimate (from 560 versus 200 g $C\ m^{-2}\ yr^{-1}$). As the previous results are over 40 years old, it is probable that during that time increased human activities have increased nutrients to this site thus causing an increase in primary production rates. The considerable increase may also be due to the more sophisticated techniques used to measure gross primary production in the present study. During the 1959 Sapelo Island study the microelectrode technique was not available; instead the bell jar technique and a flowing air system with CO_2-absorption columns were used to estimate photosynthesis (Pomeroy 1959). The methods used in the 1959 study have been known to underestimate primary production at times by as much as 75% (Darley et al. 1976, Pinckney and Zingmark 1991). It is interesting to note that the benthic microalgae production estimate in this study (560

g C m^{-2} yr^{-1}) was roughly half of *Spartina* production (1216 g C m^{-2} yr^{-1}, whole plant production) and seven times phytoplankton production (79 g C m^{-2} yr^{-1}) (Pomeroy et al. 1981) observed in the system.

The chlorophyll *a* maximum occurred in February coinciding with the primary production maximum. The results reported here are supported by data from a study conducted from 2001 to 2002 at the MH site, where benthic chlorophyll reached its maximum in January and February (Thoresen 2004). An early spring maximum was also found in similar coastal habitats by Davis and McIntire (1983) and Pinckney and Zingmark (1993a). Other communities experience spring biomass peaks such as the phytoplankton spring bloom. Thus, intuitively one would expect the benthic microalgal biomass maximum is initiated by the same factors, including increased irradiances, day lengths, nutrients and temperatures. Increased grazing frequently causes a decrease in biomass following the spring bloom (Darley et al. 1981). In the present study sampling occurred three months after the February biomass peak in May, thus there are no biomass data from the time immediately following the February maximum. It is very probable that grazing led to a decrease in biomass following the early spring biomass peak, as fiddler crabs were present at MH during warm months but were not observed during the cool months. The reduction in irradiance in the levee and high marsh due to the growing S*partina* canopies may have contributed to biomass declines. Furthermore, changing light regimes due to seasonal changes may elicit benthic microalgae photophysiological adaptations. Benthic microalgae are capable of altering their concentrations of primary and accessory pigments in order to rapidly (minutes to hours) photoacclimate to their changing light environment (Pinckney et al. 1995); for this reason, chlorophyll *a* content may vary per cell, per volume, per unit of organic carbon and per date (de Jonge and Colijn 1994).

Determining chlorophyll *a* concentrations is not only valuable for estimating the biomass of phototrophs but also for calculating the chlorophyll specific primary production (production divided by biomass). Chlorophyll specific primary production values revealed the bank and high marsh zones to be more efficient than the levee marsh (in regards to production per unit chlorophyll *a*). A study conducted in a similar habitat reported the bank had the highest chlorophyll specific primary production, which concurs with this study, followed by the *Spartina* zones (Pinckney and Zingmark 1993a). The chlorophyll specific primary production value may be used as an index of the physiological state of algae with lower numbers being characteristic of shade adapted cells and higher values characteristic of sun-adapted cells (Darley et al. 1981). The chlorophyll specific primary production values reported in this study suggest the bank microalgal populations were inhabited by sun-adapted cells and the levee marsh microalgal populations were inhabited by shade-adapted cells, with the high marsh having an intermediate value. The data from the chlorophyll specific primary production values are supported by the P-I data. This is reasonable given that the bank is micro-vegetated and receives direct sunlight, the levee marsh occupied by tall, dense *Spartina* and the high marsh occupied by shorter sparse *Spartina*.

Pheopigments showed a similar temporal pattern to chlorophyll *a* with the concentrations of both pigments being higher in the levee marsh. Pheopigments ranged from 23% of chlorophyll *a* to concentrations equal to and occasionally exceeding the chlorophyll *a* concentrations, thus revealing the importance of determining the difference between chlorophyll *a* and its degradation products. The ratio of chlorophyll *a* to pheopigments has been used as an indicator of phytoplankton succession where a decreased ratio indicates more senescing cells or a later successional stage (Connor et al. 1982). The ratio may also be an indicator of herbivory,

with lower values illustrating a greater grazing pressure or a stressed community (Pinckney and Zingmark 1993a). In this study, the chlorophyll *a* to pheopigments ratio was highest in the levee marsh and lowest in the bank. The chlorophyll *a* to pheopigments ratios spatial and seasonal trends were similar to those observed in the chlorophyll *a* values showing increases in the ratios when chlorophyll *a* increased and decreases in the ratios when chlorophyll *a* decreased. These patterns may be suggestive of active growth periods followed by senescent or periods of increased grazing (Pinckney and Zingmark 1993a).

