Final Report

Developing and Refining Phytoplankton Index of Biotic Integrity (P-IBI) for Barnegat Bay-Little Egg Harbor Estuary

Prepared for

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INTRODUCTION

Phytoplankton are known to respond directly to changes of physical and chemical conditions in aquatic ecosystems. They are also the base of food web and dynamically interact with the organisms at higher trophic levels in aquatic systems. 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 for water quality assessment. Phytoplankton-based water quality indicators have been developed and applied extensively for identifying eutrophication in coastal and estuarine ecosystems. The applied 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, multimetric biotic indices go one step further. The 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. Phytoplankton-based indices of biotic integrity (P-IBIs) have been developed, using long-term data, and applied for assessing eutrophication in several estuarine and coastal regions (Radach 1998, Jorden and Vaas 2000; Lacouture et al. 2006). P-IBIs 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 BB-LEH (Kennish et al. 2011, Kennish and Fertig 2012). However, phytoplankton indicator and biotic indices for water quality assessment were lacking for BB-LEH until the pilot study done by Ren et al. (2017). Phytoplankton data from the 2011-2013 investigation (Ren 2013, Ren 2015), together with water quality monitoring data, were utilized as the initial datasets for biological index development. The developed P-IBI correctly classified 57-81% of the samples in the calibration data set. And P-IBI scores showed good separation between the impaired and the least-impaired for most season-salinity zones (Ren et al. 2017).

However, large inter-annual variability in phytoplankton community between 2011 and 2013 has been observed due not only to its natural variability but also greatly to the disruption by the Hurricane Sandy. As a result, phytoplankton reference communities and P-IBI development based on two years 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, particularly for fall- and winter- mesohaline zones. Indicator Species Analyses showed several individual taxa can serve as metrics for P-IBI for different nutrient regimes and season-salinity categories. However, the current database does not provide sufficient data for most of the indicator species to effectively discriminate different habitat conditions (see details in Year 3 report, Ren and Belton 2015). In particular, two harmful dinoflagellates, *Prorocentrum minimum* and *Heterocapsa rotundata,* commonly present in BB-LEH, were previously found to be associated with different forms of nitrogen nutrients and water quality conditions (Rothenberger et al. 1999). But neither species showed strong discriminatory ability between the least-impaired and impaired categories due to limited data points.

For these reasons, we carried out an additional year-round investigation on phytoplankton community in Barnegat Bay-little Egg Harbor, in coordination with NJDEP's Water Quality Monitoring program. The additional data enabled us to augment the database for the calibration and validation of the P-IBI for each season-salinity zone. The objective of the study is to reduce the uncertainty and deviation of the current P-IBI and phytoplankton references communities. It is an essential step to refine and strengthen P-IBI and to achieve better representativeness of the reference communities for the BB-LEH estuary.

METHODS

Phytoplankton Sample Collection and Analysis

Water samples for phytoplankton community analysis were collected at 6 sites from May 2016 to April 2017. The collections were synchronized with the grab samplings of the NJDEP's Long-term Barnegat Bay Water Quality Monitoring. Among the 6 sites, five sites including BB01, BB04a, BB07a, BB09 and BB12 were investigated for phytoplankton community from August 2011 to December 2014. Site BB06 was added to this investigation to increase the number of

mesohaline samples for P-IBI calculation since its salinity ranges from mesohaline to polyhaline. The locations of the sites are shown in Figure 1, and the descriptions of the sites are listed in Table 1. 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 for phytoplankton analysis 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 processing and analysis as the previous years, and detailed description of the microscopic method can be found in previous publications (Ren 2013, Ren et al. 2017). In brief, phytoplankton samples were size-fractionated by filtering through 0.2 μm, 3 μm and 8 μm pore-size filters. Whole community counting was done on all three filters using an epifluorescence microscope (Leica DM L) with blue and green excitation lights and transmitted light. 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. Phytoplankton species were identified to the lowest taxonomic level possible. 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).

Validation of P-IBI

As the first step of the P-IBI refinement, the previous P-IBI was validated using 2014 data to determine the discriminatory efficiency of the P-IBI based on the 2011-2013 data. Each sample from 2014 was classified into four habitat conditions (W+PW, MPL, MBL and BB+B) based on the Secchi depth, DIN and ortho-P measurements. Meanwhile, the P-IBI score of each sample was calculated according to the criteria of each P-IBI metric (Table 10 in Ren et al. 2017) and actual measurement. The P-IBI was evaluated by the ability of each individual metric (as

discrimination efficiency, DE) and overall P-IBI metric scores (as classification efficiency, CE) to separate between the least-impaired and impaired conditions in the 2014 samples.

Refinement of P-IBI

The general steps of refining the P-IBI follows the same procedure of the P-IBI development, which were described in detail in our previous report (Ren and Belton 2016) and a recent paper (Ren et al. 2017). The general steps include data compilation and analysis, habitat classification, reference community quantification, metrics selection, metrics scoring criteria, metrics scoring and validation. The specific analysis and calculation involved in major steps are described as follows.

Data Compilation and Analysis

In addition to the 2011-2013 data, two more years of data were compiled and integrated to the general database for the refinement of the P-IBI. These additional datasets include phytoplankton community data and the contemporaneous water quality data from May 2014 to April 2015 and May 2016 to April 2017. The previous P-IBI was developed based on the dataset from 2011-2013 (see details in our previous report (Ren and Belton 2016) and Ren et al. 2017). For the present study, 55 samples from 2014 and 96 samples from May 2016 to April 2017 were added to the database. In total, 354 data were compiled into a general database for the refinement effort. The database is composed of phytoplankton community dataset and the associated water quality dataset. Both water quality and phytoplankton were collected at the same location and same time. The phytoplankton community dataset consists of species composition and cell density, biovolume and carbon biomass of each taxonomic group and of some dominant and indicator species, and the biomass percentage of major taxonomic groups. Water quality dataset includes Chlorophyll *a* (Chl*a*) and 23 other key physical, hydrological and chemical parameters, such as water temperature, salinity, turbidity, secchi depth, total suspended solids (TSS), nutrients (different forms of N and P, biogenic Si), total nitrogen and phosphorus (TN and TP), dissolved oxygen (DO), and dissolved and total organic carbon (DOC, TOC), etc. The analysis and measurements of phytoplankton community and water quality data were carried out using consistent methods throughout the multiple years of investigation. For water quality data, the reporting detection limits and analysis labs for some water quality parameters had been changed, but the analytical methods have been consistent. The detailed information about parameters and

methods of the water quality data from previous years can be found in the QAPP of NJDEP's Barnegat Bay Long Term Ambient Monitoring Program (Barnegat Bay LMP 2013).

Harmonization of phytoplankton data from 2014 and 2016 with the previous years of 2011-2013 has been done in regards of the consistency in species identification and enumeration, biovolume measurements and calculations. One of the main issues of water quality data is of those missing data and data below limit of detection (LOD). Similar strategies as described in Year-two report (Ren 2015) were applied to deal with the data below LOD and the missing data so that information can be kept: 1) the values below LOD were arbitrarily set to half the detection limit as recommended in several references (Hornung and Reed 1990, Lambert et al. 1991). For missing data, the values were set as the mean values either averaged from nearby sites from the same sampling date or from the same site but different sampling dates in the same season.

Season and Salinity Classification

The distinction of four seasons follows that in the previous development of P-IBI and is as following: winter: December-February; spring: March-May; summer: June-September; and fall: October-November.

Two types of salinity classification were tested in the refinement work, as following:

The same two-zone classification as in the previous P-IBI was first applied, following the well-accepted Venice System of 'the classification of marine waters according to salinity' (SCBW 1958), with salinity between 5 -18 ppt as mesohaline (MH) and that ≥ 18 ppt as polyhaline (PH). Each sample was designated into one of these two zones according to the salinity measurement. In general, low salinity was observed at BB04a, near the mouth of the Toms River, and higher salinity in the south of Barnegat Bay and Little Egg Harbor.

Salinity was re-classified into three zones in the current P-IBI as an alternative, which includes 5-18 ppt as mesohaline, ≥18 to <25 ppt as lower polyhaline (LPH), and ≥ 25 ppt as upper (higher) polyhaline (UPH). The two-zone classification had grouped the northern Barnegat Bay together with Little Egg Harbor as one salinity zone. However, as studies show the hydrological and chemical conditions in northern Barnegat Bay differ largely from the southern Barnegat Bay and the Little Egg Harbor (Ren et al. 2015). The three-zone classification was able to separate the northern part of Barnegat Bay from the south and the Little Egg Harbor. With three-zone

classification, the northern Barnegat Bay was mostly classified as LPH zone with salinity between 18 ~ 25 ppt and the southern Bay and Little Egg Harbor as UPH with salinity higher than 25 ppt. Site distribution and the number of samples of each salinity zone are shown in Table 2. The mesohaline zone is usually located near the mouth of Toms River and the River plume (BB04a). However, it is noted that, different from previous years, salinity of most 2016 samples collected from BB04a appeared higher than 18 ppt, thus were categorized into the LPH zone, which may be as a result of less discharge from Toms River compared to previous years.

Habitat Classification

Two major tests were carried out in this step in order to determine the better scenario for salinity classification and light condition classification.

At first, habitat conditions were classified with respect to two-zone and three-zone salinity classifications. For two-zone salinity classification, the 2014 data was integrated into the 2011-2013 dataset to make a 2011-2014 dataset. For three-zone salinity classification, 2016 data were integrated into the 2011-2014 dataset. Secchi depth, as a measure for light condition, was used together with DIN and ortho-P to calculate the criteria of four habitat classes (worst, poor, better, and best) for each season-salinity zone, using Relative Status Method and nutrient limitation thresholds. The results of Relative Status Method and the classification criteria for DIN, ortho-P and light (as secchi depth) are shown in Tables 3 to 5 for two-zone classification based on the 2011-14 data, and Tables 6 to 8 for three-zone classification based on the datasets from 2011-2014 and 2016.

Secondly, as a further attempt to refine the P-IBI, turbidity, not Secchi depth, was used as a measure of light condition and its criteria were calculated for two-zone classification, using Relative Status Method and nutrient limitation thresholds. The criteria of turbidity for habitat classification are shown in Table 9.

Each sample was then independently classified as Worst, Poor, Better or Best based on its Secchi depth or turbidity, DIN and ortho-P measurements and respective habitat 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 PO_4. Six categories of habitat condition were created from these ten categories, including B (Best), BB (Better-Best), MBL (Mixed-Better Light), MPL (Mixed-Poor Light), PW (Poor-Worst) and W

(Worst) (Ren et al. 2017). The categories B and BB, and in some cases MBL were considered to represent the least-impaired habitat condition with desirable light conditions and nutrient concentrations below phytoplankton uptake limiting thresholds. The categories W and PW are considered to represent impaired water quality with light-impoverished condition and excessive DIN and PO_4 concentrations in the water column.

Discriminatory Ability of Metrics

As an essential step for metrics selection, forty-three phytoplankton, physical and chemical metrics were evaluated for their abilities to discriminate between the least-impaired and impaired conditions. The Kruskal-Wallis test was done on each metric to test the significance of their discriminatory ability. The discriminatory ability of each metric was evaluated three times in order to compare its performance under different salinity zone classification and Secchi depth vs turbidity for light condition criteria.

RESULTS AND DISCUSSION

Validation of Previous P-IBI

The discrimination efficiency of each individual metric and classification efficiency of the P-IBI for spring and summer mesohaline (MH) and polyhaline (PH) samples are shown in Table 10 and Table 11. Of all the tested metrics, diatom percentage (%_DT), dinoflagellate percentage (%_Dino) and cryptophyte percentage (%_Crypt) showed reasonable discriminatory ability in the least-impaired in spring and summer mesohaline (MH) and summer polyhaline (PH). Picoplankton biomass percentage (%_Pico) is an important metric for the least-impaired condition in summer (Table 10). Overall, the P-IBI did not perform as well as that for 2011-2013 data, though better classification efficiency was achieved for the summer compared to spring season (Table 11), especially in the least-impaired conditions. The uncertainty in the P-IBI performance was somewhat predicted due to the large inter-annual variation in phytoplankton community related to hurricane Sandy disruption. Moreover, it is noted that, of all 2014 samples, the number of samples for most of habitat conditions was low (2 to 4 samples), which greatly limited the ability of P-IBI validation. The numbers of samples of summer MH (10) and PH (18) were higher than other categories, and the P-IBI was able to discriminate 60% and 94% of the

samples in 2014 (Table 11). Actual separation of the P-IBI score for the least-impaired and impaired communities in spring and summer is shown in Fig. 2.

Comparison of Habitat Classification Criteria

Secchi depth vs. Turbidity

Light condition in water column is essential for phytoplankton growth; therefore water clarity is one of the primary parameters, in addition to DIN and ortho-P, to classify habitat conditions. Secchi depth has been used as the measure of light condition and to establish light criteria for habitat classification in previous studies (Buchanan et al. 2005, Johnson and Buchanan 2014, Ren et al. 2017). Secchi depth in the Barnegat Bay monitoring is reported in feet (ft), and most data are whole numbers in ft. On the other hand, turbidity, a measure of water clarity, was determined using Nephelometry. The criteria of Secchi depth and turbidity based on the database composed of 2011-2014 samples are shown in Table 3 and Table 9. In addition, the criteria of DIN and ortho-P are shown in Table 4 and 5. Each sample was classified according to the criteria of Secchi depth, DIN and ortho-P, and of turbidity, DIN and ortho-P. After the classification, each sample was then put into four habitat categories, W+PW, MPL, MBL and BB+B from impaired to least-impaired, based on the combination of light, DIN and ortho-P classes.

Forty physical-chemical and phytoplankton metrics were tested for their discriminatory ability between the least-impaired and impaired conditions. Secchi depth-based and turbidity-based results of discriminatory abilities for spring and summer are shown in Table 12. The comparison showed that the turbidity-based classification resulted in more metrics with the ability of discriminating between the least-impaired and impaired, particularly for mesohaline zone in spring and summer. In general, metrics including Chl*a*, % diatoms, % cryptophytes, TSS, TP and ratio of diatom/non-diatom biomass exhibited significant discriminatory ability in most of spring and summer MH and PH. In addition, % picoplankton and average cell size were strong metrics in summer (Table 12).

Salinity: Two-zone vs. Three-zone

The criteria of Secchi depth, DIN and ortho-P for three salinity zones (MH, LPH and UPH) are shown in Tables 6 to 8.

