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Assessing Respiration Rates and Nutrient Dynamics of Aritifical Reef Biofilms and Bacterioplankton in the Mississippi Sound

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ASSESSING RESPIRATION RATES AND NUTRIENT DYNAMICS OF ARTIFICIAL REEF BIOFILMS AND BACTERIOPLANKTON IN THE MISSISSIPPI SOUND

by

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ABSTRACT

ASSESSING RESPIRATION RATES AND NUTRIENT DYNAMICS OF ARTIFICIAL REEF BIOFILMS AND BACTERIOPLANKTON IN THE MISSISSIPPI SOUND

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Artificial reefs are primarily used to provide a suitable habitat for target fish populations, but the structures can also improve water quality and benefit non-target organisms. Laboratory incubation experiments were conducted in the presence of biofilm on rubble and in its absence to examine bacterial growth, community respiration, and nutrient dynamics at four artificial reef habitats in the Mississippi Sound. Biofilm samples were also collected from settlement plates deployed at each site and were analyzed for $\delta^{13}C$ and $\delta^{15}N$ stable isotope content. Respiration rates were always higher in the presence of biofilm but bacterial abundance often declined over time, and rates of decline were higher in the presence of biofilm. This suggests that heterotrophic activity was high but bacterial abundance was limited by some factor, such as grazing pressure. PO_4 and NH_4 production were often observed in incubation experiments, and production rates were higher in the presence of biofilm, indicating that the benthic community supplements microbial water column nutrient regeneration. Respiration, PO_4 production, and NH_4 production were higher in low profile reef incubations than high profile reef incubations when biofilm was present, which reflected the higher biofilm growth observed at low profile reefs. Seasonal effects were also observed. Respiration and nutrient production rates were positively correlated with temperature, and $\delta^{13}C$ and $\delta^{15}N$

values were enriched during warmer seasons, all of which indicate higher benthic and water column productivity. Further studies are needed to compare productivity and nutrient regeneration at other artificial reefs and natural reefs.

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BA	Bacterial abundance
BOD	Biological oxygen demand
DAPI	4', 6 diamidino-2-phenyndole
DI	Deionized water
DMR	Mississippi Department of Marine Resources
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
Sq. Hand.	Square Handkerchief reef
TDN	Total dissolved nitrogen

CHAPTER I

INTRODUCTION

Artificial reefs have become a popular tool in fisheries management to provide additional habitats for fish populations. Reefs are usually constructed of repurposed scrap material, most often from automobile tires and concrete blocks, and are often constructed in an effort to improve recruitment and succession for target populations, although additional purposes can include tourism or shoreline protection (Bohnsack and Sutherland 1985; Pickering et al. 1999). Although the goal of most artificial reefs is to improve fish stocks, they can facilitate the success of many other trophic levels. Benthic invertebrates and smaller non-target nekton benefit from the structures, and these organisms rapidly colonize the reefs after construction (Davis et al. 2002; Bohnsack and Sutherland 1985). The increase in availability of hard substrate availability favors benthic primary producers, allowing greater colonization on the surface of the reef (Buckley and Hueckel 1985; Bohnsack and Sutherland 1985). Productive benthic filter feeders can improve water quality of artificial reefs, remineralizing inorganic nutrients that can then be taken up by primary producers (Miller 2002). Bacterial populations are strongly influenced by primary production, so microbial activity is expected to increase at artificial reefs as well.

The role of heterotrophic bacteria in aquatic systems has been recognized as an important contributor to the net community respiration and metabolism. Research shows that bacteria can be responsible for as much as 50% of total community respiration, and are among the most important primary consumers due to their high assimilation efficiency of primary production and high biomass (Admiraal et al. 1985; Larsson and

Hagstrom 1982; Fuhrman and Azam 1980; Fuhrman and Azam 1982; Payne 1970; Pomeroy 1974). Thus, bacteria play an essential role in the biogeochemical cycling of nutrients and organic matter. Bacteria can incorporate organic matter and nutrients into biomass for consumption by higher trophic levels or remineralize complex compounds and particulates to CO_2 and inorganic nutrients that can be utilized by primary producers (Sherr and Sherr 1988; Lancelot and Billen 1984). As a result, there has been increasing emphasis on the role of bacteria in carbon and nitrogen budgets in aquatic systems (Bronk et al. 2007).

Bacterial abundance (BA) in the water column is measured by visually counting cells via epifluorescent microscopy (Hobbie et al. 1977). The epifluorescent dye binds to any DNA, so this method allows active and inactive bacteria to be counted, which when measured over time illustrates population growth. However, when measuring bacterial biomass in soil or sediment samples, direct cell counts are less effective. Elemental analyzers provide percent carbon and percent nitrogen of samples, which can be used to determine C:N. In this study, the use of epifluorescent dye was used to estimate bacterial biomass in the water column, while elemental analysis was used to determine biomass in biofilm samples.

Winkler titration is an accurate method to measure dissolved oxygen concentrations in water (Carpenter 1965), making it effective in bacterial incubation experiments to observe changes in oxygen concentration over time (Coffin et al. 1993; Obernoster et al. 2008; Alonso-Saez et al. 2007). Concentrations are measured periodically over the duration of the incubation, and can be plotted against time to establish respiration rates (Coffin et al. 1993). Oxygen probes are another tool for

measuring dissolved oxygen concentrations in water samples and are often considered more favorably because measurements can be taken without interruption over time (Briand et al. 2004). Such probes can have issues with drift, however, and are expensive so in cases of multiple treatments in experiments their use can be cost-prohibitive. Therefore the Winkler titration method was applied for the purposes of this study. For incubation results, oxygen flux was determined periodically over the course of the incubation and divided by the total time elapsed to calculate community respiration rates.

Bacterial growth and respiration are dependent on many factors, such as the "quality" (i.e. small labile molecules vs large refractory molecules) and quantity of available organic matter, nutrient availability, and environmental conditions including seasonal variation (del Giorgio and Cole 1998). Researchers have found that as organic matter becomes more labile, bacterial production often increases (Coffin et al. 1993), however limited experimental research in Apalachicola Bay suggest that bacteria are more productive when feeding on refractory organic matter rather than labile organic matter (Dillon unpublished data). These seemingly opposing results reflect the importance of spatial and temporal variance in bacterial activity and population dynamics, particularly when considering the utilization of autochthonous versus allochthonous sources of organic matter (Ram et al. 2003; Coffin et al. 1993). In coastal systems there is often a tight coupling of phytoplankton and bacterial production, with diel variation in microbial activity that increases during daylight hours and decreases at night (Coffin et al. 1993). Estuarine systems in the Northern Gulf of Mexico tend to be dominated by allochthonous inputs of organic matter (Coffin et al. 1993). Autochthonous carbon sources in estuaries are primarily derived from phytoplankton and are considered

to be labile, with elemental stoichiometries close to the Redfield ratio (Redfield 1934; Bronk et al. 2007; Ram et al. 2003). Terrestrial and wetland plants are the primary sources of allochthonous carbon. Terrestrial-derived sources have a higher C:N than phytoplankton (i.e. the Redfield ratio), and a study by Perdue and Koprivnjak (2007) on four estuaries in the Gulf of Mexico showed that the fraction of terrestrially derived organic matter has been previously underestimated by as much as 0.05 to 0.24.

Nutrient availability also plays an important role in bacterial population dynamics and heterotrophic bacteria often compete directly with phytoplankton for inorganic nutrients (Thingstad and Rassoulzedagon 1999). Studies in the Mississippi River have shown that nitrogen is the limiting nutrient for phytoplankton and microbial growth (Turner et al. 2006), although phosphorus can also act as the limiting nutrient at certain times of the year in the Mississippi River plume (Pakulski et al. 2000). Lohrenz et al. (2008) concluded that nitrogen enrichment contributes significantly to phytoplankton production, but at certain times of the year phosphorus acts as the limiting nutrient. Bacteria can be carbon limited as well. Nutrient enrichment experiments in the Chesapeake Bay area showed that nitrogen and phosphorus enriched treatments had little effect on bacterial activity, but when treatments were enriched with organic carbon sources, respiration rates increased significantly (Smith and Kemp 2003). Chin-Leo and Benner (1992) performed nutrient enrichment experiments in the Mississippi River and Mississippi River plume and found that bacteria in low salinity areas showed signs of carbon limitation, suggesting that although high DOC concentrations were present in the river, the autochthonous DOM in intermediate salinity areas was more labile. Carpenter (2010) found that bacteria populations in Mississippi Sound are also carbon limited due

to limited amounts of labile DOM and large amounts of refractory allochthonous carbon sources, which are inaccessible to microbes. The relationship between autochthonous organic matter and microbial populations emphasizes the importance of better understanding the interactions between phytoplankton and bacteria.

Biofouling of substrate is influential in community development. Density and age of the biofilm as well as composition can affect invertebrate larval settlement and metamorphosis, even if the organism is not in direct contact with the biofilm (Huang and Hadfield 2003; Rodriguez and Epifanio 2000; Unabia and Hadfield 1999; Wieczorek and Todd 1997; Snyder et al. 2005). Wobus et al. (2003) found that bacterial population composition differed in eutrophic reservoirs when compared to dystrophic reservoirs, indicating the influence that nutrients and phytoplankton can have on bacterial populations. A study in the Baltic Sea found that microbial composition is most influenced by water depth, dissolved organic carbon, oxygen, salinity, and silicate, and that populations were not significantly influenced by nitrate, ammonium, or phosphate concentrations (Edlund et al. 2006). By measuring water quality parameters such as salinity and dissolved oxygen (DO), and examining nutrient concentrations, these factors can then be compared to the bacterial populations to determine potential interactions. Seasonal effects also play a role in biofilm growth and community composition. Moss et al. (2006) found that biofilm community structure varied seasonally as a result of changes in nutrient availability, light, and temperature. Substrate material is also an important factor in biofilm development. Nocker et al. (2004) found that species richness was significantly higher at oyster reefs than in muddy sand bottom locations, indicating the importance substrate quality can have on biofilm growth and diversity.

Stable isotopes can help elucidate the primary source of nitrogen and carbon in a system (Montoya 2007; Voss and Struck 1997; Brandes and Devol 1997; Michener and Kaufman 2007; Chaloner et al. 2002). Several studies have shown that consumers in marine and estuarine environments prefer organic matter produced *in situ* over terrestrial sources, and favor consumption of microalgae over vascular plant detritus, which can often be shown using the stable isotope values of consumers (Deegan and Garritt 1997; France 1998). According to Chaloner et al. (2002), $\delta^{13}C$ values of marine organic matter are higher than those of terrestrial and freshwater organic matter and biofilm incorporation of marine-derived nitrogen and carbon may be dependent on community composition. Heterotrophic-dominated biofilms can utilize and transform particulate organic matter to nutrients, while autotrophs cannot. Therefore, autotrophic-dominated biofilms should have lower carbon uptake than nitrogen uptake relative to heterotrophic-dominated biofilms (Chaloner et al. 2002; Fenchel et al. 1998). Chaloner et al. (2002) concluded that examining the stable carbon and nitrogen isotope values of biofilm samples can shed light on the metabolic contribution of heterotrophic bacteria versus phytoplankton. Furthermore, CO_2 availability can affect the $\delta^{13}C$ signatures of biofilm, indicating the influence of primary production and carbon cycling on stable isotope values (Staal et al. 2007; Voss and Struck 1997). $\delta^{15}N$ signatures reflect differences in trophic levels, with higher ^{15}N enrichment in higher trophic levels (Montoya 2007).

Comparison of microbial activity among different artificial reefs can illustrate how productive the reefs are relative to each other and determine seasonal differences in the production and consumption of organic matter (Caffrey 2003). Temperature and nutrient availability are typically the most important factors affecting bacterioplankton.

Higher temperatures tend to favor enhanced heterotrophic metabolic rates (Caffrey 2003). Typically nutrient-rich freshwater runoff is expected to produce autotrophic conditions, while organic-rich freshwater runoff is expected to favor heterotrophic conditions (Caffrey 2003; Caffrey 2004). However, other factors such as turbidity must be taken into consideration as well. Mississippi Sound is an organic-rich estuary that has been shown to be carbon limiting for heterotrophic microbial populations (Carpenter 2010), but artificial reef structures may enhance benthic algal populations by providing elevated hardened substrate surfaces near the water column surface which in turn may improve availability of labile carbon sources, facilitating bacterial growth.

Bacterial growth, community respiration, and nutrient dynamics were examined at four artificial reefs within the Mississippi Sound in the presence of biofilm and in the absence of biofilm. Respiration rates and nutrient remineralization were expected to be higher in the presence of biofilm. Nutrient concentrations (PO_4, NH_4, NO_3, and NO_2) and bacterial abundance were also measured along transects with increasing distances from the reef, and nutrient concentrations and bacterial abundance were predicted to be higher at high relief reefs only due to higher nutrient regeneration, enhanced bacterial growth, and limited mixing effects. Stable carbon and stable nitrogen isotopes were measured in artificial reef biofilms. $\delta^{13}C$ signatures were expected to reflect *in situ* sources of benthic microalgae and marine phytoplankton production, and $\delta^{15}N$ signatures were expected to reflect benthic production in several trophic levels. $\delta^{13}C$ and $\delta^{15}N$ signatures of artificial reef biofilms were expected to be similar to organisms at natural reefs with similar ecological roles.

CHAPTER II

METHODS AND MATERIALS

Study Sites

Sample collection was conducted at four artificial reef sites located within the Mississippi Sound (Figure 1). All reefs are maintained by Mississippi Department of Marine Resources (DMR), and were chosen based on location (2 eastern reefs and 2 western reefs) as well as reef structure (2 high relief reefs, 2 low relief reefs) and total area of hard substrate. High relief reefs are exposed at the surface and low relief reefs are completely submerged in the water column. The 4 reef sites are named as follows: Katrina (eastern high relief), Legacy (eastern low relief), USM (western low relief), and Square Handkerchief (western high relief). High relief reefs are constructed primarily of large concrete debris while low relief reefs are composed of smaller limestone rocks and oyster shells. USM is the oldest reef and was constructed in 1996, Square Handkerchief was built in 2003, and Katrina and Legacy were established in 2006 and 2007, respectively. Katrina has been replenished with additional concrete material at the eastern end of the reef since its construction, and this area of the reef was excluded from sampling due to expected differences between newly constructed artificial reef substrate and climax communities at established parts of the reef.

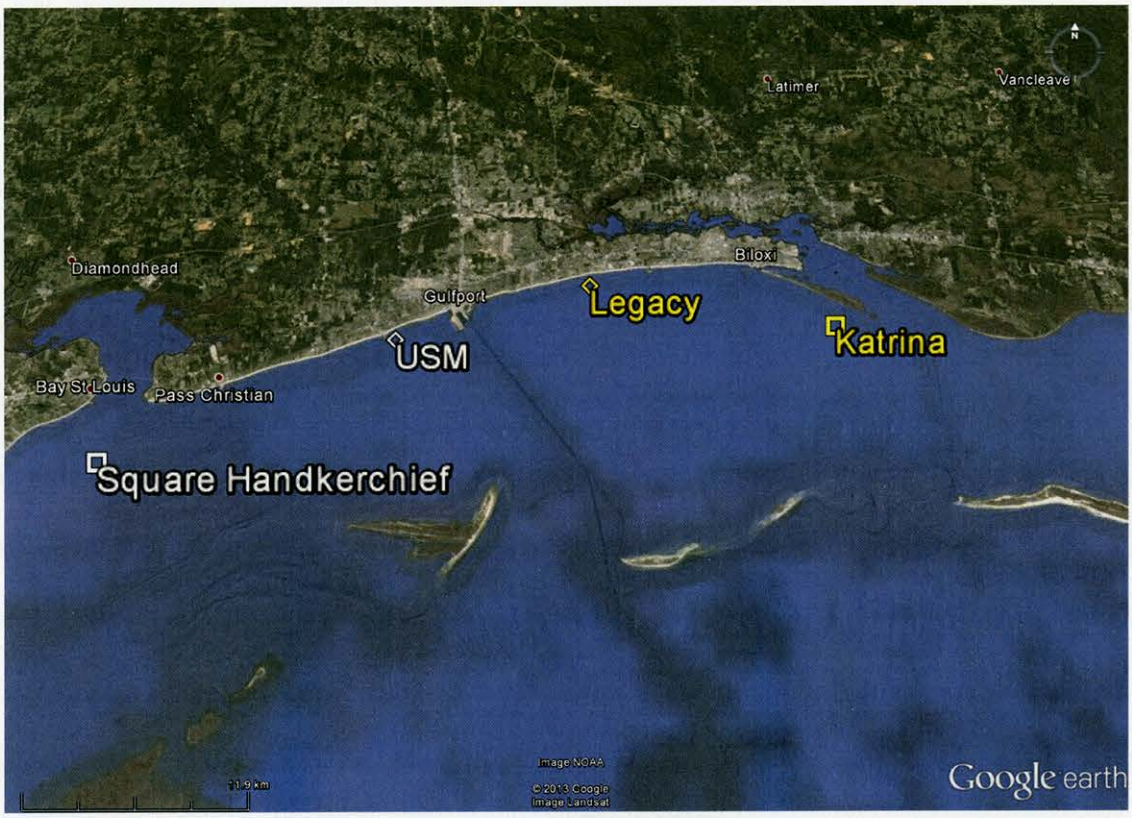

Figure 1. Artificial reef locations in Mississippi Sound. White labels indicate western reefs and yellow labels indicate eastern reefs. Square icons represent high profile reefs and diamond icons represent low profile reefs.

Experimental Design

At each reef site five substrate cages containing three pieces of crushed concrete varying in size (small, medium, large) and five plexigas settlement plates were deployed on a monthly basis for five seasons (Winter 2012, Spring 2012, Summer 2012, Fall 2012, and Winter 2013). Samples collected in January through March are considered winter samples, samples collected in April and May are considered spring samples, samples collected in June through August are considered summer samples, and samples collected in October through December are considered fall samples. The crushed concrete used for deployment in the substrate cages was the same used to construct the reefs and was obtained from DMR. Substrate cages approximately 12 inches in length were made of ½

inch mesh PVC-coated hardware cloth wrapped around 2 pieces of 6 inch PVC pipe that had been cut approximately 2 inches wide. Additional hardware cloth was cut and fastened to either end of the cages to enclose them, and each cage was secured to a concrete anchor so the cages rested just above the reef bottom. Each substrate cage was tied to a buoy and assigned a number for easy identification. A plexiglas settlement plate (6x6 inches) was also suspended from each buoy rope to collect additional biofilm samples for stable isotope analysis. To ensure settlement plates did not fall into the sediment they were secured at a depth of 6 feet for high relief reefs, which have a depth of 7-8 feet, and 3 feet for low relief reefs, which have a depth of 3-6 feet. GPS coordinates for each substrate cage were recorded at the time of deployment. Reef sites were divided into 5 transect sections to ensure the substrate cages and settlement plates were evenly distributed across each reef. Substrate cages and settlement plates were deployed for a target soak period of 2-4 weeks before being retrieved, although soak periods sometimes exceeded that time frame (Table 1).

Laboratory incubation chambers were employed following sample collection. The incubation chambers were 12 inches long and made of 6 inch clear acrylic tubes with a large silicone stopper on both ends. Bottom stoppers had one piece of Tygon tubing inserted through them to collect water subsamples with a metal tubing clamp to control flow, and the top stoppers had 2 pieces of Tygon tubing inserted through them to facilitate pressure release while filling the chambers, with plastic tubing clamps attached to close each piece of tubing. Silicone sealant was applied where each piece of tubing meets the stopper to achieve an air-tight seal. Chambers were arranged in groups of three,

on hand-made wooden rotisseries that allowed the chambers to be rotated and mixed periodically (Figure 2).

Figure 2. Incubation chamber set-up, with chambers with rubble from the substrage cages pictured on the left and control chambers without rubble on the right.

Incubation Experiments

Water temperature, salinity, and DO measurements were determined using a calibrated YSI model 6600 or YSI Pro 20 at each site. Water was pumped pneumatically from a depth at least 13 cm below the surface with the pump pressure below 20 psi to avoid cell lysis (Fuhrman and Bell 1985), and dispensed into two 20 L carboys pre-washed with 10% HCl and rinsed three times with sample water at each reef. Carboys were brought back to the lab and stored overnight in a dark temperature-controlled incubation room set at the *in-situ* water temperature at the time of collection. The

following morning the carboys were gently shaken and then the water was distributed into 2 L glass acid washed (10% HCl) incubation bottles and 60 ml glass acid washed biological oxygen demand (BOD) bottles, hereafter referred to as BOD incubation experiments, and into 2 L acid washed acrylic incubation chambers with silicone stoppers, hereafter referred to as biofilm incubation experiments. The incubation chambers were treated with rubble, and following the incubation period, water from each incubation chamber was distributed into 60 ml glass acid washed BOD bottles for dissolved oxygen analysis and glass acid washed beakers for bacterial abundance and nutrient analysis. See Figure 3 for a diagram of sample collection for each analysis. Incubation containers remained in the dark incubation room at *in-situ* temperature for the duration of the experiment. See Table 1 for incubation experiment temperatures and lengths of exposure.

Table 1

Sampling Dates and Incubation Temperatures for Incubation Experiments.

Experiment	Deploy Date	Retrieve Date	Reef	Temp (°C)	Exposure (days)
1	1/27/2012	2/28/2012	Sq Hand USM	15	32
		3/7/2012	Legacy Katrina	18	40
2	4/12/2012	5/8/2012	Sq Hand USM	27	26
		5/14/2012	Legacy Katrina	27	32
3	5/30/2012	6/21/2012	Sq Hand	28	22
	6/2/2012		USM		19
	6/2/2012	6/26/2012	Legacy Katrina	30	24
4	7/11/2012	7/24/2012	Sq Hand USM	30	13
		7/30/2012	Legacy Katrina	32	19
5	8/7/2012	8/21/2012	Sq Hand USM	29	14
		9/11/2012	Legacy Katrina	28	35

Table 1 (continued).

Experiment	Deploy Date	Retrieve Date	Reef	Temp (°C)	Exposure (days)
6	9/27/2012	10/17/2012	Sq Hand USM	23	20
		10/23/2012	Legacy Katrina	23	26
7	11/5/2012	11/15/2012	Sq Hand USM	14	10
		12/2/2012	Legacy Katrina	17	27
8	2/1/2013	3/14/2013	Sq Hand USM	15	41
		4/2/2013	Legacy Katrina	19	60

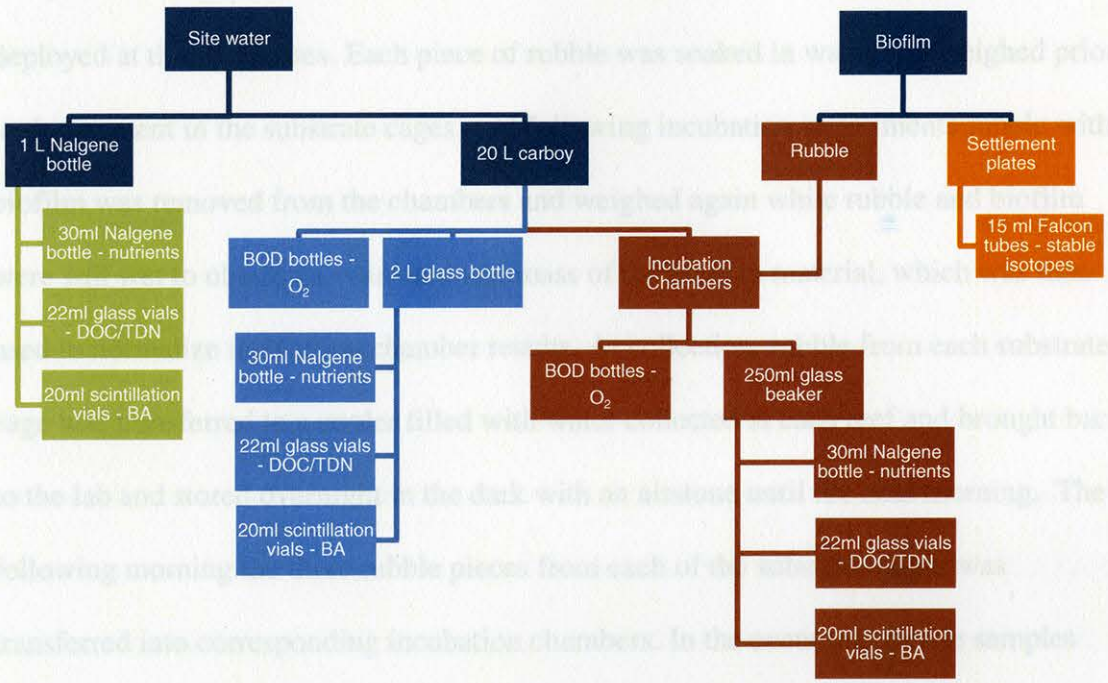

Figure 3. Flow chart of sample collection for each analysis. Dark blue boxes represent samples collected in the field, green boxes represent samples used for transect analysis, medium blue boxes represent samples used in BOD incubation experiments, red boxes represent samples used for biofilm incubation experiments, and orange boxes represent samples used for stable isotope analysis.

BOD bottles were gravity-filled from the bottom of the bottle with Tygon tubing and allowed to overflow three times to eliminate any air bubbles. The inside and outside of the Tygon tubing was acid washed and triple rinsed with deionized (DI) water, then flushed thoroughly with sample water before filling incubation bottles to ensure all contaminants are removed (Price et al. 1986). The 2 L incubation bottles were triple rinsed with sample water before being filled, and the incubation chambers were acid washed and triple rinsed with DI water.

A control incubation chamber containing only sample water was run parallel to biofilm incubations to characterize water column respiration and bacterial growth. Sample water from each reef was treated with biofilm growing on rubble in cages deployed at the study sites. Each piece of rubble was soaked in water and weighed prior to deployment in the substrate cages, and following incubation experiments rubble with biofilm was removed from the chambers and weighed again while rubble and biofilm were still wet to obtain an estimated wet mass of the biofilm material, which was then used to normalize incubation chamber results. At collection, rubble from each substrate cage was transferred to a cooler filled with water collected at each reef and brought back to the lab and stored overnight in the dark with an airstone until the next morning. The following morning the three rubble pieces from each of the substrate cages was transferred into corresponding incubation chambers. In the event that rubble samples could not be recovered due to cages being lost in the field, a control chamber was set up in its place, using water collected from its respective site. Control and biofilm treatments were randomly assigned to incubation chambers to minimize any environmental variation within the walk-in incubation room. Incubation chambers were then filled with water

from the site where rocks were deployed and clamped close. Rubble and sample water were incubated in the chambers for 2-8 hours, depending on temperature and amount of biofilm on the rubble. Incubation chambers were gently mixed every 2-5 hours by turning the rotisserie rack during incubation experiments to prevent any spatial gradients from forming within the chambers.

Sample Analysis

Triplicate BOD bottles were collected and pickled at each time point to determine microbial respiration rates. Oxygen concentrations were determined within 48 hrs of collection using an automated amperometric oxygen Winkler titrator (Langdon Enterprises). Sampled time points were at approximately 0, 1, 2, 4, 6, 8, 12, 24, and 48 hrs for BOD bottle incubations. Incubation chambers were sampled at 2-8 hrs, with a time zero sample being obtained from the carboy at the time the chambers are filled. Incubation chambers were only sampled once due to the resulting head space in the chamber after sampling, as well the limited volume each chamber could hold. A linear regression was generated for oxygen uptake and the t_n oxygen concentration was subtracted from the t_0 oxygen concentration to find oxygen consumption, where t_n is the time at which the sample was collected. A 0.01 N KIO_3 standard solution was used to measure standards and blanks. Standards were treated with 10 ml KIO_3 standard solution in DI water, and 0.5 ml of Winkler reagents were added according to the procedure in Strickland and Parsons (1984) and the mixture was titrated. Blanks were treated with 1 ml KIO_3 standard solution in DI water, and 0.5 ml of Winkler reagents were added and the mixture was titrated. Then another 1 ml of KIO_3 was added and the mixture was titrated again. The value of the reagent blank is the difference between the two titrations.

Bacterial abundance (BA), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and inorganic nutrient samples were collected from the 2 L incubation bottles over the duration of the BOD incubation experiments and were collected from cleaned and acid washed glass beakers in biofilm incubation experiments. BA, DOC, TDN, and nutrient samples for the transect samples were collected from the 1L Nalgene bottles containing the original sample. Sample water was gently mixed prior to collection. BA samples for BOD bottle incubations were collected at approximately 0, 1, 2, 4, 6, 8, 12, 24, and 48 hrs, while DOC, TDN and nutrient samples were collected at approximately 0, 6, 12, 24, and 48 hrs. BA, DOC, TDN, and nutrient samples for the incubation chambers were collected at time zero from the carboy at the time the chambers are filled, and from the chambers at a determined sampling time. BA samples were distributed into sterile 20 ml scintillation vials with a cone cap and preserved with 200 µl Lugols solution that had been filtered through a 0.22 um pore size, which preserves the samples for several years if kept in the dark and fresh Lugols solution is added occasionally (Nollet 2000). To minimize problems from decreasing cell volume due to storage with Lugols solution samples were counted within two months of collection (Hawkins et al. 2005). Samples were stored in a dark refrigerator (10°C) until prepared for counting. To prepare slides, samples were pipetted into sterile plastic Falcon tubes and the Lugols solution was cleared with 0.22 µm filtered $Na_2S_2O_3$ solution. A 1% concentration per sample of 0.1 mg ml^{-1} 4', 6 diamidino-2-phenyndole (DAPI) was added to each Falcon tube and kept in the dark for at least 30 minutes to stain. Samples were then vacuum filtered onto 25 mm black polycarbonate membrane filters (pore size 0.22 µm), oil plated onto glass slides, and hand counted on a 100X Nikon fluorescent

microscope. At least 400 bacteria and 8 fields were counted for each sample (Hobbie et al. 1977; Fry 1990). Changes in bacterial abundance over time were then used to calculate biomass and growth efficiencies, as described previously.

Triplicate samples for DOC, TDN and nutrient analysis were syringe filtered through pre-combusted (500°C for 2 hrs) 25 mm Whatman GF/F filters with acid washed stainless steel filter holders and acid washed glass syringes that had been triple rinsed with DI water into pre-combusted (500°C for 4 hrs) glass DOC vials and acid washed 30 ml Nalgene bottles for DOC, TDN and nutrient analysis, respectively. Nalgene bottles were triple rinsed with DI water before being filled. DOC vials were capped with TFE lined caps and DOC/TDN and nutrient samples were immediately frozen at -20°C until analysis.

DOC and TDN samples were thawed slowly at room temperature and acidified with 35 µl concentrated HCl. DOC and TDN concentrations were measured using a Shimadzu TOC-V analyzer (high temperature combustion, platinum catalyst) equipped with a total nitrogen detector. Standard curves were generated with known standard solutions of potassium hydrogen phthalate (1000 mg C L^{-1} stock solution) for DOC and potassium nitrate (1000 mg N L^{-1} stock solution) for TDN. Concentrations of NO_3, NO_2, and NH_4 were subtracted from TDN concentrations to determine DON concentrations.

Nutrient analysis included detection of NO_x, NO_2, NH_4, and PO_4. Sample bottles were thawed slowly at room temperature for each analysis. For NO_2 analysis, samples were measured colorimetrically on a Shimadzu UV-visible spectrophotometer (Strickland and Parsons 1984). Standard curves were generated with a known standard solution of 10 mM sodium nitrite stock solution. The minimum detection limit for NO_2 analysis is 0.10

μM. For NO_x analysis, samples were measured with a Thermo Model 42i Chemiluminescence NO-NO_2-NO_x analyzer to determine NO_x concentrations using a procedure adapted from Braman and Hendrix (1989). Standard curves were generated with a known standard solution of 10 mM potassium nitrate stock solution, and the analysis has a minimum detection limit of 0.50 μM. NO_2 concentrations were subtracted from NO_x concentrations to determine NO_3 concentrations.

NH_4 concentrations were measured colorimetrically on a Shimadzu UV-visible spectrophotometer using a procedure adapted from Bower and Holm-Hanson (1980). Standard curves were generated using a known standard solution of 10 mM ammonium sulfate stock solution. PO_4 concentrations were also measured colorimetrically on a Shimadzu UV-visible spectrophotometer according to methods outlined in Strickland and Parsons (1984). Standard curves for PO_4 analysis were generated using a known standard solution of 10 mM KH_2PO_4 stock solution. The minimum detection limit for PO_4 and NH_4 is 0.50 μM. For a summary of each type of sample and its corresponding analyses and hypothesis, see Table 2.

Respiration, bacterial production, and nutrient production rates calculated from biofilm incubations were normalized to the surface area of the rubble. The surface area for each piece of crushed concrete was measured using a Next Engine 3D Scanner HD, and the measurements were applied to the corresponding incubation chamber that contained the rubble. Rocks were scanned using a NextEngine Scan Studio HD program using 360° positioning, 8 divisions, 2000 points in^{-2}, with a wide range and neutral target settings. The scanner was calibrated using a NextEngine calibration object.

Table 2

Summary of Types of Samples Collected and Their Corresponding Hypotheses.

Type of Sample	Analysis	# Samples	Hypothesis
Transect	NH_4, PO_4, NO_x, NO_2, DOC/TDN	Sq Hand – 8 USM – 4 Legacy – 4 Katrina – 7	Expect nutrient levels to be higher on or near the reef than farthest sites, expect greater difference in transect samples at high relief reefs than at low relief reefs
Transect	Bacterial abundance	Sq Hand – 8 USM – 4 Legacy – 4 Katrina – 7	Expect higher bacterial abundance on or near the reef than populations at farthest sites
BOD Incubation	O_2 concentration	9 time points per incubation per site	Expect difference in respiration rates by reef and season
BOD Incubation	Bacterial abundance	9 time points per incubation per site	Expect difference in bacterial abundance by reef and season
BOD Incubation	NH_4, PO_4, NO_x, NO_2, DOC/TDN	5 time points per incubation per site	Expect difference in nutrient regeneration by reef and season
Incubation chambers	O_2 concentration	2 time points per incubation chamber, 6 incubation chambers per site (1 control, 5 with rubble)	Expect higher oxygen consumption in chambers with biofilm, expect difference by reef and season
Incubation chambers	Bacterial abundance	2 time points per incubation chamber, 6 incubation chambers per site (1 control, 5 with rubble)	Expect higher bacterial abundance in chambers with biofilm, expect difference by reef and season
Incubation chambers	NH_4, PO_4, NO_x, NO_2, DOC/TDN	2 time points per incubation chamber, 6 incubation chambers per site (1 control, 5 with rubble)	Expect higher nutrient regeneration in chambers with biofilm, expect difference by reef and season
Biofilm	Stable isotopes (^{13}C and ^{15}N)	5 samples per site	Expect ^{13}C signatures similar to benthic microalgae and ^{15}N signatures similar to benthic microalgae and invertebrates

The results of a pilot study conducted in the fall of 2011 were used to determine appropriate replication and statistical analysis in biofilm incubations. The following equation was used to determine sample size for the number of incubation chambers:

$$n \approx 2(z_\alpha + z_\beta)^2 * s^2/d^2$$

where n is the desired sample size, z_α is the standard normal deviate for α level probability, z_β is the standard normal deviate for β level probability, s^2 is the variance of measurements, and d is the difference between the means of measurements, in this case the difference between the means of control and rubble treatments. Using an α level of 0.01, and a β level of 0.01, it was determined that one control incubation chamber for each reef was sufficient due to the low variance of the pilot study results, leaving 5 remaining chambers per reef for treatments with rubble. Although results for chambers with rubble also had low variation, they were observed to have more variation than results from control chambers so higher replication was deemed more important for amendments.

All incubation chamber results were normalized to surface area of the rubble, as well as wet mass of biofilm per unit surface area prior to statistical analysis, and control incubation chambers were used as a correction factor for chambers with rocks. A relationship between time deployed and growth of biofilm was difficult to determine due to seasonal effects and limited replication within seasons, as well as patchiness in biofilm growth that was observed at the reef sites, so results were not normalized to time of exposure. Figure 4 depicts biofilm growth varability by exposure time. By normalizing results to biomass, distinctions can be made about mass-specific respiration rates, in order to infer contributions of different types of organisms composing the biofilm material

(microbial vs macrofaunal influence) relative to total biomass. Two-way ANOVAs were used to compare respiration, bacterial abundance, DOC, DON, and nutrient (PO_4, NH_4, NO_3, and NO_2) results for BOD incubations and biofilm incubation experiments. Respiration rates, bacterial growth rates, and nutrient flux rates for BOD bottle incubations were based on 48 hr incubation periods for all experiments. Main factors were represented by reef type (high vs low profile) and season, by reef location (east vs west) and season, or by individual reef and season. Interaction terms were included in two-way ANOVAs. Games-Howell post-hoc tests were conducted for biofilm incubation and BOD incubation two-way ANOVAs to examine differences between seasons. Where data were not normally distributed or had unequal variance Kruskal-Wallis tests were also used to confirm the findings of the ANOVAs. BA data were log transformed to improve the distribution of the data, and all other responses were analyzed as raw data. Some PO_4, NH_4, NO_3, and NO_2 measurements were below the level of detection, and these data were assigned a minimum value below the level of detection, to avoid the assumption that these nutrients were completely depleted (i.e. zeros). For PO_4, NH_4, and NO_3, this minimum value was 0.45 μM, and for NO_2 the minimum value assigned was 0.05 μM. Any calculated rates of nutrient remineralization that did not show a discernible difference over time were assigned a minimum rate value of 1.00 E-06 μM hr^{-1}, which is well below any detectable rates of change.

