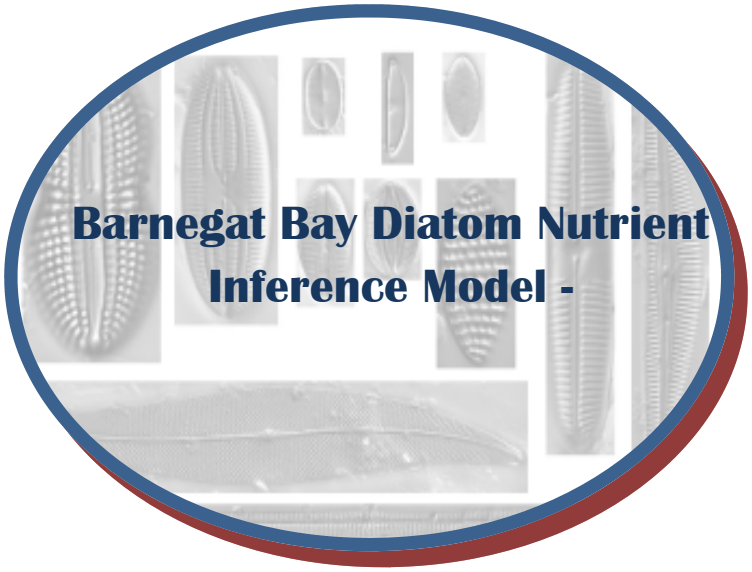
Barnegat Bay—Year 3



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February 2016

Barnegat Bay Nutrient Inference Model

FINAL REPORT

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September 8, 2015

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Executive Summary

There is an on-going discussion on whether cultural eutrophication is causing algal blooms and other deleterious effects in Barnegat Bay, New Jersey (e.g., anoxia, loss of submerged aquatic vegetation, increase in jelly fish, decreases in fish and crab population, etc). The discussion revolves around the fact that Barnegat Bay has historically been poorly drained and that what we may see as current eutrophication effects is only a part of natural conditions exacerbated by current nitrogen loading in conjunction with other stressors such as increased boat and jet ski traffic, bulkhead increases, loss of freshwater flows due to regionalization of upstream river sewage treatment plants and loss through municipal ocean outfall.

The New Jersey Department of Environmental Protection (NJDEP) is evaluating appropriate biologically-based indices to be used to measure the ecosystem health of Barnegat Bay. The federal government (USEPA and NOAA) has already developed a suite of indicators (e.g., EPA 2012 and NOAA's National Estuarine Eutrophication Assessment update) and has applied them to New Jersey's coastal bays with mixed results due to both geographic scale issues and the inability of the USEPA and NOAA metrics to identify proximate sources and causes of impairments.

Further information is needed for NJDEP to develop water quality management tools. This study is designed to directly assist NJDEP in the development of estuarine nutrient criteria. Together with macroinvertebrates, diatoms, which are microscopic siliceous algae, are commonly used as indicators of environmental conditions in aquatic ecosystems. Diatoms are widely used to monitor ecosystem health in freshwater ecosystems (lakes and streams) but less frequently in coastal, estuarine systems.

The overall goal of this project was to develop diatom indicators of ecosystem health in Barnegat Bay and to assess their utility in the development of estuarine nutrient (i.e., total nitrogen and total phosphorus) criteria . To develop nutrient criteria using diatom assemblages, three

approaches were used in our study. First, the responses of diatom assemblages to environmental factors were studied using a calibration dataset collected from 100 sites in Barnegat and Great Bays. Exceptionally diverse and abundant diatom assemblages were found in all studied habitats. Benthic diatom assemblages showed a statistically significant variation along environmental gradients, such as salinity, depth, habitat, grain size, nutrients, land-use, and sediment contaminants. Salinity was the strongest determinant of the diatom assemblage composition and a confounding factor complicating statistical modeling of the diatom responses to nutrients. The best model developed for an environmental parameter likely associated with eutrophication was for the Nitrogen content of sediments, but since sediment Nitrogen and Carbon were highly correlated, we could not exclude the possibility that diatom assemblage was responding to the total amount of organic matter in sediment, rather than to its nitrogen content alone. Since at the time of the sampling Total Nitrogen in the water column did not vary sufficiently, we were unable to demonstrate a direct link between water nitrogen and sediment diatoms that could be useful for developing nutrient criteria. At the same time, sediment nitrogen and sediment organic matter in general have been shown to increase as a consequence of nutrient pollution (e.g., Folger 1972) and therefore, diatoms indicative of sediment nitrogen increase may serve as indicators of a long-term eutrophication of the ecosystem.

We studied individual responses of diatom species to several nutrient parameters using non-parametric regression and indicator species analysis and identified species indicative of low and high nutrient concentrations. We also identified thresholds of TP, TN and N-sediment values where diatom assemblages abruptly change their species composition. Although it would be premature to recommend using these threshold values as nutrient criteria, they represent a starting point for further investigations of biological responses to eutrophication.

The second approach was to conduct nutrient enrichment experiments in vegetated marshes and intertidal mudflats of the Barnegat Bay. The goal of the experiments was to investigate how diatom assemblage composition would change as a result of nutrient increase. Nutrient additions did not cause significant changes in diatom and algal assemblage composition. Apparently, the

spatial and/or temporal scale of the experiment was insufficient to cause shifts in the assemblages.

The third approach was to apply the salinity and sediment nitrogen content inference models to marsh sediment cores which allowed us to compare diatom algal communities deposited recently to those deposited in past before Barnegat Bay was suburbanized (i.e., background) These analyses showed that the loading of nutrients deposited on the marshes increased over time in relation to increasing population and that the diatom assemblages in the marshes also shifted over time towards increased abundance of N-tolerant species. Our analysis revealed the composition of diatom assemblages before these dramatic changes took place and sets of diatom taxa were identified that we recommend for use as indicators of the "reference" or non-eutrophic condition in New Jersey marshes.

A. Introduction

A1: Background

The New Jersey Department of Environmental Protection (NJDEP) is evaluating the appropriate indicators to be used to measure the ecosystem health of New Jersey's shallow, lagoon-type estuaries such as Barnegat Bay and Great Bay. The Barnegat Bay water quality is affected by persistent pollution impacts (eutrophication, algal blooms, low dissolved oxygen) (Kennish et al. 1984, 2007, Olsen & Mahony 2001). A suite of indicators have been developed by the USEPA and NOAA [(US EPA's National Coastal Assessment Report 2005 and NOAA's National Estuarine Eutrophication Assessment update (http://ian.umces.edu/neea/pdfs/dldo.pdf)] and have been applied to New Jersey's coastal bays with mixed results, due to geographic scale issues and the inability of the USEPA and NOAA metrics to identify proximate sources and causes of impairments (Velinsky et al. 2010).

Bioindicators (e.g., phytoplankton, zooplankton, phytobenthos, zoobenthos) provide a powerful tool for water quality assessment in coastal regions under the influence of multiple stressors (e.g., urbanization, industrial and agricultural land use). Because of their sensitivity to such environmental stressors, they can be used successfully for monitoring the impact of human activities in coastal ecosystems. Extensive residential development increased the nutrient supply to Barnegat Bay (i.e., caused cultural eutrophication) which resulted in numerous adverse effects such as loss of biodiversity, episodic occurrences of algae blooms and brown tides, decline of hard clams and increasing number of invasive species (Kennish 2001). Despite the fact that nutrients from sewage have been diverted out of the Bay, the condition of Barnegat Bay has worsened over the last two decades. The impact of human-induced stressors and the biological, chemical, and physical processes responsible for habitat alteration in Barnegat Bay ecosystems are not fully understood. Thus, it is necessary to characterize the Barnegat Bay biota in terms of different kinds and degrees of impairment that are affecting its ecosystems.

Diatoms are photosynthetic protists found in nearly every freshwater and marine habitat and producing from 1/3 to 1/5 of the earth's atmospheric oxygen and organic matter (Armburst 2009). Assemblages of diatoms are proven robust indicators of stressors such as nutrients, acidification, and climate change. Diatoms are taxonomically distinct, abundant in most aquatic environments, and respond quickly to changing conditions. Because their silica shell, called frustule, preserves in sediment deposits, diatoms are also widely used in assessing long-term environmental changes and the impacts of anthropogenic activities on aquatic systems and their watersheds. Diatom species are differentiated by their shape and characteristics of their siliceous skeleton. The main forms are centric (i.e., circular, radial symmetry), and pennate (i.e., having bilateral symmetry). They exhibit two main living modes in the environment: planktonic and benthic (i.e., living on or in the bottom substrate).

Diatoms inhabiting surface layers of sediments in estuaries and shallow coastal bays are important contributors to primary production in these ecosystems (Jonge & Van Beusekom 1992, 1995, Shaffer & Sullivan 1988, Varela & Penas 1985). They are also known to be sensitive to nutrients and other factors related to eutrophication (e.g., Admiraal 1977a, b, 1984, Underwood 2000). Diatoms from surface sediments of coastal areas have been successfully used to construct inference models and reconstruct eutrophication history (e.g., Cooper et al. 2010, Wekstrom 2006). Our initial study of sediment diatoms in surface sediments from Barnegat and Great Bays revealed major patterns of variation in diatom assemblage composition and diatom responses to nutrients (Potapova et al. 2013) and an investigation the sediment cores from Barnegat Bay marshes demonstrated a consistent temporal change in diatom species composition during the last 400 years (Potapova et al. 2014). The Year 3 of the project was dedicated to conducting nutrient enrichment experiments, additional statistical analyses of the data collected during this three-year investigation and combining the results of the previous studies to better understand how diatom assemblages are changing as a result of eutrophication and how they can be used to monitor environmental conditions in Barnegat Bay.

A2: Objectives of Study

The overall goal of this project was to develop estuarine nutrient criteria for nitrogen and phosphorus. This includes development and evaluation of diatom metrics indicating the condition of key ecological characteristics of the bay.

The objectives of the Year 1 project were to create a calibration set of diatom assemblages for developing nutrient inference models for the Barnegat Bay tidal wetland, embayment and offshore ecosystems and to investigate the relationship between diatom indicators and anthropogenic influences in the watershed, such as urban and agricultural land use.

The first objective of the Year 2 project was to further analyze strata from the four marsh sediment cores collected across the Barnegat Bay in 2009 (Velinsky et al. 2010) and collect a new one in the Great Bay in order to reconstruct the history of environmental changes that occurred in the bays after European settlement. This work included application of the diatom transfer functions developed in the Year 1 project and using other data on diatom ecology obtained in Year 1 as well as pollen data for paleoecological reconstructions. The second objective was to investigate the relationships between diatom assemblages and contaminants in bay sediments using previously collected surface sediment samples.

The objectives of the Year 3 project were to ensure that diatoms identified in Year 1 and 2 studies as associated with nutrient enrichment are indeed robust indicators of eutrophication in Barnegat Bay. To this end, we conducted a series of field experiments aimed at investigating the effects of nutrient enrichment on microphytobenthos in Barnegat Bay and carried out additional statistical analyses of the data collected in Year 1 and 2 of the project. This was done by sub setting the data in a variety of ways and repeating analyses conducted in year 1 using these subsets of data and also by investigating response of individual taxa to nutrients using non-parametric regression approach and indicator species analysis.

A3: Study Area

The Barnegat Bay-Little Egg Harbor estuary (BB; Barnegat Bay) is located along the central New Jersey coastline in the Atlantic Coastal Plan province. Barnegat Bay is a barrier beach/back-barrier lagoon system from Point Pleasant south to Little Egg Inlet. The variety of highly productive shallow water and adjacent upland habitats found in this system include barrier beach and dune, submerged aquatic vegetation (SAV) beds, intertidal sand and mudflats, salt marsh islands, fringing tidal salt marshes, freshwater tidal marsh, and palustrine swamps.

The Barnegat Bay-Little Egg Harbor estuary is composed of three shallow bays (Barnegat Bay, Manahawkin Bay and Little Egg Harbor) and is approximately 70 km in length and varies from 2- to 6-km wide and up to 7-m deep. The watershed covers an area of approximately 1700 km2 and has been extensively developed over the past 70 years. The tidal waters cover approximately 280 km2 with a ratio of watershed area to water area of 6.1. The Bay is a back barrier island lagoon system with three connections to the ocean (Manasquan, Barnegat, and Beach Haven inlets. The current land use (2006) of the watershed is agriculture (~1%), wooded/forest (~28%), tidal and non-tidal wetlands (~18%), urban areas (~20%) and open water (30%) (Lathrop and Haag 2007). Importantly, watershed development (urban area) has increased over time. From 1986 to 2006 the amount of urban land cover increased from 15 to up to 21% of the land area, while forested land cover has decreased (NJ DEP, see www.state.nj.us/dep/bmw/ReportOcean.htm; Lathrop, R.G. 2004). The population of the watershed has increased substantially from the 1940s (40,000) to over 570,000 year round resident currently (US Census Reports). During the height of the summer season the population can rise to approximately 1,000,000.

The Great Bay is located south from the Little Egg Harbor and is connected to the ocean via the Little Egg Inlet. The Great Bay is the estuary of the Mullica River and is comprised of open water, intertidal marshes, mudflats and sandflats. In comparison to the Barnegat Bay-Little Egg Harbor estuary, the Great Bay watershed is considerably less developed. Average water depth in the Great Bay is 1.5m. Extensive areas of the bay bottom are covered by benthic algae and seagrasses. The Mullica River - Great Bay estuary is a large, relatively pristine, unaltered

estuarine system. It is believed to be the cleanest estuary in the corridor from Boston to Washington, D.C., owing in large part to the fact that the majority of the watershed is protected by the Pinelands Management Area, several large federal and state wildlife management areas, and state forests. This productive estuary supports a high diversity of aquatic and terrestrial habitats and species, especially marine and estuarine fisheries populations, colonial nesting waterbird colonies on the salt marsh islands, migrating and wintering waterfowl, rare brackish and freshwater tidal wetland communities, plants, and invertebrates (Dowhan et al. 1997). Samples from the Great Bay were collected to represent reference conditions in contrast to samples from the Barnegat Bay-Little Egg Harbor that has considerably more developed watershed.

B. Field and Laboratory Methods

B1: Sampling design

B1.1. Calibration dataset

Sampling design of the calibration dataset has been described in Potapova et al. (2013). Briefly, 100 sampling sites were organized in 33 transects positioned across the Barnegat and great Bays. Each transect included one mash sample and two open-water samples. Sediment cores were those collected in 2009 in the Barnegat Bay (Velinsky et al. 2010) and one additional core was collected in the Great Bay marsh in 2014.

B1.2. Field experiments

Nutrient enrichment experiments were conducted on August 6-20, 2014 at Tuckerton (southern) site and on August 17-31, 2014 at the Cattus Island County Park (northern) site. At Tuckerton the mudflat plots were set up on manmade ditches; two of the treatment series were located about 39.577533°N, 74.329611°W and the last series was set up at about 39.577169°N, 74.329901°W; while the vegetated marsh plots were set up on the bank of the ditches, two treatment series were located at 39.578044°N, 74.329971°W and one series was located at 39.577159°N, 74.329829°W. At Cattus Island (northern) site the mudflat plots were set up on

natural mudflats which were located at 39.981746°N, 74.125936°W and the vegetated marsh plots were set up at 39.984541°N, 74.127175°W (Figs 1-2).

At each site, 48 sampling plots arranged in 6 series were established. 42 tomato support rings were erected on the marsh. Eight nutrient diffusing tubes were then tied to the rings using plant twist ties. The rings with tubes were planted in mudflats and marshes as shown in Fig. 3. Legs of tomato supports were driven in sediments and nutrient diffusing tubes were immersed into sediment at 45° angle as illustrated in Fig. 4. At the vegetated marsh plots nutrients in dry form were also sprinkled inside the rings.

Three series were established in the mudlfats and another three in vegetated marshes. Each series included the following treatments: N, P, Si, N+P, N+Si, P+Si, N+P+Si, and Control. The concentrations of nutrients in the agar preparations were: $NaNO_3$ – 2 M, KH_2PO_4 -1.3 M, $Na_2O_3Si \bullet 9H_2O$ – 1 M. All preparations except those containing Si were based on 2% agar solutions, those containing Si were based on 4% agar solution.

B2: Field Experiment Sampling

B2.1 Collection of algal samples

Surface sediment samples for algal enumeration and chlorophyll-a analysis were collected from each sampling plot after two weeks of exposure of nutrient-diffusing tubes. A plastic petri dish cover (60 mm in diameter, 8 mm deep) was driven into sediment inside the support ring, then a spatula was slid underneath the petri dish cover and lifted. The sample enclosed in the Petri dish was wrapped with parafilm, placed on ice, and transported to the lab where it was kept frozen.

B2.2 Pore water sample collection

Pore water samples were collected from each sampling plot after two weeks of exposure of nutrient-diffusing tubes by filling 50 ml falcon tubes with the 0.8 cm surface sediment layer. All samples were stored on ice and transported to the ANSDU laboratory.

B3: Laboratory Methods

B3.1: Nutrient diffusion tubes

Nutrient-diffusing tubes were prepared by filling the plastic 50-ml Falcon tubes with 2% agar enriched by nutrients. The tube walls were perforated by small (0.5-1 mm in diameter) holes, at a distance of 4-6mm of each other. Tubes filled with nutrient-enriched agar were tied to the rings of plant supports, which were used to anchor the tubes in the sediments (Figs 2a, 2b; 3).

KH2PO4 was used for making 1.3 mole P agar; NaNO3 was used for preparing 2 mole N agar; and Na2O3Si • 9H2O was used for making 1 mole Si-enriched agar.

B3.2: Pore water sample preparation

Pore water was extracted by centrifugation at 4°C, 4000 rpm for 15 minutes. Pore water was then collected using syringes, filtered, placed into 20 ml plastic vials and frozen until the chemical analyses were conducted.

B3.3: Pore water chemistry analysis

Dissolved Ammonia+Ammonium-Nitrogen was determined by an Alpkem Autoanalyzer (RFA 300), utilizing the colorimetric phenate method (U.S. EPA, 1993; Method 350.1. Rev. 2.0). Dissolved Nitrate and Nitrite-Nitrogen was determined by an Alpkem Autoanalyzer (RFA 300), utilizing cadmium reduction of nitrate to nitrite, followed by diazotization. (U.S. EPA 1993; Method 353.2, Rev. 2.0). Dissolved phosphorus was determined by persulfate digestion. The resulting orthophosphate concentration was measured on the Alpkem Auto-analyzer (RFA 300) by the ascorbic acid colorimetric method (U.S. EPA 1993; Method 365.1, Rev. 2.0). Soluble reactive silica was measured spectrophotometrically following the formation of silico- molybdic acid from the reaction of ammonium molybdate and silica at low pH.

B3.4: Algal and diatom sample preparation

Surface sediment samples inside petri dishes were divided into eight equal portions. One portion was then weighted in a 100 ml beaker glass for algal analysis. The subsample was then diluted 10 times by adding RO water and was mixed thoroughly. 1 mL of the subsample was pipetted into 20 mL glass vial and was diluted 15 times by adding 12 mL of RO water. 3 mL of 10% buffered formalin was then added into the glass vial and was mixed thoroughly. Subsamples were then pipetted into Palmer-Maloney chamber and 400 X magnifications were used to count diatoms and soft-algae until 200 cells of diatoms were reached. Unit of algae per area, unit of algae per gram sediment and algae biovolume of the live algae samples were calculated. The remaining diluted samples were digested using nitric acid and diatom permanent slides were made for diatom analysis. 400 diatom valves were counted and identified to species level using LM at 1000X magnifications and SEM.

B4: Data analysis

B4.1 Field experiments

Permutational multivariate analysis of variance using distance matrices (PERMANOVA) and Canonical Correspondence Analysis (CCA) were used to determine whether diatom and all-algae assemblage composition and also algal density was influenced by nutrient additions. The independent variables used in the first PERMANOVA were nutrient additions (phosphorus (P), nitrogen (N), silica (Si) and their combinations and habitat type (mudflats (muds) and vegetated marsh (marsh)) and their combination with nutrient additions variables from both mudflats and marshes datasets from Cattus Island and Tuckerton Bay. In the second PERMANOVA, the independent variables were the nutrient additions (N, P and Si) for mudflats only and marshes only datasets from Cattus Island and Tuckerton Bay. Diatom relative abundance data were square root transformed prior to PERMANOVA. Pairwise distances were calculated and significance tests were carried out using F-test, based on sequential sums of squares from permutation raw data. Kruskal's nonmetric multidimensional scaling (nMDS) was performed to visualize the effect of each factor via ordination diagrams. PERMANOVA was carried out in R

(3.2.1; R project for statistical computing, Vienna, Austria), nMDS and CCA were carried out in CANOCO 5.0.

A series of CCA analyses was performed to determine the significance of differences in diatom community between habitat types and compares effects of habitat types and nutrients addition by decomposing the total variance into different components (variation partitioning). Diatom relative abundance data were square-root transformed and rare taxa were down-weighted prior to CCA. The first CCAs were conducted on the combined "mudflats plus marshes" datasets separately from Cattus Island and Tuckerton Bay. Variation for each group of factors, in this case grouped habitat types (mudflats and marshes) and grouped nutrients addition (N, P and Si) were calculated based on partial ordinations to test the unique effects of each group (habitat types and nutrient additions). The second group of CCAs were conducted by separating nutrient addition factors. Variation for each factor: N, P and Si addition were calculated based on partial ordinations to test the unique effect of each group (N, P and Si). The third group of CCAs were conducted on four separate datasets each reprenting only one habitat: mudflats or marshes and one site: Cattus Island ore Tuckerton Bay.

B4.2 The 2012 calibration dataset

B4.2.1 Detrended Correspondence Analyses (DCA)

Series of Detrended Correspondence Analyses (DCA) were performed on a number of 2012 calibration set data subsets: Barnegat Bay-only, marsh-only, open-water, high-salinity sites, low-salinity sites, rare taxa exclusion, planktonic taxa exclusion sample sets. DCA is based only on species data, but correlations of environmental variables with DCA axes may be calculated and plotted (DCA with passive variables) as it was done here to visualize the correspondence between variation in species data and the environment. DCA were carried out in CANOCO 5.0.

B4.2.2 Canonical Correspondence Analysis (CCA)

Canonical Correspondence Analyses (CCA) were carried out to determine the strength of the relationships between diatom assemblage composition and specific environmental variables, either one at a time, or several selected by the forward selection procedure. Unlike DCA, which is a strictly exploratory analysis, CCA allows statistical testing of effects of environmental parameters on biological assemblages. We tested for the strength of the relationships between diatom assemblages and environmental variables using several subsets of data: Barnegat Bay-only excluding Great Bay sites, marsh-only, open-water, high-salinity sites, low-salinity sites, with rare taxa excluded, and with planktonic taxa excluded. Each analysis was run twice with diatom data square-root-transformed and log-transformed. Rare diatom taxa were down-weighted. CCAs were carried out in CANOCO 5.0.

B4.2.3 Transfer functions

Inference models also known as transfer functions were computed for selected variables and diatom datasets pairs where CCAs recovered response significant at p= 0.002. This significance level was chosen because it is the strictest criterion allowed by the software. These models used the following species datasets: Barnegat Bay-only, marsh-only, open-water, rare taxa exclusion, and planktonic taxa exclusion sample sets. Five kinds of modeling approaches were used: (1) Weighed Averaging with classical de-shrinking, (2) Weighed Averaging with inverse de-shrinking, (3) Weighed Averaging- Partial Least Squares, (4) Maximum Likelihood regression and calibration, and (5) Modern Analog Technique. Bootstrapping was used to validate the models. The measures of model performance are the bootstrapped coefficient of determination (R2boot) and the root-square mean error of prediction (RMSEP). Inference models were constructed using C2 software (Juggins 2003).

B4.2.4 Generalized Additive Models

Non-parametric regression methods were used to investigate species responses to nutrients while avoid making assumptions about the possible shape of the species response curves. Generalized additive models (GAMs) were used to explore species distributions in relation to each individual chemistry characteristic. GAMs assume that the mean of the dependent variable depends on an additive predictor through a nonlinear link function (Hastie & Tibshirani 1990, Ponader & Potapova 2007), which is a log-link for Poisson distributed data, such as diatom relative abundance. The process of fitting a GAM is essentially an estimation of non-parametric smoothing functions. R 3.2.1 (R project for statistical computing, Vienna, Austria) was used to fit GAMs.

B4.2.5 Threshold Indicator Analysis (TITAN)

Diatom assemblages response to TN, TP, N sediment and TIN were also analyzed using Threshold Indicator Analysis (TITAN). TITAN is useful to identify whether biological assemblages exhibit a threshold type response to environmental stressors. This is accomplished by identifying at which values of an environmental parameter there are abrupt changes in occurrence and relative abundance of individual taxa, and by quantifying uncertainty around locations of abrupt change and estimating the relative synchrony and uncertainty of those changes as a nonparameteric indicator of the community threshold (Baker & King 2010). This approach combines the Indicator Species Analysis (Dufrêne and Legendre 1997) and multivariate portioning algorithm to determine the most reliable indicator values (maximum indicator values/IndVals) for each taxon at each candidate change-point along the stress gradient and to retain change-points with maximum IndVals. A perfect indicator taxon will occur only at sites in the same categories (i.e. it exhibits perfect *specificity* for example for high TN, TP, TIN, N sediment), and is observed in every site in these categories (i.e. demonstrates complete *fidelity* for these categories). Permutation of sites across environmental gradients is used to determine the significance of each Indicator Values (IndVal). Objective partitioning of taxa categories is achieved by accounting for the uncertainty of taxon-specific change points with bootstrap

replicates. Bootstraping is further used to characterize the purity of indicator taxa by the consistency with which they are assigned to a particular group and the *reliability* (consistency of significant IndVals scores across bootstrap replicates). TITAN uses standardized IndVals (*z* scores) instead of raw IndVals scores to facilitate consideration of relatively infrequent taxa that exhibit strong responses to nutrients-gradients (Baker & King 2010).

A community threshold is identified at the point along nutrients-gradients at which one observes synchronous change in abundance of many taxa. The synchronous change is ascertained by summing all standardized individual taxa IndVals by response group for each candidate change point. The change points with maximum sums of standardized IndVals are then designated as community thresholds. This method differentiates between taxa responding positively ($_{Z+}$) or negatively ($_{Z-}$) to nutrients gradients. We repeated analyses using several datsets: the first included all sites and species, the second included all sites and only benthic species, the third included all species and only marsh sites, the fourth contained all species and only open-water sites. In each dataset taxa with occurrence in less than 3 samples were omitted from the analysis. The resulting datasets contained 380 diatom taxa in dataset of all diatom taxa, 356 taxa for benthic species dataset, 249 taxa in marsh dataset and 318 taxa in the open water dataset. The relative abundance of these taxa were log(x)-transformed prior to analyses. TITAN was conducted in R (3.2.1; R project for statistical computing, Vienna, Austria). The stability of thresholds solutions was evaluated by examining the shape of cumulative threshold frequencies, the width of quantile intervals around change-point locations, and response shapes of individual taxa. Responses consistent with community threshold were expected to have synchronous changes in many taxa represented by sharp cumulative responses (cumulative threshold frequencies) for many species, with narrow quantile interval/QI around individual species change-points and biologically meaningful taxon responses. Results were compared among different datasets.

C. Results and Discussion

C1:Pore water analysis

Thirty-two out of the total of 96 collected pore water samples from mudflats and vegetated marsh from experimental plots were analyzed for nitrate + nitrite, ammonia, dissolved phosphorus and dissolved silicate (Appendix III). These 32 samples represented four experimental plots, one for each site (northern-southern)/habitat (marsh-mudflat) combination. Although nutrients in general increased in pore-water within those plots were they have been added, there was a high variability in the observed concentrations (Fig. 29) among treatments.

Figure 30 shows that habitat type influenced the final concentrations of nutrients in experimental plots. For example, nitrate only increased in vegetated marshes, while phosphorus increased more in mudflats compared to vegetated marshes and silica showed opposite trend, with a higher increase in vegetated marshes. It appears that concentrations of ammonia and silica were naturally higher in the southern (Tuckerton) site and nitrogen additions were not effective in the northern (Cattus Island) site.

C2:Algal analysis

All 96 algal samples were enumerated. Blue-green algae (cyanobacteria) and diatoms dominated the microscopic algal community in both locations, the Tuckerton Bay and Cattus Island (Figs 31 a, b). The density of cyanobacteria ranged from 2.16×10^5 to 1.72×10^8 cells/cm^3. The density of diatoms ranged from 2.59×10^4 to 2.56×10^6 cells/cm^3. The cell density of green algae varied from 2.58×10^4 to 1.91×10^5 cells/cm^3 (Appendix IV). Six taxa of blue-green algae were recorded in Tuckerton Bay*: Leptolyngbya* sp. 1 TB, *Leptolyngbya* sp. 2 TB, *Phormidium* sp. 1 TB, *Planktothrix* sp. 1 TB, *Pseudoanabaena* sp. 1 TB, and *Merismopedia* spp. (Figs 5-21) and three taxa of microscopic green algae were found: *Ankistrodesmus* spp. (Fig. 22) , *Monoraphidium* spp. and *Scenedesmus* spp. (Figs 34-35). More taxa were found at Cattus Island: *Gomphosphaeria* spp., *Microcystis* spp., *Chroococcus* spp. and *Aphanocapsa* spp. (Figs 37-46). Figure 33 shows that habitat type influenced the total algal abundance in experimental

plots: the algal cell density was higher in the mudflats compared to the vegetated marsh habitat at both experimental sites

Algal cell densities were relatively higher in the southern site (Tuckerton Bay) than in the northern site (Cattus Island) (Fig. 33). The PERMANOVA analysis for differences in cell densities of the four algal divisons within each of two experimental sites showed that only the habitat was a statistically significant factor in both sites (Tables 1 and 2). CCA revealed a significant effect of nitrogen addition on algal divisions' density in the northern site/Cattus Island as N addition explained 7% of total variance of 0.183 (adjusted explained variation is 4.9%) in the algae density data, Si accounts for 4.1%, P explained 3.4% and substrate explained only 2.4% (Table 3). CCA results for southern site/ Tuckerton Bay data showed the similar result with PERMANOVA as substrate accounted for 6.8% of the total variance of 0.126 (adjusted explained variation is 6.8%), Si explained 3.9%, and N explained 1.8% and P explained 0.3% of the variance (Table 4).

C3:Diatom analysis

A total of 421 diatom taxa were found in this study (Appendix II). The Cattus Island diatom community was dominated by *Achnanthes submarina, Fragilaria amicorum, Navicula salinicola, Nitzschia microcephala, Nitzschia pusilla, Planothidium frequentissimum* and *Pseudostaurosiropsis* sp. 4 COAST; while Tuckerton Bay diatom community was dominated by *Adlafia* sp. 4 COAST, *Chamaepinnularia* sp. 4 COAST, *Navicula consentanea*, *Navicula cf. phylleptosoma*, *Navicula salinicola*, *Navicula* sp. 63 COAST, *Nitzschia laevissima*, *Skeletonema* spp. and *Thallasiosira proschkinae* (Fig. 48, Fig. 49).

Results of the PERMANOVA analyses of diatom data either showed that nutrient additions did not have significant influence on diatom assemblage composition both in Cattus Island (Table 5) and Tuckerton Bay sites (Table 12). The same result was obtained by carrying out the CCA analyses. At the Cattus Island site habitat alone accounted for 10.1% and nutrient additions alone for 4.8% of the variance in diatom data (Table 8); at the Tuckerton Bay site the corresponding values were 9.6 and 5.3% (Table 15). The influence of the habitat and the lack of the effect of the nutrient additions are visualized by nMDS plots in Figs 51-54.

The PERMANOVA analyses of diatom subsets for each individual site/habitat combination also did not reveal any significant influence of nutrient additions (Tables 6-7, 11-12). CCA results showed that addition of N explained 3.7% of total variation in species data, P explained 3.9% and Si explained 3.9% on mudflats at Cattus Island site (Table 9). The addition of N explained 3.7% of total variation in species data, P explained 4.3% and Si explained 4.1% on marshes at Cattus Island site (Table 10). In the Tuckerton Bay site the addition of N explained 3.8% of total variation in species data, P explained 3.8% and Si explained 3.6% on mudflats (Table 13). The addition of N explained 3.9% of total variation in diatom data, P explained 3.9% and Si explained 4.4% in the marshes at Tuckerton Bay site (Table 14). Figs 55 to 60 show the nMDS ordinations of the four site/habitat diatom datasets and demonstrate the lack of nutrient effects on the diatom assemblages as the polygons encompassing samples from treatment versus non-treatment plots largely overlap.

C4: Summary of the experimental results

The experiments conducted in Tuckerton Bay and Cattus Island provided important information on the diversity and ecology of algae in the intertidal mudflats and salt marshes in the area, but did not reveal significant response of the algal community to nutrient enrichment. The nutrient diffusion tubes were releasing nutrients to the sediments, although the final concentrations of nutrient in pore water were highly variable and nitrate eventually increased only in vegetated marshes in the southern site. The vegetated marshes of the Tuckerton/southern sites were the least inundated by tides, so one of the reasons for the low observed nitrate concentration in other plots may have resulted from the intense flushing of nitrogen out of sediments.

The microphytobenthos communities in the vegetated and unvegetated marsh habitats of Barnegat Bay were dominated by blue-green algae and diatoms. Blue-green algae had higher cell density compared to diatoms; green algae were present, but in low abundance. Similar findings were reported in a 2-year study of microphytobenthos conducted in Chesapeake Bay (Semcheski

2014). The latter study showed seasonal fluctuation of blue-green algae with particularly high density during warm seasons. Despite of the high abundance of blue-green algae in almost in all samples, diatoms had the highest species richness in our study.

Filamentous blue green algae, such as *Leptolyngbya* sp. 1 TB were common and dominated mudflats as well as vegetated marsh habitats in Tuckerton Bay and Cattus Island. Blue green algae community in Cattus Island was more diverse compared to Tuckerton Bay's community. The filamentous blue green algae were more abundant in mudflats than in vegetated marsh habitat. Higher density of blue green algae in Tuckerton Bay might be associated with higher ammonia concentration measured in the site. Blue green algae prefer to utilize ammonia than any other nitrogen source, such as nitrate (Ohmori et al. 1977, Markou & Georgakakis 2011).

Diatom assemblages of the Tuckerton Bay marshes were dominated by *Adlafia* sp. 4 COAST, *Chamaepinnularia* sp. 4 COAST, *Navicula consentanea*, *Navicula cf. phylleptosoma*, *Navicula salinicola*, *Navicula* sp. 63 COAST, *Nitzschia laevissima*, *Skeletonema* spp. and *Thallasiosira proschkinae.* Little is known about the ecology of these diatoms and some of them have not been described in the literature. The mudflats and salt marshes of Cattus Island were dominated by small diatoms, such as *Achnanthes submarina, Fragilaria amicorum, Navicula salinicola, Nitzschia microcephala, Nitzschia pusilla, Planothidium frequentissimum* and *Pseudostaurosiropsis* sp. 4 COAST. These are common diatoms found in the brackish water, but further study is needed to investigate the identity of *Pseudostaurosiropsis* sp. 4 COAST and other small fragilarioid diatoms found in the samples, such as *Fragilaria* sp. 10 COAST which resembles *Rhaponeis crinigera*. Diatom assemblages in the northern and southern sites are quite different, most probably due to the differences in salinity and grain size of the sediments. The northern site is characterized by lower salinity and higher sand content of the marsh sediments.

Nutrient additions in our experiments did not cause significant changes in diatom and algal assemblage composition. Apparently, the spatial and/or temporal scale of the experiment was insufficient to cause shifts in diatom assemblages. The area of nutrient-enriched plots could have been too small for a community to be sufficiently distinct as it could have been

overwhelmed by cells migrating from adjacent area. The intense flushing of sediments by the tides may have caused insufficient enrichment by nitrogen. It is also possible that much longer chronic enrichment is necessary to cause shift in diatom assemblages. Previous studies of the effects of nutrient enrichment on microphytobenthos conducted in short- and long-term experiments in natural habitat showed variable results. Often, microphytobenthos community composition changes were not obvious, but there was a significant increase of biomass (Sullivan & Daiber 1975, Sullivan 1976, Coleman & Burkholder 1995, Hillebrand & Sommer 1997, Sullivan & Currin, 2000, Grinham, et al. 2011). However, laboratory experiment on unialgal cultures showed that different species respond differently to changes in nutrient concentrations (Admiraal 1977, Admiraal & Peletier 1980).

Considering the lack of the clear diatom response to nutrient additions in the first experiment, the decision was made in consultation with project managers to change the course of this investigation. Instead of the planned additional field experiments, the funds will be used to further analyze to data collected during Year 1 and Year 2 project. Part 3 is describing this work.

C5: Development of diatom nutrient indicators

In coordination with NJ DEP project managers, the following work was performed for the remaining project time. It was decided to (1) conduct the grain-size analysis and then include this parameter into analyses of nutrient and contaminant effects on diatom assemblages, (2) to repeat the analyzes after subsetting data using a variety of criteria, and (3) to identify species with high and low-nutrient affinities using other approaches in addition to inference models.

C5.1 *Environmental conditions influencing diatom assemblages in Barnegat and Great Bays*

Major environmental characteristics of the Barnegat and Great Bays were previously summarized in the Year 1 final report (Potapova et al. 2013). These data were collected in June-July 2012, once from each of 100 sampling sites. When such one-time data are used to draw

conclusions about ecological preferences of diatoms, the question of seasonal and between-year environmental variability inevitably arises. Diatom frustules in sediments accumulate and therefore, integrate environmental conditions over considerable time periods. Water quality parameters, such as nutrient concentrations, may however fluctuate rapidly. Therefore, to exclude spurious results, it is important to evaluate temporal variability of environmental characteristics. Here we used data from the New Jersey DEP Barnegat Bay Targeted Water Monitoring Project (BBTWMP) (June 2011 - June 2013) to assess temporal variability of water quality characteristics in the study area. Data were collected bi-weekly for the BBTWMP project and thus allow for among-months and among-years comparisons. For the four consecutive days in July and another four consecutive days in August 2012, water quality parameters were measured five times throughout the day for the BBTWMP program. These data allow estimating temporal variability of water quality within a day.

The strong north-south environmental gradient in the study area was evident in both calibration dataset collected in June-July 2012 (Figs 61, 62) and in the BBTWMP 2011-2013 dataset (Figs 63, 64). These patterns were consistent throughout the year: for example the north-to-south increase of salinity was consistently observed in various years (Fig. 65) and months (Fig. 66). Fig. 67 shows that there are no significant daily salinity fluctuations at the monitoring sites. The north-south salinity gradient in the study area coincides with the gradient of orthophosphate concentration. This trend was observed in both 2012 calibration dataset (Fig. 62) and in the BBTWMP dataset (Fig. 64).

Inorganic nitrogen concentrations measured as nitrate plus nitrite were generally higher in the northern part of Barnegat Bay, while ammonia and Total Kjeldahl Nitrogen (ammonia and organic nitrogen) were generally higher in the southern part of Barnegat Bay (Figs 61-64). The higher concentration of nitrate plus nitrite were also observed the northern tributaries from Metedeconk River to Toms River in comparison with southern tributaries (Fig. 68). Total inorganic nitrogen (TIN) at the BBTWMP stations of the northern part of the Barnegat Bay was consistently higher than in the rest of the bay (Fig. 69) and there were more pronounced

fluctuations of TIN throughout the day at stations located in the southern part of Barnegat Bay (Fig. 70).

Concentrations of chlorophyll A were typically higher in the northern part of the Bay (Figs 71-72), although relatively high Chlorophyll A values were observed in the southernmost part of the study area (Little Egg Harbor and Great Bay) in June 2012 (Fig. 61). Throughout the year Chlorophyll A values were considerably higher during the second half of the year with the highest values observed in July and August (Fig. 72).

Silica is a major element required for the growth of diatoms. As BBTWMP data indicate, concentrations of dissolved silica were typically higher in the southern part of the Barnegat Bay (Figs 64 and 73) and within-day fluctuations were not extremely large (Figs 64; 73-74).

C5.2 *Re-analysis of the 2012 calibration dataset*

The first recommendation for the re-analysis of the 2012 calibration set data was to subset the data to minimize the effect of environmental factors other than nutrients on diatom assemblage composition. Several ways of subsetting the data were suggested. One was to exclude Great Bay sites that experience most oceanic influence and conduct all analyses for Barnegat bay sites only. The second was to separate marsh and open-water sample sets. The third was to separate high-and low-salinity sites. Another suggestion was to exclude rare taxa using various abundance criteria and also to exclude planktonic taxa, since benthic diatoms presumably are better indicators of environmental conditions at a given site.

C5.2.1 *Exclusion of the Great Bay sites*

The results of the exploratory multivariate analyses (DCA, Figs 75-76) showed that the exclusion of the Great Bay sites did not have a noticeable effect on the relative importance of measured environmental parameters on diatom assemblage composition. As with the entire dataset, the Barnegat Bay-only diatom assemblages were ordinated mostly along the salinity

gradient, which correlated with TDP, Chlorophyll A, and the developed Land-Use gradients. As Table 19 demonstrates, the variables that exerted the highest influence on diatom assemblage composition in the Barnegat Bay-only analysis were the same as in the whole-dataset analysis: in both datasets these were the salinity, TDP, and Chlorophyll A, followed by carbon and nitrogen sediment content. In fact, the influence of salinity became even stronger in the Barnegat Bay-only dataset analysis. This result obtained by CCA analysis was further confirmed by the predictive power of inference models. Although the exclusion of the Great Bay sites slightly improved the strength of the salinity models, it did not improve the quality of TN and TP models (Table 20).

One possible explanation for the lack of a strong response to water nitrogen is its generally low concentrations. For instance, the average concentration of nitrates plus nitrites in the calibration dataset was about 0.007 mg/l (range 0.0003 - 0.14460 mg/l) and the average concentration of ammonia was 0.017 mg/l (range 0.003 - 0.088). Organic nitrogen constituted the largest part of the total nitrogen that had an average value of 0.6 mg/l and the maximum and exceeded 1 mg/l in four samples only (range 0.362 - 2.894 mg/L).

C5.2.2 *Open-water and marsh datasets*

A comparison of ordinations (DCA) between open-water (Fig. 77) and marsh sample sets (Fig. 78) showed that major environmental gradients determining composition of diatom assemblages were the same for both types of environment, The major species gradient in both subsets correlated mostly with salinity, TDP, Chlorophyll A, and the developed Land-Use. The strength of the relationships between diatom assemblages and diatom assemblages declined in both datasets in comparison to 100 sites datasets (compare tables 19 and 21) and the same environmental parameters were had the strongest relationships with diatom assemblages. This CCA analysis result was further confirmed by the predictive power of the inference models. The predictive power of all models, including those for TN and TP declined in both subsets, (Table 22) in comparison with the whole dataset (Table 20), most likely because of the lower number of observations.

C5.2.3 *High- and low-salinity datasets*

The reason of separating data into low- and high-salinity subsets was to minimize the effect of salinity, which exerts the highest influence on diatom assemblage composition. The set of 100 sites was divided into the high- and low-salinity subsets of 50 sites each. The median salinity values separating these subsets was 26.06 ppt. The main result of the subsetting was an increase of the relative importance of the second major gradient in the dataset, which was the gradient of depth, Nsed, Csed and marsh/open-water sites. In the high-salinity subset this secondary gradient became the most important (Fig. 79), while in the low salinity subset its strength became comparable to the other gradients (Fig. 80). The subsetting, did not increase, however, the strength of the diatom assemblage responses to nutrients and other variables associated with human impact (Table 23), therefore, it did not make sense to develop inference models for high- and low salinity data subsets.

C5.2.4 *Exclusion of rare taxa:*

Exclusion of rare taxa that could generate "noise" in the data analyses is often recommended for increasing the strength of the relationships between biological assemblages and environmental variables. The DCA (Figs 81-82) and CCA (Table 24) showed that the exclusion of rare taxa did improve the strength of the relationship between diatom assemblages and some environmental parameters, although the relative importance of various environmental factors did not change. As with the dataset that includes all species, the diatom assemblages were ordinated mostly along the salinity gradient. Although exclusion of rare taxa positively affected the strength of the relationship between diatom assemblages and environmental factors, it did not improve the quality of the salinity, TN and TP inference models (Table 25).

