PHYTOPLANKTON RESPONSES TO MASS CORAL SPAWNING IN THE FLOWER GARDEN BANKS, GULF OF MEXICO

A Thesis

by

COURTNEY LEIGH HORNE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2011

Major Subject: Oceanography

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Approved by:

Chair of Committee,	Gilbert T. Rowe
Committee Members,	Antonietta S. Quigg
	Douglas Biggs
Head of Department,	Piers Chapman

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ABSTRACT

Phytoplankton Responses to Mass Coral Spawning in the Flower Garden Banks, Gulf of Mexico. (May 2011)

Courtney Leigh Horne, B.S., University of Maryland Baltimore County

Chair of Advisory Committee: Dr. Gilbert Rowe

Mass coral spawning represents a nutrient input to coral reef systems that for Pacific reefs has been shown to stimulate pelagic and benthic processes. If phytoplankton in the water column over the reef are able to utilize this annual nutrient input, this could potentially alter phytoplankton biomass and community composition, in what is normally a very oligotrophic system. Sampling was performed at East Flower Garden Bank (EFGB), Gulf of Mexico during May, July, and August 2009. The annual coral spawning event occurred there August 11-14, 2009. Samples were collected morning and evening at three depths and analyzed for nutrients, chlorophyll *a*, accessory pigments, phytoplankton species composition, and carbon, hydrogen, and nitrogen (CHN).

During spawning, only small changes in nutrient concentrations were detected. Dissolved inorganic nitrogen (DIN) peaked on the second day of spawning and N:P ratio was highest on 5/28, likely due to particularly phosphate concentration. Chl *a* biomass was significantly different between sample dates and the biomass increased steadily throughout the spawning period. The contribution of different phytoplankton classes to total chlorophyll *a* was determined using known pigment algorithms. Prokaryotes were the dominant class across the entire sampling period with 60-80% abundance. *Trichodesmium* spp. was the dominant genus throughout the study and genus specific changes per sample date were seen. On 8/11 and 8/13 two genera contributed the majority of chl *a* (*Trichodesmium* spp. and *Ceratium* spp.; *Cylindrotheca* spp. and *Trichodesmium* spp., respectively). Abundance showed variability during spawning with

a peak at 11 cells/ml on 8/12. The high abundance of *Trichodesmium* spp. could indicate N limitation is alleviated at the Flower Garden Banks (FGB).

Current literature on coral spawning is limited to studies performed in the Great Barrier Reef, with assessment areas close to a major shoreline. Genera found at EFGB were similar to those found in other reef systems. It cannot be determined if nutrient input increased diversity, as diversity was high prior to spawning as well. Greater increase in available forms of nitrogen would have likely been found several days post major spawning.

The FGB were a unique system to study, as they are coral reefs, but are located 200 km offshore. This study provided a snapshot into phytoplankton dynamics as a result of spawning. Changes across the short time scale were seen in biomass and community composition.

DEDICATION

I dedicate this to my parents for their endless encouragement, support, and love.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Gilbert Rowe, for supporting me throughout this project and offering great advice and encouraging me to think about things in different ways.

I would also like to thank my committee member, Dr. Antonietta Quigg for her very helpful suggestions and putting up with countless questions. I also thank her for use of her laboratory equipment for analysis. I thank Dr. Douglas Biggs for much help via email on my proposal and great suggestions for my final document. I would like to thank G.P. Schmahl and Emma Hickerson with the Flower Garden Banks National Marine Sanctuary for permitting me to carry out this project and for ship time on board the R/V Manta. I also thank them, as well as the R/V Manta crew, for assistance with collecting the samples.

Thanks also go to my friends at Texas A&M including (but not limited to) Clif and Chi-Lin for help with statistical analysis, the entire Quigg lab for help and suggestions on sample analysis, and Sam for the R script. I also want to extend my gratitude to the Texas A&M University at Galveston Marine Biology Department, which provided the funding for this study.

Finally, thanks to my family and friends for their support and encouragement.

NOMENCLATURE

ADONIS	Permutational Multivariate Analysis of Variance
ANOVA	Analysis of Variance
BLD	Below Limit of Detection
C	Carbon
CDT	Central Daylight Time
Chl *a*	Chlorophyll *a*
CHN	Carbon, Hydrogen, and Nitrogen
CTD	Conductivity, Temperature, and Depth
DIN	Dissolved Inorganic Nitrogen
EFGB	East Flower Garden Bank
FGB	Flower Garden Banks
FGBNMS	Flower Garden Banks National Marine Sanctuary
GBR	Great Barrier Reef
GF/F	Glass Fiber Filter
GMT	Greenwich Mean Time
GOM	Gulf of Mexico
HPLC	High Performance Liquid Chromatography
MANOVA	Multivariate Analysis of Variance
N	Nitrogen
N:P	Nitrogen to Phosphorus
NWGOM	North West Gulf of Mexico
OM	Organic Matter
POC	Particulate Organic Carbon
PON	Particulate Organic Nitrogen
TPC	Total Particulate Carbon

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1. INTRODUCTION

Coral reefs are biologically productive ecosystems in subtropical oceans (Glud et al. 2008). Their species diversity, coupled with information about the mortality, settling rates, and reproductive success of coral planula larvae that are released from these ecosystems, has been well documented (Gittings et al. 1992; Lugo-Fernandez et al. 2001; Bassim et al. 2002; Shearer and Coffroth 2006). These spawning events represent short-term nutrient and organic carbon enrichment of the entire reef community. Reef fauna consume this input of energy rich material (Glud et al. 2008). Only a few recent studies have focused on the phytoplankton response to increased nutrients from the recycling of spawn material (Eyre et al. 2008, Glud et al. 2008, Wild et al. 2004, Wild et al. 2004b). This nutrient addition has the potential to stimulate water column primary productivity over the coral reef. Recent studies have shown that macrofauna-regenerated nutrients are responsible for stimulating benthic primary production in/around reefs (Uthicke and Klumpp 1998; Glud et al. 2008). In fact, benthic consumption of spawn material has been suggested to lead to a distinct bloom of dinoflagellates at Heron Island, Great Barrier Reef (Glud et al 2008).

The present paradigm is that coral spawning events stimulate biological activity in both the seafloor and water column. Chlorophyll *a* concentrations at the Great Barrier Reef have been shown to increase substantially after coral spawning (Guest 2008), suggesting an increase in phytoplankton biomass. (Glud at al. 2008). The addition of nutrient rich larvae from the reefs, during spawning, can have large effects on the reef carbon system and alter other nutrient concentrations. It has been shown that the phytoplankton rapidly exploits the available nutrient pools provided by spawn material (Wild et al. 2004; Eyre et al. 2008; Glud et al. 2008).

This study examined phytoplankton responses to mass coral spawning in the Flower Garden Banks National Marine Sanctuary (FGBNMS) in the Gulf of Mexico.

This thesis follows the style of Limnology and Oceanography.

Spawning events introduce large amount of organic material to the ecosystem and it is believed that this material will influence the community composition of phytoplankton in the Flower Garden Banks (FGB). Within the ecosystem there were small scale changes in the species of phytoplankton present in response to spawning. Spawning studies in the Great Barrier Reef have shown a shift in the phytoplankton community from pre-spawning months to spawning events (Glud et al. 2008; Eyre et al. 2008). Eyre et al. (2008) and Glud et al. (2008) suggest that this is due to the ability of certain species to more efficiently take advantage of the short-term increase in nutrients.

1.1 *Coral Reef Ecosystems*

Odum and Odum (1955), in their pioneer work on coral reef ecosystems, suggested that coral reef communities of the world are varied associations of plants and animals growing luxuriantly in tropical waters of impoverished plankton content. These communities exist in oligotrophic seawater, but are among the most productive of coastal marine ecosystems (Furnas 1991; Ferrier-Pages and Gattuso 1997). Most researchers now agree that this high productivity is a result of nutrients being intensely cycled through the microbial loop (Risk and Muller 1983; Paul et al. 1986). Depending on the location of the reef system, organisms in coral reefs vary largely. There are important regional differences in the species richness, functional composition, and biological communities. Although the Caribbean and Great Barrier Reef broadly share the same suite of functional groups, the species richness and taxonomic composition among functional groups is very different in the two regions. Caribbean reefs have only a fraction of the number of species found on the Great Barrier Reef, approximately 28% for fishes and 14% for corals. (Bellwood et al. 2004).

The two main products of the ecological functions in coral reefs are production of organic and inorganic carbon. McClanahan et al. (2002) show a conceptual carbon pathway model in coral reef ecosystems (Fig. 1). The rate of carbon accumulation in

organic and inorganic compartments is a function of solar radiation, its rate of attenuation through the water column and incoming nutrients and organic matter. All of

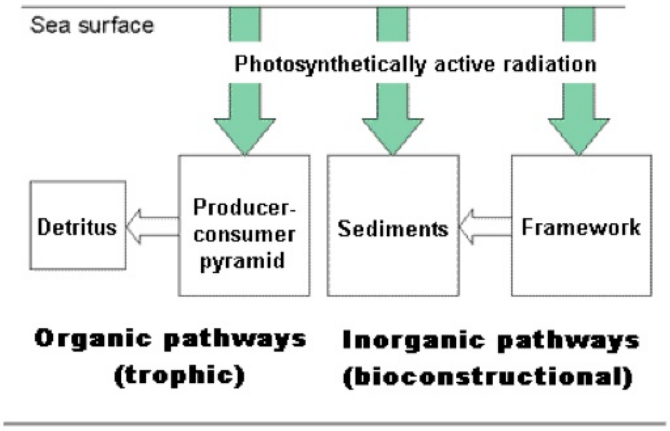

Fig.1: Carbon pathways in coral reef ecosystems (From McClanahan et al. 2002). Individual components will vary with unique reef systems.

these components vary with reef location and with depth on an individual reef. Reef organisms fix carbon to produce and maintain their physical structures (McClanahan et al. 2002). These organisms produce inorganic carbon in the form of calcium carbonate through the building of reef skeletal structure. Coral framework can be broken down into sediments and make up an important fraction of the inorganic carbon pathway. Reef systems also provide ecosystem services such as sustaining commercial fisheries, protecting beaches and coastlines from storm surges, and supporting nurseries and cultural services.

Coral reefs are critically important for the ecosystem goods and services they provide to maritime tropical and subtropical nations. Reefs are in serious decline; an estimated 30% are already severely damaged, and close to 60% may be lost by 2030 (Hughes et al. 2003). There has been a major loss of coral cover and diversity (Hoegh-Guldberg 1999; Wilkinson 2000), coupled in many areas with an increase in algal

biomass and shift in algal community structure (McCook 1999; Szmant 2002). Corals are impacted not only by natural causes such as climate change, but also by human pressures. Destructive fishing practices and coastal pollution are just two of the important anthropogenic stresses that are presently affecting community structure and reef health and contribute to present day coral reef decline (Johannes 1970; Hatcher et al. 1989; Glynn 1998; McCook 1999). Anthropogenic eutrophication is a major problem for coastal reef systems. Natural nutrient enrichment events have been studied in coral systems in the Gulf of Eilat (Genin et al. 1995) where an unusual upwelling event caused a sudden algal bloom. Natural events, however, are far less regular than the anthropogenic input of nutrients from sewage and other runoff. Elevated nutrients from eutrophication are associated with higher levels of water column productivity (Birkeland 1977,1987) and this enrichment can reduce light penetration to the reef due to nutrient stimulated phytoplankton growth (Dubinsky and Stambler 2006).

1.2 *Phytoplankton in Reefs*

Many of the pelagic components of a coral reef ecosystem are dependent on the phytoplankton associated with the reef. Early studies of benthic-pelagic coupling in tropical coral reefs focused on zooplankton rather than phytoplankton as the principal source of prey (Tranter and George 1969; Glynn 1973; Johannes and Gerber 1974; Hamner et al. 1988; Erez 1990, Yahel et al. 1998), although it is currently understood that numerous members of a coral reef community are known to feed on particles within the size range of phytoplankton (Yahel et al. 1998). More recent studies have found that phytoplankton biomass in coral reefs commonly exceeds that of zooplankton by an order of magnitude (Roman et al. 1990; Yahel et al. 1998).

Little is known about the phytoplankton community in coral reef waters and whether its composition differs from that in the adjacent ocean (Van Duyl et al 2002). Both Richter et al (2001) and Van Duyl et al (2002) have found that filter feeders in reef environments can keep phytoplankton densities in check and can cause a depletion of

bacteria sized cells in coral reef waters. Van Duyl et al (2002) compared coastal reef sites with open ocean (3km from shore) sites and found that the relative amount of the pigments fucoxanthin (indicative of diatoms) and peridinin (indicative of dinoflagellates) was usually higher in reef waters than in the ocean sites.

Studies determining genus or species of coral reef phytoplankton have been carried out primarily in the Great Barrier Reef, however, several studies carried out in fringing reef systems of the Red Sea and Japan have found that ultraphytoplankton (<8μm) contribute up to 78% of total chlorophyll *a* in upper waters (Yahel et al. 1998) and cells smaller than 10μm dominate cell abundance (Ferrier-Pages and Gattuso 1997). In the Bora Bay study of Japan (Ferrier-Pages and Gattuso 1997), microphytoplankton was dominated by *Nitszchia* sp, *Rhizosolenia* sp., *Skeletonema* sp., and *Coscinodiscus* sp. This is comparative to common genera found in the Great Barrier Reef, which include *Nitzschia*, sp. *Bacteriastrum* sp., *Rhizosolenia* sp., *Chaetoceros* sp. and *Thalassionema* sp. (Furnas and Mitchell 1986). It is not currently known how phytoplankton species shift in response to nutrient inputs from coral reproduction.

1.3 *Coral Mass Spawning*

Mass spawning is the synchronous release of gametes by multiple species of corals (Harrison and Wallace, 1990). Mass spawning has been documented in the Pacific Ocean and Caribbean reefs, as well as the Flower Garden Banks in the NW Gulf of Mexico. This reproductive strategy is visually impressive and predictably related to annual lunar cycles (Gittings et al. 1992). Hermaphroditic broadcast spawning involves the release of buoyant, lipid-rich gametes (known as egg-sperm bundles) into the water column for external fertilization (Guest 2008). Synchronous spawning within species is crucial to ensure cross-fertilization (Guest 2008) and enhances reproductive success. Mulitple species of corals often display an over-lap in spawning times. The first such multi-species spawning event was documented on the Great Barrier Reef in the early 1980's. Over-lapping release can be attributed to the difference in coral species

response to timing cues (Harrison et al. 1984; Babcock et al 1986; Oliver et al 1988; Guest 2008). The released egg-sperm bundles result in spawn slicks, which cover the water surface, resulting in large inputs of nutrients and organic carbon to the system.

Coral mass spawning has been shown to have a range of impacts on the ecosystem. Synchronous multispecies spawning releases a large volume of eggs and sperm into the reef system over a relatively short time-span. This results in episodic input of labile carbon (and associated nitrogen and phosphorus) to the coral reef ecosystem (Eyre et al. 2008). For example, an estimated 310,000 kg of C and 18,000 kg of N were released from coral eggs alone during a spawning event at Heron Island Reef in 2001 (Wild et al. 2004). This same spawning event was shown to stimulate sedimentary oxygen consumption rates for up to 9 days after spawning (Figure 2) (Wild et al. 2004). This suggests degradable organic matter from the spawn enhanced metabolism on the reef. A larger spawning event in the same location in November 2005 had an immediate, but short-term effect on the concentration of particulate organic matter detected in the water column and sediment (Guest 2008). Particulate organic nitrogen (PON) concentrations in the water column peaked after spawning. Isotopic signatures of the PON reflected that of coral gametes. These concentrations remained high for 17 days post spawning. (Eyre et al. 2008; Glud et al. 2008; Wild et al. 2008). A 2008 study done at the same site showed that mass spawning by corals measurably affected the oligotrophic reef ecosystem for at least 3 weeks, with considerable changes in water column organic matter availability, chlorophyll *a* concentrations and biological oxygen demand (Wild et. al. 2008). These data imply that the reef ecosystem exhibits a large response to the increase in nutrients and rapidly processes the spawn material.

