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**Papers from
the
SUMMER UNDERGRADUATE RESEARCH FELLOWSHIP
PROGRAM IN OCEANOGRAPHY**

at

**THE UNIVERSITY OF RHODE ISLAND
Graduate School of Oceanography
Department of Ocean Engineering
College of Environment and Life Sciences**

NARRAGANSETT, RHODE ISLAND

June – August 2013

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GSO Technical Report No. 13-03

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SUMMER UNDERGRADUATE RESEARCH
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PREFACE

This report presents the papers written by the thirteen participants in the 2013 Summer Undergraduate Research Fellowships in Oceanography (SURFO) program at the Graduate School of Oceanography (GSO), University of Rhode Island (URI). This past summer represented the 29th year in which the program has been coordinated and extended through the several disciplines in oceanography and ocean engineering at URI's Narragansett Bay Campus. The 2013 program will continue excellence beyond the official duration of the program with at least three projects presented at the AGU Fall 2013 and ASLO/AGU Ocean Science 2014 meetings.

The participants in the 2013 SURFO program are grateful to the Department of Defense ASSURE Program and the National Science Foundation REU program for their support of the program through grant OCE-1156520.

The SURFO program would like to thank all of those individuals at URI who contributed to the program's success including those who advised the students and who gave SURFO seminar presentations. In addition, our thanks to Kim Carey for their assistance in the preparation of this report as well as the administrative, financial and recruitment tasks. Finally, we would like to thank Kellen Rosburg who served as a graduate coordinator for the program.

Kathleen A. Donohue
David Smith
SURFO Site Directors

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Testing an Arducopter for Sea Ice Mapping in the Arctic

Gabe Amon, Brice Loose, Arash Bigdeli

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Abstract. Sea ice mapping in the Arctic is a critical variable for contemporary polar science. Currently, most satellite-derived maps of sea ice concentration resolve sea ice cover at 5 to 25 km² - a resolution that is not adequate for some applications. For models of air-sea exchange we need to resolve ice floes at 10 m length scales. Our research involves testing an Arducopter 3DR QUAD D and an APM 3DR Y6 to observe and quantify these features. The Arducopter is an inexpensive autonomous vehicle; it carries up to 2 kg of payload, and can fly a predetermined waypoint pattern. It calculates its geographic position and orientation, measures tilt, roll, and yaw and carries a digital camera for aerial photography. This is a feasibility study to establish whether the Arducopter can accomplish sea ice mapping missions. Specifically, we tested the stabilization of the Arducopter by tuning the proportional-integral-derivative (PID) coefficients, the ability of the copter to withstand cold temperatures and the difficulty associated with image mosaicking over featureless open water. The PID tuning proved essential to the evolution of this project because it allowed for more stabilization of the Arducopter during flights. The effect of cold conditions on battery life was tested at the Bay Campus Environmental Chamber Facility at temperatures of 23, 2 and -23 degrees Celsius. At these temperatures, the maximum flight duration was 15:00, 13:55 and 10:03 respectively. In order to successfully take pictures from autonomous flights, with approximately 60% overlap for feature tracking, we had to program the Canon cameras with CHDK to add an intervalometer of one picture taken every second. At this stage, we have proven to ourselves the capability of autonomous flight. The Arducopter UAVs have the capability for making aerial image surveys in summer Arctic-like conditions. The project will be ongoing with a cruise to the Arctic in August and subsequent method development and analysis.

1. Introduction

The ocean is a natural carbon sink and with recent climate change discoveries, questions have arose as to how it will continue to function. The ocean absorbs anthropogenic carbon daily. Of the amount of anthropogenic carbon released into the atmosphere, approximately 30% has been absorbed into the ocean in the past few decades (Feely et al, 2004). The amount of anthropogenic carbon currently absorbed in the ocean is close to one third of its holding capacity (Sabine et al 2004). This absorbed carbon is not spread out equally throughout the oceans; for example, the Southern Ocean's carbon sink has stayed steady for two decades while the North Atlantic's carbon sink has decreased over the last two decades (Else 2011). For this reason, more areas of the ocean need to be studied.

To get a better understanding of the amount of carbon in various areas of the ocean, scientists can look at the air-sea gas exchange. The area that will be specifically studied is the Arctic, focusing on ice-covered regions. One way to study the air-sea gas exchange is by using images, particularly by maps. Most current maps do not work adequately enough for this micro scale application. For studying air-sea gas exchange sufficiently, it needs to be on a scale of 10 meters of length. These micro scale maps are used to determine the percent cover of sea ice. By looking at the percent cover, the effect of ice on the rate of gas exchange can be determined. Scientists are specifically studying the effects of varying covers of sea ice on the rate of gas exchange. The increasing amount of melting ice in the Arctic makes percent cover of sea ice an important area of study. There is no definitive answer yet, but there is a difference in rate from open water to ice coverings. By creating these maps, it will further advance the study of

rates of gas exchange with varying percent of ice cover. To obtain this scale we tested an Arducopter APM 3DR QUAD D and APM 3DR Y6 and attached a Canon PowerShot D20 that took the images. These Arducopters are much more cost effective than other options available to get micro scale images.

This research project was used as a feasibility study to test whether the Arducopters would be capable of performing autonomous ice mapping missions in the Arctic. We were able to see that it would be successful by testing it at the University of Rhode Island Graduate School of Oceanography campus.

2. Methods

We used the Arducopter APM 3DR QUAD D and APM 3DR Y6 as part of our feasibility study to test that we have an inexpensive way to map sea ice. Each of these Arducopters contains an APM 2.6 Autopilot System, 3DR GPS uBlox LEA-6 w/ external compass and 3DR Radio Telemetry kit. In order to use the Arducopters in the Arctic, we had to configure them through Mission Planner, test battery life, tune the Proportional Integral Derivative controller, and orthorectify distorted images.

2.1 Mission Planner

Prior to flying the Arducopters, there is required software to download called Mission Planner, created by Michael Osborne. Mission Planner has the ability to set GPS waypoints for use during flight, monitor sensor outputs such as voltage, altitude, pitch, roll and yaw, analyze flight logs, set flight modes, configure the hardware for each APM and monitor the Arducopter during each flight. It is completely free software that needs to be run on a Windows system. We were able to communicate in many ways with the

Arducopters, whether it was through USB connection in the lab or wirelessly during flight, through Mission Planner.

Each Arducopter needed to have its APM configured through Mission Planner. In order to set up the APM 3DR QUAD D, there were 5 steps to go through. The initial step was to select the frame type. Since it has no arms that are straight in front of the face of the APM, it is in the X configuration. For enabling the compass we had to rotate the Arducopter on all four axes, away from metal, for calibration. Calibration of the accelerometer was the next step. The Arducopter needed to be placed on its left side, right side, nose down, nose up and on its back in order to calibrate the accelerometer. Radio calibration was done by using the transmitter and putting all channels to their maximum and minimum values. We calibrated 6 channels, Channel 1: Roll, Channel 2: Pitch, Channel 3: Throttle, Channel 4: Yaw, Channel 5: Flight Modes and Channel 6: PID tuning. Since we added a sonar sensor to this Arducopter we had to enable that under the additional hardware tab.

The APM configuration for the APM 3DR Y6 was essentially the same but there were a few differences. It was a X configuration for frame type and the accelerometer and compass calibration was identical to the APM 3DR Quad D. The difference came while calibrating the radio. During radio calibration, we noticed the channels were inversed. When we tried going to a maximum value that was for the Quad D, it was a minimum value for the Y6. In order to solve this problem we had to reverse it at the RC transmitter. Once that was solved, the calibration of the radio and the Y6 was completed. We did not add a sonar sensor to it because the barometric altitude readings were working properly.

After each Arducopter had its APM configured, it needed to have its electronic speed controls (ESCs) calibrated. We chose to do the automatic ESC calibration because it was quicker; they were all calibrated at once rather than individually. The ESC calibration was identical for both models. The process followed a step by step instructional. Initially it started by having no power to the Arducopter and then the transmitter was turned on and the throttle was set to maximum. Next, the Lipo battery was connected to the Arducopter and a musical tone came from the ESCs followed by a slow and continuous flashing of blue and red lights. While keeping the throttle at full, the Lipo was then disconnected and reconnected. Once the battery was reconnected, the ESCs emitted 2 beeps and in between the 2 beeps the throttle was put at its minimum. There was confirmation beeps from the ESCs and after the beeps the throttle was slowly moved up, causing the motors to spin. The Lipo was disconnected and then reconnected. Holding the throttle to the far right until there was a solid red light on the APM armed the Arducopter. When the throttle initiated the motors to spin, we checked to make sure each motor was spinning its proper way. After that step the Arducopter is completely calibrated.

2.2 Flight Modes

One of the main reasons these Arducopters were chosen for this study is their ability to perform autonomous flights.

We were able to achieve autonomous flight by using different flight modes. In order to set a flight mode, the Arducopter needed to be connected to Mission Planner. In Mission Planner, we had to go to the Configuration tab and choose Setup/Modes. Once under there we were able to go to Flight Modes. Each mode was assigned to stabilize mode initially. To change to a new flight mode all that we had to do was select one from the drop down menu. For the Arducopters, Flight Mode 1, 4 and 6 were the ones that correlated to the flap switch on the transmitter. The up switch on the transmitter was Mode 6, the middle switch was Mode 4 and the down switch was mode 1. We tested the Arducopters with flight modes such as, Auto (autopilot where the Arducopter flies to the GPS waypoints set up in Mission Planner), RTL (return to launch), Takeoff (an automatic takeoff), Loiter (Arducopter maintains the current location, altitude and direction), Acro (used for tuning PIDs) and Alt Hold (goes to a certain altitude and stays there).

2.3 Battery Life

Battery life of the Arducopters needed to be calculated prior to their use in the Arctic. By knowing the battery life we can get an estimate of how long the flights can be. The APM 3DR QUAD D was tested in three different temperatures, 23, 2 and -20 degrees C. Each temperature test consisted of two trials, one at 50% and the other at 100% throttle. This is because during the flight that is where the throttle will most likely be placed at. To keep the copter constrained and free of any crashes, it was bungee corded down to a table during the trials. What this data gave us was a time range for flight duration at different temperatures.

2.4 Proportional Integral Derivative Controller

The Proportional Integral Derivative (PID) Controller accounts for much of the stabilization of the Arducopters during their flight. To attempt to make them as stable as possible, the PID coefficients needed to be tuned. There are 3 different coefficients, the Kp (deals with Proportional), Ki (Integral) and Kd (Derivative). Each copter was bungee corded to secure it at a low altitude for observation and to keep it from crashing. During the tuning, we were looking for coefficient values that were keeping it from oscillating. The coefficients were continuously changed until we found the ideal combination that minimized oscillation.

2.5 Orthorectification

Orthorectification of distorted images is an essential part to producing aerial maps. We used a Matlab code that was created in the Loose lab to perform this (A. Bigdeli, unpublished data). A picture was taken from a measured altitude and had a certain degree of pitch, roll or yaw distortion that was measured from the Land Meter App. All the data was inputted into the Matlab code and the orthorectified image was generated.

2.6 Canon Hack Development Kit (CHDK)

Figure 1. Displays 6 different trials of battery life in 3 different temperatures (23, 2 and -20 C) at 50% and 100% throttle. The range of time between the 50% and 100% throttle times gives the range of flight time at each temperature.

Figure 2a. Kp coefficient for Alt Hold Mode set to .369. Desired altitude of 15 meters is the red line. The high amplitude of error and fairly frequent oscillation above and below 15 meters shows the Kp coefficient is too low.

To obtain the desired amount of image overlap (60%) for image mosaicking, we needed a camera with an intervalometer of 1 second. This means that the camera needs to be able to take a new picture every second. To obtain this feature, a Canon point and shoot camera was used. However, to utilize this feature the program CHDK had to be installed on the SD card and booted into the camera. We had to figure out the firmware version of the camera to download the correct version of CHDK. We decided to upgrade the firmware manually instead of going for a bootable update. The CHDK files were put on a SD card and then we updated the firmware manually from there.

3. Results and Discussion

3.1 Battery Trials

Since we were unable to discover the battery life in our preliminary research, these trials were very important to our project. It led us to come up with a time range that the Arducopters can be in flight safely without the risk of the battery dying and having a crash. The time range for safe flight at GSO was from 7:00 to 14:50. The data collected from our 2 trials at 3 different temperatures was collected and analyzed. At 23 C and 50% throttle the flight lasted 14:50 and at 100% throttle the flight lasted 7:00 giving a safe flight range of 7:00 to 14:50. At 2 C and 50% throttle the flight lasted 13:35 and at 100% throttle the flight lasted 6:51 giving a safe flight range of 6:51 to 13:35. At -20 C and 50% throttle the flight lasted 9:58 and at 100% throttle

the flight lasted 5:50 giving a safe flight range of 5:50 to 9:58. The battery voltage logs for these 6 trials are graphed in Figure 1.

By looking at Figure 1, the battery life looks to depend more on throttle than on the temperature. At 23 C and 2 C, the 50% and 100% values are very similar. However, once it is placed in the -20 C chamber the 50% value changes significantly. We feel this may be in part due to initially when the Arducopter was placed in the chamber and turned on it would not initiate the motors. In order to get it to function in this temperature, the battery needed to be connected and then placed in the chamber. The longer it was in the chamber the ESCs, APM or Lipo battery might get cold thus giving a shorter flight time. This did not occur for the 2 C chamber. Overall, these trials were very beneficial and gave us accurate flight times for the Arducopter. Also, it showed that it was capable of flying in 2 C with little change in battery life and 2 C is most like the temperature that it will encounter in the Arctic in August 2013.

3.2 PID Tuning

We tuned the Rate/Roll coefficients of the PID controller to make a more stable flight for better pictures and higher control of the Arducopters. The APM 3DR QUAD D was tuned to have a Kp of .1870, Kd of .009 and a Ki of .7880. During the flight trials to calculate these coefficients we came across important information to help with the tuning. We noticed that a low Kp value gets the copter to the desired altitude slow and the error amplitude is high but does not occur frequently. With too high of a Kp value it gets to the altitude fast and has a smaller error in the amplitude but it oscillates more frequently. When we have gotten to the most accurate Kp value, the copter has a small error in amplitude and it does not occur frequently. To help further dampen the amplitude error the Kd is tuned and to have it oscillate less the Ki is tuned. When all these are tuned as accurately as possible, the Arducopter will fly stable.

When the APM 3DR Y6 was tuned, it had a Kp of .128, Ki of .011 and Kd of .004. For the Y6, we also tuned the Throttle Rate and Altitude Hold. Initially the Throttle Rate Kp was 6 and we found that .3 worked much better. As for Altitude Hold, it was set to 4 and we tuned it to .41. These factory settings that the Y6 came with were way off and led

Figure 2b. Kp coefficient for Alt Hold Mode set to .41. Desired altitude of 15 meters is the red line. The low amplitude of error and small frequency of oscillation above and below 15 meters shows the Kp coefficient works well.

Figure 2c. Kp coefficient for Alt Hold Mode set to .499. Desired altitude of 15 meters is the red line. The medium amplitude of error and very frequent oscillation above and below 15 meters shows the Kp coefficient is too high.

Figure 3. Image taken in the Loose lab to test the image orthorectification code developed by Arash Bigdeli. This image has a distortion in pitch of 27 degrees and an altitude of .472 meters.

Figure 4. Orthorectified image of Figure 2. It was run through the code in Matlab and was generated.

us to initial troubles with flying this Arducopter. However, once we delved into the PID coefficients and tuned them accurately, these problems were gone and it led to much smoother flights.

By tuning the PIDs for Rate/Roll, we also noticed that the PIDs for the different flight modes needed to be tuned. This was a grueling process because one small mistake in the coefficients could cause a giant crash. For example, when tuning the PIDs for Loiter mode we had it set too high which caused it to shoot up in the air, spin in circles and then took a nose dive crash. This experience opened our eyes to the importance of tuning the PIDs in the flight modes.

One particular PID that was of interest to us was the Alt Hold Kp value. We noticed that the APM 3DR QUAD D was fluctuating its altitude during Alt Hode mode. We performed 3 trials of changing the Kp value to see if it decreased the fluctuation in altitude. Figure 2a, 2b and 2c display the desired altitude value along with the actual altitude of the Arducopter during the 3 different trials. We set the Kp to .369, .41 and .499 to test. As stated earlier when dealing with tuning Kp coefficients, too low of a value gives a larger amplitude of error and less frequent, while too large of a value gives a smaller amplitude with more frequent errors. By looking at this figure, when the Kp was .369 it was far too low because of the large amplitude and when it was .499 it was too large because it is frequently oscillating above and below the desired altitude. When .41 was used it provided a small amplitude, never

exceeding 20 meters while the other two were consistently over 20 meters, with a less frequent oscillation. This led us to set the Kp to .41, the original was set to 4, which explains the huge errors we were witnessing when using Alt Hold. When using Alt Hold after PID tuning, it went noticeably smoother.

FLIGHT IMAGES

Figure 5. 3 simultaneous flight images that were captured by the Y6. They were taken while flying around the GSO Bay campus.

Figure 6. This is a simple mosaic of the three images from Figure 5. It was put together by matching up features that were similar in each image and then they were layered on top of each other.

3.3 Orthorectified Images

When taking pictures from an Arducopter, they do not turn out accurate like we would want. We need uniformly accurate images to make the mosaic. Instead, they come with distortions. These distortions are due to changing altitudes and pitch, roll and yaw angles. When these values for the distorted parameters are inputted into our orthorectification code, we get an orthorectified image.

Figure 3 shows a distorted image that we used the code on. Qualitatively one can see it is a distorted image being that the sides of the paper are not parallel, it has a trapezoid shape rather than a square and the bottom of the paper has a

greater length than the top. Quantitatively the distortion comes from having a pitch of 27 degrees. Inputting the 27 degree distortion for pitch and the altitude of .472 meters into the code created a orthorectified image, Figure 4. The image now has parallel sides that is true of a sheet of paper, is rectangular and is the same length at the top as the bottom. During the orthorectification, the scale is also kept uniform. The distorted image has a length of .15 while the orthorectified image has a length of .15 also; these lengths are determined by looking at the scale on the y-axis of the images. By comparing these dimensions staying constant and the removal of the distortions, we were able to see the accuracy that was kept from the orthorectification process. This proves that we have a way to generate uniformly accurate images to be used for producing aerial maps.

3.4 Flight Images

We obtained excellent images from our flights around the GSO campus. The images had approximately 60% overlap like we desired, however, they also had lots of distortions. The Canon cameras with the CHDK programmed to them worked well with the intervalometer set to 1 second. Figure 5 contains 3 successive flight images that display the success of using these cameras. These images can then be roughly overlapped on top of each other by finding similar points in order to make a small mosaic, Figure 6. These images will be adequate enough, once they have been orthorectified, to produce aerial maps.

4. Conclusion/Future Work

Many important discoveries and conclusions were made in this feasibility study to test the ability of Arducopters to be used for sea ice mapping missions. We came across the importance of tuning PIDs to create a stable and safe flight. We also found out the lifetime of the battery to account for the flight time of the Arducopters in different temperatures. We were able to program autonomous flight waypoints for the Arducopters to fly around to in autopilot. Lastly we implemented CHDK on Canon cameras to add the intervalometer feature.

The Arducopters work to successfully take pictures from autonomous flights with approximately 60% overlap for image mosaicking. Being that they were successful in their tests at the GSO Bay Campus we are hopeful they will be able to operate in the Arctic to map the sea ice. Currently they are in the Arctic right now being used for mapping missions. Once that task has been completed the images will be orthorectified and mosaicked together to study the air-sea gas exchange of that area of the Arctic. This will prove that we have a new method for producing accurate and low cost maps of sea ice in the Arctic.

Before we are able to generate maps of sea ice, there are still things that need to be accomplished. The flight images will need to be orthorectified and mosaicked in Matlab. We have accomplished this with photographs taken in the lab, so the next step is to make sure it also works for the flight images. Once the images have been orthorectified, they will be stitched together using GPS and the IMU data to correct for copter motion. For the mosaicking of the images we

have considered using the Helmert transformation. To quantify the accuracy of the mosaicked images we will compare the position of known features with GPS-derived ground control points. When these last steps have been completed, the sea ice maps will be constructed and then analyzed.

We are very hopeful that everything will go as planned. However, there may be some difficulties that are encountered. Once the whole study is completed, we will prove that we have a new method for producing accurate and low cost maps of sea ice in the Arctic to study the rate of gas exchange with varying percent cover of sea ice.

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Long Term Changes in Winter-Spring Bloom Carbon, Nitrogen, and Silica in Lower Narragansett Bay (1959-2011)

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Abstract. The winter-spring diatom bloom is a dominant feature of phytoplankton cycles in temperate estuaries, including Narragansett Bay. I used a multidecadal (1959-2011) phytoplankton time series and related physical (temperature, light) and chemical (C, N, Si nutrients) habitat data to evaluate long-term patterns of the winter-spring bloom in lower Narragansett Bay. My study applied a database of Narragansett Bay C, N, and Si cellular content to a time series of phytoplankton abundance to evaluate long-term changes in dominant winter-spring bloom diatom (*Skeletonema spp.*, *Thalassiosira nordenskiöldii*, and *Detonula confervacea*) C, N, and Si. Mean winter-spring phytoplankton carbon decreased from 349 milligrams/m³ (1960's) to 128 milligrams/m³ (2000's). The long-term declines in carbon (63% decline), nitrogen (62% decline), and silica (40% decline) can be explained by both decreasing phytoplankton abundance and changes in community composition. *Detonula confervacea*, which contributed 22% of winter-spring carbon production in the 1960's, produced only 2% of the winter-spring carbon in the 2000's. Synchronously, the contribution of *Thalassiosira nordenskiöldii* to winter-spring carbon increased from 23% (1960's) to 44% (2000's) while *Skeletonema spp.* contribution to winter-spring carbon remained close to 55%. Winter-spring bloom carbon and nitrogen concentration had moderate negative correlation with temperature, while winter-spring silica showed no relation to temperature suggesting temperature is one of multiple drivers of long-term winter-spring bloom change. From the 1960's to the present, the disappearance of *Detonula confervacea* has left C, N, and Si nutrients formerly taken up by these cells to be assimilated by *Thalassiosira nordenskiöldii*. While this study was based on temperature, many other environmental factors (light, stratification, grazer abundance) contribute to the winter-spring bloom changes over time.

1. Introduction

Over time, coastal communities and their associated processes show great variation. One of the most important processes, primary production, is controlled by one of the smallest organisms: phytoplankton. These microscopic plants display variation not only in abundance but in cellular nutrient levels due to many physical parameters (temperature, light, stratification, grazer abundance) as well as changes in chemical nutrient availability (Smayda, 1980; Reynolds, 1997; Longhurst, 1998). These changes in phytoplankton abundance and cellular variation can be characterized over time and correlated with not only local, regional, but also worldwide climate changes.

The gradually warming and constantly changing climate has had a negative effect on phytoplankton and their ability to provide organic matter for other organisms. Although these tiny, rapidly changing organisms make up less than one percent of the World's biomass they account for almost half of the World's primary production (Falkowski, 2012). With less phytoplankton and therefore less nutrients cycling through the benthic-pelagic coupling process the oceans are undergoing a major community change.

Narragansett Bay (Rhode Island, USA) has been a heavily studied coastal system since the 1950's. In 1959 a long term phytoplankton time series of weekly observations of phytoplankton abundance, community composition, and related physical (temperature, light) and chemical (C, N, P, Si nutrients) habitat variables began (Smayda, 1957; Pratt, 1959). During this time, coastal water temperatures have increased, with local winter water temperature increasing about 1.7°C since 1970 (Nixon et al., 2003, 2004; Oviatt,

2004). The phytoplankton blooms and dominant species have been verified and documented (Karentz and Smayda, 1984, 1998). Considered one of the most important blooms for the area is the winter-spring bloom which generally takes place during the months of January, February, March, and April. This bloom shows a spike in abundance of phytoplankton during this time. It is one of the most important phytoplankton blooms because it occurs during a time of the year when temperature and light measurements are low meaning the pelagic grazers are not as active or as abundant when compared to the other months of the year. It is a time when phytoplankton can grow with little interference and convert inorganic matter to organic matter usable for grazers. When grazing and Bay activity are low, this organic matter falls to the bottom of the bay and becomes an available source of energy for the benthic filter and suspension feeders during the summer.

Benthic-pelagic coupling is an interaction that comprises the entire water column and is important for the success and survival of all species. This interaction is one of the many ways that organisms interact and coexist in Narragansett Bay. There is evidence for a recent decline in the winter-spring bloom in Narragansett Bay (Fulweiler et al. 2009; Keller et al. 1999; Nixon et al. 2009) that may negatively impact benthic-pelagic coupling. The three key winter-spring bloom diatoms in the Bay are *Skeletonema spp.*, *Thalassiosira nordenskiöldii*, and *Detonula confervacea*. Long-term patterns in the C, N, Si content of these key winter-spring bloom diatoms was modeled by applying experimentally determined temperature-dependent C, N, Si content of these species to the long-term phytoplankton abundance time series. Long-term patterns in combined winter-spring bloom C, N, Si content, changes

Figure 1: The open circle represents the long-term phytoplankton sampling site. Figure from Fulweiler and Nixon (2009).

in each species contribution to winter-spring bloom standing stock, and winter-spring bloom pattern relationships with winter temperature are described in this study.

2. Materials and Methods

2.1. Study Site and Phytoplankton Time Series

A combination of Narragansett Bay (NBay) phytoplankton abundance data and experimentally derived phytoplankton C, N, Si content data were combined to synthesize estimates of winter-spring (WS) phytoplankton bloom C, N, Si standing stock in lower NBay during 1959 to 2011. Samples for estimation of phytoplankton abundance and habitat parameters were collected weekly since January 1959 at Station II in the West Passage of lower NBay (41°34'07"N, 71°23'31"W; Figure 1). The station is approximately 9m deep. Surface water samples (1959 to 1997; 2011) or pooled water (surface and bottom water) samples (1999-2008) were collected and analyzed for phytoplankton abundance and species composition. Water samples were returned to the lab within ca. 1 hour of collection and counted live using a 1 ml Sedgwick-Rafter counting chamber and Zeiss or Olympus microscopes equipped with phase contrast illumination. Phytoplankton were counted at 250X magnification and the 1mL chamber was counted yielding a 1 cell mL⁻¹ detection limit.

Phytoplankton and related methods are further described in Karentz and Smayda (1984, 1998), Smayda (1976, 1984) and Smayda *et al.* (2004). While the whole phytoplankton community was quantified in the weekly counts, the current research focuses on the dominant phytoplankton during the WS bloom period, here defined as weeks 1-18. This period was chosen to include the months of January-April of each year capturing the entirety of the WS bloom growth for all species. There are data gaps in 6 years (1987-1988; 1992-1994; 1998). Three diatoms dominate phytoplankton numerical abundance during the WS bloom in NBay: *Detonula confervacea*, *Thalassiosira nordenskiöldii* and *Skeletonema* spp. (Smayda, 1957; Pratt, 1959, 1965; Karentz & Smayda, 1998; Smayda *et al.*, 2004; Durbin *et al.*, 1975; Borkman & Smayda, 2009). The numerical abundance of these three dominant diatoms during the WS bloom was used in estimating the C, N, Si standing stock of the WS bloom.

Figure 2: Average surface temperature of Narragansett Bay during the WS bloom period (weeks 1-18). Linear regression showed a positive relationship with an increase of about 2°C.

Figure 3: WS (weeks 1-18) combined cellular carbon content of the three key WS bloom species. Linear regression shows a statistically significant decline over time.

2.2. Phytoplankton C, N, Si Content

A database of experimentally derived C, N, Si content of NBay Bay clones of the three species of interest (*Detonula confervacea*, *Thalassiosira nordenskiöldii*, *Skeletonema*

spp.) was compiled from the literature. Sources of NBay specific phytoplankton C, N, and Si content measurements included Fontneau (1973) and Mitchell-Innes (1973) for *D. confervacea* carbon and silica content respectively; Durbin, 1976 & 1977 for *T. nordenskiöldii* C,N, Si content and Deason (1980) and Yoder (1979) for *Skeletonema* C, N, Si content. Where possible, temperature-dependent C, N, Si responses for each species were determined by using GraphPad Prism v. 3.0 software to determine the best fit to each temperature - versus cell content relationship. Temperature-dependent cell content relationships were derived for five of nine species-cell content relationships:

D. confervacea carbon (pg cell^{-1}) = $[(-0.85 \cdot T) + 63.25]$ where T is temperature (derived from Fontneau, 1973)

T. nordenskiöldii clone TN2 carbon (pg cell^{-1}) = $[(-0.048 \cdot T^3) + (0.8509 \cdot T^2) + (9.2757 \cdot T) + 190.98]$ where T is temperature (derived from Durbin, 1977).

T. nordenskiöldii clone TN2 silica (pg cell^{-1}) = $[(-0.3257 \cdot T^2) + (13.457 \cdot T) + 155.86]$ where T is temperature (derived from Durbin, 1976).

Skeletonema (clone SK6C) carbon = $[(-0.0516 \cdot T^2) + (1.0629 \cdot T) + 42.344]$ where T is temperature (derived from Deason (1980), Yoder (1979) and Langdon (1988)).

Skeletonema (clone SK6C) nitrogen = $[(-0.0001 \cdot T^4) - (0.0039 \cdot T^3) + (0.0306 \cdot T^2) + (0.1709 \cdot T) + 5.0955]$ where T is temperature (derived from Deason (1980) and Yoder (1979)).

Fixed, temperature independent cellular content values were used for species-cell content relationships not having temperature-dependent relationships (listed above). Mean C, N, Si content of each species yielded by the above equations at a mean WS bloom temperature of 3.5°C are shown in Table 1.

Figure 4: The percent carbon contributions to the total biomass of the three species. The contribution of *Detonula confervacea* and that of *Thalassiosira nordenskiöldii* showed a statistically significant relationship.

Figure 5: WS (weeks 1-18) combined cellular nitrogen content of the three key WS bloom species. Linear regression shows a statistically significant decline over time.