C5.2.5 *Exclusion of planktonic taxa:*

Prior to data analyses, several taxa from planktonic genera such as *Chaetoceros*, *Cyclotella, Thalassiosira* and others were removed from the dataset. Relative abundances of the remaining taxa were re-calculated. As with the entire dataset, the diatom assemblages were ordinated mostly along the salinity gradient, which was highly correlated with TDP, Chlorophyll A, and the developed Land-Use gradients (Fig. 83). The variables that exerted the highest influence on diatom assemblage composition were the same as in the analysis that includes all species, but the strength of the relationships slightly deteriorated. The quality of the salinity, TN and TP inference models did not improve (Table 27).

C5.2.6 *Environmental reconstructions using benthic- only and marsh-only data subsets*

Transfer functions developed from the calibration dataset were applied previously to core data to reconstruct environmental conditions in the marshes (Potapova et al. 2014). One problem with the reconstruction was a large difference in species composition between diatom assemblages found in the calibration dataset in the marsh cores. This negatively affects accuracy of reconstructions. In an attempt to increase the proportion of species common for both calibration and core datasets, we applied here diatom transfer functions for salinity and N sediment developed from the marsh sites subset and benthic diatoms subset. Exclusion of the planktonic taxa did not increase the overlap between calibration and core samples (Fig. 84): the first DCA axis clearly separated the calibration samples that were on average from deeper sites from the core samples that represented high marsh habitats. Therefore, the salinity and nitrogen sediment reconstructions using benthic only datasets showed similar results with the previous reconstruction using original dataset (Figs 86-89, Potapova et al. 2014). When open-water sites were excluded from the calibration dataset, the first DCA axis separated sites along the salinity gradient, so that cores from Barnegat Bay taken from the high vegetated marshes were positioned to the left and the Great Bay core taken form a mudflat was on the right, while the calibration marsh samples were mostly in between these two (Fig. 85). The exclusion of the open-water sites thus led to a better overlap of the calibration and core sample datasets, but the

low number of samples in the marsh calibration datasets did not allow for the development of strong inference models (Table 22). Reconstructions using marsh datasets show slightly different results from those using the inference models based on the 100 sites calibration dataset (Figs 90-93).

C5.2.7 *Comparison of the diatom-inferred N sediment with measured %N in the core samples*

Good correspondence between inferred and measured N sediment was reported in Year 2 final report (Potapova et al. 2014). Correlation coefficient between observed and inferred N sediment for the core BB1 is 0.65 and for the core BB2 it was 0.31 (Fig. 94).

C5.2.8 *Comparison of the diatom-inferred N sediment with water nitrogen concentration monitoring data collected since 1970*

We examined the Barnegat Bay water quality monitoring data provided by NJDEP for the stations located relatively close to the core locations and found only a few data points in 1972 and no observations between 1972 and 1998 (Fig. 95). Therefore, the suggested comparison of the historical water nitrogen concentrations to the inferred nitrogen sediment could not be accomplished.

C5.3 *Diatoms indicative of eutrophication*

C5.3.1 *GAM modeling*

The most abundant diatoms in the surface sediment samples collected in 2012 in the Barnegat and Great Bays were planktonic taxa, such as *Chaetoceros* spp., *Cyclotella atomus* var. *gracilis*, *C. choctawatcheeana*, *Thalassiosira proschkinae* and two benthic diatoms, *Navicula salinicola* and *Planothidium delicatulum* (Fig. 96). GAM models with salinity and TIN as two independent variables were developed for these six species in order to evaluate the importance of inorganic nitrogen in explaining their distribution patterns. *Chaetoceros* spp. and *Cyclotella*

choctawatcheeana showed affinity to the high concentration of TIN and low salinity; *Cyclotella atomus* var. *gracilis*, *Thalassiosira proschkinae* and *Navicula salinicola* preferred high concentration of TIN and high salinity; while *Planothidium delicatulum* was likely to be found in low concentration of TIN and high salinity (Fig. 97).

GAM modeling was also used to study responses of 603 individual diatom species to total nitrogen. Six taxa, *Bacillaria paradoxa, Biremis lucens, Navicula* cf. *korzeniewskii, Nitzschia frigida, Opephora* sp. 13 COAST and *Opephora* sp. 9 COAST, linearly increased with TN. Three taxa, *Achnanthes danica, Cocconeis peltoides* and *Navicula* sp. 33 COAST showed linear decreasing response. There were 33 taxa with sigmoid increasing response and 20 taxa with sigmoid decreasing response, 23 taxa showed unimodal asymmetric response and 22 taxa showed unimodal symmetric response; 12 taxa had bimodal distribution (Appendix IV). Other taxa did not have any definite response to TN. GAM models describing distribution of several taxa that were found to be indicative if either increasing or decreasing Total Nitrogen are also presented in the graphic form in Figs 98 and 99.

C5.3.2 *Indicator species analysis and TITAN*

100 sites dataset

In the 100 site dataset the Total Nitrogen varied from 362 to 2,894 µg/l. TITAN identified 30 taxa from the whole dataset with synchronous decline in response to TN concentration between 450 µg/l and 650 µg/l with the resulting sum ($_{Z-}$) change point of 563.80 µg/l (Figs. 100a, 100c, Table 28). Positive ($_{Z+}$) indicators increased sharply between 700 µg/l to 1400 µg/l resulting in a distinct sum ($_{Z+}$) peak at 766.45 µg/l. Most individual taxa change points overlapped considerably in the 500 to 800 µg/l range, thus providing evidence in support of an ecological assemblages' threshold associated with a transition from a low TN to a high TN community.

Total Phosphorus varied from 19 to 95 µg/l. Nineteen taxa from the whole dataset had asynchronous declines in response to TP concentration between 24 µg/l and 50 µg/l. The

asynchronous distribution of negative taxa change points meant that corresponding maximum of their sum (z-) showed a relatively weak (poorly defined) peak at 23.59 µg/l (Figs. 100b, 100d Table 28). Positive ($_{Z+}$) indicators increased at a wider range from 40 µg/l to 80 µg/l; resulting a relatively weak sum ($_{Z+}$) peak at 73.50 µg/l. Twenty-six taxa had positive association with increasing TP, but taxa change points were widely distributed along the TP gradient.

Nitrogen sediment content varied between 137 and 16347 µg/g of the dry weight. Forty-eight taxa from the whole dataset had relatively asynchronous declines in response to N sediment concentration between 450 µg/g and 4300 µg/g with a relatively weak peak of sum ($_{Z-}$) at 1398.17 µg/g (Figs. 101a, 101c, Table 29). Fewer taxa had positive association with increasing N sediment. Those that did were widely distributed along N sediment gradients; resulting in a poorly defined peak at 6476.20 µg/g.

Total inorganic nitrogen varied from 3.3 to 189.9 µg/l. Twenty-seven taxa from the whole dataset showed synchronous decline in response to TIN concentration between 8 µg/l and 14 µg/l with a sum ($_{Z-}$) change point of 10.75 µg/l (Figs. 101b, 101d, Table 29). The strong synchrony of change in those taxa at low concentration of TIN was consistent with an ecological community threshold. Fewer taxa showed asynchronous increase in response to TIN, with a poorly-defined peak of sum ($_{Z+}$) peak at 63.05 µg/l.

Open-water dataset

In the 66-sites open-water dataset the Total Nitrogen varied from 362 to 1,813 µg/l. Results of TITAN using open water dataset were similar to the results from the whole dataset. The threshold estimate for sensitive taxa was 563.80 µg/l TN and that for the tolerant taxa was 779.30 µg/l. Negative and positive indicator taxa identified using this dataset were the same as identified using the whole dataset (Figs 102a, 102c, Table 30).

Total Phosphorus varied from 19 to 72 µg/l and sediment Nitrogen from 137 to 10,591 µg/g of the dry weight. As with the whole dataset, negative and positive indicators for TP and N sediment had asynchronous declines and increases. Poorly-defined sum (z-) for TP was 34.19 µg/l and sum ($_{Z+}$) was 57.80 µg/l (Figs 102b, 102d, Table 30). There were considerably fewer

sensitive taxa identified for N sediment using this dataset. N sediment sum (z-) showed a poorly defined peak at 445.45 µg/g; sum (z+) at 6929.30 µg/g (Figs 103a, 103c, Table 31).

Total inorganic nitrogen varied from 3.3 to 160.8 µg/l. TITAN identified fewer sensitive and tolerant taxa for the TIN and these taxa were the same as in the previous analysis results using the whole dataset. Strong synchrony of change in sensitive taxa at low concentration of TIN was consistent with the previous analysis results using the whole dataset with the same peak of sum (z-) of 10.80 µg/l (Figs 101b, 101d, Table 31). The results failed to show a distinct threshold for positive or tolerant indicator taxa, the peak value for sum (z+) is the same as sum (z-) at 10.80 µg/l (Figs 103b, 103d, Table 31).

Marsh dataset

In the 34 site marsh dataset the Total Nitrogen varied from 391 to 2,894 µg/l. There were only four negative indicator taxa from the marsh dataset for TN and these taxa were not identified by the previous analysis using the whole dataset and open water dataset, except *Navicula* sp. 102 COAST (Fig.104c). The lack of response of diatom taxa to TN showed in this analysis was obviously due to the low number of samples. There were 12 taxa identified as positive indicator taxa and these taxa formed the threshold at 765.85 µg/l TN (Figs 104a, 104d, Table 32). There were only several negative and positive indicator taxa from the marsh dataset for N sediment identified by TITAN which had asynchronous declines and increases. Poorly-defined sum (z-) for N sediment was 5025.25 µg/l and sum ($_{Z+}$) was 7288.02 µg/l (Figs 105a, 105c, Table 33). There were only two negative indicator taxa and two positive indicator taxa from the marsh dataset for TIN identified by TITAN (Fig. 105d).

Benthic dataset

Results of TITAN using dataset from which the planktonic taxa were excluded showed similarity with the results from using the whole dataset. The assemblage-level threshold estimate for sensitive taxa was slightly higher at 617.5 µg/l TN and that for the tolerant taxa was slightly lower at 729.50 µg/l. Twenty-five sensitive taxa were identified (Figs 106a, 106c, Table 34). As with the whole dataset, negative and positive indicators for TP and N sediment had asynchronous

declines and increases. Poorly-defined sum (z-) for TP was 23.59 µg/l and sum ($_{Z+}$) was 73.50 ug/L (Figs 106b, 106d, Table 34). N sediment sum (z-) showed a poorly defined peak at 913.83 µg/g; and sum (z+) was 6476.20 µg/l (Fig. 107a 107c, Table 31).

TITAN identified 21 sensitive and 14 tolerant taxa for TIN. These were the same taxa as those found in the whole dataset analysis. Strong synchrony of change in sensitive taxa at low concentration of TIN was consistent with the previous analysis results using the whole dataset with a similar peak of sum (z-) of 11.15 µg/l (Figs 107b, 107d, Table 35). Tolerant (positive indicator) taxa showed asynchronous increase in response to TIN. The poorly-defined peak of sum (Z+) peak identified in this analysis was the same as in the whole dataset analysis, at 63.05 µg/l (Figs 107b, 107d, Table 35).

D. Summary and Conclusions

The main objective of this project was to determine how the surface sediment diatom assemblages may be used as indicators of ecosystem health in Barnegat Bay and if they can be used for developing nutrient criteria. In the course of the three-year project we studied variation of diatom assemblage composition along major environmental gradients observed in the study area and determined that salinity had an overriding effect on diatoms. The salinity gradient also coincided with the north-south gradient of human impact. This complicated development of the inference models for inferring nutrient concentrations from diatom assemblages. The best model developed for an environmental parameter likely associated with eutrophication was for the Nitrogen content of sediments, but since sediment Nitrogen and Carbon were highly correlated, we could not exclude possibility that diatom assemblage was responding to the total amount of organic matter in sediment, rather than to its nitrogen content.

Nutrient enrichment experiments conducted in vegetated marshes and intertidal mudflats of the Barnegat Bay failed to produce a noticeable shift in the composition of diatom assemblages. It is likely that the temporal and spatial extent of these experiments was insufficient to significantly shift composition of diatom assemblages in this study.

We studied individual responses of diatom species to several nutrient parameters using non-parametric regression and indicator species analysis and identified species indicative of low and high nutrient concentrations. We also identified TP, TN and N-sediment values where diatom assemblages change their species composition with the most abrupt change consistent with the threshold-type response observed in relation to water TN. Although it would be premature to recommend using this threshold value as nutrient criterion, these results represent a starting point for further investigations of biological responses to eutrophication.

We constructed inference models for inferring salinity and sediment nitrogen content from diatom data and applied them to marsh sediment cores. The diatom assemblages in the Barnegat Bay marshes shifted over time towards increased abundance of N-tolerant species. Our analysis revealed composition of diatom assemblages before these dramatic changes took place and the sets of diatom taxa were established that we recommend using as indicators of the "reference" or eutrophic conditions in New Jersey marshes. Shifts were concurrent with pollen zones and likely reflect impacts from European post-settlement activities.

Besides insights into relationships of diatom assemblages to eutrophication, this study generated a wealth of information about diatom flora and ecological preferences of coastal diatoms. These data still await publication in peer-reviewed journals and will serve as the basis of future, more detailed investigations of the ecological patterns and processes in the New Jersey coastal waters.

<u>Major findings of this study include:</u>

- Extremely diverse and abundant diatom assemblages were found in all studied habitats. The total number of species is more than 600 and taxonomic investigations are ongoing to determine the status of many of those taxa.
- Benthic diatom assemblages showed a statistically significant variation along environmental gradients such as salinity, depth, habitat, grain size, nutrients, land-use, and sediment contaminants. Salinity was the strongest determinant of the diatom

assemblage composition and a confounding factor complicating statistical modeling of the diatom responses to nutrients.

- Diatom-based models for inferring salinity and sediment nitrogen content were constructed. Lists of diatom taxa indicative of relatively low and high nutrients and sediment contaminant concentrations were developed.
- The threshold of water TN identified as the value where diatom assemblages changed their composition most abruptly have been identified at about 0.5-0.8 mg/L.
- Microfossils such as diatoms and pollen were analyzed in five sediment cores collected from marshes of Barnegat and Great Bays, in addition to sediment chemistry previously measured. There was a noticeable increase of Ambrosia pollen in the core depth intervals corresponding to 1860s. This increase is a marker of maximal deforestation that is known to occur in New Jersey approximately around 1860s.
- The analysis of diatom data showed that diatom assemblages were changing in all cores towards the prevalence of nitrogen-tolerant species in upper intervals, with major shifts in the mid-19th century and again around 1940s and often in 1980s. Nitrogen trend inferred from diatoms well tracked the actual nitrogen sediment values and we concluded that our ecological characterization of diatom species can be used for reconstructing past and monitoring current environmental conditions in New Jersey lagoonal estuaries. Lists of diatom species indicative of the "reference" and impaired conditions have been developed.

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F. Tables

Table 1. Results of PERMANOVA analyses for differences in cell densities of the four algal divisions among habitat types and nutrient addition treatments at the Cattus Island site.

Variable	Df	Sums of Squares	Mean Squares	F. Model	R^2	Pr(>F)
N	1	0.1785	0.178460	2.1576	0.04247	0.099 .
P	1	0.0209	0.020903	0.2527	0.00497	0.817
Si	1	0.0310	0.031008	0.3749	0.00738	0.694
Habitat types	1	0.2940	0.293958	3.5540	0.06995	0.032 *
N:P	1	0.0161	0.016112	0.1948	0.00383	0.870
N:Si	1	0.0505	0.050477	0.6103	0.01201	0.527
P:Si	1	0.1857	0.185747	2.2457	0.04420	0.111
N:Habitat types	1	0.0694	0.069394	0.8390	0.01651	0.417
P: Habitat types	1	0.0232	0.023225	0.2808	0.00553	0.796
Si: Habitat types	1	0.2472	0.247223	2.9890	0.05883	0.058 .
P:N:Si	1	0.1081	0.108130	1.3073	0.02573	0.248
Residuals	36	2.9776	0.082711		0.70858	
Total	47	4.2022			1.00000	

Table 2. Results of PERMANOVA analyses for differences in cell densities of the four algal divisions among habitat types and nutrient addition treatments at the Tuckerton Bay site.

Variable	Df	Sums of Squares	Mean Squares	F. Model	R^2	Pr(>F)
N	1	0.0149	0.01492	0.1624	0.00369	0.842
P	1	-0.0032	-0.00324	-0.0353	-0.00080	0.992
Si	1	0.0809	0.08094	0.8807	0.02001	0.373
Habitat types	1	0.3624	0.36243	3.9438	0.08962	0.039 *
N:P	1	0.0820	0.08204	0.8928	0.02029	0.397
N:Si	1	0.0466	0.04657	0.5068	0.01152	0.563
P:Si	1	0.0253	0.02529	0.2752	0.00625	0.740
N: Habitat types	1	0.0067	0.00673	0.0733	0.00167	0.938
P: Habitat types	1	0.0394	0.03943	0.4290	0.00975	0.619
Si: Habitat types	1	0.0673	0.06735	0.7329	0.01665	0.459
P:N:Si	1	0.0134	0.01335	0.1453	0.00330	0.860
Residuals	36	3.3084	0.09190		0.81806	
Total	47	4.0442			1.00000	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3. Results of the CCA analysis for differences in cell densities of the four algal divisions among habitat types and nutrient addition treatments at the Cattus Island site.

Explanatory variables	Total variation	Explained variation	Adjusted explained variation	Pseudo-F ratio	P-value
Habitat types	0.183	2.4 %	0.3 %	1.1	0.32
Si	0.183	4.1 %	2.0 %	2.0	0.108
P	0.183	3.4 %	1.3 %	1.6	0.196
N	0.183	7 %	4.9 %	3.4	0.014

Table 4. Results of the CCA analysis for differences in cell densities of the four algal divisions among habitat types and nutrient addition treatments at the Tuckerton Bay site.

Explanatory variables	Total variation	Explained variation	Adjusted explained variation	Pseudo-F ratio	P-value
Habitat types	0.126	8.77 %	6.8 %	4.4	0.004
Si	0.126	3.90 %	1.8 %	1.9	0.154
P	0.126	0.30 %	0 %	0.1	0.930
N	0.126	1.80 %	0 %	0.9	0.432

Table 5. Results of PERMANOVA analyses for differences in diatom assemblage composition among habitat types and nutrient addition treatments at the Cattus Island site.

Variable	Df	Sums of Squares	Mean Squares	F. Model	R^2	Pr(>F)
P	1	0.0782	0.07819	0.7016	0.01307	0.857
N	1	0.0659	0.06585	0.5909	0.01100	0.957
Si	1	0.0830	0.08302	0.7450	0.01387	0.806
Habitat types	1	1.0100	1.01000	9.0628	0.16876	0.001 ***
P:N	1	0.1110	0.11099	0.9959	0.01855	0.398
N:Si	1	0.0829	0.08292	0.7441	0.01386	0.809
P:Si	1	0.1328	0.13282	1.1918	0.02219	0.213
N: Habitat types	1	0.0961	0.09610	0.8623	0.01606	0.625
P: Habitat types	1	0.0946	0.09456	0.8485	0.01580	0.635
Si: Habitat types	1	0.1243	0.12429	1.1153	0.02077	0.265
P:N:Si	1	0.0940	0.09395	0.8431	0.01570	0.667
Residuals	36	4.0120	0.11144		0.67038	
Total	47	5.9847			1.00000	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 6. Results of PERMANOVA analyses for differences in diatom assemblage composition among nutrient addition treatments in the mudflats at the Cattus Island site.

Variable	Df	Sums of Squares	Mean Squares	F. Model	R^2	Pr(>F)
N	1	0.06734	0.067336	0.56554	0.02697	0.982
P	1	0.08329	0.083290	0.69954	0.03336	0.878
Si	1	0.09276	0.092763	0.77910	0.03715	0.739
N:P	1	0.10118	0.101178	0.84977	0.04052	0.615
N:Si	1	0.07367	0.073667	0.61872	0.02950	0.959
P:Si	1	0.10466	0.104656	0.87899	0.04191	0.587
N:P:Si	1	0.06912	0.069120	0.58053	0.02768	0.986
Residuals	16	1.90503	0.119065		0.76292	
Total	23	2.49705			1.00000	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 7. Results of PERMANOVA analyses for differences in diatom assemblage composition among nutrient addition treatments in the marshes at the Cattus Island site.

Variable	Df	Sums of Squares	Mean Squares	F. Model	R^2	Pr(>F)
N	1	0.09461	0.094614	0.85073	0.03819	0.689
P	1	0.08947	0.089468	0.80446	0.03611	0.777
Si	1	0.11455	0.114552	1.03000	0.04623	0.385
N:P	1	0.09489	0.094885	0.85317	0.03830	0.700
N:Si	1	0.07514	0.075140	0.67563	0.03033	0.931
P:Si	1	0.11694	0.116943	1.05150	0.04720	0.358
N:P:Si	1	0.11263	0.112628	1.01270	0.04546	0.402
Residuals	16	1.77944	0.111215		0.71819	
Total	23	2.47767			1.00000	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 8. Results of the CCA analysis for differences in diatom assemblage composition among habitat types and nutrient addition treatments at the Cattus Island site.

Analyses without covariates

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
Habitat types ignoring Nutrients addition	0.149	10.2 %	5.2	0.002
Nutrient addition ignoring Habitat types	0.072	4.9%	0.8	1.000
Habitat types and Nutrient addition	0.220	15.0 %	1.9	0.002
Shared: 0.149+0.072-0.220	0.001	0.1%		
Total inertia	1.465			

Analyses adjusted for covariates

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
Habitat types adjusted for Nutrients addition	0.149	10.1 %	5.1	0.002
Nutrient addition adjusted for Habitat types	0.072	4.8%	0.8	0.992
Habitat types and Nutrient addition	0.220	15.0 %	1.9	0.002

Variation decomposition of the effect of substrate types and nutrients addition

Component	Source	Explained variation	
a	Unique Habitat types	0.149	10.1 %
b	Unique Nutrients addition	0.072	4.8%
c	Shared	0.220	0.1%
d	Residual	0.441	15.1 %
Total		1.465	

Table 9. Results of the CCA analysis for differences in diatom assemblage composition among nutrient addition treatments at the Cattus Island site.

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
N+P addition	0.048	3.3%	0.8	0.988
N+Si addition	0.046	3.2%	0.7	0.998
P+Si addition	0.049	3.3%	0.8	0.976
N addition	0.023	1.5%	0.7	0.990
P addition	0.025	1.7%	0.8	0.896
Si addition	0.024	1.6%	0.8	0.952
N+P+Si addition	0.072	4.9%	0.8	1.000

Table 10. Results of the CCA analysis for differences in diatom assemblage composition among nutrient addition treatments on the mudflats at the Cattus Island site.

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
N+P addition	0.092	7.6%	0.9	0.904
N+Si addition	0.093	7.6%	0.9	0.916
P+Si addition	0.095	7.8%	0.9	0.850
N addition	0.045	3.7%	0.8	0.878
P addition	0.047	3.9%	0.9	0.728
Si addition	0.048	3.9%	0.9	0.730
N+P+Si addition	0.140	11.5 %	0.9	0.940

Table 11. Results of the CCA analysis for differences in diatom assemblage composition among nutrient addition treatments on the marshes at the Cattus Island site.

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
N+P addition	0.096	8.1 %	0.9	0.754
N+Si addition	0.093	7.8 %	0.9	0.840
P+Si addition	0.100	8.4 %	1.0	0.574
N addition	0.044	3.7 %	0.8	0.844
P addition	0.052	4.3 %	1.0	0.420
Si addition	0.049	4.1 %	0.9	0.650
N+P+Si addition	0.145	12.1%	0.9	0.794

Table 12. Results of PERMANOVA analyses for differences in diatom assemblage composition among habitat types and nutrient addition treatments at the Tuckerton Bay site.

Variable	Df	Sums of Squares	Mean Squares	F. Model	R^2	Pr(>F)
P	1	0.0873	0.08734	0.7121	0.01376	0.800
N	1	0.0734	0.07341	0.5985	0.01157	0.921
Si	1	0.0812	0.08116	0.6617	0.01279	0.843
Habitat types	1	1.1860	1.18603	9.6697	0.18688	0.001 ***
P:N	1	0.0703	0.07026	0.5728	0.01107	0.934
N:Si	1	0.0633	0.06326	0.5157	0.00997	0.977
P:Si	1	0.0894	0.08936	0.7285	0.01408	0.765
N: Habitat types	1	0.0659	0.06595	0.5377	0.01039	0.961
P: Habitat types	1	0.0633	0.06329	0.5160	0.00997	0.976
Si: Habitat types	1	0.0685	0.06848	0.5583	0.01079	0.951
P:N:Si	1	0.0825	0.08253	0.6729	0.01300	0.851
Residuals	36	4.4156	0.12265		0.69574	
Total	47	6.3466			1.00000	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 13. Results of PERMANOVA analyses for differences in diatom assemblage composition among nutrient addition treatments in the mudflats at the Tuckerton Bay site.

Variable	Df	Sums of Squares	Mean Squares	F. Model	R^2	Pr(>F)
N	1	0.06391	0.063911	0.48311	0.02433	0.974
P	1	0.06171	0.061713	0.46649	0.02350	0.981
Si	1	0.06457	0.064568	0.48807	0.02458	0.972
N:P	1	0.07429	0.074286	0.56153	0.02828	0.923
N:Si	1	0.05720	0.057204	0.43241	0.02178	0.993
P:Si	1	0.11330	0.113300	0.85644	0.04314	0.494
N:P:Si	1	0.07497	0.074968	0.56669	0.02854	0.913
Residuals	16	2.11666	0.132291		0.80585	
Total	23	2.62661			1.00000	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 14. Results of PERMANOVA analyses for differences in diatom assemblage composition among nutrient addition treatments in the marshes at the Tuckerton Bay site.

Variable	Df	Sums of Squares	Mean Squares	F. Model	R^2	Pr(>F)
N	1	0.07544	0.075444	0.61944	0.02977	0.950
P	1	0.08892	0.088919	0.73007	0.03509	0.856
Si	1	0.08507	0.085067	0.69845	0.03357	0.889
N:P	1	0.07508	0.075083	0.61648	0.02963	0.955
N:Si	1	0.06390	0.063900	0.52465	0.02522	0.994
P:Si	1	0.07462	0.074620	0.61267	0.02945	0.951
N:P:Si	1	0.12225	0.122245	1.00370	0.04824	0.422
Residuals	16	1.94871	0.121794		0.76903	
Total	23	2.53399			1.00000	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '

Table 15. Results of the CCA analysis for differences in diatom assemblage composition among habitat types and nutrient addition treatments at the Tuckerton Bay site.

Analyses without covariates

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
Habitat types ignoring Nutrients addition	0.147	9.6%	4.9	0.002
Nutrient addition ignoring Habitat types	0.081	5.3%	0.8	0.968
Habitat types and Nutrient addition	0.229	14.9%	1.9	0.002
Shared: 0.147+0.081-0.229	-0.001	0%		
Total inertia	1.533			

Analyses adjusted for covariates

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
Habitat types adjusted for Nutrients addition	0.147	9.6%	4.9	0.002
Nutrient addition adjusted for Habitat types	0.081	5.3%	0.8	0.968
Habitat types and Nutrient addition	0.229	14.9 %	1.9	0.002

Variation decomposition of the effect of substrate types and nutrients addition

Component	Source	Explained variation	
a	Unique Habitat types	0.147	9.6%
b	Unique Nutrients addition	0.081	5.3%
c	Shared	-0.001	0%
d	Residual	0.229	14.9 %
Total		1.533	

Table 16. Results of the CCA analysis for differences in diatom assemblage composition among nutrient addition treatments at the Tuckerton Bay site.

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
N+P addition	0.053	3.4%	0.8	0.960
N+Si addition	0.054	3.5%	0.8	0.932
P+Si addition	0.055	3.6%	0.8	0.916
N addition	0.026	1.7%	0.8	0.920
P addition	0.027	1.8%	0.8	0.866
Si addition	0.028	1.9%	0.9	0.746
N+P+Si addition	0.081	5.3%	0.8	0.968

Table 17. Results of the CCA analysis for differences in diatom assemblage composition among nutrient addition treatments in the mudflats at the Tuckerton Bay site.

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
N+P addition	0.094	7.6%	0.9	0.878
N+Si addition	0.092	7.4%	0.8	0.926
P+Si addition	0.092	7.4%	0.8	0.936
N addition	0.047	3.8%	0.9	0.780
P addition	0.048	3.8%	0.9	0.752
Si addition	0.045	3.6%	0.8	0.882
N+P+Si addition	0.139	11.2%	0.8	0.960

Table 18. Results of the CCA analysis for differences in diatom assemblage composition among nutrient addition treatments in marshes at the Tuckerton Bay site.

Explanatory variables	Explained variation		Pseudo-F ratio	P-value
N+P addition	0.103	7.8%	0.9	0.836
N+Si addition	0.108	8.2%	0.9	0.674
P+Si addition	0.109	8.3%	0.9	0.682
N addition	0.051	3.9%	0.9	0.744
P addition	0.052	3.9%	0.9	0.714
Si addition	0.058	4.4%	1.0	0.466
N+P+Si addition	0.160	12.1%	0.9	0.790

Table 19. Strength of the relationships between diatom assemblage composition and environmental variables as measured by the significance of the first CCA axes, the dataset of 100 samples from the Barnegat + Great (100 sites) and Barnegat Bay only (84 sites). Bold: significant at $p < 0.05$.

Environmental variable	All 100 sites		84 Barnegat Bay sites	
	F-ratio	P-value	F-ratio	P-value
Marsh/Open site	**4.0**	**0.002***	**3.2**	**0.002***
Depth, m	**4.0**	**0.002***	**3.3**	**0.002***
Dissolved Oxygen, Log mg/L	**2.1**	**0.002***	**2.7**	**0.002***
pH, Log	**2.1**	**0.002***	**2.6**	**0.002***
Salinity, Log psu	**6.8**	**0.002***	**6.9**	**0.002***
Conductivity, Log µS/cm	**6.8**	**0.002***	**6.8**	**0.002***
Turbidity, Log	**2.3**	**0.002***	**2.0**	**0.002***
Total Suspended Solids, Log mg/L	**1.5**	**0.014**$^{+}$	1.2	0.104^{+}
Chlorophyll A, Log µg/L	**4.1**	**0.002***	**5.3**	**0.002***
Particulate Phosphorus, Log µg P/L	**3.0**	**0.002**$^{+}$	**3.6**	**0.002***
Total Dissolved Phosphorus, Log µg P/L	**5.8**	**0.002***	**5.2**	**0.002***
Total Phosphorus, Log µg P/L	**2.5**	**0.002***	**1.5**	**0.022***
Ammonia, Log µg N/L	**3.0**	**0.002***	**2.8**	**0.002***
Nitrate + Nitrite, Log µg N/L	**2.9**	**0.002***	**3.4**	**0.002**$^{+}$
Total Kjeldahl Nitrogen, Log µg N/L	**1.8**	**0.002**$^{+}$	**1.7**	**0.010**$^{+}$
Total Inorganic Nitrogen, Log µg N/L	**2.1**	**0.002***	**2.1**	**0.002***
Total Nitrogen, Log µg N/L	**1.9**	**0.002**$^{+}$	**1.8**	**0.008**$^{+}$
Carbon sediment, sqrt % µg/g	**4.7**	**0.002***	**4.4**	**0.002***
Nitrogen sediment, sqrt % µg g/g	**4.5**	**0.002***	**4.1**	**0.002***
Phosphorus sediment, sqrt % µg g/g	**2.6**	**0.002***	**2.4**	**0.002***
"Developed" land-use, sqrt %	**2.7**	**0.002***	**3.3**	**0.002**$^{+}$
"Forest" land-use, sqrt %	**1.4**	**0.042**$^{+}$	**1.6**	**0.010**$^{+}$
"Grassland" land-use, sqrt %	1.1	0.176^{+}	1.2	0.094^{+}
"Wetland" land-use, sqrt %	**2.3**	**0.002***	**2.3**	**0.002***
"Agricultural" land-use, sqrt %	1.2	0.128^{+}	1.1	0.212^{+}
"Undeveloped" land-use, sqrt %	**2.9**	**0.002***	**2.6**	**0.002**$^{+}$
"Developed+agricultural" land-use, sqrt %	**2.5**	**0.002***	**3.4**	**0.002**$^{+}$
"Silt", sqrt %	**1.9**	**0.004**$^{+}$		
"Gravel/Sands", sqrt %	1.2	0.142		

Note: *Diatom data log transformed.
$^{+}$Diatom data squared-root transformed.

Table 20. Performance of diatom inference models as estimated by R^2_{boot} value. R^2_{boot} values equal or greater than 0.5 are in bold. WA -Weighed Averaging model, WA-PLS - Weighed Averaging- Partial Least Squares model, ML- Maximum Likelihood model, MAT- Modern Analog Technique model.

Dataset/Variable	**Barnegat Bay + Great Bay dataset**					**Barnegat Bay only dataset**				
	WA		**WA-PLS**	**ML**	**MAT**	**WA**		**WA-PLS**	**ML**	**MAT**
	Inverse	**Classic**				**Inverse**	**Classic**			
Depth	0.42	0.43	0.42	0.44	0.42	0.42	0.43	0.41	0.42	0.38
Salinity, psu	**0.77**	**0.77**	**0.81**	**0.79**	**0.79**	**0.84**	**0.84**	**0.84**	**0.82**	**0.85**
Chlorophyll A, Log µg/L	**0.47**	**0.48**	**0.59**	**0.56**	**0.49**	**0.66**	**0.66**	**0.65**	**0.69**	**0.67**
Total Dissolved Phosphorus, Log µg P/L	**0.65**	**0.65**	**0.65**	**0.69**	**0.70**	**0.61**	**0.61**	**0.60**	**0.65**	**0.65**
Total Phosphorus, Log µg P/L	0.28	0.28	0.28	0.28	0.29	0.06	0.07	0.06	0.14	0.12
Total Nitrogen, Log µg N/L	0.18	0.19	0.19	0.16	0.29	0.18	0.19	0.19	0.16	0.29
Nitrate + Nitrite, Log µg N/L	0.38	0.39	0.39	0.40	0.28	0.38	0.39	0.39	0.40	0.28
Total Inorganic Nitrogen, Log µg N/L	0.27	0.27	0.27	0.25	0.18	0.27	0.27	0.27	0.25	0.18
Particulate Phosphorus, Log µg P/L	0.28	0.29	0.38	0.36	0.25	0.36	0.37	0.37	0.41	0.36
Carbon sediment, Log µg/g	**0.51**	**0.52**	**0.59**	**0.53**	0.47	**0.53**	**0.54**	**0.53**	**0.57**	**0.54**
Nitrogen sediment, Log µg/g	0.49	**0.50**	**0.57**	**0.56**	**0.53**	0.48	0.49	**0.63**	**0.55**	0.49
Phosphorus sediment, Log µg/g	0.29	0.30	0.35	0.35	0.31	0.31	0.32	0.41	0.39	0.28

Table 21. Strength of the relationships between diatom assemblage composition and environmental variables as measured by the significance of the first CCA axes in open-water and marsh datasets. Bold: significant at $p < 0.05$.

Environmental variable	66 open-water sites		34 marsh sites	
	F-ratio	P-value	F-ratio	P-value
Depth, m	**1.4**	**0.028**$^{+}$	2.1	**0.002***
Dissolved Oxygen, Log mg/L	**2.7**	**0.002***	1.2	0.180*
pH, Log	1.4	0.036*	**1.6**	**0.008***
Salinity, Log psu	**5.3**	**0.002***	**3.0**	**0.002***
Conductivity, Log µS/cm	**5.4**	**0.002***	**2.9**	**0.002***
Turbidity, Log	**1.7**	**0.012***	1.3	0.062^{+}
Total Suspended Solids, Log mg/L	1.2	0.106^{+}	1.0	0.312^{+}
Chlorophyll A, Log µg/L	**3.9**	**0.002***	1.6	**0.010**$^{+}$
Particulate Phosphorus, Log µg P/L	**3.1**	**0.002***	1.4	**0.030**$^{+}$
Total Dissolved Phosphorus, Log µg P/L	**4.3**	**0.002***	**2.9**	**0.002***
Total Phosphorus, Log µg P/L	**2.0**	**0.002***	**1.5**	**0.014**$^{+}$
Ammonia, Log µg N/L	**3.1**	**0.002***	1.1	0.260*
Nitrate + Nitrite, Log µg N/L	**2.0**	**0.002***	**2.1**	**0.002***
Total Kjeldahl Nitrogen, Log µg N/L	1.4	0.056^{+}	1.2	0.176*
Total Inorganic Nitrogen, Log µg N/L	**1.9**	**0.002***	1.4	**0.038***
Total Nitrogen, Log µg N/L	**1.4**	**0.044**$^{+}$	1.2	0.142^{+}
Carbon sediment, sqrt % µg/g	**2.8**	**0.002***	**1.8**	**0.002***
Nitrogen sediment, sqrt % µg g/g	**2.7**	**0.002***	**2.0**	**0.002**$^{+}$
Phosphorus sediment, sqrt % µg g/g	**2.2**	**0.002***	1.0	0.580^{+}
"Developed" land-use, sqrt %	**3.0**	**0.002***	**1.7**	**0.002**$^{+}$
"Forest" land-use, sqrt %	1.4	**0.016**$^{+}$	1.2	0.090^{+}
"Grassland" land-use, sqrt %	1.0	0.470^{+}	1.0	0.344*
"Wetland" land-use, sqrt %	**1.8**	**0.006***	**1.5**	**0.026**$^{+}$
"Agricultural" land-use, sqrt %	1.4	**0.030**$^{+}$	0.8	0.808*
"Undeveloped" land-use, sqrt %	**2.7**	**0.002***	**1.8**	**0.002**$^{+}$
"Developed+agricultural" land-use, sqrt %	**2.8**	**0.002***	**1.7**	**0.004**$^{+}$
"Silt", sqrt %	**2.0**	**0.004***		
"Gravel/Sands", sqrt %	**2.1**	**0.002***		

Note: *Diatom data log transformed.
$^{+}$Diatom data squared-root transformed.

Table 22. Performance of diatom inference models as estimated by R^2_{boot} value. Values equal or greater than 0.5 are in bold. WA - Weighed Averaging model, WA-PLS - Weighed Averaging- Partial Least Squares model, ML- Maximum Likelihood model, MAT- Modern Analog Technique model.

Dataset/Variable	**Open water**					**Marsh**				
	WA		**WA-PLS**	**ML**	**MAT**	**WA**		**WA-PLS**	**ML**	**MAT**
	Inverse	**Classic**				**Inverse**	**Classic**			
Depth	0.17	0.18	0.17	0.20	0.17	0.27	0.28	0.29	0.34	0.29
Salinity, psu	**0.80**	**0.80**	**0.80**	**0.77**	**0.83**	**0.66**	**0.66**	**0.73**	**0.69**	**0.56**
Chlorophyll A, Log µg/L	**0.62**	**0.63**	**0.62**	**0.65**	**0.68**	**0.19**	**0.19**	**0.25**	**0.17**	**0.04**
Total Dissolved Phosphorus, Log µg P/L	**0.58**	**0.58**	**0.58**	**0.57**	**0.56**	**0.72**	**0.72**	**0.78**	**0.80**	**0.67**
Total Phosphorus, Log µg P/L	0.18	0.18	0.17	0.22	0.20	0.21	0.22	0.28	0.27	0.14
Particulate Phosphorus, Log µg P/L	0.41	0.42	0.41	0.35	0.47	0.13	0.14	0.17	0.08	0.04
Carbon sediment, Log µg/g	0.46	0.46	0.46	0.29	0.45	0.25	0.26	0.27	0.28	0.17
Nitrogen sediment, Log µg/g	0.45	0.45	0.45	0.35	0.41	0.26	0.26	0.27	0.27	0.15
Phosphorus sediment, Log µg/g	0.40	0.41	0.42	0.25	0.39	0.11	0.11	0.04	0.10	0.25
“Developed” land-use, sqrt %	0.36	0.35	0.35	0.35	0.44	0.11	0.12	0.13	0.24	0.21
“Developed” land-use, sqrt %	0.33	0.33	0.33	0.35	0.31	0.32	0.32	0.32	0.32	0.39

Table 23. Strength of the relationships between diatom assemblage composition and environmental variables as measured by the significance of the first CCA axes, two datasets from 50 high salinity sites and 50 low salinity sites . Bold: significant at $p < 0.05$.

Environmental variable	High salinity		Low salinity	
	F-ratio	P-value	F-ratio	P-value
Marsh/Open site	**1.9**	**0.002***	**3.5**	**0.002***
Depth, m	**2.0**	**0.002***	**3.5**	**0.002***
Dissolved Oxygen, Log mg/L	1.2	0.116^{+}	1.2	0.210*
pH, Log	1.0	0.416^{+}	**2.0**	**0.002***
Salinity, Log psu	**1.3**	**0.008**$^{+}$	**2.9**	**0.002***
Conductivity, Log µS/cm	1.3	0.078^{+}	**2.9**	**0.002***
Turbidity, Log	**1.6**	**0.012**$^{+}$	**2.9**	**0.002***
Total Suspended Solids, Log mg/L	1.8	0.918^{+}	**2.4**	**0.002***
Chlorophyll A, Log µg/L	**2.1**	**0.002***	**1.7**	**0.006**$^{+}$
Particulate Phosphorus, Log µg P/L	**1.5**	**0.016**$^{+}$	**2.1**	**0.002***
Total Dissolved Phosphorus, Log □g	**1.6**	**0.008**$^{+}$	**3.3**	**0.002***
Total Phosphorus, Log µg P/L	**1.6**	**0.018**$^{+}$	**2.4**	**0.002***
Ammonia, Log µg N/L	0.9	0.658^{+}	**2.1**	**0.002***
Nitrate + Nitrite, Log µg N/L	1.1	0.218^{+}	**2.4**	**0.002***
Total Kjeldahl Nitrogen, Log µg N/L	1.3	0.070^{+}	**2.4**	**0.002***
Total Inorganic Nitrogen, Log µg N/L	0.9	0.746^{+}	**2.2**	**0.002***
Total Nitrogen, µg Log N/L	1.3	0.076^{+}	**2.6**	**0.002***
Carbon sediment, Log µg/g	**2.1**	**0.002***	**3.1**	**0.002***
Nitrogen sediment, Log µg/g	**1.9**	**0.002***	**3.1**	**0.002***
Phosphorus sediment, Log µg/g	**2.0**	**0.002***	**1.7**	**0.004***
"Developed" land-use, sqrt %	1.4	0.094^{+}	**3.1**	**0.002***
"Forest" land-use, sqrt %	1.3	0.082^{+}	1.2	0.156^{+}
"Grassland" land-use, sqrt %	1.2	0.144^{+}	1.0	0.498^{+}
"Wetland" land-use, sqrt %	1.2	0.188^{+}	**2.2**	**0.002***
"Agricultural" land-use, sqrt %	1.1	0.182^{+}	**1.4**	**0.024**$^{+}$
"Undeveloped" land-use, sqrt %	1.1	0.314^{+}	**2.0**	**0.002***
"Developed+agricultural" land-use, sqrt %	1.3	0.142*	**2.9**	**0.002***

Note: *Diatom data are log transformed.
$^{+}$Diatom data are squared-root transformed.

Table 24. Strength of the relationships between diatom assemblage composition and environmental variables as measured by the significance of the first CCA axes, the dataset of 100 samples from the Barnegat and Great bays and 84 samples from Barnegat Bay only. Bold: significant at $p < 0.05$. CCAs with species dataset that included only species that reached 1% relative abundance in at least 5 samples.

