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

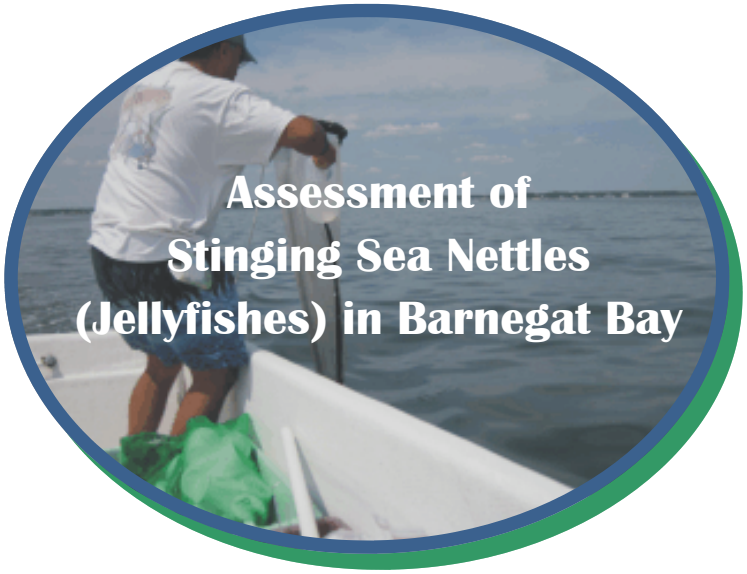
Baseline Characterization of Zooplankton in Barnegat Bay

Multi-Trophic Level Modeling of Barnegat Bay

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

Ecological Evaluation of Sedge Island Marine Conservation Zone

Barnegat Bay—Year 1



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Assessment of the Distribution and Abundance of Stinging Sea Nettles (Jellyfish) in Barnegat Bay

Final Project Report: 2013

Submitted to the New Jersey Department of Environmental Protection

Submitted by Montclair State University

Principal Investigators: Paul Bologna and John Gaynor

Acknowledgements

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List of Site Abbreviations

ME	Metedeconk River East
MW	Metedeconk River West
SBE	Silver Bay East
SBW	Silver Bay West
TRE	Toms River East
TRW	Toms River West
CCE	Cedar Creek East
CCW	Cedar Creek West
FRE	Forked River East
FRW	Forked River West
DCE	Double Creek East
DCW	Double Creek West
HCW	Harvey Cedars East
HCW	Harvey Cedars West
MBE	Manahawkin Bay East
MBW	Manahawkin Bay West
WE	Westeconk Creek East
WW	Westeconk Creek West
TCE	Tuckerton Creek East
TCW	Tuckerton Creek West

Executive Summary

During the 2012 field season, we collected samples from 16 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. During the research, 384 plankton tow samples were collected, 1152 filtered water samples were collected, and approximately 1394 lift net samples were collected during eight sampling events commencing in May and concluding in September.

The northern portion of Barnegat Bay had lower salinity compared to middle and southern regions of the bay, but no trends in oxygen or temperature were observed. Lift net samples indicted substantially higher densities of sea nettles in northern regions of the bay, while the comb jelly *Mnemiopsis leidyi* was dominant in the middle and southern regions of the bay. Seasonally, the peak in sea nettle abundance occurred in early July. Juvenile sea nettles were collected throughout the bay, but their densities were much higher in northern regions of the bay and were highly correlated with adult distributions. Water samples assessing *C. quinquecirrha* DNA indicate the presence of small individuals (i.e., early stage ephyra) throughout the bay, despite the limited number of juveniles and lack of adults from southern regions of Barnegat Bay. It is possible that the ebbing tide sufficiently flushes these individuals out of the bay, but their presence indicates proximal sources suggesting that populations of polyps exist within Little Egg Harbor. Consequently, it may just be a matter of time while polyp populations become better established that we will begin to see adult sea nettles in southern Barnegat Bay. Collection of significant quantities of *C. quinquecirrha* DNA from the 100 μm filters in all regions of Barnegat Bay indicate that the reproductive potential of the adults is wide spread in the bay and polyp colonization of new habitats in Barnegat Bay and beyond is high.

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).

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).

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.

Project Design and Methods

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. Ten paired sampling sites were established in the Barnegat Bay study area (see Figures 1, 2). 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. Figure 1 represents the sites which received settling sampling apparatus (N=10 paired sites; Objective 4), While Figure 2 represents the sites which were sampled for planktonic stages of *C. quinquecirrha* and other zooplankton species (N=8 paired sites, Objectives 1 & 2).

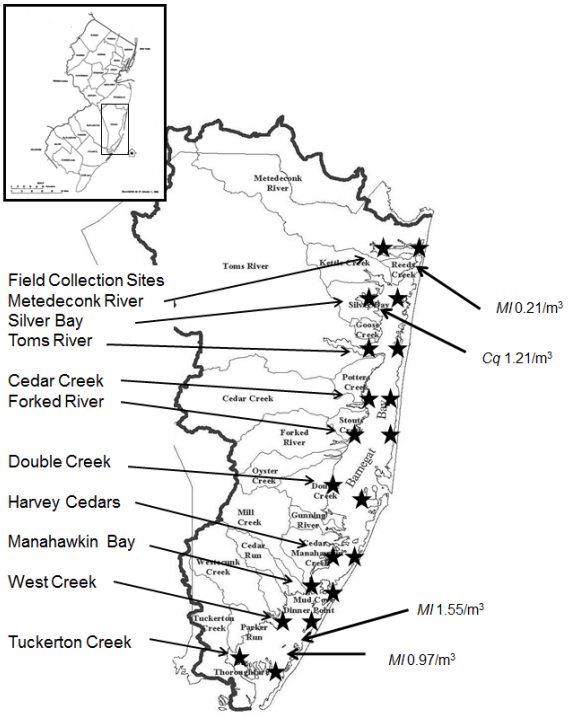

Figure 1. Research Sites for polyp settlement assessment and assessment of gelatinous zooplankton in 2011. Values represent density of *Chrysaora quinquecirrha (Cq)* and *Mnemiopsis leidya (MI)* in Barnegat Bay (N=66 lift net samples).