2.3. Data Synthesis and Data Analysis

Estimates of cellular C, N, Si content (both temperature-dependent or -invariant) were calculated and multiplied by species numerical abundance at monthly time steps to yield estimates of WS bloom C, N, Si during the WS bloom periods of 1959 to 2011. C, N, Si calculations were completed in Microsoft Excel spreadsheets. Linear trends in total WS bloom C, N, Si and for trends in each species contribution to total C, N, and Si were quantified statistically using linear regression in JMP software. Linear trends were deemed statistically significant at a p-value threshold of 0.05.

3. Results

Figure 6: The percent nitrogen contributions to the total biomass of the three species. The contribution of *Detonula confervacea* shows a statistically significant negative relationship (decline) and *Thalassiosira nordenskiöldii* a positive relationship (increase) over time.

3.1 Winter-Spring Temperature

We know that climate is changing and has changed over time. The mean WS (weeks 1-18) temperature over the interval (1959-2011) is 3.5°C. The temperature has increased by about 2 degrees over this time interval and shows a statistically significant increasing trend over time (Figure 2). This change in temperature has had effect on the phytoplankton species in NBay, specifically the WS bloom species. The abundance of *Skeletonema spp.*, *D. confervacea*, and *T. nordenskiöldii* has shown a decrease over time. The WS bloom of *D. confervacea* has been nonexistent over the past decade.

3.2 Winter-Spring Bloom Carbon

Although it is understood that the WS bloom has seen a decline in phytoplankton species abundance and an overall decline in their cellular nutrients, the individual species trends have never been looked at. Each of the three key WS species showed different patterns themselves while working together to make up the NBay phytoplankton community as a whole. The mean WS phytoplankton carbon has shown a statistically significant decline over the time series (Figure 3). The mean carbon concentration has decreased from 349 milligrams/m³ (1960's) to 128 milligrams/m³ (2000's). This is a 63% decline in the cellular carbon concentration leaving only about 1/3 of the phytoplankton carbon in the bay recently when compared to the 1960's.

The individual species contribution to the carbon biomass has changed as well. The three key WS bloom species have shown different patterns of contribution, although overall the trend is downward. *Skeletonema spp.* represented about 55% of the carbon biomass throughout the entirety of the time series (1959-2011), with no trend. *Detonula confervacea* contributed a linearly decreasing amount of carbon from 22% (1960's) to 2% (2000's). *T. nordenskiöldii* displayed no linear trend but an increase in percent contribution from 23% (1960's) to 44% (2000's) (Figure 4).

3.3 Winter-Spring Bloom Nitrogen

The combined WS nitrogen of the phytoplankton species displayed a linear decline over time (Figure 5). This decrease was of the magnitude of 62% from a mean of 62 milligrams/m³ (1960's) to 24 milligrams/m³ (2000's). The individual species contributions to this overall decline once again showed differing patterns (Figure 6). *Skeletonema spp.* contributed about 40% of the WS nitrogen, with little variation. *Detonula confervacea* showed a linear decline from 27% (1960's) to 2% (2000's). *T. nordenskiöldii* displayed a mildly significant increase in nitrogen contribution with a 24% increase from 33% (1960's) to 57% (2000's).

Figure 7: WS (weeks 1-18) combined cellular silica content of the three key WS bloom species. Linear regression shows a statistically significant decline over time.

Figure 8: The percent silica contributions to the total biomass of the three species. The contribution of *Detonula confervacea* shows a statistically significant negative relationship over time.

3.4 Winter-Spring Bloom Silica

Cellular silica concentration of the WS bloom species showed a decline over time (Figure 7). This decline was not as drastic as the other nutrients; but still at 40% less silica in the most recent decade compared to the 1960's a significant decrease has occurred. The species contributions shown in Figure 8 reiterate a pattern displayed within the other nutrient concentrations. The contribution of *D. confervacea* declined linearly to an insignificant amount as its WS bloom has disappeared. *Skeletonema* spp. stayed around an average of 16% of the silica over time. *T. nordenskiöldii* witnessed a 16% increase in contribution from a mean of 67% (1960's) to 83% (2000's) dominating the Si-flux.

3.5 Bloom-Temperature Relationship

When WS C, N, and Si combined cellular concentrations were compared with the WS mean temperature over the time series an interesting result is found. Both C and N showed a statistically significant negative relationship with increasing temperature, and Si concentration displayed no relationship. With r-squared values close to 12% it is clear that temperature only explains part of the WS variation. Temperature alone only accounts for about 12% of the long-term variation in the winter spring bloom C, N, and Si.

4. Discussion

The WS bloom in Narragansett Bay has been declining in magnitude since the 1980's (Oviatt 1994). With an increase in WS temperature of about 2°C since 1959 a change in the NBay phytoplankton community may be expected. Research has shown that warmer winters are associated with the reduced size of the WS phytoplankton bloom (Keller et al., 1999; Oviatt, 1994). This study correlated cellular C, N, and Si concentrations of the three key WS NBay phytoplankton species over time to show a decline in nutrient standing stock. Each nutrient standing stock was significantly depleted (C- 63%, N-62%, Si-40%) in the relatively warm 2000's compared to the relatively cool 1960's. The individual species contributions to the standing stock also changed. The contribution of *D. confervacea* was almost completely nonexistent in the most recent decade (2000's) due in large part to a major depletion in its WS bloom abundance. The contribution of *Skeletonema* spp. was relatively unchanged remaining at a constant average contribution to the standing stock for each nutrient. *T. nordenskiöldii* displayed an increase in its contribution to all three nutrient concentrations suggesting the possibility that it took up nutrient that was previously used by *D. confervacea*. These unique results demonstrate that temperature is likely one of multiple drivers of WS bloom change in NBay and that the response to warming is species-specific.

No matter what has driven the WS bloom change the overall decline of phytoplankton abundance and phytoplankton cellular nutrients has been studied and documented. Consistent with this study which showed about 1/3 of the WS phytoplankton C and N concentration remains from the 1960's, it has been suggested that the mean annual chlorophyll in the lower NBay system has decreased by about 70% since 1970 (Li and Smayda, 1998; Fulweiler et al., 2009). Also the species *Skeletonema* is known to have shown a decline over the period of 1959-2007 in NBay. A greater decline of this species was shown specifically in the WS bloom period going from ca. 3300 cells mL^{-1} to ca. 700 cells mL^{-1} over time (Borkman and Smayda, 2009). This decline of the WS bloom has been seen not just in NBay, but also regionally. In the New England area, the WS bloom in Massachusetts Bay and

Species	Mean volume (mm ³)	Mean C (pg cell ⁻¹)	Mean N (pg cell ⁻¹)	Mean Si (pg cell ⁻¹)
Skeletonema spp.	200	39	5.5	2.7
Thalassiosira nordenskiöldii	5,518	295	55	140
Detonula confervacea	778	59	14	9

Table 1: Mean volume, carbon, nitrogen, and silica content of three species used in evaluation of winter-spring bloom C, N, Si content. Temperature-dependent cellular constituents calculated at temperature of 3.5°C.

Boston Harbor was diminished or nonexistent through the mid 1990's (Keller et al., 2001).

With a clear idea of the decline of the phytoplankton bloom and the lack of a complete explanation of WS bloom change by temperature, other variables must be accounted for. Zooplankton abundance and grazing rate is one possible additional factor describing the change. Zooplankton play a vital role in the abundance of phytoplankton, reducing the bloom, and also the amount of nutrients that sink down to the benthic zone. As temperature has increased, the peak abundance of the phytoplankton and zooplankton could be coming closer which contributes to the result of a decreased bloom (Keller et al., 1999). Another aspect of the coastal system that can cause changes in the bloom is weather (light, wind, stratification). The abundance of *Skeletonema* has been related to the position of the NAO and GSNW suggesting a potential decrease in the WS bloom when the GSNW is located further south (Borkman and Smayda, 2009). A final factor that could be contributing to the reduction of the WS phytoplankton bloom is nutrient reduction that has taken place in NBay over the past 50 years. A change in sewage treatment and regulations has drastically changed the nutrient, particularly the nitrogen, being pumped into NBay (Nixon et al., 2008). However, even with the sediment switch from denitrification to N fixation it is uncertain how this has or will affect the WS bloom (Nixon et al. 2009).

This study has scratched the surface on a single WS bloom limiting factor in NBay: temperature. With many other factors involved in describing the variation of the WS bloom this work has given a basis for future work. Although the three species used make up a large percentage of the bloom in this area it does not describe the entire story where other species interactions could be taking place. Using samples from different areas around the bay could also provide a more advanced knowledge about the bloom as a whole and variation between different sections of the area. A future study could measure the particulate C, N, Si, and P levels and compare them to predicted levels represented by the model in this research to test for accuracy. Also other variables could be measured more

intensely along with temperature as a primary focus of variation.

5. Conclusion

The WS bloom in Narragansett Bay has been studied over a 52 year time series. An increase in WS temperature over that time period is strongly statistically related to the decrease in phytoplankton abundance. In this study temperature dependent cellular C, N, and Si concentrations of the three key WS Narragansett Bay phytoplankton species (*Skeletonema spp.*, *D. confervacea*, *T. nordenskiöldii*) were analyzed. A decline in nutrient standing stock was concluded at various magnitudes for C, N, and Si for the three species combined. Individually, the species contributions to the standing stock showed varying species-specific patterns. The contribution of Arctic-Boreal diatom *D. confervacea* has been diminished to almost nothing from the 1960's to the 2000's. The contribution of *Skeletonema spp.* was unchanged supplying the same percentage of biomass for each nutrient through time. *T. nordenskiöldii* was the only species to show an increase in its contribution to all three nutrient concentrations. This indicates that it may be taking up the nutrients that were previously taken by *D. confervacea*. Temperature explains some of the variation of the WS bloom but other factors play significant roles as well and future work will hopefully be able to explain all the variation causing the decline of the WS bloom in Narragansett Bay.

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Examining *Thalassiosira* Community Composition in the Costa Rica Upwelling Dome

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Abstract. Diatoms play an important role in the biological and chemical processes of the world's oceans. These and other phytoplankton form the base of the marine food web and are key in the ocean-atmosphere exchange of carbon dioxide. *Thalassiosira* is a genus of centric diatoms with cell walls of silica that contains the first diatom to have its genome mapped, making them important model organisms. They are found throughout the ocean, but flourish in upwelling regions. The Costa Rica Upwelling Dome (CRUD), formed during the summer months by the interactions between surface winds and equatorial currents, brings nutrient rich water to the surface. This dome is unique because the typical diatom bloom that occurs in upwelling regions does not form; instead, cyanobacteria dominate the surface. An investigation was launched to discover what was limiting the diatom population in the CRUD. The hypothesis that was tested was that there is a lack of trace metals at the surface, causing competition between the cyanobacteria and the diatoms. To test this, samples were collected from the dome and treated with nutrient additions. The growth levels were measured and it was concluded that there is a co-limitation of trace metals and silica in the dome. In addition to grow out experiments, there was also a transect through the dome where a variety of biological and chemical parameters were sampled. The flow cytometry analysis of the samples from the transect confirmed that the surface water of the dome was dominated by the cyanobacteria, with lower populations of diatoms, but in deeper waters the relationship inverted and diatoms dominated. This study focused on examining the diatom community composition from the transect samples. It was also found that there is greater variety in diatom community composition in the deep waters of the dome, and the least diversity in diatoms at the surface water in the center of the dome. Analyzing the natural diatom community and how that community changes in response to alleviation of nutrient limitation of diatoms in the dome will afford us with a better understanding of the population dynamics of the area, and what factors constrain the diatoms' ability to fix carbon. This is an important process that may be affected in the future as climate changes and shifts the ocean's chemistry.

1. Introduction

Diatoms are microscopic phytoplankton with cell walls of silica that are responsible for up to 20% of the photosynthesis on the planet. They are found in many bodies of water, from freshwater rivers to the open ocean, from the poles to the equator (Armburst, 2009). Their role in the ocean is vital- they form the base of the marine food web, thus acting as a major food source for many larger organisms in the ocean. They also are integral in the ocean's carbon pump, which is the process that summarizes the exchange of carbon dioxide between the atmosphere and the ocean. In the system phytoplankton are responsible for the uptake of carbon dioxide and its conversion to biomass carbon through photosynthesis in the surface ocean. When they die and sink to the bottom of the ocean, they can get buried in the marine sediment, which, over time, builds up and eventually forms oil and gas, the main fuel sources of the planet (Armburst, 2009). Due to their ubiquitous nature and the processes they support, diatoms are highly studied organisms. Changes in their populations and behavior can have global impacts, therefore understanding what conditions they need to survive is extremely important (Arrigo, 2005).

Thalassiosira is a genus of centric diatoms that contains the first diatom, *Thalassiosira pseudonana*, chosen to have its entire genome mapped (Armburst, 2004). Many physiological and genomic studies have focused on this genus, and some of its members are considered to be model

organisms. *Thalassiosira* ssp. and other diatoms flourish in water that is nutrient rich. In upwelling regions, winds deliver nutrient rich waters to the surface ocean, promoting diatom blooms and high photosynthetic productivity. At one particular upwelling region, however, this is not the case. The Costa Rica Upwelling Dome (CRUD) is formed off the western edge of Costa Rica during the summer months. The interaction between surface winds and equatorial currents causes a shoaling of the thermocline that brings colder, nutrient rich water to the surface (Fielder 2002). The surface water in the CRUD is teeming with cyanobacteria, some of the highest ocean concentrations of species such as *Synechococcus* have been found here (Saito, 2005). It is currently a mystery as to why diatoms are not a dominant member of the phytoplankton in the CRUD. One hypothesis is that nutrient supply in the CRUD is different than in other coastal upwelling regions and that diatom growth is limited by one or more nutrients. Preliminary data from the Jenkins lab supports this hypothesis. Previous research in the region showed that while the upwelling was bringing certain nutrients to the surface, the area was still lacking in several vital nutrients and metals. A research cruise embarked in May of 2010 to examine ocean conditions and collect samples from various locations and depths in the dome. Samples were treated with different nutrient additions to see how the diatom assemblages reacted. These samples were compared to the natural condition ones to test if the added nutrients fostered growth.

Figure 2: The graph shows the relative response from the nutrient addition conditions, as compared to the control. The x's indicate that at that particular cycle the nutrient amendment was not conducted. Cycles 2 and 4 come from the same parcel of surface water from the center of the dome, and thus are both blue. The co-additions had the greatest effect.

Figure 2: Map of Research Cruise. The locations of incubation experiments are boxed.

What the Jenkins lab found in deckboard manipulations of the ambient mixed layer phytoplankton community in the center of the CRUD was a large response to zinc and silica and iron and silica additions (Figure 1). Looking at cycle 4, which was from surface water in the center of the dome and it is the only cycle that contained data from all nutrient amendment condition some interesting patterns emerge. While the initial hypothesis was that metals were the main limiting nutrient, compared to the control of no addition, the individual iron and zinc conditions had the lowest responses, with growth of 1.4 and 1.8 times that of the natural assemblage, respectively. The plain silica addition had a modest response; the samples from that condition had growth 2.8 times that of the control. After seeing this response at cycle 2, cycle 4 additions included zinc and silica together as a condition, as well as iron and silica. These conditions showed the greatest growth rate, fostering growth 5.5 times that of the control for the zinc and silica together and 4.5 times that of the control for the iron and silica together. These results indicate that instead of a single metal limiting the diatom populations, as was previously expected, there appears to be an effect of co-limitation. This effect is likely due to the fact that zinc and iron act as co-factors for silica-uptake machinery in

Thalassiosira and other diatoms. So, while there was a moderate response from adding the silica alone, when the co-factor trace metals are available the diatoms are able to absorb and use the silica much more efficiently (Franck et al. 2003).

After compiling and analyzing the preliminary data, secondary analysis was done to determine what *Thalassiosira* species are present in the region, how shifts in their communities relate to ambient conditions, and how they changed in response to the nutrient manipulations.

2. Methods

2.1 Sample Collection

The month long research cruise to the CRUD took place in May of 2010 on board the R/V Melville (Figure 2). The ship sailed along a transect to obtain data and samples from the edges of the dome to the center. At every point marked along the transect the following measurements were taken: total metals (iron and zinc), nutrients (nitrogen, phosphorous, silica), flow-cytometry, microscopy, zooplankton counts, *Synechococcus* and *Prochlorococcus* clade specific abundance, intracellular metal quotas of phytoplankton, and CTD profile data of salinity, temperature, and chlorophyll fluorescence. DNA and RNA samples for analysis of diatom abundance and gene expression were taken at two to four different depths at each transect point, ranging from 10 m to 80 m. At the areas marked cycles 2, 3, and 4 the same measurements were taken and nutrient amendments experiments were conducted. These experiments were performed as incubations, 2L of sample water from the surface was placed in polycarbonate bottles and treated with one of the following conditions: no addition (control), +2 nM Zn, +4 nM Fe, +1 μ M Si, +2 nM Zn & +1 μ M Si, and +4 nM Fe & +1 μ M Si. The bottles were sealed and incubated at surface light intensities in large containers on the deck of the ship for 72 hours to give the diatom assemblages time to react to the condition they were given. During this time ocean water was constantly cycled in and out of the deckboard incubators to mimic the natural ocean conditions as much as possible. After the three days were up, the water was filtered in duplicate onto filters with a 3 μ m pore size using a peristaltic pump. Generally, 300-400 mL was filtered for DNA and 400-500 mL was filtered for RNA. Filters were placed into 2mL containing RNA or DNA buffers, filtered, flash frozen, and stored at -80°C.

2.2 DNA Extraction

The DNA was extracted from the samples by following the protocol of the QIAGEN DNeasy® Plant DNA extraction kit. To assist in the lysing of the cells, 0.1 μ m and 0.5 μ m silica beads were added to the tube with the RNaseA enzyme before heating the samples for 10 minutes at 65°C. After heating the samples were bead beaten for 1 minute before adhering to the protocol. At the end the samples were eluted into 50 μ L of buffer EB prior to quantifying them using a Qubit® 2.0 Fluorometer.

Figure 3: Silicate Profile in the CRUD. The center of the dome is marked as locations 5, 11, and 13 on the plot. The silicate concentrations at the surface water there are lower than at the outskirts of the dome.

Figure 4: Profile of cyanobacteria in the CRUD. The profile shows that there are high *Synechococcus* concentrations in the surface water at the center of the dome, and the high concentrations extend 20 m deep. South of the dome the surface does not have such high abundances of the cyanobacteria.

2.3 PCR Amplification

The PCR primers were designed to amplify the ITS2 region of the *Thalassiosira* DNA. A 5' fluorescent FAM label forward primer (5'-RCGAAYTGCAGAACCTCG-3') and a non-fluorescent reverse degenerate ITS2 primer (5'-TACTYAAATCTGAGATYCA-3') were used in the reaction. Each sample was diluted to 3 ng/ml before making a solution containing 2.5 μ l of sample, 1.25 μ l of each primer, 12.5 μ l of 1-x BIO-X-ACT™ Short Mix, and

7.5 μ l of PCR grade water. The samples were run at 95°C for 5 min, followed by 32 cycles of: 95°C for one min, 46°C for one min, and 72°C for 30 seconds; they ended with an extension at 72°C for 10 min and cooling at 4°C. This process was run using Eppendorf's Mastercycler gradient PCR machine.

2.4 Ethanol Precipitation

Figure 5: Profile of Photosynthetic Eukaryotes in the CRUD. At the center of the dome the surface water does not have high concentrations of photosynthetic eukaryotes, which include diatoms. The higher concentrations are found deeper in the water column, below the cyanobacteria.

Following PCR amplification, ethanol precipitations were done to purify the samples. The samples were transferred to 1.5 ml tubes and 2.5 μ l of 3M sodium acetate was added, along with 50 μ l of 100% ethanol. The samples were vortexed and incubated overnight at -20°C. After the incubation period was over the samples were centrifuged at 2°C for 30min. The supernatant was then decanted and the sample tubes were washed with 100 μ l of 70% ethanol before centrifuging again for 5 min. The 70% ethanol rinse was repeated. To speed up drying of the pellet, a final wash was done with 150 μ l of 100% ethanol. After centrifuging and decanting, the samples were dried on a heat block. The samples were finally resuspended in 25 μ l of water. To prepare the samples for fragment 11 μ l of Hi-Di Formamide and 0.3 μ l of GeneScantm 600 LIZ size standard were added to 1 μ l of sample. These samples were submitted to the Rhode Island Genomics and Sequencing Center for fragment analysis.

3. Results and Discussion

3.1 Ocean Condition Profiling

Plots were constructed using the program Ocean Data View (ODV) to create profiles of the nutrient and organism concentrations at different locations and depths of the dome (Figures 3-5). A zoomed in map, included with the plots, shows the transect sample locations (Figure 6). The silica plot (Figure 3) shows that while compared to the waters south of the dome area, which has extremely low silica, the surface water in the center of the dome (labeled 5, 11, and 13 on the x axis) has relatively high silica. However, when compared to the immediately surrounding areas, especially those just north of the dome, where transect sample 7 was taken, the center of the dome has low surface silica. The profile shows that at the center of the dome, silicate

concentrations of at least 5 μ M aren't found until depths of 18m. North of the dome, silicate concentrations of 5 μ M are found right at the surface. Based on flow cytometry data, the center of the dome has extremely high *Synechococcus* concentrations in the surface water compared to the water south of the dome (Figure 4). The high concentration extends deep into the water column at the center, to about 30m. To the north the depth to which the high concentration extends lessens to 20m. The flow cytometry data for the photosynthetic eukaryotes, which contains the diatoms this study is interested in, shows that at the center of the dome, where the surface water has high concentrations of cyanobacteria, the diatoms are less

Figure 6: Transect Sample Map. The transect, marked as the red line, had samples taken at each numbered mark. Samples taken at sites 5, 11, and 13 are in the center of the dome.

Figure 7: Species in Samples. The diagram shows the concentration of each base pair length species in each sample from the transect. Certain base pair lengths, such as 388 are found in high concentrations throughout the dome. Other lengths, such as 402 are found at a low concentration in a single sample. The key below the diagram shows the colors that represent the concentrations.

abundant (Figure 5). There is, however, a surge in photosynthetic eukaryotes abundance in the water below 20m. Also, in the northern edge of the dome (Station 7), the diatom populations begin to show up closer to the surface.

In the surface water at the center of the dome, where the silicate concentrations are low, the cyanobacteria are able to thrive. Normally, *Synechococcus* is found at very low concentrations in the ocean's surface water, but with the absence of diatoms they can flourish (Waterbury, 2006). These cyanobacteria have a large surface area to volume ratio that allows them to be successful when nutrients are limited. They also do not need silicate and therefore are not at all limited by that nutrient. The photosynthetic eukaryotes, like diatoms, do not possess this ability and are thus more impacted by a lack of certain metals. They cannot compete well enough for what is available, and are limited to deeper water.

3.2 Community Composition

The fragment analysis data for all of the samples taken in the transect was compiled and arranged in a diagram (Figure 7) that shows the relative concentration of each base pair length in the mixed assemblages. Table 1 shows what species correspond to the various base pair lengths of the ITS2 region. It appears that certain species are much more prevalent in the dome than others. For instance, the species with 388 base pairs in its ITS2 region (likely *T. anguste-lineata*) makes up more than 50% of the population for over half of the samples. It is abundant both in shallow and deeper waters, at the center and on the outskirts of the dome. Several other species are much more affected by the scarcity of certain nutrient and are limited to deeper water.

Species with base pair lengths of 386, 390, 396, 402, and 404 are each found in only one or two of the samples, and make up less than 10% of the population in them.

Some species of diatoms are better equipped to deal with scarcities in nutrients, several have adapted to low iron conditions (Armburst, 2009). These organisms are better suited to inhabit an ecological niche with limited nutrients, and therefore can inhabit that region. Given this, it is perhaps not surprising that only a few species of *Thalassiosira* seemed to exist in the center of the CRUD.

Figure 8 shows a Bray Curtis diagram that compares similarity of community compositions between samples.

Size of fragment (bases)	Species
348-349	<i>T. oceanica</i>
357-358	N/A
362-363	N/A
366-367	d
368-369	<i>Skeletonema</i>
374-375	<i>Mimodiscus</i> , <i>Skeletonema</i>
377-378	CCMP1616, <i>Skeletonema</i>
384-385	e, g, <i>T. minuscula</i>
386-387	f, i, j, <i>T. punctigera</i> , <i>T. rotula</i> , <i>Skeletonema</i>
388-389	h, k, <i>T. anguste-lineata</i> , CCMP1004
390-391	<i>T. aestivalis</i>
393-394	<i>T. guillardii</i> , <i>T. pseudonana</i>
396-397	<i>T. weissflogii</i>
402-403	N/A
404-405	c
409-410	<i>C. atlanticus</i> , a, b

Table 1: Species Identification. The table shows which species correspond to the various base pair lengths of the ITS2 region of *Thalassiosira* DNA.

Figure 8: Community Composition Similarity. The cluster diagram shows that samples to the right with the most similar communities are taken from the surface water in the center of the dome. The less similar communities are to the left, and come from deep waters in the center of the dome, where cyanobacteria are not located.

The samples with the most similar communities came from surface water at the center of the dome, where the detailed analysis up above showed only a few species were found. The deeper water in the center of the dome had samples with the least similar community compositions. A wide variety of species made up the populations in these samples. It appears that the limited nutrients in the dome can hinder diatom community diversity. Where there are lower cyanobacteria concentrations and more nutrients it appears that a wider variety of diatom species can survive.

4. Conclusions

The CRUD has low concentrations of trace metals and silica the surface water in the center of the dome, which appears to be hindering the diatom populations there. Cyanobacteria, especially *Synechococcus*, seem to be more suited for the specific limiting nutrient conditions in the CRUD and can out compete the diatoms. Trace metals can act as biological facilitators and with additional iron and zinc in the presence of silica, the diatom assemblages might be able to flourish and better compete with the cyanobacteria (Franck, 2003). Only a few species of *Thalassiosira* appear to be able to compete with the cyanobacteria successfully in the CRUD, so the regions

with an abundance of cyanobacteria have low diatom community variety. In deeper waters of the dome, where the diatoms dominate, the communities are much more diverse. As the planet's climate changes the ocean chemistry can shift. If the shift favors small organisms, like cyanobacteria, the ocean's carbon pump may not function smoothly. Thus far, it has been noted that a more complex effect of colimitation is present in the dome and that some *Thalassiosira* species are equipped to compete with cyanobacteria than others. As analysis of the samples from the nutrient amendment conditions continue, each species' reaction to the specific additions will be studied. The shifts in community composition that occur when the diatoms are no longer limited by nutrient availability will be seen.

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Reconstructing Past Plate Motions with Abyssal Hill Topography

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Abstract. The seafloor spreading history of oceanic plates is primarily reconstructed by using a combination of dated seafloor magnetic anomalies and fracture zone trends to locate Euler poles and determine rotation rates. In some regions, these conventional measures of determining spreading history do not exist and alternative methods are required. Abyssal hills are elongate, topographic highs that are created at and form parallel to the ridge axis of spreading centers. Once formed, the abyssal hills are transported onto the ridge flank and provide a record of ridge axis orientation. We propose using the orientation of abyssal hill topography to supplement existing plate motion reconstruction methods and to serve as a stand-alone method for regions where conventional methods will not apply. Our new method uses high-resolution bathymetry grids created with multibeam data with a grid-node spacing of 100 m. The bathymetric grids are detrended to remove depth-age relationships and spatially filtered to remove anomalous regions where seafloor age is unknown. The detrended grids are then clipped to remove anomalously deep (e.g., fracture zones) or anomalously shallow topography (e.g., seamounts). Abyssal hill orientations are determined by calculating the modal frequencies of slope azimuth for pre-defined sub-regions (e.g., 50 km x 50 km) of these modified grids. The Euler Pole is then found by an iterative least-squares method from a grid of potential Euler Poles; first a coarse grid and then a fine grid are utilized to avoid local minima. At each potential Euler Pole, the sum of the differences between predicted and observed abyssal hill trends is squared and the minimum value identifies the location of the best-fit Euler Pole. Our method was applied to the well-mapped Cocos-Pacific plate boundary along the northern East Pacific Rise for the past 3 Ma. The results for the northern East Pacific Rise are very encouraging and our calculated Euler Poles are within 4 degrees of the NUVEL 1a and 2 degrees of the MORVEL global relative motion models. We also applied our method to the EMAG2 magnetic anomaly intensity grids, resulting in a 17 degree difference for NUVEL 1a & 11 degrees for MORVEL. The method effectively identifies Euler Poles by means of abyssal hill topography and may be used as a supplement to existing models or as an independent method in regions without conventional measures.

1. Introduction

The relative motion of lithospheric plates on the Earth's surface can be described by an Euler Pole and a rotation rate about the pole. The location of the Euler Pole is often determined by a combination of fracture zone trends, seafloor magnetic lineations, earthquake slip vectors, and even GPS measurements (e.g., DeMets et al., 1994; 2010). The rotation rates are typically determined with dated seafloor isochrons associated with seafloor magnetic anomalies. The focus of this project is to use abyssal hill orientations in lieu of conventional methods to calculate Euler Pole locations.

Abyssal hills are ubiquitous seafloor features, covering approximately 80% of the seafloor (Menard & Mammerrickx, 1967). Abyssal hills are elongate bathymetric highs that form parallel to the ridge axis at divergent plate boundaries. As the young seafloor moves away from the ridge axis, the orientation of the abyssal hills provide a record of past ridge axis orientations (Figure 1). The great circle projection of these abyssal hill trends should intersect at the location of the Euler Pole that defines the relative plate motion.

Several previous studies have used abyssal hill morphology to infer past spreading rates and spreading directions. Goff and Jordan (1988) and Malinverno and Pockalny (1990) described the relationship between abyssal relief and spreading rate. Bird and Pockalny (1994) further characterized this relationship by quantifying the role of sediment thickness. Kriner et al. (2006) demonstrated that

abyssal hill orientation and related slope distributions can be used to constrain both past spreading rates and spreading directions

A new method is presented here which uses abyssal hill orientations measured from high-resolution bathymetry data to determine Euler Pole locations. The method is tested on the well-mapped Cocos-Pacific plate boundary along the northern East Pacific Rise where the recent (< 3 Ma) relative motion of the region is very well constrained by previous studies (DeMets et al., 1994; 2010).

2. Data & Methods

The general approach for this study was to use archived multibeam data to create high-resolution bathymetric grids of the entire Cocos-Pacific plate boundary. The abyssal hill orientations were calculated for pre-defined subregions of the bathymetric grid, and these orientations were used to calculate an Euler Pole.

