Barnegat Bay—Year 2

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Impacts of Invasive Sea Nettles (*Chrysaora quinquecirrha*) and Ctenophores on Planktonic Community Structure and Bloom Prediction of Sea Nettles Using Molecular Techniques.

Final Report 2013

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List of Abbreviations

BAY-WIDE SITE	ABBREVIATION
Metedeconk W	MW
Metedeconk E	ME
Silver Bay W	SBW
Silver Bay E	SBE
Toms River W	TRW
Toms River E	TRE
Forked River W	FRW
Forked River E	FRE
Double Creek W	DCW
Double Creek E	DCE
Harvey Cedars W	HCW
Harvey Cedars E	HCE
Westeconk W	WW
Westeconk E	WE
Tuckerton W	TW
Tuckerton E	TE

LAGOON SITES	ABBREVIATIONS
Point Pleasant Lagoon	PPL
Kettle Creek Lagoon	KCL
Chadwick Isle Lagoon	CIL
Toms River Lagoon	TRL
Cedar Creek Lagoon	CCL
Harvey Cedars Lagoon	HCL
Beach Haven Lagoon	BHL
Tuckerton Lagoon	TL

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Appendix A: CTAB/NaCl DNA Extraction Protocol
Appendix B: QAPP as electronic Attachment
Appendix C: Summarized Data Files, as electronic Attachment
Appendix D: Blast Search Summary

Statement of the Problem: Gelatinous zooplankton are increasing in marine ecosystems worldwide as a result of climate change, species introductions, and a number of anthropogenic alterations to coastal food webs that favor jellyfish and ctenophores (Sullivan et al., 2001; Purcell and Decker, 2005; Hay, 2006; Kirby and Beaugrand, 2009; Kirby et al., 2009; Richardson et al., 2009). One important driver of the shift towards greater abundance of gelatinous zooplankton is the construction of hard surfaces such as bulkheads, docks, and other shoreline modifications that provide suitable habitat for scyphozoan polyps (Hoover and Purcell 2009). Another anthropogenic action that favors gelatinous zooplankton is the increase in eutrophication resulting from coastal nutrient loading, which fuels bottom hypoxia in relatively shallow systems. Jellyfish are highly tolerant of low dissolved oxygen conditions and therefore benefit from the impacts of hypoxia on their prey species which are either easier to catch in hypoxic waters or are more concentrated in the overlying normoxic waters. In either situation, jellyfish experience elevated energy intake and reproductive capacity, which ultimately contributes to population growth (Purcell et al., 2001; Grove and Breitburg, 2005; Purcell et al., 2007). Both of these drivers of gelatinous zooplankton increases are prevalent in the Barnegat Bay system.

Scope of Project

During this project we are evaluating the following Objectives:

1. **Assess early stage *Chrysaora* ephyrae and planktonic larvae through molecular identification of species specific DNA in water samples.**
2. **Assess the distribution of gelatinous zooplankton and impacts on planktonic community structure.**
3. **Assess the distribution and density of settling *Chrysaora* polyps and development of resting podocysts.**
4. **Assess the diet of *Chrysaora* through dissection and molecular analysis.**

Project Methods

Objective 1: Assess early stage *Chrysaora* ephyrae and planktonic larvae through molecular identification of species specific DNA in water samples.

Our methods for this objective include sampling triplicate 20 liter water samples from sites in Barnegat Bay. Samples are field sieved through 500 µm and 100 µm filters. Samples are then identified using molecular PCR reactions to identify *Chrysaora* DNA. This sampling approach allows us to assess early ephyra (500 µm) and larvae and eggs (100 µm). In 2013, we collected 312 water samples (20 liters each) for DNA analysis which were split into 500 µm and 100 µm fractions using stacked filtering units. Of these 312 water samples, 240 were from bay-wide collections and 72 were from lagoonal collections. Detection and analysis of DNA occurred in the detailed methods laid out in the QAPP.

Objective 2: Assess the distribution of gelatinous zooplankton and impacts on planktonic community structure.

To complete Objective 2, we have divided our sampling approach into monthly bay-wide surveys, and targeted coastal development sampling (i.e., coastal lagoons developments).

a. Monthly assessment in Barnegat Bay

We have established 16 sampling sites in Barnegat Bay which we sampled monthly between May and September 2013 (Fig. 1). This provides essential data regarding the spatial and temporal distribution throughout Barnegat Bay. A combined sampling program of lift nets and plankton tows were used to assess the distribution of adult and juvenile sea nettles, as well as other zooplankton comprising the pelagic community. These data provide the broad based distribution of organisms and were used to identify potential relationships among various zooplankton groups (e.g., food web interactions: sea nettles vs. comb jellies, copepods, fish larvae, etc…).

b. Targeted coastal development sampling

Sea nettle polyps are known to settle on various hard substrates, and increasing coastal development has increased the amount of available surface for settling larvae. As these polyps are the ultimate source of new adults, we sampled developed regions in Barnegat Bay to gain an understanding of the distribution within these communities. Eight sites were sampled in June, July, and August using lift nets and plankton nets to assess adult and juvenile sea nettles (Fig. 1). Water samples (Objective 1) were also collected at these locations to assess ephyra and competent larvae, which are generated and/or preparing to settle in these lagoonal communities. Our eight sampling regions from north to south included Point Pleasant, Kettle Creek, Chadwick Island, Toms River, Cedar Creek, Harvey Cedars, Beach Haven West, and Tuckerton Creek (Fig. 1). These sites cover all of Barnegat Bay and provide information about the presence of *Chrysaora* within these communities and potential export of ephyrae into Barnegat Bay.

Objective 3: Assess the distribution and density of settling *Chrysaora* polyps and development of resting podocysts.

As part of the Targeted Coastal Development sampling, we deployed small settling plates within these coastal developments to assess the settlement distribution patterns. Plates will be deployed in June and July and retrieved during the following sampling period (July and August) in these same communities. Since this is the likely source of sea nettle populations, this will allow us to identify and quantify polyp settlement and distribution. Additionally, a short-term broad settlement plate experiment was carried out to get a larger spatial distribution during the summer.

Objective 4. Assess the diet of *Chrysaora* through dissection and molecular analysis.

4a. Field-Based Diet Assessment

Juvenile and adult sea nettles were collected within Barnegat Bay including near shore and open water. Individuals were collected in June, July, and August to provide a temporal assessment of diet and samples were collected from several regions within the bay to provide spatial assessment of potential diet. Field collected individuals were measured and immediately preserved in ethanol to stop digestion and allow the oral cavity and oral arms to be investigated for prey items. All food items present were identified to lowest possible taxonomic level and enumerated. Prey selection from samples was then compared to available prey collected in the plankton tows to assess whether selection was occurring or whether feeding was indiscriminant. This was done in relation to month of collection and location in the Bay. Some of the dissected organisms were used for DNA sequencing, using PCR to amplify and detect the 16S rDNA gene

of *Mnemiopsis leidyi* in *Chrysaora quinquecirrha* guts. A sub-sample of dissected prey taxa was isolated and subsequently analyzed using the Next Generation Sequencing Protocol described below in the Metagenomic Analysis.

4b. Molecular Analysis of the Gut Contents of *Chrysaora*

Often, gut contents of organisms can be substantially degraded due to digestion and limits the assessment of their impacts on prey species, because identification is relatively impossible using tradition techniques. Through molecular techniques, we can collect gastrovascular cavity contents via syringe and assay them against known DNA markers for specific organisms. In particular, the potential declines associated with hard clams and blue crabs may be due in part to the rise and dominance of these pelagic predators. Essentially, they consume larval phases of these commercially and recreationally important species. Additionally, we will be assessing the presence of bay anchovies using molecular techniques. Bay anchovies are considered to be one of the most important estuarine fish species linking pelagic primary production into fish biomass, which is consumed by higher order consumers such as weakfish, blue fish, striped bass, summer flounder, and numerous bird species.

Metagenomic Analysis

Although it is impossible to predict the diet of *Chrysaora*, a metagenomic analysis may provide the best and most comprehensive answer to this important question. The extracted stomach contents of *Chrysaora* will undoubtedly contain DNA from all of the organisms that it has recently ingested. By extracting this mixture of DNA molecules we can utilize Next-Generation Sequencing (NGS) methodologies to provide a comprehensive list of all DNA sequences that can be used to putatively identify which organisms were present based on unique matches to DNA databases (Genbank).

In order to determine the diet of *Chrysaora quinquecirrha* in Barnegat Bay, we employed two different gut-sampling protocols that utilized the Illumina HiSeq2500 next generation DNA deep sequencing platform. Next generation sequencing (NGS) methodologies allow the researcher to attain thousands of DNA sequences for a fraction of the cost of more traditional Sanger sequencing. The NGS DNA sequences can subsequently be identified by searching against publicly available DNA databases [e.g. Barcode of Life Data Systems (BOLDSystems), National Center for Biotechnology Information (NCBI)].

Traditional gut content studies rely on visual identification. However, NGS methodologies may prove to be more advantageous. First, NGS methods can detect the DNA of food items even when visual recognition is not possible due to the stage of digestion. Secondly, NGS methods allows for species level identification of food items that are morphologically indistinguishable from one another (e.g. fish eggs). Lastly, NGS methods require less hands-on time than more traditional labor-intensive visual identification methods.

Gastric lavage

We sampled 8 adult jellyfish from 3 localities (2 from Forked River West; 3 from Toms River West; and 3 from Silver Bay East) using buckets to prevent damage and compression to jellyfish that may occur with nets. To remove any bycatch, specimens were subsequently rinsed three times with sterile artificial seawater (salinity = 19 ppt) filtered through 0.45 μm filters.

Specimens were then placed upside down on clean dissecting trays and bell diameter measured. Gut contents were aspirated using approximately 3 ml of sterile seawater pipetted into the mouth to wash out the gastric pouches. Contents were immediately sucked back up and placed into sterile 15 ml tubes with 100% ethanol to yield a final concentration of 70% (v/v) ethanol. This procedure was repeated three times per jellyfish with all samples pooled. Sample tubes in the field were placed on ice and subsequently stored in a -80°C laboratory freezer until DNA isolation. Gut contents from the eight jellyfish were extracted separately.

Macroscopic Gut/tentacle Dissection

During field collections of *Chrysaora* for dissection (described above) particles of identified food items were removed from the ethanol preserved individuals and separated. Food items included fish eggs, copepods, fish larvae, polychaetes, as well as unidentifiable matter present in the gastric cavity. These items were then pooled together for DNA isolation (see below).

DNA Isolation

Total genomic DNA was isolated from the gut contents of each individual jellyfish (gastric lavages and gut washes) using a CTAB/NaCl method (see Appendix A). Field harvested gut samples (stored @-80˚C in 70% (v/v) ethanol) were centrifuged @16,000 x g for 30 minutes. Ethanol was carefully decanted and pellets were briefly dried in a Speed-Vac to remove traces of ethanol. Pellets were then extracted using the CTAB/NaCl method detailed in Appendix A with the exception that final pellets were resuspended in sterile deionized water instead of TE. Total yield and quality of DNA extracted is provided in Table 1.

Table 1. Total yield and quality of DNA extracted. Samples I1 through I4 were pooled picked samples from tentacles and gastric pouches.

Sample	DNA Conc. [ng/µl]	260/280 Ratio	Final volume (µl)	Total DNA (µg)
TRW1	2175.7	2.08	20	43.5
TRW2	826.8	2.03	20	16.5
TRW3	1020.3	2.11	20	20.4
FRW1	337.9	2.05	10	3.4
FRW2	1144.6	2.01	20	22.9
SBE1	1069	2.13	10	10.7
SBE2	1086.1	2.12	10	10.9
SBE3	1745.4	2.13	20	34.9
I1	447.8	1.92	10	4.5
I2	89	1.92	10	0.9
I3	502.6	1.95	10	5
I4	429.2	1.88	20	8.6
D1*	1160.4	2.06	24	27.84 µg total
D2*	365.4	1.97	10	3.65 µg total

*D1. 3 µl each of TRW1, TRW2, TRW3, FRW1, FRW2, SBE1, SBE2, and SBE3. Total of 24 µl (1.16 µg/µl) sent for NGS analysis.
*D2. 2.5 µl each of I1, I2, I3 and I4 were pooled for NGS analysis. Total of 10 µl (0.365 µg/µl).

NGS library preparation and sequencing

The pooled DNA samples obtained from each of the pooled gut content samples (gastric lavage and gut wash) were sent to GENEWIZ, Inc. (http://www.genewiz.com/) for library preparation and sequencing. Each library was prepared using the Illumina NEBNext® Ultra™ DNA Library Prep Kit. DNA shearing to ~250 bp was accomplished using the Covaris S220. End repair and A-tailing, adapter ligation, and PCR-mediated indexing, and enrichment then followed. The two gut content DNA libraries were multiplexed with a RNA library and paired-end sequenced (2 x 100) on the Illumina HiSeq2500 platform. This resulted in 64,134,235 (gastric lavage) and 50,670,651 (gut and tentacle picked samples) paired end reads.

Filtering and Assembly

Raw reads were quality filtered using the NGSQCToolkit_v2.3.2 (Patel and Jain 2012). We kept only full-length reads with PHRED quality scores >30. Consequently, 61,075,232 (gastric lavage) and 48,136,837 (gut wash) paired end reads were retained for further analyses.

Three separate assemblies were performed: Gastric lavage, Gut picked, and Combined. Gastric lavage and gut picked consisted of only reads associated with the given library. The combined analysis consisted of all quality-filtered paired end reads (109,201,158 reads) from each library (gastric lavage and gut wash). Paired-end reads were assembled using the CLC Genomics Workbench (http://www.clcbio.com/products/clc-genomics-workbench/) de novo assembler. Word size and bubble size were automatically calculated with a minimum contig length of 200 base pairs (bp). Once the initial contigs were assembled, each of the reads were then mapped back to the contigs (Mismatch cost = 2; Insertion cost = 3, Deletion cost = 3; Length fraction = 0.5; Similarity fraction = 0.8) which were subsequently updated.

Annotation

The combined build contigs were BLAST (Altschul et al. 1990) searched against the NCBI nucleotide sequence database using standalone blastn 2.2.29+ and the preformatted nt database (downloaded 4/13/14). BLAST searches used default settings except for: outfmt = 5 (xml) and max_target_seqs = 5. More stringent and less stringent searches were performed but did not alter prey item identification (not shown). The contigs from gastric lavage and gut wash builds were BLAST searched (same settings as above) against the combined contigs for annotation and comparative purposes.

Results

For this research, we conducted research within two defined habitats within Barnegat Bay, 'Bay-wide' and 'Lagoon' communities. Bay-wide samples represent monthly collections of sites used during the 2012 research program (Fig. 1), while Lagoon communities were identified as the probable source of polyps and ephyrae so targeted sampling of eight lagoon communities was conducted (Fig. 1).