Reef spawning represents a significant trophic link between corals and reef fish. Mass spawning provides a large seasonal pulse of high energy prey that potentially benefits reef fish that are capable of capturing and digesting coral propagules (Pratchett et al. 2000). Consumption of these coral propagules can represent a trophic link between

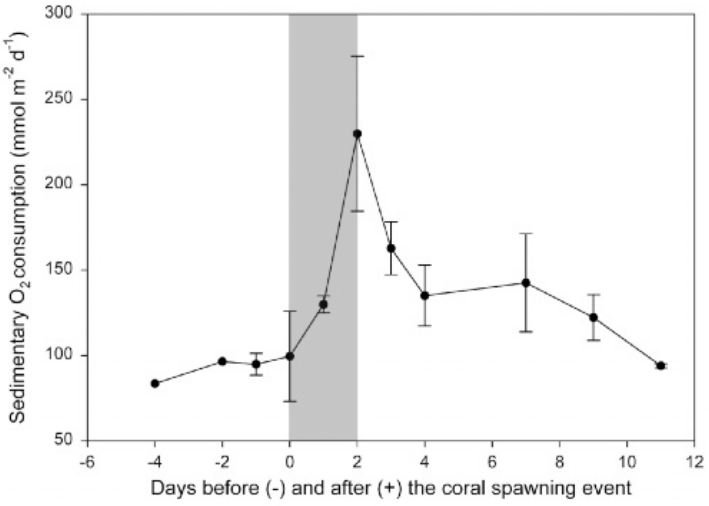

Fig. 2: Sedimentary oxygen consumption measured in chamber experiments before, during, and after *Acropora* spawning (From Wild et al. 2008).

corals and higher consumers, as corals, rather zooxanthallae contained within corals, account for 1.5% of global marine productivity (Muscatine 1980). Coral propagules are more easily consumed by higher trophic levels, as they are large, colorful, and lack the obvious morphological and physical defenses that benthic adults possess (Pratchett et al 2000). Pratchett et al (2000) showed that numerous fish species exploit the pulse of lipid-rich prey from the coral spawn in the Great Barrier Reef. One in particular, *Abudefduf whitleyi,* showed a marked shift in dietary composition during the mass spawning of corals, with clearly recognizable coral eggs in stomach content post spawning. Additionally, they found that fish feeding extensively on coral propagules amassed considerable lipid stores, which could greatly improve the quality and survivorship of their progeny.

Additional trophic links have been found between products of coral mass spawning and bacteria and viruses. In coral reef waters and sediments, bacteria constitute a significant proportion of the microbial biomass and display fluctuations in cell numbers and productivity relative to the availability of organic substrates within the

reef system (Moriarty et al. 1985, Hansen et al. 1992, Wild et al. 2004b). Patten et al (2008) found that the input of highly labile organic matter induced significant shifts in bacteria and virus-like particle abundances within reef waters and sediments. Bacterial abundances increased 2.1 fold, three days following the first major spawning at Heron Island on the Great Barrier Reef. This increase coincided with a planktonic algal bloom and elevated water column chlorophyll *a* concentration.

1.4 *Hypotheses*

A. H0: There is no change in water column nutrient levels at the Flower Garden Banks due to the release of egg sperm bundles.

H1: Nutrient levels at the Flower Garden Banks will be affected as a result of egg sperm bundles released into the water column.

B. H0: There is no effect of coral spawning on the phytoplankton biomass and community composition at the Flower Garden Banks.

H1: Coral spawning affects phytoplankton biomass and community composition at the Flower Garden Banks.

2. METHODS

2.1 *Study Site*

The Flower Garden Banks National Marine Sanctuary protects three separate areas; the East and West Flower Garden Banks (27'54.5'N, 93'36.0'W and 27'52.5'N, 93'49.0W, respectively (Fig. 3) are located 170 km southeast of Galveston, Texas. Stetson Bank is located northwest of East and West bank and sits approximately 70 km from shore. East and West Bank are two major topographic highs in the northwest Gulf of Mexico resulting from diapirism of Jurassic-age salt (Gittings et al. 1992). They rise to within 18m of the sea surface from shelf depths of 100 to 150 m. The lower 35 m of both banks are periodically covered by a nepheloid layer (resuspended muddy sediment) that limits coral growth, however, the upper 50 m are exposed to oceanic conditions favoring reef growth (Rezak et al. 1985; Deslarzes 1998).

The reef communities between 15 and 36 m are dominated by *Montastrea annularis* (~30% cover), *Diploria strigosa* (~6%), *Colpophyllia natans* (~5%), *Montastrea cavernosa* (~4%), the hydrozoan *Millepora alcicornis* (~4%), and *Porites astreoides* (~2%) (Bright et al. 1984). Total coral cover on the reef averages about 55%. Many species present at East and West bank are also present at Stetson and nearby Sonnier Bank, but on these middle-shelf banks there is lower percent cover (Rezak et al. 1985).

The Flower Garden Banks provide a unique environment to study. These isolated environments harbor coral reefs very near the northward physiological limits for tropical hermatypic corals. Less than 50 km northward, winter temperatures are too low for reef development (Rezak et al. 1990). While reduced diversity is attributed to the northerly location of the reefs, abundance and growth rates compare favorably with those in more tropical locales at similar depths (Rezak et al. 1985). Mass spawning at the Flower Garden Banks is synchronous with spawning at other Caribbean reefs and is correlated with lunar phase as well as maximum water temperature (Gittings et al. 1994; Hagman et

al. 1998; Lugo-Fernandez et al. 2001). The first direct observation of mass coral spawning at the Flower Garden Banks was made in August 1990.

Fig. 3: Location map of the Flower Garden Banks. Red box indicates specific study location.

2.2 *Sample Collection*

Water samples were collected in May, July, and August, and September 2009 at East Flower Garden Banks, NW Gulf of Mexico (27'54.6463'N; 93'36.0668'W). In May, samples were collected on board the M/V Fling and in July and August from the R/V Manta. In September 2009, samples for nutrients and species identification only were collected from Sonnier Bank. In May, July, and August, samples were collected via

Niskin bottle and transferred to acid-washed 3.7L plastic gas cans for transport back to Texas A&M Galveston (Table 1). Samples were collected from surface, mid-depth (9 m) and deep (18 m). During the May sampling dates, mechanical difficulties with the Niskin bottle allowed only surface water to be collected. For the July and August samples, only PM samples were able to be collected. The majority of samples were filtered onto 25mm Whatman GF/F filters on board within 6 hours of collection.

Table 1. Dates and times of all samples with type of parameters measured at each occasion.

Date	Time	Community Composition	Nutrients	Pigments (HPLC & chl *a*)	CHN
28-May	8:00	x		x	x
28-May	12:00	x	x	x	x
28-May	8:00	x	x	x	x
8-Jul	21:00	x	x	x	x
8-Jul	21:00	x	x	x	x
8-Jul	21:00	x		x	x
11-Aug	8:30		x	x	x
11-Aug	8:30	x	x	x	x
11-Aug	8:30	x	x	x	x
11-Aug	21:00	x		x	x
11-Aug	21:00	x	x	x	x
11-Aug	21:00	x	x	x	x
12-Aug	9:00		x	x	x
12-Aug	9:00	x	x	x	x
12-Aug	9:00	x	x	x	x
12-Aug	21:30	x	x	x	x
12-Aug	21:30	x	x	x	x
12-Aug	21:30	x	x	x	x
13-Aug	21:00	x		x	x
13-Aug	21:00	x		x	x
13-Aug	21:00	x		x	x

Those that could not be processed on board were filtered immediately upon arrival back to the laboratory. Samples were filtered for pigments, Chl *a* biomass, and CHN and were frozen at -80°C until analysis. For each depth, small aliquots of water were taken for identification of phytoplankton to genus level. Three liters of sample water was poured through a 20μm phytoplankton net and preserved with formalin to

make a final solution of 3%. Samples were stored at room temperature in the dark until analysis. Additional 30 ml samples were frozen for nutrient analysis.

2.3 *Sample Analysis*

2.3.1. Accessory Pigments- Phytoplankton contain photosynthetic pigments that are used to harvest light energy for photosynthesis. Chlorophyll *a* is most commonly used as a biomass indicator, but there are also accessory pigments that can be used to characterize a phytoplankton community. The seven major accessory pigments are shown in Table 2. The quantification of these pigments provides the basis for calculating the contribution of individual phytoplankton groups to the total amount of chl *a* (Henriksen et al. 2002). Pigments were from samples using reverse phase high performance liquid chromatography (HPLC) (Letelier et al 1993). This method provides a detailed description of a phytoplankton assemblage over the whole size range by determining the concentration of chlorophyll *a* and various accessory pigments specific to taxonomic groups (Claustre 1994).

Water samples were filtered onto 25mm Whatman GF/F filters for pigment analysis and immediately frozen at -80° C. The GF/F filters were extracted in 100% acetone (0.75 ml), sonicated and stored at -20C for 15-20 hours after extraction. Filtered extracts were spiked with 1 M ammonium acetate ion-pairing solution (final concentration 0.2 M) and 375 ul of sample was injected into a Shimadzu HPLC equipped with a single monomeric (Rainin Microsorb-MV, 0.46x10cm, 3 um) and one polymeric (Vydac 201TP, 0.46x 25cm, 5 um) reverse-phase C18 column in series. A non-linear, binary gradient was used for pigment separation (Pinckney et al. 1996). Pigment peaks were identified by comparison of retention times and absorption spectra with pure crystalline standards of chlorophylls a, b, β-carotene (Sigma Chemical Company), fucoxanthin, lutein, canthaxanthin and zeaxanthin (Hoffman-LaRoche and Company). Other pigments were identified by comparison to extracts from phytoplankton cultures (Wright et al. 1991). Photopigment concentrations were

quantified using chromatogram peak area and the appropriate extinction coefficients (Rowan 1989; Jeffrey et al. 1997). All HPLC analyses were performed at the Photopigment Analysis Laboratory at the University of South Carolina.

Table 2: Seven diagnostic accessory pigments for characterizing phytoplankton groups in the ocean. Source: Claustre 1994.

Table 1. Diagnostic accessory pigments used to characterize the main phytoplankton groups in the ocean.

Diagnostic pigment	References	Phytoplankton group
Fucoxanthin	Jeffrey 1980	Diatoms
Peridinin	Jeffrey 1980	Dinoflagellates
19′-HF and 19′-BF*	Wright and Jeffrey 1987	Nanoflagellates†
Chlorophyll *b*‡	Jeffrey 1980	Green flagellates
Alloxanthin	Gieskes and Kraay 1983	Cryptophytes
Zeaxanthin	Guillard et al. 1985	Cyanobacteria
Zeaxanthin, divinyl-chlorophyll *b*‡	Goericke and Repeta 1992	Prochlorophytes§

* 19′-HF: 19′-hexanoyloxyfucoxanthin; 19′-BF: 19′-butanoyloxyfucoxanthin.
† The term nanoflagellates refers essentially to chrysophytes and prymnesiophytes which are characterized by 19′-BF and 19′-HF, respectively.
‡ Chlorophyll *b* and divinyl-chlorophyll *b* are regrouped as "Chl *b*" in this study as they coelute on reverse-phase HPLC.
§ Zeaxanthin is an accessory pigment in surface prochlorophytes while divinyl-chlorophyll *b* is an accessory pigment in deeper populations (Morel et al. 1993).

2.3.2. Chlorophyll a- Frozen 25mm Whatman GF/F filters were analyzed for chlorophyll *a*. Chlorophyll was extracted using 3.6 ml of acetone per filter and placed in the dark for 24 hours. Samples were then analyzed to determine chlorophyll a biomass using a Shimadzu UV-2450 spectrophotometer following the methodology of Jeffrey and Humphrey (1975). Concentrations of chl *a* (ug/L) were determined using the following equations from Jeffrey and Humphrey (1975):

$$\text{Chl } a = 11.85\ A_{664} - 1.54\ A_{647} - 0.08\ A_{630}\ (\text{v } L^{-1}\ V^{-1})$$

2.3.3. Nutrients- Samples for nutrient analysis were filtered through a 0.25 µm syringe filter and frozen for later analysis. Filtered nutrient samples were processed by the Geochemical and Environmental Research Group (GERG) at Texas A&M University. A Technicon II Autoanalyzer was used to perform the analyses. Nitrate and nitrite analyses were based on the methodology of Armstrong et al. (1967) and utilized a ground Cd column for reduction of NO_3^- to NO_2^-. Orthophosphate was measured using chemistry

based on the investigations of Bernhardt and Wilhelms (1967) with the modification of hydrazine as reductant. Ammonium analysis was based on the method of Harwood and Kuhn, (1970). Total nitrogen and total phosphorus analyses were based on the utilization of potassium persulfate ($K_2S_2O_8$) with pressure and heating to accomplish the complete decomposition and oxidation of elemental components in organic matter to a detectable form. The autoanalyzer method was modeled after those developed and commonly used for seawater analyses (Valderrama, 1981). Dissolved inorganic nitrogen (DIN) was determined by combining concentrations of nitrate, nitrite, and ammonia.

2.3.4. CHN- Samples were frozen for later analysis of carbon, hydrogen, and nitrogen content. Filters were cut in half for measurement of total particulate carbon (TPC) and particulate organic carbon (POC). All filters were dried at 50°C for 24 hours and POC samples were placed in a dessicator to remove all inorganic carbon from the sample. POC filters were acidified for 24 hours and dried again at 50°C for 24 hours. Prepared filters were then run on a Perkin-Elmer Series II CHNS/O Analyzer (2400). Acetanilide (71.09% C, 6.71% H and 10.36% N) was used as an analytical standard.

2.3.5. Phytoplankton Identification- Samples for genus (and when possible, species level) identification were settled using Utermöhl chambers for 24-36 hours and examined using a Motic A130 Inverted Microscope. All organisms in all fields of view were counted.

3. RESULTS

3.1 *Spawning Observations*

The corals at the Flower Garden Banks spawned from August 11 to August 13, 2009. The corals at the FGB spawn 7-10 days after the August full moon, which fell on August 6, 2009. The 2009 spawning event was difficult to predict, however, due to the fact that the full moon was August 6th 055GMT, which, local time, was August 5th at 7:55pm CDT. Species seen spawning included *Montastrea cavernosa, M. franksi, M. faveolata, Stephanocoenia intersepta*, and *Diploria strigosa.* The night of August 12th saw 34 colonies of *M. franksi* spawn and on August 13th 59 colonies of *M. franksi*, 31 colonies of *D. strigosa*, and 20 colonies of *M. faveolata* spawned (2009 FGBNMS Coral Spawn Cruise Report). Spawning during 2009 was not a prolific as previous years and this is believed to be due to the timing of the full moon. Another small spawning event was reported in early September by recreational divers aboard the *M/V* Fling and was reported to be of similar magnitude to the August spawning.

3.2 *Water Quality Parameters*

Water column temperature and salinity were measured using a SeaBird CTD by the Flower Garden Banks National Marine Sanctuary staff during the 3 month sampling period. All data were checked by FGBNMS for accuracy before being published. Data are presented as daily averages for each sampling month. In May 2009 (Fig. 4A), average daily temperature ranged from 23.7 to 26.3ºC. Average daily salinity ranged from 34.7 to 36.4 ppt. Temperature began to increase on 5/19 corresponding to a drop in salinity from 36.0 to 35.2 ppt. The lowest reading for salinity occurred one day prior to sample collection on 5/27. Samples were collected on 5/28 where temperature measured 35.8ºC and salinity measured 25.7 ppt. Temperature and salinity both reached maximum values one day after sample collection on 5/29. In July 2009 (Fig. 4B), average daily

temperature ranged from 27.2 to 29.3ºC. Average daily salinity ranged from 36.2 to 36.8 ppt. Both temperature and salinity reached maximum values on date of sample collection 7/8. In August 2009 (Fig. 4C), only 12 days worth of data was collected. Average daily temperature ranged from 27.7 to 28.3ºC and average daily salinity ranged from 36.7 to 36.7 ppt. Highest values of temperature and salinity occurred 6 days prior to start of coral spawning (8/10).