Organic content of the MH sediments did not reveal a pronounced seasonal pattern although it was continually higher in the levee and high marshes. Despite the increased primary production in February there was no corresponding increase in sediment organic matter, suggesting that grazers are limited by their food supply. If food supply was not limiting to grazers, organic matter would accumulate. Generally, only small proportions of organic matter accumulate in comparison to the amount produced by photosynthesis however, in areas of very high primary production and shallow depths there may be considerable organic matter accrual (Valiela 1984). Although an organic matter peak was not observed in the present study, a summer maximum was documented in a Pacific coast estuary (with spatial patterns similar to chlorophyll *a*) (Davis and McIntire 1983). In a Dutch estuary low organic content was observed in the winter and relatively high values the remainder of the year (De Jonge and Colijn 1994). Seasonal and spatial variability of organic matter can be related to rates of consumption or decomposition by heterotrophs, sedimentation rates and the aerobic status of the sediment (Valiela 1984). Organic content measured more than two decades ago in the Duplin River marsh sediments averaged 10-20% (Pomeroy and Imberger 1981). These previous organic matter averages are equivalent to averages in the present study of 8-22%. Although Sapelo Island and

the surrounding area (including the Duplin River and MH) is considered a pristine site there is no denying population growth upstream and the resultant increase in nutrient run-off. Eutrophication stimulates primary production which can disrupt the balance between the production and metabolism of organic matter in coastal zones (Cloern 2001) and can lead to an enrichment of organic matter in the sediments (Meyer-Reil and Köster 2000). Thus equal organic matter two decades ago and at present suggests that the buffer capacity, a fundamental function of estuaries, is intact. (Buffer capacity is the property of an ecosystem to compensate disturbances through internal regulation mechanisms. (Meyer-Reil and Köster 2000))

Given that DB is a developed site that receives elevated nutrient inputs via septic derived materials, we did not expect nutrients to stimulate benthic primary production at this site. The experiment results suggest that production was not limited by nutrients, at least not at this time of year. *In situ* nutrient concentrations often exceeded those used in the experiment. Nevertheless, chlorophyll *a* significantly increased with nutrient additions; which agrees with the results of other studies (Nilsson et al. 1991, Camacho and de Wit 2003). It is possible that the unchanged pheopigment concentrations are a result of the benthic microalgae already under the chronic stress of pollution as suggested by the similar chlorophyll *a* and pheopigment concentrations seen in the control. With the increase in chlorophyll *a* the chlorophyll *a* to pheopigments ratio also increased from 0.9 to 1.5 in the high treatment.

On the contrary, the MH nutrient experiment displayed a significant increase in primary production, chlorophyll *a* and pheopigments after the nutrient additions. Although nitrogen is available in the MH marsh, the nutrient may be limiting relative to its demand, as shown by the stimulation of production after nutrient additions. The results indicate benthic primary production was nutrient limited during the summer. It is possible that the fairly undisturbed and

undeveloped environment of this site had an influence on the results. Compared to DB the MH site did not have the bordering community that may have provided additional nutrients to the benthic microalgal community. As previously noted, the increase in biomass has been observed in other studies as has the production increase after nutrient additions (Nilsson et al. 1991, Camacho and de Wit 2003). The pheopigment increase is reasonable, as the MH benthic community was assumed not to be under the stress of pollution and elevated nutrient additions may have altered this. In the control treatment pheopigments were approximately 76% of the chlorophyll *a* and increased to an equal amount in the low treatment further demonstrating the stressed condition. The MH high treatment displayed a comparable chlorophyll *a* to pheopigments ratio to the DB high treatment. With an increase in biomass and photosynthesis, an analogous increase was anticipated in the organic content although one was not observed. It is possible that if the duration of the experiment had been extended an increase in organic content would have occurred as very high production for an extended length of time would increase the organic matter content relative to demand.

An unexpected outcome was the reduction of primary production observed at the end of both the MH and DB nutrient experiments. The decrease may be due to the increased bubble formation disrupting or partly removing the sediment surface after the many days of nutrient addition. Sustained high photosynthetic activity caused O_2 bubbles to form directly below the surface and once bubbles penetrated the surface that area was no longer favorable for microelectrode measurements. It was difficult to locate an undisturbed area to measure with the microelectrode, therefore, it does not signify that production decreased, but perhaps only areas with lower production (no or little bubble formation) were able to be measured. This partial removal of the producing layer by O_2 bubbles was also experienced by Nilsson et al. (1991).

Additionally, it has been reported that the O_2 microelectrode method greatly underestimates primary production in sediment with extensive bubble formation (Revsbech et al. 1981). The decrease may also be related to a change in grazing pressure. It is possible that the release of predatory pressure on the infauna by the elimination of larger grazers placed an increased grazing pressure on the microalgae.

