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

at

**THE UNIVERSITY OF RHODE ISLAND
Graduate School of Oceanography
and
Department of Ocean Engineering
NARRAGANSETT, RHODE ISLAND**

June – August 2012

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GSO Technical Report No. 11-03

PARTICIPANTS IN THE 2012 SUMMER UNDERGRADUATE RESEARCH FELLOWSHIP PROGRAM IN OCEANOGRAPHY

FELLOWS

Cindy Cesar	Rhode Island College
Lydia Curliss	Oberlin College
Margo Davis	Colorado College
Nikiforos Delatolas	Cornell University
Kayla Flynn	Lyndon State College
Josephine Fong	University of California at Berkeley
Maureen Haynes	Manhattan College
Rachel Hickcox	Worcester Polytechnic Institute
Michelle Jenssen	University of Massachusetts Amherst
Samantha Maness	Pfeiffer University
Jonathan Rempel	Binghamton University
Julie Warner	American University

ADVISORS

David Borkman
Dreux Chappell
Gang Chen
Peter Cornillon
Jason Dahl
Tetsu Hara
Bethany Jenkins
Rainer Lohmann
Brice Loose
Susanne Menden-Deuer
John Merrill
Robert Pockalny
Jan Rines
Lewis Rothstein
Tatiana Rynearson
Ted Smayda

PROGRAM ASSISTANTS

Annie Foppert & Kim Carey

PREFACE

This report presents the papers written by the nine participants in the 2012 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 28th 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 2012 program will continued excellence beyond the official duration of the program with at least three projects presented at the AGU Fall 2012 and ASLO Spring 2013 meetings.

The participants in the 2012 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-0851794 and 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 Annie Foppert who served as a graduate coordinator for the program.

Kathleen A. Donohue
David Smith
SURFO Site Directors

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Identification of prey species in *Acartia tonsa* gut contents in Narragansett Bay, RI

Cindy Cesar, Edward Durbin, Alison Cleary

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. *Acartia tonsa* is the dominant copepod in Narragansett Bay, RI during the summer, and serves as an important source of nutrients and carbon for species of upper trophic levels. *A. tonsa* has a diverse diet ingesting different species of phytoplankton and microzooplankton. During the summer of 2012, the types of plankton species *A. tonsa* consume *in situ*, as well as the abundance of these prey species in the environment were analyzed using molecular techniques. Samples of adult female *A. tonsa* were collected weekly from early-June to late-July, their DNA were extracted, Peptide Nucleic Acid mediated Polymerase Chain Reaction (PNA-PCR) was used to amplify prey DNA, and clone libraries were produced and sequenced. The *A. tonsa*-specific PNA-PCR was used to exclude predator DNA from being amplified in excess. Samples from June 25th, 2012 and on July 2nd 2012 were the only samples sequenced. Sequence results for June 25th, 2012 showed copepod species' DNA including *Centropages* species, *Oithona* species, and *Temora turbinata*. The abundance of these copepods' DNA found in the sequences as opposed to phytoplankton have been attributed to picking errors made when collecting *A. tonsa* from Narragansett Bay plankton tow samples, and the possible ingestion of copepod nauplii. Sequences from July 2nd, 2012 contained double peaks indicating contamination in PCR steps involving clones. When these samples were sequenced once more, other species of copepods and the dinoflagellate *Prorocentrum minimum* were apparent. Though none of the sequences analyzed showed an abundance of prey phytoplankton ingested by *A. tonsa*, these findings helped to provide ideas on how to improve methods of identifying prey species in the guts of zooplankton.

1. Introduction.

Acartia tonsa are the dominant calanoid copepods of Narragansett Bay during the summer season (Durbin & Durbin 1981). They play a vital role in carbon cycling within marine ecosystems as they serve as a trophic link between phytoplankton and higher trophic level organisms including fish and sea birds. *A. tonsa* gain their nutrients from phytoplankton, and are in turn consumed by large predators that gain their energy from the copepod (Durbin & Durbin 1981). The feeding behaviors of copepods can affect the composition of phytoplankton in marine systems (Koehl & Strickler 1981). *A. tonsa* have been observed to consume the *Thalassiosira weissflogii* and *Heterocapsa triquetra* (Durbin, Casas, & Rynearson 2012).

It has been shown that copepods such as the *A. tonsa* are selective feeders, but that they do exhibit adaptability to changes in their environments in regards to their feeding habits (Koehl & Strickler 1981). There are studies concerning the feeding selection habits exhibited by copepods that show how selective they can be as predators (e.g. Poulet 1974; Kjørboe et al. 1996; Cowles 1979; Skiver 1980). Though this is so, studies suggest that some *Acartia* species exhibit non-selective feeding habits when placed with *Alexandrium* species, dinoflagellates unpalatable to zooplankton grazers (Teegarden et al. 2001). Light has been shed on their omnivorous

nature (e.g. Lonsdale et al. 1979; Gifford & Dagg 1988) also supporting their non-selective feeding habits. Discovering what *A. tonsa* consume as well as their feeding selectivity will help oceanographers better understand the movement of nutrients and carbon from phytoplankton to copepods to higher trophic level predators in marine systems.

In this study, the types of plankton species that *A. tonsa* consume *in situ*, as well as the abundance of these prey species in Narragansett Bay was determined using molecular techniques including *Acartia*-specific Peptide Nucleic Acid mediated Polymerase Chain Reaction (PNA-PCR). This procedure amplifies prey DNA found in the gut contents of the *A. tonsa* while blocking the excess amplification of predator DNA. A PNA probe was designed to recognize the 18S gene of the *A. tonsa* in order to block polymerase elongation thus creating the inhibition of its DNA replication (Ørum 2000).

A. tonsa gut contents revealing the copepod's prey species were sequenced and compared to plankton identified in their natural environment in order to look at the feeding selectivity that is exhibited by the *A. tonsa*. The results gathered from this study helped with the development of ideas on how to improve methods of identifying prey species in the guts of zooplankton such as the *A. tonsa*, while also providing insight to possible feeding habits exhibited by this copepod.

2. Experimental Setup.

2.1 Zooplankton sampling from Narragansett Bay

Acartia tonsa were collected on a weekly basis by plankton vertical tows in Narragansett Bay, Rhode Island from mid-June to late-July. At Station 2 (Figure 1) a 200 μ m mesh net was used to collect live samples from the bay. Two tows were performed on the boat to obtain live and preserved samples. Collecting preserved samples saved what the copepods consumed in the last several minutes before they were caught. Samples meant for preservation were filtered through a 40 μ m seive with 95% ethanol. Both samples were brought back to the lab to be surveyed for *A. tonsa*.

Figure 1. Station 2 in Narragansett Bay where plankton tows for *A. tonsa* took place.

2.2 Surveying Plankton Tows for *A. tonsa*: June 8th, 2012 & June 18th, 2012

Zooplankton were collected from Narragansett Bay using the above protocol (Section 2.1). Upon return to the laboratory, live samples were surveyed and the dominant copepod was identified to be the *Acartia hudsonica*. When preserved samples were surveyed, the same conclusion was established, and both samples were subsequently discarded.

2.3 Surveying Plankton Tows for *A. tonsa*: June 25th, 2012 – July 23rd, 2012

Zooplankton were collected from Narragansett Bay using above protocol (Section 2.1); dates and surface temperatures can be found in Table 1. Live and preserved samples were surveyed and the dominant copepods were identified as *A. tonsa*. From the preserved samples, three groups of 20 female adult *A. tonsa* were picked and stored in 95% ethanol in preparation for DNA extraction. Dates that samples were collected from Narragansett Bay, surface temperatures, when *A. tonsa* were picked, and when DNA extraction took place can be found in Table 1.

3. Methods.

3.1 DNA Extraction and Amplification

DNA extraction was performed on samples following a DNeasy Blood and Tissue Kit spin-column protocol (Qiagen). After obtaining the DNA from the samples, *A. tonsa*- specific Peptide Nucleic Acid mediated Polymerase Chain Reaction (PNA-PCR) (Ørum 2000) was performed. Each PNA-PCR reaction contained 1X GoTaq Green Master Mix (Promega), 0.5 μ M each of Universal 18S-550 forward and reverse primers (PrimerQuest), 20 μ M PNA, 1 μ l water, and 1 μ l DNA template. Krill *Meganyctiphanes norvegica* 13 eyes 10⁻² served as the positive control and water was the negative control. The PNA was warmed up at 65°C for 1 minute before being added to each solution (positive and negative controls did not receive PNA). Samples underwent a thermocycling regime (Table 2) that would perform the initialization, denaturation, PNA annealing, primer annealing and elongation processes of PNA-PCR. 5 μ l of each PCR sample and All Purpose Hi-Lo DNA Marker (Bionexus) were added to a 0.8% electrophoresis gel to undergo electrophoresis. Each gel was run for about 30 minutes at 75 Volts. PNA-PCR samples were then purified by method of ethanol precipitation (Qiagen), and DNA amplicons of each were quantified spectrophotometrically using NanoDrop 1000 software.

3.2 Cloning

Ligation step of the cloning of the prey species' DNA took place following the pGem-T Easy Vector system (Promega). The PCR product (3 μ l), 1X rapid ligation buffer, pGem[®]-T easy vector (1 μ l), and T4 DNA Ligase (1 μ l) are mixed to prepare recombinant DNA that will be ready to be introduced to the host bacteria *Escherichia coli* JM109 High Efficiency Competent Cells (Promega). Transformation was performed following pGem-T Easy Vector system procedure outline by manufacturers (Promega). Luria-Bertani broth (LB) plates with ampicillin, isopropyl β -D-1-thiogalactopyranoside (IPTG), and 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Gal) were made, transformants were spread across plates,

Plankton Tow Dates	Surface Temperatures (°C)	<i>A. tonsa</i> Survey	DNA Extraction
June 25	19.88	June 25	June 26 - June 27
July 2	20.5	July 2	July 2- July 3
July 16	22.0	July 19	July 23 - July 24
July 23	20.0	July 23	July 23 - July 24

Table 1. Plankton tow data and DNA extraction dates

Temperature	Time	Number of Cycles
95°C	30 seconds	1 Cycle
95°C	30 seconds	25 Cycles
65°C	30 seconds	
58°C	30 seconds	
70°C	45 seconds	
70°C	5 minutes	1 Cycle

Table 2. Thermocycling regime for PNA-PCR. Thermal cycling consisted of 1 cycle of 95°C for 30s (denaturing), 25 cycles of 95°C for 30s (denaturing), 65° for 30s (PNA binding), 58° for 30s (primer binding), and 70° for 45s (polymerase extension). End point fluorescence detection occurred for 1 cycle at 70°C. A low extension temperature (4°C) prevented disassociation of the PNA during polymerase extension.

Sample Date	# of White Colonies	# of Blue Colonies
June 25	46	1
July 2 (first run)	46	1
July 2 (second run)	28	0
July 16	n/a	n/a
July 23	n/a	n/a

Table 3. Colonies picked for PCR.

and individual prey species clone colonies were grown overnight in a 37°C incubator.

3.3 PCR clone library analysis

Groups of up to 8 to 47 white and blue colonies were picked for each sample date (Table 3) for PCR amplification. Each colony picked represented individual gut content DNA. PCR reactions contained 1X GoTaq Green Master Mix, 0.5µM each of M13 forward and reverse primers (Sambrook et al. 1989), and 12µl of water. A water blank was used as a negative control. The thermal cycle consisted of one cycle for 30 seconds at 95°C, 35 cycles in a series at 94°C for 30 seconds, 58°C for 1 minute, and 72°C for

2 minutes, and one cycle for 10 minutes at 72°C. A final hold period occurred at the end of PCR at 4°C. A 0.8% electrophoresis gel was run for PCR samples (white, blue, and water blanks) and HiLo ladder for 30 minutes at 75 Volts. PCR products were purified by ethanol precipitation, and DNA amplicons were quantified using NanoDrop 1000.

3.4 Gut Content Sequencing

A. tonsa gut contents' PCR products with high NanoDrop numbers were diluted with water. 2µl of each sample was added to a 1.5ml microcentrifuge tube with 0.5µM M13 forward primer, and 21µl of water. These samples were then sequenced on a

3130xl genetic analyzer (ABI). Only white colony samples went on to sequencing. Sequences results found were entered into Geneious Basic 5.6.4

and identified to be the same organism. Sequences would be entered into GenBank BLAST (National Library of Medicine) to be identified and were recorded as results.

Figure 2. Methods Outline

(Drummond et al. 2010) to be read and aligned. Alignments of all sequences were lined up according to how similar the sequence was to other sequences,

4. Results.

4.1 June 25th, 2012 Sample Sequences

There were three distinctive sequence patterns for June 25 samples. Each sequence pattern was put into GenBank BLAST and were most closely identified as calanoid copepod species *Centropages*, *Oithona*, and *Temora* at 97% and higher. No phytoplankton species were found among these sequence results.

4.2 July 2nd, 2012 Sample Sequence

4.2.1 First Sequence

In sequence results double peaks appeared in abundance and served as an indication of contamination in the PCR of colonies. The electrophoresis gel from these samples was revisited and negative control (water blank) was seen to contain traces of DNA. The presences of double peaks made identification of gut content species difficult and not plausible. PCR was sequenced again with new clones, and protocol following PCR of clone colonies was followed shortly after (Figure 2).

4.2.2 Second Sequence

July 2nd samples matched GenBank BLAST hits at 96% or higher with the sequences of copepod species including *Centropages*, *Oithona*, and *Temora*. One sequence matched dinoflagellate *Prorocentrum minimum* at 99%.

4.3 July 16th 2012 and July 23rd, 2012 Sample Sequence and Analysis

Samples underwent PNA-PCR, but negative controls (water blanks) were observed to contain traces of DNA. Due to time constraints these samples did not undergo any processes after PNA-PCR. Sequencing for these samples did not occur.

5. Discussion.

5.2 *A. tonsa* feeding habits

Acartia tonsa have been observed to ingest the nauplii of other copepods when presented as prey (Lonsdale et al. 1979). This idea concerning carnivorous feeding habits exhibited by *A. tonsa* could serve as an explanation to why gut content sequences matched those of calanoid copepods.

The presence of copepod DNA within sequence results can also be contributed to picking errors during the early surveying of *A. tonsa* from original plankton

tows. Calanoid copepods are generally distinct in shape and size, but are characterized as “calanoid” due to their streamlined shape and long antennae (Johnson

5.3. Improving Methods

Column1	Column2	surface	depth
	volume counted (mL)	1	1
<i>Pseudo-nitzschia</i>	<i>sp</i>	4	
<i>Dino</i>	<i>gyrodinoid</i>	2	
<i>Dino</i>	<i>gumnodinoid</i>		
<i>Skeletonema</i>	<i>sp</i>	17	9
<i>Chaetoceros</i>	<i>lorenzianus</i>		
<i>Prorocentrum</i>	<i>sp</i>	1	
<i>Dactyliosolen</i>	<i>fragillissimus</i>	4	
<i>Chaetoceros</i>	<i>sp</i>	7	
<i>Pennate</i>	<i>sp</i>		1
<i>Thalassiosira</i>	<i>sp</i>	1	
<i>Ciliate</i>	<i>sp</i>		
<i>Proboscica</i>	<i>alata</i>		4

Table 4. Phytoplankton Count from July 2nd, 2012 collected from Station 2, Narragansett Bay. Volume of water sample was 1mL taken from surface and at depth. *Prorocentrum* sp. was identified as a possible prey species for *Acartia tonsa*.

& Allen 2005). These similar characteristics can serve as explanation for picking errors. An overabundance of these copepod species' DNA in sequence data may have overwhelmed *A. tonsa* gut contents' DNA as adult copepod species have more DNA. Also, PNA-PCR was *A. tonsa* specific so would not block excess DNA amplification of any stray copepods found in samples.

5.2 Prorocentrum and phytoplankton counts

The presence of dinoflagellate *Prorocentrum minimum* could mean that *A. tonsa* may have fed on this species of dinoflagellates in addition to copepod nauplii found in sequence, or that any one of the stray copepods picked during *A. tonsa* surveying may have ingested it. When plankton counts were reviewed, an individual (1) *Prorocentrum* species was counted in a 1mL sample from Station 2, Narragansett Bay (Table 4). The low count for *Prorocentrum* species in this phytoplankton sample and its presence in sequence results may represent evidence to support the idea that *Acartia tonsa* are selective feeders based on the abundance of other phytoplankton species present in our counts (Figure 4). This is a conclusion that can only be made when further studies are conducted as it remains uncertain based on our results whether or not *Acartia tonsa* consumed the *Prorocentrum*.

Methods used to identify prey species within the gut contents of *Acartia tonsa* include bottle incubations (Kiørboe & Viitasalo 1996), gut fluorescence (REF) (Kiørboe & Tiselius 1987), and the DNA-based methods (Durbin et al. 2012). Characterizing the diversity of prey ingested using the first two methods do not provide a direct *in situ* measurement of consumption and may not accurately represent all species *A. tonsa* consume in their natural environment. DNA-based methods have been used instead as they have been shown to provide more accurate results concerning *in situ* consumption (Durbin et al. 2012). In this study, errors made caused results of prey species consumed by *Acartia tonsa* to vary from what has been seen in past research (Durbin et al. 2012). With the sequence results received, researchers were able to go back through their procedures and determine where contamination may have occurred, and identify minor errors that may have been made. With this came the development of ideas on how to improve methods used to identify prey species of zooplankton including checking for correct predator species within samples, as well as avoiding contaminants.

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E. Durbin, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (edurbin@gso.uri.edu)

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Characterization of seafloor backscatter of seamount moats

Lydia Curliss¹, Robert Pockalny

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. Enigmatic bathymetric depressions are observed around the base of seamounts in a range of oceanographic settings. The origin of these moat-like depressions is unknown but various mechanisms have been proposed, including sediment erosion and sediment dissolution. The processes associated with these mechanisms are predicted to have unique seafloor reflection characteristics (e.g., small scale roughness differences and grain size). To identify these potential reflection characteristics, we used bathymetry and backscatter data from the Equatorial Pacific and Drake Passage collected with hull-mounted EM120, EM122 and SeaBeam 2100 multibeam systems. Bathymetry and backscatter data were processed using MB-systems to normalize and remove the affects of the topographic slope, grazing angle and differences in multibeam systems. Our results indicate that seamounts without moats have high reflectivity across the central seamount that diminishes to background regional values away from the seamount. Seamounts with moats tended to follow one of two reflectivity patterns. The first pattern is similar to that of seamounts without moats with any identifiable reflectivity where the moat is located. Other seamounts with moats have high reflectivity across the seamount but have a lower reflective character at the moat than the regional values. Differences in patterns may be indicative of active and non-active processes rather than of formation mechanisms.

1. Introduction.

Enigmatic moat-shape depressions form around seamounts and local topographic highs over a range of oceanographic and geographic settings (Figs. 1, 2). The origin of these moats is unknown, but previous studies have suggested a number of scenarios from sediment scour to biogenic sediment dissolution (Normark and Speiss, 1976; Mayer, 1981; Moore et al., 2007; Bekins et al., 2007; Pockalny et al., 2007).

Moats are shallow depressions around the base of some seamounts (Fig. 1). Cross-sections of the moat around the seamount are symmetrical, with two depressions on either side of the seamount. Moats are typically one to three kilometers wide and one hundred meters deep. Moats have been observed in both the Atlantic and Pacific oceans, but they are not uniformly distributed (Fig. 2). Concentrations of moats occur in the Equatorial Pacific and higher latitudes in both the Atlantic and Pacific.

Two general mechanisms have been proposed to explain the origin or formation of moats: sediment erosion and sediment dissolution (Fig. 3). Erosional formation is based upon water flow of bottom currents. In this model, bottom-flow increases around the base of the seamount in order to get around it causing the scouring of the sediments. This scoured area then is continually eroded down and out to form the moat (Figure 3b) (Normark and Speiss, 1976; Mayer, 1981). Sediment dissolution is based upon pore fluid in the underlying bedrock eroding sediment. In this scenario, carbonate-poor fluid flows up through

fractured bedrock causing the dissolution of the overlying biogenic sediments. Dissolution of finer sediments hypothetically would occur first leaving behind angular and larger sediments (Fig. 3a.) (Bekins et al., 2007; Pockalny et al., 2007).

In this study we use archived and recently collected high-resolution multibeam data to address these three questions: Do moats have a unique backscatter character? Does backscatter character differ from region to region? Can we determine origin from differences in backscatter?

2. Data.

Multibeam data are a combination of seafloor bathymetry and backscatter data. Multibeam is collected via a hull-mounted echosounder. The echosounder uses sonar or sound waves in order to collect information on the physical properties of the seafloor. Bathymetry is the measure of travel time from the ship to the seafloor and measures the topography. Backscatter data is the measure of the strength of return of the sound wave, and can be used to determine seafloor texture (i.e. grain size).

Bathymetry and backscatter data from multiple cruises from the Drake Passage and Equatorial Pacific were analyzed (Table 1). The Drake Passage (-58.5803° N, 62.9094° W) is an area of high latitude, whereas the Equatorial Pacific (0° N, 155° W) is lower latitude. Data from three cruises in the Drake Passage and three from the Equatorial Pacific were used.

Backscatter data from both regions were chosen to use as a proxy for the texture of the seafloor. The reflection character or strength of the backscatter should vary for different observable textures. Bathymetry was used in relation to the backscatter in order to see if morphological and topographic features had any relation to backscatter seen. Both areas with and without seamounts were selected in order to determine a control for our results.

Figure 1. Examples of moat-like depressions surround seamounts.

Figure 2. Distribution of identified moats. The different colored dots indicate different possible formation mechanisms.

3. Methods.

Backscatter data are processed and normalized to use as a proxy for changes in reflectivity. The multibeam processing software, MB-system (Caress and Chayes, 2006; 2008), was utilized to process and image swaths of collected bathymetry and backscatter data. Several MB-System programs were used to pre-process, correct, filter and plot the various multibeam data from the various cruises.

The initial step of multibeam processing was to identify and remove bad sonar "pings" from the raw multibeam data. The two programs used to do this is MBCLEAN and MBPROCESS. MBCLEAN is a program used to identify and flag the bad "pings"

Figure 3. Perspective and cross-sectional views of proposed formation mechanisms of seamount moats, (a) biogenic sediment dissolution and (b) sediment erosion.

within the data set. MB-PROCESS is then run in order to remove the flagged "pings" and to create a cleaner data set.

Several corrections were made with MBBACKANGLE to normalize backscatter data in order to use it to characterize seafloor character (Fig 4). Differences caused by bathymetry or the topography is corrected, so that the result assumes a flat bottom. The angle at which the outgoing sound pulse hits the bottom must also be corrected. The resulting transmission loss affects the data due to the fact the further out the sound energy is the decreased. In addition, since the data being collected are not on a flat surface the grazing angle or angle at which the sonar hits the seafloor must be also be corrected.

The next step is to filter the backscatter data with MBFILTER to remove the high frequency noise (Fig. 4). MBFILTER works by finding the average backscatter value for a user-defined region about each pixel. Filtering the data removes the "speckling" pattern typical of backscatter systems to make it easier to observed longer wavelength regional trends in the data.

The processed and filtered data are then used to create gridded data files with the programs MBGRID and MBMOSAIC. These programs create

postscript plots of the data in both 2-D and 3-D image. The 2-D images were analyzed for patterns in backscatter data and compared to the expressed

Equatorial Pacific (2), in the North-Central Pacific (1), and in the Drake Passage (1).

A preliminary inspection of the backscatter

Figure 4. Example of steps used to process backscatter data: (left) raw, (center) normalized, (right) filtered data.

Figure 5. Representative backscatter character of seamount without a moat (top) with a simplified interpretation of the cross-sectional geology (middle) and the backscatter pattern (bottom).

topography. 3-D images were created as well by using GRDIMAGE by draping the backscatter over the topography (e.g., Figs 5-7).

4. Results.

Eight different regions of multibeam bathymetry were analyzed to characterize the backscatter signature of seamounts with and without moats (Figs. 5-8). The seamounts-only examples are located in the Pacific Ocean and were used as a control. The moated seamounts are located in the

data revealed three general patterns associated the seamounts and moats. In all cases, the backscatter was highest (10 dB above background) in the region of the central seamount or bathymetric high. The primary differences occurred in the region of the moat where in some cases the backscatter faded into the regional background or decrease by about 10 dB and then returned to background values.

Seamounts without moats were characterized by high backscatter at the seamount fading into the lower regional backscatter character (Fig. 5). This model of high to low was used as a control to see if there is a difference between seamounts with and without moats. The change in this region ranged by about a 10 dB difference between the high at the seamount and lower regional character. The high backscatter on the seamount likely indicates hard rocky surface that has not been covered by a layer of sediment. The lower surrounding regional character likely indicates sedimentation.

Seamounts with moats are separated into two types: Type 1 and 2. Type 1 seamounts with moats are characterized by a backscatter high at the seamount, a low intensity where the moat is increasing to a medium regional value (Fig. 6). The change in intensity here changed by about 10 dB from the high at the seamount to the low of the moat. The high at the seamount again reflects a more rocky texture, and in this case extends into the moat, which likely reflects mass wasting of the seamount. The lower backscatter character of the moat may show that there is finer sediment or texture than the surrounding seafloor. Type 1 seamounts were observed in the moats studied at higher latitudes (Fig. 8).

Type 2 seamounts are characterized by their similarity to the control examples, where the seamount is high and the moat is a medium backscatter intensity

to be studied to determine whether or not these patterns only occur in our areas studied or are larger regional patterns.

Figure 6. Same as Figure 5, but for a seamount with a moat with a backscatter character termed Type 1.

fading into the lower regional average (Fig. 7). Again the change from the high to the low is about 10 dB. This pattern appears in the seamounts seen in the Equatorial Pacific nearest the equator (Fig. 8).

5. Discussion.

From our various models and examples, we can begin to address our key questions. Our results indicate that some of the moats observed have a unique backscatter character that differs from the seamount and the regional seafloor (e.g., Type 1 moats). The areas where we observed this unique character was in the data from the Drake Passage cruises and higher latitude area in the North-Central Pacific (Fig. 8). The unique character observed shows lower backscatter intensities than the seamount and the surrounding regional seafloor (Fig. 6). However, in other cases the seamounts with moats along the equator (e.g., Type 2 moats) have backscatter characteristics that are similar to the seamount-only control group. (Figs. 5, 7)

From the two general areas studied, it cannot be determined whether there is a regional difference between the Drake Passage and Equatorial Pacific. However, globally there seems to be a pattern between higher and lower latitude (Fig. 8). In the Drake Passage and North-Central Pacific, we did observe a unique character. This differs from the equator examples where there seemed to be no change between backscatter seen here and in the control. However more areas within these regions would need

Our results do not seem to show any clear indication of formation mechanisms. If a moat were formed by sediment dissolution, we would expect to have lower backscatter intensity due to the fact the smaller material would likely be dissolved. However, from our results a lower intensity is not observed. Erosional processes cannot be determined as an origin either. A possible explanation for what is observed is that the backscatter character may indicate active versus non-active processes. In the higher latitudes, where bottom currents are faster, the moats are likely being continually eroded, whereas in Equatorial Pacific the moats may have been filled in with sediments. In order to prove this or potential origins, sediment cores are required to ground truth the backscatter observations.

Overall our results give us some insight into the answers of our questions. In some circumstances, there is a unique backscatter character of moats. However a ground truth, and more areas would need to be studied to determine regional patterns and possible origin formations. Our study is also focused on more qualitative results, a more quantitative approach could give greater insight.

