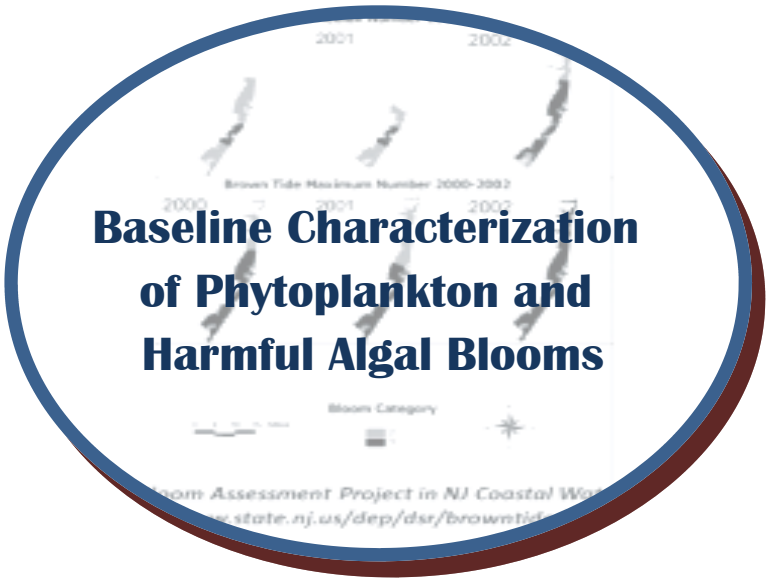
Barnegat Bay—Year 3



Baseline Characterization of Phytoplankton and Harmful Algal Blooms

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Barnegat Bay Phytoplankton Year 3:

Phytoplankton Reference Communities and Index of Biotic Integrity

FINAL REPORT

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EXECUTIVE SUMMARY

Barnegat Bay-Little Egg Harbor (BB-LEH) is very susceptible to human-induced eutrophication due to its shallow depth, relatively long flushing time and highly developed surrounding watershed. The Estuary has been classified as a highly eutrophic system (Nixon 1995, Bricker et al. 2007), experiencing episodic recurrences of brown tides and other microalgal blooms, loss of submerged aquatic vegetation, and decline of hard clam stock and harvest.

We carried out a three-year research on the phytoplankton community in BB-LEH estuary in coordination with New Jersey Department of Environmental Protection (NJDEP)'s Bureau of Marine Monitoring from 2012 to 2015. In the first two years of study we investigated phytoplankton community in BB-LEH from August 2011 to August 2013, characterized species composition and spatial and temporal distribution of phytoplankton, including bloom patterns, dominant species succession, and occurrence of Harmful Algal Bloom (HABs) species. In addition, multivariate analyses were conducted to understand the temporal changes of phytoplankton between Year-one and Year-two, and the relationship between the phytoplankton changes and environmental conditions. More details can be found in the project reports (Ren 2013, 2015).

This report presents the major results from the Year-three study. During the study period, phytoplankton reference communities and index of biotic integrity were quantified and are being developed for Barnegat Bay-Little Egg Harbor. Phytoplankton data from the first two years of investigation, together with water quality monitoring data, were used as the initial datasets for biological index development. Habitat conditions were classified for different season-salinity zones based on the nutrients and light measurements. Phytoplankton reference communities and their supporting habitat (least impaired) conditions were quantified for each season-salinity category. Phytoplankton metrics were evaluated for their ability to discriminate between the desirable (least impaired) and undesirable (impaired) conditions. The metrics which showed high discriminatory ability were selected to compose season-salinity specific IBIs. The work is the first attempt on phytoplankton reference community quantification and phytoplankton index of biotic integrity development for BB-LEH system. The calculated reference conditions and derived criteria are scientifically based and region specific, and expected to facilitate the assessment, restoration and rehabilitation efforts in water quality management in BB-LEH.

Major findings in the study are summarized below.

All 205 samples from the first two years of study were classified into 8 season-salinity categories, 4 seasons and 2 salinity zones. 18% of total samples were mesohaline, mainly from near the mouth of Toms River and the south of the Silver Bay. The majority of the samples were polyhaline from the north end of Barnegat Bay and south BB and LEH.

Secchi depth, DIN and Ortho-P (PO4) classification criteria were established, derived from 2011-2013 water quality monitoring data, for four water quality classes of Worst, Poor, Better, and Best. According to the classification criteria, phytoplankton habitat conditions of all sampling events (samples) were categorized into four major categories, Poor-Worst (including Worst, PW+W), Mixed-Poor Light (MPL), Mixed-Better Light (MBL), and Better-Best (including Best, BB+B).

In total, near 60% of the sampling events (samples) were categorized as Poor-Worst and Mixed-Poor Light (impaired or undesirable) conditions, indicating that the present-day water quality are often undesirable. Only 20% of the samples were identified as Better-Best (least-impaired) conditions.

Phytoplankton reference communities were calculated for each season-salinity category based on the samples in Better-Best category. The characteristic metrics for reference communities included Chl*a*, nano- and micro-phytoplankton (NM) abundance, NM phytoplankton biomass, average NM cell size, picoplankton biomass and Chla:C ratios, as well as DO, TSS, TN and TP.

Reference communities, in comparison to impaired habitat, were characterized with lower Chl*a*, lower Chl:C ratio, lower TN, TP and TSS, but higher DO, and in summer with lower picoplankton biomass. Chl*a*, TN, and NM phytoplankton and biomass of the mesohaline reference community were generally higher than those of polyhaline.

The boundaries of the reference Chl *a* concentration in summer and fall polyhaline, 1.3-11.6 µg/L, were comparable to the Chl *a* in 1970, but were lower than the average Chl *a* from summer 1987-1998. The upper boundary of reference Chl *a* in fall-polyhaline was slightly higher than Chl*a* in fall 1970. The comparison may suggest nutrient over-enrichment during 80s-90s and that the BB-LEH has the potential to return to relatively healthier levels near half century ago.

Thirty-four phytoplankton metrics were evaluated for their discriminatory ability between the least-impaired and impaired habitats. Metrics exhibited significant discrimination included Chl *a*, Chl*a* : C ratio, total NM phytoplankton abundance, average NM phytoplankton cell size, % diatoms, % dinoflagellate biomass, % cryptophyte biomass, summer % picoplankton and % cyanobacteria biomass, DO, DOC/TOC, and TSS. These metrics were selected to compose the season-salinity specific P-IBI.

Phytoplankton IBI metrics for spring and summer and scoring criteria of each metric were established. Calculated metric scoring criteria showed high percentage of one particular group, e.g. for summer diatoms, > 54% in mesohaline and > 67.9% in polyhaline, could be an indication of phytoplankton losing diversity, therefore indicating an impaired community. In summer, high abundance or percentage of picoplankton (> 1.2 x10^8 cells/L, or >43% in Table 10) indicates impaired habitat in mesohaline and polyhaline. Over dominance of picoplankton often associated with very low percentage of other taxonomic groups in summer. A least-impaired community, however, was more balanced in community composition.

Several key water quality parameters, such as Chl *a*, DO, TSS, TN and TP, showed significant difference between the least-impaired and impaired habitats. The calculated median and interquartile values of those parameters in the least-impaired and impaired conditions, as shown in respective tables and plots in this report, can be useful information for water quality assessment. In particular, TN and TP are two primary nutrient causal variables of eutrophication designated by EPA for nutrient criteria in estuarine and coastal ecosystem. These results provide valuable information for nutrient criteria development in the region, and guidance for water quality standards in nutrient loading management.

Recommendations

Phytoplankton data from 2011-2013, together with the synchronized water quality data, provided ideal datasets for phytoplankton IBI development. However, large inter-annual variability in phytoplankton community has been observed due not only to it natural variability but also greatly to the disruption by the Hurricane Sandy. As a result, phytoplankton reference communities and P-IBI development based on the two-year of data may have inevitably exhibited considerable uncertainty. The calculation and comparison of the reference

communities and P-IBI were largely constrained due to insufficient data particularly for some season-salinity categories, such as for mesohaline, and seasons of fall and winter. More investigations and monitoring on phytoplankton community, along with the water quality monitoring, are essential to reduce the uncertainty and deviation therefore to further refine and the reference communities and strengthen the P-IBIs for BB-LEH system.

Water quality classification is the primary step for reference community quantification and PIBI development. Light and nutrient criteria used for the classification are critical to refine the reference conditions. In this study, we used data from 9 sites from 2011 to 2013 to calculate light and nutrient thresholds using the Relative Status Method. It may be necessary to include data from all 14 monitoring sites by NJDEP to augment the data pool for each season-salinity habitat. In addition, the study used literature data (experimental results from other regions) as nutrient criteria for the classes of Poor and Better. Well-designed experiments, using natural assemblages from the BB-LEH, are suggested to be conducted to help establish region specific nutrient and light limitation thresholds for phytoplankton growth.

The reference conditions were quantified from the Better/Best categories derived from data 2011-2013, which represent the best of the present-day (best of what-is-left) conditions in BB-LEH. We compared chlorophyll *a* and phytoplankton composition in the reference communities with those from 1970. More historical data, if available, should be carefully examined and compared with the present-day reference communities. In addition, historical and present watershed development and land use, freshwater discharge and atmospheric inputs of nutrients need to be considered and incorporated to further refine the reference conditions.

INTRODUCTION

Phytoplankton is known to respond directly to changes of physical and chemical conditions in aquatic ecosystems. They are also the base of the food web and dynamically interact with the organisms at higher trophic levels in the system. Therefore, the change of phytoplankton assemblages constitutes a good integrated measure of the state of the system, reflecting both internal interactions within the system and external inputs to the system. These roles make phytoplankton an important group to consider as a valuable bio-indicator of water quality. For example, under European Water Framework Directive (EU WFD), phytoplankton has been specifically identified as a biological quality component and its monitoring is required to include elements such as taxonomic composition, abundance, biomass and blooms (European Commission 2000, Devlin et al. 2007). In the US, EPA Clean Water Act (CWA) mandates the use of biological data to assess the ecological conditions of aquatic ecosystem. The USEPA Estuarine and Coastal Technical Guidance (Gibson et al. 2000), as the implementation of CWA, recommends phytoplankton assessment with inclusion of chlorophyll *a* measurements and enumeration of bloom/dominant species.

Phytoplankton-based water quality indicators have been developed and applied extensively for identifying eutrophication in coastal and estuarine ecosystems. The indicators include phytoplankton biomass, production and pigment composition (Kauppila 2007, Paerl et al. 2003, 2007). When a bioindicator is usually an organism or a set of organisms, biotic indices go one step further. Biotic indices summarize features of different elements of the ecosystem (e.g. several bioindicators, community level information) into a single value, integrating relevant ecological information into an overall expression of biotic integrity. Recent review by Martinez-Crego et al. (2010) showed that several approaches have been used to develop biotic indices, of which the approach based on structural attributes at the community level has been frequently used. The principles of the type of biotic indices were first developed in freshwater systems (Karr 1986). More recently they have been used in coastal and estuarine systems to assess the integrity of different communities such as fish, benthic marcroinvertebrate, phytoplankton or zooplankton, seagrass and macroalgae. With the exception of benthic macroinvertebrates, phytoplankton is one of the most commonly used groups for developing this type of biotic indices. Multiple phytoplankton metrics have been used in biotic indices in several estuaries

including the German Bight (Radach 1998) and Chesapeake Bay (Jorden and Vaas 2000; Lacouture et al. 2006). Reference conditions are established based on phytoplankton elements such as chlorophyll *a*, productivity, biomass and cell sizes, for several regions (Buchanan et al. 2005; Devlin et al. 2007). The multimetric indices comprised of these phytoplankton elements are proven to be more sensitive to the environmental changes than the individual element (Buchanan et al. 2005, Martinez-Crego et al. 2010, Johnson and Buchanan 2014).

Recently, bioindicators using benthic macroalgae and seagrass, particularly eelgrass have been developed to assess eutrophication and nutrient pollution in the Barnegat Bay-Little Egg Harbor system (BB-LEH) (Kennish et al. 2011, Kennish and Fertig 2012). Other bioindicators, including benthic invertebrates and benthic diatoms, are currently being developed under the New Jersey Department of Environmental Protection's Barnegat Bay Comprehensive Plan of Action for Barnegat Bay. Studies on phytoplankton indicator and biotic indices for water quality in Barnegat Bay were lacking. The main objective of this third year in a three year study of phytoplankton dynamics in BB-LEH is to develop a phytoplankton index of biotic integrity (P-IBI), using the similar approaches as for Chesapeake Bay (Buchanan et al. 2005; Lacouture et al. 2006). Phytoplankton data from the first two years of investigation, together with water quality monitoring data, were used as the initial datasets for biological index development. Habitat conditions were classified for different season-salinity zones based on the nutrients and light measurements. Phytoplankton reference communities and their supporting habitat (least impaired) conditions were quantified for each season-salinity category. Phytoplankton metrics were evaluated for their ability to discriminate between the desirable (least impaired) and undesirable (impaired) conditions. The metrics which showed high discriminatory ability were selected to compose season-salinity specific IBIs. The project is a pilot study, aiming to provide useful information on water quality evaluation in the BB-LEH. The reference conditions and derived criteria are scientifically based and region specific, and expected to facilitate the assessment, restoration and rehabilitation efforts in water quality management in BB-LEH.

METHODS

The general steps for developing an IBI are well established (National Research Council 2000, Gibson et al. 2000). In this study, similar methods used in the Chesapeake Bay (Buchanan et al.

2005, Lacouture et al. 2006) were applied for the quantification of the reference communities and the development of P-IBIs for BB-LEH. The main steps included data compilation and analysis, habitat classification, reference community quantification, metrics selection, metrics scoring criteria, metrics scoring and validation.

Data Compilation and Analysis

Phytoplankton data generated from the first two years of this project and the contemporaneous water quality data generated by NJDEP are used as starting datasets for the development of P-IBIs. The two-year datasets cover sampling events dating from August 2011 to June 2013 and are distributed from North to South of BB-LEH spatially (Fig. 1). The phytoplankton dataset includes species composition, species abundance (cell density), and biovolume and carbon biomass of major taxonomic groups. The water quality dataset contains key parameters such as salinity, temperature, Secchi depth, DIN (dissolved inorganic nitrogen), orthophosphate (PO4), dissolved oxygen (DO), dissolved organic carbon (DOC), total organic carbon (TOC), chlorophyll *a* (Chl*a*), total nitrogen (TN) and total phosphorus (TP). Detailed information and data on phytoplankton can be found in Year-one and Year-two project reports (Ren 2014, 2015). Detailed information on water quality monitoring and data can be found at: http://www.state.nj.us/dep/barnegatbay/bbmapviewer.htm. The analysis and measurements of phytoplankton samples and water quality data were carried out by the same laboratories using the consistent methods throughout the multiple years of investigation, the Phycology lab at the Academy of Natural Sciences of Drexel University (ANS) and Marine Water Monitoring Lab at NJDEP, respectively. The reporting limit and analysis lab for several water quality parameters were changed, but the analysis methods have been consistent. Please see more detailes in the Quality Assurance Plan of the NJDEP Barnegat Bay Long Term Ambient Monitoring Program (LMP) (Barnegat Bay LMP QAPP 2013).

Season and Salinity Classification

Canonical correspondence analysis (CCA) performed to explore the relationships between the change in phytoplankton and environmental variables showed significant effects of temperature and salinity on species composition (Fig. 2). Seasons and salinity regimes were classified for developing the season and salinity reference community and IBIs in the BB-LEH. Cluster analysis was carried out based on the phytoplankton data collected from the same site to focus on

the seasonal changes in species composition (Fig. 3). In combination of the water temperature variation (Fig. 4), the distinction of four seasons was as followings: winter: December-February; spring: March-May; summer: June-September; and fall: October-November.

The classification of salinity regimes follows the well-accepted Venice System 'for the classification of marine waters according to salinity' (SCBW 1958). Salinity in the BB-LEH, based on the Water Quality Monitoring data, ranged from >10 ppt to < 33 ppt, and most of the data fell into two salinity zones: mesohaline (5 -18 ppt) and polyhaline (>18 ppt). Each sample was designated, according to its salinity, into one of the two zones. Mesohaline samples were frequently from sites BB04a, BB02, and occasionally BB01 and BB05a; polyhaline samples included those from BB07, BB09, BB10, BB12, BB14, and frequently BB01. Generally, low salinity was observed at BB04a, near the mouth of the Tom River, and higher salinity in the south of Barnegat Bay and Little Egg Harbor.

Habitat Classification

Light and nutrient availability are the primary factors for phytoplankton growth and development (Cloern 1999). A combination of light and nutrients, primarily dissolved inorganic nitrogen (DIN) and ortho-P (PO4), was used to classify the habitat condition of each sample as least-impaired (desirable) and impaired (undesirable) for phytoplankton growth. Prior to the classification, the criteria for Secchi depth, DIN and PO4 had to be set for each season-salinity category using approaches as described in this section. The nutrient limiting thresholds on phytoplankton growth, derived from bioassay experiments (e.g. Ren 2002, Fisher and Gustafson 2003), were used as the criteria to separate the better and poor classes for DIN (Table 2) and PO4 (Table 3). Phytoplankton growth is limited when the concentrations of DIN and PO4 in the system are below the thresholds, 0.07 mg/L and 0.007 mg/L, respectively, as shown in Table 2 and 3. In this case, the addition of nutrients will significantly stimulate the phytoplankton growth. On the other hand, when nutrient concentrations exceed the limiting thresholds, the additions of nutrients do not stimulate the growth at significance level. Such condition is generally undesirable (poor) with excess nutrients and insensitive to external nutrient input. The classes of best and worst were added as the extreme subsets of the better and poor classes to further classify DIN and PO4 concentrations. The DIN criteria for the best class was arbitrarily set to <0.03 mg/L (about 40% of the DIN limiting threshold). The PO4 criteria for the best class

was arbitrarily set to <0.002 mg/L (about 30% of the PO4 limiting threshold), primarily to accommodate the values under detect limit, similar strategy used in the Chesapeake Bay (Buchanan et al. 2005). The criteria for the worst class of DIN and PO4 were established using the Relative Status Method (Alden and Perry 1997, Olson 2002). The Relative Status Method is an assessment tool to evaluate a site based on the water quality conditions obtained from the current monitoring data. Scoring criteria for a particular water quality parameter are derived from the natural distribution of the data of the parameter at the monitoring sites. In our analysis, the method was applied to all sampling events. All data for these least-impaired samples were extracted, and their quartiles were used to establish threshold criteria for the worst DIN and PO4 classes. The Relative Status Method was also used to establish the worst, poor, better and best classes of Secchi depth in each season-salinity category (Table 4).

