

Plan 9: Research

Benthic Invertebrate
Community Monitoring &
Indicator Development for
the Barnegat Bay-Little Egg
Harbor Estuary -

Barnegat Bay Diatom
Nutrient Inference Model

Hard Clams as
Indicators of Suspended
Particulates in Barnegat Bay

Assessment of Fishes &
Crabs Responses to
Human Alteration
of Barnegat Bay

Baseline Characterization
of Phytoplankton and
Harmful Algal Blooms

Zooplankton
Baseline Characterization of
Zooplankton in Barnegat Bay

Multi-Trophic Level
Modeling of Barnegat
Bay

Ecopath with Eco

Ecological Evaluation of Sedge
Island Marine Conservation
Zone

Tidal Freshwater &
Salt Marsh Wetland
Studies of Changing
Ecological Function &
Adaptation Strategies

Barnegat Bay— Year 3

Assessment of Stinging Sea Nettle (Jellyfishes) in Barnegat Bay

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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 Project Report

Submitted to

New Jersey Department of Environmental Protection

by

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List of Site Abbreviations

BAY-WIDE SITE

Metedeconk W

Metedeconk E

Silver Bay W

Silver Bay E

Toms River W

Toms River E

Forked River W

Forked River E

Double Creek W

Double Creek E

Harvey Cedars W

Harvey Cedars E

Westeconk W

Westeconk E

Tuckerton W

Tuckerton E

ABBREVIATION

MW

ME

SBW

SBE

TRW

TRE

FRW

FRE

DCW

DCE

HCW

HCE

WW

WE

TW

TE

LAGOON SITES

Point Pleasant Lagoon

Kettle Creek Lagoon

Chadwick Isle Lagoon

Toms River Lagoon

Cedar Creek Lagoon

Harvey Cedars Lagoon

Beach Haven Lagoon

Tuckerton Lagoon

ABBREVIATIONS

PPL

KCL

CIL

TRL

CCL

HCL

BHL

TL

List of Molecular Abbreviations

MOLECULAR TERM

Polymerase Chain Reaction
Quantitative Polymerase Chain Reaction
Ribosomal DNA
Chrysaora quinquecirrha Forward Primer
Chrysaora quinquecirrha Reverse Primer
Environmental DNA
Quantitative Reverse-Transcriptase Polymerase Chain
Reaction
Base Pair
Next-Generation Sequencing
Basic Local Alignment Search Tool
National Center for Biotechnology Information
Nucleotide-nucleotide BLAST Extensible Markup Language
MEtaGenome ANalyzer
Lowest Common Ancestor
Cytochrome c oxidase subunit I
Nucleotide
TE

ABBREVIATION

PCR
qPCR
rDNA
CQF
CQR1
eDNA
qRT-PCR

bp
NGS
BLAST
NCBI
Blastn XML
MEGAN
LCA
COI
nt
Tris-EDTA (10 mM Tris; 1
mM EDTA)

Executive Summary

During the three year study, we collected samples from 16 bay sites in Barnegat Bay to assess the distribution of gelatinous zooplankton, with emphasis on all life history stages of sea nettles (*Chrysaora quinquecirrha*) and potential impacts on the pelagic zooplankton communities. Additionally, beginning in 2013 we added 8 lagoon sites within Barnegat Bay to assess the distribution of gelatinous zooplankton, because these regions are the proximal source of sea nettles in Barnegat Bay.

Overall, several key distributional patterns were observed. *C. quinquecirrha* adult distribution is strongly skewed to regions in the northern portion of Barnegat Bay where lower salinity occurred, but they were observed throughout the Bay, indicating that the potential exists for continued expansion throughout Barnegat Bay. Secondly, the other dominant gelatinous zooplankton species Leidy's comb jelly (*Mnemiopsis leidy*) was common throughout the bay, but their distribution was inversely proportional to the relative abundance of *C. quinquecirrha*. Since *C. quinquecirrha* is a known predator of *M. leidy*, when sea nettles are present they reduce the abundance of *M. leidy* in Barnegat Bay. This inverse relationship was present during all years, but *C. quinquecirrha* densities significantly declined in 2013 and *M. leidy* densities significantly increased, suggesting a predation release after Super-storm Sandy. However, in 2014, *M. leidy* densities once again declined to levels similar to 2012, even though *C. quinquecirrha* densities had not rebounded. This seemingly unexplainable finding may be the result of community changes observed by a substantial increase in the density of other gelatinous zooplankton species observed in 2013 and 2014, which could be acting as competitors. The unusual part of this community change is that many of the species are common in coastal and open oceanic communities and are less abundant generally in lagoon communities (e.g., salps).

Results from our bay-wide sampling of *C. quinquecirrha* DNA demonstrated similar patterns to the relative distribution of ephyrae collected in plankton tow samples. One critical finding was the presence of larval DNA throughout Barnegat Bay suggesting that reproduction and larval transport is occurring in Barnegat Bay, but tidal flushing through Barnegat Inlet and Great Bay Inlet may be exporting larvae out of the system. This is positive for minimizing settlement in Barnegat Bay, but also may signal long- distance dispersal to neighboring estuarine systems, and we have verified populations in the Navesink-Shrewsbury Estuary. However, despite the export potential, we have identified numerous regions within Barnegat Bay which show active recruitment of polyps. This is critical, as it is the polyp-podocyst populations which overwinter and create the following years' adult, medusa populations in the Bay.

Our diet analysis of *C. quinquecirrha* provides valuable data regarding the potential impact that sea nettle predation may have on structuring the pelagic and benthic communities. While copepods, fish eggs and larvae, crab larvae, and gelatinous zooplankton species are known parts of their diet, the identification of numerous benthic organisms (e.g., Polychaeta, benthic gobies) and field observations of individuals traveling to the benthos and dragging their tentacles along the sediment surface demonstrates that sea nettles may be having a bigger role in both pelagic and

benthic communities. Additionally, gastric (i.e., digestive system) dissections demonstrated selectivity of prey by *C. quinquecirrha* when compared to available prey. Consequently, sea nettles and other gelatinous zooplankton species play an intricate role in the Barnegat Bay Ecosystem by controlling pelagic community structure, linking benthic and pelagic food webs, and impacting recreational use of the waters.

With increasing development and replacement of building materials which are non-toxic, *C. quinquecirrha* populations will continue to have the opportunity to expand in Barnegat Bay and other coastal New Jersey lagoonal systems. Although Hurricane Sandy destroyed significant polyp and podocyst habitat, our data demonstrate widespread presence of *C. quinquecirrha* larvae in the water and active settlement in numerous regions of the bay. Ultimate control of sea nettles can only be accomplished by addressing polyp populations including habitat requirements and water quality which favor jellyfish over other species.

Project Introduction

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. Large populations of jellyfish are detrimental to fisheries because the jellyfish are voracious feeders on zooplankton and ichthyoplankton and are therefore competitors and predators of fish (Brodeur et al., 2008).

Increasing coastal development has created environments which favor species that are tolerant of various pollutants and degraded water quality. Additionally, the hardening of shorelines and elimination of natural vegetated regions create the potential that tolerant fouling organisms can colonize and expand in these degraded systems. Many coastal estuaries are plagued by poor water quality and increasing inclusion of non-native species. As such, developed coastal estuaries are being defined by lowered species richness and diversity as invaders monopolize available space (Ruiz et al., 1997; Cohen and Carlton, 1998), quantity and toxicity of pollutants (Long et al., 1996), loss of natural habitats (Lathrop and Bogner, 2001), and simplification of food webs through redirection of energy, species introduction, and overfishing (Byrnes et al., 2007). In particular, the relative increase in gelatinous zooplankton in many regions of the ocean has led to a phase shift from 'text-book' planktonic communities dominated by zooplanktivorous fish and higher apex predators (Reid et al., 2000), to ones dominated by ctenophores, cnidarians, and pelagic tunicates (Purcell et al., 2007). While the apparent global increase in gelatinous zooplankton is actively debated (see Brotz et al., 2012; Condon et al., 2013), many specific regional locations have strong documentation of elevated abundances (Fuentes et al., 2010) often leading to food web disruption and fisheries crashes (Roohi et al., 2010).

Perhaps the strongest evidence of gelatinous zooplankton impacted communities comes from the invasion of *Mnemiopsis leidyi* in the Black, Caspian, Mediterranean and North Seas. In all of these systems, human influences relating to eutrophication and overfishing have fueled the persistence and control of these taxa in altering food webs (Llope et al., 2011; Finenko et al., 2013). Despite this, the interaction of *M. leidyi* with native and introduced predators often mediates their overall impact. In the last several decades, *M. leidyi* has demonstrated its ability to

significantly impact pelagic food webs, devastate fisheries and alter community structure through invasions and expansions. Specifically, the introduction to the Black Sea has demonstrated rapid community shift and depletion of fish stocks (Oguz and Gilbert, 2007). More recently their proliferation in the North and Baltic Seas has generated substantial research into the potential top-down and competitive structuring forces in pelagic food webs (Javidpour et al., 2009; Hosia and Titelman, 2011; Kellnreitner et al., 2013; but see Hamer et al., 2011; Jaspers et al., 2011) and species interactions between *M. leidy* and other gelatinous zooplankton species (Riisgard et al., 2010; 2012). Within its native range, *M. leidy* has always played an important role in pelagic food webs (Nelson, 1925; Mountford, 1980; Deason and Smayda, 1982), but with increasing anthropogenic stresses related to eutrophication and overfishing, their relative abundance and influence on communities has led to broader structuring effects on coastal communities (McNamara et al., 2010; 2013). Often, it is the interactions and predator-prey relationships of other gelatinous zooplankton which seem to balance communities or minimize the impacts of *M. leidy* as a keystone species (Purcell and Cowan, 1995; Hosia and Titelman, 2011; Tilves et al., 2013).

In a similar manner, scyphozoan jellyfish have had regional increases impacting food webs and recreational use of coastal waters. The recent increases in many of these species have been hypothesized to result from eutrophication and coastal development (Duarte et al., 2012; Purcell, 2012). Blooms of *Pelagia noctiluca* in the Mediterranean (Ferraris et al., 2012), *Nemopilema nomurai* in the west Pacific (Uye, 2008), and *Chrysaora quinquecirrha* and *Aurelia aurita* in the Gulf of Mexico (Graham, 2001) have been favored under eutrophic conditions and food web simplification from overfishing. These have accentuated the impacts that humans are having on the oceans on a global level (Duarte et al., 2012) and highlighted their impacts on local and regional levels. It is now recognized that the expansion and continuation of gelatinous zooplankton blooms have the potential to significantly impact communities, as well as prospective introduction to non-native systems; their potential to permanently disrupt natural food webs has been demonstrated (Oguz et al., 2012). Concurrently, the long-term impacts to commercially important fisheries species is occurring through direct consumption of fish eggs, larvae, and juveniles (Finenko et al., 2013), as well as indirect impacts through competitive interactions for planktonic food resources with these same life history stages (Purcell et al., 2001).

Barnegat Bay, New Jersey is a coastal lagoon system in the mid-Atlantic region of the United States with significant urbanization and an extensive history of land use changes in its watershed (Lathrop and Bogner, 2001). While *M. leidy* has been reported as an important component of the pelagic community for the last century (Nelson, 1925; Mountford, 1980; Sandine, 1984), the recent expansion by the scyphozoan *C. quinquecirrha* during the last decade may be a result of the development and eutrophication of the system (sensu Duarte et al., 2012), since prior investigations do not even list this species as prevalent in the bay (Nelson, 1925; Mountford, 1980; Sandine, 1984), although they are listed in the known distribution of the species (Morandini and Marques, 2010). Bologna (2011) showed that larval recruitment to settling plates was highly localized in northern portions of the bay where development is high and salinity is reduced from two large rivers. As these jellyfish have become established in the region over the

last decade, their impacts at the community level have yet to be evaluated; even though their increasing abundance has led to reduced recreational use of some parts of the bay where their abundance has created challenges for swimming. Conceptually, the expansion by *C. quinquecirrha* could initiate a trophic cascade as they exert top-down pressure on the native *M. leidy* populations similar to the finding of Purcell and Decker (2005) in the Chesapeake Bay where both species are native.

This research project aims to evaluate sea nettle (*Chrysaora quinquecirrha*) populations at early pelagic life history stages through molecular identification, to assess medusa stage distributions within the bay, and then predict the development of adult blooms. Additionally, as sea nettles and other gelatinous zooplankton populations have increased, their distribution and abundance has the potential to shape planktonic food webs (e.g., copepods) and larvae of benthic organisms (e.g., oysters and hard clams).

Overall Project Objectives

- 1 Assess Gelatinous Zooplankton Populations in Barnegat Bay
- 2 Assess Impacts of Gelatinous Zooplankton on Pelagic Community Dynamics
- 3 Utilize Molecular Approaches for assessing Bloom conditions and distribution of Larvae
- 4 Utilize Molecular Approaches for assessing Diet Analysis of Sea Nettles

This report summarizes and synthesizes the research activities of the completed project from 2012 to 2014. All yearly objectives are summarized with specific methodologies and results. Yearly project QAPPs were submitted and approved by New Jersey Department of Environmental Protection and are provided as electronic supplemental files. QAQC data files have been provided electronically to the New Jersey Department of Environmental Protection and will be publicly available upon final release of the project from the NJDEP to the public.

Study Area

The area of geographic focus for this project is Barnegat Bay, from the mouth of the Point Pleasant Canal south to Tuckerton Creek. Eight paired sampling sites were established in the Barnegat Bay study area (Figure 1a). Site selection incorporated stratified samples throughout the bay, but focusing on major freshwater inputs which may provide preferred habitat for settling *C. quinquecirrha*. For each site, locations were sampled on the western side of the bay closer to the freshwater outflow and then the mid- to eastern side as the pair. These sites were selected to be representative of the various environmental conditions that exist in the bay and comprehensive coverage of sites within the bay. Sampling occurred at these 16 sites in 2012, 2013, and 2014. Beginning in 2013, an additional 8 sites were added to assess the distribution of zooplankton in lagoon communities around the bay (Figure 1b). These stations were added in 2013, as they were the likely sites for *C. quinquecirrha* polyp populations which generate the adult medusa in Barnegat Bay. Coordinates for sampling stations is provided in Table 1. Additionally, in 2013 and 2014 we shifted our polyp settling plate research locations to correspond to lagoon communities where polyp habitat is abundant and reflects the probable polyp populations generating medusa.

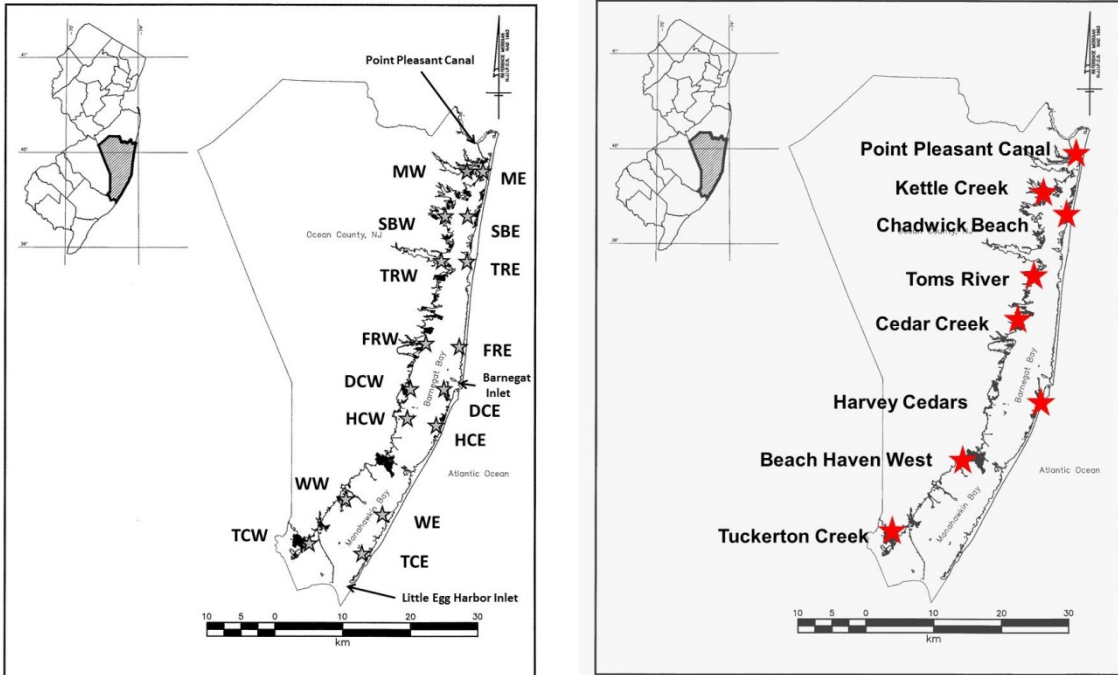


Figure 1. Sampling stations in Barnegat Bay, NJ. a. Left Panel: represents our bay-wide sampling stations collected during all three project years. b. Right Panel: represents our lagoon stations which were sampled monthly from June to August in 2013 and 2014.

Table 1. Names and coordinates for the sites being used to collect data for this project. *Cedar Creek and Manahawkin Bay sites received settling plates only in 2012, but were not sampled further. Lagoon sites were added in 2013 to assess populations and community structure.

Bay Wide Sampling Sites	Abbreviation	GPS Coordinates N	GPS Coordinate W
Metedeconk River W	MW	40.050983	74.064300
Metedeconk River E	ME	40.045183	74.054117
Silver Bay W	SBW	39.992217	74.119350
Silver Bay E	SBE	39.933683	74.092100
Toms River W	TRW	39.989917	74.107567
Toms River E	TRE	39.925833	74.084733
Cedar Creek W*	CCW	39.865567	74.129000
Cedar Creek E*	CCE	39.863650	74.102500
Forked River W	FRW	39.821333	74.159667
Forked River E	FRE	39.815767	74.122883
Double Creek E	DCE	39.787550	74.153833
Double Creek W	DCW	39.786100	74.182700
Harvey Cedars W	HCW	39.700733	74.166050
Harvey Cedars E	HCE	39.698917	74.146000

Manahawkin Bay W*	MBW	39.667833	74.212733
Manahawkin Bay E*	MBE	39.663550	74.192100
Westeconk Creek W	WW	39.620117	74.259400
Westeconk Creek E	WE	39.598800	74.229750
Tuckerton Creek W	TCW	39.578983	74.324283
Tuckerton Creek E	TCE	39.556400	74.254433
Lagoon Sampling Sites			
Point Pleasant Lagoon	PPL	40.063067	74.058817
Kettle Creek Lagoon	KCL	40.019850	74.103100
Chadwick Isle Lagoon	CIL	39.991267	74.068817
Toms River Lagoon	TRL	39.912033	74.128350
Cedar Creek Lagoon	CCL	39.839400	74.149983
Harvey Cedars Lagoon	HCL	39.696350	74.143067
Beach Haven Lagoon	BHL	39.655800	74.220683
Tuckerton Lagoon	TL	39.579533	74.338100

2012 Project Objectives

1. Assess the distribution of gelatinous zooplankton and impacts on planktonic community structure.
2. Assess the relative concentration of *C. quinquecirrha* DNA from water samples.
3. Create a field-sample predictive model for *C. quinquecirrha* blooms using real-time qPCR.
4. Assess the distribution and density of settling *C. quinquecirrha* polyps and development of resting podocysts.

2012 Project Sampling Events

Field research activities began in May 2012 with the deployment of field settling plates at the 20 locations identified in the project for jellyfish polyps (see Table 1). Sampling also began at this time at the designated 16 sites (Fig. 1a) for adult (lift nets), juvenile (lift nets, zooplankton tows), and ephyra/larvae (filtered water samples). Field sampling was completed in September 2012 resulting in eight sampling events (Table 2).

Table 2. Research Sampling Event Collection Dates. To complete sampling, it was necessary to break up field efforts into two days, due to the daily work load. Only the first sampling event required three days to collect samples, as settling plate deployment also occurred at this point. For ease of temporal display of results, a single date was chosen to represent the sampling collection event.

Sampling Event	Dates of Collection	Collection Date Designation
Collection I	5/31, 6/1, and 6/5/2012	6/1/2012
Collection II	6/14 and 6/15/2012	6/15/2012
Collection III	6/26 and 6/28/2012	6/28/2012
Collection IV	7/11 and 7/12/2012	7/12/2012
Collection V	7/26 and 7/30/2012	7/30/2012
Collection VI	8/7 and 8/8/2012	8/8/2012
Collection VII	8/21 and 8/22/2012	8/22/2012
Collection VIII	9/10 and 9/14/2012	9/14/2012

2012 Project Methodology

Objective 1: Assess the Distribution of Gelatinous Zooplankton and Impacts on Planktonic Community Structure

Gelatinous zooplankton

Gelatinous zooplankton, (e.g., pelagic cnidarians and ctenophores), tend to be rather delicate and are frequently mangled and destroyed in standard plankton tows. Gelatinous zooplankton were sampled bi-weekly from May through September at the 8 designated paired stations (Figure 1). Ten to twelve lift net samples were collected from each station during each sampling event by allowing the lift net to settle to the benthos and remain undisturbed for 30 seconds. Lift nets were then raised directly through the water column and all organisms were lifted to the surface. Once on deck, samples are transferred to a holding bin where all gelatinous zooplankton were identified and enumerated. Water depth was recorded for each lift net sample and the lift net area (0.836m^2) was then multiplied to determine the volume of water sampled. All samples were then standardized to number m^{-3} and compared among sites and dates of collection. While lift net samples were being collected, the triplicate water samples described under Objective 2 were collected to assess the PCR quantified sea nettle presence.



Image 1. Lift net being brought up with sample concentrated at center of net.

Pelagic Zooplankton Sampling

In addition to the lift nets, triplicate 363µm zooplankton nets were towed at each location. Tows were conducted at minimally engaged engine speed for one minute. Length of tow was standardized using a mechanical flow meter to assess the distance traveled. As such, the known cross section of the net with a known speed for the tow duration allowed a volume quantification for each sample. After collection in the field, ctenophores were counted while the sample was field sieved since they do not preserve well and this was the only way to get an accurate assessment of their distribution in the plankton tows. Zooplankton were preserved in ethanol and stained with Rose Bengal for ease of identification and quantification in the laboratory. Each sample was returned to the lab for identification and enumeration. All samples were standardized to #m⁻³ and compared among sites and dates of collection.



Image 2. Plankton tow sample being washed down to the cod-end of the net.

Objective 2. Assess the relative concentration of *C. quinquecirrha* DNA from water samples.

Qualitative Predictive Assessment of *C. quinquecirrha* Blooms

During the last year, Principal Investigator Gaynor has modified the Bayha and Graham (2009) qPCR technique to detect scyphozoan jellyfish to assess *C. quinquecirrha*. Data have shown that the 16S rDNA gene found in Barnegat Bay populations is unique and will readily allow identification by molecular techniques (Gaynor & Tare, 2009). This method allows qualitative detection (screening) of larval (planula, ephyra) stages and gametes (egg and sperm) of *C. quinquecirrha* found in the water column.

Quantitative Real Time qRT-PCR Analysis

Triplicate 20.0-liter water samples were collected in plastic pails and field sieved through a coarse nylon mesh pre-filter (2 mm) to remove large debris (Component Supply Company, Industrial & Scientific Supplies). Sequentially, water was filtered through 500 µm and 100 µm

nylon mesh filters (Component Supply Company, Industrial & Scientific Supplies) and 0.45 µm (Nalgene®, cellulose nitrate, Fisher Scientific) filters to remove suspended particles by operational design. The 500 µm filter will retain sea nettle ephyra; the 100 µm filter will retain planula larvae and *C. quinquecirrha* eggs; the smallest filter will retain *C. quinquecirrha* sperm and released DNA (if present). Nylon mesh filters will be new and used only once to prevent contamination between samples; 0.45 µm filters will be sterile and used only once (Fisher Scientific, 50 Fadem Rd., Springfield, NJ 07081). All filters were stored in sealed plastic bags (Whirl-Pak®, various suppliers) labeled to indicate filter pore size, location, date, time, and researcher. All filters were then stored on ice and transported to the laboratory and stored in an ultra-low freezer at -80°C until processed. See QAPP Section 10.2 (Monitoring Methods) for a complete description of the filtration device and details on our protocol. 0.45µm filters were extracted by boiling for 10 minutes in a minimal amount of Tris buffer with Chelex® 100 (Sigma Aldrich Chemicals) (Walsh et al., 1991), centrifuged to remove insoluble material, and the supernatant removed for qPCR analysis.

qPCR reactions were run using primers specific for the 16S rDNA gene of *C. quinquecirrha* (CQF 5'-TGTCACCTAATTAGTGAATGGT-3'; CQR1 5'-GCCCCAACCAAACCTGTCTTA-3'). Standard qPCR reactions were generated using the protocol listed in Table 3. Typically master mixes were generated to ensure uniformity in all reaction wells. Standard qPCR reactions were assessed by agarose gel electrophoresis with appropriate controls (no DNA, no primers, positive controls containing known amounts of cloned *C. quinquecirrha* 16S rDNA, etc.). We also routinely selected PCR products for DNA sequence analysis to verify that we have amplified the 16S rDNA gene of *C. quinquecirrha*.

TABLE 3. Setup of qPCR Reactions.

	Single RXN	100 RXN
Sterile deionized water	4.5 µl	450 µl
Forward primer 5 µM - CQF	1.5 µl	150 µl
Reverse primer 5 µM - CQR1	4.5 µl	450 µl
Power SYBR GREEN 2X Master Mix	12.5 µl	1.25 ml
Template (eDNA from sample)	2 µl	--
Total Volume	25 µl	2.3 ml

Since this study required the processing of a large number of samples and assessment of quantitative levels of larval forms of *C. quinquecirrha* in the water column, we employed quantitative real time PCR (qRT-PCR) analysis of these samples (Bayha & Graham, 2009). We have demonstrated that this technique is sensitive enough to detect as few as 10 copies of the 16S rDNA gene from *C. quinquecirrha* (Figures 2 and 3). In these experiments we have used the incorporation of SYBR Green fluorescent dye to detect the production of these amplicons in real time using our *C. quinquecirrha* specific primers for the 16S rDNA gene. Since we have the *Chrysaora* 16S rDNA gene cloned into a plasmid (pUC19), we utilized dilutions of the cloned gene to establish a standard curve for absolute quantitation of this gene in our samples (Nailis et al., 2006; Dhanasekaran et al., 2010). We have also demonstrated that this primer pair does not amplify the 16S rDNA gene of related Cnidarians (*Aurelia aurita* and *Cyanea capillata*)

sometimes found in Barnegat Bay (Gaynor and Tare, unpublished). So, we are confident that this technique is both quantitative and specific for *C. quinquecirrha* DNA.

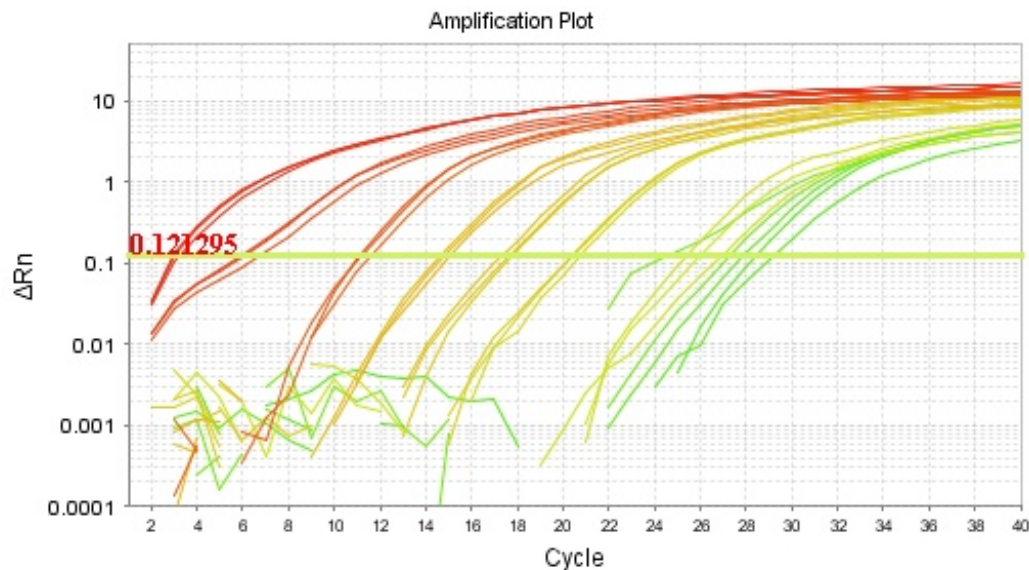


Figure 2. qPCR amplification plot of *C. quinquecirrha* DNA (Rn vs. cycle number). Nine concentrations of a cloned fragment of the 16S rDNA gene from *Chrysaora quinquecirrha* were run in triplicate under standard qPCR conditions described above. Concentrations cover a 9-log range (from 120 billion to 120 copies of 16S rDNA gene in a 2 μ l sample). C_T threshold is calculated automatically by software (ABI StepOnePlus). NTC (no template controls) were negative and showed no amplification in 40 cycles.

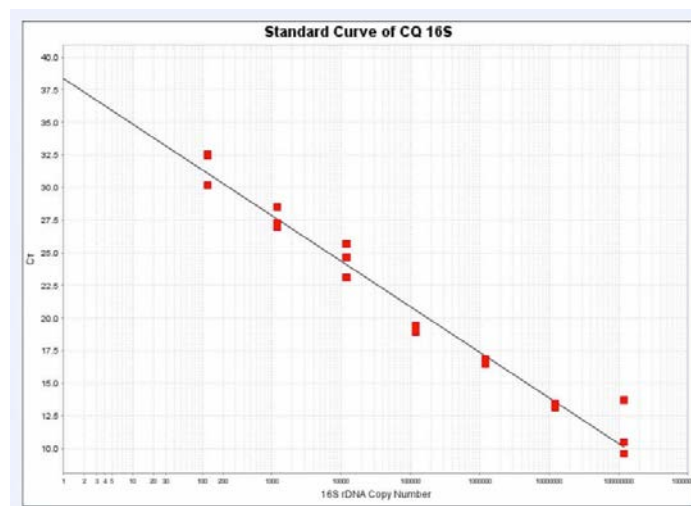


Figure 3. Standard Curve generated using known quantities of *C. quinquecirrha* 16S rDNA ranging from 12 billion copies to 120 copies of this gene. The curve shows that the reaction is linear over the 9-log range, and is highly reproducible (Slope = -3.343; y-Intercept = 37.9; $R^2 =$

0.929; % Efficiency = 99.124).

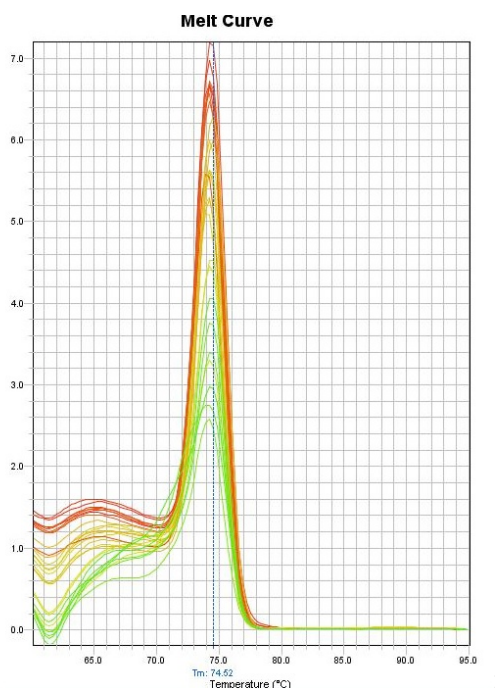


Figure 4. Melting Curve generated from 9 standards used in the *Chrysaora quinquecirrha* specific qPCR reaction above. The single peak, $T_M = 74.52^\circ\text{C}$, identifies that only a single molecular species has been amplified in the qPCR assay. Melting curves are generated for all qPCR runs to ensure that we are only amplifying the 208 bp fragment of the 16S rDNA gene from *Chrysaora quinquecirrha*.

Objective 3: Predictive Modeling

Based on the quantification of the real-time PCR data, we created a time-lag predictive model which incorporates the distribution and abundance of DNA, ephyra, juveniles, and adult medusa. Our model incorporates the molecular quantification of the ephyra discussed above, coupled with adult medusa stages. Lift net samples (Memphis Net and Twine Company, Inc., P.O. Box 80331, Memphis, TN 38108-0331) from each station were collected to assess adult and other gelatinous zooplankton. Lift nets were deployed on the benthos, then allowed to sit for 30 seconds. Net samples were then hauled through the water column to assess relative distribution (see Objective 2: Gelatinous zooplankton for full description). We coalesced the field and molecular data to determine the time lag based on bi-monthly sampling frequency.

Given that Barnegat Bay is often a wind driven system, the distribution and relative abundance of planktonic organisms (e.g., *C. quinquecirrha*) is heavily dependent upon the wind and tide conditions. While the individual samples for sites may not provide the exact predictive end-point for any given site in the bay because of these meteorologic and tidal forces, our temporal time-lag model will provide a prediction about the severity of a bloom and the estimated timing (e.g., 2-6 weeks after initial screening of ephyra) within the bay. This will allow decision makers

and the general public an opportunity to assess the management options and economic impacts of an on-coming bloom.

Objective 4: Settlement of *Chrysaora quinquecirrha* polyps

We created settling habitat by using cages filled with oyster shells and floating PVC plates to simulate a floating dock. *Chrysaora quinquecirrha* is known to settle on oyster shell (Cargo and Rabenold 1980) and PVC plates (Buesser 2011) and this would allow us to monitor and assess the settlement potential within the bay. Settlement cages were constructed of 5cm vexar mesh filled with 12 oyster shells, attached to a cinder block, and deployed at the ten paired sampling locations (Figure 1). Floating PVC plates were constructed using pressure treated lumber supported in the water column by extruded foam to simulate a floating dock. Below the structure, two rows of PVC settling plates (2.5cm x 10cm) were attached below to allow settlement of larvae in the system (See Image 3b). These floating structures were also attached to the cinder block, thereby allowing us to assess the settlement of polyps to natural and anthropogenic structures. Settlement experimental units were deployed in late May and Early June during sampling event One and collected at the end of August. During the summer, several sampling units were lost due to theft/removal or potentially being struck by errant boaters. In the end, we relocated 12 of the original 20 sampling units in usable condition to generate settlement results.



Image 3. Settlement Platform to assess larval settlement. A) Surface view floating on the water, B) Individual PVC settlement plates attached to the lower surface.

2013 Research 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.

2013 Project Methodology

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. 1a). 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. 1b). 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. 1b). 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.

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 (Tris-EDTA). Total yield and quality of DNA extracted is provided in Table 4.

Table 4. 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.

2014 Project Objectives

1. Create a field-sample predictive model for *Chrysaora* blooms and reproductive efforts using species specific DNA signatures.
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.