The multibeam data used to create the bathymetric grids was obtained from the NOAA Multibeam Portal (Multibeam Bathymetry, 2013) and was restricted to 12 kHz full-ocean multibeam systems. The multibeam data were processed using MB System (Caress and Chayes, 1995) to merge the data and create bathymetric grids with an 100-m grid spacing. The grid spacing interval is chosen to best represent the acoustic footprint for the characteristic depths in our study area.

The bathymetric grid initially encompassed the area from 2-18°N and 100-107°W to span the Cocos-Pacific

Figure 1. Abyssal hills form on newly created seafloor at divergent boundaries, shown here as grey dashed lines. The trends of the abyssal hills projected along a great circle will intersect at the Euler Pole that defines the plate motions.

boundary, but was then masked to only include seafloor younger than 3 Ma—the upper age limit of the existing models used for comparison, NUVEL 1A and MORVEL (DeMets et al., 1994; 2010). The bathymetric grid was then detrended to remove the depth versus age relationship and clipped to remove anomalously shallow or anomalously deep topography (corresponding to seamounts or fracture zones, respectively) (Figure 2).

The bathymetric grid was subsampled into regions of consistent size. The edges of the subregion were varied from 25 to 50 km to examine the effects on the standard deviation of the abyssal hill orientations. 50 km subregions resulted in the lowest standard deviation while still providing a high enough resolution to accurately determine the abyssal hill orientations.

Abyssal hill orientations for each subregion were determined using the gradient of the bathymetry. The gradient vector is computed with the Matlab gradient function, which generates scalar gradient values in the x- and y-direction from a matrix of bathymetric data. From these scalar values the trend of the gradient was calculated.

The trend, or orientation, of the abyssal hills is the parameter of interest and is normal to the trend of the gradient (Figure 3). For each subregion, a histogram was plotted of the calculated trends, a median filter of the 9th order was applied, and the local maxima were found from the filtered data. A 9th order filter is used because it provides a best fit to the frequency of the data. Two local maxima are expected with $\sim 180^\circ$ between them. Two calculated trends per subregion are a result of the eastward- and westward-facing slopes. Rotating the trend that corresponds to the westward-facing slope 180° ensures that both trends are in the same range (Figure 4).

The calculated abyssal hill orientations for a given subregion were used only if they met two criteria. First, at least 10% of the grid nodes within a subregion must represent real bathymetry data. The 10% limit marks the transition between large and consistent variance on plots abyssal hill orientation variance versus percentage of true bathymetric coverage. Second, abyssal hills orientations exceeding 25° from the modal value of all sub-regions were assumed to be non-representative of the abyssal hill fabric

Figure 2. Left- original multibeam bathymetry of the northern East Pacific Rise. Right- modified multibeam bathymetry.

and were removed from further calculations. The 25° limit was determined by varying the angular limits and noting when calculated Euler Pole locations began to stabilize.

The Euler Pole for the region was calculated with a best-fit least-squares method (equation below).

$$\sum_{i=1}^n (T_c(i) - T_{ex}(i))^2$$

Where T_c is the calculated abyssal hill orientation for a sub-region, T_{ex} is the predicted orientation for a given Euler Pole, and n is the number of calculated abyssal hill trends from the subregions that meet our predefined criteria (e.g., 10% of region is real bathymetry data and the trend is within 25° of the modal value).

Both a coarse and fine grid of potential Euler Pole locations was used to avoid local minima. The coarse grid ranges 20° in every direction with the initial predicted Euler Pole location as the center and increments by 1°. The result is a sum of the difference squared value for every Euler Pole location. The Euler Pole location with the minimum sum of the difference squared value is then used as the initial prediction for the final best-fit iteration, using the same approach as the first but with a search grid of 2° in every direction from the initial prediction and .125° increments. The Euler Pole with the minimum sum of the difference squared value after the fine grid search is the best fit Euler Pole.

Figure 3. Left- An example of a subregion with the gradient vector calculated from one data point. Right- The gradient trend is shown, with the trend of the abyssal hills calculated as θ and represented by a grey dashed line.

3. Results & Discussion

Preliminary results indicate that abyssal hill orientations yield very reasonable estimates for Euler Pole locations. The comparison of previously calculated Euler Poles and the results from this study are in very good agreement. These results suggest that abyssal hill orientations may provide useful supplemental data for future plate motion models.

The application of the method on the abyssal hill topography of the Cocos-Pacific plate boundary resulted in an Euler Pole at 40°N , 109°W (Figure 5). The method was also applied to a magnetic anomaly intensity grid. The magnetics grid was obtained from EMAG2, a combination of satellite, marine, aeromagnetic, and ground magnetic surveys with a 2 arc minute resolution (Maus et al., 2009). The Euler Pole found from the magnetics grid was located at 53°N , 117.5°W .

The results of this study are compared with two existing models, NUVEL 1A and MORVEL (DeMets et al., 1994, 2010). Both models are multivariant, combining ridge spreading rates, transform fault trends, and earthquake slip vectors to create best fit rigid plate motions on a global scale. MORVEL differs from NUVEL 1A in that it employs a more extensive database and focuses more on present day GPS data. NUVEL 1A places the Euler Pole for the Cocos-Pacific plate boundary at 37°N , 109°W , a 4° difference from this study's calculated abyssal hill Euler Pole. The MORVEL model results in an Euler Pole at 42°N , 112°W , a 2° difference from the abyssal hill Euler Pole. A 17° difference from NUVEL 1A exists for the magnetics Euler Pole and an 11° difference from MORVEL.

The difference between the existing models and the abyssal hill topography method is quite small (e.g., 2° - 4°). A source of discrepancy could be that this study capitalizes on a single parameter, abyssal hill orientation, while the existing models, NUVEL 1A and MORVEL, use a multivariant approach. The NUVEL 1A and MORVEL models also solve for a global best fit while this study focused on a best fit for a single region. MORVEL also has a strong emphasis on recent tectonic activity, by means of present day GPS and earthquake slip vectors, while

Figure 4. Every theta value for the subregion comprises the blue histogram bars. The pink line is a best-fit median filter and the local maxima, plotted as yellow circles, correspond to the abyssal hill orientation for the subregion.

Figure 5. Study area highlighted in yellow with the ridge axis delineated in red. Euler Pole locations: purple diamond is MORVEL, yellow diamond is NUVEL 1A, blue-filled circle is the abyssal hill result, and the dark green-filled circle is the magnetics result.

NUVEL 1A does not account well for activity younger than 0.7 Ma.

The Euler Pole derived from the magnetics data does not agree as well with the existing models (e.g., 11° and 17° difference for MORVEL and NUVEL 1A, respectively). The average magnetic reversal period is 500 thousand years while the average time for abyssal hill formation is 10 thousand years. This suggests that the magnetic anomaly grid might not have sufficient resolution to identify smaller scale tectonic events that are preserved by the abyssal hills, accounting for the difference in their Euler Pole

calculations. These results show that magnetic anomalies might not be the most effective seafloor feature for tectonic reconstruction because of the scale of time for formation.

The proximity of the results from the abyssal hill topography method to the existing models demonstrates that the method has potential as a supplement to existing models. The method also has potential as a stand-alone approach to tectonic reconstructions in areas where the tectonic history is poorly constrained. Abyssal hills are pervasive and can provide an alternative method to reconstructions in areas where magnetic anomalies and fracture zones, the typical features used in reconstructions, are absent.

	Euler Pole Latitude (°N)	Euler Pole Longitude (°W)
NUVEL 1A	36.80	108.60
MORVEL	42.17	112.77
Abyssal Hills	40.38	109.38
Magnetics	53.00	117.50

Table 1. Euler Pole locations for the existing models, using abyssal hill orientations, and using a magnetic intensity grid.

4. Conclusion

As the results confirm, abyssal hill topography is an effective tool for plate reconstructions by means of calculating Euler Poles. For the northern East Pacific Rise segment of the Cocos-Pacific boundary, the newly derived method came within 4° of the NUVEL 1A model Euler Pole location and 2° of the MORVEL location. The application of the method on the magnetic anomaly grid of the same area did not produce as accurate as results, with a 17° difference from the NUVEL 1A Euler Pole and an 11° difference from MORVEL. Our results provide encouraging evidence that abyssal hill topography is an

effective method for tectonic reconstructions and has the potential to support and strengthen existing models. There is also the possibility of abyssal hill topography providing a stand-alone method for tectonic reconstructions in areas where magnetic anomalies or fracture zones do not exist.

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Experimental Verification of Numerically Predicted Optimal Efficiency Energy Extraction Hydrodynamics for a Flapping Foil

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Abstract. Bio-inspired flapping foils have recently emerged as a viable offshore renewable energy source. For the flapping foil, the hydrodynamic energy extraction efficiency can be altered by varying different motion parameters, such as the heaving and pitching motion and the non-dimensional flapping frequency of the foil. A previous two-dimensional numerical analysis determined how the energy extraction efficiency of a NACA 0015 foil changed as a function of the non-dimensional heave amplitude (h_0/c), the pitching angle (θ) and the Strouhal number (St). One goal of the numerical analysis was to determine which combinations of the aforementioned parameter values resulted in optimal theoretical hydrodynamic efficiencies. This study was an experimental verification of the results obtained from the numerical analysis. The foil motion was modeled using a NACA 0012 foil mounted to a force sensor on a motor driven carriage. The carriage was equipped with X-Y actuators for driving the heave motion of the foil and a rotational motor for driving the pitch motion of the foil. The speed of the tow, heaving and pitching motions of the foil, and flapping frequency were controlled using a Delta Tau PMAC2 motion controller. One-hundred and forty experiments, consisting of different combinations of h_0/c , θ and St values, were performed, and experimental motion and force data were obtained for each. The motion and force data were used to estimate the energy extraction efficiency based on the phasing of the measured forces with respect to the foil motion. The experimentally determined efficiencies were then compared to the efficiencies obtained by the numerical analysis. The experimental efficiencies calculated showed to differ slightly in value from those of the numerical analysis; however, both exhibited the same trend overall. The maximum efficiency from experiments was found to be 49.2% occurring for $\theta_0 = 70^\circ$ and $St = 0.15$ for $h_0/c = 0.50$, while the maximum efficiency from numerical simulation was found to be 43% occurring for $\theta_0 = 90^\circ$ and $St = 0.50$ for $h_0/c = 1.25$. Accounting for expected discrepancies with the experimental study and inherent differences between the two studies, the results of the experimental study provide a good validation for the numerical model. Consequently, the combined results of these studies could now aid in providing a basis for developing control systems that would allow flapping foil energy harvesters to be more competitive with existing renewable and non-renewable energy sources.

1. Introduction

As the world continues to grow so too does the amount of energy humans use on an everyday basis. Consequently, the demand for energy is always increasing, but with current primary energy sources like oil proving to be environmentally harmful and progressively more limited, interest has turned to researching new and improved ways of harnessing energy from renewable sources. Wind and solar energy have already proven to be effective renewable sources (Persichetti, 2013), but recently ocean energy harvesting from waves and tidal driven currents has emerged as another viable renewable energy option.

One such energy extraction system that is currently being explored involves the use of biologically-inspired flapping (oscillating) foils. Research has shown that fish can use their streamlined shape (which resembles a foil) and structural properties to efficiently extract useful energy from surrounding fluid flows, consequently allowing them to move effortlessly through the water (Liao, 2003). Aside from their ability to harness energy from ocean currents, flapping foils are relatively small, versatile and, unlike conventional turbine-based designs, have the potential to have very little negative impact on the surrounding marine environment if designed and implemented appropriately. Similar to turbine-based designs, though, the flapping foil energy harvester is governed by Betz's Law in that the maximum possible achievable energy extraction efficiency for the device is the Betz limit—59.3%. However, since water is a denser medium than air, flapping foils and other

energy harvesters in water can have a much higher energy density. For a given power output, an energy harvester in water can be much smaller than a wind turbine while achieving similar efficiencies. Thus, improving the efficiencies of these devices is of primary focus for researchers since high energy extraction efficiencies coupled with the low manufacturing costs of these foils would immensely reduce the total energy cost per kW-hour of the devices.

For the flapping foil, hydrodynamic energy extraction efficiency can be altered and possibly improved by varying hydrodynamic parameters, such as the heaving and pitching motion and the flapping frequency of the foil. One study found that properly-timed leading edge vortices can actually help to transfer energy back into the system by contributing to the foil's pitching motion (Zhu, 2011). The larger the pitching motion of the foil, the bigger leading edge vortices become, which consequently increases the lift force and thus the total power output and efficiency of the foil. A separate study forced the pitch and heave motion of a symmetric NACA 0012 foil at various heave-to-chord ratios and found that efficiencies as high as 45% could be achieved for specific foil hydrodynamic parameter combinations (Simpson, 2009). Furthermore, a previous two-dimensional numerical analysis investigated how the energy extraction efficiency of a NACA 0015 foil changed as a function of the non-dimensional heave amplitude (h_0/c), the pitching angle (θ) and the non-dimensional flapping frequency or Strouhal number (St) (Persichetti, 2013). θ_0 and St were varied from 30° to 90° (10° increments) and

0.10 to 0.60 (0.10 increments), respectively, for h_0/c 's of 0.50, 0.75, 1.00, and 1.25. Efficiencies were calculated for each parameter combination and it was found that a maximum efficiency of 43% occurred for $\theta_0 = 90^\circ$ and $St = 0.50$ for $h_0/c = 1.25$.

This study was an experimental verification of the results obtained in the aforementioned two-dimensional numerical analysis. A similar parameter space was used in order to obtain efficiency contour plots that could be compared with those from the numerical analysis. Aside from the three-dimensionality as well as the inherent differences and complications present within a real, experimental study, the experimental study was designed to mimic the numerical analysis as closely as possible. It was hoped that the combined results of these studies would provide a basis for developing control systems that would allow flapping foil energy harvesters to be more competitive with existing renewable and non-renewable energy sources.

2. Experimental Method

2.1. The Small Tank and Carriage

The small tow tank used to perform the experiments measured 426.72 cm (length) x 91.44 cm (width) x 77.47 cm (height), and was made of a rigid external aluminum frame that held together an inner glass frame in which water could be held. The glass of the inner frame was 1.27 cm thick. The external frame also provided the surface on which the motor-driven carriage was towed length-wise using a belt drive. The carriage was equipped with X-Y actuators for driving the heave motion of the foil and a rotational motor for driving the pitch motion of the foil. A load cell (the force sensor) was attached to the rotational motor and was connected to a voltage amplifier mounted to the carriage. End-limit lasers were mounted on each end of the external frame to prevent the carriage from running off the track while in motion.

2.2. Motion Control and the Force Sensor

The speed of the tow, heaving and pitching motions of the foil, and flapping frequency were controlled using a Delta Tau PMAC2 motion controller. When the motions were run, the PMAC2 controller card would output a signal that allowed for the actual positions of the heave, pitch and carriage to be gathered and saved as a delimited text (.txt) file via the PMAC Plot Pro2 tool. PMAC2 program calibration values were used to change the position data from units of encoder counts to units of either meters or radians.

The force sensor was a six-axis ATI Gamma SI-130-10 Industrial Automation load cell. Moments (M) and forces (F) were measured via strain gauges located within the force sensor. The force sensor had a ± 10 V limit. The sensing ranges and the resolution of the force sensor were as follows: respectively, 130 N and 1/20 N for F_x and F_y ; 400 N and 1/20 N for F_z ; and 10 N·m and 1/800 N·m for M_x , M_y and M_z . The applied loads were required to be within the sensing ranges for each of the six axes for the load cell to give correct measurements. The resolutions were the effective resolutions after dropping four counts of noise and could be improved further by filtering the obtained force

and moment signals. The resolutions provided a characterization for the error in the experimental measurements through comparison to the level of noise in the raw force and moment signals. Voltage data representing the measured moments and forces from the load cell were sent through a voltage amplifier and then collected on National Instruments USB DAQ platform such that the data could then be viewed on a LabView program and saved as a LabView measurement (.lvm) file. The DAQ board also collected data for the tow velocity and motion-start/-stop times, which could also be viewed and saved through the same LabView program. Since the forces and moments were measured in volts (V), a factory-supplied calibration was used to convert the forces from V to N and the moments from V to N·m. The accuracy of the factory calibration was verified by a simple weight test in which a known mass was hung from the load cell and force and moment voltage data were acquired, calibrated and then compared to the known force value for verification. This calibration matrix was used to calibrate forces and moments in later experiments and tests.

2.3. The Foil

The foil used in this study was a hollow NACA 0012 profile and was made from extruded aluminum. A connector piece (also aluminum) with an emerging threaded rod and two adjustable nuts was used to mount the foil to the load cell. The foil had a chord length (c) of 6.985 cm and an average approximate submerged span (s) of 36.5 cm (aspect ratio of approximately 5.1).

Figure 1. Definitions of motion with respect to foil orientation for a foil oscillating in a two-degrees-of-freedom system. U is the free stream velocity of the fluid, $h(t)$ is the heave motion, $\theta(t)$ is the pitching motion, and $\alpha(t)$ is the nominal angle of attack.

2.4. Foil Centering and Alignment

In order to ensure that the forced pitching angles observed were actually those commanded by the PMAC2 motion program, it was necessary to determine the zero-degree pitching point of the foil prior to running any experiments. After a reference zero-degree pitching point was physically chosen at random, nineteen individual runs were performed at pitching angles varying from -45° to 45° (5° increments). Force voltage data were obtained for each run, zeroed, and calibrated. The average lift force for each

Figure 2a. Comparison between efficiency contour plots of the experimental study (left) and the 2-D numerical analysis (right) (Persichetti, 2013) for heave-to-chord ratios (h_0/c) of 0.5, 0.75, respectively. Negative efficiencies have been eliminated for comparative and visual purposes. Grey areas indicate data that was unusable. Although the contour plots and efficiency values are not identical between the two studies, it is clear that the efficiencies of the experimental study exhibit the same trends as those of the numerical analysis.

run was then plotted against its corresponding pitching angle. The point at which the average lift force was approximately zero was assumed to correspond to a zero-degree angle of attack in reference to the original zero-degree pitching point chosen at random. To get a better estimate, additional 1° increment runs were performed using angles that encompassed both the newest and the original zero-degree pitching-point estimates. Once the offset angle was successfully established, the foil was set to the most accurate zero-degree pitching point. To avoid having to redo the centering procedure each time, a laser sensor indicating the determined zero-degree pitching point location was used for homing the foil to that location for future experiments. It should be noted that although the zero-degree pitching point of the foil was determined, the foil was mounted to the force sensor differently each time it was removed and reattached such that it was never truly aligned with the local x-axis of the load cell.

2.5. Equations of Motion

Heave, $h(t)$ —the vertical displacement of the foil with respect to the direction of the free stream velocity, and pitch, $\theta(t)$ —the rotation about the pitching point of the foil, were used to define the oscillating foil motions. When these motions are combined in a two-dimensional system, though, a third independent variable, the angle of attack, $\alpha(t)$, emerges, resulting in more variables than degrees of freedom. As such, and to mimic what was done in the numerical analysis, the angle of attack was allowed to react to the fluid forces produced by the forced harmonic heave and the pitching motions:

$$\theta(t) = \theta_0 \sin(\omega t + \Psi) \quad (1)$$

$$h(t) = h_0 \sin(\omega t) \quad (2)$$

where θ_0 is the maximum pitch amplitude in radians (rad), ω is the frequency of the oscillation in rad/s, t is the time in s, Ψ is the phase angle in rad between the heave and pitch motion, and h_0 is the maximum heave amplitude in m. Figure 1 is a visible representation of these motion parameters. A phase angle of $\frac{\pi}{2}$ rad was used for Ψ in

Equation (1) based on previous work (Zhu, 2011) as well as to limit the total number of variables in the experiment.

2.6. Experimental Runs

A parameter space of h_0/c from 0.50 to 1.25 (0.25 increments), St from 0.15 to 0.60 (0.1 increments, except for the 0.05 increment from 0.15 to 0.20), and θ_0 from 30° to 90° (10° increments) was used to create the 140 different experiments that were run in the tow tank. There were 28 experiments, which included all θ_0 -values for $St = 0.4, 0.5$ and 0.6 of $h_0/c = 0.5$ as well as all θ_0 -values for $St = 0.6$ of $h_0/c = 0.75$, that were not run because the frequencies of these motions were above the 2 Hz allowable limit of the experimental apparatus. These same experiments were also not performed in the numerical analysis because efficiency patterns indicated that they would yield negative efficiencies. Although tests with $St = 0.1$ were performed in the numerical analysis, they were not performed in the experimental study as some runs resulted in less than two complete motion cycles. For each of the 140 different experiments, local force and moment voltage data and global position (motion) data were gathered and saved for later data processing and calculations. To allow for the water to properly settle, there was a 10 minute wait period in between each experiment. As such, the experiments were spread out into four different days. The foil was removed each night to be dried and cleaned to avoid corrosion and then remounted the following day to resume the

experiments. Before each new run, the foil was properly homed to the zero-degree pitching point and the tow-motor and Y-motor were properly homed to the global x and y axes, respectively.

2.7. Data Processing

Prior to performing any calculations, it was necessary to process the data obtained during each experiment into usable forms. First, using MatLab, the force and moment voltage data were zeroed such that the mean of each signal was approximately zero. This was done by taking the average values of the data recorded before the actual start of the motion and subtracting it from the total values of each force and moment signal. Once the force and moment signals were zeroed, the position data and force and moment voltage data were calibrated such that they were in the proper units (meters for heave and carriage positions, radians for pitch positions, Newtons for forces, and Newton-meters for moments). In order to have equal time-steps that allowed for correct comparisons between data, the position data were re-sampled such that they had the same sampling frequency as the force data (200 Hz). Lastly, the data needed for the efficiency calculations were properly clipped such that they were all the same length in time-steps. The heave position data were used as the reference signal and were clipped from their first heave amplitude peak to their last heave amplitude peak. The pitching motion data, the force data, and the tow velocity data were

	0.15	0.2	0.3	0.4	0.5	0.6
30°	0.042	-0.111	-0.677	N/A	N/A	N/A
40°	0.197	0.069	-0.524	N/A	N/A	N/A
50°	0.343	0.233	-0.269	N/A	N/A	N/A
60°	0.461	0.357	-0.145	N/A	N/A	N/A
70°	0.492	0.375	-0.309	N/A	N/A	N/A
80°	0.448	0.244	-0.660	N/A	N/A	N/A
90°	0.307	-0.020	-1.500	N/A	N/A	N/A

Table 1. All Efficiency Values for $h_0/c = 0.5$. Strouhal numbers (St) are shown horizontally across the top while pitch amplitudes (θ_0) are shown vertically on the left.

	0.15	0.2	0.3	0.4	0.5	0.6
30°	0.044	-0.065	-0.267	-0.253	0.116	N/A
40°	0.171	0.100	-0.128	-0.282	-0.080	N/A
50°	0.312	0.254	0.042	-0.300	-0.287	N/A
60°	0.373	0.398	0.200	-0.244	-0.446	N/A
70°	0.371	0.468	0.360	-0.101	-0.373	N/A
80°	0.256	0.446	0.368	0.005	-0.213	N/A
90°	0.167	0.379	0.207	-0.093	0.007	N/A

Table 2. All Efficiency Values for $h_0/c = 0.75$. Strouhal numbers (St) are shown horizontally across the top while pitch amplitudes (θ_0) are shown vertically on the left.

Figure 2b. Comparison between efficiency contour plots of the experimental study (left) and the 2-D numerical analysis (right) (Persichetti, 2013) for heave-to-chord ratios (h_0/c) of 1.0, 1.25, respectively. Negative efficiencies have been eliminated for comparative and visual purposes. Grey areas indicate data that was unusable. Although the contour plots and efficiency values are not identical between the two studies, it is clear that the efficiencies of the experimental study exhibit the same trends as those of the numerical analysis.

then clipped from the same starting point to the same ending point. It is important to note that although these signals were periodic, they were not necessarily peak-to-peak like the reference signal.

2.8. Efficiency Calculations

The theoretical hydrodynamic efficiency (η) of the flapping foil was defined as the average power output from the system ($\bar{P}_{avg}$) divided by the total power available ($P_{available}$)

$$\eta = \frac{\bar{P}_{avg}}{P_{available}} \quad (3)$$

or

$$\eta = \frac{\bar{P}_{avg}}{\frac{1}{2} \rho U^3 2h_0s} \quad (4)$$

where ρ is the density of the water at an average temperature of approximately 24°C—approximately 997 kg/m³, U is the average tow velocity measured (the free stream velocity of the water passing the foil)—approximately 0.3 m/s, and s is the measured submerged span of the foil—approximately 0.365 m. In the two-dimensional numerical analysis, only the chord length and the thickness of the foil were considered; therefore, s was set to 1. The governing equation that quantifies the average power output from the system is as follows:

$$P(t) = P_L(t) + P_\theta(t) = F_L \dot{h}(t) + M_\theta \ddot{\theta}(t) \quad (5)$$

Obviously, the power, $P(t)$, is time-dependent, and is the sum of the power due to heave motion, $P_L(t)$, and the power due to a moment about the pitching point of the foil, $P_\theta(t)$. The power due to heave motion for a particular time can be further broken down into the product of the hydrodynamic lift force in the heave direction, F_L , and the heave velocity (the derivative of the heave position), $\dot{h}(t)$, at a particular time. Additionally, the power due to a moment about the pitching point of the foil (the center of mass of the foil in this case) for a particular time can be further broken down

into the product of the hydrodynamic moment about the pitching point of the foil, M_θ , and the angular velocity in the pitch direction, $\dot{\theta}(t)$, (the derivative of the pitch position) for a particular time. As such, the total average power output from the system is the average of the sum of all $P(t)$ for each time, t .

Since only the hydrodynamic forces and moments are of interest in these calculations, the inertial terms must be subtracted from the total force and moment values obtained during the experiments

$$F_L = F_{y,total} - m\ddot{h}(t) \quad (6)$$

$$M_\theta = M_{\theta,total} - I\ddot{\theta}(t) \quad (7)$$

where $F_{y,total}$ and $M_{\theta,total}$ are the total force and moment in the heave and pitch directions, respectively, and $m\ddot{h}(t)$ and $I\ddot{\theta}(t)$ are the inertial terms in the heave and pitch directions, respectively— m is the total mass of the foil and its attachments (approximately 0.85 kg), $\ddot{h}(t)$ is the acceleration of the foil in the heave direction in m/s^2 (the second derivative of the heave position), I is the mass moment of inertia about the center of mass of the foil (approximately $0.0006 \text{ kg}\cdot\text{m}^2$), and $\ddot{\theta}(t)$ is the angular acceleration in the pitch direction in rad/s^2 (the second derivative of the pitch position).

The final item that must be accounted for in the efficiency calculations involves coordinate systems. While the position data obtained during the experiments were in reference to a global (world) coordinate system, the force and moment data obtained during the experiments were in reference to the local coordinate system of the rotating load cell. Therefore, before any of the above calculations could be performed, a transformation of the force and moment data from the local to the global coordinate system had to be done. Taking into account proper directionality of the two axes, the transformation equations were as follows:

$$F_{x,Global} = -F_{x,Local} \cos(\theta) - F_{y,Local} \sin(\theta) \quad (8)$$

$$F_{y,Global} = -F_{x,Local} \sin(\theta) + F_{y,Local} \cos(\theta) \quad (9)$$

$$M_{\theta,Global} = -M_{\theta,Local} \quad (10)$$

Once the necessary forces and moments were in the same global coordinate system as the other necessary values, the efficiency calculations were performed as described by Equations (3)-(7).

3. Results

All of the aforementioned calculations were performed in MatLab. Efficiencies were obtained for each of the different foil motion parameter combinations (Tables 1-4), except for the set of all θ_0 -values of $h_0/c = 0.75$, $St = 0.15$ due to incongruities in the position data obtained. Efficiency contour plots were created to better compare the experimental efficiencies obtained in this study with the efficiencies obtained in the numerical analysis. These comparisons are shown in Figures 2a,b. Negative efficiencies indicate that the average power input to the system that is needed to perform the forced motions is larger than the average power output by the system as a result of energy extraction. Negative efficiencies were not included in the contour plots.

4. Discussion

4.1. Analysis of Efficiency Results

The results of the experimental study found that the most efficient motion occurred for $\theta_0 = 70^\circ$ and $St = 0.15$ for $h_0/c = 0.50$, which is, in fact, reasonable. Not only does separation for a NACA 0012 foil not yet occur at this pitching point, but also lower heave-to-chord ratios have lower available powers. In other words, because the frequency of this motion was low and separation was not occurring, the foil was able to harvest more of the less available power from the fluid flow within the range of the heave motion. Observably, though, the contour plots for the experimental study are not identical to those of the numerical analysis. For the lower heave-to-chord ratios (0.50 and 0.75) the efficiencies of the experimental study are on average greater than those of the numerical analysis. The reverse is true for the higher heave-to-chord ratios (1.00 and 1.25). Furthermore, the efficiencies of the experimental study appear to be shifted left compared to those of the numerical analysis such that the lower St have the higher efficiencies. Nevertheless, it is apparent that the efficiencies of the experimental study overall exhibit the same trend as those of the numerical analysis exhibit.

