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***Thalassiosira* Community Composition and Diversity in Narragansett Bay**

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THALASSIOSIRA COMMUNITY COMPOSITION AND DIVERSITY IN
NARRAGANSETT BAY

BY
SARAH FLICKINGER

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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OF

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ABSTRACT

Diatoms contribute 40-45% of marine primary production. Understanding the contributions of individual species to diatom communities is important in light of warming ocean waters and changing food webs. Here, the diatom genus *Thalassiosira*, which exhibits irregular pulses in abundance in Narragansett Bay, is examined using data from the Narragansett Bay Long Term Phytoplankton Time Series. High-throughput sequencing (HTS) at the highly-variable V4 region of the 18S ribosomal gene identified fourteen *Thalassiosira* taxa in monthly samples over a six-year time period (December 2008-December 2014), seven of which had not previously been identified in this time series. During the same time period, only four *Thalassiosira* taxa were identified through light microscopy (LM) counts. Many of the newly identified species for this location are small in diameter and difficult to identify using LM. However, they appear to be very frequent and may have a significant ecological role. 18S gene copy number was shown to greatly effect sequence abundance for this locus and this genus, and therefore only presence data was analyzed. *Thalassiosira* species grouped into four seasonal assemblages, although only 25% of this pattern was explained by temperature, suggesting that other factors such as competition or prey selection may drive *Thalassiosira* community structure. Comparisons to historical records show possible shifts in frequency and abundance of *Thalassiosira* species.

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TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iii
TABLE OF CONTENTS.....	v
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
INTRODUCTION.....	1
METHODS.....	7
RESULTS.....	15
DISCUSSION.....	24
TABLES.....	37
FIGURES.....	44
APPENDICES.....	59
BIBLIOGRAPHY.....	70

LIST OF TABLES

TABLE	PAGE
Table 1. Diatom Barcode Genes.....	37
Table 2. Reference 18s V4 Database.....	38
Table 3. Mock Community Species.....	40
Table 4. <i>Thalassiosira</i> Species in Narragansett Bay.....	41
Table 5. <i>Thalassiosira</i> Cell Diameters and Ranges.....	42
Table 6: BEST Environmental Correlations.....	43

LIST OF FIGURES

FIGURE	PAGE
Figure 1. Map of Sampling Station	44
Figure 2. <i>Thalassiosira</i> Abundance in Narragansett Bay.....	45
Figure 3. Rarefaction Curves 18S.....	46
Figure 4. V4 Maximum Likelihood Tree.....	47
Figure 5. Whole 18S Maximum Likelihood Tree.....	48
Figure 6. Mock Community Sequence % Composition.....	49
Figure 7. Field Sample <i>Thalassiosira</i> Presence/Absence.....	50
Figure 8. <i>Thalassiosira</i> Species Frequency 2008-2014.....	51
Figure 9. Bray-Curtis Dendrogram of Species.....	52
Figure 10. MDS Plot of Monthly Species Compositions.....	53
Figure 11. Bray-Curtis Dendrogram of Samples.....	54
Figure 12. Occurrence Frequency of 8 Most Frequent Taxa.....	55
Figure 13. Temperature and Salinity Ranges.....	56
Figure 14. Principal Components Analysis of Environmental Factors.....	57
Figure 15. <i>Thalassiosira</i> spp. Images.....	58

INTRODUCTION

Abbreviations used in this thesis in order of occurrence:

HTS: High Throughput Sequencing

LM: Light Microscopy

ITS2: Internal Transcribed Spacer 2

NCBI: National Center for Biotechnology Information

ML: Maximum Likelihood

BIC: Bayesian Information Criterion

PCR: Polymerase Chain Reaction

MC: Mock Community

MED: Minimum Entropy Decomposition

OTU: Operational Taxonomic Unit

DIN: Dissolved Inorganic Nitrogen

DIP: Dissolved Inorganic Phosphorus

Si: Silicate

PCA: Principal Components Analysis

SSU: Small Subunit

Phytoplankton account for over 45% of global primary production despite comprising only 1% of photosynthetic biomass (Field et al. 1998), underscoring the importance of these organisms. Within the phytoplankton, diatoms account for roughly 50% of oceanic primary production (Rousseaux and Gregg 2014). Diatoms are unicellular, photosynthetic eukaryotes characterized by a siliceous frustule. There are an estimated 30,000 to 200,000 extant diatom species (Mann and Droop 1996, Mann and Vanormelingen 2013), indicating the great diversity within this class of phytoplankton. In coastal systems, diatoms play a crucial role in the silica cycle and in carbon flux (Treguer and De La Rocha 2013). Understanding the ecology of individual diatom species is essential for generating estimates of phytoplankton community composition, production, export and phenology. Diatom species traditionally are differentiated based on morphology (reviewed in Mann 1999), although a lineage-

based species approach has gained support recently (reviewed in Alverson 2008), in which molecular and morphological characteristics are used to differentiate phylogenies. Here, we examine the diatom genus *Thalassiosira*, a common genus consisting of dozens of species that are often seen coexisting, using data from the Narragansett Bay Long Term Phytoplankton Time Series.

The Narragansett Bay Long-Term Time Series was initiated by D. Pratt and T. Smayda (Pratt 1965, Smayda 1998) in 1959 and weekly sampling has been conducted by graduate students since 1999. Narragansett Bay is a highly productive 234 square kilometer estuary (Nixon et al. 2009) characterized by a phytoplankton community dominated by diatoms (Pratt 1965, Karentz and Smayda 1984, Oviatt et al. 2002). Understanding the ecology and community dynamics of this estuary are important due to the role of diatoms in affecting water quality, biogeochemical cycling, and production (Legendre 1990, Treguer and De La Rocha 2013). Time series samples are collected from a station in the southern west passage of Narragansett Bay, which experiences seasonal cycling of physical, chemical and biological signals, including temperature, nutrients, and plankton abundances and diversity (Pilson 1985, Nixon et al. 2009). Since 1959, nutrient inputs to Narragansett Bay have changed and the average winter temperature has increased by 2.2°C from 1960-2007 (Nixon et al. 2009, Smith et al. 2010). The scale, timing and even the occurrence of the spring bloom have also been in flux (Oviatt et al. 2002). Both bulk diatom counts and chlorophyll *a* concentrations have declined (Smayda 1998, Li and Smayda 1998), and the chlorophyll *a* decline is correlated with warmer water temperatures (Oviatt et al. 2002).

The dynamics and changes of some diatom genera in Narragansett Bay have been studied. The genus *Skeletonema* has been a significant focus of study since it historically composed 48-50% of the phytoplankton community in Narragansett Bay (1959-1979), and more recently composed 33% of the phytoplankton community in Narragansett Bay (1980-1997) (Borkman and Smayda 2009). Overall *Skeletonema* abundance in Narragansett Bay has decreased by almost half in the period from 1959-1997 and variations in abundance correlate strongly with environmental factors, including temperature and irradiance (Borkman and Smayda 2009). Recent high throughput 28S sequencing of cryptic *Skeletonema* species in Narragansett Bay discovered unknown diversity and seasonal patterns (Rynearson and Canesi, in press). From 1959 through 1980, some *Thalassiosira* species were identified as important contributors to the Narragansett Bay phytoplankton community. In particular, *T. nordenskioeldii* was identified as the 5th most abundant phytoplankton species and was an important winter phytoplankton species, with a 5 year abundance fluctuation cycle (Karentz and Smayda 1984). Investigating potential changes in abundance or timing in *Thalassiosira* species is valuable given that diatom genera with larger cell sizes may contribute more significantly to carbon fixation than smaller cells such as *Skeletonema*, due to the positive relationship between carbon content and cell volume in diatoms (Strathmann 1967, Menden-Deuer and Lessard 2000). Therefore, it is important to determine whether *Thalassiosira* species timing or frequencies have changed.

Thalassiosira is a centric diatom genus that can be chain forming and is very common in Narragansett Bay. At least 11 species within this genus have been

observed in Narragansett Bay since the initiation of the Long Term Time Series in 1959, including *T. eccentrica*, *T. rotula*, *T. gravida*, *T. pseudonana*, *T. punctigera*, *T. pacifica*, *T. anguste-lineata*, *T. nordenskiöldii*, *T. kushirensis*, *T. decipiens* and *T. hyalina* as well as unidentified *Thalassiosira* spp. Cells from this genus can be very abundant in Narragansett Bay, reaching cell numbers of >1.6 million cells/L.

Not only is *Thalassiosira* an important diatom genus in Narragansett Bay but various *Thalassiosira* species are also an important component of phytoplankton communities across the globe, including estuarine, coastal and open ocean regions [e.g. Narragansett Bay (Pratt 1965, Smayda 1998), the Argentinean coast (Popovich and Gayoso 1999), a Scottish sea-loch (Harris et al. 1995), the North Sea (Hoppenrath et al. 2007), German and Dutch estuaries (Muylaert and Sabbe 1996), the subarctic Pacific (Yoshie et al. 2010)]. *Thalassiosira* species have been abundant for at least the past century in British Columbia estuaries (McQuoid and Hobson, 1997). With respect to the changing climate, understanding the diversity, community composition of *Thalassiosira* and factors that drive these patterns is increasingly important.

Environmental factors are principal drivers of diatom ecology and specifically, *Thalassiosira* ecology. Many *Thalassiosira* species exhibit temperature, nutrient, and light sensitivity, and therefore occur cyclically in conjunction with nutrient cycles and seasons. In lab studies, *Thalassiosira weissflogii* stopped cell division in low silicon concentrations (De la Rocha et al. 2010). In contrast, the species continues dividing but produces cells with low N, P and Si compositions when nitrogen limited (De la Rocha et al. 2010). There are also observed differences in responses to nutrient stress between *Thalassiosira* species; *T. oceanica* maintains chlorophyll *a* levels when

grown at lower Fe concentrations whereas *T. weissflogii* demonstrates a 60% decrease in chlorophyll *a* when grown at low Fe (Sunda and Huntsman 1995). Some of these physiological differences between the species have been observed in the field as well. Chappell et al. (2013) found that *Thalassiosira* community composition in the Northeast Pacific was correlated with temperature and iron concentrations.

Despite compelling evidence that this genus deserves further study, there are a few challenges with studying the *Thalassiosira* genus, primarily that identification of *Thalassiosira* species is known to be difficult, especially using light microscopy (LM) due to subtle differences in frustule morphology (Kaczmarska et al. 2009). Some *Thalassiosira* species are unidentifiable with LM methods (Hasle 1978). Complicating matters, the morphology of *Thalassiosira* species can vary, especially in the variable environmental conditions of estuaries, making LM identification all the more difficult (Hevia-Orube et al. 2015). *Thalassiosira* identification can be done using scanning electron microscopy (Hoppenrath et al. 2007), but this requires time and expertise. Recently, molecular barcoding has been used to identify *Thalassiosira* species as well as other diatom species (e.g. Hamsher et al. 2011, Moniz and Kaczmarska 2010, Zimmerman et al. 2011, Luddington et al. 2012, Hamsher et al. 2013). This new approach has allowed for the discovery of previously unrecorded local species in North Sea estuaries (Hoppenrath et al. 2007). Hamsher et al. (2013) compared morphological and molecular sampling to estimated *Thalassiosira* species richness in the Bay of Fundy and found additional rare and difficult to identify *Thalassiosira* species using molecular methods over morphological methods. Furthermore, small volume sampling methods, such as the 1mL sample counted weekly for the

Narragansett Bay Long-Term Phytoplankton Time Series, can underestimate species counts by 20-45% (Rodriguez-Ramos et al. 2013). Species richness may be more accurately obtained through molecular methods, such as high-throughput sequencing.

