University of Rhode Island

DigitalCommons@URI

SURFO Technical Reports

Graduate School of Oceanography

2011

SURFO Technical Report No. 11-01

SURFO

Follow this and additional works at: https://digitalcommons.uri.edu/surfo_tech_reports

Recommended Citation

SURFO, "SURFO Technical Report No. 11-01" (2011). *SURFO Technical Reports*. Paper 5.
https://digitalcommons.uri.edu/surfo_tech_reports/5

This Periodical is brought to you for free and open access by the Graduate School of Oceanography at DigitalCommons@URI. It has been accepted for inclusion in SURFO Technical Reports by an authorized administrator of DigitalCommons@URI. For more information, please contact digitalcommons-group@uri.edu.

**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 2011

This program was supported by
The National Science Foundation REU Program (OCE-0851794).

GSO Technical Report No. 11-01

**PARTICIPANTS IN THE 2011
SUMMER UNDERGRADUATE RESEARCH
FELLOWSHIP PROGRAM IN OCEANOGRAPHY**

FELLOWS

John Bruce	Manchester College, North Manchester, IN
Adam Darer	Oberlin College, Oberlin, OH
Erika Gibson	University of Miami, Coral Gables, FL
Ben Heath	Northwestern University, Evanston, IL
Thomas Hespeler	University of Rhode Island, Kingston, RI
Arianna Jesanis	San Diego State University San Diego, CA
Megan McDonald	University of Southern California, Los Angeles, CA
Andrea Reis	Worcester Polytechnic Institute, Worcester, MA
Emily Rhoades	Whitman College, Walla Walla, WA
Kellen Rosburg	Western Washington University, Bellingham, WA

ADVISORS

Dreux Chappell
Jeremy Collie
Kathleen Donohue
Edward Durbin
Tetsu Hara
Bethany Jenkins
John King
Susanne Menden-Deuer
Tatiana Ryneearson
David Smith
David Ullman

PROGRAM ASSISTANTS

Brandon Reichl & Kim Carey

PREFACE

This report presents the papers written by the nine participants in the 2011 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 27th 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 2011 program continued excellence beyond the official duration of the program with at least four projects presented at the Ocean Sciences 2012 national meeting.

The participants in the 2011 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.

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 Brandon Reichl who served as a graduate coordinator for the program.

Kathleen A. Donohue
Robert A. Pockalny
SURFO Site Directors

TABLE OF CONTENTS

	<u>Page Number</u>
Participants in the 2011 Fellowship Program	i
Site Directors' Preface	ii
1. Stoke's Drift Due to Ocean Surface Waves under Tropical Cyclone Conditions <i>John Bruce, Tetsu Hara</i>	1
2. Metabolic Response of <i>Thalassiosira Oceanica</i> to Iron <i>Adam Darer, Dreux Chappell, Bethany Jenkins</i>	8
3. Identification of the Two <i>Acartia Tonsa</i> Populations Located in Narragansett Bay and Analysis of the Biological Differences Present Between Them <i>Erika Gibson, Edward G. Durbin</i>	14
4. Eddy Formation and Possible Sinking in the Northwest Corner <i>Ben Heath, Dave Ullman, Christian Buckingham</i>	20
5. Subseafloor Sedimentary Fungi and their Viability at High Pressure <i>Thomas Hespeler</i>	25
6. Amplifying Microsatellite Loci in the Marine Diatom <i>Ditylum brightwellii</i> <i>Arianna Jesanis and Tatiana Rynearson</i>	28
7. Statistical Analysis of Replicate Grab Samples for Ground-truthing Benthic Habitat Maps <i>Megan McDonald, John King, Monique LaFrance, Dane Sheldon</i>	35
8. Effects of Copepods on Phytoplankton Growth and Heterotrophic Protist Grazing Rates in Narragansett Bay <i>Andrea Reis, Caitlyn Lawrence, Susanne Menden-Deuer</i>	41
9. Dietary Guild Structure of the Fish Community in Rhode Island and Block Island Sounds <i>Emily Rhoades, Anna Malek, Jeremy Collie</i>	48
10. A Performance Evaluation of the HYCOM Gulf of Mexico Model <i>Kellen Rosburg, Kathleen Donohue</i>	55

Stokes Drift Due to Ocean Surface Waves Under Tropical Cyclone Conditions

John Bruce, Tetsu Hara

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

Abstract. Hurricanes pose a serious threat to coastal regions along the Atlantic and Gulf. In the last few decades significant efforts have been made to understand the hurricane dynamics and to improve hurricane track/intensity forecasts. However, the complex physical mechanism by which they extract and dissipate energy at the air-sea interface is not fully understood. The primary energy source of hurricanes is warm surface water. When hurricanes apply large wind stress at the air-sea interface, it enhances turbulent mixing in the ocean surface layer and increases the entrainment of cool deep water at the base of the mixed layer into the ocean surface layer. This near surface ocean cooling, in turn, reduces air-sea heat fluxes that drive hurricanes. Recently, it has been found that this upper ocean turbulent mixing may be significantly modified due to ocean surface waves. Surface waves introduce net mass transport (Stokes drift) near the water surface. This Stokes drift interacts with turbulent eddies in a complex manner, depending on different wind and wave conditions (Langmuir turbulence). As a first step to study the effect of Langmuir turbulence, we investigated the vertical profile of Stokes drift under a wide range of wind and wave conditions. First, investigations were made with growing and fully grown wave fields generated by uniform wind speeds from 10 to 50 ms^{-1} . The results show that the vertical profile of the Stokes drift is well described by two parameters: a depth that is inverse of the peak wave number (wave number at the wave spectral peak) and the Stokes drift value at that depth. Next, the Stokes drift calculations were performed for more complex wave fields under a stationary tropical cyclone, as well as storms moving at 5 and 10 ms^{-1} translation speeds. Such simulations demonstrate good directional agreement between wind and surface Stokes drift vectors as well as between dominant wave direction and deep Stokes drift vectors. For the translating tropical cyclones strong asymmetries were present in the Stokes drift field. Both cases showed significant regions where the wind and the deep Stokes drift were misaligned by more than 90° , suggesting significant weakening of near surface turbulence due to surface waves.

1. Introduction.

Tropical cyclones (TC) or hurricanes are characterized by extreme winds and are accompanied by heavy rains and storm surge that pose a serious threat to coastal communities around the world. Therefore, it is important to accurately predict a TC track and intensity in order to minimize property damage and loss of life by streamlining evacuation efforts.

The primary source of energy for a TC is thermal energy contained in warm sea surface waters (Fan et. al. 2010). High sea surface temperature (SST) tends to increase TC intensity while cooler SST reduces intensity by limiting the energy flux. The upper ocean consists of a warm mixed layer that contrasts abruptly with the cooler waters below. When TCs move over the ocean the strong winds induce turbulent mixing of the upper ocean by applying high wind stress upon the sea surface. The mixing, in turn, causes cool water entrainment at the bottom of the mixed layer, leading to a cooler sea surface temperature (SST) and consequently lower TC intensity (Price 1981, Emanuel 2004).

Recent studies suggest that surface waves, which are also generated by the TC wind, may significantly modify the turbulence intensity and the thermal structure of the upper ocean. Surface wave motions introduce net mass

transport called “Stokes drift”. It is known that the Stokes drift has the effect of tilting and organizing the turbulent eddies in the direction of the drift. The resulting turbulence is called “Langmuir turbulence”. When wind and waves are aligned, the Stokes drift tends to enhance the Langmuir turbulence. Of particular interest is the complex case in which wind is misaligned with waves at angles greater than 90° . In such cases the Stokes drift may diminish the Langmuir turbulence and the consequent cool water entrainment and the sea surface cooling (D’Asaro et. al.).

2. Procedure.

In order to investigate the Stokes drift a number of numerical simulations are performed, including wave field simulations with steady uniform wind and TC wind conditions. For all simulations NOAA’s Wave Watch III [version 3.14] was used to predict wave fields after 72 hours of simulation in the form of directional frequency wave spectrum $\psi(f, \theta)$ data, where f is the wave frequency and θ is the wave direction. The simulation space is a 18° (~ 2000 km in west-east) by 30° (~ 3340 km in south-north) virtual sea with a depth of 4000 m and a spatial resolution of 0.5° (~ 56 km) and $1/12^\circ$ (~ 9 km) for the lower and higher resolution simulations, respectively (Figure 1). The directional frequency wave spectrum is then converted to

the directional wave number spectrum $\psi(k, \theta)$ using the linear dispersion relation, $\psi(k, \theta) = (f/2k) \cdot \psi(f, \theta)$ where k is the wave number.

Figure 1. A visual representation of the 18° by 30° numerical simulation space. The central south to north transect used for investigating steady uniform wind conditions is indicated by the dashed line.

The significant wave height (H_s) is one of the standard output data provided by Wave Watch III. It is proportional to the double integral of the directional wave number spectrum with respect to wave number k and direction θ .

We first calculated the significant wave height using equation 1. The numerical integration in k was made using the trapezoid rule with the first trapezoid being from 0 to the lowest resolved wave number k_l . We then ascertained that the results are identical to the direct output from Wave Watch III. This step was taken to test the accuracy of our numerical integration program.

The Stokes drift (U_s) due to a single sinusoidal traveling wave (equation 2) is given by equation 3. Here, d is the surface elevation (relative to the still water level), x is a horizontal coordinate, z is the depth ($z=0$ is at the

$$Sig.Wv.Ht = 4 \sqrt{\int_0^\infty \int_0^{2\pi} \psi(k, \theta) d\theta dk}$$

$$d(x, t) = a \sin(kx - \omega t)$$

$$\bar{U}_s \approx \omega k a^2 \exp(-2kz)$$

$$\bar{U}_s = \int_0^\infty \int_0^{2\pi} 2\psi(k, \theta) \omega k \exp(-2kz) d\theta dk$$

$$\theta = \arccos\left(\frac{\vec{a} \cdot \vec{b}}{|\vec{a}| |\vec{b}|}\right)$$

Equations 1 through 5.

still water level), t is time, a is the wave amplitude, and $\omega = 2\pi f$ is the angular frequency.

The Stokes drift (U_s) due to a spectrum of waves follows from the discrete integration in equation 4. This quantity was calculated at 10 cm increments in depth z from the surface down to 240 m.

In order to examine the Stokes drift due to dominant waves, we focused on the Stokes drift at a depth that is inverse of the peak wave number k_{peak} , $U_s(z = k_{peak}^{-1})$, where k_{peak} is calculated from the peak frequency f_{peak} (frequency corresponding to the frequency spectral peak) and the deep water dispersion relation, $k_{peak} = (2\pi f_{peak})^2 / g$. Here, the peak frequency f_{peak} is a standard output from the Wave Watch III simulation.

2.1. Steady Uniform Wind Conditions.

For the steady uniform wind simulations directional wave spectral data were generated at locations spaced 0.5° apart along the middle of the simulation space in the 0° to 30° direction (from south to north) aligned with wind blowing from south. Wind speeds from 10 ms⁻¹ to 50 ms⁻¹ at 10 ms⁻¹ increments were used.

In each case, the significant wave height and the Stokes drift profiles were calculated as a function of fetch at 0.5° intervals. Here, the fetch is distance over which waves have grown.

Figure 2. A vector plot of a TC wind field with a 0.5° spatial resolution.

2.2. TC (Hurricane) Conditions.

Next a series of simulations were performed under TC wind conditions (Figure 2). In all cases the radius of maximum wind was 70 km and the maximum wind speed was 45 ms⁻¹. The first case considered was a stationary

Figure 3. Significant wave height (m) as a function of fetch (with 0.5° increments) under steady uniform wind conditions. Note that significant wave height displays strong fetch dependence for lower fetch values. Such fetch dependence diminishes as waves become fully grown. Also note that wave height correlates with wind speed.

Figure 4. Stokes drift velocity as a function of depth and fetch under 50 ms^{-1} steady uniform wind conditions.

TC. The other two cases included translating TCs, at translation speeds of 5 ms^{-1} and 10 ms^{-1} .

The Stokes drift profiles were initially calculated at 0.5° intervals (in both south-north and west-east directions). Later, they were calculated with a higher resolution ($1/12^\circ$ intervals) inside the radius of maximum wind.

The angle differences $\Delta\theta$ between wind and the Stokes drift were calculated by using the well-known

method of solving for an angle θ between any two vectors **a** and **b** (equation 5).

3. Results and Discussion.

3.1. Steady Uniform Wind Conditions.

The significant wave height is plotted versus fetch for all uniform wind cases (Figure 3). Notice that for the lower wind speed cases, namely the 10 ms^{-1} and 20 ms^{-1} cases, a maximum significant wave height (a fully grown state) is reached within the computational domain, while in the high wind speed cases the final fully grown state is not reached. Furthermore, note that in all cases, significant wave height is strongly fetch dependent for lower fetch values. The magnitude of significant wave height is strongly correlated with increasing wind speeds.

Figure 4 displays U_s as a function of depth and fetch for the 50 ms^{-1} wind case. Notice how U_s increases its magnitude at greater depths with increasing fetch but appears to be relatively constant very near to the sea surface.

The U_s depth profiles at both a small fetch (0.5° , $\sim 56 \text{ km}$) and a large fetch (29.5° , $\sim 328 \text{ km}$) for all wind speeds were normalized in Figure 5. Here, the depth was scaled by the dominate wave number (k_{peak}) and the $U_s(z)$ was normalized by Stokes drift at a depth that is inverse of the dominate wave number, i.e., $U_s(k_{\text{peak}}^{-1})$. This scaling makes all the profiles pass a point where the normalized depth and the normalized Stokes drift are both 1. Notice that overall the depth profiles are well collapsed by this scaling.

3.2. TC Wind Conditions.

The significant wave height (m) for a 10 ms^{-1} translating TC (translating upward or northward) is displayed in Figure 6. Notice the high degree of asymmetry with higher waves concentrated to the right of the storm tract. Such wave height asymmetry is less pronounced in the 5 ms^{-1} translating TC simulation.

The direction of the Stokes drift at the surface is always close to the wind direction as expected. However, the direction of $U_s(k_{\text{peak}}^{-1})$, the Stokes drift at the depth that is inverse of the dominate wave number, can be significantly different from the wind direction and is relatively close to the direction of the dominant waves.

The angle difference ($\Delta\theta$) between the wind direction and the direction of $U_s(k_{\text{peak}}^{-1})$ is shown in Figure 7. The left panels show the large domain results with 0.5° spatial resolution, and the right panels show more detail with $1/12^\circ$ spatial resolution near the TC center.

For the stationary TC (a and d), such misalignment is minimal near the center and gradually increases with radius but does not exceed 90° . The misalignment in the 5 ms^{-1} translating TC simulation (b and e) has a small region exceeding 90° in magnitude which peaks to the lower left, very near to the TC center within the RMW. Such a feature is more pronounced in the 10 ms^{-1} translating TC simulation (c and f) with the misalignment approaching

Figure 5. Normalized stokes drift $[U_s/U_s(k_{\text{peak}}^{-1})]$ as a function of the normalized depth (product of depth and the peak wave number) at short and long fetches (0.5° , ~ 56 km and 29.5° , ~ 328 km) under steady uniform wind conditions.

Figure 6. Significant wave height (color bar in meters) for a hurricane moving at 10 ms^{-1} . The direction of storm translation is upward. The center of the storm is at the center of the figure.

180° . Misalignment outside the RMW tends to increase with radius from the TC center and is more pronounced to the left of the TC track. There are regions where misalignment magnitude exceeds 90° in the 5 ms^{-1} translating TC simulation. For the 10 ms^{-1} translating TC simulation, misalignment outside the RMW is much greater to the left where it approaches 180° .

Normalizations of the depth profile were performed for all TC simulations, at both 0.5° and $1/12^\circ$ resolutions up to ~ 500 km and ~ 160 km respectively by longitude and latitude from TC centers. As before, the depth was scaled by the peak wave number (k_{peak}) and the U_s was normalized by $U_s(k_{\text{peak}}^{-1})$.

Figure 8 displays such normalization for the 10 ms^{-1} translating TC simulation with the low resolution data (0.5°). Depth profile normalizations from steady uniform wind simulations contained in Figure 5 are superimposed upon those of TC for reference. Notice that the normalized profiles are fairly well collapsed, except for one outlier, but not to the same degree as the uniform wind simulations. Figure 9 displays the result with the high resolution inside the RMW. For depth profiles near the TC center, we find that the normalizations do not hold, suggesting that the Stokes drift profile is much more complex.

Next, angular misalignment between wind and U_s at different depths are normalized. The depth is scaled by the peak wave number k_{peak} as before, and the angle ($\Delta\theta$) between wind and $U_s(z)$ is normalized by the $\Delta\theta$ at $z=k_{\text{peak}}^{-1}$ (Figure 10). In all TC simulations (10 ms^{-1} displayed) the vertical variation of $\Delta\theta$ overall does not collapse well with this normalization. However, they show a systematic trend. As the angle difference at $z=k_{\text{peak}}^{-1}$ increases, the more uniform the $\Delta\theta$ profile becomes except near the surface.

Figure 7. Angle difference ($\Delta\theta$) between wind direction and the direction of $U_s (k_{\text{peak}}^{-1})$, for wave fields under stationary (a) as well as 5 ms^{-1} (b) and 10 ms^{-1} (c) translating TC wind conditions. Right panels show higher resolution regions sampled at $1/12^\circ$ intervals. The TC for the translating cases is moving upward.

Figure 8. Normalized stokes drift $[U_s/U_s(k_{\text{peak}}^{-1})]$ as a function of the normalized depth (zk_{peak}) for a wave field under 10 ms^{-1} translating TC wind conditions at a 0.5° resolution. Superimposed are the normalized stokes drift profiles for the uniform wind condition cases for both far and near fetch.

Figure 9. Normalized stokes drift $[U_s/U_s(k_{\text{peak}}^{-1})]$ as a function of the normalized depth (zk_{peak}) for a wave field under 10 ms^{-1} translating TC wind conditions at $1/12^\circ$ resolution inside the RMW. Superimposed are the normalized stokes drift profiles for the uniform wind condition cases for both far and near fetch.

4.1 Steady Uniform Wind Conditions.

The Stokes drift near the surface tends to respond quickly to wind as is visible in Figure 4, however it slowly penetrates deeper. The Stokes drift magnitude at depth tends to be fetch dependent initially and also dependent upon wind speed. The normalized U_s profiles shown in Figure 5 are well collapsed and demonstrate that the Stokes drift profile is well described by the proposed two parameters.

Figure 10. Normalized angle between wind and Stokes drift (angle difference normalized by its value at $z=k_{\text{peak}}^{-1}$) as a function of the normalized depth for a wave field under a 10 ms^{-1} translating TC wind condition at a 0.5° degree resolution. The results are separated by color depending on the magnitude of the angle difference at $z=k_{\text{peak}}^{-1}$.

4.2 TC (Hurricane) Wind Conditions.

According to Figure 7, there exists regions of significant angular misalignment (exceeding 90°) between wind direction and $U_s(z=k_{\text{peak}}^{-1})$ direction for both the 5 ms^{-1} and 10 ms^{-1} translating TC simulations. Such regions, in both simulations, occur to the left of the storm translation direction both within and outside of the RMW. Such a finding lends merits to further investigation of its effect on the Langmuir turbulence.

Depth profile normalizations, displayed in both Figures 8 and 9, for the hurricane simulations suggest that in general they can be well described by the same two parameters. However, when the translation speed of the TC becomes large, as is the case with the 10 ms^{-1}

4. Conclusions.

translating TC, such normalization no longer hold for depth profiles near the eye of the hurricane.

For the $\Delta\theta$ between wind and U_s , the proposed normalization does not work well. Still, there exists some systematic trend. As $\Delta\theta$ between $U_s(k_{peak}^{-1})$ and wind increases, $\Delta\theta$ profile becomes more uniform except near the surface.

Acknowledgements. I would like to thank Rob Pockalny and Kathleen Donohue for organizing and coordinating the 2011 SURFO program, Isaac Ginis for providing the computational resources, and everyone else involved with making this program what it is.

References.

- D'Asaro E. A., T. B. Sanford, P. P. Niiler, and E. J. Terrill. 2007. Cold Wake of Hurricane Frances. *Geophys. Res. Lett.*, 34.
- Emanuel, K., 2004. Tropical Cyclone Energetics and Structure. Atmospheric Turbulence and Mesoscale Meteorology, ed. E. Fedorovich, R. Rotunno, B. Stevens. Cambridge, UK: Cambridge Univ. Press. 165-191.
- Fan, Y., I. Ginis, and T. Hara. 2010. Momentum Flux Budget Across the Air-Sea Interface Under Uniform and Tropical Cyclone Winds. *J. Phys. Oceanogr.*, **40**, 2221-2242.
- Price, J. F., 1981. Upper Ocean Response to a Hurricane. *J. Phys. Oceanogr.*, **11**(2), 153-175.
-
- T. Hara, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (thara@uri.edu)
- J. Bruce, Manchester College, North Manchester, IN, 46962. (JSBruce@spartans.manchester.edu)
-

Metabolic Response of *Thalassiosira Oceanica* to Iron Limitation

Adam Darer, Dreux Chappell, Bethany Jenkins

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

Abstract. The iron-sulfur containing protein, ferredoxin, is vital to the photosynthetic processes of many marine phytoplankton. Several species of diatoms are known to synthesize flavodoxin, a functionally similar iron-free protein, when stressed by low iron levels. From previous work in the Jenkins laboratory, it was shown that some flavodoxin copies are controlled by light and others iron. The diatom isolated in the open ocean, *Thalassiosira oceanica*, has three flavodoxin gene copies: two that show similarities to iron-responsive flavodoxins and one with similarity to the light-responsive flavodoxin. For the present study, the expressions of the three flavodoxin copies and ferredoxin were determined for *T. oceanica* using laboratory cultures grown in iron limiting conditions under continuous light and a 14:10 light: dark cycle. Quantitative polymerase chain reaction methods (qPCR) were employed to follow the amplification of cDNA and a set of standards allowed for the determination of the initial concentration of gene expression. We confirmed that one of the putative iron-responsive flavodoxins was sensitive to iron: this copy of flavodoxin was induced ca. 3000 fold in iron limited cells grown in a light: dark cycle and ca. 1000 fold in iron limited cells grown in continuous light. The expression of the one other flavodoxin copy and ferredoxin did not change significantly under the experimental conditions. We are using these methods to determine the expression of flavodoxin genes in field samples known to contain *T. oceanica*, collected from iron rich and iron limited areas within the Northeast Pacific aboard R/V Thomas G. Thompson in May 2007.