Prior to beginning the experimental study, it was expected that the experimental data would provide results that were not identical to the results of the numerical analysis as there were inherent differences between the two. First of all, the foil used in the numerical model was a NACA 0015 foil, while the foil used in the experimental study was a NACA 0012 foil. Because of this, separation occurs at smaller angles of attack and pitching angles in the experimental study than it does at those in the numerical analysis. Consequently, smaller Strouhal numbers would produce larger lift forces (ultimately indicating higher efficiencies) in the experimental study while larger Strouhal numbers would produce larger lift forces and efficiencies in the numerical analysis (due to separation occurring later for a NACA 0015 foil). As such, it was expected that the efficiencies for the experimental study would at least be shifted due to the use of a smaller foil. Additionally, the numerical analysis was a two-dimensional study, while the experimental study was inherently three-dimensional (even though the efficiency calculations performed were for a two-dimensional system—this was for verification purposes). Because of the inherent three-dimensionality, the foil's span along the z -axis was taken into account for efficiency calculations; however, the foil was not long enough to reach the bottom of the tank. As a result, end effects occurred at the bottom tip of the foil, which led to a reduced lift force, thus explaining the smaller output power and efficiencies occasionally observed. Furthermore, because the model used in the numerical analysis did not have a high enough resolution and was dealing with primarily pressure-dominated flow, viscous stresses were not integrated around the foil. As such, these forces were not included in the force calculations. In actuality, however, viscous effects exist and can have a negative effect on the total power output of the system. Actually, the efficiencies obtained in this study were closer in some instances to those

	0.15	0.2	0.3	0.4	0.5	0.6
30°	Not Usable Data	-0.046	-0.313	-0.781	-1.664	-2.818
40°	Not Usable Data	0.074	-0.122	-0.443	-1.050	-1.938
50°	Not Usable Data	0.189	0.055	-0.158	-0.560	-1.130
60°	Not Usable Data	0.283	0.219	0.072	-0.244	-0.591
70°	Not Usable Data	0.304	0.333	0.241	0.036	-0.296
80°	Not Usable Data	0.274	0.364	0.307	0.078	-0.150
90°	Not Usable Data	0.210	0.314	0.193	-0.118	-0.474

Table 3. All Efficiency Values for $h_0/c = 1.00$. Strouhal numbers (St) are shown horizontally across the top while pitch amplitudes (θ_0) are shown vertically on the left.

	0.15	0.2	0.3	0.4	0.5	0.6
30°	0.023	-0.034	-0.252	-0.679	-1.240	-2.207
40°	0.091	0.065	-0.091	-0.351	-0.741	-1.449
50°	0.147	0.158	0.059	-0.124	-0.383	-0.851
60°	0.160	0.215	0.209	0.088	-0.099	-0.387
70°	0.147	0.219	0.308	0.263	0.158	-0.036
80°	0.122	0.180	0.325	0.335	0.249	0.101
90°	0.077	0.120	0.270	0.297	0.227	0.074

Table 4. All Efficiency Values for $h_0/c = 1.25$. Strouhal numbers (St) are shown horizontally across the top while pitch amplitudes (θ_0) are shown vertically on the left.

of a separate numerical study that took into account viscous effects (Zhu, 2011).

Of course, there are other factors that would contribute to the differences in the observed efficiencies that were not previously thought of. For example, it is true that properly timed leading edge vortices can, in fact, contribute in a positive way to the foil's pitching motion such that the power output of the system is increased according to Equation (5). However, if the leading edge vortices are not timed correctly, then they can actually be detrimental to the overall power output of the system. For this study, it was assumed that energy could be extracted from both the heave and pitching motions; therefore, if the leading edge vortices were not properly timed, then this could have contributed to smaller observed efficiencies. With regards to the experimental study's larger observed efficiencies for smaller heave-to-chord ratios and smaller observed efficiencies for larger heave-to-chord ratios, however, the reason could simply be due to an inclusion of inertia (the numerical simulation only considered the hydrodynamic terms). At larger heave-to-chord ratios, the inertial term of the heave force (or pitching moment) increases, which then causes the hydrodynamic term of the heave force (or pitching moment) to slightly decrease. This in turn decreases the overall power output from the system, which ultimately yields smaller efficiencies. The reverse is true for

smaller heave-to-chord ratios. Lastly, the numerical study also had hard boundaries at the top and bottom of the simulated motion, which could have caused an increase in lift force (and thus efficiency) due to ground effects when the foil was near the wall. All things considered, though, and even taking into account each of the aforementioned factors, the fact that the efficiencies still followed the same trend as those of the numerical analysis is enough to provide a validation for the model and methods used in the numerical analysis.

4.2. Suggestions for Future Work

For a better validation of the two-dimensional numerical analysis of Persichetti, steps could be taken to eliminate some of the factors that were assumed to contribute to the differences in efficiencies observed in this study. For consistencies sake and to avoid any undesirable end effects, a smooth foil of NACA 0015 profile or larger that is long enough to just about reach the bottom of the tow tank should be used. Furthermore, to avoid alignment issues, it is recommended that a foil with a three- or four-piece attachment to the load cell be used rather than one with a one-piece attachment. This, coupled with a laser sensor, would allow for identical reattachment of the foil each time it is removed as well as proper alignment with the local axes of the load cell.

Aside from physical changes, changes could also be made to the numerical portions of this study. For example, not incorporating the idea that energy can also be extracted from the pitching motion of the foil into the power equation would allow one to observe the actual theoretical power available from solely the hydrodynamic lift force. Additionally, Equation (4) indicates that the total available power is dependent on the maximum heave amplitude of the foil. However, this position is only the maximum position that the center point of the foil reaches and not the actual maximum position that the foil reaches with its trailing edge during its oscillatory motion. If the latter value were used instead in Equation (4), then the total available power term would be increased, which would have a direct effect on the final efficiencies calculated. This method was actually used by Zhu in a separate numerical analysis that showed, on average, smaller efficiencies than those of Persichetti's numerical analysis (Zhu, 2011).

5. Conclusion

This experimental study ultimately sought to verify the efficiency results of a two-dimensional numerical analysis, which determined how the theoretical hydrodynamic energy extraction efficiency of a flapping foil changed as a function of the non-dimensional heave amplitude (h/c), the pitching angle (θ) and the Strouhal number (St). Due to inherent differences between the intrinsically three-dimensional experimental study and the two-dimensional numerical analysis, the efficiencies obtained in the experimental study were not identical to those obtained in the numerical analysis. For the lower heave-to-chord ratios (0.50 and 0.75) the efficiencies of the experimental study were on average greater than those of the numerical analysis, while for the higher heave-to-chord ratios (1.00 and 1.25) the efficiencies of the experimental study were on average smaller than those of the numerical analysis. What is more, the efficiencies of the experimental study appear to be shifted left compared to those of the numerical analysis such that the lower St had the higher efficiencies. Nevertheless, the efficiencies of the experimental study still followed the same trends as those of the numerical analysis, thus providing a good verification for the results of the numerical analysis. Therefore, the results of both studies could potentially be used in future studies to help develop control systems for flapping foil energy harvesting devices so as to help further improve their efficiencies and make them more

competitive with existing renewable and nonrenewable sources of energy.

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Investigating Plume-Influenced Mid-Ocean Ridges: Iron Redox Conditions and Tungsten Variation at the Galapagos Spreading Center

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Abstract. Mantle plumes are chemically distinct intrusions of ductile rock that rise from depth through the mantle to the earth's surface. Plumes may originate near the core mantle boundary, and may be fed by subducted materials sinking from the earth's surface. Despite these suggestions, both the depth at which plumes originate and the source of their unique geochemical signature are still unknown. Iron exists throughout the mantle as a combination of Fe^{2+} and Fe^{3+} . The ratio of $[\text{Fe}^{3+}]$ to $[\text{Fe}^{2+} + \text{Fe}^{3+}]$ (expressed $\text{Fe}^{3+}/\Sigma\text{Fe}$) in a material reflects its total level of oxidation, and subsequently whether that material came from the earth's surface. Tungsten (W) is an indicator of core-mantle interaction because the majority of W found on earth is concentrated in the core. Select isotopic ratios ($^{87}\text{Sr}/^{86}\text{Sr}$, $^{208}\text{Pb}/^{204}\text{Pb}$) measured in glasses from fresh pillow basalts collected at the Galapagos spreading center suggest that the spreading center is fed by both the upper mantle and a mantle plume. Along strike of the spreading ridge, plume influence is high at the center, decreasing to normal mid-ocean ridge composition at the east and west ends. We analyzed 28 mid-ocean ridge basalt (MORB) glasses that span the transect of the Galapagos spreading center using LA-ICP-MS (laser ablation inductively coupled plasma mass spectroscopy) to determine [W] along the transect, and therefore determine if Galapagos plume has interacted with material near the core-mantle boundary. We also determined $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios in 10 of these samples using μ -XANES (micro X-ray absorption near edge structure) spectroscopy, in order to determine whether the plume mantle is more oxidized than the ambient upper mantle and may thus have originated at the earth's surface. All 28 samples in our suite fall within expected [W] values for the modern mantle, indicating that the plume likely does not entrain tungsten enriched material as it rises. Our study does reveal a positive correlation between higher $\text{Fe}^{3+}/\Sigma\text{Fe}$ and isotopic ratios considered strong tracers of plume influence, $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{208}\text{Pb}/^{204}\text{Pb}$. Despite this correlation, 7 of our 10 measured $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios fall within the range expected for MORB, indicating that plume mantle is not more oxidized than the upper mantle. Further analysis of the Galapagos spreading center and other plume influenced mid-ocean ridges will be required to determine the extent of the correlation between plume influence and iron oxidation state, and whether that correlation translates into $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios that are observably higher than those expected for MORB.

1. Introduction

The Galapagos Spreading Center is a divergent plate boundary located off the west coast of South America that lies north of the Galapagos Islands, a volcanic island chain formed by a mantle plume. Mantle plumes are intrusions of ductile rock that rise from depth through the mantle to the surface of the earth and cause volcanic activity.

Both the depth at which plumes begin their rise through the mantle and the origin of the source material that feeds plumes are unknown. Studies of the geochemical differences between mantle plume and mid-ocean ridge volcanism reveal that mid-ocean ridge basalt (MORB), and ocean island basalt (OIB) erupted by mantle plumes must originate from separate, chemically distinct source regions in a heterogeneous mantle (Sun and McDonough, 1989). Radiogenic isotopic ratios are effective tracers of these source regions, and high ratios of $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ relative to ratios seen at normal mid-ocean ridges have been identified as a characteristic of mantle plume sources (Schilling et al., 2003; Sun and McDonough, 1989; Verma and Schilling, 1982). $^{208}\text{Pb}/^{204}\text{Pb}$ and $^{87}\text{Sr}/^{86}\text{Sr}$, when measured across the transect of the Galapagos Spreading Center, are lowest at the ends of the ridge, located at

101°(W) and 83° (W), and increase towards the center of the ridge until the highest ratios are reached between 93° and 89°(W) (Fig. 1; Kelley et al., 2013). This variation in radiogenic isotopic ratios indicates that the Galapagos Spreading Center is a plume-influenced mid-ocean ridge being fed by two unique source regions, the ambient upper mantle and a mantle plume (Schilling et al., 2003; Verma and Schilling, 1982).

Mantle plumes may originate near the core mantle boundary, and may be fed by subducted materials sinking from the earth's surface. This study utilizes iron redox and tungsten abundance as tools to investigate these questions at the Galapagos Spreading Center. The amount of tungsten suggested to exist on earth by chondrite (meteorites that accreted to form the early earth and represent the bulk composition of the modern earth) is much higher than the amount of tungsten that has actually been observed in the earth's mantle and crust (Arevalo and McDonough, 2008). The missing tungsten responsible for this deficit between the expected and observed amount of tungsten on earth is believed to have partitioned into the core as it formed, because tungsten is a siderophile (iron-loving) element and the core is made of metallic iron (Arevalo and McDonough, 2008). It has been suggested that >90% of the earth's total

Figure 1. Variation in radiogenic isotopic ratios $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ across the transect of the Galapagos Spreading Center. Enrichment patterns seen in both ratios across the ridge indicate that plume influence ranges from approximately $-95.5^\circ(\text{E})$ to $-87^\circ(\text{E})$ overall, with the highest influence occurring between -93° to -89° (E). Isotope ratios from Kelley et al. (2013).

tungsten resides in the core (Arevalo and McDonough, 2008). This makes tungsten a good tracer of core mantle interaction, because any material with elevated tungsten

relative to expected levels in the mantle is most likely to have picked up that extra tungsten from the core. Subduction forces oceanic crust at convergent plate margins into the mantle, where it is recycled. Subducted material may gain an oxidized signature at the surface while in contact with oxygen. Iron exists in the mantle as a combination of two oxidation states, Fe^{2+} and Fe^{3+} . The ratio of $[\text{Fe}^{3+}]$ to $[\text{Fe}^{2+} + \text{Fe}^{3+}]$, or $\text{Fe}^{3+}/\Sigma\text{Fe}$, can be used as a measure of how oxidized a material is, and therefore how much oxygen it has come in contact with, because oxygen oxidizes Fe^{2+} into Fe^{3+} and raises the $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratio of the material as a result. Because of this, $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios may potentially be a usable tracer of subducted materials that are oxidized at the surface, recycled into the mantle and later erupted.

We used laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) and micro X-ray absorption near edge structure spectroscopy ($\mu\text{-XANES}$) to determine the tungsten concentrations of 28 basaltic glasses that span the transect of the Galapagos Spreading Center, and the $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios in a subset of 10 of these glasses (Fig. 2). The purpose of this study was to (a) determine whether the plume mantle influencing the Galapagos Spreading Center is more oxidized than the ambient upper mantle feeding the ends of the ridge, indicating that subducted material from the surface may be feeding the plume, and (b) determine if tungsten levels are elevated relative to its baseline in the ambient upper mantle as a

Figure 2. Map of the Galapagos Islands (circled in black), the Galapagos Spreading Center ridge (black dashed line), and the locations of the 28 seafloor basalt samples analyzed in this study. All glasses were analyzed for tungsten, red stars indicate samples that were also analyzed for $\text{Fe}^{3+}/\Sigma\text{Fe}$ in this study, black circles with white stars indicate samples with pre-existing data for $\text{Fe}^{3+}/\Sigma\text{Fe}$ from Cottrell and Kelley (2011). A trail of old volcanic material from the Galapagos mantle plume can be seen to the north of the Galapagos Islands intersecting the ridge from approximately 92° - 89° (W). Base bathymetry map generated using GMRT (Global Multi-Resolution Topography) synthesis from <http://www.geomapapp.org>.

function of proximity to plume influence, indicating that the Galapagos plume could be entraining material from near the core mantle boundary as it rises. On a larger scale, these analyses may ultimately assist in constraining both the depth at which plumes begin to rise to the surface and where their source material might originate.

2. Samples and Methods

2.1. Seafloor glasses from the Galapagos Spreading Center

28 fresh pillow basalts dredged from the Galapagos spreading center between 102°-84° (W), were chosen for this study (Fig.2; TR164, CTW, DST41, KK78). Glass chips were sampled from the quenched rims of each pillow basalt. All chips were doubly polished to a nominal thickness of 150 μm , though some chips with an abundance of micro-crystalline content were polished as thin as 30 μm to create pools of crystal free glass large enough to analyze. The wafered samples were then photographed in plane-polarized, cross-polarized, and reflected light so that crystal free glass could be selected for *in-situ* analysis. This allowed for the exact liquid composition of the magma erupted at each dredge site to be determined, while eliminating the potential for inclusion of micro-crystalline content in the analyses that could skew liquid compositions.

2.2. Laser Ablation Inductively Coupled Plasma Mass Spectroscopy (LA-ICP-MS)

Tungsten and uranium concentrations were determined in submarine glasses by laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) at the Graduate School of Oceanography, University of Rhode Island on a Thermo X-Series II quadrupole ICP-MS coupled with a New Wave UP 213 Nd-YAG laser ablation system, following the techniques outlined by Kelley et al. (2003), modified to include W and U. The laser was operated at 70% energy output and 5 Hz, with spot sizes for each analysis ranging from 40 μm - 80 μm in diameter. Collected counts for ^{182}W , ^{184}W , and ^{238}U were converted to concentration data using linear calibration curves (R^2 reported in Appendix Table 1) through ≥ 4 of United States Geological Survey glass standards BCR-2g, BHVO-2g, BIR-1g, and Max Planck Institute glass standards GOR-132G, StHls-G, T1-G, ML3B-G, and KL2-G (Jochum et al., 2006; Kelley et al., 2003). Glass chips were analyzed in triplicate and concentrations were reproducible to within 4% rsd for both W and U. All analyses that were below the detection limit of the instrument were removed from the data set. Remaining analyses for each glass chip were averaged to arrive at the final concentrations (reported in Appendix Table 2). Results of propagation of error analyses performed for each reported value of W/U are reported in Appendix Table 3.

2.3. Micro X-Ray Absorption Near Edge Structure Spectroscopy (μ -XANES)

A subset of 10 samples (Fig.2) were analyzed *in-situ* to determine their $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios via micro X-ray

absorption near edge structure spectroscopy (μ -XANES), following the methods and techniques of Cottrell and Kelley (2009) at beamline X26a, National Synchrotron Light Source, Brookhaven National Laboratory. Spectra were collected using a nominal beam size of 9x5 microns, between 7020 eV and 7220 eV, using a Si[311] monochromator. Submarine glasses were analyzed in triplicate and calculated $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios were averaged (Appendix Table 2). Calculated standard deviations for each sample are reported in Appendix Table 3.

Figure 3. Crystal fractionation correction for measured for $\text{Fe}^{3+}/\Sigma\text{Fe}$ data, modeling the removal of olivine±plagioclase±clinopyroxene from a cooling melt (Cottrell and Kelley, 2011). Original $\text{Fe}^{3+}/\Sigma\text{Fe}$ data is plotted vs. MgO, then forced along a slope of -.006 (approximately parallel to the solid black line through the data above, until it intersects MgO wt.% 8, represented by the dashed line. Values of $\text{Fe}^{3+}/\Sigma\text{Fe}$ plotted on this line are considered corrected. This model is known to be effective in samples containing between ~5 and ~10 wt.% MgO.

2.4. Correcting for the effects of crystal fractionation

All $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios were corrected back to MgO = 8 wt % (MgO_8 , Fig. 3) using the methods presented in Cottrell and Kelley (2011) in order to 1) remove any influence that crystal fractionation (formation and removal of solid crystals from a cooling melt) may have had on the measured liquid composition, and 2) allow the relative difference between measured $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios to be directly compared across all samples. Measured $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios for samples that fell between ~5 and ~10 wt% MgO were modeled along a pathway representative of olivine±plagioclase±clinopyroxene fractionation. Along this pathway, $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios rise from ~.15 to ~.18 as MgO drops from ~10 to ~5 wt% (Fig. 3; Cottrell et al., 2011).

3. Results

3.1. Variations in tungsten at the Galapagos Spreading Center

Tungsten concentration alone can be influenced by both the original composition of a melt's source as well as that source's melting and crystallization history. In order to avoid the effects that fractional crystallization (removal of elements from a cooling melt via the formation of solid crystals) and melting processes have on tungsten abundance, we will consider the W/U ratio in our samples because tungsten and uranium have similar partitioning behaviors in silicate systems (Arevalo and McDonough, 2008). This will allow us to identify elevated tungsten relative to uranium that occurs as a result of differences in source mantle composition. All samples in this study fall within the range of W/U expected for the mean modern mantle ($W/U_{MMM} = .65 \pm .45$) Fig. 4; Arevalo and McDonough, 2008). Tungsten concentrations, uranium concentrations, and W/U ratios are reported in Appendix Table 2.

3.2. Variations in $Fe^{3+}/\Sigma Fe$ at the Galapagos Spreading Center

Newly determined $Fe^{3+}/\Sigma Fe$ ratios are considered along with published $Fe^{3+}/\Sigma Fe$ ratios (Appendix Table 2; Cottrell and Kelley, 2011; Cottrell and Kelley, 2013) in order to create a comprehensive sample collection that spans the length of the Galapagos Spreading Center. Three samples fall outside of the expected range for normal mid-ocean ridge basalt from the East Pacific Rise between 9° - 15° (N), $Fe^{3+}/\Sigma Fe_{EPR} = .16 \pm .004$ (Appendix Table 4; Fig. 5; Cottrell and Kelley, 2011). Samples TR164-9D-1g and DS D-1Ag are more oxidized than the expected range, while DS D-2Ag is more reduced. These three samples do not form any visible trend related to plume influence longitudinally. However, a positive correlation exists between $Fe^{3+}/\Sigma Fe$ ratios and isotopic indicators of plume influence $^{87}Sr/^{86}Sr$ and $^{208}Pb/^{204}Pb$ (Fig. 6). Linear regressions through $Fe^{3+}/\Sigma Fe$ versus $^{87}Sr/^{86}Sr$ and $^{208}Pb/^{204}Pb$ both have $R^2 \geq .5$ (Fig. 6), the threshold for what is considered a strong correlation in natural sample data.

4. Discussion

4.1. Influence of core material on W/U ratios in Galapagos Spreading Center glasses

If a sample is enriched in tungsten relative to the mean modern mantle, it can be extrapolated that the sample must have interacted with the core or an area near the core mantle boundary, since the core is believed to be the only available source of material more enriched in tungsten than the mantle. The W/U ratios in all samples from this study fall within the expected range of W/U for the mean modern mantle and show little variation from sample to sample, which is consistent with existing literature data (Arevalo and McDonough, 2008). No trends that relate W/U and proximity to plume influence exist within this expected range. This leads us to the conclusion that the Galapagos

Figure 4. W/U from this study plotted across the length of the Galapagos Spreading Center, compared to the expected range of W/U for the mean modern mantle (marked in light gray) ($W/U_{MMM} = .65 \pm .45$; Arevalo McDonough, 2008). Medium gray areas indicate the approximate outer boundary of the longitudes where plume influence is seen according to radiogenic isotopic ratios $^{87}Sr/^{86}Sr$ and $^{208}Pb/^{204}Pb$ (Kelley et al. 2013), while dark gray indicates longitudes with highest plume influence according to these ratios (Fig. 1). Error plotted for each sample is reported in Appendix Table 3.

Figure 5. $Fe^{3+}/\Sigma Fe$ corrected for crystal fractionation ($Fe^{3+}/\Sigma Fe_{MgO=8}$) from this study and Cottrell and Kelley (2011), plotted across the length of the Galapagos Spreading Center. Data is plotted overlaying the average range of $Fe^{3+}/\Sigma Fe_{MgO=8}$ ($.16 \pm .004$) calculated for samples between 9° - 15° (N) at the East Pacific Rise, a section of mid-ocean ridge considered to be a good representative of a normal (non plume-influenced) ridge sourced from the ambient upper mantle (Appendix Table 4; Cottrell and Kelley 2011). Three samples plot outside of this range, however one of these (DS D-1Ag) is not included on this corrected data plot because its wt.% MgO falls outside of the 5%-10% range for MgO wt.% that can be corrected for crystal fractionation. Medium gray areas indicate approximate outer boundary of the longitudes where plume influence is seen according to radiogenic isotopic ratios $^{87}Sr/^{86}Sr$ and $^{208}Pb/^{204}Pb$ (Kelley et al., 2013), while dark gray indicates longitudes with highest plume influence according to these ratios (Fig. 1). Error plotted for each sample is reported in Appendix Table 3.

Figure 6. $\text{Fe}^{3+}/\Sigma\text{Fe}$ corrected for crystal fractionation ($\text{Fe}^{3+}/\Sigma\text{Fe}_{\text{MgO}=8}$) plotted versus radiogenic isotopic ratios indicative of plume influence, $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ (Kelley et al., 2013). Both plots show strong correlations ($R^2 \geq .5$) between increasing plume influence and increasing iron oxidation. Areas encompassing normal MORB (averaged from East Pacific Rise data between 9° - 15° (N)) are shown as light gray regions (Appendix Table 4; Cottrell and Kelley, 2013). One of the 10 samples analyzed for $\text{Fe}^{3+}/\Sigma\text{Fe}$ is not included here because its wt.% MgO falls outside of the 5%-10% range for MgO wt.% that can be corrected for crystal fractionation.

plume likely does not entrain tungsten enriched material derived from near the core-mantle boundary as it rises from depth.

This is in contrast to observations made by Kelley et al. (2013), that elevated W/U ratios, measured on the same samples, can be explained by >1% of core material entrainment in plume-related magmas. A significant difference between this study and that of Kelley et al. (2013) is the methods of trace element analysis used. Kelley et al. (2013) used high-resolution inductively coupled plasma mass spectroscopy (HR-ICP-MS), which requires that glass chips are powdered and analyzed in bulk, a process that inevitably includes micro-crystals present in seafloor glass. LA-ICP-MS (this study) analyzes crystal-free glass *in situ* on small scales ($\ll 1$ mm), to avoid the complications in bulk analyses caused by crystal content. Figure 7 compares tungsten concentrations collected from both studies to quantitatively assess the differences in data produced by HR-ICP-MS and LA-ICP-MS methods. Data unaffected by systematic differences should plot on a 1 to 1 line. Samples plotted in Figure 7 produce a line with a slope of <1, which suggests that systematic differences exist between the data sets, perhaps due to differences in analytical methods, that must be resolved.

4.2. Variation of $\text{Fe}^{3+}/\Sigma\text{Fe}$ with plume influence

If the mantle feeding the Galapagos plume were more oxidized than the ambient upper mantle, samples

classified as being plume influenced because of their isotopic ratios would most likely also have $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios higher than the range expected for normal mid-ocean ridges. The majority of our samples fall within the expected range of $\text{Fe}^{3+}/\Sigma\text{Fe}$ for normal mid-ocean ridges ($0.16 \pm .004$) (Appendix Table 4). Despite this observation, there remains a strong positive correlation between $\text{Fe}^{3+}/\Sigma\text{Fe}_{\text{MgO}=8}$ and isotopic ratios indicative of higher plume influence ($^{87}\text{Sr}/^{86}\text{Sr}$, $^{208}\text{Pb}/^{204}\text{Pb}$; Fig. 6). This is counter to the observation that, within mid-ocean ridge basalts that are unaffected by plume influence, samples with more radiogenic isotopic ratios (i.e. elevated $^{87}\text{Sr}/^{86}\text{Sr}$) are more reduced (Cottrell and Kelley, 2013). This indicates that the Galapagos plume is most likely affecting iron's oxidation state across the Galapagos spreading center, however this study alone does not provide enough information to constrain exactly what the mechanism driving this relationship may be.

The hypothesis that subducted material is incorporated into plumes may be supported by the elevated $^{87}\text{Sr}/^{86}\text{Sr}$ ratios that accompany plume influence. Elevated $^{87}\text{Sr}/^{86}\text{Sr}$ seen across the center of the Galapagos Spreading Center is considered a tracer of higher influence from the Galapagos plume, while lower ratios of $^{87}\text{Sr}/^{86}\text{Sr}$ seen at the ends of the ridge are indicative of an upper mantle, mid-ocean ridge source (Verma and Schilling, 1982). $^{87}\text{Sr}/^{86}\text{Sr}$ is elevated in sediments derived from continental crust, which may translate into an elevated $^{87}\text{Sr}/^{86}\text{Sr}$ signature in the mantle where sediments were subducted along with the oceanic crust on which they were deposited. The positive correlation between $^{87}\text{Sr}/^{86}\text{Sr}$ and increasing $\text{Fe}^{3+}/\Sigma\text{Fe}$ may support the argument that the material feeding plumes is derived from subducted crustal components. However, further research at the Galapagos Spreading Center and

Figure 7. Tungsten concentrations collected on LA-ICP-MS (this study), vs. tungsten concentrations reported in Kelley et al. (2013) collected on HR-ICP-MS for the same sample set. Data collected using different methods that produce no systematic offset should plot on a 1 to 1 line (signified above with a dashed line), and start at (0,0). The glass analyses are extremely well correlated ($R^2 = .99$), produce a linear regression with a slope of .65, and do not start at (0,0), all indicators of systematic offset between the two methods used to collect the analyses.

other plume-influenced mid-ocean ridges will be required to evaluate the likelihood that the trends seen in our data are related to the recycling of subducted materials.

5. Conclusions

In summary, both W/U ratios and $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios in samples from the Galapagos Spreading Center fall within their respective expected ranges at normal (non plume-influenced) mid-ocean ridges. This indicates that, (a) tungsten does not vary in any way as a function plume influence, and (b) the mantle feeding the Galapagos plume is not more oxidized than the ambient upper mantle that feeds normal mid-ocean ridges. W/U ratios measured across the transect of the Galapagos spreading center fall within the range of W/U expected for the mean modern mantle ($\text{W}/\text{U}_{\text{MMM}} = 65 \pm 45$; Arevalo and McDonough, 2008), indicating that the Galapagos plume likely does not entrain material from near the core mantle boundary with an enriched tungsten signature as it rises. $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios measured along the transect fall mostly within the range of $\text{Fe}^{3+}/\Sigma\text{Fe}$ determined to be normal for the East Pacific Rise ($\text{Fe}^{3+}/\Sigma\text{Fe}_{\text{EPR}} = 16 \pm 0.04$) Appendix Table 4; Fig.5). We find that $\text{Fe}^{3+}/\Sigma\text{Fe}$ and isotopic tracers of plume influence $^{208}\text{Pb}/^{204}\text{Pb}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ are positively correlated, with higher oxidation signatures corresponding with more plume influence. This positive correlation between increasing oxidation and higher plume influence may support the idea that subducted material is incorporated into mantle plumes. Further research on other plume-influenced mid-ocean ridges could further constrain the strength of this correlation, and determine whether this correlation ever translates into $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios which differ from those of normal mid-ocean ridges. Future determination of $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios and W/U ratios in the main Galapagos plume (the Galapagos Islands) will also help further define the role that the Galapagos plume plays in producing plume-influenced mid-ocean ridge basalts.