This study will use a high throughput sequencing community genetics approach to investigate *Thalassiosira* dynamics in Narragansett Bay and will address the following questions: 1) What *Thalassiosira* species are present in Narragansett Bay? 2) Are there annual and or seasonal patterns of *Thalassiosira* species composition in the Narragansett Bay? 3) Do fluctuating environmental conditions drive these putative patterns of species composition? To gain a more thorough understanding of *Thalassiosira* diversity and seasonal patterns in Narragansett Bay, we sequenced the 18S V4 locus for 80 field samples taken over the course of six years. With this approach, *Thalassiosira* species not previously identified in Narragansett Bay were identified and correlated with environmental conditions.

METHODS

Field Sampling Methods

As part of the Narragansett Bay Long-Term Phytoplankton Time Series, weekly water samples were collected from Station II in the southern west passage of Narragansett Bay, at 41.57°N and 71.39°W (Figure 1). Surface water samples were collected and filtered through 0.22 µm pore size, 25µm diameter Millipore ExpressPlus filters. The volume filtered was dependent on the Secchi depth measured for the sampled water; 100mL of water were filtered per each meter of Secchi depth. Three replicate filters were collected each week and stored at -80°C for later DNA extraction and analysis.

Molecular Barcoding

Potential DNA barcodes were selected from current literature, including the variable V4 region of the 18S ribosomal DNA coding gene, the V9 region of the 18S, and the internal transcribed spacer 2 (ITS2) ribosomal gene (Table 1) (Chappell et al. 2013, Gast et al. 2004, Hamsher et al. 2011, Hamsher et al. 2013, Luddington et al. 2012, Moniz and Kaczmarska 2010, White et al. 1990, Zimmerman et al. 2011). Candidate loci were assessed using DNA sequences for *Thalassiosira* species available from the National Center for Biotechnology Information (NCBI) GenBank database. Potential barcodes were evaluated based on the number of identifiable *Thalassiosira* species available in the NCBI database (more species were preferable), its ability to distinguish among species (higher inter-specific variation was preferable) and the suitability of the locus for Illumina MiSeq Sequencing (amplicon length <

500bp). Two 18S regions (V4, V9) had the highest number of *Thalassiosira* sequences available in GenBank. Sequences for 30 *Thalassiosira* species were available in GenBank for 18S V9 region amplified by primers in Gast et al. (2004), but 18 of these species were indistinguishable at this locus. At the V4 region, 31 *Thalassiosira* species were available in GenBank and at least 17 of them could be distinguished from one another. Primers to amplify this region (Zimmerman et al. 2011) were selected for this study (forward primer ATTCCAGCTCCAATAGCG and reverse primer GACTACGATGGTATCTAATC). These primers were used successfully by Luddington et al. (2012) to differentiate 30 *Thalassiosira* species. Primers were assessed in silico with sequences representing all taxa that might be found in the Narragansett Bay phytoplankton community and found to amplify diatoms and raphidophytes only. The primers were not expected to amplify dinoflagellates.

To assess the power of the 18S V4 locus in resolving *Thalassiosira* species, genetic trees were constructed using the maximum likelihood (ML) method. Forty-five 18S V4 *Thalassiosira* sequences representing 20 *Thalassiosira* species from GenBank were compiled with two outgroup species (*Skeletonema marinoi* and *Skeletonema menzelsii*) to create a curated reference database (Table 2). This curated database included taxonomically verified sequences only. Sequences representing a 420 bp V4 amplicon from Zimmerman et al. (2011) were aligned using Clustal.w (Larkin et al. 2007). The HKY+G model (Hasegawa et al. 1985) was selected to be the best fit nucleotide substitution model based on Bayesian Information Criterion (BIC) using jModelTest2 (Darriba et al. 2012, Guindon and Gascuel 2003). This model allows for variable nucleotide base frequencies and has one transition rate and one transversion

rate. The best ML tree was created with Paup* version 4.0b10 (Swofford 2003) and its reliability was tested using 100 bootstrap replicates. An ML tree was also constructed using the same methods for a 1552bp partial 18S gene with the same sequence accession numbers as the reference database. The best fit nucleotide substitution model chosen with BIC for this locus was the TIM1+I+G model, which has a variable nucleotide base frequency, variable transition rates and two transversion rates.

Mock Community Design

To test and validate the community high throughput sequencing approach used for this study, four mock communities were constructed to provide insight into the effects of 18S gene copy number variation, the amount of polymerase chain reaction (PCR) bias, and the amount of sequencing bias inherent to this method. The mock communities were comprised of varying amounts of four *Thalassiosira* species, three other diatom species, a dinoflagellate and a raphidophyte (Table 3). To obtain mock communities, monocultures of different species were maintained in exponential growth in F/2 media. Concentrations were assessed by counting 1 mL samples of each strain using a Sedgwick-Rafter counting chamber (Graticules S52) under 100-200x magnification, depending on species cell size. Cell concentrations were used to calculate the volume of each culture need to achieve the target species proportions. The calculated volumes of each strain were filtered simultaneously on 0.2µm pore size, 25µm diameter Millipore ExpressPlus filters and stored at -20°C for up to 1 month, until DNA extraction.

DNA Extraction and Amplification Protocols

Previously extracted DNA from 68 monthly surface water samples from the Narragansett Bay Long-Term Phytoplankton Time Series were selected from the period of December 2008 to December 2014 (Canesi and Rynearson, in press) providing samples containing a range of *Thalassiosira* concentrations, from zero detected cells to 1,683,000 cells/L. An additional twelve monthly samples from January 2014-December 2014 were selected based on maximum *Thalassiosira* cell abundance, for a total of 24 semi-monthly samples from 2014 and 80 field sampling dates overall (Appendix 1). Genomic DNA from the additional 2014 samples and the mock community samples was extracted from frozen filters using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) with the addition of approximately 15 triple-pure high impact 0.5 mm zirconium beads (KSE Scientific) during the lysis step prior to vortexing and incubating. Genomic DNA from each of the four mock communities was extracted in triplicate.

A 420 bp fragment within the variable V4 region of the 18S rDNA gene was amplified using the primers described by Zimmerman et al. (2011) modified by the addition of Illumina specific adaptors: D512_illumina: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATTCCAGCTCCAATAGCG 3' and D978_illumina: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACGATGGTATCTAATC 3'. PCR reactions contained the following reagents: 1x Bio-x-Act Short Mix (Bioline USA Inc.), 0.5 μ M each forward and reverse primer and approximately 0.3-2.7 ng DNA template. Reactions were amplified using a multi-step thermocycler protocol, which consisted of a two minute denaturing step at 94°C, followed by 20 cycles of 30 seconds at 94°C, 30 seconds at 49°C and 30 seconds at 72°C followed by 15 cycles of 30 seconds at 94°C,

30 seconds at 67°C and 30 seconds at 72°C, followed by 10 minutes at 72°C.

Technical variation of this PCR reaction was measured through triplicate amplification of one randomly selected field sample (12/30/2014) and one mock community sample (MC1), using identical template DNA concentrations for the triplicate PCR reactions. PCR amplicons were cleaned with Ampure XP beads (Beckman Coulter, Inc.), quantified with the Qubit High Sensitivity DNA Assay Kit (Thermo Fisher Scientific, Inc.), amplified for an additional five cycles to add Nextera indices and adaptors (Illumina, Inc.) and cleaned again with Ampure XP beads.

Sequencing and Sequence Processing

PCR products were pooled with the KAPA qPCR kit (Kapa Biosystems) and sequenced on the Illumina MiSeq platform with V2 chemistry (2x250bp reads; Illumina, Inc.) at the University of Rhode Island Genomics and Sequencing Center. The resulting paired end sequencing reads were first trimmed to remove low quality bases and Illumina adaptors using Trimmomatic PE (Bolger et al., 2014) using a 4 base pair sliding window quality score of 20 and a minimum read length of 200 basepairs. Paired end sequences were merged using the BBmerge function from BBMap (Bushnell, [Sourceforge.net/projects/bbmap/](https://sourceforge.net/projects/bbmap/)) using the default settings. Trimmed and merged sequences were first filtered to a minimum length of 380 basepairs and a maximum expected error (based on phred quality scores) of 1 and then concatenated and dereplicated in USEARCH (Edgar 2010). The resulting sequences formed the final dataset for the community genetic analysis.

To identify taxa in the samples, filtered sequences were decomposed into minimum entropy decomposition (MED) nodes (Eren et al. 2015) using the default

settings. Chimeric sequence nodes were identified and removed from results using the UCHIME denovo program (Edgar 2011) with all default settings. Taxonomy was assigned to remaining MED nodes using the blast method within the assign_taxonomy.py script from QIIME (Caporaso et al. 2010) and a reference database of *Thalassiosira* sequences curated from GenBank (Table 2). MED nodes are equivalent to operational taxonomic units (OTUs), which were used here to assign sequences to one species or to a group of species for cases in which a sequence was indistinguishable between two species. UCHIME denovo was run separately with only mock community samples and then separately with only field samples as expected chimeric sequences are higher for a lower diversity community, as in the mock communities. Nodes that contained fewer than 0.04% of total sequence reads for that sample were removed as possible sequencing error (Quail et al. 2012).

Statistical and Environmental Analyses

Thalassiosira species presence-absence data were obtained from the taxonomy assigned to MED nodes per the 0.04% cutoff noted above and used to assess species composition of the water samples and mock communities. Variation within triplicate extraction and triplicate PCR reactions was assessed using ANOVA. Observed mock community compositions were compared with the expected compositions through χ square goodness-of-fit-tests.

Bray-Curtis similarity was computed between all field samples and between all species identified in the field samples in PRIMER v6.1.6 (PRIMER-E Ltd.). To examine temporal variation of species composition on yearly and monthly time scales, likelihood of species occurrence was calculated by summing species data by year or

month, respectively, and normalizing by sampling effort. Bray-Curtis similarity was then calculated for the likelihood of both yearly and monthly species presence. Cluster dendrograms and multidimensional scaling plots (MDS) based on Bray-Curtis similarity were created in PRIMER v6.1.6 (PRIMER-E Ltd.).

Correlations of community composition with environmental conditions were analyzed using environmental data obtained from the Narragansett Bay Long-Term Phytoplankton Time Series (<http://www.gso.uri.edu/phytoplankton>; surface temperature, surface salinity, chlorophyll *a*, dissolved inorganic nitrogen [DIN], dissolved inorganic phosphorus [DIP], and silicate [Si]). For a small subset of field samples (seven dates) for which chlorophyll *a* data was not available from the time series, chl *a* data were obtained from the Marine Ecosystems Research Laboratory (<http://www.gso.uri.edu/merl/data.html>) Tank 98 bay sample dataset, which is collected weekly from the University of Rhode Island Graduate School of Oceanography dock (41.49°, -71.42°). This sample site is located approximately 9.2 kilometers south of Station II in the west passage of Narragansett Bay and thus shares a very similar physical environment with Station II.