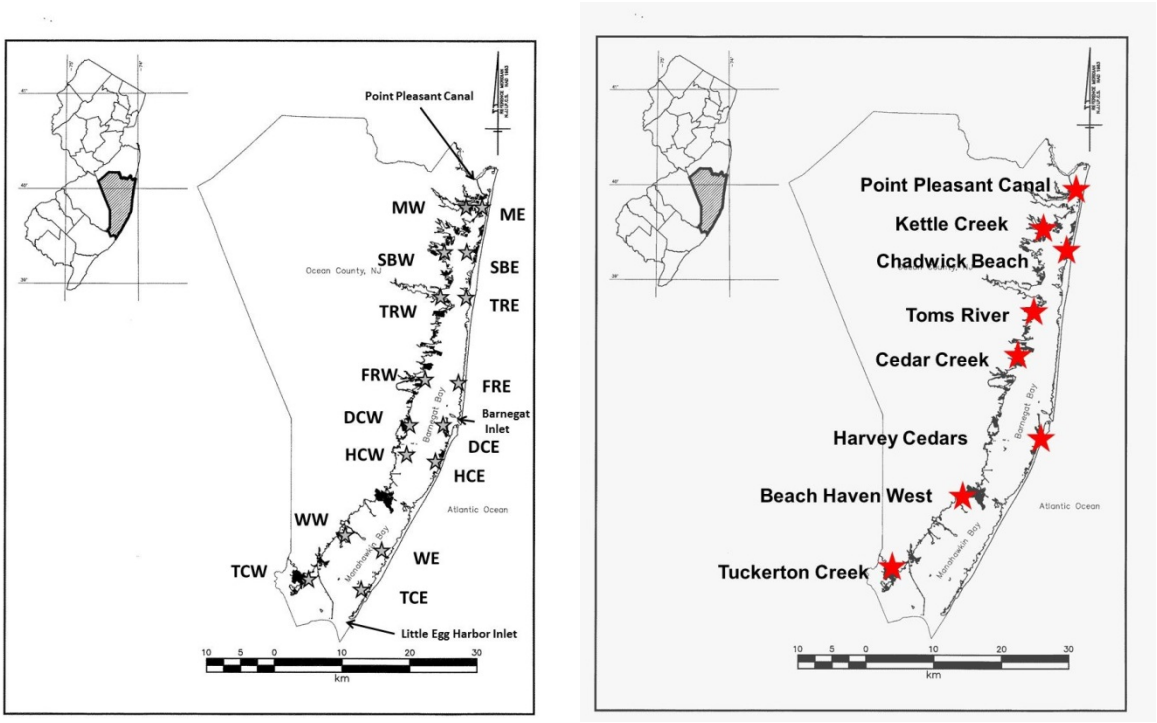

Figure 1. 2013 sampling stations in Barnegat Bay, NJ. Left panel represents our bay-wide sampling stations, while the right panel represents our lagoon sample stations.

Summary of Sampling Events

Bay-wide samples were collected monthly from May to September, while lagoon sites were sampled monthly between June and August. Generally, two days of sampling were necessary for completion of work and occurred on consecutive days when possible (Table 2).

Table 2. Monthly sampling dates for bay-wide and lagoon sampling events.

Bay Wide Sampling Events	Specific dates
May	May 22 and 29, 2013
June	June 18, 19, 20, 2013
July	July 16, 17, 2013
August	August 14, 15, 2013
September	September 21, 2013

Lagoon Sampling Events	Specific dates
June	June 18, 19, 2013
July	July 9, 10, 2013
August	August 7, 15, 2013

Water Quality Summary

During sampling events, water quality data were collected for salinity, temperature, and dissolved oxygen. Unfortunately, we had several issues arise with our YSI-85® instruments including total failure of one meter during the middle of the field season and total failure of the other during the last sampling event. Water quality data are presented for the Bay-wide sampling events in Table 3, while water quality data for lagoon sampling events is presented in Table 4.

Table 3. Bay-wide Water quality data for 2013 collected during sampling events using a YSI-85® handheld meter. Meter failure occurred during the sampling period and MF in the table represents points where we encountered these events. A) Temperature for all site and date combinations (˚C), B) Salinity (ppt), and C) Dissolved Oxygen (mg/l). Sites are abbreviated in the top row.

Temperature	MW	ME	SBW	SBE	TRW	TRE	FRW	FRE	DCW	DCE	HCW	HCE	WW	WE	TW	TE
May	20.9	21	21.7	21	20.9	21.2	23.1	20.9	18	18.5	18.3	18.8	16.7	17.1	17	15.2
June	24	23.7	24.5	23.8	24.4	24.7	22.9	22.5	22.4	17.4	22	MF	23.2	23.2	23.5	22.2
July	28.8	29	30.2	29.2	29	28.9	29.1	28	29.8	29.2	29	29.8	29.1	30.1	29	28.5
August	23.5	23.7	24.7	23.7	24.5	24.3	24.6	23.8	25.5	24.2	23.1	22.8	22.5	23.2	23.6	22.8
September	19.9	MF	MF	19.4	MF	19.4	MF	18.1	MF	MF	MF	MF	MF	MF	MF	MF
Salinity																
May	18.8	19.6	19.1	17.4	19.1	20.4	26.7	26.8	29	29.4	26.9	27.4	27	29.9	29.2	30.3
June	14.4	16.4	16.2	16.1	14.7	14.7	22.7	23.6	24.9	29.3	24.6	MF	24.8	27.8	28.2	28.5
July	17.6	18.3	14.8	16.2	16	17.9	25.6	25.7	28.2	25.8	28.4	28.8	27.6	29.2	29.2	29.7
August	14.5	19	16.2	17.4	12.7	19.3	26.7	26.8	27.7	27.1	27.1	28.3	25.2	27.2	27.6	28.4
September	24.7	MF	MF	20.4	MF	21.2	MF	29.4	MF	MF	MF	MF	MF	MF	MF	MF
Dissolved Oxygen																
May	7.72	7.29	9.4	7.85	7.83	7.74	7.39	8.43	10.6	11.6	8.6	9.1	8.2	10.86	8.99	9.57
June	8.47	7.13	6.98	6.98	6.1	7.87	6.55	7.75	5.96	6.34	7.65	MF	7.6	9.27	8.17	7.12
July	11.27	9.55	9.56	9.2	11.1	8.87	10.3	9.3	6.25	9.58	3.26	5.94	5.06	6.3	5.07	4.57
August	7.7	7.3	7.4	7.26	8.2	6.8	7.2	6.9	6.5	8.0	7.2	6.1	5.97	7.35	6.4	6.4
September	6.78	MF	MF	7.35	MF	7.33	MF	6.54	MF	MF	MF	MF	MF	MF	MF	MF

Table 4. Lagoon Water quality data for 2013 collected during sampling events using a YSI-85® handheld meter. A) Temperature for all site and date combinations (˚C), B) Salinity (ppt), and C) Dissolved Oxygen (mg/l). Sites are abbreviated in the top row.

Temperature	PPL	KCL	CIL	TRL	CCL	HCL	BHL	TL
June	23.9	24.4	24	24.1	23.8	23.5	23.4	27.7
July	26.4	28.5	28.4	29.2	27.8	27	28.1	24.9
August	23.8	24.6	24	25.5	24.9	25.2	25	24.5
Salinity								
June	17.3	15.4	17	15.7	25	27.6	24.3	22.7
July	22.8	13.5	14.5	17.3	25.2	27.7	26.3	28.4
August	23.3	17	17.6	19.8	25.6	28.6	27	28.1
Dissolved Oxygen								
June	8.61	6.2	6.7	7.4	9.5	8.9	9.5	4.17
July	7.8	5.37	4.6	6.5	4.7	5.7	6.02	3.8
August	6.7	6.17	6.25	6.37	7.52	7.94	6.13	5.52

Objective Specific Results

Objective 1: Assess early stage *Chrysaora* ephyrae and planktonic larvae through molecular identification of species specific DNA in water samples.

Bay-wide Collections: Ephyrae.

Although ephyra 16S rDNA (collected on 500 µm filters) was seen widely distributed throughout Barnegat Bay (north, central and southern regions), we clearly measured the highest values in the north (Fig. 2). The highest value recorded for the 2013 season was at our Silver Bay East site with over 2200 copies (in a 2 µl sample) in mid-June (6/18-20/2013). Other sites also gave strong signals, all of them north of Forked River (TRE, TRW, SBE, SBW, MRE, MRW). In the central and southern regions of the bay we were able to detect lower, but persistent, levels of ephyra by qPCR. The largest signals in these regions came from Double Creek East (DCE) and Harvey Cedars East and West (HCE, HCW); however, they represent at least a 5-fold reduction of signals measured in the northern part of Barnegat Bay in 2013.

It is also interesting to note that there may be a temporal shift in ephyra appearance during the season between these different regions of the bay. In the northern section, from the Metedeconk down to Toms River, the peak of ephyra signals appears between mid-June and mid-July. In contrast, from Forked River on south, 9 of the 10 locations showed peak signals about a month later (mid-July to mid-August). The only southern location not peaking later was Double Creek West (DCW), which peaked in mid-June.

These data suggest that strobilation is occurring bay-wide but that the timing of the pulses may be slightly asynchronous between northern and southern regions. It is not clear why the signals are so much stronger in the northern part of the bay, but this fact is in general agreement with higher values for medusa found in lift-nets and plankton tows in this region.

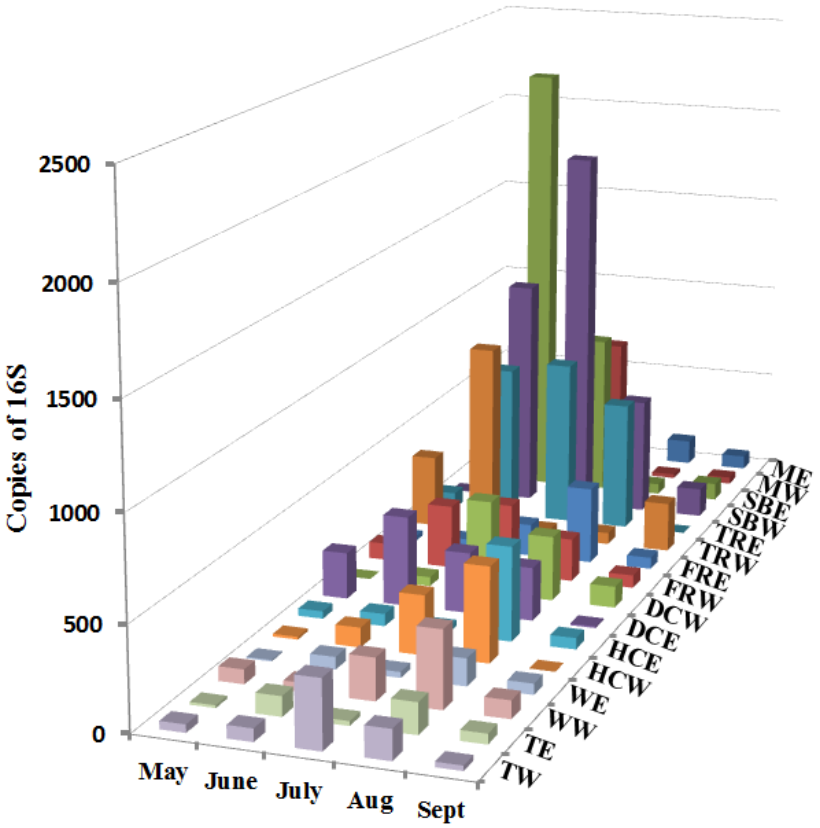

Figure 2. Molecular Assessment of DNA concentrations (16S rDNA) collected on the 500µm filters from monthly bay-wide sampling events.

Bay-wide Collections: Planula larvae.

Similar to the distribution of ephyra, planula larva 16S rDNA (collected on 100 µm filters) showed a broad, bay-wide distribution from the Metedeconk in the north to Tuckerton in the south (Fig. 3). Signal strength in qPCR assays was lower however, with peaks of planula DNA about 3-fold lower than ephyra captured on 500 µm filters of the same water samples. We do not see the strong bias of planula DNA distribution that was seen with ephyra DNA distribution in 2013. Whereas ephyra showed a strong cluster of signals in the northern part of the bay, planula DNA was more uniformly distributed throughout the bay, with equally strong signals at many sites in different regions of the bay. Strong peaks were detected at Metedeconk East and West, Silver Bay East & West, Toms River West, Forked River West, Double Creek West, Harvey Cedars West and Westeconk Creek West. Likewise, all of these peaks, except one, occurred during our 3rd or 4th collection (mid-July to mid-August). Only the Silver Bay West (SBW) site showed a significant peak in mid-June. Since sexually mature adults of *Chrysaora quinquecirrha* are required for the production of planula larva in the water column, it is reasonable to expect peaks of planula to appear later in the season than ephyra.

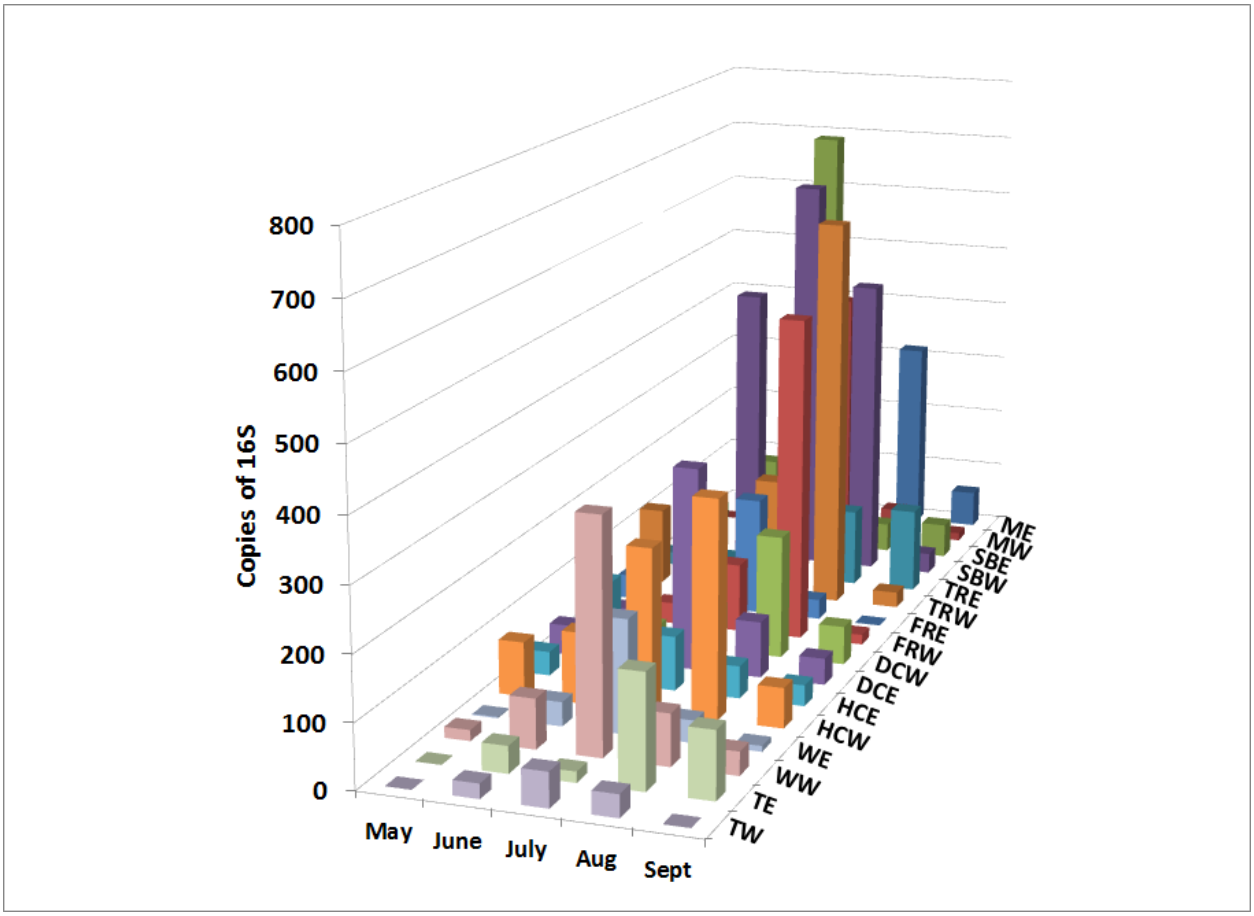

Figure 3. Molecular Assessment of DNA concentrations (16S rDNA) collected on the 100µm filters from monthly bay-wide sampling events.

Lagoon Collections

New to our sampling protocols for 2013 was the inclusion of 8 lagoonal sites in Barnegat Bay. These were selected to provide a wide coverage of lagoons in the bay from north to south. These were included this year in response to anecdotal reports of large smacks of jellyfish present during the previous summer (2012). We wanted to verify and quantify these reports as well as examine the possibility that these lagoonal communities were serving as sources for *Chrysaora quinquecirrha* ephyrae and recruitment habitat for larvae.