Table 13 shows the discriminatory ability of forty-three physical-chemical and phytoplankton metrics between the least-impaired and impaired conditions. Compared to two-zone classification, three-zone classification resulted in more metrics showing significant discriminatory ability in spring MH. However, less metrics showed significant discrimination in other season-salinity zones, particularly for spring and summer LPH and UPH, in comparison to those in the two-zone classification. This is possibly due to the reduced number of samples for each season-salinity (PH) category in the three-zone classification compared to the two-zone classification. And as a result, the significance of discrimination was reduced due to a smaller data pool.

Refining and Developing P-IBI

Phytoplankton Habitat Conditions

Phytoplankton habitat conditions in 2014 and 2016 were evaluated using the same Secchi depth, DIN and ortho-P criteria derived from 2011-2013 data and with two-zone salinity classification (Fig. 3). There was increased percentage (9%) of the category W+PW (impaired) and decreased percentage (5%) in the category of BB+B (least impaired), in comparison to the same habitat conditions in 2011-2013 which were 6% and 20%, respectively (Ren et al., 2017). However, samples in the category of MBL (mixed better-light) were higher than those in the MPL (mixed poor-light) category, accounting for 47% and 38%, respectively. Overall, there is an increase of better habitat condition (including B+BB+MBL) in 2014 and 2016 samples, accounting for 56%, compared to that in 2011-2013 of 40%. Since the classification criteria were calculated based on the 2011-2013 data, there is uncertainty when those criteria were applied to 2014 and 2016 data because of the large inter-annual changes in water quality and biological conditions in the first two years and limited number of data points for each season-salinity category.

As necessary steps for P-IBI refinement, 2014 data were integrated to the 2011-2013 dataset, and the Secchi depth, DIN and ortho-P criteria for two-zone salinity classification were re-established with the augmented data. Furthermore, 2016 data were added to the 2011-2014 database and the BB-LEH area was classified to three salinity zones. The criteria of Secchi depth, DIN and ortho-P were therefore re-calculated again for three-zone classification. Based on these two different sets of criteria, samples from 2011-2014 and 2016 altogether were classified and categorized again for its habitat conditions. Figure 4 shows the percentage of phytoplankton

habitat categories at each individual site and of all sites in BB-LEH under two-zone salinity classification. Figure 5 shows the habitat conditions of each site and of all sites resulted from the three-zone salinity classification. For most sites and over all samples, percentages of different phytoplankton habitat categories were comparable under these two salinity classifications. The category MPL accounted for nearly half of the samples collected from 2011-2016. More percentage of P and PW samples were found at BB10 and BB12 in comparison to other areas of the Bay.

Phytoplankton Reference Communities

Reference communities were re-calculated based on the data from the least-impaired (reference) habitat conditions for 6 season-salinity category, Spring MH, LPH and UPH, and Summer MH, LPH and UPH. The same representative metrics of phytoplankton communities were selected, chlorophyll *a* (Chl*a*), Chl*a*/C ratio, nano- and micro-phytoplankton (NM) abundance, NM biomass, average NM cell size, and summer picoplankton biomass. In addition, parameters which are related to phytoplankton growth and biomass were included, dissolved oxygen (DO), total suspended solids (TSS), total nitrogen (TN) and total phosphorus (TP). The maximum, minimum and median values of each metric as well as the significance of difference between the reference communities and the degraded ones are listed in Tables 14 to 16.

For most season-salinity zones, the number of samples used for the calculation of reference communities increased compared to the previous P-IBI (Tables 7 and 8 in Ren et al. 2017) despite the division of polyhaline. For Spring-LPH, Spring-UPH and Summer UPH, the calculation was able to focus on the samples from the Best and Better-Best categories. Statistical significance of differences, tested by one-way ANOVA, showed that the reference values of Chl*a*, TSS, TN and TP were significantly lower compared to the values in impaired communities for most season-salinity zones, same as the previous P-IBI. On the other hand, the concentration of DO and average NM cell size were statistically higher in reference communities than those in the impaired ones for most of the season-salinity zones. Compared to spring, more metrics in summer showed statistically significant differences between the reference and impaired conditions in all three salinity zone. Note that samples were collected twice a month in summer months from June-September while fewer samples were collected in spring, once in April and twice in May. As a result, the number of summer samples is much more than that of Spring

samples in current datasets. In comparison to the previous P-IBI, more metrics showed significant differences between the reference and impaired communities for Spring-MH (Table 14), owing possibly to the supplemented samples from 2014 and 2016.

The median values of most metrics were comparable to the previous P-IBI, particularly for mesohaline zone (MH) (Table 14 vs. Table 7 in Ren et al. 2017). However, the median values for most metrics were more specific for the LPH and UPH zones compared to the previous P-IBI. The PH in the previous P-IBI covers most of BB-LEH, while in the current P-IBI, LPH zone specific to northern BB including sites BB01, BB05a and BB06, and UPH zone is specific to southern BB and Little Egg Harbor (BB07a, BB09, BB10, BB12 and BB14 (Table 1, Figure 1). The current P-IBI showed significant difference in some key metrics of the reference communities between these two salinity zones. The concentrations of Chl*a* and TN in LPH reference communities were higher than those in the UPH reference communities (Table 15 and 16). The results are consistent with the previous and on-going monitoring data which shows the gradient from north to south in phytoplankton biomass and nutrients particularly nitrogen concentration (Olsen et al. 2001, Ren 2015). In addition, phytoplankton investigations since 2011 showed that picoplankton was more abundant and dominant in summer in the northern BB than in southern BB and Little Egg Harbor, where the phytoplankton community was dominated with more marine species. We conclude that the three-salinity-zone classification is more suitable for the BB-LEH than the previous two-salinity-zone classification.

P-IBI Metrics and Scoring Criteria

Table 13 listed 43 metrics tested for their ability in discriminating between the least-impact (L-Imp) and impact (Imp) conditions in three salinity zones. The metrics showing strong significant discriminatory ability were selected to form P-IBI metrics for each season-salinity zone. Several metrics showed good discriminatory ability in more than one season-salinity zones. These metrics included Chl*a*, Chl/C ratio, total abundance of nano- and micro- (NM) phytoplankton, and average NM phytoplankton cell size in summer, prasinophyte abundance, % diatoms, % cryptophyte biomass, summer % picoplankton and biomass, and % cyanobacteria biomass. In addition, some physiological and chemical parameters, including DO, total nitrogen and total phosphorus, and total suspend solids (TSS) were selected as well for their strong discriminatory

ability. Phytoplankton IBI metrics for spring and summer mesohaline and polyhaline zones, together with scoring criteria of each metric, are summarized in Tables 17 to 19.

Compared to the previous P-IBI, more metrics merged to be indicative in Spring-Mesohaline zone (Table 17). In addition to dissolved oxygen and % diatom biomass, turbidity, carbon biomass and Chl*a*: C ratio were included in the current P-IBI. Dinoflagellates detected from spring-MH were mainly *Katodinium rudantum* and *Prorocentrum minimus*, and the biomass percentage of dinoflagellates (%_Dino) was tested to be discriminative, and therefore was included in the P-IBI. P-IBI metrics for Summer-Mesohaline were comparable to the previous P-IBI, showing that diatoms, picoplankton and nano-microplankton (NM phytoplankton) are the major components to discriminate habitat conditions.

The Polyhaline zone (PH) in the previous P-IBI was divided into two categories, lower polyhaline (LPH) and upper polyhaline (UPH) zones in the current P-IBI. Despite the re-classification, phytoplankton metrics such as % diatom biomass, NM phytoplankton, chrysophyte abundance, summer picoplankton biomass and cyanobacteria still appeared to be strong indicators in the current P-IBI metrics. Cryptophytes were indicative in the previous P-IBI, however they were not in the current one. Instead, Prasinophytes became discriminative in both spring and summer (Tables 17 and 18).

The median values, interquartile ranges, and 25th and 75th percentile of Chl*a*, TN and TP in the reference (least-impaired) and impaired conditions for the six season-salinity zones are shown in Figures 6 to 8. The concentrations of TN and TP in the reference condition were significantly lower than those in the impaired, except for spring-mesohaline when there were few data available for statistical comparison (Fig. 6). The results showed significant differences of several phytoplankton metrics between the reference and impaired conditions (Figures 6 to 8).

P-IBI Scores and Classification Efficiencies

Actual separation of the P-IBI scores for the least-impaired and impaired communities is shown in Figure 9. Except for the Spring-LPH, the 5th percentile of P-IBI scores in the least-impaired were higher than the 95th percentile of P-IBI scores in the impaired distributions. The relatively weaker discriminatory ability of the refined P-IBI for Spring-LPH can be attributed to the following two possible reasons: 1) significant alteration of phytoplankton community in spring 2013 due to the hurricane Sandy, as shown by the baseline investigation (Ren 2015); 2) relatively

less number of samples in comparison to, such as Spring-UPH, and Summer-LPH and UPH. Overall, the refined P-IBI showed better separation than the previous P-IBI between the least-impaired and impaired communities (Fig. 3 in Ren et al. 2017). It is worth mentioning, that the previous P-IBI scores were calculated based on limited data points, especially for Spring and Summer-MH (2 to 4 data points). The refined P-IBI scores are more reliable and solid because of the augmented data. The high degree of separation in the P-IBI scores demonstrates the strong discriminatory ability of the refined P-IBI for spring and summer communities in BB-LEH.

The classification efficiencies (CE) of the refined P-IBI for the least-impaired and impaired communities are listed in Table 19. The refined P-IBI was able to correctly classify 35-87% of the impaired samples from six season-salinity zones. The classification efficiencies were higher for the least impaired samples in the calibration dataset, ranging from 80-100%. Higher classification efficiency was achieved for the impaired samples in summer than spring for the MH and PH zones, which is similar to the previous P-IBI. Overall, the refined P-IBI correctly classified 64-86% of spring samples and 79-84% of summer samples in the calibration dataset, showing generally higher classification ability than the previous P-IBI.

SUMMARY

The previously developed Phytoplankton Index of Biotic Integrity (P-IBI) (Ren et al. 2017) was refined with the supplemental datasets from the 2014 and 2016 investigations. A refined P-IBI was developed and the phytoplankton reference communities were re-calculated using all the data from the previous studies. Three datasets, in total 354 samples were used in the refined P-IBI in comparison to 203 samples in the previous one. The three datasets include the baseline dataset from August 2011-August 2013 (203 samples), April 2014-April 2015 (55 samples), and May 2016-May 2017 (96 samples).

The major steps and results of the P-IBI refinement during the study are summarized as following.

1) The previous P-IBI was validated using the data from April 2014-April 2015. The previous P-IBI did not efficiently classify the least-impaired and impaired communities for the most of the spring and summer samples. The previous P-IBI showed weak classification ability in discriminating the least-impaired and impaired communities as indicated by the separation of the P-IBI scores. The validation results suggested it is necessary to evaluate and refine the previous P-IBI as suggest by Ren et al. (2017).

2) Secchi depth-based and turbidity-based classifications were compared in terms of the discriminatory ability of forty physical-chemical and phytoplankton metrics. The comparison showed that the turbidity-based classification resulted to slightly more metrics with discriminatory ability for mesohaline zone in spring and summer, however it also resulted in less significance of discrimination in some metrics for spring and summer polyhaline zones. As a result, Secchi depth remains, in the refined P-IBI, as one of the three parameters for the classification of habitat conditions, together with dissolved inorganic nitrogen (DIN) and ortho-P (PO4).

3) Two-zone and three-zone salinity classifications were compared in terms of the discriminatory ability of the forty-three physical-chemical and phytoplankton metrics. The comparison showed that the three-zone classification resulted in more metrics with significant discriminatory ability in spring MH. Some metrics showed slightly less significant discrimination in LPH and UPH in three-zone classification. This may be a result of less number of data for respective LPH and UPH in comparison to that of PH in the two-zone system. The

calculation of reference communities showed significant discrimination between LPH and UPH in some key metrics, such as Chl*a*, TN and TP. The concentrations of Chl*a* and TN were generally higher in LPH than UPH, while the concentration of TP was generally lower in LPH than UPH. These results are consistent with the Chl*a* and nutrient gradients from the previous and on-going monitoring data. Therefore, three-zone salinity classification is more suitable for the BB-LEH and applied in the refined P-IBI.

4) Based on the above results, P-IBI was re-calculated and developed following the general steps outlined in Ren et al. (2017). As the previous P-IBI, the focus was given on the spring and summer categories because more data is available for these two seasons. Secchi depth, DIN and PO4 classification criteria were established for six season-salinity zones, including Spring-MH, Spring-LPH, Spring-UPH, and Summer-MH, Summer LPH and Summer-UPH. The criteria were calculated based on the above-mentioned three datasets, in total 354 samples.

5) Based on the criteria of Secchi depth, DIN and ortho-P, each sample was classified into one of the following four categories of habitat conditions: Poor-Worst (including Worst, PW+W), Mixed-Poor Light (MPL), Mixed-Better Light (MBL), and Better-Best (including Best, BB+B). Habitat conditions of each site and of all sites were calculated (Fig. 5). For all the sites, half or more samples were in the category of MPL. Generally northern sites (BB01, BB04a and BB07a) exhibited higher percentage of better habitat conditions including MBL and BB categories. BB04a had the highest percentage of Worse and Poor-Worst samples among the three northern sites. Southern sites, such as BB10 and BB12 had more Poor-Worst samples compared to the north. Overall, in total near 60% of the 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 15.8% of the samples were identified as Better-Best (least-impaired) conditions.

6) Phytoplankton reference communities were calculated for each of the six season-salinity category based on the samples in Better-Best category. The characteristic metrics for the reference communities include chlorophyll a (Chl*a*), Chl*a*/C ratio, nano- and micro-phytoplankton (NM) abundance, NM biomass, average NM cell size, and summer picoplankton biomass. In addition, parameters related to phytoplankton growth and biomass were also included, such as dissolved oxygen (DO), total suspended solids (TSS), total nitrogen (TN) and

total phosphorus (TP). Reference communities, in comparison to the impaired habitat, were characterized with lower Chl*a*, lower Chl*a*:C ratio, lower TN, TP and TSS, and in summer with lower picoplankton biomass.