Additionally, temperature and salinity data for 9/15/2014 and nutrient data for 10/5/2014 were obtained from the MERL dataset. Photosynthetically active radiation (PAR) data were obtained from the Narragansett Bay National Estuarine Research Reserve's System Wide Monitoring Program (41.64°, -71.34°). PAR data were downloaded from the National Estuarine Research Reserve's Centralized Data Management Office (<http://cdmo.baruch.sc.edu/>). Variation in environmental data (surface temperature, surface salinity, DIN, DIP, Si, chl *a*, PAR) was analyzed with a

Principal Components Analysis (PCA) also in PRIMER v6.1.6 based on Euclidean distance. Values for salinity, chlorophyll *a*, DIN, DIP, and Si were log transformed prior to PCA construction. A multivariate trend correlation (BIOENV) analysis was used to correlate environmental factors with the community composition also in PRIMER v6.1.6.

Cell Imaging

Thalassiosira cells from one Naphrax preserved sample from the Long-Term Time Series were imaged using a Nikon E800 microscope at 200x to verify presence of species identified in the HTS results. The chosen sample data was January 11, 2011.

RESULTS

Thalassiosira in the Narragansett Bay Long-Term Phytoplankton Time Series, December 2008 to December 2014

Based on microscopy records in the long-term dataset, *Thalassiosira* species were present in 85% of the weekly cell counts of Station II surface water collected between December 2008 and December 2014 (Figure 2). The cell abundance of the genus ranged from zero observed cells to 1,683,000 cells/liter, with a median abundance of 14,000 cells/liter (Appendix 1). During the same time period, four *Thalassiosira* species or groups of species were recorded in the long-term data set. Two were identified to the species level (*T. nordenskioeldii*, *T. punctigera*) and two represented multiple species (*T. rotula*/*T. gravida* and *Thalassiosira* spp.). The last two categories represent species that are difficult to distinguish at the resolution of light microscopy.

Thalassiosira varied in occurrence and abundance (Appendix 2). *T. punctigera* was the least abundant and the least common taxa; it was present in 9.3% of samples during the time period with a maximum abundance of 51,000 cells/liter. It appeared in the months of September through January and was only recorded in three of the six years. *T. nordenskioeldii* was present in 14.2% of samples from this period and reached a maximum abundance of 1,575,000 cells/liter. It occurred primarily in the winter-spring. The *T. rotula*/*T. gravida* group was more common but less abundant than *T. nordenskioeldii*. It was present in 16.8% of samples with a maximum abundance of 689,000 cells/liter. This taxa was recorded every year and was more commonly seen in the late fall, winter and spring. Finally, the unidentified

Thalassiosira spp. was the most common and abundant group observed. It was present in 78.0% of the weekly samples, with a maximum abundance of 1,683,000 cells/liter. This group was recorded every year and was observed year round. The abundance and ubiquity of this group are likely a result of this group representing multiple species, which could not be identified due to LM limitations or counter ability.

Sequencing Results

16,157,090 sequence read pairs were obtained for this study. Sequences per sample ranged from 58,448 sequence read pairs to 281,234 sequence read pairs, with an average of 168,303 sequences per sample (Appendix 3). An average of 11,098 sequences per sample survived (6.6%) the filtering and merging steps, with a range of 172 to 26,869 sequences per sample (Appendix 3). Despite a few samples with very low sequence survival rates, a majority of OTU rarefaction curves and species rarefaction curves terminated with an asymptote, indicating that sampling depth was appropriate for this study (Figure 3).

Reference Database

The ML tree for the 1552 bp 18S gene in *Thalassiosira* species showed moderate species resolution and bootstrap support (Figure 4). In contrast, the ML tree for the 420 bp V4 region of the 18S showed weaker support and lower species resolution (Figure 5). Some species were clearly resolved in both the V4 region and the full length 18S. For example, both the 18S and 18S V4 analyses supported a distinct clade comprised of *T. guillardii*, *T. weissflogii* and *T. pseudonana*. These three species are highly resolved and distinguishable at the 18S and 18S V4 fragments.

Other species were not resolved as clearly in the 18S V4 tree, either due to variable sequences for one species not clustering together or due to multiple species sharing one common sequence. The first pattern is seen for *T. anguste-lineata*, *T. minima*, *T. profunda* and *T. tenera*, each of which has sequences which appear on more than one branch in the 18S V4 ML tree. The *T. tenera* sequences cluster together at the whole 18S, indicating that this is one species. However, for *T. anguste-lineata* and *T. profunda*, the sequences also appear on different branches of the whole 18S tree, meaning that the taxonomies for these species can't be clarified. The second pattern appears when there are no differences at the 18S V4 locus between or among species, meaning that the two species are unable to be resolved at this locus. These unresolved species pairs include *T. nordenskioeldii* and *T. aestivalis*, *T. pacifica* and *T. aestivalis*, and *T. rotula* and *T. gravida*.

Mock Community Results

Sequencing of the mock communities recovered all 9 species that had been added, including all four *Thalassiosira* species, as well as the other diatom species (*D. brightwelli*, *S. marinoi*, *S. menzellii*,) and the raphidophyte (*H. akashiwo*). The dinoflagellate (*H. triquetra*) did not appear in sequencing results, as the primers do not amplify dinoflagellates. Initial results included OTUs for *T. guillardii* and *T. oceanica*, neither of which was added in the mock communities. Manual examination demonstrated that these sequences were comprised of sequence fragments from two species that were in the mock communities, meaning that they were chimeric. The purported *T. oceanica* OTU was comprised of a 120 bp segment of the *T. weissflogii*

sequence appended to a 260 bp segment of *T. anguste-lineata* sequence. After chimeric species were filtered and removed using UCHIME denovo (Edgar 2011), all final sequences from the mock communities were identified as species which had been added to the mock communities. There were no sequences with unassigned taxonomy, verifying that the analysis methods accurately captured community composition of samples.

Among the DNA extraction triplicates for each of the four mock communities, composition did not vary significantly (ANOVA, $p > 0.05$; Figure 6). Composition did not vary significantly among the three amplification triplicates performed on either the mock community sample (mock community 1) or the field sample (12/30/2014) (ANOVA, $p > 0.05$). In contrast, observed mock community percent OTU composition was significantly different than the expected percent species composition used to create the mock communities (χ square goodness-of-fit-test, $P < 0.001$; Figure 6). Since OTU composition did not adequately represent the actual species composition of the mock communities, sequence abundance of each OTU could not be used as an estimate of species abundance. As a result, species composition of the field samples was analyzed using presence/absence data.

Field Sample Sequencing Results

Fifteen *Thalassiosira* species were identified in the field samples (Table 4) in comparison to the three species identified through LM counts. In contrast to the mock community results, there were unidentified species remaining in the field sample sequences after taxonomy was assigned. These unidentified sequences were not used in downstream community analysis, since primers amplified all diatoms and

raphidophytes in the samples. Of the fifteen *Thalassiosira* species identified in the sequence data, eight had not been identified historically using light microscopy in the Narragansett Bay Long-Term Phytoplankton Time Series: *T. oceanica*, *T. guillardii*, *T. minima*, *T. tenera*, *T. angulata*, *T. aestivalis*, *T. tumida*, *T. concaviuscula* and *T. profunda*. Five of these new records for Narragansett Bay have cell diameters under 15µm (Table 5), making them difficult to identify using light microscopy at 100x.

The fifteen *Thalassiosira* species observed in the HTS results had different occurrence patterns (Figure 7), frequencies (Figure 8), and coexistence patterns (Figure 9). Some of the species only occurred during some months or years while others were more persistent. Seven of the least frequent species (*T. aestivalis*/*T. nordenskiöldii*, *T. anguste-lineata*, *T. concaviuscula*, *T. eccentrica*, *T. oceanica*, *T. tenera*, *T. tumida*) were not present every year. The remaining eight species (*T. angulata*, *T. guillardii*, *T. minima*, *T. pacifica*/*T. aestivalis*, *T. profunda*, *T. pseudonana*, *T. punctigera*, *T. rotula*/*T. gravis*) were present each year.

While 15 total species were observed, species richness varied from 1-8, meaning that some species did not co-occur. A clear seasonal pattern in richness was not observed. A maximum of eight species were observed in both January 2011 and May 2014. A species richness of 1 was most often observed in August (*T. pseudonana*, 2009; *T. tenera*, 2010; *T. guillardii*, 2012). Some species were more likely to co-occur than others but these patterns were not especially strong (Figure 8). At a 45% Bray Curtis similarity, one group of four species emerged: *T. guillardii*, *T. minima*, *T. profunda* and *T. pseudonana*. These species were all frequent and have small cell sizes (Table 5). *T. guillardii* and *T. minima* were the most likely species to co-occur, with a 70% Bray Curtis similarity.

The majority of species were not very frequent (Figure 8); only two species (*T. guillardii*, *T. pseudonana*) had frequencies above 40%. *Thalassiosira guillardii* was the most frequent, appearing in 78.75% of field samples, followed by *T. pseudonana* (*T. minima* and *T. pseudonana* were also quite frequent (61.25% and 47.5% respectively). All other species had frequencies under 30%. The least frequent species were *T. tumida* and *T. eccentrica*.

Seasonality of Thalassiosira community composition

To determine if there were similarities among species assemblages collected at different times of year, the Bray-Curtis similarity of species composition between months was calculated. A multi-dimensional scaling plot of the monthly pair-wise comparisons revealed three distinct assemblages at a similarity value of 55%: a winter-spring assemblage (January, February, March, April), a summer assemblage (May, June, July, August, September) and a fall assemblage (October, November, December) (Figure 10). These seasonal groupings roughly corresponded with two groups of field samples that diverge at a 35% Bray-Curtis similarity (Figure 11). One grouping at this 35% similarity level consists mainly of the winter-spring samples and the other grouping consists primarily of the summer samples. The fall samples seem to be scattered between both groups.

Several species were observed more often during warmer months. *T. angulata* occurred mostly from March-July. *T. oceanica* was most likely to occur in April - May, but occurred sporadically in other months (Figure 12). *T. tenera* occurred in July through October in all years except 2012 and 2014. *T. rotula*/*T. gravis* occurred

most often in April-June and never in the months of February and September through November (Figure 12).

Other species were more often seen during the fall, winter and spring (Figure 12). When the *T. nordenskiöldii*/*T. aestivalis* OTU was present, it occurred during the months of December-April. *T. eccentrica* only occurred during the months of October-December. The *T. pacifica*/*T. aestivalis* OTU never occurred in the summer months (from May - September). *T. punctigera* also never appeared in the summer months (from June - August) or in March. It was most likely to occur in late fall (October - December).

However, seasonal patterns were not apparent for all of the species studied, including *T. anguste-lineata*, *T. concaviuscula* and *T. profunda*. A final subset of the annually occurring species were persistent throughout the year. *T. pseudonana* occurred year round with the exception of the month of February (Figure 12). It was most likely to occur from June - September. *T. guillardii* was observed in samples from all twelve months, although its probability of occurrence was slightly higher in the winter. Likewise, *T. minima* was also observed in samples from all twelve months, with a higher occurrence probability in the winter (100% for December and January during the study period).

