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OCEAN ACIDIFICATION: UNDERSTANDING THE COASTAL CARBON PUMP IN A HIGH CO2 WORLD

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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List of Abbreviations

APase: Alkaline Phosphatase

BGase: β-1,4 glucosidase

BGE: Bacterial Growth Efficiency

BP: Bacterial Production

BR: Bacterial Respiration

CBase: 1, 4-B-Cellobiosidase

CCM: Carbon Concentrating Mechanism

C:N: Carbon to Nitrogen Ratio

DIC: Dissolved Organic Carbon

DOC: Dissolved Organic Carbon

HCl: Hydrochloric acid

IPCC: Intergovernmental Panel on Climate Change

LAPase: Leucine Aminopeptidase

pCO_2: Partial pressure CO_2

POC: Particulate Organic Carbon

ppm: Parts Per Million

Abstract

OCEAN ACIDIFICATION: UNDERSTANDING THE COASTAL CARBON PUMP IN A HIGH CO_2 WORLD

By Rachel D. Cooper, Master of Science

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2012

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Since the 1800s, carbon dioxide emissions due to human activities have contributed significantly to the amount of carbon in the atmosphere. Approximately a third of this carbon is absorbed by the ocean, through air-sea fluxes at the ocean surface (Sabine, 2004). Increased CO_2 has changed the carbon chemistry of the ocean and hence the pH. pH is expected to drop by 0.4 by the year 2100. It is unclear how this lower pH will affect carbon cycling and sequestration with respect to the biological carbon pump. Most studies have focused on open ocean phytoplankton or bacterial communities in large, stationary mesocosms. Few studies have coupled both phytoplankton and bacterial processes and even fewer have investigated coastal communities, where pH and pCO_2 can vary drastically. This study focused first on developing and evaluating a mesocosm and alternative method for elevating pCO_2. The second goal was to determine how potential changes in phytoplankton DOC release and community structure and the resulting

carbon pool may affect bacterial secondary production and ectoenzyme activity in a natural coastal community. Mesocosms aimed to mimic natural pCO_2 fluctuations by maintaining CO_2 concentration of 1250 ppm in the headspace, as aqueous pCO_2 may change with biological processes. Six mesocosms were filled with 40L of water from the Chesapeake Bay (three ambient pCO_2 and three 1250 ppm) and monitored over 15 days. Chlorophyll *a*, DOC, bacterial respiration, bacterial production, and enzyme activity were measured. Bacterial production and respiration were used to calculate bacterial growth efficiency (BGE). Results showed that there was no significant difference between the ambient and elevated groups with respect to chlorophyll *a*, DOC, BGE and enzymes activity. However, differences in bacterial respiration and bacterial production during the first four days of the experiment may suggest that bacteria require time to acclimate to elevated pCO_2. Phytoplankton and bacteria in coastal areas are exposed to a wide range of abiotic factors such as seasonal temperature variations, salinity, mixing, and terrestrial inputs. The pH of the Chesapeake Bay ranges between 7.5 and 8.3, and it is possible that the phytoplankton and bacteria are adapted to cope with a wide range of pH (Wong, 2012). This study suggests that the biological carbon pump may not be significantly altered in our future ocean.

INTRODUCTION

Since the Industrial Revolution around the 1800s, carbon dioxide emissions due to human activities have contributed significantly to the amount of carbon in the atmosphere. Approximately a third of this carbon is absorbed by the ocean, through air-sea fluxes at the ocean surface (Sabine, 2004). This exchange forms carbonic acid, as well as carbonate and bicarbonate, in different proportions, in the following reaction:

$$H_2O+CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad \text{(Equation 1)}$$

Increased hydrogen ions produced in this reaction from the dissociation of carbonate and bicarbonate decreases pH, most dramatically at the surface. Ocean surface pH has decreased by 0.1 since the beginning of the Industrial Revolution and is expected to drop by approximately 0.4 by 2100. This is the fastest rate of decrease and the lowest pH in millions of years. (Meehl et al., 2007; Turley, 2006). Since the pH scale is logarithmic, a decrease of one pH unit results in an order of magnitude increase in acidity. The partial pressure CO_2 (pCO_2) of today's ocean is approximately 380 parts per million (ppm). The Intergovernmental Panel on Climate Change (IPCC) has predicted two possible scenarios for pCO_2 levels by the year 2100: 750 ppm (business as usual scenario) or 1050 ppm (worst case scenario) (IPCC, 2001). More recent predictions suggest levels of approximately 1250 ppm (Caldeira and Wickett, 2005; Findlay et al., 2008) for 2100.

It is unclear how this change in chemistry will affect the dynamics of the ocean carbon cycle. Phytoplankton and marine bacteria are integral components of the ocean's carbon cycle and drive the biological carbon pump (Ducklow, 1995). The term "biological pump" refers to the

algal mediated uptake and fixation of CO_2 in the surface ocean and the subsequent sequestration of this organic carbon in the deep ocean. A substantial portion of phytoplankton biomass is transformed into dissolved organic carbon (DOC) via zooplankton "sloppy feeding", viral lyses, and senescence, all of which fuels the microbial loop (Azam et al, 1983). DOC is characterized by a range of bioavailability (labile, semi-labile, or refractory) which controls its metabolism or persistence. Turnover times for the highly labile DOC pool is on the order of minutes to hours versus thousands of years for the most refractory pool (Hansell, 1998, Davis, 2007). Organic carbon that is not consumed by bacteria in the surface layers may then be exported and sequestered in the deep ocean.

A decrease in surface ocean pH may impact phytoplankton abundance, stoichiometry, and community composition as well as the amount and quality of DOC and therefore its processing by bacteria. In general, open ocean and fjord studies of this concept have demonstrated an increase in phytoplankton production with an increase in pCO_2 (Schippers, 2004; Egge, et al., 2009). Other studies found changes in algal community composition, stoichiometry, and increases in the proportion of DOC relative to POC (Tortell, et al., 2002; Kim et al, 2011). With respect to the microbial loop, multiple changes have been reported including increased bacterial production, elevated bacterial ectoenzyme activity and enhanced polysaccharide degradation (Grossart, 2006, Piontek, 2010, Yamada, 2010). Collectively these studies suggest that increased pCO_2 will affect the fate of oceanic carbon. However, it is unclear how these changes will manifest themselves in terms of the biological pump and ocean carbon storage. Most studies on ocean acidification have focused solely on either the phytoplankton or microbial communities and have not assessed the potential links among alterations in the

phytoplankton community composition, the amount and quality of the resulting DOC, and its metabolic fate.

Few ocean acidification carbon cycling studies have investigated effects in ecosystems such as estuaries and the coastal ocean which have significant seasonal and annual variation in pCO_2 and pH (Joint, 2012). Recent studies have suggested that coastal phytoplankton communities are fairly resilient to pH change (Nielsen et al., 2012; Berge et al., 2012). Even though there has been speculation that estuaries and coastal systems will not be impacted by increased pCO_2, particularly with respect to the functioning of the microbial loop, there have been no experimental studies which directly test this.

The impact of increased pCO_2 on the biological pump has been primarily studied in large mesocosms, positioned at fixed locations, thus prohibiting comparative investigations across varying environmental regimes (Allgaier et al., 2008; Egg et al., 2009; Grossart et al., 2006; Riebesell et al., 2007). Furthermore, while pCO_2/pH manipulation across experimental studies have varied, many investigations have employed the bubbling of pre-mixed CO_2 into the aqueous medium, which may result in production of transparent exopolymer particles (TEP), cellular lysis, and unnecessary stress on cells. It is unclear how these potential consequences of constant bubbling of CO_2 affect factors such as carbon lability, phytoplankton community composition, and other associated factors.

This study had four objectives:

1. Develop and evaluate a new method system and alternative method for pCO_2 manipulation.
2. Determine changes in chlorophyll *a* concentrations and subsequent DOC release in response to elevated pCO_2 levels.

3. Determine changes in bacterial production, bacterial respiration, and bacterial growth efficiency in response to potential changes in DOC as well as elevated pCO_2 levels.
4. Determine changes in extra-cellular enzyme activity in response to changes in DOC composition and elevated pCO_2 levels.

By coupling the phytoplankton and bacterial communities, it can be determined how a change in one community may affect the other. Determining these changes will help to determine how the coastal carbon pump will function in a future high CO_2 world.

METHODS

Site Description

The Chesapeake Bay is the largest estuary in the United States (total watershed area of 172, 000 km^2) and is fed by the Susquehanna, Potomac, and James Rivers as well as many smaller tributaries. The Susquehanna and Potomac Rivers deliver approximately 50% and 18% total input to the northern Bay, respectively. In the southern portion of the Bay, the James River adds approximately 16% of the total freshwater input before the Bay connects to the Atlantic (Pritchard, 1952). The retention time of the Bay is approximately 42 days (Baird and Ulanowicz, 1989). Phosphorus is the limiting phytoplankton nutrient in the spring, whereas nitrogen is limiting in the summer and early autumn (Prasad et al, 2012). Dissolved organic carbon (DOC) and particulate organic carbon (POC) concentrations vary with season, primary production and salinity but average 172 ± 19 μM and 43 ± 6 μM, respectively, at the mouth that connects to the Atlantic (Fisher et al, 1998). Surface temperature varies from 21.4º to 28.9ºC in the summer and 2.3º to 5.7ºC in the winter (Ritchie and Genys, 1975). Alkalinity in the southern Chesapeake and mouth is linearly related to salinity, and pH ranges between 7.5 and 8.3 (Wong, 2012).

Water collection

Water was collected aboard *The Gabriel* the morning of April 25, 2012 at approximately 26º59'38"N, 76º19'35'W, near the mouth of the Bay (Figure 1). At the time of collection, water pCO_2 was 461 ppm, and air pCO_2 was 427 ppm. Water was pumped from approximately 0.5 meters below the surface using a fountain pump and garden hose. Water was collected in fourteen acid washed 19-liter polycarbonate jugs. Temperature variations in water samples were minimized by placing them in the shade. Samples were transported from Virginia Beach to

Richmond (approximately one hour) and pumped into mesocosms upon arrival to the greenhouse in Virginia Commonwealth University Trani Life Science building.