7) Phytoplankton IBI metrics were selected based on their discriminatory abilities and the criteria of each metric were calculated and established for each of the six season-salinity zones. The refine P-IBI was able to correctly classify 35-87% of the impaired samples. The classification efficiencies were higher for the least impaired samples in the calibration dataset, ranging from 80-100%. Higher classification efficiency was achieved for the impaired samples in summer than spring, especially for MH and LPH, which is similar to the previous P-IBI. Overall, the refined P-IBI was able to correctly classify 64-86% of spring samples and 79-84% of summer samples in the calibration dataset, showing generally higher classification ability than the previous P-IBI.

8) Chl*a*, TN and TP showed significant difference between the least-impaired and impaired habitats. The calculated median and interquartile values of these parameters in the least-impaired and impaired conditions, together with the phytoplankton reference communities, can be useful information for water quality assessment and the potential guidance in nutrient criterion development and nutrient management.

9) In total 96 samples were analyzed for phytoplankton community composition collected from 6 sites between May 2016 and April 2017. Phytoplankton species and cell density were identified and enumerated for each sample, and biovolume and carbon biomass were calculated, following the same methods as the previous studies. Phytoplankton biovolume composition and carbon biomass for all 6 sites are shown in Figures 10 to 15.

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Table 19: Classification efficiencies (CE) of the refined P-IBI.

Table 1: Description of the sites for phytoplankton sample collection and analysis from May 2016 to May 2017.

Name	description	latitude	longitude
BB01	Barnegat Bay at Mantoloking	40.0400000	-74.052222
BB04a	Barnegat Bay near the Mouth of Toms River	39.93289	-74.14069
BB06	Barnegat Bay below Cedar Creek and above Forked River	39.85262	-74.10208
BB07a	Barnegat Bay below Oyster Creek and above Barnegat Inlet	39.8012861	-74.1571172
BB09	Barnegat Bay below Barnegat Inlet and close to Long Beach	39.7426200	-74.147920
BB10	Barnegat Bay by Route 72 Bridge	39.6609500	-74.206530
BB12	Barnegat Bay in Little Egg Harbor	39.5815100	-74.268750

Table 2: Sample information for classified three salinity zones in Barnegat Bay-Little Egg Harbor. In total 354 Samples were collected from 2011-2013, 2014-2015 and 2016-2017. MPH: mesohaline, 5~18 ppt; LPH: lower polyhaline, 18~25 ppt; and UPH: upper polyhaline, >=25 ppt.

	MPH	LPH	UPH
Sites	BB04(a), BB02, BB01 (occasional)	BB01, BB05(a), BB04a (only from 2016), and BB06	BB07, BB09, BB10, BB12, BB14
# of samples	61	99	194

Table 3. Secchi depth (m) classification criteria for water quality classes of Worst, Poor, Better, and Best for different seasons and salinity zones, derived from 2011-2014 dataset. Spring: March-May; Summer: June-September; Fall: October-November; Winter: December-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	<0.914	≤1.524	>1.524	>1.829	0.914	1.524	1.829
PH	<1.219	≤1.372	>1.372	>1.829	1.219	1.372	1.829
Summer							
MH	<0.762	≤0.991	>0.991	>1.219	0.762	0.991	1.219
PH	<0.762	≤0.914	>0.914	>1.486	0.762	0.914	1.486
Fall							
MH	<0.914	≤0.991	>0.991	>1.143	0.914	0.991	1.143
PH	<0.914	≤1.219	>1.219	>1.494	0.914	1.219	1.494
Winter							
MH	<1.029	≤1.295	>1.295	>1.524	1.029	1.295	1.524
PH	<0.914	≤1.219	>1.219	>1.402	0.914	1.219	1.402

Table 4: 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, derived from 2011-2014 dataset. Spring: March-May; Summer: June-September; Fall: October-November; Winter: December-February. Salinity: mesohaline (MH), and polyhaline (PH). * 95 percentile value.

	Habitat Classification Criteria				Relative Status Method		
DIN (mg/l)	Worst	Poor	Better	Best	75%	median	25%
Spring							
MH	>0.274	>0.07	≤0.07	<0.016	0.274	0.222	0.016
PH	>0.110*	>0.07	≤0.07	<0.020	0.076	0.037	0.020
Summer							
MH	>0.122	>0.07	≤0.07	<0.020	0.122	0.039	0.020
PH	>0.187*	>0.07	≤0.07	<0.016	0.058	0.032	0.016
Fall							
MH	>0.202	>0.07	≤0.07	<0.03	0.202	0.104	0.093
PH	>0.214	>0.07	≤0.07	<0.051	0.214	0.129	0.051
Winter							
MH	>0.311	>0.07	≤0.07	<0.092	0.311	0.193	0.092
PH	>0.138*	>0.07	≤0.07	<0.014	0.067	0.025	0.014

Table 5: Ortho-P (mg/l) classification criteria for water quality classes of Worst, Poor, Better, and Best for different seasons and salinity zones, derived from 2011-2014 dataset. Spring: March-May; Summer: June-September; Fall: October-November; Winter: December-February. Salinity: mesohaline (MH), and polyhaline (PH). -: 95% value<0.007. * 95 percentile value.

	Habitat Classification Criteria				Relative Status Method		
Ortho-P (mg/l)	Worst	Poor	Better	Best	75%	median	25%
Spring							
MH	-	>0.007	≤0.007	≤0.0006	0.0025	0.0025	0.0006
PH	>0.112*	>0.007	≤0.007	≤0.0006	0.004	0.001	0.0006
Summer							
MH	>0.017*	>0.007	≤0.007	≤0.0006	0.0057	0.0025	0.0006
PH	>0.026	>0.007	≤0.007	≤0.005	0.0262	0.0137	0.0046
Fall							
MH	>0.011*	>0.007	≤0.007	≤0.002	0.0068	0.0028	0.0006
PH	>0.026	>0.007	≤0.007	≤0.002	0.0262	0.0111	0.005
Winter							
MH	-	>0.007	≤0.007	≤0.0006	0.0025	0.0025	0.0006
PH	>0.0139	>0.007	≤0.007	≤0.0024	0.0139	0.0025	0.0024

Table 6. Secchi depth (m) classification criteria for water quality classes of Worst, Poor, Better, and Best for different seasons and salinity zones, derived from 2011-2013, 2014 and 2016 data. Spring: March-May; Summer: June-September; Fall: October-November; Winter: December-February. Salinity: mesohaline (MH, <=18 ppt), low polyhaline (LPH, 18~25 ppt), and upper polyhaline (UPH, >=25 ppt).

	Habitat Classification Criteria				Relative Status Method		
Secchi depth (m)	Worst	Poor	Better	Best	25%	median	75%
Spring							
MH	<0.80	≤1.14	>1.14	>1.52	0.80	1.14	1.52
LPH	<1.22	≤1.40	>1.40	>1.55	1.22	1.40	1.55
UPH	<0.94	≤1.22	>1.22	>1.79	0.94	1.22	1.79
Summer							
MH	<0.80	≤0.99	>0.99	>1.22	0.80	0.99	1.22
LPH	<0.76	≤0.91	>0.91	>1.10	0.76	0.91	1.10
UPH	<0.91	≤1.22	>1.22	>1.10	0.91	1.22	1.52
Fall							
MH	<0.91	≤0.99	>0.99	>1.14	0.91	0.99	1.14
LPH	<0.75	≤0.91	>0.91	>1.26	0.75	0.91	1.26
UPH	<0.80	≤1.14	>1.14	>1.49	0.80	1.14	1.49
Winter							
MH	<1.07	≤1.22	>1.22	>1.52	1.07	1.22	1.52
LPH	<0.61*	≤1.22	>1.22	>1.49	1.22	1.22	1.49
UPH	<0.61*	≤0.91	>0.91	>1.40	0.88	0.91	1.40

Table 7: 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, derived from 2011-2013, 2014 and 2016 data. Spring: March-May; Summer: June-September; Fall: October-November; Winter: December-February. Salinity: mesohaline (MH, <=18 ppt), low polyhaline (LPH, 18~25 ppt), and upper polyhaline (UPH, >=25 ppt).

	Habitat Classification Criteria				Relative Status Method		
DIN (mg/l)	Worst	Poor	Better	Best	75%	median	25%
Spring							
MH	>0.2617	>0.07	≤0.07	<0.03	0.2617	0.1833	0.0812
LPH	>0.1762*	>0.07	≤0.07	<0.03	0.0636	0.0251	0.0118
UPH	>0.1054*	>0.07	≤0.07	<0.03	0.0576	0.0280	0.0150
Summer							
MH	>0.1212	>0.07	≤0.07	<0.03	0.1212	0.0339	0.0239
LPH	>0.0892*	>0.07	≤0.07	<0.03	0.0361	0.0165	0.0125
UPH	>0.1800*	>0.07	≤0.07	<0.03	0.0622	0.0404	0.0171
Fall							
MH	>0.1528	>0.07	≤0.07	<0.03	0.1528	0.0928	0.0675
LPH	>0.0591*	>0.07	≤0.07	<0.03	0.0554	0.0332	0.0152
UPH	>0.2138*	>0.07	≤0.07	<0.03	0.2138	0.1292	0.0506
Winter							
MH	>0.2858	>0.07	≤0.07	<0.03	0.2858	0.1763	0.1124
LPH	>0.1030	>0.07	≤0.07	<0.03	0.1030	0.0499	0.0108
UPH	>0.1156*	>0.07	≤0.07	<0.03	0.0339	0.0250	0.0125

Table 8: Ortho-P (mg/l) classification criteria for water quality classes of Worst, Poor, Better, and Best for different seasons and salinity zones, derived from 2011-2013, 2014 and 2016 data. Spring: March-May; Summer: June-September; Fall: October-November; Winter: December-February. Salinity: mesohaline (MH, <=18 ppt), low polyhaline (LPH, 18~25 ppt), and upper polyhaline (UPH, >=25 ppt).

	Habitat Classification Criteria				Relative Status Method		
Ortho-P (mg/l)	Worst	Poor	Better	Best	75%	median	25%
Spring							
MH	>0.0081*	>0.007	≤0.007	≤0.002	0.0059	0.0055	0.0025
LPH	>0.0117*	>0.007	≤0.007	≤0.002	0.0094	0.0057	0.0048
UPH	>0.0171*	>0.007	≤0.007	≤0.002	0.0094	0.0055	0.0045
Summer							
MH	>0.0188*	>0.007	≤0.007	≤0.002	0.0081	0.0050	0.0006
L PH	>0.0198	>0.007	≤0.007	≤0.002	0.0198	0.0050	0.0006
UPH	>0.0351	>0.007	≤0.007	≤0.002	0.0351	0.0196	0.0107
Fall							
MH	>0.0110*	>0.007	≤0.007	≤0.002	0.0067	0.0278	0.0006
LPH	>0.0143	>0.007	≤0.007	≤0.002	0.0143	0.0050	0.0017
UPH	>0.0263	>0.007	≤0.007	≤0.002	0.0263	0.0154	0.0101
Winter							
MH	>0.0176*	>0.007	≤0.007	≤0.002	0.0035	0.0025	0.0006
LPH	>0.0122	>0.007	≤0.007	≤0.002	0.0122	0.0025	0.0010
UPH	>0.0120	>0.007	≤0.007	≤0.002	0.0120	0.0025	0.0025

Table 9: Turbidity (NTU) classification criteria for water quality classes of Worst, Poor, Better, and Best for different seasons and salinity zones, derived from 2011-2014 dataset. Spring: March-May; Summer: June-August; Fall: September-October; Winter: November-February. Salinity: mesohaline (MH), and polyhaline (PH).

	Habitat Classification Criteria				Relative Status Method		
Turbidity (NTU)	Worst	Poor	Better	Best	75%	median	25%
Spring							
MH	>2.71	≥2.20	<2.20	<1.68	2.71	2.20	1.68
PH	>5.44	≥3.45	<3.45	<2.06	5.44	3.45	2.06
Summer							
MH	>5.61	≥3.92	<3.92	<3.29	5.61	3.92	3.29
PH	>6.60	≥5.50	<5.50	<3.55	6.60	5.50	3.55
Fall							
MH	>3.74	≥3.31	<3.31	<2.81	3.74	3.31	2.81
PH	>7.32	≥4.31	<4.31	<3.20	7.32	4.31	3.20
Winter							
MH	>3.60	≥2.48	<2.48	<2.28	3.60	2.48	2.28
PH	>7.75	≥4.91	<4.91	<2.30	7.75	4.91	2.30

Table 10: Discrimination efficiencies of individual metrics in least-impaired (L-imp) and impaired (imp) conditions for spring and summer mesohaline (MH) and polyhaline (PH) zones.

Habitat Condition		# of Samples	% DT	DO	Chla	%_dino	%_crypt	TOC	DOC	TSS	Chl:C	%_pico	Avrg NM cell-size	Total NM abund.	Chryso. Abund.	Cyano abund.
Spring MH	L-imp	2	100%	50%												
	imp	3	33%	0%												
Spring PH	L-imp	3	33%	100%	0%	67%	33%			67%						0%
	imp	4	75%	0%	100%	50%	75%			0%						33%
Summer MH	L-imp	10	50%	100%	50%	60%	70%	n.d	n.d			67%	100%			
	imp	0														
Summer PH	L-imp	18	67%	100%	78%		72%				100%	50%	78%	100%	37%	
	imp	2	50%	0%	0%		0%				0%	0%	0%	50%	0%	

Table 11: Classification efficiencies of the P-IBI in least-impaired (L-imp) and impaired (imp) conditions for spring and summer mesohaline (MH) and polyhaline (PH) zones.

Habitat Condition		# of Samples	Overall P-IBI	Classification Efficiency
Spring MH	L-imp	2	50%	25%
	imp	3	0%	
Spring PH	L-imp	3	0%	43%
	imp	4	25%	
Summer MH	L-imp	10	60%	50%
	imp	0	0%	
Summer PH	L-imp	18	94%	90%
	imp	2	0%	

Table 12: Comparison of discriminatory ability of phytoplankton metrics between Secchi depth based and turbidity based classifications (Kruskal-Wallis test, *p=0.05-0.1; ** p=0.01-0.05; *** p<0.01, ns: not significance).

