Barnegat Bay—Year 1

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Barnegat Bay Nutrient Inference Model

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TABLE OF CONTENTS

Page

List of Tables iii
List of Figures iv
Executive Summary v

A)
Introduction 1
A1 Background 1
A2 Objectives of Study 3
A3 Study Area 4

B)
Field and Laboratory Methods 5
B1 Sampling design 5
B2 Field Sampling 6
B3 Laboratory Methods 7
B3.1 Sediment Total Organic Carbon, Total Nitrogen and Total Phosphorus 7
B3.2 Water chemistry 7
B3.3 Watershed analysis 8
B3.4 Diatom identification and enumeration 9
. B3.5 Data analysis 10

C)

Results and Discussion 11
C1 Sediment Chemistry and Water Quality 11
C1.1 Sediment Organic Carbon, Total Nitrogen and Total Phosphorus 11
C1.2 Water Quality Parameters 12
C2 Land-use 13
C3 Diatom Assemblages 14
C4 Relationships between diatom assemblages and environmental parameters 15
C4.1 Correlations among environmental variables 15
C4.2 Distribution patterns of diatom assemblages in space and along environmental gradients 16
C4.3 Strength of diatom response to environmental factors 17
C3.4 Diatom inference models 18

D)
Summary and Conclusions 23

TABLE OF CONTENTS (cont)

Page

E)
Acknowledgments 27

F)
References 28

G)
Tables 34

H)
Figures 40

I)
Appendices 58

LIST OF TABLES

Table 1: Summary of sediment chemistry, 110 samples from 100 sites in Barnegat and Great Bays 34
Table 2: Summary of water quality parameters measured at 100 sites in Barnegat and Great Bays.....34
Table 3: Strength of the relationships between diatom assemblage composition and environmental variables as measured by the significance of the first CCA axes 35
Table 4: Strength of the relationships between diatom assemblage composition and environmental variables with effect of salinity partialled out, as measured by the significance of the first CCA axes.....36
Table 5: Performance of diatom inference models as estimated by R^2_{boot} value.....37
Table 6: Performance of diatom inference models based on data sub-setting by salinity, as estimated by R^2_{boot} value.....39

LIST OF FIGURES

Figure 1: Location of wetland sampling sites, corresponding watersheds and elevation data used for the watershed analysis 40
Figure 2: Collecting sediment samples in wetlands 41
Figure 3: Extruding the upper layer of a core 41
Figure 4: Collecting sediment samples in open water with a Glew corer 42
Figure 5: Collecting surface sediment sample with an Ekman Grab sampler 42
Figure 6. Land-cover classes used to quantify land-use in the watersheds 43
Figure 7: Maps showing distribution of % Total Organic Carbon (upper left), % Total Nitrogen (upper right), % Total Phosphorus (lower left) and C/N ratio in 100 sediment samples from the Barnegat and Great Bays 44
Figure 8: Difference in sediment C, N, and P concentrations and C/N ratio between marsh and open-water sites 45
Figure 9: Maps showing distribution of salinity, Nitrate and Nitrite, Ammonia Nitrogen, and Total Kjeldahl Nitrogen in 100 water samples from the Barnegat and Great Bays 46
Figure 10: Maps showing distribution of Total Dissolved Phosphorus, Particulate Phosphorus, Total Suspended Solids and Chlorophyll A in 100 water samples from the Barnegat and Great Bays 47
Figure 11: Land-use quantified for 34 “marsh” sites. 48
Figure 12: Results of the Principal Component Analyses of environmental variables in sample sets from Barnegat and Great Bay 49
Figure 13: Maps showing major variation is diatom assemblage composition across study area as revealed by the Detrended Correspondence Analysis 50
Figure 14: Correspondence between major gradients in diatom species composition revealed by the Detrended Correspondence Analysis and measured environmental parameters in the dataset of 100 sites from the Barnegat and Great Bays 51
Figure 15. Correspondence between major gradients in diatom species composition revealed by the Detrended Correspondence Analysis (DCA axes 1 and 2) and measured environmental parameters in the dataset of 34 marsh sites from the Barnegat and Great Bays 52
Figure 16. Correspondence between major gradients in diatom species composition revealed by the Detrended Correspondence Analysis (DCA axes 1 and 2) and measured environmental parameters in the dataset of 66 open-water sites from the Barnegat and Great Bays 53
Figure 17. Biplot of species and environmental variables showing result of the forward variable selection in CCA, dataset of 100 sites from the Barnegat and Great Bays 54
Figure 18. Plots showing performance of the chlorophyll A WA-PLS (3rd component) inference model 55

Executive Summary

There is an on-going discussion on whether eutrophication is causing algal blooms and increased macrophyte growth, which are presumably causing documented secondary detrimental side effects (i.e., anoxia, loss of submerged aquatic vegetation, increase in jelly fish, decreases in fish and crab population, etc) in the Barnegat Bay, New Jersey. 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 (i.e., with some of the negative affects coming from other stressors such as increased boat and jet ski traffic, bulkhead increases, loss of freshwater flows due to regionalization of upstream river sewerage treatment plants and loss through MUA ocean outfall, etc).

The New Jersey Department of Environmental Protection (NJDEP) is evaluating the appropriate indices to be used to measure the ecosystem health of New Jersey's shallow, lagoonal estuaries or coastal bays. These would include bays such as Great Bay, Great Egg Harbor Bay, Absecon Bay, Ludlam Bay and Barnegat Bay. The federal government (USEPA and NOAA) has already developed a suite of indicators (e.g., EPA's National Coastal Assessment Report 2005 and NOAA's National Estuarine Eutrophication Assessment update) and has applied them to NJ's coastal bays with mixed results, especially as state level management tools, 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 and/or enhancement of its nutrient criteria. There is growing consensus that the traditional macroinvertebrate indices used in EPA's EMAP and National Coastal Assessment may not be adequate to fully characterize the ecosystem health of shallow lagoonal estuaries such as those along the New Jersey coast. 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, but investigations have been started to develop diatom-based environmental indicators in coastal systems, too.

The main objective of this proposal was to determine whether surface sediment diatom assemblages may be used as indicators of ecosystem health in Barnegat Bay. This was accomplished by constructing a calibration set of diatom and water-quality data from the Barnegat Bay and the adjacent Great Bay and by quantifying responses of diatoms to environmental parameters of interest, first of all, nutrients. An additional objective of this study was an assessment of the responses of diatom assemblages to land-use.

Surface sediment samples were collected from 100 sites across tidal wetland, embayment and offshore areas in Barnegat Bay. Water-quality characteristics, such as salinity, pH, nutrients in water and sediments, total suspended solids, turbidity and chlorophyll A content were measured in the field and laboratory. Land-use characteristics, such as percentage of land in the “developed”, “agricultural”, “forest”, or “wetland” categories was quantified using GIS methods. A rich diatom flora consisting of 402 species was found. The diatom assemblages were dominated by a few small-celled *Cyclotella* species, such as *C. choctawhatcheeana* and *C.*

atomus, several species of *Chaetoceros*, *Amphora*, *Cocconeis*, *Fallacia*, *Navicula*, *Planothidium*, *Thalassiosira*, and *Opephora*. Diatom assemblages were highly diverse: the Shannon Diversity Index ranged from 2.3 to 4.2 with a mean of 3.5.

Multivariate statistical analyses were conducted to evaluate the relationships between diatom distribution and environmental variables. The major gradient in the composition of diatom assemblages corresponded to the north-south gradient of land-use, salinity and other associated environmental variables. The second most important gradient in diatom species data was between the marsh sites and open-water sites and correlated with water depth. The strongest diatom response was to salinity, which is expected in an estuarine environment. Other environmental parameters that had statistically significant effect on the composition of diatom assemblages i were Depth, Dissolved Oxygen, Turbidity, Chlorophyll A, Total Dissolved Phosphorus, Particulate Phosphorus, Total Phosphorus, Nitrate+Nitrite, Ammonia, Total Inorganic Nitrogen, Sediment Organic Carbon, Sediment Total Nitrogen, Sediment Total Phosphorus, and land-use characteristics

Inference models were constructed for those environmental parameters that were found to exert significant influence of the diatom assemblage composition. The models with the highest predictive power were those for Salinity, Total Dissolved Phosphorus, and Chlorophyll A. The inference models for Salinity may be useful for historical reconstruction of sea-level rise and other events related to climate change. Total Dissolved Phosphorus models, although highly statistically significant, may reflect a natural gradient of this compound related to salinity. Since an increased chlorophyll A level is a symptom of eutrophication and the chlorophyll gradient positively correlates with watersheds development, Chlorophyll A inference models seem to be most promising for reconstruction history of eutrophication in Barnegat Bay and adjacent areas. Diatom species optima and tolerances calculated for all models will be useful for environmental assessments in lagoonal estuaries in New Jersey.

This initial study demonstrated that surface sediment diatom assemblages are sensitive to a number of water and sediment chemistry parameters, and can be used to track eutrophication in its diverse manifestations, such as elevated nutrients and algal blooms. This is the first study that investigated in detail composition of benthic diatom assemblages in New Jersey lagoonal estuaries and quantified responses of individual diatom species to a number of environmental factors. This information may be further analyzed to develop even more robust diatom indicators and lessons learned from this project should be used to design further studies of responses of benthic communities to environmental stress in coastal areas.

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 and 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. 2010c).

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 colonize virtually every aquatic microhabitat and many diatom species have very strict ecological requirements, with well-defined optima and tolerances for environmental variables such as pH, nutrient concentrations, salinity, water transparency and physical habitat. Diatom assemblages have been shown to be important indicators of nutrient concentration within freshwater and marine environments (Janousek 2009; Sullivan 1975a; Sullivan and Currin 2000). Due to the fact the diatoms respond quickly and directly to nutrients, they have been used for many years as indicators of nutrient changes in aquatic systems (Potapova et al. 2004; Potapova and Charles 2007; Ponader et al. 2008). Because of their strong relationships with environmental conditions, diatoms are used to derive inference models for many environmental factors. The inference models are developed using calibration sets of both diatoms and measured environmental variables for specific geographic regions and types of water bodies. To produce robust quantitative models, the calibration sets require at least 30 sampling sites that maximize the gradient length covered by the variable of interest (e.g., phosphorus concentration, pH, etc.). These models can then be used to infer environmental parameters of interest, and have been used successfully to reconstruct reference conditions and assess the impact of anthropogenic activities on aquatic systems (Smol 2008).

Diatoms inhabiting surface layers of sediments in estuaries and shallow coastal bays are important contributors to primary production in these ecosystems (Jonge and Van Beusekom 1992, 1995, Shaffer and Sullivan 1988, Varela and 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). Previous investigations of sediment diatoms in four cores from Barnegat Bay wetlands revealed dramatic changes in species composition that are consistent with residential development and related anthropogenic activities (Velinsky et al. 2010c). Despite the study area being protected by both federal and state laws, sediment cores revealed an increase in pollution-tolerant diatom species over the last few decades. Unfortunately, many species identified in the Barnegat Bay study sites have not yet been described and their autecology is unknown, limiting ecological interpretations. It was concluded, therefore, that a study aimed at determining ecological preferences of diatoms found in sediments of the Barnegat Bay is needed.

In order to develop effective indicators of ecological condition it is necessary that indicators be calibrated to identify their responses to environmental stressors. The calibration process consists of quantifying environmental optima and tolerances of indicator taxa that allow defining biological systems that respond in similar ways to anthropogenic stressors. This has been rarely accomplished for diatoms living in sediments of estuaries and is the major step on the way of using these assemblages to assess ecosystem health (Trobajo and Sullivan 2010).

A2: *Objectives of Study*

The objective of this project was to incorporate a new biological component (diatoms) into ongoing water quality monitoring of Barnegat Bay coastal environments. This includes development and evaluation of diatom metrics indicating the condition of key ecological characteristics of the bay.

The first objective of this proposal was to create a regional calibration set of diatom assemblages and develop inference models for the Barnegat Bay tidal wetland, embayment and offshore ecosystems. Biological metrics derived from this calibration dataset will provide quantitative information on nutrient and other environmental parameters that can be used to characterize the health status and impact of human-related stressors on Barnegat Bay ecosystems. Because diatoms are continuously subject to water quality changes in these environments, they provide time-integrated water quality information that cannot be obtained by snapshot measurements. Our goal was to develop diatom indicators that can be successfully incorporated in future Barnegat Bay monitoring programs.

The second objective of this proposal was to investigate the relationship between diatom indicators and anthropogenic influences in the watershed, such as urban and agricultural land use. Diatom-inferred water quality characteristics may provide a more holistic indicator of the potential impacts of a wide variety of stressors, beyond the measured water quality parameters that directly influence the indicator assemblages. In order to reach this objective we obtained detailed data on watershed land use and related them to diatom assemblage composition. To date, no such assessment has been performed for Barnegat Bay.

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 km^2 and has been extensively developed over the past 70 years. The tidal waters cover approximately 280 km^2 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*

The sampling design of this project was based on that of the coastal Great Lakes diatom calibration dataset (Reavie et al. 2006) as it was outlined in the project description. It was initially proposed to select representative sites along gradients of land use and habitat types. This task was achieved by collecting samples of surface sediments approximately every 2 kilometers along the shore (**Figure 1**) rather than stratifying sampling along gradients of land-use using Geographic information system (GIS). Specifically, we proposed to use GIS to select these sites along gradients of land use and habitat types, and to relate wetland conditions to diatom communities. Following this approach, surface sediment cores would be collected from sites spanning the coastal ecosystems of Barnegat Bay. We decided that our analysis was exploratory and had enough sampling sites that we could sample every 2 kilometers and achieve our goal of

capturing the range of gradients in land-use. Our new design built a dataset that could be used to discover correlation of diatoms to land-use that may not have been captured in the proposed stratified sampling.

Surface sediment samples were thus collected from sites spanning the whole range of coastal ecosystems of Barnegat Bay. In addition, in order to develop indicators for more pristine conditions, sites from Great Bay were included in the sampling design. Subsets of surface sediment samples were collected along transects from tidal wetlands to tidal river/littoral and offshore locations within the bay. There were three samples in each transect: one on a marsh/wetlands, and two in open water. One open-water site was closer to the shore and another was further offshore. Only one transect in the Great Bay had 2 marsh sites and one open-water site. There were 33 transects with three samples in each. One more sample was also collected approximately from the center of Great Bay. Thus, the total number of sampling sites was 100. These sampling transects represent the range of natural and anthropogenic conditions present along the coastal Barnegat Bay/Great Bay region. A total of 110 samples from 100 sites were collected. Locations of the sampling sites are given in **Appendix I**.

B2: ***Field Sampling***

Sediment samples were collected using a variety of different devices depending on location and nature of sediment. Wetland cores (i.e., short cores) were collected using a ~ 8 cm diameter acrylic core barrel (**Figure 2**). The barrel was slowly pushed into the sediment to minimize compaction. The upper 1 cm-layer of the core was extruded (**Figure 3**) and placed into a pre-cleaned bottle and stored according to parameter of interest (diatoms or chemistry). In offshore locations, a Glew-modified gravity corer (**Figure 4**) or an Ekman Grab (**Figure 5**) was used depending upon sediment consistency (organic or unconsolidated).