1. Introduction.

As abundant primary producers in marine ecosystems, diatoms play an integral role in the cycling of nitrate, phosphate, carbon and silica (Arrigo 2005). There are a number of regions in the oceans with high concentrations of macronutrients but little diatom growth often referred to as high nitrate, low chlorophyll (HNLC) areas. Low atmospheric iron inputs may be limiting diatom productivity in these HNLC regions (Moore et al. 2004). Iron (Fe) is a key element involved in many cellular processes including photosynthesis. In iron depleted oceanic waters, many phytoplankton replace the iron-sulfur protein ferredoxin, which is involved in the electron transport chain of photosynthesis, with the iron-free protein flavodoxin (Erdner et al. 1999). The extent of flavodoxin expressed may be a suitable detection of iron limitation in field samples of eukaryotic phytoplankton, without requiring the addition of nutrients to field samples, or further grow-out experiments (La Roche et al. 1995). Methods such as the western blot analysis (LaRoche et al. 1995) or high performance liquid chromatography (HPLC) (Doucette et al. 1996, Erdner et al. 1999) have been used to detect flavodoxin proteins in marine diatoms under laboratory-induced iron limitation and within field samples. Here we present an assay to determine flavodoxin gene expression in field samples of *T. oceanica* that avoids the species separation steps included in protein analysis methods.

Marine phytoplankton utilize ferredoxin as a redox element in cellular processes (Zurbiggen et al. 2008).

Ferredoxin proteins are found in photosystem I of photosynthesis as an electron transport carrier to reduce NADP^+ and H^+ to NADPH. Ferredoxin proteins also transfer electrons of photosynthetic origin to enzymes involved in other cellular functions such as the incorporation of nitrogen and sulfur into the cell and the metabolism of lipids and amino acids (Zurbiggen et al. 2008). Additionally, ferredoxin can combine with thioredoxin to generate FTR reductase that regulates cellular processes such as the Calvin cycle (Schumann and Buchanan 2008). Many open ocean marine phytoplankton substitute the functionally equivalent, iron-free protein flavodoxin, for ferredoxin under conditions of low iron levels (Erdner et al. 1999). Flavodoxin proteins are used in many of the same cellular capacities as flavodoxin proteins including the reduction of NADP^+ , the metabolism of amino acids, and Calvin cycle processes (Zurbiggen et al. 2008).

Previous studies within the Jenkins laboratory have suggested that the flavodoxin copy found in the coastal diatom *Thalassiosira pseudonana* may be affected by light instead of iron (Whitney et al. in Prep). These studies also indicate that at least one flavodoxin copy in an oceanic isolate of *Thalassiosira weissflogii* is affected by iron limitation. *Thalassiosira oceanica* has one flavodoxin protein (1) similar in sequence to the *T. pseudonana* light-responsive flavodoxin and two flavodoxin proteins (2a, 2b) that show similarities to the *T. weissflogii* iron-responsive flavodoxin (Chappell unpublished). The motivation behind the present study was to determine which if any of the flavodoxin gene copies of *T. oceanica* is responsive to iron limitation.

To answer this question, we cultured *T. oceanica* under low iron levels and determined the three flavodoxin gene copies and ferredoxin gene copy expressions using quantitative real-time polymerase chain reaction methods (qRT-PCR). With the results of the laboratory induced iron limitation experiments, we analyzed gene expression from several field sites in the Northeast Pacific Ocean known to contain *T. oceanica*.

2. Materials and Methods.

Culture Conditions and Sampling *Thalassiosira oceanica* (CCMP 1005) isolates were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (West Boothbay Harbor, ME, USA). Trace metal limitation experiments were performed using a modified version of f/2 made in 0.2 μm filtered and microwave-sterilized Sargasso seawater (Guillard and Hargraves, 1993). The only deviation from the f/2 media recipe was in the amount of Fe added to the media. Replete cultures received 400 nM Fe and Fe limited cultures were grown from two successive 1:10 dilutions of 400 nM Fe f/2 media with f/2 media that had no added Fe, thus resulting in f/2 with at most 4 nM Fe. All macronutrient stocks were processed through an ion-exchange column (Chelex 100, Biorad) containing resin prepared according to Price et al. (1989) and filter-sterilized (0.2 μm Acrodisc). All media preparation and culture growth was done in acid-cleaned and microwave-sterilized polycarbonate bottles and flasks. All media preparation and culture transferring was performed in a

Class-100 HEPA filtered hood. *T. oceanica* cultures were grown at a light level of 140 $\mu\text{E}/\text{m}^2/\text{s}$ under a 24 hour continuous light as well as 14:10 light: dark cycling and incubated at 25 °C (Percival incubator). The iron limited 14:10 light: dark cycle was grown in quadruplet, all other setups were grown in triplicate.

Growth rates were monitored over a study period of four days to ensure mRNA was extracted during exponential growth phase. Representative samples (~1.5 mL) of both iron limited and replete cultures were obtained daily, in triplicate, and chlorophyll a fluorescence (Turner fluorometer) was measured. After four days of iron limitation, cellular biomass was extracted onto 25 mm filters with a pore size of 3 μm . The filters were placed in vials with RLT buffer, flash frozen in liquid nitrogen, and stored at -80°C prior to RNA extraction.

Field Sample Preparation Surface water samples were obtained along an iron gradient within the Northeast Pacific Ocean (R/V T. G. Thompson Cruise, May 12-June 8, 2007 Figure 1, Table 1). These samples were examined for the genetic expression of three flavodoxin copies and one ferredoxin copy of *T. oceanica*. One liter of surface water was collected from the CTD rosette and filtered onto 25mm filters with a pore size of 0.2 μm held in swinex filter holders using a masterflex pump system. Filters were removed from the holders, added to 2ml tubes pre-loaded with 0.1 and 0.5 μm zirconia beads and 500ml RLT buffer. The samples were then flash frozen in liquid nitrogen and stored at -80°C prior to RNA extraction.

Figure 1. Field sample sites of surface water examined for expression of flavodoxin 2b in *T. oceanica*.

Sample Site	Latitude (°N)	Longitude (°W)	Fe Concentration (nM) ^a
1	51.998	135.002	0.12
2	53.757	133.328	
3	53.167	132.786	
4	51.749	131.001	1.45

Table 1. Geographic coordinates and iron concentration of field sites analyzed. ^aData from Xiu et al. 2011

Laboratory-Induced Iron Limitation RNA Extraction and cDNA Synthesis

RNA was isolated using RNeasy Midi Kit (Qiagen) kit according to the instructions provided. The concentration of RNA in the samples was determined using Qubit (Quant-iT dsDNA assay) was diluted to achieve a final concentration of 6 ng/ul in a volume of 10 ul. Extracted RNA was converted to cDNA using (Invitrogen High Capacity cDNA) with a master mix of 10 ul added directly to the 10 ul of sample from the RNA extraction. Reactions were performed in an Eppendorf Mastercycler ep thermocycler (Eppendorf North America) using the following parameters: 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 minute. As a negative control for qRT-PCR, an additional set of samples were made using all of the reagents from cDNA synthesis except reverse transcriptase. All the samples were diluted 1:3 (20 uL sample to 40 ul nuclease free water) prior to qPCR analysis.

Field Sample RNA Extraction and cDNA Synthesis

The RNA extraction and cDNA synthesis from field samples were performed identically to the laboratory grown samples (including the no reverse transcriptase control). The only difference was an RNA concentration of 22 ng/ul was used in the first step of cDNA synthesis.

Synthesis of Standards

Cultures of *T. oceanica* were grown in iron replete and deplete f/2 media. Almost full-length copies of the genes of flavodoxin 1, flavodoxin 2a, flavodoxin 2b, ferredoxin, and actin were isolated from cDNA samples using PCR. PCR products were cleaned up using the QIAquick PCR purification kit (QIAGEN®, Germany). Purified amplicons (3 µL) were ligated into the pGEM®-T vector (Promega Corporation, Madison, WI) using an overnight ligation at 4°C. Ligations were used to transform Z-Competent™ *E.coli* (Zymo Research Corporation, Irvine, CA) and plated onto LB agar plates with 100µg/ml carbenicillin, X-gal, and IPTG and incubated overnight at

37°C. White colonies were picked onto another LB agar/cabencillin/X-gal/IPTG plate and incubated overnight again at 37°C to confirm blue-white screening. The plasmids were purified using the QIAprep Spin Miniprep kit (Qiagen) and subsequently sequenced on the Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Purified plasmids were linearized using the restriction endonuclease, SpeI (New England Biolabs). The concentration of plasmid DNA was determined using Qubit (Quant-iT dsDNA assay) and enough linearized plasmid was added to Tris-EDTA to generate a concentration of 5 ng/ul in 200 ul. The stock solutions were serially diluted in ten-fold increments (from 5×10^{-2} to 5×10^{-9} ng/ul) with nuclease-free water (Light Cycler 480 Sybr I Green Master mix version 11.0) to generate the set of eight standards used in qRT-PCR analysis.

Quantitative real-time PCR The qRT-PCR method was used to determine the quantity of the target gene expressed (LaRoche 480, Light Cycler 480 version SW 1.5, Light Cycler 480 Sybr I Green Master mix version 11.0). 2 ul of standard or sample was added to 18 ul of master mix comprised of specific primers (Table 2), Sybr I Green probe and nuclease-free water. Primers were diluted from a stock concentration of 5 uM to the final concentration indicated in Table 3. These values were obtained from a primer dilution optimization matrix and qRT-PCR analysis to uniquely determine the optimal primer concentrations for each target gene. 10 ul of Sybr I Green dye was added per reaction. The volume of water added was calculated as 20 ul (total reaction volume) – volume of primers (forward and reverse) added.

To ensure that no target gene was amplified due to a primer region sequence similar to another gene we performed a qRT-PCR run using flavodoxin 2a primers on flavodoxin 2b standards, and flavodoxin 2b primers on flavodoxin 2a standards.

The qRT-PCR conditions were 1 cycle at 95°C for 5

Target Gene	Forward Primer	Reverse Primer
Flavodoxin 1	5'-CGGCCTGGGCGACTCGGTAT	5'-CGCGGATGGCCTTGGACGAG
Flavodoxin 2a	5'-CGCTGATGCAGTTGGCGAGC	5'-CTTCGTCGCACAGCAGCCCA
Flavodoxin 2b	5'-CGACCTTTTCGAAGCAGCCGGT	5'-GCGCTCGGCCTTTGAACCCT
Ferredoxin	5'-CCGCCGAGGAGGAGGGAGTT	5'-TCTCGCAGTCCGACTTGGGGT
Actin	5'-GTGACGAGGCCAGGCGAAG	5'-CCTCGGTGAGGAGGACCGGG

Table 2. qRT-PCR Primer sequences for specific gene expressions in *Thalassiosira. oceanica*

minutes, followed by 40 cycles at 95°C for 10 seconds and 60°C for 30 seconds. The amplification efficiency of the standards was calculated by plotting the log of the standard concentration against the cycle threshold value (Ct) and using the formula: $E = 10^{(-1/\text{slope})}$. Once the efficiency of the standards was considered to be optimal (over 90%), samples were concurrently run with the standards. The standards and samples were examined in triplicate. All of the qRT-PCR runs contained a no cDNA template control with nuclease-free water replacing cDNA. The sample runs additionally contained a no reverse transcriptase control.

Target Gene	F Primer (nM)	R Primer (nM)
Flavodoxin 1	400	300
Flavodoxin 2a	400	200
Flavodoxin 2b	300	400
Ferredoxin	400	400
Actin	400	400

Table 3. Gene specific primer concentrations used in qRT-PCR experiments. F stands for forward primer, R for reverse.

Analysis of qRT-PCR Abs Quant/fit Points for All Samples analysis was used to determine the Ct cycle of each sample. The number of molecules of each standard was calculated using the size of the both the vector plasmid and the insert along with the molar mass. A calibration curve was generated by plotting the Ct values of the standards versus the log of the initial quantity of molecules. The initial number of molecules of sample was back-calculated using the calibration curve of the standards and the averaged Ct of the triplicate samples. Standard deviation of the laboratory-sample gene expression was determined from the average of the target gene expression qRT-PCR triplicate runs and the standard deviation among the cultures grown under the same experimental conditions. The field sample standard deviations were calculated as the standard deviation among the target gene expression from triplicate samples of the same field site.

The gene expression of each of the four target genes was normalized to the expression of actin. Actin is an important component of the cytoskeleton and was determined not to vary significantly under the experimental conditions. Normalizing the target gene expression to this reference gene removed variability in the expression analysis due to experimental procedures.

3. Results.

Growth Curve from Iron Limitation in *T. oceanica*.

Exponential growth was observed in cultured *T. oceanica* throughout the study period of four days for both the replete and iron limited samples under 14:10 light: dark cycling and continuous light incubation. The growth rate of the iron-limited samples was significantly less than the replete (Figure 2).

Target Gene Expression in Iron Limited *T. oceanica*.

The output of the qRT-PCR runs was analyzed as detailed in the materials and methods section. We present the gene expression data in Table 4. Flavodoxin 2b was significantly up-regulated under iron limitation, with an induction fold of 2941 ± 17 for the light: dark cycling and 970.8 ± 7.3 under continuous light. To more easily visualize this up-regulation, we show data presented in Table 4 on a logarithmic scale in Figure 3. We did not observe a significant change in flavodoxin 2a and ferredoxin gene expression under iron limitation. At the present time the efficiency of our flavodoxin 1 standards is below 90%, and so we have decided to exclude flavodoxin 1 data until we can more accurately report our results.

Figure 2. Growth curves of *T. oceanica* under A. Light Dark cycling B. 24 hour continuous light. The red boxes, RE, are the replete samples (400nM Fe). The black boxes, -Fe, are the iron limited samples (<4 nM Fe).

Target Gene	Replete LD	Replete 24 hr	Iron limited LD	Iron limited 24 hr
Flavodoxin 2a	0.68 +/- 0.16	0.81 +/- 0.29	0.87 +/- 0.45	0.75 +/- 0.17
Flavodoxin 2b	0.063 +/- 0.020	0.052 +/- 0.012	186 +/- 60	50 +/- 11
Ferredoxin	26.7 +/- 5.7	13.1 +/- 3.0	15.6 +/- 5.1	10.3 +/- 3.1

Table 4. Normalized gene expression for Flavodoxin 2a, Flavodoxin 2b and Ferredoxin genes of *T. oceanica* under laboratory induced iron limitation. LD is the light: dark cycling experiments, 24 hr is the continuous light experiments.

Figure 3. Normalized gene expression data from laboratory iron limited experiments plotted on a logarithmic scale. (Same data as Table 4).

Figure 4. Normalized flavodoxin 2b gene expression data from field sample sites plotted on a logarithmic scale. For reference, the normalized flavodoxin 2b

1	5.8 +/- 0.61
2	5.8 +/- 0.96
3	1.8 +/- 0.38
4	0.092 +/- 0.040

Table 5. Normalized gene expression of *T. oceanica* Flavodoxin 2b from field samples sites.

Specificity of Flavodoxin 2 primers We tested flavodoxin 2a qRT-PCR primers on flavodoxin 2b standards as a check that our primers were gene specific and were not amplifying another flavodoxin gene copy.

Flavodoxin 2b primers were also tested on flavodoxin 2a standards. No amplification of DNA was observed under these mismatched conditions, confirming that our flavodoxin primers were gene specific (data not shown).

Field Sample Gene Expression One goal of this project was to develop a reliable assay to examine *T. oceanica* gene expression in field samples containing the RNA of several diatom species. We applied the same methods and analysis towards 16 samples collected from a region in the Northeast Pacific Ocean with a known dissolved iron concentration gradient. However, due to trouble amplifying the cDNA in qRT-PCR we only present the results of flavodoxin 2b expression from 4 field sites (Table 5, Figure 4).

4. Discussion.

The purpose of the present work was to identify flavodoxin copies of *T. oceanica* that were responsive to iron limitation. Based on a phylogenetic tree analysis of flavodoxin proteins of different diatom species, we hypothesized that two *T. oceanica* flavodoxin copies (2a, 2b) would respond to iron limitation in the same manner as the closely related *T. weissflogii* flavodoxin protein does. We also expected that flavodoxin 1 would not be affected by iron limitation, due to its similarity in sequencing to the light-responsive flavodoxin copy of *T. pseudonana*. Here, we show that flavodoxin 2b gene expression was significantly up-regulated under iron limitation in laboratory studies. However, we did not observe a significant increase in flavodoxin 2a gene expression under iron limitation (1.28 ± 0.73 fold and 0.92 ± 0.39 fold in light-dark cycling and 24 hour continuous light respectively). While flavodoxin 2a and flavodoxin 2b are quite similar in sequencing, it may well be possible that these proteins serve different physiological tasks to *T. oceanica*. The open ocean isolate of *T. weissflogii* (CCMP 1010) has only one flavodoxin copy (Whitney et. al, in prep). Perhaps *T. oceanica* originally had one flavodoxin copy that evolved into two with separate functions. Additional studies are needed to determine the factors that up-regulate *T. oceanica* flavodoxin 2a gene expression. The gene expression level of ferredoxin did not change significantly under the experimental conditions. One may

expect a down-regulation of ferredoxin expression under iron limitation due to less dissolved iron available. Here, we observed a decrease of 1.52 ± 0.66 fold and 1.27 ± 0.79 fold for the iron limited light-dark cycling and iron limited 24-hour continuous light experiments respectively. We infer that the ferredoxin gene may be constitutively expressed in *T. oceanica*. Alternatively, *T. oceanica* may have a substantially low iron requirement. To test these hypotheses, it would be necessary to enhance the iron limitation in *T. oceanica*, however severe iron limitation greatly hinders exponential growth required during the culturing phase making such an experiment difficult to carry out.

We also tested field samples known to contain *T. oceanica* for flavodoxin 2b gene expression from a region with a 10 fold iron concentration gradient (Table 1). Sites 1 is considered to be iron limited with a concentration of 0.12 nM Fe, while site 4 is closer to the coast and has a higher iron concentration of 1.45 nM Fe (Xiu et. al 2011). We found the same general trend observed in the laboratory experiments in our analysis of the field samples; flavodoxin 2b expression was inversely related to iron concentration. The normalized flavodoxin gene expressions were 5.79 ± 0.61 , and 0.092 ± 0.040 for sites 1 and 4 respectively. There is not yet published data for the dissolved iron concentration of the remaining field sites. In addition to obtaining iron concentration values for all of the sampling sites, more work is needed to analyze the other field sites for flavodoxin 2b gene expression to be able to correlate gene expression with iron levels.

5. Conclusion.

Here we demonstrate a significant up-regulation of flavodoxin 2b gene expression in *T. oceanica* under iron limitation, and hence provide a viable genetic marker to determine iron limitation in field samples. It is of significant importance that our qRT-PCR assay was successful in uniquely amplifying *T. oceanica* genes in the presence of the complicated matrix of several diatom species from field sample sites. Further work is necessary to determine the response of flavodoxin 1, flavodoxin 2a, and ferredoxin gene expression from these field sample sites, although based on the laboratory results, we do not expect a significant change due to iron concentration. Additionally we have shown that ferredoxin may be

constitutively expressed in *T. oceanica*. Again further iron limitation experiments are necessary.

Acknowledgments: I would like to thank Dreux Chappell for mentorship and assistance in the laboratory and Bethany Jenkins for useful conversations. I would also like to acknowledge the SURFO program, Kathleen Donohue, Rob Pockalny and Kim Carey.

References.

- Arrigo (2005) "Marine microorganisms and global nutrient cycles." *Nature* **437**: 349-355.
- Doucette, G.J., D.L. Erdner, et al. (1996) "Quantitative analysis of iron-stress related proteins in *Thalassiosira weissflogii*: measurement of flavodoxin and ferredoxin using HPLC." *Marine Ecology Progress Series* **130**: 269-276.
- Erdner D.L., N. M. Price et al. (1999) "Characterization of ferredoxin and flavodoxin as markers of iron limitation in marine phytoplankton." *Marine Ecology Progress Series* **184**: 43-53.
- Guillard R. R. L., and P. E. Hargraves. (1993). "Stichochrysis immobilis is a diatom, not a chrysophyte." *Phycologia* **32**: 234-236.
- La Roche, J., H. Murray et al. (1995) "Flavodoxin expression as an indicator of Iron limitation in Marine Diatoms." *Journal of Phycology* **31**: 520-530.
- More, J.K., S. C. Doney, K. Lindsay. (2004) "Upper ocean ecosystem dynamics and iron cycling in a global three dimensional model." *Global Biogeochemical Cycles* **18**: 21.
- Price, N. M., G. I. Harrison et al. (1989) "Preparation and Chemistry of the Artificial Algal culture medium Aquil." *Biological Oceanography* **6**: 443-461.
- Schumann, P., B.B. Buchanan (2008) "The ferredoxin/thioredoxin system of oxygenic photosynthesis." *Antioxidants and Redoxin Signaling* **10**: 1235-1274.
- Whiteney et al in prep
- Xiu, P., A. P. Palacz et al. (2011) "Iron flux induced by Haida eddies in the Gulf of Alaska." *Geophysical Research Letter* **38**: 5.
- Zurbiggen, D. M., V. B. Tognetti et al. (2008) "Combating stress with flavodoxin: a promising route for crop improvement." *Trends in Biotechnology* **26** (10) 531-537.
- A. I. Darer, Oberlin College, Oberlin OH, 44074, (adarer@oberlin.edu).
- B. Jenkins and P.D. Chappell, Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI, 02881, (bjenkins@uri.edu, dreux@mail.uri.edu).