Metrics	Secchi Depth Based				Turbidity Based			
	Spring		Summer		Spring		Summer	
	MH	PH	MH	PH	MH	PH	MH	PH
Chlorophyll a (µg/L)	ns	**	*	***	ns	**	**	*
Chl*a* : C ratio	ns	**	ns	ns	ns	*	ns	***
Total carbon biomass (ug/L)	ns	ns	**	ns	ns	ns	*	***
Total abundance nano-micro phytoplankton (cells/L)	ns	ns	ns	***	ns	ns	ns	***
Total biovolume nano-micro phytoplankton (μm^3/L)	ns	ns	**	ns	ns	ns	*	ns
Average cell size nano-micro phytoplankton	ns	ns	**	*	ns	ns	**	**
Diatom biomass (µg C/L)	ns	**	ns	ns	ns	**	ns	ns
Diatom abundance (cells/L)	ns	**	*	**	ns	ns	ns	ns
% diatoms to total phytoplankton biomass	ns	**	ns	*	ns	**	**	**
C_DT/C_non-DT	ns	**	ns	*	**	*	**	ns
Chlorophyte biomass (µg C/L)	ns	ns	ns	**	*	ns	*	***
Chlorophyte abundance (cells/L)	ns	ns	ns	ns	*	ns	ns	*
% chlorophyte to total phytoplankton biomass	ns	ns	ns	*	*	ns	ns	*
Dinoflagellate biomass (µg C/L)	ns	ns	ns	ns	ns	ns	ns	**
Dinoflagellate abundance (cells/L)	ns	ns	ns	ns	ns	ns	ns	ns
% dinoflagellates to total phytoplankton biomass	ns	**	**	ns	ns	**	**	ns
Cryptophyte biomass (µg C/L)	ns	ns	ns	ns	ns	ns	ns	ns
Cryptophyte abundance (cells/L)	ns	*	ns	ns	ns	**	ns	ns
% cryptophytes to total phytoplankton biomass	ns	**	***	ns	*	***	***	ns
Chrysophyte biomass (µg C/L)	ns	*	ns	ns	*	ns	ns	ns
Chrysophyte abundance (cells/L)	ns	*	*	ns	ns	*	*	ns
% chrysophytes to total phytoplankton biomass	ns	ns	ns	ns	ns	ns	ns	ns
Picoplankton biomass (µg C/L)	ns	ns	**	ns	ns	ns	*	**
Picoplankton abundance (cells/L)	ns	ns	***	ns	ns	ns	*	**
% picoplankton to total phytoplankton biomass	ns	ns	***	**	ns	*	***	*
Cyanobacteria biomass (µg C/L)	ns	ns	ns	ns	ns	ns	ns	ns
Cyanobacteria abundance (cells/L)	ns	ns	*	ns	*	ns	ns	ns
% cyanobacteria to total phytoplankton biomass	ns	ns	ns	ns	ns	ns	ns	*
Prorocentrum minimum abundance (cells/L)	ns	ns	ns	ns	ns	ns	ns	ns
Prorocentrum minimum biomass (µg C/L)	ns	ns	ns	ns	ns	ns	ns	ns
Total suspended solids (TSS, mg/L)	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	ns	*	**
Total nitrogen (TN, mg/L)	ns	ns	**	***	ns	ns	*	*
Total phosphorus (TP, mg/L)	ns	***	***	***	ns	***	***	***

Table 13. Phytoplankton metrics examined for discriminatory ability for spring and summer in three salinity zones (MH, LPH and UPH) (Kruskal-Wallis test, *p=0.05-0.1; ** p=0.01-0.05; *** p<0.01, ns: not significance).

Metrics	Spring			Summer			Fall			Winter		
	MH	LPH	UPH	MH	LPH	UPH	MH	LPH	UPH	MH	LPH	UPH
Chlorophyll a (µg/L)	ns	*	***	*	ns	*	-	ns	**	ns	*	ns
Chl/C ratio	*	ns	ns	ns	ns	ns	-	ns	*	**	*	ns
Total abundance nano-micro phytoplankton (cells/L)	ns	ns	ns	**	ns	**	-	ns	ns	ns	ns	ns
Total biovolume nano-micro phytoplankton (μm^3/L)	ns	ns	ns	**	*	ns	-	ns	ns	ns	ns	ns
Average cell size nano-micro phytoplankton (μm^3/cell)	ns	*	ns	***	ns	ns	-	ns	*	**	*	ns
Diatom biomass (cells/L)	ns	*	ns	*	*	ns	-	ns	ns	*	*	ns
Diatom abundance (cells/L)	ns	ns	ns	**	ns	ns	-	ns	ns	ns	*	ns
% diatoms to toal phytoplankton biomass	**	ns	**	**	*	ns	-	ns	**	**	*	ns
Chlorophyte biomass	ns	*	ns	ns	**	ns	-	ns	ns	ns	ns	ns
Chlorophyte abundance (cells/L)	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
% chlorophytes to toal phytoplankton biomass	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
Dinoflagellate biomass	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	*	ns
Dinoflagellate abundance (cells/L)	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	*	ns
% dinoflagellates to toal phytoplankton biomass	**	ns	*	ns	ns	ns	-	ns	ns	**	*	ns
Cryptophyte biomass	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
Cryptophyte abundance (cells/L)	ns	*	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
% cryptophytes to toal phytoplankton biomass	ns	ns	*	ns	ns	ns	-	ns	ns	ns	ns	ns
Chrysophyte biomass	ns	ns	***	ns	ns	ns	-	ns	ns	ns	ns	ns
Chrysophyte abundance (cells/L)	ns	ns	***	ns	ns	ns	-	ns	ns	ns	ns	ns
% chrysophytes to toal phytoplankton biomass	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
Picoplankton biomass	ns	ns	ns	**	ns	ns	-	ns	ns	ns	ns	ns
Picoplankton abundance (cells/L)	ns	ns	ns	*	*	ns	-	ns	ns	ns	ns	ns
% picoplankton to toal phytoplankton biomass	ns	ns	**	**	ns	ns	-	ns	ns	ns	ns	ns
Cyanobacteria biomass	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
Cyanobacteria abundance (cells/L)	ns	ns	ns	ns	**	ns	-	ns	ns	ns	ns	ns
% cyanobacteria to toal phytoplankton biomass	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
Prorocentrum minimum abundance (cells/L)	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
Prorocentrum minimum biomass	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
Total suspended solids (TSS, mg/L)	ns	ns	**	ns	**	***	-	*	***	ns	*	*
Dissolved organic carbon (DOC, mg/L)	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
Total organic carbon (TOC, mg/L)	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
Dissolved oxygen (DO, mg/L)	ns	ns	**	**	*	***	-	*	*	ns	ns	ns
DO saturation (%)	**	ns	ns	ns	*	***	-	ns	ns	ns	*	ns
Total nitrogen (TN, mg/L)	ns	ns	ns	ns	***	*	-	ns	***	ns	ns	***
Total phosphorus (TP, mg/L)	ns	ns	***	***	ns	***	-	*	**	ns	**	***
C biomass (ug/L)	**	ns	ns	*	ns	**	-	ns	ns	**	ns	ns
Katadinium rotundatum	ns	*	ns	ns	ns	ns	-	ns	ns	ns	ns	*
Prasinophytes biomass	ns	ns	*	ns	**	**	-	ns	*	ns	ns	ns
Prasinophytes abundance (cells/L)	ns	ns	ns	ns	**	ns	-	ns	ns	ns	ns	ns
C DT/non-DT	ns	ns	ns	*	ns	ns	-	ns	ns	**	*	ns

Table 14. Phytoplankton reference communities and their supporting habitat conditions for spring and summer mesohaline zone (5~18 ppt). p: Significance of difference, ANOVA test. ** $p < 0.01$, * $p < 0.05$; ns: not significant; na: not applicable. Δ: Reference community values higher than impaired community values; ∇: Reference community values lower than impaired community values.

Metrics	Spring			Summer			Units
	B+BB+MBL (n=6)			B+BB+MBL (n=15)			
	Max/Min	Median	p	Max/Min	Median	p	
Chl*a*	7.15/0.42	4.2	ns	17/0.2	8.8	*∇	µg/L
Chl*a*/C ratio	0.006/0.003	0.017	*∇	0.11/0.001	0.03	ns	
NM abundance	6.63/0.94	2.17	*∇	30.5/0.35	3.2	**∇	10^7 cells/L
NM biomass	8.77/0.12	0.34	*∇	20.2/0.52	3.5	*∇	10^9 um^3/L
Avg NM cell size	214/99	119	ns	180/3.6	106	**Δ	um^3/cell
Pico abundance	2.47/0	0.96	*∇	16.5/0.011	6.75	**∇	10^7 cells/L
Pico biomass	113/0	14	ns	572/0	142	*∇	µg C/L
TN	0.68/0.36	0.53	*∇	0.85/0.39	0.62	ns	mg/L
TP	0.025/0.007	0.013	*∇	0.052/0.01	0.03	**∇	mg/L
DO	9.3/8.1	8.8	ns	9.18/6.45	7.3	**Δ	mg/L
TSS	16/7	11	*∇	20/4.5	12.5	ns	mg/L
DIN	0.28/0.014	0.16	na	0.23/0.011	0.05	na	mg/L
PO_4	0.01/<0.0011	<0.0011	na	0.02/0.007	0.014	na	mg/L
Secchi depth	2.13/1.37	1.68	na	1.8/1.1	1.22	na	m

Table 15: Phytoplankton reference communities and their supporting habitat conditions for spring and summer lower polyhaline zone (LPH, <=18~25 ppt). p: Significance of difference, ANOVA test. ** $p < 0.01$, * $p < 0.05$; ns: not significant; na: not applicable. Δ: Reference community values higher than impaired community values; ∇: Reference community values lower than impaired community values.

Metrics	Spring			Summer			Units
	B+BB (n=8)			B+BB+MBL (n=13)			
	Max/Min	Median	p	Max/Min	Median	p	
Chl*a*	13.0/0.42	2.1	*∇	21.0/0.42	13.5	**∇	µg/L
Chl*a*/C ratio	0.145/0.002	0.026	ns	0.17/0.012	0.022	ns	
NM abundance	6.62/0.63	2.49	ns	26.2/0.16	2.3	ns	10^7 cells/L
NM biomass	20/0.6	3.97	ns	42.5/0.13	1.68	ns	10^9 um^3/L
Avg NM cell size	702/82	163	*Δ	165/25	85	*Δ	um^3/cell
Pico abundance	1.03/0.29	0.18	ns	33.3/0.02	7.06	**∇	10^7 cells/L
Pico biomass	27/0.98	4.8	ns	851/9	54	*∇	µg C/L
TN	0.54/0.24	0.38	ns	0.72/0.11	0.42	**∇	mg/L
TP	0.047/0.005	0.018	*∇	0.07/0.02	0.03	*∇	mg/L
DO	9.8/5.6	8.6	ns	7.87/5.29	6.93	**Δ	mg/L
TSS	19/10.5	15.5	*∇	32/6	13.5	**∇	mg/L
DIN	0.23/0.003	0.048	na	0.07/0.005	0.016	na	mg/L
PO_4	0.015/0.008	<0.009	na	0.033/0.004	0.019	na	mg/L
Secchi depth	2.1/1.5	1.6	na	1.83/0.91	1.22	na	m

Table 16: Phytoplankton reference communities and their supporting habitat conditions for spring and summer upper polyhaline zone (UPH, >=25 ppt). p: Significance of difference, ANOVA test. ** $p < 0.01$, * $p < 0.05$; ns: not significant; na: not applicable. Δ: Reference community values higher than impaired community values; ∇: Reference community values lower than impaired community values.

Metrics	Spring			Summer			Units
	B+BB (n=13)			B+BB (n=8)			
	Max/Min	Median	p	Max/Min	Median	p	
Chl*a*	4.2/0.2	0.84	**∇	3.4/0.8	2.1	*∇	µg/L
Chl*a*/C ratio	0.32/0.002	0.025	*∇	0.08/0.002	0.04	ns	
NM abundance	1.6/0.03	0.33	ns	7.11/0.23	1.8	**∇	10^7 cells/L
NM biomass	41/0.2	1.77	ns	3.9/0.45	1.4	ns	10^9 um^3/L
Avg NM cell size	978/63	230	ns	701/9	87	*Δ	um^3/cell
Pico abundance	0.20/0.014	0.11	ns	5.7/0.36	0.54	ns	10^7 cells/L
Pico biomass	6.08/0.5	3.2	ns	167/0	7.0	*∇	µg C/L
TN	0.49/0.14	0.26	*∇	0.46/0.20	0.27	*∇	mg/L
TP	0.036/0.005	0.018	**∇	0.05/0.01	0.03	**∇	mg/L
DO	11.1/4.7	8.0	*Δ	8.81/5.71	6.98	**Δ	mg/L
TSS	28/4.3	19.8	*∇	27/5.5	14	**∇	mg/L
DIN	0.05/0.013	0.027	na	0.04/0.002	0.02	na	mg/L
PO_4	0.007/0.001	0.004	na	0.007/0.001	0.006	na	mg/L
Secchi depth	2.4/1.5	1.8	na	1.7/1.4	1.5	na	m

Table 17: P-IBI metrics and the scoring criteria for Spring MH, LPH and UPH.

Metrics	Metric scoring criteria			Units
	1	3	5	
Mesohaline (MH)				
DO sat %	<86.6	>86.6 and <97.3	>97.3	%
Turbidity	>2.74	>1.77 and <2.74	<1.77	NTU
Carbon biomass	<25 and >130	>71 and <130	>25 and <71	µg/L
Chl*a*:C ratio	>0.07	>0.02 and <0.07	<0.02	
%_DT	<6.6 and >59	>38 and <59	>6.6 and <37	%
%_dino	>33	>15 and <33	<15	%
Lower Polyhaline (LPH)				
Chlorophyll *a*	>5.2	>2.2 and <5.2	<2.2	µg/L
Diatom biovolume	$>1.0 \times 10^{10}$	$>2.3 \times 10^{9}$ and $<1.0 \times 10^{10}$	$<2.3 \times 10^{9}$	μm^3
%_DT	<0.7 and >87.3	>30.8 and <87.3	>0.7 and <30.8	%
TN	>0.44	>0.287 and <0.44	<0.287	mg/L
Upper Polyhaline (UPH)				
DO	<7.63	>7.63 and <9.72	>9.72	mg/L
TSS	>30	>24 and <30	<24	mg/L
TN	>0.366	>0.245 and <0.366	<0.245	mg/L
TP	>0.0451	>0.0225 and <0.0451	<0.0225	mg/L
Chlorophyll a	>4.2	>2.1 and <4.2	<2.1	µg/L
Chl*a* :C	>0.143	>0.024 and <0.143	<0.024	
NM abundance	$>1.23 \times 10^{7}$	$>2.6 \times 10^{6}$ and $<1.23 \times 10^{7}$	$<2.6 \times 10^{6}$	cells/L
Chrysophyte_biovolume	$>3.1 \times 10^{7}$	$>6.4 \times 10^{6}$ and $<3.1 \times 10^{7}$	$<6.4 \times 10^{6}$	µm3
Chrysophyte_abundance	$>3.07 \times 10^{6}$	$>2.05 \times 10^{5}$ and $<3.07 \times 10^{6}$	$<2.05 \times 10^{5}$	%
%diatom biomass	<7.5 and >89	>63.3 and <89	>7.5 and <63.3	%
Prasinophyte abundance	$>1.37 \times 10^{6}$	$>1.63 \times 10^{5}$ and $<1.37 \times 10^{6}$	$<1.63 \times 10^{5}$	cells/L

Table 18: P-IBI metrics and scoring criteria for summer MH, LPH and UPH.