In the field, the samples for sediment chemistry were kept in the dark and on wet ice. Once in the laboratory, the samples were stored frozen until preparation and analysis. Samples for diatom analysis were stored in the dark in a refrigerator and not frozen. Sediment chemistry and diatom slide preparation started as soon as the sediment samples arrived at the ANSP.

Surface water samples were collected from the adjacent waterway nearest the marsh site or sub-tidal site by hand dipping a pre-cleaned HDPE bottle. In the field the water was stored on wet ice in a cooler until returning to the shore-based laboratory or facility for filtration.

B3: *Laboratory Methods*

Published laboratory clean-techniques were used throughout (US EPA 1997; APHA, AWWA and WEF, 1995) using protocols as outlined in standard operating procedures (SOPS) at the Academy of Natural Sciences and University of Delaware (ANS 2012). All materials coming in contact with the samples were either glass or metal and were cleaned of any contaminants prior to use. Sample ID forms were used and each sample was given a unique laboratory number for sample tracking. Below are brief descriptions of each chemical, biological, or physical method.

B3.1: Sediment Total Organic Carbon, Total Nitrogen and Total Phosphorus

Total organic carbon and total nitrogen were measured using a CE Flash Elemental Analyzer following the guidelines in EPA 440.0, manufacturer instructions and ANSP-PC SOP. Samples were pre-treated with acid to remove inorganic carbon.

Total sediment phosphorus was determined using a dry oxidation method modified from Aspila et al. (1976) and Ruttenberg (1992). Solubilized inorganic phosphorus was measured with standard phosphate procedures using an Alpkem Rapid Flow Analyzer. Standard reference material (spinach leaves) and procedural blanks were analyzed periodically during this study. All concentrations were reported on a dry weight basis.

B3.2: Water Quality Parameters

Water temperature, salinity, conductivity and pH were measured with the YSI 556 hand held meter just below the surface. Water was collected using a pitcher, just below the surface,

and placed into pre-cleaned 4L cubitainers. Water was filtered through pre-rinsed and pre-weighed Whatman GF/F filters (47 mm diameter, 0.7 µm nominal pore size) for total suspended solids and through pre-combusted 25mm GF/F filters for particulate organic carbon and nitrogen. Turbidity was determined by nephelometric method using a HACH 2100P turbidimeter (U.S. EPA 1993; Method 180.1, Rev. 2.0). Total Suspended Solids were determined gravimetrically after drying the residue retained on a glass fiber filter at 103-105°C, SM20; Method 2540 D. Suspended Chlorophyll a was determined on a Turner Design fluorometer after extraction with acetone: water (90:10), SM20; Method 10200 H.

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). Total Kjeldahl Nitrogen was determined by Alpkem Autoanalyzer (RFA 300), utilizing semi-automated block digester and colorimetric phenate method. (U.S. EPA, 1993; Method 351.2, 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). Total phosphorus and total dissolved phosphorus were 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).

B3.3. Watershed analysis

Our spatial analysis of land-use for marsh/wetland sampling sites used existing 30 meter resolution maps of land cover (NLCD 2006 because 2011 has not been released), 30 meter resolution maps of geology, and new 2 meter resolution maps of land cover. We proposed to analyze land cover at several intervals of distance from each raster grid cell in the bay and the marsh. This technique was modified to analyzing watershed conditions. Once watershed conditions were analyzed, we related watershed condition to the composition of diatom communities.

We attempted to delineate watersheds using 3-meter resolution maps of elevation (DEM).

However, the 3 meter resolution maps would not yield complete watersheds for the sample locations, even though the DEM was conditioned using standard ArcHydro procedures (Maidment and Morehouse 2002). Hence, we used 10-meter resolution maps of elevations to perform watershed analyses. To delineate watersheds, we burned or embedded medium-resolution map of streams and other human made channels (National Hydrography Dataset) into the 10-meter resolution map of elevation. Burning streams into the elevation model corrected artificial "water dams", such as bridges, and did not disrupt the flow of water to the known common drainage point. Next, we filled all minor depressions in the elevation model using the ArcGIS sink procedure. Once, the DEM had been precondition we inspected each wetland sampling location and moved the point to the nearest major watershed drainage point. Each watershed was delineated separately using the ARCGIS watershed function. Initially, all watersheds were delineated simultaneously, but ARCGIS could not handle the computations and produced incomplete delineations. Therefore, watersheds were delineated individually and inspected carefully to verify that the computer did not fail to make computations due to memory problems and the sample point was capturing the proper watershed. The tasks of checking and then adjusting the delineation took several iterations to feel confident in our analyses.

We used 200 training points to classify 2010, high resolution NAIP imagery (http://www.fsa.usda.gov/FSA/apfoapp?area=home&subject=prog&topic=nai) rather than the proposed GeoEye satellite images. The NAIP imagery is 1-meter resolution and collected during the growing season ever year. Our training points came from onscreen digitization. Training data captured ten types of land-use (**Figure 6**). The land-use classes were an aggregation of the standard land-use classes used in the government's NLCD landuse maps (Fry et al. 2011). Maps with a higher resolution will enhance the accuracy of spatial analyses of the watersheds.

B3.4: Diatom identification and enumeration

About 1g of sediment from each sediment sample was used for diatom sample processing. The organic component was oxidized with 70% nitric acid while heated in a CEM microwave (165ºC) for 1.5 h. Diatoms were repeatedly allowed to settle for 24 hours and the supernatant was decanted until it reached a neutral pH. A measured amount of digested sample was dripped onto a microscope cover slip and dried. Cover slips were then mounted onto slides

using a high refractive index mounting medium (Naphrax™). Diatoms were counted and identified using a Nikon Eclipse 80i microscope equipped with DIC optics. Five hundred valves were counted for each slide at 1000x magnification. More details on standard operating procedures for diatom analysis can be found in "Protocols for the analysis of algal samples collected as part of the USGS National Water Quality Assessment Program" (Charles et al. 2002; http://diatom.ansp.org/nawqa/protocols.asp). Diatom species identifications were made using the extensive diatom library at ANSP Diatom Herbarium. The references that were most commonly consulted were diatom floras of the marine coasts and brackish waters (Cooper 1995b, Snoejis 1993, Snoejis and Balashova 1998, Snoejis and Kasperovicene 1996, Snoejis and Potapova 1995, Snoejis and Vilbaste 1994, Witkovsky et al. 2000). Scanning electron microscopy (SEM) was used to identify the smallest diatoms and to clarify taxonomic placement of many unknown species. For SEM samples were air-dried on aluminum stubs, sputter-coated with platinum-palladium using a Cressington 208HR sputter coater and examined with a Zeiss Supra 50VP SEM operated at 10 kV at the Centralized Research facility, Drexel University.

B3.5: Data Analysis

In order to elucidate patterns of environmental variation in the study area, a correlation analysis was carried out with all environmental parameters. To visualize major gradients in environmental variation, a Principal Component Analysis (PCA) with a matrix of environmental variables was carried out.

To find out major gradients in the diatom species dataset, a series of Detrended Correspondence Analyses (DCA) were performed. DCA is based only on species data, but correlations of environmental variables with DCA axes may be correlated and plotted as it was done here to visualize the correspondence between variation in species data and the environment.

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.

PCA, DCA, and CCA were carried out with the CANOCO software (Ter Braak and Smilauer 1998). Species data were square-root arcsine transformed as it is usually done for proportional data. Environmental variables that had skewed distributions were log-transformed. These included all nutrient and chlorophyll A data. Land-use variables were square-root transformed because they were expressed as percentages. All analyses that included species data were repeated with all-species datasets (with and without down-weighing of rare species) and with a dataset that included only species that reached 1% relative abundance in at least 5 samples. The latter analyses were carried out to decrease noise in the species data.

Inference models were constructed for all 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 analyses used 2 species datasets: (1) all species and (2) only those species that reached 1% relative abundance in at least 5 samples and three sites datasets: all 100 sites, marsh sites, and open-water sites for a total of 6 pairs of datasets. 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 (R^2_{boot}) and the root-square mean error of prediction (RMSEP). Inference models were constructed using C2 software (Juggins 2003).

B) Results and Discussion

C1: *Sediment Chemistry and Water Quality*

Data on water and sediment chemistry and other water-quality parameters are given in **APPENDICES I and II**.

C1.1: Sediment Organic Carbon, Total Nitrogen and Total Phosphorus

Concentrations of Sediment Total Organic Carbon for the 110 samples from 100 sites ranged from 0.11% to 31.60% on a dry weight basis (dw) with an average of 4.77 ± 5.63% (±

1σ); **Table 1, Figure 7, upper left**). Total sediment nitrogen ranged from 0.00 to 1.67% N with an overall average of 0.38 ± 0.34%; whereas total sediment phosphorus ranged from 0.01 to 0.21% with an overall average of 0.07±0.03% (**Table 1, Figure 7, upper right and lower left).**

Sediment C, N, and P concentrations were generally highest in the marsh sites in comparison to open-water sites and in river estuaries (**Figures 7 and 8).** These concentrations are generally higher than those found in tidal wetlands (salt and freshwater) in the Delaware River and Bay (Velinsky and Sommerfield, unpublished data; Velinsky et al. 2007, 2010a, b).

The carbon to nitrogen ratio (C/N; atomic units) can be used as a tracer of the source of organic matter to a location and sources such as terrestrial organic matter versus aquatic organic matter can be distinguished. For example, terrestrial material (e.g., trees) are rich in cellulose (i.e., higher C) compared to algae or marsh plants that have less structural material and are higher in proteins (i.e., higher N). Typical marine plants have C to N ratios of ~ 4-10 whereas terrestrial material can have C to N values > 15-20. In studied sediment samples the C to N ratio (atomic) ranged from 0.7 to 26 (**Table 1, Figure 7**) as was consistently higher in marshes compared to open-water sites **(Figures 7 and 8).**

C1.2: Water Quality Parameters

A summary of water-quality parameters measured at 100 sites in Barnegat and Great Bays is shown in Table 2. Salinity varied from 8.7 to 32.1 psu (**Table 2**) and was relatively low in the Northern part of the Barnegat Bay and in river estuaries (**Figure 9**). The highest salinities were observed in the Little Egg Harbor (**Figure 9, upper left**). There was little variation in pH values which ranged from 7.04 to 8.08 (**Table 2**).

Nitrate plus nitrite concentrations ranged from 0.3 to 144.6 μg/L N with an average of 7.2 μg/L. They were highest in the northern part of the Barnegat Bay, especially in marsh sites, and in the Great Bay (**Figure 9, upper right**). Ammonia-nitrogen ranged from 2.9 to 87.7 μg/L N with an average of 18.2 μg/L and was generally lower in the northern part of the Barnegat Bay and higher in the Little Egg Harbor and in the Great Bay (**Figure 9, lower left**). Concentrations of inorganic nitrogen found in this study (**APPENDIX II**) are similar to those reported earlier for

Barnegat Bay (Durand 1984, Seitzinger et al. 2001). Total Kjeldahl Nitrogen values were relatively low in the central part of the Barnegat Bay in comparison to other areas (**Figure 9, lower right**). Much higher levels of nitrogen in Total Kjeldahl Nitrogen (362.6-2866.0 with an average of 285.4 μg/L N) in comparison to inorganic nitrogen shows that most nitrogen in water column was in the organic form. Total Nitrogen values ranged from 363 to 2894 μg/L N with a mean of 694 μg/L or 50 μmol/L N, which is an average value for estuarine and coastal marine systems (Smith 2006).

Concentrations of Total Dissolved Phosphorus ranged from 3.8 to 40.6 μg/L P with an average of 16.3 μg/L P and were considerably lower in the northern part of the Barnegat Bay in comparison to the Little Egg Harbor and the Great Bay (**Figure 10, upper left**). Such an increase in Total Dissolved Phosphorus with increased salinity may be attributed to a phenomenon described by Jordan et al. (2008) who found that the phosphate release from terrigenous sediments in the saline portions of estuaries may contribute to the switch from phosphorus limitation in freshwaters to nitrogen limitation in marine water. At the same time, only additional detailed studies of nutrient dynamics between water and sediments in Barnegat Bay would clarify why Total Dissolved Phosphorus is increasing southward in this area. Particulate phosphorus ranged from 8.9 to 67.9 μg/L P with an average of 25.7 μg/L P and was highest in the northern part of the Barnegat Bay and in the Great Bay and lowest in the Little Egg Harbor (**Figure 10, upper right**). Total Phosphorus values ranged from 19 to 95 μg/L P with a mean of 42 μg/L P or 1.4 μmol/L P, which is below average value for estuarine and coastal marine systems (Smith 2006). Molar N/P ratio ranged between 17 and 124 if calculated from Total Nitrogen and Total Phosphorus values. This is consistently higher than the Redfield ratio of 16 and thus may indicate phosphorus limitation across the study area. If calculated from TDP and Total Inorganic Nitrogen values, the N/P ratio varied from 0.8 to 72.5 with a mean value of 5.4. This shows a wide variety of trophic conditions in the study area.

Distribution patterns of Total Suspended Solids and chlorophyll A were in general similar, with the highest values in the northern part of the Barnegat Bay and in the Great Bay and the lowest in the Little Egg Harbor, thus resembling the pattern of the Particulate Phosphorus (**Figure 10**). Chlorophyll A concentrations varied from 2.4 to 38.2 μg/L, the values

corresponding to the low to high levels reported for a wide range of US estuaries (Bricker et al. 2003).

C2: *Land-use*

Land-use was calculated for 34 marsh sampling sites following the study design described by Reavie et al. (2006). Quantification of watershed influences on nutrient levels and aquatic communities in open-water sites requires sophisticated modeling of hydrologic data, which was outside the original scope of this project. A total of 14 land-use categories were quantified (**APPENDIX III**). For the subsequent analyses, these categories were further aggregated into larger categories: four categories "Developed- open space", "Developed-low intensity", "Developed-medium intensity", and "Developed-high intensity" were aggregated into a single "Developed" category (DEV). Three categories "Deciduous forest", "Evergreen Forest", and "Mixed forest" were aggregated into the "Forest" category. Four categories "Barren land", "Shrub/Scrub", "Grassland/Herbaceuous", and "Pasture/hay" were aggregated into the "Grassland" category. Two categories, "Woody wetlands" and "Emergent wetlands" were aggregated into the "Wetland" category. Further, the new categories "Forest", "Grassland", and "Wetland" were aggregated into the "Undeveloped" category. As can be seen from **Figure 11**, there was a gradient from predominantly "Developed" watersheds in the northern part of the study area to predominantly "Undeveloped" in the southern part, especially in the Great Bay. Highly intensive agricultural land-use, represented by a single category "Cultivated croplands" played a minor role in the study area. Land-use characteristics obtained for the marsh sites were also extrapolated to the open-water sites of the same transects. For the open-water site in the middle of Great Bay that did not belong to any transect, an average land-use for all great Bay marsh sites was used.

