NJ Department of Environmental Protection

Division of Science and Research

Characterization of Phytoplankton Community Changes in Barnegat Bay Related to the Closure of Oyster Creek Nuclear Generating Station, Combining Next Generation Sequencing and Microscopic Analyses

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What was the purpose of the study?

The Oyster Creek Nuclear Generating Station (OCNGS) in Forked River, Lacey Township, NJ, closed in September of 2018. The plant exerted significant stresses on the ecological and biological communities of Barnegat Bay during its 50 years of operation. Closure of the OCNGS provided the NJDEP the opportunity to engage with researchers to investigate ecosystem and biotic community (i.e., phytoplankton, zooplankton, benthic invertebrates, fish, and crabs) response pre- and post-closure. This project examined how the OCNGS closure influenced phytoplankton community composition and dynamics using both light microscopy observations and DNA analyses.

What was the general approach to the study (methods)?

To understand the impact of OCNGS decommissioning on phytoplankton, species composition and abundances were examined from 2018 to 2021 and compared with results from previous phytoplankton studies performed under the Barnegat Bay Comprehensive Research Initiative. Three study sites were located close to the OCNGS: 1661A, 1663A, and BB07a. Two more sites, BB01, located at the northern end of Barnegat Bay, and BB09, close to Barnegat Inlet, were used as reference sites since both sites are located further from the OCNGS (Fig. 1). The impact of the OCNGS shutdown was assessed by comparing water quality and phytoplankton composition changes at 1661A, 1663A, and BB07a before and after the beginning of decommissioning in the week of September 17, 2018. Phytoplankton composition was analyzed using both light microscopy and metabarcoding developed using 16S rRNA and 18S rDNA gene markers. Water quality data was retrieved from EPA's Water Quality Portal (Water Quality Data | US EPA).

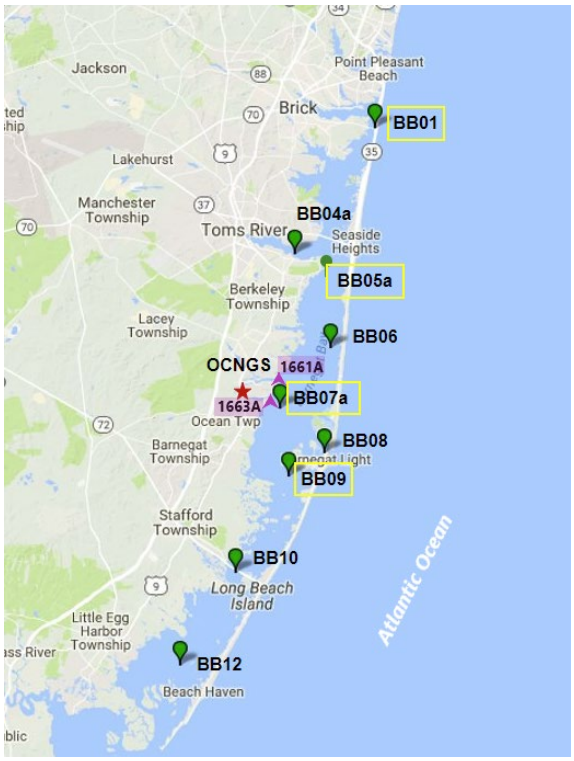


Fig. 1. Location of sampling sites. Sites with yellow border were sampled starting in 2012 when the Barnegat Bay phytoplankton baseline study began. Pink highlighted sites, 1661A and 1663A, are located closest to OCNGS (star symbol) and together with site BB07a represent OCNGS impacted sites. 1661A and 1663A were sampled starting August 2018. BB01 and BB09 represent reference sites in this study.