Metrics	Metric scoring criteria			Units
	1	3	5	
Mesohaline (MH)				
Chlorophyll *a*	> 13.8	> 8.2 and <13.8	< 8.2	µg/L
picoplankton biomass	>356	>186 and <356	<186	µgC/L
% picoplankton biomass	< 1.6 or > 94	>74.2 and <94	> 1.6 and < 74.2	%
NM biovolume	<1.84x 10^9	>1.84x 10^9 and <4.88x 10^9	>4.88x 10^9	μm^3
Average cell size NM phytoplankton	< 16	> 16 and <106	>106	μm^3/cell
Total organic carbon (TOC)	> 7.2	> 6.6 and < 7.2	< 6.6	mg/L
Total phosphorus	>0.0526	>0.033 and <0.0526	<0.033	mg/L
Total Carbon biomass	> 491	> 264 and < 491	<264	%
Dissolved oxygen (DO)	< 6.2	> 6.2 and < 7.7	> 7.7	mg/L
Lower Polyhaline (LPH)				
Chlorophyll *a*	> 10.1	> 6.3 and < 10.1	< 6.3	µg/L
Prasinophyte abundance	>2.8x 10^6	>1.1x 10^6 and <2.8x 10^6	<1.1x 10^6	cells/L
Picoplankton biomass	> 306	> 165 and < 306	< 165	µg/L
Cyanoplankton abundance	>1.54x 10^9	>2.55x 10^7 and <1.54x 10^9	<2.55x 10^7	cells/L
TN	> 0.632	> 0.354 and < 0.632	< 0.354	mg/L
TP	> 0.0584	> 0.0424 and < 0.0584	< 0.0424	mg/L
TSS	> 19.5	> 13.5 and < 19.5	< 13.5	mg/L
Dissolved oxygen (DO) %	< 79.4	> 79.4 and < 91.5	>91.5	%
Dissolved oxygen (DO)	< 5.7	> 5.7 and < 7.3	> 7.3	mg/L
Upper Polyhaline (UPH)				
Chlorophyll a	> 7.04	> 2.9 and < 7.04	< 2.9	µg/L
Chl*a*:C ratio	> 0.08	> 0.04 and < 0.08	< 0.04	
Carbon biomass	> 69.8	> 37.6 and < 69.8	< 37.6	µg/L
Picoplankton biomass	> 506	> 13.6 and < 506	< 13.6	µg/L
NM abundance	<5.97x 10^6	>5.97x 10^6 and <1.62x 10^7	>1.62x 10^7	cells/L
TN	> 0.481	> 0.278 and < 0.481	< 0.278	mg/L
TP	> 0.0740	> 0.0323 and < 0.0740	< 0.0323	mg/L
TSS	> 28.1	> 14.9 and < 28.1	< 14.9	mg/L
Average NM cell size	< 153	> 153 and < 286	> 286	μm^3/cell
Dissolved oxygen (DO) %	< 81.2	> 81.2 and <91.2	> 91.2	%
Dissolved oxygen (DO)	< 5.5	> 5.5 and <6.7	> 6.7	mg/L

Table 19: Classification efficiencies (CE) of the refined P-IBI in impaired, least-impaired (L-Imp) and impaired (Imp) conditions for spring and summer mesohaline (MH), lower polyhaline (LPH) and upper polyhaline (UPH) zones.

Season-salinity zones	Habitat condition	# of Samples	CE	Overall CE
Spring MH	L-Imp	6	83%	86%
	Imp	8	87%	
Spring LPH	L-Imp	16	81%	56%
	Imp	20	35%	
Spring UPH	L-Imp	13	100%	64%
	Imp	35	49%	
Summer MH	L-Imp	15	80%	79%
	Imp	18	78%	
Summer LPH	L-Imp	13	85%	79%
	Imp	11	73%	
Summer UPH	L-Imp	8	100%	84%
	Imp	16	69%	

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Figure 1. Map of phytoplankton sites from May 2016 to April 2017.

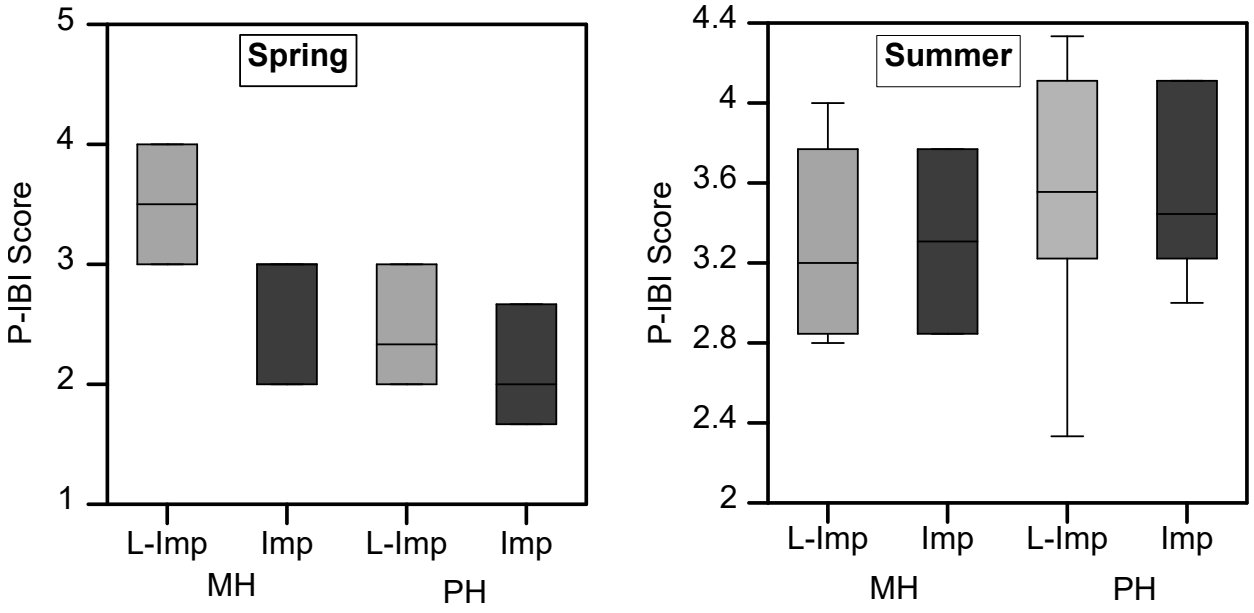

Figure 2. Distribution of P-IBI scores for the least-impaired (reference) and impaired (degraded) communities in 2014 spring and summer. The interquartile range, median value (lines within the bars), and 5th and 95th percentiles (lines below and above each bar) are displayed for summer and spring mesohaline (MH) and polyhaline (PH) zones.

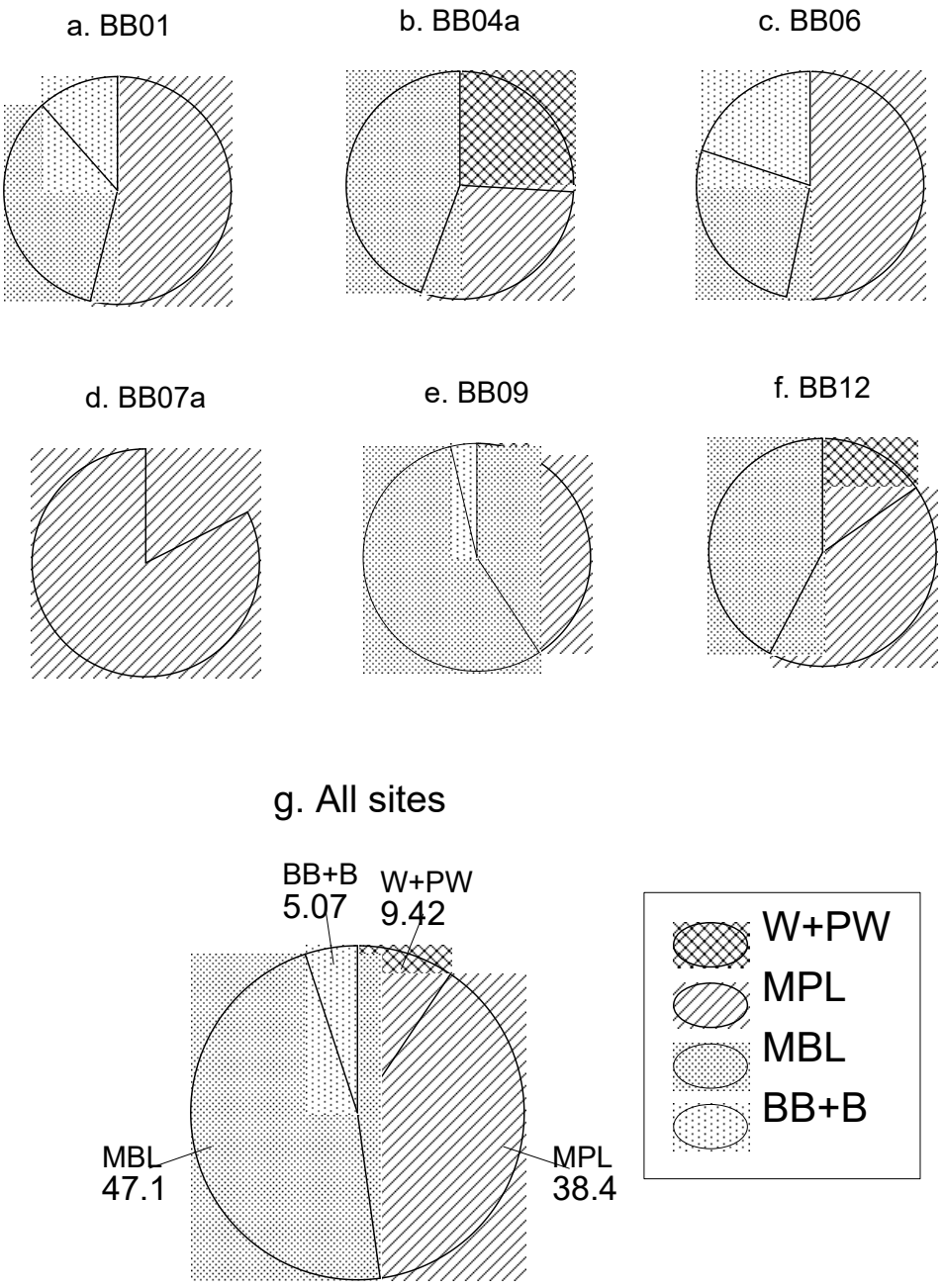

Figure 3. Percentage of phytoplankton habitat categories at each individual site and of all sites in BB-LEH based on 2014 and 2016 data and under two-zone salinity classification.

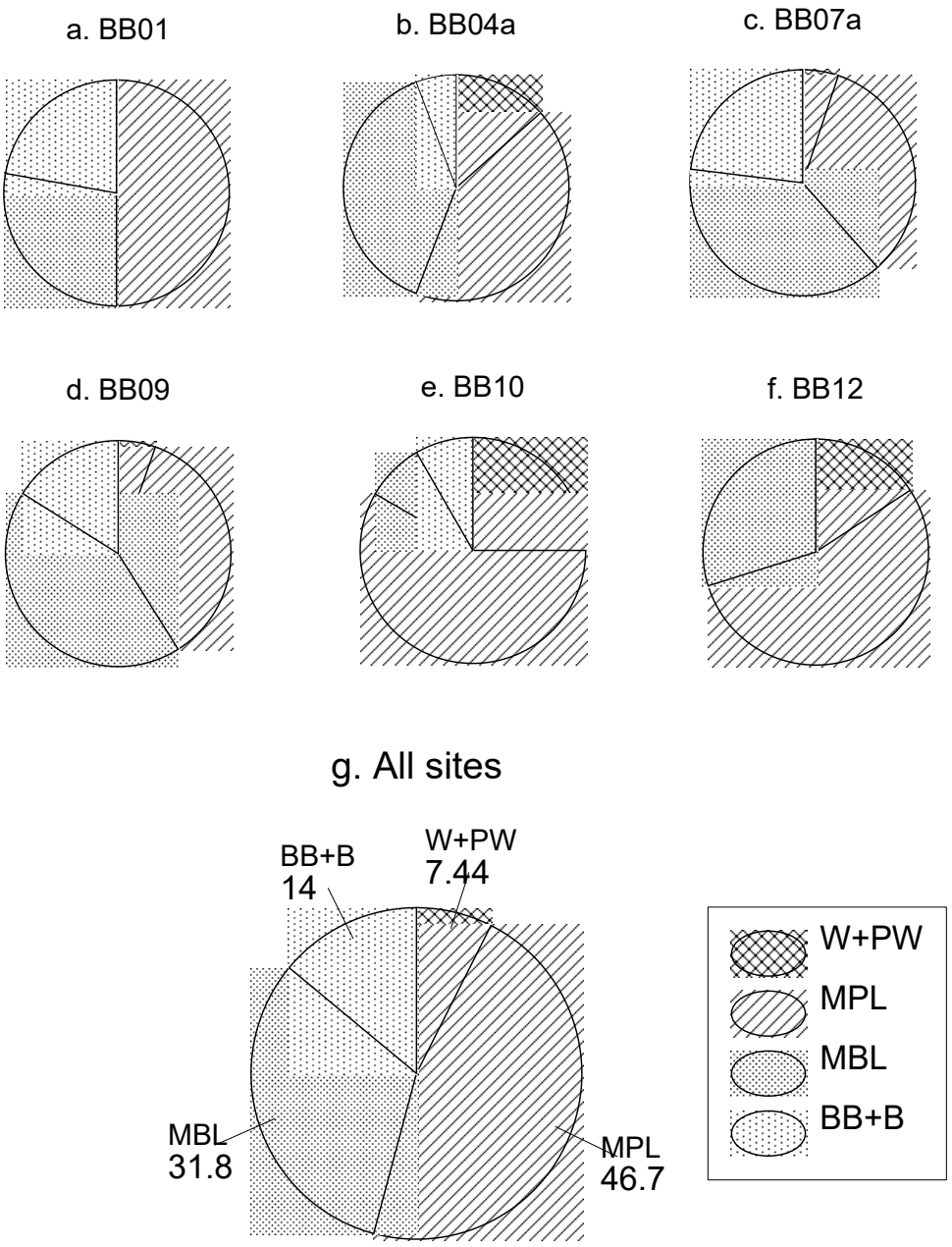

Figure 4. Percentage of phytoplankton habitat categories at each individual site and of all sites in BB-LEH based on four years of data (2011-2014 and 2016) under two-zone salinity classification.

