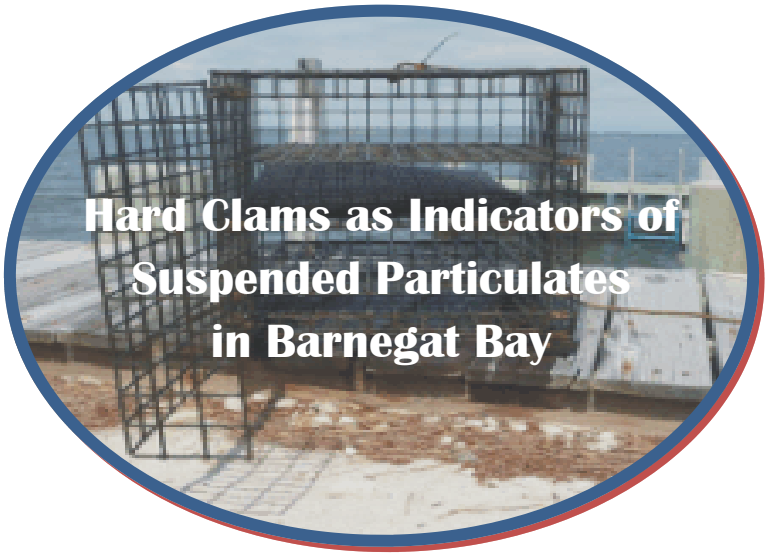
Barnegat Bay—Year 3



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

Final Report for the Project: "Assessment of summer-fall conditioning of hard clams, *Mercenaria mercenaria*, in the Barnegat Bay estuary in relation to clam size and environmental conditions"

Project period: April 1, 2014 to July 31, 2015

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

Lead Principal Investigator: V. Monica Bricelj, Research Professor,
Department of Marine and Coastal Sciences and Haskin Shellfish Research Laboratory,
Rutgers University, 6959 Miller Avenue,
School of Environmental and Biological Sciences
Port Norris, New Jersey 08349-3167.
Ph.**:** (732) 882-5874; Fax**:** 856.785.1544;
e-mail: mbricelj@marine.rutgers.edu or monica.bricelj@gmail.com

Co-PI: John Kraeuter, Visiting Scientist,
Haskin Shellfish Research Laboratory
(HSRL)**,** Rutgers University, 6959 Miller
Avenue, Port Norris, New Jersey 08349-
3167.
Ph.**: (**856) 785-0074, ext. 4331; Fax**:** 856.785.1544;
e-mail: kraeuter@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

Additional participants: Ryan Fantasia, Carola Noji, technicians, Department of Marine and Coastal Sciences, Rutgers University, 71 Dudley Road, New Brunswick, NJ 08901, and Emily McGurk, HSRL, Rutgers University.

September 14, 2015

Executive Summary

This study examined the seasonal (summer/fall), size-specific reproductive conditioning, natural mortality and spawning pattern of adult hard clams, *Mercenaria. mercenaria*, at two contrasting sites in the Barnegat Bay-Little Egg Harbor (BB-LEH) estuary relative to environmental conditions and food supply.

The present study developed a Visceral Mass Index (VMI) that was found to be more sensitive than the standard Condition Index (CI) used in the bivalve literature, as a reliable, real-time measure of female hard clam reproductive condition. The VMI used in concert with two other reproductive metrics (% oocytes and % germinal tissue) confirmed that clam reproductive allocation was significantly lower at Sedge Island than at Island Beach State Park (IBSP). Low salinity, and low juvenile growth rates were documented at IBSP in 2012-2013 and the even lower salinities with episodic reductions that approached the clams' tolerance level were documented during the 2014 study. In contrast, summer high salinities, (mean ~30 psu, max. 34 psu) were typical of the Sedge Island site throughout the 2012-2014 study period. Consistently high daily temperature fluctuations (up to 10-16 ^{o}C) were also documented at Sedge Island from mid-April to the end of August over the three consecutive years. Gonadal re-conditioning in large clams (cherrystones and chowder size classes) was observed in July at IBSP and not at Sedge Island.

There was considerable asynchrony in reproductive conditioning among clams of different size classes. Smaller clams (littleneck commercial size class) were characterized by a significantly lower reproductive condition than larger clams at both sites. Throughout the present study, littlenecks showed on average a 31-33% lower VMI than cherrystone and chowders, respectively; this difference in reproductive allocation was even greater at IBSP, where the VMI of littlenecks was on average 40% lower than that of larger size classes.

The VMI values alone (starting June 30) showed no evidence of spawning of littleneck clams at Sedge Island while histological slides from clams collected on June 16 showed some evidence of earlier spawning of this size class. None of the reproductive indices measured were able to detect any major spawning activity of littlenecks at Sedge Island. In contrast, both the VMI and % oocytes showed evidence of spawning of this size class at IBSP. Large clams (cherrystones and chowders) showed evidence of fairly protracted spawning activity throughout the entire summer at IBSP. As determined by both VMI and % oocyte indices, spawning appeared to occur earlier at Sedge Island than at IBSP. A reduction in these indices observed during the October sampling may be attributable more to gamete resorption than late spawning.

Phytoplankton composition was determined from photopigment analysis, confirmed in 2012 and 2013 by microscopic taxonomic identification. Data from this study confirmed previous results (2012, 2013) indicating that hard clam food quality at IBSP is characterized by a unique phytoplankton assemblage which is likely associated with the lower salinities and differences in nutrient loading and composition resulting from the influence of the Toms River plume. Mean total summer Chl *a* concentrations were particularly low at Sedge island during the 2014 reproductive study (1.95 µg l^{-1}, 2.5x lower than at IBSP). They were also generally low at Sedge relative to other BB-LEH study sites in previous years. The mean concentration of diatoms, generally considered a good food source for hard clams, at IBSP during the 2014 study period (= 1.28 µg Chl *a* l^{-1}) was comparable to the total Chl *a* concentration at Sedge Island (= 1.87 µg l^{-1}), including all microalgal

taxa. Although IBSP was characterized by a higher % contribution of chlorophytes and cyanobacteria to total Chl *a*, groups which are known to provide a poor food source for hard clams, the better reproductive performance may be attributable to the higher food availability at IBSP than at Sedge Island.

Concentrations of the harmful "brown tide" alga *Aureococcus anophagefferens* (up to ~440 cells μl^{-1}) were documented at Sedge in June 2013. There was a strong linear relationship between the density of *A. anophagefferens* and the concentration of the pigment 19 butanoyloxyfucoxanthin. No brown tide was detected in 2014 based on this pigment, but the occurrence of brown tide at two out of four sites in the BB-LEH in 2013 indicates that monitoring of *A. anohagefferens* should be reinstated in this estuary.

Some larger clams (cherrystones and chowders) but not littlenecks revealed a varying degree of discolored, grey viscera. Histological sections of these "grey clams" showed a degree of proliferation of brown cells in the connective tissue surrounding gonadal follicles. Brown cells are known to play a role in detoxification and constitute a stress response in bivalves. We found however, no clear relationship between the external grey appearance of clams and the prevalence of these brown cells in their tissues. This discoloration may lead to poor market acceptance and requires further investigation.

The finding of poor clam reproductive performance at the Sedge Island site is important given that this site lies in the Marine Conservation Zone (MCZ), and area that has experienced considerable state investment in hard clam stock enhancement (seeding). Our one year study of clam reproduction is useful in assessing the suitability of the Sedge Island area as a spawner sanctuary site and in identifying the environmental conditions suitable for reproduction of hard clams. The low reproductive performance of clams documented at Sedge Island in 2014 may be caused by several factors acting singly or in concert that require future investigation: a) low food supply at Sedge Island during the 2014 study period, b) the consistently lower summer temperatures at Sedge Island [the minimum temperature for spawning of *M. mercenaria* (~ 24°C) was only attained by mid-August, a month later than at IBSP during 2014, c) the Sedge Island site, based on three years of study, is characterized by high temperature fluctuations that may result in dribble-spawning and disrupt the reproductive cycle, given that temperature change is known to induce spawning of hard clams and other bivalves, d) clams for this study were transplanted in May 2014 from other locations in BB-LEH and may thus not have been adapted to local conditions at Sedge Island. The contributing role of substrate in explaining site differences documented in this study cannot be excluded as bottom plots established at Sedge Island were in finer-grained sediment than at IBSP.

At the same time, it is necessary to establish if the high overwinter mortality at Sedge Island is a typical or rare event. The cumulative losses indicated by our 2014 summer/fall sampling are generally in line with data from other studies. Calculations using the survey abundance data to provide an overall size-specific natural mortality loss for the harvestable sizes, and assuming that sublegal clams are recruits, suggest that clams in BB may not be recruiting fast enough to keep up with natural losses, but that the recent increase in recruitment in LEH more that offsets natural losses. These estimates should be viewed with extreme caution because of very limited date. The disappearance of clams from the western side of LEH and higher mortalities of adult clams determined in the NJDEP survey data in LEH, and the increasing numbers of stations with low clam densities in BB need investigation. The MCZ could potentially provide a broodstock sanctuary area

as part of a clam stock enhancement management strategy in this estuary. Future, multi-year studies are needed, however, to determine whether the low reproductive performance of hard clams at the Sedge Island location was an annual occurrence or occurs consistently, and whether it is characteristic of the MCZ ecosystem as a whole. More extensive studies conducted over several years are required to determine whether this result can be generalized and identify the causes for this outcome.

I. Background and Justification.

Our research effort in the first two years (2012 and 2013) in response to the NJ Governor's 10 Point Action Plan to fill knowledge gaps identified in the Barnegat Bay-Little Egg Harbor estuary, focused on determining growth rates (and mortalities) of juvenile hard clams, *Mercenaria mercenaria*. These were related to key environmental parameters, including temperature, salinity and food quality and quantity that are known to be important in controlling hard clam somatic production, at four representative sites along the estuary. Rapid growth during this early life history stage is key to attaining a size refuge from predators and enabling recruitment to commercial sizes. The third year of the study (2014) focused on determining the size-specific reproductive performance (condition and spawning patterns) and natural mortalities at two contrasting sites in the BB-LEH, and to relate these to environmental conditions important in controlling reproductive production. Relevance of results to management of the hard clam resource is addressed at the end of this report.

Our knowledge of hard clam, *Mercenaria. mercenaria*, reproduction in the BB-LEH estuary, despite the importance of the clam resource in this ecosystem, derives solely from a 5-yr study conducted in the late 40s in LEH (Carriker 1961). This early, seminal study determined the timing and duration of the hard clams' reproductive period from the capacity to induce spawning of field-collected adults in the laboratory, and the presence and abundance of larvae in the field. According to Carriker spawning typically started in late June, peaked in July, and some spawning continued into late August and the 1st week of September. Yet laboratory induction of spawning was achieved throughout July but was not successful for clams collected in August.

Thus, no direct information is available on the seasonal reproductive conditioning of hard clams in BB-LEH**.** While the data from the 1940s showed that LEH once favored hard clam spawning, there is no information about the magnitude of size-specific reproductive output under current environmental conditions in any portion of the BB-LEH estuary**.** This is important to determine given that this ecosystem has experienced extensive changes in past decades, i.e., increased urbanization, eutrophication, loss of habitat and marked reduction of hard clam populations (e.g., Kennish 2007). Reproductive performance may become particularly important in areas with very low clam population densities [$\leq$ 0.8 clams m^{-2} ($\leq$ 0.074 clams ft^{-2})] over a large portion of LEH in a 2001 survey) (reviewed by Bricelj et al. 2012), and thus below the density threshold suggested to be required for the maintenance of self-sustaining populations in Great South Bay, NY (Kraeuter et al. 2005). A 2011 survey of LEH showed no statistically detectable change in hard clam abundance per station relative to the 2001 survey, with a geometric mean density of 0.10 clams ft^{-2} in 2011 vs. 0.08 clams ft^{-2} in 2001 (Celestino 2003, 2013). A survey of Barnegat Bay in 2012 and a follow-up survey after Superstorm Sandy in 2013 showed few differences between the two years, but a 23 % decline in overall abundance from the 1985/86 survey (0.15 clams ft^{-2} vs. 0.10 clams ft^{-2}) (Joseph, 1986; Joseph, 1987; Dacanay, 2015) Furthermore, normal gametogenesis has

been described for clams over the salinity range 25 to 30, but little is known about reproductive development above and below this range (reviewed by Eversole 2001), and the BB-LEH estuary experiences considerable spatial gradients in salinity.

Condition

The commonly used condition index of adult hard clams, *Mercenaria mercenaria*, [CI = (tissue weight, DW x 100)/internal shell cavity capacity, where the internal shell cavity = Total live wet weight – Shell DW] (Crosby and Gale 1990) provides a measure of nutritional state, and has also been used in the published literature as an index of reproductive condition. A sharp decline in this condition index was argued to reflect spawning, typically occurring in June-July in Long Island, NY, bays, and is preceded by an increase in CI during the spring (Doall et al. 2008, Newell et al. 2009). Hard clams have typically been considered opportunistic bivalves that reproduce primarily at the expense of the phytoplankton they feed on during the spring when they undergo gametogenesis (Eversole 2001). However, multi-year studies of both native and transplanted clams into Great South Bay, NY, by The Nature Conservancy (TNC) found that the late summer-fall period of post-spawning conditioning of hard clams appears to be of critical importance in controlling their reproductive performance the following spring-summer (Doall et al. 2008, LoBue 2010)**.** Thus, the condition at the end of the fall explained ~89% of the variance in peak spring condition. This is consistent with the results from an existing model simulating population dynamics of hard clams (Hofmann et al. 2006). Furthermore, while the CI declined during late summer/fall in some estuaries (and years), it increased over this period in others (LoBue 2010), reflecting variable environmental conditions.

The condition index of adult hard clams is thus known to vary greatly among estuaries and among locations within an estuary: it was generally lower in Great South Bay than in other Long Island, NY, bays (Doall et al. 2008, LoBue 2010, Newell et al. 2009). The latter study also found that the clams' reproductive output was generally lower in south shore Long Island bays than in Sandy Hook Bay, NJ (3-fold maximum difference among sites). Large inter-annual variability in reproductive potential in NY bays was also documented in the above studies. Both food and temperature are key factors affecting hard clam reproductive performance.

Temperature (absolute values and rate of decrease) and food supply (quantity and quality) during the fall can influence the condition of hard clams at the time when they enter the winter quiescent period. Thus, the onset of temperatures >10ºC in the spring, and <10ºC the previous fall were found to affect adult condition in Long Island south shore estuaries (LoBue et al. 2009).

Clam condition is also greatly influenced by the species composition and size structure of the phytoplankton assemblage, the main food supply for suspension-feeding *M. mercenaria*. Lower condition and reproductive performance have been associated with the dominance of small (< 5 µm) microalgae as a % of total Chl *a* in the water column during the clams growing season (spring, summer fall) (Newell et al. 2009, LoBue 2010), as well as with low concentrations of centric diatoms. Poor clam condition in GSB has also been associated with the occurrence of brown tide (*Aureococcus anophagefferens*) (LoBue 2010). Peak densities of this alga in mid-Atlantic estuaries typically occur between mid-May and early June, but high levels that inhibit clam feeding can also occur in the fall.9 Intense brown tide was documented in BB-LEH prior to 2004 when routine monitoring for *A. anophagefferens* ceased (reviewed by Bricelj et al. 2012). Moderate cell densities of *A. anophagefferens* (up to 158 x 10^3 cells ml^{-1}) putatively based on algal pigment analysis were also documented more recently, in Aug. 2010 in LEH (Wei et al. 2011). Furthermore, densities (up

to 5.3 x 10^3 cells ml^{-1}) were confirmed by immunofluorescence in LEH and lower Barnegat Bay in July 2012 (L. Ren, Academy of Nat. Sci. of Drexel Univ, PA, pers. comm.). This species thus remains present in the BB-LEH system and other picoplankters (cyanobacteria, chlorophytes) of poor nutritional value (Bricelj et al. 1984, Bass et al. 1990), make an important seasonal contribution to the phytoplankton assemblage in BB-LEH (Olsen and Mahoney 2001, Bricelj et al. 2012 and 2013 unpublished data).

The condition index of *M. mercenaria* may also be influenced by clam size, although the evidence to date is contradictory. No effect of clam size was detected in Newell et al's (2009) study, but Doall et al. (2018) found that whereas the seasonal pattern of conditioning was comparable among clam sizes, larger clams (mean shell length, SL = 88-89 mm) consistently showed a higher CI than smaller clams (mean SL = 63 mm). The hard clam population dynamics model (Hofmann et al. 2006) also predicts that reproductive output reflects and interaction between clam size and food supply. While the largest clams have the greatest potential gamete output, they may contribute a lower reproductive output per unit size than smaller clams in years of below average or inadequate food supply. It is therefore important to evaluate size-specific effects on seasonal condition and reproductive performance.

Natural Mortalities

It is important to note that an increase in the estimated mortality between the clam surveys conducted in the BB-LEH in the 1980s and 2001 suggests that, in addition to lower recruitment, an increased mortality rate is also reducing these populations (Joseph, 1985, 1986; Celestino, 2002, 2013; Bricelj et al 2012). A recent survey (Dacanay 2015) in Barnegat Bay also shows a decline in the stocks, and although there is an overall trend for more stations with lower density than before, there has been a drop in the mortality rate since the 1986 survey (Joseph, 1985, 1986). The magnitude and cause/s of the additional adult mortality (e.g. predation, QPX disease, and/or senescence) remain unknown and need to be further evaluated. Although clams >30 mm are known to attain size-refuge from most predators, they remain vulnerable to gastropod (whelk), starfish fish and bird predation (Kraeuter, 2001; Table 6 in Bricelj et al. 2012). A previous study conducted in Raritan Bay in unprotected plots in the low intertidal zone that did not exclude predators, suggested that adult clam survival (for clams ranging in size from 26 to > 66 mm SL) was size-specific (Kraeuter et al. 2009). It was difficult, however, to unequivocally determine size-specific mortalities from this study because of the occurrence of large numbers of missing clams in these unprotected plots, which resulted in underestimates of natural mortality rates. It was assumed in this study that processes controlling mortality rates were comparable under intertidal and subtidal conditions, although this remains to be verified. A high percentage of missing clams from experimental plots (up to 40%) was also reported by Peterson and Beal (1989). In turn, mortality rates derived from surveys of natural populations are typically based on box counts (number of empty-attached shells out of total recovered) (e.g. Celestino 2003) which have their own inherent bias, as there is limited information on the lifespan of paired shell valves under varying environmental conditions (e.g. substrate, temperature).