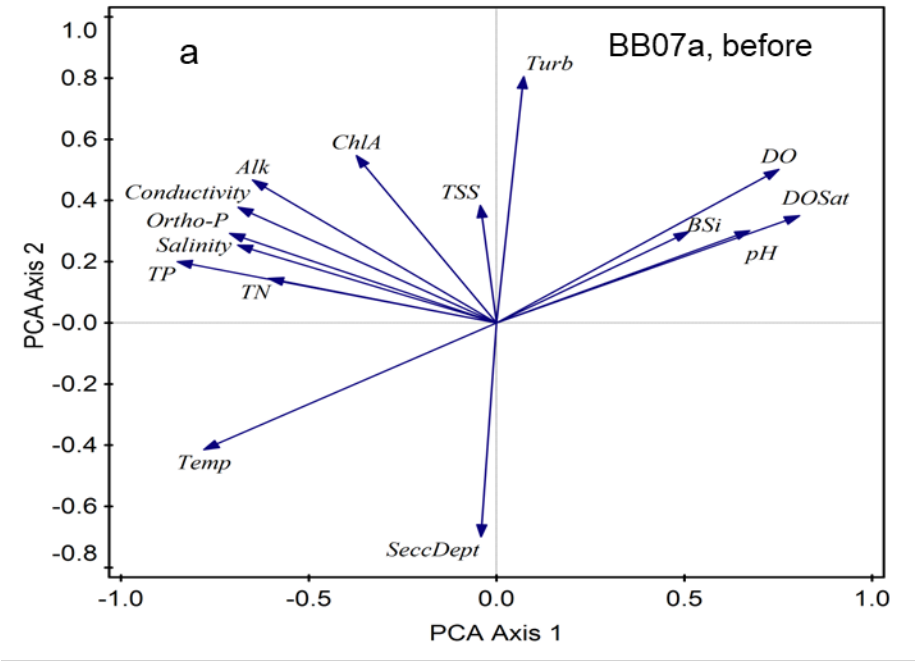
Monthly and inter-annual changes of phytoplankton species composition and relationships to environmental variables were explored using multivariate analyses to understand the immediate effects of the OCNGS decommission at the sites located near the mouths of Forked River (1661A) and Oyster Creek (1663A), as well as the long-term effects using monitoring data available for BB07a, BB01, and BB09 since 2012. Results from these analyses were used to assess the changes that occurred 'before' and 'after' the OCNGS closure using two data sets: a first data set comprises short-term water quality and phytoplankton measurements performed from August 2018 to December 2021 for the two closest OCNGS stations 1661A, 1663A; and a second dataset contains long-term measurements from April 2012 to December 2021 divided into before closure (data up to August 2018), and after closure (September 2018 to December 2021) for stations BB07a, BB01 and BB09.

Overall, what did the studies show?

Overall, the study revealed the magnitude and duration of changes in water quality characteristics, the impacts of water quality changes exerted on phytoplankton communities before and after the September 2018 OCNGS decommissioning, and the potential of phytoplankton DNA analyses in future applications.

a) *Changes in water quality characteristics*. The construction and operations of OCNGS altered the hydrology, chemistry, and biology of the Forked River, Oyster Creek, and adjacent Barnegat Bay (Summers et al. 1989). The construction of the facility changed the flow of the water in Forked River and Oyster Creek. It made them a physical extension of Barnegat Bay with salinity ranges around ~25 ppt, similar to that in the center of the Bay (Summers et al. 1989). Evident changes in response to the OCNGS shutdown were found in this study. Water quality data showed a salinity drop of 4-8 ppts as well as a gradual drop in temperature during the 2018 stepwise OCNGS decommissioning at the three closest sites 1661A, 1663A, and BB07a. The salinity decrease persisted over the winter of 2018 to early spring of 2019 at all three sites, suggesting a greater influence of freshwater input in the area. This trend was followed by a slight increase and overall high salinity variability in the following 2019-2021 time period. Although no permanent decrease in salinity was found near the mouths of Forked River and Oyster Creek, multivariate analyses of long-term data for stations BB07a, BB01, and BB09 revealed a shift in salinity and nutrients relationships for the post-OCNGS closure period, suggesting an increased effect of freshwater input in the salinity-nutrients dynamics.

Correlations between the water quality parameters were analyzed using an ordination method called principal component analysis (PCA). PCA is a statistical procedure that reduces the dimensionality of a large data set by producing new variables called 'principal components' that explain the maximal amount of variance. A PCA graph produces new axes that provide a simplified way to see and evaluate the data so that relationships between observations are better visible. A PCA conducted on water quality parameters revealed a positive relationship between salinity and nutrients before the shutdown at all three long-term stations. After the shutdown, nutrients were in reverse relationship to salinity at BB07a (Fig. 2) and BB09, whereas at BB01, which is furthest from the OCNGS, salinity and nutrients showed no or weak relation. This finding suggests that the shutdown of OCNGS resulted in freshwater inflows from Oyster Creek, which were higher in nutrient concentration than the Bay waters, thus increasing nutrients in the Barnegat Bay area near stations BB07a and BB09.