The Secchi depth, DIN and PO4 from each sample record were then independently classified as worst, poor, better or best based on the classification criteria. After the classification, each sample was grouped into one of the ten phytoplankton habitat categories depending on the combination of its class scores of Secchi depth, DIN and PO4. Table 4 explains the ten habitat categories and the combination of Secchi depth, DIN, and PO4 class scores for each category. These ten categories were then binned into six categories representing a condition range from better (including best) to poor (including worst) water quality conditions. These binning categories were assigned in order to facilitate the identification of various phytoplankton habitat conditions from the stand point of DIN, PO4 and light (Buchanan et al. 2005). The assigned six binning categories included B (best), BB (Better-Best), MBL (Mixed-Better Light), MPL (Mixed-Poor Light), PW (Poor-Worst) and W (Worst) (Table 5). The habitat categories and their frequency, as the number of samplings, for each site were summarized in Table 6.

Quantification of Reference Communities

Several characteristic metrics of phytoplankton community, including Chl*a*, Chl*a* :C ratios, nano- and micro (NM) phytoplankton abundance, average NM phytoplankton cell size were plotted for all the season-salinity-habitat categories based on available data from 2011-2013 (Fig. 7).

Of all the six habitat categories (Table 5), the categories B and BB represent the least-impaired (desirable) habitat conditions. Reference communities were calculated and quantified from the least-impaired samples. In those season-salinity habitats when categories B and BB were not present, MBL were used as the 'surrogates' for the least-impaired habitat condition (Buchanan et al. 2005). Phytoplankton populations in the least-impaired habitats were considered the best representatives of the phytoplankton communities that are not impaired by excess nutrients and poor water clarity. These populations are considered as the present-day minimally impacted communities in each specific season-salinity habitat. Table 7 and 8 summarized the reference communities and the supporting water quality conditions in mesohaline and polyhaline zones. The significance of differences between the reference communities and impaired populations were tested by ANOVA. Twenty-five percentile and 75 percentile values were used as the boundaries of the reference community (Table 7, 8).

Metric Selection

To develop phytoplankton index, thirty-four phytoplankton metrics (Table 9) were evaluated for their ability to discriminate between least-impaired and impaired conditions. The least-impaired conditions included the BB and B categories. The impaired conditions included PW and W categories. In the cases where data from those categories were lacking or insufficient, data from the MBL or MPL were included to augment the number of data, or used as surrogates if BB+B or PW+W were absent, for the least-impaired or impaired conditions. Metrics were not examined for the Fall Mesohaline as data were sparse, with only 2 data points from each of MBL and MPL. Kruskal-Wallis test was done on each metric to test the significance level of their discriminatory ability.

Metric Scoring Criteria and Index Scoring

Phytoplankton metrics showing significant discriminatory ability between the least-impaired and impaired conditions were selected to compose the phytoplankton index of biotic integrity (P-IBI). The list of P-IBI metrics for each season-salinity categories for spring and summer are shown in Table 10. The scoring criteria for each metric were established using similar approaches as described in previous studies (Weisberg et al. 1997, Gibson et al. 2000, Lacouture et al. 2006). The criteria were calculated based on the distribution of each phytoplankton metric in the reference community. For metrics showing a clear separation in their reference and

degraded distributions, values in the most desirable half (50%) of the reference distribution are scored 5. Values in and beyond the tail of the reference distribution are scored 1. The remaining values are scored 3. There may be other scenarios (e.g. bimodal or other non-normal) of the metrics distributions; the metric criteria were set by using the similar methods as for the Chesapeake Bay (Lacouture et al. 2006). Because of the limited number of data available from fall and winter, efforts were made primarily on the spring and summer P-IBI because relatively more data are available for spring and summer. The scoring criteria for each metric in the season-salinity specific IBIs were given in Table 10.

Before being incorporated into phytoplankton index, each phytoplankton metric was scored separately but on the same scale of 5, 3, or 1 in order to avoid unintended biases. Each metric was scored depending on whether its value approximated, deviated slightly, or deviated greatly from the distribution of the metric in the reference community, respectively. Individual metric scores were then averaged to generate a P-IBI score for each sampling event. The index scores were used to determine classification efficiency, or the ability of the overall index to discrimination between reference and degraded conditions (Gibson 2000, Lacouture et al. 2006).

Validation of P-IBI

A set of independent data collected from April 2014 to April 2015 are to be used to validate the P-IBIs. Water quality data were obtained from the continuous monitoring by the Bureau of Marine Water Monitoring, NJDEP (Barnegat Bay LMP QAPP 2013). Synchronized phytoplankton data were collected biweekly from April to September and monthly from other months. Sample analysis followed the same protocols as the previous years, and is described in the following Sections.

Phytoplankton Indicator Species Analysis

Indicator species analysis was performed for different seasons, salinity zones and TN:TP molar ratios, using the method described by Dufrene and Legneres (1997) and the software PC-ORD (McCune and Grace 2002). The method combines information on the concentration of species abundance in a particular group and the faithfulness of occurrence of a species in a particular group. The method requires two or more a priori groups of sample units, and data on species abundance or species presence in each of the sample unit. Before analysis, sample units are first

grouped into different categories of seasons, salinity zones or different TN:TP ratios . For each species, several steps are taken in the implementation in PC-ORD, 1) calculate the proportional abundance of a particular species in a particular group relative to the abundance of that species in all groups; 2) calculate the proportional frequency of the species in each group; 3) combine the two proportions calculated in steps 1 and 2 by multiplying them; 4) save the highest indicator values (IV*max*) for a given species; and 5) evaluate statistical significance of IV*max* by a Monte Carlo method. The analyses were carried out based on the species abundance data from 2011-2013, and for four seasons (spring, summer, fall and winter), three salinity zones (mesohaline MH, lower polyhaline LPH and higher polyhaline HPH) and three ranges of TN/TP molar ratios (>16, 8~16 and <8). The significance of the indicator values was tested using Monte Carlo method. The results of the indicator species, their highest indicator values, and statistical significance were shown in Table 11-13.

Phytoplankton Sample Collection and Analysis 2014-2015

Biweekly and monthly phytoplankton samples were collected and analyzed at four sites from April 2014 to April 2015. The location of the sites is listed in Table 1. Sample collections are coincident with grab samples from the NJDEP water quality monitoring program. Samples were collected two times a month from May to September 2014, and once a month in April, October and December 2014 and April 2015. More information about the sites and the water quality parameters can be found in the QAPP of the Barnegat Bay Long Term Monitoring Program (Barnegat Bay LMP QAPP 2013). Sample collections were carried out by the field crew of NJDEP-Leeds Point. Water samples were preserved with glutaraldehyde (final concentration ~1% v/v) shortly after collection, and stored in cold (4 °C) and dark before sample processing.

The same protocols and methods were used for sample process and analysis as the previous years. Phytoplankton samples were size-fractionated by filtering through 0.2 μm, 3 μm and 8 μm pore-size filters. The latter two fractions were stained with 0.03% proflavine hemisulfate. The 0.2 to 3 μm fraction was counted immediately after filtration. The >8 μm fraction was frozen and counted later. Algal identification and enumeration, including soft-algae and diatoms, were done under an epifluorescence microscope (Leica DM L) with blue and green excitation lights and transmitted light. For 0.2 and 3 μm pore-size filters, observations were done under ×1000 magnification. For each filter, at least 5 random fields were counted or until at least 100 cells

were counted. If the filter was very sparse, then 50 random fields were counted before stopping. For 8 μm pore-size filters, each filter was observed under three magnifications: First, under ×1000 magnification for phytoplankton < 20 μm with the same counting strategy in terms of finishing point; second, under ×400 magnification for larger (> 20 μm) phytoplankton with a maximum of 25 random fields when it was sparse; Third, under ×100 magnification to catch some large organisms, which might not have been able to be counted under higher magnifications due to either their large size or sparse density. The method allowed us to be able to examine small size phytoplankton (< 20 μm) under higher magnification (×1000) compared to other methods, e.g. using Palmer-Maloney and/or Sedgewick-Rafter counting cells. The blue and green excitation helps us to differentiate groups of algae when stained with dyes (Dortch et al. 1997, Ren et al. 2009). For samples with high abundance and diversity of diatoms, diatom slides were made separately. Diatoms were analyzed to get the percentage of dominant diatoms, especially the small centric diatoms. Phytoplankton species were identified to the lowest taxonomic level possible. In addition, each common taxon (at least 5% of total cell counts) was documented with images. Biovolumes of common taxa were calculated based on microscope measurements of dimensions and geometric models of phytoplankton (Hillebrand et al. 1999, Olenina et al. 2006). Carbon biomass was calculated based on the biovolume measurements and the cell carbon content for diatoms and non-diatoms from literature (Eppley et al. 1970).

RESULTS AND DISCUSSION

Physical Classification

In total 205 samples from 2011-2013 were used for the development of PIBIs. About 18% of the samples were mesohaline (5-18 ppt), and the rest were polyhaline (>18). Mesohaline samples were collected from a relatively narrow segment in Barnegat Bay, mostly within the plume of the Toms River (as shown by salinity values at site BB04a). Salinity north of the Toms River to the Silver Bay varied from mesohaline in 2011 and 2012 summer to polyhaline in 2013. Samples from BB01 near Mantoloking were polyhaline during most of the study period. A salinity gradient from north to south has been observed in BB-LEH. Despite both areas being polyhaline, salinity in northern BB (BB01 and BB05) ranged from 18 to 25 ppt, while salinity in south and LEH (sites 07, 09, 10, 12 and 14) was generally higher than 25 ppt and up to 33 ppt. The highest salinity was detected around Barnegat Inlet (Fig. 1, map, and Table 1 for the description of the

sites). Generally, salinity gradient and distribution in Barnegat Bay is affected by freshwater discharge, mainly from the Toms River, water circulation and exchange with the adjacent oceanic water (Defre and Ganju 2014).

Phytoplankton Habitat Categories

Among ten habitat categories, Category 1 (W) and 2 (PW) represent most impaired (undesirable) habitat which is a light-impoverished condition with excessive DIN and PO4 concentrations in the water column (Table 5). Category 1 is the extreme subset of Category 2 with light, DIN and PO4 values all falling into the class of Worst (Table 2-4). In total about 6% of the total samples had undesirable Poor-Worst and Worst habitat conditions during 2011-2013 (Fig. 5). Most of them were collected from the southern Barnegat Bay and Little Egg Harbor in summer and fall (Table 6, Fig. 6). On the other hand, Category 9 (BB) and 10 (B) represent the least-impaired habitat with desirable light condition and the nutrient concentrations below phytoplankton uptake limiting thresholds. Category 10 is the extreme subset of Better, with light, DIN and PO4 values falling into the class of Best in Tables 2-4. Approximately 20% of total samples fell into the Better-Best and Best Categories, showing the desirable habitat conditions for phytoplankton (Fig. 5). Compared to other sites, BB01 in spring and BB07a in summer (including BB07 before 5/23/2012) had high frequency of the BB (and B) category (Table 6, Fig. 6), mainly attributed to higher Secchi depth and lower PO4 concentration. Both sites were in polyhaline (PH) zone most of the time, the same salinity class as the southern BB and LEH, although salinity in north has been slightly lower than the south. BB01 is located at the northern end of the Barnegat Bay with relatively slow flushing and circulation rates. The site may have exhibited some degree of water clarity improvement due to less suspended solids in comparison to the southern sites with high flushing and circulation. Similarly, site BB07a is deeper than other sites and the circulation is relatively low, which may contribute to better light condition around the area (Defne and Ganju 2014). Therefore, both sites may have elevated light condition compared to other polyhaline sites likely due to their specific hydro-physical features. The classification criteria for light condition, however, were calculated based on all the data points from polyhaline samples, of which approximately 1/3 was from BB01 and BB07 and more than 2/3 was from sites BB09, 10, 12 and14. It is possible the current light criterion for the Better/Best may be slightly lower for sites BB01 and BB07 than that for the other polyhaline sites.

The majority of samples (over 73%) fell into the six mixed categories (Combo# 3-8 in Table 6, Fig. 5). These six categories were further grouped into two categories, Mixed Poor Light (MPL) and Mixed Better Light (MBL), differentiated by light conditions. In MPL, Secchi depth values were low and usually fell into the criteria of Poor (undesirable). DIN and PO4 concentrations are either individually (category 3 and 4) or jointly (category 5) below the phytoplankton growth limitation thresholds. In MBL, the light condition is desirable and Secchi depth values meet the criteria of Better, but one or both nutrients exceed their limitation thresholds. The grouping reflects the fact that light is one of the most important factors for phytoplankton growth and provides energy source for photosynthesis (Cloern 1999). On the other hand, technically the grouping is necessary in order to obtain sufficient numbers of data for each habitat category and to 'lessen the possibility that data from one or two sites dominate a given category' (Buchanan et al. 2005). Near 50% of total samples belong to the MPL category, and about 20% fell into MBL. Overall more samples were in the MPL category than in MBL in all seasons, indicating MPL a stable condition for most of BB-LEH, especially in spring and summer. Category 6 in the MBL exhibits high water column transparency and excess DIN and PO4, a favorable condition for possible phytoplankton growth and biomass increase. Category # 6 was more often found at southern sites (e.g. BB09 and 12, Table 6, Fig. 6) in summer. Such condition can easily change to the Mixed Poor category or even to Poor category if provided with continuous nutrient replenishment from external sources.

Phytoplankton Reference Communities

Reference communities were quantified based on the data from the least-impaired habitat in all seasons for mesohaline and polyhaline zones (Table 7 and 8). For polyhaline zone, the least-impaired habitat used for the calculation included the samples from BB and B categories. For mesohaline, less available samples and those in B or BB categories were sparse; therefore samples in MBL category were included to augment the number of data for the calculation (Table 7). The characteristic metrics to describe the reference communities included chlorophyll *a* (Chl*a*), Chl*a*:C ratios, nano- and micro-phytoplankton (NM) abundance, NM biomass, average NM cell size, picoplankton biomass, as well as dissolved oxygen (DO), total suspended solids (TSS), total nitrogen (TN) and total phosphorus (TP). The latter parameters are related with phytoplankton growth and biomass in some ways. Median, the maximal and minimum values of each metric in the reference communities for mesohaline and polyhaline were listed in the Table

7 and 8, respectively. In addition, statistical significance of difference was calculated between the least-impaired habitats and impaired habitats for metrics, such as Chl*a*, Chl*a*:C ratios, DO, TSS, TN and TP in spring and summer, and picoplankton biomass in summer (Table 7 and 8). In general, more metrics showed significant difference in polyhaline than in mesohaline. And higher significance of difference (p) was obtained for polyhaline zone than for mesohaline. This is more likely a result from 1) very few, 2 to 7 data points were available for mesohaline; and 2) the MBL category was included to BB and B in mesohaline. Reference communities, in comparison to the impaired habitats, were characterized with lower chlorophyll *a*, lower Chl:C ratio, lower TN, TP and TSS, but higher DO, and in summer lower picoplankton biomass. These results are in agreement with studies on the relationship between nutrient enrichment, phytoplankton growth and water quality in various estuarine and coastal ecosystems (Hoyer et al. 2002, Nielsen et al. 2002, Rabalais and Nixon 2002, Turner et al. 2007, Smith and Schindler 2009). Reference values for some metrics, such as Chl*a*, Chl*a* :C, average NM cell size, and DO, were comparable to those in the same salinity zones in Chesapeake Bay (Buchanan et al. 2006). TSS was generally higher than that in Chesapeake Bay both in mesohaline and polyhaline due to shallow water depth and high suspended matter in BB-LEH. For pico-biomass, the boundaries of reference biomass in summer were comparable to those in Chesapeake Bay. But the median value of pico-biomass in mesohaline was only one third of that in Chesapeake Bay, because most of the BB+B samples were collected in June when picoplankton abundance was usually one or two magnitudes lower compared to its peak in August/September.

Reference conditions are essential for assessment and restoration efforts of water quality for any specific waterbody. Several approaches may be applied to establish reference conditions/sites for coastal and marine waters with different degrees of human impacts (Gibson et al. 2000, NRC 2000). The Barnegat Bay-Little Egg Harbor Estuary (BB-LEH) is a shallow, poorly flushed system bordered by a highly developed watershed. The ecological health of the Estuary has deteriorated over the last few decades, and the Estuary has been classified as a highly eutrophic system (Nixon 1995, Kennish et al. 2010). The calculated reference communities and their supporting conditions represent the present-day least-impaired water quality in BB-LEH, and may not be the same as ‘historical’ or ‘pristine’ condition/sites (Gibson et al. 2000). The Toms River is the largest river with high discharge of freshwater and nutrients to the Estuary. Chl*a* concentration in mesohaline reference condition was generally higher than that in polyhaline

indicating elevated phytoplankton biomass due to riverine nutrient input. The boundaries of the reference Chl *a* concentration in summer and fall polyhaline, 1.3-11.6 µg/L, were comparable to the Chl *a* in 1970 from the southern Barnegat Bay (Mountford 2013). But the upper boundary of summer reference Chl *a* was lower than the average concentrations of Chl *a* from summer 1987-1998 (Olsen and Mahoney 2001). The upper boundary of reference Chl *a* in fall-polyhaline was slightly higher than Chl*a* in fall 1970. The comparison may suggest nutrient over-enrichment during 80s-90s and that the BB-LEH has the potential to return to relatively healthier levels than near half century ago.

The preliminary results of reference communities were derived from limited number of data points. For mesohaline, only very few data of BB+B were available for the calculation. Increased number of data points used for polyhaline, especially in spring and summer, but still limited compared to available datasets for similar estuarine regions (e.g. Chesapeake Bay, 18 years of data were used for reference communities and P-IBI development). Additional data is essential to refine the water quality criteria and to achieve better representativeness of the reference communities in each salinity-season category for BB-LEH.

Phytoplankton IBI Metrics and Scoring Criteria

Table 9 presents the 34 phytoplankton metrics evaluated for their discriminatory ability. In polyhaline spring and summer, up to half of the metrics showed significant distinction between the least-impaired and impaired categories (Kruskal-Wallis test). Mesohaline had less metrics showing applausive discriminatory power, mainly due to 1) mesohaline in BB-LEH is a relatively small area but receiving high discharge of freshwater and nutrients; 2) few data points were available in the dataset to compare and reach statistical significance. Several metrics showed good discriminatory ability in more than one season-salinity categories. Those metrics can be more reliable to be used for the P-IBI development. Those metrics included Chl *a*, Chl*a* : C ratio, total nano- and micro (NM) phytoplankton, and average NM phytoplankton cell size, % diatoms, % dinoflagellate biomass, % cryptophyte biomass, summer % picoplankton and % cyanobacteria biomass, DO, DOC and TOC, and TSS.