II. Study Objectives

The primary objective of this study was to determine the summer/fall, size-specific reproductive conditioning of hard clams, *M. mercenaria*, at two contrasting sites in the BB-LEH estuary in relation to environmental conditions, primarily food supply (quantity and quality), temperature and salinity. Since temperature differential is a key factor in triggering bivalve spawning, we speculated

that the high daily temperature fluctuations (up to 10-16°C) previously documented at Sedge Island (Bricelj et al. unpublished data, one of the two study sites selected in 2014), may induce a different spawning pattern ("dribble-spawning") at this site. A further objective was to determine size-specific natural mortalities of adult hard clams at the two study sites.

III. Materials and Methods

a) Experimental design and study sites

Adults of three commercial size classes (chowders, cherrystones and littlenecks) were deployed on the bottom in four 14 x 20 ft plots (= 280 ft^2 = 26 m^2 per plot) in relatively shallow water (≤ 2 m) at each of two sites in Barnegat Bay in mid-April (Fig. 1).

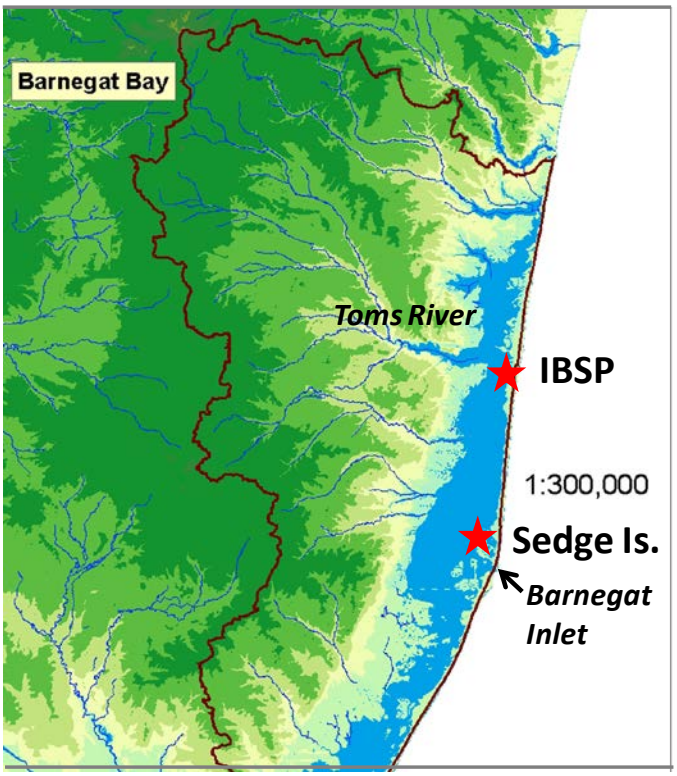
Wild clams were harvested from Tuckerton Cove, Barnegat Bay-Little Egg Harbor (BB-LEH) by a commercial grower and were spray-painted (color-coded by size class) prior to deployment to differentiate between planted and any native clams. Clam sizes ranged from 38 to 55 mm shell length (SL) for littlenecks, 56 to 76 mm SL for cherrystones and >76 mm SL for chowders. Thus all littlenecks exceeded the size of first sexual maturity for *M. mercenaria*, established at 30 to 35 mm SL (Eversole 2001). Clams (~20 to 24 per size class and per sampling date) were collected using a handheld rake with ~7/8" (22.2 mm) basket mesh opening. Clams were sampled from each of the 4 plots on a rotational basis in order to maintain a constant stocking density among plots at each site. Clam plots were covered with ½" mesh screens to minimize access by predators. Screens were staked into the sediment at all 4 corners and also in the middle of the longest dimension of the screen to keep the screen flush with the bottom. Clams of each size class were released haphazardly along 3 rope lines held taunt within each plot at the time of deployment, to facilitate their recovery during the study period.

Both study sites selected for the present study were used in two previous NJDEP-supported projects conducted in 2012 and 2013 to determine growth rates of juvenile hard clams in relation to environmental parameters, and thus provide a useful prior database on temperature, salinity and seston characteristics.

The two study sites were:

- Island Beach State Park (IBSP), northern BB, southeast of Toms River. Based on our 2012/2013 sampling this site is characterized by lower salinities due to the influence of the Toms River plume (summer average of ~22 over the previous two years), and a relatively high contribution of cyanobacteria to the phytoplankton assemblage (Bricelj et al. unpublished, Fantasia et al. in prep.).
- Sedge Island lies within the Sedge Is. Fish and Wildlife National Resource Education Center, Marine Conservation Zone (MCZ), central BB, where NJDEP hard clam stock enhancement activities have been conducted in the past. The bottom of the MCZ is covered with eelgrass, *Zostera marina*. This site typically exhibits higher salinities (summer average of ~30), due to oceanic exchange via Barnegat Inlet, consistently lower summer temperatures as well as high tidal temperature fluctuations (10 to 16°C summer variation within a day based on averages of 2 hr-daily records in
- 2012/2013), and a high relative contribution of diatoms to the phytoplankton community (2012 and 2013 unpublished data).

Figure 1. Barnegat Bay- Little Egg Harbor (BB-LEH) ecosystem, NJ, Red stars indicate our two selected sites for deployment of adult hard clams. Latitude/longitude coordinates for field sites are as follows: IMBS field site: 39º54' 20.2818"N/74º05'16.209"W; Sedge Island site: 39º 47' 40.5"N/-74º07' 06.8"W.



Sampling of the clams and of the water column to determine seston characteristics and phytoplankton composition from photopigment analysis (see below), started in mid-June 2014, and was conducted approximately every two weeks. Water samples from the two sites were processed at the IBSP Forked River Interpretive Center (see outreach section below) in one day, within ~ 1-2 hrs of sample collection and following transport in a cooler on ice.

b) Water column parameters

Discrete water column salinities were determined approximately every two weeks with a refractometer. Onset HOBO® data loggers (one per site) were deployed and programmed to record temperatures every 15 min. These were retrieved at the end of the October 2014 sampling period. An Onset HOBO® conductivity probe was also deployed at IBSP to obtain a continuous record of conductivity/salinity, given that our previous work at this site indicated that it can experience transient, low salinities (a minimum of 16 in 2013) associated with heavy rainfall events. The water column was sampled biweekly using a Masterflex® battery-powered peristaltic pump, to determine particulate inorganic and organic matter (PIM and POM, respectively), Chlorophyll *a* (Chl *a*) and diagnostic photopigment concentrations of key functional taxonomic groups (FTGs) (duplicate samples for all analyses) were used to determine phytoplankton abundance and composition, following methods used in our juvenile clam 2012-2013 studies. Size-fractionated Chl *a* (< 5 µm fraction) was also determined by running seawater samples through a 5 µm Nitex screen inserted into a 50 ml plastic syringe.

Characterization of phytoplankton functional taxonomic groups at the two sites was conducted from the analysis of photopigments by high-performance liquid chromatography (HPLC) coupled with in-line photodiode array spectrophotometry (Paerl et al 2003). Pigments were extracted in 100% acetone at 20°C, filtered on a 0.45 µm filter, and injected into an HPLC system equipped with a series of C18 reverse-phase columns. Pigments were detected by absorbance in the range of 380-700 nm and identified by comparing retention times and peak areas with pigment standards. All HPLC analyses were conducted in Hans Paerl's analytical laboratory at the Institute of Marine Sciences, University of North Carolina Chapel Hill. The relative contribution of phytoplankton classes to the phytoplankton community was calculated using CHEMTAX software (Mackey *et al* 1996). This employs factor analysis and a steepest descent algorithm to optimize the contribution of phytoplankton groups to Chl *a* and other measured photopigment concentrations, based on initial estimates of accessory pigment:Chl *a* ratios for each class. No microscopically-determined phytoplankton taxonomy data were available at these two sites in 2014, but had been conducted at these two sites in 2012 and 2013. Therefore initial pigment: Chl *a* values for CHEMTAX analysis (Appendix I) were based on those determined during our 2013 phytoplankton study.

c) Clam processing/methods development

To determine the clams' overall condition (CI) at each sampling date, data were collected on all male clams sampled. This data included: clam shell width (SW or thickness), shell length (SL), measured with digital calipers, total body wet weight (WW) measured on a top-loading balance, and soft tissue dry weight (DW), measured with an analytical balance. The following formula was used to determine the clams' overall condition index (CI) as:

CI = [Total dry weight of soft tissues (DW)/(Total live body wet weight (WW) - shell DW)] x 100

Dry tissue weight was determined by oven-drying at 75°C to constant weight (drying time varied between ~3 and 6 days depending on clam size class). It is important to note that clam pallial fluids of live clams were drained onto a paper towel prior to tissue dissection and drying for determination of the CI.

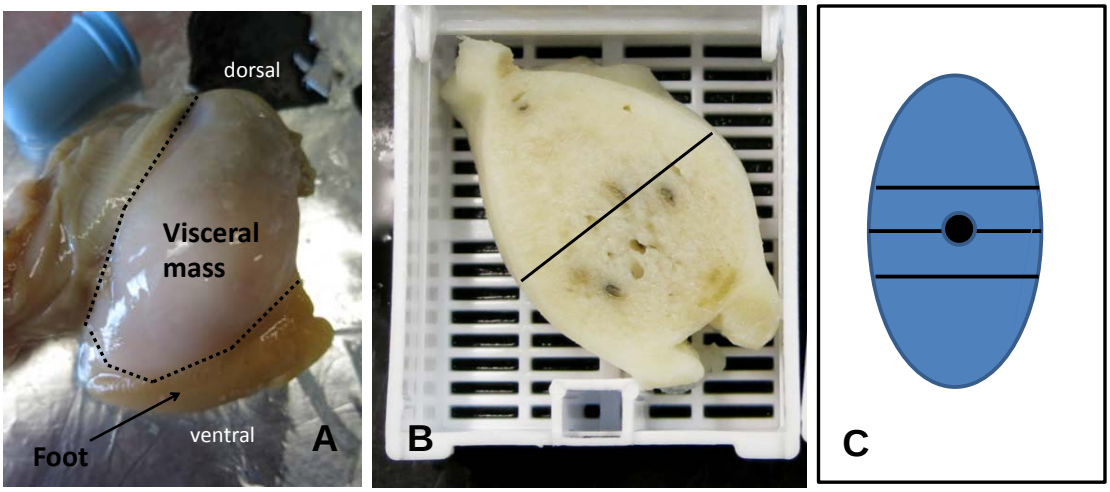
The visceral mass wet weight, VWW (following excising of the foot, gills, palps) (Fig. 2) was determined for all females, and the visceral mass index, VMI = VWW/SL^3, calculated as a measure of reproductive condition. Determination of this index was not originally proposed but was a metric newly developed as part of this study starting on June 30 sampling. It provides an additional real-time measure of reproductive status, without relying only on the more labor-intensive histological analysis.

The gender of each clam was confirmed at the time of dissection by microscopic analysis at 10x magnification, i.e., examination for the presence of eggs or sperm, and motility of sperm was also assessed at 40x magnification, following addition of a drop of 0.2 µm-filtered seawater. Viscera (foot removed) of female specimens were fixed in Davidson's fixative to process samples for histology and determine a histological reproductive index. Stereological analysis of visceral sections required preliminary methods development by M. Bricelj and Emily McGurk at the Haskin Shellfish Research Laboratory (HSRL). Histological tissue slides were analyzed under the microscope by the stereological point-counting method, with a Weibel point-counting reticule (n = 42 points) superimposed, along the mid-section of each individual (Fig. 2) using the impact point method (modified from Lowe and Moore, 1985). Two parameters were measured: a) % impact on

gonadal follicles vs other tissue (connective, muscular or digestive), and b) % impact on oocytes relative to other tissue including empty gonadal follicle space. The latter metric was used as a measure of spawning activity, in that it would indicate a lower number of oocytes per follicle following a spawning episode.

Only female clams were used for calculation of histologically determined metrics (% germinal tissue and % oocytes) as we could not assume a priori that reproductive conditioning and spawning of clams did not differ between sexes. This was tested by comparing the VMI of male an female clams in cherrystones starting on July 28 and through the end of the study, at the two sites.

Figure 2. A. Dissected visceral mass of a *Mercenaria mercenaria* female specimen showing (dashed line) the removal of the foot prior to determination of the visceral wet tissue weight (VVW), used in plots shown in Fig. 4 below. Other tissues attached to the visceral mass (gills, palps, etc were also excised. **B:** transverse ventral cross section of the visceral mass of a 5 mm-thick tissue section (ripe specimen); the line indicates the direction of microscope observations using the Weibel reticule point-counting method, also shown in schematic **C**.



Processing of clams for histological analysis was modified somewhat from that used by Newell et al. (2009) after the first few sampling dates, as we found that large chowders in very ripe reproductive condition did not fix adequately with the fixation times (48 hrs) and tissue section thickness (1 cm) recommended. Therefore some of these larger specimens were lost for histological analysis from initial sampling dates and required modification of protocols. We extended the fixation time (up to 10-14 days in Davidson fixative for larger clams) and used ~0.5 cm-thick transverse tissue sections rather than 1 cm sections as previously recommended.

During clam sampling we also recorded the number of boxes (dead clams with both empty valves attached) as well as single valves recovered, and measured all dead planted clams to provide an estimate of size-specific mortality at each sampling date. Percent mortality at each sampling date and cumulative mortalities at the end of the fall sampling (October 20) were calculated using only color-coded dead and live clams recovered over the study period.

d) Statistical Analysis

The effects of clam size class and location on measures of reproductive condition were compared using two-way ANOVA. Since significant differences were found between the two sites, changes in reproductive metrics (VMI, % oocyte and % germinal tissue) over time were compared within each site and size class using ANOVA followed by a posteriori multiple comparisons (Tukey's tests). These analyses served to identify periods of gonadal reconditioning and spawning. Differences in the VMI between males and female (data were obtained for both sexes for cherrystone clams only) were compared using three-way ANOVAs with sex, location and clam size as variables followed by Tukey's pairwise comparisons. Using Statistix 10.0 software all analyses were conducted following arcsine transformation to normalize ratio and percentage data. Correlation coefficients were calculated to determine the association (linear fit) between the VMI and histological parameters, and between % oocyte and % germinal tissue.

IV. Results

The field plots and clam deployment were successfully completed in mid-April 2014 (Fig. 3). The timing of clam deployment was designed to ensure that clams were exposed to local environmental conditions at the two study sites in Barnegat Bay before they underwent gametogenesis/gonadal development. This development typically occurs in May in mid-Atlantic estuaries, Long Island, NY south shore bays and Raritan Bay, NJ (Newell et al. 2009).We were able to obtain the full range of clam sizes needed via harvesting by a commercial clammer. The conductivity probe was deployed on May 30, whereas temperature probes, which were already available from our 2012/2013 project, were deployed at the time of clam planting in mid-April.

An unexpected difficulty encountered during early stages of this project involved access of the dock at the IBSP marina for water sampling, as was conducted in 2012/2013 (access was barred in 2014 due to unsafe conditions of the dock since Superstorm Sandy and until the dock is repaired). We therefore used the ReClam The Bay boat to access clam plots at IBSP and conduct water sampling from the boat in order to complete sampling at both sites in one day. Access to clean the conductivity probe on a regular basis required wading from the shore rather than access from the dock. Additionally, plots at Sedge Island were found to exhibit considerable fouling by the macroalga ("sourweed", presumably *Desmarestia viridis*) and other attached seaweeds. Fouling was visually estimated at ~30% cover on May 30 by snorkeling, which required removal/control of seaweed fouling from the screens at this time. This alga declined during subsequent June sampling as it typically proliferates during early spring.

Figure 3. Establishment of field plots at the IBSP study site, including deployment of adult clams and covering of plots with screens to minimize predation on April 17 (water temperature = 11.4^{o}C).

Clam (Fig. 4) and water sampling were initiated on June 16 at the time of expected peak reproductive development, and conducted every two weeks through September 22, and 28 days thereafter on October 20.

Figure 4. Raking of clams at the time of sampling at the Sedge Island study site.

a) Water column physical parameters

Summer mean daily temperatures were generally lower at Sedge from mid-April to early September , i.e., on average 3.4ºC below those determined at IBSP (Fig. 5), as was observed in 2012 and 2013 during our study of juvenile clam growth (Bricelj et al. unpublished). Although both sites had a similar water depth, the Sedge Island site was characterized by much more pronounced, short-term temperature fluctuations than IBSP (as reflected in 2 hr-means; maximum temperature differential = 11.7ºC, Fig. 6). This marked temperature differential, however, was no longer apparent in late summer-fall (early August through mid-October).

Figure 5. Temperatures (daily means) obtained at the two study sites with HOBO® probes programmed to record every 15 min from mid-April to mid-October 2014.