Identification of the Two *Acartia Tonsa* Populations Located in Narragansett Bay and Analysis of the Biological Differences Present Between Them

Erika Gibson and Edward G. Durbin

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

Abstract. Calanoid copepods are important secondary producers in the marine environment because they take in energy from phytoplankton and provide energy for higher level predators such as larger zooplankton and fish. Copepods have developed adaptations for living in harsh environments as a result of their movements being controlled by currents. One of these factors is the production of resting eggs that settle into sediment layers. The estuarine copepod, *Acartia tonsa*, lays resting eggs in the fall when the temperature drops below 15°C and these eggs remain in the sediment until the following summer when they hatch out. Genetically distinct clades have been reported within this species and these clades may have specific habitat or culturing requirements. The purpose of this study was to identify the two *A. tonsa* populations present in Narragansett Bay and determine if there are biological differences between these two populations. We hypothesized that the adults of the two populations would not be able to interbreed and that resting eggs from the two populations of *A. tonsa* would have different hatching temperatures. *A. tonsa* resting eggs were collected from Narragansett Bay sediment in April and hatched out at a series of increasing temperatures. These larvae were raised in the laboratory and then fixed for DNA extraction and sequencing using the mitochondrial cytochrome c oxidase I gene (mtCOI). From this sequence information, we determined to which population the larvae belong. Cultures of each clade were established. Juvenile stages were isolated and reared to adult. Mating experiments were set up where adults of the same clade and opposite clades were placed together and viability of eggs produced determined. This study showed that the two populations of *A. tonsa* had very similar hatching temperatures; therefore, we were able to reject our hypothesis. The findings from the mating experiment support our hypothesis that adults from different clades cannot interbreed. These findings support the suggestion that the two populations of *A. tonsa* present in Narragansett Bay represent reproductively isolated cryptic species. This information proves useful in understanding the geographic origin and genetic lineage of *A. tonsa* strains and aiding in aquaculture applications and ecosystem modeling.

1. Introduction.

Calanoid copepods are marine holoplankton, which means they drift freely through the water column throughout their entire life cycles. Different species of copepods are distinguished from one another through differences within the shape, size, and orientation of their appendages. Calanoid copepods are extremely important in the marine estuary food web because they are important secondary producers (Durbin & Durbin 1981). They repackage energy produced by marine phytoplankton and make it available for higher trophic level organisms. Since copepods are unable to move freely into more favorable conditions, they have adapted survival strategies to cope with harsh environments created by factors such as cooler temperatures, increased predation, and decreasing food availability. One of these survival strategies is the production of resting eggs. Resting eggs enter developmental dormancy and settle into sediment layers until favorable environmental conditions are reached (Marcus 1996). *Acartia tonsa* are estuarine calanoid copepods that are widely distributed in the coastal waters of the world's oceans (Chen & Hare 2008). *A. tonsa* have a generation time of 7-25 days and an average adult

longevity of 26 days (Chen & Hare 2011). *A. tonsa* is often a seasonally dominant species that lay resting eggs in the fall when the temperature drops below 15°C and these eggs remain in the sediment until the following summer when they hatch out (Sullivan et al. 2007). The taxonomy of this species is known to be incomplete and genetically distinct clades that may have specific habitat or culturing requirements have been reported within this species (Drillet et al. 2008). Previous studies suggest that *A. tonsa* may represent a cryptic species complex with multiple lineages co-occurring within estuaries (Chen & Hare 2008). The purpose of this study was to identify the two *A. tonsa* populations present in Narragansett Bay and determine if there are biological differences between these two populations. We hypothesized that the adults of the two populations would not be able to interbreed and that resting eggs from the two populations of *A. tonsa* would have different hatching temperatures.

2. Methods.

2.1. Copepod Sampling

Sediment was collected with a small Ponar grab at Conimicut Light (Providence River) and off of Davisville

on April 23, 2011. The surface 1 cm of the sediment was retained. Samples were placed in 8 oz plastic jars, filtered seawater (FSW) added, and kept on ice during the return to the lab. In the lab, the samples were rinsed with FSW into 20 L polycarbonate containers and 4 L FSW was added. These containers were placed in a walk-in incubator at 10°C under low light and left so that any immediately hatching *Acartia hudsonica* eggs could hatch. On April 25, 2011, no nauplii were present in the overlying water (i.e. no more immediately hatching eggs). The incubator temperature was then changed to 15°C. On April 27, 2011, half of the water was siphoned off, FSW was added, and the containers mixed. The water siphoned from the container was used to start copepod cultures Davisville 1 and Providence River 1. On May 2, 2011, the

temperature of the incubator was increased to 20°C to see if a different group of copepods would hatch. On May 5, 2011, all of the water was siphoned off of the sediments and FSW was added. The water siphoned from this container was used to start the copepod culture Davisville 3. *A. tonsa* was also collected from the dock (41.49°N, 71.42°W) at the Graduate School of Oceanography (GSO), Rhode Island, USA, in lower Narragansett Bay on June 21, 2011 and June 29, 2011.

2.2. *Acartia tonsa* Cultures

Beakers with 400 mL of seawater were used to start the Davisville 1 culture, giving a total of ~400 nauplii. Beakers with 3 L of seawater were used to start the

Figure 1. A maximum likelihood phylogenetic tree that shows the two populations of *Acartia tonsa* in comparison to *Acartia hudsonica*. On the bottom is a scale of 0.02 or 2% which shows population divergence.

Providence River culture, giving a total of ~450 nauplii. Phytoplankton (*Heterocapsa triquetra* and *Isochrysis galbana*) was added to each copepod culture as food. These were reared at 18°C through 20°C. On May 9, 2011, copepods in both cultures had reached the adult stage. In the Davisville 1 culture, about half were *A. hudsonica* and half *A. tonsa*. In the Providence River cultures, nearly all were *A. tonsa*. *A. tonsa* adult females were harvested from both cultures and placed in 95% alcohol. On May 9, 2011, ~400 nauplii were collected from the Davisville 3 culture and phytoplankton was added. These were reared to maturity and harvested on May 18, 2011. Very few nauplii hatched from the Providence River sediments on May 9, 2011 and no further rearing was done with these.

2.3. DNA Extraction, amplification, and sequencing

Single individuals were isolated and put into microcentrifuge tubes. Genomic DNA was purified using the DNeasy® tissue kit (Qiagen) following the manufacturer's protocol. The final product was eluted from the DNeasy® columns with two 100-µl rinses of buffer AE. An ~650-bp segment of the mtCOI gene was amplified from the genomic DNA with the universal primers L1384-COI and HCO-2198. PCR amplifications were performed in 30 µl volumes containing 15 µl Taq PCR Master Mix (Qiagen), 0.6 µl of each primer, and 4 µl of template. The thermal regime was; 1 cycle of 94°C for 60 s; 40 cycles of 94°C for 30 s, 49°C for 60 s, and 72°C

for 60 s followed by 1 cycle of 72°C for 5 min before holding at 4°C. A gel electrophoresis was run with the PCR products in order to test for the presence of the ~650-bp mtCOI gene. Next, the samples were cleaned through ethanol precipitation and DNA in the samples was quantified using a spectrophotometer (NanoDrop ND-1000). If the appropriate concentration of DNA was present, the samples were prepared for sequencing using the amplified mtCOI gene.

2.4. Data Analysis

A BLAST search was performed in order to compare the obtained sequences to the sequences on the GenBank database. Then, sequences were uploaded to Mega, aligned according to the Clustal W method, and cropped down to 524-bp segments. A maximum likelihood phylogenetic tree was created using Mega. Two samples of *A. hudsonica* were included in the data analysis as a means of comparison between species. A network analysis was performed using the program Network 4.600 and each sample was identified according to the population it belonged to.

The JMP 9 program was used to perform a one-way analysis of variance (ANOVA) test in order to compare the mean hatching temperatures of each *A. tonsa* population.

Figure 2. Network analysis showing inter-species divergence between the two *Acartia tonsa* populations.

2.5. Mating Experiment

On June 29, 2011, *A. tonsa* was collected from GSO. There were very few *A. tonsa* present. On July 5, 2011, adult females were placed in individual petri dishes with 10 ml FSW and phytoplankton. On July 6, 2011, the adult females were removed, photographed for size, and fixed for DNA (mtCOI) analysis. On July 7, 2011, all the eggs had hatched in all but one of the petri dishes. The nauplii were separated into individual dishes and reared until they reached an appropriate age of reproductive maturity. Next, 10 individuals were placed in separate dishes and each was randomly combined with one individual from the opposite population. 7 individuals were placed in separate dishes and each was randomly combined with one

individual from the same population. These dishes were analyzed for 4 days for the production of viable offspring.

3. Results.

After the *A. tonsa* samples were sequenced, a maximum likelihood phylogenetic tree was created (Figure 1) using the program MEGA v5 and two distinct populations of *A. tonsa* were identified and labeled. Analysis of the phylogenetic tree showed a 28% difference between *A. hudsonica* and population 1 of *A. tonsa*. In order to determine the exact number of base pair differences, the total number of base pairs in the sequences (524 base pairs) was multiplied by the scale difference (0.28 or 28%). Therefore, there were 147 base

Figure 3. ANOVA test performed in order to compare the mean hatching temperatures of each population of *Acartia tonsa*.

pair changes between *A. hudsonica* and population 1 of *A. tonsa*. There was a 26% difference or 137 base pair changes between *A. hudsonica* and population 2 of *A. tonsa*. There was a 14% difference or 74 base pair changes between population 1 and population 2 of *A. tonsa*. The divergence between the two *A. tonsa* populations located in Narragansett Bay was also shown through a network analysis (Figure 2).

After determining to which population each individual belonged, the individual hatching temperatures were analyzed using an ANOVA test (Figure 3). The null hypothesis for this ANOVA test stated that the mean hatching temperatures for populations 1 and 2 are equal ($\mu_1 = \mu_2$). After calculating a P-value of 0.9099, the null hypothesis could not be rejected because the P-value was greater than the significance level of 0.05. Therefore, the ANOVA test allowed us to reject our hypothesis that the resting eggs from the two populations of *A. tonsa* would have different hatching temperatures.

During the mating experiment, eggs were produced in almost all of the petri dishes. These eggs were analyzed for the production of viable offspring over the course of 4 days. Eggs from dishes that contained parents from the same population produced viable offspring if both parents were still living when they were removed from the dishes. It was assumed that the eggs from dishes that contained a dead male parent were not fertilized. None of the eggs from dishes that contained parents from opposite populations produced viable offspring.

4. Discussion.

The two distinct populations of *A. tonsa* located in Narragansett Bay appear to be the same morphologically, but they differ biologically. Even though these populations have the same hatching temperatures, they appear to be reproductively isolated as shown through the mating experiment. These findings strongly support the idea that these two populations are reproductively isolated cryptic species. This strengthens and expands the evidence for cryptic species diversity of *A. tonsa* observed in Hill (2004), Caudill & Bucklin (2004), and Chen & Hare (2011).

Implications for future research include analyzing sequences using both the mitochondrial cytochrome c oxidase I gene (mtCOI) and the nuclear ribosomal internal transcribed spacer (nITS), running mating experiments on a larger scale, and expanding studies to areas outside of Narragansett Bay. The ITS region is a neutrally evolving region that is not subject to selective pressures. It is commonly used in phylogenetic analysis because of its ability to capture finer scale divergence. The mtCOI gene is maternally inherited and is subject to selective pressures. When both of these regions are analyzed, there agreement can help confirm reproductive isolation and dramatic divergence (Chen & Hare 2008). Running the mating experiments on a larger scale would present more significant results in order to support the presence of

reproductively isolated cryptic species within *A. tonsa*. Expanding this study to areas outside of Narragansett Bay would help identify greater divergence between *A. tonsa* populations and help us gain a better understanding of *A. tonsa* taxonomy.

This research is significant because it helps us understand the geographic origin and genetic lineage of *A. tonsa* strains, aids aquaculture applications, and improves ecosystem modeling. A better understanding of *A. tonsa* taxonomy can help us understand how *A. tonsa* originated, what location the species originated from, and how the species evolved and adapted to different environments. Copepods are a very important live food source for fish because they have a high nutritional value. They have been shown to be of greater value than traditional live feeds. The use of copepods in aquaculture can be increased if we better understand the culturing requirements and egg storage methods necessary for copepod survival (Drillet et al. 2008). Lastly, understanding *A. tonsa* taxonomy can help with ecosystem modeling because we will be better able to identify the exact population of *A. tonsa* present in a particular environment and the effects it has on its surrounding environment.

Acknowledgements. I would like to thank Kathy Donohue, Rob Pockalny, and Kim Carey for organizing and coordinating the 2011 SURFO program and I would like to thank Ted Durbin for allowing me to work with him over the summer. Also, I would like to thank Jennifer Bailey, Brandon Reichl, NSF, and the entire SURFO program.

References.

- Caudill CC, Bucklin A. 2004. Molecular phylogeography and evolutionary history of the estuarine copepod, *Acartia tonsa*, on the Northwest Atlantic coast. *Hydrobiologia* **511**: 91-102.
- Chen G, Hare MP. 2008. Cryptic ecological diversification of a planktonic estuarine copepod, *Acartia tonsa*. *Molecular Ecology* **17**: 1451-1468.
- Chen G, Hare MP. 2011. Cryptic diversity and comparative phylogeography of the estuarine copepod *Acartia tonsa* on the US Atlantic coast. *Molecular Ecology* **20**: 2425-2441.
- Drillet, G., Goetz, E., Jepsen, P.M., Højgaard, J.K., and Hansen, B.W. 2008. Strain-specific vital rates in four *Acartia tonsa* cultures, I: Strain origin, genetic differentiation and egg survivorship. *Aquaculture* **280**: 109-116.
- Durbin, A.G., and Durbin, E.G. 1981. Standing stock and estimated production rates of phytoplankton and zooplankton in Narragansett Bay, R.I. *Estuaries* **4**: 24-41.
- Hill RS. 2004. Genetic diversity and structure of calanoid copepods: molecular evolutionary patterns in coastal estuaries (*Acartia tonsa*) and the open ocean (*Calanus* spp.). PhD thesis, University of New Hampshire.
- Marcus, N.H. 1996. Ecological and evolutionary significance of resting eggs in marine copepods: past, present, and future studies. *Hydrobiologia* **320**: 141-152.
- Sullivan, B.K., Costello, J.H., and Van Keuren, D. 2007. Seasonality of the copepods *Acartia hudsonica* and *Acartia tonsa* in Narragansett Bay, RI, USA during a period of

climate change. *Estuarine, Coastal and Shelf Science* **73**:
259-267.

E. Gibson, University of Miami, Coral Gables, FL 33124
(e.gibson2@umiami.edu).

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

Eddy Formation and Possible Sinking in the Northwest Corner

Ben Heath, Dave Ullman, Christian Buckingham

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

Abstract. Eddy formation and subsequent sinking in the Northwest Corner, the area in which the North Atlantic Current (NAC) turns eastward, is investigated using satellite and shipboard observations. It has been hypothesized (Prater and Rossby, 1999) that an eddy formed in this region could be the source of a subsurface lens with anomalous temperature and salinity properties observed near the Bahamas in 1976 (McDowell and Rossby, 1978). Originally believed to be a Mediterranean eddy, or meddy, because of the similarity of the temperature and salinity to the Mediterranean, the eddy is now believed to originate in the Northwest Atlantic, avoiding the difficulty of crossing the Mid-Atlantic ridge. Satellite altimetry data, in conjunction with an eddy tracking database (Chelton et al., 2011), were used to search for evidence of eddy formation/dissipation. A statistical study of eddies that originated in the Northwest Corner was conducted. Analysis of the data supported previous results showing a seasonal cycle in eddy sinking. These data lend support to the hypothesis that the Northwest Corner could be the source of lenses of high temperature and salinity water which subsequently drift equatorward.

1. Introduction

A large eddy was discovered off the coast of the Bahamas in 1976 at a depth of 1000 meters. On the order of hundreds of meters thick and tens of kilometers wide, the origin of the eddy was a mystery. McDowell and Rossby (1978) suggested that the origin of the eddy could be the Mediterranean, one of the reasons being that the eddy had similar temperature and salinity characteristics to the Mediterranean water. Other studies have brought to light the difficulty of an eddy crossing the Mid-Atlantic Ridge. Prater and Rossby (1999) have suggested an alternate hypothesis for the source of the McDowell/Rossby eddy. Lack of observations of eddies traveling from the Mediterranean to the Bahamas and recent observations of eddies in the Northwest Corner (NWC) of the North Atlantic Current suggested that the source of the observed Bahamas eddy could be the NWC, near Flemish Cap (Prater and Rossby, 1999). Eddies formed by the turning of the North Atlantic Current (NAC) in the NWC may sink and then be transported southward. Evidence of eddy sinking in the NAC could be a mechanism for the transport of kinetic energy and warm water southward towards the equator. Woityra (2005) found evidence of eddy sinking and also detected seasonality of eddy sinking, with more eddy sinkings observed in winter/early spring.

2. Data

Sea level anomaly (SLA) data were obtained from AVISO. This satellite altimetry data is measured with respect to a geoid model with a time-averaged mean (over the period 1993-1999) removed (Fig. 1). The data were available in 7-day increments from late 1992 to 2010. The

satellite itself passes over a region every 10 days and the data is interpolated to produce the 7-day increments.

A database of eddies detected using the algorithm of Chelton et al (2011) applied to the AVISO SLA data (1992-2010) was also used. This global database tracked eddies and provided eddy location, radius, amplitude, swirl speed, eddy polarity (anticyclonic vs cyclonic), as well as an eddy identifier (a number). This identification feature permitted specific eddies to be tracked through time, allowing for a statistical analysis of where/how/when eddies formed and disappeared. The eddy database was the source of all statistics done in this experiment. A visual inspection of eddies stored in the database plotted over the SLA for the NWC region showed that the eddies in the database adequately represented the eddies visually observed.

A comparison between Modular Ocean Data Assimilation System (MODAS), SSH, used in the Woityra and Rossby study, and the AVISO data set was conducted. A visual comparison showed the data sets were similar with few major discrepancies. This visual analysis provided a method to check the Woityra statistics (conducted visually on the MODAS dataset) against the statistics derived from the AVISO dataset that are presented here.

3. Methods

For the purpose of this study, eddies whose initial position was within a box encompassing the NWC (latitude range [48-54 N], longitude range [314-320 E], see Fig. 2) were selected for further study. This provided a time series of eddy properties for all eddies that originated within the NWC region. Examination of time series of eddy amplitudes or radii suggested no definitive signature, such as decreased amplitude or change in size, associated

Figure 1. The AVISO time-averaged mean field, plotted with predicted currents. Box denotes the region of interest for this study.

with eddy disappearance (Fig 3). In fact, since all eddies ultimately disappeared from the database, it was not possible to determine the fate of eddies, be it sinking or reabsorption into the NAC.

Statistical analysis of eddy disappearance using only the eddy-tracking algorithm did not show the same seasonal cycle as Woityra (2005) (Fig.4). To isolate sinking and rejoining, a visual analysis of the eddies found in the database was conducted. Sinking was limited to cases where the decrease in amplitude of the eddy was large and the eddy did not appear to be attached to the NAC. Rejoining occurred when the eddy disappeared when attached to the NAC or a much larger eddy. Eddies not fitting either description were not included in the analysis. Ultimately, sinking was used sparingly and the definition was conservative. All analysis was conducted on the SLA + Mean dataset and on anticyclonic eddies that started in the NWC for the time interval 1993-2007.

4. Results

Examination of eddy trajectories suggested that anticyclonic eddies tended to disappear inside the box (when plotting eddies first detected in the box) (Fig. 2). Perhaps even more noticeably, anticyclonic eddies seemed to roughly trend from the southeast to the northwest inside the box. This occurrence also coincides with the fact that, of anticyclonic eddies deemed to be sinking, most occurred in the northwest of the box. Rejoining occurrences tended to be roughly in the middle/southeast of the box. This trend suggests that there is some physical reason differentiating the location of sinking events and rejoin events.

Maximums in anticyclonic eddy sinking for the time period 1993-2007 were the months November-April (having plotted in two month increments). Also, rejoining

Figure 2. Start and stop locations of anticyclonic eddies that started in the region of interest, 1993-2007. Black contours illustrate lines of constant ocean depth. A bathymetric high point, Flemish Cap, is seen in the lower left corner.

events were at a maximum in the analysis during the May-June months. This appeared to coincide with a minimum in the sinking events (Fig. 5).

Anticyclonic eddies seemed to disappear preferentially in the May-June time period. This corresponds to an observed maximum in rejoin events and minimum in sinking events. The ratio of anticyclonic eddy disappearance to total disappearance was greatest in May-June with a maximum of near 60% of eddies disappearing being anticyclonic. This suggests that, because eddy lifetime is on the order of months, anticyclonic eddies may be preferentially formed in the winter months.

5. Discussion

Special focus was given to anticyclonic eddies and their seasonal disappearance times. Further study might look at eddy averages and properties (amplitude, radius, swirl speed) through different, longer time scales. Ultimately, determination of eddy sinking compared to a rejoin event was difficult. This subjectivity, when looking at satellite altimetry data, means objective proof of eddy sinking is difficult to observe. Future studies may solve

this problem with *in-situ* data. This would alleviate any concern over whether or not an eddy was sinking.

Comparing the Chelton et al. database eddy properties with the eddies that were deemed to have sunk may provide insight into a sinking signature. A hypothesis of decreased eddy amplitude and possibly increased eddy radius before disappearance may ultimately prove useful in creating an objective algorithm to detect eddy sinking events. How the complex dynamics actually work is hard to determine and it may be proven that eddies deemed to have rejoined the NAC had actually sunk.