O_2 penetration into the sediment may be used as a measure of benthic health; for this reason depths of the steady state O_2 profiles were analyzed. Increased eutrophication may cause reduced O_2 penetration due to sulfate respiration dominating over O_2 respiration (Meyer-Reil and Köster 2000). As eutrophication can cause organic matter accumulation in the sediments, this increases the demand for O_2 for the decomposition of the organic matter. The increased aerobic decomposition then creates O_2 depletion thus resulting in increased sulfate respiration and the release of sulfide. The presence of sulfide is evident at the DB marsh site by its odor and along with the sediment's black color (indicating anoxia and the presence of acid-volatile sulfides) one can deduce the site is eutrophic. Comparisons of O_2 penetration between DB and MH revealed that light steady state depths were significantly deeper in the DB sediments and dark steady state depths were deeper in the MH sediments (t-test, $p<0.05$). The results suggest when light and increased nutrients are available the increased production of O_2 through photosynthesis causes deeper penetration of O_2 into the sediment. Increased O_2 penetration was noted in the MH low nutrient treatment indicating the increased production due to addition of nutrients. On the other hand, in the dark and thus in the absence of oxygenic photosynthesis, nutrient additions will cause a decrease in O_2 penetration into the sediment. Decreased O_2 penetration in the dark may be due to increased sulfate respiration. The only decrease in O_2 penetration observed was in the MH high treatment on the last day of the experiment, the microalgae perhaps just beginning to

show the negative effects of increased nutrients. Essentially, the depths followed similar patterns to their respective steady state O_2 concentrations. In the presence of light the O_2 penetration depth can be indicative of the photic zone thus it is reasonable that the light depths had patterns similar to those of the corresponding light O_2 steady state concentrations. The photic zone depth represents the amount of microalgae receiving adequate illumination for photosynthesis.

SUMMARY

The purpose of this study was to provide insight into the adaptation of benthic microalgae to their light environment and how the availability of certain nutrients impacts rates of photosynthetic O_2 production in benthic microalgae. Primary production, chlorophyll *a* and pheopigments were quantified, important ratios and sediment characteristics such as organic content calculated, P-I curves constructed in order to examine the microalgal response to illumination. The results showed temporal and spatial variability in photosynthetic rates and indicated benthic microalgae are well adapted to the light environment encountered on the marsh. Although light was an overriding factor, there was evidence of other biological, physical and chemical environmental factors influencing benthic primary production rates.

As MH is an undeveloped site, a developed site (DB) was selected to compare responses of benthic microalgae to experimental nutrient additions. These two sites supply benthic microalgae with contrasting nutrient concentrations and as a result the two benthic microalgal communities provided opposing results after the experimental nutrient additions. Primary production, steady state O_2 concentrations in light and dark, chlorophyll *a* and pheopigments were quantified, ratios calculated, sediment characteristics and depths of O_2 penetration measured in order to examine the benthic microalgal response to nutrient additions. The results suggested that the *in situ* nutrient status is a deciding factor in the benthic microalgal nutrient response. The benthic microalgae of DB were supplied with increased nutrients in their marsh habitat and thus nutrient limitation was not observed in this microalgal community. Compared

to DB, MH receives lesser concentrations of nutrients and nutrient limitation was observed in the MH benthic microalgae.

The information collected in this study is an addition to the LTER data in which MH is a main site and primary production is a focal point. The results obtained may be compared over time to track changes in the relatively pristine estuary or may be utilized as complementary data to other studies in order to form a more comprehensive picture. Examining a system's photosynthetic populations is crucial in understanding the function of an ecosystem. In the past benthic microalgae have been overlooked but their importance is now recognized as they can contribute more than 50% of the primary production in a coastal system (Laursen et al. 2002) and are a major food source for many organisms. Not only are benthic microalgae an important food source for herbivores and detritivores but also suspension feeders, thus forming important trophic linkages. A Duplin River study proved that the diets of filter feeding bivalves are composed partially of benthic diatoms which have been resuspended by the flood tide (Thoresen 2004). Intertidal marshes occupied by benthic microalgae comprise 80% of the Duplin River watershed (Pomeroy and Imberger 1981) therefore; the contribution of benthic microalgal production to the salt marsh ecosystem is clearly significant.

Although estuaries are some of the richest and most productive ecosystems in the world they are fragile and threatened (Söderbaum 1996). Historically, low concentrations of nutrient run-off naturally limited phytoplankton growth (and therefore benthic microalgal growth) preventing it from overaccumulating (D'Elia 1987). Presently, coastal areas clearly are receiving an excess of nutrients resulting in eutrophication. Determining patterns of nutrient limitation can be used for management strategies in order to prevent negative impacts on these ecosystems.

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