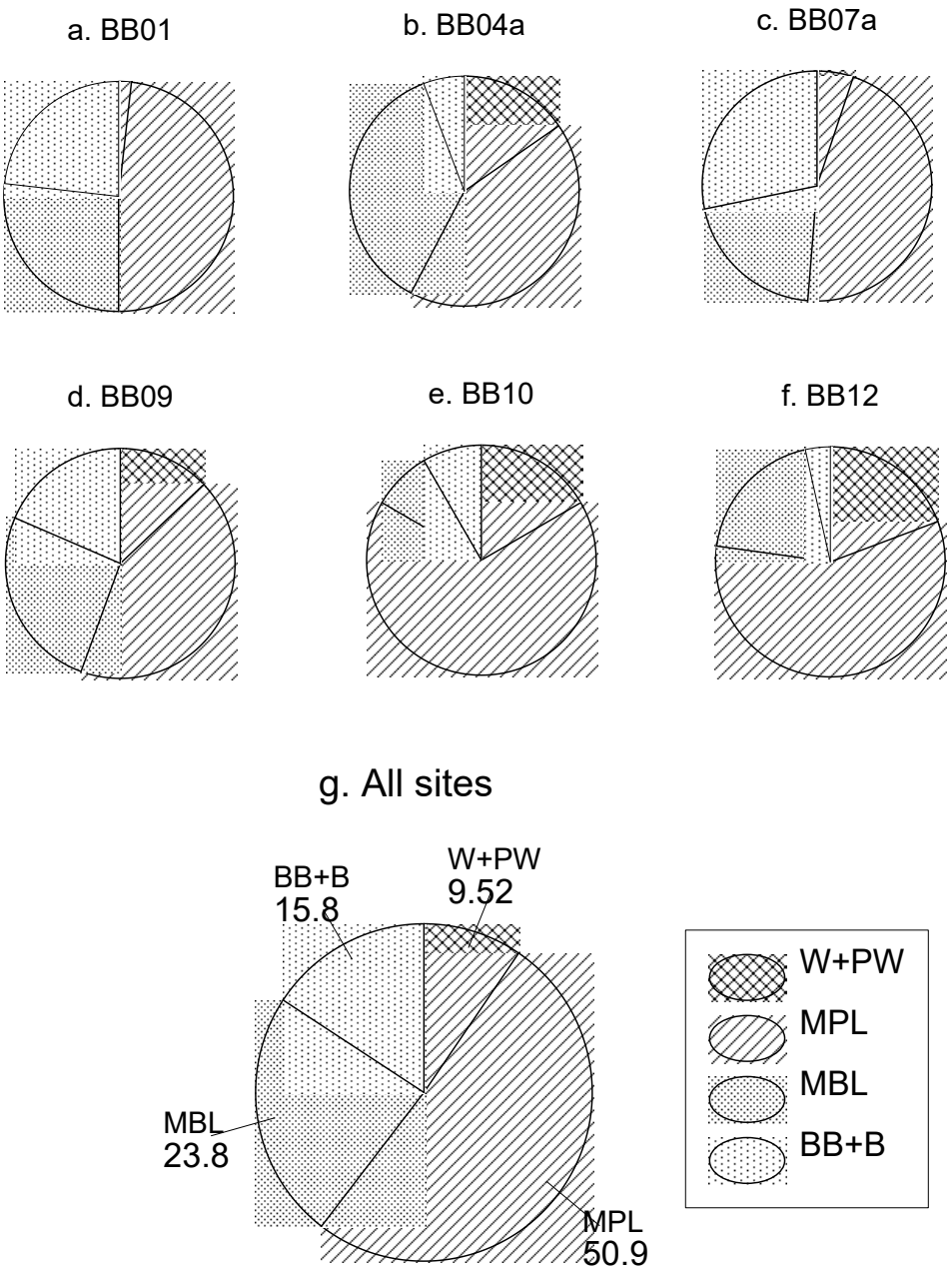

Figure 5. Percentage of phytoplankton habitat categories at each individual site and of all sites in BB-LEH based on four years of data (2011-2014 and 2016) under three-zone salinity classification.

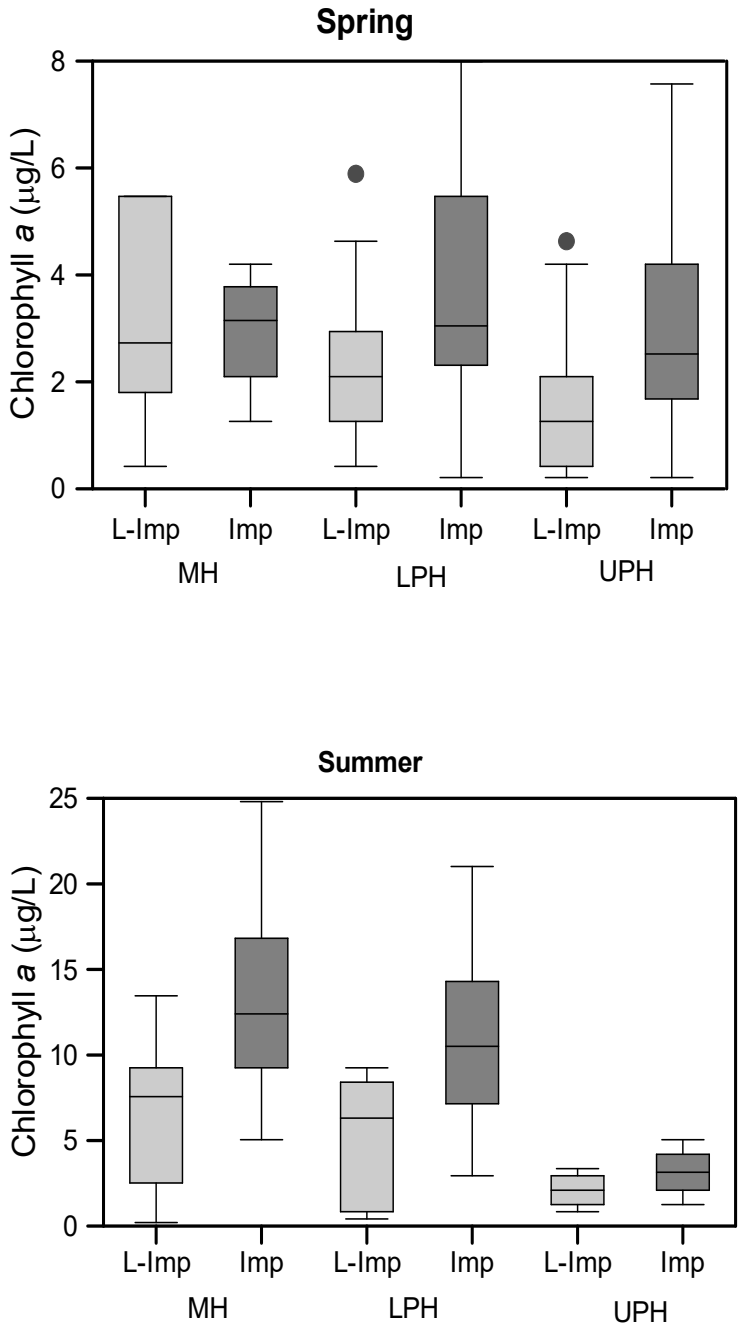

Figure 6. Median values, interquartile ranges, and 25th and 75th percentile of Chlorophyll *a* (Chl*a*) in the least-impaired and impaired conditions for the six season-salinity zones. L-Imp: least-impaired; Imp: Impaired; MH: mesohaline (5-18 ppt); LPH: lower polyhaline (>=8~25 ppt); and UPH: upper polyhaline (>= 25 ppt).

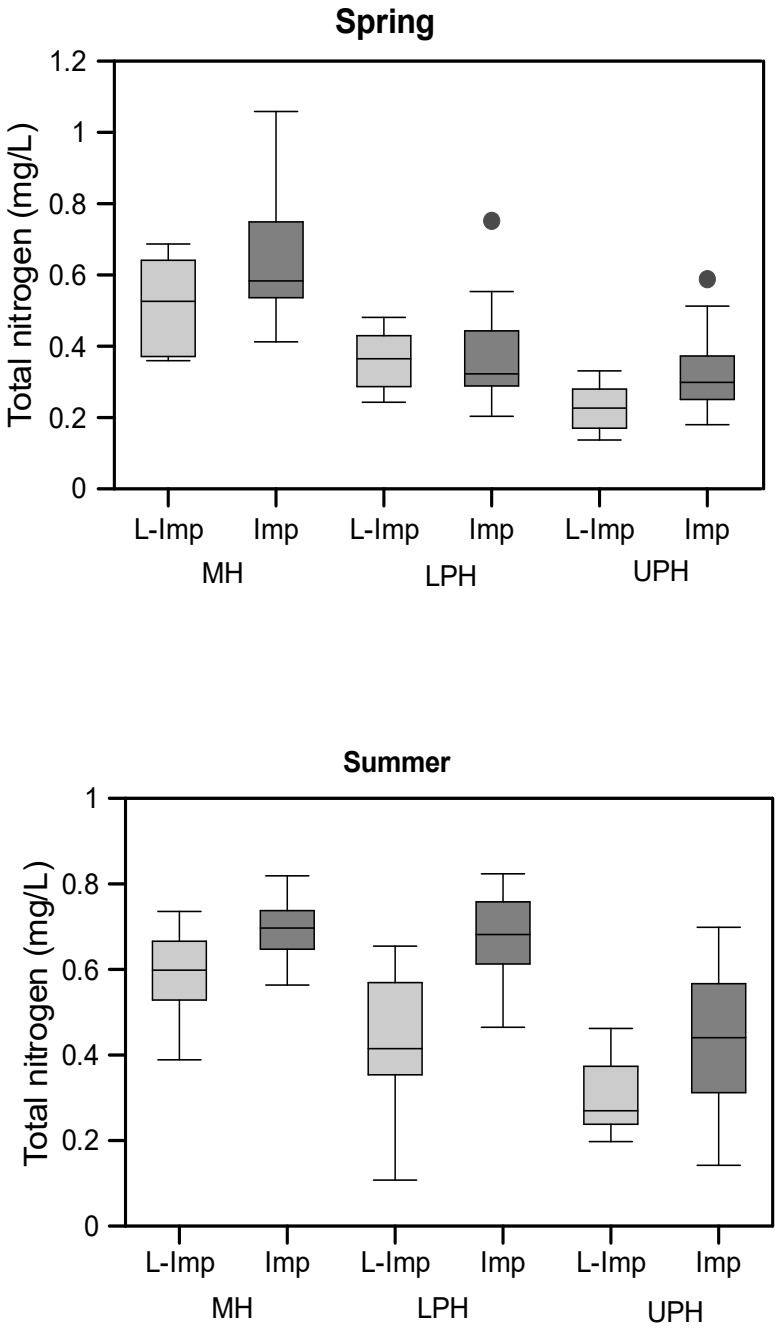

Figure 7. Median values, interquartile ranges, and 25th and 75th percentile of total nitrogen (TN) in the least-impaired and impaired conditions for the six season-salinity zones. L-Imp: least-impaired; Imp: Impaired; MH: mesohaline (5-18 ppt); LPH: lower polyhaline (>=8~25 ppt); and UPH: upper polyhaline (>= 25 ppt).

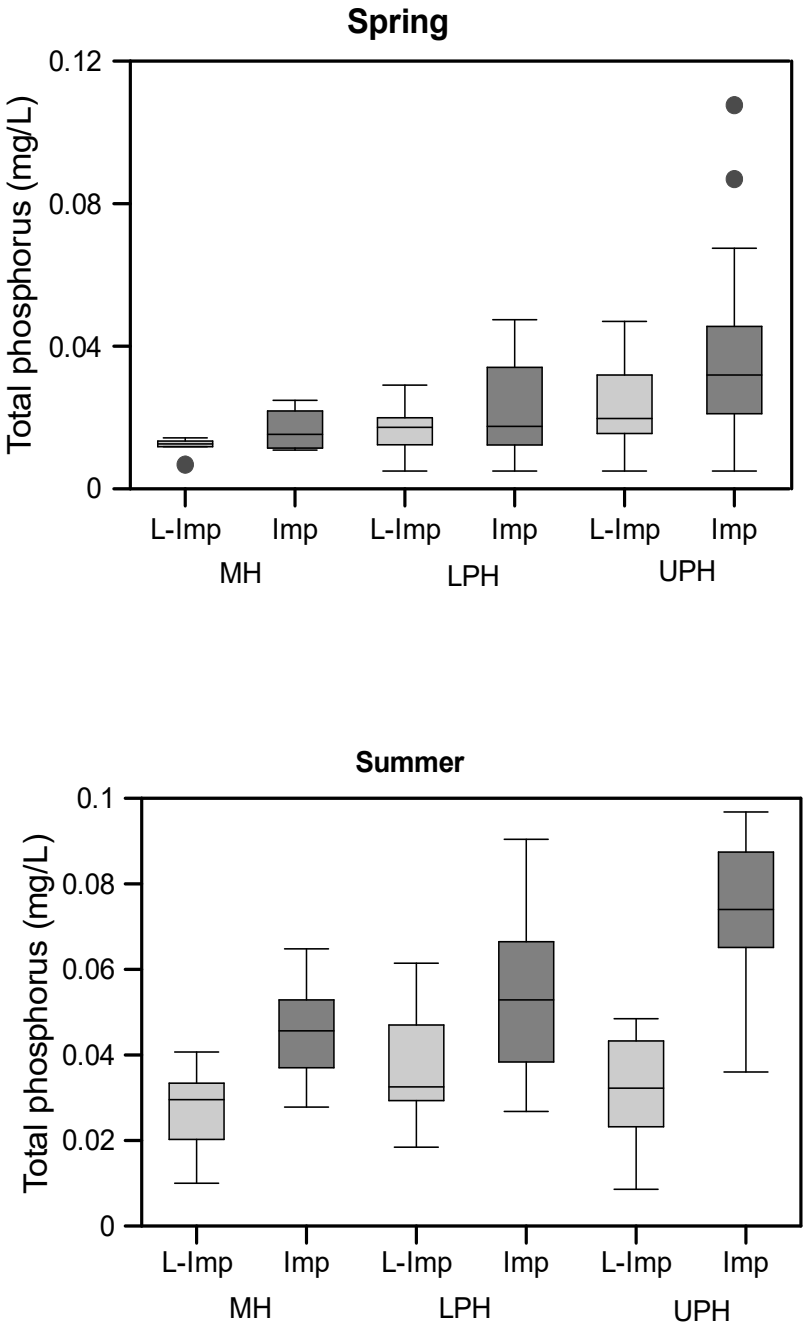

Figure 8. Median values, interquartile ranges, and 25th and 75th percentile of total phosphorus (TP) in the least-impaired and impaired conditions for the six season-salinity zones. L-Imp: least-impaired; Imp: Impaired; MH: mesohaline (5-18 ppt); LPH: lower polyhaline (>=8~25 ppt); and UPH: upper polyhaline (>= 25 ppt).