Ephyra

We were able to detect a strong signal for ephyra 16S rDNA in many of these lagoonal sites (Fig. 4). In comparison with quantitative signals seen in our bay-wide samples for 2013, the highest signals measured in some lagoons was 4-fold higher, suggesting a significantly higher number of ephyra in some lagoons than found in the bay. We detected the highest qPCR signals at Kettle Creek, Cedar Creek, Harvey Cedars, and Beach Haven West Lagoons. All of these sites peaked in early- to mid-July. In contrast to what was seen with bay-wide ephyra distribution, only the Kettle Creek lagoon is in the northern region of the bay. Forked River, Harvey Cedars, and Beach Haven West lagoons are all in the southern half of Barnegat Bay. Considering the poor turnover of water in most lagoons, this suggests that polyps may be

resident in these lagoons and are not only the source of ephyra that are so abundant in these locations, but may also be the source of ephyra found in the southern region of the bay as well.

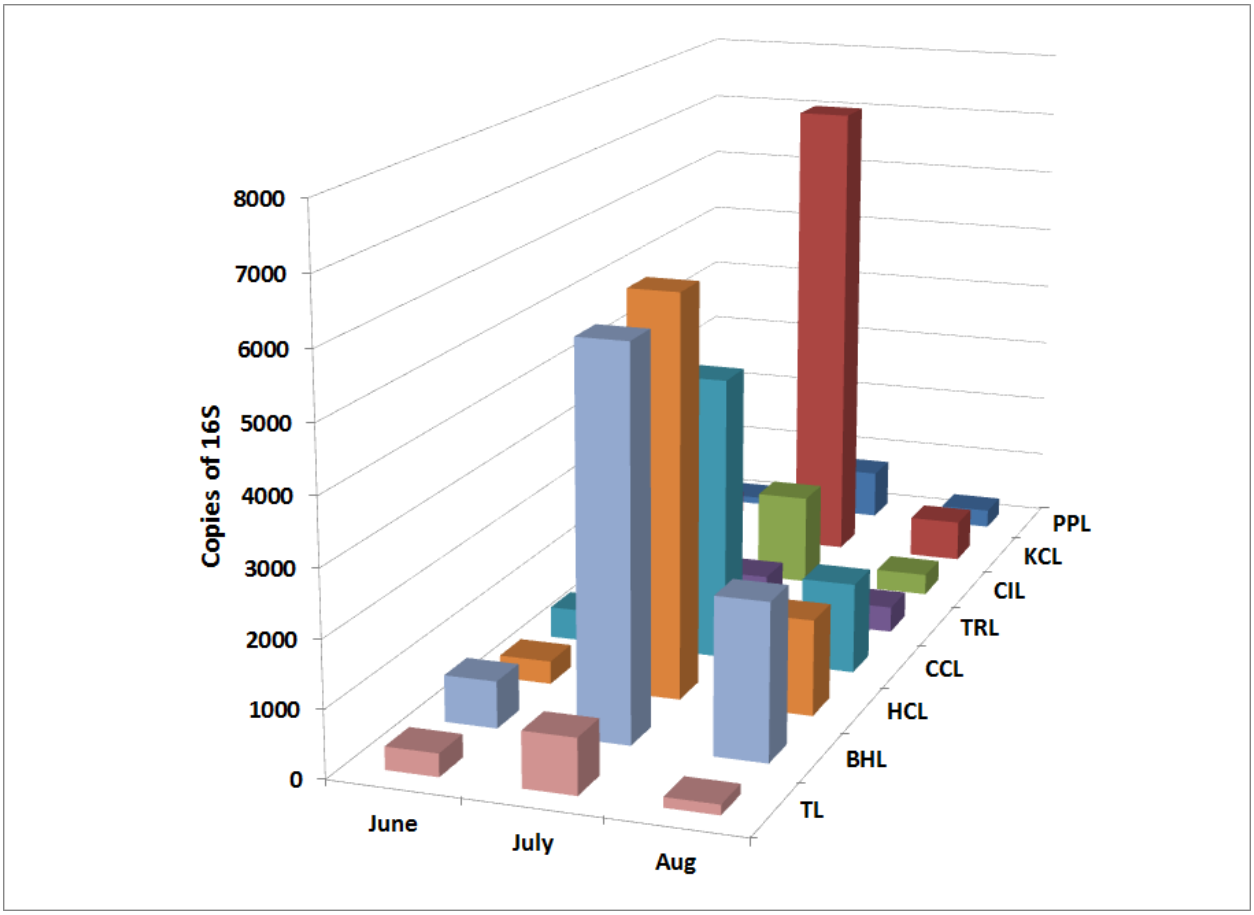

Figure 4. Molecular Assessment of DNA concentrations (16S rDNA) collected on the 500µm filters from monthly lagoon sampling events.

Planula larvae

We were also able to detect a strong signal for planula 16S rDNA in a number of these lagoonal sites (Fig. 5). Similar to what we detected for ephyra DNA in these lagoons, these signals for planula DNA were higher than those found in the bay-wide collections. In fact, the highest planula DNA signals were an order of magnitude greater in lagoons *vs*. bay-wide (8400 *vs*. 700 copies of 16S rDNA). We detected the highest qPCR signals for planula at Kettle Creek, Toms River, and Forked River Lagoons. All of these sites peaked in early- to mid-July, although some sites still had strong signals in August (Cedar Creek and Beach Haven West Lagoons). The higher numbers of planula in these lagoons may be due again to poor flushing of these structures, and planula produced then to stay within these areas and settle on the relatively high concentration of hard structures available within lagoons that are preferred by this species (vinyl bulkheads, plastic floating docks).

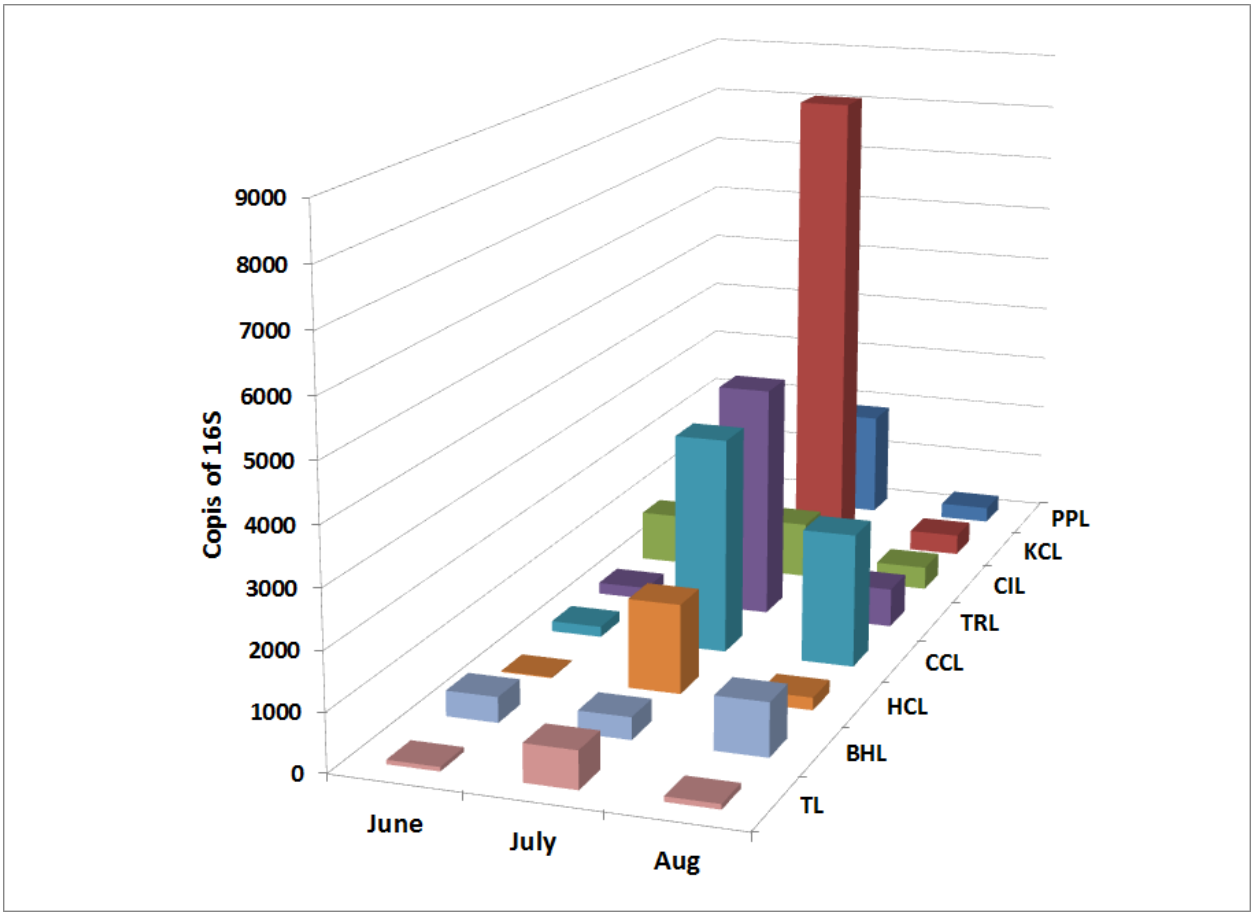

Figure 5. Molecular Assessment of DNA concentrations (16S rDNA) collected on the 100µm filters from monthly lagoon sampling events.

Objective 2: Assess the distribution of gelatinous zooplankton and impacts on planktonic community structure.

Lift Net Results Bay-wide Sampling

Samples for both bay-wide and lagoon events have been QAQC'd and standardized. Results from the bay-wide samples indicate significant spatial and temporal differences in the density of both *Chrysaora* and *Mnemiopsis* throughout the bay (Fig. 6). Specifically, significant differences in density were observed for *Chrysaora* among sites ($F_{15,803} = 4.5$, $P < 0.001$) with our Silver Bay East site having significantly greater densities compared to all sites except for Tom River East. However, no other significant site differences were observed. For dates of collection, significant differences among dates were observed ($F_{4,803} = 5.38$, $P < 0.0003$) with June having significantly more individuals than other months except for July, where there was no difference in density between these months, but no other differences were observed. *Mnemiopsis* also showed significant differences among sites ($F_{15,\ 803} = 23.48$, $P < 0.0001$) and dates of collection ($F_{4,\ 803} = 35.06$, $P < 0.0001$). Specifically, density was significantly highest at our Forked River East site (>15 m^{-3}), then Double Creek West (>10 m^{-3}). These two sites were significantly different from each other and from all other sites. The remaining 14 sites showed broad overlap in similarity in densities (non-significant), with Silver Bay East and West having

the lowest density and being significantly lower than Harvey Cedars West, Westeconk West, and Forked River West. *Mnemiopsis* density comparisons among months showed that density was significantly different among months, with a density overlap for June (5.6 m^{-3}) with both May (>6.7 m^{-3}) and July (4.8 m^{-3}). However, all other months showed significant differences. While *Chrysaora* and *Mnemiopsis* showed inverse patterns in abundance among sites and there was a negative correlation between the distributions of these two species, it was not significant (r = -0.058, P> 0.09).

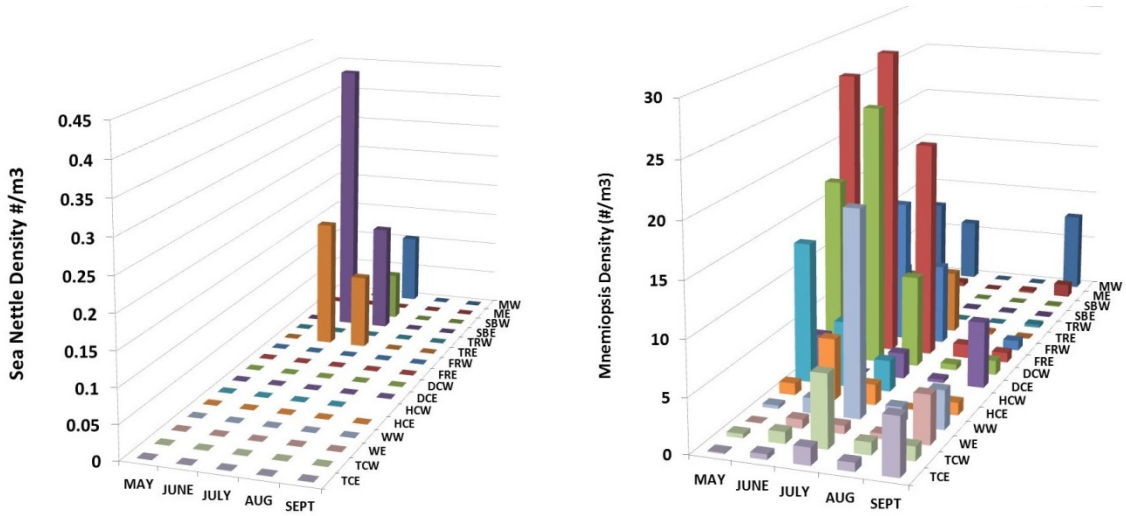

Figure 6. Distribution of *Chrysaora* (left panel) and *Mnemiopsis* (right panel) collected during bay-wide sampling events.

Lift Net Results Lagoon Sampling

Lift net results from lagoon samples showed a similar disjoint distribution between the species (Fig. 7), but *Chrysaora* was observed further south than collections made within Barnegat Bay. *Chrysaora* showed significant differences among sites ($F_{7,232} = 7.1$, $P < 0.0001$), but not difference among months ($F_{2,232} = 2.99$, $P = 0.052$). Specifically, Toms River Lagoon site had significantly greater densities than all other sites (0.182 m^{-3}), but no other sites showed any difference. For *Mnemiopsis*, they showed significant differences among sites ($F_{7,232} = 13.25$, $P < 0.0001$) and months ($F_{2,232} = 9.7$, $P < 0.0001$). Specifically, Beach Haven Lagoon density was significantly greater than all other sites (>5.37 m^{-3}). Additionally, Cedar Creek Lagoon and Harvey Cedar Lagoon were significantly greater than Chadwick Island Lagoon, Toms River Lagoon, and Kettle Creek Lagoons. For temporal patterns, August was significantly lower than both June and July sampling periods. Overall, these patterns of abundance are complimentary to the distribution of *Chrysaora* and a negative, but not significant, correlation existed between these two species (r = -0.117, P = 0.068).

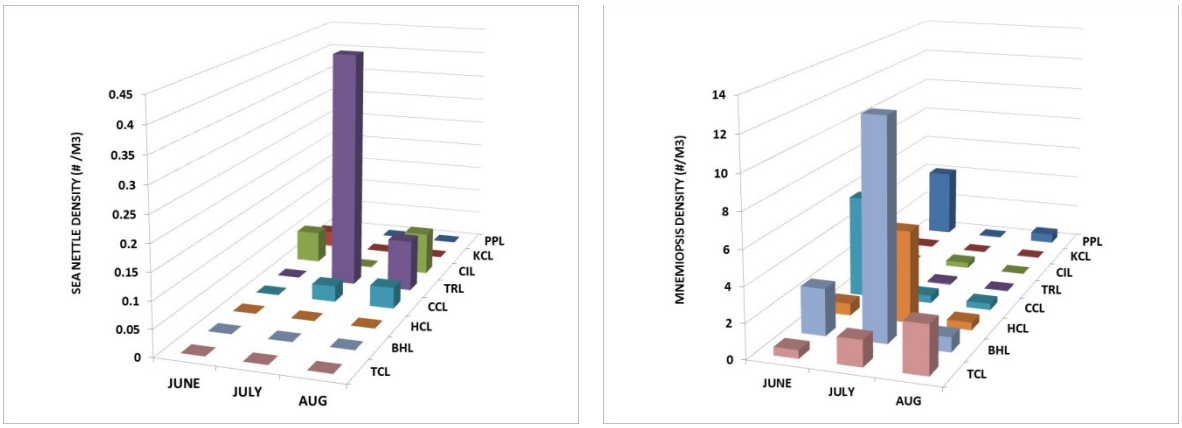

Figure 7. Distribution of *Chrysaora* (left panel) and *Mnemiopsis* (right panel) collected during Lagoon sampling events.