2014 Project Methodology

Objective 1: Create a field-sample predictive model for *Chrysaora* blooms and reproductive efforts using species specific DNA signatures.

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 with 1 liter of sampled water returned to the laboratory filtered through 0.45 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).

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.

a. Monthly assessment in Barnegat Bay

We have established 16 sampling sites in Barnegat Bay which we will sample monthly between May and October 2014. This will provide essential data regarding the spatial and temporal distribution throughout Barnegat Bay. A combined sampling program of lift nets and plankton tows will allow us to assess the distribution of adult and juvenile sea nettles, as well as zooplankton. These data will provide the broad based distribution and identify potential relationships among various zooplankton groups demonstrating food web relations (i.e., sea nettles vs. 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 are planning to sample developed regions in Barnegat Bay to gain an understanding of the distribution within these communities. Multiple sites will be sampled in June, July, and August using lift nets and plankton nets to assess adult and juvenile sea nettles. Water samples (Objective 1) will also be collected at these locations to assess ephyra and competent larvae which are generated and/or preparing to settle in these lagoonal communities. We are looking to target eight lagoon communities including Bay Head, Kettle Creek, Silver Bay, Berkley Shores, Forked River, Barnegat Township, Beach Haven West, and Tuckerton Beach.

These sites cover Barnegat Bay and will provide information about the presence of *Chrysaora* within these communities.

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 were 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 allows us to identify and quantify polyp settlement and distribution.

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

4a. Field-Based Diet Assessment

We collected juvenile and adult sea nettles throughout Barnegat Bay including near shore and open water. Individual organisms will be immediately identified, measured, and their gut contents dissected out. Location of each individual will be recorded using a hand-held GPS unit and water quality data will be recorded using a YSI-85 for salinity, temperature, and dissolved oxygen. Samples were preserved in ethanol and returned to the laboratory for gut content analysis. All food items present were identified to lowest possible taxa and enumerated. We assessed whether differences exist in feeding preference among individuals compared to available prey identified through our plankton sampling of nearby sites.

4b. Genetic 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

We sampled 53 adult jellyfish from 3 localities using the gastric lavage protocol established in 2013. Additionally, we isolated picked and identified prey items from the dissected individuals for molecular analysis. Identified food items were removed from the ethanol preserved individuals collected. Visually identified 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.

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. Library preparation was nearly identical to 2013. However, the two gut content DNA libraries were multiplexed and run on a single lane on the Illumina HiSeq2500 platform. This resulted in 235,527,252 (gastric lavage) and 228,359,016 (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) using identical protocols as 2013. 263,602,962 (gastric lavage) and 202,389,828 (gut wash) paired end reads were retained for further analyses. Similar to 2013, three separate assemblies were performed: Gastric lavage, Gut picked, and Combined. Paired-end reads were assembled using the CLC Genomics Workbench (<http://www.clcbio.com/products/clc-genomics-workbench/>) de novo assembler. Settings were identical to 2013.

Annotation

Given that the combined build yielded 1,998,271 reads BLAST searching all contigs was not a possibility. Instead these contigs were BLAST searched to identify mitochondrial genome genes and ribosomal genes (5.8S, 18S, and 28S) that were subsequently BLAST searched (blastn 2.2.29+; Altschul et al. 1990) against the preformatted nt database (downloaded 6/3/15). These genes were chosen given their ability to accurately identify prey species. BLAST searches used settings identical to 2013.

2012 Project Results

Water Quality

Results from the sampling events indicate a generalized seasonal rise in temperature among all sites, although temperature varied within sites among dates of collection (Table 5). Salinity showed patterns of lower salinity recorded in the northern portions of the bay which are greatly affected by river flow from the Metedeconk and Toms Rivers (Table 6). Oxygen varied among sites and dates of collection but generally exceeded 5 mg/l. Exceptions were seen occasionally, but only once did oxygen drop below 4 mg/l at Forked River East on 9/10 (Table 7).

Table 5. Temperatures recorded for each sampling event at the 16 sampling sites. Values are given in degrees C.

	6/1	6/15	6/26	7/11	7/30	8/7	8/22	9/10
ME	23.8	21.8	22.2	27	25.3	27	23.9	23.3
MW	25.5	21.8	22.3	26.8	24.9	26.1	28.3	22.7
SBE	26.7	22.3	24.7	28.2	25.2	27	23.9	22.2
SBW	27	21	24.7	28.3	25.7	28.4	26.4	22.3
TRE	26.6	22	23.5	27.4	25.5	28.3	25	21.2
TRW	26.7	29	23	27.1	25.6	27.3	24.5	21.6
FRE	23.3	24	23.5	26.4	26.2	27.3	25	21.4
FRW	23.9	21.5	23.7	26.8	28.4	29	26	23.7
DCE	22.9	21.6	23.9	27.9	25.7	27.3	25.1	24.3
DCW	23.7	21.3	23.1	29.9	28.1	28.8	24.9	24.8
HCE	23.4	22.2	23.7	26.8	27.6	28.4	26	23.5
HCW	19.3	21.5	23.2	28.0	26.7	27.2	26.4	23.3
WE	18.9	21.9	23.3	27.5	27.7	27.7	25	23
WW	19.7	21.3	22.4	27.1	26.9	27.4	25	23.7
TCE	18.6	21.7	22.8	26.8	26.7	26.7	24.3	22.8
TCW	19.4	21.6	23.8	27.2	27	27.6	24.9	23.6

Table 6. Salinity measurements for the eight sampling events. Values represent concentration expressed as parts per thousand (‰).

	6/1	6/15	6/26	7/11	7/30	8/7	8/22	9/10
ME	21.6	22.5	20.5	23.3	21.3	20.2	20.7	18.8
MW	18.3	22.7	19.1	22.3	24.6	17.8	20	23.3
SBE	17.1	17.9	17	17.5	18.5	19	19	16.5
SBW	17.1	16.9	16.5	16.6	17.8	18	17.3	15.8
TRE	18.5	18.8	17.5	18.8	21.1	21.2	20.5	17
TRW	18.2	16.6	15	17.5	20.7	15.2	18.9	14.6
FRE	26.6	25.9	25.4	27.7	29.8	28.6	30	26.5
FRW	25.7	25.5	26.2	24.6	27.4	28	28.8	27.9

DCE	27.1	27.2	26.8	27.8	33	29.6	30.3	24.8
DCW	22.7	25.2	26.9	26.1	29.2	28.6	30.6	25.3
HCE	28.1	27.6	29.3	23.5	28.3	29.4	29.3	25.4
HCW	27.3	26.9	24.2	24.8	27.4	29.1	28.5	24
WE	29	22	27.2	27.2	29.1	30.3	30.7	24.5
WW	28.4	23.7	19.6	24.9	27.2	26.5	28.4	27.3
TCE	29.7	28.6	27.3	28.3	30.4	30.7	31.3	29.1
TCW	29	27.9	26.4	27.6	29.1	29.1	29.9	28

Table 7. Dissolved oxygen measurements for the eight sampling events. Values represent concentration expressed as mg/l.

	6/1	6/15	6/26	7/11	7/30	8/7	8/22	9/10
ME	5.29	7.92	6.15	65.86	5.29	5.14	6.2	8.65
MW	6.21	9.7	6.23	6.39	6.17	5.7	6.27	5.68
SBE	6.98	7.75	5.24	8.09	6.29	5.66	5.43	7.13
SBW	6.75	7.24	7.25	7.25	6.89	6.58	6.39	7.3
TRE	6.84	6.85	5.25	6.47	6.62	6.13	6.6	6.25
TRW	6.16	7.45	6.71	6.45	6.1	6.74	6.06	7.04
FRE	5.99	7.78	7.36	6.73	5.61	6.32	9.1	3.39
FRW	5.77	6.81	5.8	6.9	4.7	5.6	5.29	4.16
DCE	5.45	6.8	9.38	11.09	7.27	7.93	6.23	8.15
DCW	6.11	6.71	6.49	9.39	7.38	5.58	4.23	6.65
HCE	6.33	6.19	5.57	nd	6.6	6.87	7.54	6.68
HCW	6.93	5.85	5.36	6.41	4.75	6.82	5.4	5.85
WE	6.47	6.0	7.61	9.41	6.76	7.06	6.1	7.44
WW	6.04	6.0	4.41	5.21	4.85	6.04	4.58	6.98
TCE	6.37	5.99	5.9	6.06	6.2	7.83	5.46	8.25
TCW	5.8	6.07	5.25	5.97	6.31	6.67	5.85	7.15

Lift Net Results

During sampling, six species of gelatinous zooplankton were collected and include *C. quinquecirrha*, *M. leidy*, *Beroe*, *Pleurobranchia*, *Aurelia*, and *Cyanea*. ANCOVA results indicate significant differences among sites of collection for *C. quinquecirrha* density ($F_{15, 1377} = 7.67$, $P < 0.0001$), but no difference among dates; while *Beroe*, *M. leidy*, and *Pleurobranchia* showed significant differences among sites and dates of collection: *Beroe* ($F = 4.45$, $P < 0.0001$; $F_{1,1377} = 11.21$, $P < 0.001$), *M. leidy* ($F = 17.64$, $P < 0.0001$; $F = 69.4$, $P < 0.0001$), and *Pleurobranchia* ($F = 1.81$, $P < 0.03$; $F = 16.6$, $P < 0.0001$). *Aurelia* and *Cyanea* showed no difference among sites because each was encountered only once during sampling. Numerically, *Mnemiopsis* and *C. quinquecirrha* were one to three orders of magnitude greater in abundance than the other four

species and showed disjunct distributions in the bay, with *C. quinquecirrha* dominating in the northern portion of the bay (Fig. 5), but relatively absent from the southern region which was dominated by *M. leidy* (Fig. 6). Integrally, this suggests that the disjoint distribution may be related to predator-prey interactions as a significant negative correlation occurred between these two groups ($P < 0.005$). Analysis of Covariance showed a significant difference among sites for *C. quinquecirrha* with significantly greater densities occurring at Silver Bay East and West compared to other sites (Fig. 5). *Mnemiopsis leidy* distribution reflects proximity to Little Egg Harbor Inlet and Barnegat Inlet (Fig. 6) showing significantly greater densities at Tuckerton Creek East and West, Forked River East and West, and Harvey Cedars West compared to other sites and lowest densities occurring in regions where *C. quinquecirrha* densities were high.

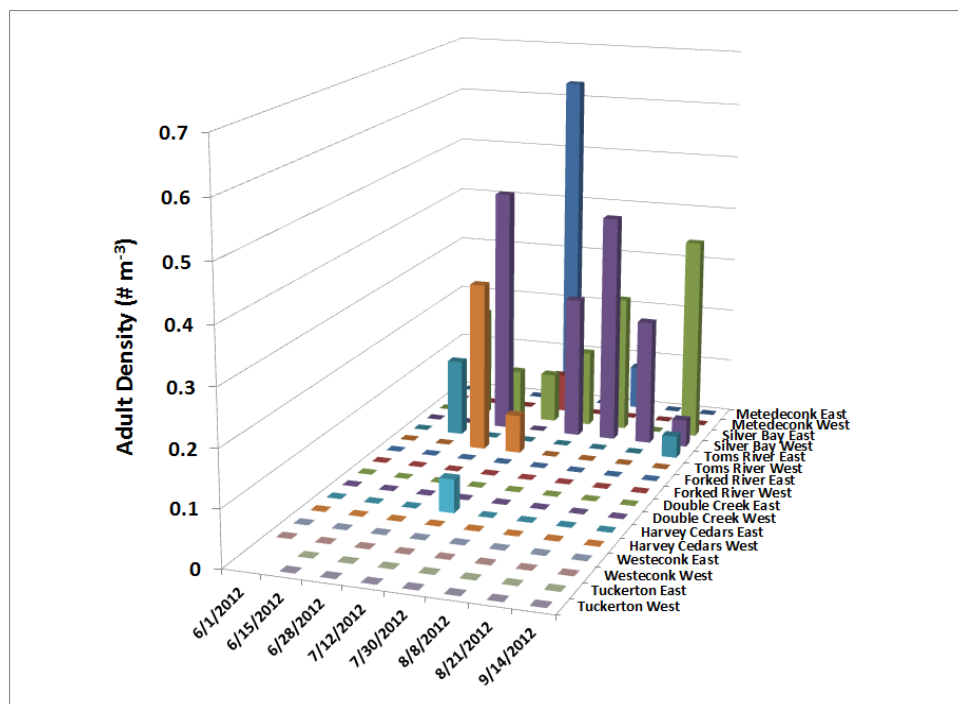


Figure 5. Spatial and temporal distribution of adult *Chrysaora quinquecirrha* in Barnegat Bay collected from lift nets during the summer of 2012 for the eight sampling events.

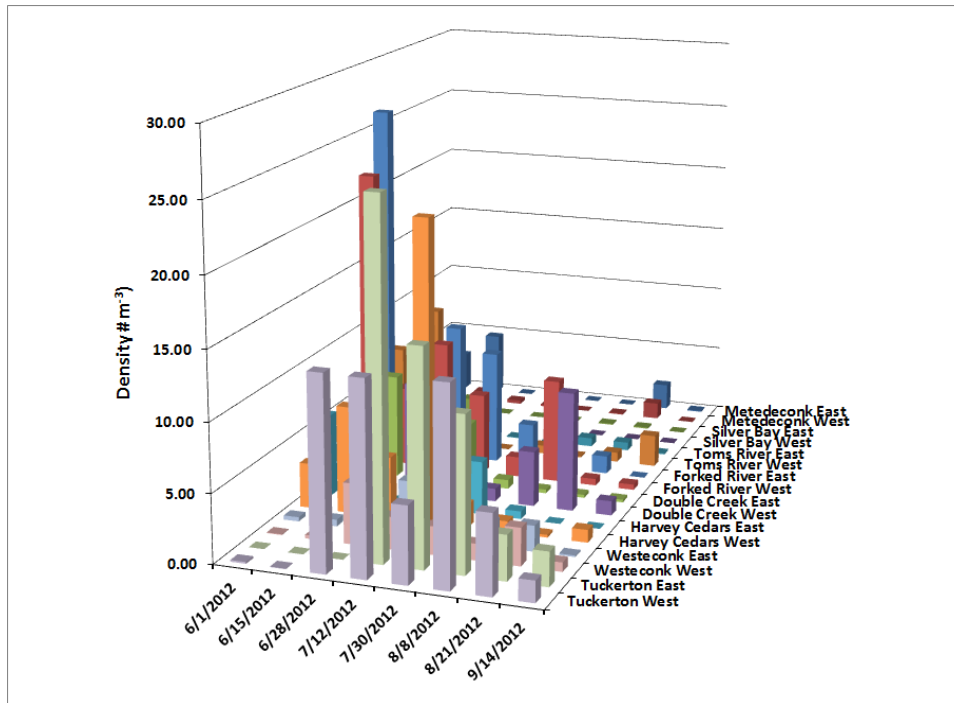


Figure 6. Spatial and temporal distribution of adult *Mnemiopsis leidyi* in Barnegat Bay collected from lift nets during the summer of 2012 for the eight sampling events.

Plankton Tow Results

A total of 64 taxonomic groups was identified from net samples and included two distinct life history stages of *C. quinquecirrha* (i.e., juvenile (Fig. 7), ephyra (Fig. 8)). Average density for the most numerically dominant groups include *Callinectes sapidus* larvae (Fig. 9, 830 m⁻³), Calanoid copepods (27.9 m⁻³), fish eggs (4.8 m⁻³), Caridea larvae (4.1 m⁻³), and *M. leidyi* (2.4 m⁻³). *Callinectes sapidus* density was driven by samples collected on July 30th from our Westeconk Creek West site, where average density for that date was 99,873 m⁻³!

Similar to lift nets, *C. quinquecirrha* densities were greater in the northern portion of the bay, but juveniles were present in the southern regions of the bay, even though adults were not (Fig. 7). Several taxa collected in the plankton nets showed significant difference in density among sites. Specifically, *C. quinquecirrha* ($F_{15,361} = 5.98$, $P < 0.0001$), *M. leidyi* ($F = 5.6$, $P < 0.0001$), *Turritopsis* ($F = 4.49$, $P < 0.0001$), Cladocera ($F = 1.83$, $P < 0.03$), Calanoid copepods ($F = 3.67$, $P < 0.0001$), *C. sapidus* larvae ($F = 2.24$, $P < 0.005$), Caridea larvae ($F = 4.3$, $P < 0.0001$), Fish eggs ($F = 3.43$, $P < 0.0001$), and Fish larvae ($F = 1.88$, $P < 0.03$) showed significant differences among sites. *Chrysaora quinquecirrha* was significantly greater from our Silver Bay East with an average density of 0.11 m⁻³, with a maximum density of 0.56 m⁻³. In regards to ephyra, they were identified throughout the bay and there appears to be several pulses into the system including mid-June, then sporadic pulses throughout the bay in July and August, but these were site specific (Fig. 8). Correlation analysis indicated that *C. quinquecirrha* was negatively correlated with most of its prey items, but none were significant, most likely because of the spatial

and temporal variability of all organisms collected in the plankton nets. Eighteen taxa had significant differences in density among sites (Table 8) with 12 of these also exhibiting significant temporal differences.

Table 8. Average densities (#m⁻³) of taxa exhibiting significant differences among sites. Significance convention *= 0.05, ** = 0.01, *** = 0.0001.

Species	MW	ME	SBW	SBE	TRW	TRE	FRW	FRE	DCW	DCE	HCW	HCE	WW	WE	TCW	TCE
<i>C. sapidus</i> ***	0.83	1.55	0.97	0.98	0.58	0.85	2.12	1.25	0.82	2.08	4.33	1.37	41.00	3.63	5.86	2.23
Calanoida ***	2.36	3.46	1.19	2.06	2.52	0.69	1.14	0.83	1.72	2.46	5.07	2.44	5.41	2.26	5.91	5.82
Fish Eggs ***	0.10	1.52	1.69	1.63	0.90	1.24	1.08	1.35	1.14	1.18	1.26	0.67	1.45	0.59	2.69	1.36
Caridea larvae***	1.32	1.87	0.51	0.83	0.34	0.68	0.79	1.90	1.10	2.19	1.95	1.64	1.39	2.29	2.12	2.01
<i>M. leidy</i> ***	0.53	0.27	0.13	0.16	0.47	0.54	0.96	1.25	1.30	0.95	1.11	0.96	0.87	0.98	2.41	1.75
Cladocera*	0.30	0.48	0.05	0.06	0.06	0.00	0.11	0.12	0.05	0.40	0.07	0.13	0.07	0.08	0.24	0.57
<i>T. nutricula</i> ***	0.01	0.00	0.00	0.00	0.04	0.00	0.01	0.06	0.09	0.16	0.59	0.63	0.25	0.46	0.09	0.13
Mellitidae***	0.06	0.08	0.02	0.13	0.07	0.07	0.03	0.27	0.33	0.56	0.22	0.11	0.31	0.44	0.24	0.40
Fish Larvae ***	0.10	0.04	0.17	0.05	0.21	0.13	0.08	0.09	0.16	0.07	0.40	0.16	0.29	0.15	0.48	0.39
<i>I. baltica</i> ***	0.06	0.02	0.00	0.02	0.00	0.02	0.16	0.38	0.25	0.50	0.13	0.09	0.08	0.41	0.14	0.35
Ostrocodia**	0.03	0.14	0.07	0.14	0.05	0.02	0.06	0.15	0.04	0.52	0.17	0.07	0.05	0.28	0.10	0.11
<i>Gammarus</i> **	0.00	0.00	0.05	0.22	0.03	0.15	0.08	0.16	0.04	0.26	0.09	0.05	0.20	0.32	0.24	0.24
Polychaeta larvae*	0.03	0.05	0.09	0.24	0.04	0.11	0.08	0.22	0.14	0.30	0.23	0.14	0.14	0.26	0.11	0.10
Caprellidae***	0.00	0.03	0.00	0.16	0.00	0.00	0.02	0.02	0.00	0.30	0.04	0.02	0.07	0.01	0.00	0.14
Pycnogonidae* **	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.09	0.11	0.09	0.07	0.03	0.17	0.04	0.04
<i>C. quinquecirrha</i> * **	0.02	0.11	0.16	0.23	0.03	0.03	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.03	0.01	0.02
<i>S. fuscus</i> *	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.04	0.00	0.00	0.10	0.00	0.05
<i>Eutima</i> **	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.02	0.04	0.02	0.10	0.02	0.03	0.02	0.00	0.08

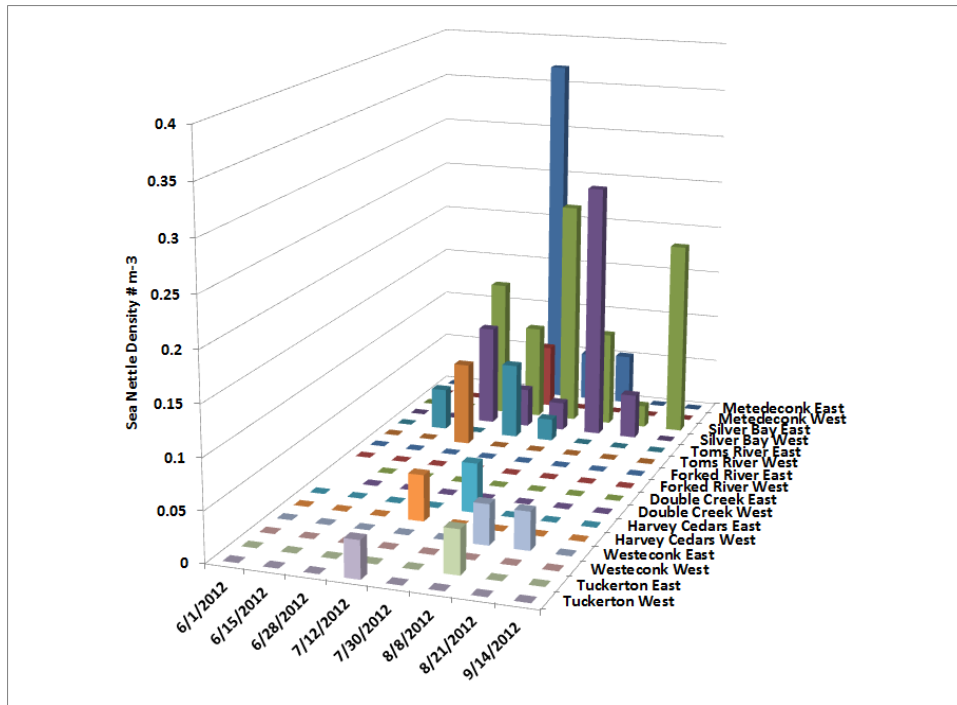


Figure 7. Density of juvenile *C. quinquecirrha* collected in plankton tows in 2012.

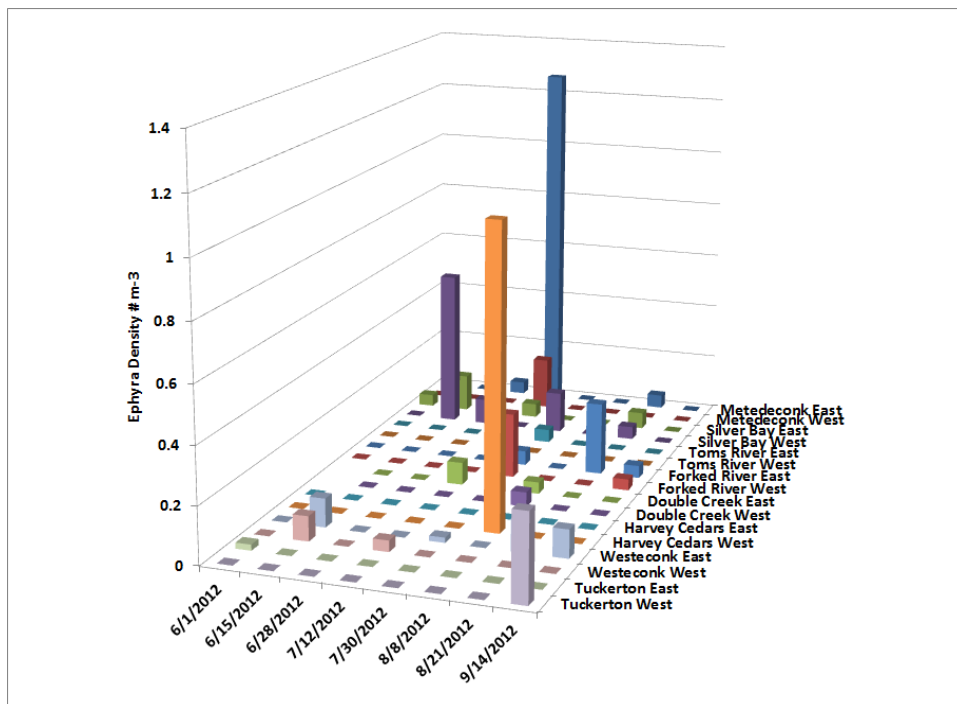


Figure 8. Density of *C. quinquecirrha* ephyra collected in plankton tows.

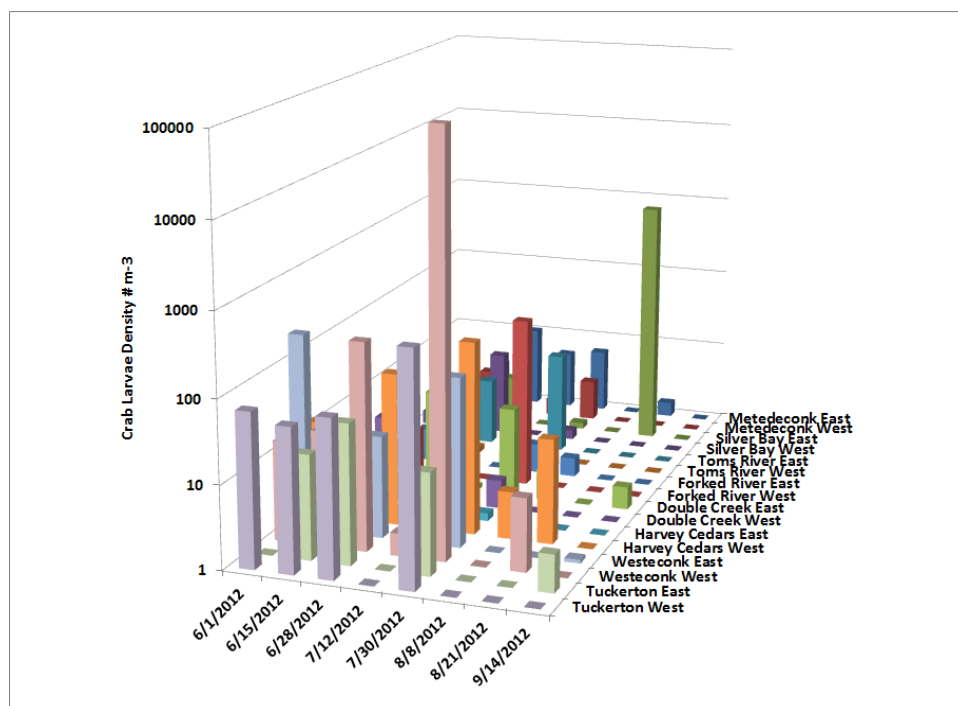


Figure 9. Crab larvae density among sites collected from plankton nets. Vertical Y-axis is presented in logarithmic scale to accommodate the extremely high density which occurred at the Westeconk Creek West site on July 30, 2012.

Correlation analysis showed some interesting patterns. Specifically, *M. leidyi* showed significant top-down impacts on several identified groups including Calanoid copepods ($P < 0.0005$), cladocerans ($P < 0.04$), fish eggs ($P < 0.0002$), and fish larvae ($P < 0.04$), but *C. quinquecirrha* showed no pattern of prey structuring, except for their potential impact on *M. leidyi* ($P < 0.07$). Many organisms were positively correlated and relate to generalized pelagic communities and larval distribution (e.g., Calanoid copepods with crab larvae ($r = 0.25$), Caridea larvae ($r = 0.5$)), as well as life history stages (e.g., fish eggs and larvae ($r = 0.44$, $P < 0.0001$)), *C. quinquecirrha* ephyra and juveniles ($r = 0.14$, $P < 0.007$), but one unique group of organisms displaying highly correlated distributions were benthic peracarida associated with floating wrack in open water. When the eleven most abundant organisms in this group were analyzed using correlation analysis 45 of 55 possible combinations showed significant positive correlations (Table 9). As these organisms are benthic in nature but are collected within floating wrack, it suggests a strong benthic-pelagic coupling for these organisms. Some of these taxa were also important in defining the site similarities in the SIMPER analysis.

Table 9. *Peracarida* correlation analysis. Values in table represent the Pearsons ‘r’ with significance indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Taxonomic abbreviations as follows: MEL = Mellitidae, IB = *Idotea baltica*, GAM = *Gammarus* spp., CAP = Caprellidae, PYC = Pycnogonidae, ER = *Erichsonella* spp., AOR = Aoridae, LJ = Lilljeborgiidae, PX = Phoxocephalidae, AML = *Ampelisca* spp., AMT = Ampithodae.

	IB	GAM	CAP	PYC	ER	AOR	LJ	PX	AML	AMT
MEL	0.52***	0.33***	0.34***	0.21***	0.2***	0.37***	0.3***	0.45***	0.19***	0.17**
IB		0.2***	0.26***	0.27***	0.3***	0.2***	0.1*	0.38***	0.15**	0.14**
GAM			0.06	0.08	0.03	0.15**	0.08	0.09	0.09	-0.01
CAP				0.22***	0.11*	0.38***	0.34***	0.17***	0.42***	0.11*
PYC					0.01	0.13*	0.16**	0.17**	0.25***	0.11*
ER						0.31***	0.06	0.21***	-0.02	0.16**
AOR							0.36***	0.36***	0.34***	0.33***
LJ								0.17***	0.18***	0.16**
PX									0.14**	0.32***
AML										0.19***

Results from the SIMPER analysis indicate average similarities ranging from 35% to 53% with between four and eight taxa contributing to >90% of the group similarity (Table 10). The two-way ANOSIM indicated a global R of 0.628 ($P < 0.001$) for differences among sites and a Global R of 0.706 ($P < 0.001$) for differences among dates. For all but one assessment between individual sites and dates, significance was < 0.001 ($P < 0.007$ for SBW and TRE) and individual R Statistics ranged from 0.338 to 0.942 for sites and 0.4 and 0.94 for dates. Collectively, these results indicate relatively unique plankton community characteristics within this system driven temporally by egg/larval production of *C. sapidus*, Caridea, fish, and polychaete larvae, as well as seasonal spikes in Calanoid copepods and naupli; and spatially through the disjunct distributions of *M. leidy* and *C. quinquecirrha* within the bay, the relative abundance of various peracarida associated with floating seagrass wrack in the system and pulses of more oceanic organisms near the sites adjacent to tidal inlets.