Chl*a* and Chl*a* : C are two metrics indicating phytoplankton photosynthesis activity in particular area. Chl*a* is one of the key parameters in routine water quality monitoring as phytoplankton

biomass because it is relatively easy to measure. Cellular Chl*a* contents in the same species may vary with light, temperature and nutrient composition and availability in waterbody. The total concentration of Chl*a* is considered a good indicator for total phytoplankton biomass. Different to Chl*a*, carbon biomass is usually calculated by multiplying the cell biovolume by carbon: biovolume ratios. The carbon: biovolume ratios vary with different species or taxonomic groups (Mullin et al. 1966, Stramski 1999). There may be de-coupling between Chl*a* biomass and biovolume-based biomass because of the effects of light, temperature, nutrients and taxonomic composition on Chl*a* content (Geider 1987, Felip and Catalan 2000). It is suggested that having both Chl*a* and Chl*a* :C in the PIBI creates a stronger overall index (Lacouture et al. 2006).

Several metrics of phytoplankton community composition turned out to be important for P-IBI. Nano- and micro- phytoplankton is defined as species and specimens with cell size in the range of 2 to 200 µm. In BB-LEH, phytoplankton in this category includes most of the common species except for the pico-coccoids (2-3 µm diameter) and cyanobacteria (mostly *Aphanocapsa* sp., < 1 µm). Other taxonomic groups such as diatoms, dinoflagellates and cryptophytes, and their biomass percentage were important components of the index. These metrics reflect the biodiversity of phytoplankton in the system, which is an essential indicator for heathy environment.

DOC and TOC are the metrics indicative for physiological status of phytoplankton. The production of DOC and TOC from biological processes includes phytoplankton extracellular and cellular excretion and lysis due to zooplankton grazing and other stresses, and mortality of phytoplankton/zooplankton (TOC). The concentration of DOC/TOC is partially a reflection of the presence of phytoplankton biomass prior to the sample being collected. DOC/TOC is also an important parameter related to microbial metabolism and dissolved oxygen level in water column (Lacouture et al. 2006). Dissolved oxygen is a metric reflecting biological metabolism of an aquatic system including phytoplankton photosynthesis, respiratory and microbial decomposition. It is a key indicator for nutrient over-enrichment in coastal ecosystems (Rabalais and Nixon 2002).

Phytoplankton IBI metrics for spring and summer and scoring criteria of each metric are summarized in Table 10. We focused primarily on spring and summer for P-IBI because more

data are available for these seasons compared to fall and spring. Calculated metric scoring criteria showed that in class 1 (as impaired community), the biomass percentage of each major taxonomic group could be either very low or very high. High percentage of one particular group, e.g. for summer diatoms, > 54% in mesohaline and > 67.9% in polyhaline could be an indication of losing biodiversity phytoplankton community, thus a criterion for impaired community. A least-impaired community, however, was more balance in community composition as showed in the criteria for class 5 (Table 10). In summer, high abundance or percentage of picoplankton (> 1.2 x10^8 cells/L, or >43% in Table 10) indicates impaired habitat in mesohaline and polyhaline. Over dominance of picoplankton often associated with very low percentage of other taxonomic groups in summer. High percentage of picoplankton, including cyanobacteria in summer is an indicator for nutrient over-enrichment in estuarine and coastal ecosystems. Picoplankton and cyanobacteria are less favorable food sources for shellfish and clams (Bricelj 1999). Summer phytoplankton was abundant in picoplankton, cryptophytes and dinoflagellate, more likely indicating silica limitation in the water column, especially in northern Barnegat Bay (Ren 2015).

Several individual taxa can serve as metrics for the P-IBI for different nutrient regimes and season-salinity categories as shown by the indicator species analysis (Table 11-13). However, the current database does not provide sufficient data for most of the indicator species to effectively discriminate different habitat conditions. Phytoplankton community showed significant year-to-year differences between 2011-2012 and 2012-2013, resulting from both natural inter-annual variabitlity and disruption by the Hurricane Sandy (Ren 2015). In addition, dinoflagellates *Prorocentrum minimum* and *Heterocapsa rotundata* can be important for P-IBI metrics because both species are harmful and reached fairly high abundance in BB-LEH. *P. minimum* was detected at high abundance (2.5 x 10^6 cells /L) in northern part of the Bay in winter 2011, and *H. rotundata* (=*Katodinium rotundatum,* 1.3 x10^6 cells /L) off the mouth of Toms River in the following 2012 winter. The growth of these two dinoflagellates was found to be associated with different forms of nitrogen nutrient and water quality conditions. *P. minimum* was positively related to high dissolved organic nitrogen and TSS, and high TN:TP ratios as shown from indicator species analysis (Table 13) . *H. rotundata* was positively related to inorganic nitrogen and is closely more related to high density of diatoms in winter-spring (Rothenberger et al. 1999). This may explain why *P. minimum* was abundant in the first year while *H. rotundata* was abundant in the second year when the hydrological and chemical conditions in Northern part of

the Bay had been altered by the Hurricane Sandy. *P. minimum* appeared as an indicator species in winter from the Indicator Species Analysis (Table 11). But neither species showed strong discriminatory ability between the least-impaired and impaired categories based on the current dataset (only 4 data points for *P. minimum* in winter from mesohaline, and 5 for polyhaline; and less data for *H. rotundata*). Both species need be considered in further work on P-IBI.

The median, interquartile, maximal and minimum values of major P-IBI metric from the least-impaired and impaired conditions for 4 season-salinity categories are shown in Fig. 8-11. The concentrations of DOC/TOC and TSS were significantly higher in the impaired condition than those in the least-impaired condition. In contrast, DO in the impaired condition was often lower than that in the least-impaired in most season-salinity zones. Except for spring-polyhaline, larger variability of DO and outliers in the impaired and least-impaired data, mostly due to those from northern BB likely resulted from the Hurricane disturbance. Chl*a* concentration in the least-impaired condition was lower and its variation was smaller compared to those in the impaired habitats. The concentration of Chl*a* appeared more variable in the impaired habitats than the least-impaired ones (Fig. 10-11). The median values and boundaries of the metrics in the least-impaired and impaired habitats, though preliminary, can be helpful for water quality evaluation in the BB-LEH system.

In summer-polyhaline, despite overlap of the ranges and data outliers, total abundance of NM phytoplankton in the least-impaired condition appeared lower than that from the impaired condition. The average cell size of the NM phytoplankton, however, tended to be smaller in the degraded environment. Biomass (biovolume-based) percentage of picoplankton was higher in the impaired habitats than in the least-impaired ones (Fig. 11). These results are coincident with those from Chesapeake Bay (Buchanan et al. 2005). The percentage of the major phytoplankton groups (diatoms, cryptophytes and dinoflagellates) showed large data overlap between the least-impaired and impaired and outliers despite the significant difference in median values. This uncertainty is likely caused by data insufficiency, and may be largely lowered with additional data input.

TN and TP are considered primary nutrient causal variables for estuarine and coastal eutrophication (EPA 2001). Positive relationship between TN and TP and chlorophyll *a* has been

revealed in several studies (Smith et al. 1999, Guildford and Hecky 2000). Dual reduction of N and P and careful management for both N and P loading were recommended as essential to control eutrophication and water quality (Pearl 2009, Smith and Schindler 2009). The data showed significant difference in TN and TP concentrations (median and interquartile) between the least-impaired and impaired in most season-salinity categories (Fig. 12 and 13), except for spring-mesohaline when few data available for statistical comparison. The comparison and the TN and TP values in the least-impaired and impaired habitat from this study can be useful information for nutrient criteria development in the region, and guidance for nutrient loading control in water quality management.

Index Scoring and Validation of PIBI

To be completed

Post-Sandy Phytoplankton Community

Seasonal changes of phytoplankton species abundance and compositions from April-December 2014, as well as the comparison with 2011-2013 data, are to be added in the report.

SUMMARY

Phytoplankton reference communities and index of biotic integrity were quantified and are being developed for Barnegat Bay-Little Egg Harbor in this study, using the phytoplankton community data generated from this project, and contemporaneous NJDEP water quality data from August 2011 to June 2013. The work is the first attempt on phytoplankton reference community quantification and phytoplankton index of biotic integrity development for BB-LEH system.

Major Findings

All 205 samples were classified into 8 season-salinity categories, 4 seasons and 2 salinity zones. 18% of total samples were mesohaline, mainly from near the mouth of Toms River and the south of the Silver Bay. The majority of the samples were polyhaline from the north end of Barnegat Bay and south BB and LEH.

Secchi depth, DIN and Ortho-P (PO4) classification criteria were established for water quality classes.

In total near 60% of the sampling events (samples) were classified as Poor-Worst and Mixed-Poor conditions, indicating that the present-day water quality are often undesirable. Only 20% of the samples were identified from the least-impaired condition. Sites BB01 and BB07 showed better water quality condition more often than other sites because of improved light condition probably due to their specific physical-hydrological features.

Phytoplankton reference communities were quantified for all season-salinity categories. The characteristic metrics for reference communities included Chl*a*, nano- and micro-phytoplankton (NM) abundance, NM phytoplankton biomass, average NM cell size, picoplankton biomass and Chla:C ratios, as well as DO, TSS, TN and TP.

Reference communities, in comparison to impaired habitat, were characterized with lower Chl*a*, lower Chl:C ratio, lower TN, TP and TSS, but higher DO, and in summer with lower picoplankton biomass. Chl*a*, TN, and NM phytoplankton and biomass of the mesohaline reference community were generally higher than those of polyhaline.

The boundaries of the reference Chl *a* concentration in summer and fall polyhaline, 1.3-11.6 µg/L, were comparable to the Chl *a* in 1970, but were lower than the average Chl *a* from summer 1987-1998. The upper boundary of reference Chl *a* in fall-polyhaline was slightly higher than Chl*a* in fall 1970. The comparison may suggest nutrient over-enrichment during 80s-90s and that the BB-LEH has the potential to return to relatively healthier levels near half century ago.

Thirty-four phytoplankton metrics were evaluated for their discriminatory ability between the least-impaired and impaired habitats. Metrics exhibited significant discrimination included Chl *a*, Chl*a* : C ratio, total NM phytoplankton abundance, average NM phytoplankton cell size, % diatoms, % dinoflagellate biomass, % cryptophyte biomass, summer % picoplankton and % cyanobacteria biomass, DO, DOC/TOC, and TSS. These metrics were selected to compose the season-salinity specific P-IBI.

Phytoplankton IBI metrics for spring and summer and scoring criteria of each metric were established. Calculated metric scoring criteria showed high percentage of one particular group, e.g. for summer diatoms, > 54% in mesohaline and > 67.9% in polyhaline, could be an indication of phytoplankton losing diversity, therefore indicating an impaired community. In summer, high abundance or percentage of picoplankton (> 1.2 x10^8 cells/L, or >43% in Table 10) indicates impaired habitat in mesohaline and polyhaline. Over dominance of picoplankton often associated with very low percentage of other taxonomic groups in summer. A least-impaired community, however, was more balanced in community composition.

Several key water quality parameters, such as Chl *a*, DO, TSS, TN and TP, showed significant difference between the least-impaired and impaired habitats. The calculated median and interquartile values of those parameters in the least-impaired and impaired conditions, as shown in respective tables and plots in this report, can be useful information for water quality assessment. In particular, TN and TP are two primary nutrient causal variables of eutrophication designated by EPA for nutrient criteria in estuarine and coastal ecosystem. This study provides valuable information for nutrient criteria development in the region, and guidance for water quality standards in nutrient loading management.

Recommendations

Phytoplankton data from 2011-2013, together with the synchronized water quality data, provided an ideal dataset for phytoplankton IBI development. However, large inter-annual variability in phytoplankton community has been observed due not only to it natural variability but also greatly to the disruption by the Hurricane Sandy. As a result, phytoplankton reference communities and P-IBI development based on the two-year of data may have inevitably exhibited uncertainty. The calculation and comparison of the reference communities and P-IBI were largely constrained due to insufficient data particularly for some season-salinity categories, especially for mesohaline, and fall and winter. More investigations and monitoring on phytoplankton community, along with the water quality monitoring, are essential to reduce the uncertainty and deviation therefore to further refine the reference communities and developed P-IBIs in this study.

Water quality classification is the primary step for reference community quantification and PIBI development. Light and nutrient criteria used for the classification are critical to refine the reference conditions. In this study, we used data from 9 sites from 2011 to 2013 to calculate light and nutrient thresholds using the Relative Status Method. It may be necessary to include data from all 14 monitoring sites by NJDEP to augment the data pool for each season-salinity habitat. In addition, the study used literature data (experimental results from other regions) as nutrient criteria for poor and better. Well-designed experiments, using natural assemblages from the BB-LEH, are suggested to be conducted to help determine the nutrient limitation thresholds on phytoplankton growth.

The reference conditions were quantified from the Better/Best categories derived from data 2011-2013, which represent the best of the present-day (best of what-is-left) conditions in BB-LEH. We compared chlorophyll *a* and phytoplankton composition in the reference communities with those from 1970. More historical data, if available, should be carefully examined and compared with the present-day reference communities (EPA 2001). In addition, historical and present watershed development and land use, freshwater discharge and atmospheric inputs of nutrients need to be considered and incorporated to refine the reference conditions.

RESPONSES TO CHARGE QUESTIONS

1. *Does your research support the development of indicators or models to assess and protect aquatic life?*

   Yes. Our study provided valuable information for the development of water quality indicators for Barnegat Bay-Little Egg Harbor (BB-LEH). The three years of phytoplankton data, in combination of water quality data, provided baseline datasets for the development of biotic indices for water quality assessment. In the Yr3 study, we evaluated water quality conditions, calculated salinity-season specific phytoplankton reference communities and their habitat conditions, compared phytoplankton communities and several key water quality parameters (DO, TN, TP, DOC, etc.) between least-impaired and impaired conditions. These results are valuable and helpful for the water quality assessment and nutrient criterion development for BB-LEH. In addition, as ecosystem modeling became important tool for assessment and prediction of water quality changes, carbon (C) is one general currency in biological models (Glibert et al. 2010). Phytoplankton, as a primary producer, is an essential compartment in the models. Unlike chlorophyll, there is no direct in-situ measurement for phytoplankton carbon biomass. This study provides three-year dataset of phytoplankton carbon biomass, estimated specifically based on species composition and abundance, biovolume from BB-LEH sites.

2. *Can the collection of data be reduced or streamlined from the research methodology in a cost-effective manner (e.g., fewer sites and/or sampling times) to support an annual routine monitoring and assessment protocol by the state?*

   It is recommended the phytoplankton collection coordinated with water quality monitoring for the cost-effectiveness in terms of field workload and data usage. Yes, it is possible to reduce collection sites and/or sampling times. Cluster analysis on phytoplankton community data from 8 sites collected from Yr 1 showed significant similarity among BB01 and BB02, BB05a and BB7a, and BB12 and BB14 (Figure in Yr2 report). Therefore Yr2 collection was reduced to 6 sites. Unfortunately same cluster analysis on 6 sites from Yr2 did not show significant grouping, largely due to the disturbance of Hurricane Sandy to the phytoplankton community. If continuous data collection is possible, the sites might be possible to further reduce by 1 or 2 sites. And the reduction of sampling times may be considered in two possible ways, either to reduce the frequency to monthly, or to just focus on spring and summer since there have been more spring-summer samples collected than fall-winter ones. However, since the data collection may be coordinated with water quality monitoring, and the phytoplankton data

may be useful for modeling study, it would be may be better to consider the data collection reduction in combination with capacity/requests from those two groups.

3. *Has the increasing human population density and urbanization of the bay had an effect on the abundance and diversity of phytoplankton?*

   We did not do actual correlation, but data showed that there was increase of abundance and biomass from south to north in phytoplankton biomass, especially in summer months. This trend is coincident with the human population density and urbanization in the BB-LEH watershed. In addition, phytoplankton abundance at BB04a, near the mouth of Tom River, was generally higher compared to other nearby sites while salinity was lower, indicating the effects of freshwater discharge mainly riverine nutrient input.

   Diversity of phytoplankton showed seasonal variations, being higher in winter months and lower in spring and summer (Figure as attached). In spring and summer, phytoplankton diversity was relatively lower in northern area than that in south, which is again coincident with the higher urbanization in north and lower in south.

4. *Are there tends in phytoplankton abundance and diversity? Are key species declining, stable, or improving?*

   It is hard to see any tends in phytoplankton abundance and diversity from these three-year data. The comparison was made difficult particularly due to the effects of Hurricane Sandy when second year's community had been changed especially in the norther area. Summer picoplankton dominance has been observed from northern area with peak in August-September. During the study, we used the method of polyclonal antibody labeling and fluorescence microscopic observation and detected low density of *Aureococcus anophagefferens* in southern Barnegat Bay and Little Egg Harbor. An incidence of *Aureococcus anophagefferens* bloom, however, was detected near Sedge Island on June 19, 2013 (4.5×10^8 cells/L, Bricelj et al. unpublished data). In addition, several other HAB species were recorded with considerably high abundance including *Prorocentrum minimum*, *Heterocapsa rotundata* (=*Katodinium rotundatum*), *Dinophysis acuminate*, *Pseudo-nitzschia* spp. and *Chaetoceros* spp. Even though the detected species and their abundance varied year to year, the study showed their presence in BB-LEH, which is a primary factor indicating the potential for harmful blooms.

5. *Is there a documented change in water quality in the bay that may have effects on phytoplankton abundance and distributions? If so, are these in measurable decline?*

From these three-year data, we have not noticed any overall decline in phytoplankton abundance and distribution related to water quality change. However, significance of difference of several phytoplankton metrics between the least-impaired and impaired conditions has been calculated based on the first two years of data and documented in Yr3 report. This should provide guidance for future observations on what particular phytoplankton metrics for us to focus on in relation to water quality changes.

6. *What is the long term perspective on sustainable phytoplankton populations in the bay? What is the long term ecological perspective for a balanced food web, carbon cycling, habitat resilience, etc.?*

   Human caused nutrient enrichments (or eutrophication) are a major factor for phytoplankton development in an estuarine ecosystem like Barnegat Bay, especially concerning harmful algal blooms. In addition, global warming and related see level rise are particularly concern in long-term since water temperature and salinity are primary factors for phytoplankton production and species compositions. Changes of phytoplankton components often have significant effects on the organisms at higher trophic levels in the food web. For instance, fish kills and/or reduction of some important fishery resources are often linked to, directly or indirectly, some specific algae, especially harmful algal blooms. It is very important to fully understand the complex interactions between nutrient loadings, temperature and salinity changes, phytoplankton responses and food web alteration in such a coastal system like Barnegat Bay, particularly in terms of long-term changes.