Mesocosm Design and Set-up

Mesocosms were constructed from a clear polyethylene (pp 5 plastic) Kis Omnibox® containers (50 liters, 23"x 15"x 11 2/3", acid leached with 10% HCl) with a sealed lid made from a sheet of Optix Plaskolite acrylic (0.08 inches thick) to allow for transmission of visible light and approximately 20 % of UV light (based on manufacturer statistics). The container and the plexiglass were sealed together with 3M^{TM} 5200 sealant and 32 stainless steel bolts (Figure 2). One sampling port (Beckson® Marine 4" clear screw in deck plate, Model # 11743028 | Manuf. # C13102WM) was installed in the plexiglass to allow for sampling and cleaning purposes, as well as two one-way air valves for air sampling (air- intake valve: 5 ml syringe stop cock, air out valve: 3M Niosh respirator, TC-84A-1229). Mesocosms were tested for air and water tightness by filling mesocosms with water and checking for water leaks and submerging the mesocosm in water to check for air leaks. Completed mesocosms were acid leached with 10% HCl and rinsed prior to the start of the experiment. Mesocosms were incubated in the VCU Trani Life Science building greenhouse in circulating water baths to maintain water temperature. Water baths were constructed of wood and a pond liner, and were large enough to hold three mesocosms (Figure 3).

pCO_2 manipulation

An air-gas mixture of 1250 ppm CO_2 (Air-Gas welders) was utilized in experimental mesocoms based on studies by Caldeira and Wickett (2005) and Findlay et al. (2008). At time 0,

approximately 40L of the collected water was placed in each mesocosm. Pure CO_2 was bubbled into the water in three of the mesocosms using an aeration stone (Top Fin Mini Air Stone) for approximately three minutes, until the target pCO_2 of 1250 ppm was reached. The aeration stone was chosen to minimize bubbling to reduce phytoplankton mortality and transparent exopolymer particles (TEP) (Hurd et al., 2009, Gattuso et al., 2009). Mesocosms were not bubbled again. Constant pCO_2 was maintained during the duration of the experiment by monitoring pCO_2 in the head space (air/water equilibrium) using the air-gas mixture. pCO_2 measurements were taken daily from both the headspace and the water in each mesocosm using an Environmental Gas Monitor (EGM). To measure the pCO_2 of the water a mini-module, which strips the water of CO_2 was attached to the EGM (Cole and Prairie, 2009). Dri-rite was used to dry the air before it entered the EGM.

Mesocosm Sampling

At time 0, nutrients were added to each mesocosm (final concentrations: $NaNO_3$=25 µmol/L, KH_2PO_4=2 µmol/L, $Na_2SiO_3 \cdot H_2O$=12 µmol/L) to stimulate a phytoplankton bloom. Mesocosms were stirred once a day using a peristaltic pump before sampling. Those mesocosms designated at "elevated" were connected to the air-gas mixture and those that were designated as "ambient" were left open to equilibrate with the ambient pCO_2, which was between 420 ppm and 450 ppm. Chlorophyll *a*, DOC, bacterial production (BP), bacterial respiration (BR), and C:N ratio were taken at times 0, 1, 2, 3, 4, 6, 8, 10, 12, and 15 days, in triplicates and extra-cellular enzymes were sampled at times 1, 6, and 15 days between April 25, 2012 and May 10, 2012. A total of 15 of the 40 liters in each mesocosm were removed for sampling during the duration of the experiment.

Analytical Methods

Chlorophyll *a*

Chlorophyll *a* samples (120 mL) were collected on a 25 mm (0.7 nominal pore size) Whatman glass-fiber filters and stored frozen (4ºC) until analysis. After thawing filters were placed in 15 mL vials with 10 mL of acetone for 24 hours. Fluorescence was measured using a Turner System Fluorometer (TD-700). Samples were run in the VCU Environmental Analysis Lab.

Dissolved Organic Carbon (DOC)

Samples were filtered through a 47 mm (0.7 nominal pore size) pre-combusted (525°C, 4 hours) Whatman glass-fiber filter. 35 mL of sample was placed in a 40 mL amber DOC vial (previously acid leached in 10% hydrochloric acid (HCl) and combusted at 525°C for 4 hours). Samples were acidified to a pH of 2 with 200 µl of concentrated HCl to remove any inorganic carbon and refrigerated in the dark until processed. Samples were analyzed by high temperature combustion on a Shimadzu TOC analyzer by the Environmental Analysis Lab (VCU).

Bacterial Production (BP)

Bacterial production was measured by ^{3}H-leucine incorporation. A 1.5 mL aliquot of sample was added to a microcentrifuge tube, followed by the addition of 40 nM ^{3}H-leucine and incubated in the dark for approximately an hour. Incubations were terminated with the addition of 100 µl of 100% trichloroacetic acid (TCA) and samples were stored in the dark at 4ºC until processing (within 24 hours) by the method outlined by Schultz (1999). Blanks were run each

day using the same method. Samples were run on a liquid scintillating counting with a Beckman 6000 IC after the addition of 1 ml scintillation cocktail.

Bacterial Respiration (BR)

Unfiltered water from each mesocosm was placed in biological oxygen demand bottles (350 ml) with Presense Sensors attached. The bottles were incubated in the dark in a water bath (approximately 21ºC) for the duration of the sampling period. At hour 0, 12, 24, and 48 an oxygen reading was taken using the PreSens FIBOX 3 Fiber-optic oxygen meter and computer program. Oxygen consumption was determined by subtracting the t=24 oxygen level from the t=0 oxygen level (mg/L) or as shown in Equation 2:

$$\text{Oxygen (mg/L)}_{24}\text{-Oxygen (mg/L)}_{0}\text{=Oxygen consumed over 24 hours} \quad \text{(Equation 2)}$$

Oxygen concentration was converted to carbon using a respiratory quotient of 1 (McCallister & del Giorgio, 2008).

Bacterial Growth Efficiency (BGE)

Bacterial production was converted from an instantaneous rate to a cumulative rate using Equation 3, cited by Roland & Cole (1999):

$$BPI\ (\mu gC\ l^{-1}d^{-1}) = [(BP_{T1}/k)e^{kT1}] - [(BP_{T0}/k)e^{kT0}] \quad \text{(Equation 3)}$$

In which $k=(\ln BP_{T1}-\ln BP_{T0})/T1$, BPI stands for the integrated bacterial production, BP_{T0} is the instantaneous bacterial production at time zero and BP_{T1} is the instantaneous bacterial production at the end of the incubation. Bacterial respiration was calculated using the carbon consumption rates over 24 hours. BGE was calculated using the following equation:

$$BGE=BP/(BP+BR)\ x100 \quad \text{(Equation 4)}$$

Extra-cellular enzymes

The activity of four extra-cellular enzymes were determined in this experiment: Leucine Aminopeptidase (LAPase), Alkaline Phosphatase (APase), B-1,4 glucosidase (BGase), and 1, 4-B-Cellobiosidase (CBase) (Table 1).

Enzymes assays were performed in Costar 96 Non-Treated, Flat Bottom, Non sterile Assay Plates (Costar #3915) at concentrations of 0.4 mM for BGase and LAPase and 1.0 mM for APase and CBase. Reaction time for BGase was approximately five hours, while CBase, APase, and LAPase were incubated for approximately two hours (Table 1). All activity measurements were made using BioTekSynergy II microplate reader and Gen5 software version 1.07 located in the Franklin Lab. Wavelengths were read on the Biosynergy II (excitation 360 nm and emission at 460 nm).

Statistical analysis

All statistical analysis was done in JMP (version 9) statistical software. Significance was established at $\alpha=0.05$. A repeated measures ANOVA was conducted for chlorophyll *a*, DOC, BR, BP and BGE measurements to determine if the two treatments differed in their response over time. Since the assumption that time intervals are equally spaced was not met, the time course was divided into two sets. For the first five days of the experiment samples were equally spaced at 24 hour intervals, and hence were grouped together. The remaining 10 days of the experiment were sampled approximately every other day (day 6, 8, 12, 15) and therefore combined. A student's two tailed t-test was conducted to compare ambient and elevated means on individual days.

A two-way ANOVA was conducted for extra-cellular enzyme activity data. To determine correlations, a multivariate correlation was conducted if the distribution was normal while a Spearman's test was done for non-normal distributions.

RESULTS

Assessment of Mesocosm and Experimental Design

The goal of the mesocosms and the experimental design was to initiate a phytoplankton bloom and subsequent senescence via the addition of inorganic nutrients as well as first hit initial target pCO_2 (1250 ppm) on Day 0 and then maintain target headspace pCO_2 levels in the mesocosms. The aqueous pCO_2 was similar among triplicate treatments and ambient mesocosms with standard deviations less than 45 ppm in both groups. Initial and secondary phytoplankton blooms were detected on Days 3 and 6, respectively, through elevated chlorophyll *a* concentrations followed by a chlorophyll *a* decline and bloom senescence on Day 8. Aqueous pCO_2 concentrations were altered by the phytoplankton CO_2 draw down on days 3 and 6 (Figure 4). However, the elevated aqueous pCO_2 decreased dramatically going from 1330 ± 0 ppm on Day 0 to 738 ± 44 ppm, whereas the ambient went from 496 ± 0 ppm on Day 0 to 385±4.5 ppm by Day 4. Elevated and ambient mesocosm pCO_2 was significantly different each day (two-tailed t-test $p<0.05$). The headspace CO_2 concentrations in the elevated and ambient groups were also successfully maintained over the 15 days. The temperature variation between all six mesocosms was less than 1ºC.

