

# Plan 9: Research

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

Barnegat Bay Diatom  
Nutrient Inference Model

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

Assessment of Stinging Sea  
Nettles (Jellyfishes) in  
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

Hard Clams as Indicators of  
Suspended Particulates  
in Barnegat Bay

Dr. V. Monica Bricelj, Rutgers University,  
Principal Investigator

John Kraeuter , Rutgers University, Co-Investigator  
Gef Flimlin, Rutgers University, Co-Investigator

Project Manager:

Bruce Ruppel, Division of Science, Research  
and Environmental Health

Thomas Belton, Barnegat Bay Research Coordinator

Dr. Gary Buchanan, Director—Division of Science,  
Research & Environmental Health

Bob Martin, Commissioner, NJDEP

Chris Christie, Governor



**Benthic-pelagic coupling: hard clams as indicators of suspended particulates  
in the Barnegat Bay – Little Egg Harbor estuary**

**Final Report prepared for the New Jersey Department of Environmental  
Protection (NJDEP)**

**October 2013**

**Principal Investigators:**

Lead PI: **V. Monica Bricelj**, Research Professor,  
Institute of Marine and Coastal Sciences (IMCS),  
Rutgers University (RU), 71 Dudley Road,  
New Brunswick, NJ 0801  
Ph.: (848) 932-9709; Fax: (732) 932-8578;  
E-mail: [mbricelj@marine.rutgers.edu](mailto:mbricelj@marine.rutgers.edu)

Co-PI: **John Krauter**, Visiting Scientist,  
Haskin Shellfish Research Laboratory,  
Rutgers University, 6959 Miller Avenue,  
Port Norris, NJ 08349-3167  
Ph.: (856) 785-0074, ext. 4331; Fax: (856) 785-1544  
E-mail: [krauter@hsrl.rutgers.edu](mailto:krauter@hsrl.rutgers.edu)

Co-PI: **Gef Flimlin**, Professor,  
Marine Extension Agent Cooperative Extension of Ocean County,  
1623 Whitesville Road, Toms River,  
New Jersey 08755-1199.  
Ph.: (732) 349-1152; e-mail:  
E-mail: [flimlin@aesop.rutgers.edu](mailto:flimlin@aesop.rutgers.edu)

## **Final Report for the project titled “Benthic-pelagic coupling: hard clams as indicators of seston in the Barnegat Bay-Little Egg Harbor estuary”**

Prepared for the New Jersey Department of Environmental Protection (NJDEP), Office of Science, P.O. Box 420, 428 E. State St., Trenton, NJ 08625-0420.

By V. Monica Bricelj<sup>1</sup>, Gef Flimlin<sup>2</sup> and John Kraeuter<sup>3</sup>

<sup>1</sup>Institute of Marine and Coastal Sciences, Rutgers University 71 Dudley Road, New Brunswick, NJ 08901

<sup>2</sup>Marine Extension Agent Cooperative Extension of Ocean County, 1623 Whitesville Road, Toms River, NJ 08755-1199.

<sup>3</sup>Visiting Scientist, Rutgers University Haskin Shellfish Laboratory, Port Norris, NJ 08349

### **Background**

Mid-Atlantic shallow coastal bays have experienced progressive eutrophication, and environmental degradation, as evidenced by increased macroalgal growth, harmful algal blooms (brown tide), proliferation of gelatinous zooplankton, and loss of bottom habitat (e.g. submerged aquatic vegetation, SAV). These effects may result in shifts in food web structure, loss of fisheries, serious decline in ecosystem services, and declining human uses of estuaries.

The Barnegat Bay-Little Egg Harbor (BB-LEH) estuary, NJ, has experienced a historical decline in stocks of the suspension-feeding hard clam (=quahog), *Mercenaria mercenaria*, (Gastrich and Celestino 2003; reviewed by Bricelj et al. 2012), and in SAV, especially eelgrass *Zostera marina* (Kennish et al. 2010, 2012). Hard clam populations have also experienced a dramatic decline in other Atlantic coastal lagoonal ecosystems, such as Long Island’s south shore estuaries (SSE), NY, and inland MD bays (Chincoteague and Assawoman Bay, MD). The precipitous decline of hard clams in SSE in the 1980s was clearly attributed to overfishing (Kraeuter et al. 2008), but continued decline of this population, despite markedly reduced fishing pressure in recent decades, has led to postulate other potential contributing factors, which could also be operating in BB-LEH. These include potential changes in the food supply that may lead to poor recruitment, growth and compromised reproductive success of hard clams (Bricelj 2009). Therefore, characterization of food quantity and quality of suspended particulates and their relationship to bivalve somatic and reproductive growth is required. This characterization has often required measurement of multiple parameters (Newell et al. 2009; Powell et al. 2012), as single metrics are often inadequate. Total Chlorophyll *a* concentrations alone tend to underestimate the food supply for suspension-feeding bivalves.

Recent short-term studies indicate that there are strong spatial gradients in food quality/quantity across Long Island SSE, NY, and Sandy Hook Bay, NJ, during years of no or low brown tide, that are associated with marked differences in hard clam production (Newell et al. 2009; reviewed by Bricelj 2009). Empirical data have shown that the food supply for benthic suspension-feeders such as *M. mercenaria* remains ill-defined, and larval model simulations showed that variation in food quality had much greater effects on hard clam larval metamorphic success than changes in temperature and food quantity (Bricelj 2009).

Specific algal species, classes and/or size groups are known to play a role in limiting the production of suspension-feeding bivalves. The BB-LEH estuary has experienced toxic brown

tides of the picoplankter *Aureococcus anophagefferens* (Pelagophyceae), especially in the southern portion of BB and in LEH (Olsen and Mahoney 2001; Mahoney et al. 2006). *Aureococcus anophagefferens* attained high peak bloom densities exceeding  $1 \times 10^6$  cells  $\text{ml}^{-1}$  in 1995, and over four consecutive years between 1999 and 2003. More moderate cell densities ( $\leq 200,000$  cells  $\text{ml}^{-1}$ ) were documented in 1988, 1997, 2003 and 2004, but routine monitoring for brown tide in BB-LEH ceased after that. Feeding inhibition of juvenile hard clams occurs at *A. anophagefferens* densities  $\geq 35,000$  cells  $\text{ml}^{-1}$  (Bricelj et al. 2001), and growth ceases above a threshold density of 400,000 cells  $\text{ml}^{-1}$  (Bricelj et al. 2004). Peak densities in mid-Atlantic estuaries typically occur between mid-May and early June, although lower-intensity blooms can also occur in the fall. The BB-LEH estuary is also characterized by high abundances of other “small forms” such as the chlorophyte *Nannochloris atomus* and blue green alga *Synechococcus* sp. which are poorly captured and digested by hard clams (Bricelj et al. 1984a). Picoplankters (here defined as bacteria or microalgae in the 0.2 to 2  $\mu\text{m}$ -size range), are poorly retained by *M. mercenaria*, as gill retention efficiency in post-metamorphic stages of this species declines rapidly below a particle size of  $\sim 3\text{-}4$   $\mu\text{m}$ . Above this threshold hard clams retain particles with 100% efficiency (Grizzle et al. 2001). The impact of brown tide, picoplankton in general, and cyanobacteria and chlorophytes of poor nutritional value on suspension-feeding bivalves, including hard clams and oysters, in the BB-LEH system in recent years remains unknown.

The value of bivalves as indicators of pollution, water quality and environmental perturbation, due to their sessile habit and high filtration rates, is well established by NOAA’s Mussel Watch Program (Kimbrough et al. 2008). Hard clams, especially during juvenile stages when their growth response is most rapid, can thus provide an ideal indicator of the environmental conditions in shallow Atlantic estuaries that, due to their relatively long residence times, are particularly susceptible to the effects of nutrient enrichment. Due to its importance as a commercial native shellfish species, *M. mercenaria* has been the focus of population enhancement efforts in the mid-Atlantic, e.g. in Great South Bay, Long Island, NY (Doall et al. 2008) and to a lesser extent in BB-LEH (Barnegat Bay Shellfish Restoration/ReClam the Bay, through Rutgers Cooperative Extension of Ocean County). Future investment and management decisions on the value, scale and siting of shellfish restoration efforts relies on demonstrating that present environmental conditions (especially the food supply) in these bays is adequate to support self-sustaining populations. Characterization of spatial patterns in growth will also be useful in selecting sites for hard clam stock enhancement.

## Objectives

Our overall goal was to characterize the seasonal quality and quantity of suspended particulate matter (seston) for bivalve suspension-feeders in the BB-LEH estuary using the hard clam, *Mercenaria mercenaria*, a shellfish species that once supported major commercial and recreational fisheries in this ecosystem, as a biosensor. Specific objectives of the present study were a) to assess temporal (weekly) and spatial variability in growth of juvenile hard clams over a range of environmental conditions at 4 contrasting field sites in LEH-BB, and b) to attempt to relate the clams’ *in situ* growth rates to key environmental conditions, temperature, salinity and key characteristics of the seston/food supply. The latter included Chlorophyll *a*, a measure of phytoplankton biomass, particulate organic carbon and nitrogen (POC, PON), total suspended solids, particulate organic matter (POM) and inorganic matter (PIM), and particulate organic and inorganic matter (POM, PIM).

This project was supplemented via a parallel study funded by the Barnegat Bay Partnership (BBP) titled: “**Characterization of phytoplankton functional taxonomic groups in relation to juvenile hard clam production in the Barnegat Bay-Little Egg Harbor Estuary (BB-LEH)**”. The main objective of the latter was to determine the composition of key phytoplankton functional groups (FTGs) via analysis of photopigments by high-performance liquid chromatography (HPLC) from split water samples collected at the same 4 sites where clams were deployed as part of the current study. Phytoplankton photopigments have been used in other Atlantic estuaries as indicators of eutrophication and environmental conditions (Paerl et al. 2003). This analysis also allowed direct comparison of FTGs with microscopically-determined phytoplankton species composition at two of the 4 sites (Sedge Is. and IBSP) on selected sampling dates. Some of our preliminary results are presented in this report as they are helpful in interpreting spatial and temporal pattern in observed clam growth rates.

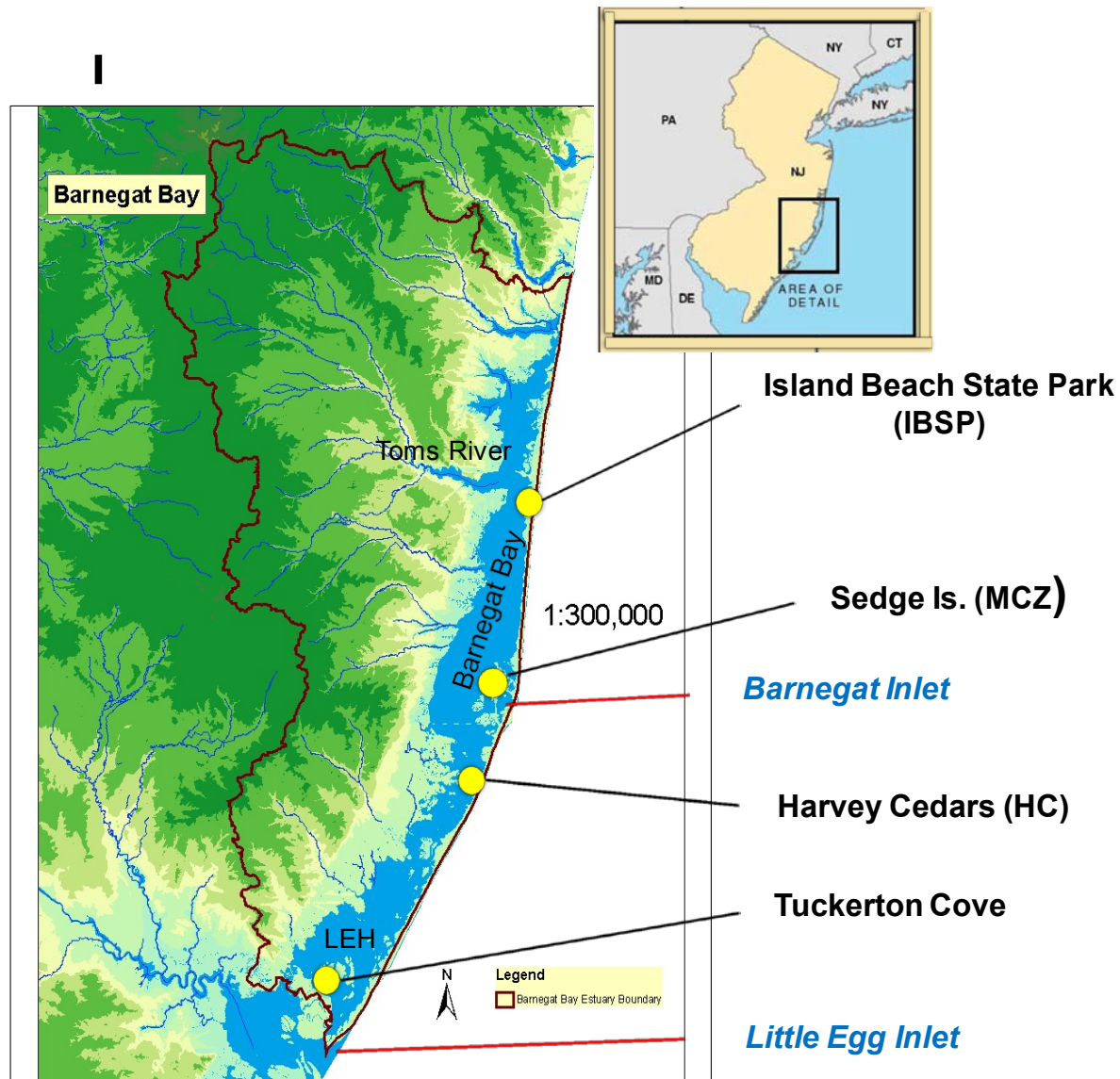
## Methods

### Study sites

Juvenile hard clams were deployed at the following four sites in BB-LEH, listed from north to south (Fig. 1):

- Island Beach State Park (IBSP), northern BB, southeast of Toms River. Here we compared the performance of juvenile clams at the approved field location and at the adjacent land-based Barnegat Bay Shellfish Restoration Program (BBSRP) upweller system. This provided useful future information on whether growth in the upweller systems is representative of that obtained at a nearby field site.
- Sedge Island Marine Conservation Zone (MCZ), central BB, where NJDEP hard clam stock enhancement activities have been conducted in the past. This area differs in characteristics from mid-Bay stations due to its proximity to Barnegat Inlet. The bottom is covered with eelgrass, *Zostera marina*.
- Harvey Cedars (HC), Long Beach, southern BB, off Long Beach Island.
- Tuckerton Cove, on the western shore of LEH. This is a site of past clam relaying activities and provided highly productive habitat in the past (Carriker 1961).

**Figure 1.** Barnegat Bay-Little Egg Harbor (BBLEH) estuary, NJ, and its watershed. Inset shows the location of the study area in the mid-Atlantic. MCZ = Marine Conservation Zone. Yellow circles indicated the four selected field study sites for off-bottom deployment of juvenile hard clams. Latitude/longitude coordinates for field sites are as follows: IMBS field site: 39°54' 20.2818"N/74°05'16.209"W; Sedge site: 39° 47' 40.5"N/-74° 07' 06.8"W; Harvey Cedars: 39°42' 30.45"N/74°08'16.24"W; Tuckerton Cove: 39°33'48.51N/74°20'23.07"W.