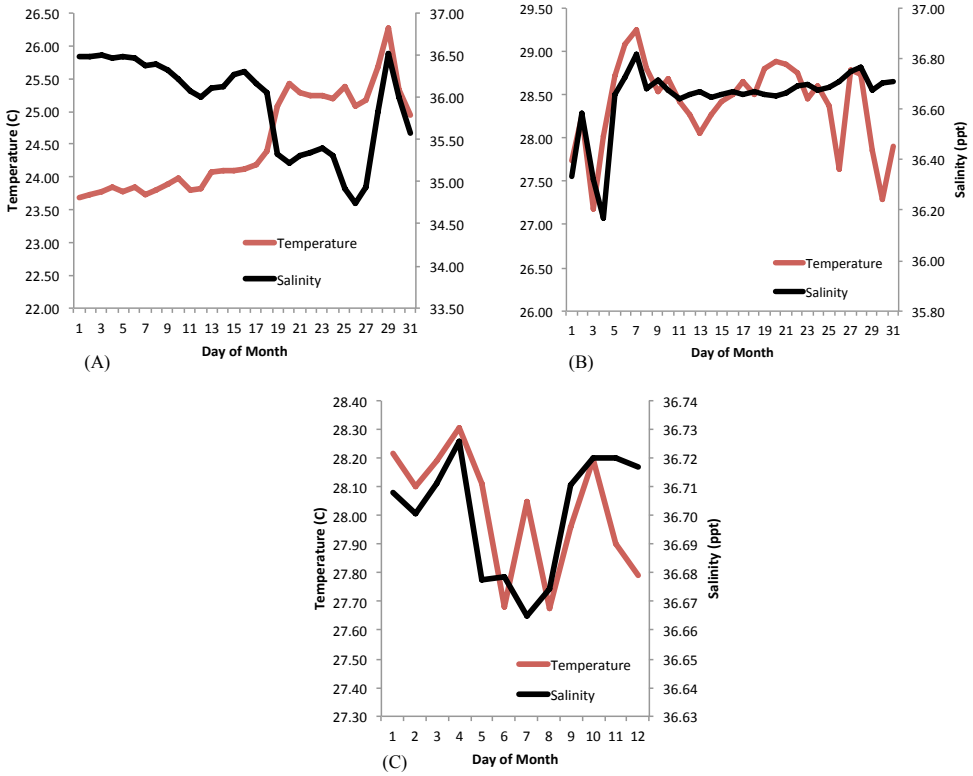

Fig 4. Daily averages of temperature and salinity in (A) May 2009, (B) July 2009, and (C) August 2009.

3.3 *Water Column Parameters*

For all parameters measured during the study period, time and depth factors were combined for each sample date and mean values are presented. Means for phytoplankton community composition, accessory pigments, chl *a* and CHN are presented in Table 3. Nutrient means are presented in Table 4.

Table 3. Times and depths for each date combined to produce means (+/- 1 standard deviation).

Sample Date	**Cells per 30 ml**	**Number of phytop. Genera**	**Chl *a* biomass (μg/L)**	**C:N Ratio**
5/28 (n=2)	34.2 (+/-2.2)	14 (+/-1)	0.269 (+/-0.181)	N/A
7/8 (n=3)	92.5 (+/-10.3)	17 (+/-4)	0.098 (+/-0.023)	13 (+/-2)
8/11 (n=6)	130.1 (+/-40.4)	19 (+/-5)	0.071 (+/-0.017)	8 (+/-4)
8/12 (n=6)	22.5 (+/-2.1)	24 (+/-4)	0.097 (+/-0.050)	6 (+/-3)
8/13 (n=3)	336.7 (+/-60.0)	14 (+/-3)	0.151 (+/-0.065)	8 (+/-1)

3.3.1. Chl a Biomass- Chl *a* was highest on 5/28 with a mean of 0.269 μg/L (σ_2= +/- 0.175). The lowest value of mean chl *a* was 0.071 μg/L (σ_6= +/- 0.011) on 8/11. Sample dates 7/8 and 8/12 had similar average chl *a* with values of 0.098 μg/L (σ_3= +/- 0.023) and 0.097 μg/L (σ_6= +/- 0.052).

3.3.2 Phytoplankton Community- Mean abundance was highest at 336 individuals (σ_3= +/- 60) on 8/13 and had a range of 30 to 798. Mean abundance was lowest on 8/12 with 22 individuals (σ_6= +/- 2) and a range of 10 to 47; however showed the highest number of genera present with 24 identified. Number of genera was lowest on 5/28 and 8/13 both with 14 present.

3.3.3 Nutrients- Several nutrient sample bottles broke upon analysis and thus σ = 2, 5, and 5 for 7/8, 8/11, and 8/12 respectively. No nutrient samples were collected on 8/13.

Mean nitrate (NO_3^-) was highest on the second day of coral spawning (8/12) at 0.89 µM (σ_5= +/-0.86) and lowest on 7/8 with 0.09 µM (σ_2= +/-0.01). The second highest value of NO_3^- occurred on the first day of spawning (8/11) at 0.68 µM (σ_5= +/-0.53).

Table 4. Times and depths for each date to produce mean value for each nutrient measured (+/- 1 standard deviation).

Date	NO_3^- (µM)	HPO_4^- (µM)	SiO_2 (µM)	NH_4^+ (µM)	NO_2^- (µM)	$NO_3^- + NO_2$ (µM)	Urea (µM)
5/28	0.48 (+/-0.49)	0.05 (+/-0.05)	1.76 (+/-0.72)	1.64 (+/-0.41)	0.09 (+/-0.08)	0.57 (+/-0.41)	2.67 (+/-0.36)
7/8	0.09 (+/-0.01)	0.47 (+/-0.41)	1.92 (+/-0.27)	1.10 (+/-0.11)	0.09 (+/-0.06)	0.18 (+/-0.07)	0.67 (+/-0.14)
8/11	0.68 (+/-0.53)	0.20 (+/-0.06)	1.10 (+/-0.13)	1.49 (+/-1.26)	0.10 (+/-0.07)	0.78 (+/-0.52)	1.51 (+/-1.44)
8/12	0.89 (+/-0.86)	0.41 (+/-0.15)	1.15 (+/-0.47)	1.08 (+/-0.62)	0.18 (+/-0.06)	1.07 (+/-0.84)	2.50 (+/-2.10)

Nitrite (NO_2^-) remained relatively constant on the first 3 sample dates and doubled in value on 8/12 with a mean 0.18 µM (σ_5= +/-0.06). Mean nitrate plus nitrite (NO_3^- + NO_2^-) was lowest on 7/8 with 0.18 µM (σ_2= +/-0.07) and highest on 8/12 with 1.07 µM (σ_5= +/-0.84). The highest mean value of ammonia (NH_4^+) occurred on 5/28 with 1.64 µM (σ_2=+/-0.41) detected and lowest on 8/12 at 1.08 µM (σ_5= +/- 0.62). Mean phosphate (HPO_4^-) was highest on 7/8 at 0.47 µM (σ_2= +/-0.41) and lowest on 5/28 at 0.05 µM (σ_2= +/-0.05). Silica (SiO_2) was higher during non-spawning sample dates 5/28 and 7/8 with 1.76 µM (σ_2= +/-0.72) and 1.93 µM (σ_2= +/-0.27), respectively. SiO_2 was lowest on 8/11 with 1.10 µM (σ_5= +/-0.13). Mean urea peaked at 2.67 µM (σ_2= +/-0.36) on 5/28 with a second highest value at the end of the sampling period on 8/12 with 2.50 µM (σ_5= +/-2.10). The lowest mean for urea occurred on 7/8 at 0.67 µM (σ_2= +/- 0.14). The CN ratio was highest in July at 13 and equal on 8/11 and 8/13 at 8. On 8/12, the CN

ratio was at 6, close to the Redfield ratio of 6:6:1 for healthy phytoplankton. No CN data was available for May.

3.4 *Phytoplankton Biomass Indicated by Chl a*

Chlorophyll *a* was analyzed at 3 depths throughout the water column using HPLC and spectrophotometric methods. Chl *a* values from the HPLC method are presented in this section. Concentrations ranged from 0.145 to 0.393 µg/L in May, 0.071 to 0.112 µg/L in July, and 0.025 to 0.221 during the coral spawn in August (Fig. 5). In May, the lowest value of chl *a* occurred in surface waters in the morning, while the highest occurred in surface waters in the evening. The variations in chl *a* by time and depth is illustrated in Fig 6 A-E. No samples were collected at mid or deep depth on 5/28 and only PM samples were collected on 7/8 and 8/13. On 5/28 chl *a* was higher in PM water with 0.393 µg/L (σ_2=+/- 0) than in AM waters. This value is at least 3 fold higher than any other measurement of chl *a* throughout the entire sampling period. On 7/8, chl *a* was highest at mid depth with 0.112 µg/L (σ_2=+/-0.02). Mid depth and deep water measurements only varied by 0.001 ug/L. During the first day of coral spawning on 8/11, chl *a* was highest in the AM at the surface with 0.084 µg/L (σ_2=+/-0.01) detected. Of the evening samples, the highest chl *a* was found a mid depth with 0.078 µg/L (σ_2=+/-0.01) . The lowest value of chl *a* was in PM surface waters at 0.05 µg/L (σ_2=+/-0.01). On the second day of spawning (8/12), chl *a* was highest in PM surface waters instead of in the morning at 0.153 µg/L (σ_2=+/-0.03). The highest morning value was also measured at the surface with 0.121 µg/L (σ_2=+/-0.05).The lowest measurement overall was found in the morning in mid depth waters with 0.025 µg/L (σ_2=+/-0.05).

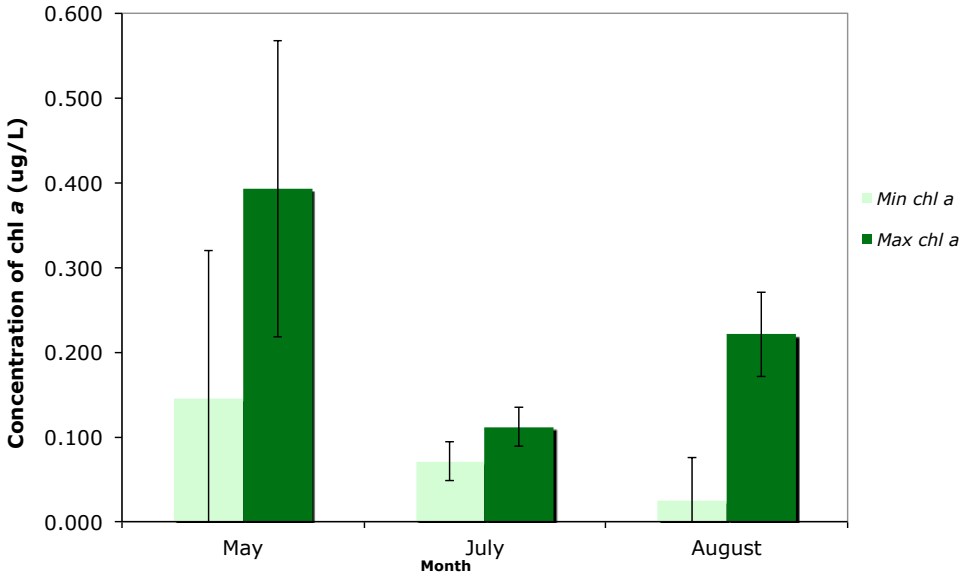

Fig 5. Range of chl *a* concentration per month. Sampling dates 8/11, 8/12, 8/13 were combined into August .

On 8/13, chl *a* was highest at the surface at 0.221 µg/L (σ_2=+/-0.06) which is 2 fold higher than the lowest value 0.098 µg/L (σ_2=+/-0.06) at mid depth. Chl *a* biomass was significantly different ($P<0.01$) between sample dates, but not significantly different between the depth or time at which the sample was collected. Chl *a* concentrations were significantly correlated (as shown in the table on pg. 26) with chl *b* ($P<0.01$) and silicate ($P<0.05$).

3.5 *Phytoplankton Community Structure*

3.5.1. Accessory Pigments- Carotenoid pigments were analyzed at 3 depths (0 m, 9 m, and 18 m) using HPLC. The contribution of different classes of phytoplankton to total chlorophyll *a* was determined using known equations (Kana et al. 1988; Letelier et al. 1993; Lambert et al. 1999; Qian et al. 2003) (Table 5). The algorithms shown have been used with success in Gulf of Mexico phytoplankton studies (Lambert et al. 1999, Qian et al. 2003).

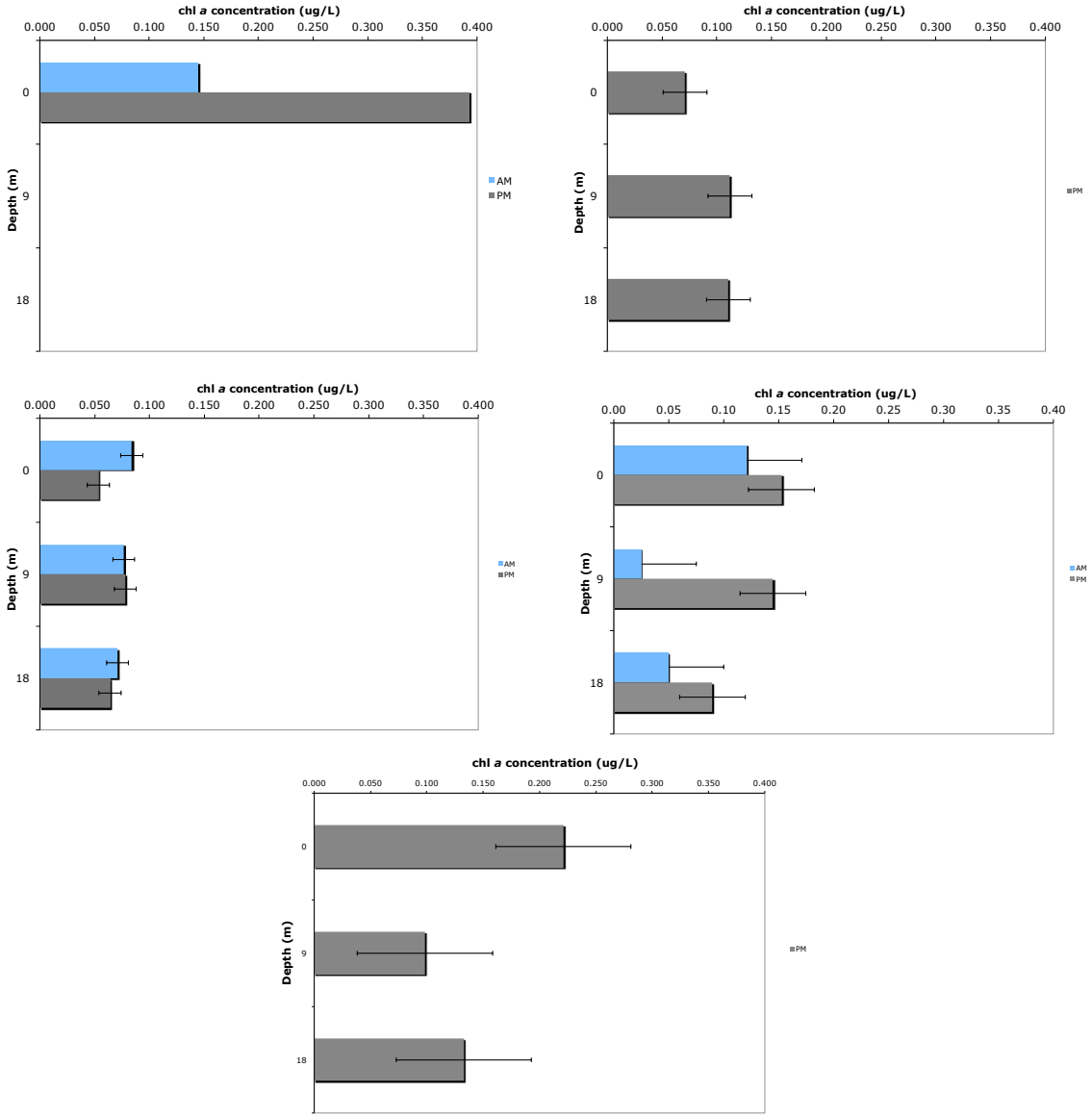

Fig.6. Concentration of chl *a* on (A) 5/28, (B) 7/8, (C) 8/11, (D) 8/12, and (E) 8/13.