C3: *Diatom Assemblages*

A total of 402 diatom species belonging to 89 genera were found in 110 studied surface sediment samples (**APPENDIX IV**). The most diverse genus was *Navicula* (112 species), followed by *Nitzschia* (42 species), *Amphora* (23 species), *Cocconeis* (15 species), and *Fallacia* (14 species). Other genera were comprised of fewer than 10 species each. 176 taxa were

considered to be undescribed species, while eight of them could not be identified even to genus level. Taxonomic investigations of these undescribed taxa are being conducted with LM and SEM methods with a goal of establishing their identity and species limits. The most common taxa (i.e., those found in the highest number of samples) were *Planothidium delicatulum* (99 samples), *Amphora coffeaeformis* (96 samples), *Cyclotella atomus* var. *gracilis* (95 samples), *Opephora mutabilis* (90 samples), *Cyclotella choctawhatcheeana* (88 samples), *Cocconeis californica* (86 samples), *Opephora olseni* (85 samples), *Berkeleya rutilans* (84 samples), and *Navicula perminuta* (84 samples). Taxa that reached the highest relative abundance were *Chaetoceros* spp. (43%), *Thalassiosira proschkinae* (37%), *Navicula* sp. 5 (37%), and *Amphora coffeaeformis* (27%). Several common taxa from the genera *Chaetoceros, Cyclotella*, and *Thalassiosira* are usually considered planktonic rather than benthic, but we did not exclude them from the analyses as most neritic diatoms are known to spend at least some part of their life cycle in bottom sediments. Currently, there is no sufficient evidence to ascertain that these species are not playing essential role in benthic communities.

The number of taxa per sample varied from 37 to 108, with a mean of 69, which indicates highly diverse assemblages considering relatively low number of counted valves (500). The high diversity of the assemblages is also indicated by relatively high values of Shannon Diversity Index that ranged from 2.3 to 4.2 with a mean of 3.5. Approximately half of all taxa found in this study were reported in a study conducted by Cooper (1995a, b) who studied diatoms from four sediment cores from the Chesapeake Bay. Most common species found in this study were also recorded by Hein and Koppen (1979) who studied benthic diatoms in the canal at the Oyster Creek Nuclear Station, although direct comparison is impossible because of taxonomic uncertainties and lack of species documentation by Hein and Koppen. In studies conducted by Sullivan (1971, 1975a, b) on mud-flat diatoms from a salt marsh in Delaware Bay and by Wilderman (1984) who studied diatoms from surface sediments in the River Severn Estuary, Chesapeake Bay, many common species (e.g., *Skeletonema costatum, Amphora coffeaformis, Planothidium delicatulum*) were the same as in the Barnegat Bay, but considerable differences also existed among these areas. Additional investigations of original collections made in 1970s and 1980 are necessary to be able to make direct comparisons with our findings.

C4: *Relationships between diatom assemblages and environmental parameters*

C.4.1. Correlations among environmental variables

Prior to evaluating relationships between diatoms and environmental characteristics, it is important to reveal correlation patterns in the environmental dataset. **Appendix V** contains a worksheet with correlations coefficients among variables, while **Figure 12** demonstrates the main patterns of co-variation among variables determined by the Principal Component Analysis (PCA). Salinity, which is known to be a major environmental gradient in estuaries strongly affecting biological communities, was negatively correlated with Chlorophyll A and the amount of the development in the watersheds. The variable most strongly positively correlated with salinity (besides conductivity) was Total Dissolved Phosphorus, which may be explained by the findings of Jordan et al. (2008). A number of variables indicative of relatively high organic content in sediments, such as Total Sediment Phosphorus, Sediment Organic Carbon and Total Sediment Nitrogen, or with abundance of phytoplankton, such as Particulate Phosphorus, Total Suspended Solids, Turbidity, Chlorophyll A, and Total Kjeldahl Nitrogen, were inter-correlated.

C4.2: Distribution patterns of diatom assemblages in space and along environmental gradients

In order to reveal major patterns of surface sediment diatom assemblage composition in Barnegat and Great Bays, a series of Detrended Correspondence Analyses (DCA) were performed. DCA uses only species data to elucidate major axes of variation in sample sets. We ran DCA using three different sets of sites: (1) 100 samples from all 100 sites, (2) 34 marsh sites, and (3) 66 open-water sites. Three versions of species datasets were used for all multivariate analyses: (1) included all species weighted equally, (2) used down-weighting of rare species, and (3) used only species that were found at 1% relative abundance in at least 5 samples. The DCAs with species datasets that included only species that reached 1% relative abundance in at least 5 samples had the largest amount of variation explained by the ordination axes, so these results are presented here. **Figure 13** shows a map with samples scores for DCA axes 1 and 2 for 100-sites analysis. DCA 1 ordinates sites accordingly to the main direction of variation in species composition. In the studied dataset the most differences were observed between up-bay and down-bay sites (**Figure 13**) and the pattern was very similar to the pattern of Salinity and Total

Dissolved Phosphorus (**Figures 9 and 10, upper left**). This gradient in species composition also corresponded to a gradient in land-use: from the most developed watershed in the north to the least developed in the south. The second DCA axis extracts a major pattern of variation after the variation along the first axis is taken into account. This second direction of variation did not have such a clear spatial pattern as DCA 1, but the DCA 2 loadings were in general higher in off-shore sites compared to marsh sites and sites positioned closer to the shore. These patterns are also illustrated by ordinations diagrams showing an overlay of environmental factors on DCA axes 1 and 2 (**Figure 14**). DCA axis 1, corresponding to major dimension of variation in species data is positively correlated with Salinity, Total Dissolved Phosphorus, Total Phosphorus, Ammonia and Total Inorganic Nitrogen, and negatively correlated to Chlorophyll A, Total Nitrogen, Dissolved Oxygen, Nitrate + Nitrite, and Particulate Phosphorus. DCA 2 was most strongly correlated with Depth.

In datasets of 34 "marsh" and 66 "open-water" sites, the major directions of variation in species data were similar to that in the 100-sites dataset: they also corresponded to gradients in Salinity, Total Dissolved Phosphorus and other variables with north-south general variation pattern, but relationships between diatoms and nutrients were more detached from relationships to salinity than in the 100-sites analysis (**Figures 15 and 16**). This is an expected result since separating the dataset into marsh and open-water sites somewhat decreased variation in salinity, which was generally lower in the marsh in comparison to the open-water sites. Salinity is usually an overriding environmental factor determining diatom assemblage composition in estuaries and bays (e.g. Cooper et al. 2010, Juggins 1992, Ulanova and Snoejis 2006, Wekstrom and Juggins 2005). This is the main reason why the effects of nutrients are often masked (Wachnicka 2009). The next section addresses the question of whether nutrients significantly affect diatoms in surface sediments of the studied area.

C4.3: Strength of diatom response to environmental factors

The strength of the relationships between composition of diatom assemblages and environmental variables was measured by testing significance of the first canonical axes in a series of Canonical Correspondence Analyses (CCA). In each CCA, multivariate response variable was diatom assemblage composition, and a single explanatory variable was an environmental variable of interest. Significance of the response was measured by a Monte-Carlo

permutation procedure, which was carried out simultaneously with CCA in CANOCO program. As in the case of DCA, all analyses were carried out with three different sets of sites and three sets of species. The summary of these analyses carried out with the species sets that included only species found at 1% relative abundance in at least 5 samples is given in Table 3. In all three datasets of sites, which included 100-sites dataset, marsh-sites dataset, and an open water-sites dataset, the strongest response of the diatom assemblages was to Salinity, followed by Total Dissolved Phosphorus. The highest number of environmental variables was significantly related to diatom assemblage composition in 100-sites dataset, and the lowest – in the 34 marsh-sites dataset. This shows that the response is easier to elucidate from larger datasets. In the 100-sites dataset, most variables except pH, Total Kjeldahl Nitrogen and Total Suspended Solids were significantly related to diatom assemblage composition at 0.002 p-value. In the smallest marsh-sites dataset, the response was significant at this level only for salinity, Total Dissolved Phosphorus, sediment Total Nitrogen and Sediment Total Organic Carbon.

It is notable that the strongest response in diatom assemblage composition in all three datasets was found to variables that were most strongly positively or negatively correlated to salinity. This underlines the difficulty in revealing the effect of nutrients in the presence of a strong response to salinity. In order to determine whether an independent response to nutrients existed, a series of partial CCAs were conducted where environmental variables that showed significant effect in previous series of CCAs were used as constraints and salinity was used as a covariable. The results of these analyses are shown in Table 4. In the 100-sites dataset the response to Total Dissolved Phosphorus, Particulate Phosphorus, and Total Phosphorus, sediment Total Nitrogen and sediment Total Organic Carbon was still significant at p-level of 0.002, but it became weaker. The response to sediment Total Phosphorus, Turbidity and Depth became slightly stronger because these factors were more or less orthogonal to salinity and their effect became clearer when the salinity effect was taken out. In marsh sites dataset all responses became weaker, while in the open-water dataset effects of nutrient became slightly weaker, but were still significant at p=0.002 level for Total Dissolved Phosphorus, Total Phosphorus, Total Inorganic Nitrogen, sediment Total Nitrogen, sediment Total Phosphorus, and Chlorophyll A.

Another way of determining which environmental variables independently contribute into explaining significant variation in species data is to carry out a CCA with forward selection of

variables. The result of this analysis carried out with a set of all 100 sites and species that reached 1% relative abundance in 5 samples is shown in **Figure 17**. All variables that significantly contributed to the explanatory power of the model at p=0.05 and did not have inflation factor higher than 10 were included and are shown in the diagram. Eleven variables that together most parsimoniously explained variation in diatom assemblage composition were: Salinity, Marsh/Open site, Depth, Total Dissolved Phosphorus, sediment Total Nitrogen, chlorophyll A, "Developed" land-use, Dissolved Oxygen, Particulate Phosphorus, Total Kjeldahl Nitrogen, and Nitrate + Nitrite. These variables are most likely to produce meaningful inference models.

C4.4: Diatom inference models

Inference models were constructed for all variables and diatom datasets pairs where CCAs recovered response significant at p= 0.002. These analyses used 2 species datasets: (1) all species and (2) only those species that reached 1% relative abundance in at least 5 samples and three sites datasets: all 100 sites, marsh sites, and open-water sites for a total of 6 pairs of datasets. 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 ultimate measures of model performance are the bootstrapped coefficient of determination (R^2_{boot}) and the root-square mean error of prediction (RMSEP). Models constructed for the all- and reduced-species datasets did not differ significantly in their performance, and therefore, only models based on all-species datasets are reported here (**Table 5**).

The R^2_{boot} higher than 0.50 was observed only for models constructed for Salinity and Total Dissolved Phosphorus in all three datasets and also for Chlorophyll A in the all-sites and the open-water datasets (**Table 5, Figure 18).** One Depth and one Sediment Total Nitrogen model had R^2_{boot} around 0.5. Conductivity models had high R^2_{boot}, but they are almost identical to salinity models as conductivity is essentially a measure of salinity and therefore they are not shown here. The Total Dissolved Phosphorus model thus appears as the best one among all nutrient models in terms of its predictive power. The problem, however, is that considerable part

of the variation in species data was explained by the interactions of Total Dissolved Phosphorus and Chlorophyll A with Salinity. For example, in the 100 sites dataset, Salinity alone explained 4.7% variance in species data; Total Dissolved Phosphorus explained 3.1 %, while their interaction explained 4.8%. In the open-water dataset Salinity alone explained 6.9%, Chlorophyll A – 3.1%, and their interaction – 4.2% of variance in diatom species data. This makes it somewhat difficult to ensure an independent response of diatoms to factors other than salinity. The increase in total dissolved phosphorus in the Barnegat Bay seems to be associated with increased salinity and is opposite in its direction to the gradient of human impact captured by the land-use analysis. As it was discussed earlier, the increase of total dissolved phosphorus may be a natural consequence of the increased salinity that leads to phosphate release from the sediments to water column.

The gradient of chlorophyll A is, however, in line with the human impact gradient and therefore, the response of the benthic diatom assemblage to this variable can be useful for reconstructing eutrophication history in the area. Successful use of diatom inference models to reconstruct levels of chlorophyll A has been demonstrated earlier for lakes (Jones and Juggins 1995). Chlorophyll concentrations depend on the development of phytoplankton, and therefore may be a better, symptom-based measure of eutrophication, while dissolved nutrients in the water column may be more volatile indicators as they may be consumed by an algal bloom (Bricker et al. 2003). Diatom species that had relatively high chlorophyll A optima in surface sediment diatom assemblages were not necessarily planktonic, although relative abundances of such planktonic diatoms as *Chaetoceros* spp., *Thalassiosira cedarkeyensis, Cylindrotheca closterium, Cyclotella meneghiniana,* and *C. choctawhatcheeana* were higher at the higher end of the chlorophyll A gradient. Most species with high chlorophyll A optima were benthic diatoms, thus showing that change in the assemblage corresponding to the increase in chlorophyll A was not caused only by the increased amount of planktonic diatoms sinking to the bottom, but also by the intrinsic change in benthic species proportion due to effects of eutrophication. The eutrophication effects that cause shifts in benthic diatom assemblage composition can be diminished DO levels in interstitial sediment water due to bacterial degradation of bloom algae settling to the bottom, or other factors associated with algal blooms. An increase in relative abundance of *Chaetoceros* and *Cyclotella choctawhatcheeana* in sediments due to eutrophication has been demonstrated earlier for the Chesapeake Bay by

Cooper (Cooper 1995a, b), and Olsen and Mahoney (2001) listed *Cylindrotheca closterium* among bloom-producing planktonic diatoms in the Barnegat Bay. On the other hand, such species as *Skeletonema costatum* listed by Mountford (1971) and Olsen and Mahoney (2001) as bloom-producers did not increase in abundance in surface sediment from sites associated with higher chlorophyll A in our study.

Inference models for other variables were not as strong as for the salinity, TDP, and chlorophyll A, but some of them, especially for the depth and sediment nutrients, may be useful and have potential for further development. Usefulness of diatoms for reconstructing depth and, therefore, sea-level change has been amply demonstrated (Kemp et al. 2009, Patterson et al. 2005). Relatively low predictive power of depth models obtained in this project in comparison to the abovementioned studies can be explained by much higher diversity of sampled habitats and low number of samples in each particular habitat. For example, our "marsh" dataset of 34 sites included samples from high marsh, mud and sand intertidal flats, tidal creeks, salt pans, etc., with every habitat represented by a few samples only. Datasets of such a diverse nature may be useful for developing inference models, but they have to be based on much large number of samples.

A complex environmental and spatial north-south gradient in the studied area obviously makes it difficult to disentangle responses of diatom assemblages to individual water-quality parameters. One way of dealing with such a problem is to attempt to minimize variation in environmental factors other than the factor of interest. This can be done, for example, by sub-setting data. We attempted to shorten the salinity gradient by cutting out from the analyses species that had extreme salinity optima (20% highest and 20% lowest) and also by dividing our dataset into two of equal size: low- and high-salinity subsets. In some cases, such as for Sediment Total Nitrogen and Sediment Total Organic Carbon in the low-salinity dataset performance of diatom-nutrient models increased, but decreased size of the datasets contributed to the overall diminished predictive power (**Table 6**). The predictive power of the models became especially low in the high-salinity dataset.