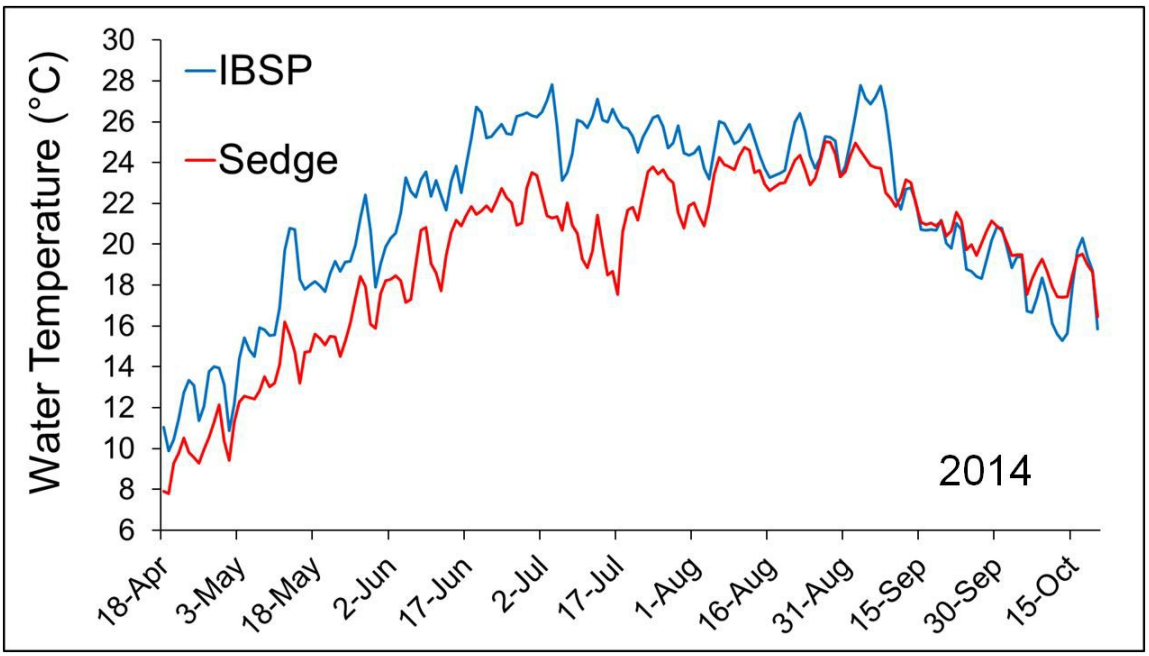
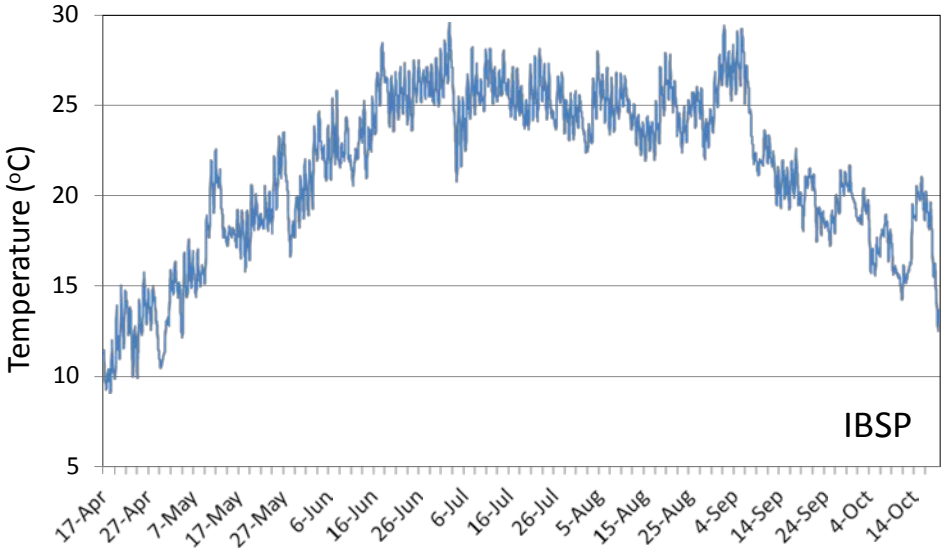
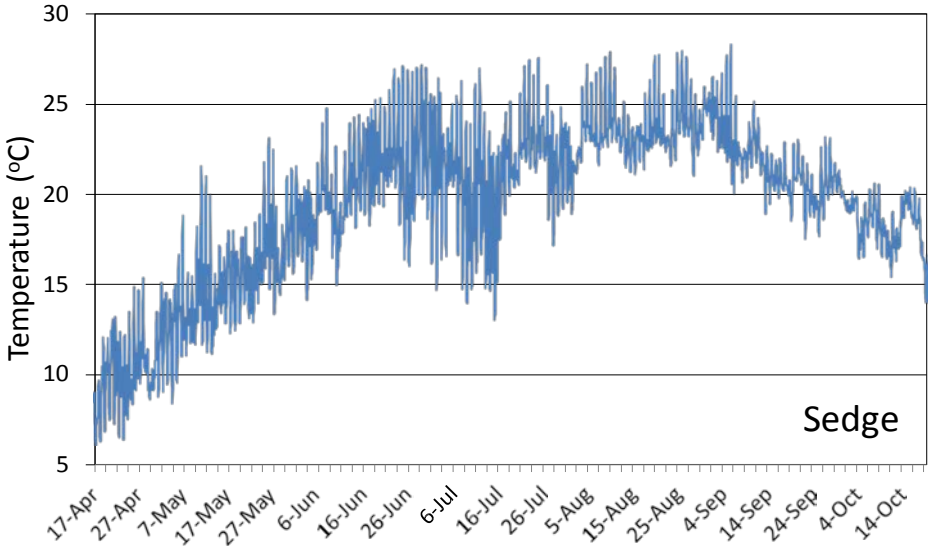


Figure 6. Water temperature showing fluctuations (2 hr-means) determined from records obtained every 15 min, at the two study sites mid-April to mid-October 2014.



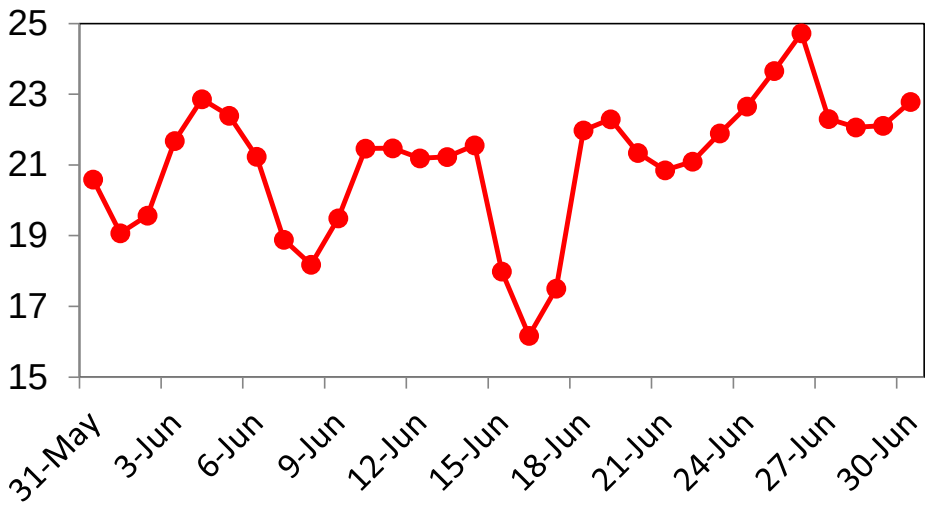


Summer salinities were consistent with patterns observed in the two previous years and generally higher at Sedge Island than at IBSP. After more than a week of deployment, the continuous HOBO® conductivity probe proved unreliable due to high sensitivity to fouling. We were only able to recover reliable daily salinities (calibrated against discrete salinities determined with a refractometer) for the month of June (Fig. 7), and do not recommend use of this probe where fouling may exist. Data reported in Figure 7 show that mean daily salinities showed pronounced short-term variation at this site ranging from ~16 to 25 psu, thus approaching the minimum salinity tolerance level of about 15 psu for *M. mercenaria* (reviewed by Bricelj et al 2012).

Table 1. Salinities (mean and range) measured at the two study sites in 2014, compared to results obtained in 2012 and 2013.

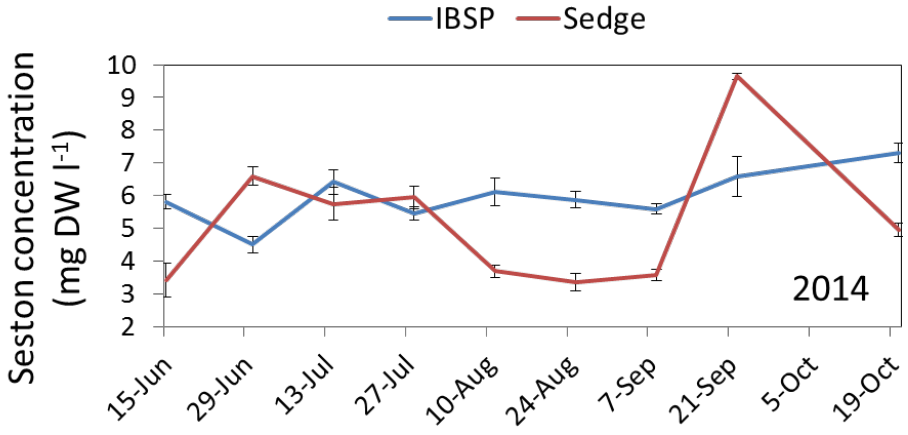
		2012	2013	2014
Sedge	Mean	**30.9**	**30.4**	**31.8**
	Range	28-33	26.5-33	28-35
IBSP	Mean	**22.4**	**22.8**	**23.7**
	Range	19-26	16-27	16-30

Figure 7. Daily salinity means during June 2014 at IBSP (continuous readings with HOBO® conductivity probe) (see text)



b) Seston characterization

Figure 8. Water column seston concentration, in mg dry weight (DW) l^{-1} (= total suspended solids, TSS) at the two study sites.



Total suspended solid (TSS) concentrations were relatively low at the two study sites, remaining below 10 mg l^{-1} throughout the study period, Concentrations of particulate organic matter (POM), a measure of the food supply, were generally higher at IBSP than at Sedge Island, and, as in the two previous years, IBSP was characterized by a high % of organic matter in seston, with a maximum of up to 69% (Fig. 9). Concentrations of particulate inorganic matter (PIM), a measure of suspended sediments, were generally low at the two study sites, attaining a maximum of ~8 mg l^{-1} at Sedge Island in late September (not shown). They thus remained well below levels (~ 40 mg l^{-1}) known to inhibit growth of juvenile clams (Bricelj and Malouf 1984).

Mean chlorophyll *a* concentrations throughout the study period were 2.5x higher at IBSP than at Sedge Island (IBSP: mean ± standard deviation, SD = 4.76 ± 1.76; Sedge island: 1.95 ± 0.62) (Fig. 10). These values were lower than those measured at these sites in 2013 and more comparable to those measured in 2012. The <5 µm size fraction contributed a greater percentage to the total phytoplankton biomass at IBSP (mean ± SD = 92.7% ± 13.4) than at Sedge Island (mean = 74.8% ± 21.0).

Figure 9. Concentration of particulate organic matter (POM, in mg l^{-1}) (upper graph) and percent of total seston comprised of POM at the two study sites (lower graph) at Sedge Island and IBSP.

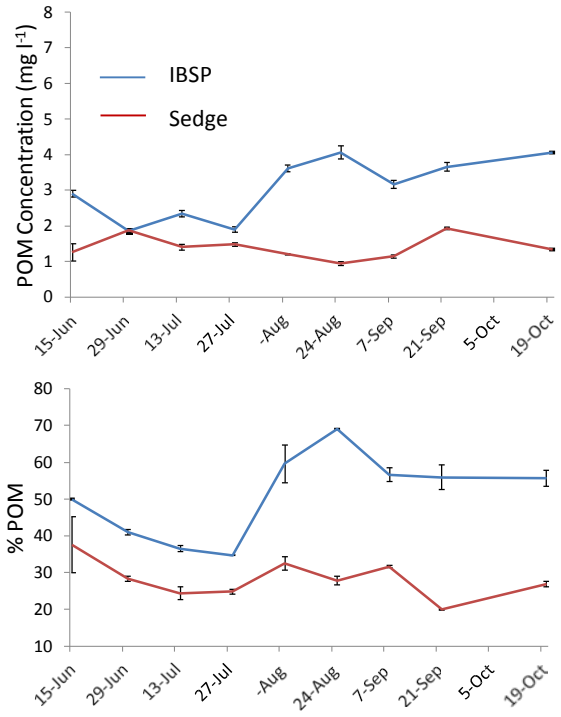
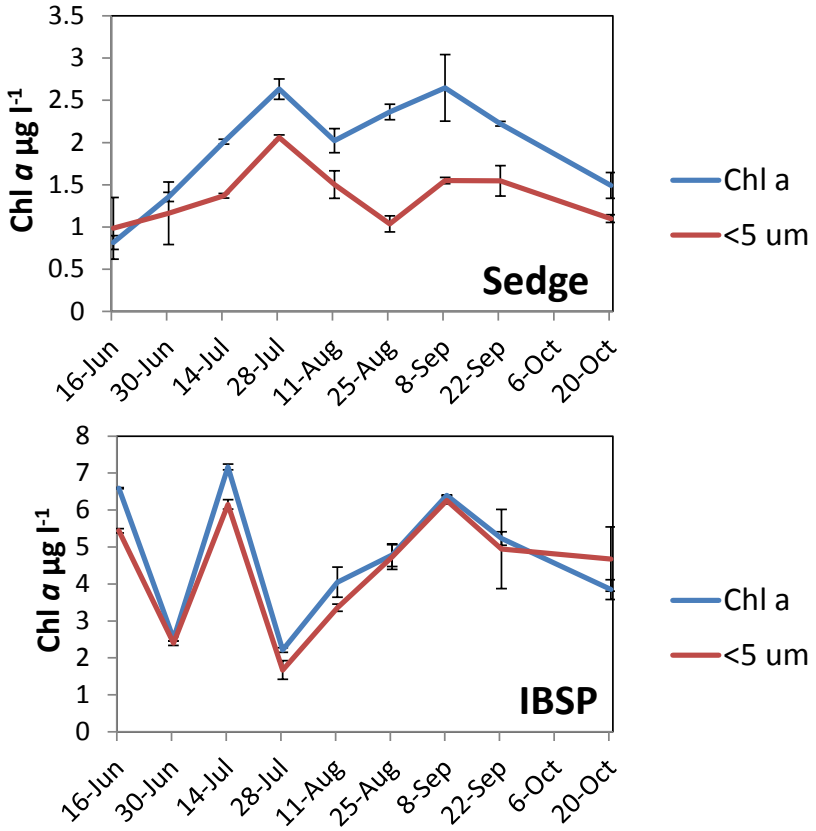


Figure 10. Total Chlorophyll *a* concentrations and the fraction <5 µm at the Sedge Island and IBSP study sites. Note the difference in scales of the Y axis.



As in the two previous study years (Bricelj et al. 2012 and 2013 unpublished) the phytoplankton assemblage was characterized by a very different composition at Sedge than at IBSP (Fig. 11). Multiple and more pronounced peaks in Chl *a* were observed at IBSP that were not evident at Sedge Island. Diatoms made a major contribution to total phytoplankton biomass at Sedge Island (maximum = 72.5%, mean = 47.1%) especially during late summer, early fall 2014, but attained much lower peak concentrations (1.61 µg l^{-1}) in 2014 than in the two previous years (maximum of ~ 7 µg l^{-1} in August 2012). At IBSP, diatoms made a maximum contribution of 57.6% to total Chl *a*, and averaged only 26.8% over the 2014 study period (Table 3),

In 2014 chlorophytes made an important contribution total Chl *a* at IBSP between early August and mid-October (up to 60.4%), while this taxonomic group made a much more limited contribution to total phytoplankton biomass (<10%) at Sedge Island (Fig. 12). Similarly cyanobacteria, also an algal functional group that provides a poor food source for hard clams, made a greater contribution to Chl *a* at IBSP (mean = 15.9%) than at Sedge Island (mean = 6.8%) (Table 3). Cyanobacteria, however, contributed a greater % of total Chl *a* at IBSP in 2013 (mean = 28.3%) than in 2014. In contrast, at IBSP chlorophytes contributed a greater fraction of the total phytoplankton biomass in 2014 (averaging 29.0%) than they did in 2013 (mean = 17.9% over the same period). Cryptophytes made a mean contribution to total phytoplankton biomass at Sedge Island of 17.5%, maximum = 30.7%), and averaged 11.5% at IBSP in 2014 (Fig. 12). As in the two prior years, during the summer-early fall 2014, dinoflagellates made a relatively minor contribution

to total Chl *a* at both study sites, averaging 6.7% and 4.8% at Sedge and IBSP, respectively.

Bloom concentrations of 19'butanoyloxyfucoxanthin (19'but), previously used as an indicator of *Aureococcus anophagefferens* in US Atlantic estuaries (Trice et al 2004), were documented at Sedge Island in 2013. Our 2014 photopigment analysis, however, did not reveal any detectable concentrations of this diagnostic pigments, at either study site.

Figure 11. Contribution of phytoplankton classes to the total Chlorophyll *a* concentration at the two study sites in 2014, as estimated by CHEMTAX.

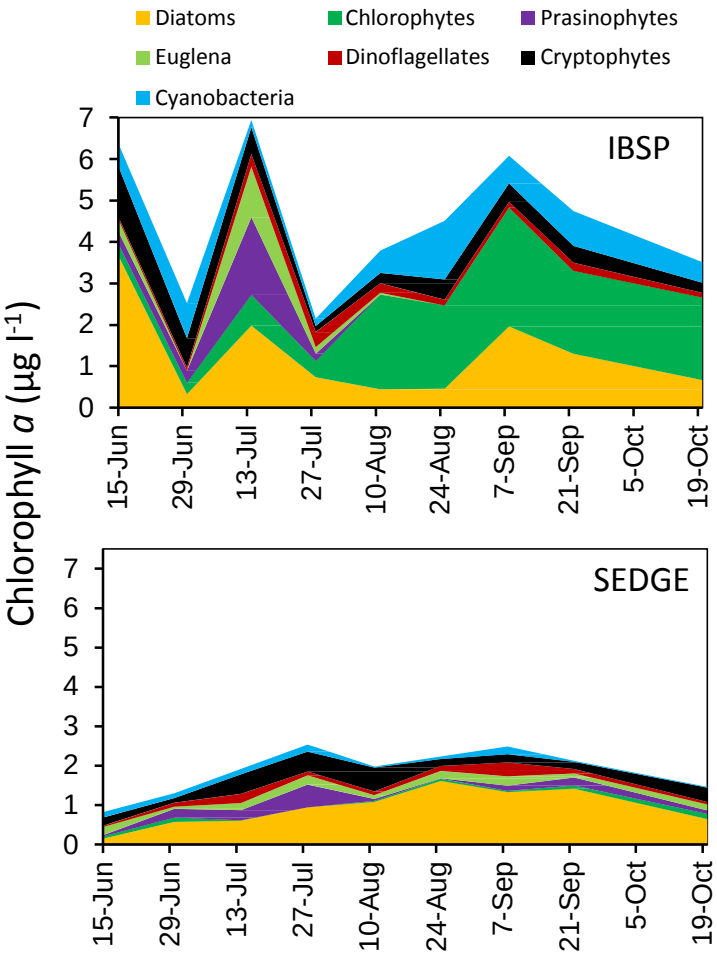
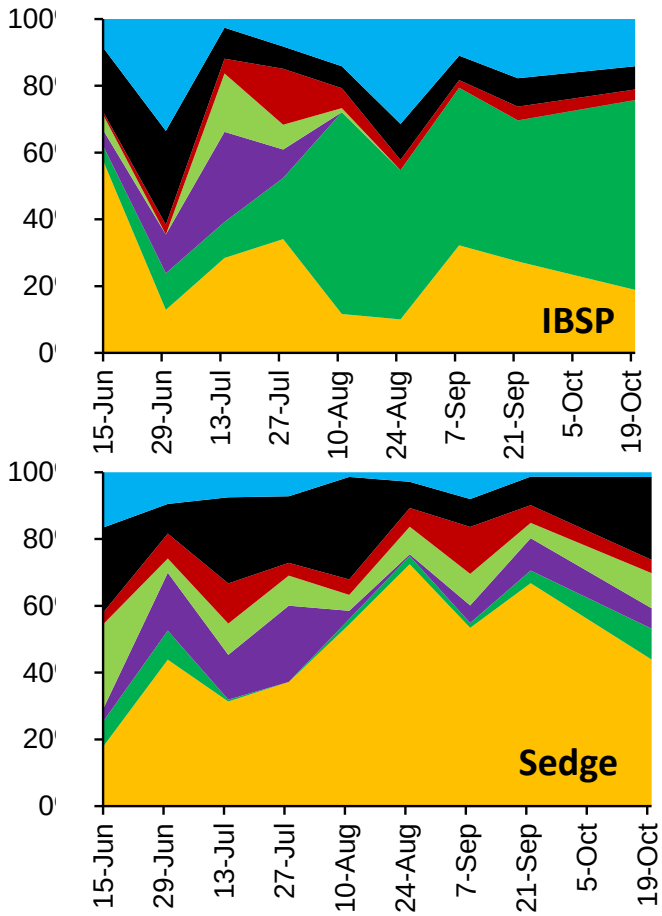


Figure 12. Percent contribution of phytoplankton classes to total Chlorophyll *a* at the two study sites in 2014, as estimated by CHEMTAX.