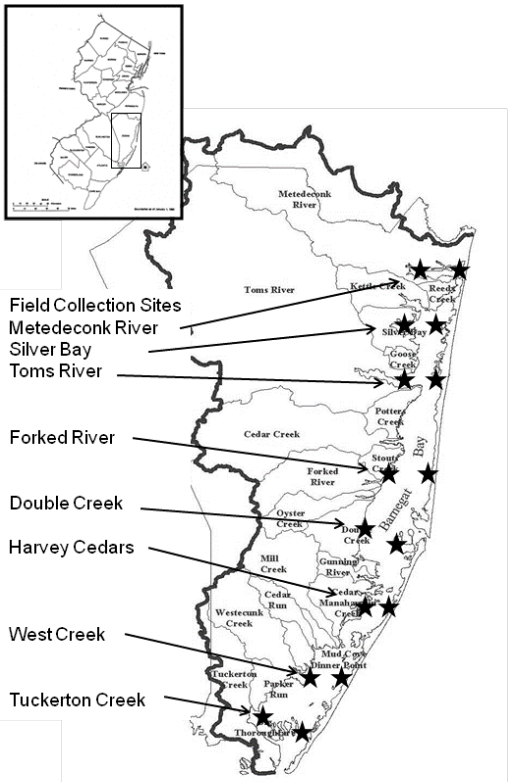

Figure 2. Research Sites for the assessment of gelatinous zooplankton.

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 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 1. Names and coordinates for the 20 sites being used to collect data for this project. Cedar Creek and Manahawkin Bay sites received settling plates only.

Site Name	GPS Coordinates N	GPS Coordinate W
Metedeconk River W	40.050983	74.064300
Metedeconk River E	40.045183	74.054117
Silver Bay W	39.992217	74.119350
Silver Bay E	39.933683	74.092100
Toms River W	39.989917	74.107567
Toms River E	39.925833	74.084733
Cedar Creek W	39.865567	74.129000
Cedar Creek E	39.863650	74.102500
Forked River W	39.821333	74.159667
Forked River E	39.815767	74.122883
Double Creek E	39.787550	74.153833
Double Creek W	39.786100	74.182700
Harvey Cedars W	39.700733	74.166050
Harvey Cedars E	39.698917	74.146000
Manahawkin Bay W	39.667833	74.212733
Manahawkin Bay E	39.663550	74.192100
Westeconk Creek W	39.620117	74.259400
Westeconk Creek E	39.598800	74.229750
Tuckerton Creek W	39.578983	74.324283
Tuckerton Creek E	39.556400	74.254433

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

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 2). 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'-GCCCCAACCAAACTGTCTTA-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 CQ 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 3 and 4). 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.

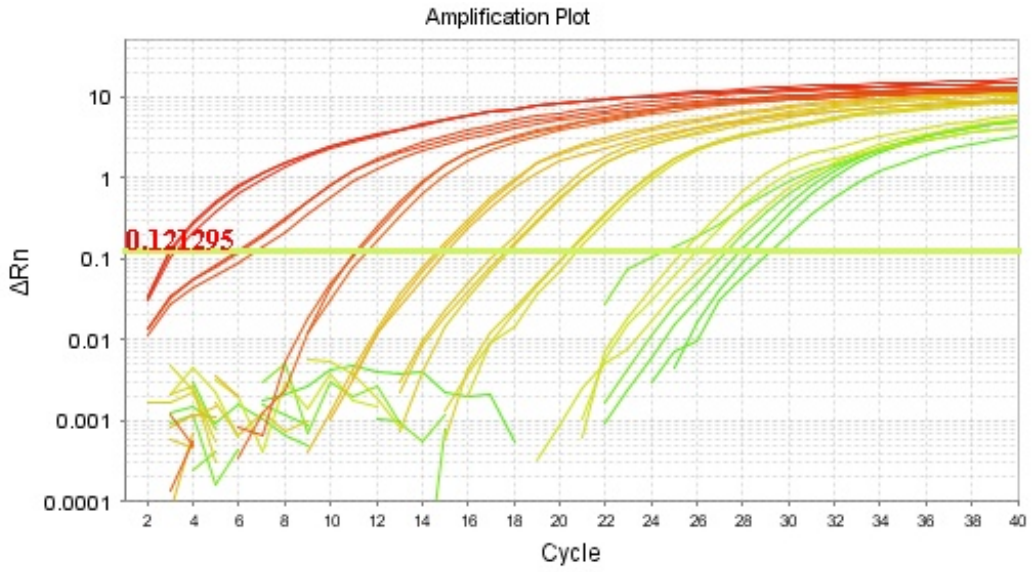

Figure 3. qPCR amplification plot of *C. quinquecirrha* DNA (Rn *vs*. cycle number). Nine concentrations of a cloned fragment of the 16S rDNA gene from *Chrysaora quinquecirrha* were run in triplicate under standard qPCR conditions described above. Concentrations cover a 9-log range (from 120 billion to 120 copies of 16S rDNA gene in a 2 µl sample). C_T treshold is calculated automatically by software (ABI StepOnePlus). NTC (no template controls) were negative and showed no amplification in 40 cycles.

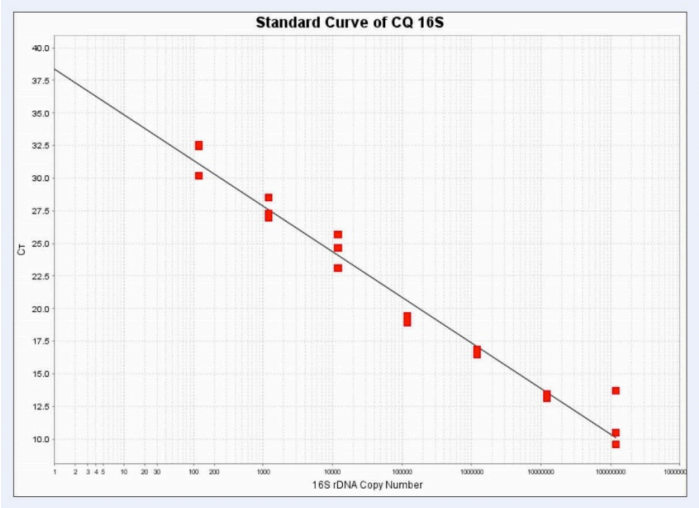

Figure 4. Standard Curve generated using known quantities of CQ 16S rDNA ranging from 12 billion copies to 120 copies of this gene. The curve shows that the reaction is linear over the 9-log range, and is highly reproducible (Slope = -3.343; y-Intercept = 37.9; R^2 = 0.929; % Efficiency = 99.124).