Phytoplankton Responses to Ambient and Elevated pCO_2 levels

The addition of phosphate, nitrogen, and silicate stimulated an initial phytoplankton bloom on Day 3, followed by a second bloom on Day 6 in both the elevated and ambient groups (Figure 5). Concentrations of chlorophyll *a* during the initial bloom for the elevated and ambient treatments were 7.1 ± 0.5 µg/L and 7.6 ± 0.4 µg/L, respectively, and 8.7 ± 4.1 µg/L and 7.9 ±0.4 µg/L, respectively, in the second bloom. The second Chlorophyll *a* bloom was slightly larger

than the first in both the treatment and control incubations but was not significantly different (two-tailed t-test $p>0.05$). Post bloom concentrations between Days 6 and 15 dramatically decreased by 7.8 mg/L and 7.1 mg/L in the elevated and ambient groups, respectively. However, a repeated measures ANOVA determined that there was no significant difference between the two groups ($p>0.05$).

The DOC concentrations in both the elevated and ambient mesocosms followed the same trends over the 15 days (Figure 6). From Day 0 to Day 3 DOC increased in both groups and peaked at 2.9 ± 0.2 mg/L for the elevated group and 2.8 ± 0.1 mg/L for the ambient. DOC concentrations were lowest on Day 6 in the elevated and ambient incubations with values of 2.6 ± 0.2 mg/L and 2.5 ± 0.1 mg/L, respectively. From Day 8 through Day 15, DOC average concentrations increased steadily in both incubations, 0.07 $mgL^{-1}d^{-1}$ in the elevated and 0.08 $mgL^{-1}d^{-1}$ in the ambient, which is consistent with the phytoplankton die-off after the second bloom. The p-value generated by repeated measures ANOVA was not significantly significant in the two groups over time ($p>0.05$).

Bacterial Responses to Ambient and Elevated pCO_2 levels

Bacterial production was relatively low between Days 0 and 6 for both groups, peaked at Day 8 and then declined between Days 10 and 15. Both the ambient and elevated pCO_2 groups had the highest bacterial production on day 8, 101.49 ± 35.76 µgC $L^{-1}d^{-1}$ and 106.27± 11.92 µgC $L^{-1}d^{-1}$, respectively (Figure 7). Smaller peaks occurred on Days 2 and 4 in both groups (Figure 7), which correspond to the slight increase in DOC between Days 0 and 3. According to a repeated measures ANOVA, there were no significant differences in the two groups over time

($p>0.05$). Additionally, the two groups were significantly different according to a student's t-test on day 0 ($p<0.005$), day 3 ($p<0.05$) and day 6 ($p<0.005$).

Bacterial respiration (BR) in the elevated incubations was relatively low and constant between Days 0 and 4, peaked at Days 6 and 8 and then decreased between Days 10 and 15. The ambient incubations followed a similar trend but was highly variable between Days 0 and 4. BR was highest at Day 6 and Day 8 for both treatments (Figure 8). At Days 0, 2, and 3 carbon respired was noticeable higher in the ambient group than the elevated group by 210.8 µg/L/d ($p<0.005$), 220 µg/L/d ($p>0.05$) and 202.5 µg/L/d respectively ($p<0.05$). However, only days 0 and 3 were significantly different. Results from a repeated measures ANOVA indicated that there was a significant difference between days 0 and 4 ($p=0.042$) but not between days 6 and 15 ($p>0.05$).

Bacterial growth efficiency (BGE) in the two incubations was similar over time. BGE at Day 0 for the ambient group was 15 ± 2% and 22 ± 5% for the elevated group. After a dip at Day 2 (essentially the rate between days 2 and 3), BGE increased for the remainder of the experiment and by day 10 BGE was 61 ± 8% in the ambient and 64 ± 2% in the elevated (Figure 9). The p-values resulting from a repeated measures ANOVA were not low enough to establish significance ($p>0.05$).

Enzyme Activity at Ambient and Elevated pCO_2 Levels

In general, activity for all enzymes was lowest at Day 1 and highest at day 15. At Day 1 1, 4-B-Cellobiosidase (CBase) had the least activity (ambient: $5x10^{-3}\pm 8x10^{-4}$ nmol/h^{-1}/ml^{-1}, elevated: $4x10^{-3} \pm 4x10^{-4}$ nmol/h^{-1}/ml^{-1}) and Leucine Aminopeptidase (LAPase) had the most activity (ambient: 0.62 ± 0.04 nmol/h^{-1}/ml^{-1} elevated: 0.54±0.02 nmol/h^{-1}/ml^{-1}). LAPase had the

highest activity by Day 15 (ambient: 0.03±0.01 nmol/h^{-1}/ml^{-1} elevated: 1.045±0.083 nmol/h^{-1}/ml^{-1}) and BGase had the least activity (ambient: 0.04±0.005 nmol/h^{-1}/ml^{-1}, elevated: 0.03±0.01 nmol/h^{-1}/ml^{-1}). LAPase activity steadily increased over the fifteen days of the experiment (Figure 10a). The elevated group increased from 0.54 to 1.05 nmol/h^{-1}/ml^{-1} from Day 1 to Day 15, while the ambient group increased from 0.62 to 0.89 nmol/h^{-1}/ml^{-1}. Between Day 1 and Day 6 CBase activity increased slightly whereas there was a dramatic increase of eleven-fold in the ambient group and fifteen-fold in the elevated (Figure 10b). B-1,4 glucosidase (BGase) slowly increased between Days 1 and 6 and then rapidly increased from Day 6 to Day 15 by 0.01 nmol/h^{-1}/ml^{-1} in both incubations (Figure 10c). Alkaline Phosphatase (APase) production remained relatively constant between Days 1 and 6 but increased six fold between days 6 and 15 (Figure 10d). There were no significant difference between the elevated and ambient groups according to a two-way ANOVA test for any of the enzymes ($p>0.05$).

DISCUSSION

Few ocean acidification studies couple phytoplankton and bacterial responses and only phytoplankton have been previously studied in samples collected from a coastal location. Simultaneous assessment of both phytoplankton and bacterial responses to ocean acidification allows for a more complete understanding of the potential impacts to the coastal carbon pump in a changing climate. Since estuaries and coastal systems are dynamic and economically important ecosystems, it is of top priority to understand how they may respond to fluctuations in pCO_2 associated with ocean acidification. This study utilizes an innovative mesocosm method for testing potential alterations in phytoplankton, bacteria, and carbon dynamics resulting from ocean acidification.

Mesocosm Evaluation

There are many variables to consider when designing an ocean acidification study such as type of pCO_2/pH manipulation, culture versus natural algal and bacterial communities, laboratory experiment versus mesocosm, etc. Researchers must assess and determine the method that best suits their objectives. Currently, the consequences of ocean acidification on phytoplankton and/or bacterial communities and carbon cycling have been studied using a variety of methods. The inconsistencies in these various methodologies have often been blamed for conflicting or equivocal results, such as bubbling versus acid addition effects on maintaining alkalinity and calcification debated by Iglesias-Rodriguez et al. (2008) and Riebesell et al. (2008). This study developed and employed a novel mesocosm model that directly elevated aqueous pCO_2 with minimal turbulence and could be deployed at multiple field sites. This

mesocosm design minimizes pCO_2 bubbling, which may cause cellular lysis or increased TEPs (Hurd et al., 2009, Gattuso et al., 2009). To better mimic natural systems, the aqueous pCO_2 was initially elevated in experimental mesocosms to the designated level using pure CO_2 and then the headspace was maintained at the same concentration throughout the experiment. Thus, while there may be fluctuations in the aqueous pCO_2, the atmosphere is maintained at a constant value. To accomplish this, elevated mesocoms were bubbled with pure CO_2 at Day 0 to reach the target pCO_2 of 1250 ppm.

The differences in elevated and ambient BR and BP between Day 0 and 4 may have been due to bubbling on Day 0. Thus, other studies may consider eliminating or further reducing the amount of bubbling. The headspace in each elevated mesocosm was continuously flushed with an air-gas mixture of 1250 ppm while the ambient mesocosms were left open to equilibrate with the ambient atmosphere CO_2 which ranged between 420 and 450 ppm and was well within the range of pCO_2 measured at the collection site (427 ppm). Elevated pCO_2 mesocosms were constantly flushed with a small stream of 1250 ppm air-CO_2 mixture. When pressure built up, air was released through a one way valve (Figure 2). The one-way valve minimized the escape of elevated pCO_2, alleviating potential concerns that flushing elevated tanks would alter the overall pCO_2 of the greenhouse.

Not only did this mesocosm system mimic natural processes but it was inexpensive and portable. Construction of each mesocosm costs less than $50 and each air-gas mixture tank was $70. The mesocosms were made out of materials that can be found at any local hardware store and were relatively simple to assemble, in contrast to most mesocosms used in ocean acidification studies which are large and unwieldy, fixed in one location, and inaccessible to

many scientists. This method offers a smaller, cost efficient, portable method that can be used in any aquatic or marine system.

Chlorophyll *a* and DOC release

Ribulose-1, 5-bisphohsphate carboxylate/oxygenase (RubisCO) is the main algal enzyme that fixes carbon during photosynthesis. However, RubisCO has a low CO_2 affinity and high affinity for O_2 and is thought to have evolved when the oceans had low levels of O_2 and high levels of CO_2 (Giordano et al., 2005). In today's ocean, the dissolved inorganic carbon (DIC) concentration is lower than the half-saturation constant of RubisCO. To combat the low CO_2 affinity coupled with the relatively low DIC concentration in today's ocean, many photosynthesizing organisms have developed carbon concentration mechanisms (CCMs) that concentrate CO_2 in the vicinity of RubisCO. Since ocean acidification increases the DIC concentration, primary production will likely increase because of reduced energetic constraints associated with CCM activity. Thus the energy and maintenance required to utilize the CCMs can be allocated elsewhere in the cell. Those organisms that do not use CCMs may also increase production simply because the higher DIC concentration will decrease the CO_2/O_2 competition for RubisCO. However, even if more DIC is fixed due to the higher concentrations, phytoplankton are rarely considered carbon-limited, and therefore the availability of other nutrient inputs may ultimately affect the primary production more strongly than DIC concentration. Changes in ocean chemistry could potentially alter enzyme form and function as well as the ability of organisms such as coccolithophores to build calcium carbon skeletons. The resulting hypothesized change in primary production, community composition, and function may also increase and/or alter the amount, quality and subsequent lability of DOC released by

phytoplankton. Some open ocean studies on phytoplankton have suggested this will be true of the future ocean (Riebesell et al, 2007; Schippers et al., 2004; Engel et al., 2005) whereas other studies have concluded the opposite (Tortell et al., 2002).