Table 10. 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: 45.86

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Caridea	1.22	13.35	1.69	29.12	29.12
<i>C. sapidus</i>	1.11	11.67	1.53	25.45	54.57
Calanoida	1.44	9.92	1.11	21.64	76.21
Fish Eggs	0.95	6.54	0.74	14.26	90.47

Metedeconk River West: Average similarity: 38.76

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Caridea	0.95	10.20	1.15	26.32	26.32
Calanoida	1.17	9.32	0.89	24.03	50.35
Fish Eggs	0.80	6.68	0.71	17.23	67.58
<i>C. sapidus</i>	0.69	5.74	0.72	14.81	82.39
<i>M. leidy</i>	0.47	4.82	0.42	12.44	94.84

Silver Bay East: Average similarity: 41.22

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Caridea	0.82	9.88	1.26	23.96	23.96
<i>C. sapidus</i>	0.88	9.11	1.26	22.10	46.06
Calanoida	1.02	6.86	1.12	16.63	62.69
Fish Eggs	0.99	6.65	0.78	16.13	78.83
<i>C. quinquecirrha</i>	0.34	2.69	0.55	6.53	85.35
Polychaeta	0.32	1.63	0.45	3.96	89.32
Larvae					
<i>Gammarus</i> spp.	0.27	1.17	0.33	2.83	92.15

Silver Bay West: Average similarity: 35.05

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Fish Eggs	1.05	11.24	0.97	32.06	32.06
<i>C. sapidus</i>	0.78	8.03	0.89	22.92	54.99
Calanoida	0.77	6.42	0.79	18.32	73.31
Caridea	0.49	3.05	0.58	8.71	82.02
<i>C. quinquecirrha</i>	0.24	2.46	0.31	7.01	89.03
Fish Larvae	0.25	1.23	0.38	3.50	92.53

Toms River East: Average similarity: 38.55

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Caridea	0.68	9.19	1.00	23.84	23.84
<i>C. sapidus</i>	0.74	8.04	0.90	20.85	44.69
Calanoida	0.64	6.84	0.79	17.75	62.44
Fish Eggs	0.82	6.84	0.66	17.74	80.17
<i>M. leidy</i>	0.49	4.26	0.51	11.06	91.24

Toms River West: Average similarity: 39.46

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Calanoida	1.24	18.86	1.33	47.79	47.79
<i>C. sapidus</i>	0.60	7.07	0.82	17.92	65.71
Fish Eggs	0.67	4.93	0.62	12.50	78.21

<i>M. leidy</i>	0.47	4.04	0.54	10.25	88.46
Caridea	0.41	3.14	0.56	7.95	96.41

Forked River East: Average similarity: 47.39

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Caridea	1.23	11.38	1.72	24.01	24.01
<i>C. sapidus</i>	1.03	10.72	2.25	22.61	46.63
<i>M. leidy</i>	0.87	7.11	0.73	15.00	61.63
Calanoida	0.75	6.16	0.94	12.99	74.62
Fish Eggs	0.82	4.43	0.65	9.35	83.97
<i>Idotea baltica</i>	0.47	2.76	0.70	5.83	89.80
Mellitidae	0.32	1.21	0.42	2.55	92.35

Forked River West: Average similarity: 47.82

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
<i>C. sapidus</i>	1.18	12.88	1.78	26.94	26.94
<i>M. leidy</i>	0.85	9.90	1.13	20.70	47.65
Caridea	0.78	9.03	1.34	18.88	66.52
Calanoida	0.87	8.87	1.09	18.55	85.07
Fish Eggs	0.74	4.87	0.58	10.17	95.25

Double Creek East: Average similarity: 45.90

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
<i>C. sapidus</i>	1.33	10.92	2.10	23.80	23.80
Calanoida	1.30	7.83	1.09	17.07	40.86
Caridea	1.22	7.56	1.09	16.46	57.32
<i>M. leidy</i>	0.82	6.03	1.01	13.14	70.46
Fish Eggs	0.87	4.50	0.92	9.79	80.25
Ostrocooda	0.48	1.79	0.50	3.89	84.15
<i>Idotea baltica</i>	0.45	1.58	0.44	3.44	87.59
Mellitidae	0.49	1.50	0.48	3.26	90.85

Double Creek West: Average similarity: 43.07

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
<i>M. leidy</i>	1.04	15.62	1.28	36.27	36.27
Caridea	0.90	9.07	1.18	21.06	57.34
Calanoida	1.04	8.66	1.03	20.10	77.44
<i>C. sapidus</i>	0.67	4.19	0.74	9.73	87.17
Fish Eggs	0.63	2.22	0.38	5.16	92.32

Harvey Cedars East: Average similarity: 49.09

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Caridea	1.24	15.48	3.93	31.53	31.53
<i>C. sapidus</i>	1.03	10.24	1.85	20.85	52.38
Calanoida	1.19	7.41	0.82	15.10	67.48
Fish Eggs	0.72	7.14	1.40	14.55	82.03
<i>M. leidy</i>	0.70	4.98	0.61	10.15	92.18

Harvey Cedars West: Average similarity: 53.28

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Calanoida	2.01	14.22	2.61	26.70	26.70
<i>C. sapidus</i>	1.79	11.43	1.57	21.45	48.15
Caridea	1.31	10.77	2.02	20.22	68.37
Fish Eggs	0.93	5.69	1.09	10.67	79.04
<i>M. leidy</i>	0.78	4.16	0.74	7.81	86.85
Fish Larvae	0.46	1.97	0.60	3.70	90.55

Westeconk East: Average similarity: 45.94

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
<i>C. sapidus</i>	1.61	11.93	2.75	25.98	25.98
Caridea	1.39	11.52	3.45	25.08	51.06
<i>M. leidy</i>	0.77	5.69	0.73	12.38	63.44
Calanoida	1.14	5.25	0.84	11.42	74.86
<i>Idotea baltica</i>	0.50	3.51	0.77	7.63	82.49
Fish Eggs	0.54	2.26	0.63	4.91	87.41
Polychaeta larvae	0.33	1.25	0.44	2.73	90.14

Westeconk West: Average similarity: 38.82

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
<i>C. sapidus</i>	3.65	10.03	1.07	25.83	25.83
Calanoida	1.97	9.57	1.03	24.65	50.48
<i>M. leidy</i>	0.74	6.56	0.61	16.90	67.37
Caridea	1.02	5.99	1.00	15.42	82.79
Fish Eggs	0.84	2.80	0.59	7.20	89.99
Fish Larvae	0.37	1.21	0.51	3.12	93.11

Tuckerton Creek East: Average similarity: 42.31

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Calanoida	1.90	10.48	1.73	24.76	24.76
Caridea	1.23	8.00	1.44	18.90	43.66

<i>C. sapidus</i>	1.22	6.81	1.19	16.10	59.76
<i>M. leidy</i>	0.99	6.15	0.66	14.53	74.29
Fish Egg	0.82	3.44	0.63	8.13	82.43
Fish Larvae	0.44	1.74	0.61	4.12	86.55
Mellitidae	0.43	1.72	0.54	4.05	90.60

Tuckerton Creek West: Average similarity: 42.89

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
<i>M. leidy</i>	1.33	13.83	0.81	32.25	32.25
Caridea	1.26	8.76	1.45	20.42	52.67
<i>C. sapidus</i>	1.82	7.77	0.95	18.12	70.79
Calanoida	1.78	6.90	0.84	16.09	86.88
Fish Egg	1.03	2.55	0.45	5.94	92.82

Molecular Analyses

We collected 384 water samples for DNA analysis which were split into 500 μm and 100 μm fractions using stacked filtering units. Each sample was assayed in triplicate, with appropriate controls and internal standards. Our first analysis was to determine whether DNA from the 500 μm filter was cascading onto the 100 μm filter from disruption of tissues/cells from the filtering process. A correlation analysis between DNA copies quantified on the 500 μm and 100 μm filters showed no relationship ($r = -0.044$, $P > 0.42$), so each DNA size fraction from samples is independent and allowed further analyses. Results from the molecular analyses indicate substantial differences in relative copies of the 16S gene among sites ($F_{15, 326} = 1.95$, $P < 0.02$) and dates of collection ($F_{7, 326} = 7.5$, $P < 0.0001$). The biggest surprise related to the samples collected from our Westeconnk West site which demonstrated extremely high DNA concentrations on June 28th (Fig. 10). This value discriminated this site as significantly different in the analyses. Additionally, for samples filtered with the 500 μm , ephyra were present in several pulses throughout the summer including prior to our first sampling event with two major peaks in mid-late June depending upon location in the bay and a peak on August 8th (Fig. 10). The pulse on August 8th was significantly greater than all other dates except for June 28th, where the high concentrations were identified from Westeconnk West. This second peak was also greater than samples from sampling events 1, 4, 7 and 8. In fact, for sampling events 7 and 8, DNA presence was extremely limited throughout the bay, suggesting that strobilation had essentially ceased at this point. Although molecular identification of ephyra occurred throughout the bay, adults were only abundant in the northern portion of the bay (Fig. 5). This indicates several strobilation events occurring in the bay yielding new recruits into the planktonic community, but other physical forces may limit the development of adult blooms in Barnegat Bay.

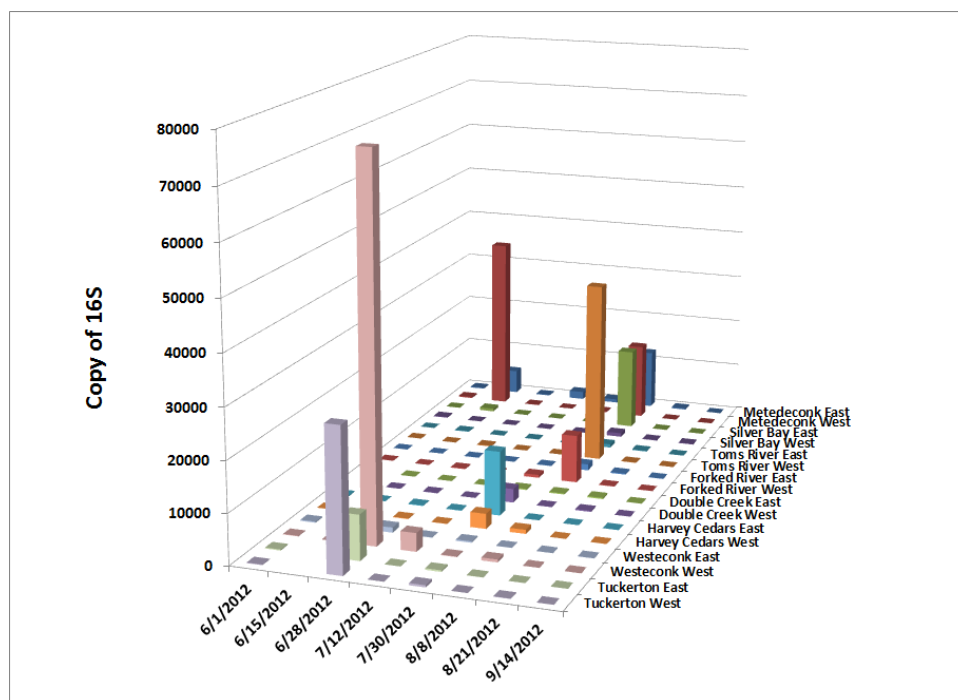


Figure 10. Relative abundance of sea nettle DNA on 500 µm filters in water samples.

For samples collected on the 100 µm filters, a small pulse of larvae occurred in mid-July, but the reproduction peaked during the end of summer with peaks in the southern regions of the bay in late August, while the highest concentration were identified in September in the northern portion of the bay (Fig. 11). Statistical analyses indicate no differences among sites ($F_{15,334} = 1.4$, $P > 0.14$), but significantly higher larval DNA concentrations from the September sampling event ($F_{7,334} = 6.04$, $P < 0.0001$). Interestingly, there appears to be several pulses of reproduction, but generally dominated by this late summer pulse.

One pattern present in the data is multiple pulses of reproduction and larval development in the northern regions of the bay. It appears that three events occurred at about two-week intervals starting in early July. The abundance of DNA on the 100 µm filters occurred throughout the bay. Consequently, physical transport of larvae through the water column is the likely mechanisms through which larvae were distributed. It also may indicate small populations of adults in regions of the bay which have not been sampled.

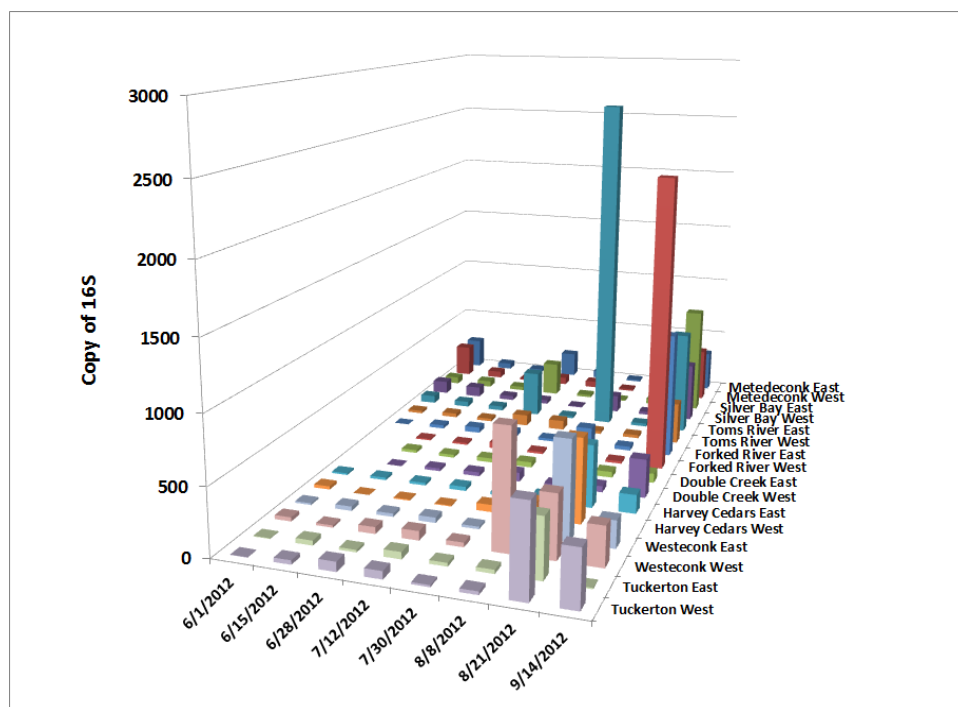


Figure 11. Relative abundance of sea nettle DNA on 100 µm filters in water samples.

Results from the 0.45 µm fraction indicate considerably less 16S rDNA copy number in these samples (compared to the 500 µm and 100 µm filters). The highest value measured was 567 copies (in a 2 µl sample) at our Forked River West site in Collection 1 (Fig. 12). DNA copy number on our 500 µm and 100 µm filters are orders of magnitude greater (see Figs. 10, 11). Additionally, the spatial and temporal fluctuation of *C. quinquecirrha* DNA on these filters is unusual. For example, we detect virtually no signal across Barnegat Bay for the last two collections (7 & 8). We also see generally higher signals in the southern and central parts of the bay and relatively low amounts in the north. Since this fraction represents all DNA between 100 µm and 0.45 µm, we predicted that we should be capable of detecting *C. quinquecirrha* sperm in this fraction. Although the size of *C. quinquecirrha* planula larva and eggs was known (Littleford, 1939), the size of sperm cells from this organism is unknown. However, based on the size of other Cnidarian sperm cells in the literature, we estimated that *Chrysaora quinquecirrha* sperm should be captured onto 0.45 µm filters. What is unexpected from these data is the lack of correlation of these signals with adult medusa (from lift net data) and planula larva (from 100 µm filter). Since sexually mature male medusa would be the source of these gametes, we anticipated that we would see general agreement of the distribution with this population. In addition, we see a marked absence of signals at the end of the reproductive season (Collections 7 & 8) when one would expect these to be relatively high and unexpectedly high signals at the beginning of the season (Collection 1).

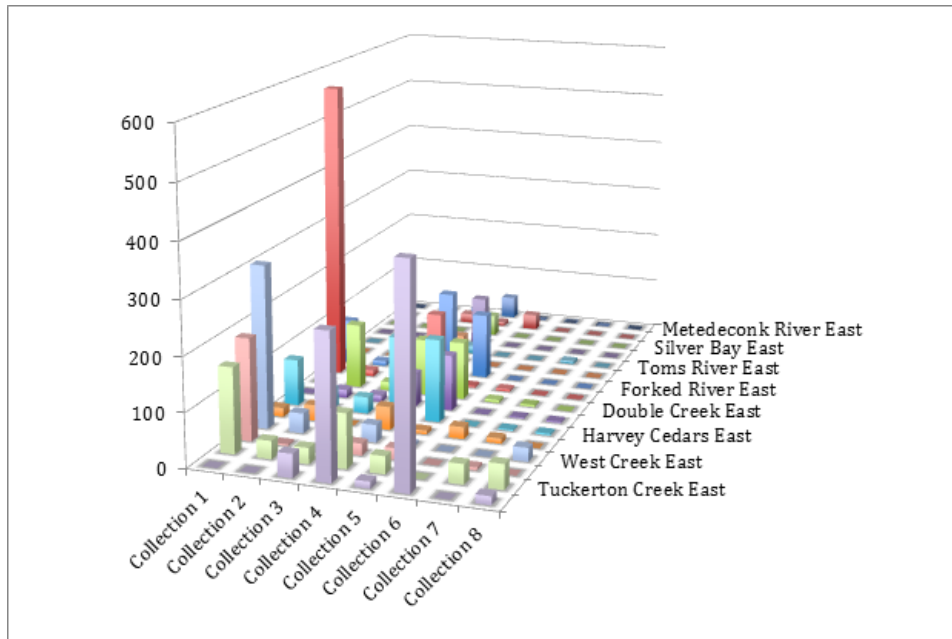


Figure 12. Relative abundance of sea nettle DNA on 0.45 µm filters in water samples.

Predictive Bloom Model

Based on the collected data, our basic model prediction suggests a two-week time lag from appearance of ephyra to presence of juveniles and an additional two-week lag for juveniles to mature into adults. Because of the spatial variability for the distribution of both juveniles and adults, we expected that the bay-wide model has limited power at this point. However, results from the AUTOREG analysis indicate that for the seven possible combinations, (e.g., week 1 juveniles to week 2 adults) six demonstrated significant relationships between collection of juveniles and their appearance as adults two weeks later (Table 11). For the analyses related to ephyra to juveniles and the DNA analysis for the 500 µm fraction to juveniles, only the ephyra analysis indicated a significant regression, but for only the peak in abundance (Table 12), while DNA copy number provided no significant results (Table 13). This last result is due to the large presence of ephyra identified in these analyses from the southern portion of Barnegat Bay, which must be transported out of the system through tidal exchange prior to these individuals growing to juvenile and adult life history stages. These results are also reflected in the assessment of the relationship between adult *C. quinquecirrha* and molecular DNA collected on the 100 µm filters, which demonstrate no relationship (Table 14).

Table 11. Results from the AUTOREG analysis between *C. quinquecirrha* juveniles collected during a sampling event and adults collected two-weeks later. T-value is provided regarding the parameter estimate in the equation analysis.

Time Lag Series	Equation Intercept	Parameter Estimate	t-value	P-value	R ²
1 → 2	0.0136	3.105	2.25	0.04	0.28
2 → 3	0.008	2.25	5.39	0.0001	0.69
3 → 4	-0.015	1.539	10.38	0.0001	0.89
4 → 5	0.0099	0.6375	2.13	0.053	0.26
5 → 6	-0.0049	1.69	12.89	0.0001	0.92
6 → 7	-0.004	2.66	4.10	0.0012	0.56
7 → 8	0.0058	1.8375	21.57	0.0001	0.97

Table 12. Results from the AUTOREG analysis between *C. quinquecirrha* ephyra collected during a sampling event and juveniles collected two-weeks later. T-value is provided regarding the parameter estimate in the equation analysis. The only significant relationship is bolded and relates to the second pulse of ephyra into the system.

Time Lag Series	Equation Intercept	Parameter Estimate	t-value	P-value	R ²
1 → 2	0.003	-0.009	-0.45	0.66	0.015
2 → 3	0.015	0.67	1.44	0.17	0.13
3 → 4	0.0178	0.276	10.29	0.0001	0.89
4 → 5	0.0276	-0.0425	-0.54	0.59	0.02
5 → 6	0.0032	-0.0235	-0.34	0.7	0.009
6 → 7	0.0044	0.066	1.18	0.25	0.097
7 → 8	0.015	-0.0775	-0.45	0.66	0.015

Table 13. Results from the AUTOREG analysis between *C. quinquecirrha* molecular DNA on the 500 µm filter collected during a sampling event and juveniles collected in plankton nets two-weeks later. T-value is provided regarding the parameter estimate in the equation analysis.

Time Lag Series	Equation Intercept	Parameter Estimate	t-value	P-value	R ²
1 → 2	0.0036	-1.1 E-7	-0.31	0.7	0.009
2 → 3	0.023	-4.0 E-7	-0.66	0.5	0.03
3 → 4	0.036	0.000025	1.0	0.3	0.07
4 → 5	0.02	1.06 E-6	0.21	0.8	0.003
5 → 6	0.032	-4.46 E-7	-0.27	0.8	0.005
6 → 7	0.009	-0.00003	-0.63	0.5	0.03
7 → 8	-0.0058	0.00048	1.15	0.27	0.09

Table 14. Results from the AUTOREG analysis between *C. quinquecirrha* adult distribution and molecular DNA on the 100 μm filter collected during a sampling event representing sexual reproduction. T-value is provided regarding the parameter estimate in the equation analysis.

Time Lag Series	Equation Intercept	Parameter Estimate	t-value	P-value	R ²
1 $\rightarrow$ 2	28.7	107.89	1.1	0.29	0.08
2 $\rightarrow$ 3	28.01	6.0	0.18	0.86	0.002
3 $\rightarrow$ 4	76.25	229.6	1.41	0.18	0.12
4 $\rightarrow$ 5	36.6	-116.3	-1.56	0.14	0.15
5 $\rightarrow$ 6	302.6	-684.9	-0.45	0.66	0.014
6 $\rightarrow$ 7	247.7	-975.9	-0.75	0.46	0.039
7 $\rightarrow$ 8	498.5	1065	0.66	0.52	0.03

Settling Plates

During the summer, several logistical problems occurred with our settling plates ranging from lines being cut, to active vandalism and movement. The settlement slides were evaluated, but no polyps or podocysts were identified. For most plates, barnacles covered approximately 100% of the surface and allowed few other organisms to settle. On average, barnacle settling density was 20,576 m^{-2} , *Diadumene lineata* settling density was 328.6, *Ciona intestinalis* average density was 58.9 m^{-2} and Polychaeta density was 33.7 m^{-2} . Barnacle density ranged from 3100 m^{-2} to 47480 m^{-2} (Table 15) and differed significantly among sites ($F_{11, 172} = 22.97$, $P < 0.0001$) and *Diadumene lineata* density ranges between 0-1356 m^{-2} and significantly differed among sites ($F_{11,173} = 2.8$, $P < 0.002$). Other organisms were identified on the settling plates including *Membranipora membranacea*, crustose coralline algae, hydroids, and Ascidiacea (colonial tunicates), which included *Botryllus* and *Botrylloides*; these were not identified individually, but rather grouped. Overall, settling plates were massively covered in barnacles which excluded most other organisms from settling, with the exception of non-native species (e.g., *D. lineata*, *C. intestinalis*).

Table 15. Density and prevalence of organisms settling on experimental settlement platforms. Densities for barnacles, *Diadumene lineata*, *Ciona intestinalis*, and Polychaeta given # m^{-2} + SE, while prevalence of *Membranipora membranacea*, coralline algae, hydroids, and colonial tunicates are provided as the percent of individual settling plates showing the presence of these organisms within each site. Table Abbreviations: BAR = Barnacles, Diad = *Diadumene lineata*, Ciona = *C. intestinalis*, POLY = Polychaeta, MEM = *Membranipora membranacea*, COR = Crustose coralline algae, ASC = Ascidiarians.

Site	BAR	Diad	Ciona	POLY	MEM	COR	Hydroid	ASC
ME	15213 $\pm$ 2138	388 $\pm$ 224	97 $\pm$ 97	0	100%	6.25%	0	0
SBW	14632 $\pm$ 2238	581 $\pm$ 240	0	0	100%	0	0	0
TRE	22384 $\pm$ 2520	291 $\pm$ 211	97 $\pm$ 97	0	100%	0	0	0
FRW	25872 $\pm$ 3273	291 $\pm$ 211	0	0	18.75%	0	18.75%	0
FRE	16860 $\pm$ 2817	0	0	0	100%	0	0	0
DCW	6007 $\pm$ 774	678 $\pm$ 244	194 $\pm$ 132	0	6.25%	56.25%	87.5%	37.5%

DCE	27519±3141	0	0	388±300	75%	0	0	12.5%
HCE	32267±3095	0	0	0	87.5%	0	0	12.5%
MBW	26260±3226	1357±598	291±291	0	43.75%	0	0	0
WW	47481±4124	194±194	0	0	50%	0	12.5%	6.25%
TCW	3101±939	0	0	0	6.25%	25%	0	6.25%
TCE	7461±1352	0	0	0	0	0	0	0

2013 Project 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 (see Fig. 1), while Lagoon communities were identified as the probable source of polyps and ephyrae so targeted sampling of eight lagoon communities was conducted (see Fig. 1).

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 16).

Table 16. 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 17, while water quality data for lagoon sampling events is presented in Table 18.

Table 17. 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.

	MW	ME	SBW	SBE	TRW	TRE	FRW	FRE	DCW	DCE	HCW	HCE	WW	WE	TW	TE
Temperature																
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 18. 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

2013 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. 13). The highest value recorded for the 2013 season was at our Silver Bay East site with over 2200 copies (in a 2 μ l 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.

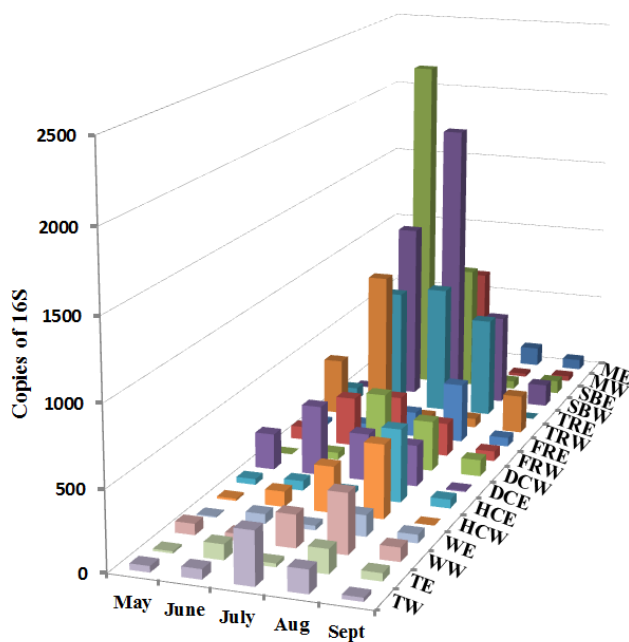


Figure 13. Molecular Assessment of DNA concentrations (16S rDNA) collected on the 500 μ m filters from monthly bay-wide sampling events.

Bay-wide Collections: Planula larvae.

Similar to the distribution of ephyra, planula larva 16S rDNA (collected on 100 μ m filters) showed a broad, bay-wide distribution from the Metedeconk in the north to Tuckerton in the south (Fig. 14). 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.

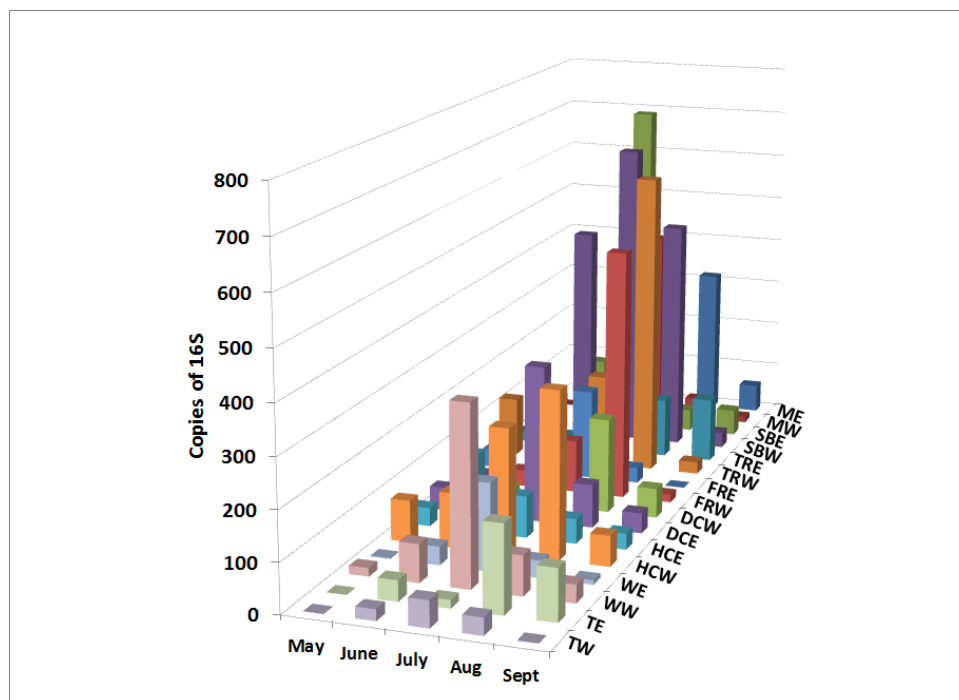


Figure 14. Molecular Assessment of DNA concentrations (16S rDNA) collected on the 100 μm filters from monthly bay-wide sampling events.

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. 15). 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.

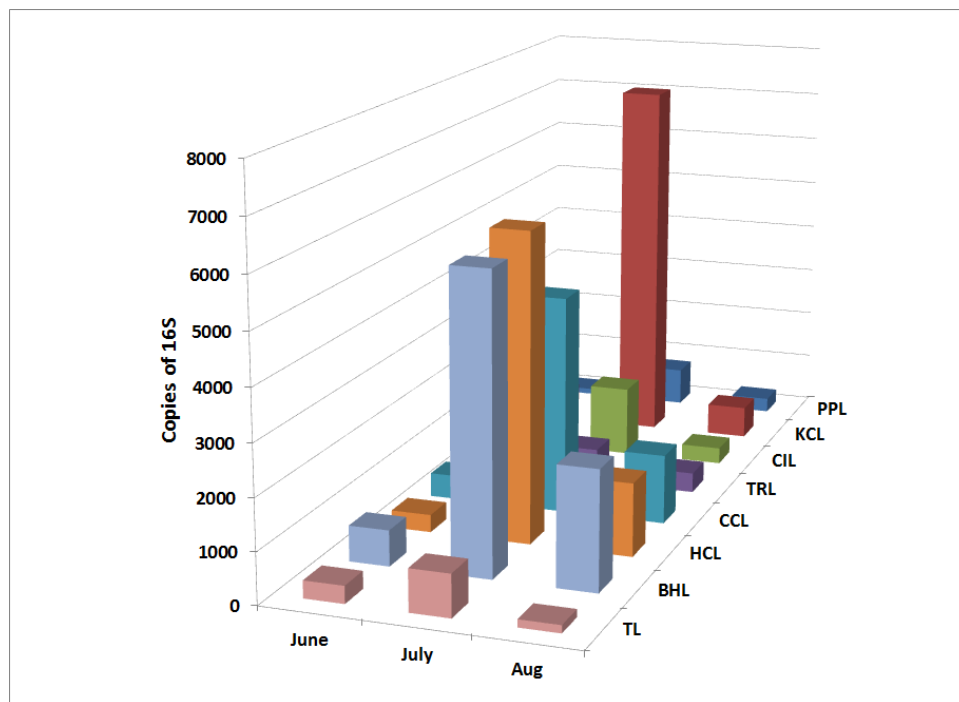


Figure 15. Molecular Assessment of DNA concentrations (16S rDNA) collected on the 500 μ m filters from monthly lagoon sampling events.

Planula larvae

We were also able to detect a strong signal for planula 16S rDNA in a number of these lagoonal sites (Fig. 16). 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).

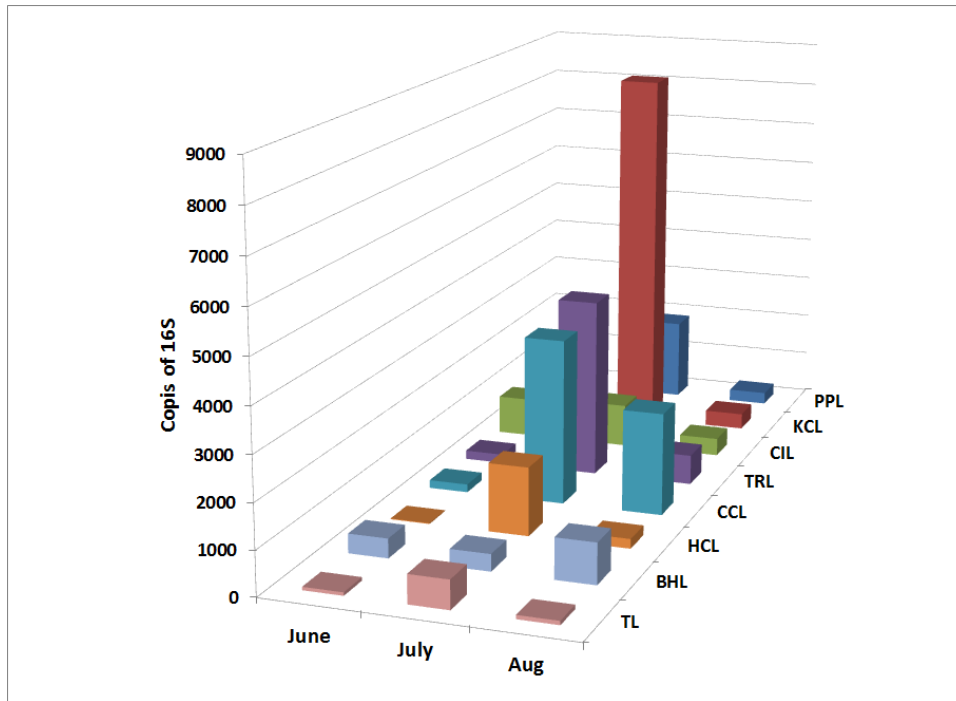


Figure 16. Molecular Assessment of DNA concentrations (16S rDNA) collected on the 100µm filters from monthly lagoon sampling events.

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. 17). 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 ($>15 \text{ m}^{-3}$), then Double Creek West ($>10 \text{ m}^{-3}$). 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 ($>6.7 \text{ m}^{-3}$) 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 ($r = -0.058$, $P > 0.09$).

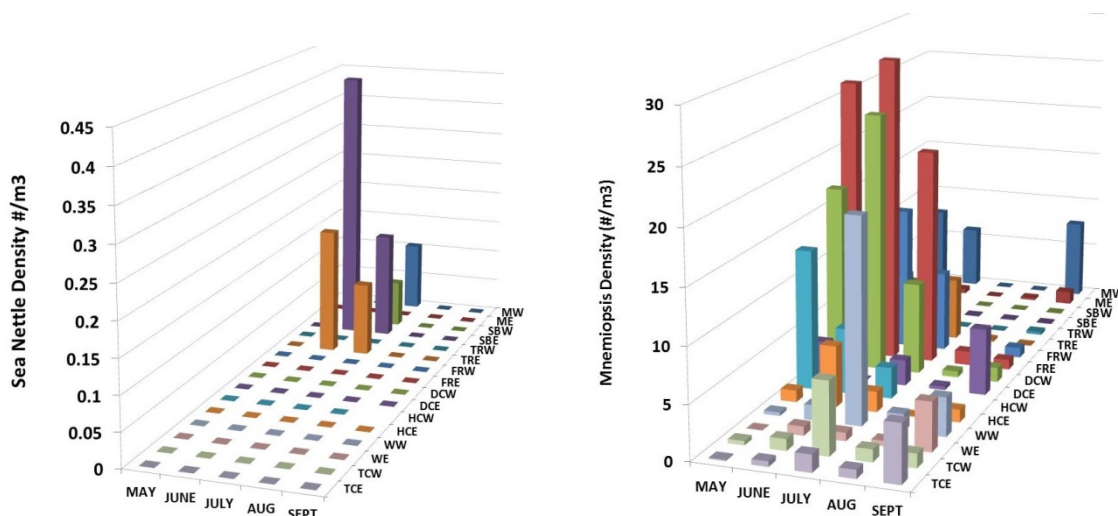


Figure 17. Distribution of *Chrysaora quinquecirrha* (left panel) and *Mnemiopsis leidyi* (right panel) collected during bay-wide sampling events in 2013.

Lift Net Results Lagoon Sampling

Lift net results from lagoon samples showed a similar disjoint distribution between the species (Fig. 18), 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 ($>5.37 \text{ m}^{-3}$). 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 ($r = -0.117$, $P = 0.068$).

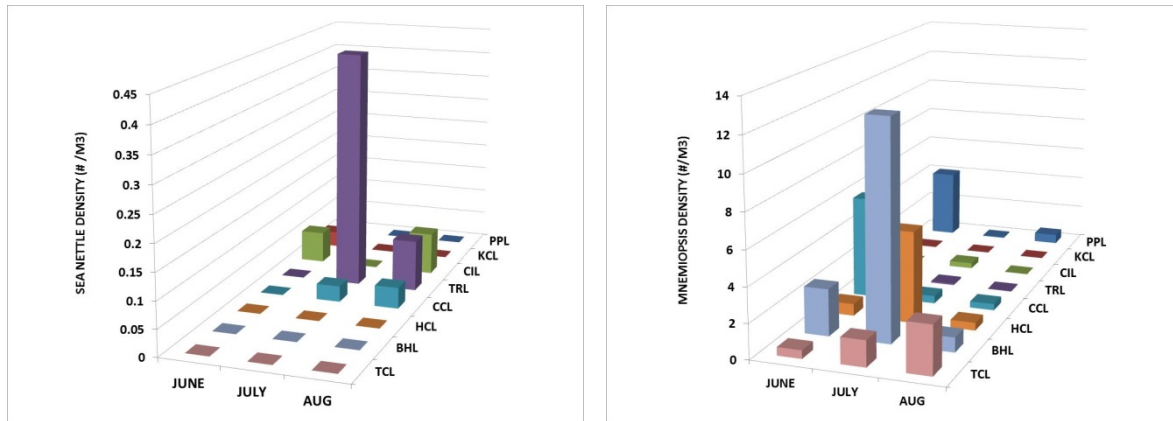


Figure 18. Distribution of *Chrysaora quinquecirrha* (left panel) and *Mnemiopsis leidyi* (right panel) collected during lagoon sampling events in 2013.