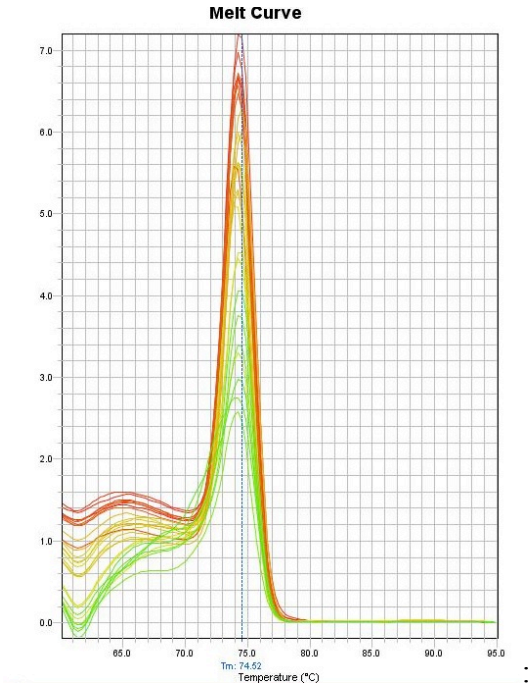

Figure 5. Melting Curve generated from 9 standards used in the *Chrysaora quinquecirrha* specific qPCR reaction above. The single peak, T_M = 74.52C, identifies that only a single molecular species has been amplified in the qPCR assay. Melting curves are generated for all qPCR runs to ensure that we 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 gelatenous 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 planktoninc 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 b). 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.

Quality Assurance

The Quality Assurance Project Plan was approved on . The completed document is included as Appendix I.

Results and Discussion

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 4). 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 5). 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 6).

Table 4. 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 5. 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 6. 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. leidyi*, *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. leidyi*, 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. leidyi* ($F = 17.64$, $P < 0.0001$; $F = 69.4$, $P < 0.0001$), and *Pleurobranchia* ($F = 1.81$, $P < 0.03$; $F = 16.6$, $P < 0.0001$). *Aurelia* and *Cyanae* 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. 6), but relatively absent from the southern region which was dominated by *M. leidyi* (Fig. 7). 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. 6). *Mnemiopsis leidyi* distribution reflects proximity to Little Egg Harbor Inlet and Barnegat Inlet (Fig. 7) 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.

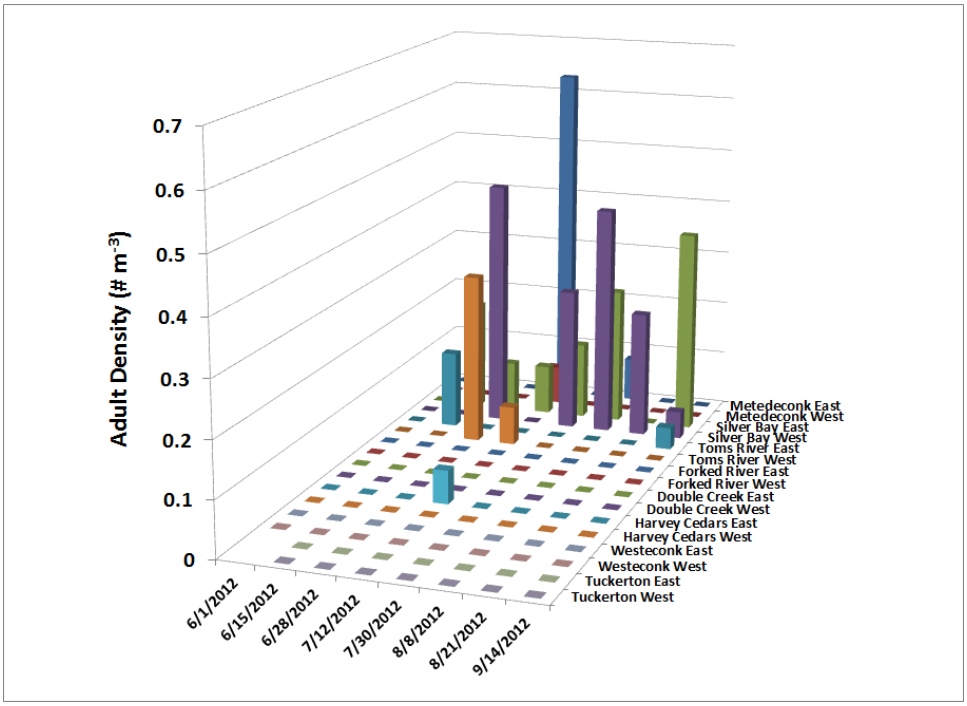

Figure 6. Spatial and temporal distribution of adult *Chrysaora quinquecirrha* in Barnegat Bay collected from lift nets during the summer of 2012 for the eight sampling events.