Plankton Tows

Dominant Gelatinous Zooplankton Bay-Wide Samples

Distribution and density of gelatinous zooplankton from plankton tow samples reveals similar results to the lift net results, but this was not unexpected. Results from both bay-wide (Fig. 8) and lagoon sampling events (Fig. 9) show similar patterns of higher abundances of *Chrysaora* in northern portions of the bay with *Mnemiopsis* abundance more equally distributed.

Chrysaora showed significant differences among sites ($F_{15,200}$ = 3.06, P < 0002) and months ($F_{4,200}$ = 5.09, P < 0.0001). Specifically, significantly more *Chrysaora* were collected from our Toms River East, Silver Bay East, and Silver Bay West sites compared to others and temporally density was significantly higher in June compared to all other months. *Mnemiopsis* showed significant differences among sites ($F_{15,200}$ = 4.19, P < 0.0001) and dates of collection ($F_{4,200}$ = 10.88, P < 0.0001), with the highest density occurring at the Double Creek West Site (>17.6 m^{-3}) and very few occurring at our Toms River West site (0.86m^{-3}) and Silver Bay West (0.0). While these two species showed disjoint distributions and a negative correlation between densities, it was not significant (r=-0.09, P = 0.17).

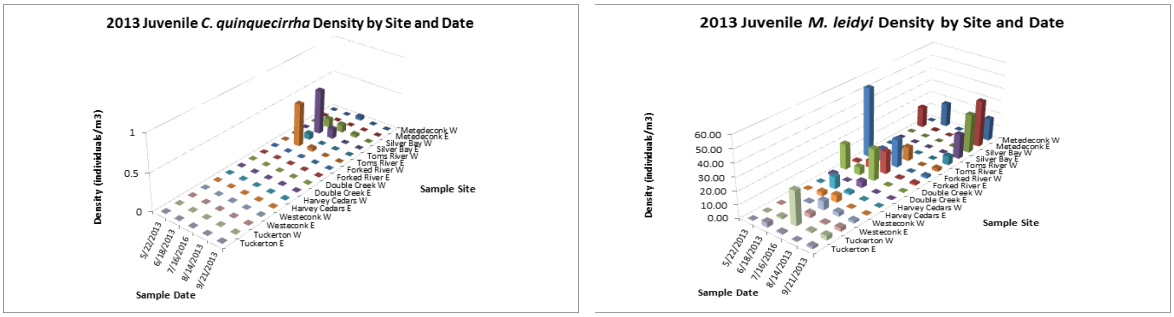

Figure 8. Distribution of *Chrysaora* (left panel) and *Mnemiopsis* (right panel) collected during bay-wide sampling events with plankton nets.

Dominant Gelatinous Zooplankton Lagoon Samples

The distribution of the two major gelatinous zooplankton species within the lagoons (Fig. 9) showed somewhat similar patterns seen in the lift nets (Fig. 7). *Chrysaora* showed significant density differences among sites ($F_{7,58}$= 5.82, P < 0001) and months ($F_{2,58}$= 6.64, P < 0.003). Specifically, significantly more *Chrysaora* were collected from Kettle Creek Lagoon (0.25 m^{-3}) compared to all other sites. No *Chrysaora* were collected from Beach Haven Lagoon, Tuckerton Creek Lagoon, or Harvey Cedars Lagoon in 2013. Densities were also significantly greater in July compared to either June or August. *Mnemiopsis* showed no significant differences among sites ($F_{7,58}$= 1.75, P > 0.1), but significant differences among dates of collection existed ($F_{2,58}$= 6.02, P < 0.005). The highest average densities were recorded for Cedar Creek Lagoon (6.5m^{-3}) and Kettle Creek Lagoon (6.3m^{-3}) and densities were significantly greater in June compared to July and August. While these two species showed somewhat disjoint distributions and a negative correlation between densities, it was not significant (r=-0.09, P = 0.46).

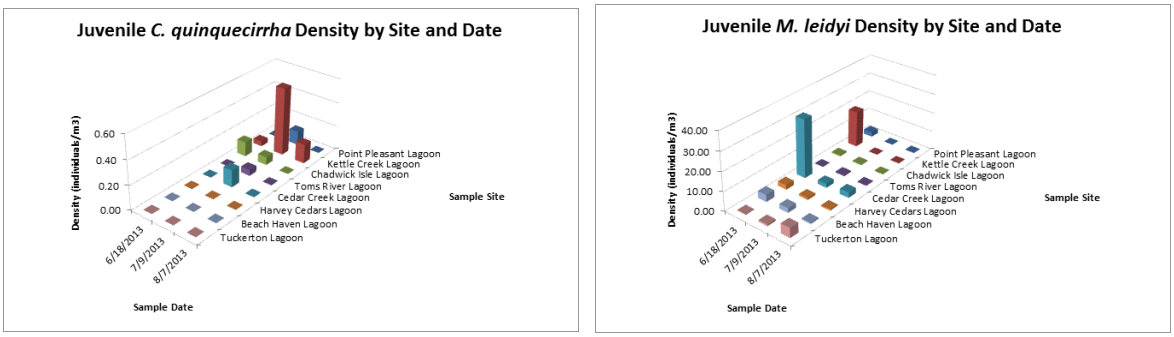

Figure 9. Distribution of *Chrysaora* (left panel) and *Mnemiopsis* (right panel) collected during lagoon sampling events with plankton nets.

Planktonic Organism Distributions: Bay-Wide Samples

In addition to the dominant gelatinous zooplankton collected (i.e., *Mnemiopsis* and *Chrysaora*), several other species were present and abundant in plankton tows. These include hydrozoans, scyphozoans, ctenophores, and salps; many showing significant differences in density among sites or months of collection (Table 5). Specifically, *Pleurobranchia* showed significant differences among sites ($F_{15,200}$=1.99, P < 0.02) and months ($F_{4,200}$ = 4.8, P < 0.001), *Turritopsis nutricula* showed significant differences among sites ($F_{15,200}$=2.3, P < 0.005) and months ($F_{4,200}$ = 2.66, P < 0.04), *Aurelia aurita* showed significant differences among months ($F_{4,200}$ = 3.6, P < 0.008), *Eutima* spp. showed significant differences among months ($F_{4,200}$ = 3.5, P < 0.009), and *Salpa salpa* showed significant differences among sites ($F_{15,200}$=2.7, P < 0.0007) and months ($F_{4,200}$ = 10.07, P < 0.0001). The increase in species richness between 2012 and 2013 may indicate greater oceanic influence in Barnegat Bay, as many of these species are more indicative of coastal and open ocean pelagic communities (e.g., *Salpa salpa*), compared to back-bay lagoons like Barnegat Bay.

Table 5. Average densities (#m^{-3}) of gelatinous zooplankton taxa exhibiting significant differences in density. Significance convention *= 0.05, ** = 0.01, *** = 0.0001, or ns =not significant. First value indicates significance level for differences among sites, second value indicates significant temporal variation in the ANCOVA model. Differing letters adjacent to density values indicate significant differences in means among sites for taxa. Taxa abbreviations: ML = *Mnemiopsis leidyi*, PB = *Pleurobranchia* , CQ = *Chrysaora quinquecirrha*, TN = *Turritopsis nutricula*, AA = *Aurelia aurita*, ES = *Eutima* spp., SS = *Salpa salpa.*

Site	ML***	PB *,**	CQ ***,***	CQ ephyrae ns,***	TN **,*	AA ns,**	ES ns,**	*Clytia* ***,ns	SS ***,***
MW	12.9AB	0.155B	0.01B	0.0697	0B	0	0	0B	5.08AB
ME	7.6ABC	2.07A	0B	0.043	0B	0	0	0B	2.08AB
SBW	0C	0B	0.06AB	0.086	0B	0	0	0.011B	0B
SBE	3.2BC	0.13B	0.15A	0.006	0.01B	0	0	0B	0B
TRW	0.86C	0.13B	0.013B	0.023	0B	0	0	0B	0B
TRE	2.8BC	0B	0.16A	0	0B	0	0	0B	0B
FRW	8.5ABC	0B	0B	0	0.017B	0	0	0B	0.06B
FRE	6.8BC	0B	0B	0	0.02B	0	0	0.043A	0.19B
DCW	17.7A	0.048B	0B	0	0.02B	0.011	0.011	0B	0.026B
DCE	2.1BC	0B	0B	0	0.008B	0	0	0B	0.03B
HCW	7.6ABC	0B	0B	0	0.03B	0	0.0106	0B	0B
HCE	2.3BC	0.008B	0B	0	0.25B	0.009	0.0087	0B	0B
WW	3.1BC	0.029B	0B	0	0.13B	0	0	0B	0B
WE	2.2BC	0.046B	0B	0	0.8A	0.03	0	0B	0.68B
TCW	5.9BC	0B	0B	0	0B	0	0	0B	0.45B
TCE	1.04C	0B	0B	0	0B	0	0.017	0B	8.13A

In terms of dominant zooplankton groups, Calanoid copepods showed the highest average densities amongst sites and dates in bay-wide collections (Fig. 10), with significant difference among sites ($F_{15,200}$= 3.1, P < 0.0002) and months ($F_{4,200}$ = 8.9, P < 0.0001). Other numerically dominant taxa include Caridea, Brachyura, and Cladocerans. Caridea showed significant differences among sites ($F_{15,200}$= 2.6, P < 0.001) and months ($F_{4,200}$ = 6.8, P < 0.0001), Brachyura showed significant differences among sites ($F_{15,200}$= 2.7, P < 0.001) and months ($F_{4,200}$ = 4.9, P < 0.001), and Cladocerans showed significant differences among sites ($F_{15,200}$=3.8, P < 0.001). A complete summary of taxa identified from the plankton tows occurs in the Appendix C.

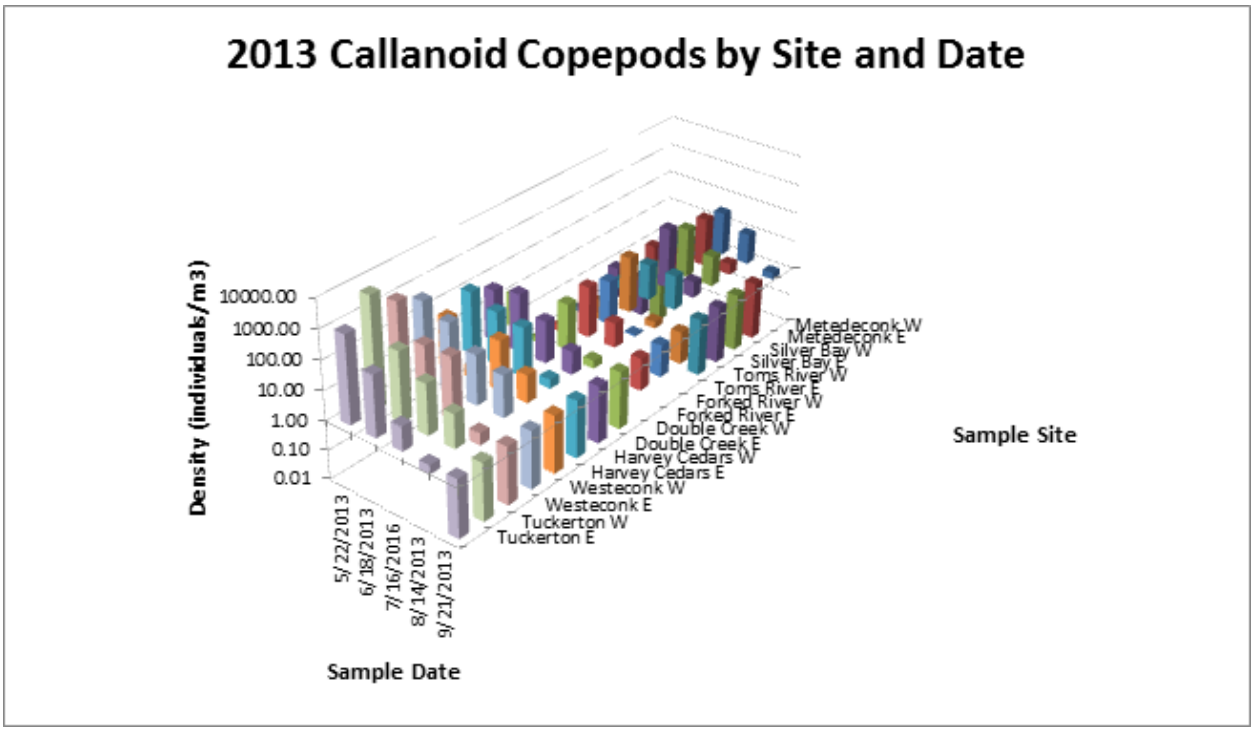

Figure 10. Density of calanoid copepods among sites and dates of collection in 2013 for bay-wide samples. Y-axis is given in exponential scale to address the significant densities which occurred early in the season in the southern regions of the bay.

Correlation analysis indicated that 18 taxa showed some significant correlation between species (Table 6). One interesting feature, which was present in the results, was that no significant correlations existed between *Chrysaora* and any other taxa. However, all interactions between *Chrysaora* and other taxa were negative, suggesting that these planktonic organisms are potential prey and our diet analysis demonstrates that for numerous taxa (see Diet Analysis Section).

Table 6. Significant Correlations among taxa collected from Plankton Tow samples. Table is broken into two parts (A, B) to accommodate the essential information. Taxa abbreviations as follows: MN: *Mnemiopsis leidyi*, CLAD: Cladocera, BRCH: Brachyura larvae, CARD: Caridea larvae, POLY: Polychaeta, IBAL: *Idotea baltica*, COP: Calanoid copepod, MELIT: Mellitidae, FLRV: Fish Larvae, GAM: *Gammarus* spp., ERIC: *Erichsonella* spp., AOR: Aoridae, OSTR: Ostrocoda, CAPR: Caprellidae, CQ: *Chrysaora quinquecirrha*, TTOP: *Turritopsis*, OBEL: *Obelia*, BOUG: *Bougainvillea*, PLBR: *Pleurobranchia*.