Sources of error for the experiment include subjectivity in determining eddy sinking events, the Chelton et al database recording disappearance times and the SLA field interpolation. The largest error is subjectivity. It is very difficult to objectively observe eddies sinking. Error introduced by a premature disappearance of an eddy as tracked by the Chelton et al database would not lead to a sinking/rejoining designation for the eddy, possibly diminishing the number of true sinking and rejoining events. Data for the SLA field was interpolated allowing for the possibility of distorting SLA in such a way to either create eddies where there are none or distort eddies when they exist.

Figure 3. Plot of amplitude, radius and swirl speed (azimuthal velocity) as functions of time for anticyclonic eddies that started in the region of interest and that disappeared in during the November-December months. Only parameters of eddies from 1993 to 2007 are shown.

Figure 4. Plot of frequency of disappearance of anticyclonic eddies that started in the region of interest. The x-axis corresponds to the time of year during which eddies disappeared.

Figure 5. Plot of anticyclonic eddies that started in the box and deemed to be sinking or rejoining. Seasonality can be observed by defining winter and summer bins as “Nov.-Apr.” and “May-Oct.”, respectively and computing the number of eddies falling in each bin.

6. Conclusions

This study used 15 years of altimeter-derived sea level anomaly and the Chelton et al (2011) eddy database to investigate the fate of eddies formed in the NWC of the NAC. Evidence of eddy sinking was observed, and this sinking was determined to occur more frequently during winter. This finding is consistent with the results of Woityra (2005), who used an analysis sea level product (MODAS) over a shorter time period and who did not have the objective eddy detection algorithm. However, because eddy fate could not be unambiguously determined from the eddy database, the results presented here have an element of subjectivity associated with them. Further and more definitive evidence that the NWC is indeed the source of deep subsurface lenses in the subtropics will likely require intensive *in-situ* measurements in addition to the SLA/SSH data analyzed here. Analysis of such an *in situ* data set, obtained during the 2011 winter/spring is presently in progress.

Acknowledgments: I would like to thank Kathleen Donohue, Rob Pockalny and Kim Carey for organizing the SURFO program and NSF for funding the program. I would also like to thank Dave Hebert, Tom Rossby and George Sutyrin for their help.

References

- Chelton, Dudley B., M. Schlax, R. Samelson (2011), Global observations of nonlinear mesoscale eddies, *Progress in Oceanography*, **91**(2): 167-216
- McDowell, S.E., and H.T. Rossby (1978), Mediterranean water: an intense mesoscale eddy off the Bahamas, *Science*, **202**: 1085-1087.
- Prater, M.D., and T. Rossby (1999), An alternative hypothesis for the origin of the "Mediterranean" salt lens observed off the Bahamas in the Fall of 1976, *Journal of Physical Oceanography* **29**(8): 2103-2109
- Woityra, W., and T. Rossby (2008), Current broadening as a mechanism for anticyclogenesis at the Northwest Corner of the North Atlantic Current, *Geophys. Res. Lett.*, **35** L05609, doi:10.1029/2007GL033063
- Woityra, W. (2005), A View of the Northwest Corner: Using Satellite Altimetry to Estimate Kinetic Energy Flux Convergence in the Region of the North Atlantic Current-Subpolar Front Transition, Master of Science Thesis, University of Rhode Island, US

B. Heath, Northwestern University, Evanston, IL 60201
(benjaminheath2008@u.northwestern.edu).
D. Ullman and C. Buckingham, Graduate School of
Oceanography, University of Rhode Island, Narragansett RI
02882 (d.ullman@gso.uri.edu, cbuckingham@gso.uri.edu).

Subseafloor Sedimentary Fungi and their Viability at High Pressure

Thomas Hespeler

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

Abstract. Fungi are found in diverse habitats throughout the world. While terrestrial fungi have been extensively studied, there is a very limited understanding of the diversity and role of fungi in the marine environment, especially in the deep sea. The focus of this study is to determine if the fungi isolated from deep sea sediment cores are capable of growth at *in situ* pressures. The samples were collected in the South Pacific Gyre (SPG) during the Integrated Ocean Drilling Program (IODP) Expedition 329. Pure cultures of fungi were specifically chosen based on their antibacterial properties. Each fungus was initially isolated on agar plates at 1 atmosphere. Samples were transferred to liquid media to verify growth in the liquid media prior to conducting the experiment. After growth in the liquid media was verified, the inoculated media was then placed in several sealed bags for pressurization. Each isolate was subjected to atmospheric pressure and 500 atm. Bags were then opened on selected days and O₂ concentration was measured to quantify growth rates. Finally, the data collected from the atmospheric bags was compared to that of the pressurized bags to determine if growth had occurred in the pressurized samples. For isolates grown at 1 atm, the minimum dissolved O₂ concentration was reached on day 7. For the 500 atm isolates, the minimum O₂ concentration was reached on day 14. Growth rates were calculated for each isolate at 1 atm and 500 atm. It was determined that all three isolates showed no significant growth compared to the negative control at 500 atm. The lack of significant growth of the isolates at 500 atm indicates that the isolates may be a contaminant or that the fungi were dormant during their time in the sediment column prior to isolation.

1. Introduction

From the guts of fish to glacial ice in the Arctic Ocean, fungi are found in extreme environments throughout nature. Low temperature, high pressure, and reduced nutrient availability make the deep sea a formidable environment for even the most adaptive creatures. While the role of fungi in terrestrial environments has been extensively studied, the role of fungi in the deep sea is of limited understanding. This extensive environment, marine sediments cover two-thirds of the earth's surface (Snelgrove et al. 1997), is an untapped resource for understanding of a large portion of Earth's biomass. Previous studies have identified piezophilic fungi in the Central Indian Basin, whereas this study will focus on fungi isolated from cores samples taken from the South Pacific Gyre (SPG). In the experiment fungal isolates will be subjected to *in situ* pressures using a high pressure reactor whether or not they are viable at simulated *in situ* pressures.

2. Methods

Sediment samples were obtained from cores isolated on Integrated Ocean Drilling Program Expedition 329 in the South Pacific Gyre. Five pure cultures were selected for their antimicrobial properties and used for the initial pressure experiment. Prior to conducting the initial pressure experiment liquid media (YPM) (1 g yeast

extract, 1 g peptone, 2 g mannitol per 500 mL seawater) was inoculated in sterile tubes to confirm growth of isolates in the liquid media. Each isolate was then inoculated in 10 mL of YPM, using a flame sterilized loop, and 5 ml was placed into a metalized polyester barrier film bag. Two bags were used for each isolate, one bag to be maintained at 1 atmosphere (atm) and the other bag to be maintained at 500 atm. The 500 atm bags were then placed into a pressure reactor (HIP OC-15) and pressurized to 500 atm for 5 days. After the fifth day the reactor was depressurized and each bag was sampled for dissolved O₂ concentration. Isolates SPG11-F42, F49, and F-57 were selected based on their large change in O₂ concentration after the five day incubation period.

For the second experiment 150 ml of YPM were inoculated for each isolate. 5 ml of the inoculated media were then placed in a heat sealed bag. A total 15 bags for the 1 atm samples and 6 bags for the 500 atm samples were inoculated for each isolate. The atmospheric bags were sampled on days 1, 2, 4, 7, and 14 following inoculation for O₂ concentration. The 500 atm bags were sampled on days 7 and 14 following inoculation. Dissolved O₂ concentration was measured using an optode with each bag immersed in an ice water bath to stabilize sampling temperature and prevent growth of the 500 atm samples when depressurized. Samples were measured for two minutes each to ensure a stable measurement. Each isolate was measured in triplicate on their respective sampling days. Dissolved O₂ concentration was quantified in the non-inoculated bags during each sampling day to provide negative control.

Growth rate was calculated for each isolate and the negative control at 1 atm by comparing an average of the day 1 O₂ concentration to that of the day 14 O₂ concentration using the exponential growth rate equation. Growth rate for each isolate and the negative control at 500 atm by comparing an average of the day 7 O₂ concentration to that of the day 14 O₂ concentration using the exponential growth rate equation. Finally a non-paired T-test was used to determine if there was a significant difference between the growth rate of the negative control and the isolates at both 1 and 500 atm.

3. Results

After completion of the second experiment, SPG11-F42, F49, and F57 the O₂ concentration decreased after the fourteen day incubation period. Figure 1 shows the decreasing O₂ concentrations for each isolate at 1 atm. Minimum O₂ concentration was reached for each 1 atm isolate during day 7 sampling. Each isolate also showed decreased O₂ concentration after the fourteen day incubation period for the elevated pressure samples. Figure 2 shows the decreasing O₂ concentration for each isolate at 500 atm. Minimum O₂ concentration was reached at day 14 for each isolate at 500 atm. Each isolate had a positive growth rate and growth rate was significant compared to the negative control at 1 atm. Growth rate was not significant for each isolate at 500 atm compared to the negative control.

4. Discussion

While all isolates showed significant growth at 1 atm, none of the isolates exhibited significant growth at 500 atm therefore disproving the initial hypothesis. The lack of growth may be caused by three possibilities. First the isolates are contaminants and not actually found in the sediment column. The YPM was inoculated with spores versus hyphae impeding growth. Finally, the fungi were dormant in the sediment.

The first reason for lack of growth in the high pressure samples initially seems like the most plausible

reason but the possibility of the isolates being terrestrial

Figure 2. Dissolved O₂ concentration versus time for isolates subjected to atmospheric pressure

Figure 1. Dissolved O₂ concentration versus time for isolates subjected to 500 atm.

contaminants is unlikely since the YPM media utilized seawater which may impede growth of terrestrial fungi. Since each isolate demonstrated growth in seawater it is more likely that these are marine contaminants found in

Isolate	Growth Rate (days ⁻¹)	Isolate	Growth Rate (days ⁻¹)
1 atm Control	-0.018	500 atm Control	0.033
1 atm F42	0.185	500 atm F42	0.060
1 atm F49	0.236	500 atm F49	0.039
1 atm F57	0.205	500 atm F57	0.050

Table 1. Growth rate of isolates F42, F49, F57 at 1 atm and 500 atm compared to growth rate of the negative control.

the water column when the sample was brought to the surface on the drilling ship. This scenario also seems unlikely due to the aseptic techniques used when processing the sediment cores aboard the drill ship. Also cores were inspected visually for cracks in the sediment and a chemical tracer was used to verify no drill water contamination had occurred.

The second reason for lack of growth is also a distinct possibility since the plates used to inoculate the YPM media were seven days old. If the YPM media was inoculated with spores then the mycostatic effect of seawater (Kirk 1980) coupled with the stress of the high pressure environment could have hindered the growth of the fungi at pressure. Media inoculated with spores have demonstrated growth at high pressure, but when the stress of the elevated pressures was combined with low temperature has in the past prevented growth. This combined stress theory may explain why the 1 atm bags overcame the mycostatic effects of the seawater but when combined with the stress of the elevated pressure prevented significant growth.

The final reason for inhibited growth seems to be the most unlikely but the most intriguing. The oldest known fungi was found to be 0.43 million years old (Ma) and isolated from the Central Indian Basin (Raghukumar 2004). Based on the location of the core samples from which isolates SPG11-F42, F49, and F57 and the depth below the sea floor the sediment samples were removed from, their age would be from 35-50 Ma. The likely hood of these fungi remaining dormant and viable (once the proper environmental conditions existed) seems highly unlikely.

5. Conclusion

The fungal isolates showed no significant growth under simulated in situ conditions, while growth was significant for all three isolates at atmospheric pressure. Further investigation is necessary to determine which of the three possibilities is the most relevant. To determine whether or not the fungi were terrestrial contaminants, each isolate could be cultured in distilled water and the

growth compared to that of a culture with YPM media. To determine whether or not the media was inoculated with spores the second experiment could be repeated and the inoculation material could be visually verified under microscope prior to inoculating the YPM media. The final reason for lack of growth requires the most thorough of experiment where the sediment in which the fungi was isolated from be studied for size and a pore water characteristics and then the material surround the fungi dated in order to determine the actual age of the fungal isolates. Each of these three experiments will provide a better understanding of where these fungi originate and what is their purpose in the environment.

References

- Kirk P.W. Jr. 1980. The mycostatic effect of seawater on spores of terrestrial and marine higher fungi. *Botanica Marina*, **23**, 233-238.
- Raghukumar C., Raghukumar S., Sheelu G., Gupta S.M., Nagender Nath B., Rao B.R. 2004. Buried in time: culturable fungi in a deep-sea sediment core from the Chagos Trench, Indian Ocean. *Deep-sea Research I*, **51**, 1759-1768.
- Raghukumar C., Damare S.R., Singh P. 2010. A review on deep-sea fungi: occurrence, diversity and adaptations. *Botanica Marina*, **53**, 479-492.
- Raghukumar C., Damare S.R., Raghukumar S. 2006. Fungi in deep-sea sediments of the Central Indian Basin. *Deep-sea Research I*, **53**, 14-27.
- Snelgrove P.V.R., Blackburn T.H., Hutchings P., Alongi D., Grassle J.F., Hummel H., King G., Koike I., Lambshead P.J.D., Ramsing N.B., Solis-Weiss V. and Freckman D.W. 1997. The importance of marine sediment biodiversity in ecosystem process. *Ambio*, **26**, 578-582.

T.Hespeler, University of Rhode Island, Kingston, RI 02881, (thomas_hespeler@my.uri.edu).

Amplifying Microsatellite Loci in the Marine Diatom *Ditylum Brightwellii*

Arianna Jesanis and Tatiana Rynearson

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

Abstract. Microsatellites are tandem repeats of DNA nucleotides that are numerous within the genome. Because they exhibit high amounts of polymorphism, meaning they are highly variable among both individuals and populations, microsatellites are useful as genetic markers in determining population differentiation. These regions can be amplified using a technique known as polymerase chain reaction, which uses primers of a known sequence to exponentially amplify DNA regions during repeated heating and cooling cycles. One major challenge to amplifying microsatellites is variability amongst individuals at priming sites, leading to poor or non-specific amplification of the loci. In order to have successful amplification of microsatellites PCR amplification needs to be optimized for each locus. This study worked to optimize PCR protocols to amplify five different known microsatellite loci in the diatom species, *Ditylum brightwellii*. Diatoms, a type of marine phytoplankton, are biogeochemically significant in that they are responsible for 20-25% of globally fixed carbon and atmospheric oxygen and in turn it is of interest to assess their genetic structure and associated physiological qualities. Alterations in reagent concentrations, temperature, and thermal cycles were made in order to successfully amplify the five microsatellite regions. The results were analyzed using polyacrylamide gel electrophoresis. The amplified DNA was then sequenced to confirm the identity of each microsatellite region. Optimizing the PCR conditions for these loci will make them useful tools for identifying populations of *Ditylum brightwellii* over space and time in order to better understand the genetic connectivity of this species. Understanding the extent of genetic connectivity between populations can inform our understanding of the environmental, physical, and behavioral drivers of evolution and adaptation in this species.

1. Introduction

Diatoms are unicellular phytoplankton that are responsible for 20-25% of the globally fixed carbon and atmospheric oxygen (Mann, 1999). They are extremely diverse organisms with as many as 200,000 different species living today (Mann and Droop, 2006). They are found in waters all over the globe and have large population sizes leading to the suggestions that their dispersal is ubiquitous (Finlay, 2002). In contrast, more recent studies have suggested that micro-organisms such as diatoms can also have restricted ranges (Foissner, 2006). This restricted gene flow can then lead to high population diversity given the right environmental and geographic conditions (Casteleyn *et al.*, 2010).

Physiological diversity has been studied in diatom populations and their high physiological diversities both between and within species likely contributes to their high success in reproduction and adaptive potential (Rynearson and Armbrust, 2000). The high physiological diversity may relate to underlying genetic diversity (Brand 1990), however little is known in regards to the factors affecting diatom intraspecific diversity and their global distribution (Mann, 1999). In order to understand the genetic diversity within and between diatom species, higher resolution molecular tools need to be developed.

One of these species specific molecular markers is microsatellites. Microsatellites are tandem repeats of DNA nucleotides that are numerous within the genome

and have a high amount of polymorphism (Goldstein and Pollock, 1997). They form due to slippage of the DNA polymerase during replication of the repeating units (Schlötterer and Tautz, 1992) and therefore the number of repeats can vary dramatically between individuals (Rynearson and Armbrust, 2000). This can then serve as a high-resolution DNA fingerprint for individuals; due to their high variability, microsatellites are useful in distinguishing between species, populations within a species, and individuals. The more microsatellite regions that are analyzed the more accurate the DNA fingerprint (Rynearson and Armbrust, 2000); therefore, it is critical to the study of micro-organism genetic diversities to be able to analyze multiple loci.

The goal of this study was to develop new microsatellite markers for the diatom species, *Ditylum brightwellii*, as tools to explore intra-specific genetic diversity and connectivity between populations. *Ditylum brightwellii* was used as the study organism because it is one of the larger diatoms and also has a unique morphology making it easy to isolate from other plankton. A recent study used two microsatellite loci, Dbr4 and Dbr9, in order to study genetic diversities within a single field population of *Ditylum brightwellii* in the Puget Sound, Washington and found high genetic and phenotypic diversity within the population (Rynearson and Armbrust, 2000). Another study revealed high genetic diversity within a single bloom of *Ditylum brightwellii* using three microsatellite loci, Dbr4, Dbr9, and Dbr10 (Rynearson and Armbrust, 2005). Analyzing

more microsatellite loci leads to more precise DNA fingerprinting and more accurate calculations of genetic diversity. Therefore, this study worked to optimize the conditions necessary to analyze additional microsatellite loci both within and between populations of *Ditylum brightwellii*.

In order to analyze microsatellite loci they need to first be successfully amplified before being sequenced. The technique used to amplify DNA is the polymerase chain reaction technique. It was invented by Kary Mullis in order to amplify specific regions of DNA (Mullis *et al.*, 1986). Due to the sensitivity of the technique in amplifying single DNA molecules there is no single protocol that works in all situations (Innis & Gelfand, 1990). This then leads to the need for optimizing PCR conditions for each new application.

Due to PCR's high sensitivity and specificity difficulties often arise when optimizing PCR conditions. Some of these include: no or low product yield, non-specific amplification, presence of "primer-dimers" that compete for amplification against the actual product, and contamination (Innis and Gelfand, 1990). PCR's ability to amplify such small amounts of DNA makes it extremely prone to contamination (Rochelle *et al.*, 1997), and therefore precautions against contamination were taken.

PCR conditions were optimized by investigating a few of the many variables in the reaction including: primer annealing temperature (T_a), number of cycles, and $MgCl_2$ concentration. Other variables that could be investigated for a PCR include: denaturation and extension temperature as well as dNTP, Taq polymerase, and primer concentrations. Previous studies have concluded that increasing the primer annealing temperature decreases the amount of non-specific amplification and suggested annealing temperatures are 5°C below the T_m of the primers (Innis and Gelfand, 1990). Achieving the optimal number of cycles in a PCR is necessary; too few cycles will yield low concentrations of DNA, and too many cycles may result in background amplification, making results difficult to interpret (Innis and Gelfand, 1990). Lastly, it is beneficial to optimize the magnesium concentration in that it affects many components of the reaction including: primer annealing and strand disassociation temperatures, primer-dimer formation, product specificity and enzyme activity. Too low of a magnesium concentration results in little or no product yield while high concentrations may result in non-specific amplification (Innis and Gelfand, 1990).

Through investigating these variables of the PCR, the study aimed to amplify microsatellite regions of the marine diatom *Ditylum brightwellii* in order to increase the precision of DNA fingerprints created for both individuals and populations of this species. This work was successful in developing multiple tools to accurately determine genetic diversity not only between populations but between individuals within populations?

2. Methods

2.1 Isolate Collection and Culture

Four isolates of the marine diatom *Ditylum brightwellii* were obtained in three different ways. Water samples were collected on 7 October 2008 by Martha's Vineyard Coastal Observatory from the coast of Martha's Vineyard (MV2) (41°34'55"N, 70°54'85"W) and *Ditylum brightwellii* was isolated on 9 October 2008. Two isolates were obtained from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton (CCMP). One isolate, CCMP 358, was collected from the Gulf of Mexico (28°90'20"N, 89°48'53"W) on 1 February 1980. The second isolate, CCMP 2227, was collected from Avery Point, Connecticut (41°31'96"N, 72°06'70"W) on 16 November 2001. The fourth isolate was obtained from the Stazione Zoologica Anton Dohrn (SZN). The isolate, SZN 340, was collected from the Gulf of Naples, Italy in October 2007. All isolates were cultured in f/2 media at 14°C with a 16:8 light : dark cycle.

2.2 DNA extraction

Cultures of exponentially growing isolates were filtered after visual inspection of cell health and growth. Total genomic DNA was then extracted using a Qiagen DNeasy kit and stored at -20°C.

2.3 PCR primers and conditions

This study used investigated different previously sequenced microsatellite regions, DBR1-5 for which primers had previously been designed. All PCR primers were synthesized by a commercial laboratory (Integrated DNA Technologies, Inc., Coralville, IA) and known to recognize the flanking regions of the microsatellite loci (Table 1). In this study primers were named according to the microsatellite regions they recognized, Dbr1-5, and both reverse and forward primers were unique to each of the microsatellite region. Suggested annealing temperatures (Table 1) for each of the five microsatellite regions were given by the commercial laboratory as well (IDT, Inc.). These temperatures were determined based on the base composition, length, and primer concentration of the different microsatellite loci (Innis and Gelfand, 1990).

PCR amplifications were performed in 10 μ L volumes initially containing 2.00 μ L 5x Mango reaction buffer, 0.08 μ L Mango Taq polymerase, 0.20 μ L 10mM dNTPs, 0.75 μ L 50mM $MgCl_2$, 0.25 μ L 10 μ M reverse primer, 0.25 μ L 10 μ M forward primer, and 5.47 μ L H_2O . The initial PCR conditions consisted of an initial denaturation 94°C for 20 s, followed by 33 cycles of 94°C for 20 s, suggested annealing temperature for 20 s, and 72°C for 20 s. This was all followed by a final 10 minute extension at 72°C.