7. *Are there measurable environmental stressors affecting phytoplankton in BBay (e.g., nutrients, build out, boating, etc.)? Management suggestions?*

   Multivariate analysis showed significant relationships between phytoplankton species composition and the environmental variables. In addition to salinity and temperature, several nutrient variables were significantly related to the change of phytoplankton community, including total nitrogen (TN), dissolved silica (DSi), total phosphorus (TP), TN:TP ratio, dissolved and total organic carbon (DOC and TOC), as well and dissolved oxygen (DO) and total suspended solids (TSS). High abundance of diatoms was negatively related to DSi in the water column in both years, indicating Si limitation in spring and summer. The dominance of picoplankton and cyanobacteria in summer was significantly related to high nutrients, particularly TN and dissolved organic matter, and low concentration of dissolved oxygen in the water column. The results further confirmed that the change in species composition was sensitive to nutrient input in BB-LEH, and that the phytoplankton community is an important component of water quality monitoring.

8. *What impact, if any, did Super Storm Sandy have on phytoplankton abundance and distribution in BB-LEH?*

   Yes. Ordination analysis showed that phytoplankton community composition was significantly influenced by Hurricane Sandy. The largest change in the phytoplankton community was found at BB01 where the water residence time is the longest. Consequently, the 2013 winter and spring phytoplankton assemblages after the Hurricane were significantly different than those from the previous year. Hurricane Sandy had affected the phytoplankton composition especially in northern Bay. How the resulting phytoplankton changes related to associated food web changes, and how long it takes the system to recover to the pre-storm condition, if recover even happens, are questions of interests.

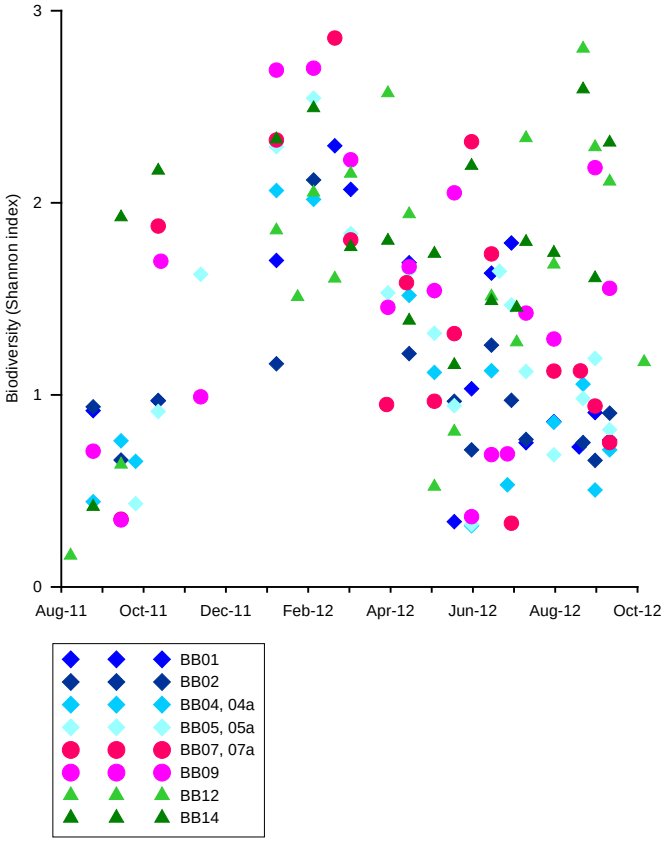

Figure: Biodiversity (Shannon index) of phytoplankton from 2011-2012.

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LIST OF TABLES

Table 1: Barnegat Bay Phytoplankton sample collection sites, 2011-2013. 9 sites were analyzed in Yr 1 (2011-2012); 6 sites (highlighted in grey) were analyzed in Yr 2 (2012-2013), and 5 sites, labelled with X were analyzed in 2014-2015.

Site ID	**longitude**	**latitude**	**site description**
BB01 (x)	-74.052222	40.04	Barnegat Bay at Mantoloking
BB02	-74.09847	39.97762	Barnegat Bay between Silver Bay and Goose Creek
BB04a (x)	-74.14069	39.93289	Barnegat Bay near the Mouth of Toms River
BB05a	-74.1094237	39.9157764	Barnegat Bay above Cedar Creek
BB07a	-74.1571172	39.8012861	Barnegat Bay below Oyster Creek and above Barnegat Inlet
BB09 (x)	-74.14792	39.74262	Barnegat Bay below Barnegat Inlet and close to Long Beach
BB10	-74.20653	39.66095	Barnegat Bay by Route 72 Bridge
BB12 (x)	-74.26875	39.58151	Barnegat Bay in Little Egg Harbor
BB14	-74.29737	39.51123	Little Egg Harbor Inlet near Beach Haven Heights

Table 2: DIN (mg/l, NO_3+NO_2+NH_4) classification criteria for water quality classes of Worst, Poor, Better, and Best for different seasons and salinity zones. Spring: March-May; Summer: June-August; Fall: September-October; Winter: November-February. Salinity: mesohaline (MH), and polyhaline (PH)

	Habitat Classification Criteria				Relative Status Method		
DIN (mg/l)	Worst	Poor	Better	Best	75%	median	25%
Spring							
MH	>0.262	>0.07	d0.07	<0.03	0.262	0.152	0.033
PH	>0.078	>0.07	d0.07	<0.03	0.078	0.042	0.025
Summer							
MH	>0.113	>0.07	d0.07	<0.03	0.113	0.032	0.018
PH	>0.055	>0.07	d0.07	<0.03	0.055	0.028	0.017
Fall							
MH	>0.153	>0.07	d0.07	<0.03	0.153	0.072	0.036
PH	>0.214	>0.07	d0.07	<0.03	0.214	0.129	0.051
Winter							
MH	>0.283	>0.07	d0.07	<0.03	0.283	0.193	0.113
PH	>0.052	>0.07	d0.07	<0.03	0.052	0.025	0.016

Table 3: Ortho-P (mg/l) classification criteria for water quality classes of Worst, Poor, Better, and Best for different seasons and salinity zones. Spring: March-May; Summer: June-August; Fall: September-October; Winter: November-February. Salinity: mesohaline (MH), and polyhaline (PH)

	Habitat Classification Criteria				Relative Status Method		
Ortho-P (mg/l)	Worst	Poor	Better	Best	75%	median	25%
Spring							
MH	>0.01	>0.007	d0.007	d0.002	-	-	-
PH	>0.01	>0.007	d0.007	d0.002	0.009	0.005	0.003
Summer							
MH	>0.005	>0.007	d0.007	d0.002	0.005	0.0005	0.002
PH	>0.023	>0.007	d0.007	d0.002	0.023	0.014	0.007
Fall							
MH	>0.01	>0.007	d0.007	d0.002	-	-	-
PH	>0.027	>0.007	d0.007	d0.002	0.027	0.015	0.011
Winter							
MH	>0.01	>0.007	d0.007	d0.002	-	-	-
PH	>0.01	>0.007	d0.007	d0.002	0.005	0.005	0.004

Table 4. Secchi depth (m) classification criteria for water quality classes of Worst, Poor, Better, and Best for different seasons and salinity zones. Spring: March-May; Summer: June-August; Fall: September-October; Winter: November-February. Salinity: mesohaline (MH), and polyhaline (PH)

	Habitat Classification Criteria				Relative Status Method		
Secchi depth (m)	Worst	Poor	Better	Best	25%	median	75%
Spring							
MH	<1.30	d1.52	>1.52	>1.75*	1.30	1.52	1.52
PH	<1.11	d1.52	>1.52	>1.83	1.11	1.52	1.83
Summer							
MH	<0.76	d0.91	>0.91	>1.14	0.76	0.91	1.14
PH	<0.62	d0.91	>0.91	>1.22	0.62	0.91	1.22
Fall							
MH	<0.91	d0.99	>0.99	>1.07	0.91	0.99	1.07
PH	<0.91	d1.14	>1.14	>1.49	0.91	1.14	1.49
Winter							
MH	<0.99	d1.37	>1.37	>1.52	0.99	1.37	1.52
PH	<0.91	d1.22	>1.22	>1.40	0.91	1.22	1.40

Table 5: Explanation of the phytoplankton habitat category classification. Combo#: combination number in consideration of the classes of light, DIN and ortho-P. Low = poor and worst light classes, High = better and best light classes, Excess = poor and worst nutrient classes, Limiting = better and best nutrient classes. The Poor habitat category represents impaired conditions; the Better category and some Mixed-Better Light represent Least-impaired conditions. The Worst category is a subset of Poor (with worst DIN, ortho-P and Secchi depth); the Best category is a subset of Better (with best DIN, ortho-P, and secchi-depth).

Combo #	Light	DIN	ortho-P	category	category code
1	Low	Excess	Excess	Worst	W
2	Low	Excess	Excess	Poor-Worst	PW
3	Low	Limiting	Excess	Mixed-Poor Light	MPL
4	Low	Excess	Limiting	Mixed-Poor Light	MPL
5	Low	Limiting	Limiting	Mixed-Poor Light	MPL
6	High	Excess	Excess	Mixed-Better Light	MBL
7	High	Excess	Limiting	Mixed-Better Light	MBL
8	High	Limiting	Excess	Mixed-Better Light	MBL
9	High	Limiting	Limiting	Better-Best	BB
10	High	Limiting	Limiting	Best	B

Table 6: Frequency (as number of sampling events) of phytoplankton habitat categories in spring and summer for all phytoplankton sites, derived from data 2011-2013. See Table 5 for the explanation of category code and combination #.

Category code	W	PW	MPL			MBL			BB	B
Combo#	1	2	3	4	5	6	7	8	9	10
Spring										
BB01				1	1		1		6	
BB02					2					
BB04(a)				1	3		1		1	
BB05(a)					3		1			1
BB07(a)				3	5			1	1	1
BB09			1	3	3		1		1	1
BB10				1		1				
BB12		1	1	2	3	1		2		
BB14					2			1	1	1
Summer										
BB01			1	1	7					2
BB02					6				1	1
BB04(a)			1	4	2	1	2		2	
BB05(a)		1	1	1	6				1	
BB07(a)			1		2			1	5	1
BB09			2		1	4		2	2	
BB10		2								
BB12	1	2	4		1	1		2		
BB14			2		1	2		4	1	

Table 6 (cont.): Frequency (as number of sampling events) of phytoplankton habitat category in fall and winter for all phytoplankton sites, derived from 2011-2013 data. See Table 5 for the explanation of category code and combination #.

Category code	W	PW	MPL			MBL			BB	B
Combo#	1	2	3	4	5	6	7	8	9	10
Fall										
BB01					2	1	1			
BB02			1					1		
BB04(a)				1			1			
BB05(a)									1	
BB07(a)		1				1				1
BB09		2						1	1	
BB10			1	3	1					
BB12		2						1		
BB14					1					
Winter										
BB01				1	1		2			
BB02				1					1	
BB04(a)			1	2			2			
BB05(a)			1				2			
BB07(a)		1			1				3	
BB09					1				2	1
BB10					2				1	
BB12				2	5					
BB14					1				1	

Table 7: phytoplankton reference communities and the habitat conditions support them for mesohaline zone (5~18 ppt). p: Significance of difference, ANOVA test. ** $p < 0.01$, * $p < 0.05$; ns: not significant; blank: not applicable. Δ: Reference community values higher than impaired community values; ∇: Reference community values lower than impaired community values.

	Spring			Summer			Fall			Winter			
	BB+MBL (n=4)			B+BB+MBL (n=7)			MBL (n=2)			BB+MBL (n=3)			
	Max/Min	Median	p	Max/Min	Median	p	Max/Min	Median	p	Max/Min	Median	p	units
Chl a	11.8/0.8	4.2	ns	12/2.2	5.47	*	14.3/6.7	10.5		7.4/1.7	5.5	ns	ug/L
Chl:C	0.12/0.004	0.031	ns	0.18/0.008	0.05	ns	0.056/0.028	0.042		0.035/0.029	0.03	ns	ratio
DIN	0.28/0.02	0.17		0.03/0.015	0.03		0.3/0.04	0.17		0.45/0.04	0.11		mg/L
PO4		<0.0011			<0.0011		<0.0005	<0.0011		<0.0005	<0.0011		mg/L
Secchi depth	1.8/1.5	1.52		1.6/1.1	1.22			1.07		1.66/1.52	1.52		m
DO	9.3/8.9	9.09	*Δ	8.5/6.9	7.8	*Δ	10.7/7.3	9		12.9/11.2	12.62	ns	mg/L
TSS	12.3/8.2	10.25	ns	13.0/7.4	9.4	**∇	19.5/10	15		14/10.3	13.5	ns	mg/L
NM abundance	6.1/1.2	2.37	ns	28.8/4.7	11.7	ns	2.5/1.0	1.75		0.8	0.4	ns	10^7 cells/L
NM biomass	6.3/1.6	3.77	ns	17.9/1.6	3.44	ns	3.5/2.8	3.22		3.9/1.2	2.4	ns	10^9 um^3/L
avg NM Cell Size	175/102	133	ns	99.1/18.0	39.1	*Δ	374/112	243.6		597/278	508	*Δ	um^3/cell
TN	0.68/0.38	0.53	** ∇	0.68/0.49	0.55	*∇		0.78		0.55/0.35	0.52	ns	mg/L
TP	0.028/0.014	0.019	*∇	0.03/0.01	0.03	**∇		0.03		0.013/0.005	0.007	ns	mg/L
pico biomass	109/41	75	ns	571/6.6	119	* ∇		174			0	na	ug/L

Table 8: phytoplankton reference communities and the habitat conditions support them for polyhaline zone (> 18 ppt). p: Significance of difference, ANOVA test. ** $p < 0.01$, * $p < 0.05$; ns: not significant; blank: not applicable. Δ: Reference community values higher than impaired community values; ∇: Reference community values lower than impaired community values.

	Spring			Summer			Fall			Winter			
	B+BB (n=13)			B+BB (n=12)			B+BB (n=3)			B+BB (n=8)			
	Max/Min	Median	p	Max/Min	Median	p	Max/Min	Median	p	Max/Min	Median	p	units
Chl a	3.5/0.3	1.26	*∇	11.6/1.3	2.9	**∇	11.4/2.4	4.6	ns	9.9/0.8	2.73	ns	ug/L
Chl:C	0.122/0.002	0.022	* ∇	0.14/0.006	0.041	* ∇	0.14/0.07	0.13	ns	3.6/0.01	0.052	ns	ratio
DIN	0.06/0.01	0.035	na	0.04/0.01	0.017	na	0.08	0.07		0.012/0.008	0.01		mg/L
PO4	0.0045	<0.0011	na	0.0075	0.006	na		<0.01			<0.005		mg/L
Secchi depth	2.3/1.7	1.9	na	1.86/1.09	1.52	na	1.52/1.25	1.52		2.2/1.0	1.4		m
DO	8.9/5.2	7.9	*Δ	8.65/5.75	7.17	**Δ	9.5/7.6	7.91	ns	12.5/10	11	ns	mg/L
TSS	25.5/11.0	16.7	*Δ	25.4/9.35	14.75	* ∇	23.1/15	19.5	*Δ	32/15	21	ns	mg/L
NM abundance	4.6/0.6	1.3	ns	10.8/0.7	3.6	ns	5.1/0.2	0.4	ns	0.86/0.13	0.38	*Δ	10^7 cells/L
NM biomass	8.2/0.2	2.2	ns	7.95/0.6	2.4	ns	3.3/0.3	0.53	ns	9.1/0.3	4.2	ns	10^9 um^3/L
avg NM Cell Size	774/77	164	ns	441/17	77	ns	1785/18	133	ns	3046/155	1094	ns	um^3/cell
TN	0.48/0.22	0.3	ns	0.48/0.24	0.32	**∇	0.44/0.21	0.27	**∇	0.27/0.15	0.19	**∇	mg/L
TP	0.023/0.008	0.018	* ∇	0.05/0.02	0.04	**∇	0.03/0.01	0.02	* ∇	0.039/0.005	0.017	* ∇	mg/L
Pico biomass	12.0/7.2	3.3	ns	730.4/9.4	127	*∇	75.2/7	15.1	ns				ug/L

Table 9: Phytoplankton metrics and their discriminatory ability for significant difference between least-impaired and impaired communities examined by Kruskal-Wallis test. * p: 0.05-0.1; ** P: 0.01-0.05; *** p <0.01; ns: not significant; -: not applicable.

Metrics	spring		summer		Fall		Winter	
	MH	PH	MH	PH	MH	PH	MH	PH
Chlorophyll a (ug/l)	ns	***	**	***	-	ns	ns	**
Chla : C ratio	ns	ns	ns	**	-	ns	ns	*
Total abundance nano-micro phytoplankton (cells/l)	ns	ns	ns	**	-	ns	ns	**
Total biovolume nano-micro phytoplankton (um ^{3}/l)	ns	ns	*	**	-	ns	ns	ns
Average cell size nano-micro phytoplankton (um ^{3}/cell)	ns	ns	*	***	-	ns	ns	ns
Diatom biomass (cells/l)	ns	ns	*	ns	-	ns	ns	ns
Diatom abundance (cells/l)	ns	ns	**	ns	-	ns	ns	*
% diatoms to toal phytoplankton biomass	*	**	*	**	-	ns	ns	*
Chlorophyte biomass	ns	ns	ns	*	-	ns	ns	ns
Chlorophyte abundance (cells/l)	ns	ns	ns	ns	-	ns	ns	ns
% chlorophytes to toal phytoplankton biomass	ns	ns	ns	ns	-	ns	ns	ns
Dinoflagellate biomass	ns	*	ns	ns	-	ns	ns	*
Dinoflagellate abundance (cells/l)	ns	ns	ns	ns	-	ns	ns	**
% dinoflagellates to toal phytoplankton biomass	ns	**	**	ns	-	ns	ns	ns
Cryptophyte biomass	ns	***	ns	ns	-	ns	ns	ns
Cryptophyte abundance (cells/l)	ns	***	ns	ns	-	ns	ns	ns
% cryptophytes to toal phytoplankton biomass	ns	**	***	**	-	ns	ns	ns
Chrysophyte biomass	ns	**	ns	**	-	ns	ns	ns
Chrysophyte abundance (cells/l)	ns	**	ns	**	-	*	ns	ns
% chrysophytes to toal phytoplankton biomass	ns	ns	ns	ns	-	ns	ns	ns
Picoplankton biomass	ns	ns	**	ns	-	ns	ns	ns
Picoplankton abundance (cells/l)	ns	ns	***	ns	-	ns	ns	ns
% picoplankton to toal phytoplankton biomass	ns	ns	***	**	-	ns	ns	ns
Cyanobacteria biomass	ns	ns	ns	ns	-	ns	ns	ns
Cyanobacteria abundance (cells/l)	ns	*	ns	ns	-	ns	ns	ns
% cyanobacteria to toal phytoplankton biomass	ns	ns	ns	***	-	ns	ns	ns
Prorocentrum minimum abundance (cells/l)	ns	ns	ns	ns	-	ns	ns	ns
Prorocentrum minimum biomass	ns	ns	ns	ns	-	ns	ns	ns
Total suspended solids (TSS, mg/l)	ns	**	***	**	-	***	ns	ns
Dissolved organic carbon (DOC, mg/l)	ns	ns	ns	***	-	ns	ns	*
Total organic carbon (TOC, mg/l)	ns	ns	***	***	-	*	ns	ns
dissolved oxygen (DO, mg/L)	*	**	**	***	-	*	ns	ns
Total nitrogen (TN, mg/- sl)	ns	ns	**	***	-	***	ns	**
Total phosphorus (TP, mg/l)	ns	***	***	***	-	**	ns	**

Table 10: Phytoplankton IBI metrics scoring criteria for spring (upper table) and summer (lower table).