Table 5. Pigment algorithms used for partitioning chlorophyll a biomass among algal groups. Abbreviations include chlorophyll *a* (Chl *a*), zeaxanthin (Zeax), chlorophyll b (Chl b), fucoxanthin (Fuco), 19'hexanoyloxyfucoxanthin (19'Hex), 19'butanoyloxyfucoxanthin (19'But), and peridinin (Perid).

Algal Groups	Pigment Equation	Source
Prokaryotes	[Chl *a*]prok= [Zeax]-+0.5[Chl *b*]	Letelier et al. 1993 Qian et al. 2003
Prymnesiophytes	[Chl *a*]prymnes= 1.3[19'Hex]-0.1[19'But+Fuco]	Letelier et al. 1993
Pelagophytes	[Chl *a*]pela= 0.9[19'But]	Letelier et al. 1993
Dinoflagellates	[Chl *a*]= 1.5[Perid]	Letelier et al. 1993
Diatoms	[Chl *a*]diat= 0.8{[Fuco]-(0.02[19'Hex]+0.14[19'But]}	Letelier et al. 1993

This approach assumes that (1) the species of the taxonomic groups present in the study area produce pigments in approximately the same ratios as those in culture and (2) any unknown algal groups in the sample do not contribute significantly to total phytoplankton biomass (Qian et al. 2003). Cyanobacteria, based on zeaxanthin and chlorophyll b concentrations, dominate the chl *a* pool at EFGB throughout the study period (as shown in the figure on pg. 27). Abundance was highest on 5/28 with 80% and lowest on 8/12 at 66%. Dinoflagellates were the 2nd most abundant class making up between 17-26% of total phytoplankton. A peak in dinoflagellate abundance occurred on the second day of coral spawning (8/12) at 26%. Prymnesiophytes, as indicated by the biomarker 19'hexanoloxy, contributed between between 6-8% of the total chl *a*. Diatom (based on fucoxanthin, 19'hex, and 19'but) and pelagophyte (based on 19'but) abundance were consistently low (<5%) throughout the study period (Figure 7).

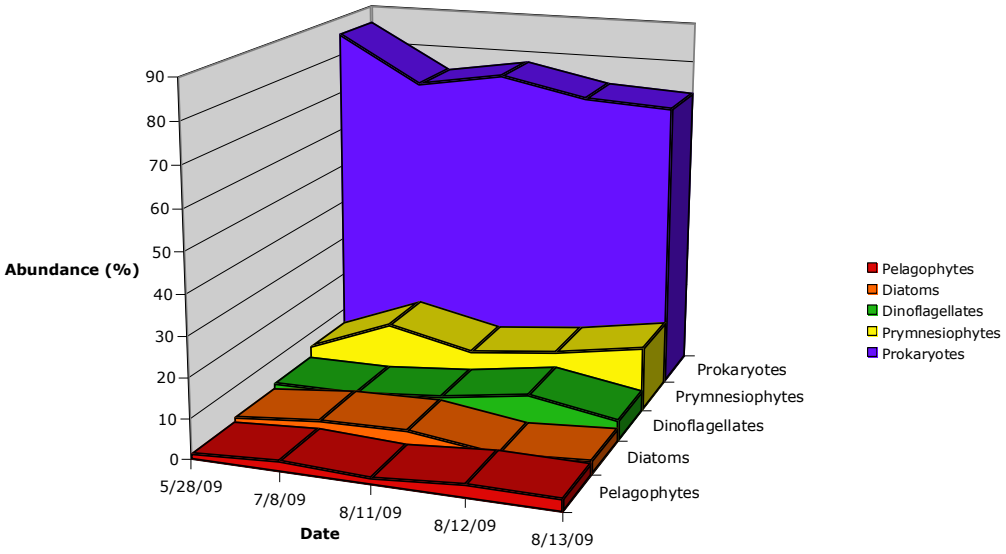

Fig. 7. Class abundance (%) of phytoplankton at EFGB. Abundances determined using algorithms from Letelier et al. 1993.

R Software (version 2.1.1) was used to perform multivariate statistical analyses on pigment data. Specifically, permutational multivariate analysis of variance using distance matrices (ADONIS) was used to determine correlations between pigment data and factors of date, time, and depth. ADONIS is a function for the analysis and partitioning sums of squares using semi-metric and metric distance matrices and is analogous to MANOVA. Prior to performing the test, pigments were normalized to chl *a* and chl *a* was removed from the data matrix. Results of the test can be seen in Table 6. Treatment refers to grouping of data by pre (5/28 and 7/8) and during (8/11, 8/12, and 8/13) spawning observations. One-way analysis of variance (ANOVA) was performed on individual pigments and nutrients to determine if significant difference between pre and spawn samples existed. Significance was taken at $p<0.05$.

Table 6. P-values generated by multivariate analysis of pigment data.

Factor	Date+ Time	Treatment	Depth	Time	Depth+Time	Depth+Time +Treatment
All Pigments	0.02	0.03	0.175	0.08	0.08	N/A

The dominant carotenoids throughout the study period were zeaxanthin, fucoxanthin, peridinin, and 19'HF. Average concentrations of zeaxanthin, a biomarker for cyanobacteria, ranged from a maximum at 0.104 µg/L on 5/28 (σ_2=+/-0.078) to a minimum at 0.046 µg/L (σ_2=+/-0.018) on 8/12. On 5/28 (Fig. 8A), zeaxanthin concentration was highest in PM water at 0.159 µg/L (σ_2=+/-0.00, n=2). This value is also the highest concentration found among all sample dates and is 3 fold higher than the AM concentration of zeaxanthin on 5/28. On 7/8 (Fig. 8B), zeaxanthin concentration varied only slightly across depth with the maximum found at mid depth (0.058 µg/L; σ_2=+/-0.007, n=3). Concentrations were near equal on 8/11 (Fig. 8C) at mid depth with 0.066 µg/L(σ_2=+/-0.013, n=6) in AM and 0.067 µg/L (σ_2=+/-0.016) in PM . The PM mid depth concentration was also the highest found on this date. On 8/12 (Fig. 8D), AM concentration of zeaxanthin was higher than PM concentration at the surface, but lower than PM concentration at mid and deep depth. The highest concentration on this date was found in AM surface water at 0.071 µg/L (σ_2=+/-0.026, n=6). On the last day of coral spawning (8/13), zeaxanthin concentrations only varied slightly with depth at 0.057, 0.055, and 0.062 µg/L, respectively (σ_2=+/-0.004, n=3). Pearson correlation coefficients and corresponding P-values were determined for individual dominant pigments, nutrients, and depth (Table 7). Zeaxanthin was significantly correlated with depth ($P<0.05$) , but not with other variables. Zeaxanthin was not significantly different between pre-spawn and spawn samples ($P>0.05$).

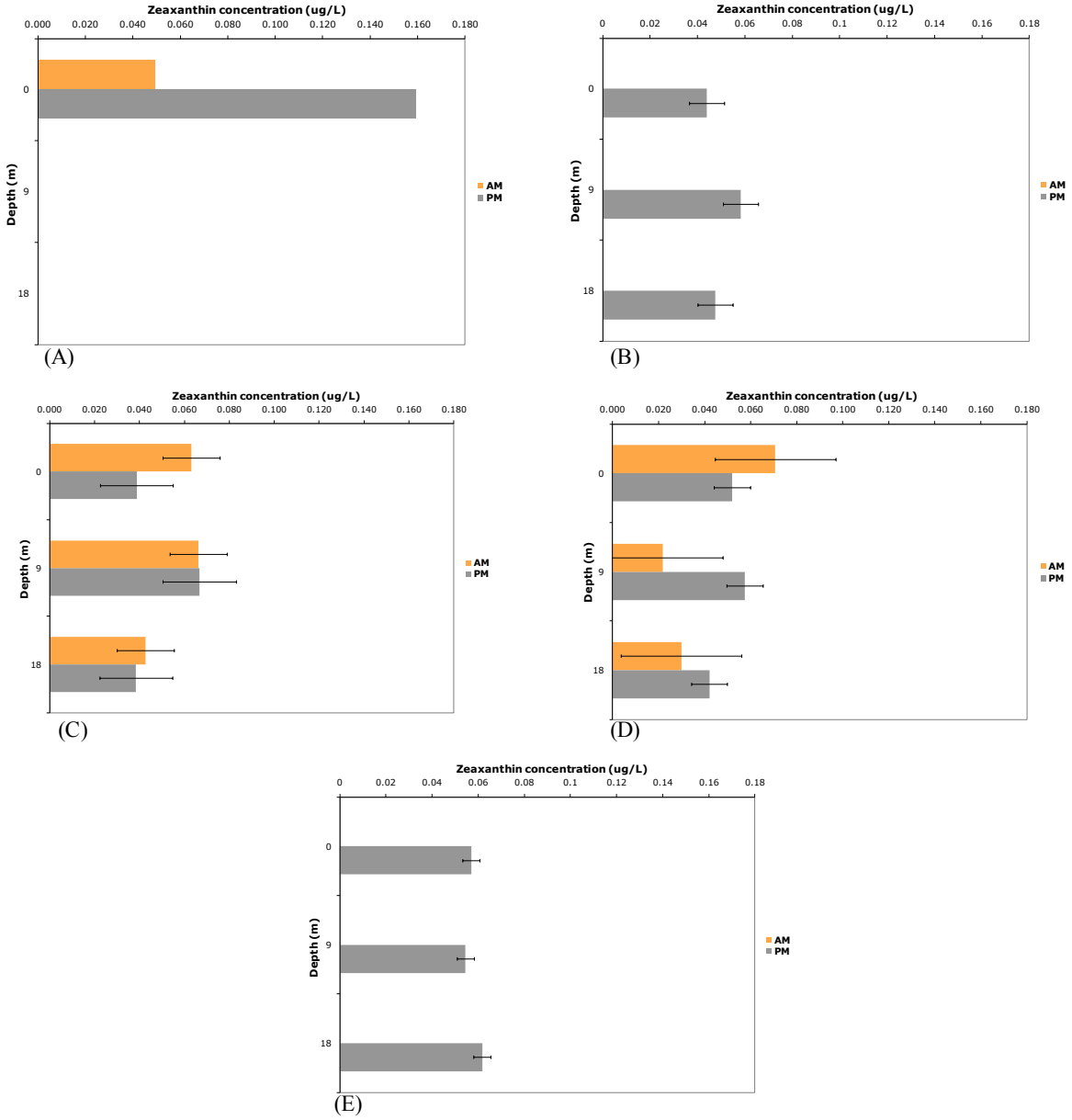

Fig. 8. Concentration of zeaxanthin on (A) 5/28, (B) 7/8, (C) 8/11, (D) 8/12, and (E) 8/13.

Table 7. Correlations between phytoplankton pigment concentration, nutrient concentrations, and depth. Top row numbers indicate correlation coefficient and numbers in parenthesis represent P-value. N=15.

	Chl b	Zeax	Fuco	Perid	19'hex	DIN	Phos	Silicate	Depth
Chl *a*	0.64	0.91	0.10	0.14	0.36	0.21	-0.33	0.57	-0.45
	(0.0095)	(0.0001)	(0.7188)	(0.6200)	(0.1879)	(0.4623)	(0.2255)	(0.0252)	(0.0907)
Chl b		0.67	-0.05	0.01	0.24	-0.14	-0.11	0.52	-0.56
		(0.0065)	(0.8578)	(0.9580)	(0.3847)	(0.6255)	(0.6896)	(0.0477)	(0.0284)
Zeax			0.01	0.05	0.25	0.23	-0.43	0.49	-0.51
			(0.9581)	(0.8608)	(0.3646)	(0.4100)	(0.1088)	(0.0688)	(0.0429)
Fuco				0.49	0.11	0.12	-0.14	0.10	0.4
				(0.0629)	(0.6850)	(0.6716)	(0.6190)	(0.7106)	(0.1344)
Perid					0.11	0.03	0.13	-0.34	0.049
					(0.7041)	(0.4271)	(0.6498)	(0.2084)	(0.8544)
19'HF						0.43	0.13	0.37	-0.33
						(0.1063)	(0.6511)	(0.1692)	(0.2246)
DIN							0.13	0.30	0.20
							(0.6467)	(0.2711)	(0.4768)
Phos								0.18	-0.12
								(0.5137)	(0.6577)
Silicate									-0.28
									(0.3100)

Fucoxanthin, a biomarker for diatoms, had a range of below limit of detection (BLD) to 0.015 µg/L (σ_2=+/-0.003, n=20) on 8/11. On 5/28 (Fig. 9A), fucoxanthin concentration was higher in PM water at 0.006 µg/L (σ_2=+/-0.00, n=2). On 7/8 (Fig.9B), concentrations were equal at mid and deep depth at 0.006 µg/L (σ_2=+/-0.001, n=3) and lowest at the surface. Fucoxanthin was BLD on 8/11 (Fig. 9C) in AM surface water and highest on this date in AM deep water (0.015 µg/L; σ_2=+/-0.008, n=6). This sample date had the largest range of fucoxanthin concentration throughout the study. On 8/12 (Fig. 9D), concentration was also BLD in AM surface water, with the highest concentration found in PM surface water (0.005 µg/L; σ_2=+/-0.001, n=6). PM concentration of fucoxanthin decreases with increased depth. 8/13 (Fig. 9E) samples had the equal concentration of fucoxanthin at surface and mid depth (0.006 µg/L; σ_2=+/-0.002, n=6). Fucoxanthin concentration was not significantly different between pre-spawn and spawn ($p>0.05$).