Diatom-based salinity and depth inference models for estuaries and coastal environments have been constructed and successfully used in various parts of the world (e.g., Gehrels et al. 2001, Juggins 1992, Kemp et al. 2009, Patterson et al. 2005, Sawai et al. 2004, Zong and Horton

1998, 1999). In both cases, these functions are based on a strong response of diatoms to salinity gradient that exists in estuaries and in coastal areas. Responses to nutrients are masked by these strong salinity gradients and are often difficult to detect. Diatom-nutrient transfer functions were developed for the Gulf of Finland, Baltic Sea (Wekstrom et al. 2004), but their application to sediment cores showed some disagreements with actual nutrient levels recorded for the same area (Wekstrom 2006). Several diatom-based nutrient inference models were created by Wachnicka (2009) for the Florida and Biscayne Bays, Florida, but only apparent coefficients of determination were reported. They were 0.75 for Total Water Nitrogen and Total Water Phosphorus, which is similar to apparent coefficients of determination for some nutrients in our analyses, but may be misleading as the models have to be validated by the bootstrapping or other validation procedure for the realistic assessment of their predictive abilities.

Although diatoms inhabiting surface sediments in estuaries are obviously extremely responsive to many water-quality and physical habitat parameters (Admiraal 1977a, b, 1984, Underwood 2000, Underwood et al. 1998), several factors hamper development of very precise diatom-based methods for monitoring of environmental conditions in estuaries and shallow coastal areas. Perhaps the most important is the uncertainty of which diatoms are autochthonous for a location, and which are allochthonous, brought there by currents (Vos and DeWolf 1993). The dynamic nature of tidal ecosystems certainly introduces considerable noise into numerical models describing response of biotic assemblages to environment. Still, a strong response to some environmental variables shows that diatoms are useful indicators in these ecosystems. In comparison to efforts spent to develop diatom-based indicators in fresh waters, the studies of coastal diatoms are quite limited (Trobajo and Sullivan 2010). As this investigation shows, about one third of the species found in the area do not fit any published descriptions. Publication of formal descriptions of these species together with autecological information for each species contained in the constructed calibration dataset will be an important step forward to future use of sediment diatoms for ecosystem health assessment in the Barnegat Bay.

D) Summary and Conclusions

The main objective of this project was to determine whether surface sediment diatom assemblages may be used as indicators of ecosystem health in Barnegat Bay. We collected surface sediment and water samples from 100 marsh, and open-water sites across Barnegat Bay-Little Egg Harbor and Great Bay, and analyzed nutrients in sediments and water column, land-use in the watersheds, and composition of diatom assemblages in sediments. We found highly significant responses of benthic diatom assemblage composition to a variety of environmental factors, including concentrations of nutrients in sediments and water column, chlorophyll A in water column, and land-use. Our data show that surface sediment diatoms have a great potential for monitoring ecosystem health in the Barnegat Bay and can be used to reconstruct past environmental condition in sediment cores. The calibration set of diatom and environmental data constructed in this project should serve as the basis of these applications.

Major findings of this study include:

- Total sediment nitrogen ranged from 0.00 to 1.67% N whereas total sediment phosphorus ranged from 0.01 to 0.21% of the dry weight. Sediment C, N, and P concentrations were generally highest in the marsh sites in comparison to open-water sites and in river estuaries.
- Salinity varied from 8.7 to 32.1 psu and was relatively low in the Northern part of the Barnegat Bay and in river estuaries. The highest salinities were observed in the Little Egg Harbor.
- Nitrate plus nitrite concentrations in water column ranged from 0.3 to 144.6 μg/L N with an average of 7.2 μg/L and were highest in the northern part of the Barnegat Bay, while ammonia ranged from 2.9 to 87.7 μg/L N and was generally lower in the northern part of the Barnegat Bay and higher in the Little Egg Harbor and in the Great Bay. Relatively high Total Kjeldahl Nitrogen values (362.6-2866 μg/L N) in comparison to inorganic dissolved nitrogen show that most nitrogen in water column was present in the organic form.
- Concentrations of Total Dissolved Phosphorus ranged from 3.8 to 40.6 μg/L P and were considerably lower in the northern part of the Barnegat Bay in comparison to the Little

Egg Harbor and the Great Bay. Total Dissolved Phosphorus was a variable most strongly positively correlated with salinity in the study area. Particulate Phosphorus ranged from 8.9 to 67.9 μg/L P and was the highest in the northern part of the Barnegat Bay and in the Great Bay, and lowest in the Little Egg Harbor.

- Total Suspended Solids and chlorophyll A had the highest values in the northern part of Barnegat Bay and in Great Bay and the lowest in Little Egg Harbor, thus resembling the pattern of the Particulate Phosphorus. Chlorophyll A concentrations varied from 2.4 to 38.2 μg/L, the values corresponding to the low-to high levels for US estuaries.
- Land-use analysis of the watersheds contributing to the Barnegat Bay-Little Egg Harbor and the Great Bays revealed a gradient from predominantly "developed" or urban watersheds in the northern part of the study area to predominantly "undeveloped" ones (dominated by forests, grasslands and wetlands) in the southern part, especially in the Great Bay.
- A rich diatom flora consisting of 402 species was found in 110 analyzed diatom samples. This species list includes some species reported earlier for the mid-Atlantic coast, and many new species, including those new for science. Diatom assemblages were highly diverse: the Shannon Diversity Index ranged from 2.3 to 4.2 with a mean of 3.5. The major gradient in the composition of diatom assemblages corresponded to the north-south gradient of land-use, salinity and other associated environmental variables. The second most important gradient in diatom species data was between the marsh sites and open-water sites and correlated with water depth.
- Composition of diatom assemblages was significantly affected by a number of measured environmental parameters. The strongest response was to salinity, which is expected in an estuarine environment. Other environmental parameters that had statistically significant effect on the composition of diatom assemblages in 100 sites dataset were Depth, Dissolved Oxygen, Turbidity, Chlorophyll A, Total Dissolved Phosphorus, Particulate Phosphorus, Total Phosphorus, Nitrate+Nitrite, Ammonia, Total Inorganic Nitrogen, Sediment Organic Carbon, Sediment Total Nitrogen, Sediment Total Phosphorus, percent of land-use classified as "Developed", "Wetland", "Undeveloped" (sum of Forest, Grassland, Wetland, etc.), and "Developed + Agriculture". Responses to fewer environmental parameters were significant for the subsets of 34 "marsh" and 66 "open-

water" sites because of the low number of observations in each subset. These multivariate analyses may be used in the future to infer past environmental conditions by including core diatom samples as passive samples in existing ordinations.

- Inference models were constructed for those environmental parameters that were found to exert significant influence of the diatom assemblage composition. The models with the highest predictive power were those for Salinity, Total Dissolved Phosphorus, and Chlorophyll A. The inference models for Salinity may be useful for historical reconstruction of sea-level rise and other events related to climate change. Total Dissolved Phosphorus models, although highly statistically significant, may reflect a natural gradient of this compound related to salinity. Since an increased chlorophyll A level is a symptom of eutrophication and the chlorophyll gradient positively correlates with watersheds development, Chlorophyll A inference models seem to be most promising for reconstruction history of eutrophication in Barnegat Bay and adjacent areas. Diatom species optima and tolerances calculated for all models will be useful for environmental assessments in lagoonal estuaries in New Jersey.

Recommendations for Future Steps

- We produced a high-resolution map of land-use in 2010, but the accuracy of the map has not been assessed. We will digitize additional points of land-use in the watersheds to evaluate the accuracy of our classification. We will use this new data for examining relations among land-use and diatom assemblages once the map has been assessed. Our second task will be to link wetland and bay cores to bay water. We will do this by analyzing land-use and water quality loadings at inlets to the sites. The recently available models of flow directions and high and low tides will be used to look "upflow" at various distance intervals.

- We will continue taxonomy work to publish descriptions of new species discovered in this project. The goal is to produce a diatom flora of the Barnegat Bay accompanied by autecological data for most species. This work will be useful for those wishing to use diatoms as environmental indicators along mid-Atlantic coasts.

- More precise inference models may be constructed for less heterogeneous sets of

habitats, such as high marshes, intertidal mudflats and sandflats, and subtidal sites. Studying these habitats separately, in detail, and in conjunction with other studies carried out on these systems (e.g., wetlands nutrient dynamics, or macroinvertebrate assemblages studies in open-water sites across the Bay coupled with phytoplankton and water-quality monitoring) will considerably enhance our ability to use diatoms as ecosystem health indicators in Barnegat Bay and adjacent areas. It is important to note that the current study only used a one-time water chemistry measurements, while it would be beneficial to assess the accumulated effect of water chemistry over time on biotic communities.

- Including other environmental parameters known to influence diatom assemblage composition, such as benthic chlorophyll A, sediment grain size, etc. in the analyses would be helpful to get more accurate estimates of ecological niches of sediment diatoms and therefore, would help in developing better diatom-based indicators.

E) Acknowledgments

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F) References

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G) Tables

Table 1. Summary of sediment chemistry, 110 samples from 100 sites in Barnegat and Great Bays.

Parameter	Minimum	Mean	Maximum	Standard Deviation
% C	0.11	4.77	31.6	5.63
% N	0	0.38	1.67	0.34
% P	0.01	0.07	0.21	0.03
C/N	0.7	12.3	26.4	4.8

Table 2. Summary of water quality parameters measured at 100 sites in Barnegat and Great Bays.

Parameter	Minimum	Mean	Maximum	Standard Deviation
Water temperature, C	19.2	23.1	28.2	1.6
Dissolved Oxygen, mg/L	4.57	7.47	10.26	1.07
Salinity, psu	8.74	24.2	32.11	5.71
Conductivity, µS/cm	21492	38017	49072	7937
pH	7.04	7.76	8.08	0.23
Chlorophyll A, µg/L	2.4	11.0	38.2	8.4
Total Suspended Solids, mg/L	3.5	13.4	44.9	8.3
Turbidity, NTU	1.2	5.2	26.1	3.5
Particulate Phosphorus, µg P/L	8.9	25.7	67.9	10.9
Total Dissolved Phosphorus, µg P/L	3.8	16.3	40.6	10.8
Total Phosphorus, µg P/L	18.8	42.0	95.0	15.3
Nitrate + Nitrite, µg N/L	0.3	7.2	144.6	20.3
Ammonia, µg N/L	2.9	18.2	87.7	14.9
Total Inorganic Nitrogen, µg N/L	3.3	25.4	189.9	27.9
Total Kjeldahl Nitrogen, µg N/L	362.6	686.4	2866.0	285.4
Total Nitrogen, µm N/L	362.9	693.5	2894.2	287.9
N/P ratio, mol TN-N/mol TP-P	17.4	39.0	124.0	14.3

Table 3. Strength of the relationships between diatom assemblage composition and environmental variables as measured by the significance of the first CCA axes. Bold: significant at p=0.002. CCAs with species dataset that included only species that reached 1% relative abundance in at least 5 samples.

Environmental variable	All 100 sites		34 marsh sites		66 open-water sites	
	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Marsh/Open site	**5.0**	**0.002**				
Depth, m	**4.4**	**0.002**	**2.1**	**0.002**	1.6	0.088
Dissolved Oxygen, mg/L	**3.4**	**0.002**	1.4	0.086	**5.1**	**0.002**
pH	2.3	0.004	1.9	0.006	0.9	0.520
Salinity, psu	**13.8**	**0.002**	**5.0**	**0.002**	**11.6**	**0.002**
Turbidity	**2.6**	**0.002**	1.3	0.160	1.6	0.076
Total Suspended Solids, mg/L	1.8	0.026	1.1	0.314	1.3	0.140
Chlorophyll A, Log μg/L	**7.3**	**0.002**	2.2	0.004	**7.0**	**0.002**
Particulate Phosphorus, Log μg P/L	**4.5**	**0.002**	1.2	0.142	**5.1**	**0.002**
Total Dissolved Phosphorus, Log μg P/L	**11.6**	**0.002**	**5.1**	**0.002**	**8.7**	**0.002**
Total Phosphorus, Log μg P/L	**4.0**	**0.002**	1.9	0.006	**3.3**	**0.002**
Ammonia, Log μg N/L	**5.1**	**0.002**	1.1	0.284	**6.4**	**0.002**
Nitrate + Nitrite, Log μg N/L	**3.4**	**0.002**	2.0	0.010	2.5	0.004
Total Kjeldahl Nitrogen, μg N/L	2.0	0.010	1.0	0.362	1.6	0.068
Total Inorganic Nitrogen, μg N/L	**2.5**	**0.002**	0.9	0.614	**3.3**	**0.002**
Total Nitrogen, μm N/L	2.1	0.010	1.1	0.336	1.6	0.056
Carbon sediment, Log μg/g	**5.9**	**0.002**	**2.3**	**0.002**	**4.0**	**0.002**
Nitrogen sediment, Log μg/g	**6.0**	**0.002**	**2.4**	**0.002**	**3.9**	**0.002**
Phosphorus sediment, Log μg/g	**2.9**	**0.002**	0.8	0.752	**2.7**	**0.002**
“Developed” land-use, sqrt %	**7.5**	**0.002**	**2.8**	**0.002**	**6.3**	**0.002**
“Forest” land-use, sqrt %	1.8	0.022	1.1	0.258	1.7	0.046
“Grassland” land-use, sqrt %	1.2	0.198	0.8	0.862	1.2	0.220
“Wetland” land-use, sqrt %	**4.0**	**0.002**	1.7	0.020	**3.0**	**0.002**
“Agricultural” land-use, sqrt %	1.3	0.212	0.9	0.516	1.0	0.418
“Undeveloped” land-use, sqrt %	**6.0**	**0.002**	**2.4**	**0.002**	**4.8**	**0.002**
“Developed+agricultural” land-use, sqrt %	**7.4**	**0.002**	**2.8**	**0.002**	**6.2**	**0.002**

Table 4. Strength of the relationships between diatom assemblage composition and environmental variables with effect of salinity partialled out, as measured by the significance of the first CCA axes. Bold: significant at p=0.002. CCAs with species dataset that included only species that reached 1% relative abundance in at least 5 samples.

Environmental variable	All 100 sites		34 marsh sites		66 open-water sites	
	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Marsh/Open site	**5.6**	**0.002**				
Depth	**4.9**	**0.002**	1.2	0.172		
Dissolved Oxygen, mg/L	1.4	0.060			1.8	0.026
Turbidity	3.0	0.002				
Chlorophyll A, Log μg/L	**2.9**	**0.002**			**2.6**	**0.002**
Particulate Phosphorus, Log μg P/L	**2.4**	**0.002**			1.7	0.032
Total Dissolved Phosphorus, Log μg P/L	**4.1**	**0.002**	**1.9**	**0.002**	**3.5**	**0.002**
Total Phosphorus, Log μg P/L	**3.7**	**0.002**			**3.4**	**0.002**
Ammonia, Log μg N/L	1.9	0.012			2.4	0.004
Nitrate + Nitrite, Log μg N/L	2.3	0.010				
Total Inorganic Nitrogen, μg N/L	2.2	0.004			**2.4**	**0.002**
Carbon sediment, Log μg/g	**3.9**	**0.002**	1.0	0.460	1.8	0.026
Nitrogen sediment, Log μg/g	**4.5**	**0.002**	1.2	0.188	2.7	0.004
Phosphorus sediment, Log μg/g	**3.1**	**0.002**			**3.2**	**0.002**
“Developed” land-use, sqrt %	**3.2**	**0.002**	**1.9**	**0.002**	2.4	0.004
“Wetland” land-use, sqrt %	1.7	0.016			1.3	0.152
“Undeveloped” land-use, sqrt %	1.5	0.060	0.9	0.492	1.1	0.326
“Developed+agricultural” land-use, sqrt %	**2.8**	**0.002**	**1.8**	**0.002**	1.9	0.018

Table 5. Performance of diatom inference models as estimated by R^2_{boot} value. Values equal or greater than 0.5 are in bold.