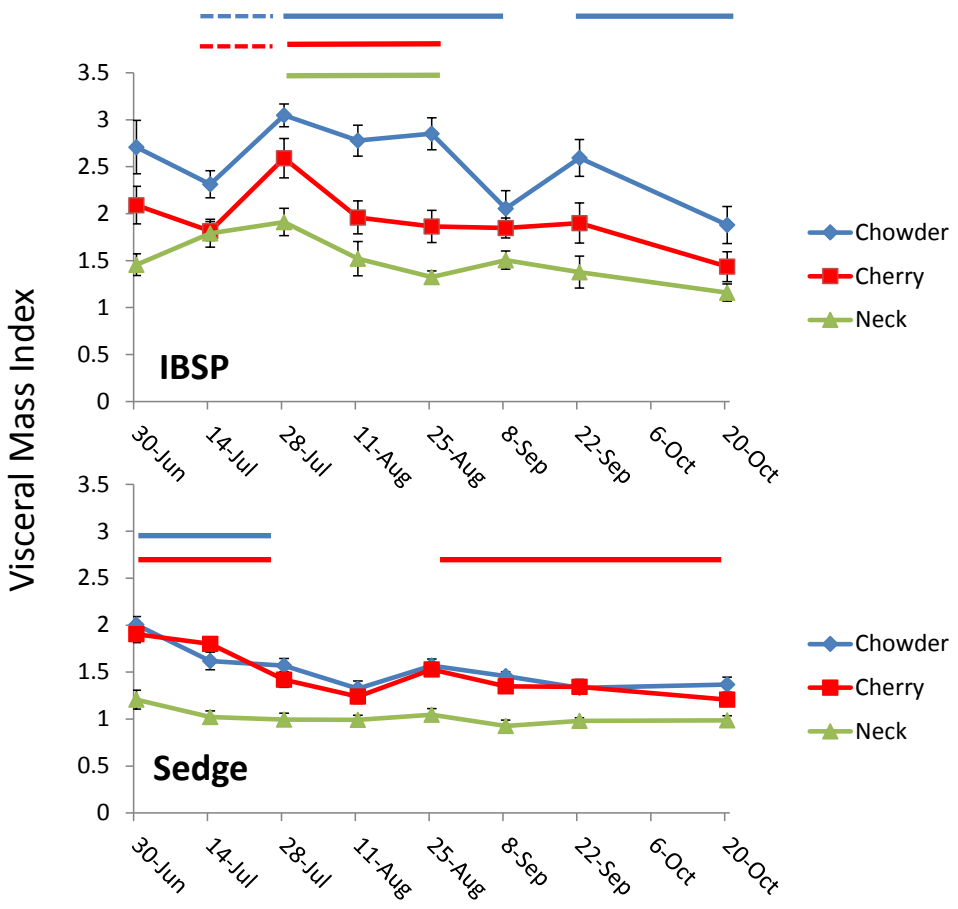


c) Clam reproductive condition

As indicated earlier, initial processing of clams from the June 16 sampling suggested the use of the visceral mass as a viable index of reproductive development, which has not been used in previous studies. A power equation of the data generated provided a good fit to the relationship between the visceral mass (-foot) and clam shell length (not shown), with an exponent (slope) approximating 3 as expected from an allometric relationship between a weight and length measure. This provided justification for using the visceral mass index, VMI, as $(VVW \times 10^5)/SL^3$, where VVW = visceral mass wet weight in g and SL in mm, to correct for differences in clam size.

Temporal patterns in the VMI at the two study sites are shown in Figure 13 and show marked differences in reproductive conditioning between the two study sites, as well as among clam size classes. Statistical analysis of VMI results between June 30 and October 20 indicated that the **allocation to reproduction was greater at IBSP than at Sedge Island** (as illustrated by the VMI in Fig. 13). This was especially apparent for chowder size clams. There was a significant effect of site ($p<0.01$) and of clam size class ($p<0.001$) on the visceral mass index, but no significant size x site interaction (two-way ANOVA). This was found despite the fact that the IBSP site was characterized by lower salinities approaching the clams' lower limit of tolerance, and by lower food quality, reflected in lower summer growth rates and condition of juvenile clams in 2012-2013. Additionally, reconditioning of larger clams (chowders and cherrystones) was observed at IBSP (July 14 to 18) but not at Sedge Island (Fig. 13).

Figure 13. Temporal pattern in the visceral mass index (VMI mean ± standard error, SE) of female hard clams by size class (littlenecks, cherrystones and chowders) collected at the two study sites (n = 7 to 14 chowders, 7 to 15 cherrystone clams, and 3 to 13 littlenecks per site/sampling date). The index is calculate as (VWW x 10^5)/SL^3] where VWW = visceral mass wet weight, and SL = shell length. Horizontal solid lines above each plot indicate a period of significant reduction in the VMI indicative of spawning (or potentially gamete resorption during October), and dashed lines a period of significant increase in the VMI, indicative of reconditioning.



- **Littlenecks showed significantly lower allocation to gamete production than larger clams (cherrystones and chowders) at both sites** even when data were corrected for differences in size via calculation of the visceral mass index (Fig. 13).

During the present study, sexing of smaller clams (littlenecks) was generally reliable at IBSP, but not always possible at Sedge, due to either the more immature condition of small clams and/or reduced allocation to reproduction of smaller clams at this site. A reduction in the VMI during the summer is indicative of spawning, although the reduction observed in October may be attributable to secondary spawning and/or gamete resorption. Stereological analysis of % germinal or gonadal cover and % oocyte cover from histological slides provided complementary information helpful in

interpreting results.

A comparison of the VMI between males and females at the two sites (Fig. 14) demonstrated that we cannot assume that both sexes show comparable reproductive patterns or allocation to reproduction. Indeed, the VMI of female cherrystone clams was significantly greater than that of male cherrystones at IBSP (ANOVA and a posteriori multiple comparisons, $p < 0.05$). In contrast, no statistical difference was detected between the VMI of both sexes at Sedge, the site where reproduction allocation was relatively low.

Figure 14. Comparison of the visceral mass index (VMI) between male (M) and female (F) cherrystone *M. mercenaria* at the two study sites. Letters indicate results of a two-way ANOVA followed by a posteriori Tukey's comparisons ($p < 0.05$): different letters indicate significant differences in the VMI.

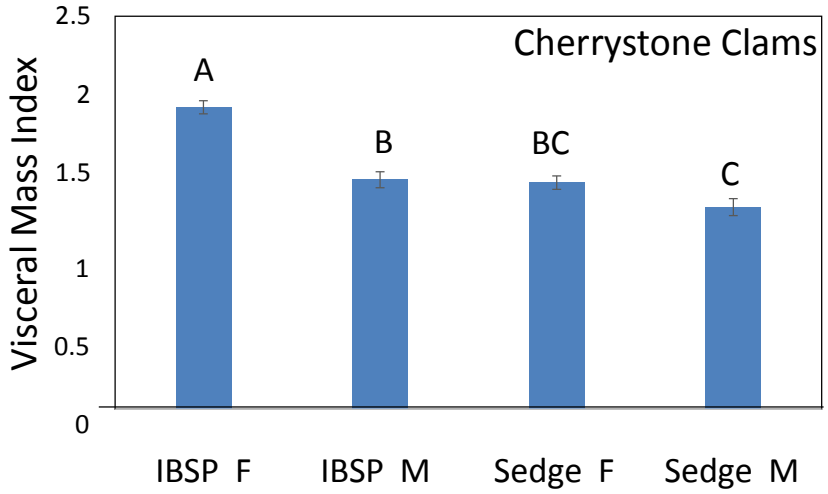


Figure 15. Temporal pattern in the % germinal or gonadal cover (mean ± standard error, SE), as determined by the grid point-counting method, of female hard clams by size class (necks, cherrystones and chowders) collected at the two study sites (n = 7 to 14 chowders, 7 to 15 cherries, and 3 to 13 necks per site/sampling date). As in Figure 13, a significant reduction in this metric is indicative of spawning, the period marked by horizontal lines. Note that histological metrics were determined from the first sampling date on June 16, whereas the VMI, a new metric developed during this study, was first determined on June 30. The interrupted, dashed horizontal line for chowders indicates that the % germinal cover differed significantly between end member dates, June 16 and July 14, but not between consecutive dates, as determined by Tukey's a posteriori multiple comparisons. There were no statistically significant temporal patterns at IBSP.

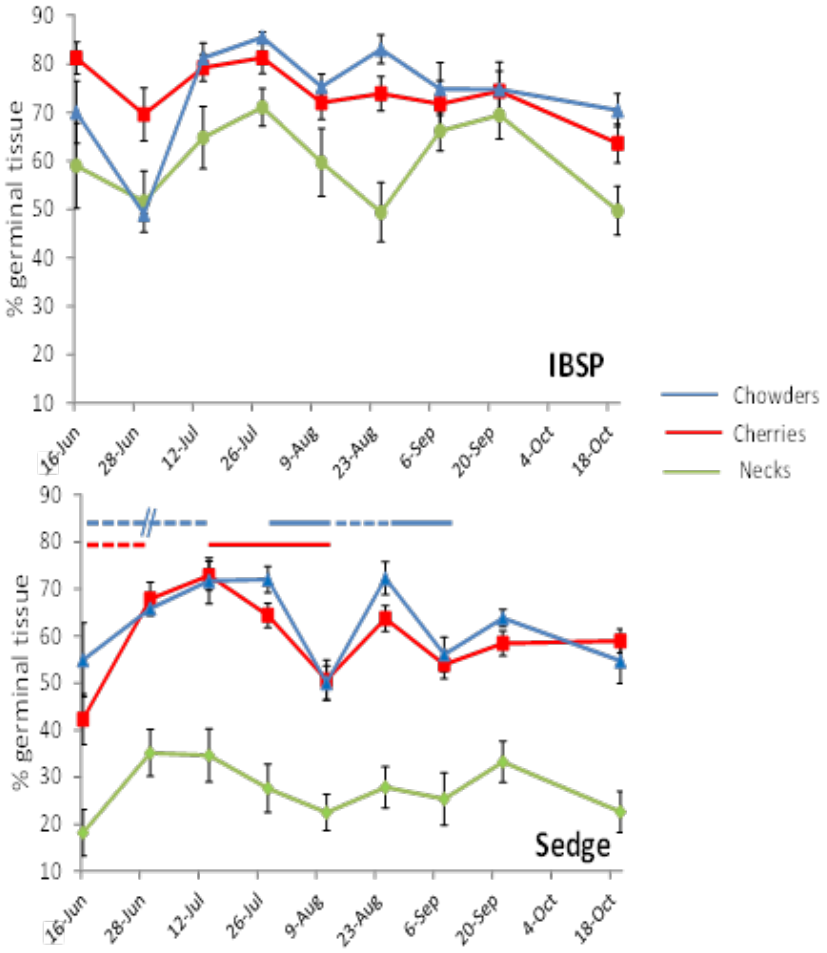
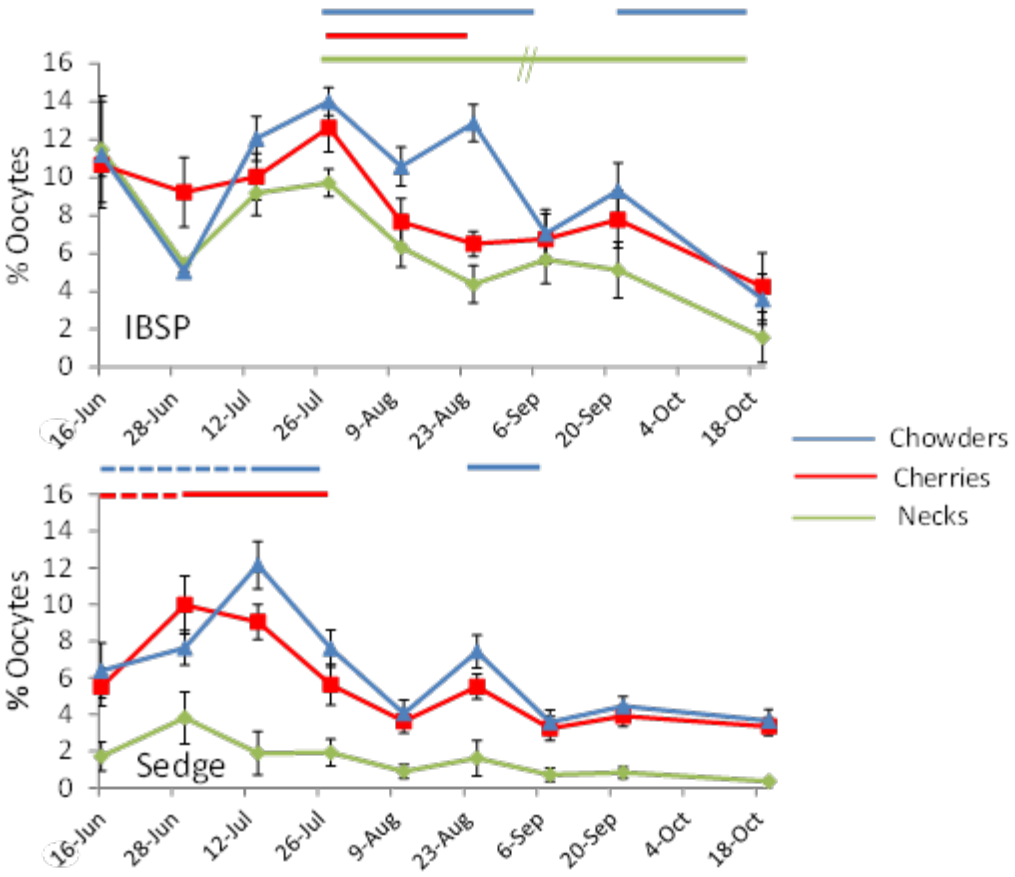

% germinal tissue
90
80
70
60
50
40
30
20
10
IBSP
16-Jun
28-Jun
12-Jul
26-Jul
9-Aug
23-Aug
6-Sep
20-Sep
4-Oct
18-Oct
Chowders
Cherries
Necks
% germinal tissue
90
80
70
60
50
40
30
20
10
Sedge
16-Jun
28-Jun
12-Jul
26-Jul
9-Aug
23-Aug
6-Sep
20-Sep
4-Oct
18-Oct

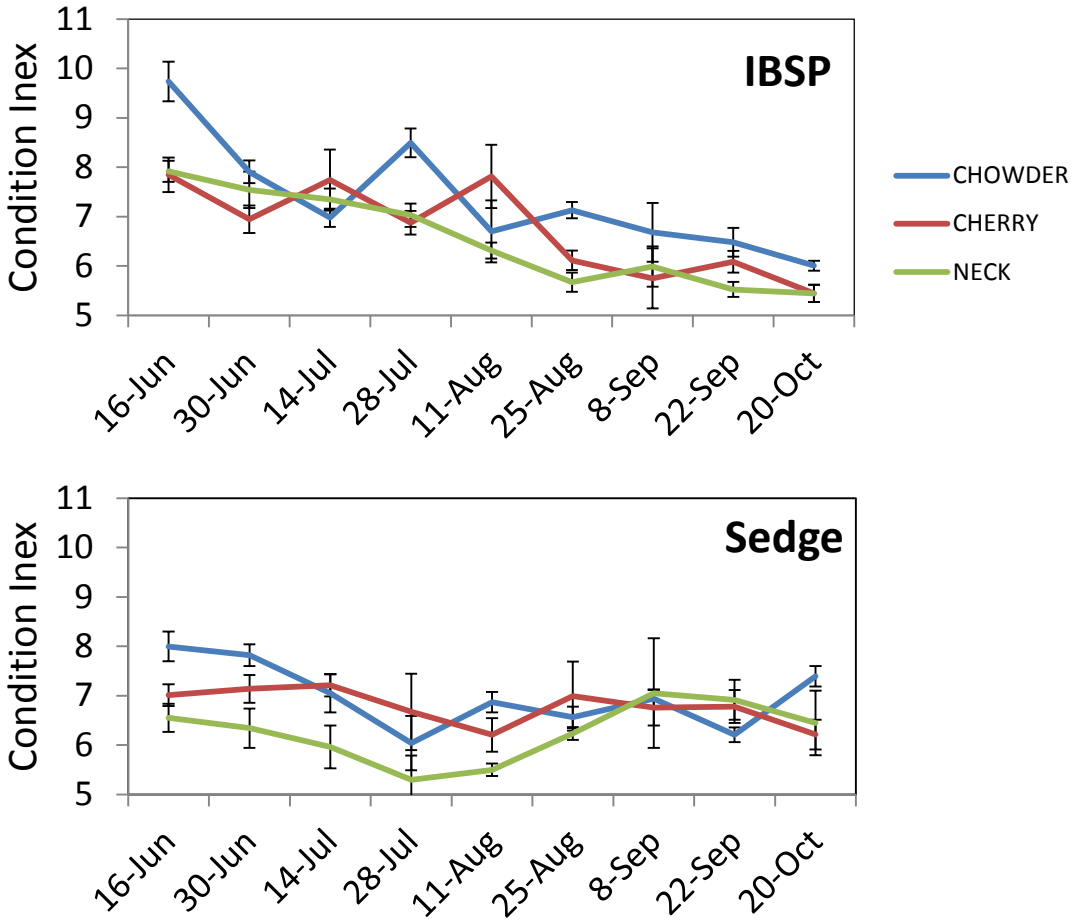
Figure 16. Temporal pattern in the % oocyte cover (mean ± standard error, SE), as determined by the grid point-counting method, of female hard clams by size class (littlenecks, cherrystones and chowders) collected at the two study sites, IBSP and Sedge Island (n = 7 to 14 chowders, except for June 28 at IBSP where only one individual was available, 7 to 15 cherries, and 3 to 13 necks per site/sampling date). Solid and dashed horizontal lines as in Figures 13 and 15. The interrupted horizontal line for littlenecks indicates that the % oocyte cover differed significantly between July 28 and October 20, but not between consecutive dates, as determined by Tukey's a posteriori multiple comparisons.