Appendix

Table 1 LA-ICP-MS W and U calibration information

Element	R ²	Standard glasses used in calibration*			
²³⁸ U	0.999	GOR13		ML3B	KL2-
		2-G	T1-G	-G	G
		BHVO-		ML3B	KL2-
¹⁸² W	0.988	2G	T1-G	-G	G
		BHVO-		ML3B	KL2-
¹⁸⁴ W	0.987	2G	T1-G	-G	G

*accepted concentration data and other information about the standard glasses used in this study can be found in Jochum et al. (2006)

Table 2 Physical data for samples in this study

Sample name	Lat. (°N)	Long. (°E)	FeO ^{Total} (wt%)*	MgO (wt%)*	Fe ³⁺ /ΣFe	Fe ³⁺ /ΣFe _{MgO=8}	W (ppm)	U (ppm)	W/U
DS D-2Ag	1.74	-85.12	8.5	8.66	0.148	0.152	0.012	0.045	0.269
CTW 06D-1g	0.85	-86.55	12.84	6.72	-	-	0.084	0.139	0.600
CTW 07D-1g	0.86	-87.09	8.65	8.56	0.153	0.157	0.004	0.019	0.220
TR164 03D-1g	0.73	-88.24	11.49	7.27	-	-	0.070	0.117	0.600
CTW 09D-1g	0.78	-89.23	10.6	7.52	-	-	0.099	0.147	0.677
TR164 06D-1g	0.84	-89.59	10.37	6.29	0.174	0.164	0.418	0.638	0.655
TR164 09D-1g	0.93	-90.5	9.75	7.48	0.176	0.173	0.207	0.373	0.556
TR164 26D-3g	1.9	-90.95	9.84	5.79	0.175	0.162	0.655	1.058	0.619
TR164 25D-2g	1.95	-91.18	9.63	8.43	-	-	0.154	0.261	0.590
CTW 10D-1g	1.99	-91.61	11.09	6.9	0.169	0.163	0.135	0.227	0.597
TR164 11D-1g	2.09	-92.24	11.01	6.56	0.173	0.165	0.186	0.342	0.546
TR164 13D-1	2.42	-93.34	11.48	7.34	-	-	0.106	0.172	0.620
TR164 16D-1g	2.56	-94.45	11.9	6.91	-	-	0.081	0.136	0.596
DS D-8g	2.6	-95.33	11.42	7.74	-	-	0.074	0.130	0.569
DS D-5g	2.44	-95.62	8.87	8.91	-	-	0.033	0.054	0.604
TR164 18D-3g	2.29	-96.18	9.4	8.33	0.159	0.161	0.015	0.028	0.539
TR164 20D-1g	2.24	-97.86	9.33	8.78	-	-	0.020	0.039	0.504
TR164 21D-4g	2.31	-98.54	10.03	8.01	-	-	0.014	0.047	0.305
TR164 22D-1g	2.3	-99.56	11.09	7.38	0.164	0.161	0.029	0.063	0.451
DS D-1Ag	0.71	-85.5	18.45	4.31	0.225	0.225	0.123	0.234	0.525
<i>Existing Data^(a)</i>									
K 10-34	2.635	-95.49	8.54	8.99	0.149	0.155	0.014	0.031	0.458
K 14-3	2.75	-95.24	11.59	6.19	0.167	0.156	0.099	0.147	0.673
K 18-21	2.6	-95.43	11.18	7.08	0.168	0.162	0.083	0.130	0.640
KK78-D17-4	2.43	-95.55	9.19	9.90	0.146	0.157	0.011	0.029	0.379
KK78-D17-14	2.43	-95.55	9.19	9.72	0.151	0.161	0.011	0.019	0.598
KK78-D17-32	2.43	-95.55	9.24	9.76	0.144	0.155	0.089	0.025	0.583
KK78-D17-3	2.43	-95.55	9.28	9.92	0.141	0.152	0.011	0.027	0.423
KK78-D21-2	1.64	-94.97	9.58	9.04	0.154	0.161	0.013	0.033	0.393

*from Kelley et al. (2013)

^(a) Fe³⁺/ΣFe values reported for these samples are from Cottrell and Kelley (2011), and are included in this study for the sake of completeness

Table 3 Error analyses of W/U and $\text{Fe}^{3+}/\Sigma\text{Fe}$

Sample name	Standard deviation (W/U)	Standard deviation ($\text{Fe}^{3+}/\Sigma\text{Fe}$)
DS D-2Ag	0.251	0.0021
CTW 06D-1g	0.562	-
CTW 07D-1g	0.229	0.0041
TR164 03D-1g	0.568	-
CTW 09D-1g	0.551	-
TR164 06D-1g	0.518	0.0031
TR164 09D-1g	0.465	0.0032
TR164 26D-3g	0.496	0.0050
TR164 25D-2g	0.471	-
CTW 10D-1g	0.465	0.0024
TR164 11D-1g	0.512	0.0026
TR164 13D-1	0.541	-
TR164 16D-1g	0.066	-
DS D-8g	0.018	-
DS D-5g	0.065	-
TR164 18D-3g	0.334	0.0031
TR164 20D-1g	0.233	-
TR164 21D-4g	0.069	-
TR164 22D-1g	0.100	0.0031
DS D-1Ag	0.135	0.0045
K 10-34	0.099	0.0045
K 14-3	0.059	0.0045
K 18-21	0.031	0.0045
KK78-D17-4	0.110	0.0045
KK78-D17-14	0.321	0.0045
KK78-D17-32	0.173	0.0045
KK78-D17-3	0.069	0.0045
KK78-D21-2	0.224	0.0045

Table 4 Data from Cottrell and Kelley (2011, 2013) used to calculate expected the ranges for $\text{Fe}^{3+}/\sum\text{Fe}_{\text{MgO}=8}$ and radiogenic isotopic ratios in East Pacific Rise normal mid-ocean ridge basalt (NMORB) referenced in this study

Sample name	Lat. (°N)	Long. (°E)	$\text{FeO}^{\text{Total}}$ (wt%)*	MgO (wt%)*	$\text{Fe}^{3+}/\sum\text{Fe}$	$\text{Fe}^{3+}/\sum\text{Fe}_{\text{MgO}=8}$	W (ppm)	U (ppm)	W/U
DS D-2Ag	1.74	-85.12	8.5	8.66	0.148	0.152	0.012	0.045	0.269
CTW 06D-1g	0.85	-86.55	12.84	6.72	-	-	0.084	0.139	0.600
CTW 07D-1g	0.86	-87.09	8.65	8.56	0.153	0.157	0.004	0.019	0.220
TR164 03D-1g	0.73	-88.24	11.49	7.27	-	-	0.070	0.117	0.600
CTW 09D-1g	0.78	-89.23	10.6	7.52	-	-	0.099	0.147	0.677
TR164 06D-1g	0.84	-89.59	10.37	6.29	0.174	0.164	0.418	0.638	0.655
TR164 09D-1g	0.93	-90.5	9.75	7.48	0.176	0.173	0.207	0.373	0.556
TR164 26D-3g	1.9	-90.95	9.84	5.79	0.175	0.162	0.655	1.058	0.619
TR164 25D-2g	1.95	-91.18	9.63	8.43	-	-	0.154	0.261	0.590
CTW 10D-1g	1.99	-91.61	11.09	6.9	0.169	0.163	0.135	0.227	0.597
TR164 11D-1g	2.09	-92.24	11.01	6.56	0.173	0.165	0.186	0.342	0.546
TR164 13D-1	2.42	-93.34	11.48	7.34	-	-	0.106	0.172	0.620
TR164 16D-1g	2.56	-94.45	11.9	6.91	-	-	0.081	0.136	0.596
DS D-8g	2.6	-95.33	11.42	7.74	-	-	0.074	0.130	0.569
DS D-5g	2.44	-95.62	8.87	8.91	-	-	0.033	0.054	0.604
TR164 18D-3g	2.29	-96.18	9.4	8.33	0.159	0.161	0.015	0.028	0.539
TR164 20D-1g	2.24	-97.86	9.33	8.78	-	-	0.020	0.039	0.504
TR164 21D-4g	2.31	-98.54	10.03	8.01	-	-	0.014	0.047	0.305
TR164 22D-1g	2.3	-99.56	11.09	7.38	0.164	0.161	0.029	0.063	0.451
DS D-1Ag	0.71	-85.5	18.45	4.31	0.225	0.225	0.123	0.234	0.525
<i>Existing Data^(a)</i>									
K 10-34	2.635	-95.49	8.54	8.99	0.149	0.155	0.014	0.031	0.458
K 14-3	2.75	-95.24	11.59	6.19	0.167	0.156	0.099	0.147	0.673
K 18-21	2.6	-95.43	11.18	7.08	0.168	0.162	0.083	0.130	0.640
KK78-D17-4	2.43	-95.55	9.19	9.90	0.146	0.157	0.011	0.029	0.379
KK78-D17-14	2.43	-95.55	9.19	9.72	0.151	0.161	0.011	0.019	0.598
KK78-D17-32	2.43	-95.55	9.24	9.76	0.144	0.155	0.089	0.025	0.583
KK78-D17-3	2.43	-95.55	9.28	9.92	0.141	0.152	0.011	0.027	0.423
KK78-D21-2	1.64	-94.97	9.58	9.04	0.154	0.161	0.013	0.033	0.393

*from Kelley et al. (2013)

^(a) $\text{Fe}^{3+}/\sum\text{Fe}$ values reported for these samples are from Cottrell and Kelley (2011), and are included in this study for the sake of completeness

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Optimal Growth Conditions of *Rhodococcus* Isolated from South Pacific Gyre Sediments

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Abstract. In order to be an active member of the deep-sea microbial community, a bacterium must have the ability to perform in the extreme conditions of that environment. A bacterium isolated from deep-sea (5,127 m) sediment (IODP Site U1366; 24 mbsf) from the South Pacific Gyre was examined for growth under a range of temperature, salinity, and pressure conditions to determine if this isolate could be an active member of the deep-sea community. An isolate of *Rhodococcus* was chosen because this genus is known to produce secondary metabolites of biomedical interest. This isolate was grown in all possible combinations of four temperatures (4 °C, 10 °C, 14 °C, and 20 °C) and four salinities (7.91 ‰, 15.57 ‰, 23.29 ‰, and 30.85 ‰). Optical density measurements (600 nm) through time were used to quantify growth in each treatment, indicating that this isolate of *Rhodococcus* is both eurythermal and euryhaline. No significant differences in growth rate or total biomass were seen among salinity treatments ($P = 0.843$ and $P = 0.373$, respectively). The 10 °C temperature treatment showed a significantly higher growth rate over the 20 °C treatment ($P = 0.049$), and 4 °C treatments yielded a significantly lower total biomass than the other temperature treatments ($P = 0.002$). A High Pressure Liquid Chromatography (HPLC) test on 10 °C and 19 °C extracts from cultures revealed the need for further optimization of HPLC conditions in order to test for differences in compound production among the isolate grown at different temperatures. No significant growth was recorded at the *in situ* pressure of 50.662 MPa, indicating the need for a longer study, allowing for the full completion of all growth phases in each pressure treatment. The activity level of this *Rhodococcus* strain in the deep-sea community is not currently known.

1. Introduction

Prokaryotes residing in deep-sea sediments vary globally in abundance (Kallmeyer et al., 2012) and play a critical role in global biogeochemistry (D'Hondt et al., 2009). The global abundance of seafloor microbes is estimated to be 2.9×10^{29} cells (Kallmeyer et al. 2012). Even though studies of marine microbes, including those manipulating marine bacterial growth conditions such as pressure, have been undertaken since the 1880's (e.g. Certes, 1884), the deep sea is still largely unknown. The South Pacific Gyre (SPG) sediments, from which samples in the study were isolated, are one end member on the spectrum of abundance and activity. The very low sedimentation rates and low organic carbon content (D'Hondt et al., 2009) may be used to characterize other low productivity sites (e.g. oceanic gyres). Low productivity sites such as the subtropical gyres represent ~48 % of global seafloor (D'Hondt et al., 2009) and the five main subtropical gyres are all located in deep water: North and South Atlantic, North and South Pacific, and the Indian Ocean.

Antibiotic resistance in pathogenic bacteria is increasing worldwide (Boucher & Corey, 2008). Lack of deep-sea studies geared toward microbial characteristics has encouraged the pharmaceutical industry to look closer at marine bacteria for new antibiotics (Deming, 1998). At this time, whether deep-sea sediments underlying gyres are potential sources of bacteria that produce secondary metabolites for the development of antibiotics is unknown. However, it is known that the adaptive characteristics of marine bacteria are different than that of terrestrial bacteria (Carvalho & Fernandes, 2010). In addition, past studies indicate that bacteria are able to produce secondary metabolites grown under certain conditions, such as disturbance and temperature (Carvalho & Fernandes, 2010).

Optimal growth conditions of *Rhodococcus* residing in these sediments, and whether this isolate is adapted to grow at high pressure, have not been previously studied. In this study, the growth of one isolate of *Rhodococcus* previously isolated from SPG sediments below 5200 meters of seawater and 24 meters below seafloor (mbsf; Forschner–Dancause, 2012) was characterized. Characterization included the determination of optimal growth at different salinities, temperatures, and pressures. Pathogenic microbes have developed resistance against secondary metabolites produced by terrestrial *Rhodococcus* that society has used for antibiotics in the past (Jensen & Fenical, 2000). Therefore, an attempt to observe relative differences of production with temperature as the parameter was also completed.

2. Methods

2.1. Sample Collection

The Integrated Ocean Drilling Program (IODP) recovered sediment cores from the South Pacific Gyre (SPG) on Expedition 329 in 2010 (Figure 1; D'Hondt et al. 2011). A core from Site U1366 was taken in 5,127 m of water. Samples were extracted from the core at a depth of 24 mbsf using sterile syringes with the luer-lock end removed. Bacteria from the sample were isolated on marine agar by Forschner–Dancause (2012). DNA sequence analysis of one isolate identified it as *Rhodococcus erythropolis* (Mavis Zhu, personal communication).

2.2. Growth Curves

To determine the optimal growth conditions, cultures were subjected to different temperature and salinity

Figure 1. Sampling sites from 2010 Integrated Ocean Drilling Program (IODP) EXP 329. Samples used in this study were collected from Site U1366.

treatments. Seawater collected from Narragansett Bay was diluted with 18 MΩ-cm deionized (DI) water to obtain 25 %, 50 %, 75 %, and 100 % seawater concentrations. These dilutions resulted in salinity treatments of 7.91 ‰, 15.57 ‰, 23.29 ‰, and 30.85 ‰. For each dilution, 100 mL of seawater was amended with 0.5 g peptone and 0.1 g yeast extract. The chlorinity of each diluted media was measured using a 794 Basic Titrimo to ensure that salinity matched the ratio of seawater to DI water. All media was autoclaved prior to culture to remove contaminating bacteria.

The isolate was streaked on marine agar plates and a colony was transferred to 4 mL of 75 % marine broth. The culture was incubated at room temperature on a shaker for two days. *Rhodococcus* was cultured using two replicates of sixteen treatments utilizing all combinations of salinities (7.91 ‰, 15.57 ‰, 23.29 ‰, and 30.85 ‰) and controlled temperatures (4 °C, 10 °C, 14 °C, and 20 °C). The respective media (8 mL) and 100 µL of the 4 mL culture were transferred into Falcon tubes. All transfers were completed under a NUAIR laminar flow hood to prevent contamination.

Optical density (OD) measurements (600 nm) were made at least once a day on all treatments using a SpectraMax M5 microplate reader until stationary phase was reached or all sample was utilized. Two points representing the steepest portion of the exponential growth phase were used to calculate growth rate (μ) equation below.

$$\mu = \frac{\ln(OD_2) - \ln(OD_1)}{t_2 - t_1}$$

Maximum biomass was found for each treatment by obtaining the maximum values from OD readings. Growth curves were created for raw OD readings, growth rates, and maximum biomass values. One-way ANOVA tests were run for growth rates and maximum biomass values to find significant differences among salinity and temperature treatments, which could give an indication of optimal growth conditions.

2.3. Growth at High Pressure

A first attempt to grow bacteria under pressure at room temperature (approx. 21 °C) was completed using a small HiP pressure rig. This trial compared the *in situ* pressure (56.662 MPa) to a control sample at 1 atmosphere (0.101 MPa) with samples held in wide-mouth amber vials with a pliable Teflon septum to transmit the pressure. A second trial was conducted using sealed pliable transfer pipette bulbs to hold cultures, and with pressure and temperature conditions identical to the first trial. For HPLC analysis of bacteria grown at different pressures, a larger volume (250 mL) of sample was needed. To achieve a larger yield, Mylar bags were filled with culture using 100 % marine broth treatments. Bags were subjected to pressures of 0.101 and 56.662 MPa (utilizing the large HiP pressure rig) at 10 °C.

2.4. High Performance Liquid Chromatography (HPLC) analysis

Figure 2. *Rhodococcus* isolate grown at 10 °C on shaker. Individual cells are approximately 2 - 3 μ m.

HPLC tests of bacteria grown at varied temperatures required a larger volume (250 mL) of sample. To acquire a larger sample, Erlenmeyer flasks were arranged on shakers in controlled 10 °C and 19 °C rooms. These flasks contained culture using 100 % marine broth treatments. Culture extraction was completed when growth could be visually confirmed. Samples were extracted with ethyl acetate, which was removed using a Rotovap after extraction. HPLC grade methanol aided the transfer of sample organic matter into tarred vials, and was thereafter removed using a SpeedVac. Methanol was again added to samples, followed by sonication and transfer (via 0.2 μ m filter attached to a syringe) of the solution to an amber vial. HPLC analysis was conducted on a C18 column using EZChrom ELITE Chromatography Data System. Time constraints allowed for only one HPLC analysis, which was completed using the pump method (Table 1).

3. Results and Discussion

3.1. Growth Curves

No visual trends were observed in growth rates of different salinity treatments over varying temperature, suggesting that salinity does not play a role in average growth rate (Figure 5). This point was backed by the absence of a significant difference in average growth rate among different salinity treatments ($P = 0.843$; Figures 3 & 5). A lack of significant difference was also seen in total biomass achieved among salinity treatments ($P = 0.373$;

Figure 7), suggesting that salinity does not play a role in the total biomass produced during the incubations. However, total biomass decreased with higher salinity for all temperature treatments (Figure 8), implying that treatments with higher salinities could either not reach stationary phase or had a lower stationary phase than treatments with lower salinity.

A one-way ANOVA test revealed that the average growth rate of 10 °C treatments was significantly higher than that of 20 °C treatments ($P = 0.049$; Figures 4 & 6), indicating that 10 °C is the optimal temperature of the study temperatures. Significance was also observed in total biomass among temperature treatments (Figure 8). The biomass in the 4 °C treatments was significantly lower ($P = 0.002$) than that of 10 °C, 14 °C, and 20 °C treatments (Figure 8). Visible trends include increased total biomass with higher temperature for all salinity treatments (Figure 7).

Based on these data, bacteria were able to grow under all temperature and salinity conditions, with optimal growth at 10 °C. Due to their ability to grow under a wide range of temperatures and salinities, the bacteria may be considered euryhaline and eurythermal as opposed to stenohaline and stenothermal. It is interesting that the *Rhodococcus* isolated from low temperature sediments (~2 °C) grew faster at 10 °C than at 4 °C. This phenomenon has been documented for bacteria in the upper ocean (Fuhrman and Azam, 1983).

3.2. Growth at High Pressure

Figure 3. Growth curves via optical density (OD) measurements for the four salinity treatments at 10 °C.

Figure 5. Growth rates for the four salinity treatments versus temperature. No significant difference in average growth rate was found among the salinity treatments for all temperatures ($P = 0.843$).

Figure 4. Growth curves via optical density (OD) measurements for the four temperature treatments at 30.85 ‰ salinity.

Figure 6. Growth rates for different temperature treatments versus salinity. The average growth rate for 10 °C treatments was found to be significantly greater than the average growth rate for 20 °C treatments ($P = 0.0488$). Other average growth rates for temperature treatments were not significant.

No growth was observed during the first trial using wide-mouth amber vials under 0.101 MPa or 50.662 MPa. Growth was observed in the pipette bulbs during the second trial at both pressures (Figure 9). Control samples growing at 0.101 MPa in pipette bulbs grew at the same rate as samples growing at 0.101 MPa in Falcon tubes during the salinity/temperature experiment. Samples grown at 50.662 MPa did not grow at a high rate (Figure 9), indicating one of two things: this strain of *Rhodococcus* may not be able to grow under high pressure or the bacteria did not have sufficient time to acclimate to 50.662 MPa due to initial

culturing at 0.101 MPa. It is unknown at this time whether this isolate of *Rhodococcus* is an active member of the deep-sea microbial community or has the ability to produce secondary metabolites that are of biomedical importance.

No growth was observed in the Mylar bags at 0.101 MPa or 50.662 MPa, so new culturing techniques for growing large volumes under pressure must be developed. Due to the lack of bacterial growth in the Mylar bags, a comparison

Figure 7. Total biomass for different salinity treatments versus temperature. No significant difference in total biomass was found among the salinity treatments for all temperatures ($P = 0.373$).

Figure 8. Total biomass for different temperature treatments versus salinity. The total biomass for 4 °C treatments was found to be significantly lower than total biomass for all other temperature treatments ($P = 0.00204$).

of production at different pressures using HPLC techniques could not be completed.

Our results indicate that it may be informative to grow these bacteria under lower pressures. This strain has also been isolated from 1000 m below sea level, so the strain may grow optimally at pressures between 0.101 MPa and 50.662 MPa. HPLC analyses would determine if this

Rhodococcus isolate produces a different set of compounds under different pressures. To run HPLC analyses, enough biomass of *Rhodococcus* must be cultured at a variety of pressures between 0.101 and 50.662 MPa.

3.3. High Performance Liquid Chromatography (HPLC) analysis

The HPLC conditions were not optimized to produce distinct peaks for each compound to determine relative differences between the 10 °C and 19 °C sample extractions (Figure 2). Optimization of HPLC conditions must be completed in order to obtain distinct peaks for each compound produced at 10 °C and 19 °C.

Figure 9. Growth curves of 0.101 MPa in falcon tubes (blue diamonds), 0.101 MPa in pipette bulbs (green triangles), and 50.662 MPa in pipette bulbs (red squares) at 20 °C.

Table 1. HPLC settings for % H₂O, % Methanol, and Flow Rate over time.

Time (minutes)	% H ₂ O	% Methanol	Flow Rate (mL/min)
0	95	5	0.5
4	95	5	0.5
24	0	100	0.5
28	0	100	0.5
28	95	5	0.5
32	95	5	0.5

4. Conclusion

This study gave insight to the growth characteristics of a *Rhodococcus* isolate. *Rhodococcus* grew to the same

biomass and at the nearly same rate for all salinity and temperature treatments, indicating that this strain is both euryhaline and eurythermal. Of the four tested salinities and temperatures, no optimal salinity was found, and the optimal temperature was 10 °C. Extended incubation times for *Rhodococcus* under pressures between 0.101 and 56.662 MPa are needed in order to determine optimal pressure for the *Rhodococcus* isolate. Although growth could not be achieved at high pressure in this study, this strain of *Rhodococcus* may still grow at high pressure after acclimation and may have the capacity to produce secondary metabolites. Past studies indicate bacteria's ability to produce secondary metabolites grown under certain conditions, such as disturbance and temperature. Therefore, further experimentation could include running HPLC analyses on cultures grown under a variety of salinities (including *in situ* salinity), disturbance levels, and temperatures.

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Near Surface Ozone Transport In Rhode Island

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Abstract: Ozone outbreaks can occur in Southern New England on hot summer days with prevailing southwesterly winds. These outbreaks can have deleterious effects on the population as well as plants and animals. The National Ambient Air Quality Standard for ozone is 0.075 ppm, applied to a running eight-hour average. On many occasions, the concentration of ozone in Rhode Island has exceeded this standard and continues to do so. Estimating the transport of ozone can be an important factor in bettering our knowledge of when these outbreaks will occur. Past studies have quantified the transport of ozone using complex meteorological trajectories. In this study we utilized wind data from sonic detection and ranging (SoDAR) instruments and ozone concentration data measured by the Environmental Protection Agency (EPA) to estimate the net ozone advection rate across the western and southern borders of the state during the summer of 2013. In May, strong northerly winds led to a flux of ozone out of the state while in June, there was a positive flux of ozone into the state. However, the occurrence and magnitude of the fluxes in relation to ozone outbreaks do not suggest that transport is a controlling factor. This suggests that local ozone production is a key contributor to ozone outbreaks. There are limits to this study including a lack of representation of the ozone flux across the northern and southern borders of the state.

1. Introduction

Ambient ozone is a secondary pollutant that forms when nitrogen oxide, nitrogen dioxide, and volatile organic compounds (VOCs) are released, commonly during fuel combustion processes, and react with natural sunlight in the troposphere (Spiro *et al.*, 2012). VOCs are also produced naturally by vegetation and in industrial processes. A study by Shen *et al.* (2010) found that anthropogenic sources contribute more to VOC creation than natural sources in the Coastal New England Region. Ozone has oxidative properties that make it very destructive (Spiro *et al.*, 2012). For example, ozone can cause eye, throat, respiratory irritation (US Environmental Protection Agency, 2012) as well as damage to vegetation (Dugger and Ting, 1970).

The US Environmental Protection Agency (US EPA, 2008) set a National Ambient Air Quality Standard of 0.075 ppm for the pollutant, which is applied to a running eight-hour time period and cannot be exceeded by the fourth highest annual maximum. Areas where the ozone levels exceed the standard are deemed non-attainment zones and must implement plans to reduce ozone precursors. On the national level, programs like the 1990 Clean Air Act, the Clean Air Act's Acid Rain Program, and EPA's Clean Air Interstate Rule have been implemented to reduce ozone levels. In general, ozone trends levels are decreasing (US EPA, 2010); however, there are still many days in numerous areas on which ozone levels exceed the standard and are hazardous to human health and the environment. Rhode Island is just one area in the nation where the standard is regularly exceeded. High ozone levels in Rhode Island are caused, in part, by local ozone formation, but also by the transport of ozone into the state (Air Quality Summary: State of Rhode Island, 2011).

Transport of ozone in Rhode Island during the summer is dominated by the prevailing winds. The prevailing winds are caused by the Bermuda high and come from the southwest (Knorr, 2013). Locations near the shore experience the land breeze/sea breeze cycle, which is driven

by heat differences between the land and water. The cycle causes powerful southerly winds to come from the ocean during the afternoon and slightly weaker northerly winds come from the land at night (Knorr, 2013).

Wind speed and direction data along with ozone concentration data can be used to estimate the transport of ozone. A study conducted by Cleveland *et al.* (1974) utilized wind and air pollutant data from multiple sites in New England and found that air pollutants created in New York City were transported into New England by the summer southwesterly winds. This transport caused elevated levels of air pollution in Connecticut and Massachusetts. However, the study did not calculate air pollutant transport into Rhode Island. Other studies have used complex meteorological trajectories to follow the transport of ozone (Moody *et al.*, 1996). Our study focused on estimating ozone transport across the western and southern borders of Rhode Island using wind data and ozone concentration data collected from two sites within the state.

2. Data Collection and Methods

Wind and ozone concentration data were collected from two sites within Rhode Island from April 1st, 2013 to July 16th, 2013, as part of two continuous monitoring programs. Site one is located at the University of Rhode Island's Graduate School of Oceanography (GSO) in Narragansett (41.29°N 71.25°W). Wind data were collected near the water on Aquarium Road, while ozone data were collected from the EPA Narragansett Lab, north of South Ferry Road. GSO lies on the western edge of the Narragansett Bay. Due to its southeastern location within the state, the data collected from GSO served as a proxy for the southern border of Rhode Island in this study. Site two occupies the area north of site one on the western border of the state. Wind data at site two were collected in Coventry (41.41°N 71.34°W), while ozone concentration data from the EPA were taken from nearby West Greenwich at the

Alton Jones Campus (41.34°N 71.44°W). Site two served as a proxy for the western border of the state.

2.1 Wind Data

Wind speed (meters/second) data and wind direction (degrees) data were collected from both sites using sonic detection and ranging instruments (SoDAR). SoDAR works by sending three short acoustic chirps in different directions into the air and measuring the return pulse sent back as the chirps bounce off of turbulence in the atmosphere (Merrill, *et al.*, 2012). The frequency of the return pulse provides information on the speed of the wind. The principles of the Doppler Effect are used in combination with the different chirps sent out to provide the u, v, and w components of the wind. Depending on how much turbulence there is, a SoDAR instrument can collect wind information for heights up to 200 meters (Merrill, *et al.*, 2012). For the purposes of this study, data were collected at a height of 30 meters.

2.2 Ozone Data

Ozone data were collected from two EPA monitoring sites in Narragansett and West Greenwich. Both sites use Thermo Scientific Model 49i ozone analyzers and report hourly average values in parts per billion (ppb). To measure ozone concentrations, air samples are collected from a manifold extended above a tower, and travel through a series of glass and teflon tubing that lead to the analyzer, which resides inside a protected shelter. Then the sample runs through an ultraviolet detector set at 254 nm. The ozone concentrations measured at this step are averaged over an hour and sent to airnowtech.org where we collected the data. In this form, the data were still unchecked by EPA experts. However, “challenges” are performed on the instrument twice a week to check the analyzer output

Figure 1: Hourly average wind speeds at Narragansett during May and June of 2013 represented by a conventional meteorological wind rose. The lengths of the bins represent the frequency of wind that came from that azimuth. The colors of the bins represent speed, where orange and red are the fastest speeds.

against known ozone concentrations in order to look for deficiencies in the instrument. The instrument also uses paired cells that allow an internal check for drift and changes in sensitivity.

2.3 Flux Calculations

Estimated ozone fluxes across the borders were calculated using the product of wind speed (denoted as U in the equations) and ozone concentration. The wind directions were broken into north-south (meridional) or east-west (zonal) components by multiplying by $-\cos(\theta)$ and $-\sin(\theta)$ respectively.

$$\text{Meridional Flux} = -\cos(\theta) \cdot [\text{O}_3] \cdot U$$

$$\text{Zonal Flux} = -\sin(\theta) \cdot [\text{O}_3] \cdot U$$

Meteorological wind roses differ from the standard unit circle in that 0° meteorologically is located where 90° degrees would be on the unit circle. Therefore, the meridional component of the flux is given by $-\cos(\theta)$. Positive values occur with southerly winds while negative values come from northerly winds. Similarly opposite, $-\sin(\theta)$ gives the zonal component of the flux. Positive values arise from westerly winds and negative values from easterly winds. Once the meridional and zonal flux components were calculated they were normalized and an aggregate flux was calculated for the state by adding the zonal flux from site two (Coventry/W. Greenwich) to the meridional flux from site one (Narragansett). This process was done for both May and June.

Figure 2: Hourly average wind speeds at Narragansett during May and June of 2013 represented by a conventional meteorological wind rose. The lengths of the bins represent the frequency of wind that came from that azimuth. The colors of the bins represent speed, where orange and red are the fastest speeds.

3. Results and Discussion

3.1 Wind Observations

Figure 3: Color plot of ozone concentrations in West Greenwich for May and June of 2013. Units for the color bar are ppbv. Time in Julian Days is on the x-axis and hour of day is on the y-axis.

Figure 4: Color plot of ozone concentrations in Narragansett for May and June of 2013. Units for the color bar are ppbv. Time in Julian Days is on the x-axis and hour of day is on the y-axis.

usually fell between 20-40 ppbv. Periods of extremely high ozone concentrations, which exceeded the NAAQS, were observed around Julian Days 152 and 176. These ozone outbreak events occurred in the afternoon and the recorded concentrations were between 75 and 100 ppbv.