Figure 8. Location of seamounts (triangles) and seamount moats (circles) analyzed in this study. The filled circles indicate moats with Type 1 backscatter patterns and the open circles indicate moats with Type 2 backscatter patterns.

Cruise	Ship	Multibeam System	Year
NBP0710	R/V Palmer	Simrad EM 120	2007
NBP0908	R/V Palmer	Simrad EM 120	2009
NBP0812	R/V Palmer	Simrad EM 120	2008
Knox01RR	R/V Knorr	Simrad EM 120	2006
MGL1208	R/V Langseth	Simrad EM 122	2012
KN195-3	R/V Knorr	SeaBeam 2100	2009

Table 1. Cruise information for all data processed and used.

6. Conclusion.

Moats found around seamounts in some cases have a different backscatter character than seamounts without moats. However in other circumstances there is no significant difference between the control and seamounts with moats. In order to determine regional trends, more areas where seamounts with moats in the previously studied areas would need to be looked at. From our current results the origins of seamounts cannot be determined by differences in patterns we see in the backscatter.

Determining whether our results accurately depict seafloor character and textures, a series of

quantitative testing would need to be done. A series of sediment cores in these areas could provide insight into what types of sediment or rock types exist in these areas. As well a ground truth for these areas would provide us with visual information that could confirm our observations.

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- R. Pockalny, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (Robp@gso.uri.edu)
- L. Curliss, Oberlin College, Oberlin, OH, 44074. (lcurliss@oberlin.edu)

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Long-term bloom patterns of the diatom *Thalassiosira nordenskiöldii* in Narragansett Bay

Margo Davis¹, David Borkman, Ted Smayda

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. Phytoplankton perform a crucial role in ecosystems, as they are responsible for over half of global oxygen production and serve as the starting point of biogeochemical cycles. Long-term trends, bloom patterns, and environmental drivers of the marine diatom *Thalassiosira nordenskiöldii* were studied. *Thalassiosira nordenskiöldii* is abundant in Narragansett Bay seasonally, representing up to 44% of winter diatoms some years, being most prevalent in water from -1 to 1 °C. Water temperature in Narragansett Bay during the *T. nordenskiöldii* bloom window has increased over 1°C since 1959, which may push winter water temperature past *T. nordenskiöldii*'s optimal in-situ habitat conditions. As climate change continues, increasing water temperatures may alter *T. nordenskiöldii* bloom patterns. The data analyzed came from a time-series of weekly observations in lower Narragansett Bay, spanning from 1959-2011. Long-term trends show elevated abundance in 1960s and 1970s, followed by declining abundance through 1980s and 1990s. Populations increased in 2000s, but not to the same magnitude seen early in the time-series. Embedded in the long-term pattern were 53-month cycles, with an apparent disappearance in recent years. Cardinal characters were assigned to bloom characteristics (initiation, peak, duration, etc.) and used for analysis. Perhaps most noteworthy was the high variation exhibited, with blooms initiating anywhere from early December to early April and maximum bloom magnitude ranging from 96 to 8137 cells/ml. Multivariate statistical analyses identified three bloom types: an early, moderate bloom; a later intense bloom; and a late bloom with low abundance. Intense blooms came in winters with reduced river flow (37.3 m³/sec) and cold surface water temperatures (3.8°C), compared to smaller blooms occurring in winters with increased river flow (42.4-49.6 m³/sec) and warmer water (4.2-4.6°C). Understanding trends and bloom parameters of *T. nordenskiöldii* will allow for appropriate analysis of climate effects and prediction of future impacts.

1. Introduction.

Despite the overwhelming consensus within the scientific community regarding the importance of phytoplankton to ecosystems, relatively little is known about long-term bloom patterns of individual species. *Thalassiosira nordenskiöldii* is a centric marine diatom that is an important component of the winter-spring bloom in Narragansett Bay, representing up to 44% of all diatoms in some winters. The winter-spring bloom is of particular interest because the high levels of production resulting from the seasonal bloom fuel higher trophic levels (Townsend *et al.* 1994, Nixon *et al.* 2008). In cold water, zooplankton are less active to consume organic production, thus allowing these depositions to reach the benthos (Townsend *et al.* 1994, Nixon *et al.* 2008). Nixon *et al.* (2008) further point out that the nutritional quality of the winter depositions will be higher because they have a greater nitrogen content. *Thalassiosira nordenskiöldii* is a key source of production and nutrient input in the winter-spring bloom in Narragansett Bay (Karentz and Smayda 1984, Smayda 1957, Pratt 1965) and in boreal-temperate waters globally (Cleve 1873, Hasle 1976, Smith *et al.* 2001). Studying long-term bloom

patterns of *T. nordenskiöldii* to determine if changes have occurred and what may be driving potential changes will allow for a greater understanding of the critical winter-spring bloom in Narragansett Bay and other temperate estuaries. Initial observations displayed a wide range of variance between years. Identifying the trends embedded in this variance and the factors influencing *T. nordenskiöldii* bloom variability were main questions of this research.

Thalassiosira nordenskiöldii is found seasonally in Narragansett Bay, being found primarily during winter and spring when water temperature is between -2 to 5°C (Figure 1). This narrow temperature range in which *T. nordenskiöldii* has been successful suggests that temperature may be one potential factor determining the long-term bloom patterns. Average surface water temperatures in Narragansett Bay during the *T. nordenskiöldii* bloom window (early December-early April) have increased by just over 1°C since 1959 (Figure 2). Continuing warming trends could impact the ability of *T. nordenskiöldii* to thrive in Narragansett Bay. The winter-spring bloom in Narragansett Bay has already deviated from the historical norm, which typically had the *T. nordenskiöldii* bloom preceded by a *Detonula confervacea* bloom (Smayda 1957, Pratt 1959, Pratt

1965). However, while *D. confervacea* was once highly abundant, its bloom magnitude has diminished to a level that is trivial relative to its historical abundance (Smayda *et al.* 2004). Analysis of *T. nordenskiöldii* population trends may show a parallel decline in abundance.

Figure 1. *Thalassiosira nordenskiöldii* abundance distribution in Narragansett Bay during 1959-2011 as a function of water temperature. Temperature records were grouped into one degree bins.

Given the specific temperature range of *T. nordenskiöldii* in Narragansett Bay and the rising surface water temperature, a decrease in *T. nordenskiöldii* abundance is hypothesized. It is important to note that, while *T. nordenskiöldii* has a highly constrained temperature range in its habitat, these are not its physiological limitations. In laboratory settings, it can have cellular growth at both 10 and 15°C (Durbin 1974). Therefore, while temperature is the measured factor changing, there are other factors influencing *T. nordenskiöldii* abundance for which temperature is a proxy. Phytoplankton populations are under multivariate control (Smayda 1998). Nutrient concentrations, irradiance, salinity, and predation are all important factors that may be driving potential changes in *T. nordenskiöldii* abundance and bloom pattern across time.

2. Methods.

2.1. Data and Study Site

All data analyzed came from one site in Narragansett Bay, a well-mixed, shallow estuary. Narragansett Bay is located at a biogeographical jump between boreal and temperate waters. Thus, it is near the southern boundary of *T. nordenskiöldii* distribution. Long-term trends can be assessed because the phytoplankton time-series in Narragansett Bay is one of the longest running time-series, with weekly observations from 1959-2011. Each observation includes the phytoplankton species distribution and relevant environmental data (climatic factors, nutrient concentrations, etc.). The sampling and phytoplankton counting methodologies are

summarized in Smayda and Borkman (2008) and in Borkman and Smayda (2009). The earliest first appearance of *T. nordenskiöldii* in a given bloom cycle was in week 40, and the latest disappearance was in week 23. Weeks 40-52 were pushed forward to the next calendar year in order to incorporate full duration of presence. Thus each *T. nordenskiöldii* annual observation runs from week -12 to week 25. Each year of data was assigned cardinal characters describing different bloom characteristics (Table 1). These cardinal characters were used for analysis of *T. nordenskiöldii* bloom patterns following the methodologies applied by Maberly *et al.* (1994) to a multidecadal diatom abundance time-series. In order to characterize *T. nordenskiöldii* blooms, a working definition of a bloom was required. For the purposes of this study, bloom initiation occurs when there is a two-fold increase in abundance that is sustained to the peak, or any decreases hold abundance at over two times the average abundance of that year. Bloom termination occurs at the peak. Other bloom describing cardinal characters included metrics of bloom timing and bloom abundance (Table 1).

Of the 52 years during the observation period (1959-2011), nine full years are missing phytoplankton observations. In addition, 229 weeks in other years are missing observations. This leaves 1443 weeks of *T. nordenskiöldii* abundance data. Raw data was used whenever possible, but when gaps were not acceptable for the type of statistical analysis, fill methods were implemented for both abundance and environmental data. Linear interpolation filled individual missing weeks. From 1959-1997, gaps greater than one week were filled with the monthly average from the aforementioned time period. Gaps greater than one week occurring from 2000-2011 were filled with weekly averages from the full time-series. The exception to this fill method was for the missing river flow data, which could be filled with monthly averages from USGS records. Additionally, missing nutrient data from 1964-1969 was filled with the annual decadal average to account for higher pre-Clean Water Act values.

2.1 Data Analysis

2.1.1 Time-series analysis

Multiplicative time-series analysis was performed to observe long-term trend. This particular type of time-series analysis was chosen because the method specifically accommodates for time-series that have many zeros due to seasonality (Broekhuizen and McKenzie 1995). Briefly, this method removes the seasonal trend and uses a 20 percent smoother of the de-seasonalized data as an indicator of long-term trend for the 1959-1997 and 1999-2011 data segments separately. The time-series analysis methods applied to *T. nordenskiöldii* abundance data are similar to

Cardinal Character	Definition	Range of Values	Mean
N1	Maximum abundance	96 - 8137 cells ml ⁻¹	1528.7 cells ml ⁻¹
N2	Average abundance (weeks -12 to 25)	13.3 - 575.9 cells ml ⁻¹	150.3 cells ml ⁻¹
N3	First half average abundance (weeks -12 to 6)	0 - 451.1 cells ml ⁻¹	82.8 cells ml ⁻¹
N4	Second half average abundance (weeks 7 to 25)	7.4 - 943.9 cells ml ⁻¹	216.4 cells ml ⁻¹
W1	Week of first appearance	Week -12 - 18	Week 1.7
W2	Week of maximum abundance	Week 1 - 19	Week 10.0
W3	Week of bloom initiation	Week -3 - 17	Week 7.2
W4	Bloom duration (W3-W2)	1 - 9 weeks	2.8 weeks
W5	Week of disappearance	Week 12 - 23	Week 17.7
W6	Duration of presence (W5-W1)	6 - 31 weeks	16.0 weeks
W7	Number of weeks present	2 - 25 weeks	12.7 weeks

Table 1. Cardinal characters utilized for *T. nordenskiöldii* analysis, with their means and ranges.

Variable	Average			p-value
	Bloom Type 1	Bloom Type 2	Bloom Type 3	
Total river flow (m ³ sec ⁻¹)	42.44	37.30	49.63	< 0.0001
Wind (m sec ⁻¹)	4.67	4.68	4.48	0.0111
Surface Temperature (°C)	8.04	7.52	8.51	0.0528
Bloom window temperature (°C)	4.22	3.80	4.59	0.0639
Irradiance	137.42	138.74	126.38	0.0662
Precipitation prior week (cm)	2.19	2.23	2.60	0.0834
Si (μM)	11.35	8.00	12.35	< 0.0001
NO ₃ (μM)	2.89	3.00	4.82	< 0.0001

Table 2. Environmental variables having statistically significant differences between *T. nordenskiöldii* bloom patterns, as determined by Mann-Whitney tests or post-hoc tests.

Cardinal Character	Average			p-value
	Bloom Type 1	Bloom Type 2	Bloom Type 3	
N1: Maximum abundance	1083.3	4550.0	991.3	0.009
N2: Average abundance	127.2	377.0	64.6	0.003
N3: First half average abundance	110.6	98.3	1.1	<0.0001
N4: Second half average abundance	143.8	643.5	122.7	0.0005
W1: Week of first appearance	-1.1	0	8.3	<0.0001
W2: Week of maximum abundance	8.7	11.5	12.2	0.0312
W3: Week of bloom initiation	5.8	7.5	10.2	0.0417
W4: Bloom duration	2.9	4	2	0.0424
W5: Week of disappearance	17.8	21.0	16.1	0.009
W6: Duration of presence	18.9	21	7.8	<0.0001
W7: Number of weeks present	14.4	18.8	7.2	<0.0001

Table 3. Cardinal characters having statistically significant differences between *T. nordenskiöldii* bloom patterns, as determined by Mann-Whitney tests. N cardinal characters are abundance values in cells ml⁻¹, W cardinal characters relate to bloom timing in weeks.

those described by Turner *et al.* (2011) in their analysis of coastal zooplankton abundance trends. To detect cyclical behavior embedded in the *T. nordenskioldii* abundance time-series, the spectral density of the raw observations was calculated using JMP software (JMP Version 9, SAS Institute Inc.).

2.1.2 Statistical Analysis

Cluster analysis was used on a matrix of cardinal character data (11 cardinal characters by 45 years) to identify different bloom types. Cluster analysis used the average linkage method unweighted pair-group method using arithmetic averages (or UPGMA). Once bloom types were identified, environmental data were coded with bloom type and analyzed with a Similarity Percentage analysis (SIMPER) in PRIMER-E software (PRIMER_E Version 5), which identified the primary sources of dissimilarity between bloom types. SIMPER is traditionally used to recognize species that create variance between sites. However, by using cluster codes as sites and environmental data as the ‘species’ creating variance, the exploratory technique can be applied here to identify potential drivers of *T. nordenskioldii* abundance. The findings suggested with SIMPER were then analyzed with hypothesis testing statistical tests for significant differences in environmental factors. Environmental data was tested for normality and the null hypothesis was rejected in almost all cases. The nonparametric hypothesis test Mann-Whitney U-test was utilized to detect differences in environmental variables between bloom types.

3. Results.

Thalassiosira nordenskioldii long-term bloom patterns and sources of variation were studied through time-series analysis, multivariate statistical analysis, and nonparametric hypothesis testing. Time-series analysis showed long-term trends of *T. nordenskioldii* in Narragansett Bay from 1959 to 2011 (Figure 3). Population levels were high in the 1960s, and remained at an elevated level through late 1968. Here, the trend begins to decline, but abundance is still over the long-term mean. Populations hover around the long-term mean through the end of the 1970s, when populations decline. In 2000s, the trend is again above the long-term mean, although even at its peak in 2003, the trend is of a lesser magnitude than observed in the 1960s. This elevated abundance is also for a shorter time period, as it begins a general decline in trend in late 2003 that is sustained through the end of the time-series. Spectral density suggested that in addition to annual cycles, *T. nordenskioldii* also exhibits 53-month cycles. Fisher’s Kappa statistic indicated that this cycle was significantly different than a result produced by

random variation. This was the case for the entirety of the time-series, but different results were found when data was divided into two segments, 1959-1997 and 1999-2011. Only the older data had a similar cyclical pattern, with a 52-month cycle. A cycle with approximately this period was absent from 1999-2011.

Figure 2. Average water temperature in Narragansett Bay during *T. nordenskioldii* bloom window. Linear regression had a significant positive slope of 0.0265, indicating a long-term temperature increase of approximately 1.4°C.

Figure 3. Long-term trend of *T. nordenskioldii* abundance from 1959-2011, as derived from time-series analysis (solid line). Long-term mean indicated by dashed line.

The cardinal characters showed that bloom characteristics had wide annual variation (Table 1). Cluster analysis on each year’s set of cardinal characters identified three bloom types (Figure 4). Bloom type one was a moderate bloom, with an average maximum of 1083 cells ml⁻¹, peaking in February. Bloom type two was of higher magnitude, with an average maximum of 4450 cells ml⁻¹, which occurred early March. Bloom type three peaked slightly later, in mid-March, and was the least intense bloom, with the average maximum abundance at 991 cells ml⁻¹. Table 2 shows the complete set of average cardinal characters for each bloom type.

SIMPER identified both sources of similarity within groups and sources of dissimilarity between groups. In all three-bloom types, the environmental factors that were most responsible for similarity with

the group were irradiance, total river flow, and salinity. Environmental factors causing dissimilarity between groups were NO_3 , Si, and total river flow. These results help illuminate important factors, but do not show statistical significance.

Hypothesis testing determined factors that were statistically different between bloom types (Table 2). According to Mann-Whitney U-tests, there were statistically significant differences in both NO_3 and Si concentrations between small and moderate blooms as well as between small and large blooms. Small blooms had significantly more NO_3 ($4.82 \mu\text{M}$) than both moderate ($2.89 \mu\text{M}$) and large ($3.00 \mu\text{M}$) blooms ($z = 4.57$, $p < 0.0001$; $z = 2.26$, $p = 0.0241$, respectively). Small blooms similarly had significantly more Si ($12.35 \mu\text{M}$) than moderate ($11.35 \mu\text{M}$) and large ($8.00 \mu\text{M}$) blooms ($z = 3.83$, $p = 0.0002$; $z = 4.32$, $p < 0.0001$, respectively). Large and moderate blooms had no statistical difference in nutrient concentration. Quarter 3 (prior to *T. nordenskioldii* appearance) nutrient concentrations were also analyzed. Pre-bloom PO_4 levels were lower in small blooms ($1.49 \mu\text{M}$) than in moderate blooms ($1.82 \mu\text{M}$, $z = -4.22$, $p = 0.0084$) and large blooms ($1.95 \mu\text{M}$, $z = -3.96$, $p = 0.0001$). Similarly, small blooms had less initial NO_3 ($0.45 \mu\text{M}$) than either moderate ($0.67 \mu\text{M}$, $z = -2.29$, $p = 0.0220$) or large ($0.97 \mu\text{M}$, $z = -2.51$, $p = 0.0119$) blooms.

Figure 4. Monthly mean of *T. nordenskioldii* abundance for three bloom patterns, identified by cluster analysis during its period of presence in Narragansett Bay of October (month -2) to May (month 5).

Total river flow was significantly different between all three bloom types. Bloom type two, the most intense bloom, occurred in winters with an average river flow of $37.3 \text{ m}^3 \text{ sec}^{-1}$, lower than either the moderate bloom type two ($42.44 \text{ m}^3 \text{ sec}^{-1}$, $z = -3.34$, $p = 0.0008$) or bloom type three, the bloom of least magnitude ($49.63 \text{ m}^3 \text{ sec}^{-1}$, $z = 4.85$, $p < 0.0001$). The difference in river flow between bloom type one and bloom type three was also significantly different, with $z = 2.80$ and $p = 0.0051$. Bloom type three years had significantly less wind than bloom type one ($z = -2.80$, $p = 0.0051$) and bloom type two ($z = -2.31$, $p =$

0.0207). There was no statistical difference in wind speed between bloom type one and two.

Temperature showed statistically significant differences between bloom types two and three. Temperature during the bloom window of *T. nordenskioldii* was 3.8°C in bloom type two years, significantly colder than the 4.6°C of bloom type three years ($z = 2.11$, $p = 0.0351$). Average water temperature for the duration of *T. nordenskioldii* presence was also cooler in bloom type two years than in bloom type three years ($z = 2.37$, $p = 0.0176$). Neither bloom type two nor three showed significant differences in water temperature from bloom type one.

4. Discussion.

The wide range of *T. nordenskioldii* bloom characteristics and the annual variation in bloom magnitude both in the short and long-term time scale were assessed to determine what types of changes were occurring and potential factors affecting these changes. Time-series analysis showed a long-term trend that did not remain consistent throughout the time-series, with trend being elevated early in the time-series, declining through the 1990s, then briefly increasing before a final downward trend. Because the long-term trends are not entirely in one direction, it is somewhat difficult to determine the exact nature of the changes in *T. nordenskioldii* abundance. However, the high populations observed in the 1960s and running into 1970 were never duplicated. *Thalassiosira nordenskioldii* abundance levels did increase from the trough in the 1990s, but this recovery was only slight. The results of the time-series analysis do not provide a concrete prediction of future populations. While the results are not conclusive, it does suggest that *T. nordenskioldii* abundance is declining and *T. nordenskioldii* may be becoming a less important source of production in the winter-spring bloom in Narragansett Bay.

Another component of the long-term trend is the cyclical pattern of *T. nordenskioldii* abundance. In addition to the expected annual cycles, spectral density analysis showed approximately 53-month patterns. The causes of this cycle are not entirely known. Sea surface temperature also seemed to follow a similar cycle, although whether this is causative cannot be said. Spectral analysis on separate parts of the time-series suggested that, in recent years, this 53-month cycle has disappeared. Interestingly, Narragansett Bay historically has iced approximately once every five years, but this pattern has also desisted. At this point, citing warming waters and the disappearance of icing in Narragansett Bay as influences on the disappearance of the 53-month *T. nordenskioldii* cycle is entirely speculative. Nonetheless, the pattern of elevated abundance and disproportionate number of large (type 2) blooms

during the colder 1960s is consistent with the expected association between *T. nordenskiöldii* and cold water. The disappearance of a 53-month peak in spectral density during the latter segment of the time-series (1999-2011) may be a manifestation of the short duration of this series, possibly making detection of 53-month cycles improbable. However, it does seem that this 53-month cycle is no longer a component of *T. nordenskiöldii* bloom patterns.

The role of temperature in the cyclical pattern of *T. nordenskiöldii* is plausible but has not been fully explored. While there have not been conclusive findings regarding the drivers of cycles in the long-term trend, temperature has been found to be a significant factor in *T. nordenskiöldii* blooms. When comparing the three bloom types, the large bloom had significantly colder water temperatures than the small bloom. This difference, while statistically significant, was only slight. The average temperatures differed by approximately 1°C (depending on the window temperature is being observed). From a physiological standpoint, this temperature change should not have a significant impact on *T. nordenskiöldii* blooms. Therefore, temperature is likely serving as a surrogate climatic factor that incorporates other concurrent factors affecting *T. nordenskiöldii*.

One of these interacting factors to consider is predation. In cold waters, zooplankton have lower metabolic activity and thus lower grazing rates. In warmer waters, predation will play a greater role in reducing phytoplankton biomass (Keller *et al.*, 1999). It may be that at temperatures seen in high magnitude blooms were too low for active zooplankton, while the temperatures in the small bloom allowed for zooplankton grazing. Zooplankton data for this study was limited to 18 years of monthly zooplankton dry weight. Zooplankton dry weight was not a statistically significant factor in determining bloom type ($p = 0.1213$), although since dry weight is not the best measurement and bloom types were not uniformly represented in the 18-year sample, grazing rates are still an important factor for further consideration.

Other external drivers, in addition to temperature, played a role in bloom type. Both wind and river flow were statistically different between bloom types. Freshwater river flow alters salinity, residence time, nutrient flux, and turbidity, among other factors. *Thalassiosira nordenskiöldii* had larger blooms in seasons with low river flow. Similar results have been found in an estuary in Portugal, attributed to the long residence times that were conducive to growth and establishment of phytoplankton (Gameiro and Brotas, 2009). Wind speed was higher in years with large (type 2) blooms. Wind can affect phytoplankton blooms in a number of ways that will produce varying results. The impact of wind can also depend on the direction of the wind. One possible

reason that windy winters have large blooms is because the wind has brought *T. nordenskiöldii* into Narragansett Bay. *Thalassiosira nordenskiöldii* does not form cysts in Narragansett Bay, and therefore each year requires new recruitment of cells. In addition to relying on wind speeds, wind direction will play a role in bringing *T. nordenskiöldii* cells to Narragansett Bay. Without directional data, the way in which wind has a positive influence on *T. nordenskiöldii* abundance is unclear. According to Pilon (2008), wind speeds near Narragansett Bay have been declining. This may also contribute to the decline in *T. nordenskiöldii* abundance, given the significantly lower wind speeds observed in small bloom years.

Large blooms came in years with significantly less nitrate and silica. Intuitively, this would be due to the high rate of consumption from the thriving bloom. However, analysis of nutrient concentration slopes showed that in all nutrients measured, the large bloom did not have any significantly non-zero slopes. This is somewhat difficult to interpret, but perhaps has to do with nutrient flux from river flow and wind or the recycling of nutrient.

There is no single identifiable factor influencing *T. nordenskiöldii* bloom patterns. Rather, there is a cohort of factors interacting with one another that will drive populations in various ways. Different factors will be more or less important in different years and even from week to week. Temperature and the factors changing with temperature seem to be key drivers in *T. nordenskiöldii* abundance, based on attributes of high magnitude blooms and the *T. nordenskiöldii* distribution as a function of water temperature. Internal to Narragansett Bay bottom-up (i.e., nutrients) and top-down (i.e., zooplankton abundance) forces did not result in conclusive findings, with most significant factors being external drivers. That being said, as the external drivers change, the internal drivers will also respond to these factors in ways that could affect *T. nordenskiöldii* bloom characteristics.

5. Conclusion.

Thalassiosira nordenskiöldii bloom patterns have exhibited a high level of variance throughout the time-series. However, recent changes in abundance seem to have deviated from long-term patterns and tend to be dominated by declining trends. The important role *T. nordenskiöldii* has historically played in the winter-spring bloom in Narragansett Bay may be diminishing. The extreme decline in *D. confervacea* abundance has not been seen with *T. nordenskiöldii*, although a similar pattern could occur in the future. The elevated abundance of *T. nordenskiöldii* in the 1960s has not been replicated, and environmental conditions associated with large

blooms such as cold temperatures and high wind speeds are becoming less prevalent, suggesting *T. nordenskiöldii* populations will become less dominant in Narragansett Bay. This alteration in the winter-spring bloom could affect carbon cycling and nutrient deposition. On a larger scale, these changes in Narragansett Bay may be indicative of future trends in boreal waters if climatic changes continue.

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D. Borkman and T. Smayda, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (dborkman@gso.uri.edu, tsmayda@gso.uri.edu)
Margo Davis, Colorado College, Colorado Springs, CO, 80907 (margo.davis@coloradocollege.edu)

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Developing a low-cost demonstration ocean wave energy converter

Nikiforos Delatolas

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. A resonance-type wave energy converter was designed, built, and tested. The device was made from inexpensive hardware store materials that may be used for physics demonstrations in high school classrooms. To illustrate energy conversion, the resonant device was designed to generate enough electricity to light a small bulb. A program was developed to determine the operational frequency of the energy converter based on armature mass, buoy characteristics, and typical wave frequencies. Using a wave frequency of 0.6 Hz, which maximizes oscillation amplitude of the energy converter, a maximum amplitude, root-mean square velocity, and root-mean square voltage were estimated. Knowing the resistance of the device the power output was found to be 2.2 Watt based on the design assumptions. The energy converter was tested in a wave tank to observe the actual power output. Experimental observations show that with a wave height of 6 cm and a wave frequency of 0.6 Hz, there is a power output of 0.013 to 0.056 Watts. The power output obtained experimentally was lower than the theoretical model due to non-vertical motions of the buoy, non-sinusoidal voltage outputs, and frictional forces not taken into consideration in the theoretical model. The device successfully illuminates a 0.05 Watt LED bulb using relatively inexpensive, off-the-shelf hardware store materials. The device can be used to demonstrate energy conversion to students, specifically showing how the kinetic and potential energy of ocean waves can be converted to electricity under the theme of clean and renewable energy.

1. Introduction.

In a world that must adapt to renewable energy recourses, ocean waves have been of great interest because of the immense amounts of energy they carry. This project uses the principles of a resonant wave energy convertor and develops a demonstrational device showing the conversion to electricity. Being a demonstration the objectives are different from an actual energy convertor. The design must be as simple as possible, it has to be made from inexpensive materials and the conversion to electricity must be visible. As an outcome, students developing the device would learn the underlying physics principles that make it work.