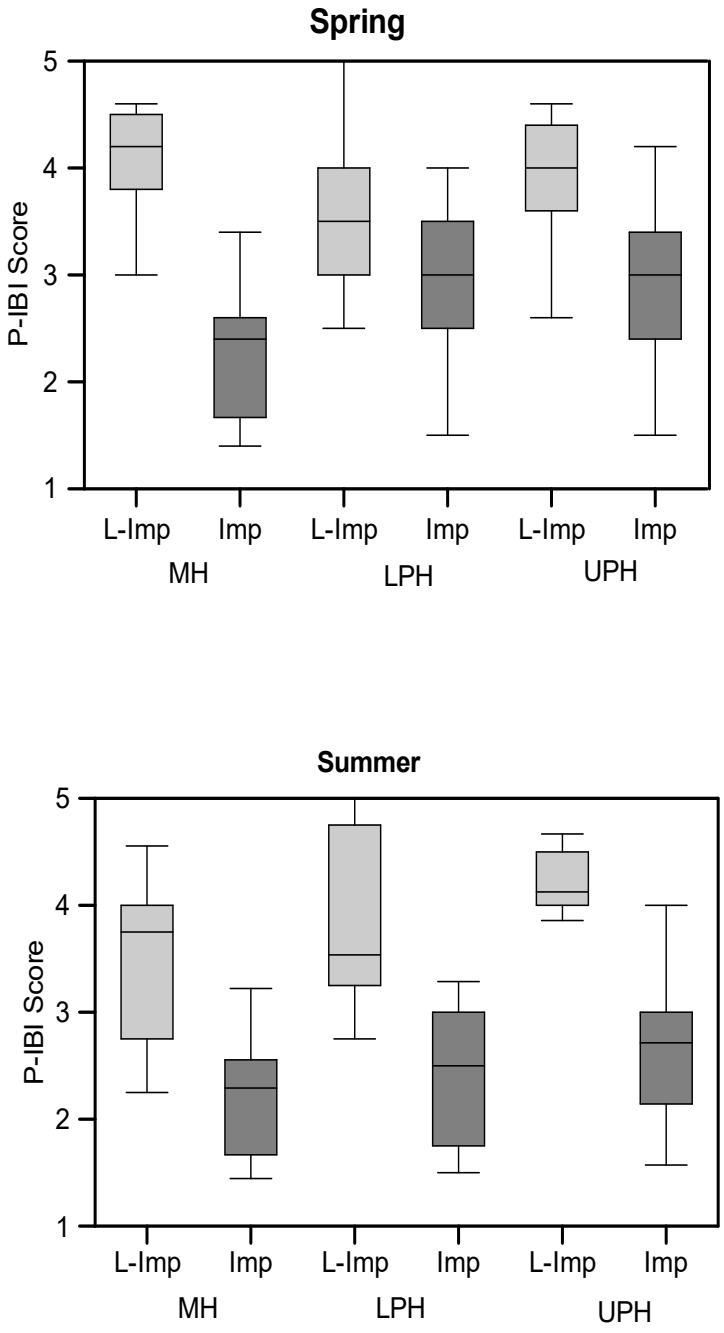

Figure 9. Distribution of P-IBI scores for the least-impaired (reference) and impaired (degraded) communities. The interquartile range, median value (lines within the bars), and 5th and 95th percentiles (lines below and above each bar) are displayed for summer and spring mesohaline (MH) and lower and upper polyhaline (LPH and UPH) zones.

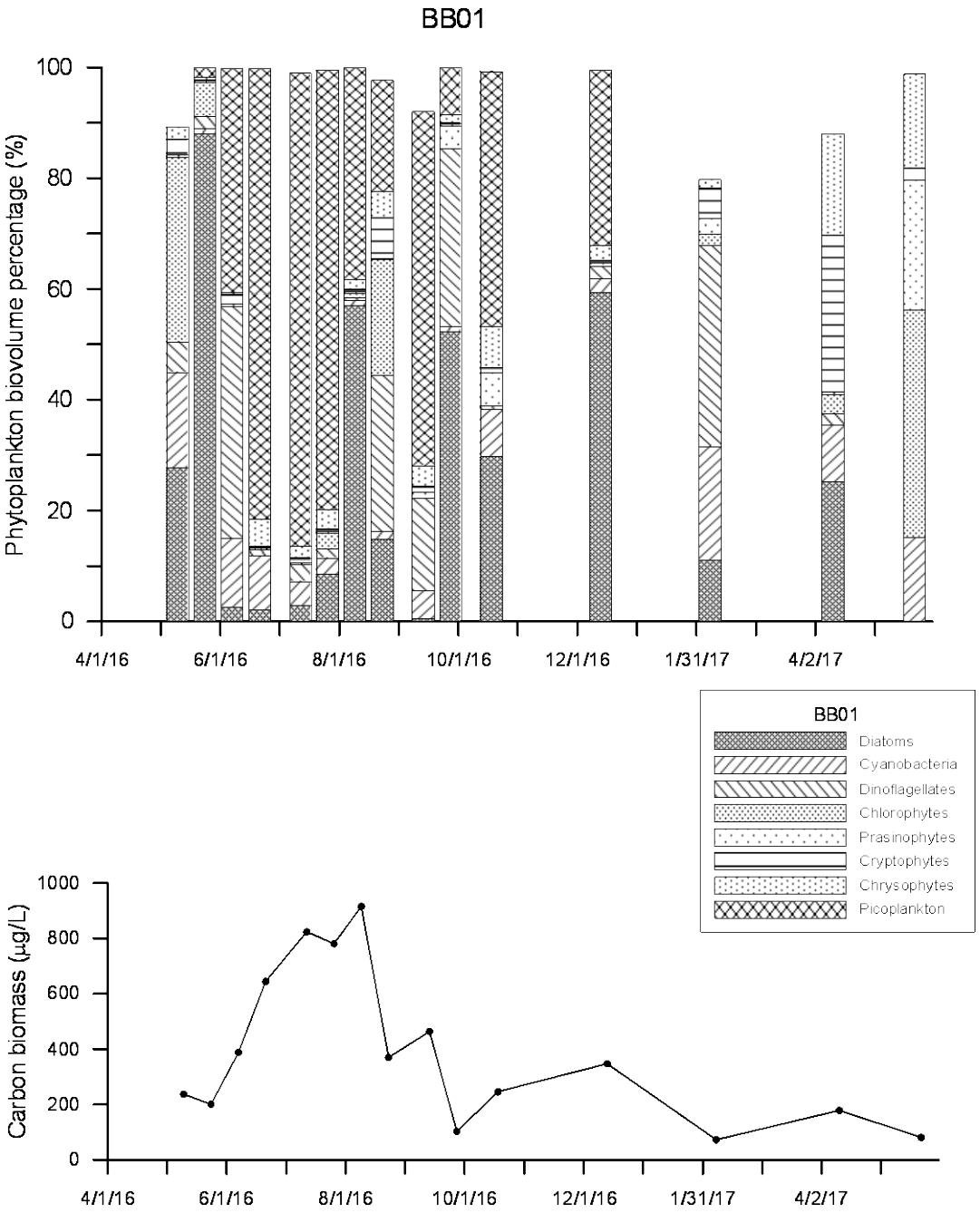

Figure 10. Biovolume percentage of each major phytoplankton groups, and carbon biomass estimation of phytoplankton at site BB01 from May 2016 to May 2017.

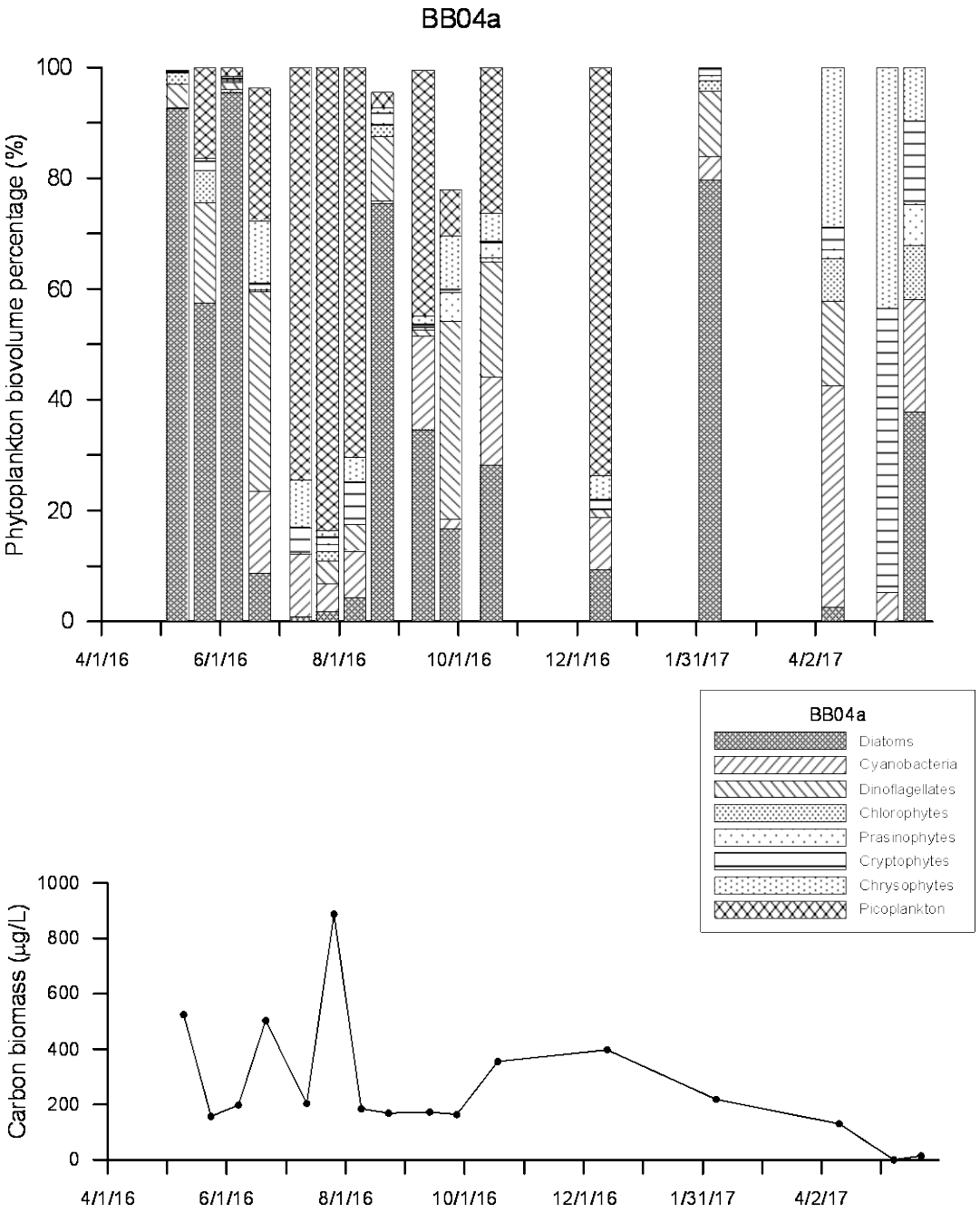

Figure 11. Biovolume percentage of each major phytoplankton groups, and carbon biomass of phytoplankton at site BB04a from May 2016 to May 2017.