This study found no significant difference in chlorophyll *a* between the elevated and ambient treatments (Figure 5). There were peaks on Days 3 and 6 in both groups, the first stimulated by a nutrient addition at Day 0, the second conceivably by the regeneration of nutrients via the microbial loop. Post-bloom concentrations between Days 6 and 15 dramatically decreased by 7.8 mg/L and 7.1 mg/L in the elevated and ambient groups, respectively, presumably due to nutrient limitation. Elevated alkaline phosphatase activity at Day 15 supports this interpretation because production and activity of APase increases with $(PO_4)_3$ limitations (Tanaka et al., 2008). It is also interesting to note that the draw down in CO_2 was higher in the elevated treatment than the ambient (Figure 4). This is consistent with the findings of Riebesell et al. (2007), in which pCO_2 draw down was much higher in the elevated group. This was linked to a higher C:N ratio in the elevated group (Riebesell et al., 2007; Nielsen et al., 2012), which may be the case in this experiment. An increased C:N ratio would lower the nutritional value of organic carbon fixed by primary producers and could ultimately affect the efficiency of bacteria degradation (Liu et al., 2010). This could lead to an excess of CO_2 sequestration potential in the ocean because more carbon is fixed by photosynthesis but less is degraded and remineralized to DIC due to a less efficient bacterial loop.

DOC concentrations (Figure 6) follow the chlorophyll *a* trend, with the lowest DOC concentrations mirroring the peak in chlorophyll *a* on Day 6 and then increasing by 50% during the final chlorophyll *a* decline between days 10 and 15. DOC concentrations were similar between the elevated and the ambient group over the fourteen day experiment. In situ DOC

concentrations (3.1 mg/L) were consistent with what has previously been observed in the Chesapeake Bay (Fisher et al, 1998). Collectively, the chlorophyll *a* and DOC trends suggest that coastal phytoplankton chlorophyll *a*, as well as DOC release, will not be altered by pCO_2 levels of 1250 ppm. Coastal phytoplankton is frequently exposed to a wider range of pH than marine bacteria due to riverine discharge, nutrient inputs, algal blooms, seasonal temperature variation, and pollutions. Hence their enzymes may be resilient to changes in pH and their CCMs are not significantly altered. However, it is unclear from the current data whether there was a change in phytoplankton community structure or DOC molecular characteristics.

Bacterial Respiration, Production, and BGE

Potential changes in the amount and lability of DOC released by phytoplankton due to ocean acidification may have profound effects on bacterial production, respiration, extra-cellular enzyme production and overall efficiency of carbon utilization. The allocation of DOC to either respiration or bacterial biomass production controls the role of the microbial loop in regeneration of bioavailable carbon and carbon sequestration (source versus sink). Therefore a fundamental understanding of these processes is critical to evaluate carbon cycling in coastal zones. Bacterial growth efficiency (BGE) is a measure of the amount of bacterial biomass created per unit of carbon consumed (Carlson et al., 2007). Generally, BGE is low when nutrients and/or energy is limiting or pollutants and stressors are high. When low BGE occurs, cells allocate less carbon to biomass and growth and more towards maintenance and respiration (Carlson et al., 2007). Ocean acidification may alter multiple parameters governing BGE including: amount and quality of DOC released, nutrient availability and energetic demands associated with enzyme production which may have positive or negative feedbacks on BGE.

The elevated incubation had lower BP and BR than the ambient during the beginning of the experiment (Figure 7 and Figure 8). Since no differences were observed in chlorophyll *a* concentrations, DOC concentration, and enzyme production, this could suggest that bacteria required time to acclimate to the change in pCO_2. Although pCO_2 may increase to 1250 ppm by 2100, this will occur slowly over time. In this experiment, the pCO_2 concentration was changed dramatically in a short amount of time. As there was no significant differences among treatments between Days 8 and 15, it suggest that after acclimation, the bacteria function and process carbon much like they do in today's ocean. Hence future studies may

Both BR and BP can be altered in response to DOC concentrations, lability, and availability because DOC is the major carbon source for bacteria. BR peaks in both groups on Days 6 and 8, when DOC is relatively low and decrease as DOC concentrations increase for the remainder of the experiment. However, bacterial production peaks on day 8 slightly after the phytoplankton bloom on day 6 when DOC is beginning to increase. Bacterial production decreases slowly over the remainder of the experiment, despite an increase in DOC, perhaps due to nutrient limitation or the release of more recalcitrant DOC. Bacteria allocate the carbon to biomass when DOC concentrations are high, and to maintenance of the cell when DOC is low. Bacterial production and bacterial respiration were not significantly correlated (Elevated: r^2=0.7124, p=0.0713, Ambient: r^2=0.7143, p=0.0713) in either of the two groups, which is consistent with other studies (Del Giorgio and Cole, 1998, Russell and Cook, 1995).

BGE ranges previously found in coastal regions and estuaries are between 10% and 60% but are highly variable (del Giogio & Cole, 1998). BGE is typically higher in coastal compared to marine areas due to relatively high nutrient availability, and DOC availability and quality in coastal ecosystems. Generally, BGE increased over time in both treatments except at Day 2 and

Day 8 (Figure 9). By the end of the experiment, BGE was as high as 60.0 ± 7.9% in the ambient and 64.2 ± 2.2% indicating that over half of DOC consumed was allocated to growth. This is consistent with high DOC concentration (Figure 6). However, BGE was not correlated with DOC concentrations in either of the groups (Ambient: r^2=0.071 $p>0.05$, Elevated: r^2=0.095 $p>0.05$). Several studies have suggested that BGE is driven more by nutrient availability than DOC as well as the source of available DOC, as DOC exudates from cells that are actively growing may promote BGE better than growth on cellular constituents (del Giogio & Cole, 1998). BGE was positively correlated with bacterial production, as expected (Ambient: r^2=.9643, $p=0.0005$, Elevated: r^2=0.8396, $p<0.05$) (Kroer, 2012).

Extra-cellular enzyme Activity and BGE

Extra-cellular enzymes are secreted by phytoplankton and bacteria and are either located in the periplasmic space or associated with the cell surface. Enzymes cleave larger organic molecules into smaller organic or inorganic constituents (Chrost and Rai, 1993). Since this breakdown of larger molecules to smaller molecules (less than 600 Daltons) is critical for transport across the cell membrane, the rate of substrate conversion by extra-cellular enzymes is considered a rate-limiting step in carbon and nutrient assimilation (Sinsabaugh, 1997; Weiss, 1991). Enzymatic hydrolysis is critical in the regeneration of dissolved organic matter and organic aggregates. However, a change in hydrogen ion concentration may alter the ionization state of enzymes thus potentially impacting its tertiary structure and activity. (Piontek, 2010).

Studies on extra-cellular enzyme activity in response to ocean acidification have been conflicting. Although there have been no studies on the effects of ocean acidification on CBase, past studies suggest that ocean acidification in the open ocean will not have a significant effect

on BGase activity (Yamada et al., 2010, Grossart et al., 2006; Liu et al., 2010) whereas others have suggested that BGase activity will increase in response to increased photosynthetically derived labile DOC (Piontek et al., 2010). Yamada et al. demonstrated that under low pH conditions, LAPase activity decreases while Grossart et al. results suggest that LAPase will increase in lower pH (2006). Yamada et al. attributes these inconsistencies to direct chemical effects on enzymes and the suggests the need for the more research. Studies have consistently shown that APase will not be significantly affected by the predicted change in pH because the predicted future pH is in the range of optimal range for maximum hydrolysis (Tanaka et al., 2008; Yamada et al., 2012).

The slow increase observed in LAPase activity over the 15 days demonstrates that there was a slight increase in peptides and proteins composing the DOC pool. The spike in CBase and BGase on day 15 suggests a high amount of semi-labile DOC at day 15 compared to the other two days, which corresponds to the total available DOC on day 15. Conceivably, the spike in APase on day 15 is linked to a phosphate limitation, which induced the production and activity of APase. These results suggest there was not a significant change in the composition of the DOC pool and resulting enzyme activity between the elevated and ambient groups.

The production and activity of extra-cellular enzymes does not contribute to biomass but rather the maintenance of the cell. Therefore it is expected that BGE will decrease as extra-cellular enzyme activity increases (Del Giorgio and Cole, 1998; Russell and Cook, 1995). However, this was not the case as BGE and the activity of all four enzymes was highest by the end of the experiment for both treatments (Figure 9 and Figure 10). This could suggest that even though extra-cellular enzymes were produced, there were sufficient macromolecules to permit enzymes to be efficient enough for energy and carbon to be allocated to biomass.