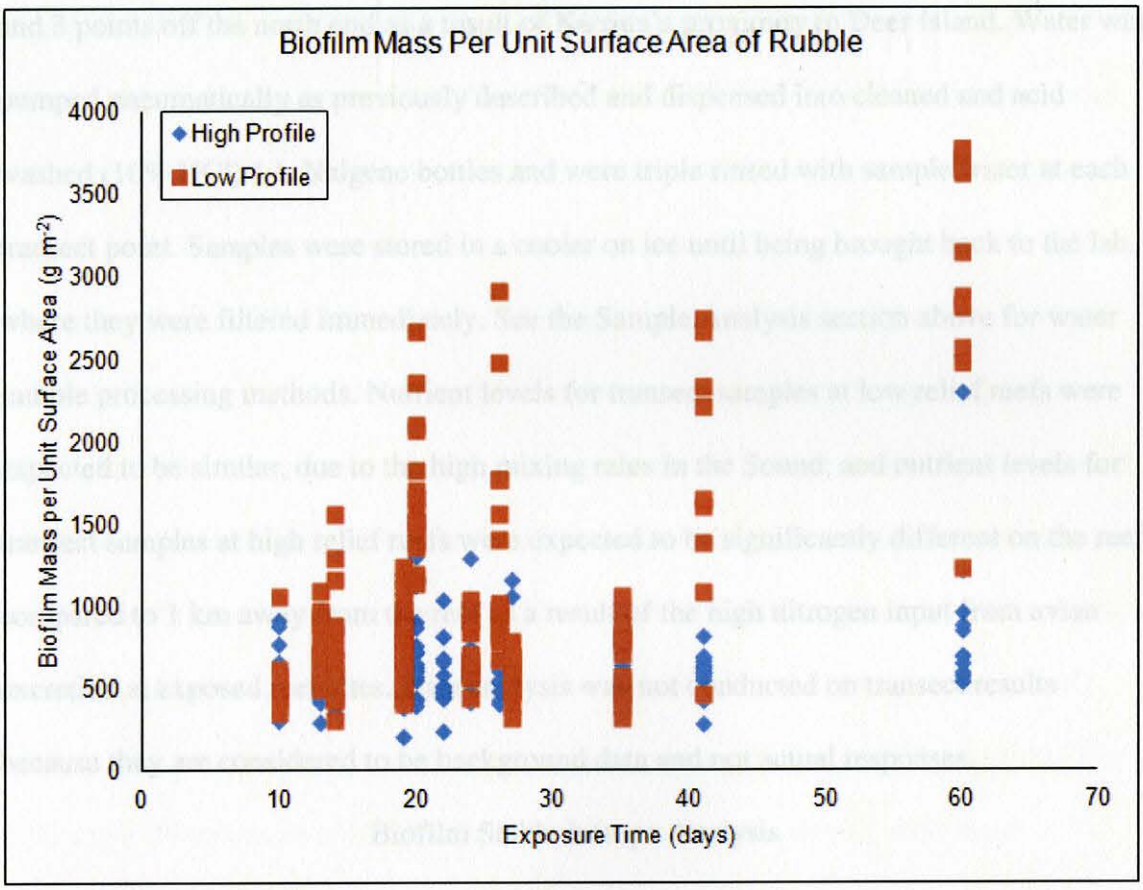

Figure 4. Estimated wet mass of biofilm growth on rubble normalized to surface area of the rubble by time of exposure. Blue symbols represent high profile reef biofilm growth, and red symbols represent low profile reef biofilm growth.

Transects

Transects were sampled at each reef to measure surface water nutrient concentrations and bacterial abundances at increasing distances from the reef locations (0, 0.3, 0.6, and 1 km). All transects were conducted in a north-south direction, with the exception of Square Handkerchief which had east/west transects due to its north-south orientation. Square Handkerchief reef had a total of 8 points where water was collected: 4 points off the west end of the reef and 4 points off the east end of the reef. Due to their close proximity to shore, USM and Legacy reefs only had 4 transect points off the southern end of the reefs. Katrina reef had 4 transect points off the south end of the reef,

and 3 points off the north end as a result of Katrina's proximity to Deer Island. Water was pumped pneumatically as previously described and dispensed into cleaned and acid washed (10% HCl) 1 L Nalgene bottles and were triple rinsed with sample water at each transect point. Samples were stored in a cooler on ice until being brought back to the lab, where they were filtered immediately. See the Sample Analysis section above for water sample processing methods. Nutrient levels for transect samples at low relief reefs were expected to be similar, due to the high mixing rates in the Sound, and nutrient levels for transect samples at high relief reefs were expected to be significantly different on the reef compared to 1 km away from the reef as a result of the high nitrogen input from avian excretion at exposed reef sites. Data analysis was not conducted on transect results because they are considered to be background data and not actual responses.

Biofilm Stable Isotope Analysis

Biofouled samples that contain total community growth, including microbial and invertebrate populations, hereafter referred to as biofilm, were collected from the settlement plates. The use of settlement plates in lieu of rubble for stable isotope analysis was to ensure collection of sufficient amounts of biofilm sample. Collecting biofilm samples from rubble was more difficult due to the uneven surface of the substrate. Settlement plates were not utilized until Experiment 4, and therefore stable isotope data is unavailable for Experiments 1, 2, and 3. Each plate was scraped into a 250 ml Nalgene bottle pre-washed with 10% HCl, triple rinsed with DI water, and filled with approximately 50 ml 0.2 μm filtered seawater, using an acid washed razor that had been triple rinsed with 0.2 μm filtered seawater. The scraped biofilm sample was then kept on ice until brought back to the lab for processing. Isotope samples from the biofilm were

transferred to 15 ml Falcon centrifuge tubes and frozen until ready to be analyzed. Sample preparation for isotope analysis was conducted based on methods adapted from Cifuentes et al. (1988). Biofilm was thawed and then dried to a constant weight in an oven at 70°C. Samples were then ground into a powder using a mortar and pestle and then suspended in 10% HCl to remove inorganic carbon for at least 15 minutes or until bubbling from the dissolution of inorganic carbon stopped, before being centrifuged at 2500 rpm for 15 minutes and then the acid was decanted. Samples were then rinsed with DI water and centrifuged (2500 rpm for 15 minutes) three times to remove any acid residue. Biofilm was then dried again at 70°C, reground and homogenized with a mortar and pestle and then stored in sterile 20 ml glass scintillation vials in a dessicator until analysis. Prior to analysis, samples were packed into tin capsules and then analyzed for $\delta^{13}C$ and $\delta^{15}N$ values on a Thermo Delta V Advantage stable isotope ratio mass spectrometer coupled to a Costech elemental analyzer. A series of acetanilide standards of varying weights (0.1 to 0.6 mg) were analyzed with each sample run to ensure accurate analysis of both isotopes across a range of sample weights. The acetanilide standard isotopic values of $\delta^{13}C$ and $\delta^{15}N$ were obtained by running the samples against certified standards (USGS-40, USGS-41, and urea) obtained from the National Institute of Standards and Technology (NIST). Elemental analysis provided total carbon and nitrogen concentrations of the biofilm samples, which was used to estimate biofilm community biomass.

Stable isotope data were analyzed using one-way ANOVAs to compare in relation to reef, profile, location, and season as main factors. Because the majority of the data were not normally distributed, and often had unequal variances, Kruskal-Wallis tests

were also conducted to verify the results of the ANOVAs. Games-Howell post-hoc tests were conducted for ANOVAs with season and individual reef as main factors to determine differences between seasons and reefs.

CHAPTER III

RESULTS

Incubation Experiments

Community respiration and bacterial abundances were expected to vary by reef site and season. Nutrient regeneration was expected to differ by reef site and season due to the hypothesized differences in biological productivity. For incubation chamber experiments, respiration rates were expected to be higher in chambers in the presence of biofilm compared to chambers without biofilm, and the presence of biofilm was expected to increase BA and nutrient regeneration. See Appendix A for a list of calculated ranges measured for each response for all BOD and chamber incubation experiments.

Winter 2012

Experiment 1. Oxygen consumption in low relief reef BOD incubations was lower than in high relief reef BOD incubations, and western reefs had lower respiration rates than eastern reefs (Figure 5a). BA often decreased over time in BOD incubations, with the exception of Square Handkerchief, which had positive growth rates (Figure 5b). Growth rates declined more rapidly in low profile BOD incubations than high profile incubations. PO_4 concentrations at high relief BOD incubations increased over 48 hrs, and remained undetectable in low relief incubations (Figure 5c). Undetectable concentrations were observed in the USM BOD incubation for NH_4 production, while the Legacy incubation and the two high relief incubations all increased in NH_4 concentration (Figure 5d). Eastern reefs had higher NH_4 production than western reefs. NO_3 concentrations were undetectable in all BOD incubations except the Square Handkerchief incubation, which declined in concentration over time (Figure 5e). NO_2 concentrations

declined in low profile reef BOD incubations, and concentrations declined in the Katrina incubation and remained constant in the Square Handkerchief incubation (Figure 5f). However, rates of decline in the USM, Legacy, and Katrina incubations are not accurate because concentrations were below detection at the 48 hr time point. DOC concentrations declined in high profile reef BOD incubations, while concentrations in low profile incubations decreased to a lesser degree or increased in concentration (Figure 5g). Changes in DON concentration declined in the Katrina BOD incubation and increased in the Square Handkerchief, USM, and Legacy incubations (Figure 5h). Low profile reef BOD incubations had higher rates of DON production than high profile reef incubations.

Figure 5. Winter 2012 – Experiment 1 BOD incubation results for Square Handkerchief (Sq Head, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_2 concentration versus time, F) NO_3 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

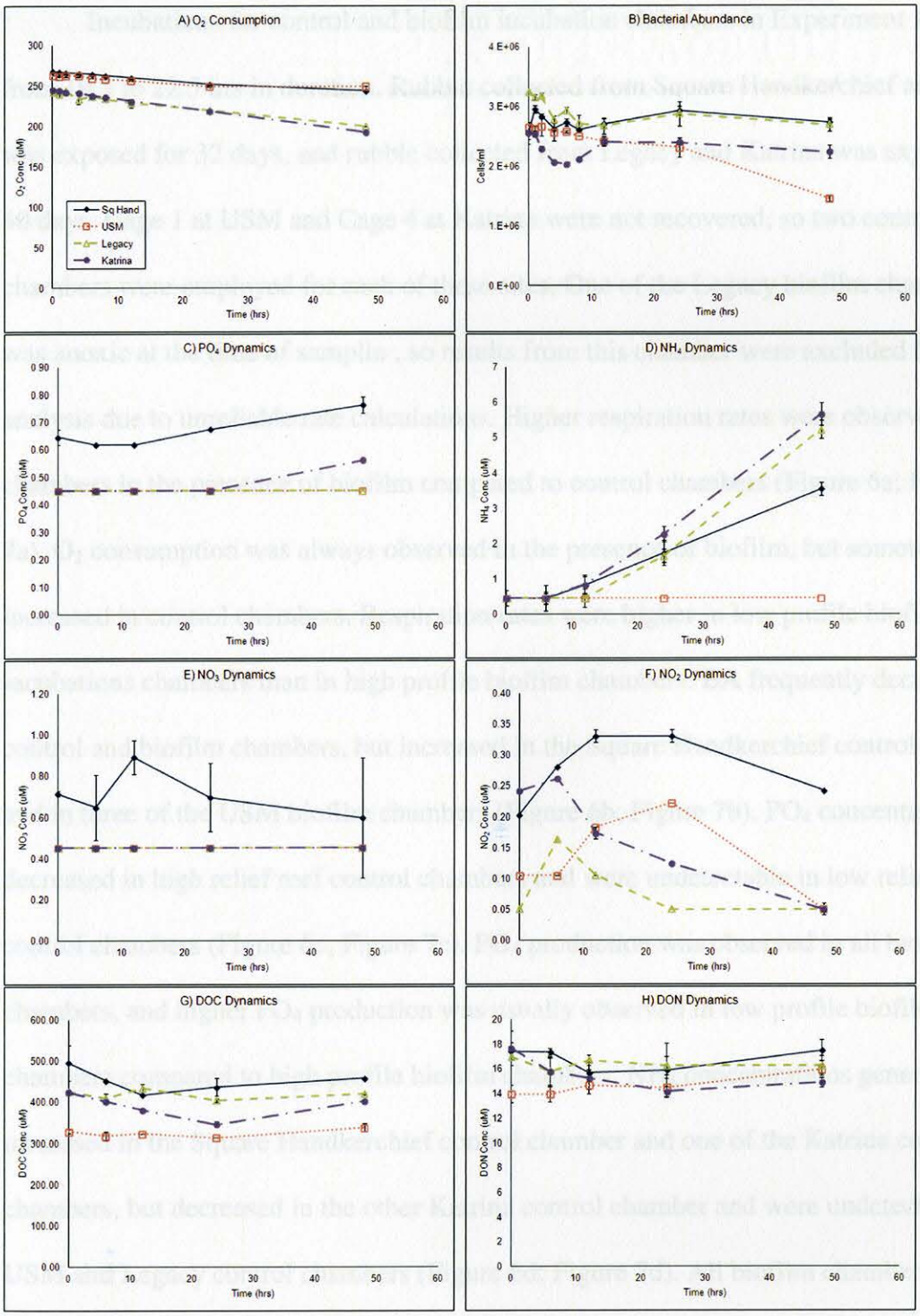

Figure 5. Winter 2012 – Experiment 1 BOD incubation results for Square Handkerchief (Sq Hand, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Incubations for control and biofilm incubation chambers in Experiment 1 ranged from 19.5 to 22.5 hrs in duration. Rubble collected from Square Handkerchief and USM was exposed for 32 days, and rubble collected from Legacy and Katrina was exposed for 40 days. Cage 1 at USM and Cage 4 at Katrina were not recovered, so two control chambers were employed for each of these sites. One of the Legacy biofilm chambers was anoxic at the time of samplin , so results from this chamber were excluded from data analysis due to unreliable rate calculations. Higher respiration rates were observed in chambers in the presence of biofilm compared to control chambers (Figure 6a; Figure 7a). O_2 consumption was always observed in the presence of biofilm, but sometimes increased in control chambers. Respiration rates were higher in low profile biofilm incubations chambers than in high profile biofilm chambers. BA frequently decreased in control and biofilm chambers, but increased in the Square Handkerchief control chamber and in three of the USM biofilm chambers (Figure 6b; Figure 7b). PO_4 concentrations decreased in high relief reef control chambers and were undetectable in low relief reef control chambers (Figure 6c; Figure 7c). PO_4 production was observed in all biofilm chambers, and higher PO_4 production was usually observed in low profile biofilm chambers compared to high profile biofilm chambers. NH_4 concentrations generally increased in the Square Handkerchief control chamber and one of the Katrina control chambers, but decreased in the other Katrina control chamber and were undetectable in USM and Legacy control chambers (Figure 6d; Figure 7d). All biofilm chambers increased in NH_4 concentration, and low relief biofilm chambers often had higher NH_4 production rates than high relief biofilm chambers. The highest PO_4 and NH_4 production rates were correlated with hypoxic and anoxic chambers. NO_3 concentrations declined in

the Square Handkerchief control chamber, and increased in both the Katrina control chambers, while Legacy and USM control chambers had concentrations below detection (Figure 6e; Figure 7e). Legacy and USM biofilm chambers also had undetectable NO_3 concentrations. Three of the Square Handkerchief biofilm chambers decreased in NO_3 concentration, while two chambers increased in concentration, and one Katrina biofilm chamber decreased in concentration while the remaining three chambers increased in concentration. Square Handkerchief and Legacy control chambers increased in NO_2 concentration, while USM and Katrina control chambers decreased in concentration (Figure 6f; Figure 7f). Of the low profile biofilm chambers, NO_2 concentrations always declined in USM chambers, while only one of the four Legacy biofilm chambers declined in concentration and the rest showed an increase. High profile biofilm chambers always exhibited an increase in NO_2 concentration. DOC concentrations increased in three control chambers, and decreased in the other three control chambers (Figure 6g; Figure 7g). High profile biofilm chambers all declined in DOC concentration, and low profile biofilm chambers all increased in DOC concentration. DON concentrations generally decreased in control chambers, although the Square Handkerchief and one of the USM control chambers increased in concentration (Figure 6h; Figure 7h). All biofilm chambers declined in DON concentration, usually at higher rates than control chambers.

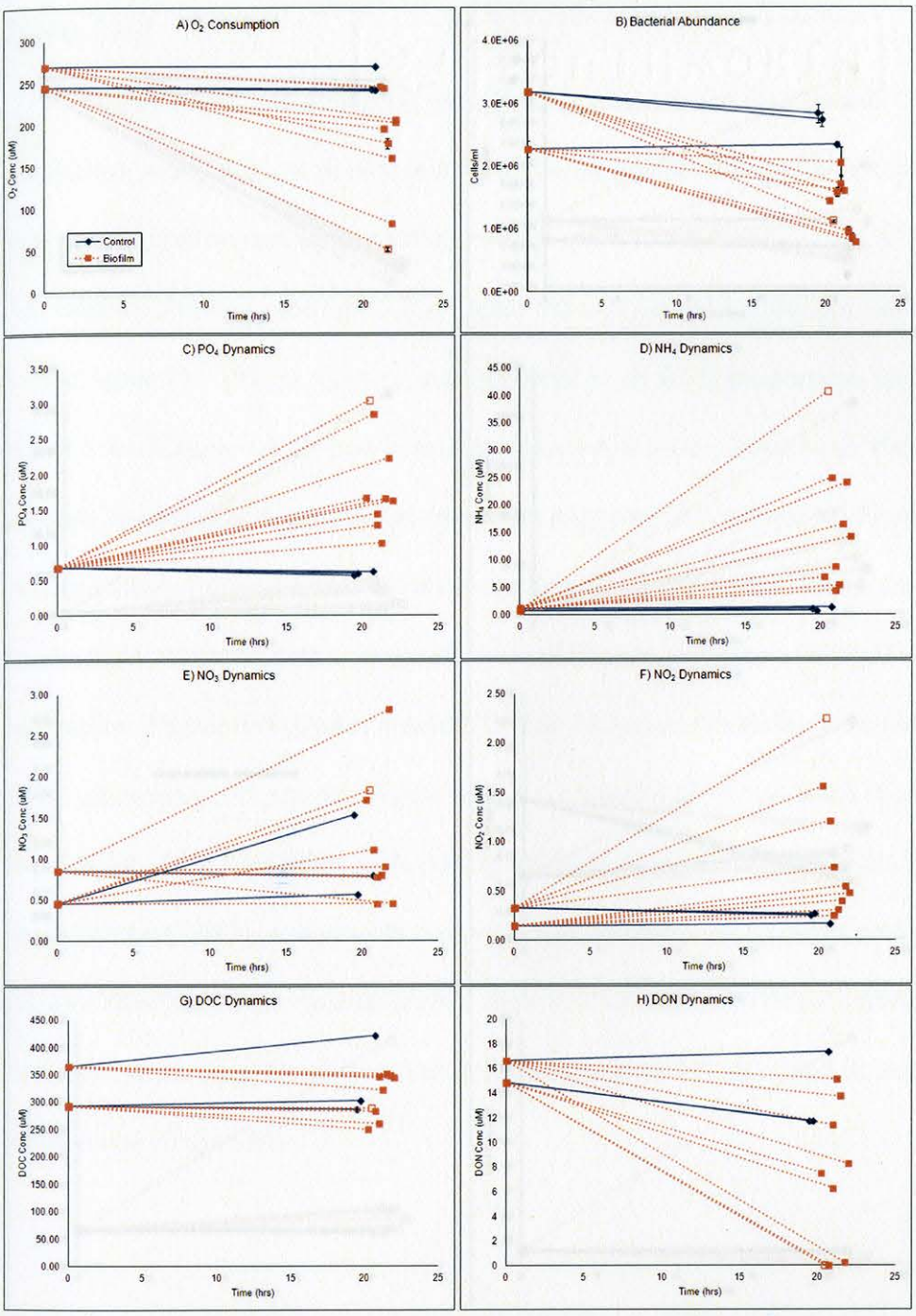

Figure 6. Winter 2012 – Experiment 1 high profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 μM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

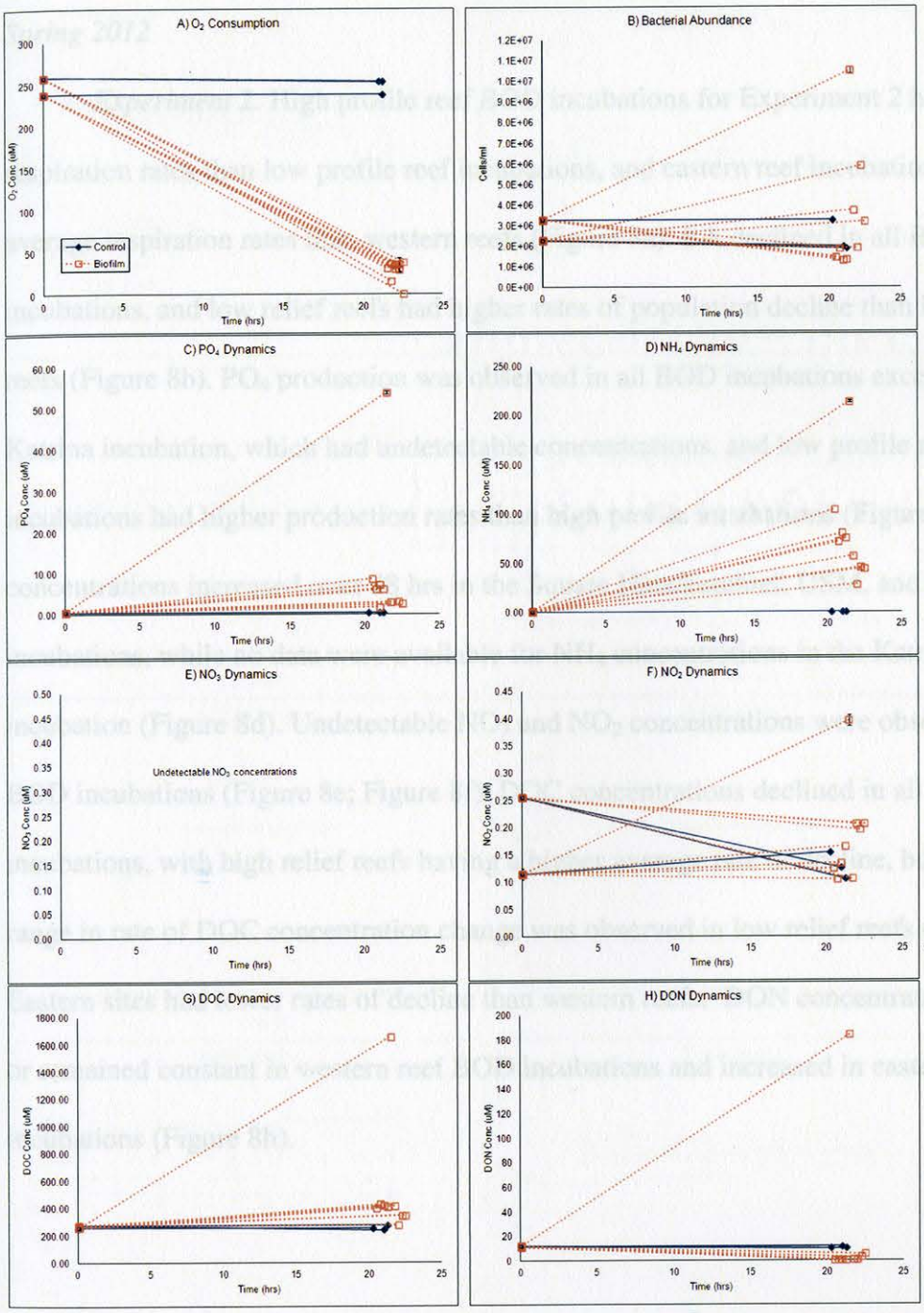

Figure 7. Winter 2012 – Experiment 1 low profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 μM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Spring 2012

Experiment 2. High profile reef BOD incubations for Experiment 2 had lower respiration rates than low profile reef incubations, and eastern reef incubations had higher average respiration rates than western reefs (Figure 8a). BA declined in all BOD incubations, and low relief reefs had higher rates of population decline than high relief reefs (Figure 8b). PO_4 production was observed in all BOD incubations except the Katrina incubation, which had undetectable concentrations, and low profile reef incubations had higher production rates than high profile incubations (Figure 8c). NH_4 concentrations increased over 48 hrs in the Square Handkerchief, USM, and Legacy BOD incubations, while no data were available for NH_4 concentrations in the Katrina incubation (Figure 8d). Undetectable NO_3 and NO_2 concentrations were observed in all BOD incubations (Figure 8e; Figure 8f). DOC concentrations declined in all BOD incubations, with high relief reefs having a higher average rate of decline, but a greater range in rate of DOC concentration change was observed in low relief reefs (Figure 8g). Eastern sites had lower rates of decline than western reefs. DON concentrations declined or remained constant in western reef BOD incubations and increased in eastern reef incubations (Figure 8h).

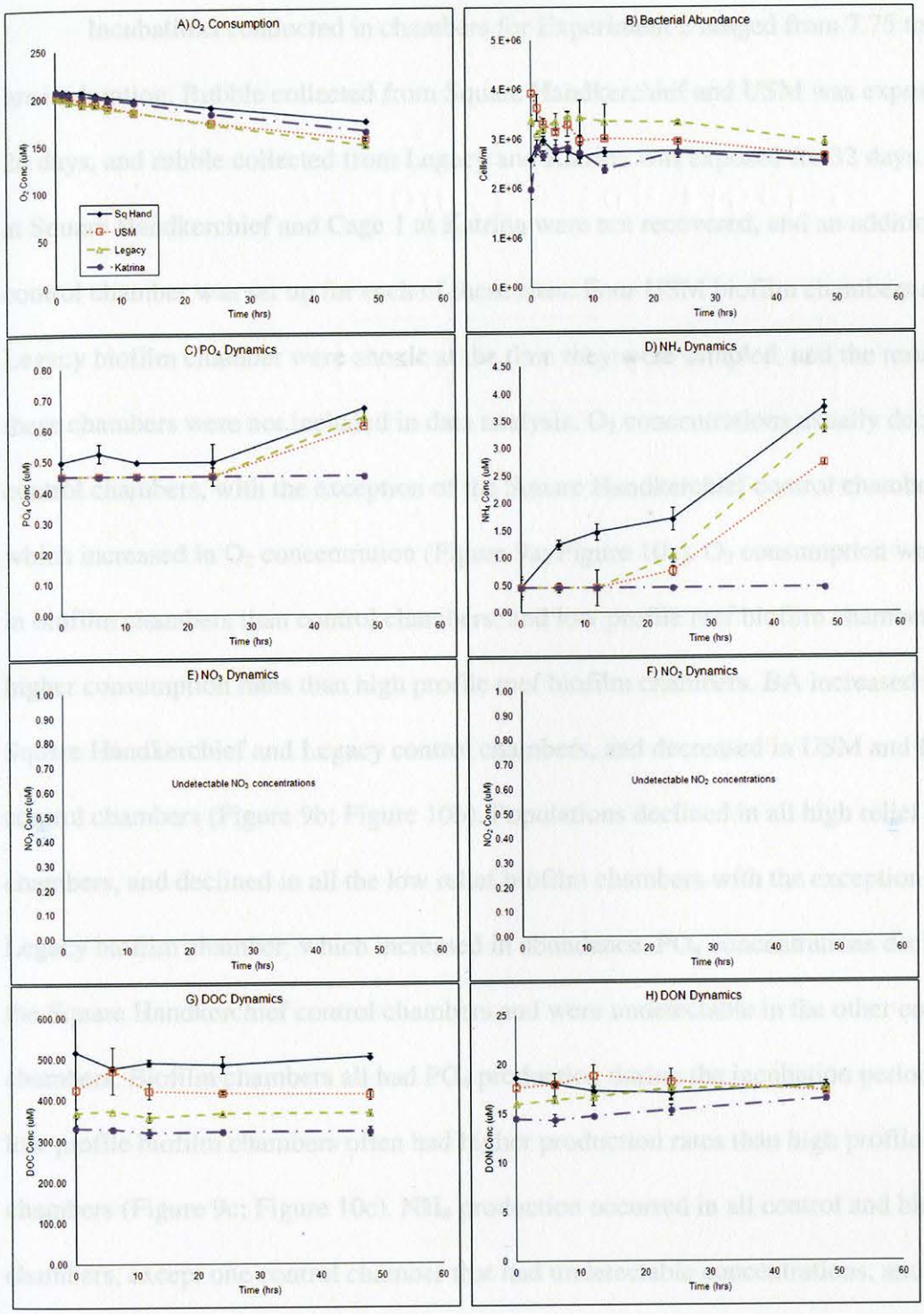

Figure 8. Spring 2012 – Experiment 2 BOD incubation results for Square Handkerchief (Sq Hand, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Incubations conducted in chambers for Experiment 2 ranged from 7.75 to 14.25 hrs in duration. Rubble collected from Square Handkerchief and USM was exposed for 26 days, and rubble collected from Legacy and Katrina was exposed for 32 days. Cage 4 at Square Handkerchief and Cage 1 at Katrina were not recovered, and an additional control chamber was set up for each of these sites. Four USM biofilm chambers and one Legacy biofilm chamber were anoxic at the time they were sampled, and the results from these chambers were not included in data analysis. O_2 concentrations usually decreased in control chambers, with the exception of the Square Handkerchief control chambers, which increased in O_2 concentration (Figure 9a; Figure 10a). O_2 consumption was higher in biofilm chambers than control chambers, and low profile reef biofilm chambers had higher consumption rates than high profile reef biofilm chambers. BA increased in Square Handkerchief and Legacy control chambers, and decreased in USM and Katrina control chambers (Figure 9b; Figure 10b). Populations declined in all high relief biofilm chambers, and declined in all the low relief biofilm chambers with the exception of one Legacy biofilm chamber, which increased in abundance. PO_4 concentrations declined in the Square Handkerchief control chambers and were undetectable in the other control chambers. Biofilm chambers all had PO_4 production during the incubation period, and low profile biofilm chambers often had higher production rates than high profile biofilm chambers (Figure 9c; Figure 10c). NH_4 production occurred in all control and biofilm chambers, except one control chamber that had undetectable concentrations, and higher production rates were observed in biofilm chambers than controls (Figure 9d; Figure 10d). Low relief biofilm chambers usually had higher NH_4 production rates than high relief biofilm chambers. Hypoxic and anoxic chambers had the highest PO_4 and NH_4

release rates. NO_3 concentrations were below detection in control chambers, as well as in low profile biofilm chambers (Figure 10e). Most high profile biofilm chambers had increases in NO_3 concentrations (Figure 9e). NO_2 concentrations were undetectable in all the control chambers with the exception of one of the Katrina controls, which increased in NO_2 concentration over the incubation period (Figure 9f). Low profile biofilm chambers typically had NO_2 concentrations below detection, with the exception of one Legacy biofilm chamber, which increased in concentration, and all high profile biofilm chambers increased in concentration (Figure 9f; Figure 10f). NO_2 production rates were higher in high profile biofilm chambers than low profile biofilm chambers. DOC concentrations decreased in all control chambers except the USM control chamber, which increased in concentration (Figure 9g; Figure 10g). Low profile biofilm chambers all increased in DOC concentrations, while high profile biofilm chambers generally decreased in concentration, with the exception of two Katrina biofilm chambers, which increased in DOC concentration. DON concentrations decreased over time in control chambers, except the USM control chamber, which increased in concentration (Figure 9h; Figure 10h). Four high relief biofilm chambers decreased in DON concentration, while the other four chambers increased in concentration, and all of the low relief biofilm chambers increased in DON concentration, often at greater rates than the high relief biofilm chambers.

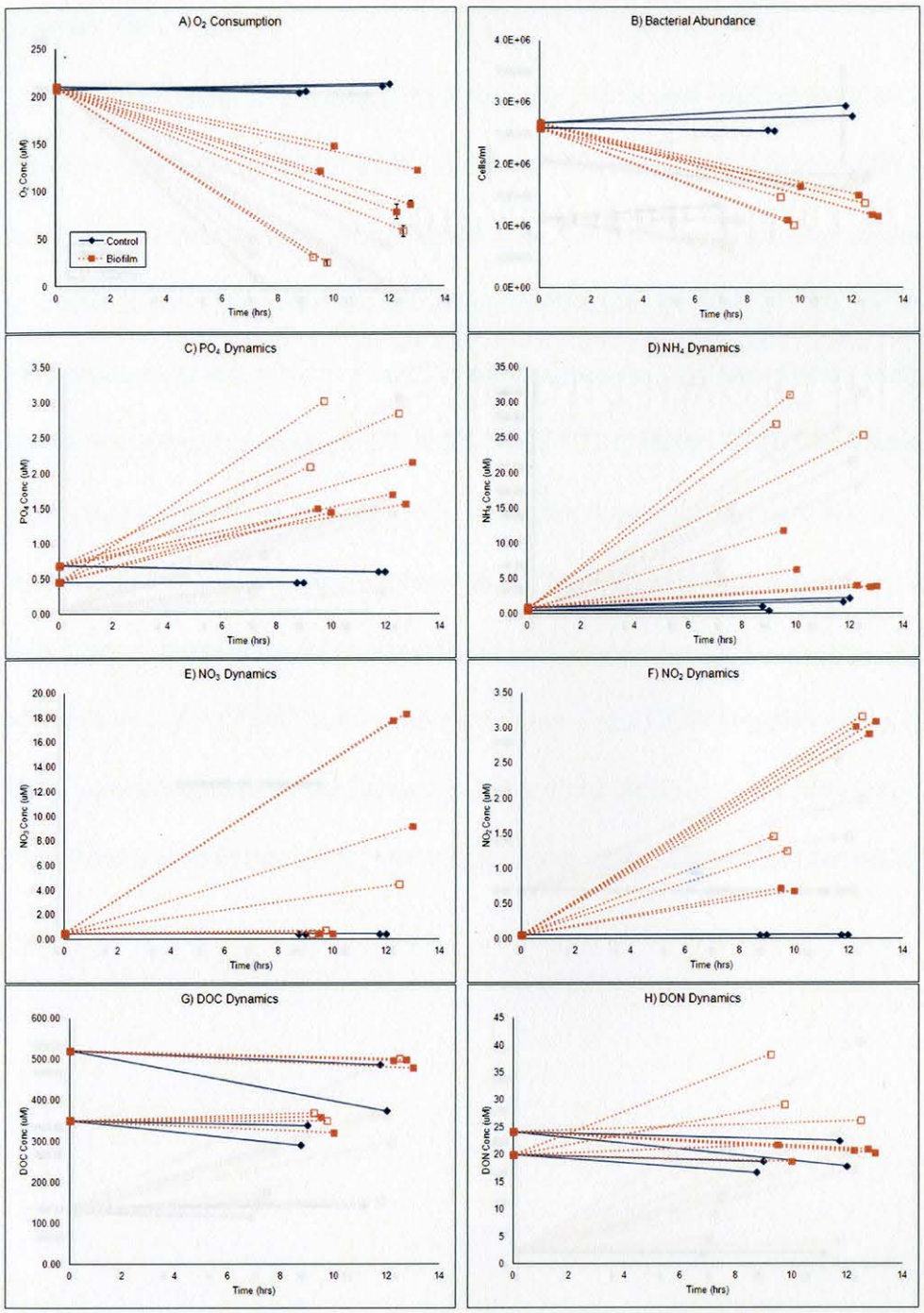

Figure 9. Spring 2012 – Experiment 2 high profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 μM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

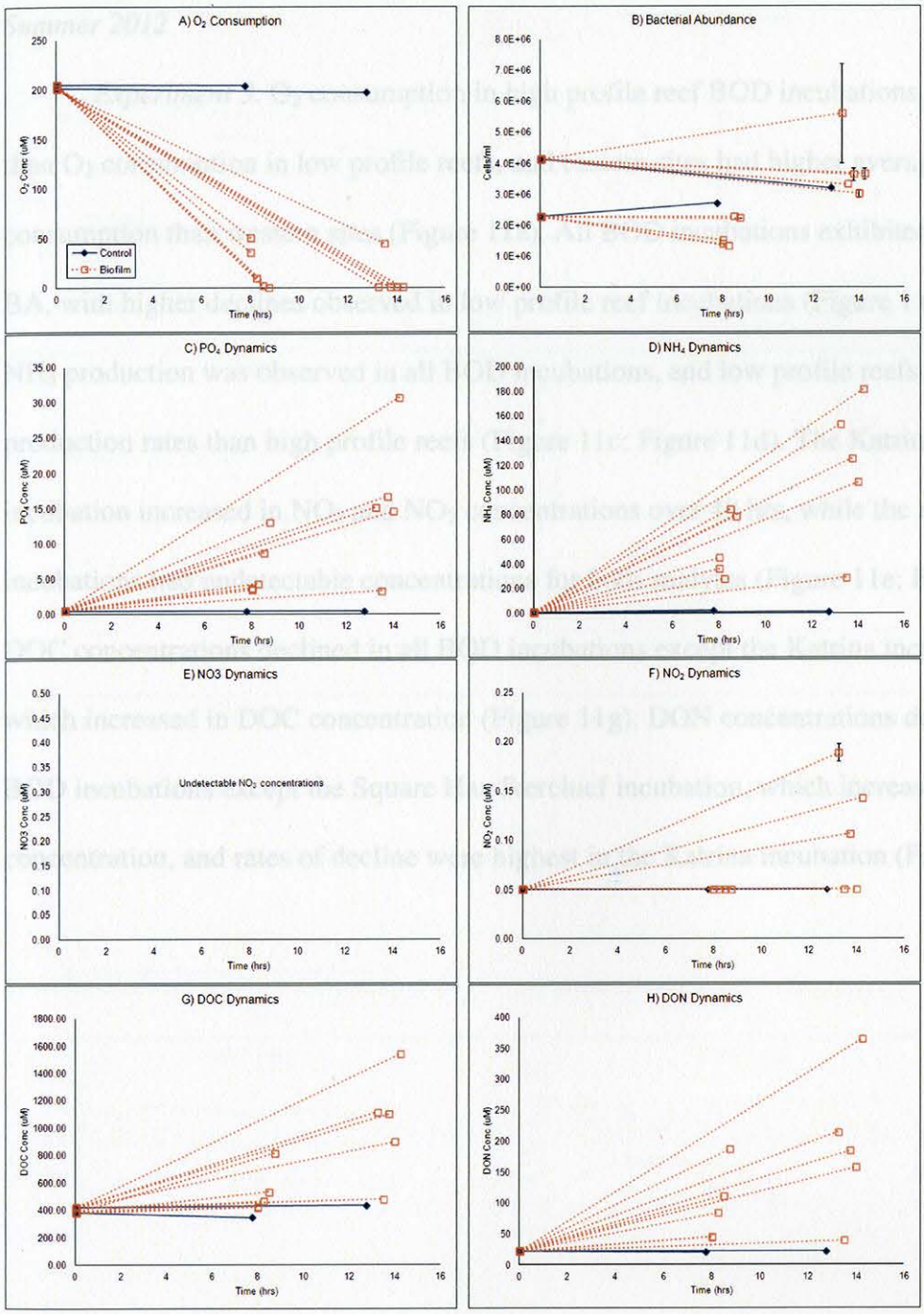

Figure 10. Spring 2012 – Experiment 2 low profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 µM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Summer 2012

Experiment 3. O_2 consumption in high profile reef BOD incubations was lower than O_2 consumption in low profile reefs, and eastern sites had higher average O_2 consumption than western sites (Figure 11a). All BOD incubations exhibited a decline in BA, with higher declines observed in low profile reef incubations (Figure 11b). PO_4 and NH_4 production was observed in all BOD incubations, and low profile reefs had higher production rates than high profile reefs (Figure 11c; Figure 11d). The Katrina BOD incubation increased in NO_3 and NO_2 concentrations over 48 hrs, while the rest of the incubations had undetectable concentrations for both analytes (Figure 11e; Figure 11f). DOC concentrations declined in all BOD incubations except the Katrina incubation, which increased in DOC concentration (Figure 11g). DON concentrations declined in all BOD incubations except the Square Handkerchief incubation, which increased in concentration, and rates of decline were highest in the Katrina incubation (Figure 11h).