2.4 Optimizing PCR conditions

PCR was optimized by making alterations in thermal cycling conditions as well as reagent proportions. Common alterations included changing the number of cycles, annealing temperature, and $MgCl_2$ concentration. Touchdown amplification was introduced in order to increase the specificity and sensitivity of the PCR reaction (Zuma'rraga *et al.*, 2005). Five cycles beginning at 5°C above the annealing temperature and decreasing 1°C over each cycle were introduced to all five microsatellite PCR reactions. Temperature gradients were then performed to achieve the optimal annealing temperature for each microsatellite. Gradients started at 4°C below the suggested annealing temperature of the microsatellite and increased by 2°C until 4°C above the suggested T_a (Figure 1). Once an optimal T was determined, cycle number was optimized. Optimal cycle number was determined using

cycle gradients during which the number of cycles increased by 2 cycles with each reaction. After achieving the optimal cycle number, magnesium chloride concentration was altered. Increasing the amount of magnesium chloride was performed on microsatellite regions and DNA strains that exhibited low PCR product yield but high specificity. Decreasing the magnesium chloride was performed on reactions that exhibited non-specific amplification in order to increase the specificity. PCRs were analyzed for expected length fragments through gel electrophoresis with 6% polyacrylamide gels. HyperLadder V (25bp to 500bp range) (Bioline, Randolph, MA) was used as a molecular weight standard. Gels were stained with SYBR Green and imaged using UV imager (Syngene, Frederick, MD). If optimized 20 μ L reactions were conducted on a 2% agarose gel in order to be extracted and sequenced to confirm the correct microsatellite region was optimized.

Figure 1. Photograph of the polyacrylamide gel taken after a temperature gradient was performed. Lanes 1 and 8 contain 2.5 μ L of Hyperladder V. Lane 7 contains the negative control. Lane 2-6 contain PCR products of amplifying microsatellite loci Dbr2 with an expected length of 200 bp in DNA isolate, CCMP 358 with decreasing temperature. Lane 2 has a T_a of 51°C, lane 3 has a T_a of 49°C, lane 4 has a T_a of 47°C, lane 5 has a T_a of 45°C and lane 6 has T_a of 43°C. Lane 3 is the optimal T_a with surrounding lanes containing more non-specific amplification.

Microsatellite	Primer sequence (5' to 3')	Core motif	Expected length (bp)	MgCl ₂ (μL)	Cycle Number	T _a (°C)
Dbr1	F:GTGTATAGAAGCAAGGAGCTGTC R:AGGCGATTCAATGTAAAGGACG	(AAC) ²⁷	166	0.55	37	47
Dbr2	F:GGGTAATTCTGTGGCAGCG R:GCCCAAGACCTTGTAAGTTAGG	(AC) ¹⁵	200	0.50	33	60
Dbr3	F:AGTGCCGTTTGATTGGGC R:TGCATACGACTTGGCTCTG	(ACG) ¹¹	340	0.70	33	53
Dbr4	F:GAAAGAAATGTAAGGTGTACATAC R:AGGGCGTGAAGGGAGTATC	(AG) ¹¹	240	0.75	35	50
Dbr5	F:ACGCATGTATCAGCATTGAGG R:GCGGTACAATACCCTTCGTTG	(AG) ¹²	152	0.60	37	49

Table 1: Characteristics of the five microsatellite loci developed for *Ditylum brightwellii* isolated in the Gulf of Mexico. Forward and reverse primer sequences, core motifs, expected lengths, and concluded primer annealing temperatures (T_a), cycle numbers, and MgCl₂ volumes per 10 μL reaction are given.

3. Results

3.1 PCR Optimization

The first round of PCR began with the suggested PCR conditions given by Integrated DNA Technologies, Inc. for each microsatellite region and initial results showed multiple-banding patterns for each DNA field population with some amplified fragments being at the expected length of the microsatellite region. The addition of the touch-down amplification led to increasing

Figure 2. Photograph of the polyacrylamide gel taken after a cycle gradient was performed. Lane 5 contains 2.5 μL of Hyperladder V. Lane 1-4 contain PCR products of amplifying microsatellite loci Dbr1 with an expected length of 152bp in DNA isolate, CCMP 358 with decreasing number of cycles. Lane 1 has 37 cycles, lane 2 has 35 cycles, lane 3 has 33 cycles, and lane 4 has 31 cycles. Lane 2 is the optimal number of cycles with the largest product and minimal non-specific amplification

specificity. Results from the temperature gradients revealed an increase in non-specific amplification at both sub and super-optimal annealing temperatures (Figure 1). The optimal cycle number gave the greatest PCR product without increasing the amount and complexity of background products. At sub-optimal cycle numbers the PCR product decreased while at super-optimal numbers more non-specific amplification occurred (Figure 2). Increasing the MgCl₂ concentration yielded more product as well as more non-specific amplification. Through performing these alterations of touchdown amplification, altering annealing temperature, cycle number, and magnesium chloride concentration some polyacrylamide gels exhibited both specific and high amounts of amplification for DNA populations while others have yet to be optimized.

3.2 Optimized conditions

DNA field population, CCMP 358 was successfully amplified for all five microsatellite regions while CCMP 2227 was never successfully amplified. Optimized conditions in terms of MgCl₂ concentration, annealing temperature, and cycle number varied between all five microsatellite regions (Table 1). Dbr1 was successfully amplified for CCMP 358, while SZN 340 showed two bands, and CCMP 2227 showed strongest amplification at a different base pair length (Figure 3). Optimal conditions for Dbr1 include .55 μL MgCl₂, 37 cycles and an annealing temperature of 47°C. Dbr2 was successfully amplified for CCMP 358, while SZN 340 and MV2 expressed some non-specific amplification (Figure 4). Optimal conditions for Dbr2 include .50 μL MgCl₂, 33 cycles and an annealing temperature of 60°C. Dbr3 was successfully amplified for CCMP 358, and MV2 while CCMP 2227 showed strongest amplification at an incorrect base pair length (Figure 5). Optimal conditions for Dbr3 include .70 μL MgCl₂, 33 cycles and an annealing temperature of 53°C. Dbr4 was successfully

Figure 3. Photograph of the polyacrylamide gel taken under optimal conditions for Dbr1. Lane 4 contains 2.5 μ L of Hyperladder V. Lane 1 has CCMP 358 DNA, lane 2 has SZN 340 DNA, and lane 3 has CCMP 2227 DNA.

amplified for MV2, CCMP 358, and SZN 340 while CCMP 2227 showed strongest amplification at an incorrect base pair length (Figure 6). Optimal conditions for Dbr4 include .75 μ L $MgCl_2$, 35 cycles, and an annealing temperature of 50°C. Dbr5 was only successfully amplified for CCMP 358 (Figure 7). Optimal conditions for Dbr5 include .60 $MgCl_2$, 37 cycles, and 49°C.

4. Discussion.

The process of PCR optimization is challenged by the number of variables impacting the amplification. However, optimizing PCR conditions for microsatellites provides a useful tool to explore intra-specific diversity and its connection to and evolution. Microsatellites are high resolution molecular markers that can determine distances not only between species but between and within populations of the same species. Therefore optimizing the PCR conditions for microsatellite regions will provide even more precise genetic characteristics (Rynearson and Armbrust, 2000).

In this study microsatellites were successfully optimized by making alterations to the suggested PCR conditions. Reducing the $MgCl_2$ concentration increased the specificity and thereby reduced the non-specific amplification of all reactions performed. It was also noted

Figure 4. Photograph of the polyacrylamide gel taken under optimal conditions for Dbr2. Lane 6 contains 2.5 μ L of Hyperladder V. Lane 1 is the negative control. Lane 2 has CCMP 358 DNA, lane 3 shows unsuccessful amplification of CCMP 2227, lane 4 shows SZN 340 DNA, and lane 5 has MV2 DNA.

that reducing the $MgCl_2$ concentration reduced the primer-dimer formation at the bottom of the gel in all cases even without altering the primer concentration which supports the conclusions of previous studies (REF). In general, the $MgCl_2$ concentration necessary for successful amplification was less than the suggested .75 μ L per 10 μ L reaction. Some microsatellite loci required a different concentration than others (Table 1). Drawing in the knowledge that $MgCl_2$ affects primer annealing

Figure 5. Photograph of the polyacrylamide gel taken under optimal conditions for Dbr3. Lane 4 contains 2.5 μ L of Hyperladder V. Lane 1 has CCMP 358 DNA, lane 2 has SZN 340 DNA, and lane 3 has MV2 DNA.

Figure 6. Photograph of the polyacrylamide gel taken under optimal conditions for Dbr4. Lane 1 contains 2.5 μ L of Hyperladder V. Lane 2 contains the positive control. Lane 3 has CCMP 227 DNA with unsuccessful amplification. Lanes 4-6 have SZN 340 DNA, MV2 DNA, and CCMP 358 DNA respectively with successful amplification.

temperatures and enzyme activity the difference in magnesium requirements could suggest differences in flanking region sequences. It also could suggest differences in length of the microsatellite region and how many base pairs the DNA polymerase is replicating. However, more data needs to be collected to support this hypothesis.

Another general trend revealed through gel electrophoresis analysis was that lower cycle numbers yielded a lower concentration of PCR product and higher cycle numbers yielded more background amplification (Figure 2). Although this was not expressed in all gels this was the general trend shown in this study. This again supports previous conclusions and the optimal cycle numbers achieved in this study were often more than the suggested 33 cycles (Table 2). This could be that the quantity or quality of DNA used in this study was lower than expected. The optimal cycle number remained consistent between DNA field strains and only altered between microsatellite regions suggesting that the quality of DNA was similar between these field populations including with CCMP 358 from which the microsatellite regions were derived.

A third general trend shown was that temperature gradients performed in order to achieve an optimal primer annealing temperature did not always reveal increasing specificity with increasing temperature. For instance, in Figure 1 both sub-optimal and super-optimal T_a non-specific products were formed. This trend has also been revealed in previous studies (Rychlik *et al.*, 1990) but the reasoning behind non-specific product formation at higher than optimal T_a has yet to be concluded. Optimal annealing temperatures are generally found to be about

5°C below the melting temperatures of the primers (Innis and Gelfand, 1990) but this study revealed different results. Dbr1 T_a was about 10°C below the T_m , Dbr2 T_a was about 5°C below the T_m , Dbr3 T_a was about 3°C below the T_m , while Dbr4 and Dbr5 were both about 7°C below the T_m . These results suggest that optimal annealing temperature must be achieved experimentally, although the 5°C below the T_m is a good starting point. It also reveals that the touchdown cycling can begin higher than the T_m of the primers as in Dbr3 with a touchdown cycle starting at 57°C and the T_m being at 54.9°C.

Having the PCR optimization guidelines expressed in previous studies definitely provided a good starting point as well as general expectations of what results might reveal when altering certain variables. On the other hand, this study revealed that experimental application of these suggested guidelines is necessary to achieve the optimal conditions. With PCR being such a sensitive technique the application to different DNA regions and even between populations of the same species can vary dramatically and therefore experimental derivation or confirmation of optimal conditions is necessary.

For the DNA field strains and microsatellite regions within these that have yet to be optimized, other variables might be investigated such as altering the extension temperature, denaturing time, or other reagent concentrations. Though many of these strains and microsatellite loci show promise for optimization more changes to the PCR conditions still need to be made in order to gain more precision in creating the DNA fingerprint both between and within populations of *Ditylum brightwellii*.

Figure 7. Photograph of the polyacrylamide gel taken under optimal conditions for Dbr5. Lane 3 contains 2.5 μ L of Hyperladder V. Lane 1 contains successful amplification of CCMP 358 DNA and lane 2 contains the negative control.

Optimizing PCR conditions is the first step using microsatellites as a high-resolution molecular tool and providing more information on diatom ecology and genetic characteristics. Once these conditions are optimized the regions can be measured using fragment analysis. Then further research can use this information to better understand the behavioral, physical, and environmental drivers behind the evolution and adaptation behind diatom species. This can then help to predict how these species and their role in the carbon cycle might react to a changing environment.

Acknowledgements. We would like to thank Kathy Donohue and Rob Pockalny for their directorship in the 2011 SURFO program and Kim Carey for her hard work and support of the SURFO program at the Graduate School of Oceanography. We would also like to thank Kerry Whittaker, Jeremy Lins, Katie McCusker, and Dan Roche for their laboratory assistance as well as the National Science Foundation for their funding in this project.

References.

- Brand, L.E. 1990. Review of genetic variation in marine phytoplankton species and the ecological implications. *Biological Oceanography*, **6**, 397-409.
- Castelyleyn, G. *et al.* 2010. Limits to gene flow in a cosmopolitan marine planktonic diatom. *PNAS*, **107**, 12952-12957.
- Finlay, B. 2002. Global Dispersal of Free-Living Microbial Eukaryote Species. *Science*, **296**, 1061-1063.
- Foissner, W. 2006. Biogeography and Dispersal of Micro-organisms: A Review Emphasizing Protists. *Acta Protozoologica*, **45**, 111-136.
- Innis, M. and Gelfand, D. 1990. PCR Protocols: A Guide to Methods and Applications. Harcourt Brace Jovanovich, Publishers.
- Mann, D.G. and Droop, S.J.M. 1996. Biodiversity, biogeography, and conservation of diatoms. *Hydrobiologia*, **336**, 19-32.
- Mann, D.G. 1999. The species concept in diatoms. *Phycologia*, **38**, 437-495.
- Rochelle, P. *et al.* 1997. Comparison of Primers and Optimization of PCR Conditions for Detection of *Cryptosporidium parvum* and *Giardia lamblia* in Water. *Applied and Environmental Microbiology*, **63**, 106-114.
- Rynearson, T.A. and Armbrust, V.E. 2000. DNA fingerprinting reveals extensive genetic diversity in a field population of the centric diatom *Ditylum brightwellii*. *Limnol Oceanography*, **45**, 1329-1340.
- Rynearson, T.A. and Armbrust, V.E. 2005. Maintenance of clonal diversity during a spring bloom of the centric diatom *Ditylum brightwellii*. *Molecular Ecology*, **14**, 1631-1640.
- Rynearson, T.A. *et al.* 2006. Spring bloom development, genetic variation, and population success in the planktonic diatom *Ditylum brightwellii*. *Limnol oceanography*, **51**, 1249-1261.
- Schlötterer and Tautz, 1992. Slippage synthesis of simple sequence DNA. *Nucleic Acids Research*, **20**, 211-215.
- Zumrraga, M. *et al.* 2005. Use of touch-down polymerase chain reaction to enhance the sensitivity of *Mycobacterium bovis* detection. *J Vet Diagn Invest*, **17**, 232-238.

A. Jesanis, San Diego State University, San Diego, CA 92182 (ajesanis-08@sandiego.edu).

T. Rynearson, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882 (rynearson@gso.uri.edu).

The Statistical Analysis of Replicate Grab Samples for Ground-truthing Benthic Habitat Maps

Megan McDonald, John King, Monique LaFrance, Dane Sheldon
Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Abstract. Benthic habitat mapping is vital to marine management; including, but not limited to: identifying marine protected areas, fisheries management, wind-farm site locations, and human impact assessments. The purpose of this study is to assist Ocean SAMP and BayMap, benthic habitat mapping programs in Rhode Island waters, by developing efficient methods for ground-truthing acoustic data via replicate grab samples. One implication of this study is the standardization of replicate grab sample numbers needed to accurately characterize different bottom types. In researching previous investigations, a historical study from Block Island Sound (Stiemle, 1982) showed, through species accumulation plots and biotope classification, that three replicate grab samples are not adequate to correctly identify a macrofaunal assemblage. This approach served as a reference point for the on-going study, which will collect 15 replicate grab samples at two stations of distinctly different bottom types in Rhode Island Sound. The 15 samples at each station will undergo the same data analysis of species accumulation plots and biotope characterizations to pinpoint the shift from additive to redundant information with successive grab samples.

1. Introduction.

The focus of this research is to determine an efficient and effective manner of ground-truthing benthic habitats within Block Island Sound (BIS) and Rhode Island Sound (RIS) through replicate grab samples. This goal will be realized using statistical analyses on two data sets to determine the number of replicate grab samples needed to adequately represent the composition of benthic macrofaunal communities. The first data set is historical, conducted by Stiemle in BIS in 1982, and is the only study using replicate samples. Stiemle chose eight stations, each representing a different bottom type, and grab sampled them in triplicate to generate the overall benthic macrofaunal assemblage of each station. The second data set collected in RIS is on-going and will consist of collecting 15 grab samples at two stations with different bottom types. The bottom types were identified from side scan sonar. The lighter area in the side scan is characterized by 90 % medium to very coarse sand, whereas the darker area is comprised of 98 % clay to fine sand bottom type.

The data from both data sets will be analyzed using the following three methods: species accumulation plots, additive biotope classification, and individual biotope classification, creating a cohesive and comparative study.

2. Materials and Methods.

The data collection methods apply to the two data sets, the historical data collected in February of 1982 by Stiemle and on-going sampling being collected by the King Lab.

2.1 Collection.

Figure 1.1- Zoomed in backscatter intensity map of new sample area in the RIS.

A Smith-McIntyre grab sampler, having an area of 0.05m^2 and a grab radius of $.16\text{m}$, was used to collect bottom samples. The predetermined bottom sites (as described above) were located and referenced by latitude and longitude. The number of replicate samples is the only variant, three samples collected in the Stiemle study and 15 in the on-going study.

The acquired sample is put in a tagged bucket, and the log was noted for latitude and longitude, sample volume, depth and cursory appearance. The collected samples were brought back to the lab, and sieved on a 1.0mm mesh. The animals were separated from the biologic detritus, sorted and counted. The species counts (i.e. abundance) serve as the basis for the following data analysis.

Figure 1.2- The map depicts the locations of the grab stations collected by Stiemle (1982), as well as of the 15 grabs collected in the dark side scan station. The light side scan station samples have yet to be collected.

The on-going study to date has been able to collect 15 replicate grabs from one of the stations, the 98% clay to fine sand. Of these replicate grab samples five grab samples have been sorted, counted and identified, these five are depicted in the on-going research preliminary findings.

2.2 Data Analysis.

The first data analysis method was the creation of species accumulation plots. The multivariate statistical analysis program, PRIMER 6, is used to formulate species accumulation plots. The option to permute 999 times is chosen to randomize the grab sample order, plotting the resulting average of all the permutations (Clark & Gorley, 2006). These plots delineate the trend of number of species found with increased sampling; the ideal plot shows an exponentially decreasing slope, going to zero. The zero slope indicates that all species are accounted for, and additional sampling garners no new information. Each species is only accounted for on the basis of absence or presence therefore the plots are to be supplemented by additional data analysis to determine to dominant species.

The second analysis was to look at the data with regard to biotope classification, in both an additive and individual process. The additive process of biotope classification indicates dominant species and develops an average biotope. The replicates are averaged to create a biotope that is an overall picture of the macrofaunal assemblage represented at that station. This analysis allows for possible relationships to be drawn between environmental data (i.e. bottom type, grain size) and the biologic data (i.e. benthic communities). To perform the additive process, the first step was to select the top three species per station, determined by a comprehensive look at all replicate grabs, selecting the three species that showed the highest summation overall. The next step was to successively add the replicate grabs of each station of those top three species,

$$\text{Grab 1} = \text{Sum 1} \quad \text{Eq. (2.1)}$$

$$\text{Grab 1} + \text{Grab 2} = \text{Sum 2} \quad \text{Eq. (2.2)}$$

$$\text{Grab 1} + \text{Grab 2} + \text{Grab 3} = \text{Sum 3} \quad \text{Eq. (2.3)}$$

to see if the dominant species shifted between summations.

Station 2

Station 3

Station 5

Figure 3.1- Species accumulation plots for stations 2, 3, and 5 of Stiemle's 1982 February data

Thirdly, the samples were examined individually for biotope classification, which dictates the degree of patchiness within a sample area to be assessed. If the dominant biotope is different for each of the grab samples

at a given station, then it is likely that the bottom type is heterogeneous. If the biotope is consistent, then it is possible it is a homogenous bottom area. The degree of patchiness will dictate the necessary number of grab samples to account for the rapid shift in benthic communities.

3. Historic Data.

3.1 Results.

Stations 2, 3 and 5 were chosen as representatives, as they were indicative of the 8 total stations.

(a) Species Accumulation: Figure 3.1.

(b) Additive Biotope Classification: Table 3.1.

(c) Individual Biotope Classification: Table 3.2.

3.2 Discussion.

Overall, the Stiemle data results show that triplicate sampling is inadequate for the purposes of ground-truthing BIS benthic habitat maps. The distinct inadequacy is most evident in the species accumulation plots. The plots (Fig. 3.1), all show a steep slope with little to no decrease in the period between the second and third sample. This result indicates that there are species yet to be collected at each of these stations and, therefore, more sampling is needed.

The additive biotope analysis showed a shift in biotope at each of the eight stations except for Station 2; (i.e., the dominant species changed between summations (Eq. 2.1-2.3)). Station 2 remained consistent throughout, showing a biotope of *Nucula proxima*. This result indicates, to determine average biotope, three replicate grab samples are enough to satisfactorily represent Station 2. Stations 1, 3, and 5 show a shift within genus (for example, Station 5 shifts to different species within the *Ampelisca* genus). Station 5 can adequately be characterized by genus, but more sampling would be required to form a determination of dominant species. The remaining stations, 4, 6, 7 and 8 show no tendency towards any species or genus, and therefore no conclusion can be drawn about dominant species.

The individual biotope classifications depict a similar trend to the additive classification. None of the eight stations show the same dominant species over the triplicate grabs. Stations 1, 3, 7 and 8 show the same dominant species in 2 of 3 grabs. Station 5 shows the same genus in 2 of the 3 grabs. This result portends the possibility that the habitats where the samples were taken are heterogeneous over a small spatial scale and additional sampling is needed to develop a better idea of community structure.