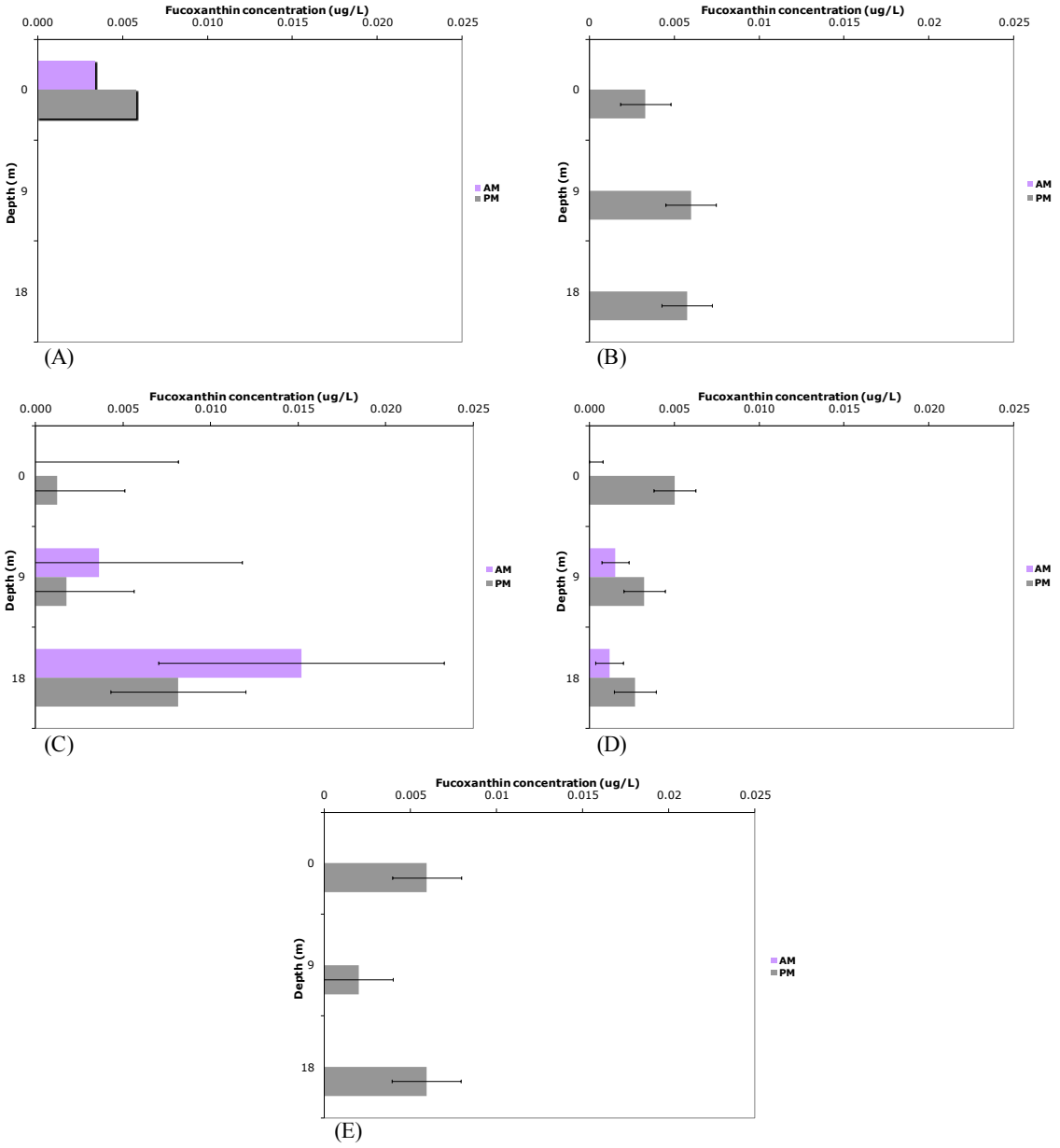

Fig. 9. Concentration of fucoxanthin on (A) 5/28, (B) 7/8, (C) 8/11, (D) 8/12, and (E) 8/13.

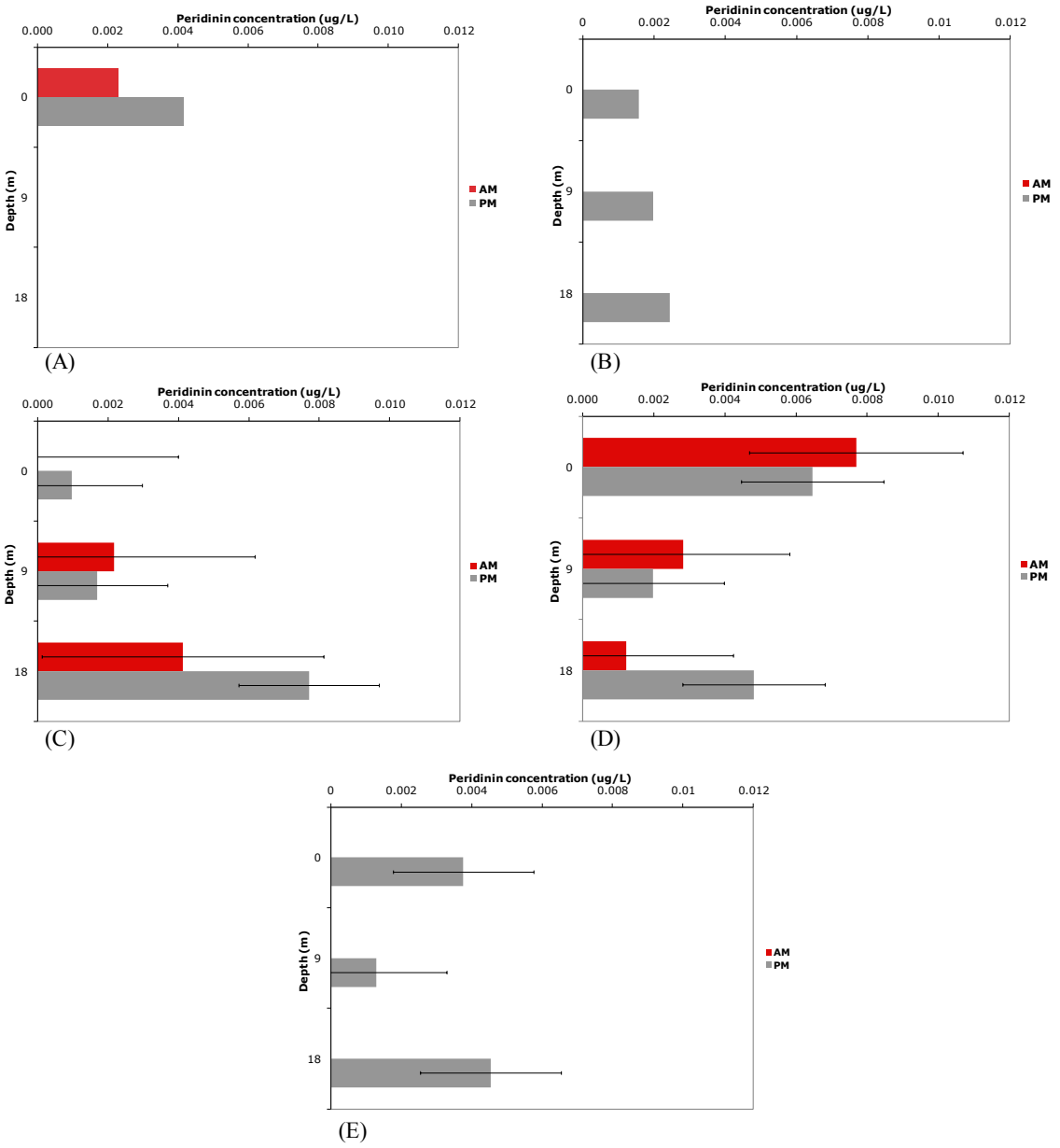

Fig. 10. Concentration of peridinin on (A) 5/28, (B) 7/8, (C) 8/11, (D) 8/12, and (E) 8/13.

The pigment peridinin, a biomarker for dinoflagellates, ranged in concentration from below limit of detection (BLD) to 0.008 µg/L across the sampling period. On 5/28 (Fig. 10A), the concentration was highest in PM water at 0.0045 µg/L. On 7/8 (Fig. 10B), peridinin concentrations were nearly equal across depth ranging from 0.0015 µg/L

at surface to 0.002 µg/L at depth (σ_2=+/-0.00, n=3). Concentration was BLD in AM surface water on 8/11 (Fig. 10C), with the highest concentration found in PM deep water (0.008 µg/L; σ_2=+/-0.004, n=6). The following day (8/12), the highest concentration of peridinin was found in AM surface water at 0.008 µg/L (σ_2=+/-0.003, n=6) (Fig. 10D). Concentrations in AM water decreased with increased depth, while PM values were staggered throughout the water column. On 8/13 (Fig. 10E), mid depth water had the lowest concentration of peridinin at 0.001 µg/L (σ_2=+/-0.002) and deep water the highest at 0.005 µg/L. Peridinin was not significantly different between pre-spawn and spawn samples ($p>0.05$).

19'Hex is a biomarker for prymnesiophytes and concentrations ranged from 0.002 µg/L to 0.014 µg/L (σ_2=+/-0.003, n=20). On 5/28 (Fig. 11A), AM and PM surface concentration were equal at 0.007 ug/L(σ_2=+/-0.00, n=2). Concentration of 19'Hex increased with depth on 7/8 (Fig. 11B) with maximum of 0.012 µg/L (σ_2=+/-0.003, n=3). On 8/11 (Fig. 11C), mid depth concentrations were near equal at 0.008 µg/L (σ_2=+/-0.002, n=6). The lowest concentration of 19'Hex on this date was found in PM surface water with 0.004 µg/L (σ_2=+/-0.002). On 8/12 (Fig. 11D), PM concentrations decreased with increased depth with a maximum in surface water at 0.014 µg/L (σ_2=+/-0.003, n=6). Lowest concentration was found in AM mid depth water at 0.002 µg/L (σ_2=+/-0.002, n=6). The following evening (8/13), the lowest concentration of 19'Hex was seen in mid depth water at 0.009 ug/L and the highest in deep water with 0.013 µg/L (σ_2=+/-0.002, n=3) (Fig. 11E). 19'hex was not significantly different between pre-spawn and spawn samples ($p>0.05$).

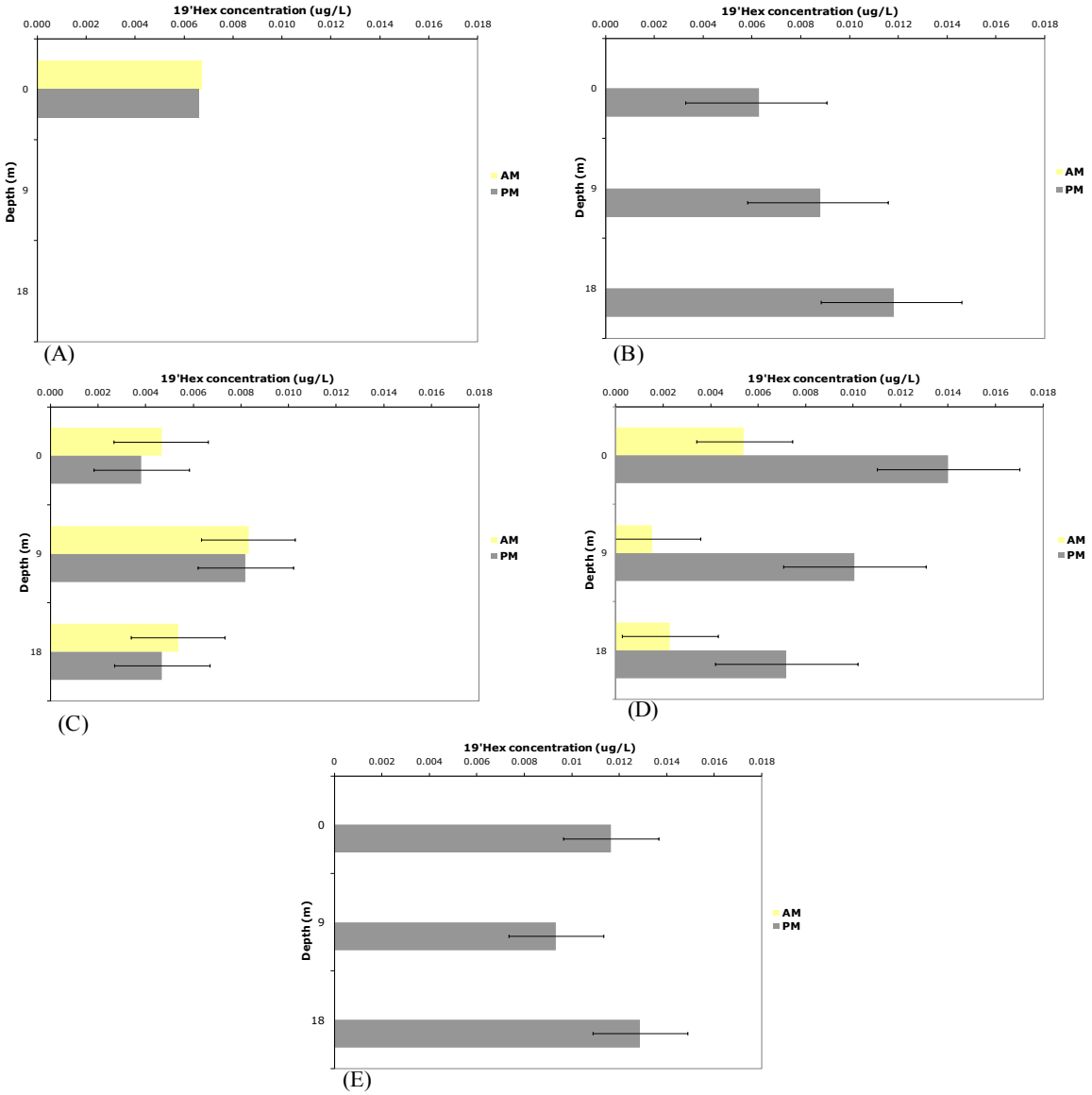

Fig 11. Concentration of 19'Hex on (A) 5/28, (B) 7/8, (C) 8/11, (D) 8/12, and (E) 8/13.

3.5.2- Total Cell Counts- Microscopic counts of phytoplankton were performed for each sample. Organisms were identified to genera and to species when possible. No statistical significant difference was found when performing multivariate analysis of variance (ADONIS) between date, time, depth, or treatment (pre vs. during spawning).

Abundance of all cells (cells/mL) per sampling date ranged from 0.75 cells/mL on 8/12 to a sharp peak on the last day of coral spawning (8/13) at 11.2 cells/mL (Fig. 12). On 8/13, there were particularly high counts of the diatom *Cylindrotheca* sp. (>500 cells) and the cyanobacterium *Trichodesmium* sp (>300 cells). The numbers of genera belonging to the diatom and dinoflagellate algal groups were summed per sample date (Fig. 13). A maximum of 15 diatom genera were found on 7/8 and 8/12 (n=3; n=5). The lowest number of dinoflagellate genera also occurred on 7/8. On the first and second day of spawning (8/11 and 8/12), the highest presence of dinoflagellate genera occurred at 7.

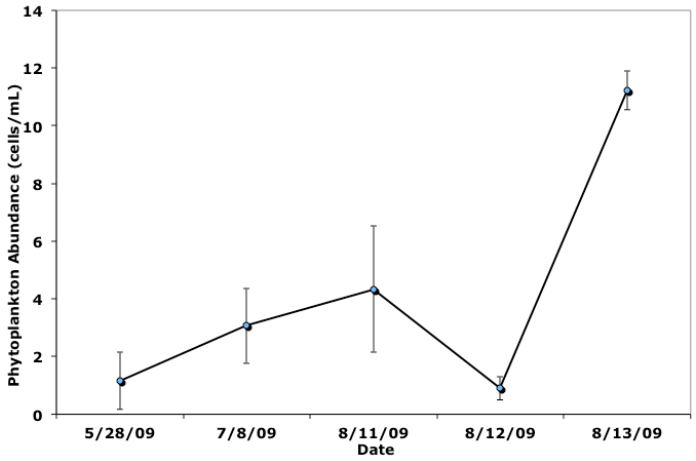

Fig. 12. Abundance (cells/mL) of all phytoplankton (n=2,3,6,5, and 3 for sample dates, respectively).

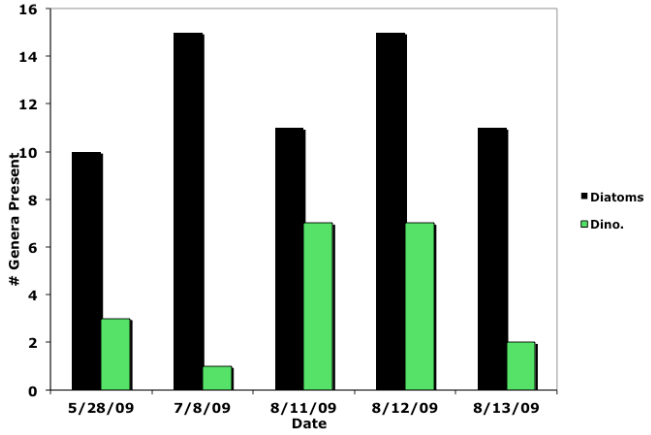

Fig 13. Number of genera belonging to diatom and dinoflagellate algal groups.