Metrics	Metric scoring criteria			unite
	1	3	5	
Mesohaline (MH)				
% diatom biomass	<5.0 or >80.1	> 32.2 and < 80.1	> 5.0 and <32.2	%
Dissolved Oxygen (DO)	< 8.5	> 8.5 and < 9.1	> 9.1	mg/l
Polyhaline (PH)				
Chlorophyll a	> 2.48	> 1.26 and < 2.48	<1.26	mg/l
% diatom biomass	<1.7 or >57.4	>12.9 and < 57.4	>1.7 and < 12.9	%
% dinoflagellate biomass	<2.5 or >54.0	> 54.0 and < 20.0	>2.5 and < 20.0	%
%Cryptophyte biomass	< 2.3 or > 27	> 7.1 and < 27.0	> 23 and <7.1	%
Cyanobacteria abundance	> $1.7x10^6$	< 7.3×10^5 and $1.7x10^6$	< 7.3×10^5	cells/l
TSS	> 25.5	>19.5 and <25.5	< 19.5	mg/l
Dissolved Oxygen (DO)	< 7.82	> 7.82 and < 8.73	> 8.73	mg/l

Metrics	Metric scoring criteria			
	1	**3**	**5**	**unit**
Mesohaline (MH)				
Chlorophyll a	> 8.5	> 5.5 and < 8.5	< 5.5	ug/l
% diatom biomass	< 0.5 or > 24.8	>8.1 and < 24.8	> 0.5 and < 8.1	%
% dinoflagellate biomass	< 2.6 or > 54.4	>23.8 and < 54.4	> 2.6 and < 23.8	%
% cryptophyte biomass	< 1.1 or > 21.9	> 10.8 and < 21.9	> 1.1 and < 10.8	%
Picoplankton abundance	> 1.2×10^8	>2.6×10^7 and <1.2×10^8	< 2.6×10^7	cells/l
Total organic carbon (TOC)	> 7.2	> 6.6 and < 7.2	< 6.6	mg/l
TSS	> 12.4	> 9.5 and < 12.4	< 9.5	mg/l
DO, mg/l	< 33.1	> 33.1 and < 55.9	> 55.9	mg/l
Polyhaline (PH)				
Chlorphyll a	> 6.3	> 2.9 and < 6.3	< 2.9	ug/l
Chla : C	> 0.076	> 0.041 and < 0.076	< 0.041	
Total abundance of NM phytoplankton	> 2.9×10^7	> 1.3×10^7 and < 2.9×10^7	< 1.3×10^7	cells/l
Average cell size of NM phytoplankton	< 62	> 62 and < 119	> 119	um^3/cell
% diatom biomass	< 4.1 or > 67.9	> 23.1 and < 67.9	> 4.1 and < 23.1	%
% cryptophyte biomass	< 1.4 or > 33.3	> 14.2 and < 33.3	> 1.4 and < 14.2	%
Chrysophyte abundance	> 6.6×10^5	> 1.5×10^5 and < 6.6×10^5	< 1.5×10^5	cells/l
% picoplankton biomass	> 43.4	> 19 and < 43.4	< 19	%
Dissolved organic carbon (DOC)	> 4.5	> 3.1 and < 4.5	< 3.1	mg/l
Dissolved oxygen (DO)	< 5.1	> 5.1 and < 6.4	> 6.4	mg/l

Table11: Indicator species for different seasons, their indicator values and statistical significance (p). Spring: March-May; Summer: June-September; Fall: October-November; and winter: December-Februray. IVmax: maximum indicator value.

Species	**season**	**IVmax**	**p**
Dactyliosolen fragilissimus	spring	44.1	0.0002
Leptocylindrus minimus	spring	25.9	0.012
Chlamydomonas sp. 'c' Campbell	spring	15.8	0.026
Leucocryptos marina	spring	19.1	0.054
Planktolyngbya sp.	spring	14.9	0.012
Minutocellus scriptus	summer	38.8	0.001
Gyrodinium flagellare	summer	26	0.007
Plagioselmis sp.	summer	30.9	0.032
pico-coccoids	summer	38.9	0.0006
Aphanocapsa sp.	summer	33.4	0.002
Gyrodinium estuariale	summer	17.5	0.035
Cyclotella atomus	fall	31.2	0.0002
Cylindrotheca closterium	fall	37.6	0.0018
Thalassiosira nordenskioeldii	fall	20.8	0.004
Hemiselmis sp.	fall	32.9	0.011
Asterionellopsis glacialis	winter	36.3	0.002
Cyclotella choctawhatcheeana	winter	34.3	0.019
Guinardia flaccida	winter	12.9	0.025
Skeletonema costatum	winter	53.4	0.0002
Thalassionema nitzschioides	winter	43.3	0.0002
Prorocentrum minimum	winter	15.2	0.045

Table 12: Indicator species for different TN:TP ratios, their indicator values and statistical significance (p). TN:TP groups: 1: TN:TP>16 (sample # 160); 2: TN:TP <=16 and >8 (sample # 41); 3: TN:TP<=8 (no samples). IVmax: maximum indicator value; IVmean: mean indicator values.

Species	Indicator Values			P
	TN:TP Groups	IVmax	IVmean	
Asterionellopsis glacialis	2	32.1	21.4	0.016
Chaetoceros cf. *tenuissimus*	1	10.6	7.4	0.08
Cyclotella choctawhatcheeana	1	61.2	39	0.0004
Cylindrotheca closterium	2	42.7	26.6	0.003
Leptocylindrus minimus	2	21.7	14.9	0.0386
Minutocellus polymorphus	2	7.1	2.8	0.0224
Skeletonema menzelli	2	24.3	5.5	0.0002
Thalassiosira proschkinae	2	82.6	21.5	0.0002
Thalassiosira spp.	2	34.5	22.6	0.013
Chlamydomonas sp. 'c' Campbell	1	16.9	10.3	0.0276
Prorocentrum minimum	1	23	13.8	0.0146
Aphanocapsa sp.	1	37.1	23.4	0.006

Table 13: Indicator species for different salinity categories, their indicator values and statistical significance (p). Salinity category: 1: 5-18 ppt (# of samples 38); 2: 19-25 ppt (# of samples 60; 3: 26-33 ppt (# of samples 103). IVmax: maximum indicator value.

Species	**Indicator Values**		p
	Salinity	**IVmax**	
Chaetoceros spp.	1	46.1	0.0028
Cyclotella choctawhatcheeana	1	42.7	0.0038
Hemiselmis spp.	1	39	0.0032
Rhodomonas sp.	1	22.6	0.0002
Calycomonas ovalis	1	52.2	0.0002
Pico-coccoids	1	45.9	0.0002
Aphanocapsa sp.	1	39	0.0002
Prorocentrum minimum	1	17.8	0.038
Pseudoscourfieldia marina	2	28.8	0.0082
Paralia sulcata	3	16	0.0032
Rhizosolenia imbricata	3	26.8	0.0012
Thalassiosira proschkinae	3	50	0.0002
Asterionellopsis glacialis	3	37.4	0.0006
Psuedo-nitzschia spp.	3	17.2	0.043
Leptocylindrus minimus	3	18.9	0.048

Table 14: gradient of salinity and nutrient in BB-LEH, averaged from Aug 2011 to Oct 2012.

site	Salinity (ppt)	TN (mg/l)	NO3+NO2 (mg/l)	TP (mg/l)	Orth-P (mg/l)
BB01	21.737	0.537	0.036	0.043	0.001
BB04a	14.206	0.654	0.095	0.038	0.005
BB05a	20.892	0.566	0.027	0.045	0.008
BB07a	28.051	0.408	0.013	0.044	0.006
BB09	27.072	0.408	0.010	0.049	0.014
BB10	26.448	0.433	0.014	0.058	0.018
BB12	28.737	0.357	0.012	0.063	0.025
BB14	30.059	0.273	0.018	0.050	0.021

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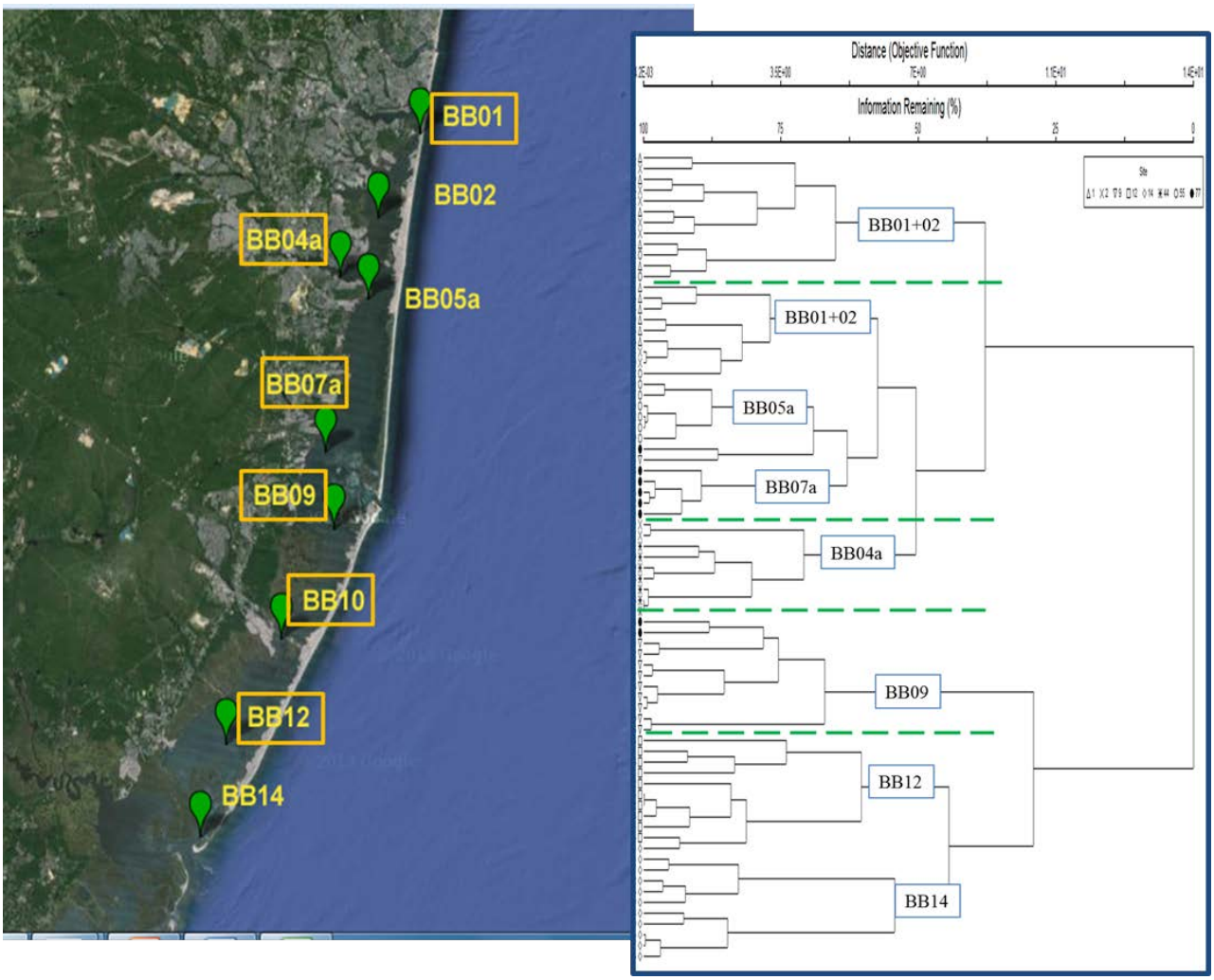


Fig. 1. Map of sites for phytoplankton sample collection 2011-12. Samples from six sites, as framed, were collected from 2012-2013. In addition, BB01, BB04a, BB07, BB09 and BB12 were analyzed from April 2014 to April 2015.

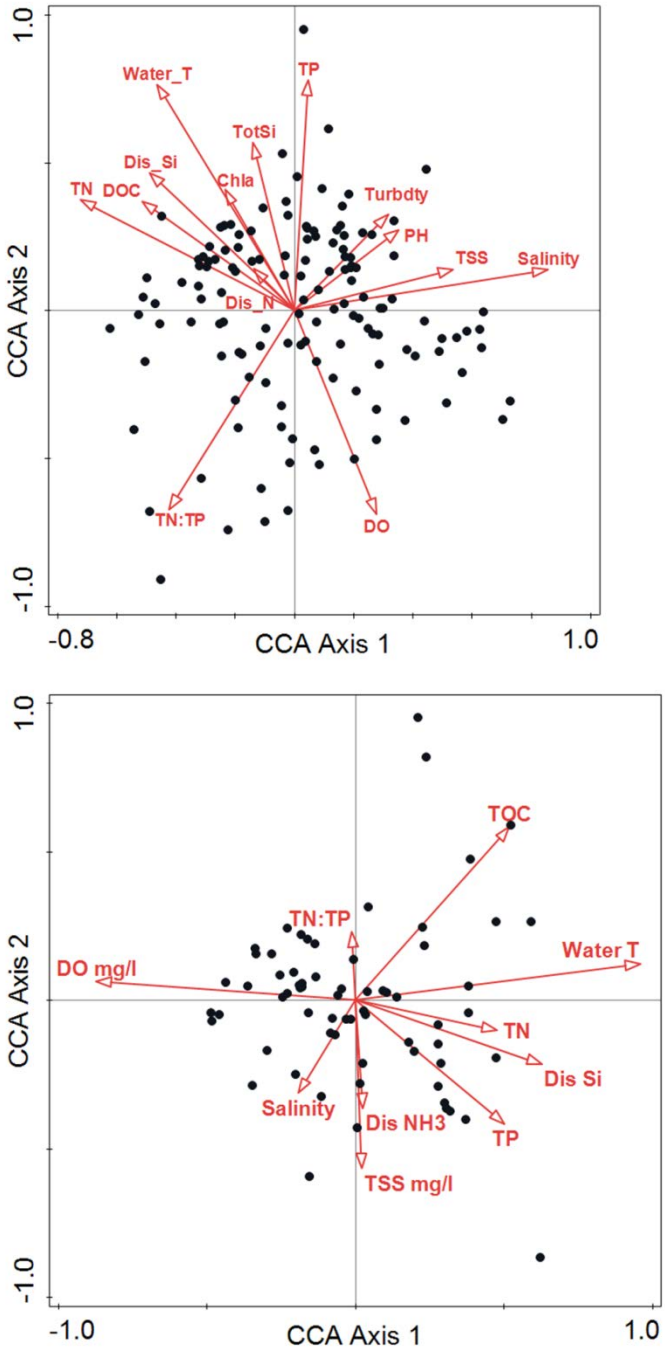
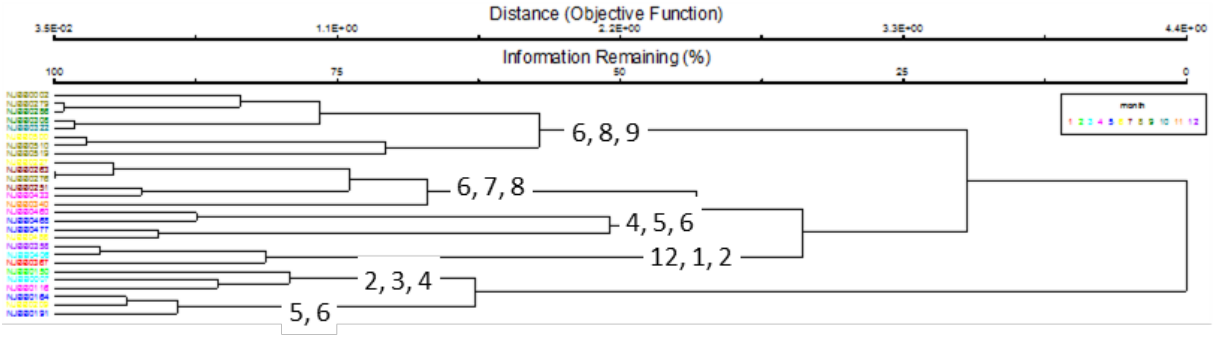
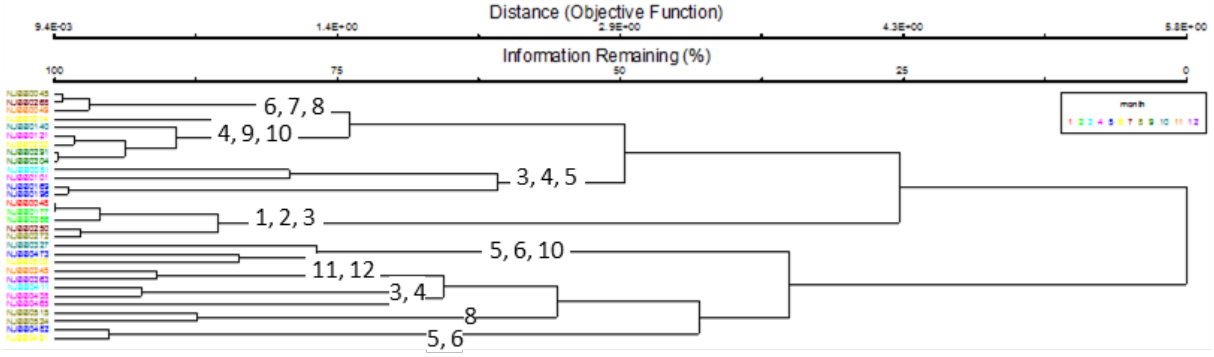


Fig. 2. Canonical correspondence analysis (CCA) on the relationship between phytoplankton community and environmental variables from Yr1 (upper panel) and Yr2 (lower panel).