Table 6A

	MN	CLAD	BRCH	CARD	POLY	IBAL	COP	MELIT	FLRV	GAM
MN	1.00	-0.06 0.36	-0.05 0.44	-0.05 0.45	-0.06 0.41	-0.03 0.68	-0.07 0.27	-0.08 0.26	0.05 0.45	-0.07 0.31
CLAD	-0.06 0.36	1.00	0.02 0.73	0.13 0.06	0.67 <.0001	-0.03 0.62	0.00 0.99	0.58 <.0001	-0.04 0.53	0.00 0.98
BRCH	-0.05 0.44	0.02 0.73	1.00	0.97 <.0001	0.04 0.59	0.08 0.22	0.06 0.37	0.10 0.14	0.15 0.03	0.24 0.00

CARD	-0.05	0.13	0.97	1.00	0.12	0.08	0.02	0.18	0.12	0.24
	0.45	0.06	<.0001		0.07	0.26	0.76	0.01	0.08	0.00
POLY	-0.06	0.67	0.04	0.12	1.00	0.12	0.00	0.56	0.02	0.11
	0.41	<.0001	0.59	0.07		0.08	0.97	<.0001	0.76	0.10
IBAL	-0.03	-0.03	0.08	0.08	0.12	1.00	-0.03	0.42	-0.01	0.27
	0.68	0.62	0.22	0.26	0.08		0.67	<.0001	0.86	<.0001
COP	-0.07	0.00	0.06	0.02	0.00	-0.03	1.00	-0.02	0.40	0.02
	0.27	0.99	0.37	0.76	0.97	0.67		0.78	<.0001	0.80
MELIT	-0.08	0.58	0.10	0.18	0.56	0.42	-0.02	1.00	0.03	0.50
	0.26	<.0001	0.14	0.01	<.0001	<.0001	0.78		0.63	<.0001
FLRV	0.05	-0.04	0.15	0.12	0.02	-0.01	0.40	0.03	1.00	0.22
	0.45	0.53	0.03	0.08	0.76	0.86	<.0001	0.63		0.00
GAM	-0.07	0.00	0.24	0.24	0.11	0.27	0.02	0.50	0.22	1.00
	0.31	0.98	0.00	0.00	0.10	<.0001	0.80	<.0001	0.00	
ERIC	-0.05	0.02	0.02	0.03	0.02	0.03	-0.01	0.07	0.05	0.18
	0.50	0.79	0.73	0.65	0.75	0.69	0.92	0.31	0.51	0.01
AOR	-0.03	0.19	-0.01	0.01	0.39	0.62	-0.02	0.65	-0.03	0.26
	0.70	0.01	0.83	0.83	<.0001	<.0001	0.72	<.0001	0.70	0.00
OSTR	-0.03	-0.01	-0.01	-0.01	0.03	-0.01	0.00	-0.02	-0.02	-0.01
	0.70	0.87	0.87	0.93	0.71	0.83	0.96	0.79	0.73	0.87
CAPR	-0.06	0.77	0.03	0.12	0.83	-0.04	-0.01	0.49	-0.05	0.02
	0.38	<.0001	0.68	0.07	<.0001	0.59	0.93	<.0001	0.47	0.77
CQ	-0.09	-0.03	-0.03	-0.04	-0.02	-0.04	-0.03	-0.04	-0.06	-0.03
	0.17	0.69	0.63	0.56	0.73	0.53	0.64	0.52	0.37	0.64
TTOP	-0.06	-0.01	0.00	0.01	0.03	0.02	-0.02	0.15	-0.01	0.00
	0.39	0.83	0.98	0.89	0.67	0.81	0.72	0.02	0.89	0.97
OBEL	0.05	-0.01	-0.01	-0.01	-0.01	0.08	-0.01	0.26	-0.02	0.02
	0.47	0.88	0.86	0.91	0.84	0.22	0.93	<.0001	0.72	0.79
BOUG	-0.04	-0.01	-0.01	0.01	0.07	0.11	-0.01	0.03	0.06	-0.01
	0.54	0.84	0.83	0.94	0.27	0.11	0.88	0.66	0.39	0.87
PLBR	0.34	-0.01	-0.02	-0.01	0.00	0.00	0.04	0.00	0.12	-0.02
	<.001	0.84	0.77	0.84	1.00	0.96	0.59	0.95	0.08	0.77

Table 6B

	ERIC	AOR	OSTR	CAPR	CQ	TTOP	OBEL	BOUG	PLBR
MN	-0.05	-0.03	-0.03	-0.06	-0.09	-0.06	0.05	-0.04	0.34
	0.50	0.70	0.70	0.38	0.17	0.39	0.47	0.54	<.0001
CLAD	0.02	0.19	-0.01	0.77	-0.03	-0.01	-0.01	-0.01	-0.01
	0.79	0.01	0.87	<.0001	0.69	0.83	0.88	0.84	0.84
BRCH	0.02	-0.01	-0.01	0.03	-0.03	0.00	-0.01	-0.01	-0.02
	0.73	0.83	0.87	0.68	0.63	0.98	0.86	0.83	0.77
CARD	0.03	0.01	-0.01	0.12	-0.04	0.01	-0.01	0.01	-0.01
	0.65	0.83	0.93	0.07	0.56	0.89	0.91	0.94	0.84
POLY	0.02	0.39	0.03	0.83	-0.02	0.03	-0.01	0.07	0.00
	0.75	<.0001	0.71	<.0001	0.73	0.67	0.84	0.27	1.00
IBAL	0.03	0.62	-0.01	-0.04	-0.04	0.02	0.08	0.11	0.00
	0.69	<.0001	0.83	0.59	0.53	0.81	0.22	0.11	0.96
COP	-0.01	-0.02	0.00	-0.01	-0.03	-0.02	-0.01	-0.01	0.04
	0.92	0.72	0.96	0.93	0.64	0.72	0.93	0.88	0.59
MELIT	0.07	0.65	-0.02	0.49	-0.04	0.15	0.26	0.03	0.00
	0.31	<.0001	0.79	<.0001	0.52	0.02	<.0001	0.66	0.95
FLRV	0.05	-0.03	-0.02	-0.05	-0.06	-0.01	-0.02	0.06	0.12
	0.51	0.70	0.73	0.47	0.37	0.89	0.72	0.39	0.08

GAM	0.18 0.01	0.26 0.00	-0.01 0.87	0.02 0.77	-0.03 0.64	0.00 0.97	0.02 0.79	-0.01 0.87	-0.02 0.77
ERIC	1.00	0.23 0.00	0.43 <.0001	0.03 0.69	-0.01 0.83	0.14 0.04	-0.01 0.88	0.47 <.0001	-0.02 0.78
AOR	0.23 0.00	1.00	-0.01 0.93	0.25 0.00	-0.03 0.62	0.05 0.50	0.34 <.0001	0.16 0.02	-0.02 0.78
OSTR	0.43 <.0001	-0.01 0.93	1.00	0.10 0.13	-0.01 0.84	0.00 0.99	0.00 0.99	0.02 0.78	-0.01 0.90
CAPR	0.03 0.69	0.25 0.00	0.10 0.13	1.00	-0.03 0.69	-0.02 0.75	-0.01 0.88	-0.01 0.84	-0.02 0.80
CQ	-0.01 0.83	-0.03 0.62	-0.01 0.84	-0.03 0.69	1.00	-0.03 0.65	-0.01 0.85	-0.02 0.79	-0.02 0.73
TTOP	0.14 0.04	0.05 0.50	0.00 0.99	-0.02 0.75	-0.03 0.65	1.00	0.03 0.71	0.39 <.0001	-0.02 0.80
OBEL	-0.01 0.88	0.34 <.0001	0.00 0.99	-0.01 0.88	-0.01 0.85	0.03 0.71	1.00	-0.01 0.93	-0.01 0.90
BOUG	0.47 <.0001	0.16 0.02	0.02 0.78	-0.01 0.84	-0.02 0.79	0.39 <.0001	-0.01 0.93	1.00	-0.01 0.87
PLBR	-0.02 0.78	-0.02 0.78	-0.01 0.90	-0.02 0.80	-0.02 0.73	-0.02 0.80	-0.01 0.90	-0.01 0.87	1.00

Planktonic Organism Distributions: Lagoon Samples

38 different taxa were identified from plankton samples. Several taxa were only collected once in a large algal mass within a single plankton tow. This sample was removed from the analyses as being unrepresentative of plankton communities. Among the dominant taxa encountered were Calanoid copepods, Brachyura larvae, fish eggs, Caridea larvae, and ephyrae. Many taxa (excluding *Mnemiopsis* and *Chrysaora* which were presented earlier) showed significant spatial and temporal differences in density among sites and months. Specifically, calanoid copepods were the numerically most abundant organisms encountered in plankton tows (Fig. 11) and showed significant differences among sites ($F_{7,58} = 2.7$, $P < 0.02$) and months ($F_{2,58} = 13.3$, $P < 0.0001$). Other abundant organisms showing significant differences include fish eggs and fish larvae which showed significant differences among sites ($F_{7,58} = 3.1$, $P < 0.008$; $F=2.6$, $P< 0.02$, respectively), Caridea showed significant differences among sites ($F_{7,58} = 2.7$, $P < 0.02$). For other gelatinous zooplankton, *Chrysaora* ephyrae showed significant differences among sites ($F_{7,58} = 3.85$, $P < 0.002$) and months ($F_{2,58} = 12.84$, $P < 0.0001$), while *Turritopsis* and *Aurelia aurita* showed significant differences among sites ($F_{7,58} = 3.26$, $P < 0.006$, $F = 2.57$, $P < 0.03$, respectively).

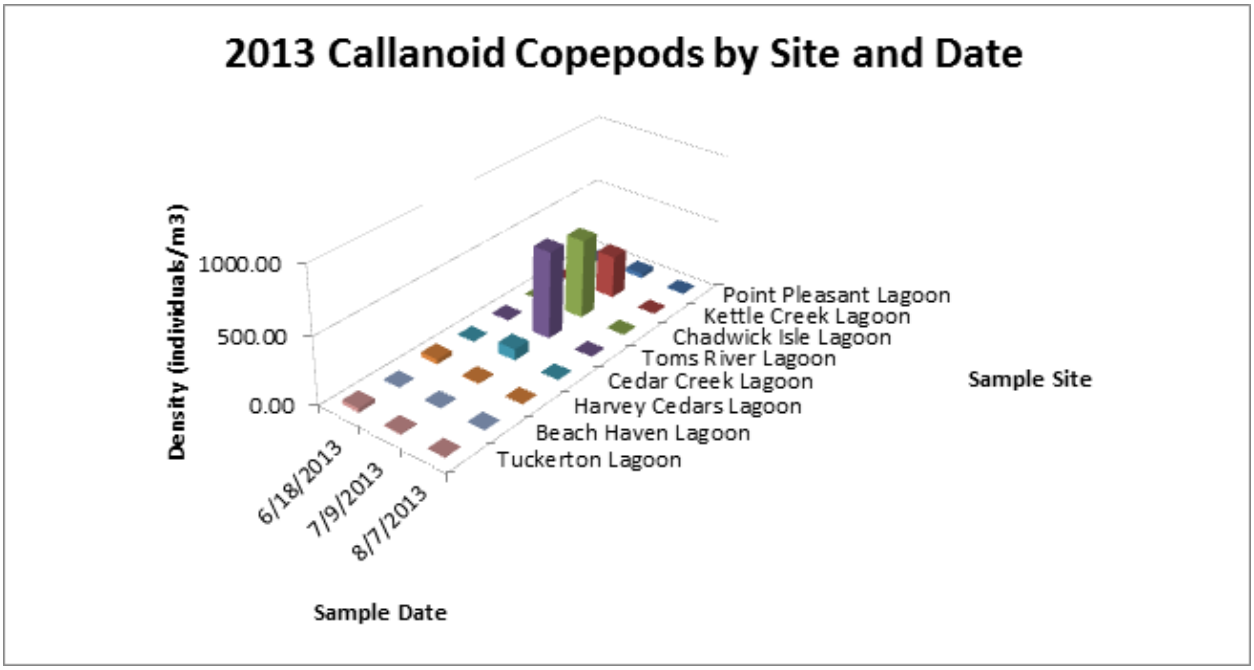

Figure 11. Density of calanoid copepods among sites and dates of collection in 2013 for lagoon samples.

Planktonic Community Structure Assessment: Bay-Wide Samples

Results from the SIMPER analysis indicate average similarities ranging from 49% to 74% with between four and six taxa contributing to >90% of the group similarity (Table 7). In particular, six taxa were important in structuring most of the planktonic communities observed in Barnegat Bay and include: *M. leidyi*, Fish Eggs, Calanoida, *Salpa salpa*, Caridea, and Brachyura. The two-way ANOSIM indicated a global R of 0.647 ($P < 0.001$) for differences among sites and a Global R of 0.822 ($P < 0.001$) for differences among dates. In all pairwise comparisons among the 16 sites, only one (Double Creek West and Forked River West) showed no significant differences in the planktonic community. This was not surprising, given that these sites are both on the western side of the bay and are essentially next to each other. Pairwise comparisons for all monthly comparisons were significantly different suggesting generalized temporal communities present in the Bay.

Table 7. Contributing taxa defining the planktonic community associated with plankton tow samples based upon SIMPER Analysis. Similarity Percentages and species contributions provided for each site.

Metedeconk River East: Average similarity: 72.50

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
M. leidyi	1.68	13.53	1.03	18.66	18.66
Fish Eggs	2.1	12.28	0.94	16.94	35.6
Calanoida	1.76	11.37	0.81	15.68	51.28
Salpa salpa	0.64	11.12	0.48	15.34	66.62
Caridea	1.31	10.29	1.65	14.19	80.8
Brachyura	0.69	6.79	0.97	9.36	90.17

Metedeconk River West: Average similarity: 71.64

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
M. leidyi	2.8	29.13	1.13	40.66	40.66
Calanoida	1.66	11.62	0.63	16.22	56.88
Caridea	0.9	8.98	1.2	12.53	69.41
Fish Eggs	1	7.79	1.04	10.87	80.29
Salpa salpa	0.98	7.38	0.47	10.3	90.59

Silver Bay East: Average similarity: 63.51

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Fish Eggs	2.06	20.59	1.05	32.42	32.42
Calanoida	2.62	16.12	0.93	25.38	57.8
M. leidyi	0.86	8.8	0.52	13.86	71.66
Caridea	0.71	7.16	0.93	11.28	82.94
Brachyura	0.59	4.57	0.95	7.2	90.13

Silver Bay West: Average similarity: 61.74

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	2.72	25.83	1.14	41.84	41.84
Fish Eggs	0.88	18.92	0.64	30.65	72.49
Caridea	0.6	6.5	0.89	10.53	83.02
Brachyura	0.88	6.15	0.88	9.97	92.99

Toms River East: Average similarity: 49.28

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Fish Eggs	1.72	20.28	1.1	41.15	41.15
Calanoida	2.43	10.85	0.69	22.01	63.16
Brachyura	0.84	5.3	0.6	10.76	73.92
Caridea	0.95	4.36	0.63	8.85	82.77
M. leidyi	0.98	2.96	0.55	6.02	88.79
C. quinquecirrha	0.21	2.15	0.49	4.36	93.15

Toms River West: Average similarity: 73.07

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	2.09	23.66	0.95	32.38	32.38
Fish Eggs	1.45	19.57	0.66	26.78	59.16
M. leidyi	0.56	18.43	0.56	25.22	84.38
Brachyura	0.54	6.85	0.86	9.38	93.76

Forked River East: Average similarity: 69.52

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%

M. leidyi	2.08	18.17	0.97	26.14	26.14
Calanoida	2.59	15.97	0.96	22.97	49.11
Caridea	2.11	15.05	1.06	21.65	70.75
Brachyura	0.77	6.08	0.92	8.75	79.5
Salpa salpa	0.22	4.73	0.52	6.8	86.31
Fish Eggs	0.88	4.61	0.67	6.63	92.94

Forked River West: Average similarity: 70.36

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
M. leidyi	2.36	27.21	1.17	38.68	38.68
Calanoida	1.8	12.35	1.2	17.56	56.24
Caridea	1.28	12.09	1.02	17.19	73.42
Brachyura	0.99	8.29	0.9	11.79	85.21
Fish Eggs	1.07	6.49	0.88	9.23	94.44

Double Creek East: Average similarity: 65.65

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	5.29	25.63	1.6	39.04	39.04
Fish Eggs	2.05	10.27	0.9	15.65	54.69
Cladocera	4.53	9.44	0.63	14.38	69.07
Caridea	2.64	7.12	1.61	10.84	79.91
M. leidyi	1.01	5.79	0.63	8.82	88.73
Brachyura	1.39	4.86	1.7	7.41	96.14

Double Creek West: Average similarity: 64.10

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
M. leidyi	3.06	26.31	1.37	41.06	41.06
Calanoida	2.22	14.63	1.19	22.83	63.88
Brachyura	0.7	7.36	0.9	11.48	75.37
Caridea	0.74	5.87	1.03	9.16	84.52
Salpa salpa	0.07	4.14	0.52	6.46	90.99

Harvey Cedars East: Average similarity: 59.08

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
M. leidyi	1.3	19.6	0.79	33.17	33.17
Calanoida	3.32	14.4	1.28	24.38	57.55
Caridea	1.63	12.96	1.22	21.93	79.48
Brachyura	1.49	7.56	1	12.79	92.28

Harvey Cedars West: Average similarity: 69.41

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	6.27	30.49	1.84	43.93	43.93

M. leidyi	2.27	13.72	1.59	19.77	63.7
Brachyura	1.61	13.17	2.07	18.98	82.67
Caridea	1.31	9.97	1.68	14.37	97.04

Westeconk East: Average similarity: 68.34

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	10.48	19.75	1.1	28.9	28.9
M. leidyi	1.18	14.15	0.7	20.71	49.61
Brachyura	2.49	11.61	0.96	16.98	66.59
Caridea	2.87	9.33	1.1	13.66	80.24
Salpa salpa	0.34	5.68	0.48	8.31	88.55
Turritopsis	0.5	3.26	0.63	4.78	93.33

Westeconk West*:* Average similarity: 65.05

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	8.81	22.95	0.98	35.28	35.28
M. leidyi	1.4	16.87	0.69	25.94	61.22
Brachyura	2.28	8.69	1.25	13.37	74.58
Caridea	2.44	8.52	1.41	13.09	87.68
Fish Eggs	2.12	5.66	0.67	8.71	96.38

Tuckerton Creek East*:* Average similarity: 66.48

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	8.77	22.95	0.92	34.52	34.52
Salpa salpa	1.25	13.27	0.48	19.96	54.48
Caridea	3.05	11.14	1.08	16.76	71.24
Brachyura	1.85	6.55	0.85	9.86	81.09
Fish Eggs	1.84	4.74	0.69	7.13	88.22
M. leidyi	0.73	4.31	1.11	6.48	94.7

Tuckerton Creek West*:* Average similarity: 74.91

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	19.46	28.7	1.12	38.32	38.32
M. leidyi	1.71	12.77	1.01	17.05	55.36
Caridea	5.01	11.67	1.11	15.58	70.95
Salpa salpa	0.29	10.23	0.48	13.66	84.61
Brachyura	4.4	8.95	1.14	11.95	96.56

When a non-metric Multidimensional Scatter Plot was generated based on the spatial and temporal distribution of the taxa present in samples, two patterns emerge. First, when points are plotted based on the collection sites, significant overlap occurs given that the dominant taxa are greatly overlapping among all sites (Fig. 12). However, when points are plotted based on the

month of collection, strong clustering of samples is evident (Fig. 13). Collectively, these demonstrate both spatial and temporal differences among sites within the bay, but more importantly the planktonic community in 2013 seemed to respond similarly among sites, possibly due to the reduction of *Chrysaora* from stations in the northern bay.