Environmental variable	All 100 sites		Barnegat Bay Sites	
	F-ratio	P-value	F-ratio	P-value
Marsh/Open site	**5.6**	**0.002***	**4.5**	**0.002***
Depth, m	**5.7**	**0.002***	**4.6**	**0.002***
Dissolved Oxygen, Log mg/L	**2.6**	**0.002***	**3.8**	**0.002***
pH, Log	**2.5**	**0.002***	**3.2**	**0.002**$^{+}$
Salinity, Log psu	**9.7**	**0.002**$^{+}$	**9.7**	**0.002**$^{+}$
Conductivity, Log µS/cm	**9.7**	**0.002**$^{+}$	**9.6**	**0.002**$^{+}$
Turbidity, Log	**2.7**	**0.002***	**2.4**	**0.002***
Total Suspended Solids, Log mg/L	**1.6**	**0.024**$^{+}$	1.3	0.102^{+}
Chlorophyll A, Log µg/L	**5.4**	**0.002**$^{+}$	**7.7**	**0.002**$^{+}$
Particulate Phosphorus, Log µg P/L	**3.7**	**0.002**$^{+}$	**4.9**	**0.002**$^{+}$
Total Dissolved Phosphorus, Log µg P/L	**8.3**	**0.002**$^{+}$	**7.1**	**0.002**$^{+}$
Total Phosphorus, Log µg P/L	**3.3**	**0.002**$^{+}$	**1.7**	**0.026***
Ammonia, Log µg N/L	**3.8**	**0.002***	**3.5**	**0.002***
Nitrate + Nitrite, Log µg N/L	**3.6**	**0.002**$^{+}$	**4.4**	**0.002**$^{+}$
Total Kjeldahl Nitrogen, Log µg N/L	**2.0**	**0.012**$^{+}$	**1.9**	**0.010**$^{+}$
Total Inorganic Nitrogen, Log µg N/L	**2.4**	**0.004***	**2.3**	**0.002***
Total Nitrogen, µm Log N/L	**2.1**	**0.006**$^{+}$	**2.1**	**0.008**$^{+}$
Carbon sediment, sqrt % µg/g	**6.4**	**0.002**$^{+}$	**6.1**	**0.002**$^{+}$
Nitrogen sediment, sqrt % µg/g	**6.2**	**0.002***	**5.6**	**0.002**$^{+}$
Phosphorus sediment, sqrt % µg/g	**3.3**	**0.002***	**3.0**	**0.002***
"Developed" land-use, sqrt %	**3.8**	**0.002**$^{+}$	**4.4**	**0.002**$^{+}$
"Forest" land-use, sqrt %	1.3	0.138^{+}	**1.7**	**0.034**$^{+}$
"Grassland" land-use, sqrt %	1.1	0.340*	1.3	0.144*
"Wetland" land-use, sqrt %	**3.0**	**0.002**$^{+}$	**2.9**	**0.002**$^{+}$
"Agricultural" land-use, sqrt %	1.4	0.108^{+}	1.1	0.250*
"Undeveloped" land-use, sqrt %	**3.8**	**0.002***	**3.3**	**0.002**$^{+}$
"Developed+agricultural" land-use, sqrt %	**3.4**	**0.002**$^{+}$	**4.6**	**0.002**$^{+}$

Note: *Diatom data are log transformed.
$^{+}$Diatom data are squared-root transformed.

Table 25. Performance of diatom inference models as estimated by R^2_{boot} value. Rare species were excluded from the datasets. Values equal or greater than 0.5 are in bold. WA -Weighed Averaging model, WA-PLS - Weighed Averaging- Partial Least Squares model, ML- Maximum Likelihood model, MAT- Modern Analog Technique model.

Dataset/Variable	**Barnegat Bay + Great Bay dataset (rare taxa excluded)**					**Barnegat Bay only dataset (rare taxa excluded)**				
	WA		**WA-PLS**	**ML**	**MAT**	**WA**		**WA-PLS**	**ML**	**MAT**
	Inverse	**Classic**				**Inverse**	**Classic**			
Depth	0.35	0.36	0.35	0.43	0.33	0.42	0.43	0.41	0.42	0.38
Salinity, psu	**0.74**	**0.75**	**0.79**	**0.78**	**0.79**	**0.84**	**0.84**	**0.84**	**0.82**	**0.85**
Chlorophyll A, Log µg/L	**0.43**	**0.44**	**0.52**	**0.46**	**0.51**	**0.66**	**0.66**	**0.65**	**0.69**	**0.67**
Total Dissolved Phosphorus, Log µg P/L	**0.74**	**0.74**	**0.77**	**0.77**	**0.76**	**0.61**	**0.61**	**0.60**	**0.65**	**0.65**
Total Phosphorus, Log µg P/L	0.23	0.24	0.23	0.26	0.30	0.06	0.07	0.06	0.14	0.12
Total Nitrogen, Log µg N/L	0.15	0.16	0.14	0.13	0.11	0.18	0.19	0.19	0.16	0.29
Nitrate + Nitrite, Log µg N/L	0.29	0.31	0.29	0.34	0.28	0.38	0.39	0.39	0.40	0.28
Total Inorganic Nitrogen, Log µg N/L	0.12	0.14	0.12	0.19	0.18	0.27	0.27	0.27	0.25	0.18
Particulate Phosphorus, Log µg P/L	0.25	0.26	0.25	0.31	0.28	0.36	0.37	0.37	0.41	0.36
Carbon sediment, Log µg/g	0.49	0.49	0.49	**0.54**	0.39	**0.53**	**0.54**	**0.53**	**0.57**	**0.54**
Nitrogen sediment, Log µg/g	0.49	0.49	**0.58**	**0.54**	0.36	0.48	0.49	**0.63**	**0.55**	0.49
Phosphorus sediment, Log µg/g	0.28	0.29	0.27	0.36	0.29	0.31	0.32	0.41	0.39	0.28

Table 26. Strength of the relationships between diatom assemblage composition and environmental variables as measured by the significance of the first CCA axes, the dataset of 100 samples from the Barnegat and Great Bays with planktonic diatoms excluded. Bold: significant at $p < 0.05$.

Environmental variable	F-ratio	P-value
Marsh/Open site	**3.7**	**0.002***
Depth, m	**3.7**	**0.002***
Dissolved Oxygen, mg/L	**2.0**	**0.002***
pH, Log	**2.1**	**0.002***
Salinity, Log psu	**5.9**	**0.002***
Conductivity, Log µS/cm	**5.8**	**0.002***
Turbidity, Log	**2.3**	**0.002***
Total Suspended Solids, Log mg/L	**1.4**	**0.026**$^{+}$
Chlorophyll A, Log µg/L	**3.8**	**0.002***
Particulate Phosphorus, Log µg P/L	**2.9**	**0.002**$^{+}$
Total Dissolved Phosphorus, Log µg P/L	**5.0**	**0.002***
Total Phosphorus, Log µg P/L	**2.5**	**0.002***
Ammonia, Log µg N/L	**2.5**	**0.002***
Nitrate + Nitrite, Log µg N/L	**2.4**	**0.002**$^{+}$
Total Kjeldahl Nitrogen, Log µg N/L	**1.8**	**0.002**$^{+}$
Total Inorganic Nitrogen, Log µg N/L	**1.9**	**0.002***
Total Nitrogen, µg Log N/L	**1.9**	**0.002**$^{+}$
Carbon sediment, Log µg/g	**4.4**	**0.002***
Nitrogen sediment, Log µg/g	**4.3**	**0.002***
Phosphorus sediment, Log µg/g	**2.4**	**0.002***
"Developed" land-use, sqrt %	**2.3**	**0.002***
"Forest" land-use, sqrt %	**1.3**	**0.040**$^{+}$
"Grassland" land-use, sqrt %	1.1	0.166^{+}
"Wetland" land-use, sqrt %	**2.2**	**0.002***
"Agricultural" land-use, sqrt %	1.2	0.132^{+}
"Undeveloped" land-use, sqrt %	**2.6**	**0.002***
"Developed+agricultural" land-use, sqrt %	**2.2**	**0.002***

Note: *Diatom data are log transformed.
$^{+}$Diatom data are squared-root transformed.

Table 27. Performance of diatom inference models as estimated by R^2_{boot} value. Values equal or greater than 0.5 are in bold. WA -Weighed Averaging model, WA-PLS - Weighed Averaging-Partial Least Squares model, ML- Maximum Likelihood model, MAT- Modern Analog Technique model.

Dataset/Variable	**Barnegat Bay + Great Bay dataset (benthic)**				
	WA		**WA-PLS**	**ML**	**MAT**
	Inverse	**Classic**			
Salinity, psu	**0.78**	**0.79**	**0.78**	**0.82**	**0.78**
Chlorophyll A, Log µg/L	**0.51**	**0.52**	**0.55**	**0.55**	**0.48**
Total Phosphorus, Log µg P/L	0.26	0.27	0.27	0.28	0.29
Nitrogen sediment, Log µg/g	**0.52**	**0.53**	**0.56**	**0.57**	**0.54**
"Developed" land-use, sqrt %	0.33	0.33	0.33	0.35	0.31

Table 28. TITAN community-level thresholds estimated from diatom taxa responses to TN and TP

Method	All sites (TN, ug/L)						All sites (TP, ug/L)					
	Obs.	5%	10%	50%	90%	95%	Obs.	5%	10%	50%	90%	95%
Titan sum(z-)	563.80	448.15	463.10	573.35	649.30	663.25	23.59	23.29	23.58	29.11	47.84	48.49
Titan sum(z+)	766.45	727.54	729.49	766.45	1364.30	1379.15	73.50	40.50	40.51	65.02	76.46	79.89

Table 29. TITAN community-level thresholds estimated from diatom taxa responses to N sediment and TIN

Method	All sites (N sediment, ug/L)						All sites (TIN, ug/L)					
	Obs.	5%	10%	50%	90%	95%	Obs.	5%	10%	50%	90%	95%
Titan sum(z-)	1398.17	445.45	805.29	1320.51	3246.88	4323.25	10.75	8.05	9.70	10.75	14.15	14.30
Titan sum(z+)	6476.20	4978.82	5048.83	6476.20	9160.52	9624.22	63.05	10.49	11.15	36.30	99.55	125.75

Table 30. TITAN community-level thresholds estimated from diatom taxa responses to TN and TP

Method	Open water sites (TN, ug/L)						Open water sites (TP, ug/L)					
	Obs.	5%	10%	50%	90%	95%	Obs.	5%	10%	50%	90%	95%
Titan sum(z-)	563.80	446.35	455.85	534.90	622.26	642.80	34.19	21.95	23.59	32.99	40.78	46.28
Titan sum(z+)	779.30	658.65	661.85	744.90	1053.85	1195.40	57.81	38.75	40.51	57.81	62.54	64.08

Table 31. TITAN community-level thresholds estimated from diatom taxa responses to N sediment and TIN

Method	Open water sites (N sediment, ug/L)						Open water sites (TIN, ug/L)					
	Obs.	5%	10%	50%	90%	95%	Obs.	5%	10%	50%	90%	95%
Titan sum(z-)	445.45	336.80	421.55	880.70	1573.60	2037.43	10.80	6.64	8.05	10.80	12.65	15.15
Titan sum(z+)	6929.30	2512.95	2512.95	3762.60	6929.30	7466.55	10.80	10.15	10.40	14.65	58.16	87.48

Table 32. TITAN community-level thresholds estimated from diatom taxa responses to TN and TP

Method	Marsh sites (TN, ug/L)						Marsh sites (TP, ug/L)					
	Obs.	5%	10%	50%	90%	95%	Obs.	5%	10%	50%	90%	95%
Titan sum(z-)	709.25	527.90	560.70	672.50	721.85	725.30	29.29	27.24	27.24	32.24	48.20	51.32
Titan sum(z+)	765.85	739.06	749.10	766.25	865.80	907.70	72.55	40.41	40.45	65.89	79.89	80.47

Table 33. TITAN community-level thresholds estimated from diatom taxa responses to N sediment and TIN

Method	Marsh sites (N sediment, ug/L)						Marsh sites (TIN, ug/L)					
	Obs.	5%	10%	50%	90%	95%	Obs.	5%	10%	50%	90%	95%
Titan sum(z-)	5025.25	2325.47	4312.62	5025.25	5200.01	5339.97	10.45	8.15	8.20	12.00	19.45	20.13
Titan sum(z+)	7288.02	6111.27	6406.10	7288.02	10259.33	10438.32	36.30	19.45	22.20	36.30	45.25	57.50

Table 34. TITAN community-level thresholds estimated from diatom taxa responses to TN and TP

Method	All sites-benthic only (TN, ug/L)						All sites-benthic only (TP, ug/L)					
	Obs.	5%	10%	50%	90%	95%	Obs.	5%	10%	50%	90%	95%
Titan sum(z-)	617.5	463.10	465.70	573.35	673.85	649.30	23.59	23.15	23.59	28.51	47.84	47.84
Titan sum(z+)	729.5	706.04	726.75	765.85	1239.35	1379.15	73.50	39.85	40.53	65.02	76.46	78.89

Table 35. TITAN community-level thresholds estimated from diatom taxa responses to N sediment and TIN

Method	All sites-benthic only (N sediment, ug/L)						All sites-benthic only (TIN, ug/L)					
	Obs.	5%	10%	50%	90%	95%	Obs.	5%	10%	50%	90%	95%
Titan sum(z-)	913.83	371.53	447.84	1029.08	3079.25	4312.62	11.15	8.05	9.09	10.75	12.84	14.15
Titan sum(z+)	6476.20	4529.47	5047.05	6476.20	9145.93	9474.01	63.05	11.55	19.65	46.90	99.55	104.05

G. Figures

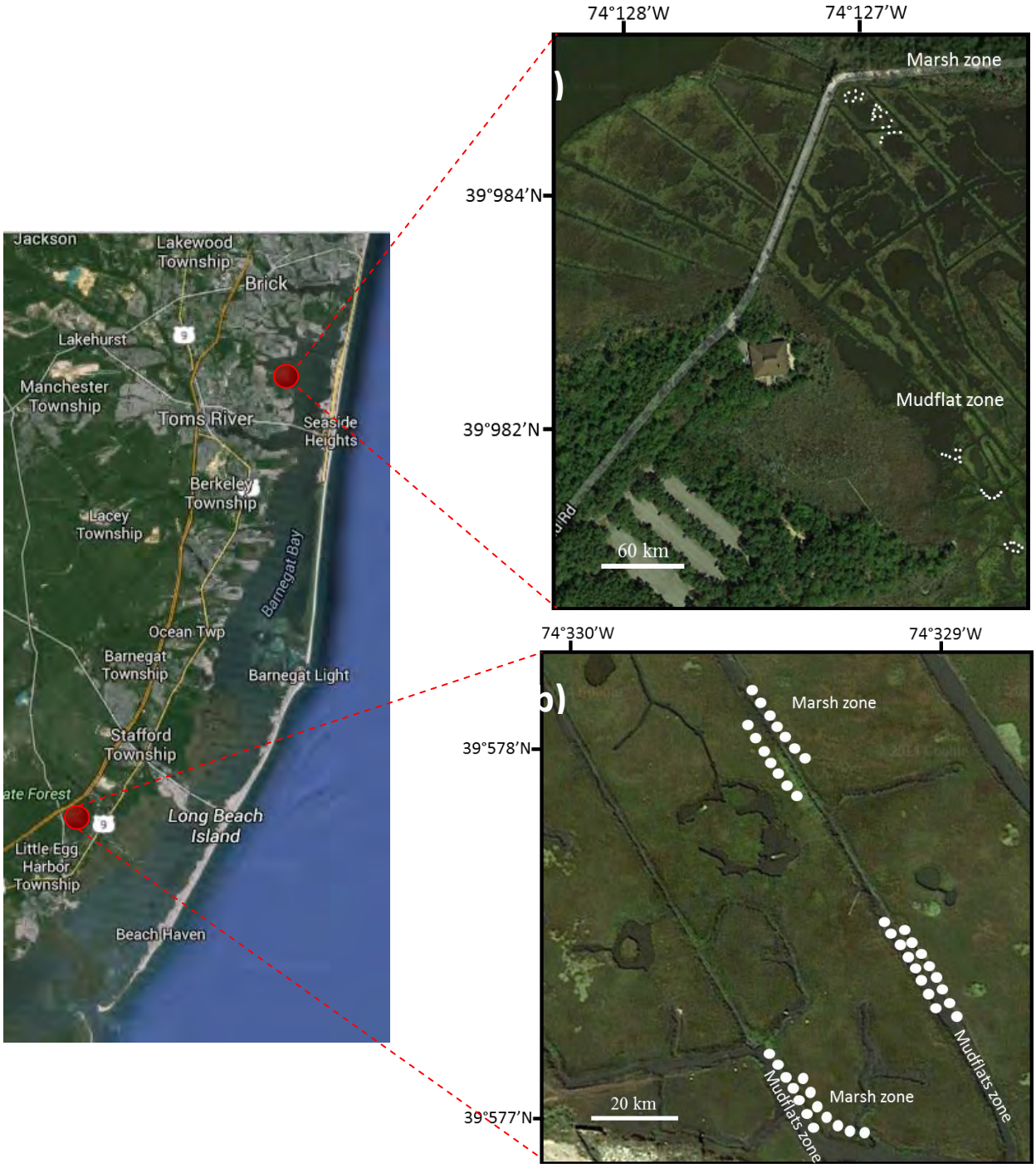


Figure 1. Locations of experimental plots at the Tuckerton (southern) site, August 6-20, 2014 (a) and Cattus Island (northern) site, August 17-31, 2014 (b).

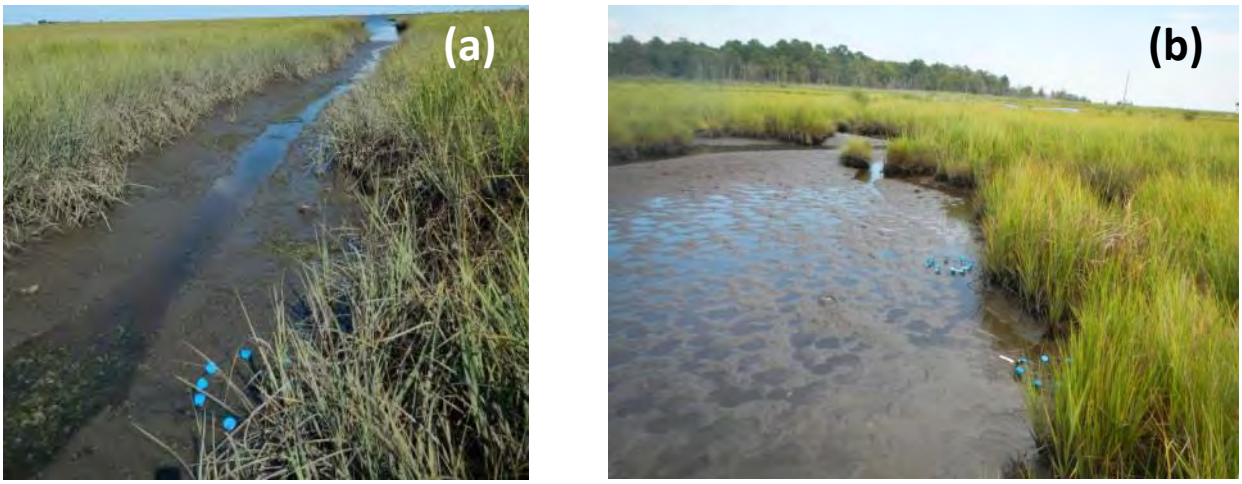
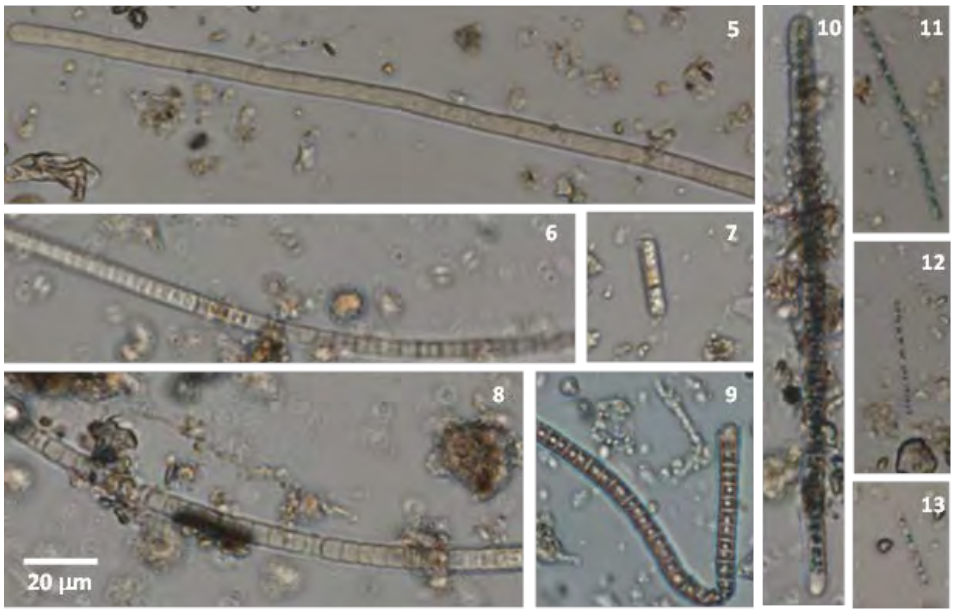


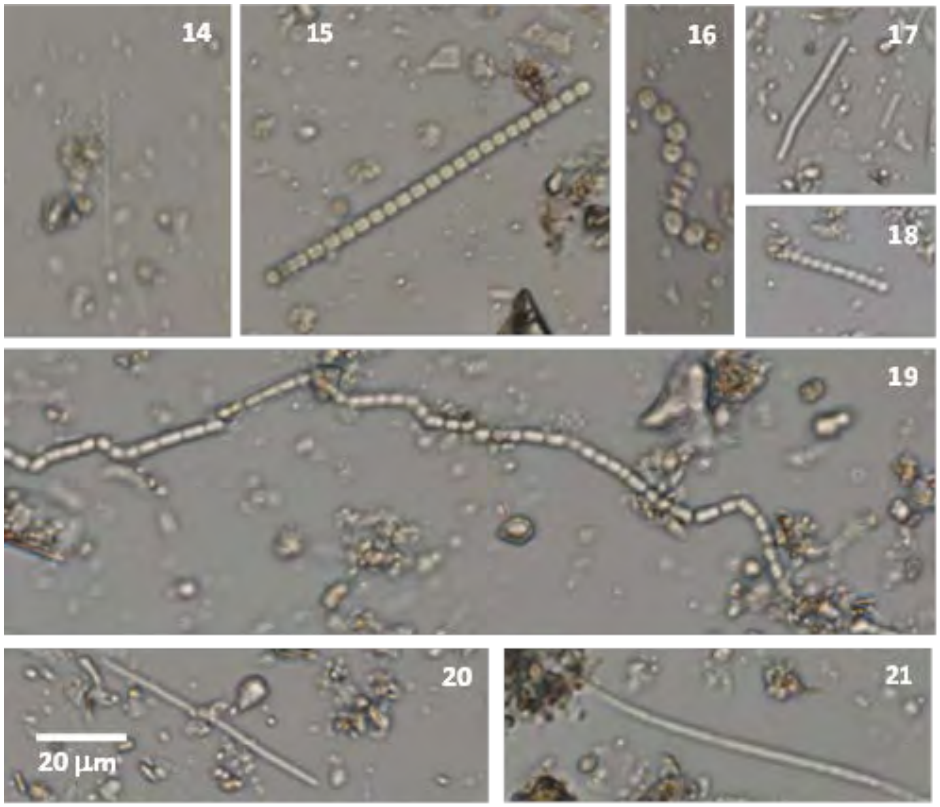
Figure 2. Marsh and ditches with mudflat bottom at the Tuckerton (southern) experimental site (a). Marsh and mudflat at the Cattus Island (northern) experimental site, during low tide (b).

Figure 3. Setting up the experiment at the Tuckerton site, August 6, 2014. Preparation of plant supports with nutrient-diffusing substrates.

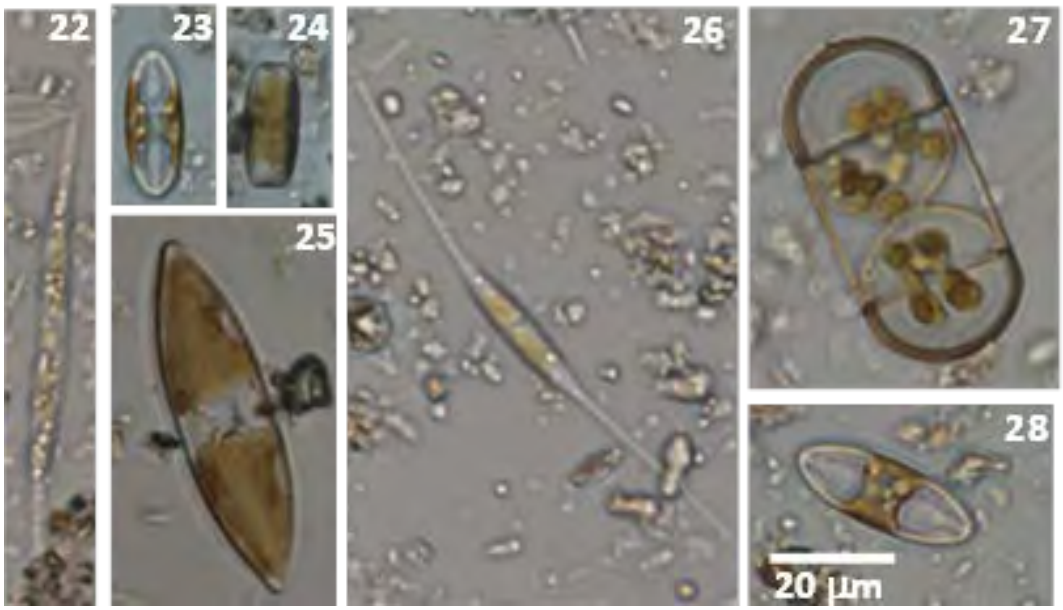
Figure 4. Plant support with attached tubes containing nutrient-enriched agar, anchored in the mudflat at the Tuckerton experimental site, at the end of 2-week exposure, July 2014.



Figures 5-13. Blue-green algae collected from the mudflat at the Tuckerton experimental site in June and July 2014. Figs 5-9. *Phormidium* sp. 1 TB. Figs 10-13. *Planktothrix* sp. 1 TB.



Figures 14-21. Blue-green algae collected from the mudflat at the Tuckerton experimental site in June and July 2014. Figs 14. *Spirulina* sp. 1 TB. Figs 15. *Pseudoanabaena* sp. 2 TB. Figs 16-18. *Pseudoanabaena* sp. 1 TB. Fig. 19. *Leptolyngbya* sp. 2 TB. Figs 20-21. *Leptolyngbya* sp. 1 TB.



Figures 22-28. Green and diatom algae collected from the mudflat at the Tuckerton experimental site in June and July 2014. Fig. 22. *Ankistrodesmus* sp. 1 TB. Figs 23-24, 28. *Fallacia* spp. Fig. 25. *Caloneis* spp. Fig. 26. *Nitzschia closterium.* Fig. 27. *Melosira nummuloides.*

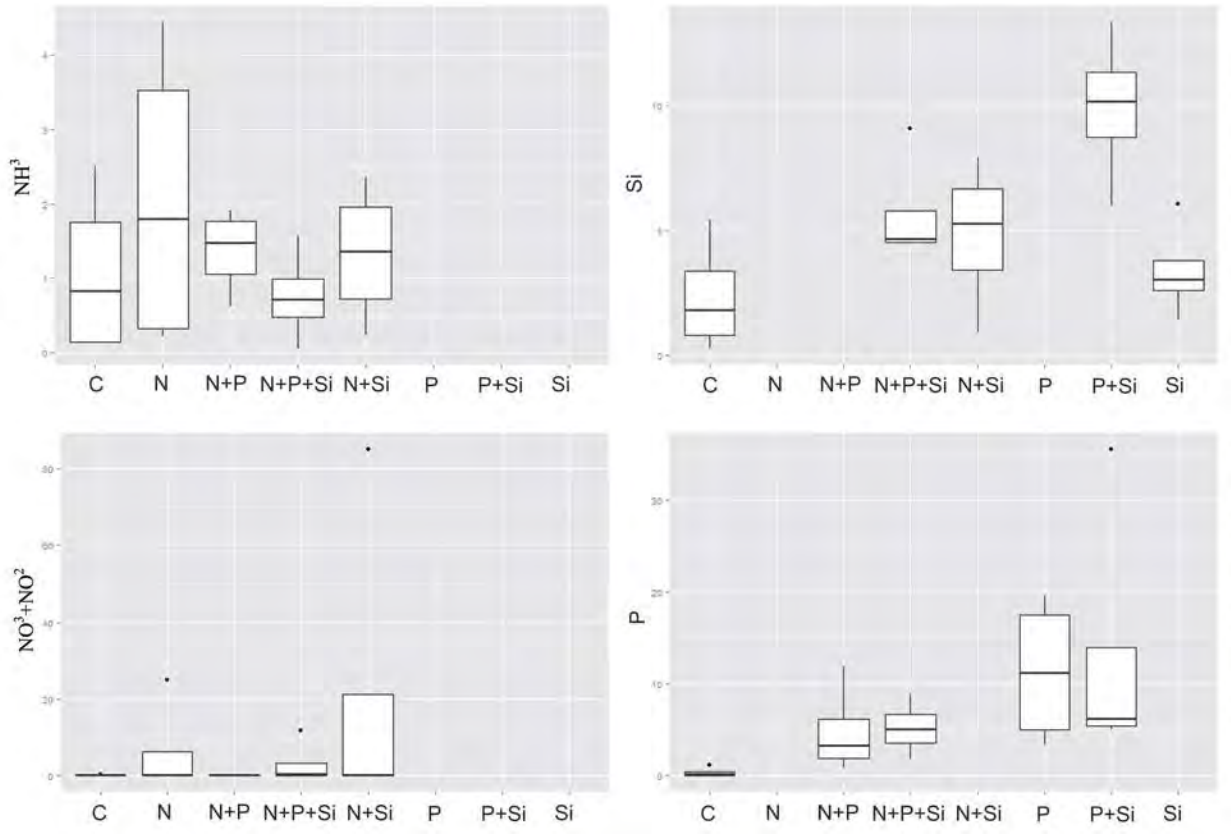


Figure 29. Nutrient concentrations in treatment plots.
Upper left: ammonia (mg/L, NH3); upper right: dissolved silicate (mg/L, Si); lower left: nitrate + nitrite (mg/L, NO3.NO2); lower right: dissolved phosphorus (mg/L, P). Control plots (C), nitrogen (N), phosphorus (P), silica (Si) addition plots, combination of nitrogen and phosphorus (N+P), combination of phosphorus and silica (P+Si), combination of nitrogen and silica (N+Si), combination of nitrogen, phosphorus and silica (N+P+Si) plots; n=4. Samples were collected from mudflats and vegetated marshes from Tuckerton Bay (on 20 August 2014) and Cattus Island (on 31 August 2014). Horizontal line: mean value, box lower and upper limits: 25th and 75th percentiles, vertical lines: range of values.

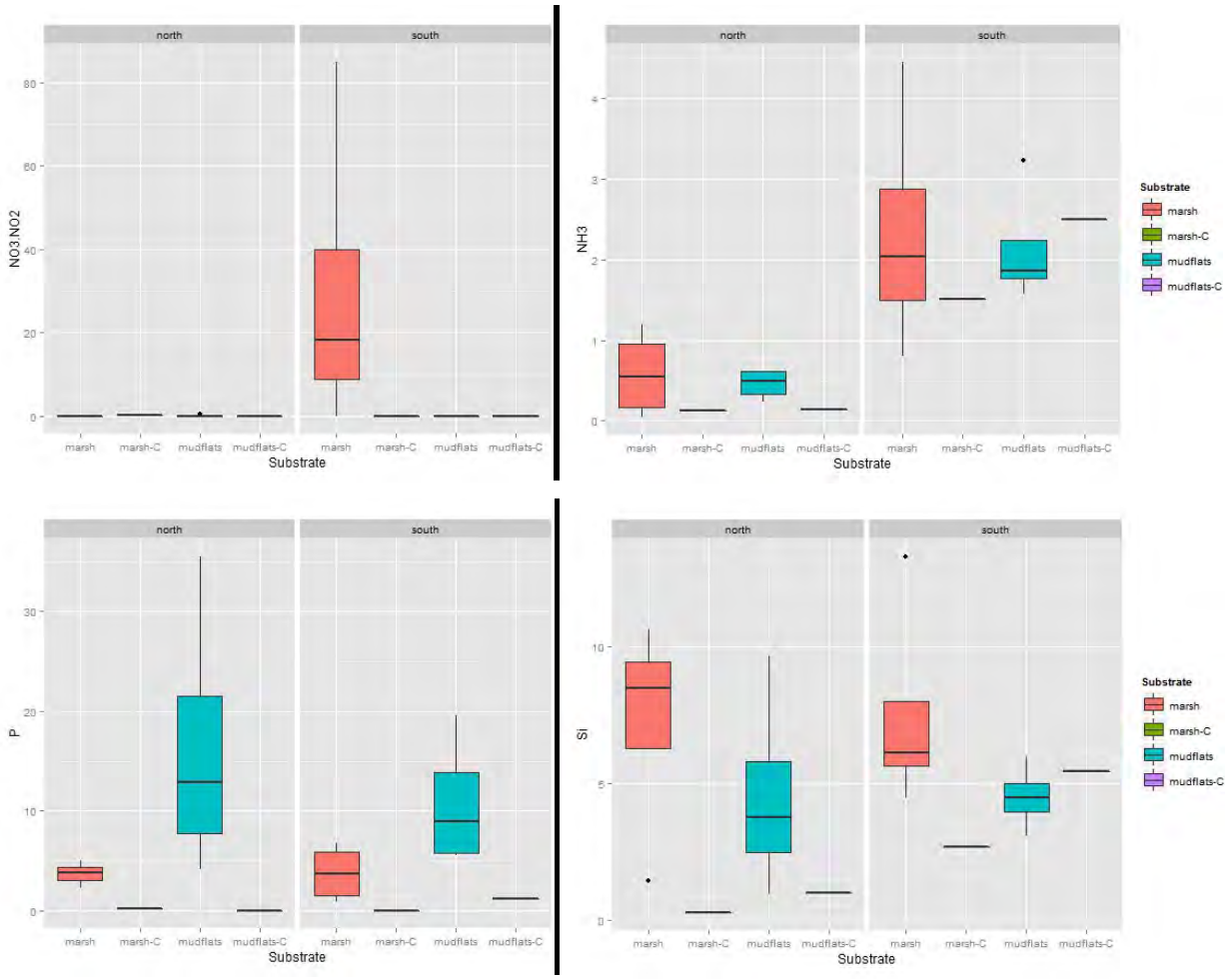


Figure 30. Comparison of pore-water nutrient concentrations among treatment plots in vegetated marshes (marsh), treatment plots in mudflats (mudflats), control plot in vegetated marsh (marsh-C), control plot in mudflats (mudflats-C) in Cattus Island (North) and Tuckerton Bay (South): (a) nitrate + nitrite (mg/L)(NO3.NO2) in nitrogen addition plots (n=4) and a control plot (n=1); (b) ammonia (mg/L) (NH3) in nitrogen addition plots (n=4) and a control plot (n=1); (c) dissolved phosphorus (mg/L)(P) in phosphorus addition plots (n=4) and a control plot (n=1); and (d) dissolved silicate (mg/L) (Si) in silicate addition plots (n=4) and a control plot (n=1). Samples were collected from mudflats in Tuckerton Bay (on 20 August 2014) and Cattus Island (on 31 August 2014) experimental sites.

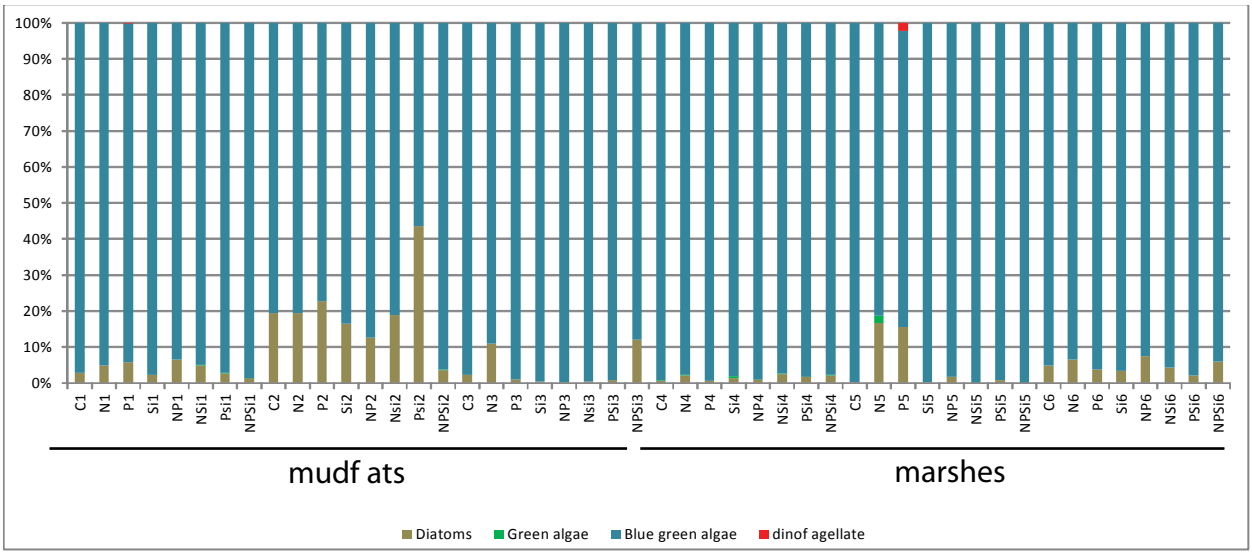


Figure 31 a. Relative abundance of diatoms, green algae, blue green algae and dinoflagellates at the Tuckerton Bay experimental site in control (C), nitrogen addition (N), phosphorus addition (P), silicate addition (Si) and different combination of N, P, Si addition plots. Samples were collected on August 20, 2014.

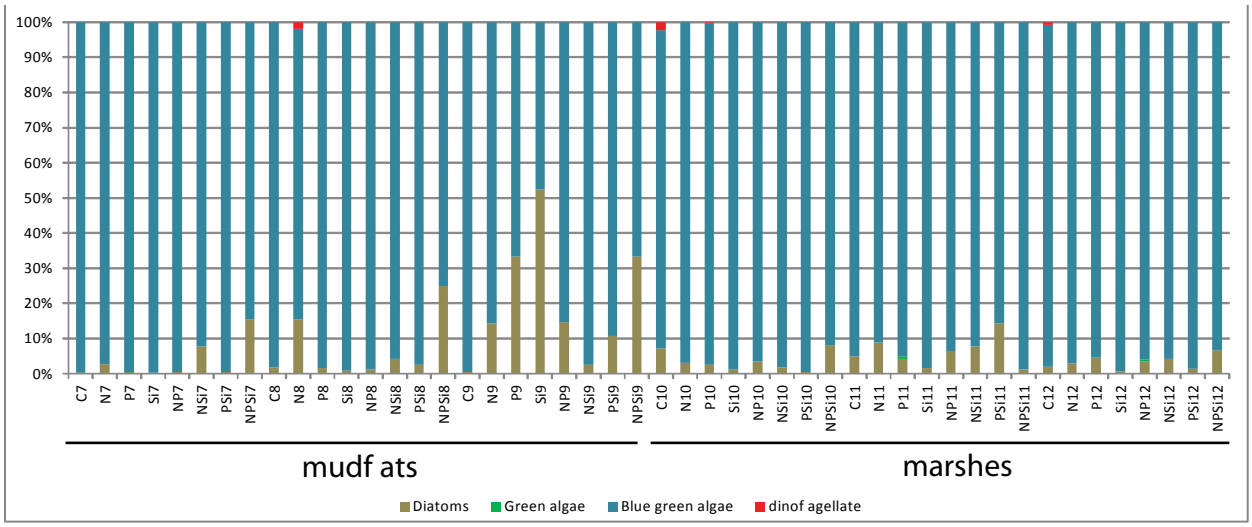


Figure 31 b. Relative abundance of diatoms, green algae, blue green algae and dinoflagellates at the Cattus Island experimental site in control (C), nitrogen addition (N), phosphorus addition (P), silicate addition (Si) and different combination of N, P, Si addition plots. Samples were collected on August 31, 2014.

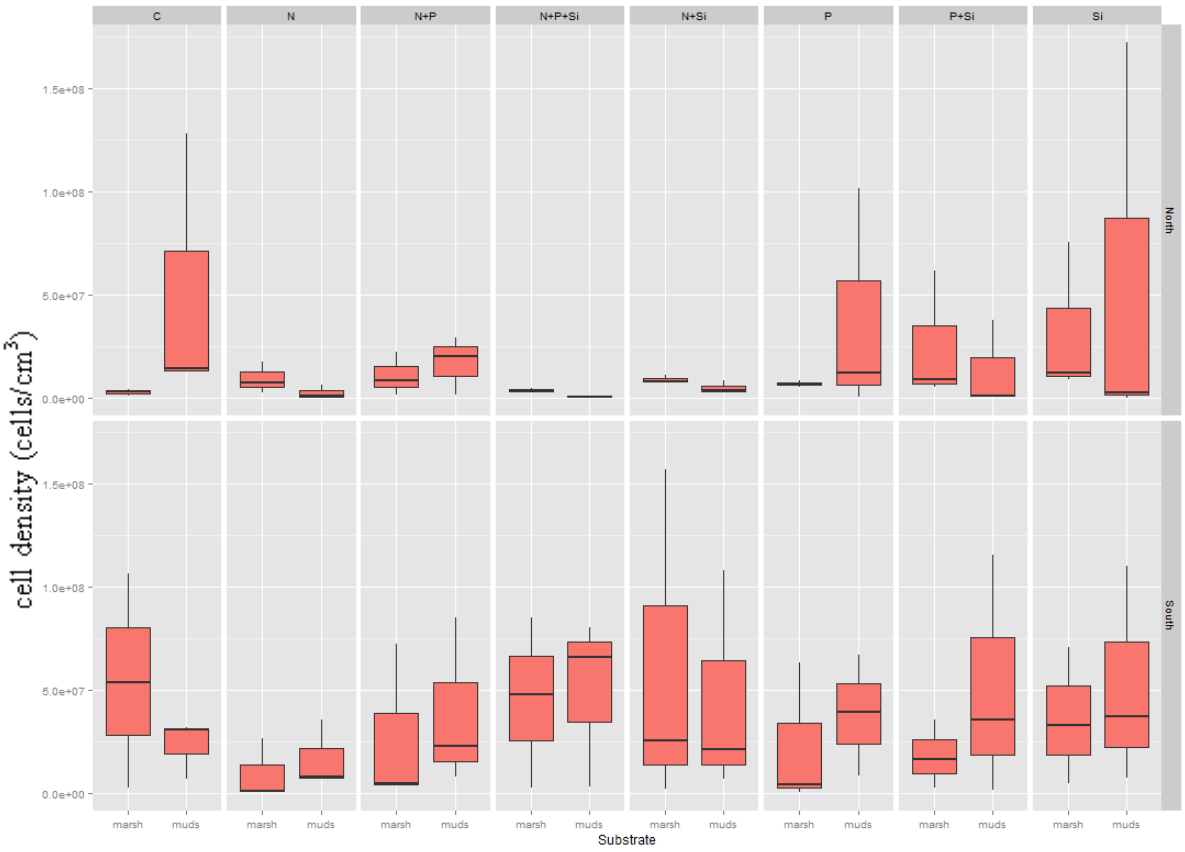


Figure 32. Cell density (cells/cm^3) of blue-green algae in Control, N, N+P, N+P+Si, N+Si, P, P+Si, Si plots in mudflats and vegetated marsh habitats in Cattus Island (North) and Tuckerton Bay (South). N=3.