### Clam deployment and sampling.

Juvenile clams (~8-10 mm shell length, SL) were obtained from local, NJ hatchery sources and deployed in bottom cages (18" x 18" x 18" high; n = 4 cages per site), that are divided into 3 levels (Fig. 2). Cages were deployed in relatively shallow waters (<2 m) marked by surface buoys and weighed with concrete blocks inserted in the bottom compartment. Clams were contained in mesh bags placed in the middle shelf. Each mesh bag contained 300 to 500 juvenile clams depending on initial size, a low density that precludes density-dependent growth inhibition; at each sampling date ~30 to 50 clams were removed without replacement from each of the cages. Cages were deployed twice (deployments are referred to as Trial I and II) for a total of 11 weeks from early June to mid-September. Deployment of clams above bottom was selected for the purposes of this study, to preclude the confounding effects on clam growth of substrate type, associated near-bottom sediment resuspension, and also to reduce potential access by bottom predators.



**Figure 2.** Vinyl-coated wire cages used for clam deployment in mesh bags held off-bottom.



Clam survival/recovery, and shell and tissue growth rate were determined on a weekly basis during each deployment period. Clam mortalities were determined in situ based on the number of empty valve pairs, but any additional dead individuals not identified by this method were confirmed by prying open the shells upon arrival to the laboratory.

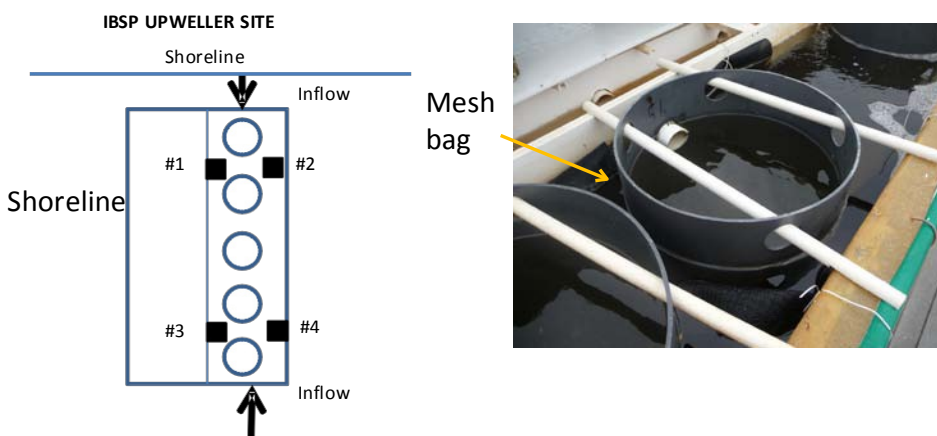
Both absolute and relative growth rates, the latter calculated as the instantaneous growth coefficient (% change day<sup>-1</sup>) were determined as:  $k = [\ln X_f - \ln X_o] / \text{time interval in days} \times 100$ , where  $X_f$  and  $X_o$  are the mean final and initial shell length (SL), or soft-tissue dry weights (DW) of clams in each cage. This parameter is suitable for the calculation of juvenile growth as the latter is typically exponential during this life history stage. It provides a relative measure of growth and reduces the confounding effect of initial clam size. Shell length, the greatest antero-posterior dimension, was determined with digital calipers ( $\pm 0.01$  mm). Tissue DW was determined following dissection of tissues and oven-drying to a constant weight (24 to 48 h depending on size) at  $\sim 60^\circ\text{C}$ . Fifty clams for DW and 100 clams for SL were sampled initially. Tissue mass is a more sensitive parameter to measure growth rates than the increase in SL, and can also reflect weight loss during periods of poor food supply. Individual tissue DW was determined with a Cahn electrobalance ( $\pm 0.1$   $\mu\text{g}$ ). Condition index was determined for individual clams as: soft tissue DW (mg)/SL (mm)<sup>3</sup>  $\times 1000$ .

**Trial I.** At all sites a cohort of juvenile clams (mean shell length, SL = 10.97 mm, standard deviation, SD = 1.65, n = 100) were deployed in modified window screen mesh bags (1x2 mm square mesh). At 2 of the 4 field sites, IBSP and Sedge Is., we compared the growth performance of these clams to that of a somewhat larger cohort from the same hatchery source (mean SL = 13.15, SD = 1.224, n = 47) held in Vexar bags ( $\sim 6$  mm square mesh) to assess the effect of mesh

size on growth rates. A flow chart is included in Appendix 1 to describe the details of deployments.

During both trials clam performance at the IBSP field site was compared to that of clams held vertically in mesh bags ( $n = 3$ ) in the land-based upweller system (Fig. 3). The nursery unit consisted of a 2.44 x 1.22 m tank, containing ten to sixteen silos 45.7 cm in diameter. Ambient seawater was pumped at  $\sim 227 \text{ L min}^{-1}$  directly from the bay. The position of mesh bags between the silos is shown in Fig. 3, and was such that the experimental clams directly received ambient, inflowing seawater and were not affected by food depletion of shellfish seed contained in the silos.

**Figure 3.** Schematic, top-down view (left) showing the positioning of experimental clams in mesh bags (represented by black squares) at the BBSRP land-based upwellers, operated by ReClam The Bay Inc. volunteers. Circles indicate silos containing oyster and clam seed (also shown in the photo on the right).



Weekly sampling of clams and of the water column continued for a total period of 4 wks ( $n = 5$  sampling dates). Fouling of cages and mesh bags by macroalgae, encrusting polychaetes, solitary and colonial tunicates, mussels, etc, and presence of potential clam predators were tracked over time; cages were brushed clean of fouling organisms on a weekly basis at the time of sampling.

**Trial II.** A new batch of clams (initial mean SL = 9.06 mm, standard deviation, SD = 0.016,  $n = 50$ ) was obtained from a local commercial hatchery and re-deployed at the same 4 sites listed above on July 23, 2012 (see Appendix I). This cohort contained on average 66% of “wild”, “unselected clams” and 34% of the notata variety. Weekly sampling of cages and suspended particulates (seston) continued through September 11-12, i.e. for a total duration of 7 wks. Thus, a longer experimental period was used rather than undertaking a 3<sup>rd</sup> deployment as originally proposed, given that initial size variability was lower than in Trial I, and clam mortalities were negligible, such that a sufficient number of clams was available for the more extended observation period. The sample size per cage was increased from 30 to 50 clams per cage based on the variability observed during Trial I, and generally clams from 3 cages were processed ( $n = 6$  at Harvey Cedars). For Trial II all clams were deployed in new, custom-made bags with a 4 mm square mesh (Fig. 2B), given that our results from Trial I indicated that the finer 1x2 mm



mesh bags resulted in significant flow obstruction and thus yielded reduced clam growth rates relative to the larger mesh bags (see Results).

Cages and mesh bags were power-washed prior to re-using them for Trial II, and brushed on a weekly basis to remove macroalgae and fouling invertebrates. Encrusting organisms (e.g. barnacles) were removed by scraping with a knife.

#### **Water column sampling of particulates.**

Seawater samples were collected weekly with a battery-powered, Masterflex peristaltic pump ~ 20 cm off-bottom, i.e., from approximately the same off-bottom height as that of clam deployment, in plastic containers. Collected seawater was sieved *in situ* through the 153  $\mu\text{m}$  mesh and split for the various seston analyses reported: Particulate Inorganic Matter (PIM), Particulate Organic Matter (POM), total seston or Suspended Solids (TSS = PIM + POM), determined gravimetrically, particulate organic carbon (POC) and nitrogen (PON), and Chlorophyll *a*. Sampling of seawater with a peristaltic pump minimized damage/disruption of algal cells and disturbance/sediment resuspension from the bottom.

*In situ* growth of juvenile clams was related to the quantity and quality of the suspended food supply. Additional information on the composition of the phytoplankton, based on analysis of functional taxonomic groups (FTGs) by high-performance liquid chromatography (HPLC) was provided via a concurrent project supported by the Barnegat Bay Partnership (BBP) that ends in spring 2014 (data analysis is ongoing). Preliminary results are presented for two of the study sites, IBSP and Sedge Is. Collected seawater in plastic containers was transported in coolers on ice to the Rutgers University Jacques Cousteau facility, Tuckerton, for sampling of seawater collected at the two southern stations, and at the IBSP Forked River Interpretive Center for sampling at the two northern stations. There it was processed by low-vacuum filtration (5-10 mm) of known volumes of the suspension (measured with a graduated cylinder) on 2.4 cm diameter Whatman glass-fiber filters using a multi-port filtration setup, following sieving through a coarse 153  $\mu\text{m}$  Nitex mesh sieve to remove large zooplankton and detrital particles.

For PIM (ash weight) and POM (AFDW) samples were filtered through pre-combusted (overnight at 470°C), pre-weighed Whatman GF/C glassfiber filters (24 mm diameter, 1.2  $\mu\text{m}$  nominal pore size), and filters were rinsed twice *in situ* with an isotonic ammonium formate solution to remove salts that contribute to the DW. Dry weight and ash weight were determined following oven-drying at 60°C for 24 h, and overnight combustion in a muffle furnace at 480°C respectively to allow calculation of AFDW. Samples for Chl *a* analysis (and FTGs) were filtered on 2.4 cm diameter Whatman GF/F glass-fiber filter (0.7  $\mu\text{m}$  nominal pore size). Filters (GF/C) containing samples for POC/PON analysis were dried at 60°C and shipped overnight to the Horn Point Analytical Laboratory, University of Maryland. CHN analysis was conducted using acetanilide as standard.

Temperature was determined continuously (every 15 min) at the study sites with *in situ* temperature HOBO Onset data loggers directly attached to the cages. Discrete temperatures and salinities were also determined at the time of sampling with a hand-held thermometer and refractometer, respectively

#### **Statistical analysis.**

Hard clam growth rates among stations were compared using analysis of variance (ANOVAs) and Tukey's a posteriori multiple comparisons. All data expressed in percentages, e.g. % instantaneous growth coefficients, were arcsine transformed to meet the assumptions of normality prior to conducting ANOVAs. We also explored relationships between clam weekly growth rates (k based on DW) and various environmental factors (temperature and seston metrics) by fitting linear regressions for each variable at each site (with k as the dependent variable Y and the environmental factor as the independent variable X). For this purpose, since the growth rates were computed weekly, a mean of the environmental variable at the beginning and end of the weekly period was used in the regressions. Regression analysis was carried out for each site, as the contrasting nature of the 4 selected study sites indicated that merging of all parameters across sites would be of limited value. ANOVAs comparing clam growth rates (k) were carried out with SPSS software and significance tests of fitted linear regressions used Statistix 10. The latter analysis included a) t-tests of the significance of the regression coefficient (slope) to determine whether it deviated significantly from zero in either a positive or negative direction, and b) ANOVA F tests of significance of the linear regression to determine whether each environmental factor could explain a significant portion of the variation in weekly clam growth rates at any given site (Sokal and Rohlf 1995).

## **Results**

### **a) Water column physical parameters**

Discrete water column salinities determined weekly during the two Trials are shown in Table 1. The lowest mean salinity (22.4) was obtained at IBSP as this site is influenced by the Toms River plume. The highest mean salinity was obtained at Sedge Island, which is influenced by its proximity to the Barnegat Bay Inlet. Discrete temperatures and salinities were determined at the 4 field sites from seawater drawn with a peristaltic pump from the same height off-bottom (~ 20 cm) at which juvenile clams were deployed. Means and ranges are also reported.

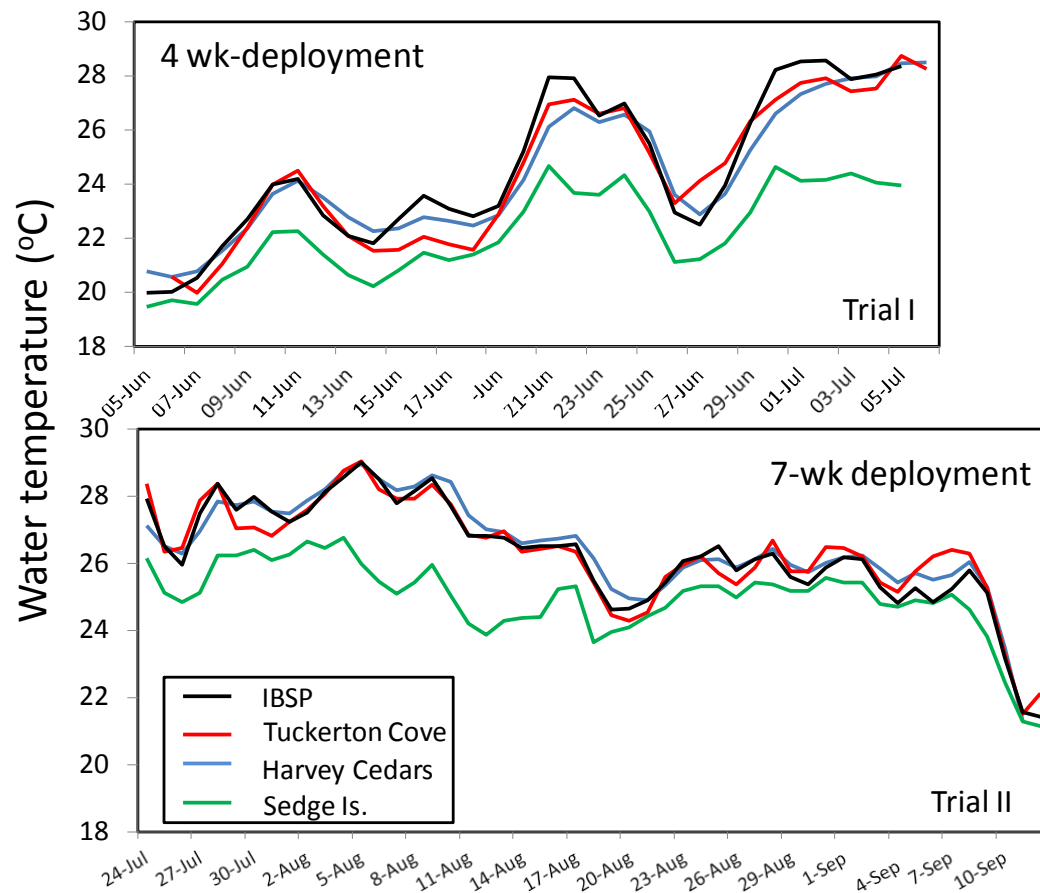
**Table 1.** Weekly salinities determined at the four study sites during Trials I and II. Averages and ranges are also shown.