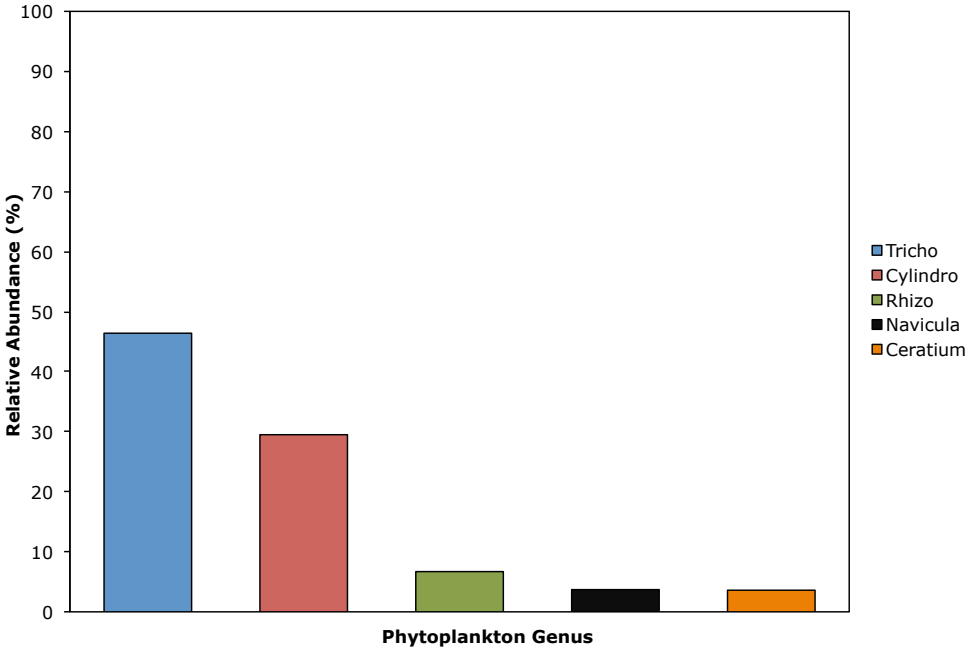

Fig 14. Percent relative abundance of phytoplankton genera across all dates.

A discrepancy exists between HPLC and microscopy method for diatoms in the samples. HPLC pigment concentration plugged into pigment algorithms, yielded low class abundance for diatoms (based on fucoxanthin), a yet microscopic count for diatoms were very high.

Relative abundance was calculated for each genus across all sample dates by determining the contribution (%) of each genus to the total population. The 5 most dominant genera during the study period were *Trichodesmium* sp., *Cylindrotheca* sp., *Rhizosolenia* sp., *Navicula* sp., and *Ceratium* sp. (Fig. 14). *Trichodesmium* sp. had the highest relative abundance with 46% and *Cylindrotheca* the second highest at 30%. The same relative abundance estimates were performed for the dominant genera per sample date. Only genera contributing greater than 5% to the total population were considered. On 5/28 (n=2), genera were well distributed with *Trichodesmium* sp. accounting for 22% (σ_2=+/-6.36) relative abundance and the diatom *Thalassiosira* sp. contributing 14% (σ

$_2$=+/-4.24) (Fig. 15A). The sample date in May was the only time that *Bacteriastrum* sp. made up greater than five percent of the total population (11.8%, σ_2=+/-0.00). The only dinoflagellate genus on this date was *Ceratium* sp., contributing 10.3% relative abundance (σ_2=+/-3.54). On 7/8 (n=3), *Cylindrotheca* sp. had a relative abundance of 38% (σ_2=+/-30.98) with the diatom *Rhizosolenia* at 32% abundance (Fig. 15B). This is the only sample date where *Trichodesmium* spp. does not contribute a high relative abundance to the total population. Samples collected on the first day of coral spawning (8/11; n=6) showed a less evenly distributed relative abundance of genera than earlier sample dates (Fig. 15C). Two genera (*Trichodesmium* sp. and *Ceratium* sp.) accounted for 88% of total relative abundance, with 83% and 5%, respectively. Among all sample dates, *Trichodesmium* sp. contributed the highest percent relative abundance on 8/11. On 8/12, the abundance is more evenly distributed with the diatom *Navicula* sp. accounting for 20% (σ_2=+/-5.41) relative abundance and *Trichodesmium* sp. contributing 17.8% (σ_2=+/-5.89) (Fig. 15D). *Cylindrotheca* sp. abundance drops on this date to <10% from an abundance that was three times higher on 7/8. On the last day of coral spawning (8/13; n=3), two genera once again accounted for 88% of the total abundance in all samples (Fig. 15E). *Cylindrotheca* sp. contributed 53% (σ_2=+/-2.44) and *Trichodesmium* sp. contributed 35% (σ_2=+/-2.05). Zeaxanthin concentration (marker for cyanobacteria) was highest on 5/28, when *Trichodesmium* spp. abundance was low (20%), and lower on 8/11 when *Trichodesmium* spp. abundance was at 83%.

The diversity of the phytoplankton community on each sample date was estimated using Simpson's Index of Diversity and Shannon-Weiner Index of Diversity (Table 8). Simpson's Index takes into account the number of species present as well as relative abundance. As species richness and evenness increase, so diversity increases (Simpson 1949). The Shannon-Weiner Index is also commonly used for diversity and considers the number of species and evenness of species.

Table 8. Diversity indices used to evaluate phytoplankton community

Diversity Index	Equation	Description	Range
Simpson	$D = \sum_{i=1}^{s} p_i^2$	Where s is the number of species encountered P*i* is the fraction of the population made up of species *i*, and ∑ is the sum of species 1 to species S	Index range from 0 to 1, with 1 being highest diversity
Shannon-Weiner	$H = \sum_{i=1}^{s} (p_i \ln p_i)$	Where s is the number of species encountered Pi is the fraction of the population made up of species *i*, ln p*i* is the natural logarithm of that fraction, and ∑ is the sum of species 1 to species S	Index range normally from 1.5 to 3.5, with 3.5 being highest diversity

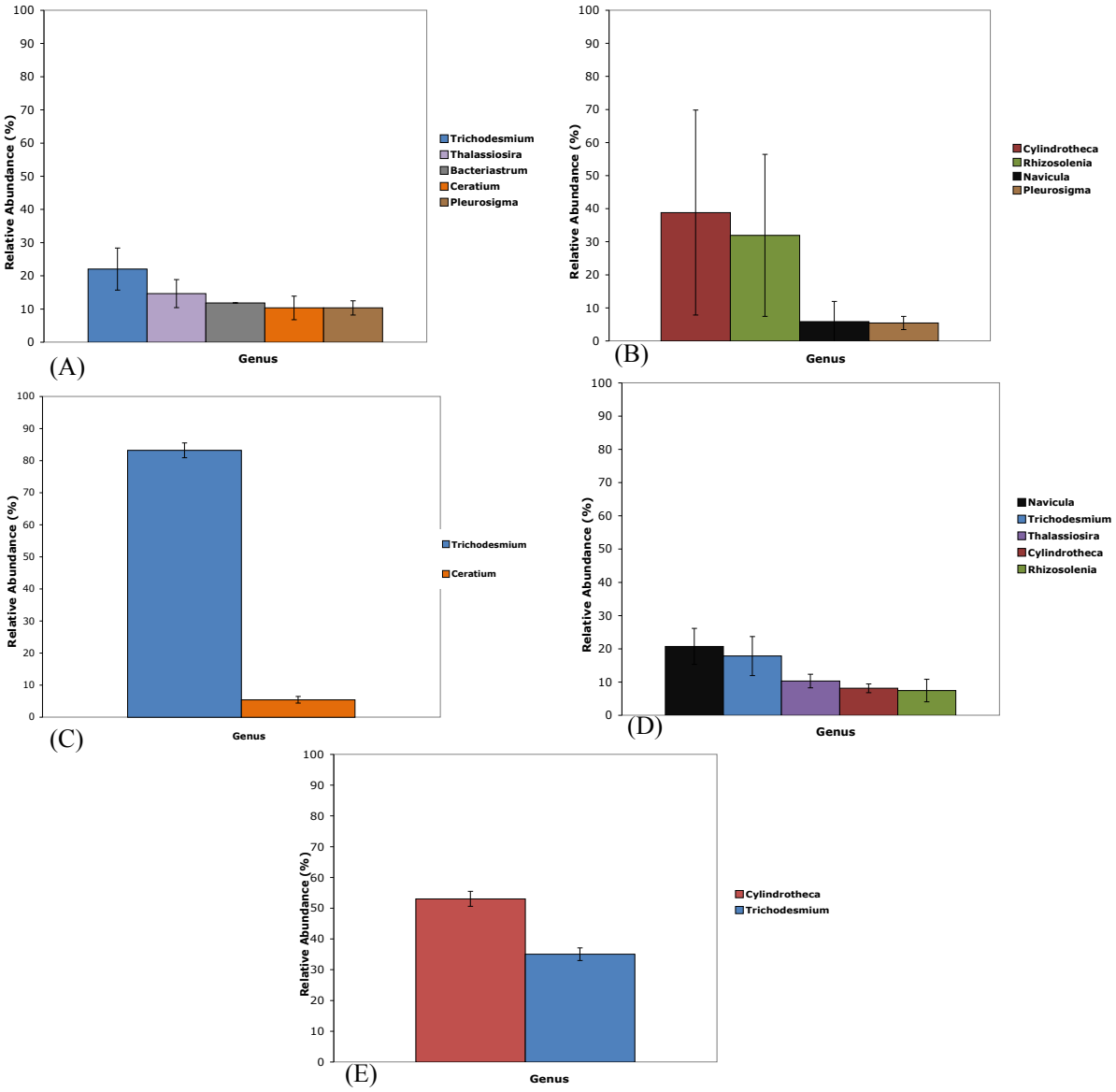

Fig 15. Relative abundance of phytoplankton genera on (A) 5/28, (B) 7/8, (C) 8/11, (D) 8/12, and (E) 8/13.

Diversity index values for the phytoplankton community ranged from 0.303 to 0.893 (Simpson) and 0.85 to 2.63 (Shannon- Weiner) (Fig. 16). The phytoplankton community on 8/12 had the highest estimated diversity using both Simpson and Shannon-Weiner indices (1-D= 0.893 and H= 2.63, respectively) (Fig. 16). Lowest diversity was seen on 8/11 and is also in agreement between both indices (1-D=0.303 and H=0.85). There was a 3-fold difference between the sample dates with lowest and highest diversity although both dates occurred within the coral spawning window.

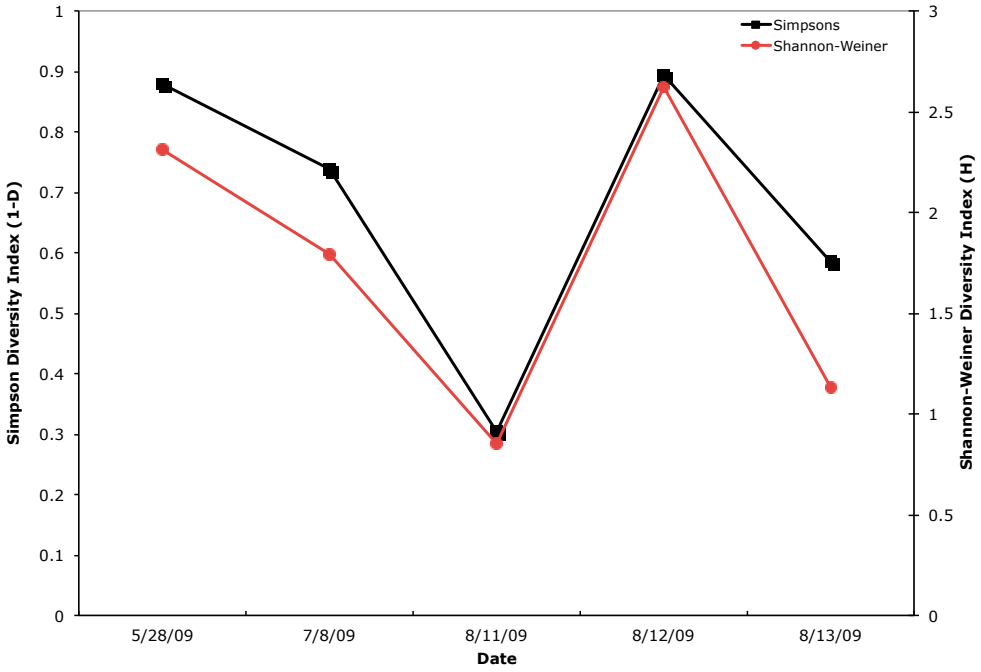

Fig 16. Simpson (1-D) and Shannon-Weiner (H) indices of diversity for the phytoplankton population.

Table 9. P-values generated from multivariate analysis of nutrient data.

Factor	Date+Time	Treatment	Depth	Time	Depth+Time	Depth+Time +Treatment
All Nutrients	0.01	0.155	0.168	0.04	0.04	0.04

3.6 *Water Column Nutrients*

Nutrient analyses were performed on samples collected on 5/28, 7/8, 8/11, and 8/12. Absence of bars does not indicate a zero value, but indicates no measurements taken. All nutrients combined were significantly correlated with date+time, depth+time, depth+time+treatment, and time alone (P-value <0.05 for all, ADONIS) (Table 9). Dissolved inorganic nitrogen (DIN) was highest throughout the sampling period on 8/11 in AM mid depth waters at 4.31 µmol/L and lowest on 8/12 in AM deep waters with 0.47 µmol/L (σ_2=+/-1.17, n=15). On 5/28 (Fig. 17A), DIN was higher in PM waters than AM, with 2.79 µmol/L (σ_2=+/-0.00, n=2). On 7/8 (Fig. 17B), only PM samples were collected and DIN was higher at mid depth than surface at 1.40 µM (σ_2=+/-0.181, n=2). The mid depth sample on 8/11 (Fig. 17C) had highest value of DIN at 4.31 µM and a very low value in AM surface water with 0.68 µM (σ_2=+/-1.89, n=5). On 8/12 (Fig. 17D), DIN was highest in PM deep water at 3.88 µM (σ_2=+/-0.979, n=6). At mid depth, PM value of DIN was 2 fold higher than AM value. DIN concentrations were not significantly different (ANOVA, P>0.05) across date, time, or depth.