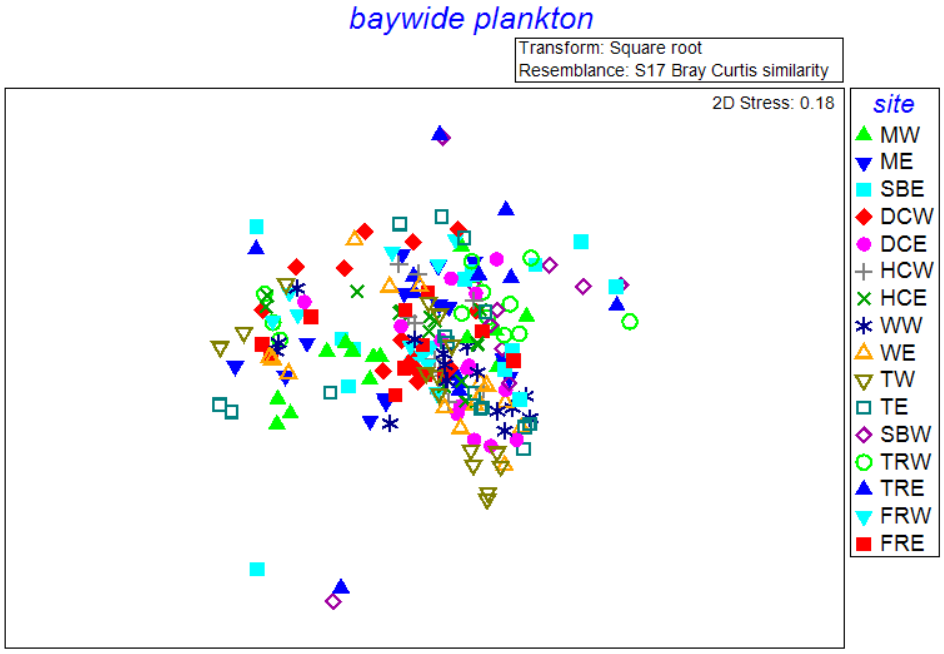

Figure 12. MDS plot of planktonic community structure from bay-wide samples plotted based upon the sites of collection. Clustering of samples indicates similarity of community structure. .

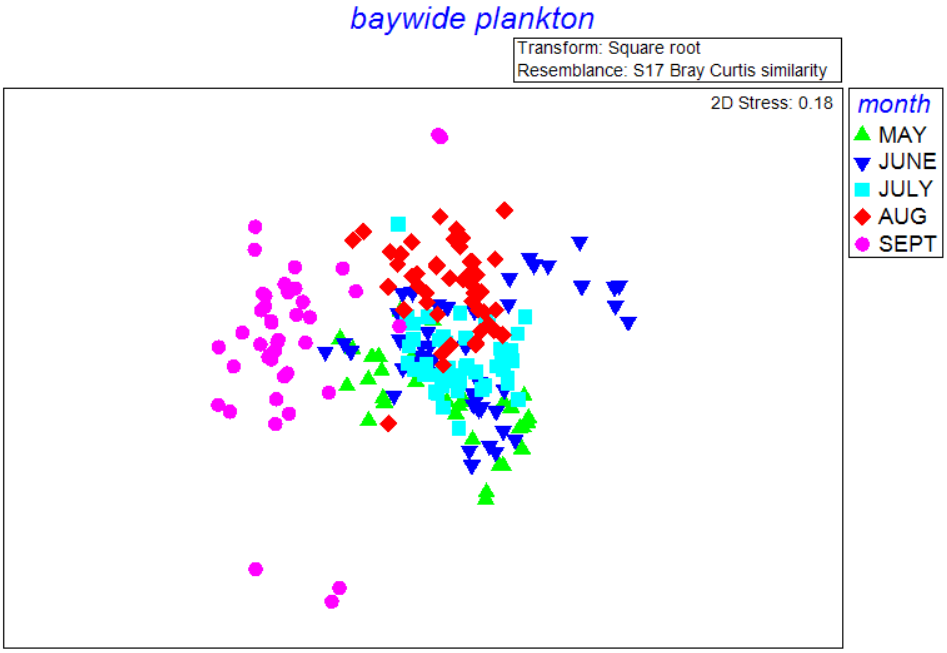

Figure 13. MDS plot of planktonic community structure from bay-wide samples plotted based upon the month of collection. Clustering of samples indicates similarity of community structure. .

Lagoon Planktonic Community

When lagoon samples were analyzed for species contributing to defining the planktonic community, the SIMPER analysis showed an average similarity between 28-51% for all sites. Of the 27 identified taxa from samples, defining community composition was dictated by five dominant groups including Calanoida, Brachyura, Caridea, Fish Eggs, and *M. leidyi* (Table 8). Only Kettle Creek Lagoon showed the relative importance of both juvenile and ephyrae of *Chrysaora*, while *Turritopsis* was important for the Harvey Cedars Lagoon site. When site and month of collection were analyzed using a Two-Way Crossed ANOSIM, significant differences were observed among all sites and for each month. Overall analysis for site differences showed a Global R statistic of 0.79 ($P< 0.001$) and all sites combinations were different from each other (Table 9). For monthly comparisons, significant differences were observed for each monthly comparison and a Global R statistic of 0.87 ($P< 0.001$). Combining the results from the SIMPER and ANOSIM analyses demonstrates both significant spatial and temporal variation among the lagoons sampled and that the relative abundance of the other 21 taxa created relatively unique planktonic communities among the lagoons.

Table 8. Contributing taxa defining the planktonic community associated with plankton tow samples collected in the lagoon sites based upon SIMPER Analysis. Similarity Percentages and species contributions provided for each site.

Point Pleasant Lagoon*:* Average similarity: 41.70

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	2.82	16.11	1.14	38.62	38.62
Brachyura	0.71	7.56	3.4	18.13	56.75
Caridea	0.71	6.29	0.88	15.09	71.84
Fish Eggs	1.41	4.57	0.61	10.95	82.79
M. leidyi	0.57	3.95	0.37	9.46	92.25

Kettle Creek Lagoon*:* Average similarity: 28.75

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	6.33	12.19	0.67	42.38	42.38
Brachyura	1.44	7.93	1.25	27.56	69.95
C. quinquecirrha	0.39	2.46	0.43	8.55	78.5
C. quinquecirrha ephyrae	0.72	2.01	0.37	7	85.5
Caridea	0.27	1.47	0.71	5.12	90.62

Chadwick Island Lagoon*:* Average similarity: 32.87

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	2.56	17.23	0.98	52.42	52.42
Fish Eggs	1.66	5.54	0.58	16.84	69.27
Calanoida	7.7	4.8	0.34	14.61	83.88
Caridea	0.62	2.37	0.77	7.22	91.1

Toms River Lagoon*:* Average similarity: 30.66

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	9.04	17.86	1.33	58.26	58.26
Calanoida	9.17	6.11	0.43	19.91	78.17
Fish Eggs	1.42	2.64	0.75	8.61	86.78
Caridea	1.04	2.32	0.96	7.55	94.33

Cedar Creek Lagoon*:* Average similarity: 51.28

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	4.88	28.72	1.29	56.01	56.01
M. leidyi	2.12	12.64	2.05	24.65	80.65
Calanoida	4.11	7.23	0.51	14.09	94.74

Harvey Cedars Lagoon*:* Average similarity: 44.12

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	1.11	12.97	2.35	29.41	29.41
Calanoida	2.55	10.43	0.81	23.65	53.06
M. leidyi	0.98	7.9	1.4	17.9	70.96
Fish Eggs	0.78	4.46	0.76	10.1	81.06
Turritopsis	0.4	3.17	0.59	7.19	88.25

Beach Haven West Lagoon*:* Average similarity: 47.28

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	1.98	20.98	1.18	44.38	44.38
M. leidyi	1.29	16.78	1.48	35.49	79.87
Calanoida	0.69	5.87	0.86	12.42	92.29

Tuckerton Creek Lagoon*:* Average similarity: 48.26

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
M. leidyi	1.43	16.69	1.41	34.58	34.58
Brachyura	2.15	13.96	1.07	28.93	63.51
Calanoida	2.44	11.67	2.18	24.18	87.69
Caridea	0.5	4.78	1.5	9.91	97.6

Table 9. Results from the ANOSIM statistical test for Pair wise comparisons between lagoon sites for plankton community structure.

Pairwise Tests		
Groups	R Statistic	P-value
PPL, KCL	0.796	0.005
PPL, CIL	0.975	0.001

PPL, TRL	0.988	0.002
PPL, CCL	1	0.003
PPL, HCL	0.914	0.001
PPL, BHL	0.752	0.009
PPL, TL	0.965	0.001
KCL, CIL	0.512	0.005
KCL, TRL	0.611	0.002
KCL, CCL	0.696	0.005
KCL, HCL	0.821	0.002
KCL, BHL	0.799	0.003
KCL, TL	0.757	0.002
CIL, TRL	0.506	0.001
CIL, CCL	0.763	0.005
CIL, HCL	0.975	0.002
CIL, BHL	0.855	0.007
CIL, TL	0.979	0.001
TRL, CCL	0.867	0.003
TRL, HCL	0.741	0.003
TRL, BHL	0.778	0.003
TRL, TL	0.82	0.002
CCL, HCL	0.896	0.003
CCL, BHL	0.657	0.003
CCL, TL	1	0.003
HCL, BHL	0.66	0.002
HCL, TL	0.667	0.002
BHL, TL	0.919	0.003

When a non-metric Multidimensional Scatter Plot was generated based on the spatial and temporal distribution of the taxa present in samples a tremendous amount of overlap occurred in both the structure associated with sites (Fig. 14) and months of collection (Fig. 15). These similarities may reflect fewer taxa being identified in these samples (taxa poor relative to bay-wide collections) and general similarities with water quality/habitat.

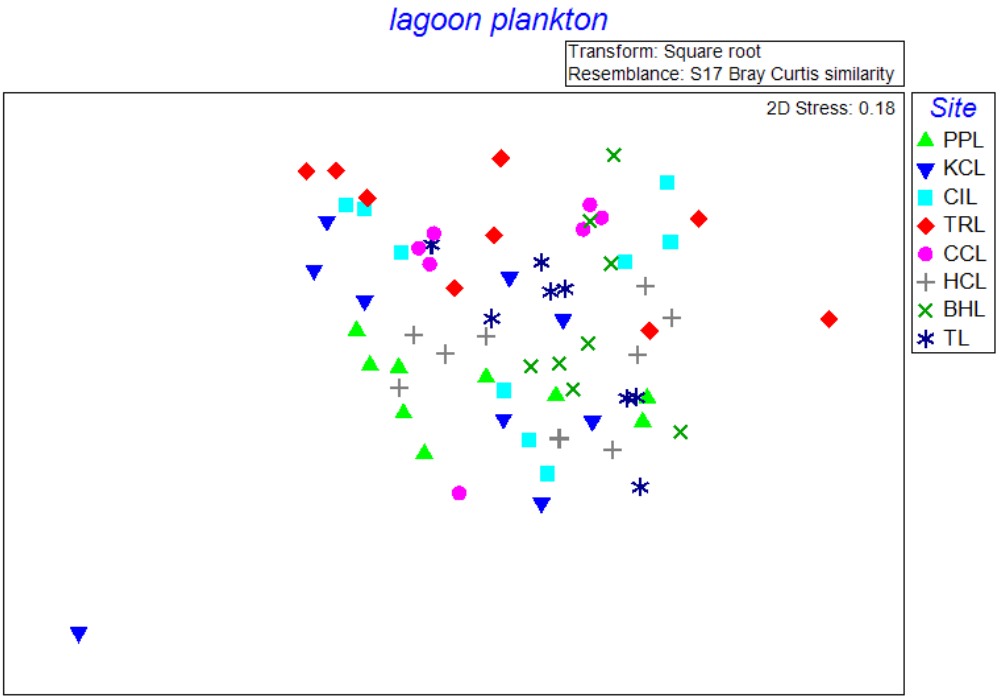

Figure 14. MDS plot of planktonic community structure from lagoon samples plotted based upon the sites of collection. Clustering of samples indicates similarity of community structure. Outlying point for Kettle Creek indicates a sample with high densities of *Chrysaora* in August.

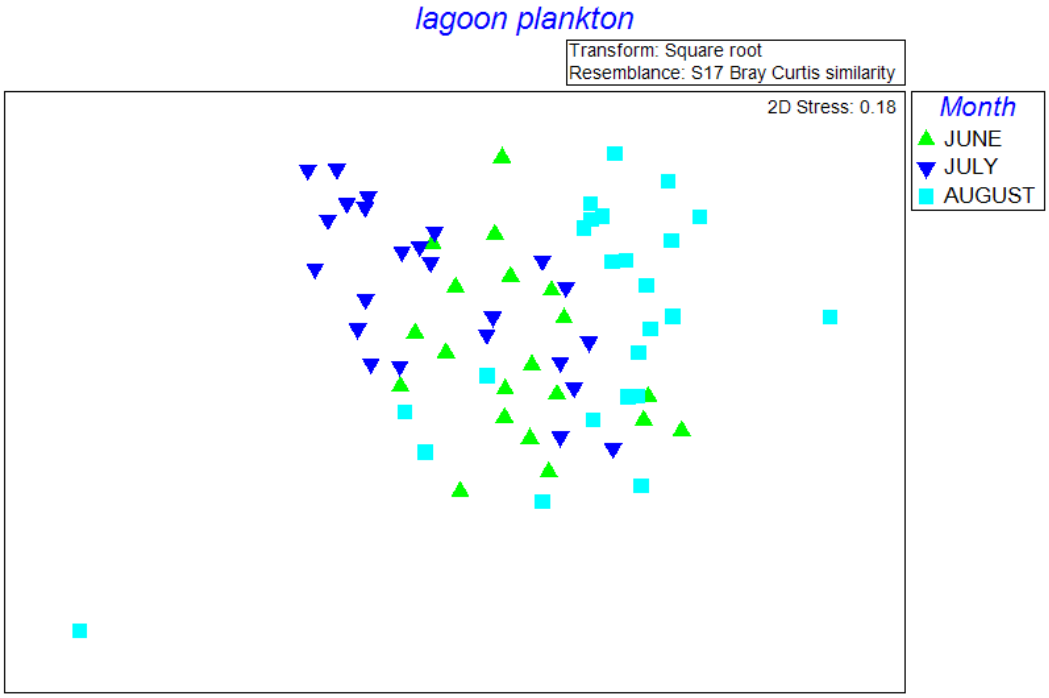

Figure 15. MDS plot of planktonic community structure from lagoon samples plotted based upon the month of collection. Clustering of samples indicates similarity of community structure. Outlying point for August indicates a sample with high densities of *Chrysaora* in Kettle Creek.