Species Specific Environmental Ranges

In addition to variation in seasonal occurrence patterns, species also had variable observed temperature and salinity ranges (Figure 13). Ranges are not reported for *T. tumida*, as this species was only observed on one occasion (conditions were 14.76°C and 30.7 ppm). Many species had broad temperature ranges, but a few species

had more specific temperature ranges. The *T. nordenskioeldii*/*T. aestivalis* OTU had a lower temperature range (0.5-8.5°C). *T. eccentrica* was observed only in mid-temperatures (5.5-17.07°C), while *T. tenera* was only observed in warm temperatures (18.1-24.6°C).

More variation was observed in salinity ranges. Some of the most frequent species had the broadest salinity ranges, including *T. guillardii* (18.6-32 ppt), *T. minima* (18.6-32 ppt) and *T. pacifica*/*T. aestivalis* (18.6-31.41 ppt). Half of the species were only observed at salinities over 24 ppt (*T. concavuscula*, *T. eccentrica*, *T. nordenskioeldii*/*T. aestivalis*, *T. oceanica*, *T. profunda*, *T. punctigera*, *T. tenera* and *T. tumida*). All species were observed at salinities of over 30 ppt.

Environmental Factors

Environmental factors in Narragansett Bay varied widely from December 2008 - December 2014. Surface temperature was strongly seasonal and ranged from 0.5 - 24.6°C. Average daily PAR was also seasonal, varying from 16.21 - 572.05 mmol/m². Salinity averaged 29 ppt, with a range of 18.6 - 32.39 ppt. Chlorophyll *a* had a minimum value of 0.23 µg/L and a maximum of 29.8 µg/L during a large phytoplankton bloom in April 2010. Dissolved inorganic nitrogen (DIN) ranged from 0.14 - 15.8 µM, dissolved inorganic phosphorus (DIP) ranged from 0.04 - 1.87 µM and silicate ranged from 0.05 - 38.12 µM.

A principal components analysis (PCA) of the environmental factors separated the sample dates roughly by season (Figure 14). The first PC axis explained 40.1% of the variation and separated samples primarily by DIP, but also by DIN, Si, Chl *a*, and surface salinity. Higher nutrients and lower chl *a* were associated with summer and

fall samples. The second PC axis explained 25.4% of the variation and differentiated samples by surface temperature and average daily PAR. Higher temperatures and average daily PAR were associated with summer samples, while lower temperatures and PAR were associated with winter samples. Surface temperature was the most highly correlated environmental factor and explained 24.6% of the *Thalassiosira* community variation through BEST analysis (Table 6).

Morphological Variation of Thalassiosira Cells

A preserved sample was examined to verify species presence when unresolved species OTUs were present in the HTS results. One sample from January 11, 2011 was chosen when both the *T. nordenskioeldii*/*T. aestivalis* and the *T. pacifica*/*T. aestivalis* OTUs were present in the HTS sequence results (Figure 15). Hexagonal shaped cells with depressed valve faces that appear to be *T. nordenskioeldii* are visible (Figures 14a, 14b). Cells with several closely spaced marginal processes are also visible, which is indicative of *T. aestivalis* or *T. pacifica* (Figures 14c, 14d). Identifications are based on Hasle (1978).

DISCUSSION

Use of the V4 region as a barcoding gene for the genus Thalassiosira

The HTS results uncovered greater diversity than found through traditional light microscopy methods and this research supports using the 18S V4 locus as a barcode gene for the *Thalassiosira* genus of diatoms. As in Luddington et al. (2011), the 18S V4 barcode here differentiated between several species within a closely related diatom genus. The 18S V4 maximum likelihood tree resulted in one strongly supported clade consisting of *T. weissflogii*, *T. guillardii*, and *T. pseudonana*. This is similar to results in Hoppenrath et al. (2007), which found one clade consisting of *T. pseudonana* and *Detonula confervacea*, and another clade consisting of *T. weissflogii*, *T. guillardii* and *T. oceanica* (AF374479). The 18S V4 ML tree constructed here shows a different *T. oceanica* strain (DQ514878) which does not cluster with *T. weissflogii* and *T. guillardii*. This is in accordance with a phylogeny constructed from both SSU and partial LSU nuclear DNA by Alverson et al. (2007). Finally, the larger clade found in the ML tree contains all other *Thalassiosira* species, in accordance with Kaczmarek et al. (2006), Hoppenrath et al. (2007), and Alverson et al. (2007). Overall, the ML tree constructed using the reference database sequences used here appears to accurately represent the paraphyly of this genus.

Understanding the limitations of a method is important. The reference database used here does not perfectly resolve all *Thalassiosira* species, since some species have identical sequences at this locus (*T. nordenskioldii* and *T. aestivalis*, *T. pacifica* and *T. aestivalis*), while other species are very closely related and therefore, indistinguishable (*T. rotula* and *T. gravida*). Additionally, just 31 *Thalassiosira*

species have 18S V4 sequences at this locus available in GenBank, meaning that dozens of *Thalassiosira* species do not yet have sequences at this locus. This limitation is present for all barcoding loci and will improve as more sequencing is completed for taxonomically identified species.

The mock community experiment was a valuable way to verify this method and validate the bioinformatics pipeline. While the trimming performed here was very stringent, the mock community results validated the accuracy of the sequence processing pipeline, particularly with regards to removal of chimeric sequences, thus enabling accurate interpretation of these field sample data. Not only did this verification experiment shed light on 18S copy number variation, but it allowed for easy exploration of chimera contamination in the results and demonstrated the amount of technical variation inherent to this method.

A significant potential limitation of the HTS approach is variation in 18S copy number variation. 18S gene copy number can vary from 1 to 12,000 in phytoplankton species, and more specifically, can vary by an order of magnitude between *Thalassiosira* species (Zhu et al. 2005) and is not known for the large majority of *Thalassiosira* species. Copy number variation was seen in the mock community results. Mock community sequence percent composition did not equal known species abundances in each community. While variation in DNA extraction or amplification could cause these differences in expected versus actual results, the triplicate extraction samples and triplicate amplification samples were not significantly different from each other. Therefore, small subunit (SSU) rDNA copy number is likely partially responsible for this inequality. While triplicate variation in mock communities 3 and 4

is not statistically significant, it is greater than triplicate variation seen in mock communities 1 and 2, which is minimal. This variation is too large to be easily explained by SSU rDNA copy number, and a satisfactory alternate explanation is not readily apparent. Other factors such as PCR bias and sequencing bias, or methodological error may be at play. As a result, care is necessary in interpreting SSU rDNA sequence abundance for diatoms, as sequence abundance is not likely to reflect species abundance.

Chimeric sequences were found in the mock community sequences results. When species were identified which had not been added, it was very easy to pinpoint the issue. If a mock community control did not exist, this problem may have been overlooked in field samples. Chimeric sequences can arise during PCR (Smyth et al. 2010) if the elongation step is aborted while a sequence has only partially been extended. If this partial sequence misprimers another sequence during a following PCR cycle, the resulting sequence can be partially from one species and partially from another. Therefore, it is valuable to maintain a control when sequencing field samples to help screen for issues such as chimeric sequences and running final OTUs through a chimera filter such as UCHIME is recommended.

Finally, the mock community demonstrated the technical replication of this method. The triplicate amplifications of a mock community sample and a field sample all resulted in identical species compositions. Additionally, our triplicate mock community extractions also all resulted in identical species compositions. When compared to other next generation desktop sequencers, Illumina MiSeq generates the most data with the lowest error rate (Loman et al. 2012). Canesi and Ryneearson (in

press) tested triplicate extraction, amplification and Illumina Miseq sequencing with 28S ribosomal DNA from *Skeletonema* diatoms and found low levels of variation (coefficient of variance less than 15%) were associated with Illumina sequencing, which is why triplicate sequencing tests were not completed in this study.

Thalassiosira diversity

The diversity of *Thalassiosira* species in Narragansett Bay is greater than previously realized. The fifteen species found through HTS represents a five-fold increase in number of *Thalassiosira* species found using LM during the same time period. Eight of the fifteen species identified using HTS had not been historically documented in the Narragansett Bay Long-Term Phytoplankton Time Series. Realistically, seven new *Thalassiosira* species are identified here for Narragansett Bay and one new species record (*T. tumida*) is questionable, which will be discussed below.

If all *Thalassiosira* species recorded using both light microscopy and high throughput sequencing in the Phytoplankton Time Series from 1959 through the present are tallied, 18 *Thalassiosira* species are known to exist in this estuary. This is comparable to *Thalassiosira* species diversity found in other global estuaries. A study by Hoppenrath et al. (2007) using light and scanning electron microscopy methods found 21 *Thalassiosira* species in the North Sea. Similarly, using light and scanning electron microscopy methods, Harris et al. (1995) identified 18 *Thalassiosira* taxa in a Scottish sea loch. The results here are comparable to both the results from Hoppenrath et al. (2007) and Harris et al. (1995) but were obtained without using scanning

electron microscopy. The 18S V4 barcode can assess diversity within a closely related diatom genus similarly to SEM methods, but less time and expertise is required.

Several of the newly recorded species in Narragansett Bay are small (<15µm) (*T. guillardii*, *T. minima*, *T. oceanica*, *T. profunda*; Table 5) which may explain why they were not identified using LM at 100x. Hasle (1978) states that *T. profunda* is not identifiable through LM. However, their presence in an estuary is not unprecedented. *T. guillardii*, the most frequent species seen in these results, is very frequent and abundant in a Spanish estuary (Trigueros et al. 2001). *T. guillardii* is also recorded in several British estuaries (Belcher and Swale 1986). *T. oceanica* has been identified in Loch Creran in Scotland (Harris et al. 1995). Some of the larger newly recorded species for Narragansett Bay are also observed in other estuaries. *T. profunda*, *T. minima*, and *T. tenera* are recorded in British estuaries (Belcher and Swale 1986) and *T. angulata*, and *T. concaviuscula* have been collected in Loch Creran, Scotland (Harris et al. 1995).

The presence of *T. tumida* in Narragansett Bay is surprising. It was only present in 1 out of 80 field samples and was the least frequent species in the HTS results. This species is known to only exist in the Southern Ocean (Tomas 1997). While this could suggest that the range of this species is greater than previously thought, the sample with *T. tumida* was collected in the summer (6/19/2013) when water temperatures was 20.0°C, well above the recorded tolerance of this species. It is more likely that another *Thalassiosira* species shares the same 18S V4 sequence as *T. tumida* at this locus, similar to the very closely related 18S V4 sequences for *T. rotula*

and *T. gravida* also in these results. Since 18S V4 sequences are lacking for many *Thalassiosira* species, it is likely that this OTU was incorrectly assigned here.