Initially we will describe the equations of motion that govern the convertor, and explain how from those we can determine the components necessary to light a small bulb. Then we will explain the experiments done to test our theories and see if the device actually works followed by the results, a discussion of our findings and the conclusion.

2. Experimental Method.

2.1. Energy Converter Design and Cost

The device is 0.914 m long, it consists of an outer clear tube with diameter 0.0762 m that provides the buoyancy and an inner concentric clear tube that is stabilized by insulation foam rings (Figure 1). On the outside surface of the inner tube there is a 0.114 m

long solenoid made off about 2000 turns of magnet copper wire. On the top of this tube there is a spring attached, also concentric to the vertical axis and on the other end of the spring is a Neodymium cylindrical magnet. The bottom of the outer tube is sealed with a plug and 1.27 kg of reused lead shot provide ballast for stability. As the energy converter (buoy) oscillates, energy is transferred to the spring-magnet system from the kinetic and potential energy of the waves. The magnet travels through the solenoid creating an electric field by the change in magnetic field, forcing a current through the wire and illuminating a Light Emitting Diode (LED)

The cost of the demonstration is a very important component of this project. There is no real application of this device, even though it might work, if the cost is well above the budget of a high school laboratory or an undergraduate research project. The calculated cost per device was estimated to \$86.00 but the actual cost of materials purchased was \$151.00. This difference in price is because some of the materials bought were in greater quantity than needed. Looking at the table below we see the most expensive components are the magnet and the outer clear tube.

2.2. Theoretical Model

The motion of the armature-buoy system was treated as an undamped two degree of freedom mechanical vibration (Figure 2). The schematic below indicates the forces acting on the masses and the corresponding displacements. The change in buoyant force (k_b) was calculated from equation (1) sited in

Table 2 and can be seen as a spring constant because it has units of (N/m). The total buoy mass without the armature mass is (m_b).

Figure 1. Wave energy convertor components.

Figure 2. Free body diagram for buoy and armature.

A system of differential equations describes the motion of the coupled system. No driving force was taken into consideration in order to solve for the natural frequencies of the buoy-armature system.

$$\begin{bmatrix} m_b & 0 \\ 0 & m_a \end{bmatrix} \begin{bmatrix} \frac{\partial^2 x_b}{\partial t^2} \\ \frac{\partial^2 x_a}{\partial t^2} \end{bmatrix} + \begin{bmatrix} k_s + k_b & -k_b \\ -k_b & k_b \end{bmatrix} \begin{bmatrix} x_b \\ x_a \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

with solutions :

$$x_b(t) = A_b \cos(\omega t + \phi) \quad x_a(t) = A_a \cos(\omega t + \phi)$$

Substituting the solutions in the differential equations and solving for omega we end up with a fourth degree polynomial that can be solved for ω_a^2 and ω_b^2 which are the natural frequencies of armature and buoy, dependent only on the corresponding masses and spring constants. The general equation for resonant natural frequency of a spring-mass system

could also be used and does not require derivation. Given equations (2.a) and (2.b) in Table 2 the natural frequency of the buoy and armature can be calculated immediately. Equation (3), expresses the relationship between the frequency of the waves and the natural frequencies of the buoy and armature that could be derived from the system of differential equations. Instead, it was found in a technical report for linear wave generators by Bastien and others (2011) and was used directly. An additional relation that connects the natural frequency of the buoy and armature is equation (11) forcing them to be linearly proportional to each other by the (ratio) factor.

Holding (m_b) and (k_b) constant, due to their dependence to the physical characteristics of the device, while changing (m_a) and the (ratio) we obtained a matrix of wave frequencies in correspondence to different armature masses and ratios. Resonance Period (eqn. 4) vs. Mass of armature was then plotted. The graph indicates the appropriate mass of armature needed for a given wave period and ratio. Once a mass and corresponding period are chosen the spring constant (k_s) can be found using equation (5) in Table 2.

A static free body diagram of the mass-spring system helped indicate the forces and solve for the displacement (x) from equation (12). The length of the spring plus the displacement indicates the equilibrium position of the magnet in relation to the body of the device. The length of the spring had to be chosen such that the magnet would be centered inside the solenoid and the amplitude of oscillation would not exceed the distance between the equilibrium position and the bottom of the buoy. A spring could then be purchased with the above spring constant and appropriate length determined by the design.

From the amplitude response of the armature at the resonance frequency, the corresponding maximum velocity could be found by equation (6). Then, the root mean square (RMS) velocity was calculated by equation (7), the RMS voltage output from equation (8), and the RMS current from equation (9). Finally the Power output directly related to the amplitude of the magnet was found by equation (10).

2.3. Wave Tank Testing

The theoretical method described above determines the appropriate mass and spring characteristics necessary for a given wave frequency. Incorporating these values into the design, the energy converter was then tested in a wave tank. In this setup there were three main instruments that had to work simultaneously.

The paddle that generates the waves was controlled by a LabView program in which the period and amplitude of waves were inputted.

Not knowing the accuracy of the actual waves generated in relation to the values typed in,

Product	Price	Price/device
Polycarbonate Round Tube 3" OD	23.370	23.37
Polycarbonate Round Tube 1-1/4" OD	11.280	11.28
Copper Magnet Wire	15.82	3.16
Neodymium Cylinder Magnet	30.65	30.65
Reclaimed Lead Shot	36.99	4.11
0.05W LED Light Bulb	3.50	1.75
Steel Extension Spring Length 11.0"	3.31	3.31
Eye Hook	4.50	4.50
Low-Pressure Plug	2.81	2.81
Lightweight Polystyrene Foam	17.18	1.43
Electrical Tape	2.00	0.20
Total	151.410	86.38

Table 1. Table of products and prices

another device was used that measures surface elevation. This instrument is a capacitor that records change in potential difference due to the change the device is emerged in water. Within a range the potential difference is linearly proportional to surface elevation and while obtaining data at a rate of 30 Hz we could graph the actual wave amplitude. Using this data, we can find with better accuracy the wave period.

The third instrument was a data acquisition board (NI-USB-6353) that recorded the output voltage from the magnet wire of the solenoid. As the magnet was oscillating in response to a specific wave period the corresponding voltage was measured and graphed in LabView. Using equations (6) through (10) of Table 2, voltage could be converted to (RMS) power output.

The waves were initially set to propagate with a 1.40 second period then voltage data was recorded for 45 seconds and stored in a file. For every 0.05 seconds the above procedure was followed up to 2.00 seconds. In Matlab the voltage data was converted to RMS power output and plotted against the corresponding period.

3. Results.

An expression for the amplitude in terms of frequency can be found by solving the differential

equations in section 2.2. A range of wave periods can be inputted and the corresponding magnet response plotted as seen below. The amplitudes can be converted to power output, as done in equations (6) to (10) and plotted against period. The two peaks in each graph represent the natural frequencies of the system, which are directly derived from the system of differential equations. Maximum power is generated at a period of 1.6 seconds.

The matrix of period data described in paragraph 3 of section 2.2 is graphed below. The different color lines represent the (ratio) coefficient starting at 1 with intervals of 0.25 and up to 3. The ratio used was 1.5 (orange line); with an armature mass of 0.1488 kg the resonance period is 1.67 s. Using equation (5) it resulted to a spring constant of 5.2.

The experimental RMS power output is represented by the continues line. The dashed line represents the standard deviation for each datum point. The separation of the two curves shows the difference between the instantaneous and the RMS power output. We observe a RMS peak of 0.013W, an instantaneous peak of 0.056 W, and the range of periods that outputs a high enough wattage is 0.04 seconds.

Variable Name	Expression	Number
Buoy Mass	m_b	
Armature Mass	m_a	
Cross-Sectional Area of buoy	Area	
Water density	ρ	
Gravitational Acceleration	g	
Change in Buoyant Force	$k_b = \text{Area} \cdot \rho \cdot g$	Eqn. 1
Displacement of armature	x_a	
Displacement of buoy	x_b	
Buoy frequency	$\omega_b = \sqrt{\frac{k_b}{m_b}}$	Eqn. 2.a
Armature frequency	$\omega_a = \sqrt{\frac{k_s}{m_a}}$	Eqn. 2.b
Wave frequency	$\omega_w = \frac{\sqrt{(\omega_b + \omega_a)^2 + \omega_a^2 \cdot \frac{m_a}{m_b}}}{2} - \frac{\sqrt{(\omega_b - \omega_a)^2 + \omega_a^2 \cdot \frac{m_a}{m_b}}}{2}$	Eqn. 3
Resonance Period	$T_{\text{resonance}} = \frac{2 \cdot \pi}{\omega_w}$	Eqn. 4
Spring Constant	$k_s = \frac{4 \cdot \pi^2 \cdot m_a}{T_{\text{resonance}}^2}$	Eqn. 5
Maximum Velocity of oscillation	$ V_{\text{max}} = \omega \cdot A_{\text{max}}$	Eqn. 6
Root mean square Velocity	$V_{\text{rms}} = \frac{ V_{\text{max}} }{\sqrt{2}}$	Eqn. 7
Magnetic Field	B	
Length of solenoid wire	L	
Root mean square Voltage	$\text{Volts} = V_{\text{rms}} \cdot B \cdot L$	Eqn. 8
Resistance	R	
Root mean square Current	$I_{\text{rms}} = \frac{V_{\text{rms}}}{R}$	Eqn. 9
Root mean square Power	$W_{\text{rms}} = V_{\text{rms}} \cdot I_{\text{rms}}$	Eqn. 10
Ratio of armature to buoy natural frequency	$\text{ratio} = \frac{\omega_a}{\omega_b}$	Eqn. 11
Initial Tension of Spring	T_i	
Static Displacement of Spring	$x = \frac{m_a \cdot g \cdot T_i}{k_s}$	Eqn. 12

Table 2. Table of Variables and Equations

4. Discussion.

4.1. Analysis of theoretical method

The theoretical model as seen schematically in figure (2) and expressed by the set of differential equations that follow it, is oversimplified. There are no frictional forces of any sort in the free body diagram nor does the system oscillate in the vertical

Graph 1.a Armature amplitude response for undamped system.

Graph 1.b. Corresponding power output, of armature amplitude for undamped system.

direction, therefore not including gravitational forces. The Matlab program that generated graph (1.a) and (1.b) simply inputs and outputs values through the set of differential equations not taking into account physical restrictions of the design, such as length of device and spring specifications. As this is an undamped vibration, the amplitude and power output reach infinity as the system oscillates at the natural frequencies. The largest armature response expected while testing, was influenced by the information in graph (1.a) showing the greatest peak around 1.6 seconds of wave period. These graphs also brought some concerns when observing the narrow range of wave periods necessary for lighting the LED. Although the data of graph (1.a) and (1.b) do not represent reality, the curves give good insight of the trends and behavior of the system.

Graph 2 is the direct result of equation (3) with two unknowns, (m_a) and (ratio). Wanting to work only with one, armature mass was kept constant at 145 g thus having 9 corresponding resonance periods for the different ratios. Ideally we would like the resonance frequency of the buoy and armature to have a ratio of 1. When substituting the period value for

ratio 1 and 145 grams into equation (5) it resulted to a (k_s) value of approximately 1.9 N/m. But these equations do not take into account the physical limitations of the spring nor the device design. For example if a 2 N/m spring would be used with a 145 gram mass it would deform resulting to a non-linear relationship between displacement and load. In addition, the spring would not have the capacity to resonate at velocities that would produce a satisfying power output. But also, when solving equation (12), the displacement (x) would exceed the length of the entire device. For all the reasons above a ratio of 1.5 was used, which is the closest ratio to 1 that results to a high enough spring constant (5.2 N/m) overcoming the physical constraints.

4.2. Analysis of Wave Tank Testing

The theoretical power output calculated by equation (10) in section (2.2) was 2.2 W. From Graph (3) we see an instantaneous power output of 0.067 W. Calculating the percent decrease from the theoretical to the maximum experimental power output we find a change of 96.95 %. Damping forces are an essential part of the analysis and the major reason for the great difference. More specifically, the axis of motion is not only constrained to the vertical, as theoretical models assume, but there is rolling involved which has its own natural frequency and amplitude. The rolling increases friction between the walls of the inner tube and the magnet as well as viscous forces between the outer body of the buoy and the water. The experimental voltage output is far from being sinusoidal as a result of the hand coiled magnet wire the solenoid is made off. Typical generators include multiple coils in order to condition the form of the output voltage signal, however since this is a demonstration device, efforts were not made to maximize the efficiency of energy conversion. Equation (6) in Table 2 that gives the velocity, which is proportional to voltage, is for sinusoidal signals only. This difference also increases the gap of results between the theoretical model and the experiment.

RMS Power data represented by the continues line in Graph (3) was calculated by taking the absolute value of the voltage signal and then the mean, for a given experimental run. With this RMS voltage the power was found as before. The dashed line represents error; it is the standard deviation for each voltage signal converted to power. This graph also explains why the LED blinks. Around the 1.65 s period, there is a difference of 0.02 W between the RMS power output and the instantaneous power, which is reached once or twice every cycle. Lastly we see the very narrow range of periods the buoy responds in order to generate more than 0.05 W. This indicates that in the ocean environment, which has a plethora of wave periods, this resonant device will not work.

Graph 2. Equation 3 of Table 2 graphed for different armature masses and ratios.

Graph 3. RMS and STD experimental power output for different wave periods.

4.3. Future Work Suggestions

As a next step to this project, it would be great if the device could work in the ocean, fully demonstrating clean, sustainable energy conversion. To do this there are several ways it might be done. Initially damping forces must be included in the numerical models. For example, if the frictional force acting on the armature is linear proportional to its RMS velocity, the damping coefficient should be found. The dynamic stability of the buoy should be increased by adding fins to the sides of the tube maximizing drag in the x-y plane. In addition, the bottom of the buoy should have a hydrodynamic shape that would decrease added mass and viscous forces in the z-axis. The combination of design changes would decrease rolling and increase the response amplitude of the buoy. Lastly, a way should be found to increase the range of wave periods in which the power output is high enough.

To make this device even more appealing, the cost could be decreased further by using white PVC tubing as well as a series of smaller less expensive magnets. Looking at this device in perspective, the success and transition to wave energy generators lays on how competitive the cost per kWh is, compared to fossil fuels.

5. Conclusion.

Harnessing energy from sustainable resources, while gaining cost effective power is one of the most challenging engineering and technological problems of our time. Ocean waves represent our world's last untapped renewable energy resource that could offer a vast source of clean electricity. There are many designs that convert wave energy to electricity, but our focus was on resonant devices. The objective of this project was to develop a simple, inexpensive device that would demonstrate energy conversion and would teach the physics principals that allow this to happen while going through the process of making it. The process not only would solidify physics concepts, but hopefully inspire students to get involved in renewable energy.

Overall the project goals were achieved. The resonant device demonstrates energy conversion by generating an instantaneous power output of 0.056 Watt at a wave period of 1.65 seconds, resulting to the periodic lighting of the LED. The numerical models helped determine the components needed to make this happen although the experimental power output was lower than calculated by 96.95%. This indicates that including damping forces to future analysis would close the gap between theory and experiment. The design is as simple as possible making it easy to reconstruct, while being below the \$100 budget line. Lastly, physics concepts such as vibrations, electromagnetism, and buoyancy can be taught through the construction of this buoy, making it an ideal science project.

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Analysis of coastal surface winds

Kayla Flynn, John Merrill, Kelly Knorr

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. In this study, we present current analyses of surface wind speeds (SWS) for Buzzards Bay, Massachusetts and T. F. Green Airport, Rhode Island. Analyses focus on temporal trends, with a peek at spatial variation in the long term datasets. The datasets come from the National Oceanic and Atmospheric Administration (NOAA), which observes wind speed at a single height level. Gap years and missing data are taken into consideration and are quite common. The two-parameter Weibull distribution is used to characterize the mean hourly wind data at the surface for Buzzards Bay. Seasonally at Buzzards Bay, it is shown that mean winds are stronger in the autumn months than in summer months. The terrestrial site of T.F. Green Airport show annual trends of SWS decreasing in the 1984-2007 dataset. Off shore and on shore observations will also be compared in this context. Further analyses of more stations with longer datasets will increase knowledge of the distance scale of land and sea breezes on coastal surface winds, which could lead to more accurate air temperature and cloud coverage forecasts.

1. Introduction.

Surface winds are a fundamental meteorological variable, driven by pressure gradients, the Coriolis force, and surface friction (He et al., 2010). Winds are central to the global climate system which describes climate change and variability, and influences key aspects of the terrestrial environment. A reflection of atmospheric circulation, surface winds transfer heat and moisture between the earth's surface and the atmosphere, and from one place to another (Wan et al., 2010). Near-surface winds also drive ocean currents, initiate convection through surface convergence and divergence, and impart stress onto the underlying surface (Capps and Zender, 2008).

Several studies in climate literature have already analyzed SWS. For example, Klink (2002) characterized near-surface wind climatology, variability, and long-term trends; while Smits et al. (2005) and Yan et al. (2002) have examined extreme SWS, which could be directly linked to intense storms and even natural disasters. There are also several studies of SWS for purposes of wind energy generation (Wan et al., 2010).

One aspect of wind variability that we know little about is how and why wind speeds vary from one year to the next. Research studies have shown using both climatic model simulations (Yin, 2005) and surface observations (Klink, 2002) that the position of the main storm tracks that cross Northern America, which are linked to the jet stream, have moved northward (Iacono, 2009). This research may lead to a better understanding of the decrease in SWS. Another aspect of wind variability is seasonal change. According to Klink (2006), in Minnesota at 70 meters above the ground, regression on wind speed residuals (pressure gradient effects removed) shows that an additional 6%-15% of the variation can be related to

the Arctic Oscillation and sea surface temperature anomalies. In this study, the terrestrial site (T.F. Green Airport) and the marine site (Buzzards Bay) in coastal New England are compared to analyze the trends at two relatively close stations. Significantly, T.F. Green data shows a decrease in yearly wind speeds, with reasons unknown. This decrease occurs in all seasons, with the maximum decrease during the autumn months. The analyses of these statistics will be important for wind power estimation, wind risk assessment, and surface climatology. Thus, it is of great interest to analyze the historical record of coastal New England's SWS, to see whether these changes are consistent with the reported trends in other climate variables during the same period. These questions motivate the current study.

2. Wind data.

Anemometers at two locations in coastal New England (Buzzards Bay, Massachusetts and T.F. Green Airport, Rhode Island) measure SWS in meters per second (m/s). SWS are often transmitted in real time to the operational numerical weather prediction centers for incorporation into their global analyses (Large et al. 1995). NOAA's branch, National Climatic Data Center (NCDC), receives these observations and is the world's largest active archive of weather data. NOAA's public data for surface winds are used for the current analyses.

The National Data Buoy Center (NDBC) provided the long term hourly data for the Buzzards Bay SWS. From 1985-1994, continuous wind speed observations were recorded once per hour, while from 1997-2011 data was recorded six times per hour (every 10 minutes). Although there are some gaps in the time series, this 22 year off shore dataset has undergone thorough quality checks and represents a substantial quantity of wind information. A propeller

Figure 1. Location of the two towers. (o) T.F. Green in Providence, Rhode Island (+) Buzzards Bay, Massachusetts.

anemometer is used to survey the SWS at this tower, which is located 24.8 meters above the ocean surface; this is much higher than the World Meteorological Organization (WMO) standard height for wind measurement devices of 10 meters.

NCDC collected 24 years of continuous wind speed data for T.F. Green Airport from 1984–2007. The measurements were recorded at a 6.1 meter height from 1984–1995, but the height increased to 10 meters high from 1995–2007. Representative measurements require the anemometer be located at a height where wind will not be affected by nearby objects. This change in height will be discussed further when talking about decreasing annual trend at this site. Also, we had easy access to the observed daily mean SWS in this dataset, which will be compared to the hourly mean SWS data observed at the Buzzards Bay site.

The two locations, Buzzards Bay, Massachusetts and T.F. Green Airport in Providence, Rhode Island, were chosen because their continuous data consists of more than twenty years and both are located in coastal Southern New England. Although the period of record is short relative to typical climatologically analyses (30 yr of data or more), it is comparable to record lengths used for wind energy analysis (Klink, 2006).

3. Methods.

In this paper, data from T.F. Green and Buzzards Bay are analyzed using MATLAB programming. Figure 1 shows a Google map of the locations. T.F.

Green Airport (o) has a tower located on land in Providence, Rhode Island close to the coast. In Buzzards Bay, Massachusetts (+), there is a tower in the ocean collecting hourly SWS. When analyzing seasons in this study, we decided each season should consist of three entire months starting at the beginning of each year. For example, the winter season months are January, February, and March.

For Buzzards Bay hourly mean dataset, seasonal trends were analyzed using the probability density function (PDF). The PDF, Weibull distribution, is displayed with the histogram of the hourly SWS for the specific season. The two-parameter Weibull distribution has been suggested as a good empirical representation of surface wind speed PDF over land and sea. Currently, the Weibull distribution is the most widely used PDF for applications in observational based wind power estimation over the globe, wind risk assessment in the United Kingdom, hurricane wind intensity estimation in the United States, and downscaling of wind speed in northern Europe (He et al. 2010). A time series plot with a mean value line brings focus to the variation of seasonal SWS at Buzzards Bay. The study focuses on seasonal trends at the Buzzards Bay site.

The T.F. Green dataset supplied mean daily SWS in m/s. SWS are observed at different heights, which is not expected to introduce important variations when plotting. A box and whisker plot shows the dominant wind speeds from a long time series of observations from 1984–2007. This initial box and whisker plot

Figure 2. Comparison of 2004 seasons at Buzzards Bay. (a) summer Weibull distribution (b) autumn Weibull distribution (c) summer time series with mean (d) autumn time series with mean.

shows a slight decrease in annual SWS. To examine this trend closer, a time series plot with a trend line is graphed using daily mean values for each year in the dataset. To take this one step further, another time series with a trend line is analyzed using annual mean values for each season.

The analysis of T.F. Green differs from Buzzards Bay. We use the observed daily means at T.F. Green, while hourly means are used at Buzzards Bay. Thus, there are more data points for the Buzzards Bay site than the T.F. Green site. An hourly mean box and whisker plot and a daily mean box and whisker plot are analyzed to find how this affects the accuracy of graphs. Missing data points in both datasets were discarded to create a more efficient working program in MATLAB. Also, means were calculated only when missing data composed fewer than 25 percent of the hourly SWS. The Buzzard's Bay years of 1985 and 1994-1997 were discarded completely for this study.

4. Results and Discussion.

4.1. Buzzards Bay, MA

Data from the NDBC was used to analyze the seasonal trends at the off shore Buzzards Bay tower from 1985-2011, discarding 1985 and 1994-1997. An example of seasonal trends at this site is shown in Figure 2. The top Weibull plots (a&b) are examples of

comparing seasonal trends for a year. SWS in m/s are located on the x-axis, while frequency is on the y-axis for both plots for the 2004 year. Observed SWS during the summer months are the lowest, while autumn months tend to have the strongest wind speed at this off shore tower. The bar graphs represent the histogram or how many data points occur at that speed. The maximum of the Weibull fit represents the average value for the x-axis. Summer (a), has a maximum at 6.09 m/s, while the maximum for autumn (b) is 8.52 m/s. In Figure 2, the time series plots with seasonal SWS are on the bottom, which is another way to focus on seasonal trends, but with an emphasis on the mean value (line). Julian days are located on the x-axis, while wind speed m/s is on the y-axis. These time series plots show the autumn mean SWS to be 2.43 m/s greater than the mean for the summer months. This is due to the movement of the jet stream from season to season. The summer season has lower SWS on average because the jet stream is poleward. When the jet stream moves south of New England, more Low Pressure systems develop. Stronger winds are associated with Low Pressure systems, so autumn and winter months, specifically December and January, tend to have higher SWS at this site.

Future steps at this site should be conducted. The seasonal trends at Buzzards Bay should be studied continually. A change in SWS may bring about other changes, such as the Jet Stream or sea surface

Figure 3. Annual trend at T. F. Green Airport. (top) box and whisker (bottom) mean annual values as bullets with trend line.

temperatures. In the next decade, additional data will be available to find trends, which may alter these results or verify this study.

4.2. T.F. Green Airport, RI

Analyzed data from NCDC show an annual trend at the on shore T.F. Green airport tower from 1984-2007. In Figure 3, the top plot is a box and whisker, where the red line represents the median, the

black line with a bullet represents the mean, and the red plus signs are the outliers. Years are on the x-axis, while wind speeds (m/s) are on the y-axis. The plot displays a slight decrease in the mean and median values from 1984-2007. So, to take a closer look at the decrease, a time series using daily mean SWS is plotted below the box and whisker in Figure 3. The bullets represent the mean value, while the line represents the trend. The trend shows a decrease of .852 m/s. From 1960-1995 the anemometer was at 6.1 meters

Figure 4. Blue Hill Observatory annual mean wind speed and running means.

above the ground, but the height changed to 10 meters from 1995 onward. The closer the anemometer is to the ground, the slower the wind speeds will be due to an increase in friction. This study hypothesizes that the annual decrease may be greater than .852 m/s, and possibly reaches 1 m/s. The reasons for the decrease are unknown. Iacono (2009) hypothesized that the annual mean decrease at the Blue Hill Observatory in Figure 4 was due to an increase in vegetation and the northward movement of the jet stream. Blue Hill Observatory is a terrestrial site located in Milton, Massachusetts. The years are on the x-axis and wind speed in m/s and miles per hour are located on the y-axis. The focus on this picture is the decrease speeds, and also that he has a longer data set than this study, but has similar results. This dataset had a slightly higher decrease from 1980-2008 of 1.2 m/s.

Are the annual SWS decreasing for every season, or is there one season that has a dramatic decrease in SWS? In Figure 5, a time series plot for each season shows that SWS are decreasing for every season. The bullets represent the annual mean wind speed for that season, while the line represents the annual trend for that season. The x-axis holds the years for the entire dataset, and wind speed in m/s is on the y-axis. Winter (a) has the lowest decrease of .737 m/s for the 24 year time scale. Winter month also have the strongest SWS at this site. Spring (b) has a decrease of .826 m/s, while summer months (c) decrease .864 m/s for this time scale. The SWS decreased the most during autumn months (d) at .955 m/s; also, SWS show the greatest deviation from the trend line during the autumn months of October,

November, and December. This decrease in annual wind speed should be studied more closely with additional years as time passes. This trend will have a great impact on the weather and wind farms around coastal New England in the future.

4.3. Terrestrial vs. Marine

A comparison of a terrestrial site (T.F. Green Airport) and a marine site (Buzzards Bay) in coastal New England is studied to contrast the difference in apparent trends at nearby stations. The pressure gradient force is linked to temperature, which changes more on land than sea because of land's specific heat is much smaller than the ocean's. This causes diurnal SWS to vary more at the terrestrial site. Analyses results show strong winds at the marine site due to less surface friction impacting SWS. Buzzards Bay data shows steady, stronger winds, while T.F. Green SWS are weaker and more variable.