Station 2					
*Silt to Clay, 37 m	Grab 1	Grab 2	Sum 2	Grab 3	Sum 3
<i>Nucula proxima</i>	<u>42</u>	20	<u>62</u>	50	<u>112</u>
<i>Casco bigelowi</i>	23	26	<u>49</u>	0	49
<i>Nephtys incisa</i>	9	12	<u>21</u>	10	31

Station 3					
*Coarse to medium gravel, 47 m	Grab 1	Grab 2	Sum 2	Grab 3	Sum 3
<i>Byblis Serrata</i>	206	256	462	407	<u>869</u>
<i>Ampelisca Vadorum</i>	<u>218</u>	294	<u>512</u>	134	646
<i>Clymenella Zonalis</i>	68	110	178	144	322

Station 5					
*Silty sand, 19m	Grab 1	Grab 2	Sum 2	Grab 3	Sum 3
<i>Ampelisca Vadorum</i>	10	101	<u>111</u>	6	<u>117</u>
<i>Ampelisca Agassizi</i>	0	23	23	10	33
<i>Ampelisca Verrilli</i>	<u>18</u>	0	18	0	18

Table 3.1. Additive biotope tables, the bolded and underlined numbers show the dominant species of that summation. The additive process is based on Eqs. (2.1) – (2.3), these summations track the shift of average biotope. The top left hand corner of each station describes the sediment type and water depth (m) found at each station.

Station 2		
Grab 1	Grab 2	Grab 3
<i>Nucula proxima</i> - 42	<i>Casco bigelowi</i> - 26	<i>Nucula proxima</i> - 50

Station 3		
Grab 1	Grab 2	Grab 3
<i>Ampelisca vadorum</i> - 218	<i>Ampelisca vadorum</i> - 294	<i>Byblis serrata</i> - 407

Station 5		
Grab 1	Grab 2	Grab 3
<i>Ampelisca verrilli</i> -18	<i>Ampelisca vadorum</i> -101	<i>Scoloplos acutus</i> - 10

Table 3.2. - Individual biotope tables, where each grab is classified by the dominant species. The number next to each species is the abundance.

4. On-going research.

Since the results from Stiemle's data conclusively indicate that triplicate grab samples at a station are

insufficient with respect to species accumulation plots, additive biotope and individual biotope classifications, new data collection was undertaken. In order to effectively diagnose the correct number of samples needed to adequately characterize a habitat, it was decided

to take 15 grab samples at two physically different bottom types. The two stations were chosen because of their close proximity to each other and the apparent homogeneity of the physical environment (in attempt to rule out patchiness), as interpreted from the side scan mosaic (Fig.1.1).

4.1 Preliminary results.

The abundance data for the first five grab samples of the dark station were made available for analysis. These results will be built on with the remaining ten grabs but show interesting possibilities.

(a) Species accumulation plots: Figure 4.1

(b) Additive biotope classification: Figure 4.2.

(c) Individual biotope classification: Figure 4.3.

4.2 Discussion.

The species accumulation plot for the dark station showed the slope exponentially decreasing. This decrease indicates that the number of additional species found with successive samples dwindles, with a high possibility of going to zero before the requisite sample number of 15.

The additive biotope classification shows the average biotope does not shift, and remains *Ampelisca agassizi* throughout. This preliminary finding indicates that this particular soft sediment bottom type (comprised 98% of clay to fine sand) can show an average biotope in five grab samples or less.

The individual biotope classification shows a promising trend, with four of the five grabs showing

Figure 4.1- Species accumulation plot for dark station

dominance by *Ampelisca agassizi*. This result indicates that the bottom type at the dark station is fairly homogenous. In addition, when the species dominance does shift, the functional group stays the same, as both *Ampelisca agassizi* and *Byblis serrata* are tube-building amphipods.

5. Comparison of historical and current research.

A cursory look at the on-going research preliminary results in relation to the historical data shows an interesting corollary. The dark station in the new data has a similar soft sediment composition to station 2 of the historical data (clay to fine sand). The additive biotope

Dark Station									
	Grab 1	Grab 2	Sum 2	Grab 3	Sum 3	Grab 4	Sum 4	Grab 5	Sum 5
*Clay to fine sand, 149 m									
<i>Ampelisca agassizi</i>	29	18	47	63	110	61	171	0	171
<i>Byblis serrata</i>	0	0	0	0	0	0	0	6	6
<i>Lumbrieneries hebes</i>	18	13	31	13	44	15	59	8	68
								1	60

Figure 4.2- Dark station additive biotope classification

Dark Station				
Grab 1	Grab 2	Grab 3	Grab 4	Grab 5
<i>Ampelisca agassizi</i> -29	<i>Ampelisca agassizi</i> -18	<i>Ampelisca agassizi</i> -63	<i>Ampelisca agassizi</i> -61	<i>Byblis serrata</i> -68

Figure 4.3- Dark station individual biotope classification

classifications of the two show strong ties, both maintain the same dominant species throughout the summations. The individual biotopes for the two stations show a less perfect comparison but also of interest; the dominant species was the same in four out the five grabs at the new station and two of the three grabs in the historical data. Overall, these results show commonality, which develops the hypothesis that similar bottom types require the same level of ground-truthing.

6. Future research.

The future research relies on the still in-coming data, dependent on the necessary replicate number for the two bottom types lies within the first 15 grab samples. If it does not lie in that range, then it will be necessary to return to the stations and collect more grab samples, possibly 18-25 grabs. With the replicate number determined, the study can investigate if bottom types similar to the two in the on-going research are consistent with our results. This line of inquiry would dis/allow a distinct correlation between replicate sampling number and bottom type. This standardization can be applied to stations of different bottom types, collecting the determined replicate number to in/validate the idea that different bottom types require differing replicate numbers

to adequately characterize the biotic community of the station.

References.

- Clarke, K.R., Gorley, R.N., 2006. PRIMER v6 User Manual/Tutorial. PRIMER-E: Plymouth.
- Clements, A., Strong, J., & Service, M. (2009). Objective stratification and sampling-effort allocation of ground-truthing in benthic-mapping surveys. *Journal of Marine Science*, 628-637.
- Colwell, R., & Coddington, J. (1994). Estimating terrestrial biodiversity through extrapolation. *The Royal Society*, 101-118.
- Stiemle, F. (1982). The Benthic Macroinvertebrates of the Block Island Sound. *Estuarine, Coastal and Shelf Science*, 1-16.
- Webster, R., & Oliver, M. (2007). *Geostatistics for Environmental Scientists*. West Sussex, England: Wiley.

J. King and M. LaFrance, Graduate School of Oceanography,
University of Rhode Island, Narragansett, RI 02882.
(jking@gso.uri.edu, lafrance.monique@gmail.com)
M.McDonald, University of Southern California, Los Angeles,
CA, 90089. (meganmcd@usc.edu)

Effect of Copepods on Phytoplankton Growth and Heterotrophic Protist Grazing Rates in Narragansett Bay

Andrea Reis, Caitlyn Lawrence, Susanne Menden-Deuer

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

Abstract. Phytoplankton are the primary producers of oceanic ecosystems. As such, phytoplankton biomass, affected by factors including predation and nutrient limitation, determines the amount of energy provided to higher trophic level organisms in estuarine ecosystems such as Narragansett Bay. Calbet and Landry (2004) concluded, based on data from a meta-study, that heterotrophic protists are the dominant predators of phytoplankton, consuming on average 60% of daily primary production in estuarine systems. Traditionally, mesozooplankton, such as copepods, have been thought to be dominant grazers on plankton, including diatoms and heterotrophic protists. This study explored how copepods affect phytoplankton growth rates and heterotrophic protist grazing rates in Narragansett Bay. Seven dilution experiments were conducted over three months, from June to August 2011, with seawater filtered through a 200-micron mesh to remove copepods. Five and fifteen copepods were added to 1 L seawater samples to determine the change in phytoplankton growth and heterotrophic protist grazing rates. Each experiment was treated with and without the addition of nutrients to ameliorate effects of nutrient limitation. Phytoplankton growth rates were calculated by measuring changes in chlorophyll *a* concentration, a phytoplankton biomass indicator, from T_0 to T_f (24 hr incubation in simulated in situ conditions). Average phytoplankton growth rates were 1.73 day^{-1} (± 0.07) for non-nutrient treated samples and 1.96 day^{-1} (± 0.04) for all copepod treatments. Average heterotrophic protist grazing rates ranged from 0.35 day^{-1} to 1.71 day^{-1} (± 0.07) with different treatments. A paired t-test showed a significant difference in heterotrophic protist grazing rates and phytoplankton growth rates between nutrient treated and untreated samples, indicating natural nutrient limitation in Narragansett Bay. Alteration in copepod density did not have a significant effect on phytoplankton growth rates, while there was a 35% decrease in average protistan grazing rate with the addition of 5 copepods, presumably through consumption of heterotrophic protists by copepods, thus an indication of a trophic cascade. These results indicate that average concentrations of copepods graze preferentially on heterotrophic protists while increased concentrations of copepods graze on both heterotrophic protists and phytoplankton. Despite the change in protistan grazing rate, the phytoplankton growth rates were not significantly altered, indicating that the change in grazing preference is not a significant factor in limiting phytoplankton blooms.

1. Introduction.

As primary producers in the ocean, phytoplankton provide energy for oceanic ecosystems. The amount of biomass they produce, determines how much energy can be transferred to other organisms that cannot produce their own energy. In order to undergo photosynthesis to produce energy and grow, phytoplankton require nutrients, including nitrate and phosphate, and light. Phytoplankton growth is controlled not only by nutrient availability but also by top-down predation. Phytoplankton blooms, large increases in net growth of phytoplankton, occur when phytoplankton growth rates exceed predation (Behrenfeld 2010). Bloom magnitude is reduced as nutrients are depleted, thus limiting growth, and predation increases.

Microzooplankton, or heterotrophic protists, have been suggested to be the dominant consumers of phytoplankton (Sherr & Sherr 2009). Calbet and Landry (2004) pooled data from different research projects on the consumption of phytoplankton to create a meta-study. From this meta-study, they concluded that heterotrophic protists consume an average of 60% of daily primary

production in estuarine systems like Narragansett Bay. Due to the large magnitude of protistan grazing, it was thought that protists may be able to prevent formation of phytoplankton blooms. Conversely, studies such as Sherr & Sherr (2009) reasoned that the protists are unable to suppress a phytoplankton bloom because protistan growth rates are low before bloom conditions when the prey concentrations are also low. Protist populations are also top-down controlled by predators and would not reach the necessary abundance levels to graze down a bloom before it forms (Sherr & Sherr 2009). Therefore, formation of phytoplankton blooms is not expected to be prevented despite considerable grazing pressure from heterotrophic protists.

Copepods, a type of mesozooplankton, are predators of not only phytoplankton but also heterotrophic protists. These mesozooplankton were once thought to be dominant grazers on plankton, including diatoms and heterotrophic protists. Studies conducted on copepods have shown a trophic link between protists and copepods, copepods preferring larger heterotrophic protists (Levinsen, et al. 2000). Studies have also been conducted on the trophic link between copepods and phytoplankton. In Narragansett Bay, Durbin et al. (1983) found that the

dry weight and egg production of *Acartia tonsa*, the dominant calanoid copepod during the summer months, was correlated to the seasonal concentration of phytoplankton in the bay. Overall, the trophic links between zooplankton, protists and phytoplankton are important to the trophic cascade of the food web because zooplankton and protists provide the link between the phytoplankton and higher level organisms that cannot eat the phytoplankton. Therefore, it is important to understand how zooplankton affect plankton dynamics. In order to determine if copepods affect phytoplankton and heterotrophic protist community dynamics, this study used dilution experiments to compare phytoplankton growth and heterotrophic protist grazing rates in seawater samples, from Narragansett Bay, with and without the presence of copepods.

One drawback to using dilution experiments is that different growth media can affect the growth increases in dilution experiments (Landry & Hassett 1982). A deficiency in nutrients can skew calculated grazing rate. This is because in less dilute samples more phytoplankton are competing for the nutrients. Therefore, phytoplankton growth in these samples will not be as maximized as in more dilute samples, skewing the linear relationship between growth and dilution level and also the grazing

rate derived from the change in phytoplankton growth. Narragansett Bay is known to be nutrient limited (Nixon et al. 2009). Thus in this study, each experiment conducted contained a set of samples without added nutrients and a set of samples with added nutrients to determine how copepods would affect protistan grazing rates without nutrient limitation. From the nutrient amended samples, protistan grazing rates and phytoplankton growth rates with and without the presence of copepods were compared to determine the impact of copepods on plankton community dynamics.

2. Methods.

2.1. Dilution Experiments.

Seven dilution experiments, as described by Landry and Hassett (1982), were conducted from June 22 to August 4, 2011, using surface seawater collected from Station 2 in the Narragansett Bay Time Series. A 0.2 micron filter was used to create filtered seawater which was then mixed with whole seawater to create dilutions of whole seawater. Two dilution points, 10% and 100% whole seawater were used, based on research by Landry et al. (2008) and Strom & Fredrickson (2008) which found that there was no significant difference between results from a 2-point dilution versus a multi-level dilution experiment.

Figure 1. Average protistan grazing rate for 4 triplicated copepod addition experiments on June 30, July 11, July 27 and August 4 of 2011. White bars indicate samples without added nutrients that were sieved through 200-micron mesh, light gray bars indicate samples with added nutrients that were sieved through 200-micron mesh, medium gray bars indicate samples with added nutrients that were sieved through 200-micron mesh with 5 *A. tonsa* added, and black bars indicate samples with added nutrients that were sieved through 200-micron mesh with 15 *A. tonsa* added. Error bars indicate standard deviation between triplicate measures. Asterisks indicate significant difference between results. Average concentrations of *Acartia tonsa* (5^{-L}) decreased protistan grazing rates by an average of 35%.

Figure 2. Average protistan grazing rate for 3 triplicated non-sieve experiments on June 22, July 5 and July 18 of 2011. Light gray bars indicate samples without added nutrients that were sieved through 200-micron mesh, medium gray bars indicate samples with added nutrients that were sieved through 200-micron mesh and black bars indicate samples with added nutrients that were not sieved. Error bars indicate standard deviation between triplicate measures. Asterisks indicate significant difference between results. Natural assemblage of copepods did not have a significant effect on protistan grazing rates.

Figure 3. Average phytoplankton growth rate for 4 triplicated copepod experiments. White bars indicate samples without added nutrients that were sieved through 200-micron mesh, light gray bars indicate samples with added nutrients that were sieved through 200-micron mesh, medium gray bars indicate samples with added nutrients that were sieved through 200-micron mesh with 5 *A. tonsa* added, and black bars indicate samples with added nutrients that were sieved through 200-micron mesh with 15 *A. tonsa* added. Error bars indicate standard deviation between triplicate measures. Asterisks indicate significant difference between results. Average and increased concentrations of *Acartia tonsa* did not significantly affect phytoplankton growth rates.

In each set of experiments, a 200-micron mesh was used to remove large copepods from samples. Natural copepod assemblage experiments were conducted in which half the samples were not sieved through the 200-micron mesh, thus retaining the natural copepod concentrations from the bay. In copepod addition experiments, after the seawater was filtered through the 200-micron mesh, 5 *A. tonsa* were added to 1 L samples to simulate average concentrations in the bay while 15 *A. tonsa* were added to 1 L samples to simulate increased concentrations of copepods. In each experiment, nutrients, phosphate and nitrate, were added to a final concentration of 2 μ M and 10 μ M respectively, to one set of triplicate samples of 10% and 100% dilutions.

Triplicate samples of 10% and 100% dilutions each with and without nutrients were poured into 1 L polycarbonate bottles for each experiment. For the non-sieve experiments, triplicate samples of 10% and 100% dilutions were also made. For the copepod addition experiments, triplicate samples of 100% whole seawater with 5 and 15 *A. tonsa* were made. All non-sieved and copepod addition samples were given the same concentration of nutrients as above. Bottles were placed

on the 'plankton wheel' full of Narragansett Bay seawater that was constantly rotated and had in situ light and temperature conditions. After 24 hours, bottles were removed from the plankton wheel to be filtered for chlorophyll which was used as an indicator of phytoplankton biomass.

Triplicates of initial (T_0) chlorophyll samples were taken when the dilutions were poured and gathered through vacuum filtering on GF/F filters. Triplicates of final (T_f) chlorophyll samples were taken from each bottle. Filters were placed in tubes with 10 mL of acetone, covered in aluminum foil to block out light, and stored in a -20°C freezer for 24 hours. After 24 hours, the samples were centrifuged, vortexed, and run on a Turner AUTO fluorometer. Phytoplankton growth was determined using the equation, $k = \ln(P_f/P_0)/t$, in which k represents the net phytoplankton growth rate, P_0 and P_f are the initial and final chlorophyll concentrations, and t is time, which in this experiment is one day. The protistan grazing rates were calculated as the difference between k of the two dilution samples for each treatment. For the copepod addition experiments, in which only 100% dilutions were made, the 10% net growth rate from the non-copepod addition with nutrients was used to calculate the protistan grazing rates.

Figure 4. Average phytoplankton growth rate for 3 triplicated non-sieve experiments. Light gray bars indicate samples without added nutrients that were sieved through 200-micron mesh, medium gray bars indicate samples with added nutrients that were sieved through 200-micron mesh and black bars indicate samples with added nutrients that were not sieved. Error bars indicate standard deviation between triplicate measures. Asterisks indicate significant difference between results. Natural assemblage of copepods did not alter phytoplankton growth rates significantly.

2.2. Specimen Counts.

Time initial and time final cell counts of specimens were made by storing seawater samples in 3% Acid Lugol's. 50 mL of each sample were settled in Utermöhl chambers for 24 hours. Then samples were counted for heterotrophic protists using a Nikon Diaphot 300 inverted light microscope. The different species of heterotrophic protists counted included aloricate ciliates, tintinnids, *Gyrodinium* spp., *Protoperidinium* spp., and *Dinophysis* spp.

Similarly, initial and final cell counts were made for phytoplankton species from samples without copepods for each experiment, and counted using 1mL of sample and a Nikon Eclipse E800 light microscope. The species that were most abundant throughout the course of experiments were counted: *Ceratulina pelagica*, *Chaetoceros* spp., *Chaetoceros socialis*, *Cylindrotheca closterium*, *Prorocentrum gracile*, *Skeletonema* spp., *Thalassiosira* spp., *Leptocylindrus minimus*, *Pseudo-nitzschia* spp., *Eucampia zodiacus*, and *Dactyliosolen blavyanus*. Phytoplankton counts and heterotrophic counts were then converted into net growth rates for each species.

2.3. Data Analysis.

Paired t-tests were used to determine if the addition

of the natural assemblage of copepods significantly altered either protistan grazing rates (day^{-1}) or phytoplankton growth rates (day^{-1}). Differences between phytoplankton growth and protistan grazing rates of non-nutrient added samples and nutrient added samples were also analyzed using paired t-tests. A single factor analysis of variance (ANOVA) was used to determine if there were significant differences ($p < 0.05$) among protistan grazing rates and phytoplankton growth rates of the different copepod additions. Two-way ANOVAs with a significance level of 0.05 were also used to determine if there was a significant change in grazer community over the dilution experiments and if there was a significant change in grazer community based on copepod additions.

3. Results and Discussion.

3.1. Phytoplankton Growth Rates and Protistan Grazing Rates.

Nutrient limitation skewed protistan grazing rates, making the rates appear larger than nutrient-added grazing rates (Figures 1 and 2), and caused a decrease in phytoplankton growth rates (Figure 3). Average protistan grazing rates for samples with nutrients ranged from 0.35 to 0.54 ± 0.08 (day^{-1}) while average phytoplankton growth

Figure 5. Change in protistan net growth rates from T_0 to T_f for 2 natural copepod assemblage experiments on July 5 and July 18 of 2011. All samples were 100% whole seawater. Solid red bars indicate samples from July 5th that were sieved through 200-micon mesh, crosshatched red bars indicate samples from July 5th that were not sieved, solid blue bars indicate samples from July 18th that were sieved through 200-micron mesh, and crosshatched blue bars indicate samples from July 18th that were not sieved. Grazer community did not change significantly over the course of these experiments.

rates for samples with nutrients ranged from $1.84 \pm 1.97 \pm 0.05$ (day^{-1}) (Table 1). For samples without added nutrients, the average protistan grazing rate was 1.46 ± 0.07 (day^{-1}) and the average phytoplankton growth rate was 1.75 ± 0.06 (day^{-1}) (Table 1). Nutrient limitation was most apparent when examining the difference in growth between the 100% whole seawater samples with and without the addition of nutrients. The 100% whole seawater samples without nutrients showed an average increase in chlorophyll of about 95% less than that of 100% whole seawater samples with nutrients. Each experiment showed evidence of nutrient limitation in Narragansett Bay which is a factor in controlling the net phytoplankton biomass available to higher level organisms.

Sieving the seawater samples did not significantly affect the protistan grazing and phytoplankton growth rates that occurred in the bay at the time of these experiments. No significant differences were found in protistan grazing rates between sieved and non-sieved samples (Figure 2) and no significant differences were found in phytoplankton growth rates of these samples as well (Figure 4). Therefore natural copepod assemblage

did not significantly alter net phytoplankton biomass produced over the course of the experiments.