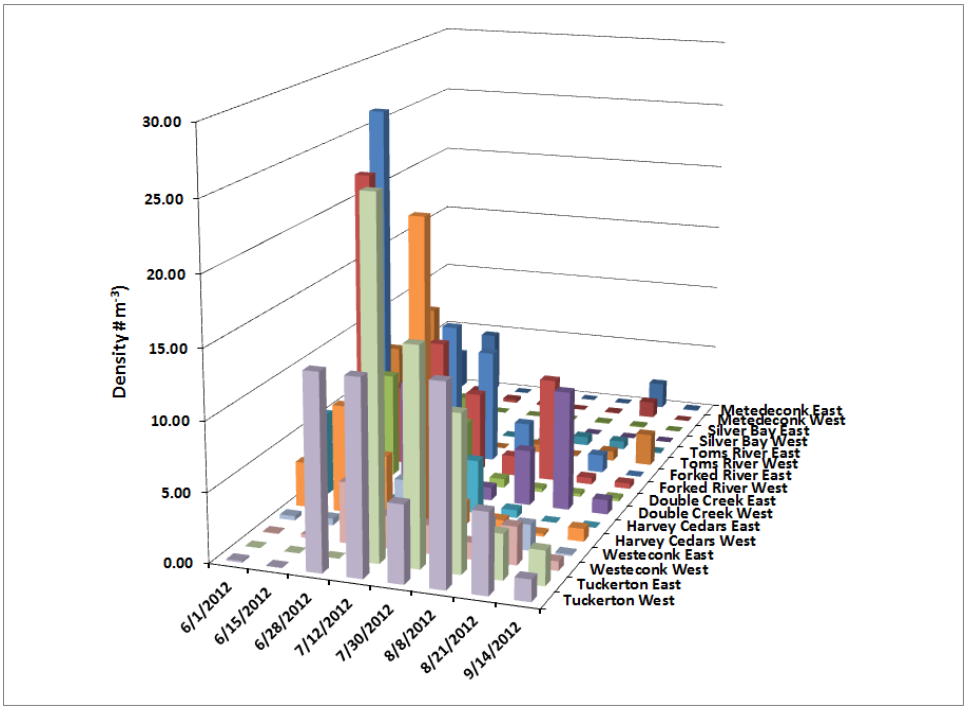

Figure 7. Spatial and temporal distribution of adult *Mnemiopsis leidyi* in Barnegat Bay collected from lift nets during the summer of 2012 for the eight sampling events.

Plankton Tow Results

A total of 64 taxamomic groups was identified from net samples and included two distinct life history stages of *C. quinquecirrha* (i.e., juvenile (Fig. 8), ephyra (Fig. 9)). Average density for the most numerically dominant groups include *Callinectes sapidus* larvae (Fig. 10, 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. 8). 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 and average density of 0.11 m^{-3}, with a maximum density of 0.56m^{-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 7) with 12 of these also exhibiting significant temporal differences.

Table 7. 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
*C. sapidus***	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
*M. leidyi****	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
*T. nutricula****	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. baltica****	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
Ostrocoda**	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
*Gammarus***	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
*C. quinquecirrha****	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
*S. fuscus**	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
*Eutima***	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

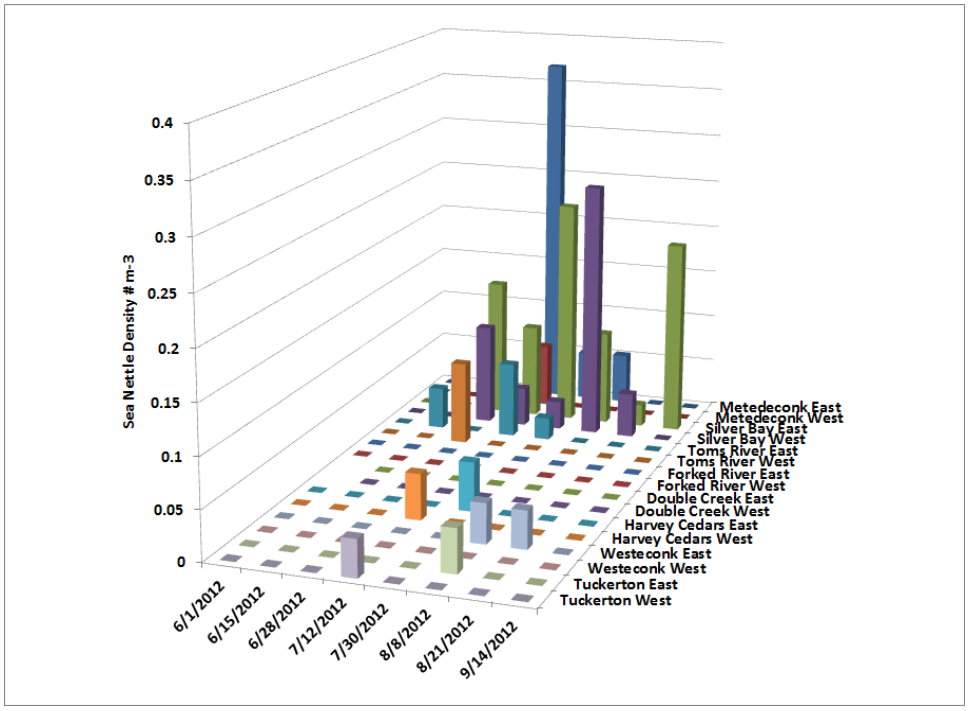

Figure 8. Density of juvenile *C. quinquecirrha* collected in plankton tows.

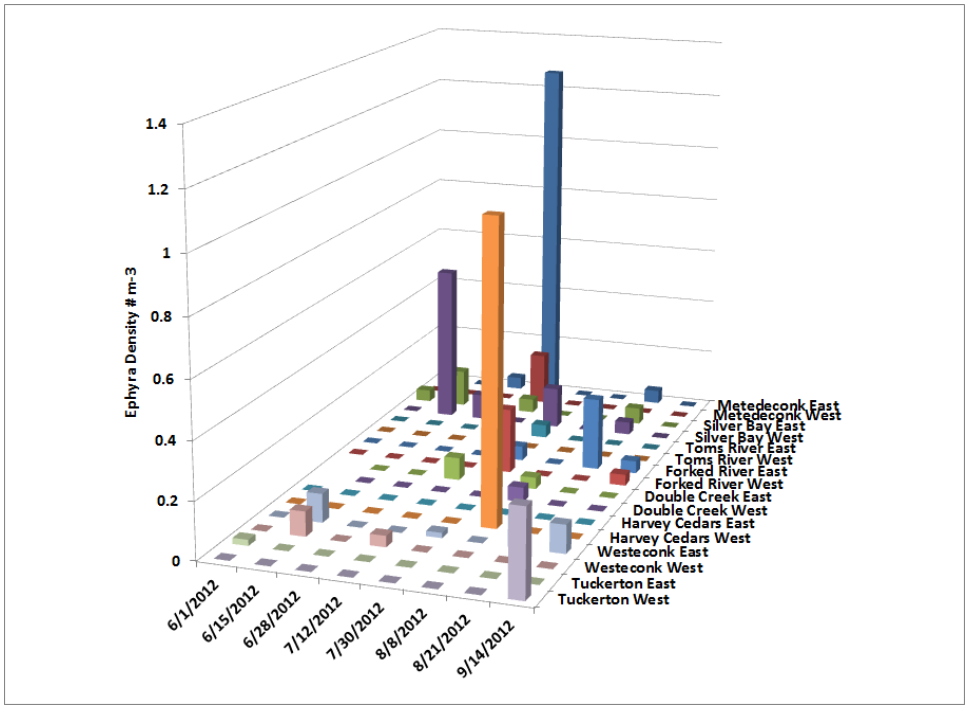

Figure 9. Density of *C. quinquecirrha* ephyra collected in plankton tows.