In Figure 6, Windographer is used to create another plot, which displays the difference between a terrestrial and marine site. Windographer is a good tool in wind resource assessment. The marine site is located at Camp Cronin in Narragansett, Rhode Island, while the terrestrial site example is located at Fields Point in Providence, Rhode Island. Even though these sites are not at the exact locations of the two towers used in this study, they can be used to represent the average diurnal changes. The x-axis represents hours, and the y-axis is wind speed in m/s. The marine site is indicated by blue bullets and has higher SWS, but less variability in near-surface wind speeds. The terrestrial

Figure 5. Seasonal trends at T.F. Green using mean values as bullets with trend line. (a) winter (b) spring (c) summer (d) autumn.

site is indicated by red bullets and has a lower SWS and more variability, which is due to the explanation above.

4.4. Averaging wind data

The anemometer doesn't record calm winds, because the instrument needs a strong enough force from the wind to move the propellers to record observations. A dataset will have a recording of 0 m/s, which indicates no wind or calm winds. It is unusual to have an anemometer record wind speeds of 0 m/s for an entire day; thus, the box and whisker error bars will be shorter than the error bars for hourly data. There is a significant difference between daily means and hourly means, which affect the appearance of most plots. The daily mean values smooth out SWS, which decrease the number of outliers. In Figure 7 we compare the averaging. The example box and whisker plots are taking from the year 2005 at Buzzards Bay. With a focus on the central plot (b), there are more outliers (red plus signs) and the error bar reaches the bottom of the y-axis to represent 0m/s SWS. The central plot (b), hourly means, has more data points than (a) daily means. Plot (c) compares (a&b) by overlapping them. It is clear that the blue box, which represents the 25th to 75th percentile, increases in size for hourly mean SWS, which occurs because there are more data points. Accuracy increasing in a plot with more data points.

Figure 6. Diurnal profile. Camp Cronin in Narragansett, Rhode Island and Fields Point in Providence, Rhode Island.

Figure 7. Box and Whisker comparison of daily means and hourly means. (a) daily means; (b) hourly means, (c) combination of the two.

5. Conclusion.

Analyses of trends at T.F. Green Airport in Providence, Rhode Island and Buzzards Bay, Massachusetts were conducted. Using long records of observations from NOAA, seasonal trends at Buzzards Bay are shown using the two-parameter Weibull distribution and time series plots. SWS for autumn are greater than in the summer months of July, August, and September due to the movement of the jet stream. T.F. Green box and whisker plot and the mean value plot with trend lines show seasonal and annual trends. From 1984-2007, SWS at T.F. Green have decreased by .852 m/s. Seasonally, SWS for autumn have the greatest decrease of .9552 m/s. The reasons for the decrease in SWS are still not completely understood. Hypotheses include: jet stream movement to the north, an increase in afforestation, and climate change.

For coastal regions, SWS affect regional wave conditions and coastal erosion processes, and contributes to surges, which may cause flooding along coastlines (Wan et al., 2010). Changes in winds imply associated changes in atmospheric circulation, which are an integral part of climate variability and climate change. This study of SWS gives evidence of a decrease in annual SWS in Providence Rhode Island. Other regional decreases in the United States have been hypothesized (Klink, 2002; Iacono 2009), but more studies with longer datasets and at different locations must be conducted in the future.

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- J. Merrill and K. Knorr, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (jmerrill@gso.uri.edu, kellyireneknorr@gmail.com)
- K. Flynn, Lyndon State College, Lyndonville, VT, 05851. (kayla.flynn@lyndonstate.edu)

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Using Kinect to measure wave spectrum

Josephine Fong, Brice Loose, and Ann Lovely

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. Gas exchange at the air-sea interface is enhanced by aqueous turbulence generated by capillary-gravity waves, affecting the absorption of atmospheric carbon dioxide by the ocean. The mean squared wave slope $\langle S^2 \rangle$ of these waves correlates strongly with the gas transfer velocity. To measure the energy in capillary-gravity waves, this project aims to use the Microsoft Xbox Kinect to measure the short period wave spectrum. Kinect is an input device for the Xbox 360 with an infrared laser and camera that can be used to map objects at high frequency and spatial resolution, similar to a LiDAR sensor. For air-sea gas exchange, we are interested in the short period gravity waves with a wavenumber of 40 to 100 radians per meter. We have successfully recorded data from Kinect at a sample rate of 30 Hz with 640x480 pixel resolution, consistent with the manufacturer specifications for its scanning capabilities. At 0.5 m distance from the surface, this yields a nominal resolution of approximately 0.7 mm with a theoretical vertical precision of 0.24 mm and a practical 1σ noise level of 0.91 mm. We have found that Kinect has some limitations in its ability to detect the air-water interface. Clean water proved to be a weaker reflector for the Kinect IR source, whereas a relatively strong signal can be received for liquids with a high concentration of suspended solids. Colloids such as milk and $\text{Ca}(\text{OH})_2$ in water proved more suitable media from which height and wave spectra were detectable. Results from monochromatic wind waves yield predictable wave speeds and a concentrated power at low frequencies. In comparison, laboratory generated wind-waves produced a broader power spectrum at capillary and gravity frequencies.

1. Introduction.

The ocean acts as a natural carbon sink, absorbing about 35% of anthropogenic carbon dioxide emissions annually. However, the mechanisms by which carbon dioxide gets into the ocean are still poorly constrained, making it difficult to predict how ocean absorption will change over time. Air-sea gas exchange is one such important mechanism, and the rate of exchange is highly variable and difficult to predict. Many challenges are faced in measuring gas exchange in the open water. Factors such as turbulence, waves, and bubbles enhance gas transfer, whereas surfactants make it more difficult for gases to move across the air-sea interface. For this reason, precise measurements of gases in the ocean are needed to calculate the gas transfer velocity of carbon dioxide.

To better estimate the rate of air-sea gas exchange, we need to measure processes that are most directly related to the production of turbulence in the water. Small deformations in the water surface cause such turbulence and can be used to explain the rate of gas exchange. However, these waves have proven difficult to observe, and currently there are no acceptable methods for measuring small surface waves. To address this observation gap, we have attempted to use Microsoft's Xbox Kinect. Kinect is an input device for the Xbox 360, a video game console developed by Microsoft that can be used to map objects at high frequency and spatial resolution, similar to a LiDAR sensor. Kinect was used to

measure waves and obtain depth data from its infrared sensor and camera. Recordings of depth allowed us to generate the height spectrum of the waves, from which we can calculate the slope spectrum. This will allow us to calculate the mean square slope $\langle S^2 \rangle$, which is strongly correlated with the gas transfer velocity (Frew et al., 2004).

2. Methods.

We used the Microsoft Xbox Kinect to obtain depth measurements from the sensor. Kinect, an input device for the video game console Xbox 360, contains an infrared projector, an RGB camera, and an infrared camera (Figure 1). Scanning at 30 Hz, Kinect has a high resolution of 640x480 pixels and a resolution of approximately 0.7 mm for objects half a meter away (Mankoff et al., 2011). Moreover, Kinect has a theoretical precision of 0.24 mm, storing data in 2^{11} bits, and a practical 1σ noise level of 0.91 mm found by taking the standard deviation over 69 frames in 2.3 seconds. Kinect works by sending a beam of infrared light into the domain of view and measuring the time it takes to return after bouncing or reflecting off objects. Using time-of-flight, Kinect can generate a depth map of the viewing domain. Since Kinect's infrared system can capture data at high frequency and spatial resolution, Kinect can be used as a LiDAR sensor for a fraction of the cost of LiDAR.

Kinect was connected to a standard USB port on the computer to capture data directly from the sensors.

Figure 1. Microsoft's Xbox Kinect contains an infrared projector, an RGB camera, and an infrared camera. This project uses the depth recordings generated by the infrared projector and camera.

Figure 2. This is the setup of the experiment, where Kinect is fixed 0.5 m above the tank. On the left is the panel used to generate waves, and on the right we added sand to create a beach-like effect to minimize reflection of waves from the other end of the tank.

The advantage of using Kinect is the open source software available after Microsoft open sourced the device, which led to new applications using Kinect. This resulted in free software and open source libraries that have been made available by developers to use and record directly from the sensors. We used the software libfreenect from Openkinect to view and record data. With libfreenect, we were able to view both the infrared and RGB cameras using a program called glview. To record data at the sample rate of 30 Hz, a solid state hard drive or temporary ramdisk must be created due to issues with memory. We looked at recordings of the depth files, which were in binary, and converted them to distance in millimeters using the source code for offline registration to obtain a frame of view with pixel values in 640x480 matrices, consistent with the resolution. This allowed us to read the data into MATLAB, which was used to process

and analyze the data.

Prior to measuring waves, we accounted for noise and distortion from Kinect by setting up the device 0.5 m away from the floor. After leveling the Kinect to the best of our abilities, we recorded a baseline reading. We were able to capture the noise from Kinect by taking standard deviations of each pixel in the domain in time (Tetsu Hara, personal communication). For distortion, we placed an object at various locations on the floor to observe whether the object would be distorted throughout the domain.

Afterwards, we fixed the Kinect 0.5 m away from a 200 cm x 17 cm x 20 cm tank (Figure 2). On one side of the tank, we had a paddle that was used to generate waves. On the opposite side of the tank, we tried to remove some of the reflection from the opposite side of the tank by adding sand to create a beach effect. In the tank, we generated waves in the

milk since water and other liquids proved too reflective and transparent for Kinect to obtain a clear signal (See Section 3.2). We used the paddle of the tank to generate monochromatic waves and a fan to produce wind waves.

3. Results and Discussion.

3.1. Noise and Distortion

Since electronic devices can be very noisy, we observed how values in each scan changed over time. To estimate the noise, we used the 1σ standard deviation in time for each pixel of the domain. An example of this is shown in Figure 3, where the standard deviation was calculated over 69 scans in 2.3 seconds. These figures indicated relatively low noise. The center of the Kinect field of view showed the lowest standard deviations close to 0 with the majority of surrounding values around 1 mm. This can also be seen in the histogram in Figure 4, where there is a bell-shaped curve around 1 mm. Toward the borders of the domain of view, the standard deviations increased to 2 mm, and around the edges we saw a maximum standard deviation of 5 mm.

Figure 3. This is the 2-dimensional standard deviation in time of the floor using 69 frames in 2.3 seconds. The middle of the domain shows an average standard deviation of 1 mm and increases towards the edges of the domain, which indicates the Kinect works best for objects directly under the sensor. The white area in the center indicates pixels of no data due to the blind spot in Kinect.

In the recordings for Kinect, we often observed areas of no data. The last eight columns of Kinect always had no data, which were removed. In the center of the domain, there was typically a blind spot of a circular shape about 2 cm in radius. There were also pixels of no data randomly dispersed throughout the domain. To account for these, we took the column average to replace the missing data points since the column average is close to the actual value since the waves were propagating in the horizontal direction.

Although we used a level to make sure that Kinect was perpendicular to the ground, recordings of flat surfaces indicated slight slopes. Kinect is very difficult to level exactly and therefore requires detrending. To detrend the data, we assumed a linear relationship between the depth data (z) and the horizontal x and y directions. During each unique measurement circumstance, we obtained a baseline for the domain of view and then subtracted the data from the baseline for each recording to remove any slopes.

To check for distortion, we moved a cylinder of constant height in the domain to see how Kinect read the height of the object and whether the apparent height varied depending on the position of the object within the view frame. We moved a jar around the domain, from the center to each of the corners, to check for possible distortion. After making each of these recordings, we detrended the data and observed no clear pattern indicating distortion. For a jar about 9 cm tall, we obtained recordings of 9.5, 9.8, 9.6, 9.1, and 9.8 cm, showing a maximum of about 6 mm differences in height when measured at various locations but no general trends. The lower and upper left showed a 6 mm difference, while the lower and upper right showed no difference. Between the left and right, we found a 3 mm difference. We did not find any obvious trends in the x or y directions.

3.2. Test Solutions

When the tank was filled with clear water, Kinect was unsuccessful in capturing data. Water was too transparent and reflective for Kinect to obtain a clear signal of the surface. When the water was shallow and still, Kinect saw through the water and recorded the distance to the bottom of the tank rather than the distance to the surface of the water. In deeper water, Kinect had no return signal. Yet when an object was dropped in shallow water, Kinect was able to see some of the ripples of the water. This may be a result of absorption of the laser in water or some sort of scattering or reflection that occurs on multiple returns and will require further study.

Adding dye to the water did not improve Kinect's signal, as the water was still too transparent. We then added lime to the water to create a more opaque solution, and it was apparent that particles suspended in the water tended to enhance the signal return. Kinect was able to see the surface of the lime water solution, but the particles settled quickly, making it difficult to capture the waves before the water became clear. Similarly, cornstarch added to water made a cloudy solution, but resulted in a noisy signal due to particles settling quickly.

Milk was a successful test liquid. Kinect was able to see the milk surface in the tank and generated a relatively clean signal due to the particle suspension. Particles in milk remain distributed evenly throughout the liquid, making it a great colloid for the experiment.

Figure 4. The histogram shows the distribution of standard deviations across the same domain and frames as Figure 3. In the histogram, there is a normal distribution about 1 mm.

Figure 5. The left panels show the plan view of the wave after detrending. Next to these figures on the right are the corresponding side views of the waves.

3.3. Monochromatic Waves

We generated monochromatic waves in the tank using the paddle attached to one end of the tank. In Figure 5, we show frames of the waves propagating horizontally to the left through Kinect's domain of

view at 0.5, 1, and 1.5 seconds. The left panels were obtained after detrending the data and accounting for areas of no data, as described in Section 3.1. The figures on the left show a plan view with color shading to indicate depth in millimeters, using depth

Figure 6. The 2-dimensional FFT is shown with the highest power in the first wavenumber. The power drops in the second and third wavenumber, and there is almost no power elsewhere.

measurements from Kinect. In the right panels, we see the side view of the waves at these instants.

Given the difficulty interpreting the results of the Kinect measurements in seeing ripples in shallow water (as discussed in Section 3.1), we wanted to confirm the depth output of Kinect with the basic physical properties of waves. To check with the theoretical properties of waves, we created a wave crest-tracking algorithm in order to estimate the velocity using the travel time approach. From this, we calculated a velocity of 0.76 m/s. The theoretical velocity is calculated using the shallow water wave speed equation, $c = \sqrt{gh}$, where g is gravity and h is the height of the water. We calculated a theoretical velocity of 0.95 m/s, which indicates a 20% difference compared to the velocity calculated from measuring wave frames and may be due to the fact that we only used the paddle once to generate the waves, so they may not be fully developed gravity waves.

To develop a physical intuition of the information in a multi-dimensional Fourier transform, we applied a 2-dimensional discrete Fourier transform (DFT) on

wave frames that were detrended to obtain the wave height power spectrum. As expected, we found that most of the power is concentrated in low frequencies. In Figure 6, we have a 2-dimensional FFT plot taken at 0.5 s, which captures a frame of the monochromatic wave. All of the power lies in the x direction since the wave is propagating in one direction in a narrow tank, which confirms that the power of the wave spectrum is higher than the floor baseline. We see that the most power lies in the first wavenumber. There is also noticeable power at the second wavenumber and almost no power elsewhere. Where the most power lies, we calculate a wavenumber of 19.6 and 39.3 rad/m, corresponding to wavelengths of 0.32 m and 0.16 m, respectively.

3.4. Wave Spectrum Under Wind Conditions

Our final step in evaluating Kinect's ability to capture waves was to measure wind waves. We placed a fan above one end of the tank to generate wind waves in the capillary-gravity range. The velocity of the wind was 3 m/s, which was measured using a

Figure 7. The left panels show the plan view of the wind waves after detrending. Next to these figures on the right are the corresponding side views of the waves.

Figure 8. The 2-dimensional FFT shows power in both the x and y directions. The power is at higher frequency than for that of the monochromatic wave FFT.

sonic anemometer. We produced high frequency wind waves in the capillary range with the hope that Kinect would be able to record these wind waves and not interpret them as noise. In Figure 7, we see frames of the wind waves at 0.5, 1, and 1.5 seconds. However, there are anomalies in the data at 100 and 450 pixels

Figure 9. This is the 1-dimensional FFT obtained by taking the power in the x-direction, which shows four large peaks that indicate wavenumbers in the capillary-gravity range.

that require further investigation.

In Figure 8, we see the 2-dimensional DFT of a frame that has been detrended, and we can see power in all directions. If we take a look at the horizontal 1-dimension DFT, we found in Figure 9 the wavenumbers the four highest powers: 78.50, 137.4,

186.3, and 235.6 rad/m, which all fall in the range of 40 to 800 rad/m for capillary-gravity waves (Frew et al., 2004). This corresponds to wavelengths of 8.0, 4.6, 3.4, and 2.7 cm.

In comparison to monochromatic waves, we see a much larger range of frequencies. The power is much higher for wind waves than from monochromatic waves, and we also see power distributed in the y-direction whereas power was only along the x-axis for monochromatic waves.

4. Summary.

Kinect shows relatively low noise and has a sample rate sufficient to measure capillary-gravity waves. However, Kinect is limited in its capacity to measure water waves, which indicates that it may not be practical for field experiments. However, we can apply the same method of calculation to find the wavenumbers and power spectrum. At the same time, we must take into account the slight difference in viscosity between milk and water. Moreover, Kinect has low infrared power due to safety issues, as the main objective of Kinect is to be used for video games that constantly scan movement of players in the game.

We hope to extend these studies to make simultaneous measurements of gas exchange and capillary-gravity wave spectrum to observe whether we can reproduce prior correlations (Frew et al, 2004). Moreover, we hope to continue to investigate other instruments that are similar and could be used as LiDAR. We will transfer these methods by taking observations and calculating expected gas exchange with mean squared slope. While we have confirmed

that Kinect is successful in its ability to sample at high temporal and spatial resolution, the problem of directly measuring wind waves in the field remains unsolved and will require further study.

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- B. Loose and A. Lovely, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (brice@gso.uri.edu, ann_lovely@my.uri.edu)

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The role of pressure on polycyclic aromatic hydrocarbons' partitioning into polyethylene passive samplers

Maureen Haynes, Zoe Ruge, Rainer Lohmann

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. Persistent organic pollutants are a growing problem in the environment, specifically polycyclic aromatic hydrocarbons (PAHs). PAHs are hazardous atmospheric pollutants, known to be carcinogenic, mutagenic, and teratogenic. A variety of factors, including temperature and salinity, affect the partitioning of PAHs between polyethylene (PE) and water. Passive samplers will be used to accumulate PAHs for analysis. Passive samplers are deployed for an extended amount of time based on accumulation of PAHs in PE strips due to molecular diffusion. However, the effect that depth and pressure play on partitioning of PAHs is unknown. This project will explore differences in partitioning of PAHs under various pressures to see if samplers deployed at greater depth display differences from those at the surface. PAHs in the sample will be analyzed by gas chromatography coupled with mass spectroscopy (GC/MS).

Studying the impact pressure has on the partitioning of PE is important to understand behaviors of contaminants, such as PAHs, at various depths with passive samplers. An initial hypothesis is that, as pressure increases concentrations of PAHs would decrease because of compression of the polymer matrix. By performing this experiment, we hope to draw conclusions on the impact pressure has on polyethylene passive samplers and PAHs. We found that for several PAHs, the concentration in the PE increased from 4000 psi to 5000 psi. Yet, a few of PAHs did not follow this trend and compared to the ambient pressure samples the partitioning was not consistent. Overall, there was no significant effect of pressure observed, suggesting that the partitioning of PAHs is not affected by pressure. In this experiment, the temperature of the water inside the pressure device was not taken into account, so in the future temperature should be incorporated into the analysis of the data.

1. Introduction

There are over one hundred types of polycyclic aromatic hydrocarbons (PAHs) found in the environment. This experiment will look at eleven, naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, retene, benz(a)anthracene, and chrysene. The growing presence of PAHs in the environment has caused an increase in air pollution and negative implications for aquatic life and humans. PAHs can enter the air by forest fires or car exhaust. With increasing exposure, both manmade and natural, humans and aquatic life are more at risk. In aquatic life, PAHs can cause liver problems, decreased growth, and increased death rate. The impacts PAHs have on humans depend on the time of exposure and concentration (Buha 2011). The U.S. Environmental Protection Agency (2008) has listed seven different PAHs as possible human carcinogens.

In this experiment, the role of pressure on PAHs partitioning in polyethylene (PE) passive samplers will be studied at four different, 14.7, 2000, 4000, and 5000 psi. Six samples will be tested under each pressure experiment, five samples containing PAHs and one blank. Each experiment will run for 16 days

to ensure equilibrium between the PE and the solution. The desired pressure will be acquired by the use of a pressure device. The PE will then be extracted with ethyl acetate, evaporated using a nitrogen turbo-vap and analyzed by gas chromatography coupled with mass spectrometry (GC/MS).

In the past, experiments have been done seeing how temperature affects the partitioning of PAHs. It was determined that as the temperature decreases, the partitioning of PAHs increased (Khairy 2012). Pressure and depth though have never been analyzed. Pressure is important to consider because pressure is not constant throughout the ocean, for every ten meters below sea level one atmosphere of pressure is added. In the past, experiments with passive samplers have only been done on the surface or shallow depths, but we want to deploy at greater depths. With this in mind, we need to know if pressure has any impact on the partitioning of PAHs, specifically in regard to the PE matrix and how susceptible it is to pressure increases. Such effects could change the water-PE partitioning value, which would need to be considered when calculating actual water concentrations

2. Experimental Setup.

In this experiment, four different pressures were tested, 14.7, 2000, 4000, and 5000 psi. Their corresponding depths were 0, 1360, 2720, and 3400 meters below sea level, respectively. Three main points went into creating this experiment, the use of polyethylene passive samplers, the preparation of the samples, and the use of a pressure device.

Two main methods when researching organic contaminants are, active samplers and passive samplers. In this experiment, polyethylene (PE) passive samplers were used. Passive samplers enable a longer deployment and do not require an energy source. Passive samplers are based on the accumulation of PAHs in the sample into the PE strip, which continues until equilibrium is reached. Performance reference compounds (PRCs) were used to determine when equilibrium was reached (Vrand 2005). PRCs are deuterated compounds that are manmade and not found in the environment. In general, the PRCs are added before deployment and equilibrium is reached when all PRCs have exchanged into the water body indicating that what is on the PE was equivalent to what is in the water. This experiment though had a fixed sample volume, meaning the PRCs would still be present in the PE strips and milli-Q water.

Figure 1. Amber and clear bottles used for pressure experiments.

Figure 1 shows the vials used to prepare the passive samplers, the amber bottles were used for the ambient pressure experiment while the clear bottles were used for the pressure experiments. Amber bottles were used for ambient pressure because PAHs can photodegrade when exposed to ultraviolet radiation or sunlight. Clear bottles were used for the pressure experiments because of the lids that accompanied the bottles. Figure 1 shows how the blue lids had a septum enabling the bottles pressure to equilibrate with the outside pressure. When the amber bottles were first tested, the bottoms would break off when pressure was applied. The volumes of the amber and clear bottles were 2.26 in³ and 2.94 in³, respectively. In each

experiment, the bottles were filled to the top, ensuring no air bubbles were present. Six samples were run in each experiment, five containing PAHs and one blank containing plain milli-Q water. 0.014 g of PE with a thickness of 17.78 mm was the desired amount of PE needed for each bottle; the corresponding area of PE needed was 1.20 in², giving dimensions of approximately 3.75 in x 0.375 in. The PE strips were pierced onto a metal wire to ensure maximum surface contact between the PE and the PAH solution.

Figure 2. Pressure device used to hold samples at desired pressures.

After preparing the samples, they were placed in the pressure device. Figure 2 shows a depiction of the pressure device. The six samples were placed in the silver tube and the remaining of the tube was filled with water. The valve on the left was then opened and the wheel was spun to the left adding water into the system. Once the wheel was completely rotated that valve was closed and the valve on the right was opened. The wheel was then spun in the reverse direction adding water to the silver tube and increasing the pressure. This process was repeated until the desired pressure was reached. The samples remained under that pressure for sixteen days for the 4000 and 5000 psi experiments, but only twelve days for the 2000 psi experiment. The first pressure experiments initially ran for sixteen days because simultaneously the first ambient pressure experiment was running so a curve could be developed comparing

the concentrations of PAHs versus the day in the experiment. When the concentrations leveled out, equilibrium was achieved.

3. Methods.

Before beginning any experimentation with pressure, the PAH native solution needed to be made. The PAH native solution consists of equal amounts of the eleven PAHs studied in a solution of methanol. The solution was made in methanol because all the PAHs are soluble in methanol. The desired final concentration of the PAH native was 10 $\mu\text{g/mL}$. To begin, 0.01 g of each PAH was measured and put in separate bottles. 100 mL of methanol was added to each, where the solid PAHs completely dissolve giving a final concentration of 100 $\mu\text{g/mL}$. From those solutions, 0.1 mL was measured from each and combined into one vial, making the final volume 1.1 mL. The final mass of each PAH was calculated in nanograms per milliliter (ng/mL) by multiplying the volume of the PAH solution by the corresponding starting concentrations. The final concentration of each PAH in the native was calculated by dividing the final mass by the total volume of the PAH native solution. The concentration of the PAH native was approximately 9 $\mu\text{g/mL}$.

From the PAH native solution, 0.125 mL was added to one liter of milli-Q water, giving an approximate concentration of 1.1 $\mu\text{g/L}$ of each PAH. This solution was used for all samples used throughout the experiment. The bottles used were 30 mL, making the mass of each PAH originally in solution approximately 34,000 pg. The samples were prepared with pre-measured PE strips and either placed under pressure or in a box to avoid sunlight and decomposition of the PE strip.

After the samples had reached their desired number of days, the PE strips were extracted with ethyl acetate. Ethyl acetate was used because it is a strong organic solvent able to remove the PAHs from the PE and put them in solution with the ethyl acetate. The PE was only extracted overnight because if left for a longer period of time the ethyl acetate may start to decompose the PE. Following the extraction, each solution was spiked with 10 μL of a 5 $\text{ng}/\mu\text{L}$ surrogate. The surrogate was composed of four deuterated compounds, acenaphthene d-10, phenanthrene d-10, chrysene d-12, and perylene d-12. The purpose of the surrogate is to act as an internal standard for PAH analysis. This enabled the recovery from the lab extraction to be calculated. The internal standard is a known concentration of the deuterated compounds that are present in each of the samples being analyzed and improves the precision of quantitative analysis. The internal standard behaves similar to the PAHs but provides a signal that is distinguishable from the PAHs. The PE strips were weighed after the extraction

in order to calculate the concentration/g PE for each PAH.

Following the extraction, the ethyl acetate solutions were evaporated using a nitrogen turbo-vap to reduce the volume making it more concentrated. Each sample was spiked with the injection standard, containing p-terphenyl. The samples were analyzed with gas chromatography coupled with mass spectrometry (GC/MS). The GC separated the compounds and the MS identified the ions or ion pairs enabling the identification of the PAHs based on the present ions.

Figure 3. Standard curve for acenaphthene, comparing the ratios of responses and concentration between acenaphthene and acenaphthene d-10.

The PAH standards were the first samples run. Five different standards were run, each with known concentrations of the internal standard, surrogates, PRCs, and natives. The results from the GC/MS were analyzed using ChemStation, where the time and response of each compound was calculated. The retention times are acquired for each of the ions in the standards so when analyzing unknown samples the correct PAH can be identified based on the compound's ion being present at the correct retention time. The responses of the internal standard, surrogates, PRCs, and natives were obtained by integrating their peaks using ChemStation. After acquiring all the retention times, graphs were created comparing the ratio between the response of the surrogate and injection and the ratio between their concentrations. The standard curve of acenaphthene is shown in Figure 3. The response of acenaphthene is compared to the response of the deuterated compound, acenaphthene-d10; concentration is calculated with the same comparison. A linear trendline is applied to the points; the desired R^2 value is 1, for acenaphthene, $R^2 = 0.9972$, implying that the comparison between the ratios will continue to follow this straight line. The slope of the line is essential when calculating the unknown concentrations of the samples. From using ChemStation the responses of both the analyte and the surrogate and the concentration of the surrogate is a

known value, leaving the concentration of the analyte as the only unknown. By rearranging the original equation, the concentration of the analyte can easily be calculated.