Ozone concentrations recorded in Narragansett were more variable (See Figure 4). The defined sections of early morning, afternoon, and evening ozone concentrations were not observed consistently at this site. Concentrations at almost all hours of the day were between 25-45 ppbv. Occasionally, concentrations reached 60 ppbv during the afternoon. Local atmospheric chemistry processes may be responsible for this uneven pattern, brought on by the close proximity to the water. However, even with the large contrasts to West Greenwich, the same outbreak events appear in Narragansett during the same time periods.

3.3 Ozone Flux

In May, a flux of ozone out of the state was observed. The sum of the flux estimates from site two was +3059

Meteorological wind roses generated using the wind data from both sites show that southwesterly winds prevailed during the months of May and June. This was expected given the dominance of the southwesterly winds, especially during the summer. Wind speeds in Coventry were, on average, between 1-3 m/s (See Figure 1). The occurrence of a storm in June brought stronger wind speeds from the north, above 7 m/s, observed at a very small frequency.

Wind speeds were higher at the Narragansett Site, and more frequently reached speeds between 3-5 m/s from the southwest direction (See Figure 2). Faster wind speeds are common at this site because of its close proximity to the water. The effects of the land-breeze/sea-breeze cycle are evident at the Narragansett site; the cycle forces greater wind speeds than at the land-locked Coventry site. Frequent winds from the north with speeds that reached over 7 m/s were also observed at the Narragansett site. The high frequency of northerly winds recorded is most likely a representation of a series of storms that passed through the area. When the wind data for the site are further broken down into individual months, almost all of the northerly winds recorded occurred during the month of May.

3.2 Near-Surface Ozone

EPA ozone concentration data collected in West Greenwich during May and June show ozone concentrations increasing throughout the day and peaking in the afternoon (See Figure 3). In the early hours from 0000 to 0900, ozone concentrations were around 20 ppbv. During the sunny afternoon hours of 1000 to 1800, concentrations increased to around 60 ppbv. In the evening to late-night hours, concentrations were less uniform across the two months, but

$\text{ppbm}^{-1}\text{s}^{-1}$, suggesting more ozone crossed the western border into the state during this month. However, the total meridional flux at site one was $-3429 \text{ ppbm}^{-1}\text{s}^{-1}$, resulting in a total negative flux for the month at $-370 \text{ ppbm}^{-1}\text{s}^{-1}$. A negative total flux for the month, according to our method, means that more ozone left the state. The series of storms that came through the Narragansett site during this month could be responsible for this flow of ozone out of the state.

In June, less frequent northerly winds at the southern border led to an influx of ozone into the state. The flux across the western border was $+1231 \text{ ppbm}^{-1}\text{s}^{-1}$, while the flux across the southern border was $+756 \text{ ppbm}^{-1}\text{s}^{-1}$. The total flux for the month was $+1987 \text{ ppbm}^{-1}\text{s}^{-1}$.

Even though a flux of ozone into the state is observed, the timing and magnitude of the fluxes in relation to the ozone outbreaks do not match, as might be expected. In June, the outbreak of high ozone concentrations occurs at the end of the month around the 25th. Looking at this same date on both the zonal and meridional fluxes for the month, no significant peak in flux is found (See Figure 5). When the magnitude of the fluxes does increase, no increase in ozone concentration is observed. These observations suggest that transport of ozone is not a controlling factor in ozone outbreaks and local ozone production is the main source. Limitations to this study could, however, affect the results observed.

Figure 5: Composite plot of flux and ozone concentration data for June 2013. Flux data is hourly. The Meridional Flux data come from Narragansett and the Zonal flux data come from Coventry. Positive flux values represent ozone going into the state, while negative values represent ozone leaving the state. Units for the color concentration figures are ppbv.

Limitations for this study include a lack of consideration of the northern and eastern borders of the state. The vertical changes in ozone concentration were also not accounted for. Local atmospheric chemistry processes could affect the rates at which ozone is created and destroyed at some scale. Lastly, there were some gaps in the data for the limited time frame used in this project. In the future, to reduce these limitations and improve the study, multiple steps could be taken. Wind and ozone concentration measuring instruments could be placed on the northern and eastern borders to more adequately represent the all of the borders of the state. Additionally, a vertical assessment of the local changes in ozone chemistry could be done and applied to a more sophisticated calculation for the flux. An intermediate step would involve examination of simulations using numerical models accounting for transport and chemistry. Finally, due to the continuous nature of the monitoring programs utilized for data collection, a long-term analysis of ozone concentrations and flux could be carried out in order to calculate the long-term trends.

4. Conclusion

Analyses of wind and ozone data collected from two sites within Rhode Island were performed. Both the Narragansett and Coventry sites were dominated by prevailing winds from the southwest. A series of storms that passed through the Narragansett site in May caused an outflow of ozone from the state. No such events were observed in June and there was a total inflow of ozone into the state. Ozone concentrations at both sites recorded two ozone outbreaks, one at the end of May and one in mid June. However, the occurrence of these outbreaks did not match the timing of the peaks in the flux data as had been expected. These results suggest that local ozone production is the controlling factor in ozone outbreaks during the period considered. These are import implications considering both the health effects associated with ozone and the resources put forth to reduce ozone precursors. If transport of ozone is not as large of a factor as previously thought, interests may have to shift. Due to limitations in this study, further research including both the northern and

eastern borders would have to be done to verify the flux versus outbreak results found in this study.

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An Evaluation of Polycyclic Aromatic Hydrocarbon Uptake into Polyethylene Samplers

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Abstract. Polyethylene passive samplers (PEs) are simple reliable tools that have been widely used in the detection of hydrophobic organic compounds. Thick ($>200\ \mu\text{m}$) PEs have important applications to specific sampling scenarios including biological assays, deployment on ships and aircraft (towing) and long term sampling, however little is known about their uptake kinetics. This study aimed to develop an accurate understanding of the uptake kinetics of these thick PEs. PEs of equal surface area, but differing thicknesses were co-deployed in the surface water and air of lower Narragansett Bay in 2013 to characterize differences in their uptake of polycyclic aromatic hydrocarbons (PAHs). PE samplers of approximately 50, 800, and 1600 μm thicknesses were analyzed for 38 parent and alkylated PAHs, with replicate sampler reproducibility mostly within 25%. A number of smaller PAHs (typically those with a molecular weight less than 180) equilibrated over a 4 week deployment, while the larger molecules remained in the linear or curvilinear uptake stages. Results from a second, 24 week deployment of 800 μm and 1600 μm samplers in surface waters suggest that all 38 compounds studied remained in the linear uptake stage. The PE-weight normalized concentration ratio of 1600 μm to 800 μm sampler fell below 1 for all analytes, implying equilibrium had not been established.

1. Introduction

The accurate sampling of hydrophobic organic compounds (HOCs) has been a problem facing environmental and analytical chemists for years. Traditional approaches utilize high volume air and water samplers, however these are expensive to operate and require onsite electricity as well as a technician in the event of mechanical problems. These limitations led to the development of alternative sampling approaches, originally primarily through the use of biota, such as mussels and other filter feeders (Farrington et al. 1983). Still further advancement led to the development of synthetic passive samplers, which were designed to emulate the structure (typically a semipermeable membrane around a lipid like substance) of the previously used biota samplers (Huckins et al. 1990). More recently, the field of passive sampling is turning to solid nonpolar matrix samplers, such as polyethylene. Polyethylene samplers are nothing more than thin strips of low density polyethylene that provide a simple and reliable way to measure truly dissolved concentrations of hydrophobic organic compounds. If the sampler is at equilibrium, the concentration in the water equals the concentration in the sampler divided by a compound specific partitioning coefficient.

$$C_w = \frac{C_{PE}}{K_{PE-W}} \quad (1)$$

where C_{PE} (mol/kg_{PE}) is the concentration in the PE, C_w (mol/L) is the ambient concentration in the water, and K_{PE-W} is the polyethylene-water partitioning coefficient.

Typically polyethylene used for environmental sampling ranges between 25 and 200 μm . Thick PE samplers ($> 200\mu\text{m}$) offer a number of distinct advantages over their traditional counterparts. First given their thicker nature they avoid some of the “boundary layer problems” described by

Lohmann (2012). Additionally these samplers, given their greater volume, could be used to simplify the process of bioassays (Allan et al. 2012), and could increase sensitivity, effectively lowering limits of detection as described by Bartkow et al (2004). Despite these marked advantages very little is known about these thicker samplers or how they accumulate various compounds.

This study investigates polycyclic aromatic hydrocarbons (PAHs) as a model HOCs to investigate thick PE samplers. PAHs are known carcinogens and mutagens with a relative wealth of knowledge available regarding their transport and toxicological effects. (Nisbet and LaGoy 1992)

2. Materials and Methods

2.1 Sampler Prep

PE samplers of $\sim 8 \times 20$ cm were cut from sheets of 51 μm polyethylene (Berry Plastics, Franklin, MA, USA) as well as 800 μm and 1600 μm sheets (US Plastic, Lima, OH, USA). Holes (~ 5 mm) were punched into the 800 μm and 1600 μm samplers, while the 51 μm samplers were woven onto stainless steel wire. They were then pre-extracted by soaking for 24 hours in DCM before being changed to hexane for another 24hrs. After preparation all samplers were wrapped in muffled aluminum foil and stored in a freezer until deployment.

2.2 Deployment

The study was conducted over two separate deployments in lower Narragansett Bay. The first deployment (12/28/12-6/11/13) consisted of two pairs of co-deployed 800 μm and 1600 μm samplers in the surface water of lower Narragansett Bay. Samplers were placed into either a cylindrical mesh cage constructed of stainless steel hardware cloth, or a stainless steel tube with regularly spaced holes. These devices allow water flow while still protecting the samplers from a number of biotic and abiotic interferences. The second deployment (6/12/13-7/8/13)

Figure 1: Concentrations of selected PAHs during consecutive extractions of xx μm thick PE samplers.

consisted of two pairs of co-deployed 51 μm and 800 μm samplers in the bay, as well as two sets of co-deployed 51 μm and 800 μm samplers in air. The water samplers were deployed as above. The air samplers were deployed approximately 3 meters off the ground in inverted mixing bowls to protect them from any sunlight degradation and precipitation.

2.3 Extraction

Samplers were extracted in hexane three times. Fouling was removed from the samplers using a Kimwipe before the samplers were cut (using solvent rinsed scissors) into 8 sections to affect easier transfer. Samplers were placed in muffled and solvent rinsed amber jars, and approximately 100 mL of hexane was added along with 10 μL of a surrogate cocktail consisting of 5 deuterated PAHs (acenaphthene- d_{10} , phenanthrene- d_{10} , chrysene- d_{12} , perylene- d_{12} at 5 ng/ μL), this was repeated three times. After each extraction the extract was concentrated to about 300 μL under nitrogen. Before analysis all samples were injected with 10 μL of p-terphenyl d_{14} (at 5 ng/ μL) to act as an injection standard. After extraction the samplers were dried and weighed.

2.4 Analysis and Quality control/Assurance

All samples were analyzed on an Agilent 6890 series gas chromatograph coupled with a 5973 network mass spectrometer operated in selective ion monitoring mode (70eV). The concentrations were then quantified against an 8-point calibration curve of 38 PAHs (0.01-10 ng/ μL). Instrumental calibration checks were run every 10 samples to assure that data was being analyzed consistently. Field and fabrication blanks were produced and carried through the complete analytical process; data was not corrected from blanks as in all cases the concentrations represented less than 10% of the total sampled concentrations.

3. Results and Discussion

3.1 Reproducibility

Reproducibility between replicate samplers was calculated as the relative percent difference (absolute value of the difference divided by the mean times one hundred). Reproducibility in both air and water were good, mostly below 25% in air, and 30% in water for all compounds. If the lower weight compounds which are strongly affected by contamination in the laboratory (and far greater more variability) are excluded, reproducibility fell below 25% for both sampling media.

3.2 Sampler Extraction

To evaluate the required extraction time for thicker PE samplers we focused on 3 compounds: naphthalene, pyrene and dibenz(a,h)anthracene. These three PAHs represent a range of molecular weight and sizes and should adequately represent low, middle and high molecular weight PAHs. The concentrations in each of the three sequential extracts plotted against time are presented in Figure 1. The PEs

Figure 2: Ratio of weight normalized concentrations (2a) and sampled amounts (2b) of PAHs in 800 and 1600 μm samplers as a function of their molecular weights.

Figure 3: Ratio of weight normalized concentrations of PAHs against molecular weight for air samplers.

showed exponential loss of all compounds over the initial hours followed by a marked decrease in the extract concentration after approximately 40 hours. The limits of detection for all compounds used were reached by the 39-hour mark, suggesting that all usable contaminants (at least all those detectable using this study's procedures) were extracted at that point.

3.3 Sampler uptake

During the first deployment only 800 and 1600 μ m samplers were deployed. A ratio of PE-weight normalized concentrations was taken ($\text{Conc.}_{\text{thick}}/\text{Conc.}_{\text{thin}}$), and plotted against molecular weight (Figure 2a). The ratio for all compounds fell below the equilibrium line of 1. However if the weight correction is removed and the ratio of amounts collected by each sampler is plotted (Figure 2b) the points average around the one line suggesting that the samplers acted as replicates, this can only occur during the initial linear stage where neither sampler has yet reached its curve linear or equilibrium stage. This suggests that over the initial 24 week deployment neither the 800 nor the 1600 μ m samplers achieved equilibrium, or even approached their "capacity".

The second deployment utilized both air and water samplers over a four-week period. First examining the air data a number of significant trends stand out. When the ratio of the weight-normalized concentrations is again plotted against molecular weight (Figure 3), a clear decrease in the ratio is seen as the molecular weight increases. This suggests that for the lighter weight compounds (which can

diffuse more readily into the sampler) the sampler was acting as an equilibrium sampler (those compounds had reached equilibrium), whereas compounds with heavier molecular weights fell firmly below the equilibrium line suggesting that given their slower uptake rate they remained in linear or kinetic uptake. A similar analysis done on two different sets of water data looks much the same (Figures 4a and 4b), with lighter compounds having reached equilibrium while heavier compounds remained in linear uptake.

Within the water data there is far more variability as well as several compounds displaying a higher weight normalized concentration in the thicker sampler. The most likely cause of this anomalous data is a variance in the ambient water concentration during the length of the deployment. The area in which the water samplers was deployed is characterized by light commercial activity as well as being the homeport for the R/V Endeavor. These factors may have lead to fluctuating HOC concentrations over the period of deployment that might explain the ratios greater one for the lower molecular weight compounds. If equilibrium had already been reached in the thinner samplers before the concentration fluctuated it may have affected the concentrations in the thin samplers while not affecting the thick sampler due to their higher capacity.

Future studies should investigate the uptake of these samplers over time, perhaps taking weekly extracts over a long-term deployment to examine the uptake kinetics over time.

4. Conclusion

As the presence of PAHs in the environment grows as well as the risks from new emerging contaminants, a need for an accurate sampling scheme to monitor them becomes important. In future work it should be possible to deploy thick samplers in remote locations where they may not be retrievable for significant periods of time. Samplers of this type may also be useful in simplifying biological assays conducted with concentrated contaminants from samplers, and it may be possible to deploy these samplers in situations where boundary layers are essentially non-existent such as in towing scenarios. Overall the results from this study suggest that thick PE samplers show promise, particularly in specified and unique sampling scenarios.

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Figure 4: Ratio of weight normalized concentrations of PAHs against molecular weight for water samplers.

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The Effects of pCO₂ on Growth Rate and Community Structure of a Natural Plankton Assemblage from Narragansett Bay, RI

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Abstract: Ever-increasing amounts of anthropogenic carbon dioxide are being sunk into the oceans, changing sea surface chemistry. These changes may alter community structure, nutrient cycling, and carbon export of marine organisms. One group influenced by these fluctuations is phytoplankton, which are important, as they comprise the base of the aquatic food chain, account for half of all photosynthetic activity on earth, and are a vital component in oceanic nutrient cycling. This project used a continuous culture system known as an Ecostat to examine the effects of ambient (380 ppm) and high pCO₂ concentrations (750 ppm) on plankton growth and grazing rates, and community structure. To further investigate the effects on a single predator *Oxyrrhis marina* and prey *Isochrysis galbana*, laboratory incubation experiments were conducted. In the whole-plankton assemblage, increased initial pCO₂ at 750 ppm resulted in increased chlorophyll *a* concentrations and a population shift, with early dominance of *Skeletonema spp.*, followed by an increase in larger diatoms, and later dominance of *Chaetoceros spp.* In the lab culture incubations, at a pH difference of 0.04 to 0.1 units, *O. marina* grew significantly faster, up to 461% at higher pH. Average growth rate of *O. marina* at 750 ppm was 0.04 cells d⁻¹, and 0.20 cells d⁻¹ at 380 ppm. This difference suggests a significant sensitivity to ambient pH. Our results suggest that even a hardy heterotroph, such as *O. marina* may be affected by changes in pCO₂ concentrations and ambient pH, which may have top-down effects on natural plankton assemblages. Given the amount of CO₂ that continues to be absorbed into the ocean, monitoring the effects on plankton growth and community structure as the base of the marine food chain will become increasingly important.

1. Introduction

As a result of human activity, increasing amounts of carbon dioxide are being emitted into the atmosphere, with a large portion ending up in the ocean. It is estimated that 118 ± 19 pG of carbon emitted from the combustion of fossil fuels have been absorbed into the ocean, accounting for a staggering 30% of the total CO₂ emission for the time period 1800 to 1994 (Sabine *et al.* 2004). This addition of CO₂ has influenced sea-surface carbonate chemistry by increasing the concentration of carbonic acid (H₂CO₃) and decreasing pH and the carbonate ion (CO₃²⁻). pH has decreased 0.12 units since 1800, and is predicted to decrease another 0.3 units by 2100 (Riebesell 2004). These changes have in turn resulted in a decrease in the saturation state of aragonite and biogenic calcite (Riebesell 2004, Kim *et al.* 2006).

These changes in sea surface carbonate chemistry are having profound effects on the organisms that live there, particularly on plankton, though not all taxonomic groups respond similarly. One area in which there are large differences between phytoplankton groups is in CO₂ specificity of the main carboxylating enzyme ribulose biphosphate carboxylase/oxygenase (Rubisco) (Badger *et al.* 1998, Riebesell 2004). Specificity of Rubisco (which is its affinity to CO₂ relative to that of O₂) decreases with increasing evolutionary age of phytoplankton, meaning that the highest specificity is found in the most recently evolved (youngest) group: the diatoms. Specificity decreases in coccolithophorids, dinoflagellates, and cyanobacteria respectively (Tortell 2000). This means that an increase in pCO₂ in the oceans would be expected to result in a competitive advantage in diatoms over other genera.

One aspect of the phytoplankton life cycle that is affected by changing carbonate chemistry and within which groups differ is photosynthesis. Riebesell (2004) notes that dominant phytoplankton species have different CO₂ requirements—some species preferentially use CO₂ as their carbon source while others obtain carbon from HCO₃⁻. Differences among groups in CO₂ sensitivity also carry over to carbon metabolism. For example, the photosynthetic carbon fixation rates of all diatom species are at or close to present day CO₂ saturation (Burkhardt *et al.*, 1999, 2001; Rost *et al.*, 2003; Riebesell 2004). Coccolithophorids such as *Emiliania huxleyi* and *Gephyrocapsa oceanica*, on the other hand, are below saturation at modern levels (Riebesell 2000). Taxa that are sensitive to CO₂, such as those that are calcifying, should therefore respond better to increasing CO₂ concentrations than the non-calcifying diatoms (Riebesell 2004). This conclusion, however, does not take into account the effects of increased CO₂ on calcification.

Calcification is also an area of concern in response to the present-day increase in CO₂, as it may result in population shifts, such as the shift away from *Phaeocystis* toward diatoms in the Tropical Pacific shown in Tortell *et al.* (2002). Kim *et al.* (2006) found that in a natural phytoplankton assemblage from the southern coast of Korea, only the populations of two diatom species (*Skeletonema costatum* and *Nitzschia spp.*) increased substantially in response to increased pCO₂. As further evidence for the specialization even within taxonomic groups, only *S. costatum* showed an increase in growth rate with increased pCO₂ levels, indicating that not all diatom species are equally sensitive to pCO₂.

Many of the studies investigating the effects of changing pCO₂ on phytoplankton have been conducted

using mesocosm experiments. For example, mesocosm experiments have investigated the elemental composition of phytoplankton (Burkhardt *et al.* 1999; Engel *et al.* 2002), the calcification rates of certain organisms (Engel *et al.* 2005), the accumulation of dissolved organic matter (Rochelle-Newall *et al.* 2004), and others (Engel *et al.* 2004). Mesocosm experiments are popular and useful in that they are similar to environmental conditions in terms of light and temperature fluctuations (Kim *et al.* 2006). However, the problem with these approaches is that they create changing chemical conditions over time that can create many different stressors on growth rates and competitive interactions (Rhee 1980, Pickell *et al.* 2009).

A relatively new method of experimentation is a continuous culture system known as an Ecostat, as presented by Hutchins *et al.* (2003) and refined by Pickell *et al.* (2009). The Ecostat maintains a mixed plankton assemblage under a 24-h light cycle while maintaining controlled carbonate chemistry and nutrient conditions. The Ecostat provides refined insight into the responses of phytoplankton assemblages to minute changes in seawater chemistry, such as pH, temperature, and nutrient manipulations. It is a more analogous to natural processes and more realistically simulates community responses (Pickell *et al.* 2009). Most Ecostat experiments filter out zooplankton grazers and as a result do not investigate whole plankton assemblage dynamics. Rose *et al.* (2009) did however use a whole plankton assemblage in an Ecostat to investigate the effects of climate change variables (temperature and pCO₂) on trophic dynamics and found that after an initial increase in microzooplankton, by the end of the experiment total abundance decreased, which occurred concurrently with the growth of an unpalatable phytoplankton assemblage dominated by coccolithophores. Their results suggest that indirect, bottom-up effects on the microzooplankton resulting from CO₂ and temperature impacts on the phytoplankton community were more important than direct physiological impacts on the microzooplankton.

For our study we utilized the Ecostat to examine the effects of high (750 ppm) pCO₂ concentrations on the growth rate and population dynamics of a natural plankton assemblage from Narragansett Bay, RI. We also conducted lab incubation experiments to investigate the effects of ambient (380 ppm) and high pCO₂ concentrations on a single predator (*Oxyrrhis marina*) and prey (*Isochrysis galbana*). We hypothesized that in the high pCO₂ treatments, growth rates would increase in both the lab incubation and Ecostat experiments and that there would be a population shift in the Ecostat experiments resulting in the dominance of a few more CO₂-sensitive species.

2. Materials and Methods

2.1 Ecostat Experiment

2.1.1 Seawater acidification

Seawater manipulation was achieved by aeration with custom air mixtures pre-mixed to CO₂ concentrations of 380 and 750 ppm, purchased from AirGas ($\pm$ 10% blend tolerance). Gas was bubbled into 10 L polycarbonate carboys containing 0.2 μ m filtered seawater through a 2 μ m

ultra-fine stone gas sparge (Kim *et al.*, in press). Seawater for manipulation was not autoclaved because autoclaving results in degassing of the water, which leads to changes in carbonate chemistry (Riebesell *et al.* 2004). pH was observed periodically throughout the manipulation process using a pH meter (Radiometer analytical HANNA HI98150, precision $\pm$ 0.01, accuracy $\pm$ 0.01) calibrated with pH 7.01 and 10.01 NBS buffers. The water was manipulated until it reached a stable pH, indicating equilibrium with pCO₂ of the gas mixtures.

2.1.2 Experimental Design

In order to simulate a more natural environment, experiments were conducted using a continuous seawater culture system known as an 'Ecostat' (Hutchins *et al.* 2003; Hare *et al.* 2005, 2007; Pickell *et al.* 2009; Rose *et al.* 2009). Whole seawater (WSW) was collected with a 20 L polycarbonate carboy from the surface off the dock of the University of Rhode Island Graduate School of Oceanography (41°29'31.64"N, 71°25'8.17"W) and filtered through a 150 μ m mesh to remove mesozooplankton. Seawater was put directly into 3, 8L acid-cleaned polycarbonate bottles. There were three treatments, summarized in Table 1. After WSW was added to each bottle, the 750 ppm-manipulated seawater was added, taking care to avoid bubbles and create as little disturbance as possible to avoid gas escape. Bottles were placed in rocking cradles in an outdoor tub with recirculating ambient seawater to control temperature. The rolling cradles were attached to a 1/20 horsepower motor that rocked the bottles at a speed of 2 rpm. Each bottle was attached to 0.64 mm inflow and outflow tubes. The inflow tubes were attached to a 10 L polycarbonate carboy containing 750 ppm manipulated seawater via a 16 channel ISMATEC IPC peristaltic pump. The flow rate was set at 0.58 mL min⁻¹ but when measured it was $\sim$ 3 mL min⁻¹.

Bottle	% WSW	% MSW
1	10	90
2	30	70
3	50	50

Table 1. A summary of the Ecostat treatments, including bottle (treatment), % whole seawater (WSW), and % manipulated seawater (MSW).

2.1.3 Sample Collection and analysis

During sampling times, each outflow tube was screwed into a 45 mL falcon tube until the appropriate amount was collected. At 8:00 am, 11:00 am and 3:00 pm, 10 mL samples were collected for pH and Coulter counter analysis. pH readings were taken immediately after collection, and all samples were run on the Coulter counter within 60 minutes of collection. At 3:00 pm, an additional 68-70 mL were collected for chlorophyll analysis. Following the Chl a extraction methods of Graff &

Prey Concentration (cells mL ⁻¹)	pCO ₂ (ppm)	mL <i>I. galbana</i>	mL <i>O. marina</i>	mL MSW
120,000	750	38	106	106
80,000	750	25	106	106
30,000	750	10	106	106
120,000	380	52	96	102
80,000	380	35	96	102
30,000	380	13	96	102
120,000 prey control	750	38	106	106
80,000 prey control	750	25	106	106
30,000 prey control	750	10	106	106
120,000 prey control	380	52	96	102
80,000 prey control	380	35	96	102
30,000 prey control	380	13	96	102

Table 2. A summary of *O. marina* and *I. galbana* laboratory incubation treatments. Includes target concentrations of the prey *I. galbana*, the pCO₂ concentration of the manipulated seawater added (750 ppm or 380 ppm), the amount (mL) of *I. galbana* and *O. marina* added to achieve target concentrations, and the amount of manipulated seawater added.

Rynearson (2011) samples were filtered onto GF/F filters at <5mm Hg vacuum pressure and extracted in 90% acetone for 20.5 hours in the dark at -20°C and read on a 10 AU Turner Designs fluorometer. The chlorophyll reading from bottle 1 at T4 was lost, but the value was estimated by multiplying average initial chlorophyll measured in 100% whole seawater by 0.10 (the percentage of WSW in the bottle). On the final day, samples were taken as usual, and then the bottles were inverted and duplicate samples were taken directly from the bottles. Chlorophyll readings from 52 hours were discarded due to experimental error. Samples from the initial whole seawater and each of the bottles at 69 hours (T_{final}) were settled for 24 hours and observed under a dissecting microscope.

2.2 Lab Culture Experiment

2.2.1 Culture Conditions

The prymnesiophyte *I. galbana* (CCMP 1323) (prey) was cultured in 1 L polycarbonate bottles at 16°C in 0.2 µm sterile-filtered autoclaved seawater (FSW) collected from Narragansett Bay, Rhode Island and enriched with f/2 nutrients (Guillard, 1975) minus silica. Cultures were grown at a light intensity of 75-90 µmol photons m⁻²s⁻¹ with cool and warm white lights on a 12:12 h light:dark cycle. Cells were grown to concentrations of 570,000-786,000 cells mL⁻¹. The heterotrophic protist *O. marina* was also cultured in 1 L polycarbonate bottles at 16°C in 0.2 µm sterile-filtered autoclaved seawater (FSW) collected from Narragansett Bay, Rhode Island. The cultures were maintained at a light intensity of 18 µmol photons m⁻²s⁻¹ and fed *I. galbana* every other day until they reached constant concentrations between 4,000-5,000 cells mL⁻¹.

2.2.2 Experimental Setup

To investigate the effects of varying prey concentrations and the addition of manipulated seawater (MSW), twelve treatments were set up in 250 mL

polycarbonate bottles, summarized in Table 2. *I. galbana* (prey) was added to the bottles in target concentrations of 120,000, 80,000 or 30,000 cells mL⁻¹ with *O. marina* (predator) at a 1,500 cells mL⁻¹ target concentration. Then manipulated seawater at 750 ppm or 380 ppm was added. The 380 ppm treatments were set up on the first day, and the 750 ppm treatments were set up the following day, which is important because the stock culture pHs were different on the different starting days and could effect the pHs measured throughout the course of the experiment. The amount of *O. marina* and manipulated seawater in the bottles were constant in all treatments with the same pH. Each treatment containing predator, prey, and manipulated seawater was done in triplicate. Single prey control bottles were also prepared containing the same amounts of *I. galbana* and MSW, with the remaining portion filled with sterile filtered seawater. Triplicate 10 mL samples were taken from each bottle (72 total) and initial pH readings were taken using the same pH meter used for seawater manipulation. Each sample was run on a Beckman Coulter Multisizer 3 Coulter Counter to measure cell abundance and size distribution. The prey *I. galbana* was evident in a peak around 2-6 µm and everything from 10-40 µm was counted as *O. marina*. Cultures were incubated at 16°C at a light intensity of 18 µmol photons m⁻²s⁻¹ on a 12:12 h light:dark cycle. Triplicate pH and Coulter counter measurements were taken within 30 minutes of the time of the initial reading. Samples were taken for three consecutive days.

2.3 Statistical analysis.

Chlorophyll *a* concentration (µg L⁻¹), pH, and specific growth rate (µ, d⁻¹) as functions of differing initial amounts of pCO₂-manipulated seawater in the three different bottles in the Ecostat were compared using a two-way, Analysis of Variation (ANOVA) with repetition in MatLab. Specific growth rates over time between the 380 and 750 ppm treatments in the *O. marina* and *I. galbana* laboratory incubation experiments and the pH over time

Figure 1. (Top) pH over time in the 3 Ecostat bottles. Bottle 1 was 90% manipulated seawater (MSW) at 750 ppm, 10% whole seawater (WSW); bottle 2 was 70% MSW, 30% WSW, and bottle 3 was 50% MSW, 50% WSW. Sample times are marked by star (T4, T28, T52, T69). (Middle) Chlorophyll *a* (µg L⁻¹) over time (Bottom) Specific growth rate, µ d⁻¹.

between 380 and 750 ppm treatments were also compared using a two-way ANOVA. For all tests, significance was accepted if $p < 0.05$.