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. 19) and lagoon sampling events (Fig. 20) 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 ($F_{15,200} = 3.06$, $P < 0.0002$) 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 ($>17.6 \text{ m}^{-3}$) and very few occurring at our Toms River West site (0.86 m^{-3}) and Silver Bay West (0.0). While these two species showed disjoint distributions and a negative correlation between densities, it was not significant ($r = -0.09$, $P = 0.17$).

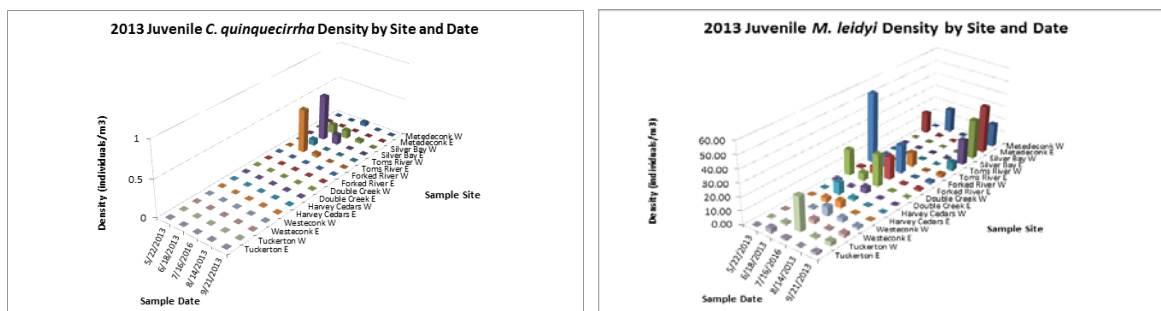


Figure 19. Distribution of *Chrysaora quinquecirrha* (left panel) and *Mnemiopsis leidyi* (right panel) collected during bay-wide plankton net sampling events.

Dominant Gelatinous Zooplankton Lagoon Samples

The distribution of the two major gelatinous zooplankton species within the lagoons (Fig. 20) showed somewhat similar patterns seen in the lift nets (Fig. 18). *Chrysaora* showed significant density differences among sites ($F_{7,58} = 5.82$, $P < 0.001$) 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 (6.5 m^{-3}) and Kettle Creek Lagoon (6.3 m^{-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 ($r = -0.09$, $P = 0.46$).

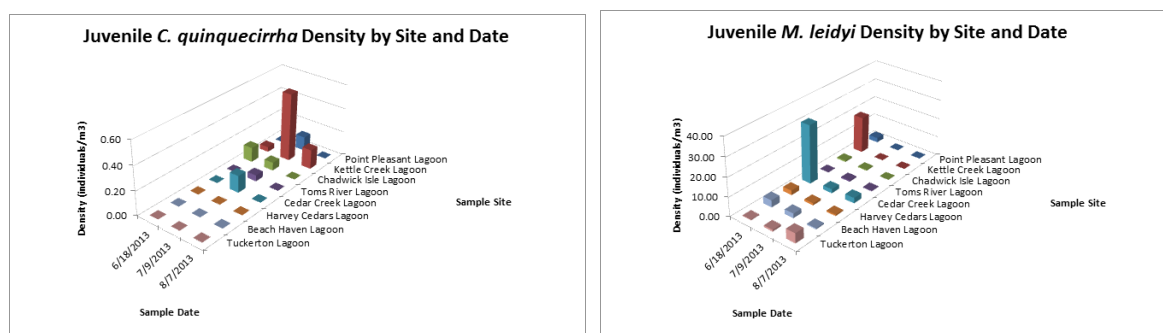


Figure 20. Distribution of *Chrysaora* (left panel) and *Mnemiopsis* (right panel) collected during lagoon sampling events with plankton nets.

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 19). 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 19. 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,**	<i>Clytia</i> ***,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. 21), 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 Supplemental Data (provided electronically).

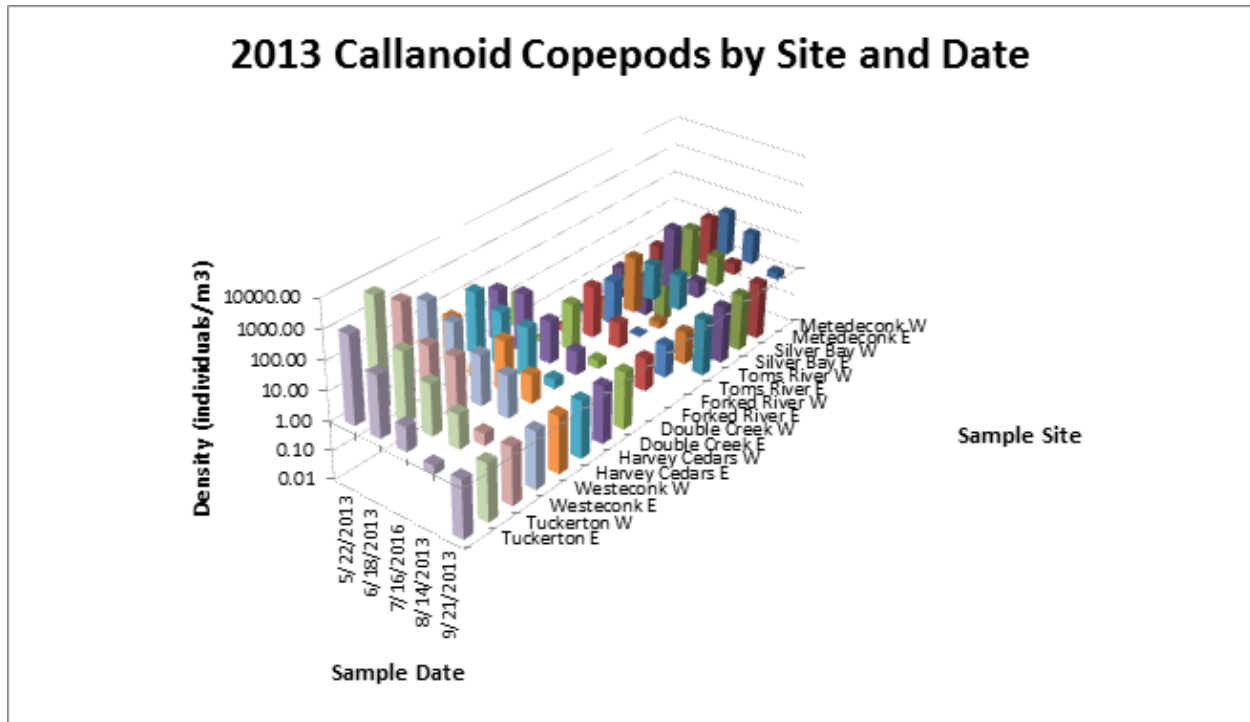


Figure 21. Density of calanoid copepods among sites and dates of collection in 2013 for bay-wide samples. Y-axis is given in exponential scale to address the significant densities which occurred early in the season in the southern regions of the bay.

Correlation analysis indicated that 18 taxa showed some significant correlation between species (Table 20). 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 20. 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: Ostrocods, CAPR: Caprellidae, CQ: *Chrysaora quinquecirrha*, TTOP: *Turritopsis*, OBEL: *Obelia*, BOUG: *Bougainvillea*, PLBR: *Pleurobranchia*.

Table 20A

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

BRCH	-0.05	0.02	1.00	0.97	0.04	0.08	0.06	0.10	0.15	0.24
	0.44	0.73		<.0001	0.59	0.22	0.37	0.14	0.03	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 20B

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

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. 22) 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).

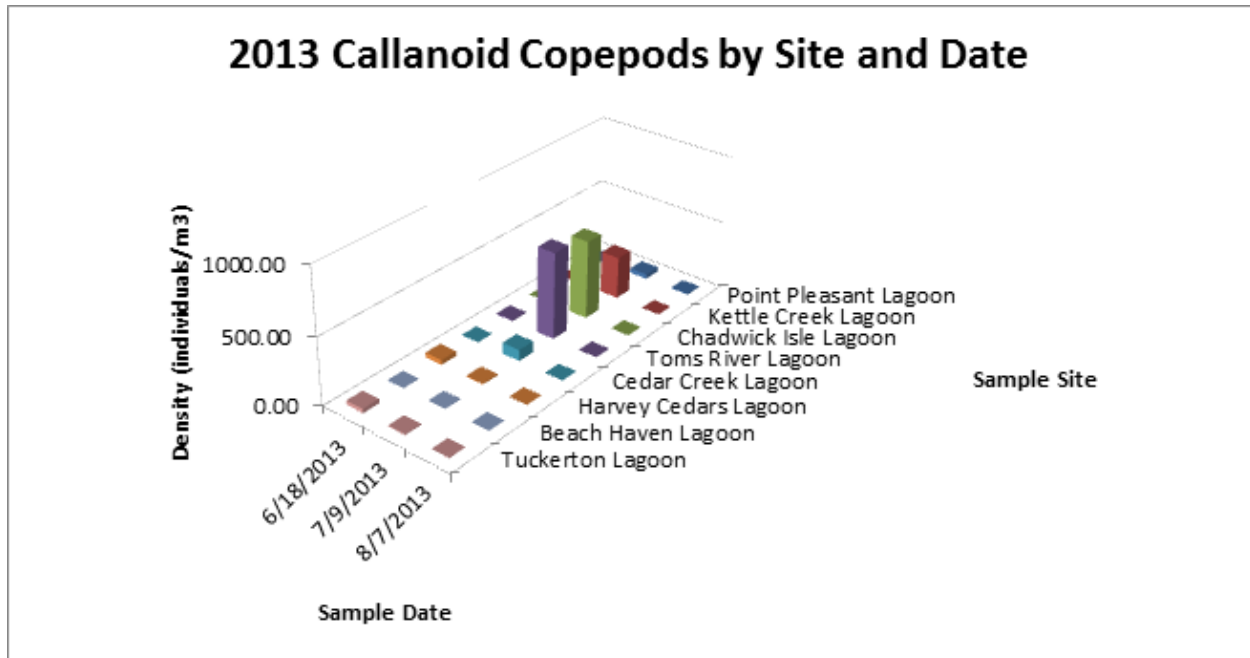


Figure 22. Density of calanoid copepods among sites and dates of collection in 2013 for lagoon samples.

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 21). In particular, six taxa were important in structuring most of the planktonic communities observed in Barnegat Bay and include: *M. leidy*, 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 21. 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.%
<i>M. leidy</i>	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
<i>Salpa salpa</i>	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.%
<i>M. leidy</i>	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
<i>Salpa salpa</i>	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
<i>M. leidy</i>	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
<i>M. leidy</i>	0.98	2.96	0.55	6.02	88.79
<i>C. quinquecirrha</i>	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
<i>M. leidy</i>	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.%
<i>M. leidy</i>	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
<i>Salpa salpa</i>	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.%
<i>M. leidy</i>	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
<i>M. leidy</i>	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.%
<i>M. leidy</i>	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
<i>Salpa salpa</i>	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.%
<i>M. leidy</i>	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
<i>M. leidy</i>	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
<i>M. leidy</i>	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
<i>Salpa salpa</i>	0.34	5.68	0.48	8.31	88.55
<i>Turritopsis</i>	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
<i>M. leidy</i>	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
<i>Salpa salpa</i>	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
<i>M. leidy</i>	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
<i>M. leidy</i>	1.71	12.77	1.01	17.05	55.36
Caridea	5.01	11.67	1.11	15.58	70.95
<i>Salpa salpa</i>	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. 23). However, when points are plotted based on the month of collection, strong clustering of samples is evident (Fig. 24). 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.

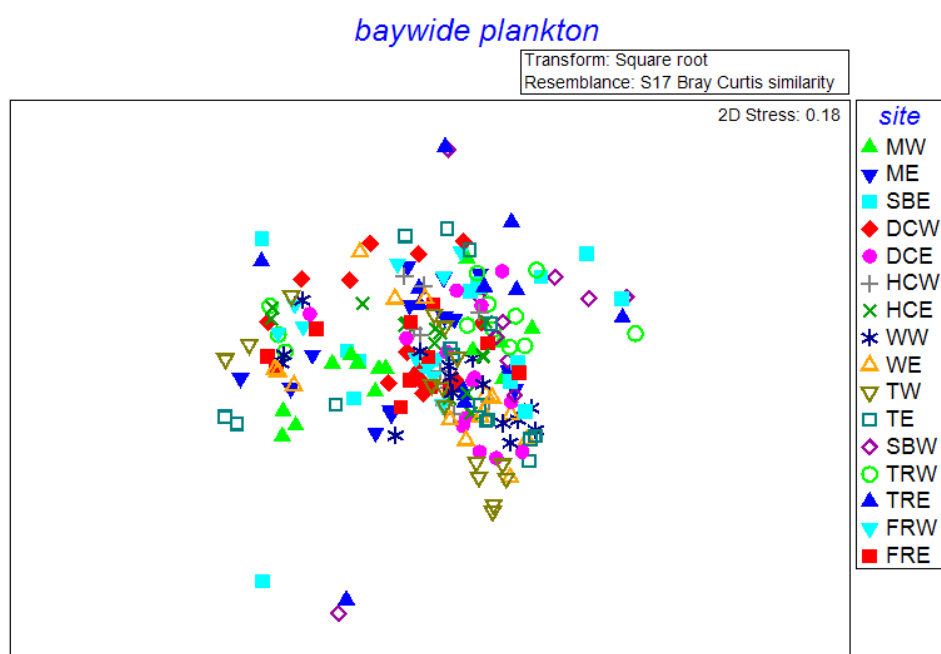


Figure 23. MDS plot of planktonic community structure from bay-wide samples plotted based upon the sites of collection. Clustering of samples indicates similarity of community structure.

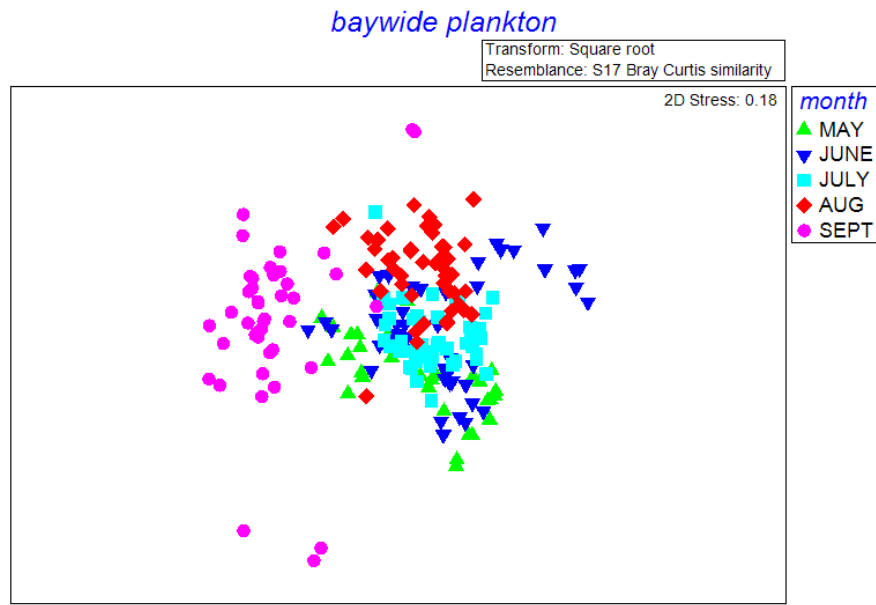


Figure 24. MDS plot of planktonic community structure from bay-wide samples plotted based upon the month of collection. Clustering of samples indicates similarity of community structure.

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. leidy* (Table 22). 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 23). 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 22. 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
<i>M. leidy</i>	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
<i>C. quinquecirrha</i>	0.39	2.46	0.43	8.55	78.5
<i>C. quinquecirrha</i> 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
<i>M. leidy</i>	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
<i>M. leidy</i>	0.98	7.9	1.4	17.9	70.96
Fish Eggs	0.78	4.46	0.76	10.1	81.06
<i>Turritopsis</i>	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
<i>M. leidy</i>	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.%
<i>M. leidy</i>	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 23. 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. 25) and months of collection (Fig. 26). 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.

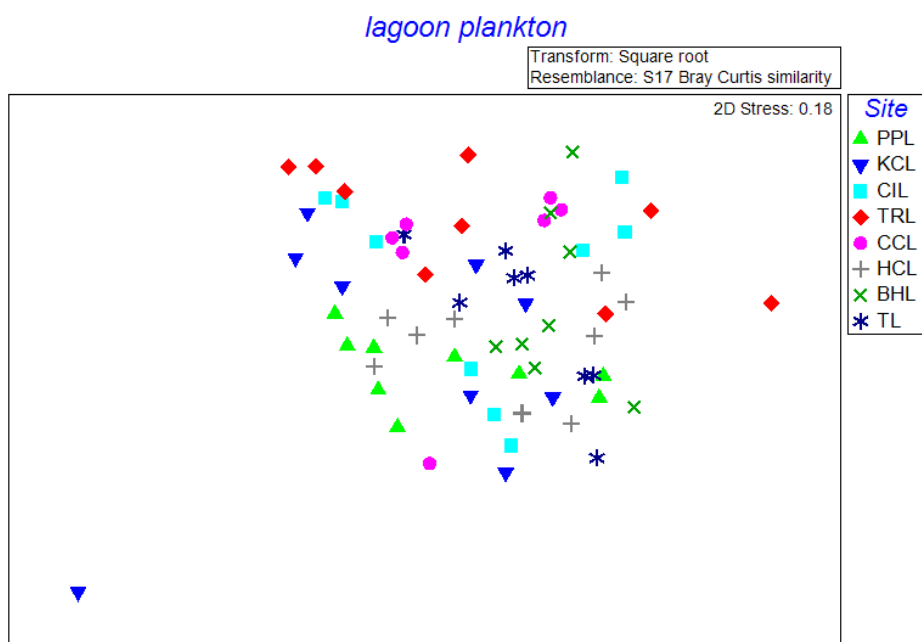


Figure 25. MDS plot of planktonic community structure from lagoon samples plotted based upon the sites of collection. Clustering of samples indicates similarity of community structure. Outlying point for Kettle Creek indicates a sample with high densities of *Chrysaora* in August.

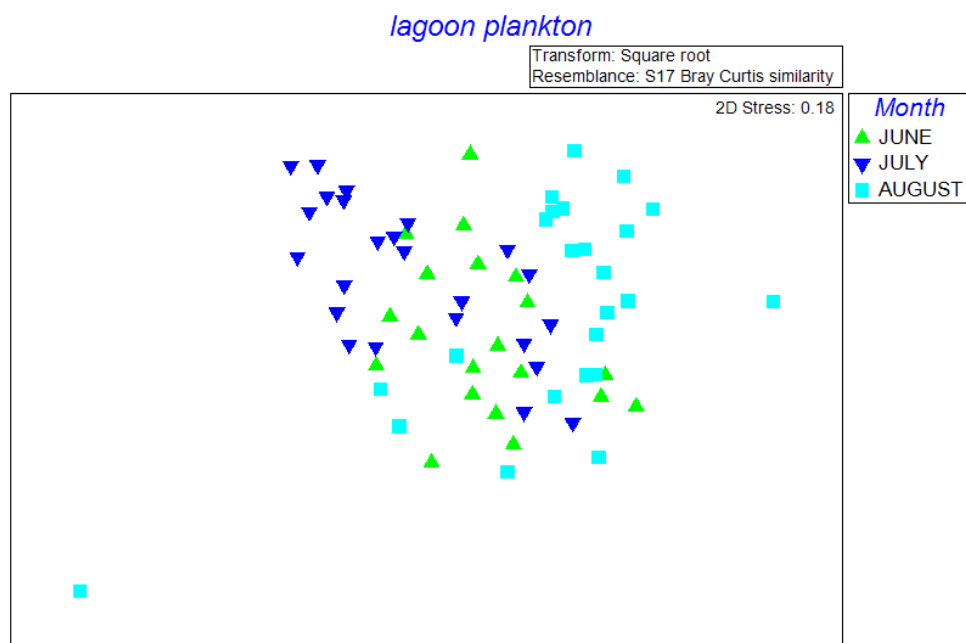


Figure 26. MDS plot of planktonic community structure from lagoon samples plotted based upon the month of collection. Clustering of samples indicates similarity of community structure. Outlying point for August indicates a sample with high densities of *Chrysaora* in Kettle Creek.

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 24.

Table 24. 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
<i>Mogula</i> spp.	Sea Squirt	0.85
<i>Semibalanus balanoides</i>	Barnacles	27.94
<i>Spiroibis</i> spp.	Polychaeta	55.93
<i>Crepidula</i> spp.	Slipper shell	0.023
<i>Diadumene leucolena</i>	White anemone	0.162
<i>Diadumene lineata</i>	Orange Stripped Anemone	0.2129
<i>Metridium senile</i>	Clonal Plumose Anemone	0.0138
<i>Chrysaora</i> Podocyst	Sea Nettle Podocyst	8.939
<i>Chrysaora</i> Polyps	Sea Nettle Polyps	0.0601
<i>Bugula turrita</i>	Spiral Tufted Bushy Bryozoa	8.393

Scientific Name	Common Name	Mean Percent Cover
<i>Membranipora membranacea</i>	Lacy Bryozoa	38%
<i>Flustrellidra hispida</i>	Bristly Bryozoa	0.7%
<i>Scruparia ambigua</i>	Bryozoa	5%
<i>Crisia</i> spp.	Jointed-tubed Bryozoa	0.004%
<i>Eucretea loricata</i>	Eucretea Bryozoa	0.02%
<i>Botryllus schlosseri</i>	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 Figs. 17-19). 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. 27) 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 harpacticoid copepods, which are both benthic prey, compared to their availability in the water column (Fig. 27). 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 harpacticoids 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.

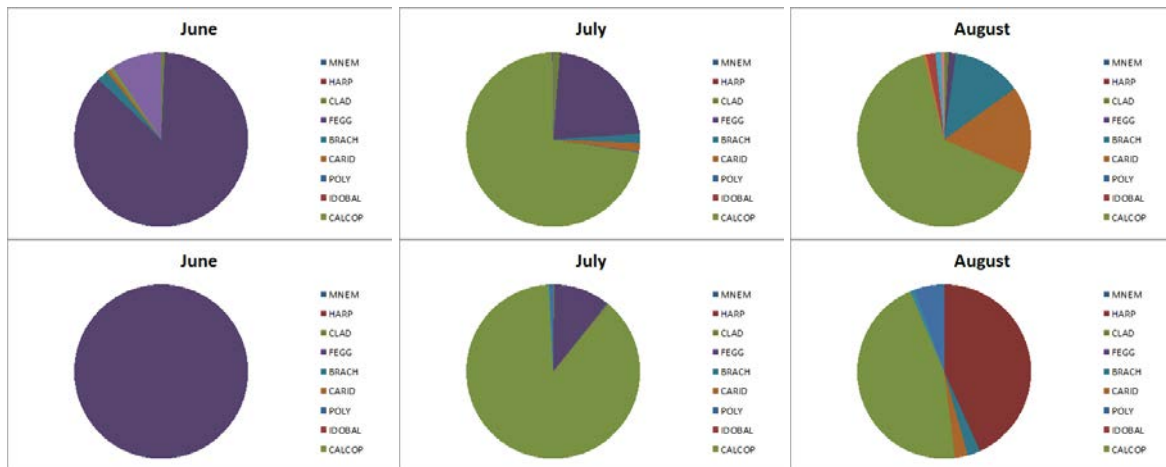


Figure 27. Comparisons of Potential Available Prey (upper Panels) collected in plankton tows with Prey Selected (lower panels) by *Chrysaora* for the months of collection from the Northern Region of the Bay where *Chrysaora* is present.

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. 28). Similar to the August results from the northern region, polychaetes and harpacticoid copepods were strongly represented in the diet, but not in plankton tows. This again suggests benthic targeting by *Chrysaora* in this region.

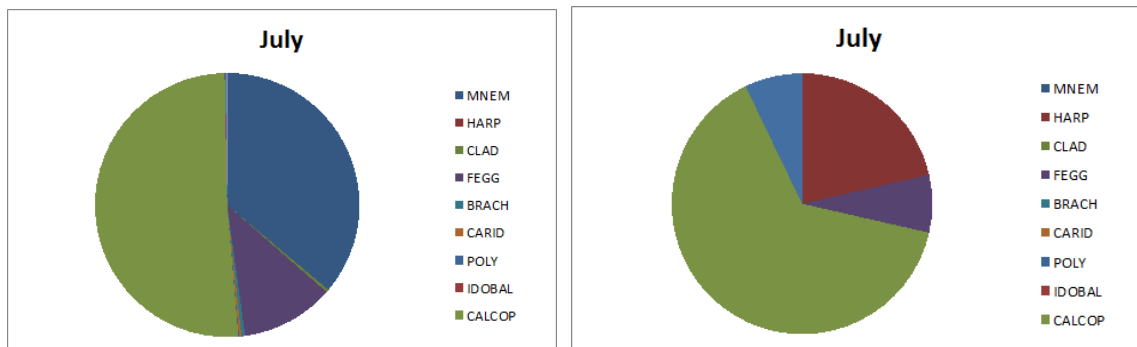


Figure 28. Comparisons of Potential Available Prey (Left Panel) collected in plankton tows with Prey Selected (Right Panel) by *Chrysaora* for July from the Middle Region of the Bay where *Chrysaora* is present.

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. 29), but diet was again over represented in harpactacoids and polychaetes.

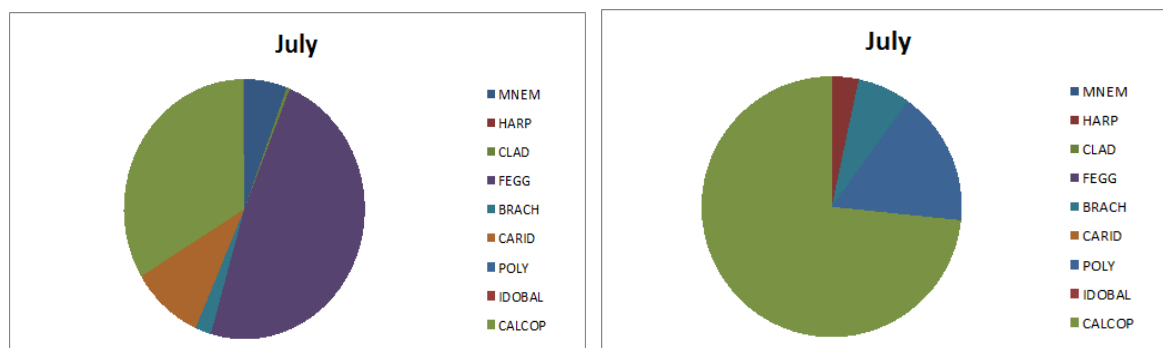


Figure 29. Comparisons of Potential Available Prey (Left Panel) collected in plankton tows with Prey Selected (Right Panel) by *Chrysaora* for July from the Southern Region of the Bay where *Chrysaora* is present.

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. 30) for 2 of the sampled individuals.

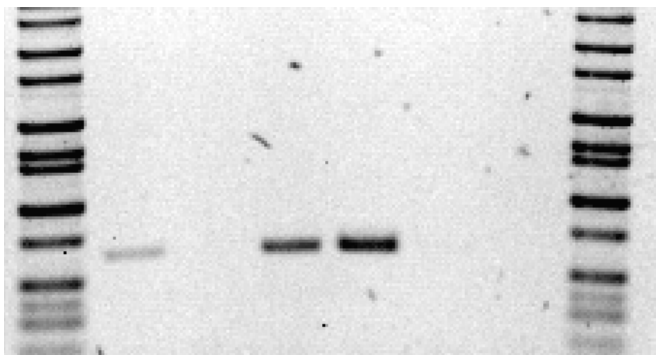


Figure 30. PCR amplification of *Mnemiopsis leidyi* DNA from the gut of *Chrysaora quinquecirrha*. Using PCR primers that specifically amplify a region of the 16S rDNA gene of *M. leidyi* we amplified a 682 bp fragment of the comb jelly mitochondrial genome which was verified by Sanger sequencing (data 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 (see supplemental data on CD-ROM). Of these, 371 sequences were assigned in MEGAN (Table 25). Manual inspection of these sequences identified 100 contig sequences, which

were confidently assigned to twenty-three taxa (Table 26) that were not bacteria, viruses, or tetrapods. Of these twenty-three taxa, we were able to identify 9 to the species level (Table 26). 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. See supplemental data disk for further information on our final taxonomic assignments for the 100 identifiable contigs.

Table 25. Number of contigs assigned by MEGAN from samples collected in 2013.

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
<i>Chrysaora</i> (sea nettles)	5
Ctenophora (comb jellies)	20

Table 26. *C. quinquecirrha* prey items identified in both the gut lavage and tentacle picks.

Final Classification	Common Name	Gene(s)	Gut lavage	tentacle pick
<i>Ampithoe valida</i>	amphipod	COI; 18S	X	
<i>Acartia tonsa</i>	copepod #1	ITS1, 5.8S rRNA, ITS2; COI	X	X
Crangonyctidae	copepod #2 (Not Cyclopoida)	28S	X	
Cyclopoida	copepod #3 (Not Crangonyctidae)	18S	X	
<i>Pseudodiaptomus coronatus</i>	copepod #4	18S; 28S	X	X
Cirripedia	barnacle	28S	X	
<i>Americamysis bahia</i>	opossum shrimp	18S	X	X
<i>Diadumene leucolena</i>	white anemone	28S	X	
Nynantheae	anemone possibly white anemone	COI and other mitochondrial genes	X	
Actiniaria possibly <i>Nematostella vectensis</i>	starlet sea anemone	Miscellaneous nuclear genes	X	X
<i>Mnemiopsis leidyi</i>	sea walnut	18S; 28S; COI and other mitochondrial genes	X	X
<i>Alitta</i> (=Nereis) <i>succinea</i>	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
<i>Mercenaria mercenaria</i>	hard clam	COI; 18S		X
Veneridae other than <i>Mercenaria</i>	clam #2	COI		X
Hemichordata	possibly acorn worm	BAC sequences	X	X
<i>Anchoa mitchilli</i>	bay anchovy	COI and other mitochondrial genes		X

2014 Project Results

For 2014, 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 and 2013 research programs that continued in 2014 (Fig. 1), while Lagoon communities were identified in 2013 as the probable source of polyps and ephyrae so targeted sampling of eight lagoon communities was continued in 2014.

Summary of Sampling Events

Bay-wide samples were collected monthly from May to October, 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 27).

Table 27. Monthly sampling dates for bay-wide and lagoon sampling events.

Bay Wide Sampling Events	Specific dates
May	May 29, 2014
June	June 16, 17, 2014
July	July 16, 17, 2014
August	August 14, 15, 2014
October	October 3, 2014
Lagoon Sampling Events	Specific dates
June	June 16, 17, 2014
July	July 8, 9, 2014
August	August 7, 8, 2014

Water Quality Summary

During sampling events, water quality data were collected for salinity, temperature, and dissolved oxygen using a YSI-Pro2030® water quality meter. Water quality data are presented for the Bay-wide sampling events in Table 28, while water quality data for lagoon sampling events is presented in Table 29.

Table 28. Bay-wide Water quality data for 2014 collected during sampling events using a YSI-Pro2030® handheld meter. A) Temperature for all site and date combinations (°C), B) Salinity (ppt), and C) Dissolved Oxygen (mg/l). Due to logistical constraints, data were not collected in May from the four most southerly sites (WW, WE, TW, and TE).

Temperature	MW	ME	SBW	SBE	TRW	TRE	FRW	FRE	DCW	DCE	HCW	HCE	WW	WE	TW	TE
May	16.3	15.3	17.6	16.8	17.8	18	17.6	17	18.8	15.5	17.8	19				
June	23.3	25.3	25.9	24.2	25.8	23.8	28.2	22.5	25	23.8	22.9	24.4	23.5	23.8	23.6	22.4
July	23.9	24.3	26.0	24.8	26.5	25.4	26.6	24.3	25.6	17.7	26.1	26	25.6	24.5	25	21.6
August	23.5	23.6	24	23.2	24.3	23.1	24.6	23.5	24.4	24.5	22.8	23.2	22.8	22.9	23.5	23

October	18.6	18.8	18.7	18.2	19.1	18.4	19.1	18.1	18.4	20.2	18.7	19.7	19.4	19.5	19.8	19.6
Salinity																
May	21.3	23.3	17.7	17.6	16.7	16.6	23.1	21.6	23.8	25.2	23.7	24				
June	19.2	17.7	15.5	15.8	16.1	16.1	25.2	26	26.3	23.8	22.9	26.2	26.2	28.6	28.4	29.3
July	9.5	13.8	17.4	19.3	18.6	21.5	27.6	20	28.2	30.3	28.4	29.6	28.2	30.3	28.9	30.7
August	17.3	19.3	16.5	17.3	14.6	18.4	25.8	24.3	27	27	29.4	25.6	21.2	26.5	26.7	27
October	24.5	27.1	20.4	20.9	19	21	27.1	26.3	28.5	29	27.6	27.2	25	25.6	28.3	29.3
Dissolved Oxygen																
May	7.09	7.24	7.82	7.84	7.81	7.50	6.84	7.59	7.18	8.79	7.85	7.42				
June	7.99	9.05	7.51	7.21	7.24	7.17	7.57	6.80	9.12	9.86	6.57	7.51	6.24	8.16	6.72	6.67
July	7.70	8.23	8.33	7.14	8.23	6.99	7.67	7.20	10.12	9.87	6.84	7.20	7.15	8.41	8.23	8.23
August	7.40	8.14	7.87	6.84	8.34	6.96	6.67	6.67	6.38	10.89	5.61	5.89	5.53	8.32	6.15	6.50
October	7.02	6.45	7.55	7.40	7.99	6.29	6.96	6.63	7.49	12.04	6.52	7.60	5.85	7.97	6.81	19.6

Table 29. Lagoon Water quality data for 2014 collected during sampling events using a YSI-Pro2030® handheld meter. A) Temperature for all site and date combinations (°C), B) Salinity (ppt), and C) Dissolved Oxygen (mg/l)

Temperature	PPL	KCL	CIL	TRL	CCL	HCL	BHL	TL
June	24.9	25.5	23.9	26.9	26.8	24.5	23.6	24.1
July						25.8	26.6	26.5
August	24	25.6	25	26.3	26.8	26	25.7	26.2
Salinity								
June	21.2	16.3	16.3	16.5	23.7	26.3	24.2	27.7
July						29.2	26.9	25.5
August	25.9	17.9	19.1	20.1	27.0	27.9	25.9	28.2
Dissolved Oxygen								
June	7.96	7.09	6.62	6.97	7.22	6.91	6.34	6.79
July						6.91	7.90	4
August	8.05	5.42	5.82	7.71	7.02	7.69	7.60	5.71

Objective Specific Results

2014 Molecular Analyses of *Chrysaora quinquecirrha* from Water Samples

Our sampling protocol for molecular analysis in 2014 was identical to that used in the summer of 2013. Bay-wide we sampled monthly from May through September, at 16 sites, in triplicate at each site. So we collected and processed 240 samples for real-time qPCR analysis from the bay in the summer of 2014. For the lagoonal collection, we sampled a total of 72 times (8 lagoonal sites, for 3 months (June, July, August) in triplicate at each site). Total samples processed for qPCR was 312 (with each sample assayed in triplicate along with appropriate controls and internal standards). All samples have been processed and qPCR results indicate the presence of *Chrysaora* ephyrae DNA throughout Barnegat Bay with peaks in July. Additionally, in northern portions of the bay, their presence is noted earlier in the summer, but by July and into August qPCR results demonstrate a broad distribution throughout the bay. For samples collected in the lagoons, Ephyrae DNA was identified during June samples in the northern-most section of Barnegat Bay, but showed a broad distribution during our July sampling event and ephyra DNA was observed in August in the Tuckerton Creek Lagoon system. Given the reduced total abundance in DNA, initial modeling results are not strongly correlated.