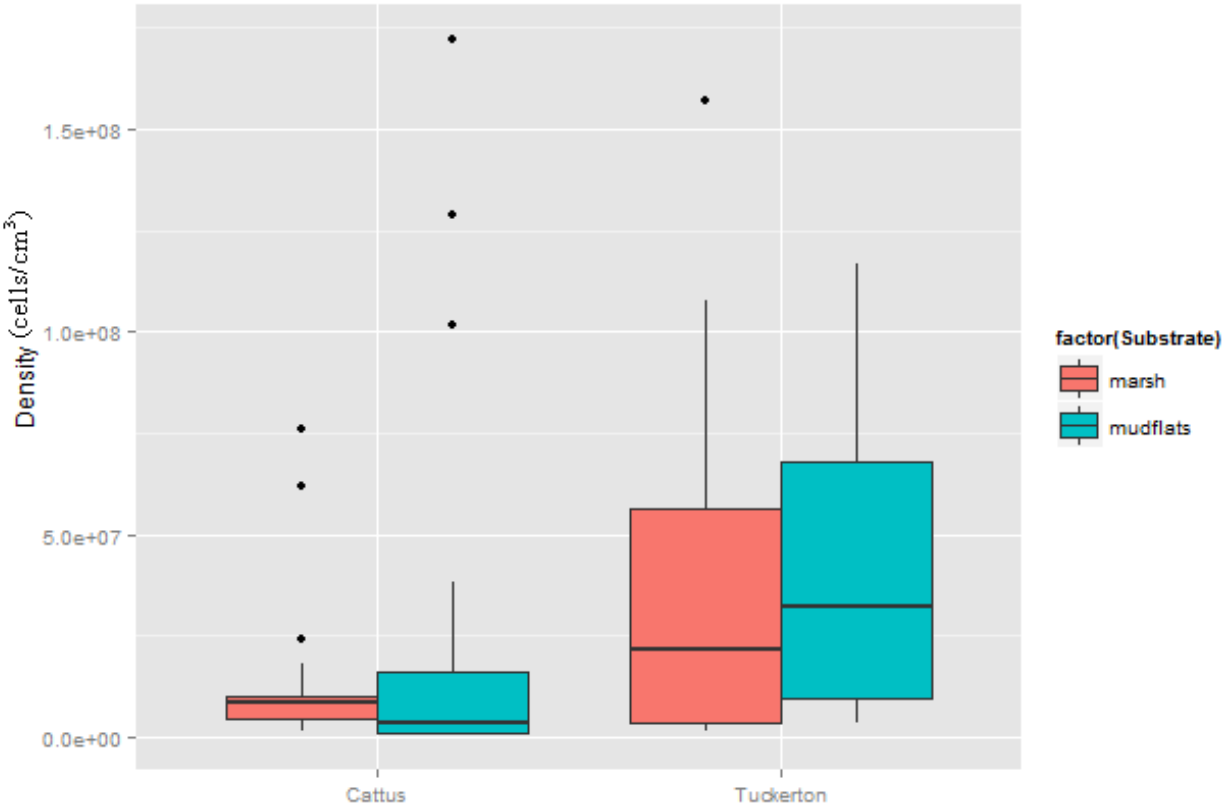
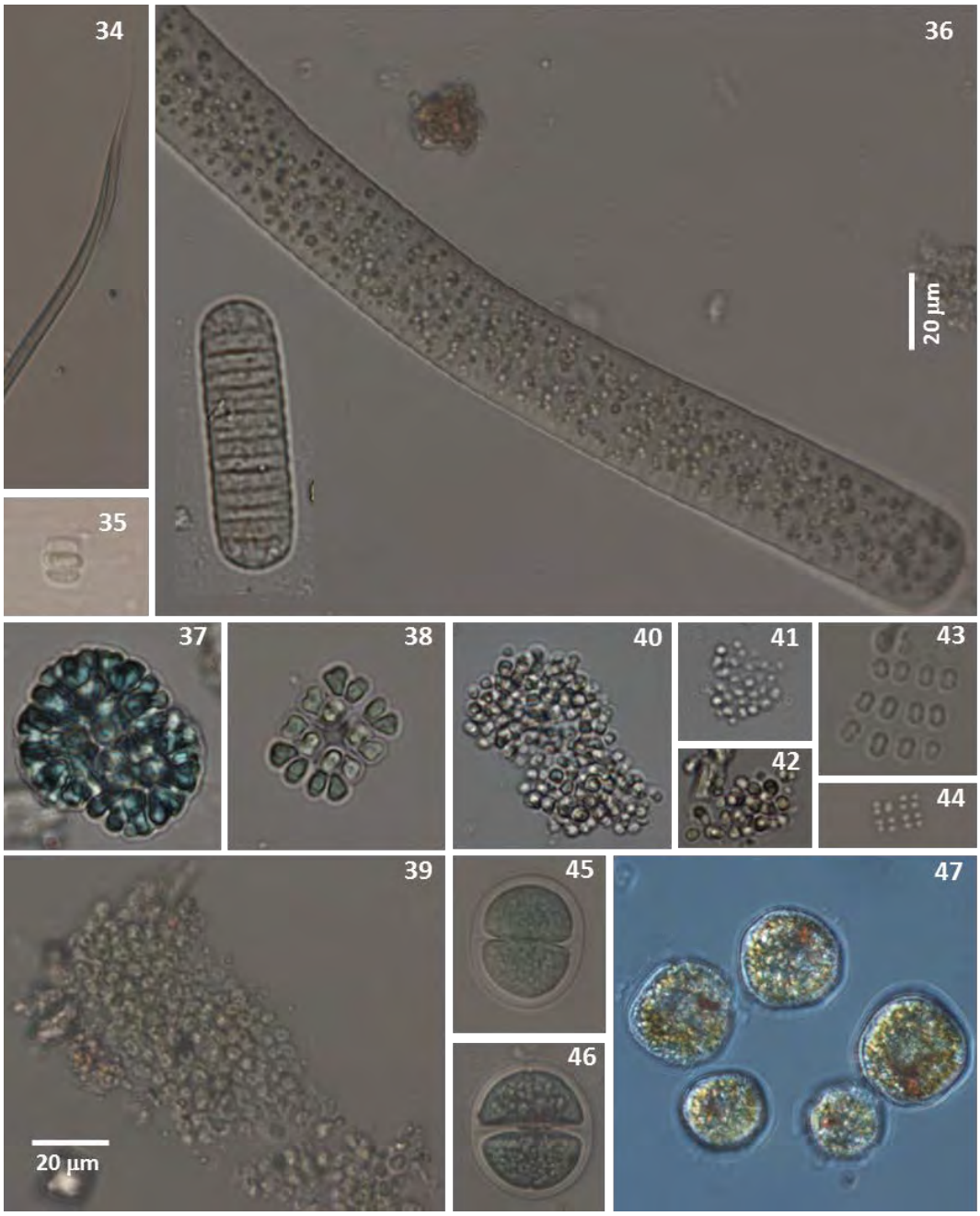


Figure 33. Comparison of microphytobenthos cell density (cells/cm^3) between mudflats and vegetated marsh in Cattus Island (North) and Tuckerton Bay (South). N=24.



Figures 34-47. Green, blue-green and dinoflagellate algae collected from the mudflat at the Cattus Island experimental site on 31 August 2014. Fig. 34. *Monoraphidium* spp. Fig. 35. *Scenedesmus* spp. Fig. 36. *Oscilatoria* spp. Fig. 37-38. *Gomphosphaeria* spp. Figs 39-40. *Microcystis* spp**.** Figs 41-42. *Aphanocapsa* spp**.** Figs 43-44. *Merismopodia* spp. Figs 45-46. *Chroococcus* spp. Fig. 47. *Alexandrium* spp.

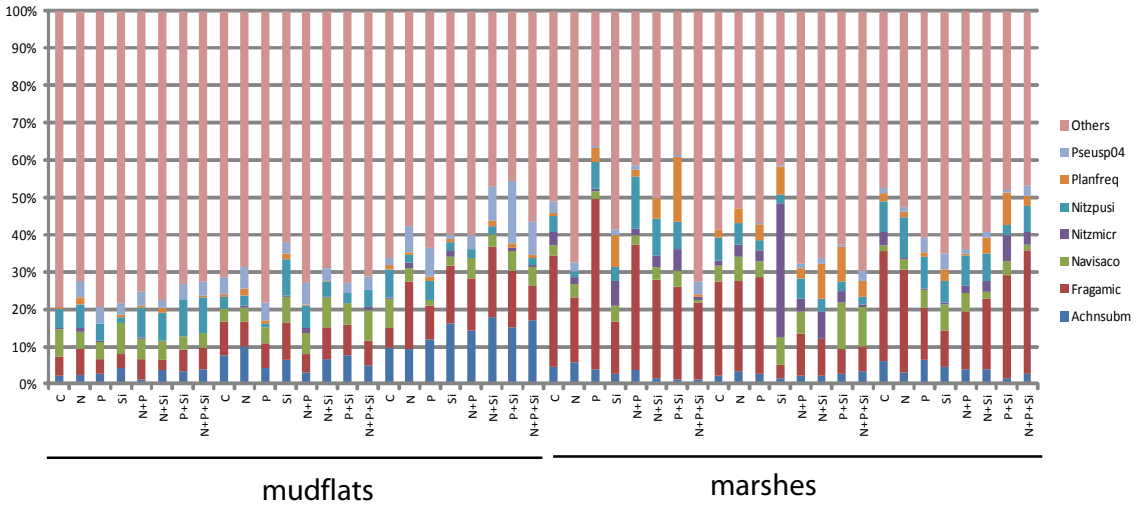


Figure 48. Relative abundance of diatoms (more than 5 percent in at least 7 samples): *Achnanthes submarina* (Achsbm), *Fragilaria amicorum* (Fragamic), *Navicula salinicola* (Navisaco), *Nitzschia microcephala* (Nitzmicr), *Nitzschia pusilla* (Nitzpusi), *Planothidium frequentissimum* (Planfreq), *Pseudostaurosira* sp. 4 COAST (Pseusp04) and other species in the experiment plots in Cattus Island. Samples were collected in 31 August 2014. Three replicates of each treatment were established in each habitat, mudflat or marsh. Control (C), nitrogen addition (N), phosphorus addition (P), silicate addition (Si) and different combinations of N, P, Si additions.

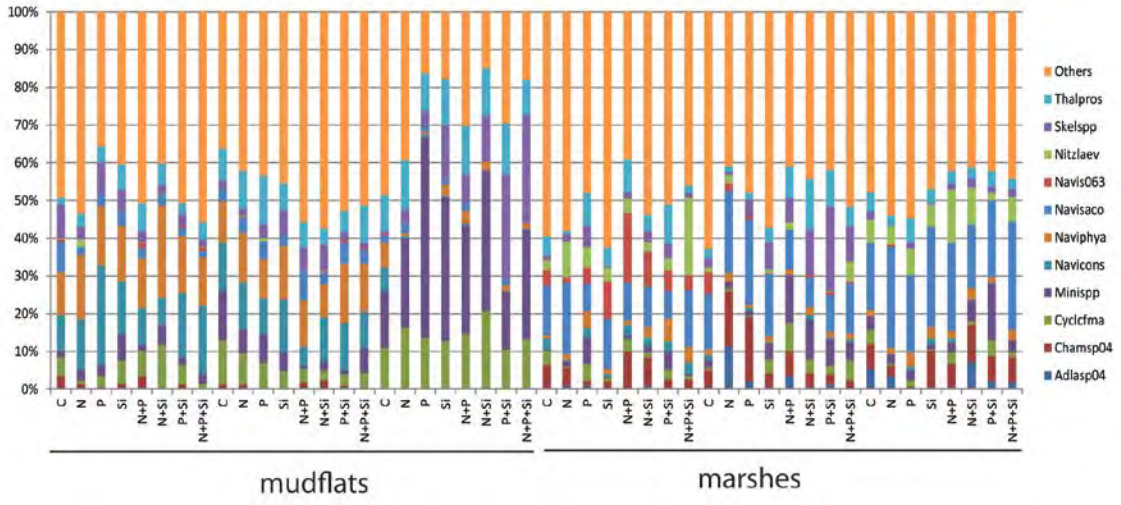


Figure 49. Relative abundance of diatoms (more than 10 percent in at least one sample): *Adlafia* sp. 4 COAST (Adlasp04), *Chammaepinnularia* sp. 4 COAST (Chamsp04), *Navicula consentanea* (Navicons), *Navicula* cf. *phylleptosoma* (Naviphya), *Navicula salinicola* (Navisaco), *Navicula* sp. 63 COAST (Navis063), *Nitzschia laevissima* (Nitzlaev), *Skeletonema* spp. (Skelspp), *Thallasiosira proschkinae* (Thalpros) and other species in the experiment plots in Tuckerton Bay. Samples were collected in 20 August 2014. Three replicates of each treatment were established in each habitat, mudflat or marsh. Control (C), nitrogen addition (N), phosphorus addition (P), silicate addition (Si) and different combinations of N, P, Si additions.

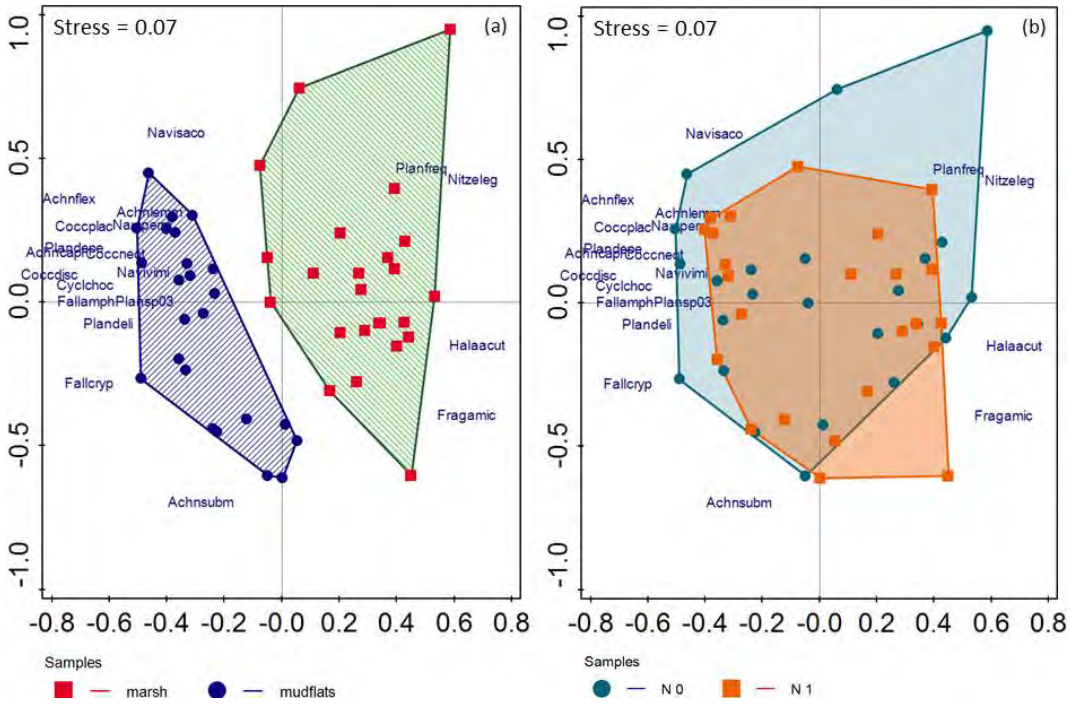


Figure 51. NMDS plots of the 48 samples from the Cattus Island site,
Polygons are drawn around samples from (a) mudflats and marshes and (b) nitrogen addition (N1)vs.non-nitrogen addition plots (N0) (b). Horizontal axis – NMDS 1, Vertical axis- NMDS 2.

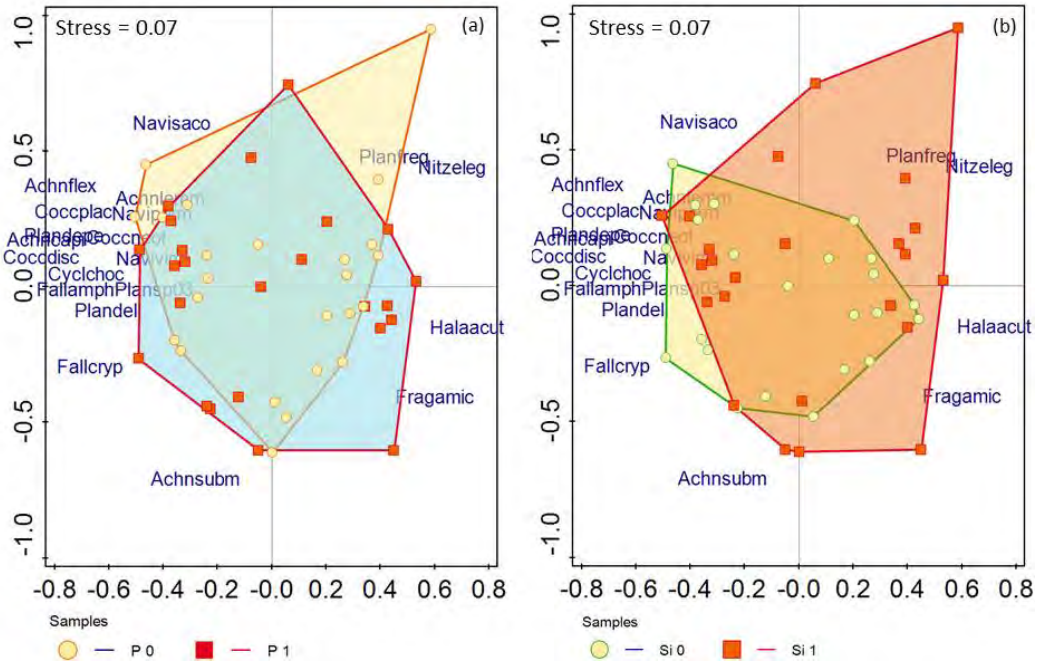


Figure 52. NMDS plots of the 48 samples from the Cattus Island site,
Polygons are drawn around samples (a) from phosphorus addition (P1) vs. non-phosphorus addition plots (P0) and (b) from silica addition \(Si1) vs. no silica addition plots (Si0). Horizontal axis – NMDS 1, Vertical axis- NMDS 2.

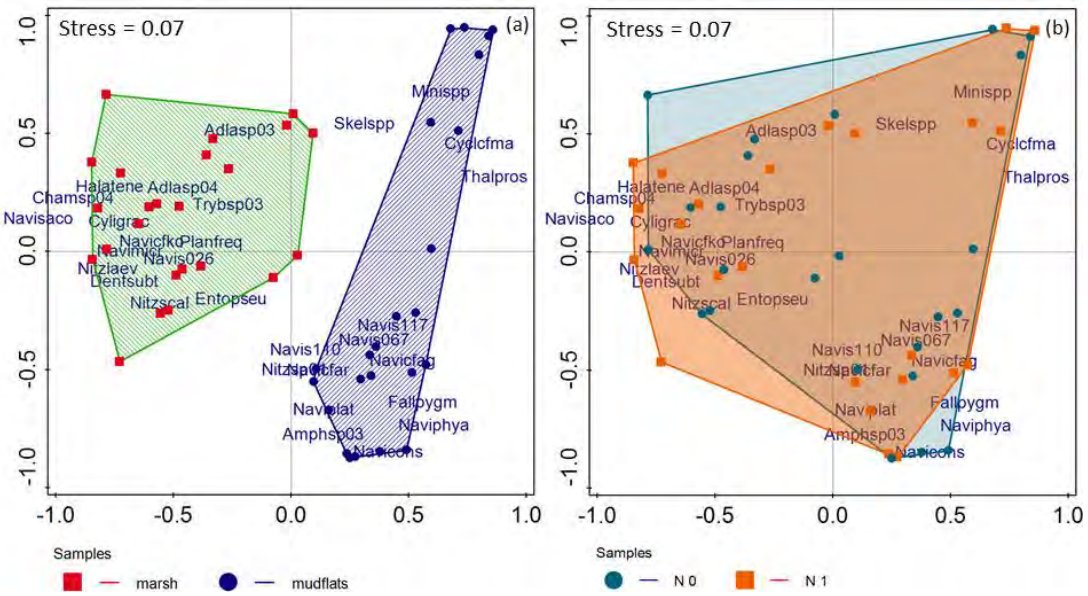


Figure 53. NMDS plots of the 48 samples from the Tuckerton Bay site,
Polygons are drawn around samples from (a) mudflats and marshes and (b) nitrogen addition (N1)vs. non-nitrogen addition plots (N0) (b). Horizontal axis–NMDS 1, Vertical axis- NMDS 2. Species short codes correspond to those in Appendix 6.

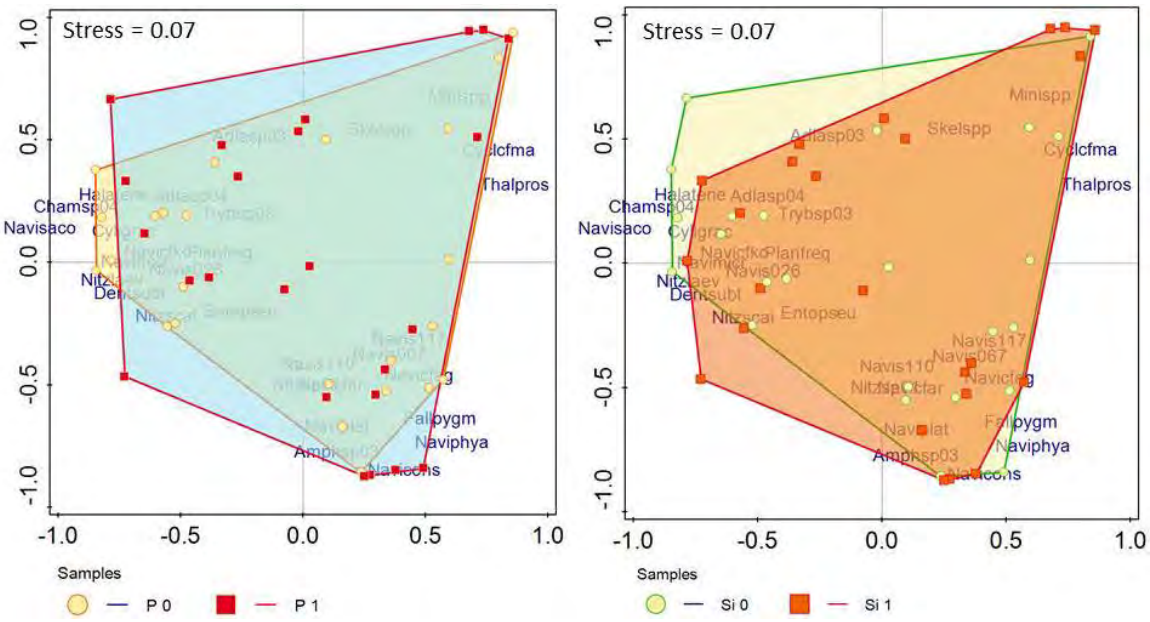


Figure 54. NMDS plots of the 48 samples from the Tuckerton Bay site,
Polygons are drawn around samples (a) from phosphorus (P1) vs. no phosphorus addition plots (P0) and (b) from silica addition \(Si1) vs. no silica addition plots (Si0). Horizontal axis – NMDS 1, Vertical axis- NMDS 2. Species short codes correspond to those in Appendix 6.

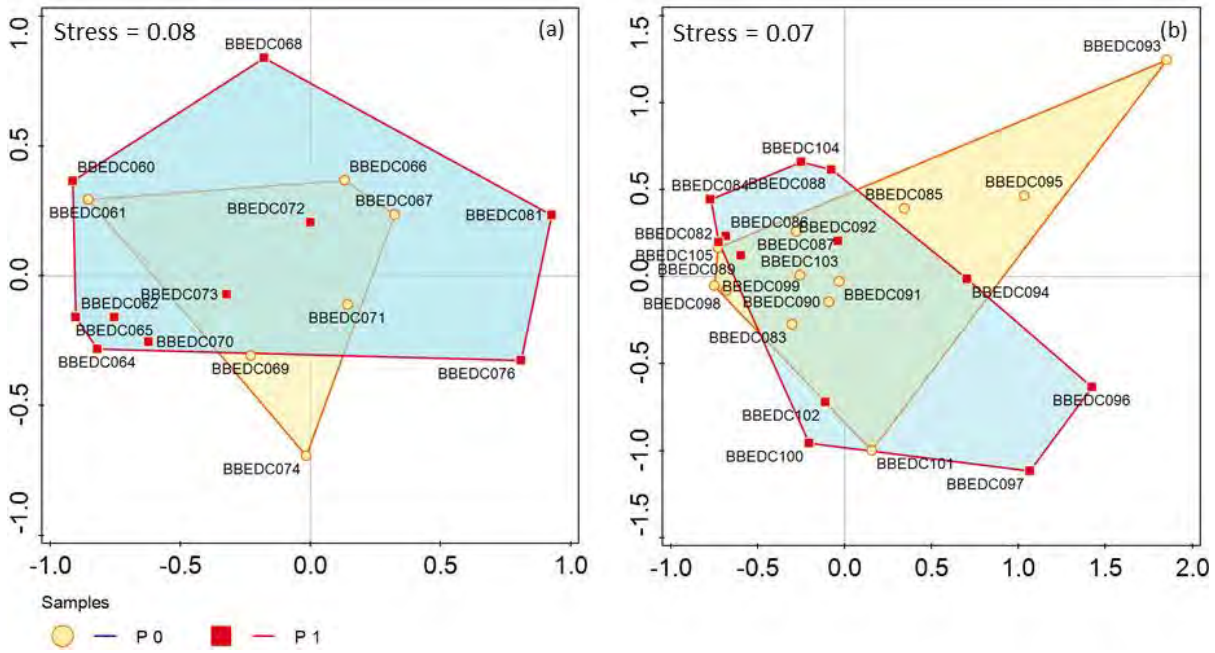


Figure 55. NMDS plots of the 24 samples from the mudflats (a) and marshes (b) at the Cattus Island site. Polygons are drawn around samples from phosphorus (P1) vs. no phosphorus addition plots (P0). Species short codes correspond to those in Appendix 6.

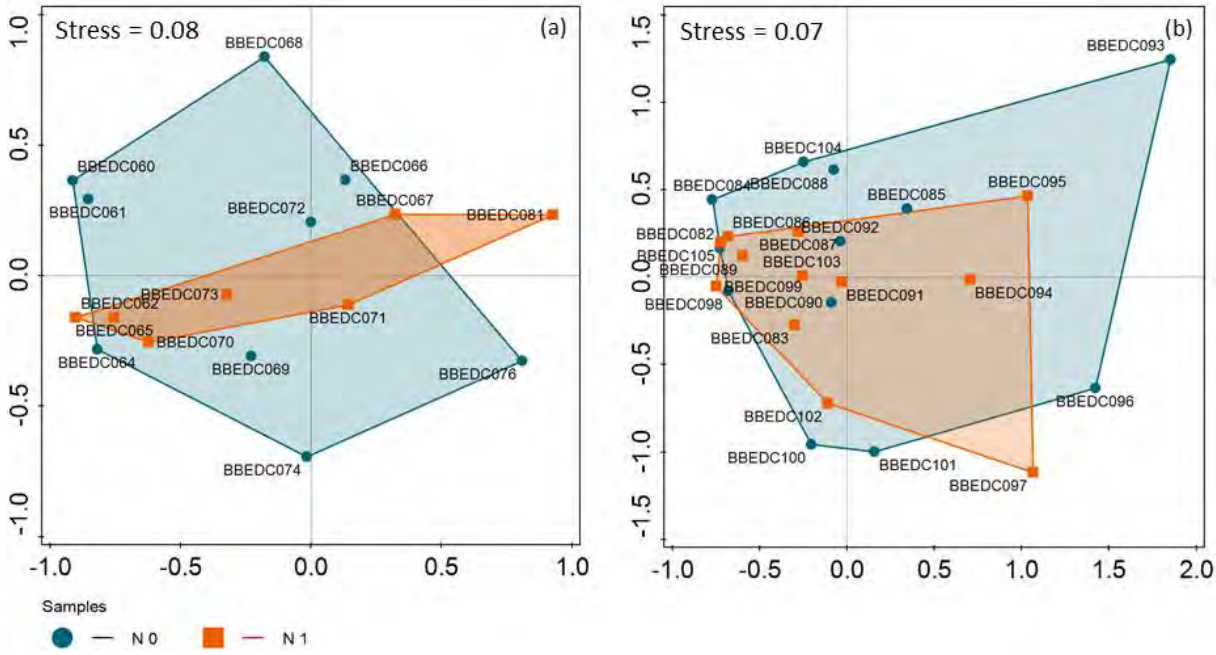


Figure 56. NMDS plots of the 24 samples from the mudflats (a) and marshes (b) at the Cattus Island site. Polygons are drawn around samples from nitrogen (N1) vs. no nitrogen addition plots (N0). Species short codes correspond to those in Appendix 6.

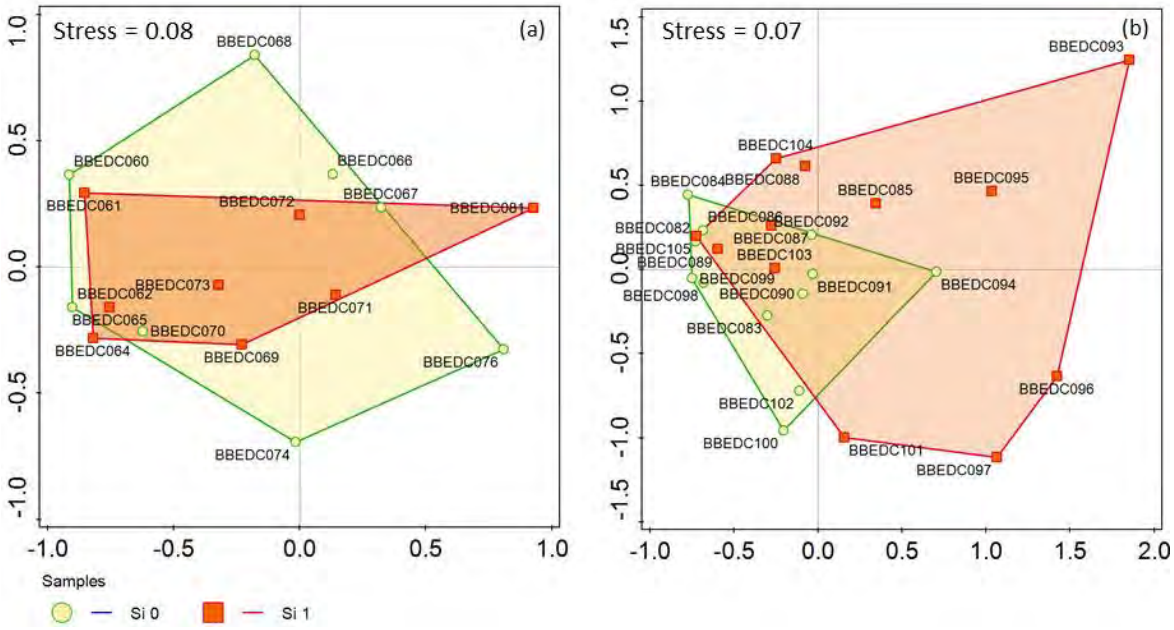


Figure 57. NMDS plots of the 24 samples from the mudflats (a) and marshes (b) at the Cattus Island site. Polygons are drawn around samples from silica (Si1) vs. no silica addition plots (Si0). Species short codes correspond to those in Appendix 6.

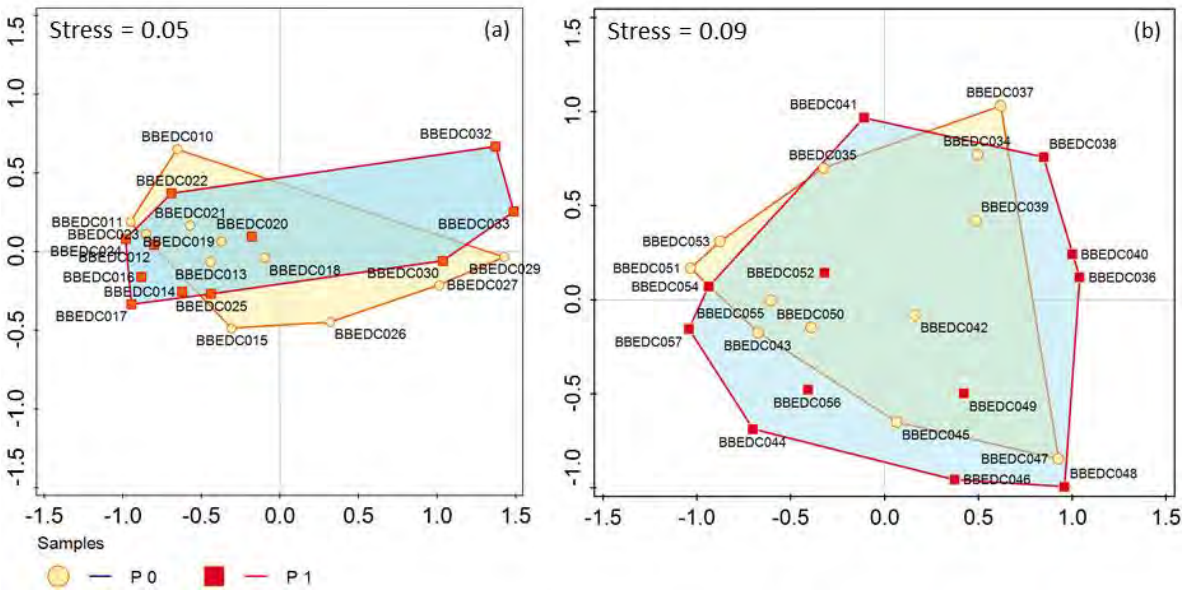


Figure 58. NMDS plots of the 24 samples from the mudflats (a) and marshes (b) at the Tuckerton Bay site. Polygons are drawn around samples from phosphorus (P1) vs. no phosphorus addition plots (P0). Species short codes correspond to those in Appendix 6.

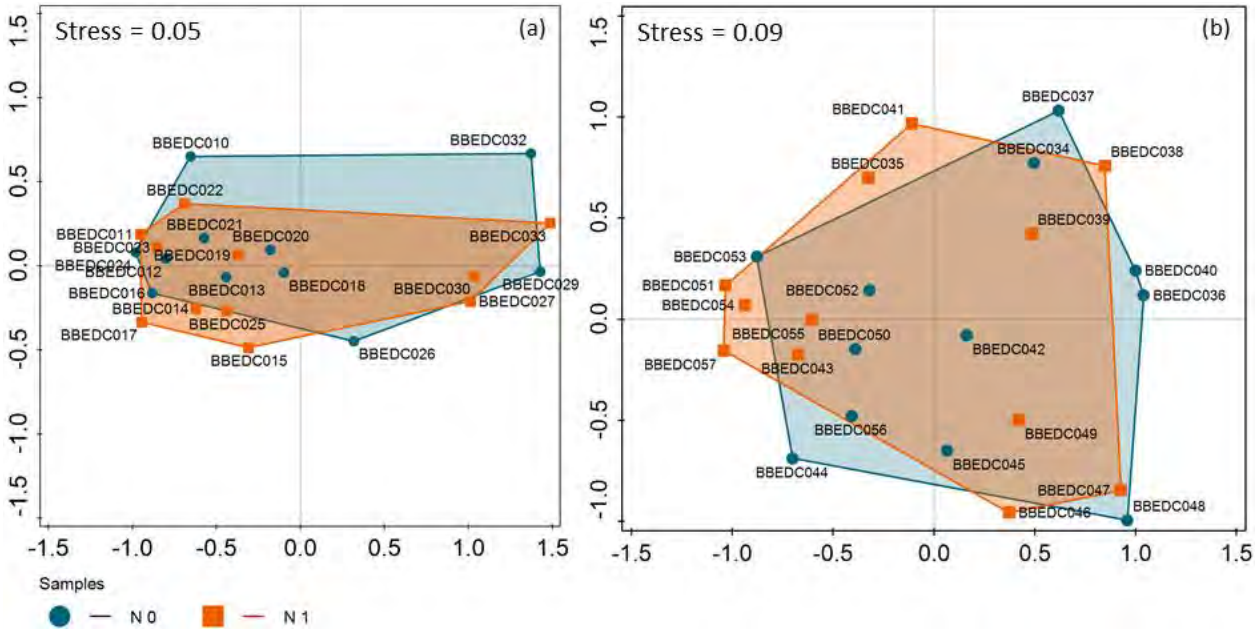


Figure 59. NMDS plots of the 24 samples from the mudflats (a) and marshes (b) at the Tuckerton Bay site. Polygons are drawn around samples from nitrogen (N1) vs. no nitrogen addition plots (N0). Species short codes correspond to those in Appendix 6.

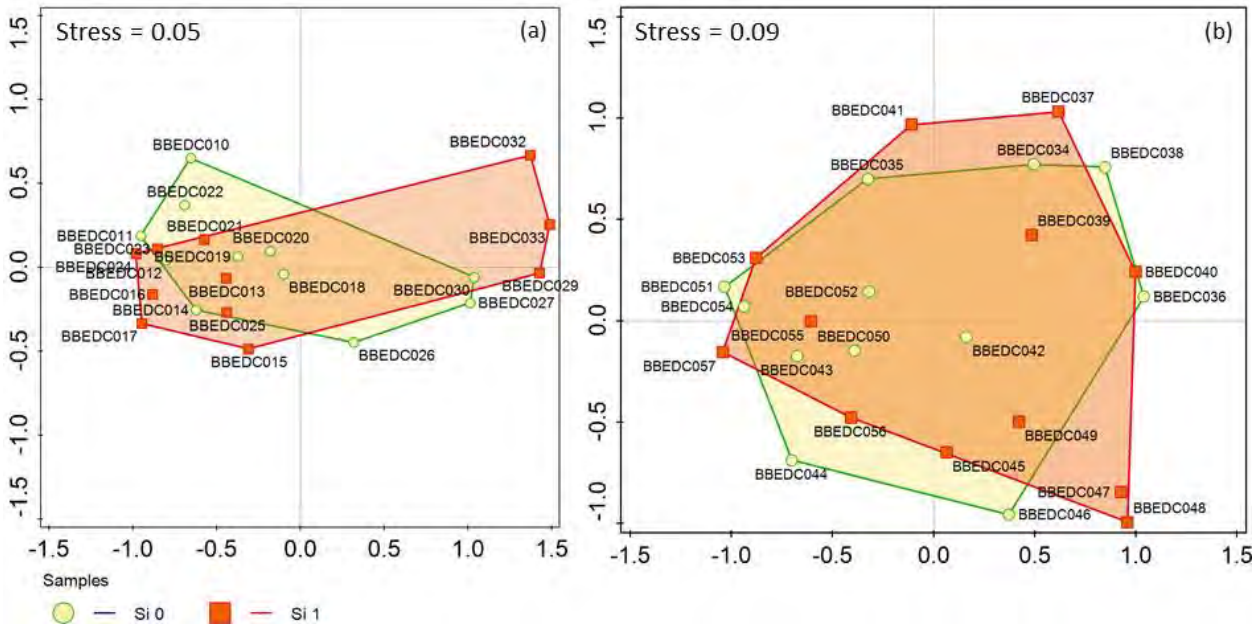


Figure 60. NMDS plots of the 24 samples from the mudflats (a) and marshes (b) at the Tuckerton Bay site. Polygons are drawn around samples from silica (Si1) vs. no silica addition plots (Si0). Species short codes correspond to those in Appendix 6.

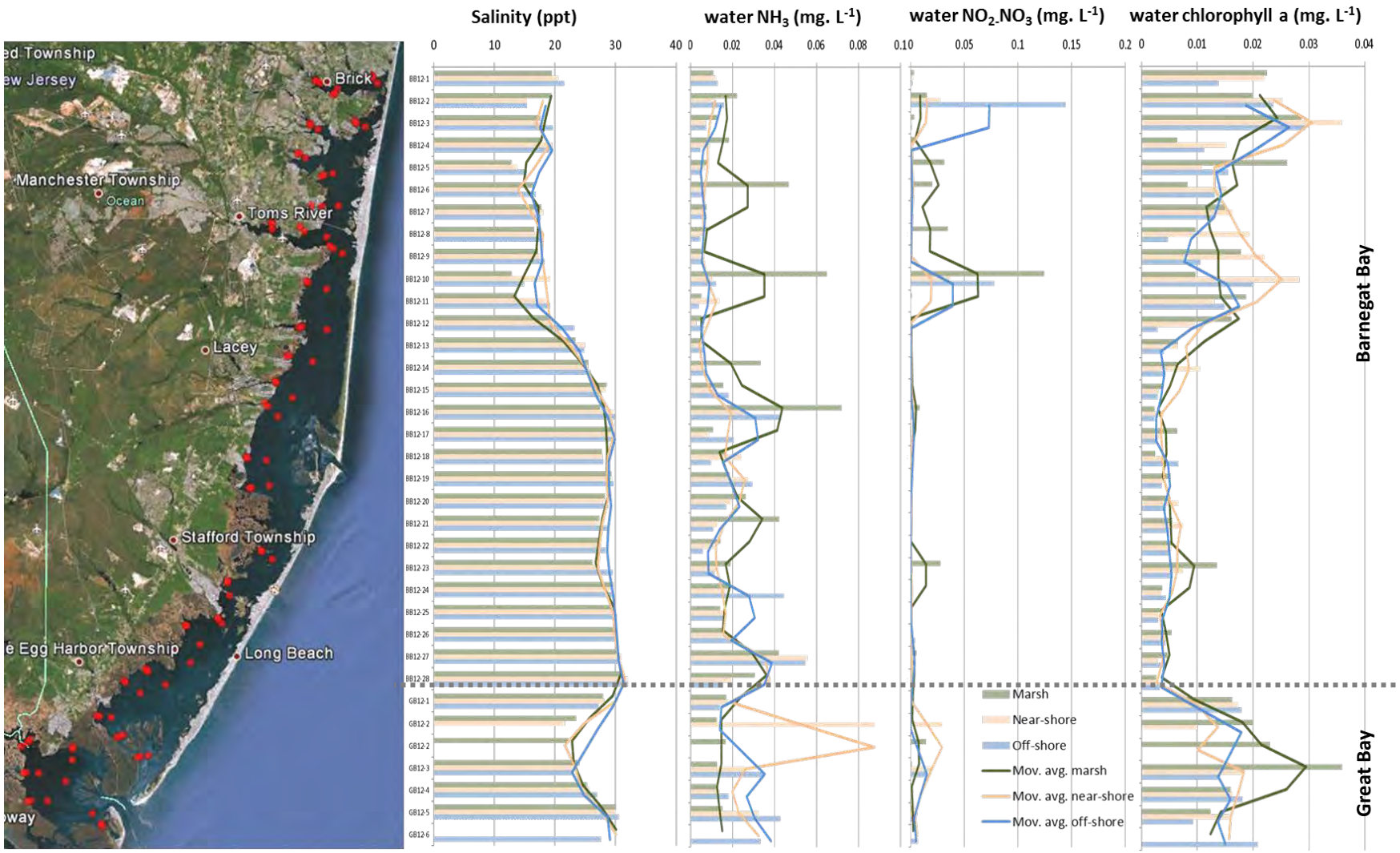


Figure 61. Salinity and concentrations of ammonia (NH_3), nitrite+nitrate ($NO_2.NO_3$) chlorophyll a in surface water at marsh, near-shore, off-shore sites in Barnegat Bay and Great Bay (100 sites) in 2012.

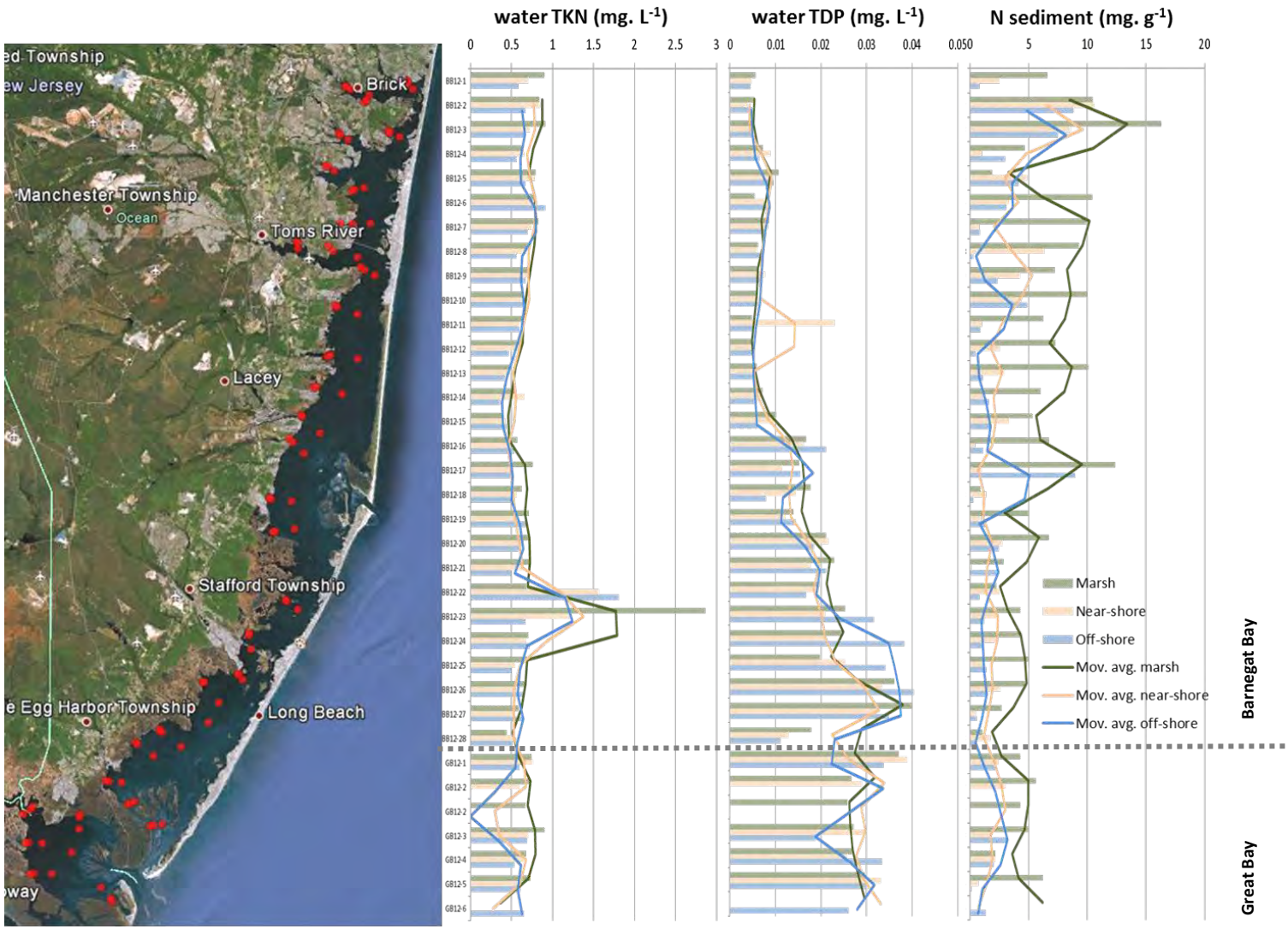


Figure 62. Concentrations of Total Kjeldahl Nitrogen (TKN), total dissolved phosphorus (TDP), nitrogen sediment (N sediment) in surface water at marsh, near-shore, off-shore sites in Barnegat Bay and Great Bay (100 sites) in 2012.