Historical data comparisons

Since this study builds upon a long running time series, it is valuable to compare these most recent HTS data with the historical record. Our recent data both include species that were important in the past and exclude some historically recorded species. Three species (*T. hyalina*, *T. kushirensis* and *T. decipiens*) recorded historically in the Narragansett Bay Long-Term Time Series were not detectable using our 18S HTS methods (Table 4). Two of these species (*T. kushirensis* and *T. decipiens*) do not have reference sequences at the 18s V4 locus and were therefore not included in the reference database used in this study. A 333bp partial 18S V4 sequence does exist in GenBank for *T. hyalina* (JX437383.1), which is shorter than the amplicon used here. If reference sequences for these species are added in GenBank, this data can be tested for their presence. Our results did include sequences which were not assigned taxonomy from the reference database. These unidentified sequences may include additional *Thalassiosira* species, but also contain other phytoplankton species. The primers used here amplify other diatoms and raphidophytes (as in the mock community results presented here), and Narragansett Bay is home to numerous diatom genera, which were also amplified in the field samples but not analyzed for this study.

Of the historically recorded species that were found in the recent HTS results, *T. nordenskioeldii* is perhaps the most notable. As the fifth most abundant diatom

species from 1959-1980, Karentz and Smayda (1984) identified *T. nordenskioeldii* as moderately persistent and as a characteristic winter species in Narragansett Bay. From 1959-1980, *T. nordenskioeldii* occurred every year, although annual abundance appeared to vary on a 5 year cycle. This persistent pattern contrasts with more recent results. From 1999-2008, *T. nordenskioeldii* was the eighth most abundant species in LM counts (Windecker 2010) and 20th most frequent species. *T. nordenskioeldii* no longer appears every year; it is absent from LM counts in 1999, 2006 and 2013.

Our HTS data can provide additional information regarding the recent frequency and seasonality of *T. nordenskioeldii* from December 2008-December 2014. Unfortunately, while our 18S V4 data could not be used to distinguish between *T. nordenskioeldii* and *T. aestivalis*, the environmental data associated with these sequences does help differentiate the species to some degree. The temperature ranges for *T. nordenskioeldii* and *T. aestivalis* do not overlap very much. *T. nordenskioeldii* is a cold water species (3.1-15°C; Karentz and Smayda 1984) while *T. aestivalis* is a temperate species. The temperature range for the *T. nordenskioeldii*/*T. aestivalis* OTU was 0.5-8.5°C, suggesting that the species observed here may be *T. nordenskioeldii*. The preserved cell images (Figure 14) also include *T. nordenskioeldii*. While our results are unable to definitively distinguish *T. nordenskioeldii* from *T. aestivalis*, these additional data make it more plausible that this OTU may be only *T. nordenskioeldii*. Since *T. aestivalis* has not historically been identified in the Phytoplankton Time Series, we are unable to confirm its identification and occurrence in Narragansett Bay.

In our HTS results, the *T. nordenskioeldii*/*T. aestivalis* OTU was not present from 2012-2013, and was only the eighth most frequent species identified in the *Thalassiosira* genus. The absence from 2012-2013 is in accordance with the LM count data, validating the decrease in persistence for this species. While this genetic analysis does not include cell abundance, it is known that mean total diatoms in the winter at Station II have displayed a downward trend from 1959-1980 (Smayda 1998). Windecker (2010) notes that several notable diatom species, including *Skeletonema* and *Thalassiosira*, declined dramatically between 1959-1980 and 1999-2008. It is possible that this trend continued through 2014, and is reflected by the decreased frequency of *T. nordenskioeldii*.

Other species with historic records in Narragansett Bay are *T. rotula*, *T. pseudonana*, and *Thalassiosira* sp. *T. rotula* was the 18th most abundant phytoplankton species and 3rd most abundant *Thalassiosira* species from 1959-1980 (Karentz and Smayda 1984). From 2008-2014, it was still very common and the *T. rotula*/*T. gravida* OTU appeared annually in the HTS results and was more likely to occur in the spring and summer. *T. rotula* historically displayed a yearly maximum in the fall. *T. gravida* was less abundant than *T. rotula* in the 1959-1980 time series records (36th most abundant phytoplankton; 5th most abundant *Thalassiosira*) but no seasonality information is given in Karentz and Smayda (1984). The LM counts from December 2008 – December 2014 include records of *T. rotula*/*T. gravida* during all months with the exception of February with the highest abundances occurring in December and January. So, while this OTU is more likely to occur in the spring and summer, highest abundances are now seen in the winter. This appears to be an

example of a species with a broad thermal tolerance that can maintain a low population year round, punctuated by opportunistic, winter blooms. These winter *T. rotula*/*T. gravida* blooms do not appear to be annual and are recorded in the LM counts only for 2011, 2012, and 2014. This contrasts with the historic fall abundance peaks, and perhaps represents a shift in the timing of this species. Alternatively, since seasonality is not specified historically for *T. gravida*, this possibly may not represent a shift but rather the presence of *T. gravida* with *T. rotula*.

T. pseudonana occurred frequently in the HTS results and is also an important model organism with a fully sequenced genome (Armbrust et al. 2004). It was the 39th most abundant phytoplankton species and 6th most abundant *Thalassiosira* species recorded in the time series from 1959-1980 (Karentz and Smayda 1984). Since *T. pseudonana* was not one of the top numerically dominant species observed from 1959-1980, there is no published data regarding the timing or seasonality of this species. However, this is the last published occurrence of *T. pseudonana* in the time series. Likely due to its small size, it has not been recorded in the time series LM counts since 1999. Globally, earlier references only recorded this species rarely (i.e. Muyllaert and Sabbe 1996, Belcher and Swale 1986). More recently, however, *T. pseudonana* was recorded as a bloom forming species in the Bilbao estuary (Hevia-Orube et al. 2015) and a dominant spring taxon in Chesapeake Bay (Marshall et al. 2006). Our results combined with these more recent observations suggest that this species could be an important or at least a more frequent contributor to estuarine phytoplankton communities than currently recognized.

The final historic *Thalassiosira* record of note is *Thalassiosira* sp., the sixth most abundant phytoplankton species (2nd most abundant *Thalassiosira*) from 1959-1980 (Karentz and Smayda 1984). This species was characterized as a small (~10µm in diameter), unidentified *Thalassiosira* species that appeared in Narragansett Bay beginning in 1967 and became both frequent and common. Our HTS results identified several small, very frequent *Thalassiosira* species that had not been previously identified in the Phytoplankton Time Series, including *Thalassiosira guillardii* (4-14µm) and *Thalassiosira minima* (5-15µm), either of which may be the species discussed historically.

Factors affecting species composition

Seasonal patterns are apparent when looking at individual species (as in the discussions of *T. nordenskioeldii* and *T. rotula/T. gravida* above) but as whole, seasonality does not fully explain *Thalassiosira* community composition (Figure 10). Field samples grouped into three rough seasonal assemblages by month, but when comparing by sample day, these patterns are difficult to ascertain. The dendrogram based on Bray-Curtis similarity breaks the samples down into two main groups at only a 35% similarity level. One group contains most of the summer sample dates while the other contains most of the winter/spring sample dates. Fall sample dates are spread between both groups. This pattern is not very strong and may be driven by the seasonality of a few key species, while other sporadic species and persistent species may dilute the seasonality signal of the entire community. The contributions of the seasonal species (*T. nordenskioeldii*, *T. eccentrica*, *T. punctigera*, *T. rotula/T. gravida*) are perhaps more apparent when samples are grouped by month, explaining why the

three seasonal groups are more apparent in Figure 10 than in Figure 11, which considers samples individually. Temperature explains 24.6% of the variation in community composition according to the BEST analysis, which leaves the majority of the variation due to other factors.

If seasonality does not entirely explain the community composition of the *Thalassiosira* species seen in Narragansett Bay, then what factors control the patterns reported here? Additional factors not analyzed in this study include large-scale climatic processes, top-down control, other loss factors and biological competition. For example, larger scale climatic processes including the Gulf Stream and the North Atlantic Oscillation are known to impact size and timing of Narragansett Bay summer *Skeletonema* blooms (Borkman 2009, Smayda and Borkman 2009). These regional ocean-atmospheric patterns may also impact other diatoms, including *Thalassiosira* species. On a much smaller scale, microzooplankton grazers consume an average of 96% of primary production in Narragansett Bay (Lawrence and Menden-Deuer 2012). Grazers also preferentially select particular prey which in turn can structure the phytoplankton community. Microzooplankton in Chesapeake Bay selectively graze on picophytoplankton ($<2\ \mu\text{m}$) (Sun et al. 2007). The species in this study range in size from $1.8\mu\text{m}$ - $186\mu\text{m}$, and may be under differential grazing pressure. Another noteworthy annual grazer in Narragansett Bay is *Mnemiopsis leidyi*. *M. leidyi* exerts significant control on the plankton community in Narragansett Bay. In four years out of a six year study (1972-1977), a summer *M. leidyi* bloom was correlated with rapid declines in both the zooplankton and phytoplankton communities and variations in the summer abundance of diatom *Skeletonema* sp. correlated with the size of the *M. leidyi*

pulse (Deason and Smayda 1981). *M. leidy* blooms may be occurring earlier in the spring and increasing in abundance as water temperatures rise with climate change (Sullivan et al. 2001), which may impact *Thalassiosira* species.

Other loss factors affecting diatom communities are cell lysis, sinking and advection (as reviewed in Sarthou et al. 2004). These three factors may be structuring the *Thalassiosira* population in Narragansett Bay as these three processes can differentially affect species based on factors including size, density, and morphology (Smayda 1969, Veldhuis et al. 2001, Miklasz and Denny 2010). Spore formation in particular contributes to *Thalassiosira* loss through sinking (as reviewed by McQuoid and Hobson 1996). Advection can also seed diatom populations from surrounding water masses. Benthic diatom populations and pelagic diatom populations are both able to seed diatom blooms, but may result in divergent population structures (McQuoid and Godhe 2004), which may explain the lack of clear seasonal *Thalassiosira* assemblages observed here.

Finally, only one diatom genus was examined here while at least 30 diatom genera have been observed in the Long-Term Phytoplankton Time Series, along with at least 10 dinoflagellate genera and 10 flagellate genera (Windecker 2010). The additional species that make up the entire phytoplankton community could affect the *Thalassiosira* community structure through resource competition. Alternately, diatoms may co-occur through resource partitioning. For example, metatranscriptome data from diatoms in Narragansett Bay revealed that *Skeletonema* sp., the most abundant diatom in the bay, and *T. rotula* utilize different nitrogen and phosphate metabolic pathways, perhaps allowing these species to partition resources (Alexander et al.

2015). These complex environment-species-species interactions were not examined in this study, but clearly are a part of the Narragansett Bay ecology. Future work on community wide processes in Narragansett Bay may provide insight into additional controls on *Thalassiosira* populations.

Conclusions

The high throughput sequencing approach using the 18s V4 locus is a powerful tool for exploring diversity and seasonal occurrence patterns for diatom genera such as *Thalassiosira* that harbor great species diversity and are morphologically similar. *Thalassiosira* species diversity in Narragansett Bay is greater than previously recognized. Much of this newly found diversity can be attributed to several smaller sized species that are very frequent and have been overlooked using light microscopy methods. *Thalassiosira* species have a rough seasonal pattern grouped by winter-spring, summer and fall, and only 25% of the *Thalassiosira* species composition is explained by the physical and chemical environmental factors in this study. Other factors, such as large scale climactic processes, predation effects, sinking rates, autolysis and community competition, may be contributing to *Thalassiosira* species composition in Narragansett Bay. In comparison to historical data, the frequency of important bloom species such as *T. nordenskioeldii* may be in decline, while *T. rotula* timing may have shifted.