The data generated often revealed agreement in the seasonal patterns of the VMI and % oocytes metrics. For example, chowders and cherrystone clams showed evidence of spawning, marked by a significant reduction in these two parameters between July 28 and later August or Sept. 8 (Figs 13 and 16). Reconditioning of chowders between June 16 and July 12 was detected at Sedge Island using both % oocyte and % germinal tissue histological indices (note that the VMI was not measured until June 30). The data for littleecks showed no significant reduction in the three reproductive indices at Sedge Island, whereas they showed evidence of spawning at IBSP. A larger sample size is recommended in future studies to provide improved detection of temporal changes in reproductive condition.

The overall temporal pattern in the condition index (CI) of clams of the three size classes used at the two study sites is shown in Figure 17 to allow comparison with previous studies. We find that this index, commonly used in prior studies of hard clam reproduction in mid-Atlantic estuaries (e.g. LoBue 2010) does not provide a sensitive indicator of reproductive condition and we have therefore focused our analysis on the new metrics developed during the course of this study. The CI at IBSP was generally greater than that at Sedge Island, and this difference was most pronounced for necks. Additionally, the CI declined monotonically over the whole study period at IBSP, while at Sedge Island it declined through late July, and remained relatively constant during September and October. Therefore, there was no clear pattern at either study site of an increase in the clams' overall CI in the fall.

Figure 17. Condition index (CI) of male hard clams (mean ± SE) by size class throughout the 2014 study period at the two study sites, $\mathrm{CI} = \frac{\text{Tissue Dry Weight(g)} * 100}{\text{Total Body Wet Weight (g)} - \text{Shell Dry Weight(g)}}$ (n = 8 to 14 chowders, 11 to 12 cherries, and 11 to 16 littlenecks).



Observation of 2-3 representative clam histological sections within each size class on June 14 (a date for which no VMI values were available), and August 25 are shown in Figures 18 and 19, respectively. These clams were selected among those that approached the mean reproductive condition for that date and site. These observations reveal that ripe oocytes were present in larger clams (both cherrystones and chowders shown in Fig. 11) on these two dates. In contrast, littleneck clams at IBSP and especially at Sedge Island showed ripe oocytes on June 16, our first sampling date (Fig. 11), but were rare (at IBSP) or absent in gonadal follicles at Sedge Island. **These observations suggest that littleneck clams spawned earlier and over a more limited period than larger clams**. This is supported by the fact that the visceral mass index remained relatively constant throughout the June 30-Oct. 20 study period. Ripe oocytes were still present, although in variable numbers per follicle depending on the individual as well as the size class, in gonadal follicles of clams of all three size classes sampled at IBSP on September 22 (Fig. 20), indicating that they were still spawning at this time. By the end of the study period (October 20), however, gonads of clams of all size classes at this site appeared in spent condition, with empty follicles or only very few (typically 1-2) ripe oocytes per follicle, and little evidence of atresic, resorbed oocytes (Fig. 21). Thus clams completed spawning sometime between late September and mid-October. The same pattern was observed at Sedge Island (ripe eggs in follicles on Sept. 22 and spent gonadal condition by Oct. 20) except that fewer oocytes were observed per follicle (not shown), as expected

given the lower allocation to reproduction at this site.

Figure 18. Histological sections of the visceral mass of a cherrystone and littleneck hard clam on June 16, 2014 at the two study sites (Fig. 5). All photos taken at the same magnification (60x).

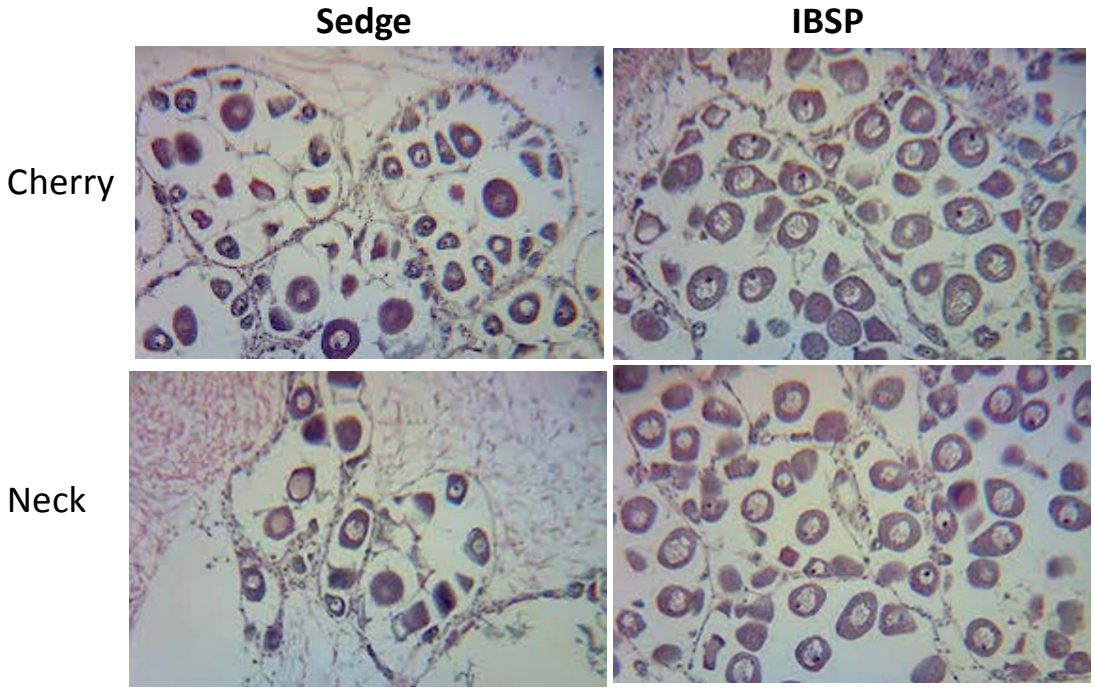
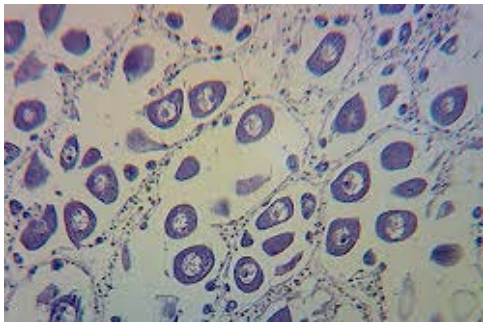
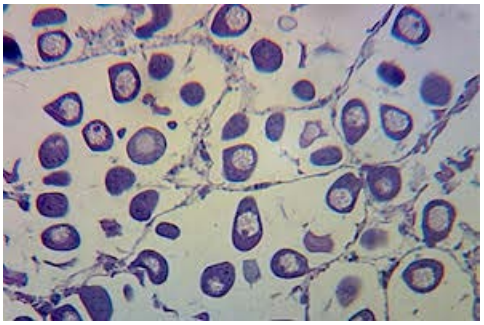


Figure 19. Histological sections of the visceral mass of a representative chowder, cherrystone and littleneck hard clam on August 25, 2014 at the two study sites. All photos taken at the same magnification (60x).

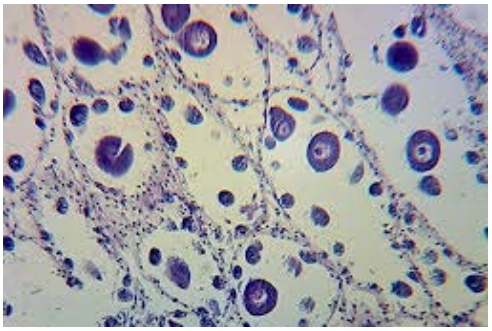
Sedge Aug. 25, 2014 IBSP

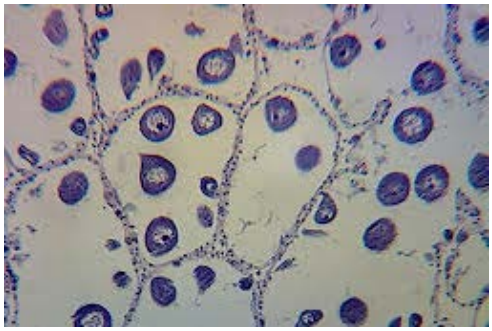
Chowders



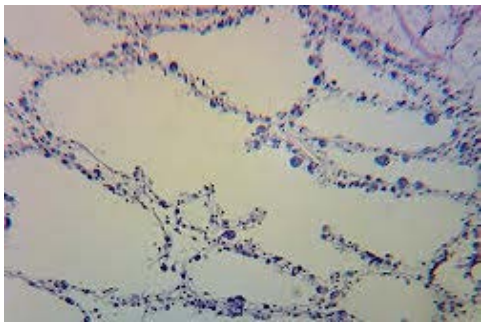


Cherries





Littlenecks



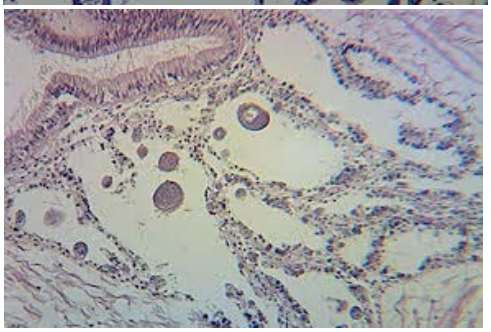
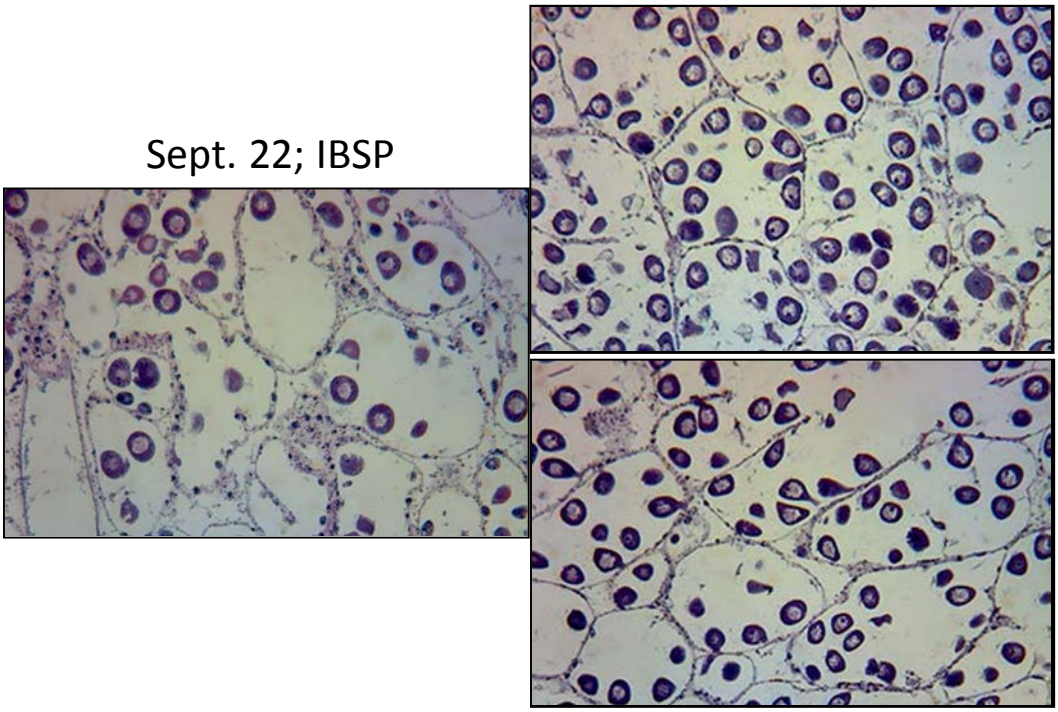


Figure 20. Micrographs showing gonadal follicles of a representative clam of each size class at IBSP, illustrating the presence of ripe eggs in the follicles on September 22. Left: neck; upper right: cherry; lower right: chowder. All photos taken at the same magnification (60x).



The observation of histological sections of clams sampled on October 20 indicated that clams of all three size classes were found in spent gonadal condition. This was characterized by empty follicles containing either no oocytes, or only very few ripe oocytes (Fig. 21, upper right). A closer examination of 4 specimens from each size class/site revealed the presence of resorbed oocytes exhibiting signs of atresia (Fig. 22).

Figure 21. Gonadal follicles of a representative clam of each size class at IBSP illustrating their spent condition (no or very few ripe oocytes in the follicle lumen). Left: neck; upper right: cherry; lower right: chowder. All photos taken at the same magnification 60x.

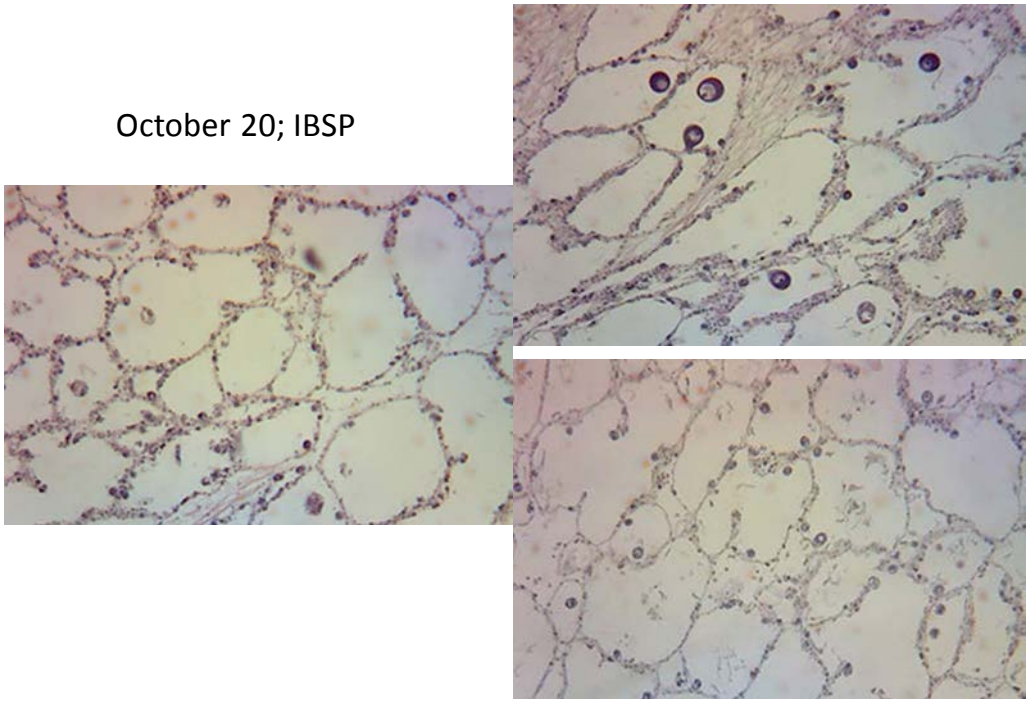
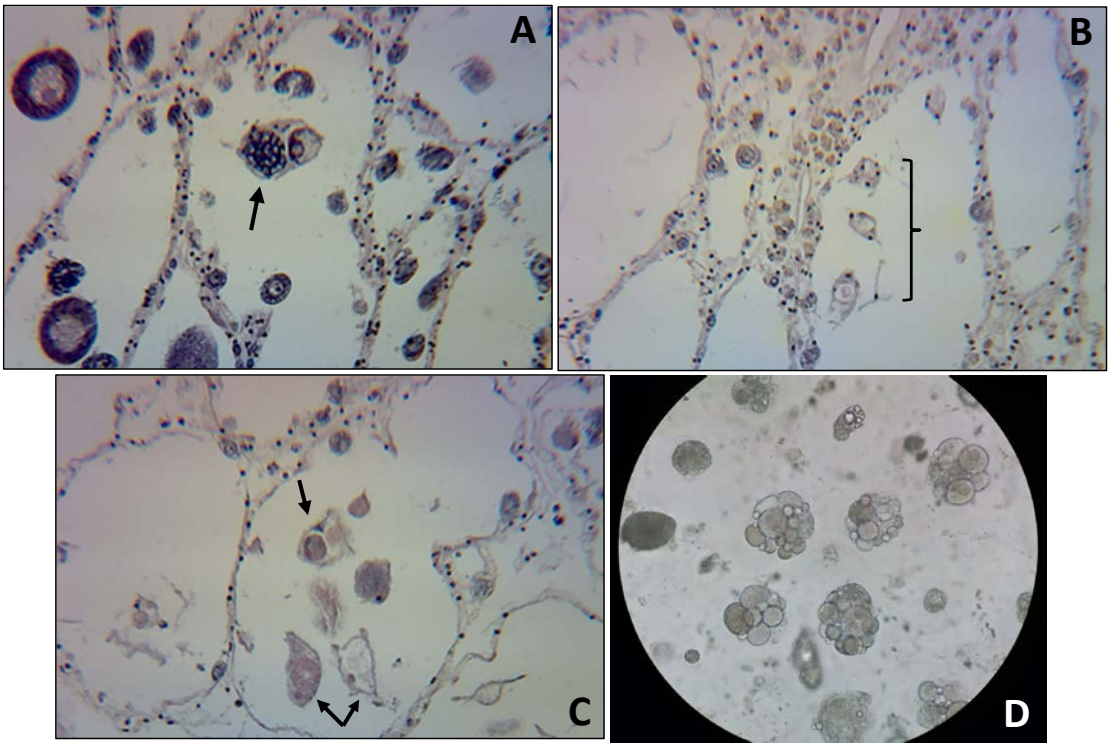


Figure 22. Gonadal follicles of representative clams collected on October 20 (last sampling date) showing signs of resorption and atresia of oocytes, marked by the black arrows. **A-C**. Micrographs of histological sections of clams sampled on October 20, 2014. **A.** Sedge Island chowder. **B & C.** IBSP chowders. All photos taken at the same magnification (100x). **D.** Anomalous oocytes sampled at the time of in vivo dissection of clams and withdrawal of gametes with a Pasteur pipette for microscopic sex determination.