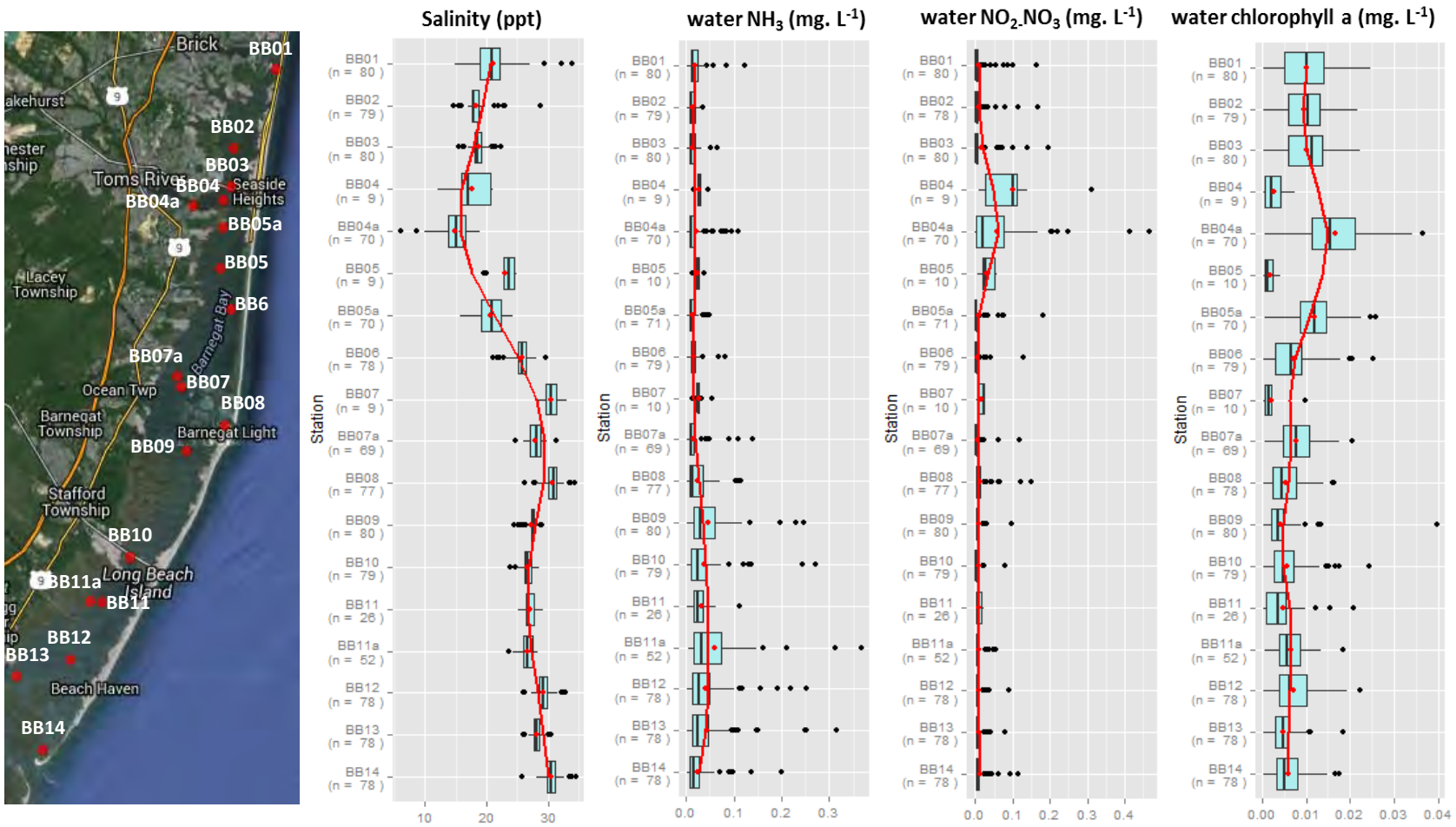


Figure 63. Salinity and concentrations of ammonia (NH3), nitrite+nitrate ($NO_2.NO_3$) and chlorophyll a in surface water at 18 BBTWMP sites from January to December 2012.

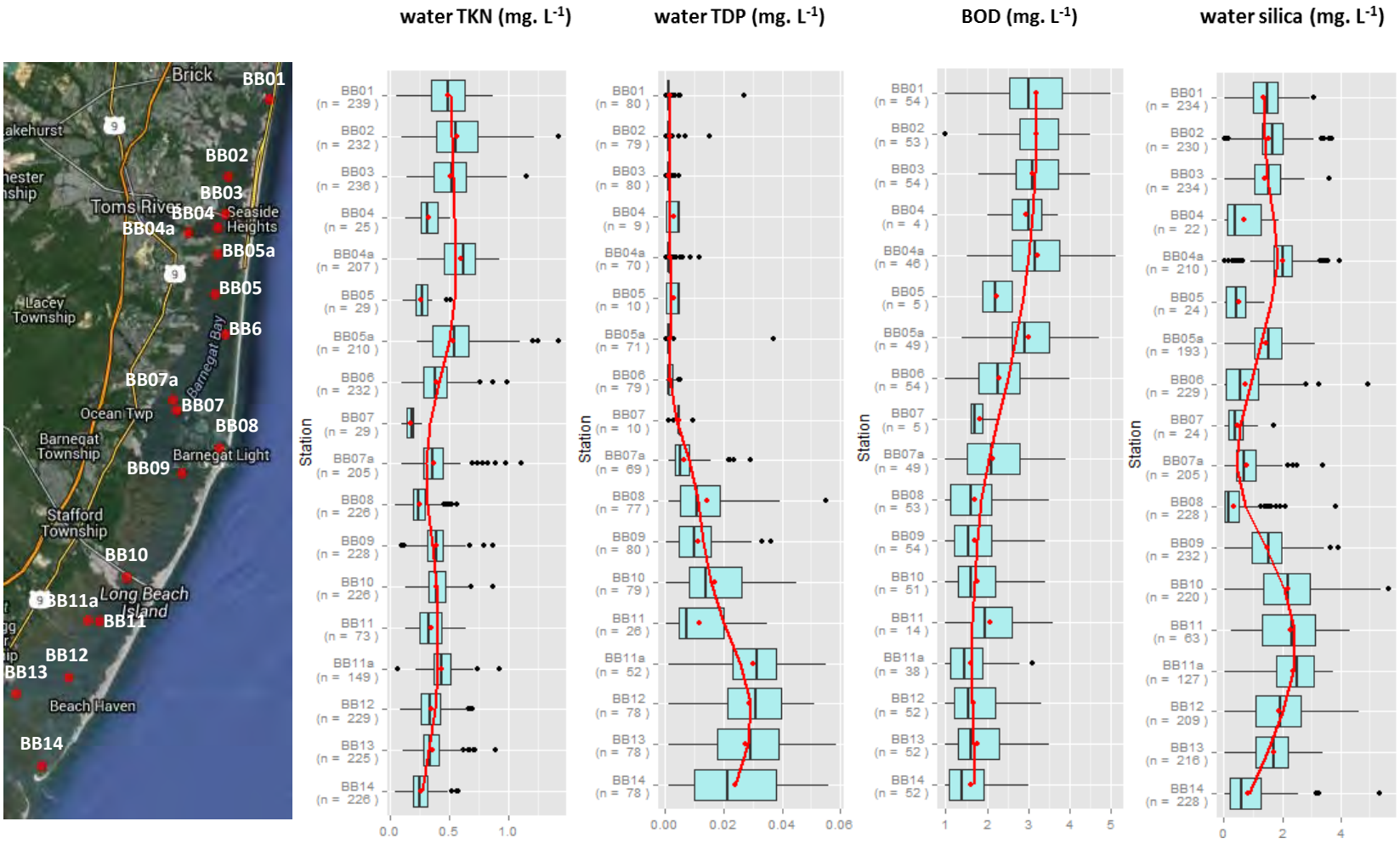


Figure 64. Concentrations of Total Kjeldahl Nitrogen (TKN), total dissolved phosphorus (TDP), biochemical oxygen demand (BOD) and silica in surface water at 18 BBTWMP sites from January to December 2012.

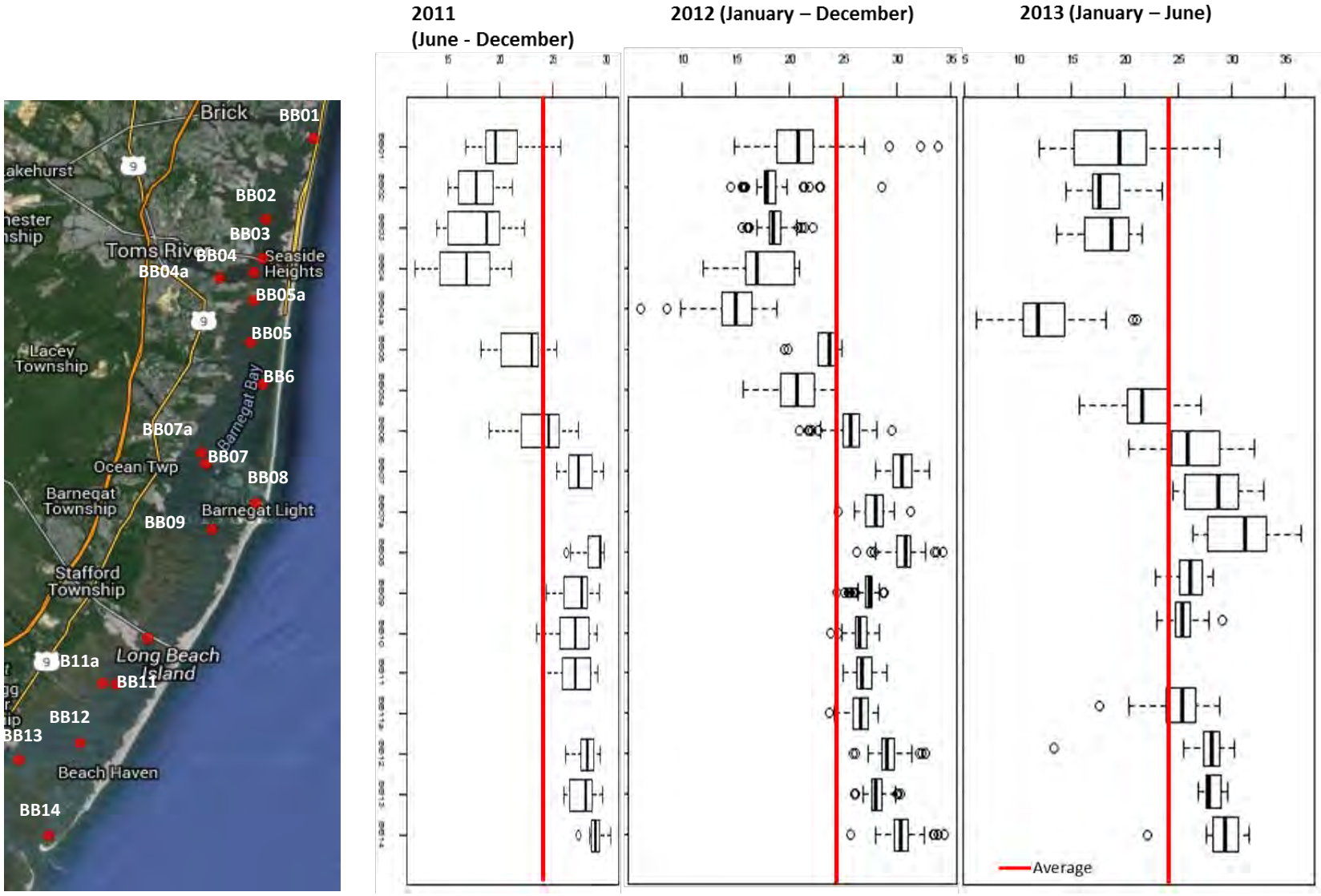


Figure 65. Salinity (ppt) at BBTWMP sites in 2011 (14 sites), 2012 (18 sites) and 2013 (14 sites).

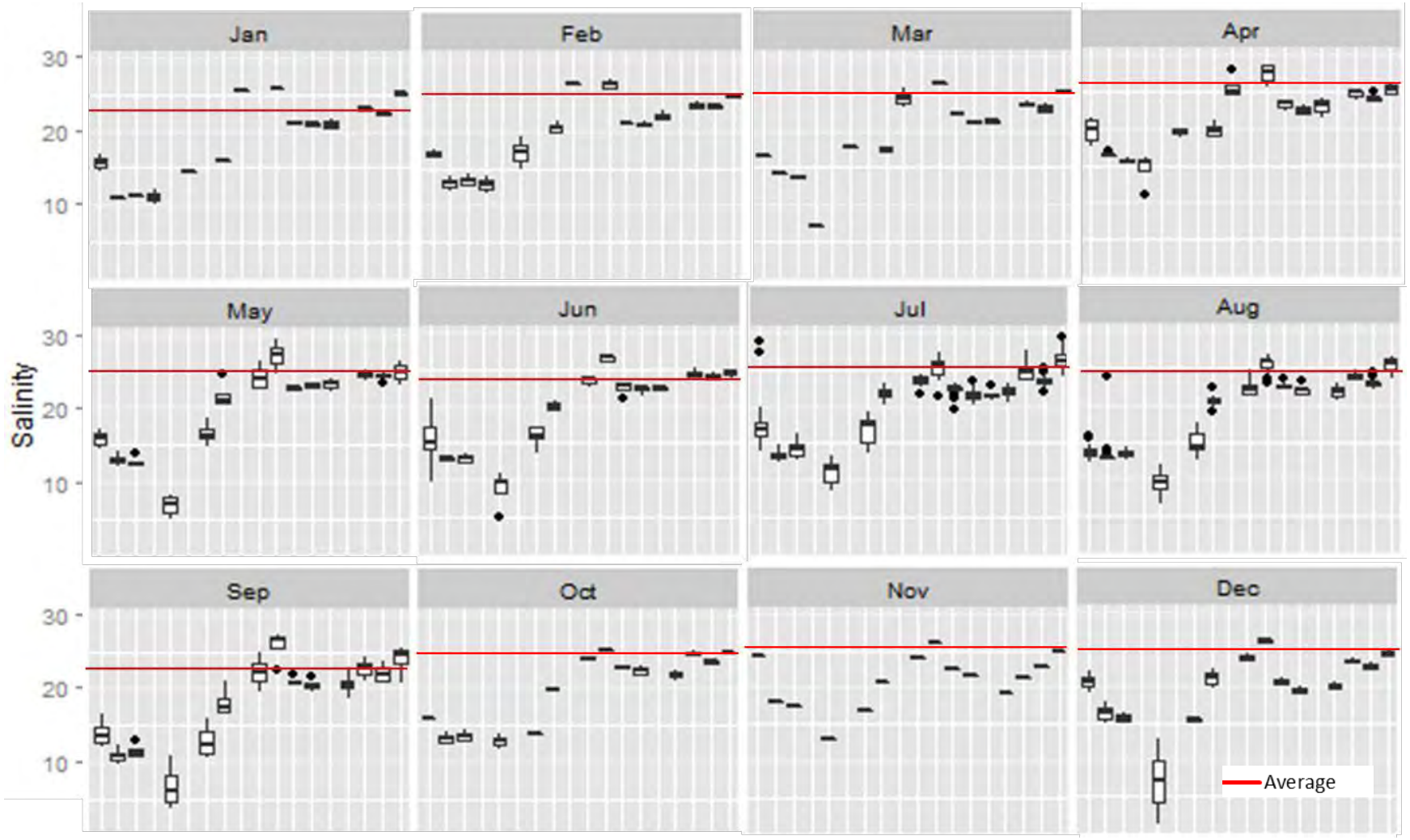


Figure 66. Salinity (ppt) at 18 BBTWMP sites from January to December 2012.

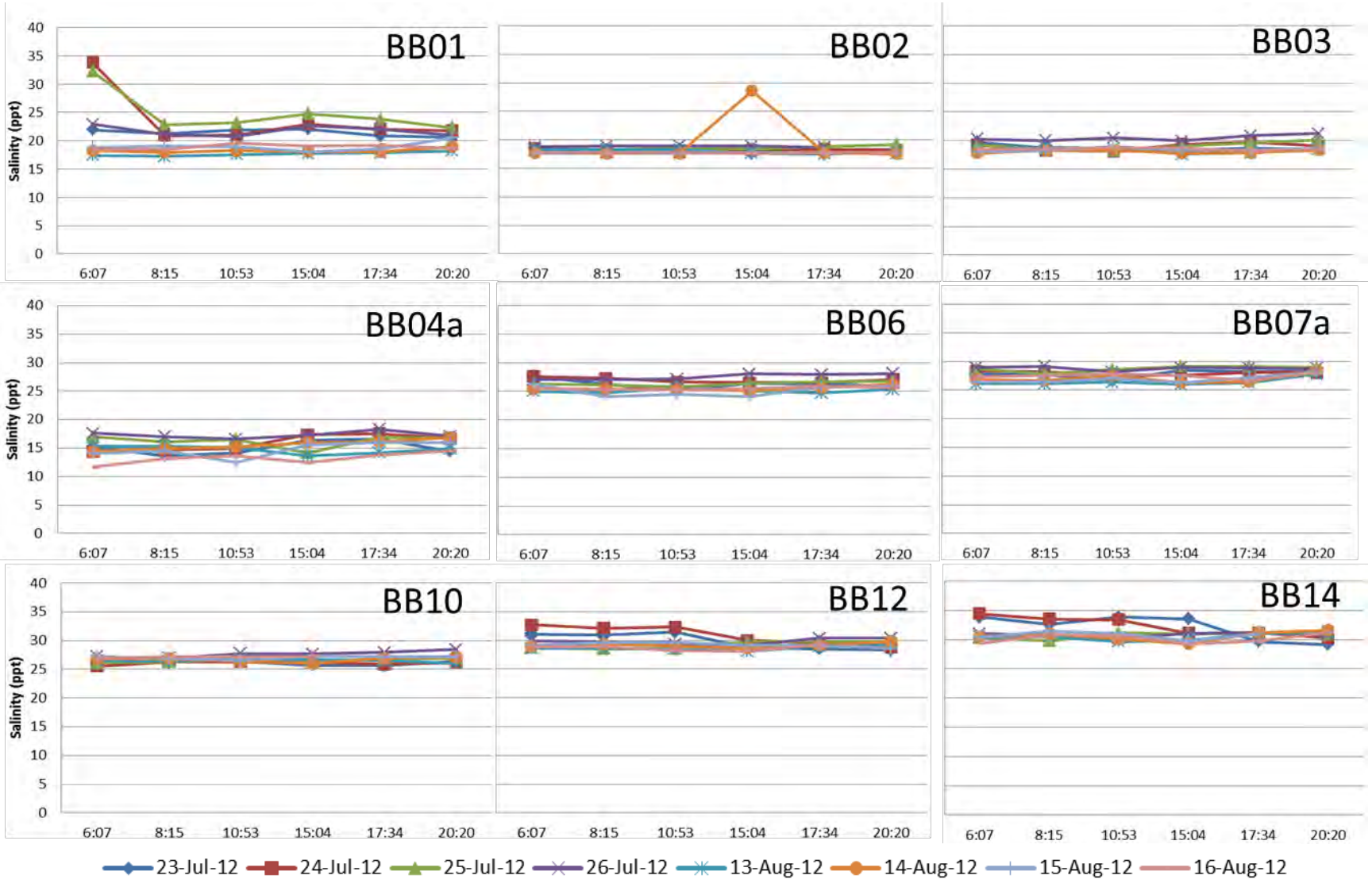


Figure 67. Salinity at 12 BBTWMP sites from 6:07 am to 8:20 pm in July and August 2012.

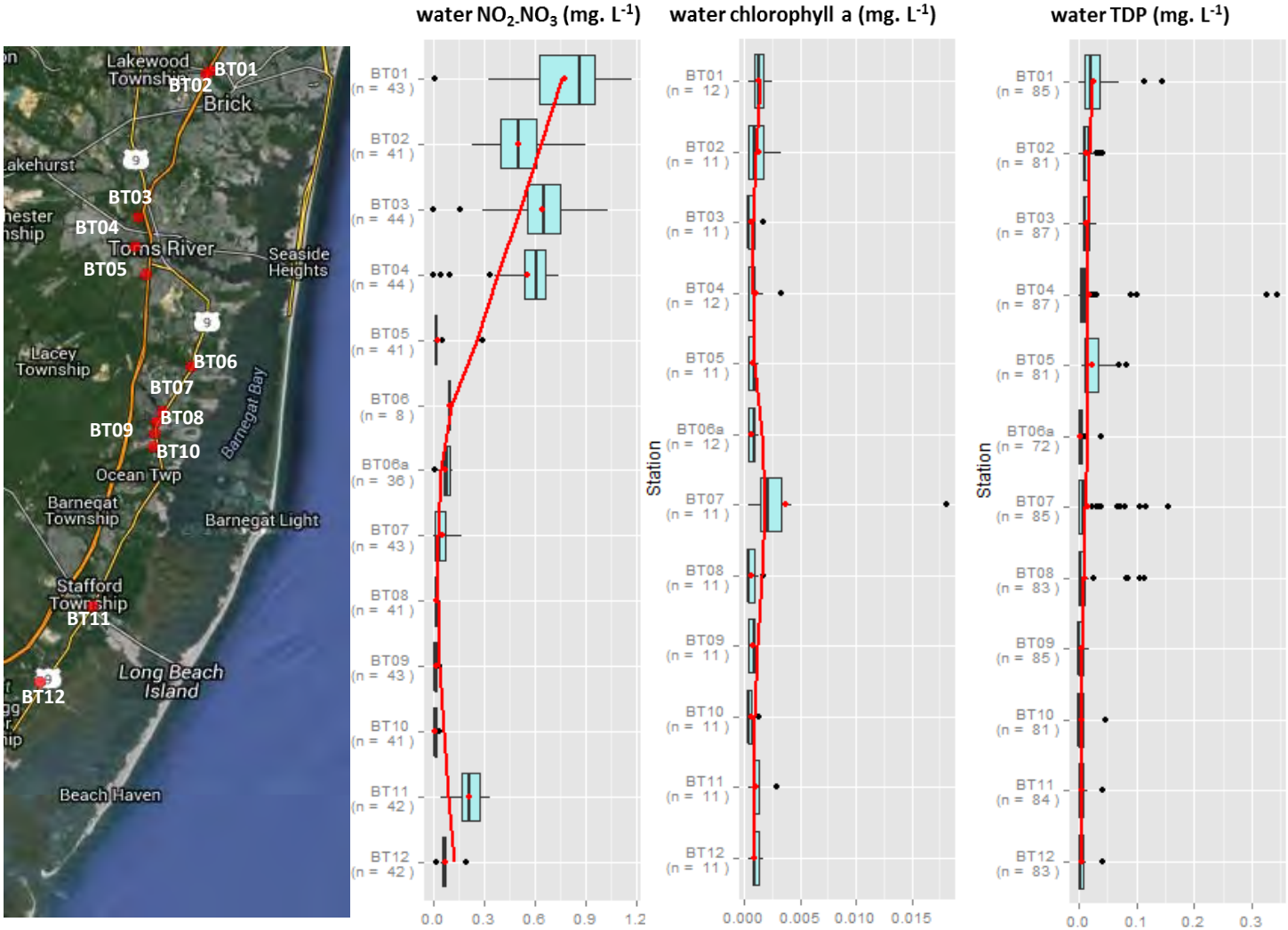


Figure 68. Concentrations of nitrate + nitrite (NO3.NO2), chlorophyll-a and phosphate (TDP) in surface water at 12 BBTWMP-BB tributaries sites from January to December 2012.

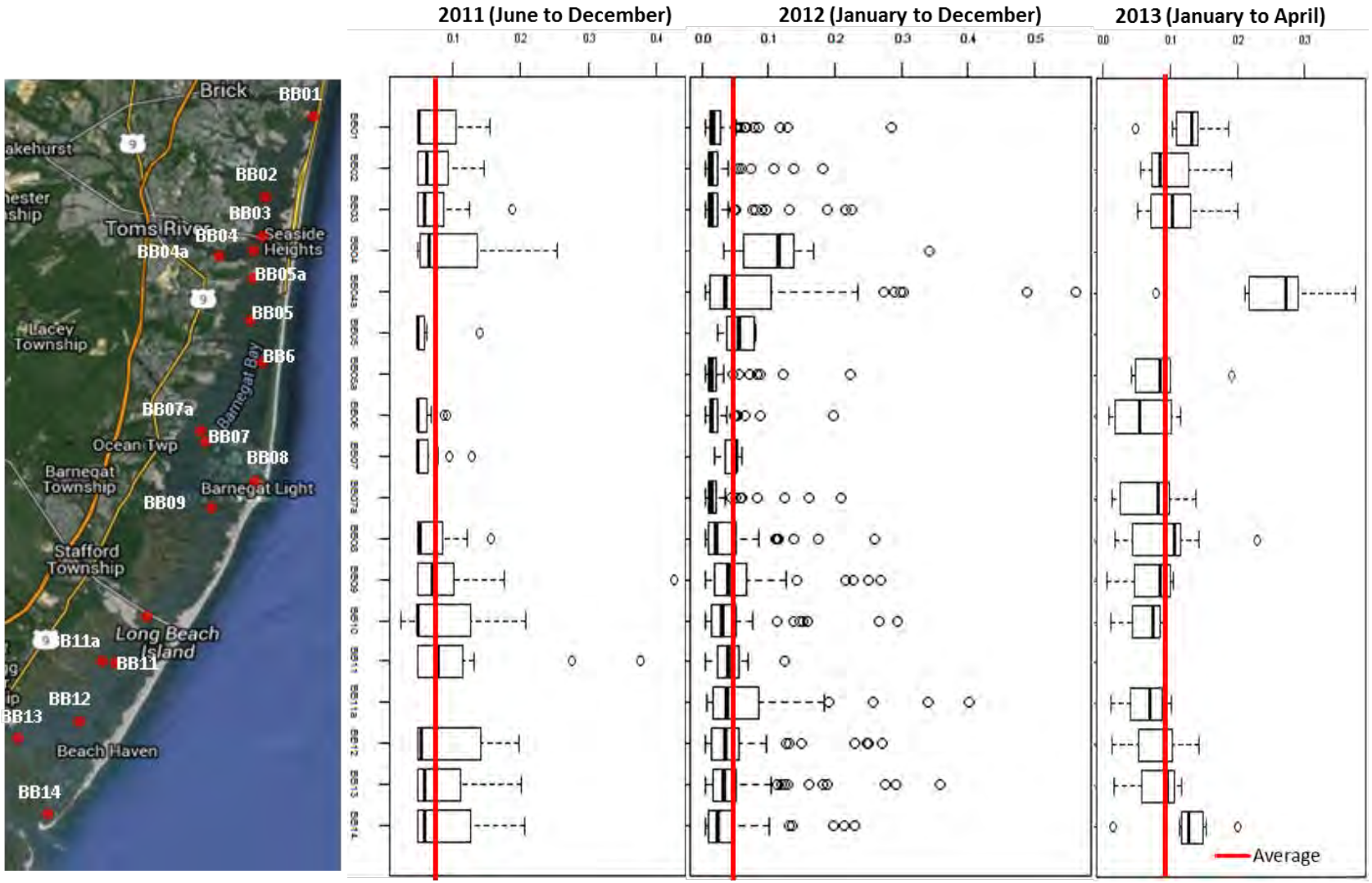


Figure 69. Concentrations of total inorganic nitrogen (TIN in mg.L^{-1}) in surface water at BBTWMP sites in 2011 (14 sites), 2012 (18 sites) and 2013 (14 sites).

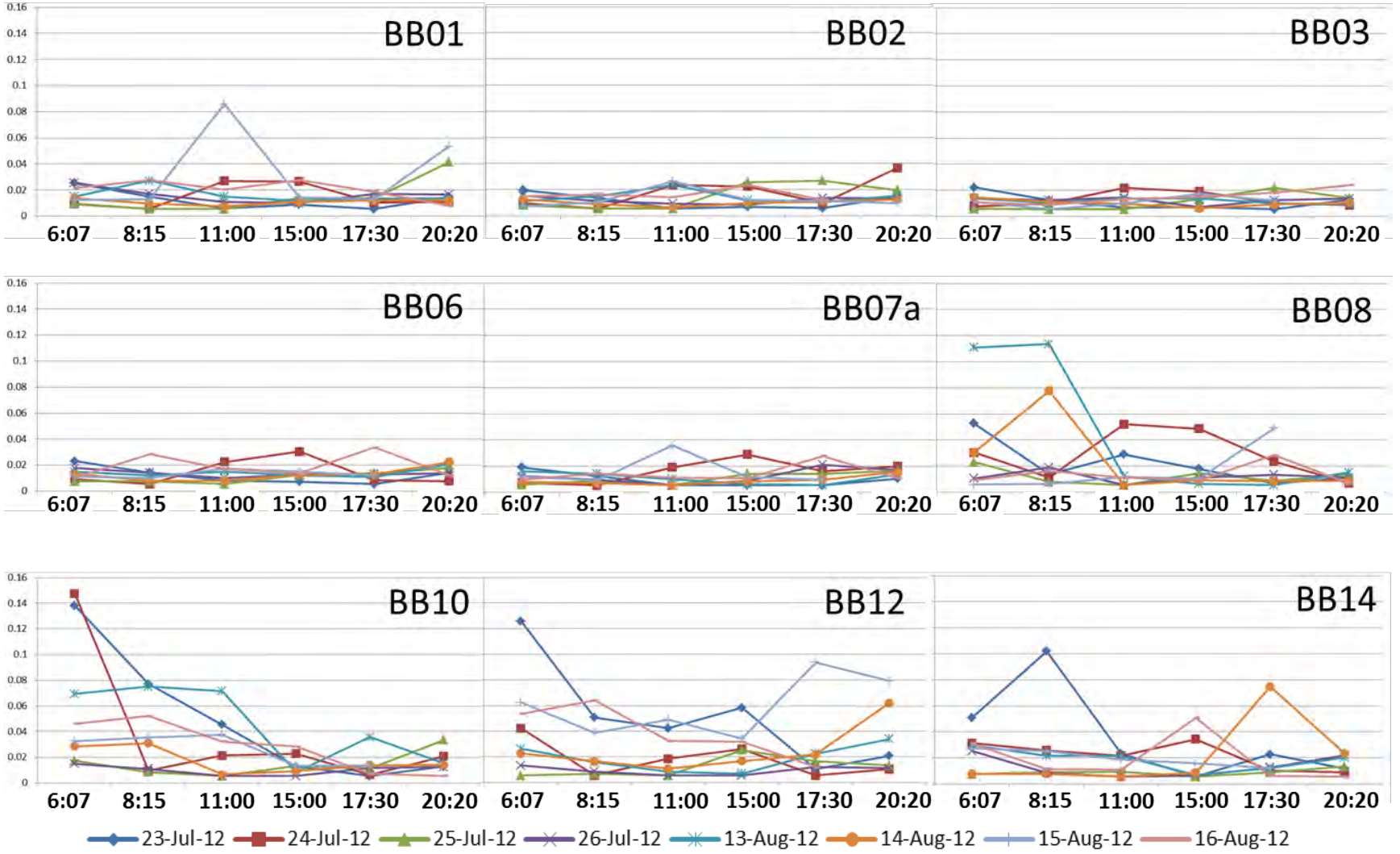


Figure 70. Concentrations of total inorganic nitrogen (TIN in in mg.L^{-1}) in surface water at 12 BBTWMP sites from 6:07 am to 8:20 pm in July and August 2012.

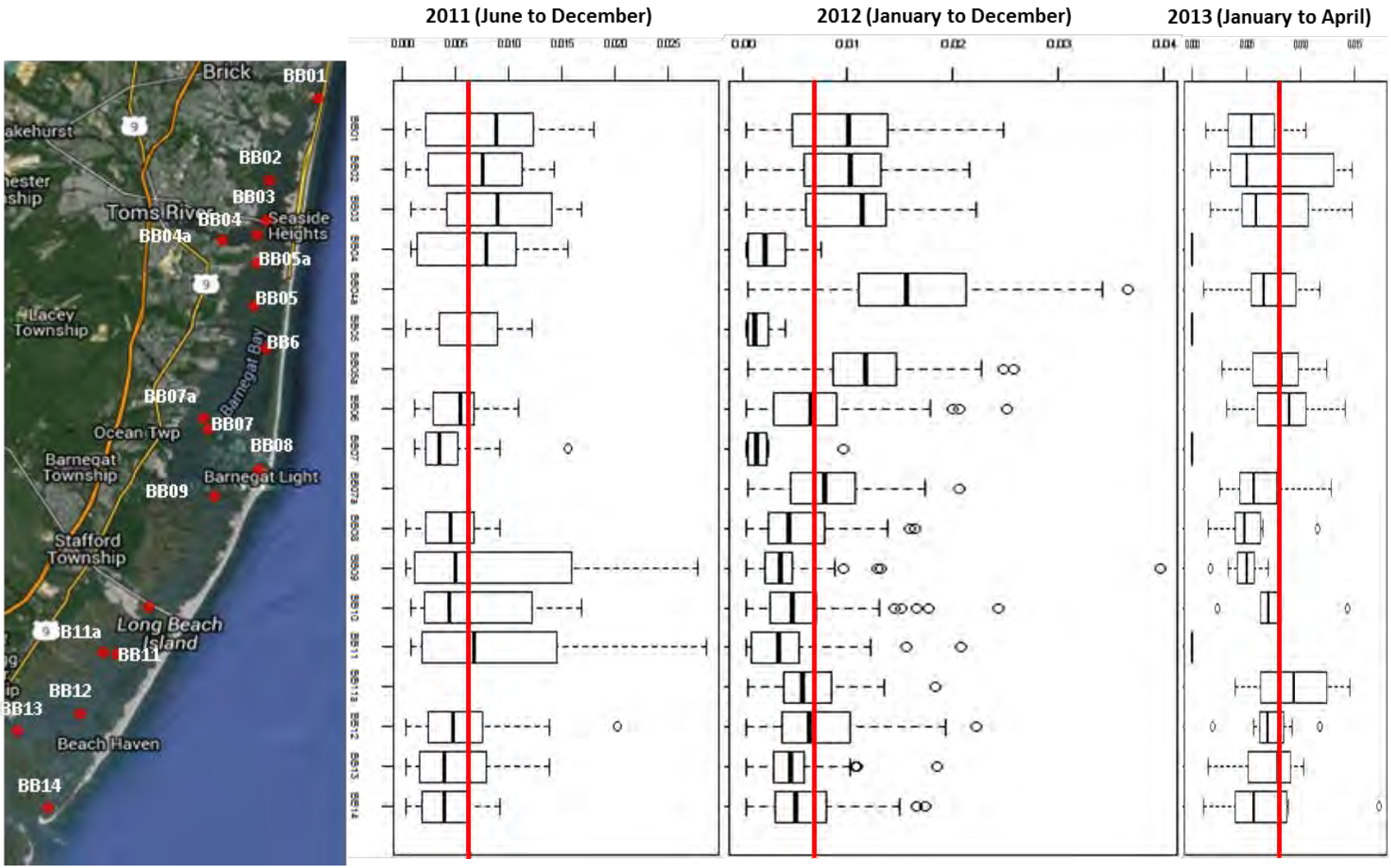


Figure 71. Concentrations of chlorophyll a (mg.L-1) in surface water at BBTWMP sites in 2011 (14 sites), 2012 (18 sites) and 2013 (14 sites).

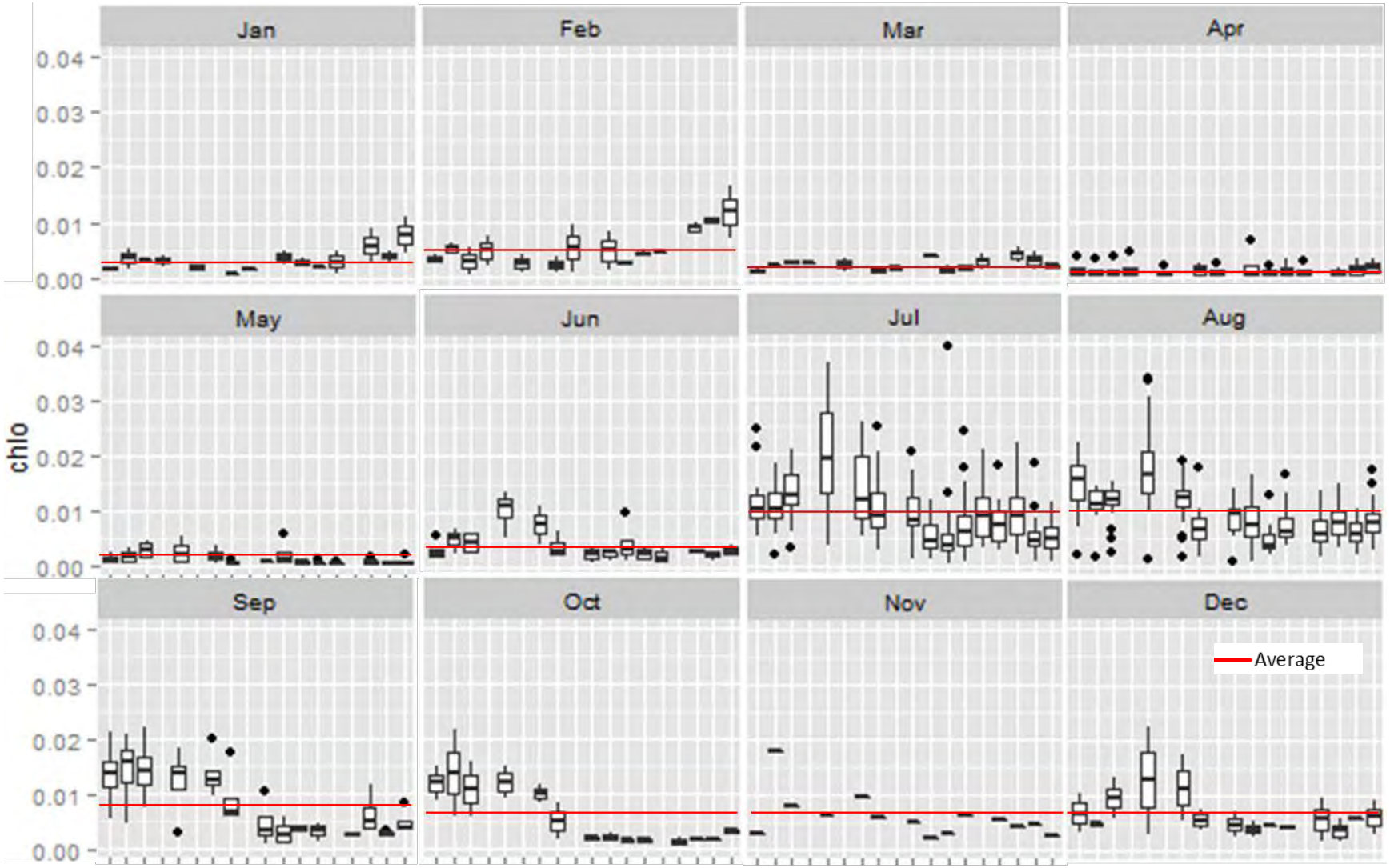


Figure 72. Chlorophyll a (chlo in mg.L^{-1}) at 18 BBTWMP sites from January to December 2012.

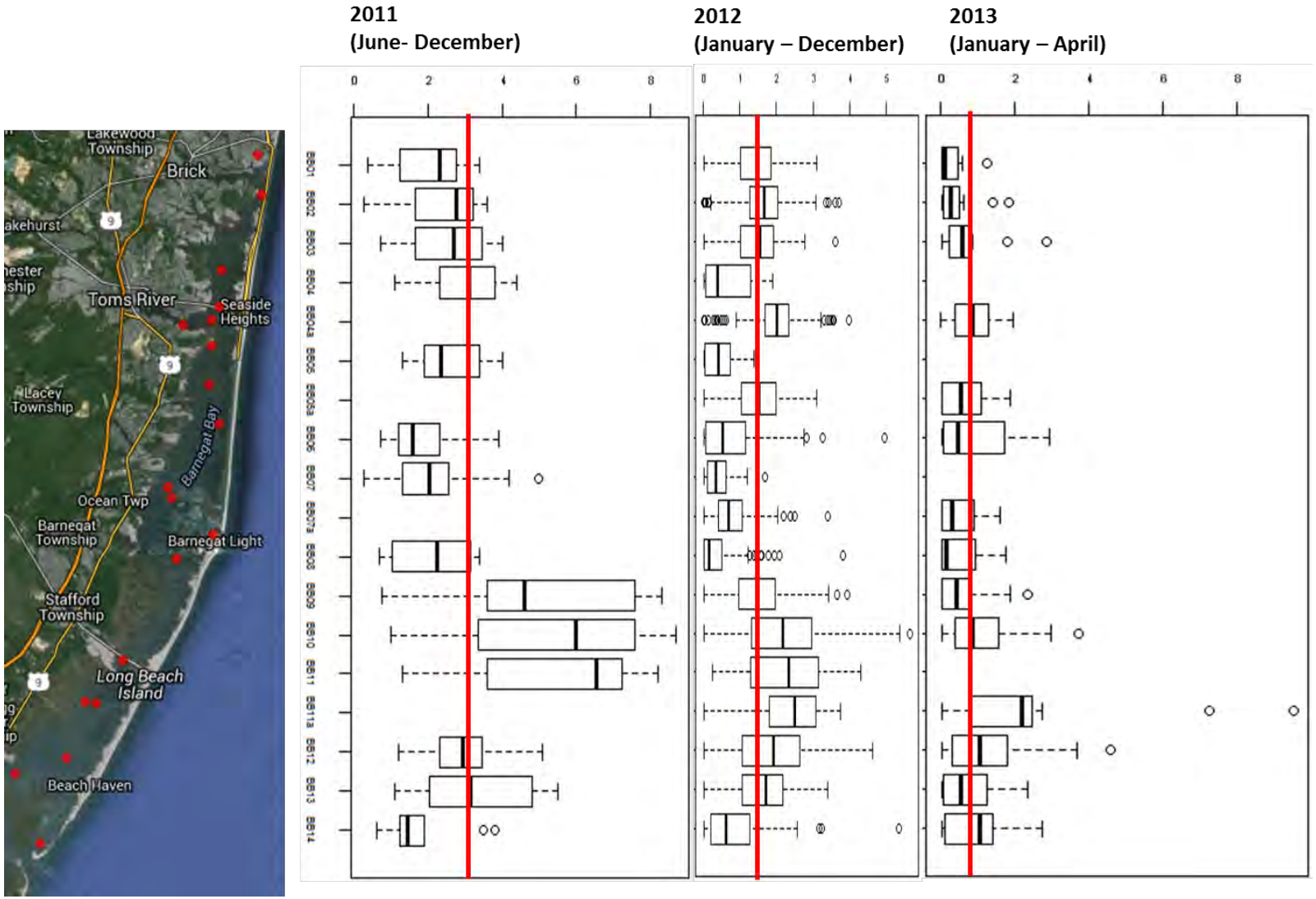


Figure 73. NJDEP biweekly monitoring of silica from 14 to 18 stations in Barnegat Bay from 2011 to 2013.