A Site BB01



B. Site BB09



C. Site BB12

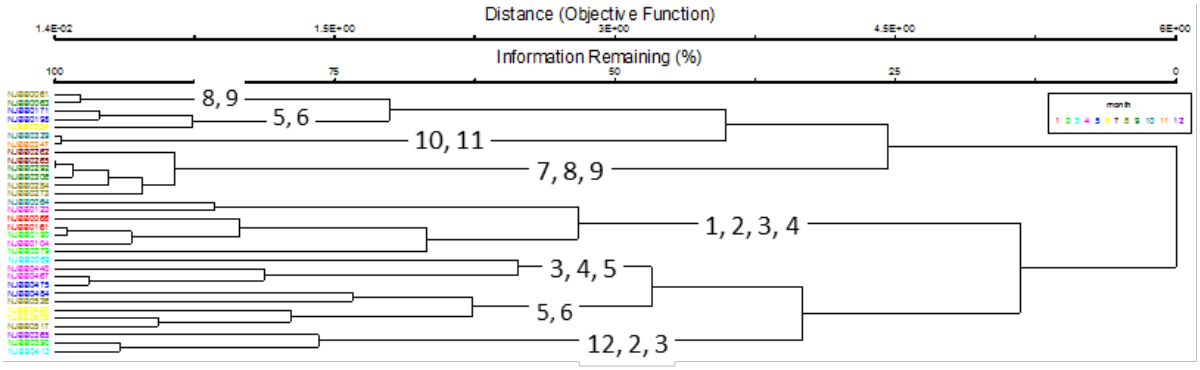


Fig. 3. Cluster analysis on phytoplankton community data from 2011-2013 at sites BB01, BB09 and BB12 (Label: months).

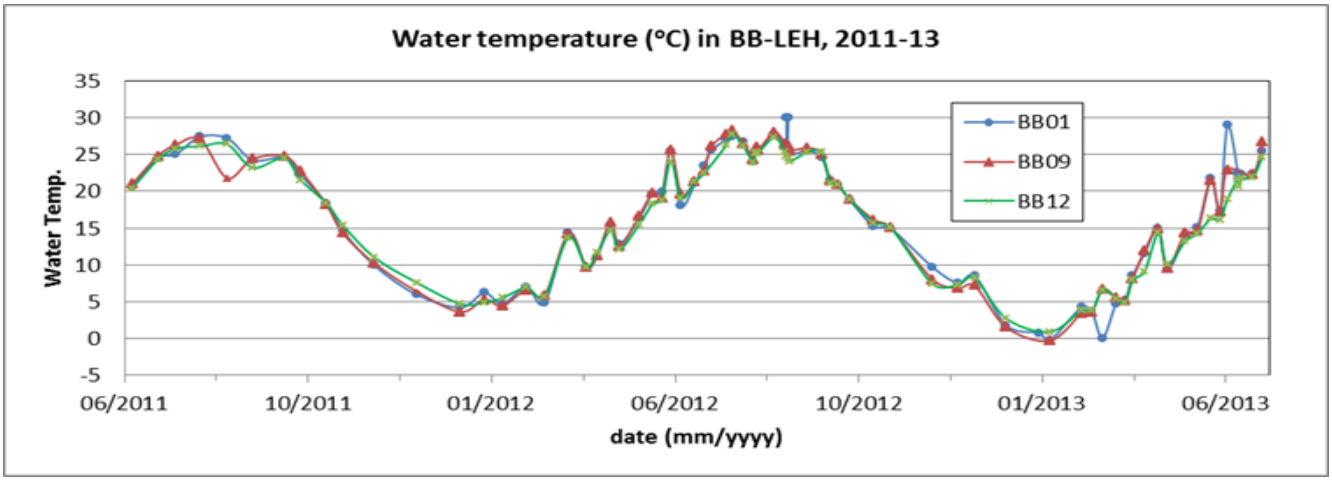


Fig. 4. The variability of water temperature (^{o}C) at sites BB01, BB09 and BB12 from 2011 to 2013.

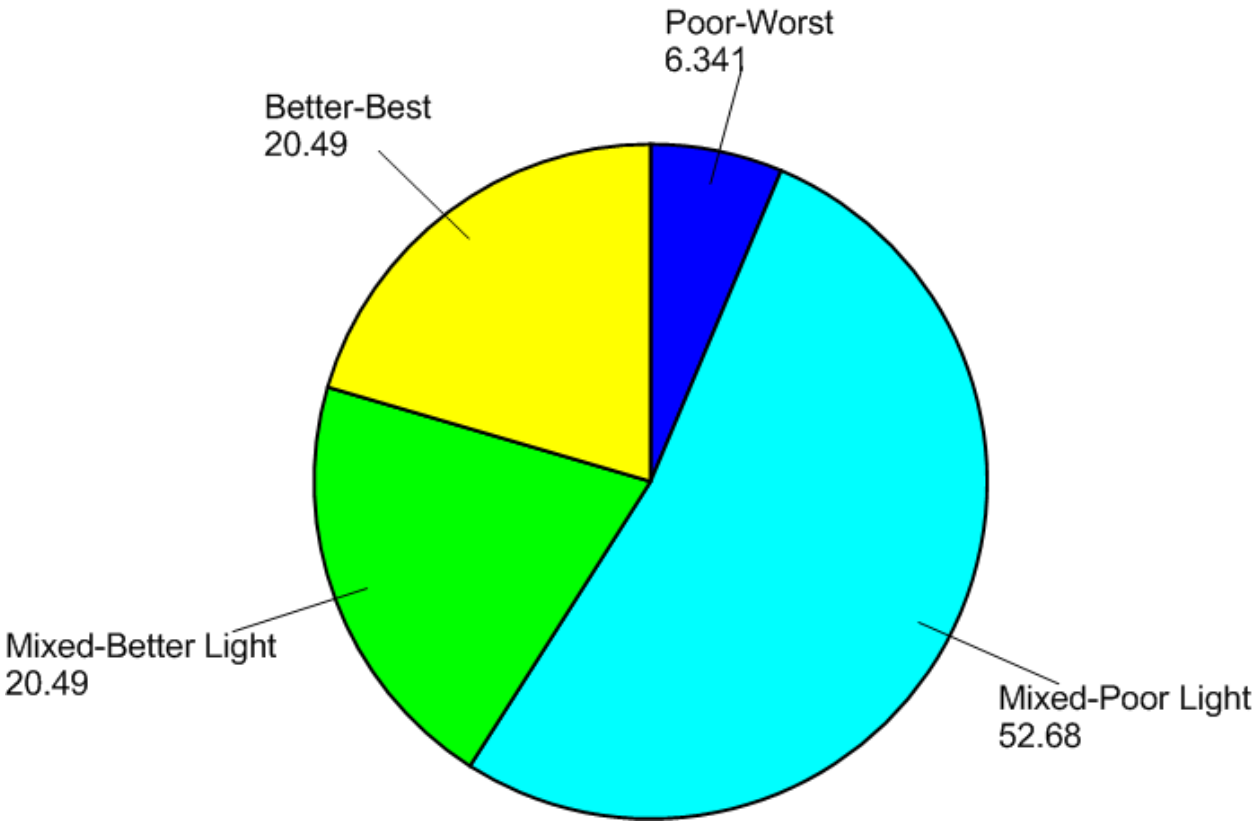


Fig. 5. Percentage of each major habitat category of all 205 samples collected from 2011-2013. PW: Poor-Worst (including Worst); MPL: Mixed-Poor Light; MBL: Mixed-Better Light; and BB: Better-Best (including Best). See Table 5 for the explanation on classification of the habitat categories, and Table 6 for the detailed data for each site and each category that the plot is based on.

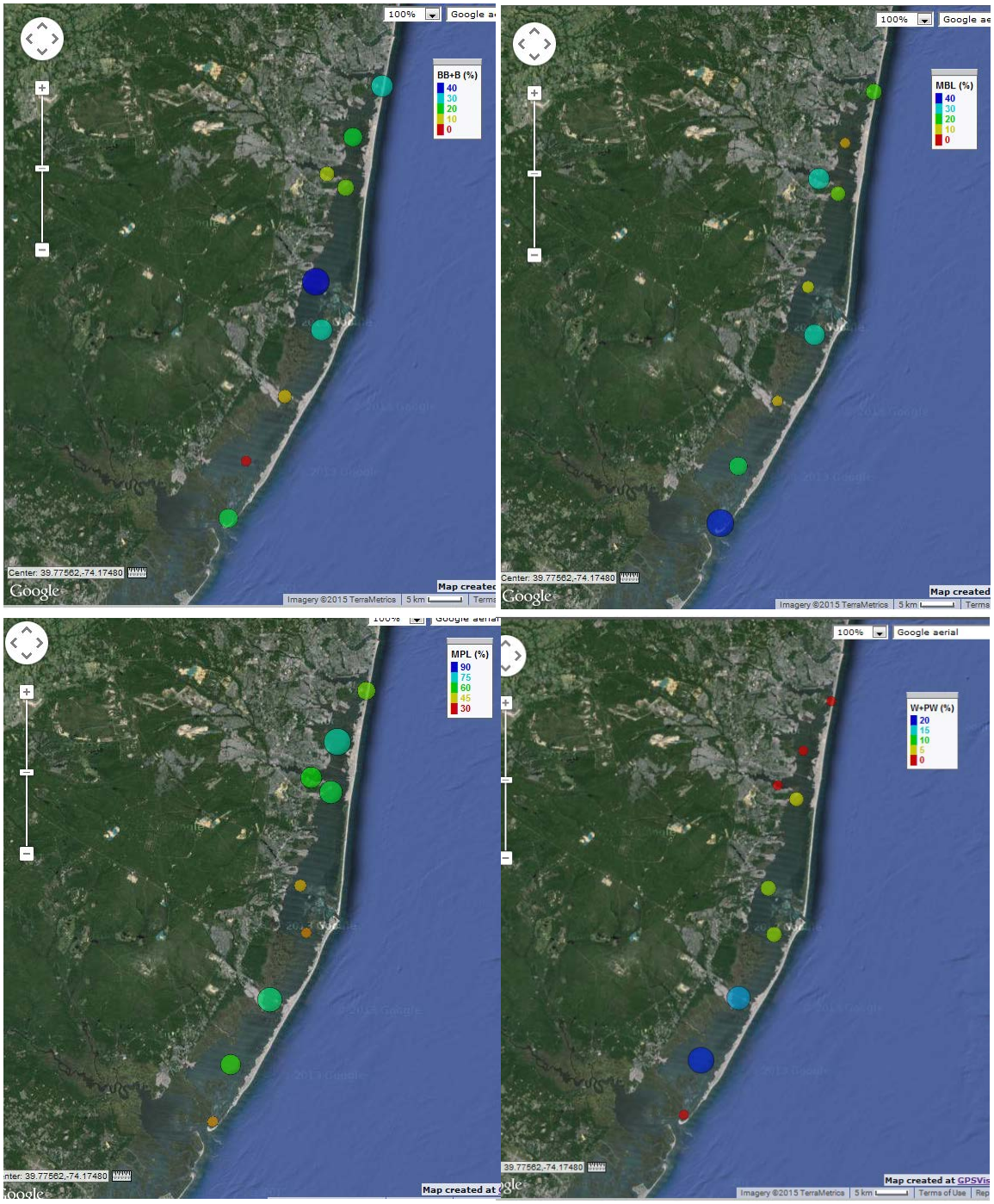


Fig. 6. Percentage of each of the four major habitat categories (BB+B, MBL, MPL and P+PW) at each phytoplankton site (from north to south: BB01, BB02, BB04a, BB05a, BB07a, BB09, BB10, BB12, and BB14), derived from 205 sampling events from 2011-2013. BB+B: better-best; MBL: mixed-better light; MPL: mixed-poor light; and P+PW: poor-worst. See table 5 for more detailed explanation on habitat categories, and Table 6 for the frequency of sample events for each category and each season for each site.

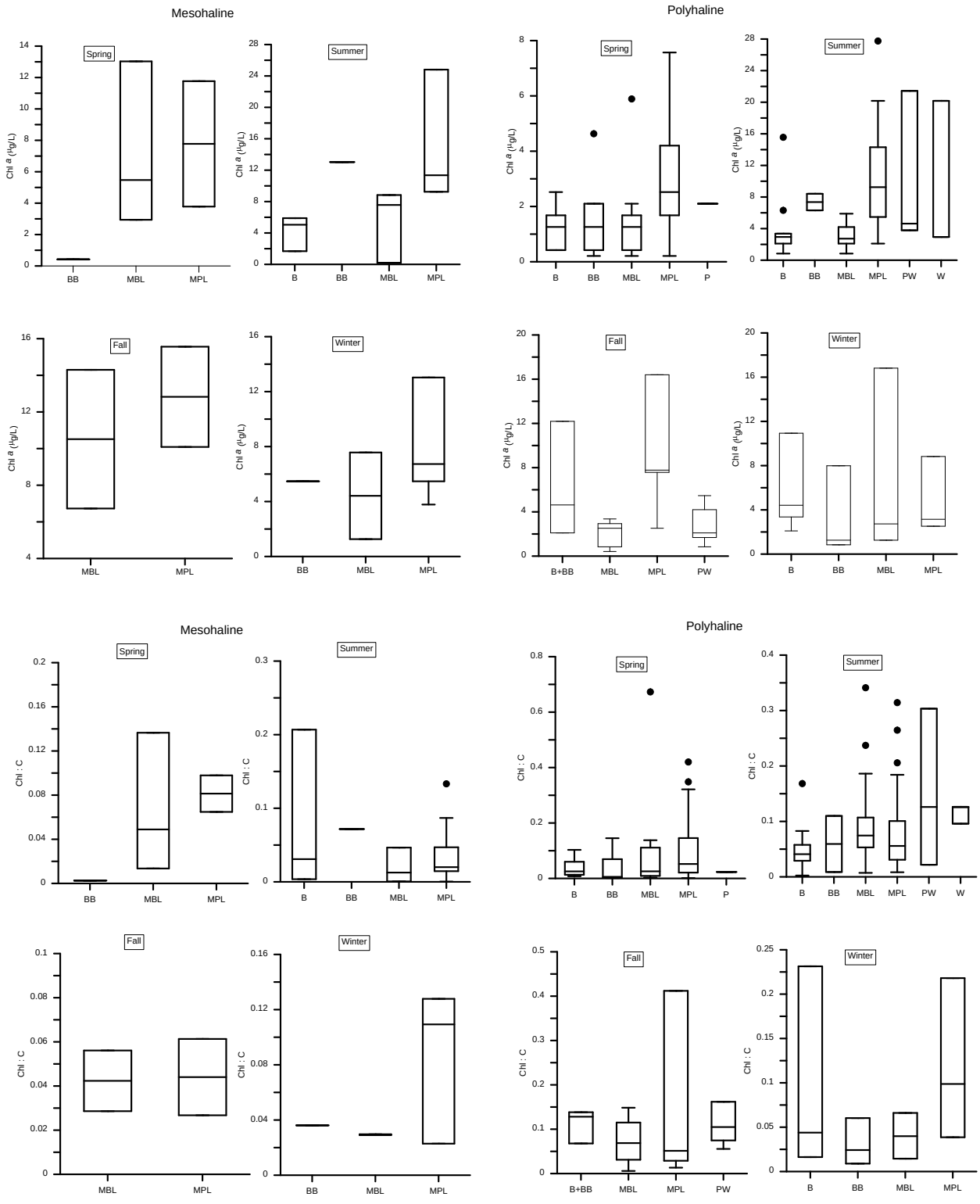


Fig. 7. Selected characteristic parameter from all season-salinity-habitat categories based on the data from 2011-2013: Chlorophyll *a* and Chla : C ratios.

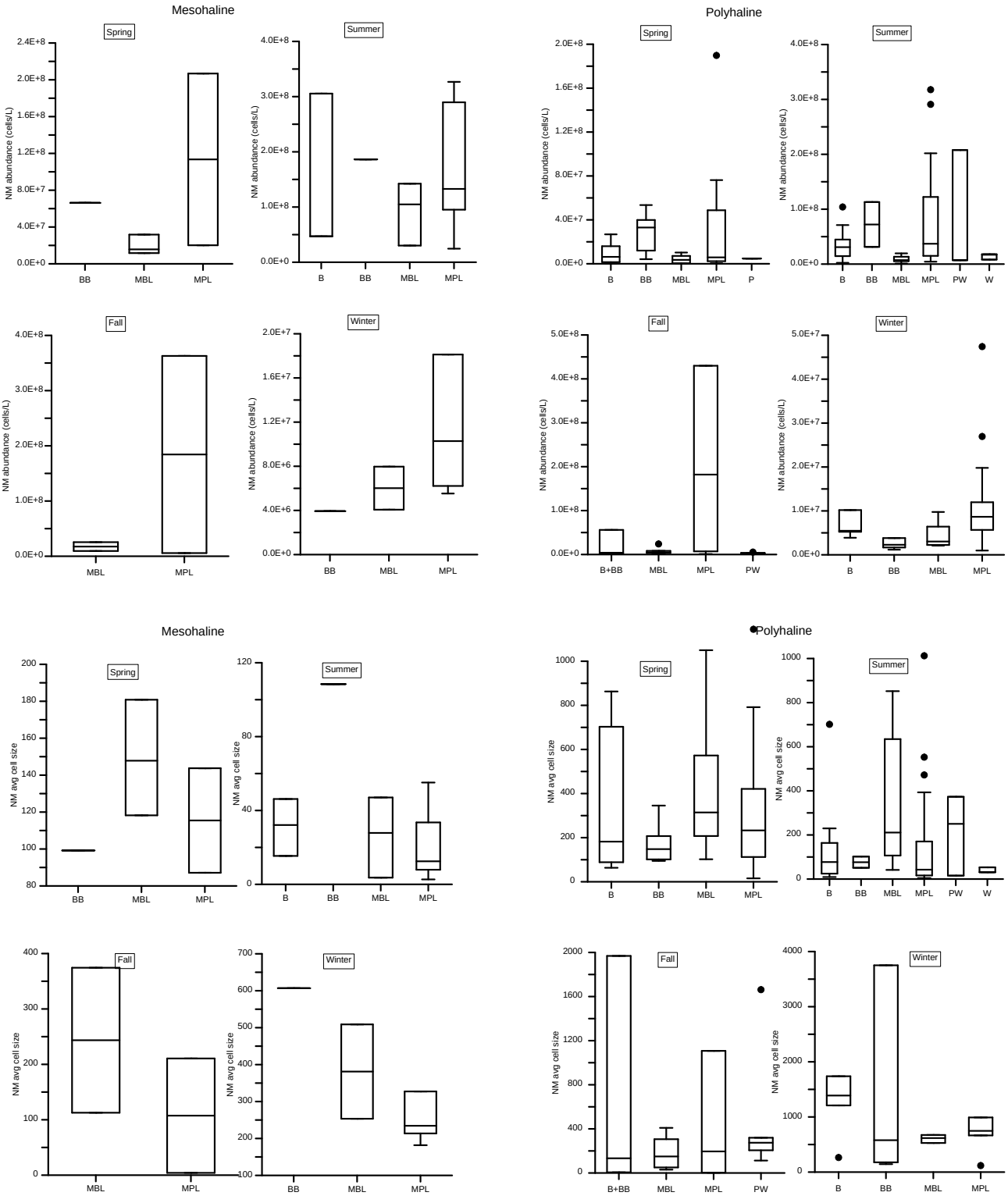


Fig. 7 (cont.): Selected characteristic parameters from all season-salinity-habitat categories based on the data from 2011-2013: Nano- and micro- (NM) phytoplankton and average cell size of NM phytoplankton