Phosphate concentrations ranged from 0.02 µM in PM surface waters on 5/28 to 0.75 µM on 7/8 at surface (σ_2=+/-0.208, n=15). On 5/28 (Fig. 18A), phosphate was 4 fold higher (0.09 µM) in AM surface water than in PM surface water (0.02 µM). On 7/8 (Fig. 18B), samples were collected from PM surface and mid water and phosphate was 3 fold higher at the surface (0.75 µM ; σ_2=+/-0.407, n=2). At the start of spawning (8/11),

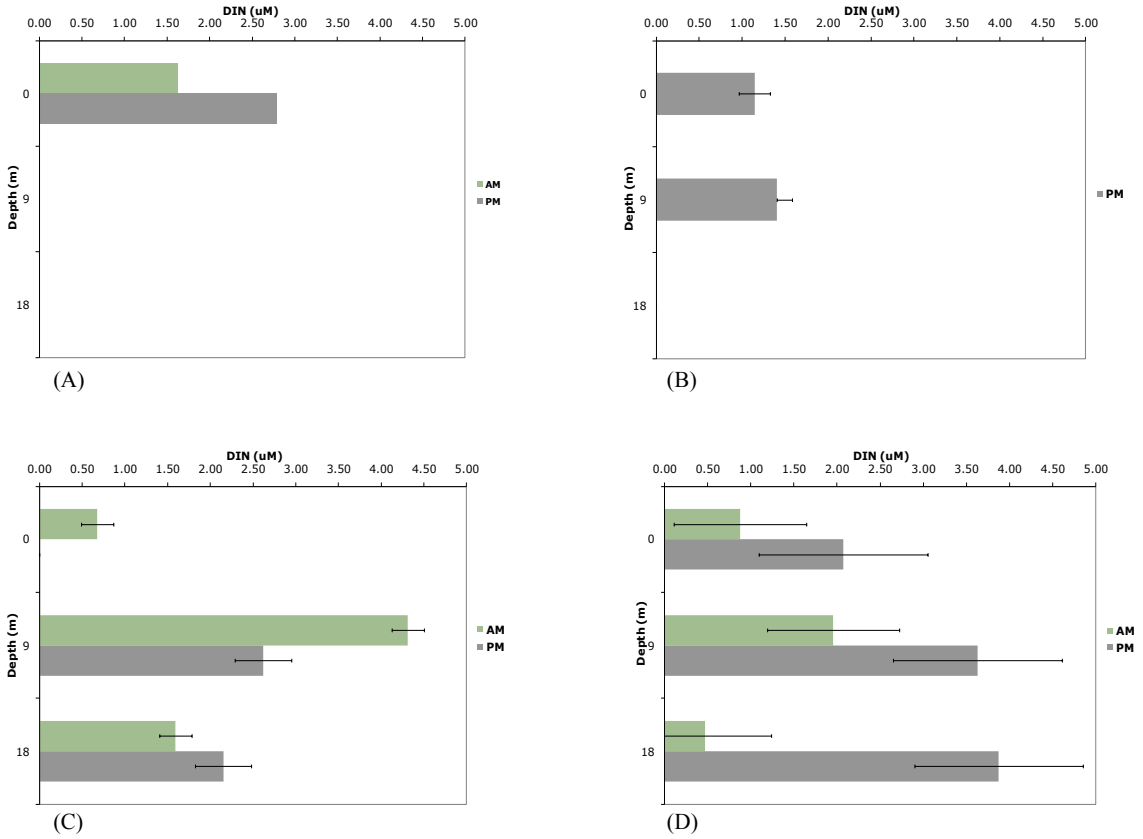

Fig 17. Concentration of DIN on (A) 5/28, (B) 7/8, (C) 8/11, (D) 8/12.

the highest concentration of phosphate was found in AM deep water at 0.27 µM (σ_2=+/-0.04, n=5). The lowest concentration on this date was in AM surface water at 0.12 µM (σ_2=+/-0.08) (Fig. 18C). On 8/12, PM concentrations of phosphate were consistently higher across all depths with the highest concentration at 0.60 µM (σ_2=+/-0.094, n=6) at mid depth (Fig. 18D).

The ratio of average dissolved inorganic nitrogen to average phosphorus (DIN:P) was highest in May at 78 and lowest in July at 4. DIN:P dropped during spawning from 11 on 8/11 to 5 on 8/12.

Concentrations of silicate across the entire sampling period ranged from 0.48 µmol/L to 2.27 µM (σ_2=+/-0.483, n=15). On 5/28 (Fig 19A), silicate concentration was highest in PM water with 2.27 µM (σ_2=+/-0.00). On 7/8 (Fig. 19B), there was small

variation in concentration between surface and mid depth with surface higher at 2.10 µM (σ_2=+/-0.265, n=2). The highest silicate concentration on 8/11 (Fig. 19C) was found in AM mid depth water at 1.24 µM (σ_2=+/-0.168, n=5) and the lowest in AM deep water at 0.91 µM . On 8/12 (Fig. 19D), PM concentrations of silicate were consistently higher throughout the water column with the highest found at mid depth (1.90 µM).

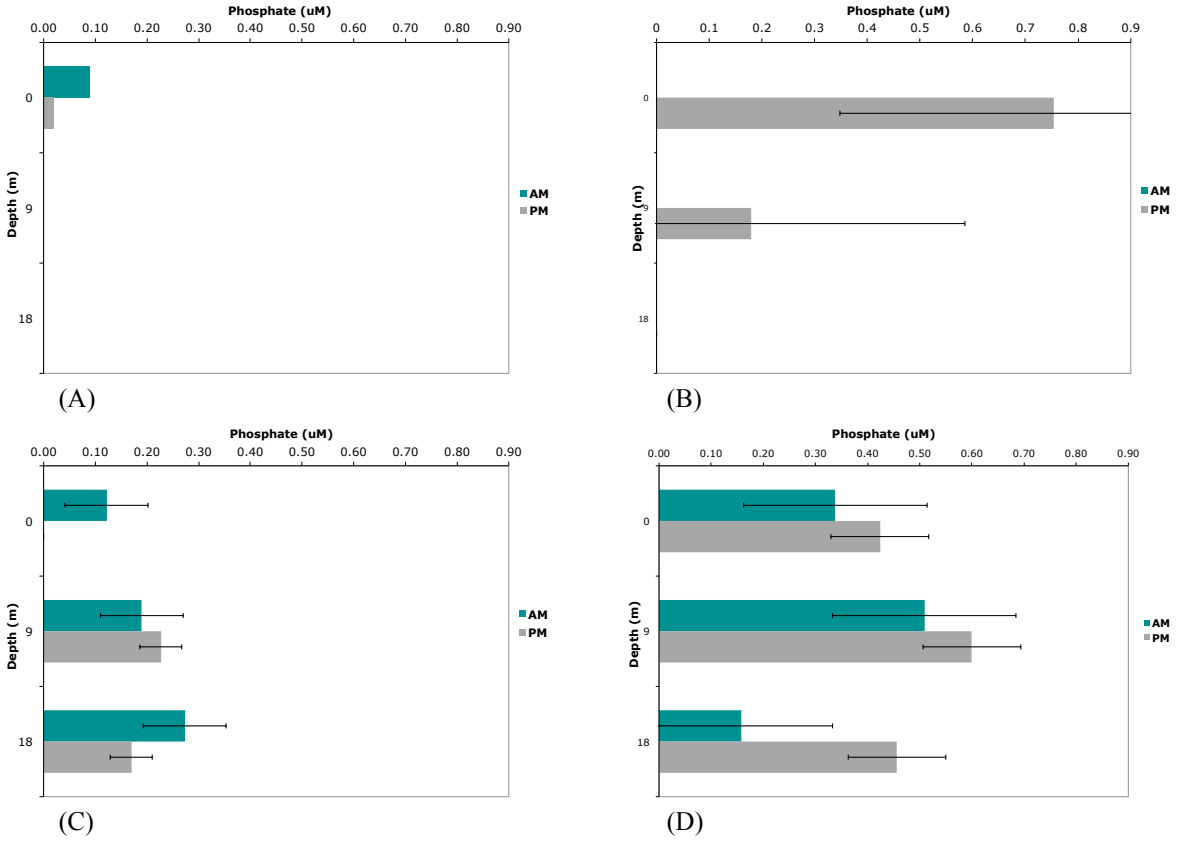

Fig 18. Concentration of phosphate on (A) 5/28, (B) 7/8, (C) 8/11, and (D) 8/12.

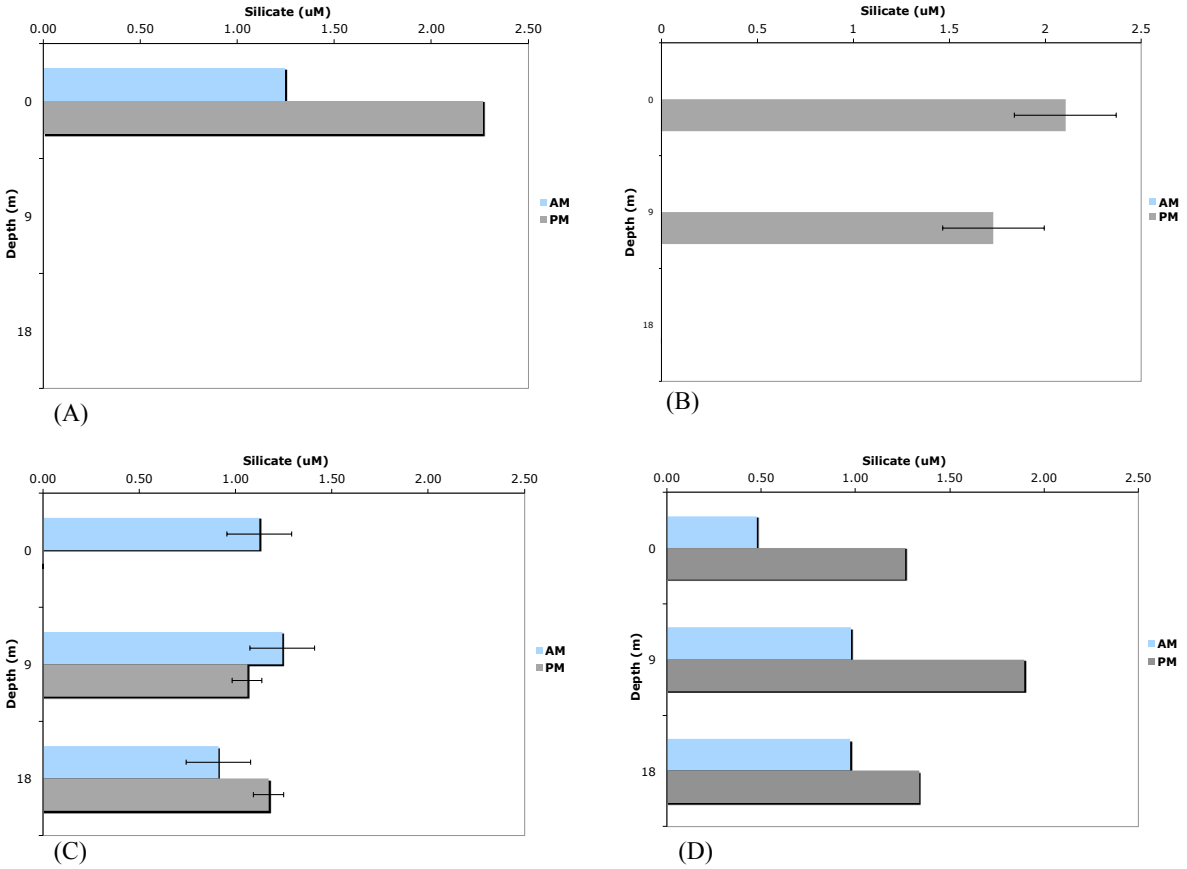

Fig 19. Concentration of silicate on (A) 5/28, (B) 7/8, (C) 8/11, and (D) 8/12.

4. DISCUSSION

The current literature regarding the impact of coral spawning on phytoplankton biomass is limited to studies performed in the GBR, with assessment areas approximately 70 km or closer from the major shoreline. These sites likely see higher amounts of freshwater, sediment, and nutrients from the coast (Furnas et al. 2005) than the FGB, located 3 times the distance offshore (200 km). This could explain far higher chl *a* concentration found during spawning at GBR sites than the study site at EFGB. Furnas and Mitchell (1986) conducted a study on the effect of intrusive nutrient activity on GBR phytoplankton and found that individually, single samples gave little indication of the responses of phytoplankton, but series of transects over seasons allowed for better reconstruction of phytoplankton dynamics. This suggests a longer study period may create a clearer picture of phytoplankton variation. No samples were collected post spawning; it is highly probable that had samples been collected one to two weeks post spawning at EFGB, values higher than 0.2 μg/L would have been recorded, as coral gametes would have had more time to be mineralized and thus release nutrients that could be utilized by phytoplankton (Wild et al. 2008). Satellite chlorophyll *a* data indicate that, while waters directly within EFGB do not show a detectable change in concentration from August to September 2009 (Figs 20 and 21), water immediately surrounding the bank has lower concentration in August and a higher concentration post-spawning in September.

The dominant accessory pigments found in this study were zeaxanthin, fucoxanthin, peridinin, and 19'hex. Zeaxanthin concentration at EFGB was significantly correlated with depth ($P<0.05$). The average concentration of this pigment during the study period was 0.056 μg/L, which is similar to the concentration at a non-eutrophic reef station in the Southern Caribbean at 0.061 μg/L (Van Duyl et al. 2002). Similar concentrations have also been reported in the N. Pacific at 0.041 μg/L, whereas N. Atlantic values are lower at 0.006 μg/L (both measurements from top 20 m of water

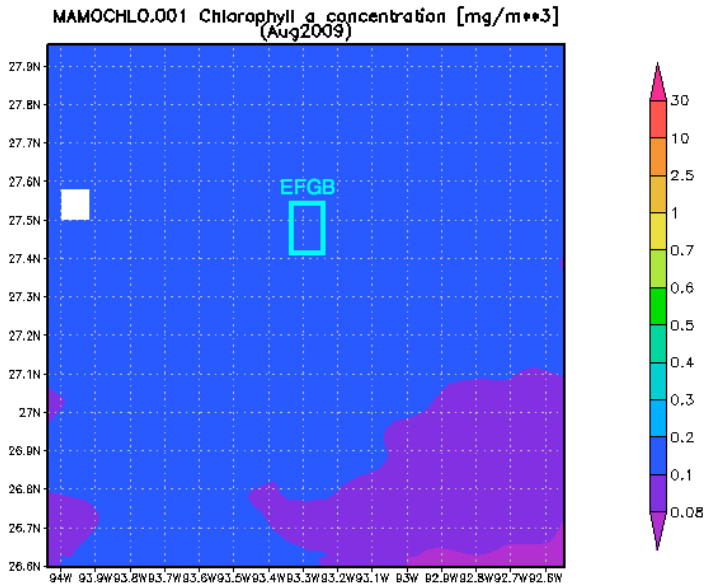

Fig 20. Chlorophyll *a* concentration in August 2009 from MODIS Aqua Satellite. Blue box indicates study site at East Flower Garden Bank (EFGB).

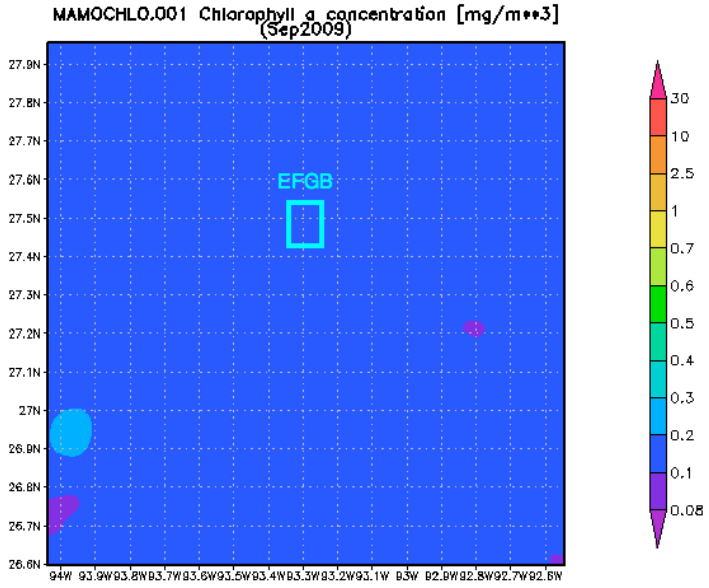

Fig 21. Chlorophyll *a* concentration in September 2009 from MODIS Aqua Satellite. Blue box indicates study site at East Flower Garden Bank (EFGB).

column) (Andersen et al. 1996; Letelier et al. 1993). Lambert et al. (1999) have also shown in the North West Gulf of Mexico (NWGOM) average zeaxanthin concentration of 0.05 μg/L at offshore stations and a maximum concentration of 0.10 μg/L (maximum found at EFGB was 0.104 μg/L). Highest concentration of zeaxanthin (in May) did not coincide with highest abundance of *Trichodesmium* spp. *Trichodesmium* spp was highest when average zeaxanthin was below 0.06 μg/L. The samples taken in May could have had high abundance of smaller cyanobacteria that would contribute to increased zeaxanthin concentration.