October 20



Strong correlation, as measured by the correlation coefficient (R^2), was found between the % oocytes and % germinal tissue, the two histologically determined parameters, at both study sites, and for all three size classes, although the value was consistently lower at Sedge and for necks than larger clams (Table 2, boldfaced values). Therefore, these two parameters may provide redundant information. A higher correlation was found at both sites and for all three size classes between the VMI and the % oocytes, than between VMI and % germinal tissue.

Table 2. Pearson's **c**orrelation coefficient (R^2) determined from linear relationships between the three metrics of reproductive condition used in the present study, the Visceral Mass Index (VMI), % oocytes (O) and % germinal or gonadal tissue (G) (see Methods) at IBSP and Sedge Island, by clam size class. All individual clams of a given size class were used for analysis at each site (pooled for all sampling dates; untransformed data).

	Clam size class	IBSP	Sedge
a) **% O vs % G**	Chowders	**0.9461**	**0.8708**
b) % O vs VMI		0.4703	0.1622
c) % G vs VMI		0.322	0.1335
a) **% O vs % G**	Cherrystones	**0.9375**	**0.857**
b) % O vs VMI		0.452	0.3011
c) % G vs VMI		0.3878	0.1832

a) **% O vs % G**		**0.8845**	**0.7724**
b) % O vs VMI	Necks	0.5315	0.3865
c) % G vs VMI		0.392	0.1807

d) "Grey" clams

A number of larger clams (up to 9% of cherrystone and chowder clams at any sampling date), showed anomalous discoloration/grey color of the visceral mass (Fig. 23). This condition never appeared in littlenecks. Occasionally the discolored specimens ("grey" clams) also exhibited dark mantle tissue (Fig. 23A) and were evident from gross observation of tissues and/or following cutting of the transverse section used for histological processing (Fig. 24). This condition ("grey clams" was described for clams collected from Little Egg Harbor, NJ by Kraeuter et al. (1997). These authors assumed that it was caused by gut contents but clam depuration did not change the clams' discolored condition.

Proliferation of brown cells within the connective tissue of gonadal tissue was also observed in some clams (Fig. 25), but was not clearly correlated with the macroscopically determined "grey" appearance of the visceral mass.

Figure 23. Discoloration of the visceral mass in a *Mercenaria mercenaria* (so-called "grey" clams): **A.** Chowder clam collected at Sedge Island on July 14, 2014. Note associated dark mantle marked by the arrow; **B.** Cherrystone collected at Sedge Island September 8, 2014. **C.** Normal specimen collected at IBSP July 14.

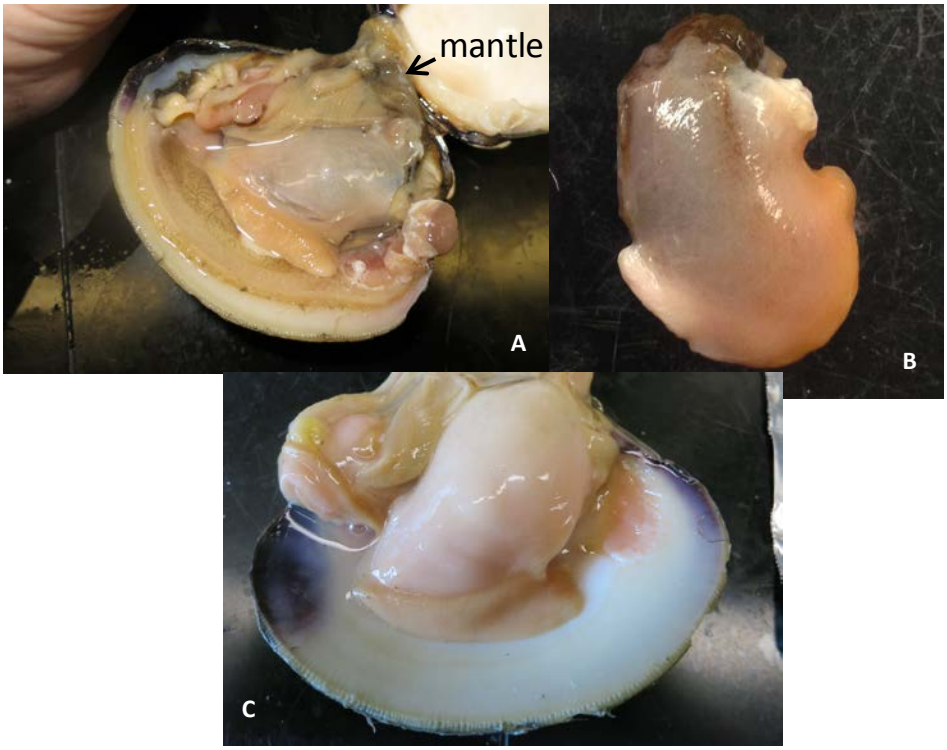


Figure 24. Visceral mass in ventral cross-section showing anomalous dark clam (left) and a normal specimen (right) collected at Sedge Island Sept. 8, 2014. Both are cherrystones.

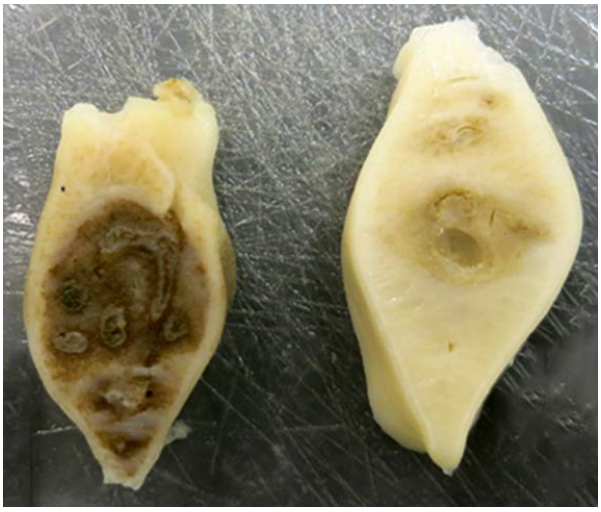
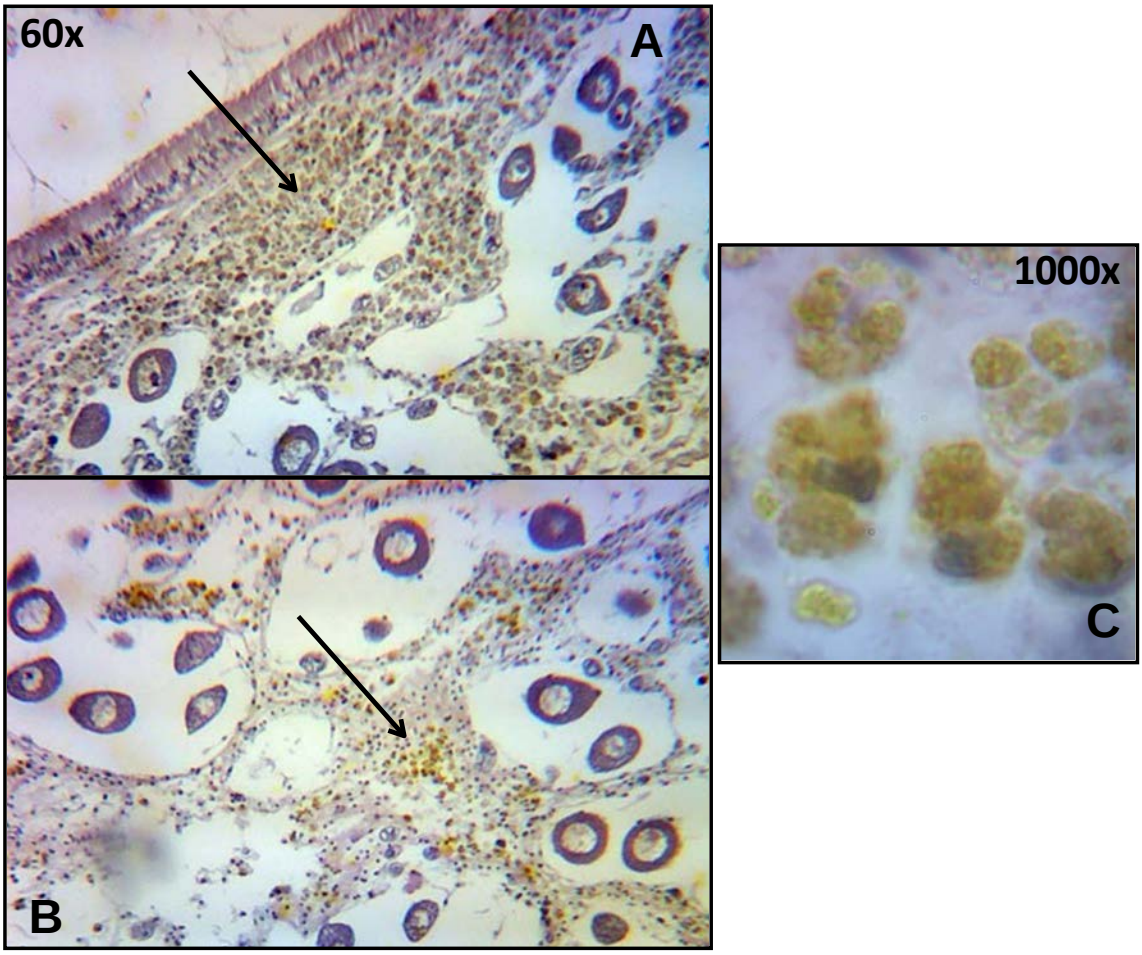


Figure 25. Histological sections showing varying degrees of the proliferation of brown cells in the connective tissue surrounding gonadal tissues (marked by the arrows in **A, B**) (see text). **C**. Closeup of brown cells at x1000 magnification.

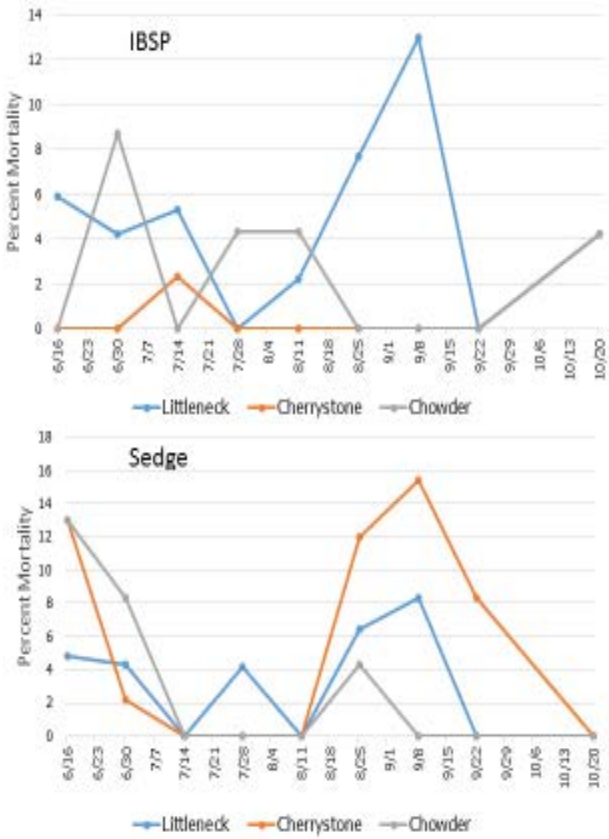


e) Clam mortalities

Maximum clam mortalities on any sampling date at IBSP were: 8.7% for chowders 4.2% for cherrystone, and 13% for littlenecks (Fig. 26). At Sedge Island maximum mortality was 13% for chowders, 15.4% for cherrystones, and 8.3% for littlenecks at Sedge Island. For this study the paired valves are assumed to last for the entire length of the study, and thus individual samples were considered to be the result of the cumulative mortality. These individual samples have been averaged to obtain a mortality estimate. The cumulative mortalities (%) are at IBSP chowder 3.1, cherrystone 7.1, littleneck 4.0, and at Sedge Island chowder 2.0, cherrystone 1.5, littleneck 6.3. These mortalities, while relatively low are similar to those found in Raritan Bay (Kraeuter et al. 2009), but that study utilized unprotected plots. If we assume that the size-specific mortality rates for IBSP and Sedge Island we observed are typical for the entire system and then use the numbers of clams in each size class from the latest surveys (littleneck, cherrystone and chowder clams), we can estimate the number of clams dying in each of these three size classes. Summing these data and dividing by the total clams in these size classes yields the number and average percent mortality of the harvestable population. These estimates are: Barnegat Bay 5,298,100 clams and average mortality of 3.9%, Little Egg Harbor Bay 2,932,349 and average mortality of 3.7%.

Some mortality observed in the current study may be partly due to initial handling stress, as a number of the clams provided by the commercial harvester were found cracked and removed prior to initial deployment. The estimated mortality rates should be interpreted with caution because of the above caveats, and the lack of year round data. Overall, although this study was not intended as a rigorous quantitative study of natural mortalities, there was no clear evidence of differential size-specific mortality or of greater mortalities of chowders associated with senescence.

Figure 26. Percent clam mortalities by size class at the IBSP (upper graph) and Sedge (lower graph) sampling sites (note the difference in scale of the Y axis).



Mortalities at the time of final plot retrieval in early June 2015 were very low or moderate at IBSP (0% for cherrystone and chowder clams and 13% for littlenecks), but much higher at Sedge for all 3 size classes (50% for cherrystones and chowders, and 81% for littlenecks.) Shells of dead clams were intact and did not show signs of predation (cracked or bored shells). The higher mortality at Sedge than at IBSP may indicate a winter mortality event (exposure at low tide) at Sedge Island that did not occur at IBSP.

.

V. Discussion and Conclusions

The Visceral Mass Index (VMI) provided a reliable, real-time measure of female reproductive condition that was more sensitive than the standard Condition Index (CI) used in prior published studies

of hard clam reproduction. Reproductive condition, as measured by the VMI and histological indices (% germinal tissue and % oocytes), was significantly greater at IBSP than Sedge Island, despite low salinities, and low juvenile growth rates documented at IBSP in 2012-2013. Lower salinities with episodic reductions to levels that approach the clams' tolerance were also documented at IBSP during the present study. In contrast, high salinities, with maxima of up to 34 psu and means of ~30 psu during the summer, were also typical of the Sedge Island site throughout our 2012-2014 studies. A summary of key environmental parameters during the three study years is provided in Table 3, and that of phytoplankton functional groups in Table 4.

Table 3. Summary table comparing key environmental water column parameters recorded during the three study years in summer. Only data from June-September sampling dates are included. Note that the sampling schedule left large gaps in weekly data for the 2012 and 2013 study years (July 6-23 and July 10-August 13 for 2012, respectively between juvenile clam trials), while sampling was conducted biweekly without interruption during the 2014 reproductive study.

	IBSP				Sedge Island		
Salinity	2012	2013	2014		2012	2013	2014
Mean	22.4	22.8	23.7		30.9	30.4	31.
STDEV	2.0	3.2	3.4		1.9	2.3	1.9
Range	19.0-26.0	16.0-27.0	16.0-30.0		28.0-33.0	26.5-34.0	29.0-34.0
n	13	11	7		13	11	6
Temp ºC							
Mean	25.64	24.66	25.00		23.94	21.56	22.10
STDEV	2.25	2.06	1.45		1.90	1.67	1.86
Range	20.00-29.02	21.05-29.74	21.68-27.82		19.50-26.77	17.57-24.42	17.17-25.01
n	82	64	100		82	64	100
PIM (mg/L)							
Mean	6.04	8.58	2.86		6.01	6.11	3.85
STDEV	2.69	3.05	0.70		2.90	1.72	1.89
Range	3.23-12.11	5.35-13.71	1.82-4.08		2.32-12.13	3.55-8.53	2.17-7.71
n	12	8	8		13	8	8
POM (mg/L)							
Mean	6.45	8.24	2.94		2.30	2.66	1.40
STDEV	2.98	2.45	0.84		1.12	0.94	0.35
Range	2.57-13.98	5.95-11.78	1.85-4.06		0.86-3.87	1.35-3.69	0.94-1.93
n	12	8	8		13	8	8
Chl a (ug/L)							
Mean	8.65	12.12	4.87		4.58	5.78	2.01
STDEV	5.10	2.943	1.851		3.401	4.033	0.634
Range	3.08-23.10	7.26-15.87	2.12-7.17		0.54-10.64	2.30-17.15	0.81-2.65
n	12	11	8		13	11	8

Table 4. Summary table comparing mean CHEMTAX estimates of (a) chlorophyll *a* concentration (μg l^{-1}) and (b) % chlorophyll *a* contributed by major phytoplankton classes during three consecutive study years. Only data from June-September sampling dates included in this comparison. Note that the sampling schedule left large gaps in weekly data for the 2012 and 2013 study years (July 6-23 and July 10-August 13 for 2012 and 2013, respectively), while sampling was routinely conducted biweekly during 2014.