SALINITY VALUES FOR BARNEGAT BAY FIELD SITES				
DATE	HARVEY CEDARS	TUCKERTON COVE	SEDGE ISLAND	IBSP
June 5/6	30	30	29	22
June 12/13	30	30	28	21
June 19/20	30	30	31	20
June 26/27	28	25	30	22
July 5/6	28	29	33	22
July 24/25	28	27	33	26
July 31/Aug 1	29	ND	33	24
Aug 7/8	29.5	27	32.2	25
Aug 14/15	30.5	28	31	23
Aug 21/22	ND	30	31	23
Aug 29/30	29	28	33	21
Sept 4/5	29	26	31	23
Sept 11/12	28	27	28	19
<b>Mean</b>	<b>29.1</b>	<b>28.1</b>	<b>31.0</b>	<b>22.4</b>
<b>Range:</b>	28 - 30.5	27 - 30	28 - 32.2	19 - 26

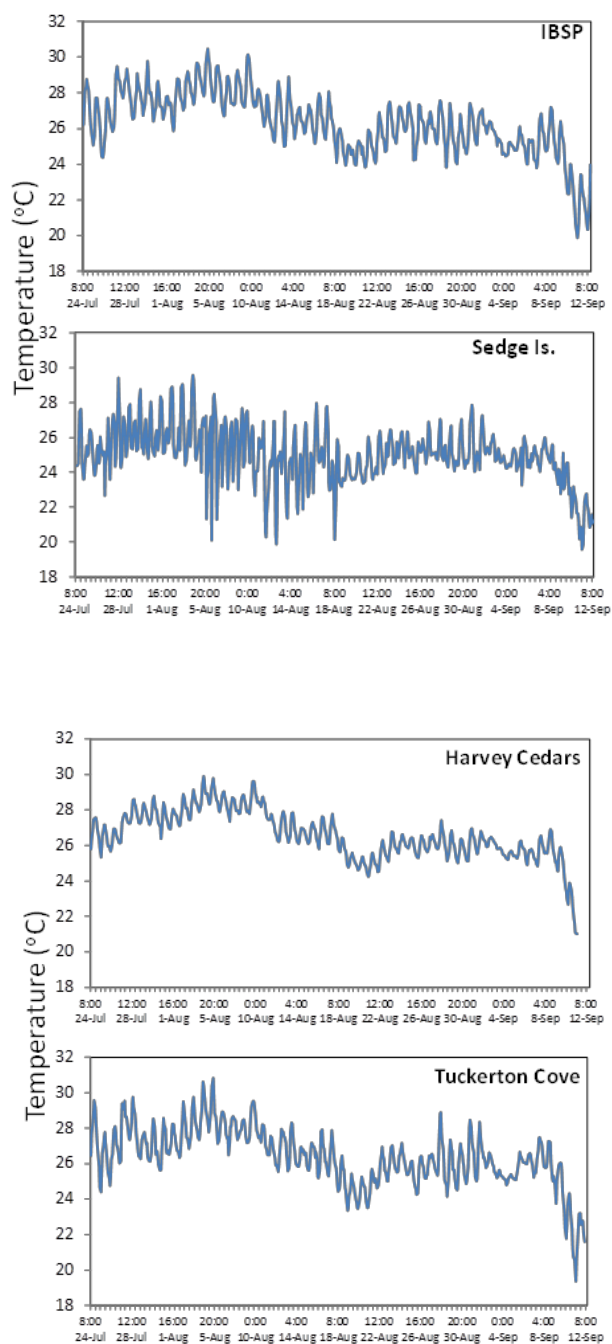
ND = not determined

Continuous temperature records (daily means) during Trials I and II are shown in Figure 4. Consistently lower temperatures were measured at the Sedge Is. site, ~ 2°C lower than at the other 3 field sites, again due to the exchange of oceanic water through Barnegat Inlet. Short-term temperature variability (2 h-averages) is illustrated in Figure 5. Maximum daily temperature fluctuations (up to ~10°C in the first week of July) were recorded at the Sedge Is. site (Fig. 4) comparable to those measured during Trial I (not shown), and were dampened in late August/September. In contrast, temperature variability was least pronounced at Harvey Cedars site (maximum daily temperature differential = 2.9°C). Intermediate temperature fluctuations were found at IBSP and Tuckerton sites, with a maximum daily differential of 3.5-3.6°C. Water temperatures within the IBSP nursery system closely tracked that measured at the adjacent field site (Fig. 6), reflecting the high flow rate pumped through this system

**Figure 4.** Continuous temperature records obtained with Onset HOBO<sup>®</sup> data loggers attached to one of the 4 cages at each site. Although readings were recorded every 15 min to determine short-term variability, the values plotted represent mean daily temperatures at the 4 field sites.

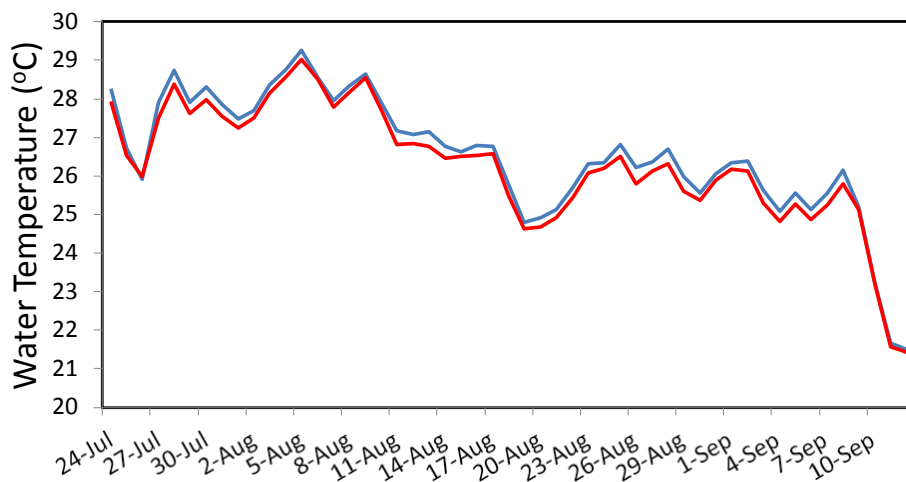


**Figure 5.** Temperature fluctuations, as determined by 2 h-averages during Trial II at the 4 study sites.





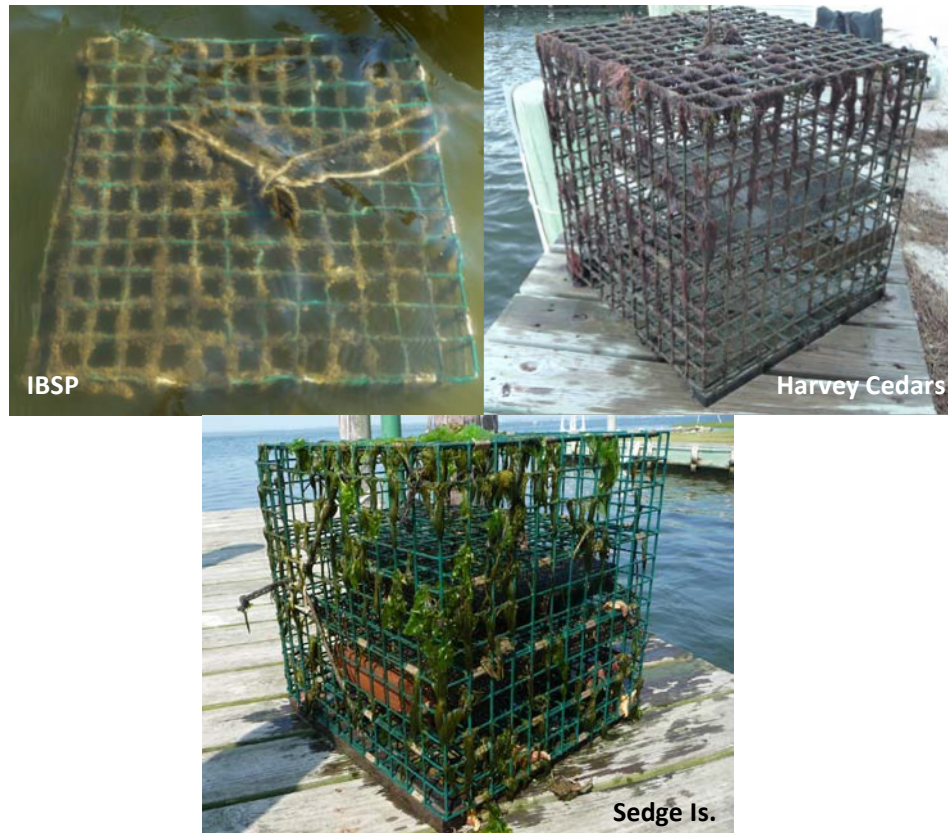
**Figure 6.** Comparison of water temperatures at the IBSP land-based upweller and the adjacent field site based on continuous records.



#### b) Fouling community

Overall, fouling of cages was relatively limited at all sites during both Trials (Fig. 7), with a few exceptions (e.g. macroalgal fouling at Tuckerton Cove on the last day of Trial I). Heavy fouling of cages by mussel, *Mytilus edulis*, set was observed in late May and only at the Sedge Is. site, but was only moderate by the start of Trial I in the 1<sup>st</sup> week of June.

**Figure 7.** Examples of macroalgal fouling of cages at three of the study sites as observed on the last wk of August, ~5 wks following cage deployment during Trial II. Different dominant macroalgae were observed at the 3 sites: putatively *Desmarestia viridis* (sourweed), the red alga *Gracilaria tikvahiae* and *Ulva lactuca* (sea lettuce) at IBSP, Harvey Cedars and Sedge Is, respectively.



### c) Seston characterization

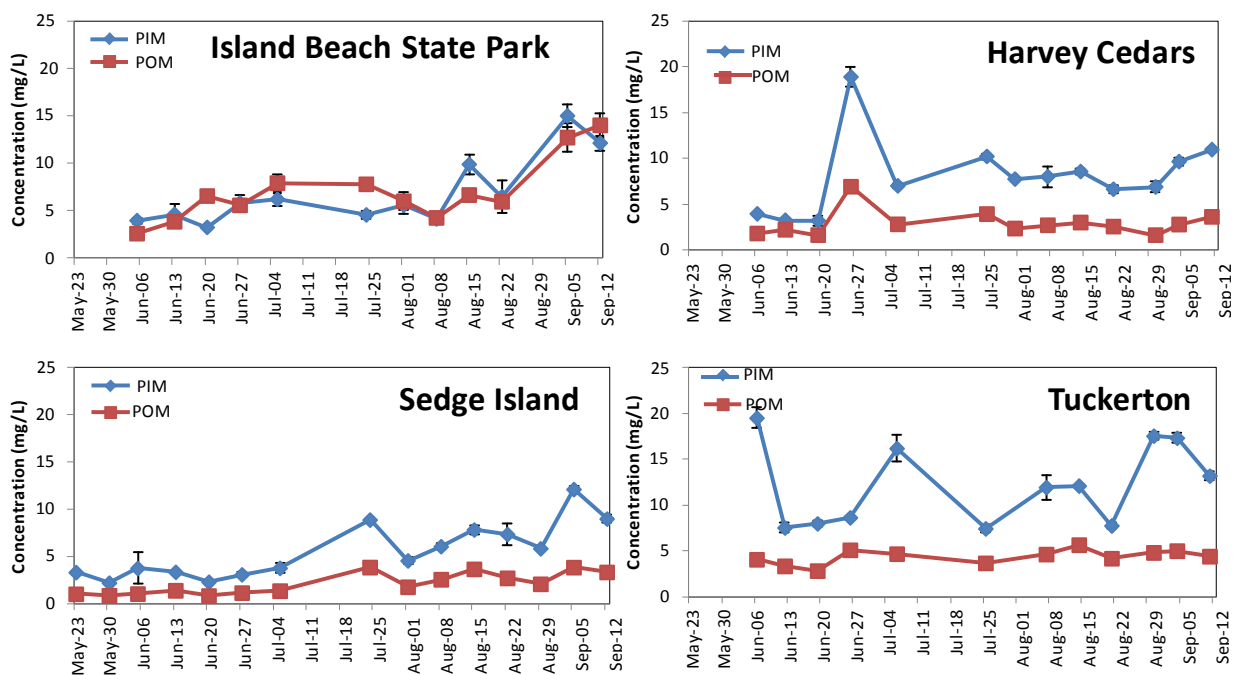
Off-bottom PIM concentrations are largely representative of suspended sediment concentrations. Levels consistently remained below  $20 \text{ mg DW L}^{-1}$  (Fig. 8) and thus below the threshold known to significantly inhibit growth of juvenile hard clams [ $\geq 25 \text{ mg PIM L}^{-1}$ , Bricelj et al. (1984b)]. The two northern stations (IBSP and Sedge) showed lower concentrations of suspended sediments (reflected in PIM values) than the two southern stations (Harvey Cedars and Tuckerton).

It is noteworthy that POM concentrations, one of the proxies used for food quantity available for clams, contributed a greater proportion of the total seston at the IBSP site (Fig. 8) (mean % POM =  $51.4\% \pm 0.26$  standard error, SE), than at the other 3 sites (% POM  $\pm$  SE =  $27.7 \pm 0.07$ ,  $28.1 \pm 0.15$ , and  $27.6 \pm 0.07$  at Sedge, Harvey Cedars and Tuckerton respectively). Organic matter absolute concentrations, POM, POC and PON were also highest at IBSP, where POC and PON attained maxima of  $\sim 3,500 \mu\text{g L}^{-1}$  and  $\sim 450 \mu\text{g L}^{-1}$ , respectively (Fig. 8) (see discussion).

Seston concentrations (PIM + POM) can be used as a proxy for turbidity, caused by organic (microalgal and detrital) and/or inorganic (suspended sediment) sources. Sedge Is. exhibited the lowest seston concentrations of the 4 study sites, with weekly mean concentrations ranging from  $2.81$  to  $15.99 \text{ mg DW L}^{-1}$ , and averaging  $8.48 \text{ mg L}^{-1}$  over the period May 30 to Sept 12. Peak seston concentrations attained maxima of  $27.68$ ,  $22.40$  and  $25.76 \text{ mg L}^{-1}$  at IBSP, Harvey Cedars and Tuckerton, respectively, and averaged  $14.63$  at IBSP,  $13.08$  at Harvey Cedars and  $15.55 \text{ mg}$

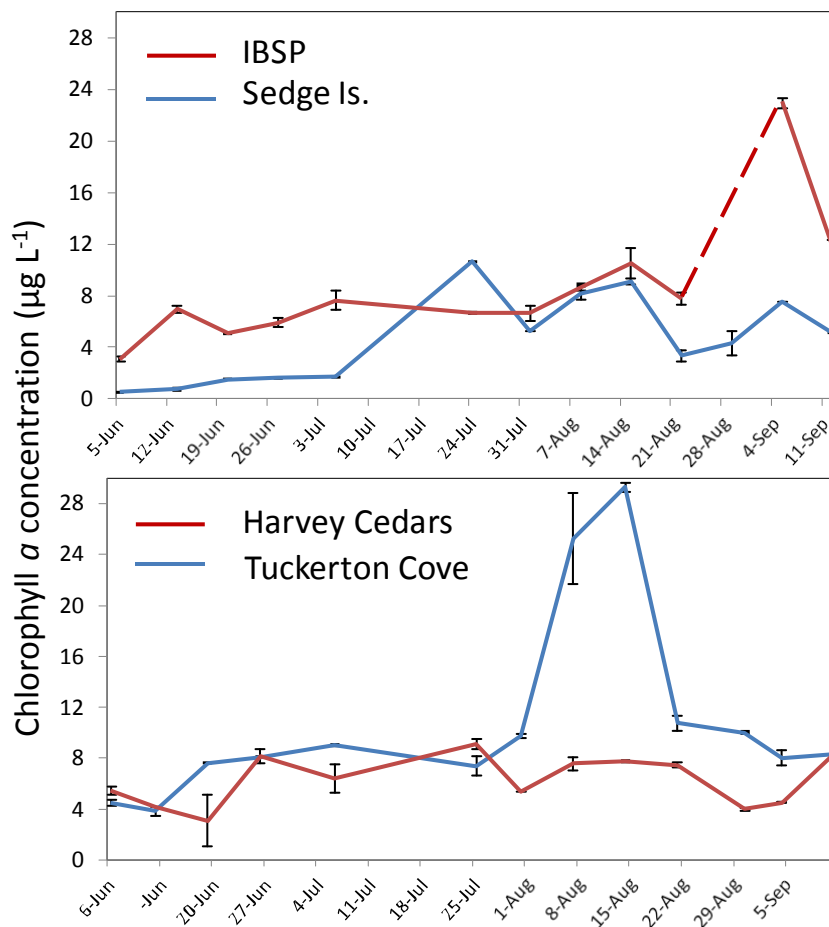
$L^{-1}$  at Tuckerton Cove over the study period. Although seston levels were comparable among these 3 sites, Tuckerton generally exhibited the highest mean PIM levels and a higher frequency of PIM peaks (Fig 8).