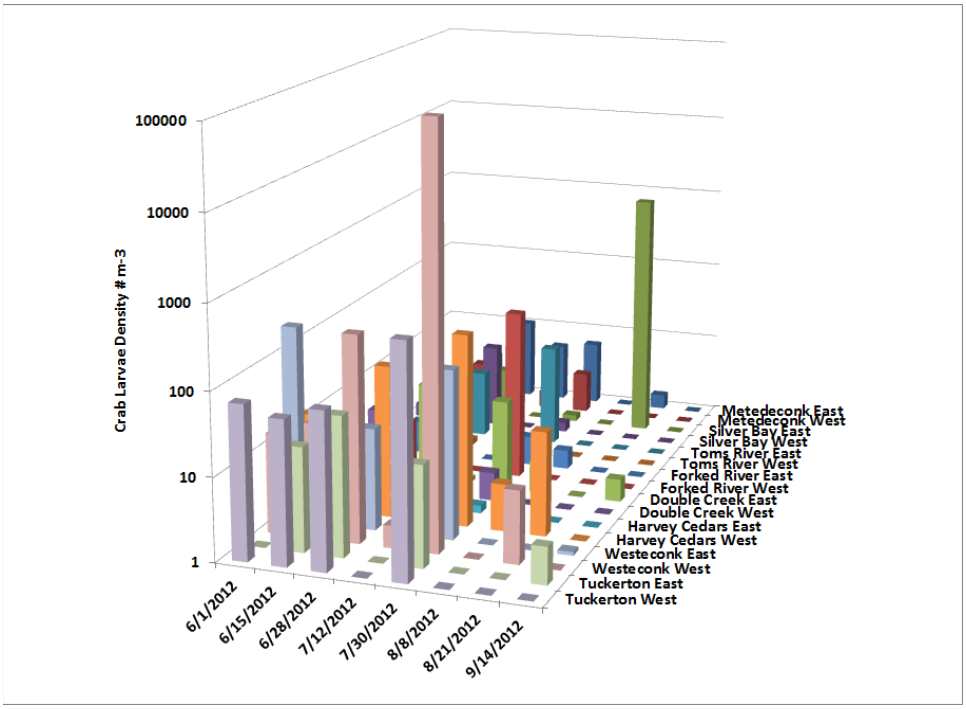

Figure 10. Crab larvae density among sites collected from plankton nets. Vertical Y-axis is presented in logarithmic scale to accommodate the extremely high density which occurred at the Westeconk Creek West site on July 30, 2012.

Correlation analysis showed some interesting patterns. Specifically, *M. leidyi* showed significant top-down impacts on several identified groups including Calanoid copepods ($P < 0.0005$), cladocerens ($P < 0.04$), 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 8). 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 8. 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 9). 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. leidyi 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 9. 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
C. sapidus	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
C. sapidus	0.69	5.74	0.72	14.81	82.39
M. leidyi	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
C. sapidus	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
C. quinquecirrha	0.34	2.69	0.55	6.53	85.35
Polychaeta Larvae	0.32	1.63	0.45	3.96	89.32
Gammarus 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
C. sapidus	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
C. quinquecirrha	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
C. sapidus	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
M. leidyi	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
C. sapidus	0.60	7.07	0.82	17.92	65.71
Fish Eggs	0.67	4.93	0.62	12.50	78.21

M. leidyi	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
C. sapidus	1.03	10.72	2.25	22.61	46.63
M. leidyi	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
Idotea baltica	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.%
C. sapidus	1.18	12.88	1.78	26.94	26.94
M. leidyi	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.%
C. sapidus	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
M. leidyi	0.82	6.03	1.01	13.14	70.46
Fish Eggs	0.87	4.50	0.92	9.79	80.25
Ostrocoda	0.48	1.79	0.50	3.89	84.15
Idotea baltica	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.%
M. leidyi	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
C. sapidus	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
C. sapidus	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
M. leidyi	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
C. sapidus	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
M. leidyi	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.%
C. sapidus	1.61	11.93	2.75	25.98	25.98
Caridea	1.39	11.52	3.45	25.08	51.06
M. leidyi	0.77	5.69	0.73	12.38	63.44
Calanoida	1.14	5.25	0.84	11.42	74.86
Idotea baltica	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.%
C. sapidus	3.65	10.03	1.07	25.83	25.83
Calanoida	1.97	9.57	1.03	24.65	50.48
M. leidyi	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
C. sapidus	1.22	6.81	1.19	16.10	59.76
M. leidyi	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.%
M. leidyi	1.33	13.83	0.81	32.25	32.25
Caridea	1.26	8.76	1.45	20.42	52.67
C. sapidus	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 Westeconk West site which demonstrated extremely high DNA concentrations on June 28th (Fig. 11). 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. 11). The pulse on August 8th was significantly greater than all other dates except for June 28th, where the high concentrations were identified from Westeconk 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. 6). 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.