Dataset/Variable	WA		WA-PLS	ML	MAT
	Inverse	Classic			
100 sites					
Depth	0.36	0.37	0.46	0.40	**0.52**
Salinity, psu	**0.83**	**0.83**	**0.85**	**0.82**	**0.82**
Dissolved Oxygen, mg/L	0.14	0.15	0.14	0.15	0.16
Turbidity	0.13	0.13	0.19	0.18	0.09
Total Dissolved Phosphorus, Log µg P/L	**0.67**	**0.67**	**0.68**	**0.74**	**0.74**
Chlorophyll A, Log µg/L	**0.52**	**0.53**	**0.65**	**0.54**	**0.53**
Particulate Phosphorus, Log µg P/L	0.26	0.27	0.33	0.31	0.28
Total Phosphorus, Log µg P/L	0.25	0.26	0.26	0.26	0.28
Nitrate + Nitrite, Log µg N/L	0.27	0.28	0.27	0.17	0.23
Ammonia, Log µg N/L	0.29	0.29	0.34	0.32	0.31
Total Inorganic Nitrogen, µg N/L	0.22	0.22	0.27	0.16	0.21
Total Nitrogen, µm N/L	0.24	0.25	0.23	0.17	0.24
Carbon sediment, Log µg/g	0.39	0.39	0.44	0.34	0.37
Nitrogen sediment, Log µg/g	0.41	0.41	**0.50**	0.34	0.43
Phosphorus sediment, Log µg/g	0.21	0.22	0.26	0.24	0.22
"Developed" land-use, sqrt %	0.41	0.42	0.44	0.51	0.46
"Wetland" land-use, sqrt %	0.23	0.23	0.32	0.24	0.36
"Undeveloped" land-use, sqrt %	0.32	0.33	0.31	0.31	0.37
"Developed+agricultural" land-use, sqrt %	0.38	0.39	0.39	0.45	0.45
34 marsh sites					
Salinity, psu	**0.77**	**0.77**	**0.79**	**0.74**	**0.73**
Total Dissolved Phosphorus, Log µg P/L	**0.75**	**0.76**	**0.79**	**0.81**	**0.76**
Carbon sediment, Log µg/g	0.16	0.17	0.16	0.19	0.13
Nitrogen sediment, Log µg/g	0.18	0.19	0.16	0.19	0.13
"Developed" land-use, sqrt %	0.36	0.38	0.46	0.45	0.39
"Undeveloped" land-use, sqrt %	0.25	0.26	0.25	0.25	0.31
"Developed+agricultural" land-use, sqrt %	0.37	0.38	0.39	0.39	0.36
66 open-water sites					
Salinity, psu	**0.84**	**0.84**	**0.84**	**0.82**	**0.81**
Dissolved Oxygen, mg/L	0.29	0.30	0.28	0.34	0.25
Total Dissolved Phosphorus, Log µg P/L	**0.61**	**0.62**	**0.59**	**0.65**	**0.65**
Chlorophyll A, Log µg/L	**0.60**	**0.61**	**0.69**	**0.68**	**0.59**
Particulate Phosphorus, Log µg P/L	0.41	0.42	0.41	0.48	0.43
Total Phosphorus, Log µg P/L	0.27	0.28	0.29	0.28	0.26
Nitrate + Nitrite, Log µg N/L	0.18	0.19	0.17	0.19	0.17
Ammonia, Log µg N/L	0.43	0.44	0.42	0.47	0.45
Total Inorganic Nitrogen, µg N/L	0.19	0.19	0.23	0.24	0.23

Total Nitrogen, μm N/L	0.19	0.19	0.17	0.15	0.18
Carbon sediment, Log μg/g	0.29	0.31	0.29	0.24	0.20
Nitrogen sediment, Log μg/g	0.34	0.35	0.47	0.25	0.29
Phosphorus sediment, Log μg/g	0.30	0.31	0.37	0.35	0.25
"Developed" land-use, sqrt %	0.41	0.42	0.41	**0.51**	0.39
"Wetland" land-use, sqrt %	0.21	0.21	0.26	0.24	0.21
"Undeveloped" land-use, sqrt %	0.31	0.32	0.29	0.36	0.28
"Developed+agricultural" land-use, sqrt %	0.37	0.38	0.38	0.47	0.37

WA -Weighed Averaging model, WA-PLS - Weighed Averaging- Partial Least Squares model, ML- Maximum Likelihood model, MAT- Modern Analog Technique model.

Table 6. Performance of diatom inference models based on data sub-setting by salinity, as estimated by R^2_{boot} value. Values equal or greater than 0.5 are in bold.

Dataset/Variable	WA		WA-PLS	ML	MAT
	Inverse	Classic			
100 sites, species with extreme salinity optima deleted					
Chlorophyll A, Log µg/L	**0.51**	**0.51**	**0.55**	**0.55**	0.41
Particulate Phosphorus, Log µg P/L	0.31	0.32	0.37	0.33	0.24
Total Dissolved Phosphorus, Log µg P/L	**0.61**	**0.61**	**0.65**	**0.75**	**0.67**
Ammonia, Log µg N/L	0.28	0.29	0.33	0.28	0.26
Nitrate + Nitrite, Log µg N/L	0.10	0.10	0.10	0.17	0.19
Carbon sediment, Log µg/g	0.38	0.39	0.42	0.37	0.36
Nitrogen sediment, Log µg/g	0.44	0.45	**0.51**	0.47	0.42
Phosphorus sediment, Log µg/g	0.22	0.23	0.21	0.30	0.24
"Developed" land-use, sqrt %	0.39	0.39	0.39	0.47	0.39
50 sites with salinity lower than 28 psu					
Chlorophyll A, Log µg/L	0.17	0.19	0.19	0.20	0.24
Particulate Phosphorus, Log µg P/L	0.22	0.23	0.23	0.27	0.28
Total Dissolved Phosphorus, Log µg P/L	**0.65**	**0.66**	**0.70**	**0.75**	**0.71**
Ammonia, Log µg N/L	0.22	0.23	0.22	0.22	0.19
Nitrate + Nitrite, Log µg N/L	0.37	0.38	0.43	0.24	0.30
Carbon sediment, Log µg/g	**0.51**	**0.52**	**0.54**	0.45	**0.50**
Nitrogen sediment, Log µg/g	**0.56**	**0.57**	**0.59**	**0.55**	**0.53**
Phosphorus sediment, Log µg/g	0.19	0.20	0.19	0.18	0.23
"Developed" land-use, sqrt %	0.58	0.59	0.57	0.55	0.56
50 sites with salinity greater than 28 psu					
Chlorophyll A, Log µg/L	0.31	0.32	0.44	0.32	0.18
Particulate Phosphorus, Log µg P/L	0.04	0.05	0.13	0.11	0.01
Total Dissolved Phosphorus, Log µg P/L	0.30	0.31	0.35	0.36	0.39
Ammonia, Log µg N/L	0.03	0.03	0.01	0.00	0.00
Nitrate + Nitrite, Log µg N/L	0.02	0.03	0.01	0.05	0.04
Carbon sediment, Log µg/g	0.10	0.11	0.09	0.05	0.03
Nitrogen sediment, Log µg/g	0.13	0.15	0.20	0.08	0.11
Phosphorus sediment, Log µg/g	0.16	0.17	0.17	0.15	0.16
"Developed" land-use, sqrt %	0.10	0.11	0.10	0.11	0.11

WA -Weighed Averaging model, WA-PLS - Weighed Averaging- Partial Least Squares model, ML- Maximum Likelihood model, MAT- Modern Analog Technique model.

H) Figures

Figure 1. Location of marsh sampling sites, corresponding watersheds, and elevation data used for the watershed analysis.

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Figure 3. Extruding the upper layer of a core..

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Figure 13. Maps showing major variation is diatom assemblage composition across study area as revealed by the Detrended Correspondence Analysis. A – sites scores along DCA1, B- sites scores along DCA2.

Figure 14. Correspondence between major gradients in diatom species composition revealed by the Detrended Correspondence Analysis (DCA axes 1 and 2) and measured environmental parameters in the dataset of 100 sites from the Barnegat and Great Bays Biplot of species and passive environmental variables. Only centroids for species with highest weights (20-100%) are shown. Abbreviations for environmental variables are as in Figure 12. Green circle: centroid of marsh sites, blue circle: centroid of open-water sites.

Figure 15. Correspondence between major gradients in diatom species composition revealed by the Detrended Correspondence Analysis (DCA axes 1 and 2) and measured environmental parameters in the dataset of 34 marsh sites from Barnegat and Great Bays. Biplot of species and passive environmental variables. Only centroids for species with highest weights (20-100%) are shown. Abbreviations for environmental variables are as in Figure 12.

Figure 16. Correspondence between major gradients in diatom species composition revealed by the Detrended Correspondence Analysis (DCA axes 1 and 2) and measured environmental parameters in the dataset of 66 open-water sites from Barnegat and Great Bays. Biplot of species and passive environmental variables. Only centroids for species with highest weights (20-100%) are shown. Abbreviations for environmental variables are as in Figure 12.

Figure 17. Biplot of species and environmental variables showing the result of a forward variable selection in CCA, dataset of 100 sites from the Barnegat and Great Bays. Only centroids for species with highest weights (20-100%) are shown. Abbreviations for environmental variables are as in Figure 12. Variables that were significant at p=0.05 and independently added to the explanatory power of the model were selected ad are shown in the plot.

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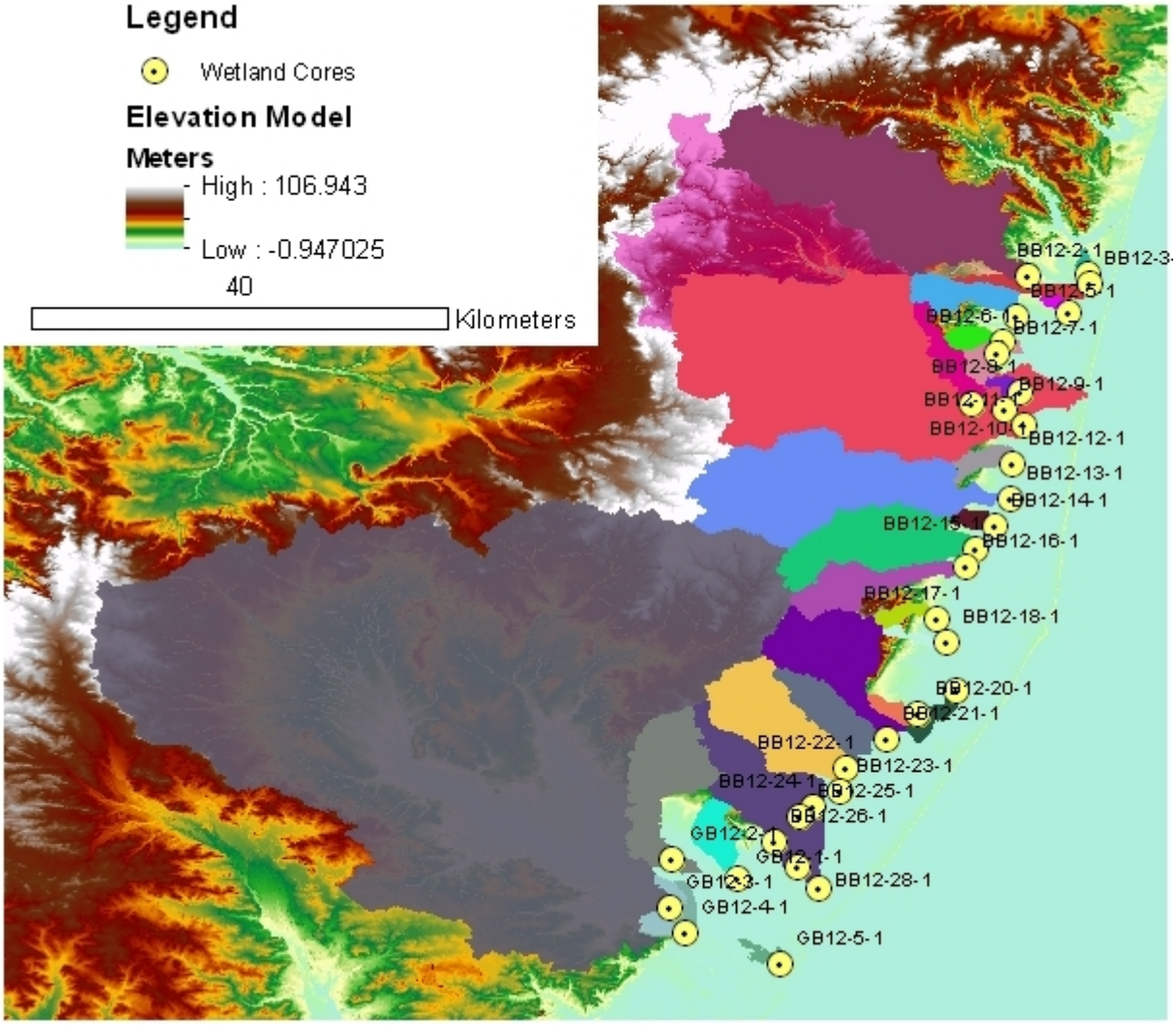

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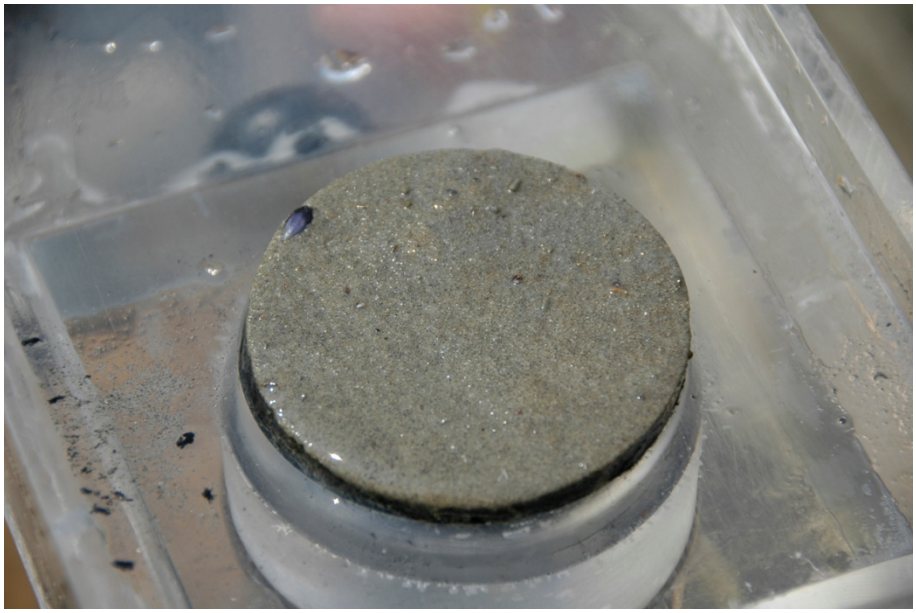