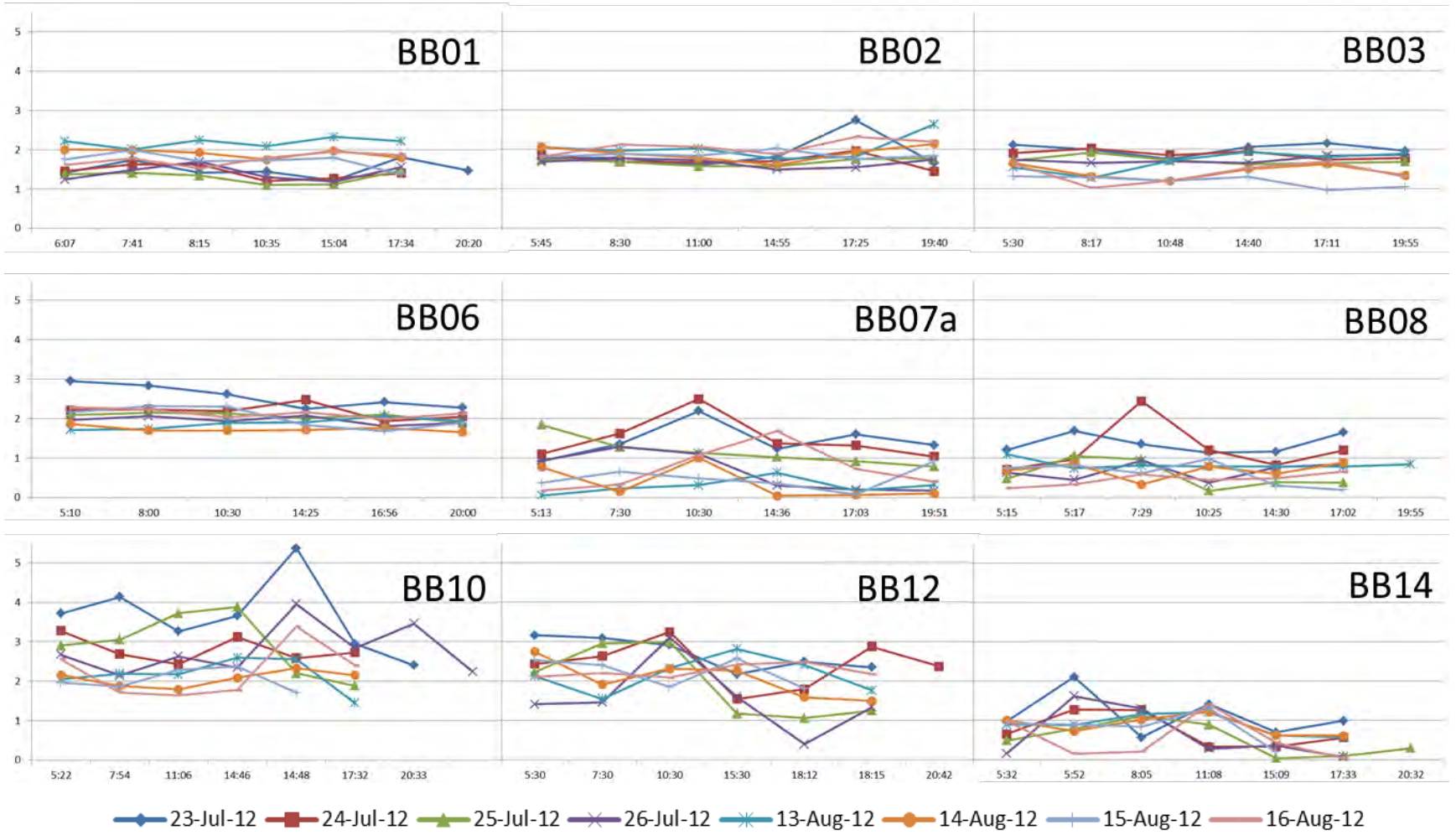


Figure 74. Concentrations of silica ($mg.L^{-1}$) in surface water at 12 BBTWMP sites from 6:07 am to 8:20 pm in July and August 2012.

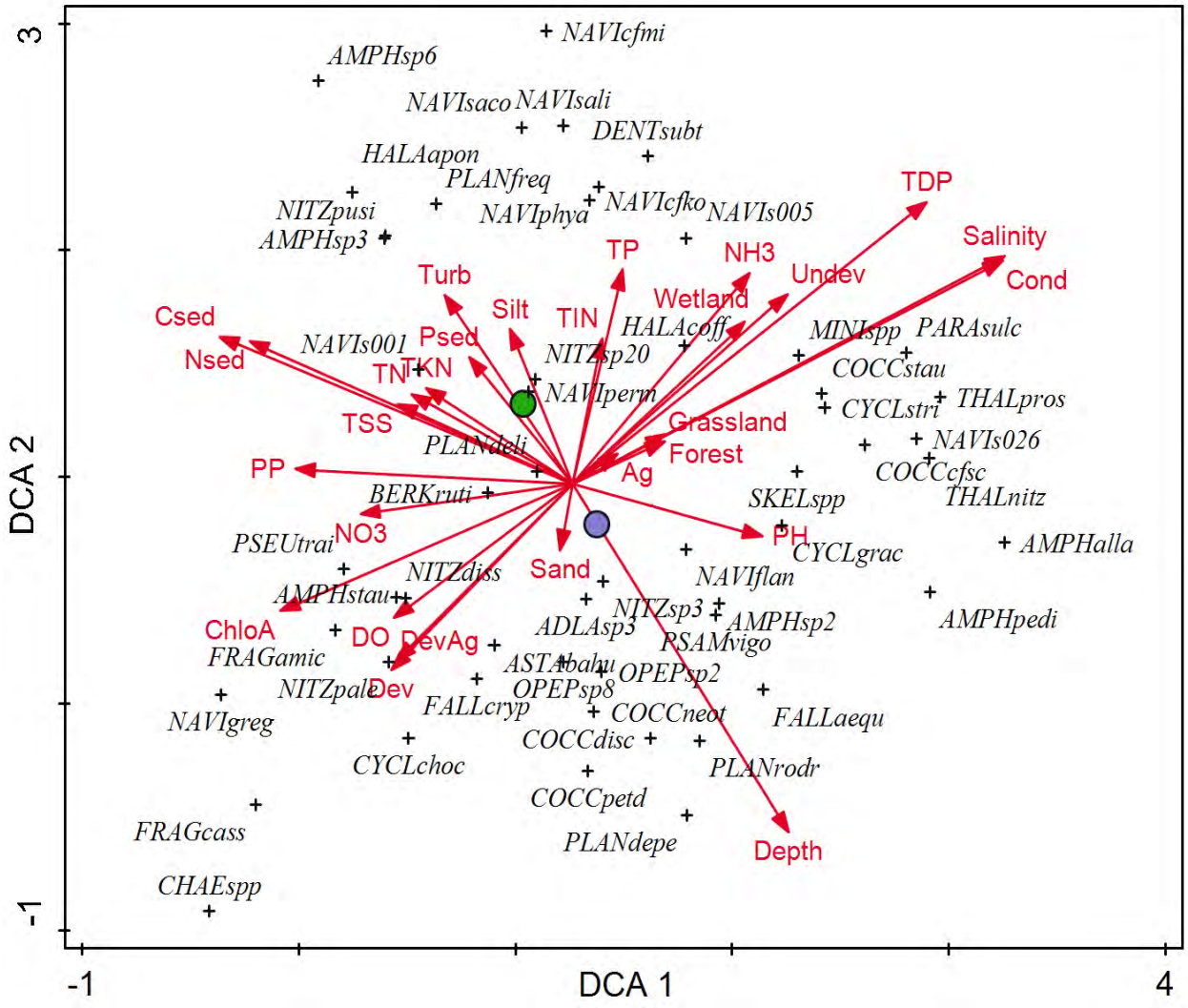


Figure 75. DCA results using Barnegat + Great Bay dataset (100 sites/603 taxa).
Biplot of species and environmental variables showing the result of a DCA exploring relationships between diatom assemblages and water-quality and land-use parameters. Only centroids for species with highest weights (20-100%) are shown. Species short codes correspond to those in Appendix 6. Green circle is the centroid of marsh sites and blue circle is the centroid of open-water sites.

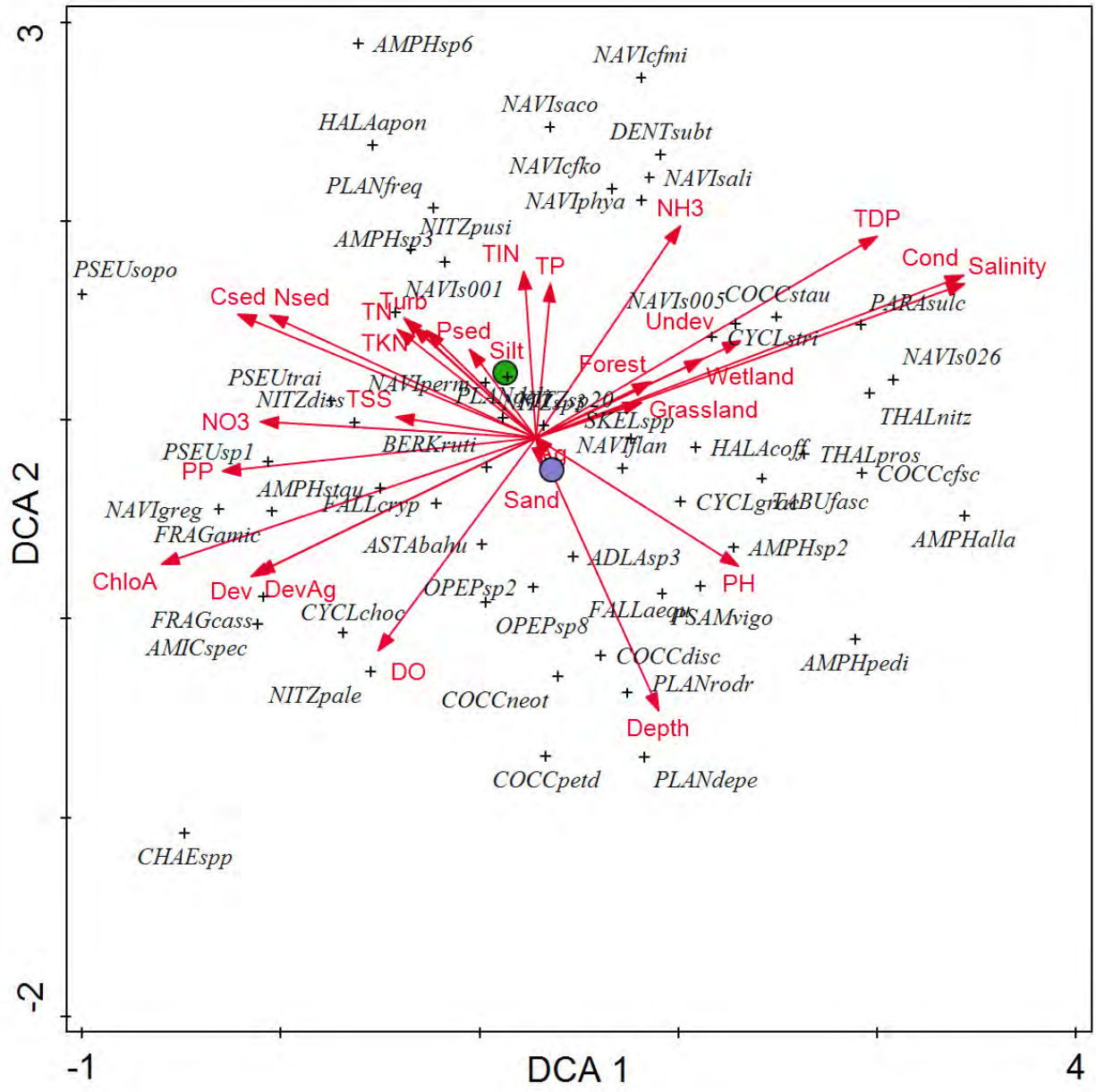


Figure 76. DCA results using Barnegat Bay only dataset (84 sites/569 taxa).
Biplot of species and environmental variables showing the result of a DCA exploring relationships between diatom assemblages and water-quality and land-use parameters. Only centroids for species with highest weights (20-100%) are shown. Species short codes correspond to those in Appendix 6. Green circle is the centroid of marsh sites and blue circle is the centroid of open-water sites.

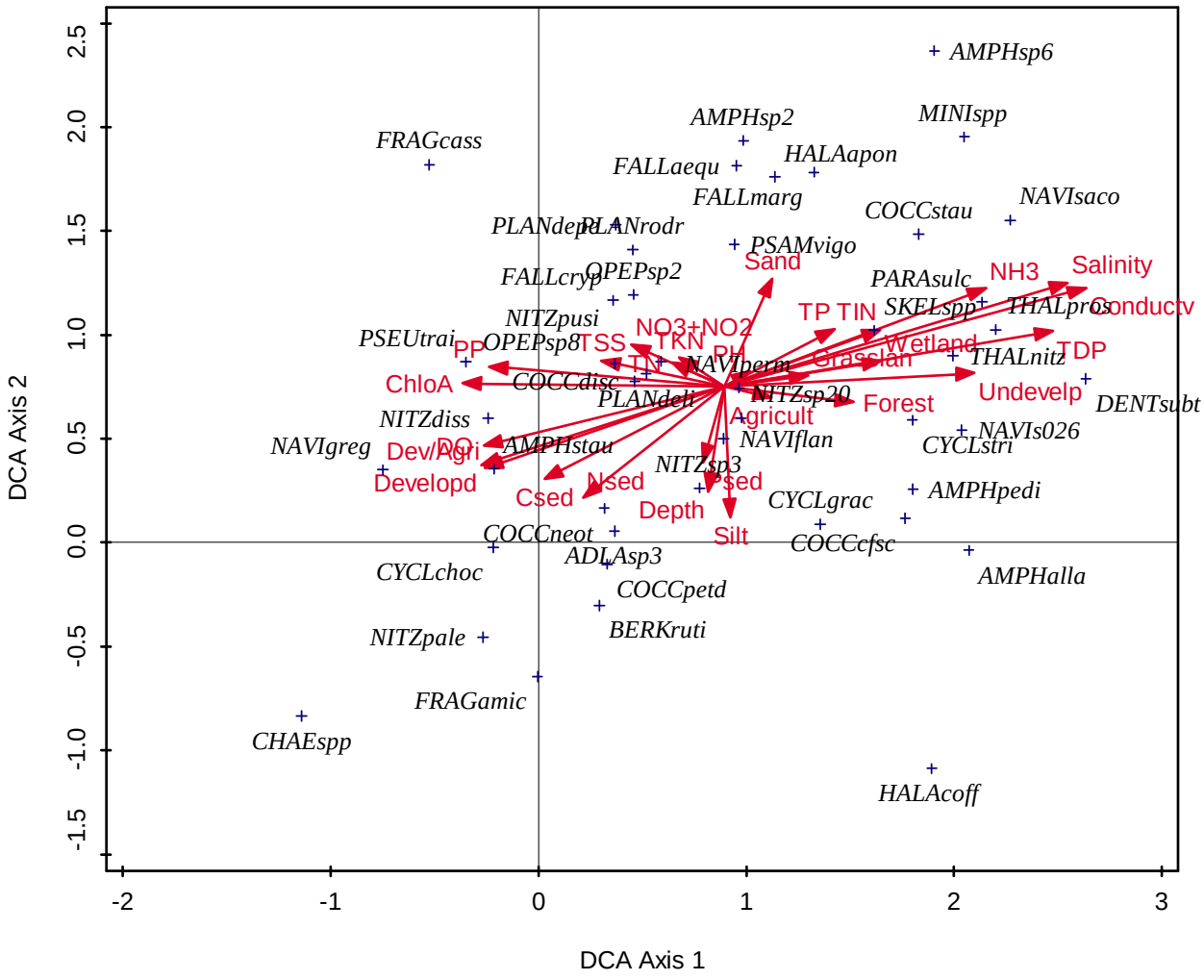


Figure 77. DCA results using open water sites dataset (66 sites/536 taxa).
Biplot of species and environmental variables showing the result of a DCA exploring relationships between diatom assemblages and water-quality and land-use parameters. Only centroids for species with highest weights (20-100%) are shown. Species short codes correspond to those in Appendix 6.

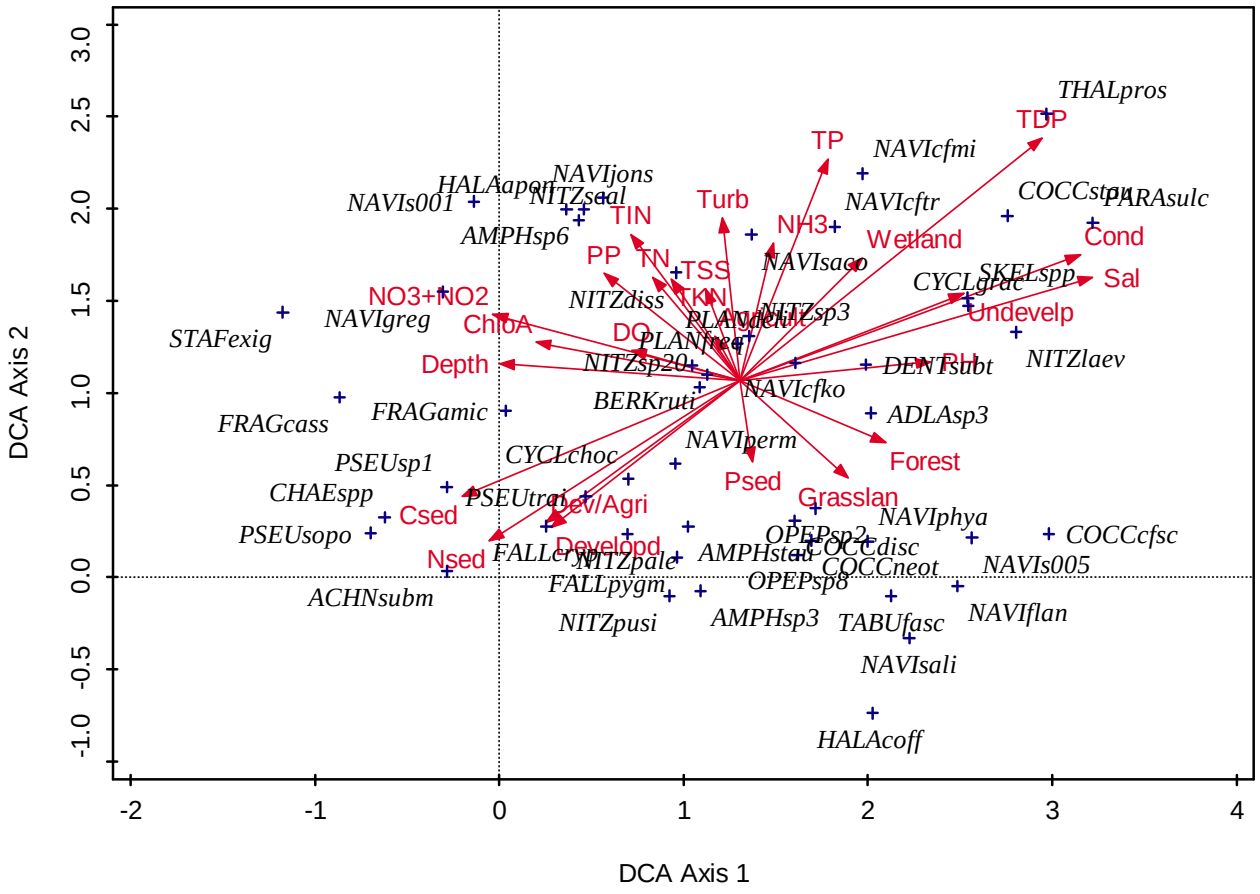


Figure 78. DCA results using marsh sites dataset (34 sites/442 taxa).
Biplot of species and environmental variables showing the result of a DCA exploring relationships between diatom assemblages and water-quality and land-use parameters. Only centroids for species with highest weights (20-100%) are shown. Species short codes correspond to those in Appendix 6.

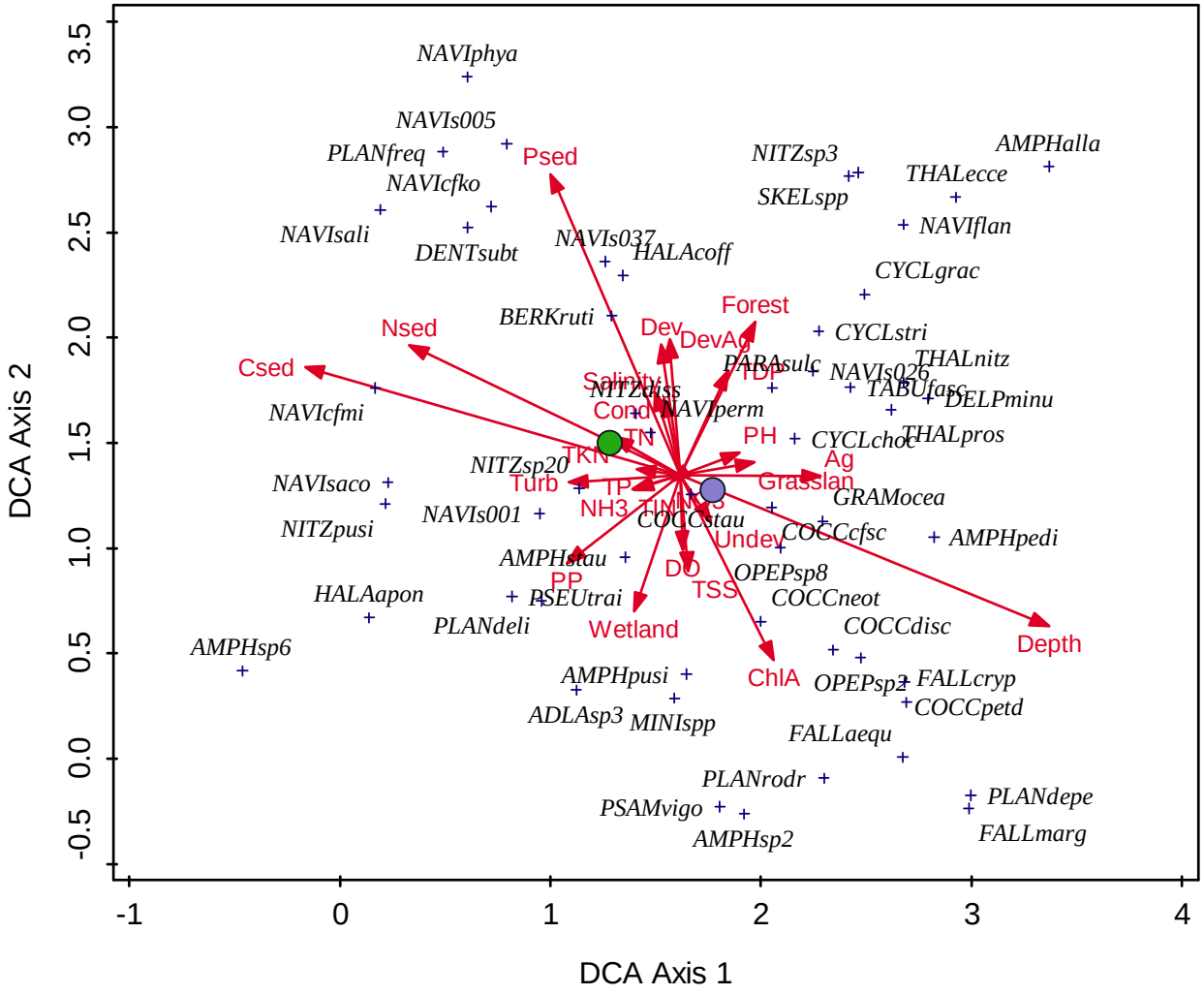


Figure 79. DCA results using high salinity sample sets in Barnegat + Great Bay (50 high salinity sites/458 species).

Biplot of species and environmental variables showing the result of a DCA exploring relationships between diatom assemblages and water-quality and land-use parameters. Only centroids for species with highest weights (20-100%) are shown. Species short codes correspond to those in Appendix 6. Green circle is the centroid of marsh sites and blue circle is the centroid of open-water sites.

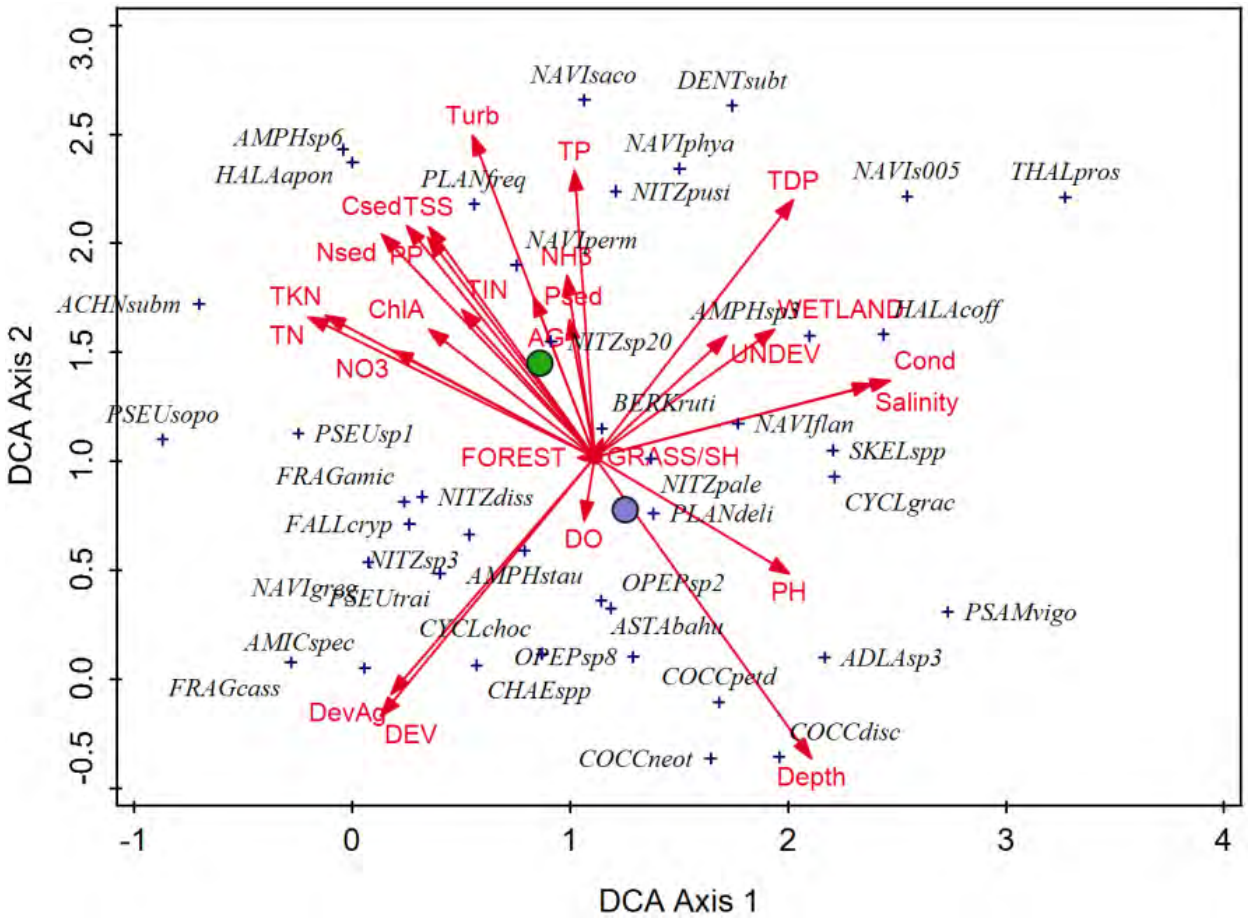


Figure 80. DCA results using low salinity sample sets in Barnegat + Great Bay (50 low salinity sites/504 species, right) datasets. Biplot of species and environmental variables showing the result of a DCA exploring relationships between diatom assemblages and water-quality and land-use parameters. Only centroids for species with highest weights (20-100%) are shown. Species short codes correspond to those in Appendix 6. Green circle is the centroid of marsh sites and blue circle is the centroid of open-water sites.

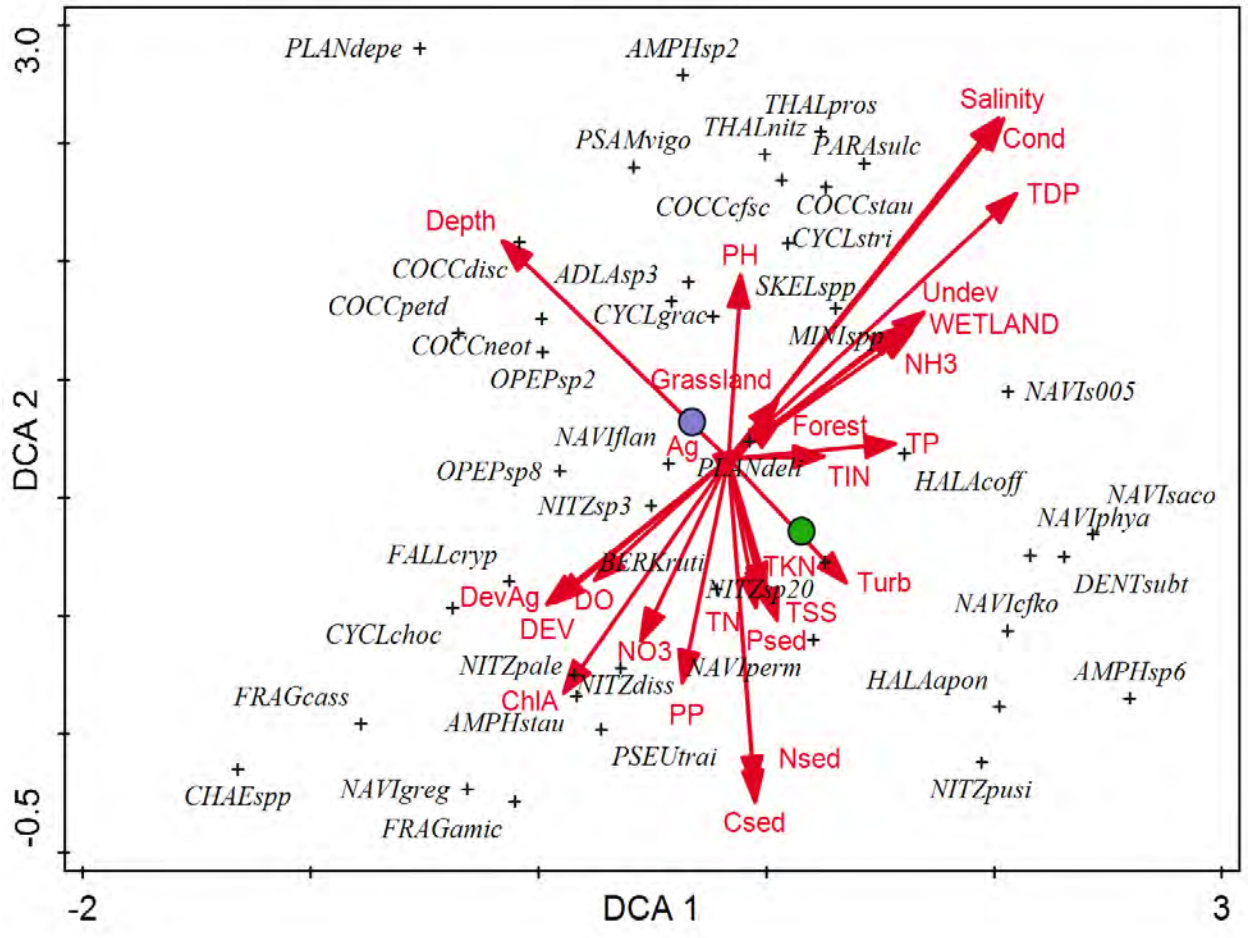


Figure 81. DCA results using Barnegat + Great Bay dataset with exclusion of rare species (>1% in 5 samples; 100 sites/110 species). Biplot of species and environmental variables showing the result of a DCA exploring relationships between diatom assemblages and water-quality and land-use parameters. Only centroids for species with highest weights (20-100%) are shown. Species short codes correspond to those in Appendix 6. Green circle is the centroid of marsh sites and blue circle is the centroid of open-water sites.

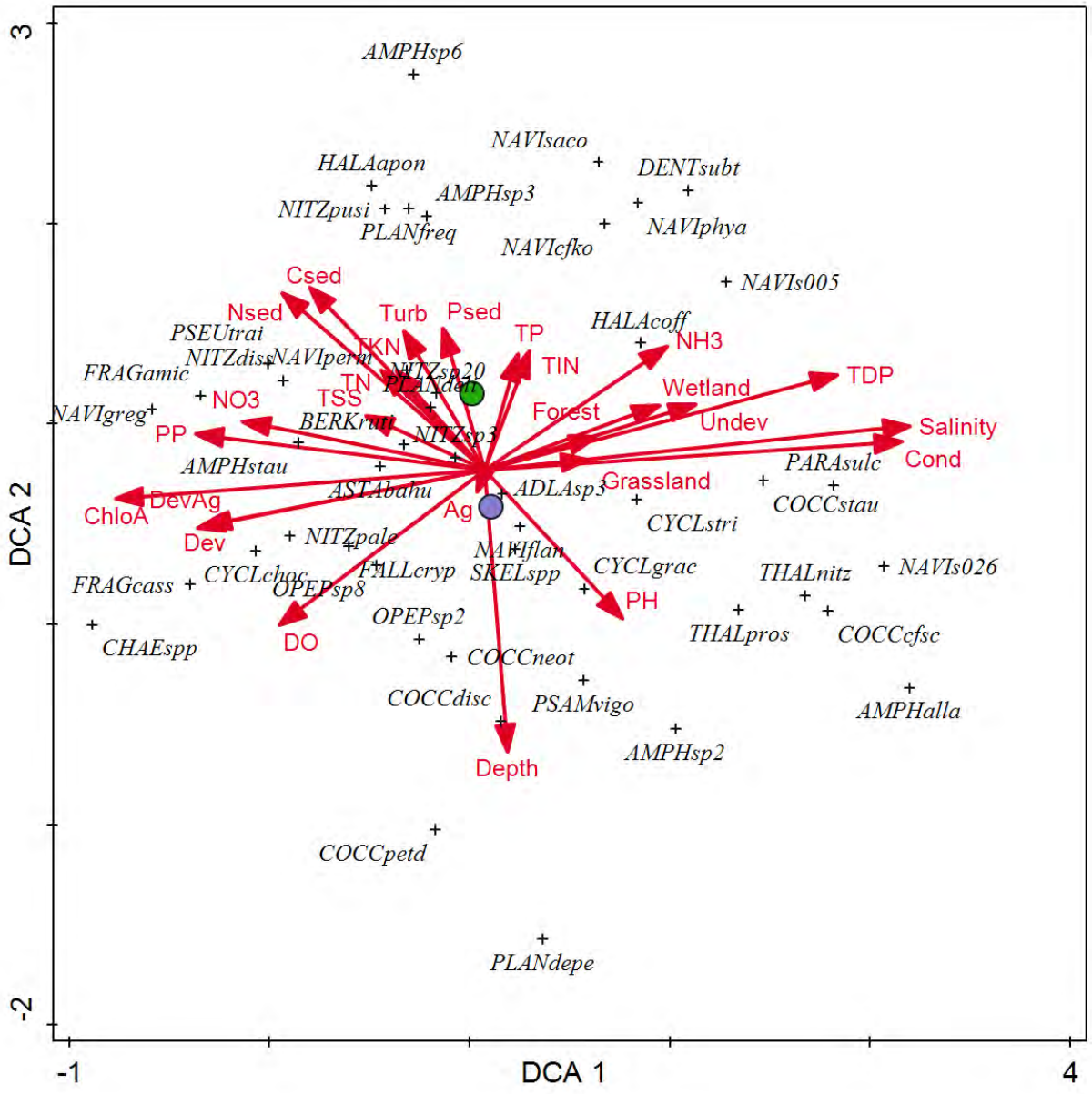


Figure 82. DCA results using Barnegat Bay only with exclusion of rare species (>1% in 5 samples; 84 sites/105 species). Biplot of species and environmental variables showing the result of a DCA exploring relationships between diatom assemblages and water-quality and land-use parameters. Only centroids for species with highest weights (20-100%) are shown. Species short codes correspond to those in Appendix 6. Green circle is the centroid of marsh sites and blue circle is the centroid of open-water sites.

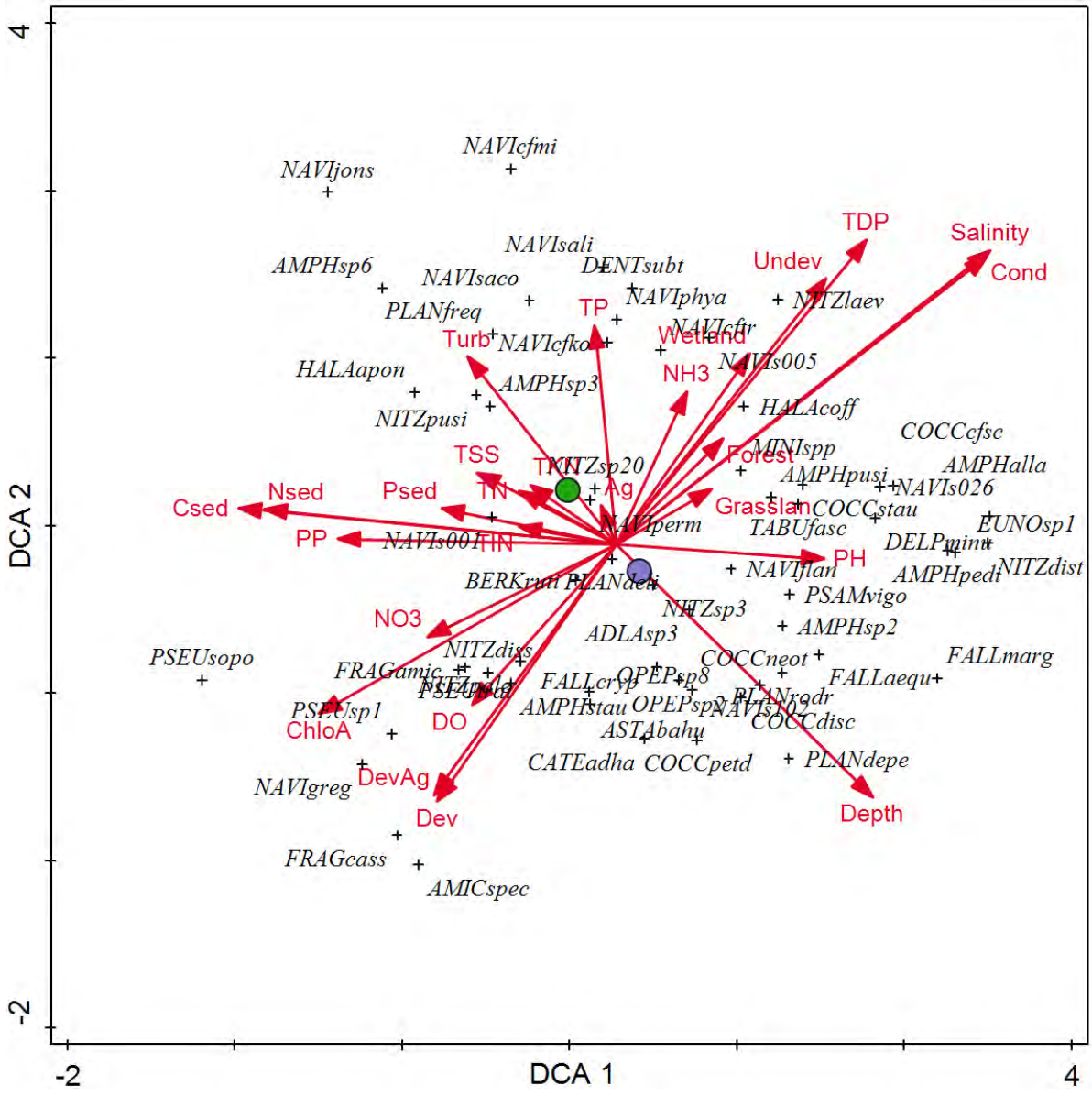


Figure 83. DCA results using benthic species- all planktonic taxa excluded dataset (100 sites/566 species). Biplot of species and environmental variables showing the result of a DCA exploring relationships between diatom assemblages and water-quality and land-use parameters. Only centroids for species with highest weights (20-100%) are shown. Species short codes correspond to those in Appendix 6. Green circle is the centroid of marsh sites and blue circle is the centroid of open-water sites.

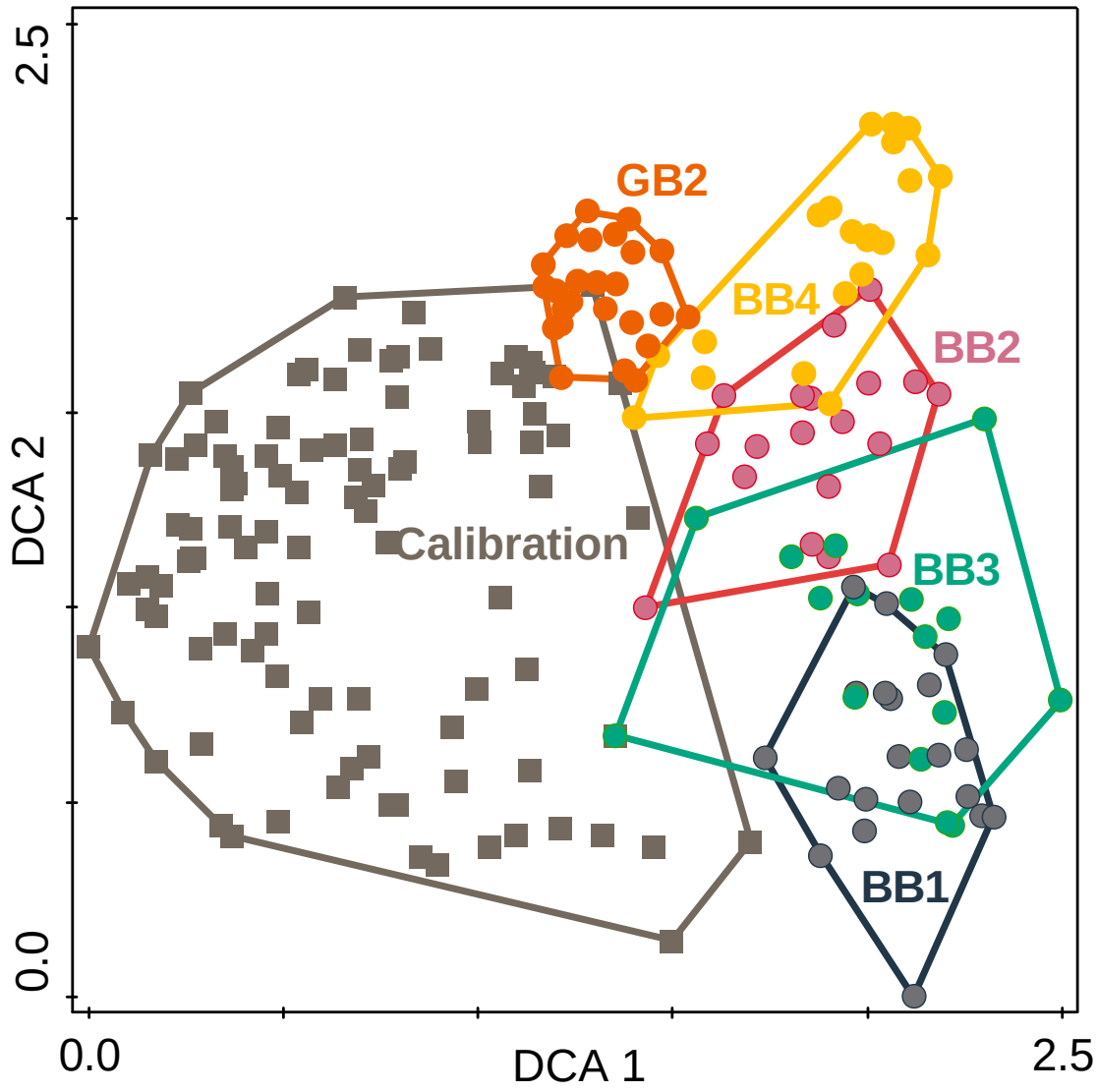


Figure 84. Plot of sample scores in the ordination space of the first and second DCA axes. Samples from the calibration set from which the planktonic taxa were excluded are shown by brown squares, core samples – by circles of different colors corresponding to four cores. There is some overlap between calibration dataset and BB3, BB2 and GB 2 core sample sets.