Figure 11. Summer 2012 – Experiment 3 BOD incubation results for Square Handkerchief (Sq Hand, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

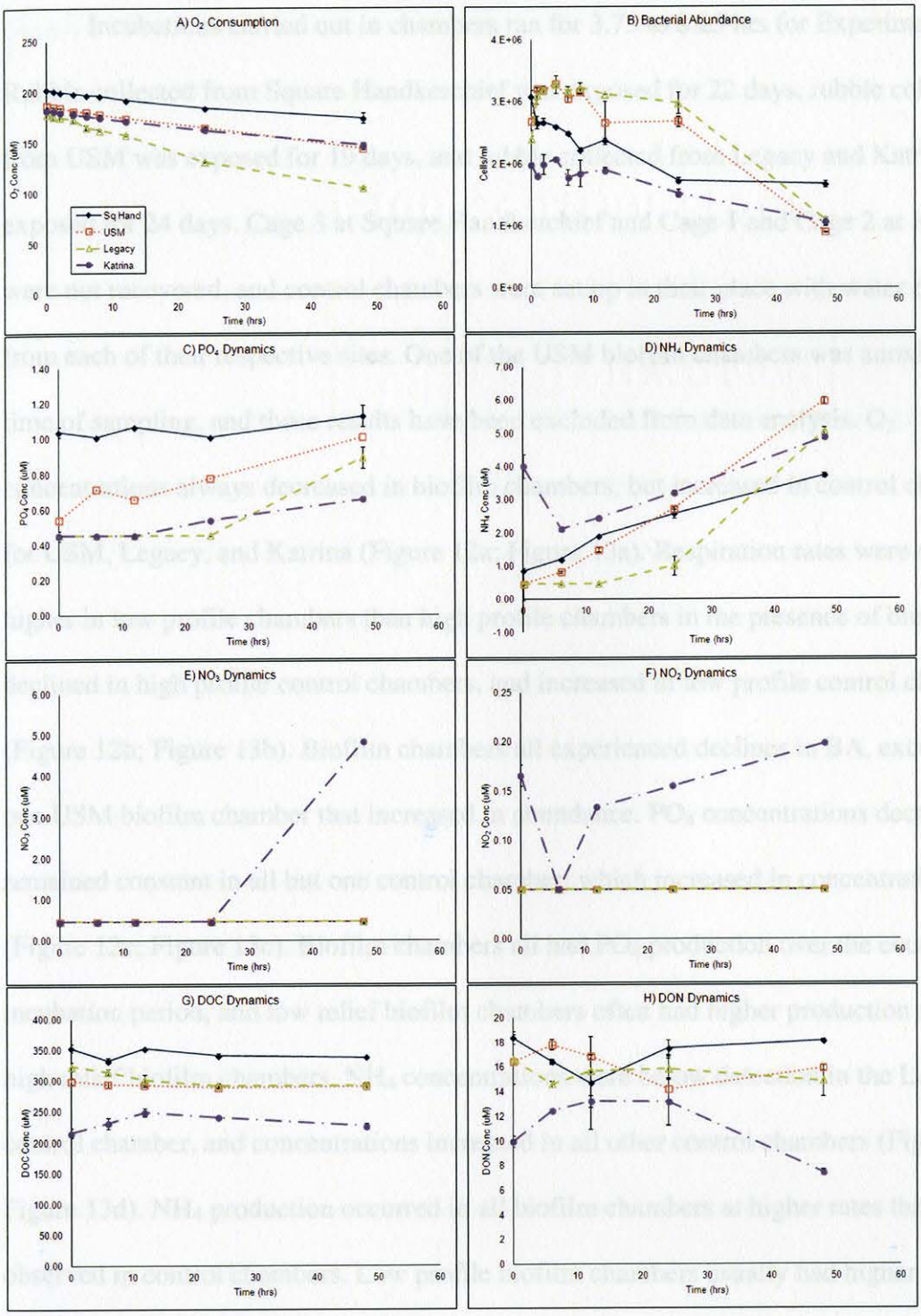

Figure 11. Summer 2012 – Experiment 3 BOD incubation results for Square Handkerchief (Sq Hand, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Incubations carried out in chambers ran for 3.75 to 8.25 hrs for Experiment 3. Rubble collected from Square Handkerchief was exposed for 22 days, rubble collected from USM was exposed for 19 days, and rubble collected from Legacy and Katrina was exposed for 24 days. Cage 3 at Square Handkerchief and Cage 1 and Cage 2 at Katrina were not recovered, and control chambers were set up in their place with water collected from each of their respective sites. One of the USM biofilm chambers was anoxic at the time of sampling, and those results have been excluded from data analysis. O_2 concentrations always decreased in biofilm chambers, but increased in control chambers for USM, Legacy, and Katrina (Figure 12a; Figure 13a). Respiration rates were often higher in low profile chambers than high profile chambers in the presence of biofilm. BA declined in high profile control chambers, and increased in low profile control chambers (Figure 12b; Figure 13b). Biofilm chambers all experienced declines in BA, except for one USM biofilm chamber that increased in abundance. PO_4 concentrations decreased or remained constant in all but one control chamber, which increased in concentration (Figure 12c; Figure 13c). Biofilm chambers all had PO_4 production over the course of the incubation period, and low relief biofilm chambers often had higher production rates than high relief biofilm chambers. NH_4 concentrations were below detection in the Legacy control chamber, and concentrations increased in all other control chambers (Figure 12d; Figure 13d). NH_4 production occurred in all biofilm chambers at higher rates than observed in control chambers. Low profile biofilm chambers usually had higher NH_4 production rates than high profile biofilm chambers. The highest NH_4 and PO_4 production rates were associated with hypoxic and anoxic chambers. NO_3 concentrations were frequently undetectable in control and biofilm chambers. The Legacy control

chamber was the only control that had a detectable increase in NO_3 at the time of sampling, and NO_3 production was observed in some biofilm chambers for each reef, but was more often observed in high profile biofilm chambers (Figure 12e; Figure 13e). NO_2 concentrations were undetectable for all control chambers except one Katrina control chamber, which had an increase in NO_2 concentration over time. High profile biofilm chambers all increased in NO_2 concentration, while only some low profile biofilm chambers increased in NO_2 concentrations, and the remaining low profile biofilm chambers had undetectable concentrations (Figure 12f; Figure 13f). Biofilm chambers that had NO_2 production all had higher rates than those observed in control chambers. DOC concentrations declined in three control chambers, and increased in four control chambers (Figure 12g; Figure 13g). Two Square Handkerchief biofilm chambers decreased in DOC concentrations, and all other biofilm chambers increased in DOC concentration, usually at higher rates in low profile reef biofilm chambers than in high profile reef biofilm chambers. DON concentrations declined in three control chambers and increased in four control chambers (Figure 12h; Figure 13h). Low profile biofilm chambers all increased in DON concentration over time, while three high profile biofilm chambers increased in DON concentration and the remaining high profile biofilm chambers decreased in concentration. Of the high profile biofilm chambers that had DON production, their rates were usually lower than the production rates observed in low profile biofilm chambers.

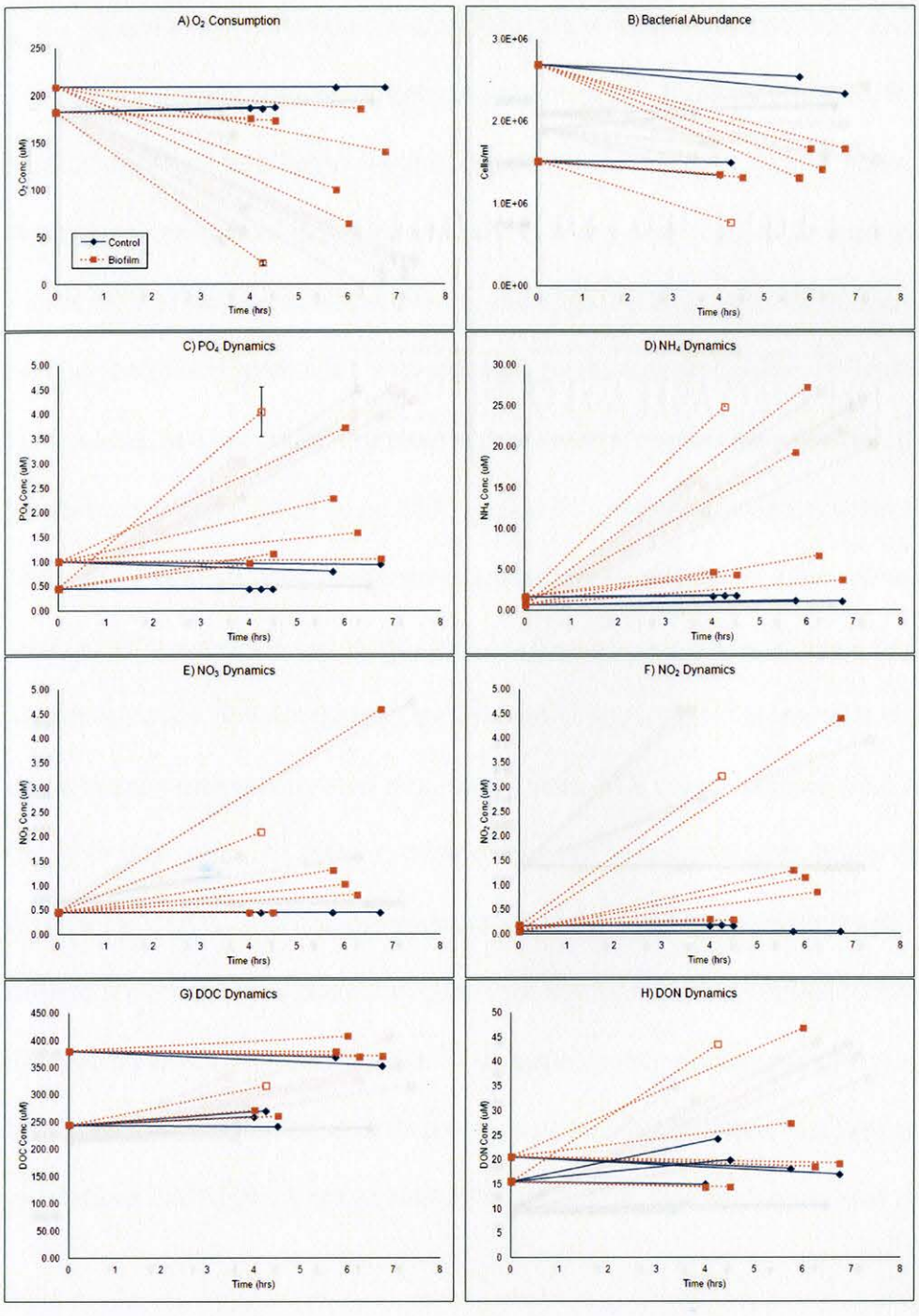

Figure 12. Summer 2012 – Experiment 3 high profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 μM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

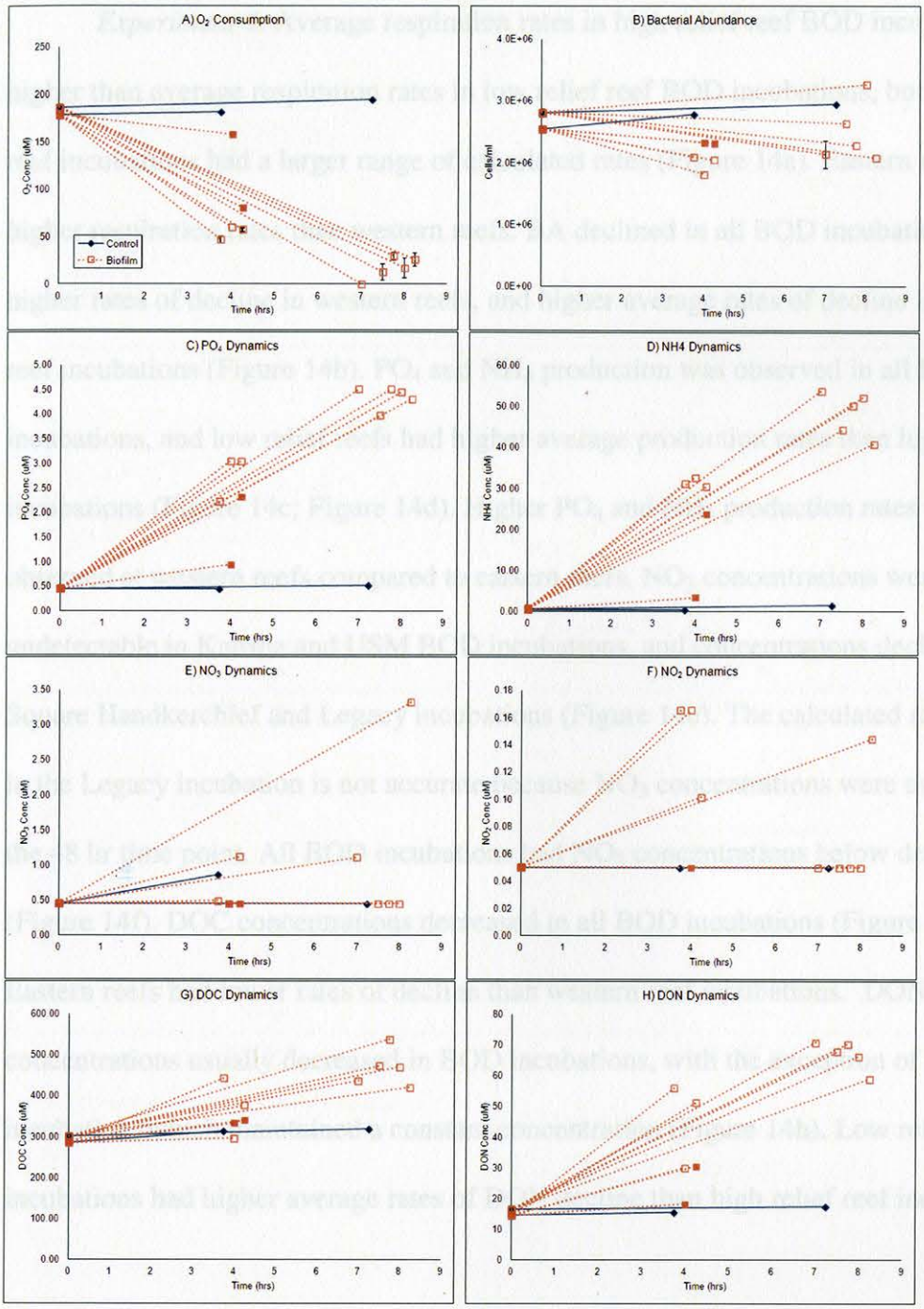

Figure 13. Summer 2012 – Experiment 3 low profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 μM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Experiment 4. Average respiration rates in high relief reef BOD incubations were higher than average respiration rates in low relief reef BOD incubations, but high relief reef incubations had a larger range of calculated rates (Figure 14a). Eastern reefs had higher respiration rates than western reefs. BA declined in all BOD incubations, with higher rates of decline in western reefs, and higher average rates of decline in high profile reef incubations (Figure 14b). PO_4 and NH_4 production was observed in all BOD incubations, and low relief reefs had higher average production rates than high relief reef incubations (Figure 14c; Figure 14d). Higher PO_4 and NH_4 production rates were observed at western reefs compared to eastern reefs. NO_3 concentrations were undetectable in Katrina and USM BOD incubations, and concentrations declined in the Square Handkerchief and Legacy incubations (Figure 14e). The calculated rate of decline in the Legacy incubation is not accurate because NO_3 concentrations were undetectable at the 48 hr time point. All BOD incubations had NO_2 concentrations below detection (Figure 14f). DOC concentrations decreased in all BOD incubations (Figure 14g). Eastern reefs had lower rates of decline than western reef incubations. DON concentrations usually decreased in BOD incubations, with the exception of the Katrina incubation, which maintained a constant concentration (Figure 14h). Low relief reef BOD incubations had higher average rates of DON decline than high relief reef incubations.

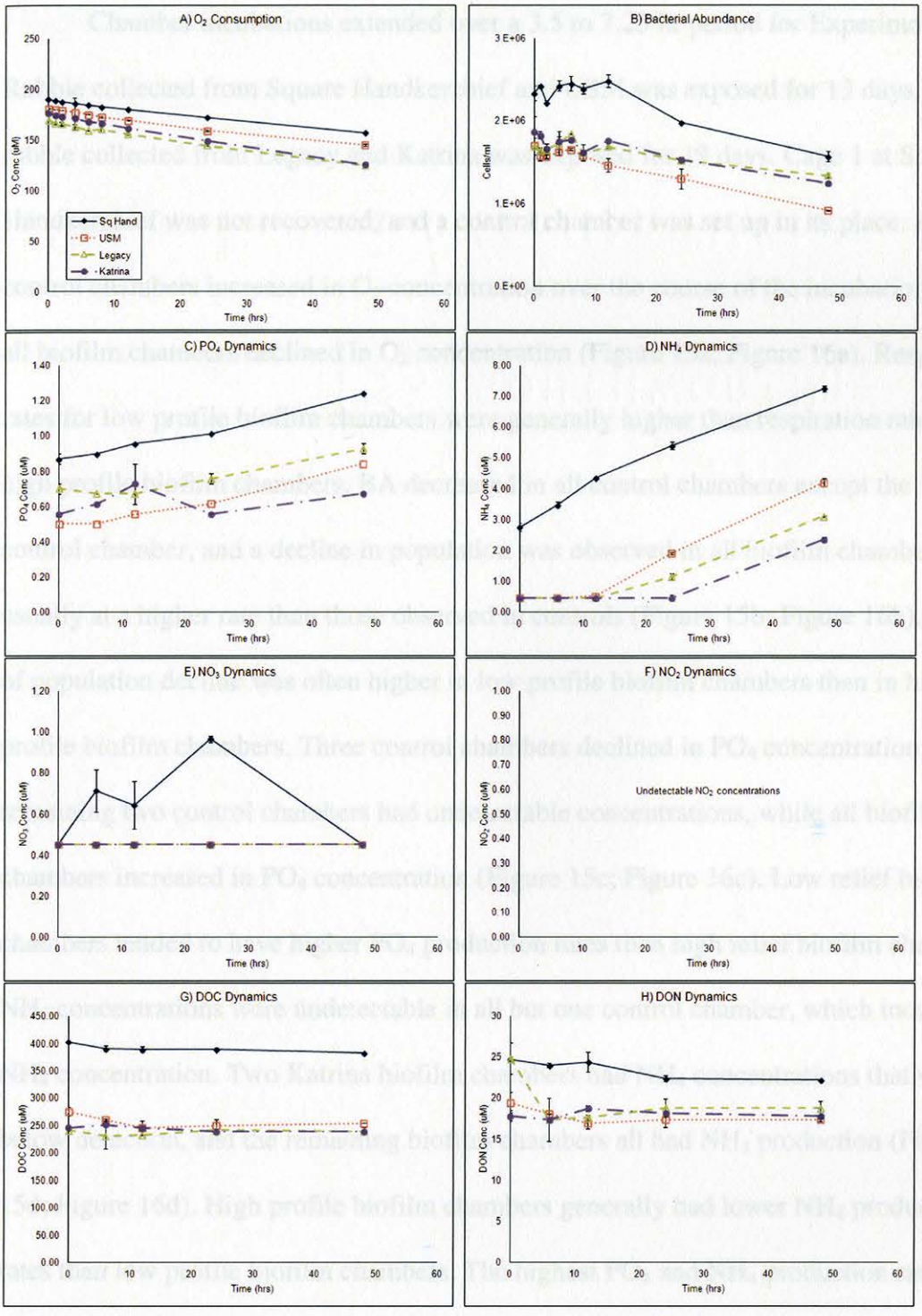

Figure 14. Summer 2012 – Experiment 4 BOD incubation results for Square Handkerchief (Sq Hand, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Chamber incubations extended over a 3.5 to 7.25 hr period for Experiment 4. Rubble collected from Square Handkerchief and USM was exposed for 13 days, and rubble collected from Legacy and Katrina was exposed for 19 days. Cage 1 at Square Handkerchief was not recovered, and a control chamber was set up in its place. All control chambers increased in O_2 concentration over the course of the incubation, while all biofilm chambers declined in O_2 concentration (Figure 15a; Figure 16a). Respiration rates for low profile biofilm chambers were generally higher than respiration rates for high profile biofilm chambers. BA decreased in all control chambers except the Katrina control chamber, and a decline in population was observed in all biofilm chambers, usually at a higher rate than those observed in controls (Figure 15b; Figure 16b). The rate of population decline was often higher in low profile biofilm chambers than in high profile biofilm chambers. Three control chambers declined in PO_4 concentration and the remaining two control chambers had undetectable concentrations, while all biofilm chambers increased in PO_4 concentration (Figure 15c; Figure 16c). Low relief biofilm chambers tended to have higher PO_4 production rates than high relief biofilm chambers. NH_4 concentrations were undetectable in all but one control chamber, which increased in NH_4 concentration. Two Katrina biofilm chambers had NH_4 concentrations that were below detection, and the remaining biofilm chambers all had NH_4 production (Figure 15d; Figure 16d). High profile biofilm chambers generally had lower NH_4 production rates than low profile biofilm chambers. The highest PO_4 and NH_4 production rates were positively correlated with respiration rates and were therefore highest in hypoxic chambers. NO_3 concentrations in control chambers were undetectable. The majority of biofilm chambers had undetectable NO_3 concentrations, with the exception of one Square

Handkerchief biofilm chamber, which had an increase in NO_3 concentration over time (Figure 15e; Figure 16e). NO_2 concentrations usually remained below detection in control chambers, but declined in the Square Handkerchief control chambers. With the exception of one Katrina biofilm chamber that had undetectable NO_2 concentrations, all biofilm chambers had NO_2 production during the incubations, and high profile biofilm chambers often had higher production rates than low profile biofilm chambers (Figure 15f; Figure 16f). DOC concentrations increased in all control and biofilm chambers, with the exception of one Square Handkerchief biofilm chamber, which decreased in concentration (Figure 15g; Figure 16g). Biofilm chambers usually had higher rates of DOC production than control chambers, and low profile biofilm chambers usually had higher rates of production than high profile biofilm chambers. DON concentrations declined in high profile control chambers, and increased in low profile control chambers (Figure 15h; Figure 16h). Three Square Handkerchief biofilm chambers decreased in DON concentration, and the remaining biofilm chambers all increased in DON concentration. Low profile biofilm chambers often had higher DON production rates than high profile biofilm chambers.

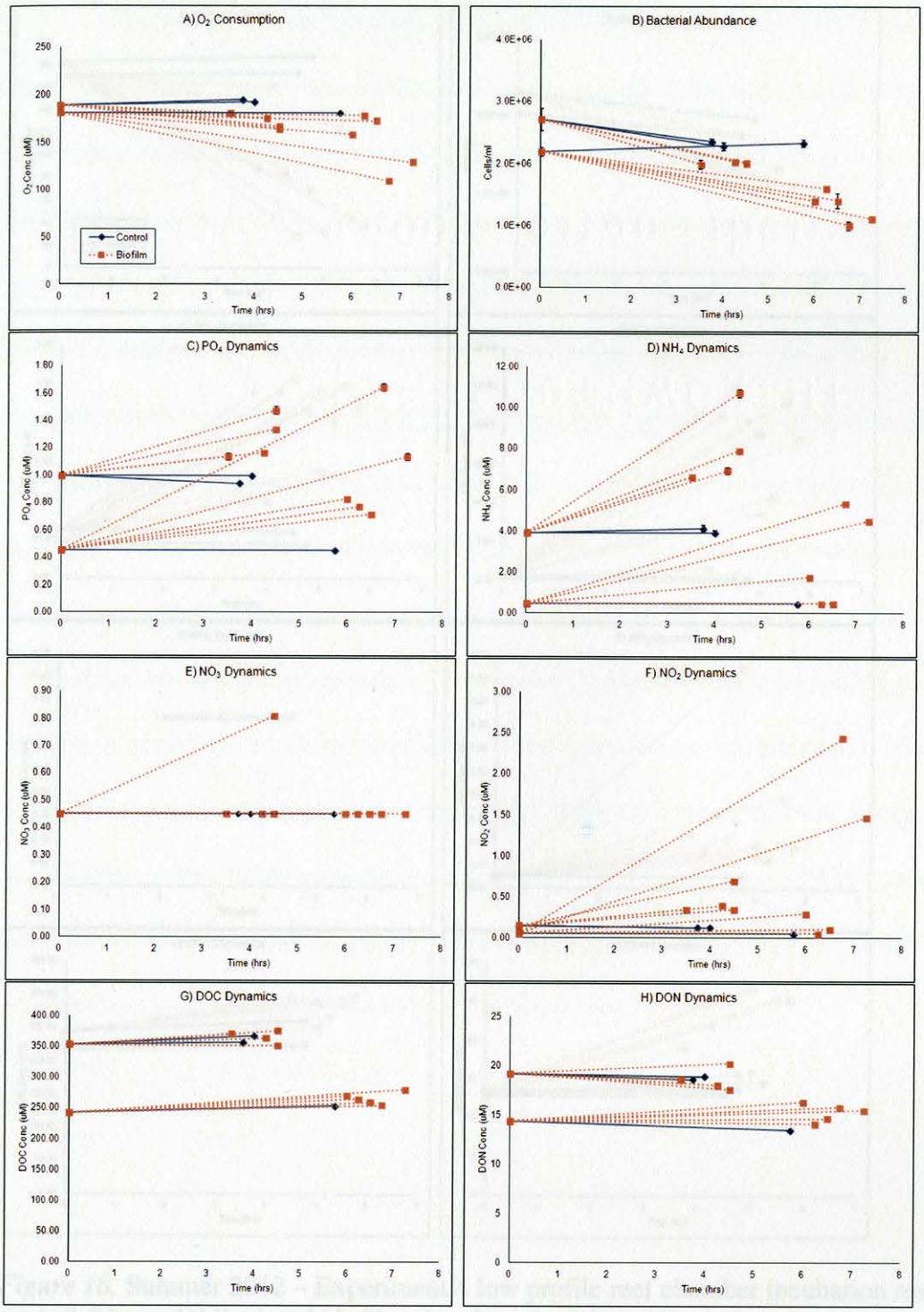

Figure 15. Summer 2012 – Experiment 4 high profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

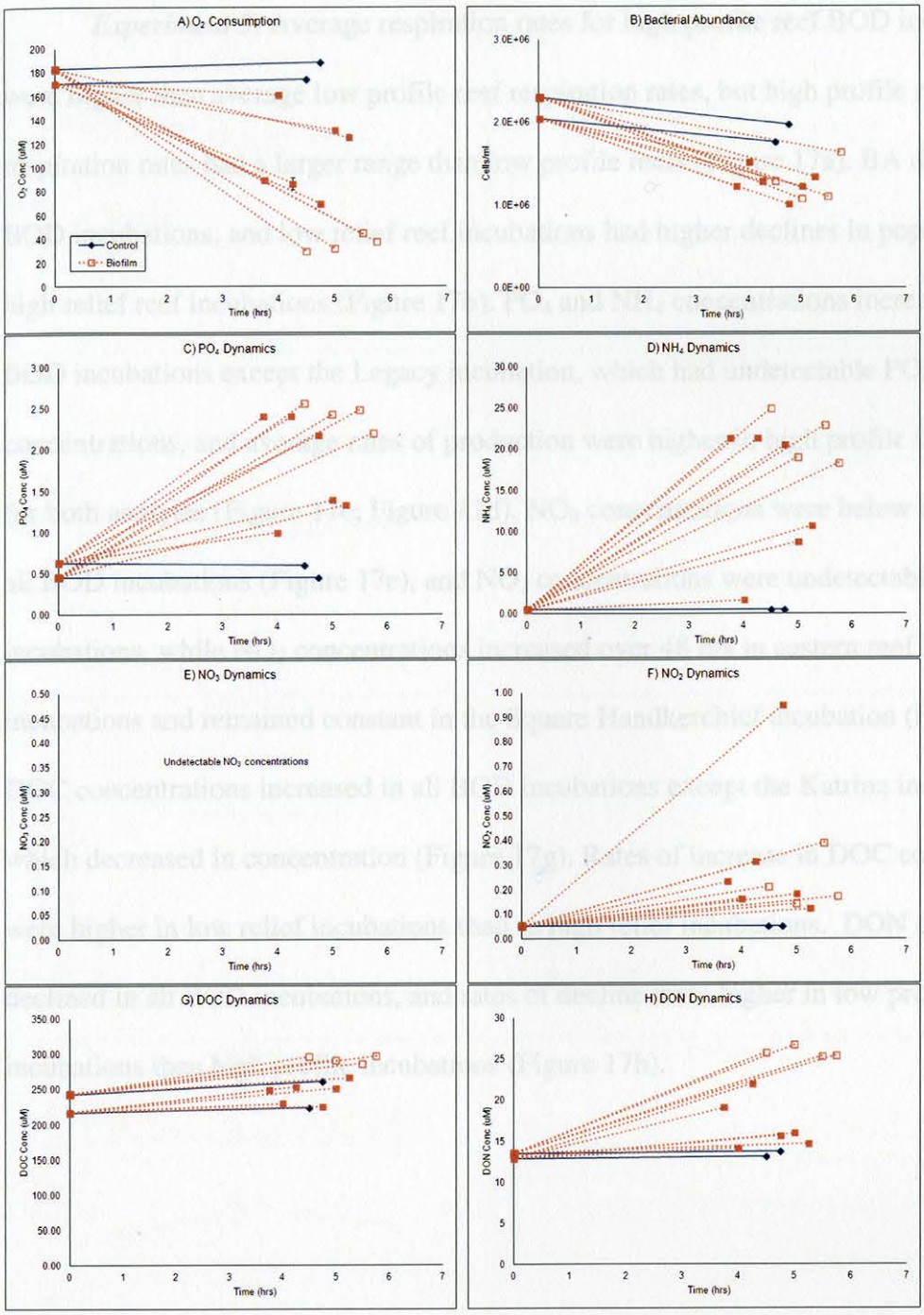

Figure 16. Summer 2012 – Experiment 4 low profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 µM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Experiment 5. Average respiration rates for high profile reef BOD incubations were higher than average low profile reef respiration rates, but high profile reef respiration rates had a larger range than low profile reefs (Figure 17a). BA declined in all BOD incubations, and low relief reef incubations had higher declines in population than high relief reef incubations (Figure 17b). PO_4 and NH_4 concentrations increased in all BOD incubations except the Legacy incubation, which had undetectable PO_4 concentrations, and average rates of production were higher in high profile incubations for both analytes (Figure 17c; Figure 17d). NO_3 concentrations were below detection in all BOD incubations (Figure 17e), and NO_2 concentrations were undetectable in USM incubations, while NO_2 concentrations increased over 48 hrs in eastern reef BOD incubations and remained constant in the Square Handkerchief incubation (Figure 17f). DOC concentrations increased in all BOD incubations except the Katrina incubation, which decreased in concentration (Figure 17g). Rates of increase in DOC concentration were higher in low relief incubations than in high relief incubations. DON concentrations declined in all BOD incubations, and rates of decline were higher in low profile incubations than high profile incubations (Figure 17h).

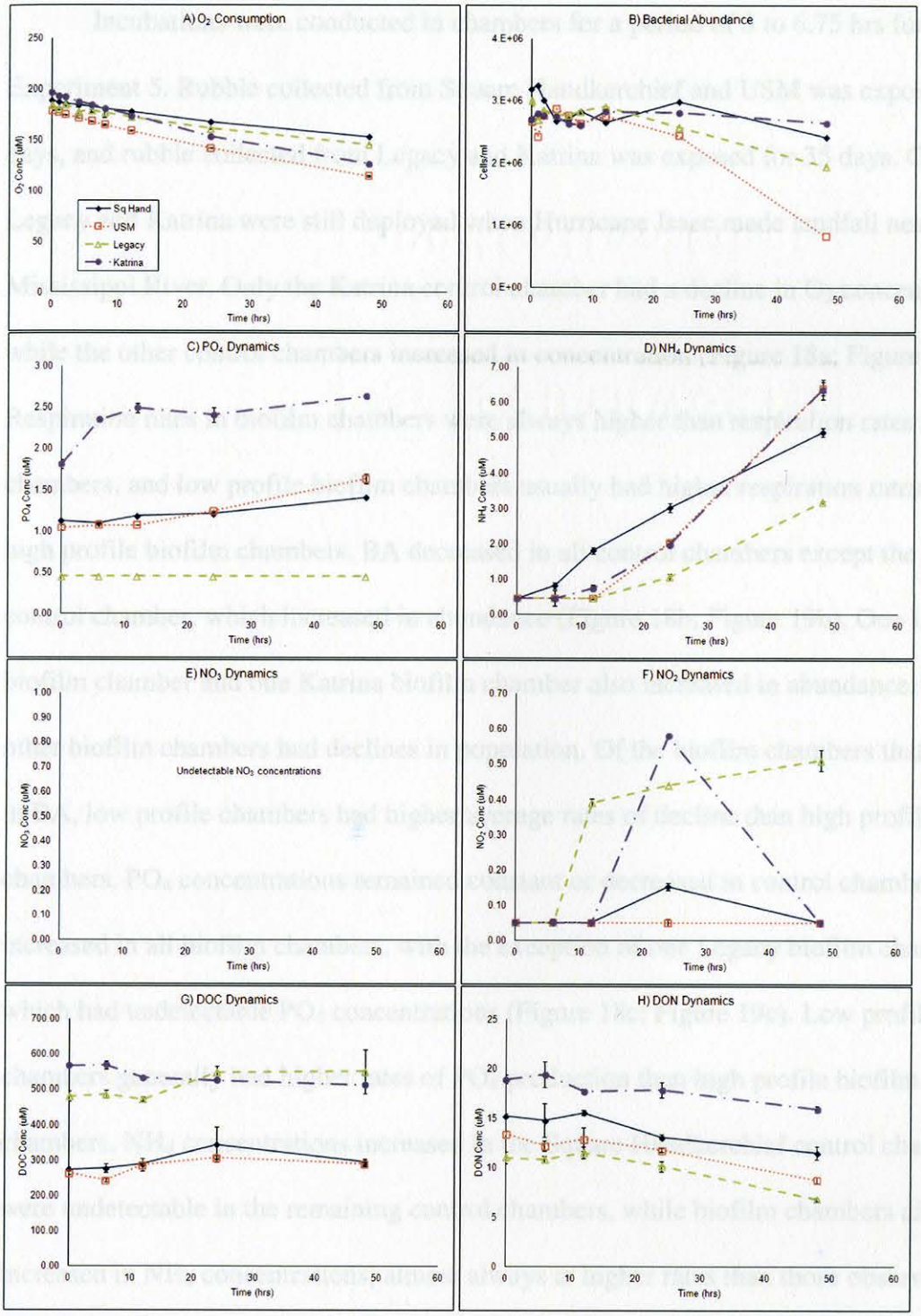

Figure 17. Summer 2012 – Experiment 5 BOD incubation results for Square Handkerchief (Sq Hand, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Incubations were conducted in chambers for a period of 3 to 6.75 hrs for Experiment 5. Rubble collected from Square Handkerchief and USM was exposed for 14 days, and rubble collected from Legacy and Katrina was exposed for 35 days. Cages at Legacy and Katrina were still deployed when Hurricane Isaac made landfall near the Mississippi River. Only the Katrina control chamber had a decline in O_2 concentration, while the other control chambers increased in concentration (Figure 18a; Figure 19a). Respiration rates in biofilm chambers were always higher than respiration rates in control chambers, and low profile biofilm chambers usually had higher respiration rates than high profile biofilm chambers. BA decreased in all control chambers except the Katrina control chamber, which increased in abundance (Figure 18b; Figure 19b). One USM biofilm chamber and one Katrina biofilm chamber also increased in abundance, while all other biofilm chambers had declines in population. Of the biofilm chambers that declined in BA, low profile chambers had higher average rates of decline than high profile chambers. PO_4 concentrations remained constant or decreased in control chambers, and increased in all biofilm chambers, with the exception of one Legacy biofilm chamber, which had undetectable PO_4 concentrations (Figure 18c; Figure 19c). Low profile biofilm chambers generally had higher rates of PO_4 production than high profile biofilm chambers. NH_4 concentrations increased in the Square Handkerchief control chamber and were undetectable in the remaining control chambers, while biofilm chambers always increased in NH_4 concentrations, almost always at higher rates than those observed in controls (Figure 18d; Figure 19d). NH_4 production rates were usually higher in low relief biofilm chambers than in high relief biofilm chambers. High PO_4 production rates were typically correlated with hypoxic chambers, and high NH_4 production rates were always

correlated with hypoxic chambers. NO_3 concentrations were below detection in all control chambers. Two low profile biofilm chambers had NO_3 production while the other low profile reef biofilm chambers had undetectable concentrations, and all but three high profile reef biofilm chambers had NO_3 production while the remaining three chambers had undetectable concentrations (Figure 18e; Figure 19e). High profile biofilm chambers often had higher NO_3 production rates than low profile biofilm chambers. NO_2 concentrations were undetectable in control chambers. All biofilm chambers increased in NO_2 concentration except for one USM biofilm chamber, which had undetectable concentrations (Figure 18f; Figure 19f). High profile biofilm chambers often had higher NO_2 production rates, although the highest rate was observed in a Legacy biofilm chamber. DOC concentrations declined in all control chambers, and often declined in high profile biofilm chambers as well (Figure 18g; Figure 19g). All low profile biofilm chambers increased in DOC concentration, usually at higher rates than the high profile biofilm chambers that also increased in concentration. DON concentrations declined in all control chambers except the Katrina control chamber, which increased in concentration (Figure 18h; Figure 19h). Biofilm chambers usually increased in DON concentration, and low relief biofilm chambers usually had higher rates of DON production than those observed in high relief biofilm chambers.