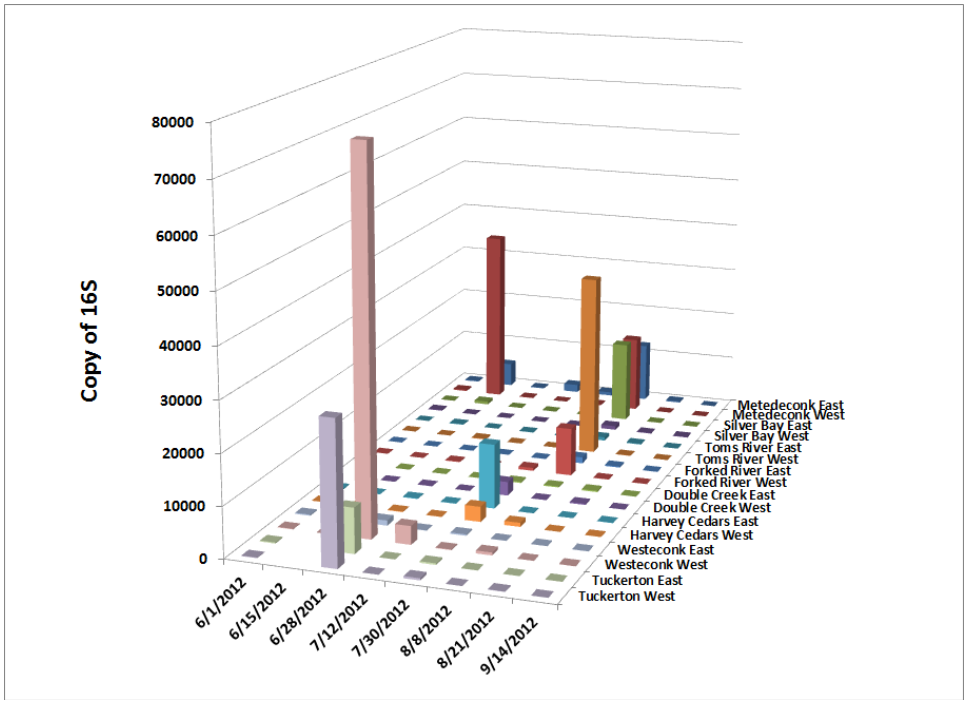

Figure 11. Relative abundance of sea nettle DNA on 500 µm filters in water samples.

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. 12). 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.

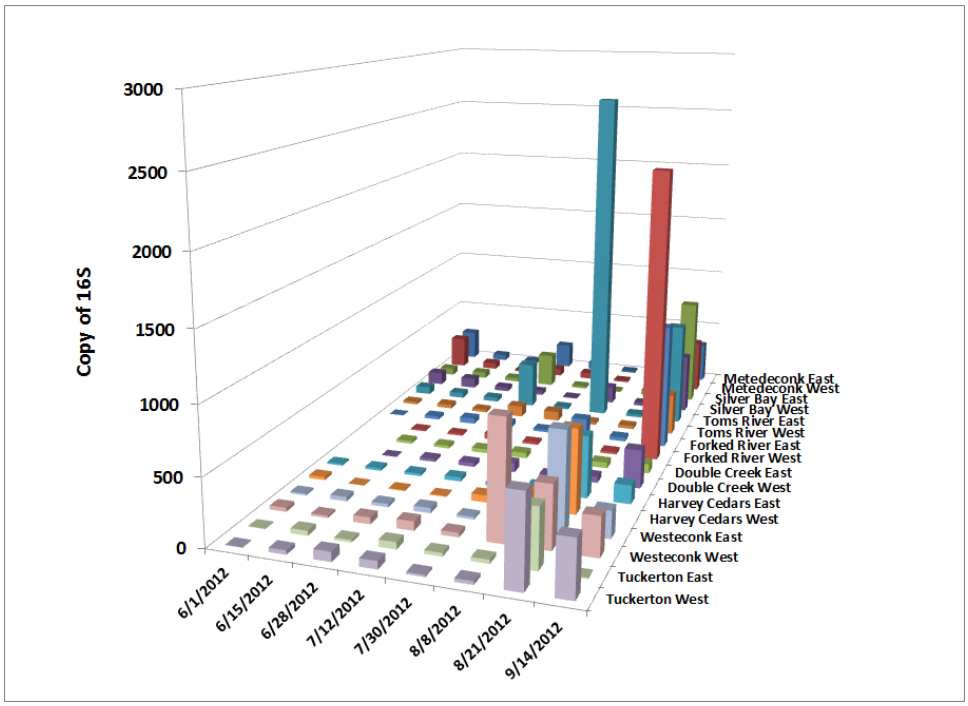

Figure 12. Relative abundance of sea nettle DNA on 100 µm filters in water samples.

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. 13). DNA copy number on our 500 µm and 100 µm filters are orders of magnitude greater (see Figs. 11, 12). 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).

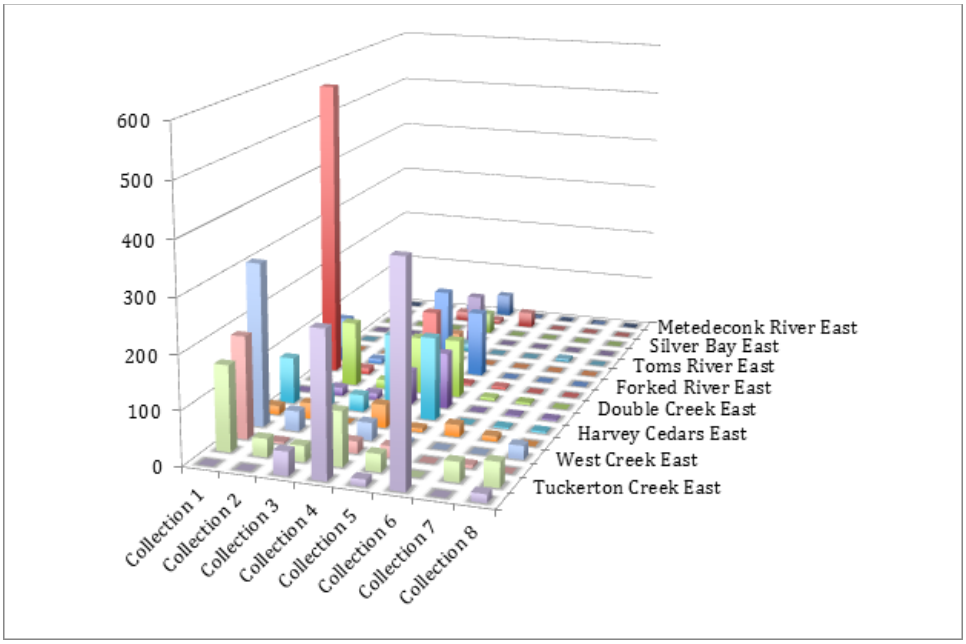

Figure 13. Relative abundance of sea nettle DNA on 0.45 µm filters in water samples.