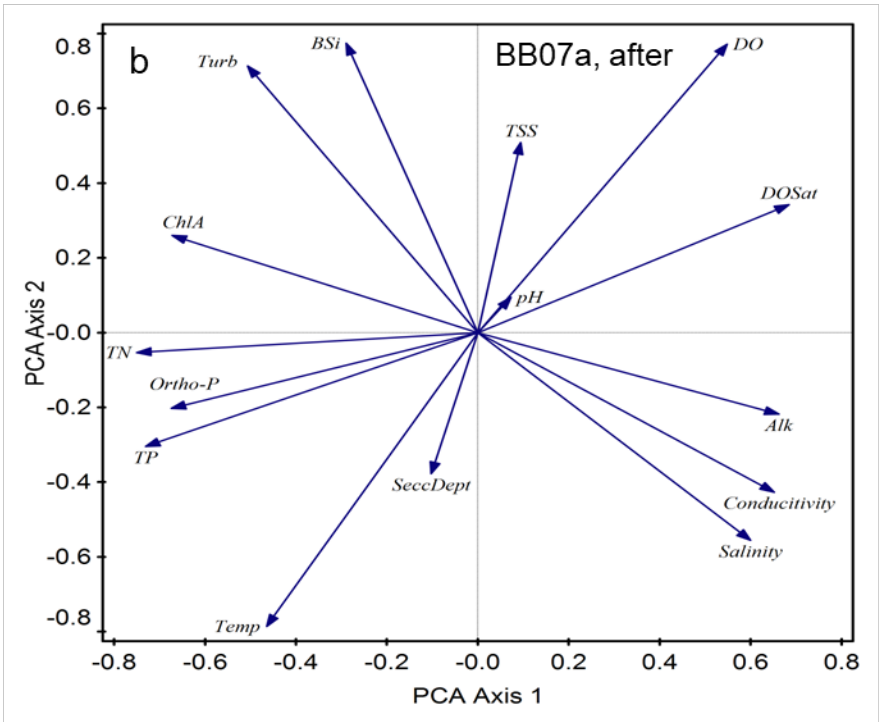


Fig. 2. Correlations of environmental variables derived from PCA at BB07a (the station closest to OCNGS) before and after the closure of OCNGS. The 'before' dataset includes monthly data from April 2014 to August 2018; the 'after' dataset includes monthly data from September 2018 to December 2021. A reverse relationship between nutrients and salinity is visible in the before/after diagrams. *Note that environmental parameters (vector arrows) increase in the direction of the arrow and decrease in the opposite direction; angles between vector arrows approximate their correlations.*

b) *Impacts of water quality changes on phytoplankton communities*. Phytoplankton growth and composition can change quickly in response to hydrological, physical, and chemical modification due to their nature of floating, tiny sizes, and fast-growing rate (O'Boyle and Silke 2010). In this study, a large dissimilarity was found between the phytoplankton communities at the mouth of Forked River (site 1661A) and the mouth of Oyster Creek (site 1663A) in August 2018 compared to the October 2018, coinciding with the first step shutdown of OCNGS. Subsequently, more similarity was recorded by December after the shutdown of the secondary circulation pumps in November suggesting a move towards equilibrium between Oyster Creek and Forked River phytoplankton communities. Occasional high variations in the following months/years were observed in the similarity/dissimilarity of these two stations, sometimes exceeding the August-October dissimilarity.

A comparison of the phytoplankton at BB07a was made based on samples collected in August from 2012 to 2021 to reflect changes related to the OCNGS decommissioning. The dissimilarity of the August samples in 2018 vs. 2019 was comparable to that observed in August 2012 vs. August 2013 when the latter was heavily impacted by Hurricane Sandy. For the September samples, the largest dissimilarity was detected from 2018 vs. 2019. However, similar or larger dissimilarities were occasionally observed in phytoplankton assemblages for other months during the entire sampling period.

A multivariate analysis named canonical correspondence analysis (CCA) was used to explore relationships between phytoplankton community changes and water quality parameters. A CCA is a direct gradient analysis that allows the biological community variation to be directly related to the environmental variation. It is an ordination technique that can display patterns of variation in community composition that are best explained by the environmental variables and provides diagrams that effectively summarize relationships between biological communities and environment. The CCA showed that changes in phytoplankton communities were significantly related to environmental variables over the entire study period. However, there was an increase of the explanatory power of the environmental variables for all three long-term sites after the OCNGS closure, with BB07a increasing the most and BB01 the least, coincident with their distance from the OCNGS (Table 1).

Table 1: Explanatory power of environmental variables showing changes of phytoplankton before and after OCNGS closure at stations BB07a, BB01, and BB09, derived from CCA analyses. Environmental variables with high significance level are listed.