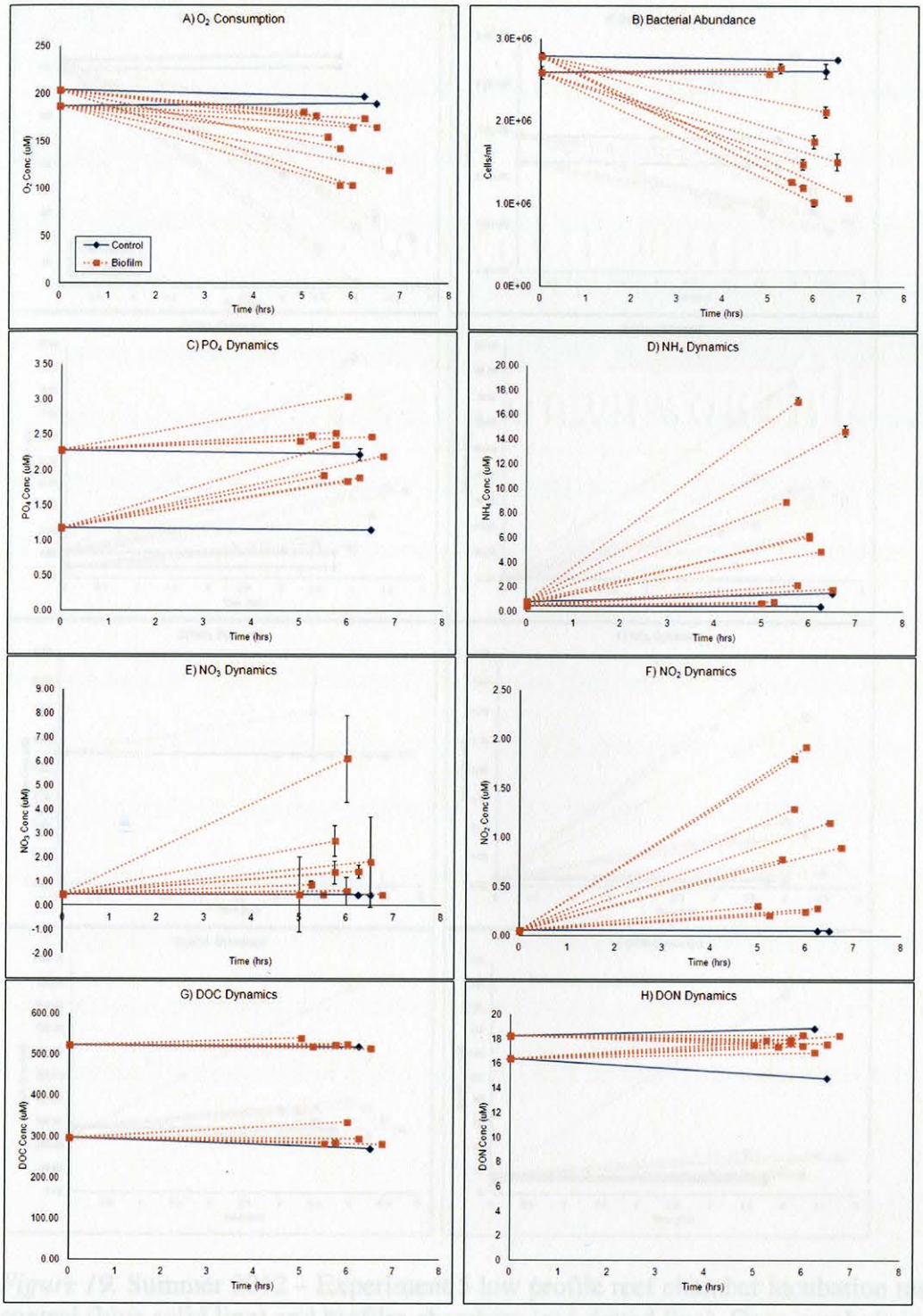

Figure 18. Summer 2012 – Experiment 5 high profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

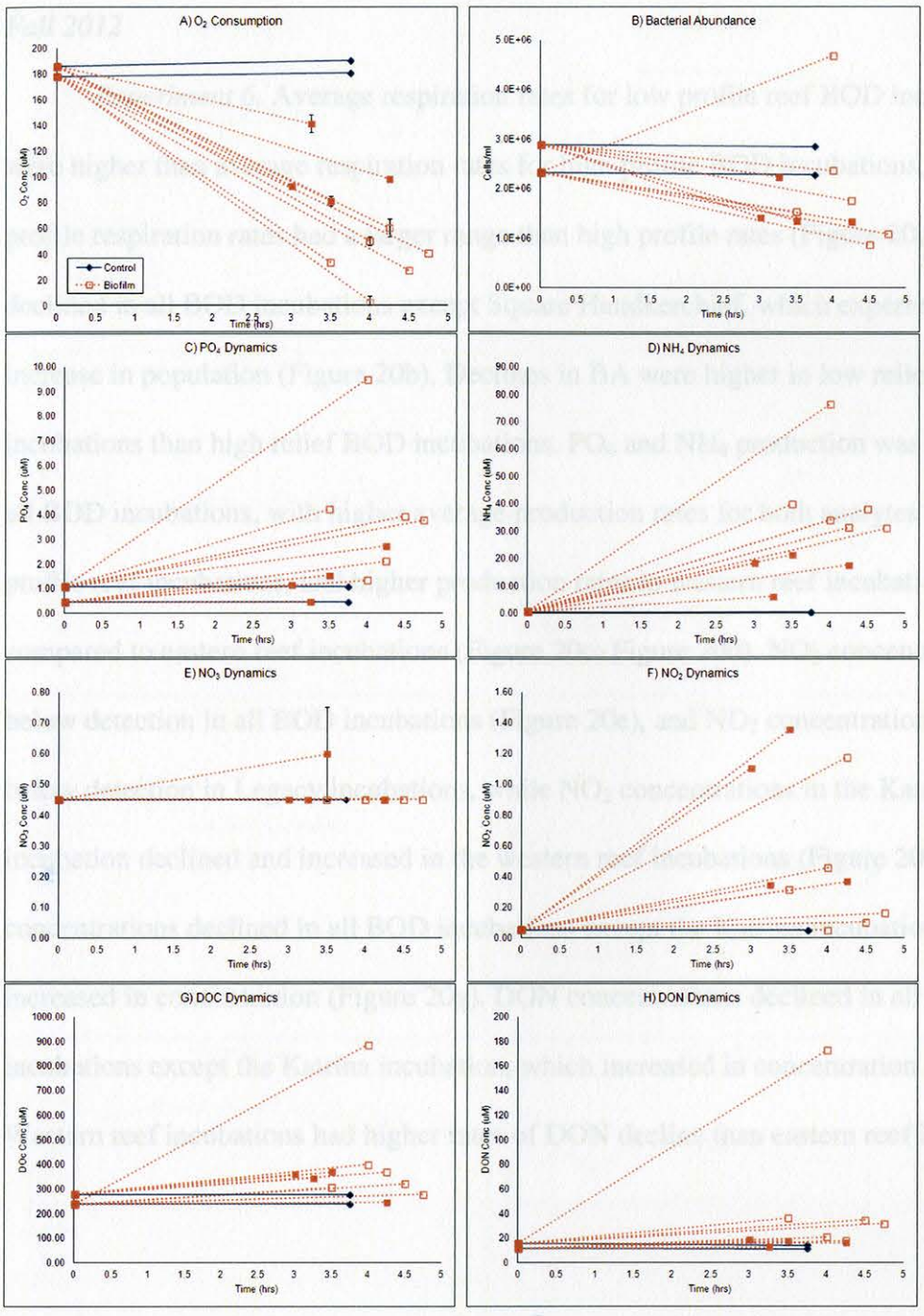

Figure 19. Summer 2012 – Experiment 5 low profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 μM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Fall 2012

Experiment 6. Average respiration rates for low profile reef BOD incubations were higher than average respiration rates for high profile BOD incubations, but low profile respiration rates had a larger range than high profile rates (Figure 20a). BA declined in all BOD incubations except Square Handkerchief, which experienced an increase in population (Figure 20b). Declines in BA were higher in low relief BOD incubations than high relief BOD incubations. PO_4 and NH_4 production was observed in all BOD incubations, with higher average production rates for both analytes in low profile reef incubations, and higher production rates in western reef incubations compared to eastern reef incubations (Figure 20c; Figure 20d). NO_3 concentrations were below detection in all BOD incubations (Figure 20e), and NO_2 concentrations were below detection in Legacy incubations, while NO_2 concentrations in the Katrina incubation declined and increased in the western reef incubations (Figure 20f). DOC concentrations declined in all BOD incubations except the Katrina incubation, which increased in concentration (Figure 20g). DON concentrations declined in all BOD incubations except the Katrina incubation, which increased in concentration (Figure 20h). Western reef incubations had higher rates of DON decline than eastern reef incubations.

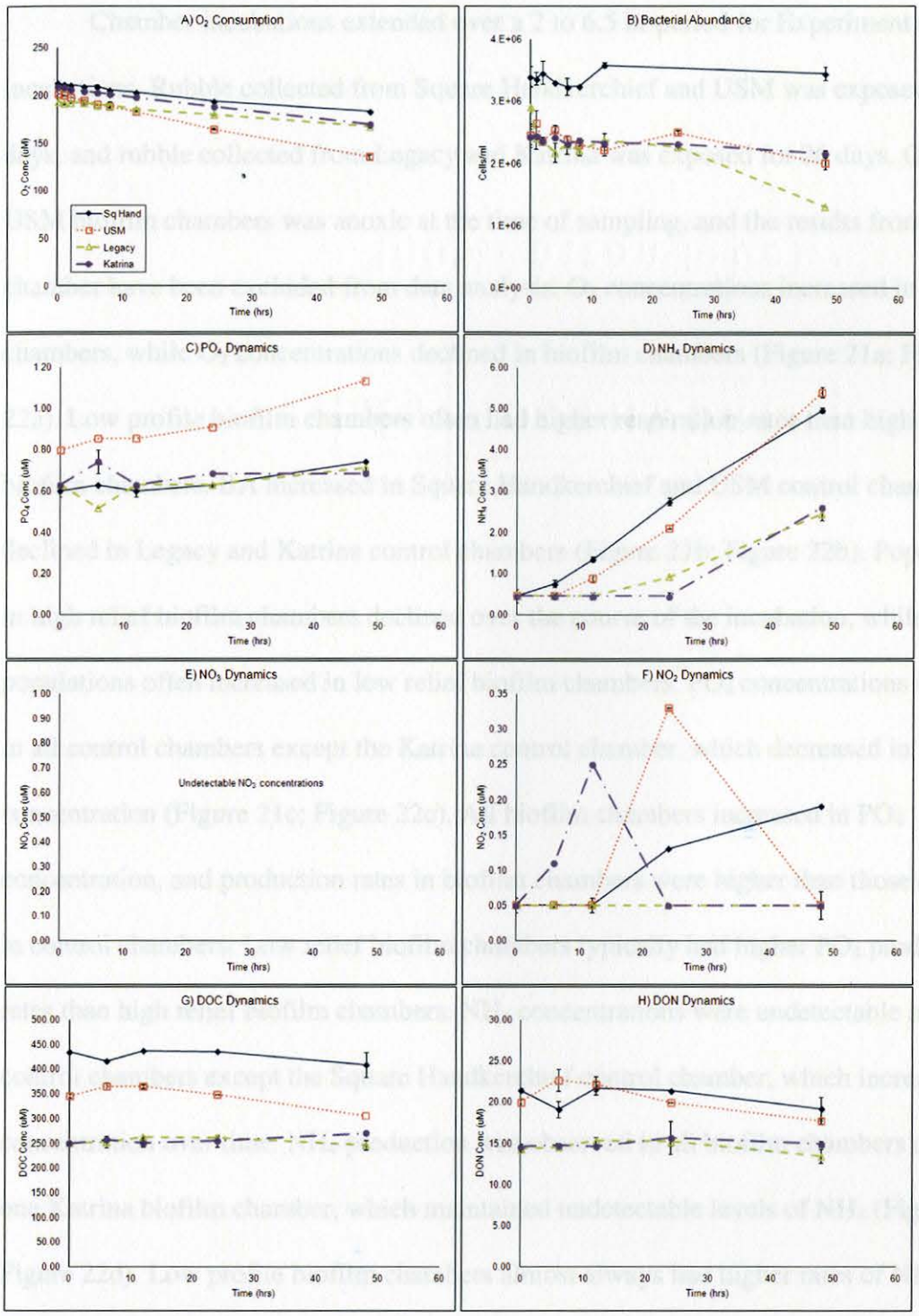

Figure 20. Fall 2012 – Experiment 6 BOD incubation results for Square Handkerchief (Sq Hand, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Chamber incubations extended over a 2 to 6.5 hr period for Experiment 6 incubations. Rubble collected from Square Handkerchief and USM was exposed for 20 days, and rubble collected from Legacy and Katrina was exposed for 26 days. One of the USM biofilm chambers was anoxic at the time of sampling, and the results from this chamber have been excluded from data analysis. O_2 concentrations increased in control chambers, while O_2 concentrations declined in biofilm chambers (Figure 21a; Figure 22a). Low profile biofilm chambers often had higher respiration rates than high profile biofilm chambers. BA increased in Square Handkerchief and USM control chambers, and declined in Legacy and Katrina control chambers (Figure 21b; Figure 22b). Populations in high relief biofilm chambers declined over the course of the incubation, while populations often increased in low relief biofilm chambers. PO_4 concentrations increased in all control chambers except the Katrina control chamber, which decreased in concentration (Figure 21c; Figure 22c). All biofilm chambers increased in PO_4 concentration, and production rates in biofilm chambers were higher than those observed in control chambers. Low relief biofilm chambers typically had higher PO_4 production rates than high relief biofilm chambers. NH_4 concentrations were undetectable in all control chambers except the Square Handkerchief control chamber, which increased in concentration over time. NH_4 production was observed in all biofilm chambers except one Katrina biofilm chamber, which maintained undetectable levels of NH_4 (Figure 21d; Figure 22d). Low profile biofilm chambers almost always had higher rates of NH_4 production than high profile biofilm chambers. PO_4 and NH_4 production rates were highest in hypoxic and anoxic chambers. Concentrations for NO_3 were all below detection in control chambers, and most of the low relief biofilm chambers also had

undetectable NO_3 concentrations (Figure 22e). NO_3 production was observed in the majority of high relief biofilm chambers, although two high relief biofilm chambers had undetectable concentrations as well (Figure 21e). One low relief biofilm chamber had NO_3 production during the incubation period, but this rate was lower than most observed production rates in the high relief biofilm chambers. NO_2 concentrations were undetectable in all but one control chamber, which decreased in concentration. The rate calculated in the control chamber with NO_2 uptake is not accurate due to concentrations being undetectable at the time of sampling. Most biofilm chambers had an increase in NO_2 concentration, and the remaining biofilm chambers maintained undetectable levels of NO_2 (Figure 21; Figure 22f). NO_2 production rates were usually higher in high profile biofilm chambers than low profile biofilm chambers. DOC concentrations decreased in all control chambers except the Legacy control chamber, which increased in concentration (Figure 21g; Figure 22g). Most of the Square Handkerchief biofilm chambers increased in DOC concentration, while most of the Katrina biofilm chambers decreased in concentration. The majority of low profile biofilm chambers increased in DOC concentration, and the production rates observed in low profile biofilm chambers were higher than those observed in high profile biofilm chambers. DON concentrations decreased in all control chambers and all high relief biofilm chambers (Figure 21h; Figure 22h). Low profile biofilm chambers often increased in DON concentration over time.

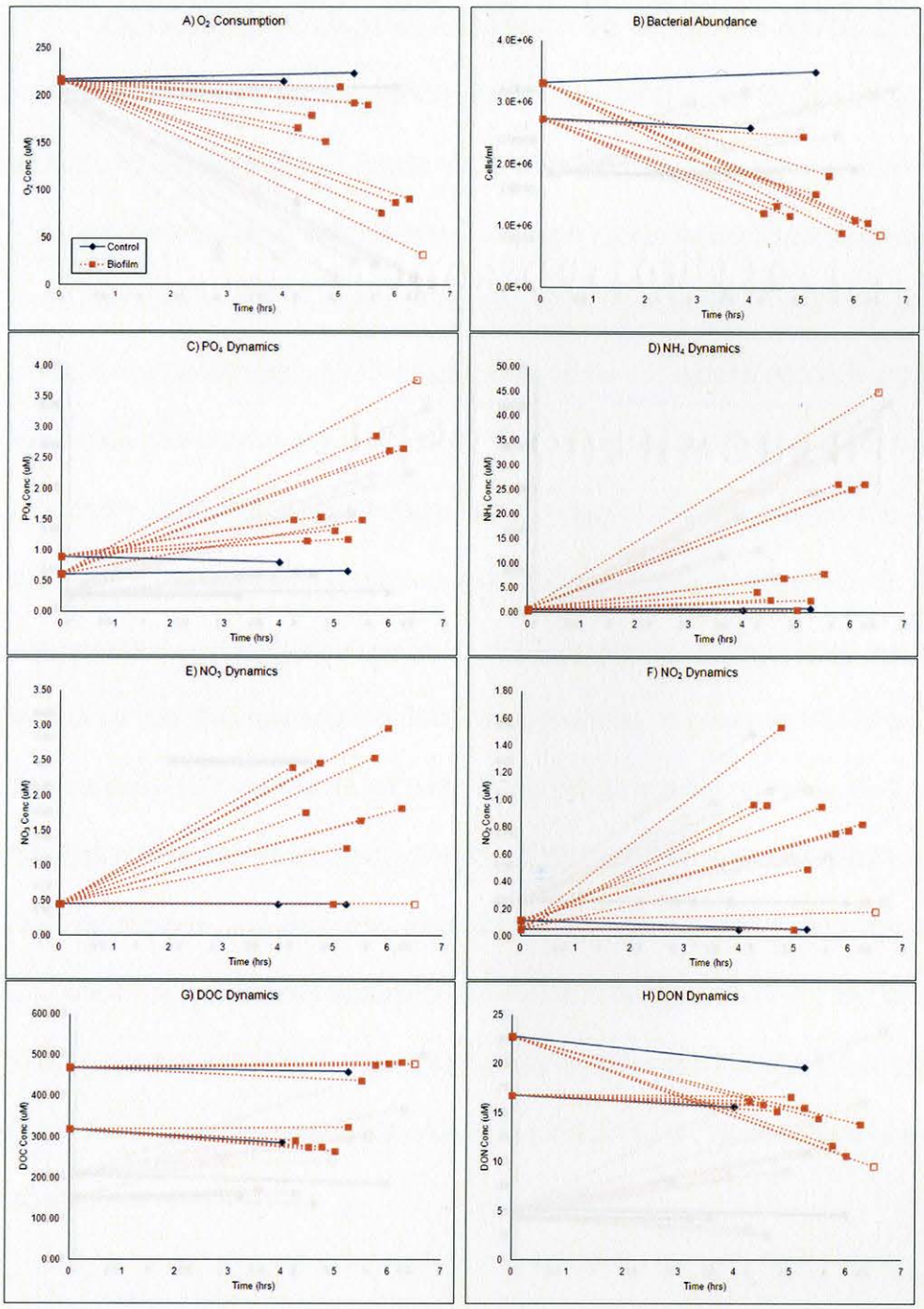

Figure 21. Fall 2012 – Experiment 6 high profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 μM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

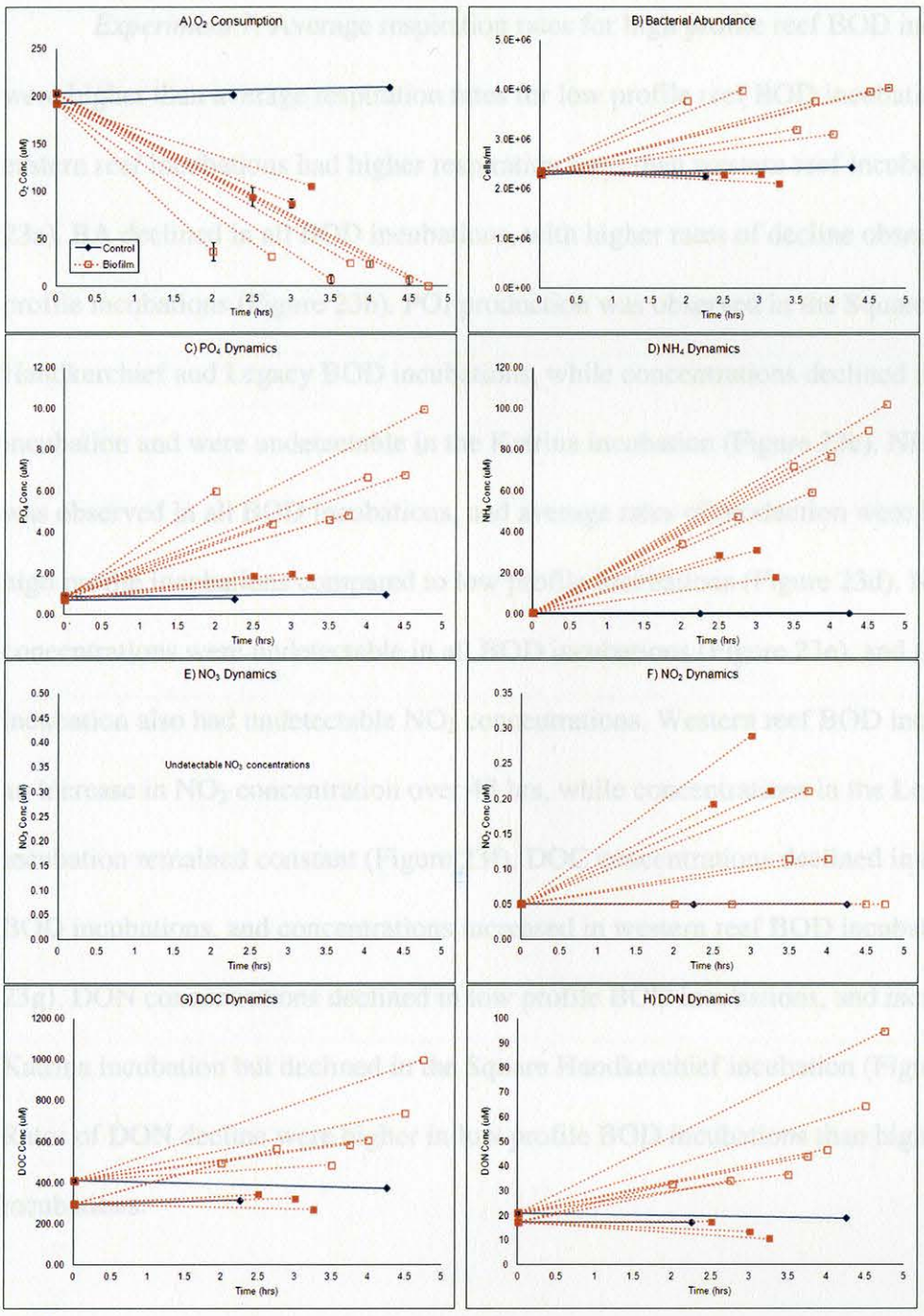

Figure 22. Fall 2012 – Experiment 6 low profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 µM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Experiment 7. Average respiration rates for high profile reef BOD incubations were higher than average respiration rates for low profile reef BOD incubations, and eastern reef incubations had higher respiration rates than western reef incubations (Figure 23a). BA declined in all BOD incubations, with higher rates of decline observed in low profile incubations (Figure 23b). PO_4 production was observed in the Square Handkerchief and Legacy BOD incubations, while concentrations declined in the USM incubation and were undetectable in the Katrina incubation (Figure 23c). NH_4 production was observed in all BOD incubations, and average rates of production were higher in high profile incubations compared to low profile incubations (Figure 23d). NO_3 concentrations were undetectable in all BOD incubations (Figure 23e), and the Katrina incubation also had undetectable NO_2 concentrations. Western reef BOD incubations had an increase in NO_2 concentration over 48 hrs, while concentrations in the Legacy incubation remained constant (Figure 23f). DOC concentrations declined in eastern reef BOD incubations, and concentrations increased in western reef BOD incubations (Figure 23g). DON concentrations declined in low profile BOD incubations, and increased in the Katrina incubation but declined in the Square Handkerchief incubation (Figure 23h). Rates of DON decline were higher in low profile BOD incubations than high profile incubations.

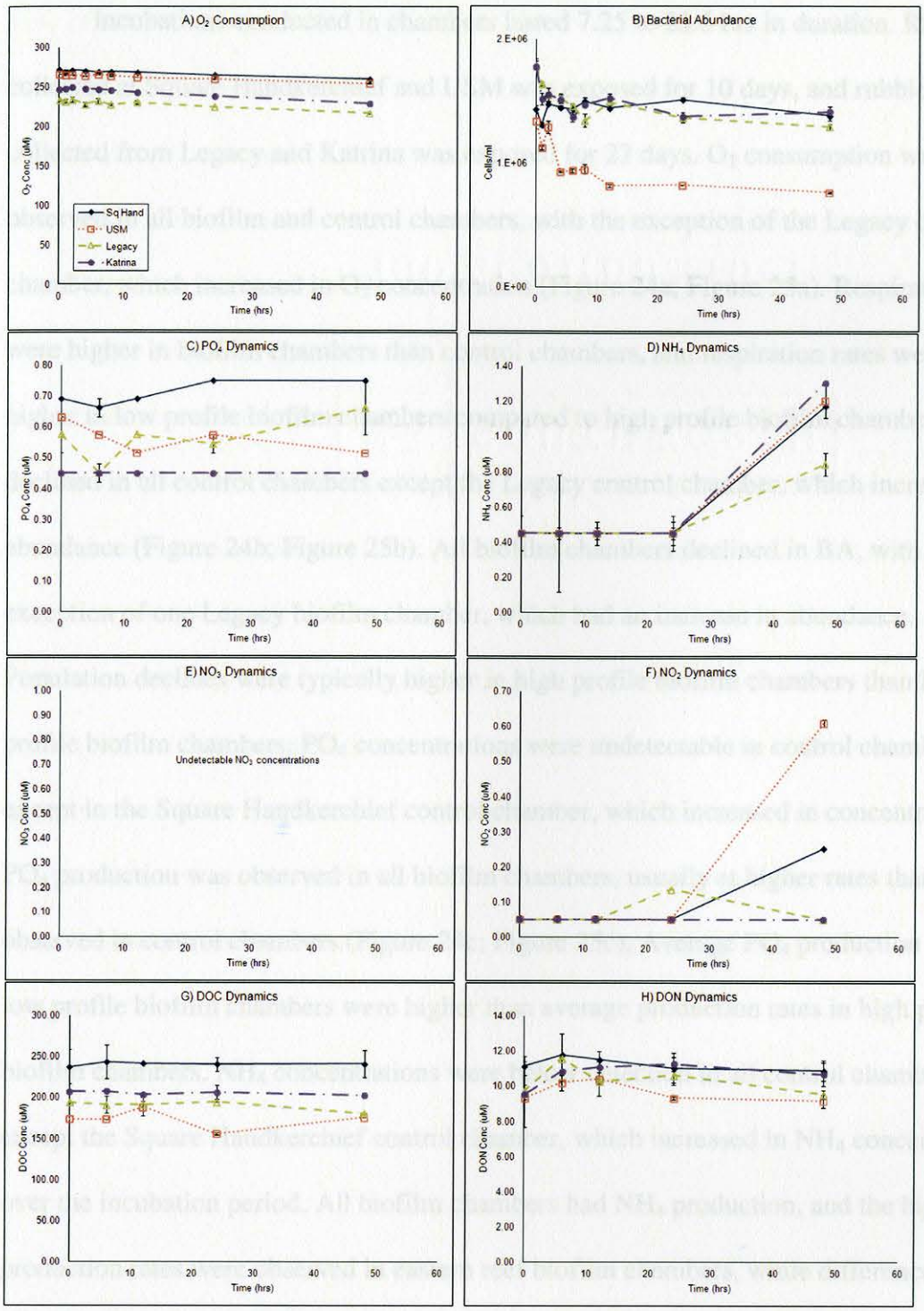

Figure 23. Fall 2012 – Experiment 7 BOD incubation results for Square Handkerchief (Sq Hand, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Incubations conducted in chambers lasted 7.25 to 25.5 hrs in duration. Rubble collected at Square Handkerchief and USM was exposed for 10 days, and rubble collected from Legacy and Katrina was exposed for 27 days. O_2 consumption was observed in all biofilm and control chambers, with the exception of the Legacy control chamber, which increased in O_2 concentration (Figure 24a; Figure 25a). Respiration rates were higher in biofilm chambers than control chambers, and respiration rates were often higher in low profile biofilm chambers compared to high profile biofilm chambers. BA declined in all control chambers except the Legacy control chamber, which increased in abundance (Figure 24b; Figure 25b). All biofilm chambers declined in BA, with the exception of one Legacy biofilm chamber, which had an increase in abundance. Population declines were typically higher in high profile biofilm chambers than low profile biofilm chambers. PO_4 concentrations were undetectable in control chambers except in the Square Handkerchief control chamber, which increased in concentration. PO_4 production was observed in all biofilm chambers, usually at higher rates than those observed in control chambers (Figure 24c; Figure 25c). Average PO_4 production rates in low profile biofilm chambers were higher than average production rates in high profile biofilm chambers. NH_4 concentrations were below detection in all control chambers except the Square Handkerchief control chamber, which increased in NH_4 concentration over the incubation period. All biofilm chambers had NH_4 production, and the highest production rates were observed in eastern reef biofilm chambers, while differences in production were less discernible in low relief biofilm chambers compared to high relief biofilm chambers (Figure 24d; Figure 25d). The highest NH_4 and PO_4 production rates were observed in a hypoxic chamber. NO_3 concentrations were all undetectable at the

time of sampling (Figure 24e; Figure 25e). However, the initial concentration for the Square Handkerchief incubations was detectable. Therefore, NO_3 was taken up in control and biofilm Square Handkerchief chambers, but because concentrations were below detection at the time of sampling the rates calculated are not accurate. Control chambers all had undetectable levels of NO_2, as did low profile biofilm chambers (Figure 25f). Two high profile biofilm chambers had NO_2 production, and the remaining chambers had NO_2 concentrations below detection (Figure 24f). DOC concentrations increased in control chambers, except the Square Handkerchief control chamber, which decreased in concentration (Figure 24g; Figure 25g). Six high relief biofilm chambers decreased in DOC concentration, and the remaining four high relief biofilm chambers increased in DOC concentration. Low relief biofilm chambers almost always increased in DOC concentration, with the exception of one Legacy biofilm chamber, which decreased in concentration. DON concentrations always increased in control chambers, and low relief biofilm chambers usually increased in DON concentration while high relief biofilm chambers usually decreased in DON concentration (Figure 24h; Figure 25h).

Figure 24. Fall 2012 – Experiment 7 high profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_2 concentration versus time, F) NO_3 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

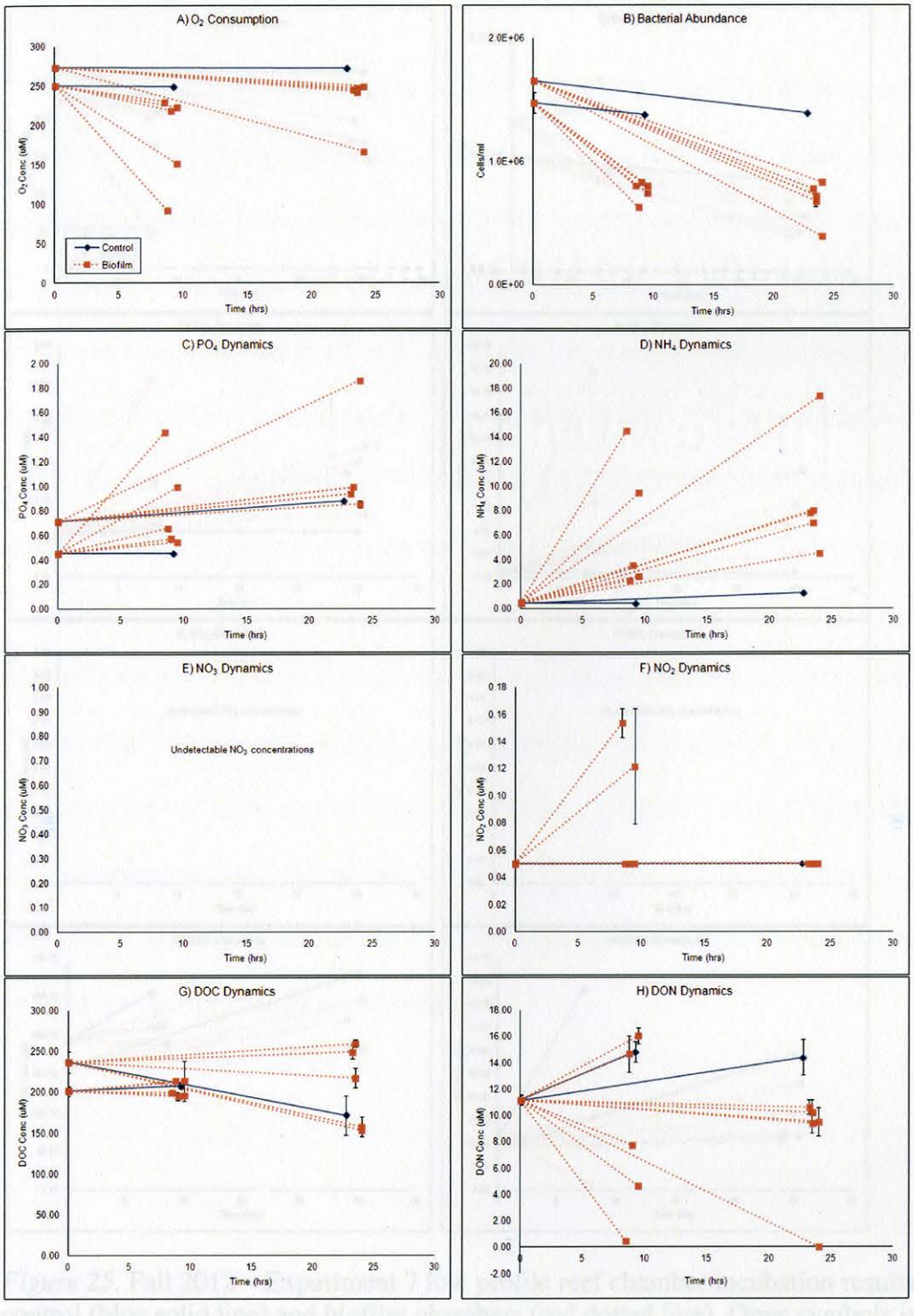

Figure 24. Fall 2012 – Experiment 7 high profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

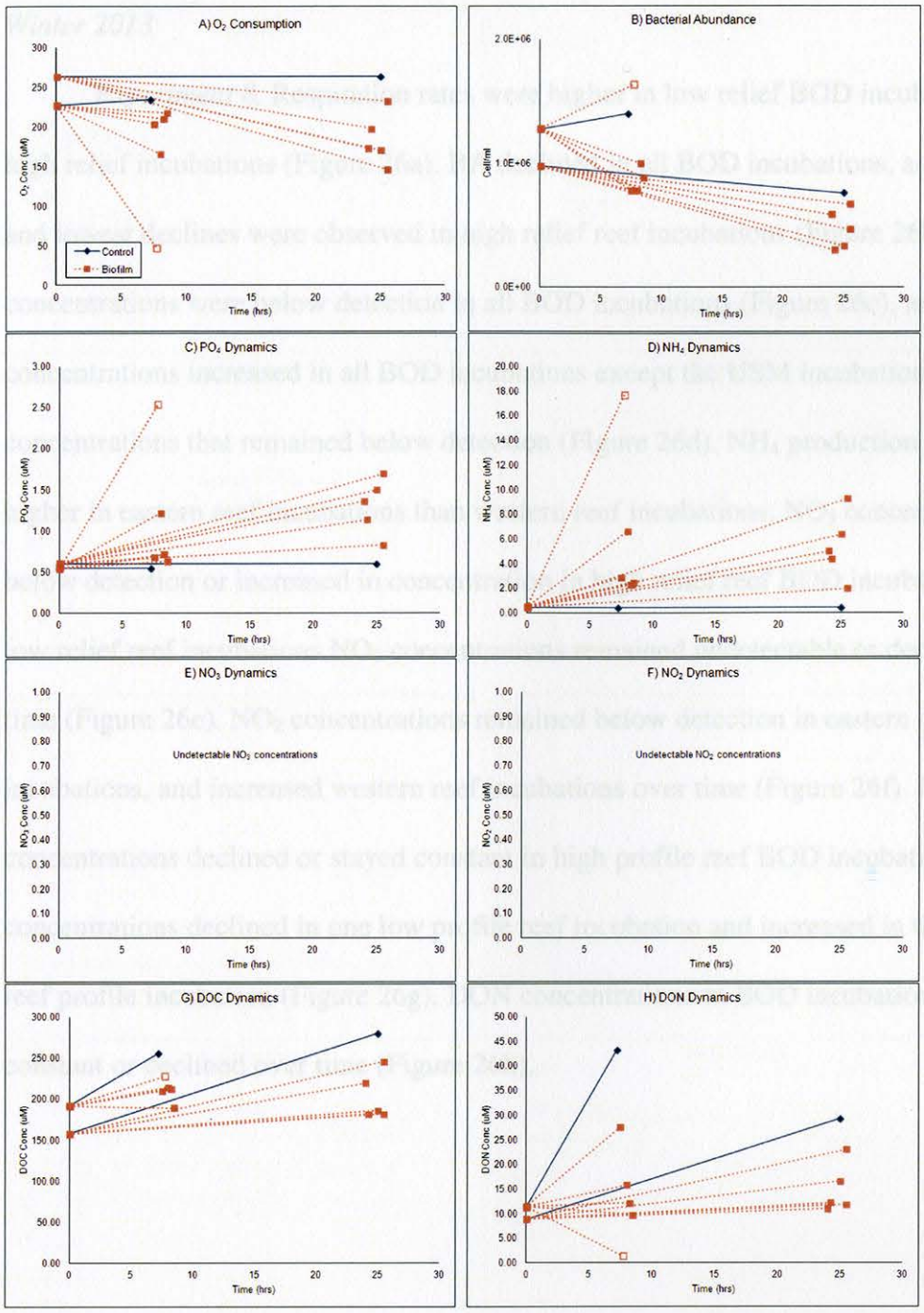

Figure 25. Fall 2012 – Experiment 7 low profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 µM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Winter 2013

Experiment 8. Respiration rates were higher in low relief BOD incubations than high relief incubations (Figure 26a). BA declined in all BOD incubations, and the highest and lowest declines were observed in high relief reef incubations (Figure 26b). PO_4 concentrations were below detection in all BOD incubations (Figure 26c), and NH_4 concentrations increased in all BOD incubations except the USM incubation, which had concentrations that remained below detection (Figure 26d). NH_4 production rates were higher in eastern reef incubations than western reef incubations. NO_3 concentrations were below detection or increased in concentration in high relief reef BOD incubations, and in low relief reef incubations NO_3 concentrations remained undetectable or decreased over time (Figure 26e). NO_2 concentrations remained below detection in eastern reef BOD incubations, and increased western reef incubations over time (Figure 26f). DOC concentrations declined or stayed constant in high profile reef BOD incubations, and concentrations declined in one low profile reef incubation and increased in the other low reef profile incubation (Figure 26g). DON concentrations in BOD incubations remained constant or declined over time (Figure 26h).