TABLES

Table 1: Five candidate diatom barcodes were considered for this study. For each potential barcode, the reference, primer pair and number of *Thalassiosira* species available in GenBank are given.

Barcode	Reference	Forward Primer	Reverse Primer	# Species in GenBank
18S (V4 region)	Zimmerman et al. 2011, Luddington et al. 2012	D512 ATTCCAGCTCCAAT AGCG	D978 GACTACGATGGTAT CTAATC	31
ITS2	Chappell et al. 2013	RCGAAYTGCAGAA CCTCG	TACTYAATCTGAGA TYCA	5
ITS2	Moniz and Kaczmaraska 2010, White et al. 1990	TCCGTAGGTGAAC CTGCGG	TCCTCCGCTTATTG ATATGC	5
18S (V9 region)	Amaral-Zettler et al. 2007	1380F CCCTGCCHTTTGTA CACAC	1510R CCTTCYGCAGGTTC ACCTAC	21
18s (V7 region)	Gast et al. 2004	960F GGCTTAATTTGACT CAACRCG	Diatom1R ATGCAGATTGATG ATCTGCG	30

Table 2: Accession numbers, references, and strain (if known) are given for all of the reference 18S V4 genes used in this study. Sequences were accessed through NCBI GenBank. Numbers given after *Thalassiosira* species with >1 sequence in this reference database are used to differentiate sequences.

Species	Accession Number	Reference	Strain (if known)
<i>D. brightwellii</i>	FJ266033	Rynearson et al., 2009	
<i>H. akashiwo</i>	KT163009	Harvey et al., 2015	
<i>S. marinoi</i>	AJ632216	Sarno et al., 2005	
<i>S. menzellii</i>	AJ535168	Medlin and Kaczmarek, unpublished, 2002	CCMP 790
<i>T. aestivalis 1</i>	DQ093369	Kaczmarek et al., 2006	CCMP 975
<i>T. aestivalis 2</i>	DQ514873	Alverson et al., 2007	CCMP976
<i>T. angulata</i>	DQ514867	Alverson et al., 2007	BEN02-35
<i>T. anguste-lineata 2</i>	DQ514865	Alverson et al., 2007	BEN02-30
<i>T. anguste-lineata 1</i>	AJ810854	Hoppenrath et al., 2007	
<i>T. antarctica</i>	DQ514874	Alverson et al., 2007	CCMP982
<i>T. concavuscula 1</i>	AJ810857	Hoppenrath et al., 2007	
<i>T. concavuscula 2</i>	HM991689	Park, Jung, Ki and Lee, unpublished, 2010	
<i>T. eccentrica 1</i>	DQ514868	Alverson et al., 2007	BER02-9
<i>T. eccentrica 2</i>	X85396	Medlin et al., 1996	
<i>T. gravida 1</i>	JX069323	Whittaker et al., 2012	
<i>T. gravida 2</i>	JX069325	Whittaker et al., 2012	
<i>T. gravida 3</i>	JX069333	Whittaker et al., 2012	
<i>T. guillardii 1</i>	AF374478	Armbrust and Galindo, 2001	CCMP988
<i>T. guillardii 2</i>	DQ514869	Alverson et al., 2007	CC-03-04
<i>T. guillardii 3</i>	DQ514875	Alverson et al., 2007	CCMP988
<i>T. minima 1</i>	DQ514876	Alverson et al., 2007	CCMP990
<i>T. minima 2</i>	JN934676	Balzano, Fourvil, Siano, Percopo, Chanoine, Sarno and Vaulot, unpublished, 2011	
<i>T. minuscula 1</i>	DQ514887	Alverson et al., 2007	FB02-31
<i>T. minuscula 2</i>	HM991694	Park, Jung, Ki and Lee, unpublished, 2010	
<i>T. nordenskiöldii 1</i>	DQ093365	Kaczmarek et al., 2006	CCMP997
<i>T. nordenskiöldii 2</i>	JF794045	Balzano et al., 2012	
<i>T. oceanica</i>	DQ514878	Alverson et al., 2007	CCMP1001
<i>T. cf. pacifica</i>	DQ514888	Alverson et al., 2007	FB02-35
<i>T. profunda 1</i>	AM235383	Kaczmarek et al., 2006	POMX
<i>T. profunda 2</i>	KC284713	Alverson 2013	
<i>T. pseudonana 1</i>	AF374481	Armbrust and Galindo, 2001	CCMP1335
<i>T. pseudonana 2</i>	DQ093367	Kaczmarek et al., 2006	
<i>T. punctigera</i>	AJ810856	Hoppenrath et al., 2007	CCMP1007
<i>T. rotula 1</i>	AF374480	Armbrust and Galindo, 2001	

<i>T. rotula</i> 2	AF462058	Cordevant and Lange, unpublished, 2001	CCMP1647
<i>T. rotula</i> 3	DQ514884	Alverson et al., 2007	CCMP1647
<i>T. rotula</i> 4	JX069326	Whittaker et al., 2012	CCMP1812
<i>T. rotula</i> 5	JX069328	Whittaker et al., 2012	
<i>T. rotula</i> 6	X85397	Medlin et al., 1996	CCMP1018
<i>T. tenera</i> 1	AJ810858	Hoppenrath et al., 2007	
<i>T. tenera</i> 2	HM991701	Park, Jung, Ki and Lee, unpublished, 2010	
<i>T. tumida</i>	DQ514890	Alverson et al., 2007	
<i>T. weissflogii</i> 1	AF374477.2	Armbrust and Galindo, 2001	LA09-20
<i>T. weissflogii</i> 2	KF733529	Hu et al., 2014	
<i>T. weissflogii</i> 3	GU594641	Cleary et al., 2012	
<i>T. weissflogii</i> 4	AY485445	Damste et al., 2004	

Table 3: Percent composition of each mock community (MC1-4). In each mock community, 9 species were added in varying percentages of cell abundance. The strains and location of isolation are given for each species.

Species	Strain	Location	MC1	MC2	MC3	MC4
<i>T. anguste-lineata</i>	NBN	Narragansett Bay	9.1	18.4	13.9	2.5
<i>T. weissflogii</i>	CCMP 1336	Long Island Sound	9.1	5.1	13.9	2.5
<i>T. rotula</i>	NBA	Narragansett Bay	18.2	20.4	27.8	47.5
<i>T. pseudonana</i>	CCMP 1007	Chincoteague, Virginia	9.1	5.1	13.9	22.5
<i>D. brightwellii</i>	MV1	Martha's Vineyard	10.9	10.2	13.9	5.0
<i>S. marinoi</i>	CCMP 1332	Connecticut	10.9	10.2	4.2	5.0
<i>S. menzelii</i>	CCMP 793	Falmouth, Massachusetts	10.9	10.2	4.2	5.0
<i>H. akashiwo</i>	CCMP 3374	Narragansett Bay	10.9	10.2	4.2	5.0
<i>H. triquetra</i>	CCMP 448	Falmouth, Massachusetts	10.9	10.2	4.2	5.0

Table 4: *Thalassiosira* species identified historically (1959-1980) through LM counts and recently (2008-2014) using light microscopy (LM) and high throughput sequencing (HTS). X = identified; * = indistinguishable from other species by this method; - = unable to be detected (sequence not available in reference database); blank = not detected by these methods. Note: *T. tumida* was recovered in sequence results but this record is questionable and not included in the total species count for Narragansett Bay. See discussion on page 27.

	Time Series 1959 - 1980 and 1999-2008	Time Series Dec. 2008 - Dec. 2014	HTS Dec 2008 - Dec 2014
<i>T. aestivalis</i>			*
<i>T. angulata</i>			X
<i>T. anguste-lineata</i>	X		X
<i>T. concaviuscula</i>			X
<i>T. decipiens</i>	X		-
<i>T. eccentrica</i>	X		X
<i>T. gravida</i>	X	*	*
<i>T. guillardii</i>			X
<i>T. hyalina</i>	X		-
<i>T. kushirensis</i>	X		-
<i>T. minima</i>			X
<i>T. nordenskioeldii</i>	X	X	*
<i>T. oceanica</i>			X
<i>T. pacifica</i>	X		X
<i>T. profunda</i>			X
<i>T. pseudonana</i>	X		X
<i>T. punctigera</i>	X	X	X
<i>T. rotula</i>	X	*	X
<i>T. spp</i>	X	X	
<i>T. tenera</i>			X
<i>T. tumida</i>			X

Table 5: Cell diameter in microns and species range is presented for all *Thalassiosira* species recorded in the 2008-2014 sequence data. All information is from Tomas (1997) except where noted.

Species	Cell diameter (μm)	Range
<i>T. aestivalis</i>	14-56	Warm water region to temperate
<i>T. angulata</i>	12-39	Cold to temperate waters (Hoppenrath et al. 2007)
<i>T. anguste-lineata</i>	14-78	Cosmopolitan
<i>T. concaviuscula</i>	14-56 (Hoppenrath et al. 2007)	Neritic, cold to temperate waters (Hoppenrath et al. 2007)
<i>T. eccentrica</i>	15-110	Cosmopolitan (exclusive polar zones)
<i>T. guillardii</i>	4-14 (Hasle 1978)	Inland and marine/brackish coastal waters (Hasle 1978)
<i>T. minima</i>	5-15	Cosmopolitan (exclusive polar zones)
<i>T. nordenskiöldii</i>	10-50	Cold to temperate, northern hemisphere
<i>T. oceanica</i>	3-12	Mainly warm water region
<i>T. pacifica</i>	7-46	Cosmopolitan (exclusive polar zones)
<i>T. profunda</i>	1.8-5 (Belcher and Swale 1986)	Cosmopolitan (Hasle 1978)
<i>T. pseudonana</i>	2.3-5.5	Cosmopolitan
<i>T. punctigera</i>	40-186	Warm water region to temperate
<i>T. rotula</i>	8-55	Cosmopolitan
<i>T. tenera</i>	10-29	Cosmopolitan (Hasle and Fryxell 1977)
<i>T. tumida</i>	21-137	Southern cold water region

Table 6: Multivariate correlation of *Thalassiosira* species composition with seven environmental factors (surface temperature, surface salinity, average daily PAR, DIP, DIN, Si and chlorophyll *a* concentration) analyzed with BIOENV BEST using PRIMER v6.1.6 (PRIMER-E Ltd.).

Number of Variables	Correlation	Environmental Variables
1	0.246	Surface Temperature
2	0.232	Surface Temperature, log (DIP)
3	0.224	Surface Temperature, Average daily PAR, log (Si)
2	0.223	Surface Temperature, Average daily PAR
3	0.223	Surface Temperature, Average daily PAR, log (DIP)
2	0.219	Surface Temperature, Surface Salinity
2	0.217	Surface Temperature, log (Si)
3	0.208	Surface Temperature, Surface Salinity, log (DIP)
4	0.207	Surface Temperature, Average daily PAR, log (DIP), log (Si)
4	0.200	Surface Temperature, Surface Salinity, Average daily PAR, log (DIP)

FIGURES

Figure 1: The geographic location of the sampling site for the Narragansett Bay Long Term Phytoplankton Time Series is located in the West Passage of Narragansett Bay, south of Quonset Point and east of Wickford Harbor. Water depth at this location is about 8m.