Bay-wide Collections: Ephyrae

Similar to 2012 and 2013, ephyra 16S rDNA (collected on 500 μm filters) was seen widely distributed throughout Barnegat Bay (north, central and southern regions). However, the highest value for the season was seen at our Harvey Cedars West (HCW) site in July (over 1700 copies in a 2 μl sample; see Fig. 31). In the northern portion of the bay, the presence of ephyra is noted earlier in the summer, but by July and into August qPCR results demonstrate a broad distribution throughout the bay. In contrast to the 2013 season our highest signal was detected further south in the bay (Silver Bay in 2013 vs. Harvey Cedars in 2014). A comparison of the overall distribution pattern shows higher signals moving to central and even southern areas of the bay in 2014. In addition to Harvey Cedars, other sites that also produced strong signals included Double Creek West, Harvey Cedars East, and the Westeconk Creek sites. By the end of September the ephyra signal had virtually disappeared suggesting a halt in strobilation. It is important to note although these signals are comparable in copy number to 2013 values, both 2013 and 2014 are significantly reduced from the 2012 data.

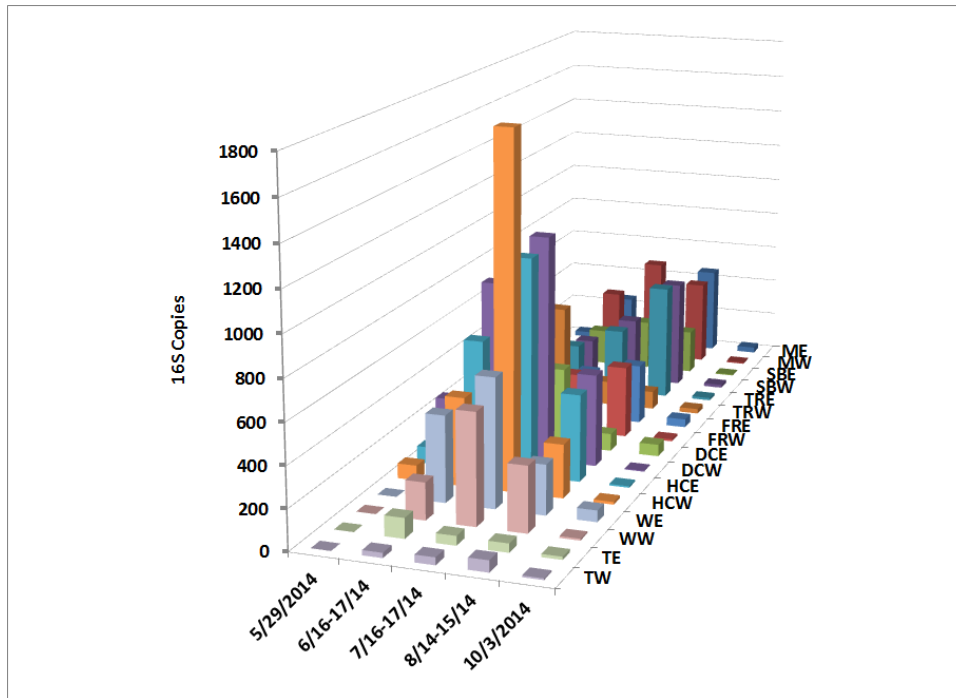


Figure 31. Distribution of ephyral DNA detected using qPCR for *Chrysaora* from water samples collected during bay-wide sampling events.

Bay-wide Collections: Planula larvae.

As with the distribution of ephyra, planula larvae 16S rDNA (collected on 100 μ m filters) showed a broad, bay-wide distribution from the Metedeconk in the north to Tuckerton in the south (Fig. 32). Peaks of larvae occurred in June, July or August, depending on the site. Harvey Cedars East peaked in mid-June, Silver Bay and Forked River sites peaked in July, and Toms River peaked in August. The highest signal seen was from Silver Bay in July (>900 copies of 16S rDNA). Although signal intensity for planula larvae was lower than ephyra signals bay-wide, planula DNA was more uniformly distributed throughout the bay, showing strong signals at many sites in different regions of the bay. 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.

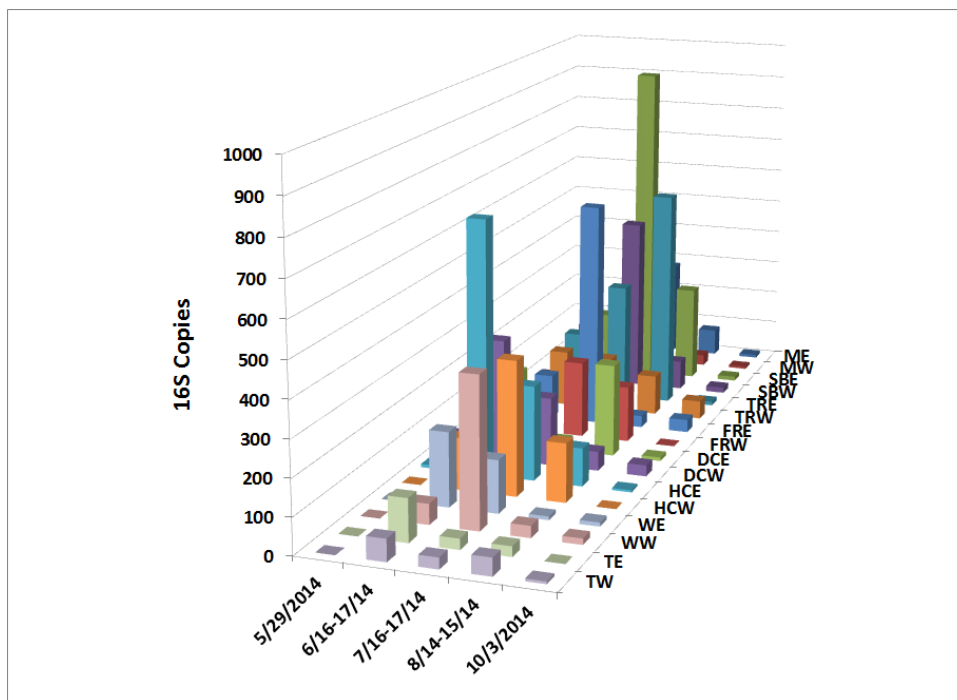


Figure 32. Distribution of larval DNA detected using qPCR for *Chrysaora* from water samples collected during bay-wide sampling events.

Lagoon Collections

In 2014 we continued our sampling of lagoonal sites initiated in 2013. These were selected to provide a broad distribution of lagoons in the bay and were included in response to anecdotal reports of large smacks of jellyfish present in these areas. Our 2013 results supported the possibility that these lagoonal communities were serving as sources for sea nettle ephyrae and recruitment habitat for larvae.

Lagoon Collections: Ephyra

We were able to detect a significant signal for ephyra 16S rDNA in a number of lagoonal sites (Fig. 33). Similar to what we measured in 2013, a comparison with quantitative signals seen in our bay-wide samples, we measured signals in some lagoons that were twice as high, suggesting a higher number of ephyra in some lagoons than found in the bay in 2014. We detected the highest qPCR signals at Harvey Cedars, Beach Haven West, Cedar Creek, Toms River, and Kettle Creek Lagoons. Although most of the sites peaked in mid-July (Toms River, Beach Haven West, and Cedar Creek), some peaked in June (Kettle Creek), and others in August (Harvey Cedars, Tuckerton). The turnover of water in lagoons is likely poor, and this again suggests that polyps are resident in these locations. Furthermore, these polyps may not only be the source of ephyra in these lagoons, but may also be the source of ephyra found in the southern region of the bay as well.

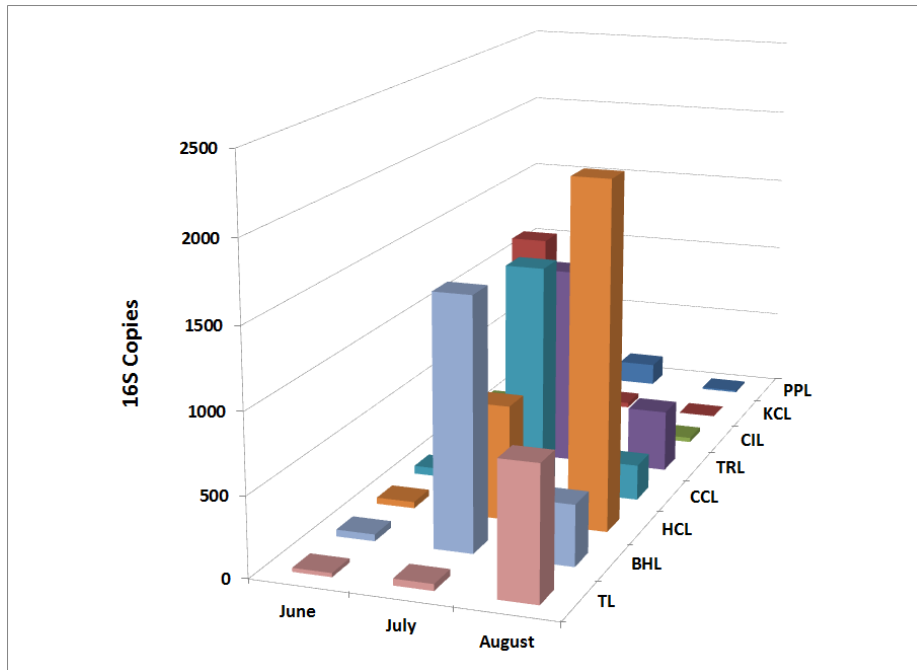


Figure 33. Distribution of ephyral DNA detected using qPCR for *Chrysaora* from water samples collected during lagoon sampling events.

Lagoon Collections: Larvae

In contrast to what was detected for ephyrae distribution in lagoons, we detected strong signals for planula larvae in only two locations, Harvey Cedars and Cedar Creek lagoons (Fig. 34). Similar to what we detected for ephyra DNA in these lagoons, these signals for planula DNA were roughly twice as high than those found in the bay-wide collections. Although some of these sites peaked in June and July, the two largest signals peaked in August (Harvey Cedars and Cedar Creek lagoon). However, the copy number of 16S rDNA was less in 2014 (and 2013) compared to pre-Sandy values of 2012 possibly due to lower numbers of adults detected in the southern portion of the bay.

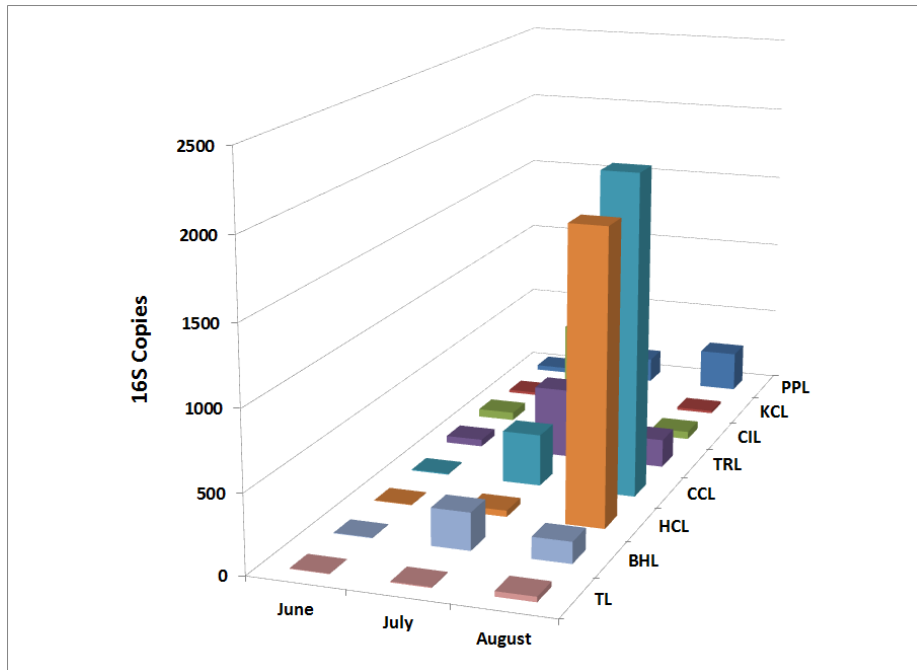


Figure 34. Distribution of larval DNA detected using qPCR for *Chrysaora* from water samples collected during lagoon sampling events.

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

Lift Net Results

Results from the bay-wide samples indicate temporal differences in the density of both *Chrysaora* and *Mnemiopsis* throughout the bay (Fig. 35). *Mnemiopsis* densities in the southern portion of the bay have continued to decrease from previous years (Fig. 35). Lift net results from lagoon samples continued to show a similar disjoint distribution between the species (Fig. 36), but *Mnemiopsis* densities were much lower than those in 2013. *Chrysaora* densities showed similar spatial and temporal patterns to the previous year (Fig. 35).

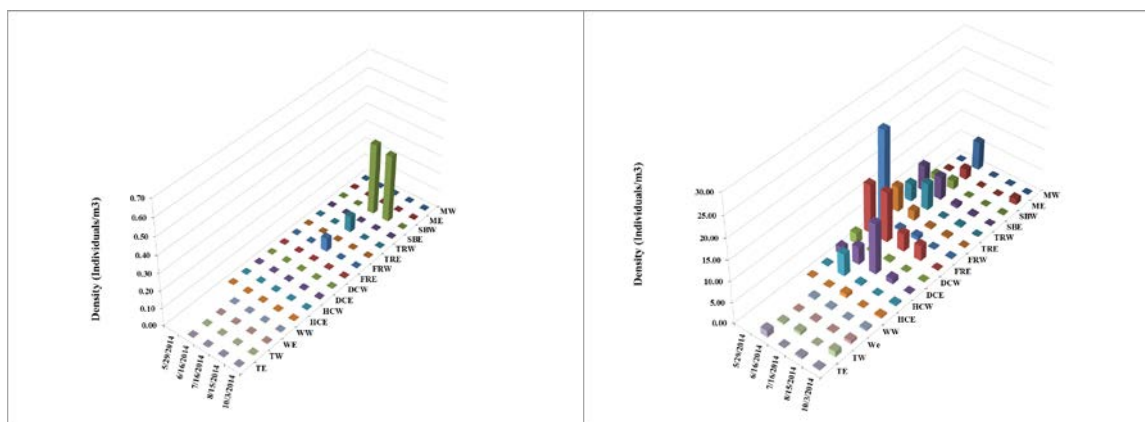


Figure 35. Distribution of *Chrysaora* (left panel) and *Mnemiopsis* (right panel) collected during bay-wide sampling events.

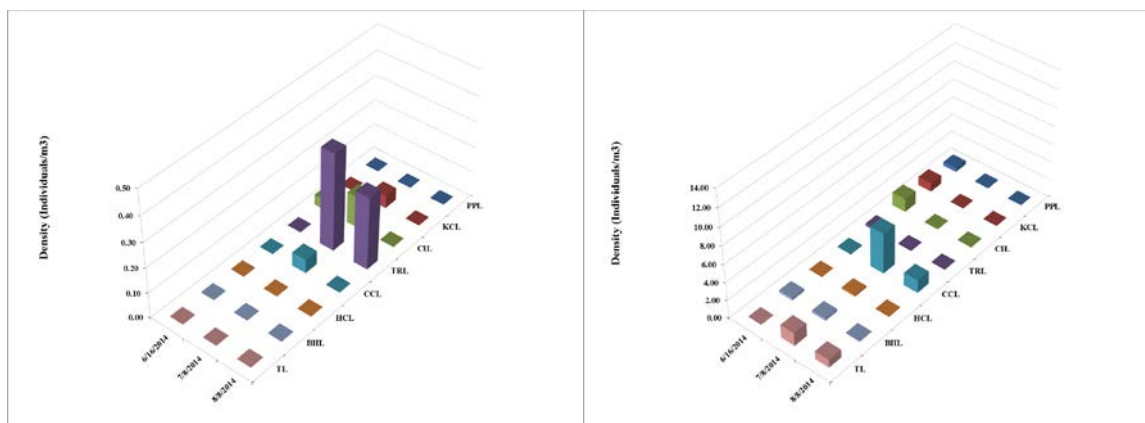


Figure 36. Distribution of *Chrysaora* (left panel) and *Mnemiopsis* (right panel) collected during lagoon sampling events.

Plankton Tows

Dominant Gelatinous Zooplankton Bay-Wide Samples

Bay-wide sampling events showed lower densities of *Chrysaora* in the northern portion of the bay that remained temporally disjointed from *Mnemiopsis*, though *Chrysaora* were found near Barnegat Inlet (Fig. 37). Similar to lift net findings, *Mnemiopsis* densities in the southern bay have decreased from previous years (Fig. 37). Lagoon sampling events showed similar abundances of *Chrysaora* in the northern sites to those found in the previous year, while *Mnemiopsis* had lower densities than seen in 2013 across all sites (Fig. 38). The density and distribution of both species are consistent with the results from the lift nets in the bay-wide surveys.

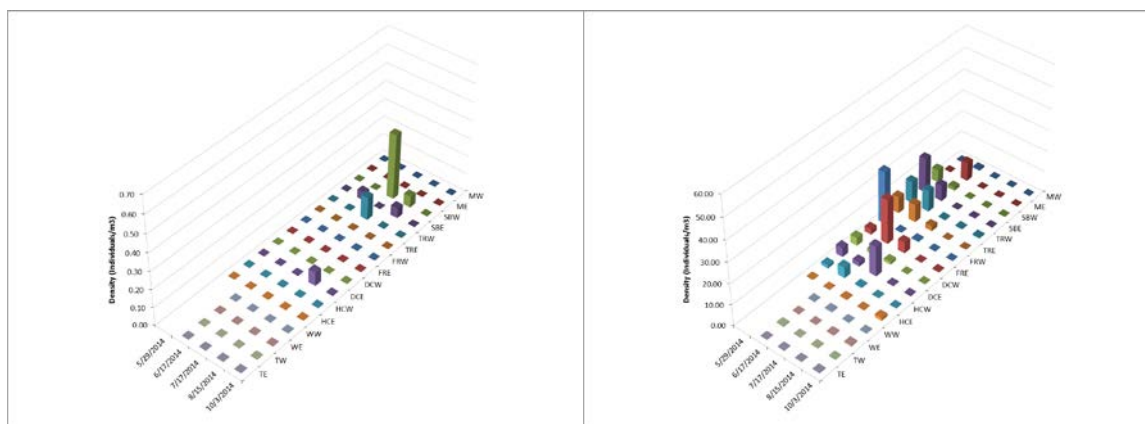


Figure 37. Distribution of *Chrysaora* (left panel) and *Mnemiopsis* (right panel) collected during bay-wide sampling events with plankton nets.

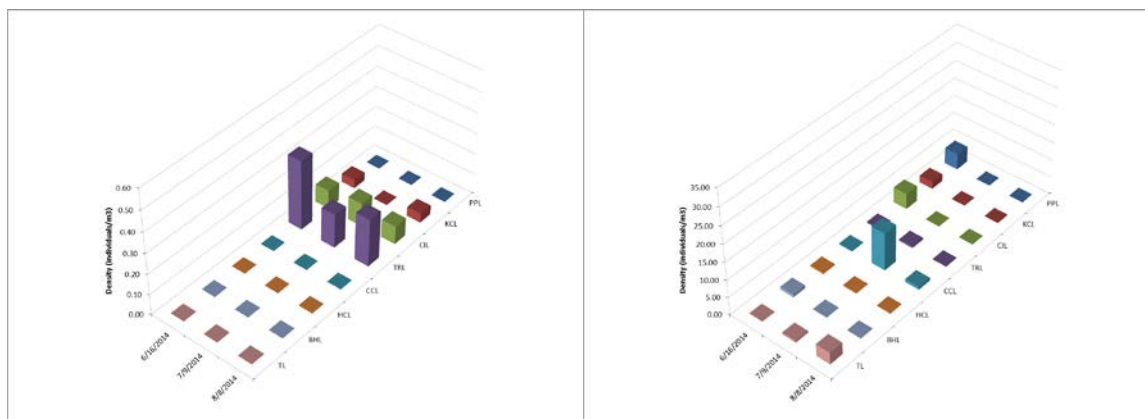


Figure 38. Distribution of *Chrysaora* (left panel) and *Mnemiopsis* (right panel) collected during lagoon sampling events with plankton nets.

Planktonic Community Structure Assessment: Bay-Wide Samples

Results from the SIMPER analysis indicate average similarities ranging from 42% to 82% with between four and six taxa contributing to >90% of the group similarity (Table 30), while dissimilarities between sites ranged between 31-69%. In particular, five taxa were important in structuring most of the planktonic communities observed in Barnegat Bay and include: *M. leidy*, Fish Eggs, Calanoida, Caridea, and Brachyura (Table 30). The two-way ANOSIM indicated a global R of 0.463 ($P < 0.001$) for differences among sites and a Global R of 0.512 ($P < 0.001$) for differences among dates. In all 120 pairwise comparisons among the 16 sites, only 7 showed no significant differences in community structure. These include: MW vs. TRW, MW vs. FRW, MW vs. DCW, MW vs. WW, MW vs. TE, SBE vs. TRE, and TW vs. TE. The non-significant pairings were dominated by similar fauna and distributions on one side of the bay (i.e., west or east) for both sites, with the exception of the paired east-west sites in the most southern region (TW vs. TE). Pairwise comparisons for all monthly comparisons were significantly different, suggesting generalized temporal communities present in the Bay.

Table 30. Contributing taxa defining the planktonic community associated with plankton tow samples collected in the bay-wide surveys based upon SIMPER Analysis. Similarity Percentages and species contributions provided for each site.

Metedeconk West: Average similarity: 51.85

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.38	19.04	1.61	36.71	36.71
Fish Eggs	1.14	10.32	0.65	19.91	56.63
Caridea	0.74	8.27	1.03	15.95	72.57
Brachyura	0.77	7.49	0.95	14.44	87.01
<i>M. leidy</i>	0.52	4.06	0.59	7.83	94.84

Metedeconk East: Average similarity: 65.37

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.88	24.32	1.85	37.21	37.21
Caridea	1.23	16.54	3.4	25.3	62.5
Brachyura	0.91	9.97	1.06	15.25	77.75
Fish Eggs	0.83	8.24	0.82	12.6	90.35

Silver Bay West: Average similarity: 63.00

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.52	21.11	1.31	33.51	33.51
<i>M. leidy</i>	0.57	10.07	0.7	15.98	49.49
Caridea	0.74	9.46	1.22	15.02	64.52
Fish Eggs	0.42	8.62	0.64	13.68	78.2
Brachyura	0.64	8.41	1.05	13.35	91.55

Silver Bay East: Average similarity: 50.20

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.37	13.59	1.16	27.07	27.07
Fish Eggs	1.19	12.24	1.06	24.38	51.46
<i>M. leidy</i>	0.85	11.25	0.81	22.41	73.86
Caridea	0.58	5.59	1.16	11.14	85
Brachyura	0.59	4	0.55	7.97	92.97

Toms River West: Average similarity: 57.27

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.64	16.19	1.05	28.27	28.27
Fish Eggs	1.1	11.99	1.06	20.94	49.21
<i>M. leidy</i>	0.91	10	0.98	17.45	66.67
Caridea	0.64	8.29	0.99	14.47	81.14
Brachyura	0.54	3.8	0.57	6.64	87.78
<i>C. quinquecirrha</i>	0.21	2.32	0.53	4.05	91.83

Toms River East: Average similarity: 60.03

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.73	24.54	2.1	40.88	40.88
Fish Eggs	1.34	11.69	1.16	19.48	60.36
<i>M. leidy</i>	1.05	11.31	0.97	18.83	79.19
Caridea	0.86	6.3	1.09	10.49	89.68
Brachyura	0.66	3.09	0.62	5.14	94.82

Forked River West: Average similarity: 65.63

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.37	23.72	2.37	36.14	36.14
Caridea	0.94	14.72	1.93	22.43	58.58
Brachyura	0.73	9.82	1.28	14.96	73.54
<i>M. leidy</i>	0.8	9.63	0.76	14.67	88.21
Fish Eggs	0.61	6.22	0.98	9.48	97.69

Forked River East: Average similarity: 69.30

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.63	20.13	2.03	29.05	29.05
Caridea	1.32	14.02	1.62	20.23	49.28
Brachyura	1.17	11.46	1.2	16.53	65.81
Fish Eggs	1.16	10.44	1.21	15.06	80.88
<i>M. leidy</i>	0.94	8.3	0.85	11.97	92.85

Double Creek West: Average similarity: 67.51

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.78	19.62	1.37	29.06	29.06
Caridea	1.08	13.1	1.98	19.4	48.46
Brachyura	1.08	10.8	1.21	16	64.46
<i>M. leidy</i>	0.87	9.35	1.3	13.85	78.31
Fish Eggs	0.88	8.43	1.25	12.49	90.8

Double Creek East: Average similarity: 69.97

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.83	20.95	2.36	29.95	29.95
Caridea	1.35	14.24	3.25	20.35	50.29
<i>M. leidy</i>	0.92	9.1	1.03	13.01	63.3
Brachyura	0.97	7.83	1.07	11.19	74.49
Fish Eggs	0.85	7.76	1.27	11.1	85.59
<i>Rathkea</i>	0.31	3.35	0.48	4.79	90.37

Harvey Cedars West: Average similarity: 60.52

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	2.43	19.37	1.83	32	32
Brachyura	1.47	12.67	1.32	20.93	52.92
Caridea	0.95	7.69	1.49	12.71	65.64
<i>M. leidy</i>	0.76	6.3	1.02	10.41	76.05
Fish Eggs	0.54	3.48	0.56	5.75	81.81
Fish Larvae	0.53	3.27	0.72	5.4	87.2

Aoridae	0.2	1.1	0.37	1.82	89.02
Gammaridae	0.17	1.09	0.48	1.81	90.82

Harvey Cedars East: Average similarity: 66.53

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.72	17.65	3.08	26.53	26.53
Caridea	1.04	11.64	4.59	17.5	44.03
Brachyura	1.33	10.79	1.14	16.22	60.25
Fish Eggs	0.97	9.04	1.1	13.59	73.83
Fish Larvae	0.65	5.72	0.89	8.59	82.43
Melitidae	0.58	4.7	0.8	7.07	89.5
<i>M. leidy</i>	0.38	2.62	0.66	3.94	93.43

Westeconk West: Average similarity: 64.41

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.9	16.71	1.48	25.94	25.94
Brachyura	1.43	13.53	1.49	21	46.94
Fish Eggs	1.15	11.29	1.38	17.52	64.46
Caridea	1.16	10.75	1.92	16.69	81.15
<i>M. leidy</i>	0.59	5.88	0.76	9.14	90.29

Westeconk East: Average similarity: 64.23

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	2.04	18.72	1.4	29.15	29.15
Callanoida spp.	1.51	15.52	1.17	24.16	53.31
Caridea	1.43	13.84	1.96	21.54	74.85
Fish Eggs	1.09	9.08	1.08	14.14	88.99
Fish Larvae	0.28	1.82	0.55	2.83	91.82

Tuckerton West: Average similarity: 68.82

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.74	20.12	2.45	29.24	29.24
Brachyura	1.48	15.87	1.15	23.06	52.3
Caridea	1.39	15.66	3.9	22.75	75.05
Fish Eggs	0.99	8.49	0.83	12.33	87.39
<i>M. leidy</i>	0.68	7.47	1.43	10.85	98.24

Tuckerton East: Average similarity: 76.51

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.73	21.81	2.33	28.51	28.51
Brachyura	1.72	18.3	1.26	23.92	52.42

Caridea	1.39	17.71	4.16	23.15	75.58
<i>M. leidy</i>	0.47	8.11	0.92	10.61	86.18
Fish Eggs	0.81	7.11	1.06	9.3	95.48

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. 39) and months of collection (Fig. 40). 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. However, strong similarities exist within communities collected during the same monthly sampling (Fig. 40).

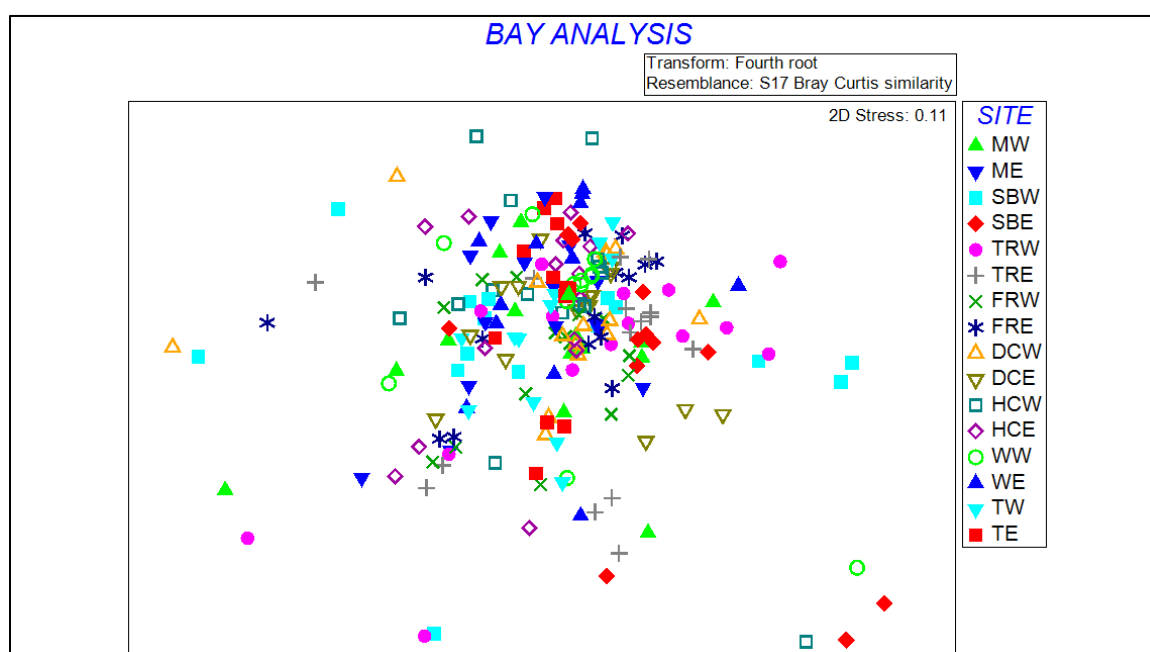


Figure 39. MDS plot of planktonic community structure from bay-wide samples plotted based upon the sites of collection. Clustering of samples indicates similarity of community structure.

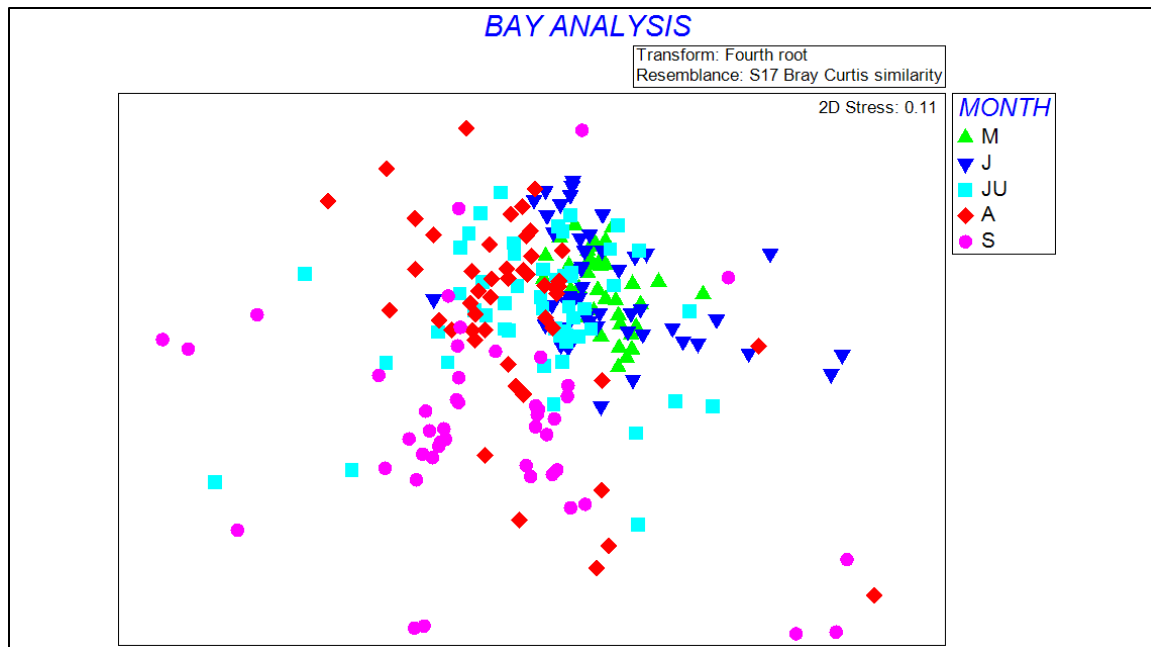


Figure 40. MDS plot of planktonic community structure from bay-wide samples plotted based upon the months of collection. Clustering of samples indicates similarity of community structure.

Lagoon Sampling

Planktonic Community Structure Assessment: Bay-Wide Samples

Results from the SIMPER analysis indicate average similarities ranging from 61% to 68% with between four and six taxa contributing to >90% of the group similarity (Table 31), while dissimilarities between sites ranged between 40-74%. In particular, five taxa were important in structuring most of the planktonic communities observed in Barnegat Bay and include: *M. leidy*, Fish Eggs, Calanoida, Caridea, and Brachyura (Table 31). Two sites (Toms River and Chadwick Island) had substantial representation of *C. quinquecirrha* in their communities which were contributing members of the dominant taxa. However, no other sites showed a strong presence in driving community structure. The two-way ANOSIM indicated a global R of 0.621 ($P < 0.001$) for differences among sites and a Global R of 0.648 ($P < 0.001$) for differences among dates. In all pairwise comparisons among the 8 sites, only one showed no significant difference in community structure (Table 32) and these sites are roughly straight across from each other (KCL vs. CIL), while all monthly comparisons were significantly different from each other showing strong seasonal signals in community structure.