Once all of the PAH concentrations have been calculated, the averages and standard deviations of the samples under pressure containing PAHs were determined. The standard deviation is essential in comparing the results from the different pressure experiments. The coefficient of variation for the 5000 pressure experiment was 75%, 45% for 4000 pressure experiment, and 11% for 2000 pressure experiment.

4. Results and Discussion.

The first experiment run was at ambient pressure or sea level. Figure 4a shows the concentration of each PAH in $\mu\text{g/g}$ PE compared to the day the PE was extracted from the sample. For a majority of the PAHs, their concentrations began to level out between days four and ten. Although some of the PAHs did not follow this trend. For instance, benz(a)anthracene had an unusually high concentration at day eight of 8.45 $\mu\text{g/g}$ PE. The concentrations for days four and sixteen had a more linear relationship with concentrations of 2.66 and 1.73 $\mu\text{g/g}$ PE, respectively. Pyrene and fluoranthene both had abnormally high peaks day one, being approximately 8 $\mu\text{g/g}$ PE over the average of the nine other PAHs. This could be due to an incorrect integration of the peaks on ChemStation or contamination in the lab processes.

The ambient pressure experiment was repeated a second time to ensure equilibrium was accomplished and to make sure the PRCs were present. Figure 4b shows a depiction of eight PAHs and their concentration in $\mu\text{g/g}$ PE compared to the experimental day. Only eight PAHs are graphed because the solutions used to make the standard curve did not contain all of the PAHs, but since a majority of the PAHs were present the curves and trends demonstrated are reliable. Aside from anthracene, the other PAHs follow the same trend. The concentration of each increases until day four and then begins to level out. Anthracene though does not follow this trend, and has overall concentrations more than double any other PAH. The concentration peaks at day two with a concentration of 8.38 $\mu\text{g/g}$ PE but then has a drastic drop at day four and levels out by day eight. From this it was concluded that the experiments did not need to be run for sixteen days but instead could be run for twelve days since equilibrium was reached earlier. Therefore, when running the 2000 pressure and second ambient pressure experiments, the experiments were only run for twelve days. The abnormal concentration of anthracene could be due to error in the integration via ChemStation. Phenanthrene

and anthracene have the same molecular weight, 178, implying that they will come through the MS at around the same. But from running standards, we know that phenanthrene will be the first peak to appear followed by anthracene. The peaks though are not very distinguishable from one another and the anthracene peak is more difficult to identify and integrate making a larger margin for error when calculating the final concentration.

Figure 4a. Concentrations of all individual PAHs over the course of the first ambient pressure experiment. Concentrations ($\mu\text{g/g}$ PE) were measured on days 1, 2, 4, 8, and 16.

Figure 4b. Concentrations of a majority of individual PAHs over the course of the second ambient pressure experiment. Concentrations ($\mu\text{g/g}$ PE) were measured on days 1, 2, 4, and 8.

Pressure experiments were run and integrated using ChemStation, their concentrations per gram PE could be calculated. When first comparing the results from the 5000 psi experiment and the sixteenth day of the ambient pressure experiment, little variation occurred between the two concentrations of each PAH. Figure 5a shows a table displaying the molecular weight of the corresponding PAH. Figure 5b is a demonstration of the differences in concentration ($\mu\text{g/g}$ PE) compared to the molecular weight of the PAH. The diamonds represent the measured concentration of the PAHs on the sixteenth day of the ambient pressure experiment. The squares represent the average concentration of each PAH at 5000 psi. There is no continuous relationship between the ambient and 5000 psi concentrations. The black

lines show the standard deviation between the 5000 psi pressure concentration averages. Almost all 5000 psi concentrations had roughly the same concentration as the ambient pressure experiment. Phenanthrene had a slightly higher concentration, 5.17 $\mu\text{g/g PE}$, compared to its ambient pressure concentration, 3.47 $\mu\text{g/g PE}$. When considering the standard deviation of the 5000 psi average the difference is no longer significant. The high standard deviation of phenanthrene at 5000 psi, 4.76, created an overlap between the two pressures proving no significant change from the application of pressure.

Name	Major Ion/MW
Naphthalene	128
Acenaphthylene	152
Acenaphthene	153
Fluorene	166
Phenanthrene	178
Anthracene	178
Fluoranthene	202
Pyrene	202
Retene	219
Benz(a)anthracene	228
Chrysene	228

Figure 5a. Table of PAHs with corresponding major ion/molecular weight.

The comparison between the 5000 psi experiment and the ambient pressure experiment found no conclusive statement of how pressure affects the partitioning of PAHs in PE passive samplers. The 2000 and 4000 psi experiments also confirmed this conclusion. Figure 5c shows the relationship between the three different pressure experiments, 2000, 4000, and 5000 psi. The graph shows the concentration ($\mu\text{g/g PE}$) based on each PAHs molecular weight. The triangles represent the 2000 psi experiment, the squares signify the 4000 psi experiment, while the diamonds were used for the 5000 psi experiment. For a majority of the PAHs, little variation was observed over the three pressures, although some did demonstrate an impact of pressure on the concentrations. Anthracene showed an increase in concentration from 5000 to 2000 psi of 1.45 to 8.45 $\mu\text{g/g PE}$. Even when considering the standard deviation of the two, 1.32 and 0.72, respectively, a clear distinction is clear between the two. The 8.45 $\mu\text{g/g PE}$ concentration of anthracene at 2000 psi though is the only PAH with a concentration higher than five; this could have been done to error in the lab. Figure 5d shows the same graph as Figure 5c but incorporates the standard deviations for each PAHs average concentration. All the standard deviations proved that pressure did not have an impact on the partitioning. The standard deviations lines showed a

Figure 5b. Comparison between concentrations ($\mu\text{g/g PE}$) of all individual PAHs taken on day 16 of ambient pressure experiment and the averages from 5000 psi experiment. Standard deviation lines are shown for the average 5000 psi concentrations. Concentration is compared to the molecular weight of each PAH found in Figure 5a.

Figure 5c. Comparison between the average concentrations ($\mu\text{g/g PE}$) of individual PAHs from 2000, 4000, and 5000 psi experiments. Concentration is compared to the molecular weight of each PAH found in Figure 5a.

lot of overlap between the three pressure experiments, implying no significant effect of pressure.

5. Conclusion.

The growing presence of PAHs in air, water, and sediments has raised concerns for the potential impacts on the environment and humans. The study of PAHs is essential because the seriousness of the consequences they can have on the environment are directly proportional to the concentration of PAHs and the exposure time. PAHs enter the environment both naturally and by human influences. For instance from oil spills, volcanoes, petroleum, and electric power generators. Because of the influence PAHs have studying their presence in different environments is very important.

In the past experiments have been done to see how temperature affects the partitioning of PAHs. It

was found that as the temperature decreased, the partitioning of PAHs increased. (Khairy 2012) Pressure had never been incorporated into the analysis of the partitioning of PAHs into PE passive samplers. In order to be able to deploy at greater depths, the impact of pressure needed to be known so the data could be analyzed effectively.

Figure 5d. Comparison between the average concentrations (ug/ g PE) of individual PAHs from 2000, 4000, and 5000 psi experiments including calculated standard deviations. Concentration is compared to the molecular weight of each PAH found in Figure 5a.

Testing eleven different PAHs at four different depths, 0, 1360, 2720, and 3400 meters below sea level, concluded that pressure and depth do not have a significant effect on the partitioning of PAHs. Although a few instances of specific PAHs showed variations in concentrations with depth, the variations were not substantial because of the standard deviation of the average concentrations. Comparing the ambient pressure experiment to the experiment under pressure, after reaching equilibrium the ambient pressure concentrations were within 30% of the PAHs concentrations under pressure.

In the future, it would be important to incorporate temperature into the PAH analysis because of the known role temperature plays on PAH partitioning and in the ocean as you go deeper, the temperature drops. Also when going deeper into the ocean, less

ultraviolet and sunlight is present. PAHs and PE are both reactive with ultraviolet radiation, so it would be beneficial to consider the amount of ultraviolet radiation present at different depths and the possible implications. Overall, pressure and depth do not extensively impact the partitioning of PAHs into PE passive samplers at room temperature or under the current experimental conditions.

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- R. Lohmann and Z. Ruge, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (lohmann@gso.uri.edu , zruge@my.uri.edu)
- M. Haynes, Manhattan College, Riverdale, NY, 10471. (mhaynes.student@manhattan.edu)

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Environmental parameters affecting the halo-tolerance of the toxic raphidophyte *Heterosigma akashiwo*

Rachel Hickcox, Elizabeth Harvey, Susanne Menden-Deuer

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. *Heterosigma akashiwo* is a toxic, halo-tolerant raphidophyte known to cause harmful algal blooms (HABs) worldwide. The mechanism for bloom formation in this species is largely unknown. Previous research has shown that the growth rate of *H. akashiwo* is constant across a range of salinities given ample light and nutrients. To understand *H. akashiwo*'s halo-tolerance in non-ideal conditions, we measured the growth rates of two different strains of *H. akashiwo* in 5 salinities, 3 light intensities, and 3 temperatures. We also measured cellular biomass, photosynthetic efficiency, and environmental stress responses based upon chlorophyll abundance and fluorescence. Data showed that the highest growth rates of strain 2809 for light and temperature treatments were $0.43 \pm 0.01 \mu \text{ day}^{-1}$ in high light ($100 \mu \text{mol photon m}^{-2} \text{s}^{-1}$) at 15°C . Strain NB grew 9% faster at $0.52 \pm 0.02 \mu \text{ day}^{-1}$ in high light ($100 \mu \text{mol photon m}^{-2} \text{s}^{-1}$) and 15°C . For both strains, growth rate did not differ significantly at 15, 25, and 30 psu while 5 psu had a significantly lower growth rate ($p < 0.001$) and 3 psu had little to no growth ($p < 0.001$). Data also suggests *Heterosigma akashiwo* is unable to survive at high temperatures. Irrespective of other conditions, *H. akashiwo* had little to no growth at 7°C and became resting cysts in low light ($10 \mu \text{mol photon m}^{-2} \text{s}^{-1}$). These results show that despite variable environmental conditions, *H. akashiwo* still maintains broad halo-tolerance and the ability for *H. akashiwo* to withstand a wide range of salinities will provide this HAB alga with an advantage in bloom formation and survival.

1. Introduction.

Harmful algal blooms (HABs) are accumulated toxic or destructive phytoplankton biomass in freshwater or marine environments. When population growth rates surpass loss rates, a bloom can form. Factors that are hypothesized to induce bloom formation include vertical migration, alleopathy, eutrophication, and low grazing pressure (Harvey & Menden-Deuer 2011). Similarly, the physical factors in the marine environment including nutrient concentrations, water temperature, irradiance, and salinity are predicted to affect the growth of harmful algae (Martinez 2010).

Heterosigma akashiwo, a toxic raphidophyte, has been found to form lethal and ichthyotoxic blooms which harm fish, oyster, and mammalian populations (Harvey & Menden-Deuer 2011). This phytoplankton forms protective resting cysts in adverse conditions (Itakura 1996). *H. akashiwo* is known to be extremely halo-tolerant within a range of 30 to 5 psu. Research has shown that growth rate across this range of salinities is relatively constant when provided with sufficient light and nutrients (Strom et. al. in review). Under non-ideal conditions, phytoplankton growth rates are hypothesized to decrease when conditions are unfavorable for growth. Salinity, water temperature, and irradiance conditions are hypothesized to play an important role in the growth of *Heterosigma akashiwo*. Furthermore, genetically different *H. akashiwo* strain isolates from a variety of geographic areas are thought to differ in halo-tolerance and growth rate under certain conditions (Smayda 1998; Fredrickson et al. 2011).

The following experiments determined the variability in growth rate between two geographically and genetically different strains of *H. akashiwo* along a range of salinities under specific irradiance and temperature gradients. Cellular biomass, growth rate, and photosynthetic efficiency were measured over time. The cumulative results will aid in understanding what conditions affect the halo-tolerance of *Heterosigma akashiwo* and support its growth.

2. Methods.

Culture of phytoplankton- The two toxic strains of *Heterosigma akashiwo* compared in these experiments were collected in 2006 from the Salish Sea or nearby coastal waters in WA, USA (NCMA 2809 strain) and Narragansett Bay in 2010 from Newport, RI, USA (NB strain). Each strain of *H. akashiwo* was grown in $0.2\text{-}\mu\text{m}$ sterile-filtered autoclaved seawater (FSW) enriched with F/2 nutrients without silicate. The stock cultures were maintained in exponential growth on a 12L: 12D cycle at 15°C , salinity of 29.7 psu, and a light intensity of $90\text{-}100 \mu \text{mol photon m}^{-2} \text{s}^{-1}$. These cultures were transferred during mid-exponential growth phase into FSW diluted with distilled water to specific salinities 30, 25, 15, 5, and 3 psu with F/2 nutrients added.

Irradiance and temperature experiments were cultured in 6-well plates (Figure 1) to measure fluorescence over time. Wells contained 6 mL FSW at specific salinities and $100 \mu\text{L}$ of culture (approximately 500 cells per well). Each experiment was performed in triplicate (3 well plates per treatment) with the first two plates containing one

blank well and the final plate containing one control well with 6 ml FSW at 30 psu with no phytoplankton. The light experiment plates were incubated at 15°C under high light ($100 \mu\text{mol photon m}^{-2}\text{s}^{-1}$), medium light ($60 \mu\text{mol photon m}^{-2}\text{s}^{-1}$), and low light ($10 \mu\text{mol photon m}^{-2}\text{s}^{-1}$). Temperature plates were incubated at 22°C, 15°C, and 7°C at $50\text{--}60 \mu\text{mol photon m}^{-2}\text{s}^{-1}$.

Abundance and productivity experiments were cultured in 16 1-L bottles. Each bottle contained 400 mL FSW at specific salinities and 9 mL of 2809 culture or 12 mL of NB culture (approximately 500 cells per mL initially). Each salinity was cultured in triplicate (3 bottles per salinity). One control bottle for each strain containing 400 mL FSW at 30 psu was also prepared. All bottles were incubated at 15°C and $100 \mu\text{mol photon m}^{-2}\text{s}^{-1}$.

Figure 1. 6-well culture plate. Each well contained a different salinity and a blank or control well. These plates were incubated and read on the plate reader. 6-well plates were chosen as culture vessel because the multiple salinity treatments at the same light and temperature treatments could be incubated at the same time.

Data collection- The fluorescence of each 6-well cell well plate was measured each day using a SpectraMax M5 well plate reader (Molecular Devices, Sunnyvale, CA, USA). Each well was bottom read 6 times and all measurements averaged using SoftMax® Pro software. Cell abundance and photosynthetic efficiency of aliquots from each 1-L culture bottle was measured every other day. For the 2809 strain, a Beckman Coulter Counter (Beckman Coulter, Inc., Danvers, MA, USA) measured average cell counts and size per 500 μL of sample. A Fluorescence Induction and Relation (FiRe) System (Satlantic LP, Halifax, NS, Canada) was used to measure photosynthetic efficiency (F_v/F_m), or chlorophyll fluorescence during photosynthesis. For the NB strain, the microplate reader was used to measure fluorescence (equivalent to abundance). Cell size was estimated once a week by counting a 1 mL live sample from each salinity on Sedgwick-Rafter counting chambers (SPI Supplies, West Chester, PA, USA) on a Nikon Eclipse E800 light microscope magnified at 200x. Cellular diameter

was measured thereafter using ImageJ (National Institute of Health, Bethesda, MD, USA) image processing software. When all growth curves for light, temperature, and productivity experiments exhibited growth rates that did not change significantly, the cultures were considered in stationary growth and the experiment finished.

Data analysis- Firepro (FiRe System), SpectraMax M5 (microplate reader), and Multisizer 3 (Coulter Counter) computer software measured photosynthetic efficiency, fluorescence, and cell count of the samples. All data was entered into Microsoft Excel 2010 and analyzed using MatLab R 7.10 (Natick, MA, USA). Two-way analysis of variance (ANOVA) and Tukey's HSD post-hoc analysis were conducted to determine statistical significance ($p < 0.05$). Growth rate was estimated from the rate of change in fluorescence over the exponential phase $\mu = \ln(\text{fluorescence final/fluorescence initial})/\Delta \text{time}$ (μ, d^{-1}).

Figure 2. HA 2809 average growth rates (μday^{-1}) in 5 salinity treatments between 3 and 30 psu for 3 light levels ($\mu\text{mol photon m}^{-2}\text{s}^{-1}$) (top) and 3 temperatures ($^{\circ}\text{C}$) (bottom). Shown are the averages of triplicate cultures with error bars showing standard error of the mean.

3. Results.

3.1. Strain 2809 *H. akashiwo* strain 2809 had the highest growth rate of $0.43 \pm 0.01 \mu \text{day}^{-1}$ in high light

($100 \mu\text{mol photon m}^{-2}\text{s}^{-1}$) at 15 psu and 15°C (Figure 2). For both irradiance and temperature treatments, there was a significant difference between growth rates for 3 and 5 psu salinities ($p < 0.001$) but no significant difference in growth rates between 15, 25, and 30 psu. 5 psu had a reduced, positive growth rate while 3 psu had negative growth with cysts observed.

Figure 3. HA 2809 Fluorescence Induction and Relation (FiRe) System optimal chlorophyll efficiency (Fv/Fm) for 5 salinity treatments between 3 and 30 psu. Each growth stage is represented (lag, peak exponential, and stationary) to show change of efficiency over time. Shown are the averages of triplicate cultures with error bars showing standard error of the mean.

Light treatments had significantly different growth rates ($p < 0.001$). Low light had lower growth rates (only $0.05 \pm 0.005 \mu\text{day}^{-1}$ at 15 psu) compared to both high and medium light intensities and were negative when salinity was less than 15 psu. After 15 days, plates were moved from low light at $10 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ to high light at $100 \mu\text{mol photon m}^{-2}\text{s}^{-1}$. Compared with all other light intensities, *H. akashiwo* had the highest growth rate at all salinities. At 3 psu, these plates had a positive growth rate of $0.005 \pm 0.01 \mu\text{day}^{-1}$ instead of the 3 psu negative growth rates of the other irradiance levels. Growth rates differed significantly depending on temperature treatments ($p < 0.001$) as well. 22°C and 15°C sustained *H. akashiwo* growth, the highest growth rate being $0.3065 \pm 0.02 \mu\text{day}^{-1}$ at 15°C . 7°C had reduced growth rates and 5 and 3 psu resulted in negative growth ($-0.06 \pm 0.01 \mu\text{day}^{-1}$ and $-0.005 \pm 0.001 \mu\text{day}^{-1}$, respectively). The interaction between salinity and light as well as salinity and temperature were significantly different ($p < 0.001$), indicating strain 2809 *Heterosigma akashiwo* growth is interdependent on irradiance, temperature, and salinity condition interactions.

Optimal chlorophyll efficiency (Fv/Fm) at all salinities was significantly different ($p < 0.001$) but not significantly different based on growth stages ($p > 0.005$). During lag phase, Fv/Fm at 30, 25, 15 psu had similar Fv/Fm values and 5 psu had a lower efficiency compared with other salinities (Figure 3).

By exponential phase, 5 psu had acclimated and its efficiency matched other salinities. 15 psu had the highest Fv/Fm at $0.57 \pm 0.004 \mu\text{day}^{-1}$ on day 9. 3 psu had no photosynthetic efficiency and formed resting cysts.

3.2. Strain NB *H. akashiwo* strain NB had the highest growth rate of $0.52 \pm 0.02 \mu\text{day}^{-1}$ in high light ($100 \mu\text{mol photon m}^{-2}\text{s}^{-1}$) at 15°C and 25 psu (Figure 4). For irradiance treatments, there was a significant difference between growth rates for 3 and 5 psu ($p < 0.001$) but no significant difference in growth rates between 15, 25, and 30 psu. 5 psu had a reduced, positive growth rate while 3 psu had little to no growth. For temperature treatments, 3 and 5 psu had higher growth rates at 22°C while 25 and 30 psu had higher growth rates at 15°C , all in medium light. 15 psu had similar growth rates at both 22°C and 7°C which slightly decreased at 15°C . There was no significant difference between growth rates for 5, 15, 25, and 30 psu ($p < 0.001$). 3 psu had positive growth, excluding 7°C .

Figure 4. HA NB average growth rates (μday^{-1}) in 5 salinity treatments between 3 and 30 psu for 3 light levels ($\mu\text{mol photon m}^{-2}\text{s}^{-1}$) (top) and 3 temperatures ($^\circ\text{C}$) (bottom). Shown are the averages of triplicate cultures with error bars showing standard error of the mean

Light treatments resulted in significantly different growth rates ($p < 0.001$). Low light had lower growth rates (only $0.07 \pm 0.007 \mu\text{day}^{-1}$ at 15 psu) compared to both high ($0.77 \pm 0.002 \mu\text{day}^{-1}$ at 15 psu) and medium ($0.23 \pm 0.002 \mu\text{day}^{-1}$ at 15 psu) light intensities and were negative when salinity was less than 5 psu. After

23 days, plates were moved from low light at $10 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ to high light at $100 \mu\text{mol photon m}^{-2} \text{s}^{-1}$. 3 psu and 5 psu cultures had survived, and with optimal lighting conditions the cells proliferated and grew. At 5 psu, *H. akashiwo* had the highest growth rate of $0.31 \pm 0.02 \mu \text{day}^{-1}$. These growth rates were at least four times larger than the other light conditions. Cells at 15, 25, and 30 psu proliferated but the growth rates were smaller when compared to low salinity treatments.

Growth rates differed significantly depending on temperature treatments ($p < 0.001$). 22°C and 15°C sustained *H. akashiwo* growth, the highest growth rate being $0.32 \pm 0.02 \mu \text{day}^{-1}$ at 15°C and 25 psu. At 3 psu, 22°C had the highest growth rate out of all three temperatures. 7°C had reduced growth rates at 3, 5, 25, and 30 psu but had a similar growth rate as 15°C . The interaction between salinity and light growth rates as well as salinity and temperature growth rates were all significantly different ($p < 0.001$), indicating strain NB *Heterosigma akashiwo* growth is dependent on the irradiance, temperature, and salinity condition interactions.

Figure 5. HA NB Fluorescence Induction and Relation (FiRe) System optimal chlorophyll efficiency (Fv/Fm) for 5 salinity treatments between 3 and 30 psu. Each growth stage is represented (lag, peak exponential, and stationary) to show change of efficiency over time. Shown are the averages of triplicate cultures with error bars showing standard error of the mean.

Optimal chlorophyll efficiency (Fv/Fm) at all salinities and growth stages were significantly different ($p < 0.001$). During lag phase, Fv/Fm at all salinities ranged from 0.38 to $0.48 \pm 0.06 \mu \text{day}^{-1}$ (Figure 5). By exponential phase, Fv/Fm for 3 psu had declined while the Fv/Fm for all other salinities had increased. 25 psu had the highest Fv/Fm at $0.58 \pm 0.003 \mu \text{day}^{-1}$ on day 6. Compared to strain 2809, 3 psu cultures grew considerably better during all phases of growth. No resting cysts were observed.

4. Discussion.

Harmful algal blooms of the toxic phytoplankton species *Heterosigma akashiwo* are known to cause large fish kills, harm marine life, and damage to the aquaculture industry (Harvey & Menden-Deuer 2011). These specific harmful algal blooms are extremely halo-tolerant (Strom et al. in review). Previous research has hypothesized that the halo-tolerance of *Heterosigma akashiwo* is affected by temperature and irradiance (Martinez 2010). From this research, *H. akashiwo* growth is affected by the interdependence of irradiance, temperature, and salinity. Halo-tolerance in a range of salinities from 3 psu to 30 psu was confirmed. In addition, conditions in which phytoplankton growth rate was highest and lowest, depending on irradiance and the temperature, were determined. These results also indicate a difference in the halo-tolerance between genetically different strains of the phytoplankton.

Maximal observed growth rates varied between these two strains by approximately $1.0 \mu \text{day}^{-1}$ with strain NB having the higher growth rate. Both strains grew at approximately the same rate (peak exponential growth reached around day 5). Strain NB had a larger halo-tolerance and survived better in lower salinities. Conversely, strain 2809 survived better in higher salinities and had an overall smaller, but still broad, halo-tolerance. The growth rate difference and overall salinity range must be recognized and taken into account when using any strain of *H. akashiwo*.

Low irradiance, temperature, and salinity are known to prevent *Heterosigma akashiwo* growth. In this experiment, both strains survived long periods of darkness and were able to recover and grow at a higher rate than other irradiance experiments. In strain 2809, when the irradiance of the cultures was changed from low to high after a 15 day period and after protective resting cysts was observed, each salinity recovered from cyst form and had a higher growth rate than other irradiance levels. In strain NB, cysts were not observed but the cells remaining in low light culture after 23 days recovered in high light as well. *H. akashiwo*'s ability to survive long periods of darkness and recover when conditions are more favorable indicates the species' extreme resilience and ability to adapt to extremely intolerable environments.

Results suggested the environmental conditions in the location of isolation influences the halo-tolerance of each strain. Each strain was isolated from different bodies of water and natural environments. Thus, *Heterosigma akashiwo*'s ideal *in vitro* conditions should be similar to *in situ* conditions. *H. akashiwo* strain 2809 was able to grow the most *in vitro* in 15°C between 15 and 30 psu. According to the National Oceanographic Data Center at the National Oceanic and Atmospheric Administration (NOAA), the historical yearly difference in average coastal water temperatures of Port Townsend, Washington,

USA (NOAA station nearest Salish Sea) is about 5°C, ranging from 7°C to 12°C. Conversely, strain NB *in vitro* sustained growth equally well in 3,5, and 15 psu at 22°C or 15, 25, and 30 psu at 15°C. In Newport, RI, USA (NOAA station in Narragansett Bay), historical yearly range in average coastal water temperatures is 2°C to 22°C, a difference of 20°C. Strain 2809 was isolated from water with a relatively small change in water temperature per year compared with strain NB, who survives in water with much larger temperature variability. The data from these experiments showed similar results; strain NB had multiple temperatures that supported similar high growth rates while strain 2809 had the highest growth rate at a single temperature 15°C. Both strains showed little growth in extremely low salinity (less than 5 psu) or temperatures (7°C), but under these conditions, strain NB had slightly higher growth rates compared with strain 2809.

These data suggest *Heterosigma akashiwo* is unable to survive at high temperatures. Both strains had a slight decreased growth rate at 22°C compared with 15°C. Strain NB tolerated higher temperatures (22°C) at lower salinities but at the ideal salinity range between 25 and 30 psu, *H. akashiwo* grew the most in temperatures below 25°C. The decreased growth rate at higher temperatures indicates that over time, as water temperatures are expected to increase, *H. akashiwo* may not create blooms or be as successful as it has been in the past. Even so, high evidence of acclimatization in certain conditions (specifically salinity) suggests the phytoplankton could adapt to the new water temperatures and maintain population size.