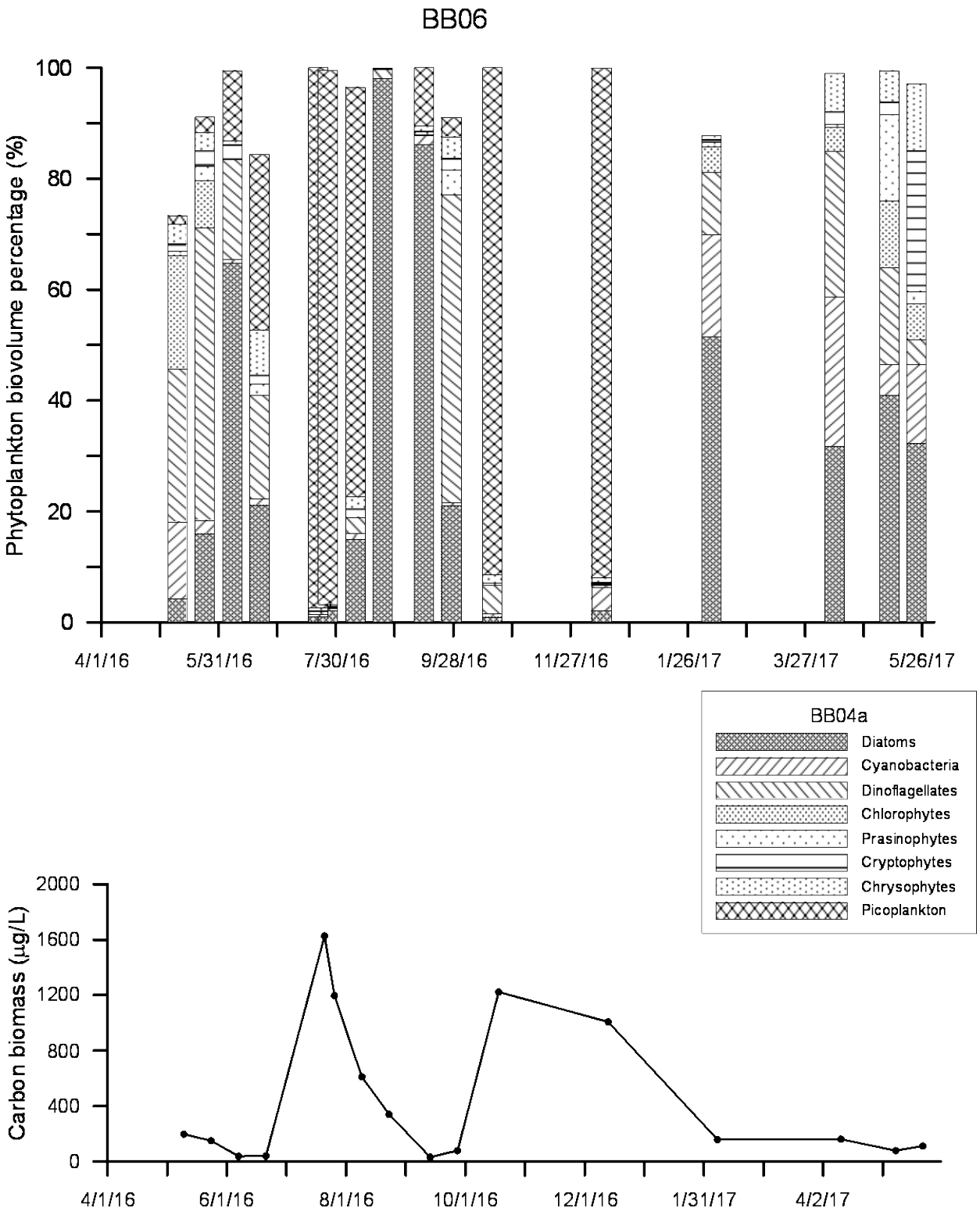

Figure 12. Biovolume percentage of each major phytoplankton groups, and carbon biomass of phytoplankton at site BB06 from May 2016 to May 2017.

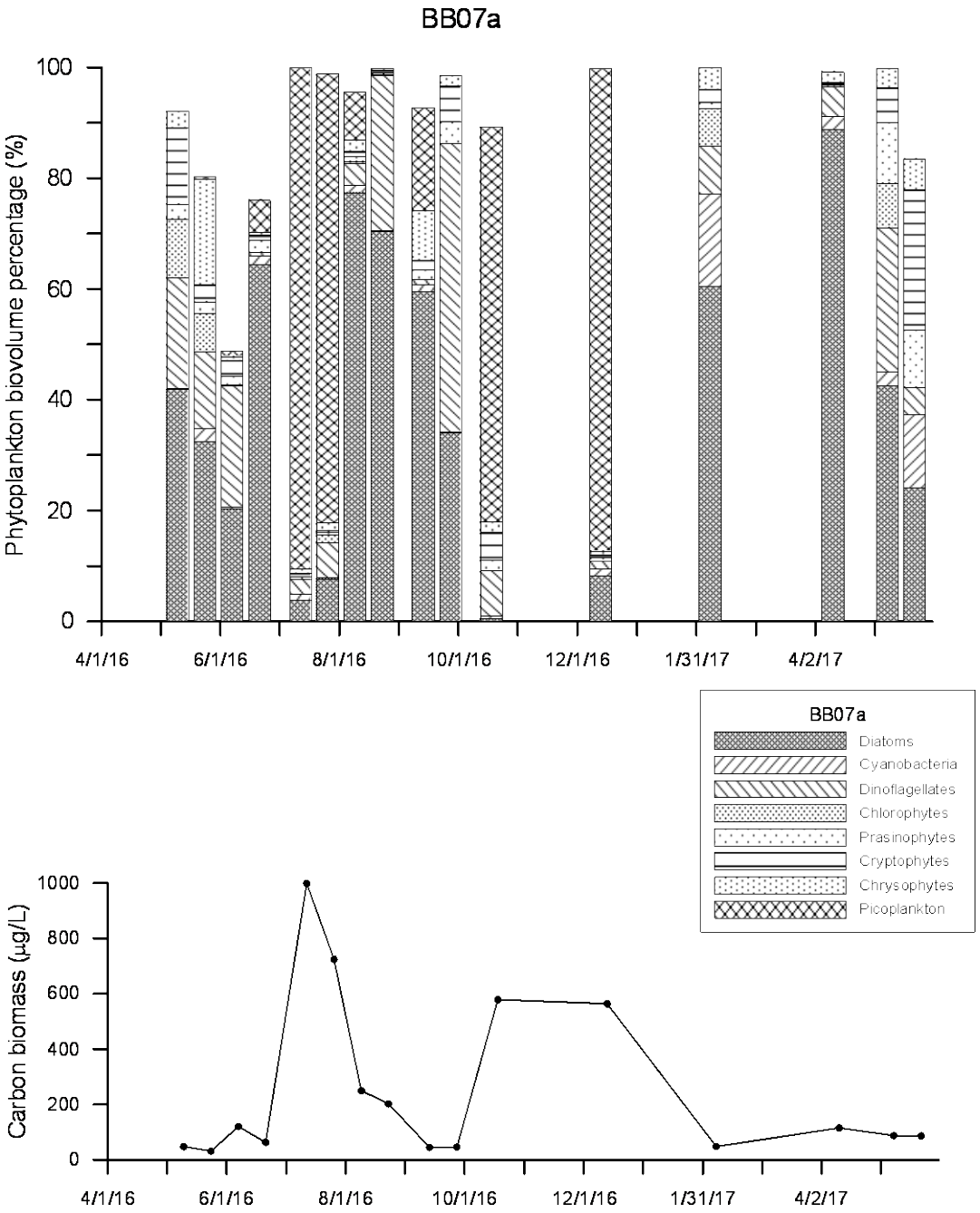

Figure 13. Biovolume percentage of each major phytoplankton groups, and carbon biomass of phytoplankton at site BB07a from May 2016 to May 2017.

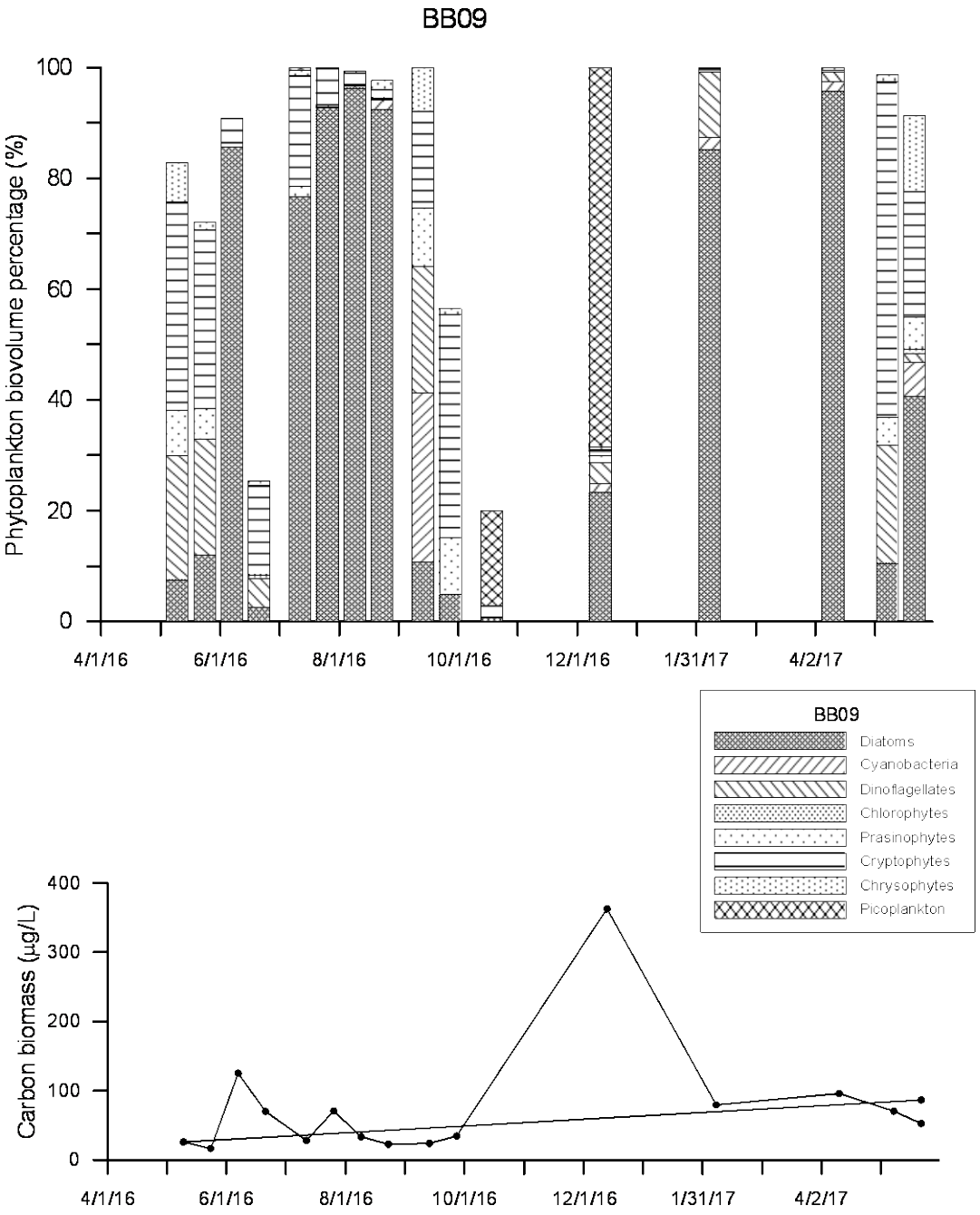

Figure 14. Biovolume percentage of each major phytoplankton groups, and carbon biomass of phytoplankton at site BB09 from May 2016 to May 2017.

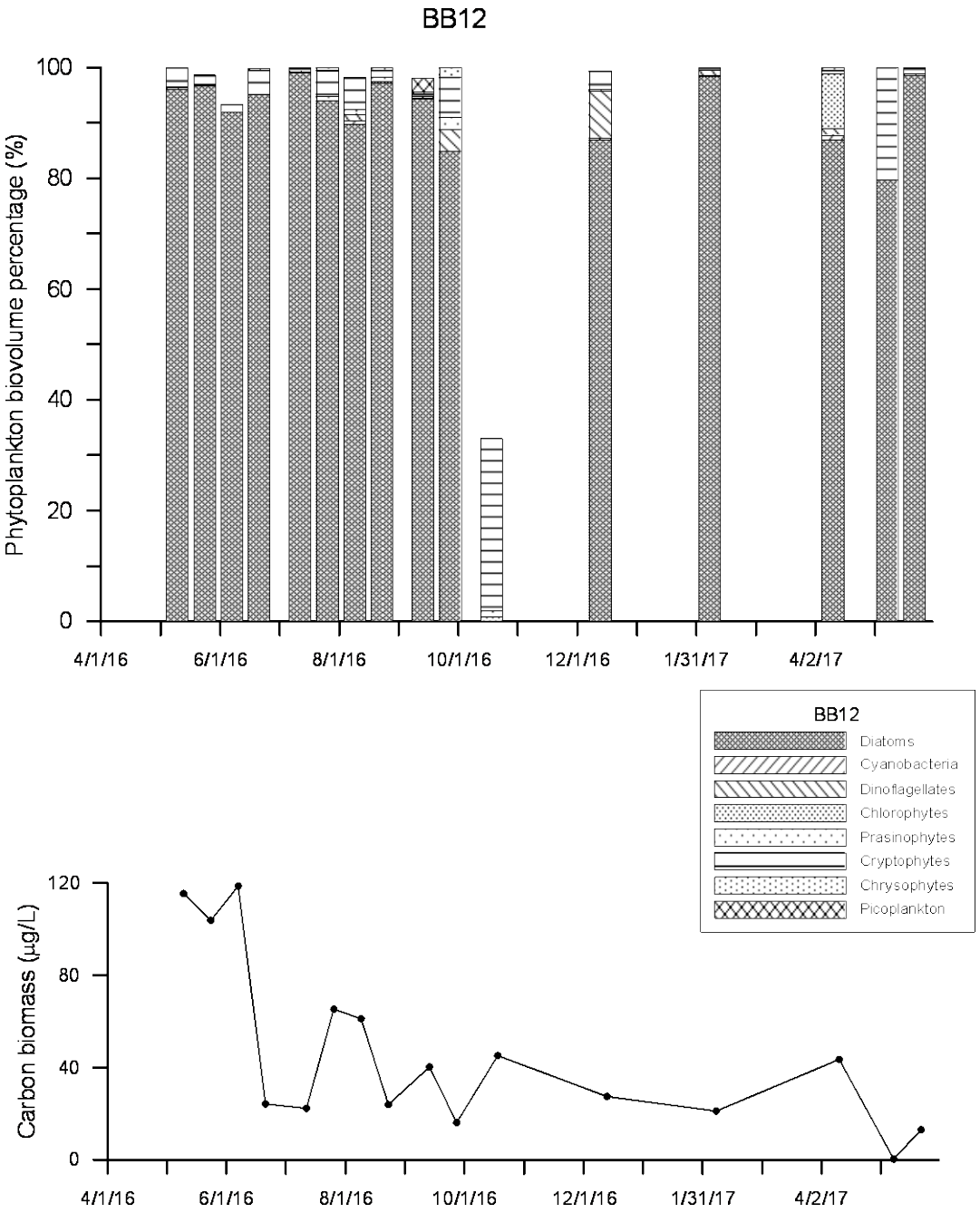

Figure 15. Biovolume percentage of each major phytoplankton groups, and carbon biomass of phytoplankton at site BB12 from May 2016 to May 2017.