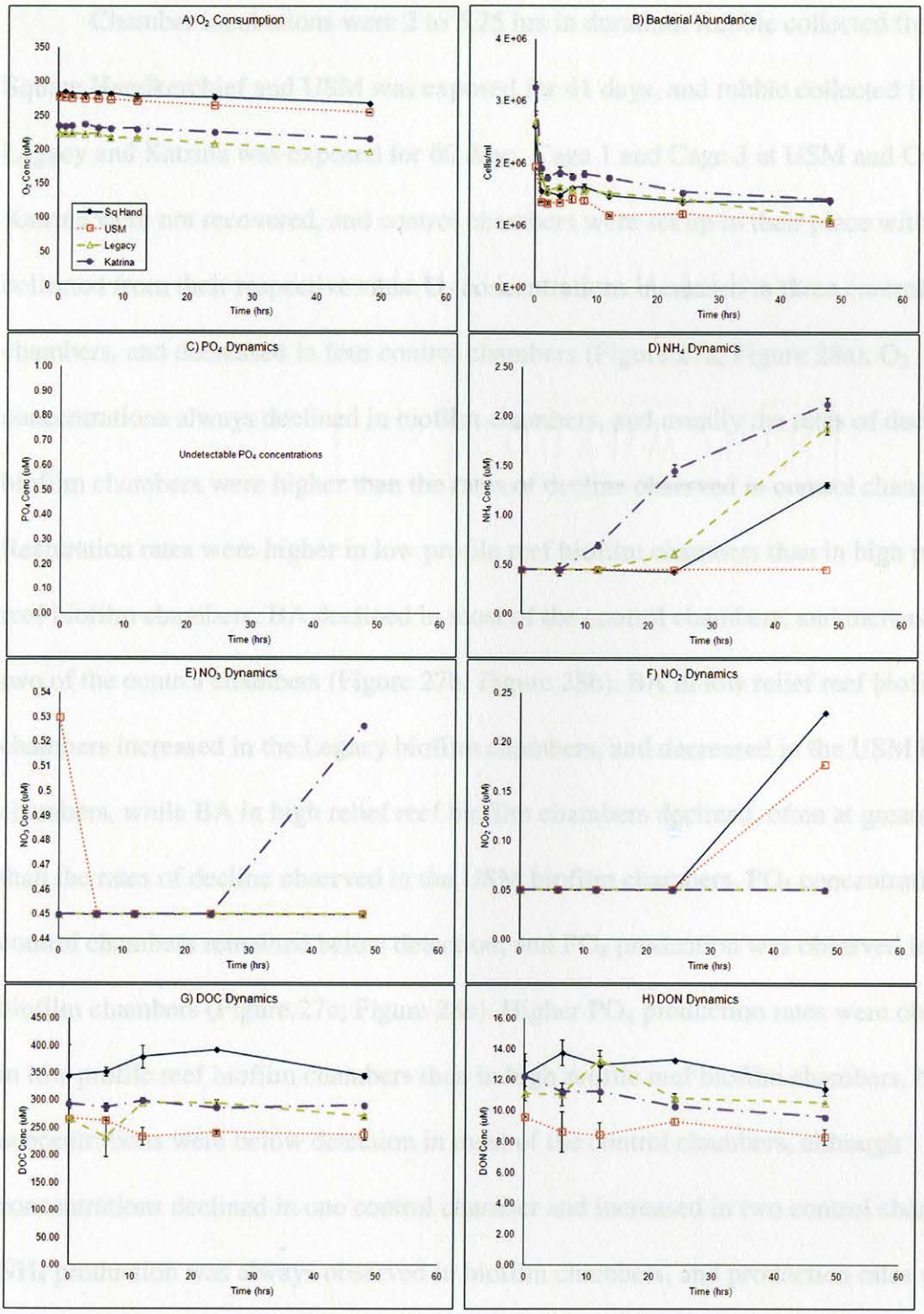

Figure 26. Winter 2013 – Experiment 8 BOD incubation results for Square Handkerchief (Sq Hand, dark blue), USM (red), Legacy (green), and Katrina (purple). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Chamber incubations were 2 to 5.25 hrs in duration. Rubble collected from Square Handkerchief and USM was exposed for 41 days, and rubble collected from Legacy and Katrina was exposed for 60 days. Cage 1 and Cage 3 at USM and Cage 4 at Katrina were not recovered, and control chambers were set up in their place with water collected from their respective sites. O_2 concentrations increased in three control chambers, and decreased in four control chambers (Figure 27a; Figure 28a). O_2 concentrations always declined in biofilm chambers, and usually the rates of decline in biofilm chambers were higher than the rates of decline observed in control chambers. Respiration rates were higher in low profile reef biofilm chambers than in high profile reef biofilm chambers. BA declined in most of the control chambers, and increased in two of the control chambers (Figure 27b; Figure 28b). BA in low relief reef biofilm chambers increased in the Legacy biofilm chambers, and decreased in the USM biofilm chambers, while BA in high relief reef biofilm chambers declined, often at greater rates than the rates of decline observed in the USM biofilm chambers. PO_4 concentrations in control chambers remained below detection, and PO_4 production was observed in all biofilm chambers (Figure 27c; Figure 28c). Higher PO_4 production rates were observed in low profile reef biofilm chambers than in high profile reef biofilm chambers. NH_4 concentrations were below detection in most of the control chambers, although concentrations declined in one control chamber and increased in two control chambers. NH_4 production was always observed in biofilm chambers, and production rates were higher in low profile reef biofilm chambers than in high profile reef biofilm chambers (Figure 27d; Figure 28d). The highest PO_4 and NH_4 productions rates were observed in hypoxic chambers, with the exception of one high PO_4 and NH_4 production rate that were

observed in a normoxic chamber. NO_3 concentrations were largely undetectable in control and biofilm chambers, although NO_3 production was observed in one control chamber and five biofilm chambers (Figure 27e; Figure 28e). High relief reef biofilm chambers had higher NO_3 production rates than the rate observed in the low relief reef biofilm chamber that also had NO_3 production. NO_2 concentrations were mostly undetectable in control chambers, with the exception of the Square Handkerchief control chamber, which declined over time, but the rate calculated is not accurate because concentrations were undetectable at the time of sampling. NO_2 concentrations were largely undetectable in biofilm chambers at the time of sampling, although NO_2 production was observed in several biofilm chambers (Figure 27f; Figure 28f). The highest NO_2 production rates were observed in high profile reef biofilm chambers. DOC concentrations declined in four control chambers and increased in three control chambers, and concentrations often increased in biofilm chambers, with the exception of three Square Handkerchief biofilm chambers, which decreased in concentration (Figure 27g; Figure 28g). Rates of DOC production were higher in low profile reef biofilm chambers than in high profile reef biofilm chambers. DON concentrations decreased in high profile control chambers, and increased in low profile control chambers (Figure 27h; Figure 28h). DON concentrations declined in all but one high relief reef biofilm chamber, which increased in concentration, and concentrations increased in all low relief reef biofilm chambers.

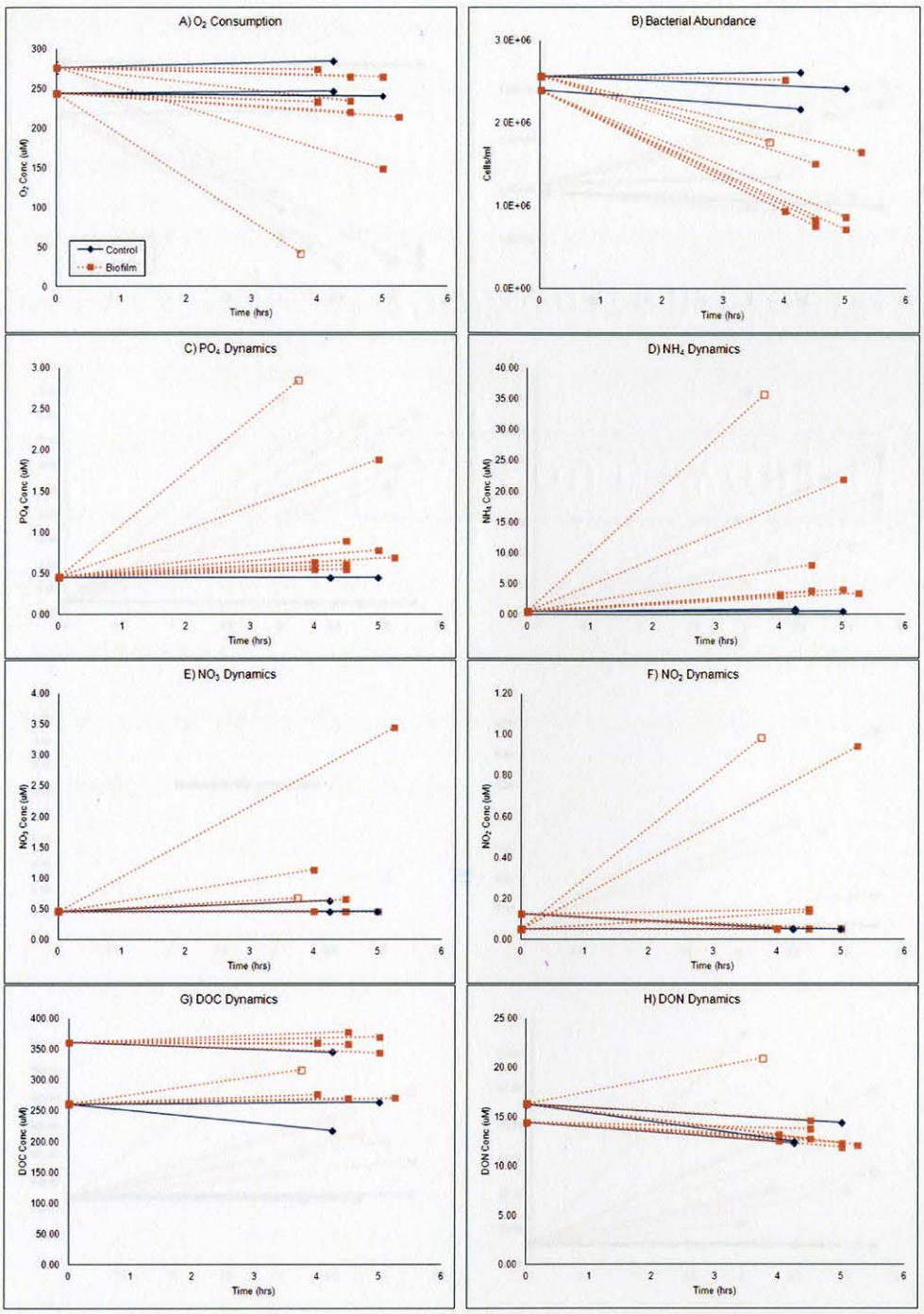

Figure 27. Winter 2013 – Experiment 8 high profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic chambers (O_2 < 62.5 µM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

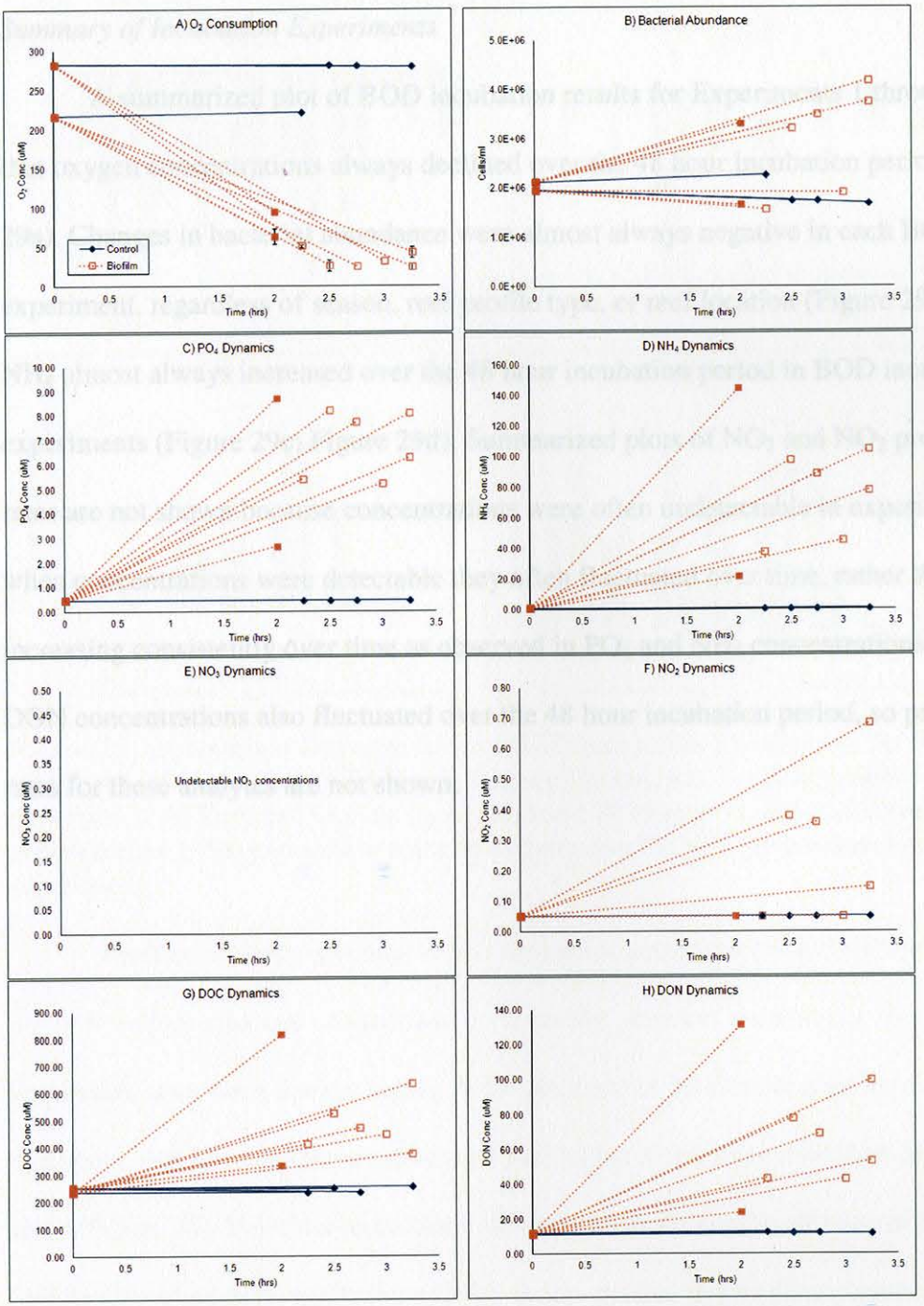

Figure 28. Winter 2013 – Experiment 8 low profile reef chamber incubation results for control (blue solid line) and biofilm chambers (red dotted line). Open symbols indicate hypoxic conditions (O_2 < 62.5 µM). Error bars represent ± SEM. A) Oxygen concentration versus time, B) bacterial abundance versus time, C) PO_4 concentration versus time, D) NH_4 concentration versus time, E) NO_3 concentration versus time, F) NO_2 concentration versus time, G) DOC concentration versus time, H) DON concentration versus time.

Summary of Incubation Experiments

A summarized plot of BOD incubation results for Experiments 1 through 8 show that oxygen concentrations always declined over the 48 hour incubation period (Figure 29a). Changes in bacterial abundance were almost always negative in each BOD experiment, regardless of season, reef profile type, or reef location (Figure 29b). PO_4 and NH_4 almost always increased over the 48 hour incubation period in BOD incubation experiments (Figure 29c; Figure 29d). Summarized plots of NO_3 and NO_2 production rates are not shown because concentrations were often undetectable in experiments, and when concentrations were detectable they often fluctuated over time, rather than increasing consistently over time as observed in PO_4 and NH_4 concentrations. DOC and DON concentrations also fluctuated over the 48 hour incubation period, so production rates for these analytes are not shown.

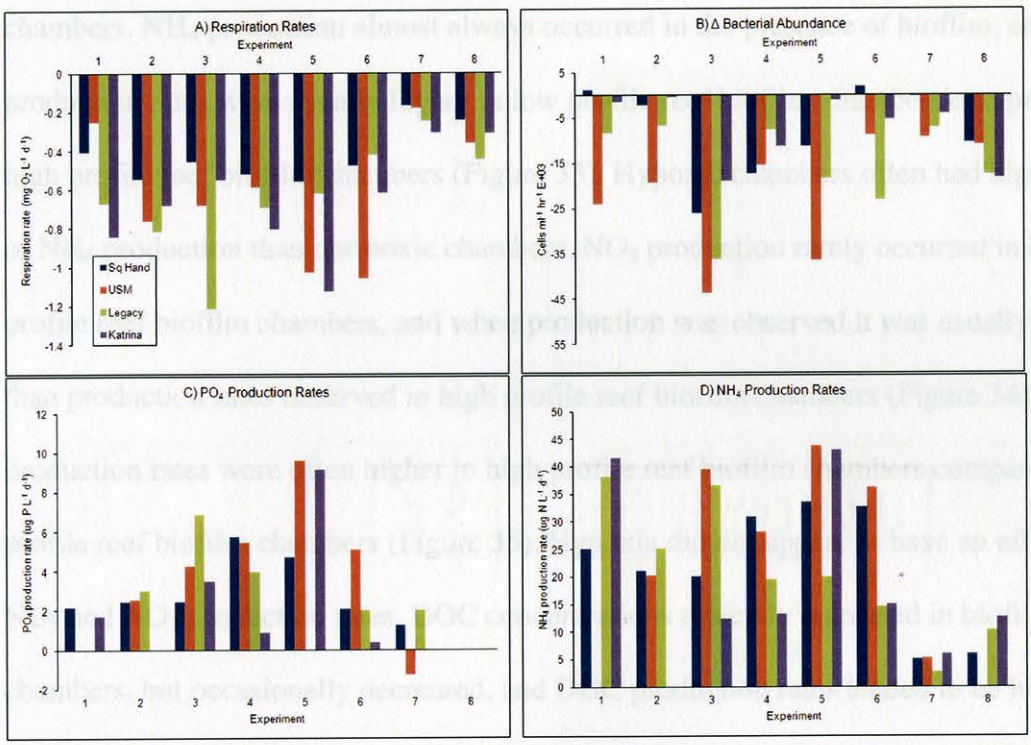

Figure 29. Summarized response rates of BOD incubation experiments for Square Handkerchief (dark blue), USM (red), Legacy (green), and Katrina (purple) by experiment. A) Respiration rates by experiment, B) change in bacterial abundance by experiment, C) PO_4 production rates by experiment, D) NH_4 production rates by experiment.

Biofilm incubation results were control-corrected and normalized to biofilm mass per unit surface area and summarized by response rates and experiment. Normalized respiration rates were always higher in the presence of biofilm relative to control chambers, and low profile reef chambers had higher respiration rates than high profile reefs (Figure 30). Bacterial abundance almost always declined over time in high profile reef biofilm chambers, while abundances in low profile reef biofilm chambers sometimes increased, especially in hypoxic chambers (Figure 31). PO_4 production almost always occurred in the presence of biofilm, and production rates were typically higher in low profile reef biofilm chambers compared to high profile reef biofilm chambers (Figure 32). Hypoxic chambers often had higher rates of PO_4 production than normoxic

chambers. NH_4 production almost always occurred in the presence of biofilm, and production rates were usually higher in low profile reef biofilm chambers compared to high profile reef biofilm chambers (Figure 33). Hypoxic chambers often had higher rates of NH_4 production than normoxic chambers. NO_3 production rarely occurred in low profile reef biofilm chambers, and when production was observed it was usually lower than production rates observed in high profile reef biofilm chambers (Figure 34). NO_2 production rates were often higher in high profile reef biofilm chambers compared to low profile reef biofilm chambers (Figure 35). Hypoxia did not appear to have an effect on NO_3 and NO_2 production rates. DOC concentrations typically increased in biofilm chambers, but occasionally decreased, and DOC production rates tended to be higher in low profile reef biofilm chambers (Figure 36). Changes in DON concentrations in biofilm chambers were more variable and showed increases and decreases in concentration during incubations (Figure 37). Larger responses in changes in DON concentrations were typically seen in low profile reef biofilm chambers. Hypoxic chambers usually exhibited DOC and DON production. Each of the summarized response rate graphs illustrate the variability observed in each experiment and within each study site.

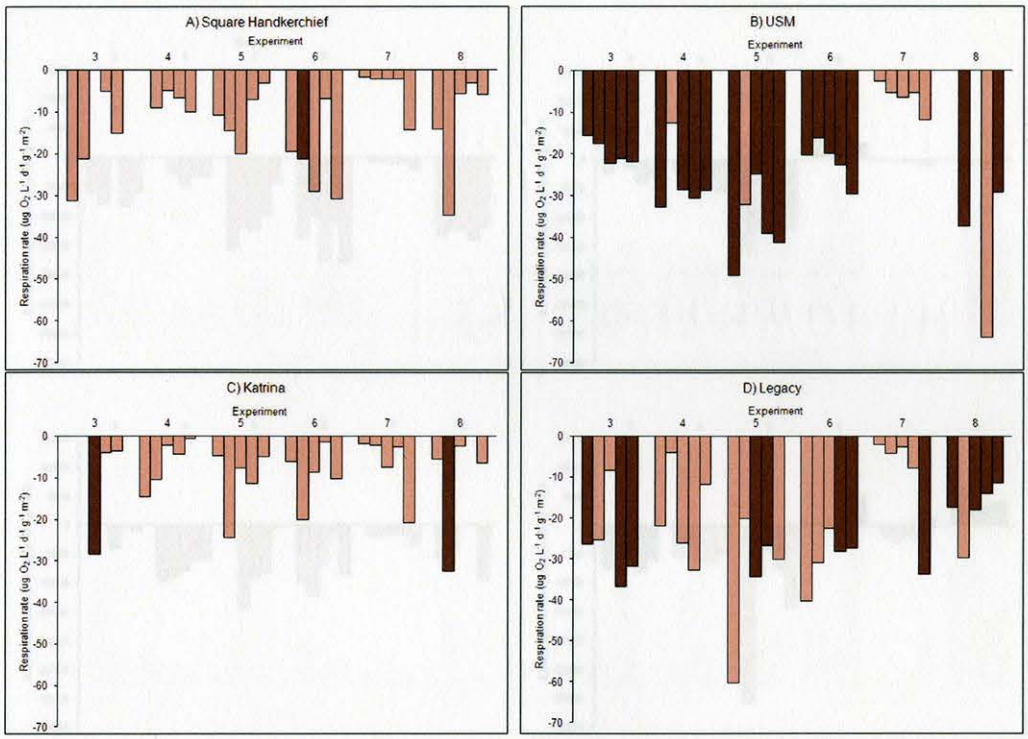

Figure 30. Summarized respiration rates of individual control-corrected biofilm chambers normalized to biofilm mass per unit surface area by experiment. Pink bars represent normoxic chambers and red bars represent hypoxic chambers. A) Respiration rates of Square Handkerchief biofilm chambers, B) respiration rates of USM biofilm chambers, C) respiration rates of Katrina biofilm chambers, D) respiration rates of Legacy biofilm chambers.

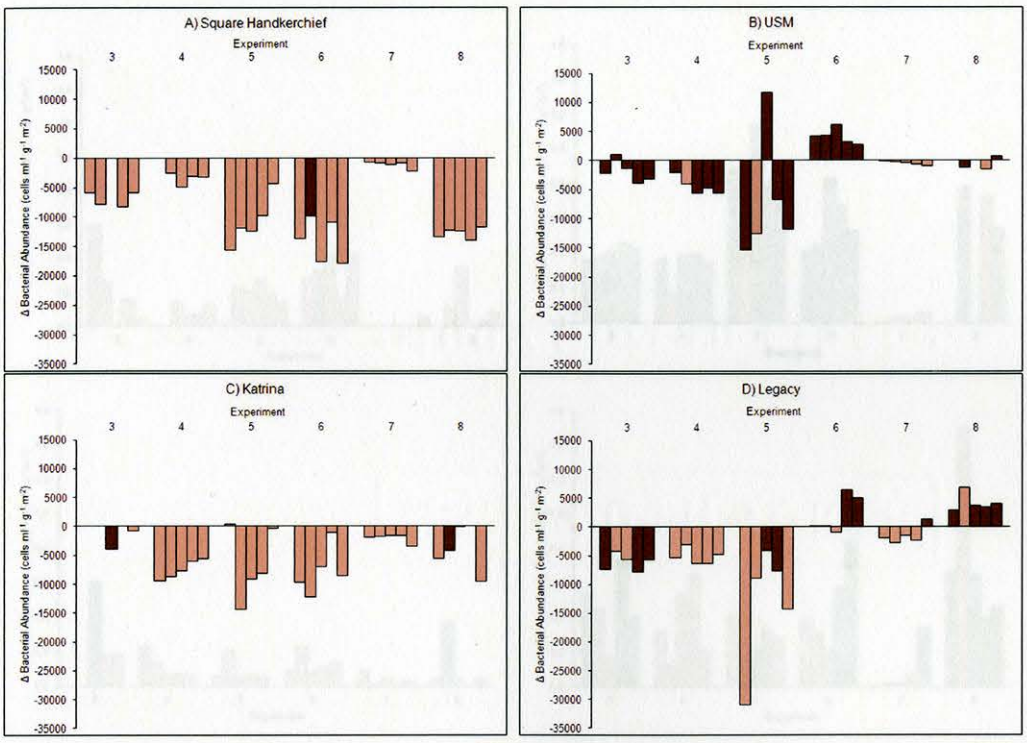

Figure 31. Summarized rates of change in bacterial abundance of individual control-corrected biofilm chambers normalized to biofilm mass per unit surface area by experiment. Pink bars represent normoxic chambers and red bars represent hypoxic chambers. A) Change in BA rates of Square Handkerchief biofilm chambers, B) change in BA rates of USM biofilm chambers, C) change in BA rates of Katrina biofilm chambers, D) change in BA rates of Legacy biofilm chambers.

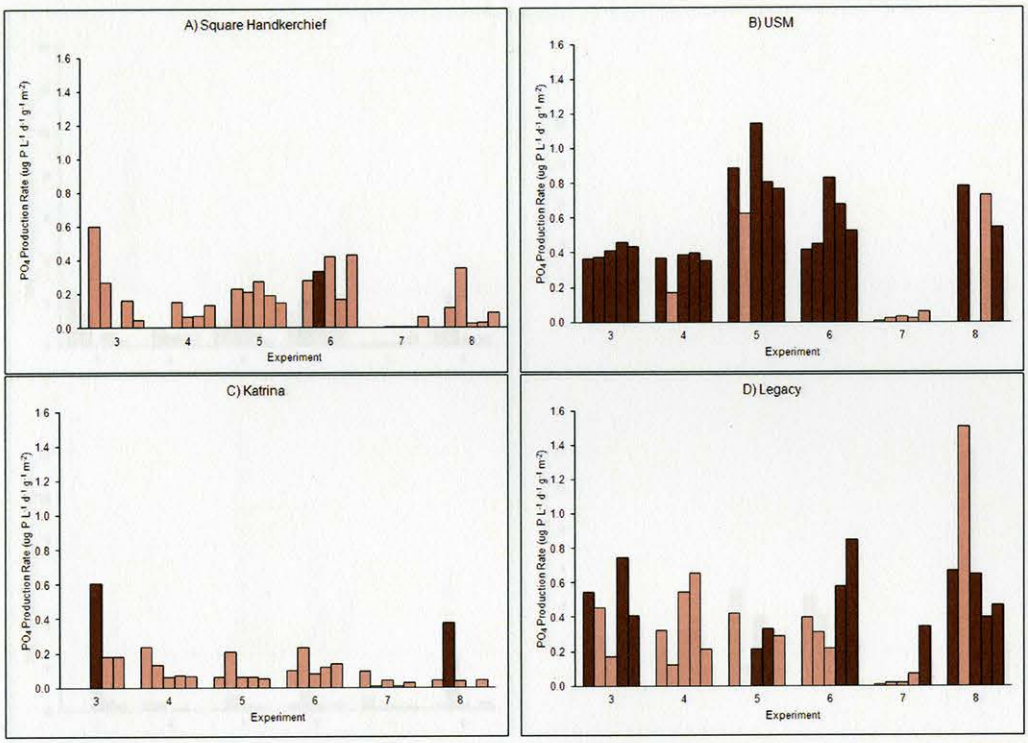

Figure 32. Summarized PO_4 production rates of individual control-corrected biofilm chambers normalized to biofilm mass per unit surface area by experiment. Pink bars represent normoxic chambers and red bars represent hypoxic chambers. A) PO_4 production rates of Square Handkerchief biofilm chambers, B) PO_4 production rates of USM biofilm chambers, C) PO_4 production rates of Katrina biofilm chambers, D) PO_4 production rates of Legacy biofilm chambers.

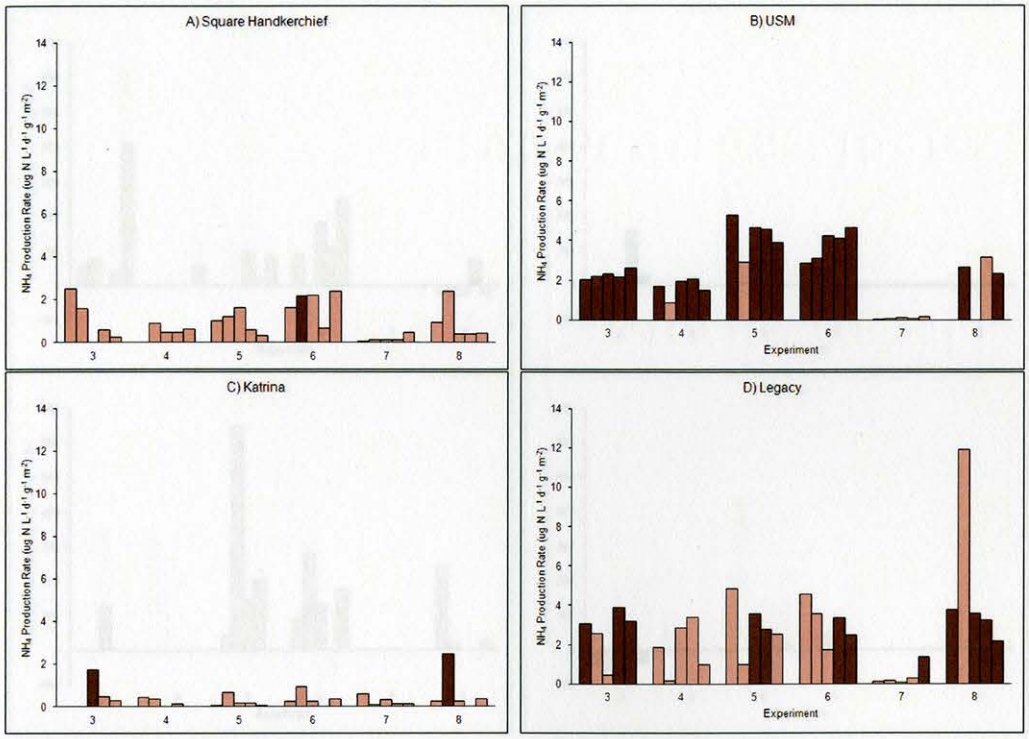

Figure 33. Summarized NH_4 production rates of individual control-corrected biofilm chambers normalized to biofilm mass per unit surface area by experiment. Pink bars represent normoxic chambers and red bars represent hypoxic chambers. A) NH_4 production rates of Square Handkerchief biofilm chambers, B) NH_4 production rates of USM biofilm chambers, C) NH_4 production rates of Katrina biofilm chambers, D) NH_4 production rates of Legacy biofilm chambers.

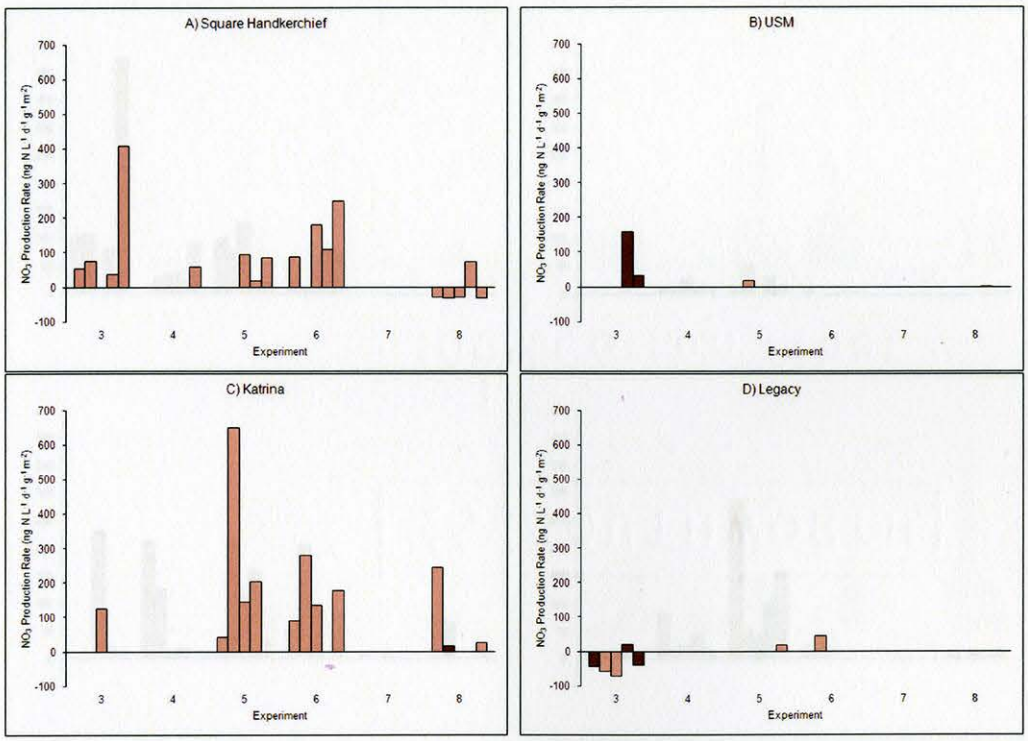

Figure 34. Summarized NO_3 production rates of individual control-corrected biofilm chambers normalized to biofilm mass per unit surface area by experiment. Pink bars represent normoxic chambers and red bars represent hypoxic chambers. A) NO_3 production rates of Square Handkerchief biofilm chambers, B) NO_3 production rates of USM biofilm chambers, C) NO_3 production rates of Katrina biofilm chambers, D) NO_3 production rates of Legacy biofilm chambers.

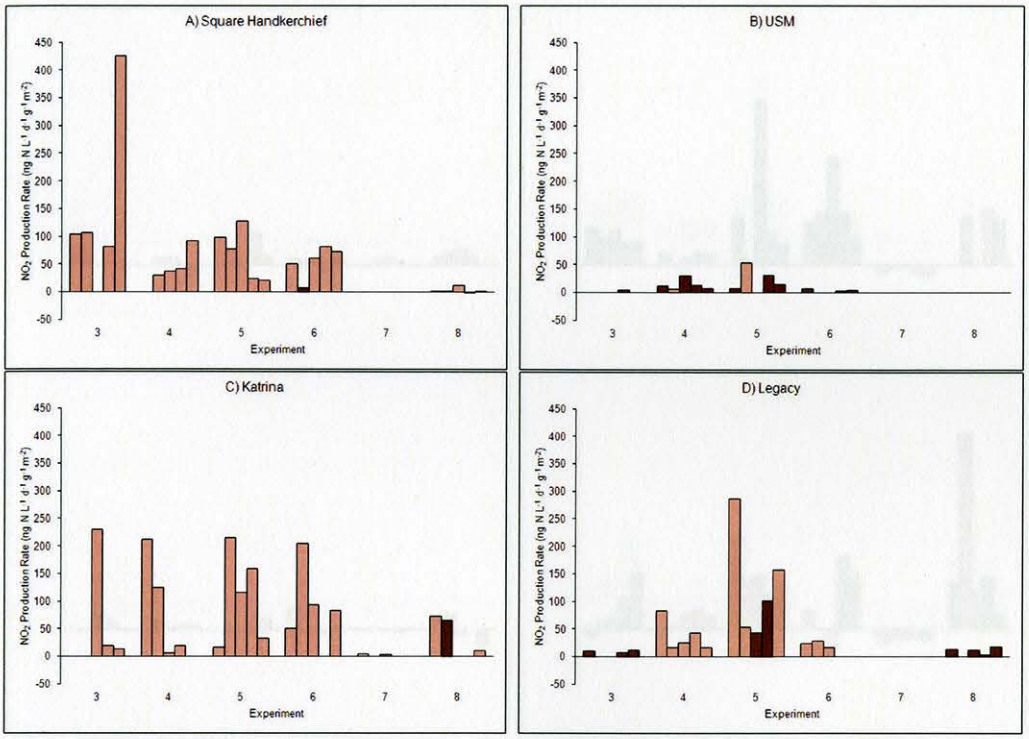

Figure 35. Summarized NO_2 production rates of individual control-corrected biofilm chambers normalized to biofilm mass per unit surface area by experiment. Pink bars represent normoxic chambers and red bars represent hypoxic chambers. A) NO_2 production rates of Square Handkerchief biofilm chambers, B) NO_2 production rates of USM biofilm chambers, C) NO_2 production rates of Katrina biofilm chambers, D) NO_2 production rates of Legacy biofilm chambers.

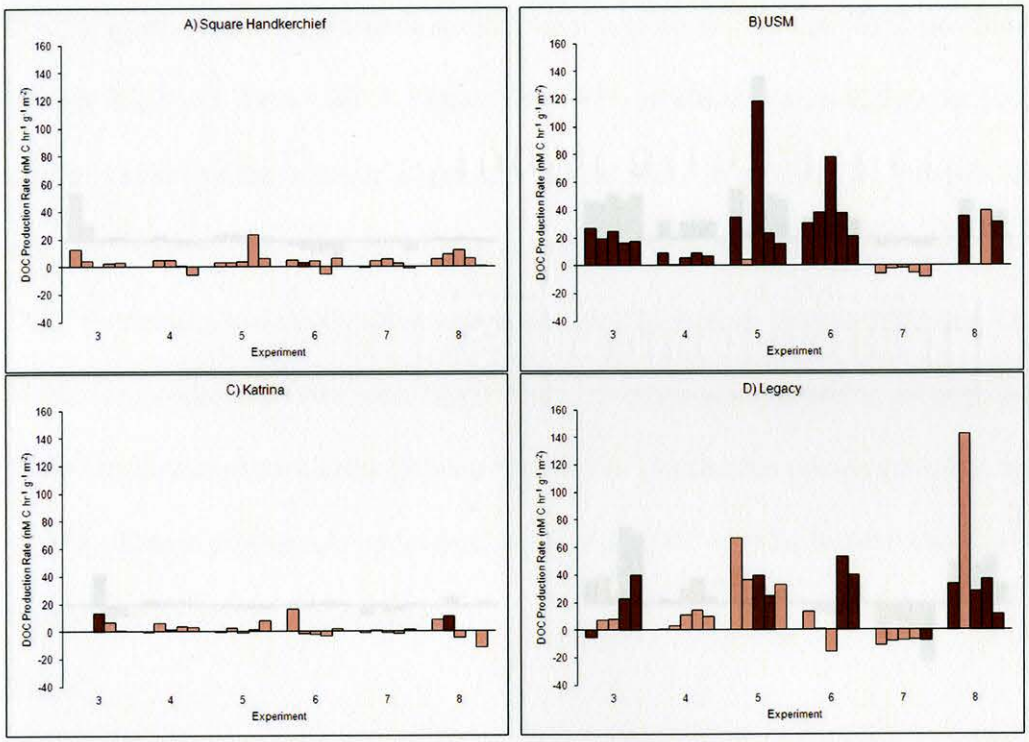

Figure 36. Summarized DOC production rates of individual control-corrected biofilm chambers normalized to biofilm mass per unit surface area by experiment. Pink bars represent normoxic chambers and red bars represent hypoxic chambers. A) DOC production rates of Square Handkerchief biofilm chambers, B) DOC production rates of USM biofilm chambers, C) DOC production rates of Katrina biofilm chambers, D) DOC production rates of Legacy biofilm chambers.