	IBSP			Sedge Island		
	(a) Chlorophyll *a* concentration (µg l^{-1})					
Chlorophytes	2012	2013	2014	2012	2013	2014
Mean	2.03	2.26	1.36	0.28	0.61	0.05
STDEV	1.28	1.70	1.04	0.29	0.58	0.04
Range	0.05-3.67	0.00-5.55	0.28-2.87	0.00-1.03	0.00-1.54	0.00-0.11
N	12	11	8	13	11	8
Cyanobacteria	2012	2013	2014	2012	2013	2014
Mean	2.23	3.66	0.65	0.28	0.64	0.11
STDEV	1.05	2.48	0.40	0.30	0.51	0.07
Range	0.28-4.38	0.27-7.87	0.18-1.42	0.02-1.08	0.11-1.67	0.03-0.20
n	12	11	8	13	11	8
Diatoms	2012	2013	2014	2012	2013	2014
Mean	2.63	3.80	1.36	2.97	2.43	0.96
STDEV	3.04	1.36	1.15	2.92	1.31	0.50
Range	0.80-11.98	1.94-6.31	0.32-3.68	0.22-8.59	1.02-5.53	0.15-1.61
n	12	11	8	13	11	8

	(b) % Chlorophyll *a*					
Chlorophytes	2012	2013	2014	2012	2013	2014
Mean	23.66	17.92	29.88	6.33	2.661	3.25
STDEV	14.72	13.15	21.07	5.20	0.941	3.22
Range	1.68-46.02	0.00-39.86	4.46-60.43	0.00-20.22	1.347-3.687	0.00-8.70
n	12	11	8	13	11	8
Cyanobacteria	2012	2013	2014	2012	2013	2014
Mean	27.63	28.26	15.89	7.59	12.01	6.79
STDEV	13.09	16.10	11.16	6.05	6.49	5.07
Range	9.06-56.24	3.42-57.88	2.59-33.49	2.14-21.18	2.75-24.30	1.30-16.57
n	12	11	8	13	11	8
Diatoms	2012	2013	2014	2012	2013	2014
Mean	28.67	34.09	26.81	53.30	49.11	47.17
STDEV	16.80	19.52	15.76	18.78	12.22	18.28
Range	10.49-68.44	18.45-80.22	10.05-57.65	22.74-82.46	32.61-68.38	17.79-72.47
n	12	11	8	13	11	8

Our 2014 study confirmed results of studies conducted in 2012 and 2013 indicating that IBSP is characterized by a unique phytoplankton assemblage likely associated with lower salinities and

different nutrient loading and composition related to the influence of the Toms River plume. Additionally, mean total summer chlorophyll *a* concentrations were generally low at Sedge Island relative to other BB-LEH study sites in previous years (4 to 6 µg l^{-1} in 2012 and 2013, respectively), and particularly low during the 2014 reproductive study (2 µg l^{-1}, ~2.5x lower than at IBSP) (Table 3). Concentrations of the harmful alga *Aureococcus anophagefferens* (up to 440 cells µl^{-1}) were also documented at Sedge in June 2013 (R. Fantasia, V.M. Bricelj and L. Ren, in prep.), although historical surveys showed that brown tide occurrence was more prevalent in southern portions of the BB-LEH (Olsen and Mahoney 2001). No brown tide was detected in 2014, but the occurrence of brown tide at two out of four sites in the BB-LEH in 2013 indicate that monitoring of *Aureococcus anohagefferens* should be reinstated in this estuary.

- **Our characterization of summer physical parameters (temperature and salinity), and seston metrics, including phytoplankton biomass and composition, before and after Superstorm Sandy impacted the region in fall 2012, do not point to major changes that can be attributed to this event.**
- **All three reproductive metrics used (VMI, % oocytes and % germinal tissue confirmed that clam reproductive allocation was significantly lower at Sedge than at IBSP**.

Furthermore, gonadal reconditioning, as evidenced by an increase in the VMI occurred in the large size classes at IBSP in July (e.g. July 14 to 28, Fig. 13), but was not observed at Sedge Island, again indicating that conditions at IBSP were better suited for reproductive conditioning than at Sedge Island at least during our 2014 study year This may be attributable to the higher food availability at IBSP than at Sedge Island in 2014. Although IBSP was characterized by a higher % contribution of chlorophytes and cyanobacteria to total Chl *a* (microalgae known to provide a poor food source for hard clams), total food levels as measured by both POM and total Chl *a* were much higher at Sedge Island than at IBSP in 2014 (Figs. 9 and 11). In this context, the mean concentration of diatoms, generally considered a good food source for hard clams, at IBSP during the 2014 study period (= 1.281 µg Chl *a* l^{-1}) was comparable to the total Chl *a* concentration at Sedge Island (= 1.872 µg l^{-1}), including all microalgal taxa. Furthermore, bivalves, including hard clams, are able to selectively ingest and absorb algae of high nutritional quality out of a mixed phytoplankton assemblage, thus allowing enrichment of the available food supply (Bricelj et al. 1984; Ward and Shumway 2004). Mean Chl *a* and POM concentrations during the summer were higher at IBSP than at Sedge Island in all three consecutive study years (2012 to 2014) (Table 3). This suggests that despite the relatively high food quality at Sedge Island, as measured by the % of algal functional groups that support clam production, this site may offer food limited conditions.

The above finding, i.e, low clam reproductive performance at Sedge Island, is important given that this site lies in the Marine Conservation Zone (MCZ), and area that has experienced considerable state investment in hard clam stock enhancement, primarily through seeding (Bricelj et al. 2012). Two of the most common hard clam stock enhancement practices involve seeding and the establishment of spawner sanctuaries in protected areas closed to commercial fishing. In Great South Bay, NY, sustained planting of adults by the TNC was attributed as the cause of recruitment of a strong 2007 cohort (LoBue 2010). Our one-year study of clam reproduction is useful in assessing the suitability of the Sedge Island area as a spawner sanctuary site and in identifying the

environmental conditions suitable for reproduction of hard clams. More extensive studies, however, conducted over several years are required to determine whether this result can be generalized and identify the causes for this outcome. At the same time, it is necessary to establish if the high overwinter mortality at Sedge Island is a typical or rare event. The cumulative losses indicated by the summer/fall sampling are also still very high and need further investigation.

The MCZ could potentially provide a broodstock sanctuary area as part of a clam stock enhancement management strategy in this estuary, if local hard clams are shown to reproduce successfully under local environmental conditions. Future, multi-year studies are needed to determine whether the low reproductive performance of hard clams at the Sedge Island location was an annual occurrence or occurs consistently, and whether it is characteristic of the MCZ ecosystem as a whole.

The relatively low reproductive performance of clams documented at Sedge Island in 2014, may be caused by several factors acting singly or in concert that will require future investigation: a) low food supply at Sedge Island during the 2014 study period, b) the Sedge Island site was characterized by lower summer temperatures and during the present study the minimum temperature for spawning of *M. mercenaria* [~ 24°C (reviewed by Bricelj et al 2012)] was only attained by mid-August (Fig. 5), a month later than at IBSP, c) the Sedge Island site, based on three years of study, was also characterized by high daily temperature fluctuations that may result in dribble-spawning and disrupt the clams'reproductive cycle, given that temperature change is known to induce spawning of hard clams and other bivalves, d) clams for this study were transplanted in May 2014 from other locations in BB-LEH and may thus not have been adapted to local conditions at Sedge Island. The contributing role of substrate in explaining site differences documented in this study cannot be excluded as bottom plots established at Sedge Island were in finer-grained sediment than at IBSP, where they were characterized by coarse sand.

- **Size-specific effects were documented in the present study even when metrics used were corrected for size. Smaller clams (littleneck commercial size class) were characterized by a lower reproductive condition than larger clams at both sites.**

This supports Peterson's (1983) assertion that smaller/younger hard clams partition more energy to somatic rather than gonadal growth than larger ones, allowing smaller individuals to more rapidly achieve size refuge from predation. Throughout the present study, littlenecks showed on average a 31-33% lower VMI than cherrystone and chowder clams, respectively. This difference in reproductive allocation was even greater at IBSP, where the VMI of littlenecks was on average 40% lower than that of larger size classes. Newell et al (2009) found no significant relationship between the CI of hard clams and shell size over the range ~40 to 125 mm SL. Their study, however did not evaluate the effect of clam size on the % gamete volume fraction, a metric more comparable to the measures of reproductive condition used in our present study. Additionally, we found no evidence of spawning of littlenecks at Sedge Island, based on VMI values, although this parameter was only measured starting June 30. Observation of histological slides from clams collected on June 16 and compated with those collected on August 25, however, showed evidence of earlier spawning in littlenecks than in the larger size classes and thus a reduced spawning period at both Sedge Island and IBPS.

- **There is thus considerable asynchrony in reproductive conditioning among clams of different size classes.**

- **Large clams (cherrystones and chowders) showed evidence of fairly protracted spawning activity throughout the summer at IBSP (July 28 to at least September 8)**, as indicated by a significant reduction in both the VMI and % oocytes.

The above result agrees with findings by Carriker (1961) in Little Egg Harbor in the 1940s, where spawning, as indicated by the presence of larvae in the water column, typically started in late June, and attained a maximum in July, with some spawning continuing into late August and the first week of September. In the current study clams were generally characterized by 2 or 3 peaks in reproductive condition followed by multiple spawning, rather than a single, major spawning event. A single major peak in the reproductive condition of hard clams, as measured by the % gamete volume fraction, was found at five south shore Long Island, NY, bays; a dual peak was only found in Middle Bay, western Long Island with a secondary, late summer peak in Sandy Hook Bay (Newell et al 2009). Hard clams in their study typically attained peak reproductive condition in early June and spawned thereafter, into September.

Spawning appeared to occur earlier at Sedge Island than at IBSP (Fig. 13), as determined by both VMI and % oocyte indices. The reduction in these indices during October may have been attributable to gamete resorption rather than/or in addition to late spawning. None of the three reproductive indices measured were able to detect significant spawning of littlenecks at Sedge Island. In contrast, both the VMI and % oocytes showed evidence of spawning of this size class at IBSP.

- **Clams with varying degrees of discolored, grey viscera were observed in larger clams (cherrystones and chowders) but not in littlenecks, with a maximum prevalence of 9% of large clams sampled at any sampling date. This discoloration may lead to poor market acceptance.**

Histological sections of larger clams (cherrystones and chowders) showed varying degree of proliferation of brown cells in the connective tissue surrounding gonadal follicles. We initially speculated that there was a relationship between the external grey appearance of clams and the prevalence of brown cells in their tissues. More in depth analysis, however, indicated that there was no clear association, between the abundance/density of brown cells based on histological analysis and that of discolored, “grey clams”, both determined qualitatively. Brown cells are known to play a role in detoxification and constitute a stress response in bivalves (Jeffries 1972, Zaroogian et al 1989). Their presence in different substrate types and functional significance in the BB-LEH estuary deserves further attention.

Kennish (1978) examined mortality in Barnegat Bay hard clams that had been placed in cages. While mortality was different on his two sites, in both areas losses were greatest in summer. At one site the highest loss was during the first summer (34.91%) with a cumulative loss for the year of 54%. At the second site the first summer loss was 13.53% and the cumulative loss was only 30%, but this site was maintained for a second summer and mortality during that summer was 28.6% (Kennish 1978). Our studies also indicate summer mortality, and although some of these losses could be due to handling, both the Kennish (1978) and now our study indicate potentially very high mortality rates of adult clams. Our size adjusted average mortality rates, however, were well below the rates estimated by Kennish (1978) and our final sampling of experimental plots.

- **Our estimates of size-adjusted average loss rates of about 3.8% by the end of the**

summer/early fall, suggest that recruitment must be at least at this level to maintain the existing hard clam population in BB-LEH. Coupled with the survey data indicating that few if any clams occur in large areas along the western portion of the bay, where they historically were present, these data suggest the need for further studies of adult clam mortalities.

VI. Management implications

- **A key finding of this study was that the relative allocation to reproduction [as measured by a visceral mass condition index and histologically] was significantly lower at Sedge Island for all 3 clam size classes**, although this site supported good to moderate growth of juveniles in 2012-2013.

Environmental conditions that support somatic production of bivalves, however, may differ from those that sustain reproduction (e.g. Santos et al. 2011). Our finding of poor clam reproductive conditioning at Sedge Island was unexpected, as IBSP is characterized by lower salinities that can at times approach the lower tolerance limit for hard clams, and a high % contribution of "small forms" to the phytoplankton assemblage. This result is especially important given that MCZ-protected waters have experienced extensive plantings of clam seed over the years (Calvo, 2012; reviewed by Bricelj et al. 2012), and would provide a likely location for establishment of a broodstock sanctuary as part of a clam stock enhancement management strategy in this estuary. Lack of success of early attempts to establish a spawner sanctuary in the mid-1980s in southern BB were attributed to clam poaching, limited scale of plantings and lack of a sustained effort. In contrast, supression of gamete production presumably due to poor environmental or nutritional factors was suggested as the main responsible factor at the LEH site (McCay 1988). The value of maintaining spawner sanctuaries in the BB-LEH and their siting needs further consideration.

- **There was no evidence based on the metrics used in the present study that chowder clams exhibited reproductive senescence. Both the VMI and % oocyte were generally higher for chowders than cherrystone clams at IBSP, although this was not the case at Sedge Island.**

The current research was conducted within a state-designated MCZ characterized by unique hydrographic and environmental conditions within the BB-LEH estuary. The MCZ supports an unknown magnitude of recreational fishing for hard clams but no commercial effort, and has sustained multi-year, clam seeding activities by the Barnegat Bay Shellfish Restoration Program (BBSRP) and the NJDEP Division of Fish & Wildlife (Bricelj et al. 2012). This stock enhancement effort is expected to continue/expand in future but its success remains to be evaluated.

The MCZ/Sedge Islands provide a unique and highly dynamic hydrographic system within the BB-LEH estuary that is strongly influenced by changes in configuration of the Barnegat Inlet and consequently experiences changing patterns in flow and bottom sedimentation (Kennish 2000). Our prior 2012-2013 study showed that Sedge Island is characterized by relatively low daily summer temperatures (on average 2.2 to 4.1 lower than at 3 other representative BB-LEH study sites in 2012 and 2013, respectively). This lower temperature regime was confirmed in the present 2014 study. Indeed, in 2014 the minimum temperature for hard clam spawning (~24°C) (Malouf

and Bricelj (1989) was only attained as a mean temperature at Sedge Island in mid-August, yet local clam populations may have adapted to these lower summer temperatures. High daily temperature fluctuations during early summer (up to 16ºC day^{-1} in 2013) are also characteristic of Sedge Island (Bricelj et al. unpublished). This may influence reproduction at this site given that spawning in *M. mercenaria* (and bivalves in general) is triggered by rapidly changing temperatures (Carriker 1961).

- **Another key finding of our present 2014 study in BB-LEH was that reproductive allocation (corrected for size) was significantly lower for littlenecks than larger clams at both study sites. This is an important result as it suggests that the minimum size for legal harvesting may not allow a significant contribution of littlenecks to the population's reproductive output. While this might suggest a larger minimum size, it is more important to maintain a sufficiently large population of all sizes of clams. It is also important to consider that clams remain in the littleneck size class for only a few years and thus this size class is thus more transient than the larger size classes.**

Littlenecks made up a lower % contribution to total clam numerical abundance than larger clams during sampling conducted in the 1980s in Barnegat Bay (reviewed by Bricelj et al. 2012). As determined histologically, littlenecks also showed earlier and a narrower window of spawning than larger clams (not shown). The latter supports anecdotal observations made by Dale Parsons (Parsons Seafood Inc.) based on hatchery-spawning induction of clams (2014 personal communication). The recruitment success of fertilized oocytes spawned earlier vs. later in the reproductive season also needs further investigation.

Following the winter of 2014, cumulative mortalities of clams of all size classes were higher at Sedge Island than at IBSP, and this result could not be attributed to predation. It is possible that this higher mortality at Sedge Island was caused by exposure to low temperatures and ice at low tide during the winter.

- **The magnitude and causes of mortalities of adult *M. mercenaria* in the BB-LEH estuary require additional study as they are a major factor influencing the population dynamics of this species**.

We address the following charge questions below:

1. *What is the long term perspective on sustainable commercial fisheries in the bay? Long term ecological perspective for a balanced food web, carbon cycling, habitat resilience, etc.?*

As long as the bay does not suffer increased eutrophication so that oxygen levels near the bottom do not decline or brown tides (caused by *Aureococcus anophagefferens*) do not increase in frequency, magnitude or spatial extent, and that fishing (commercial and recreational) harvest is appropriately managed, the prospects for sustaining a limited clam fishery in the bay are good. Proper siting of clam aquaculture and its attendant production will go a long way to providing the social/economic infrastructure needed to sustain the wild harvest. If there is a desire to manage the resource, which we highly recommend to ensure its long-term sustainabiliyt, it will require development of some form of management plan based on an adequate data stream.

Minimum data would require a stock survey from Barnegat Bay and Little Egg Harbor Bay every 2 years (perhaps alternating between the two). It is impossible to estimate landings at present, but an annual survey of landings (commercial and recreational) by size (littleneck, cherrystone and chowder) and some indication of where the landings are coming from will be required for resource management.

If the MCZ is to be utilized as a clam rehabilitation area, harvest will have to be restricted, at least in the portion of the area where seed are planted, and a broodstock sanctuary established. Area management of the resource throughout the BB-LEH system should be considered. The area management model being utilized in Delaware Bay for the oyster fishery should be examined for its utility (with some significant modifications due to major differences between these two bivalve species) to the hard clam resources of BB-LEH. This areal management should take into account aquaculture leases, areas closed to harvest and commercial harvest. Maintenance of broodstock at densities around 5-10 m^{-2} in spawner sanctuaries of several acres in size scattered throughout the system would help to assure that recruitment will be maintained. This bet-hedging strategy is better than focusing all enhancement efforts in a single area. Seeding of juvenile clams is not recommended in IBSP given that our 3-yr studies showed that this site experiences transient low salinity events that can suppress growth of juveniles and thus extend the time when they are exposed to predators.