**Figure 8.** Mean concentrations ( $\pm$  standard error, SE,  $n = 2$  to 4 filters) of suspended Particulate Inorganic Matter (PIM) and Organic Matter (POM) at the 4 study sites in BB-LEH (in mg dry weight  $L^{-1}$ ). Note that May values were obtained at Sedge Is. although clam deployment at all sites and thus the start of Trial I did not occur until June 6.



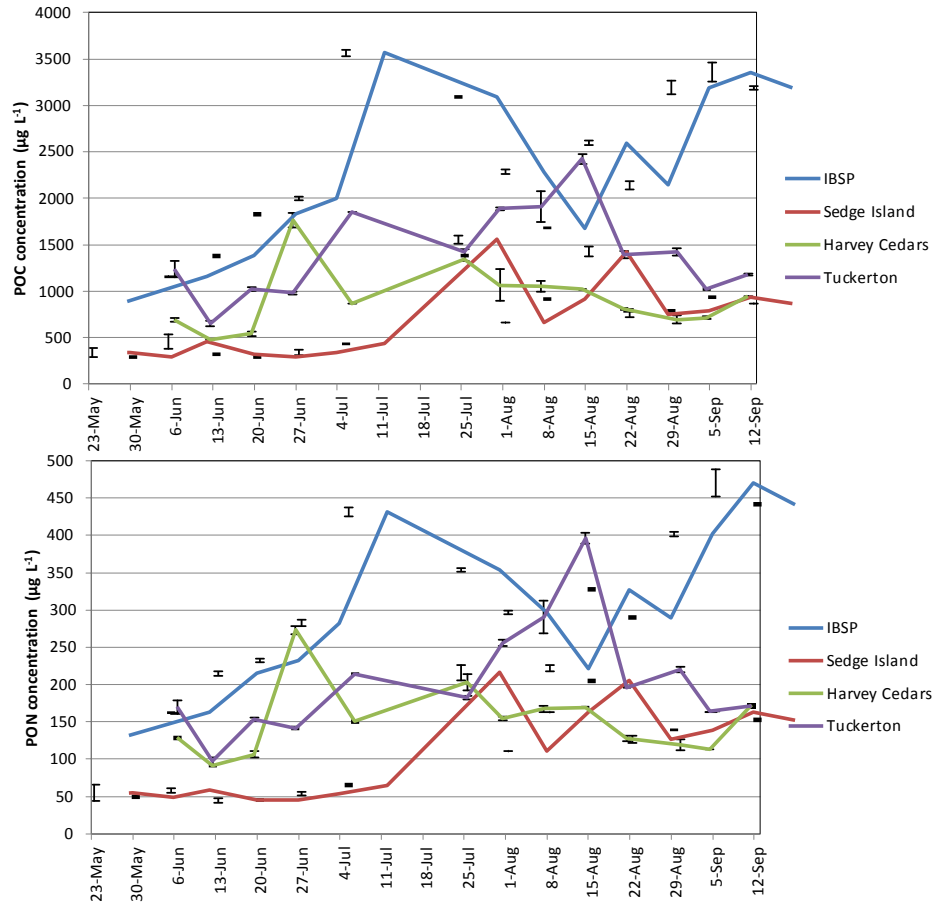
Chlorophyll *a* concentrations were highest at IBSP and at Tuckerton Cove, where they peaked at  $\sim 22$  and  $30 \mu g L^{-1}$  respectively (Fig. 9). The Chl *a* maximum occurred in early August in Tuckerton, was less pronounced and extended between late July and mid-August at Sedge, and was delayed until early September at IBSP. Harvey Cedars experienced a relatively constant and low mean Chl *a* concentration over the study period, averaging  $6.27 \mu g L^{-1}$  ( $\pm SE = 1.94$ ). At IBSP the highest concentrations of Chl *a* on Sept. 4 (Fig. 9) coincided with the highest PIM concentration (both  $> 2x$  the average value for this site), and the 2<sup>nd</sup> highest POM concentration (Fig. 8). This peak in Chl *a*, PIM and POM coincided with an episode of high precipitation from Sept. 3 to 5, based on records at Toms River, totaling 6.0" of rainfall (RISE <http://climate.rutgers.edu/njwxnet/>).

**Figure 9.** Total water column Chlorophyll *a* concentrations (mean  $\pm$  SE,  $n = 3$ ) determined by HPLC at the two northern and two southern study sites (upper and lower graphs respectively). The dashed line serves to indicate loss of samples at one sampling date.



Concentrations of POC and PON throughout the study period (Trials I and II) are shown in Figure 10. Patterns in these two parameters generally tracked each other closely. Highest PON and POC concentrations were typically observed at IBSP, and thus consistent with the high POM concentrations found at this site.

**Figure 10.** Water column particulate organic carbon (POC) and nitrogen (PON) concentrations (mean  $\pm$  SE,  $n = 2$ ) determined at the 4 study sites during summer 2012.

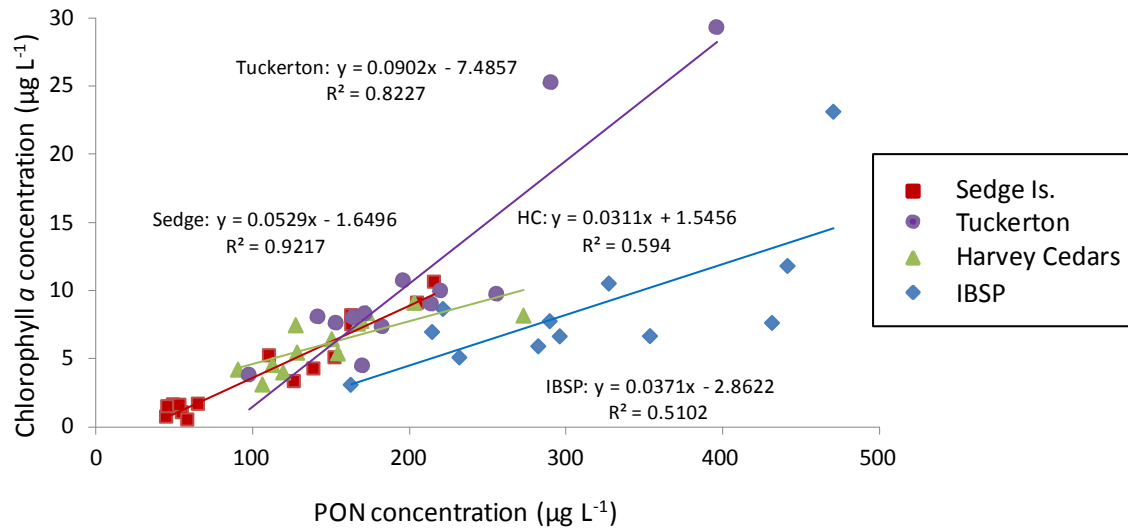


A positive, significant relationship between Chl *a* and PON concentrations (Fig. 11), as well as between Chl *a* and POC concentrations (not shown) was found at all study sites. The highest correlation between weekly Chl *a* and PON (and POC) concentrations was found at Sedge and Tuckerton, the two sites where overall clam growth rates ranked highest (Fig. 15 and 16;  $R^2 = 0.82$  and  $0.92$ , respectively). Lowest Chl *a*/PON and Chl *a*/POC ratios were found at IBSP, potentially reflecting the higher detrital contribution to total particulate organic matter at this site (see discussion). Overall, the relationships between seston parameters differed considerably at IBSP compared to the other three sites, indicating that the food supply had unique characteristics at the northernmost study site.

The food supply at the Tuckerton Cove, LEH, study site is expected to be most influenced by tidal changes. This site experienced the highest tidal range (~70 cm between MLLW and MHHW), whereas this tidal range was only ~12 cm at the IBSP, Sedge Is. and Harvey Cedars study sites.

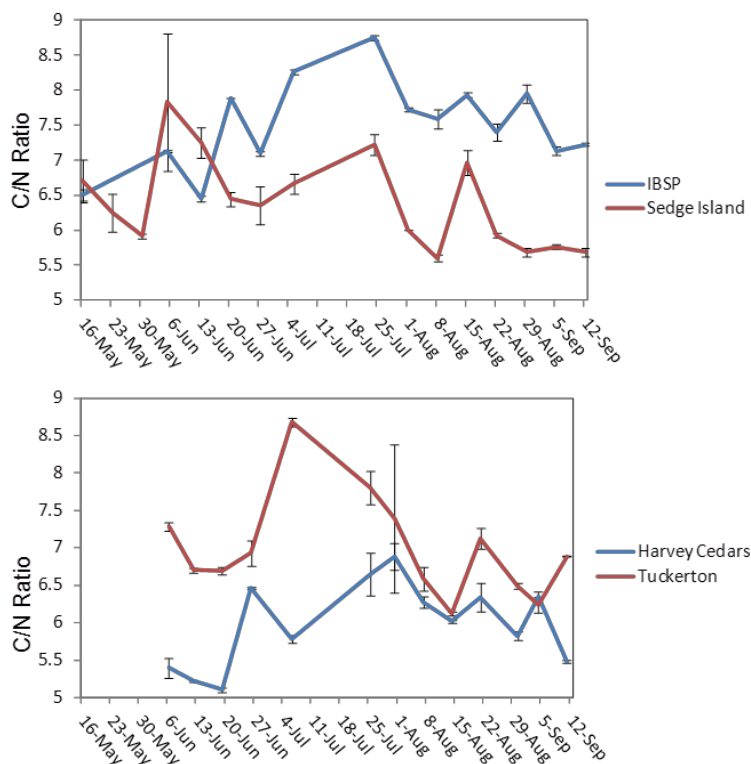
**Figure 11.** Relationship between weekly Chlorophyll *a* and particulate organic nitrogen (PON) concentrations in the water column (mean of initial and final values each week) at the 4 study sites. Fitted linear regressions with  $R^2$  = coefficient of determination are shown.





The particulate C/N ratio has been frequently used in previous studies as an indicator of the nutritional quality of seston for suspension-feeders, with lower ratios indicating a higher quality food source. C/N ratios were generally higher at IBSP than Sedge Is. (Fig. 12), consistent with the previous statement (overall clam growth > at Sedge than Harvey Cedars, Fig. 15 and 16), but they were higher at Tuckerton than Harvey Cedars, although the opposite was found for overall clam growth rates at these two sites. These ratios also generally showed a declining pattern starting in the 1<sup>st</sup> wk of July (at Tuckerton) and from the 3<sup>rd</sup> wk of July at the other 3 sites.

**Figure 12.** Water column particulate organic carbon to nitrogen (C/N) ratios (mean  $\pm$  SE,  $n = 2$ ) determined at the two northern and two southern study sites (upper and lower graphs respectively).

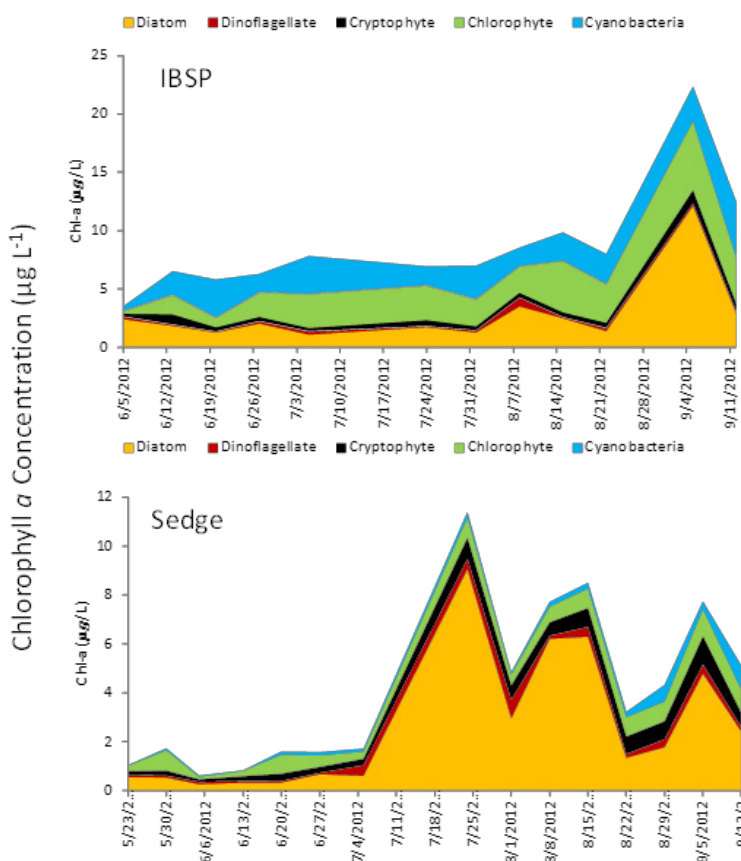


Preliminary data is included in Fig. 13 on the composition of the phytoplankton assemblage at two sites, IBSP and Sedge, for which we have taxonomic genus/species identification (unpublished data from a BBP-supported project conducted in parallel to this study). We refer to detailed methods, analysis of FTG data including assumptions invoked, to an upcoming report for that project.

At the IBSP and Sedge Is. sites, microscopically determined phytoplankton abundance and composition for selected sampling dates were available in summer 2012 (provided by Ling Ren, Philadelphia Academy of Sciences as collaborator on the BBP-supported project), thus allowing groundtruthing for preliminary determination of phytoplankton classes based on analysis of photopigments. This analysis revealed that the IBSP site is characterized by a consistently much greater contribution of cyanobacteria (blue-green algae) to the phytoplankton community during the summer compared to Sedge Is. (averaging 34% and 25% during Trial I and II, respectively at IBSP, and only 6% at Sedge throughout the study period) (Fig. 13). Overall picoplankton (chlorophytes + cyanobacteria) made a dominant contribution to total phytoplankton biomass at IBSP (mean = 61 to 64%), whereas at Sedge picoplankton contributed high levels during Run I (mean = 50%) but these were lower throughout Trial II (mean = 29%). At Sedge Is., diatoms

(Bacillariophyceae) increased in relative abundance from a mean of 31% during Trial I to 56% during Trial II, thus becoming the dominant phytoplankton class at this site where they attained a maximum of 67% during the first 2 weeks of August. Dinoflagellates, often a poor food source for hard clams (Weiss et al. 2007 and references therein), were a minor component of the phytoplankton assemblage during the present study.

**Figure 13.** Total Chlorophyll *a* and the predicted contribution of key phytoplankton taxonomic classes, based on the concentration of diagnostic photopigments at the IBSP and Sedge Is. study sites. No sampling was conducted between July 5 (end of Trial I) and July 23 (start of Trial II). The vertical line in the Sedge graph indicates the start of Trial I (two earlier water column samplings were conducted at this site although no concurrent clam growth data are available). Preliminary analysis of FTGs was generated via a parallel project sponsored by the BBP, following methods described by Goericke and Montoya (1998). The contribution to Chl *a* was derived from diagnostic photopigment:Chl *a* ratios common to prevalent species known to occur in BB-LEH: Chl *b*/Chl *a* = 0.380 for chlorophytes, zeaxanthin/Chl *a* = 0.800 for cyanobacteria, alloxanthin/Chl *a* = 0.250 for cryptophytes, and fucoxanthin/Chl *a* = 0.600 for diatoms. Note the difference in scales of the Y axis between the 2 plots.