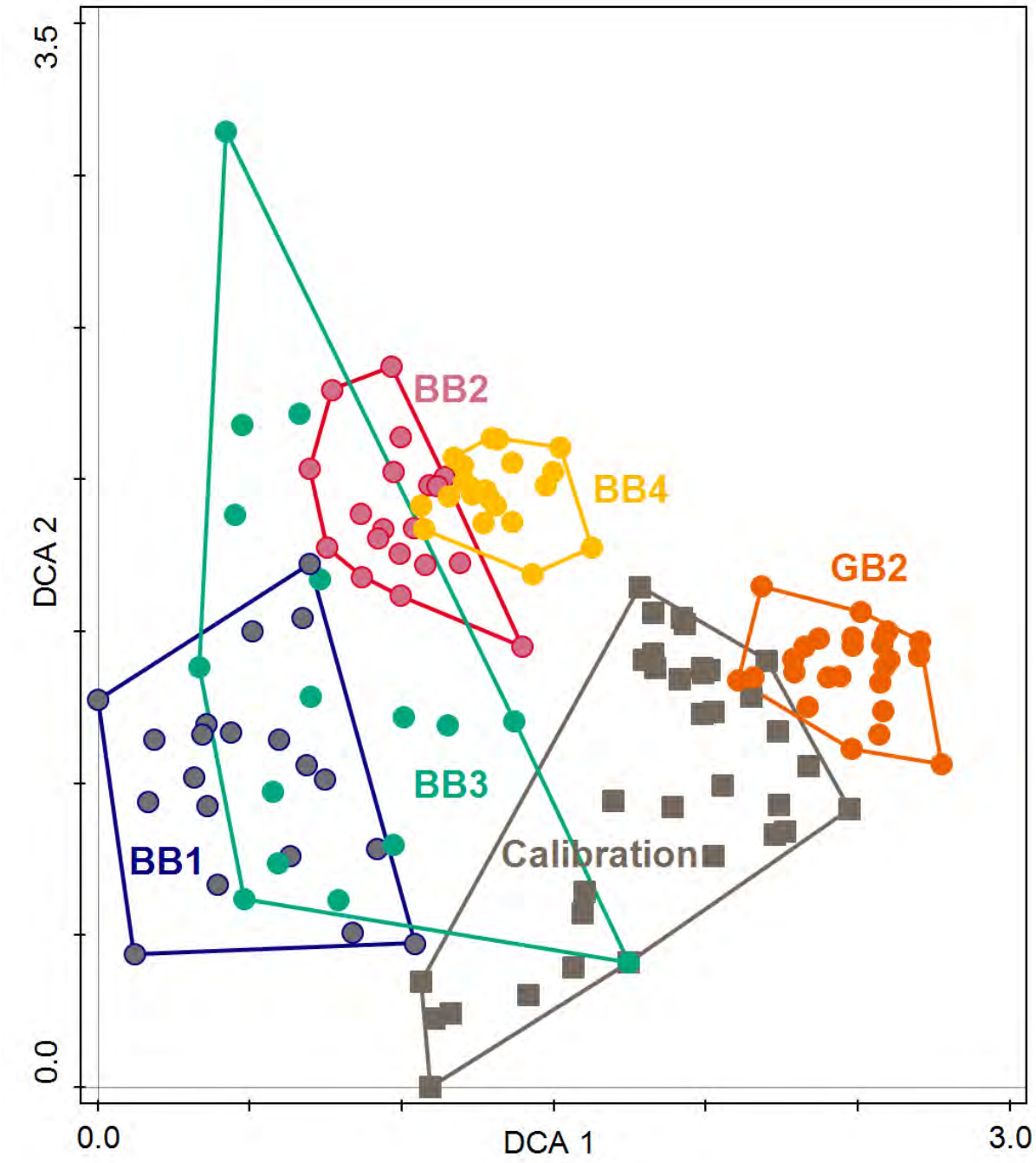


Figure 85. Plot of sample scores in the ordination space of the first and second DCA axes. Samples from the marsh sites of the calibration set are shown by brown squares, core samples – by circles of different colors corresponding to four cores. There is some overlap of samples from calibration dataset and from the cores BB3 and GB 2.

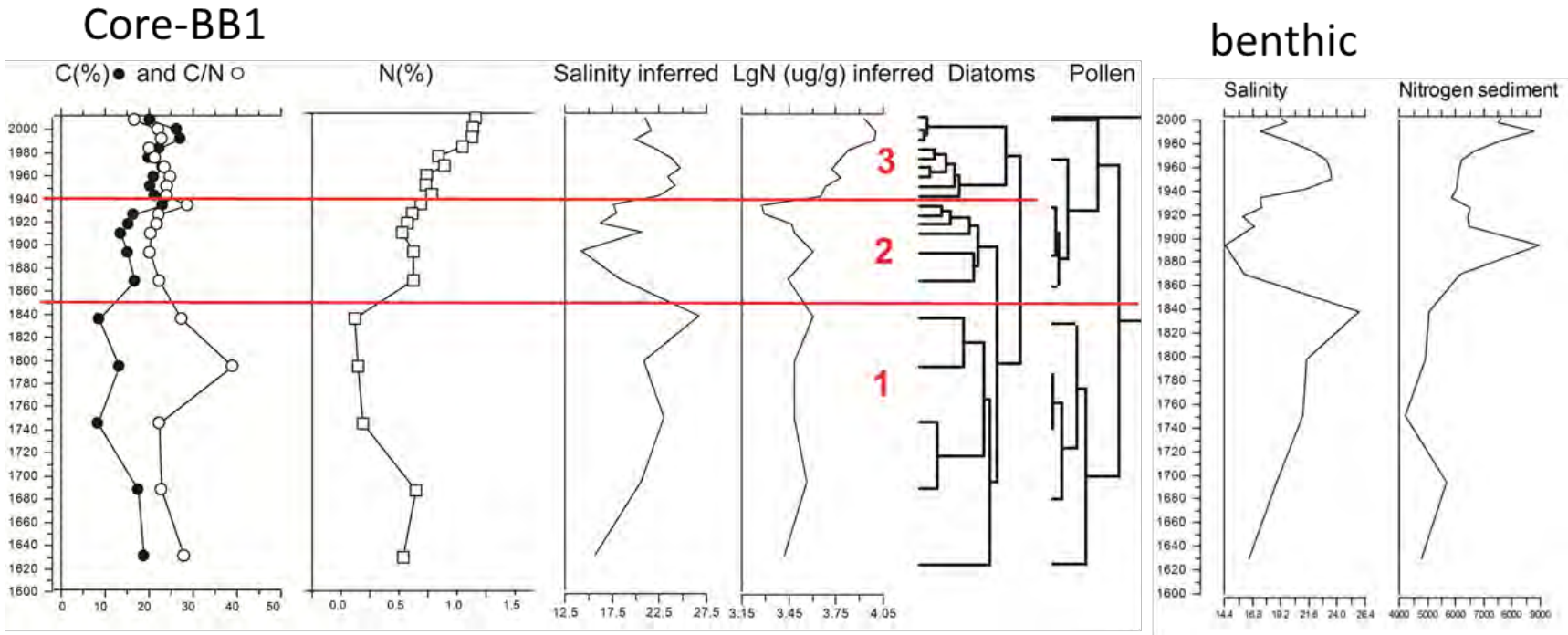


Figure 86. Comparison of stratigraphic diagram showing changes along the BB1 core in sediment organic carbon content (C, %), nitrogen content (N, %), major diatom- and pollen base zones resulting from the constrained hierarchical clustering (CONISS) and salinity and nitrogen inferred from diatoms using original dataset (left) and using benthic taxa dataset.

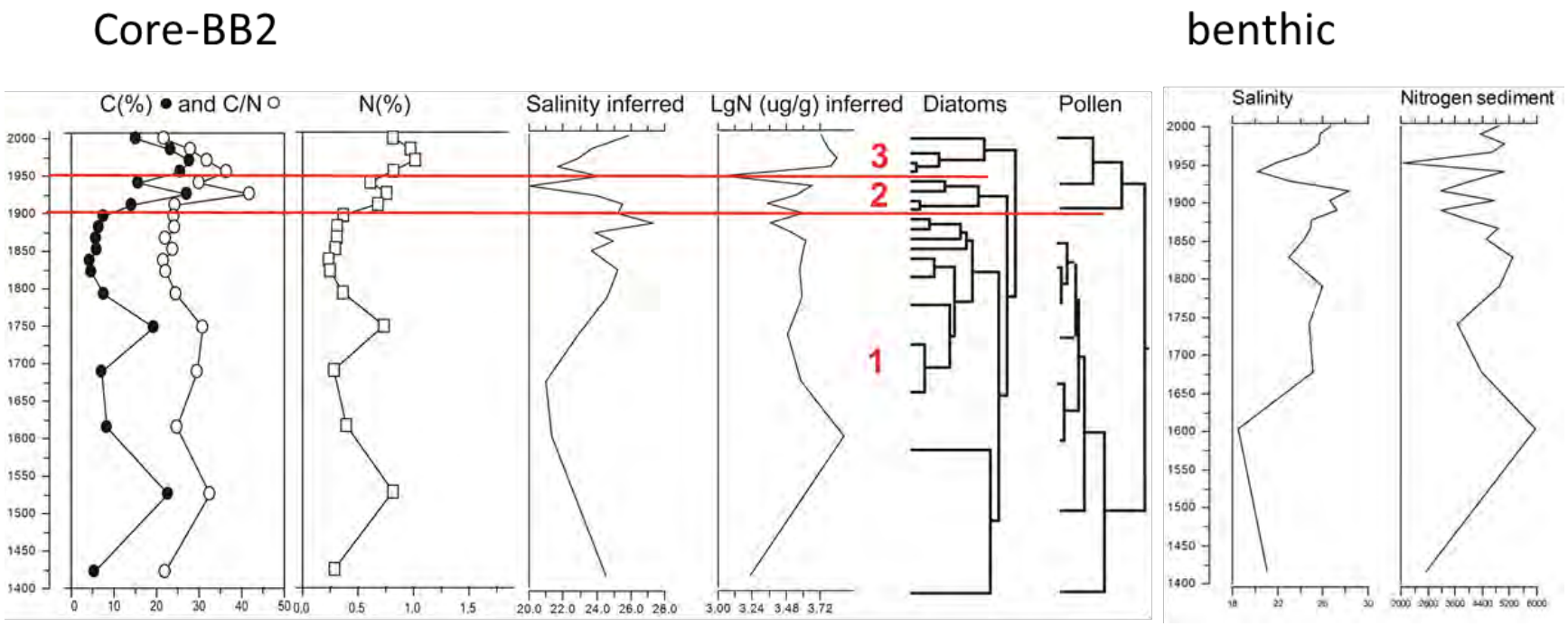


Figure 87. Comparison of stratigraphic diagram showing changes along the BB2 core in sediment organic carbon content (C, %), nitrogen content (N, %), major diatom- and pollen base zones resulting from the constrained hierarchical clustering (CONISS) and salinity and nitrogen inferred from diatoms using original dataset (left) and using benthic taxa dataset.

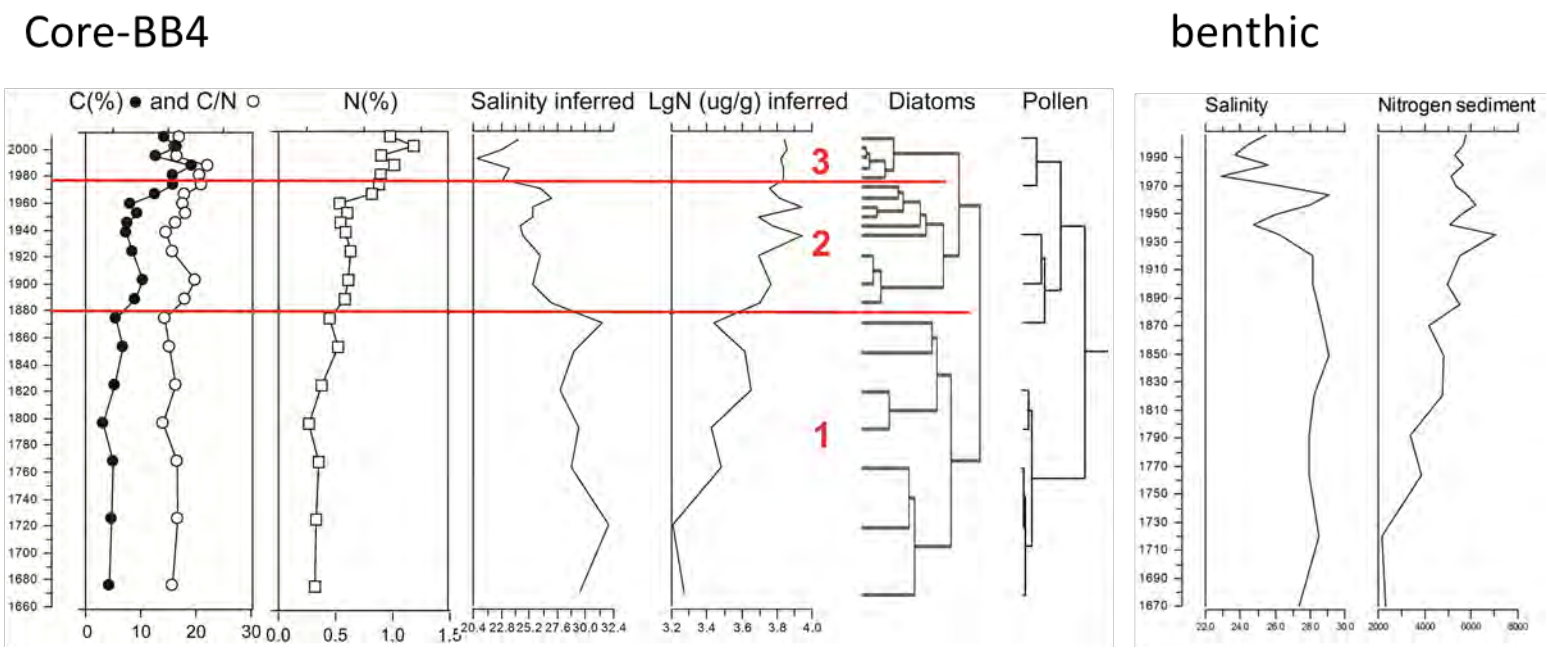


Figure 88. Comparison of stratigraphic diagram showing changes along the BB4 core in sediment organic carbon content (C, %), nitrogen content (N, %), major diatom- and pollen base zones resulting from the constrained hierarchical clustering (CONISS) and salinity and nitrogen inferred from diatoms using original dataset (left) and using benthic taxa dataset.

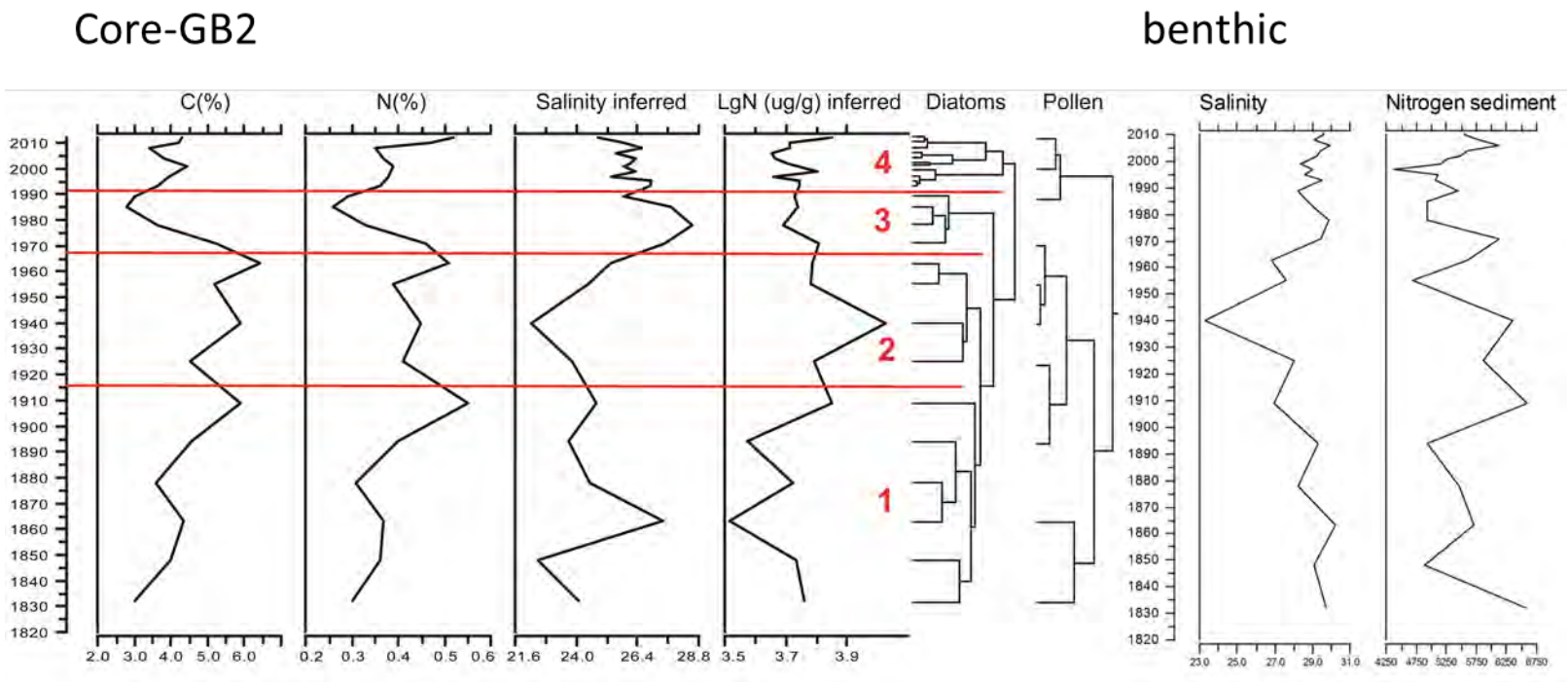


Figure 89. Comparison of stratigraphic diagram showing changes along the Great Bay core in sediment organic carbon content (C, %), nitrogen content (N, %), major diatom- and pollen base zones resulting from the constrained hierarchical clustering (CONISS) and salinity and nitrogen inferred from diatoms using original dataset (left) and using benthic taxa dataset.

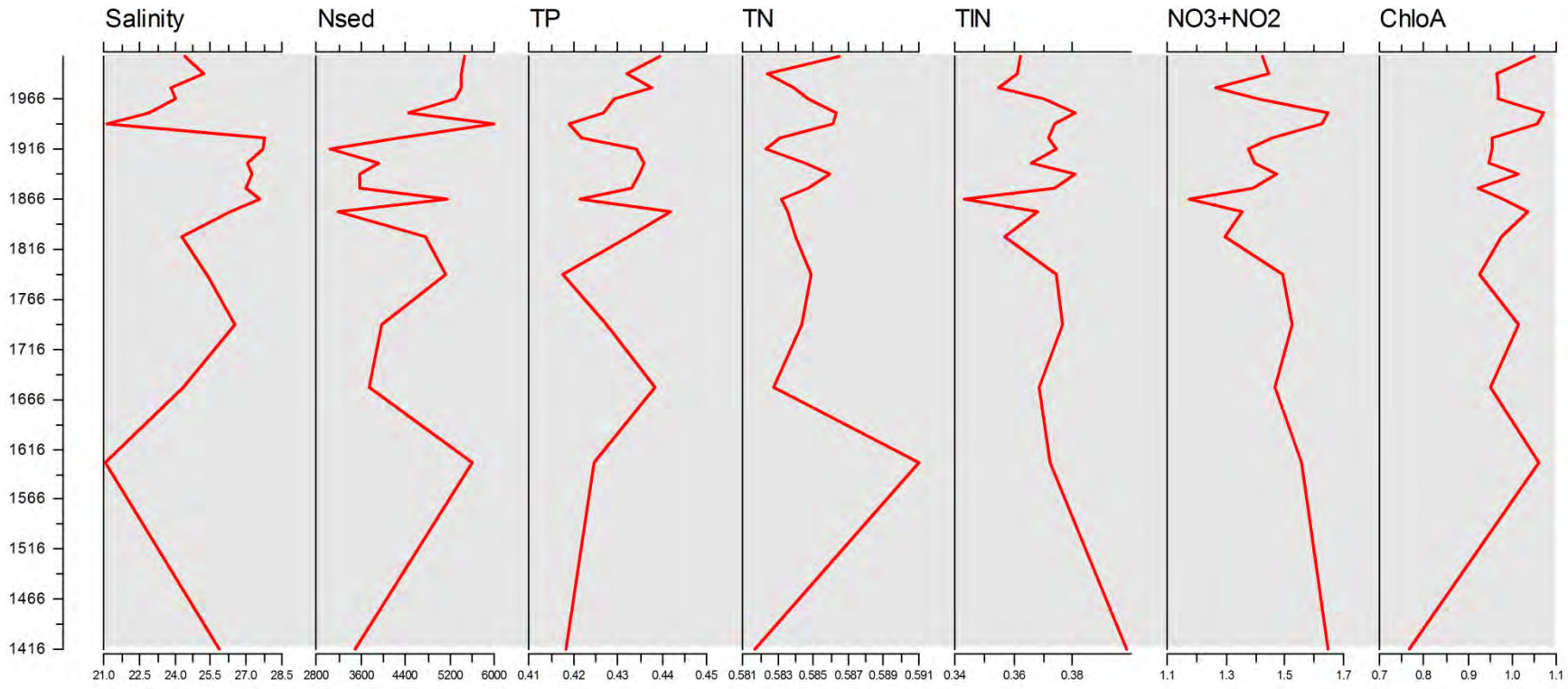


Figure 90. Stratigraphic diagram showing changes along the BB1 core in salinity, nitrogen sediment, total phosphorus (TP), total nitrogen (TN), total inorganic nitrogen (TIN) nitrate plus nitrite (NO3+NO2) and chlorophyll-a inferred from diatoms using marsh sites dataset.

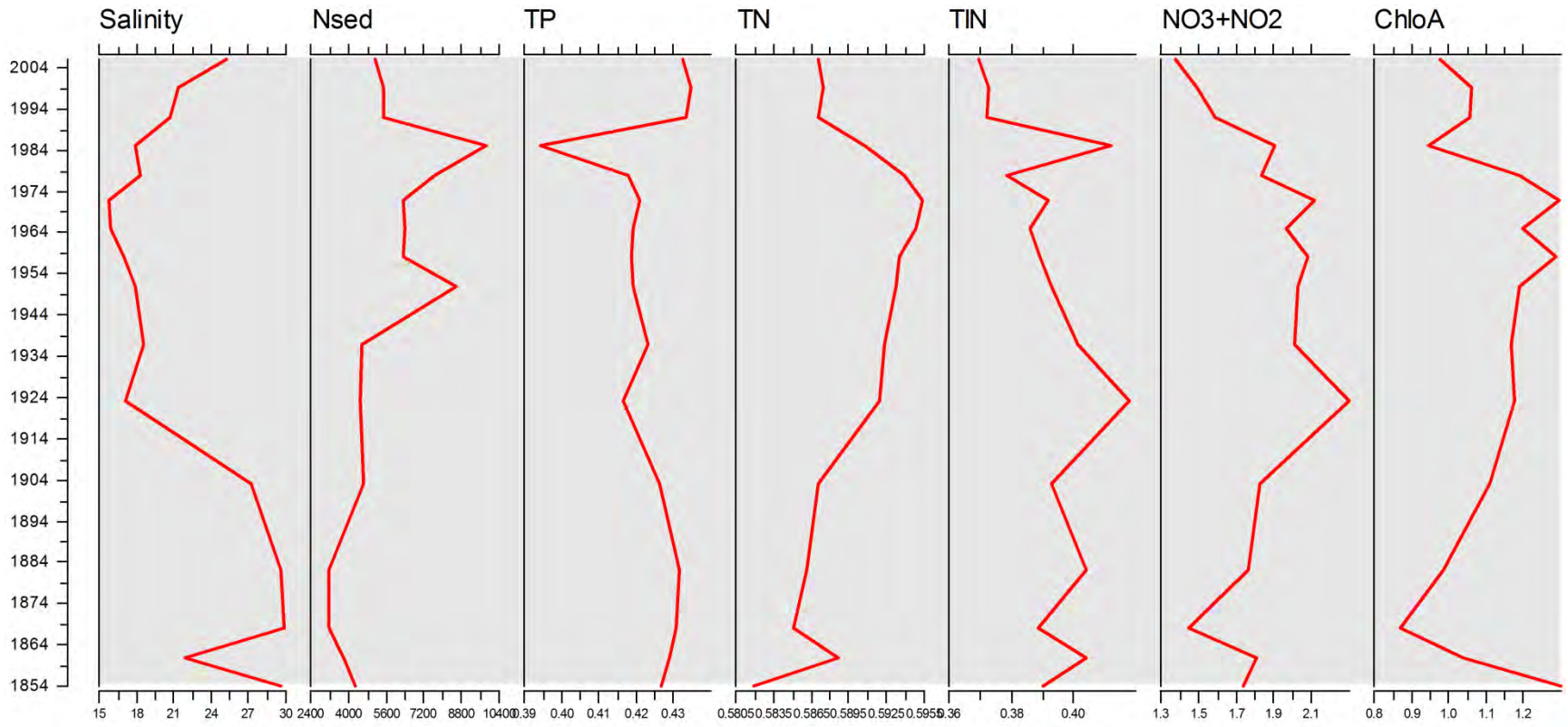


Figure 91. Stratigraphic diagram showing changes along the BB2 core in salinity, nitrogen sediment, total phosphorus (TP), total nitrogen (TN), total inorganic nitrogen (TIN) nitrate plus nitrite (NO3+NO2) and chlorophyll-a inferred from diatoms using marsh sites dataset.

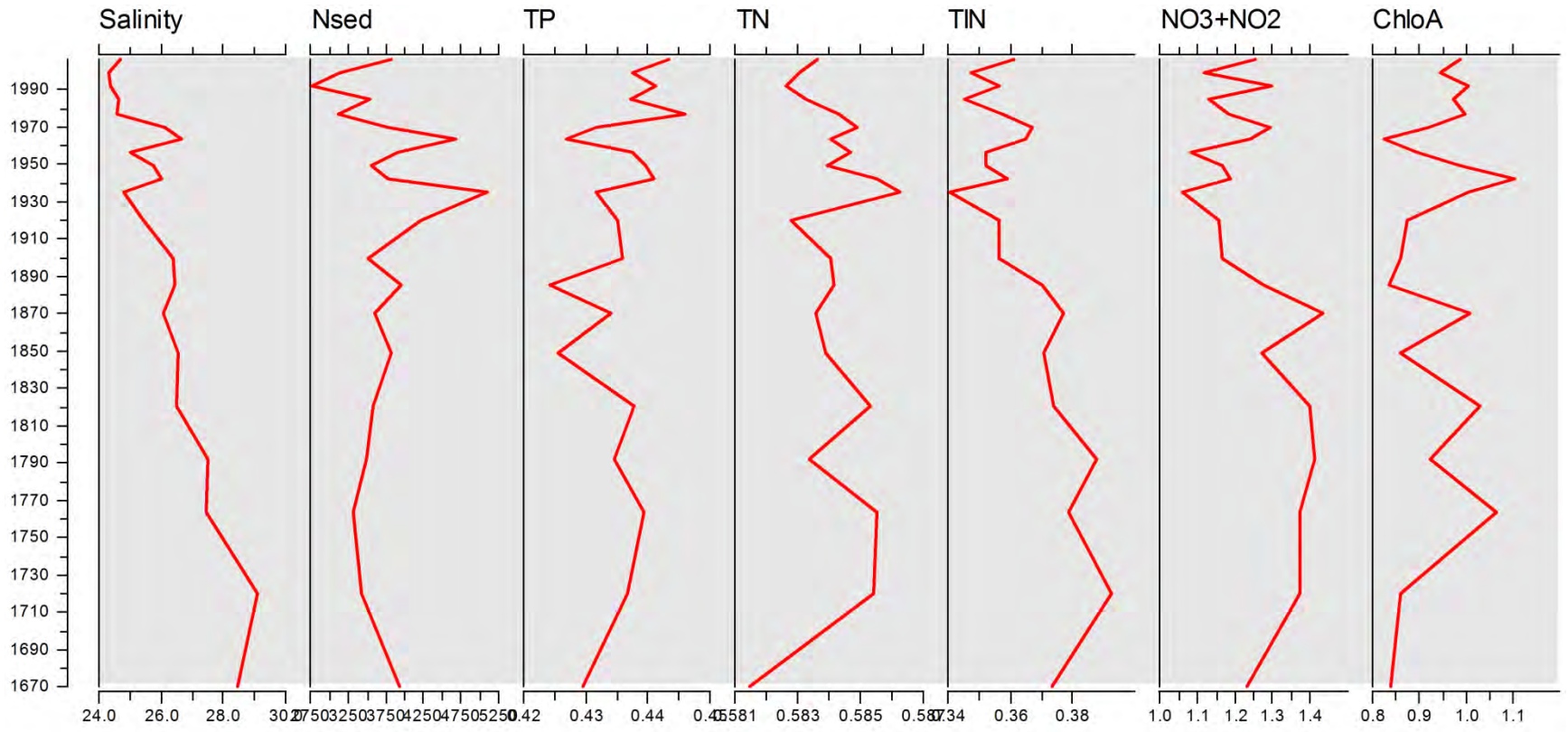


Figure 92. Stratigraphic diagram showing changes along the BB4 core in salinity, nitrogen sediment, total phosphorus (TP), total nitrogen (TN), total inorganic nitrogen (TIN) nitrate plus nitrite (NO3+NO2) and chlorophyll-a inferred from diatoms using marsh sites dataset.

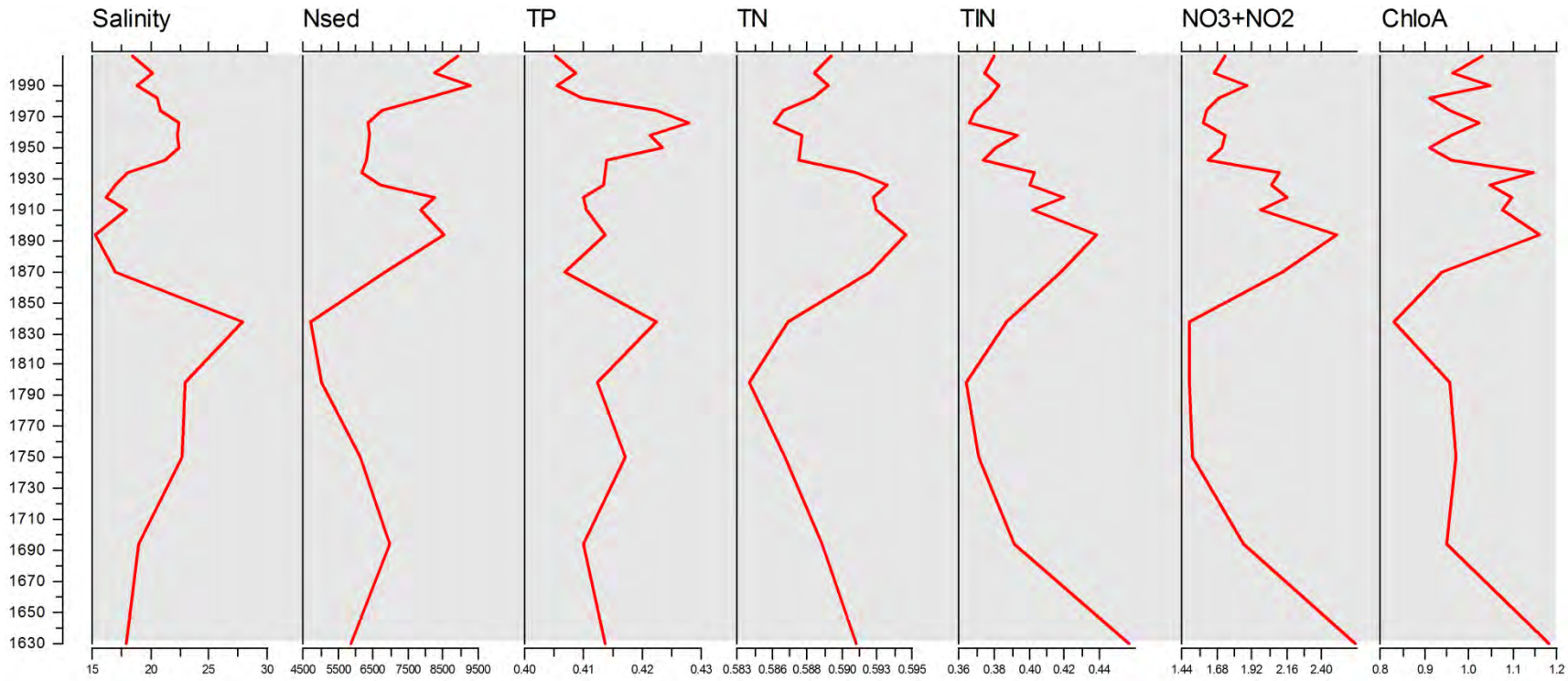


Figure 93. Stratigraphic diagram showing changes along the Great Bay core in salinity, nitrogen sediment, total phosphorus (TP), total nitrogen (TN), total inorganic nitrogen (TIN) nitrate plus nitrite (NO3+NO2) and chlorophyll-a inferred from diatoms using marsh sites dataset.

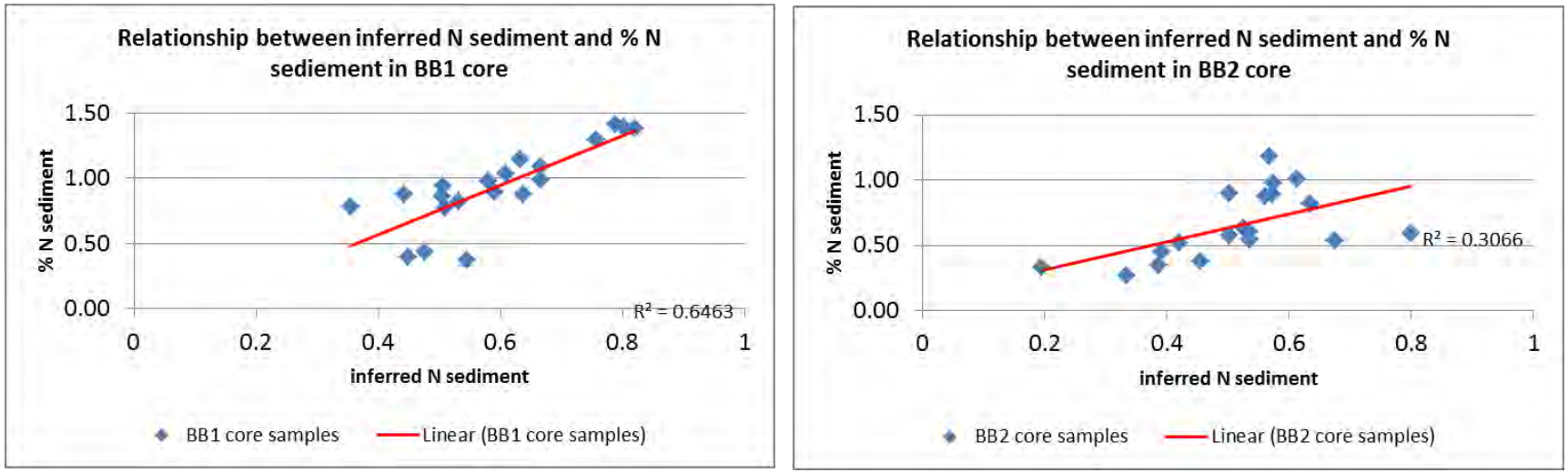


Figure 94. Relationships between diatom-inferred and observed N sediment in the core BB1 (left) and in the core BB2 (right).

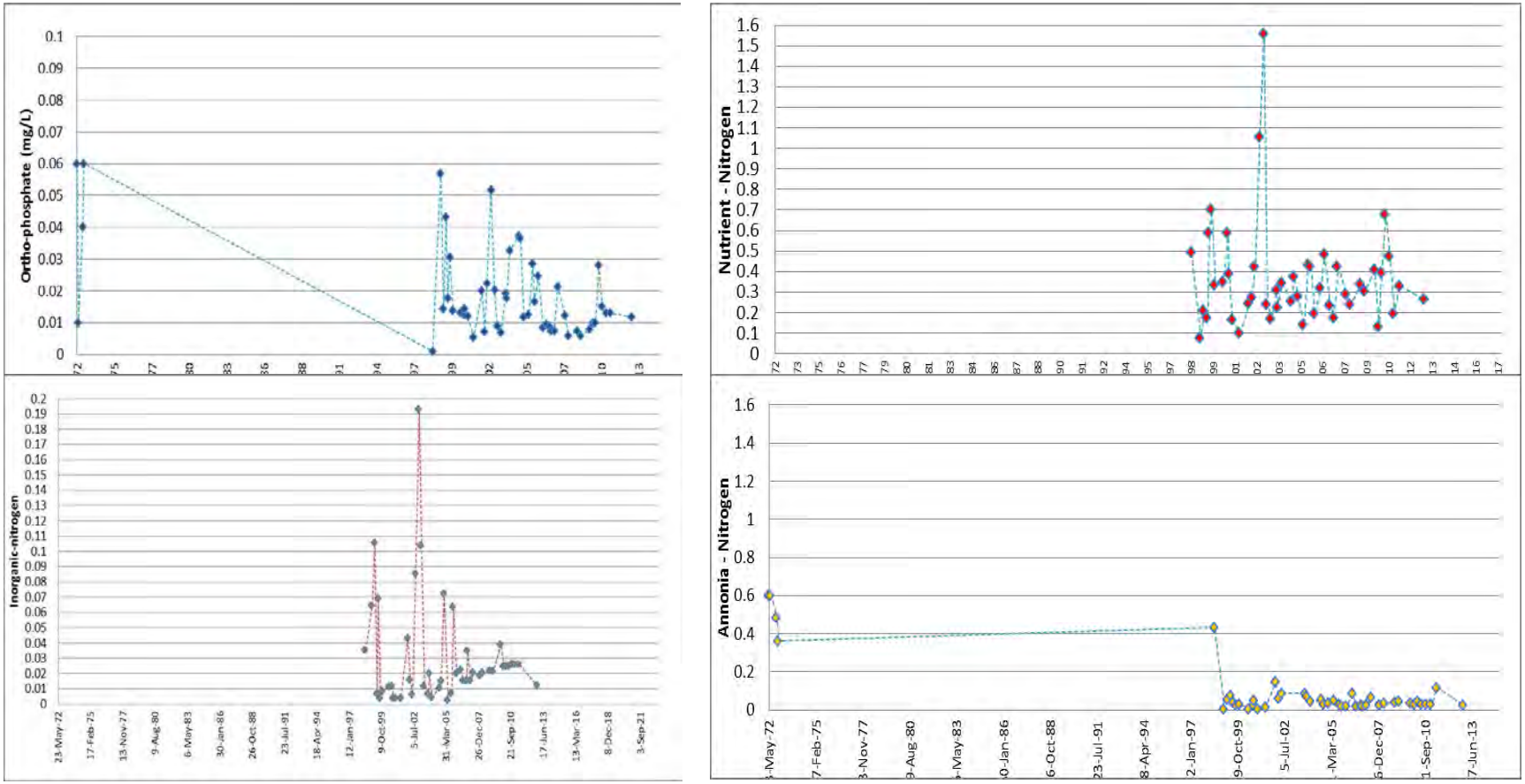


Figure 95. Monitoring of nutrients results at the R14A station (near to core BB3 location) from 1972 to 2013.

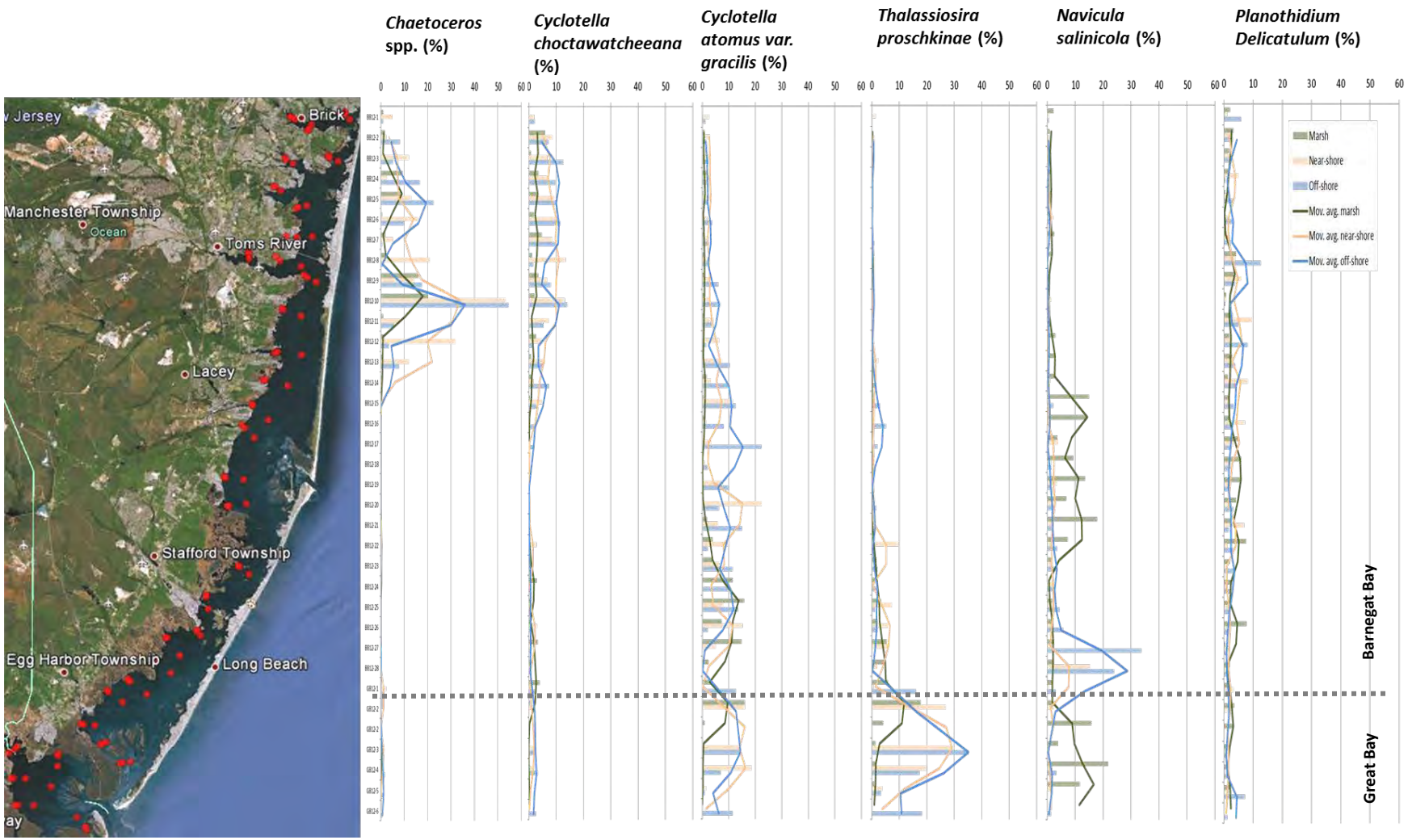


Figure 96. Relative abundance of six most common diatom taxa in Barnegat Bay and Great Bay.