Table 31. 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: 65.12

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.27	23.49	1.55	36.07	36.07

Brachyura	0.6	14.09	0.98	21.64	57.71
Caridea	0.48	7.65	0.73	11.75	69.46
Gelatinous Ephyra	0.45	7.2	0.77	11.06	80.51
Fish Eggs	0.52	6.43	0.83	9.88	90.39

Kettle Creek Lagoon: Average similarity: 64.90

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	1.2	21.81	1.52	33.6	33.6
Callanoida spp.	1.02	13.57	0.84	20.91	54.51
Gelatinous Ephyra	0.98	13.29	1.22	20.47	74.98
<i>M. leidy</i>	0.4	7.83	0.62	12.06	87.05
Fish Eggs	0.53	4.64	0.67	7.16	94.21

Chadwick Island Lagoon: Average similarity: 61.23

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	0.78	13	1.26	21.24	21.24
Gelatinous Ephyra	0.82	11.49	1.32	18.76	40
<i>M. leidy</i>	0.61	8.35	0.66	13.63	53.63
Gastropoda spp. Larvae	0.69	8.03	0.66	13.11	66.74
Callanoida spp.	0.78	7.82	0.84	12.77	79.52
Caridea	0.48	5.27	0.81	8.61	88.13
<i>C. quinquecirrha</i>	0.41	4.51	0.6	7.36	95.49

Toms River Lagoon: Average similarity: 62.35

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	1.48	17.27	1.54	27.7	27.7
Brachyura	1.4	13.57	1.27	21.77	49.47
<i>C. quinquecirrha</i>	0.49	11.04	0.72	17.7	67.17
Gelatinous Ephyra	0.71	7.72	0.79	12.39	79.56
Fish Eggs	0.46	4.07	0.66	6.53	86.08
Caridea	0.55	3.79	0.68	6.08	92.16

Cedar Creek Lagoon: Average similarity: 68.94

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	0.91	15.84	1.76	22.97	22.97
<i>M. leidy</i>	1.05	15.8	1.02	22.91	45.89
Fish Eggs	0.46	11.67	0.78	16.94	62.82
Callanoida spp.	0.94	10.37	1.05	15.05	77.87
Gastropoda spp.	0.64	9.48	0.8	13.75	91.63

Larvae	
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Harvey Cedars Lagoon: Average similarity: 65.88

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Callanoida spp.	2.02	19.52	3.2	29.63	29.63
Fish Eggs	1.46	16.21	1.21	24.61	54.24
Brachyura	1.5	15.43	3.44	23.42	77.66
Caridea	0.83	5.24	1.04	7.96	85.62
<i>M. leidy</i>	0.39	3.44	0.84	5.23	90.84

Beach Haven Lagoon: Average similarity: 64.25

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	1.21	23.72	3.09	36.91	36.91
Callanoida spp.	1.44	21.42	1.07	33.33	70.24
<i>M. leidy</i>	0.52	9.64	1.12	15.01	85.24
Fish Eggs	0.54	4.28	0.66	6.66	91.91

Tuckerton Creek Lagoon: Average similarity: 63.50

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>M. leidy</i>	0.8	19.06	0.91	30.01	30.01
Brachyura	1.13	15.82	1.61	24.91	54.92
Callanoida spp.	1.25	13.96	1.21	21.99	76.91
Caridea	0.89	10.32	1.27	16.25	93.16

Table 32. Results from the ANOSIM statistical test for Pair wise comparisons between lagoon sites for plankton community structure. Bolded values indicate no significant differences.

Pairwise Tests	R	Significance
Groups	Statistic	Level %
PPL, KCL	0.444	1.2
PPL, CIL	0.469	1.7
PPL, TRL	0.691	0.1
PPL, CCL	0.612	0.4
PPL, HCL	0.691	0.3
PPL, BHL	0.642	0.6
PPL, TL	0.753	0.1
KCL, CIL	0.16	20.8
KCL, TRL	0.543	0.4
KCL, CCL	0.751	0.5
KCL, HCL	0.815	0.2
KCL, BHL	0.63	0.8

KCL, TL	0.84	0.3
CIL, TRL	0.469	0.7
CIL, CCL	0.758	0.9
CIL, HCL	0.778	0.2
CIL, BHL	0.642	0.4
CIL, TL	0.827	0.1
TRL, CCL	0.772	0.4
TRL, HCL	0.864	0.1
TRL, BHL	0.642	0.3
TRL, TL	0.778	0.3
CCL, HCL	0.841	0.1
CCL, BHL	0.792	0.3
CCL, TL	0.314	5
HCL, BHL	0.407	1
HCL, TL	0.63	0.2
BHL, TL	0.593	0.2

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. 41) and months of collection (Fig. 42). While no broad patterns of similarity are present among sites, there is strong monthly similarity for all sites. This may reflect the presence of different larval taxa which occur during defined time periods within Barnegat Bay, regardless of region (Fig. 42).

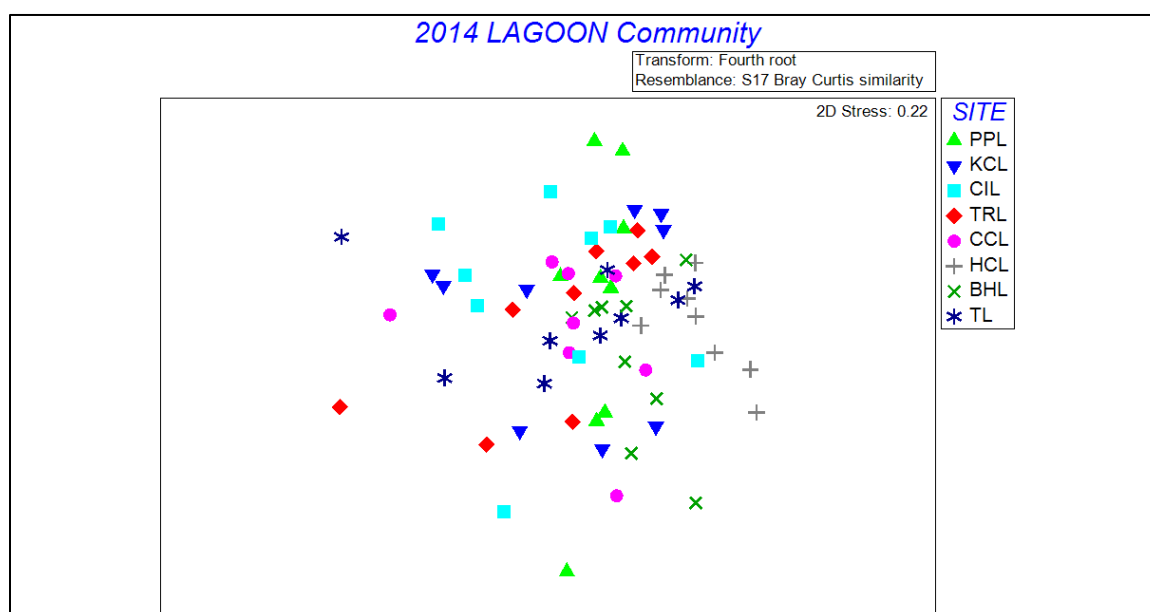


Figure 41. MDS plot of planktonic community structure from lagoon samples plotted based upon the sites of collection. Clustering of samples indicates similarity of community structure.

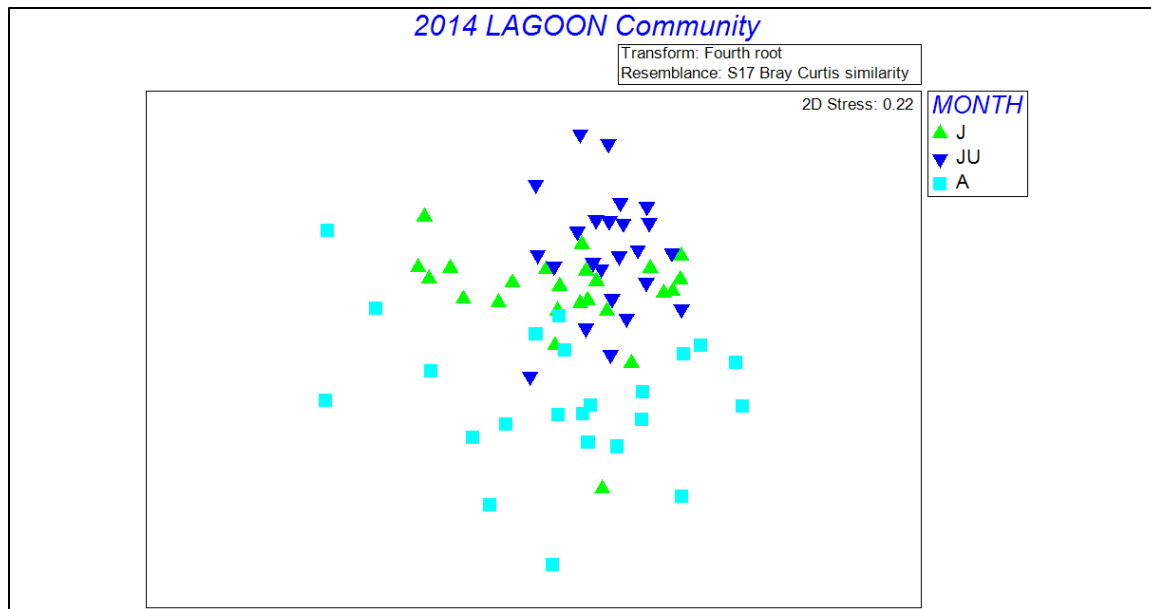


Figure 42. MDS plot of planktonic community structure from lagoon samples plotted based upon the months of collection. Clustering of samples indicates similarity of community structure.

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

We sampled 10 locations within Barnegat Bay to assess the distribution of settling polyps throughout the summer (Fig. 43). Results indicate five sites which showed the recruitment of polyps and include regions ranging from Kettle Creek in the north to Beach Haven West in the southern portion of the Bay (Fig. 44). This suggests reproduction and settlement is occurring throughout the bay. Temporal trends indicate peaks in recruitment during the end of July and early August, which corresponds with peaks in adults in July (Fig. 44).

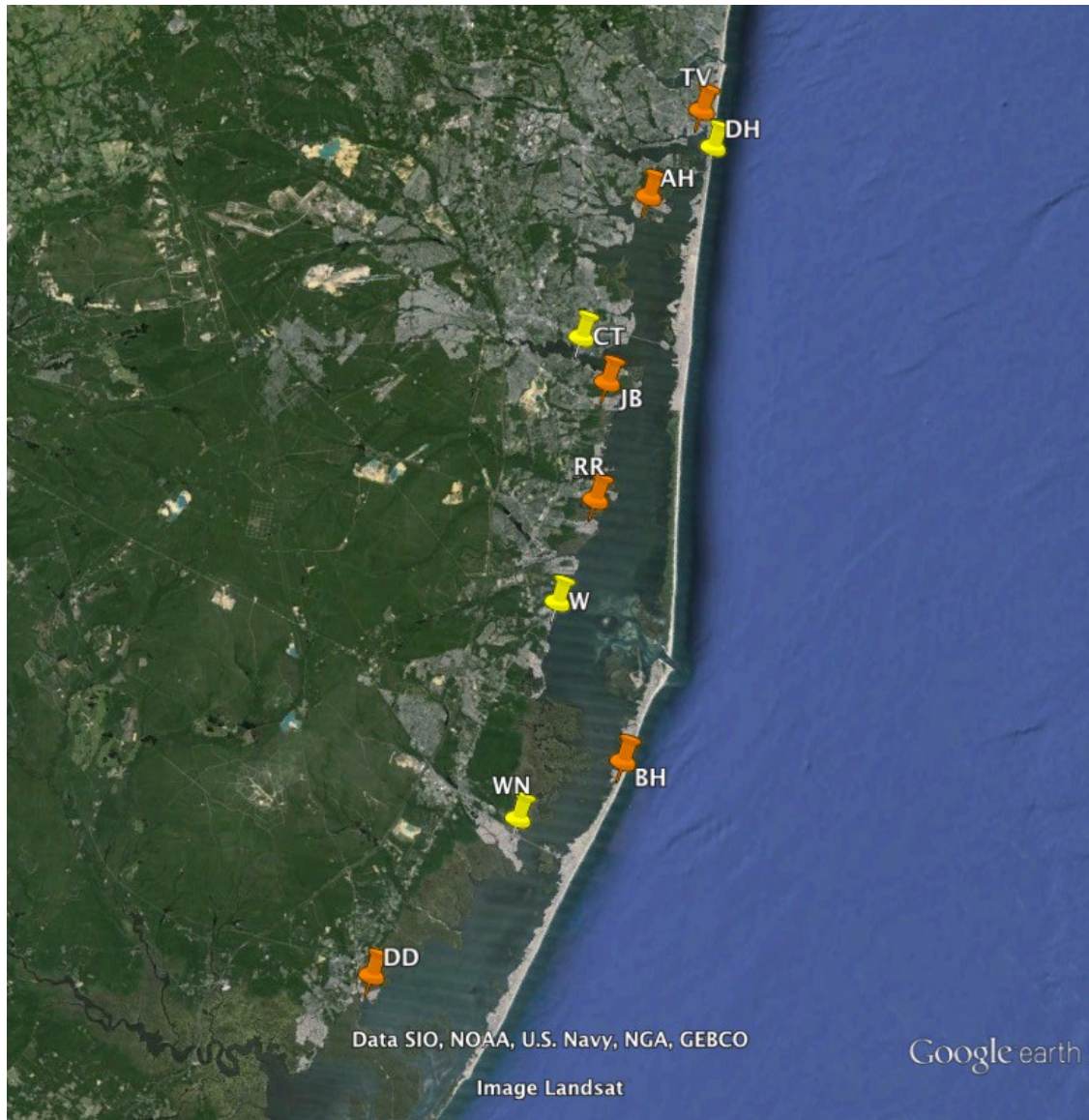





Figure 43. Settling Plate locations in Barnegat Bay, NJ for 2014

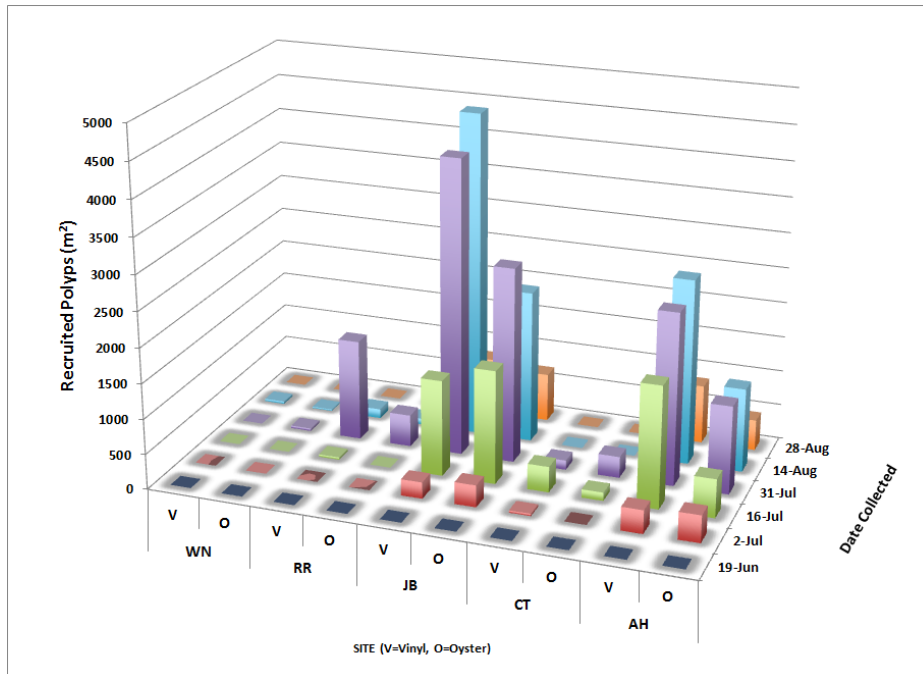


Figure 44. Average polyp settlement at the five lagoon sites which showed the presence of settling individuals in 2014. Axis indicates whether settling material was comprised of vinyl bulkhead material (V) or natural oyster shell (O).

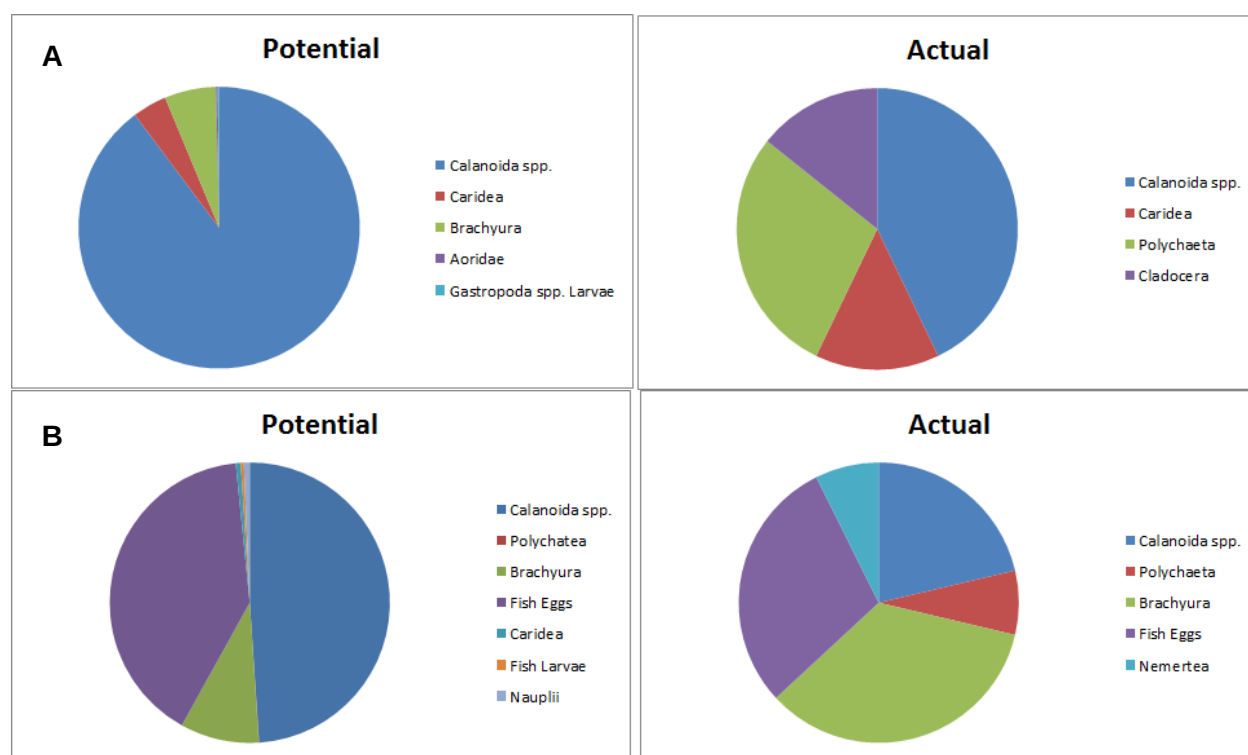
Assess the diet of *Chrysaora* through dissection and molecular analysis.

We completed the dissection of *Chrysaora* digestion tracks from 65 individuals and have identified several groups of taxa. From the dissections, 18 groups were identified (Table. 33), but some groups overlap (e.g., crab larvae and crab megalope; fish eggs and larvae). Prey items include pelagic/planktonic organisms (e.g., Callanoid copepods), but also include numerous benthic species such as large polychaetes, benthic gobies, and sea anemones. This clearly indicates that *C. quinquecirrha* is actively targeting benthic prey. When we compare the prey items where we have collected numerous individuals in a sampling trip with those identified from the equivalent plankton tows, we have identified some similarities, but also strong selection against what is merely abundant. Specifically, when we compared the average diet contents with the available prey items from the plankton samples, we saw similar results to 2013, where *C. quinquecirrha* showed a pattern of differential selection of prey items versus available prey (Fig. 45).

Table 33. Identified prey items from dissected *C. quinquecirrha* collected in 2014. Values in table represent the average abundance of individual prey items identified. N indicates the sample size upon which the averages are based.

Site	CIL	DCW	SBW	SBE	FRL	SBE	TRL	CCL	FRL	SBE
Date	7/8	7/9	7/17	7/22	7/30	8/7	8/7	8/7	8/8	8/14
N	1	12	9	7	7	5	2	1	3	18
Prey										

Calanoida	17.00	0.00	1.00	1.86	3.00	6.80	1.00	6.00	0.00	3.28
Harpactechoida	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00
Cladocera	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caridea	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brachyura	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.06
Brachyura Megalopa	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00
Polychaeta	1.00	0.00	0.67	0.43	0.00	0.2	0.00	0.00	0.00	1.67
Platyhelminthes	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nemertea	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.00	0.00	0.17
Lilijboridae	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00
Melitidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Fish Eggs	0.00	0.00	0.00	1.71	0.00	0.00	0.00	0.00	0.00	0.67
Fish Larvae	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gobiidae	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00
Argulidae	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00
Anemone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00
Bryozoa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Egg Sack	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06



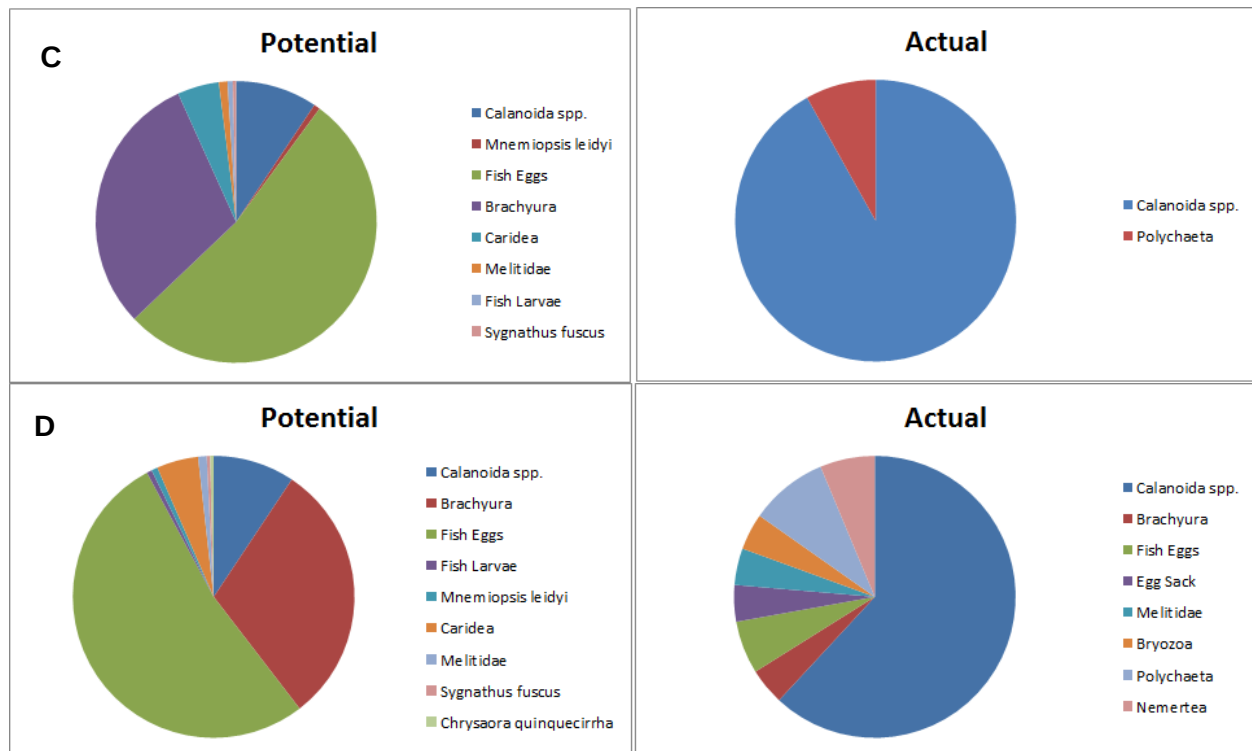


Figure 45. Comparison of diet selection of *C. quinquecirrha* from available prey resources identified through plankton tows (left panel) from similar sites and dates with that actually identified in dissections (right panel). A. Silver Bay collected July 17, 2014; B. Silver Bay collected July 22, 2014; C. Silver Bay collected August 7, 2014; D. Silver Bay collected August 14, 2014.

Molecular Diet Analysis: Gut content Taxon Identification

Similarly to 2013, we record the identity of prey items to lowest possible taxonomic unit. Blastn XML results were visually inspected in MEGAN 5.10.3 (Huson et al. 2011) with 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).

Species level identification is indicated for contigs with >98% sequence similarity for genes that are well represented in the NCBI database. If the gene regions identified were only present in a limited number of taxa they 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 for mitochondrial genes, 28S, 16S, 5.8S, and 18S identified 4897 contigs that were assigned in MEGAN. Similar to 2013, tetrapod, bacteria, and virus assignments were not looked at. Manual inspection of the remaining sequences identified 44 contig sequences, which were confidently assigned 19 taxa (Table 34). Of these taxa, we were able to identify 6 to the species level (Table 34), but as greater DNA data are generated in the databases, greater resolution will be possible in the future.

Table 34. *C. quinquecirrha* prey items identified in the combined gut lavage and tentacle pick build.

Final Classification	Common Name	Gene(s)
Goniadidae	Polychaete worm	18S
<i>Alitta (=Nereis) succinea</i>	Polychaete worm	18S; 28S
Phyllodocidae	Polychaete worm	28S
<i>Acartia tonsa</i>	Copepod	5.8S
<i>Americamysis bahia</i>	opossum shrimp	18S
<i>Gammarus</i>	Amphipod	18S
<i>Palaemonetes pugio</i>	Daggerblade grass shrimp	28S
<i>Stylochus</i>	Flatworm	5.8S; 28S
Holothuroidea	Sea cucumber	5.8S
Apodida	Holothurian	18S
Styelidae	Sea squirt	5.8S
<i>Botryllus schlosseri</i>	Star ascidian	18S
Nynantheae	Anemone	18S; Mitochondrial genes
Hydrozoa	Hydrozoan	28S
<i>Cerebratulus lacteus</i>	Nemertean worm	18S; 28S
Scaphopoda	Scaphopod	28S
<i>Cuthona</i>	Nudibranch	18S
Eubranchidae	Sea slug	18S
Gobiidae	Goby	Mitochondrial genes

Results: Project Summary and Synthesis 2012-2014

During the course of the research project, substantial changes occurred in Barnegat Bay. Specifically, Hurricane Sandy impacted coastal New Jersey in October 2012 and substantially changed the physical structure of the bay through island breaches, storm surge, rainfall, and collateral damage of property and habitats. Consequently, when addressing the trends observed in data from 2012 and comparing them to 2013 and 2014, this provides insight into the impacts that Sandy had on Barnegat Bay, its' water quality, and its' community structure.

Water Quality

Assessment of water quality revealed some unexpected results. When we averaged salinity, temperature, and dissolved oxygen readings for each year there are no differences among years for salinity (Fig. 46). However, after Sandy both temperature and dissolved oxygen showed very different patterns. Specifically, average temperature significantly declined each year ($F_{2,273}=17.1$, $P < 0.0001$) from an average maximum of 24.7C in 2012 to a minimum of 22.1C in 2014 (Fig. 47). Similarly, and most likely related to the lowered temperatures, dissolved oxygen was significantly greater ($F_{2,273}=27.7$, $P < 0.0001$) in both 2013 and 2014 compared to 2012 (Fig. 48). Given that overall salinity in the bay did not differ among years, but both temperature and dissolved oxygen did, it is probable that Hurricane Sandy had an impact on water quality in the bay, possibly through elevated tidal activity from the coastal ocean. This possibility would support our findings regarding the distribution and abundance of Gelatinous Zooplankton observed during the whole project (see below).

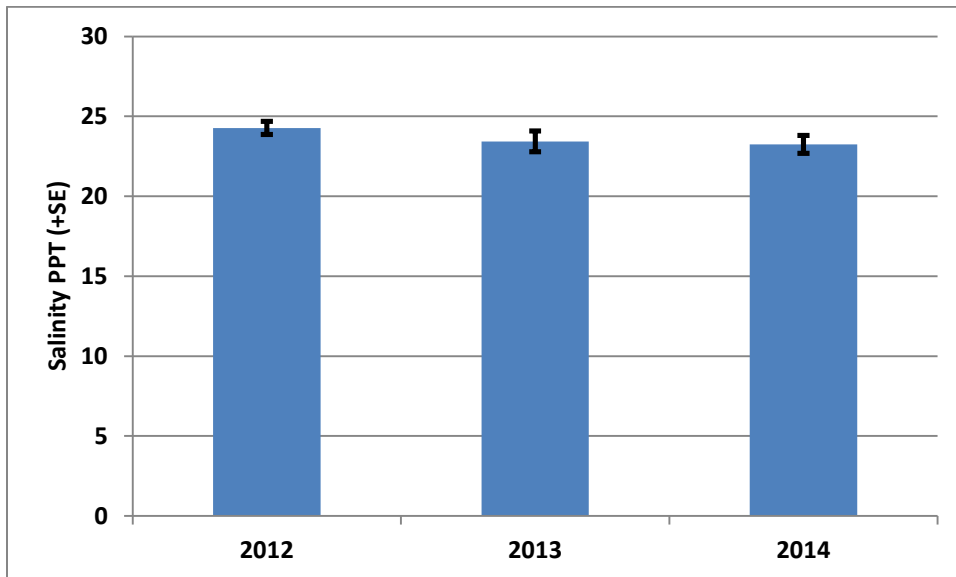


Figure 46. Yearly averaged salinity from the combined 16 sampling stations in Barnegat Bay.

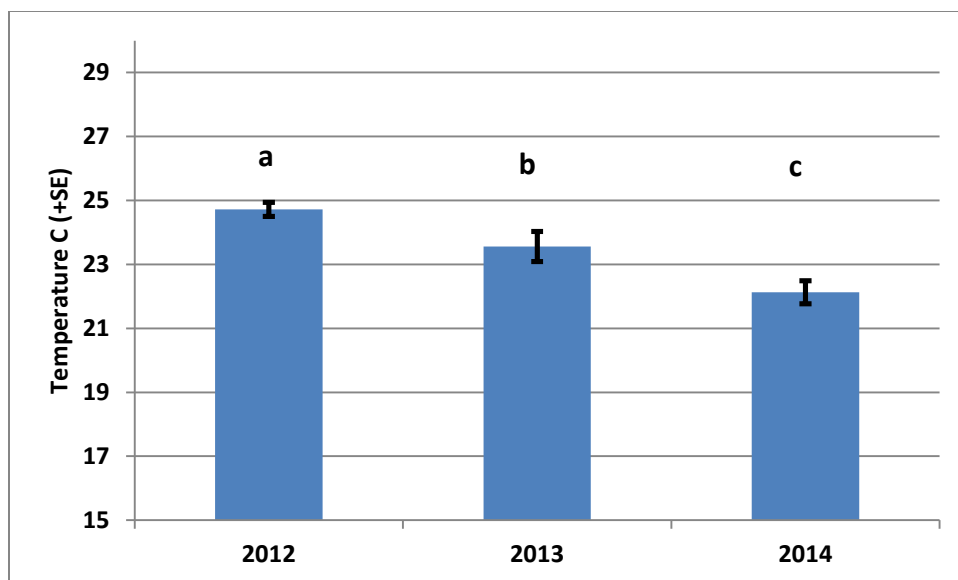


Figure 47. Yearly averaged temperature from the combined 16 sampling stations in Barnegat Bay. Differing letters above mean indicate significant differences ($P < 0.05$).

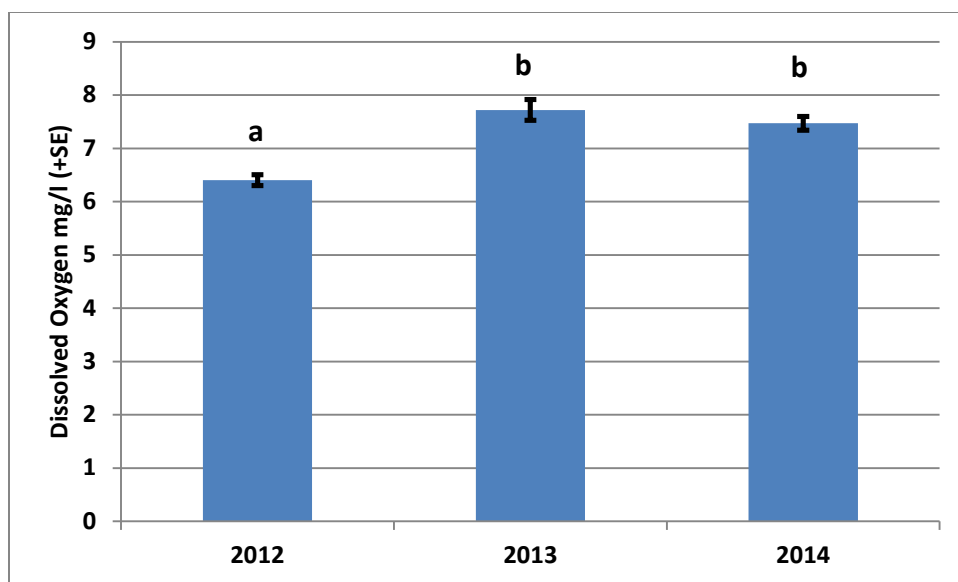


Figure 48. Yearly averaged dissolved oxygen concentration from the combined 16 sampling stations in Barnegat Bay. Differing letters above mean indicate significant differences ($P < 0.05$).

Gelatinous Zooplankton Distributions

Lift Net Summary of Gelatinous Zooplankton

During our investigation, results from the distribution and abundance of *C. quinquecirrha* and *M. leidy* changed dramatically among years. For *C. quinquecirrha*, density was substantially higher in 2012, and greatly reduced in 2013 and 2014 (Fig. 49). However, these differences were

not significant. Densities were similar between 2013 and 2014, but it appears that the physical destruction of polyp habitat associated with floating docks and other structures from Hurricane Sandy may have limited the podocyst populations causing a temporary decline in sea nettle populations. Continued monitoring of this life stage is essential for evaluating future jellyfish blooms. One feature of the decline in sea nettle populations in 2013 is the dramatic increase in *M. leidyi* densities during that year (Fig. 50). However, in 2014, *M. leidyi* densities significantly declined, but were no different than densities in 2012 ($F_{2,278} = 3.52$, $P < 0.031$). It is probable that the rise in *M. leidyi* density in 2013 was a result of predation pressure release from the decreased sea nettle densities, but the significant decline in 2014 can't be explained directly by this mechanism. Rather, it appears that the declines in 2014 are more likely related to some other change in the system, potentially related to water quality (i.e., significant reduction in temperature) or other system changes. One possibility relates to changes in other gelatinous zooplankton which also occurred in 2013 and 2014.