Figure 2: Relative abundance of *Thalassiosira* (percentage of the total phytoplankton cell counts) was calculated from LM cell counts of weekly surface phytoplankton samples from the Long Term Phytoplankton Time Series from December 2008 – December 2014. *Thalassiosira* relative cell abundance varies from 0% to over 75% of the total phytoplankton community in Narragansett Bay and peaks in abundance vary seasonally and annually.

Figure 3: Rarefaction curves of operational taxon unit (OTU) richness (A) and species richness (B) for each of the 82 field sample sequence libraries. Figure A gives all OTUs generated in this study whereas Figure B is only showing only *Thalassiosira* species richness. The difference in figures is due to multiple OTUs corresponding to one species and the fact that the primers used in the study amplified all diatoms and raphidophytes in the samples, not just *Thalassiosira* species.

Figure 4: A maximum likelihood tree of a 1552bp fragment of *Thalassiosira* 18s sequences was constructed with Paup* 4.0 b10 (Swofford 2003). Numbers following species correspond to records in Table 2. Two *Skeletonema* species were used as an outgroup. Sequences were downloaded from GenBank and trimmed to the amplicon region used in this study. Bootstrap values (>50) are given at the nodes. Genetic distance in substitutions per site is provided by the scale bar at the bottom. Asterisks denote reference sequences recovered from field HTS results.

Figure 5: A maximum likelihood tree of *Thalassiosira* 18S V4 sequences included in the curated reference database was constructed with Paup* 4.0 b10 (Swofford 2003). Numbers following species names correspond to records in Table 2. Two *Skeletonema* species were used as an outgroup. Sequences were downloaded from GenBank and trimmed to the amplicon region used in this study. Bootstrap values (>50) are given at the nodes. Genetic distance in substitutions per site is provided by the scale bar at the bottom. Asterisks denote reference sequences recovered from field HTS results. Brackets with asterisks denote groups of sequences from different species that are indistinguishable at this locus.

Figure 6: Expected and high-throughput sequence results for all four mock community experiments are shown as % composition. Expected community composition from cell counts of each species is given in the leftmost column of each graph. Triplicate extraction sequence results for each mock community are shown as A, B and C in each graph. Observed mock community sequence data is significantly different than the expected composition (χ square goodness-of-fit test, $p < 0.001$). Within all four of the triplicate mock communities (A, B, C), composition did not vary significantly (ANOVA, $p > 0.05$).

Figure 7: Time series occurrence of 15 *Thalassiosira* species detected by high throughput sequencing. Samples are monthly for 2008-2013 and bi-weekly for 2014. Sample dates are represented in rows. Years are labeled and shaded in grey. Species are given in columns, where the label is at the top of the chart and shading indicates presence. White boxes indicate absence of that species at that sampling time point. Seasons are represented by color (see key). Species are arranged according to the Bray Curtis Similarity dendrogram constructed in PRIMER v6.1.6 (PRIMER-E Ltd.) shown in Figure 8.

Figure 8: A Bray-Curtis similarity dendrogram is given for species composition constructed in PRIMER v6.1.6 (PRIMER-E Ltd.), indicating which species are likely to co-occur. Similarity is displayed in the y-axis of the dendrogram, from 0-100, where 100 indicates complete similarity and 0 indicates no similarity. Species names are abbreviated as follows: T. aest./nord. = *T. nordenskioldii*/*T. aestivalis* OTU; T. pac./aest. = *T. pacifica*/*T. aestivalis* OTU; T. rot./grav. = *T. rotula*/*T. gravida* OTU.

Figure 9: The frequency of occurrence in field samples of each species identified in the HTS results. Ten species have a frequency of <0.2 and two species have a frequency >0.4. Species names are abbreviated as follows: *T. nord./aest.* = *T. nordenskiöldii/T. aestivalis* OTU; *T. pac./aest.* = *T. pacifica/T. aestivalis* OTU; *T. rot/T. grav.* = *T. rotula/T. gravida* OTU.

Figure 10: Multi-Dimensional Scaling plot (MDS) based on Bray-Curtis similarity of monthly averaged field sample presence/absence data averaged and normalized by sampling effort. Green rings denote 60% Bray-Curtis similarity. Four seasonal groups are apparent at the 60% similarity level: winter (January-April), spring (May-June), summer (July-September), fall (October-December).

Figure 12: Occurrence frequency by month is presented for eight most frequent taxa in the HTS results as labeled in each panel. Number of years each individual taxon was present in each month was summed and divided by number of years sampled over the 6 year study period. All months were sampled 7 times each, with the exception of February, March and July, which were each sampled 6 times each.

Figure 13: Observed temperature ranges (top panel) and salinity ranges (bottom panel) for fourteen of the *Thalassiosira* species recovered from the HTS data. The range for *T. tenera* is omitted since that species was only observed in one sample and therefore, does not have enough data for a range. Species names are abbreviated as follows: T. nord./aest. = *T. nordenskioeldii*/*T. aestivalis* OTU; T. pac./aest. = *T. pacifica*/*T. aestivalis* OTU; T. rot./T. grav. = *T. rotula*/*T. gravis* OTU.

Figure 14: Principal components analysis of environmental data associated with each sample date for this study. Environmental variables included are surface temperature ($^{\circ}\text{C}$), surface salinity (ppt), surface chlorophyll a (μL), average daily PAR (mmol/m^2), DIN (μM), DIP (μM), and Si (μM). Values for salinity, chlorophyll a , DIN, DIP, and Si were log transformed. Colors indicate the season of each sample (winter is January through April, spring is May through June, summer is July through September, fall is October through December). Surface temperature and average daily PAR contribute to PC2, separating winter samples from summer samples. PC1 explains 40.1% of the variance in these environmental parameters while PC1 and PC2 together explain 65.4% of the variance in the environmental parameters plotted here.

Figure 15: *Thalassiosira* spp. images from preserved slides of a sample collected on January 11, 2011. *Thalassiosira nordenskiöldii* was present in the LM cell counts from this sample and both *T. aestivalis*/*T. nordenskiöldii* and *T. pacifica*/*T. aestivalis* sequences were present in HTS results. All images taken at 200x. Scale bars (white) in lower right corners are 10µm. A. Cells in this image are likely *T. nordenskiöldii*. Hexagonal shaped cells indicate *T. nordenskiöldii* or *T. aestivalis*. Slight depressions on the valve face are visible in girdle view cell, indicative of *T. nordenskiöldii*. B. Valve faces in girdle view cells may be concave, as in *T. nordenskiöldii*. C and D. A single central process is visible in these valve view cells, which appears in *T. aestivalis*, *T. nordenskiöldii* and *T. pacifica*. Several marginal processes are seen around the diameter of all cells. Closer marginal process spacing, as in the right cell in image C, is indicative of *T. aestivalis* or *T. pacifica*.

APPENDICES

Appendix 1: Dates for 80 field sampling dates in Narragansett Bay from December 2008 through December 2014 are given with the total phytoplankton abundance and *Thalassiosira* genus abundance for that day as quantified by light microscopy cell counts. Blanks in abundance columns indicate dates that were sequenced but for which no abundance data is available. Monthly samples were chosen for 2008-2013, with the exceptions of July 2011 and February-April 2012, when samples were not collected. Bi-weekly samples were sequenced for 2014.

Date	Total Phytoplankton Abundance (cells/liter)	Thalassiosira Abundance (cells/liter)
12/9/2008	1866000	135000
1/27/2009	242000	31000
2/2/2009	207000	110000
3/3/2009	921000	717000
4/13/2009	5147000	533000
5/27/2009	1153000	0
6/23/2009	4680000	306000
7/27/2009	10784000	70000
8/10/2009	25849000	0
9/4/2009	17383000	16000
10/8/2009	541000	35000
11/23/2009	438000	67000
12/31/2009	728000	22000
1/26/2010	19612000	34000
2/2/2010	10633000	23000
3/29/2010	6085000	140000
4/13/2010	49346000	112000
5/28/2010	4942000	13000
6/15/2010	12709000	84000
7/19/2010	18144000	75000
8/20/2010	8015000	1683000
9/15/2010	599000	6000
10/5/2010	2101000	107000
11/16/2010	1474000	6000
12/30/2010	1179000	91000
1/11/2011	4991000	514000
2/1/2011	3539000	14000
3/9/2011	2840000	2000
4/20/2011	14207667	262000
5/31/2011	4361000	2000

6/6/2011	19456000	29000
8/19/2011	1861000	43000
9/6/2011	3590000	0
10/3/2011	2460000	2000
11/7/2011	454000	33000
12/7/2011	165000	17000
1/4/2012	699000	11000
5/23/2012		
6/25/2012		
7/2/2012		
8/1/2012		
9/5/2012		
10/5/2012		
11/2/2012		
1/2/2013		
1/2/2013	45200	700
2/12/2013	8272800	31000
3/12/2013	1157000	0
4/2/2013	807800	0
5/28/2013	478000	2500
6/19/2013	15745200	815000
7/15/2013	48604100	0
8/16/2013	2769200	4000
9/9/2013	5512100	147000
10/28/2013	199700	400
11/20/2013	851900	40000
12/2/2013	809000	71000
1/21/2014	987700	501000
1/27/2014	1239500	479000
2/3/2014	944000	175000
2/24/2014	889300	610000
3/10/2014	409500	0
3/31/2014	4297900	45000
4/4/2014	12428800	84000
4/21/2014	6082100	39000
5/6/2014	746000	0
5/27/2014	93800	1000
6/16/2014	6100267	0
6/23/2014	19872200	21000
7/21/2014	4286100	18000
7/28/2014	13656400	819000
8/19/2014	10642500	4000
8/25/2014	5256900	819000

9/9/2014	446000	4000
9/15/2014	900000	224000
10/6/2014	2469000	4000
10/27/2014	565000	0
11/19/2014	435000	3000
11/25/2014	288000	4000
12/8/2014	192100	32200
12/30/2014	609000	20000

Appendix 2: Dates for 80 field sampling dates in Narragansett Bay from December 2008 through December 2014 are given with the *Thalassiosira* species abundance for that day quantified by light microscopy cell counts. Blanks in abundance columns indicate dates that were sequenced but for which no abundance data is available. Monthly samples were chosen for 2008-2013, with the exceptions of July 2011 and February-April 2012, when samples were not collected. Bi-weekly samples were sequenced for 2014.