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 10). 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 11), while DNA copy number provided no significant results (Table 12). 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 13).

Table 10. 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 11. 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 12. 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 13. 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 → 2	28.7	107.89	1.1	0.29	0.08
2 → 3	28.01	6.0	0.18	0.86	0.002
3 → 4	76.25	229.6	1.41	0.18	0.12
4 → 5	36.6	-116.3	-1.56	0.14	0.15

5 → 6	302.6	-684.9	-0.45	0.66	0.014
6 → 7	247.7	-975.9	-0.75	0.46	0.039
7 → 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 14) 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 (Table 6). 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 14. 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 = Ascidians.

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

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 ostrocods, 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* predates 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.

If the fundamental goal of studying the gelatinous zooplankton is to develop management strategies to combat their increase, greater research is needed to understand the distribution and abundance of the polyp stage of *C. quinquecirrha*. Increasing development, continued eutrophication, and depleted oxygen levels in coastal waters favor *C. quinquecirrha* over other

organisms. As such, they can out-compete other fouling species for space and asexually spread and expand. Since this life-history stage is critical for overwintering, understanding the dynamics and survival of polyps is necessary to develop reasonable management strategies to limit their expansion or reduce their numbers.

Recommendations and Application for the NJ Department of Environmental Protection

Currently, gelatinous zooplankton are abundant and important components to the planktonic community. They have the potential to exert top-down pressure in these communities and simultaneously act as competitors and predators of commercially and recreationally important fish and invertebrates through consumption of shared food resources (e.g., copepods) and direct consumption of eggs, larvae and early juveniles. If populations continue to increase in Barnegat Bay, it is possible that they may become the top seasonal predators and potentially disrupt food webs leading to 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.

List of Presentations

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. PI Bologna also provided a public lecture entitled "*Chrysaora quinquecirrha* in Barnegat Bay" to Save the Bay at their annual meeting. This lecture highlighted the preliminary research findings as well as biology and ecology of sea nettles in Barnegat Bay. We also presented a paper at the 4th International Jellyfish Bloom Conference in Hiroshima, Japan discussing the time-lag assessment of the appearance of DNA to juvenile, adults and then adults to larvae. Additionally, our students presented findings from our research as part of the Master's Degree research.

October 2012, Atlantic Estuary Research Society Meeting
October 2012, MACUB Meeting
March 2013, Benthic Ecology Meeting
April 2012, 2013, New Jersey Academy of Sciences Meeting

We also presented our research at the 4th International Jellyfish Bloom Symposium, June, 2013.

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

Abstract: 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.

Bibliography

Bayha, K.M. and W. M. Graham. 2009. A new Taqman PCR-based method for the detection and identification of scyphozoan jellyfish polyps. Hydrobiologia. 616:217–228.

Bednarski, M. and A. Morales-Ramirez. 2004. Composition, abundance and distribution of macrozooplankton in Culebra Bay, Gulf of Papagayo, Pacific Coast of Costa Rica and its value as a bioindicator of pollution. Revista de Biologia Tropical. V.52, supl.2,

Boero, F. Bouillon J., Gravili C., Miglietta M. P, Parsons T., and Piraino S. 2008. Gelatinous plankton: irregularities rule the world (sometimes). Marine Ecology Progress Series. 356: 299–310.

Bridge, D., C. W. Cunningham, B. Schierwater, R. Desalle, and L. W. Buss. 1992. Class-level relationships in the phylum Cnidaria - evidence from mitochondrial genome structure. Proceedings of the National Academy of Science. 89:8750-8753.

Brodeur, R., Decker, M., Ciannelli, L., Purcell, J., Bond, N., Stabeno, P., Acuna, E., Hunt, G., 2008. Rise and fall of jellyfish in the eastern Bering Sea in relation to climate regime shifts. Progress in Oceanography 77:103-111

Cargo, D. and Rabenold, G. 1980. Observation on the asexual reproductive activities of the sessile stages of the sea nettle *Chrysaora quinquecirrha* (Scyphozoa). Estuaries 3: 20-27.

Champalbert, G., M. Pagano, P. Sene, and D. Corbin. 2007. Relationships between meso- and macro-zooplankton communities and hydrology in the Senegal River Estuary. Estuarine, Coastal and Shelf Science 74: 381-394.

Clesceri, L., A. Greenberg, A. Eaton, and M.A. Franson. 1998. Standard Methods for the Examination of Water and Wastewater. Prepared and published jointly by American Public Health Association, American Water Works Association and Water Environment Federation. American Public Health Association, Washington, D.C.

de Almeida e Silva, T., S. Neumann-Leitao, R. Schwamborn, L.M. de Oliveira, and D.A do Nascimento-Vieira. 2003. Diel and seasonal changes in the macrozooplankton community of a Tropical Estuary in Northeastern Brazil. Revista Brasileira de Zoologia 20: 439-446.

Dhanasekaran, S., Doherty, T.M., and J. Kenneth. 2010. Comparison of different standards for real-time PCR-based absolute quantification. Immunological Methods. 354 (1–2): 34–39.

Gaynor, J.J., and Tare. A. 2009. The 16S rRNA gene of *Chrysaora quinquecirrha*. Genbank #GU300724 http://www.ncbi.nlm.nih.gov/nuccore/283826627

GO. 2006. General Oceanics Digital Flow Meter Mechanical and Electronic Operators Manual. General Oceanics, Inc. Miami, FL.

Grove, M.W. and Breitburg, D.L. 2005. Growth and reproduction of gelatinous zooplankton exposed to low dissolved oxygen. Marine Ecology Progress Series 301: 185-198.

Hay, S. 2006. Marine ecology: gelatinous bells may ring change in marine ecosystems. Current Biology 16: R679-R682.