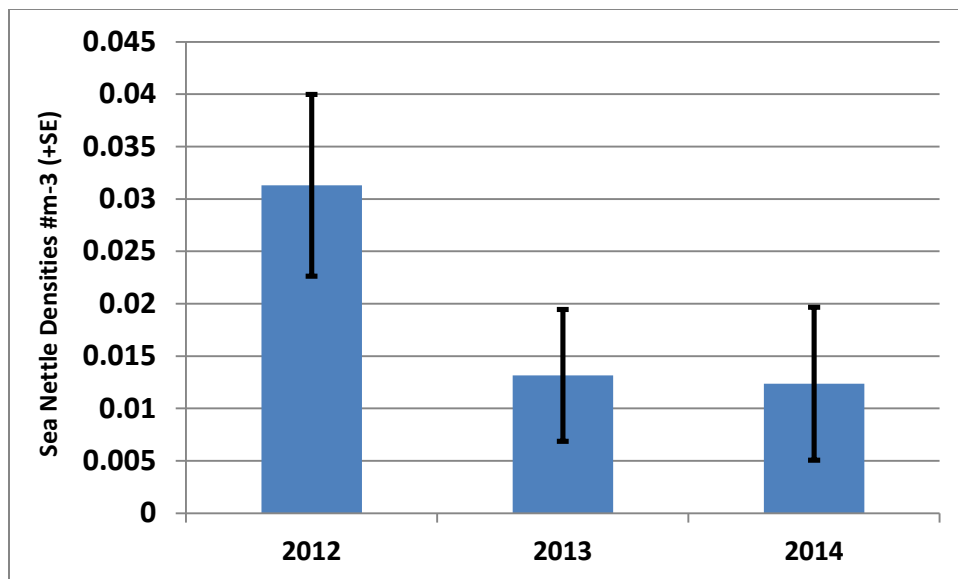


Figure 49. Yearly averaged *C. quinquecirrha* densities from the combined 16 sampling stations in Barnegat Bay collected from lift nets. Differing letters above mean indicate significant differences ($P < 0.05$).

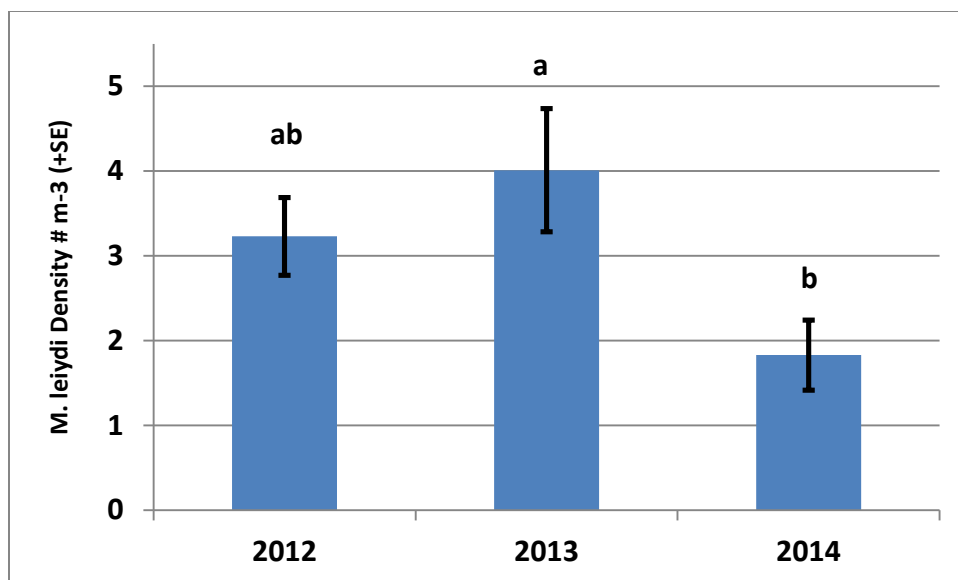


Figure 50. Yearly averaged *M. leidy* densities from the combined 16 sampling stations in Barnegat Bay collected from lift nets. Differing letters above mean indicate significant differences ($P < 0.05$).

Plankton Tow Summary of Gelatinous Zooplankton

Results from the plankton net samples show similar trends in abundance for both *C. quinquecirrha* and *M. leidy*. Comparably, no difference was observed in *C. quinquecirrha* densities among years (Fig. 51), but the lowest density was recorded in 2014. For *M. leidy*, we observed a significant increase in 2013 ($F_{2,810} = 14.18$, $P < 0.0001$) and then a dramatic decline in 2014 to a similar density to 2012 (Fig. 52). This response is mostly likely due to predation release in 2013. The change in 2014 may relate to the relative distribution of other gelatinous species which also showed increases in 2013 and 2014 (Fig. 53). One outstanding result from 2013 was the massive and significant increase in *Salpa*, which is a coastal/open ocean species rarely encountered in sheltered lagoon systems ($F_{2,811} = 10.6$, $P < 0.0001$). This high abundance indicates greater offshore fluxes of individuals and species post-Hurricane Sandy. However, this was not the only species which showed significant increases in the years following Sandy. A similar pattern was observed with *Pleurobrachia pileus* with a significant increase in density in 2013 ($F_{2,811} = 3.7$, $P < 0.03$), which is also a known prey item for *C. quinquecirrha*. *Turritopsis nutricula*, *Nemopsis bachei*, and *Bougainvillea muscus* both showed significant increases following Sandy ($F = 5.12$, $P < 0.007$; $F = 8.25$, $P < 0.0003$; $F = 3.82$, $P < 0.025$, respectively) with consistent increases following in 2014 (Fig. 53). Some species did show declines following Sandy including *Cladonema* spp., *Clytia* spp., Hydroid medusa, *Aurelia aurita*, and *Aequora* spp., but none were significant.

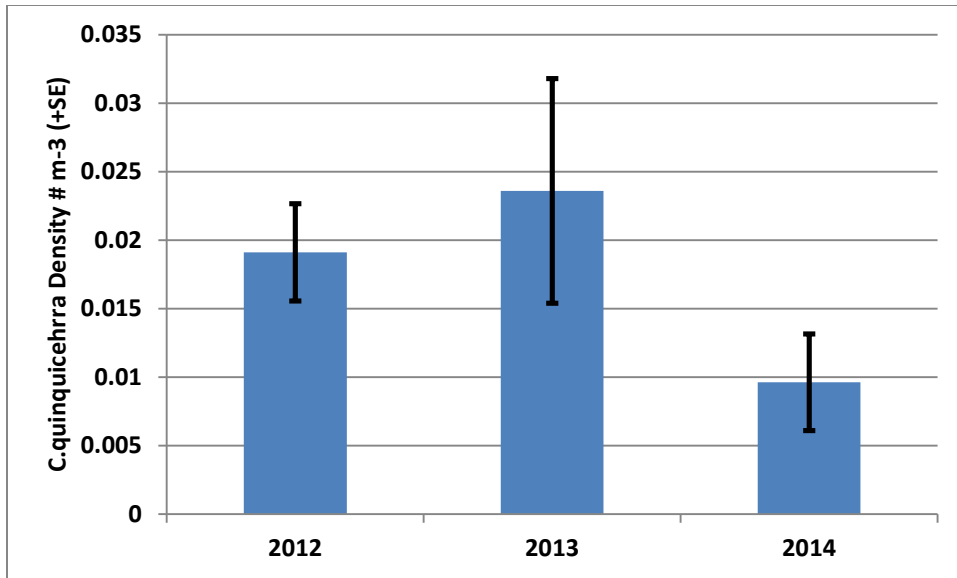


Figure 51. Yearly averaged *C. quinquecirrha* densities from the combined 16 sampling stations in Barnegat Bay collected from plankton nets.

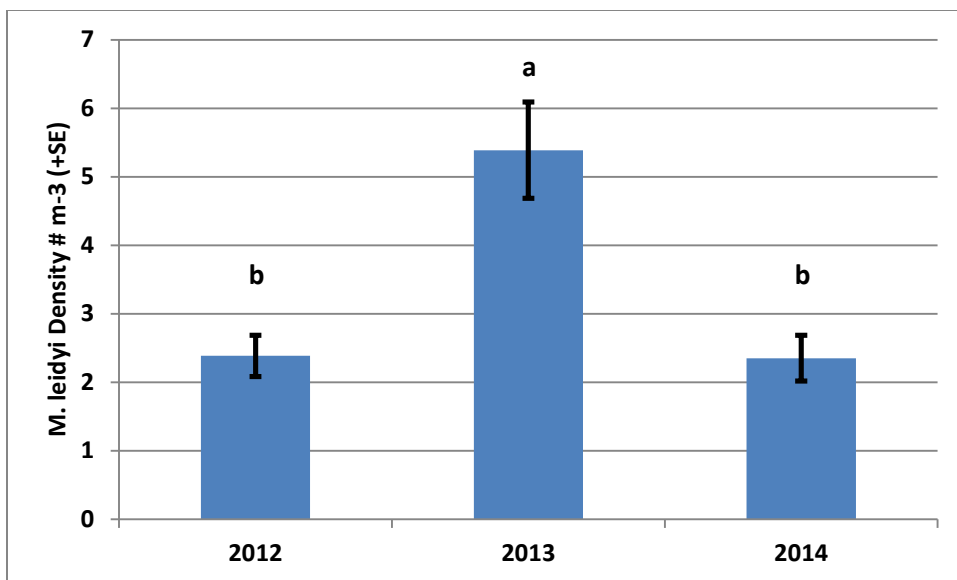


Figure 52. Yearly averaged *M. leidyi* densities from the combined 16 sampling stations in Barnegat Bay collected from plankton nets. Differing letters above mean indicate significant differences ($P < 0.0001$).

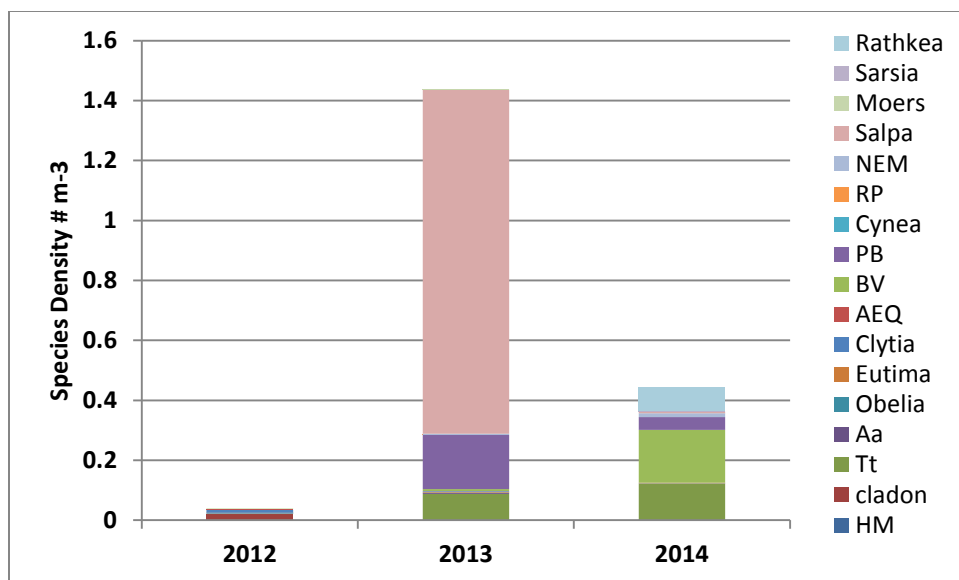


Figure 53. Yearly averaged gelatinous zooplankton densities from the combined 16 sampling stations in Barnegat Bay collected from plankton nets.

Community Dynamics

Planktonic Community Structure Assessment: Bay-Wide Samples

Results from the SIMPER analysis indicate average similarities ranging from 44% to 52% with approximately five taxa contributing to >88% of the group similarity (Table 35). In particular, the five taxa that were important in structuring most of the planktonic communities observed in Barnegat Bay include: Calanoida, Caridea, Brachyura, *M. leidy*, and Fish Eggs. When comparing the Dissimilarity among years, average dissimilarity ranged between 52.5 and 56.2% (Table 35). On average, contributions to dissimilarity between years involved 24 different taxa, suggesting that within year taxonomic similarity is common, but communities differ across years. This may reflect the impacts of Hurricane Sandy altering the overall community given the significant differences in density seen among years for *M. leidy*, Caridea, and Calanoida.

Table 35. Contributing taxa defining the planktonic community associated with plankton tow samples collected among years based upon SIMPER Analysis. Similarity Percentages and species contributions provided for each site.

2012: Average Similarity = 44.84%

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brachyura	0.88	9.96	1.32	22.22	22.22
Caridea	0.85	9.88	1.3	22.03	44.25
Calanoida	0.85	9	1.13	20.07	64.31
Fish Eggs	0.65	5.35	0.74	11.93	76.24
<i>M. leidy</i>	0.58	5.22	0.61	11.65	87.89
Fish Larvae	0.27	0.91	0.29	2.03	89.93

Melitidae	0.25	0.72	0.27	1.61	91.54
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2013: Average Similarity 45.77%

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	0.9	9.44	1.1	20.63	20.63
Caridea	0.83	9.41	1.17	20.55	41.18
<i>M. leidy</i>	0.75	9.06	0.85	19.79	60.97
Brachyura	0.77	8.46	1.04	18.48	79.45
Fish Eggs	0.63	5.23	0.71	11.42	90.87

2014: Average Similarity = 52.24%

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Calanoida	1.06	15.73	1.96	30.1	30.1
Caridea	0.91	12.72	1.71	24.34	54.44
Brachyura	0.77	7.89	0.99	15.11	69.55
<i>M. leidy</i>	0.65	6.5	0.78	12.44	81.99
Fish Eggs	0.69	6.16	0.8	11.79	93.78

2012 vs. 2013 Comparison: Average dissimilarity = 56.21

Species	Group 2012		Group 2013		Contrib%	Cum.%
	Av.Abund	Av.Abund	Av.Diss	Diss/SD		
Fish Eggs	0.65	0.63	4.98	0.98	8.85	8.85
Calanoida	0.85	0.9	4.96	0.92	8.83	17.68
<i>M. leidy</i>	0.58	0.75	4.91	0.93	8.74	26.43
Brachyura	0.88	0.77	4.36	0.83	7.75	34.18
Caridea	0.85	0.83	4.1	0.8	7.29	41.47
Cladocera	0.17	0.38	3.27	0.82	5.82	47.29
Fish Larvae	0.27	0.21	2.8	0.74	4.97	52.26
Polychaeta	0.23	0.16	2.34	0.67	4.17	56.43
Melitidae	0.25	0.13	2.28	0.68	4.06	60.49
<i>Gammarus</i> spp.	0.19	0.15	2.14	0.61	3.81	64.3
<i>I. baltica</i>	0.22	0.09	2.02	0.61	3.6	67.9
Ostrocodea	0.16	0.08	1.63	0.51	2.91	70.81
<i>S. salpa</i>	0	0.12	1.32	0.32	2.35	73.16
Nauplia	0.16	0	1.24	0.43	2.21	75.37
<i>C. quinquecirrha</i>	0.09	0.07	1.22	0.4	2.17	77.55
Gastropoda	0.15	0	1.21	0.4	2.15	79.7
Ephyrae	0.09	0.05	1.04	0.37	1.84	81.54
Caprellidae	0.08	0.07	0.95	0.4	1.7	83.23
Harpacticoida	0.09	0.03	0.81	0.36	1.44	84.67
Aoridae	0.04	0.07	0.74	0.34	1.32	86

Cladonema	0.09	0	0.72	0.31	1.28	87.28
<i>Turritopsis</i>	0	0.09	0.71	0.32	1.27	88.54
<i>Hippocampus</i>	0.09	0	0.63	0.31	1.12	89.66
<i>Clytia</i>	0.05	0.02	0.58	0.26	1.03	90.69

2012 vs. 2014 Comparison: Average dissimilarity = 53.18

	Group 2012		Group 2014			
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Fish Eggs	0.65	0.69	4.9	0.99	9.21	9.21
<i>M. leidy</i>	0.58	0.65	4.7	0.97	8.84	18.05
Brachyura	0.88	0.77	4.48	0.89	8.43	26.48
Calanoida	0.85	1.06	4.09	0.81	7.69	34.17
Caridea	0.85	0.91	3.37	0.72	6.35	40.52
Fish Larvae	0.27	0.3	3.18	0.83	5.98	46.5
Melitidae	0.25	0.21	2.68	0.72	5.04	51.54
Cladocera	0.17	0.14	2.02	0.59	3.8	55.34
<i>I. baltica</i>	0.22	0.08	1.94	0.6	3.66	59
<i>Gammarus</i>	0.19	0.1	1.88	0.57	3.54	62.53
Polychaeta	0.23	0.03	1.82	0.58	3.42	65.96
Gastropoda	0.15	0.1	1.78	0.51	3.35	69.3
Nauplia	0.16	0.07	1.6	0.51	3.01	72.31
Ostrocooda	0.16	0.02	1.29	0.45	2.42	74.73
<i>C. quinquecirrha</i>	0.09	0.05	1.06	0.38	2	76.73
Aoridae	0.04	0.1	0.98	0.4	1.84	78.57
Ephyrae	0.09	0.02	0.83	0.34	1.56	80.13
Caprellidae	0.08	0.05	0.79	0.37	1.48	81.61
Harpacticoida	0.09	0.02	0.79	0.35	1.48	83.09
<i>Bougainvillea</i>	0	0.09	0.76	0.31	1.42	84.51
Cladonema	0.09	0	0.71	0.31	1.33	85.84
<i>Turritopsis</i>	0	0.08	0.66	0.29	1.24	87.09
<i>Hippocampus</i>	0.09	0	0.62	0.32	1.17	88.25
<i>Pleurobrachia</i>	0	0.07	0.57	0.28	1.07	89.32
<i>Erischsonella</i>	0.03	0.05	0.57	0.28	1.06	90.39

2013 vs. 2014 Comparison: Average dissimilarity = 52.53

	Group 2013		Group 2014			
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Fish Eggs	0.63	0.69	5.22	0.99	9.94	9.94
<i>M. leidy</i>	0.75	0.65	4.92	0.93	9.36	19.3
Brachyura	0.77	0.77	4.82	0.91	9.18	28.48
Calanoida	0.9	1.06	4.73	0.79	9	37.48
Caridea	0.83	0.91	3.99	0.76	7.59	45.07

Cladocera	0.38	0.14	3.3	0.81	6.28	51.35
Fish Larvae	0.21	0.3	3.13	0.78	5.96	57.31
Mellitidae	0.13	0.21	2.24	0.61	4.27	61.58
<i>Gammarus</i>	0.15	0.1	1.71	0.51	3.26	64.85
<i>S. salpa</i>	0.12	0.02	1.53	0.36	2.92	67.77
Polychaeta	0.16	0.03	1.35	0.47	2.57	70.34
<i>Turritopsis</i>	0.09	0.08	1.3	0.42	2.47	72.81
Aoridae	0.07	0.1	1.21	0.43	2.31	75.12
<i>I. baltica</i>	0.09	0.08	1.16	0.43	2.21	77.33
<i>C. quinquecirrha</i>	0.07	0.05	1.02	0.34	1.94	79.28
<i>Pleuronectes</i>	0.06	0.07	0.98	0.37	1.87	81.15
Gastropoda	0	0.1	0.84	0.32	1.6	82.75
<i>Bougainvillea</i>	0.01	0.09	0.82	0.32	1.56	84.31
Caprellidae	0.07	0.05	0.82	0.36	1.55	85.86
Ostrocooda	0.08	0.02	0.69	0.32	1.31	87.18
<i>S. fuscus</i>	0.05	0.03	0.61	0.31	1.15	88.33
Ephyrae	0.05	0.02	0.58	0.26	1.1	89.43
Nauplia	0	0.07	0.55	0.27	1.05	90.48

The ANOSIM indicated a global R of 0.025 ($P < 0.003$) for differences among years. In pairwise comparisons among the years, significant differences were seen in community structure between 2012 and 2013 ($R=0.031$, $P < 0.014$) and 2013 and 2014 ($R=0.051$, $P < 0.001$). However, no difference was observed between 2012 and 2014, suggesting that community structure was becoming reestablished and stable by 2014. The MDS plot indicates substantial overlap in community structure among years with only two outliers (Fig. 54).

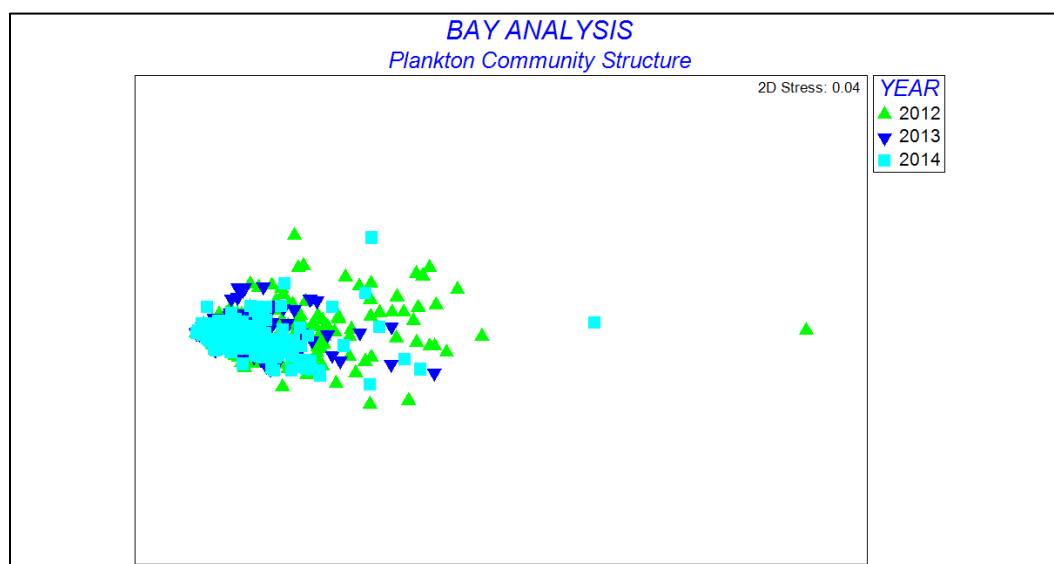


Figure 54. MDS plot of planktonic community structure from bay wide samples plotted based upon the year of collection. Clustering of samples indicates similarity of community structure. Outlying point for 2012 indicates a sample with high densities of crab larvae.

Planktonic Community Structure Assessment: Lagoon Sampling

Results from the SIMPER analysis indicate average similarities ranging from 46% to 50% with five taxa contributing to >89% of the group similarity. In particular, the five taxa that were important in structuring most of the planktonic communities observed in Barnegat Bay lagoons include: Calanoida, Caridea, Brachyura, *M. leidy*, and Fish Eggs, which are identical to those from the bay-wide surveys (Fig. 55). When comparing the dissimilarity between years, average dissimilarity was 53.98%. On average, contributions to dissimilarity between years involved 14 different taxa, suggesting that within year taxonomic similarity is common, but communities differ across years, dominated by the influx of several other gelatinous zooplankton species including *Turritopsis* and *Bougainvillea*. This may reflect the impacts of Hurricane Sandy altering the overall community given the significant differences in density seen among years for *M. leidy*, Caridea, and Calanoida. Results from a Two-Way ANOSIM demonstrate significant differences between years (Global $R=0.09$, $P < 0.001$) and months ($R=0.268$, $P < 0.001$) with significant differences between all monthly comparisons (range: $R = 0.154$ to 0.389 , $P < 0.001$), suggesting that both seasonal and annual variability exist within the planktonic communities and driving factors such as the significantly greater densities of *M. leidy* in 2013 and greater species richness of other gelatinous species in 2014 (Fig. 53) strongly influenced these relationships. When lagoons were visually assessed through an MDS (Fig. 54) strong temporal differences are observed, but also strong overlap and similarity when looking at both years during monthly sampling.

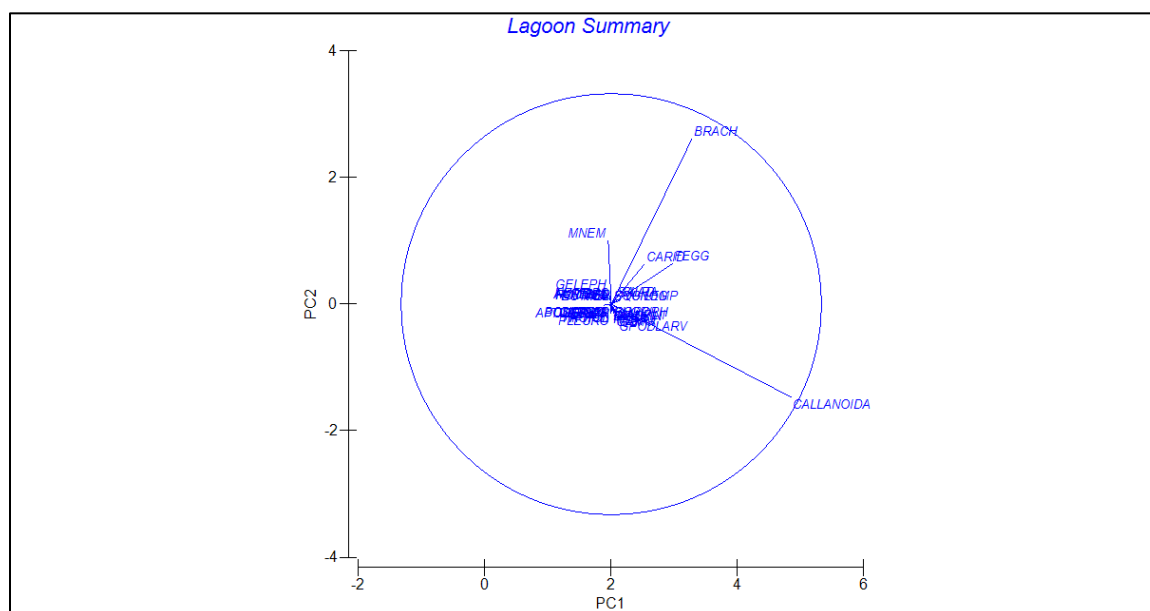


Figure 55. Principle Component Analysis plot demonstrating dominant taxa driving community structure in lagoons.

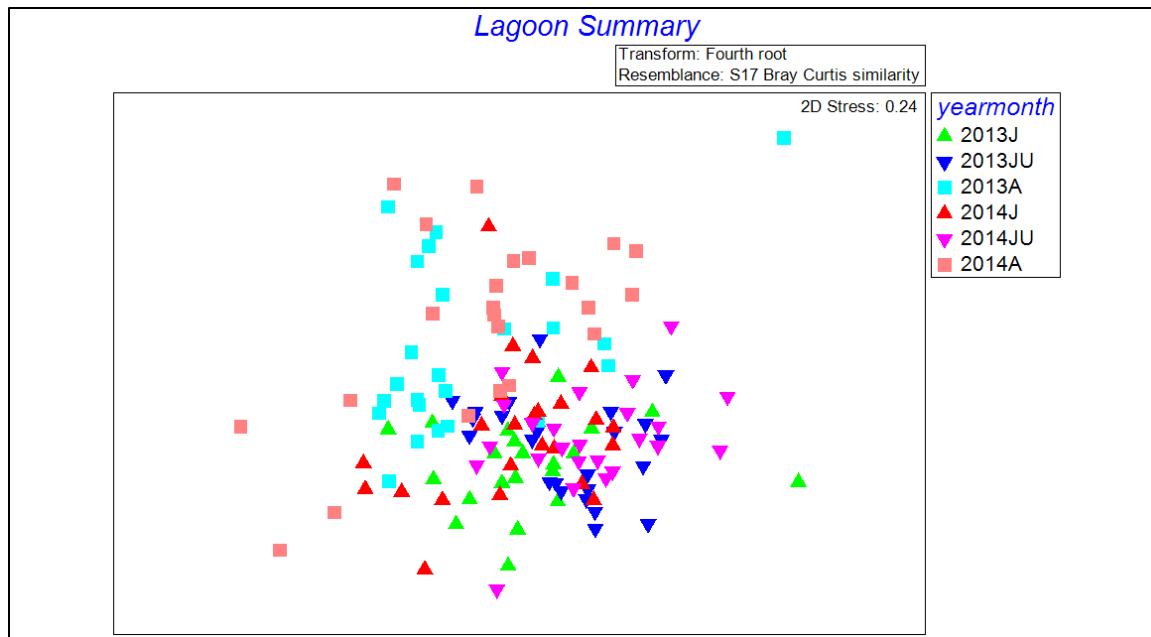


Figure 56. MDS plot of planktonic community structure from lagoon samples plotted based upon the year of collection. Clustering of samples indicates similarity of community structure.

Molecular Diet Analyses: Comparison between 2013 and 2014.

In general, the prey items recovered for both years were similar at higher taxonomic levels. Even though fewer taxa were recovered in 2014, twelve new taxa were identified (Table 36) bringing the total number of prey species to 36. As compared to 2013, the 2014 diet content contained more worms but less cnidarians and no pelecypods, flatworms, barnacles, starfish, or echinoderms. However, as data become available in DNA libraries, greater taxonomic resolution will be possible in the future as well as identification and resolution of unidentified sequence information.

Table 36. Comparison of *C. quinquecirrha* prey items identified in the combined gut lavage and tentacle pick builds for 2013-2014.

Final Classification	Common Name	Gene(s)	2013	2014
<i>Ampithoe valida</i>	Amphipod #1	COI; 18S	X	
<i>Gammarus</i> sp.	Amphipod #2	18S		X
<i>Acartia tonsa</i>	Copepod #1	ITS1, 5.8S rRNA, ITS2; COI	X	X
Crangonyctidae	Copepod #2 (Not Cyclopoida)	28S	X	
Cyclopoida	Copepod #3 (Not Crangonyctidae)	18S	X	
<i>Pseudodiaptomus coronatus</i>	Copepod #4	18S; 28S	X	
Cirripedia	Barnacle	28S	X	

<i>Americamysis bahia</i>	Opossum shrimp	18S	X	X
<i>Palaemonetes pugio</i>	Daggerblade grass shrimp	28S		X
<i>Diadumene leucolena</i>	White anemone	28S	X	
Nynantheae	Anemone possibly white anemone	18S; COI and other mitochondrial genes	X	X
Actiniaria possibly <i>Nematostella vectensis</i>	Starlet sea anemone	Miscellaneous nuclear genes	X	
<i>Botryllus schlosseri</i>	Star ascidian	18S		X
Hydrozoa	Hydrozoan	28S		X
<i>Mnemiopsis leidyi</i>	Sea walnut	18S; 28S; COI and other mitochondrial genes	X	
<i>Cerebratulus lacteus</i>	Nemertean worm	18S; 28S		X
<i>Alitta (=Nereis) succinea</i>	Polychaete clam worm	18S; 28S; COI	X	X
Goniadidae	Polychaete worm #2	28S	X	X
Phyllodocidae	Polychaete worm	28S		X
Lepocreadiidae	Trematode	18S	X	
Stylochidae	Flatworm	28S	X	
<i>Stylochus</i>	Flatworm	5.8S; 28S		X
Asterozoa	Starfish or brittle star	28S	X	
Echinozoa	Echinoderm	28S	X	
Nudibranchia	Nudibranch	18S	X	
<i>Cuthona</i>	Nudibranch	18S		X
Eubbranchidae	Nudibranch	18S		X
Holothuroidea	Sea cucumber	5.8S		X
Apodida	Sea cucumber	18S		X
Scaphopoda	Scaphopod	28S		X
Gastropoda possibly <i>Euthyneura</i>	Gastropod possibly <i>Euthyneura</i>	heat shock protein	X	
<i>Mercenaria mercenaria</i>	Hard clam	COI; 18S	X	
Veneridae other than <i>Mercenaria</i>	Clam #2	COI	X	
Hemichordata	Possibly acorn worm	BAC sequences	X	
Styelidae	Sea squirt	5.8S		X
Gobiidae	Goby	Mitochondrial genes		X
<i>Anchoa mitchilli</i>	Bay anchovy	COI and other mitochondrial genes	X	

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 ephyra. This suggests that polyp populations in the southern regions of the bay may be currently small, but growing. Additionally, the higher abundances of these small stages observed in the southern most region of the Bay indicates that tidal export of larvae and juveniles may be occurring and these individuals are being exported into coastal waters. With this export, the potential exists for sea nettles to expand to other estuaries in New Jersey. Our molecular techniques would be the best approach to track and identify these stages in estuaries to assess the potential threat to other marine communities.

The distribution of sea nettles is significantly negatively correlated with the other dominant gelatinous zooplankton species *Mnemiopsis leidyi*, demonstrating strong top-down food web structuring. Sea nettles were also negatively correlated with major prey items including cladocerans, fish eggs, fish larvae, and ostracods, but showed positive correlations with nauplii and barnacle larvae. The positive relationships may be due to the fact that these organisms are planktonic and are widely distributed through physical forces including tidal and wind driven currents, but it could also suggest potential size selectivity for sea nettles against the smaller larval organisms.

Based on the findings of our research, it appears that there is about a two week time-lag between major life history stages of *C. quinquecirrha*. This may be related to our sampling scheme, but the correlation does seem to provide credence to this finding. One area of research which needs to be fully explored is the role of developed coastal communities (i.e., lagoon developments) on the distribution of polyps which generate ephyra. If the early stages are being generated within these highly developed regions, then early sampling and detection would allow us to ascertain the potential strength of jellyfish blooms.

One key component that is essential to understand is the inverse relationship between the comb jelly *Mnemiopsis leidyi* and the sea nettle *C. quinquecirrha*. It is known that *C. quinquecirrha* predate upon *M. leidyi* and may control their populations. As *M. leidyi* is also a voracious predator of fish eggs and larvae, there may be beneficial outcomes with increasing *Chrysaora* populations. Control of the numerically dominant *M. leidyi* could reduce their top-down influence, but further research into these interactions is needed, especially since the results suggest that in 2014 competitive interactions among numerous gelatinous zooplankton may have limited the overall density of *M. leidyi*.

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. Given their strong presence and increasing distribution throughout Barnegat Bay, understanding, assessing, and controlling polyp populations will be critical for minimizing the medusa stage of sea nettles in the bay. Currently, two regions which appear to have large or increasing polyp populations include the lagoon communities in Forked River, Waretown, and Beach Haven West. These populations will most likely lead to elevated numbers in the future in the vicinity of Barnegat Inlet, Manahawkin Bay, and Little Egg Harbor, unless polyp population control is addressed.

Impacts of Hurricane Sandy

- Substantial reduction in sea nettle density following the storm, with continued low densities in 2014.
- Reduction in predation by *C. quinquecirrha* on *M. leidy* in 2013 allowed a significant increase in *M. leidy* density
- Changes in gelatinous zooplankton species distribution suggests changes in tidal exchange with the coastal ocean, increasing the occurrence of oceanic species in the bay.
- Increasing competition from these other gelatinous zooplankton species reduced *M. leidy* density in 2014.
- Significant decline in sea horse (*Hippocampus erectus*) larvae in 2013 and 2014 suggests that loss of seagrass habitat impacted their populations and reduced reproductive output.