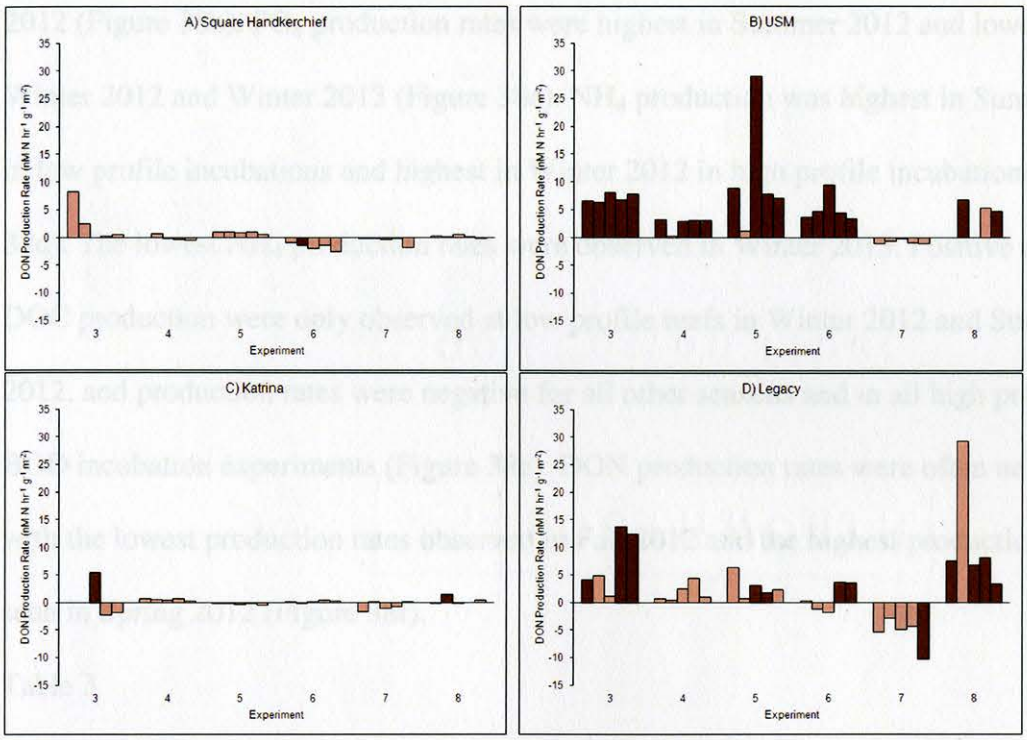

Figure 37. Summarized DON production rates of individual control-corrected biofilm chambers normalized to biofilm mass per unit surface area by experiment. Pink bars represent normoxic chambers and red bars represent hypoxic chambers. A) DON production rates of Square Handkerchief biofilm chambers, B) DON production rates of USM biofilm chambers, C) DON production rates of Katrina biofilm chambers, D) DON production rates of Legacy biofilm chambers.

Statistical Analysis

BOD incubation experiments. Significant results for BOD incubations were only observed in ANOVAs by season and profile, so these are the only results discussed (Table 3). NO_3 and NO_2 rates of change were not analyzed due to the results being largely undetectable. Significant seasonal effects were observed in respiration, BA, PO_4, and DON responses. Significant profile effects were only observed in BA. No significant interactions were present in any of the ANOVA analyses. Respiration rates were highest in Summer 2012 for low and high profile reefs, and the lowest rates were observed in Winter 2013 (Figure 38a). Bacterial abundance declined at higher rates in low profile incubations, and the lowest rates of decline were observed in Winter 2012 and Spring

2012 (Figure 38b). PO_4 production rates were highest in Summer 2012 and lowest in Winter 2012 and Winter 2013 (Figure 38c). NH_4 production was highest in Summer 2012 in low profile incubations and highest in Winter 2012 in high profile incubations (Figure 38d). The lowest NH_4 production rates were observed in Winter 2013. Positive rates of DOC production were only observed at low profile reefs in Winter 2012 and Summer 2012, and production rates were negative for all other seasons and in all high profile BOD incubation experiments (Figure 38e). DON production rates were often negative, with the lowest production rates observed in Fall 2012 and the highest production rates seen in Spring 2012 (Figure 38f).

Table 3

ANOVA Results for BOD Incubations, Winter 2012 – Experiment 1 through Winter 2013 – Experiment 8.

Response	Factor	Type III Sum of Square	df	F value	p value
Respiration	Season	1.277	4	2.881	0.047
	Profile	0.058	1	0.522	0.477
	Interaction	0.130	4	0.293	0.879
BA	Season	7.97E-05	4	4.182	0.011
	Profile	3.83E-05	1	8.032	0.010
	Interaction	6.10E-06	4	0.320	0.861
PO_4	Season	185.691	4	4.767	0.006
	Profile	0.703	1	0.072	0.791
	Interaction	14.042	4	0.361	0.834
NH_4	Season	0.016	4	2.816	0.051
	Profile	0.000	1	0.176	0.679
	Interaction	0.003	4	0.490	0.743
DOC	Season	0.359	4	0.268	0.896
	Profile	0.012	1	0.036	0.852
	Interaction	1.140	4	0.850	0.509
DON	Season	0.007	4	3.747	0.018
	Profile	0.000	1	0.103	0.751
	Interaction	0.003	4	0.739	0.576

Note. $\alpha = 0.05$.

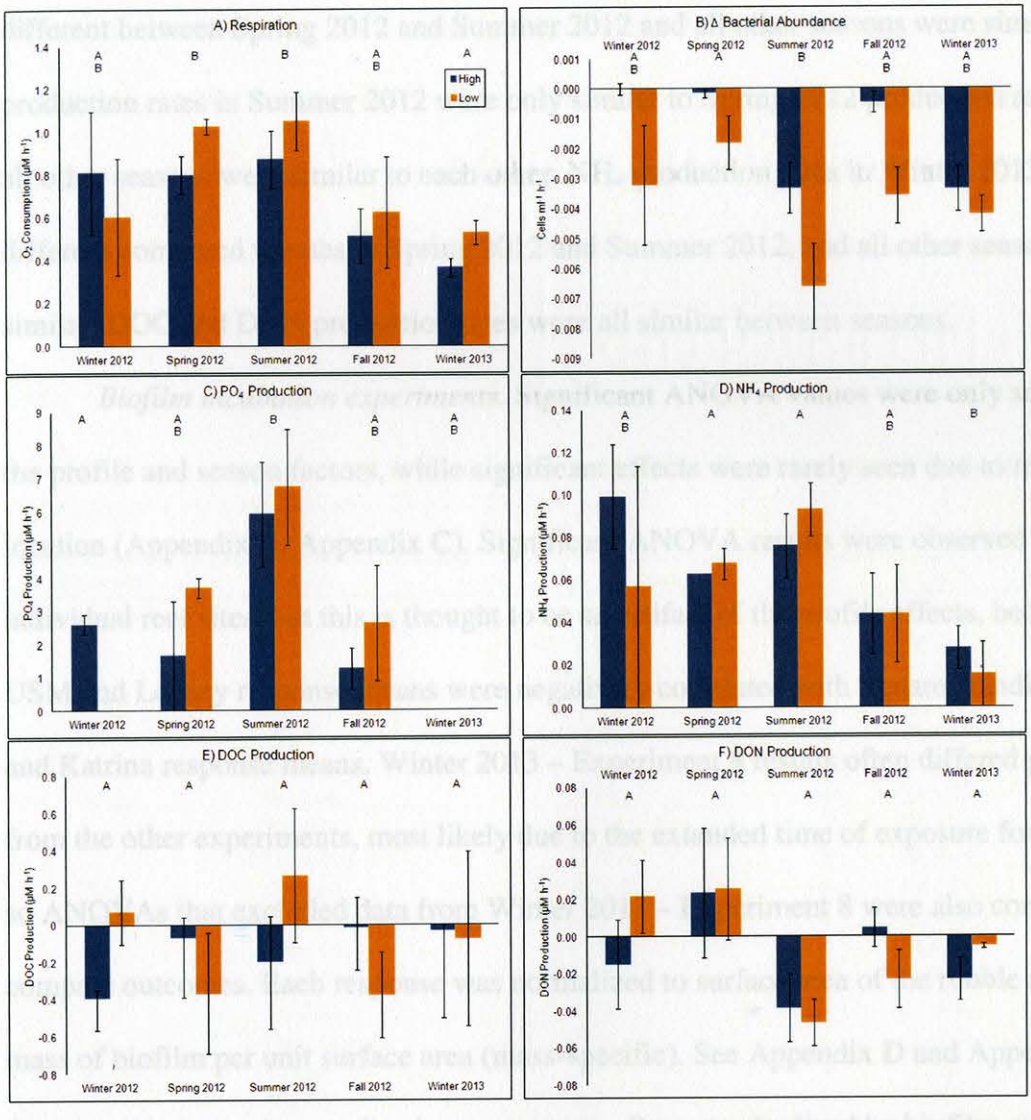

Figure 38. Estimated marginal means of BOD incubations in high profile reef chambers (blue) and low profile reef chambers (orange) for each season. Letter groupings indicate overall means (high and low profile combined) of seasons that are similar to each other. Error bars represent ± SEM. A) Respiration rates versus season, B) change in bacterial abundance versus season, C) PO_4 production rates versus season, D) NH_4 production rates versus season, E) DOC production rates versus season, F) DON production rates versus season.

Games-Howell post-hoc test results compared differences in season between total means (high and low profile reef responses combined), and showed that total respiration rates in Winter 2013 were different from respiration rates in Spring 2012 and Summer 2012, and all other seasons were similar to each other. Bacterial production rates were

different between Spring 2012 and Summer 2012 and all other seasons were similar. PO_4 production rates in Summer 2012 were only similar to Spring 2012 production rates and all other seasons were similar to each other. NH_4 production rates in Winter 2013 were different compared to rates in Spring 2012 and Summer 2012, and all other seasons were similar. DOC and DON production rates were all similar between seasons.

Biofilm incubation experiments. Significant ANOVA values were only seen for the profile and season factors, while significant effects were rarely seen due to reef location (Appendix B; Appendix C). Significant ANOVA results were observed between individual reef sites, but this is thought to be an artifact of the profile effects, because the USM and Legacy response means were negatively correlated with Square Handkerchief and Katrina response means. Winter 2013 – Experiment 8 results often differed greatly from the other experiments, most likely due to the extended time of exposure for rubble, so ANOVAs that excluded data from Winter 2013 – Experiment 8 were also conducted to compare outcomes. Each response was normalized to surface area of the rubble and to mass of biofilm per unit surface area (mass-specific). See Appendix D and Appendix E for control-corrected normalized response rates. Data standardized by biofilm mass were unavailable for Experiments 1 and 2, so these experiments have been excluded from ANOVAs of mass-specific responses. Responses were almost always significantly different by season and profile type, whether normalized to surface area or mass of biofilm per unit surface area (Table 4; Table 5).

In some cases, the Kruskal-Wallis test did not support the ANOVA results, which is most likely because the data are not normally distributed and had unequal variances, and the significance of these ANOVA results should therefore be treated with caution.

Several of the ANOVA results also had significant interactions between season and profile, even when data from Experiment 8 were excluded from the analysis. However, these interactions reflect a difference in trends by profile type during different seasons, and the profile type trends do no interact with each other, so no further analysis was conducted on these results.

Table 4

ANOVA Results for Biofilm Incubations Normalized to Surface Area, Winter 2012 – Experiment 1 through Winter 2013 – Experiment 8.

Response	Factor	Type III Sum of Squares	df	F value	p value
Respiration	Season	3849721	4	20.789	0.000
	Profile	3727328	1	80.511	0.000
	Interaction	2829308	4	15.278	0.000
BA	Season	94	4	3.624	0.008
	Profile	155	1	23.813	0.000
	Interaction	424	4	16.303	0.000
PO_4	Season	3598	4	21.149	0.000
	Profile	3255	1	76.521	0.000
	Interaction	3525	4	20.720	0.000
NH_4	Season	590519	4	19.241	0.000
	Profile	520679	1	67.860	0.000
	Interaction	511983	4	16.682	0.000
NO_3*	Season**	135	4	3.160	0.016
	Profile	186	1	17.386	0.000
	Interaction	136	4	3.162	0.016
NO_2*	Season	37	4	3.238	0.014
	Profile	22	1	7.577	0.007
	Interaction	33	4	2.883	0.025
DOC	Season	7550706	4	10.542	0.000
	Profile	5887453	1	32.880	0.000
	Interaction	6962767	4	9.721	0.000
DON	Season	386884	4	13.201	0.000
	Profile	259907	1	35.474	0.000
	Interaction	342111	4	11.673	0.000

Note. $\alpha = 0.05$. * denotes a significant interaction between profile and season, even when Experiment 8 is excluded from the ANOVA analysis, and ** denotes a significant result that is not supported by the Kruskal-Wallis test. Main effects were always significant even when Experiment 8 was excluded from the ANOVA analysis.

Table 5

ANOVA Results for Chamber Incubations Normalized to Mass of Biofilm Per Unit Surface Area, Summer 2012 – Experiment 3 through Winter 2013 – Experiment 8.

Response	Factor	Type III Sum of Squares	df	F value	p value
Respiration	Season**	0.001	2	3.081	0.050
	Profile	0.006	1	27.459	0.000
	Interaction	0.001	2	2.501	0.087
BA	Season	1.39E-06	2	7.641	0.001
	Profile	1.80E-06	1	19.734	0.000
	Interaction	2.09E-06	2	11.450	0.000
PO_4	Season	1.19E-06	2	7.060	0.001
	Profile	4.44E-06	1	52.655	0.000
	Interaction	1.12E-06	2	6.648	0.002
NH_4	Season	0.000	2	5.080	0.008
	Profile	0.001	1	49.085	0.000
	Interaction	0.000	2	3.568	0.032
NO_3	Season	7.40E-08	2	0.543	0.583
	Profile	5.88E-07	1	8.619	0.004
	Interaction	7.42E-08	2	0.544	0.582
NO_2	Season	5.47E-07	2	8.277	0.000
	Profile	2.22E-07	1	6.701	0.011
	Interaction	7.30E-08	2	1.104	0.335
DOC	Season	0.005	2	6.395	0.002
	Profile	0.010	1	27.936	0.000
	Interaction	0.003	2	4.777	0.010
DON*	Season	0.000	2	12.806	0.000
	Profile	0.000	1	25.206	0.000
	Interaction	0.000	2	7.068	0.001

Note. $\alpha = 0.05$. * denotes a significant interaction between profile and season, even when Experiment 8 is excluded from the ANOVA analysis, and ** denotes a significant result that is not supported by the Kruskal-Wallis test. Main effects were always significant even when Experiment 8 was excluded from the ANOVA analysis.

Respiration rates normalized to surface area were highest in Winter 2013 and lowest in Winter 2012 for low profile chambers, and high profile chambers also had the lowest respiration rates in Winter 2012 but the highest rates were observed in Spring 2012 (Figure 39a). Mass-specific respiration rates were highest in Summer 2012 and Winter 2013 for low profile chambers, and lowest in Fall 2012, while high profile chambers had similar mass-specific rates between Summer 2012 and Fall 2012 and higher rates in Winter 2012 (Figure 40a). BA normalized to surface area were highest in Winter 2013 for low profile chambers and in Winter 2012 for high profile chambers, but

when Winter 2013 data were removed from the ANOVA analysis profile effects were no longer significant (Figure 39b). Low profile chambers always had higher BA than high profile chambers when normalized to surface area. Mass-specific BA was highest during Fall 2012 for high profile reefs and during Winter 2013 for low profile reefs, and were always higher in low profile chambers (Figure 40b).

PO_4 production rates normalized to surface area were always higher in low relief chambers, and production rates in low relief chambers were highest in Winter 2013 and lowest in Winter 2012 (Figure 39c). High relief chambers had similar PO_4 production rates between seasons. Mass-specific PO_4 production rates were higher in low profile chambers, and the highest production rates in low profile chambers were observed in Winter 2013 and Summer 2012, while high profile chambers had higher production rates in Summer 2012 (Figure 40c). NH_4 production rates normalized to surface area exhibited similar patterns in low and high profile reefs as those seen in PO_4 rates normalized to surface area (Figure 39d). Mass-specific NH_4 production rates also had similar trends as mass-specific PO_4 rates, although high profile chambers had the highest NH_4 production rates in Winter 2013 (Figure 40d). NO_3 production was typically observed in high profile chambers only, and rates normalized to surface area were highest in Spring 2012 and lowest in Winter 2012 for high profile chambers (Figure 39e). Mass-specific NO_3 production rates were highest in Summer 2012 and lowest in Winter 2013 in high profile chambers, while rates in low profile chambers were relatively similar (Figure 40e). NO_2 production rates normalized to surface area were usually higher in high profile chambers, with the highest rates in high profile chambers observed in Spring 2012 and the highest rates in low profile chambers observed in Winter 2012 (Figure 39f). Mass-specific NO_2

production rates were always higher in high profile reefs, and profile types had similar patterns across seasons, with the highest rates for both observed in Summer 2012 (Figure 40f).

DOC production rates normalized to surface area were always higher in low relief chambers and the highest rates in low relief chambers were observed in Winter 2013 (Figure 39g). Most DOC production rates were positive, but Winter 2012 high profile chambers declined in DOC concentration over time. Mass-specific DOC production rates were also higher in low profile chambers, and the highest rates in low profile chambers were observed in Winter 2013, while the lowest rates for low and high profile chambers were seen in Fall 2012 (Figure 40g). DON production rates normalized to surface area were always higher in low relief chambers, and the highest DON production was observed in Winter 2013 and the lowest rates were seen in Winter 2012 in low relief chambers (Figure 39h). Mass-specific rates of DON production were also highest in low profile chambers, and the highest rates in low profile chambers were seen in Winter 2013 while the lowest rates for both profile types were seen in Fall 2012 (Figure 40h).

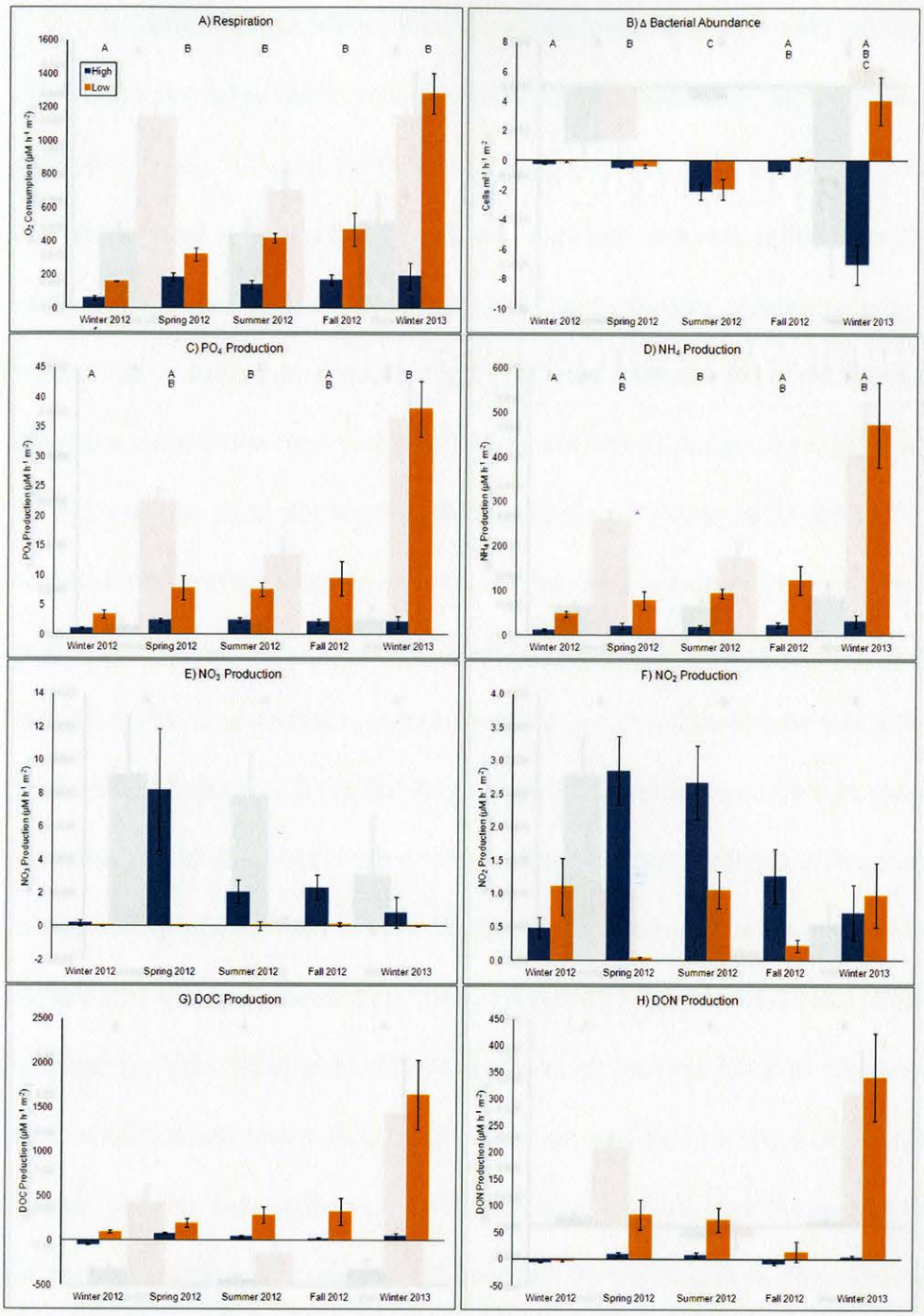

Figure 39. Estimated marginal means normalized to surface area in high profile reef chambers (blue) and low profile reef chambers (orange) for each season. Letter groupings indicate overall means (high and low profile combined) of seasons that are similar to each other. Error bars represent ± SEM. A) Respiration rates versus season, B) change in bacterial abundance versus season, C) PO_4 production rates versus season, D) NH_4 production rates versus season, E) NO_3 production rates versus season, F) NO_2 production rates versus season, G) DOC production rates versus season, H) DON production rates versus season.

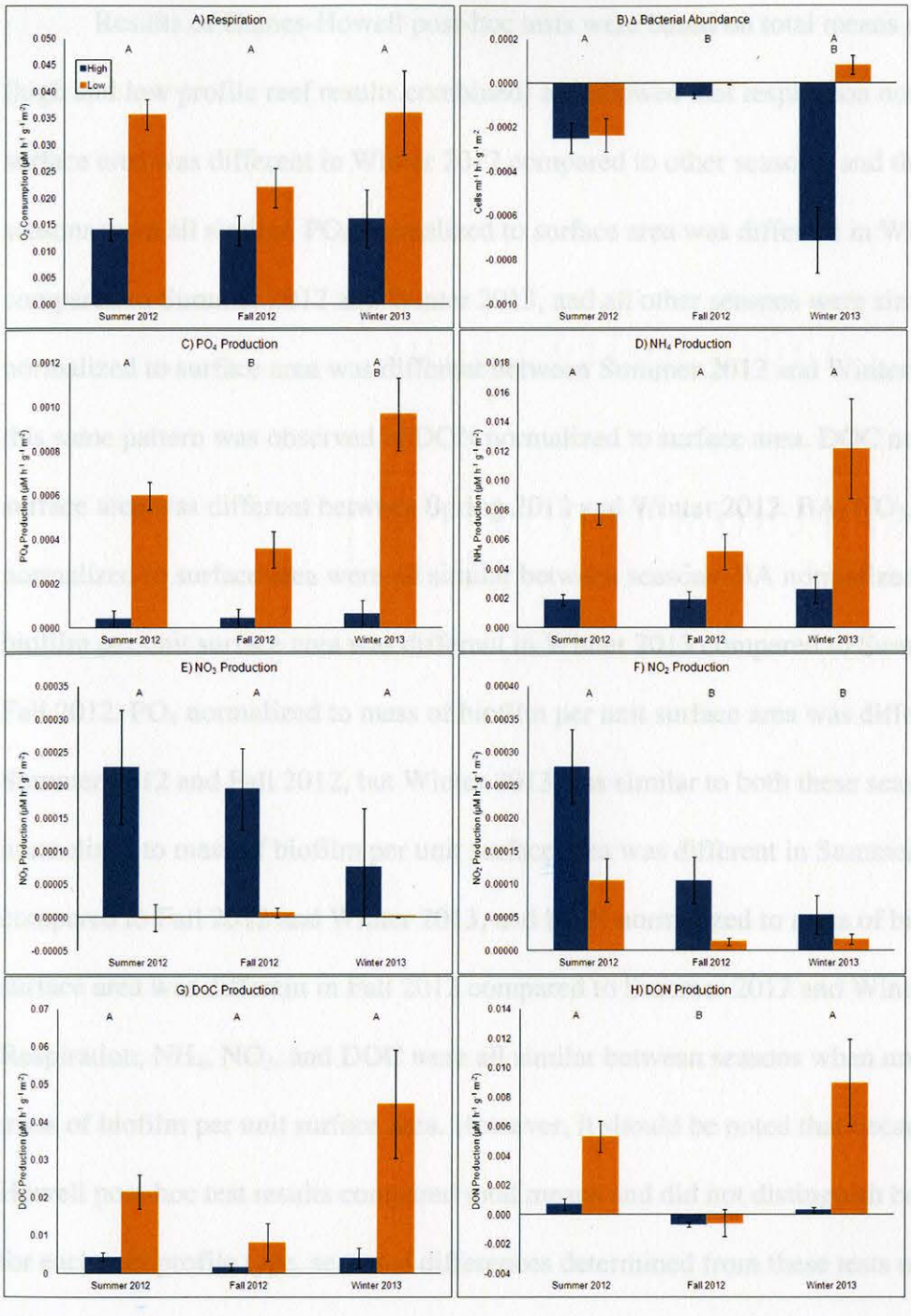

Figure 40. Estimated marginal means normalized to mass of biofilm per unit surface area in high profile reef chambers (blue) and low profile reef chambers (orange) for each season. Letter groupings indicate overall means (high and low profile combined) of seasons that are similar to each other. Error bars represent ± SEM. A) Respiration rates versus season, B) change in bacterial abundance versus season, C) PO_4 production rates versus season, D) NH_4 production rates versus season, E) NO_3 production rates versus season, F) NO_2 production rates versus season, G) DOC production rates versus season, H) DON production rates versus season.

Results of Games-Howell post-hoc tests were based on total means of responses (high and low profile reef results combined) and showed that respiration normalized to surface area was different in Winter 2012 compared to other seasons, and the remaining seasons were all similar. PO_4 normalized to surface area was different in Winter 2012 compared to Summer 2012 and Winter 2013, and all other seasons were similar. NH_4 normalized to surface area was different between Summer 2012 and Winter 2012, and this same pattern was observed in DON normalized to surface area. DOC normalized to surface area was different between Spring 2012 and Winter 2012. BA, NO_3, and NO_2 normalized to surface area were all similar between seasons. BA normalized to mass of biofilm per unit surface area was different in Winter 2013 compared to Summer 2012 and Fall 2012. PO_4 normalized to mass of biofilm per unit surface area was different between Summer 2012 and Fall 2012, but Winter 2013 was similar to both these seasons. NO_2 normalized to mass of biofilm per unit surface area was different in Summer 2012 compared to Fall 2012 and Winter 2013, and DON normalized to mass of biofilm per unit surface area was different in Fall 2012 compared to Summer 2012 and Winter 2013. Respiration, NH_4, NO_3, and DOC were all similar between seasons when normalized to mass of biofilm per unit surface area. However, it should be noted that because Games-Howell post-hoc test results compared total means and did not distinguish between means for each reef profile type, seasonal differences determined from these tests may not reflect seasonal differences between each reef profile type.

Transects

Nutrient concentrations and bacterial abundance were expected to be similar along transects at low profile reefs due to high rates of water column mixing while high

profile reefs were expected to have higher nutrient and bacterial abundances that would decline moving away from the reef due to the presence of excessive amount of guano and reduced water column mixing.

Transect data for Winter 2012 – Experiment 1 were only collected at the time of substrate cage retrieval, and transect data for Spring 2012 – Experiment 2 were collected on a day between substrate cage deployment and retrieval, and were also collected at the time of substrate cage retrieval. Transect data for all other experiments (Summer 2012 through Winter 2013) were collected at the time of substrate cage deployment and retrieval. PO_4 concentrations at transects were often above the level of detection but were usually relatively low (< 2 μM), and NH_4, NO_3, and NO_2 concentrations were typically undetectable, with detectable concentrations rarely exceeding 2 μM (Table 6). See Appendix F for specific nutrient concentrations along transects by sampling date and individual reef. BA ranged from 1.19 ± 0.02 to 3.57 ± 0.26 E+06 cells ml^{-1} at transect sites, and abundances were similar between reefs and along transects within each collection date. DOC concentrations ranged from 165 ± 26 to 1570 ± 28 μM, but concentrations > 1000 μM are likely due to an error in analysis, and the actual high concentration is probably 607 ± 0 μM, which is the highest observed DOC concentration when the excessively high concentrations are excluded. DON concentrations ranged from 7.13 ± 1.43 to 21.17 ± 0.22 μM, and both DOC and DON concentrations were similar between reefs and along transects within each collection date. DOC:DON ratios ranged from 10.29 ± 0.06 to 137.64 ± 0.18, but the highest ratios are a reflection of the excessively high DOC concentrations observed, and the highest ratio value is likely 34.73

± 0.27. See Appendix G for specific BA, DOC, and DON concentrations and DOC:DON ratios for transect results by sampling date and individual reef.

Table 6

Transect Nutrient Data.

Collection Date	PO_4	NH_4	NO_3	NO_2
2/28/2012	s	s	s	su
3/9/2012	-	k	K	lk
4/27/2012	sulk	s	-	-
5/8/2012	s	-	U	-
5/14/2012	-	-	-	-
5/30/2012	s	-	-	-
6/2/2012	ulk	-	-	-
6/21/2012	su	s	-	-
6/26/2012	-	-	-	k
7/11/2012	su	SL	sk	sl
7/24/2012	su	S	s	-
7/30/2012	lk	k	-	-
8/7/2012	sulk	S	-	s
8/21/2012	su	s	-	su
9/11/2012	K	k	-	k
9/27/2012	slk	-	s	-
10/17/2012	su	-	-	-
10/23/2012	lk	k	-	lk
11/5/2012	sulk	-	-	-
11/15/2012	su	u	-	-
12/2/2012	lk	-	-	-
2/1/2013	s	sk	S	sk
3/14/2013	-	-	s	-
4/2/2013	-	k	-	-

Note. Letters indicate which reef had detectable nutrient concentrations. S = Square Handkerchief, U = USM, L = Legacy, and K = Katrina. Lower case letters indicate concentrations < 2 μM, upper case letters indicate concentrations > 2 μM, and dashes indicate no detectable concentrations. No concentrations greater than 4 μM were measured on any reef transect.

DOC:DON ratios for transect samples were plotted over time with daily precipitation data to examine the effects of rainfall on DOC:DON (Figure 41). Excessively high DOC:DON values (> 70) were excluded from the comparison due to the likely inaccurate results they represent, as discussed previously. Higher DOC:DON ratios were often positively correlated with daily precipitation amount. Rainfall data were obtained from the Weather Underground weather service (www.wunderground.com).

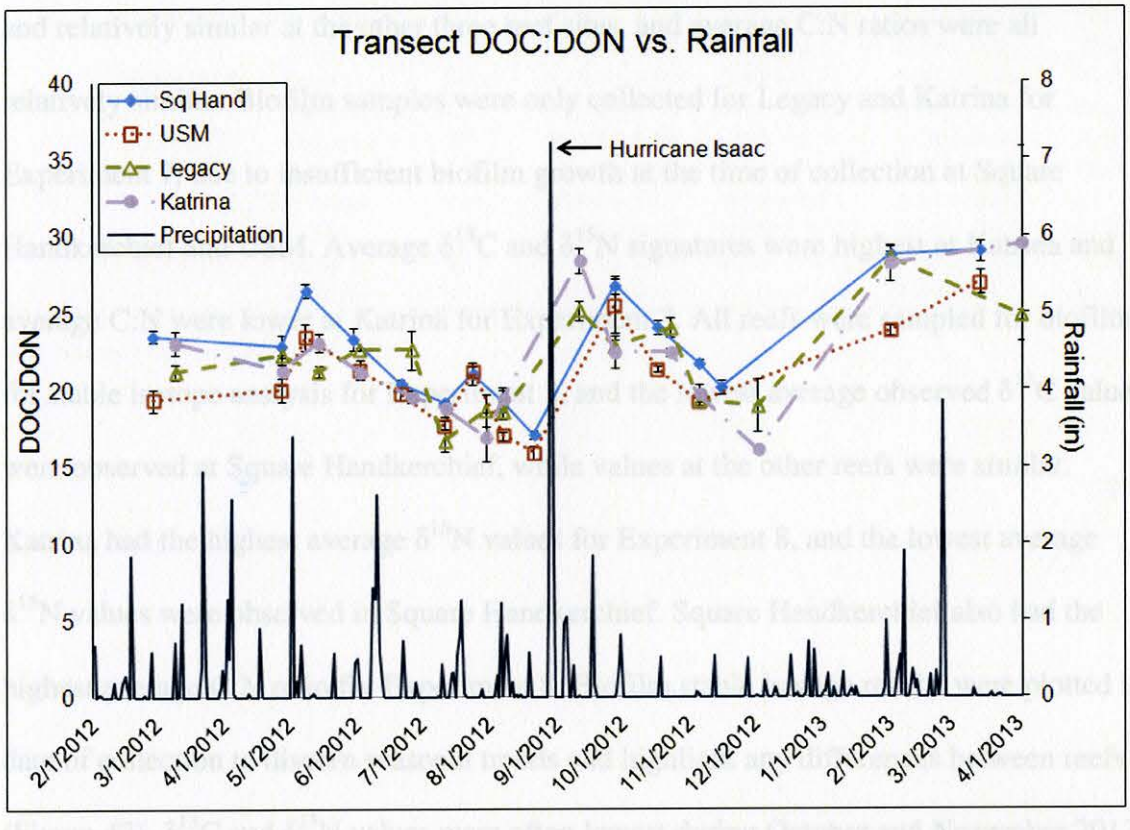

Figure 41. DOC:DON ratios for Square Handkerchief (blue), USM (red), Legacy (green), and Katrina (purple) transect samples over time compared to daily rainfall amount in inches (dark blue). Error bars represent ± SEM, arrow indicates rainfall from Hurricane Isaac.

Biofilm Stable Isotope Analysis

Biofilm samples for stable isotope analysis were only collected for Legacy and Katrina reefs for Experiment 4. Values for average $\delta^{13}C$ and average $\delta^{15}N$ were similar at both reefs for Experiment 4 samples, and average C:N ratios were also similar (Table 7).

Biofilm samples for stable isotope analysis were collected from all reefs for Experiment 5. The lowest average C:N ratios were observed at USM for Experiment 5, and USM had the highest average values for $\delta^{13}C$ and $\delta^{15}N$, while the other three reef sites had similar values. All reefs were sampled for stable isotope analysis of biofilm for Experiment 6, and average $\delta^{13}C$ values were lowest at Square Handkerchief and increased steadily going east in location. Average values for $\delta^{15}N$ in Experiment 6 were highest at USM, and relatively similar at the other three reef sites, and average C:N ratios were all relatively similar. Biofilm samples were only collected for Legacy and Katrina for Experiment 7, due to insufficient biofilm growth at the time of collection at Square Handkerchief and USM. Average $\delta^{13}C$ and $\delta^{15}N$ signatures were highest at Katrina and average C:N were lower at Katrina for Experiment 7. All reefs were sampled for biofilm for stable isotope analysis for Experiment 8, and the lowest average observed $\delta^{13}C$ values were observed at Square Handkerchief, while values at the other reefs were similar. Katrina had the highest average $\delta^{15}N$ values for Experiment 8, and the lowest average $\delta^{15}N$ values were observed in Square Handkerchief. Square Handkerchief also had the highest average C:N ratio for Experiment 8. Biofilm stable isotope results were plotted by date of collection to discern seasonal trends and highlight any differences between reefs (Figure 42). $\delta^{13}C$ and $\delta^{15}N$ values were often lowest during October and November 2012, and highest in July and August 2012 and March and April 2013, while C:N ratios typically showed an inverse trend to those seen in $\delta^{13}C$ and $\delta^{15}N$ values.

Table 7

Stable Isotope Analysis Results for Biofilm Samples, Summer 2012 – Experiment 4 through Winter 2013 – Experiment 8.

Expt.	Date	Reef	Exposure days	Average $\delta^{13}C$ ‰	Average $\delta^{15}N$ ‰	Average C:N	Salinity ppt
4	7/30/2012	Legacy	19	-21.25 ± 0.08	10.51 ± 0.29	5.16 ± 0.23	24.1
		Katrina		-21.48 ± 0.05	9.97 ± 0.24	5.45 ± 0.39	24.4
5	8/21/2012	Sq. Hand.	14	-23.28 ± 5.11	9.39 ± 0.49	5.14 ± 0.07	NA
		USM		-20.75 ± 5.06	13.14 ± 0.09	4.83 ± 0.02	NA
	9/11/2012	Legacy	35	-23.71 ± 4.24	9.72 ± 0.47	5.66 ± 0.06	NA
		Katrina		-23.69 ± 7.52	8.70 ± 0.39	5.57 ± 0.07	NA
6	10/17/2012	Sq. Hand.	20	-25.91 ± 0.04	8.72 ± 0.50	5.40 ± 0.07	NA
		USM		-24.10 ± 0.07	9.74 ± 0.22	5.19 ± 0.03	NA
	10/23/2012	Legacy	26	-23.53 ± 0.09	8.97 ± 0.21	5.93 ± 0.06	21.4
		Katrina		-22.95 ± 0.08	8.57 ± 0.23	5.39 ± 0.06	24.2
7	12/2/2012	Legacy	27	-23.85 ± 0.05	7.93 ± 0.39	6.74 ± 0.04	28.5
		Katrina		-22.48 ± 0.10	8.24 ± 0.51	6.42 ± 0.05	28.0
8	3/14/2013	Sq. Hand.	41	-23.04 ± 0.07	8.94 ± 0.35	5.34 ± 0.05	11.1
		USM		-21.70 ± 0.07	10.00 ± 0.77	4.91 ± 0.04	19.7
	4/2/2013	Legacy	60	-21.10 ± 0.08	10.08 ± 0.11	4.95 ± 0.06	21.6
		Katrina		-21.16 ± 0.07	11.18 ± 0.09	4.80 ± 0.02	20.1

Note. Average values are reported with ± SEM.