2. *What is the current population level of hard clams in the BB-LEH system? Recruitment status? Scenarios for short and long term stock enhancement versus stressors (e.g., habitat loss, overfishing)?*

   Based on the limited population data points that are available the current clam population appears to be significantly lower than during the mid-1980s in the entire system, but there has been a recent encouraging increase in the population, and a decrease in mortality rate in Little Egg Harbor Bay (2012/13 survey). If our size-specific mortality rates for the harvestable portion of the population are approximately correct, and we use the sublegal category for an estimate of recruitment to the harvestable classes, then the population in Barnegat Bay is not recruiting fast enough to cover losses (1.9% recruitment vs 3.9% mortality), and the Little Egg Harbor population should be growing (7.5% vs 3.7%). **These estimates, however, should be used with extreme caution because they are based on very limited data from a portion of a year in protected plots and could easily be in error.** The most disconcerting factor is the disappearance of clams from the western portion of Little Egg Harbor Bay (2011 survey) and a trend toward more stations with low clam density in Barnegat Bay (2012 survey). The fact that the cause/s of the area devoid of clams in LEH is unknown (although it appears that clams can grow well in the area), will require additional investigation.

3. *Is there evidence that potential changes in food quality (phytoplankton) and/or supply may have led to poor recruitment, growth and compromised reproductive success of hard clams in Barnegat Bay? If so, are there measurable environmental stressors (e.g., eutrophication,*

overfishing) driving this change in food quality/quantity for hard clams? Management suggestions?

During our 2012-2013 study when funding was available to undertake concurrent microscopic analysis of phytoplankton, via support from the Barnegat Bay Partnership and the NJDEP in 2012 and 2013, respectively, three dinoflagellate species known to be harmful to bivalve mollusks were occasionally found at our IBSP and Sedge Island sites: *Scripsiella trochoidea*, *Akashiwo sanguinea*, and *Prorocentrum minimum*. There is no evidence, however, that toxic species of dinoflagellates (e.g. producers of paralytic shellfish toxins) or toxic diatoms (domoic-acid producing Pseudo-nitzschia spp.) are present in the BB-LEH estuary. Yet the documented presence of *A. anophagefferens*, the causative agent of brown tides, at levels known to inhibit growth of juvenile clams and that may affect reproduction, is of concern. The densities documented do not necessarily cause water discoloration and thus cannot be recognized visually. Visual discoloration of waters is not a sufficient criterion to prompt follow-up, ground-truthing to determine potentially detrimental concentrations of *A. anophagefferens*. We therefore recommend that monitoring for *A. anophagefferens* at key sites using species-specific methods (immunofluorescence assay) be reinstated in BB-LEH.

It is of interest that our 2012-2013 studies documented brown tide for the first time in Sedge Is., MCZ at levels of up to 440,000 cells ml^{-1}. Sampling for *A.anophagefferens* had not been conducted in these waters in previous studies. The Sedge Islands area is under direct oceanic influence due to exchange through the Barnegat Bay Inlet, and is characterized by relatively high salinities. In turn, *A.anophagefferens* is considered a species of oceanic origin that requires relatively high salinities for growth, so it is perhaps not surprising that it was found in this section of the Bay.

Our 2012-2013 results showed that the pigment 19' butanoylfucoxanthin (19'but) was useful as an indicator of *A. anophagefferens* and could be used in future as a pre-screening method to reduce the number of water samples required to run the immunofluorescence assay. Recent advances using flow-cytometry to quantify fluorescent cells (Stauffer *et al* 2008) allow more rapid analysis and increased throughput of samples. Overall, the use of photopigment analysis should be considered as a more cost-effective method (~$16 vs. ~$300 cost per sample) to provide real-time, spatial and temporal characterization of the phytoplankton community in the BB-LEH system. This should be accompanied by validation by microscopic taxonomical species identification at a subset of key sites during the summer/fall when brown tide is known to occur.

Total summer phytoplankton biomass as measured by Chl *a* concentrations, remained at moderate levels at the four study sites in 2012 and 2013 (= 8.6 and 12 1 $\mu g\ l^{-1}$ at IBSP, 4.2 and 5.8 $\mu g\ l^{-1}$ at Sedge Island, 6.3 and 10.3 $\mu g\ l^{-1}$ at Harvey Cedars and 10.9 to 13.1 $\mu g\ l^{-1}$ at Tuckerton, respectively), and was even lower at IBSP and Sedge in 2014 (4.8 and 2.0 $\mu g\ l^{-1}$, respectively). These levels are at or below those reported in BB-LEH between 1999 and 2010 (reviewed by Bricelj et al 2012). Our 2012-2013 studies showed that phytoplankton species composition was more important in predicting growth of juvenile hard clams that total biomass. Similarly, Newell

et al (2009) found that this was also the case in predicting reproductive output of hard clams in mid-Atlantic US bays. It will therefore be important in future to characterize the phytoplankton composition and abundance of various functional microalgal groups, and especially of small forms (= picoplankton, including cyanobacteria and chloropytes that are poorly retained and/or digested by hard clams), in areas that undergo seeding of cultured bivalve seed (hard clams, oysters or bay scallops) or that are used as a broodstock sanctuary during the period of growth and reproductive development. This information will be useful to identify the locations with the best food supply to support clam production for these stock enhancement activities.

Low reproductive performance of clams at the Sedge Island site in 2014, however, was attributed to low algal biomass (low food quantity rather than poor food quality) and/or the anomalous temperature regime at this site (relatively low mean temperature and high daily fluctuations in temperature), suggesting that if this may not be an optimum site to establish a spawner sanctuary. It remains unknown whether these environmental features can be extended to the MCZ as a whole. Multi-year characterization of clam reproductive performance at more than one MCZ site, as well as at other locations in the BB-LEH estuary (e.g. Tuckerton Cove, and area that historically supported high clam larval production), is needed.

More rigorous determination of growth rates of juvenile hard clams in ReClam The Bay upwellers could also provide early warning of deficiencies in food quality and/or quantity at specific locations throughout the bay. We demonstrated that this is possible in our 2012 study in which we obtained comparable clam growth rates in land-based upwellers receiving pumped water from the bay, and at an adjacent field site in IBSP, using bags of sufficient mesh size (4 mm square mesh) that did not inhibit flow and thus food delivery to the clams. Improved supervision, coordination and training of ReClam The Bay volunteers would be required to achieve this goal

VI. **Personnel involved:**

Co-investigators John Kraeuter and Gef Flimlin assisted in selection of the locations used to establish field plots and also participated in clam deployment at the two study sites in late April 2014 (with Jeffrey Silady, ReClam The Bay). J. Kraeuter also participated in statistical analysis of data. Sampling of clams was conducted primarily by J. Silady and Ryan Fantasia (RF), hired to participate in this project during the summer/fall of 2014. Sampling of the water column and in situ filtration for seston analysis were conducted by the PI, M. Bricelj (MB), Carola Noji (CN), part-time technician at IMCS/RU, and RF. Noji and Fantasia were responsible for laboratory preparation of filters (ashing and weighing) prior to use in field-sampling, and with MB, for processing of clams in the laboratory (i.e., measuring of SL and SW, dissection and weighing of soft tissues, and processing of samples for histological analysis). R. Fantasia was also responsible for CHEMTAX analysis of phytoplankton photopigments. Emily S. McGurk, Haskin Shellfish Research Laboratory, Rutgers University, was responsible for preparation of histological slides and microscopic quantification of investment in reproduction. Romi Patel, undergraduate work-study at RU, assisted in data entry and analysis.

VII. Outreach/Education

We interacted with a number of organizations over the course of this project as follows:

- **Barnegat Bay Shellfish Restoration Program/ReClam the Bay (**BBSRP/RCTB**):** provided boat access to the Sedge Island Education Center and IBSP every 2 wks, primarily via Jeffrey Silady, who also led the clam field sampling.
- **Island Beach State Park Forked River Interpretive Center:** as in previous year**s,** we btained authorization from IBSP personnel to process water samples from our two field sites at the Interpretive Center. They provided suitable space with an electrical outlet required for operation of our portable vacuum pump, and access to a freezer for transient holding of filters. This facility is frequently visited by the public (all age groups) and we have routinely informed them of our research activities when questioned during their visits.
- **Sedge Island Fish & Wildlife National Resource Education Center, Marine Conservation Zone:** we have interacted with staff at this facility, and occasionally briefed the students visiting this facility about our research activities.

VIII. Presentations

Dec. 20 2014. **V.M. Bricelj**, R. Fantasia, C. Noji, E. McGurk, G. Flimlin, J. Kraeuter. "Growth and reproduction of hard clams, *Mercenaria mercenaria*, in relation to environmental conditions in Barnegat Bay, a NJ coastal lagoonal ecosystem". 2014 NJDEP Barnegat Bay Workshop, Bordentown, NJ.

Oct. 2014. **V.M. Bricelj**, G. Flimlin, J. Kraeuter, R. Fantasia, Carola Noji. "Growth and reproduction of hard clams, *Mercenaria mercenaria*, in relation to environmental conditions in Barnegat Bay", Cooperative Extension of Ocean County, Toms River, NJ.

IX. References

Bricelj, V.M**.**, R.E. Malouf, 1984. Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam, *Mercenaria mercenaria*. *Mar. Biol.* 84: 155-165.

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

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., 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-T-09-001, 43 pp. http://www.seagrant.sunysb.edu/hclam/article.asp?ArticleID=308.

Dacanay, K. 2015. Inventory of New Jersey's estuarine shellfish resources: Hard clam stock assessment. Barnegat Bay (Survey year 2012) with Post-Superstorm Sandy investigation (2013). NJDEP Marine Fish Admin. Bureau of Shellfish. 54 pp.

Doall, M.H., Padilla, D.K., LoBue, C.P., Clapp, C., Webb, A.R. and Hornstein J. 2008. Evaluating northern quahog (= hard clam, *Mercenaria mercenaria* L.) restoration: are transplanted clams spawning and reconditioning? *J. Shellfish Res.* 27(5): 1-12.

Calvo, G., 2012. Hard clam enhancement activities in Great Bay. 2011 Cumulative Activity Report submitted to the NJ Bureau of Shellfisheries, 10 pp.

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.

Celestino, M. 2003. Shellfish stock assessment of Little Egg Harbor (2001), 20003. NJDEP Report, Nacote Creek Research Station, Port Republic, NJ. 41 pp.

Celestino, M. 2013. Shellfish stock assessment of Little Egg Harbor (2011), 2013. NJDEP Report, Nacote Creek Research Station, Port Republic, NJ. 37 pp.

Crosby, M.P. and L.D. Gale, 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. *J. Shellfish Res*. 9:233-237.

Eversole, A.G. 2001. Reproduction of *Mercenaria mercenaria*. Chapter 5 In: Biology of the hard clam. J.N. Kraeuter and M. Castagna (eds.). Developments in aquaculture and fisheries science 31, Elsevier, New York.

Glibert, P.M., C.E. Wazniak, M.R. Hall, B. Sturgis. 2007. Seasonal and interannual trends in the nitrogen and brown tide in Maryland coastal bays. *Ecol. Appl.* 17:S79-S87.

Jeffries, H.P., 1972. A stress syndrome in the hard clam, *Mercenaria mercenaria*. *J. Invert. Pathol*. 20: 242-251.

Joseph, J. W., 1986. Inventory of New Jersey's Estuarine Shellfish Resources. US. Department of Commerce. Project No. 3-405-R: 1, 35 pp. + Attachment A.

Joseph, J. W., 1987. Inventory of New Jersey's Estuarine Shellfish Resources. US. Department of Commerce. Project No. 3-405-R: 2, 79 pp.

Kennish, M.J., 1978. Effects of thermal discharges on mortality of *Mercenaria mercenaria* in Barnegat Bay, New Jersey. *Env.Geology* 2: 223-254.

Kennish et al., 2007. Barnegat Bay-Little Egg Harbor Estuary: case study of a highly eutrophic coastal bay system. *Ecol. Appl.* 17(5) Suppl. S3-S16.

Kraeuter, J.K., G. Flimlin, M.J. Kennish, R. Macaluso, J. Viggiano, 2009. Sustainability of northern quahogs (hard clams) *Mercenaria mercenaria*, Linnaeus in Raritan Bay, New Jersey: assessment of size specific growth and mortality. *J. Shellfish Res*. 28(2): 273-287.

Kraeuter, J.N., Flimlin, G.E., Canzonier, W.J., Ford, S.E., Zodl, J., Parsons, D., 1997. Declining quality of hard clams in Little Egg Harbor Bay: Effects of a long-term salinity reduction?, in:Flimlin, G.E., Kennish, M.J. (Eds.), The Barnegate Bay Workshop. Barnegat Bay Estuary Program and Rutgers University, pp. 123-138.

LoBue, C., 2010. Restoring hard clams to Great South Bay. Final Completion Report from the Nature Conservancy to Suffolk County, Hauppauge, NY, 48 pp.

Lowe, D.M. and M.N. Moore, 1985. Cytological and cytochemical procedures. Chapter 8 In: The effects of stress and pollution on marine animals, Bayne B.L., D.,A. Brown, K. Burns, D.R. Dixon DR et al. (Eds). Praeger, Westport, CT, pp. 179-204.

Malouf, R.E., V.M. Bricelj, 1989. Comparative biology of clams: environmental tolerances, feeding and growth. In: Clam mariculture in North America, J. Manzi, M. Castagna (eds.) Elsevier, New York: pp. 23-73.

Mackey, M.D., Mackey, D.J., Higgins, H.W., Wright, S.W. 1996. CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of

phytoplankton. *Mar. Ecol. Prog. Ser., 144*, 265-283.

McCay, B.J., 1988. Muddling through the clam beds: cooperative management of New Jersey's hard clam spawner sanctuaries. *Journal of Shellfish Research* 79(2): 327-340.

Newell, R.I.E, S. T. Tettlebach, 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. In: M. Kennish (ed.), Barnegat Bay-Little Egg Harbor, New Jersey: estuary and watershed assessment. *J. Coastal Res.* Sp. Issue (32): 115-143.

Paerl, H.W., L.M. Valdes, Pinckney, J.L., F. Piehler, J. Dyble, and P.H. Moisander, 2003. Phytoplankton photopigments as indicators of estuarine and coastal eutrophication. *Biosci.* 53:953-964.

Peterson, C.H., 1983. A concept of quantitative reproductive senility: application to the hard clam *Mercenaria mercenaria* (L)? *Oecologia* 58: 164-168.

Santos, S., J.F.M.F. Cardoso, C. Carvalho, P.C. Luttikhuizen, H.W. van der Veer, 2011. Seasonal variability in somatic and reproductive investment of the bivalve *Scrobicularia plana* (da Costa 1778) along a latitudinal gradient. *Estuarine Coastal Shelf Sci.* 92: 19-26.

Stauffer, B.A., R.A. Schaffner, C. Wazniak and D.A. Caron, 2008. Immunofluorescence flow cytometry technique for enumeration of the brown-tide alga, *Aureococcus anophagefferens. Appl. Environm. Microbiol*. 74(22): 6931-6940.

Trice, T., Gilbert, P., Lea, C., and L. Van Heukelem, 2004. HPLC pigment records provide evidence of past blooms of *Aureococcus anophagefferens* in the coastal bays of Maryland and Virginia, USA. *Harmful Algae, 3*(4), 295-304.

Ward, J.E. and S.E. Shumway, 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. *J. Exp. Mar. Biol. Ecol*. 300: 83-130.

Zaroogian, G., P. Yevitch, S. Pavignano, 1989. The nature and function of the brown cell in *Crassostrea virginica*. *Mar. Env. Res*. 28: 447-450.

Appendix I. Initial and final pigment/chlorophyll *a* ratios used in CHEMTAX predictions of microalgal class contribution to total Chl *a*. Abbreviations are as follows: Peri = peridinin, Fuco = fucoxanthin, Neo = neoxanthin , Viola = violaxanthin, Allo = alloxanthin, Lut = lutein, Zea = zeaxanthin.

Initial Pigment Ratio Matrix – 2014									
Class / Pigment	Peri	Fuco	Neo	Chl *c*	Viola	Allo	Lut	Zea	Chl *b*
Prasinophytes			0.036		0.099		0.041	0.026	0.638
Dinoflagellates	0.900			0.200					
Cryptophytes				0.107		0.222			
Chlorophytes			0.060		0.060		0.211	0.030	0.350
Cyanobacteria								0.675	
Diatoms		0.550		0.115					
Euglena			0.077				0.020	0.022	0.858

IBSP Final Pigment/Chlorophyll *a* ratios – 2014									
Class / Pigment	Peri	Fuco	Neo	Chl *c*	Viola	Allo	Lut	Zea	Chl *b*
Prasinophytes	0.0000	0.0000	0.0366	0.0000	0.0995	0.0000	0.0416	0.0263	0.4437
Dinoflagellates	0.9000	0.0000	0.0000	0.2000	0.0000	0.0000	0.0000	0.0000	0.0000
Cryptophytes	0.0000	0.0000	0.0000	0.0404	0.0000	0.2897	0.0000	0.0000	0.0000
Chlorophytes	0.0000	0.0000	0.0661	0.0000	0.0550	0.0000	0.2323	0.0300	0.3296
Cyanobacteria	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.1917	0.0000
Diatoms	0.0000	0.6035	0.0000	0.0802	0.0000	0.0000	0.0000	0.0000	0.0000
Euglena	0.0000	0.0000	0.0770	0.0000	0.0000	0.0000	0.0200	0.0220	0.8580

Sedge Final Pigment/Chlorophyll *a* ratios – 2014									
Class / Pigment	Peri	Fuco	Neo	Chl *c*	Viola	Allo	Lut	Zea	Chl *b*
Prasinophytes	0.0000	0.000	0.0645	0.0000	0.0937	0.0000	0.0416	0.0263	0.3961
Dinoflagellates	0.9000	0.000	0.0000	0.2537	0.0000	0.0000	0.0000	0.0000	0.0000
Cryptophytes	0.0000	0.000	0.0000	0.0364	0.0000	0.1553	0.0000	0.0000	0.0000
Chlorophytes	0.0000	0.000	0.0600	0.0000	0.0600	0.0000	0.2110	0.0300	0.3500
Cyanobacteria	0.0000	0.000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7733	0.0000
Diatoms	0.0000	0.598	0.0000	0.0421	0.0000	0.0000	0.0000	0.0000	0.0000
Euglena	0.0000	0.000	0.0482	0.0000	0.0000	0.0000	0.0200	0.0220	0.8580