The Coastal Biological Pump: Tying the two together

High pCO_2 levels had little to no impact on coastal phytoplankton and bacterial carbon processes as well as little impact on the interaction between the two. The only significant differences were in the BP and BR during the first four days of the experiment as well as the amount of CO_2 drawdown during the phytoplankton blooms. These results are consistent with the limited knowledge gained from other studies of the effects of ocean acidification on coastal communities. Nielsen et al.(2010) found that coastal phytoplankton communities were highly resilient to lower pH as they found no significant difference in primary production (similar to this study) as well as no differences in total POC. A separate study by Nielson et al.(2012), found that phytoplankton communities were not significantly affected by a lower pH with respect to photosynthesis, nutrient uptake, and biomass build-up. Although there are limited studies on coastal bacterial communities, changes in bacterial respiration and/or bacterial production, and hence BGE, could have significant effects on the allocation of carbon (biomass or respiration) and fate of organic carbon. These processes also determine if an ecosystem is a source or sink for carbon, which determines the rate of global carbon cycling. Phytoplankton and bacteria in coastal ecosystems are naturally exposed to many abiotic and biotic factors such as seasonal temperature changes, salinity and terrestrial inputs that constantly change the pH of their environment. For this reason, phytoplankton and bacteria that are found in these areas may be resilient to future predicted changes in pH/pCO_2 for 2100.

Broader Impacts and Future Directions

All processes involved in the biological pump are important in carbon cycling and processing. Coastal ecosystems and estuaries are different from the open ocean, and thus results from dissimilar ecosystems cannot be extrapolated. Coastal ecosystems are also important economically, as they act as nurseries for larval and juvenile marine species, are permanent habitats for many birds, fish, oysters etc., provide recreation for tourists, and are waterways for trade and transportation. The health of estuaries can be severely affected by changes in the biological carbon pump. Decreased primary production could disrupt the food chain while increased primary production could lead to hazardous algal blooms and decreased carbon sequestration. Increased bacterial processes could lead to larger oxygen-depleted dead zones.

It is clear that the biological pump needs to be studied more intensely in estuaries, as well as in other dynamic ecosystems such as mangroves, in-land waters, and wetlands. Many recent studies, even those beyond the biological pump, have theorized that ocean acidification will affect each species differently and each unique ecosystem will react differently to ocean acidification. Hence there has been a need for many different organisms to be studied both individually and within their natural ecosystem (Garrard et al, 2012). Additionally, ocean acidification is just one of many global changes that will occur due to carbon emissions. Studies that include temperature change, sea level rise, and extreme weather would be beneficial in understanding the carbon pump in our future ocean.

Finally, public awareness and knowledge concerning ocean acidification are difficult to gauge, though they seem to have been increasing since around 2006, because of magazine/news articles as well as social media (Logan, 2010). Public knowledge and attitudes are critical in altering the causes of ocean acidification, as well as the support of those in governmental decision making. Therefore it is necessary that everyone, from children to adults to politicians to

voters, should be aware and educated on the consequences of ocean acidification. In collaboration with the Rice Center and several VCU scientists, a Carbon Awareness Partnership (CAP) was established to better educate high school students and teachers about carbon cycling and the consequences of carbon emission in both aquatic and terrestrial environments, such as ocean acidification. CAP promotes critical thinking and problem-based learning to promote scientific thinking and collaboration.

In summary, like other aquatic and marine ecosystems, coastal regions will experience ocean acidification due to carbon emissions in the near future. Any changes in the coastal biological carbon pump due to lower pH/higher pCO_2 could have significant impacts on the overall health of the ecosystem. This study suggests that chlorophyll *a* and DOC concentrations will likely not change, possibly due to coastal phytoplankton's ability resistant to natural changes in pH/pCO_2 due to riverine discharge, nutrient inputs, algal blooms, seasonal temperature variation, and pollutions. However, there were significant differences in BR and BP during the first four days of the experiment, which could have been caused by acclimation to the rapid change in pCO_2, which suggests that bacteria may need time to acclimate to changes in acidity. However, BGE and extra-cellular enzymes production was not different between the two treatments suggesting that the coastal microbial loop with function much like it does in today's ocean, possibly due to the frequent changes in pH in coastal regions. Future studies should include temperature and salinity alterations, as well as attempt to educate the public about the consequences of ocean acidification.

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Literature Cited

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Table 1: Summary of substrates, solvents, concentrations, and reaction times used to assess extracellular enzyme activity (Courtesy Franklin Lab).

Enzyme	EC#	Substrate	Sigma-Aldrich Catalog Number	Solvent	Assay Concentration (mM)	Total Reaction Time (H)
β-1,4-glucosidase	3.2.1.21	4-MUB β-D-glucopyranoside	M3633	Bicarbonate Buffer 5mM	0.40	5.08
1,4- β –cellobiosidase	3.2.1.91	4-MUB β-D-cellobioside	M6018	Bicarbonate Buffer 5mM	1.0	1.95
Leucyl aminopeptidase	3.4.11.1	L-Leucine-7-amido-4-methylcoumarin HCl	L2145	Bicarbonate Buffer 5mM	0.40	1.95
Alkaline Phosphatase	3.1.3.2	4-MUB-phosphate	M8883	Bicarbonate Buffer 5mM	1.0	2.10

AMC (7-amino-4-methylcoumarin) standard was used to form the Quench for Leucyl aminopeptidase (Sigma # A9891)
MUB(4-methylumbelliferone) standard was used for all other enzymes tested. (Sigma # M1381)
A 5mM, pH 8 bicarbonate buffer was used to make all substrates and standards. (Fisher # S233)

Plates were Costar 96 Non-Treated, Flat Bottom, Non Sterile Assay Plates (Costar #3915)

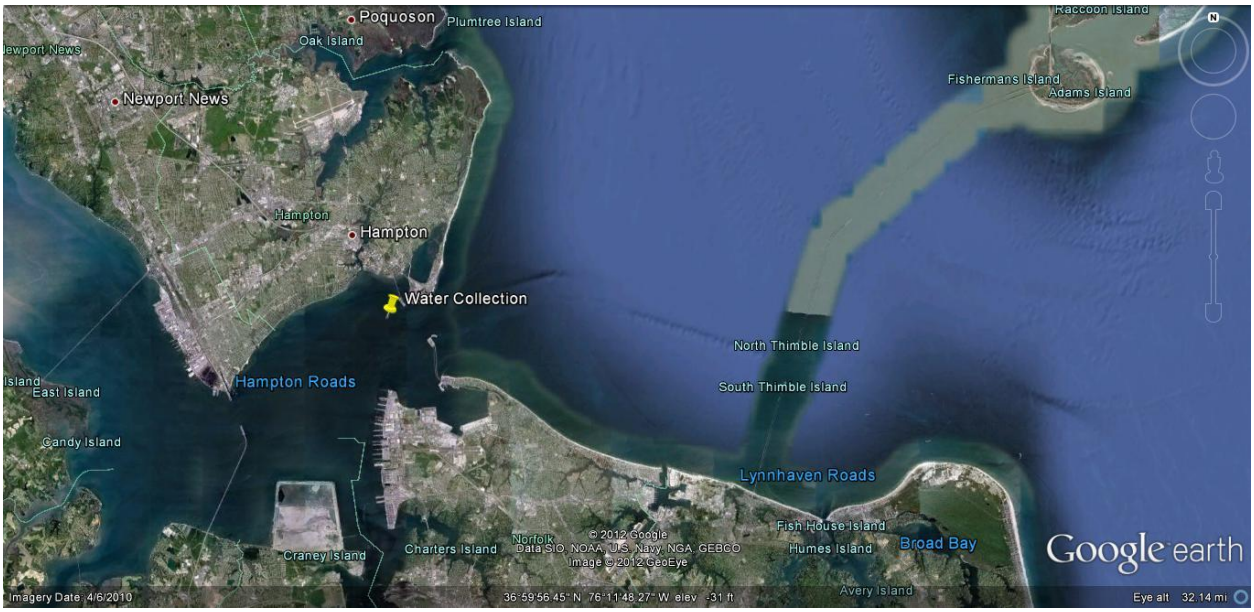

Figure 1: Site of water collection at approximately 26º59’38”N, 76º19’35”W, near VA Beach, Virginia. (Googleearth.com)