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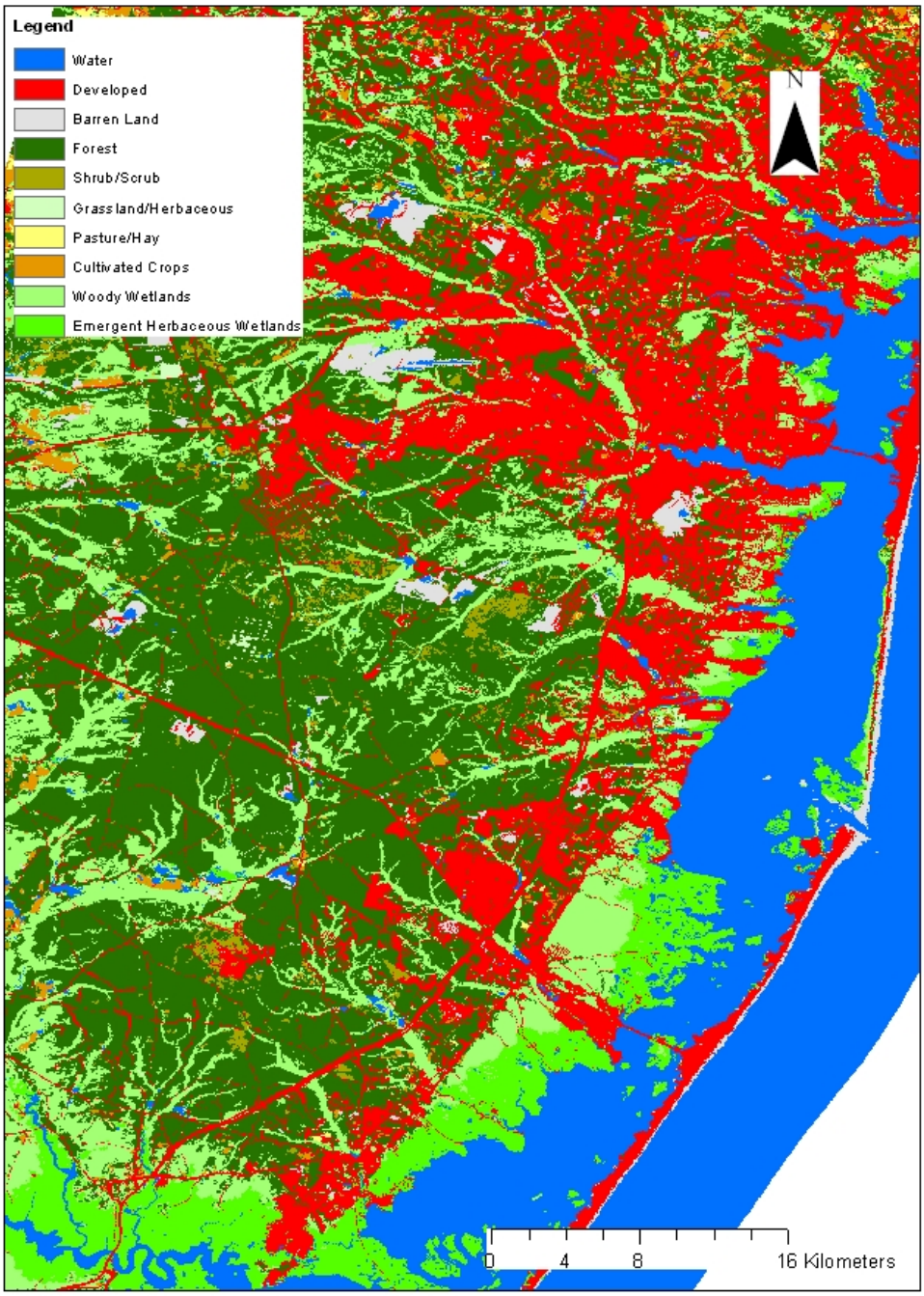

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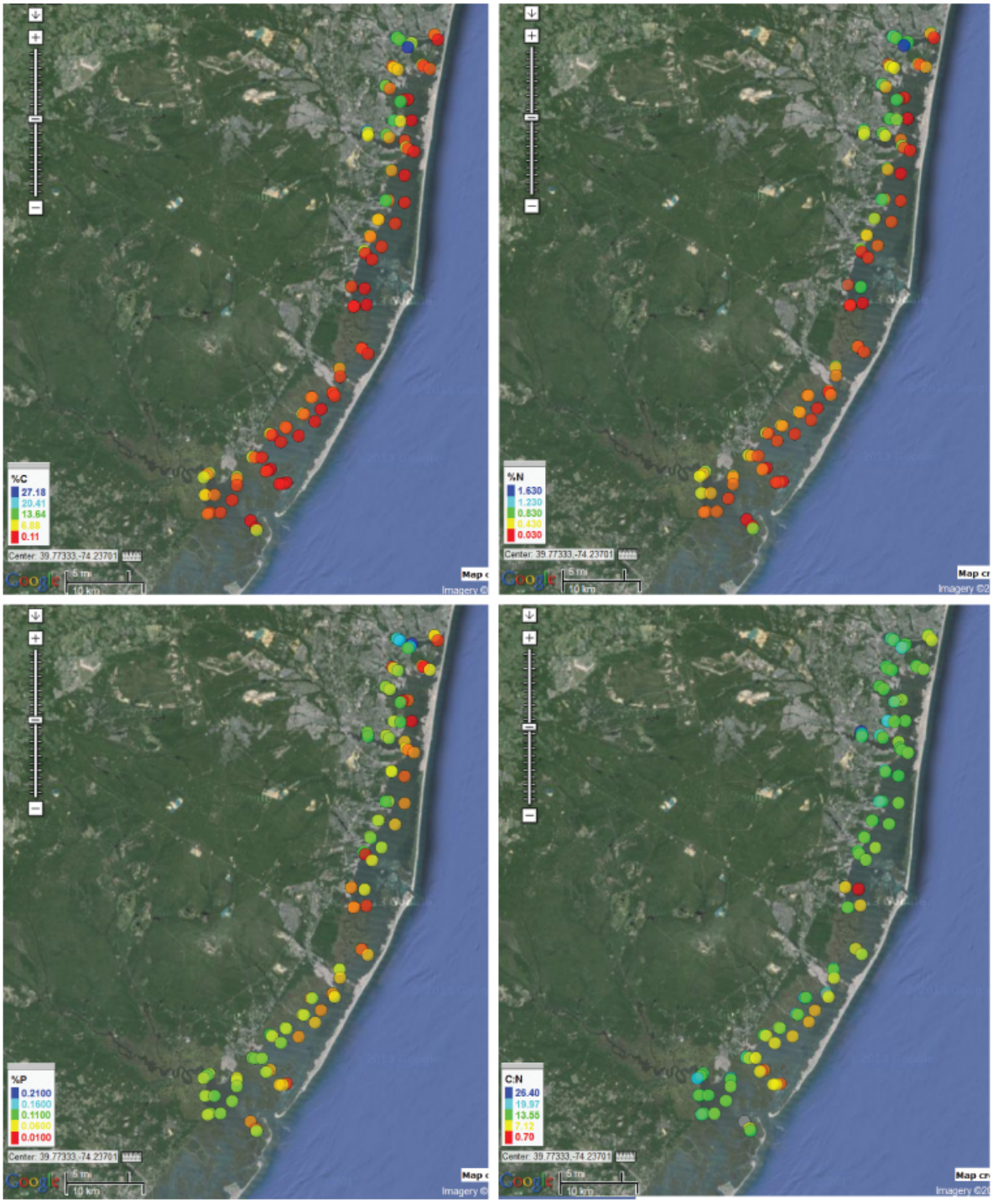

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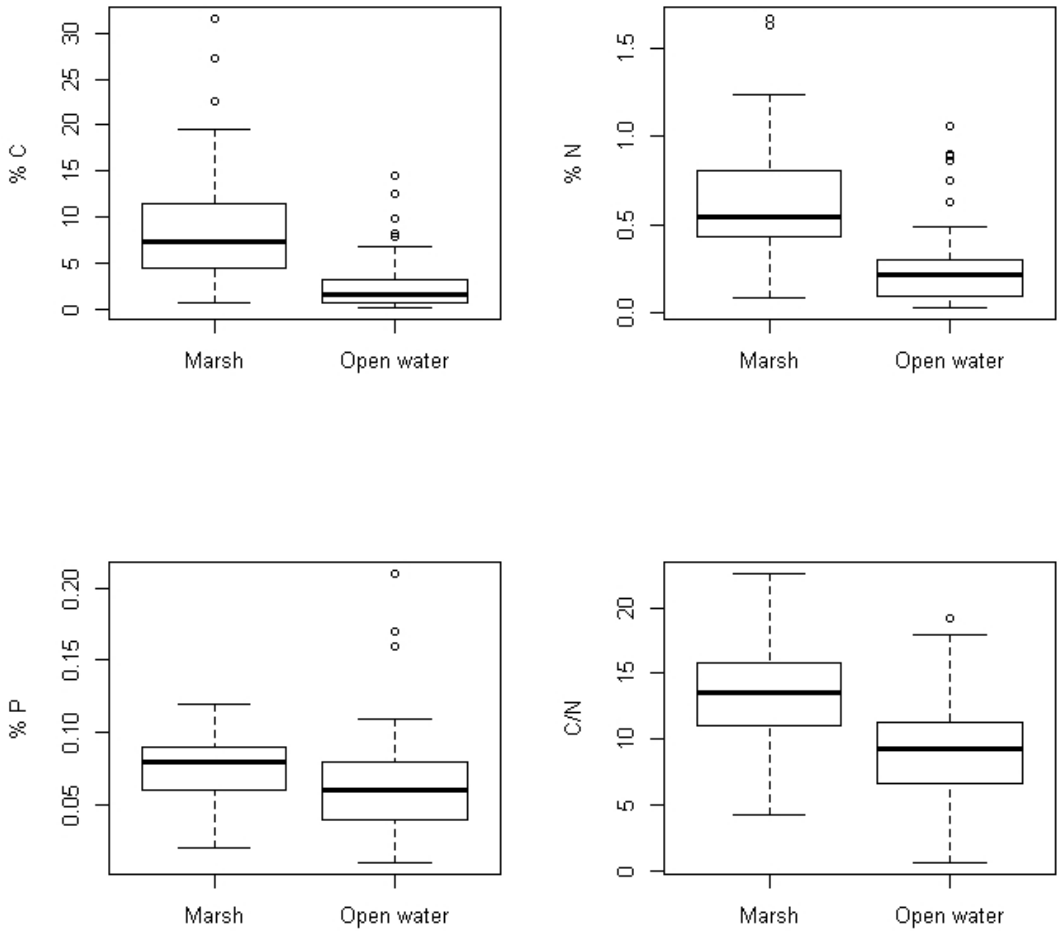

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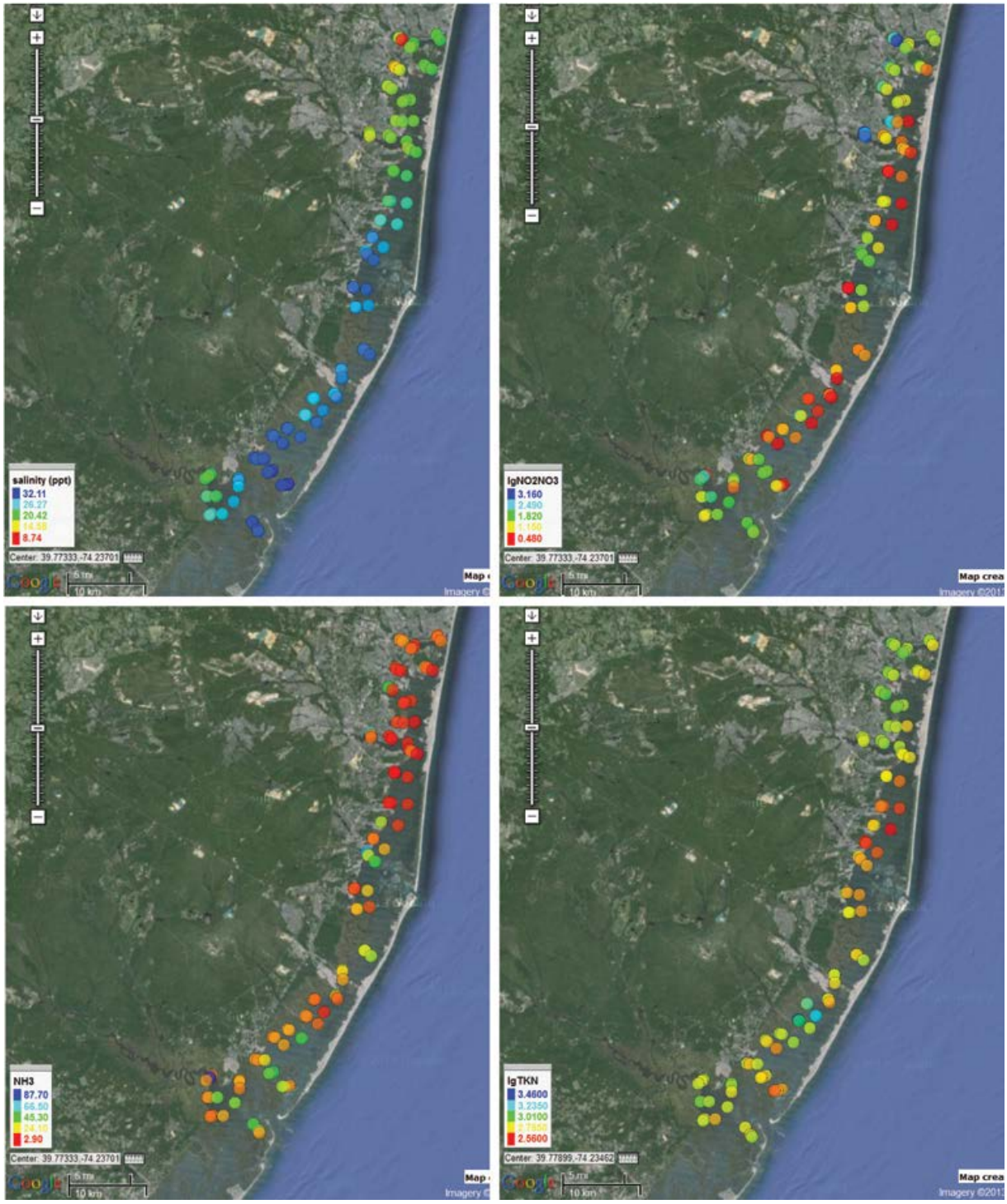