BB07a # explanatory variables : 12

Before decommission accounted for 38% of community variations					After decommission accounted for 50% of community variations				
Name	**Explains %**	**Contribution %**	**pseudo-F**	**P**	**Name**	**Explains %**	**Contribution %**	**pseudo-F**	**P**
Temp	6.2	16.5	2.3	0.002	DO	8.7	17.4	2.5	0.002
Secchi depth	4.4	11.7	1.6	0.008	Temp	6.3	12.6	1.8	0.002
DO	4.5	11.9	1.7	0.002	Salinity	5.1	10.2	1.5	0.006
TP	3.8	9.9	1.4	0.014	Secchi depth	3.8	7.7	1.2	0.172

BB01 # explanatory variables : 11

Before decommission accounted for 42% of community variations					After decommission accounted for 46% of community variations				
Name	**Explains %**	**Contribution %**	**pseudo-F**	**P**	**Name**	**Explains %**	**Contribution %**	**pseudo-F**	**P**
Temp	7	16.8	2.4	0.002	TN	9.6	19.2	2.6	0.002
Secchi depth	5.3	12.7	1.9	0.002	Salinity	5.8	11.7	1.7	0.004
Salinity	4.6	11	1.6	0.004	DO	5.4	10.9	1.6	0.006
pH	4.4	10.5	1.6	0.006	Ortho-P	4.4	8.9	1.3	0.044
TP	3.9	9.3	1.5	0.016	Turbidity	4.2	8.4	1.3	0.1
TN	3.4	8	1.3	0.064					

BB09 # explanatory variables: 14

Before decommission accounted for 44% of community variations					After decommission accounted for 53% of community variations				
Name	**Explains %**	**Contribution %**	**pseudo-F**	**P**	**Name**	**Explains %**	**Contribution %**	**pseudo-F**	**P**
Ortho-P	5.3	12	1.9	0.002	DO	8.2	15.3	2.3	0.002
Secchi depth	4.3	9.7	1.6	0.01	Temp	4.7	8.9	1.4	0.046
Chl. *a*	4.4	9.8	1.6	0.018	pH	4.5	8.5	1.3	0.108
TP	3.4	7.7	1.3	0.064	Salinity	4.3	8.1	1.3	0.112

The changes observed at the study sites resulting from the OCNGS decommission are difficult to disentangle from the regular and very dynamic hydrological, chemical, and biological processes taking place in Barnegat Bay. In addition, the impact of weather conditions, such as wind and precipitations, tides, small-scale water advection, and circulation, are not considered throughout the sampling periods.

c) *Phytoplankton DNA analyses*. Phytoplankton metabarcoding was developed using 16S rRNA and 18S rDNA gene markers and compared with the microscopy method. The combination of 16S rRNA marker and NextGen Sequencing generated high-throughput data and provided information on photosynthetic cyanobacteria and eukaryotic phytoplankton, its community diversity and composition, as well as the relative abundance of major taxonomic groups. A significant correlation between microscopic counts and DNA-based relative abundance of four diatom classes (Skeletonemaceae, Cymatosiraceae, Chaetocerotaceae, and Melosirales) was obtained from the 2018-2019 data. Although limitations exist in the classification resolution for nano-phytoplankton and dinoflagellates, the method has advantages over light microscopy for picoplankton and was able to distinguish major groups in picoplankton complex, which are often challenging by the traditional microscopy methods. The test of the three sets of 18S rDNA primer sets showed that each primer set has its strengths and weaknesses in phytoplankton classification. 18S-Dino gave better resolution in dinoflagellates but was comparable to PhytoREF in terms of the classifications of other phytoplankton groups. The V3 87 Oxy primer set did not appear to be advantageous in most taxonomic groups; however, it was able to distinguish Prymnesiophytes and the genus *Aureococcus*, which contain *Prymnesium parvum* and brown tide *A. anophagefferens*, respectively, both classified as harmful algae.

How will DEP use the data?

Phytoplankton is a microscopic group of organisms of major importance for environmental condition assessment in aquatic ecosystems. This project proved that phytoplankton can be successfully used to assess changes in Barnegat Bay related to the OCNGS closure, capturing changes in water quality parameters and their interactions with phytoplankton species composition. This finding highlights the potential use of the phytoplankton for future assessments of anthropogenic disturbances on Barnegat Bay and subsequent impacts on higher trophic levels, such as zooplankton, fish, and filter feeders, which depend on their species composition and quantity. Changes recorded in phytoplankton composition and abundances provided by this project complement the results provided by other projects dedicated to the assessment of the OCNGS closure impacts and contribute to a more holistic assessment of the Bay changes related to this event. The study also highlighted that the impacts of the OCNGS closure on phytoplankton were rather local and short-lived, while climate and other anthropogenic factors have the potential to induce higher magnitude and larger scale changes in the Barnegat Bay phytoplankton. Another major highlight of this project is that it demonstrated the utility of phytoplankton DNA analyses and the potential application in future

condition assessments and monitoring efforts in New Jersey coastal ecosystems. In addition, this study identified some existing gaps in the phytoplankton DNA databases and provided a foundation for further research in developing metabarcoding and its environmental condition applications.

Please review the full report for more detailed information at
https://hdl.handle.net/10929/137567

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Who to contact with further questions.

For additional information or questions regarding this study, please contact Mihaela Enache (mihaela.enache@dep.nj.gov) in the NJDEP Division of Science and Research.

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