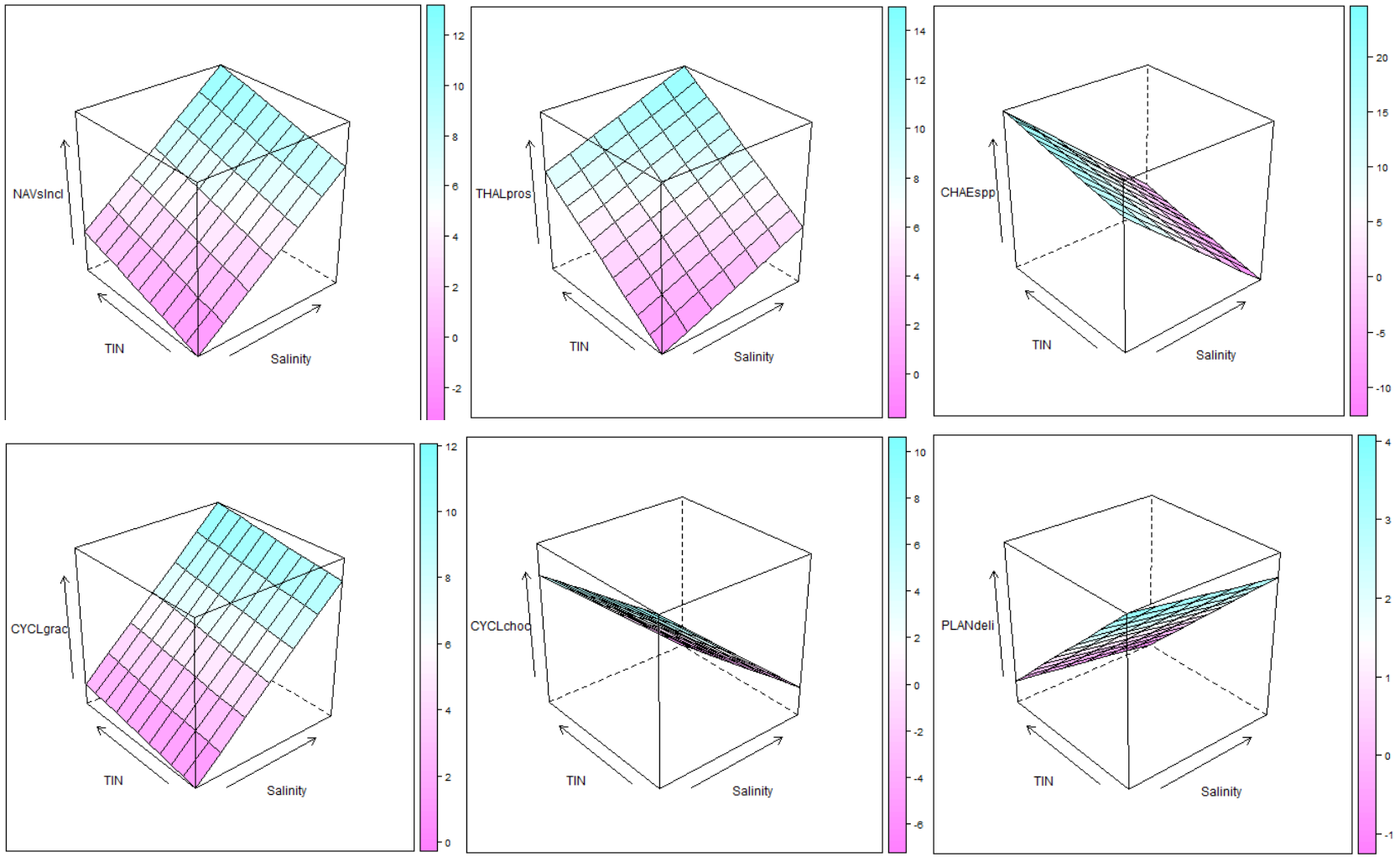


Figure 97. GAM models showing relative abundance of six most common diatom taxa in Barnegat Bay and Great Bay as response variables and water TIN and salinity as predictor variables.

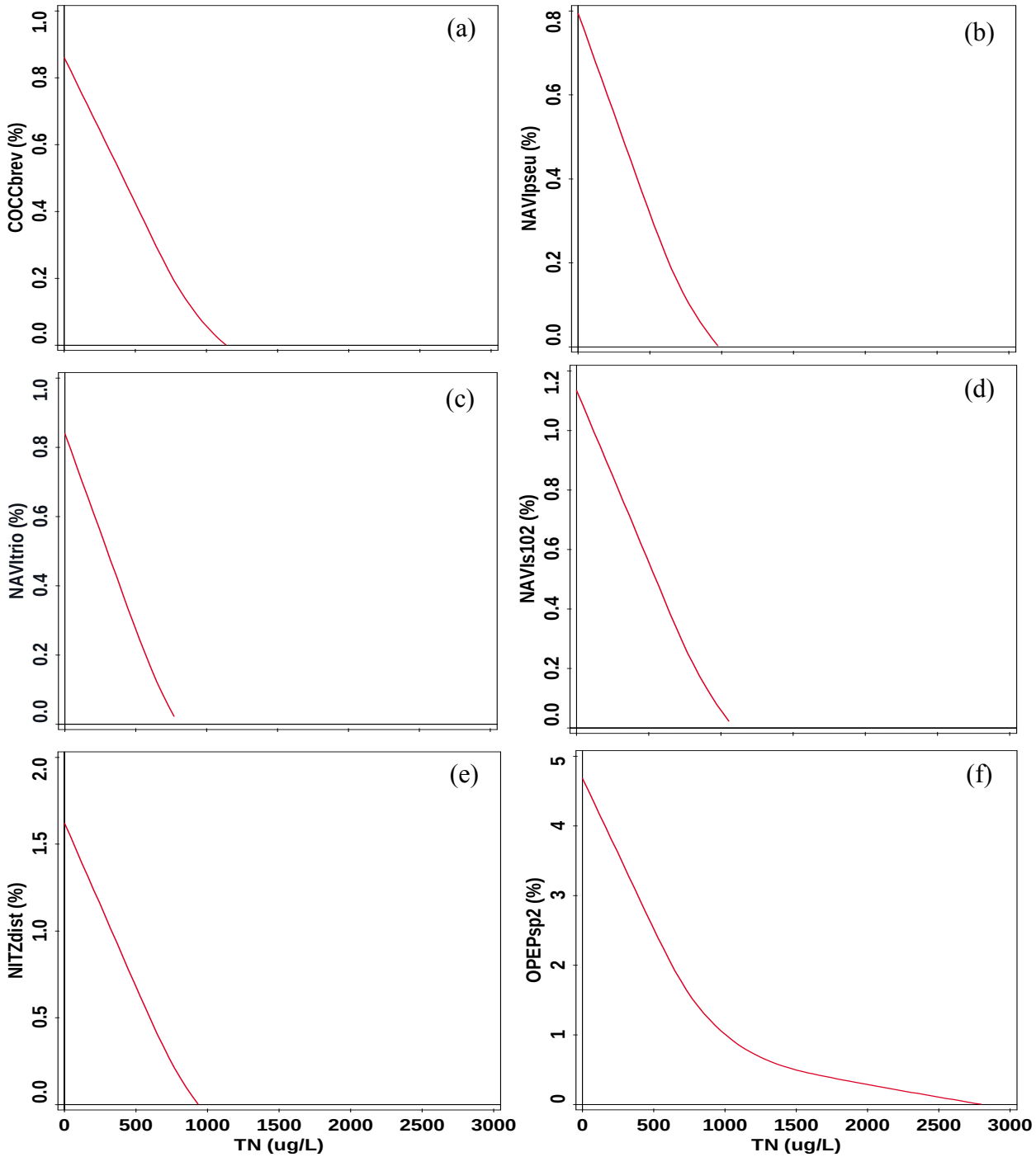


Figure 98. GAM modeling of relative abundance of selected TN-sensitive taxa in relation to TN. (a) *Cocconeiopsis breviata*, WA optimum = 595.34 ± 147.94 µg/L; i.env = 445.45 µg/L; (b) *Navicula pseudosalinarioides*, WA optimum = 572.243 ± 137.92 µg/L; i.env = 574.65 µg/L; (c) *Navicula transistantioides*,WA optimum = 491.25 ± 87.46, i.env = 6929.30 µg/L; (d) *Navicula* sp. 102 COAST, WA optimum = 592.12 ± 102.86 µg/L; i.env = 574.65 µg/L; (e) *Nitzschia distans*, WA optimum = 553.12 ± 93.69 µg/L, ; ienv = 445.45µg/L; (f) *Opephora* sp. 2 COAST, WA optimum = 619.87 ± 189.55 µg/L, ; ienv = 6929.3 µg/L.

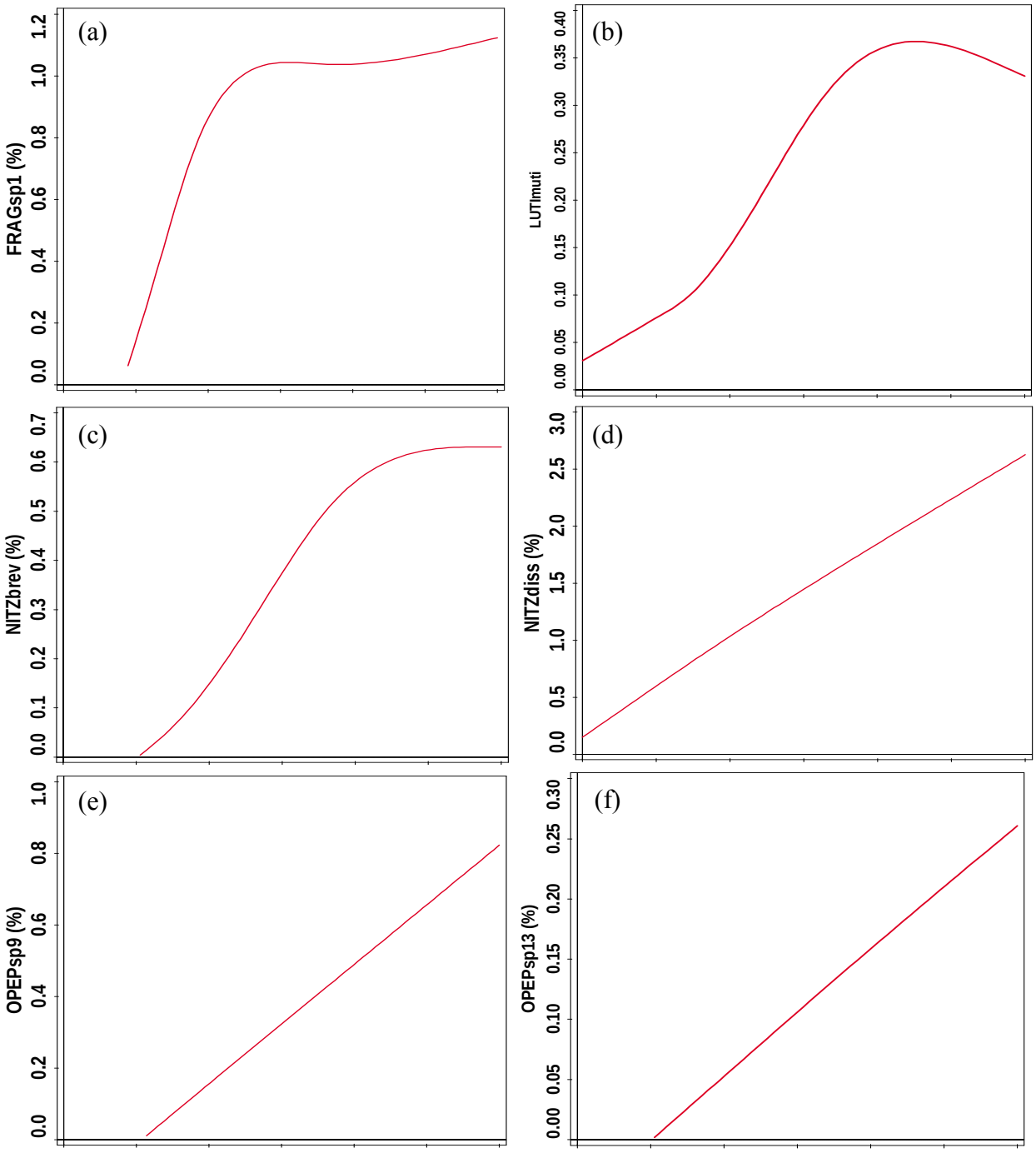


Figure 99. GAM modeling of relative abundance of selected TN-tolerant taxa in relation to TN. (a) *Fragilaria* sp. 1 COAST, WA optimum = 843.81 ± 369.89 µg/L; i.env = 3096.4 µg/L; (b) *Luticola mutica*, WA optimum = 820.90 ± 452.13 µg/L; i.env = 421.55 µg/L; (c) *Nitzschia brevissima*,WA optimum = 1195.99 ± 679.60, i.env = 2544.1 µg/L; (d) *Nitzschia dissipata*, WA optimum = 794.119 ± 388.98 µg/L; i.env = 3762.6 µg/L; (e) *Opephora* sp. 9 COAST, WA optimum = 1219.97 ± 648.63 µg/L, ; ienv = 6929.3µg/L; (f) *Opephora* sp. 13 COAST, WA optimum = 1159.35 ± 647.18 µg/L, ; ienv = 2562.2

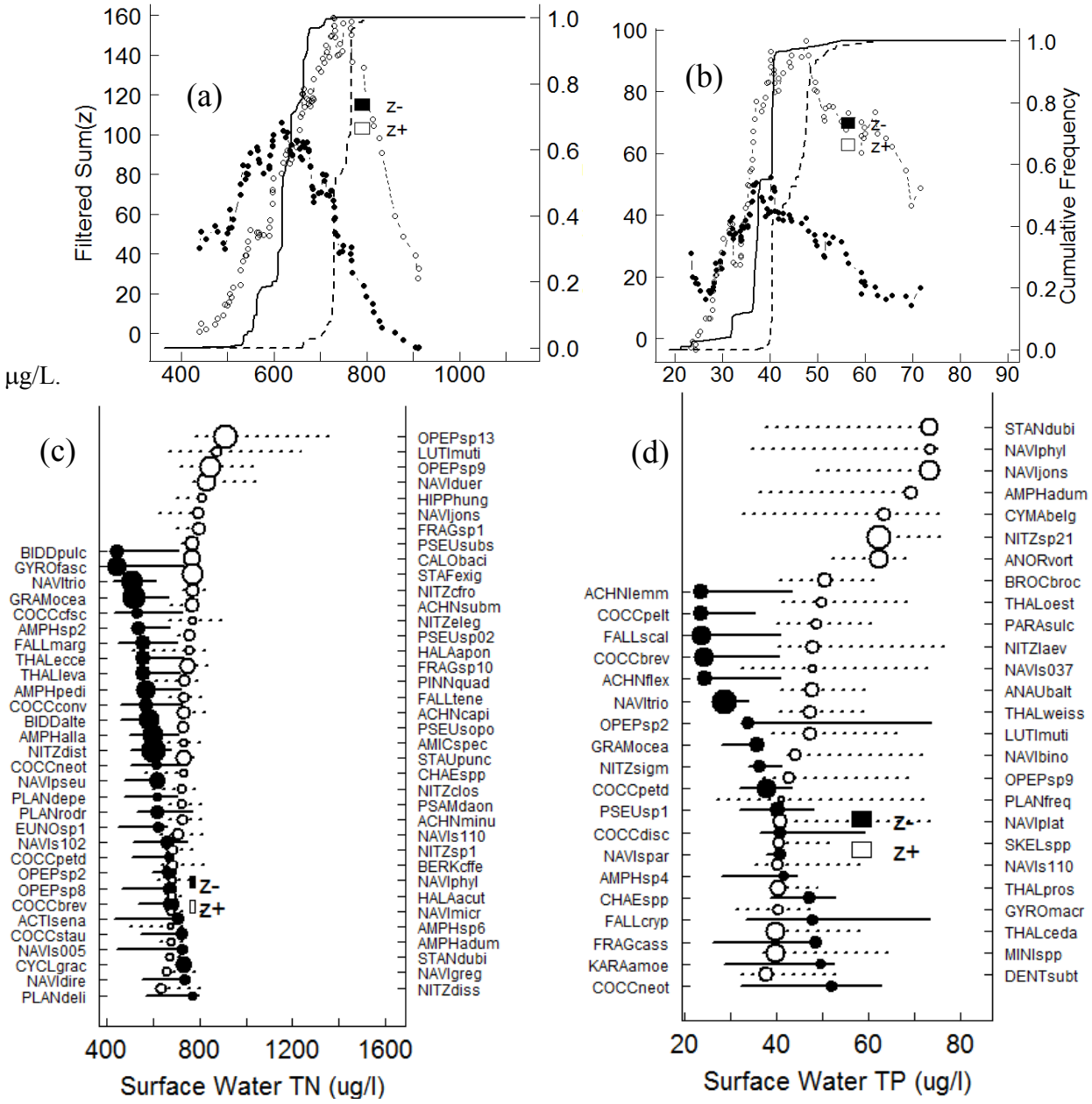


Figure 100. Results of the TITAN analyzes for TN (plots a and c) and TP (plots b and d) using the 100 sites dataset. The sum of the negative (z-) (aggregate response of negative indicator taxa, black symbols) and the sum of the positive (z+) (positive indicator taxa, open symbols) scores are shown in response to TN (a) and TP (b). Significant (purity ≥ 0.95, reliability ≥ 0.95, P ≤0.05) indicator taxa are plotted in increasing order with respect to 90% confidence in their observed change point (plots c and d). Solid symbols correspond to negative (z-) indicator taxa, whereas open symbols correspond to positive (z+) indicator taxa. Symbols are sized in proportion to magnitude of the response (z scores). Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 1000 bootstrap replicates with actual values available in Appendix 5. Species short codes correspond to those in Appendix 6.

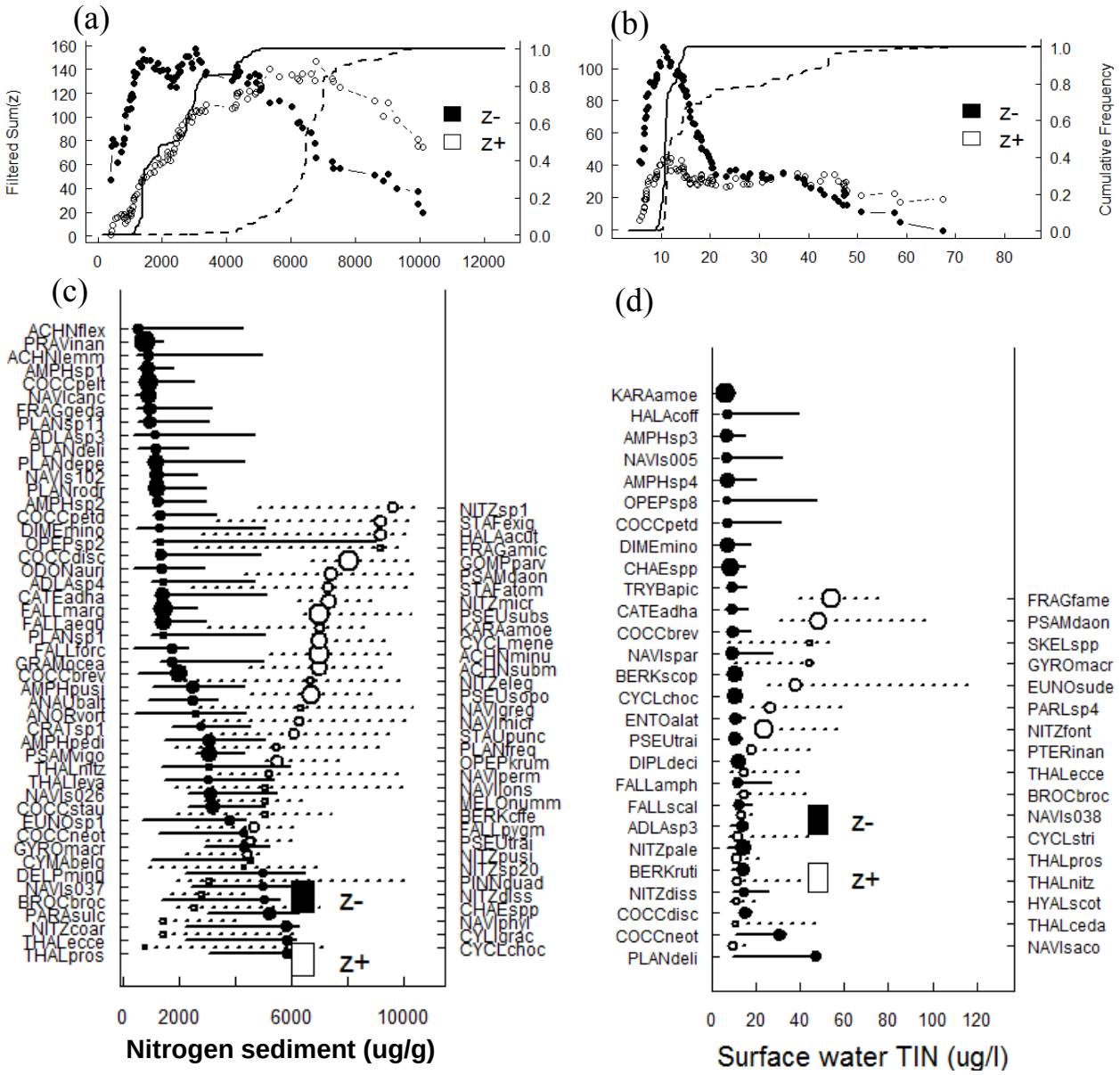


Figure 101. Results of the TITAN analyzes for N sediment (plots a and c) and TIN (plots b and d) using the 100 sites dataset. See Fig. 100 for explanation of plots and legend.

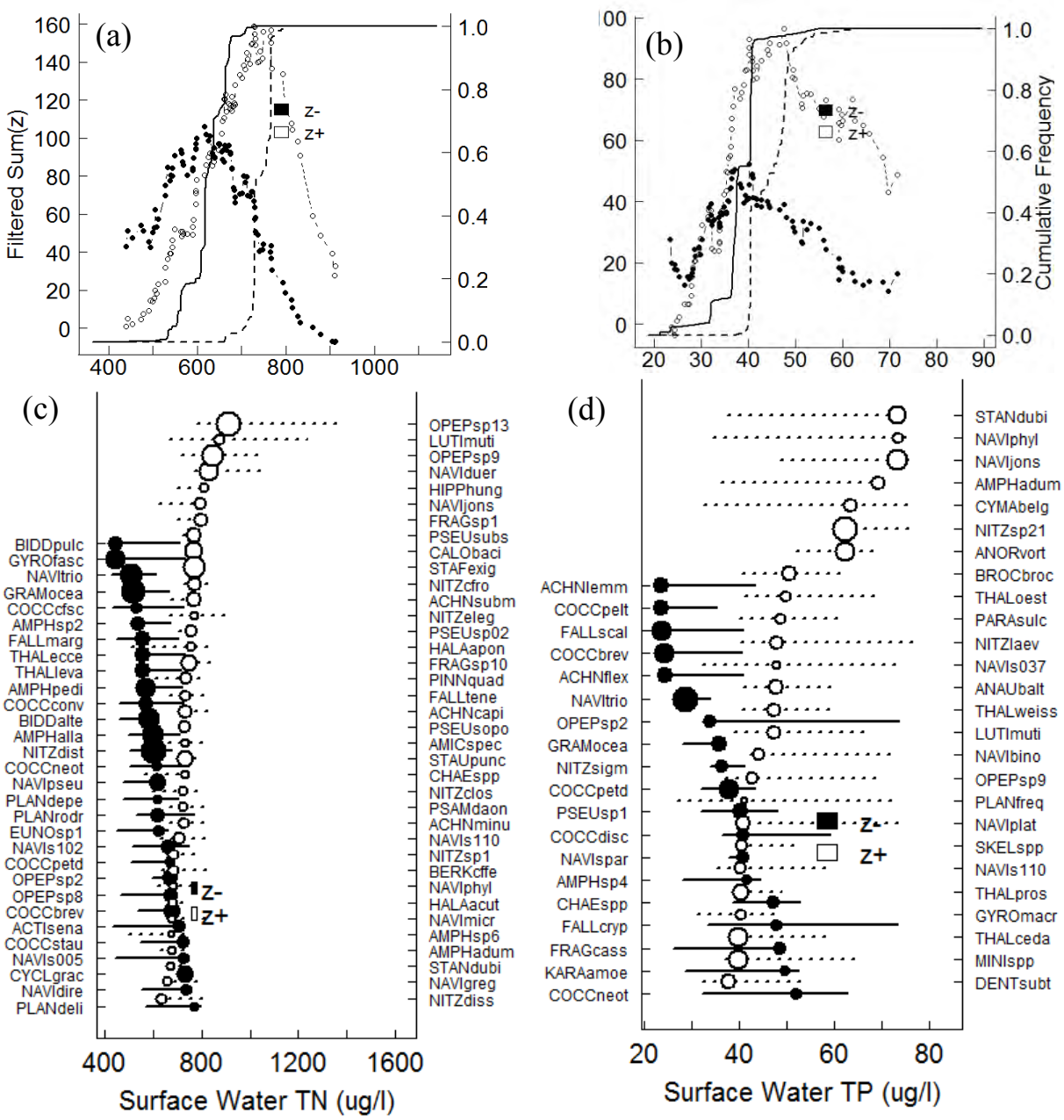


Figure 102. Results of the TITAN analyzes for TN (plots a and c) and TP (plots b and d) using the 66-sites open-water sites dataset. See Fig. 100 for explanation of plots and legend.

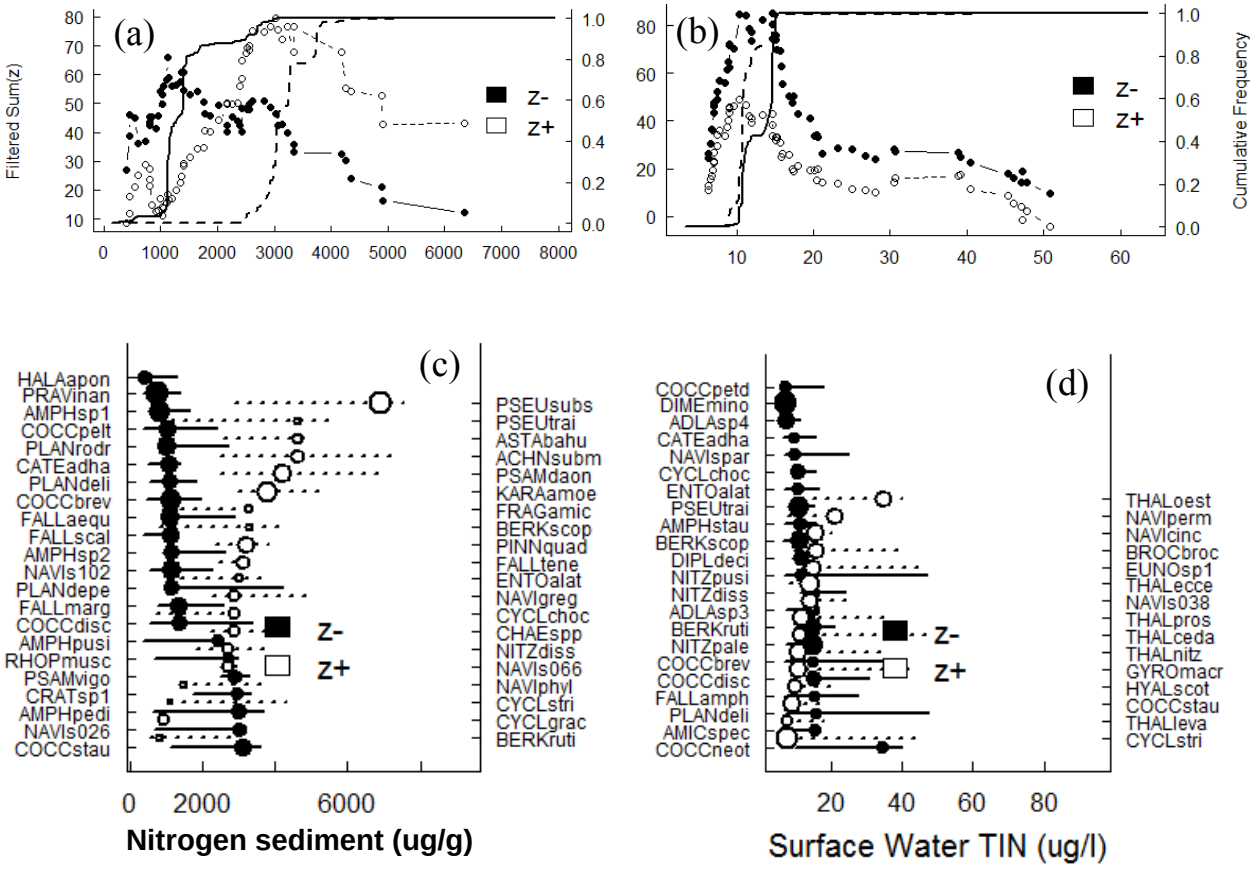


Figure 103. Results of the TITAN analyzes for N sediment (plots a and c) and TIN (plots b and d) using the 66 open-water sites dataset. See Fig. 100 for explanation of plots and legend.

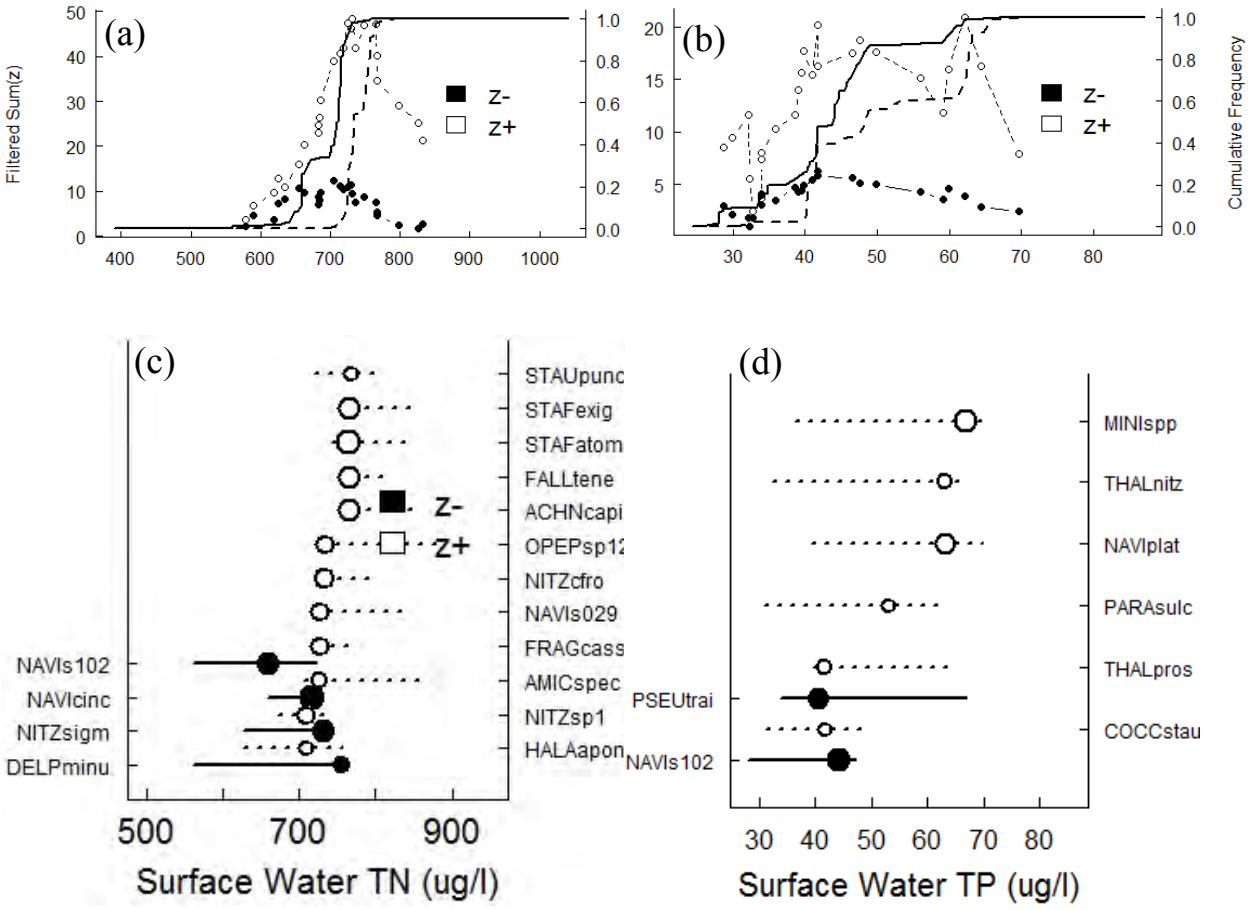


Figure 104. Results of the TITAN analyzes for TN (plots a and c) and TP (plots b and d) using 34 marsh sites datasets. See Fig. 100 for explanation of plots and legend.

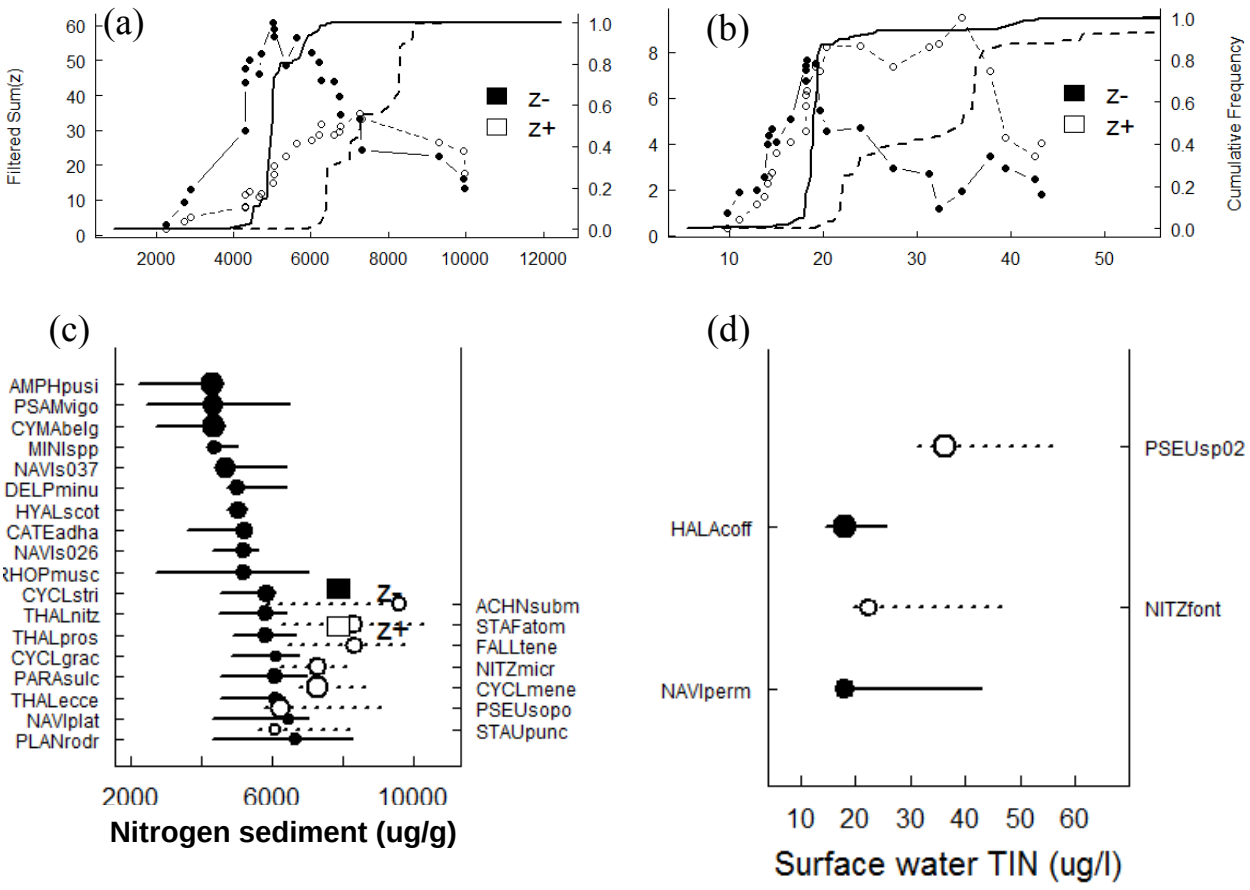


Figure 105. Results of the TITAN analyzes for N sediment (plots a and c) and TIN (plots b and d) using 34 marsh sites datasets. See Fig. 98 for explanation of plots and legend.

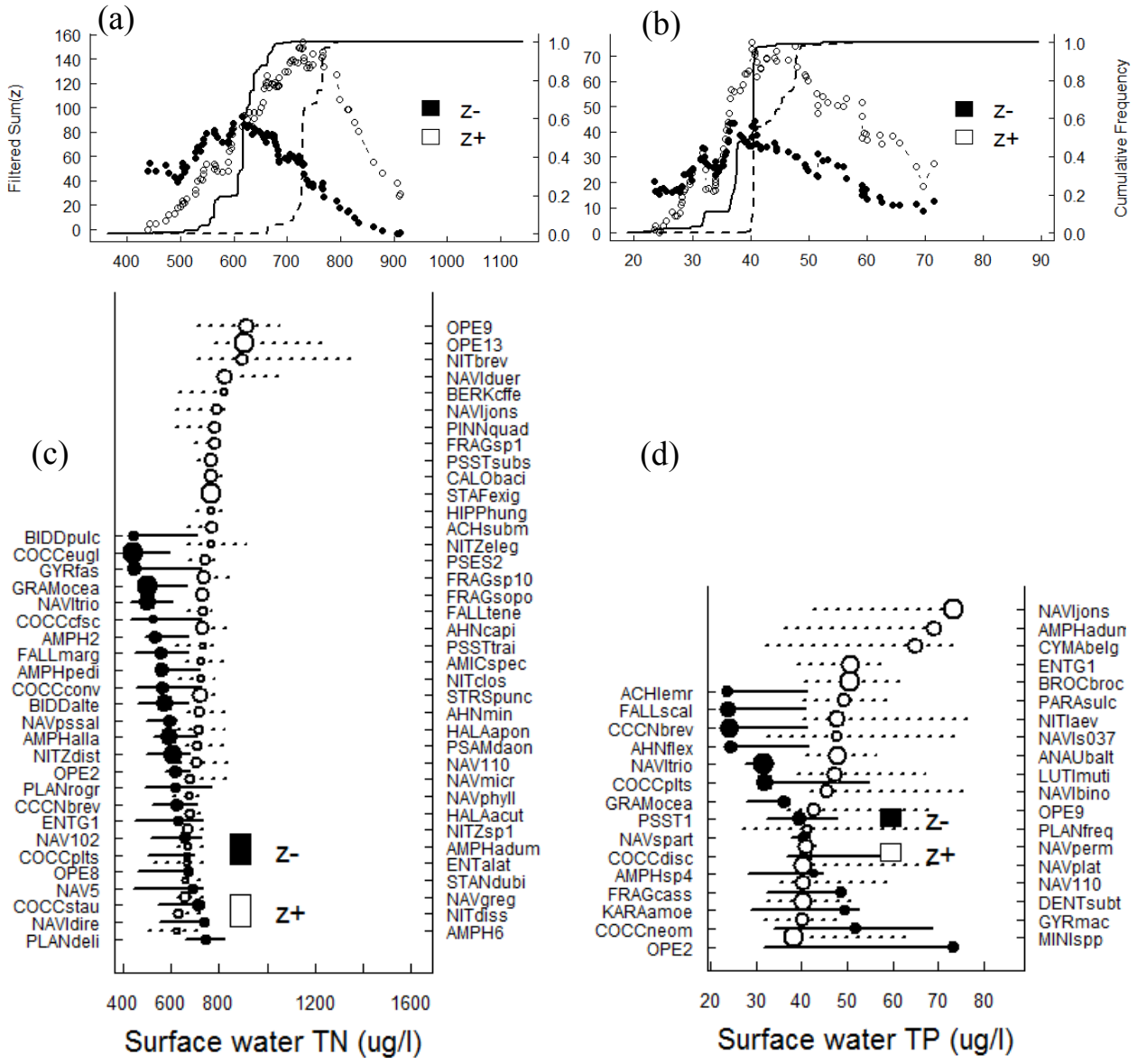


Figure 106. Results of the TITAN analyzes for TN (plots a and c) and TP (plots b and d) using 100 benthic datasets. See Fig. 98 for explanation of plots and legend.

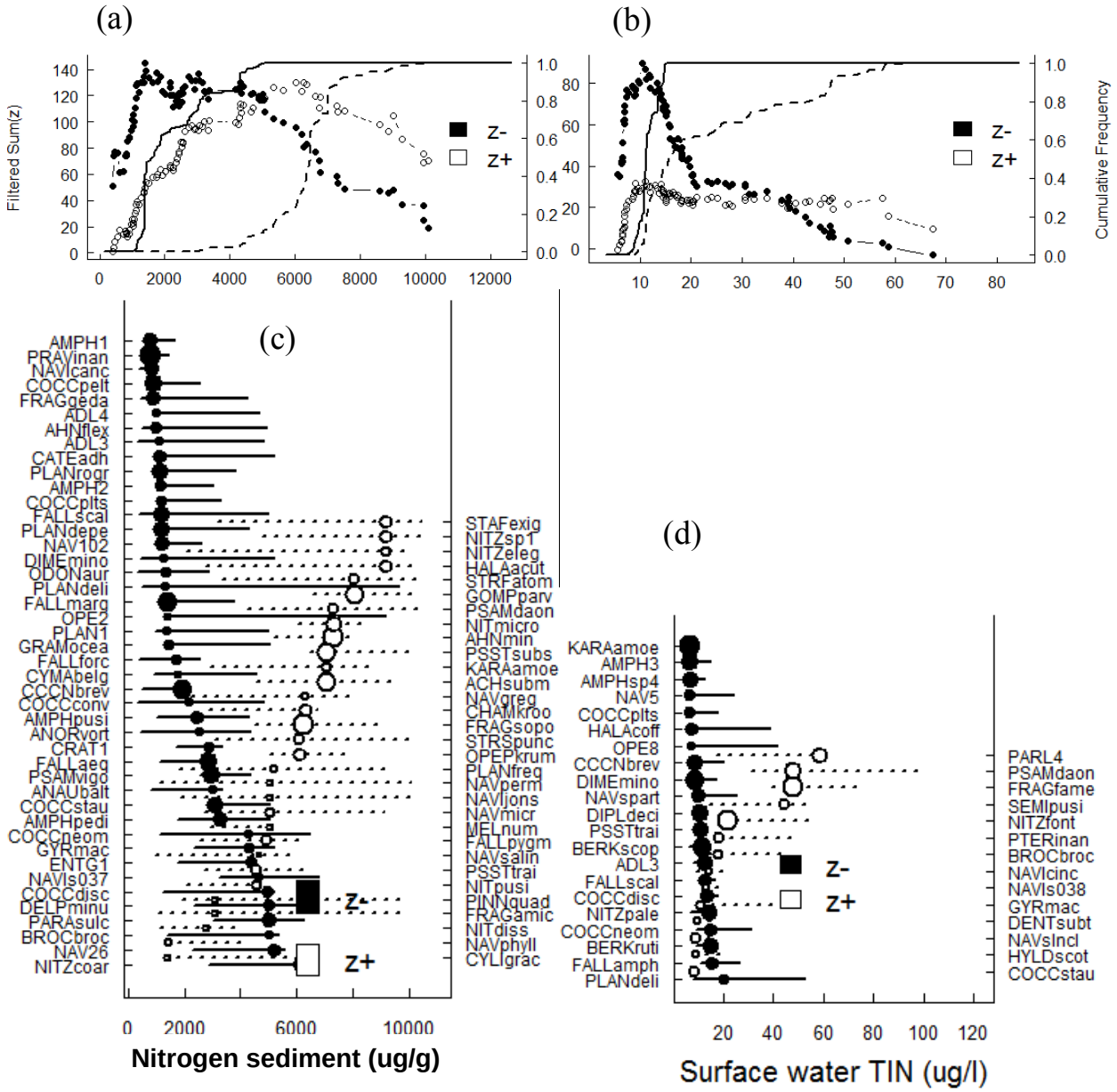


Figure 107. Results of the TITAN analyzes for N sediment (plots a and c) and TIN (plots b and d) using 100 benthic datasets. See Fig. 98 for explanation of plots and legend.

H. Appendices

Appendix I. Diatom count data, 96 sediment samples from two experimental sites. Relative abundance data and species richness. Excel File.

Appendix II. Algae count data, 96 sediment samples from two experimental sites. Absolute cell densities. Excel File.

Appendix III. Pore-water chemistry, 32 samples from two experimental sites.

Appendix IV. Modeled responses of individual diatom taxa to Total Nitrogen, results of the GAM modeling and Weighted Average optima and tolerances. Excel File

Appendix V. Results of the TITAN analyses. Excel File

Appendix VI. Diatom count data, 2012 calibration dataset of 100 surface sediment samples. Excel File