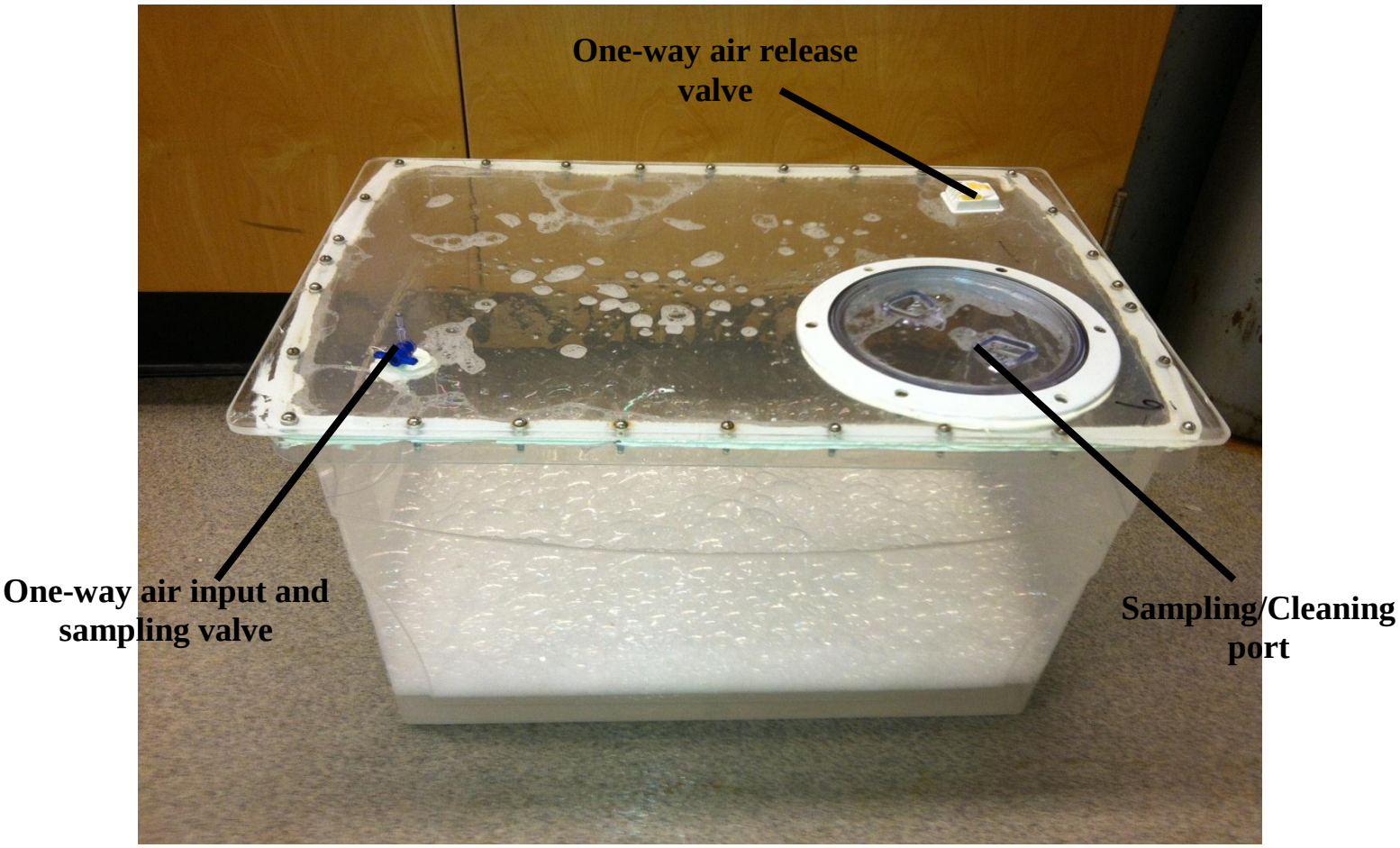

Figure 2: Mesocosm design consisting of a storage container with a plexiglass lid fitted with two one-way valves and a sampling/cleaning port.

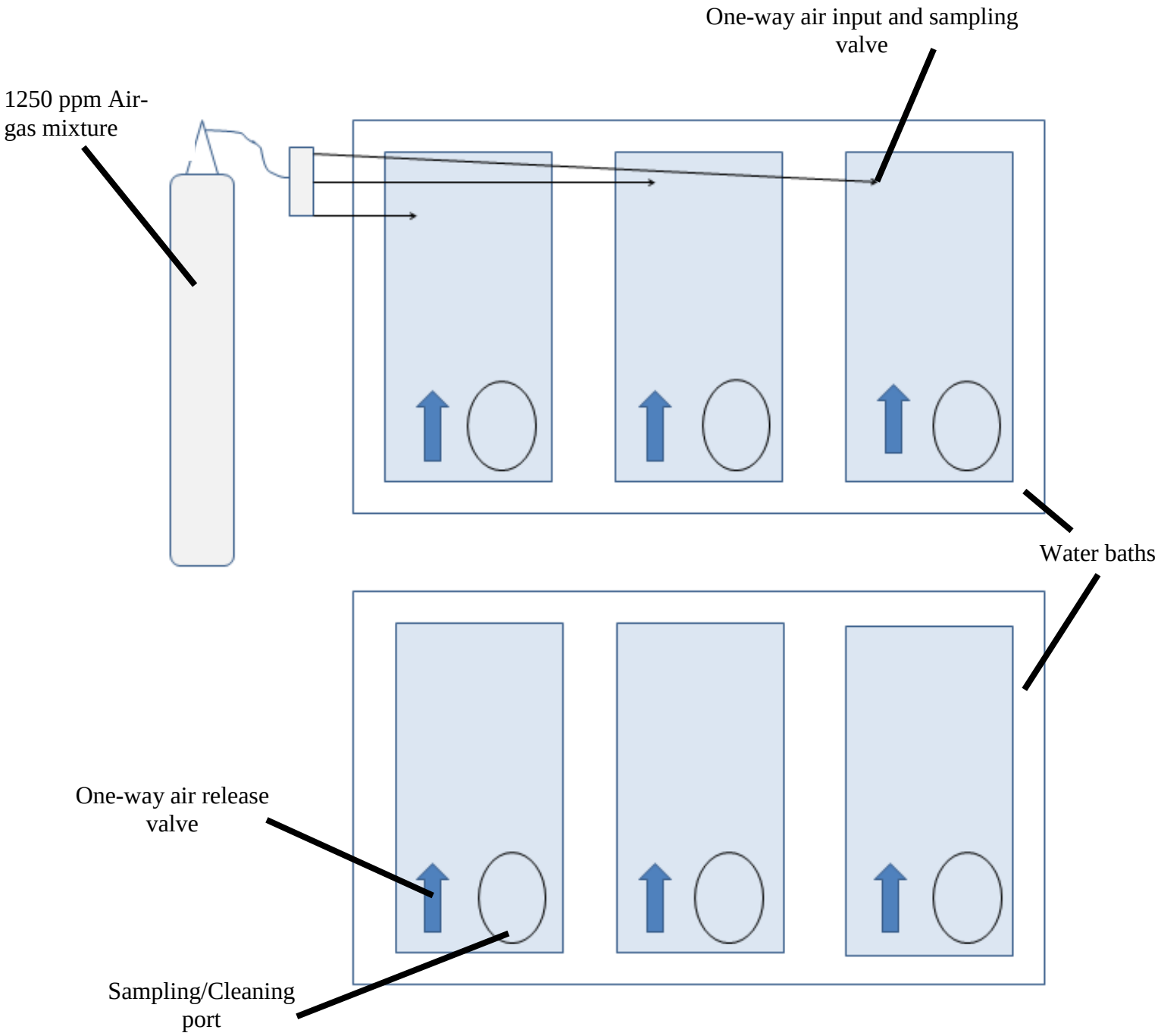

Figure 3: Experimental set-up. There are a total of six mesocosm placed in water baths. Three of the mesocosms are connected to the air-gas mixture.

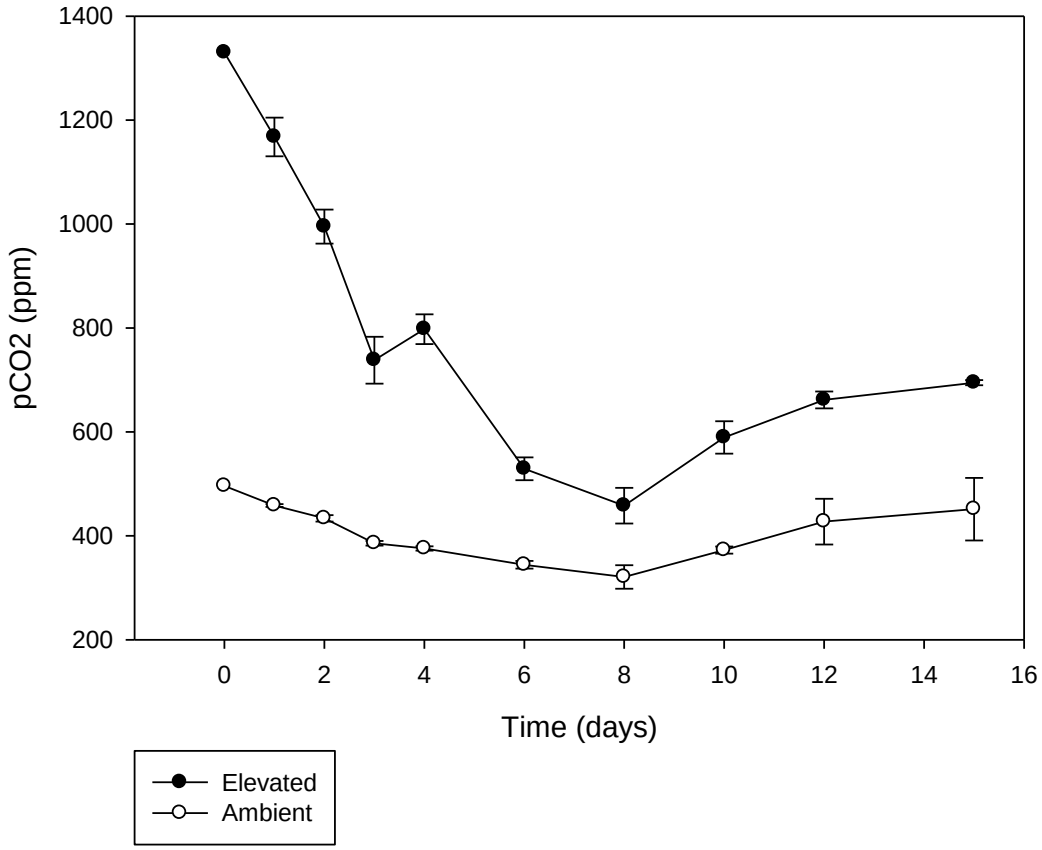

Figure 4: pCO_2 levels in elevated and ambient mesocosms. Averages were derived from triplicate measurements in each mesocosm. Error bars represent standard deviations.