3. Results

3.1 Ecostat Experiment

Initial pH was highest in bottle 1 (90% MSW, 10% WSW) (7.99) and lowest in bottle 3 (50% MSW, 50% WSW) at 7.95 (Figure 1 top panel). pH was fairly constant up until 28 hours after the start of the experiment, at which point it climbed in all three bottles to a maximum of 8.23 in Bottles 2 and 3 at 52 hours. From 24 to 52 hours, bottle 1 had the lowest pH. Bottles 2 and 3 had very close pHs between 28 and 52 hours. Between 52 and 69 hours, the pH in bottle 2 dropped from 8.23 to 8.13. The pH varied significantly with time in all three bottles ($p=0$, $F=36.64$, $DF=8$) but not significantly among the three bottles with different proportions of whole seawater treatments ($p=0.6695$, $F=0.41$, $DF=2$). At all times during the 69-hour Ecostat incubation, pH was higher in all bottles than the pH of the 100% whole seawater, which was 7.70.

Initial chlorophyll was highest in bottle 3, as would be expected given it started with the most whole seawater. By the end of the experiment, chlorophyll *a* was highest in bottle 1, with a value of $13.34 \mu\text{g L}^{-1}$ (Figure 1 middle panel). At all points during the experiment. Bottle 2 had intermediate amounts of chl *a*. Bottle 3 was the only treatment to decrease in chlorophyll, with a maximum at 28 hours of $10.64 \mu\text{g L}^{-1}$, which dropped to $6.72 \mu\text{g L}^{-1}$ at 69 hours. Despite observations, the changes in chlorophyll were not significantly related to time or treatment bottle ($p=0.525$, $F=6.73$, $DF=2$).

Growth rate as calculated by chlorophyll *a* between 4 and 28 hours was highest in bottle 1 with a μ value of 3.22 d^{-1} . Between 4 and 28 hours, the growth rates of all bottles were extremely high, with the lowest being 2.73 d^{-1} in bottle 3. Between 28 and 69 hours, the growth rates dropped in all 3 bottles (Figure 1 bottom panel). Growth rates were still positive in bottles 1 and 2, with the highest rate still being in bottle 1, at 1.60 d^{-1} . Bottle 3 had a negative growth rate of -0.59 d^{-1} . Growth rate varied significantly with time ($p = 0.0366$, $F=25.86$, $DF=1$) but not with treatment bottle ($p = 0.2892$, $F=2.46$, $DF=2$).

3.2 Laboratory Incubations

The differences in pH in the 380 ppm and 750 ppm treatments were small but significant throughout the course of the experiment ($p = 2.56149 \times 10^{-15}$, $F=77.17$, $DF=1$). The pH was always higher in the 380 ppm treatment, and pH decreased over the course of the experiment. The average pH in the 380 ppm treatments started at 8.58 and ended 8.51. The average pH of the 750 ppm treatments started at 8.53 and ended at 8.45 (Figure 4 top panel). The starting pH of the stock *I. galbana* culture was 9.23 and the pH of the stock *O. marina* culture was 8.78 when added to the 380 ppm treatments. The starting pH of the stock *I. galbana* culture one day later was 9.49 and the pH of the stock *O. marina* culture was 8.76 when added to the 750 ppm cultures. The pH of the 380 ppm manipulated seawater added to the incubations 8.13 and the pH of the 750 ppm manipulated seawater was 7.98. In the 380 ppm treatment, *O. marina* growth rate increased with increased *I. galbana* prey concentration, but in the 750 ppm treatments, the *O. marina* growth rate did not increase with

Figure 2. Particle size distribution and abundance over time. Bottle 2 (30% WSW) shown but all bottles followed same pattern. Early in the experiment, the population was dominated by smaller plankton, whereas the ending plankton assemblage was dominated by larger plankton, indicating a population shift.

increasing prey concentration (Figure 4 middle panel). Splitting that data, the average growth rate of *O. marina* at prey concentrations less than 50,000 cells mL⁻¹ was 0.08 d⁻¹ in the 380 ppm treatment and 0.02 d⁻¹ in the 750 ppm treatment. Average *O. marina* growth rate at prey concentrations greater than 50,000 cells mL⁻¹ rose to 0.45 d⁻¹ in the 380 ppm treatment and 0.07 in the 750 ppm treatment (Figure 4 bottom panel). The growth rates varied significantly both with time and between the 380 ppm and 750 ppm treatments ($p = 0.0077$, $F = 7.3940$, $DF = 1$).

4. Discussion and Conclusion

4.1 Ecostat Experiment

By the end of the experiment, chlorophyll *a* was highest in Bottle 3 (Figure 1 middle panel), which corresponded to the highest growth rate (Figure 1 bottom panel). This did support the hypothesis that more pCO₂ would result in increased growth rates. The growth rates that were observed however were extremely high. The growth rate between 4 and 28 hours was 3.22 d⁻¹, which would mean 4.7 cell divisions per day. These are almost unheard of growth rates. One possible explanation, other than experimental error could be the population shift that was observed. Early in the experiment, the samples in all three bottles were dominated by *Skeletonema spp.* and other small phytoplankton species in the 4-5 μm range. As early as 28 hours into the experiment, the population started to

shift toward larger species around 10 μm (Figure 2). Samples were observed under a dissecting microscope and the growth of even larger species such as *Ditylum brightwellii* and *Chaetoceros spp.* was seen (Figure 3). One division of these larger cells would account for the same biomass as many divisions of the smaller cells.

The increased growth observed under increased pCO₂ conditions agrees with previous studies. Kim *et al.* (2006) showed an increase in the abundance and growth rate of the diatom *Skeletonema costatum* under increased seawater pCO₂. They also propose that not all diatom species respond to pCO₂ changes the same, which we also observed. Furthermore, when the settled samples from T_{final} were observed under the microscope, while not counted, qualitatively, the greatest number of predators and ciliates were seen in the bottle 3, which had the least amount of manipulated seawater added. This contradicts the findings of Rose *et al.* (2009), which were that at the end of the experiment, microzooplankton abundance was highest in the highest pCO₂ treatment. However, their experimental incubations were maintained for 2 weeks vs. the 3-day incubations used here. Thus, temporal shifts in species composition and dominance are likely.

Figure 3. A summary of the observed effects of adding 750 ppm CO₂ - manipulated seawater. The natural assemblage that was added to the sample bottles was dominated by smaller plankton, such as *Skeletonema* spp. At the end of the experiment, there were fewer predators such as ciliates (shown) which may have allowed increased plankton growth. The final plankton assemblage was dominated by large and/or pointy diatoms such as *Ditylum brightwellii* (top) and *Chaetoceros* spp. (bottom).

Figure 4. (Top) pH over time in the 750 ppm and 380 ppm treatments in the lab incubation experiments. Standard error bars included. (Middle) Specific growth rate μ (d⁻¹) of the predator *O. marina* as a function of the prey *I. galbana* concentration (cells mL⁻¹). (Bottom) Average specific growth rate of *O. marina* at *I. galbana* concentrations less than 50,000 cells mL⁻¹ and greater than 50,000 cells mL⁻¹.

To the best of our knowledge, while there have been Ecostat experiments that have investigated the effects of pCO₂ on plankton growth and grazing (Rose *et al.* 2009, Hare 2007), none have attempted to maintain cultures at constant pH. The pH did not remain as constant as expected (Figure 1 top panel). pH was expected to remain relatively constant due to the high flow rate counteracting the effects of photosynthesis and respiration on pH. One possible reason for the variation could be that in order to avoid collapsing the carboys that contained the 750 ppm manipulated seawater being pumped into the bottles, the tops had to remain loosened. This could have caused the manipulated water to re-equilibrate to the atmosphere. However, the seawater before manipulation had a pH of 7.89, which was lower than that of the manipulated seawater so it may have been caused by some other reason. In future experiments, self-collapsing carboys could be used to prevent some of these issues.

4.2 Lab Incubation experiments

In the 380 ppm and 750 ppm incubations of *O. marina* and *I. galbana*, it was found that increasing pCO₂ decreased and even led to negative growth rates of *O. marina* (Figure 4B). This does not support our hypothesis that elevated pCO₂ levels would increase predator growth rate. We expected that increasing pCO₂ would result in an increase in the prey *I. galbana* growth rate, which would in turn lead to faster growth of *O. marina*. This sensitivity of *O. marina* to relatively small variations in pH was surprising, as *O. marina* is well known as a hardy species (Montagnes *et al.* 2011).

Taking into account the increased plankton growth rate, species shift, and the decrease in grazer abundance at increased quantities of high pCO₂ manipulated seawater seen in the Ecostat experiment along with the decrease in predator growth rate seen in the *O. marina* lab incubations, we have arrived at a new hypothesis: the increase in phytoplankton growth rate seen in the Ecostat may have in part been caused by a decrease in predator growth rate as a result of increased pCO₂ (Figure 3). Perhaps pCO₂ has a stronger effect on predators, leading to top-down population shifts.

This experiment provides only preliminary results. There is still much work to be done to investigate the effects of pCO₂ on the organismal and ultimately ecosystem level. There is still so much that is unknown. A similar experimental setup could be used to investigate the role of pCO₂ on grazing by adding dilution experiments. As a further extension of our own experiment, ciliates and other predators could be counted to provide more quantitative evidence. In the future, the Ecostat could also be used to investigate the effects of combining stressors, such as increased pCO₂ and increased temperature (similar to Rose *et al.* (2009)) or pCO₂ and nutrient addition on the plankton of Narragansett Bay. Furthermore, nanophytoplankton could also be measured or observed, to provide even more information about trophic dynamics (Hare *et al.* 2007).

Anthropogenic carbon dioxide continues to be absorbed into the ocean, possibly influencing everything from plankton grazing rates to calcification. Phytoplankton account for over half of all photosynthetic activity on earth and comprise the base of the marine food chain. As such it

is important that we continue working toward understanding the impacts of human activity on sea surface chemistry.

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Marine Macroinvertebrates and Greenhouse Gas production: Effects of Anthropogenic Nutrient and Temperature Stressors on Coastal Filter Feeders

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Abstract. The current global warming situation has many of its origins in human activity. Anthropogenic stressors such as nutrient loading and pollution in Narragansett Bay, Rhode Island have been suggested to potentially cause coastal marshes to become sources rather than sinks of greenhouse gases (GHGs). Although nutrient-stressed macroinvertebrates have been seen to release GHGs in isolated laboratory settings due to incomplete denitrification by bacteria in their innards, their emissions have not often been studied in an environment closely mimicking their natural habitat. This project was conducted using subtidal blue mussels (*Mytilus edulis*) collected from the dock adjacent to University of Rhode Island, Bay Campus, which is low in background nutrient levels. The increased stress of nitrogen loading and higher temperatures were hypothesized result in a larger amount of GHG emissions due to the incomplete denitrification of ingested bacteria by the mussels. To test this hypothesis, the mussels were placed in glass mesocosms filled with sediment and raw seawater from Narragansett Bay, or raw seawater spiked with ammonium nitrate to achieve a target concentration of 100 μM . The mesocosms were maintained in temperature-controlled chambers at either 20 or 24°C, and then sealed and samples of the water and headspace gases were taken about every 3 hours over 10 hours, and analyzed for content of nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) using a gas chromatograph (GC). GHGs were also measured using a Picarro cavity-ring down spectrometer. Levels of N_2O increased over the sampling period, while levels of CH_4 remained constant. N_2O production rates were much higher at elevated temperatures, and slightly higher at elevated nutrient levels. This project will hopefully add to the knowledge of coastal GHG fluxes, allowing for the ability to further classify the GHG fluxes observed in coastal areas by Dr. Moseman-Valtierra, and potentially allow for future experiments to utilize the Picarro CRDS for biological gas production analysis.

1. Introduction

At the current stage of scientific knowledge, it is widely regarded that significant changes in atmospheric concentrations of greenhouse gases (GHGs) are responsible for a majority of issues surrounding global warming and the climate change crisis. Although GHGs are necessary for maintaining Earth's atmosphere and habitable climate, the increasing levels associated with post-industrial society are contributing to the trend of higher temperatures and the breakdown of the "ozone layer" (IPCC 2007). Although carbon dioxide (CO_2) is often the GHG given the most attention in popular media, nitrous oxide (N_2O) is in fact one of the most dangerous, with about 300 times the potential for global warming per molecule than CO_2 (radiative forcing), compared to methane (CH_4), which is about 25 times as dangerous in terms of radiative forcing (Forster *et al.* 2007, Ravishankara *et al.* 2009). Recent work done by Dr. Moseman-Valtierra suggests that anthropogenically-impacted coastal ecosystems such as the salt marshes found in Narragansett Bay, Rhode Island can become notable sources of GHGs, despite usually being observed as sinks of GHGs in normal conditions (Moseman-Valtierra *et al.* 2011). The reason for this anomaly is not fully understood at this stage; however it is thought that anthropogenic nutrient loading could be at fault, due to increased use of fertilizers and sewage disposal (Calabretta and Oviatt 2008). The nitrogen levels in Narragansett Bay are observed to exist in a gradient, with the highest levels being in the northern bay, and the lowest in the southern bay near the Graduate School of Oceanography (GSO) (Calabretta and Oviatt 2008;

Fulweiler *et al.* 2010; Berounsky and Nixon 1993; Nixon and Pilson 1983).

These elevated levels of nitrogen are relevant to this research because of their observed effects on the augmentation of GHG emissions in coastal ecosystems. Although this is a topic that has not been studied in great depth thus far, Dr. Moseman-Valtierra has been looking at the effects of nitrate addition to wetland ecosystems on the levels of N_2O fluxes from the area (Moseman-Valtierra *et al.* 2011). The reason behind these coastal ecosystems becoming sources rather than sinks of GHGs lies in the fact that these increased fluxes are able to offset between one-third and half of the rates of carbon sequestration in the areas (Moseman-Valtierra *et al.* 2011). Similar problems with increased N_2O fluxes have also been seen in other nutrient loaded coastal areas around the world (Barnes and Upstill-Goddard 2011, Zhang *et al.* 2010). Essentially, GHG fluxes are bound to increase given anthropogenic nutrient loading, and this increase in the emissions of harmful GHGs could become more dangerous as the threat of global warming looms.

Macroinvertebrates such as the blue mussel (*Mytilus edulis*) are known to be established core species in coastal marshes due to their large total biomass and rapid rates of secondary production (Kautsky and Evans 1987). Blue mussels are also filter feeders, which are known to be good indicators of ecosystem health, given that they ingest any particles present in the water column. Because of this, mussels ingest many microorganisms that are responsible for nitrogen-related processes such as nitrification and denitrification, which are the cause for most production of N_2O in these areas (Mosier *et al.* 1998). Denitrification, or the respiratory reduction of nitrate or nitrite to nitrogenous

gases (Heisterkamp *et al.* 2010) usually involves N_2O solely as an intermediate, but this process has been shown to prematurely stop before its complete reduction to N_2 gas (Zumft 1997). N_2O emissions by terrestrial and freshwater filter feeders have been shown to increase with nitrate concentration and temperature (Heisterkamp *et al.* 2010, Steif *et al.* 2009), and given the major roles of marine filter feeders such as *Mytilus edulis* in coastal ecosystems, there is a strong reason to examine how anthropogenic effects could influence the amount of GHGs they emit.

2. Materials and Methods

2.1. Sampling and Experimental Setup

Blue mussels (*Mytilus edulis*) were collected by SCUBA divers from subtidal areas around the dock at GSO for each trial. Collection occurred here because of its location in the lower end of the nutrient gradient in Narragansett Bay (Calabretta and Oviatt 2008), so the mussels would theoretically be healthier and more affected by exposure to higher nutrient levels and temperatures. Mussels were cleaned of all epiphytes such as limpets and barnacles. Mussels were then placed in 18 cm glass cube tanks, filled approximately 12 cm high with raw seawater to create a volume of about 2.6 liters, with the remaining top 6 cm of the cube being about 1.3 liters of air. Airstones were placed in each tank to ensure the mussels were exposed to enough oxygen, and the mussels were allowed to acclimate to their new environment overnight. Trials were initially done to establish a timeframe for the experiment in which gas production rates would vary at little as possible over time, and to identify methodological adjustments that needed to be made (data available upon request). For the experiment, half of the tanks were stored in a fixed-temperature chamber set to 20°C to create a water temperature of 19°C , the average summer temperature for Narragansett Bay, with the other half of the tanks being stored in a replicate chamber at 24°C to create a 23°C water temperature, an EPA average summer temperature estimate for the area given future GHG increases. Approximately 20 minutes before the first gas samples were taken the next morning, half of the tanks were spiked with ammonium nitrate to create an approximate concentration of $100\ \mu\text{M}$ in the tanks, according to what was determined to be a “high” value of nitrogen loading in Vadeboncoeur *et al.* (2010), and sealed to be airtight. Tanks were then left for 15 minutes with stir bars running before the first samples were taken. Four samples were taken over a 24 hour period, at times 0, 4 hours, 9 hours, and 24 hours, and sampling methods included taking water samples, gas samples of the headspace (the space between the water and the top of the tank), and real-time gas analysis done using a Picarro Cavity Ring Down Spectrometer (CRDS) (Model G2508, Santa Clara, CA: Picarro Inc.) which determines levels of N_2O , CH_4 and CO_2 in the headspace approximately every second. Water and headspace samples were taken using syringes, with water samples being equilibrated using helium to extract the gas from the water, following standard methods (Stainton 1973) and then both types of samples were analyzed using a gas chromatograph (GC) (GC-2014, Kyoto, Japan: Shimadzu Corp.).

Figure 1. Example of the final mesocosm design. Top for the tank was hand-cut from foam and the acrylic top was glued with silicone to the foam top. Hole were drilled to allow for the water input tube (back) and the water sampling tube (front, extends 4.75 inches into the tank from the top). Holes were also drilled for the gold bolts on the top, which are used for the Picarro CRDS input and output. Magnetic stir bar was suspended from the top using zipties that are silicone glued to the inside of the top, about 5 inches from the top in order to ensure proper mixing around the sampling port.

2.2. Data Analysis

For headspace gas and water samples analyzed using the GC, N_2O , CH_4 , and CO_2 concentrations were plotted over time to quantify the GHG production rate created by the mussels, although CO_2 data was not utilized in this analysis. In order to normalize the gas data, all mussels were weighed and measured for height, width, and length, then dissected into shell and wet tissue. This was done so that each component could be weighed and dried at 70°C for at least 48 hours, then weighed again, according to Chintala *et al.* (2006). However, normalization was simply done by individual for this project, with the size and weight data being left for future work with the data. Gas concentrations were analyzed from the CRDS-data (Picarro) using MATLAB (version 8.1.0.604, Natick, Massachusetts: The MathWorks Inc., 2013) to determine total average amount of gas produced over the time the analyzer was measuring the headspace of the tank. Frozen water samples from a few of the tanks were also thawed and analyzed for ammonium presence using methods explained in Solorzano (1969), which utilized several reagents and a photospectrometer to plot absorbance versus concentration.

A 2-factor ANOVA testing, with the two factors being temperature and nitrogen level, was used to test for differences in gas production rates in response to either warming or nitrogen amendments. Statistical analysis was done using the JMP software (JMP Version 10.0.0, Cary, NC: SAS Institute Inc., 2012). For tanks in the low temperature-low nutrient class, mesocosms 1-3 (Figure 3), were used for final data, with mesocosm 4 being excluded

Figure 2. Histogram of total production rate in picomoles per individual per hour for each of four treatments, calculated by subtracting concentrations at $t = 0$ hours ($t = 2$ hours for the warmer tanks) from concentrations at $t = 9$ hours. Concentrations were determined by taking samples of the mixed water column and equilibrating with helium to extract the gases in the water and analyze them with a gas chromatograph. The x axis labels are ordered so that the first number is the exposed temperature, and the second number followed by “N” is the approximate concentration in μM of nitrogen in the water. $F_{3,9} = 3.77$, $p = 0.05$.

because of a dead mussel and murky water uncertainties, as well as outliers. All data points were included for the low temperature-high nutrient class (Figure 3). For the high temperature-low nutrient class, mesocosms 9-11 (Figure 4) were used for final data, with mesocosm 12 being excluded because of a malfunctioning stirrer and outliers. For the high temperature-high nutrient class, mesocosms 13-15 (Figure 4) were used for final data, with mesocosm 16 being excluded because of extreme outliers. All raw data was examined in Microsoft Excel, and graphs were made using Microsoft Excel.

3. Results

Greater average N_2O production rates were observed in the tanks exposed to warmer conditions than the control temperature tanks (Figure 2). This difference between treatments was significant ($F_{3,9} = 3.77$, $p = 0.05$). Of the two manipulated factors, temperature had a stronger effect on the N_2O production rates (Figure 2, $p = 0.0085$) than nitrogen ($p = 0.6791$). These results were achieved by excluding the data from three tanks as outliers from the 16 tanks total, explained in greater detail in the discussion.

There was no significant interaction between the nutrient factor and the temperature factor ($p = 0.9960$).

As expected, the concentrations of dissolved N_2O in water samples were generally observed to increase as time passed, with the most extreme increases of the control-temperature tanks seen in the nutrient-spiked tanks (Figure 3). This trend was not as obvious in the warmer temperature tanks, as increases in the control nutrient tanks were comparable to those seen in nutrient-spiked tanks (Figure 4).

No CH_4 was observed dissolved in the water samples, although CH_4 data for the headspace air analyzed by GC displayed that CH_4 levels stayed relatively constant throughout the course of the experiment. GHG levels seen in headspace samples match those observed from real-time Picarro readings (data available upon request) and all three methods correlated in data trends seen for N_2O . Given the lack of evidence that methane was being influenced by invertebrates, further results and discussion will focus henceforth on the N_2O analyses.

4. Discussion

Figure 3. A scatterplot of the concentration of N_2O (pmol) versus the time (hours) for the 19°C control temperature-exposed tanks. Concentrations were determined by taking samples of the mixed water column and equilibrating with helium to extract the gases in the water and analyze them with a gas chromatograph. Points that are solid shapes (Mesocosms 1-3) are the tanks exposed to unaltered raw seawater as a control, and the points that are lines or dashes (Mesocosms 5-8) are the tanks exposed to an increased nitrogen load ($\sim 100 \mu M$).

Based on the statistically significant average production rate data, it does seem that temperature is the more significant contributor to N_2O production (Figure 2). This finding could potentially prove to be an important factor in future knowledge about global warming and marine ecosystems, given the positive feedback cycle that is created in this case. Since N_2O is a greenhouse gas that causes thinning of the ozone layer and in some manner, an increase in the global climate, if warmer temperatures cause the increased production of N_2O by mussels in these coastal environments, the N_2O will continue to cause the climate increase, which will cause further N_2O production, and so on. Since other factors of global warming can also be responsible for increased N_2O production, such as eutrophication and ocean acidification, it is crucial that the extent of temperature's effect on N_2O production is understood independently of other forms of N_2O production (Codispoti 2010).

A technical error could be at fault for increased values for the warmer experimental tanks however. The building containing the temperature chambers experienced an electrical malfunction, which caused the airstones in the warmer temperature chamber to shut off at some point between leaving them in during the afternoon and the following morning when the problem was discovered. This

may have caused the mussels to be exposed to unhealthy anoxic conditions for up to 14 hours before the experiment even started, and might have caused premature death and abnormal amounts of gas production during the course of the experiment. Since the electricity stayed on in the control temperature chamber and those mussels were exposed to oxic conditions for the entire incubation period, there is an element of uncertainty in those results. However, based on statistics from previous trials, where temperature was also seen to be more significant of a factor than nutrient loading, it is likely that the temperature does in fact have a more substantial effect on N_2O production than the excess nutrients do.

Looking at the changes in the dissolved N_2O concentrations over time, it does seem that by looking at just the tanks kept at a control temperature, that the addition of nitrogen caused the amount of N_2O produced over the first 9 hours to increase, as the tanks without a nitrogen addition (Figure 3; Mesocosm 1-3) tended to stay at a constant level of N_2O , while those with the nitrogen addition (Figure 3; Mesocosms 5-8) were seen to increase over the 9 hours, despite starting at a similar level as those without the nitrogen added (Figure 3). The data for the tanks in the warmer temperature chamber seemed to be much more variable, as is explained below, but the tanks without a

Figure 4. A scatterplot of the concentration of N_2O (pmol) versus the time (hours) for the 23°C elevated temperature-exposed tanks. Concentrations were determined by taking samples of the mixed water column and equilibrating with helium to extract the gases in the water and analyze them with a gas chromatograph. Points that are solid shapes (Mesocosms 9-11) are the tanks exposed to unaltered raw seawater as a control, and the points that are lines or dashes (Mesocosms 13-15) are the tanks exposed to an increased nitrogen load (~100 μM).

nitrogen addition (Figure 4; Mesocosms 9-11) did not stay as steady as they did in the control temperature chamber, which could most likely be attributed to temperature effects causing increased activity in terms of denitrification (Bachand and Horne 1999; Maag and Vinther 1996). The fact that the temperature had more of an effect on N_2O production than nutrients could be seen through the indiscernible differences in N_2O changes over time between those without the nitrogen addition and those that did (Figure 3; Mesocosms 13-15).

The absence of CH_4 in water samples, as well as the relatively stable CH_4 levels seen in Picarro and headspace sample analysis is expected, as methane production in marine environments is noted to be absent due to the methane producing methanogens being outcompeted in the ocean water. In areas such as coastal marshes, sulfate reduction is the dominant form of anaerobic decomposition (Fenchel and Blackburn 1979) and it has been shown that sulfate-reducing bacteria will outcompete methanogens for organic matter in estuarine areas (Oremland and Polcin 1982). In addition, nitrous oxide is known to be one of the more effective inhibitors of methanogenesis in salt marshes (Fenchel and Blackburn 1979).

In order to analyze use of the Picarro CRDS for future applications with macroinvertebrate GHG production, raw

data for GHG concentrations from the Picarro software were roughly compared to GHG concentrations received from GC data. The correlation of the headspace samples analyzed by GC and the levels seen by the Picarro readings indicate that both methods of data collection were functioning correctly, and for future experiments, this measure of accuracy does not need to be taken. Because the model of this Picarro CRDS is relatively new, allowing for analysis of N_2O in addition to CO_2 and CH_4 , the use of this instrument for an application such as this is something that has not been done yet, and could prove significant for further research on filter feeding organisms.

5. Conclusion

Blue mussels exposed to a combination of higher temperatures and elevated nitrogen levels are shown to produce higher levels of the GHG N_2O , with temperature having a larger effect than the nutrient load on the gas production. This increase is most likely due to rates of denitrification increasing with increased nitrogen presence, or more bacteria that incompletely denitrify nitrogen to N_2O , which occurs in overly oxic conditions (it is an obligate anaerobic process) or the bacteria are unhealthy

(Steif *et al.* 2009). This is relevant because of its addition to the knowledge in the currently under-researched field of coastal GHG fluxes, and because of it being the first to use a Picarro CRDS to take real-time gas measurements. In addition, with major wastewater treatment plants in Narragansett Bay beginning to upgrade to tertiary treatment and reduce the amount of nitrogen released to the bay in the summer of 2005, this data is important as a baseline for future research on the bay as nitrogen levels decrease over time (30-40% decrease with full implement) (Fulweiler *et al.* 2010).

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Physiological and Genetic Diversity in Nitrogen-Limited *Skeletonema* Species

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Abstract. Narragansett Bay is a dynamic estuary in which nutrient levels are constantly changing. Waste pollution is largely responsible for these shifts. Recently, efforts have been made to reduce the amount of sewage water and waste entering the bay. This decrease in runoff has resulted in lower nitrogen levels. While pollution reduction is an important step to protect marine ecosystems, phytoplankton rely on nitrogen to survive. *Skeletonema*, a key phytoplankton genus in Narragansett Bay, is one particularly abundant phytoplankton group that may be affected by this decrease in nitrogen. *Skeletonema* was previously thought to be a single species; however, it was recently discovered that *Skeletonema* is a complex comprised of several genetically distinct species. Six species of *Skeletonema* were grown in high, medium, and low nitrogen treatments to identify low-nitrogen tolerances of each species of *Skeletonema*. Nitrogen was shown to be a limiting factor for growth of every species. Significant differences in the growth rates of three different species in both high and low nitrogen treatments indicate an evolutionary tradeoff between rapid growth and ability to tolerate limited nutrients. Additionally, three types of genes related to the diatom nitrogen cycle (nitrate transporter, ferredoxin NADP reductase, and nitrate reductase ferredoxin-dependent fragment) were aligned to further explore interspecies variation in nitrogen assimilation. The phylogenetic trees produced from the alignments show that similar genes within each function evolved before the various species of *Skeletonema* diverged. We hope to further investigate the physiology and genetics of *Skeletonema* to identify correlations between nitrogen-limited growth rates and genetic relatedness. With a better understanding of inter-species differences in limited nitrogen tolerance, we will be more aware of the potential implications of a phytoplankton species shift in Narragansett Bay.

1. Introduction

Diatoms are tiny, eukaryotic, unicellular organisms also known as phytoplankton. They contain photosynthetic pigments that harvest energy from sunlight and carbon dioxide. Diatoms use silica to create their cell walls and other nutrients to support cell processes. They live in either benthic or open ocean environments, and are essential to all marine ecosystems (Round, et al. 1997). As the primary producers at the bottom of the food web, they are responsible for the wellbeing of virtually all marine life. *Skeletonema*, one type of diatom, is a genus of photosynthetic algae that is ecologically critical to marine ecosystems worldwide (Round, et al. 1997). *Skeletonema* was previously defined as a single species, but was recently discovered to be a species complex (Sarno, et al., 2007). There is much to learn about the different ways each species responds to environmental change.