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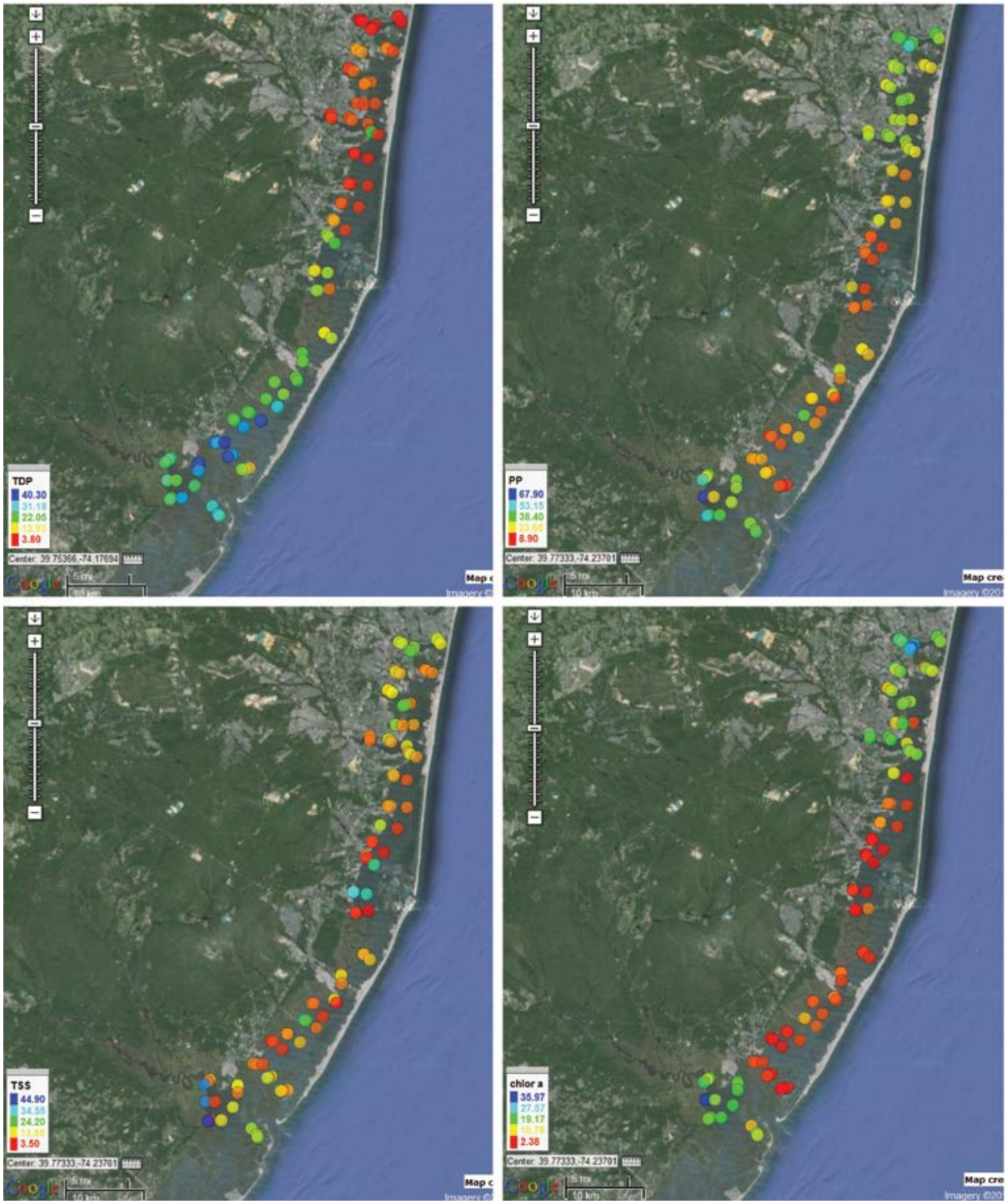

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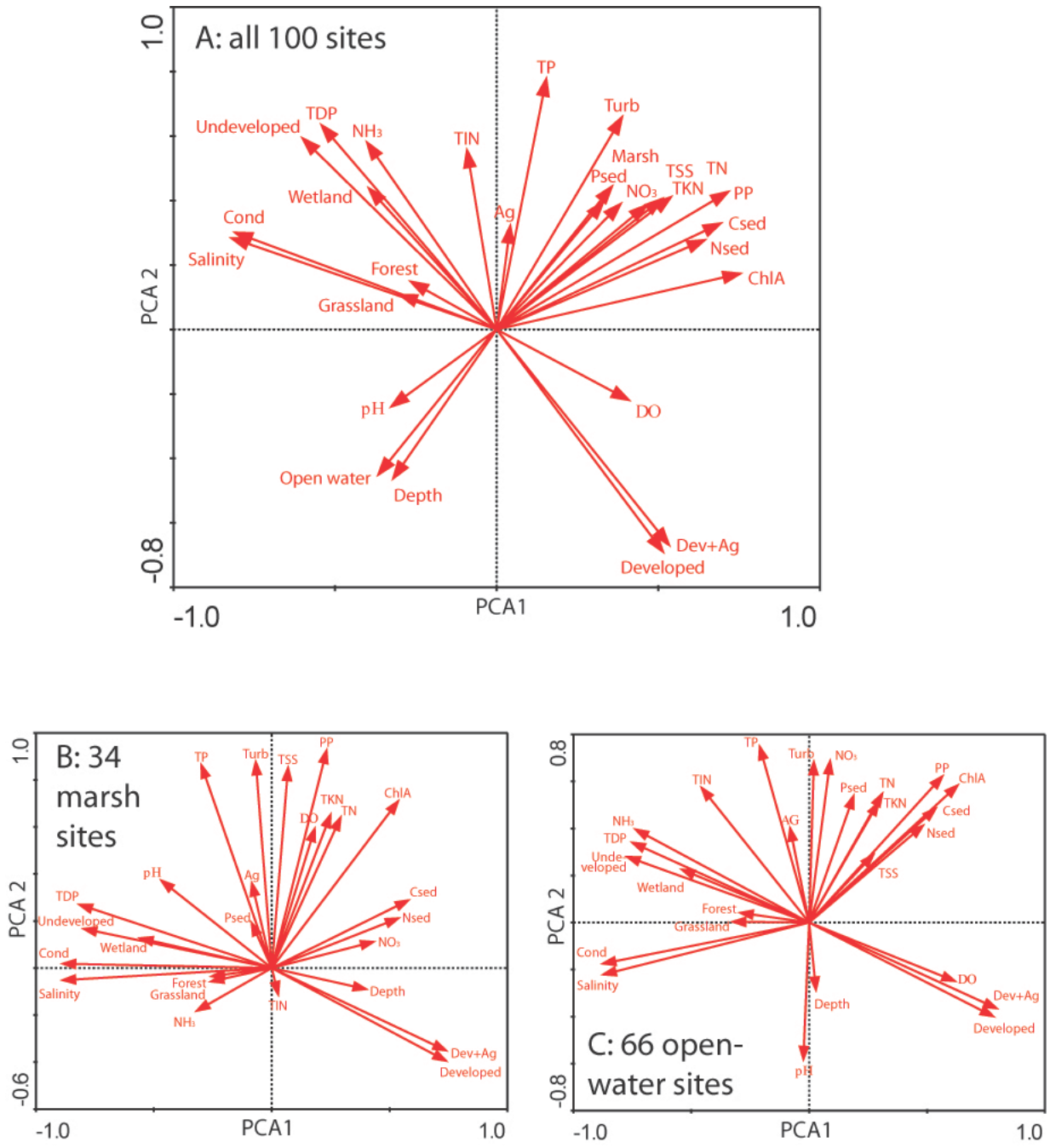

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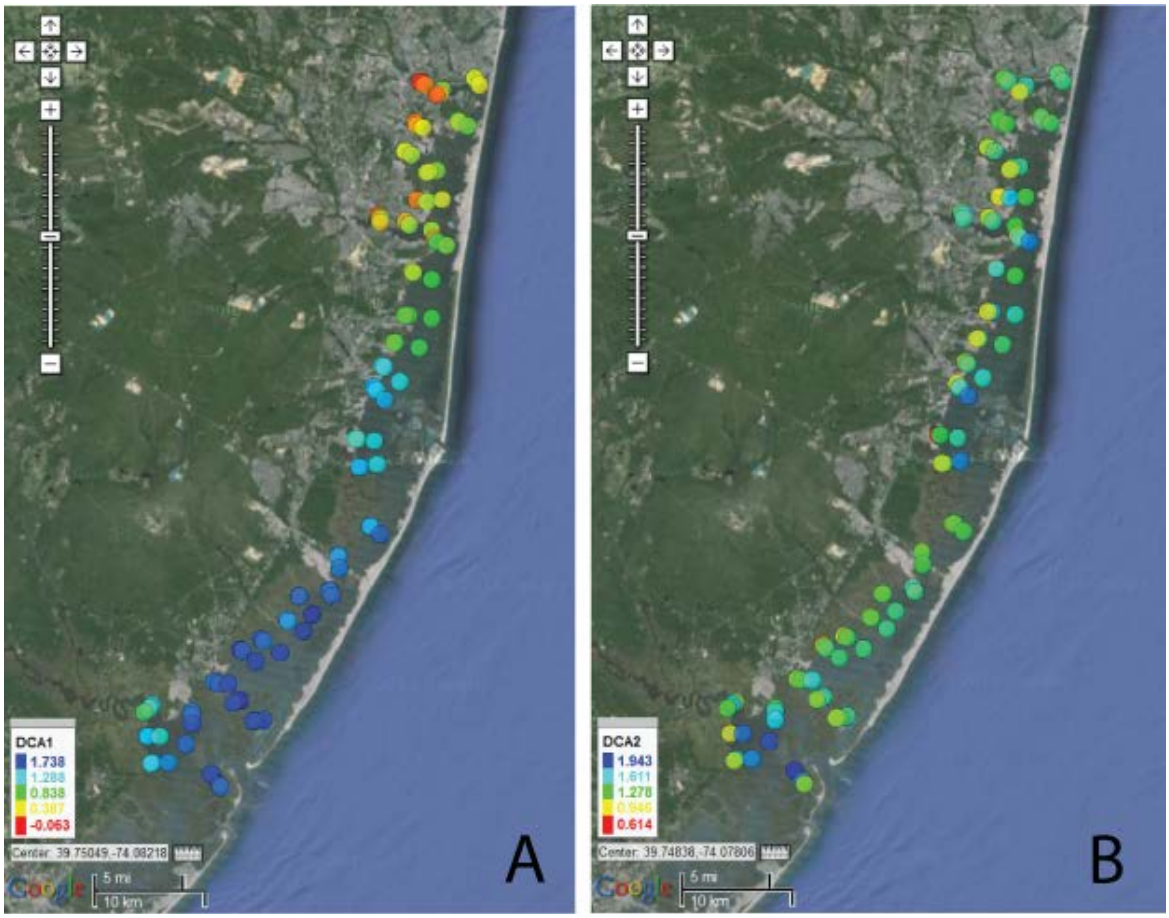

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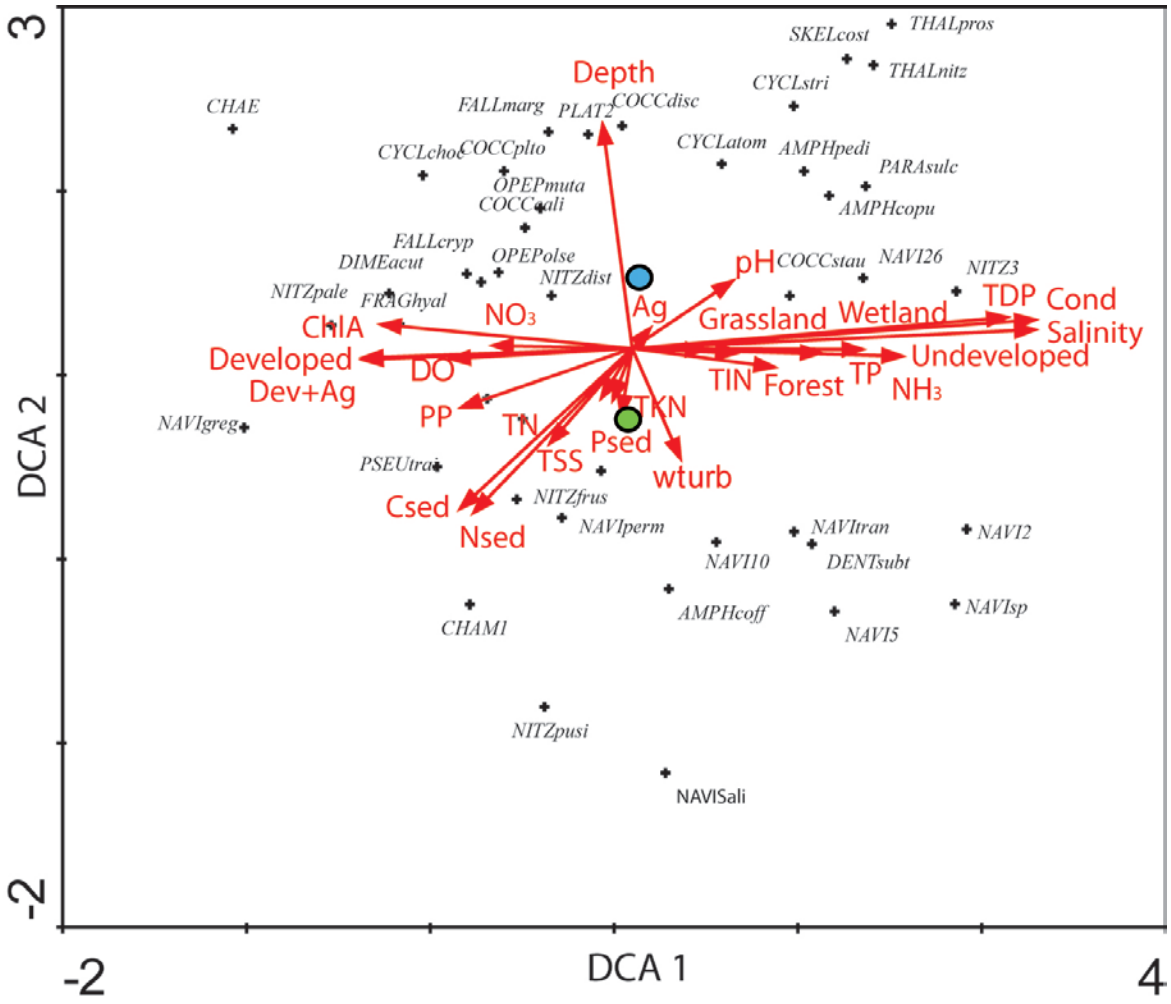