Including past and current data regarding the halo-tolerance of *Heterosigma akashiwo* and the impact of interdependent environmental conditions, researchers and industry will be able to predict, locate, and track blooms using actual irradiance and water temperature data from the oceans. Harmful algal bloom formation and the conditions that initiate growth will be better understood. Furthermore, the aquaculture and marine industry will be able to monitor for excess phytoplankton growth to be aware of possible fish kills and eutrophication. Understanding *Heterosigma akashiwo* halo-tolerance based upon environmental conditions will increase the possibility of identifying and controlling blooms in

order to protect habitats, species, and marine environments from destruction and depletion.

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- S.Menden-Deuer and E. Harvey, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (smenden@gso.uri.edu, harveyel@gso.uri.edu)
- R. Hickcox, Worcester Polytechnic Institute, Worcester, MA 01609. (rhickcox@wpi.edu)

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Genetic diversity of *Thalassiosira* diatoms

Michelle Jenssen, Gang Chen, Tatiana Rynearson

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. Marine planktonic diatoms are major primary producers in the ocean, responsible for 20–25% of all organic carbon fixation. Their important roles in marine ecology are presumably associated with their long evolutionary history, rich species diversity, and strong adaptive potentials. Unfortunately, we have very limited knowledge of their contemporary biodiversity, adaptability, and species history. This project investigated genetic variation and diversity of globally distributed, ecologically important marine *Thalassiosira* diatom species. Two gene loci, nuclear ITS and mitochondrial COII, were examined from individual isolates representing geographic populations in both the Atlantic and Pacific oceans. More than 300 isolates were PCR-amplified, 206 were RFLP-genotyped, and 60 were sequenced for distinct genotypic lineages. A total of ten unique mtCOII haplotypes were discovered, forming 7 distinct mtCOII lineages. Sequence divergence among lineages was between 4.7–13.9%, while less than 0.8% was found within lineages. Nine unique ITS1 sequences from this study combined with reference sequences from GenBank and previous lab work (K. Whittaker) were associated with 6 nominal *Thalassiosira* species. Results showed unexpected relationships between mtCOII lineages and associated species, suggesting a substantial difference in phylogenetic and taxonomic species identification. Geographic distribution of mtCOII lineages was uneven among different locations. Except for one lineage that was only found in Norway, all lineages showed a wide regional or global distributional pattern, but with varied compositions. In the future, more geographic samples will be further tested to confirm the observed genetic patterns in this study.

1. Introduction

Diatoms are microscopic uni-cellular photosynthetic algae that play an important role in many biological processes. Recent estimates report that there are over 200,000 species of diatoms in both marine and freshwater environments, which are responsible for 20–25% of global primary production and atmospheric oxygen (Mann & Droop 1996; Mann 1999). These organisms drift with tides and currents and have shown to have a high dispersal potential, however little information is known about the factors responsible for their geographic distribution and species diversity (Chepurnov et al. 2004).

The diatoms in the family Thalassiosiraceae can be used as a model system for diatom research because of their high diversity and large geographic range. There are approximately 500 fossil and living species of *Thalassiosira* diatoms, with their classification being majorly based on phenetics (Alverson et al. 2007). The diatoms in this family are found in large rivers and reservoirs, freshwater lakes, brackish and estuarine waters, coastal marine environments as well as the open ocean (Hasle and Syvertsen, 1997; Stoermer and Julius, 2003). *Thalassiosira* diatoms not only serve as a good model geographically, but can also serve as an important genetic model. One species in the *Thalassiosira* genus, *T. pseudonana*, is one of only two diatoms with its entire genome completely sequenced (Armbrust et al 2004). This information can provide an opportunity to gain insight into the history and evolutionary

potentials of members of the *Thalassiosira* genus, as well as many other species of diatoms.

Because of their large species diversity and important roles in many biogeochemical cycles, species identification of diatoms is very important. Taxonomy on the species level has become a successful way to use diatoms in biomonitoring and environmental change research (Smol & Stoermer, 2010). However, diatom delineation has shown to be a difficult task, with many researchers disagreeing about the taxonomy and identification of diatom species (Mann et al. 2010). Historically, classification and taxonomic species identification of diatoms has largely been based on the characteristics of their intricate silica cell wall, called the frustle (Round et al. 1990), however studies have shown that morphological characterization alone is not an accurate or effective way in determining species identification (Sar et al. 2011). The high physiological diversity of diatoms may relate to a large underlying genetic diversity (Brand 1990), and in order to understand the intra- and inter-specific diversity within and between species, new molecular tools must be developed.

A potential technique to determine species identification is by DNA barcoding. This method uses a genetic marker in an organisms DNA to identify it as a specific species. DNA barcoding has been proven as an effective solution to species identification in plants and animals (Hebert et al. 2003), and has now recently been introduced as a way to identify diatom species (Moniz & Kaczmarek 2009). Many different genes have shown potential as molecular markers for DNA

barcoding, one of which is the ribosomal Internal Transcribed Spacer Region 1 (ITS-1) gene (Evans et al. 2007). This gene is a ribosomal gene that has been widely used in DNA barcoding for both plants and animals, and has also shown the potential to uncover cryptic species within diatoms of the *Thalassiosira* genus (Wittaker et al. Submitted). This study will include sequence data from the ITS-1 gene, which will be then used in conjunction with a mitochondrial gene. A mitochondrial gene will be used in this research because of its potential as a strong DNA barcode for diatom species. The small size and high polymorphism of the genes in mitochondrial genomes, which are present in diatoms, make these genes good subjects for phylogenetic studies (Funk and Omland 2003; Alverson 2008). Mitochondrial genes are present in all eukaryotic cells, and all have a common ancestor. This study focused on a mitochondrial gene, cytochrome *c* oxidase subunit II, a gene that has not been widely tested for DNA barcoding in diatoms, but has been shown successful in animal studies (Beckenwith 1993). Preliminary research has shown that these two genes are able to be successfully amplified and sequenced in laboratory culture strains of *Thalassiosira gravida* and *T. rotula* (G. Chen, unpublished).

The objective of our research was to use a molecular genetics approach to examine the variation and diversity within and between *Thalassiosira* lineages/species, as well as examine the global

distribution of each lineages/species based on their mtCOII DNA sequence. We hypothesized that genetically distinct lineages existed within *Thalassiosira* diatoms, and that inter and intra-specific genetic variations can be revealed at two tested genetic loci, the ribosomal ITS-1 gene and mtCOII gene. These two genetic loci may be used as powerful molecular markers for DNA barcoding, diatom species delineation as well as population genetics studies.

2. Materials and Methods

2.1 Diatom sampling and DNA extraction

Diatom samples used in this study include field collections of 8 geographic populations, one culture strain from Culture Collection of Algae and Protozoa (CCAP, UK), and two strains from the National Centre for Marine Algae and Microbiota (formerly CCMP, Bigelow Laboratory for Ocean Sciences, USA). Water samples were collected either from surface or layers of maximum Chlorophyll. *Thalassiosira* diatoms were isolated from water samples under microscope and cultured on board during research cruises for 1–2 weeks. Surviving cultures were harvested through filters (0.2 µm pore size) and frozen at -20°C or -80°C. Genomic DNA was extracted from each isolate using Qiagen Dneasy Plant Kits and stored at -80°C before genetic analysis.

Figure 1. Schematic banding patterns for RFLP genotyping of *Thalassiosira* mtCOII genetic lineages. The first lane on the left is the DNA ladder (Bioline Hyperladder V) mixed with un-digested PCR amplicons of a 691-bp fragment size. The endonuclease *Hinc* II selectively cut the amplicons from different lineages, yielding a range of fragment size from 90 bp to 575 bp, forming distinct banding patterns for different lineages or lineage groups.

2.2 PCR amplification and sequencing of ITS-1 and mtCOII genes

One region of the ribosomal DNA, the internal transcribed spacer region I (ITS-1), along with one region of the mitochondrial DNA, cytochrome *c* oxidase subunit II (mtCOII), were amplified using Polymerase Chain Reaction and later sequenced. To amplify these regions, a mixture comprised of 0.5 µl DNA template was combined with 0.2 mM dNTPs, 2.5 mM MgCl₂, 0.2 mg/ml Bovine Serum Albumin, 0.015 U/µl Mango *Taq* polymerase, 1X Mango reaction buffer, 0.2 µM each of forward and reverse primers, in a final volume of 10–20 µl. The forward primer 1645F (5'CTTATCATTTAGAGGAAGGTGAAGTCGT3') and the reverse primer Rot5.8SR (5'CTGCGTTCTTCATCGTTGTGG3') were used to amplify the ITS-1 region of the DNA. PCR to amplify this region consisted of a 2 min denaturation step at 95°C, 35 cycles of 30 sec at 94°C, 30 sec at 60°C, and 50 sec at 72°C followed by a 7 min extension at 72°C. The forward primer Thaco2F (5'ATGATTTTATAATACAATGTGA3') and reverse primer ROTCO2R2 (5'GCATAAAACCATGATTTACACCAC3') were used to amplify the mtCOII region. The PCR reaction to amplify the COII region consisted of a 2 min denaturation step at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 50°C, and 50 sec at 72°C, and a 10 min at extension at 72°. 8 µl of the PCR products were loaded into each well of a large GE rig containing 1% agarose gel and run at 90 volts for 45 minutes. Gels were stained with SYBR green for 15 minutes and imaged using a UV imager. Those DNA samples with a positive product were further used for sequencing and RFLP genotyping. A third type of PCR reaction was performed to create a ladder for RFLP genotyping. The mtCOII region on two known samples, GMP14c1 and GSO101, was amplified in a 50 µl reaction. The resulting products were mixed together and used as a ladder for enzyme digestion.

For sequencing, PCR products were first purified using the ethanol precipitation method and then quantified spectrophotometrically using Nanodrop model 1000 (Thermo Scientific). Purified DNA with a concentration of >20 ng/µl and a 260/280 ratio between 1.6 and 2.0 was submitted to the Genomic Sequencing Center at the University of Rhode Island for sequencing. PCR primers were used for priming in all sequencing reactions.

2.4 Phylogenetic and genetic diversity analyses

All high quality sequences were edited using CodonCode Aligner (CodonCode Corporation). Mitochondrial COII sequences were aligned directly using CodonCode Aligner, while ITS-1 sequences using Clustal X with default parameter settings

(Larkin et al. 2007). DNA polymorphism and sequence divergence were examined and computed for both ITS-1 and COII genes using computer program MEGA5 (Tamura et al. 2011) and DnaSP v5.10 (Librato & Rosas 2009). Nucleotide diversity (π) and haplotype diversity (HD) were calculated for mtCOII at both the lineage level and the nominal species level. Tajima's *D* was calculated to test evolutionary neutrality of mtCOII gene. Due to limited sample size, only genetic divergence among lineages and species was examined for the ITS-1 gene.

Phylogenetic relationships among sequences were analyzed for both ITS-1 and COII genes. Neighbor-joining gene trees based on uncorrected *p*-distance were constructed using MEGA5 program. For mtCOII, two GenBank sequences (Accession no. GU002153 for *Synedra acus* and NC007405 for *Thalassiosira pseudonana*) were included. For ITS-1, 20 references, including 16 sequences from Ryneerson Lab (K. Whittaker, unpublished data) and 4 GenBank sequences (no. EU600777 for *T. rotula*, EF208799 for *T. minuscula*, EF208800 for *T. anguste-lineata*, and EF208797 for *T. aestivalis*) were included. Both tree topologies were tested using bootstrap method with 1,000 pseudo-replicates.

2.5 RFLP-genotyping of Major Mitochondrial Lineages

Phylogenetic analysis of sequence data resolved several distinct mitochondrial genetic lineages (see results). These lineages were further genotyped using Restriction Fragment Length Polymorphism technique. This technique uses a restriction enzyme to cut a DNA segment when a specific sequence is encountered. When the products of enzyme digestion are viewed in a polyacrylamide gel, bands of DNA of different lengths are visible at resulting locations within the gel. The enzyme that was used in this experiment is *Hinc* II, an endonuclease that cuts a DNA segment wherever "GTYRAC" occurs (Figure 1). To genotype each isolate, 5–10 µl of PCR products were mixed with 1X NE Buffer 3, 100 µg/ml Bovine Serum Albumin, 2 U *Hinc* II enzyme in a final volume of 15 µl. Enzyme digestion was set for 37°C for more than 2 hours. The digested products were loaded into 6% Polyacrylamide gels, run for 30 minutes at 200 volts and visualized with SYBR green staining. The genotyping was based on predicted banding patterns from sequence data (Figure 1).

Figure 2. The mtCOII gene tree of *Thalassiosira* lineages. The tree was constructed based on a 501-bp fragment alignment of 62 mtCOII sequences, using uncorrected *p*-distance and Neighbor-joining algorithm. The tree was rooted using the mid-point method. Numbers on branches denote bootstrap support values. Only values greater than 70 were presented. The full sequence data include a total of 8 unique lineages with 16 unique *Thalassiosira* haplotypes. The 53 colored squares denote individual sequences and their geographic origins as keyed in the legend (See Table 1 for population abbreviations). A “*” symbol denotes a sequence that has ambiguous base calls and was excluded from the sequence diversity analysis (Table 2-4). The species names associated with the lineages are determined based on the ITS-1 gene tree (Figure 3).

3. Results

3.1 Phylogenetic analysis

Neighbor-joining gene trees for both mtCOII and ITS-1 revealed several distinct genetic lineages. For mtCOII, seven deeply diverged lineages were resolved with net genetic distances of 0.0471–0.1391 substitutions/site among lineages, whereas <0.001 within lineages (Table 2-3, Figure 2). Based on the ITS gene tree and grouping structure of unknown

lineages with known species, we associated all lineages to 4 *Thalassiosira* species and one unknown species (*Thalassiosira* sp.) (Figure 2). Genetic distances calculated at species level resulted in 0.0563–0.4727 substitutions/site and up to 0.0441 within species.

Similarly, 8 genetic lineages were found in the ITS-1 gene tree, associated with 5 nominal species and one unknown species (Figure 3). Genetic distances were between 0.0491–0.4913 substitutions/site among lineages, 0.0563–0.4727 among species, <0.0154

Figure 3. The ITS gene tree of *Thalassiosira* lineages and species. The tree was constructed based on an alignment of 335-bp ITS gene fragment, using *p*-distance and Neighbor-joining algorithm. The tree was rooted using the mid-point method. High bootstrap support values (>70) for major clades were presented on the branches. The full sequence data include a total of 9 unique allele sequences from present study (in black box), 16 sequences from Ryneerson Lab (Open circles; K. Whitacker, unpublished), and 4 GenBank sequences with known species identity (labeled with species names). Two pseudo-alleles (ITSp1, ITSp2) predicted from a double-heterozygous-site sequence were also included. A total of 8 genetic lineages and 6 *Thalassiosira* species, including one unknown (*T. sp.*) were recognized based on genetic distances.

Figure 4. *Thalassiosira* mtCOII lineage composition in five well sampled geographic populations based on RFLP-genotyping. Four lineages were coded with colors, including Lineage 1 (in red), Lineage 2 (in orange), Lineage 5 (in pink), and Lineage 6 (in blue). Samples size (n) and percentage composition values were presented with each pie chart.

within lineages, and up to 0.0636 within species (Table 5-6).

3.2 Genetic diversity within lineages and species

Excluding 8 ambiguous sequences from the data set for phylogenetic analysis, 53 mtCOII sequences were used for genetic diversity analysis. A total of 14 unique haplotypes were found. Within-lineage nucleotide diversities were between 0.0000 and 0.0082, while within-species between 0.0000 and 0.0441. Haplotype diversities were between 0.000 and 1.000 within lineages and 0.000–0.593 within species (Table 2). No apparent deviations from 0 were found in Tajima's *D* estimates.

3.3 RFLP genotyping

RFLP genotyping was successfully performed on a total of 198 individuals in 5 geographic populations. Four lineages were genotyped and unevenly distributed among populations (Figure 4). The mtCOII Lineage 1 was mainly found in Atlantic populations with high percentage compositions (52–100%) in samples. In contrast, Lineage 5 was exclusively found in North Pacific populations. Lineage 2 mainly occurred in North Pacific populations but also had a small percentage showing in Disko Bay. Lineage 6 appeared to be a cosmopolitan one, showing in both North Pacific and Atlantic populations. Three geographic populations had at least three lineages coexisting together. Interestingly, two Atlantic populations, Gulf of Maine and Iceland, were dominated by one single lineage (L1, Figure 4).

4. Discussion

4.1 A novel mitochondrial DNA marker

A novel mitochondrial DNA marker for molecular ecology studies was successfully developed and applied in this study. Although mitochondrial gene markers has been used widely in many other organisms, we experienced certain difficulties developing and applying them, especially for the barcoding gene mtCOII in marine diatoms, due to its complex gene structure interfered with multiple introns (Evans et al. 2007). In this study, we applied a novel mtDNA marker, the mtCOII developed in Rynearson Lab (G. Chen, unpublished data), to examine the genetic diversity of *Thalassiosira* diatoms. Multiple genetic lineages with deep sequence divergences (4.7–13.9%) were revealed in global samples, suggesting that the mtCOII gene can be a powerful, rapidly evolving marker and ideal for detecting genetic diversity at both interspecific and intraspecific levels. The DNA polymorphisms found in this study were mostly synonymous, commonly seen in neutral protein coding genes. Overall neutrality test was not apparently deviated from zero (Tajima's *D* = 0.08, Table 2).

4.2 Mitochondrial genetic lineages in *Thalassiosira*

In all the samples we examined, 7 mtDNA lineages were found deeply diverged in an average of 11.2% (4.7–13.9%). Some of these lineages represent single nominal species with little diversities in both ITS-1 and mtCOII data, while others show drastic difference between the two genes. The most striking

Population name	Location	Longitude	Latitude	Sampling time	Source	Sample size (N)		
						RFLP	ITS-1	mtCOII
GME	Gulf of Maine	67.77–70.33°W	41.86 – 43.09°N	April 6–10, 2011	Field isolation	20	7	7
ICL	Iceland	25.35–27.59°W	60.63–61.53°N	May 2–13, 2008	Field isolation	68	6	6
BCC	British Columbia Coast	127.44°W	49.65°N	May 13, 2007	Field isolation	56	0	15
DKB	Disko Bay	52.06°W	69.12°N	May 7, 2011	Field isolation	25	3	9
PGS	Puget Sound	122.42°W	47.74°N	May 12, 2007	Field isolation	29	0	14
JPN	Seto Inland Sea, Japan	133.33°E	34.17°N	February, 2007	Field isolation	1	0	1
GON	Gulf of Naples, Italy	14.25°E	40.96°N	?, 2008	CCAP1085_21	1	0	1
NB	Narragansett Bay	71.38°W	41.53°N	October 10, 2008	Field isolation	1	1	1
GOA	Gulf of Alaska	145.43°W	59.73°N	May 16, 2011	Field isolation	2	0	2
NOR	Troms, Norway	18.97°W	69.67°N	August 1, 1978	CCMP986	2	0	2
SO	Southern Ocean	77.83°W	163.00°S	February 1, 1991	CCMP1462	1	0	1

Table 1. Geographic sampling information and sample sizes for sequencing and RFLP genotyping. A total of 11 geographic populations were sampled during Aug.1978–May 2011. A total of 17 ITS and 60 mtCOII sequences were obtained and 206 diatom isolates were RFLP genotyped for distinct mtDNA lineages.

case is the two *T. gravida* lineages (Lineage 1 and 7), shown identical ITS sequences but as deep as 11.4% in mtCOII divergence. Similarly, two *T. aestivalis* lineages (Lineage 3 and 5) show 9.8% and 9.6% divergence in mtCOII and ITS-1 respectively, although we defined them all as *T. aestivalis* based on clade structure of ITS gene tree (Figure 3). These findings, given limited sample size, potentially confound our determination of species identity based on either gene marker. To well resolve *Thalassiosira* species-lineage relationships, we need to further look at more conserved genes, such as 18S, 28S, and also examine more samples that may cover all possible genetic compositions worldwide.

In this study, we paid particular attention to two closely related species, *T. gravida* and *T. rotula*. A recent study has extensively examined their morphological traits, concluding no substantial difference between them and regarding them as one single species with heteronymous names (Sar et al. 2011). However, our molecular data showed a deep divergence in both mtCOII (Lineage 1 vs. 2: 4.7%; Table 3) and ITS-1 gene (5.6%, Table 2). These divergences were much greater than at within-lineage level (0.6% for mtCOII and 0.7% for ITS-1). It is worth noting that all genetic distances in this study were calculated as uncorrected *p*-distance (proportions

of nucleotide differences between pairwise sequences). These distances were possibly underestimated for the real nucleotide changes due to substitution saturation. Because sequence divergences reached as high as 13.9%, we may expect the occurrence of substitution saturations among deeply diverged lineages. Further studies using model corrected distances will not only provide more accurate genetic distances among and within lineages/species, but also resolve the cladogenesis for these lineages, which was ambiguous with *p*-distance (Figure 2).

4.3 Geographic distribution of mtDNA genetic lineages

We surveyed five geographic populations using RFLP genotyping methods for their genetic compositions and distribution patterns of major lineages. Four mtDNA lineages were found unevenly distributed among geographic regions. Interestingly, except Lineage 6 that occurred in both North Pacific and Atlantic oceans, all three other lineages showed more or less a regional distribution pattern: Lineage 1 in North Atlantic while Lineage 2 and 5 in North Pacific, though a low percentage of Lineage 2 was also found in Disko Bay (Figure 4).

Lineage or Species	Sample size (N)	Nucleotide diversity (π)	Number of haplotypes (H)	Haplotype diversity (HD)	Tajima's <i>D</i>
<i>Mitochondrial genetic lineages</i>					
Lineage 1	17	0.0005	3	0.228	-1.50
Lineage 2	12	0.0032	4	0.561	-1.25
Lineage 3	6	0.0000	1	0.000	n/a
Lineage 4	2	0.0000	1	0.000	n/a
Lineage 5	2	0.0082	2	1.000	n/a
Lineage 6	11	0.0063	2	0.509	1.94
Lineage 7	2	0.0000	1	0.000	n/a
<i>Nominal Thalassiosira species</i>					
<i>T. gravida</i>	19	0.0231	4	0.380	-1.20
<i>T. rotula</i>	14	0.0023	5	0.593	-1.50
<i>T. aestivalis</i>	8	0.0441	3	0.464	0.41
<i>T. sp.</i>	2	0.0000	1	0.000	n/a
<i>T. anguste-lineata</i>	11	0.0063	2	0.509	1.94
Full data set	53	0.0765	15	0.871	0.08

Table 2. Genetic diversity of *Thalassiosira* diatoms at mtCOII gene locus. Nucleotide and haplotype diversities based on a 489-bp fragment of 53 unambiguous sequences were examined for both mitochondrial genetic lineages and associated nominal species. Tajima's *D* was tested for neutrality of mtCOII evolution. n/a – not applicable.

	COII-L1	COII-L2	COII-L3	COII-L4	COII-L5	COII-L6	COII-L7	COII-L8
COII-L1		0.0092	0.0117	0.0128	0.0134	0.0131	0.0139	0.0144
COII-L2	0.0471		0.0118	0.0139	0.0135	0.0126	0.0138	0.0135
COII-L3	0.0775	0.0839		0.0143	0.0128	0.0137	0.0132	0.0142
COII-L4	0.1000	0.1172	0.1268		0.0143	0.0143	0.0148	0.0148
COII-L5	0.1041	0.1088	0.0982	0.1247			0.0143	0.0144
COII-L6	0.1059	0.0979	0.1038	0.1318	0.1038		0.0142	0.0146
COII-L7	0.1140	0.1155	0.0982	0.1391	0.1329	0.1242		0.0148
COII-L8	0.1243	0.1150	0.1207	0.1350	0.1247	0.1276	0.1329	

Table 3. Mitochondrial COII genetic distances between resolved *Thalassiosira* lineages (See Figure 2 for lineage definition). *P*-distances between pairs of mtCOII sequences were calculated as the genetic distance using MEGA5. Mean values and standard errors of between-lineage distances were presented in bottom left and up right of the matrix respectively. The GenBank sequence of *T. pseudonana* was retrieved (Accession no. NC007405) and included as Lineage 8 for between-lineage comparisons. Both the highest and lowest values are highlighted in bold.

Multiple lineages tend to coexist in one sample as shown in three populations (British Columbia Coast, Puget Sound, and Disko Bay). Two populations, Gulf of Maine and Iceland, were dominated by one single lineage, which is most likely due to its blooming when sampled. The four lineages represent four nominal species (*T. gravida*, *T. rotula*, *T. aestivalis*, and *T. anguste-lineata*). So, the lineage distribution pattern reflected the species distribution pattern in this study. Should we survey more samples from more

geographic locations, we may be able to find diverse sublineages within species and resolve their phylogeographic history.

	Tgra	Tang	Tsp	Trot	Taes	Tpse
<i>T. gravida</i> (Tgra)		0.0119	0.0119	0.0082	0.0083	0.0130
<i>T. anguste-lineata</i> (Tang)	0.0965		0.0146	0.0131	0.0110	0.0147
<i>T. sp.</i> (Tsp)	0.0927	0.1318		0.0137	0.0125	0.0145
<i>T. rotula</i> (Trot)	0.0430	0.0979	0.1172		0.0101	0.0134
<i>T. aestivalis</i> (Taes)	0.0541	0.0827	0.1052	0.0691		0.0117
<i>T. pseudonana</i> (Tpse)	0.1138	0.1276	0.1350	0.1150	0.1006	

Table 4. Mitochondrial COII genetic distance between tested *Thalassiosira* nominal species. Net *P*-distances between pairs of species were calculated using MEGA5. Mean values and standard errors of between-species distances were presented in bottom left and up right of the matrix respectively. The GenBank sequence of *T. pseudonana* was retrieved (Accession no. NC007405) and included for between-lineage comparisons. Both the highest and lowest values are highlighted in bold.

Lineage	ITS-L1	ITS-L2	ITS-L3	ITS-L4	ITS-L5	ITS-L6	ITS-L7	ITS-L8
ITS-L1	0.0154	0.0138	0.0258	0.0282	0.0268	0.0266	0.0269	0.0311
ITS-L2	0.0563	0.0000	0.0218	0.0249	0.0232	0.0225	0.0266	0.0280
ITS-L3	0.2446	0.1969	0.0033	0.0167	0.0203	0.0218	0.0236	0.0292
ITS-L4	0.2948	0.2464	0.0969	0.0000	0.0243	0.0256	0.0266	0.0310
ITS-L5	0.2635	0.2206	0.1831	0.2331	0.0060	0.0225	0.0236	0.0286
ITS-L6	0.2476	0.2101	0.1681	0.2207	0.1759	0.0000	0.0188	0.0293
ITS-L7	0.2665	0.2747	0.1945	0.2468	0.1887	0.0955	0.0000	0.0340
ITS-L8	0.4727	0.3793	0.4237	0.4913	0.4368	0.4319	0.0491	0.0034

Table 5. Genetic distances within and between *Thalassiosira* ITS lineages (See Figure 3. for lineage definition). *P*-distances between pairs of ITS sequences were calculated as the genetic distance using MEGA5. Mean values and standard errors of between-lineage distances were presented in bottom left and up right of the matrix respectively. Values in bold along the diagonal line are within-lineage distances. The highest and lowest between-lineage distance values are shaded.