There was a copepod density-dependent effect on protistan grazing rate that suggests a switch from grazing on heterotrophic protists at lower copepod densities to grazing on both heterotrophic protist and phytoplankton prey at higher copepod densities. Samples with average copepod concentrations ($5 A. tonsa L^{-1}$) showed a 35% decrease in protistan grazing rate (Figure 1). However, there was no significant difference in phytoplankton growth rates between *A. tonsa* additions (Figure 3). No significant difference was found for protistan grazing or phytoplankton growth rates when 0 *A. tonsa* L^{-1} and 15 *A. tonsa* L^{-1} were added. This would suggest that the 5 *A. tonsa* L^{-1} samples are grazing down the protists preferentially, which lowers the grazing upon the phytoplankton while the 15 *A. tonsa* L^{-1} are grazing on both prey, the protists and phytoplankton, restoring the grazing rate upon the phytoplankton. Despite this change in grazing however, phytoplankton growth rates were not significantly altered in any of the copepod additions. As a result, average and increased concentrations of *Acartia tonsa* did not significantly affect the net amount of

Figure 6. Change in protistan net growth rates from T_0 to T_f for 2 sets of copepod addition experiments on July 27 and August 4 of 2011. All samples were 100% whole seawater, sieved through 200-micon mesh. Red bars indicate samples from July 27th while blue bars indicate samples from August 4th. Solid bars indicates that no *A. tonsa* were added, striped bars indicates that 5 *A. tonsa* were added and crosshatched bars indicate that 15 *A. tonsa* were added. Grazer community was not significantly altered over the course of experiments, or by copepod additions.

Figure 7. Change in net growth rates of prey species for six dilution experiments from June 30 through August 4 of 2011. Each colored/crosshatched bar indicates a different sample date. All samples were 100% whole seawater, sieved through a 200-micron mesh with no *A. tonsa* added. Species with negative net growth rates over the course of experiments may have been preferred prey by heterotrophic protists.

biomass that was being produced each day.

3.2. Grazer and Prey Community Composition.

Copepods did not cause a significant shift in grazer community. The net growth rate of heterotrophic protists of each species counted did not significantly change over the course of the experiments. The grazer community also was not significantly affected by the different copepod treatments. This indicates that the different concentrations of *A. tonsa* did not significantly alter heterotrophic protist populations. While the change in net growth rate of heterotrophic protists over the experiments was not significantly different, the changes in each species' growth rates were averaged for each type of experiment (Figures 5 and 6). On average the dinoflagellates, *Dinophysis* spp., *Gyrodinium* spp., and *Prorocentrum* spp. net growth rates decreased from the beginning to end of the experiments while aloricate ciliates and tintinnids increased in net growth rates. Changes in net growth rates could have been due to grazing or the species' ability to grow within the environmental conditions of the bottles. These small changes in heterotrophic protist net growth rates did not likely have a significant effect upon phytoplankton community dynamics.

A shift in phytoplankton community species composition over the course of the experiments may indicate that heterotrophic protists exhibited prey preference of phytoplankton species (Figure 7). A decrease in net growth rate of a species over each experiment indicates mortality of the cells, whether by predation or other causes. Species with negative change in net growth rate may indicate prey preference of heterotrophic protists. *Thalassiosira* spp., *Chaetoceros* spp. net growth rates decreased the most on average over the course of the experiments, indicating they may have been grazed down. *Skeletonema* spp. net growth rate decreased on July 27th and August 4th, however in earlier dates there was a large bloom of *Skeletonema* spp. which may mask any appearance of grazing. Species such as *Eucampia zodiacus* and *Ceratulina pelagica* increased in net growth (day⁻¹) during most of the experiments, indicating that they may have been grazed the least, or less preferred by heterotrophic protists. Phytoplankton counts should be conducted on samples with copepods in the future to compare the species' change in net growth rates with the samples without copepods and discover if copepods show prey preference. While these results suggest there is prey preference it cannot conclusively be called prey preference because it was too difficult to have

a control in this part of the experiment. A good control would be a sample of phytoplankton with no heterotrophic protists to see difference the predators make upon the phytoplankton growth rates. It is also unknown if some phytoplankton species were able to grow more easily than others based upon the bottled environment. More work would be required to further support the implication that heterotrophic protists showed prey preference. The addition of copepod preference data would allow conclusions to be drawn as to whether copepods may affect phytoplankton community composition.

4. Conclusion.

The natural assemblage of copepods found in Narragansett Bay, through the natural copepod assemblage experiments, did not significantly affect protistan and phytoplankton community dynamics. Phytoplankton blooms are therefore not likely to be prevented or controlled by average concentrations of copepods present in Narragansett Bay. In the copepod addition experiments, average concentrations of *Acartia tonsa* appeared to prefer heterotrophic protists over phytoplankton prey, decreasing the protistan grazing rate by 35%, while increased concentrations of *Acartia tonsa* appeared to prey on both protists and phytoplankton, which resulted in a decreased protistan grazing rate by only 0.2%. Copepods did not show specific prey preference for the different types of heterotrophic protists. There did appear to be prey preference by heterotrophic protists which could impact the individual prey species' growth rates, however, we do not know if there is phytoplankton prey preference by copepods. If increased concentrations of *Acartia tonsa* occurred in Narragansett Bay, we would expect to see a small increase in grazing rates upon phytoplankton but minimal change in phytoplankton growth rates and community. Increased concentrations of *Acartia tonsa* would not likely be able to prevent or control a phytoplankton bloom in Narragansett Bay.

Acknowledgements. I would like to thank Kathy Donohue, Rob Pockalny, and Kim Carey for running the 2011 SURFO program and I would like to thank Caitlyn Lawrence and

Susanne Menden-Deuer for guiding me in my summer research project. I would also like to thank NSF for funding this program.

References.

- Behrenfeld, M.J. 2010. Abandoning Svedrup's Critical Depth Hypothesis on phytoplankton blooms. *Ecology* **91**: 977-989.
- Calbet, A. and Landry, M.R. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnology and Oceanography* **49**: 51-57.
- Durbin, E.G., Durbin, A.G., Smayda, T.J., and Verity, P.G. 1983. Food limitation and production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. *Limnology and Oceanography* **28**: 1199-1213.
- Landry, M.R. and Hassett, R.P. 1982. Estimating the grazing impact of marine micro-zooplankton. *Marine Biology* **67**: 283-288.
- Landry, M.R., Brown, S.L., Rii, Y.M., Selph, K.E., Bidigare, R.B., Yang, E.J., and Simmons, M.P. 2008. Depth-stratified phytoplankton dynamics in Cyclone *Opal*, a subtropical mesoscale eddy. *Deep-Sea Research Part 11* **55**: 1348-1359.
- Levinsen, H., Turner, J.T., Nielson, T.G., and Hansen, B.W. 2000. On the trophic coupling between protists and copepods in arctic marine ecosystems. *Marine Ecology Progress Series* **204**: 65-77.
- Nixon, S.W., Fulweiler, R.W., Buckley, B.A., Granger, S.L., Nowicki, B.L. and Henry, K.M. 2009. The impact of changing climate on phenology, productivity, and benthic-pelagic coupling in Narragansett Bay. *Estuarine, Coastal and Shelf Science* **82**: 1-18.
- Sherr, E.B. and Sherr, B.F. 2009. Capacity of herbivorous protists to control initiation and development of mass phytoplankton blooms. *Aquatic Microbial Ecology* **57**: 253-262.
- Strom, S.L. and Fredrickson, K.A. 2008. Intense stratification leads to phytoplankton nutrient limitation and reduced microzooplankton grazing in the southeastern Bering Sea. *Deep-Sea Research* **55**: 1761-1774.

A. Reis, Worcester Polytechnic Institute, Worcester, MA 01609-2280 (areis@wpi.edu).

C. Lawrence and S. Menden-Deuer, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882 (caitlynl@gso.uri.edu, smenden@gso.uri.edu).

Dietary Guild Structure of the Fish Community in Rhode Island and Block Island Sounds

Emily Rhoades, Anna Malek, Jeremy Collie

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

Abstract. Dietary guild analysis groups species based on dietary overlap, identifying functionally similar species within a fish community. This study investigated the dietary guild structure of the demersal fish community in Rhode Island and Block Island Sounds. Bottom trawl surveys and stomach-content analysis were used to determine the diet composition of 15 predator species. Six predator species were divided into size classes to account for ontogenetic diet shifts resulting in 21 total predator categories. The contents of 980 stomachs were identified to the lowest possible taxon and the prevalence of 36 prey groups was determined as a proportion of total stomach contents. Multivariate analysis was applied to the diet composition data, resulting in 6 significant dietary guilds: planktivores, benthivores, generalists, crab eaters, piscivores, and amphipod, shrimp, and fish eaters. Dietary guilds were distinguished by primary prey type and foraging location. Results suggest that ontogenetic diet shifts do not lead to a shift in guild membership throughout life history. The results of this work provide a better understanding of the complex relationships amongst the demersal fish of Rhode Island and Block Island Sounds. For example, findings indicate that competitive interactions could occur within the benthivore guild between scup, a seasonally proliferating species, and winter flounder, a species of concern. Competition for resources between these two species may limit the efficacy of stock recovery efforts. Black sea bass and smooth dogfish, members of the crab eater guild, may also exhibit such competitive interactions. In this way, dietary guild analysis can inform ecosystem based management by reducing a complex and highly connected ecosystem into groups of organisms that exploit resources in a similar fashion. Reduction in dimensionality of the community improves management efforts by limiting the focus to one or more guilds instead of many individual species.

1. Introduction

A guild is a group of species that exploit the same class of environmental resources in a similar way, and thus overlap significantly in their niche requirements (Root 1967). By investigating dietary guilds, species with similar dietary composition, which potentially exhibit strong competitive interactions, are identified. This is a useful first step in defining functionally similar groups of species within an ecosystem (Garrison and Link 2000).

Studies of resource overlap have generally not taken ontogenetic diet shifts into account, though shifts in diet often occur in association with changes in predator morphology and habitat throughout the life history of a species. Different size-classes of a species may play functionally different roles within an ecosystem; thus by defining multiple size-classes to be used in dietary guild analysis, different sizes of one species may fall into different guilds (Garrison and Link 2000).

Guilds can be used to inform ecosystem based fisheries management (Garrison and Link 2000). This form of management is needed for sustainability and continued availability of marine resources (Pauly and Chuenpagdee 2007, Malek et al. 2010). High functional redundancy within certain guilds allows for ecosystem function to be maintained, though abundance of individual

guild members may fluctuate. Thus by identifying dietary guilds, management efforts can define catch levels for functional guilds, rather than single species, and focus on preserving ecological roles (Auster and Link 2009).

While dietary guild analysis has previously been performed on fish species of the Northeast United States continental shelf ecosystem (Garrison and Link 2000), it has not yet been applied to the demersal fish species of Rhode Island Sound (RIS) and Block Island Sound (BIS). This is an ecologically and economically important area, yet it has been relatively understudied until recently because it is too far offshore for state surveys, and too far inshore for federal surveys (Malek et al. 2010). Long-term studies of Narragansett Bay, which has a similar community composition to that of RIS and BIS, have indicated a shift from benthic to pelagic species over the last four decades (Collie et al. 2008). For example winter flounder, once a dominant species in Narragansett Bay, RIS, and BIS reached a peak abundance in 1979, but has declined since then, being replaced in abundance by pelagic species such as scup and butterfish (Jeffries and Terceiro 1985, Collie et al. 2008). This decrease in winter flounder abundance and shift towards pelagic species can be attributed largely to increases in sea surface temperature and changes in chlorophyll concentration (Collie et al. 2008). By investigating the dietary guilds of the area we can determine sources of competition within guilds that may have contributed to this observed shift as

well.

By analyzing the dietary guild structure of fish in RIS and BIS, this study identifies complex relationships amongst members of a previously understudied ecosystem, and possible sources of competition in the area. This study uses similar methods to those used by Garrison and Link (2000), confirming the validity of their groupings.

2. Methods

2.1. Sources of Data

Data used in this study were obtained from bottom trawl surveys conducted in conjunction with the Northeast Monitoring and Assessment Program in fall 2009 and fall 2010. All sampling was performed aboard the 90' F/V *Darana R* using a 400 cm, three-bridle, four-seam bottom trawl, paired with a set of Thyboron, Type IV 6" trawl doors. A 12 cm stretch mesh cod-end was used with a 2.43 cm knotless nylon liner so as to collect both juvenile and adult fishes. This gear was towed for 20 minutes at a target speed of 3.1 knots, resulting in a tow distance of approximately 1.0 nautical mile.

The catch was sorted by species and size class. Weights, counts, and individual lengths were recorded for all species collected. From each catch a random subsample of 5 fish per size class per species was collected for diet analysis. Fish stomachs were extracted immediately after capture, and preserved in Normalin, a non-carcinogenic preservative. In the laboratory, the contents of each stomach were identified to the lowest possible taxon and the wet weight of each prey group was recorded as a proportion of the total stomach contents.

2.2. Dietary guild analysis

Data from 15 predator species and 980 stomach samples were used in the dietary guild analysis (Table 1). Predator species were divided into size classes to account for known ontogenetic diet shifts (Bowman and Michaels 1984, Garrison and Link 2000). Only predator categories with adequate stomach samples (>10 stomachs) were included in the analysis, resulting in 21 predator categories (Table 1).

Prey items were grouped based on dietary prevalence and digestive state. Abundant prey items were grouped at lower taxonomic levels, while less abundant items were grouped at higher levels. The resulting prey classification contained 36 categories (Table 2).

The Schoener (1970) similarity index (Equation 1) was used to assess the dietary overlap, D_{ij} , between predator pairs (Garrison and Link 2000):

$$(1) D_{ij} = 1 - 0.5 (\sum |p_{ik} - p_{jk}|)$$

where p_{ik} is the mean proportional mass of prey k in predator i and p_{jk} is the mean proportional volume of prey k in predator j . The statistical software package, PRIMER 6.0, was used to create a resemblance matrix containing the diet similarity (D_{ij}) of each predator category pair.

Figure 1. Multi-dimensional scaling plot (MDS plot) depicting the pattern of diet composition amongst demersal fish species and size classes in Rhode Island Sound and Block Island Sound. Each point represents one predator category, and the distance between points is an indication of similarity of diet composition of the predators, with closer points being more similar.

Figure 2. Cluster analysis groupings based on similarity of predator category diet composition. Significant dietary guilds are defined by the last solid black branching point. Members of the planktivore guild are indicated by green triangles (SIMPROF: $\pi = 1.27$, $p = 0.496$), members of the benthivore guild are indicated by purple circles (SIMPROF: $\pi = 1.96$, $p = 0.185$), members of the generalist guild are indicated by red diamonds (SIMPROF: $\pi = 3.93$, $p = 0.123$), members of the crab eater guild are represented by blue triangles (SIMPROF: $\pi = 0$, $p = 0.636$), members of the amphipod, shrimp, and fish eater guild are represented by grey plus signs (SIMPROF: $\pi = 0$, $p = 1$), and members of the piscivore guild are represented by light blue squares (SIMPROF: $\pi = 2.82$, $p = 0.136$).

Figure 3. Multi-dimensional scaling plot (MDS plot) depicting the pattern of dietary guilds amongst demersal fish species and size classes in Rhode Island Sound and Block Island Sound. Each point represents one predator category, and the distance between points is an indication of similarity of diet composition of the predators, with closer points being more similar. Green triangles represent members of the planktivore guild, purple circles represent benthivore guild members, red diamonds represent generalists, blue triangles represent crab eater guild members, grey pluses represent amphipod, shrimp and fish eaters, and light blue squares represent piscivore guild members.

Figure 4. Mean proportional composition (by weight) of prey categories in the planktivore guild.

derived from the dietary resemblance matrix to ordinate the predator categories in two dimensions such that the relative distance between points represents the degree of dietary similarity between predator categories (Clarke and Gorley 2006). Accordingly, points that are close together represent predator categories that have similar diet composition, while points that are far apart indicate predator categories with dissimilar diets (Figure 1).

The CLUSTER function in PRIMER 6.0 was used to group the predator categories based on similarity of diet composition (Figure 2). The CLUSTER analysis was carried out with the SIMPROF routine, which determines statistically significant dietary clusters within an a-priori ungrouped set of samples. The basis for the SIMPROF test is the summed absolute distances (π) between a similarity profile created from the analysis, and a simulated similarity profile based on the possibility that groupings are the result of chance occurrence. A significance level ($p < 0.05$) indicates whether the difference between the real and simulated π is large enough that the clustering is significant.

3. Results

The CLUSTER and SIMPROF analysis defined six groups of predator categories with significant dietary overlap. These groups were interpreted as individual dietary guilds and were broadly categorized based on the dominant prey types and foraging locations shared by the guild members (Figure 3).

The planktivore guild clustered at 79% similarity and consisted of American shad, alewife, Atlantic herring, and butterfish (SIMPROF: $\pi = 1.27$, $p = 0.496$). A high proportion of zooplankton in their diets distinguished these species. Animal remains, the prey category that contained zooplankton in this study, accounted for 73% of the diets of these species (Figure 4).

The benthivore guild clustered at 68% similarity and consisted of small and medium scup, and small and medium winter flounder (SIMPROF: $\pi = 1.96$, $p = 0.185$). Their diets consisted mainly of bottom dwelling prey, including amphipods, isopods, echinoderms, polychaetes, and animal remains (Figure 5).

The generalist guild consisted of little skates, and small and medium winter skates and clustered at 53% similarity (SIMPROF: $\pi = 3.93$, $p = 0.123$). These species relied on a wide variety of prey types, including both benthic invertebrates and pelagic fish and squid, and prey items from 31 of the 36 prey categories used in this study (Figure 6).

The crab eater guild consisted of black sea bass and smooth dogfish, which clustered at 46% similarity (SIMPROF: $\pi = 0$, $p = 0.636$). Together, cancer crabs and unclassified decapod crabs accounted for 59% of their diets, while bony fish contributed to another 17% (Figure 7).

The amphipod, shrimp and fish eater guild clustered at 67% and consisted of small and medium silver hake

A Multi-dimensional scaling plot (MDS plot) was

(SIMPROF: $\pi = 0$, $p = 1$). Their diets consisted of 23% amphipods, 34% shrimp, 17% unclassified crustaceans (likely amphipods or shrimp), and 17% unidentified fish (Figure 8).

The piscivore guild clustered at 45% similarity and included small and medium bluefish, medium and large summer flounder, spiny dogfish, and weakfish (SIMPROF: $\pi = 2.82$, $p = 0.136$). Fish accounted for 64% of the diets of these species, while squid contributed an average of 18% and were present in the diets of all the species except weakfish (Figure 9).

4. Discussion

Dietary guild analysis of 15 predator species and 21 predator categories of demersal fish in RIS and BIS resulted in 6 significant dietary guilds. The species and dominant prey types within guilds were similar to those found in a previous study of the Northeast Atlantic continental shelf ecosystem, which includes the RIS and BIS study area (Garrison and Link 2000). Unlike this previous study, ontogenetic diet shifts did not have an effect on guild membership. This is due in part to a lack of data contributing to the analysis, resulting in a lack of predator species and size classes represented. In this study, there was only enough data to represent two size classes in each of six predator species, and in 5 of these 6 species only small and medium categories were represented. As a result this study did not account for diet shifts at larger sizes where changes in diet are known to occur. Future analyses should include more data so that size classes are represented in more than 6 fish species, and larger size categories are represented, instead of just two small categories per species. Results would be more accurate if more than 10 stomachs contributed to each predator category. It would also be interesting to compare this analysis of fall dietary data, with a dietary guild analysis performed on stomachs collected in the spring.

The six dietary guilds found in this study indicate groups of species, which might be competing over resources in RIS and BIS. It has been suggested that competition for food resources is not an important factor in the structure of the larger Northeast US continental shelf, of which RIS and BIS are a part. For example, piscivores can switch between a wide variety of prey, depending on which is most abundant (Garrison and Link 2000). In a highly productive ecosystem, where a significant decrease in abundance of all of these prey types is unlikely, competitive exclusion is less likely to occur. Between species such as scup and winter flounder, which are known to be shifting in abundance, understanding that they both are members of the benthivore guild is more valuable. That these species may exhibit competition over resources, however slight this competition is, contributes to our understanding of observed shifts in abundance, and reveals a potential limiting factor in stock recovery efforts. Similarly,

Figure 5. Mean proportional composition (by weight) of prey categories in the benthivore guild.

Figure 6. Mean proportional composition (by weight) of prey categories in the generalist guild.

Figure 7. Mean proportional composition (by weight) of prey categories in the crab eater guild.

Figure 8. Mean proportional composition (by weight) of prey categories in the amphipod, shrimp, and fish eater guild.

dogfish and black sea bass have a significant overlap in diet can inform fisheries management. In this case, a commercially valuable species, black sea bass, fills a functionally similar ecological role to smooth dogfish, which is not fished commercially.

This knowledge allows us to predict that should black sea bass be overfished, smooth dogfish will increase in abundance so that the functional roll of crab eaters in the ecosystem is maintained. Future studies could test whether competition is in fact occurring between guild members in RIS and BIS. To do this, in addition to finding dietary overlap between species, one must demonstrate that there is a shortage of available prey, and that the diet of one competing species will change with the loss of the species it is supposedly in competition with (Hixon 1980).

Figure 9. Mean proportional composition (by weight) of prey categories in the piscivore guild.

knowing that members of the crab eater guild, smooth

Common Name	Scientific Name	Length (mm)	Number of Individuals
Alewife	<i>Alosa pseudoharengus</i>	150 - 200	21
American shad	<i>Alosa sapidissima</i>	191 - 220	11
Atlantic herring	<i>Clupea harengus</i>	95 - 214	56
Black sea bass	<i>Centropristis striata</i>	105 - 565	42
Small bluefish	<i>Pomatomus saltatrix</i>	125 - 245	13
Medium bluefish	<i>Pomatomus saltatrix</i>	405 - 710	19
Butterfish	<i>Peprilus triacanthus</i>	71 - 182	43
Little skate	<i>Leucoraja erinacea</i>	220 - 295	74
Small scup	<i>Stenotomus chrysops</i>	57 - 255	263
Medium scup	<i>Stenotomus chrysops</i>	260 - 340	32
Small silver hake	<i>Merluccius bilinearis</i>	90 - 199	28
Medium silver hake	<i>Merluccius bilinearis</i>	200 - 339	42
Smooth dogfish	<i>Mustelus canis</i>	670 - 905	16
Spiny dogfish	<i>Squalus acanthias</i>	616 - 785	38
Medium summer flounder	<i>Paralichthys dentatus</i>	300 - 395	18
Large summer flounder	<i>Paralichthys dentatus</i>	401 - 705	56
Weakfish	<i>Cynoscion regalis</i>	130 - 395	11
Small winter flounder	<i>Pseudopleuronectes americanus</i>	180 - 209	12
Medium winter flounder	<i>Pseudopleuronectes americanus</i>	210 - 445	128
Small winter skate	<i>Leucoraja ocellata</i>	210 - 305	19
Medium winter skate	<i>Leucoraja ocellata</i>	310 - 517	38
Total Individuals			980

Table 1. Predator categories, length range of individuals sampled, and number of individuals sampled.