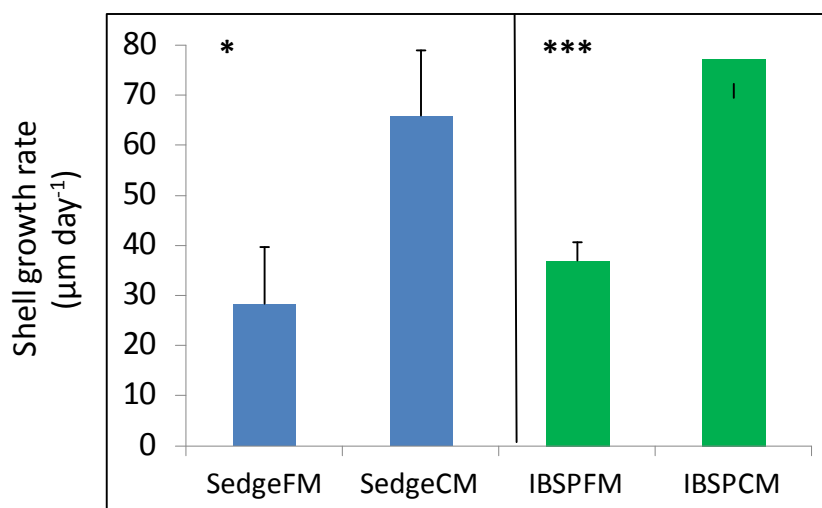
#### d) Clam growth rates and survival

Clam mortalities remained low at all field sites during Trial I (the maximum mortality averaged over all cages at any given date = 3.3% at IBSP, 9.3% at Sedge, 9.5% at Tuckerton and 14.4% at Harvey Cedars), and there was no consistent pattern of increasing cumulative mortalities over

time. Mortalities in IBSP upwellers were low (maximum, = 5.6%) and comparable to those at the IBSP field site. Losses were negligible during Trial II ( $\leq 2\%$  at all sites).

A comparison of cumulative shell growth rates of clams held in the finer-mesh and coarser-mesh bags at two field sites, Sedge Is. and IBSP during Trial I, showed that the finer mesh significantly reduced growth rates by a factor that was comparable at the two sites (2.2 to 2.1-fold reduction) (Fig. 14). This result provided the basis for using coarser mesh bags (4x4 mm square mesh) that allowed retention of clams available from a local hatchery at the time of initiation of Trial II (mean initial SL = 9 mm). Appendix 4 shows a comparison of the weekly tissue growth rates (k) of clams during Trial 1 held in the coarse mesh (6 mm) and those of a different batch of clams from the same grower used for Trial II at IBSP and Sedge (see Appendix 1 for experimental conditions used).

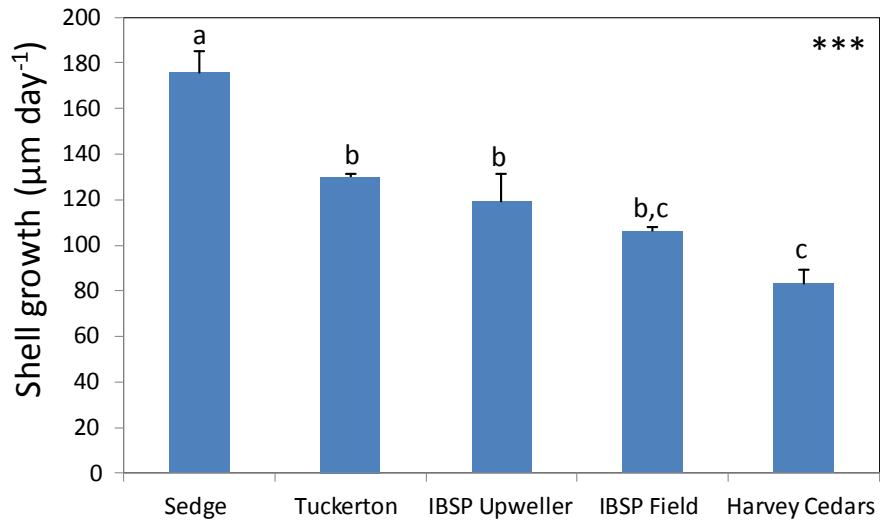
**Figure 14.** Effects of bag mesh size on clam shell growth rate (mean  $\pm$  standard error, SE) at the Sedge Is. and Island Beach State Park (IBSP) field sites (Trial I). FM: finer mesh (2x1mm rectangular mesh), CM: coarser mesh (6 mm square mesh). Differences were statistically significant based on a one-way ANOVA (\*:  $p \leq 0.05$ ; \*\*\* $0.01 \leq p < 0.001$ ).



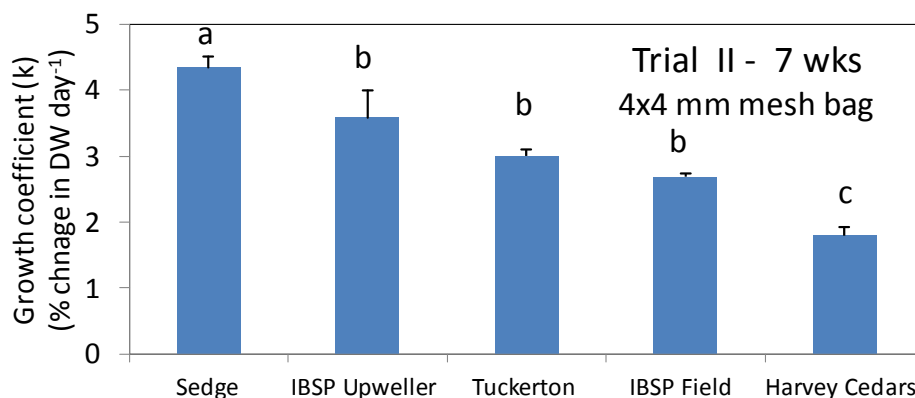
The ranking of shell growth rates at the 4 field sites over 7 wks during Trial II (late July to mid-September) was: Sedge > Tuckerton = IBSP > Harvey Cedars (Fig. 15). Thus, despite lower temperatures and higher temperature variability at the Sedge Is. deployment site, juvenile clams at this site experienced the highest growth rate out of the 4 field study sites (mean growth rate at Sedge  $\sim 182 \mu\text{m day}^{-1}$ ). There was no statistically significant difference in shell growth rate measured in  $\mu\text{m day}^{-1}$ , between clams in the land-based upwellers and those deployed in the field (Fig. 15).

Shell growth rates at Sedge and IBSP in the coarser, 6 mm mesh treatments during Trial I (Fig. 14) were considerably lower (averaging  $\sim 65$  and  $76 \mu\text{m day}^{-1}$ ) respectively. The same ranking among sites was obtained when soft tissue growth rates (k) were compared among sites (Fig. 16).

**Figure 15.** Shell growth rate of juvenile hard clams held in 4 x 4 mm mesh bags over the 7 weeks of Trial II (July 23 – Sept. 11-12 2012) at the 4 field sites and at the IBSP upweller nursery system. Mean shell length ( $\mu\text{m day}^{-1}$ ,  $n = 3$  or 4 cages,  $\pm$  SE, 50 clams per cage). Differences were statistically significant based on a one-way ANOVA and Tukey's multiple comparisons ( $***0.01 \leq p < 0.001$ ).



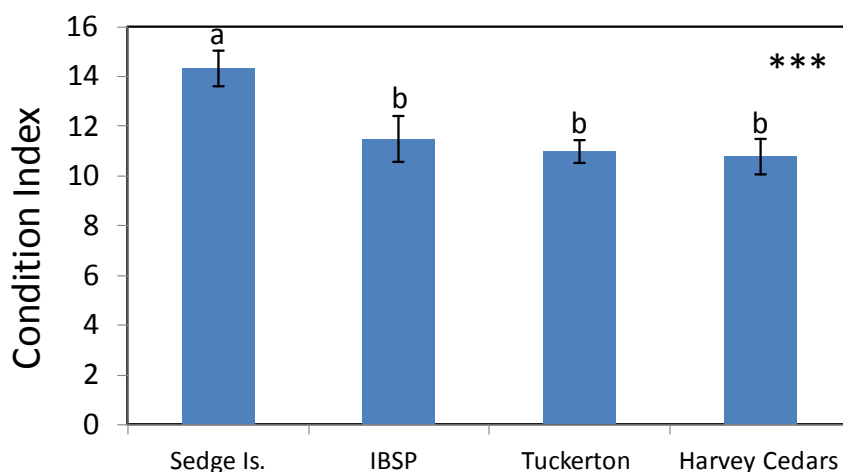
**Figure 16.** Ranking of sites in terms of growth rates in soft tissue dry weight (DW) (% change  $\text{day}^{-1}$ ), based on results integrated over 7 wks during Trial II, and measured by the instantaneous daily growth coefficient ( $k$ , % change  $\text{day}^{-1}$ ). Mean  $\pm$  SE of 3 initial and 3 final cages per site ( $n = 5$  to 6 at Harvey Cedars). Note that there was no significant difference in clam growth rate between the IBSP field site and the adjacent, land-based upweller system.





Clams at Sedge Is. not only had the highest soft tissue and shell growth rates, but they also exhibited a significantly higher condition index (CI) than clams at the other 3 field sites (Fig. 17). Thus the average over 7 wks of Trial II was 14.33 at this site, compared to 11.48, 11.00 and 10.79 at IBSP, Tuckerton and Harvey Cedars, respectively. When these same data are examined on a weekly basis, clams at Sedge Is. consistently showed the highest CI (not shown). The maximum CI (15.23) was observed at Sedge on August 22, and the minimum (9.5) at Harvey Cedars at the end of Trial II, consistent with the finding that clams were experiencing negative growth of soft tissues at this time. A comparison of the weekly mean CI of clams during Trial I and II, using a comparable coarse mesh is shown in Appendix 5.

**Figure 17.** Ranking of sites in terms of their condition index ( $= \text{soft Tissue DW}/\text{SL}^3 \times 1000$ , mean  $\pm$  SD, where DW in mg and SL in mm), averaged over 7 wks of Trial II. The initial CI (mean  $\pm$  SD) of clams at the time of deployment was  $11.98 \pm 1.34$ .



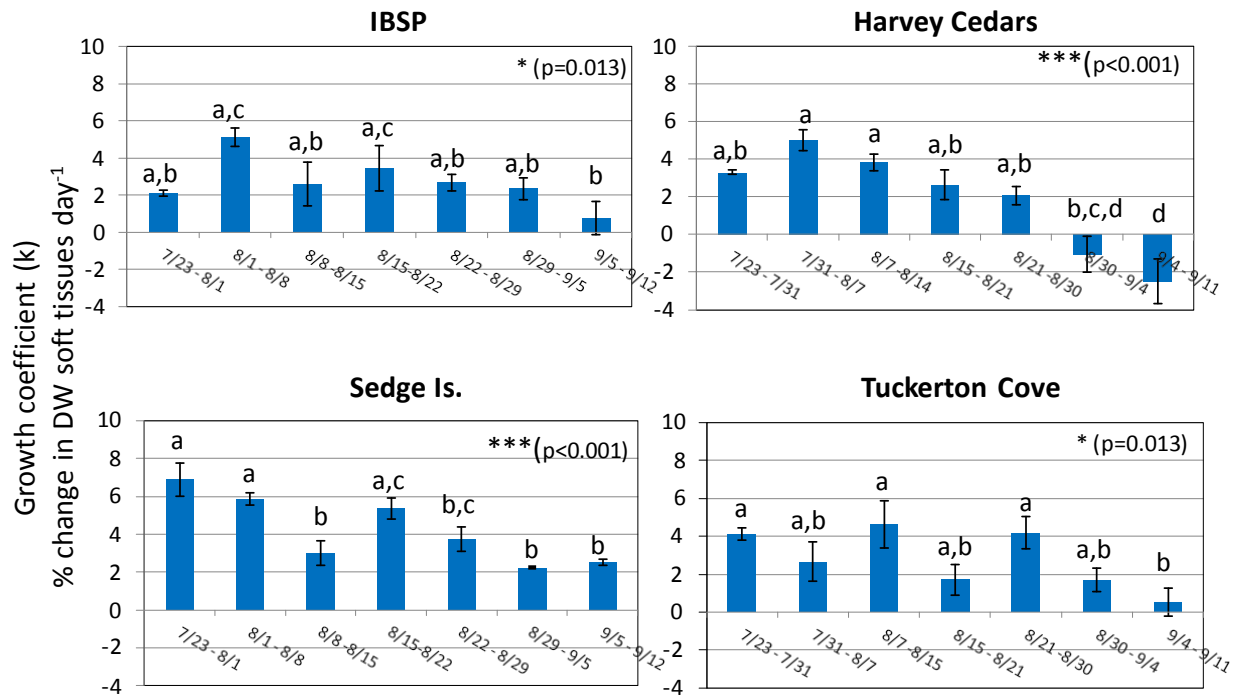
There was a significant effect of time (7 weeks) and site (4 field sites) on growth rate of clams, as measured by the instantaneous growth coefficient  $k$  (both based on soft tissue DW and shell length) (two-way ANOVA,  $p < 0.0001$ ). There was also a significant effect of week  $\times$  site interaction ( $p < 0.001$ ). Results of one-way ANOVAs followed by Tukey's a posteriori multiple comparisons of soft tissue growth rates ( $k$ ) within each site are shown in Figure 17. There are thus site-specific and seasonal differences in weekly clam growth rates, e.g: 1) clams at Harvey Cedars and Sedge showed the greatest temporal variability in growth rates (ANOVA,  $p < 0.0001$ ); 2) at IBSP and Sedge clams experienced a significant reduction in growth rates between wk 2 and wk 3 in early August, but this did not occur at Harvey Cedars or at Tuckerton. A reduction in growth rate was also observed at IBSP but was not statistically significant. In general, a pattern of declining soft tissue growth rates was observed between mid-August (wk 4) and mid-September (wk 7) at Sedge, and between late August (wk 5) and mid-September at Harvey Cedars and Tuckerton.

Lowest overall clam tissue growth rates during Trial II were observed at the Harvey Cedars site (Fig. 17), where clams actually experienced tissue weight loss during the last 2 wks (last wk of August through mid-September). Shell growth rate ceased during the same period. The pigment 19'-butanoyloxyfucoxanthin (referred to as 19'but), an indicator of pelagophytes and thus potentially indicating the presence of *Aureococcus anophagefferens*) was detected at Harvey

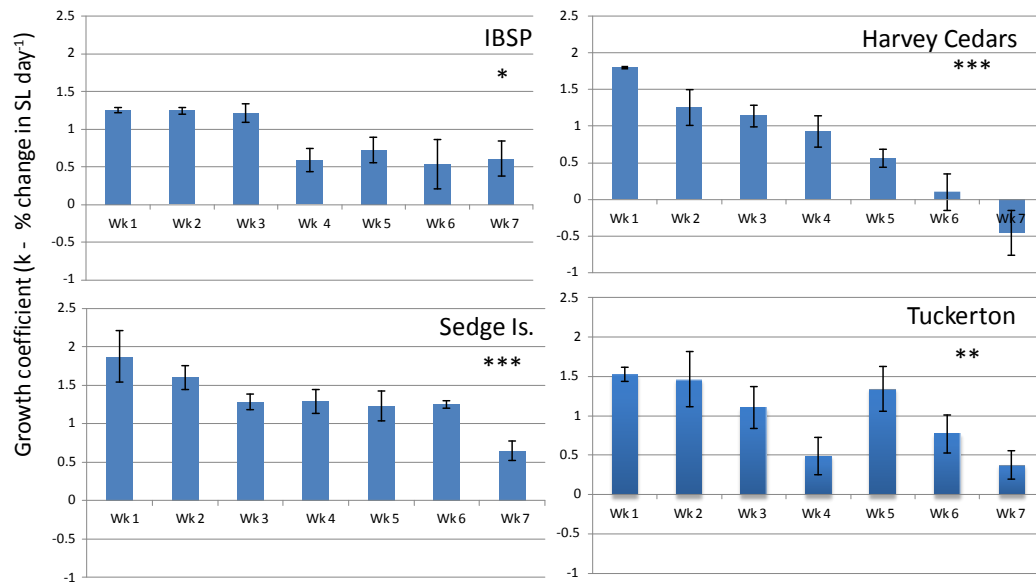
Cedars in early June at a concentration of  $0.14 \mu\text{g L}^{-1}$  (unpublished results from a concurrent project supported by the Barnegat Bay Partnership). This diagnostic pigment was also detected at the Sedge Is. site but at an order of magnitude lower concentration than at Harvey Cedars.