Species	Trot	Tgra	Tmin	Tang	Taes	Tsp
<i>T. rotula</i> (Trot)	0.0154	0.0138	0.0248	0.0271	0.0239	0.0308
<i>T. gravida</i> (Tgra)	0.0563	0.0000	0.0212	0.0229	0.0216	0.0271
<i>T. minuscula</i> (Tmin)	0.2290	0.1811	0.0668	0.0203	0.0171	0.0274
<i>T. anguste-lineata</i> (Tang)	0.2635	0.2206	0.1675	0.0060	0.0208	0.0290
<i>T. aestivalis</i> (Taes)	0.2220	0.1998	0.1831	0.1483	0.0636	0.0291
<i>T. sp.</i> (Tsp)	0.4727	0.3793	0.4147	0.4368	0.4199	0.0034

Table 6. The ITS Genetic distances within and between nominal *Thalassiosira* species. *P*-distances between pairs of ITS sequences were calculated as the genetic distance using MEGA5. Mean values and standard errors of between-species distances were presented in bottom left and up right of the matrix respectively. Values in bold along the diagonal line are within-species distances. The highest and lowest between-lineage distance values are shaded.

5. Conclusion

Diatoms play many important roles in biological oceanography, and gaining knowledge about their species history and evolutionary potentials is extremely important. Investigating the genetic variation and diversity of the ecologically important marine *Thalassiosira* diatom species can help gain insight of diatom biodiversity, adaptability, as well as species history. The two gene loci that were investigated in this study, the ribosomal ITS gene and mitochondrial COII gene, were proven to act as successful molecular markers for species delineation and genetics studies. Results of this study have showed multiple deeply diverged mtDNA lineages exist in global samples, suggesting probable cryptic speciation in *Thalassiosira*, particularly *T. gravida*

and *T. rotula*. Discordant definitions between lineage and nominal species also suggest that *Thalassiosira* genetic diversity at both species and population levels may be widely underestimated. Further investigations using mtCOII and ITS-1 for all *Thalassiosira* diatoms are needed.

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Gang Chen, Tatiana Rynearson, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (gchen@gso.uri.edu, trynearson@gso.uri.edu)

M. Janssen, University of Massachusetts, Amherst, MA 01003. (michelle.janssen@gmail.com)

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***Thalassiosira* community composition in a transect through a biodiversity “hotspot”**

Samantha Maness^{1,2}, P. Dreux Chappell¹, Bethany Jenkins^{1,2}

¹Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI

²Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. Diatoms are microscopic unicellular eukaryotic phytoplankton which carry out up to one fifth of photosynthesis on Earth. Diatoms not only produce the oxygen we breathe, but they also support many fisheries and generate the majority of the Earth’s organic carbon; thus they have been important contributors to petroleum reserves. There are over 200,000 different species of diatoms and over 100 species compose the genus *Thalassiosira*. This genus is an important contributor to marine production in temperate to polar regions. The complete genome of two species in the genus *Thalassiosira* has been sequenced and data is available for additional species, making this group an important model for studying how environmental change affects their productivity. An important first step is identifying where individual species in the genus are located in the ocean. The Jenkins lab has developed an automated ribosomal intergenic spacer analysis (ARISA) method that can identify *Thalassiosira* species in field samples. This method was used to analyze samples collected in a transect off the coast of Canada and Washington into the open ocean. There was a clear distinction in the *Thalassiosira* community composition between the coastal and oceanic samples. In addition, the fragment analysis showed a novel *Thalassiosira* species dominated the transition zone shelf break. This data was confirmed by sequence analysis. ARISA data will be compared with other ancillary data from the cruise. This may reveal the environmental factors that drive the shifts in community composition of the *Thalassiosira* genus within this region

Introduction.

Diatoms are microscopic, eukaryotic phytoplankton found in waters worldwide. Their name is derived from the Greek word *diatomos*, meaning ‘cut in half’, a reference to their two part cell walls made of silica (Armbrust, 2009). Diatoms are the most diverse group of phytoplankton containing an estimated 200,000 different species, ranging from a few micrometers to a few millimeters in size. These unicellular photosynthetic organisms are responsible for one fifth of the photosynthesis on Earth and create about as much organic carbon as all terrestrial rainforests combined (Armbrust, 2009 and references therein). Diatoms serve as a base for marine food webs and support our most productive fisheries. When diatoms escape consumption, they settle on the seafloor, which in the past has contributed to many of the petroleum reserves we have today. Diatom growth influences global climate, atmospheric carbon dioxide concentration, and marine ecosystem function thus it is important to understand how they will respond to future environmental changes.

Within the diatom genus *Thalassiosira* there are an estimated 100 different species living in habitats ranging from the tropics to the poles. This diatom genus has one of the highest species diversity in the marine, temperate phytoplankton community (Hoppenrath, 2007). During their lifecycle,

Thalassiosira species can vary in both geometry and micromorphology (Hoppenrath, 2007). The complete genomic infrastructure for two *Thalassiosira* species has already been identified (Armbrust, 2004; Lommer *et al.*, 2012). This infrastructure will assist in observing the change in *Thalassiosira* species with differing environmental factors.

The availability of iron has limited primary productivity within 30-40% of the world’s oceans (Armbrust 2009). These regions are known to contain a high concentration of essential nutrients such as nitrate, phosphate and silicic acid, but lack iron. The lack of iron has caused many of the *Thalassiosira* species to modify their photosynthetic apparatus (Sunda, 1991). These accommodations have compromised their ability to cope with rapid light fluctuations, a characteristic of coastal environments (Ribalet, 2010). Learning how these diatoms live in specific environmental situations will help us grasp a better understanding of the lives of diatoms and how they will cope with future environmental changes.

The Jenkins lab went on a cruise in May of 2012 to a “hotspot” in the Northeast Pacific Ocean (Ribalet, 2010), one of the largest bodies of atmospheric carbon dioxide. This hotspot is a narrow but seasonally persistent zone of iron-poor, nitrate-rich offshore waters transitioning to nutrient rich coastal waters, separated by a continental shelf. We are using a *Thalassiosira*-specific ARISA method (Chappell *et al.*, in prep) to analyze the samples and determine what

Figure 1. Map of station locations for May 2012 Geomics cruise depicting depth topography

species of the *Thalassiosira* genus are present throughout this region.

The Automated Ribosomal Intergenic Spacer Analysis (ARISA) method is designed to estimate community composition in natural samples and can be used to detect community shifts (Fisher, 1999). This method is highly reproducible and sensitive, allowing it to be a substantial method to use for comparing results-from field samples. The ARISA method targets rDNA cistrons with two variable internal transcribed spacer regions (ITS). When the second ITS region is amplified the fragment length varies for the differing *Thalassiosira* species, with many species having a distinct fragment length (Chappell *et al.*, in prep). The ARISA method is a useful method for detecting diatoms within field samples that are difficult to identify using light microscopy. Within our samples we found there to be a high relative abundance of *Thalassiosira oceanica* and *Minidiscus* within the open ocean waters of the Northeast Pacific Ocean. We also found a novel *Thalassiosira* species dominating the shelf break and of high relative abundance throughout the coastal samples.

2. Methods.

2.1 Sample Collection

Samples used for analysis were collected May of 2012 off the coast of Washington and Canada as part of the GEOMICS test cruise on the R/V Thomas G. Thompson. Approximately 1L of seawater was collected from various depths at each station using the shipboard conductivity, temperature, depth profiler (CTD) Rosette and was immediately run through a pressure filter. The filters were then placed in a screw cap test tube containing 500 μ L QIAGEN API buffer and flash frozen in liquid nitrogen for transportation.

Upon arrival in the lab, samples were immediately stored in -80°C freezer until analyzed.

2.2 DNA Extraction

General protocol was followed from the QIAGEN DNeasy® Plant DNA extraction kit, with a minor exception when removing the filters. A mixture of 0.1 μ m and 0.5 μ m silica beads was added to each tube with the RNaseA enzyme to lyse the cells. To enhance the cell breakup, the tubes were incubated at 65°C for 10 minutes, followed by bead beating for 1 minute. Following protocol, samples were eluted in two elution steps into a total of 200 μ L AE buffer and quantified using a NanoDrop™ 8000 spectrophotometer (Chappell *et al.*, in prep).

2.3 PCR Amplifications

PCR primers were selected to amplify all ITS2 sequences of the genus *Thalassiosira*. The reaction contained a 5' fluorescent FAM label (Life technologies Corporation, Carlsbad, CA) and a non-fluorescent reverse degenerate ITS2 PCR primer (Fwd Primer: 5'-RCGAAYTGCAGAACCTCG-3'; Rev Primer: 5'-TACTYAATCTGAGATYCA-3'0). A 25 μ L of PCR mixture was prepared for each sample using 6ng of DNA, 500nmol L⁻¹ forward and reverse primers, 1x BIO-X-ACT™ Short mix (Bioline USA Inc., Tauton, MA) and PCR grade water. The cycling conditions consisted of 95°C for 5min, followed by 32 cycles of: 95°C for one min, 46°C for one min, and 72°C for 30 sec; and ending with an extension at 72°C for 10min and cooling to 4°C. All PCR reactions were performed using a Mastercycler gradient PCR machine (Eppendorf AG, Hamburg, Germany).

2.4 Ethanol Precipitation

Ethanol precipitation was used to purify the PCR products. This was done by transferring samples into 1.5mL tubes and adding 0.1 volumes of 3mol L⁻¹ sodium acetate and 2 volumes of 100% ethanol. The

Figure 2. Temperature of each sample station in relation to depth. X's denote approximate area on map samples were collected from.

Figure 3. Fluorescence of each sample station in relation to depth. X's denote approximate area on map samples were collected from.

reaction was mixed and incubated at -20°C overnight. Samples were then removed from the -20°C freezer and centrifuged (14,000 rpm) for 30min at 3°C in an Eppendorf 5810R centrifuge (Eppendorf AG, Hamburg, Germany). The supernatant was discarded, washed with $200\mu\text{L}$ of 70% ethanol, and centrifuged (14,000rpm) for 5min at twice. A final wash was performed using 100% ethanol in place of the 70% ethanol. Samples were then dried at 40°C in a heat block and then resuspend in $50\mu\text{L}$ PCR grade water. A $12.3\mu\text{L}$ mixture of the sample, GeneScantm 600 LIZ size standard (Life Technologies Corporation, Carlsbad, CA) and Hi-Di Formamide were run on an ABI 3130xl genetic analyzer (Life Technologies Corporation, Carlsbad, CA) by the Rhode Island

Genomics and Sequencing Center. These results were then analyzed using Peak Scannertm v1.0 software.

2.5 Fragment Analysis

Comparative analysis of fragments from all samples was done in a manner similar to Nelson (2009). Peak height and size was determined for each FAM peak between 320 and 420 base pairs on every fragment run and binned into the fragment lengths previously determined by Chappell (2012, in prep). If peak heights were off-scale, the fragment was re-run at a 1:2 or 1:10 dilution. If the majority of peaks were below 1000 in peak height, the fragment was re-run with twice to four times as much sample added to the fragment analysis mixture and re-analyzed. Data for each sample were normalized to total peak height and all analyses were done using relative peak heights (the

height of each peak relative to the overall height of peaks in that sample). A relative abundance matrix was generated combining the relative peak height data for all samples at all fragment sizes where a peak was detected. If an individual sample did not have a measureable peak for any of the fragment size bins, a zero was placed in the matrix in that base pair range for that sample.

2.6 Cloning

Certain samples were selected for cloning analysis. 3 μ l of these samples were mixed with 5 μ l Rapid Ligation Buffer, 1 μ l PGEM-T Vector, and 1 μ l T4 DNA ligase and incubated at 4°C overnight. Ligations were plated onto Luria Bertani (LB) agar plates with 100 μ g/mL carbenicillin, 80 μ g/mL 5-

Figure 4. Fragment analysis results from 3 selected stations. The x-axis for each trace is the fluorescence relative in height to the largest peak for each sample. The y-axis is the base pair length associated with each peak, as determined by the 600-LIZ standard.

	Open Ocean Stations					Transition Stations							Coastal Stations			
	Station P8		Station P6			Station P5		Station P4			Station P3		Station P2		Station P1	
Size	5m	33m	5m	15m	20m	5m	55m	5m	20m	45m	5m	28m	5m	22m	5m	15m
349																
353																
363																
366																
369																
375																
384																
386																
388																
390																
393																
405																
410

Figure 5. Relative abundance of fragments from all stations. Each column represents a different sample with its station identified at the top of the column. The shading of different boxes indicate the percentage of the total peak height that each fragment group was responsible for in a given sample.

bromo-4-chloro-indolyl-galactopyranoside (X-gal), and 0.5mmol L⁻¹ isopropyl- β -D-1-thiogalactopyranoside (IPTG), and incubated overnight at 37°C. White colonies from each sample were picked onto another LB agar/carbenicillin/X-Gal/IPTG plate and incubated overnight at 37°C to confirm blue-white screening. Selected colonies were grown overnight in liquid LB/carbenicillin at 37°C and plasmids were purified using the QIAprep Spin Miniprep Kit (QIAGEN, Germany). The Rhode Island Genomics and Sequencing Center sequenced the plasmids by using the universal T7 primer (5'-TAAACGACTCACTATAGGG-3') on an ABI 3130xl genetic analyzer (Life Technologies Corporation, Carlsbad, CA).

3. Results.

3.1 Cruise Information

Figure 1 shows the samples that were evaluated on a map denoting depth topography. The cruise track included presumably nutrient rich coastal stations with depths no more than 100m to oceanic potentially iron limited stations with depths greater than 2000m, as

well as the transition zone in between. The temperature and fluorescence in relation to these stations at various depths is shown in Figure 2 and Figure 3, respectively. In the temperature graph (figure 2) the bottom of the mix layer and the beginning of the thermocline generally corresponds to the green line. The fluorescence profile is important to study because it is directly correlated to the chlorophyll intensities. The chlorophyll max zone was located around our deepest samples for each station. There was a significant spike in the chlorophyll max zone at station P5 around a depth of 55m. This was also around the base of the mix layer.

3.2 Fragment Analysis Data

ITS2 fragment lengths vary for individual species; Figure 4 shows the fragment lengths for the most coastal station (P1), main transition station (P4), and most oceanic station (P8). *Thalassiosira oceanica* is located at 349 base pairs fragment length and *Minidiscus* is located at 375 base pairs in fragment length. The open ocean station of P8 contained a large peak at 349 and 375 base pairs. This shows

Figure 6. Maximum likelihood tree of all ITS2 sequences from GenBank and field samples generated in the sequence analysis program Geneious using the PHYML algorithm with the Jukes-Cantor substitution model and 1000 bootstraps. The novel *Thalassiosira* diatom listed in this diagram indicates the sequence does not match any current information within the database.

Thalassiosira oceanica and *Minidiscus* are both prominent species within the open ocean station. In the coastal station there was also a large peak at 349 base pairs but not one at 375 base pairs. This shows *Thalassiosira oceanica* also appeared within our coastal zone but *Minidiscus* was not very detectable at those stations. The transition zone was dominated by a *Thalassiosira* species with a 393 base pair fragment length. This species is one that contains a new sequence that with homology-based searching does not match with other diatom sequences in the National Center for Biotechnology's sequence database (see below under cloning). Our coastal stations also contained a large peak at 393 base pairs, suggesting that this novel species may be a coastal species.

Figure 5 shows the relative abundance of the different fragment groups located at all stations. At most stations, results from multiple depths are shown. *Thalassiosira oceanica*, located at 349 base pairs, is abundant at all depths in the open ocean stations, but seems to only be very apparent within the shallow waters of the coastal stations. *Minidiscus* appears to dominate the open ocean station of P6. The novel species at 393 base pairs in length, clearly dominates the transition stations and the chlorophyll max of the coastal stations. Another fragment length that was found in all the open ocean stations, but was less abundant in the coastal and transition zone station was the 366 base pair fragment. This is another fragment that is not associated with any specific *Thalassiosira* species, but has been detected in field samples before (Chappell *et al*, in prep).

3.3 Cloning

The transition station of P4 was cloned and submitted for sequencing to try and classify this specific diatom. All of the sequences returned identical, grouping this specific diatom separate from the other *Thalassiosira* diatoms containing known ITS2 sequence data. A maximum likelihood tree of 5.8s-ITS2 sequences from GenBank is shown in figure 6, with the novel species not able to be classified highlighted in red.

4. Discussion.

Many factors must be taken into consideration when evaluating field samples to relate to the abundance and productivity of *Thalassiosira* diatoms including temperature, chlorophyll, depth, nutrient, and trace element values. Results indicating the nutrient and trace element values are still in progress from the field. The temperature profile in relation to depth (Figure 1) is similar to what was expected; the warmer waters were near the surface and as the depth increases the temperature decreases. The fluorescence profile (Figure 3), which is a proxy for chlorophyll abundance, showed intriguing results. Each station had a fluorescence/chlorophyll max that usually occurred at the base of the mixed layer, which is the start of the thermocline. The start of the thermocline

is often associated with the start of the nutricline; therefore the rich nutrient area may be the cause of the high chlorophyll concentration, but further research must be conducted to be sure. The transition zone is also associated with high productivity (Ribalet *et al.*, 2010), which the high chlorophyll data supports. The transition station of P5 shows a significant spike in the chlorophyll concentration at around a depth of 55m.

The PCR primers used for this experiment were designed to amplify the ITS2 regions of *Thalassiosira* diatoms within the field samples. *Minidiscus* and *Skeletonema* were diatoms that showed up within our research, but this is expected due to the *Minidiscus* species often being grouped with *Thalassiosira* species in phylogenetic trees based off of rDNA cistron components (Kaczmarek *et al.*, 2005; Alverson *et al.*, 2007). In designing the primer sets, we wanted to ensure all *Thalassiosira* species were amplified, being aware that this would result in the possibility of other non-*Thalassiosira* species being amplified as well.

A variety of *Thalassiosira* diatoms were found present within our coastal and oceanic stations. This is observed both by evaluating the fragment analysis profiles of key stations (Figure 4), and the relative abundance of fragments (Figure 5). The fragment analysis profiles of the key stations show multiple peaks within open ocean and coastal stations, whereas the transition station has one dominant peak. The low diversity within the transition zone could be the result of a specific *Thalassiosira* species bloom. The variety of other species around the coastal and ocean zones may have a correlation with the nutrient data. The relative abundance of fragments shows the different species vary not only with stations but also with depth. This is easily seen in the transition station of P5. The diatoms present at a depth of 5m vary dramatically with the diatoms present at a depth of 55m. The diatoms at a depth of 55m vary more than the diatoms present at a depth of 5m.

Our ITS2 sequences show *Thalassiosira oceanica*, *Minidiscus*, and a novel *Thalassiosira* species being most dominant throughout the field samples. *Thalassiosira oceanica* is a warm water species (Tomas, 1997; Kaczmarek *et al* 2005); which has also been reported in cold waters as low as 12°C (Aizawa *et al.*, 2005; Garcia and Odebrecht, 2009). *Thalassiosira oceanica* was found both within the coastal and ocean stations within our samples, but its main habitat seemed to be within the oceanic stations (Figure 5). *Minidiscus* was found to be very prominent within our open ocean stations as well. *Minidiscus* is difficult to identify using light microscopy; therefore the knowledge of their distribution throughout the world's ocean is limited (Tomas, 1997). Our transition zone was dominated by a *Thalassiosira* species with a fragment length of around 393 base pairs. This sequence was also high in abundance throughout the coastal stations. This species may be

one that has already been identified but is lacking an ITS2 sequence in GenBank; or alternatively, this fragment could be an unidentified *Thalassiosira* species. More research such as microscopy of samples where this species dominated will be needed to identify this species. The other diagnostic species, which does not correspond to any ITS2 sequence in GenBank, is located at 366 base pairs. This fragment length has appeared in previous research along this region (Chappell *et al.*, in prep) and its sequence also does not appear in GenBank. This species was found to be more prominent within our open ocean stations. It is possible that this sequence is either *Thalassiosira eccentrica* or *Thalassiosira pacifica*, which are likely to occur in this region (Aizawa *et al.*, 2005). It is also possible that this sequence could represent an unidentified *Thalassiosira* species. As the number of ITS2 sequences in public databases increase, these species may be identified in the future.

Analyzing the distribution of *Thalassiosira* species in relation to environmental factors will help us to predict how these species will respond to future changes. Within this paper the environmental factors taken into consideration were temperature, fluorescence, and depth. The spike within the chlorophyll max zone around station P5 was shown in the high relative abundance of our novel species. Nutrients and trace elements are known to be key factors in the distribution of diatom species; these future studies may help us better understand the composition of the *Thalassiosira* species within our field samples.

5. Conclusion.

Thalassiosira oceanica and *Minidiscus* were prominent *Thalassiosira* diatoms within our field samples collected from the Northeast Pacific Ocean. There is a distinct difference of diatoms present within the coastal and oceanic zones with a novel *Thalassiosira* diatom dominating the transition zone. Further research is necessary to classify this specific species. Additionally, comparing this information with nutrient and trace element values from the cruise may help to explain the difference between the field samples and the factors required for the survival of specific diatoms.

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- Samantha Maness, Pfeiffer University, Charlotte, NC, 28209 (SLManess@my.pfeiffer.edu).
- P.D Chappell and B. Jenkins, Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI, 02881. B. Jenkins (dreuz@mail.uri.edu, bjenkins@uri.edu).

Biological warfare in the planktonic world: Allelopathic inhibition of phytoplankton through the production of bioactive compounds

Jonathan Rempel¹, Jan Rines, Percy Donaghay

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. *Haslea* is a genus of marine pennate diatoms, comprising approximately a dozen species. The best known is *Haslea ostrearia*, which produces a blue-green polyphenol known as marennine. This pigment has been well studied, and has been shown to have numerous bioactive properties. Experiments were conducted to determine if two different *Haslea* species were able to inhibit the growth of other phytoplankton through the production of marennine or other similarly bioactive compounds. The goal of these experiments was to discover why water samples collected during an experiment in East Sound, WA contained a virtually monospecific *Haslea* sp. bloom, and why other phytoplankton that are normally quite abundant were nearly absent. Growth rates of *Skeletonema* sp. and *Thalassiosira rotula* grown in water preconditioned with both *Haslea ostrearia* and *Haslea* sp. were recorded and compared with control groups grown in Narragansett Bay water. The diatoms grown in water with a high concentration of marennine experienced severe growth inhibition, with population density remaining stagnant throughout the study. The conditions under which the East Sound *Haslea* sp. were grown did not result in the production of inhibitory chemicals, as shown in the lack of any significant difference in the growth rates of the phytoplankton versus the control group in the water preconditioned with this species. Because of the strong monospecificity of the East Sound bloom, it is clear that there are some factors that are resulting in differential survival among phytoplankton taxa, so future research should be conducted to look into why the East Sound *Haslea* has such an advantage over other diatoms.

1. Introduction

Haslea is a cosmopolitan genus of marine pennate diatoms. The genus *Haslea* was first described by Simonsen (1974) as a new designation for the genus *Vibrio* Gaillon. It was named for the famed Norwegian planktologist Grethe Hasle. Microscopical structure analysis proved that the differences were enough to warrant a new genus, possessing numerous characteristics. The cells bear a spindle-shaped outline with acute ends and convex sides. The central raphe is straight, and has approximately central pores and little developed apical pores. Their valve structure consists of transapical, parallel, and straight apical lines of areaolae on the exterior, covered by a thin, siliceous membrane with a slit of apical direction inside each areaolae (Simonsen 1974). Upon the establishment of this genus, several species were transferred into it, including the generitype (type species) *Haslea ostrearia*. *H. ostrearia* is the only known member of the genus to produce the blue-green polyphenolic pigment known as marennine, which is well known for, among other things, causing the greening of marennes oysters in oyster ponds on the French Atlantic coast. It is also known to have allelopathic effects, as well as antibacterial, antiviral, and antiproliferative applications (Pouvreau et al. 2007, Gastineau et al.

2012). The genus is quite diverse in its types of life histories, ranging from planktonic (Simonsen 1974, von Stosch 1985), to benthic and tube-dwelling (Pouvreau et al. 2007), as well as ice-dwelling.

Pouvreau et al.'s (2007) study tested the effects of purified marennine on cultures of phytoplankton that normally coexist with *Haslea ostrearia* in oyster ponds, but become less abundant during blooms of *H. ostrearia*. It was found that most of the species tested were significantly growth inhibited by the presence of marennine due to the biochemical properties of the compound, while others were inhibited only because the shading from the marennine in the water reduced the available sunlight for photosynthesis. Indeed, in most cases there was a negatively synergistic effect on growth rates due to the combination of the polyphenolic compound and the shading that results from its presence.

In 2009, Jan Rines, Percy Donaghay, and colleagues travelled to East Sound, Washington to conduct an interdisciplinary field experiment. There they found a novel bloom of a diatom that was tentatively identified as *Haslea* cf. *wawriake*, because of the overall similarities to the described species *Haslea wawriake*. Rather than the great diversity that is expected in any phytoplankton bloom, this bloom was virtually monospecific, and other genera and species of phytoplankton that are normally quite abundant were completely absent. Since the time that

the bloom was reported (Rines et al. 2010), further examination of the diatoms revealed that the taxonomic situation is more complicated than initially believed, and that *H. wawriake* is not the correct name for this diatom. For the purposes of this paper, it will be referred to simply as *Haslea* sp. Our hypothesis was that this species of *Haslea* was in a monospecific bloom because it may share the allelopathic properties of its prominent congeneric, *Haslea ostrearia*.

Figure 1. Phase contrast micrograph of *Skeletonema* sp. clone Skel1.

Figure 2. Phase contrast micrograph of *Thalassiosira rotula* clone Rot2.

The experiments we performed had several purposes. Our first experiment was conducted to try and repeat the results of Pouvreau et al.'s (2007) study testing the effects of marennine on various species of phytoplankton, as well as determine if our experimental setup was capable of testing for allelopathy. We did not explore the shading effect of marennine, only the effect of the presence of the chemical on growth rates. After the success of our first experiment, we proceeded with the second, whose purpose was to actually test for allelopathy in East Sound *Haslea* sp.

2. Experimental Setup.

This study consisted of two major experiments, both with very similar setups. The first experiment used water preconditioned with *Haslea ostrearia*, which resulted in a blue-green color due to the pigment marennine present in the exudates. The second experiment used water in which *Haslea* sp. from East Sound was grown. Because marennine was apparently not produced by this taxon, the medium appeared clear to the naked eye. Two clonal cultures of chain-forming diatoms were used as test subjects for the potential allelopathy: *Skeletonema* sp. (clone Skel1, Figure 1) and *Thalassiosira rotula* (clone Rot2, Figure 2), isolated from material collected in East Sound, Washington on June 4, 2012. For the first experiment, the two diatoms were grown as part of both the control and the experimental groups. In the control group, three replicates of each species were grown in 125 mL Erlenmeyer flasks containing 50 mL of f/2 culture medium based on water collected from Narragansett Bay, Rhode Island. In the experimental group, the same species were used, however the f/2 medium was made with water preconditioned with *Haslea ostrearia*. The cultures were inoculated with a calculated cell density of approximately 400 cells per mL and allowed to grow for several days until the populations entered their stationary phase.

The second experiment had a similar setup; the control group was the same, however instead of water preconditioned with *Haslea ostrearia*, the experimental group's culture medium was preconditioned with East Sound *Haslea* sp. The cultures were grown in triplicate, and under the same conditions. However, for this experiment the inoculation of the *Thalassiosira rotula* was reduced to 200 cells per mL, in order to prolong the exponential growth phase of the population and therefore provide better data for calculation of growth rates.

Experiments were run in a growth chamber at set at 17°C, under a 14:10 L:D (hours of light to dark) cycle of cool white illumination, for approximately one week. For both experiments, placement of the twelve culture flasks was randomized on the incubator shelf.