Assess the distribution and density of settling *Chrysaora* polyps and development of resting podocysts.

We sampled locations within Barnegat Bay to assess the distribution of settling polyps throughout the summer. Results indicate two regions with settling polyps and include regions near Lavalette, NJ and within the Forked River Lagoon region. Density of solitary organisms and percent cover of the settling plate of the most common organisms is provided in Table 10.

Table 10. Average density or percent cover of settling solitary organisms or colonial taxa encountered on settling plates deployed in Barnegat Bay. Density expressed by average number of individuals counted on the 5cm x 5cm plates.

Scientific Name	Common Name	Mean Density
Mogula spp.	Sea Squirt	0.85
Semibalanus balanoides	Barnacles	27.94
Spiroibis spp.	Polychaeta	55.93
Crepidula spp.	Slipper shell	0.023
Diadumene leucolena	White anemone	0.162
Diadumene lineata	Orange Stripped Anemone	0.2129
Metridium senile	Clonal Plumose Anemone	0.0138
***Chrysaora* Podocyst**	**Sea Nettle Podocyst**	**8.939**
***Chrysaora* Polyps**	**Sea Nettle Polyps**	**0.0601**
Bugula turrita	Spiral Tufted Bushy Bryozoa	8.393
Membranipora membranacea	Lacy Bryozoa	38%
Flustrellidra hispida	Bristly Bryozoa	0.7%
Scruparia ambigua	Bryozoa	5%
Crisia spp.	Jointed-tubed Bryozoa	0.004%
Eucretea loricata	Eucretea Bryozoa	0.02%
Botryllus schlosseri	Golden Star Tunicates	1.4%

Assess the diet of *Chrysaora* through dissection and molecular analysis.

Direct Dissection of *Chrysaora*

84 *Chrysaora* were dissected to assess diet preference. Temporally, 7 were collected in June, 55 in July, and 22 in August. Spatially, we divided collections into three sub-sections of the bay and these were nominally referred to as 'southern', 'mid', and 'northern' portions of the bay where *Chrysaora* was present. The southern region relates to individuals collected near the Double Creek sampling region (Fig. 1), the mid bay region reflects individuals collected near our Forked River sampling stations, and the northern region encompasses individuals collected near the Toms River to Silver Bay sampling stations. The minimal collections in June related to few individuals encountered during that month and August samples were minimal due to the high die-off, which occurred in the middle of July depressing overall population density in Barnegat Bay in 2013 (see Fig. 6). Spatially, 46 were collected in the north region, 19 in the mid-bay region, and 19 in the southern region of the *Chrysaora* distribution. Dissection results demonstrate some seasonal trends in food selection. Specifically, fish eggs were prominent in

June and July 2013 samples, but samples from July showed a broad range of prey items including Copepods, Polychaeta, Brachyura, Caridea, and fish larvae.

Northern Region

When diet trends are compared to available prey, strong selection by *Chrysaora* is present in the results. Specifically, if we assess individuals collected in the northern region, it is apparent that samples collected in June and July showed similar patterns of presence to those occurring in the plankton (Fig. 16) and diet was dominated by fish eggs in June and copepods and fish eggs in July. However, in August, diet selection showed a preference for polychaetes and harpactacoid copepods, which are both benthic prey, compared to their availability in the water column (Fig. 16). This suggests either novel feeding strategies targeting benthic organisms where individual *Chrysaora* swim to the benthos, trail their tentacles along the bottom, then swim back into the water column (Bologna pers. obs.) or strong selection for targeted prey. Given the small size of harpactacoids and the relatively high densities of calanoid copepods, Caridea, and Brachyura larvae in the water column, but not equally represented in the diet; the observed behavior of targeting the benthos seem the most likely answer.

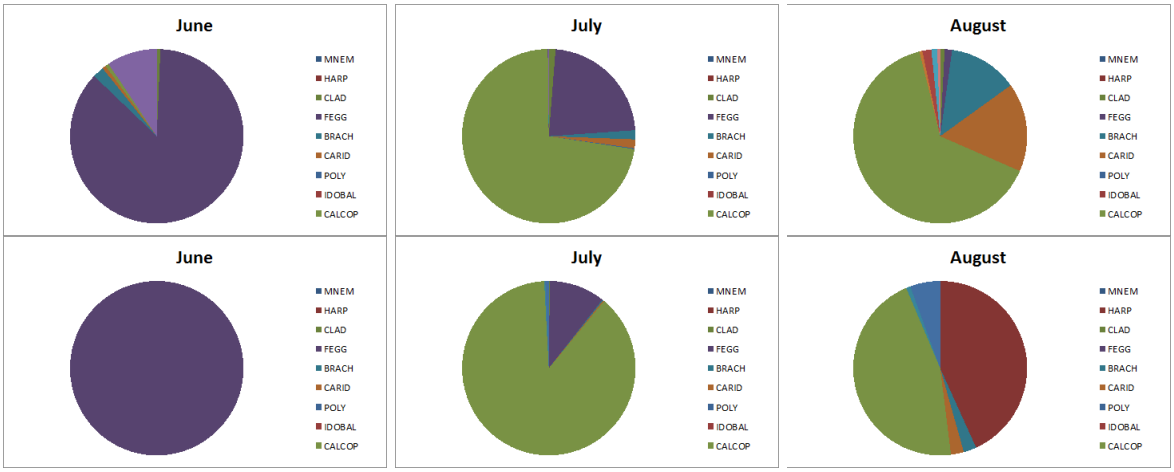

Figure 16. Comparisons of Potential Available Prey (upper Panels) collected in plankton tows with Prey Selected (lower panels) by *Chrysaora* for the months of collection from the Northern Region of the Bay where *Chrysaora* is present.

Middle-Bay Region

Samples collected from the mid-bay region were only collected in July, matching peak abundance of *Chrysaora* in Barnegat Bay. From the individuals dissected, Calanoid copepods and fish eggs were large portions of both available prey and prey chosen by *Chrysaora* (Fig. 17). Similar to the August results from the northern region, polychaetes and harpactacoid copepods were strongly represented in the diet, but not in plankton tows. This again suggests benthic targeting by *Chrysaora* in this region.

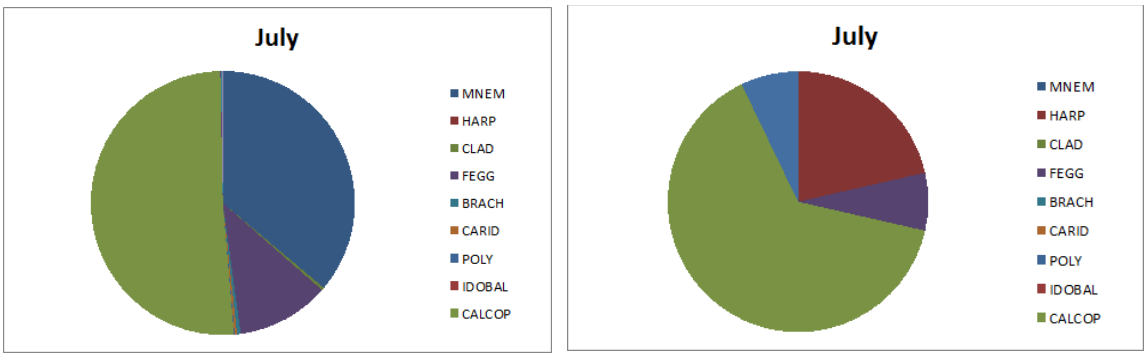

Figure 17. Comparisons of Potential Available Prey (Left Panel) collected in plankton tows with Prey Selected (Right Panel) by *Chrysaora* for July from the Middle Region of the Bay where *Chrysaora* is present.

Southern Region

Samples from the southern region were only collected in July since they were not abundant in June in this region and seemed to have died off during July and were absent in August. Diet analysis showed a strong selection for calanoid copepods and Brachyura larvae, both present in high densities in the water column (Fig. 18), but diet was again over represented in harpactacoids and polychaetes.

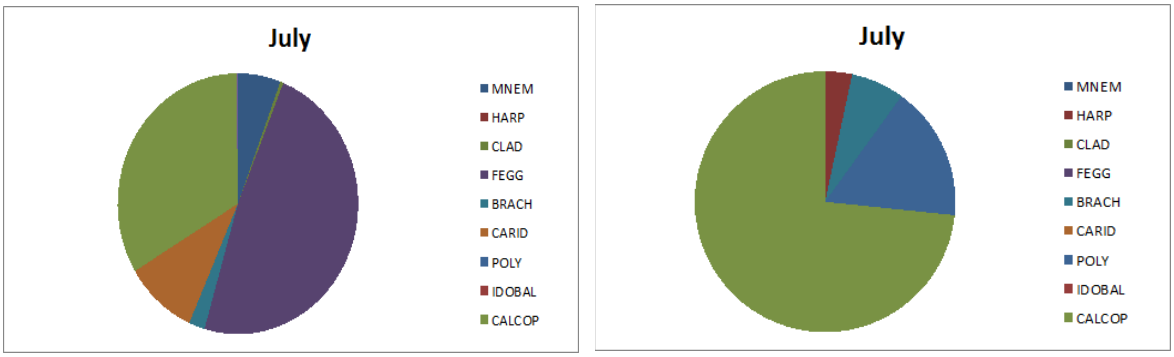

Figure 18. Comparisons of Potential Available Prey (Left Panel) collected in plankton tows with Prey Selected (Right Panel) by *Chrysaora* for July from the Southern Region of the Bay where *Chrysaora* is present.

Dissection Summary

While some organisms were abundant in both the plankton samples of available prey and in the diet assessment of dissected *Chrysaora*, it is evident that sea nettles demonstrate preference of certain organisms in their diet. In particular, *Chrysaora* in June showed only fish eggs as part of their diet and this matched their presence in the water column, but other taxa prey were not identified from any individuals. This suggests that during the early part of the summer *Chrysaora* were preferentially targeting fish eggs, a highly nutritious prey. During July and August, *Chrysaora* diet showed some similarities with available pelagic prey, but the strong preference for polychaetes and harpactacoid copepods demonstrates strong diet selection for benthic organisms, since both of these groups were poorly represented in the water column and the polychaetes in the diet were large adults, not small larval forms. Based on field observations

of *Chrysaora* actively swimming to the benthos and dragging their tentacles along the surface suggests prey targeting, specifically for large, energy rich polychaetes. The small harpactacoids are more likely incidental catch while targeting the larger polychaetes. Regardless, this demonstrates that *Chrysaora* may not only play an important role in pelagic food web structure, but they also play a role in benthic-pelagic coupling based on their feeding strategies in Barnegat Bay. While *Mnemiopsis* was abundant in plankton tows and lift nets, the dissection results did not identify any, due the fact that they preserve poorly and are digested quickly by *Chrysaora*. However, a sub-sample of individuals (N=17) was sampled for the presence of *Mnemiopsis* DNA in their guts. Specifically, a species-specific region of the 16S rDNA for *Mnemiopsis leidyi* was sequenced from these samples and they showed a positive hit for this DNA (Fig. 19) for 2 of the sampled individuals.

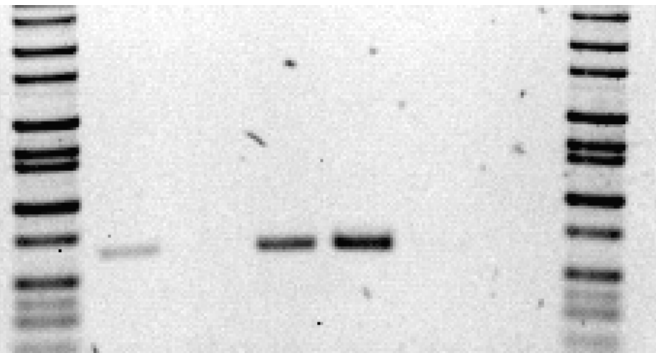

Figure 19. PCR amplification of *Mnemiopsis leidyi* DNA from the gut of *Chrysaora quinquecirrha.* Using PCR primers that specifically amplify a region of the 16S rDNA gene of *M. leidyi* we amplified a 682 bp fragment of the comb jelly mitochondrial genome which was verified by Sanger sequencing (data not shown). In this experiment we identified 2 out of 17 samples collected as positive for *M. leidyi* DNA (11%).

Molecular Diet Analysis: Gut content Taxon Identification

In the case of experimentally controlled diet analyses, the number of reads does not appear to correlate well with known diet proportions (e.g. Deagle et al. 2013). For these reasons, we did not quantify the relative abundance of prey species from the proportion of reads associated with each of the assembled contigs. Even using the frequency of occurrence in individual scats as a quantitative assessment can be problematic (see Deagle et al. 2013). We chose a conservative approach and combined multiple individual gut lavages and tentacle picks into two different library preparations that were analyzed individually and simultaneously to simply record the identity of prey items to lowest possible taxonomic unit (presence or absence data) to get at the breadth of the sea nettle's feeding ecology.

Blastn XML results were imported into MEGAN 5.5.4 (Huson et al. 2011) for visual inspection. Given our expectation of many more contigs than useful identifiable sequences we used very stringent LCA (lowest common ancestor) and analysis parameters (Min Score = 500, Max Expected = 0.01, Top Percent = 5.0, Min Support Percent = 0.0, Min Support = 1, LCA percent = 100.00, Min Complexity = 0, Use Minimal Coverage Heuristic) with hopes of more quickly identifying high probability prey item matches. Tetrapod, bacteria, and virus assignments

were not looked as these likely represent contamination, microbiome of predator or prey, and/or environmental DNA found in the bay water.

We only indicate species level identification for contigs with >98% sequence similarity for genes that are well represented in the NCBI database and are able to delineate species boundaries (e.g. 12S, 16S, 18S, 28S, COI). In many cases, the gene regions identified were only present in a limited number of taxa and as such were scored as having higher taxonomic rankings based on sequence similarity, number of available sequences, and length of BLAST hit.

BLAST Annotation

The BLAST search of the combined data set identified 49,840 contigs with blastn hits to the nr database (Appendix D). Of these, 371 sequences were assigned in MEGAN (Table 11). Manual inspection of these sequences identified 100 contig sequences, which were confidently assigned to twenty-three taxa (Table 12) that were not bacteria, viruses, or tetrapods. Of these twenty-three taxa, we were able to identify 9 to the species level (Table 12). More taxa were recovered from the gut lavage (19) than the tentacle picked samples (17). Varying the cutoff values for the removal of poor quality reads had no effect on the identification of prey items (results not shown).

Care was taken to ensure that the assignment of each of the 100 contig sequences was accurate to the lowest taxonomic level possible. For example, MEGAN assigned fifteen contig sequences to the Engraulidae (anchovies): *Coilia nasus* (1 sequence), *Anchoa* (10 sequences), and *Engraulis* (4 sequences). The *Coilia nasus* contig was blast annotated as *RYR3* with 86% sequence similarity for 553 base pairs. Other engraulid *RYR3* sequence are not available on Genbank but *RYR3* is well represented for many ray-finned fishes. The *RYR3* contig sequence was manually assigned to Engraulidae based on the paucity of engraulid *RYR3* genes sequences, overlapping sequence coverage with hundreds of different ray-finned fish, and the northwest Pacific Ocean distribution of *Coilia nasus*.

Of the 10 *Anchoa* sequences, 9 sequences had 96-100% sequence homology to *Anchoa compressa* ultra-conservative elements. The ultra-conservative sequences were assigned to Engraulidae because only one engraulid has been sequenced for ultra-conservative elements, ultra-conservative elements for many ray-finned fish families have been sequenced, and ultra-conservative elements are poor markers for species level identification given their high sequence conservation. The remaining *Anchoa* sequence was BLAST annotated as *Rag1* with 98% sequence homology to three *Anchoa* species (*A. parva*, *A. delicatissima*, *A. mitchilli*). *Rag1* has historically been used as a phylogenetic marker but by itself is a poor marker for species level identification due to its' highly conservative nature. The *Rag1* sequence was assigned to Engraulidae.