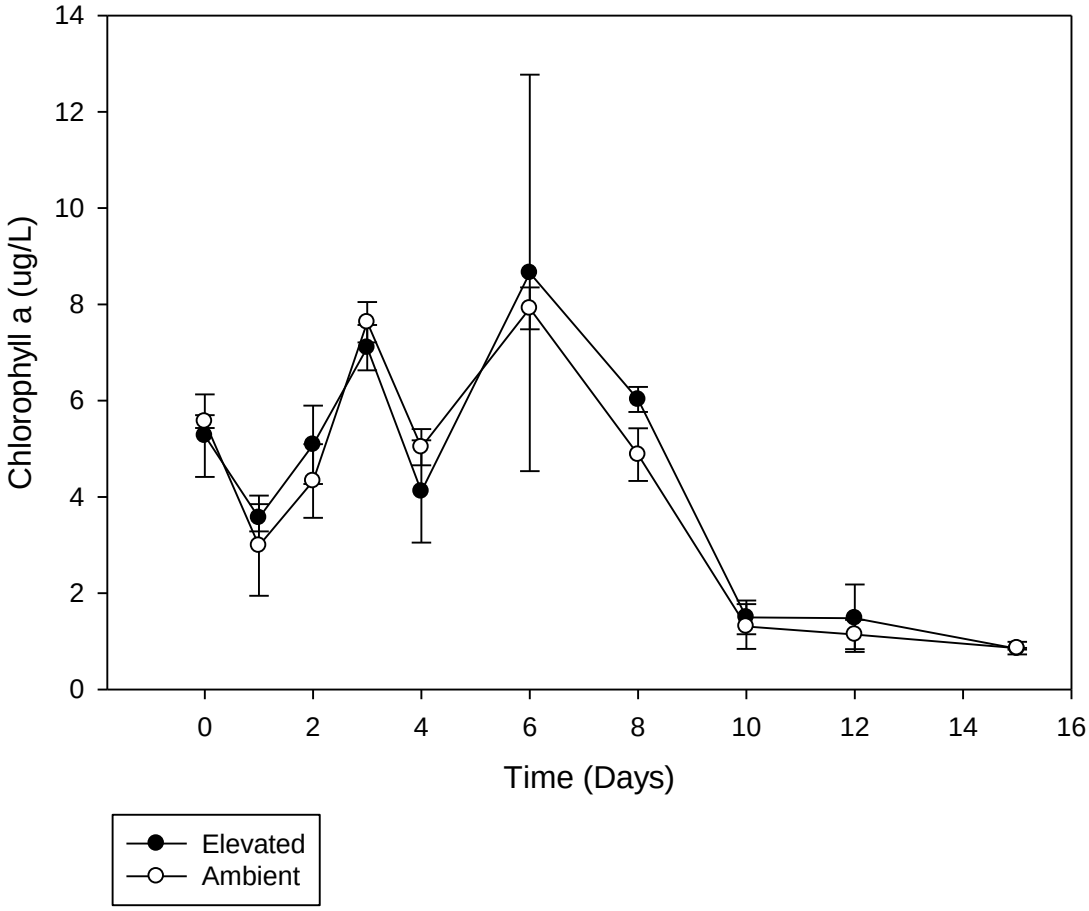

Figure 5: Chlorophyll a concentrations over fourteen days. Averages were derived from triplicate mesocosms and error bars denote standard deviations.

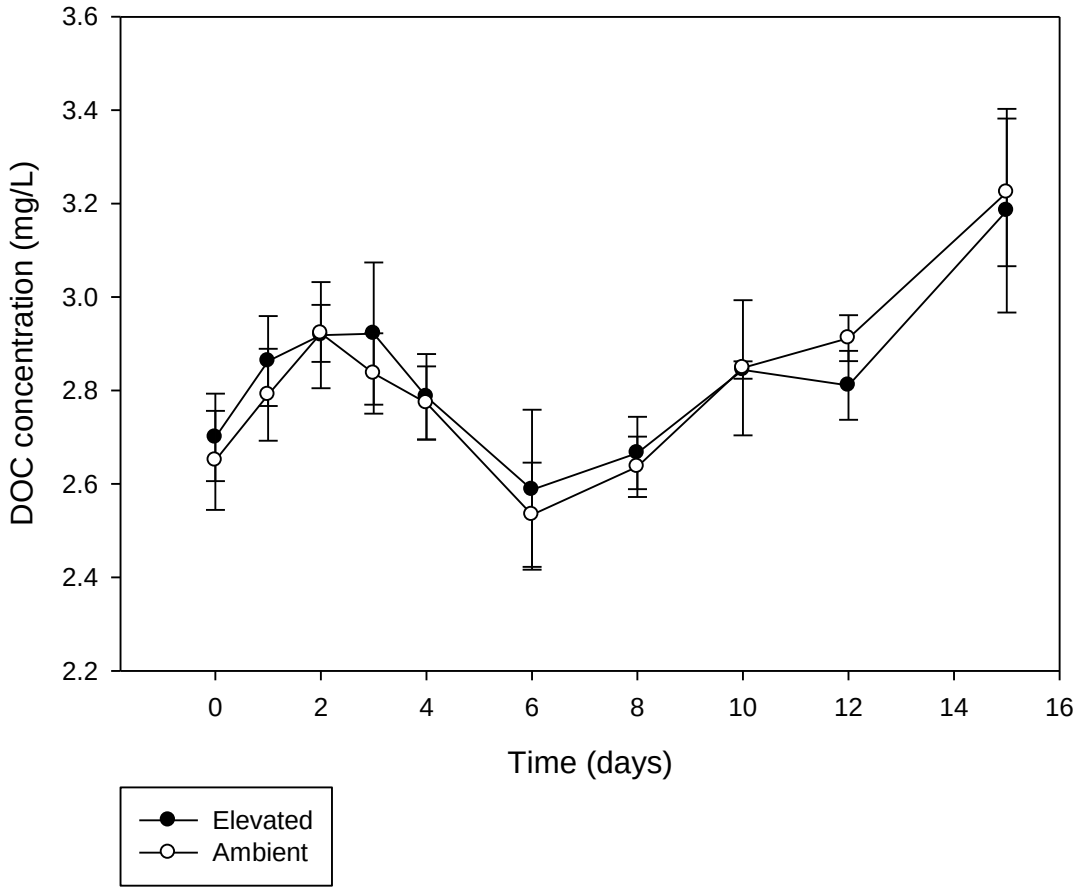

Figure 6: Average dissolved organic carbon (DOC) concentrations in elevated and ambient mesocosms over fifteen days. Averages were determined from triplicate mesocosms. Error bars denote standard deviations.

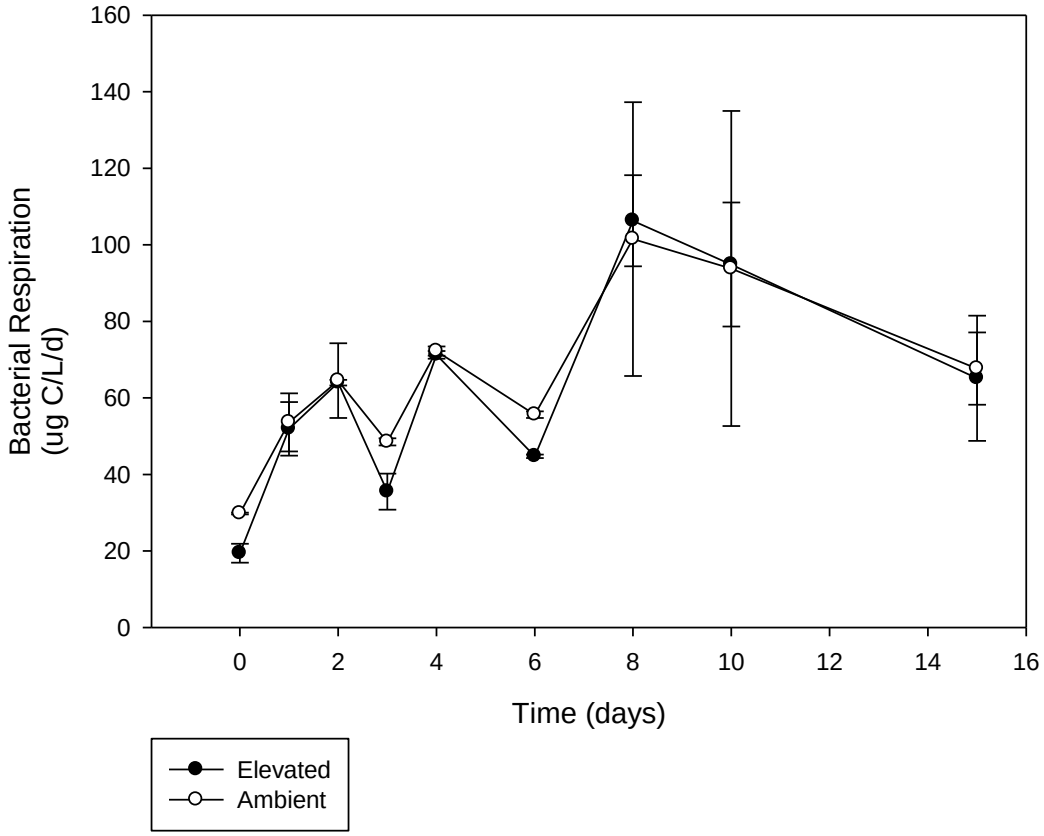

Figure 7: Average instantaneous bacterial production over fifteen days in elevated and ambient mesocosms. Averages were derived from triplicate samples from triplicate mesocosms. Error bars represent standard deviations.