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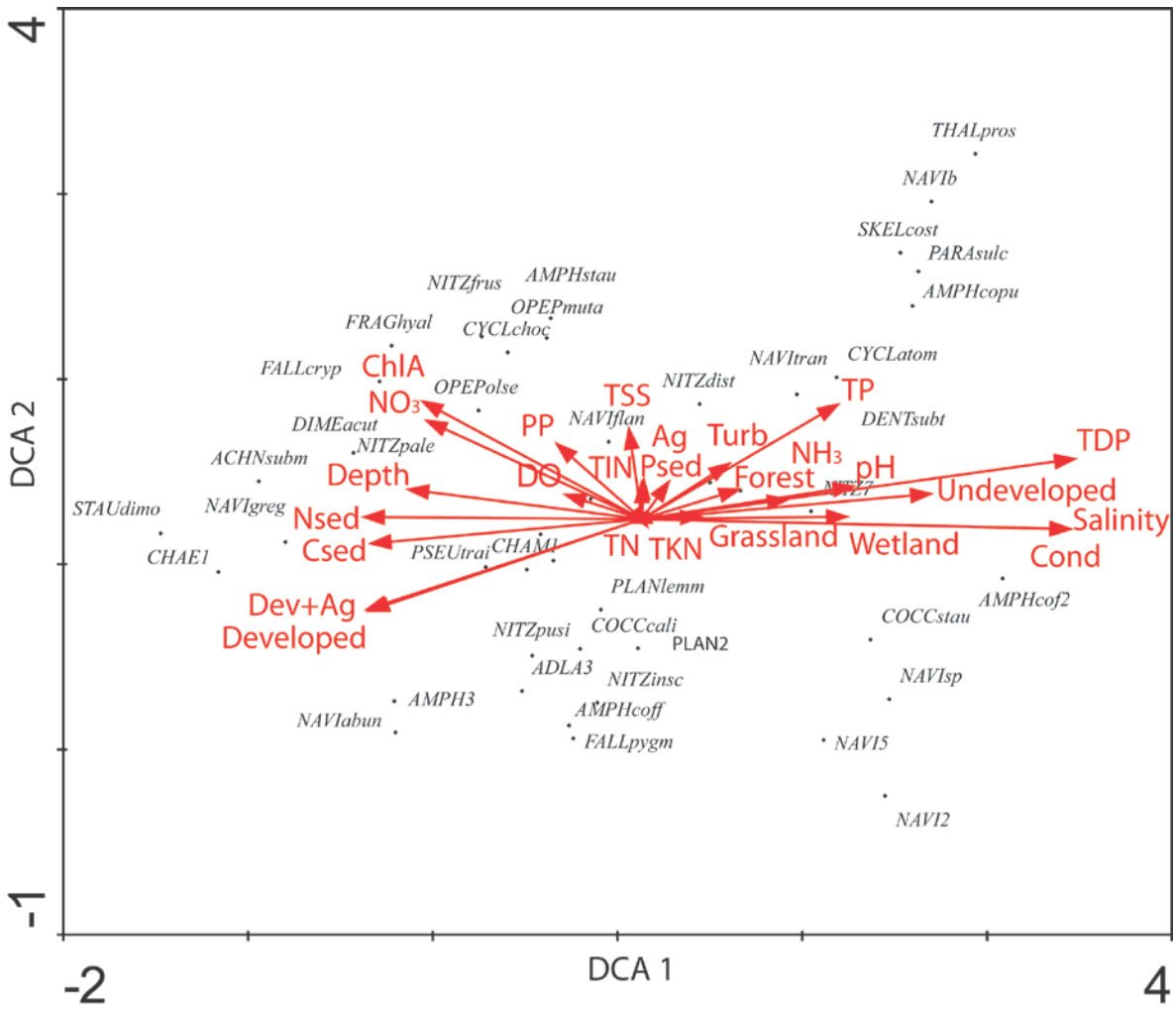

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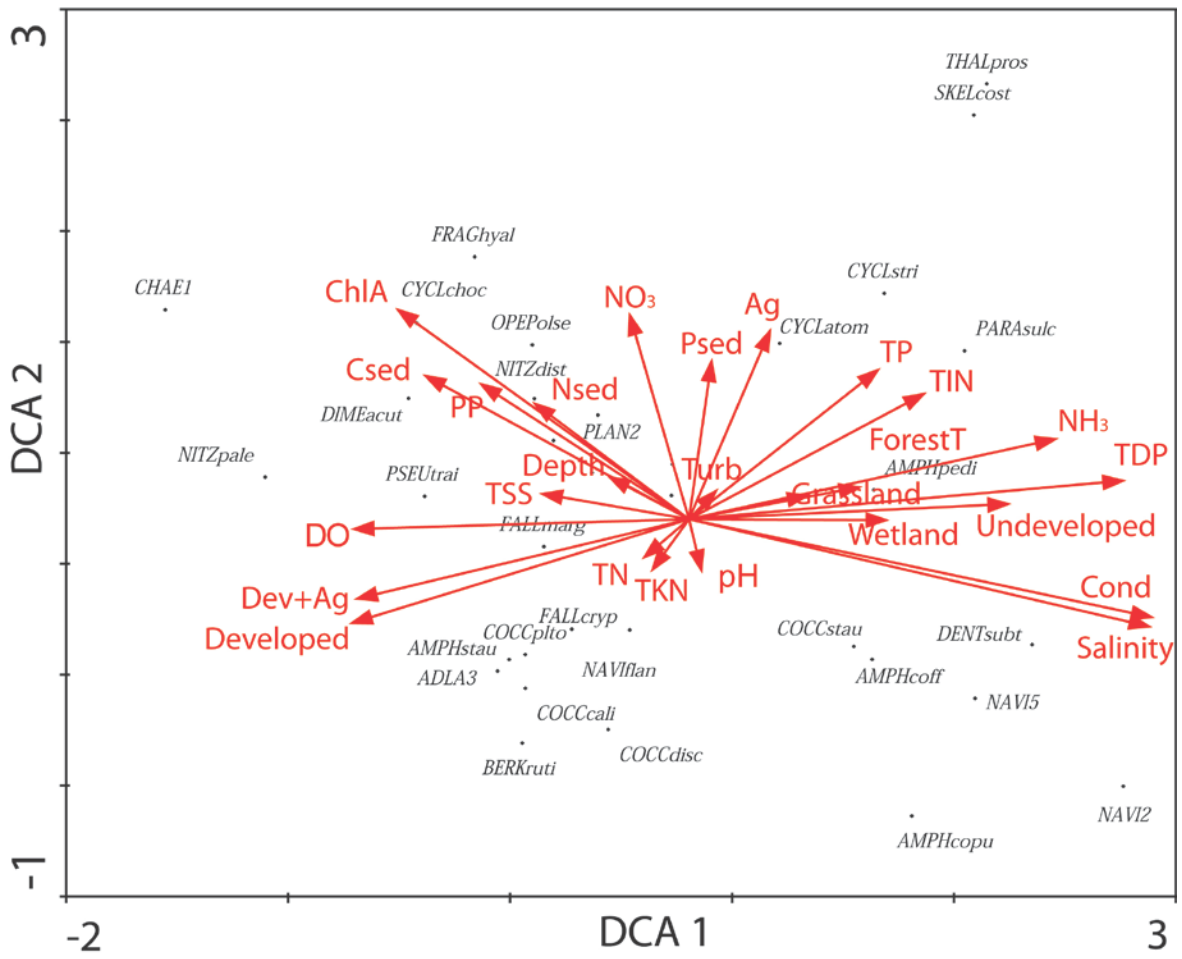

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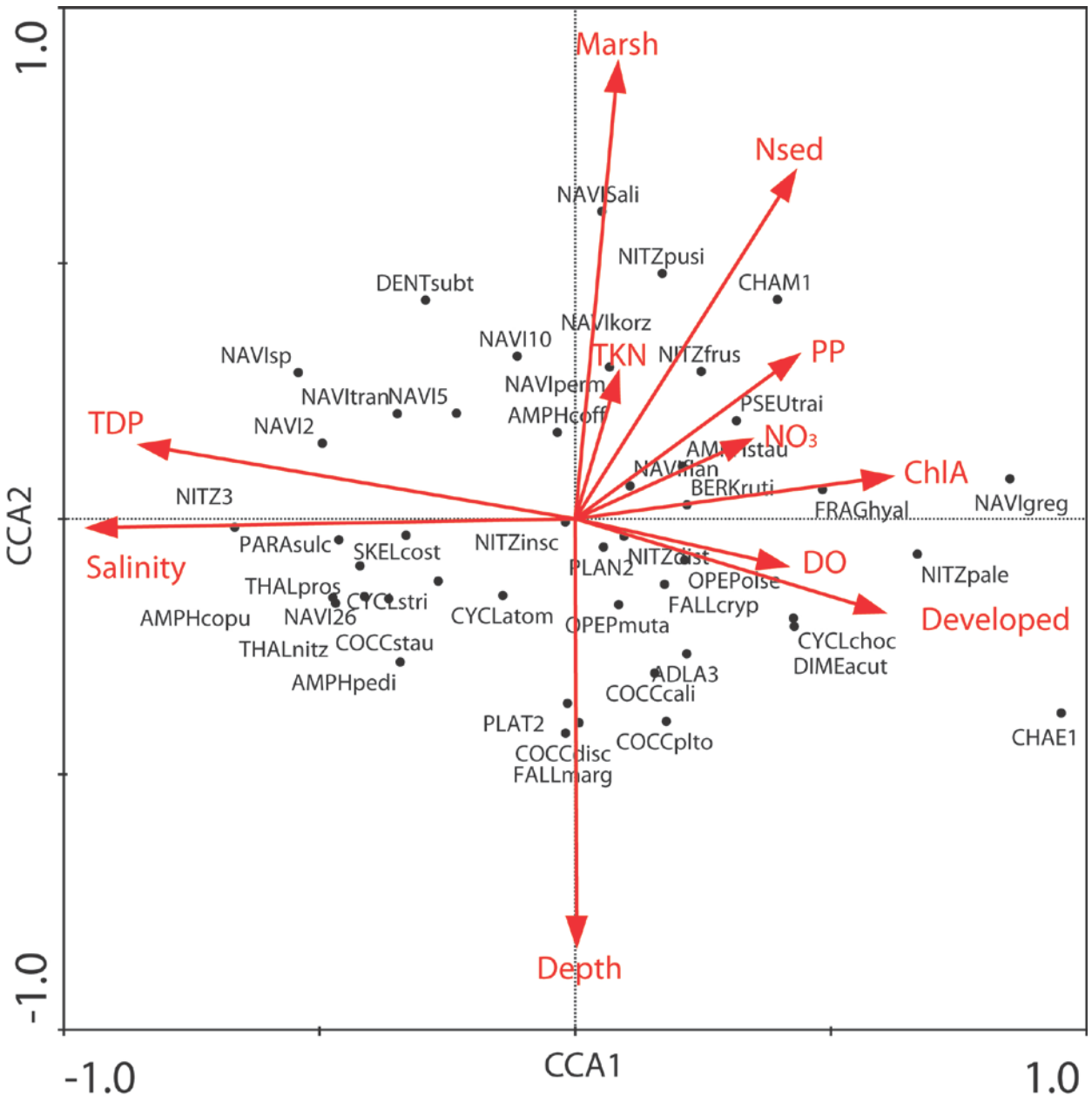

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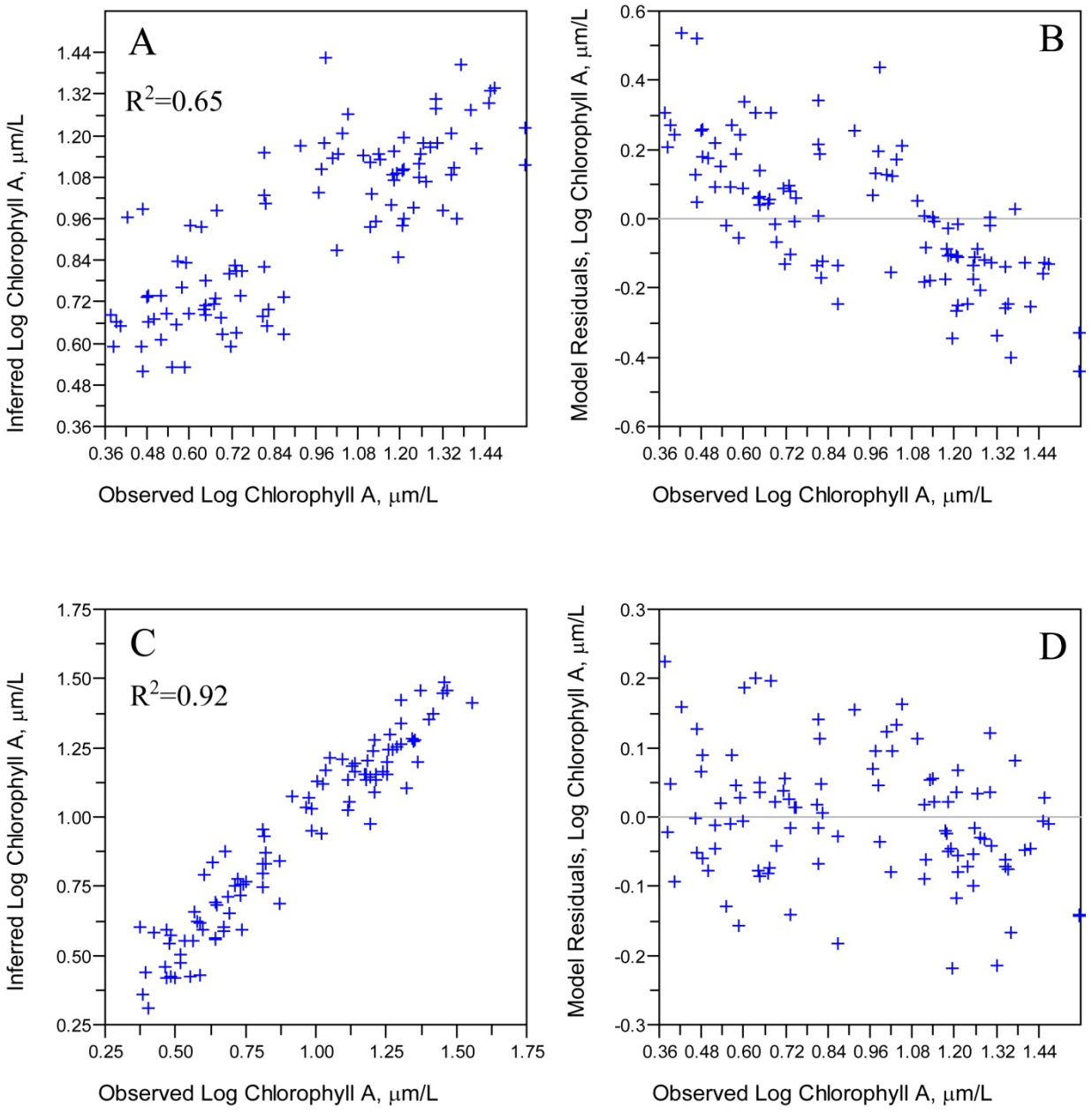

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Appendices

APPENDIX I. Excel file with sampling information and environmental (field measured) parameters.

APPENDIX II. Excel file with sediment and water chemistry data.

APPENDIX III Excel file with land-use data.

APPENDIX IV. Excel file with diatom species data, counts.

APPENDIX V Excel file with data prepared for analysis, contains several spreadsheets: environmental variables, species variables, and correlations among environmental variables.