Differences were observed in a number of cases between weekly growth patterns based on DW of soft tissues ( $k_{\text{DW}}$ ) and those based on shell length ( $k_{\text{SL}}$ ) (Figs. 17 and 18). For example, at Sedge: 1) clams suffered a much greater reduction in tissue growth (48.7%) between the 1<sup>st</sup> and 2<sup>nd</sup> wk of August than that in shell growth (19.9%), and 2) the significant increase in  $k_{\text{DW}}$  in August between week 3 and 4 of Trial II was not reflected in an increase in shell growth. The greatest mismatch or uncoupling between tissue and shell growth rates occurred at IBSP, where the  $R^2$  of linear regressions relating  $k_{\text{SL}}$  to  $k_{\text{DW}}$  was only 0.152, in contrast to higher  $R^2$  values at other sites (0.639, 0.8142 and 0.642 at Sedge, Harvey Cedars and Tuckerton, respectively).

**Figure 17.** Mean instantaneous growth coefficient  $k$  ( $= \% \text{ change in soft tissue dry weight, DW, per day} = 100 \times (\ln W_f / W_i) / [\Delta t]$ )  $\pm$  SE, of juvenile clams during Trial II at Sedge Island and Tuckerton Cove field sites in the BB-LEH estuary.  $W_f$  and  $W_i$  = final and initial tissue DW respectively,  $\Delta t$  = time interval = 7 days. Different letters indicate significantly different growth rates (ANOVA, followed by Tukey's a posteriori multiple comparisons).

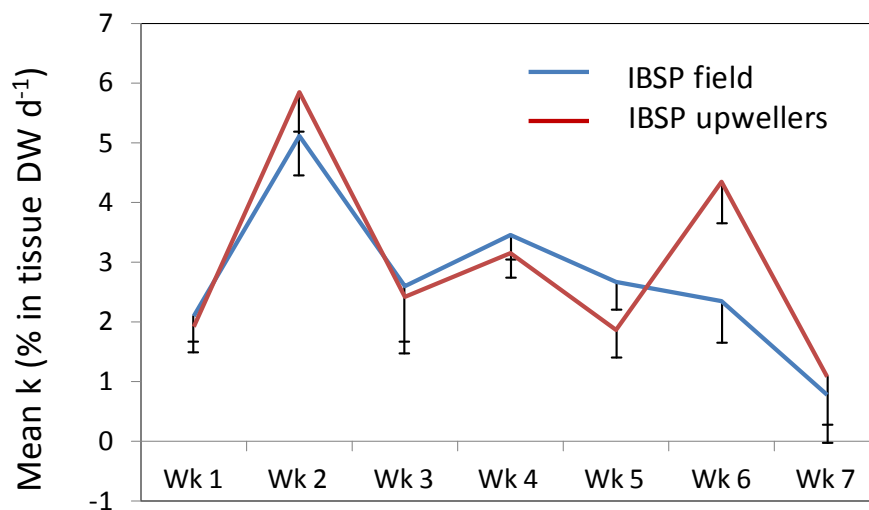


**Figure 18.** Mean instantaneous growth coefficient  $k$  ( $= \% \text{ change in shell length, SL, per day} \pm$  SE ( $n = 3$  cages except  $n = 6$  cages at Harvey Cedars), of juvenile clams during Trial II at the 4 field sites in the BB-LEH estuary.  $\Delta t$  = time interval = 7 days. The negative  $k_{\text{SL}}$  on wk 7 is attributed to a sampling artifact.



Weekly growth rates in soft tissues at the IBSP land-based upwellers generally closely tracked those at the adjacent field site, except for the one anomalous, unexplained difference during wk 6 (Fig. 19).

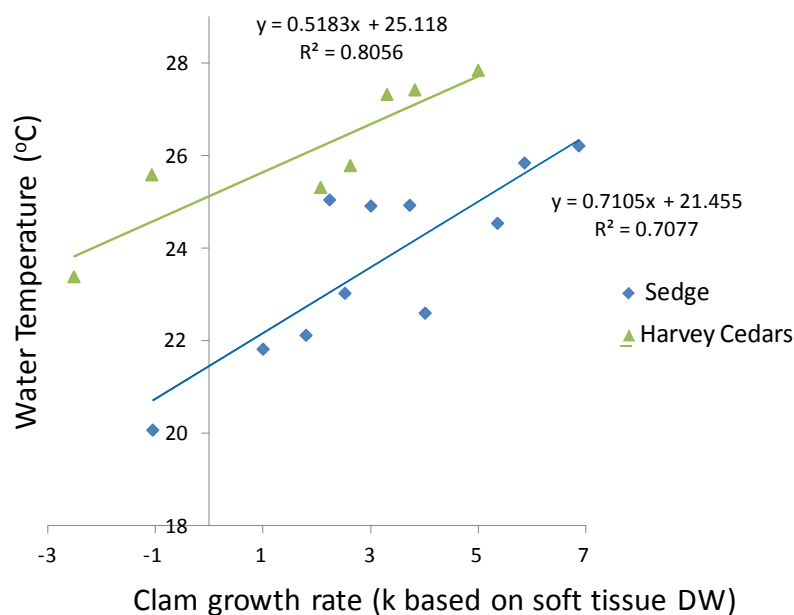
**Figure 19.** Comparison between growth rates (instantaneous growth coefficient  $k_{DW}$ ) in IBSP upwellers and the adjacent IBSP field site during Trial II over 7 wks ( $n = 3$  cages in the field or 3 mesh bags in upwellers). Growth rate determined as  $k =$  instantaneous growth coefficient (% change in soft tissue dry weight per day)



#### d) Relationship between clam growth rates and environmental parameters

A decline in clam growth rates was found at all sites between mid- to late August and mid-September, (Fig. 17) coinciding with a decline in temperatures (Fig. 6). Table 2 provides a summary of the relationship between clam growth rates ( $k_{DW}$ ) and key environmental variables of interest, including temperature and selected seston parameters, from fitted linear regressions [ $R^2$  and significance t test of the regression coefficient (slope)]. A significant, positive coefficient was found between temperature and soft tissue growth rate of juvenile clams at Sedge Is. and Harvey Cedars (Fig. 20 and Table 2,  $p < 0.01$  and  $p < 0.05$ , respectively), over the temperature range ~20 and 28°C, but was not significant at the other sites. **Overall, temperature could explain a significant amount of the variation in growth rate at these 2 sites (ANOVA,  $p < 0.01$  and  $p < 0.05$  at Sedge Is. and Harvey Cedars, respectively).** Salinity, over the range encountered, had no significant effect in explaining temporal differences in growth of clams within any of the 4 study sites.

**Figure 20.** Relationship between temperature and clam soft tissue clam growth rate (daily instantaneous growth coefficient,  $k_{DW}$ , calculated from the change in DW of soft tissues over a 7-day interval).



A significant positive regression coefficient between Chl *a* and clam  $k_{DW}$  was only observed at Sedge Is, a site where Chl *a* concentrations were typically lower than at Tuckerton. It was not significantly different from zero for regressions between POC concentrations and growth at any of the sites, except IBSP, where it was significantly different from zero (negative), thus indicative of poor food quality at this site (Table 2). Suspended sediment concentrations (measured by PIM) vs. clam growth showed a significant negative coefficient at Tuckerton, as expected given that this site exhibited more frequent resuspension events and generally higher PIM concentrations.

Relationships between absolute or relative concentrations of key diagnostic pigments for various phytoplankton classes and  $k_{DW}$  provided useful information to interpret the results of this study. They are therefore reported here (Table 2) although they were generated via a related project, and their analysis is still in progress. Noteworthy is that the zeaxanthin/Chl *a* ratio, a measure of the relative contribution of cyanobacteria to total phytoplankton biomass, was inversely related to clam growth at all 4 sites, although this effect was not statistically significant at Harvey Cedars. The regression coefficient was significantly different from zero at IBSP and Tuckerton ( $p < 0.05$ ) and at Sedge Is. ( $p < 0.001$ ). There was also a significant negative slope between the Chl *b*/Chl *a* ratio (a measure of the contribution of chlorophytes to total phytoplankton biomass) and clam growth at Sedge Is. and Tuckerton, although most evident at the former site. In contrast, the absolute concentration of fucoxanthin, a proxy for that of diatoms, was positively related to clam growth rate at IBSP and Sedge where the regression coefficient was significantly different from zero ( $p < 0.05$  at IBSP  $p < 0.01$  at Sedge).

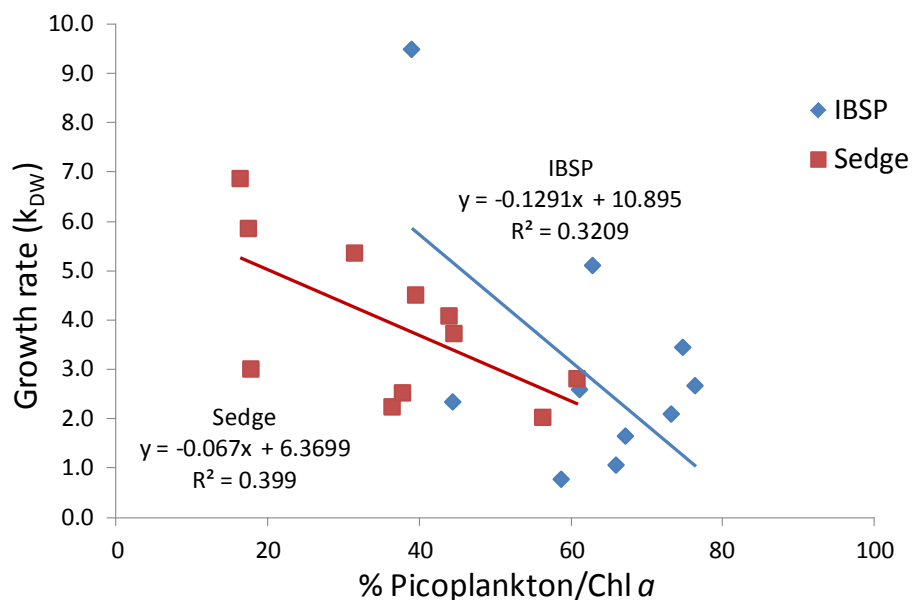
Finally, preliminary analysis of the % of various phytoplankton classes vs. clam growth (at present most reliable for Sedge and IBSP given that taxonomic data are available for selected dates at these 2 sites), supports the above findings. An inverse relationship was obtained between the % contribution of both cyanobacteria and chlorophytes and  $k_{DW}$ , but the regression coefficient was significantly different from zero only at Sedge Is. ( $p < 0.001$ ). However, when cyanobacteria and chlorophytes are combined to estimate the % contribution of picoplankton to the phytoplankton assemblage, a significant negative slope is found both at IBSP and Sedge (Fig. 22,  $p < 0.05$  at IBSP and  $p < 0.001$  at Sedge). **Overall, the % contribution of picoplankton to total Chl *a* could explain a significant portion of the weekly variation in clam growth rates only at Sedge Is. (ANOVA,  $p < 0.01$ ), however, as this relationship was only marginally significant at IBSP ( $p = 0.0693$ ).** Chlorophytes were included in the picoplankton size class based on the fact that *Nannochloris atomus* (1-2  $\mu\text{m}$  in cell size) was identified microscopically as the dominant chlorophyte at these two sites during the study period (dates for which taxonomic data are available; data generated by Ling Ren, Philadelphia Academy of Science via our joint BBP-supported research).

**Table 2.** Results of fitted linear regressions to the relationship between selected environmental parameters (temperature and seston metric, X) and clam growth ( $k_{DW}$ , Y) at each study site.  $R^2$  = coefficient of determination; p values indicate results of tests to determine whether the regression coefficient (slope) was significantly different from zero (boldfaced if significant); regressions with negative slopes are highlighted in grey. Seston metrics: Chl *a*, POC, PON, PIM, Chl *b*/Chl *a*, Zeaxanthin (zea)/Chl *a*, fucoxanthin (fuco)/Chl *a*, and fucoxanthin concentrations. ns = non significant, \* =  $p < 0.05$ ; \*\* =  $p \leq 0.01$ ; \*\*\* =  $0.01 < p \leq 0.001$ .



Site	Temperature			Chl <i>a</i>			POC			PIM		
	R <sup>2</sup>	p	Significance	R <sup>2</sup>	p	Significance	R <sup>2</sup>	p	Significance	R <sup>2</sup>	p	Significance
IBSP	0.0021	0.6915	ns	0.0448	0.0614	ns	0.3484	<b>0.0127</b>	**	0.0612	0.0628	ns
Sedge Is.	0.5946	<b>0.0014</b>	**	0.108	<b>0.0085</b>	**	0.1259	0.0537	ns	0.0003	<b>0.0228</b>	*
H. Cedars	0.7355	<b>0.039</b>	*	0.3004	0.321	ns	0.4836	0.1473	ns	0.2975	0.151	ns
Tuckerton	0.4938	0.125	ns	0.1212	0.2773	ns	0.3014	0.7766	ns	0.278	<b>0.0508</b>	*
	Chl <i>b</i> / Chl <i>a</i>			zea / Chl <i>a</i>			fuco / Chl <i>a</i>			fuco		
	R <sup>2</sup>	p	Significance	R <sup>2</sup>	p	Significance	R <sup>2</sup>	p	Significance	R <sup>2</sup>	p	Significance
IBSP	0.1646	0.0556	ns	0.166	0.0465	*	0.2251	0.7438	ns	0.0111	0.0394	*
Sedge Is.	0.3285	0.0002	***	0.3432	0.0001	***	0.3206	0.704	ns	0.2148	0.0024	**
H. Cedars	0.0153	0.5178	ns	0.2612	0.1143	ns	0.0278	0.8141	ns	0.2188	0.4882	ns
Tuckerton	0.2908	0.0338	*	0.2607	0.0305	*	0.4109	0.7898	ns	0.2742	0.2593	ns

**Figure 22.** Relationship between the percent contribution of picoplankton (chlorophytes + cyanobacteria) to total Chlorophyll *a* and growth rate of clams (*k* based on dry tissue weight,  $k_{DW}$ , % change day<sup>-1</sup> calculated over weekly intervals) at IBSP and Sedge Is. Fitted linear equations and the coefficient of determination ( $R^2$ ) are shown. The slope was significantly different from zero (negative) at IBSP (\*  $p = 0.0241$ ) and at Sedge (\*\*\*  $p < 0.001$ ).



## Discussion and Conclusions

The present study provides the first characterization of seasonal and spatial growth patterns of hard clam juveniles in the BB-LEH estuary. We demonstrated highly significant variation both spatially and temporally in growth rates of juvenile hard clams. **Maximal shell growth rates in BB-LEH in 2012 were comparable to those reported in other mid-Atlantic coastal lagoonal ecosystems (up to ~200  $\mu\text{m day}^{-1}$ ) (reviewed by Grizzle et al. 2001).**

Each of the 4 study sites selected for this study exhibited distinctive features, that are representative of different habitats within the BB-LEH estuary.