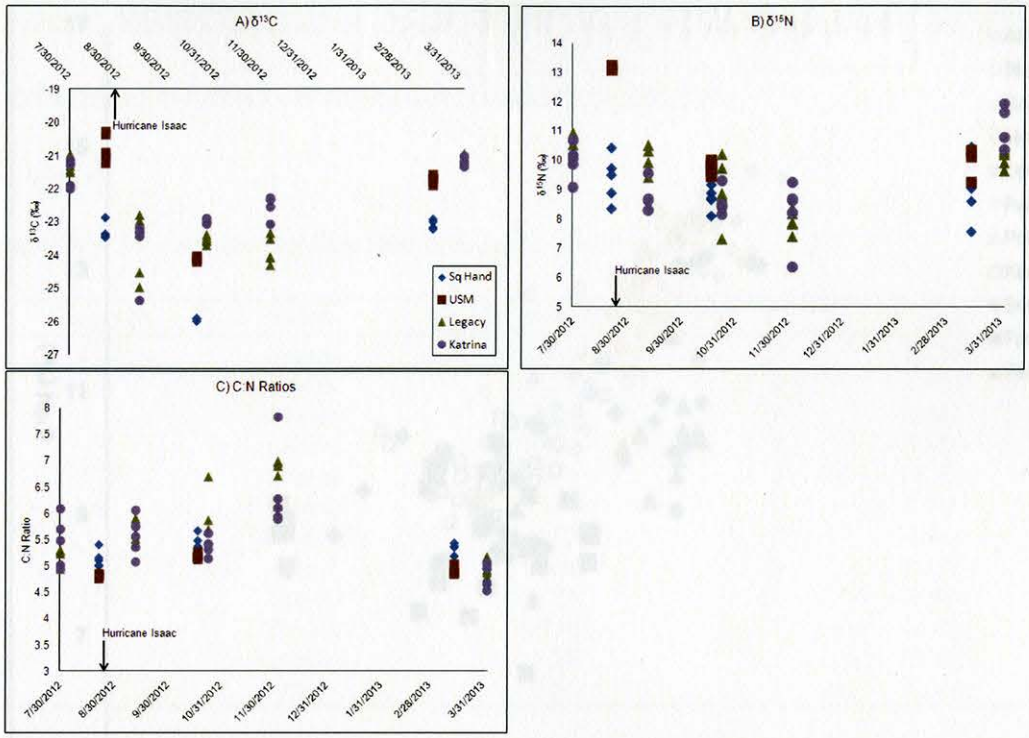

Figure 42. Biofilm stable isotope analysis results plotted over time. Arrows indicate the date at which Hurricane Isaac made landfall near the Mississippi River. A) $\delta^{13}C$ values versus date of sampling, B) $\delta^{15}N$ values versus date of sampling, C) C:N ratios versus date of sampling.

$\delta^{13}C$ and $\delta^{15}N$ values of biofilm samples were compared to values measured at an artificial oyster reef in Ocean Springs Harbor, MS (Dillon, Peterson, and Fulford unpublished data). Biofilm stable isotope signatures were typically similar to *Xanthidae* and *Alpheus* species, commonly known as mud crabs and snapping shrimp, respectively (Figure 43). However, some biofilm samples collected from Legacy and Katrina had similar isotopic signatures to *Palaemonetes* (grass shrimp), and several USM biofilm samples were similar to *Gobiidae* (gobies) and *Opsanus beta* (toadfish).

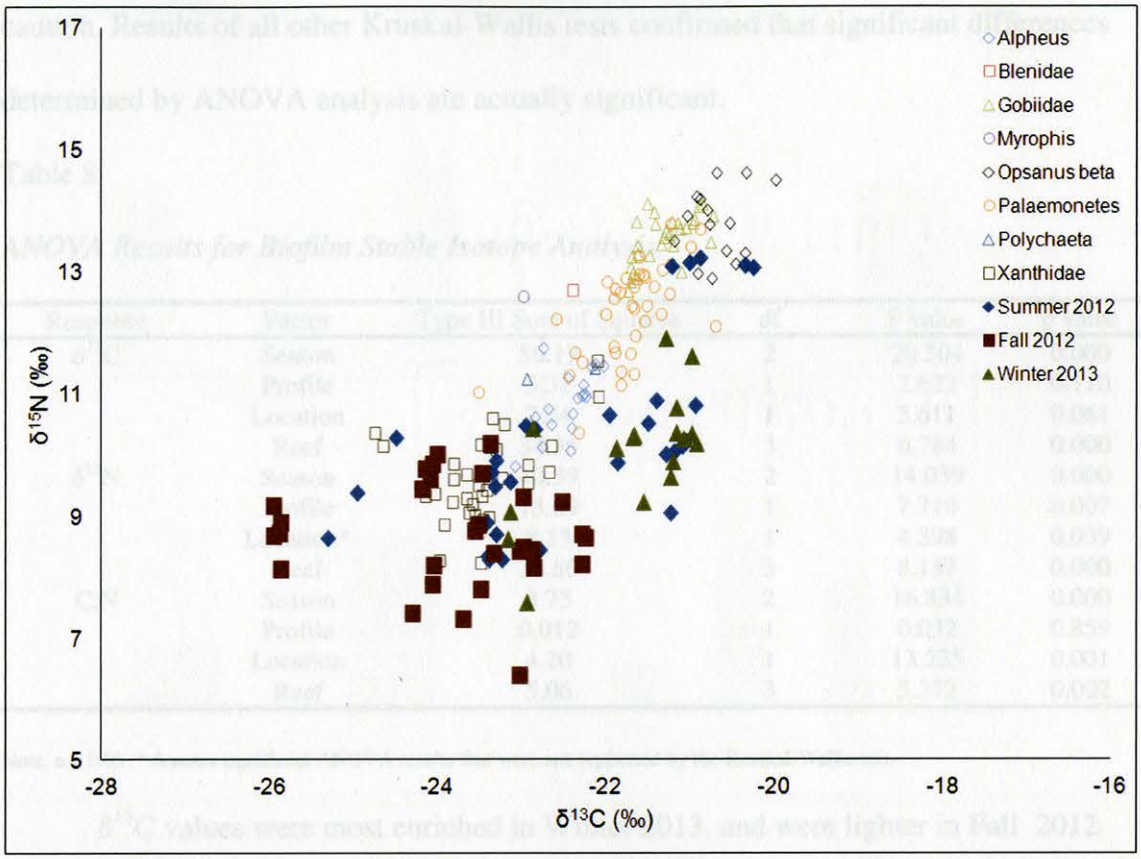

Figure 43. $\delta^{13}C$ and $\delta^{15}N$ signatures of artificial reef biofilm samples and fish and invertebrate samples from an artificial oyster reef near Ocean Springs, MS. The artificial oyster reef samples are represented by open symbols (blue diamond = *Alpheus*, red square = *Blenidae*, green triangle = *Gobiidae*, purple circle = *Myrophis*, black diamond = *Opsanus beta*, orange circle = *Palaemonetes*, blue triangle = *Polychaeta*, and brown square = *Xanthidae*) and artificial reef biofilm samples are represented by solid symbols (blue diamond = Summer 2012, red square = Fall 2012, green triangle = Winter 2013).

One-way ANOVAs were conducted on $\delta^{13}C$ values, $\delta^{15}N$ values, and C:N ratios for individual reef, profile type, location, and season as main factors. $\delta^{13}C$, $\delta^{15}N$, and C:N ratios were all significantly different by individual reef and season (Table 8). Only $\delta^{15}N$ was significantly different by profile type, and $\delta^{15}N$ and C:N ratios were significantly different by location. However, the results of the ANOVA analysis for $\delta^{15}N$ by location were not supported by the Kruskal-Wallis test, and these results should be treated with

caution. Results of all other Kruskal-Wallis tests confirmed that significant differences determined by ANOVA analysis are actually significant.

Table 8

ANOVA Results for Biofilm Stable Isotope Analysis.

Response	Factor	Type III Sum of Squares	df	F value	p value
$\delta^{13}C$	Season	56.19	2	20.504	0.000
	Profile	5.32	1	2.622	0.110
	Location	7.24	1	3.611	0.061
	Reef	34.36	3	6.784	0.000
$\delta^{15}N$	Season	40.39	2	14.039	0.000
	Profile	13.69	1	7.710	0.007
	Location*	8.13	1	4.398	0.039
	Reef	36.80	3	8.137	0.000
C:N	Season	8.75	2	16.834	0.000
	Profile	0.012	1	0.032	0.859
	Location	4.20	1	13.235	0.001
	Reef	5.06	3	5.372	0.002

Note. $\alpha = 0.05$. * denotes significant ANOVA results that were not supported by the Kruskal-Wallis test.

$\delta^{13}C$ values were most enriched in Winter 2013, and were lighter in Fall 2012 (Figure 44a), and were highest at low profile reefs (Figure 44b). Eastern reefs had higher average $\delta^{13}C$ values than western reefs (Figure 44c) and values were highest at USM and lowest at Square Handkerchief (Figure 44d). $\delta^{15}N$ values were highest in Summer 2012 and lowest in Fall 2012 (Figure 45a) and low profile reefs had higher values than high profile reefs (Figure 45b). Western reefs had higher $\delta^{15}N$ values than eastern reefs (Figure 45c), and USM had the highest values while Square Handkerchief had the lowest values (Figure 45d). C:N ratios in biofilm samples were highest in Fall 2012 and lowest in Winter 2013 (Figure 46a), and high profile reefs had higher ratios than low profile reefs (Figure 46b). Eastern reefs had higher C:N ratios than western reefs (Figure 46c), and USM had the lowest C:N ratio while Legacy had the highest C:N ratio in biofilm samples (Figure 46d).

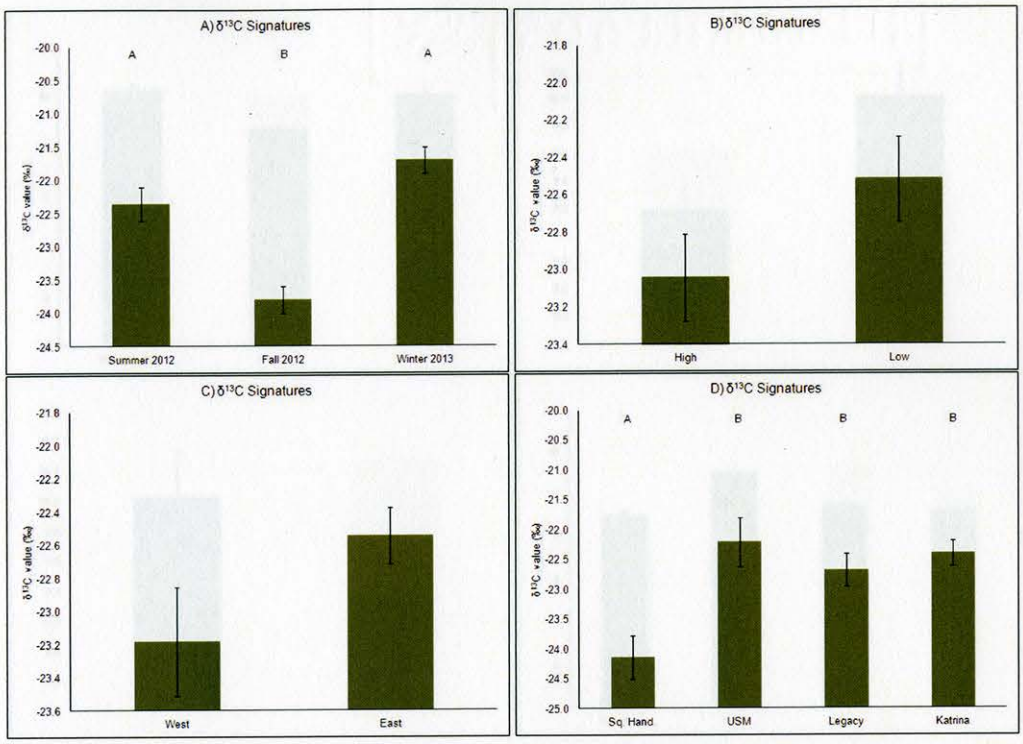

Figure 44. Estimated marginal means of $\delta^{13}C$ values in biofilm samples. Letter groupings indicate seasons and individual reefs that are similar to each other. Error bars represent ± SEM. A) $\delta^{13}C$ values versus season, B) $\delta^{13}C$ values versus reef profile type, C) $\delta^{13}C$ values versus reef location, D) $\delta^{13}C$ values versus individual reef.

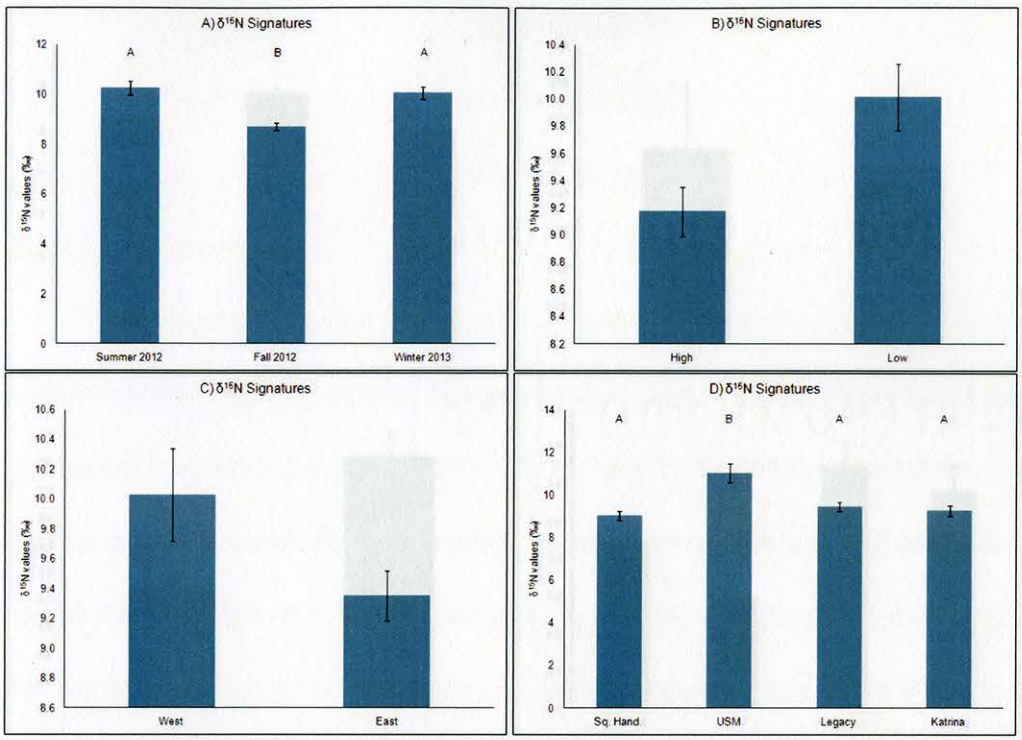

Figure 45. Estimated marginal means of $\delta^{15}N$ values in biofilm samples. Letter groupings indicate seasons and individual reefs that are similar to each other. Error bars represent ± SEM. A) $\delta^{15}N$ values versus season, B) $\delta^{15}N$ values versus reef profile type, C) $\delta^{15}N$ values versus reef location, D) $\delta^{15}N$ values versus individual reef.

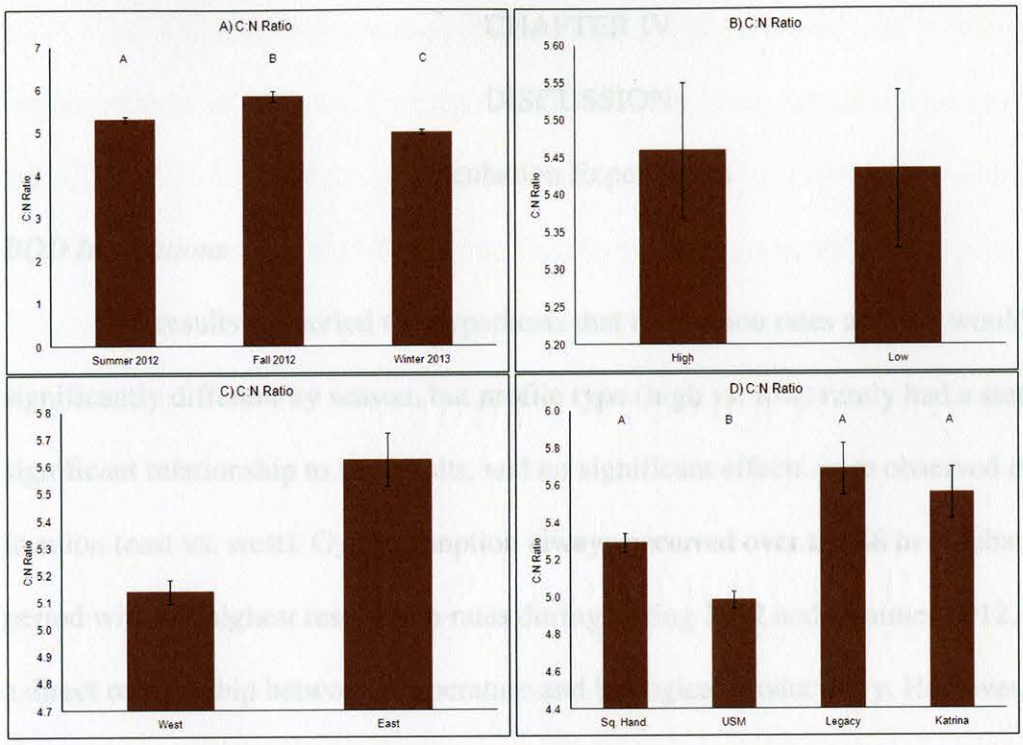

Figure 46. Estimated marginal means of C:N ratios in biofilm samples. Letter groupings indicate seasons and individual reefs that are similar to each other. Error bars represent ± SEM. A) C:N ratios versus season, B) C:N ratios versus reef profile type, C) C:N ratios versus reef location, D) C:N ratios versus individual reef.

Games-Howell test results showed that $\delta^{13}C$ and $\delta^{15}N$ values were different in Fall 2012 compared to Summer 2012 and Winter 2013, and the latter two seasons were similar to each other. $\delta^{13}C$ values were different at Square Handkerchief compared to other reefs, and the remaining three reefs were all similar to each other. $\delta^{15}N$ values were different at USM compared to other reefs, and C:N ratios were also different at USM relative to other reefs. There were no similarities between seasons for C:N ratios.

CHAPTER IV

DISCUSSION

Incubation Experiments

BOD Incubations

The results supported the hypothesis that respiration rates and BA would be significantly different by season, but profile type (high vs. low) rarely had a statistically significant relationship to the results, and no significant effects were observed due to reef location (east vs. west). O_2 consumption always occurred over the 48 hr incubation period with the highest respiration rates during Spring 2012 and Summer 2012, indicating a direct relationship between temperature and biological productivity. However, bacterial populations often declined over time, and had the highest rates of decline in Summer 2012, which was not expected. Sample water for incubations was unfiltered, so it's likely that bacteria populations declined due to grazing effects or mortality. Depending on the environment, bacterivory can affect up to 80% of the bacterial population, and different protozoans tend to graze preferentially based on cell size and type of bacteria (Sherr et al. 1989; Gonzalez et al. 1990). Grazers can also be seasonally influenced, with different protozoans dominating grazing patterns at different times of the year (Sanders et al. 1989). Given the high respiration rates observed during warmer seasons, grazer populations were likely to be more active and therefore grazed a larger percentage of the bacterial population during this time. Future research should be conducted to verify this hypothesis by setting up parallel experiments with unfiltered and 1 μm filtered water, which would eliminate grazers. Unfiltered water should be analyzed for the presence of grazers, and their population should be enumerated.

PO_4 and NH_4 concentrations increased progressively over time in most experiments in all reef sites for each experiment. This trend suggests that bacteria populations are remineralizing these nutrients in the water column and act as a source for PO_4 and NH_4. Caron et al (1988) found that the presence of bacterivorous protozoa relieved bacterial grazing pressure on phytoplankton populations and enhanced NH_4 remineralization as well. PO_4 production can also be affected by bacterivorous protozoa. Johannes (1965) found that ciliate populations were responsible for a larger fraction of PO_4 regeneration than bacteria populations. If the observed declines in bacterial abundance are due to grazing effects then it's possible the grazers are also contributing to the observed water column nutrient regeneration. PO_4 production rates were highest in Summer 2012 and lowest in Winter 2012 and Winter 2013, and NH_4 production rates were also highest in Summer 2012 but high production rates were also observed in Winter 2012. However, NH_4 production rates were lowest in Fall 2012 and Winter 2013, indicating a relationship with temperature. The high biological oxygen demand observed in the summer correlates with the high PO_4 and NH_4 production rates in the summer, further supporting the theory that the microbial community is responsible for regenerating these nutrients, and grazers may be influential in this role as well based on the high declines in bacterial abundance in the summer. NO_3 and NO_2 concentrations were often below the level of detection and when concentrations were detectable they did not show a relationship with time elapsed, indicating that these nutrients are being cycled efficiently in the water column.

Chamber Incubations

The results supported the hypothesis that respiration rates, PO_4 production rates, and NH_4 production rates would be higher in the presence of biofilm, but trends were less clear in NO_3 and NO_2 production, and bacterial abundances usually declined over time in the presence of biofilm, which was not expected. When bacterial populations did increase over time, it was most often in chambers that had become hypoxic, and there was a noticeable shift in the bacterial population from sphere-shaped cells to rod-shaped cells. This change in population structure indicates a response to the low oxygen conditions, and suggests that different bacteria strains and/or morphologies were better adapted for such conditions. However, when chambers were sampled prior to severe O_2 depletion, bacterial abundance almost always declined, and populations remained dominated by sphere-shaped cells. As discussed previously, it is possible that the presence of grazers diminished the bacterial population. Grazer populations are likely to be higher with the inclusion of biofilm in the chamber, which would explain the sharp decline often seen in bacterial abundance in biofilm-treated chambers relative to control chambers. Another explanation could be that bacteria were settling on the reef rubble in the chambers, and on the chamber surfaces as well. Bacteria counts were only obtained in the water column, so any bacteria present on the rocks are not accounted for. To test this hypothesis, further experiments should be conducted with biofilm only and no rubble, removing any settlement opportunity, to determine if bacterial populations still decline over time. Biofilm and water column samples should also be examined for grazer populations and abundance.

Respiration rates were higher in the presence of biofilm, which was expected due to the increased biomass. PO_4 and NH_4 production rates in the presence of biofilm were much higher than those observed in control chambers and in 48 hour BOD incubations. This suggests that the benthic community is supplementing bacterial remineralization of these nutrients. This is especially relevant to NH_4 production rates in low profile amended chambers, which usually had abundant barnacle growth, so NH_4 concentrations are expected to increase due to excretion. Flint and Kamykowski (1984) found that benthic fauna can be responsible for up to 50% of NH_4 remineralization and have significant contributions to sediment metabolism as well. Sediment type is influential in the role of benthic nutrient remineralization as well. A study conducted in three North Carolina estuaries found that benthic regeneration of nutrients contributed 28 to 35% of N and P needed for primary production in organic-rich depositional estuaries, while sandy sediments in a highly flushed estuary did not exhibit any nutrient contribution to the estuarine community, and the authors also found that NH_4 and PO_4 fluxes were highly correlated to each other (Fisher et al. 1982). Mississippi Sound is an organic-rich estuary, and the observed NH_4 and PO_4 production rates in the presence of biofilm support the idea that benthic interactions are a major contributor to nutrient regeneration at the artificial reef sites.

It should be noted that differences in deployment periods for different experiments likely influenced some of the results. The target deploy period was 2 weeks, but due to bad weather and boat availability issues rubble was sometimes deployed for a longer period such as Experiment 8 when rubble was left at the western and eastern reefs for 41 and 60 days, respectively. Although these samples were collected during the

Winter 2013 seasons when productivity was expected to be lower, the substrate cages were left at the sites much longer than the other experiments and there was a large amount of biofilm growth on the rubble, which likely explains why Experiment 8 results tended to be different from the rest of the experiments. However, results from Experiment 8 are still informative because they may more accurately exemplify a stable reef state, as opposed to the other experiments which were more influenced by initial recruitment and settlement at the reefs. Samples collected in the Winter 2012 and Spring 2012 seasons were also left at the sites longer than two weeks to correspond with a photosynthesis study being conducted at all sites. Most samples collected in the Summer 2012 and Fall 2012 seasons were exposed at the sites for the target length of 2 to 3 weeks. Biofilm samples collected in Experiment 7 at western reefs were collected earlier than usual (10 days), and there was little biofilm growth present at both the high and low profile western reef, most likely due to seasonal effects. Because of the low biofilm growth observed, eastern reef rubble for Experiment 7 was left in the field for a longer period (27 days) to ensure sufficient biofilm growth. During Experiment 5, the substrate cages at the eastern reefs were still deployed when Hurricane Isaac made landfall near the Mississippi River. Cages at western reefs were collected before the storm hit, but weather related boat logistics delayed transport to eastern sites and collection was delayed another 2 weeks. Therefore, the eastern reef biofilm samples were left out much longer than the western reef biofilm samples in Experiment 5 and experienced a major disturbance that may have impacted the biofilm and hence the results.

Seasonal and profile type effects were seen in almost all responses in chamber incubations. Significant seasonal effects should be considered with caution, due to the

variability in soak times that may have influenced apparent seasonal effects. However, it should be noted that most rubble deployed during Summer 2012 was exposed for a shorter period of time (roughly 2 weeks) and usually had higher biofilm mass on the rubble at the time of collection, indicating Summer 2012 likely was a productive season. The presence of biofilm has been observed to have a significant influence on the responses, so only results that have been normalized to mass of biofilm per unit surface area will be discussed here. Normalized respiration rates in low profile chambers were lowest in Fall 2012, and highest in Summer 2012 and Winter 2013. However, as discussed earlier, the Winter 2013 results are most likely skewed because of how long they were exposed at the reef sites. Respiration rates in high profile chambers were relatively similar in spite of season, although rates were slightly higher in Winter 2013, and respiration rates in low profile chambers were up to 52 times higher than rates in high profile chambers. Bacterial abundances were lowest in Winter 2013 for high profile chambers which is negatively correlated to the respiration rates observed, further supporting the theory that grazers in the water column and biofilm community are responsible for depleting the bacterial population. Low profile chambers had the highest abundances in Winter 2013 with mostly positive growth rates, and abundances were also relatively high in Fall 2012, but this reflects the hypoxic conditions that resulted in a shift in the bacterial community to rod shaped cells. Rates of change in bacterial abundance were similar between high and low profile chambers in Summer 2012 and Fall 2012, supporting the idea that seasonal effects are influencing the water column community. Normalized PO_4 and NH_4 production patterns were similar and were positively correlated with respiration rates. Low profile chambers had the lowest production rates for these

nutrients in Fall 2012, and the highest rates were observed in Winter 2013, but high rates were also seen in Summer 2012. If Experiment 8 is excluded due to the length of exposure for rubble, then the results suggest that higher productivity and nutrient regeneration is occurring in warmer seasons. Low profile reefs had PO_4 and NH_4 release rates up to 65 and 42 times higher than high profile reefs, respectively, which reflects the effects of hypoxic conditions caused by higher respiration rates. The difference in high and low profile reefs is also reflected in NO_3 and NO_2 production rates. High profile reefs had NO_3 and NO_2 production rates up to 37 and 80 times higher than low profile reefs, respectively, although seasonal effects were similar in both profile types. Summer 2012 production rates were usually higher than Fall 2012 rates, and Fall 2012 rates were higher than Winter 2013 rates, suggesting a positive relationship with temperature. High profile reef biofilms were not dominated by benthic invertebrates, so these samples may be more representative of the benthic microbial community's biological activity.

Low profile reefs had much higher abundances of benthic invertebrates, mainly barnacles, than high profile reefs. Barnacle recruitment is often a passive process that is determined by advective water transport, and there is a strong correlation between barnacle recruitment and larval concentrations in nearby waters (Gaines and Bertness 1992; Gaines et al. 1985). However, biotic factors influence benthic invertebrate recruitment as well. A study by Leonard et al. (1999) found that barnacle populations at low advection sites had lower recruitment rates, elevated mortality from abiotic stress, and increased predation by crabs. Low profile reefs may be located in areas with higher larval transport potential, and may lack species that compete with the barnacles for space.

Further studies should be conducted to determine the driving factors behind benthic invertebrate recruitment patterns at different artificial reef profile types.

Transects

The transect results showed similar nutrient concentrations and bacterial abundance at all reef sites along the transects, regardless of distance from the reef. These results could possibly be explained by tidal action. Mississippi Sound is a shallow estuary, and as the tide rises and falls it can thoroughly mix the water column, and wind driven mixing can contribute to water column mixing as well. This hypothesis is supported by water quality data collected at each transect site. Salinity and temperature were typically similar to each other between surface and bottom waters, suggesting that the water column at the reef sites is well mixed (Appendix H). Another explanation is that the remineralized nutrients are immediately recycled in the system. Heterotrophic bacteria in Mississippi Sound have been determined to be carbon-limited, but other microorganisms may be P or N limited and the introduction of these nutrients into the system would result in immediate uptake (Carpenter 2010). Further research needs to be conducted to explore these results, possibly examining offshore artificial reefs located in deeper waters to determine the effects of mixing on nutrient concentration and bacterial abundance.

DOC:DON ratios in transect samples were often positively correlated with daily rainfall amounts, and this relationship is most clearly exemplified by the effects of Hurricane Isaac. Transect samples collected at Square Handkerchief and Katrina before the storm had lower DOC:DON values than those observed in Legacy and Katrina transect samples collected after the storm. The results suggest that storm events are

introducing different types of organic matter with higher DOC:DON ratios than organic matter produced *in situ*, which may impact bacterial populations depending on whether the organic matter from stormwater inputs is more refractory or labile. Further studies should be done to examine organic matter content in relation to storm events.

Biofilm Stable Isotope Analysis

$\delta^{13}C$ values were significantly different by season and by individual reef, although profile type and location effects were not observed. Winter 2013 biofilm samples were the most enriched in $\delta^{13}C$, and Fall 2012 samples had the lowest average values. Higher $\delta^{13}C$ values are associated with marine-derived organic carbon and microalgae as carbon sources for higher trophic levels (Deegan and Garritt 1997; France 1998) so the results suggest that seasonal differences exist in the availability of these sources. Photosynthesis typically peaks in the summer, when temperature and light favor primary productivity. Voss and Struck (1997) found that higher $\delta^{13}C$ values are associated with high primary production as a result of higher fractionation during photosynthesis. Thus, $\delta^{13}C$ signatures should be compared to photosynthetic activity to determine if a relationship exists at the artificial reef sites between primary production and $\delta^{13}C$ values . Biofilm samples collected in Winter 2013 were most likely enriched due to the long exposure time which allowed more biofilm to accumulate and therefore increase ^{13}C concentrations. Winter 2013 biofilm samples are likely a closer representation to $\delta^{13}C$ signatures in climax biofilm communities at the reefs.

Average $\delta^{13}C$ values were significantly different by individual reefs, which contrasts with the chamber incubation results that were significantly different by profile type in the presence of biofilm. Stable isotope analysis may possibly shed light on

differences between sites that cannot be distinguished from the chamber incubation results. Biofilm samples for stable isotope analysis reflect heterotrophic and autotrophic interactions, while chamber incubations were designed to examine only heterotrophic activity by conducting all experiments in the dark. Another explanation is that substrate material affected biofouling activity. Anderson and Underwood (1994) found that the type of substrate used influenced recruitment of many biofouling species. The authors saw higher recruitment on concrete and plywood surfaces, and lower recruitment on fiberglass and aluminum surfaces (Anderson and Underwood 1994). Biofilm samples for stable isotope analysis were collected from Plexiglas plates, while biofilm used in chamber incubations grew on crushed concrete. Further studies should be conducted to determine if substrate material has a significant effect on biofilm growth and composition, which could in turn affect stable isotope content.

$\delta^{15}N$ values were significantly different between seasons, with higher values in Summer 2012 and lower values in Fall 2012. ^{15}N enrichment is a reflection of higher trophic levels (Montoya 2007), so these results indicate that higher trophic levels dominated biofilms during warmer periods. High $\delta^{15}N$ values were also observed in Winter 2013, but this is most likely a reflection of how long samples were exposed at the reef, allowing a greater accumulation of biofilm and increased settlement by higher trophic levels. Significant profile effects were also observed, as were significant differences by individual reef. Low profile reefs had higher $\delta^{15}N$ signatures than high profile reefs. Biofouling by benthic invertebrates, especially barnacles, was higher at low relief reefs than high relief reefs and this most likely explains the $\delta^{15}N$ enrichment seen in low relief reef biofilms. The significant differences between reef sites are likely an

artifact from the profile effects, because the highest average $\delta^{15}N$ values were observed at USM and Legacy.

Seasonal effects were observed in C:N ratios, with the highest ratios occurring in Fall 2012 and the lowest ratios occurring in Summer 2012 and Winter 2013. Primary production is expected to be higher in warmer seasons, and carbon assimilation may be lower in autotrophic-dominated biofilms compared to heterotrophic-dominated biofilms (Chaloner et al. 2002; Fenchel et al. 1998), so the lower C:N ratios observed in Summer 2012 may reflect enhanced benthic primary production. Location also played a role in C:N ratios. Higher C:N ratios were seen in eastern reefs, which is interesting because all other measured responses were similar in regards to location. However, since only eastern reef samples were collected in Experiment 4 and Experiment 7, these results may not reflect an accurate depiction of location effects. C:N ratios were also significantly different by individual reef, but these results are most likely due to location effects, because Legacy and Katrina have higher average C:N ratios than those observed at USM and Square Handkerchief.

$\delta^{13}C$ and $\delta^{15}N$ values of biofilm samples tended to be similar to stable isotope signatures in mud crabs and snapping shrimp at a nearby artificial oyster reef in Ocean Springs Harbor, Mississippi, but some biofilm samples had signatures that correlated to higher trophic levels. Legacy and Katrina biofilm samples collected in Summer 2012 and Winter 2013 had higher $\delta^{13}C$ and $\delta^{15}N$ values that were closer to those of grass shrimp at the oyster reef, and Summer 2012 USM biofilm samples had the highest observed enrichment in both isotopes, with values that were similar to gobies and toadfish. Square Handkerchief $\delta^{13}C$ and $\delta^{15}N$ values were the lowest and most closely resembled the

values of mud crabs at the oyster reef, although some values which were collected in Fall 2012 were lower than any values seen in organisms from Ocean Springs Harbor. These results indicate that biofilms are capable of supporting multiple trophic levels within the biofilm community in more productive seasons, and the low $\delta^{13}C$ and $\delta^{15}N$ values measured at Square Handkerchief suggest that this reef has the least productive biofilm community. Reef habitats are often capable of sustaining the biological community with *in situ* production and do not rely on allochthonous sources. Abeels et al (2012) found that oyster reef habitats in southwest Florida had tight coupling of $\delta^{13}C$ values and $\delta^{15}N$ values for several organisms of different trophic levels that suggested reef-dwelling organisms derived their food source from the reef. $\delta^{13}C$ values in biofilm samples at the four reef sites varied over time, and $\delta^{15}N$ values spanned a larger range of values over time, suggesting that the biofilm community utilizes different carbon sources at different times of the year and can assume higher trophic levels. Diet analysis is currently being conducted on benthic invertebrates and cryptic fishes at the four artificial reef sites to determine the trophic roles of these organisms, and this information can then be used to determine the trophic influence of benthic biofilms based on the stable isotope comparisons discussed.

Conclusion

In summary, bacterioplankton and benthic biofilms at artificial reef sites in the Mississippi Sound have been observed to be highly productive heterotrophic communities that act as a source for PO_4 and NH_4, especially at low profile reefs where biofilm growth is more pronounced and represented by relatively great abundances of biofouling macrofauna. During more productive seasons, benthic biofilm communities

may assume a higher trophic role, likely due to changes in relative proportions of microbial and macrofaunal composition over time. Further research needs to be conducted to compare the biofilm community to other benthic organisms present at the reefs and determine trophic interactions at the reefs. However, the effects of enhanced biofilm and bacterioplankton production at the reefs were not observed in transect water column samples, most likely due to tidal and wind driven mixing and shallow depths. Hypoxic conditions are strongly correlated with high PO_4 and NH_4 release, suggesting that in highly stratified water columns artificial reef biofilms may lead to hypoxic or anoxic bottom waters and toxic PO_4 and NH_4 concentrations. Additional studies need to be performed to examine benthic community dynamics in deeper waters where the water column is more stratified.

APPENDIX A

RESPONSE RATES FOR BOD AND BIOFILM INCUBATION EXPERIMENTS

Summary of raw oxygen consumption, bacterial production, PO_4, NH_4, NO_3, NO_2, DOC, and DON production rate ranges in BOD and chamber incubations. * denotes calculated rates that are imprecise due to undetectable concentrations at the time of sampling.