Skeletonema species can differ both physiologically and genetically in their tolerances of limited nutrients. When nutrient levels vary in an ecosystem, a common concern is whether or not an evolutionary tradeoff exists within each species. For example, can fast growers in conditions of high nitrogen concentrations tolerate low nitrogen concentrations equally well? The answer to this ultimately lies in the concept of cell machinery. Some species may devote energy to the production of growth and reproductive organelles, which allow them to grow quickly. However, species that devote energy to the production of

chloroplasts may be more able to sustain populations even in nutrient limited environments (Arrigo, 2005). Furthermore, these evolutionary tradeoffs may be controlled by genetic differences among the species. If a genetic mutation has caused species to diverge, we suspect that it will also lead to differing physiologies. This study attempts to address both the physiologic and genetic aspects of *Skeletonema* species via a nitrogen-limited growth experiment as well as analysis of nitrogen cycle-related genetic sequences.

One nutrient that may vary within an ecosystem and affect phytoplankton growth is nitrogen. The diatom nitrogen cycle is responsible for metabolizing environmental nitrogen for cell use (Allen, et al. 2006). Environmental nitrogen can be found as ammonium, nitrate, or nitrite (Armbrust, et al. 2004). In the diatom nitrogen cycle, nitrate is reduced to nitrite, which is then reduced to ammonium (Armbrust, et al. 2004). Identifying the inter-specific differences between genes responsible for this nitrogen metabolism system can provide another perspective on limited nutrient tolerances among *Skeletonema* species. The genomes of *Skeletonema dohrnii*, *Skeletonema marinoi*, and *Skeletonema menzelii* have all been partially sequenced, so genes from these three species are analyzed for relatedness in this study.

Skeletonema response to limited nitrogen is especially relevant because nitrogen runoff from fertilizers and faulty wastewater treatment centers affects sensitive marine ecosystems in which *Skeletonema* is common. One such ecosystem is Narragansett Bay in Rhode Island, a

Table 1. Sources for the five *Skeletonema* cultures used in the nitrogen-limited experiment. Three of the five species were isolated from Narragansett Bay, while the other two are from Japan and Italy.

Culture Name	Species	Date of Origin	isolated from
CCMP1804	<i>S. grethae</i>	8/1/1995	Narragansett Bay
CCMP2506	<i>S. japonicum</i>	10/1/2001	Hiroshima Bay, Seto Inland Sea, Japan
Skel F	<i>S. marinoi</i>	11/22/2010	Narragansett Bay
Skel B	<i>S. dohrnii</i>	11/22/2010	Narragansett Bay
CCMP2800	<i>S. tropicum</i>	10/3/2002	Bay of Naples, Tyrrhenian Sea, Italy

thriving estuary that is home to a myriad of fish, shellfish and plankton (NBEP, 2013). Due to inefficient waste treatment and sewage leaks, Narragansett Bay has a history of high nitrogen levels (RI DEM, 1998 and NBEP, 2013). However, a sewage cleanup beginning in 2006 has alleviated much of the waste, resulting in less ambient nitrogen (NOAA, 2013). The current levels in the bay range from 0.6 μM to 11 μM , depending on the season (NBC Data Report 2013). Phytoplankton rely on nitrogen to survive, so it is possible that the altered nitrogen levels will impact phytoplankton growth in Narragansett Bay and other ecosystems with reduced environmental nitrogen.

This study examines the effects of nitrogen limitation on the growth rates and genes of different species of *Skeletonema*. We hypothesize that certain *Skeletonema* species will grow better in limited nitrogen while others grow better in replete nitrogen. These differences, coupled with a changing environment, could cause a shift in the timing, abundance, and nutritional content of the phytoplankton bloom. We also hypothesize that species with similar growth patterns will likewise have similar genes. Analyzing genes for inter-species relatedness is a step in determining how species of *Skeletonema* will respond to lower nitrogen levels. The growth and genetic makeup of *Skeletonema* can be used to make predictions about how phytoplankton communities will change in the future, both in Narragansett Bay and beyond.

2. Materials and Methods

Nitrogen-limited fluorometer experiment

This component focused on the growth of *Skeletonema grethae*, *Skeletonema marinoi*, *Skeletonema dohrnii*, *Skeletonema tropicum*, and *Skeletonema pseudocostatum* under varying levels of nitrogen. Some *Skeletonema* species have been found in Narragansett Bay, but because the discovery of the species complex is so recent, it is possible that even more *Skeletonema* currently reside in the bay (Kooistra, et al. 2008). The cultures came

from a well-maintained culture library (Table 1) and had been held in a constant state of exponential growth before being transferred to the experiment tubes to avoid a lag at the beginning of the growth curve. The five species were grown in three nitrogen treatments: 883 μM (high nitrogen), 10 μM (medium nitrogen), and 0 μM (low nitrogen). These amounts were chosen because 883 μM is the replete nitrogen standard for limited nutrient experiments, 10 μM reflects current nitrogen levels in Narragansett Bay, and 0 μM represents a worst-case scenario. All other required nutrients (trace metals, silica, and phosphorous) were held at normal levels and the cultures were grown in a medium of filtered Sargasso seawater. Each species and treatment was grown in triplicate.

The parameter used to track growth of the cultures was RFU (Relative Fluorescence Units), which quantifies the amount of fluorescing chlorophyll in the cultures. The amount of chlorophyll is correlated with the number of cells in the tube, and therefore related to the growth of the cultures. Cultures were projected to start at 0.5 RFU in 20 mL of filtered Sargasso seawater and continue growth until they reached stationary phase. After measuring the RFU (Relative Fluorescence Units) of each culture library sample three times using a Turner fluorometer, the appropriate amount of culture to transfer was determined using the equation $(\text{average RFU})(x \text{ mL}) = (0.5 \text{ RFU})(20 \text{ mL})$.

The cultures were grown in a shaking Percival incubator for 9 days until they reached stationary phase. The incubator light intensity was kept at 60 microeinsteins/ m^2/s and the temperature at 14 degrees Celsius. Additionally, the incubator was on a light/dark cycle to mimic outdoor conditions. Each culture was measured three times daily using the fluorometer. The cultures were briefly vortexed in an IKA vortex before measurements for homogeneity. RFU data were entered into Excel, and growth rate was calculated using the following equations (Fogg, 1987):

$$\mu = (\ln N_t - \ln N_0) / (t - t_0)$$

where μ is the specific growth rate, or the relative increase in biomass per hour, t_0 is 0 hours, t is x hours, N_0 is the RFU reading at 0 hours, and N_t is the RFU reading at x hours.

Figure 1. An example of a growth curve for *Skeletonema dohrnii*. All species were grown in high, medium and low nitrogen. Differing nitrogen treatments significantly affected growth. Lower nitrogen levels hinder growth of *Skeletonema*.

Figure 2. Five species of *Skeletonema* in three nitrogen treatments. *S. grethae* outperforms in every treatment. *S. marinoi* grows faster than *S. pseudocostatum* and *S. tropicum* in high nitrogen but slower than both in low nitrogen.

Additionally, 500 μL of culture were isolated and preserved with Lugol's solution when cultures were at their

maximum RFU. Cells were counted for each of the low-nitrogen treatments in triplicate using a 100 μL Palmer counting chamber. Cell counts were expanded to units of cells/mL. All data were subjected to a one-way ANOVA analysis to determine significance.

Analysis of nitrogen cycle-related genes

Genes of three different functions within the diatom nitrogen cycle were chosen for investigation. The first function, nitrate transporters, bring environmental nitrate directly into diatom cells (Hildebrand et al. 2001). The second type of gene analyzed in this study is ferredoxin NADP reductase, which is detected when nitrite reductase and glutamine synthase II are present. Nitrite reductase is responsible for reducing nitrite to ammonium in the diatom cycle of nitrogen metabolism (Crete et al. 2003). Glutamine synthase II converts ammonium to glutamate, which is the usable form of nitrogen for diatoms (Arakaki et al. 2000). Ferredoxin NADP reductase is therefore closely connected with the diatom nitrogen cycle. The third type of gene analyzed in this study is a nitrite reductase ferredoxin-dependent fragment. While it is involved with nitrite reduction in the nitrogen cycle, it is only a fragment of the nitrite reductase gene. Due to the incomplete nature of these genome sequences, only a fraction of existing genes are identified and available for alignment. Consequently, no quantitative inferences about gene expression can be made until entire *Skeletonema* genomes have been sequenced.

Figure 3. Cell counts for each species in low nitrogen. *S. grethae* and *S. dohrnii* are able to reach the highest cell abundance in low nitrogen, while *S. pseudocostatum*, *S. marinoi*, and *S. tropicum* do not grow to very high abundances in low nitrogen.

Partially-sequenced genomes of *Skeletonema dohrnii*, *Skeletonema marinoi*, and *Skeletonema menzelii* were obtained from the Moore dataset. Using the CLC Genomics Workbench 6, the nitrate transporter, ferredoxin-NADP reductase, and nitrite reductase ferredoxin-dependent fragment were first aligned within each species to find the longest possible coding sequences for each individual gene. Identical genes were eliminated. Two genes involved with nitrate transport and ferredoxin NADP reduction were found in each species, while one nitrite reductase gene was found in each species. After these specific genes were identified, they were aligned intra-specifically to determine the relatedness among the three sequenced *Skeletonema* species. In cases where there is more than one gene of a specific function, one of two possibilities can occur. Multiple genes of similar function can be homologs, in which the genes are separated by the event of speciation and more similar inter-specifically, or they can be paralogs, in which there are duplicated genes within a genome that are more similar intra-specifically. The three genes were analyzed and phylogenetic trees were produced to determine relatedness and identify whether each gene is a homolog or paralog.

3. Results and Discussion

Differences in growth rate among species and nitrogen treatments

Within each of the five species (*Skeletonema grethae*, *Skeletonema marinoi*, *Skeletonema dohrnii*, *Skeletonema tropicum*, and *Skeletonema pseudocostatum*), there were significant differences among the three nitrogen treatments ($p < 0.05$). The results were consistent for each treatment. Cultures reached their highest RFU values in the high nitrogen concentrations, moderate RFU values in the medium nitrogen concentrations, and lowest RFU values in the low nitrogen concentrations (Figure 1). Growth rates were significantly different among the species within the high and low nitrogen treatments ($p < 0.05$), but there was no significant difference within the medium nitrogen treatment ($p = 0.14$) (Figure 2). *Skeletonema grethae* showed the highest growth rates in each nitrogen treatment, with *Skeletonema dohrnii* a close second. *Skeletonema marinoi* outperformed both *Skeletonema pseudocostatum* and *Skeletonema tropicum* in the high nitrogen treatment, but grew slower than both in low nitrogen. *Skeletonema pseudocostatum* and *Skeletonema tropicum* had the lowest growth rates in the high nitrogen treatment, but fared slightly better in the low nitrogen treatment than *Skeletonema marinoi*. Because of the lack of statistical software available, the one-sided ANOVA tests performed can only detect statistical differences in whole sets of data, not minute differences between certain species.

Maximum cell abundance was counted for all cultures in the low nitrogen treatment and there were significant differences among the species ($p < 0.05$). *Skeletonema grethae* and *Skeletonema dohrnii* reached the highest cell abundance in low nitrogen, while *Skeletonema marinoi*, *Skeletonema pseudocostatum*, and *Skeletonema tropicum* maintained relatively low cell abundances (Figure 3). *Skeletonema dohrnii* and *Skeletonema grethae* reached

cell abundances more than 10 times those of the other three species.

The finding that *Skeletonema marinoi* outperforms *Skeletonema pseudocostatum* and *Skeletonema tropicum* in high nitrogen and vice versa in low nitrogen hints at an evolutionary tradeoff. Since no single organism can win at every evolutionary game, some species devote more cellular machinery to reproduction, and others to limited nutrient survival. The ecological implications of this finding could involve a species shift. For example, the recent and drastic nitrogen drop in Narragansett Bay could cause a species shift from *Skeletonema marinoi* to *Skeletonema pseudocostatum*. More species were not used in this study because their identities have not yet been confirmed through PCR in the lab; further growth experiments might include more species of *Skeletonema* and more nitrogen treatments to more closely track these physiological differences amongst *Skeletonema* species.

A genetic perspective of nitrogen utilization in Skeletonema

The genetics component of this study revealed the relatedness of nitrate transporters, ferredoxin NADP reductases, and ferredoxin-dependent nitrite reductase in three *Skeletonema* species. A phylogenetic tree constructed of the nitrate transporters used in this study revealed they are homologs (Figure 4). Therefore, there are two types of

Figure 4. Phylogenetic tree of nitrate transporters. Two types of nitrate transporters were found in each species of *Skeletonema*. Nitrate transporters are more similar across species than within species of *Skeletonema*. *S.costatum* and *T.pseudonana* were included for comparison.

nitrate transporters found in each sequenced species of *Skeletonema* that are more similar to each other than to the other nitrate transporter in their respective genomes. Additionally, the nitrate transporters in *Skeletonema marinoi* and *Skeletonema dohrnii* are more related to each other than they are to *Skeletonema menzelii*.

Alignments of the ferredoxin NADP reductases revealed they are also homologs within these three species. There are two types of ferredoxin NADP reductases that are more similar inter-specifically than they are to the other ferredoxin NADP reductase in their own genomes. A pairwise comparison of the ferredoxin NADP reductases also showed that in one type of the gene, *Skeletonema marinoi* and *Skeletonema dohrnii* are almost identical. This is apparent in a phylogenetic tree constructed with the *Skeletonema* NADP ferredoxin reductases and those of *Thalassiosira pseudonana* for comparison (Figure 5). There was only one nitrite reductase ferredoxin-dependent fragment found in each *Skeletonema* species, eliminating the question of homologs and paralogs. However, the nitrite reductase ferredoxin-dependent fragment reflects the same phylogeny as shown in the other two trees (Figure 6).

The genetic component of this study reveals that both the nitrate transporters and ferredoxin NADP reductases are homologs, meaning that the two genes within each function are more related to their interspecific counterparts in other *Skeletonema* species than they are to each other. These genes duplicated and began growing apart before the speciation of *Skeletonema dohrnii*, *Skeletonema marinoi* and *Skeletonema menzelii* occurred. Additionally, in all three genes examined, *Skeletonema dohrnii* and *Skeletonema marinoi* were more related to each other than to *Skeletonema menzelii*. It is possible that future growth experiments based on this finding could confirm or reject a

Figure 5. Phylogenetic tree of NADP ferredoxin-dependent reductase. Each type was also shown to be more similar across species of *Skeletonema* than within their genomes. The NADP ferredoxin-dependent reductase for *T.pseudonana* is more related to the type on the left than on the right.

Figure 6. Nitrite reductase ferredoxin-dependent fragment for *Skeletonema* species reinforces the phylogeny shown in the other two nitrogen-related genes.

relationship between genetic relatedness and growth patterns in limited nitrogen. *Thalassiosira pseudonana*, a diatom outside of the *Skeletonema* genus, had the same types of genes as *Skeletonema* species, but the genes were more related to each other than to similar genes in *Skeletonema*, which makes them paralogs.

4. Conclusion

Evidently, nitrogen limitation negatively affects the growth rates of *Skeletonema*. While *Skeletonema grethae* is the fastest grower in every nitrogen treatment, there are some evolutionary tradeoffs among the other species of *Skeletonema*. *Skeletonema marinoi* outperforms *Skeletonema pseudocostatum* and *Skeletonema tropicum* in high nitrogen, but the opposite is true in low nitrogen. Because this tradeoff exists, changes in nitrogen levels in ecosystems such as Narragansett Bay may result in a species shift in *Skeletonema*. Phytoplankton are essential to food webs throughout the ocean, and a *Skeletonema* species shift would undoubtedly affect higher trophic levels. For example, a study in Laizhou Bay found that a decrease in phytoplankton biomass resulted in smaller-sized pelagic fishes (Jin et al. 2013). Another study in the Adriatic Sea suggested that a decrease in phytoplankton biomass caused a fish species shift (Giani et al. 2013). In addition, once more *Skeletonema* genomes are sequenced, further analysis of genes connected to the diatom nitrogen cycle may reveal a stronger link between genetic relatedness and physiological similarities amongst the various species of *Skeletonema*.

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A coordinated increase in export production and denitrification on the Costa Rica margin during the early Pleistocene

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Abstract. The oceanic carbon, nitrogen, and oxygen cycles are linked through export production, oxygen consumption, and denitrification. Denitrification, the bacterial reduction of nitrate, is an anoxic process that serves as the primary sink for fixed nitrogen in the ocean. The Eastern Tropical Pacific (ETP) houses an oxygen minimum zone (OMZ) where pelagic denitrification proceeds as a result of both large scale circulation and regionally high export of organic matter that fuels oxygen consumption in the subsurface. Here we present a 2.4 million year long record of denitrification and productivity in the ETP using ODP Site 1242. Site 1242 is located ~70 km off the west coast of Costa Rica, beneath the OMZ. Downcore records of bulk sedimentary nitrogen isotope values ($\delta^{15}\text{N}$) and opal and organic matter accumulation rates show near synchronous increases in $\delta^{15}\text{N}$ values and biogenic sediment accumulation at approximately 1.6 My. The concurrent increase in the denitrification and productivity proxies suggests that regional oxygen demand could be driving the increase in denitrification observed at Site 1242. This is in contrast to what is observed elsewhere, where export productivity and denitrification changes do not appear to be coupled locally. Moreover, the timing of this increase is distinct from a previously observed increase in $\delta^{15}\text{N}$ at California Margin Site 1012 which occurred at ~2.1 Ma (Liu et al., *G-cubed*, 2008) and is also attributed to enhanced denitrification in the OMZ. These differences suggest that the Site 1242 record reflects a purely local or regional process. Interestingly, the later increase in denitrification at 1.6 Ma observed at Site 1242 does not appear to be restricted to the ETP, but rather shows similar timing to a $\delta^{15}\text{N}$ shift in a site from the Arabian Sea OMZ (Muzuka et al., *Proceedings of the ODP*, 1991). The new data presented here will help in the development of a mechanistic understanding of relative roles of local and regional processes over global controls on intermediate water OMZ intensity.

1. Introduction

Nitrogen is an essential nutrient to life processes; however, most nitrogen is unavailable until it has been fixed by microbes in the process of nitrogen fixation. The oceanic inventory of fixed nitrogen is controlled by nitrogen fixation (source) and denitrification (sink). Denitrification is the anoxic process where bacteria reduce biologically available nitrate to the largely unavailable form N_2 . Studying the extent and variability of denitrification is essential to understanding the carbon, oxygen and nitrogen cycles as they are linked through export productivity, oxygen consumption and denitrification.

The Eastern Tropical Pacific (ETP) has a large oxygen minimum zone (OMZ) in subsurface waters that houses two large regions of denitrification which are responsible for nearly a quarter of fixed nitrogen loss from the water column. The ETP is located between 23.5°N and 23.5°S and between 140°W and the American landmass. It houses equatorial and coastal upwelling systems and accounts for 10% of the global ocean's productivity (Robinson et al., 2009). The confluence of productivity, oxygen demands and denitrification makes studying the ETP essential to gaining an understanding of denitrification.

This study sought to characterize changes in denitrification over the last 2.4 Ma in the ETP from ODP Site 1242. Site 1242 is located at 7°51.352'N, 83°36.418'W on the southern edge of the northern OMZ (Fig. 1). The composite length of the cores was 287.74 m. Site 1242 contained two lithological units that are separated by a ten million year hiatus. Only Unit 1 was studied. Upwelled water is the dominant source of nitrate to the surface above Site 1242. Annually, all the nitrate is biologically consumed so that the isotopic signature of

marine organic matter (as $\delta^{15}\text{N}$ where $\delta^{15}\text{N} = ((^{15}\text{N}/^{14}\text{N}_{\text{sample}} / ^{15}\text{N}/^{14}\text{N}_{\text{standard}}) - 1) \times 1000$ and the standard is atmospheric N_2) exported from the surface is equal to the nitrogen isotopic signature of the upwelled water. Denitrification occurs at intermediate depths in water column in the OMZ and its isotopic signature is imparted on the upwelled water. The isotopic signature is transferred to organic matter which is subsequently buried on the seafloor. In denitrification, and most other processes, ^{14}N is kinetically favored over ^{15}N during reaction, leaving the nitrate pool enriched in ^{15}N and creating an N_2 product with proportionally more ^{14}N . As nitrate is being reduced by denitrification, the $\delta^{15}\text{N}$ value of the nitrate pool increases. Water column denitrification has a strong isotope effect (19-29‰) that is imparted on the environment as the reaction greatly prefers the lighter ^{14}N (Sigman and Casciotti, 2001). This elevated signature is incorporated into organic matter and subsequently buried on the seafloor.

In a study focusing on the last deglaciation (last 30 ka) (Martinez and Robinson, 2010), denitrification at Site 1242 increased abruptly during the last deglaciation around 19 ka B.P. Export production was also at a maximum, thus increasing sub-surface oxygen demand and an intensification of water column denitrification. The proposed triggering mechanism for the increase in denitrification was an increased amount of preformed nutrients feeding the Equatorial Undercurrent (EUC) with the resumption of overturning in the Southern Ocean. The EUC is the water mass source of ETP suboxic zones as well as upwelled waters. Increased upwelling would have amplified the amount of nutrients at the sea surface (Martinez and Robinson, 2010).

This pattern has been observed in the three major OMZs over the last glacial cycle (Liu et al., 2008; Martinez and Robinson, 2010; Muzuka et al., 1991) and beyond. The

Figure 1. Sub-surface dissolved oxygen concentrations at 250 m water depth and surface nitrate concentrations. ODP Site 1242 is marked with a black dot.

longest record is 4 million years in length (Liu et al., 2008). This record shows the same glacial-interglacial pattern described at 1242, with higher denitrification during warm periods. It also shows an increase in $\delta^{15}\text{N}$ that is interpreted as intensified denitrification starting at 2.1 Ma in the Eastern Tropical North Pacific (ETNP). The suggested oceanic changes for this intensification are the shoaling of the thermocline and the development of a modern front system in high latitudes. By extending the Site 1242 record to 2.4 Ma, we can improve our understanding of how denitrification changed over the Pleistocene as the world cooled. The denitrification record at Site 1242 shows an increase in denitrification at 1.6 Ma but not at 2.1 Ma.

2. Materials and Methods

Nitrogen isotope values ($\delta^{15}\text{N}$, ‰) and total nitrogen (TN, as wt. %) were measured from dried, ground bulk sediment via combustion analysis with an elemental analyzer in line with an isotope ratio mass spectrometer at URI. The $\delta^{15}\text{N}$ generated by the EA were standardized using an in-house aminocaproic acid (ACA) standard and IAEA standards N1 and N2. The overall precision of the measurements was $<0.3\text{‰}$.

The opal content (hydrated biogenic silica) of sediment samples from ODP Site 1242 was measured by UV/vis spectrophotometry following a modified version of Mortlock and Froelich (1989). Sediment samples are first weighed out in 50 mL falcon tubes, and extracted in 40 mL 0.5 N NaOH at 80°C for 5 hours. Once solubilized, the silica is complexed with ammonium molybdate and subsequently reduced by metol. The reduced molybdate complex develops a deep blue color in proportion to the amount of silica in the sediment. The absorption is measured at 812 nm detection on the UV/vis spectrophotometer and silica content is standardized by a dilution scheme of from 0-8 mM [Si]. The silica

concentrations are then converted to opal content following Mortlock and Froelich (1989). Precision was better than 10% for all samples and better than 5% for samples with concentrations >10 weight percent opal.

Sediment opal content, expressed as weight percentage, as a proxy for diatom productivity is subject to uncertainties concerning varying dilution by carbonate and terrigenous fractions. It is for these reasons the mass accumulation rate (MAR) of a sedimentary component is developed for time-series analysis. The opal MAR is defined as the opal percentage multiplied by the dry bulk density (g/cm^3) and the linear sedimentation rate (cm/ky). The resulting units of opal MAR are ($\text{g/cm}^2/\text{ky}$), and reflect a mass flux to the seafloor. Similarly for nitrogen content, dilution is a concern. The nitrogen MAR is calculated from N% multiplied by the sedimentation rate and dry bulk density to find the mass flux over time ($\text{g/cm}^2/\text{ky}$).

The age control for this study was based on biostratigraphic age ties from calcareous nannofossil provided by the Ocean Drilling Program. Due to the limited number of biostratigraphic age ties (13 ages for a span of 2.4 Ma), the age model for this record is poorly constrained.

3. Results and Discussion

At Site 1242, the denitrification and total nitrogen MAR records reveal an increase starting ~ 1.6 Ma. Before 1.6 Ma, the baseline for both proxies was on average lower than the values after 1.6 Ma. From ~ 1.6 Ma to 1.2 Ma, TN MAR experiences a transient peak in productivity before returning to a lower level of productivity that was still higher than previous 1.6 Ma values. The denitrification record does not show the same transient peak during this time interval, but a higher average value with larger amplitudes that persists to the present. The baseline change for $\delta^{15}\text{N}$ was roughly 1-2‰. Opal MAR is more variable and experiences several transient peaks around ~ 1.6 Ma, ~ 1.3 Ma to ~ 1.5 Ma and

Figure 2. TN (top) and opal (middle) MAR and bulk sediment $\delta^{15}\text{N}$ (bottom) plotted versus age in ODP Site 1242.

~0.5 Ma to ~1.2 Ma. On the long term, Opal MAR and the denitrification record appear to track with each other such that denitrification was more intense when opal production was higher (Fig. 2).

Since the productivity at Site 1242 is limited by surface nitrate, the likely explanation for increased productivity is enhanced upwelling near or at the site, either as a result of greater volumes of nutrient rich water being upwelled or an increase in the nutrient content of the upwelled water. Regional SST records reveal a major cooling from ~1.8 to

~1.2 Ma (Martínez-García et al., 2010) with the development and expansion of the cold tongue off Peru. The expansion of the cold tongue indicates that the thermocline shoaled, causing the intersection of the colder, older water was at the surface. This may have increased the nutrient content of the upwelled water. The low latitude cooling was also accompanied by greater cooling at high latitudes, which intensified the pole-to-equator temperature gradient. Enhancing the gradient would also cause an intensification of wind driven upwelling along North America. The pattern of productivity change observed at Site 1242 is similar to what is seen in California margin site 1012, consistent with a regional intensification of upwelling. On the other hand, the productivity pattern at the two upwelling sites is quite different from what is seen in the eastern Equatorial Pacific (Lawrence et al., 2005; Etourneau et al., 2013), implying that a regional change in nutrient content is not driving productivity changes.

The concurrent increase in productivity and denitrification suggests a local event may have triggered the increase in denitrification. Increased biological export would increase the amount of respiration in the water column and oxygen consumption. The increase in respiration in turn would lead to decreased oxygen in the water column. The subsequent demand for nitrate as a terminal electron acceptor in the OMZ would increase denitrification. Since opal MAR and denitrification track together, denitrification above Site 1242 may be controlled locally by productivity. Denitrification occurs when oxygen concentrations are less than 5–10 $\mu\text{mol/L}$; Site 1242 has an average of 5–6 $\mu\text{mol/L}$ of O_2 in the subsurface at ~150–200 m, just below the threshold concentrations. In addition, it sits on the southward edge of the OMZ. It may be that O_2 concentrations before ~1.6 Ma were too high for denitrification to occur over this site. The intensification of wind driven upwelling at 1.6 Ma may have expanded the OMZ enough to include this locale.

The other denitrification record in the region that spans the Pleistocene and the late Pliocene is from ODP Site 1012 located upstream from the OMZ along the California

Figure 3. ODP Site 1242 $\delta^{15}\text{N}$ (top) and ODP Site 1012 $\delta^{15}\text{N}$ (bottom) plotted versus age.

margin (Liu et al., 2008). Nitrate bearing the high $\delta^{15}\text{N}$ signature of denitrification is carried northward in the California Undercurrent and upwelled near Site 1012. The result is a long term integrated record of denitrification in the ETP. The $\delta^{15}\text{N}$ records from Site 1242 and 1012 do not reveal similar patterns of denitrification (Fig. 3). At Site 1012, denitrification experiences a rapid increase at ~ 2.1 Ma. While there exists a small increase in denitrification at ~ 2.1 Ma at Site 1242, the most significant change in the denitrification record occurs at ~ 1.6 Ma. The long term records of denitrification for the sites are not similar until the latest one million years. From ~ 1 Ma to present, the denitrification records start to covary with each other in cyclic patterns that reflect a larger global phenomenon in climate change. Before ~ 1 Ma the denitrification records for Sites 1012 and 1242 are out of phase with each other; arguably there appears to be antiphasing from ~ 1 Ma to 2 Ma. Peaks in one record matches with troughs in the other.

The growth of the OMZ as the global climate cooled may have influenced the denitrification signal received at Site 1012. Site 1012 is located on the northern edge of the OMZ (downstream), while Site 1242 is on the southern edge (upstream). The OMZ could have experienced intensified denitrification around 2.1 Ma which Site 1012 would have captured. Site 1242, being upstream of this signal, would not record this period of intensified denitrification. As the OMZ continued to develop and expand, Site 1242 could have begun to record changes. Around 1 Ma, the denitrification records begin to reflect similar trends; possibly a regional signal is recorded with local controls losing influence.

4. Conclusion

At Site 1242, there is a change at ~ 1.6 Ma in the denitrification and TN MAR record. Baseline averages of denitrification and TN before 1.6 Ma were lower than baseline averages of $\delta^{15}\text{N}$ values after ~ 1.6 Ma. TN and denitrification did not experience the same level of increase. TN productivity experienced a peak from ~ 1.2 Ma to ~ 1.6 Ma, while denitrification slowly increased in value. Opal MAR, on the long term, tracked with denitrification changes. Intensified productivity in opal MAR occurred near simultaneously with intensified denitrification values.

Concurrent increases in denitrification and productivity seen at Site 1242 suggest increased productivity drove denitrification to intensify around ~ 1.6 Ma. From the denitrification record, and comparisons with productivity, denitrification responds to local productivity at 1.6 Ma. As

export productivity increased, the oxygen demand led to an intensification of water column denitrification. This increase in denitrification is recorded in the $\delta^{15}\text{N}$ record. Enhanced upwelling could be the cause for the increase in productivity.

The ODP Site 1012, downstream of Site 1242, experiences intensified denitrification at 2.1 Ma. However, the denitrification records appear to covary after 1 Ma. The difference in Site 1242 and 1012 could be attributed to the development of the OMZ in the early Pleistocene. The OMZ could have experienced denitrification downstream of Site 1242 that was recorded at Site 1012 and slowly expanded to encompass Site 1242 ~ 1 Ma.

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