3. Methods.

The experimental culture media were prepared by culturing each species of *Haslea* (*Haslea ostrearia*, and *Haslea* sp. from East Sound) in prepared f/2 enriched seawater medium. The seawater that formed the basis of the f/2 medium was collected from Narragansett Bay, Rhode Island on May 16, 2012. The seawater was then enriched to f/2 concentrations of nutrients and sterile filtered to produce the control medium. The nutrient concentrations were as originally described by Guillard (1975). In order to produce the medium for the first experiment, *H.*

Figure 3. *Skeletonema* sp. growth curves from Experiment #1, plotted on a log scale.

Figure 4. *Thalassiosira rotula* growth curves from Experiment #1, plotted on a log scale.

ostrearia clone SB4 was isolated from the Pettaquamscutt River Estuary in Narragansett, Rhode Island. The isolate was then grown in a Fernbach flask containing 1.5 L of f/2 at 19°C, in an incubator on a 14 hour day and 10 hour night cycle. The culture was left to grow for 25 days, after which the seawater

appeared blue in color. The *H. ostrearia* cells were removed by sequential filtration through 64 µm nitex nylon mesh, followed by 20 µm nitex, and finally through Whatman GF/F glass fiber filters rated at 0.7 µm. The water was then enriched to create the f/2 medium, and sterile filtered using a Corning 0.22 µm

Figure 5. *Skeletonema* sp. growth curves from Experiment #2, plotted on a log scale.

Figure 6. *Thalassiosira rotula* growth curves from Experiment #2, plotted on a log scale.

disposable filter unit.

For the second experiment, East Sound *Haslea* sp. clone Has9 was isolated from an enrichment of material collected in East Sound, Washington in July

2011. The clone was then grown in 10 liters of f/10 medium at 15°C in an incubator on a 12:12 L:D cycle. The water was collected while the cells were still in their active growth phase, and the cells were filtered

out using the same procedure as previously described.

It was assumed that the exudates and compounds produced by the diatoms, if any, would be at a high enough concentration in the media to exhibit allelopathic effects on other phytoplankton grown in those media.

Cell density counts were conducted daily by drawing a 0.1 mL aliquot from each culture flask using a Pasteur pipette, and placing it into a Palmer-Maloney counting chamber. Each flask was swirled prior to sampling, to allow the cells that had settled to the bottom of the flask to go into an evenly distributed suspension. All cell sampling and transfers were conducted in a laminar flow hood containing an air filter, to prevent contamination of the cultures via airborne pathogens such as bacteria and fungi. Similarly, sterile procedure was used at all steps to prevent further contamination. Cells were examined and enumerated on a Nikon Eclipse E800 microscope at 100X magnification (10X objective), using phase contrast optics. Assuming even mixing of the culture flask, this count could be used as a representative density for the rest of the culture.

The time and date, as well as number of cells, were recorded for each flask every day. The times were converted to decimal time from the standard 12-hour AM/PM format to calculate exactly how many

days since inoculation each sample was counted. Once the cell counts were compiled, they were entered into a Microsoft Excel spreadsheet and the number of cells as a function of time was plotted. The graphs of population of the control group and the experimental group were then compared to visually examine the results. Growth rates for each culture were obtained by graphing the cell counts with the independent variable on a logarithmic scale, then isolating the points on the graph pertaining to the exponential phase, rather than the lag phase or stationary phase. A line of best fit was then fitted to these points, and an exponential equation was derived from this line. This exponential equation, following the format $f(x) = e^x$, is in essence the rate of growth for that culture.

4. Results and Discussion.

In the first experiment, we saw strong growth inhibition caused by the presence of marennine in the water. While the control group exhibited normal exponential growth after an approximately two day lag phase, the experimental group remained stagnant throughout the entire study period, with a slight downward trend; there was no exponential growth whatsoever, and barely exceeded the original inoculum density (if at all). This growth inhibition was

Table 1: Growth rates of *Skeletonema* sp. and *Thalassiosira rotula* from Experiment #1.

Culture	Growth Rate Formula	R ² Value
M-Skel1-A	$y = 436.34e^{-0.212x}$	0.31788
M-Skel1-B	$y = 2006.3e^{-0.439x}$	0.46414
M-Skel1-C	$y = 2059.6e^{-0.339x}$	0.70657
C-Skel1-A	$y = 34.722e^{1.0752x}$	0.99308
C-Skel1-B	$y = 36.841e^{1.2108x}$	0.98856
C-Skel1-C	$y = 60.528e^{0.9349x}$	0.98164
M-Rot2-A	$y = 360.81e^{-0.022x}$	0.0463
M-Rot2-B	$y = 399.78e^{-0.021x}$	0.37063
M-Rot2-C	$y = 431.77e^{0.059x}$	0.42899
C-Rot2-A	$y = 226.57e^{0.844x}$	0.99893
C-Rot2-B	$y = 208.03e^{0.8216x}$	0.99525
C-Rot2-C	$y = 263.94e^{0.83x}$	0.96622

Table 2: Growth rates of *Skeletonema* sp. and *Thalassiosira rotula* from Experiment #2.

Culture	Growth Rate Formula	R ² Value
ES-Skel1-A	$y = 193.03e^{1.479x}$	0.99692
ES-Skel1-B	$y = 174.04e^{1.6286x}$	0.99592
ES-Skel1-C	$y = 211.47e^{1.5122x}$	0.98908
C-Skel1-A	$y = 184.76e^{1.5311x}$	0.99853
C-Skel1-B	$y = 149.34e^{1.5772x}$	0.98759
C-Skel1-C	$y = 146.44e^{1.6564x}$	0.9951
ES-Rot2-A	$y = 121.01e^{1.2534x}$	0.98115
ES-Rot2-B	$y = 128.26e^{1.2553x}$	0.94899
ES-Rot2-C	$y = 135.48e^{1.2006x}$	0.9408
C-Rot2-A	$y = 134.46e^{1.28x}$	0.92068
C-Rot2-B	$y = 131.24e^{1.2323x}$	0.96558
C-Rot2-C	$y = 101.02e^{1.3374x}$	0.95293

clear in both *Skeletonema* sp. and *Thalassiosira rotula*, but more so in the former species due to the much smaller cell size, and therefore greater cell density and growth rates. There was some minor variation in the growth rates among the control group, but this can be attributed to the colonial nature of these diatoms. Each colony can be thought of as a single particle in suspension, and when a chain consists of 10-20 or more cells, the presence or absence of a colony from the initial inoculation due to random sampling can mean a major difference in the number of cells after a few days of exponential growth. The growth rate formulas, as well as R^2 values, can be seen in Table 1.

In the second experiment, there was no significant difference in the growth rates between the control group and the experimental group, in both the *Skeletonema* sp. cultures and the *Thalassiosira rotula* cultures. The severe growth inhibition observed in the first experiment was nowhere to be seen. Upon inoculation, both the control and experimental group *Skeletonema* underwent exponential growth until after day 3, when the population size began to level off into stationary phase. As for the *Thalassiosira*, the cultures grew exponentially until day 4, after which they entered the stationary phase. The growth rates for the second experiment and R^2 values are described in Table 2.

If we wished to further explore the effects of marennine on diatoms, there are a few possible channels for future research. In this experiment, we tested the effects of marennine produced by a culture of *Haslea ostrearia*, and we measured the presence of marennine qualitatively rather than quantitatively, by observing the blue-green color of the medium. However, if we were to either dilute (through the addition of seawater) or concentrate the exudates in the f/2 solution, we could test the effects of varying concentrations of marennine on growth rates, and determine if there is a dose-dependent response to marennine in phytoplankton. The negative effects of allelopathy are a function of dosage; indeed, many stimulatory compounds become harmful above a certain concentration or timed exposure, and some inhibitory compounds can aid growth below a critical dosage (Cembella 2003), as seen in marennine's effect on cultures of *H. ostrearia* (Pouvreau et al. 2007). Additionally, we explored the effects of marennine on only two species of diatoms: *Skeletonema* sp. and *Thalassiosira rotula*. The allelopathic effects of marennine on other phytoplankton is not universal, and indeed some species have a much higher tolerance of its presence than others, except when shading becomes a factor (Pouvreau et al. 2007). The mechanisms by which allelopathy induces cell death or growth inhibition are numerous and diverse, but commonly include cell membrane damage, inhibiting protein activity, and modifying or activating a

physiological function (Legrand 2003). By continuing experiments similar to this one, we could learn more about what determines a phytoplankton's ability to resist the harmful effects of these compounds.

After performing these experiments, we were unable to determine the cause of the extreme monospecificity of the field bloom in East Sound, Washington. There are several possible explanations for why this was so, and several directions we could go in to further explain the unusual phenomenon. Our cultures of the East Sound *Haslea* sp. did not prove to be allelopathic, however this was merely under the conditions in which we grew them. Clearly something in the water in East Sound made it possible for only the *Haslea* to survive; perhaps if we were to more closely mimic field conditions, the allelopathic properties might emerge. The vast majority of allelopathic studies are laboratory experiments, rather than field studies involving mesocosms or microcosms (Legrand 2003). The incubator we used simulated sunlight, however the lights only emitted electromagnetic radiation in the visible light spectrum, rather than the wide range from ultraviolet rays to infrared light that the sun emits. By growing the *Haslea* in natural sunlight, and then subjecting the subject diatoms to the same conditions, allelopathic properties may result.

Alternatively, growing the *Haslea* in the same water as other phytoplankters may cause them to produce the allelopathic exudates as a defense mechanism, something that would not be seen in a laboratory monoculture setup. Additionally, for these experiments we have only been looking at interactions on the same trophic level, that of the primary producers. Perhaps trophic interactions such as predator/prey relations played some role in the composition of the bloom in East Sound, such as selective feeding by zooplankton. One possible application of allelopathy is the deterrent of potential predators, through the production of noxious compounds that induce olfactory or gustatory responses in the predator during the preingestive stage, or even through negatively affecting the fertility and viability of zooplankton and their eggs (Cembella 2003). If these *Haslea* produce compounds that deter predators, while other bloom diatoms do not, then *Haslea* would have the clear survival advantage.

Our results for the two experiments, although extremely clear, were quite different. The stark difference in cell density between the control and experimental groups in the first experiment contrasts sharply with the null effect of *Haslea* sp. on growth rates in the second experiment. The effects of the exudates from *H. ostrearia* have been well studied, but at this time it is not known whether *Haslea* sp. exudes any metabolites whatsoever. Even if something were produced in those culture flasks, the conditions under which we ran the experiment meant that those

exudates had no effect on the *Skeletonema* sp. or *Thalassiosira rotula*, and deeper exploration into the nature of the monospecific bloom is required. One thing that is important to note when discussing tests for allelopathy is that although there may be demonstrated growth inhibition, correlation does not imply causation and there may be other factors at work suppressing growth. For example, depending on the composition of the metabolites in the exudates, reactions may take place between these compounds and the nutrients in the culture medium, making the medium nutrient-limited. Therefore, it is difficult to truly test for allelopathy even in a controlled setting (Cembella 2003). Due to the clearly observable nature of marennine (with its deep blue-green coloration), as well as prior research involving the compound, we believe that our experiments are suitable tests for allelopathy.

5. Conclusion.

Even on a microscopic scale, marine ecosystems normally exhibit a great deal of diversity. So the near monospecificity of the *Haslea* sp. bloom found in East Sound, Washington raised several questions, chief among them being why *Haslea* was so successful when other common temperate diatoms were nowhere to be found. Our hypothesis was that this species of *Haslea* has similar allelopathic properties to its better known congeneric, *Haslea ostrearia*. Our first experiment showed clear growth inhibition in the cultures grown in water preconditioned with *H. ostrearia*, containing the compound marennine. This verified the results obtained by Pouvreau et al. (2007), and proved that our experimental setup was a good test for allelopathy. The second experiment, using water from *Haslea* sp. from East Sound, did not show any inhibition under the conditions we used. Because we were unable to determine the cause of the monospecificity in East Sound, further research is needed to narrow down the possibilities.

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- J.N. Rempel, Binghamton University, Binghamton, NY, 13902. (jrempe1@binghamton.edu)
- J.E.B. Rines and P.L. Donaghay, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (jrines@gso.uri.edu, donaghay@coral.gso.uri.edu)

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Exploring eddy radii in laboratory experiments

Julie Warner, Peter Cornillon, Lew Rothstein, John Salter, Dan Iwanski

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. Radii of naturally occurring eddies are dependent on both the stratification or water depth and the Coriolis force present at the eddy's specific latitude. In laboratory experiments using a rotating tank, these factors translate to an eddy radius dependent on the water height in the tank and rotation rate of the tank. However, when creating eddies by pumping water in or out of the tank, observed radii are much smaller than expected, often measuring only 10% to 20% of the expected radius. This project attempts to understand the reason for the discrepancy, in particular to examine eddy radii in the context of a theory developed to explain the differences. Due to secondary circulation within the eddy, water height in the tank is still thought to be a controlling factor in eddy radius in a barotropic fluid. However, pump flow rate rather than tank rotation rate is thought to be another factor. The theory suggests that eddy radius changes with the square root of both the water height and pump flow rate. To test this, eddies were created with varying pump flow rates and water heights. The flow of fluid inside the rotating tank was analyzed using particle image velocimetry (PIV), which produces quantitative data of the fluid flow. These data were then used to determine a radius for each eddy produced. Preliminary experiments indicate that water depth and pump flow rate do set the eddy radius. Determining the relationship between these factors and radius will be important in experiments whose aim is to study eddy behavior in a laboratory and relate this behavior to eddies in the ocean.

1. Introduction.

Creating eddies in a laboratory setting is a much-used technique in physical oceanography experiments. This project in particular was initially intended as an experiment to model ocean striations in a laboratory using eddies. It became clear from preliminary lab runs that a fundamental understanding of eddy behavior and formation was required to proceed with the experiments. A set of experiments was designed to characterize how certain important elements affect eddy behavior. In carrying out these experiments, we noticed the radii of the eddies produced were much smaller than expected.

Naturally occurring eddies in the ocean have radii that are on the order of the radius of deformation. The radius of deformation depends on the Coriolis force at the eddy's specific location, gravity, and water depth or stratification (Cushman-Roisin 2004). When creating eddies in a laboratory using a rotating tank, one would assume that the radii of these eddies would depend on the rotation rate of the tank and the height of the water in the tank. However, in our experiments, in which we used a pump as a sink to create the eddies, radii were much smaller than the expected radius of deformation based on the tank rotation rate and height and depth of the water.

Upon further research, we found this to be a common occurrence, but one with very little further investigation (Clercx and Van Heijst 2009) and so we designed a new set of experiments with the aim to determine what factors were influencing the radii of our eddies. Our initial theory to explain the discrepancy between theoretical and experimentally

determined radii concerns the secondary circulation of an eddy in which bottom friction plays a central role. This secondary circulation is a result of water flowing into the eddy through the Ekman layer at the bottom of the tank and then up through the center of the eddy. With no suction out of the tank, once the water reaches the top of the eddy, it turns over and flows back down towards the bottom and the eddy slowly collapses as the bottom friction applies a retarding stress to the eddy. However, with suction, the water flowing up through the center is sucked out through the pump (Clercx and Van Heijst 2009). The theory relates two time constants. The first is the time it takes to spin down the eddy once pumping has stopped. The second is the amount of time it takes for water flowing in to refill the volume of the eddy. This produces an equation for eddy radius where the radius is proportional to the square root Q , the pump flow rate, and one over the fourth root of the rotation rate, Ω , and independent of H , the height of water in the tank. This is the theory that fueled our experimental design.

2. Experimental Setup.

The experiments in this project all revolve around the use of a rotating table. Mounted on the table is a cylindrical, Plexiglass tank approximately 97 cm in diameter and 45 cm tall. A thin layer of black plastic covers the bottom of the tank. This cylindrical tank is surrounded by a larger, octagonal tank. The purpose for the octagonal tank is to reduce the distortion resulting from parallax when viewing the fluid in the tank from the outside. The tank also has a clear Plexiglass lid used to reduce wind effects on the

fluid inside during rotation. A small centimeter wide hole is drilled in the center of the lid through which we place a long, thin glass tube. The glass tube is also set through a rectangular metal block with a hole drilled through the top center to keep the end of the tube at the selected height in the water, approximately 14 cm above the tank bottom. This glass tube is connected by flexible plastic tubing to a pump secured to the supports on the rotating table. The tubing and pump make up the sink or source by which the eddies are created. Another piece of flexible plastic tubing is connected to the pump and is inserted into the outer, octagonal tank around the cylindrical tank. This is where water pumped out of the tank is deposited when creating eddies using a sink, and where water is pumped out of when the pump is used as a source. The glass tube inserted through the tank lid is kept in place by two adjustable lengths of string attached to the table structure. The pump is plugged in to a power strip on the rotating table. The power supply to the power strip is controlled by a switch on the wall so that the power strip and, by extension, the pump can be turned on from a distance while the table is in motion. The eddy is created at the center of the tank in order to utilize the flattest part of the water surface during rotation and thereby reduce as much as possible the unwanted β -effect from a sloping water surface. The tank setup is diagramed in Figure 1.

Figure 1. Basic diagram of tank setup. The sink is at the center of the tank.

The water height, H , that we chose for all experiments completed to date is 16 cm. In testing our theory, we varied both tank rotation rate, Ω , and pump flow rate, Q . We chose flow rates (53 mL/min, 132 mL/min, 245 mL/min, 352 mL/min, and 435 mL/min) and two rotation rates (0.5 rad/s and 1.291 rad/s) to test in our experiments. All five pump flow rates were tested with the table rotating at 1.291 rad/s, while only 132 mL/min and 352 mL/min were tested with the rotation rate of 0.5 mL/min. In addition, with a rotation rate of 1.291 rad/s, we attempted to create eddies using the pump as a source rather than a sink.

We did two experiments with this setup, using pump flow rates of 53 mL/min and 245 mL/min.

In addition to the cylindrical tank, a laser and camera are attached to the rotating table structure. The laser is attached in such a way that the light sheet it forms is perpendicular to the axis of rotation and approximately 8 cm above the bottom of the tank. The camera is attached at the top of the table structure, which is a few meters above the tank lid, and positioned such that it is aimed down towards the tank and focused on a specific rectangular area of water inside the tank. Connected to the cameras and laser and situated underneath the table is a computer that controls both of these elements and records the camera output. A laptop computer uses a wireless network to remotely access this computer and serves as a monitor through which to access the data stored on the table computer.

3. Methods.

Experiment Methods

This project is comprised of a total of 9 separate experiments, with each experiment testing a different combination of pump flow rate and table rotation rate. The experiments were done in two sets, both defined by the rotation rate of the table for the experiments in the set. However, the majority of the procedure was the same for both sets of experiments.

The first step in running the experiments was to measure the fluid flow through the pump. Marking off five equally spaced settings on the pump dial, we measured the flow rate at each by using the pump to fill a graduated cylinder, timing out a minute of flow. We took the measurement of water in the graduated cylinder to be the flow rate at that particular setting, in mL/min. The next step was to calibrate the camera using the LaVision particle image velocimetry (PIV) software DaVis, which is also the software used to collect data on the fluid flow inside the rotating tank. Calibration of the camera was conducted as follows. A laminated sheet covered by an evenly spaced grid of crosses was placed at laser-height in the tank in the camera's viewfield. The camera then took a picture of the calibration sheet in the water. The spacing between crosses in the grid as well as the height and width of each cross was measured and these measurements were entered into the PIV software. The software then used these measurements to develop a calibration setting so the DaVis software could use the images from the camera to accurately measure fluid movement in the tank. Additionally, we seeded the tank with tiny glass spheres approximately ten microns in diameter. These tiny glass spheres are integral to how the PIV software tracks the flow of the fluid.

To begin our first set of experiments, we turned on the pump at the lowest flow rate, although the power supply to the pump was kept off so the pump

wasn't active. We turned on the laser and the camera, and then started the table rotation. We spun up the tank to the desired rotation rate. Our first set of experiments used a rotation rate of 1.291 rad/s. After the table reached this rotation rate, it was left rotating for approximately 20 to 30 minutes in order for the water in the tank to reach solid body rotation, the steady state for the water with the pump off. We verified that the tank had reached solid body rotation by examining several frames of data from the PIV system to ensure there was no residual flow in the tank.

Once we felt the water had reached steady state, we turned on the power supply to the pump, which pumped water out of the tank, serving as a sink to create the eddy. The pump ran undisturbed for about six minutes. This was more than enough time for the eddy to reach state, which we verified in the subsequent analysis of the data described below. After this time period passed, we turned on the camera and laser and the PIV software took pictures of the tank for about 5 minutes. After five minutes of picture taking, in which about 600 images are taken, the camera and laser were turned off and the pump flow rate was changed to the next higher flow rate. Once again we waited about six minutes for the eddy to reach steady state and then the camera and laser were switched on for another five minutes of image-taking. This process was then repeated for the remaining pump flow rates. Once we finished testing all of the pump flow rates with the pump as a sink, the plastic tubing was rearranged to create a source. We then performed two experiments with the pump as a source pumping water into the tank, one with a pump flow rate of 53 mL/min and one with a pump flow rate of 245 mL/min. After finishing this set of experiments, we performed a set of experiments following the procedure outlined above but at a rotation rate of 0.5 rad/s and only two pump flow rates: 132 mL/min and 352 mL/min. No experiments were conducted using the pump as a source at this rotation rate

PIV Methods

An integral component of data collection was the particle image velocimetry software. The was used to record our data and perform preliminary processing of these data as follows.

The PIV software collects its data through images of the fluid. It makes use of a laser pulse and tiny glass spheres distributed throughout the fluid to collect these images. The laser is set to send out a beam of light at a certain height in the fluid. It sends out this pulse at certain rate; in our experiments this rate was set to 2 Hz. The tiny glass spheres in the fluid reflect the light from the laser in all directions. Meanwhile, the camera above the tank is set to take pictures at the same rate that the laser sends out a light beam. Therefore, the camera captures an image of the glass spheres reflecting the laser light. After an

experiment is completed and enough images are taken by the camera, the software processes the images.

Figure 2. Fitting a circle to ring of velocity vectors with magnitudes between 0.00142 and 0.00147 m/s. This technique was used to find the center of the eddy.

To do so, the PIV software tracks the movement of the glass spheres from image to image. It does a double pass cross-correlation on successive images. It uses this to create a vector field of the velocity of the fluid flow for each set of successive images. These vector fields are what we use to analyze our eddies.

Eddy Analysis Methods

The majority of data processing was done using MATLAB scripts. First PIV vector fields are imported into MATLAB using a LaVision program, which imports into matlab an x- and y-position and the x- and y-components of the velocity vector at this location. Then the following procedure is followed for each processed image. First the magnitude of each vector is computed using the x- and y-components. Then each vector with a magnitude less than 0.00001 m/s is replaced with a zero vector. In an attempt to remove any inaccurate vectors, any vector with an angle more than $\pi/6$ radians different from at least three of the vectors around it is also replaced with a zero vector.

After these preliminary computations, the maximum velocity magnitude is found for the image. Then the positions of all vectors with a magnitude between this maximum magnitude and 0.005 m/s below the maximum are identified. These points generally form a circular ring, as shown in Figure 2. A circle is then fit to the ring of points using a circle-fitting MATLAB script, which also finds the center of the circle. This process is then repeated with the lower magnitude boundary becoming the upper magnitude boundary and the lower boundary 0.005 m/s below this value. Once again a circle is fit to these points and a center found for this ring. This

continues until all vectors in the image have been fitted to a circle. Finally, the center coordinates are

Figure 3. Graph of average azimuthal velocity versus radius for a given image in one experiment. Similar graphs were plotted for each of the 600 images in an experiment.

Figure 4. Graph of eddy radius versus the square root of the pump flow rate. Circles indicate experiments performed with a rotation rate of 0.5 rad/s and crosses indicate experiments performed with a rotation rate of 1.291 rad/s. The dotted line represents the best-fit line between data from experiments with a rotation rate of 0.5 rad/s and the solid line represents the best-fit line between data from experiments with a rotation rate of 1.291 rad/s.

averaged and one center is found for the entire image. To remove any error from incorrectly fit circles, individual centers with coordinates more than one standard deviation from the mean coordinates are removed and the center recalculated. This becomes the center for the eddy for the given image, which is then used to find a radius and azimuthal velocity for each vector in the image.

The azimuthal velocities are then averaged over a 5 mm range of radii, excluding velocities below 0.0005 m/s to avoid including zero velocity vectors. This gives an average azimuthal velocity by radius, which is then graphed for the image, as shown in Figure 3.

This graph is used to find the radius of the maximum azimuthal velocity in the image. We call this the radius of the eddy in that particular image. Once this process has been completed for all of the images in an experiment, the eddy radii are averaged to obtain an average radius for the eddy for the experiment. This process is repeated for each experiment and an average radius is acquired for each experiment. These radii are then compared in the context of our initial theory.

4. Results and Discussion.

Data from our experiments were examined to see how closely our theory fit the laboratory results. To interpret our results, the eddy radius was plotted versus the square root of the flow rate used for each experiment, as shown in 4. The data points were separated into groups based on rotation rate, and only experiments using a sink were included. Comparing the radii from experiments with a rotation rate of 1.291 rad/s, there appears to be a direct linear correlation between radius and the square root of the flow rate. Although we only have two data points for experiments with a rotation rate of 0.5 rad/s, these points fall on a line that is roughly parallel to that of the first set of experiments. In this aspect, both sets of experiments agree with our theory that eddy radius should be directly proportional to the square root of the flow rate of the pump creating the eddy.

Comparing the two sets of experiments to each other reveals additional information. The experiments with a rotation rate of 0.5 rad/s have a consistently smaller eddy radius than the experiments with a rotation rate of 1.291 rad/s when identical pump flow rates are used. This indicates that eddy radius may be a weak function of tank rotation rate, also in agreement with our theory. However, there is also a slight contrast to our theory, as the theory predicts faster tank rotation rates to have a smaller radii, while we observe the opposite.

In addition to the experiments conducted using the pump as a sink, we also conducted two experiments using the pump as a source. These experiments yielded little useful data. Neither created a steady, consistent eddy that could be analyzed in the same manner as the other seven experiments, so neither could be compared in any comprehensible way to the other experiments.

The results of our experiments are preliminary in that we did not examine the relationship, if any, of eddy radius to the height of the fluid nor do we have enough data to definitively answer the question of the relationship between eddy radius and rotation rate.

5. Conclusion.

This aim of this project was to characterize the factors that are important in setting eddy radii in laboratory experiments using a rotating tank and a sink to form eddies. We wanted to understand why the radii of eddies formed in a rotating tank were so different from the radius of deformation, which is close to what one would expect if the eddy were to be formed in the ocean. We theorized that this discrepancy was caused by secondary circulation of the eddy. This theory led us to a definition of eddy radius that was proportional to the square root of the flow rate of the pump and the fourth root of the tank rotation rate while being independent of water height in the tank. Our experiments were designed to test this theory by varying the pump flow rate and the rotation rate of the tank. Our results suggested that there is a dependence on the square root of the pump flow rate, as well as a slight dependence on tank rotation rate, although of the wrong sign. Further experiments are needed to solidify these results. The development of a definite theory on what factors are influential in setting the radius for eddies in a rotating

tank will be extremely useful for experiments done in a laboratory. It will allow eddies to be made at specific radii and also allow for more accurate relations between eddies in the laboratory and eddies in the ocean.

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- P. Cornillon and L. Rothstein, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (pcornillon@gso.uri.edu, lrothstein@gso.uri.edu)
- J. Warner, American University, Washington, DC 20016 (jw3367a@american.edu)