Hong, J., He-Qin, C., Hai-Gen, X., Arreguin-Sanchez, F., Zetina-Rejon, M., Del Monte Luna, P., LeQuesne, W. 2008. Trophic controls of jellyfish blooms and links with fisheries in the East China Sea. Ecological Modeling 212: 492-503

Hoover, R., and Purcell, J. 2009. Substrate preferences of scyphozoan *Aurelia labiata* polyps among common dock-building materials. Hydorbiologia 616: 259-267.

Javidpour, J., Molinero JC, Peschutter Sommer U. 2009. Seasonal changes and population dynamics of the ctenophore *Mnemiopsis leidyi* after its first year of invasion in the Kiel Fjord, Western Baltic Sea. Biological Invasions. 11:873–882

Johnson, W. and D. Allen. 2005. Zooplankton of the Atlantic and Gulf Coasts: A Guide to Their Identification and Ecology. Johns Hopkins University Press. Baltimore, MD.

Kaufman, R., E. Fisher, W. Gill, A. King, M. Laubacher, and B. Sullivan. 2003. Temporal patterns in the bistribution, biomass and community structure of macrozooplankton and micronekton within Port Foster, Deception Island, Antarctica. Deep-Sea Research II 50: 1765-1785.

Kennish, M. 2001. Barnegat Bay – Little Egg Harbor, New Jersey: Estuary and Watershed Characterization. Journal of Coastal Research. Vol. SI, No. 32, 2001:163-167.

Kideys A.E., Roohi, A,. Bagheri, S., Finenko, G., Kamburska, L. 2005. Impacts of invasive ctenophores on the fisheries of the Black Sea and Caspian Sea. Oceanography (Black Sea Spec Issue) 18(2):76–85.

Kirby, RR and Beaugrand, G. 2009. Trophic amplification of climate warming. Proceedings of the Royal Society B 276: 4095-4103.

Kirby, RR, Beaugrand, G and Lindley, JA. 2009. Synergistic effects of climate and fishing in a marine ecosystem. Ecosystems 12: 548-561.

Lynam, C.P., Gibbons, M.J., Axelsen, B.E., Sparks, C.A.J., Coetzee, J., Heywood, B.G. and Brierley, A.S. 2006. Jellyfish overtake fish in a heavily fished ecosystem. Current Biology 16: R492-R493.

Mountford, K. 1984. Phytoplankton. Chapter in: M. Kennish and R. Lutz. Ecology of Barnegat Bay, New Jersey. Springer-Verlag. Lecture Notes on Coastal and Estuarine Studies. New York, NY.

Nelson, T. 1925. On the occurrence and food habits of ctenophores in New Jersey incland coastal waters. Biological Bulletin 48:92-111.

Newell, G. and R. Newell. 1973. Marine Plankton: A Practical Guide. Hutchinson Educational. London, WI.

Nailis H, Coenye T, Van Nieuwerburgh F, Deforce D, Nelis HJ. 2006. Development and

evaluation of different normalization strategies for gene expression studies in *Candida albicans* biofilms by real-time PCR. BMC Molecular Biology. 7: 25-32.

Newell, G. and R. Newell. 1973. Marine Plankton: A Practical Guide. Hutchinson Educational. London, WI.

Pauly, D., Christensen, V., Dalsgaard, J., Froese, R., and Torres, F. Jr. 1998. Fishing down marine food webs. Science 279: 860-863.

Pauly, D., Watson, R., and Alder, J. 2005. Global trends in world fisheries: impacts on marine ecosystems and food security. Philosophical Transactions of the Royal Society of London B. 360: 5-12.

Purcell, J.E., Arai, M.N., 2001. Interactions of pelagic cnidarians and ctenophores with fish: a review. Hydrobiologia 451, 27–44.

Purcell, J.E., Breitburg, D.L. Decker, M.B., Graham, W.M., Youngbluth, M.J. and Raskoff, K.A. 2001. In: Coastal hypoxia: consequences for living resources and ecosystems (Rabalais, N.N. and Turner, R.E., eds.) American Geophysical Union, Washington, DC.

Purcell, J.E. and Decker, M.B. 2005. Effects of climate on relative predation by scyphomedusae and ctenophores on copepods in Chesapeake Bay during 1987-2000. Limnology and Oceanography 50: 376-387.

Purcell, J., Uye, S., Lo, W., 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Marine Ecology Progress Series 350:153-174

Reeve, M. R., Walter, M. A., Ikeda, T. 1978. Laboratory studies of ingestion and food utilization in lobate and tentaculate ctenophores. Limnology and Oceanography. 23: 740-751

Richardson, A.J., Bakun, A., Hays, G.C., and Gibbons, M.J. 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends in Ecology and Evolution 24(6): 312-322.

Sandine, P. 1984. Zooplankton. Chapter in: Kennish, M. and R. Lutz. 1984. Ecology of Barnegat Bay, New Jersey. Lecture Notes on Coastal and Estuarine Studies. Springer-Verlag. New York, NY.

Smith, D. and K. Johnson. 1996. A Guide to Marine Coastal Plankton and Marine Invertebrate Larvae (second edition). Kendall/Hunt Publishing Co. Dubuque, IW.

Sullivan, BK, Van Keuren, D and Clancy, M. 2001. Timing and size of blooms of the ctenophore *Mnemiopsis leidyi* in relation to temperature in Narragansett Bay, RI. Hydrobiologica 451: 113-120.

Todd, C., M. Laverack and G Boxshall.1996. Coastal Marine Zooplankton: A Practical Manual for Students. Cambridge University Press. Cambridge, UK.

USEPA. 1994. Standard Operating Procedures for Phytoplankton Sample Collection and Preservation. U.S. Environmental Protection Agency. Grace Analytical Lab. Chicago, IL.

USEPA. 2002. Standard Operating Procedures for Phytoplankton Sample Collection and Preservation (Revised). U.S. Environmental Protection Agency. Grace Analytical Lab. Chicago, IL.

Walsh, P.G., Metzger, D.A., and R. Higuchi. 1991. Chelex R100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506-513.

Young, C. (ed) and M. Sewell and M. Rice (assoc. eds.). 2006. Atlas of Marine Invertebrate Larvae. Academic Press. Burlington, MA.

Appendices are provided in electronic formats on associated CD.