Scientific name	Common Name
Algae	Red, brown, and green algae
Gastropoda	Snails and nudibranchs
Bivalvia	Bivalves
Squid	Illex and Loligo Squid
Polychaeta	Polychaete worms
Aphroditidae	Sea mice
Other worms	Assorted worms
Crustacea	Unclassified crustaceans
Cumacea	Cumaceans
Isopoda	Isopods
Amphipoda	Unclassified amphipods
Gammaridea	Gammarid Amphipods
Ampeliscidae	Ampeliscid amphipods
Aoridae	Aorid amphipods
Unciola sp	Unciolid amphipods
Caprellidae	Caprellid amphipods
Euphausiacea	Krill
Decapoda	Unclassified decapods
Decapoda shrimp	Decapod shrimp
Palaemonetes sp	Palaemonetes shrimp
Hippolyte sp	Hippolyte shrimp
Pandalidae	Pandalid shrimp
Crangon septemspinosa	Crangonid shrimps
Homarus americanus	American lobster
Decapoda crab	Decapod crabs
Cancer sp	Cancer crabs
Echinodermata	Sea stars, sand dollars, and urchins
Osteichthyes	Unidentified fish remains and scales
Ammodytes americanus	American sand lance
Peprilus triacanthus	Butterfish
Stenotomus chrysops	Scup
Merluccius bilinearis	Silver hake
Other fish	Other fish
Other invertebrate	Other invertebrates
Animal Remains	Unidentified animal remains
Substrate	Sand, rocks, and invertebrate tubes

Table 2. Prey categories used in the dietary guild analysis.

5. Conclusion

Analysis of the dietary overlap of 15 prominent demersal fish species present in RIS and BIS defined six significant dietary guilds. Our results indicate that ontogenetic diet shifts are not significant enough to change guild membership, though analysis of more data is needed to confirm this. These guilds indicate fish species that exploit similar niches and potentially compete over resources. The simplification of the complex relationships amongst members of this community by identifying dietary guilds can improve ecosystem based management efforts by helping in interpretation of changes in the ecosystem such as shifts in abundance, and defining broader functional categories on which to focus management efforts.

Acknowledgements. I would like to thank everyone who made the 2011 SURFO program possible, especially Kathy Donohue, Kim Carey, Brandon Reichl, and Rob Pockalny for coordinating the program, and the NSF for funding.

References.

- Auster, P.J. and Link, J.S. 2009. *Marine Ecology Progress Series* 382: 163-172.
- Bowman, R.E. and Michaels, W.L. 1984. Food of seventeen species of Northwest Atlantic fish. NOAA Tech Memo, NOAA, Washington, DC, NMFS-F/NEC-28.
- Clarke, K.R. and Gorley, R.N. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.
- Collie, J.S., Wood, A.D. and Jeffries, H.P. 2008. Long-term shifts in the species composition of a coastal fish community. *Canadian Journal of Fisheries and Aquatic Sciences* 65: 1352-1365.
- Garrison, L.P. and Link, J.S. 2000. Dietary guild structure of the fish community in the northeast United States continental shelf ecosystem. *Marine Ecology Progress Series* 202: 231-240.
- Hixon, M.A. 1980. Competitive interactions between California reef fishes of the genus *Embiotoca*. *Ecology* 61: 918-931.
- Jeffries, H.P. and Terciero, M. 1985. Cycle of changing abundances in the fishes of the Narragansett Bay area. *Marine Ecology Progress Series* 25: 239-244.
- Malek, A., LaFrance, M., Collie, J. and King, J. 2010. Fisheries ecology and benthic habitat in Rhode Island and Block Island Sounds. *Rhode Island Special Area Management Plan, Volume 2*: 14.
- Pauly, D., and Chuenpagdee, R. 2007. Fisheries and coastal ecosystems: the need for integrated management. *Journal of Business Administration and Policy Analysis* 30-31: 205-222.
- Schoener, T.W. 1970. Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology* 51: 408-418.
- E. Rhoades, Whitman College, Walla Walla, WA, 99362. (rhoadeem@whitman.edu)
- A. Malek and J. Collie, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882. (amalek@gso.uri.edu, jcollie@gso.uri.edu)

A Performance Evaluation of the HYCOM Gulf of Mexico Model

Kellen Rosburg, Kathleen Donohue

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

Abstract. The 2010 Deepwater Horizon oil spill highlighted the lack of understanding about deep current circulation in the Gulf of Mexico, as well as the need for accurate forecasts of both upper and deep circulation. In 2009, a comprehensive field study began, aimed to investigate Loop Current circulation dynamics, eddy-shedding mechanisms, and formation of lower-layer flows in the Gulf of Mexico. This study utilized a mapping array centered near 26°N 87°W, which consisted of 9 full-depth and 7 near-bottom moorings as well as 25 bottom-mounted pressure equipped inverted echo sounders (PIES). Moorings were deployed in April 2009 and data recovered via rotation or telemetry in July and November 2010. Output from the 1/25th degree resolution Gulf of Mexico HYCOM run was compared to data from the array. Performance of the HYCOM model was evaluated using a suite of data metrics consisting of root-mean-square differences, correlations, as well as an examination of time-series or mean patterns of deep circulation and the vertical and spatial distribution of eddy energy. A model-to-mooring comparison revealed high temperature correlations and moderate to high correlations for both zonal and meridional velocity, with array-averaged correlations in the thermocline of 0.83, 0.71 and 0.79 respectively. Time-averaged eddy kinetic energy (EKE) showed comparable, but higher, values of deep EKE in the mooring array. A case study of upper and lower layer flows during the separation of Loop Current Eddy Franklin showed similar features between model and mooring array. In particular, both indicate that deep cyclones are generated beneath the Loop Current during the separation process. Overall, the favorable comparison indicates that dynamical analysis of the HYCOM model will improve our understanding of full-water-column circulation in the Gulf of Mexico.

1. Introduction

The Deepwater Horizon (DWH) oil spill, which began 20 April 2010 and lasted until the well was capped 86 days later on 15 July 2010, released a large amount of oil into the Gulf of Mexico (GOM). It was initially predicted that the oil would propagate southward from the spill site and enter the Loop Current (LC), which would carry the oil out of the Gulf through the Straights of Florida, polluting the waters and shores along the way (Gramling 2010; Liu et al. 2011). Fortunately, this prediction was incorrect. Oil remained within the GOM, causing less widespread environmental damage than originally suspected.

The uncertainty in oil propagation outlined two main issues in oil pathway prediction, the first being that understanding of GOM circulation is lacking. The second is that better prediction methods are desperately needed. This second point was further illustrated in a simulation comparing predicted oil distribution by four computer models performed by the Ocean Circulation Group at University of South Florida. In the animation of the May 15th 4-model run, 3 of the 4 models, WFS ROMS, SABGOM and global HYCOM, showed simulated oil entering and propagating with the LC. Oppositely, GOM HYCOM predicted oil entering a frontal eddy, which blocked the oil from entering the LC.

In 2009, a field study began in the Gulf of Mexico, which utilized a large mooring array centered near 26°N 87°W (Figure 1). The moorings were deployed for 15 months, from April 2009 to July 2010, with some

moorings being recovered in November 2010. The project, funded by BOEMRE, sought to answer questions about Loop Current circulation dynamics, eddy-shedding mechanisms, and formation of lower-layer flows in the GOM.

Figure 1. A map of the Gulf of Mexico including the locations of tall moorings (black diamonds), near-bottom moorings (gray triangles) and PIES (black dots). Bathymetry (gray-scale contours) is plotted as well.

This array produced a highly unique dataset. Spanning an area of almost 101,000 km² or roughly 7% of the surface area of the Gulf, the array provided a large

spatial coverage. 9 tall moorings allowed measurements from 75 m to ~3000 m at 48-51 different depths, resulting in full-water-column observations. An additional 7 near-bottom moorings allowed for an expanded view of deep currents. Because the array was positioned in the center of the Loop Current, and thus potentially in the middle of any LC eddies, the resultant data set is ideal for studying the formation and detachment of LC eddies.

While the array was sampling, two rings, eddies Ekman and Franklin, formed and separated from the LC. Eddy Franklin formed during the DWH incident, and detached shortly thereafter.

The need for better understanding of GOM circulation and enhanced predictive abilities is evident, and is echoed by (Liu et al. 2011) calling for better knowledge of model strengths and weaknesses in oil spill prediction. In response to the need, this study was launched to evaluate the performance of the Gulf of Mexico HYCOM model and to examine its viability for studying the LC and LC eddy dynamics. In particular, because this model assimilates only upper ocean information, it is of importance to test its ability to model deep (unassimilated) currents. The BOEMRE experiment provides the critical deep velocity information for the model evaluation.

2. Data.

2.1. Model

The model used in this study was the high resolution Gulf of Mexico Hybrid Coordinate Ocean Model

(HYCOM), more specifically, GOM HYCOM L0.04, experiment 20.1. GOM HYCOM has 20 coordinate surfaces in the vertical and is forced using the Navy Operational Global Atmospheric Prediction System (NOGAPS). The equatorial resolution is $1/25^{\text{th}}$ of a degree and latitudinal resolution is $1/25^{\text{th}}\cos(\text{lat})$, which is approximately 3.5 km at mid latitudes. Assimilation is performed by the Navy Coupled Ocean Data Assimilation (NCODA) system and includes data from satellite and in-situ sea surface height (SSH), satellite sea surface temperature (SST) and vertical temperature profiles from XBTs. Data from GOM HYCOM is made available in snapshot form, so it was low-pass filtered with a 3-day, 4th-order Butterworth filter to mimic the data processing performed on the array data.

2.2. The Mooring Array

The mooring array (Figure 1) consists of the 16 moorings, with tall moorings labeled as a, b or c and short moorings as d, followed by a number (example: a1, d3, etc.). Velocity was recorded on the 9 tall moorings by single current meters located at 600 m, 900 m, 1300 m, 2000 m, and 100 mab, and the surface currents were profiled by an upward-looking 75kHz ADCP situated at 450 m. Temperature sensors were located at 75, 150, 250, 350, 525, 600, 750, 900, 1100, 1300, 1500, and 2000 meters, as well as 100 mab. Short moorings consisted of a single current meter and temperature sensor placed at 100 mab. Pressure and round trip acoustic travel time, tau, were recorded by the 25 PIES.

Figure 2. Time-series comparison between a2 mooring (black) and modeled (gray) velocity at 100 m.

Figure 3. Time series comparison between a4 mooring (black) and modeled (gray) velocity at 100 m.

3. Results.

3.1. Upper Currents

3.1.1. Time Series

Comparison of near-surface velocity time-series at a2 show correlations of 0.61 and 0.88 and RMS differences of 21.1 and 25.8 for zonal and meridional velocity respectively at 100 m. These values are respectable, but the time-series indicates that GOM HYCOM often misses short timescale events (~ 3 days or less), as evident in (Figure 2). Around mid September 2009 and early January 2010, short bursts of high velocity are apparent in mooring a2, but not reflected in the model. Despite issues with short events, GOM HYCOM accurately models most peaks with comparable amplitudes at the correct times.

A similar comparison of near-surface temperature at a4 (150 m; Figure 3) shows that mooring temperature is consistently higher than the model. On average, a4 is 3.64°C warmer than the model at 150 m. This trend is evident in all moorings and at all depths to 900 m. Mooring a4 has a model-to-mooring temperature correlation of 0.90 and an RMS difference of 4.1. Near the end of August 2009, a4 sees a rapid decline in temperature, which also appears in the model, but over a longer time period. A similar pattern is observed in the other moorings as well.

Figure 4. Model-to-mooring temperature correlations as a function of depth. Tall moorings are shown with circles and near-bottom moorings with triangles (key is on the left side of plot). Filled markers indicate statistically significant R-values with p less than 0.05.

Figure 5. a) A map of temperature correlation coefficients at 150 m depth (scaled circles) for tall moorings. b) A similar map featuring standard deviation of each mooring's 150 m temperature record (scaled circles). Both plots are overlaid with GOM HYCOM modeled time-mean SSH (gray-scale contours) and Jason1 and Jason2 satellite paths (gray and black dashed lines). Each plot has a reference circle in the lower left.

3.1.2. Correlations

Model-to-mooring temperature correlations show good agreement between model and array. At 75 m depth, correlations are low, with the array average R of 0.61. Moorings a4 and b3 are the exception to low correlation at 75 m (Figure 4). R -values increase significantly at 150 m to an array average of 0.83. At 150 m all moorings have correlations above 0.5, and with the exception of c1 and c2, all are above $R=0.8$. If moorings a1, c1 and c2 are excluded, all temperature correlations remain above $R=0.8$ from 150 m to the extent of the correlation analysis at 900 m. That being said, a1 and c2 still have correlations above approx. 0.6 at 900 m depth.

To further pinpoint the source(s) of high temperature correlation, R -values at 150 m are superimposed onto a map of Jason1 and Jason2 satellite tracks, as well as time-

mean GOM HYCOM SSH contours to indicate the approximate location of the LC. Time-mean positioning of the LC and satellite tracks has little to no impact on correlations (Figure 5a). Instead, moorings with higher temperature correlations at 150 m tended also to have larger variances than moorings with lower correlations (Figure 5b).

Upper-ocean velocity correlations are relatively high (Figure 6). Array-averaged meridional velocity correlation is 0.79 at 96 m and 0.67 at 456 m. Above 900 m, all moorings exhibit R-values of greater than 0.5. Similar trends are observed in analysis of zonal velocity.

All model-to-mooring velocity correlations fall below 0.6 at depths greater than 900 m and remain essentially constant from 1300 m and below. Near bottom moorings, in general, have R-values of less than 0.4 (Figure 6).

Near-surface eddy kinetic energy (EKE) correlation (not shown) is less favorable. This could be anticipated

Figure 6. Model-to-mooring meridional velocity correlations plotted as a function of depth for tall moorings (circles) and near-bottom moorings (triangles). Varying colors indicate different moorings. Correlations with p values of greater than 0.05 (unfilled markers) are statistically insignificant.

because EKE goes as velocity squared, so EKE correlations will be less than velocity correlations. Overall, the majority of moorings exceed R-values of 0.5 at 96 m and 0.4 at 456 m, with array averages of 0.56 and 0.45 respectively. Moorings c1 and c2 have low correlations, around 0.2, at 96 m. Analysis of SSH movies over the 15-month data collection period show the occurrence of many events associated with frontal cyclones with small time and space scales in the area of c1

Figure 7. Plot of second deepest (1900 m) vs. deepest (2949 m) modeled b1 current meter data (dots). A linear regression fit to the data (gray line) is plotted, along with a reference line (slope = 1; black line) for comparison.

and c2. Low correlations here are likely due to the model's inability to simulate events of this scale (less than 3 days and ~20 km).

3.2. Deep Currents

3.2.1. Bottom Trapping

Plots of second deepest vs. deepest current meter speed, in which a slope of less than one signifies the existence of bottom trapping, were created for both array and model (example: Figure 7). These plots indicate a lack of bottom trapping in the mooring array and presence of slight trapping in the model. Array bottom trapping slopes range from 0.94 to 1.06 with a mean slope of 1.00 ± 0.01 and model slopes from 0.80 to 0.94 with a mean of 0.87 ± 0.03 . A visual analysis of bottom trapping slopes and the associated errors can be seen in Figure 8.

3.2.2. Deep Eddy Kinetic Energy

A time-averaged deep EKE comparison reveals peaks of high energy in the same locations and with comparable values, for example $122.9 \text{ cm}^2 \text{ s}^{-2}$ versus $120.5 \text{ cm}^2 \text{ s}^{-2}$ at d5 for the mooring and model respectively (Figure 9). Another peak in the array, near moorings d3 and c1, is echoed by similar magnitude at model c1 and ~30% less at model d3. The elevated EKE at b2 is about 25% greater in the array. Two peaks in the northwest corner of the array, at moorings d1 and d2, are not simulated in the model.

In general the mooring array exhibits higher deep EKE values than the model. This trend arises from strong currents during the two eddy separation events, which are not modeled with the same magnitude. Despite the difference in magnitudes, the spatial-energy distribution is similar between the mooring array and the model.

Figure 8. A comparison of bottom trapping analysis slopes and the associated error for array tall moorings (black) and GOM HYCOM modeled tall moorings (gray). Slopes greater than one indicate the possibility of bottom trapping, with values closest to zero most strongly trapped.

3.3. Case Study

A 12-day case study examining the separation of Eddy Franklin (Figure 10) tracks the progress of a deep cyclone in both the mooring array and GOM HYCOM. The large deep cyclone propagates to the southeast through the array from 12 May 2010 to 24 May 2010. A cyclone of similar size and magnitude is observed in the model on 12 May 2010, though this was about a half of a degree north of the cyclone seen in the array. By 20 May 2010, the cyclone has moved from approx. 27°N 86.5°W to 26.5°N 86°W. GOM HYCOM for the 20th showed the center of the same cyclone having moved to about 27°N 86°W. In both the array and model the radius reaches southward to ~25.75°N. On 24 May 2010 the meridional positioning is the same in both data sets and the zonal positioning is only different by ~0.2 degrees (22 km).

On 24 May 2010, a smaller deep anticyclone is seen in the mooring array, that is offset vertically and horizontally ~1/4° (28 km) from the model. This anticyclone does not appear in GOM HYCOM until around May 24th, but had formed in the array by May 16th.

Figure 9. Array to model comparison of time-averaged deep EKE (color contour) at the location of tall and near-bottom moorings (black diamonds and gray triangles respectively). EKE values are plotted for each mooring and have units of $\text{cm}^2 \text{s}^{-2}$.

4. Discussion and Conclusion.

Numerical models indicate that deep cyclone-anticyclone pairs appear to be the main mechanism for separation of eddies from the Gulf Loop (Hurlburt and Thompson 1980,1982; Sturges et al. 1993; Welsh and Inoue 2000). Each deep cyclone (anticyclone) tends to be paired, but offset upstream from an upper-ocean cyclone (anticyclone). This pattern is indicative of baroclinic instability (Cushman-Roisin 1994). The case study reveals that both model and data show a strong deep cyclone offset upstream from an intensifying frontal cyclone. This result coupled with high temperature and surface-velocity correlations suggest that the GOM HYCOM is well suited for the study of processes involved in eddy formation and separation. Such studies have the potential to lead to an improved dynamical understanding of GOM circulation and potentially result in better predictive skill.

Figure 10. Case study of upper and lower-layer coupling [12 May 2010 to 24 May 2010] in array (left column) and HYCOM (right column). Mapped bottom current (gray vectors) is plotted for four separate days (top to bottom panels) beneath round-trip acoustic travel time, τ , (left column, colored contours) or sea surface height (SSH; right column, colored contours). The contour interval for τ is .002 seconds and is comparable to ~ 9 cm in SSH. The contour interval for SSH is 9 cm for comparison to τ contours (Hamilton et al. 2011).

Acknowledgements. I would like to thank Rob Pockalny and Kim Carey for organizing and coordinating the 2011 SURFO program and to thank everyone else involved with making this program what it is. I would also like to thank Stefanie Zamorski and Karen Tracey for their assistance in learning MATLAB, Randy Watts and Stuart Bishop for their helpful feedback, and Maureen Kennelly for the extensive processing of PIES data.

References.

Cushman-Roisin, B. (1994). *Introduction to Geophysical Fluid Dynamics*. New Jersey, Prentice-Hall.
 Gramling, C. (2010). WaterWise: Where will the oil go. (<http://www.earthmagazine.org/earth/article/352-7da-6-e>).
 Hamilton, P., K.A. Donohue, R.R. Leben, A. Lugo-Fernandez and R.E. Green (Accepted 2011). Loop Current Observations during Spring and Summer of 2010: Description and Historical Perspective. *Monitoring and Modeling the Deepwater Horizon Oil Spill: A Record-Breaking Enterprise*. Washington, DC, American Geophysical Union.

Hurlburt, H.E. (1980). A Numerical Study of Loop Current Intrusions and Eddy Shedding. *J. Phys. Oceanogr.*, 10, 1611-1651.
 Hurlburt, H.E. and J.D. Thompson (1982). The Dynamics of Loop Current and Shed Eddies in a Numerical Model of the Gulf of Mexico. *Hydrodynamics of Semi-enclosed Seas*, edited by J.C.J. Nihoul, pp. 243-298, Elsevier Sci., New York.
 Liu, Y., R.H. Weisberg, C. Hu and L. Zheng (2011). Tracking the Deepwater Horizon Oil Spill: A Modeling Perspective. *EOS, Transactions of the American Geophysical Union* 92(6): 45-46.
 Sturges, W., J.C. Evans, S. Welsh, and W. Holland (1993). Separation of Warm-Core Rings in the Gulf of Mexico. *J. Phys. Oceanogr.*, 23, 250-268.
 Welsh, S.E. and M. Inoue (2000). Loop Current Rings and Deep Circulation in the Gulf of Mexico. *J. Geophys. Res.* 105(C7): 16951-16959.

K. Rosburg, Western Washington University, Bellingham, WA 98225 (rosburk@students.wvu.edu).
 K. Donohue, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882 (kdonohue@gso.uri.edu).