**Clam growth rates (in terms of both soft tissue and shell growth) integrated over the 7-wk summer period (Trial II) ranked as follows: Sedge > Tuckerton = IBSP > Harvey Cedars.** Clams at Sedge Is. attained the highest mean overall growth rate of  $\sim 180 \mu\text{m day}^{-1}$ , and the maximum weekly shell growth rate. Although current velocities were not measured as part of this study, we believe that they were highest at this site based on our observations (e.g. advection of drift macroalgae). **Overall clam growth over 7 wks was highest at Sedge despite consistently lower temperatures and highest daily temperature fluctuations. Thus hard clam juveniles were found to be relatively tolerant of high summer temperature variability.** We are not aware of previous studies that have documented this effect. Additionally, clams exhibited high overall growth rates at Sedge during Trial II despite the high salinities characteristic of this site (values of 33 were measured during 4 weekly samplings throughout the study period). Although the effects of high salinities on growth of juvenile and adult hard clams are poorly understood, Hamwi (1969) found that clams acclimated to experimental salinities for 4 to 7 days showed a marked reduction in pumping rates at salinities exceeding  $\sim 29$ . Juvenile hard clams also exhibited a significantly higher condition index when this metric was examined over a weekly basis or integrated over a 7 wk period (Trial II, Fig. 17).

**The Tuckerton Cove site supported the next highest overall clam growth rates (both  $k_{\text{DW}}$  and  $k_{\text{SL}}$ ) during Trial II, following Sedge Is.** However, clams held in fine mesh bags experienced the fastest growth at Tuckerton during Trial I. This reversal in ranking between Sedge Is. and Tuckerton cannot be attributed to flow limitation, given that Sedge experienced the highest current velocities based on visual observations. **The Tuckerton site showed the highest water column suspended sediment concentrations, as measured by PIM, and more frequent peaks in this parameter.** Although PIM concentrations did not exceed levels that are inhibitory for clam growth, higher levels are likely to occur at the sediment-water column interface. Higher concentrations of suspended sediments are attributed to the fact that Tuckerton Cove is characterized by fine-grained, muddy bottom, whereas at the other 3 sites clams were deployed above coarse sandy substrate. Additionally the Tuckerton site experienced the largest tidal range of the 4 sites. The Tuckerton Cove site also experienced the highest Chl *a* levels and most pronounced summer Chl *a* peak, occurring during the 2nd week of August. The latter coincided with highest weekly clam growth rate at this site, maximal POC and PON concentrations, and a minimum in the C/N ratio, a putative index of food quality. Higher Chl *a*/POC and Chl *a*/PON ratios were found at Tuckerton, indicating that phytoplankton typically made the greatest contribution to POM at this site. This was found despite the fact Tuckerton Cove is surrounded by marshes, suggesting that salt marsh-derived detritus makes a minor contribution to the food supply of hard clams relative to phytoplankton.

Lowest clam growth rates were found at the Harvey Cedars site. Temperature, salinity and seston parameters measured in this study do not appear to explain this result. Several factors may be invoked, although they remain speculative. **A pelagophyte alga (presumably *A. anophagefferens*, although this remains to be confirmed by specific immunofluorescence methods) was detected at relatively low concentrations at this site, coinciding with a 2-wk period of inhibited clam growth.** Although the estimated concentrations were below levels

known to inhibit growth of juvenile clams, higher concentrations may have occurred between weekly sampling times and contributed to the negative growth rates observed during the last 2 wks of Trial II at this site. [The diagnostic pigment for pelagophytes, 19' butanoylfucocoxanthin, attained  $0.14 \mu\text{g L}^{-1}$  at Harvey Cedars, and  $0.85 \mu\text{g L}^{-1}$  was equivalent to 35,000 *A. anophagefferens* cells  $\text{L}^{-1}$  in Maryland bays (Glibert 2007), the threshold that when exceeded is known to inhibit feeding rates of juvenile hard clams (Bricelj et al. 2004)]. It is noteworthy that Harvey Cedars was the only site where clams were deployed in the vicinity of bulkheaded shoreline, and it is also possible that physical effects (i.e., increased turbulence and wave action generated by bulkheading) may have negatively affected clam growth rates. Finally, it cannot be ruled out that proximity to boat traffic and a developed shoreline could have resulted in the presence of anthropogenic contaminants that adversely affected clam growth.

**Clams exhibited intermediate cumulative summer clam growth rates at IBSP (as determined over 7 wks during Trial II), the site which experienced highest absolute concentrations of organic matter, as measured by water column POM, POC and PON, as well as the lowest % contribution of Chl *a* to total POC and PON. These results suggest that at the detrital contribution to total organic matter is higher at this site. Photopigment data (unpublished data) also indicated that this site showed a relatively high summer % contribution of picoplankton (chlorophytes, and especially cyanobacteria) to total Chl *a*. Thus high organic matter was not necessarily associated with high food levels at this site.**

**The IBSP site is influenced by the Toms River flume, as reflected in lowest mean salinities at this site.** We did not record salinities ( $< 15\text{-}16$ ) known to be inhibit clam growth and/or limit its distribution in natural waters (Grizzle et al. 2001; Bricelj et al. 2012) during our weekly 2012 sampling at this site. However, low salinities associated with heavy precipitation in the watershed coincided with cessation of clam growth during 2013 (unpubl. results). Increased intensity of precipitation events at this latitude, as predicted by climate-driven changes, may lead to suboptimal conditions for growth of clams in this portion of the estuary via direct effects (low salinity) or indirect effects (dominance of the phytoplankton assemblage by picoplanktonic algae, chlorophytes and cyanobacteria). Both of these groups are known to be poorly assimilated by hard clams (Bricelj et al. 1984) and to support poor growth of juvenile *M. mercenaria* (Bass et al. 1990). These algal classes may proliferate at IBSP due to the consistently lower salinities and high nutrient concentrations in this sector of the bay (spatial and seasonal variation in these parameters in BB-LEH reviewed by Bricelj et al. 2012), or may be advected from the Toms River plume during periods of high precipitation and high river flow rates. Zeaxanthin levels, indicative of cyanobacteria, were generally higher at low tides at IBSP, especially near the end of ebb tides (not shown), suggesting a potential riverine source for this group. Advective transport of phytoplankton especially during periods of high river flow has been demonstrated in other temperate, more river-dominated mid-Atlantic estuaries such as the Neuse River Estuary, NC (Paerl et al. 2006).

**It is noteworthy that conditions that supported the highest growth rates of juvenile clams occurred within relatively undeveloped, protected areas of the BB-LEH estuary, namely the Marine Conservation Zone (Sedge Is.) and the Jacques Cousteau National Estuarine Research Reserve (Tuckerton Cove). These two sites are the closest to inlets (Barnegat Bay Inlet and Little Egg Inlet, respectively) and thus experienced the greatest influence from oceanic exchange. Our**

findings contrast with those reported in coastal lagoonal estuaries (Shinnecock and Great South Bay) on the south shore of Long Island, NY, where proximity to the inlets was associated with reduced summer growth of hard clam juveniles relative to central bay locations due to lower temperatures and Chl *a* limitation (Weiss et al. 2007).

Determination of clam growth rates in soft tissues, although considerably more labor-intensive to obtain, provided a more sensitive measure of the response of juvenile clams to weekly changes in environmental variables than shell growth rates. Uncoupling between tissue and shell growth rates was documented in the present study, supporting previous findings in bivalves (Hilbish 1986, Lewis and Cerrato, 1997) that resource allocation between soft tissues and shell can vary depending on conditions and is not controlled by the same limiting factors, despite the fact that there is strong selection for rapid shell growth to attain a refuge from predators during this vulnerable life history stage.

Fouling of deployed cages and mesh bags by macroalgae and other suspension-feeding invertebrates that compete with bivalves for the available food supply (e.g., solitary and colonial tunicates, barnacles, mussels) was more pronounced at the two sites that yielded highest overall clam growth rates, i.e. Sedge Is. and Tuckerton. Fouling was generally negligible at the IBSP and Harvey Cedars deployment sites. Overall, fouling was maintained in check by our weekly cage and mesh cleaning protocols.

**Temperatures and growth rates of clams in land-based upwellers at IBSP generally closely tracked those at the adjacent IBSP field site (Trial II, 4 mm mesh).** This suggests that flow rate was not limiting for clam growth at the IBSP field site. When a finer mesh (1x2 mm) was used (Trial I), however, growth rates were greater in the upwellers than the adjacent IBSP field site. This effect is attributed to the higher, forced flow maintained in the upwellers which prevented food limitation in the finer mesh bags. Our findings suggest that existing upwellers established throughout the estuary to provide a source of seed and contribute to public education and involvement in clam aquaculture, could provide useful information on spatial and temporal (seasonal and interannual) juvenile hard clam growth as an indicator of water quality in the BB-LEH. This would require, however, collection of appropriate growth data, and more rigorous control of stocking densities, clam sizes, etc. Monitoring of clam growth using standardized, controlled protocols could be incorporated into reClam The Bay practices in future.

**A significant positive effect of temperature on clam growth rates was detected in this study, at Sedge Is. and Harvey Cedars over a relatively narrow range of mean weekly temperatures (□ of 6.6°C, 21.0 to 26.2°C, and □ of only 4.5°C, 23.3 to 27.8°C at these two sites, respectively).** This temperature effect was evident although *M. mercenaria* growth rates are generally considered to be optimized and relatively constant between ~ 20 and 25°C, while declining above and below this temperature range (reviewed by Grizzle et al. 2001).

It is important to recognize that discrete, weekly measurement of water column seston metrics and salinity (continuous records were available for temperature) will likely be influenced by our sampling time in relation to the tidal cycle, whereas clam growth rates provided a time-integrated (weekly) measure of conditions experienced at the site. In most cases, however, our sampling

regime allowed representation of various stages of the tidal cycle and thus allows a synoptic characterization of conditions at this site.

The emerging relationships documented in this study between growth of hard clam juveniles and environmental parameters (most notably the positive effect of temperature and negative effect of the % contribution of picoplankton to total Chl *a*), are based on a relatively small number of weekly samples (7 to 11). Yet high-resolution (weekly) clam growth data and concurrently determined environmental parameters across a north to south gradient in BB-LEH were unavailable prior to this study. We expect that additional data generated in Yr 2 (2013) at the same 4 study sites, including the characterization of phytoplankton composition from FTG analysis as a measure of food quality, will further enhance our ability to predict growth rates from relevant environmental variables within key habitat types in this estuary.

### Acknowledgements

We thank Carola Noji, technician at IMCS/RU, and Ashley Andrews, RU undergraduate student, for their participation in field sampling, and the latter for processing of clam samples from Trial I. We thank Lisa Izzo for occasional field participation, and especially for processing of clams from Trial II and data workup, and Ryan Fantasia for his contribution to preparation of this report. He also contributed to the analysis of phytoplankton FTGs (work supported by the BBP). Jennifer Tomko, a graduate student in the Education Department at RU in the fall of 2012 occasionally assisted in field sampling. We thank Jeffrey Silady who provided boat access to Sedge Is. and his unfailing support and good humor during our project activities. We also thank Jim Merritt, Sedge Island Fish & Wildlife National Resource Education Center, Marine Conservation Zone, for his assistance during early phases of the project, Larry Murphy for providing access to his property in Harvey Cedars, and the IBSP Forked Interpretive Center for providing space at their facility and access to the Park for in situ processing of water samples.

### References

- Bass, A.E., R.E. Malouf and S.Shumway, 1990. Growth of northern quahogs (*Mercenaria mercenaria* (Linnaeus, 1758)) fed on picoplankton. *J. Shellfish Res.* 9: 299-307.
- Bricelj, V. M., 2009. The Hard Clam Research Initiative: Factors controlling *Mercenaria mercenaria* populations in South Shore Bays of Long Island, NY. New York Sea Grant, Report NYSGI-T09-001, 43 pages.
- Bricelj, V. M., J. N. Kraeuter, G. Flimlin, 2012. Status and Trends of hard clam, *Mercenaria mercenaria*, populations in Barnegat Bay, New Jersey. Technical Report prepared for the Barnegat Bay Partnership, NJ., 143 pp.  
<http://bbp.ocean.edu/Reports/Barnegat%20Bay%20Hard%20Clam%20White%20Paper%20Final.pdf>
- Bricelj, V.M., S. MacQuarrie, R. Smolowitz. 2004. Concentration-dependent effects of toxic and non-toxic isolates of the brown tide alga *Aureococcus anophagefferens* on growth of juvenile bivalves. *Mar. Ecol. Prog. Ser.* 282: 101-114.

Bricelj, V.M., S. MacQuarrie, R.A. Schaffner, 2001. Differential effects of *Aureococcus anophagefferens* isolates ("brown tide") in unialgal and mixed suspensions on bivalve feeding. *Mar. Biol.* 139: 605-615.

Bricelj, V.M., A.E. Bass, G.R. Lopez, 1984a. Absorption and gut passage time of microalgae in a suspension feeder: an evaluation of the  $^{51}\text{Cr}$ : $^{14}\text{C}$  twin tracer technique. *Mar. Ecol. Prog. Ser.* 17: 57-63.

Bricelj, V.M., R.E. Malouf, C. de Quillefeldt, 1984b. Growth of juvenile *Mercenaria mercenaria* and the effect of resuspended bottom sediments. *Mar. Biol.* 84: 167-173.

Carriker, M.R., 1961. Interrelation of functional morphology, behavior and autoecology in early life states of the bivalve *Mercenaria mercenaria*. *Journal of the Elisha Mitchell Science Society* 77:168-241.

Doall, M.H., D.K. Padilla, C.P. LoBue, C. Clapp, A.R. Webb, and J. Hornstein, 2008. Evaluating northern quahog (= hard clam, *Mercenaria mercenaria* L.) restoration: Are transplanted clams spawning and reconditioning? *J. Shellfish Res.* 27:1069–1080.

Goericke, R. and J.P., Montoya, 1998. Estimating the contribution of microalgal taxa to chlorophyll a in the field – variations of pigment ratios under nutrient and light-limited growth. *Mar. Ecol. Prog. Ser.* 169, 97-112.

Glibert, P. (2007) Seasonal and interannual trends in nitrogen and brown tide in Maryland's coastal bays. *Ecological Applications* 17.5: 79-87.

Grizzle, R. Bricelj, V.M., S.E. Shumway, 2001. Physiological ecology of *Mercenaria mercenaria*. Chapter 8 In: *Biology of the Hard Clam*. J. N. Kraeuter and M. Castagna (eds.), Elsevier Publ., New York, pp. 305-382.

Hamwi, A., 1969. Oxygen consumption and pumping rate of the hard clam *Mercenaria mercenaria* L. PhD. Dissertation, Rutgers University, New Brunswick, NJ, 177 pp.

Hilbish T.J., 1986. Growth trajectories of shell and soft tissue in bivalves: seasonal variation in *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.* 96:103-113.

Kennish, M. J. and B. Fertig, 2012. Application and assessment of a nutrient pollution indicator using eelgrass (*Zostera marina* L.) in Barnegat Bay–Little Egg Harbor Estuary, New Jersey. *Aquatic Botany*, 96: 23-30.

Kennish, M. J., S. M. Haag and G. P. Sakowicz, 2010. Seagrass decline in New Jersey coastal lagoons: A response to increasing eutrophication. In: M. J. Kennish and H. W. Paerl, eds., *Coastal Lagoons: Critical Habitats of Environmental Change*. Taylor and Francis, CRC Press, Boca Raton, Florida, pp. 167-201.

Kimbrough, K. L., W. E. Johnson, G. G. Lauenstein, J. D. Christensen and D. A. Apeti. 2008. An Assessment of Two Decades of Contaminant Monitoring in the Nation's Coastal Zone. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 74. 105 pp.