	<i>T.</i> <i>nordenskioeldii</i>	<i>T.</i> <i>punctigera</i>	<i>T. rotula/</i> <i>gravida</i>	<i>T.</i> <i>spp.</i>
12/9/2008	0	0	0	500
1/27/2009	0	0	0	0
2/2/2009	69000	0	0	0
3/3/2009	717000	0	0	0
4/13/2009	533000	0	0	0
5/27/2009	0	0	0	0
6/23/2009	0	0	0	0
7/27/2009	0	0	0	0
8/10/2009	0	0	0	0
9/4/2009	0	0	0	0
10/8/2009	0	3000	0	0
11/23/2009	0	0	0	0
12/31/2009	0	1000	0	0
1/26/2010	8000	0	0	0
2/2/2010	0	0	0	0
3/29/2010	126000	0	0	0
4/13/2010	102000	0	0	0
5/28/2010	0	0	0	0
6/15/2010	0	0	0	0
7/19/2010	0	0	0	0
8/20/2010	0	0	0	0
9/15/2010	0	0	0	0
10/5/2010	0	0	0	0
11/16/2010	0	0	0	0
12/30/2010	40000	1000	0	48000
1/11/2011	492000	0	0	15000
2/1/2011	12000	0	0	0
3/9/2011	0	0	0	0
4/20/2011	262000	0	0	0
5/31/2011	0	0	0	0
6/6/2011	0	0	0	22000
8/19/2011	0	0	0	0
9/6/2011	0	0	0	0

10/3/2011	0	0	0	0
11/7/2011	0	8000	0	0
12/7/2011	0	3000	0	0
1/4/2012	0	0	0	0
5/23/2012				
6/25/2012				
7/2/2012				
8/1/2012				
9/5/2012				
10/5/2012				
11/2/2012				
1/2/2013				
1/2/2013	0	0	0	0
2/12/2013	0	0	0	0
3/12/2013	0	0	0	0
4/2/2013	0	0	0	0
5/28/2013	0	0	0	0
6/19/2013	0	0	0	0
7/15/2013	0	0	0	0
8/16/2013	0	0	0	0
9/9/2013	0	0	0	29000
10/28/2013	0	0	0	0
11/20/2013	0	0	0	10000
12/2/2013	0	0	0	71000
1/21/2014	0	0	0	60000
1/27/2014	0	0	0	95000
2/3/2014	0	0	0	27000
2/24/2014	0	0	0	0
3/10/2014	0	0	0	0
3/31/2014	0	0	0	0
4/4/2014	0	0	0	4000
4/21/2014	39000	0	0	0
5/6/2014	0	0	0	0
5/27/2014	0	0	0	0
6/16/2014	0	0	0	0
6/23/2014	0	0	0	0
7/21/2014	0	0	0	18000
7/28/2014	0	0	0	0
8/19/2014	0	0	0	0
8/25/2014	0	0	0	0
9/9/2014	0	0	0	0
9/15/2014	0	0	0	0
10/6/2014	0	0	0	0

10/27/2014	0	0	0	0
11/19/2014	0	0	0	0
11/25/2014	0	0	0	0
12/8/2014	0	0	0	200
12/30/2014	0	0	0	0

Appendix 3: Total sequence pairs are given for each sample and the % of reads surviving after merging and filtering is given. Dates are given to identify the 80 field samples. FST = Field Sample Triplicate; MCT = Mock Community Triplicate; MC1 – MC4 = Mock Communities 1-4.

Date/Sample	# Raw Sequence Pairs	# Sequences	
		Post Processing	% Surviving
12/9/2008	255177	14521	5.69
1/27/2009	145180	8422	5.80
2/2/2009	140346	6036	4.30
3/3/2009	114711	3056	2.66
4/13/2009	196912	10008	5.08
5/27/2009	221158	7013	3.17
6/23/2009	134667	5860	4.35
7/27/2009	182865	5431	2.97
8/10/2009	120095	5502	4.58
9/4/2009	134498	5764	4.29
10/8/2009	136573	6158	4.51
11/23/2009	162485	4468	2.75
12/31/2009	129098	5985	4.64
1/26/2010	140674	10428	7.41
2/2/2010	101792	4980	4.89
3/29/2010	202189	8155	4.03
4/13/2010	146527	5081	3.47
5/28/2010	148210	4271	2.88
6/15/2010	127744	3704	2.90
7/19/2010	72164	776	1.08
8/20/2010	281234	7651	2.72
9/15/2010	137015	3447	2.52
10/5/2010	138851	3217	2.32
11/16/2010	172452	3012	1.75
12/30/2010	179573	13896	7.74
1/11/2011	245228	24069	9.81
2/1/2011	243232	19842	8.16
3/9/2011	153565	4380	2.85
4/20/2011	196164	2067	1.05
5/31/2011	197398	16015	8.11
6/6/2011	103276	172	0.17
8/19/2011	175560	20527	11.69
9/6/2011	161465	15073	9.34
10/3/2011	205795	25320	12.30
11/7/2011	212989	20778	9.76
12/7/2011	116734	7143	6.12
1/4/2012	200879	12378	6.16
5/23/2012	244447	21447	8.77
6/25/2012	181029	15415	8.52
7/2/2012	180062	23239	12.91
8/1/2012	195191	13812	7.08
9/5/2012	181376	16162	8.91
10/5/2012	198987	10935	5.50
11/2/2012	163263	5171	3.17

1/2/2013	222317	13312	5.99
2/12/2013	168643	14651	8.69
3/12/2013	155320	12097	7.79
4/2/2013	216061	11010	5.10
5/28/2013	58448	4659	7.97
6/19/2013	142475	13823	9.70
7/15/2013	178433	19408	10.88
8/16/2013	128707	3578	2.78
9/9/2013	131126	10081	7.69
10/28/2013	122585	11334	9.25
11/20/2013	122134	11088	9.08
12/2/2013	133863	10807	8.07
1/21/2014	214763	13339	6.21
1/27/2014	127159	1530	1.20
2/3/2014	170470	7509	4.40
2/24/2014	166239	7360	4.43
3/10/2014	193472	17337	8.96
3/31/2014	166761	6754	4.05
4/4/2014	202389	13906	6.87
4/21/2014	173732	12044	6.93
5/6/2014	171535	18372	10.71
5/27/2014	183364	15956	8.70
6/16/2014	161328	18090	11.21
6/23/2014	141669	11282	7.96
7/21/2014	186566	15510	8.31
7/28/2014	241130	26869	11.14
8/19/2014	186100	14919	8.02
8/25/2014	249490	23405	9.38
9/9/2014	160043	9164	5.73
9/15/2014	137876	9772	7.09
10/6/2014	115319	5202	4.51
10/27/2014	111218	3564	3.20
11/19/2014	144845	11978	8.27
11/25/2014	173187	13450	7.77
12/8/2014	124275	10316	8.30
12/30/2014	123401	5284	4.28
FST	156349	10262	6.56
FST	165973	10418	6.28
MCT	133939	9274	6.92
MCT	123498	6735	5.45
MC1	220995	25357	11.47
MC1	212757	22583	10.61
MC1	185309	21057	11.36
MC2	204383	19944	9.76
MC2	204192	11763	5.76
MC2	164451	7029	4.27
MC3	188280	3121	1.66
MC3	116071	3771	3.25
MC3	206657	15049	7.28
MC4	199419	14554	7.30
MC4	191195	12149	6.35
MC4	198349	12769	6.44

Appendix 4: Presence/absence data of all 15 *Thalassiosira* OTUs for all 80 field sample dates as obtained from 18S V4 sequencing. X's indicate presence. Species names are abbreviated as follows: *T. aest./nord* = *T. aestivalis*/*T. nordenskiöldii*; *T. angu* = *T. angulata*; *T. ang.-lin.* = *T. anguste-lineata*; *T. con.* = *T. concaviuscula*; *T. ecc.* = *T. eccentrica*; *T. guil.* = *T. guillardii*; *T. min.* = *T. minima*; *T. oce.* = *T. oceanica*; *T. pac./aest.* = *T. pacifica*/*T. aestivalis*; *T. prof.* = *T. profunda*; *T. pseu.* = *T. pseudonana*; *T. punc.* = *T. punctigera*; *T. rot./grav.* = *T. rotula*/*T. gravida*; *T. ten.* = *T. tenera*; *T. tum.* = *T. tumida*.

	<i>T. aest./nord</i>	<i>T. angu.</i>	<i>T. ang.-lin.</i>	<i>T. con.</i>	<i>T. ecc.</i>	<i>T. guil.</i>	<i>T. min.</i>	<i>T. oce.</i>	<i>T. pac./aest.</i>	<i>T. prof.</i>	<i>T. pseu.</i>	<i>T. punc.</i>	<i>T. rot./grav.</i>	<i>T. ten.</i>	<i>T. tum.</i>
12/9/2008				X	X	X	X						X		
1/27/2009						X	X		X						
2/2/2009						X	X	X	X			X			
3/3/2009	X						X		X						
4/13/2009	X						X					X			
5/27/2009						X	X				X		X		
6/23/2009						X	X			X	X		X		
7/27/2009						X	X				X		X	X	
8/10/2009											X				
9/4/2009		X				X				X	X			X	
10/8/2009							X				X	X			
11/23/2009					X	X	X					X			
12/31/2009						X	X					X			
1/26/2010						X	X								
2/2/2010						X									
3/29/2010	X					X									
4/13/2010						X	X		X						
5/28/2010				X		X	X	X				X			
6/15/2010							X				X		X		
7/19/2010		X				X					X			X	
8/20/2010														X	
9/15/2010							X			X	X				
10/5/2010				X						X	X			X	
11/16/2010						X	X				X	X			
12/30/2010	X					X	X			X	X	X	X		
1/11/2011	X					X	X		X	X	X	X	X		
2/1/2011	X					X	X					X			
3/9/2011		X				X	X				X		X		
4/20/2011	X	X				X		X							

5/31/2011					X			X		X	
6/6/2011				X	X			X	X		X
8/19/2011				X					X		
9/6/2011				X				X	X	X	X
10/3/2011					X			X	X		
11/7/2011							X			X	
12/7/2011				X	X		X			X	
1/4/2012			X	X	X		X				X
5/23/2012				X				X	X		
6/25/2012	X		X	X				X	X		
7/2/2012			X	X				X	X		
8/1/2012				X							
9/5/2012		X		X	X			X	X		
10/5/2012				X	X					X	
11/2/2012				X	X			X	X	X	
1/2/2013				X	X					X	
2/12/2013		X		X			X			X	
3/12/2013				X		X					
4/2/2013	X			X		X					X
5/28/2013	X			X		X		X			X
6/19/2013	X	X							X		X
7/15/2013	X								X		
8/16/2013				X					X		
9/9/2013		X			X				X		X
10/28/2013			X	X	X			X	X	X	
11/20/2013				X	X						
12/2/2013				X	X				X		
1/21/2014				X	X		X				
1/27/2014	X			X	X		X				
2/3/2014	X			X			X				
2/24/2014	X			X							
3/10/2014	X			X							
3/31/2014	X			X	X						
4/4/2014	X			X	X		X				
4/21/2014				X			X	X	X		X
5/6/2014											X
5/27/2014			X	X	X	X					X
6/16/2014				X	X				X		X
6/23/2014	X			X		X			X		X
7/21/2014				X	X	X					
7/28/2014	X			X					X		
8/19/2014				X	X	X			X		X
8/25/2014		X	X	X					X		

9/9/2014				X	X		X	X
9/15/2014				X	X		X	
10/6/2014			X	X	X		X	X
10/27/2014		X		X	X	X	X	
11/19/2014				X	X	X		
11/25/2014	X	X						X
12/8/2014					X			
12/30/2014				X	X			X

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