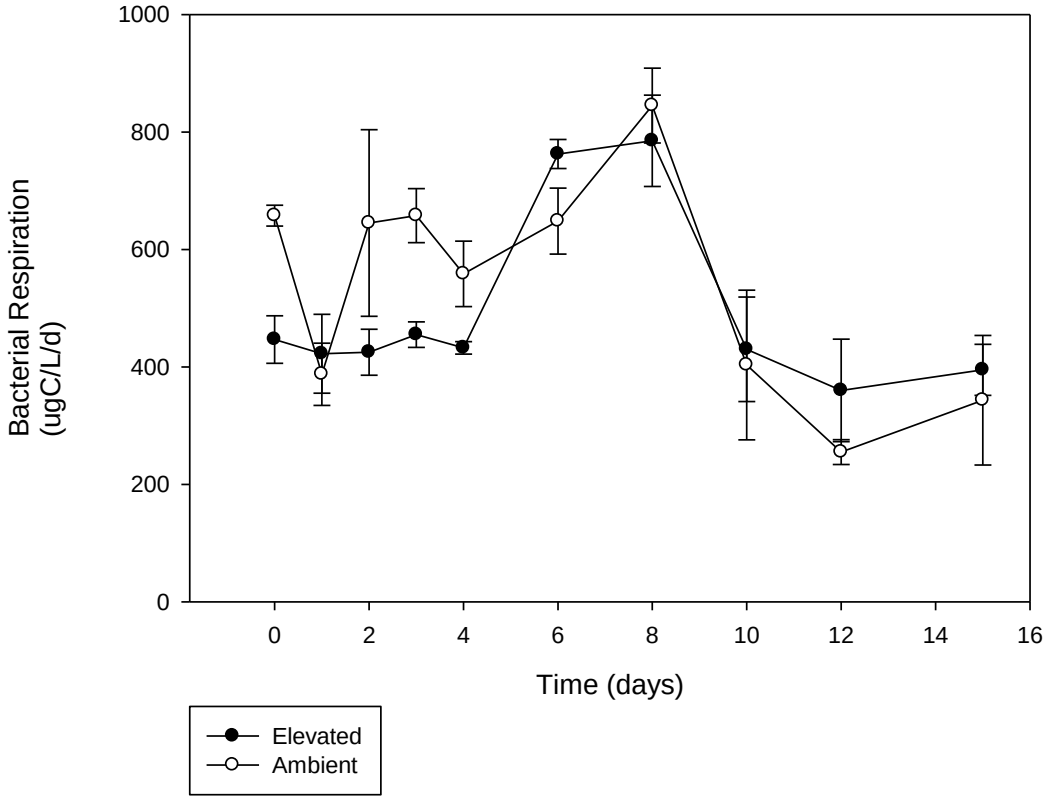

Figure 8: Bacterial respiration over fifteen days in elevated and ambient treatments. Averages were derived from triplicate incubations from triplicate mesocosms. Error bars represent standard deviations.

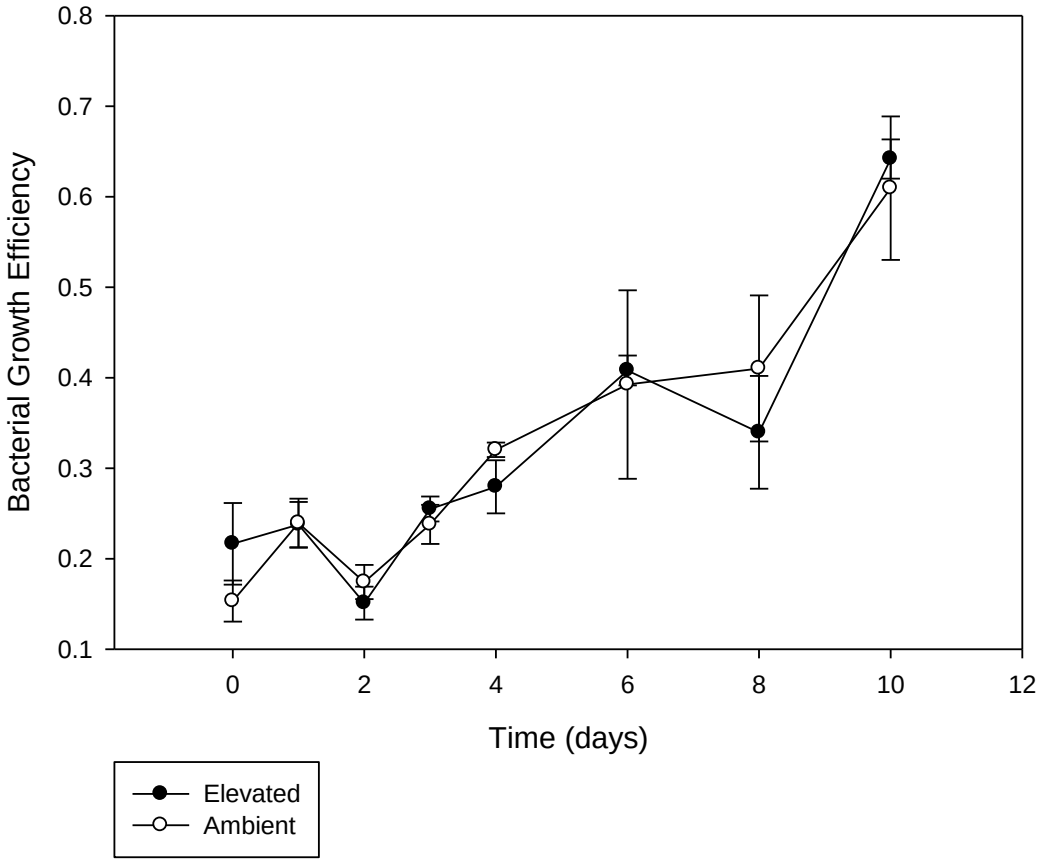

Figure 9: Bacterial Growth Efficiencies over eight days. Averages were derived from triplicate measures in triplicate mesocosms. Error bars are standard deviations.

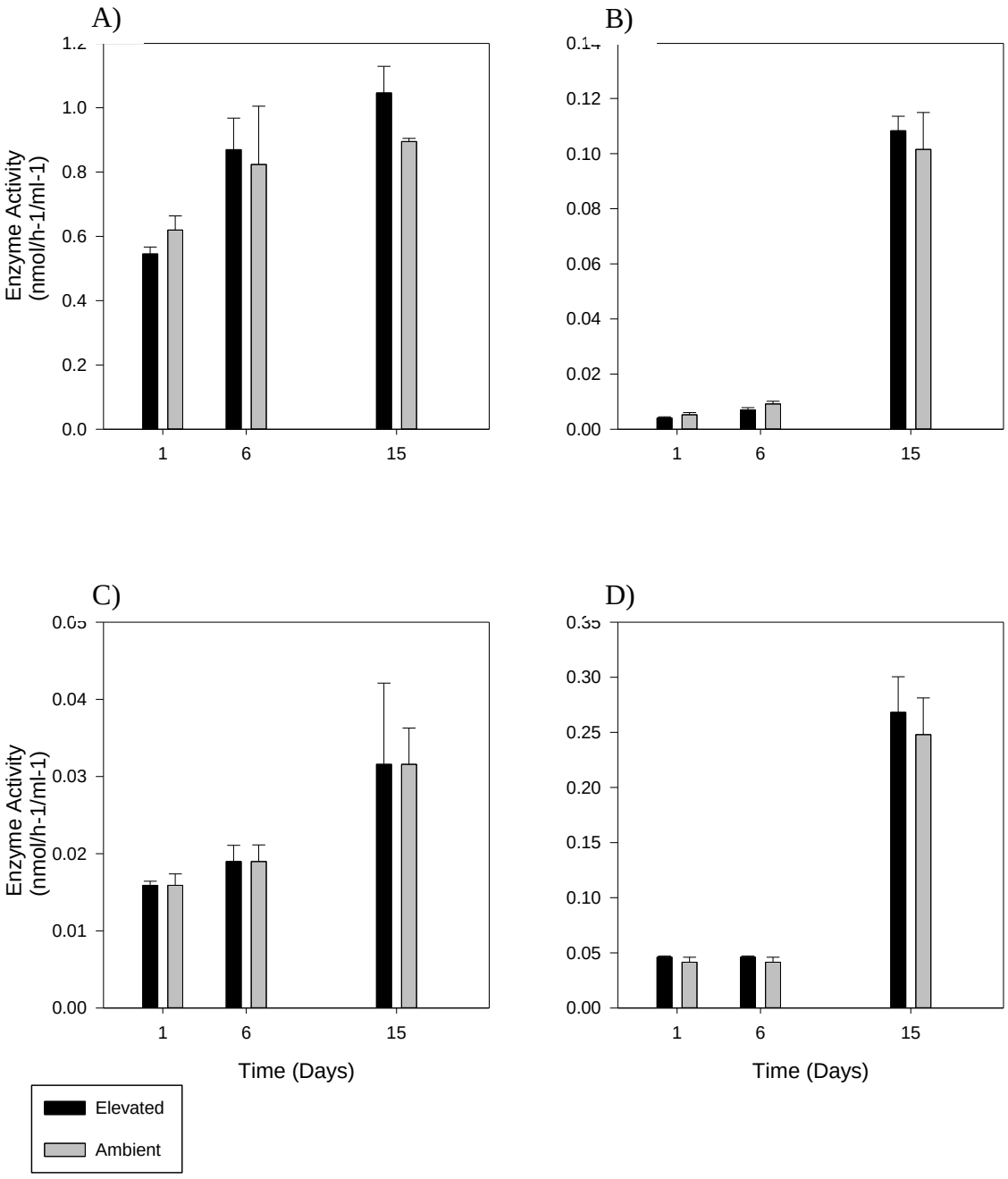

Figure 10: A)LAPase activity; B) CBase activity; C) BGase; D) APase
Average enzyme activity at days 1, 6, and 15. Averages were calculated from triplicate samples from triplicate mesocosms and error bars represent standard deviations. Note that y-axis scales are not similar.

Vita

Rachel D. Cooper was born in Apex, North Carolina on March 18, 1988. She graduated from Apex High School in May 2006. She graduated valedictorian from North Carolina State University with a B.S. in Biology and a Spanish minor in 2010. During her time at North Carolina State she worked as an educator at the NC Aquarium at Pine Knoll Shores and assisted in research focused on responses to hypoxia of juvenile spotfish (*Leiostomus xanthurus*) in NC estuaries. Rachel also was in the Scholars and Mentorship program at NC State. During her time at VCU, she has been awarded a Washington Group Explorer's Club Grant, a Rice Center Research Grant, and several travel grants. She has one publication entitled "Biological impacts of ocean acidification: a postgraduate perspective on research priorities" which has just been accepted by Marine Biology. As Graduate Outreach Coordinator for the Graduate Organization of Biology Students (GOBS), Rachel has been heavily involved in outreach education at the VCU Rice Center. Rachel completes her M.S. in Biology from Virginia Commonwealth University in August 2012 and will adjunct at VCU and John Tyler Community College.