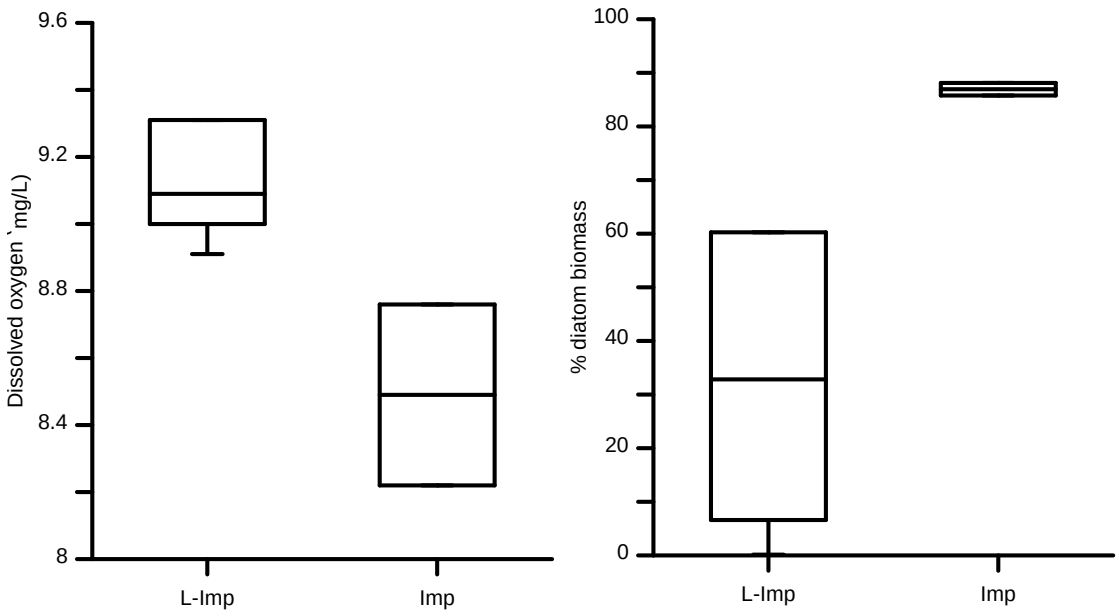


Fig. 8. Box-Whisker plots of the P-IBI metrics between the least-impaired L-Imp; and impaired Imp habitats for spring mesohaline

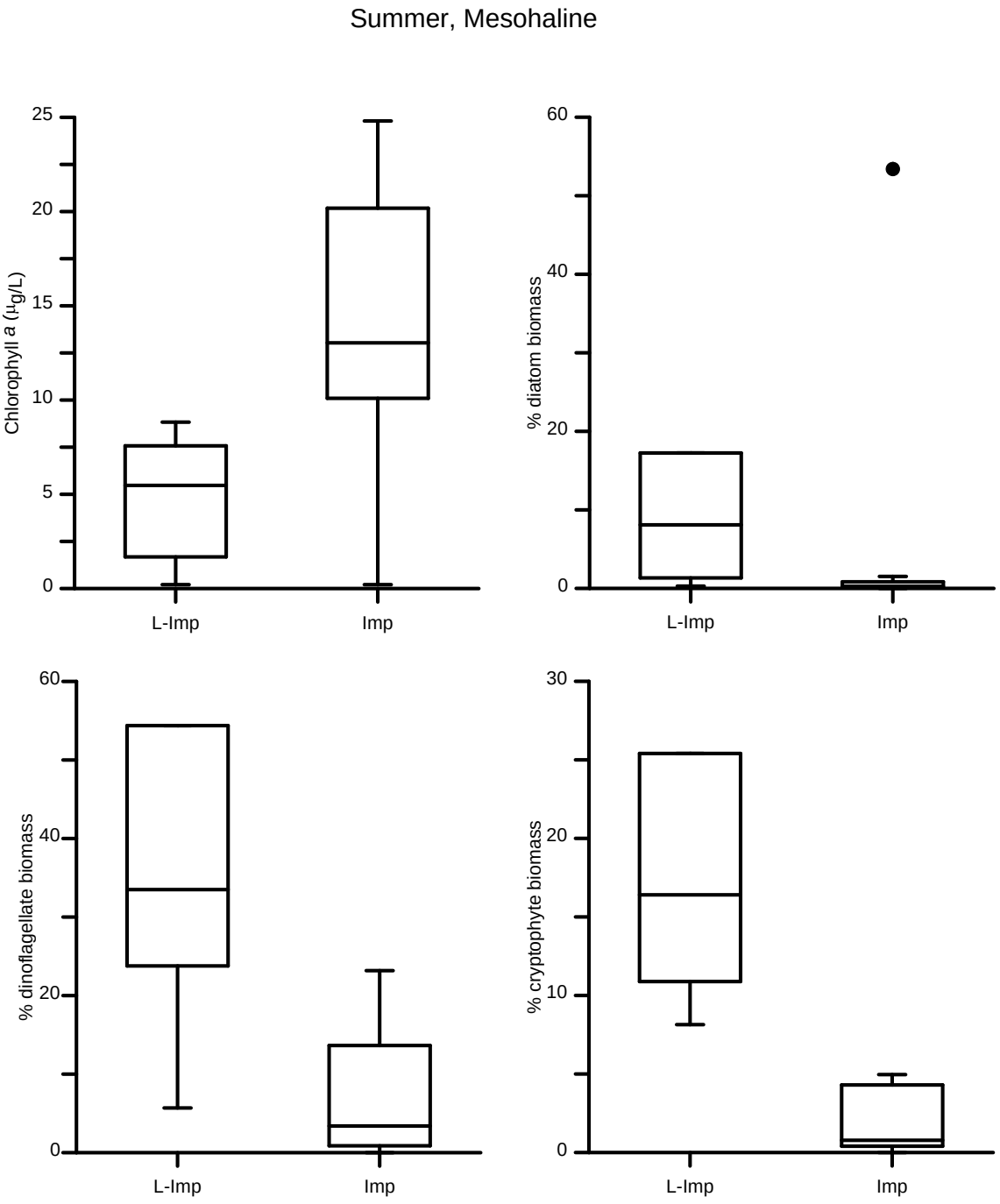


Fig.9. Box-Whisker plots of the P-IBI metrics between the least-impaired and impaired habitats for summer mesohaline.

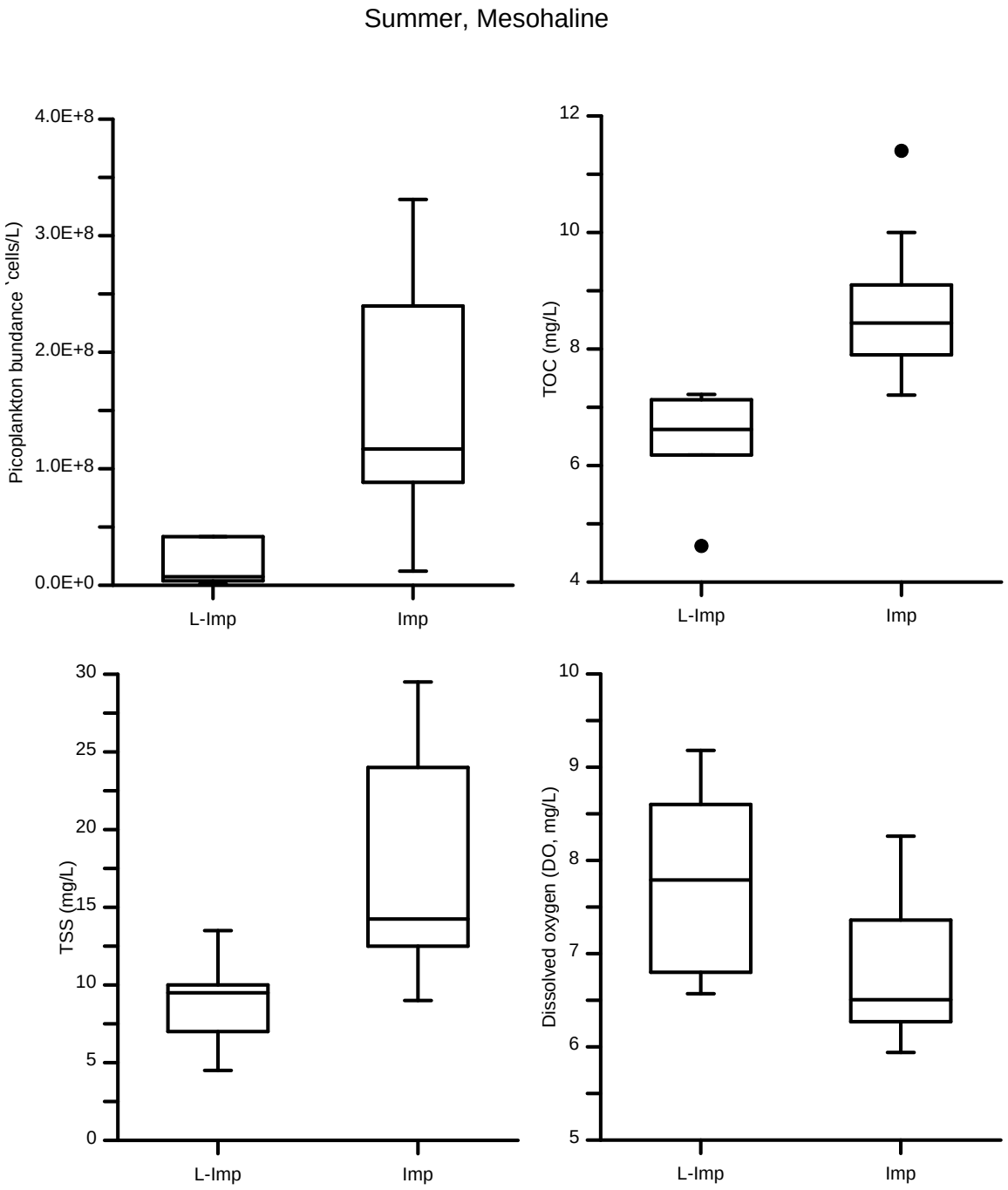


Fig.9 (cont.). Box-Whisker plots of the P-IBI metrics between the least-impaired and impaired habitats for summer mesohaline.

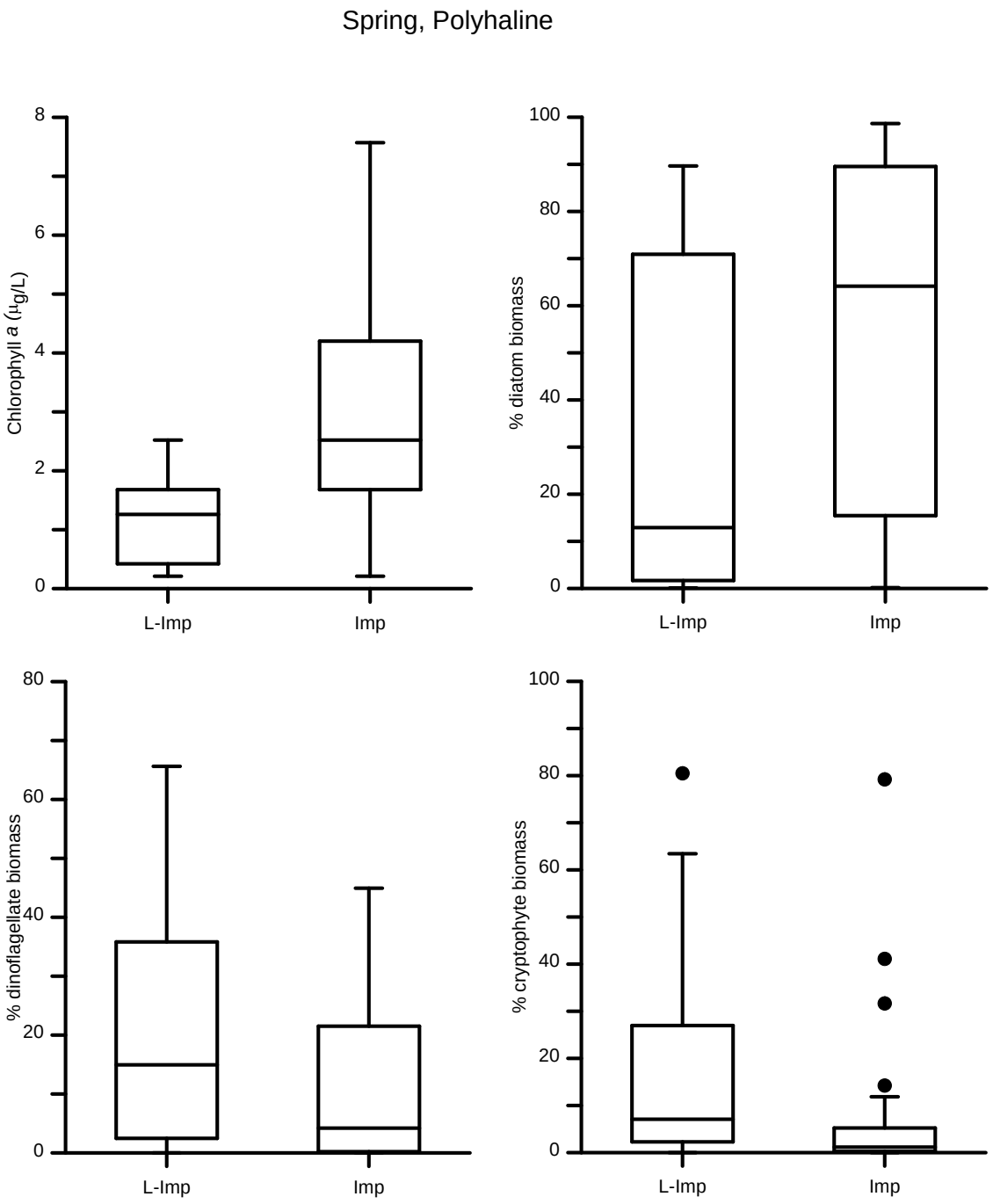


Fig. 10. Box-Whisker plots of the P-IBI metrics between the least-impaired and impaired habitats for spring polyhaline

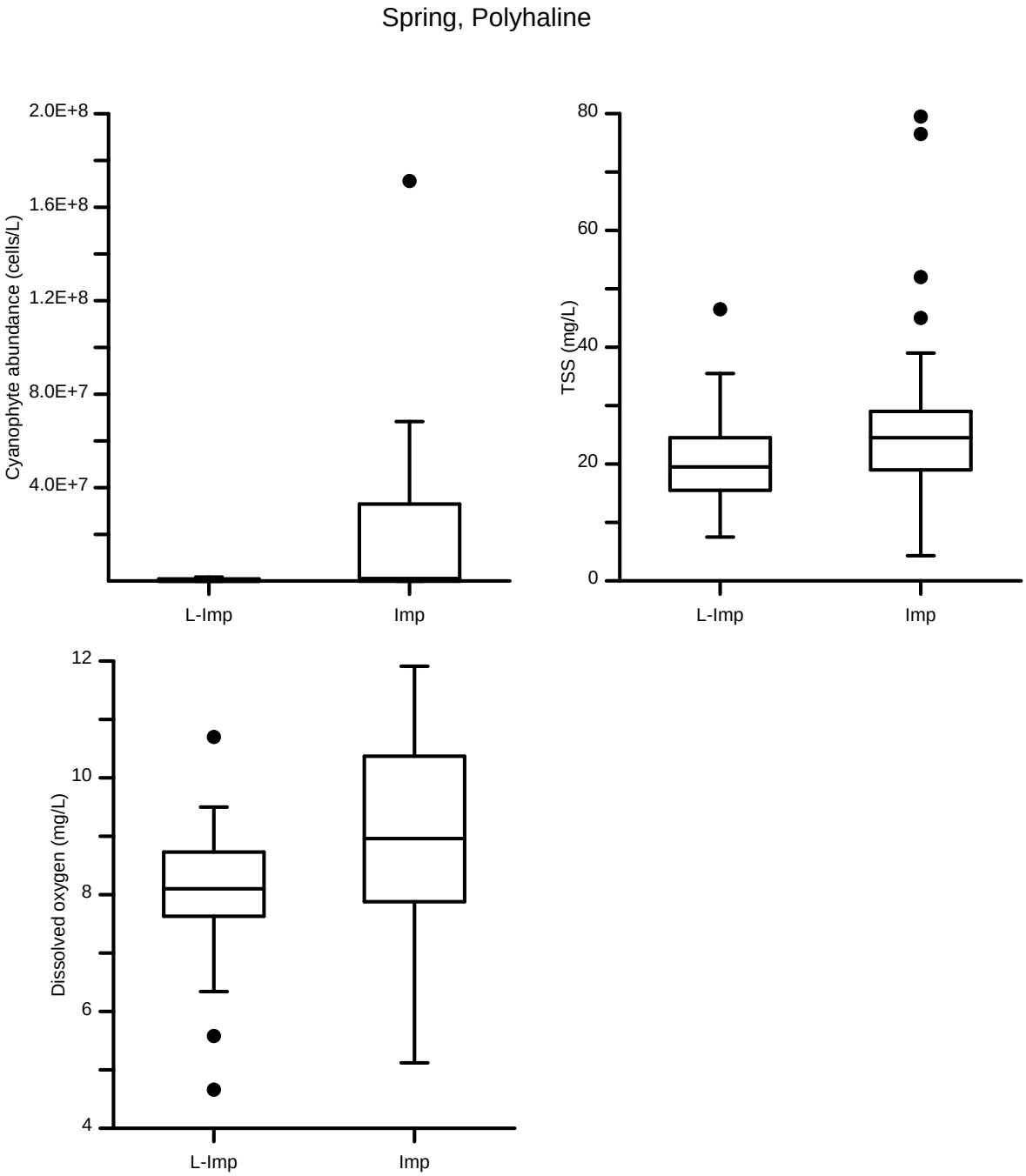


Fig. 10 (cont.). Box-Whisker plots of the P-IBI metrics between the least-impaired and impaired habitats for spring polyhaline