Expt.	Sample Type	O_2 Cons. $\mu M\ hr^{-1}$	Δ BA E+04 cells $ml^{-1}\ hr^{-1}$	PO_4 Prod. $nM\ hr^{-1}$	NH_4 Prod. $\mu M\ hr^{-1}$	NO_3 Prod. $nM\ hr^{-1}$	NO_2 Prod. $nM\ hr^{-1}$	DOC Prod. $\mu M\ hr^{-1}$	DON Prod. $\mu M\ hr^{-1}$
1	BOD (High)	0.53 to 1.10	-0.14 to 0.12	2 to 3	0.07 to 0.12	BD to -3	-5* to 0	-0.79 to -0.47	-0.05 to 0.01
	BOD (Low)	0.33 to 0.88	-2.41 to -0.84	BD	BD to 0.11	BD	-2	-0.11 to 0.19	0.02 to 0.08
	Control Chamber	-0.02 to 0.27	-2.26 to 0.33	BD to -6	-0.01 to 0.02	-3 to 54	-7 to 2	-1.68 to 2.74	-0.16 to 0.04
	Biofilm (High)	1.15 to 9.00	-8.59 to -1.00	16 to 116	0.15 to 1.95	-18* to 90	5 to 94	-2.12 to -0.24	-0.75 to -0.07
	Biofilm (Low)	9.31 to 11.17	-9.48 to 16.25	56 to 398	1.24 to 5.07	BD	-7 to 2	0.21 to 8.53	-0.58 to -0.06
2	BOD (High)	0.71 to 0.89	-0.22 to -0.04	BD to 3	0.06*	BD	BD	-0.26 to -0.21	-0.01 to 0.06
	BOD (Low)	0.99 to 1.06	-1.94 to -0.67	3 to 4	0.06 to 0.07	BD	BD	-0.28 to -0.04	0.00 to 0.05
	Control Chamber	-0.47 to 0.76	-7.41 to 2.33	BD to -7	BD to 0.26	BD	BD to 4	-12.25 to 1.46	-0.53 to 0.04
	Biofilm (High)	6.19 to 19.38	-16.16 to -9.45	68 to 264	0.23 to 3.14	BD to 14,144	63 to 249	-3.26 to 2.06	-0.30 to 1.99
	Biofilm (Low)	11.61 to 23.87	-11.53 to 0.03	209 to 968	2.08 to 9.88	BD	BD to 5	4.54 to 17.63	1.28 to 10.53
3	BOD (High)	0.60 to 0.71	-2.61 to -1.90	3 to 5	0.04 to 0.06	BD to 91	BD to 2	-0.32 to 0.22	-0.06 to 0.02
	BOD (Low)	0.89 to 1.58	-4.37 to -3.62	6 to 9	0.11 to 0.12	BD	BD	-0.66 to -0.19	-0.03 to -0.01
	Control Chamber	-1.14 to 0.08	-5.28 to 6.10	-34 to 9	BD to 0.11	BD to 229	BD to 3	-4.07 to 7.72	-0.54 to 2.08
	Biofilm (High)	1.74 to 37.51	-24.13 to -4.09	8 to 849	0.59 to 5.47	BD to 613	26 to 718	-1.68 to 17.02	-0.33 to 6.59

	Biofilm (Low)	5.19 to 35.1	-18.41 to 5.76	122 to 649	0.73 to 8.20	BD to 348	BD to 31	2.91 to 42.12	0.93 to 11.07
4	BOD (High)	0.68 to 1.05	-1.74 to -1.14	1 to 8	0.04 to 0.09	BD to -1*	BD	-0.42 to -0.15	-0.05 to 0.00
	BOD (Low)	0.77 to 0.91	-1.54 to -0.77	5 to 7	0.06 to 0.08	BD to -1*	BD	-0.45 to -0.04	-0.06 to -0.03
	Control Chamber	-1.49 to -0.06	-10.89 to 2.44	BD to -15	BD to 0.06	BD to 20	BD to -5	1.07 to 3.96	-0.16 to 0.06
	Biofilm (High)	0.44 to 10.54	-20.68 to -9.42	39 to 176	BD to 1.51	BD to 80	BD to 353	-0.34 to 5.20	-0.35 to 0.33
	Biofilm (Low)	2.41 to 34.07	-24.62 to -11.66	91 to 473	0.28 to 5.56	BD	14 to 189	1.75 to 11.94	0.22 to 2.73
5	BOD (High)	0.73 to 1.46	-1.13 to -0.11	6 to 12	0.10 to 0.13	BD	0 to 2	-1.13 to 0.47	-0.08 to -0.07
	BOD (Low)	0.81 to 1.34	-3.64 to -1.84	BD to 13	0.06 to 0.13	BD	BD to 10	0.56 to 1.49	-0.09
	Control Chamber	-1.21 to 1.08	-1.47 to 0.30	BD to -9	BD to 0.11	BD	BD	-4.14 to -0.04	-0.44 to 0.10
	Biofilm (High)	2.02 to 16.66	-27.62 to 1.04	28 to 206	0.04 to 2.84	BD to 944	31 to 312	-2.66 to 6.41	-0.13 to 0.28
	Biofilm (Low)	13.73 to 43.83	-49.12 to 58.88	BD to 2102	1.69 to 18.98	BD to 42	BD to 373	1.94 to 162	0.11 to 39.30
6	BOD (High)	0.62 to 0.81	-0.52 to 0.20	1 to 3	0.04 to 0.10	BD	-1 to 3	-0.54 to 0.43	-0.03 to 0.01
	BOD (Low)	0.55 to 1.38	-2.31 to -0.87	3 to 7	0.04 to 0.11	BD	BD to 1	-0.83 to -0.10	-0.08 to -0.02
	Control Chamber	-3.84 to -0.26	-4.96 to 3.49	-21 to 25	BD to 0.03	BD	BD to -12	-8.89 to 8.75	-0.60 to -0.13
	Biofilm (High)	1.00 to 28.42	-42.15 to -5.81	54 to 486	BD to 6.83	BD to 460	BD to 312	-10.76 to 2.33	-2.03 to -0.02
	Biofilm (Low)	26.84 to 77.92	-7.99 to 70.65	332 to 2644	5.33 to 20.48	BD to 127	BD to 80	-7.66 to 101	-2.09 to 9.67
7	BOD (High)	0.22 to 0.40	-0.40 to -0.01	BD to 2	0.02	BD	BD to 4	-0.08 to 0.08	-0.01 to 0.01
	BOD (Low)	0.24 to 0.32	-0.93 to -0.72	-2 to 3	0.01 to 0.02	BD	0 to 12	-0.28 to 0.05	-0.02
	Control Chamber	-1.07 to 0.13	-1.16 to 1.72	BD to 7	BD to 0.04	BD to -46*	BD	-2.89 to 8.91	0.14 to 4.39

	Biofilm (High)	1.01 to 18.05	-9.68 to -3.43	6 to 116	0.17 to 1.65	BD to -45*	BD to 12	-3.50 to 1.42	-1.26 to 0.51
	Biofilm (Low)	1.10 to 23.27	-6.61 to 4.61	9 to 257	0.06 to 2.22	BD	BD	-0.29 to 4.71	-1.29 to 2.14
8	BOD (High)	0.32 to 0.41	-1.83 to -1.04	BD	0.02 to 0.04	BD to 3	BD to 4	-0.06 to 0.00	-0.03 to 0.00
	BOD (Low)	0.47 to 0.58	-1.75 to -1.10	BD	BD to 0.03	BD to -2	BD to 3	-0.55 to 0.39	-0.02 to 0.00
	Control Chamber	-2.13 to 1.09	-8.31 to 6.41	BD	-0.10 to 0.11	BD to 56	BD to -17*	-10.29 to 1.52	-0.89 to 0.55
	Biofilm (High)	0.48 to 53.86	-36.49 to -1.19	22 to 640	0.56 to 9.41	BD to 570	-19* to 249	-3.36 to 14.74	-0.79 to 1.25
	Biofilm (Low)	53.40 to 102.66	-17.28 to 63.41	1100 to 4125	10.42 to 72.04	BD to 2	BD to 195	41.95 to 291.43	6.32 to 59.68

APPENDIX B

TWO-WAY ANOVA RESULTS FOR SEASON AND LOCATION NORMALIZED TO SURFACE AREA

ANOVA results for biofilm incubation chamber results normalized to surface area of the rubble for Winter 2012 – Experiment 1 to Winter 2013 – Experiment 8. * denotes significant effects due to reef location even when Experiment 8 is excluded from the analysis. $\alpha = 0.05$.

Response	Factor	Type III Sum of Squares	df	F value	p value
Respiration	Season	3453525	4	8.654	0.000
	Location	8629	1	0.086	0.769
	Interaction	92739	4	0.232	0.920
BA	Season	76	4	5.968	0.000
	Location	94	1	29.401	0.000
	Interaction	328	4	25.600	0.000
PO_4	Season	2996	4	8.848	0.000
	Location	306	1	3.615	0.059
	Interaction	897	4	2.651	0.036
NH_4	Season	484616	4	9.141	0.000
	Location	78365	1	5.912	0.016
	Interaction	268118	4	5.057	0.001
NO_3	Season	380	4	10.446	0.000
	Location*	137	1	15.084	0.000
	Interaction	494	4	13.562	0.000
NO_2	Season	42	4	3.393	0.011
	Location	0.332	1	0.107	0.745
	Interaction	37	4	2.952	0.023
DOC	Season	6263091	4	6.052	0.000
	Location	441901	1	1.708	0.194
	Interaction	2039252	4	1.971	0.103
DON	Season	322962	4	7.828	0.000
	Location	29300	1	2.841	0.094
	Interaction	172582	4	4.183	0.003

APPENDIX C

TWO-WAY ANOVA RESULTS FOR SEASON AND LOCATION NORMALIZED TO MASS OF BIOFILM PER UNIT SURFACE AREA

ANOVA results for biofilm chamber results normalized to mass of biofilm per unit surface area of the rubble for Winter 2012 – Experiment 1 to Winter 2013 – Experiment 8. $\alpha = 0.05$.

Response	Factor	Type III Sum of Squares	df	F value	p value
Respiration	Season	0.002	2	2.597	0.079
	Location	0.000	1	1.479	0.227
	Interaction	0.000	2	0.825	0.441
BA	Season	0.486	2	9.504	0.000
	Location	0.001	1	0.058	0.810
	Interaction	0.030	2	0.589	0.556
PO_4	Season	1.09E-06	2	4.311	0.016
	Location	0.000	1	0.000	1.000
	Interaction	3.47E-07	2	1.366	0.260
NH_4	Season	1.47E-04	2	2.894	0.060
	Location	1.64E-05	1	0.649	0.422
	Interaction	1.11E-04	2	2.190	0.117
NO_3	Season	6.75E-08	2	0.433	0.650
	Location	3.31E-08	1	0.424	0.516
	Interaction	4.64E-08	2	0.297	0.743
NO_2	Season	5.07E-07	2	6.816	0.002
	Location	5.02E-08	1	1.350	0.248
	Interaction	4.34E-09	2	0.058	0.943
DOC	Season	0.004	2	4.418	0.014
	Location	8.12E-05	1	0.175	0.677
	Interaction	0.001	2	0.725	0.487
DON	Season	4.24E-04	2	9.742	0.000
	Location	1.76E-06	1	0.081	0.777
	Interaction	1.19E-04	2	2.734	0.070

APPENDIX D

RESPONSE RATES FOR BIOFILM INCUBATIONS NORMALIZED TO SURFACE AREA

Summary of control-corrected oxygen consumption, bacterial production, PO_4, NH_4, NO_3, NO_2, DOC, and DON production rate ranges in biofilm incubations normalized to surface area of the rubble. * denotes calculated rates that are imprecise due to undetectable concentrations at the time of sampling.

Expt.	Profile Type	O_2 Cons. $\mu M\ hr^{-1}\ m^{-2}$	ΔBA E+06 cells $ml^{-1}\ hr^{-1}\ m^{-2}$	PO_4 Prod. $\mu M\ hr^{-1}\ m^{-2}$	NH_4 Prod. $\mu M\ hr^{-1}\ m^{-2}$	NO_3 Prod. $\mu M\ hr^{-1}\ m^{-2}$	NO_2 Prod. $\mu M\ hr^{-1}\ m^{-2}$	DOC Prod. $\mu M\ hr^{-1}\ m^{-2}$	DON Prod. $\mu M\ hr^{-1}\ m^{-2}$
1	High	19 to 129	-35.2 to -5.9	0.34 to 1.76	2.28 to 28.39	-0.51* to 1.36	0.06 to 1.42	-85 to -4	-11 to -2
	Low	140 to 173	-39.9 to 81	0.92 to 5.80	20.49 to 73.84	BD	-0.05 to 2.36	16 to 160	-9 to 4
2	High	93 to 288	-66.1 to -39.4	1.27 to 4.14	2.31 to 48.73	BD to 23.47	1.02 to 4.46	19 to 107	1 to 33
	Low	172 to 380	-75.7 to 5.1	3.20 to 15.32	31.37 to 152.32	BD	BD to 0.07	47 to 354	19 to 170
3	High	45 to 528	-97.3 to -7.1	0.50 to 11.64	6.26 to 74.54	BD to 10.39	0.41 to 10.89	10 to 191	-21 to 80
	Low	109 to 566	-101 to 16.1	2.24 to 10.05	13.37 to 129.50	BD to 6.26	BD to 0.49	-74 to 543	12 to 170
4	High	31 to 178	-92.0 to -25.1	0.75 to 2.38	BD to 31.00	BD to 1.54	BD to 5.94	-52 to 66	-5 to 10
	Low	63 to 597	-88.2 to -24.7	1.95 to 8.74	5.64 to 101.43	BD	0.29 to 3.52	4 to 143	4 to 49
5	High	41 to 242	-109 to -2.4	0.55 to 3.00	0.59 to 39.01	BD to 14.69	0.51 to 5.15	-10 to 166	-4 to 9
	Low	215 to 755	-185 to 271	BD to 35.67	24.32 to 322.11	BD to 0.58	BD to 5.15	31 to 2753	6 to 675
6	High	22 to 434	-194 to -12.6	1.39 to 6.95	BD to 99.65	BD to 11.01	BD to 5.87	-43 to 170	-22 to 5
	Low	608 to 1462	-16.2 to 207	5.35 to 46.85	92.75 to 336.91	BD to 1.99	BD to 1.25	-286 to 1712	-34 to 173
7	High	33 to 284	-36.4 to -11.1	-0.03 to 1.69	2.43 to 23.88	BD to 0.04	BD to 0.18	-25 to 73	-24 to 2

	Low	40 to 472	-34.5 to 14.9	0.17 to 4.99	1.16 to 43.14	BD	BD	-168 to -22	-110 to -4
8	High	38 to 746	-133 to -0.5	0.36 to 8.87	6.74 to 129.57	BD to 7.70	-0.02* to 3.46	-104 to 188	-2 to 26
	Low	894 to 1819	-43.1 to 280	21.11 to 59.70	200.01 to 1044	BD to 0.03	BD to 3.61	795 to 4202	113 to 861

APPENDIX E

RESPONSE RATES FOR BIOFILM INCUBATIONS NORMALIZED TO MASS OF BIOFILM PER UNIT SURFACE AREA

Summary of control-corrected oxygen consumption, bacterial production, PO_4, NH_4, NO_3, NO_2, DOC, and DON production rate ranges in biofilm incubations normalized to mass of biofilm per unit surface area. * denotes calculated rates that are imprecise due to undetectable concentrations at the time of sampling.

Expt.	Profile Type	O_2 Cons. nM hr^{-1} g^{-1} m^{-2}	ΔBA E+03 cells ml^{-1} hr^{-1} g^{-1} m^{-2}	PO_4 Prod. pM hr^{-1} g^{-1} m^{-2}	NH_4 Prod. nM hr^{-1} g^{-1} m^{-2}	NO_3 Prod. pM hr^{-1} g^{-1} m^{-2}	NO_2 Prod. pM hr^{-1} g^{-1} m^{-2}	DOC Prod. nM hr^{-1} g^{-1} m^{-2}	DON Prod. nM hr^{-1} g^{-1} m^{-2}
3	High	4.61 to 40.62	-8.24 to -0.80	58 to 812	0.73 to 7.41	BD to 1210	39 to 1269	0.90 to 12.84	-2.35 to 8.22
	Low	10.89 to 47.96	-7.80 to 1.05	225 to 1002	1.34 to 11.59	BD to 467	BD to 36	-5.41 to 39.82	1.16 to 13.71
4	High	0.88 to 18.97	-9.43 to -2.52	81 to 316	BD to 2.63	BD to 171	BD to 631	-5.78 to 6.25	-0.51 to 0.71
	Low	5.26 to 42.74	-6.38 to -2.01	163 to 878	0.47 to 10.16	BD	18 to 246	0.30 to 14.59	0.22 to 4.33
5	High	4.13 to 31.90	-15.7 to 0.31	69 to 369	0.07 to 4.79	BD to 1932	49 to 639	-1.31 to 23.53	-0.44 to 0.96
	Low	26.39 to 78.50	-30.9 to 11.7	BD to 1534	2.99 to 15.61	BD to 53	BD to 853	4.27 to 118.45	0.71 to 29.02
6	High	1.85 to 40.20	-17.9 to -1.04	108 to 575	0.66 to 7.13	BD to 828	BD to 609	-5.46 to 16.23	-2.51 to 0.39
	Low	20.97 to 52.53	-0.89 to 6.43	295 to 1138	5.11 to 13.79	BD to 132	BD to 83	-15.75 to 53.19	-1.89 to 4.73
7	High	2.31 to 27.26	-3.49 to -0.73	-2 to 123	0.16 to 1.75	BD to 5	BD to 13	-1.45 to 5.26	-1.84 to 0.12
	Low	2.57 to 43.84	-2.79 to 1.38	12 to 463	0.09 to 4.00	BD	BD	-10.88 to 1.44	--10.23 to -0.26
8	High	3.00 to 45.42	-14.0 to 0.00	35 to 503	0.64 to 7.34	BD to 725	-2* to 216	-11.65 to 10.64	-0.20 to 1.48
	Low	14.92 to 83.14	-1.55 to 7.92	537 to 2028	6.43 to 35.47	BD to 2	BD to 53	11.53 to 142.78	3.37 to 29.25

APPENDIX F

TRANSECT NUTRIENT CONCENTRATIONS

Summary of PO_4, NH_4, NO_3, and NO_2 concentration ranges for transect samples by collection date and reef site. BD indicates concentrations below detection. Concentrations are reported with ± SEM.

Collection Date	Reef	PO_4 µM	NH_4 µM	NO_3 µM	NO_2 µM
2/28/2012	Sq. Hand.	BD to 0.73 ± 0.06	BD to 0.97 ± 0.28	BD to 0.88 ± 0.11	0.11 ± 0.00 to 0.30 ± 0.00
	USM	BD	BD	BD	BD to 0.12 ± 0.01
3/9/2012	Legacy	BD	BD	BD	BD to 0.19 ± 0.00
	Katrina	BD	BD to 1.50 ± 0.18	0.68 ± 0.13 to 3.11 ± 0.16	0.17 ± 0.00 to 0.32 ± 0.00
4/27/2012	Sq. Hand.	0.71 ± 0.00 to 0.94 ± 0.00	BD to 1.21 ± 0.03	BD	BD
	USM	0.54 ± 0.00 to 0.60 ± 0.00	BD	BD	BD
	Legacy	0.54 ± 0.00 to 0.83 ± 0.00	BD	BD	BD
	Katrina	BD to 0.65 ± 0.06	BD	BD	BD
5/8/2012	Sq. Hand.	BD to 0.65 ± 0.03	BD	BD	BD
	USM	BD	BD	BD to 2.00 ± 0.10	BD
5/14/2012	Legacy	BD	BD	BD	BD
	Katrina	BD	BD	BD	BD
5/30/2012	Sq. Hand.	0.74 ± 0.00 to 0.85 ± 0.00	BD	BD	BD
6/2/2012	USM	0.82 ± 0.03 to 0.96 ± 0.00	BD	BD	BD
	Legacy	0.62 ± 0.00 to 1.02 ± 0.11	BD	BD	BD

	Katrina	0.51 ± 0.00 to 0.68 ± 0.00	BD	BD	BD
6/21/2012	Sq. Hand.	0.61 ± 0.00 to 1.05 ± 0.03	BD to 0.75 ± 0.16	BD	BD
	USM	BD to 0.55 ± 0.15	BD	BD	BD
6/26/2012	Legacy	BD	BD to 0.84 ± 0.38	BD	BD
	Katrina	BD	BD to 1.10 ± 0.13	BD	BD to 0.15 ± 0.00
7/11/2012	Sq. Hand.	1.14 ± 0.00 to 1.20 ± 0.00	BD to 2.37 ± 0.12	BD to 0.81 ± 0.17	BD to 0.10 ± 0.04
	USM	1.02 ± 0.00 to 1.37 ± 0.00	BD	BD	BD
	Legacy	BD	BD to 2.13 ± 0.00	BD	BD to 0.15 ± 0.00
	Katrina	BD	BD	BD to 0.85 ± 0.15	BD
7/24/2012	Sq. Hand.	0.53 ± 0.00 to 1.01 ± 0.00	BD to 3.47 ± 0.03	BD to 1.13 ± 0.03	BD
	USM	BD to 0.84 ± 0.00	BD	BD	BD
7/30/2012	Legacy	0.76 ± 0.03 to 0.78 ± 0.00	BD	BD	BD
	Katrina	BD to 1.38 ± 0.03	BD to 1.08 ± 0.03	BD	BD
8/7/2012	Sq. Hand.	1.08 ± 0.03 to 1.39 ± 0.00	BD to 3.10 ± 0.06	BD	BD to 0.23 ± 0.01
	USM	0.94 ± 0.00 to 1.25 ± 0.03	BD	BD	BD
	Legacy	0.52 ± 0.03 to 0.58 ± 0.03	BD	BD	BD
	Katrina	0.50 ± 0.00 to 0.94 ± 0.00	BD	BD	BD
8/21/2012	Sq. Hand.	1.09 ± 0.00 to 1.32 ± 0.00	BD to 1.31 ± 0.09	BD	BD to 0.20 ± 0.01
	USM	1.12 ± 0.03 to 1.32 ± 0.00	BD	BD	BD to 0.15 ± 0.00

9/11/2012	Legacy	BD	BD	BD	BD
	Katrina	1.90 ± 0.00 to 2.51 ± 0.03	BD to 1.03 ± 0.06	BD	BD to 0.15 ± 0.00
9/27/2012	Sq. Hand.	BD to 0.68 ± 0.11	BD	BD to 1.75 ± 0.18	BD
	USM	BD	BD	BD	BD
	Legacy	0.57 ± 0.00 to 0.63 ± 0.00	BD	BD	BD
	Katrina	0.74 ± 0.00 to 0.94 ± 0.03	BD	BD	BD
10/17/2012	Sq. Hand.	0.67 ± 0.00 to 0.81 ± 0.03	BD	BD	BD
	USM	0.96 ± 0.00 to 1.01 ± 0.00	BD	BD	BD
10/23/2012	Legacy	0.73 ± 0.00 to 1.01 ± 0.23	BD	BD	BD to 0.13 ± 0.00
	Katrina	0.67 ± 0.00 to 0.87 ± 0.03	BD to 0.80 ± 0.03	BD	BD to 0.14 ± 0.00
11/5/2012	Sq. Hand.	0.63 ± 0.00 to 0.74 ± 0.00	BD	BD	BD
	USM	0.66 ± 0.03 to 0.74 ± 0.00	BD	BD	BD
	Legacy	0.66 ± 0.03 to 0.74 ± 0.00	BD	BD	BD
	Katrina	0.57 ± 0.00 to 0.88 ± 0.03	BD	BD	BD
11/15/2012	Sq. Hand.	0.75 ± 0.00 to 0.92 ± 0.00	BD	BD	BD
	USM	0.78 ± 0.03 to 0.89 0.09	BD to 1.01 ± 0.30	BD	BD
12/2/2012	Legacy	0.66 ± 0.03 to 0.81 ± 0.00	BD	BD	BD
	Katrina	BD to 0.52 ± 0.00	BD	BD	BD
2/1/2013	Sq. Hand.	0.66 ± 0.03 to 0.83 ± 0.03	0.84 ± 0.03 to 1.28 ± 0.03	1.18 ± 0.05 to 4.08 ± 0.10	0.16 ± 0.00 to 0.29 ± 0.00

	USM	BD	BD	BD	BD
	Legacy	BD	BD	BD	BD
	Katrina	BD	BD to 0.60 ± 0.03	BD	BD to 0.16 ± 0.00
3/14/2013	Sq. Hand.	BD	BD	BD to 0.81 ± 0.14	BD
	USM	BD	BD	BD	BD
4/2/2013	Legacy	BD	BD	BD	BD
	Katrina	BD	BD to 0.76 ± 0.03	BD	BD

APPENDIX G

TRANSECT BACTERIAL ABUNDANCE, DOC, AND DON CONCENTRATIONS AND DOC:DON RATIOS

Summary of bacterial abundance (BA), DOC, and DON concentration ranges and DOC:DON ratio ranges for transect samples by collection date and reef. Concentrations are reported with ± SEM.

Collection Date	Reef	BA E+06 cells ml^{-1}	DOC µM	DON µM	DOC:DON
2/28/2012	Sq. Hand.	2.34 ± 0.10 to 2.63 ± 0.04	350 ± 26 to 462 ± 6	14.68 ± 0.58 to 18.87 ± 1.16	21.60± 0.12 to 25.23 ± 0.02
	USM	2.16 ± 0.02 to 2.71 ± 0.04	256 ± 45 to 320 ± 10	13.25 ± 0.76 to 15.15 ± 1.09	17.21 ± 0.18 to 20.53 ± 0.05
3/9/2012	Legacy	2.82 ± 0.17 to 3.22 ± 0.12	257 ± 8 to 320 ± 18	11.62 ± 0.99 to 13.44 ± 1.09	20.09 ± 0.05 to 22.46 ± 0.06
	Katrina	2.07 ± 0.13 to 2.58 ± 0.02	250 ± 17 to 344 ± 15	9.77 ± 0.86 to 13.77 ± 2.29	20.11 ± 0.07 to 25.42 ± 0.16
4/27/2012	Sq. Hand.	2.65 ± 0.05 to 3.30 ± 0.14	377 ± 28 to 499 ± 18	16.62 ± 1.17 to 20.41 ± 0.48	19.40 ± 0.09 to 25.28 ± 0.04
	USM	2.54 ± 0.05 to 2.66 ± 0.05	361 ± 3 to 401 ± 13	15.87 ± 0.84 to 20.28 ± 3.35	18.91 ± 0.16 to 22.29 ± 0.10
	Legacy	2.50 ± 0.18 to 2.65 ± 0.02	405 ± 92 to 421 ± 10	17.02 ± 1.90 to 18.67 ± 0.20	20.65 ± 0.23 to 23.17 ± 0.14
	Katrina	2.59 ± 0.05 to 2.84 ± 0.05	240 ± 7 to 398 ± 11	12.80 ± 1.98 to 16.42 ± 0.15	17.53 ± 0.15 to 24.38 ± 0.22
5/8/2012	Sq. Hand.	2.34 ± 0.05 to 3.00 ± 0.05	347 ± 27 to 468 ± 14	13.12 ± 0.78 to 16.29 ± 0.64	24.69 ± 0.10 to 28.91 ± 0.04
	USM	2.74 ± 0.26 to 3.24 ± 0.00	297 ± 7 to 319 ± 12	11.20 ± 0.71 to 13.76 ± 0.85	21.37 ± 0.06 to 25.46 ± 0.07
5/14/2012	Legacy	2.14 ± 0.03 to 2.84 ± 0.03	281 ± 22 to 317 ± 7	12.38 ± 0.28 to 13.70 ± 0.06	20.23 ± 0.04 to 21.96 ± 0.05
	Katrina	2.19 ± 0.05 to 2.41 ± 0.04	257 ± 21 to 283 ± 11	10.13 ± 1.96 to 11.62 ± 0.44	21.41 ± 0.12 to 25.10 ± 0.18
5/30/2012	Sq. Hand.	3.34 ± 0.22 to 4.23 ± 0.19	313 ± 4 to 406 ± 4	12.74 ± 0.77 to 16.71 ± 1.10	19.85 ± 0.05 to 25.72 ± 0.03
6/2/2012	USM	2.45 ± 0.10 to 2.69 ± 0.06	216 ± 7 to 260 ± 8	9.66 ± 0.40 to 10.59 ± 0.60	20.07 ± 0.11 to 22.53 ± 0.06

	Legacy	2.48 ± 0.11 to 2.65 ± 0.12	249 ± 17 to 294 ± 10	10.08 ± 0.19 to 12.03 ± 0.63	22.55 ± 0.07 to 22.93 ± 0.10
	Katrina	2.01 ± 0.08 to 2.69 ± 0.06	237 ± 3 to 263 ± 6	10.09 ± 0.42 to 11.45 ± 0.28	19.92 ± 0.02 to 21.86 ± 0.03
6/21/2012	Sq. Hand.	2.31 ± 0.17 to 3.09 ± 0.06	322 ± 30 to 363 ± 34	14.70 ± 2.70 to 17.05 ± 0.66	19.69 ± 0.08 to 21.89 ± 0.02
	USM	2.80 ± 0.03 to 2.93 ± 0.15	274 ± 9 to 308 ± 29	12.68 ± 0.10 to 15.08 ± 1.19	19.07 ± 0.16 to 20.78 ± 0.02
6/26/2012	Legacy	2.39 ± 0.27 to 2.80 ± 0.02	294 ± 11 to 334 ± 4	12.52 ± 0.43 to 14.76 ± 3.62	18.74 ± 0.23 to 24.30 ± 0.04
	Katrina	1.95 ± 0.17 to 2.50 ± 0.02	234 ± 2 to 247 ± 7	10.45 ± 1.35 to 12.19 ± 0.44	17.83 ± 0.04 to 20.61 ± 0.01
7/11/2012	Sq. Hand.	2.96 ± 0.20 to 3.64 ± 0.05	330 ± 2 to 349 ± 6	16.18 ± 0.06 to 17.50 ± 0.97	18.51 ± 0.01 to 19.97 ± 0.12
	USM	2.60 ± 0.39 ± 2.94 ± 0.08	262 ± 5 to 279 ± 1	13.84 ± 0.35 to 15.03 ± 0.23	17.26 ± 0.03 to 18.36 ± 0.03
	Legacy	1.22 ± 0.07 to 2.76 ± 0.22	237 ± 3 to 260 ± 5	12.78 ± 0.12 to 15.55 ± 1.38	14.94 ±0.08 to 17.71 ± 0.03
	Katrina	2.33 ± 0.06 to 3.11 ± 0.05	254 ± 23 to 306 ± 18	12.75 ± 0.07 to 15.76	17.33 ± 0.09 to 22.06 ± 0.06
7/24/2012	Sq. Hand.	2.84 ± 0.02 to 3.26 ± 0.05	381 ± 2 to 478 ± 11	18.35 ± 0.40 to 22.29 ± 2.15	18.47 ± 0.26 to 23.92 ± 0.12
	USM	1.96 ± 0.00 to 2.45 ± 0.02	269 ± 0 to 302 ± 5	11.73 ± 0.97 to 13.79 ± 0.39	20.43 ± 0.03 to 21.92 ± 0.02
7/30/2012	Legacy	1.95 ± 0.03 to 2.11 ± 0.11	263 ± 1 to 298 ± 46	12.84 ± 0.61 to 15.26 ± 0.16	17.07 ± 0.03 to 21.63 ± 0.16
	Katrina	1.99 ± 0.05 to 2.98 ± 0.02	218 ± 22 to 309 ± 21	10.84 ± 1.73 to 29.09 ± 1.60	10.29 ± 0.06 to 21.35 ± 0.14
8/7/2012	Sq. Hand.	2.26 ± 0.11 to 2.76 ± 0.01	247 ± 5 to 361 ± 44	13.97 ± 0.15 to 16.44 ± 1.55	16.88 ± 0.10 to 23.44 ± 0.12
	USM	3.10 ± 0.01 to 3.46 ± 0.07	230 ± 1 to 243 ± 10	12.28 ± 0.20 to 14.03 ± 0.33	16.23 ± 0.05 to 17.67 ± 0.01
	Legacy	2.26 ± 0.06 to 2.49 ± 0.03	236 ± 6 to 253 ± 2	11.56 ± 0.85 to 13.17 ± 0.38	17.08 ± 0.03 to 19.57 ± 0.01
	Katrina	2.26 ± 0.04 to 3.85 ± 0.25	230 ± 1 to 355 ± 1	11.25 ± 0.38 ± 16.89 ± 0.15	16.74 ± 0.03 to 22.05 ± 0.12
8/21/2012	Sq. Hand.	2.35 ± 0.07 to 2.68 ± 0.11	271 ± 1 to 333 ± 13	14.68 ± 0.01 to 17.85 ± 1.12	15.82 ± 0.05 to 18.61 ± 0.08

	USM	2.61 ± 0.05 to 2.82 ± 0.05	229 ± 2 to 264 ± 5	12.87 ± 0.11 to 16.84 ± 0.54	14.90 ± 0.04 to 16.75 ± 0.02
9/11/2012	Legacy	2.23 ± 0.02 to 2.63 ± 0.29	355 ± 0 to 405 ± 5	13.39 ± 0.91 to 14.99 ± 0.28	23.35 ± 0.04 to 26.49 ± 0.07
	Katrina	2.28 ± 0.06 to 2.53 ± 0.09	490 ± 10 to 607 ± 0	16.61 ± 0.16 to 19.60 ± 1.94	25.42 ± 0.10 to 31.07 ± 0.01
9/27/2012	Sq. Hand.	3.10 ± 0.10 to 3.43 ± 0.12	352 ± 13 to 474 ± 0	12.12 ± 0.22 to 16.86 ± 2.06	24.94 ± 0.05 to 29.56 ± 0.07
	USM	3.39 ± 0.04 to 3.57 ± 0.26	306 ± 17 to 406 ± 0	12.03 ± 0.71 to 15.91 ± 0.17	23.56 ± 0.08 to 30.80 ± 0.28
	Legacy	3.04 ± 0.09 to 3.37 ± 0.12	314 ± 63 to 367 ± 10	12.90 ± 2.65 to 14.57 ± 0.11	22.02 ± 0.10 to 23.67 ± 0.03
	Katrina	2.27 ± 0.00 to 2.52 ± 0.15	265 ± 83 to 376 ± 3	12.59 ± 1.17 to 15.00 ± 0.03	16.60 ± 0.31 to 24.98 ± 0.02
10/17/2012	Sq. Hand.	2.64 ± 0.02 to 3.15 ± 0.07	408 ± 36 to 483 ± 9	17.31 ± 0.43 to 19.60 ± 0.78	22.35 ± 0.09 to 25.77 ± 0.05
	USM	2.30 ± 0.07 to 2.37 ± 0.07	362 ± 9 to 415 ± 0	16.08 ± 0.51 to 18.66 ± 0.46	20.95 ± 0.02 to 21.84 ± 0.12
10/23/2012	Legacy	1.93 ± 0.01 to 2.25 ± 0.07	317 ± 0 to 362 ± 38	12.73 ± 0.95 to 13.71 ± 0.86	22.84 ± 0.04 to 25.33 ± 0.08
	Katrina	2.17 ± 0.03 to 2.47 ± 0.09	295 ± 0 to 318 ± 1	12.39 ± 0.41 to 13.89 ±0.15	21.40 ± 0.03 to 23.77 ± 0.03
11/5/2012	Sq. Hand.	2.24 ± 0.04 to 2.46 ± 0.03	247 ± 29 to 267 ± 0	10.20 ± 1.78 to 11.69 ± 0.81	20.26 ± 0.09 to 22.44 ± 0.01
	USM	2.01 ± 0.12 to 2.23 ± 0.02	190 ± 15 to 207 ± 3	8.57 ± 0.98 to 10.20 ± 0.43	18.55 ± 0.04 to 19.93 ± 0.13
	Legacy	1.87 ± 0.11 to 2.16 ± 0.05	166 ± 7 to 190 ± 2	7.53 ± 0.34 to 8.94 ± 0.33	19.00 ± 0.03 to 19.51 ± 0.06
	Katrina	1.88 ± 0.07 to 2.06 ± 0.05	176 ± 2 to 226 ± 2	8.07 ± 0.20 to 11.22 ± 2.06	15.83 ± 0.17 to 20.87 ± 0.01
11/15/2012	Sq. Hand.	1.28 ± 0.02 to 1.60 ± 0.03	165 ± 26 to 257 ± 5	7.13 ± 1.43 to 12.95 ± 0.57	18.46 ± 0.05 to 22.68 ± 0.02
	USM	1.19 ± 0.02 to 1.25 ± 0.06	790 ± 7 to 1570 ± 28	8.48 ± 0.35 to 11.57 ± 0.55	78.47 ± 0.06 to 137.64 ± 0.18
12/2/2012	Legacy	1.39 ± 0.02 to 1.81 ± 0.01	191 ± 1 to 1552 ± 14	8.48 ± 0.35 to 11.57 ± 0.55	16.54 ± 0.03 to 123.91 ± 0.04

	Katrina	1.55 ± 0.01 to 2.31 ± 0.13	165 ± 0 to 197 ± 1	9.21 ± 0.46 to 11.55 ± 0.18	15.48 ± 0.03 to 16.87 ± 0.03
2/1/2013	Sq. Hand.	2.28 ± 0.32 to 2.59 ± 0.14	525 ± 4 to 569 ± 0	18.08 ± 2.06 to 21.17 ± 0.22	26.26 ± 0.02 to 30.89 ± 0.05
	USM	2.15 ± 0.12 to 2.39 ± 0.14	385 ± 3 to 403 ± 13	15.08 ± 0.33 to 15.84 ± 1.73	23.24 ± 0.15 to 24.51 ± 0.05
	Legacy	2.08 ± 0.00 to 2.19 ± 0.00	407 ± 2 to 422 ± 8	12.97 ± 0.20 to 14.01 ± 0.35	27.71 ± 0.01 to 29.35 ± 0.02
	Katrina	2.06 ± 0.07 to 2.19 ± 0.00	374 ± 23 to 527 ± 130	12.55 ± 0.30 to 14.25 ± 0.93	26.03 ± 0.06 to 34.73 ± 0.27
3/14/2013	Sq. Hand.	2.09 ± 0.03 to 2.53 ± 0.02	352 ± 40 to 437 ± 26	11.48 ± 0.21 to 14.09 ± 1.94	25.42 ± 0.18 to 31.13 ± 0.07
	USM	2.08 ± 0.01 to 2.22 ± 0.07	221 ± 25 to 284 ± 29	7.64 ± 0.95 to 10.61 ± 2.20	25.68 ± 0.22 to 28.95 ± 0.14
4/2/2013	Legacy	2.26 ± 0.03 to 2.35 ± 0.16	263 ± 13 to 289 ± 0	9.74 ± 0.67 to 10.79 ± 0.61	24.20 ± 0.00 to 25.68 ± 0.05
	Katrina	2.72 ± 0.00 to 3.02 ± 0.16	179 ± 0 to 825 ± 240	10.73 ± 0.17 to 11.99 ± 0.22	15.18 ± 0.04 to 66.20 ± 0.29

APPENDIX H

TRANSECT WATER QUALITY DATA

Water temperature and salinity data for transect data collected from surface and bottom waters at the artificial reef sites.

Date	Reef	Surface Temp (°C)	Bottom Temp (°C)	Surface Sal. (ppt)	Bottom Sal. (ppt)
5/8/2012	Sq. Hand.	27.12	27.01	9.70	9.74
	USM	27.79	27.47	14.19	14.19
5/14/2012	Legacy	25.65	25.18	17.83	17.82
	Katrina	27.01	25.65	19.91	19.98
5/30/2012	Sq. Hand.	30.1	29.9	13.9	13.9
6/2/2012	USM	26.6	26.5	24.2	24.3
	Legacy	29.4	29.3	15.1	15.4
	Katrina	25.9	26.6	24.7	28.2
6/21/2012	Sq. Hand.	26.7	27.1	18.0	18.6
	USM	28.0	27.6	22.9	23
6/26/2012	Legacy	30	29.1	23.4	23.8
	Katrina	29.1	28.8	25.3	26.1
7/11/2012	Sq. Hand.	28.4	28.4	18.4	18.4
	USM	28.2	28.1	22.4	22.5
	Legacy	29.3	29.5	23.8	17.7
	Katrina	29.7	29.6	24.6	24.5
7/24/2012	Sq. Hand.	28.6	30.2	15.3	19.2
	USM	30.4	31.0	22.1	22.6
7/30/2012	Legacy	31.2	31.3	24.1	25.1
	Katrina	32.3	31.0	24.4	24.9
8/7/2012	Sq. Hand.	30.2	30.7	NA	NA
	USM	30.4	30.0	NA	NA
	Legacy	30.4	30.3	NA	NA
	Katrina	32.3	31.0	NA	NA
8/21/2012	Sq. Hand	28.3	28.4	NA	NA
	USM	29.0	28.7	NA	NA
9/11/2012	Legacy	27.3	27.3	NA	NA
	Katrina	28.1	28.1	NA	NA
9/27/2012	Sq. Hand.	26.4	26.4	NA	NA
	USM	27.1	26.6	NA	NA
	Legacy	28.2	27.7	NA	NA
	Katrina	28.0	27.8	NA	NA
10/17/2012	Sq. Hand.	23.4	NA	10.6	NA
	USM	27.7	NA	17.5	NA
10/23/2012	Legacy	23.5	22.7	21.4	21.7
	Katrina	23.2	23.2	24.2	24.4
11/5/2012	Sq. Hand.	19.3	19.4	21.7	22.2
	USM	20.1	20.1	27.6	27.7
	Legacy	20.1	20.1	28.4	28.5
	Katrina	20.6	20.5	27.1	28.5
11/15/2012	Sq. Hand.	14.4	14.4	23.1	23.2
	USM	14.3	14.2	28.9	29.0
12/2/2012	Legacy	16.7	16.6	28.5	28.7
	Katrina	16.6	16.5	28.0	28.1
2/1/2013	Sq. Hand.	13.5	13.6	2.8	2.9

	USM	15.0	14.9	12.3	12.4
	Legacy	14.6	14.4	12.7	12.8
	Katrina	15.1	15.3	15.2	23.9
3/14/2013	Sq. Hand.	14.3	15.4	11.1	15.5
	USM	16.0	15.4	19.7	19.8
4/2/2013	Legacy	19.3	19.0	21.6	21.8
	Katrina	19.9	18.7	20.1	26.6

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