Fucoxanthin averaged 0.003 μg/L and was substantially lower than measurements in Caribbean reefs (0.118 μg/L) (Van Duyl et al. 2002). The value found at EFGB is in best agreement with average concentrations found at both Hydrostation S (N. Atlantic) and ALOHA (N. Pacific) at 0.001 μg/L and 0.006 μg/L, respectively (Andersen et al. 1996). 19'hex was significantly different (ANOVA, $P<0.01$) between morning and evening samples, possibly indicating diurnal fluctuations in the prymnesiophyte community at EFGB. Average peridinin levels in this study were lower than concentrations found in coastal reef waters (Van Duyl et al. 2002); however, peridinin measured in oligotrophic waters of N. Atlantic and N. Pacific was extremely low and not considered in these studies to be a dominant accessory pigment (Letelier et al. 1993; Andersen et al. 1996). In the Gulf of Mexico, peridinin concentrations have also been shown to be very low (Lambert et al. 1999; Qian et al. 2003) and usually detected at only a few stations (Qian et al. 2003).

Variation in phytoplankton composition between sample dates was seen through estimation of class abundance; however the prokaryote class accounted for over 70% of the total chl *a* pool across the entire sampling period (Fig. 7). The HPLC method used to determine pigment concentrations cannot resolve divinyl chl *a*, which is a biomarker for procholophytes; therefore cyanobacteria and prochlorophytes cannot be distinguished from one another (Qian et al. 2003). The prokaryote class presented in this study represents both of these groups. It has been found that small prokaryotes typically dominate the GBR (Furnas et al. 2005; Eyre et al. 2008). It cannot be determined if small

prochlorophytes (such as *Prochlorococcus* spp. and *Synehococcus* spp. both <8 μm) or other prokaryotes, were a major component of phytoplankton biomass, as cells this small were not quantified in this study. It was found, however, that the cyanobacterium *Trichodesmium* spp. existed in high counts across the entire study and a likely represented a large contribution to the prokaryote class. *Trichodesmium* spp. is thought to be important in the fixation of N in many tropical systems (Capone et al. 1997) and is an important primary producer in tropical and subtropical seas (Carpenter et al. 2004; Capone et al. 2005; Hutchins et al. 2007). The decrease in prokaryote abundance steadily (6% from the start of coral spawning) (Fig. 7) coincided with a decrease in water temperature and a gradual decrease in salinity (Fig. 4C).

Low amounts of the cyanobacteria biomarker zeaxanthin were also found at a southern Caribbean reef (Van Duyl et al. 2002), in contrast to the high concentration found in the present study. Prymnesiophytes were the second most abundant group found at EFGB. In other open ocean environments, prymnesiophytes have been shown to contribute 40-80% of phytoplankton derived chl *a* (at Bermuda Atlantic Time Series, Hydrostation S; Andersen et al. 1996). Dinoflagellates were always in low abundance, as expected, with one exception: a peak on the second day of spawning, 8/12, at 9% abundance. Although low, this did represent a 3-fold increase from May and July samples, and a 2-fold increase from 8/11 and 8/13. A large increase (by 0.6uM) of average NO_3^- concentration in the water column on 8/11 could have been utilized by dinoflagellates; however, this group has slower growth potential than other phytoplankton groups (Furnas et al. 2005), so unless the growth rate was fast the peak in dinoflagellates may not be due to an increase of NO_3^- availability.

With the start of coral spawning, diatom abundance decreased from 8/11 to 8/12, but then slightly increased again on 8/13 (Fig. 7). SiO_2 concentration in the water column followed this same trend during spawning. The distance of EFGB from the coast (approx. 200 km) prevents direct input of river plumes or terrestrial runoff, which often carry high concentrations of silica, a required element for diatom production (Prezelin et al. 1987; Furnas et al. 2005). Pelagophytes were the least abundant phytoplankton class

at EFGB, contributing only 1.5-3.5 % chl *a*. Pelagophytes showed no appreciable variation between sample date (Fig. 9), although they can be a dominant group in both the N. Atlantic and Pacific Oceans, contributing between 15-35% abundance (Andersen et al. 1996).

The increase in cells to a high of 11 cells/ml on 8/13 suggests that after 2 nights of spawning, enough time had elapsed for organic matter (OM) from spawn material to be taken up by heterotrophic bacteria and recycled to fuel phytoplankton growth (Glud et al. 2008).

Other genera found in reef systems include *Nitzschia* spp., *Bacteriastrum* spp., *Chaetoceros* spp., and *Thalassionema* spp. (Furnas and Mitchell 1986). All of these genera were identified in EFGB waters, however, were found in very low abundance (all less than 0.08 cells/mL, n=19). Only *Bacteriastrum* spp. contributed greater than 5% relative abundance on any sample date (5/28). *Ceratium* spp. was the only dinoflagellate genus to contribute higher than 5% relative abundance (10% on 5/28 and 5.5% on 8/11). *Ceratium* spp. has been shown to exhibit mixotrophy (Jacobson and Anderson 1996; Stoecker et al. 1997; Glud et al. 2008) and assimilate organic matter directly; however, this genus did not show an increase in abundance during coral spawning. Competition for light and nutrients in an N limiting environment by *Trichodesmium* spp., may explain the high abundance during spawning (Mullholland and Capone 2000) and low abundance of other phytoplankton groups. *Trichodesmium* spp. was the most dominant genera of phytoplankton found across all sampling dates in EFGB waters. This genus has been observed in high concentrations in Gulf of Mexico (GOM) slope waters (Lambert et al. 1999) and has been shown to contribute significantly to coral reef phytoplankton composition (Revelante et al. 1982). This genus is known to aggregate in high densities at the surface on calm, sunny days (Capone et al. 1997). This could potentially explain the high abundance (83%) of *Trichodesmium* spp. on 8/11, as surface conditions were calm and the weather ideal for bloom formation. Small pelagic cyanobacteria (<2μm) were not accounted for in this study, however, both *Prochlorococcus* spp. and *Synecococcus* spp. can dominate coral reef waters in GBR

(Furnas et al. 2005) and have been shown to contribute 77-94% phytoplankton abundance in Red Sea fringing reef systems (Yahel et al. 1998).

It cannot be determined from this study if nutrient input increased diversity, as values were also very high in months prior to spawning (Fig. 17), however, during the 3 day spawning window, there was large variability. In contrast to samples from 8/12, which had 5 genera contributing greater than 5% relative abundance, 8/11 and 8/13 only had 2 dominant genera each. *Trichodesmium* spp. dominated abundance on the first day of spawning, and diversity dropped to 0.303. This suggests the potential for this genus to alleviate N limitation at the start of spawning and begin to outcompete other species (Mulholland and Capone 2000). High diversity is generally found at intermediate levels of biomass and low diversity when blooms are present or a single species dominates the community (Irigoen et al. 2004). This trend was seen during sampling at EFGB, as diversity was high at mid level biomass (~0.09 μg/L) and very low when single genera dominated the abundance (*Trichodesmium* spp. on 8/11 at 85% and *Cylindrotheca* spp. on 8/13 at 55%).

Oligotrophic surface waters are generally depleted in combined N (Mulholland and Capone, 2000) and DIN:P ratios found during this study suggest that phytoplankton at EFGB are limited by nitrogen on most dates. The average DIN:P ratio across the entire sampling period was 24 with a range from 4.6 to as high as 78 on 5/28. This very high DIN:P ratio on 5/28 could be attributed to extremely low values of phosphate on this sample date (average 0.04 μM) compared to other dates. This suggests that on 5/28, phytoplankton were phosphate limited as the DIN:P value was greater than 30 (Dortch and Whitledge, 1992; Lambert et al. 1999). Phytoplankton on the remaining sample dates appear to switch to N limitation with DIN:P ratios between 5-11. The slightly lower DIN:P range for EFGB phytoplankton could be the product of a lower amount of OM introduced by coral spawning to the water column as compared to riverine inputs or bottom water regeneration in the NWGOM shelf/slope region. Concentration of all nutrient species measured were significantly different between pre spawn and spawn at EFGB only when taking into account depth and time (AM/PM) factors. This could

indicate a distinct vertical structure present with nutrient concentration and possible day/night fluctuation in certain nutrient species.

Average concentration of readily available forms of nitrogen in other reef systems has been found to be on the order of 0.05 μM (Furnas et al. 2005). These concentrations increased during GBR spawning studies, with individual components of DIN (NH_4^+, NO_3^- , NO_2^-) below 0.6, 0.4, and 0.2 μM, respectively (Eyre et al. 2008). Increases in nutrient species were not seen as a result of spawning until approximately 4-5 days post spawning at the GBR (Eyre et al. 2008), indicating a lag time in release of available nutrients from spawn material to phytoplankton. Concentrations of NO_3^- at offshore, non-eutrophic reefs in the Caribbean have been shown to range from 0.4 to 0.8 μM (Van Duyl et al. 2002). Values at EFGB were similar for NO_2^-, however, NO_3^- was mostly below 0.8 μM and $NH_4^+$was higher with most measurements below 1.0 μM. A high metabolism rate of organic matter by heterotrophic bacteria would likely produce a large ammonium pool. When both nitrate and ammonium are available, phytoplankton will often preferentially take up ammonium (Dortch, 1990). The fluctuations in concentration seen throughout coral spawning could indicate that ammonium is being taken up as quickly as it is being produced causing a rapid turnover in the NH_4^+ pool (Furnas et al. 2005).

Silicate concentration was highest at EFGB on 7/8 at 1.92 μM. Silicate is a required nutrient for diatoms (Furnas et al. 2005) and this group is able to form blooms following nutrient input events. No spawning was occurring on 7/8, however, all 4 dominant genera belonged to the diatom class. This was also the only sample date in which *Trichodesmium* spp. was not a major component of the community, thus this may have alleviated competition on diatoms. Offshore values of silicate at GBR have been shown to range from 0.4 to 3.2 μM (Furnas et al. 2005), while NWGOM concentrations have ranged between BLD to as high as 28.5 μM (Lambert et al. 1999). The maximum silicate value at EFGB was 2.27 μM, in better agreement with GBR values. Concentrations were likely not as high as those found in the NWGOM, as the FGB are further removed from freshwater input of silica. Vertical distribution of silicate in GOM

outer shelf water is known to be higher in the upper 10 m and decrease to 50 m (Morse and Biggs, 1994). Silicate concentration at the FGB generally decreased from a depth of 9 m to 18m. Phosphate concentration was variable between sample date with an average value of 0.3 μM. Other studies have found phosphate concentrations to increase with depth, but this trend was not apparent in EFGB waters. Silicate concentration has also been show to be depleted in surface waters (Lambert et al. 1999) and increase with depth, however, this correlation can also not be made for this study site. The relatively shallow water column (60 m) and extension of photic zone could attribute to these trends being absent from EFGB waters.

5. CONCLUSIONS

The Flower Garden Banks are a unique system to study as they are a coral reef system, but are isolated in the open GOM waters, far from direct inputs from coastal Texas and Louisiana. This study provided a snapshot of how coral spawning affects the local phytoplankton community highlighting the similarities and differences from other reef systems. It is noted that the 2009 coral spawning event at the FGB was relatively small and less prolific than in previous years (FGB Coral Spawn Report 2009). Changes across a short time scale (days) during spawning window were seen; biomass steadily increased during spawning and relative abundance of phytoplankton genera exhibited shifts from pre-spawn to spawn samples. The small number of pre spawn samples (n=5) and lack of any samples post spawning, however, limit the results of this study as it cannot be determined if these samples allow for accurate reconstruction of phytoplankton variation in the area. The two pre spawn dates were 10 days apart and collected more than one month prior to the start of coral spawning. Had collection been able to be carried out on a more regular basis several months prior to and at least one-month post spawning, it is likely a clearer picture of phytoplankton dynamics at EFGB would emerge. Several studies have found that phytoplankton community did not change significantly due to a single intrusion event, but instead increased on a regional basis with enhanced biomass/species shifts being found in patches (Furnas and Mitchell 1986; Furnas et al. 2005).

Cyanobacterium *Trichodesmium* spp. was a large component of phytoplankton abundance throughout the sampling period. This genus contributes new nitrogen to tropical and subtropical marine systems (Mulholland and Capone 2000), and could indicate that N limitation is alleviated at the FGB when compared to other systems where *Trichodesmium* spp. is less abundant.

On 8/12/2009, there was approximately a 1 knot flow moving past the study site at the FGB (Fig. 22). Water movement flowing steadily past the reef could indicate a

more transient phytoplankton community and possibly explain the variability in dominant genera seen on a daily time scale.

Future studies should sample EFGB waters over an entire year to develop baseline values of biomass, community composition, and nutrient concentrations, which currently are not available in the literature. Samples taken during coral spawning can then be more accurately evaluated for changes specifically attributed to the input of OM from coral reproduction. Accounting for smaller phytoplankton than were collected in this study could also greatly change the abundance of different algal classes, as very small cells such a unicellular cyanobacteria *Prochlorococcus* spp. and *Synechococcus* spp. have been shown contribute largely to chl *a* pools in other reef systems (Eyre et al. 2008; Glud et al. 2008).

Coral reef sediments have been shown to play an important role in the degradation of organic matter (Wild et al. 2004). Future studies on how coral spawn material is recycled in both the water column and sediments could enhance understanding of where this material is going in the FGB system. Knowledge of these processes can determine if intense benthic-pelagic coupling, as is seen in other reef systems, is present in the deep-water coral reef system at the Flower Garden Banks.

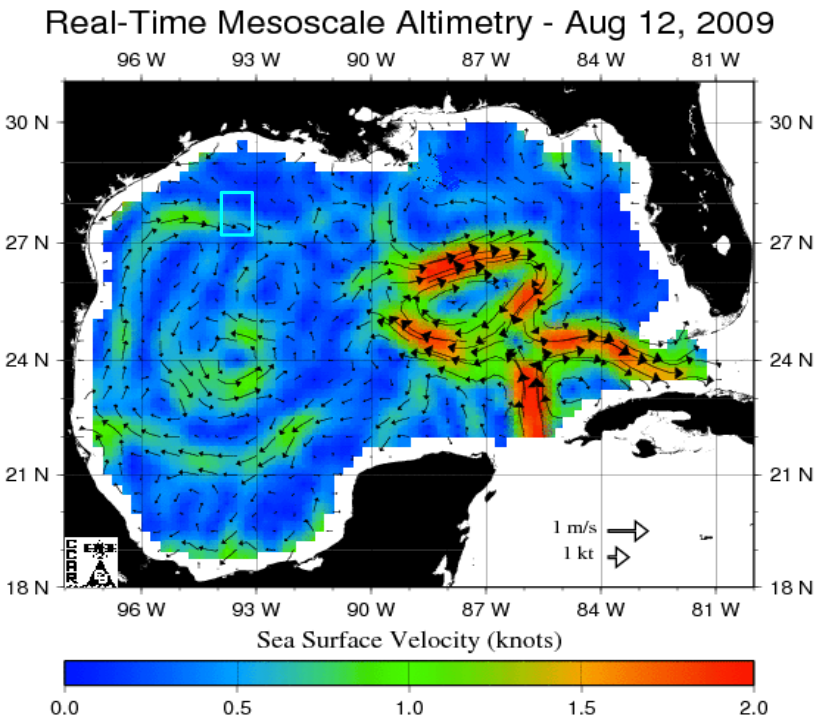

Fig 22. Altimetry map showing sea surface velocity for the Gulf of Mexico on August 12, 2009. Blue box indicates study site at FGB.

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