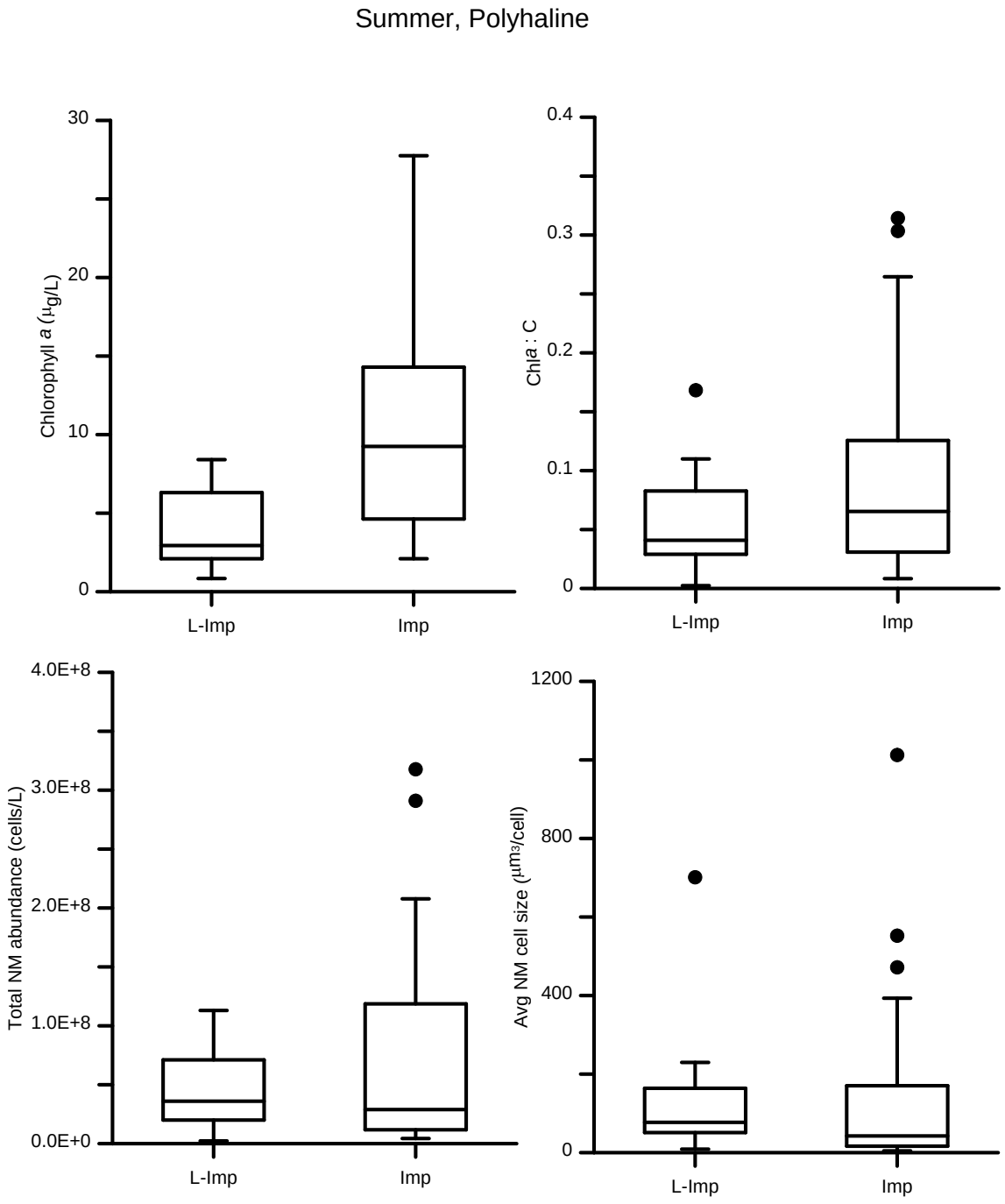


Fig. 11. Box-Whisker plots of the P-IBI metrics between the least-impaired and impaired habitats for summer polyhaline

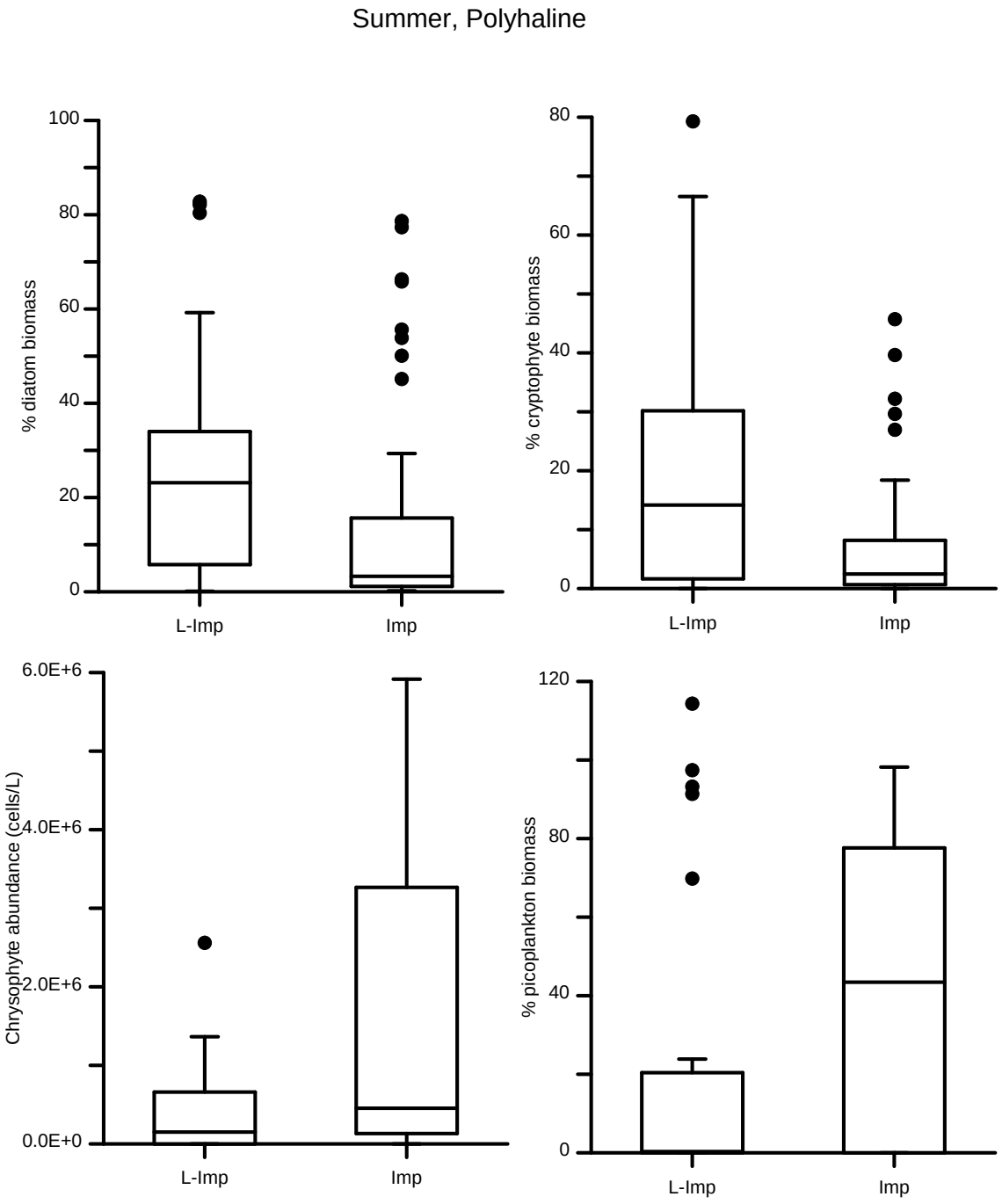


Fig. 11 (cont.). Box-Whisker plots of the P-IBI metrics between the least-impaired and impaired habitats for summer polyhaline

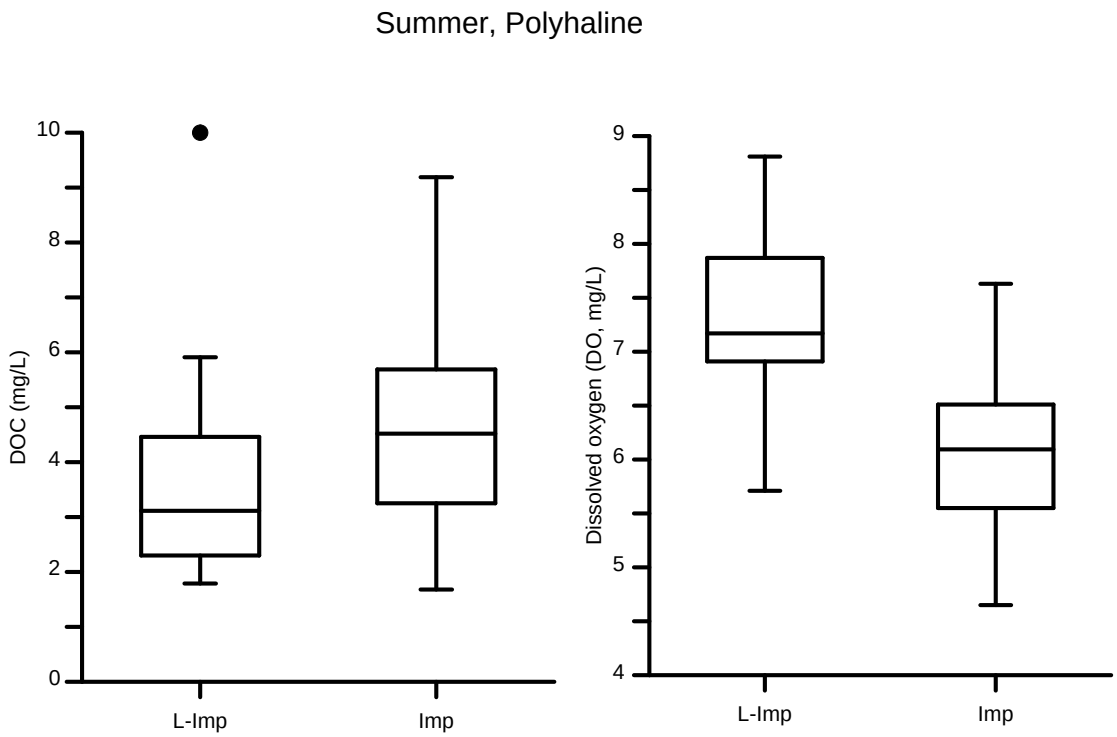


Fig. 11 (cont.). Box-Whisker plots of the P-IBI metrics between the least-impaired and impaired habitats for summer polyhaline

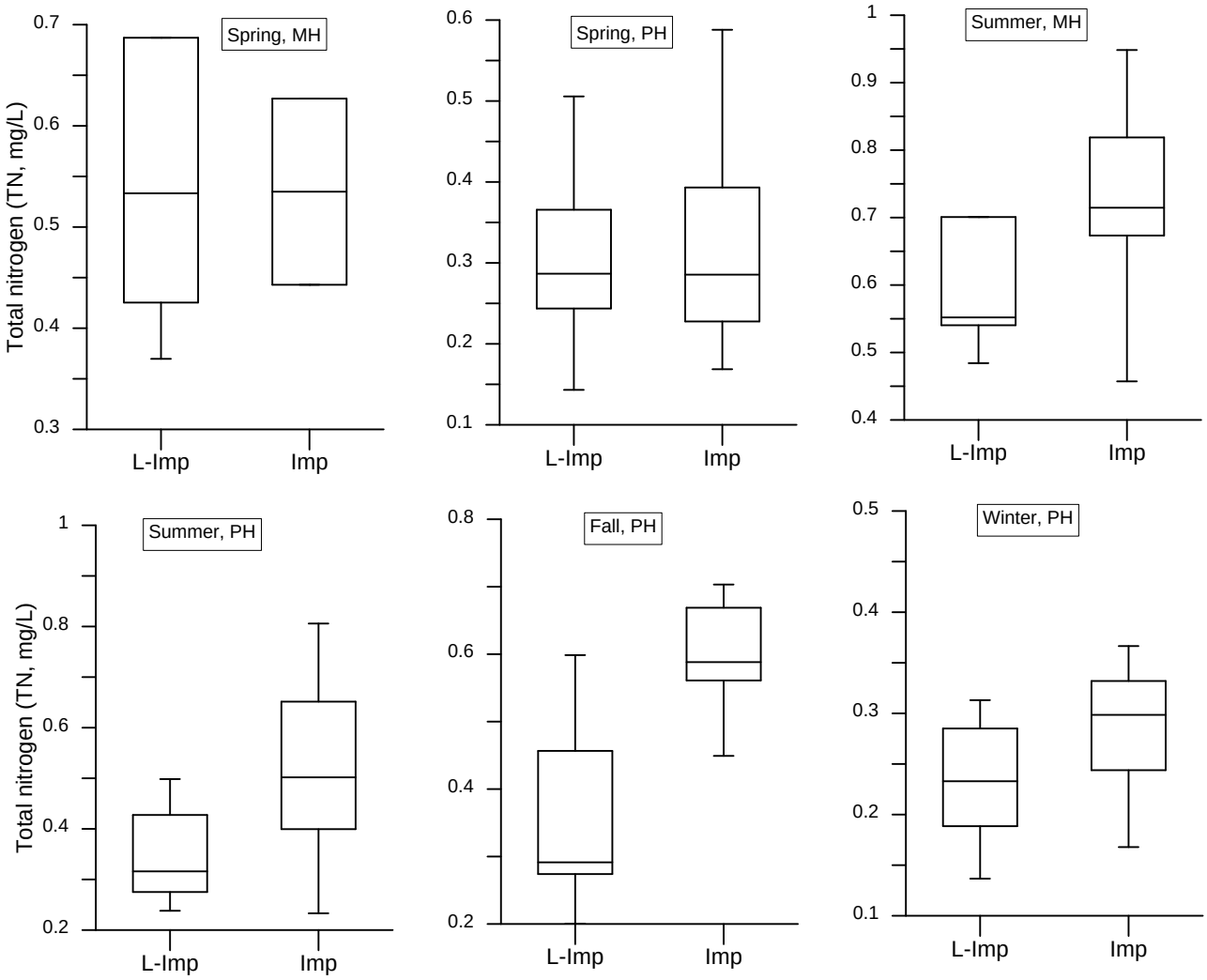


Fig. 12. Total nitrogen (TN) between the least-impaired and impaired habitats for different season-salinity categories

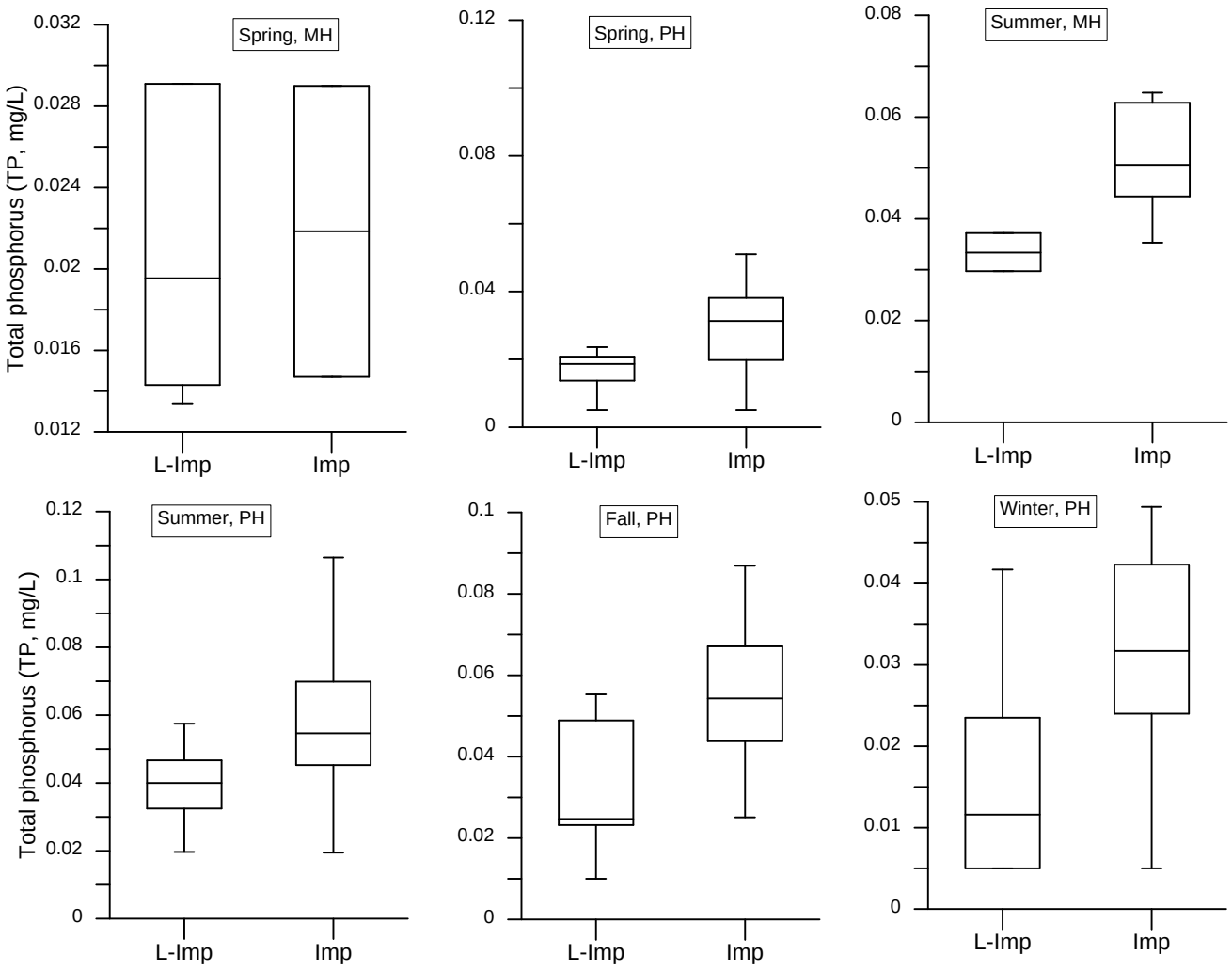


Fig. 13. Total nitrogen (TN) between the least-impaired and impaired habitats, derived from 2011-2013.