Three of the four *Engraulis* blast annotated contigs were mitochondrial genome sequences. These three mitochondrial contigs ranged in length from ~350bp to 10,000 bp. Unlike *Engraulis*, a complete mitochondrial genome of *Anchoa* is not available on Genbank. *Anchoa mitchilli* has five mitochondrial genes sequenced (*Cytb*, *16S*, *12S*, *COI*, *D-loop*). Direct comparison between the Genbank *Anchoa mitchilli* sequences and our contigs showed higher sequence similarity than to the *Engraulis* mitochondrial genome. This includes the bar coding

gene *COI*. The three mitochondrial genome sequences were assigned to *Anchoa mitchilli* given the 99% sequence homology with *COI* and the East Atlantic distribution of *Anchoa mitchilli*. The remaining *Engraulis* contig sequence was blast annotated as rhodopsin. Rhodopsin is well represented in ray-finned fish but no *Anchoa* sequences are available. The rhodopsin contig was assigned to Engraulidae. Appendix D further summarizes our final taxonomic assignments for the 100 identifiable contigs.

Table 11. Number of contigs assigned by MEGAN.

Group	MEGAN contig assignments
Bacteria	86
Viruses	2
Eukaryota	283
Amoebozoa (amoebas)	1
Actinopterygii (fish)	63
Tetrapoda (tetrapods)	116
Echinodermata (urchins, sand dollars, sea cucumbers, sea lilies)	2
Hemichordata (acorn worms)	3
Platyhelminthes (flat worms)	6
Arthropoda (arthropods)	23
Chelicerata (mites, scorpions, and relatives)	2
Crustacea (crustaceans)	17
Insecta (insects)	4
Annelida (segmented worms)	6
Mollusca (bivalves, gastropods, cephalopods)	10
Cnidaria (jellyfish, hydra, sea anemones, corals)	33
Chrysaora (sea nettles)	5
Ctenophora (comb jellies)	20

Table 12. *C. quinquecirrha* prey items identified in both the gut lavage and tentacle picks.

Final Classification	Common Name	Gene(s)	Gut lavage	tentacle pick
Ampithoe valida	amphipod	COI; 18S	X	
Acartia tonsa	copepod #1	ITS1, 5.8S rRNA, ITS2; COI	X	X
Crangonyctidae	copepod #2 (Not Cyclopoida)	28S	X	
Cyclopoida	copepod #3 (Not Crangonyctidae)	18S	X	
Pseudodiaptomus coronatus	copepod #4	18S; 28S	X	X
Cirripedia	barnacle	28S	X	
Americamysis bahia	opossum shrimp	18S	X	X
Diadumene leucolena	white anemone	28S	X	
Nynantheae	anemone possibly white anemone	COI and other mitochondrial genes	X	
Actiniaria possibly *Nematostella vectensis*	starlet sea anemone	Miscellaneous nuclear genes	X	X
Mnemiopsis leidyi	sea walnut	18S; 28S; COI and other mitochondrial genes	X	X
Alitta (=*Nereis*) *succinea*	polychaete clam worm	28S; COI	X	X
Goniadidae	polychaete worm #2	28S	X	X
Lepocreadiidae	trematode	18S	X	X
Stylochidae	flatworm	28S	X	X
Asterozoa	starfish or brittle star	28S		X
Echinozoa	echinoderm	28S		X
Nudibranchia	nudibranch	18S	X	X
Gastropoda possibly Euthyneura	gastropod possibly Euthyneura	heat shock protein	X	X
Mercenaria mercenaria	hard clam	COI; 18S		X
Veneridae other than *Mercenaria*	clam #2	COI		X
Hemichordata	possibly acorn worm	BAC sequences	X	X
Anchoa mitchilli	bay anchovy	COI and other mitochondrial genes		X

Project Summary

Results from the 2013 research demonstrate some important and fundamental findings regarding the distribution and abundance of gelatinous zooplankton and their role in community structure in Barnegat Bay. Some of the differences observed in 2013 compared to 2012 may have been due to Hurricane Sandy and this is discussed below. One critical data series which was identified during 2013 related to the very high water temperatures which occurred in July. While warming summer temperatures is expected to occur, the generalized increase of between 4-7˚C from June to July (and 12˚C for DCE!) and peak water temperatures exceeding 30˚C impacted the distribution and abundance of *Chrysaora* in Barnegat Bay with almost no individuals collected in August and September in lift nets. *Chrysaora* was collected in lagoon sampling events during August and in plankton tows from bay-wide collections, but these generally represented either ephyrae or very small individuals. With the reduction in density and distribution, the top-down impacts of *Chrysaora* on pelagic community structure was muted. Consequently, few significant interactions occurred between *Chrysaora* and other taxa in the system. The addition of sampling in lagoon communities in Barnegat Bay has yielded information relevant for understanding the life history of *Chrysaora* and has identified that even the most southerly regions of Barnegat Bay are demonstrating the presence of *Chrysaora* DNA in both bay-wide and lagoonal communities. Consequently, *Chrysaora* distributions maybe expanding within Barnegat Bay, and we now have evidence of their presence in the Navesink-Shrewsbury Estuary. Diet analysis of *Chrysaora* has also yielded critical trophic linkages in Barnegat Bay, although their muted density and distribution did not cause observable trophic cascades. Specifically, direct dissection has shown that while sea nettles do show diet patterns that reflect the relative distribution of planktonic organisms available, strong evidence shows a critical benthic-pelagic link, whereby sea nettles swim to the benthos and drag their tentacles capturing both large polychaetes and benthic harpactacoid copepods, neither of which are present in abundance in plankton tow samples. The molecular assessment of diet has demonstrated the power of the technique to not only identify prey visible through dissection, but also species which do not preserve well (e.g., comb jellies). Additionally, our findings have demonstrated that the dominant fish eggs being consumed from the 2013 samples were bay anchovies (*Anchoa mitchilli*) and that *Chrysaora* diet includes several species of copepods, several species of worms, bivalves (including hard clams, *Mercenaria mercenaria*) and numerous other taxa from several phyla. The uncertainty among identification of prey relates to the lack of sequenced data for comparisons. Consequently, as data become available, we will be able to identify more species and better develop trophic linkages. Potentially, bar-coding the Bay will yield critical information leading to a complete trophic analysis and will provide a foundation for future studies.

Potential Impacts of Hurricane Sandy: Comparisons of 2012 to 2013

In October of 2012, Hurricane Sandy hit the central coast of New Jersey and Barnegat Bay took a significant hit substantially impacting the coastal island and the bay. In particular, a new inlet was cut in the northern region near the Metedeconk River at the base of the Mantoloking Bridge. While this cut was rapidly filled, large amounts of sand and debris were deposited into Barnegat Bay. Several over-wash conditions were observed throughout the bay

and sand and debris were deposited. Additionally, damage to infrastructure (homes, docks, etc…) was large and ultimately this could create cumulative impacts to the pelagic communities present in Barnegat Bay. Based on both lift net and plankton tow samples, the density and distribution of *Mnemiopsis leidyi* were similar in distribution and density between 2012 and 2013. However, when assessing the density and distribution of *Chrysaora quinquecirrha*, the distribution was muted and density was about half in 2013 compared to 2012. These results were repeated for molecular DNA detection of ephyrae and larvae. DNA concentrations for 2013 were about half that of 2012 suggesting that both production of ephyrae was muted and the reduction in adult populations reduced reproductive output of larvae. One potential explanation of this is the destruction of numerous floating docks and piers within the bay. Based on our settlement data over the last several years, polyp populations are the source of adult medusa in the bay. If these floating structures, which can show very high densities of polyps and are the source of medusa, were lost; then their loss due to the storm would necessarily decrease the polyp population causing the reduced densities observed in 2013. It will take several years of monitoring to assess the long-term impacts of Hurricane Sandy on pelagic communities and whether the destruction of polyp habitat has long-lasting impacts on Barnegat Bay.

Conclusions and Recommendations for Future Research

Conclusions from this research indicate that there are higher densities of sea nettles in the northern portions of Barnegat Bay, with few individuals encountered south of Barnegat Inlet. However, molecular evidence shows that despite few adults being encountered, large amounts of DNA exists throughout the Bay including larvae and ephyrae. This suggests that polyp populations in the southern regions of the bay may be currently small, but growing. The addition of lagoon sampling in 2013 has provided us with greater insight into the probable source of ephyrae, which grow into the adult medusa stage, as well as the probable settling habitat for larvae. Certainly Hurricane Sandy impacted the relative abundance of polyps in Barnegat Bay by destroying docks and bulkheads, but future investigations into lagoon communities will allow us to evaluate their importance in the life history cycle of *Chrysaora* and potentially develop management strategies to minimize their impacts in Barnegat Bay.

The muted density and distribution of sea nettles in 2013 still showed negative correlations with the other dominant gelatinous zooplankton species, *Mnemiopsis leidyi*, demonstrating top-down food web structuring. Sea nettles were also negatively correlated with major prey items including cladocerans, fish eggs, fish larvae, and copepods. The diet analysis we conducted has demonstrated not only theorized trophic linkages based on literature (e.g., *Mnemiopsis leidyi*, copepods, fish eggs and larvae), but also unknown and uninvestigated trophic interactions. Specifically, the incorporation of large polychaetes and benthic copepods in the diet of *Chrysaora* indicate significant benthic-pelagic coupling which needs to be addressed. Additionally, the molecular investigations have allowed us to identify species based on their DNA (e.g., *Anchoa mitchilli* eggs) and have added new taxa and taxa groups to known diet items of sea nettles. One area which future research should begin to address is to identify DNA sequences for coastal organisms to increase the genetic data resources for understanding diets, as well as to understand community structure. One particular way to address the lack of DNA data for marine organisms is to develop a bar-coding system. In this way, targeted DNA analyses can

yield critical information in understanding not only Barnegat Bay, but all coastal regions of New Jersey.

If the fundamental goal of studying the gelatinous zooplankton is to develop management strategies to combat their increase, greater research is needed to understand the distribution and abundance of the polyp stage of *C. quinquecirrha*. Increasing development, continued eutrophication, and depleted oxygen levels in coastal waters favor *C. quinquecirrha* over other organisms. As such, they can out-compete other fouling species for space and asexually spread and expand. Since this life-history stage is critical for overwintering, understanding the dynamics and survival of polyps is necessary to develop reasonable management strategies to limit their expansion or reduce their numbers.

Recommendations and Application for the NJ Department of Environmental Protection

Currently, gelatinous zooplankton are abundant and important components to the planktonic community. They have the potential to exert top-down pressure in these communities and simultaneously act as competitors and predators of commercially and recreationally important fish and invertebrates through consumption of shared food resources (e.g., copepods) and direct consumption of eggs, larvae and early juveniles. If populations continue to increase in Barnegat Bay, it is possible that they may become the top seasonal predators and potentially disrupt food webs leading to declines in commercially and recreationally important species. The changes which occurred in Barnegat Bay during 2013 showed an increase in the diversity of gelatinous zooplankton species including numerous coastal/open water species. Consequently, it is likely that Hurricane Sandy impacted the regional geomorphology, potentially impacting tidal influxes of coastal marine species into Barnegat Bay. Understanding these changes may help to evaluate community structure and ecosystem functioning in a post-Sandy Barnegat Bay.

Our findings of early stage individuals through molecular techniques and the addition of lagoonal communities shows that small polyp populations exist throughout Barnegat Bay, but clearly the highest densities occur in the northern region of the Bay. If these small polyp populations begin to expand, it is highly likely that southern regions of Barnegat Bay may be plagued by sea nettles similar to the northern regions. As a result, if *Chrysaora* polyps expand and begin producing blooms of adults, the adult reproductive capacity would provide a positive feedback loop increasing polyp populations leading to continued sea nettle blooms in the future. Ultimately, controlling or minimizing sea nettle populations will require monitoring of polyp populations and solutions to eradicate or minimize this life history stage of *Chrysaora*.

Next Generation sequencing of *Chrysaora* diet has begun to establish the potential impacts the *Chrysaora* is having/may have on planktonic community structure, especially as it may relate to commercially and ecologically important species. *Chrysaora* is known to feed heavily on fish eggs and larval and adult *Anchoa mitchilli*. Consequently, the expansion of *Chrysaora* could have major impacts on various species directly (e.g., *A. mitchilli*) and indirectly through trophic shunting of energy away from larger fish predators to those of jellyfish. Therefore, understanding trophic linkages using Next Generation sequence analysis allows us to identify major linkages and identify linkages yet unknown. Our investigation has identified hard

clam (*Mercenaria mercenaria*) DNA in the gut analysis, suggesting that *Chrysaora* might contribute to hard clam larval losses and this could impact an already depleted hard clam population in Barnegat Bay.

Perhaps the limiting factor in fully assessing this question is the lack of marine organisms in the genetic databanks. One area that the NJDEP would benefit from is the development of a DNA bar-coding system of organisms which would allow these linkages to be identified. Given that all coastal New Jersey Waters contain a similar, almost identical suite of species, the bar-coding system would need to be developed only once, then the data are available for anyone interested in addressing these questions.

List of Presentations

PI Bologna also provided a public lecture entitled "*Chrysaora quinquecirrha* in Barnegat Bay" to Save the Bay at their annual meeting. This lecture highlighted the research findings as well as biology and ecology of sea nettles in Barnegat Bay. Additionally, our students presented findings from our research as part of the Master's Degree research at local and national meetings.

October 2013, MACUB Meeting
March 2014, Benthic Ecology Meeting
April 2014, New Jersey Academy of Sciences Meeting

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Appendix A

Appendix A**:** CTAB/NaCl DNA Extraction Protocol

1. Combine CTAB and water in sterile 15 mL plastic tube. Swirl under hot tap water until CTAB has dissolved.
2. Add other reagents in order. Move to hood to add the -mercaptoethanol.
3. Add proteinase K powder last. Cap and invert to dissolve.
4. Add 500 L mix to each sample in 1.5 mL Eppendorf tube.
5. Grind each sample separately with blue micropestle, leaving pestle in tube.
6. Incubate @ 60°C for 60 minutes. Invert tubes occasionally to mix.
7. Add 0.5 mL of chloroform:isoamyl alcohol (24:1)
8. Gently mix for 2 minutes by inverting the tube.
9. Spin for 10 minutes @ maximum speed (14,000 x g) in microcentrifuge @4°C.
10. Transfer upper aqueous phase into new 1.5 mL tube. Do not transfer any solid material at the interface to new tube.
11. Add 1 µL RNase A (10 mg/mL) and incubate 30 minutes @37°C.
12. Add 2/3 volume of isopropanol. Close cap and gently invert to mix.
13. Allow tube to sit at room temperature for 2 hours to overnight. Watch for formation of DNA fibers in solution.
14. Spin for 15 minutes @14,000 x g at 4°C to pellet the DNA.
15. Remove supernatant carefully. Then wash 2X with 500 µL of 70% EtOH. Each time spin for 15 minutes @14,000 x g at 4°C to pellet the DNA.
16. Remove supernatant and dry pellet briefly (5 min) in Speed-Vac without heating.
17. Resuspend pellet in minimum volume of TE (pH 8.0).
18. Determine concentration and purity by UV absorption with NanoDrop.
19. Store in aliquots at -20°C.
20. Run small aliquot on 1.0% agarose gel to check for quality and size of DNA.

Reagent	[Final]	Volume	# of Samples	Total Volume	Checklist
CTAB (solid)	2%	10 mg			
ddH_2O		289			
1 M Tris	100 mM	50			
5 M NaCl	1.4 M	140			
0.5 M EDTA	20 mM	20			
-mercaptoethanol (14.3 M stock)	0.2% (28.6 mM)	1			
Proteinase K	0.1 mg/mL	50			

Single = 500 □L	Total	

Potter-Elvehjem homogenizer

Appendix B: QAPP as electronic Attachment

Appendix C: Summarized Data Files, as electronic Attachment

Appendix D: Blast Search Summary