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 commercially and recreationally important species. Our findings of early stage individuals through molecular techniques demonstrates the efficacy of this technique to conduct expanded regional surveys of coastal bays in New Jersey to determine if larvae and ephyra are being advected out of Barnegat Bay and subsequently invading other estuaries. It is highly probable that the distributions seen through our sampling suggested out-welling of these individuals from Barnegat Bay into the coastal Atlantic. These individuals then have the opportunity to be transported along the coast and enter other regions. Investing in molecular identification of these individuals will allow us to track and predict the invasion potential of other

estuaries and to develop long-term strategies to combat the problem of gelatinous zooplankton blooms.

Based on our research two fundamental management strategies can be enacted or addressed. First, the abundance of sea nettles (*Chrysaora quinquecirrha*) is a growing problem in not only Barnegat Bay, but increasingly in other coastal estuaries in the state. To minimize the population within a region, emphasis needs to be placed on the polyp/podocyst life history stage. This is the critical stage for sea nettles, as this stage overwinters and produces the adult medusa which impacts food webs, community structure, and local economies based on tourism and fishing. The developed regions of the bay demonstrate settlement and production of ephyrae so minimizing sea nettle populations should focus there. One way to combat jellyfish polyps is through improving water quality, especially in lagoon communities. Specifically, the low dissolved oxygen in many of these systems limits the colonization of various hard substrates (e.g., docks, bulkheads etc.) allowing sea nettle polyps to flourish without competition for space.

Results Dissemination

During the course of this project, we have worked to disseminate the results from the research. We have submitted articles for publication, with one published in 2014 and two currently in review. We have presented our research in formal settings at local, national, and international meetings, as well as informal sessions for the general public and for teacher's workshops. Many of the students who have worked on this project have presented research at local and national meetings with 6 published abstracts occurring from presentations made at the New Jersey Academy of Sciences annual meetings and subsequently published in the Journal. A list of publications and presentations is listed below. Appendix B provides the abstracts for each of these presentations. Lastly, funding from this project has helped support research activities of several graduate and undergraduate students resulting in completion of two Masters Theses and two in progress.

One area of significant dissemination of results has occurred on an international level. We presented a paper at the 4th International Jellyfish Bloom Conference in Hiroshima, Japan (2013) discussing the time-lag assessment of the appearance of DNA to juvenile, adults and then adults to larvae. This conference is the largest international gathering of gelatinous zooplankton researchers and allowed us a platform to discuss the findings of our research. Based on the research provided in that presentation, we were subsequently invited to present at a special session on Gelatinous Zooplankton at the International Council for the Exploration of the Seas (ICES) meeting in A Coruna, Spain (2014) by the Organizing Committee. This presentation has allowed us to gain international recognition of our research and we expect to present at the 5th International Jellyfish Bloom Conference to be held in Barcelona, Spain in 2016.

In addition to formal scientific dissemination, we have engaged in public outreach and education by working with the Barnegat Bay Teacher Research Institute Teachers Workshop to engage educators and provide educational materials and ideas regarding sea nettles in Barnegat Bay and food web dynamics. Additionally, PI Bologna also provided public lectures entitled "*Chrysaora quinquecirrha* in Barnegat Bay" to several citizen groups including Save Barnegat Bay, the Monmouth SEAS group, and the Seaweeders Club. This lecture highlighted the biology and ecology of sea nettles in Barnegat Bay and research highlights from this project and previous research.

Published Manuscripts

Crum, K., Fuchs, H., Bologna, P., Gaynor, J. 2014. Model-data comparisons reveal influence of jellyfish interactions on plankton community dynamics. *Mar. Ecol. Prog. Ser.* 517: 105–119.

Manuscripts in Review

Bologna, P. Gaynor, J., Castellano, C., Restaino, D. (in review) Direct and indirect predatory impacts of gelatinous zooplankton on pelagic community structure.

Meredith, R., Gaynor, J. Bologna, P. (in review) Diet Assessment of the Atlantic Sea Nettle *Chrysaora quinquecirrha* in Barnegat Bay, New Jersey using Next Generation Sequencing.

Non-Peer Reviewed Publications

Bologna, P. Gaynor, J. and Meredith, R. 2014. Impacts of Invasive Sea Nettle (*Chrysaora quinquecirrha*) and Ctenophores on Planktonic Community Structure and Bloom Prediction of Sea Nettle Using Molecular Techniques. NJDEP Project Report. 44p.

Bologna, P. and Gaynor, J. 2013. Assessment of the Distribution and Abundance of Stinging Sea Nettle (Jellyfish) in Barnegat Bay. NJDEP Project Report. 38p.

Published Abstracts:

Aristizabal, N.*, Bologna, P., Gaynor, J. 2014. Experimental colonization of hard substrate in a coastal New Jersey lagoonal system. Bull. NJ Acad. Sci. 59:

Castellano, C.*, Bologna, P., Gaynor, J. 2014. Direct and Indirect Impacts of Gelatinous Zooplankton on pelagic community structure. Bull. NJ Acad. Sci. 59:

Rios, I.*, Bologna, P., Gaynor, J. 2014. Diet Analysis of the Atlantic Sea Nettle, *Chrysaora quinquecirrha*, in Barnegat Bay, NJ. Bull. NJ Acad. Sci. 59:

Castellano, C.*, Bologna, P. 2013. An Assessment of Gelatinous Zooplankton and Impacts on Planktonic Community Structure in Barnegat Bay, New Jersey. Bull. NJ Acad. Sci. 58 (2): 16.

Restaino, D.*, Shchegolev, G.*, Lussier, V.*, Bologna, P., Gaynor, J. 2013. qPCR-Based Assay for the Identification of Early Life History Stages of *Chrysaora quinquecirrha* in Barnegat Bay. Bull. NJ Acad. Sci. 58 (2): 19.

Castellano, C.*, Bologna, P. 2012. Effects of Sea Nettle (*Chrysaora quinquecirrha*) Presence on Populations of Zooplankton in Northern Barnegat Bay, New Jersey. Bull. NJ Acad. Sci. 57 (2): 8.

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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 μ L 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 μ L 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 ₂ O		289 μ L			
1 <u>M</u> Tris	100 mM	50 μ L			
5 <u>M</u> NaCl	1.4 M	140 μ L			
0.5 <u>M</u> EDTA	20 mM	20 μ L			
☐ mercaptoethanol (14.3 <u>M</u> stock)	0.2% (28.6 mM)	1 μ L			
Proteinase K	0.1 mg/mL	50 ☐			
Single = 500 ☐ L				Total	



Potter-Elvehjem homogenizer

Appendix B. Dissemination of Results: Presentation Abstracts

2012 Metropolitan Association of College and University Biologists

PCR-Based Detection of Sea Nettle (*Chrysaora quinquecirrha*) DNA in Environmental Samples

George Shchegolev, Dena Restaino, Victoria Lussier, Archana Tare, Paul A.X. Bologna and John J. Gaynor

The sea nettle, *Chrysaora quinquecirrha*, has become abundant in estuaries of Mid-Atlantic States and frequently blooms in warm summer months. Various factors have been attributed to the rise in numbers of sea nettles and other jellyfish including eutrophication, overfishing, global warming, construction and species introduction. Despite its abundance and frequent disruption of ecosystems, virtually nothing is known about the genome of this important species. In an effort to aid in the molecular identification of this organism, especially at early developmental stages, we have developed a PCR-based assay using the mitochondrial 16S rDNA locus. Using primers developed by Bahya & Graham (Hydrobiologia 616:217–228, 2009), we have amplified and sequenced a 209 bp fragment (Genbank #GU300724) unique to *C. quinquecirrha*. This technique is sensitive enough to detect as little as 80 copies of *C. quinquecirrha* 16S rDNA and may permit us to not only determine and quantify the abundance of early medusa stages (ephyra), but also identify gametes and planula larval stage to assess sexual reproduction in environmental samples. We plan to utilize this method to quantify *C. quinquecirrha* DNA in Barnegat Bay both temporally and spatially and correlate these data with counts of adult medusa found in the bay in warm weather. We anticipate that this method may permit us to predict blooms of adult medusa in estuaries.

2012 Atlantic Estuarine Research Society Meeting

PCR-Based Detection of Sea Nettle (*Chrysaora quinquecirrha*) DNA in Environmental Samples

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weather. We anticipate that this method may permit us to predict blooms of adult medusa in estuaries.

Spatial and Temporal Distribution of Gelatinous Zooplankton in Barnegat Bay, New Jersey

Paul A. X. Bologna, John J. Gaynor, Christie L. Castellano

Global changes in the coastal ecosystems due to anthropogenic forces, climate change and species introductions have led to the increase in populations of gelatinous zooplankton. In recent years, Atlantic sea nettle (*Chrysaora quinquecirrha*) abundance has dramatically increased in Barnegat Bay, New Jersey. Sea nettles represent important top down predators within this system, potentially impacting zooplankton and larval fish populations within the bay. Lift net sampling was used to determine abundance and distribution of sea nettle and ctenophore populations in 2012. Results showed substantial spatial and temporal variability in population density and distribution of comb jellies, with only a few sites showing sea nettles throughout the bay. This differed from results in 2011 where greater densities of nettles were observed. The high variability in density and distribution may reflect the influences of wind driven currents and tidal fluctuations within Barnegat Bay.

2012 New Jersey Academy of Sciences Meeting

Effects of sea nettle (*Chrysaora quinquecirrha*) presence on populations of zooplankton in northern Barnegat Bay, New Jersey

Christie L. Castellano; Paul A. X. Bologna

Global changes in the ocean due to anthropogenic forces have led to the increase in relative abundance in jellyfish populations. In recent years, sea nettle (*Chrysaora quinquecirrha*) abundance has dramatically increased in Barnegat Bay, New Jersey. Sea nettles represent important top down predators within this system whose effects have yet to be what investigated. Plankton nets were used to assess the distribution of juveniles, which was determined to conglomerate in the northwestern portion of the bay. Lift nets were used to assess the distribution of adults, which was determined to be clustered in the northern part of the bay, with the greatest density of 51 individuals per cubic meter within the Metedeconk River. Relationships between sea nettle juveniles and other planktonic larvae were also investigated, with a negative correlation found between sea nettles and the presence of crab zoea, shrimp and polychaete larvae and a positive correlation found between the presence of sea nettles versus copepods and snail larvae.

2012 Benthic Ecology Meeting

Effects of sea nettle (*Chrysaora quinquecirrha*) presence on populations of zooplankton in northern Barnegat Bay, New Jersey

Christie L. Castellano; Paul A. X. Bologna

Global changes in the ocean due to anthropogenic forces have led to the increase in relative abundance in jellyfish populations. In recent years, sea nettle (*Chrysaora quinquecirrha*) abundance has dramatically increased in Barnegat Bay, New Jersey. Sea nettles represent important top down predators within this system whose effects have yet to be what investigated. Plankton nets were used to assess the distribution of juveniles, which was determined to conglomerate in the northwestern portion of the bay. Lift nets were used to assess the distribution

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2013 4th International Jellyfish Bloom Symposium, Hiroshima, Japan.

Modeling Sea Nettle (*Chrysaora quinquecirrha*) Blooms through Molecular Identification of Early Stage Individuals

Paul Bologna; John Gaynor

Increasing coastal development has eliminated natural shorelines and increased substantial hard structure for cnidarian polyps. This, coupled with coastal eutrophication, has led to water quality declines for which cnidarians are minimally impacted compared to other organisms. In our system, polyp density can exceed 4,000m⁻² on non-toxic settling plates leading to potential surges of ephyra into the coastal bays. While planktonic sampling can be used to collect and quantify later stage ephyra and juveniles, we have been collecting water samples, filtering through 500 µm nylon mesh, and extracting DNA to quantitatively assess for *C. quinquecirrha* ephyra using qPCR. These data are then being used to develop a time lag model in which the presence of early stage ephyra DNA in the water can be correlated to the appearance of juveniles and adults at later periods of the year using plankton and lift nets, respectively. Additionally, assessing DNA in the water column has led to the discovery of wide scale presence of *C. quinquecirrha* in the bay, despite the absence of juveniles and adults in many regions. This suggests that polyps are established ubiquitously in the bay, but that tidal flushing and wind driven currents force these individuals out of the bay and into coastal waters where their dispersal may expand their distribution.

2013 Benthic Ecology Meeting

qPCR Based Assay for the Temporal and Spatial Distribution of Early Life History Stages of *Chrysaora quinquecirrha* in Barnegat Bay

Restaino, Dena J; Shchegolev, George; Lussier, Victoria; Bologna, Paul. A. X; Gaynor, John

The sea nettle, *Chrysaora quinquecirrha*, has become abundant in estuaries of Mid-Atlantic States and frequently blooms in warm summer months. Various factors have been attributed to the increasing localized appearance of sea nettles and other jellyfish including eutrophication, overfishing, global warming, construction and species introduction. Despite its abundance and frequent distribution within estuarine systems, very little work has been done to detect and quantify the early life history stages of this organism. Larval stages of *C. quinquecirrha* can now be detected and quantified using a qPCR assay specific for the 16S rDNA locus of the mtDNA of *C. quinquecirrha*. This assay is species specific, is linear over a 9-log range, and can detect as few as 60 copies of 16S rDNA. 20 liter field samples were filtered through 500 µm and 100 µm mesh to separate ephyra from planula larvae and gametes, respectively. Quantifiable levels of *C. quinquecirrha* 16S rDNA were detected at all eight paired locations in Barnegat Bay, with levels varying on both spatial and temporal scales. Identifying and quantifying early stage *C. quinquecirrha* using molecular techniques may allow us to develop predictive models regarding

the onset and intensity of sea nettle blooms.

An Assessment of Gelatinous Zooplankton and Impacts on Planktonic Community Structure in Barnegat Bay, New Jersey

Castellano, Christie L.; Bologna, Paul A. X.

Local populations of gelatinous zooplankton are experiencing increases in response to changes in coastal ecosystems due to anthropogenic forces. The abundance of the Atlantic sea nettle (*Chrysaora quinquecirrha*) has dramatically increased in Barnegat Bay, NJ. Lift net sampling was used to determine the density and distribution of sea nettle and ctenophore (*Mnemiopsis leidyi*) populations, while zooplankton tows were used to compare relationships between their abundance to that of other zooplankton species. Lift net results showed substantial spatial and temporal variability in density and distribution of ctenophores and sea nettles, with these patterns being inversely proportional. High variability may reflect the influences of wind driven water movement and tidal fluctuations within Barnegat Bay. Zooplankton tow results showed similar trends on spatial and temporal scales. The presence of jellyfish ephyrae was found across all sampling sites, suggesting the expansion of sea nettles in Barnegat Bay. Correlation analysis for the abundance of gelatinous zooplankton against other zooplankton suggests predation upon copepods, fish eggs, and larval fish, crab and shrimp by *C. quinquecirrha* and *M. leidyi* and is indicative of potential top-down structuring forces in the pelagic community.

2013 Montclair State University Research Symposium

qPCR Based Assay for the Temporal and Spatial Distribution of Early Life History Stages of *Chrysaora quinquecirrha* in Barnegat Bay

Restaino, Dena J; Shchegolev, George; Lussier, Victoria; Bologna, Paul. A. X; Gaynor, John. J

The sea nettle, *Chrysaora quinquecirrha*, has become abundant in estuaries of Mid-Atlantic States and frequently blooms in warm summer months. Various factors have been attributed to the increasing localized appearance of sea nettles and other jellyfish including eutrophication, overfishing, global warming, construction and species introduction. Despite its abundance and frequent distribution within estuarine systems, very little work has been done to detect and quantify the early life history stages of this organism. Larval stages of *C. quinquecirrha* can now be detected and quantified using a qPCR assay specific for the 16S rDNA locus of the mtDNA of *C. quinquecirrha*. This assay is species specific, is linear over a 9-log range, and can detect as few as 60 copies of 16S rDNA. 20 liter field samples were filtered through 500 μ M and 100 μ M mesh to separate ephyra from planula larvae and gametes, respectively. Quantifiable levels of *C. quinquecirrha* 16S rDNA were detected at all eight paired locations in Barnegat Bay, with levels varying on both spatial and temporal scales. Identifying and quantifying early stage *C. quinquecirrha* using molecular techniques may allow us to develop predictive models regarding the onset and intensity of sea nettle blooms.

2013 New Jersey Academy of Sciences Meeting

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AN ASSESSMENT OF GELATINOUS ZOOPLANKTON AND IMPACTS ON PLANKTONIC COMMUNITY STRUCTURE IN BARNEGAT BAY, NEW JERSEY

Christie L. Castellano, Paul A. X. Bologna, John J. Gaynor

Local populations of gelatinous zooplankton are experiencing increases in response to changes in coastal ecosystems due to anthropogenic forces. The abundance of the Atlantic sea nettle (*Chrysaora quinquecirrha*) has dramatically increased in Barnegat Bay, NJ. Lift net sampling was used to determine the density and distribution of sea nettle and ctenophore (*Mnemiopsis leidyi*) populations, while zooplankton tows were used to compare relationships between their abundance to that of other zooplankton species. Lift net results showed substantial spatial and temporal variability in density and distribution of ctenophores and sea nettles, with these patterns being inversely proportional. High variability may reflect the influences of wind driven water movement and tidal fluctuations within Barnegat Bay. Zooplankton tow results showed similar trends on spatial and temporal scales. The presence of jellyfish ephyrae was found across all sampling sites, suggesting the expansion of sea nettles in Barnegat Bay. Correlation analysis for the abundance of gelatinous zooplankton against other zooplankton suggests predation upon copepods, fish eggs, and larval fish, crab and shrimp by *C. quinquecirrha* and *M. leidyi* and is indicative of potential top-down structuring forces in the pelagic community.

RESTAINO THESIS ABSRACT 2013:

The sea nettle, *Chrysaora quinquecirrha*, has become abundant in estuaries of Mid-Atlantic States and frequently blooms in warm summer months. Various factors have been attributed to the increasing localized appearance of sea nettles and other jellyfish including eutrophication, overfishing, global warming, construction and species introduction. Despite its abundance and frequent distribution within estuarine systems, very little work has been done to detect and quantify

the early life history stages of this organism. Larval stages of *C. quinquecirrha* can now be detected and quantified using a qPCR assay specific for the 16S rDNA locus of the mtDNA of *C. quinquecirrha*. This assay is species specific, linear over a 9-log range, and can detect as few as 60 copies of 16S rDNA. 20-liter field samples were filtered through 500 μ M and 100 μ M mesh to separate ephyrae from planula larvae and gametes, respectively. Quantifiable levels of *C. quinquecirrha* 16S rDNA were detected at all eight paired locations in Barnegat Bay, with levels varying on both spatial and temporal scales. The use of quantitative molecular data on the distribution of early stage *C. quinquecirrha* to predict and model blooms of sea nettle medusa in Barnegat Bay will be discussed.

2014 International Council for the Exploration of the Sea Meeting, A Coruna, Spain

Range expansion of the Sea Nettle (*Chrysaora quinquecirrha*) and impacts on pelagic food webs.

Paul A. X. Bologna, John J. Gaynor, Christie Castellano, Dena Restaino

Urbanization of estuaries has dramatically altered shorelines through habitat removal and destruction, as well as construction of permanent structures like docks, bulkheads, and piers. Concomitantly, water quality degradation has occurred from various pollutants and excess nutrients derived from anthropogenic sources. Excessive nutrients result in coastal eutrophication and reduced oxygen concentrations, substantially altering the environment for living organisms. When we add in climate change and introduced species, many urban estuaries have the potential for substantial community changes. In particular, gelatinous zooplankton (ctenophores and jellyfish) can flourish under diminished water quality. Specifically, the rise in jellyfish populations on global and local scales benefit greatly by urbanized landscapes, because of their tolerance of degraded water and the increased hard substrate for their polyp life history stage. In coastal New Jersey, we have witnessed the range extension of the Atlantic Sea Nettle (*Chrysaora quinquecirrha*) from the Chesapeake Bay to a full-fledged member of the pelagic food web. Their abundance has altered pelagic community structure as they assume the role of top-predator. Our investigations have documented significant density differences among our sites, driven principally in regions where development is high, relative to regions with modest development, and those receiving substantial runoff from developed watersheds. The rise in sea nettle populations has altered the pelagic food web, as sea nettles exert substantial top-down pressure, especially upon the comb jelly, *Mnemiopsis leidyi*.

2014 Metropolitan Association of College and University Biologists

Use of qPCR to Map the Distribution and Seasonal Changes in Early Developmental Forms of the Atlantic Sea Nettle (*Chrysaora quinquecirrha*) in Barnegat Bay, New Jersey.

Maria Carvalho, Dena Restaino, Nashali Ferrara, Zachary Fetske, Isabella Pastore, Paul A.X. Bologna, and John J. Gaynor

Real-time quantitative PCR (qPCR) is rapidly becoming the method of choice for identifying species-specific DNA in environmental samples (eDNA). We have developed a real-time qPCR assay to detect the presence of sea nettle DNA in water samples from Barnegat Bay, NJ. The sea

nettle, *Chrysaora quinquecirrha*, is abundant in estuaries of Mid-Atlantic States and frequently blooms in warm summer months. Various factors may be contributing to the increasing rise of sea nettles and other jellyfish including eutrophication, overfishing, global warming, construction and species introduction. Despite its abundance and frequent distribution within estuarine systems, very little work has been done to detect and quantify the early developmental stages of this organism. Ephyra and planula larval stages of *C. quinquecirrha* can now be detected and quantified using a qPCR assay specific for the 16S rDNA locus of the mtDNA genome. This assay is species-specific, is linear over a 9-log range, and can detect as few as 10 copies of 16S rDNA. 20-liter field samples were filtered through 500 μ M and 100 μ M mesh to separate ephyra from planula larvae and gametes, respectively. Quantifiable levels of *C. quinquecirrha* 16S rDNA were detected at all 16 baywide locations and 8 lagoonal sites in Barnegat Bay, with levels varying on both spatial and temporal scales. Identifying and quantifying early stage *C. quinquecirrha* using molecular techniques may allow us to develop predictive models regarding the onset and intensity of sea nettle blooms.

2014 Atlantic Estuarine Research Society Meeting

Potential Impacts of Superstorm Sandy on Sea Nettle (*Chrysaora quinquecirrha*) Early Life History Stage in Barnegat Bay, NJ

Restaino, D.; Gaynor, J.; Bologna, P.; Carvalho, M.

Anthropogenic effects including coastline modification and eutrophication have resulted in increased populations of gelatinous zooplankton through increased polyp habitat. After Sandy, much of the potential benthic substrate (e.g, floating docks, bulkheads etc...) in Barnegat Bay, NJ was damaged or destroyed, posing the question of whether Sandy may have impacted these populations. Our 2012 molecular survey of sea nettle (*Chrysaora quinquecirrha*) ephyrae showed a broad distribution throughout all sites but a 1-2 order of magnitude reduction in DNA from 2013 samples. Planulae larvae abundance was about half compared to 2013, with peaks earlier in the summer, but generally lacking from September samples. The lack of larvae is most likely due to the collapse of the adult population in late July. While larval abundance was reduced, the substantial decline in ephyrae points to polyp habitat disruption from the storm.

Direct and Indirect Impacts of Gelatinous Zooplankton on Pelagic Community Structure in Barnegat Bay, NJ

Christie L. Castellano, Paul A. X. Bologna, John J. Gaynor

Gelatinous zooplankton populations are undergoing increases on local scales as a result of modifications to coastal systems due to anthropogenic forces such as habitat alteration and eutrophication. These changes have direct and indirect impacts on planktonic community structure that can result in strong top-down control by gelatinous species. Natural forces such as major storm events can also modify coastal systems in such a way as to also have impacts on planktonic community structure. In Barnegat Bay, NJ, the scyphozoan *Chrysaora quinquecirrha* and the ctenophore *Mnemiopsis leidyi* play important roles in planktonic food webs. Data collected in 2012 indicated significant inverse spatial and temporal distribution of these species, suggesting the exertion of top-down pressure on *M. leidyi* by *C. quinquecirrha*. In the year following Hurricane

Sandy, 2013 data showed similar distribution patterns, but with significantly lower densities of *C. quinquecirrha* while *M. leidy* densities remained comparable to the previous year. Populations of gelatinous zooplankton appeared to crash following a 2013 mid-summer heat spell, with differing density rebound afterward for each species. In both years, *C. quinquecirrha* and *M. leidy* had significant impacts on calanoid copepod populations, with *C. quinquecirrha* controlling northern populations and *M. leidy* controlling southern populations.

SEAGRASS WRACK AS A POTENTIAL BENTHIC-PELAGIC LINK IN BARNEGAT BAY, NJ

Joseph Robert McGinnis, Paul Bologna, Jack Gaynor, Robert Meredith

Seagrass wrack can result from storm surge, human interaction, or benthic sea grass shedding off excess grass blades when temperatures increase. Seagrass wrack can offer shelter and provide food for fish and invertebrates that either were on the grass previously in the benthos or found refuge within the floating grass mats. As such, seagrass wrack may offer trophic linkages between benthic and pelagic communities. The purpose of this research was to assess a potential coupling between benthic and pelagic invertebrates via *Zostera marina* seagrass wrack. Floating sea grass wrack was collected from several locations in Barnegat Bay, NJ during the summer of 2014. Results indicate that the dominant invertebrate taxa were amphipods and isopods. As these groups are typically linked to benthic communities, their presence in the wrack may represent a potential trophic link into pelagic food webs through fish consumption which has not been investigated previously. Additionally, floating wrack may also be important in transport of these organisms over longer distances influencing colonization of distant benthic habitats and regional genetic diversity.

2014 Benthic Ecology Meetings

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Direct and Indirect Impacts of Gelatinous Zooplankton on Pelagic Community Structure

Castellano, C. L.; Bologna, P.A.X.; Gaynor, J. J.

Populations of gelatinous zooplankton are undergoing increases on local scales in response to modifications in coastal systems as a result of anthropogenic forces such as pollution and habitat alteration. These changes are causing shifts in planktonic community structure that may allow for

strong-top down control by gelatinous species. In Barnegat Bay, NJ, the scyphozoan *Chrysaora quinquecirrha* and the ctenophore *Mnemiopsis leidyi* play influential roles in planktonic food webs. In 2012, Lift net sampling showed significant inverse spatial and temporal variations in density and distribution of these two species, suggesting *C. quinquecirrha* exerts top-down pressure on *M. leidyi*. However, when assessing the planktonic food webs, *C. quinquecirrha* did not appear to impact other planktonic species, while correlation analysis showed that *M. leidyi* has significant direct impacts on calanoid copepods, cladocerans, fish eggs and fish larvae. Consequently, it appears that *C. quinquecirrha* has an indirect top-down impact in this community by suppressing *M. leidyi* populations. Data collected in 2013 showed similar spatial and temporal patterns for *M. leidyi* populations, but *C. quinquecirrha* density was substantially lower with restricted temporal and spatial abundances. While the inverse distribution was similar and a negative correlation between these two species was observed, it was not significant.

2014 Montclair State University Research Symposium

Direct and Indirect Impacts of Gelatinous Zooplankton on Pelagic Community Structure

Christie L. Castellano, Dr. Paul A. X. Bologna

Populations of gelatinous zooplankton are undergoing increases on local scales in response to modifications in coastal systems as a result of anthropogenic forces such as pollution and habitat alteration. These changes are causing shifts in planktonic community structure that may allow for strong-top down control by gelatinous species. In Barnegat Bay, NJ, the scyphozoan *Chrysaora quinquecirrha* and the ctenophore *Mnemiopsis leidyi* play influential roles in planktonic food webs. In 2012, Lift net sampling showed significant inverse spatial and temporal variations in density and distribution of these two species, suggesting *C. quinquecirrha* exerts top-down pressure on *M. leidyi*. However, when assessing the planktonic food webs, *C. quinquecirrha* did not appear to impact other planktonic species, while correlation analysis showed that *M. leidyi* has significant direct impacts on calanoid copepods, cladocerans, fish eggs and fish larvae. Consequently, it appears that *C. quinquecirrha* has an indirect top-down impact in this community by suppressing *M. leidyi* populations. Data collected in 2013 showed similar spatial and temporal patterns for *M. leidyi* populations, but *C. quinquecirrha* density was substantially lower with restricted temporal and spatial abundances. While the inverse distribution was similar and a negative correlation between these two species was observed, it was not significant.

2014 New Jersey Academy of Sciences Meeting

DIRECT AND INDIRECT IMPACTS OF GELATINOUS ZOOPLANKTON ON PELAGIC COMMUNITY STRUCTURE

Christie L. Castellano; Paul A. X. Bologna; John J. Gaynor

Populations of gelatinous zooplankton are undergoing increases on local scales in response to modifications in coastal systems as a result of anthropogenic forces such as pollution and habitat alteration. These changes may result in direct and indirect impacts on planktonic community structure that can result in strong top-down control by gelatinous species. In Barnegat Bay, NJ, the scyphozoan *Chrysaora quinquecirrha* and the ctenophore *Mnemiopsis leidyi* play influential roles in planktonic food webs. In 2012, lift net sampling showed significant inverse spatial and temporal variations in density and distribution of these two species, suggesting *C. quinquecirrha* exerts top-

down pressure on *M. leidyi*. However, when assessing the planktonic food webs, *C. quinquecirrha* appears to also have an effect on calanoid copepod populations in the northern portion of the bay. Correlation analysis showed that *M. leidyi* has significant direct impacts on calanoid copepods in the southern portion of the bay. Data collected in 2013 showed similar spatial and temporal patterns for *M. leidyi* populations, but *C. quinquecirrha* density was substantially lower with restricted temporal and spatial abundances. While the inverse distribution was similar and a negative correlation between these two species was observed, it was not significant.

CASTELLANO THESIS ABSTRACT 2014:

Local populations of gelatinous zooplankton are experiencing increases in response to changes in coastal ecosystems due to anthropogenic forces. The abundance of the Atlantic sea nettle (*Chrysaora quinquecirrha*) has dramatically increased in Barnegat Bay, NJ. Lift net sampling was used to determine the density and distribution of sea nettle and ctenophore (*Mnemiopsis leidyi*) populations, while zooplankton tows were used to compare relationships between their abundance to that of other zooplankton species. Lift net results showed substantial spatial and temporal variability in density and distribution of ctenophores and sea nettles, with these patterns being inversely proportional. *Chrysaora quinquecirrha* was more abundant in north Barnegat Bay while *Mnemiopsis leidyi* was more abundant in the south. Zooplankton tow results showed similar trends on spatial and temporal scales. *Chrysaora quinquecirrha* individuals were collected in southern sample sites, suggesting the expansion of sea nettles in Barnegat Bay. Correlation analysis for the abundance of *Mnemiopsis leidyi* against other zooplankton suggests predation upon copepods, fish eggs, and larval fish, crab and shrimp and is indicative of potential top-down structuring forces in the pelagic community. Correlation analysis of *C. quinquecirrha* and *M. leidyi* suggests predation upon the ctenophore species by the scyphozoan.

2015 Benthic Ecology Meetings

Temporal and Spatial Relationship of Adult and Planula *Chrysaora quinquecirrha* Barnegat Bay, NJ

Restaino, D.*; Bologna, P.A.X.; Gaynor, J. J.; Meredith, R.

The scyphozoan *Chrysaora quinquecirrha* has become a common nuisance species in Barnegat Bay, New Jersey over the last decade. However, the number of medusa observed in the Bay after Super-storm Sandy has been considerably reduced. Two years after the storm the numbers of medusa observed in lift net samples has remained low. Molecular analysis of planula larvae via qPCR has also shown that numbers of planula larvae decreased following the storm event. This suggests that the damage caused by Sandy impacted the resident population within the Bay. The post storm removal of substrate, containing podocysts, from the Bay may account for the decrease in both adult and larval stages of this organism in 2013 and reduced planula larvae, limited polyp recruitment, and subsequent adults and larvae in 2014. The decline in larval abundance is important as it directly relates to polyp settlement and asexual reproduction of this species, as well as perpetuation of the population. Future studies will be needed to determine the lasting impacts of Super-storm Sandy on jellyfish populations.

Impacts of major storm events on gelatinous zooplankton and planktonic community structure in Barnegat Bay, NJ

Castellano, C. L.*; Bologna, P. A. X.; Gaynor, J. J.; Meredith, R. W.

Gelatinous zooplankton are experiencing distributional and abundance shifts on local scales resulting from anthropogenic forces such as eutrophication and land use alteration. These may have direct and indirect impacts on planktonic community structure that may result in strong top-down control by gelatinous species. In Barnegat Bay, NJ, the scyphozoan *Chrysaora quinquecirrha* and the ctenophore *Mnemiopsis leidyi* are the most abundant forms of gelatinous zooplankton and play important roles in planktonic food webs. Data collected in 2012 indicated significant inverse spatial and temporal distribution of these species, suggesting the exertion of top-down pressure of *C. quinquecirrha* on *M. leidyi*. However, storm events may also restructure communities in unplanned ways. Hurricane Sandy made landfall in New Jersey on October 29th, 2012. After Sandy, the relative distributions of these two species were similar, but muted. Additionally, different species of gelatinous zooplankton were observed. In 2014, this pattern repeated with even further decrease in *C. quinquecirrha* and *M. leidyi* and regular occurrence of more coastal ocean species.

2015 Montclair State University Research Symposium

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collected from several locations in Barnegat Bay, NJ during the summer of 2014. Results indicate that the dominant invertebrate taxa were amphipods and isopods. As these groups are typically linked to benthic communities, their presence in the wrack may represent a potential trophic link into pelagic food webs through fish consumption which has not been investigated previously. Additionally, floating wrack may also be important in transport of these organisms over longer distances influencing colonization of distant benthic habitats and regional genetic diversity.

2015 New Jersey Academy of Sciences Meeting

TEMPORAL AND SPATIAL RELATIONSHIP OF *Chrysaora quinquecirrha* IN BARNEGAT BAY, NJ FOLLOWING SUPERSTORM SANDY

Dena J. Restaino, John J. Gaynor, Paul A. X. Bologna, Maria Carvalho, Robert W. Meredith

The scyphozoan *Chrysaora quinquecirrha* has become a common nuisance species in Barnegat Bay, NJ due in large part to anthropogenic disturbances and increased polyp habitat. Following Superstorm Sandy much of the substrate utilized by polyps was damaged, destroyed, or removed, raising the question as to how this affected populations of *Chrysaora quinquecirrha* within the Bay. The number of medusa, ephyrae, and planula seen throughout the Bay has decreased following the storm. Molecular analysis of ephyrae and planula showed that while populations remain widely distributed there has a 1-2 order of magnitude reduction following the storm. A similar reduction of medusa has been seen in lift net sampling. These findings suggests that damage from the storm impacted the resident population through the post-storm removal of substrate containing polyps. As populations of *C. quinquecirrha* have not yet rebounded, there is no evidence to suggest larval recruitment into the system. Given that all life history stages have remained low there is evidence to support the idea that planula produced by medusa within the Bay are being flushed out of the system. Future studies will be needed to determine the lasting impacts of Superstorm Sandy on *C. quinquecirrha* in Barnegat Bay.

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