Kraeuter, J.N., J. Klinck, E.N. Powell, E. Hoffman, S.C. Buckner, R.E. Grizzle and V.M. Bricelj, 2008. Effects of the fishery on the northern quahog (= hard clam, *Mercenaria mercenaria* L.) population in Great South Bay, New York: A modeling study. *J. Shellfish Res.* 27(4): 653-666.

Lewis, D.E. and R.M. Cerrato, 1997. Growth uncoupling and the relationship between shell growth and metabolism in the soft shell clam *Mya arenaria*. *Mar. Ecol. Progress Ser.* 158: 177-189.

Mahoney, J.B., P.S. Olsen and D. Jeffress, 2006. Bloom history of picoplankter *Aureococcus anophagefferens* in the New Jersey Barnegat Bay-Little Egg Harbor system and Great Bay, 1995-1999. US Dept. Commerce, Northeast Fish Sci. Center Ref. Doc. 06-07; 54.

Newell, R.I.E., S. T. Tettelbach, C. J. Gobler and D. G. Kimmel, 2009. Relationships between reproduction in suspension feeding hard clams *Mercenaria mercenaria* and phytoplankton community structure. *Mar. Ecol. Prog. Ser.* 387: 179-196.

Olsen, P.S. and J.B. Mahoney, 2001. Phytoplankton in the Barnegat Bay-Little Egg Harbor estuarine system: species composition and picoplankton bloom development. Pp. 115-143 In: Barnegat Bay-Little Egg Harbor, New Jersey: estuary and watershed assessment. M.J. Kennish (ed.) J. Coastal Res. Special Issue #32.

Paerl, H.W., L.M. Valdes, J.L. Pinckney, M.F. Piehler, J. Dyble and P.H. Moisander. Phytoplankton photopigments as indicators of estuarine and coastal eutrophication. *BioScience* 53(3): 953-964.

Paerl, H.W., L.M. Valdes, J. Adolf, B.L. Peierls and L.W. Harding, Jr., 2006. Anthropogenic and climatic influences on the eutrophication of large estuarine ecosystems. *Limnol. Oceanogr.* 51: 448-462.

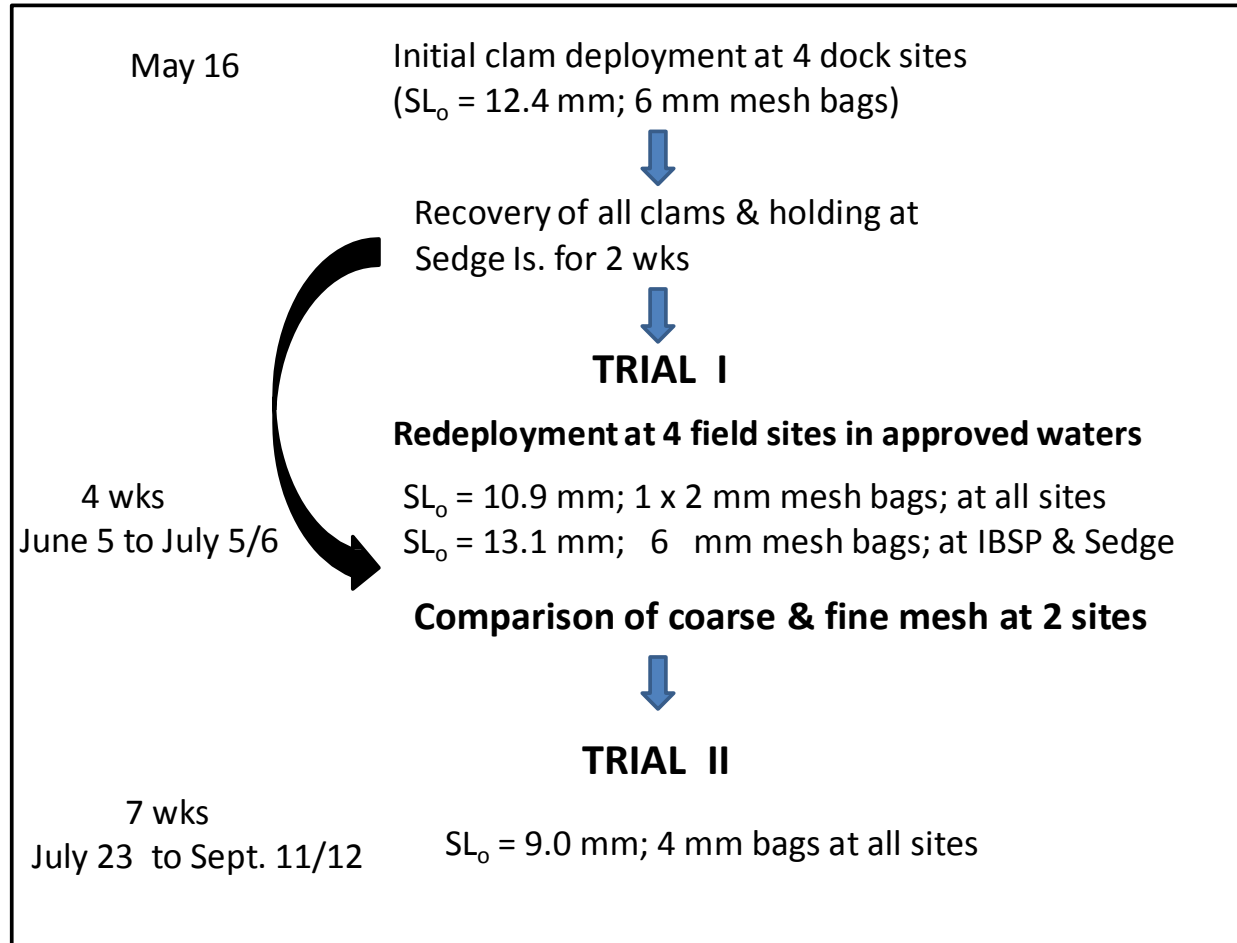
Powell, E.N., D.A. Kreeger, J.M. Morson, D.B. Haidvogel, Z. Wang, R. Thomas and J.E. Gius, 2012. Oyster food supply in Delaware Bay: estimation from a hydrodynamic model and interaction with the oyster population. *J. Mar. Res.* 70: 469-503.

Sokal, R.R. and F.J. Rohlf, 1995. Test of significance in regression. Pp.: 466-476 In: Biometry. The Principles and practice of statistics in biological research. W.H. Freeman & Co., New York.

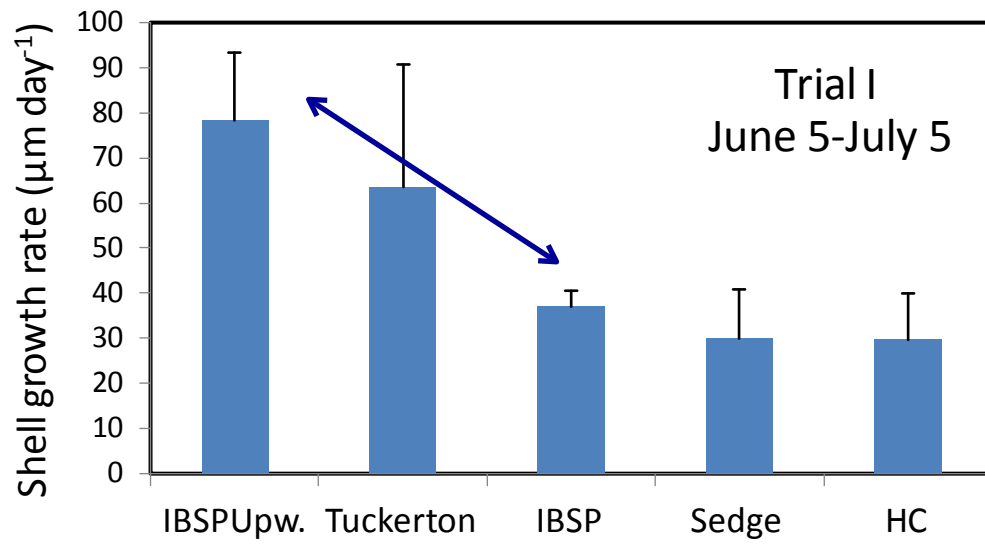
Weiss, M.B., P.B. Curran, B.J. Peterson and C.J. Gobler, 2007. The influence of plankton composition and water quality on hard clam (*Mercenaria mercenaria* L.) populations across Long Island's south shore lagoon estuaries (New York, USA). *J. Exp. Mar. Biol. Ecol.* 345:12-25.



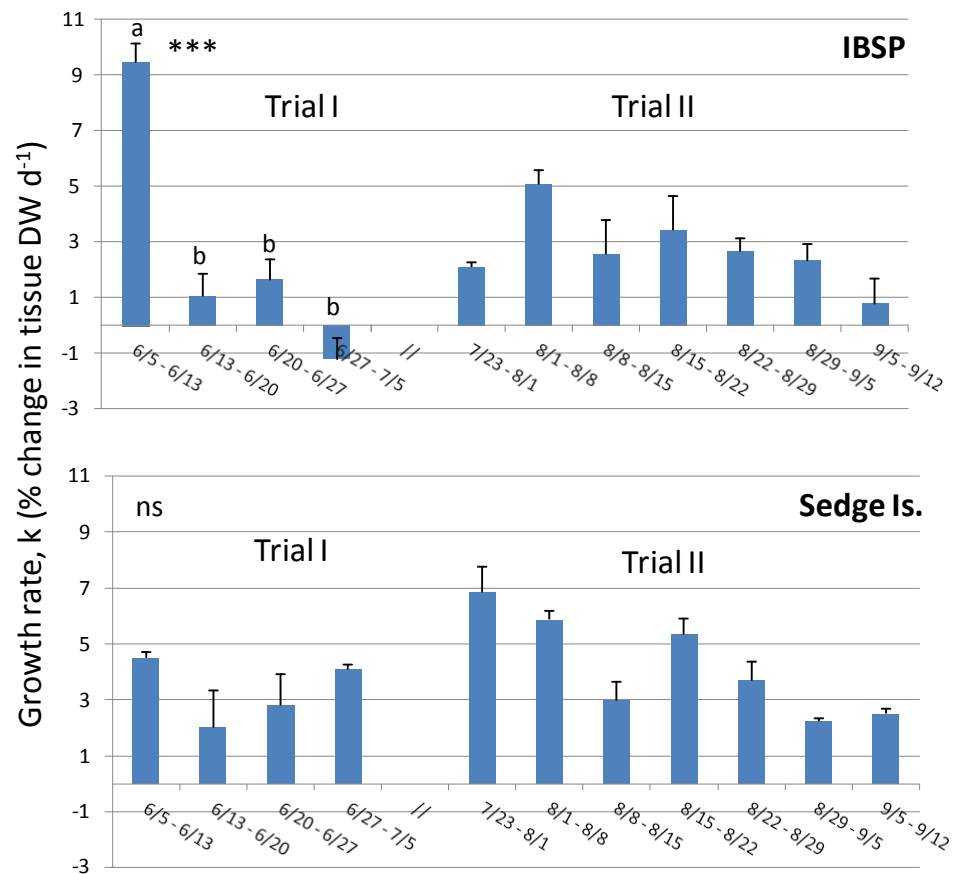
**Appendix 1.** Chronology of various trials conducted in 2012.



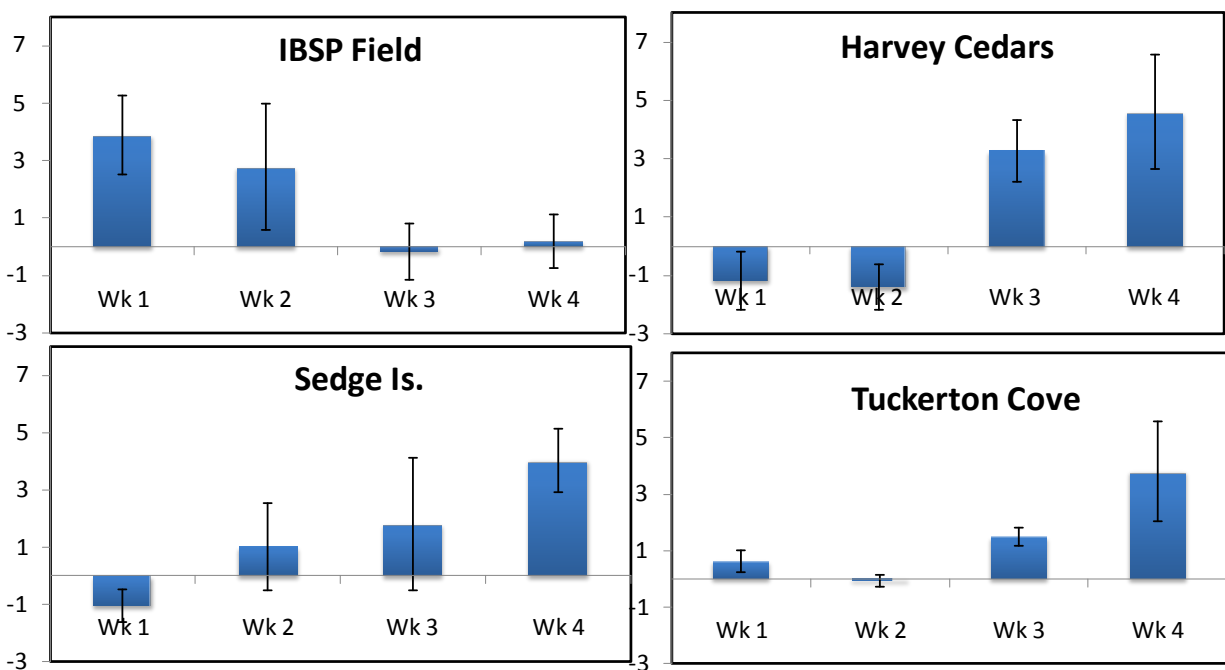
**Appendix 2.** Shell growth rate (in  $\mu\text{m day}^{-1}$ ) of juvenile clams held in 1x2 mm mesh bags over 4 wks during Trial I 2012.



**Appendix 3.** Comparison of weekly growth rates of juvenile hard clams (mean instantaneous growth coefficient, based on dry weight of soft tissues,  $\pm$  SE, during Trial I (June 5 to July 5, 2012; 6 mm mesh bags) and Trial II (July 23 to September 12, 2012; 4 mm mesh bags). Data for Trial II are the same as shown in Fig.15 but are shown here to allow direct comparison with Trial I. Note that clams from the two trials were obtained from the same commercial grower (George Mathis Inc, NJ) but originated from different spawnings. Statistical results comparing growth rates over 4 wks are shown for Trial I (those for Trial II over 7 wks are reported in Fig. 17).



**Appendix 4.** Weekly growth rate in soft tissue DW (mean of 3-4 cages  $\pm$  SE) of juvenile clams during Trial I, using 1x2 mm mesh bags that were demonstrated in the present study to significantly limit flow and thus food delivery. Therefore, these data only allow relative comparisons among sites (note that deployments during Trial II used coarser mesh bags, 4 x 4 mm to preclude this confounding effect).



**Appendix 5.** Comparison of weekly condition indices (mean CI  $\pm$  SE, n – 3 cages) of juvenile hard clams during Trial I (June 5 to July 5, 2012; 6 mm mesh bags) and Trial II (July 23 to September 12, 2012; 4 mm mesh bags). Initial condition index of clams at the time of Trial I deployment (mean  $\pm$  SD, n =50 clams) =  $6.22 \pm 2.22$ , and at the time of Trial II deployment =  $11.98 \pm 1.34$ .

